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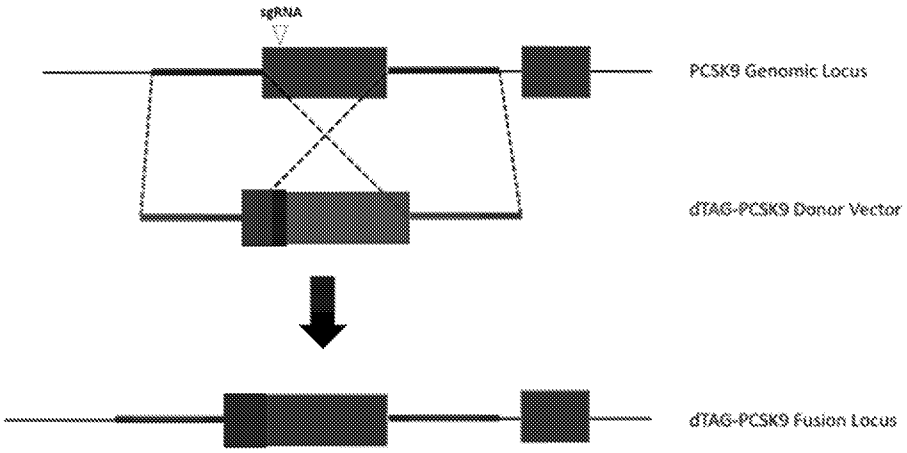

FIG. 2

(57) Abstract: The present invention provides a means to modulate gene expression in vivo in a manner that avoids problems associated with CRISPR endogenous protein knock-out or knock-in strategies and strategies that provide for correction, or alteration, of single nucleotides. The invention includes inserting into the genome a nucleotide encoding a heterobifunctional compound targeting protein (dTAG) in-frame with the nucleotide sequence of a gene encoding an endogenously expressed protein of interest which, upon expression, produces an endogenous protein-dTAG hybrid protein. This allows for targeted protein degradation of the dTAG and the fused endogenous protein using a heterobifunctional compound.

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TUNABLE ENDOGENOUS PROTEIN DEGRADATION WITH HETEROBIFUNCTIONAL COMPOUNDS

Related Applications

This application claims the benefit of provisional U.S. Patent Application No. 62/456,654, filed February 8, 2017, and provisional U.S. Patent Application No. 62/457,127, filed February 9, 2017. The entirety of these applications are hereby incorporated by reference for all purposes.

Incorporation by Reference

The contents of the text file named "16010-025WO1_sequencelisting_ST25.txt" which was created on January 26, 2018, and is 287 kilobytes in size, are hereby incorporated by reference in their entirety.

Field of the Invention

This invention describes methods, compounds, and compositions to modulate an endogenously expressed protein using targeted protein degradation.

Background

Many tools have been developed to manipulate gene expression to interrogate the function of a gene or protein of interest. For example, techniques such as RNA interference and antisense deoxyoligonucleotides are commonly used to disrupt protein expression at the RNA and DNA level. Homologous recombination or loss-of-function mutations can be accomplished using site-specific double-strand breaks using zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), or clustered regulatory interspaced short palindromic repeat (CRISPR)-Cas9 (Cheng, J.K. and Alper, H.S., "The genome editing toolbox: a spectrum of approaches for targeted modification" *Curr. Opin. Biotechnol.*, 30C, (2014): 87-94; and Graham et al., *Gen Biol*, (2015): 16:260). The CRISPR-Cas9 system has been used to modulate endogenous gene expression by incorporating specific mutations into a gene of interest (see, for example, Lo et al., Genetics, 2013; 195(2): 331-348; Yu et al., Biology Open, 2014; 3:271-280; Park et al., PLOS One, 2013; 9(4):e95101; Lackner et al., Nature Communications, 2015; 17(6): 1-7; U.S. Patent No. 8,771,945 and 9,228,208; WO 2014/204729; and U.S. Publication 2014/0273235).

For example, the CRISPR-Cas9 system was employed to mutate the human PCSK9 gene in chimeric liver-humanized mice bearing human hepatocytes (Wang, X., et al. "CRISPR-Cas9 Targeting of PCSK9 in Human Hepatocytes In Vivo." *Arteriosclerosis, Thrombosis, and Vascular Biology*, (2016).). PCSK9 was successfully mutated and the CRISPR-Cas9 system has been proposed to be useful as a way to treat human disorders in vivo. However, the long-term implications of permanent genome modification are unknown and concerns exist over the imperfect precision of genome editing, the continuous activity of virally-delivered CRISPR-Cas9, and the impact of direct correction in adults where biological compensation mechanisms may exist (Kormann et al., "Expression of therapeutic proteins after delivery of chemically modified mRNA in mice" *Nat. Biotechnol.*, 29, (2011):154-157; Cho et al., "Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases." *Genome Res.*, 24, (2014):132-141). Furthermore, CRISPR knock-out strategies may be undesirable where the protein expressed, even if imperfect, is essential for cellular function.

Efforts have been made to modulate gene expression in vitro using inducible degradation systems. For example, the auxin-inducible degradation (AID) system in plants has enabled controlled protein depletion in yeast and cultured vertebrate cells. This system relies on expression of a plant-specific F-box protein, TIR1, which regulates diverse aspects of plant growth and morphogenesis in response to the phytohormone auxin. TIR1 is the substrate recognition component of a Skp1-Cullin-F-box E3 ubiquitin ligase complex, which recognizes substrates only in the presence of auxin and targets them for degradation by the proteasome. This system has been manipulated and shown to enable conditional auxin-dependent protein depletion in Caenorhabditis elegans as well as in human HCT116 cells (see, for example, Zhang et al., Development, 2015; 142: 4374-4384 and Natsume et al., Cell Reports, 2016; 15: 210-218). However, this approach is impractical as an in vivo modulation system due to the toxicity of auxin.

An alternative approach to reversibly controlling gene expression has been the use of ligand-dependent destabilization domains and the Shield-1 ligand, which allows for reversible stabilization and destabilization of a tagged protein of interest in a dose-dependent manner (see, for example, Rakhit et al., Chemistry & Biology, 2014; 21: 1238-1252). Fusing the destabilizing domain to a gene of interest results in the expression a fused protein that is degraded by the proteasome. Shield-1 binds specifically to the destabilization domain and inactivates protein degradation. However, this system is also not viable as an in vivo modulation strategy due to the

requirement for the presence of Sield-1 in the cell cytoplasm in order to avoid degradation. Such an approach would require a constant administration of Shield-1 to maintain protein stability.

Thus, there remains an unmet need for improved systems that allow for reversible control of endogenous gene expression in vivo while providing improved treatment modalities in subjects suffering from disorders such as proteopathies.

It is therefore an object of the present invention to provide methods, compounds, and compositions to modulate gene expression in vivo in a manner that avoids problems associated with CRISPR endogenous protein knock-out or knock-in strategies.

Summary of the Invention

The present invention provides a means to modulate gene expression in vivo in a manner that avoids problems associated with CRISPR endogenous protein knock-out or knock-in strategies and strategies that provide for correction, or alteration, of single nucleotides. The invention includes inserting into the genome a nucleotide encoding a heterobifunctional compound targeting protein (dTAG) in-frame with the nucleotide sequence of a gene encoding an endogenously expressed protein of interest which, upon expression, produces an endogenous protein-dTAG hybrid protein. This allows for targeted protein degradation of the dTAG and the fused endogenous protein using a heterobifunctional compound in a controlled, tunable fashion.

A heterobifunctional compound, as contemplated herein, is a compound that binds to an ubiquitin ligase through a ubiquitin ligase binding moiety and also binds to the dTAG through its dTAG Targeting Ligand in vivo, as defined in more detail below. Heterobifunctional compounds are capable of induced proteasome-mediated degradation of the fused endogenous proteins via recruitment to E3 ubiquitin ligase and subsequent ubiquitination. These drug-like molecules offer the possibility of reversible, dose-responsive, tunable, temporal control over protein levels.

Compared to CRISPR-Cas9 genome editing that incorporates irreversible changes into a gene of interest, the use of a heterobifunctional compound to target endogenously expressed proteins with a dTAG allows for reversible control of the endogenously expressed protein of interest. Accordingly, the heterobifunctional compound can be used as a rheostat of protein expression affording the ability to turn endogenous protein expression on and off upon titration of the heterobifunctional compound. Furthermore, by genomically and stably incorporating a nucleic

acid sequence encoding a dTAG in frame, either 5'- or 3'- to the gene of the endogenous protein, side effects associated with CRISPR-Cas9 such as negative downstream consequences associated with permanently editing a gene can be avoided.

The invention provides a mechanism to control the degradation of endogenous proteins that mediate a disease by combining genome engineering with small molecule activation/modulation of degradation. The methods and compositions described herein are particularly useful for targeting endogenous proteins associated with disease due to a gain of function, toxic accumulation, overexpression, or downstream enzymatic process the protein may be involved in. Applications of this technology include, but are not limited to 1) targeted degradation of proteins where pathology is a result of gain of function mutation(s), 2) targeted degradation of proteins where pathology is a function of amplification or increased expression, 3) targeted degradation of proteins that are manifestations of monogenetic disease, 4) targeted degradation of proteins where genetic predisposition manifests over longer periods and often after alternative biological compensatory mechanisms are no longer adequate, for example, but not limited to, hypercholesterolemia and proteinopathies.

Therefore, in one embodiment, a method is provided that includes at least the steps of:

(i) transforming relevant cells of a subject, typically a human, with a nucleic acid sequence encoding a dTAG, wherein the nucleic acid sequence is integrated genomically in-frame with a nucleic acid sequence of an endogenous protein which is acting as a mediator of disease, wherein insertion of the nucleic acid encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid or fusion protein upon expression; and

(ii) administering to the subject, as needed, a heterobifunctional compound which binds to a) the inserted dTAG and b) a ubiquitin ligase in a manner that brings the dTAG (and thus the endogenous protein-dTAG hybrid protein) into proximity of the ubiquitin ligase, such that the endogenous protein-dTAG hybrid protein is ubiquitinated, and then degraded by the proteasome.

In one embodiment, the subject's cell is transformed in vivo. In one embodiment, the subject's cell is transformed ex vivo and administered back to the subject. In one embodiment, the subject's cell is a liver cell.

In one embodiment, a method is provided that includes the steps of:

administering to the subject, as needed, a heterobifunctional compound, wherein the subject has one or more cells which have been transformed with a nucleic acid sequence encoding a dTAG, wherein the nucleic acid sequence is integrated genomically in- frame in a 5' or 3' orientation with a nucleic acid sequence of an endogenous protein which is acting as a mediator of disease, wherein insertion of the nucleic acid encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid or fusion protein upon expression of the protein; and wherein the heterobifunctional compound binds to a) the inserted dTAG and b) a ubiquitin ligase in a manner that brings the dTAG (and thus the endogenous protein-dTAG hybrid protein) into proximity of the ubiquitin ligase, such that the endogenous protein-dTAG hybrid protein is ubiquitinated, and then degraded by the proteasome.

As contemplated herein, the synthetic gene encoding the endogenous protein of interest-dTAG hybrid is derived in vivo through the targeted insertion of a nucleic acid encoding the dTAG in-frame either 5'- or 3'- to the nucleic acid encoding the protein of interest. This results in an in-frame gene fusion that is susceptible to proteasome mediated degradation upon treatment with a heterobifunctional compound that is capable of binding the dTAG. In a main embodiment, the dTAG does not substantially interfere with the function of the endogenously expressed protein. In one embodiment, the dTAG is a non-endogenous peptide, which allows for heterobifunctional compound selectivity and minimizes off target effects upon administration of the heterobifunctional compound. In one embodiment, the dTAG is an amino acid sequence derived from an endogenous protein which has been modified, for example through a "bump" strategy (see, for example, (see Clackson et al., "Redesigning an FKBP–ligand interface to generate chemical dimerizers with novel specificity", *PNAS* 95 (1998):10437–10442, incorporated herein by reference), so that the heterobifunctional compound binds only to or preferentially to the modified amino acid sequence of the dTAG and not the corresponding endogenously expressed protein.

Also contemplated herein is a method for the in vitro allele-specific regulation of an endogenous protein through the targeted insertion of a nucleic acid sequence encoding a dTAG in frame either 5'- or 3'- to the genomic sequence encoding a protein of interest, wherein insertion of the nucleic acid encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid or fusion protein upon expression, wherein the endogenous protein-dTAG is capable of being degraded by a heterobifunctional compound which binds to a) the inserted dTAG and b) a ubiquitin ligase in a manner that brings the dTAG (and thus the endogenous

protein-dTAG hybrid protein) into proximity of a ubiquitin ligase, such that the endogenous protein-dTAG hybrid protein is ubiquitinated, and then degraded by the proteasome. By using the methods described herein to insert a nucleic acid encoding a dTAG in frame with a gene encoding an endogenous protein of interest, the expression of the resultant protein can be tightly controlled through the introduction of a heterobifunctional compound capable of binding the dTAG, resulting in the degradation of the endogenous protein. Importantly, by using a heterobifunctional compound, expression of the endogenous protein can be reversibly controlled, allowing for the examination of the effects of protein expression on the cell.

Accordingly, by regulating expression of endogenous proteins in this manner, downstream effects of modulating protein expression can be examined across a wide variety of proteins and cell types, and in various physiological conditions. Because the heterobifunctional compound concentration within the cell can be titrated, protein-dTAG hybrid protein concentrations within the cell can be finely tuned, allowing for the conditional alteration of protein abundance within the cell and the ability to alter phenotype within the cell on demand. In one embodiment, provided herein is a method of assessing protein expression attenuation in a cell comprising inserting a nucleic acid sequence encoding a dTAG in frame either 5'- or 3'- to a genomic sequence encoding a protein of interest, wherein insertion of the nucleic acid encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid or fusion protein upon expression, wherein the endogenous protein-dTAG is capable of being degraded by a heterobifunctional compound which binds to a) the inserted dTAG and b) a ubiquitin ligase in a manner that brings the dTAG (and thus the endogenous protein-dTAG hybrid protein) into proximity of a ubiquitin ligase, such that the endogenous protein-dTAG hybrid protein is ubiquitinated, and then degraded by the proteasome. In one embodiment, the heterobifunctional compound is administered to the cell so that the concentration of the protein-dTAG hybrid protein in the cell is partially degraded. In one embodiment, the heterobifunctional compound is administered to the cell so that the concentration of the endogenous protein-dTAG hybrid protein in the cell is completely degraded.

In one embodiment, provided herein is a method of identifying a protein target associated with a disease or disorder comprising inserting a nucleic acid sequence encoding a dTAG in frame either 5'- or 3'- to the genomic sequence encoding a protein of interest, wherein insertion of the nucleic acid encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid or fusion protein upon expression, wherein the endogenous protein-dTAG is capable

of being degraded by a heterobifunctional compound which binds to a) the inserted dTAG and b) a ubiquitin ligase in a manner that brings the dTAG (and thus the endogenous protein-dTAG hybrid protein) into proximity of a ubiquitin ligase, such that the endogenous protein-dTAG hybrid protein is ubiquitinated, and then degraded by the proteasome, and measuring the effects of protein degradation on the disorder or disease state of the cell. By using the methods described herein to insert nucleic acids encoding dTAGs in frame with a gene encoding an endogenous protein of interest, downregulation of various proteins can be examined and potential targets for treating disorders associated with a particular disease state can be identified. In addition, the current methods can be utilized to validate a potential protein being targeted as associated with a disease state.

In a particular embodiment, the dTAGs for use in the present invention include an amino acid sequence derived from an endogenous protein kinase. In one embodiment, the endogenous protein kinase amino acid sequence includes a mutation rendering the kinase inactive. In one embodiment, the mutation in the protein kinase occurs within a conserved kinase catalytic triad amino acid sequence. In one embodiment, the conserved kinase catalytic triad amino acid sequence is TVS. In one embodiment, the conserved kinase catalytic triad amino acid sequence is HRD. In one embodiment, the conserved kinase catalytic triad amino acid sequence is DFG. In one embodiment, the conserved kinase catalytic triad amino acid sequence is TRD. See Kornev et al., "Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism," PNAS 2006;103(47):17783–17788, incorporated herein by reference. In one embodiment, at least one of the catalytic triad amino acids is substituted for an alanine. In one embodiment, at least one of the catalytic triad amino acids is substituted for a glycine. In one embodiment, the heterobifunctional compound contains an allelic-specific ligand capable of selectively binding the mutant protein kinase sequence. In one embodiment, the mutant kinase is as described in Roskoski et al., "Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes," Pharmacological Research http://dx.doi.org/10.1016/j.phrs.2015.10.021, incorporated herein by reference and/or Roskoski et al., " A historical overview of protein kinases and their targeted small molecule inhibitors," Pharmaceutical Research (2015), http://dx.doi.org/10.1016/j.phrs.2015.07.10, incorporated herein by reference. In one embodiment, the dTAG is derived from a kinase that is an analog-sensitive kinase. In one embodiment, the mutant kinase is as described in Zhang et al., "Structure-guided

inhibitor design expands the scope of analog-sensitive kinase technology," ACS Chem Biol. 2013:8(9);1931-1938, incorporated herein by reference. In alternative embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from proteins selected from EGFR, BCR-ABL, ALK, JAK2, BRAF, LRRK2, PDGFRα, and RET. In one embodiment, the proteins contain one or more mutations. In one embodiment, the one or more mutations render the protein inactive.

In alternative embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from proteins selected from Src, Src, Pkd1, Kit, Jak2, Abl, Mek1, HIV integrase, and HIV reverse transcriptase.

In particular embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from endogenously expressed proteins such as FK506 binding protein-12 (FKBP12), bromodomain-containing protein 4 (BRD4), CREB binding protein (CREBBP), or transcriptional activator BRG1 (SMARCA4). In other embodiments, dTAGs for use in the present invention may include, for example, a hormone receptor e.g. estrogen-receptor protein, androgen receptor protein, retinoid x receptor (RXR) protein, or dihydrofolate reductase (DHFR), including bacterial DHFR. In other embodiments, the dTAG may include, for example, an amino acid sequence derived from a bacterial dehalogenase. In other embodiments, the dTAG, may include, amino acid sequences derived from 7,8-dihydro-8-oxoguanin triphosphatase, AFAD, Arachidonate 5-lipoxygenase activating protein, apolipoprotein, ASH1L, ATAD2, baculoviral IAP repeat-containing protein 2, BAZ1A, BAZ1B, BAZ2A, BAZ2B, Bcl-2, Bcl-xL, BRD1, BRD2, BRD3, BRD4, BRD5, BRD6, BRD7, BRD8, BRD9, BRD10, BRDT, BRPF1, BRPF3, BRWD3, CD209, CECR2, CREBBP, E3 ligase XIAP, EP300, FALZ, fatty acid binding protein from adipocytes 4 (FABP4), GCN5L2, GTPase k-RAS, HDAC6, hematopoietic prostaglandin D synthase, KIAA1240, lactoglutathione lyase, LOC93349, Mcl-1, MLL, PA2GA, PB1, PCAF, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, PHIP, poly-ADP-ribose polymerase 14, poly-ADP-ribose polymerase 15, PRKCBP1, prosaposin, prostaglandin E synthase, retinal rod rhodopsin-sensitive cGMP 3','5-cyclic phosphodiesterase subunit delta, S100-A7, SMARCA2, SMARCA4, SP100, SP110, SP140, Src, Sumo-conjugating enzyme UBC9, superoxide dismutase, TAF1, TAF1L, tankyrase 1, tankyrase 2, TIF1a, TRIM28, TRIM33, TRIM66, WDR9, ZMYND11, or MLL4. In yet further embodiments, the dTAG may include, for example, an amino acid sequence derived from MDM2.

In a particular embodiment, the dTAG is derived from BRD2, BRD3, BRD4, or BRDT. In certain embodiments, the dTAG is a modified or mutant BRD2, BRD3, BRD4, or BRDT protein. In certain embodiments, the one or more mutations of BRD2 include a mutation of the Tryptophan (W) at amino acid position 97, a mutation of the Valine (V) at amino acid position 103, a mutation of the Leucine (L) at amino acid position 110, a mutation of the W at amino acid position 370, a mutation of the V at amino acid position 376, or a mutation of the L at amino acid position 381.

In certain embodiments, the one or more mutations of BRD3 include a mutation of the W at amino acid position 57, a mutation of the V at amino acid position 63, a mutation of the L at amino acid position 70, a mutation of the W at amino acid position 332, a mutation of the V at amino acid position 338, or a mutation of the L at amino acid position 345. In certain embodiments, the one or more mutations of BRD4 include a mutation of the W at amino acid position 81, a mutation of the V at amino acid position 87, a mutation of the L at amino acid position 94, a mutation of the W at amino acid position 374, a mutation of the V at amino acid position 380, or a mutation of the L at amino acid position 387. In certain embodiments, the one or more mutations of BRDT include a mutation of the W at amino acid position 50, a mutation of the V at amino acid position 56, a mutation of the L at amino acid position 63, a mutation of the W at amino acid position 293, a mutation of the V at amino acid position 299, or a mutation of the L at amino acid position 306.

In a particular embodiment, the dTAG is derived from cytosolic signaling protein FKBP12. In certain embodiments, the dTAG is a modified or mutant cytosolic signaling protein FKBP12. In certain embodiments, the modified or mutant cytosolic signaling protein FKBP12 contains one or more mutations that create an enlarged binding pocket for FKBP12 ligands. In certain embodiments, the one or more mutations include a mutation of the phenylalanine (F) at amino acid position 36 to valine (V) (F36V) (referred to interchangeably herein as FKBP* or FKBP12*).

In one embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof from any of SEQ. ID. NOs.: 1-44. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 1. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 2. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 3. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 4. In a particular embodiment, the dTAG is derived from an

amino acid sequence, or fragment thereof of SEQ. ID. NO.: 5. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 6. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 7. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 8. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 9. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 10. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 11. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 12. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 13. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 14. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 15. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 16. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 17. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 18. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 19. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 20. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 21. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 22. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 23. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 24. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 25. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 26. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 27. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 28. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 29. In a particular embodiment,

the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 30. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 31. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 32. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 33. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 34. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 35. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 36. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 37. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 38. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 39. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 40. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 41. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 42. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 43. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 44. In a particular embodiment, the fragment thereof refers to the minimum amino acid sequence need to be bound by the heterobifunctional compound. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 62. In a particular embodiment, the dTAG is derived from an amino acid sequence, or fragment thereof of SEQ. ID. NO.: 63. In a particular embodiment, the fragment thereof refers to the minimum amino acid sequence needed to be bound by the heterobifunctional compound.

In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 1 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-1-dFKBP-5. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 2 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-6-dFKBP-13. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional

compound selected from any of dBET1-dBET18. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dBromo1-dBromo34. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 9 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dHalo1-dHalo2.

In one embodiment, the dTAG is derived from any amino acid sequence described herein, or a fragment thereof, and the dTAG is capable of being bound by a corresponding heterobifunctional compound comprising a dTAG Targeting Ligand capable of binding the dTAG described herein. In one embodiment, the dTAG is amino acid sequence capable of being bound by a heterobifunctional compound described in Figure 29, Figure 30, Figure 31, Figure 32, and Figure 33, or any other heterobifunctional compound described herein. In one embodiment, the dTAG is amino acid sequence capable of being bound by a heterobifunctional compound comprising a dTAG Targeting Ligand described in Table T. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 1 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-1-dFKBP-5. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 2 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-6-dFKBP-13. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dBET1-dBET18. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dBromo1-dBromo34. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 9 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dHalo1-dHalo2.In a particular embodiment, the dTAG is derived from CREBBP and the heterobifunctional compound contains a CREBBP dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from SMARCA4, PB1, or SMARCA2 and the heterobifunctional compound contains a SMARCA4/PB1/SMARCA2 dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from TRIM24 or BRPF1 and the heterobifunctional compound

contains a TRIM24/BRPF1 dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from a glucocorticoid receptor and the heterobifunctional compound contains a glucocorticoid dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from an estrogen or androgen receptor and the heterobifunctional compound contains an estrogen/androgen receptor dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from DOT1L and the heterobifunctional compound contains a DOT1L dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from Ras and the heterobifunctional compound contains a Ras dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from RasG12C and the heterobifunctional compound contains a RasG12C dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from HER3 and the heterobifunctional compound contains a HER3 dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from Bcl-2 or Bcl-XL and the heterobifunctional compound contains a Bcl-2/Bcl-XL dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from HDAC and the heterobifunctional compound contains a HDAC dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from PPAR and the heterobifunctional compound contains a PPAR dTAG Targeting Ligand selected from Table T. In a particular embodiment, the dTAG is derived from DHFR and the heterobifunctional compound contains a DHFR dTAG Targeting Ligand selected from Table T.

In one aspect, the synthetic gene of the present invention includes a gene of interest that is implicated in a genetic disorder. By way of a non-limiting example, a mutated gene, for example, encoding alpha-1 antitrypsin (A1AT), may be targeted for dTAG in frame insertion in a cell to produce a synthetic gene which encodes a hybrid protein capable of being degraded by a heterobifunctional compound that targets the dTAG of the endogenous A1AT-dTAG hybrid protein. By generating an A1AT-dTAG hybrid, the function of the mutated A1AT can be regulated or modulated through heterobifunctional compound administration, allowing the cell to maintain some function of the A1AT endogenous protein while reducing the effects of A1AT over-expression. Other non-limiting examples of proteins that may be targeted include β-catenin (CTNNB1), apolipoprotein B (APOB), angiopoietin-like protein 3 (ANGPTL3), proprotein convertase subtilisin/kexin type 9 (PCSK9), apolipoprotein C3 (APOC3), low density lipoprotein

receptor (LDLR), C-reactive protein (CRP), apolipoprotein a (Apo(a)), Factor VII, Factor XI, antithrombin III (SERPINC1), phosphatidylinositol glycan class A (PIG-A), C5, alpha-1 antitrypsin (SERPINA1), hepcidin regulation (TMPRSS6), (delta-aminolevulinate synthase 1 (ALAS-1), acylCaA:diacylglycerol acyltransferase (DGAT), miR-122, miR-21, miR-155, miR-34a, prekallikrein (KLKB1), connective tissue growth factor (CCN2), intercellular adhesion molecule 1 (ICAM-1), glucagon receptor (GCGR), glucorticoid receptor (GCCR), protein tyrosine phosphatase (PTP-1B), c-Raf kinase (RAF1), fibroblast growth factor receptor 4 (FGFR4), vascular adhesion molecule-1 (VCAM-1), very late antigen-4 (VLA-4), transthyretin (TTR), survival motor neuron 2 (SMN2), growth hormone receptor (GHR), dystophia myotonic protein kinase (DMPK), cellular nucleic acid-binding protein (CNBP or ZNF9), clusterin (CLU), eukaryotic translation initiation factor 4E (eIF-4e), MDM2, MDM4, heat shock protein 27 (HSP 27), signal transduction and activator of transcription 3 protein (STAT3), vascular endothelial growth factor (VEGF), kinesin spindle protein (KIF11), hepatitis B genome, the androgen receptor (AR), Atonal homolog 1 (ATOH1), vascular endothelial growth factor receptor 1 (FLT1), retinoschism 1 (RS1), retinal pigment epithelium-specific 65 kDa protein (RPE65), Rab escort protein 1 (CHM), and the sodium channel, voltage gated, type X, alpha subunit (PN3 or SCN10A). The genetic disorders include but are not limited to homozygous familial hypercholesterolemia, AGS1-AGS7, PRAAS/CANDLE, SAVI, ISG15 def., SPENCDI, hemophagocytic lymphohistiocytosis, NLRC4-MAS, CAMPS, DADA2, PLAID, Tyrosinemia type I, BSEP deficiency, MRD3 gene defect, glycogen storage disease types IV, I, Crigler-Najjar syndrome, Ornithine transcarbamylase deficiency, primary hyperoxaluria, Wilson disease, Cystic fibrosis, FIC1 deficiency, citrullinemia, cystinosis, propionic academia, ADA-SCID, X-linked SCID, lipoprotein lipase deficiency, Leber's congenital amaurosis, and adrenoleukodystrophy.

Also contemplated herein is the use of heterobifunctional compounds capable of binding to the dTAG of the endogenous protein-dTAG hybrid of the present invention and inducing degradation through ubiquination. By administering to a subject a heterobifunctional compound directed to a dTAG, the endogenous protein-dTAG hybrid can be modulated in a subject suffering from a disease or disorder as a result of the target protein's expression. The heterobifunctional compounds for use in the present invention are small molecule antagonists capable of disabling the biological function of the endogenous protein through degradation of the endogenous protein-dTAG hybrid. They provide prompt ligand-dependent target protein degradation via chemical

conjugation with, for example, derivatized phthalimides that hijack the function of the Cereblon E3 ubiquitin ligase complex. Using this approach, the endogenous protein-dTAG hybrid of the present invention can be degraded rapidly with a high specificity and efficiency.

The heterobifunctional compounds that can be used in the present invention include those that include a small molecule E3 ligase ligand which is covalently linked to a dTAG Targeting Ligand through a Linker of varying length and/or functionality as described in more detail below. The heterobifunctional compound is able to bind to the dTAG and recruit an E3 ligase, for example, by binding to a Cereblon (CRBN) containing ligase or Von Hippel-Lindau tumor suppressor (VHL) to the endogenous-dTAG hybrid for ubiquitination and subsequent proteasomal degradation.

Moreover, by combining the chemical strategy of protein degradation via the bifunctional molecules of the present application with the effectiveness of gene therapy, the activity of the endogenously expressed protein, and thus the side effects, can be regulated in a precise, temporal manner by rapidly turning on and off ubiquitination, and proteasomal degradation of the endogenous protein-dTAG hybrid.

Examples of heterobifunctional compounds useful in the present invention are exemplified further below.

In one aspect, the genomic nucleic acid sequence encodes a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG which, when expressed, results in an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound. Cells and animals, including in particular non-human animals, bearing such genetic modifications are part of the invention.

In a particular embodiment, the genomic nucleic acid sequence encodes a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG wherein the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 1 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-1-dFKBP-5. In a particular embodiment, the genomic nucleic acid sequence encodes a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG wherein the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 2 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dFKBP-6-dFKBP-13. In a particular embodiment, the genomic nucleic acid sequence encodes a synthetic gene comprising an

endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG wherein the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dBET1-dBET18. In a particular embodiment, the genomic nucleic acid sequence encodes a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG wherein the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 3 and the dTAG is capable of being bound by a heterobifunctional compound selected from any of dBromo1-dBromo34. In a particular embodiment, the genomic nucleic acid sequence encodes a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG wherein the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 9 and the dTAG is capable of being bound by a heterobifunctional compound selected from dHalo1 and dHalo2.

In one aspect, an amino acid encoded by a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG is provided, wherein the amino acid being an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound.

In one aspect, provided herein is a transformed cell comprising a genomic nucleic acid sequence encoding a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG which, when expressed, results in an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound.

In one aspect, provided herein is a cell expressing a synthetic gene comprising an endogenous gene of interest having a 5'- or 3'- in-frame insertion of a nucleic acid encoding a dTAG which, when expressed, results in an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound.

In a particular aspect, a method of modulating the activity of an endogenous protein by genomically inserting in frame a nucleic acid sequence encoding a dTAG is provided which, when expressed, results in an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound, and administering to a subject a heterobifunctional compound capable of binding the dTAG and degrading the endogenous protein-dTAG hybrid.

In a particular aspect, a method of identifying an endogenous protein associated with a disease state is provided wherein the activity of the endogenous protein is modulated by genomically inserting in frame a nucleic acid sequence encoding a dTAG which, when expressed, results in an endogenous protein-dTAG hybrid protein wherein the dTAG is capable of being bound by a heterobifunctional compound, and administering a heterobifunctional compound capable of binding the dTAG and degrading the endogenous protein-dTAG hybrid, wherein degradation of the protein results in the alteration of the disease state.

In one embodiment, provided herein is a transformed cell comprising a nucleic acid encoding SEQ. ID. NO.: 52 and a nucleic acid encoding a dTAG. In one embodiment, provided herein is a transformed cell comprising a nucleic acid encoding SEQ. ID. NO.: 52 and a nucleic acid encoding dTAG derived from an amino acid sequence, or fragment thereof, selected from SEQ. ID. NOs.: 1-44.

In one embodiment, provided herein is a first nucleic acid encoding SEQ. ID. NO.: 52 and a second nucleic acid encoding a dTAG. In one embodiment, provided herein is a first nucleic acid encoding SEQ. ID. NO.: 52 and a second nucleic acid encoding a dTAG derived from an amino acid sequence, or fragment thereof, selected from SEQ. ID. NO.: 1-44.

Other aspects of the invention include polynucleotide sequences, plasmids, and vectors encoding the synthetic genes of the present invention, and host cells expressing the synthetic genes of the present invention.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from EGFR. In certain embodiments, the dTAG is a modified or mutant EGFR protein or fragment thereof. In certain embodiments, the one or more mutations of EGFR include a substitution of Leucine (L) with Arginine (R) at amino acid position 858, a deletion of the amino acid sequence LREA in exon 19, an insertion of amino acids VAIKEL in exon 19, a substitution of Glycine (G) with Alanine (A), Cysteine (C), or Serine (S) at amino acid position 719, a substitution of Leucine (L) with Alanine (A), Cysteine (C), or Serine (S) at amino acid position 861, a substitution of Valine (V) with Alanine (A) at amino acid position 765, a substitution of Threonine (T) with Alanine (A) at amino acid position 783, a substitution of Serine (S) with Proline (P) at amino acid position 784, a substitution of Threonine (T) with Methionine (M) at amino acid position 790 M, a substitution of Threonine (T) with Alanine (A) at amino acid position 854, a substitution of Aspartic Acid (D) with Tyrosine (Y) at amino acid 761, a substitution of Leucine

(L) with Serine (S) at amino acid position 747, a substitution of Cysteine (C) with Serine (S) or Glycine (G) at amino acid position 797. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 53. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 54. In one embodiment, SEQ. ID. NO.: 54 has a Leucine at position 163. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 55. In one embodiment, SEQ. ID. NO.: 55 has a Leucine at position 163. In one embodiment, SEQ. ID. No.: 55 has a Threonine at position 95. In one embodiment, SEQ. ID. NO.: 55 has a Leucine at position 163 and a Threonine at position 95. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 56. In one embodiment, SEQ. ID. NO.: 56 has a Leucine at position 163. In one embodiment, SEQ. ID. NO.: 56 has a Threonine at position 95. In one embodiment, SEQ. ID. NO.: 56 has a Leucine at position 163 and a Threonine at position 95.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from BCR-ABL. In certain embodiments, the dTAG is a modified or mutant BCR-ABL protein or fragment thereof. In certain embodiments, the one or more mutations of BCR-ABL include a substitution of Tyrosine (T) with Isoleucine (I) at amino acid position 315. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 57. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 58.

In an alternative embodiment, the dTAGs for use in the present invention is an amino acid sequence derived from ALK. In certain embodiments, the dTAG is a modified or mutant ALK protein or fragment thereof. In certain embodiments, the one or more mutations of ALK include a substitution of Leucine (L) with Methionine at amino acid position 1196. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 59.

In an alternative embodiment, the dTAGs for use in the present invention is an amino acid sequence derived from JAK2. In certain embodiments, the dTAG is a modified or mutant JAK2 protein or fragment thereof. In certain embodiments, the one or more mutations of JAK2 include a substitution of Valine (V) with Phenylalanine (F) at amino acid position 617. In one

embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 60.

In an alternative embodiment, the dTAGs for use in the present invention is an amino acid sequence derived from BRAF. In certain embodiments, the dTAG is a modified or mutant BRAF protein or fragment thereof. In certain embodiments, the one or more mutations of BRAF include a substitution of Valine (V) with Glutamic Acid (E) at amino acid position 600. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 61.

In alternative embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from proteins selected from EGFR, BCR-ABL, ALK, JAK2, and BRAF. In one embodiment, the proteins contain one or more mutations. In one embodiment, the one or more mutations render the protein inactive.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from Src. In certain embodiments, the dTAG is a modified or mutant Src protein or fragment thereof. In certain embodiments, the one or more mutations or modifications of Src include a substitution of Threonine (T) with Glycine (G) or Alanine (A) at amino acid position 341. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 62. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 63.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from LKKR2. In certain embodiments, the dTAG is a modified or mutant LKKR2 protein or fragment thereof. In certain embodiments, the one or more mutations of LKKR2 include a substitution of Arginine (R) with Cysteine (C) at amino acid 1441, a substitution of Glycine (G) with Serine (S) at amino acid 2019, a substitution of Isoleucine (I) with Threonine (T) at amino acid 2020.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from PDGFRα. In certain embodiments, the dTAG is a modified or mutant PDGFRα protein or fragment thereof. In certain embodiments, the one or more mutations of PDGFRα include a substitution of Threonine (T) with Isoleucine (I) at amino acid 674.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from RET. In certain embodiments, the dTAG is a modified or mutant RET

protein or fragment thereof. In certain embodiments, the one or more mutations of RET include a substitution of Glycine (G) with Serine (S) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Arginine (R) with Threonine (T) at amino acid 749. In certain embodiments, the one or more mutations of RET include a substitution of Glutamic acid (E) with Glutamine (Q) at amino acid 762. In certain embodiments, the one or more mutations of RET include a substitution of Tyrosine (Y) with Phenylalanine (F) at amino acid 791. In certain embodiments, the one or more mutations of RET include a substitution of Valine (V) with Methionine (M) at amino acid 804. In certain embodiments, the one or more mutations of RET include a substitution of Methionine (M) with Threonine (T) at amino acid 918.

In alternative embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from proteins selected from Kit, Jak3, Abl, Mek1, HIV reverse transcriptase, and HIV integrase.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from LKKR2. In certain embodiments, the dTAG is a modified or mutant LKKR2 protein or fragment thereof. In certain embodiments, the one or more mutations of LKKR2 include a substitution of Arginine (R) with Cysteine (C) at amino acid 1441, a substitution of Glycine (G) with Serine (S) at amino acid 2019, a substitution of Isoleucine (I) with Threonine (T) at amino acid 2020.

In one embodiment, the dTAG has an amino acid sequence derived from a LRRK2 protein (UniProtKB – Q5S007 (LRKK2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1328 to 1511 of Q5S007. In one embodiment, the dTAG is derived from amino acid 1328 to 1511 of Q5S007, wherein amino acid 1441 is Cysteine. In one embodiment, the dTAG is derived from amino acid 1328 to 1511 of Q5S007 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U1. In one embodiment, the dTAG is derived from amino acid 1328 to 1511 of Q5S007, wherein amino acid 1441 is Cysteine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U1. In one embodiment, the dTAG is derived from amino acid 1879 to 2138 of Q5S007. In one embodiment, the dTAG is derived from amino acid 1879 to 2138 of Q5S007, wherein amino acid 2019 is Serine. In on embodiment, the dTAG is derived from amino acid 1879 to 2138 of Q5S007, wherein amino acid 2020 is Threonine. In one embodiment, the dTAG is derived from amino acid 1879 to 2138 of

Q5S007 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U2 or U3. In one embodiment, the dTAG is derived from amino acid 1879 to 2138 of Q5S007, wherein amino acid 2019 is Serine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U2. In one embodiment, the dTAG is derived from amino acid 1879 to 2138 of Q5S007, wherein amino acid 2020 is Threonine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U3.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from PDGFRα. In certain embodiments, the dTAG is a modified or mutant PDGFRα protein or fragment thereof. In certain embodiments, the one or more mutations of PDGFRα include a substitution of Threonine (T) with Isoleucine (I) at amino acid 6741.

In one embodiment, the dTAG has an amino acid sequence derived from a PDGFRα protein (UniProtKB – P09619 (PDGFR_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619, wherein amino acid 660 is Alanine. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-V1. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619, wherein amino acid 660 is Alanine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-V1.

In an alternative embodiment, the dTAG for use in the present invention is an amino acid sequence derived from RET. In certain embodiments, the dTAG is a modified or mutant RET protein or fragment thereof. In certain embodiments, the one or more mutations of RET include a substitution of Glycine (G) with Serine (S) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Arginine (R) with Threonine (T) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Glutamic acid (E) with Glutamine (Q) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Tyrosine (Y) with Phenylalanine (F) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Valine (V) with Methionine (M) at amino acid 691. In certain embodiments, the one or more mutations of RET include a substitution of Methionine (M) with Threonine (T) at amino acid 691.

In one embodiment, the dTAG has an amino acid sequence derived from a PDGFRα protein (UniProtKB – P07949 (RET_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 940 is Isoleucine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W1. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 940 is Isoleucine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W1. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W2. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W3. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W4. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W5. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949 and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W6.

In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 53 and the dTAG is capable of being bound by a heterobifunctional compound that contains an EGFR dTAG Targeting Ligand selected from Table T-P1. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 54 and the dTAG is capable of being bound by a heterobifunctional compound that contains an EGFR dTAG Targeting Ligand selected from Table T-P2. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 55 and the dTAG is capable of being bound by a heterobifunctional compound that contains an EGFR dTAG Targeting Ligand selected from Table T-P3. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 56 and the dTAG is capable of being bound by a heterobifunctional compound that contains an EGFR dTAG Targeting Ligand selected from Table T-P. In a particular embodiment, the dTAG is

derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 57 and the dTAG is capable of being bound by a heterobifunctional compound that contains a BCR-ABL dTAG Targeting Ligand selected from Table T-Q1. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 58 and the dTAG is capable of being bound by a heterobifunctional compound that contains a BCR-ABL dTAG Targeting Ligand selected from Table T-Q1. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 59 and the dTAG is capable of being bound by a heterobifunctional compound that contains a ALK dTAG Targeting Ligand selected from Table T-R1. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 60 and the dTAG is capable of being bound by a heterobifunctional compound that contains a JAK2 dTAG Targeting Ligand selected from Table T-S1. In a particular embodiment, the dTAG is derived from an amino acid sequence or fragment thereof of SEQ. ID. NO.: 61 and the dTAG is capable of being bound by a heterobifunctional compound that contains a BRAF dTAG Targeting Ligand selected from Table T-T1.

In one embodiment, the dTAG is derived from LRRK2 amino acid 1328 to 1511 (UnitPro-Q5S007) and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U1. In one embodiment, the dTAG is derived from LRRK2 amino acid 1328 to 1511 (UniProt-Q5S007), wherein amino acid 1441 is Cysteine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U1. In one embodiment, the dTAG is derived from LRRK2 amino acid 1879 to 2138 (UniProt-Q5S007. In one embodiment, the dTAG is derived from LRRK2 amino acid 1879 to 2138 (UniProt-Q5S007), wherein amino acid 2019 is Serine. In on embodiment, the dTAG is derived from amino acid 1879 to 2138 (UniProt-Q5S007), wherein amino acid 2020 is Threonine. In one embodiment, the dTAG is derived from LRRK2 amino acid 1879 to 2138 (UniProt-Q5S007) and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U2 or U3. In one embodiment, the dTAG is derived from LRRK2 amino acid 1879 to 2138 (UniProt-Q5S007), wherein amino acid 2019 is Serine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U2. In one embodiment, the dTAG is derived from LRRK2 amino acid 1879 to 2138 (UniProt-Q5S007), wherein amino acid 2020 is Threonine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-U3. In one embodiment, the dTAG is derived from PDGFR amino acid 600 to 692 (UniProt-

P09619) and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-V1. In one embodiment, the dTAG is derived from PDGFR amino acid 600 to 692 (UniProt-P09619), wherein amino acid 674 is Isoleucine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-V1. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W1-W6. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 691 is Serine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W1. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 749 is Threonine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W2. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 762 is Glutamine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W3. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 791 is Phenylalanine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W4. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 804 is Methionine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W5. In one embodiment, the dTAG is derived from RET amino acid 724 to 1016 (UniProtKB – P07949), wherein amino acid 918 is Threonine and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-W6. In one embodiment, the dTAG is derived from an JAK2, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-JJJ1. In one embodiment, the dTAG is derived from an Abl, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-KKK1. In one embodiment, the dTAG is derived from anMEK1, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-LLL1. In one embodiment, the dTAG is derived from an KIT, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-MMM1. In one embodiment, the dTAG is derived from an HIV reverse transcriptase, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-NNN1. In one

embodiment, the dTAG is derived from an HIV integrase, and the dTAG Targeting Ligand in the heterobifunctional compound is selected from a ligand in Table T-OOO1.

In a particular embodiment, the dTAG is derived from a protein selected from EGFR, ErbB2, ErbB4, VEGFR1, VEGFR2, VEGFR3, Kit, BCR-Abl, Src, Lyn, Hck, RET, c-Met, TrkB, Flt3, Axl, Tie2, ALK, IGF-1R, InsR, ROS1, MST1R, B-Raf, Lck, Yes, Fyn, HER2– breast cancer, PNET, RCC, RAML, SEGA, BTK, FGFR1/2/3/4, DDR1, PDGFRα, PDGFRβ, CDK4, CDK6, Fms, Itk, T315I, Eph2A, JAK1, JAK2, JAK3 CDK8, CSF-1R, FKBP12/mTOR, MEK1, MEK2, Brk, EphR, A-Raf, B-Raf, C-Raf and the heterobifunctional compound contains a dTAG Targeting Ligand selected from Table Z.

Heterobifunctional compounds capable of binding to the amino acid sequences, or a fragment thereof, described above can be generated using the dTAG Targeting Ligands described in Table T. In one embodiment, a nucleic acid sequence encoding a dTAG derived from an amino acid sequence described above, or a fragment thereof, is genomically inserted into a gene encoding an endogenous protein of interest which, upon expression, results in an endogenous protein-dTAG hybrid protein and is degraded by administering to the subject a heterobifunctional compound comprising a dTAG Targeting Ligand described in Table T. In one embodiment, a nucleic acid sequence encoding a dTAG derived from an amino acid sequence described above, or a fragment thereof, is genomically inserted into a gene encoding an endogenous protein of interest which, upon expression, results in an endogenous protein-dTAG hybrid protein and is degraded by administering to the subject its corresponding heterobifunctional compound, which is capable of binding to the dTAG, for example a heterobifunctional compound described in Figure 29, Figure 30, Figure 31, Figure 32, and Figure 33, or any other heterobifunctional compound described herein.

Brief Description of the Figures

FIG. 1 is a schematic representing a "bump-hole" approach for selective degradation of a dTAG fusion protein. For example, the dTAG fusion can be a version of the FK506- and Rapamycin-binding protein FKBP12 engineered with a cavity forming "hole" via an amino acid mutation (F36V). This mutant FKBP12 ("bumped" FKBP, aka FKBP* or FKBP12* (SEQ. ID. NO.: 2)) can then be selectively targeted by a heterobifunctional compound possessing a synthetic "bump" in the FKBP12 binding domain, a linker, and a cereblon targeting domain. This

heterobifunctional compound does not target native FKBP12 and thus offers selectivity against wildtype variants of the tag naturally present in human cells.

FIG. 2 is a schematic representing the genomic integration of a nucleic acid sequence encoding a dTAG into the genomic locus of the endogenous gene encoding PCSK9. Following homologous recombination, the resultant insertion results in an expression product comprising an N-terminus dTAG in frame with the proprotein convertase subtilisin/kexin type 9 (PCSK9) protein, thus providing a proprotein convertase subtilisin/kexin type 9 (PCSK9)-dTAG hybrid capable of being degraded by a heterobifunctional compound targeting the dTAG sequence.

FIG. 3 is a schematic representing the genomic integration of a nucleic acid sequence encoding a dTAG into the genomic locus of the endogenous gene encoding β-catenin (CTNNB1). Following homologous recombination, the resultant insertion results in an expression product comprising an N-terminus dTAG in frame with the β-catenin (CTNNB1) protein, thus providing a β-catenin (CTNNB1)-dTAG hybrid capable of being degraded by a heterobifunctional compound targeting the dTAG sequence.

FIG. 4 is an immunoblot of cells treated with heterobifunctional compounds described in the present invention. 293FT cells (CRBN-WT or CRBN-/-) expressing either HA-tagged FKBP12WT or FKBP* were treated with indicated concentrations of dFKBP7 for 4 hours. CRBN-dependent degradation of FKBP* and not FKBPWT confirms selective activity of dFKBP7 for mutant FKBP*.

FIG. 5A and FIG. 5B are graphs measuring the activity of a panel of dFKBP heterobifunctional compounds in cells expressing FKBP* fused to Nluc. Degradation of FKBP* is measured as a signal ration (Nluc/Fluc) between NANOluc and firefly luciferase from the same multicistronic transcript in wild type (Fig. 7A) or CRBN -/- (Fig. 7B) 293FT cells treated with indicated concentrations of dFKBPs for 4 hours. A decrease in the signal ratio indicates FKBP* (Nluc) degradation.

FIG. 6 is an immunoblot of cells treated with heterobifunctional compounds described in the present invention. Isogenic 293FT cells (CRBN-WT or CRBN-/-) expressing either FKBP12WT or FKBP* were treated with 100nM of either dFKBP7 or dFKBP13 for 4 hours. CRBN-dependent degradation of FKBP* and not FKBP12WT or endogenous FKBP12 confirms selectivity of dFKBP7 and dFKBP13 for mutant FKBP*.

FIG. 7 is an immunoblot of cells treated with heterobifunctional compounds described in the present invention. Isogenic 293FT cells (CRBN-WT or CRBN-/-) expressing HA-tagged FKBP* were treated with the indicated dose of dFKBP13 for 4 hours. These data confirm dose- and CRBN-dependent degradation of HA-tagged FKBP* by dFKBP13.

FIG. 8 is an immunoblot of cells treated with heterobifunctional compounds described in the present invention. 293FT cells (CRBN-WT) expressing HA-tagged FKBP* were treated with 100nM dFKBP13 for the indicated times. Cells were harvested and protein lysates immunoblotted to measure the kinetics of HA-tagged FKBP* degradation induced by dFKBP13.

FIG. 9 is an immunoblot of cells treated with heterobifunctional compounds described in the present invention. 293FT cells (CRBN-WT) expressing FKBP* were pretreated with 1uM Carfilzomib (proteasome inhibitor), 0.5uM MLN4924 (neddylation inhibitor), and 10uM Lenalidomide (CRBN binding ligand) for two hours prior to a 4 hour treatment with dFKBP13. Degradation of HA-tagged FKBP* by dFKBP13 was rescued by the proteasome inhibitor Carfilzomib, establishing a requirement for proteasome function. Pre-treatment with the NAE1 inhibitor MLN4924 rescued HA-tagged FKBP* establishing dependence on CRL activity, as expected for cullin-based ubiquitin ligases that require neddylation for processive E3 ligase activity. Pre-treatment with excess Lenalidomide abolished dFKBP13-dependent FKBP* degradation, confirming the requirement of CRBN engagement for degradation.

FIG. 10A and FIG. 10B are immunoblots of cells treated with heterobifunctional compounds described in the present invention. Immunoblots of MV4;11 leukemia cells expressing indicated proteins fused to mutant FKBP* with an HA tag. Cells were treated for 16 hours with indicated concentrations of FKBP* selective heterobifunctional compounds, dFKBP7 or dFKBP13 and abundance of fusion proteins measured by western immunoblot analysis.

FIG. 11 is an immunoblot of NIH3T3 cells expressing KRASG12V allele fused to FKBP* in the N-terminus or C-terminus. Cells were treated with 500nM dFKBP7 for the indicated time. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRASG12V and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest N-terminal FKBP* fusions are active and degraded upon administration of dFKBP7.

FIG. 12 is an immunoblot of NIH3T3 cells expressing FKBP* fused to the N-terminus of KRASG12V treated with 1uM of the indicated dFKBP heterobifunctional compounds for 24 hours. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRASG12V and

downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP9, dFKBP12, and dFKBP13 induce potent degradation of FKBP*-KRASG12V and inhibition of downstream signaling.

FIG. 13 is an immunoblot of NIH3T3 cells expressing FKBP* fused to the N-terminus of KRASG12V treated with the indicated concentrations of dFKBP13 for 24 hours. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRASG12V and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP13 induces potent degradation of FKBP*-KRASG12V and inhibits downstream signaling potently with an IC50 >100nM.

FIG. 14 is an immunoblot of NIH3T3 cells expressing FKBP* fused to the N-terminus of KRASG12V treated with 1uM dFKBP13 for the indicated time. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRASG12V and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). Data suggest that dFKBP13 induces potent degradation of FKBP*-KRASG12V and inhibition of downstream signaling as early as 1 hour post treatment.

FIG. 15 is an immunoblot of NIH3T3 cells expressing dTAG-KRASG12V pretreated with 1uM Carfilzomib (proteasome inhibitor), 0.5uM MLN4924 (neddylation inhibitor), and 10uM Lenalidomide (CRBN binding ligand) for two hours prior to a 4 hour treatment with dFKBP13.

FIG. 16 is an immunoblot of NIH3T3 cells expressing KRAS alleles either WT or mutant forms of amino acid glycine 12 (G12C, G12D, and G12V) treated with 1uM of dFKBP13 for 24 hours.

FIG. 17 is an immunoblot of NIH3T3 cells expressing either WT or mutant KRAS alleles (G13D, Q61L, and Q61R) treated with 1uM of dFKBP13 for 24 hours.

FIG. 18A, FIG. 18B, FIG. 18C, and FIG. 18D are panels of phase contrast images of control NIH3T3 cells or NIH3T3 expressing FKBP* fused to the N-terminus of KRASG12V treated with DMSO of dFKBP13 for 24 hours. Phase contrast images highlight the morphological change induced upon dFKBP13-dependent degradation of FKBP*-KRASG12V.

FIG. 19A, FIG. 19B, FIG. 19C, and FIG. 19D are proliferation graphs that measure the effect of dFKBP13 on the growth of NIH3T3 control cells of NIH3T3 expressing FKBP*-KRASG12V. Cells were treated with the indicated concentrations if dFKBPs for 72 hours and cell count measured using an ATPlite assay. The ATPlite 1step luminescence assay measures cell proliferation and cytotoxicity in cells based on the production of light caused by the reaction of

ATP with added luciferase and D-luciferin. A decrease in signal indicates a reduction in cell number.

FIG. 20 is a bar graph illustrating NIH3T3 cells expressing dTAG-KRASG12V treated with dFKBP7 and dFKBP13 for 48 hours to induce targeted dTAG-KRASG12V degradation. Fixed cells were stained with propidium iodide and cell cycle analysis was performed.

FIG. 21A, FIG. 21B, FIG. 21C, FIG. 21D, FIG. 21E, FIG. 21F, FIG. 21G, FIG. 21H, and FIG. 21I provide examples of Degron moieties for use in the present invention, wherein R is the point of attachment for the Linker and X is as defined herein.

FIG. 22 provides additional examples of Degron moieties for use in the present invention, wherein R is the point of attachment for the Linker and X is as defined herein.

FIG. 23 provides additional examples of Degron moieties for use in the present invention, wherein R is the point of attachment for the Linker and X is as defined herein.

FIG. 24 provides examples of Linker moieties for use in the present invention.

FIG. 25 provides additional examples of Linker moieties for use in the present invention.

FIG. 26 provides examples of heteroaliphatic Linker moieties for use in the present invention.

FIG. 27 provides examples of aromatic Linker moieties for use in the present invention.

FIG. 28A, FIG. 28B, FIG. 28C, FIG. 28D, FIG. 28E, FIG. 28F, and FIG. 28G provide dTAG Targeting Ligands for use in the present invention, wherein R is the point at which the Linker is attached.

FIG. 29A, FIG. 29B, FIG. 29C, FIG. 29D, FIG. 29E, FIG. 29F, FIG. 29G, and FIG. 29H provide specific heterobifunctional compounds for use in the present invention.

FIG. 30A, FIG. 30B, FIG. 30C, FIG. 30D, FIG. 30E, FIG. 30F, FIG. 30G, FIG. 30H, FIG. 30I, FIG. 30J, FIG. 30K, FIG. 30L, FIG. 30M, FIG. 30N, FIG. 30O, and FIG. 30P provides specific heterobifunctional compounds for use in the present invention, wherein X in the above structures is a halogen chosen from F, Cl, Br, and I.

FIG. 31A, FIG. 31B, FIG. 31C, FIG. 31D, FIG. 31E, FIG. 31F, FIG. 31G, FIG. 31H, FIG. 31I, and FIG. 31J provide specific heterobifunctional compounds for use in the present invention.

FIG. 32S, FIG. 32B, FIG. 32C, FIG. 32D, FIG. 32E, FIG. 32F, FIG. 32G, FIG. 32H, FIG. 32I, FIG. 32J, FIG. 32K, FIG. 32L, FIG. 32M, FIG. 32N, FIG. 32O, FIG. 32P, FIG. 32Q, FIG. 32R, FIG. 32S, FIG. 32T, FIG. 32U, FIG. 32V, FIG. 32W, FIG. 32X, FIG. 32Y, FIG. 32Z, FIG.

32AA, FIG. 32BB, FIG. 32CC, FIG. 32DD, and FIG. 32EE provide specific heterobifunctional compounds for use in the present invention, wherein R^{AR1} and R^{AR2} are described herein.

FIG. 33A, FIG. 33B, FIG. 33C, FIG. 33D, FIG. 33E, FIG. 33F, FIG. 33G, FIG. 33H, FIG. 33I, FIG. 33J, FIG. 33K, FIG. 33L, FIG. 33M, FIG. 33N, FIG. 33O, FIG. 33P, FIG. 33Q, FIG. 33R, FIG. 33S, FIG. 33T, FIG. 33U, FIG. 33V, and FIG. 33W provide additional heterobifunctional compounds for use in the present invention.

Detailed Description of the Invention

Practice of the methods, as well as preparation and use of the compositions disclosed herein employ, unless otherwise indicated, conventional techniques in molecular biology, biochemistry, chromatin structure and analysis, computational chemistry, cell culture, recombinant DNA and related fields as are within the skill of the art. These techniques are fully explained in the literature. See, for example, Sambrook et al. MOLECULAR CLONING: A LABORATORY MANUAL, Second edition, Cold Spring Harbor Laboratory Press, 1989 and Third edition, 2001; Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, 1987 and periodic updates; the series METHODS IN ENZYMOLOGY, Academic Press, San Diego; Wolffe, CHROMATIN STRUCTURE AND FUNCTION, Third edition, Academic Press, San Diego, 1998; METHODS IN ENZYMOLOGY, Vol. 304, "Chromatin" (P. M. Wassarman and A. P. Wolffe, eds.), Academic Press, San Diego, 1999; and METHODS IN MOLECULAR BIOLOGY, Vol. 119, "Chromatin Protocols" (P. B. Becker, ed.) Humana Press, Totowa, 1999.

Here, we describe a method that takes advantage of both gene and protein disruption to provide a highly selective and reversible method for promoting protein degradation. This methodology is of value for precise, temporal, small-molecule controlled target validation and the exploration of cellular and in vivo effects of protein of interest degradation.

In this method, a region of the target gene of interest is targeted by a guide RNA and Cas9 in order to insert (knock-in) an expression cassette for dTAG present in a homologous recombination (HR) targeting vector. The HR targeting vector contains homology arms at the 5' and 3' end of the expression cassette homologous to the genomic DNA surrounding the targeting gene of interest locus. By fusing dTAG in frame with the target gene of interest, the resulting

fusion protein upon expression will be made susceptible to proteasome mediated degradation upon treatment with a bioinert small molecule heterobifunctional compound.

Genome editing in mammalian cells offers much potential for the treatment and correction of human disease. By using short single-guide RNAs (sgRNAs) the Cas9 endonuclease can be directed to genomic positions of interest whereupon it induces DNA double strand breaks. These breaks are repaired by non-homologous end joining, which can be leveraged to produce insertions or deletions (indels) that inactivate genes. In vivo genome editing can be accomplished with CRISPR/Cas9 delivery by adeno-associated virus (AAV-), lentivirus-, particle-, hydrodynamic injection -or electroporation-mediated methods, or combinations thereof (see, for example, Kumar et al., *Hum. Gene Ther.* 12, (2001):1893-1905; Wu et al., *Mol. Ther.* 18, (2010):80-86; Ran et al., Nature 520, (2015): 186-191; Swiech et al., *Nat. Biotechnol.* 33, (2015):102-105; Zuris et al., *Nat. Biotechnol.* 33, (2015):73-80; Kauffman et al., *Nano. Lett.* 15, (2015):7300-7306; Ding et al., *Circ. Res.* 115, (2014):488-492; Maresch et al., *Nat. Commun.* 7, (2016):10770; Khorsandi et al., *Cancer Gene Ther.* 15, (2008):225-230; Yin et al., *Nat. Rev. Genet.* 15, (2014):541-555; Yin et al., *Nat. Biotechnol.* 34, (2016):328-333; and Xue et al., *Nature* 514, (2014):380-384 , incorporated herein by reference) and somatic genome editing has been applied to mouse organs such as the lung, liver, brain, and pancreas (see, for example, Xue et al., *Nature* 514, (2014):380-384; Sanchez-Rivera et al., *Nature* 516, (2014):428-431; Platt et al., *Cell* 159, (2014):440-455; Yin et al., *Nat. Biotechnol.* 32, (2014):551-553; Zuckermann et al., *Nat. Commun.* 6, (2015):7391; Chiou et al., *Genes Dev.* 29, (2015):1576-1585; and Mazur et al., *Nat. Med.* 21, (2015):1163-1171, incorporated herein by reference). However, the long-term implications of permanent genome modification are unknown and concerns exist over the imperfect precision of genome editing and the impact of direct correction in adults where biological compensation mechanisms may exist (see, for example, Fu et al., Nat. Biotechnol. 31(9), (2013):822-826, and Cho et al., Genome Res. 24, (2014):132-141, incorporated herein by reference).

Here we describe a strategy for widespread therapeutic use that is based on in vivo genome engineering to produce knock-in fusion proteins that are produced from the endogenous locus and are readily degraded in a ligand-dependent, reversible, and dose-responsive, fashion. The fusion protein contains a dTAG that is targeted by a bi- or polyvalent heterobifunctional compound. The heterobifunctional compound has the ability to bind the dTAG and recruit an E3 ligase e.g. the cereblon-containing CRL4A E3 ubiquitin ligase complex. This recruitment induces ubiquitination

of the fusion protein (on either the dTAG domain or on the cognate protein) and subsequent degradation via the UPP. Through this approach a protein of interest can be targeted for rapid ubiquitin mediated degradation with high specificity and high specificity without requiring the discovery of a de novo ligand for the protein of interest. In light of the combined use of a small molecule and genome engineering for in vivo use.

A variety of dTAGs can be used, including, but not limited to, bromodomains e.g. the first bromodomain of BRD4; hormone receptors e.g. ER, AR, RXR; FKBP12; DHFR, esp. bacterial DHFR, and other commonly used protein fusion tags that can be bound by a ligand that can be converted to a heterobifunctional compound. In some cases, there will be an advantage to using a dTAG that leverages a "bump-hole" strategy conceptually related to that developed to selectively target the ATP binding site of protein kinases. In such a case, the dTAG fusion is a version of the FK506- and Rapamycin-binding protein FKBP12 engineered with a cavity forming "hole" via an amino acid mutation (F36V). This mutant FKBP12 ("bumped" FKBP, aka FKBP* (SEQ. ID. NO.: 2) is then targeted by a heterobifunctional compound (or similar molecule) possessing a synthetic "bump" in the FKBP12 binding domain, a linker, and a cereblon targeting domain (e.g. an IMID derivative). This molecule does not target native FKBP12 and thus offers selectivity of the heterobifunctional compound against wildtype variants of the tag naturally present in human cells. An illustration representing the exemplified "bump-hole" strategy is provided for in Figure 1.

The invention described herein provides a mechanism to control the degradation of endogenous proteins of relevance to disease by combining genome engineering with small molecule activation/modulation of degradation. Applications of this technology include, but are not limited to 1) targeted degradation of proteins where pathology is a function of gain of function mutation(s), 2) targeted degradation of proteins where pathology is a function of amplification or increased expression, 3) targeted degradation of proteins that are manifestations of monogenetic disease, 4) targeted degradation of proteins where genetic predisposition manifests over longer periods and often after alternative biological compensatory mechanisms are no longer adequate, e.g. hypercholesterolemia, proteinopathies.

Definitions

The terms "nucleic acid," "polynucleotide," and "oligonucleotide" are used interchangeably and refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular

conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms are not to be construed as limiting with respect to the length of a polymer. The terms can encompass known analogues of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). In general, an analogue of a particular nucleotide has the same base-pairing specificity; i.e., an analogue of A will base-pair with T.

The terms "polypeptide," "peptide," and "protein" are used interchangeably to refer to a polymer of amino acid residues. The term also applies to amino acid polymers in which one or more amino acids are chemical analogues or modified derivatives of corresponding naturally-occurring amino acids.

"Binding" refers to a sequence-specific, non-covalent interaction between macromolecules (e.g., between a protein and a nucleic acid) or a macromolecule and a small molecule (e.g. between a protein and a drug). Not all components of a binding interaction need be sequence-specific (e.g., contacts with phosphate residues in a DNA backbone), as long as the interaction as a whole is sequence-specific.

"Recombination" refers to a process of exchange of genetic information between two polynucleotides. For the purposes of this disclosure, "homologous recombination" (HR) refers to the specialized form of such exchange that takes place, for example, during repair of double-strand breaks in cells via homology-directed repair mechanisms. This process requires nucleotide sequence homology, uses a "donor" molecule to template repair of a "target" molecule (i.e., the one that experienced the double-strand break), and leads to the transfer of genetic information from the donor to the target.

One or more targeted nucleases as described herein create a double-stranded break in the target sequence (e.g., cellular chromatin) at a predetermined site, and a "donor" polynucleotide, encoding a dTAG, having homology to the nucleotide sequence in the region of the break, can be introduced into the cell. The presence of the double-stranded break has been shown to facilitate integration of the donor sequence. The donor sequence may be physically integrated, resulting in the introduction of all or part of the nucleotide sequence as in the donor into the cellular chromatin. Thus, a first sequence in cellular chromatin can be altered and converted into a sequence present in a donor polynucleotide.

In certain methods for targeted recombination and/or replacement and/or alteration of a sequence in a region of interest in cellular chromatin, a chromosomal sequence is altered by homologous recombination with an exogenous "donor" nucleotide sequence encoding a dTAG. Such homologous recombination is stimulated by the presence of a double-stranded break in cellular chromatin, if sequences homologous to the region of the break are present.

In any of the methods described herein, the exogenous nucleotide sequence (the "donor sequence" or "transgene") can contain sequences that are homologous, but not identical, to genomic sequences in the region of interest, thereby stimulating homologous recombination to insert a non-identical sequence, i.e., the nucleic acid sequence encoding a dTAG, in the region of interest. Thus portions of the donor sequence that are homologous to sequences in the region of interest exhibit between about 80 to 99% (or any integer there between) sequence identity to the genomic sequence that is replaced. In other embodiments, the homology between the donor and genomic sequence is higher than 99%, for example if only 1 nucleotide differs as between donor and genomic sequences of over 100 contiguous base pairs. A non-homologous portion of the donor sequence contains nucleic sequences not present in the region of interest, e.g., a sequence encoding a dTAG, such that new sequences are introduced into the region of interest. In these instances, the non-homologous sequence is generally flanked by sequences of 50-1,000 base pairs (or any integral value there between) or any number of base pairs greater than 1,000, that are homologous or identical to sequences in the region of interest. In other embodiments, the donor sequence is non-homologous to the first sequence, and is inserted into the genome by non-homologous recombination mechanisms.

"Cleavage" refers to the breakage of the covalent backbone of a DNA molecule. Cleavage can be initiated by a variety of methods including, but not limited to, enzymatic or chemical hydrolysis of a phosphodiester bond. Both single-stranded cleavage and double-stranded cleavage are possible, and double-stranded cleavage can occur as a result of two distinct single-stranded cleavage events. DNA cleavage can result in the production of either blunt ends or staggered ends. In certain embodiments, fusion polypeptides are used for targeted double-stranded DNA cleavage.

"Chromatin" is the nucleoprotein structure comprising the cellular genome. Cellular chromatin comprises nucleic acid, primarily DNA, and protein, including histones and non-histone chromosomal proteins. The majority of eukaryotic cellular chromatin exists in the form of nucleosomes, wherein a nucleosome core comprises approximately 150 base pairs of DNA

associated with an octamer comprising two each of histones H2A, H2B, H3 and H4; and linker DNA (of variable length depending on the organism) extends between nucleosome cores. A molecule of histone H1 is generally associated with the linker DNA. For the purposes of the present disclosure, the term "chromatin" is meant to encompass all types of cellular nucleoprotein, both prokaryotic and eukaryotic. Cellular chromatin includes both chromosomal and episomal chromatin.

An "exogenous" molecule is a molecule that is not normally present in a cell, for example, certain dTAGs but can be introduced into a cell by one or more genetic, biochemical or other methods. An exogenous molecule can comprise, for example, a synthetic endogenous protein-dTAG hybrid.

An "endogenous" protein is one that is normally present in a particular cell at a particular developmental stage under particular environmental conditions. For example, an endogenous protein, for example, may be a transcription factor or enzyme or any other type of naturally expressed protein.

A "fusion" or "hybrid" protein is a protein in which two or more polypeptides are linked, preferably covalently. Examples of fusion proteins, for example, include a fusion between an endogenous protein and a dTAG.

A "gene," for the purposes of the present disclosure, includes a DNA region encoding a gene product, as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions.

"Gene expression" refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of an mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristilation, and glycosylation.

"Modulation" of protein expression refers to a change in the activity of a protein. Modulation of expression can include, but is not limited to, reduced protein activity or increased protein activity. For example, as contemplated herein, exposing an endogenous protein-dTAG hybrid to a heterobifunctional compound, resulting in the degradation of the endogenous protein-dTAG hybrid, may modulate the activity of the endogenous protein. Thus, protein inactivation may be partial or complete.

A "vector" is capable of transferring gene sequences to target cells. Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target cells. Thus, the term includes cloning, and expression vehicles, as well as integrating vectors.

The terms "subject" and "patient" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, dogs, cats, rats, mice, rabbits and other animals. Accordingly, the term "subject" or "patient" as used herein means any patient or subject (e.g., mammalian) having a disorder.

A. Heterobifunctional Compound Targeting Protein (dTAGs)

The present invention provides method for making knock-in fusion proteins that are produced from the endogenous locus and are readily degraded in a ligand-dependent, reversible, and dose-responsive, fashion. Specifically, a nucleic acid encoding a dTAG is inserted in frame with a target gene of interest, wherein upon expression, the resulting fusion protein contains a dTAG that is targeted by a bi- or polyvalent heterobifunctional compound. The heterobifunctional compound has the ability to bind the target protein and recruit an E3 ligase e.g. the cereblon-containing CRL4A E3 ubiquitin ligase complex. This recruitment induces ubiquitination of the fusion protein (on either the dTAG or on the cognate protein) and subsequent degradation via the ubiquitin proteasome pathway (UPP). Through this approach a protein of interest can be targeted for rapid ubiquitin mediated degradation with high specificity without requiring the discovery of a de novo ligand for the POI.

The heterobifunctional compound targeting protein of the synthetic gene is any amino acid sequence to which a heterobifunctional compound can be bound, leading to the ubiquitination and degradation of the expressed endogenous protein-dTAG hybrid protein when in contact with the heterobifunctional compound. Preferably, the dTAG should not interfere with the function of the

endogenously expressed protein. In one embodiment, the dTAG is a non-endogenous peptide, leading to heterobifunctional compound selectivity and allowing for the avoidance of off target effects upon administration of the heterobifunctional compound. In one embodiment, the dTAG is an amino acid sequence derived from an endogenous protein or fragment thereof which has been modified so that the heterobifunctional compound binds only to the modified amino acid sequence and not the endogenously expressed protein. In one embodiment, the dTAG is an endogenously expressed protein or a fragment of an endogenously expressed protein. Any amino acid sequence domain that can be bound by a ligand for use in a heterobifunctional compound can be used as a dTAG as contemplated herewith. In certain embodiments, it is preferred that the smallest amino acid sequence capable of being bound by a particular heterobifunctional compound be utilized as a dTAG.

In particular embodiments, the dTAG for use in the present invention include, but are not limited to, an amino acid sequence derived from an endogenously expressed protein such as FK506 binding protein-12 (FKBP12), bromodomain-containing protein 4 (BRD4), CREB binding protein (CREBBP), and transcriptional activator BRG1 (SMARCA4), or a variant thereof. As contemplated herein, "variant" means any variant comprising a substitution, deletion, or addition of one or a few to plural amino acids, provided that the variant substantially retains the same function as the original sequence, which in this case is providing a ligand for a heterobifunctional compound. In other embodiments, a dTAG for use in the present invention may include, for example, a hormone receptor e.g. estrogen-receptor protein, androgen receptor protein, retinoid x receptor (RXR) protein, and dihydrofolate reductase (DHFR), including bacterial DHFR, bacterial dehydrogenase, and variants.

Some embodiments of dTAGs can be, but are not limited to, those derived from Hsp90 inhibitors, kinase inhibitors, MDM2 inhibitors, compounds targeting Human BET Bromodomain-containing proteins, compounds targeting cytosolic signaling protein FKBP12, HDAC inhibitors, human lysine methyltransferase inhibitors, angiogenesis inhibitors, immunosuppressive compounds, and compounds targeting the aryl hydrocarbon receptor (AHR).

In certain embodiments, the dTAG is derived from, a kinase, a BET bromodomain-containing protein, a cytosolic signaling protein (e.g., FKBP12), a nuclear protein, a histone deacetylase, a lysine methyltransferase, a protein regulating angiogenesis, a protein regulating

immune response, an aryl hydrocarbon receptor (AHR), an estrogen receptor, an androgen receptor, a glucocorticoid receptor, or a transcription factor (e.g., SMARCA4, SMARCA2, TRIM24).

In certain embodiments, the dTAG is derived from a kinase, for example, but not limited to, a tyrosine kinase (e.g., AATK, ABL, ABL2, ALK, AXL, BLK, BMX, BTK, CSF1R, CSK, DDR1, DDR2, EGFR, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRK, FYN, GSG2, HCK, IGF1R, ILK, INSR, INSRR, IRAK4, ITK, JAK1, JAK2, JAK3, KDR, KIT, KSR1, LCK, LMTK2, LMTK3, LTK, LYN, MATK, MERTK, MET, MLTK, MST1R, MUSK, NPR1, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PLK4, PTK2, PTK2B, PTK6, PTK7, RET, ROR1, ROR2, ROS1, RYK, SGK493, SRC, SRMS, STYK1, SYK, TEC, TEK, TEX14, TIE1, TNK1, TNK2, TNNI3K, TXK, TYK2, TYRO3, YES1, or ZAP70), a serine/threonine kinase (e.g., casein kinase 2, protein kinase A, protein kinase B, protein kinase C, Raf kinases, CaM kinases, AKT1, AKT2, AKT3, ALK1, ALK2, ALK3, ALK4, Aurora A, Aurora B, Aurora C, CHK1, CHK2, CLK1, CLK2, CLK3, DAPK1, DAPK2, DAPK3, DMPK, ERK1, ERK2, ERK5, GCK, GSK3, HIPK, KHS1, LKB1, LOK, MAPKAPK2, MAPKAPK, MNK1, MSSK1, MST1, MST2, MST4, NDR, NEK2, NEK3, NEK6, NEK7, NEK9, NEK11, PAK1, PAK2, PAK3, PAK4, PAK5, PAK6, PIM1, PIM2, PLK1, RIP2, RIP5, RSK1, RSK2, SGK2, SGK3, SIK1, STK33, TAO1, TAO2, TGF-beta, TLK2, TSSK1, TSSK2, ULK1, or ULK2), a cyclin dependent kinase (e.g., Cdk1 – Cdk11), and a leucine-rich repeat kinase (e.g., LRRK2).

In certain embodiments, the dTAG is derived from a BET bromodomain-containing protein, for example, but not limited to, ASH1L, ATAD2, BAZ1A, BAZ1B, BAZ2A, BAZ2B, BRD1, BRD2, BRD3, BRD4, BRD5, BRD6, BRD7, BRD8, BRD9, BRD10, BRDT, BRPF1, BRPF3, BRWD3, CECR2, CREBBP, EP300, FALZ, GCN5L2, KIAA1240, LOC93349, MLL, PB1, PCAF, PHIP, PRKCBP1, SMARCA2, SMARCA4, SP100, SP110, SP140, TAF1, TAF1L, TIF1a, TRIM28, TRIM33, TRIM66, WDR9, ZMYND11, and MLL4. In certain embodiments, a BET bromodomain-containing protein is BRD4.

In certain embodiments, the dTAG is derived from, but not limited to, 7,8-dihydro-8-oxoguanin triphosphatase, AFAD, Arachidonate 5-lipoxygenase activating protein, apolipoprotein, baculoviral IAP repeat-containing protein 2, Bcl-2, Bcl-xL, E3 ligase XIAP, fatty acid binding protein from adipocytes 4 (FABP4), GTPase k-RAS, HDAC6, hematopoietic prostaglandin D

synthase, lactoglutathione lyase, Mcl-1, PA2GA, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, poly-ADP-ribose polymerase 14, poly-ADP-ribose polymerase 15, prosaposin, prostaglandin E synthase, retinal rod rhodopsin-sensitive cGMP 3','5-cyclic phosphodiesterase subunit delta, S100-A7, Src, Sumo-conjugating enzyme UBC9, superoxide dismutase, tankyrase 1, or tankyrase 2.

In certain embodiments, the dTAG is derived from a nuclear protein including, but not limited to, BRD2, BRD3, BRD4, Antennapedia Homeodomain Protein, BRCA1, BRCA2, CCAAT-Enhanced-Binding Proteins, histones, Polycomb-group proteins, High Mobility Group Proteins, Telomere Binding Proteins, FANCA, FANCD2, FANCE, FANCF, hepatocyte nuclear factors, Mad2, NF-kappa B, Nuclear Receptor Coactivators, CREB-binding protein, p55, p107, p130, Rb proteins, p53, c-fos, c-jun, c-mdm2, c-myc, and c-rel.

In a particular embodiment, the dTAG has an amino acid sequence derived from BRD2 ((Universal Protein Resource Knowledge Base (UniProtKB) - P25440 (BRD2_HUMAN) incorporated herein by reference), BRD3 (UniProtKB - Q15059 (BRD3_HUMAN) incorporated herein by reference), BRD4 (UniProtKB - O60885 (BRD4_HUMAN) incorporated herein by reference), or BRDT (UniProtKB - Q58F21 (BRDT_HUMAN) incorporated herein by reference) (see Baud et al., "A bump-and-hole approach to engineer controlled selectivity of BET bromodomains chemical probes", *Science* 346(6209) (2014):638-641; and Baud et al., "New Synthetic Routes to Triazolo-benzodiazepine Analogues: Expanding the Scope of the Bump-and-Hole Approach for Selective Bromo and Extra-Terminal (BET) Bromodomain Inhibition", *JMC* 59 (2016):1492-1500, both incorporated herein by reference). In certain embodiments, the dTAG is a modified or mutant BRD2, BRD3, BRD4, or BRDT protein (see Baud et al., "A bump-and-hole approach to engineer controlled selectivity of BET bromodomains chemical probes", *Science* 346(6209) (2014):638-641; and Baud et al., "New Synthetic Routes to Triazolo-benzodiazepine Analogues: Expanding the Scope of the Bump-and-Hole Approach for Selective Bromo and Extra-Terminal (BET) Bromodomain Inhibition", *JMC* 59 (2016):1492-1500, both incorporated herein by reference). In certain embodiments, the one or more mutations of BRD2 include a mutation of the Tryptophan (W) at amino acid position 97, a mutation of the Valine (V) at amino acid position 103, a mutation of the Leucine (L) at amino acid position 110, a mutation of the W at amino acid position 370, a mutation of the V at amino acid position 376, or a mutation of the L at amino acid position 381. In certain embodiments, the one or more mutations of BRD3 include a mutation of

the W at amino acid position 57, a mutation of the V at amino acid position 63, a mutation of the L at amino acid position 70, a mutation of the W at amino acid position 332, a mutation of the V at amino acid position 338, or a mutation of the L at amino acid position 345. In certain embodiments, the one or more mutations of BRD4 include a mutation of the W at amino acid position 81, a mutation of the V at amino acid position 87, a mutation of the L at amino acid position 94, a mutation of the W at amino acid position 374, a mutation of the V at amino acid position 380, or a mutation of the L at amino acid position 387. In certain embodiments, the one or more mutations of BRDT include a mutation of the W at amino acid position 50, a mutation of the V at amino acid position 56, a mutation of the L at amino acid position 63, a mutation of the W at amino acid position 293, a mutation of the V at amino acid position 299, or a mutation of the L at amino acid position 306.

In certain embodiments, the dTAG is derived from a kinase inhibitor, a BET bromodomain-containing protein inhibitor, cytosolic signaling protein FKBP12 ligand, an HDAC inhibitor, a lysine methyltransferase inhibitor, an angiogenesis inhibitor, an immunosuppressive compound, and an aryl hydrocarbon receptor (AHR) inhibitor.

In a particular embodiment, the dTAG is derived from cytosolic signaling protein FKBP12. In certain embodiments, the dTAG is a modified or mutant cytosolic signaling protein FKBP12. In certain embodiments, the modified or mutant cytosolic signaling protein FKBP12 contains one or more mutations that create an enlarged binding pocket for FKBP12 ligands. In certain embodiments, the one or more mutations include a mutation of the phenylalanine (F) at amino acid position 36 to valine (V) (F36V) (as counted without the methionine start codon) (referred to interchangeably herein as FKBP* or FKBP12*) (see Clackson et al., "Redesigning an FKBP–ligand interface to generate chemical dimerizers with novel specificity", *PNAS* 95 (1998):10437–10442) (incorporated herein by reference).

In a particular embodiment, the dTAG has an amino acid sequence derived from an FKBP12 protein (UniProtKB - P62942 (FKB1A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 1) GVQVETISP GDGRTFPKRG QTCVVHYTGM LEDGKKFDSS RDRNKPFKFM LGKQEVIRGW EEGVAQMSVG QRAKLTISPD YAYGATGHPG IIPPHATLVF DVELLKLE.

In one embodiment, the dTAG is a FKBP12 derived amino acid sequence with a mutation of the phenylalanine (F) at amino acid position 36 (as counted without the methionine) to valine

(V) (F36V) (FKBP*) having the amino acid sequence: (SEQ. ID. NO.: 2) GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRG W EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD4 protein (UniProtKB – O60885 (BRD4_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 3) MSAESGPGTRLRNLPVMGDGLETSQMSTTQAQAQPQPANAASTNPPPPETSNPNKPKRQ TNQLQYLLRVVLKTLWKHQFAWPFQQPVDAVKLNLPDYYKIIKTPMDMGTIKKRLENN YYWNAQECIQDFNTMFTNCYIYNKPGDDIVLMAEALEKLFLQKINELPTEETEIMIVQAK GRGRGRKETGTAKPGVSTVPNTTQASTPPQTQTPQPNPPPVQATPHPFPAVTPDLIVQTP VMTVVPPQPLQTPPPVPPQPQPPPAPAPQPVQSHPPIIAATPQPVKTKKGVKRKADTTTPT TIDPIHEPPSLPPEPKTTKLGQRRESSRPVKPPKKDVPDSQQHPAPEKSSKVSEQLKCCSGI LKEMFAKKHAAYAWPFYKPVDVEALGLHDYCDIIKHPMDMSTIKSKLEAREYRDAQEF GADVRLMFSNCYKYNPPDHEVVAMARKLQDVFEMRFAKMPDEPEEPVVAVSSPAVPP PTKVVAPPSSSDSSSDSSSDSDSSTDDSEEERAQRLAELQEQLKAVHEQLAALSQPQQNK PKKKEKDKKEKKKEKHKRKEEVEENKKSKAKEPPPKKTKKNNSSNSNVSKKEPAPMKS KPPPTYESEEEDKCKPMSYEEKRQLSLDINKLPGEKLGRVVHIIQSREPSLKNSNPDEIEID FETLKPSTLRELERYVTSCLRKKRKPQAEKVDVIAGSSKMKGFSSSESESSSESSSSDSED SETEMAPKSKKKGHPGREQKKHHHHHHQQMQQAPAPVPQQPPPPPQQPPPPPPPQQQQ QPPPPPPPPSMPQQAAPAMKSSPPPFIATQVPVLEPQLPGSVFDPIGHFTQPILHLPQPELPP HLPQPPEHSTPPHLNQHAVVSPPALHNALPQQPSRPSNRAAALPPKPARPPAVSPALTQT PLLPQPPMAQPPQVLLEDEEPPAPPLTSMQMQLYLQQLQKVQPPTPLLPSVKVQSQPPPP LPPPPHPSVQQQLQQQPPPPPPPQPQPPPPQQQHQPPPRPVHLQPMQFSTHIQQPPPPQGQQ PPHPPPGQQPPPPQPAKPQQVIQHHHSPRHHKSDPYSTGHLREAPSPLMIHSPQMSQFQSL THQSPPQQNVQPKKQELRAASVVQPQPLVVVKEEKIHSPIIRSEPFSPSLRPEPPKHPESIK APVHLPQRPEMKPVDVGRPVIRPPEQNAPPPGAPDKDKQKQEPKTPVAPKKDLKIKNM GSWASLVQKHPTTPSSTAKSSSDSFEQFRRAAREKEEREKALKAQAEHAEKEKERLRQE RMRSREDEDALEQARRAHEEARRRQEQQQQQRQEQQQQQQQQAAAVAAAATPQAQS SQPQSMLDQQRELARKREQERRRREAMAATIDMNFQSDLLSIFEENLF.

In one embodiment, the dTAG is derived from amino acid 75-147 of SEQ. ID. NO.: 3.

In one embodiment, the dTAG has an amino acid sequence derived from a ASH1L protein (UniProtKB - Q9NR48 (ASH1L_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 2463-2533 of Q9NR48.

In one embodiment, the dTAG has an amino acid sequence derived from a ATAD2 protein (UniProtKB - Q6PL18 (ATAD2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1001-1071 of Q6PL18.

In one embodiment, the dTAG has an amino acid sequence derived from a BAZ1A protein (UniProtKB - Q9NRL2 (BAZ1A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1446-1516 of Q9NRL2.

In one embodiment, the dTAG has an amino acid sequence derived from a BAZ1B protein (UniProtKB - Q9UIG0 (BAZ1B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1356-1426 of Q9UIG0.

In one embodiment, the dTAG has an amino acid sequence derived from a BAZ2A protein (UniProtKB - Q9UIF9 (BAZ2A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1810-1880 of Q9UIF9.

In one embodiment, the dTAG has an amino acid sequence derived from a BAZ2B protein (UniProtKB - Q9UIF8 (BAZ2B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 2077-2147 of Q9UIF8.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD1 protein (UniProtKB - O95696 (BRD1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 579-649 of O95696.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD2 protein (UniProtKB - P25440 (BRD2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 13)

MLQNVTPHNKLPGEGNAGLLGLGPEAAAPGKRIRKPSLLYEGFESPTMASVPALQLTPA
NPPPPEVSNPKKPGRVTNQLQYLHKVVMKALWKHQFAWPFRQPVDAVKLGLPDYHKII
KQPMDMGTIKRRLENNYYWAASECMQDFNTMFTNCYIYNKPTDDIVLMAQTLEKIFLQ
KVASMPQEEQELVVTIPKNSHKKGAKLAALQGSVTSAHQVPAVSSVSHTALYTPPPEIPT
TVLNIPHPSVISSPLLKSLHSAGPPLLAVTAAPPAQPLAKKKGVKRKADTTTPTPTAILAP
GSPASPPGSLEPKAARLPPMRRESGRPIKPPRKDLPDSQQQHQSSKKGKLSEQLKHCNGI
LKELLSKKHAAYAWPFYKPVDASALGLHDYHDIIKHPMDLSTVKRKMENRDYRDAQE

FAADVRLMFSNCYKYNPPDHDVVAMARKLQDVFEFRYAKMPDEPLEPGPLPVSTAMPP
GLAKSSSESSSEESSSESSSEEEEEEDEEDEEEEESESSDSEEERAHRLAELQEQLRAVHEQ
LAALSQGPISKPKRKREKKEKKKKRKAEKHRGRAGADEDDKGPRAPRPPQPKKSKKAS
GSGGGSAALGPSGFGPSGGSGTKLPKKATKTAPPALPTGYDSEEEEESRPMSYDEKRQL
SLDINKLPGEKLGRVVHIIQAREPSLRDSNPEEIEIDFETLKPSTLRELERYVLSCLRKKPR
KPYTIKKPVGKTKEELALEKKRELEKRLQDVSGQLNSTKKPPKKANEKTESSSAQQVAV
SRLSASSSSSDSSSSSSSSSSSDTSDSDSG.

In one embodiment, the dTAG is derived from amino acid 91-163 or 364-436 of SEQ. ID. NO.: 13.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD3 protein (UniProtKB - Q15059 (BRD3_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 14)

MSTATTVAPAGIPATPGPVNPPPPEVSNPSKPGRKTNQLQYMQNVVVKTLWKHQ
FAWPFYQPVDAIKLNLPDYHKIIKNPMDMGTIKKRLENNYYWSASECMQDFNTMFTNC
YIYNKPTDDIVLMAQALEKIFLQKVAQMPQEEVELLPPAPKGKGRKPAAGAQSAGTQQ
VAAVSSVSPATPFQSVPPTVSQTPVIAATPVPTITANVTSVPVPPAAAPPPPATPIVPVVPP
TPPVVKKKGVKRKADTTTPTTSAITASRSESPPPLSDPKQAKVVARRESGGRPIKPPKKD
LEDGEVPQHAGKKGKLSEHLRYCDSILREMLSKKHAAYAWPFYKPVDAEALELHDYH
DIIKHPMDLSTVKRKMDGREYPDAQGFAADVRLMFSNCYKYNPPDHEVVAMARKLQD
VFEMRFAKMPDEPVEAPALPAPAAPMVSKGAESSRSSEESSSDSGSSDSEEERATRLAEL
QEQLKAVHEQLAALSQAPVNKPKKKKEKKEKEKKKKDKEKEKEKHKVKAEEEKKAK
VAPPAKQAQQKKAPAKKANSTTTAGRQLKKGGKQASASYDSEEEEEGLPMSYDEKRQ
LSLDINRLPGEKLGRVVHIIQSREPSLRDSNPDEIEIDFETLKPTTLRELERYVKSCLQKKQ
RKPFSASGKKQAAKSKEELAQEKKKELEKRLQDVSGQLSSSKKPARKEKPGSAPSGGPS
RLSSSSSSESGSSSSSGSSSDSSDSE.

In one embodiment, the dTAG is derived from amino acid 51-123 or 326-398 of SEQ. ID. NO.: 14.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD7 protein (UniProtKB - Q9NPI1 (BRD7_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 148-218 of Q9NP11.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD8 protein (UniProtKB - Q9H0E9 (BRD8_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 724-794 or 1120-1190 of Q9H0E9.

In one embodiment, the dTAG has an amino acid sequence derived from a BRD9 protein (UniProtKB - Q9H8M2 (BRD9_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 153-223 of Q9H8M2.

In one embodiment, the dTAG has an amino acid sequence derived from a BRDT protein (UniProtKB - Q58F21 (BRDT_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 15)

MSLPSRQTAIIVNPPPPEYINTKKNGRLTNQLQYLQKVVLKDLWKHSFSWPFQRPVDAV
KLQLPDYYTIIKNPMDLNTIKKRLENKYYAKASECIEDFNTMFSNCYLYNKPGDDIVLM
AQALEKLFMQKLSQMPQEEQVVGVKERIKKGTQQNIAVSSAKEKSSPSATEKVFKQQEI
PSVFPKTSISPLNVVQGASVNSSSQTAAQVTKGVKRKADTTTPATSAVKASSEFSPTFTE
KSVALPPIKENMPKNVLPDSQQQYNVVKTVKVTEQLRHCSEILKEMLAKKHFSYAWPF
YNPVDVNALGLHNYYDVVKNPMDLGTIKEKMDNQEYKDAYKFAADVRLMFMNCYK
YNPPDHEVVTMARMLQDVFETHFSKIPIEPVESMPLCYIKTDITETTGRENTNEASSEGNS
SDDSEDERVKRLAKLQEQLKAVHQQLQVLSQVPFRKLNKKKEKSKKEKKKEKVNNSN
ENPRKMCEQMRLKEKSKRNQPKKRKQQFIGLKSEDEDNAKPMNYDEKRQLSLNINKLP
GDKLGRVVHIIQSREPSLSNSNPDEIEIDFETLKASTLRELEKYVSACLRKRPLKPPAKKI
MMSKEELHSQKKQELEKRLLDVNNQLNSRKRQTKSDKTQPSKAVENVSRLSESSSSSSS
SSESESSSSDLSSSDSSDSESEMFPKFTEVKPNDSPSKENVKKMKNECIPPEGRTGVTQIG
YCVQDTTSANTTLVHQTTPSHVMPPNHHQLAFNYQELEHLQTVKNISPLQILPPSGDSEQ
LSNGITVMHPSGDSDTTMLESECQAPVQKDIKIKNADSWKSLGKPVKPSGVMKSSDELF
NQFRKAAIEKEVKARTQELIRKHLEQNTKELKASQENQRDLGNGLTVESFSNKIQNKCS
GEEQKEHQQSSEAQDKSKLWLLKDRDLARQKEQERRRREAMVGTIDMTLQSDIMTMF
ENNFD.

In one embodiment, the dTAG is derived from amino acid 44-116 or 287-359 of SEQ. ID. NO.: 15.

In one embodiment, the dTAG has an amino acid sequence derived from a BRPF1 protein (UniProtKB - P55201 (BRPF1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 645-715 of P55201.

In one embodiment, the dTAG has an amino acid sequence derived from a BRPF3 protein (UniProtKB - Q9ULD4 (BRPF3_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 606-676 of Q9ULD4.

In one embodiment, the dTAG has an amino acid sequence derived from a BRWD3 protein (UniProtKB - Q6RI45 (BRWD3_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1158-1228 or 1317-1412 of Q6RI45.

In one embodiment, the dTAG has an amino acid sequence derived from a CECR2 protein (UniProtKB - Q9BXF3 (CECR2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 451-521 of Q9BXF3.

In one embodiment, the dTAG has an amino acid sequence derived from a CREBBP protein (UniProtKB - Q92793 (CBP_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1103-1175 of Q92793.

In one embodiment, the dTAG has an amino acid sequence derived from a EP300 protein (UniProtKB - Q09472 (EP300_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1067-1139 of Q09472.

In one embodiment, the dTAG has an amino acid sequence derived from a FALZ protein (UniProtKB - Q12830 (BPTF_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 2944-3014 of Q12830.

In one embodiment, the dTAG has an amino acid sequence derived from a GCN5L2 protein (UniProtKB - Q92830 (KAT2A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 745-815 of Q92830.

In one embodiment, the dTAG has an amino acid sequence derived from a KIAA1240 protein (UniProtKB - Q9ULI0 (ATD2B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 975-1045 of Q9ULI0.

In one embodiment, the dTAG has an amino acid sequence derived from a LOC93349 protein (UniProtKB - Q13342 (SP140_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 796-829 of Q13342.

In one embodiment, the dTAG has an amino acid sequence derived from a MLL protein (UniProtKB - Q03164 (KMT2A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1703-1748 of Q03164.

In one embodiment, the dTAG has an amino acid sequence derived from a PB1 protein (UniProtKB - Q86U86 (PB1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 63-134, 200-270, 400-470, 538-608, 676-746, or 792-862 of Q86U86.

In one embodiment, the dTAG has an amino acid sequence derived from a PCAF protein (UniProtKB - Q92831 (KAT2B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 740-810 of Q92831.

In one embodiment, the dTAG has an amino acid sequence derived from a PHIP protein (UniProtKB - Q8WWQ0 (PHIP_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1176-1246 or 1333-1403 of Q8WWQ0.

In one embodiment, the dTAG has an amino acid sequence derived from a PRKCBP1 protein (UniProtKB - Q9ULU4 (PKCB1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 165-235 of Q9ULU4.

In one embodiment, the dTAG has an amino acid sequence derived from a SMARCA2 protein (UniProtKB - P51531 (SMCA2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1419-1489 of P51531.

In one embodiment, the dTAG has an amino acid sequence derived from a SMARCA4 protein (UniProtKB - P51532 (SMCA4_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1477-1547 of P51532.

In one embodiment, the dTAG has an amino acid sequence derived from a SP100 protein (UniProtKB - P23497 (SP100_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 761-876 of P23497.

In one embodiment, the dTAG has an amino acid sequence derived from a SP110 protein (UniProtKB - Q9HB58 (SP110_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 581-676 of Q9HB58.

In one embodiment, the dTAG has an amino acid sequence derived from a SP140 protein (UniProtKB - Q13342 (SP140_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 796-829 of Q13342.

In one embodiment, the dTAG has an amino acid sequence derived from a TAF1 protein (UniProtKB - P21675 (TAF1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1397-1467 or 1520-1590 of P21675.

In one embodiment, the dTAG has an amino acid sequence derived from a TAF1L protein (UniProtKB - Q8IZX4 (TAF1L_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1416-1486 or 1539-1609 of Q8IZX4.

In one embodiment, the dTAG has an amino acid sequence derived from a TIF1A protein (UniProtKB - O15164 (TIF1A_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 932-987 of O15164.

In one embodiment, the dTAG has an amino acid sequence derived from a TRIM28 protein (UniProtKB - Q13263 (TIF1B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 697-801 of Q13263.

In one embodiment, the dTAG has an amino acid sequence derived from a TRIM33 protein (UniProtKB - Q9UPN9 (TRI33_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 974-1046 of Q9UPN9.

In one embodiment, the dTAG has an amino acid sequence derived from a TRIM66 protein (UniProtKB - O15016 (TRI66_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1056-1128 of O15016.

In one embodiment, the dTAG has an amino acid sequence derived from a WDR9 protein (UniProtKB - Q9NSI6 (BRWD1_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1177-1247 or 1330-1400 of Q9NSI6.

In one embodiment, the dTAG has an amino acid sequence derived from a ZMYND11 protein (UniProtKB - Q15326 (ZMY11_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 168-238 of Q15326.

In one embodiment, the dTAG has an amino acid sequence derived from a MLL4 protein (UniProtKB - Q9UMN6 (KMT2B_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 1395-1509 of Q9UMN6.

In one embodiment, the dTAG has an amino acid sequence derived from an estrogen receptor, human (UniProtKB - P03372-1) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 4)

MTMTLHTKASGMALLHQIQGNELEPLNRPQLKIPLERPLGEVYLDSSKPAVYNYPEGAA
YEFNAAAAANAQVYGQTGLPYGPGSEAAAFGSNGLGGFPPLNSVSPSPLMLLHPPPQLS
PFLQPHGQQVPYYLENEPSGYTVREAGPPAFYRPNSDNRRQGGRERLASTNDKGSMAM

ESAKETRYCAVCNDYASGYHYGVWSCEGCKAFFKRSIQGHNDYMCPATNQCTIDKNR
RKSCQACRLRKCYEVGMMKGGIRKDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRA
ANLWPSPLMIKRSKKNSLALSLTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLT
NLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLVWRSMEHPGKLLF
APNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSS
TLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGME
HLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEETDQSHLATAGSTSSHSLQK
YYITGEAEG FPATV.

In one embodiment, the dTAG has an amino acid sequence derived from an estrogen receptor ligand-binding domain, or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 5)

SLALSLTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKR
VPGFVDLTLHDQVHLLECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGM
VEIFDMLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKI
TDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLL
LEMLDAHRL.

In one embodiment, the dTAG has an amino acid sequence derived from an androgen receptor, UniProtKB - P10275 (ANDR_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 6)

MEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGPRHPEAASAAPPGASLLLLQQ
QQQQQQQQQQQQQQQQQQQQQETSPRQQQQQQGEDGSPQAHRRGPTGYLVLDEEQQ
PSQPQSALECHPERGCVPEPGAAVAASKGLPQQLPAPPDEDDSAAPSTLSLLGPTFPGLSS
CSADLKDILSEASTMQLLQQQQQEAVSEGSSSGRAREASGAPTSSKDNYLGGTSTISDNA
KELCKAVSVSMGLGVEALEHLSPGEQLRGDCMYAPLLGVPPAVRPTPCAPLAECKGSL
LDDSAGKSTEDTAEYSPFKGGYTKGLEGESLGCSGSAAAGSSGTLELPSTLSLYKSGALD
EAAAYQSRDYYNFPLALAGPPPPPPPPHPHARIKLENPLDYGSAWAAAAAQCRYGDLAS
LHGAGAAGPGSGSPSAAASSSWHTLFTAEEGQLYGPCGGGGGGGGGGGGGGGGGGG
GGGEAGAVAPYGYTRPPQGLAGQESDFTAPDVWYPGGMVSRVPYPSPTCVKSEMGPW
MDSYSGPYGDMRLETARDHVLPIDYYFPPQKTCLICGDEASGCHYGALTCGSCKVFFKR
AAEGKQKYLCASRNDCTIDKFRRKNCPSCRLRKCYEAGMTLGARKLKKLGNLKLQEEG

EASSTTSPTEETTQKLTVSHIEGYECQPIFLNVLEAIEPGVVCAGHDNNQPDSFAALLSSL
NELGERQLVHVVKWAKALPGFRNLHVDDQMAVIQYSWMGLMVFAMGWRSFTNVNS
RMLYFAPDLVFNEYRMHKSRMYSQCVRMRHLSQEFGWLQITPQEFLCMKALLLFSIIPV
DGLKNQKFFDELRMNYIKELDRIIACKRKNPTSCSRRFYQLTKLLDSVQPIARELHQFTF
DLLIKSHMVSVDFPEMMAEIISVQVPKILSGKVKPIYFHTQ.

In one embodiment, the dTAG has an amino acid sequence derived from an androgen receptor ligand-binding domain, or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 10)

DNNQPDSFAALLSSLNELGERQLVHVVKWAKALPGFRNLHVDDQMAVIQYSWMGLM
VFAMGWRSFTNVNSRMLYFAPDLVFNEYRMHKSRMYSQCVRMRHLSQEFGWLQITPQ
EFLCMKALLLFSIIPVDGLKNQKFFDELRMNYIKELDRIIACKRKNPTSCSRRFYQLTKLL
DSVQPIARELHQFTFDLLIKSHMVSVDFPEMMAEIISVQVPKILSGKVKPIYFHT.

In one embodiment, the dTAG has an amino acid sequence derived from a Retinoic Receptor, (UniProtKB - P19793) (RXRA_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 7)

MDTKHFLPLDFSTQVNSSLTSPTGRGSMAAPSLHPSLGPGIGSPGQLHSPISTLSSPINGM
GPPFSVISSPMGPHSMSVPTTPTLGFSTGSPQLSSPMNPVSSSEDIKPPLGLNGVLKVPAHP
SGNMASFTKHICAICGDRSSGKHYGVYSCEGCKGFFKRTVRKDLTYTCRDNKDCLIDKR
QRNRCQYCRYQKCLAMGMKREAVQEERQRGKDRNENEVESTSSANEDMPVERILEAE
LAVEPKTETYVEANMGLNPSSPNDPVTNICQAADKQLFTLVEWAKRIPHFSELPLDDQVI
LLRAGWNELLIASFSHRSIAVKDGILLATGLHVHRNSAHSAGVGAIFDRVLTELVSKMR
DMQMDKTELGCLRAIVLFNPDSKGLSNPAEVEALREKVYASLEAYCKHKYPEQPGRFA
KLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQMT.

In one embodiment, the dTAG has an amino acid sequence derived from a Retinoic Receptor ligand-binding domain, or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 11)

SANEDMPVERILEAELAVEPKTETYVEANMGLNPSSPNDPVTNICQAADKQLFTLVEWA
KRIPHFSELPLDDQVILLRAGWNELLIASFSHRSIAVKDGILLATGLHVHRNSAHSAGVG
AIFDRVLTELVSKMRDMQMDKTELGCLRAIVLFNPDSKGLSNPAEVEALREKVYASLEA
YCKHKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQMT.

In one embodiment, the dTAG has an amino acid sequence derived from a DHFR, E.coli, UniProtKB - Q79DQ2 (Q79DQ2_ECOLX) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 8)

MNSESVRIYLVAAMGANRVIGNGPNIPWKIPGEQKIFRRLTEGKVVVMGRKTFESIGKPL
PNRHTLVISRQANYRATGCVVVSTLSHAIALASELGNELYVAGGAEIYTLALPHAHGVF
LSEVHQTFEGDAFFPMLNETEFELVSTETIQAVIPYTHSVYARRNG.

In one embodiment, the dTAG has an amino acid sequence derived from a bacterial dehalogenase, or variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 9)

MAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAPTH
RCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFHWAK
RNPERVKGIAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVV
RPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKL
LFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEIARWLSTLEISG.

In one embodiment, the dTAG has an amino acid sequence derived from the N-terminus of MDM2, or variants thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 12)

MCNTNMSVPTDGAVTTSQIPASEQETLVRPKPLLLKLLKSVGAQKDTYTMKEVLFYLG
QYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTMIYRNLVVV.

In one embodiment, the dTAG has an amino acid sequence derived from apoptosis regulator Bcl-xL protein, UniProtKB – Q07817 (B2CL1_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 16)

MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEMETPSAINGNPSWHL
ADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQLHITPGT
AYQSFEQVVNELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLN
DHLEPWIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAGVVLLGSLFSR
K.

In one embodiment, the dTAG has an amino acid sequence derived from the CD209 antigen, UniProtKB – Q9NNX6 (CD209_HUMAN) (incorporated herein by reference), or a

variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 17)

MSDSKEPRLQQLGLLEEEQLRGLGFRQTRGYKSLAGCLGHGPLVLQLLSFTLLAGLLVQ
VSKVPSSISQEQSRQDAIYQNLTQLKAAVGELSEKSKLQEIYQELTQLKAAVGELPEKSK
LQEIYQELTRLKAAVGELPEKSKLQEIYQELTWLKAAVGELPEKSKMQEIYQELTRLKA
AVGELPEKSKQQEIYQELTRLKAAVGELPEKSKQQEIYQELTRLKAAVGELPEKSKQQEI
YQELTQLKAAVERLCHPCPWEWTFFQGNCYFMSNSQRNWHDSITACKEVGAQLVVIKS
AEEQNFLQLQSSRSNRFTWMGLSDLNQEGTWQWVDGSPLLPSFKQYWNRGEPNNVGE
EDCAEFSGNGWNDDKCNLAKFWICKKSAASCSRDEEQFLSPAPATPNPPPA.

In one embodiment, the dTAG has an amino acid sequence derived from E3 ligase XIAP, UniProtKB – P98170 (XIAP_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 18)

MTFNSFEGSKTCVPADINKEEEFVEEFNRLKTFANFPSGSPVSASTLARAGFLYTGEGDT
VRCFSCHAAVDRWQYGDSAVGRHRKVSPNCRFINGFYLENSATQSTNSGIQNGQYKVE
NYLGSRDHFALDRPSETHADYLLRTGQVVDISDTIYPRNPAMYSEEARLKSFQNWPDYA
HLTPRELASAGLYYTGIGDQVQCFCCGGKLKNWEPCDRAWSEHRRHFPNCFFVLGRNL
NIRSESDAVSSDRNFPNSTNLPRNPSMADYEARIFTFGTWIYSVNKEQLARAGFYALGEG
DKVKCFHCGGGLTDWKPSEDPWEQHAKWYPGCKYLLEQKGQEYINNIHLTHSLEECLV
RTTEKTPSLTRRIDDTIFQNPMVQEAIRMGFSFKDIKKIMEEKIQISGSNYKSLEVLVADL
VNAQKDSMQDESSQTSLQKEISTEEQLRRLQEEKLCKICMDRNIAIVFVPCGHLVTCKQC
AEAVDKCPMCYTVITFKQKIFMS.

In one embodiment, the dTAG has an amino acid sequence derived from baculoviral IAP repeat-containing protein 2, UniProtKB – Q13490 (BIRC2_HUMAN) (incorporated herein by reference) or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 19)

MHKTASQRLFPGPSYQNIKSIMEDSTILSDWTNSNKQKMKYDFSCELYRMSTYSTFPAG
VPVSERSLARAGFYYTGVNDKVKCFCCGLMLDNWKLGDSPIQKHKQLYPSCSFIQNLV
SASLGSTSKNTSPMRNSFAHSLSPTLEHSSLFSGSYSSLSPNPLNSRAVEDISSSRTNPYSY
AMSTEEARFLTYHMWPLTFLSPSELARAGFYYIGPGDRVACFACGGKLSNWEPKDDAM
SEHRRHFPNCPFLENSLETLRFSISNLSMQTHAARMRTFMYWPSSVPVQPEQLASAGFY
YVGRNDDVKCFCCDGGLRCWESGDDPWVEHAKWFPRCEFLIRMKGQEFVDEIQGRYP

HLLEQLLSTSDTTGEENADPPIIHFGPGESSSEDAVMMNTPVVKSALEMGFNRDLVKQT
VQSKILTTGENYKTVNDIVSALLNAEDEKREEEKEKQAEEMASDDLSLIRKNRMALFQQ
LTCVLPILDNLLKANVINKQEHDIIKQKTQIPLQARELIDTILVKGNAAANIFKNCLKEIDS
TLYKNLFVDKNMKYIPTEDVSGLSLEEQLRRLQEERTCKVCMDKEVSVVFIPCGHLVVC
QECAPSLRKCPICRGIIKGTVRTFLS.

In one embodiment, the dTAG has an amino acid sequence derived from hematopoietic prostaglandin D synthase, UniProtKB – O60760 (HPGDS_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 20)

MPNYKLTYFNMRGRAEIIRYIFAYLDIQYEDHRIEQADWPEIKSTLPFGKIPILEVDGLTL
HQSLAIARYLTKNTDLAGNTEMEQCHVDAIVDTLDDFMSCFPWAEKKQDVKEQMFNE
LLTYNAPHLMQDLDTYLGGREWLIGNSVTWADFYWEICSTTLLVFKPDLLDNHPRLVT
LRKKVQAIPAVANWIKRRPQTKL.

In one embodiment, the dTAG has an amino acid sequence derived from GTPase k-RAS, UniProtKB – P01116 (RASK_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 21)

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTA
GQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNK
CDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTP
GCVKIKKCIIM.

In one embodiment, the dTAG has an amino acid sequence derived from Poly-ADP-ribose polymerase 15, UniProtKB – Q460N3 (PAR15_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 22)

MAAPGPLPAAALSPGAPTPRELMHGVAGVTSRAGRDREAGSVLPAGNRGARKASRRSS
SRSMSRDNKFSKKDCLSIRNVVASIQTKEGLNLKLISGDVLYIWADVIVNSVPMNLQLG
GGPLSRAFLQKAGPMLQKELDDRRRETEEKVGNIFMTSGCNLDCKAVLHAVAPYWNN
GAETSWQIMANIIKKCLTTVEVLSFSSITFPMIGTGSLQFPKAVFAKLILSEVFEYSSSTRPI
TSPLQEVHFLVYTNDDEGCQAFLDEFTNWSRINPNKARIPMAGDTQGVVGTVSKPCFTA
YEMKIGAITFQVATGDIATEQVDVIVNSTARTFNRKSGVSRAILEGAGQAVESECAVLAA
QPHRDFIITPGGCLKCKIIIHVPGGKDVRKTVTSVLEECEQRKYTSVSLPAIGTGNAGKNP

ITVADNIIDAIVDFSSQHSTPSLKTVKVVIFQPELLNIFYDSMKKRDLSASLNFQSTFSMTT
CNLPEHWTDMNHQLFCMVQLEPGQSEYNTIKDKFTRTCSSYAIEKIERIQNAFLWQSYQ
VKKRQMDIKNDHKNNERLLFHGTDADSVPYVNQHGFNRSCAGKNAVSYGKGTYFAV
DASYSAKDTYSKPDSNGRKHMYVVRVLTGVFTKGRAGLVTPPPKNPHNPTDLFDSVTN
NTRSPKLFVVFFDNQAYPEYLITFTA.

In one embodiment, the dTAG has an amino acid sequence derived from Poly-ADP-ribose polymerase 14, UniProtKB – Q460N5 (PAR14_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 23)

MAVPGSFPLLVEGSWGPDPPKNLNTKLQMYFQSPKRSGGGECEVRQDPRSPSRFLVFFY
PEDVRQKVLERKNHELVWQGKGTFKLTVQLPATPDEIDHVFEEELLTKESKTKEDVKEP
DVSEELDTKLPLDGGLDKMEDIPEECENISSLVAFENLKANVTDIMLILLVENISGLSNDD
FQVEIIRDFDVAVVTFQKHIDTIRFVDDCTKHHSIKQLQLSPRLLEVTNTIRVENLPPGAD
DYSLKLFFENPYNGGGRVANVEYFPEESSALIEFFDRKVLDTIMATKLDFNKMPLSVFPY
YASLGTALYGKEKPLIKLPAPFEESLDLPLWKFLQKKNHLIEEINDEMRRCHCELTWSQL
SGKVTIRPAATLVNEGRPRIKTWQADTSTTLSSIRSKYKVNPIKVDPTMWDTIKNDVKD
DRILIEFDTLKEMVILAGKSEDVQSIEVQVRELIESTTQKIKREEQSLKEKMIISPGRYFLLC
HSSLLDHLLTECPEIEICYDRVTQHLCLKGPSADVYKAKCEIQEKVYTMAQKNIQVSPEIF
QFLQQVNWKEFSKCLFIAQKILALYELEGTTVLLTSCSSEALLEAEKQMLSALNYKRIEV
ENKEVLHGKKWKGLTHNLLKKQNSSPNTVIINELTSETTAEVIITGCVKEVNETYKLLFN
FVEQNMKIERLVEVKPSLVIDYLKTEKKLFWPKIKKVNVQVSFNPENKQKGILLTGSKTE
VLKAVDIVKQVWDSVCVKSVHTDKPGAKQFFQDKARFYQSEIKRLFGCYIELQENEVM
KEGGSPAGQKCFSRTVLAPGVVLIVQQGDLARLPVDVVVNASNEDLKHYGGLAAALSK
AAGPELQADCDQIVKREGRLLPGNATISKAGKLPYHHVIHAVGPRWSGYEAPRCVYLL
RRAVQLSLCLAEKYKYRSIAIPAISSGVFGFPLGRCVETIVSAIKENFQFKKDGHCLKEIY
LVDVSEKTVEAFAEAVKTVFKATLPDTAAPPGLPPAAAGPGKTSWEKGSLVSPGGLQM
LLVKEGVQNAKTDVVVNSVPLDLVLSRGPLSKSLLEKAGPELQEELDTVGQGVAVSMG
TVLKTSSWNLDCRYVLHVVAPEWRNGSTSSLKIMEDIIRECMEITESLSLKSIAFPAIGTG
NLGFPKNIFAELIISEVFKFSSKNQLKTLQEVHFLLHPSDHENIQAFSDEFARRANGNLVS
DKIPKAKDTQGFYGTVSSPDSGVYEMKIGSIIFQVASGDITKEEADVIVNSTSNSFNLKAG
VSKAILECAGQNVERECSQQAQQRKNDYIITGGGFLRCKNIIHVIGGNDVKSSVSSVLQE

CEKKNYSSICLPAIGTGNAKQHPDKVAEAIIDAIEDFVQKGSAQSVKKVKVVIFLPQVLD
VFYANMKKREGTQLSSQQSVMSKLASFLGFSKQSPQKKNHLVLEKKTESATFRVCGEN
VTCVEYAISWLQDLIEKEQCPYTSEDECIKDFDEKEYQELNELQKKLNINISLDHKRPLIK
VLGISRDVMQARDEIEAMIKRVRLAKEQESRADCISEFIEWQYNDNNTSHCFNKMTNLK
LEDARREKKKTVDVKINHRHYTVNLNTYTATDTKGHSLSVQRLTKSKVDIPAHWSDMK
QQNFCVVELLPSDPEYNTVASKFNQTCSHFRIEKIERIQNPDLWNSYQAKKKTMDAKNG
QTMNEKQLFHGTDAGSVPHVNRNGFNRSYAGKNAVAYGKGTYFAVNANYSANDTYS
RPDANGRKHVYYVRVLTGIYTHGNHSLIVPPSKNPQNPTDLYDTVTDNVHHPSLFVAFY
DYQAYPEYLITFRK.

In one embodiment, the dTAG has an amino acid sequence derived from superoxide dismutase, UniProtKB – P00441 (SODC_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 24)

MATKAVCVLKGDGPVQGIINFEQKESNGPVKVWGSIKGLTEGLHGFHVHEFGDNTAGC
TSAGPHFNPLSRKHGGPKDEERHVGDLGNVTADKDGVADVSIEDSVISLSGDHCIIGRTL
VVHEKADDLGKGGNEESTKTGNAGSRLACGVIGIAQ.

In one embodiment, the dTAG has an amino acid sequence derived from retinal rod rhodopsin-sensitive cGMP 3',5'-cyclic phosphodiesterase subunit delta, UniProtKB – O43924 (PDE6D_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 25)

MSAKDERAREILRGFKLNWMNLRDAETGKILWQGTEDLSVPGVEHEARVPKKILKCKA
VSRELNFSSTEQMEKFRLEQKVYFKGQCLEEWFFEFGFVIPNSTNTWQSLIEAAPESQM
MPASVLTGNVIIETKFFDDDLLVSTSRVRLFYV.

In one embodiment, the dTAG has an amino acid sequence derived from induced myeloid leukemia cell differentiation protein Mcl-1, UniProtKB – Q07820 (MCL1_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 26)

MFGLKRNAVIGLNLYCGGAGLGAGSGGATRPGGRLLATEKEASARREIGGGEAGAVIG
GSAGASPPSTLTPDSRRVARPPPIGAEVPDVTATPARLLFFAPTRRAAPLEEMEAPAADAI
MSPEEELDGYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEEEEDELYRQSLE
IISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLDIK

NEDDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESITDVLV
RTKRDWLVKQRGWDGFVEFFHVEDLEGGIRNVLLAFAGVAGVGAGLAYLIR.

In one embodiment, the dTAG has an amino acid sequence derived from apoptosis regulator Bcl-2, UniProtKB – Q07820 (BCL2_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 27)

MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPH
PAASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSS
QLHLTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIAL
WMTEYLNRHLHTWIQDNGGWDAFVELYGPSMRPLFDFSWLSLKTLLSLALVGACITLG
AYLGHK.

In one embodiment, the dTAG has an amino acid sequence derived from peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, UniProtKB – Q13526 (PIN1_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 28)

MADEEKLPPGWEKRMSRSSGRVYYFNHITNASQWERPSGNSSSGGKNGQGEPARVRCS
HLLVKHSQSRRPSSWRQEKITRTKEEALELINGYIQKIKSGEEDFESLASQFSDCSSAKAR
GDLGAFSRGQMQKPFEDASFALRTGEMSGPVFTDSGIHIILRTE.

In one embodiment, the dTAG has an amino acid sequence derived from tankyrase 1, UniProtKB – O95271 (TNKS1_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 29)

MAASRRSQHHHHHHQQQLQPAPGASAPPPPPPPLSPGLAPGTTPASPTASGLAPFASPR
HGLALPEGDGSRDPPDRPRSPDPVDGTSCCSTTSTICTVAAAPVVPAVSTSSAAGVAPNP
AGSGSNNSPSSSSSPTSSSSSSPSSPGSSLAESPEAAGVSSTAPLGPGAAGPGTGVPAVSGA
LRELLEACRNGDVSRVKRLVDAANVNAKDMAGRKSSPLHFAAGFGRKDVVEHLLQM
GANVHARDDGGLIPLHNACSFGHAEVVSLLLCQGADPNARDNWNYTPLHEAAIKGKID
VCIVLLQHGADPNIRNTDGKSALDLADPSAKAVLTGEYKKDELLEAARSGNEEKLMAL
LTPLNVNCHASDGRKSTPLHLAAGYNRVRIVQLLLQHGADVHAKDKGGLVPLHNACSY
GHYEVTELLLKHGACVNAMDLWQFTPLHEAASKNRVEVCSLLLSHGADPTLVNCHGK
SAVDMAPTPELRERLTYEFKGHSLLQAAREADLAKVKKTLALEIINFKQPQSHETALHC

AVASLHPKRKQVTELLLRKGANVNEKNKDFMTPLHVAAERAHNDVMEVLHKHGAKM
NALDTLGQTALHRAALAGHLQTCRLLLSYGSDPSIISLQGFTAAQMGNEAVQQILSESTP
IRTSDVDYRLLEASKAGDLETVKQLCSSQNVNCRDLEGRHSTPLHFAAGYNRVSVVEYL
LHHGADVHAKDKGGLVPLHNACSYGHYEVAELLVRHGASVNVADLWKFTPLHEAAA
KGKYEICKLLLKHGADPTKKNRDGNTPLDLVKEGDTDIQDLLRGDAALLDAAKKGCLA
RVQKLCTPENINCRDTQGRNSTPLHLAAGYNNLEVAEYLLEHGADVNAQDKGGLIPLH
NAASYGHVDIAALLIKYNTCVNATDKWAFTPLHEAAQKGRTQLCALLLAHGADPTMK
NQEGQTPLDLATADDIRALLIDAMPPEALPTCFKPQATVVSASLISPASTPSCLSAASSID
NLTGPLAELAVGGASNAGDGAAGTERKEGEVAGLDMNISQFLKSLGLEHLRDIFETEQI
TLDVLADMGHEELKEIGINAYGHRHKLIKGVERLLGGQQGTNPYLTFHCVNQGTILLDL
APEDKEYQSVEEEMQSTIREHRDGGNAGGIFNRYNVIRIQKVVNKKLRERFCHRQKEVS
EENHNHHNERMLFHGSPFINAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGG
GTGCPTHKDRSCYICHRQMLFCRVTLGKSFLQFSTMKMAHAPPGHHSVIGRPSVNGLA
YAEYVIYRGEQAYPEYLITYQIMKPEAPSQTATAAEQKT.

In one embodiment, the dTAG has an amino acid sequence derived from tankyrase 2, UniProtKB – O9H2K2 (TNKS2_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 30)

MSGRRCAGGGAACASAAAEAVEPAARELFEACRNGDVERVKRLVTPEKVNSRDTAGR
KSTPLHFAAGFGRKDVVEYLLQNGANVQARDDGGLIPLHNACSFGHAEVVNLLLRHGA
DPNARDNWNYTPLHEAAIKGKIDVCIVLLQHGAEPTIRNTDGRTALDLADPSAKAVLTG
EYKKDELLESARSGNEEKMMALLTPLNVNCHASDGRKSTPLHLAAGYNRVKIVQLLLQ
HGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVNAMDLWQFTPLHEAASKN
RVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQLKERLAYEFKGHSLLQAAREADVTRIK
KHLSLEMVNFKHPQTHETALHCAAASPYPKRKQICELLLRKGANINEKTKEFLTPLHVA
SEKAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQ
GFTALQMGNENVQQLLQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIE
GRQSTPLHFAAGYNRVSVVEYLLQHGADVHAKDKGGLVPLHNACSYGHYEVAELLVK
HGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGADPTKKNRDGNTPLDLVKDGDT
DIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHLAAGYNNLEVA
EYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEA

AQKGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSALPSCYKPQ
VLNGVRSPGATADALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEGASSLEKKEV
PGVDFSITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVE
RLISGQQGLNPYLTLNTSGSGTILIDLSPDDKEFQSVEEEMQSTVREHRDGGHAGGIFNR
YNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHGSPFVNAIIHKGFDERHAY
IGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCRVTLGKSFLQF
SAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRPEGMVDG.

In one embodiment, the dTAG has an amino acid sequence derived from 7,8-dihydro-8-oxoguanin triphosphatase, UniProtKB – P36639 (8ODP_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 31)

MYWSNQITRRLGERVQGFMSGISPQQMGEPEGSWSGKNPGTMGASRLYTLVLVLQPQR
VLLGMKKRGFGAGRWNGFGGKVQEGETIEDGARRELQEESGLTVDALHKVGQIVFEFV
GEPELMDVHVFCTDSIQGTPVESDEMRPCWFQLDQIPFKDMWPDDSYWFPLLLQKKKF
HGYFKFQGQDTILDYTLREVDTV.

In one embodiment, the dTAG has an amino acid sequence derived from Proto-oncogene tyrosine protein kinase Src, UniProtKB – P12931 (SRC_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 32)

MGSNKSKPKDASQRRRSLEPAENVHGAGGGAFPASQTPSKPASADGHRGPSAAFAPAA
AEPKLFGGFNSSDTVTSPQRAGPLAGGVTTFVALYDYESRTETDLSFKKGERLQIVNNTE
GDWWLAHSLSTGQTGYIPSNYVAPSDSIQAEEWYFGKITRRESERLLLNAENPRGTFLV
RESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQQLVAYYSKH
ADGLCHRLTTVCPTSKPQTQGLAKDAWEIPRESLRLEVKLGQGCFGEVWMGTWNGTT
RVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLVQLYAVVSEEPIYIVTEYMSKGSLLDF
LKGETGKYLRLPQLVDMAAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFG
LARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGM
VNREVLDQVERGYRMPCPPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEP
QYQPGENL.

In one embodiment, the dTAG includes a substitution of Threonine (T) with Glycine (G) or Alanine (A) at amino acid position 341. In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 62.

LRLEVKLGQGCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLV
QLYAVVSEEPIYIVTEYGSKGSLLDFLKGETGKYLRLPQLVDMAAQIASGMAYVERMN
YVHRDLRAANILVGENLVCKVADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRF
TIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGYRMPCPPECPESLHDLMCQC
WRKEPEERPTFEYLQAFLEDYF.

In one embodiment, the dTAG is an amino acid sequence derived from, or a fragment thereof, of SEQ. ID. NO.: 63.

LRLEVKLGQGCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLV
QLYAVVSEEPIYIVTEYASKGSLLDFLKGETGKYLRLPQLVDMAAQIASGMAYVERMN
YVHRDLRAANILVGENLVCKVADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRF
TIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGYRMPCPPECPESLHDLMCQC
WRKEPEERPTFEYLQAFLEDYF

In one embodiment, the dTAG has an amino acid sequence derived from prostaglandin E synthase, UniProtKB – O14684 (PTGES_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 33)

MPAHSLVMSSPALPAFLLCSTLLVIKMYVVAIITGQVRLRKKAFANPEDALRHGGPQYC
RSDPDVERCLRAHRNDMETIYPFLFLGFVYSFLGPNPFVAWMHFLVFLVGRVAHTVAY
LGKLRAPIRSVTYTLAQLPCASMALQILWEAARHL.

In one embodiment, the dTAG has an amino acid sequence derived from Arachidonate 5-lipoxygenase activating protein, UniProtKB – P20292 (AL5AP_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 34)

MDQETVGNVVLLAIVTLISVVQNGFFAHKVEHESRTQNGRSFQRTGTLAFERVYTANQ
NCVDAYPTFLAVLWSAGLLCSQVPAAFAGLMYLFVRQKYFVGYLGERTQSTPGYIFGK
RIILFLFLMSVAGIFNYYLIFFFGSDFENYIKTISTTISPLLLIP.

In one embodiment, the dTAG has an amino acid sequence derived from fatty acid binding protein from adipocyte, UniProtKB – P15090 (FABP4_HUMAN) (incorporated herein

by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 35)

MCDAFVGTWKLVSSENFDDYMKEVGVGFATRKVAGMAKPNMIISVNGDVITIKSESTF
KNTEISFILGQEFDEVTADDRKVKSTITLDGGVLVHVQKWDGKSTTIKRKREDDKLVVE
CVMKGVTSTRVYERA.

In one embodiment, the dTAG has an amino acid sequence derived from PH-interacting protein, UniProtKB – Q8WWQ0 (PHIP_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 36)

MSCERKGLSELRSELYFLIARFLEDGPCQQAAQVLIREVAEKELLPRRTDWTGKEHPRT
YQNLVKYYRHLAPDHLLQICHRLGPLLEQEIPQSVPGVQTLLGAGRQSLLRTNKSCKHV
VWKGSALAALHCGRPPESPVNYGSPPSIADTLFSRKLNGKYRLERLVPTAVYQHMKMH
KRILGHLSSVYCVTFDRTGRRIFTGSDDCLVKIWATDDGRLLATLRGHAAEISDMAVNY
ENTMIAAGSCDKMIRVWCLRTCAPLAVLQGHSASITSLQFSPLCSGSKRYLSSTGADGTI
CFWLWDAGTLKINPRPAKFTERPRPGVQMICSSFSAGGMFLATGSTDHIIRVYFFGSGQP
EKISELEFHTDKVDSIQFSNTSNRFVSGSRDGTARIWQFKRREWKSILLDMATRPAGQNL
QGIEDKITKMKVTMVAWDRHDNTVITAVNNMTLKVWNSYTGQLIHVLMGHEDEVFVL
EPHPFDPRVLFSAGHDGNVIVWDLARGVKIRSYFNMIEGQGHGAVFDCKCSPDGQHFA
CTDSHGHLLIFGFGSSSKYDKIADQMFFHSDYRPLIRDANNFVLDEQTQQAPHLMPPPFL
VDVDGNPHPSRYQRLVPGRENCREEQLIPQMGVTSSGLNQVLSQQANQEISPLDSMIQR
LQQEQDLRRSGEAVISNTSRLSRGSISSTSEVHSPPNVGLRRSGQIEGVRQMHSNAPRSEI
ATERDLVAWSRRVVVPELSAGVASRQEEWRTAKGEEEIKTYRSEEKRKHLTVPKENKIP
TVSKNHAHEHFLDLGESKKQQTNQHNYRTRSALEETPRPSEEIENGSSSSDEGEVVAVS
GGTSEEEERAWHSDGSSSDYSSDYSDWTADAGINLQPPKKVPKNKTKKAESSSDEEEES
EKQKQKQIKKEKKKVNEEKDGPISPKKKKPKERKQKRLAVGELTENGLTLEEWLPSTWI
TDTIPRRCPFVPQMGDEVYYFRQGHEAYVEMARKNKIYSINPKKQPWHKMELREQELM
KIVGIKYEVGLPTLCCLKLAFLDPDTGKLTGGSFTMKYHDMPDVIDFLVLRQQFDDAKY
RRWNIGDRFRSVIDDAWWFGTIESQEPLQLEYPDSLFQCYNVCWDNGDTEKMSPWDM
ELIPNNAVFPEELGTSVPLTDGECRSLIYKPLDGEWGTNPRDEECERIVAGINQLMTLDIA
SAFVAPVDLQAYPMYCTVVAYPTDLSTIKQRLENRFYRRVSSLMWEVRYIEHNTRTFNE
PGSPIVKSAKFVTDLLLHFIKDQTCYNIIPLYNSMKKKVLSDSEDEEKDADVPGTSTRKR

KDHQPRRRLRNRAQSYDIQAWKKQCEELLNLIFQCEDSEPFRQPVDLLEYPDYRDIIDTP
MDFATVRETLEAGNYESPMELCKDVRLIFSNSKAYTPSKRSRIYSMSLRLSAFFEEHISSV
LSDYKSALRFHKRNTITKRRKKRNRSSSVSSSAASSPERKKRILKPQLKSESSTSAFSTPTR
SIPPRHNAAQINGKTESSSVVRTRSNRVVVDPVVTEQPSTSSAAKTFITKANASAIPGKTI
LENSVKHSKALNTLSSPGQSSFSHGTRNNSAKENMEKEKPVKRKMKSSVLPKASTLSKS
SAVIEQGDCKNNALVPGTIQVNGHGGQPSKLVKRGPGRKPKVEVNTNSGEIIHKKRGRK
PKKLQYAKPEDLEQNNVHPIRDEVLPSSTCNFLSETNNVKEDLLQKKNRGGRKPKRKM
KTQKLDADLLVPASVKVLRRSNRKKIDDPIDEEEEFEELKGSEPHMRTRNQGRRTAFYN
EDDSEEEQRQLLFEDTSLTFGTSSRGRVRKLTEKAKANLIGW.

In one embodiment, the dTAG has an amino acid sequence derived from SUMO-conjugating enzyme UBC9, UniProtKB – P63279 (UBC9_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 37)

MSGIALSRLAQERKAWRKDHPFGFVAVPTKNPDGTMNLMNWECAIPGKKGTPWEGGL
FKLRMLFKDDYPSSPPKCKFEPPLFHPNVYPSGTVCLSILEEDKDWRPAITIKQILLGIQEL
LNEPNIQDPAQAEAYTIYCQNRVEYEKRVRAQAKKFAPS.

In one embodiment, the dTAG has an amino acid sequence derived from Protein S100-A7, UniProtKB – P31151 (S10A7_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 38)

MSNTQAERSIIGMIDMFHKYTRRDDKIEKPSLLTMMKENFPNFLSACDKKGTNYLADVF
EKKDKNEDKKIDFSEFLSLLGDIATDYHKQSHGAAPCSGGSQ.

In one embodiment, the dTAG has an amino acid sequence derived from phospholipase A2, membrane associated, UniProtKB – P14555 (PA2GA_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 39)

MKTLLLLAVIMIFGLLQAHGNLVNFHRMIKLTTGKEAALSYGFYGCHCGVGGRGSPKD
ATDRCCVTHDCCYKRLEKRGCGTKFLSYKFSNSGSRITCAKQDSCRSQLCECDKAAATC
FARNKTTYNKKYQYYSNKHCRGSTPRC.

In one embodiment, the dTAG has an amino acid sequence derived from histone deacetylase 6, UniProtKB – Q9UBN7 (HDAC6_HUMAN) (incorporated herein by reference), or

a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 40)

MTSTGQDSTTTRQRRSRQNPQSPPQDSSVTSKRNIKKGAVPRSIPNLAEVKKKGKMKKL
GQAMEEDLIVGLQGMDLNLEAEALAGTGLVLDEQLNEFHCLWDDSFPEGPERLHAIKE
QLIQEGLLDRCVSFQARFAEKEELMLVHSLEYIDLMETTQYMNEGELRVLADTYDSVYL
HPNSYSCACLASGSVLRLVDAVLGAEIRNGMAIIRPPGHHAQHSLMDGYCMFNHVAVA
ARYAQQKHRIRRVLIVDWDVHHGQGTQFTFDQDPSVLYFSIHRYEQGRFWPHLKASNW
STTGFGQGQGYTINVPWNQVGMRDADYIAAFLHVLLPVALEFQPQLVLVAAGFDALQG
DPKGEMAATPAGFAQLTHLLMGLAGGKLILSLEGGYNLRALAEGVSASLHTLLGDPCP
MLESPGAPCRSAQASVSCALEALEPFWEVLVRSTETVERDNMEEDNVEESEEEGPWEPP
VLPILTWPVLQSRTGLVYDQNMMNHCNLWDSHHPEVPQRILRIMCRLEELGLAGRCLT
LTPRPATEAELLTCHSAEYVGHLRATEKMKTRELHRESSNFDSIYICPSTFACAQLATGA
ACRLVEAVLSGEVLNGAAVVRPPGHHAEQDAACGFCFFNSVAVAARHAQTISGHALRI
LIVDWDVHHGNGTQHMFEDDPSVLYVSLHRYDHGTFFPMGDEGASSQIGRAAGTGFTV
NVAWNGPRMGDADYLAAWHRLVLPIAYEFNPELVLVSAGFDAARGDPLGGCQVSPEG
YAHLTHLLMGLASGRIILILEGGYNLTSISESMAACTRSLLGDPPPLLTLPRPPLSGALASI
TETIQVHRRYWRSLRVMKVEDREGPSSSKLVTKKAPQPAKPRLAERMTTREKKVLEAG
MGKVTSASFGEESTPGQTNSETAVVALTQDQPSEAATGGATLAQTISEAAIGGAMLGQT
TSEEAVGGATPDQTTSEETVGGAILDQTTSEDAVGGATLGQTTSEEAVGGATLAQTTSE
AAMEGATLDQTTSEEAPGGTELIQTPLASSTDHQTPPTSPVQGTTPQISPSTLIGSLRTLEL
GSESQGASESQAPGEENLLGEAAGGQDMADSMLMQGSRGLTDQAIFYAVTPLPWCPHL
VAVCPIPAAGLDVTQPCGDCGTIQENWVCLSCYQVYCGRYINGHMLQHHGNSGHPLVL
SYIDLSAWCYYCQAYVHHQALLDVKNIAHQNKFGEDMPHPH.

In one embodiment, the dTAG has an amino acid sequence derived from prosaposin, UniProtKB – P07602 (SAP_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 41)

MYALFLLASLLGAALAGPVLGLKECTRGSAVWCQNVKTASDCGAVKHCLQTVWNKPT
VKSLPCDICKDVVTAAGDMLKDNATEEEILVYLEKTCDWLPKPNMSASCKEIVDSYLPV
ILDIIKGEMSRPGEVCSALNLCESLQKHLAELNHQKQLESNKIPELDMTEVVAPFMANIP
LLLYPQDGPRSKPQPKDNGDVCQDCIQMVTDIQTAVRTNSTFVQALVEHVKEECDRLG
PGMADICKNYISQYSEIAIQMMMHMQPKEICALVGFCDEVKEMPMQTLVPAKVASKNV

IPALELVEPIKKHEVPAKSDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKMCSKLPKS
LSEECQEVVDTYGSSILSILLEEVSPELVCSMLHLCSGTRLPALTVHVTQPKDGGFCEVC
KKLVGYLDRNLEKNSTKQEILAALEKGCSFLPDPYQKQCDQFVAEYEPVLIEILVEVMD
PSFVCLKIGACPSAHKPLLGTEKCIWGPSYWCQNTETAAQCNAVEHCKRHVWN.

In one embodiment, the dTAG has an amino acid sequence derived from apolipoprotein a, UniProtKB – P08519 (APOA_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 42)

MEHKEVVLLLLLFLKSAAPEQSHVVQDCYHGDGQSYRGTYSTTVTGRTCQAWSSMTP
HQHNRTTENYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVA
PPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHS
HSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPT
VTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSR
TPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTP
VPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPE
YYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPS
LEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYP
NAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEA
PSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNA
GLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPS
EQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGL
IMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQ
APTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIM
NYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAP
TEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNY
CRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTE
QRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCR
NPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRP
GVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPD
AVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGV
QECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAV

AAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQEC
YHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAP
YCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYH
GNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYC
YTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGN
GQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYT
RDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQ
SYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRD
PGVRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSY
RGTYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPG
VRWEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRG
TYSTTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVR
WEYCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTY
STTVTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWE
YCNLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTT
VTGRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYC
NLTQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVT
GRTCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNL
TQCSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGR
TCQAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQ
CSDAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTC
QAWSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCS
DAEGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQA
WSSMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDA
EGTAVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWS
SMTPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGT
AVAPPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSM
TPHSHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAV
APPTVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPH
SHSRTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPP
TVTPVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHS

RTPEYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVT
PVPSLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTP
EYYPNAGLIMNYCRNPDAVAAPYCYTRDPGVRWEYCNLTQCSDAEGTAVAPPTVTPVP
SLEAPSEQAPTEQRPGVQECYHGNGQSYRGTYSTTVTGRTCQAWSSMTPHSHSRTPEYY
PNAGLIMNYCRNPDPVAAPYCYTRDPSVRWEYCNLTQCSDAEGTAVAPPTITPIPSLEAP
SEQAPTEQRPGVQECYHGNGQSYQGTYFITVTGRTCQAWSSMTPHSHSRTPAYYPNAG
LIKNYCRNPDPVAAPWCYTTDPSVRWEYCNLTRCSDAEWTAFVPPNVILAPSLEAFFEQ
ALTEETPGVQDCYYHYGQSYRGTYSTTVTGRTCQAWSSMTPHQHSRTPENYPNAGLTR
NYCRNPDAEIRPWCYTMDPSVRWEYCNLTQCLVTESSVLATLTVVPDPSTEASSEEAPT
EQSPGVQDCYHGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQRTTEYYPNGGLTRNY
CRNPDAEISPWCYTMDPNVRWEYCNLTQCPVTESSVLATSTAVSEQAPTEQSPTVQDCY
HGDGQSYRGSFSTTVTGRTCQSWSSMTPHWHQRTTEYYPNGGLTRNYCRNPDAEIRPW
CYTMDPSVRWEYCNLTQCPVMESTLLTTPTVVPVPSTELPSEEAPTENSTGVQDCYRGD
GQSYRGTLSTTITGRTCQSWSSMTPHWHRRIPLYYPNAGLTRNYCRNPDAEIRPWCYTM
DPSVRWEYCNLTRCPVTESSVLTTPTVAPVPSTEAPSEQAPPEKSPVVQDCYHGDGRSY
RGISSTTVTGRTCQSWSSMIPHWHQRTPENYPNAGLTENYCRNPDSGKQPWCYTTDPC
VRWEYCNLTQCSETESGVLETPTVVPVPSMEAHSEAAPTEQTPVVRQCYHGNGQSYRG
TFSTTVTGRTCQSWSSMTPHRHQRTPENYPNDGLTMNYCRNPDADTGPWCFTMDPSIR
WEYCNLTRCSDTEGTVVAPPTVIQVPSLGPPSEQDCMFGNGKGYRGKKATTVTGTPCQ
EWAAQEPHRHSTFIPGTNKWAGLEKNYCRNPDGDINGPWCYTMNPRKLFDYCDIPLCA
SSSFDCGKPQVEPKKCPGSIVGGCVAHPHSWPWQVSLRTRFGKHFCGGTLISPEWVLTA
AHCLKKSSRPSSYKVILGAHQEVNLESHVQEIEVSRLFLEPTQADIALLKLSRPAVITDKV
MPACLPSPDYMVTARTECYITGWGETQGTFGTGLLKEAQLLVIENEVCNHYKYICAEHL
ARGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKPGVYARVSRFVTWIEGM
MRNN.

In one embodiment, the dTAG has an amino acid sequence derived from lactoglutathione lyase, UniProtKB – Q04760 (LGUL_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 43)

MAEPQPPSGGLTDEAALSCCSDADPSTKDFLLQQTMLRVKDPKKSLDFYTRVLGMTLIQ
KCDFPIMKFSLYFLAYEDKNDIPKEKDEKIAWALSRKATLELTHNWGTEDDETQSYHNG

NSDPRGFGHIGIAVPDVYSACKRFEELGVKFVKKPDDGKMKGLAFIQDPDGYWIEILNP
NKMATLM.

In one embodiment, the dTAG has an amino acid sequence derived from protein afadin, UniProtKB – P55196 (AFAD_HUMAN) (incorporated herein by reference), or a variant thereof. In one embodiment, the dTAG is derived from the amino acid sequence: (SEQ. ID. NO.: 44)

MSAGGRDEERRKLADIIHHWNANRLDLFEISQPTEDLEFHGVMRFYFQDKAAGNFATK
CIRVSSTATTQDVIETLAEKFRPDMRMLSSPKYSLYEVHVSGERRLDIDEKPLVVQLNW
NKDDREGRFVLKNENDAIPPKKAQSNGPEKQEKEGVIQNFKRTLSKKEKKEKKKREKE
ALRQASDKDDRPFQGEDVENSRLAAEVYKDMPETSFTRTISNPEVVMKRRRQQKLEKR
MQEFRSSDGRPDSGGTLRIYADSLKPNIPYKTILLSTTDPADFAVAEALEKYGLEKENPK
DYCIARVMLPPGAQHSDEKGAKEIILDDDECPLQIFREWPSDKGILVFQLKRRPPDHIPKK
TKKHLEGKTPKGKERADGSGYGSTLPPEKLPYLVELSPGRRNHFAYYNYHTYEDGSDS
RDKPKLYRLQLSVTEVGTEKLDDNSIQLFGPGIQPHHCDLTNMDGVVTVTPRSMDAETY
VEGQRISETTMLQSGMKVQFGASHVFKFVDPSQDHALAKRSVDGGLMVKGPRHKPGIV
QETTFDLGGDIHSGTALPTSKSTTRLDSDRVSSASSTAERGMVKPMIRVEQQPDYRRQES
RTQDASGPELILPASIEFRESSEDSFLSAIINYTNSSTVHFKLSPTYVLYMACRYVLSNQYR
PDISPTERTHKVIAVVNKMVSMMEGVIQKQKNIAGALAFWMANASELLNFIKQDRDLS
RITLDAQDVLAHLVQMAFKYLVHCLQSELNNYMPAFLDDPEENSLQRPKIDDVLHTLT
GAMSLLRRCRVNAALTIQLFSQLFHFINMWLFNRLVTDPDSGLCSHYWGAIIRQQLGHIE
AWAEKQGLELAADCHLSRIVQATTLLTMDKYAPDDIPNINSTCFKLNSLQLQALLQNYH
CAPDEPFIPTDLIENVVTVAENTADELARSDGREVQLEEDPDLQLPFLLPEDGYSCDVVR
NIPNGLQEFLDPLCQRGFCRLIPHTRSPGTWTIYFEGADYESHLLRENTELAQPLRKEPEII
TVTLKKQNGMGLSIVAAKGAGQDKLGIYVKSVVKGGAADVDGRLAAGDQLLSVDGRS
LVGLSQERAAELMTRTSSVVTLEVAKQGAIYHGLATLLNQPSPMMQRISDRRGSGKPRP
KSEGFELYNNSTQNGSPESPQLPWAEYSEPKKLPGDDRLMKNRADHRSSPNVANQPPSP
GGKSAYASGTTAKITSVSTGNLCTEEQTPPPRPEAYPIPTQTYTREYFTFPASKSQDRMAP
PQNQWPNYEEKPHMHTDSNHSSIAIQRVTRSQEELREDKAYQLERHRIEAAMDRKSDSD
MWINQSSSLDSSTSSQEHLNHSSKSVTPASTLTKSGPGRWKTPAAIPATPVAVSQPIRTDL
PPPPPPPPVHYAGDFDGMSMDLPLPPPPSANQIGLPSAQVAAAERRKREEHQRWYEKEK
ARLEEERERKRREQERKLGQMRTQSLNPAPFSPLTAQQMKPEKPSTLQRPQETVIRELQP
QQQPRTIERRDLQYITVSKEELSSGDSLSPDPWKRDAKEKLEKQQQMHIVDMLSKEIQEL

QSKPDRSAEESDRLRKLMLEWQFQKRLQESKQKDEDDEEEEDDDVDTMLIMQRLEAER
RARLQDEERRRQQQLEEMRKREAEDRARQEEERRRQEEERTKRDAEEKRRQEEGYYSR
LEAERRRQHDEAARRLLEPEAPGLCRPPLPRDYEPPSPSPAPGAPPPPPQRNASYLKTQV
LSPDSLFTAKFVAYNEEEEEEDCSLAGPNSYPGSTGAAVGAHDACRDAKEKRSKSQDA
DSPGSSGAPENLTFKERQRLFSQGQDVSNKVKASRKLTELENELNTK.

In one embodiment, the dTAG has an amino acid sequence derived from epidermal growth factor receptor (EGFR, UniProtKB P00533(EGFR_HUMAN) incorporated herein by reference, or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 53): (L858R)

GEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKA
NKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYL
LNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGRAKLLGAEEKEYHA
EGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGE
RLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPS
PTDSNFYRALMDEEDMDDVVDADEYLIPQQG.

In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 54): (T790M)

GEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKA
NKEILDEAYVMASVDNPHVCRLLGICLTSTVQLIMQLMPFGCLLDYVREHKDNIGSQYL
LNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGRAKLLGAEEKEYHA
EGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGE
RLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPS
PTDSNFYRALMDEEDMDDVVDADEYLIPQQG. In one embodiment, SEQ. ID. NO.: 54 has a Leucine at position 163.

In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 55): (C797S)

GEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKA
NKEILDEAYVMASVDNPHVCRLLGICLTSTVQLIMQLMPFGSLLDYVREHKDNIGSQYL
LNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGRAKLLGAEEKEYHA
EGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGE
RLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPS

PTDSNFYRALMDEEDMDDVVDADEYLIPQQG. In one embodiment, SEQ. ID. NO.: 55 has a Leucine at position 163. In one embodiment, SEQ. ID. NO.: 55 has a Threonine at position 95. In one embodiment, SEQ. ID. NO.: 55 has a Leucine at position 163 and a Threonine at position 95.

In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 56): (C790G)
GEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKA
NKEILDEAYVMASVDNPHVCRLLGICLTSTVQLIMQLMPFGCGLDYVREHKDNIGSQYL
LNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGRAKLLGAEEKEYHA
EGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGE
RLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPS
PTDSNFYRALMDEEDMDDVVDADEYLIPQQG. In one embodiment, SEQ. ID. NO.: 56 has a Leucine at position 163. In one embodiment, SEQ. ID. NO.: 56 has a Threonine at position 95. In one embodiment, SEQ. ID. NO.: 56 has a Leucine at position 163 and a Threonine at position 95.

In one embodiment, the dTAG has an amino acid sequence derived from epidermal growth factor receptor (BCR-ABL, or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 57): (T315I)
SPNYDKWEMERTDITMKHKLGGGQYGEVYEGVWKKYSLTVAVKTLKEDTMEVEEFL
KEAAVMKEIKHPNLVQLLGVCTREPPFYIIIEFMTYGNLLDYLRECNRQEVNAVVLLYM
ATQISSAMEYLEKKNFIHRDLAARNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKF
PIKWTAPESLAYNKFSIKSDVWAFGVLLWEIATYGMSPYPGIDLSQVYELLEKDYRMER
PEGCPEKVYELMRACWQWNPSDRPSFAEIHQAFETMFQES. In one embodiment, SEQ. ID. NO.: 57 has a Threonine at position 87.

In one embodiment, the dTAG has an amino acid sequence derived from BCR-ABL (BCR-ABL) or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 58):
SPNYDKWEMERTDITMKHKLGGGQYGEVYEGVWKKYSLTVAVKTLKEDTMEVEEFL
KEAAVMKEIKHPNLVQLLGVCTREPPFYIITEFMTYGNLLDYLRECNRQEVNAVVLLYM
ATQISSAMEYLEKKNFIHRDLAARNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKF

PIKWTAPESLAYNKFSIKSDVWAFGVLLWEIATYGMSPYPGIDLSQVYELLEKDYRMER PEGCPEKVYELMRACWQWNPSDRPSFAEIHQAFETMFQES.

In one embodiment, the dTAG has an amino acid sequence derived from ALK (ALK, UniProtKB Q9UM73 (ALK_HUMAN) incorporated herein by reference, or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 59) (L1196M):

ELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDLKEVPRKNITLIRGLGHGAFGEVYE GQVSGMPNDPSPLQVAVKTLPEVCSEQDELDFLMEALIISKFNHQNIVRCIGVSLQSLPRF IMLELMAGGDLKSFLRETRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIAAR NCLLTCPGPGRVAKIGDFGMARDIYRAGYYRKGGCAMLPVKWMPPEAFMEGIFTSKTD TWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPPKNCPGPVYRIMTQCWQHQ PEDRPNFAIILERIEYCTQDPDVINTALPIEYGPLVEEEEK. In one embodiment, SEQ. ID. NO.: 59 has a Leucine at position 136.

In one embodiment, the dTAG has an amino acid sequence derived from JAK2 (JAK2, UniProtKB O60674 (JAK2_HUMAN) incorporated herein by reference, or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 60) (V617F):

VFHKIRNEDLIFNESLGQGTFTKIFKGVRREVGDYGQLHETEVLLKVLDKAHRNYSESFF EAASMMSKLSHKHLVLNYGVCFCGDENILVQEFVKFGSLDTYLKKNKNCINILWKLEV AKQLAWAMHFLEENTLIHGNVCAKNILLIREEDRKTGNPPFIKLSDPGISITVLPKDILQE RIPWVPPECIENPKNLNLATDKWSFGTTLWEICSGGDKPLSALDSQRKLQFYEDRHQLP APKAAELANLINNCMDYEPDHRPSFRAIIRDLNSLFTPD. In one embodiment, SEQ. ID. NO.: 60 has a valine at position 82.

In one embodiment, the dTAG has an amino acid sequence derived from BRAF (BRAF, UniProtKB P15056 (BRAF_HUMAN) incorporated herein by reference, or a variant thereof. In one embodiment, the dTAG is derived from, includes, or is the amino acid sequence: (SEQ. ID. NO.: 61) (V600E):

DWEIPDGQITVGQRIGSGSFGTVYKGKWHGDVAVKMLNVTAPTPQQLQAFKNEVGVL RKTRHVNILLFMGYSTAPQLAIVTQWCEGSSLYHHLHASETKFEMKKLIDIARQTARGM DYLHAKSIIHRDLKSNNIFLHEDNTVKIGDFGLATEKSRWSGSHQFEQLSGSILWMAPEV IRMQDSNPYSFQSDVYAFGIVLYELMTGQLPYSNINNRDQIIFMVGRGSLSPDLSKVRSN

CPKRMKRLMAECLKKKRDERPSFPRILAEIEELARE. In one embodiment, SEQ. ID. NO.: 61 has a Valine at position 152. In one embodiment, SEQ. ID. NO. 61 has a Tyrosine at position 153. In one embodiment, SEQ. ID. NO.: 61 has a Valine at position 152. In one embodiment, SEQ. ID. NO. 61 has a Lysine at position 153. In one embodiment, SEQ. ID. NO.: 61 has a Valine at position 152 and a Lysine at position 153.

In one embodiment, the dTAG has an amino acid sequence derived from a LRRK2 protein (UniProtKB – Q5S007 (LRRK2_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from LRRK2amino acid 1328 to 1511. In one embodiment, the dTAG is derived from LRRK2 amino acid 1328 to 1511, wherein amino acid 1441 is Cysteine

In one embodiment, the dTAG has an amino acid sequence derived from a PDGFRα protein (UniProtKB – P09619 (PDGFR_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619. In one embodiment, the dTAG is derived from amino acid 600 to 692 of P09619, wherein amino acid 674 is Isoleucine.

In one embodiment, the dTAG has an amino acid sequence derived from a RET protein (UniProtKB – P07949 (RET_HUMAN) incorporated herein by reference), or variant thereof. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 691 is Serine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 749 is Threonine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 762 is Glutamine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 791 is Phenylalanine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 804 is Methionine. In one embodiment, the dTAG is derived from amino acid 724 to 1016 of P07949, wherein amino acid 918 is Threonine.

In one embodiment, the dTAG has an amino acid sequence derived from a JAK3 protein (UniProtKB - P52333 (JAK3_HUMAN) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a ABL protein (UniProtKB - P00519 (ABL_HUMAN) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a MEK1 protein (UniProtKB - Q02750 (MP2K1_HUMAN) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a KIT protein (UniProtKB - P10721 (KIT_HUMAN) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a KIT protein (UniProtKB - P10721 (KIT_HUMAN) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a HIV reverse transcriptase protein (UniProtKB - P04585 (POL_HV1H2) incorporated herein by reference), or variant thereof.

In one embodiment, the dTAG has an amino acid sequence derived from a HIV integrase protein (UniProtKB - Q76353 (Q76353_9HIV1)) incorporated herein by reference), or variant thereof.

B. **Proteins of Interest**

As contemplated herein, the dTAG strategy can be utilized to produce a stably expressed, endogenous protein-dTAG hybrid in vivo, or as the case may be ex vivo or in vitro, by genomic insertion of the dTAG nucleic acid sequence either 5'- or 3' in-frame with the nucleic acid sequence encoding the protein of interest. Following the insertion of the in-frame dTAG nucleic acid sequence, the cell expresses the endogenous protein-dTAG hybrid, allowing for the modulation of the activity of the endogenous protein-dTAG hybrid through the administration of a heterobifunctional compound that is capable of binding the dTAG and thus degrading the endogenous protein-dTAG hybrid. In one embodiment, the activity of the endogenous protein-dTAG hybrid is reduced.

In certain embodiments, a nucleic acid encoding a dTAG can be genomically inserted in-frame with a gene encoding a protein that is involved in a disorder. Non-limiting examples of particular genes involved in disorders that may be targeted for dTAG insertion include by way of non-limiting example, alpha-1 antitrypsin (A1AT), apolipoprotein B (APOB), angiopoietin-like protein 3 (ANGPTL3), proprotein convertase subtilisin/kexin type 9 (PCSK9), apolipoprotein C3 (APOC3), catenin (CTNNB1), low density lipoprotein receptor (LDLR), C-reactive protein (CRP), apolipoprotein a (Apo(a)), Factor VII, Factor XI, antithrombin III (SERPINC1), phosphatidylinositol glycan class A (PIG-A), C5, alpha-1 antitrypsin (SERPINA1), hepcidin

regulation (TMPRSS6), (delta-aminolevulinate synthase 1 (ALAS-1), acylCaA:diacylglycerol acyltransferase (DGAT), miR-122, miR-21, miR-155, miR-34a, prekallikrein (KLKB1), connective tissue growth factor (CCN2), intercellular adhesion molecule 1 (ICAM-1), glucagon receptor (GCGR), glucorticoid receptor (GCCR), protein tyrosine phosphatase (PTP-1B), c-Raf kinase (RAF1), fibroblast growth factor receptor 4 (FGFR4), vascular adhesion molecule-1 (VCAM-1), very late antigen-4 (VLA-4), transthyretin (TTR), survival motor neuron 2 (SMN2), growth hormone receptor (GHR), dystophia myotonic protein kinase (DMPK), cellular nucleic acid-binding protein (CNBP or ZNF9), clusterin (CLU), eukaryotic translation initiation factor 4E (eIF-4e), MDM2, MDM4, heat shock protein 27 (HSP 27), signal transduction and activator of transcription 3 protein (STAT3), vascular endothelial growth factor (VEGF), kinesin spindle protein (KIF11), hepatitis B genome, the androgen receptor (AR), Atonal homolog 1 (ATOH1), vascular endothelial growth factor receptor 1 (FLT1), retinoschism 1 (RS1), retinal pigment epithelium-specific 65 kDa protein (RPE65), Rab escort protein 1 (CHM), and the sodium channel, voltage gated, type X, alpha subunit (PN3 or SCN10A). Additional proteins of interest that may be targeted by dTAG insertion include proteins associated with gain of function mutations, for example, cancer causing proteins.

In particular embodiments, the protein of interest for targeting is apoB-100, ANGPTL3, PCSK9, APOC3, CRP, ApoA, Factor XI, Factor VII, antithrombin III, phosphatidylinositol glycan class A (PIG-A), the C5 component of complement, Alpha-1-antitrypsin (A1AT), TMPRSS6, ALAS-1, DGAT-2, KLB1, CCN2, ICAM, glucagon receptor, glucocorticoid receptor, PTP-1B, FGFR4, VCAM-1, VLA-4, GCCR, TTR, SMN1, GHR, DMPK, or NAV1.8.

In one embodiment, the dTAG is genomically integrated in-frame, either 5' or 3', into the gene encoding for an endogenous protein associated with a proteopathy. In one embodiment the dTAG is genomically integrated in-frame, either 5' or 3', into the gene encoding for an endogenous protein associated with a disorder selected from is genomically inserted in-frame, either 5' or 3', into the gene encoding for an endogenous protein associated with Alzheimer's disease (Amyloid β peptide (Aβ); Tau protein), Cerebral β-amyloid angiopathy (Amyloid β peptide (Aβ)), Retinal ganglion cell degeneration in glaucoma (Amyloid β peptide (Aβ)), Prion diseases (Prion protein), Parkinson's disease and other synucleinopathies (α-Synuclein), Tauopathies (Microtubule-associated protein tau (Tau protein)), Frontotemporal lobar degeneration (FTLD) (Ubi+, Tau-) (TDP-43), FTLD–FUS (Fused in sarcoma (FUS) protein), Amyotrophic lateral sclerosis (ALS)

(Superoxide dismutase, TDP-43, FUS), Huntington's disease and other triplet repeat disorders (Proteins with tandem glutamine expansions), Familial British dementia (ABri), Familial Danish dementia (Adan), Hereditary cerebral hemorrhage with amyloidosis (Icelandic) (HCHWA-I) (Cystatin C), CADASIL (Notch3), Alexander disease (Glial fibrillary acidic protein (GFAP)), Seipinopathies (Seipin), Familial amyloidotic neuropathy, Senile systemic amyloidosis (Transthyretin), Serpinopathies (Serpins), AL (light chain) amyloidosis (primary systemic amyloidosis) (Monoclonal immunoglobulin light chains), AH (heavy chain) amyloidosis (Immunoglobulin heavy chains), AA (secondary) amyloidosis (Amyloid A protein), Type II diabetes (Islet amyloid polypeptide (IAPP; amylin)), Aortic medial amyloidosis (Medin (lactadherin)), ApoAI amyloidosis (Apolipoprotein AI), ApoAII amyloidosis (Apolipoprotein AII), ApoAIV amyloidosis (Apolipoprotein AIV), Familial amyloidosis of the Finnish type (FAF) (Gelsolin), Lysozyme amyloidosis (Lysozyme), Fibrinogen amyloidosis (Fibrinogen), Dialysis amyloidosis (Beta-2 microglobulin), Inclusion body myositis/myopathy (Amyloid β peptide (Aβ)), Cataracts (Crystallins), Retinitis pigmentosa with rhodopsin mutations (rhodopsin), Medullary thyroid carcinoma (Calcitonin), Cardiac atrial amyloidosis (Atrial natriuretic factor), Pituitary prolactinoma (Prolactin), Hereditary lattice corneal dystrophy (Keratoepithelin), Cutaneous lichen amyloidosis (Keratins), Mallory bodies (Keratin intermediate filament proteins), Corneal lactoferrin amyloidosis (Lactoferrin), Pulmonary alveolar proteinosis (Surfactant protein C (SP-C)), Odontogenic (Pindborg) tumor amyloid (Odontogenic ameloblast-associated protein), Seminal vesicle amyloid (Semenogelin I), Cystic Fibrosis (cystic fibrosis transmembrane conductance regulator (CFTR) protein), Sickle cell disease (Hemoglobin), and Critical illness myopathy (CIM) (Hyperproteolytic state of myosin ubiquitination).

As contemplated herein, by genomically inserting a nucleic acid encoding a dTAG in frame with particular proteins of interest, modulation of the protein of interest can be achieved by administering a heterobifunctional compound specific for the dTAG, which binds to the protein-dTAG hybrid, leading to its degradation. Because of the ability to modulate a particular protein of interest in this manner, such a strategy can be used to treat disorders wherein expression of a protein above certain threshold levels within the cell leads to a diseased state. Other applications of this technology include, but are not limited to 1.) targeted degradation of proteins where pathology is a function of gain of function mutation(s), 2) targeted degradation of proteins where pathology is a function of amplification or increased expression, 3) targeted degradation of

proteins that are manifestations of monogenetic disease, 4) targeted degradation of proteins where genetic predisposition manifests over longer periods and often after alternative biological compensatory mechanisms are no longer adequate, for example, but not limited to, hypercholesterolemia and proteinopathies.

By controlled degradation of the endogenous protein-dTAG hybrid, a favorable change in protein expression or activity kinetics may result in prevention and/or treatment of a disorder in a subject in need thereof.

Exemplary diseases and disorders capable of being treated by the currently contemplated methods are described, for example, in U.S. Application No. 20150329875 titled "Methods and Compositions for Prevention of Treatment of a Disease," incorporated herein by reference.

In certain embodiments, the target proteins are involved in lipid metabolism. For example, hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood which is known to increase the risk of coronary artery disease. Familial hypercholesterolemia, hyperlipidemia, and familial chylomicronemia are genetic conditions passed through families where an aberrant gene causes the observed symptomology. Mutations in genes encoding the LDL receptor (LDLR), Apoliprotein B (APOB), angiopoietin-like protein 3 (ANGPTL3) and proprotein convertase subtilisin/kexin type 9 (PCSK9) are involved in these diseases. The LDLR serves to remove LDL from the plasma for internalization into the cell. The LDLR is a transmembrane protein that localizes to clathrin-coated pits where it forms a complex with ApoB-100 (the longer gene product of APOB) and apoE enriched lipoproteins. Following endocytosis of this complex, it moves to the endosome where the lipoproteins are released from the complex for eventual degradation by the lysosome. The LDLR can then be recycled back to the cell surface.

Patients with defective apoB-100, termed 'Familial defective apolipoprotein B' (FDB), frequently carry a R3500Q mutation in APOB which makes LDL with reduced ability to bind to the LDLR, reducing plasma clearance, thus raising plasma levels of fatty acids (Innerarity et al, (1987) PNAS USA 84:6919). FDB is generally recognized as an autosomal dominant condition, and occurs in approximately 1:700 people of European descent (Ginsburg and Willard (2012) Genomic and Personalized Medicine, volumes 1 and 2. Academic Press, London. p. 507). Thus, in FDB patients that are heterozygous for the mutation at apoB-100, specific degradation of the defective apoB-100 allele by inserting a dTAG in-frame in the allele in liver cells and

administering a heterobifunctional compound, resulting in the gene product of an apo-100 defective protein-dTAG hybrid, can cause correction of the disease.

Similarly, angiopoietin-like protein 3 (ANGPTL3) overexpression mutations that cause elevated levels of ANGPTL3 can cause hyperlipidemia in subjects. ANGPTL3 also acts as dual inhibitor of lipoprotein lipase (LPL) and endothelial lipase (EL), and increases plasma triglyceride and HDL cholesterol in rodents. ANGPTL3 is expressed primarily in the liver and secreted, and normally acts to increase plasma levels of triglycerides, LDL cholesterol and HDL cholesterol where it acts directly on the liver to regulate hepatocellular lipoprotein secretion and clearance (Musunuru et at (2010) N Engl J Med 363:23 p. 2220). Thus, the method of the invention can be used to treat hyperlipidemia related to ANGPTL3 overexpression through the targeted degradation of the protein using the dTAG insertion strategy described herein.

PCSK9 is another gene encoding a protein that plays a major regulatory role in cholesterol homeostasis. PCSK9 binds to the epidermal growth factor-like repeat A (EGF-A) domain of LDLR, and induces LDLR degradation. Autosomal dominant, toxic gain of function mutations in PCSK9 (e.g. S127R, P216L, D374Y and N157K) have been described and are associated with hyperlipidemia and Familial hypercholesterolemia (FH) as a result of an increased rate of LDLR degradation leading to a corresponding increase in plasma LDL cholesterol (Abifadel et at (2003) Nat Gen 34(2):154). In addition, loss of function PCSK9 mutations have been identified (e.g. Y142X, C679X and R46L) that cause an increase in hepatic LDLR levels, with an associated substantial decrease in the amount of plasma LDL cholesterol, leading to an 88% reduction in the incidence of coronary heart disease (Cohen et at (2003) New Eng J Med 354(12):1264). Thus the methods and compositions of the invention can be used to treat or prevent hyperlipidemia and/or FH through the targeted degradation of the PCSK9 protein using the dTAG insertion strategy described herein.

Familial chylomicronemia syndrome, or FCS, is characterized by extremely high levels of plasma triglycerides and lead to a number of health problems such as abdominal pain, enlargement of the liver and spleen and recurrent acute pancreatitis. In addition, there are subjects with high triglyceride levels that do not have FCS, but, due to the elevated triglycerides, have similar health issues. Apolipoprotein C3, or apo-CIII, encoded by the APOC3 gene, is a component of very low lipoprotein (VLDL), LDL, HDL and chylomicrons, and normally inhibits lipolysis by inhibiting lipoprotein lipase and hepatic lipase. Apo-CIII inhibits hepatic uptake of triglyceride-rich particles

and can be elevated in patients with hyperlipidemia (Bobik, (2008) Circulation 118:702) and is an independent cardiovascular disease risk factor. Knocking out the APOC3 gene in mice results in animals with reduced plasma triglyceride levels as compared to normal (Maeda et al (1994) J Biol Chem 269(38):23610). Thus, the methods and compositions of the invention can be used to prevent or treat a subject with lipid metabolism disorders (e.g., familial hypercholesterolemia, hyperlipidemia, and familial chylomicronemia) by targeted degradation of the APOC3 protein through use of the dTAG insertion strategy described herein.

In other embodiments, the target protein(s) are involved in vascular diseases such as cardiovascular disease and coronary artery disease. Similar to the lipid metabolism disorders discussed above, coronary artery diseases can also be caused by specific genes. For example, C-reactive protein (CRP) is a protein produced in the liver that has been associated with inflammatory disease. It is an acute phase protein that binds to phosphocholine expressed on the surface of dead or dying cells where its job is to activate the complement system to help clear the cell. In chronic inflammatory disease, increased levels of CRP may exacerbate disease symptoms by contributing and amplifying an overall chronic inflammatory state. In addition, it has been shown in rat models that CRP increases myocardial and cerebral infarct size, which, when translated into human patients, maybe predicative of a more negative prognosis following heart attack. When inhibitors of CRP are introduced into these rat models, infarct size and cardiac dysfunction are decreased (Pepys et at (2005) Nature 440(27):1217). Inhibition of CRP thus may be beneficial both in inflammatory diseases and in coronary artery disease. The methods and compositions of the invention may be used to cause modulation of CRP expression by targeted degradation of the CRP protein through use of the dTAG insertion strategy described herein.

Plasma lipoprotein (Lp(a)) is a low density lipoprotein particle comprising Apolipoprotein(a) (apo(a)), and is also an independent risk factor for cardiovascular disease including atherosclerosis. Apo(a) contacts the surface of LDL through apoB-100, linked by a disulfide bond, and it has been reported that genetic polymorphisms associated with elevated Apo(a) levels are associated with an excessive rate of myocardial infarction (Chasman et at (2009) Atherosclerosis 203(2):371). Lp(a) concentration in the plasma varies widely in concentration between individuals, where these concentration differences appear to be genetically determined. The apo(a) gene comprises a number of plasminogen kringle 4-like repeats, and the number of these kringle repeats is inversely related to plasma concentration of Lp(a). A DNA-vaccine

approach, designed to mount an immune response to apo(a) and cause antibody-mediated clearance of Lp(a), demonstrated a reduction in the proatherosclerotic activity of Lp(a) in mice (Kyutoku et at (2013) Sci Rep 3 doi:10.1038/srep1600). Thus the methods and compositions of the invention can be used to reduce the expression of the ApoA protein, resulting in a decrease in plasma concentration of Lp(a), by targeted degradation of the ApoA protein through use of the dTAG insertion strategy described herein.

Clotting disorders, often referred to as thrombophilia, can have ramifications in vascular diseases. The complex network of biochemical events regulating mammalian coagulation comprises 5 proteases (factors II, VII, IX, and X and protein C) that interface with 5 cofactors (tissue factor, factor V, factor VIII, thrombomodulin, and surface membrane proteins) to generate fibrin, which is the main component of a clot. A delicate balance exists between powerful endogenous procoagulant and thromboresistant forces to ensure the fluidity of blood and maintain the readiness of these factors to induce a blood clot if an injury occurs. High plasma activity of both Factor XI and Factor VII are associated with hypercoagulation and thrombotic disease (coronary infarcts, stroke, deep vein thrombosis, pulmonary embolism) and with poor patient prognosis. It has been demonstrated that people that with severe Factor XI deficiency are protected from ischemic brain injury and stroke (Saloman et at (2008) Blood 111:4113). At the same time, it has been shown that high levels of FXI are associated with higher rates of stroke incidents in patients (Yang et at (2006) Am J Clin Path 126: 411). Similarly, high Factor VII levels are also associated with coronary artery disease although this is complicated by other considerations such as how the Factor VII is measured, and which form of the protein is analyzed (Chan et at (2008) Circulation 118:2286). Thus, the methods and compositions of the invention can be used to prevent or treat subjects with hyperthrombotic disease through selective degradation of clotting factors associated with the disease (for example, Factor VII and Factor XI) by targeted degradation of Factor XI and/or Factor VII through use of the dTAG insertion strategy described herein.

As described above, the balance of the clotting cascade is crucial. Thus, in addition to the importance of the clotting factors, the inhibitors of these factors are also critical. Patients with hemophilias are deficient in one or more components of the clotting cascade, and have a reduced clotting capacity as a consequence. In one of the last steps of this cascade, thrombin acts on fibrinogen to create fibrin which is the main component of the clot. The cascade leads up to the production of active thrombin to allow this to occur. To keep the system balanced, antithrombin

(also known as antithrombin III, encoded by the SERPINC1 gene) acts upon thrombin to inhibit its action. In many hemophilias, the factor deficiency is not absolute and there is some degree of clotting that occurs. Thus an approach based on degradation of antithrombin could allow the clotting cascade to produce sufficient clotting when the upstream factors are limited, potentially regardless of which factor is deficient. This has been demonstrated using blood derived from hemophilia A patients (see Di Micco et at (2000) Eur J Pharmacol. March 10; 391(1-2):1-9.). The methods and compositions of the invention can be used to treat patients with hemophilias such as Hemophilia A and Hemophilia B by targeted degradation of the antithrombin III protein through use of the dTAG insertion strategy described herein.

The target protein(s) may also be involved in blood disorders (hematological conditions). The complement system is a pivotal player in multiple hematological conditions. Paroxysmal nocturnal hemoglobinuria (PNH) is a hemolytic disease caused by a defect in the PIG-A gene (see Brodsky (2008) Blood Rev 22(2):65). The PIG-A gene product phosphatidylinositol glycan class A is required for the first step in the synthesis of GPI-anchored proteins. PIG-A is found on the X chromosome and mutations in PIG-A result in red blood cells that are sensitive to hemolysis by complement. Notably, these mutant cells lack the GPI-anchored proteins CD55 and CD59. CD59 interacts directly with the complement related membrane attack complex (or MAC) to prevent lytic pore formation by blocking the aggregation of C9, a vital step in the assembly of the pore. CD55 functions to accelerate the destruction of the C3 convertase, so in the absence of CD55, there is more of the C3 convertase enzyme, leading to more MAC formation. Thus, the lack of both of these proteins leads to increases lysis of the mutant red blood cells. For patients with PNH, complications due to increased thrombosis are the greatest concern (Brodsky (2008) Blood Rev 22(2):65). 40% of PNH patients have ongoing thrombosis which can lead to stroke and acute cardiovascular disease. Thus, the methods and compositions of the inventions can be used to treat and/or prevent PHN in a subject by targeted degradation of the phosphatidylinositol glycan class A (PIG A) through use of the dTAG insertion strategy described herein.

Inhibition of the C5 component of complement has been approved as a treatment for both PNH and atypical hemolytic-uremic syndrome (aHUS), validating C5 as an important therapeutic target. The hemolysis of red blood cells associated with aHUS occurs when the cells are targeted for destruction by the alternative pathway due to a dysregulation of the complement system (part of innate immunity). Normally the destructive C3bBb complex is formed on the surface of an

invading cell (e.g. a bacterium) to hasten its destruction as part of the alternative pathway in the complement system. The C3bBb complex can bind another C3b to form a C3bBbC3b complex which then acts as a C5 convertase. C5 convertase cleaves C5 to C5a and C5b, and C5b recruits C6, C7, C8 and C9 to form the MAC. A set of complement regulatory proteins (e.g. CD35, CD46, CD55 and CD59) are located on the body's own cells to inhibit the activity of these proteins and thus protect them. However, when there is an imbalance of these regulatory proteins, the C3bBb complex can form inappropriately (de Jorge et at (2011) J Am Soc Nephrol 22:137). This syndrome, in addition to the premature destruction of red blood cells can also lead to kidney disease as a result of the damaging and clogging of the glomerular filtering apparatus. C5 negative mice were shown to be protected when crossed with mice with complement regulator protein mutations, data that has been used to validate the idea of C5 as a target in aHUS (de Jorge, ibid) and other diseases related to complement dysregulation. The C5b-specific monoclonal antibody eculizamab has been successfully used to treat aHUS (Gruppo and Rother, (2009) N Engl J Med 360; 5 p 544) and other complement-mediated diseases (e.g. Paroxysmal Nocturnal Haemoglobinuria (PNH) (Hillmen et al, (2013) Br. J Haem 162:62)). Thus, the methods and compositions of the invention can be used to modulate the expression of C5 and so prevent or treat diseases associated with complement dysregulation by targeted degradation of C5 through use of the dTAG insertion strategy described herein.

Alpha-1-antitrypsin (A1AT) deficiency occurs in about 1 in 1500-3000 people of European ancestry but is rare in individuals of Asian descent. The alpha-1-antitrypsin protein is a protease inhibitor that is encoded by the SERPINA1 gene and serves to protect cells from the activity of proteases released by inflammatory cells, including neutrophil elastase, trypsin and proteinase-3 (PR-3). Deficiency is an autosomal co-dominant or a recessive disorder caused by mutant SERPINA1 genes in heterozygous individuals where reduced expression from the mutant allele or the expression of a mutant A1AT protein with poor inhibitory activity leads to chronic lack of inhibition of neutrophil elastase resulting in tissue damage. The most common SERPINA1 mutation comprises a Glu342Lys substitution (also referred to as the Z allele) that causes the protein to form ordered polymers in the endoplasmic reticulum of patient hepatocytes. These inclusions ultimately cause liver cirrhosis which can only be treated by liver transplantation (Yusa et at (2011) Nature 478 p. 391). The polymerization within the hepatocytes results in a severe decrease in plasma A1AT levels, leading to increased risk of this inflammatory disease. In

addition, A1AT deficiency is linked to pulmonary diseases including chronic obstructive pulmonary disease (COPD), emphysema and chronic bronchitis (Tuder et at (2010) Proc Am Thorac Soc 7(6): p. 381) and potentially may have a far broader reach into the inhibition of the progression of other diseases including type 1 and type 2 diabetes, acute myocardial infarction, rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, transplant rejection, graft versus host disease and multiple sclerosis (Lewis (2012) Mol Med 18(1) p. 957). Population studies have suggested a minimum ATA1 plasma threshold of approximately 0.5 mg/mL (normal plasma levels are approximately 0.9-1.75 mg/ML in a non-inflammatory state) to avoid these diseases, and current therapies mostly act to reduce symptoms through the use of bronchodilators and the like, although the use of weekly infusions of A1AT (Zemaira®) is also an option for emphysema patients with a demonstrated severe lack of plasma A1AT. Severe lung disease associated with A1AT also is ultimately treated by transplant. Clinical trials for the treatment of A1AT deficiency involve a variety of approaches including the delivery of concentrated A1AT protein, use of an AAV construct comprising an A1AT gene by IM injection, and the use of A1AT in HIV, to list just a few. Thus, the compositions and methods of the invention can be used to treat or prevent diseases related to A1AT deficiency by targeted degradation of alpha-1-antitrypsin protein through use of the dTAG insertion strategy described herein, thereby eliminating the hepatic aggregates that can lead to cirrhosis.

Another liver target of interest includes any protein(s) that is(are) involved in the regulation of iron content in the body. Iron is essential for the hemoglobin production, but in excess can result in the production of reactive oxygen species. In patients that are dependent on blood transfusions (e.g. certain hemophilias, hemoglobinopathies), secondary iron overload is common. The iron-regulatory hormone hepcidin, and its receptor and iron channel ferroportin control the dietary absorption, storage, and tissue distribution of iron by promoting its cellular uptake. The regulation of hepcidin is done at a transcriptional level, and is sensitive to iron concentrations in the plasma where increased hepcidin expression leads to lower plasma iron concentrations. Through a series of receptor-ligand interactions, involving a receptor known as hemojuvelin, the hepcidin gene is upregulated by a SMAD transcription factor. Iron-related hepcidin down regulation in turn is regulated by a protease known as TMPRSS6, which cleaves hemojuvelin and prevents the upregulation of hepcidin (Ganz (2011) Blood 117:4425). Down regulation of TMPSS6 expression by use of an inhibitory RNA targeting the TMRSS6 mRNA has been shown

to cause a decrease in iron overload in mouse models (Schmidt et al (2013) Blood 121:1200). Thus, the methods and compositions of the invention can be used to target TMPRSS6 for degradation through use of the dTAG insertion strategy described herein.

Other conditions related to iron utilization pathways in the body are porphyrias. These disorders result from a number of deficiencies in the enzymes involved in heme synthesis. Acute intermittent porphyia (AIP) is an autosomal dominant disorder and is the second most common porphyria, with an incidence of approximately 5-10 in 100,000 people. AIP is caused by a deficiency in hydroxymethylbilane synthase (HMB synthase (HMBS), also called porphobilinogen-deaminase), where the mutations in the HMBS gene are very heterogeneous, comprising missense and point mutations (Solis et al (1999) Mol Med 5:664). The potentially life-threatening AIP attacks can have gastrointestinal, neuropsychiatric, cardiovascular and nervous system manifestations. Attacks have several triggers, can last for several days, and often require hospitalization and can be precipitated by several seemingly unrelated factors including certain drugs, infection, caloric restriction, smoking, alcohol and hormonal fluctuations relating to the menstrual cycle (Yasuda et al (2010) Mol Ther 18(1):17). HMB synthase is part of the heme synthesis pathway, where glycine and succinyl-CoA are joined by delta-aminolaevulinate synthase 1 (ALAS-1) to make aminolevulinic acid, which is then acted upon by aminolevulinic acid dehydratase (ALAD) to make phosphobilinogen. Phosphobilinogen is the converted to hydroxymethylbilane by HMB synthase. The pathway continues on from there, ultimately producing the heme (Ajioka et at (2006) Biochim Biophys Acta 1762:723). Regardless of the trigger, all attacks result in an elevation of the enzyme delta-aminolevulinate synthase 1 (ALAS-1). This enzyme is the first enzyme in the hepatic heme synthesis pathway and when induced, the deficiency in HMB synthase becomes rate-limiting and the aminolevulinic acid and phosphobilinogen precursors accumulate (Yasuda, ibid). Liver transplant in AIP patients can stop the attacks, indicating that targeting the liver may be therapeutically beneficial. Additionally, in mouse models of AIP, where the mice have only 30% of normal HMB synthase levels, insertion of the transgene HMBS, encoding HMB synthase, resulted in a decrease in aminolevulinic acid and phosphobilinogen accumulation when the mice were given phenobarbital (Yasuda, ibid). Double stranded RNAs designed for the inhibition of ALAS-1 have also been shown to reduce ALAS-1 expression in vivo in a mouse AIP model and to reduce phosphobilinogen accumulation in response to phenobarbital treatment (see U.S. Patent Publication 20130281511). Thus the

methods and compositions of the invention may be used to prevent and treat AIP by targeted degradation of ALAS-1 using the dTAG insertion strategy described herein.

Non-alcoholic fatty liver disease (NAFLD) is the most common form of liver disease worldwide, with a prevalence of 15%-30% in Western populations and is caused by triglyceride accumulation within the liver. However, the prevalence increases to 58% in overweight populations and 98% in obese populations. Nonalcoholic steatohepatitis (NASH) is a more advanced form of NAFLD where liver injury has occurred, and can lead to liver failure, portal hypertension, hepatocarcinoma and cirrhosis (Schwenger and Allard (2014) World J Gastronen 20(7): 1712). Evidence appears to suggest that the hepatic triglyceride accumulation observed in NALFD is strongly associated with hepatic insulin resistance, often as a part of type 2 diabetes and metabolic syndrome (Choi et at (2017, J Biol Chem 282 (31): 22678). Acyl-CaA:diacylglycerol acyltransferase (DGAT) catalyzes the final step in triglyceride synthesis by facilitating the linkage of sn-1,2 diacylglygerol (DAG) with a long chain acyl CoA. There are two primary isoforms of DGAT, DGAT-1 and DGAT-2. DGAT-1 is primarily expressed in the small intestine while DGAT-2 exhibits primarily hepatic expression where its expression is insulin responsive. Knock down of expression of DGAT-1 or DGAT-2 using antisense oligonucleotides in rats with diet-induced NALFD significantly improved hepatic steatosis in the DGAT-2 knockdowns but not the DGAT-1 knockdowns (Choi, ibid). Thus, the materials and methods of the invention can be used to alter expression of DGAT-2 for the treatment of NASH and NALFD, and to reduce hepatic insulin resistance by targeted degradation of DGAT-2 using the dTAG insertion strategy described herein.

Further vascular targets include those involved in hereditary angioedema (HAE). HAE is an autosomal dominant disease that affects 1 in 50,000 people and is a result of decreased levels of the C1 inhibitor. Patients experience recurrent episodes of swelling in any part of the body where swelling localized to the oropharynx, larynx or abdomen carry the highest risk of morbidity and death (see Tse and Zuraw, (2013) Clev Clin J of Med 80(5):297). The disease occurs from extravasation of plasma into tissues as a result of the over production of bradykinin. The mechanism seems to involve the cleavage of prekallikrein (also known as PKK) by activate factor XII, releasing active plasma kallikrein (which activates more factor XII). Plasma kallikrein then cleaves kininogen, releasing bradykinin. The bradykinin then binds to the B2 bradykinin receptor on endothelial cells, increasing the permeability of the endothelium. Normally, the C1 inhibitor

(encoded by SERPING1) controls bradykinin production by inhibiting plasma kallikrein and the activation of factor XII. HAE occurs in three types, Type I and II that are distinguished by the amount and type of C1 inhibitor present, and Type III which is tied to a Thr309Lys mutation in factor XII (Prieto et at (2009) Allergy 64(2):284). Type I HAE has low levels of C1 inhibitor that appear to be a result of poor expression and destruction of the small amount of C1 inhibitor protein that is made. Type 1 accounts for approximately 85% of HAE patients. Type II patients have normal levels of C1 inhibitor, but the C1 inhibitor protein is ineffectual due to mutations (Tse and Zuraw, ibid). More than 250 mutations in SERPING1 have been characterized that lead to Type I HAE including small and large insertions and deletions as well as duplications (Rijavec et at (2013) PLoS One 8(2): e56712). Due to this high variability in the genetic basis of HAE, the methods and compositions of the invention can be used to prevent or treat HAE by targeting downstream players in the manifestation of HAE. For example, targeting prekallikrein (KLKB1, expressed in hepatocytes) to effect a decrease in prekallikrein (abbreviated PKK) expression can result in a decrease in bradykinin production without regard to the type of mutation upstream that is causing the HAE, and thus result in a decrease in plasma extravasation. Thus, the methods and compositions of the invention may be used to cause a decrease in the expression of KLKB1 to prevent or treat HAE by targeted degradation of KLKB1 using the dTAG insertion strategy described herein.

Target(s) may also be involved in a fibrotic disease. Fibrotic disease in various organs is the leading cause of organ dysfunction and can occur either as a reaction to another underlying disease or as the result of a predisposition towards fibrosis in an afflicted individual. The hallmark of fibrosis is the inappropriate deposition of extracellular matrix compounds such as collagens and related glycoproteins. TGF-β plays a major role in the fibrotic process, inducing fibroblasts to synthesize extracellular matrix (ECM) proteins, and it also inhibits the expression of proteins with ECM break down activity (Leask (2011) J Cell Commun Signal 5:125). There is a class of ECM regulatory proteins known as the CNN proteins (so-called because the first three members are described, namely CYR61 (cysteine-rich 61/CCN1), CTGF (connective tissue growth factor/CCN2), and NOV (nephroblastoma overexpressed/CCN3). These proteins regulate a variety of cellular functions including cell adhesion, migration, apoptosis, survival and gene expression. TGF-β strongly upregulates the CCN2 expression which acts synergistically as a co-factor with TGF-β and seems to be involved in pericyte activation, a process which appears to be

essential in fibrosis (Leask ibid). CCN2 is overexpressed in fibrotic tissue, including pulmonary tissue and is also found in the plasma of patients with systemic sclerosis (scleroderma). Also, knock down of CCN2 expression through use of antisense oligonucleotides (ASO) reduced chemical-induced liver fibrosis, ureteral obstruction-induced renal fibrosis, fibrotic scarring in cutaneous wounds, and renal interstitial fibrogenesis following partial nephrectomy (Jun and Lau (2013) Nat Rev Drug Discov. 10(12): 945-963). In addition to its pro-fibrotic role, CCN2 may be important in cancer, especially in metastasis. It may promote tumor growth by inducing angiogenesis, and high levels of CCN2 in breast cancer cells is a marker of bone metastasis potential (Jun and Lau, ibid). Experimental models that knock down CCN2 expression in various models of fibrosis, cancer, cardiovascular disease and retinopathy through the use of CCN2 modulating compounds such as monoclonal antibodies or inhibitory RNAs have shown impact of clinical progression of a number of these diseases. (Jun and Lau ibid). Thus, the methods and compositions of the invention can be used to prevent or treat fibrosis, cancer, vascular disease and retinopathy by decreasing expression of CCN2 by targeted degradation of CCN2 using the dTAG insertion strategy described herein.

In other embodiments, the target(s) are involved in an autoimmune disease. Autoimmune diseases as a class are common, and affect more than 23 million people in the United States alone. There are several different kinds with many different levels of severity and prognoses. Generally, they are characterized by the production of auto-antibodies against various self-antigens leading to an immune response against one's own body. Autoimmune disease of the gut can lead to conditions such as ulcerative colitis and inflammatory/irritable bowel disease (e.g., Crohn's disease). The cell surface glycoprotein intercellular adhesion molecule 1 (ICAM-1) is expressed on endothelial cells and upregulated in inflammatory states, serving as a binding protein for leukocytes during transmigration into tissues. Specific ICAM-1 alleles have been found to be associated with Crohn's disease (e.g. K469E allele, exon 6) or with ulcerative colitis (e.g. G241R, exon 4) and may preferentially participate in the chronic inflammatory induction found in these diseases (Braun et at (2001) Clin Immunol. 101(3):357-60). Knock out of ICAM in mouse models of vascular and diabetic disease have demonstrated the usefulness of this therapeutic approach (see Bourdillon et at (2000) Ather Throm Vasc Bio 20:2630 and Okada et at (2003) Diabetes 52:2586, respectively). Thus, the methods and compositions of this invention may be used for the general

reduction of ICAM expression in inflammatory diseases by targeted degradation of ICAM using the dTAG insertion strategy described herein.

Another common disease that has been more recently recognized as an autoimmune disease is diabetes. Glucagon, a peptide hormone released by the α-cell of pancreatic islets, plays a key role in regulating hepatic glucose production and has a profound hyperglycemic effect. In addition, glucagon activates multiple enzymes required for gluconeogenesis, especially the enzyme system for converting pyruvate to phosphoenolpyruvate, the rate-limiting step in gluconeogenesis. It has been proposed that hyperglucagonemia is a causal factor in the pathogenesis of diabetes based on the following observations: 1) diabetic hyperglycemia, from animal to human studies, is consistently accompanied by relative or absolute hyperglucagonemia; 2) infusion of somatostatin inhibits endogenous glucagon release, which in turn reduces blood glucose levels in dogs with diabetes induced by alloxan or diazoxide; and 3) chronic glucagon infusion leads to hepatic insulin resistance in humans (see Liang et at (2004) Diabetes 53(2):410). The glucagon receptor (encoded by the GCGR gene) is expressed predominantly in the liver, and treatment of diabetic (db/db) mice with antisense RNA targeting the glucagon receptor causes a significant reduction in serum glucose levels, triglycerides and fatty acids in comparison with controls (Liang et al, ibid). Similarly, glucocorticoids (GCs) increase hepatic gluconeogenesis and play an important role in the regulation of hepatic glucose output. In db/db mice, a reduction in glucocorticoid receptor (GCCR) expression through the use of targeted antisense RNAs caused ~40% decrease in fed and fasted glucose levels and ~50% reduction in plasma triglycerides (see Watts et at (2005) Diabetes 54(6):1846). Thus, the methods and compositions of the invention may be used to prevent or treat diabetes through targeting the glucagon receptor and/or the glucocorticoid receptor by decreasing expression of the glucagon receptor and/or glucocorticoid receptor by targeted degradation using the dTAG insertion strategy described herein.

Another potential target in type 2, insulin resistant diabetes is protein tyrosine phosphatase 1B (PTP-1B). Insulin resistance is defined as the diminished ability of cells to respond to insulin in terms of glucose uptake and utilization in tissues. One of the most important phosphatases regulating insulin signaling is the PTP-1B which inhibits insulin receptor and insulin receptor substrate 1 by direct dephosphorylation. Mice that are PTP-1B −/− (mutated at both alleles) are hypersensitive to insulin and resistant to weight gain on high fat diets (see Fernandez-Ruiz et at (2014) PLoS One 9(2):e90344). Thus this target may be useful for both diabetes treatment and

obesity. Developing inhibitory small molecules specific for this enzyme is problematic because of the highly conserved active site pocket, but antisense oligonucleotides directed PTP-1B has been shown to reduce PTP-1B mRNA expression in liver and adipose tissues by about 50% and to produce glucose lowering effects in hyperglycemic, insulin-resistant ob/ob and db/db mice, experiments that were repeated in non-human primates (see Swarbrick et at (2009) Endocrin 150:1670). Thus, the methods and compositions of the invention can be used to target the PTP-1B by targeted degradation of PTP-1B using the dTAG insertion strategy described herein, leading to increased insulin sensitivity.

A high risk factor for developing type diabetes insulin resistant diabetes is obesity. Worldwide, more than 1 billion people are estimated to be overweight (body mass index (BMI) ≧ 25 kg/m2, and more than 300 million of these are considered obese (BMI ≧ 30 kg/m2), meaning that obesity is one of the greatest threats to public health today (Lagerros and Rössner (2013) Ther Adv Gastroenterol 6(1):77). Obesity is highly associated with co-morbidities such as insulin resistant type II diabetes, dyslipidemia, hypertension and cardiovascular disease. Treatment of obesity typically starts with modification of diet and exercise, but often with a decrease in caloric consumption, a parallel and confounding decrease in energy expenditure by the body is observed (Yu et al, (2013) PLoS One 8(7):e66923). Fibroblast growth factor receptor 4 (FGFR4) has been shown to have an anti-obesity effect in mouse obesity models. FGFR4 is mainly expressed in the liver, and it and its ligand FGF19 (in humans) regulate bile acid metabolism. FGFR4/FGF19 regulate the expression of cholesterol 7 alpha-hydroxylase and its activity. In addition, FGFR4 and FGF19 seem to be involved in lipid, carbohydrate or energy metabolism. Hepatic FGFR4 expression is decreased by fasting, and increased by insulin. FGFR4 null mice also show changes in lipid profiles in comparison with wild type mice in response to different nutritional conditions. Treatment of obese mice with FGF 19 increased metabolic rate and improved adiposity, liver steatosis, insulin sensitivity and plasma lipid levels, and also inhibited hepatic fatty acid synthesis and gluconeogenesis while increasing glycogen synthesis. Anti-sense reduction of FGFR4 in obese mice also lead to reduced body weight and adiposity, improvement in insulin sensitivity and liver steatosis, and increased plasma FGF15 (the mouse equivalent of FGF19) levels without any overt toxicity (Yu et al, ibid). Thus, the methods and compositions of the invention can be used to treat obesity by reducing the expression of FGFR4 by targeted degradation using the dTAG insertion strategy described herein.

Multiple sclerosis (MS) is a chronic, disabling, autoimmune disease of the central nervous system that is characterized by inflammation, demyelination and axonal destruction. The flare ups associated with relapsing MS (occurring in 85-95 percent of patients) are thought to be tied to the entry of activated lymphocytes into the brain. Currently available treatments are only able to inhibit the rate of relapses by about 30%. Inflammatory responses induce the expression of vascular adhesion molecule-1 (VCAM-1) on the endothelium of the vasculature, and the adhesion of the lymphocytes to VCAM-1 is a necessary step that then allows the activated cells to pass through into the brain. VCAM-1 adherence by the lymphocytes is mediated by binding of very late antigen-4 (VLA-4, also known as α4β1 integrin) on the surface of the activated lymphocyte (Wolf et at (2013) PLos One 8(3): e58438). Disruption of this interaction has been the idea behind the therapeutic use of anti-VLA-4 specific antibodies and small molecule antagonists (Wolf et al, ibid). Thus, the materials and methods of the invention can be used to target VCAM-1 or VLA-4 expression by targeted degradation using the dTAG insertion strategy described herein.

Another disease of interest is Cushing's disease/syndrome (CS). In this disease, patients have elevated serum levels of glucocorticoid due to increased expression by the adrenal gland. CS is an uncommon condition with an incidence rate between 1.8 and 2.4 patients/million per year. The most common cause of endogenous CS is an ACTH-producing pituitary adenoma, seen in ~70% of patients with CS. Cortisol-producing adrenal adenomas and ectopic ACTH-producing tumors are less common, each accounting for ~10-15% of cases. The first-line treatment for patients with pituitary derived CS is transsphenoidal pituitary surgery (TSS) and unilateral adrenalectomy for cortisol-producing adrenal adenoma. Unilateral adrenalectomy is curative in almost all patients with cortisol-producing adrenal adenoma and permanent adrenal insufficiency is rare. Conversely, hypopituitarism is common after TSS, with a range between 13 and 81% (see Ragnarsson and Johannsson (2013) Eur J Endocrin 169:139). In some patients however, surgical resection is not successful and so pharmacological treatment is indicated. One approach is to inhibit the activity of the hypercortisolemia by targeting the glucocorticoid receptor (GCCR), for example, using Mifepristone (also known as RU 486), a GCCR antagonist (see Johanssen and Allolio (2007) Eur J Endocrin 157:561). However, RU 486 has several other activities (most notably, induction of an abortion in pregnant patients). Thus, the methods and compositions of the invention may be used to target the GCCR by decreasing expression by targeted degradation using the dTAG insertion strategy described herein.

Transthyretin Amyloidoses (TTRA) is one of several degenerative diseases suspected to be linked to misfolded and aggregated protein (amyloids). Transthyretin (TTR) is a tetramer produced in the liver and secreted into the bloodstream that serves to transport holo-retinal binding protein. However, upon conformational changes, it becomes amyloidogenic. Partial unfolding exposes stretches of hydrophobic residues in an extended conformation that efficiently misassemble into largely unstructured spherical aggregates that ultimately before cross-β sheet amyloid structures (see Johnson et at (2012) J Mol Biol 421(2-3):183). TTRA can occur in patients in both sporadic and autosomal dominant inherited forms which include familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy (FAC). These inherited forms are usually earlier onset and relate to over 100 point mutations described in the TTR gene. Generally, the more destabilizing of the protein that the mutation is, the more likely it is to have some amount of amyloid pathology. The amyloid formed causes selective destruction of cardiac tissue in FAC or peripheral and central nervous tissue in FAP. Some new therapeutic strategies for treating these diseases such as inhibitory RNA strategies center on trying to decrease the amount of TTR to decrease the aggregation potential of the protein (Johnson et al, ibid). Thus the methods and compositions of the invention can be used to target TTR in an effort to reduce the quantity of the pathological forms of the TTR protein and/or to decrease TTR concentration in general by targeted degradation using the dTAG insertion strategy described herein.

Muscular diseases can also be approached using the methods of the invention. Spinal muscular atrophy is an autosomal recessive disease caused by a mutation in the SMN1 gene which encodes the 'survival of motor neuron' (SMN) protein and is characterized by general muscle wasting and movement impairment. The SMN protein is involved in the assembly of components of the spliceosome machinery, and several defects in the SMN1 gene are associated with splicing defects that cause exon 7 of the mature mRNA to be specifically excluded. These defects are especially prevalent in spinal motor neurons, and can cause spinal muscular atrophy. The severity of SMN1 defects can be modified by a paralogue of SMN1 known as SMN2. The SMN2 gene sequence differs from SMN1 in only a few single nucleotide polymorphisms in exons 7 and 8 and several others in the intronic sequences. Thus the methods and compositions of the invention can be used to target SMN1 in an effort to reduce the quantity of the pathological forms of the SMN1 protein and/or to decrease SMN1 concentration in general by targeted degradation using the dTAG insertion strategy described herein.

Dysregulation of the secretion of growth hormone (GH) can lead to a condition known as acromegaly, a disorder of disproportionate skeletal, tissue, and organ growth which first becomes evident at about 40 years of age (Roberts and Katznelson (2006) US Endocrine Disease: 71). It occurs an annual incidence of approximately 5 cases per million, and diagnosis requires a determination of dysregulation of GH secretion and elevated IGF1 levels. The inability to suppress GH secretion during the 2 hours post an oral glucose load is generally used for diagnosis of acromegaly. Normal regulation of GH secretion is carried out by the pituitary gland. Hypothalamic GH-releasing hormone (GHRH), ghrelin and somatostatin regulate GH production by anterior pituitary somatotroph cells. The gene encoding the GH receptor or GHR is widely expressed and when a GH molecule interacts with a GHR dimer, signal proceeds via JAK2-dependent and independent intracellular signal transduction pathways (see Melmed (2009) J Clin Invest 119(11):3189). Circulating GH stimulates hepatic secretion of insulin-like growth factor-1 (IGF-1). Acromegaly occurs when benign pituitary tumors cause an increase in GH secretion and thus in IGF-1 secretion. One GHR mutation that is tied to acromegaly has an in-frame deletion in exon 3 that causes a deletion of 22 amino acids in the protein. This mutated receptor, known as d3-GHR, results in enhanced GH responsiveness. Current therapies focus on the normalization of GH and IGF-1 levels, often through surgical removal of the pituitary tumors. Since secretion of IGF-1 is induced by GH, targeting of the GHR is an attractive target for the methods and compositions of the invention. Thus, the methods and compositions of the invention may be used to target GHR by decreasing expression by targeted degradation using the dTAG insertion strategy described herein.

Another disease associated with muscle wasting is myotonic dystrophy, which is a chronic disease characterized by muscle wasting, cataracts, heart conduction defects, endocrine changes, multiorgan damage and myotonia (prolonged muscle contraction following voluntary contraction). Myotonic dystrophy occurs at an incidence rate of approximately 13 per 100,000 people, and there are two forms of the disease, Myotonic Dystrophy Type 1 (also called Steinert's disease, MMD1 or DM1, and is the most common) and Myotonic Dystrophy Type 2 (MMD2 or DM2). Both are inherited autosomal dominant diseases caused by abnormal expansions in the 3′ non-coding regions of two genes (CTG in the DMPK gene (encoding dystrophia myotonica protein kinase) for type 1, and CCTG in the ZNF9 gene (encoding cellular nucleic acid-binding protein) in type 2) and DM1 is the most common form of muscular dystrophy in adults. These mutations result in

toxic intranuclear accumulation of the mutant transcripts in RNA inclusions or foci (see Caillet-Boudin et al, (2014) Front. Mol. Neurosci doi:10.3389). Type 1 patients have CTG copy numbers greater than 50 and have variable phenotypes, ranging from asymptomatic to severe. Antisense RNA techniques have been used to cause the specific destruction of the mutant DMPK transcripts in vitro which caused no effect on the proliferation rate of DM1 myoblasts but restored their differentiation (Furling et at (2003) Gene Therapy 10:795). Thus, the methods and compositions of the invention can be used to target the dystrophia myotonica protein kinas or cellular nucleic acid binding protein by targeted degradation using the dTAG insertion strategy described herein.

Chronic pain is a major health concern affecting 80 million Americans at some time in their lives with significant associated morbidity and effects on individual quality of life. Chronic pain can result from a variety of inflammatory and nerve damaging events that include cancer, infectious diseases, autoimmune-related syndromes and surgery. Voltage-gated sodium channels (VGSCs) are fundamental in regulating the excitability of neurons and overexpression of these channels can produce abnormal spontaneous firing patterns which underpin chronic pain. There are at least nine different VGSC subtypes in the nervous system, and each subtype can be functionally classified as either tetrodotoxin-sensitive or tetrodotoxin-resistant. Neuronal sodium channel subtypes including Nav1.3, Nav1.7, Nav1.8, and Nav1.9 have been implicated in the processing of nociceptive information. The VGSC Nav1.8 is a tetrodotoxin-resistant sodium channel with a distribution restricted to primary afferent neurons and the majority of Nav1.8-containing afferents transmit nociceptive signals to pain processing areas of the spinal cord. Changes in the expression, trafficking and redistribution of Nav1.8 (encoded by PN3) following inflammation or nerve injury are thought to be a major contributor to the sensitization of afferent nerves and the generation of pain (see Schuelert and McDougall (2012) Arthritis Res Ther 14:R5). Rodent models of osteoarthritis have demonstrated that inhibition of Nav1.8 channels on peripheral nerves, with synaptic connections in the spinal cord, is a promising treatment of nociceptive sensory processing and could be helpful to achieve more pronounced and longer lasting analgesia. Thus, the methods and compositions of the invention can be used to treat chronic pain by decreasing localized expression of NAV1.8 by targeted degradation using the dTAG insertion strategy described herein.

Cancer may also be targeted as described herein. Cancer is a generic term used to describe a number of specific diseases that are united by a lack of cellular growth regulation. Since there

are so many forms, involving a myriad of different cell types, there are also numerous specific gene targets that are involved in cancer. For example, the clusterin protein (also known as apolipoprotein J), encoded by the CLU gene, is a heterodimeric protein assembled following the proteolytic cleavage into the two chains of the primary polypeptide CLU gene product. In recent years, it has been found that there are two forms of clusterin, a secretory and heavily glycosylated form (sCLU) and a nuclear form (nCLU), where nCLU is first synthesized as a pre nuclear form (pnCLU) that is found in the cell cytoplasm. The differences between the two CLU forms are tied to alternative splicing of the CLU message and the selection of the starting ATG during message translation. The translation of sCLU utilized the first AUG in the full length CLU mRNA whereas the translation of pnCLU is initiated from a second in-frame AUG following the splice-dependent removal of the transcribed leader section and Exon 1 from the full length mRNA. The sCLU form appears to promote cell survival while the nCLU form is associated with apoptosis. Overexpression of the sCLU form of the protein has been found in many tumor types, including prostate, skin, pancreatic, breast, lung, and colon tumors, as well as oesophageal squamous cell carcinoma and neuroblastoma. In addition, the progression of some cancer types towards high grade and metastatic forms leads to an elevation of sCLU levels (Shannan et at (2006) Cell Death Dif 13: 12). Use of specific antisense oligonucleotides (ASO) designed to cause silencing sCLU expression in combination with standard treatments has been carried out in Phase I studies of breast and prostate cancer, with an increase in apoptosis observed only in the patients that received both the ASO and the standard therapeutic agent (Shannan ibid). Thus, the methods and compositions of the invention can be used to treat cancers marked with an increase in sCLU expression by targeted degradation using the dTAG insertion strategy described herein.

Another protein that appears to have an oncogenic role is eukaryotic translation initiation factor 4E (eIF-4E). eIF3-4E binds to the M7GpppN cap (where N is any nucleotide) of a eukaryotic mRNA and is the rate limiting member for the formation of the eIF-4F complex. eIF-4E normally complexes with eIF-4G in the eIF-4F complex, and under normal physiologic conditions, the availability of eIF-4E is negatively regulated by the binding of a family of inhibitory proteins known as 4E-BPs which act to sequester eIF-4E from eIF-4G. Since eIF-4E is expressed normally at low levels, mRNAs compete for the available eIF-4E to be translated. mRNAs with short, unstructured 5′ UTRs are thought to be more competitive for translation since they are less dependent on the unwinding activity found in the eIF-4F complex. mRNAs

that are highly structural then are more dependent on eIF-4E binding for translation, and thus when eIF3-4E is overexpressed, these mRNAs are more easily translated. Growth-promoting gene products such as cyclin D1, VEGF, c-myc, FGF2, heparanase, ODC and MMP9 have these complex 5′ UTRs (Mamane et at (2004) Oncogene 23:3172, Fischer (2009) Cell Cycle 8(16):2535). Additionally, eIF-4E may serve a role in modification of the nuclear pore complex and cause an increase in translocation of these same mRNAs into the cytoplasm (Culjikovic-Kraljacic et at (2012) Cell Reports 2 p. 207). eIF-4E has been implicated in oncogenic cellular transformation and is overexpressed in several cancer types including acute myeloid leukemia, colon, breast, bladder, lung, prostate, gastrointestinal tract, head and neck cancers, Hodgkin's lymphoma and neuroblastoma and elevated levels are associated with increasing grade of disease. Targeting of eIF-4E has been attempted by several different approaches, including overexpression of 4E-BPs and peptides derived there from, the development of small molecule inhibitors to prevent eIF-4E:eIFG interaction, and antisense oligos (ASO) specific for eIF-4E (Jia et at (2012) Med Res Rev 00, No. 00:1-29). ASO administration has demonstrated a knock down of eIF-4E expression in tumor cells in vitro, and in xenograft tumors in mouse models in vivo. Expression levels of eIF-4E were decreased by 80% in these mouse models without any decrease in overall protein translation and without any obvious toxicity, while increasing chemosensitivity to chemotherapeutic agents, increasing cancer cell apoptosis and suppressing tumor growth (Jia ibid). Thus, the methods and compositions of the invention may be used for the treatment or prevention of various cancers. Expression of eIF-4F can be modulated by degradation using the dTAG insertion strategy described herein.

Vascular endothelial receptor (VEGF), acting via its receptor VEGFR has a role in normal development, and also in the development of pathological angiogenesis in cancer. In humans, there are five distinct VEGF family members: VEGF-A (also known as VEGF); placenta growth factor (PIGF), VEGF-B, VEGF-C and VEGF-D. VEGF-A also has three common subtypes: VEGF-121. VEGF-165 and VEGF-189. The various VEGFs have differing roles in angiogenesis with VEGF-A primarily being involved in normal angiogenesis and also in tumor growth and metastasis, while VEGF-C and VEGF-D are involved in normal lymphangiogenesis and in malignant lymph node metastasis. In addition, the VEGF-A subtypes may also have specific growth promoting activity in hormone responsive tumors. Based on this knowledge, a number of antibodies and small molecule kinase inhibitors which suppress the VEGF-VEGFR interaction

directly or the signal transduction pathways activated by the interaction. However, these therapeutics often have significant and potentially troublesome side effect profiles, such that active research is occurring to develop inhibitors with increased specificity (Shibuya, (2014) Biomol Ther 11(1):1-9). Thus, the methods and compositions of the invention may be used to prevent or treat cancer in a subject by targeting specific VEGF proteins by degradation using the dTAG insertion strategy described herein.

Another protein that plays a role in several cancers is kinesin spindle protein (KSP), encoded by the KIF11 gene. The most successful anti-cancer therapies currently in use target microtubules where these agents have been used for the treatment of breast, lung, ovarian, bladder, and head and neck cancers. Microtubules are part of the mitotic spindle, and thus targeting them is successful in inhibiting rapidly dividing cancer cells, but microtubules are also part of the cytoskeleton, such that treatment with these agents also is associated with serious side effects. Kinesin, specifically kinesin spindle protein, is a motor protein that binds to spindle fibers and serves to force the spindle fibers apart during chromosome segregation in cell division. Thus, targeting KSP using a KSP-specific anti-mitotic agent will only target dividing cells, and might have fewer side effects. Agents that deplete KSP selectively lead to cell cycle arrest in mitosis, which after a prolonged period, leads to apoptosis. KSP is also abundant in dividing tissues, and is highly expressed in tumors of the breast, colon, lung, ovary and uterus (Sarli and Giannis, (2008) Clin Cancer Res 14:7583). In addition, clinical trials are underway using RNA interference targeted to KSP and VEGF simultaneously in cancer patients with liver involvement (Tabernero et al, (2013) Cancer Discovery 3:406). Thus, the methods and compositions of the invention may be used to treat or prevent cancers by targeted degradation of the kinesin spindle protein (KSP) using the dTAG insertion strategy described herein.

Heat shock protein 27 (HSP 27, also known as heat shock protein beta-1 or HSPB1) is another protein that is implicated in cancer. HSP 27, encoded by the HSPB1 gene, is a heat shock protein that was initially characterized in response to heat shock as a small chaperonin that facilitates proper refolding of damaged proteins. However, ongoing investigation revealed that it also is involved in responses to cellular stress conditions such as oxidative stress, and chemical stress, appears to have anti-apoptotic activity, and is able to regulate actin cytoskeletal dynamics during heat shock and other stress conditions (Vidyasagar et at (2012) Fibrogen Tis Rep 5(7)). In addition, suppression of HSP 27 may play a role in long term dormancy of cancers as research has

revealed that HSP 27 is upregulated in angiogenic breast cancer cells, and suppression of HSP 27 in vivo leads to long term tumor dormancy (Straume et at (2012) Proc Natl Acad Sci USA 109(22): 8699-8704). Increased expression of heat shock proteins in tumor cells is related to loss of p53 functions and to the upregulation of proto-oncogenes such as c-myc. HSP 27's anti-apoptotic activity protects tumor cells and also has been shown to be associated with chemotherapy resistance in breast cancer and leukemia (Vidysagar ibid). Thus, HSP 27 may be a suitable target for cancer therapeutics, where inhibitors of the protein may be used in combination with known chemotherapies to enhance their activities. The HSP 27 inhibitor quercetin has been shown to significantly reduce tumor volumes in vivo when combined with traditional chemotherapeutic agents in comparison with the agents alone. In addition, HSP 27 inhibitory ASOs are currently be evaluated in clinical studies in lung, ovarian, breast and pancreatic cancers (Vidyasagar, ibid). Thus, the methods and compositions of the invention may be used to treat cancers by inhibition of HSP 27 expression through targeted degradation of HSP 27 using the dTAG insertion strategy described herein.

Several kinases have been the target of research into anti-cancer therapeutics since they are often key regulators of cell growth. However, downstream in the signaling pathway, the effect of mutant kinases is often seen in the upregulation of the Signal Transduction and Activator of Transcription 3 protein, or Stat3, encoded by the STAT3 gene. Additionally, it appears that both Hepatitis B and C activate Stat3, and both are associated with the development of hepatic cancer. Thus it may be that the HepB and HepC viruses subvert Stat3 signaling pathways and promote hepatocyte transformation (Li et al, (2006) Clin Cancer Res 12(23):7140).

RAS proteins are a family of proteins that play a role in cell differentiation, proliferation, and survival. Various members of the RAS protein family have been implicated in cancer as aberrant RAS signaling has been found to play a role in approximately 30% of all cancers. The KRAS protein (also known as V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is a GTPase that performs an essential function in normal tissue signaling. KRAS is an attractive cancer target, as frequent point mutations in the KRAS gene render the protein constitutively active. Thus, KRAS may be a suitable target for cancer therapeutics, where small molecules targeting the function of the KRAS protein may be used for therapeutic advantages, including in combination with known chemotherapies to enhance their activities. In one embodiment, the methods and

compositions of the invention may be used to treat cancers by modulation of KRAS expression through targeted degradation of KRAS using the dTAG insertion strategy described herein.

All the various Stat proteins are transcription factors that primarily mediate signaling from cytokine and growth factor receptors. For example, IL6 and IL11 bind to their respective receptor subunits and trigger homodimerization of gp130, the transmembrane receptor that triggers Stat3 activation. Following activation via phosphorylation of the growth factor receptors, Stat3 proteins dimerize and traverse into the nucleus and bind to DNA in a sequence specific manner, up regulating many genes that are involved in cell proliferation. Tumor cells of various types often have kinase mutations that lead to overexpression of Stat3 so a decrease in Stat3 expression has the potential to be beneficial in cancers of multiple origins without regard to each specific mutant kinase (Jarnicki et at (2010) Cell Div 5:14). Stat3 contributes to malignancy by several mechanisms. It inhibits apoptosis by upregulating the pro-survival/anti-apototic Bcl2 proteins and promotes proliferation primarily by stimulating expression of cyclinB1, cdc2, c-myc, VEGF, H1F1α and cyclin D1 as well as through its repression of the cell cycle inhibitor p21. Stat3 also promotes tumor metastasis through the induction of extracellular matrix-degrading metalloproteinases including MMP-2 and MMP-9. In normal physiological states, Stat3 functioning is inhibited by the transcriptional inhibitor Socs3, which is normally induced by Stat3 to maintain growth balance in the cell. However, in a malignant cell, Stat3 overexpression can overcome Socs3 inhibition. Thus, the methods and compositions of the invention can be used to inhibit Stat3 functioning and prevent or treat cancer by targeted degradation of Stat3 using the dTAG insertion strategy described herein.

Prostate cancer (PCa) is an androgen-dependent disease that remains one of the leading causes of death in the United States, and is the leading cause of death from cancer in men. While several studies have been done that suggest that up to 42% of prostate cancer cases have a genetic link (Mazaris and Tsiotras (2013) Nephro Urol Mon 5(3):792-800), several types of inheritance patterns have been observed (e.g. X-linked, autosomal dominant, autosomal recessive) suggesting that there is not one sole gene or gene mutation that leads to inheritance of PCa. This cancer is dependent upon the activity of the androgen receptor for growth and progression (Mahmoud et at (2013) PLoS One 8(10): e78479). Typically, PCa can be a slow to progress disease that can be treated using fairly conservative approaches, but in about 25-30% of the cases, the cancer can be an aggressive one leading to patient death. In the case of metastatic disease 70-80% of patients

respond initially to androgen-deprivation therapy but in later stages, the tumor becomes hormone refractory and more aggressive, leading to a worsening prognosis (Mazaris and Tsiotras ibid). Hormone refractory PCa is not dependent on circulating androgen, but rather is driven by inappropriate activation of the androgen receptor (AR, encoded by the AR gene) through such mechanisms as AR amplification, deregulation of growth factors, and co-amplification of AR co-factors. Additionally, mutations in the AR ligand binding domain can cause the AR to be supersensitive to very low circulating androgen levels or to be sensitive to an expanded set of ligands such as estrogens, progestins, adrenyl steroids and antiandrogens. Tumor cells that have undergone these types of mutations in the AR ligand binding domain may no longer be sensitive to anti-androgen therapies despite the reliance of the cancer on the activity of the AR. Normally the AR is present in the cytoplasm and is bound by heat shock proteins to prevent its activation. Upon exposure to androgen, the receptor is able to dimerize and travel into the cell nucleus to promote expression of several growth related genes. Thus the methods and compositions of the invention may be used to treat PCa at all stages by targeting degradation of the androgen receptor using the dTAG insertion strategy described herein.

C. Genomic In-Frame Insertion of dTAGs

As described above, the methods of the present invention are based on the genomic insertion of a dTAG in-frame with a gene expressing an endogenous protein of interest. As contemplated herein, the 5'- or 3' in-frame insertion of a nucleic acid sequence encoding a dTAG results, upon expression of the resultant nucleic acid sequence, in an endogenous protein-dTAG hybrid protein that can be targeted for degradation by the administration of a specific heterobifunctional compound.

In-frame insertion of the nucleic acid sequence encoding the dTAG can be performed or achieved by any known and effective genomic editing processes. In one aspect, the present invention utilizes the CRISPR-Cas9 system to produce knock-in endogenous protein-dTAG fusion proteins that are produced from the endogenous locus and are readily degraded in a ligand-dependent, reversible, and dose-responsive, fashion. In certain embodiments, the CRISPR-Cas9 system is employed in order to insert an expression cassette for dTAG present in a homologous recombination (HR) "donor" sequence with the dTAG nucleic acid sequence serving as a "donor" sequence inserted into the genomic locus of a protein of interest during homologous recombination

following CRISPR-Cas endonucleation. The HR targeting vector contains homology arms at the 5'and 3'end of the expression cassette homologous to the genomic DNA surrounding the targeting gene of interest locus. By fusing the nucleic acid sequence encoding the dTAG in frame with the target gene of interest, the resulting fusion protein contains a dTAG that is targeted by a heterobifunctional compound.

The present invention provides for insertion of an exogenous dTAG sequence (also called a "donor sequence" or "donor" or "transgene") in frame with the target gene of interest, and the resulting fusion protein contains a dTAG that is targeted by a heterobifunctional compound. It will be readily apparent that the donor sequence need not be identical to the genomic sequence where it is placed. A donor sequence can contain a non-homologous sequence flanked by two regions of homology to allow for efficient HR at the location of interest. Additionally, donor sequences can comprise a vector molecule containing sequences that are not homologous to the region of interest in cellular chromatin. A donor molecule can contain several, discontinuous regions of homology to cellular chromatin. For example, for targeted insertion of sequences not normally present in a region of interest, for example, the dTAGs of the present invention, said sequences can be present in a donor nucleic acid molecule and flanked by regions of homology to sequence in the region of interest. Alternatively, a donor molecule may be integrated into a cleaved target locus via non-homologous end joining (NHEJ) mechanisms. See, e.g., U.S. 2011/0207221 and U.S. 2013/0326645, incorporated herein by reference.

The donor dTAG encoding sequence for insertion can be DNA or RNA, single-stranded and/or double-stranded and can be introduced into a cell in linear or circular form. See, e.g., U.S. 2010/0047805, U.S. 2011/0281361, and 2011/0207221, incorporated herein by reference. The donor sequence may be introduced into the cell in circular or linear form. If introduced in linear form, the ends of the donor sequence can be protected (e.g., from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3′ terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al. *Proc. Natl. Acad. Sci.* 84, (1987):4959-4963 and Nehls et al. *Science*, 272, (1996):886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

The donor polynucleotide encoding a dTAG can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, CRISPR-Cas sequences, replication origins, promoters and genes encoding antibiotic resistance. Moreover, donor polynucleotides can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (e.g., adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)).

The present invention takes advantage of well-characterized insertion strategies, for example the CRISPR-Cas9 system. In general, the "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), and/or other sequences and transcripts from a CRISPR locus. (See, e.g., Ruan, J. et al. "Highly efficient CRISPR/Cas9-mediated transgene knockin at the H11 locus in pigs." *Sci. Rep.* 5, (2015):14253; and Park A, Won ST, Pentecost M, Bartkowski W, and Lee B "CRISPR/Cas9 Allows Efficient and Complete Knock-In of a Destabilization Domain-Tagged Essential Protein in a Human Cell Line, Allowing Rapid Knockdown of Protein Function." PLoS ONE 9(4), (2014): e95101, both incorporated herein by reference).

The Cas nuclease is a well-known molecule. For example, the protein sequence encoded by the Cas-9 nuclease gene may be found in the SwissProt database under accession number Q99ZW2 - (SEQ. ID. NO.: 52):

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA

FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL
KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTG
WGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLIT
QRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEI
VWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKK
YGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKE
VKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENI
IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD.

In some embodiments, the CRISPR/Cas nuclease or CRISPR/Cas nuclease system includes a non-coding RNA molecule (guide) RNA, which sequence- specifically binds to DNA, and a Cas protein (e.g., Cas9), with nuclease functionality (e.g., two nuclease domains). Further included is the donor nucleotide encoding a dTAG for in-frame insertion into the genomic locus of the protein of interest.

In some embodiments, one or more elements of a CRISPR system is derived from a type I, type II, or type III CRISPR system. In some embodiments, one or more elements of a CRISPR system is derived from a particular organism comprising an endogenous CRISPR system, such as Streptococcus pyogenes.

In some embodiments, a Cas nuclease and gRNA (including a fusion of crRNA specific for the target sequence and fixed tracrRNA), and a donor sequence encoding a dTAG are introduced into the cell. In general, target sites at the 5' end of the gRNA target the Cas nuclease to the target site, e.g., the gene, using complementary base pairing. In some embodiments, the target site is selected based on its location immediately 5' of a protospacer adjacent motif (PAM) sequence, such as typically NGG, or NAG. In this respect, the gRNA is targeted to the desired sequence by modifying the first 20 nucleotides of the guide RNA to correspond to the target DNA sequence.

In some embodiments, the CRISPR system induces DSBs at the target site, followed by homologous recombination of the donor sequence encoding a dTAG into the genomic locus of a protein of interest, as discussed herein. In other embodiments, Cas9 variants, deemed "nickases" are used to nick a single strand at the target site. In some aspects, paired nickases are used, e.g., to improve specificity, each directed by a pair of different gRNAs targeting sequences such that upon introduction of the nicks simultaneously, a 5' overhang is introduced.

In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence. Typically, in the context of formation of a CRISPR complex, "target sequence" generally refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between the target sequence and a guide sequence promotes the formation of a CRISPR complex, and wherein insertion of the donor sequence encoding a dTAG is to take place. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex.

Typically, in the context of an endogenous CRISPR system, formation of the CRISPR complex (comprising the guide sequence hybridized to the target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild- type tracr sequence), may also form part of the CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence. In some embodiments, the tracr sequence has sufficient complementarity to a tracr mate sequence to hybridize and participate in formation of the CRISPR complex.

As with the target sequence, in some embodiments, complete complementarity is not necessarily needed. In some embodiments, the tracr sequence has at least 50%, 60%, 70%, 80%, 90%, 95% or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned. In some embodiments, one or more vectors driving expression of one or more elements of the CRISPR system are introduced into the cell such that expression of the elements of the CRISPR system direct formation of the CRISPR complex at one or more target sites. For

example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. In some embodiments, CRISPR system elements that are combined in a single vector may be arranged in any suitable orientation, such as one element located 5' with respect to ("upstream" of) or 3' with respect to ("downstream" of) a second element. The coding sequence of one element may be located on the same or opposite strand of the coding sequence of a second element, and oriented in the same or opposite direction. In some embodiments, a single promoter drives expression of a transcript encoding a CRISPR enzyme and one or more of the guide sequence, tracr mate sequence (optionally operably linked to the guide sequence), and a tracr sequence embedded within one or more intron sequences (e.g. each in a different intron, two or more in at least one intron, or all in a single intron). In some embodiments, the CRISPR enzyme, guide sequence, tracr mate sequence, and tracr sequence are operably linked to and expressed from the same promoter.

In some embodiments, a vector comprises a regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR RNA-guided endonuclease. In some embodiments, a vector comprises a regulatory element operably linked to an enzyme-coding sequence encoding the CRISPR enzyme, such as a Cas protein. Non-limiting examples of Cas proteins include Casl, Cas IB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csxl2), CaslO, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, CsxlO, Csxl6, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, Csf4, Cpfl, homologs thereof, or modified versions thereof. (see WO 2015/200334, incorporated herein by reference). These enzymes are known; for example, the amino acid sequence of S. pyogenes Cas9 protein may be found in the SwissProt database under accession number Q99ZW2 (incorporated herein by reference).

Cas proteins generally comprise at least one RNA recognition or binding domain. Such domains can interact with guide RNAs (gRNAs, described in more detail below). Cas proteins can also comprise nuclease domains, for example endonuclease domains (e.g., DNase or RNase domains), DNA binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. A nuclease domain possesses catalytic activity for

nucleic acid cleavage. Cleavage includes the breakage of the covalent bonds of a nucleic acid molecule. Cleavage can produce blunt ends or staggered ends, and it can be single-stranded or double- stranded.

Examples of Cas proteins include Casl, Cas IB, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8al , Cas8a2, Cas8b, Cas8c, Cas9 (Csnl or Csxl2), CaslO, CaslOd, CasF, CasG, CasH, Csyl, Csy2, Csy3, Csel (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl , Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, CsxlO, Csxl6, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, Csf4, and Cul966, and homologs or modified versions thereof (see WO 2015/200334, incorporated herein by reference).

Any Cas protein that induces a nick or double-strand break into a desired recognition site can be used in the methods and compositions disclosed herein.

In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of the CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more.

Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of the CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of the CRISPR system sufficient to form the CRISPR complex, including the guide sequence to be tested, may be provided to the cell having the corresponding target sequence, such as by transfection with vectors encoding the components

of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of the CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions.

A guide sequence may be selected to target any target sequence. In some embodiments, the target sequence is a sequence within a genome of a cell, and in particular, a protein of interest targeted for controlled degradation through the engineering of an endogenous protein-dTAG hybrid. Exemplary target sequences include those that are unique in the target genome which provide for insertion of the dTAG donor nucleic acid in an in-frame orientation. In some embodiments, a guide sequence is selected to reduce the degree of secondary structure within the guide sequence. Secondary structure may be determined by any suitable polynucleotide folding algorithm.

In general, a tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the corresponding tracr sequence; and (2) formation of a CRISPR complex at a target sequence, wherein the CRISPR complex comprises the tracr mate sequence hybridized to the tracr sequence. In general, degree of complementarity is with reference to the optimal alignment of the tracr mate sequence and tracr sequence, along the length of the shorter of the two sequences.

As contemplated herein, the CRISPR-Cas system is used to insert a nucleic acid sequence encoding a dTAG in-frame with the genomic sequence encoding a protein of interest in a eukaryotic, for example, human cell. In some embodiments, the method comprises allowing the CRISPR complex to bind to the genomic sequence of the targeted protein of interest to effect cleavage of the genomic sequence, wherein the CRISPR complex comprises the CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said target polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence.

In some aspects, the methods include modifying expression of a polynucleotide in a eukaryotic cell by introducing a nucleic acid encoding a dTAG.

In some aspects, the polypeptides of the CRISPR-Cas system and donor sequence are administered or introduced to the cell. The nucleic acids typically are administered in the form of an expression vector, such as a viral expression vector. In some aspects, the expression vector is a retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression vector, or an AAV expression vector. In some aspects, one or more polynucleotides encoding CRISPR-Cas system and donor sequence delivered to the cell. In some aspects, the delivery is by delivery of more than one vectors.

Methods of delivering nucleic acid sequences to cells as described herein are described, for example, in U.S. Pat. Nos. 8,586,526; 6,453,242; 6,503,717; 6,534,261; 6,599,692; 6,607,882; 6,689,558; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the disclosures of all of which are incorporated by reference herein in their entireties.

The various polynucleotides as described herein may also be delivered using vectors containing sequences encoding one or more of compositions described herein. Any vector systems may be used including, but not limited to, plasmid vectors, retroviral vectors, lentiviral vectors, adenovirus vectors, poxvirus vectors; herpesvirus vectors and adeno-associated virus vectors, etc. See, also, U.S. Pat. Nos. 6,534,261; 6,607,882; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, incorporated by reference herein in their entireties.

Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 1991/17424 and WO 1991/16024. Delivery can be to cells (e.g. in vitro or ex vivo administration) or target tissues (e.g. in vivo administration).

In some embodiments, delivery is via the use of RNA or DNA viral based systems for the delivery of nucleic acids. Viral vectors in some aspects may be administered directly to patients (in vivo) or they can be used to treat cells in vitro or ex vivo, and then administered to patients. Viral-based systems in some embodiments include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of

target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system depends on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., *J. Virol.* 66, (1992):2731-2739; Johann et al., *J. Virol.* 66, (1992):1635-1640; Sommerfelt et al., *J. Virol.* 176, (1990):58-69; Wilson et al., *J. Virol.* 63, (1989):2374-2378; Miller et al., *J. Virol.* 65, (1991):2220-2224; and PCT/US94/05700).

In applications in which transient expression is preferred, adenoviral based systems can be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and high levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors are also used to transduce cells with target nucleic acids, e.g., in the in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy procedures (see, e.g., West et al., *Virology* 160, (1987):38-47; U.S. Pat. No. 4,797,368; WO 1993/24641; Kotin, *Human Gene Therapy* 5, (1994):793-801; Muzyczka, *J. Clin. Invest.* 94, (1994):1351. Construction of recombinant AAV vectors is described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., *Mol. Cell. Biol.* 5, (1985):3251-3260; Tratschin, et al., *Mol. Cell. Biol.* 4, (1984):2072-2081; Hermonat & Muzyczka, *PNAS* 81, (1984):6466-6470; and Samulski et al., *J. Virol.* 63, (1989):3822-3828.

At least six viral vector approaches are currently available for gene transfer in clinical trials, which utilize approaches that involve complementation of defective vectors by genes inserted into helper cell lines to generate the transducing agent.

pLASN and MFG-S are examples of retroviral vectors that have been used in clinical trials (Dunbar et al., *Blood* 85, (1995):3048-305; Kohn et al., *Nat. Med.* 1, (1995):1017-1023; Malech et al., *PNAS* 94(22), (1997):12133-12138). PA317/pLASN was the first therapeutic vector used in a gene therapy trial. (Blaese et al., *Science* 270, (1995):475-480). Transduction efficiencies of

50% or greater have been observed for MFG-S packaged vectors. (Ellem et al., *Immunol Immunother.* 44(1), (1997):10-20; and Dranoff et al., *Hum. Gene Ther.* 1, (1997):111-112).

Vectors suitable for introduction of polynucleotides described herein also include non-integrating lentivirus vectors (IDLV). See, for example, Naldini et al. *Proc. Natl. Acad. Sci.* 93, (1996):11382-11388; Dull et al. *J. Virol.* 72, (1998):8463-8471; Zuffery et al. *J. Virol.* 72, (1998):9873-9880; Follenzi et al. *Nature Genetics* 25, (2000):217-222; and U.S. 2009/0117617.

Recombinant adeno-associated virus vectors (rAAV) may also be used to deliver the compositions described herein. All vectors are derived from a plasmid that retains only the AAV inverted terminal repeats flanking the transgene expression cassette. Efficient gene transfer and stable transgene delivery are key features for this vector system. (Wagner et al., *Lancet* 351, (1998):9117 1702-3, and Kearns et al., *Gene Ther.* 9, (1996):748-55). Other AAV serotypes, including AAV1, AAV3, AAV4, AAV5, AAV6, AAV8, AAV9 and AAVrh10, pseudotyped AAV such as AAV2/8, AAV2/5 and AAV2/6 and all variants thereof, can also be used in accordance with the present invention.

Replication-deficient recombinant adenoviral vectors (Ad) can be produced at high titer and readily infect a number of different cell types. Most adenovirus vectors are engineered such that a transgene replaces the Ad E1a, E1b, and/or E3 genes; subsequently the replication defective vector is propagated in human 293 cells that supply deleted gene function in trans. Ad vectors can transduce multiple types of tissues in vivo, including non-dividing, differentiated cells such as those found in liver, kidney and muscle. Conventional Ad vectors have a large carrying capacity. An example of the use of an Ad vector in a clinical trial involved polynucleotide therapy for anti-tumor immunization with intramuscular injection (Sterman et al., *Hum. Gene Ther.* 7, (1998):1083-1089). Additional examples of the use of adenovirus vectors for gene transfer in clinical trials include Rosenecker et al., *Infection* 24(1), (1996):5-10; Sterman et al., *Hum. Gene Ther.* 9(7), (1998):1083-1089; Welsh et al., *Hum. Gene Ther.* 2, (1995):205-218; Alvarez et al., *Hum. Gene Ther.* 5, (1997):597-613; Topf et al., *Gene Ther.* 5, (1998):507-513; Sterman et al., *Hum. Gene Ther.* 7, (1998):1083-1089.

Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and ψ2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by a producer cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the

minimal viral sequences required for packaging and subsequent integration into a host (if applicable), other viral sequences being replaced by an expression cassette encoding the protein to be expressed. The missing viral functions are supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess inverted terminal repeat (ITR) sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

The vector can be delivered with a high degree of specificity to a particular tissue type. Accordingly, a viral vector can be modified to have specificity for a given cell type by expressing a ligand as a fusion protein with a viral coat protein on the outer surface of the virus. The ligand is chosen to have affinity for a receptor known to be present on the cell type of interest. For example, Han et al., *Proc. Natl. Acad. Sci.* 92, (1995):9747-9751, reported that Moloney murine leukemia virus can be modified to express human heregulin fused to gp70, and the recombinant virus infects certain human breast cancer cells expressing human epidermal growth factor receptor. This principle can be extended to other virus-target cell pairs, in which the target cell expresses a receptor and the virus expresses a fusion protein comprising a ligand for the cell-surface receptor. For example, filamentous phage can be engineered to display antibody fragments (e.g., FAB or Fv) having specific binding affinity for virtually any chosen cellular receptor. Although the above description applies primarily to viral vectors, the same principles can be applied to nonviral vectors. Such vectors can be engineered to contain specific uptake sequences which favor uptake by specific target cells.

Vectors can be delivered in vivo by administration to an individual subject, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, intrathecal, intratracheal, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells ex vivo, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells,

followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

Vectors (e.g., retroviruses, adenoviruses, liposomes, etc.) containing nucleases and/or donor constructs can also be administered directly to an organism for transduction of cells in vivo. Alternatively, naked DNA can be administered. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells including, but not limited to, injection, infusion, topical application and electroporation. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

In some embodiments, the polypeptides of the CRISPR-Cas system are synthesized in situ in the cell as a result of the introduction of polynucleotides encoding the polypeptides into the cell. In some aspects, the polypeptides of the CRISP-Cas system could be produced outside the cell and then introduced thereto. Methods for introducing a CRISPR-Cas polynucleotide construct into animal cells are known and include, as non-limiting examples stable transformation methods wherein the polynucleotide construct is integrated into the genome of the cell, transient transformation methods wherein the polynucleotide construct is not integrated into the genome of the cell, and virus mediated methods, as described herein. Preferably, the CRISPR-Cas polynucleotide is transiently expressed and not integrated into the genome of the cell. In some embodiments, the CRISPR-Cas polynucleotides may be introduced into the cell by for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, in some aspects, transient transformation methods include microinjection, electroporation, or particle bombardment. In some embodiments, the CRISPR-Cas polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in the cells.

In some embodiments, non-CRISPR-CAS viral and non-viral based gene transfer methods can be used to insert nucleic acids encoding a dTAG in frame in the genomic locus of a protein of interest in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding components of a ZFP, ZFN, TALE, and/or TALEN system to cells in culture, or in a host organism including a donor sequence encoding a dTAG for in-frame insertion into the genomic locus of a protein of interest.

Non- viral vector delivery systems include DNA plasmids, RNA (e.g. a transcript of a vector described herein), naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson, *Science* 256, (1992):808-813; Nabel & Feigner, *TIBTECH* 11, (1993):211-217; Mitani & Caskey, *TIBTECH* 11, (1993): 162-166; Dillon. *TIBTECH* 11, (1993): 167-173; Miller, *Nature* 357, (1992):455-460; Van Brunt, Biotechnology 6(10), (1988):1149-1154; Vigne, *Restorative Neurology and Neuroscience* 8, (1995):35-36; Kremer & Perricaudet, *British Medical Bulletin* 51(1), (1995):31-44; and Yu et al., *Gene Therapy* 1, (1994): 13-26.

The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, *Science* 270, (1995):404-410; Blaese et al., *Cancer Gene Ther.* 2, (1995):291-297; Behr et al., *Bioconjugate Chem.* 5, (1994):382-389; Remy et al., *Bioconjugate Chem.* 5, (1994):647-654; Gao et al., *Gene Therapy* 2, (1995):710-722; Ahmad et al., *Cancer Res.* 52, (1992):4817-4820; and U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

Additional methods of delivery include the use of packaging the nucleic acids to be delivered into EnGeneIC delivery vehicles (EDVs). These EDVs are specifically delivered to target tissues using bispecific antibodies where one arm of the antibody has specificity for the target tissue and the other has specificity for the EDV. The antibody brings the EDVs to the target cell surface and then the EDV is brought into the cell by endocytosis. Once in the cell, the contents are released (see MacDiarmid et al *Nature Biotechnology* 27(7), (2009):643).

D. Heterobifunctional Compounds

The present application includes the use of a heterobifunctional compound which has (i) a moiety that binds to a ubiquitin ligase and (ii) a targeting moiety which binds to a dTAG which has been fused to an endogenous protein intended for ubiquitination and proteasomal degradation. In one embodiment the heterobifunctional compound binds to a dTAG that is mutated to have selectivity over the corresponding endogenous protein (i.e. the dTAG Targeting Ligand binds dTAG but does not significantly bind to the naturally occurring (and in some embodiments, will not significantly bind to a mutant or variant protein expressed by the host)).

Strategies harnessing the ubiquitin proteasome pathway (UPP) to selectively target and degrade proteins have been employed for post-translational control of protein function. Heterobifunctional compounds, are composed of a target protein-binding ligand and an E3 ubiquitin ligase ligand. Heterobifunctional compounds, are capable of induced proteasome-mediated degradation of selected proteins via their recruitment to E3 ubiquitin ligase and subsequent ubiquitination. These drug-like molecules offer the possibility of reversible, dose-responsive, tunable, temporal control over protein levels. An early description of such compounds was provided in U.S. Patent 7,041,298, titled "Proteolysis Targeting Chimeric Pharmaceutical," filed in September 2000 by Deshales et al. and granted in May 2006. The publication by Sakamoto et al. (*PNAS* 98(15) (2001): 8554-8559), titled "PROTACS: Chimeric Molecules that Target Proteins to the Skp1-Cullin F Box Complex for Ubiquitination and Degradation," describes a heterobifunctional compound consisting of a small molecule binder of MAP-AP-2 linked to a peptide capable of binding the F-box protein β-TRCP, the disclosure of which is also provided in U.S. Patent 7,041,298. The publication by Sakamoto et al. (*Molecular and Cellular Proteomics* 2 (2003):1350-1358), titled "Development of PROTACS to Target Cancer-promoting Proteins for Ubiquitination and Degradation," describes an analogous heterobifunctional compound (PROTAC2) that instead of degrading MAP-AP-2 degrades estrogen and androgen receptors. The publication by Schneekloth et al. (*JACS* 126 (2004):3748-3754), titled "Chemical Genetic Control of Protein Levels: Selective *in vivo* Targeted Degradation," describes an analogous heterobifunctional compound (PROTAC3) that targets the FK506 binding protein (FKBP12) and shows both PROTAC2 and PROTAC3 hit their respective targets with green fluorescent protein (GFP) imaging. The publication by Schneekloth et al. (*ChemBioChem* 6 (2005)40-46) titled "Chemical Approaches to Controlling Intracellular Protein Degradation" described the state of the field at the time, using the technology. The publication by Schneekloth et al. (*BMCL* 18(22) (2008):5904-5908), titled "Targeted Intracellular Protein Degradation Induced by a Small Molecule: En Route to Chemical Proteomics," describes a heterobifunctional compound that consist of two small molecules linked by PEG that *in vivo* degrades the androgen receptor by concurrently binding the androgen receptor and Ubiquitin E3 ligase. WO 2013/170147 to Crews et al., titled "Compounds Useful for Promoting Protein Degradation and Methods Using Same," describes compounds comprising a protein degradation moiety covalently bound to a linker, wherein the ClogP of the compound is equal to or higher than 1.5. A review of the foregoing

publications by Buckley et al. (*Angew. Chem. Int. Ed.* 53 (2014):2312-2330) is titled "Small-Molecule Control of Intraceullular Protein Levels through Modulation of the Ubiquitin Proteasome System." WO 2015/160845 assigned to Arvinas Inc., titled "Imide Based Modulators of Proteolysis and Associated methods of Use," describes the use of Degron technology with thalidomide to utilize cereblon as the E3 ligase protein. The following publication by J. Lu et al. (*Chemistry and Biol.* 22(6) (2015):755-763), titled "Hijacking the E3 Ubiquitin Ligase Cereblon to efficiently Target BDR4," similarly describes thalidomide based compounds useful for degrading BDR4. Additional publications describing this technology include Bondeson et al. (*Nature Chemical Biology* 11 (2015):611-617), Gustafson et al. (*Angew. Chem. Int. Ed.* 54 (2015):9659-9662), Buckley et al. (*ACS Chem. Bio.* 10 (2015):1831-1837), U.S. 2016/0058872 assigned to Arvinas Inc. titled "Imide Based Modulators of Proteolysis and Associated Methods of Use", U.S. 2016/0045607 assigned to Arvinas Inc. titled "Estrogen-related Receptor Alpha Based PROTAC Compounds and Associated Methods of Use", U.S. 2014/0356322 assigned to Yale University, GlaxoSmithKline, and Cambridge Enterprise Limited University of Cambridge titled "Compounds and Methods for the Enhanced Degradation of Targeted Proteins & Other Polypeptides by an E3 Ubiquitin Ligase", Lai et al. (*Angew. Chem. Int. Ed.* 55 (2016):807-810), Toure et al. (*Angew. Chem. Int. Ed.* 55 (2016):1966-1973), and US 2016/0176916 assigned to Dana Farber Cancer Institute titled "Methods to Induce Targeted Protein Degradation Through Bifunctional Molecules."

Other descriptions of targeted protein degradation technology include Itoh et al. (*JACS* 132(16) (2010):5820-5826), titled "Protein Knockdown Using Methyl Bestatin-Ligand Hybrid Molecules: Design and Synthesis of Inducers of Ubiquitination-Mediated Degradation of Cellular Retinoic Acid-Binding Proteins," which describes a small molecule linked to a peptide that utilizes E3 ubiquitin ligase to degraded retinoic acid-binding proteins, and Winter et al. (*Science* 348 (2015):1376-1381), titled "Phthalimide Conjugation as a Strategy for *in vivo* Target Protein Degradation," describes thalidomide based targeted protein degradation technology.

Heterobifunctional compounds useful for present invention may be any heterobifunctional compound capable of binding to a dTAG to induce degradation. Heterobifunctional compounds are generally known in the art, for example, see U.S. Patent 7,041,298; Sakamoto et al. (PNAS, 2001, 98(15): 8554-8559); Sakamoto et al. (*Molecular and Cellular Proteomics* 2 (2003)1350-1358); Schneekloth et al. (*JACS* 126 (2004):3748-3754); Schneekloth et al. (*ChemBioChem* 6

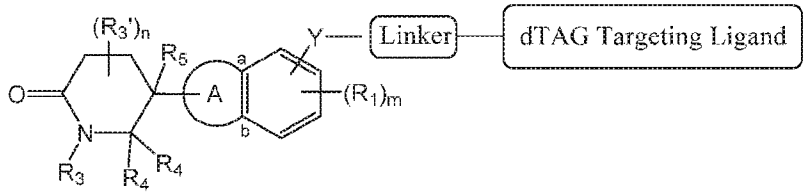
(2005):40-46); Schneekloth et al. (*BMCL* 18(22) (2008):5904-5908); WO 2013/170147; Buckley et al. (*Angew. Chem. Int. Ed.* 53 (2014):2312-2330); WO 2015/160845; Lu et al. (*Chemistry and Biol.* 22(6) (2015):755-763); Bondeson et al. (*Nature Chemical Biology* 11 (2015):611-617); Gustafson et al. (*Angew. Chem. Int. Ed.* 54 (2015):9659-9662); Buckley et al. (*ACS Chem. Bio.* 10 (2015):1831-1837); U.S. 2016/0058872 assigned to Arvinas Inc. titled "Imide Based Modulators of Proteolysis and Associated Methods of Use", U.S. 2016/0045607 assigned to Arvinas Inc. titled "Estrogen-related Receptor Alpha Based PROTAC Compounds and Associated Methods of Use", U.S. 2014/0356322 assigned to Yale University, GlaxoSmithKline, and Cambridge Enterprise Limited University of Cambridge titled "Compounds and Methods for the Enhanced Degradation of Targeted Proteins & Other Polypeptides by an E3 Ubiquitin Ligase", U.S. 2016/0176916 assigned to Dana-Farber Cancer Institute, Inc. titled "Methods to Induce Targeted Protein Degradation Through Bifunctional Molecules", Lai et al. (*Angew. Chem. Int. Ed.* 55 (2016):807-810); Toure et al. (*Angew. Chem. Int. Ed.* 55 (2016):1966-1973); Itoh et al. (*JACS* 132(16) (2010):5820-5826); and Winter et al. (*Science* 348 (2015):1376-1381), each of which is incorporated herein by reference.

In general, heterobifunctional compounds suitable for use in the present application have the general structure:

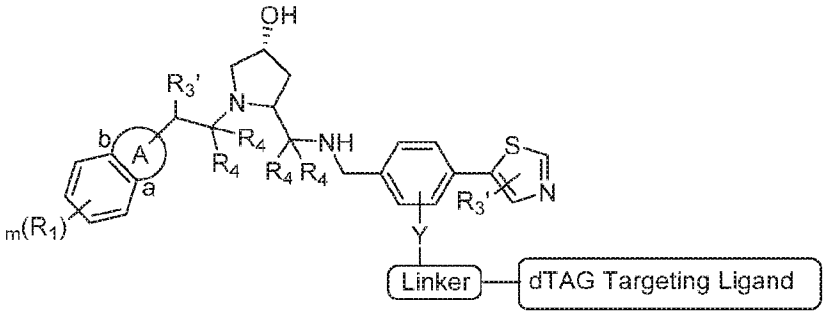
Degron–Linker–dTAG Targeting Ligand

wherein the Linker is covalently bound to a Degron and a dTAG Targeting Ligand, the Degron is a compound capable of binding to a ubiquitin ligase such as an E3 Ubiquitin Ligase (*e.g.*, cereblon), and the dTAG Targeting Ligand is capable of binding to the dTAG on the endogenous protein-dTAG hybrid protein.

In certain embodiments, the present application utilizes a compound of Formula I or Formula II:



(I)



(II)

wherein:

the Linker is a group that covalently binds to the dTAG Targeting Ligand and Y; and

the dTAG Targeting Ligand is capable of binding to a dTAG target or being bound by a dTAG target that allows tagging to occur.

In certain embodiments, the present application provides a compound of Formula (I), or an enantiomer, diastereomer, stereoisomer, or pharmaceutically acceptable salt thereof,

wherein:

the Linker (L) is a group that covalently binds to the dTAG Targeting Ligand and Y; and

the dTAG Targeting Ligand is capable of binding to or binds to a dTAG;

and wherein X1, X2, Y, R_1, R_2, R_2', R_3, R_3', R_4, R_5, m and n are each as defined herein.

In certain embodiments, the present application provides a compound of Formula (II), or an enantiomer, diastereomer, stereoisomer, or pharmaceutically acceptable salt thereof,

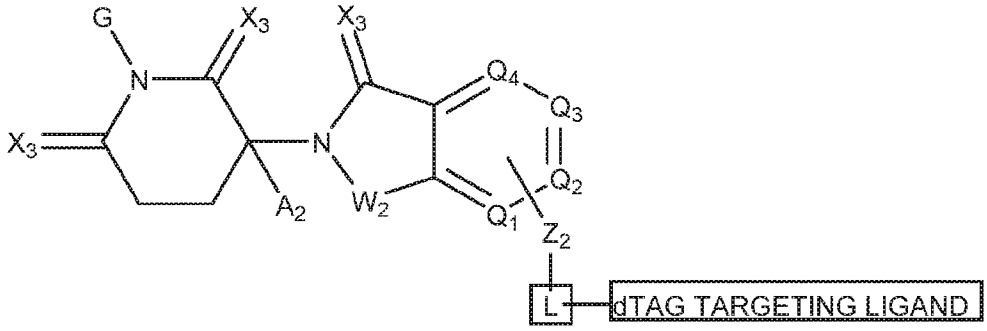
wherein:

the Linker is a group that covalently binds to the dTAG Targeting Ligand and Y; and

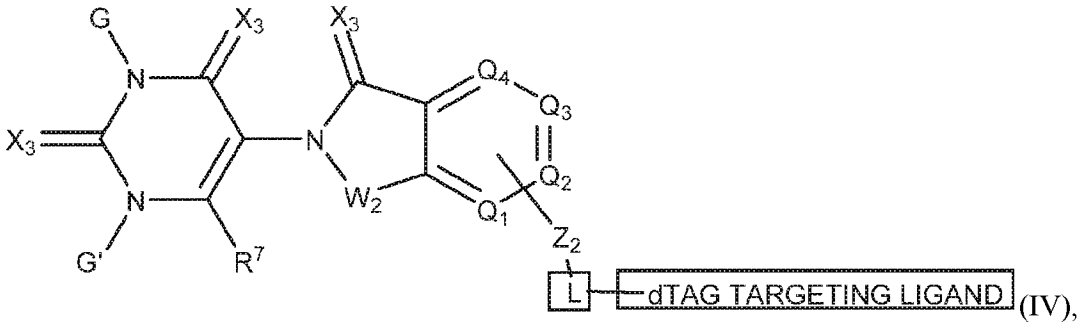
the dTAG Targeting Ligand is capable of binding to or binds to a dTAG;

and wherein X_1, X_2, Y, R_1, R_2, R_2', R_3, R_3', R_4, R_5, m and n are each as defined herein.

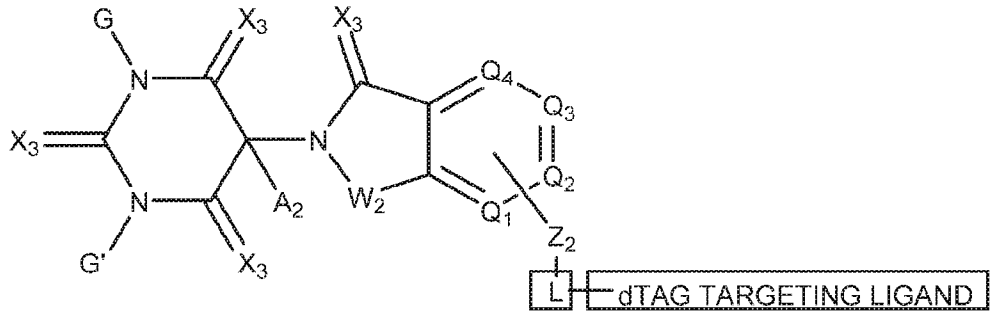
In certain embodiments, the present invention uses a compound of Formula III, Formula IV, Formula V, Formula VI, Formula VII, Formula VIII, and Formula IX:



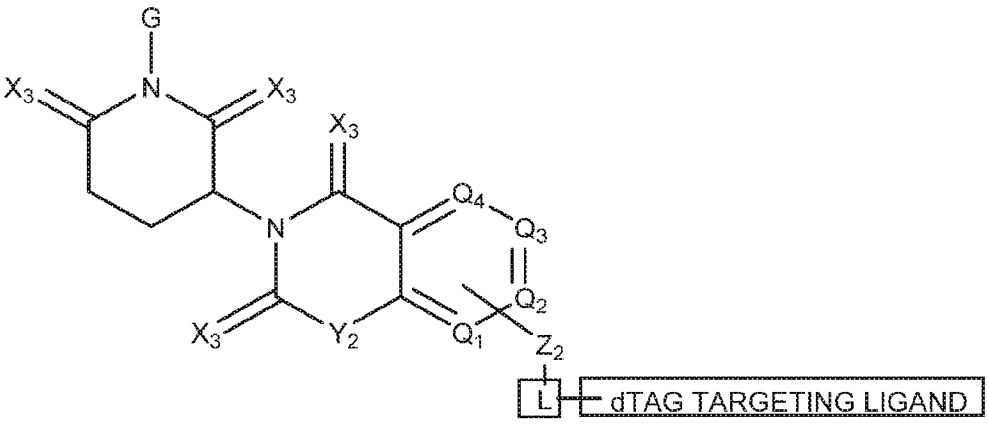
(III),



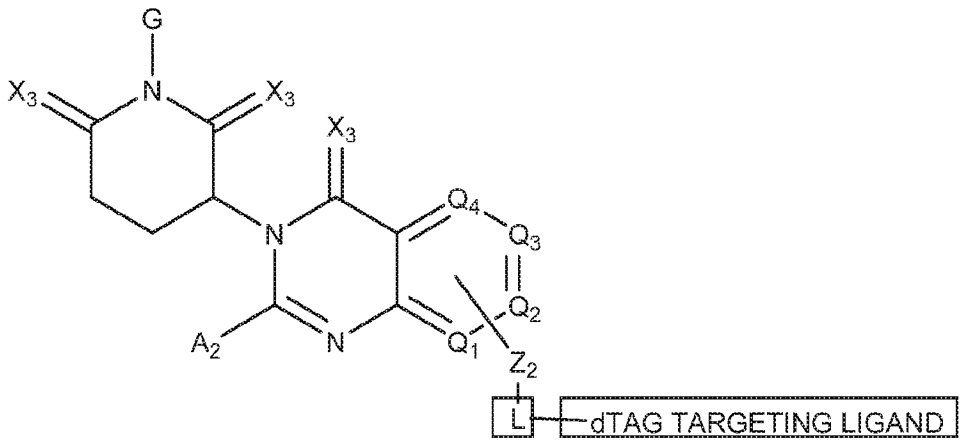
(IV),



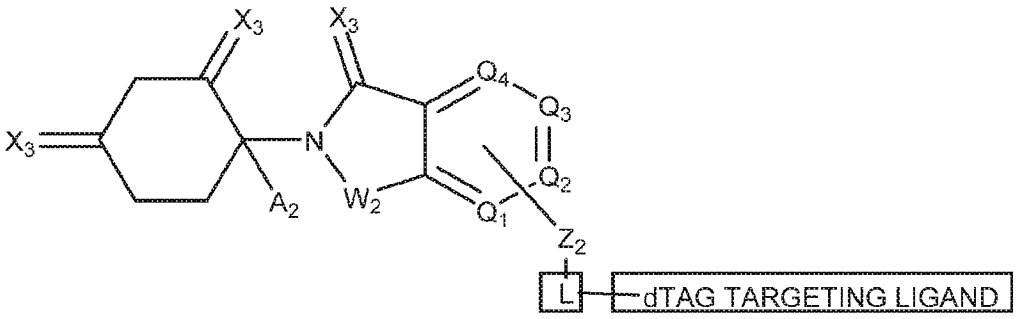
(V),



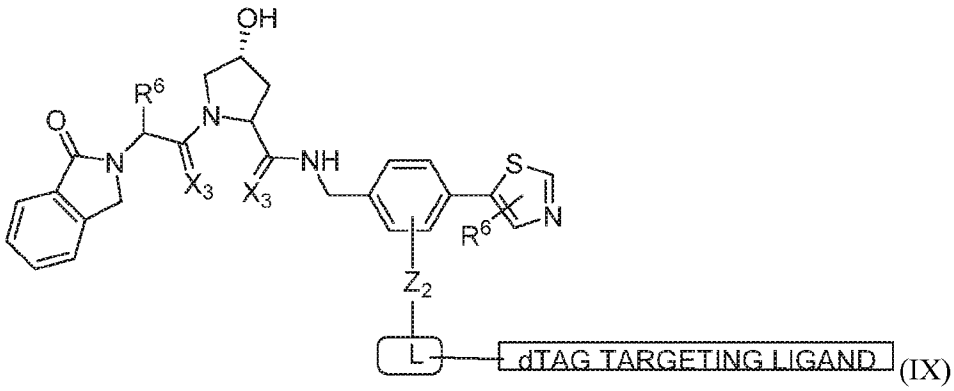
(VI),



(VII),



(VIII),



(IX)

wherein:

the Linker (L) is a group that covalently binds to the dTAG Targeting Ligand and Z_2;

the dTAG Targeting Ligand is capable of binding to a target dTAG or being bound by a target dTAG;

Z_2 is a bond, alkyl, -O, -C(O)NR_2, -NR^6C(O), -NH, or –NR^6;

R^6 is H, alkyl, -C(O)alkyl, or -C(O)H;

X_3 is independently selected from O, S, and CH_2;

W_2 is independently selected from the group CH_2, CHR, C=O, SO_2, NH, and N-alkyl;

Y_2 is independently selected from the group NH, N-alkyl, N-aryl, N-hetaryl, N-cycloalkyl, N-heterocyclyl, O, and S;

G and G′ are independently selected from the group H, alkyl, OH, CH_2-heterocyclyl optionally substituted with R′, and benzyl optionally substituted with R′;

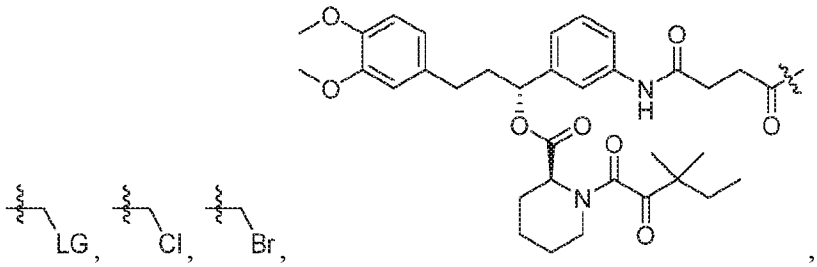
Q_1, Q_2, Q_3, and Q_4 are independently selected from CH, N, CR', and N-oxide.

A_2 is independently selected from the group alkyl, cycloalkyl, Cl and F;

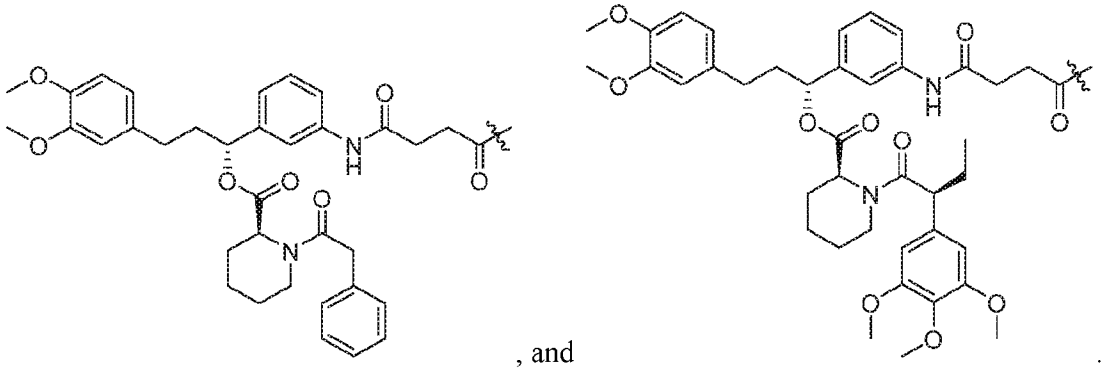
R^7 is selected from: —CONR′R″, —OR′, —NR′R″, —SR′, —SO_2R′, —SO_2NR′R″, —CR′R″—, —CR′NR′R″—, -aryl, -hetaryl, -alkyl, -cycloalkyl, -heterocyclyl, —P(O)(OR′)R″, —P(O)R′R″, —OP(O)(OR′)R″, —OP(O)R′R″, —Cl, —F, —Br, —I, —CF_3, —CN, —NR′SO_2NR′R″, —NR′CONR′R″, —CONR′COR″, —NR′C(=N—CN)NR′R″, —C(=N—CN)NR′R″, —NR′C(=N—CN)R″, —NR′C(=C—NO_2)NR′R″, —SO_2NR′COR″, —NO_2, —CO_2R′, —C(C=N—OR′)R″, —CR′=CR′R″, —CCR′, —S(C=O)(C=N—R′)R″, —SF_5 and —OCF_3

R′ and R″ are independently selected from a bond, H, alkyl, cycloalkyl, aryl, heteroaryl, heterocyclyl

Non-limiting examples of dTAG Targeting Ligands for use in the present invention include:



,



, and

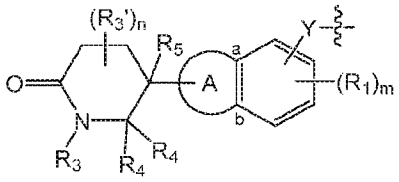
.

In some embodiments the dTAG Targeting Ligand targets a mutated endogenous target or a non-endogenous target.

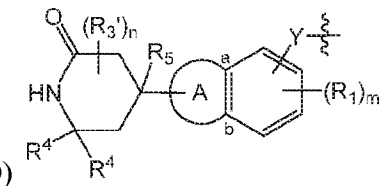
Degron

The Degron is a compound moiety that links a dTAG, through the Linker and dTAG Targeting Ligand, to a ubiquitin ligase for proteosomal degradation. In certain embodiments, the Degron is a compound that is capable of binding to or binds to a ubiquitin ligase. In further embodiments, the Degron is a compound that is capable of binding to or binds to a E3 Ubiquitin Ligase. In further embodiments, the Degron is a compound that is capable of binding to or binds to cereblon. In further embodiments, the Degron is a thalidomide or a derivative or analog thereof.

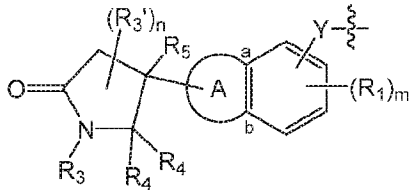
In certain embodiments, the Degron is a moiety of Formula D, Formula D0, or Formula D':



(D)

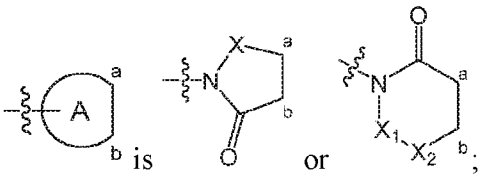


(D0) or



(D')

or an enantiomer, diastereomer, or stereoisomer thereof, wherein:



is or ;

Y is a bond, $(CH_2)_{1-6}$, $(CH_2)_{0-6}$-O, $(CH_2)_{0-6}$-C(O)NR_2', $(CH_2)_{0-6}$-NR_2'C(O), $(CH_2)_{0-6}$-NH, or $(CH_2)_{0-6}$-NR_2;

X is C(O) or $C(R_3)_2$;

X_1-X_2 is $C(R_3)$=N or $C(R_3)_2$-$C(R_3)_2$;

each R_1 is independently halogen, OH, C_1-C_6 alkyl, or C_1-C_6 alkoxy;

R_2 is C_1-C_6 alkyl, C(O)-C_1-C_6 alkyl, or C(O)-C_3-C_6 cycloalkyl;

R_2' is H or C_1-C_6 alkyl;

each R_3 is independently H or C_1-C_3 alkyl;

each R_3' is independently C_1-C_3 alkyl;

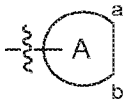
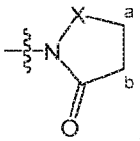
each R_4 is independently H or C_1-C_3 alkyl; or two R_4, together with the carbon atom to which they are attached, form C(O), a C_3-C_6 carbocycle, or a 4-, 5-, or 6-membered heterocycle comprising 1 or 2 heteroatoms selected from N and O;

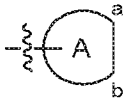
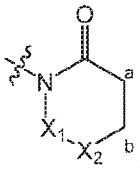
R_5 is H, deuterium, C_1-C_3 alkyl, F, or Cl;

m is 0, 1, 2 or 3; and

n is 0, 1 or 2;

wherein the compound is covalently bonded to another moiety (*e.g.*, a compound, or a Linker) via  .

In certain embodiments, the Degron is a moiety of Formula D, wherein  is  .

In certain embodiments, the Degron is a moiety of Formula D, wherein  is  .

In certain embodiments, the Degron is a moiety of Formula D, wherein X is C(O).

In certain embodiments, the Degron is a moiety of Formula D, wherein X is $C(R_3)_2$; and each R_3 is H. In certain embodiments, X is $C(R_3)_2$; and one of R_3 is H, and the other is C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, X is $C(R_3)_2$; and each R_3 is independently selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein X_1-X_2 is $C(R_3)$=N. In certain embodiments, X_1-X_2 is CH=N. In certain embodiments, X_1-X_2 is $C(R_3)$=N; and R_3 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, X_1-X_2 is $C(CH_3)$=N.

In certain embodiments, the Degron is a moiety of Formula D, wherein X_1-X_2 is $C(R_3)_2$-$C(R_3)_2$; and each R_3 is H. In certain embodiments, X_1-X_2 is $C(R_3)_2$-$C(R_3)_2$; and one of R_3 is H,

and the other three R_3 are independently C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, X_1-X_2 is $C(R_3)_2$-$C(R_3)_2$; and two of the R_3 are H, and the other two R_3 are independently C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, X_1-X_2 is $C(R_3)_2$-$C(R_3)_2$; and three of the R_3 are H, and the remaining R_3 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is a bond.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is $(CH_2)_1$, $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$, $(CH_2)_5$, or $(CH_2)_6$. In certain embodiments, Y is $(CH_2)_1$, $(CH_2)_2$, or $(CH_2)_3$. In certain embodiments, Y is $(CH_2)_1$ or $(CH_2)_2$.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is O, CH_2-O, $(CH_2)_2$-O, $(CH_2)_3$-O, $(CH_2)_4$-O, $(CH_2)_5$-O, or $(CH_2)_6$-O. In certain embodiments, Y is O, CH_2-O, $(CH_2)_2$-O, or $(CH_2)_3$-O. In certain embodiments, Y is O or CH_2-O. In certain embodiments, Y is O.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is $C(O)NR_2$', CH_2-$C(O)NR_2$', $(CH_2)_2$-$C(O)NR_2$', $(CH_2)_3$-$C(O)NR_2$', $(CH_2)_4$-$C(O)NR_2$', $(CH_2)_5$-$C(O)NR_2$', or $(CH_2)_6$-$C(O)NR_2$'. In certain embodiments, Y is $C(O)NR_2$', CH_2-$C(O)NR_2$', $(CH_2)_2$-$C(O)NR_2$', or $(CH_2)_3$-$C(O)NR_2$'. In certain embodiments, Y is $C(O)NR_2$' or CH_2-$C(O)NR_2$'. In certain embodiments, Y is $C(O)NR_2$'.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is NR_2'C(O), CH_2-NR_2'C(O), $(CH_2)_2$-NR_2'C(O), $(CH_2)_3$-NR_2'C(O), $(CH_2)_4$-NR_2'C(O), $(CH_2)_5$-NR_2'C(O), or $(CH_2)_6$-NR_2'C(O). In certain embodiments, Y is NR_2'C(O), CH_2-NR_2'C(O), $(CH_2)_2$-NR_2'C(O), or $(CH_2)_3$-NR_2'C(O). In certain embodiments, Y is NR_2'C(O) or CH_2-NR_2'C(O). In certain embodiments, Y is NR_2'C(O).

In certain embodiments, the Degron is a moiety of Formula D, wherein R_2' is H. In certain embodiments, the Degron is a moiety of Formula D, wherein R_2' is selected from methyl, ethyl, propyl, butyl, i-butyl, t-butyl, pentyl, i-pentyl, and hexyl. In certain embodiments, R_2' is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is NH, CH_2-NH, $(CH_2)_2$-NH, $(CH_2)_3$-NH, $(CH_2)_4$-NH, $(CH_2)_5$-NH, or $(CH_2)_6$-NH. In certain embodiments, Y is NH, CH_2-NH, $(CH_2)_2$-NH, or $(CH_2)_3$-NH. In certain embodiments, Y is NH or CH_2-NH. In certain embodiments, Y is NH.

In certain embodiments, the Degron is a moiety of Formula D, wherein Y is NR_2, CH_2-NR_2, $(CH_2)_2$-NR_2, $(CH_2)_3$-NR_2, $(CH_2)_4$-NR_2, $(CH_2)_5$-NR_2, or $(CH_2)_6$-NR_2. In certain embodiments, Y is NR_2, CH_2-NR_2, $(CH_2)_2$-NR_2, or $(CH_2)_3$-NR_2. In certain embodiments, Y is NR_2 or CH_2-NR_2. In certain embodiments, Y is NR_2.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_2 is selected from methyl, ethyl, propyl, butyl, i-butyl, t-butyl, pentyl, i-pentyl, and hexyl. In certain embodiments, R_2 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_2 is selected from C(O)-methyl, C(O)-ethyl, C(O)-propyl, C(O)-butyl, C(O)-i-butyl, C(O)-t-butyl, C(O)-pentyl, C(O)-i-pentyl, and C(O)-hexyl. In certain embodiments, R_2 is C(O)-C_1-C_3 alkyl selected from C(O)-methyl, C(O)-ethyl, and C(O)-propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_2 is selected from C(O)-cyclopropyl, C(O)-cyclobutyl, C(O)-cyclopentyl, and C(O)-cyclohexyl. In certain embodiments, R_2 is C(O)-cyclopropyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_3 is H.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_3 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, R_3 is methyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein n is 0.

In certain embodiments, the Degron is a moiety of Formula D, wherein n is 1.

In certain embodiments, the Degron is a moiety of Formula D, wherein n is 2.

In certain embodiments, the Degron is a moiety of Formula D, wherein each R_3' is independently C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein m is 0.

In certain embodiments, the Degron is a moiety of Formula D, wherein m is 1.

In certain embodiments, the Degron is a moiety of Formula D, wherein m is 2.

In certain embodiments, the Degron is a moiety of Formula D, wherein m is 3.

In certain embodiments, the Degron is a moiety of Formula D, wherein each R_1 is independently selected from halogen (*e.g.*, F, Cl, Br, and I), OH, C_1-C_6 alkyl (*e.g.*, methyl, ethyl, propyl, butyl, i-butyl, t-butyl, pentyl, i-pentyl, and hexyl), and C_1-C_6 alkoxy (*e.g.*, methoxy, ethoxy, propoxy, butoxy, i-butoxy, t-butoxy, and pentoxy). In further embodiments, the Degron is a

moiety of Formula D, wherein each R_1 is independently selected from F, Cl, OH, methyl, ethyl, propyl, butyl, i-butyl, t-butyl, methoxy, and ethoxy.

In certain embodiments, the Degron is a moiety of Formula D, wherein each R_4 is H.

In certain embodiments, the Degron is a moiety of Formula D, wherein one of R_4 is H, and the other R_4 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein each R_4 is independently C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein two R_4, together with the carbon atom to which they are attached, form C(O).

In certain embodiments, the Degron is a moiety of Formula D, wherein two R_4, together with the carbon atom to which they are attached, form cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein two R_4, together with the carbon atom to which they are attached, form a 4-, 5-, or 6-membered heterocycle selected from oxetane, azetidine, tetrahydrofuran, pyrrolidine, piperidine, piperazine, and morpholine. In certain embodiments, two R_4, together with the carbon atom to which they are attached, form oxetane.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_5 is H, deuterium, or C_1-C_3 alkyl. In further embodiments, R_5 is in the (*S*) or (*R*) configuration. In further embodiments, R_5 is in the (*S*) configuration. In certain embodiments, the Degron is a moiety of Formula D, wherein the compound comprises a racemic mixture of (*S*)-R_5 and (*R*)-R_5.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_5 is H.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_5 is deuterium.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_5 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl. In certain embodiments, R_5 is methyl.

In certain embodiments, the Degron is a moiety of Formula D, wherein R_5 is F or Cl. In further embodiments, R_5 is in the (*S*) or (*R*) configuration. In further embodiments, R_5 is in the (*R*) configuration. In certain embodiments, the Degron is a moiety of Formula D, wherein the compound comprises a racemic mixture of (*S*)-R_5 and (*R*)-R_5. In certain embodiments, R_5 is F.

In certain embodiments, the Degron is selected from the structures in Figure 21, wherein X is H, deuterium, C_1-C_3 alkyl, or halogen; and R is the attachment point for the Linker.

In certain embodiments, the Degron is selected from the structures in Figure 22.

In certain embodiments, the Degron is selected from the structures in Figure 23.

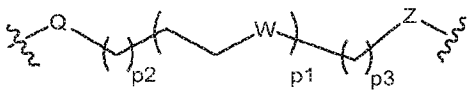
Linker

The Linker is a bond or a chemical group that links a dTAG Targeting Ligand with a Degron. In certain embodiments the Linker is a carbon chain. In certain embodiments, the carbon chain optionally includes one, two, three, or more heteroatoms selected from N, O, and S. In certain embodiments, the carbon chain comprises only saturated chain carbon atoms. In certain embodiments, the carbon chain optionally comprises two or more unsaturated chain carbon atoms (*e.g.*, C=C or C≡C). In certain embodiments, one or more chain carbon atoms in the carbon chain are optionally substituted with one or more substituents (*e.g.*, oxo, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_3 alkoxy, OH, halogen, NH_2, NH(C_1-C_3 alkyl), N(C_1-C_3 alkyl)$_2$, CN, C_3-C_8 cycloalkyl, heterocyclyl, phenyl, and heteroaryl).

In certain embodiments, the Linker includes at least 5 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises less than 20 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises 5, 7, 9, 11, 13, 15, 17, or 19 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises 5, 7, 9, or 11 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises 6, 8, 10, 12, 14, 16, or 18 chain atoms (*e.g.*, C, O, N, and S). In certain embodiments, the Linker comprises 6, 8, 10, or 12 chain atoms (*e.g.*, C, O, N, and S).

In certain embodiments, the Linker is a carbon chain optionally substituted with non-bulky substituents (*e.g.*, oxo, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_3 alkoxy, OH, halogen, NH_2, NH(C_1-C_3 alkyl), N(C_1-C_3 alkyl)$_2$, and CN). In certain embodiments, the non-bulky substitution is located on the chain carbon atom proximal to the Degron (*i.e.*, the carbon atom is separated from the carbon atom to which the Degron is bonded by at least 3, 4, or 5 chain atoms in the Linker).

In certain embodiments, the Linker is of Formula L0:



(L0),

or an enantiomer, diastereomer, or stereoisomer thereof, wherein

p1 is an integer selected from 0 to 12;

p2 is an integer selected from 0 to 12;

p3 is an integer selected from 1 to 6;

each W is independently absent, CH_2, O, S, NH or NR_5;

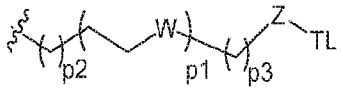
Z is absent, CH_2, O, NH or NR_5;

each R_5 is independently C_1-C_3 alkyl; and

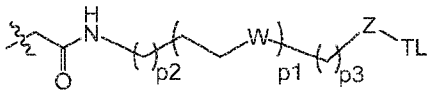
Q is absent or -$CH_2C(O)NH$-,

wherein the Linker is covalently bonded to the Degron with the  next to Q, and covalently bonded to the dTAG Targeting Ligand with the  next to Z, and wherein the total number of chain atoms in the Linker is less than 20.

In certain embodiments, the Linker–dTAG Targeting Ligand (TL) has the structure of Formula L1 or L2:



(L1),



(L2),

or an enantiomer, diastereomer, or stereoisomer thereof, wherein:

p1 is an integer selected from 0 to 12;

p2 is an integer selected from 0 to 12;

p3 is an integer selected from 1 to 6;

each W is independently absent, CH_2, O, S, NH or NR_5;

Z is absent, CH_2, O, NH or NR_5;

each R_5 is independently C_1-C_3 alkyl; and

TL is a dTAG Targeting Ligand,

wherein the Linker is covalently bonded to the Degron with  .

In certain embodiments, p1 is an integer selected from 0 to 10.

In certain embodiments, p1 is an integer selected from 2 to 10.

In certain embodiments, p1 is selected from 1, 2, 3, 4, 5, and 6.

In certain embodiments, p1 is selected from 1, 3, and 5.

In certain embodiments, p1 is selected from 1, 2, and 3.

In certain embodiments, p1 is 3.

In certain embodiments, p2 is an integer selected from 0 to 10.

In certain embodiments, p2 is selected from 0, 1, 2, 3, 4, 5, and 6.

In certain embodiments, p2 is an integer selected from 0 and 1.

In certain embodiments, p3 is an integer selected from 1 to 5.

In certain embodiments, p3 is selected from 2, 3, 4, and 5.

In certain embodiments, p3 is selected from 1, 2, and 3.

In certain embodiments, p3 is selected from 2 and 3.

In certain embodiments, at least one W is CH_2.

In certain embodiments, at least one W is O.

In certain embodiments, at least one W is S.

In certain embodiments, at least one W is NH.

In certain embodiments, at least one W is NR_5; and R_5 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, W is O.

In certain embodiments, Z is absent.

In certain embodiments, Z is CH_2.

In certain embodiments, Z is O.

In certain embodiments, Z is NH.

In certain embodiments, Z is NR_5; and R_5 is C_1-C_3 alkyl selected from methyl, ethyl, and propyl.

In certain embodiments, Z is part of the dTAG Targeting Ligand that is bonded to the Linker, namely, Z is formed from reacting a functional group of the dTAG Targeting Ligand with the Linker.

In certain embodiments, W is CH_2, and Z is CH_2.

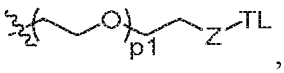
In certain embodiments, W is O, and Z is CH_2.

In certain embodiments, W is CH_2, and Z is O.

In certain embodiments, W is O, and Z is O.

In certain embodiments, the Linker–dTAG Targeting Ligand has the structure selected from Table L:

Table L



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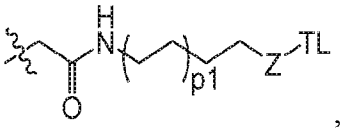

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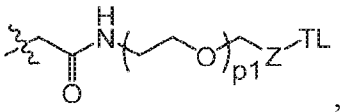

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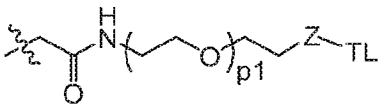

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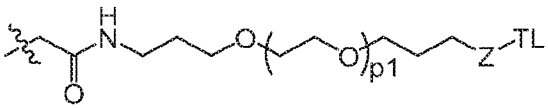

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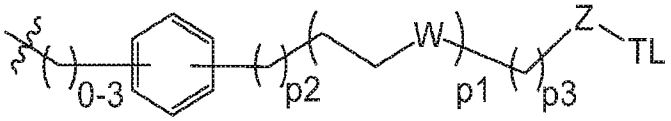
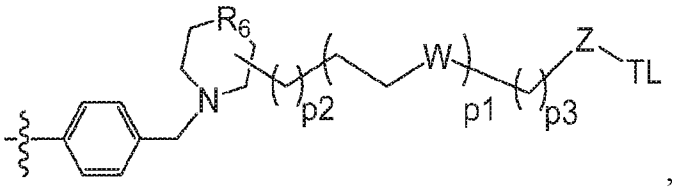


, or

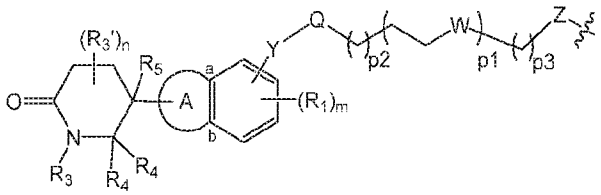


,

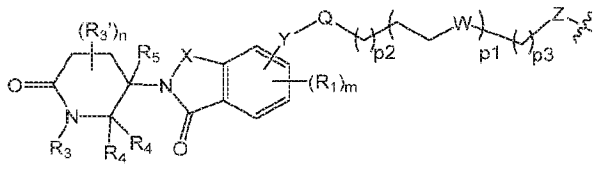
wherein Z, TL, and p1 are each as described above.

Any one of the Degrons described herein can be covalently bound to any one of the Linkers described herein.

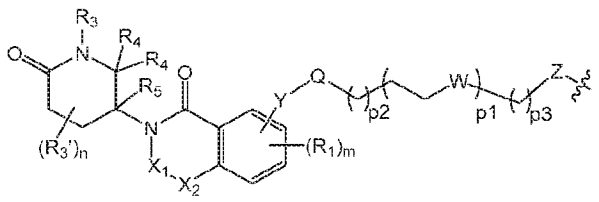
In certain embodiments, the present application includes the Degron-Linker (DL) having the following structure:



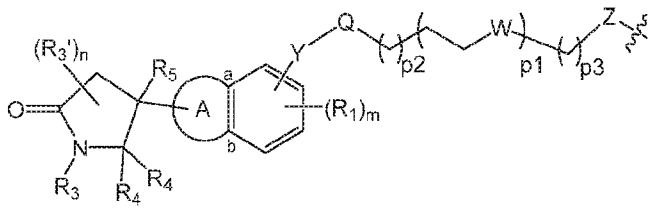
(DL),



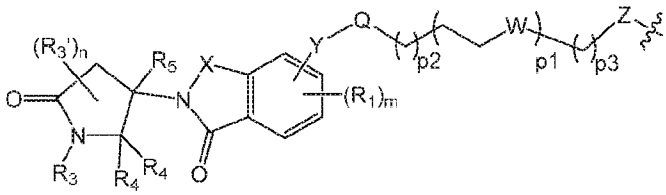
(DLa),



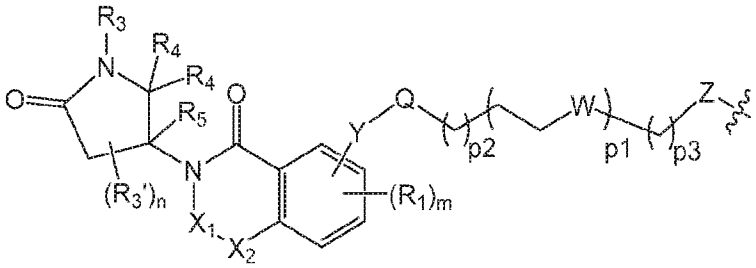
(DLb),



(DL'),



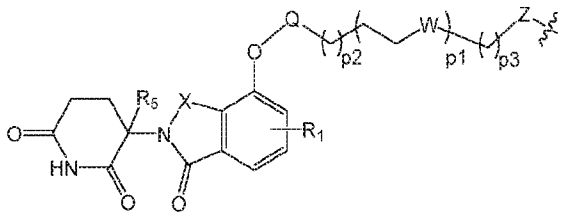
(DLa'),



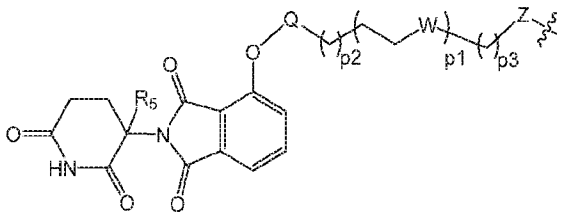
(DLb'),

wherein each of the variables is as described above in Formula D0 and Formula L0, and a dTAG Targeting Ligand is covalently bonded to the DL with the  next to Z.

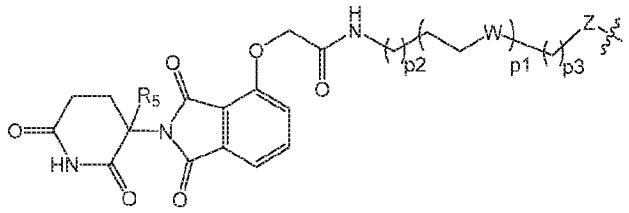
In certain embodiments, the present application includes to the Degron-Linker (DL) having the following structure:



(DLa1),



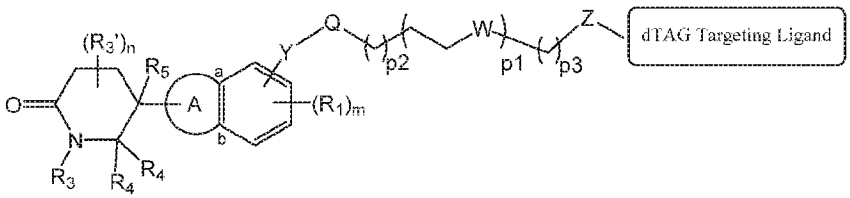
(DLa2), or



(DLa3),

wherein each of the variables is as described above in Formula D and Formula L0, and a dTAG Targeting Ligand is covalently bonded to the DL with the  next to Z.

Some embodiments of the present application relate to a bifunctional compound having the following structure:



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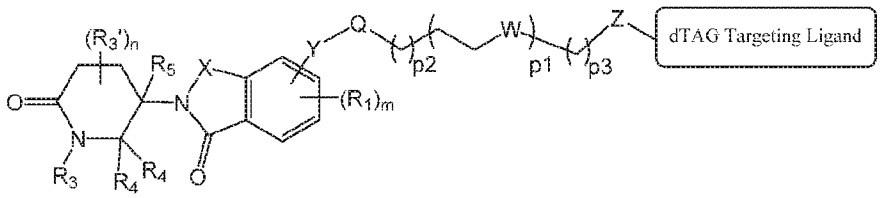

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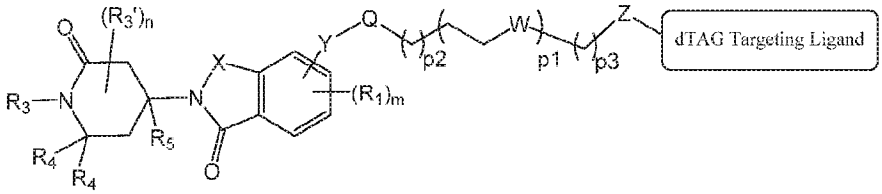
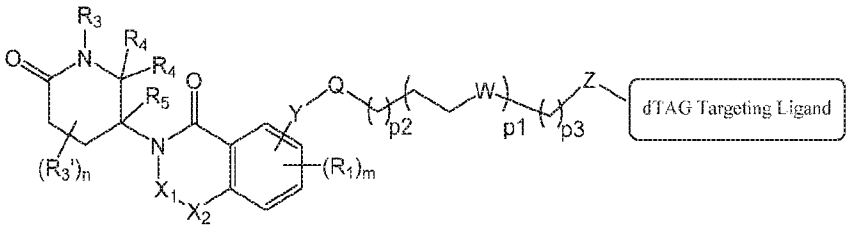


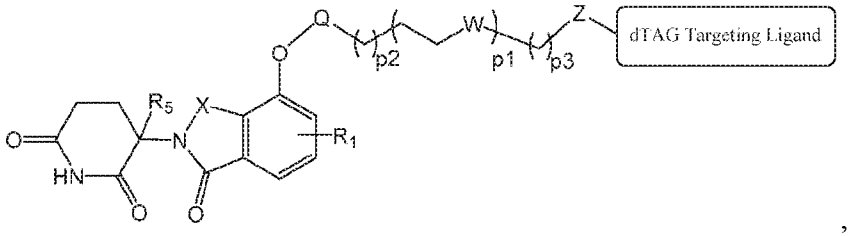
, or



,

or an enantiomer, diastereomer, or stereoisomer thereof, wherein each of the variables is as described above in Formula D and Formula L0, and the dTAG Targeting Ligand is described herein below.

Further embodiments of the present application relate to a bifunctional compound having the following structure:



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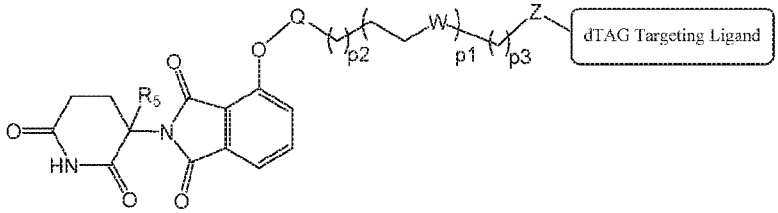
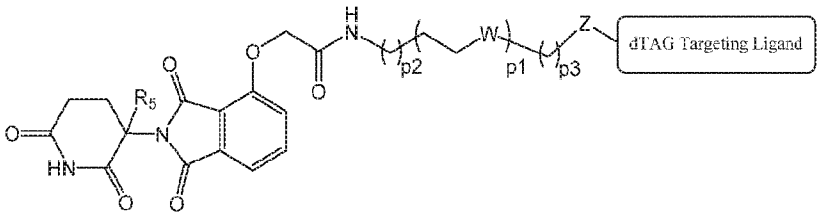
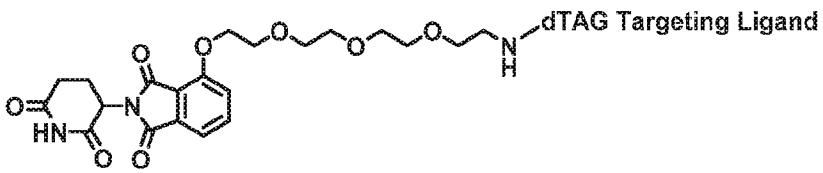


, or

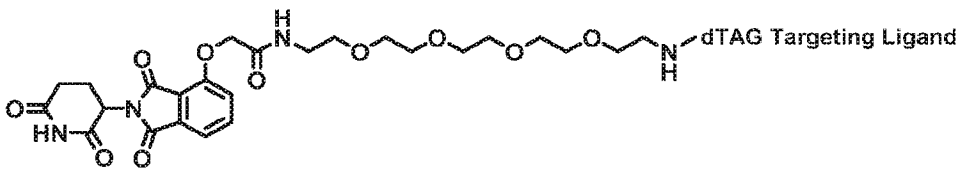


or an enantiomer, diastereomer, or stereoisomer thereof, wherein each of the variables is as described above in Formula D and Formula L0, and the dTAG Targeting Ligand is described herein below.

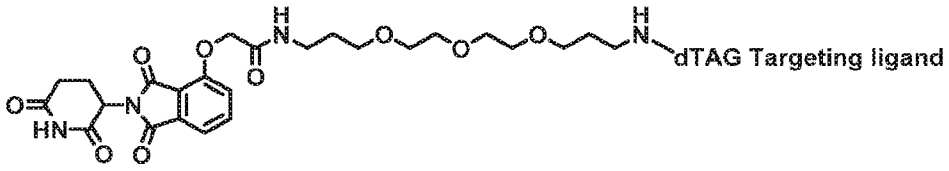
Certain embodiments of the present application relate to bifunctional compounds having one of the following structures:



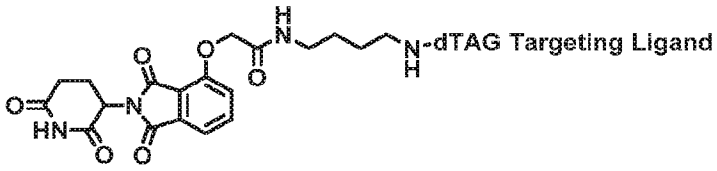
(DL1-TL),



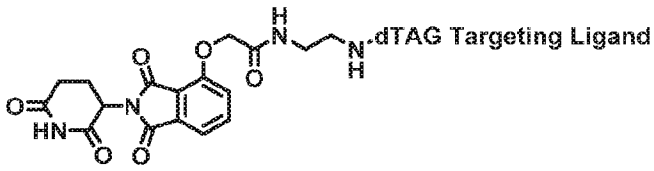
(DL2-TL),



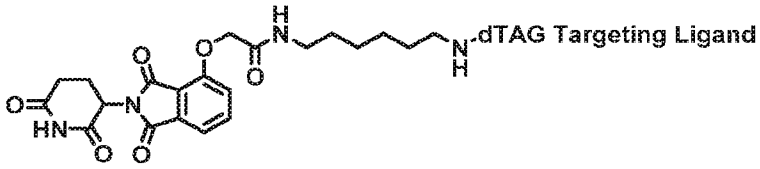
(DL3-TL),



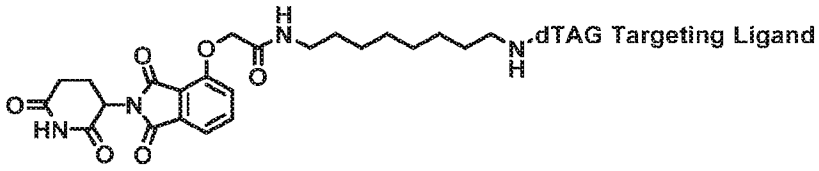
(DL4-TL),



(DL5-TL),



(DL6-TL), or



(DL7-TL).

In certain embodiments, the Linker may be a polyethylene glycol group ranging in size from about 1 to about 12 ethylene glycol units, between 1 and about 10 ethylene glycol units, about 2 about 6 ethylene glycol units, between about 2 and 5 ethylene glycol units, between about 2 and 4 ethylene glycol units.

In certain embodiments, the Linker is designed and optimized based on SAR (structure-activity relationship) and X-ray crystallography of the dTAG Targeting Ligand with regard to the location of attachment for the Linker.

In certain embodiments, the optimal Linker length and composition vary by target and can be estimated based upon X-ray structures of the original dTAG Targeting Ligand bound to its target. Linker length and composition can be also modified to modulate metabolic stability and pharmacokinetic (PK) and pharmacodynamics (PD) parameters.

In certain embodiments, where the dTAG Targeting Ligand binds multiple targets, selectivity may be achieved by varying Linker length where the ligand binds some of its targets in different binding pockets, *e.g.*, deeper or shallower binding pockets than others.

In an additional embodiment, the heterobifunctional compounds for use in the present invention include a chemical Linker (L). In certain embodiments, the Linker group L is a group comprising one or more covalently connected structural units of A (e.g., -A_1 . . . A_q-), wherein A_1 is a group coupled to at least one of a Degron, a dTAG Targeting Ligand, or a combination thereof. In certain embodiments, A_1 links a Degron, a dTAG Targeting Ligand, or a combination thereof directly to another Degron, Targeting Ligand, or combination thereof. In other embodiments, A_1 links a Degron, a dTAG Targeting Ligand, or a combination thereof indirectly to another Degron, dTAG Targeting Ligand or combination thereof through A_q.

In certain embodiments, A_1 to A_q are, each independently, a bond, $CR^{L1}R^{L2}$, O, S, SO, SO_2, NR^{L3}, SO_2NR^{L3}, $SONR^{L3}$, $CONR^{L3}$, $NR^{L3}CONR^{L4}$, $NR^{L3}SO_2NR^{L4}$, CO, $CR^{L1}{=}CR^{L2}$, C≡C, $SiR^{L1}R^{L2}$, $P(O)R^{L1}$, $P(O)OR^{L1}$, $NR^{L3}C(=NCN)NR^{L4}$, $NR^{L3}C(=NCN)$, $NR^{L3}C(=CNO_2)NR^{L4}$, $C_{3\text{-}11}$cycloalkyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, $C_{3\text{-}11}$heteocyclyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, aryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, heteroaryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, where R^{L1} or R^{L2}, each independently, can be linked to other A groups to form a cycloalkyl and/or heterocyclyl moiety which can be further substituted with 0-4 R^{L5} groups; wherein

R^{L1}, R^{L2}, R^{L3}, R^{L4} and R^{L5} are, each independently, H, halo, $C_{1\text{-}8}$alkyl, $OC_{1\text{-}8}$alkyl, $SC_{1\text{-}8}$alkyl, $NHC_{1\text{-}8}$alkyl, $N(C_{1\text{-}8}alkyl)_2$, $C_{3\text{-}11}$cycloalkyl, aryl, heteroaryl, $C_{3\text{-}11}$heterocyclyl, $OC_{1\text{-}8}$cycloalkyl, $SC_{1\text{-}8}$cycloalkyl, $NHC_{1\text{-}8}$cycloalkyl, $N(C_{1\text{-}8}cycloalkyl)_2$, $N(C_{1\text{-}8}cycloalkyl)(C_{1\text{-}8}alkyl)$, OH, NH_2, SH, $SO_2C_{1\text{-}8}$alkyl, $P(O)(OC_{1\text{-}8}alkyl)(C_{1\text{-}8}alkyl)$, $P(O)(OC_{1\text{-}8}alkyl)_2$, CC—$C_{1\text{-}8}$alkyl, CCH, $CH{=}CH(C_{1\text{-}8}alkyl)$, $C(C_{1\text{-}8}alkyl){=}CH(C_{1\text{-}8}alkyl)$, $C(C_{1\text{-}8}alkyl){=}C(C_{1\text{-}8}alkyl)_2$, $Si(OH)_3$, $Si(C_{1\text{-}8}alkyl)_3$, $Si(OH)(C_{1\text{-}8}alkyl)_2$, $COC_{1\text{-}8}$alkyl, CO_2H, halogen, CN, CF_3, CHF_2, CH_2F, NO_2, SF_5, $SO_2NHC_{1\text{-}8}$alkyl, $SO_2N(C_{1\text{-}8}alkyl)_2$, $SONHC_{1\text{-}8}$alkyl, $SON(C_{1\text{-}8}alkyl)_2$, $CONHC_{1\text{-}8}$alkyl, $CON(C_{1\text{-}8}alkyl)_2$, $N(C_{1\text{-}8}alkyl)CONH(C_{1\text{-}8}alkyl)$, $N(C_{1\text{-}8}alkyl)CON(C_{1\text{-}8}alkyl)_2$, $NHCONH(C_{1\text{-}8}alkyl)$, $NHCON(C_{1\text{-}8}alkyl)_2$, $NHCONH_2$, $N(C_{1\text{-}8}alkyl)SO_2NH(C_{1\text{-}8}alkyl)$, $N(C_{1\text{-}8}alkyl)$ $SO_2N(C_{1\text{-}8}alkyl)_2$, NH $SO_2NH(C_{1\text{-}8}alkyl)$, NH $SO_2N(C_{1\text{-}8}alkyl)_2$, NH SO_2NH_2.

In certain embodiments, q is an integer greater than or equal to 0. In certain embodiments, q is an integer greater than or equal to 1.

In certain embodiments, e.g., where q is greater than 2, A_q is a group which is connected to a Degron, and A_1 and A_q are connected via structural units of A (number of such structural units of A: q−2).

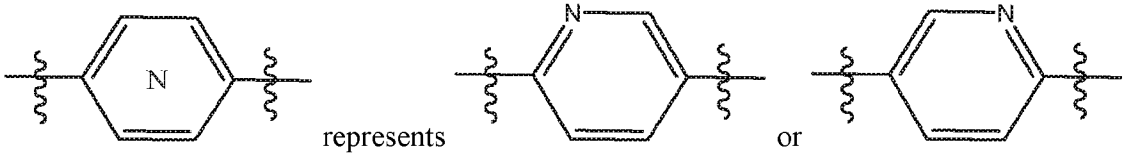
In certain embodiments, e.g., where q is 2, A_q is a group which is connected to A_1 and to a Degron moiety.

In certain embodiments, e.g., where q is 1, the structure of the Linker group L is -A_1-, and A_1 is a group which is connected to a Degron moiety and a dTAG Targeting Ligand moiety.

In additional embodiments, q is an integer from 1 to 100, 1 to 90, 1 to 80, 1 to 70, 1 to 60, 1 to 50, 1 to 40, 1 to 30, 1 to 20, or 1 to 10.

In certain embodiments, the Linker (L) is selected from the structures in Figure 24.

In other embodiments the Linker (L) is selected from the structures in Figure 25, wherein



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In additional embodiments, the Linker group is optionally substituted (poly)ethyleneglycol having between 1 and about 100 ethylene glycol units, between about 1 and about 50 ethylene glycol units, between 1 and about 25 ethylene glycol units, between about 1 and 10 ethylene glycol units, between 1 and about 8 ethylene glycol units and 1 and 6 ethylene glycol units, between 2 and 4 ethylene glycol units, or optionally substituted alkyl groups interspersed with optionally substituted, O, N, S, P or Si atoms. In certain embodiments, the Linker is substituted with an aryl, phenyl, benzyl, alkyl, alkylene, or heterocycle group. In certain embodiments, the Linker may be asymmetric or symmetrical.

In any of the embodiments of the compounds described herein, the Linker group may be any suitable moiety as described herein. In one embodiment, the Linker is a substituted or unsubstituted polyethylene glycol group ranging in size from about 1 to about 12 ethylene glycol units, between 1 and about 10 ethylene glycol units, about 2 about 6 ethylene glycol units, between about 2 and 5 ethylene glycol units, between about 2 and 4 ethylene glycol units.

Although the Degron group and dTAG Targeting Ligand group may be covalently linked to the Linker group through any group which is appropriate and stable to the chemistry of the

Linker, the Linker is independently covalently bonded to the Degron group and the dTAG Targeting Ligand group preferably through an amide, ester, thioester, keto group, carbamate (urethane), carbon or ether, each of which groups may be inserted anywhere on the Degron group and dTAG Targeting Ligand group to provide maximum binding of the Degron group on the ubiquitin ligase and the dTAG Targeting Ligand group on the target dTAG. (It is noted that in certain aspects where the Degron group targets Ubiquitin Ligase, the target protein for degradation may be the ubiquitin ligase itself). The Linker may be linked to an optionally substituted alkyl, alkylene, alkene or alkyne group, an aryl group or a heterocyclic group on the Degron and/or dTAG Targeting Ligand groups.

In certain embodiments, "L" can be linear chains with linear atoms from 4 to 24, the carbon atom in the linear chain can be substituted with oxygen, nitrogen, amide, fluorinated carbon, etc., such as the structures in Figure 26.

In certain embodiments, "L" can be nonlinear chains, and can be aliphatic or aromatic or heteroaromatic cyclic moieties, some examples of "L" include but not be limited to the structures of Figure 27, wherein X and Y are independently selected from a bond, $CR^{L1}R^{L2}$, O, S, SO, SO_2, NR^{L3}, SO_2NR^{L3}, $SONR^{L3}$, $CONR^{L3}$, $NR^{L3}CONR^{L4}$, $NR^{L3}SO_2NR^{L4}$, CO, $CR^{L1}=CR^{L2}$, C≡C, $SiR^{L1}R^{L2}$, $P(O)R^{L1}$, $P(O)OR^{L1}$, $NR^{L3}C(=NCN)NR^{L4}$, $NR^{L3}C(=NCN)$, $NR^{L3}C(=CNO_2)NR^{L4}$, C_{3-11}cycloalkyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, C_{3-11}heteocyclyl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, aryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, heteroaryl optionally substituted with 0-6 R^{L1} and/or R^{L2} groups, where R^{L1} or R^{L2}, each independently, can be linked to other A groups to form a cycloalkyl and/or heterocyclyl moiety which can be further substituted with 0-4 R^{L5} groups.

dTAG Targeting Ligand

The dTAG Targeting Ligand (TL) is capable of binding to a dTAG or being bound by a dTAG target that allows tagging with ubiquitin to occur;

As contemplated herein, the genomes of the present invention include a heterobifunctional compound targeted protein (dTAG) which locates in the cytoplasm. The heterobifunctional compound targeted protein of the genome is any amino acid sequence to which a heterobifunctional compound can be bound, leading to the degradation of the protein-dTAG hybrid protein when in contact with the heterobifunctional compound. Preferably, the dTAG should not

interfere with the function of the CAR. In one embodiment, the dTAG is a non-endogenous peptide, leading to heterobifunctional compound selectivity and allowing for the avoidance of off target effects upon administration of the heterobifunctional compound. In one embodiment, the dTAG is an amino acid sequence derived from an endogenous protein which has been modified so that the heterobifunctional compound binds only to the modified amino acid sequence and not the endogenously expressed protein. In one embodiment, the dTAG is an endogenously expressed protein. Any amino acid sequence domain that can be bound by a ligand for use in a heterobifunctional compound can be used as a dTAG as contemplated herewith.

In particular embodiments, the dTAGs for use in the present invention include, but are not limited to, amino acid sequences derived from endogenously expressed proteins such as FK506 binding protein-12 (FKBP12), bromodomain-containing protein 4 (BRD4), CREB binding protein (CREBBP), and transcriptional activator BRG1 (SMARCA4), or a variant thereof. As contemplated herein, "variant" means any variant such as a substitution, deletion, or addition of one or a few to plural amino acids, provided that the variant substantially retains the same function as the original sequence, which in this case is providing ligand binding for a heterobifunctional compound. In other embodiments, dTAGs for us in the present invention may include, for example, hormone receptors e.g. estrogen-receptor proteins, androgen receptor proteins, retinoid x receptor (RXR) protein, and dihydrofolate reductase (DHFR), including bacterial DHFR, bacterial dehydrogenase, and variants.

In one embodiment the dTAG is a portion of any of the proteins identified herein. For example, the dTAG can be the BD1 domain of BRD4 or the BD2 domain of BRD4. In one embodiment that Targeting Ligands identified herein to target the parent dTAG are instead used to target portion. In one embodiment, the BRD4 Targeting Ligands in Table T can be used to target the BD1 dTAG. In another embodiment, the BRD4 Targeting Ligands in Table T can be used to target the BD2 dTAG.

Some embodiments of the present application include TLs which target dTAGs including, but not limited to, those derived from Hsp90 inhibitors, kinase inhibitors, MDM2 inhibitors, compounds targeting Human BET bromodomain-containing proteins, compounds targeting cytosolic signaling protein FKBP12, HDAC inhibitors, human lysine methyltransferase inhibitors, angiogenesis inhibitors, immunosuppressive compounds, and compounds targeting the aryl hydrocarbon receptor (AHR).

In certain embodiments, the dTAG Targeting Ligand is a compound that is capable of binding to or binds to a dTAG derived from a kinase, a BET bromodomain-containing protein, a cytosolic signaling protein (*e.g.*, FKBP12), a nuclear protein, a histone deacetylase, a lysine methyltransferase, a protein regulating angiogenesis, a protein regulating immune response, an aryl hydrocarbon receptor (AHR), an estrogen receptor, an androgen receptor, a glucocorticoid receptor, or a transcription factor (*e.g.*, SMARCA4, SMARCA2, TRIM24).

In certain embodiments, the dTAG is derived from a kinase to which the dTAG Targeting Ligand is capable of binding or binds including, but not limited to, a tyrosine kinase (*e.g.*, AATK, ABL, ABL2, ALK, AXL, BLK, BMX, BTK, CSF1R, CSK, DDR1, DDR2, EGFR, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FRK, FYN, GSG2, HCK, IGF1R, ILK, INSR, INSRR, IRAK4, ITK, JAK1, JAK2, JAK3, KDR, KIT, KSR1, LCK, LMTK2, LMTK3, LTK, LYN, MATK, MERTK, MET, MLTK, MST1R, MUSK, NPR1, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PLK4, PTK2, PTK2B, PTK6, PTK7, RET, ROR1, ROR2, ROS1, RYK, SGK493, SRC, SRMS, STYK1, SYK, TEC, TEK, TEX14, TIE1, TNK1, TNK2, TNNI3K, TXK, TYK2, TYRO3, YES1, or ZAP70), a serine/threonine kinase (*e.g.*, casein kinase 2, protein kinase A, protein kinase B, protein kinase C, Raf kinases, CaM kinases, AKT1, AKT2, AKT3, ALK1, ALK2, ALK3, ALK4, Aurora A, Aurora B, Aurora C, CHK1, CHK2, CLK1, CLK2, CLK3, DAPK1, DAPK2, DAPK3, DMPK, ERK1, ERK2, ERK5, GCK, GSK3, HIPK, KHS1, LKB1, LOK, MAPKAPK2, MAPKAPK, MNK1, MSSK1, MST1, MST2, MST4, NDR, NEK2, NEK3, NEK6, NEK7, NEK9, NEK11, PAK1, PAK2, PAK3, PAK4, PAK5, PAK6, PIM1, PIM2, PLK1, RIP2, RIP5, RSK1, RSK2, SGK2, SGK3, SIK1, STK33, TAO1, TAO2, TGF-beta, TLK2, TSSK1, TSSK2, ULK1, or ULK2), a cyclin dependent kinase (*e.g.*, Cdk1 – Cdk11), and a leucine-rich repeat kinase (*e.g.*, LRRK2).

In certain embodiments, the dTAG is derived from a BET bromodomain-containing protein to which the dTAG Targeting Ligand is capable of binding or binds including, but not limited to, ASH1L, ATAD2, BAZ1A, BAZ1B, BAZ2A, BAZ2B, BRD1, BRD2, BRD3, BRD4, BRD5, BRD6, BRD7, BRD8, BRD9, BRD10, BRDT, BRPF1, BRPF3, BRWD3, CECR2, CREBBP, EP300, FALZ, GCN5L2, KIAA1240, LOC93349, MLL, PB1, PCAF, PHIP, PRKCBP1, SMARCA2, SMARCA4, SP100, SP110, SP140, TAF1, TAF1L, TIF1a, TRIM28, TRIM33,

TRIM66, WDR9, ZMYND11, and MLL4. In certain embodiments, a BET bromodomain-containing protein is BRD4.

In certain embodiments, the dTAG is derived from a nuclear protein to which the dTAG Targeting Ligand is capable of binding or binds including, but not limited to, BRD2, BRD3, BRD4, Antennapedia Homeodomain Protein, BRCA1, BRCA2, CCAAT-Enhanced-Binding Proteins, histones, Polycomb-group proteins, High Mobility Group Proteins, Telomere Binding Proteins, FANCA, FANCD2, FANCE, FANCF, hepatocyte nuclear factors, Mad2, NF-kappa B, Nuclear Receptor Coactivators, CREB-binding protein, p55, p107, p130, Rb proteins, p53, c-fos, c-jun, c-mdm2, c-myc, and c-rel.

In certain embodiments, the dTAG Targeting Ligand is selected from a kinase inhibitor, a BET bromodomain-containing protein inhibitor, cytosolic signaling protein FKBP12 ligand, an HDAC inhibitor, a lysine methyltransferase inhibitor, an angiogenesis inhibitor, an immunosuppressive compound, and an aryl hydrocarbon receptor (AHR) inhibitor.

In certain embodiments, the dTAG Targeting Ligand is a SERM (selective estrogen receptor modulator) or SERD (selective estrogen receptor degrader). Non-limiting examples of SERMs and SERDs are provided in WO 2014/191726 assigned to Astra Zeneca, WO2013/090921, WO 2014/203129, WO 2014/203132, and US2013/0178445 assigned to Olema Pharmaceuticals, and U.S. Patent Nos. 9,078,871, 8,853,423, and 8,703,810, as well as US 2015/0005286, WO 2014/205136, and WO 2014/205138 assigned to Seragon Pharmaceuticals.

Additional dTAG Targeting Ligands include, for example, any moiety which binds to an endogenous protein (binds to a target dTAG). Illustrative dTAG Targeting Ligands includes the small molecule dTAG Targeting Ligand: Hsp90 inhibitors, kinase inhibitors, HDM2 and MDM2 inhibitors, compounds targeting Human BET bromodomain-containing proteins, HDAC inhibitors, human lysine methyltransferase inhibitors, angiogenesis inhibitors, nuclear hormone receptor compounds, immunosuppressive compounds, and compounds targeting the aryl hydrocarbon receptor (AHR), among numerous others. Such small molecule target dTAG binding moieties also include pharmaceutically acceptable salts, enantiomers, solvates and polymorphs of these compositions, as well as other small molecules that may target a dTAG of interest.

In some embodiments the dTAG Targeting Ligand is an Ubc9 SUMO E2 ligase 5F6D targeting ligand including but not limited to those described in “Insights Into the Allosteric

Inhibition of the SUMO E2 Enzyme Ubc9."by Hewitt, W.M., et. al. (2016) Angew.Chem.Int.Ed.Engl. 55: 5703-5707

In another embodiment the dTAG Targeting Ligand is a Tank1 targeting ligand including but not limited to those described in "Structure of human tankyrase 1 in complex with small-molecule inhibitors PJ34 and XAV939." Kirby, C.A., Cheung, A., Fazal, A., Shultz, M.D., Stams, T, (2012) Acta Crystallogr.,Sect.F 68: 115-118; and "Structure-Efficiency Relationship of [1,2,4]Triazol-3-ylamines as Novel Nicotinamide Isosteres that Inhibit Tankyrases." Shultz, M.D., et al. (2013) J.Med.Chem. 56: 7049-7059.

In another embodiment the dTAG Targeting Ligand is a SH2 domain of pp60 Src targeting ligand including but not limited to those described in "Requirements for Specific Binding of Low Affinity Inhibitor Fragments to the SH2 Domain of pp60Src Are Identical to Those for High Affinity Binding of Full Length Inhibitors" Gudrun Lange, et al., J. Med. Chem. 2003, 46, 5184-5195.

In another embodiment the dTAG Targeting Ligand is a Sec7 domain targeting ligand including but not limited to those described in "The Lysosomal Protein Saposin B Binds Chloroquine." Huta, B.P., et al., (2016) Chemmedchem 11: 277.

In another embodiment the dTAG Targeting Ligand is a Saposin-B targeting ligand including but not limited to those described in "The structure of cytomegalovirus immune modulator UL141 highlights structural Ig-fold versatility for receptor binding" I. Nemcovicova and D. M. Zajonc Acta Cryst. (2014). D70, 851-862.

In another embodiment the dTAG Targeting Ligand is a Protein S100-A7 2OWS targeting ligand including but not limited to those described in "2WOS STRUCTURE OF HUMAN S100A7 IN COMPLEX WITH 2,6 ANS" DOI: 10.2210/pdb2wos/pdb; and "Identification and Characterization of Binding Sites on S100A7, a Participant in Cancer and Inflammation Pathways." Leon, R., Murray, et al., (2009) Biochemistry 48: 10591-10600.

In another embodiment the dTAG Targeting Ligand is a Phospholipase A2 targeting ligand including but not limited to those described in "Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase A2 " Schevitz, R.W., et al., Nat. Struct. Biol. 1995, 2, 458-465.

In another embodiment the dTAG Targeting Ligand is a PHIP targeting ligand including but not limited to those described in "A Poised Fragment Library Enables Rapid Synthetic

Expansion Yielding the First Reported Inhibitors of PHIP(2), an Atypical Bromodomain" Krojer, T.; et al. Chem. Sci. 2016, 7, 2322–2330.

In another embodiment the dTAG Targeting Ligand is a PDZ targeting ligand including but not limited to those described in "Discovery of Low-Molecular-Weight Ligands for the AF6 PDZ Domain" Mangesh Joshi, et al. Angew. Chem. Int. Ed. 2006, 45, 3790-3795.

In another embodiment the dTAG Targeting Ligand is a PARP15 targeting ligand including but not limited to those described in "Structural Basis for Lack of ADP-ribosyltransferase Activity in Poly(ADP-ribose) Polymerase-13/Zinc Finger Antiviral Protein." Karlberg, T., et al., (2015) J.Biol.Chem. 290: 7336-7344.

In another embodiment the dTAG Targeting Ligand is a PARP14 targeting ligand including but not limited to those described in "Discovery of Ligands for ADP-Ribosyltransferases via Docking-Based Virtual Screening." Andersson, C.D., et al.,(2012) J.Med.Chem. 55: 7706-7718.; "Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors."Wahlberg, E., et al. (2012) Nat.Biotechnol. 30: 283-288.; "Discovery of Ligands for ADP-Ribosyltransferases via Docking-Based Virtual Screening. "Andersson, C.D., et al. (2012) J.Med.Chem. 55: 7706-7718.

In another embodiment the dTAG Targeting Ligand is a MTH1 targeting ligand including but not limited to those described in "MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool" Helge Gad, et. al. Nature, 2014, 508, 215-221.

In another embodiment the dTAG Targeting Ligand is a mPGES-1 targeting ligand including but not limited to those described in "Crystal Structures of mPGES-1 Inhibitor Complexes Form a Basis for the Rational Design of Potent Analgesic and Anti-Inflammatory Therapeutics." Luz, J.G., et al., (2015) J.Med.Chem. 58: 4727-4737.

In another embodiment the dTAG Targeting Ligand is a FLAP- 5-lipoxygenase-activating protein targeting ligand including but not limited to those described in "Crystal structure of inhibitor-bound human 5-lipoxygenase-activating protein."Ferguson, A.D., McKeever, B.M., Xu, S., Wisniewski, D., Miller, D.K., Yamin, T.T., Spencer, R.H., Chu, L., Ujjainwalla, F., Cunningham, B.R., Evans, J.F., Becker, J.W. (2007) Science 317: 510-512.

In another embodiment the dTAG Targeting Ligand is a FA Binding Protein targeting ligand including but not limited to those described in "A Real-World Perspective on Molecular Design." Kuhn, B.; et al. J. Med. Chem. 2016, 59, 4087–4102.

In another embodiment the dTAG Targeting Ligand is a BCL2 targeting ligand including but not limited to those described in "ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets." Souers, A.J., et al. (2013) NAT.MED. (N.Y.) 19: 202-208.

In another embodiment the dTAG Targeting Ligand is an EGFR targeting ligand. In one embodiment the dTAG Targeting Ligand is selected from erlotinib (Tarceva), gefitinib (Iressa), afatinib (Gilotrif), rociletinib (CO-1686), osimertinib (Tagrisso), olmutinib (Olita), naquotinib (ASP8273), nazartinib (EGF816), PF-06747775 (Pfizer), icotinib (BPI-2009), neratinib (HKI-272; PB272); avitinib (AC0010), EAI045, tarloxotinib (TH-4000; PR-610), PF-06459988 (Pfizer), tesevatinib (XL647; EXEL-7647; KD-019), transtinib, WZ-3146, WZ8040, CNX-2006, and dacomitinib (PF-00299804; Pfizer). The linker can be placed on these Targeting Ligands in any location that does not interfere with the Ligands binding to EGFR. Non-limiting examples of Linker binding locations are provided in Table T below. In one embodiment the EGFR targeting ligand binds the L858R mutant of EGFR. In another embodiment the EGFR targeting ligand binds the T790M mutant of EGFR. In another embodiment the EGFR targeting ligand binds the C797G or C797S mutant of EGFR. In one embodiment the EGFR targeting ligand is selected from erlotinib, gefitinib, afatinib, neratinib, and dacomitinib and binds the L858R mutant of EGFR. In another embodiment the EGFR targeting ligand is selected from osimertinib, rociletinib, olmutinib, naquotinib, nazartinib, PF-06747775, Icotinib, Neratinib, Avitinib, Tarloxotinib, PF-0645998, Tesevatinib, Transtinib, WZ-3146, WZ8040, and CNX-2006 and binds the T790M mutant of EGFR. In another embodiment the EGFR targeting ligand is EAI045 and binds the C797G or C797S mutant of EGFR.

Any protein which can bind to a dTAG Targeting Ligand group and acted on or degraded by a ubiquitin ligase is a target protein according to the present invention. In general, an endogenous target proteins for use as dTAGs may include, for example, structural proteins, receptors, enzymes, cell surface proteins, proteins pertinent to the integrated function of a cell, including proteins involved in catalytic activity, aromatase activity, motor activity, helicase activity, metabolic processes (anabolism and catabolism), antioxidant activity, proteolysis, biosynthesis, proteins with kinase activity, oxidoreductase activity, transferase activity, hydrolase activity, lyase activity, isomerase activity, ligase activity, enzyme regulator activity, signal transducer activity, structural molecule activity, binding activity (protein, lipid carbohydrate),

receptor activity, cell motility, membrane fusion, cell communication, regulation of biological processes, development, cell differentiation, response to stimulus, behavioral proteins, cell adhesion proteins, proteins involved in cell death, proteins involved in transport (including protein transporter activity, nuclear transport, ion transporter activity, channel transporter activity, carrier activity, permease activity, secretion activity, electron transporter activity, pathogenesis, chaperone regulator activity, nucleic acid binding activity, transcription regulator activity, extracellular organization and biogenesis activity, translation regulator activity.

More specifically, a number of drug targets for human therapeutics represent dTAG targets to which protein target or dTAG Targeting Ligand may be bound and incorporated into compounds according to the present invention. These include proteins which may be used to restore function in numerous polygenic diseases, including for example B7.1 and B7, TINFR1m, TNFR2, NADPH oxidase, BclIBax and other partners in the apoptosis pathway, C5a receptor, HMG-CoA reductase, PDE V phosphodiesterase type, PDE IV phosphodiesterase type 4, PDE I, PDEII, PDEIII, squalene cyclase inhibitor, CXCR1, CXCR2, nitric oxide (NO) synthase, cyclo-oxygenase 1, cyclo-oxygenase 2, 5HT receptors, dopamine receptors, G Proteins, i.e., Gq, histamine receptors, 5-lipoxygenase, tryptase serine protease, thymidylate synthase, purine nucleoside phosphorylase, GAPDH trypanosomal, glycogen phosphorylase, Carbonic anhydrase, chemokine receptors, JAW STAT, RXR and similar, HIV 1 protease, HIV 1 integrase, influenza, neuramimidase, hepatitis B reverse transcriptase, sodium channel, multi drug resistance (MDR), protein P-glycoprotein (and MRP), tyrosine kinases, CD23, CD124, tyrosine kinase p56 lck, CD4, CD5, IL-2 receptor, IL-1 receptor, TNF-alphaR, ICAM1, Cat+ channels, VCAM, VLA-4 integrin, selectins, CD40/CD40L, newokinins and receptors, inosine monophosphate dehydrogenase, p38 MAP Kinase, RaslRaflMEWERK pathway, interleukin-1 converting enzyme, caspase, HCV, NS3 protease, HCV NS3 RNA helicase, glycinamide ribonucleotide formyl transferase, rhinovirus 3C protease, herpes simplex virus-1 (HSV-I), protease, cytomegalovirus (CMV) protease, poly (ADP-ribose) polymerase, cyclin dependent kinases, vascular endothelial growth factor, oxytocin receptor, microsomal transfer protein inhibitor, bile acid transport inhibitor, 5 alpha reductase inhibitors, angiotensin 11, glycine receptor, noradrenaline reuptake receptor, endothelin receptors, neuropeptide Y and receptor, estrogen receptors, androgen receptors, adenosine receptors, adenosine kinase and AMP deaminase, purinergic receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2X1-7), farnesyltransferases, geranylgeranyl transferase, TrkA a receptor for NGF, beta-amyloid, tyrosine

kinase Flk-IIKDR, vitronectin receptor, integrin receptor, Her-21 neu, telomerase inhibition, cytosolic phospholipaseA2 and EGF receptor tyrosine kinase. Additional protein targets useful as dTAGs include, for example, ecdysone 20-monooxygenase, ion channel of the GABA gated chloride channel, acetylcholinesterase, voltage-sensitive sodium channel protein, calcium release channel, and chloride channels. Still further target proteins for use as dTAGs include Acetyl-CoA carboxylase, adenylosuccinate synthetase, protoporphyrinogen oxidase, and enolpyruvylshikimate-phosphate synthase.

In one embodiment the dTAG and dTAG Targeting Ligand pair are chosen by screening a library of ligands. Such a screening is exemplified in "Kinase Inhibitor Profiling Reveals Unexpected Opportunities to Inhibit Disease-Associated Mutant Kinases" by Duong-Ly et al.; Cell Reports 14, 772-781 February 2, 2016.

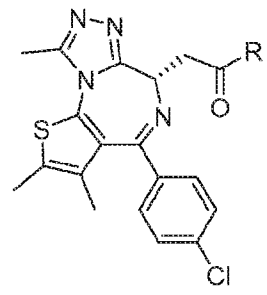
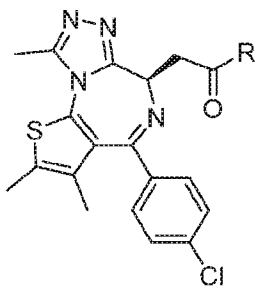
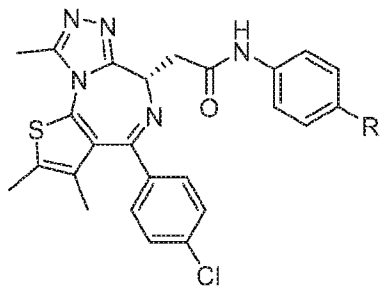
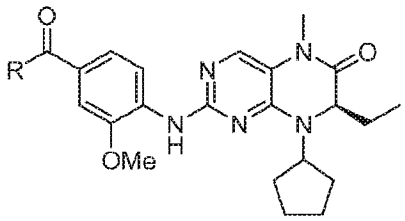
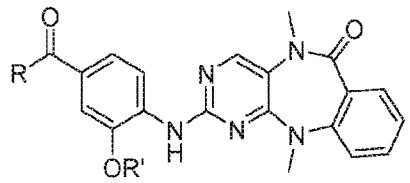
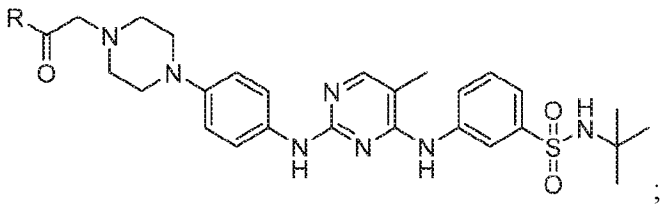
Haloalkane dehalogenase enzymes are another target of specific compounds according to the present invention which may be used as dTAGs. Compounds according to the present invention which contain chloroalkane peptide binding moieties (C1-C12 often about C2-C10 alkyl halo groups) may be used to inhibit and/or degrade haloalkane dehalogenase enzymes which are used in fusion proteins or related diagnostic proteins as described in PCT/US2012/063401 filed Dec. 6, 2011 and published as WO 2012/078559 on Jun. 14, 2012, the contents of which is incorporated by reference herein.

Non-limiting examples of dTAG Targeting Ligands are shown below in Table T and represent dTAG Targeting Ligands capable of targeting proteins or amino acid sequence useful as dTAGs.

TABLE T:

A. BRD dTAG Targeting Ligands:

BRD dTAG Targeting Ligands as used herein include, but are not limited to:

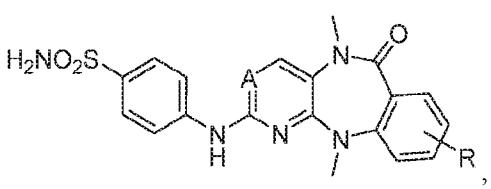
wherein:

R is the point at which the Linker is attached; and

R': is methyl or ethyl.

B. CREBBP dTAG Targeting Ligands:

CREBBP dTAG Targeting Ligands as used herein include, but are not limited to: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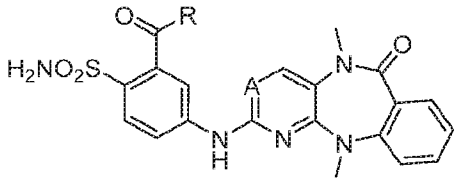
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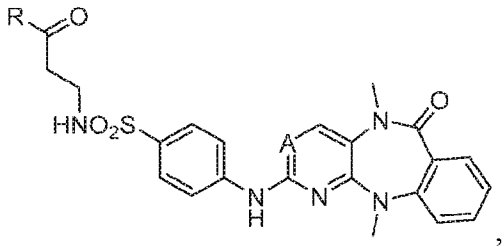
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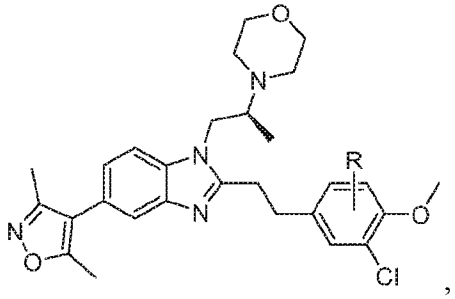
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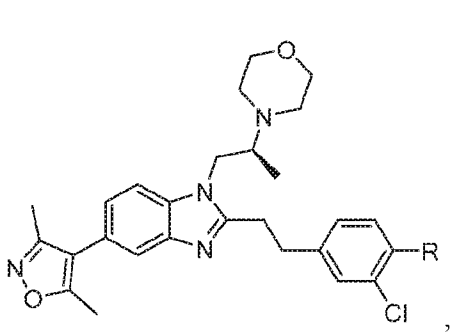
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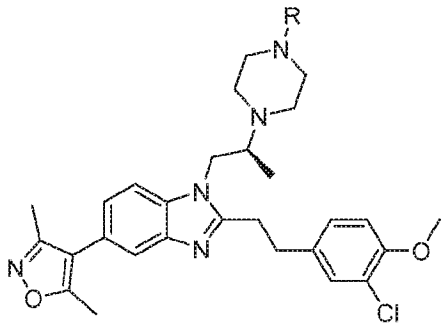
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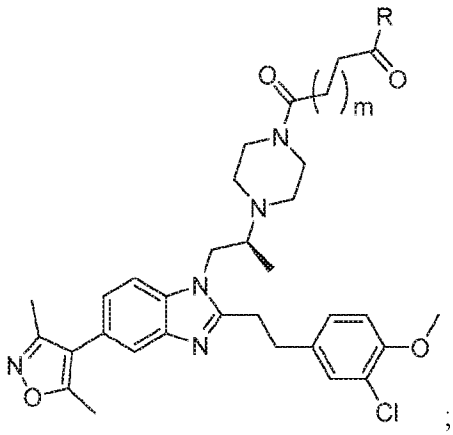

;

wherein:

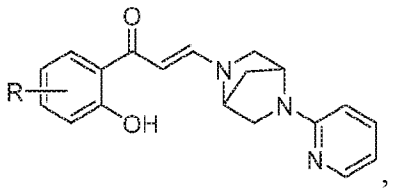
R is the point at which the Linker is attached;

A is N or CH; and

m is 0, 1, 2, 3, 4, 5, 6, 7, or 8.

C. SMARCA4, PB1, and/or SMARCA2 dTAG Targeting Ligands:

SMARCA4, PB1, and/or SMARCA2 dTAG Targeting Ligands as used herein include, but are not limited to: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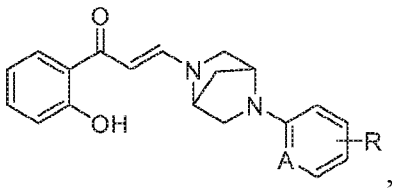
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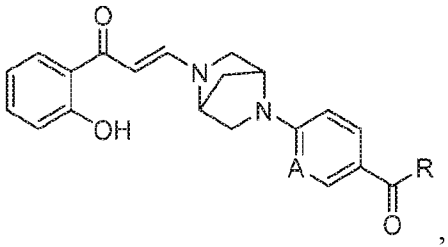
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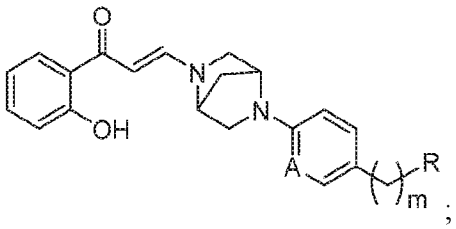

;

wherein:

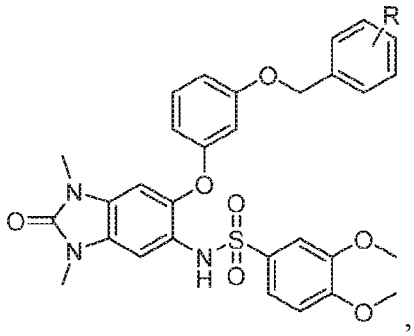
R is the point at which the Linker is attached;

A is N or CH; and

m is 0, 1, 2, 3, 4, 5, 6, 7, or 8.

D. TRIM24 and/or BRPF1 dTAG Targeting Ligands:

TRIM24 and/or BRPF1 dTAG Targeting Ligands as used herein include, but are not limited to: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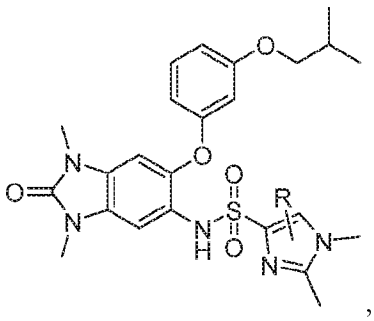
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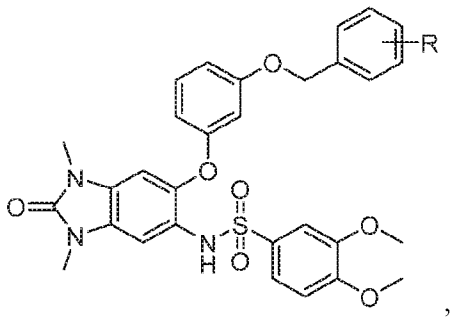
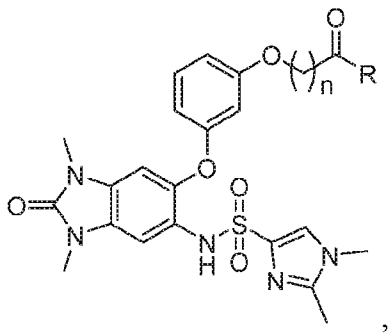
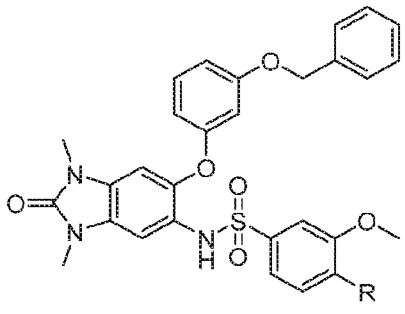
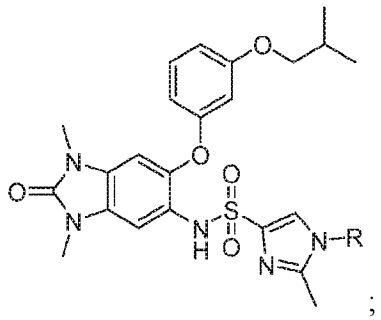





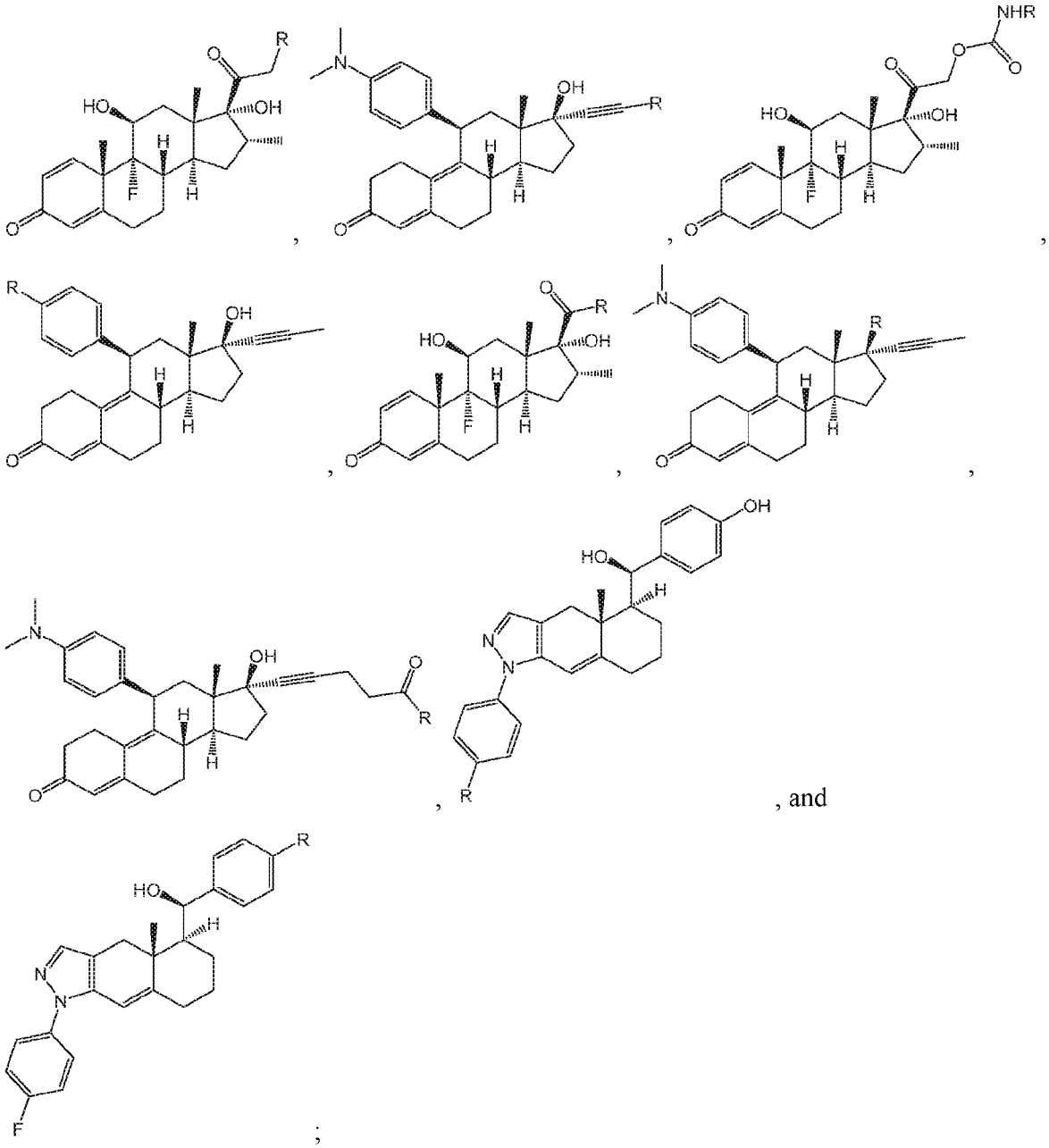
wherein:

R is the point at which the Linker is attached; and

m is 0, 1, 2, 3, 4, 5, 6, 7, or 8.

E. Glucocorticoid Receptor dTAG Targeting Ligand:

Glucocorticoid dTAG Targeting Ligands as used herein include, but are not limited to:

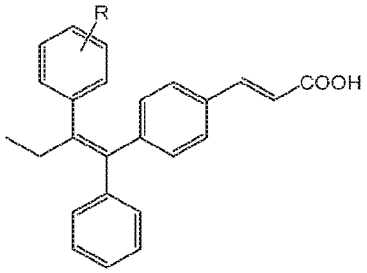


wherein:

R is the point at which the Linker is attached.

F. Estrogen and/or Androgen Receptor dTAG Targeting Ligands:

Estrogen and/or Androgen dTAG Targeting Ligands as used herein include, but are not limited to:



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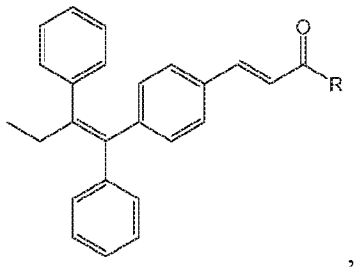

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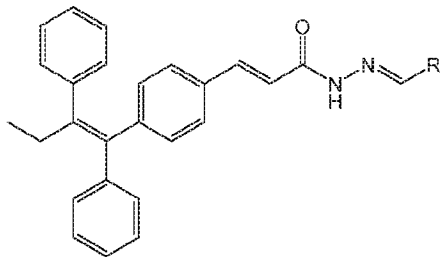

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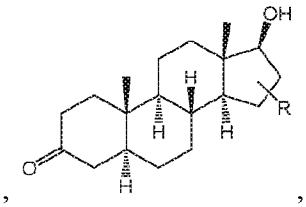

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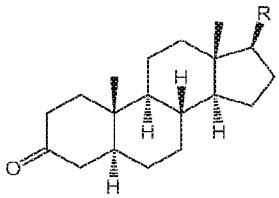

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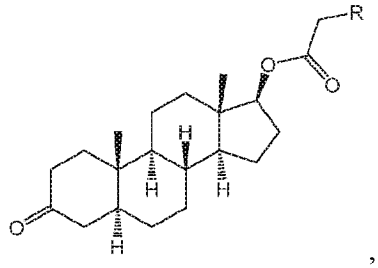

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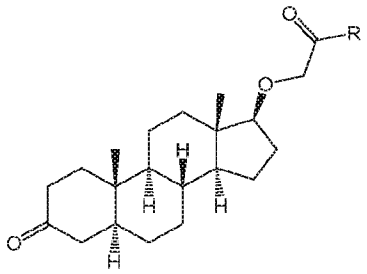

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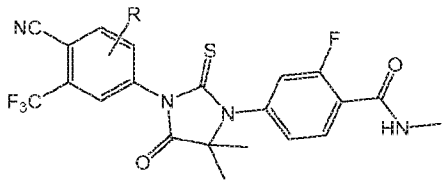
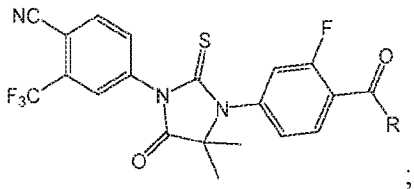


, and


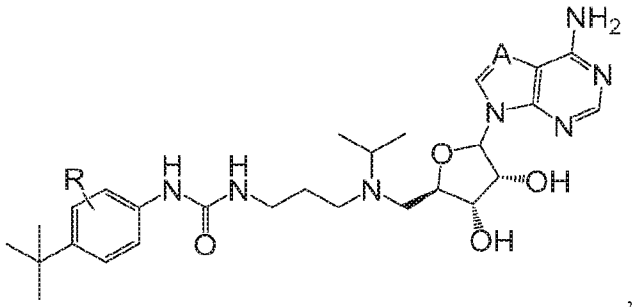
;

wherein:

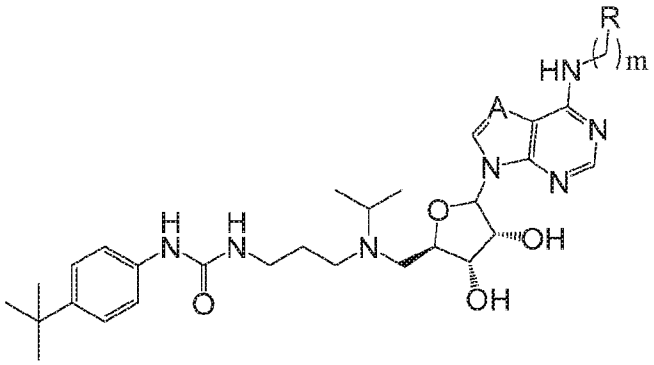
R is the point at which the Linker is attached.

G. DOT1L dTAG Targeting Ligands:

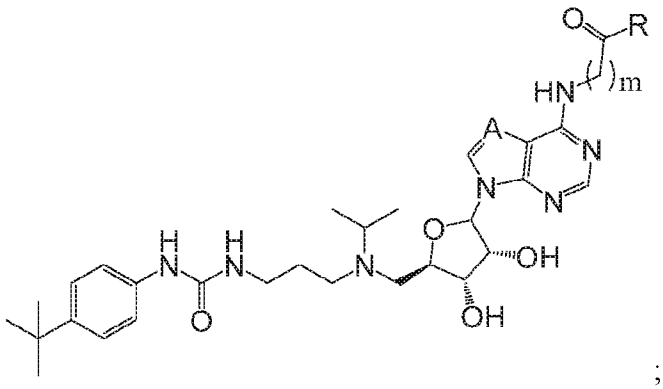
DOT1L dTAG Targeting Ligands as used herein include, but are not limited to:



,



, and



;

wherein:

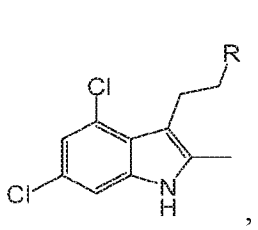
R is the point at which the Linker is attached;

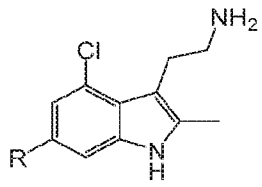
A is N or CH; and

m is 0, 1, 2, 3, 4, 5, 6, 7, or 8.

H. Ras dTAG Targeting Ligands:

Ras dTAG Targeting Ligands as used herein include, but are not limited to:



,


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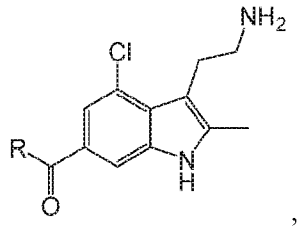
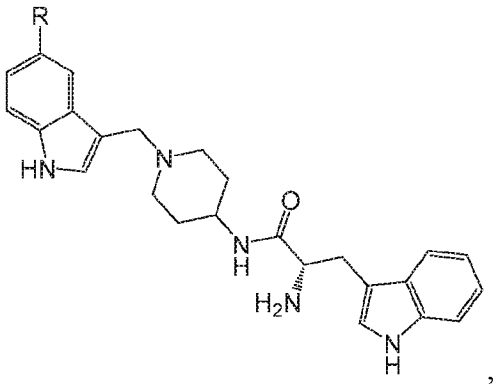


,


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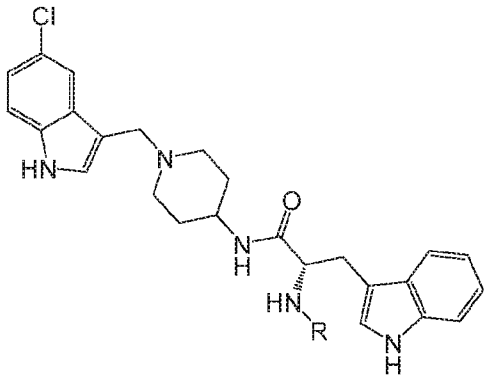

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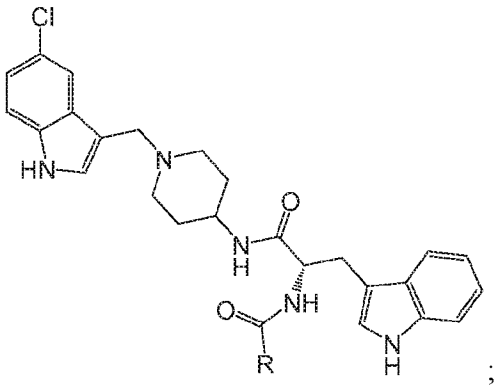


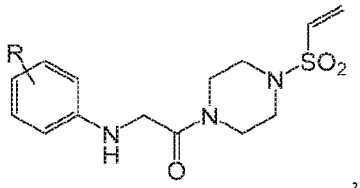
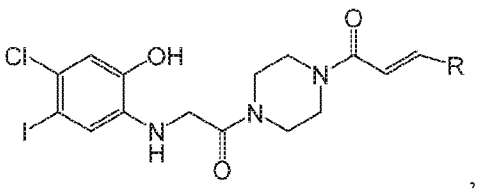
;

wherein:

R is the point at which the Linker is attached.

I. RasG12C dTAG Targeting Ligands:

RasG12C dTAG Targeting Ligands as used herein include, but are not limited to:


,

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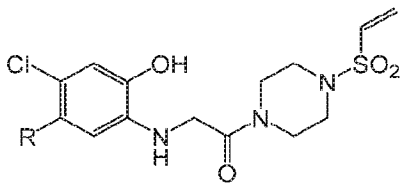
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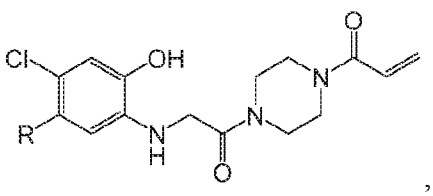
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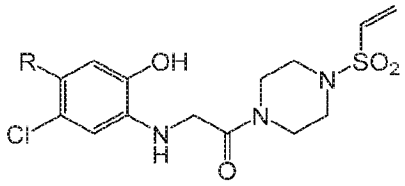
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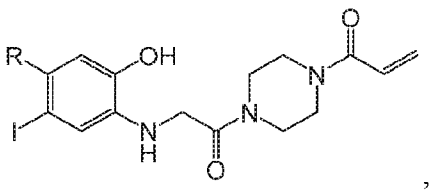
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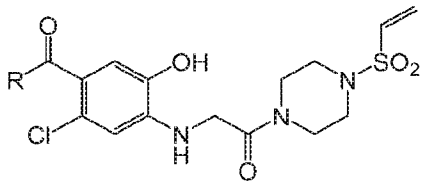
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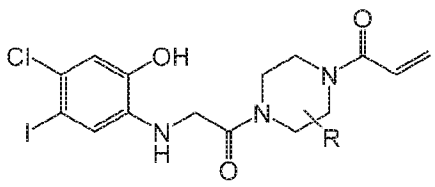
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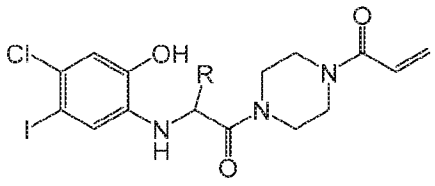
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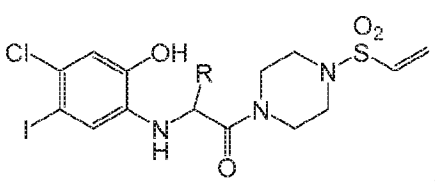
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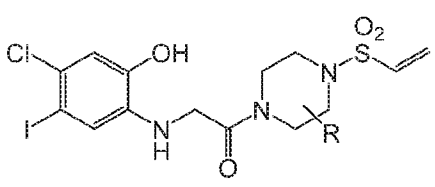
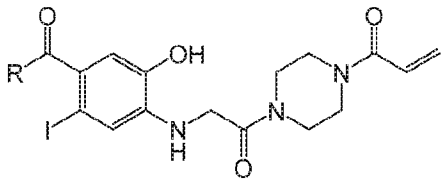
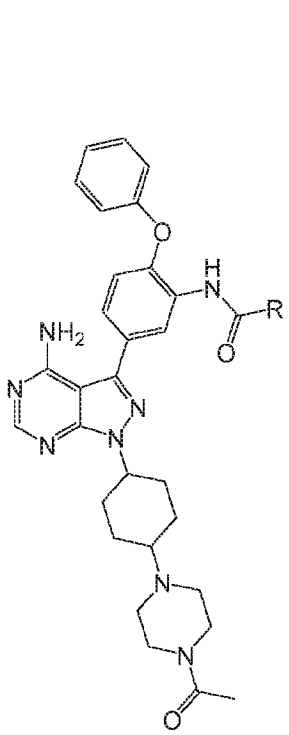
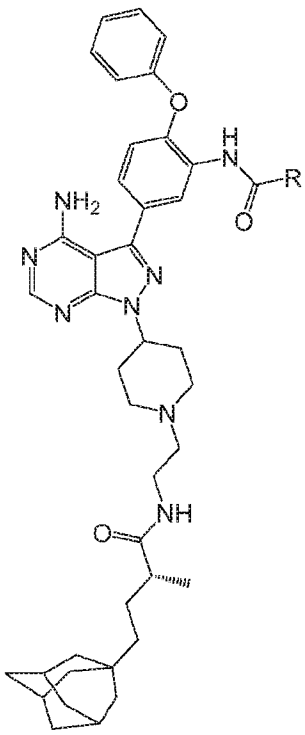
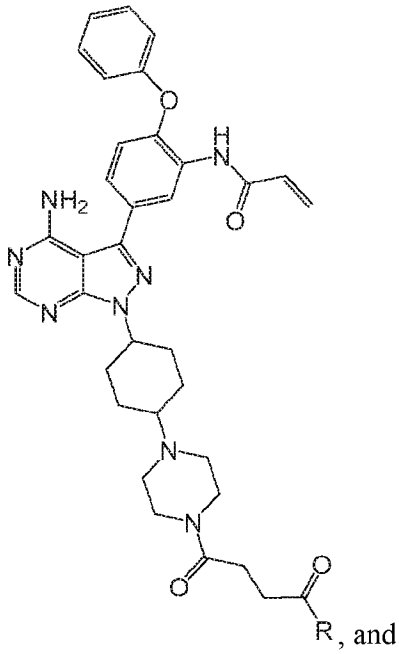

and

;

wherein:

R is the point at which the Linker is attached.

J. Her3 dTAG Targeting Ligands:

Her3 dTAG Targeting Ligands as used herein include, but are not limited to: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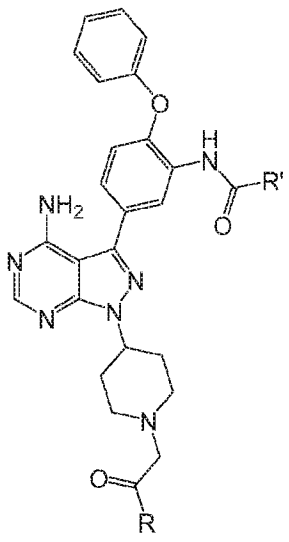

;

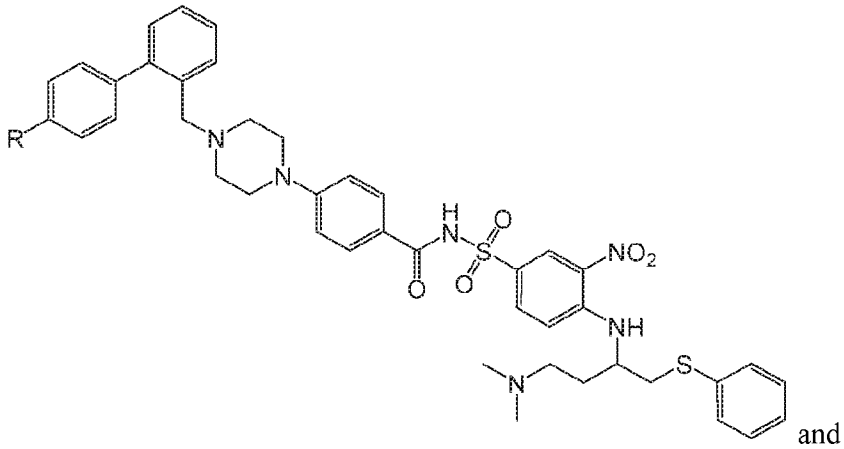
wherein:

R is the point at which the Linker is attached; and

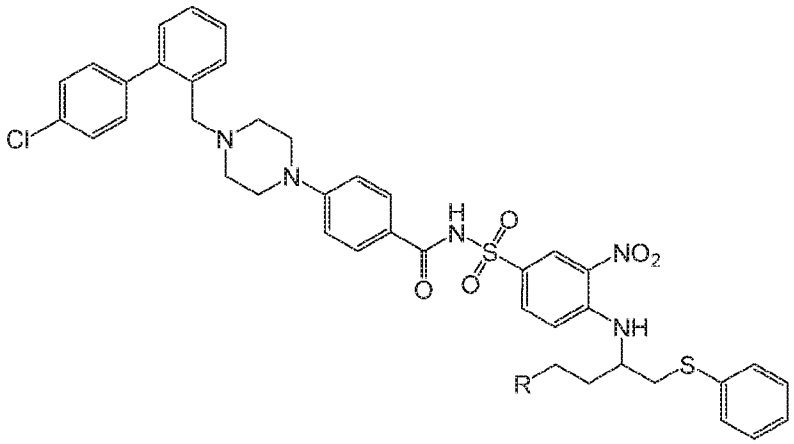
R' is  or  .

K. Bcl-2 or Bcl-XL dTAG Targeting Ligands:

Bcl-2 or Bcl-XL dTAG Targeting Ligands as used herein include, but are not limited to:



and

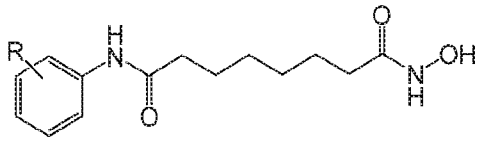
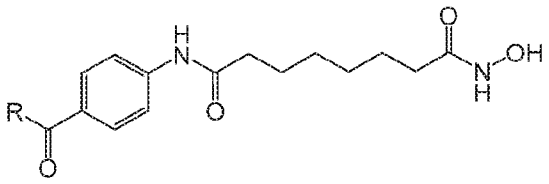


wherein:

R is the point at which the Linker is attached.

L. HDAC dTAG Targeting Ligands:

HDAC dTAG Targeting Ligands as used herein include, but are not limited to:

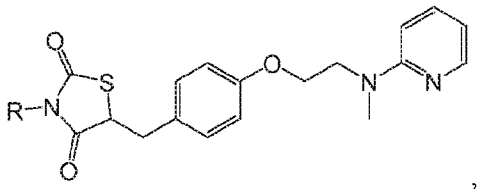

and

;

wherein:

R is the point at which the Linker is attached.

M. PPAR-gamma dTAG Targeting Ligands:

PPAR-gamma dTAG Targeting Ligands as used herein include, but are not limited to: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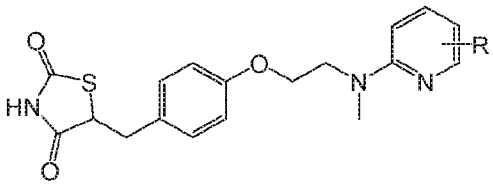
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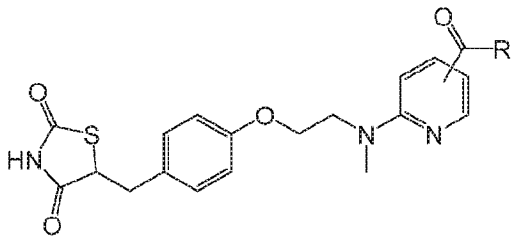
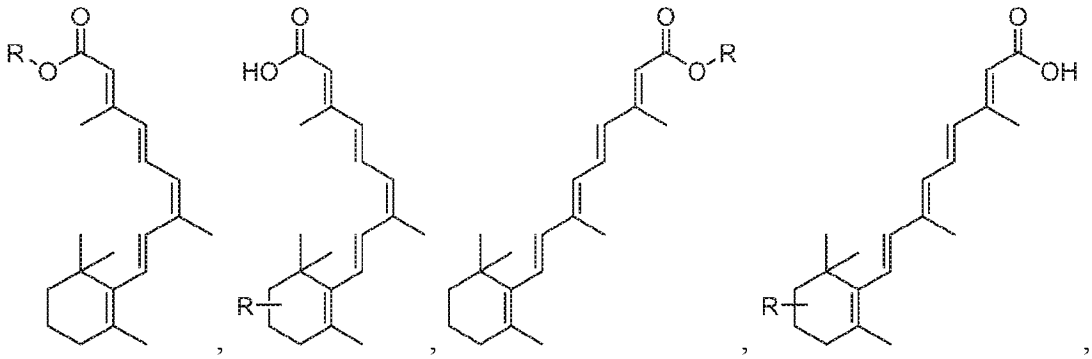
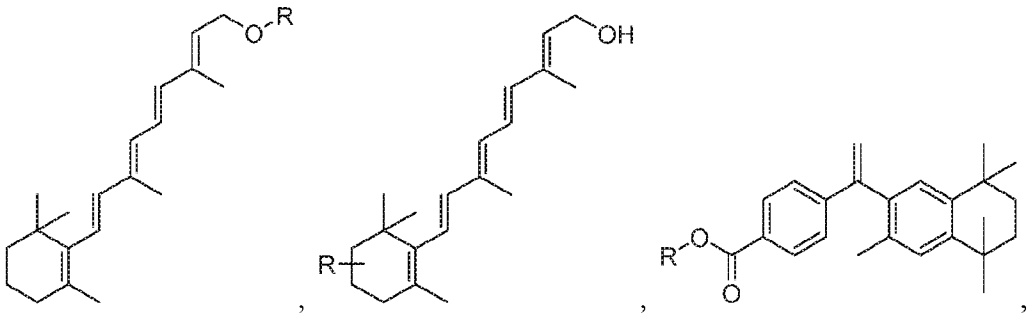
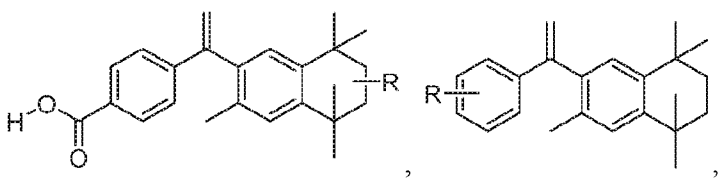

;

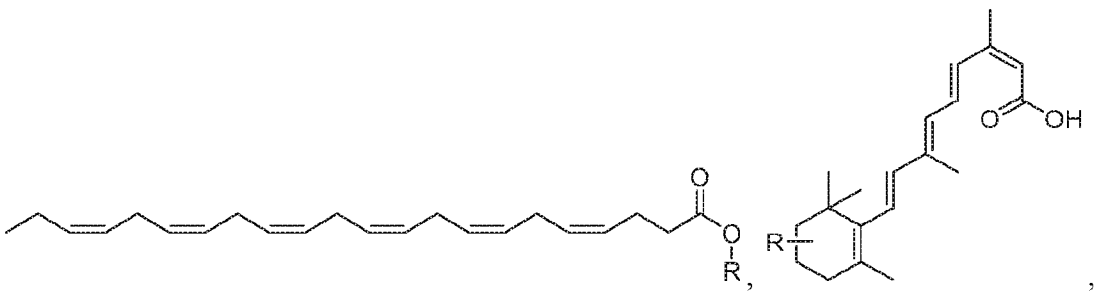
wherein:

R is the point at which the Linker is attached.

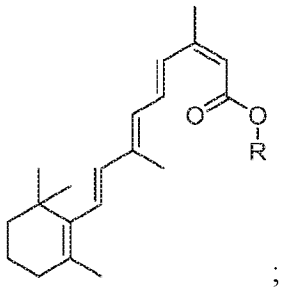
N. RXR dTAG Targeting Ligands:

RXR dTAG Targeting Ligands as used herein include, but are not limited to:



, and



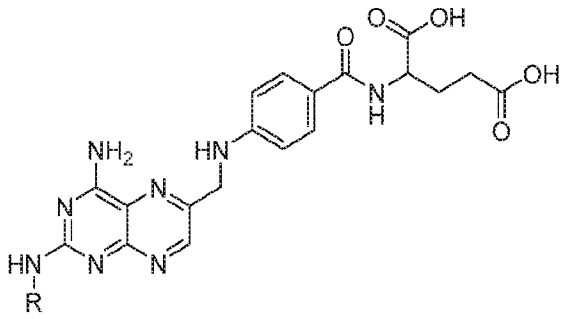
;

wherein:

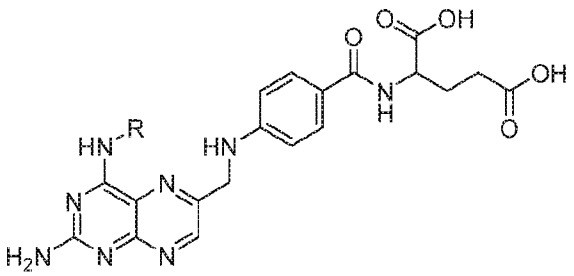
R is the point at which the Linker is attached.

O. DHFR dTAG Targeting Ligands:

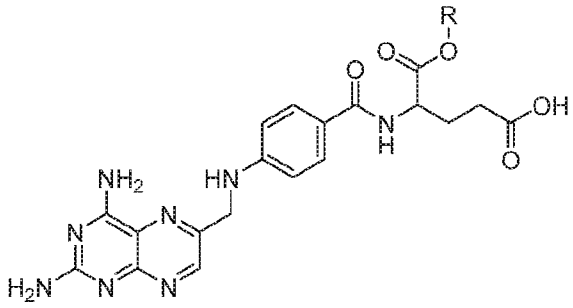
DHFR dTAG Targeting Ligands as used herein include, but are not limited to:



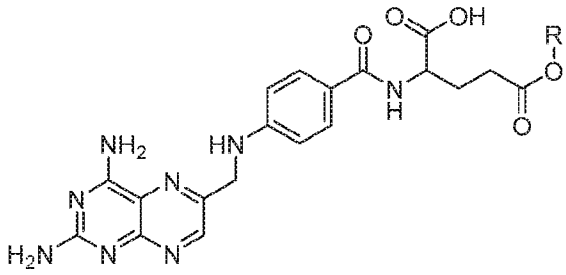
,



,



,



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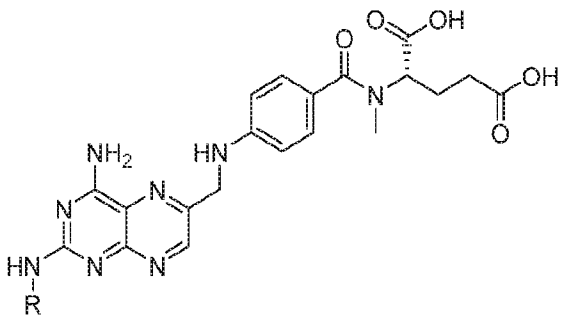
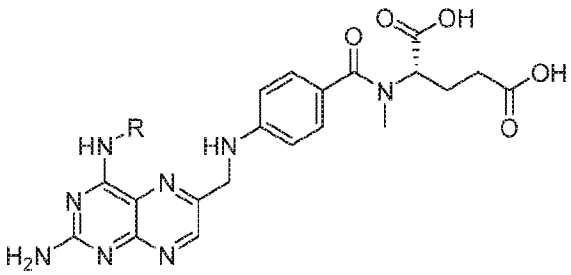
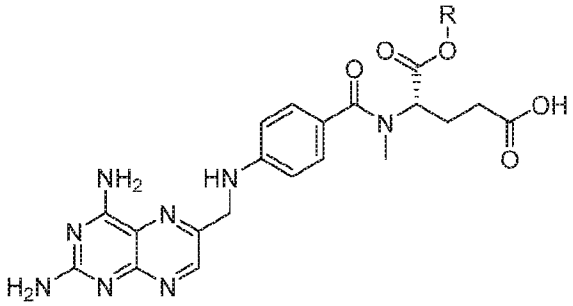
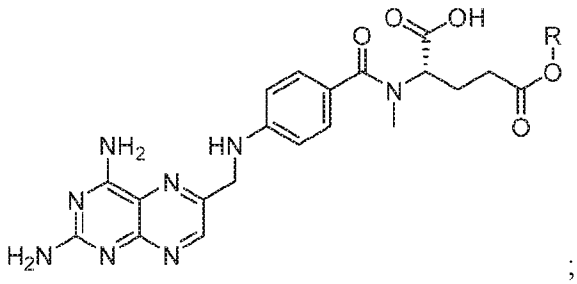



, and

;

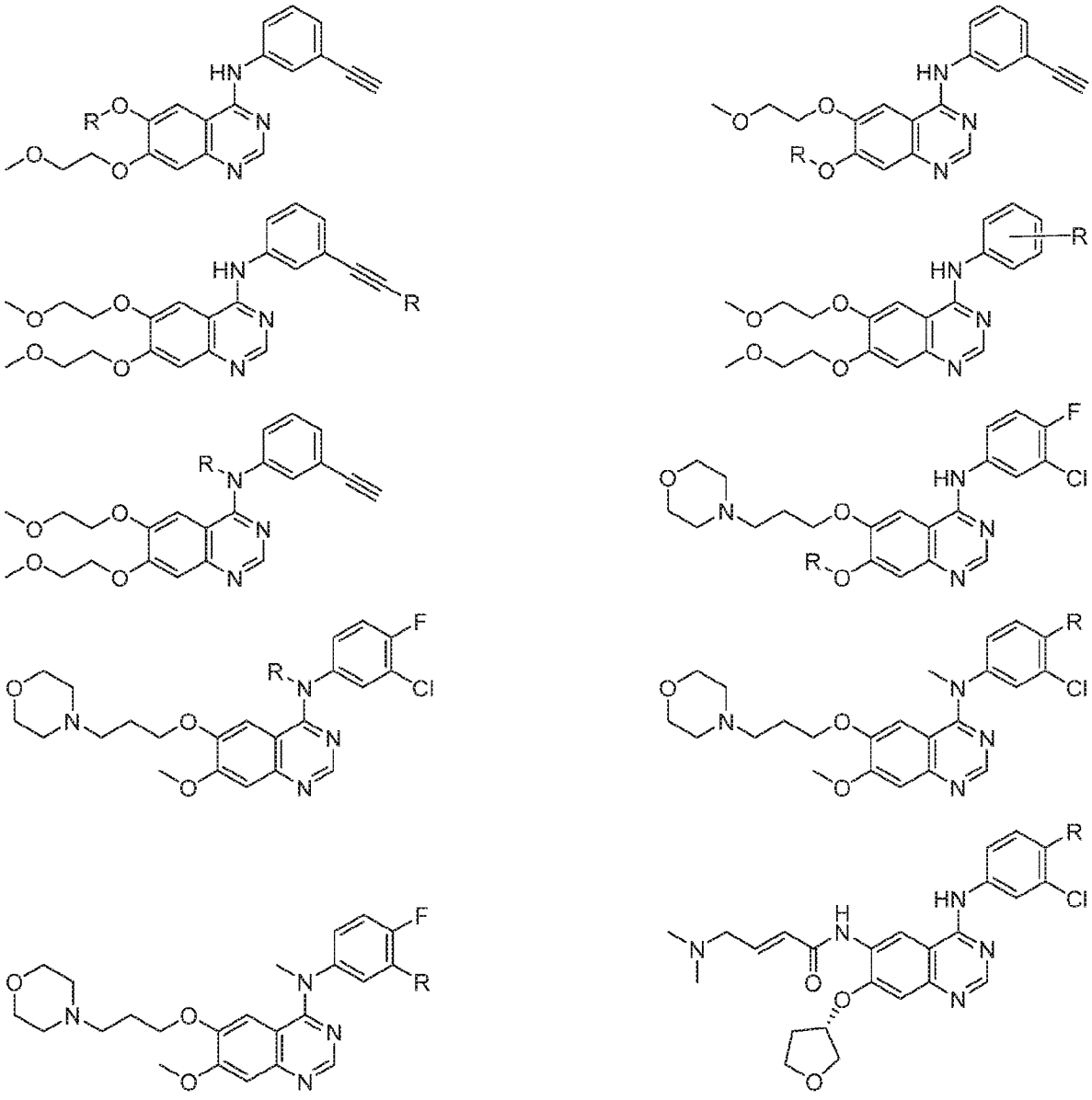
wherein:

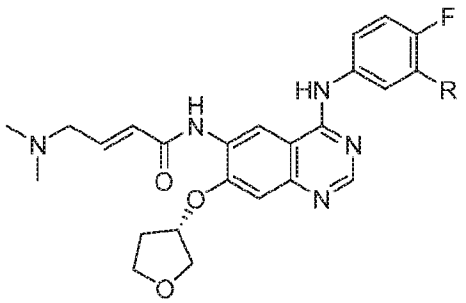
R is the point at which the Linker is attached.

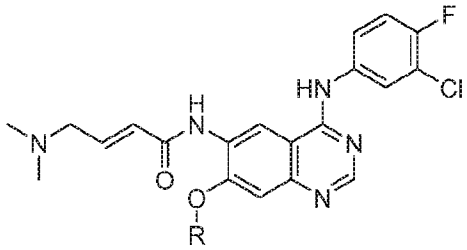
P. EGFR dTAG Targeting Ligands:

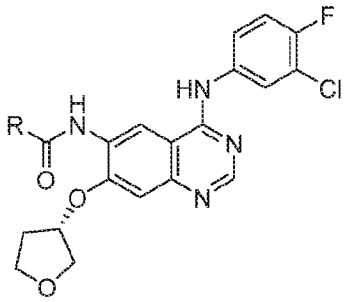
EGFR dTAG Targeting Ligands as used herein include, but are not limited to:

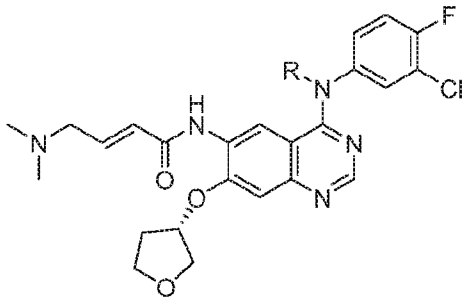
1. Targeting Ligands that target L858R mutant EGFR, including erlotinib, gefitnib, afatinib, neratinib, and dacomitinib.

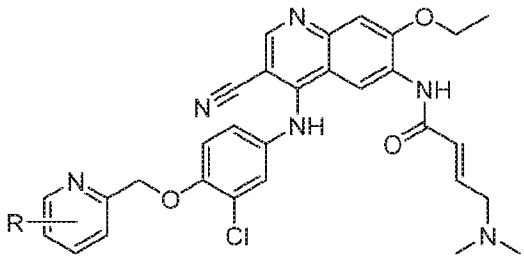


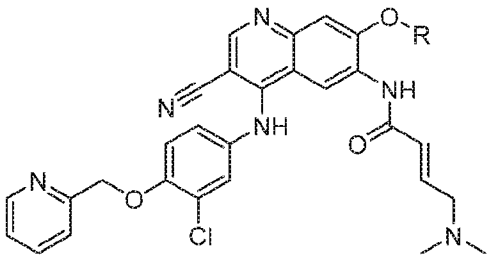


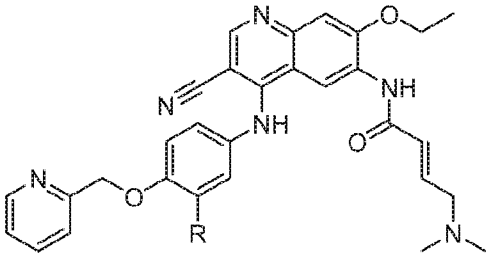


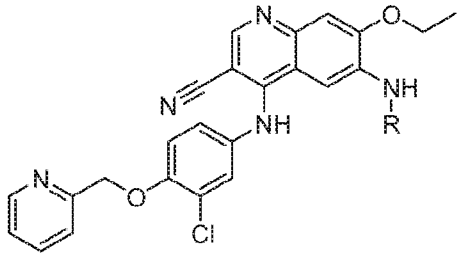


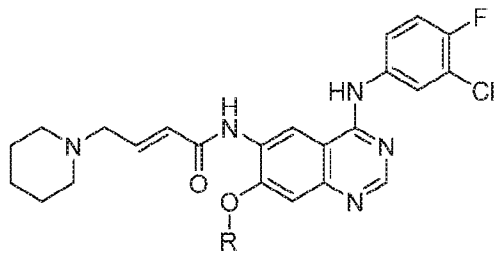
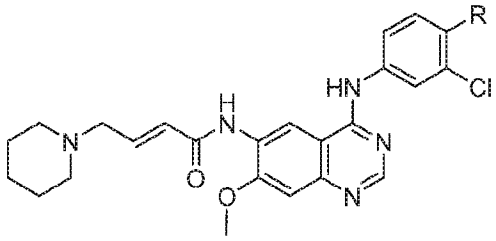
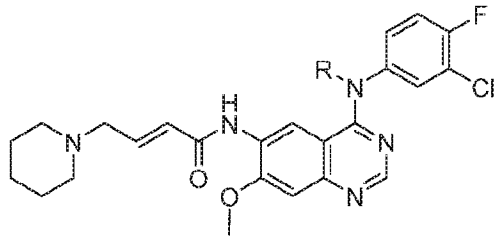
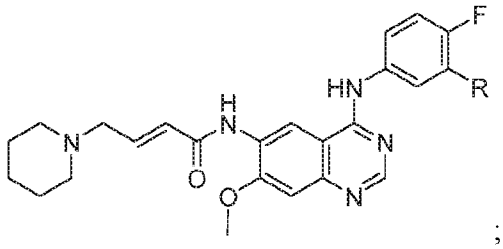








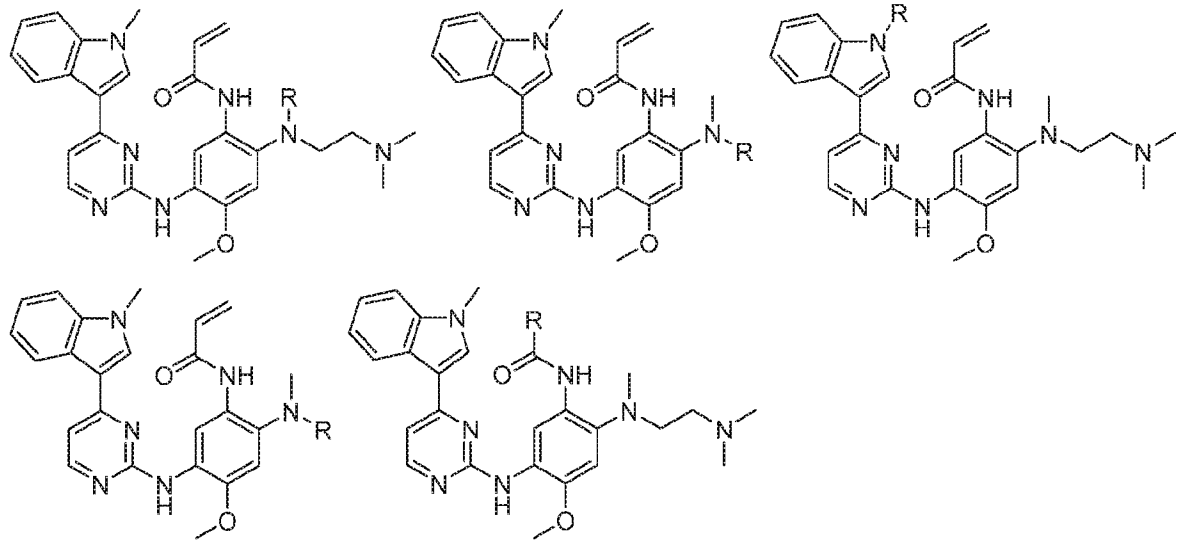


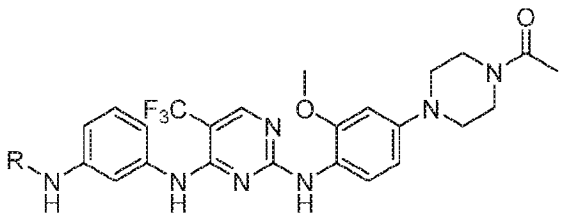




;

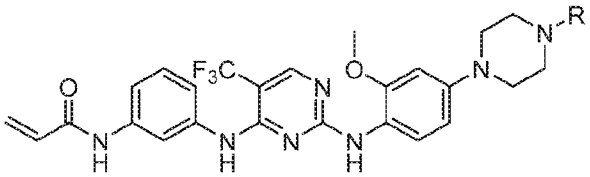
wherein:

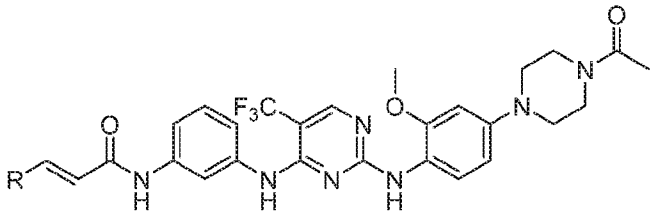
R is the point at which the Linker is attached.

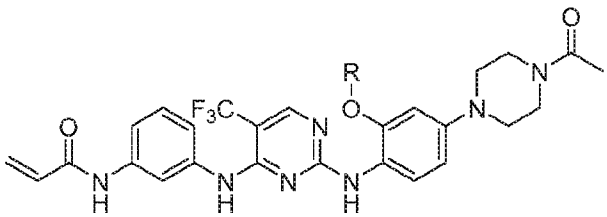
2. Targeting Ligands that target T790M mutant EGFR, including osimertinib, rociletinib, olmutinib, naquotinib, nazartinib, PF-06747775, Icotinib, Neratinib, Avitinib, Tarloxotinib, PF-0645998, Tesevatinib, Transtinib, WZ-3146, WZ8040, and CNX-2006:

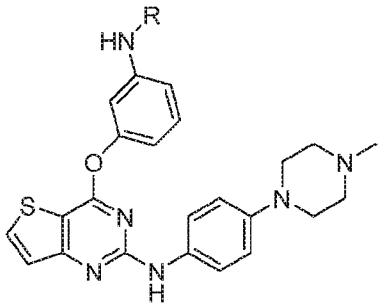


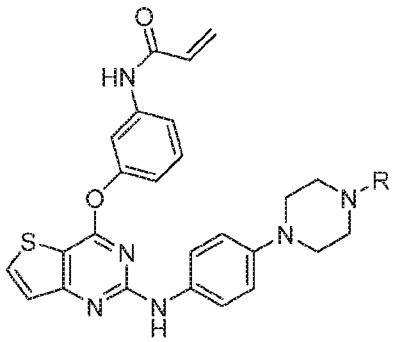


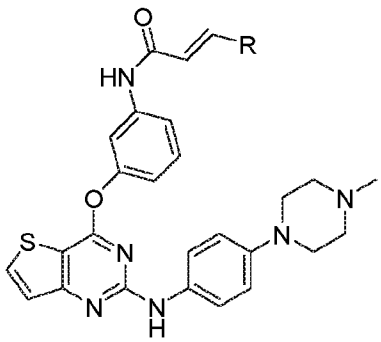


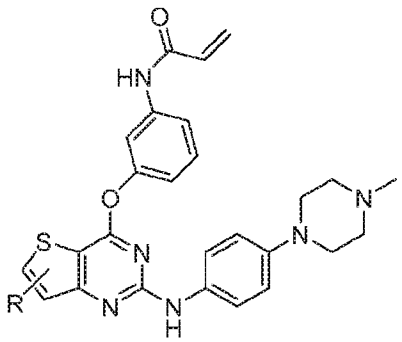












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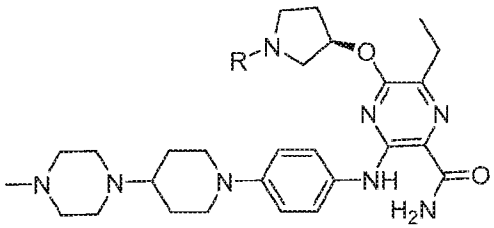

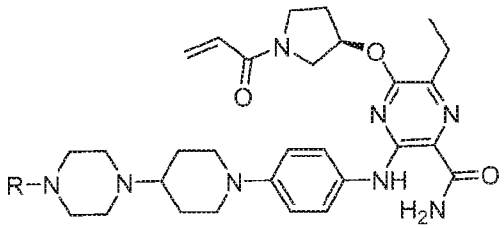

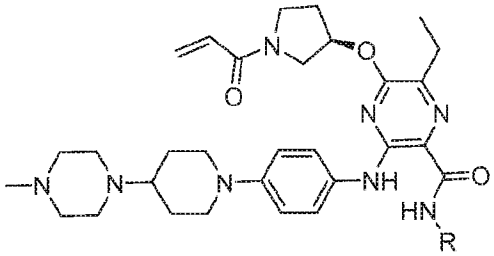

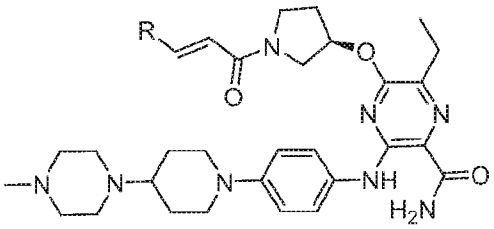

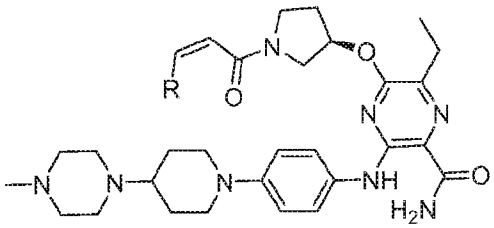

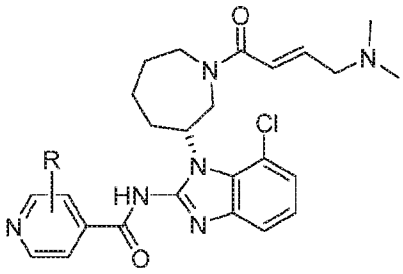

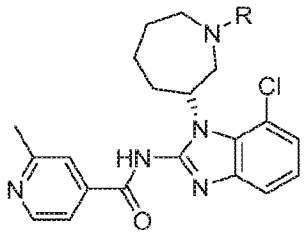

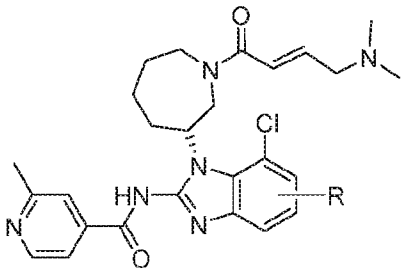

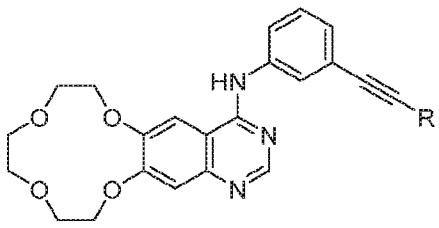

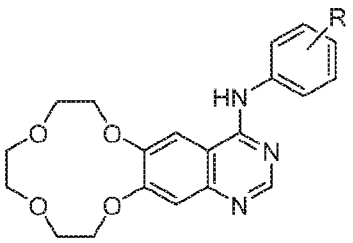

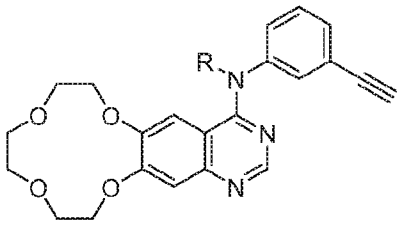

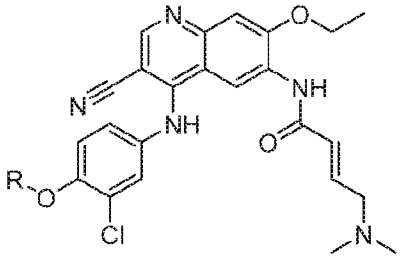

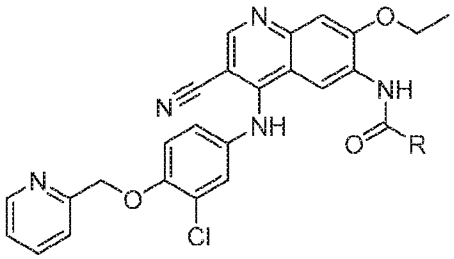

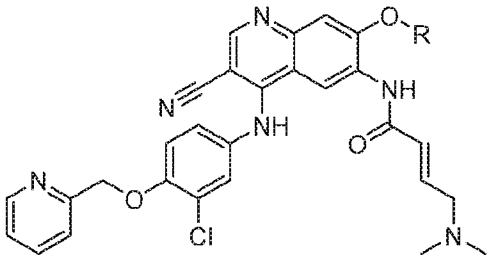

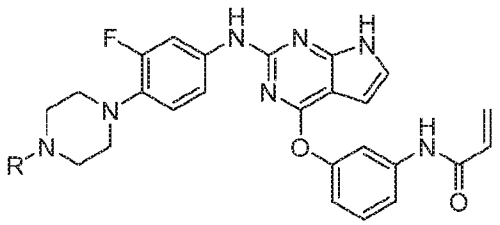
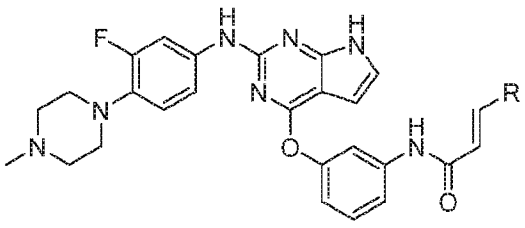
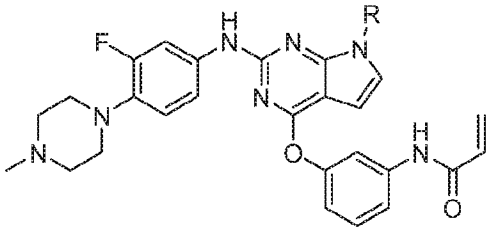
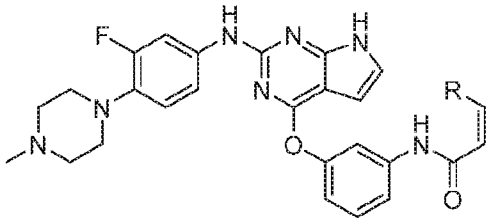
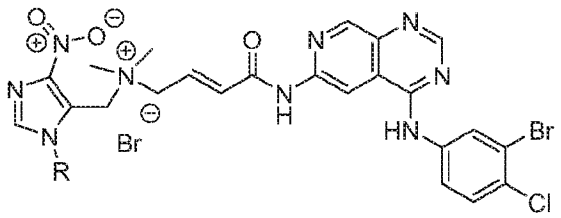
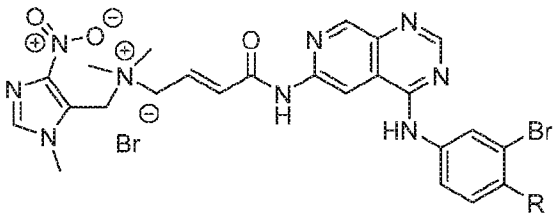
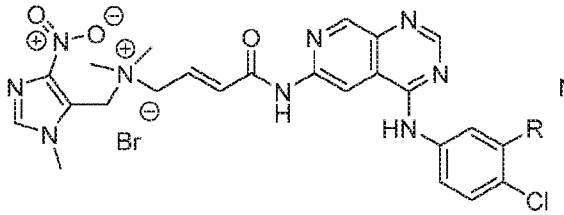
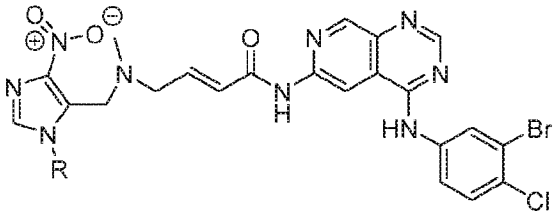
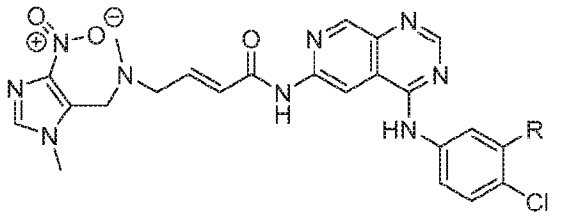
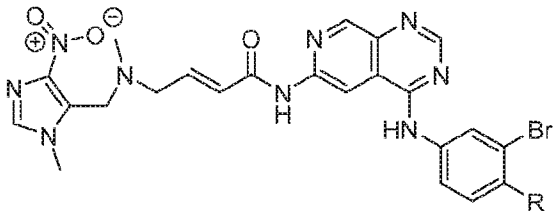
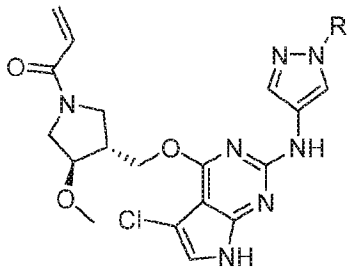
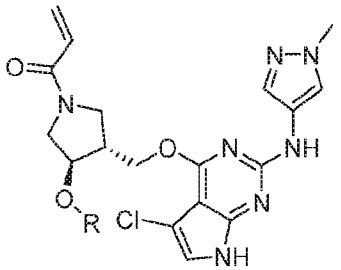
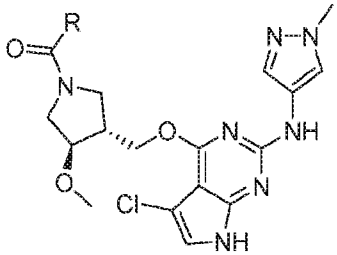

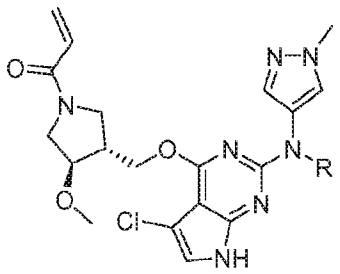

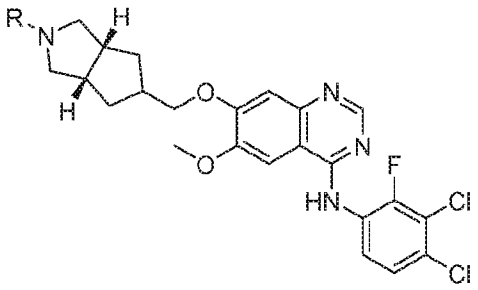

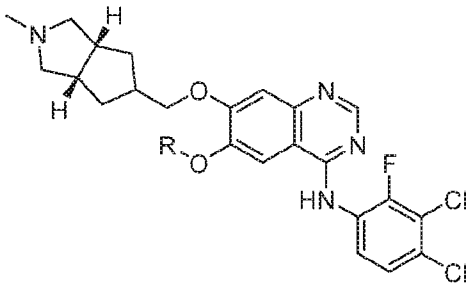

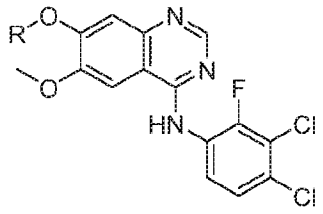

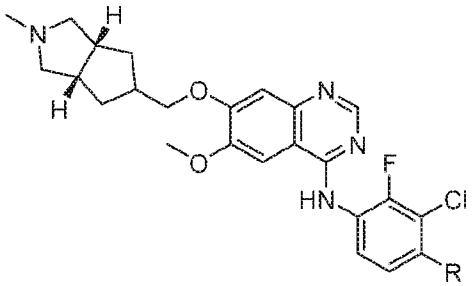

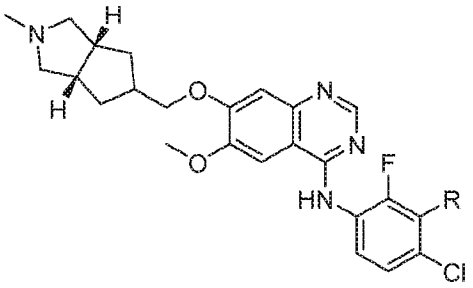

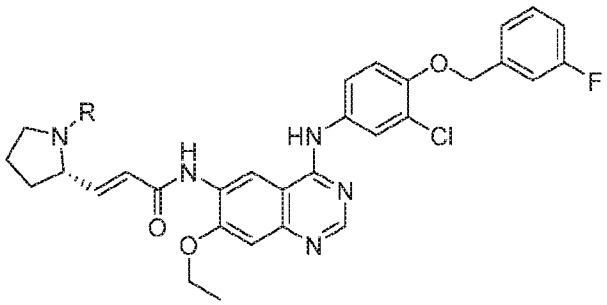

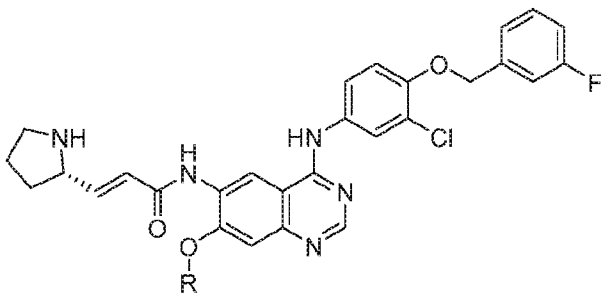

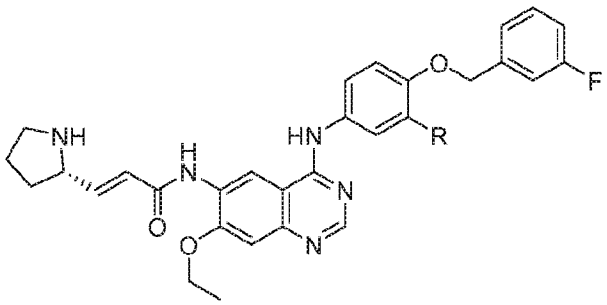

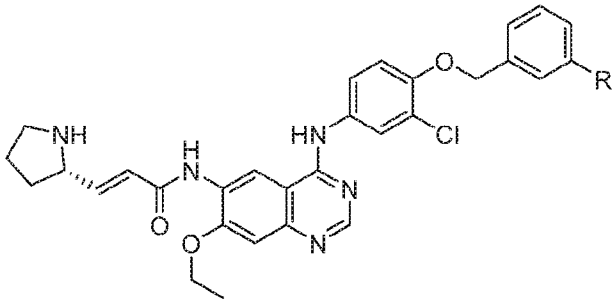

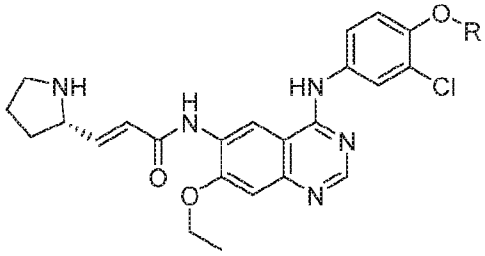

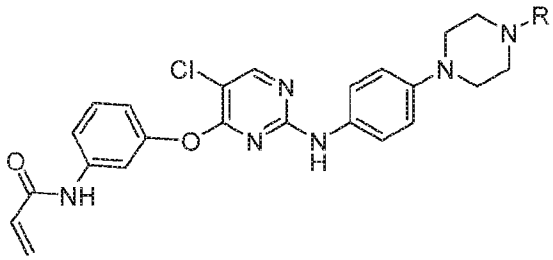

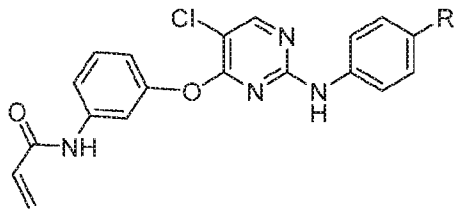

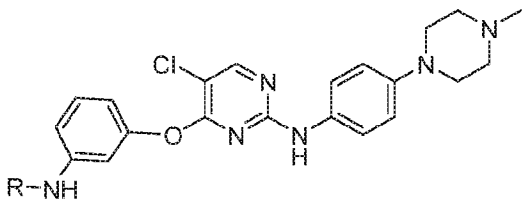

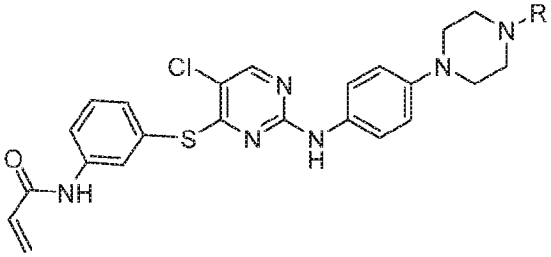

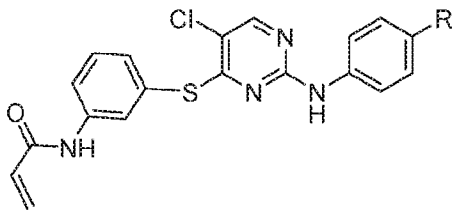

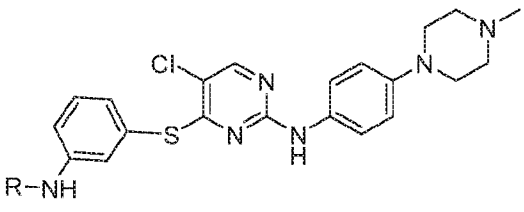

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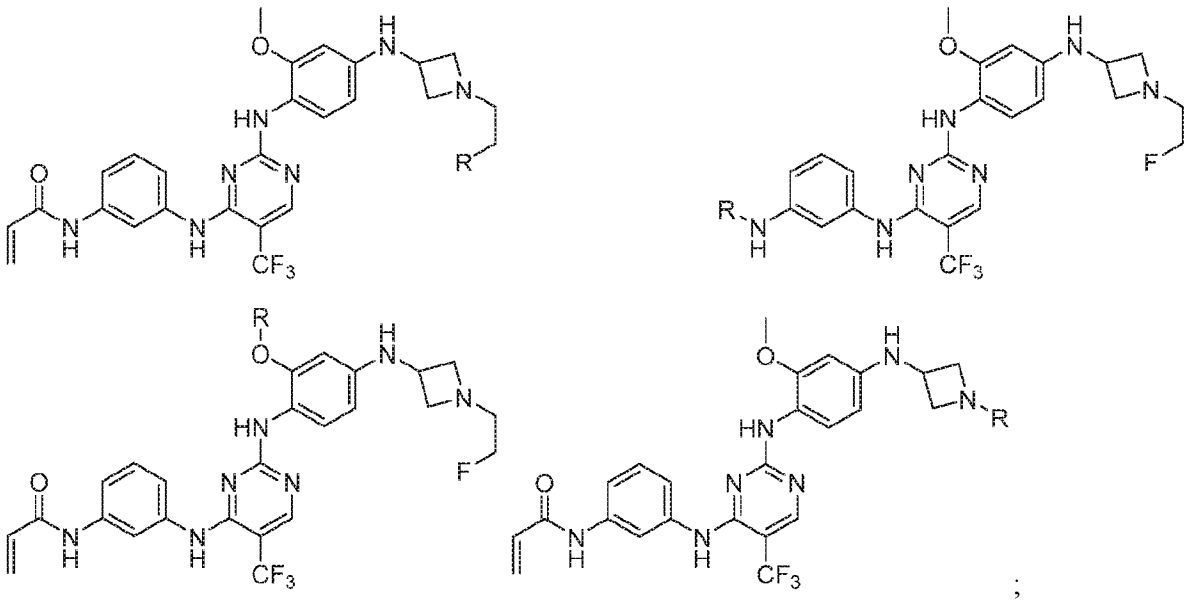
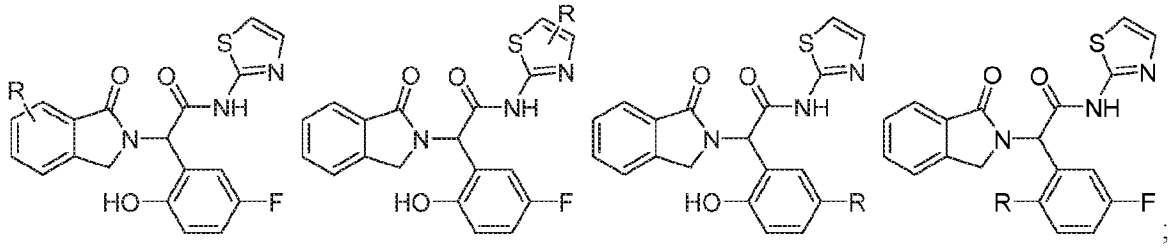


wherein:

R is the point at which the Linker is attached.

3. Targeting Ligands that target C797S mutant EGFR, including EAI045:



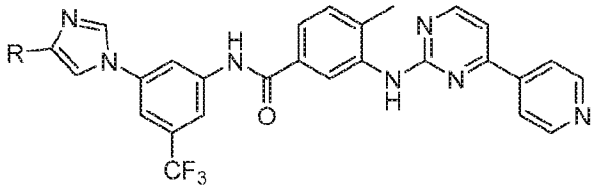
wherein:

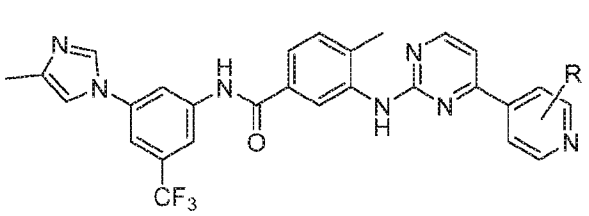
R is the point at which the Linker is attached.

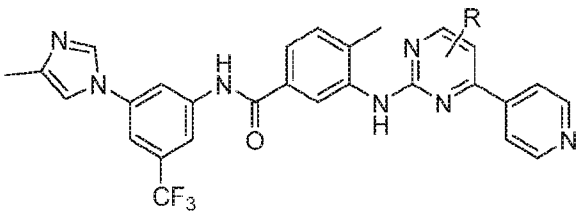
Q. BCR-ABL dTAG Targeting Ligands:

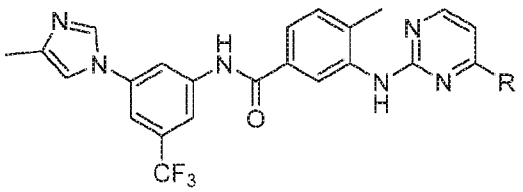
BCR-ABL dTAG Targeting Ligands as used herein include, but are not limited to:

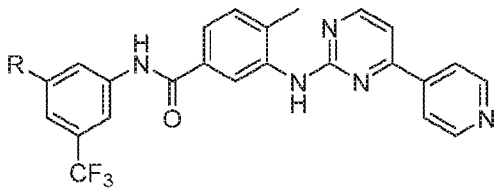
1. Targeting Ligands that target T315I mutant BCR-ABL (PDB #3CS9), including Nilotinib and Dasatinib:

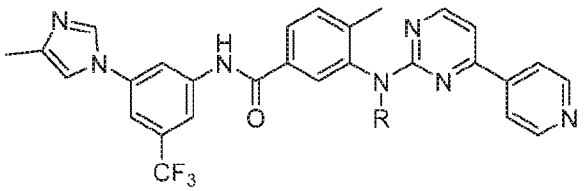


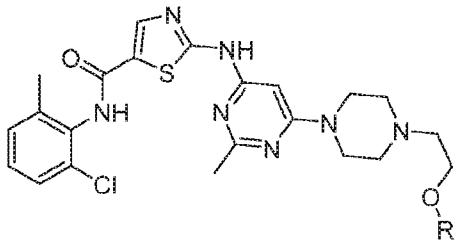


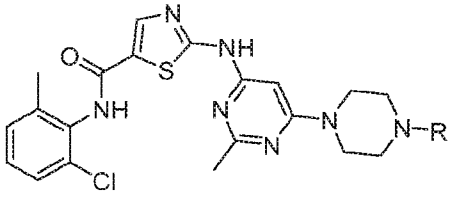


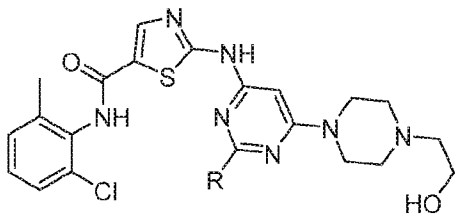


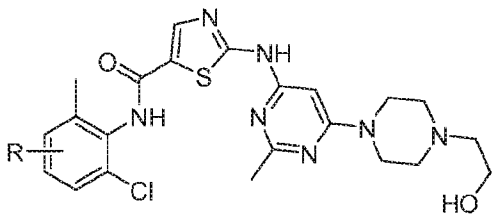








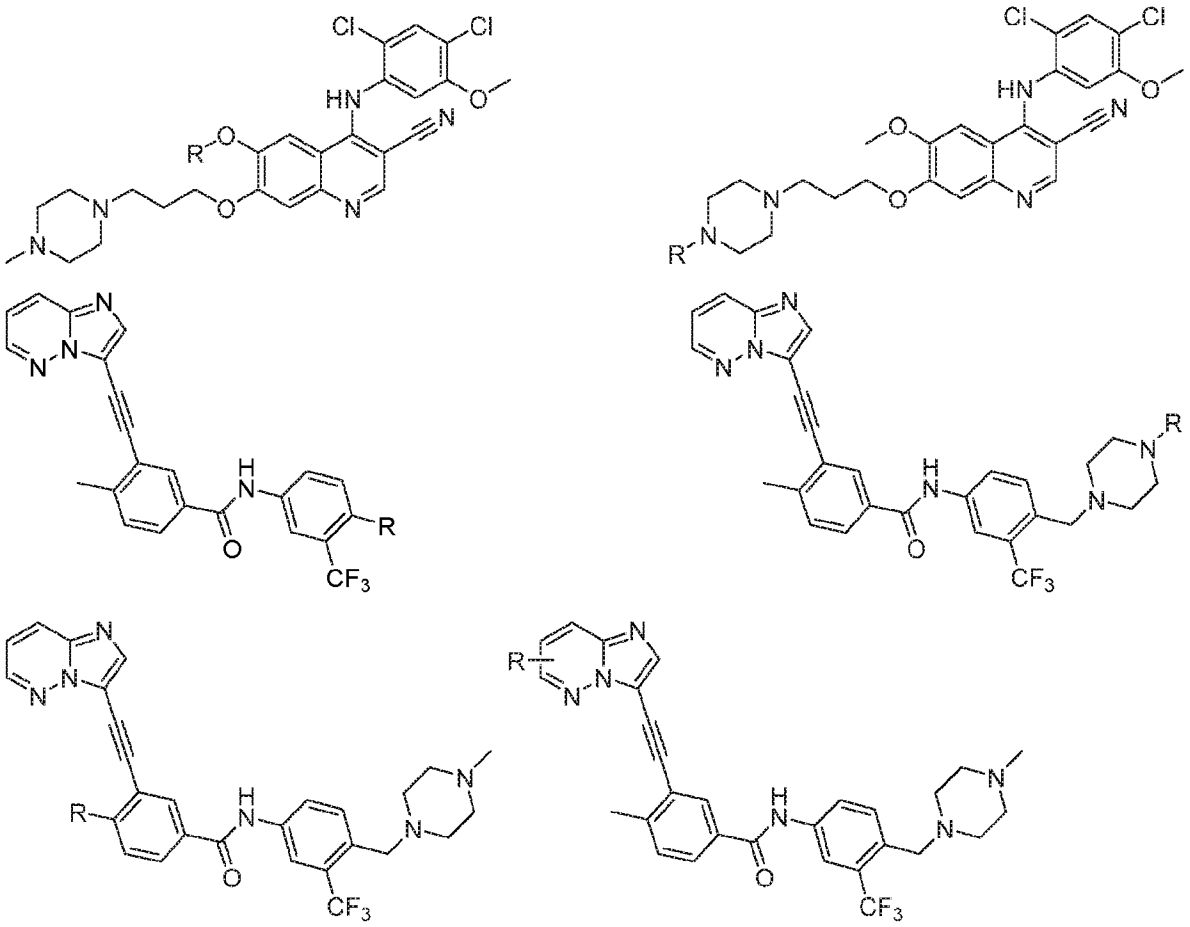




wherein:

R is the point at which the Linker is attached.

2. Targeting Ligands that target BCR-ABL, including Nilotinib, Dasatinib, Ponatinib, and Bosutinib:



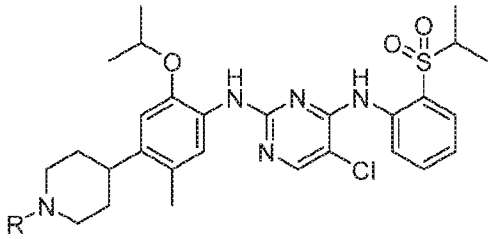
wherein:

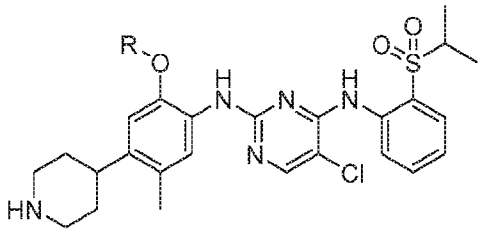
R is the point at which the Linker is attached.

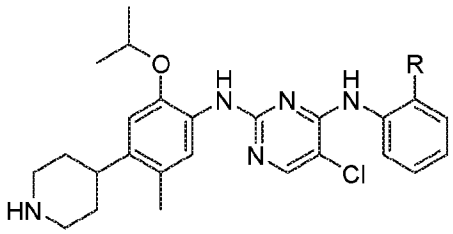
R. ALK dTAG Targeting Ligands:

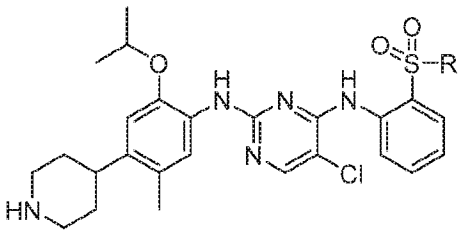
ALK dTAG Targeting Ligands as used herein include, but are not limited to:

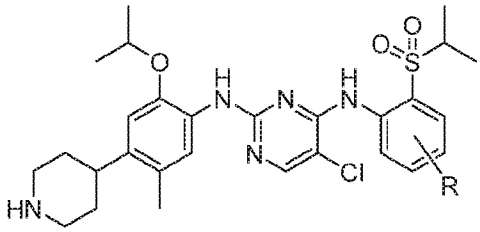
1. Targeting Ligands that target L1196M mutant ALK (PDB #4MKC), including Ceritinib:











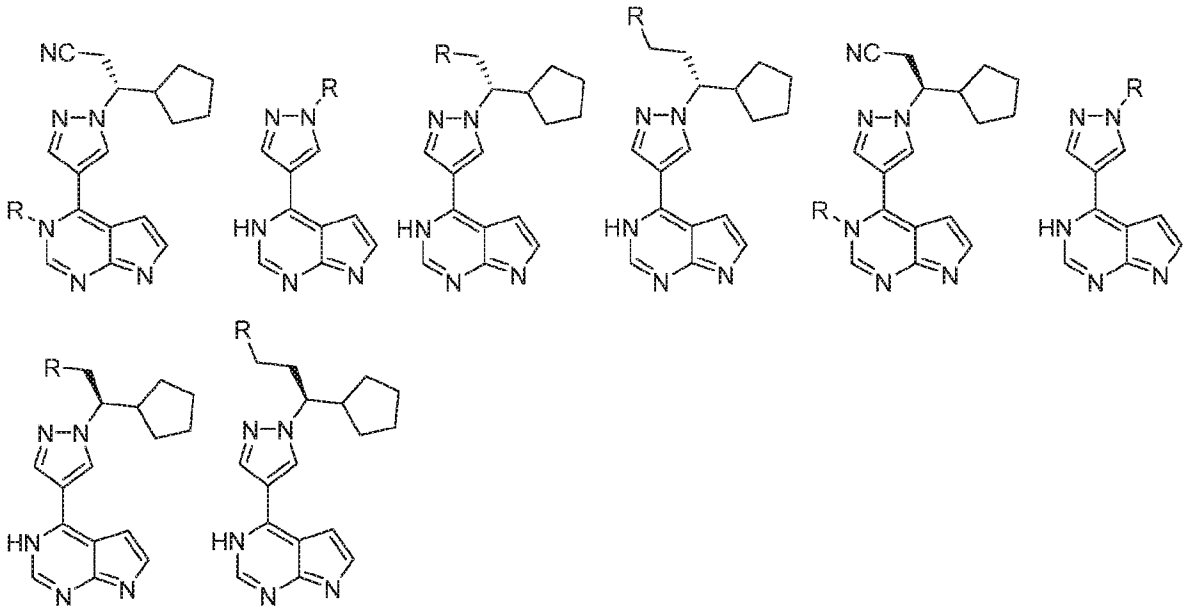
wherein:

R is the point at which the Linker is attached.

S. JAK2 dTAG Targeting Ligands:

JAK2 dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target V617F mutant JAK2, including Ruxolitinib:



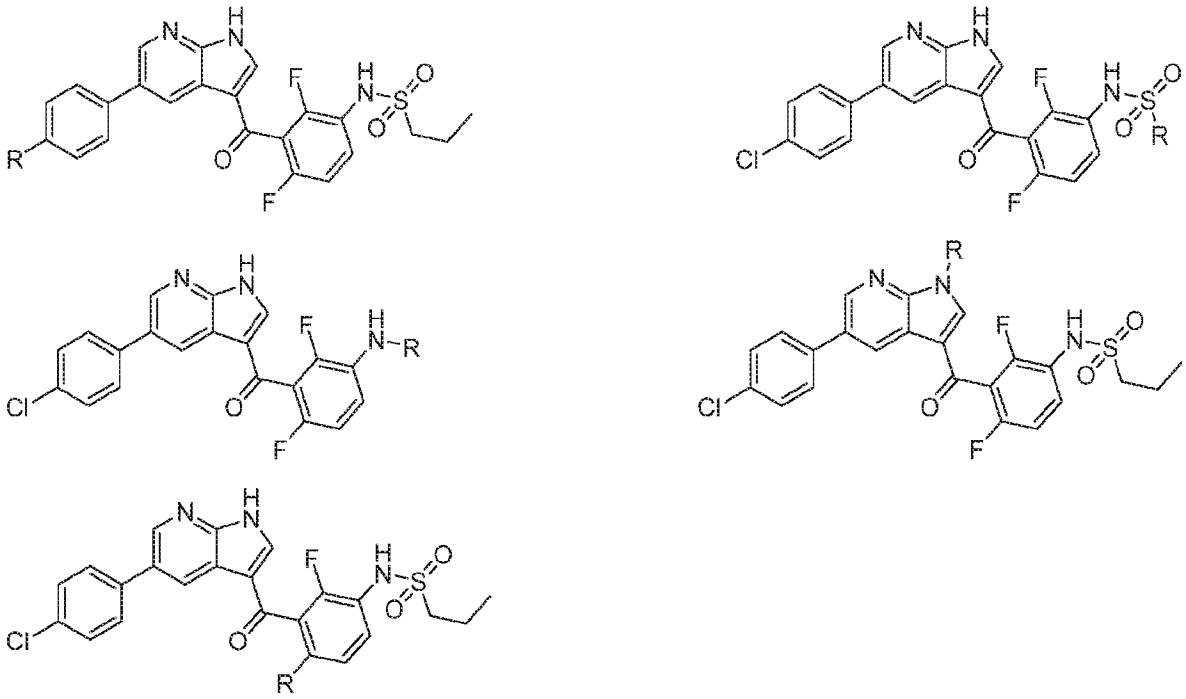
wherein:

R is the point at which the Linker is attached.

T. BRAF dTAG Targeting Ligands:

BRAF dTAG Targeting Ligands as used herein include, but are not limited to:

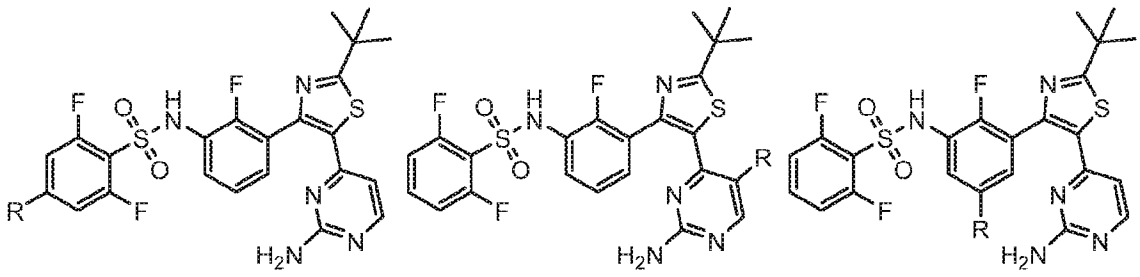
1. Targeting Ligands that target V600E mutant BRAF (PBD # 3OG7), including Vemurafenib:



wherein:

R is the point at which the Linker is attached.

2. Targeting Ligands that target BRAF, including Dabrafenib:



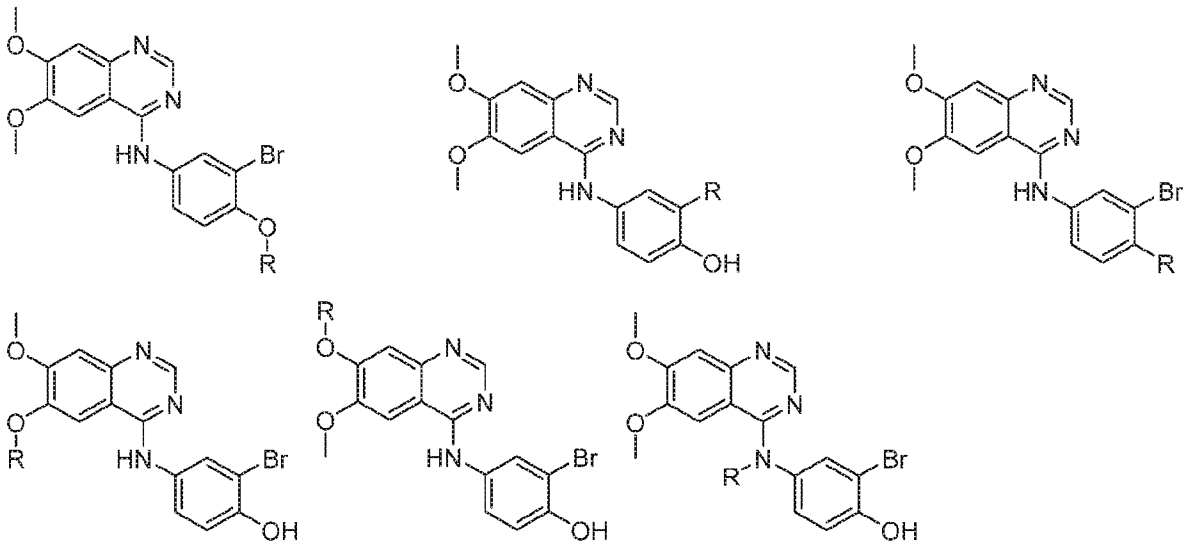
wherein:

R is the point at which the Linker is attached.

U. LRRK2 dTAG Targeting Ligands:

LRRK2 dTAG Targeting Ligands as used herein include, but are not limited to:

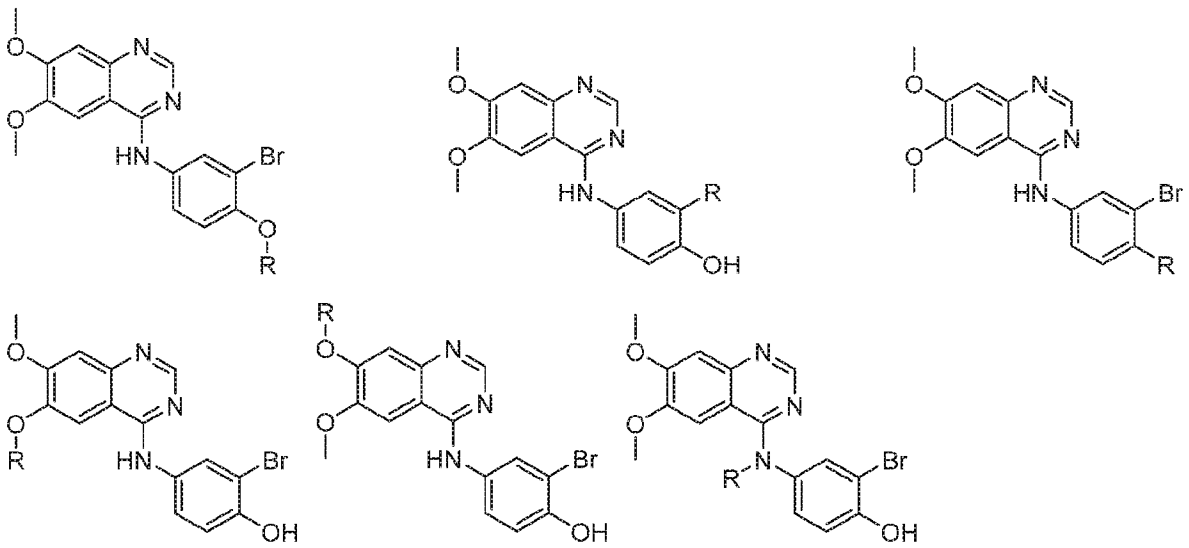
1. Targeting Ligands that target R1441C mutant LRRK2, including:



wherein:

R is the point at which the Linker is attached.

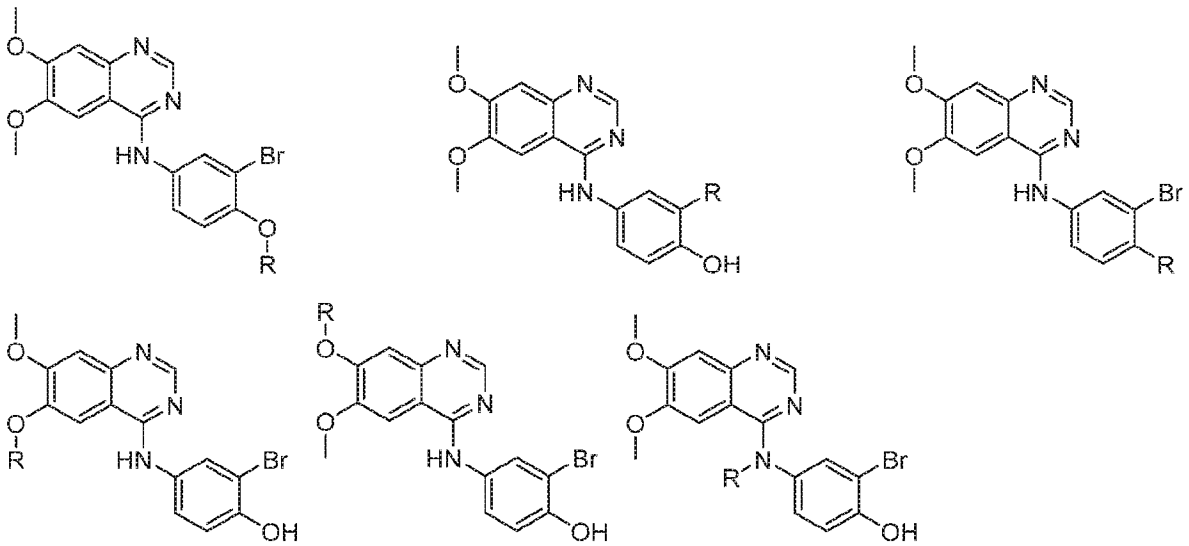
2. Targeting Ligands that target G2019S mutant LRRK2, including:



wherein:

R is the point at which the Linker is attached.

3. Targeting Ligands that target I2020T mutant LRRK2, including:



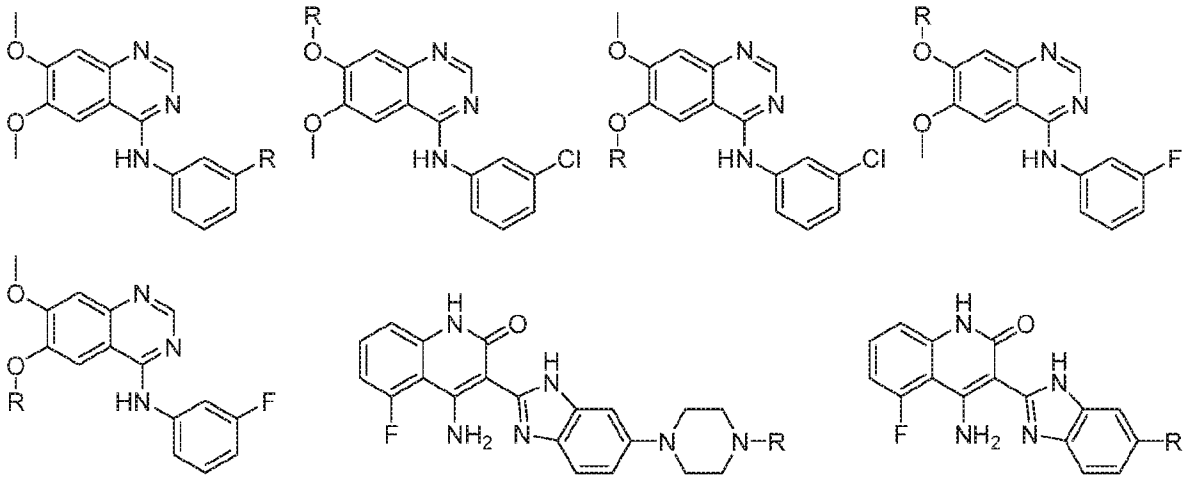
wherein:

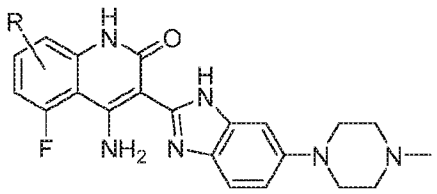
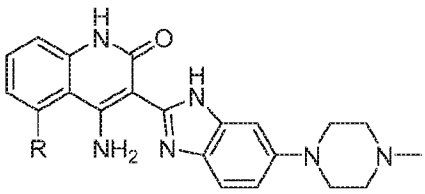
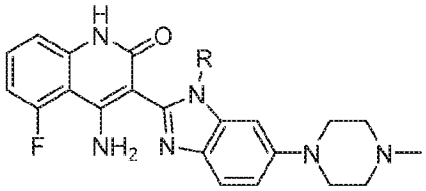
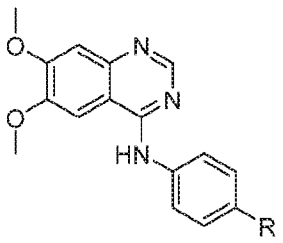
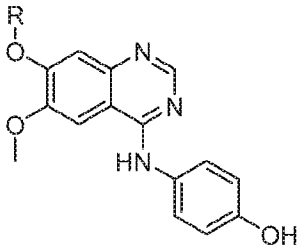
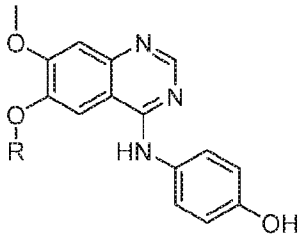
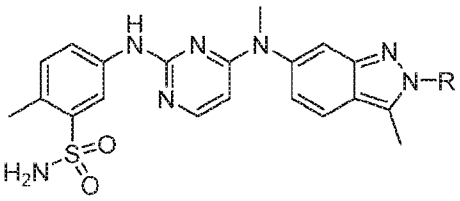
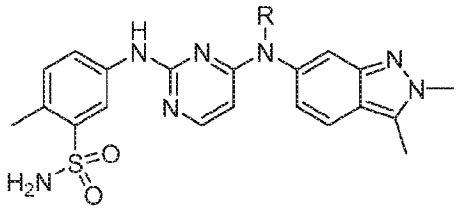
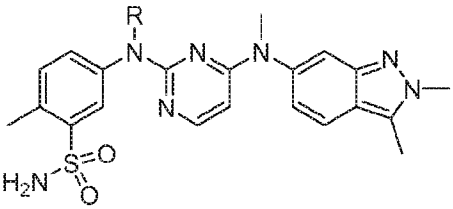
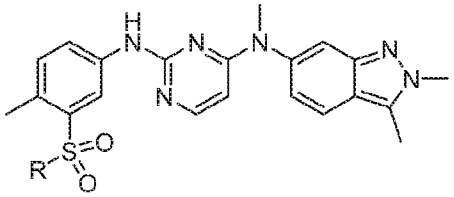
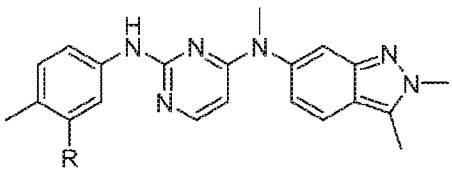
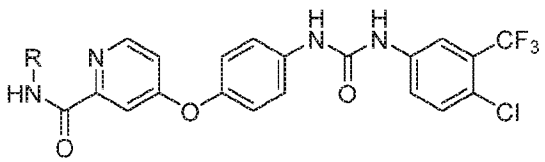
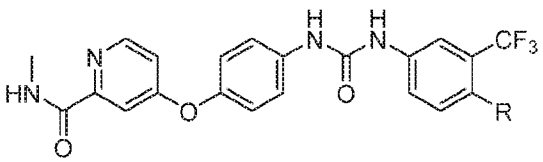
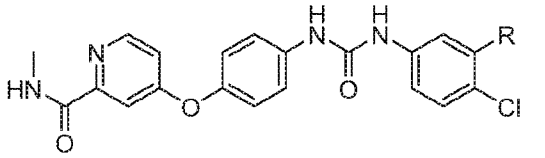
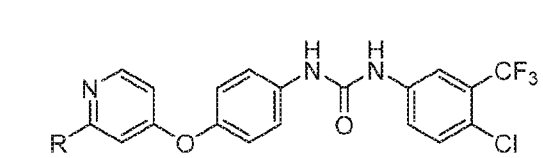
R is the point at which the Linker is attached.

V. PDGFRα dTAG Targeting Ligands:

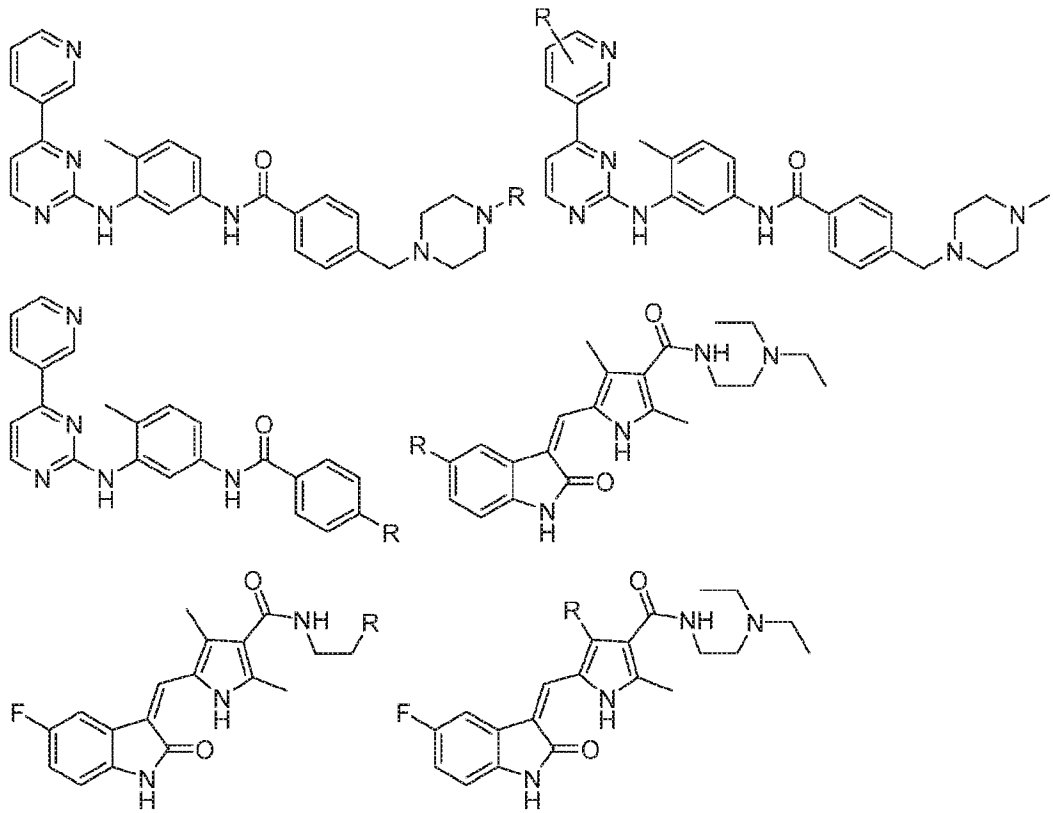
PDGFRα dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target T674I mutant PDGFRα, including AG-1478, CHEMBL94431, Dovitinib, erlotinib, gefitinib, imatinib, Janex 1, Pazopanib, PD153035, Sorafenib, Sunitinib, WHI-P180:



5



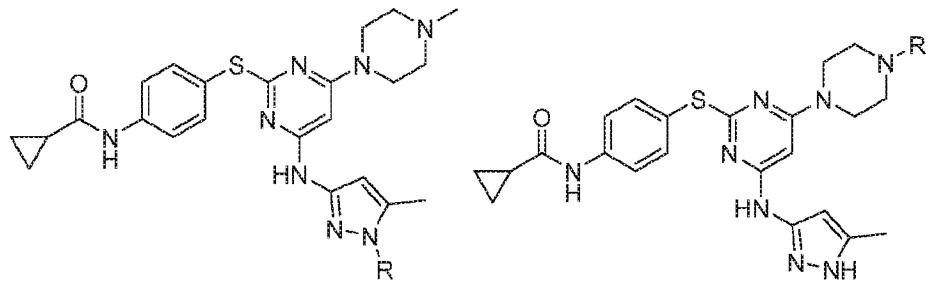
wherein:

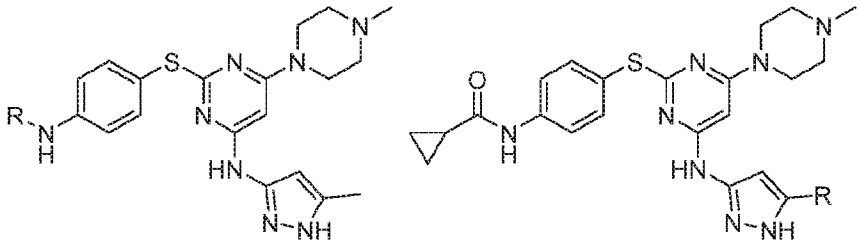
R is the point at which the Linker is attached.

W. RET dTAG Targeting Ligands:

RET dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target G691S mutant RET, including tozasertib

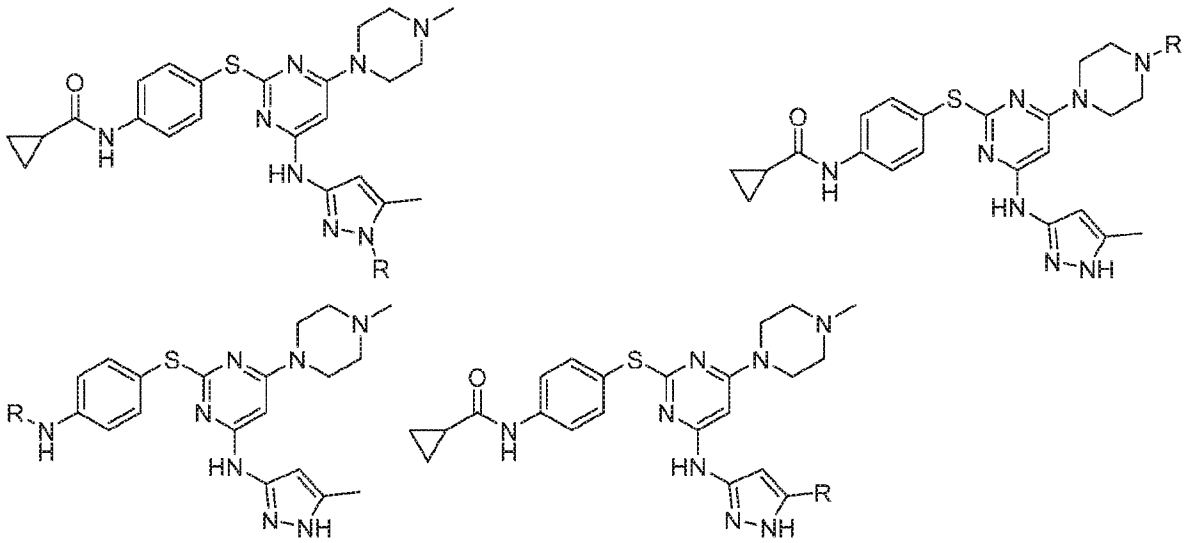




wherein:

R is the point at which the Linker is attached.

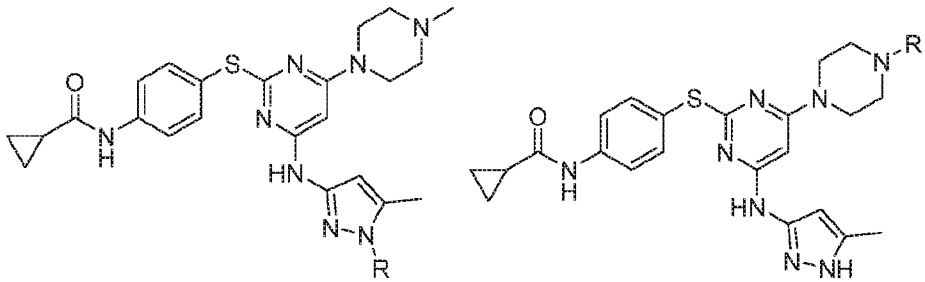
2. Targeting Ligands that target R749T mutant RET, including tozasertib

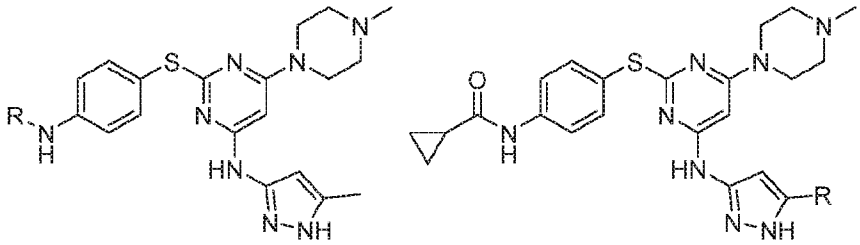


wherein:

R is the point at which the Linker is attached.

3. Targeting Ligands that target E762Q mutant RET, including tozasertib

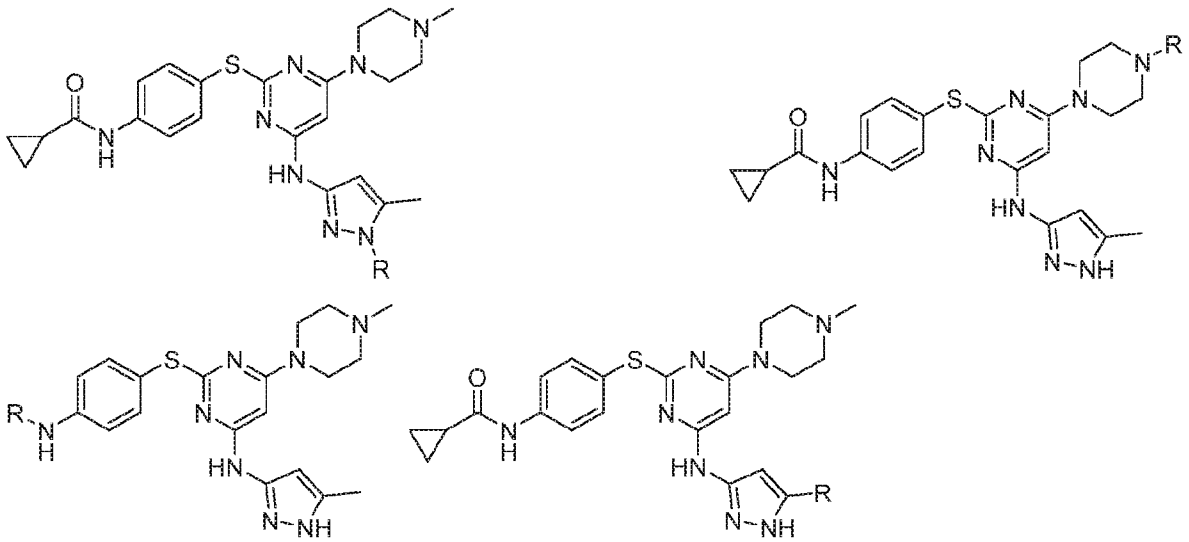




wherein:

R is the point at which the Linker is attached.

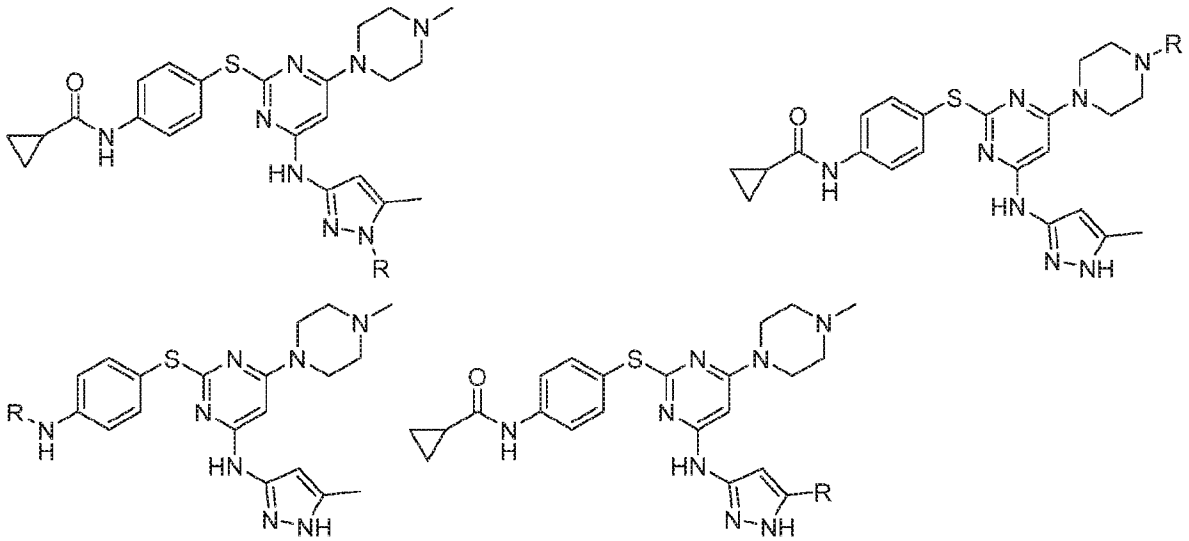
4. Targeting Ligands that target Y791F mutant RET, including tozasertib



wherein:

R is the point at which the Linker is attached.

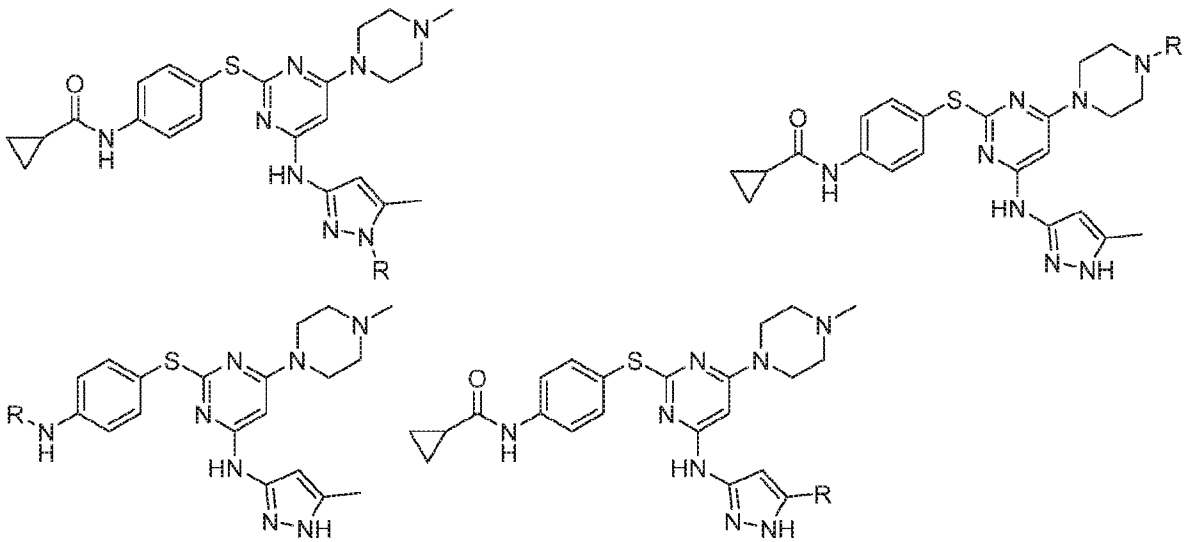
5. Targeting Ligands that target V804M mutant RET, including tozasertib



wherein:

R is the point at which the Linker is attached.

6. Targeting Ligands that target M918T mutant RET, including tozasertib



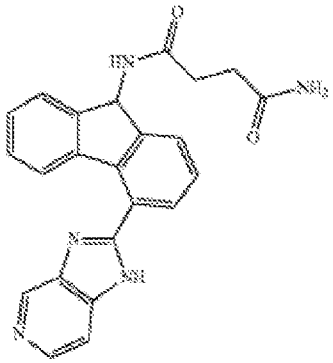
wherein:

R is the point at which the Linker is attached.

X. Heat Shock Protein 90 (HSP90) dTAG Targeting Ligands:

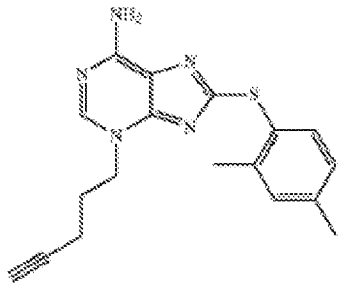
Heat Shock Protein 90 (HSP90) dTAG Targeting Ligands as used herein include, but are not limited to:

1. The HSP90 inhibitors identified in Vallee, et al., "Tricyclic Series of Heat Shock Protein 90 (HSP90) Inhibitors Part I: Discovery of Tricyclic Imidazo[4,5-C]Pyridines as Potent Inhibitors of the HSP90 Molecular Chaperone (2011) J. Med. Chem. 54: 7206, including YKB (N-[4-(3H-imidazo[4,5-C]Pyridin-2-yl)-9H-Fluoren-9-yl]-succinamide):



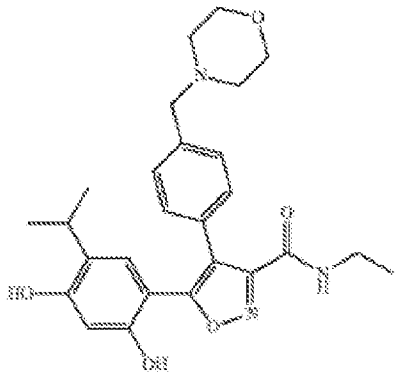
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal amide group;

2. The HSP90 inhibitor p54 (modified) (8-[(2,4-dimethylphenyl)sulfanyl]-3]pent-4-yn-1-yl-3H-purin-6-amine):



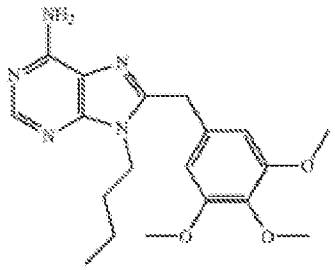
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal acetylene group;

3. The HSP90 inhibitors (modified) identified in Brough, et al., "4,5-Diarylisoxazole HSP90 Chaperone Inhibitors: Potential Therapeutic Agents for the Treatment of Cancer", J. MED. CHEM. vol: 51, page: 196 (2008), including the compound 2GJ (5-[2,4-dihydroxy-5-(1-methylethyl)phenyl]-n-ethyl-4-[4-(morpholin-4-ylmethyl)phenyl]isoxazole-3-carboxamide) having the structure:



derivatized, where a Linker group L or a -(L-DEGRON) group is attached, for example, via the amide group (at the amine or at the alkyl group on the amine);

4. The HSP90 inhibitors (modified) identified in Wright, et al., Structure-Activity Relationships in Purine-Based Inhibitor Binding to HSP90 Isoforms, Chem Biol. 2004 June; 11(6):775-85, including the HSP90 inhibitor PU3 having the structure:



derivatized where a Linker group L or -(L-DEGRON) is attached, for example, via the butyl group; and

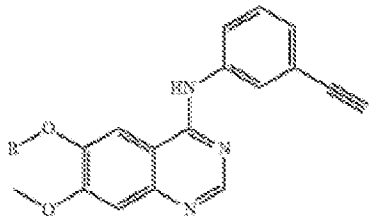
5. The HSP90 inhibitor geldanamycin ((4E,6Z,8S,9S,10E,12S,13R,14S,16R)-13-hydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1] (derivatized) or any of its derivatives (e.g. 17-alkylamino-17-desmethoxygeldanamycin ("17-AAG") or 17-(2-

dimethylaminoethyl)amino-17-desmethoxygeldanamycin ("17-DMAG")) (derivatized, where a Linker group L or a -(L-DEGRON) group is attached, for example, via the amide group).

Y. Kinase and Phosphatase dTAG Targeting Ligands:

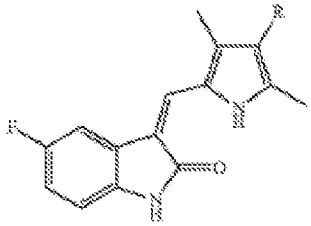
Kinase and Phosphatase dTAG Targeting Ligands as used herein include, but are not limited to:

1. Erlotinib Derivative Tyrosine Kinase Inhibitor:



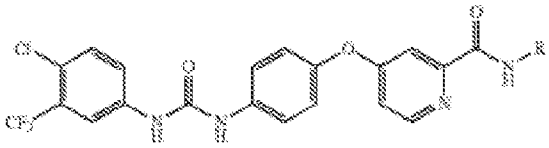
where R is a Linker group L or a -(L-DEGRON) group attached, for example, via the ether group;

2. The kinase inhibitor sunitinib (derivatized):



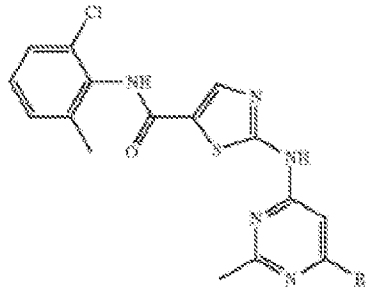
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the pyrrole moiety;

3. Kinase Inhibitor sorafenib (derivatized):



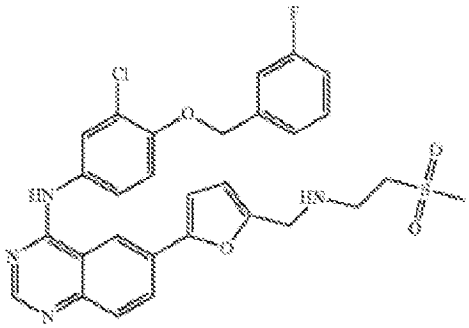
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the amide moiety;

4. The kinase inhibitor desatinib (derivatized):



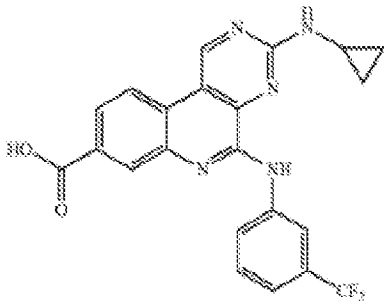
derivatized where R is a Linker group Lor a -(L-DEGRON) attached, for example, to the pyrimidine;

5. The kinase inhibitor lapatinib (derivatized):



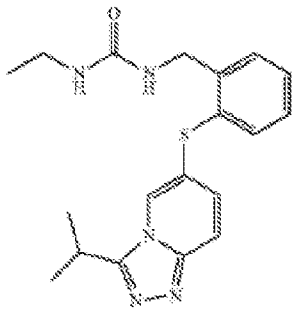
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal methyl of the sulfonyl methyl group;

6. The kinase inhibitor U09-CX-5279 (derivatized):

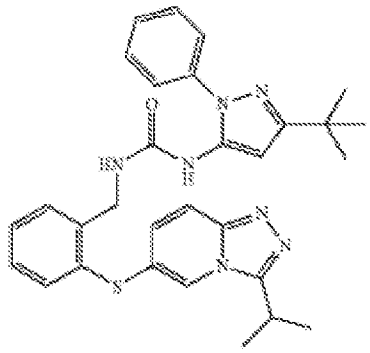


derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the amine (aniline), carboxylic acid or amine alpha to cyclopropyl group, or cyclopropyl group;

7. The kinase inhibitors identified in Millan, et al., Design and Synthesis of Inhaled P38 Inhibitors for the Treatment of Chronic Obstructive Pulmonary Disease, J. MED. CHEM. vol:54, page: 7797 (2011), including the kinase inhibitors Y1W and Y1X (Derivatized) having the structures:



Y1X(1-ethyl-3-(2-{[3-(1-methylethyl)[1,2,4]triazolo[4,3-a]pyridine-6-yl]sulfanyl}benzyl)urea, derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the i-propyl group;

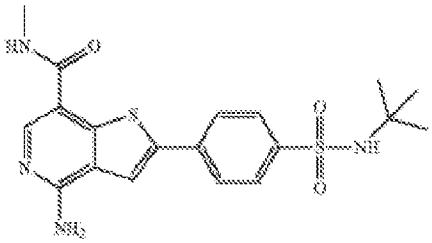


Y1W

1-(3-tert-butyl-1-phenyl-1H-pyrazol-5-yl)-3-(2-{[3-(1-methylethyl)[1,2,4]triazolo[4,3-a]pyridin-6-yl]sulfanyl}benzyl)urea

derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, preferably via either the i-propyl group or the t-butyl group;

8. The kinase inhibitors identified in Schenkel, et al., Discovery of Potent and Highly Selective Thienopyridine Janus Kinase 2 Inhibitors J. Med. Chem., 2011, 54 (24), pp 8440-8450, including the compounds 6TP and 0TP (Derivatized) having the structures:

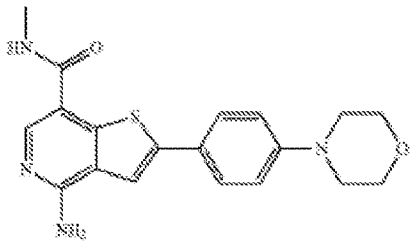


6TP

4-amino-2-[4-(tert-butylsulfamoyl)phenyl]-N-methylthieno[3,2-c]pyridine-7-carboxamide

Thienopyridine 19

derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal methyl group bound to amide moiety;



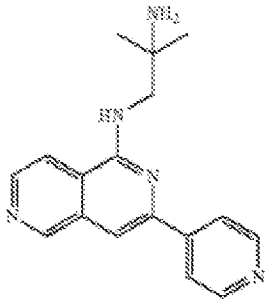
0TP

4-amino-N-methyl-2-[4-(morpholin-4-yl)phenyl]thieno[3,2-c]pyridine-7-carboxamide

Thienopyridine 8

derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal methyl group bound to the amide moiety;

9. The kinase inhibitors identified in Van Eis, et al., "2,6-Naphthyridines as potent and selective inhibitors of the novel protein kinase C isozymes", Biorg. Med. Chem. Lett. 2011 Dec. 15; 21(24):7367-72, including the kinase inhibitor 07U having the structure:

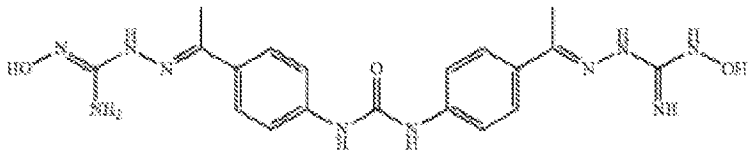


07U

2-methyl-N~1~-[3-(pyridin-4-yl)-2,6-naphthyridin-1-yl]propane-1,2-diamine

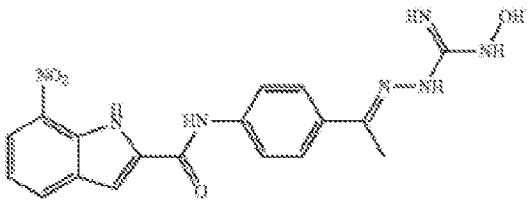
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the secondary amine or terminal amino group;

10. The kinase inhibitors identified in Lountos, et al., "Structural Characterization of Inhibitor Complexes with Checkpoint Kinase 2 (Chk2), a Drug Target for Cancer Therapy", J. STRUCT. BIOL. vol:176, pag: 292 (2011), including the kinase inhibitor YCF having the structure:



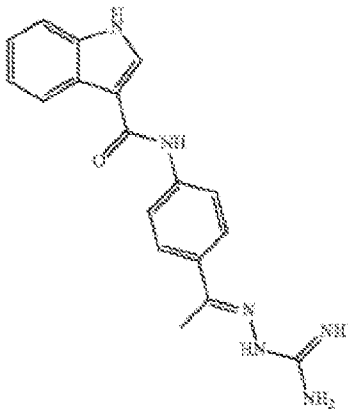
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via either of the terminal hydroxyl groups;

11. The kinase inhibitors identified in Lountos, et al., "Structural Characterization of Inhibitor Complexes with Checkpoint Kinase 2 (Chk2), a Drug Target for Cancer Therapy", J. STRUCT. BIOL. vol:176, pag: 292 (2011), including the kinase inhibitors XK9 and NXP (derivatized) having the structures:



XK9

N-{4-[(1E)-N—(N-hydroxycarbamimidoyl)ethanehydrazonoyl]phenyl}-7-nitro-1H-indole-2-carboxamide



NXP

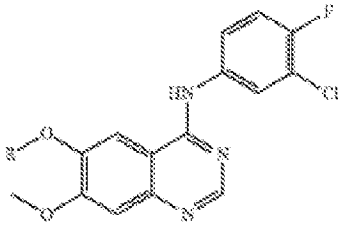
N-{4-[(1E)-N—CARBAMIMIDOYLETHANEHYDRAZONOYL]PHENYL}-1H-INDOLE-3-CARBOXAMIDE

derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the terminal hydroxyl group (XK9) or the hydrazone group (NXP);

12. The kinase inhibitor afatinib (derivatized) (N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide) (Derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the aliphatic amine group);

13. The kinase inhibitor fostamatinib (derivatized) ([6-({5-fluoro-2-[(3,4,5-trimethoxyphenyl)amino]pyrimidin-4-yl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b]-1,4-oxazin-4-yl]methyl disodium phosphate hexahydrate) (Derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via a methoxy group);

14. The kinase inhibitor gefitinib (derivatized) (N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine):



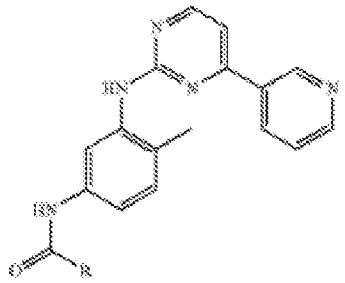
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via a methoxy or ether group;

15. The kinase inhibitor lenvatinib (derivatized) (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxy-quinoline-6-carboxamide) (derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the cyclopropyl group);

16. The kinase inhibitor vandetanib (derivatized) (N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine) (derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the methoxy or hydroxyl group);

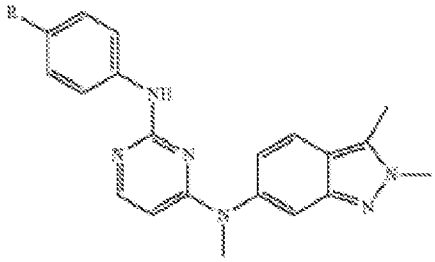
17. The kinase inhibitor vemurafenib (derivatized) (propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4-difluoro-phenyl}-amide), derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the sulfonyl propyl group;

18. The kinase inhibitor Gleevec (derivatized):



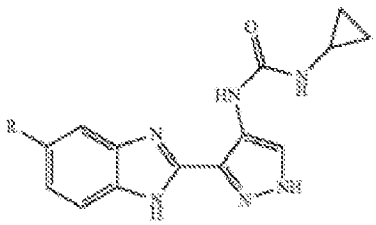
derivatized where R as a Linker group L or a -(L-DEGRON) group is attached, for example, via the amide group or via the aniline amine group;

19. The kinase inhibitor pazopanib (derivatized) (VEGFR3 inhibitor):



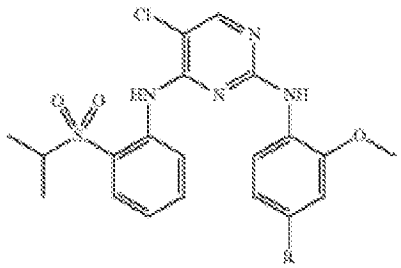
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety or via the aniline amine group;

20. The kinase inhibitor AT-9283 (Derivatized) Aurora Kinase Inhibitor



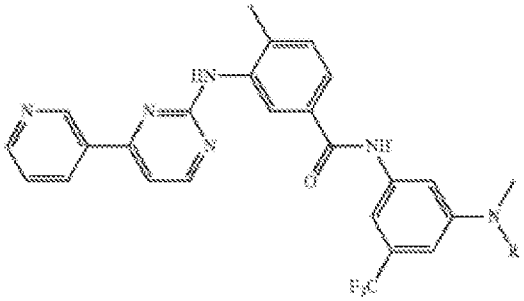
where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety);

21. The kinase inhibitor TAE684 (derivatized) ALK inhibitor



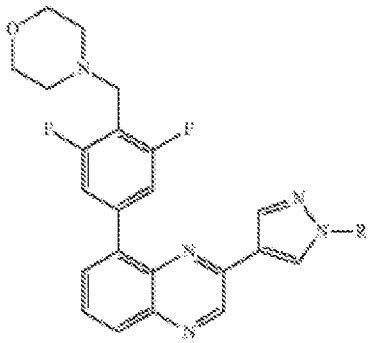
where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety);

22. The kinase inhibitor nilotanib (derivatized) Abl inhibitor:



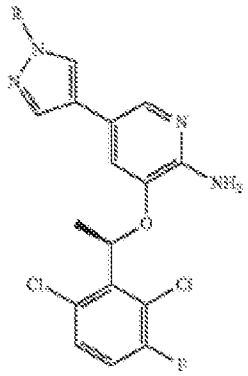
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety or the aniline amine group;

23. Kinase Inhibitor NVP-BSK805 (derivatized) JAK2 Inhibitor



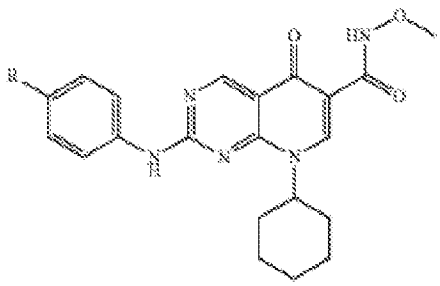
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety or the diazole group;

24. Kinase Inhibitor crizotinib Derivatized Alk Inhibitor



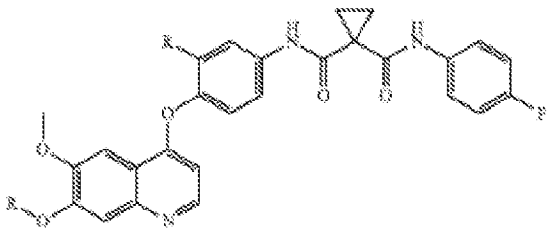
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety or the diazole group;

25. Kinase Inhibitor JNJ FMS (derivatized) Inhibitor



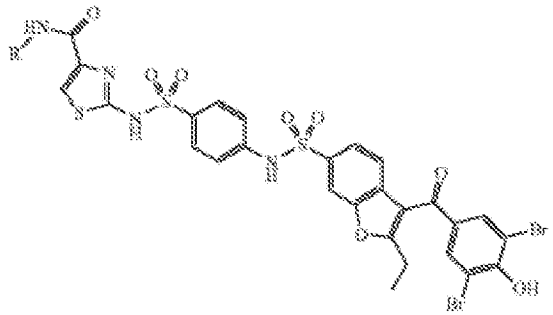
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety;

26. The kinase inhibitor foretinib (derivatized) Met Inhibitor



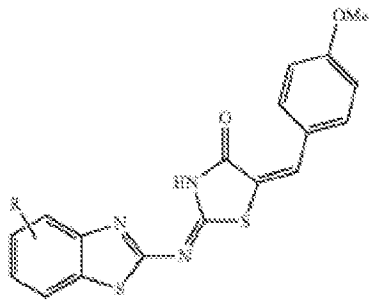
derivatized where R is a Linker group L or a -(L-DEGRON) group attached, for example, to the phenyl moiety or a hydroxyl or ether group on the quinoline moiety;

27. The allosteric Protein Tyrosine Phosphatase Inhibitor PTP1B (derivatized):



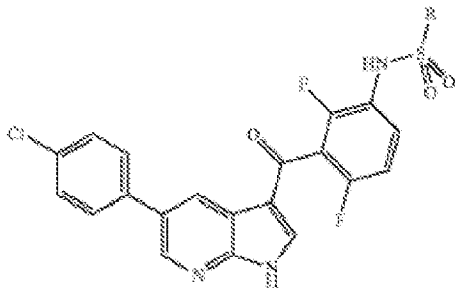
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R, as indicated;

28. The inhibitor of SHP-2 Domain of Tyrosine Phosphatase (derivatized):



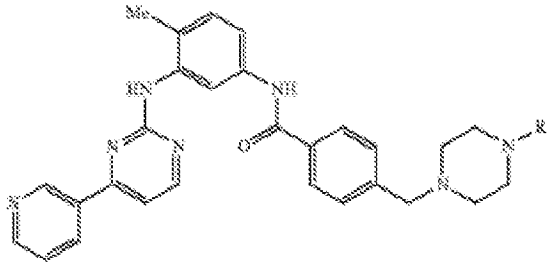
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R;

29. The inhibitor (derivatized) of BRAF (BRAFV600E)/MEK:



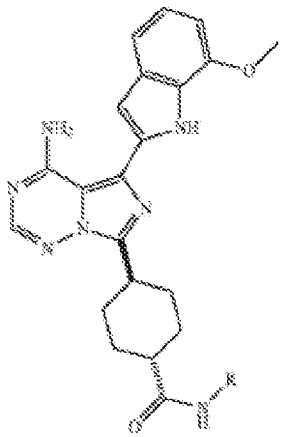
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R;

30. Inhibitor (derivatized) of Tyrosine Kinase ABL



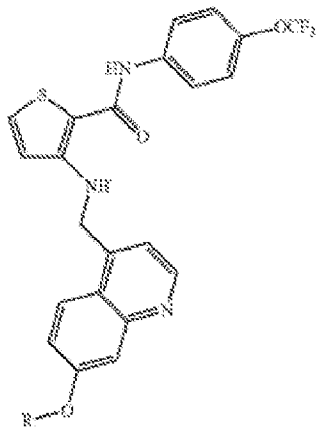
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R;

31. The kinase inhibitor OSI-027 (derivatized) mTORC1/2 inhibitor



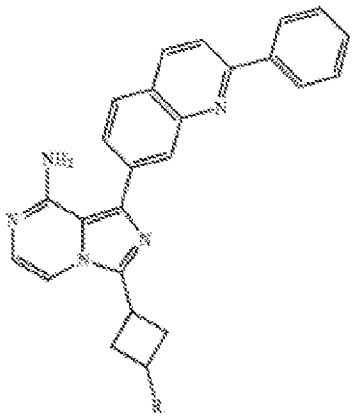
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R;

32. The kinase inhibitor OSI-930 (derivatized) c-Kit/KDR inhibitor



derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R; and

33. The kinase inhibitor OSI-906 (derivatized) IGF1R/IR inhibitor



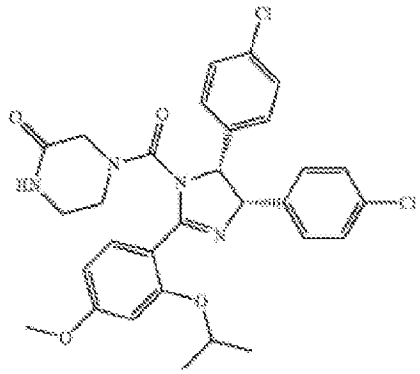
derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at R.

Wherein, in any of the embodiments described in sections I-XVII, "R" designates a site for attachment of a Linker group L or a -(L-DEGRON) group on the piperazine moiety.

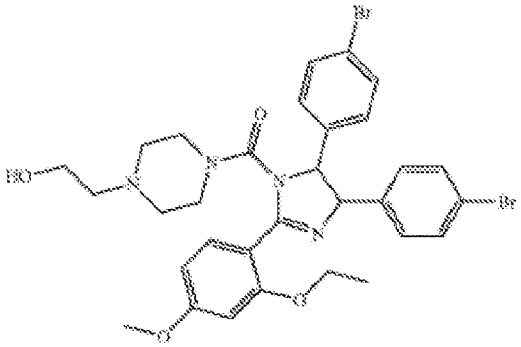
Z. HDM2 and/or MDM2 dTAG Targeting Ligands:

HDM2 and/or MDM2 dTAG Targeting Ligands as used herein include, but are not limited to:

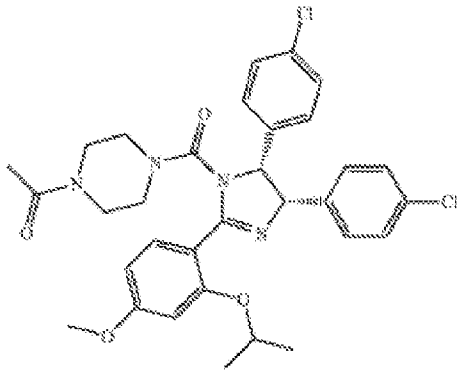
1. The HDM2/MDM2 inhibitors identified in Vassilev, et al., In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, SCIENCE vol:303, pag: 844-848 (2004), and Schneekloth, et al., Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics, Bioorg. Med. Chem. Lett. 18 (2008) 5904-5908, including (or additionally) the compounds nutlin-3, nutlin-2, and nutlin-1 (derivatized) as described below, as well as all derivatives and analogs thereof:



(derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at the methoxy group or as a hydroxyl group);

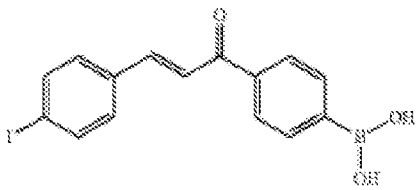


(derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, at the methoxy group or hydroxyl group);



(derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the methoxy group or as a hydroxyl group); and

2. Trans-4-Iodo-4′-Boranyl-Chalcone

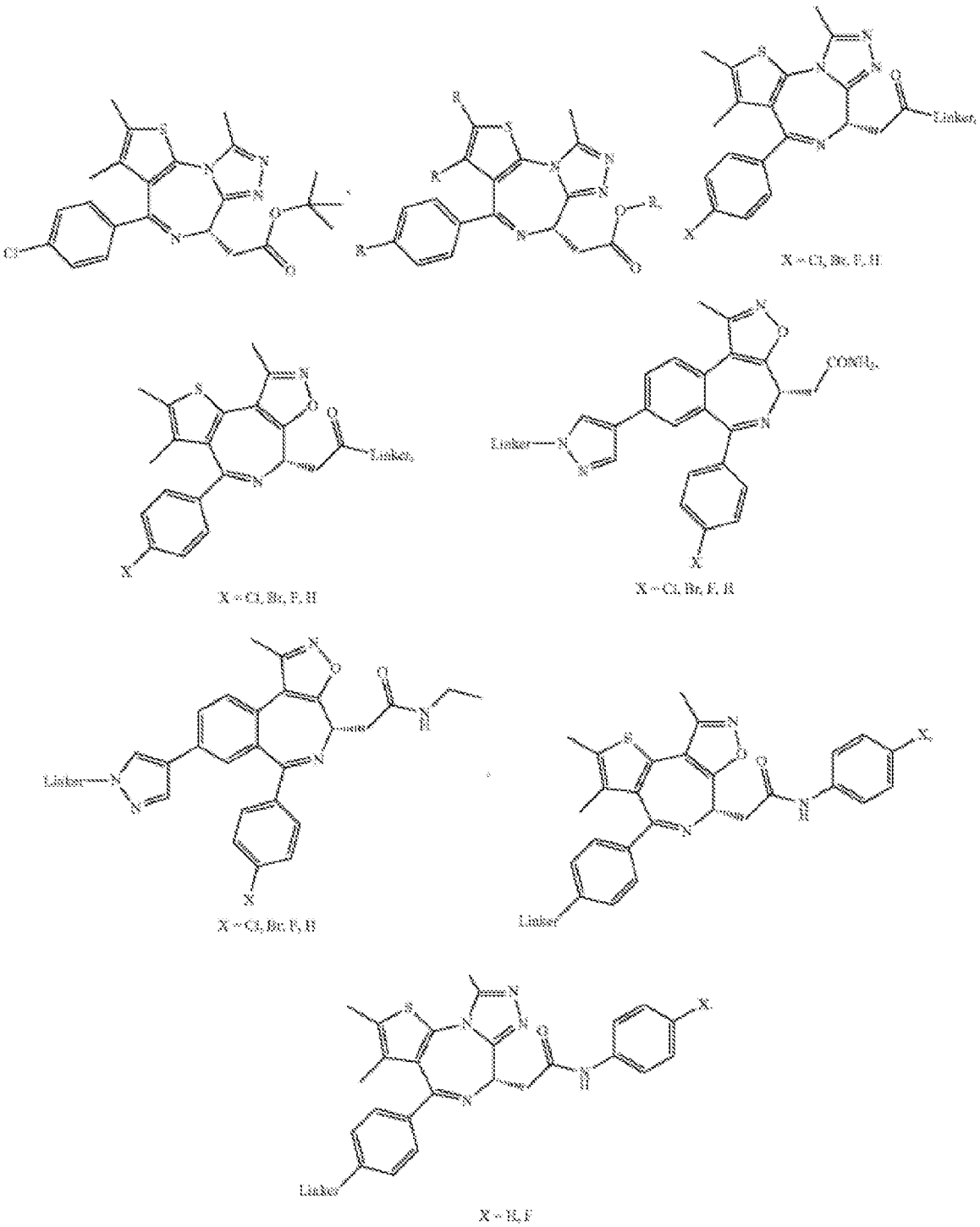


(derivatized where a Linker group L or a Linker group L or a -(L-DEGRON) group is attached, for example, via a hydroxy group).

AA. Human BET Bromodomain-Containing Proteins dTAG Targeting Ligands:

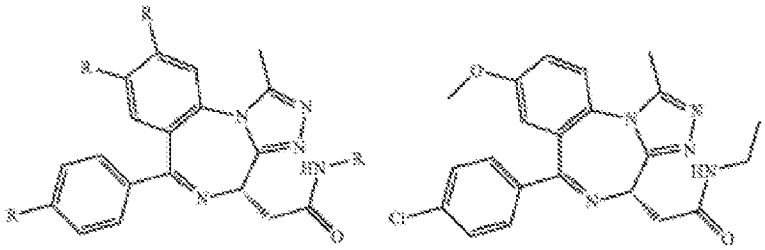
In certain embodiments, "dTAG Targeting Ligand" can be ligands binding to Bromo- and Extra-terminal (BET) proteins BRD2, BRD3 and BRD4. Compounds targeting Human BET Bromodomain-containing proteins include, but are not limited to the compounds associated with the targets as described below, where "R" or "Linker" designates a site for Linker group L or a -(L-DEGRON) group attachment, for example:

1. JQ1, Filippakopoulos et al. Selective inhibition of BET bromodomains. Nature (2010):

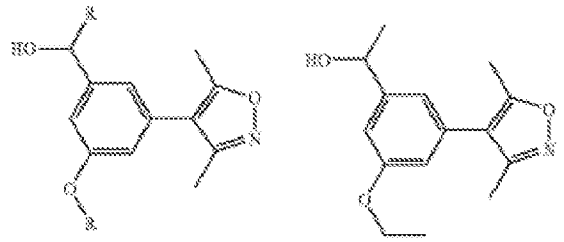


,

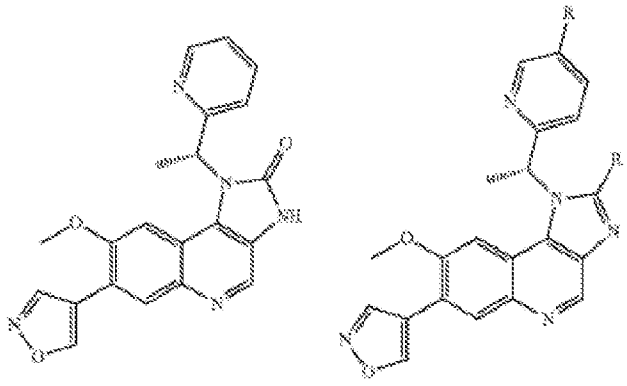
2. I-BET, Nicodeme et al. Suppression of Inflammation by a Synthetic Histone Mimic. Nature (2010). Chung et al. Discovery and Characterization of Small Molecule Inhibitors of the BET Family Bromodomains. J. Med Chem. (2011):



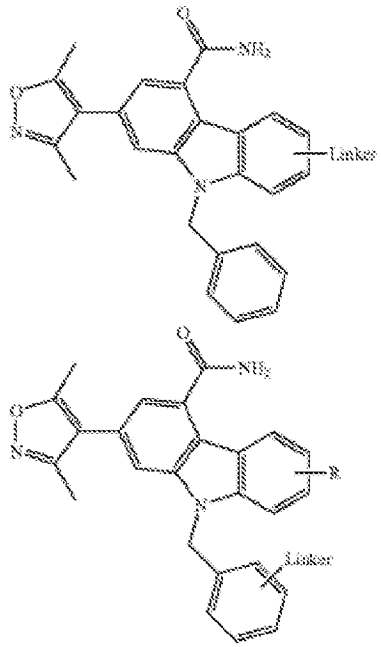
3. Compounds described in Hewings et al. 3,5-Dimethylisoxazoles Act as Acetyl-lysine Bromodomain Ligands. J. Med. Chem. (2011) 54 6761-6770.



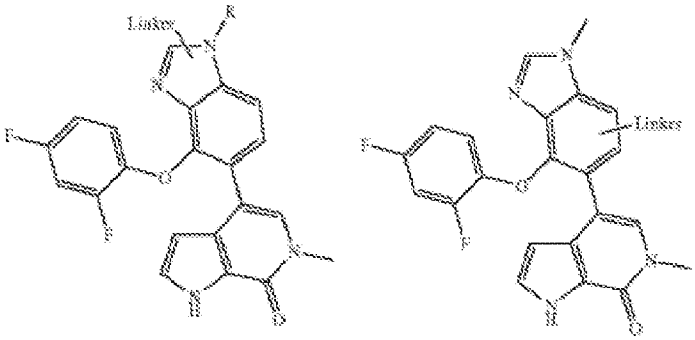
4. I-BET151, Dawson et al. Inhibition of BET Recruitment to Chromatin as an Effective Treatment for MLL-fusion Leukemia. Nature (2011):



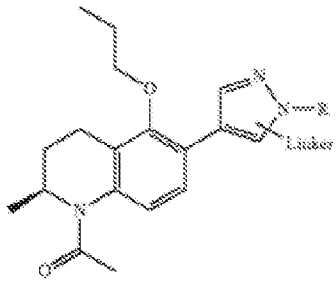
5. Carbazole type (US 2015/0256700)



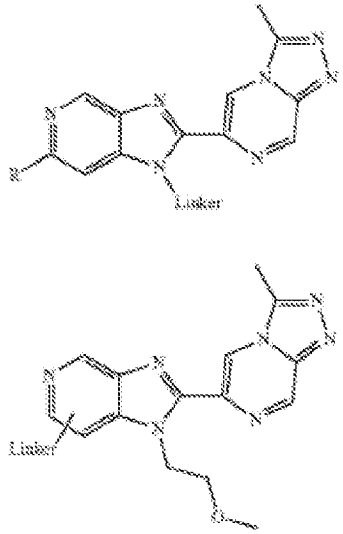
6. Pyrrolopyridone type (US 2015/0148342)



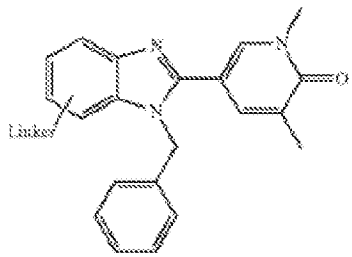
7. Tetrahydroquinoline type (WO 2015/074064)



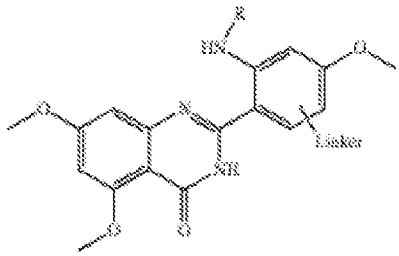
8. Triazolopyrazine type (WO 2015/067770)



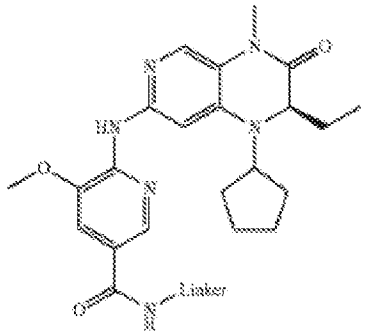
9. Pyridone type (WO 2015/022332)



10. Quinazolinone type (WO 2015/015318)



11. Dihydropyridopyrazinone type (WO 2015/011084)

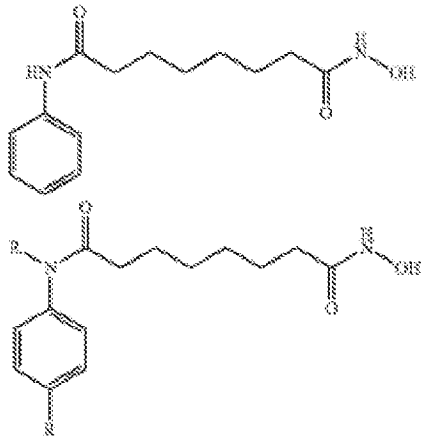


(Where R or L or Linker, in each instance, designates a site for attachment, for example, of a Linker group L or a -(L-DEGRON) group).

BB. HDAC dTAG Targeting Ligands:

HDAC dTAG Targeting Ligands as used herein include, but are not limited to:

1. Finnin, M. S. et al. Structures of Histone Deacetylase Homologue Bound to the TSA and SAHA Inhibitors. Nature 40, 188-193 (1999).



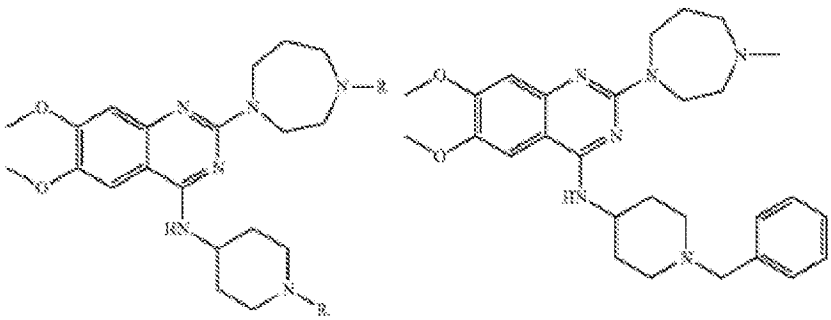
(Derivatized where "R" designates a site for attachment, for example, of a Linker group L or a -(L-DEGRON) group); and

2. Compounds as defined by formula (I) of PCT WO0222577 ("DEACETYLASE INHIBITORS") (Derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the hydroxyl group);

CC. Human Lysine Methyltransferase dTAG Targeting Ligands:

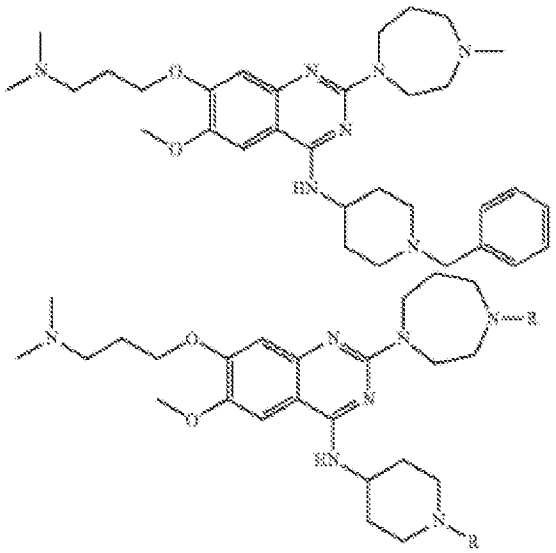
Human Lysine Methyltransferase dTAG Targeting Ligands as used herein include, but are not limited to:

1. Chang et al. Structural Basis for G9a-Like protein Lysine Methyltransferase Inhibition by BIX-1294. Nat. Struct. Biol. (2009) 16(3) 312.



(Derivatized where "R" designates a site for attachment, for example, of a Linker group L or a -(L-DEGRON) group);

2. Liu, F. et al Discovery of a 2,4-Diamino-7-aminoalkoxyquinazoline as a Potent and Selective Inhibitor of Histone Methyltransferase G9a. J. Med. Chem. (2009) 52(24) 7950.



(Derivatized where "R" designates a potential site for attachment, for example, of a Linker group L or a -(L-DEGRON) group);

3. Azacitidine (derivatized) (4-amino-1-(3-D-ribofuranosyl-1,3,5-triazin-2(1H)-one) (Derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via the hydroxy or amino groups); and

4. Decitabine (derivatized) (4-amino-1-(2-deoxy-b-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one) (Derivatized where a Linker group L or a -(L-DEGRON) group is attached, for example, via either of the hydroxy groups or at the amino group).

DD. dTAG targeting ligands organized by functionality

Angiogenesis Inhibitors:

Angiogenesis inhibitors include, but are not limited to:

1. GA-1 (derivatized) and derivatives and analogs thereof, having the structure(s) and binding to Linkers as described in Sakamoto, et al., Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation, Mol Cell Proteomics 2003 December; 2(12):1350-8;

2. Estradiol (derivatized), which may be bound to a Linker group L or a -(L-DEGRON) group as is generally described in Rodriguez-Gonzalez, et al., Targeting steroid hormone receptors for ubiquitination and degradation in breast and prostate cancer, Oncogene (2008) 27, 7201-7211;

3. Estradiol, testosterone (derivatized) and related derivatives, including but not limited to DHT and derivatives and analogs thereof, having the structure(s) and binding to a Linker group L or a -(L-DEGRON) group as generally described in Sakamoto, et al., Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation, Mol Cell Proteomics 2003 December; 2(12):1350-8; and

4. Ovalicin, fumagillin (derivatized), and derivatives and analogs thereof, having the structure(s) and binding to a Linker group L or a -(L-DEGRON) group as is generally described in Sakamoto, et al., Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation Proc Natl Acad Sci USA. 2001 Jul. 17; 98(15):8554-9 and U.S. Pat. No. 7,208,157.

Immunosuppressive Compounds:

Immunosuppressive compounds include, but are not limited to:

1. AP21998 (derivatized), having the structure(s) and binding to a Linker group L or a -(L-DEGRON) group as is generally described in Schneekloth, et al., Chemical Genetic Control of Protein Levels: Selective in Vivo Targeted Degradation, J. AM. CHEM. SOC. 2004, 126, 3748-3754;

2. Glucocorticoids (e.g., hydrocortisone, prednisone, prednisolone, and methylprednisolone) (Derivatized where a Linker group L or a -(L-DEGRON) group is to bound, e.g. to any of the hydroxyls) and beclometasone dipropionate (Derivatized where a Linker group or a -(L-DEGRON) is bound, e.g. to a proprionate);

3. Methotrexate (Derivatized where a Linker group or a -(L-DEGRON) group can be bound, e.g. to either of the terminal hydroxyls);

4. Ciclosporin (Derivatized where a Linker group or a -(L-DEGRON) group can be bound, e.g. at any of the butyl groups);

5. Tacrolimus (FK-506) and rapamycin (Derivatized where a Linker group L or a -(L-DEGRON) group can be bound, e.g. at one of the methoxy groups); and

6. Actinomycins (Derivatized where a Linker group L or a -(L-DEGRON) group can be bound, e.g. at one of the isopropyl groups).

EE. Aryl Hydrocarbon Receptor (AHR) dTAG Targeting Ligands:

AHR dTAG Targeting Ligands as used herein include, but are not limited to:

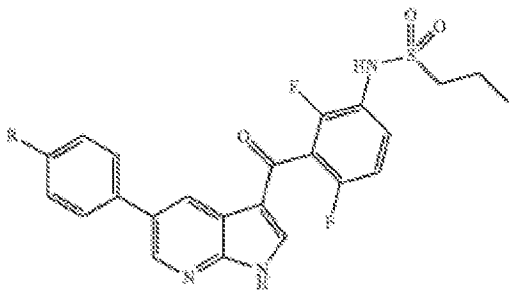
1. Apigenin (Derivatized in a way which binds to a Linker group L or a -(L-DEGRON) group as is generally illustrated in Lee, et al., Targeted Degradation of the Aryl Hydrocarbon Receptor by

the PROTAC Approach: A Useful Chemical Genetic Tool, Chem Bio Chem Volume 8, Issue 17, pages 2058-2062, Nov. 23, 2007); and

2. SR1 and LGC006 (derivatized such that a Linker group L or a -(L-DEGRON) is bound), as described in Boitano, et al., Aryl Hydrocarbon Receptor Antagonists Promote the Expansion of Human Hematopoietic Stem Cells, Science 10 Sep. 2010: Vol. 329 no. 5997 pp. 1345-1348.

FF.RAF dTAG Targeting Ligands:

RAF dTAG Targeting Ligands as used herein include, but are not limited to:

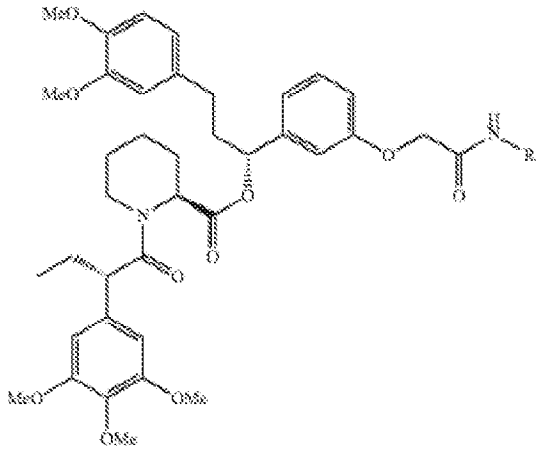


PLX4032

(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment, for example).

GG. FKBP dTAG Targeting Ligands:

FKBP dTAG Targeting Ligands as used herein include, but are not limited to:

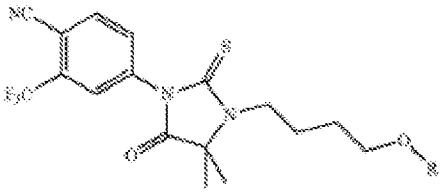


(Derivatized where "R" designates a site for a Linker group L or a -(L-DEGRON) group attachment, for example).

HH. Androgen Receptor (AR) dTAG Targeting Ligands:

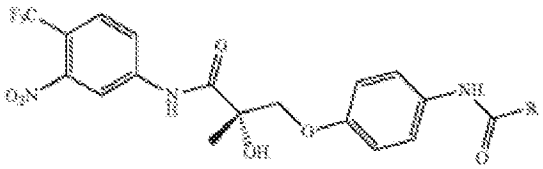
AR dTAG Targeting Ligands as used herein include, but are not limited to:

1. RU59063 Ligand (derivatized) of Androgen Receptor



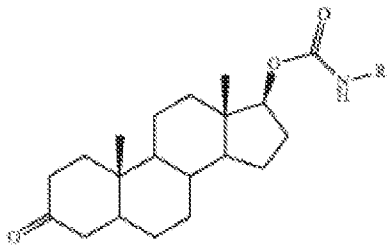
(Derivatized where "R" designates a site for a Linker group L or a -(L-DEGRON) group attachment, for example).

2. SARM Ligand (derivatized) of Androgen Receptor



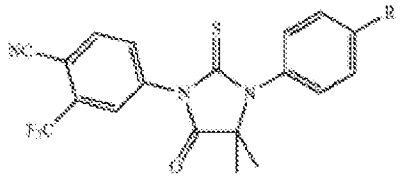
(Derivatized where "R" designates a site for a Linker group L or a -(L-DEGRON) group attachment, for example).

3. Androgen Receptor Ligand DHT (derivatized)

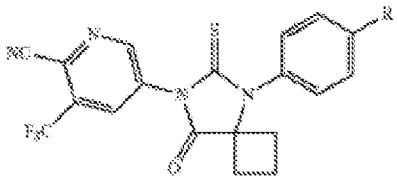


(Derivatized where "R" designates a site for a Linker group L or -(L-DEGRON) group attachment, for example).

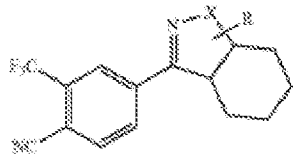
4. MDV3100 Ligand (derivatized)



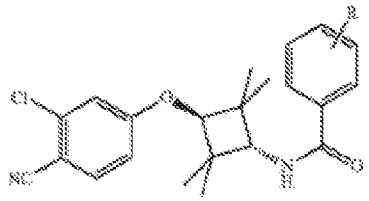
5. ARN-509 Ligand (derivatized)



6. Hexahydrobenzisoxazoles



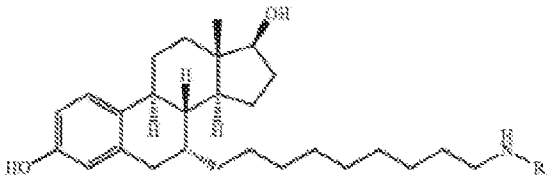
7. Tetramethylcyclobutanes



II. Estrogen Receptor (ER) dTAG Targeting Ligands:

ER dTAG Targeting Ligands as used herein include, but are not limited to:

1. Estrogen Receptor Ligand

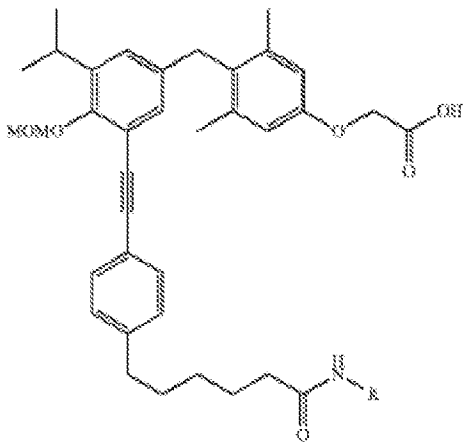


(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment).

JJ. Thyroid Hormone Receptor (TR) dTAG Targeting Ligands:

TR dTAG Targeting Ligands as used herein include, but are not limited to:

1. Thyroid Hormone Receptor Ligand (derivatized)

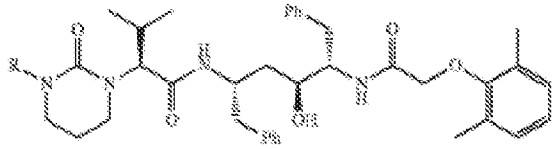


(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment and MOMO indicates a methoxymethoxy group).

KK. HIV Protease dTAG Targeting Ligands:

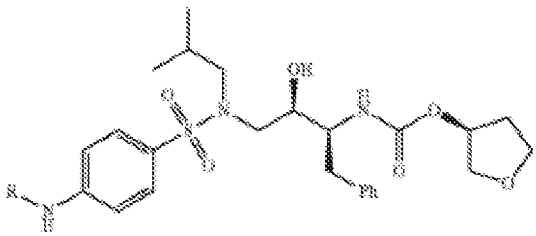
HIV Protease dTAG Targeting Ligands as used herein include, but are not limited to:

1. Inhibitor of HIV Protease (derivatized)



(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment). See, J. Med. Chem. 2010, 53, 521-538.

2. Inhibitor of HIV Protease

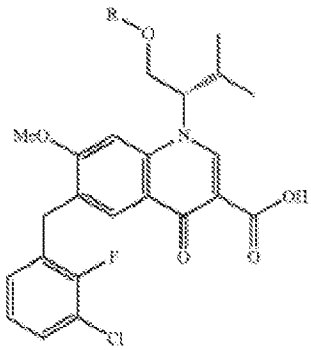


(Derivatized where "R" designates a potential site for Linker group L or -(L-DEGRON) group attachment). See, J. Med. Chem. 2010, 53, 521-538.

LL. HIV Integrase dTAG Targeting Ligands:

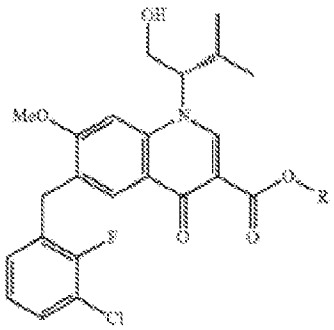
HIV Integrase dTAG Targeting Ligands as used herein include, but are not limited to:

1. Inhibitor of HIV Integrase (derivatized)

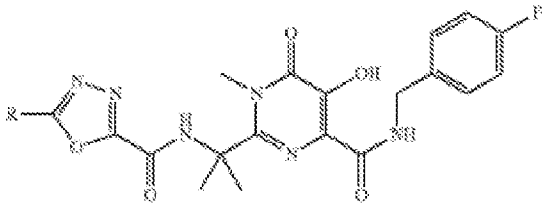


(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment). See, J. Med. Chem. 2010, 53, 6466.

2. Inhibitor of HIV Integrase (derivatized)



3. Inhibitor of HIV integrase (derivatized)

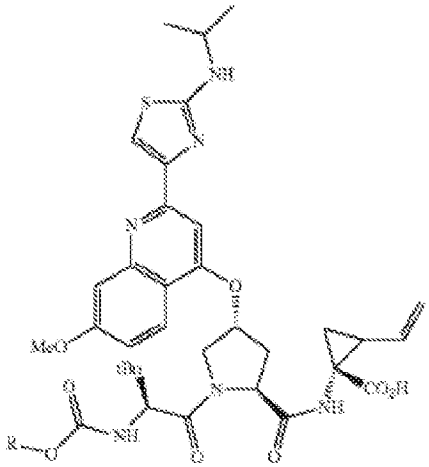


(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment). See, J. Med. Chem. 2010, 53, 6466.

MM. HCV Protease dTAG Targeting Ligands:

HCV Protease dTAG Targeting Ligands as used herein include, but are not limited to:

1. Inhibitors of HCV Protease (Derivatized)

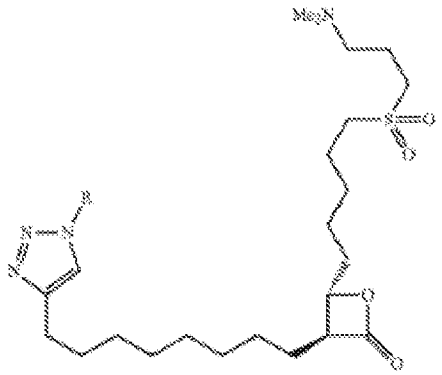


(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment).

NN. Acyl-Protein Thioesterase-1 and -2 (APT1 and APT2) dTAG Targeting Ligands:

Acyl-Protein Thioesterase-1 and -2 (APT1 and APT2) dTAG Targeting Ligands as used herein include, but are not limited to:

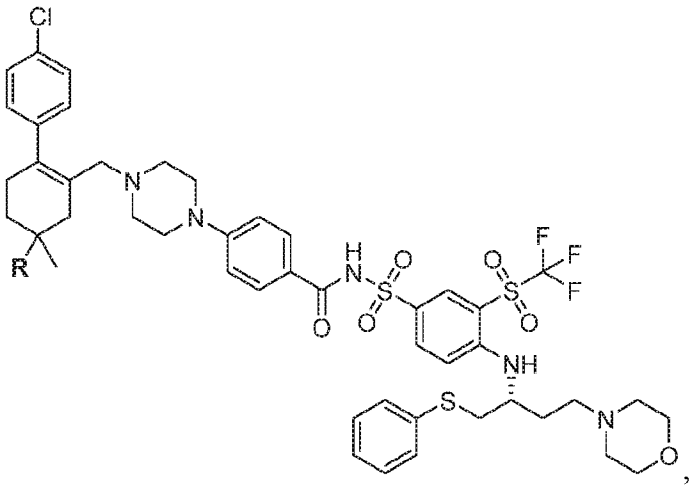
1. Inhibitor of APT1 and APT2 (Derivatized)



(Derivatized where "R" designates a site for Linker group L or -(L-DEGRON) group attachment). See, Angew. Chem. Int. Ed. 2011, 50, 9838-9842, where L is a Linker group as otherwise described herein and said Degron group is as otherwise described herein such that the Linker binds the Degron group to a dTAG Targeting Ligand group as otherwise described herein.

OO. BCL2 dTAG Targeting Ligands:

BCL2 dTAG Targeting Ligands as used herein include, but are not limited to:



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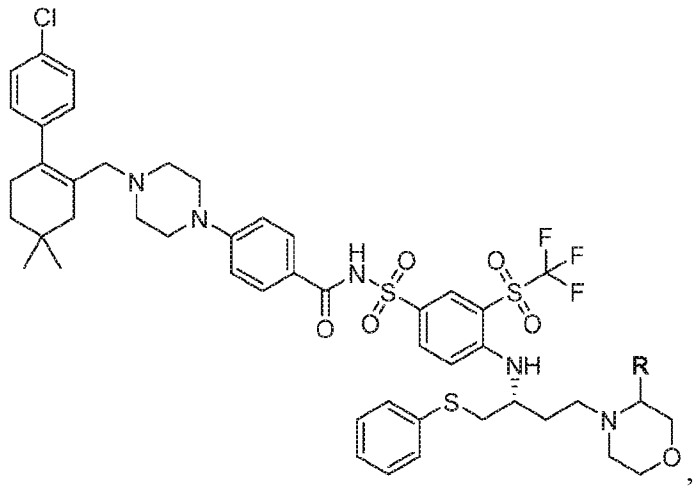
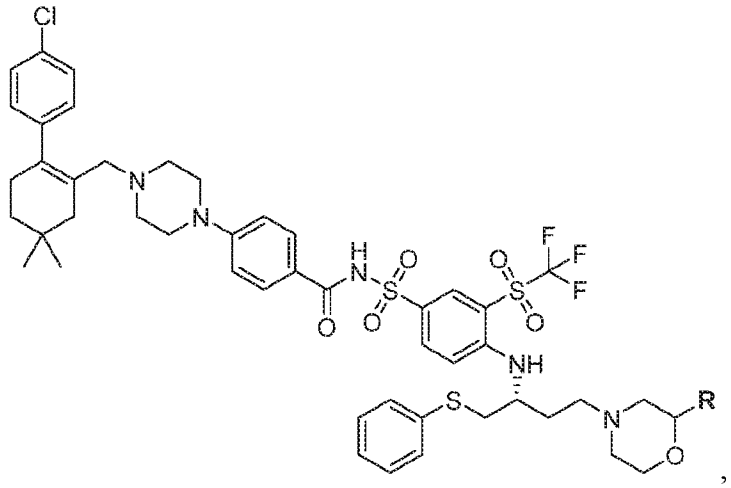
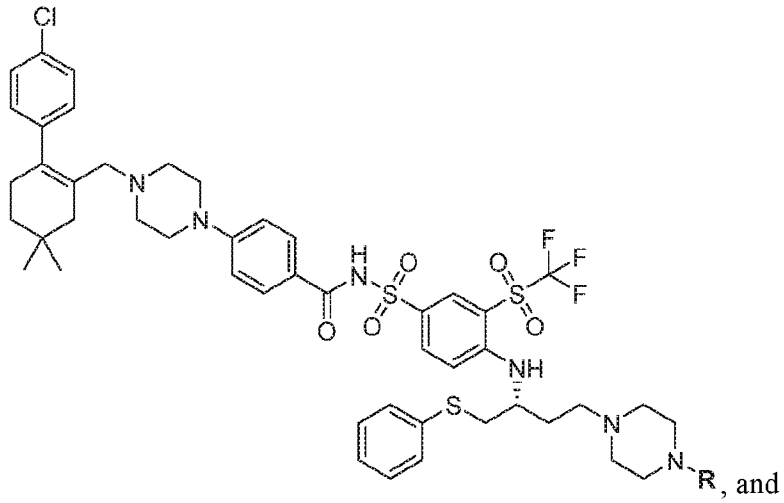
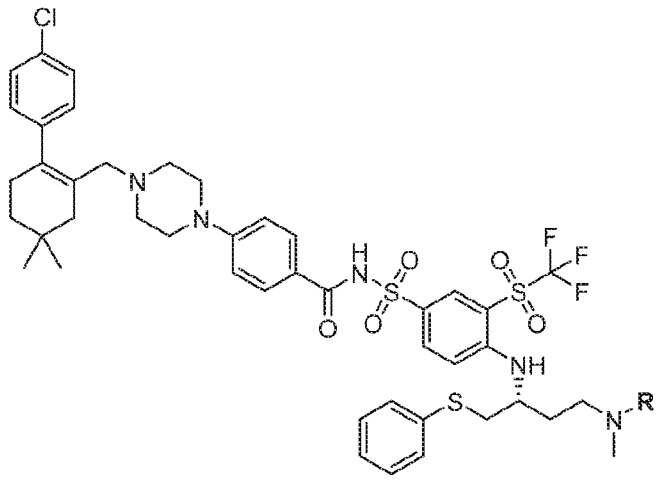






, and

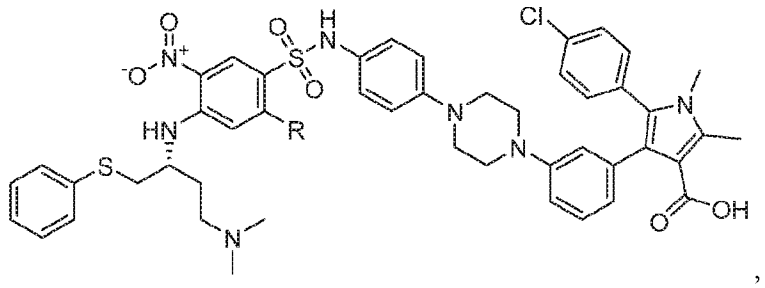


wherein:

R is the point at which the Linker is attached.

PP.BCL-XL dTAG Targeting Ligands:

BCL-XL dTAG Targeting Ligands as used herein include, but are not limited to:



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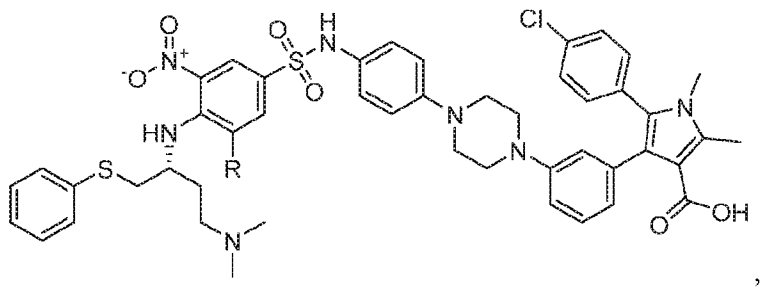

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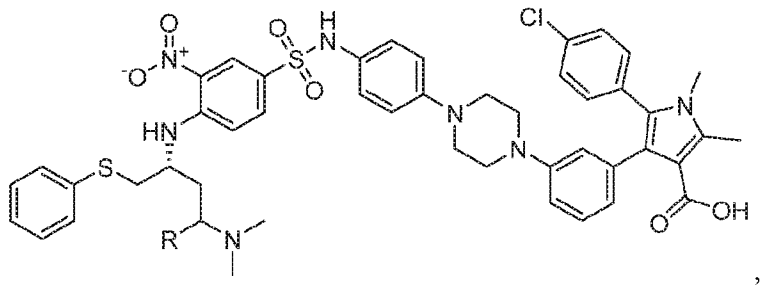

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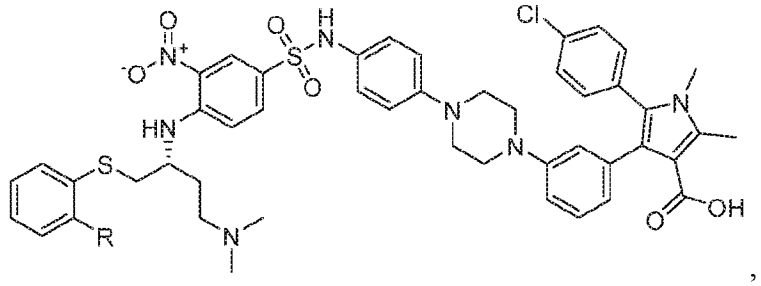

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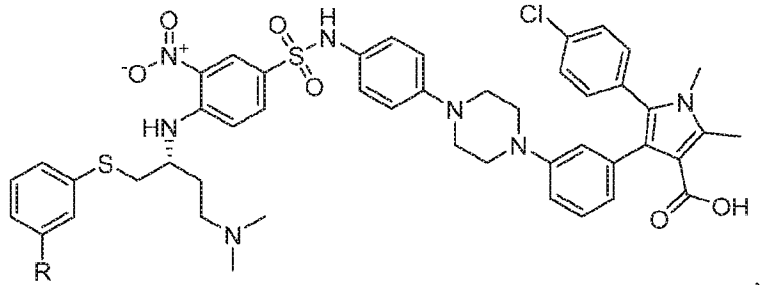

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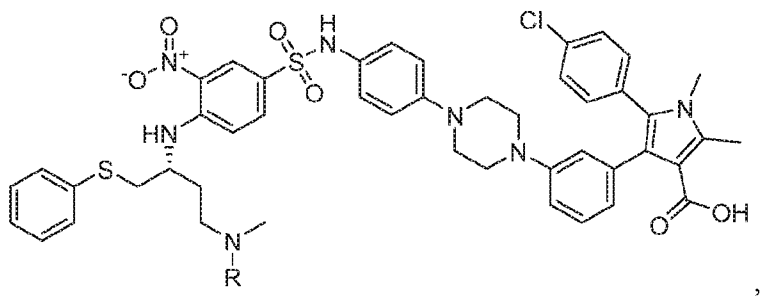

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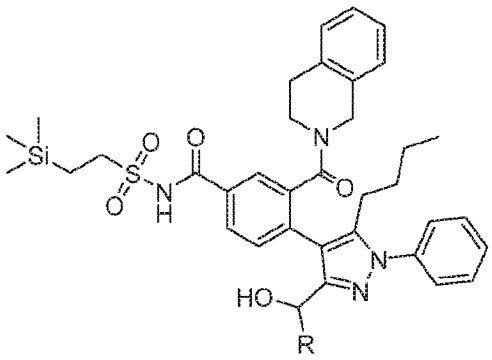
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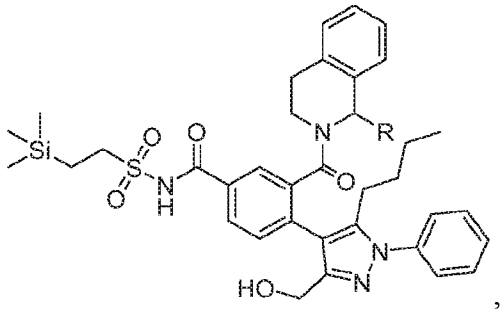
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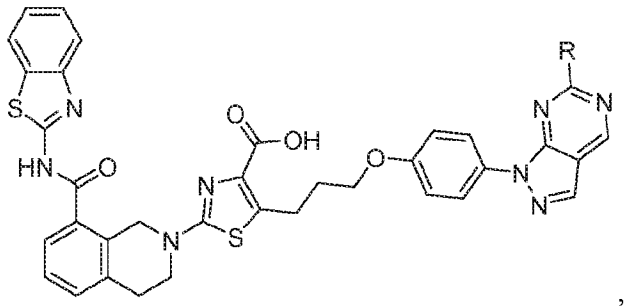
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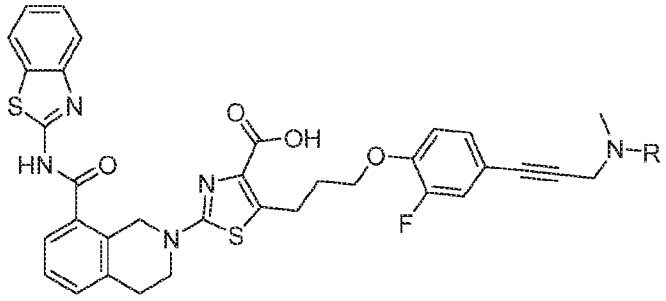
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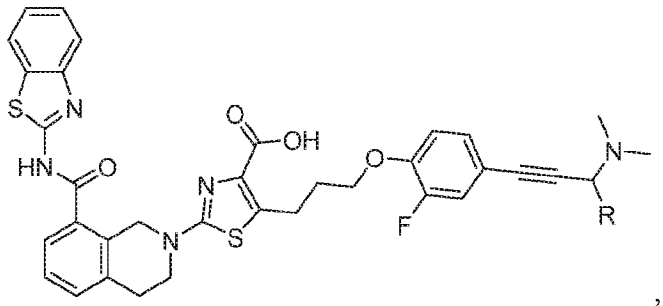
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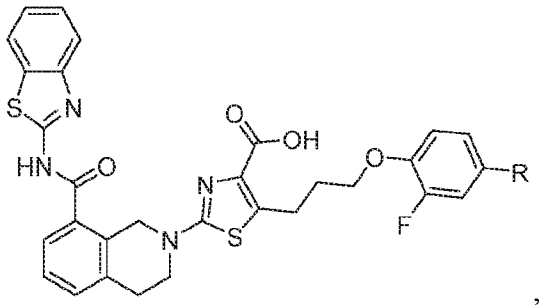

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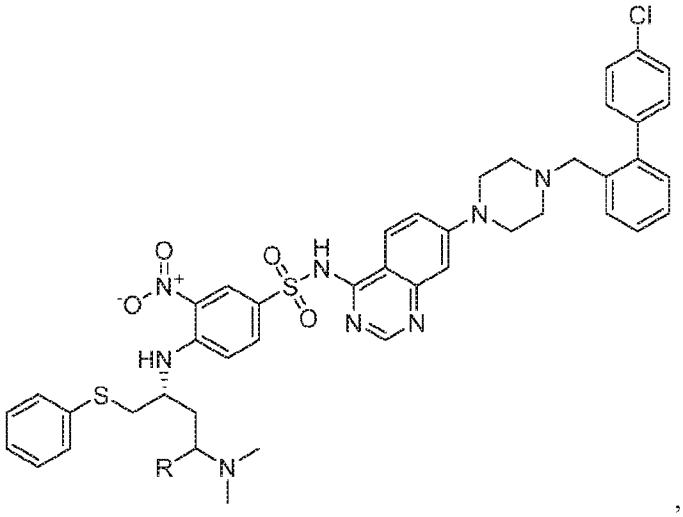

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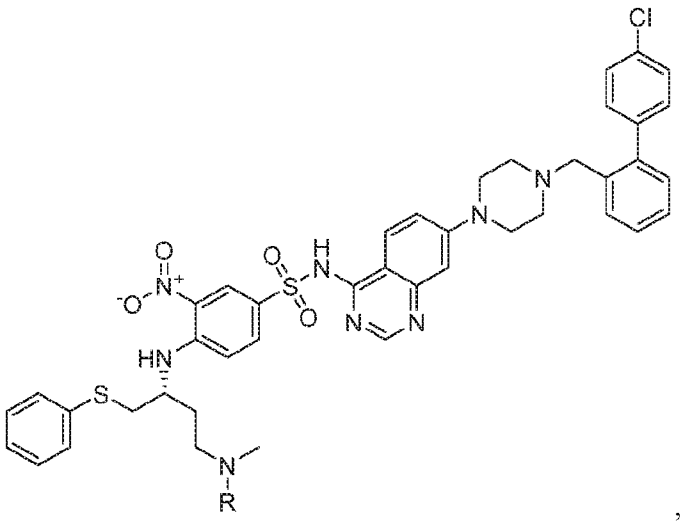

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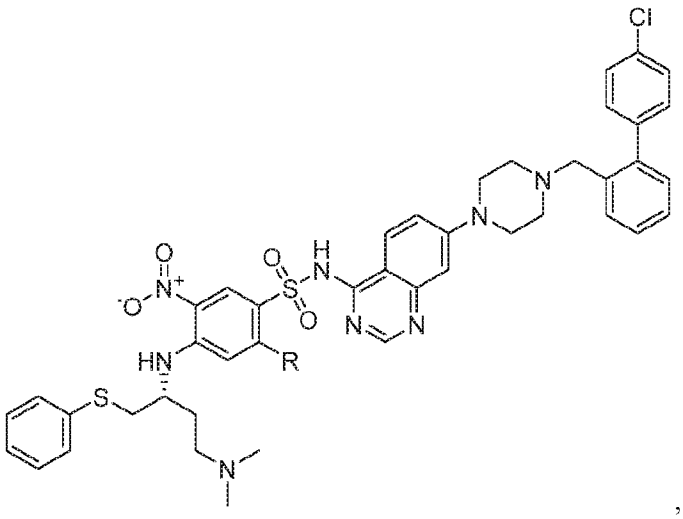

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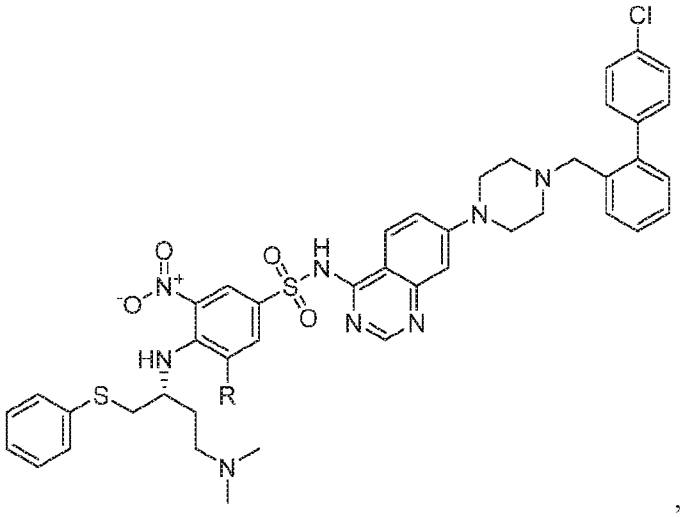

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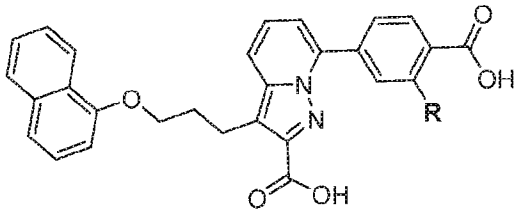

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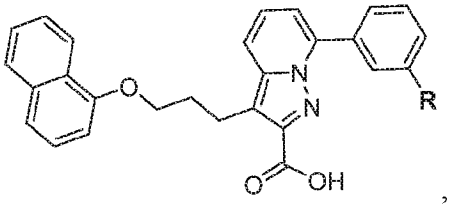

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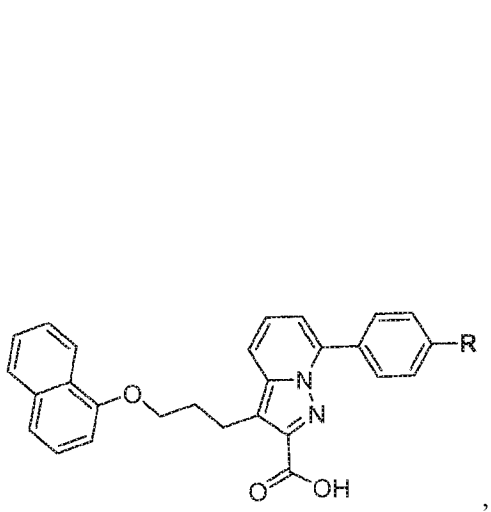
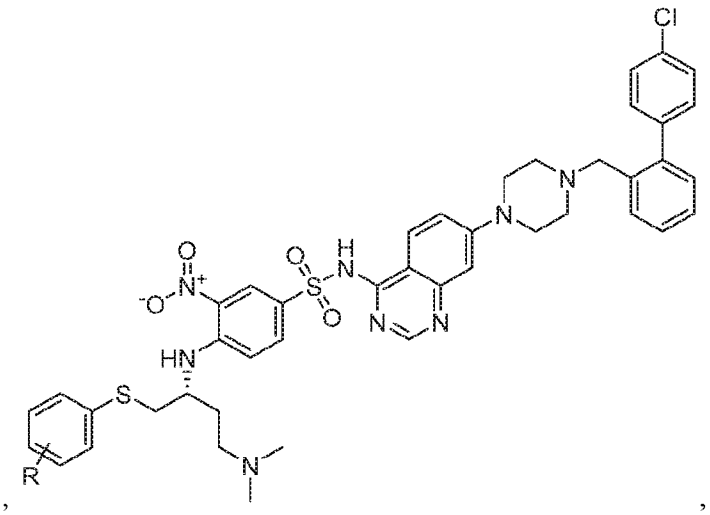
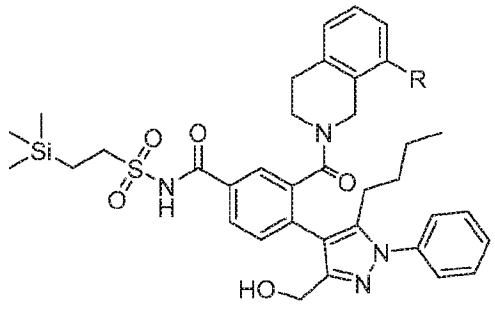




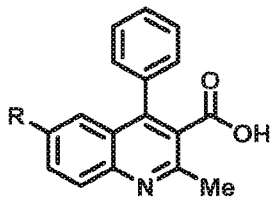
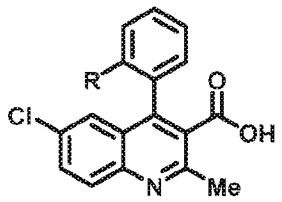
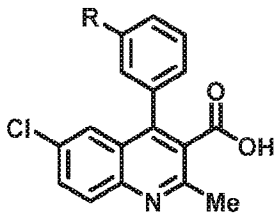
and

wherein:

R is the point at which the Linker is attached.

QQ. FA Binding Protein dTAG Targeting Ligands:

FA dTAG Targeting Ligands as used herein include, but are not limited to:

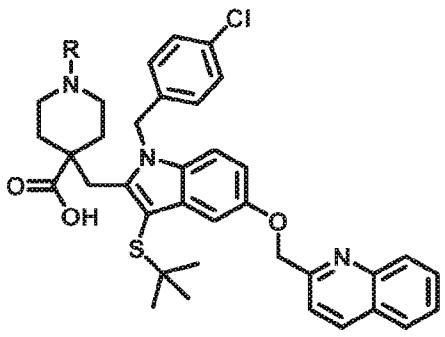
, and

wherein:

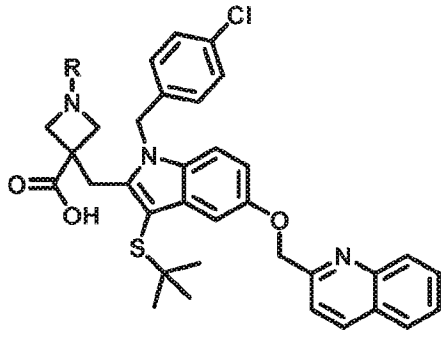
R is the point at which the Linker is attached.

RR. FLAP – 5-Lipoxygenase Activating Protein dTAG Targeting Ligands:

FLAP – 5-Lipoxygenase Activating Protein dTAG Targeting Ligands as used herein include, but are not limited to:



and

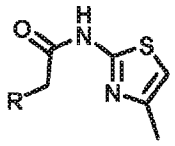


wherein:

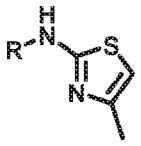
R is the point at which the Linker is attached.

SS. HDAC6 Zn Finger Domain dTAG Targeting Ligands:

HDAC6 Zn Finger Domain dTAG Targeting Ligands as used herein include, but are not limited to:



and

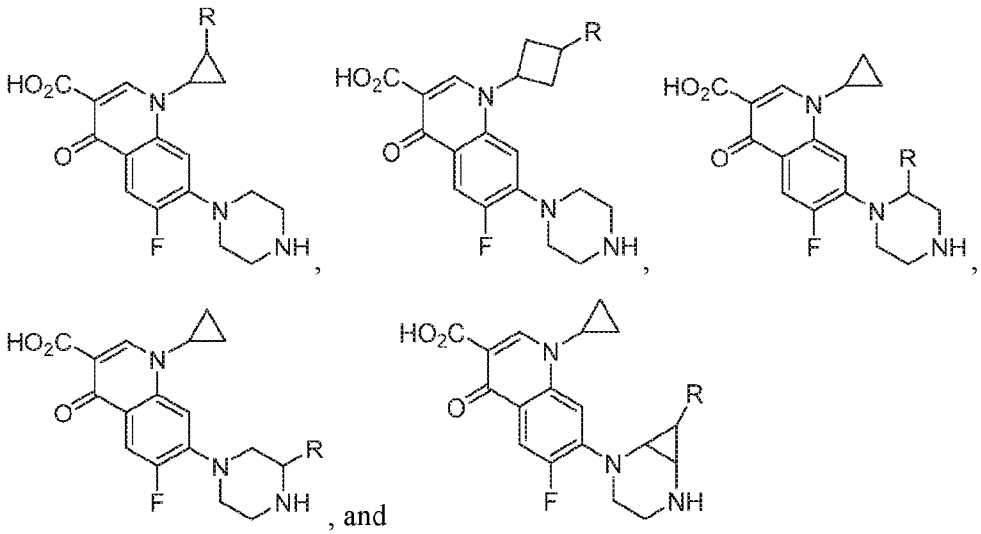


wherein:

R is the point at which the Linker is attached.

TT. Kringle Domain V 4BVV dTAG Targeting Ligands:

Kringle Domain V 4BVV dTAG Targeting Ligands as used herein include, but are not limited to:



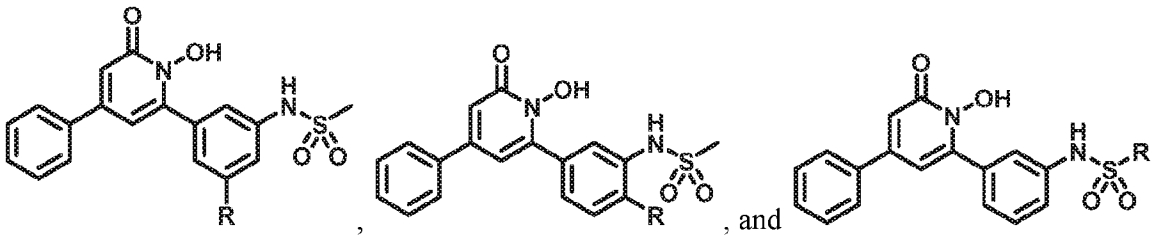
, and

wherein:

R is the point at which the Linker is attached.

UU. Lactoylglutathione Lyase dTAG Targeting Ligands:

Lactoylglutathione Lyase dTAG Targeting Ligands as used herein include, but are not limited to:



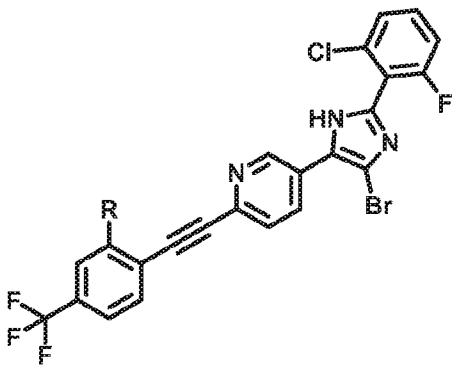
, and

wherein:

R is the point at which the Linker is attached.

VV. mPGES-1 dTAG Targeting Ligands:

mPGES-1 dTAG Targeting Ligands as used herein include, but are not limited to:



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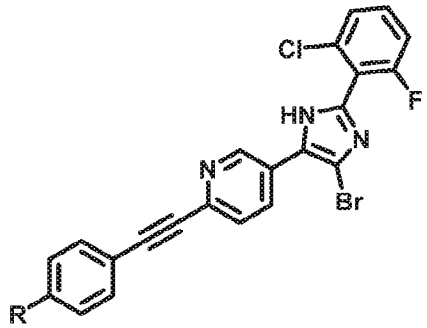

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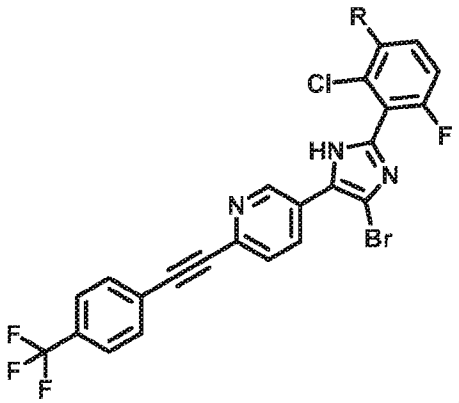

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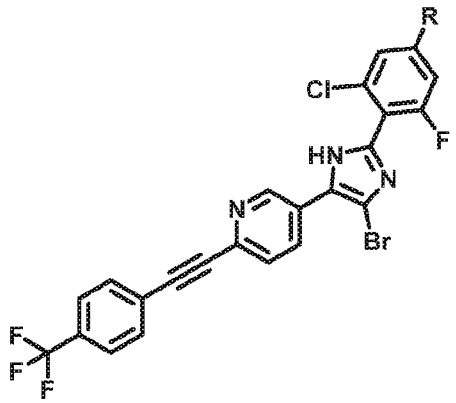

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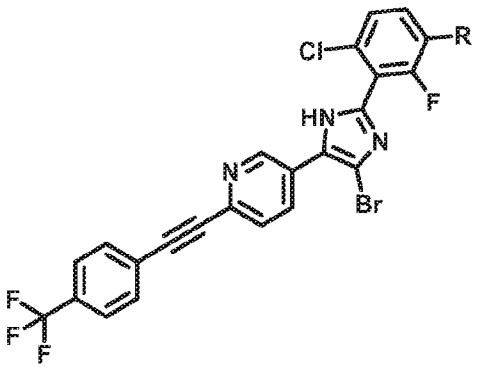
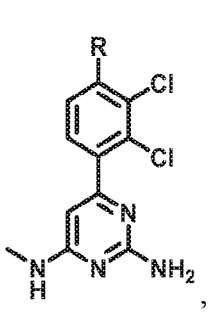
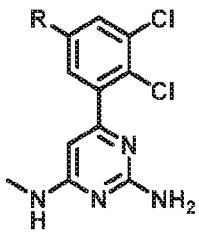
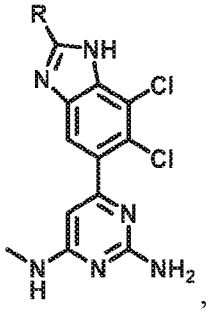
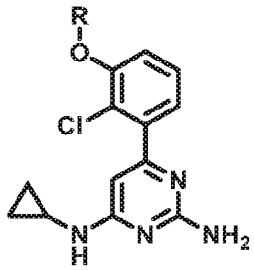


wherein:

R is the point at which the Linker is attached.

WW. MTH1 dTAG Targeting Ligands:

MTH1 dTAG Targeting Ligands as used herein include, but are not limited to: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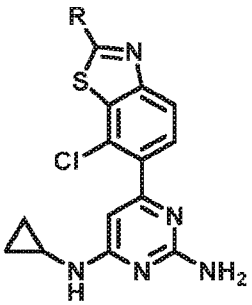
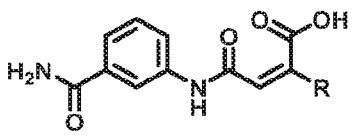
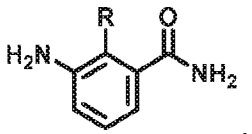
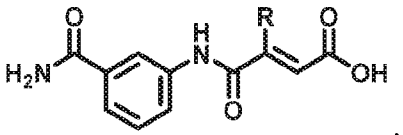


wherein:

R is the point at which the Linker is attached.

XX. PARP14 dTAG Targeting Ligands:

PARP14 dTAG Targeting Ligands as used herein include, but are not limited to: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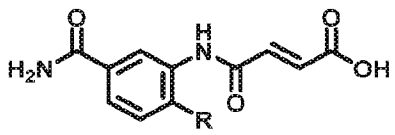
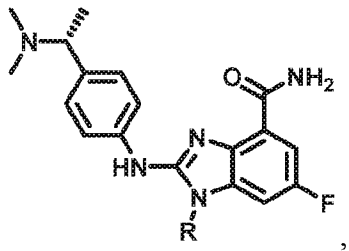
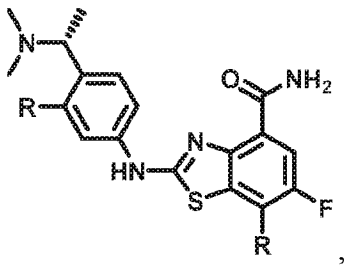
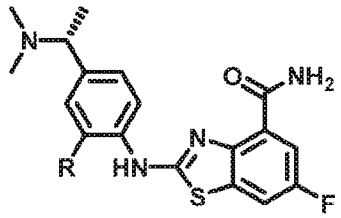



, and

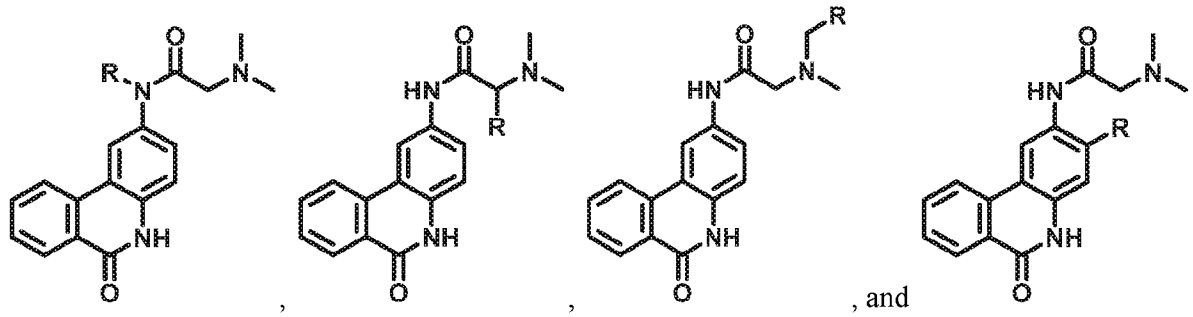


wherein:

R is the point at which the Linker is attached.

YY. PARP15 dTAG Targeting Ligands:

PARP15 dTAG Targeting Ligands as used herein include, but are not limited to:

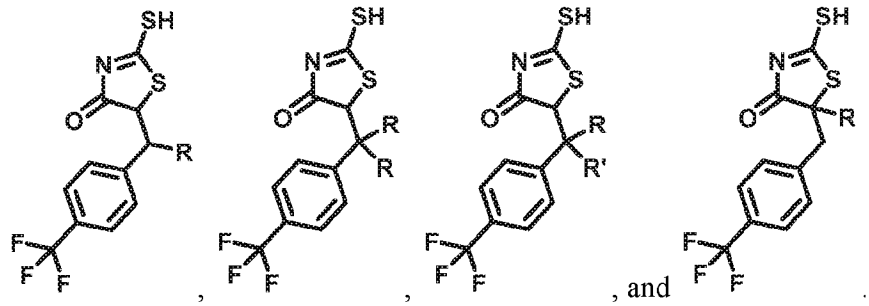


wherein:

R is the point at which the Linker is attached.

ZZ. PDZ domain dTAG Targeting Ligands:

PDZ domain dTAG Targeting Ligands as used herein include, but are not limited to:

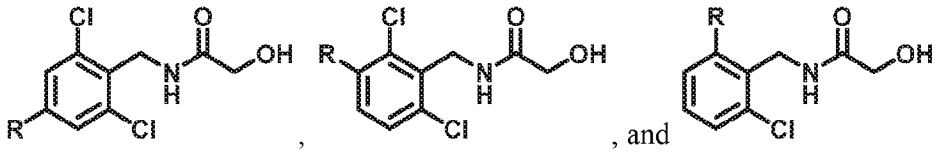


wherein:

R and R' are points at which the Linker(s) are attached.

AAA. PHIP dTAG Targeting Ligands:

PHIP dTAG Targeting Ligands as used herein include, but are not limited to:

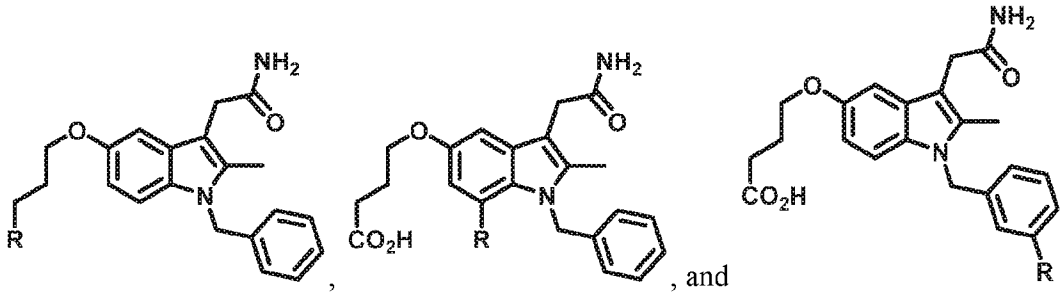


wherein:

R is the point at which the Linker is attached.

BBB. Phospholipase A2 domain dTAG Targeting Ligands:

Phospholipase A2 domain dTAG Targeting Ligands as used herein include, but are not limited to:

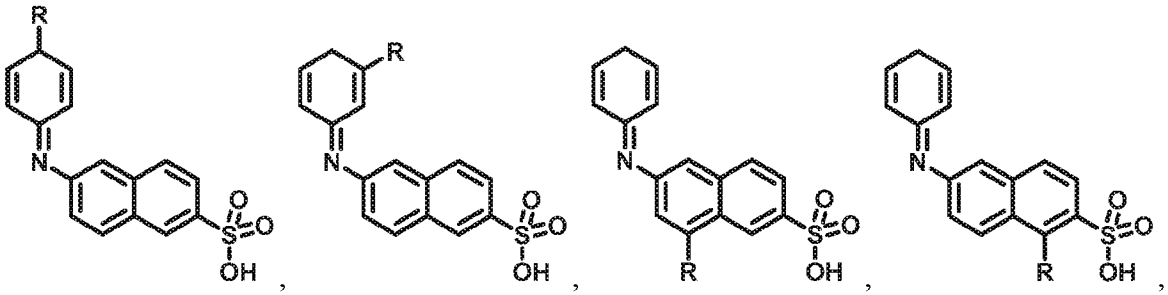


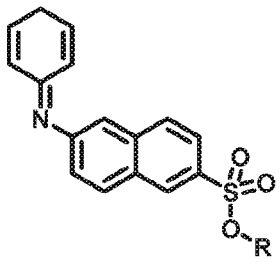
wherein:

R is the point at which the Linker is attached.

CCC. Protein S100-A7 2WOS dTAG Targeting Ligands:

Protein S100-A7 2WOS dTAG Targeting Ligands as used herein include, but are not limited to:





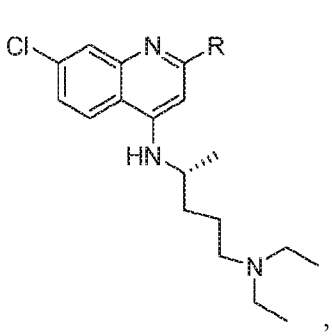
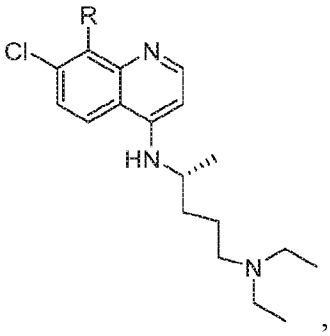
and

wherein:

R is the point at which the Linker is attached.

DDD. Saposin-B dTAG Targeting Ligands:

Saposin-B dTAG Targeting Ligands as used herein include, but are not limited to:


,

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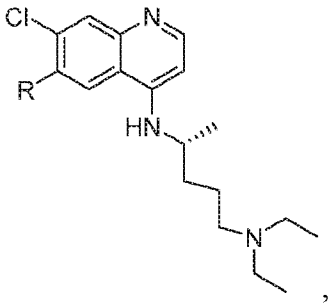
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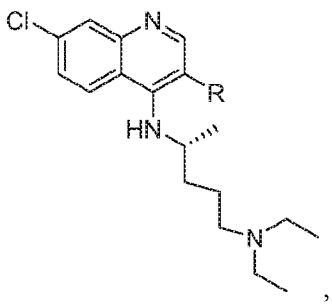
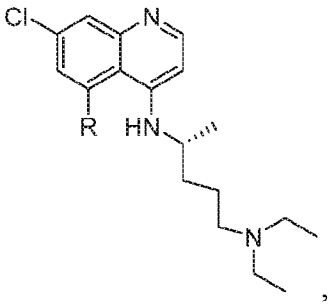
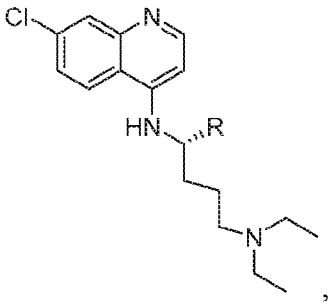

,

,

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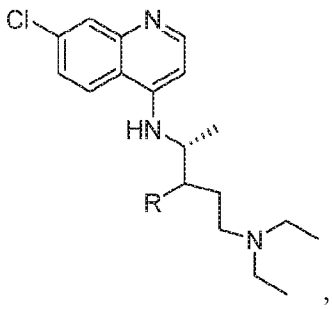
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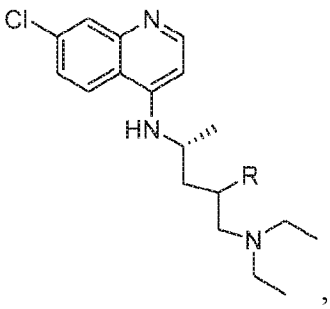
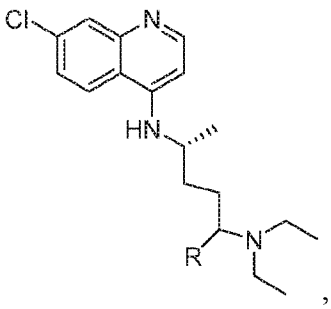

,

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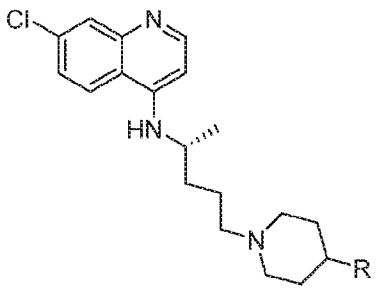
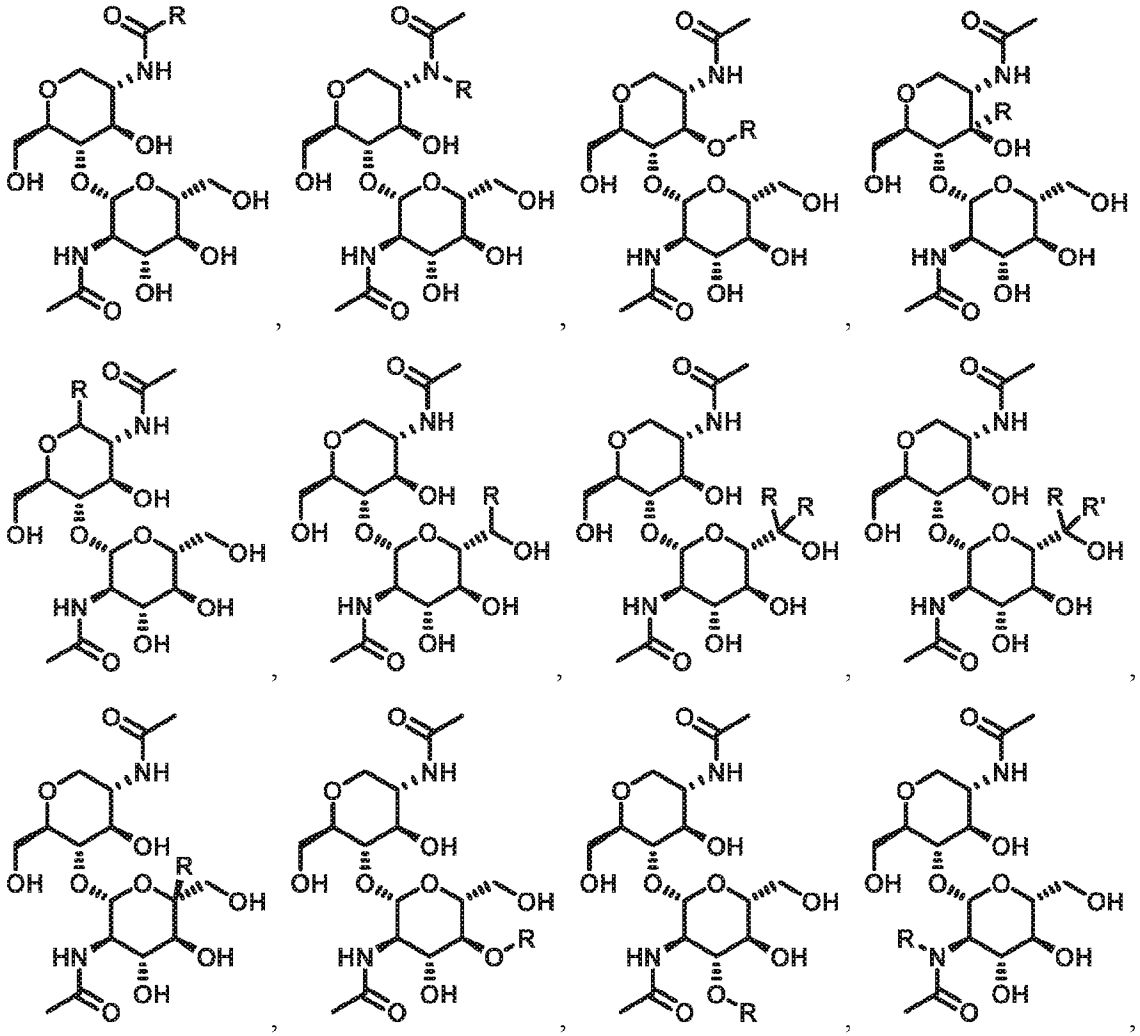


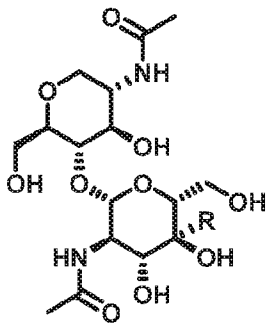
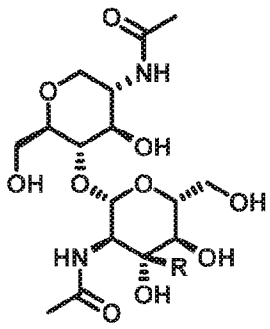
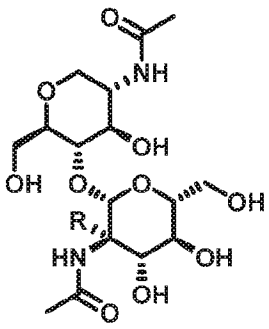
wherein:

R is the point at which the Linker is attached.

EEE. Sec7 dTAG Targeting Ligands:

Sec7 dTAG Targeting Ligands as used herein include, but are not limited to:



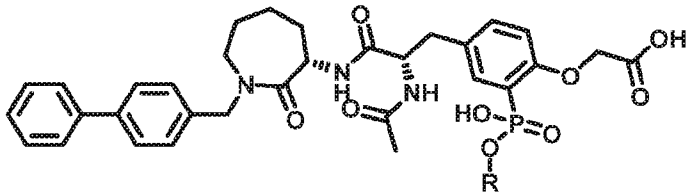
, , and

wherein:

R is the point at which the Linker is attached.

FFF. SH2 domain of pp60 Src dTAG Targeting Ligands:

SH2 domain of pp60 Src dTAG Targeting Ligands as used herein include, but are not limited to: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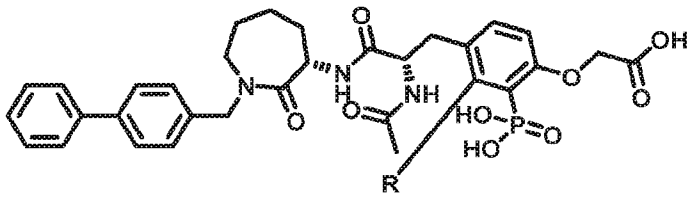
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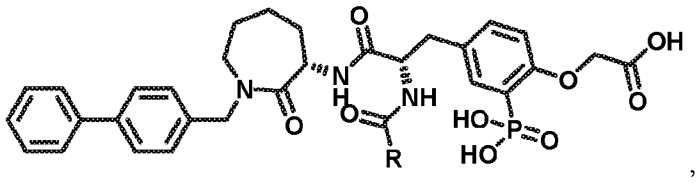
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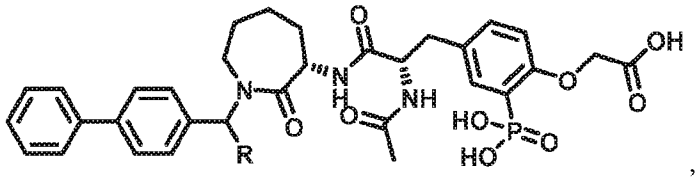
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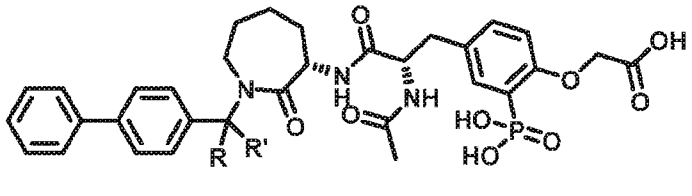
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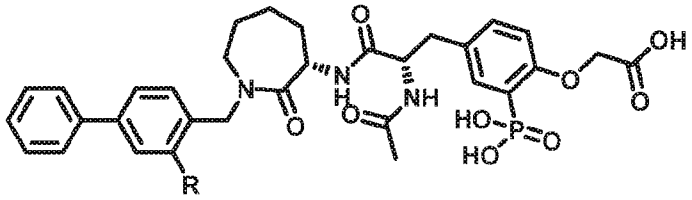
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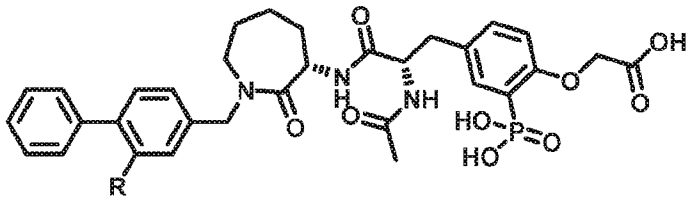
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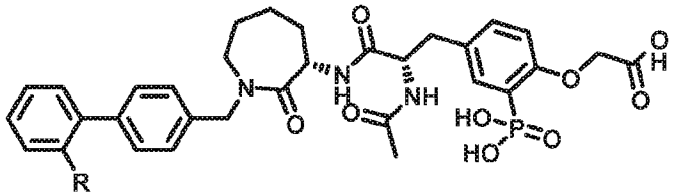
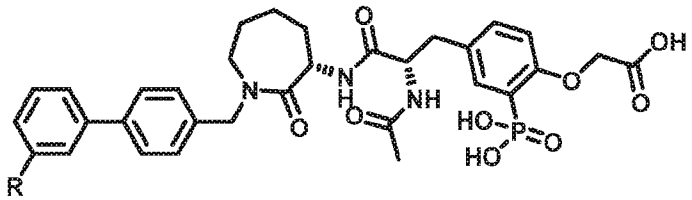

, and

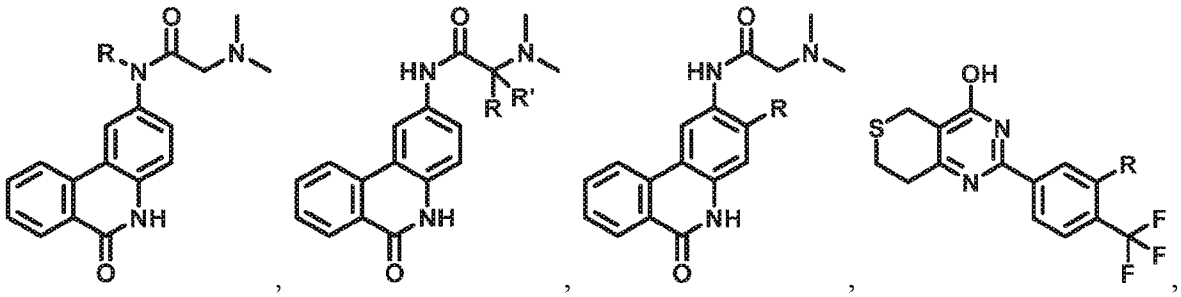


wherein:

R is the point at which the Linker is attached.

GGG. Tank1 dTAG Targeting Ligands:

Tank1 dTAG Targeting Ligands as used herein include, but are not limited to: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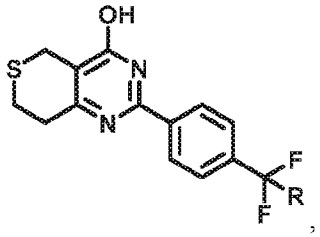
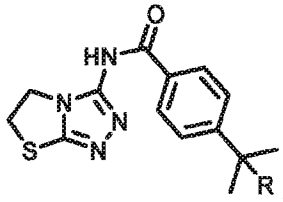
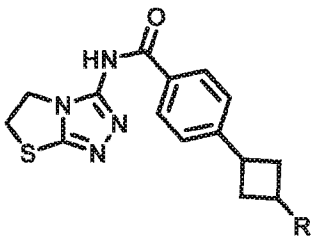
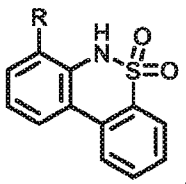
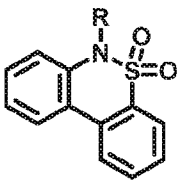
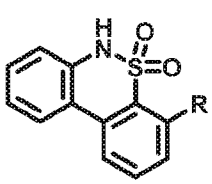
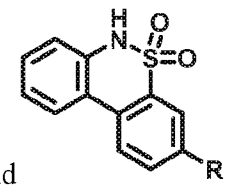




wherein:

R is the point at which the Linker is attached.

HHH. Ubc9 SUMO E2 ligase SF6D dTAG Targeting Ligands:

Ubc9 SUMO E2 ligase SF6D dTAG Targeting Ligands as used herein include, but are not limited to:

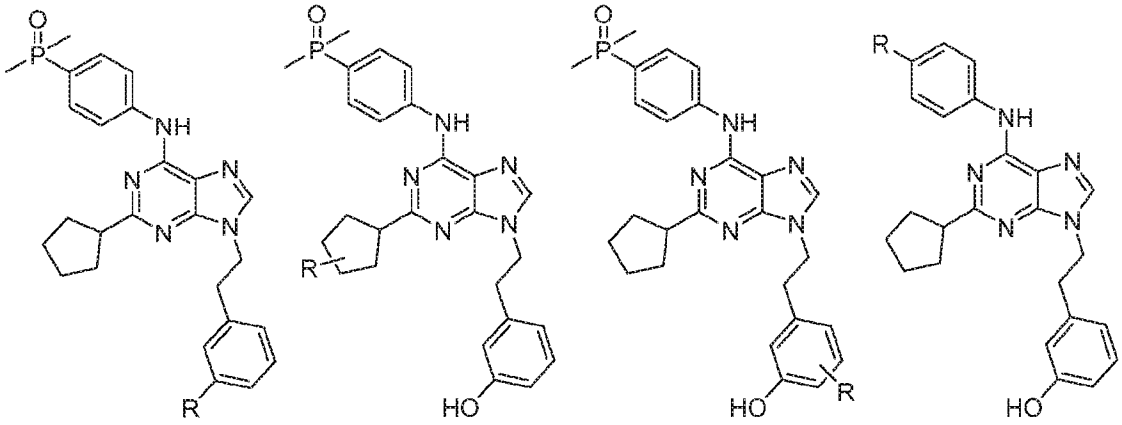
wherein:

R is the point at which the Linker is attached.

III. Src (c-Src) dTAG Targeting Ligands:

Src dTAG Targeting Ligands as used herein include, but are not limited to:

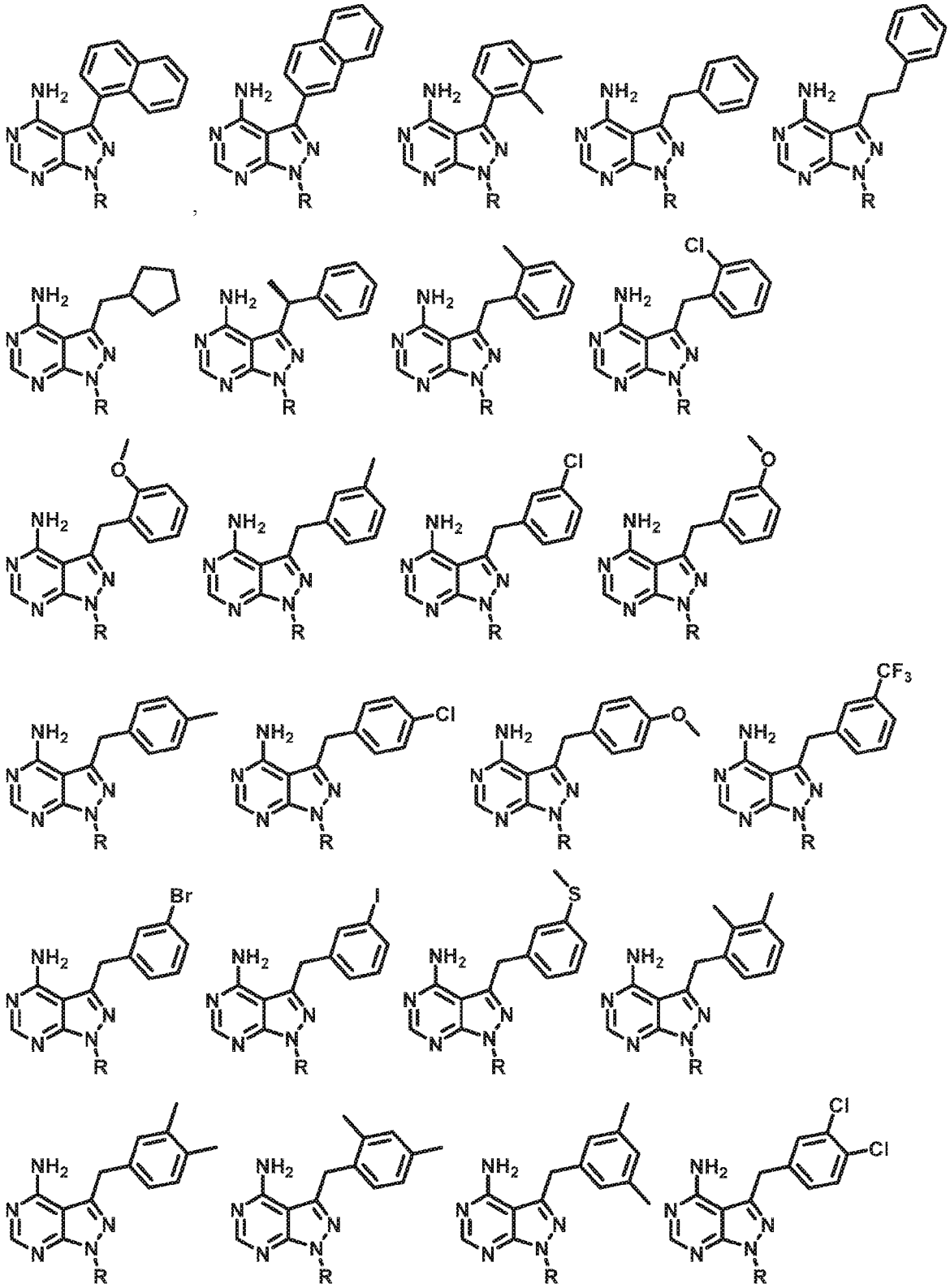
1. Src Targenting Ligands including AP23464:

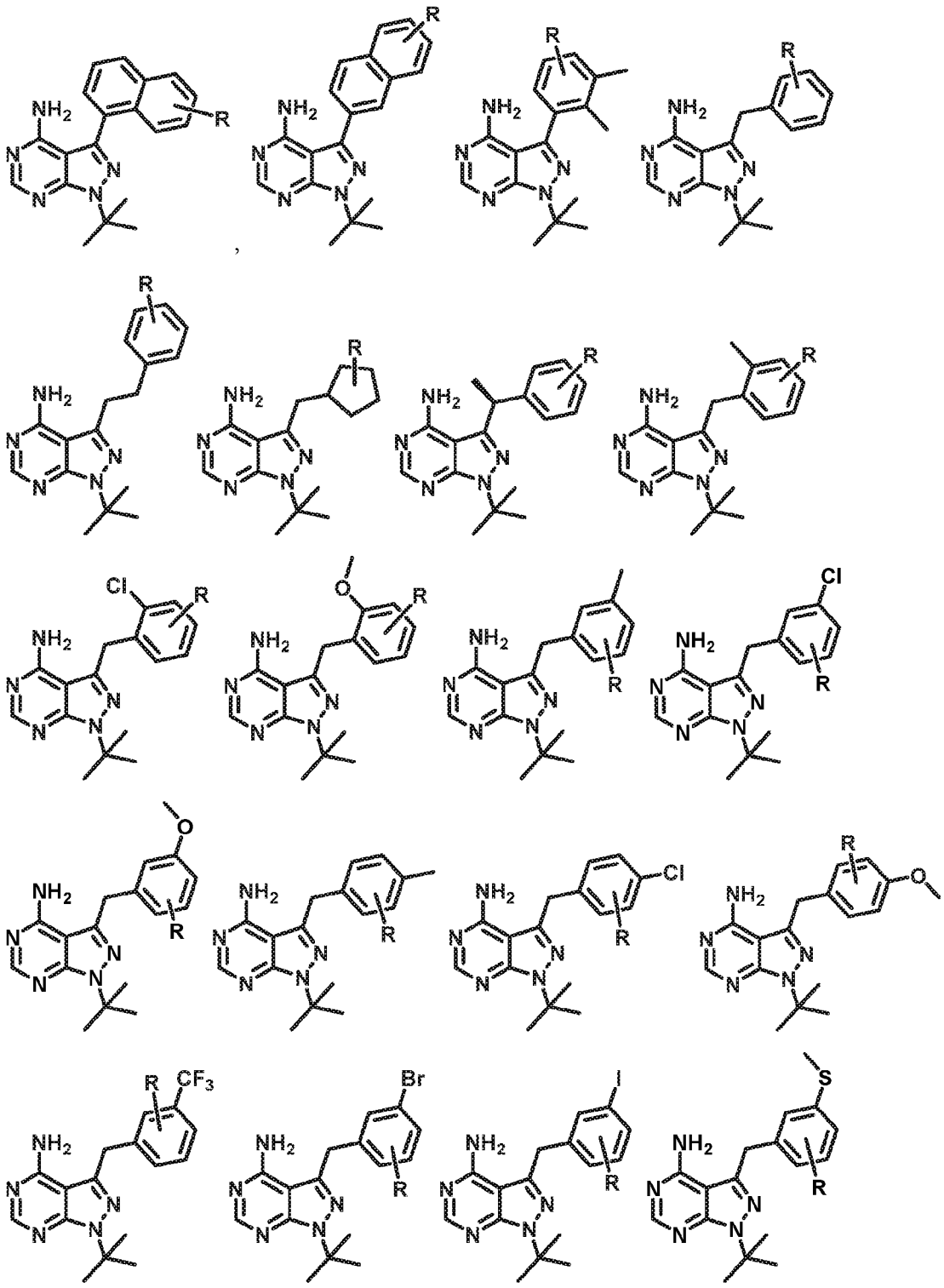


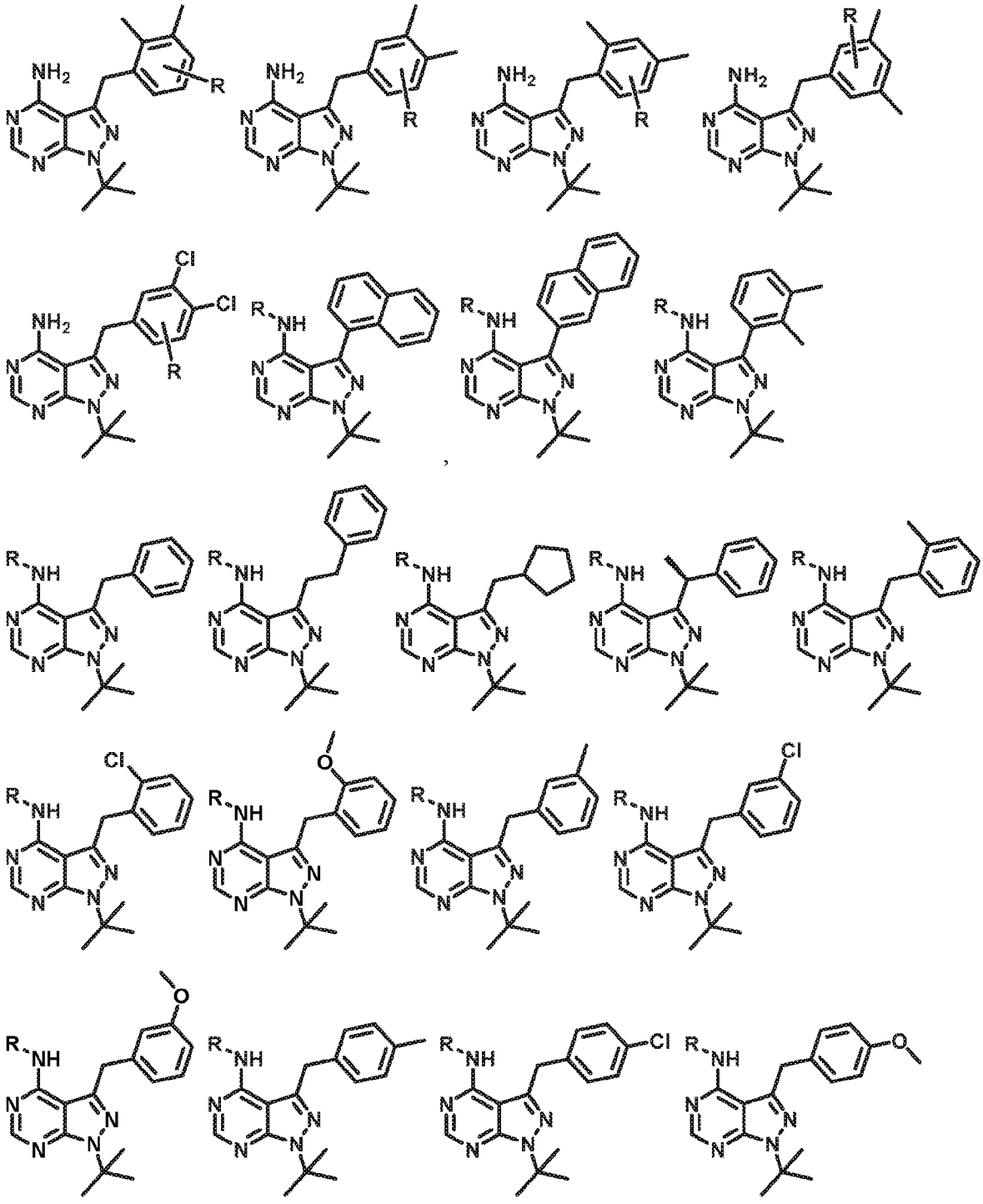
wherein:

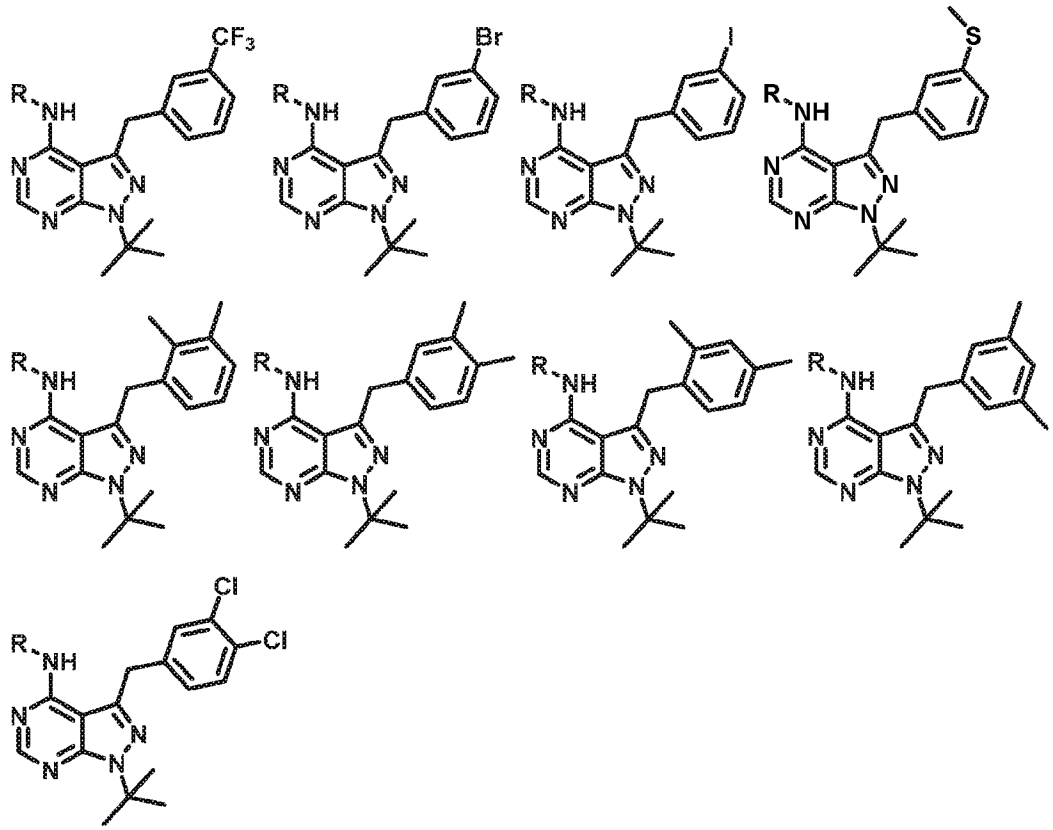
R is the point at which the Linker is attached.

2. Src-AS1 and/or Src AS2 Targeting Ligands including:









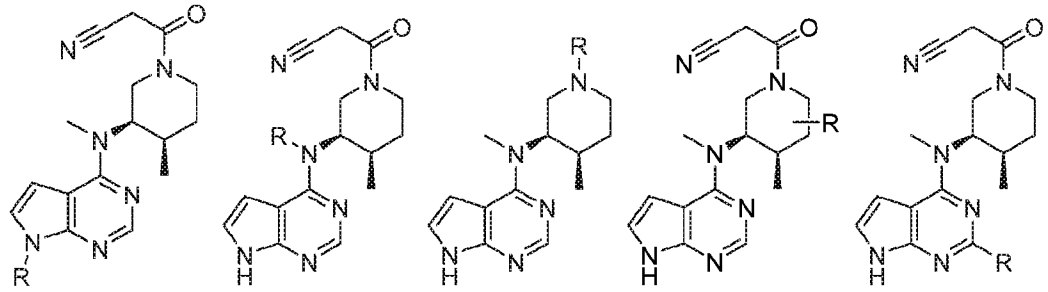
wherein:

R is the point at which the Linker is attached.

JJJ. JAK3 dTAG Targeting Ligands:

JAK3 dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target JAK3, including Tofacitinib:



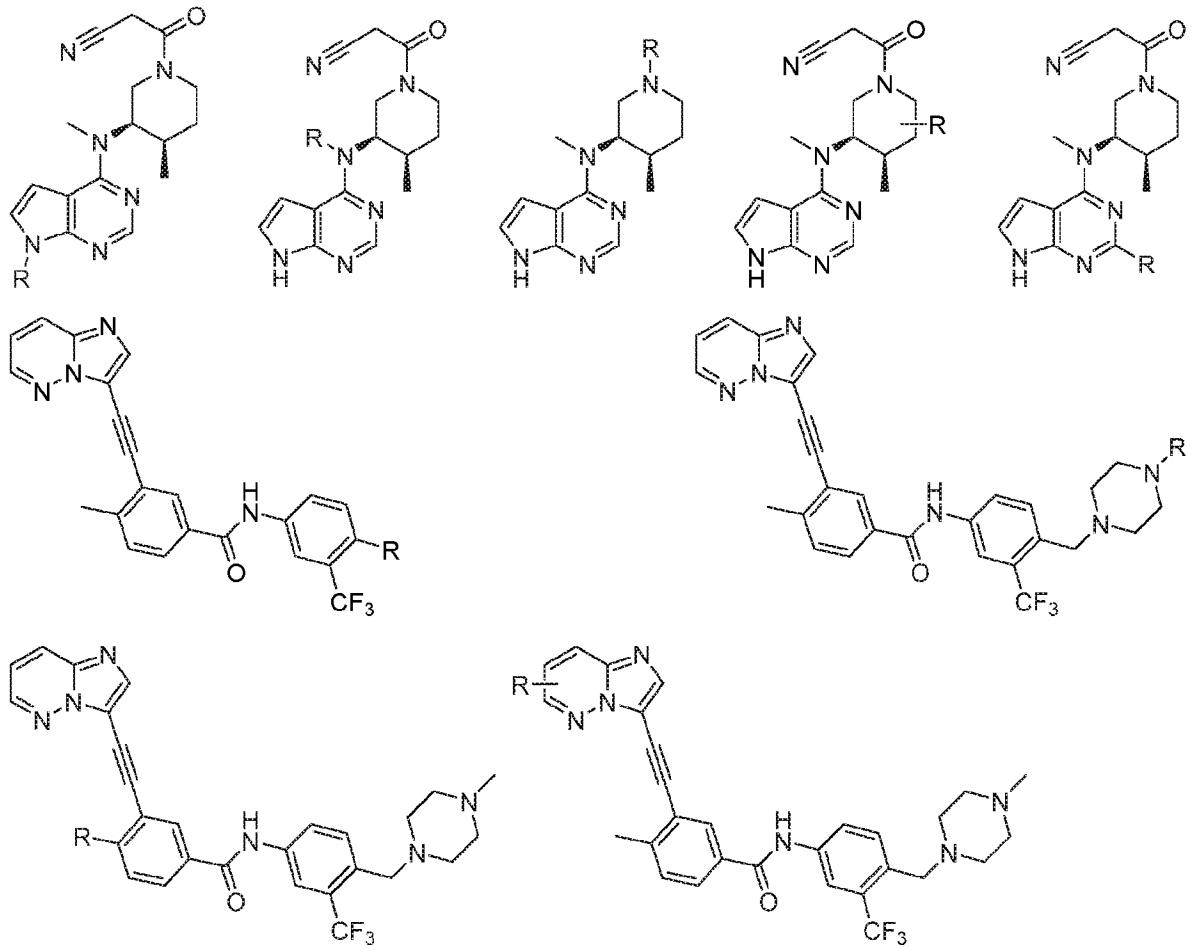
wherein:

R is the point at which the Linker is attached.

KKK. Abl dTAG Targeting Ligands:

Abl dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target Abl, including Tofacitinib and Ponatinib:



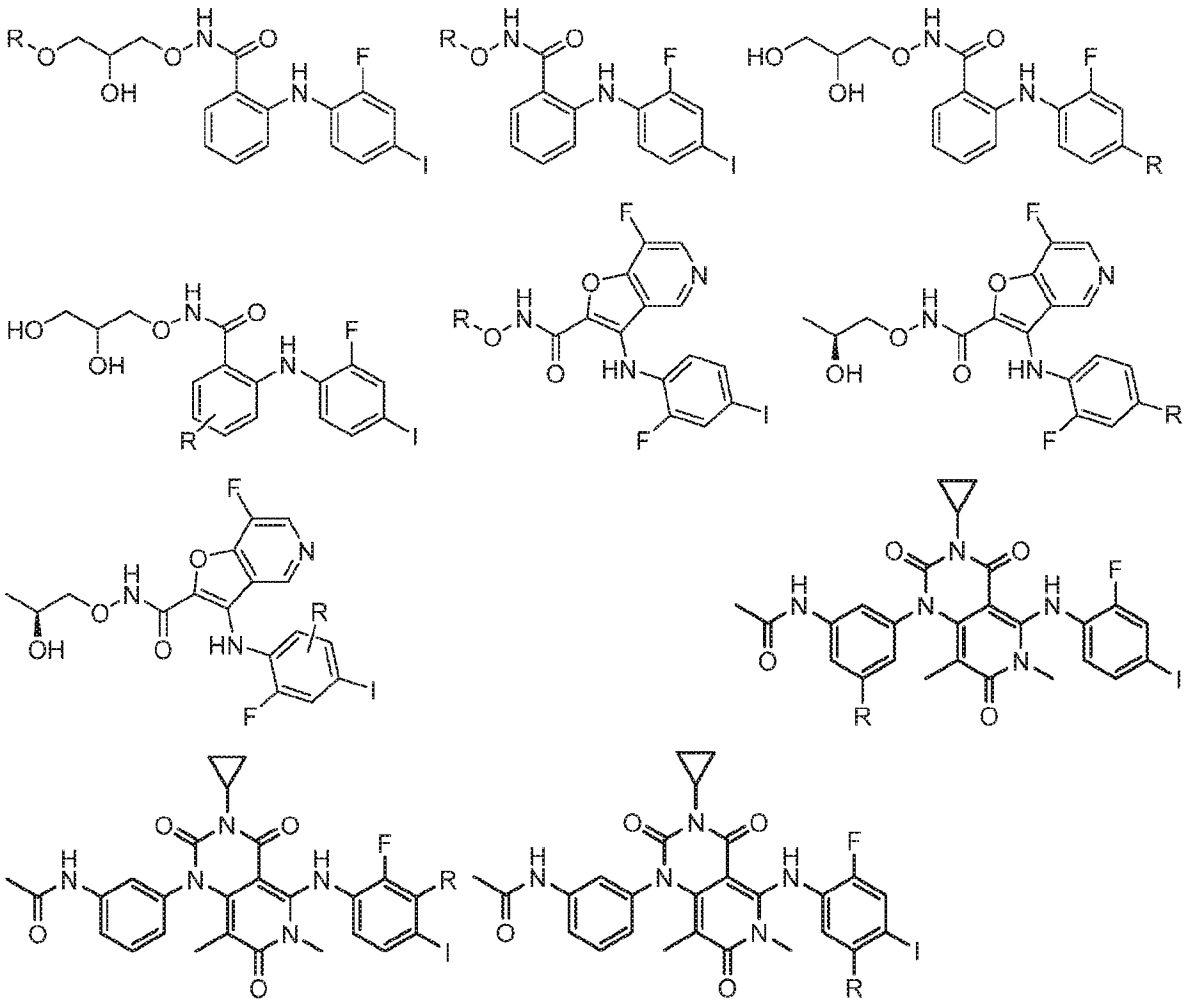
wherein:

R is the point at which the Linker is attached.

LLL. MEK1 dTAG Targeting Ligands:

MEK1 dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target MEK1, including PD318088, Trametinib, and G-573:



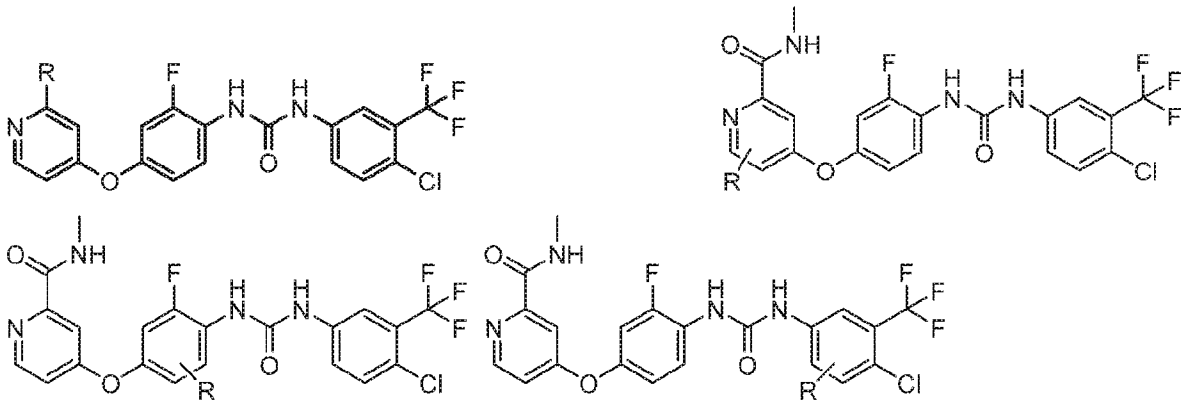
wherein:

R is the point at which the Linker is attached.

MMM. KIT dTAG Targeting Ligands:

KIT dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target KIT, including Regorafenib:



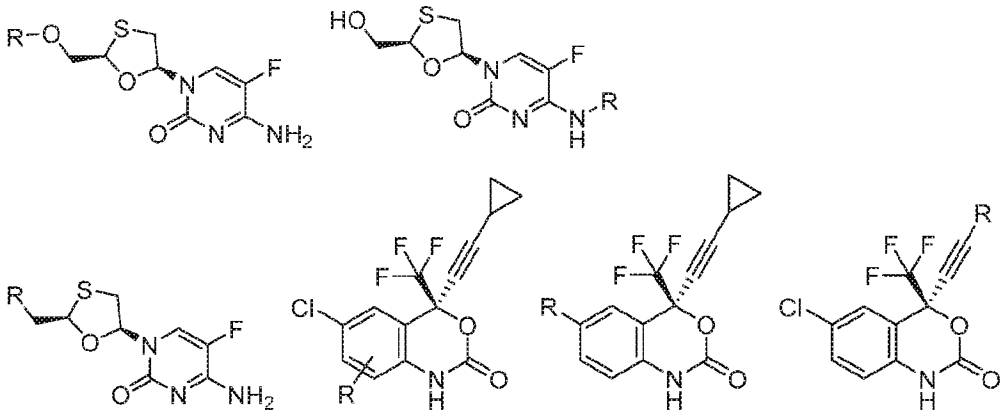
wherein:

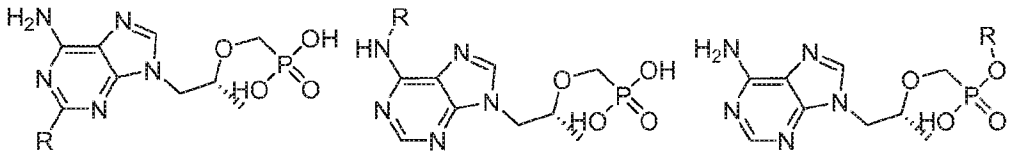
R is the point at which the Linker is attached.

NNN. HIV Reverse Transcriptase dTAG Targeting Ligands:

HIV Reverse Transcriptase dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target HIV Reverse Transcriptase, including Efavirenz, Tenofovir, Emtricitabine, Ritonavir, Raltegravir, and Atazanavir:





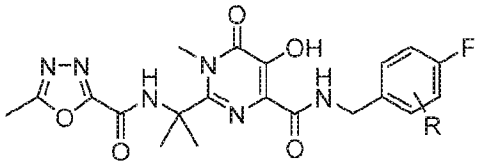
wherein:

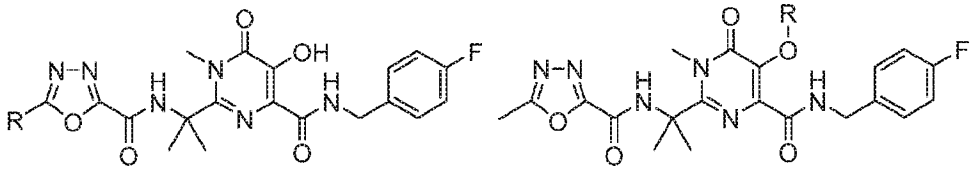
R is the point at which the Linker is attached.

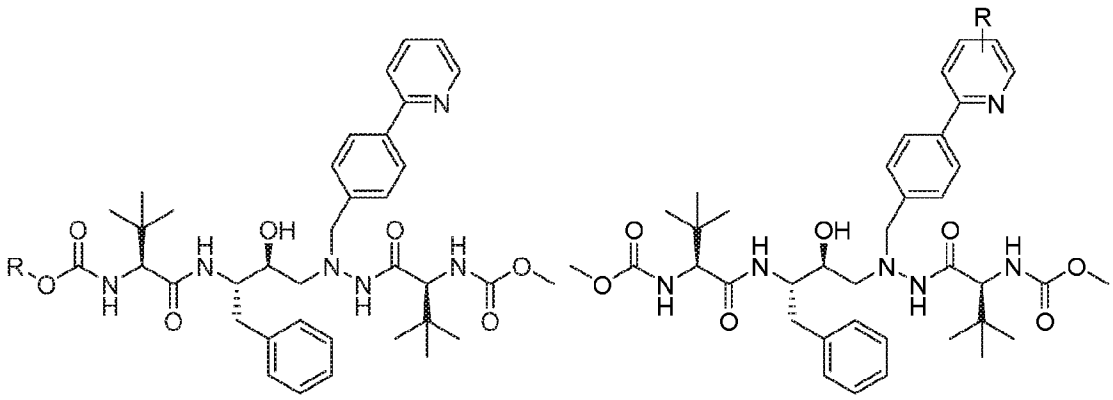
OOO. HIV Protease dTAG Targeting Ligands:

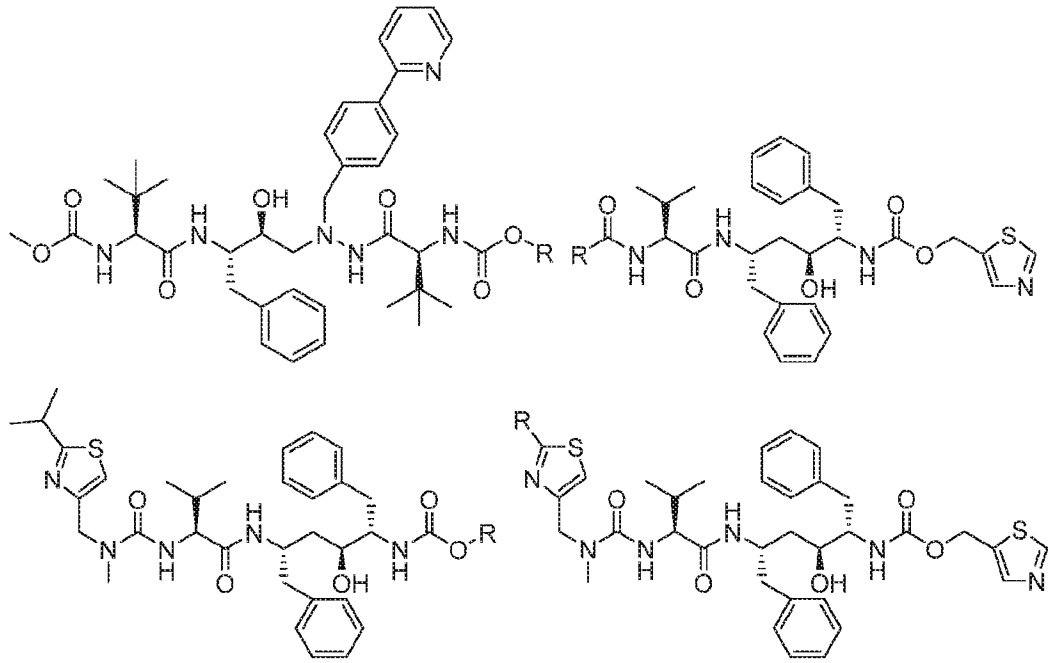
HIV Protease dTAG Targeting Ligands as used herein include, but are not limited to:

1. Targeting Ligands that target HIV Protease, including Ritonavir, Raltegravir, and Atazanavir:









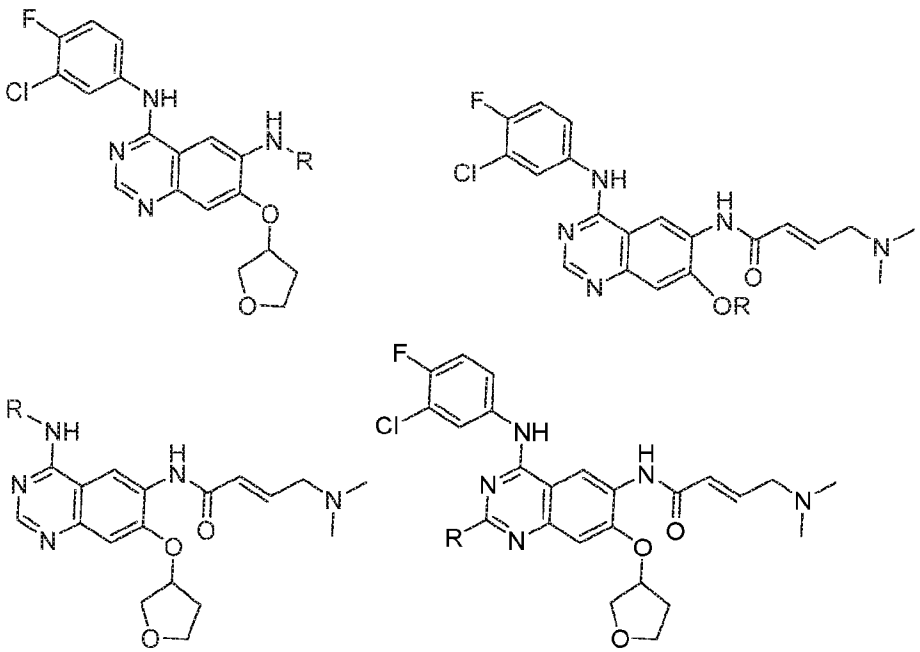
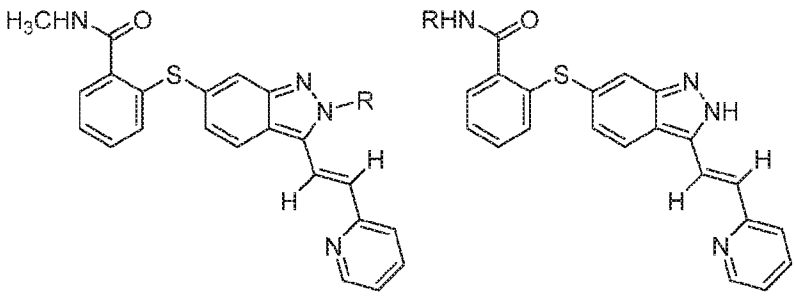
wherein:

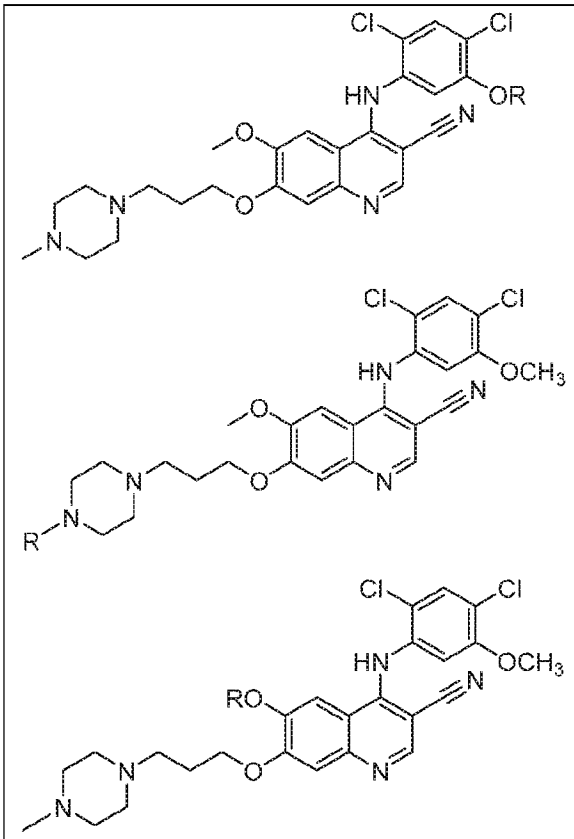
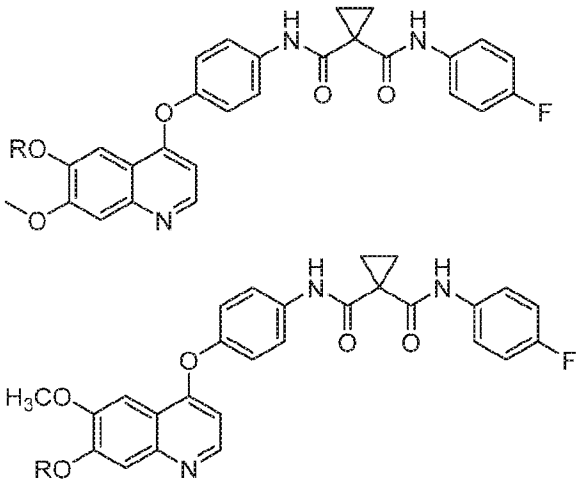
R is the point at which the Linker is attached.

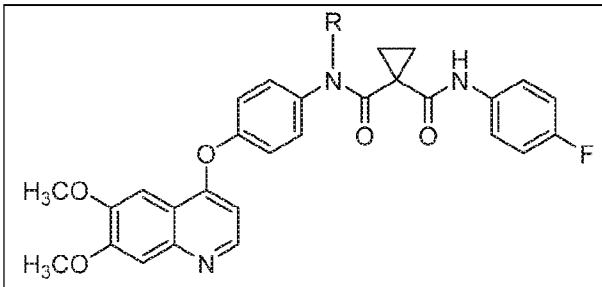
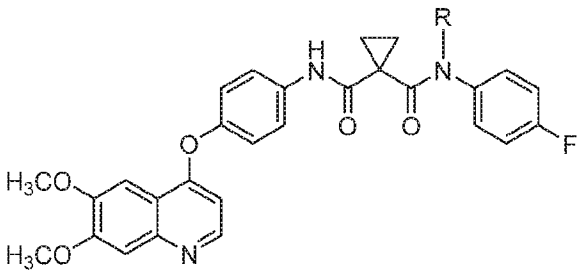
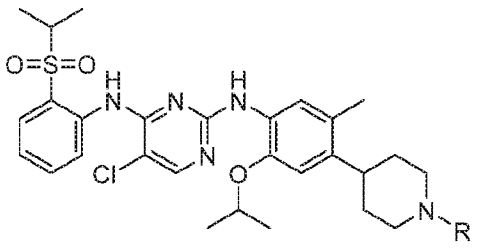
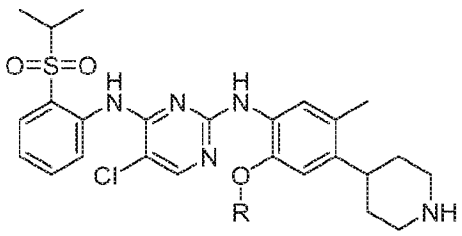
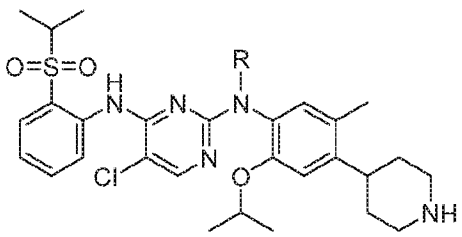
In one embodiment any of the above dTAG Targeting Ligands of Table T is used to target any dTAG described herein.

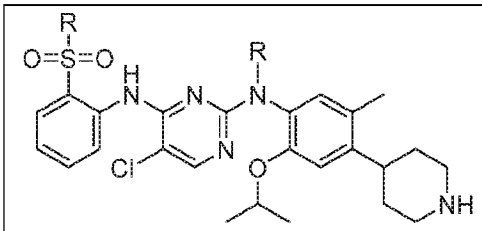
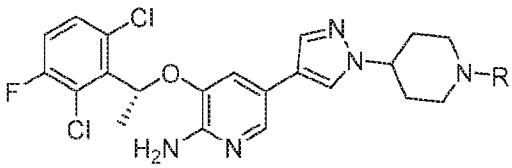
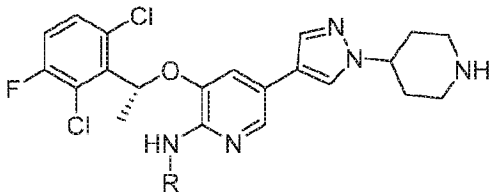
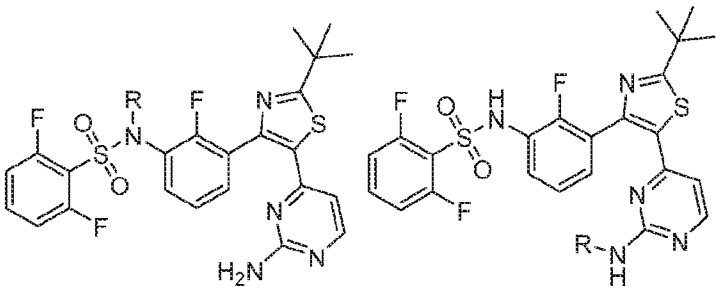
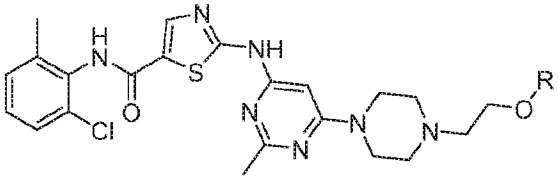
Many dTag targeting ligands are capable of binding to more than one dTAG, for example, Afatinib binds to the EGFR, the ErbB2, and the ErbB4 protein. This allows for dual attack of many proteins, as exemplified in Table Z. In one embodiment, the dTAG targeting ligand is selected from the "dTAG targeting ligand" column in Table Z and the dTAG is selected from the corresponding "dTAG" row.

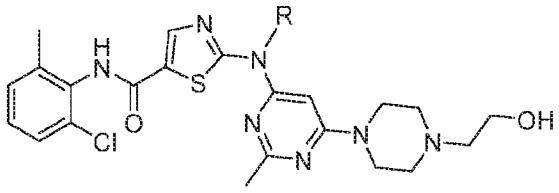
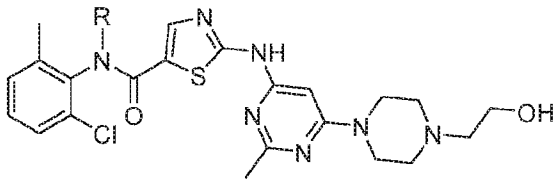
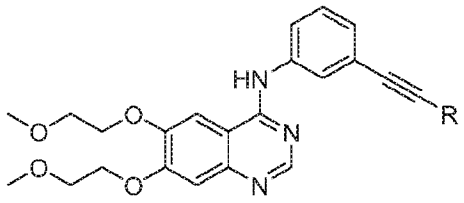
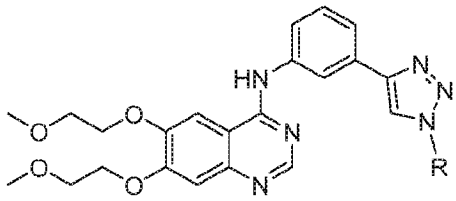
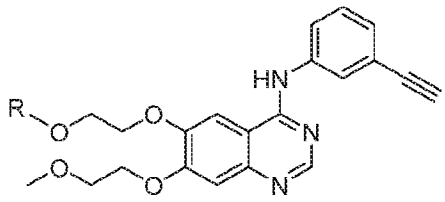
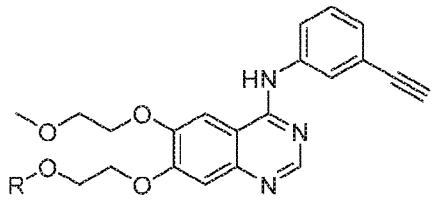
Table Z. dTAG Targeting Ligands and corresponding dTAG

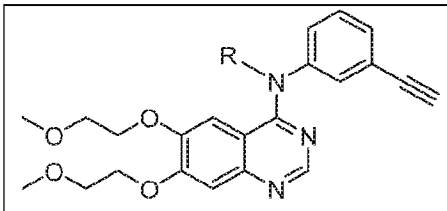
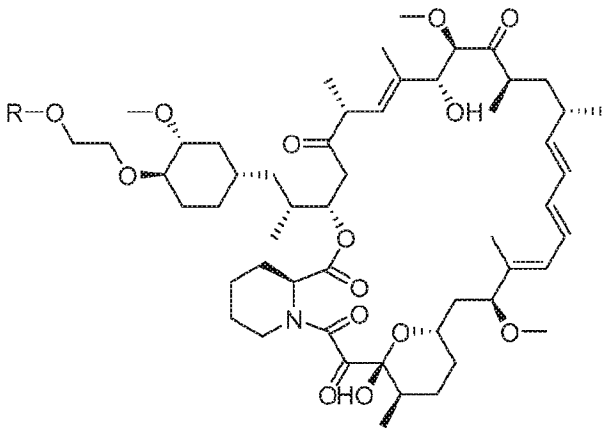
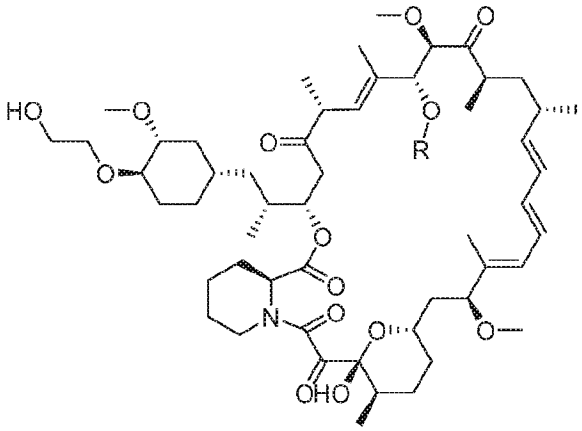
dTAG Targeting Ligand	dTAG
Afatinib 	EGFR, ErbB2/4
Axitinib 	VEGFR1/2/3, PDGFRβ, Kit
Bosutinib	BCR-Abl, Src, Lyn, Hck

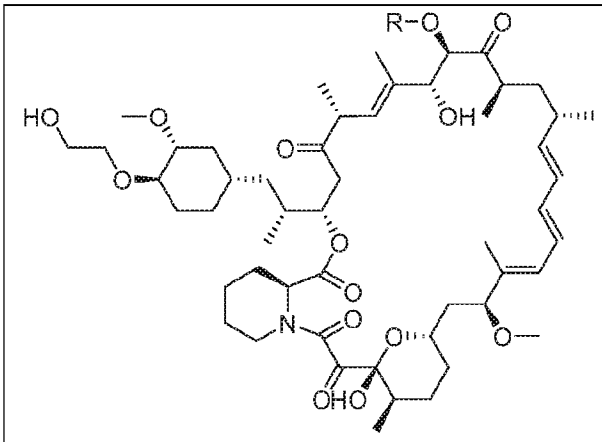
	
Cabozantinib 	RET, c-Met, VEGFR1/2/3, Kit,TrkB, Flt3, Axl, Tie2

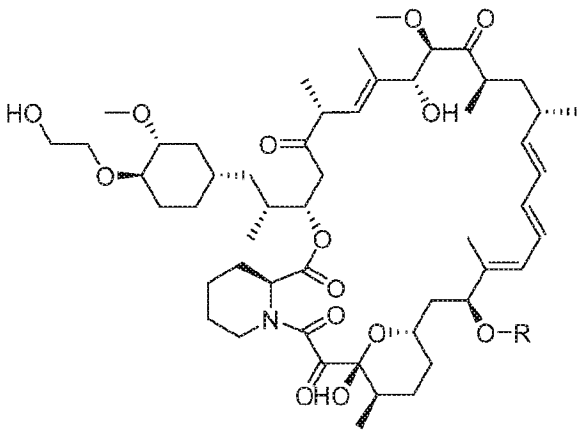
 	
Ceritinib   	ALK, IGF-1R, InsR, ROS1

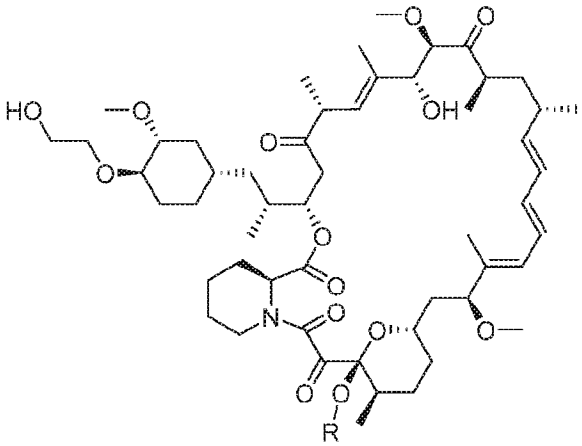
	
Crizotinib  	ALK, c-Met (HGFR), ROS1, MST1R
Dabrafenib 	B-Raf
Dasatinib 	BCR-Abl, Src, Lck, Lyn, Yes, Fyn, Kit, EphA2, PDGFRβ

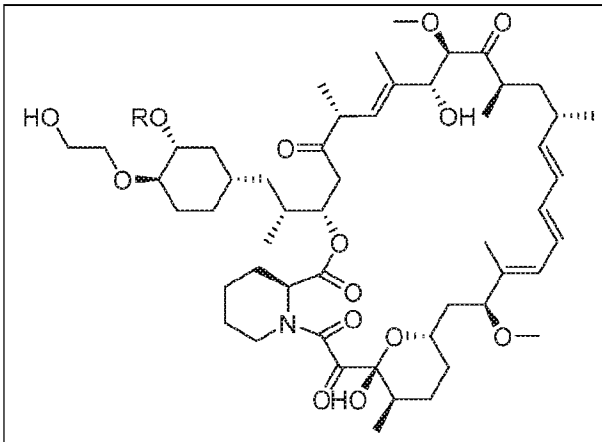
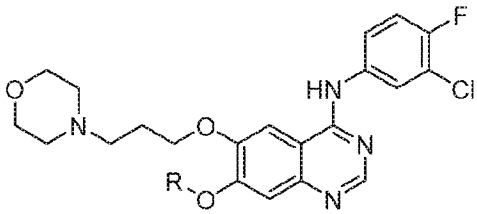
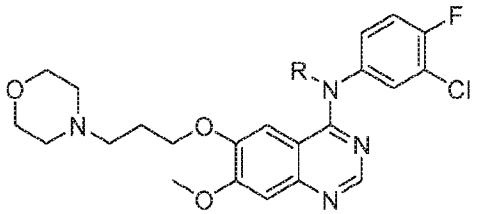
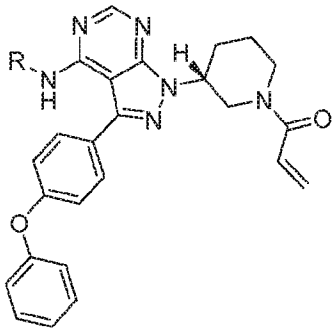
 	
Erlotinib    	EGFR

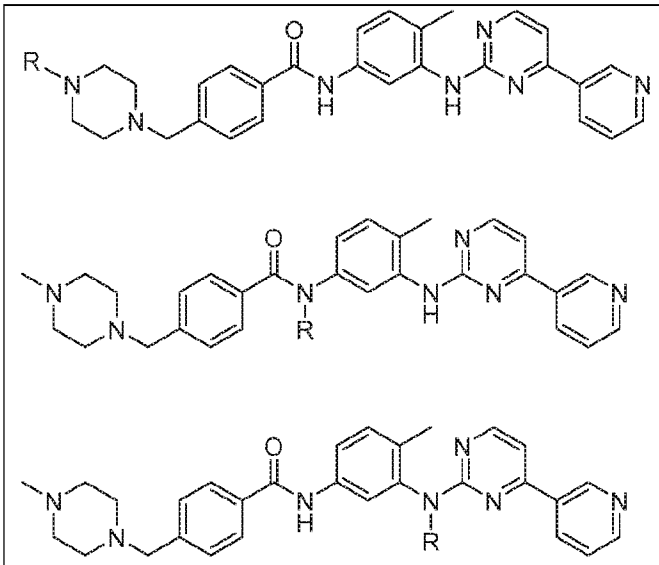
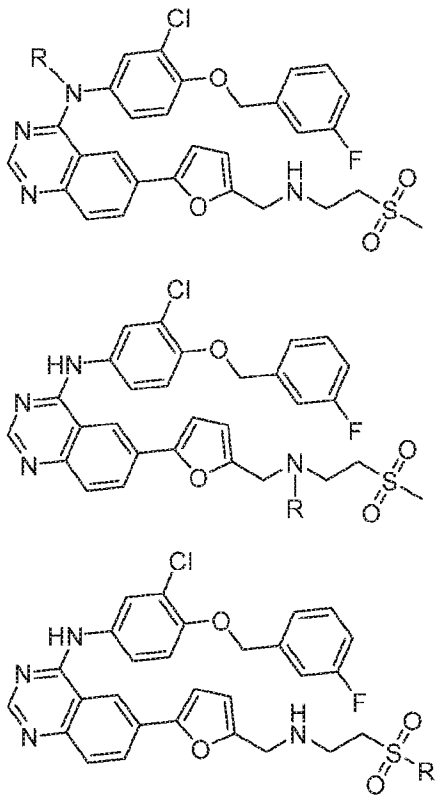
	
Everolimus  	HER2– breast cancer, PNET, RCC, RAML, SEGA

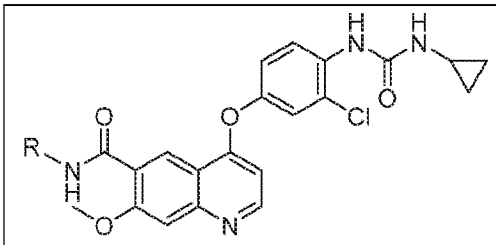
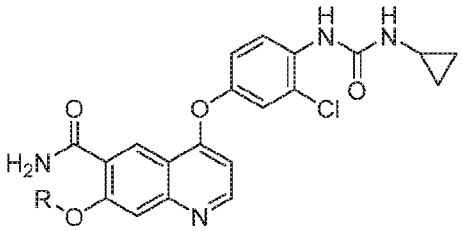
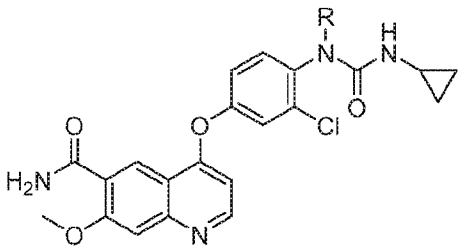
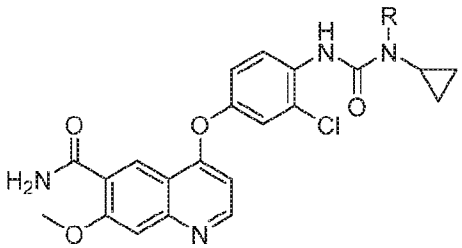
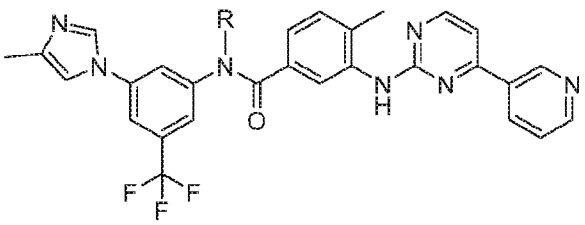

R–O
HO
—O
OH
O
N
OHO
O—

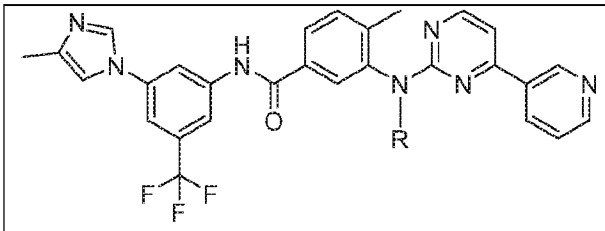
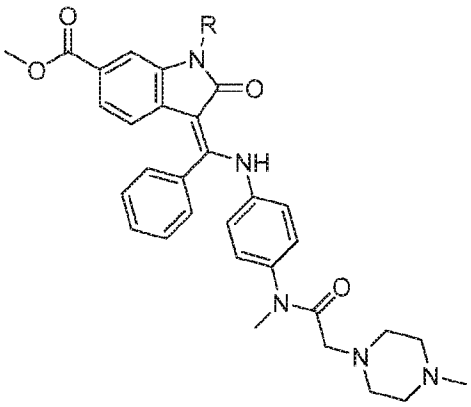
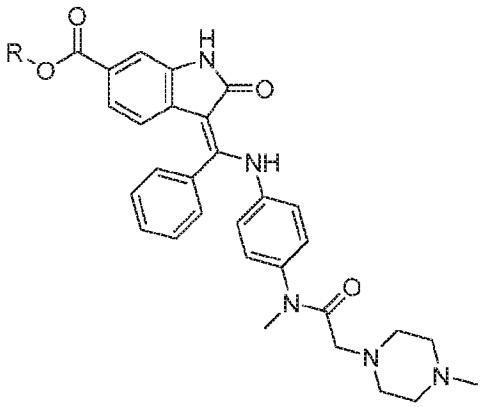

—O
HO
OH
O
N
OHO
O–R

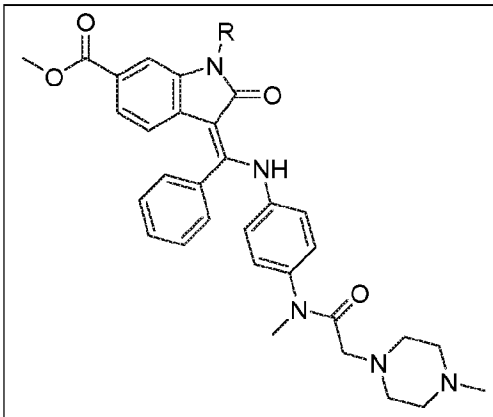

—O
HO
OH
O
N
O
R

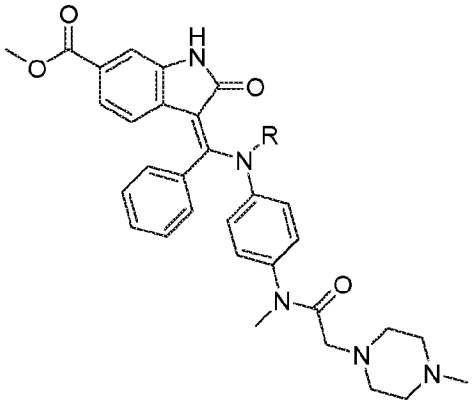
	
Gefitinib  	EGFR, PDGFR
Ibrutinib 	BTK
Imatinib	BCR-Abl, Kit, PDGFR

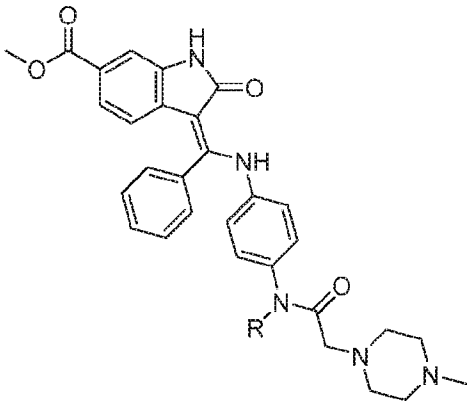
	
Lapatinib 	EGFR, ErbB2
Lenvatinib	VEGFR1/2/3, FGFR1/2/3/4,

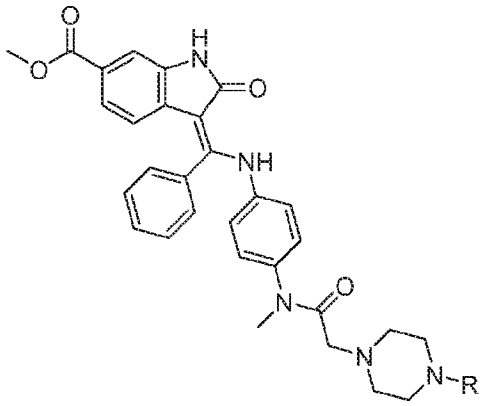
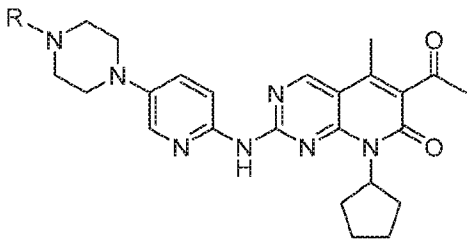
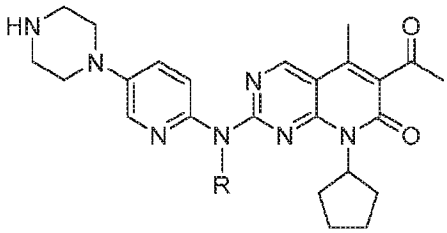
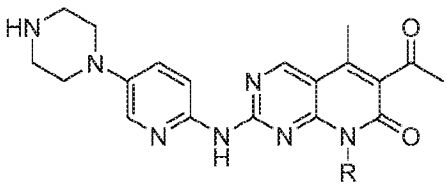
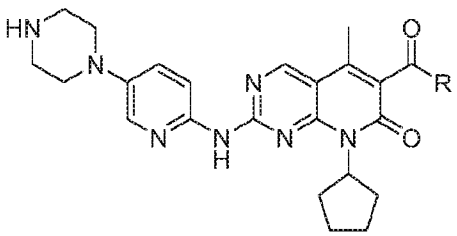
   	PDGFRα, Kit, RET
Nilotinib 	BCR-Abl, PDGFR, DDR1

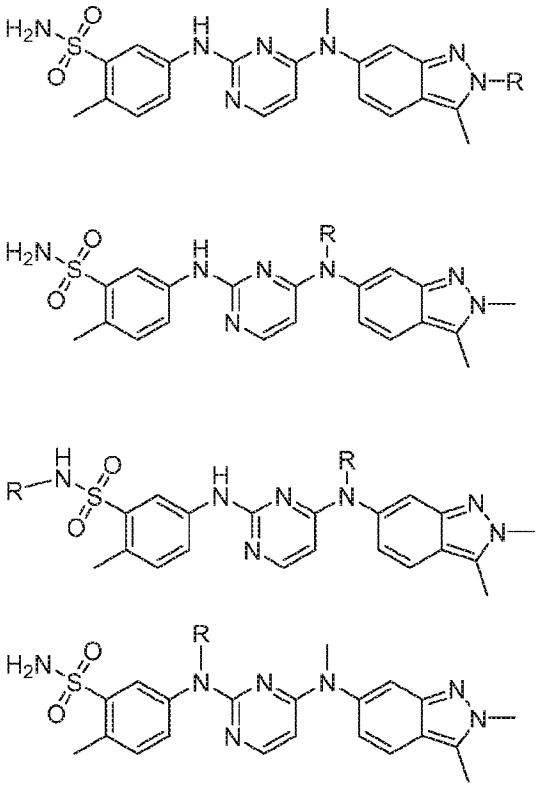
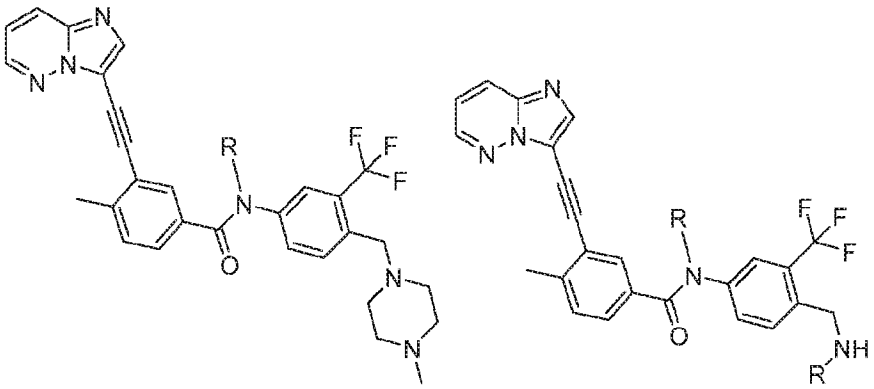
	
Nintedanib  	FGFR1/2/3, Flt3, Lck, PDGFRα/β, VEGFR1/2/3

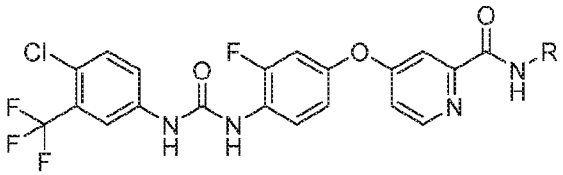
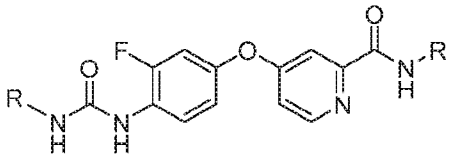
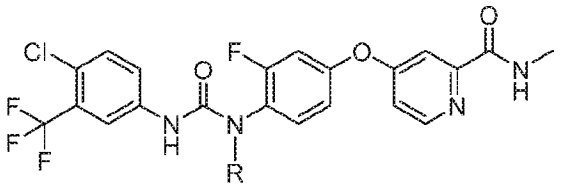
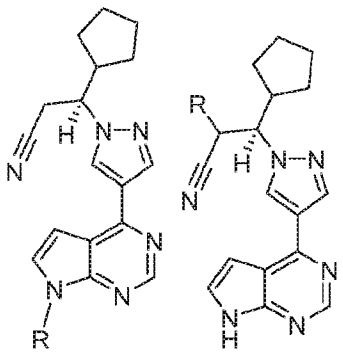
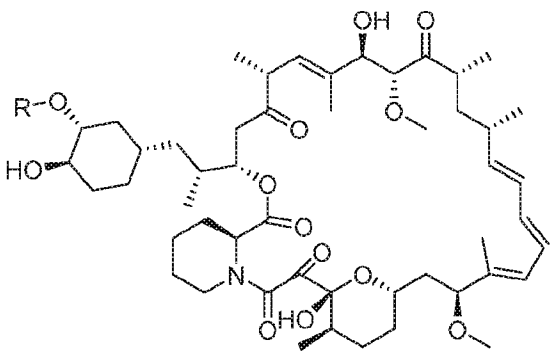


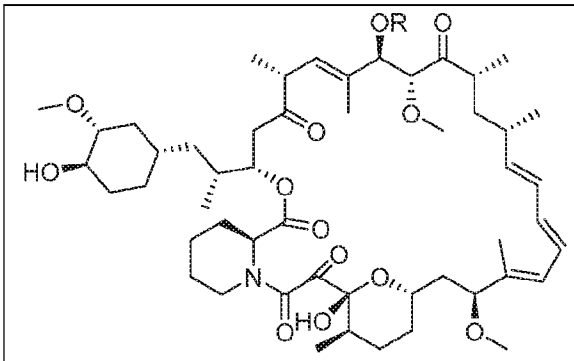


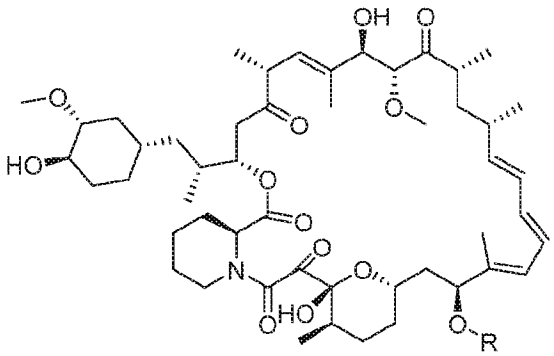


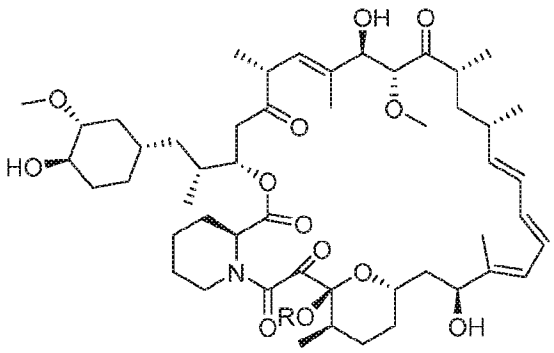
	
Palbociclib    	CDK4/6

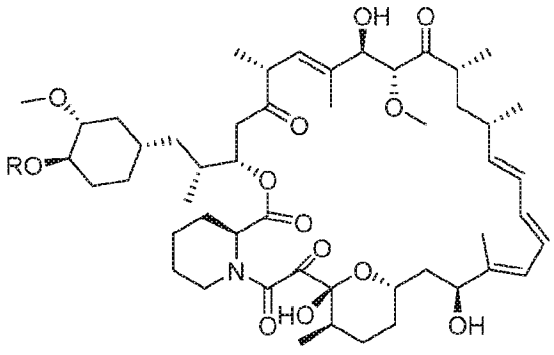
Pazopanib 	VEGFR1/2/3, PDGFRα/β, FGFR1/3, Kit, Lck, Fms, Itk
Ponatinib 	BCR-Abl, BCR-Abl T315I, VEGFR, PDGFR, FGFR, EphR,Src family kinases, Kit, RET, Tie2, Flt3
Regorafenib	VEGFR1/2/3, BCR-Abl, B-Raf, B-Raf (V600E), Kit, PDGFRα/β,

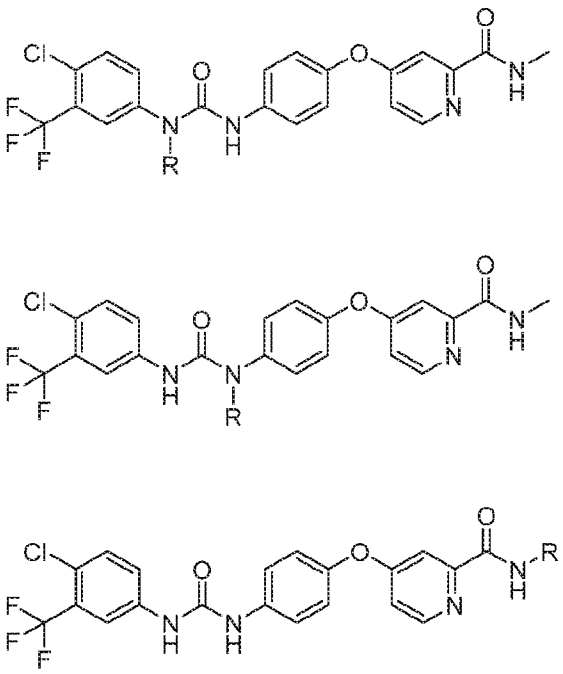
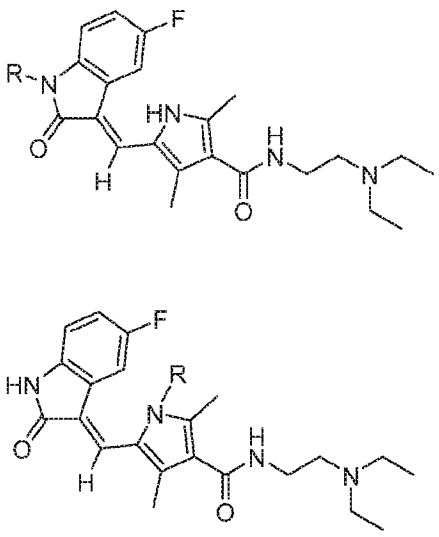
  	RET, FGFR1/2, Tie2, and Eph2A
Ruxolitinib 	JAK1/2
Sirolimus 	FKBP12/mTOR

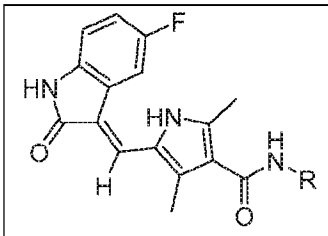
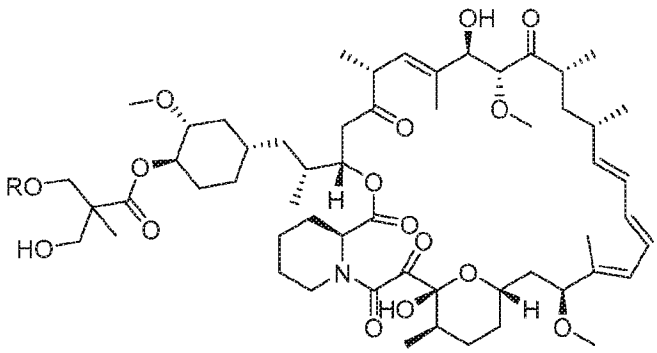
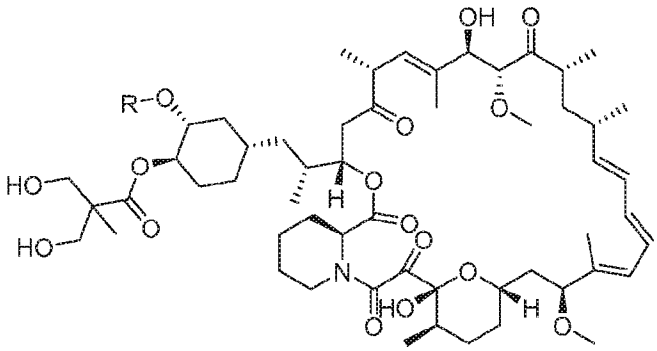
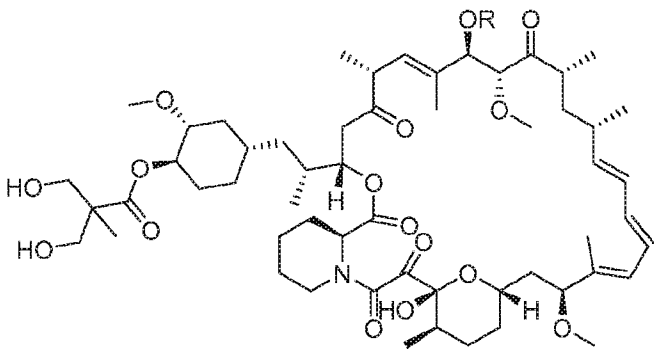


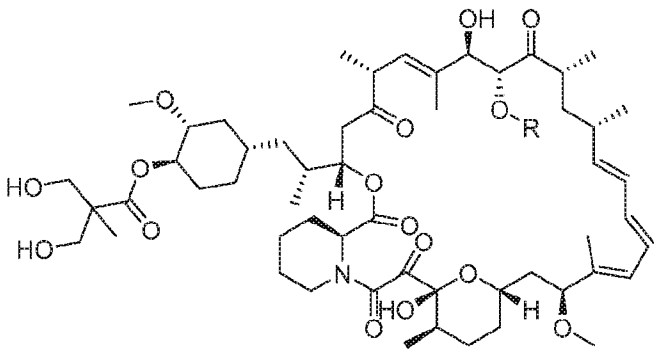
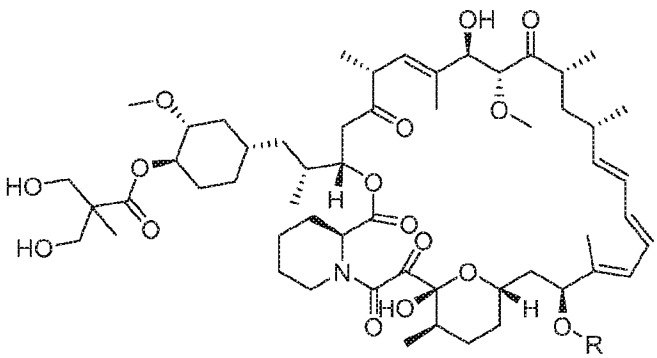
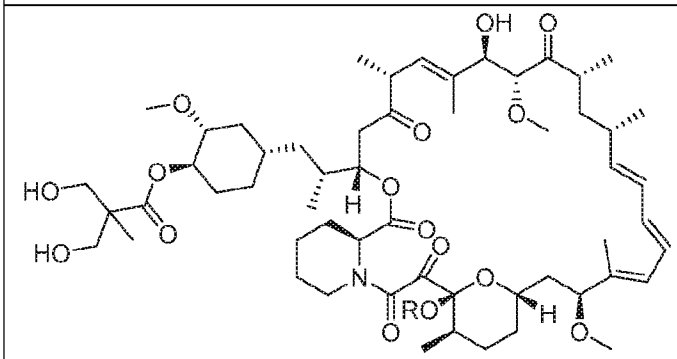


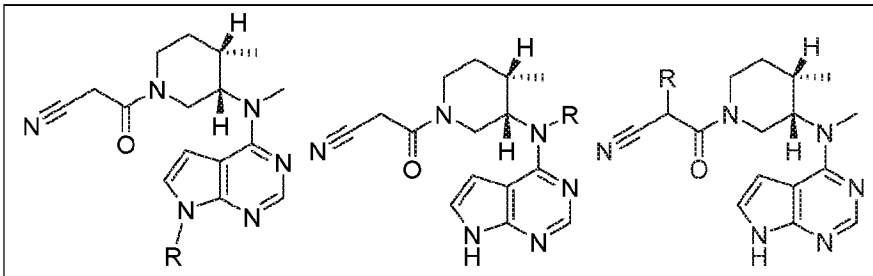
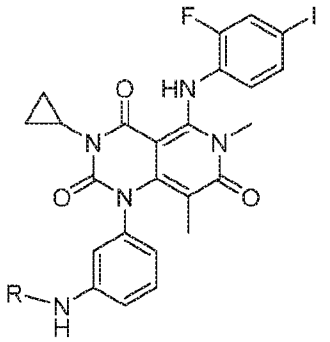
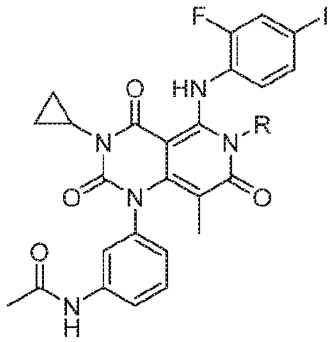
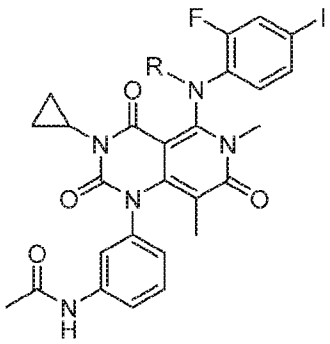
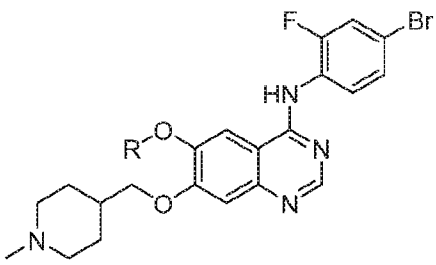


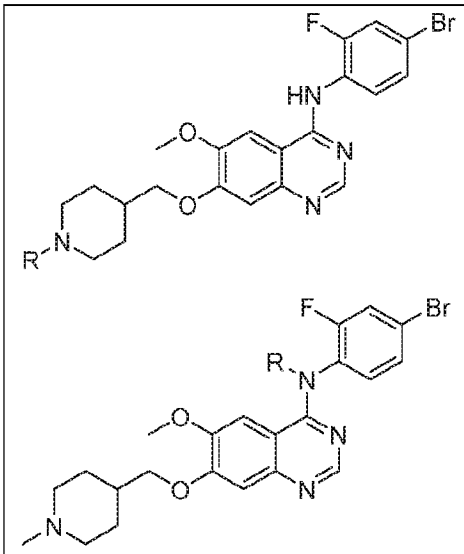
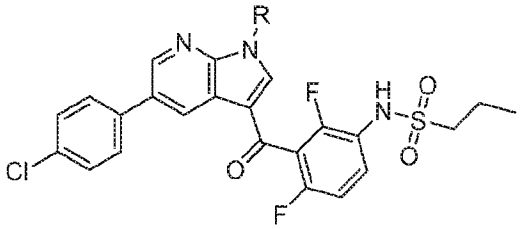
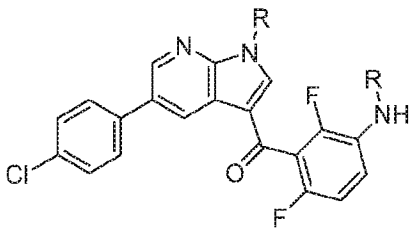


Sorafenib 	B-Raf, CDK8, Kit, Flt3, RET, VEGFR1/2/3, PDGFR
Sunitinib 	PDGFRα/β, VEGFR1/2/3, Kit, Flt3, CSF-1R, RET

	
Temsirolimus   	FKBP12/mTOR

 	
	
Tofacitinib	JAK3

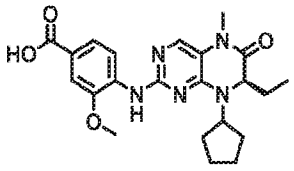
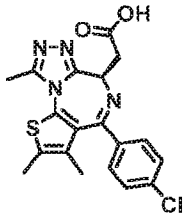
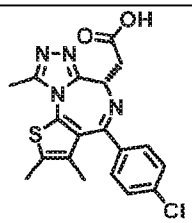
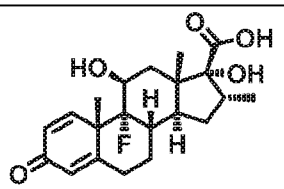
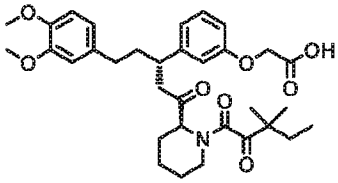
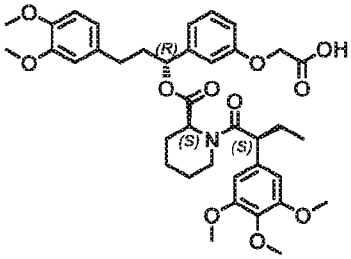
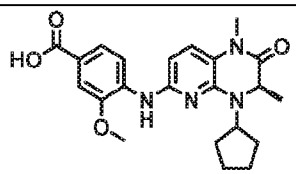
	
Trametinib   	MEK1/2
Vandetanib 	EGFR, VEGFR, RET, Tie2, Brk, EphR

	
Vemurafenib  	A/B/C-Raf and B-Raf (V600E)

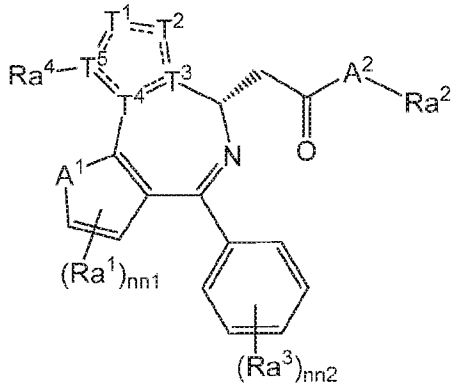
In certain embodiments, the present application includes compounds containing the dTAG Targeting Ligands shown in Table 1.

5

Table 1. dTAG Targeting Ligands 1-6

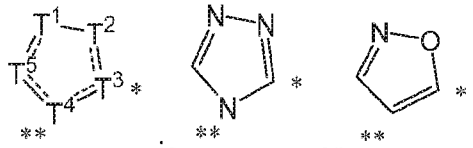
Compound	Structure	Compound	Structure
TL1	 Ang. Chem. Int'l. Ed. 50, 9378 (2011)	TL3	
TL2		TL4	
TL5	 JACS 115, 9925 (1993)	TL6	
TL7			

In certain embodiments, the dTAG Targeting Ligand is a compound of Formula TL-I:



(TL-I),

or a pharmaceutically acceptable salt thereof, wherein:



is or ;

A^1 is S or C=C;

A^2 is NRa^5 or O;

nn1 is 0, 1, or 2;

each Ra^1 is independently C_1-C_3 alkyl, $(CH_2)_{0-3}$-CN, $(CH_2)_{0-3}$-halogen, $(CH_2)_{0-3}$-OH, $(CH_2)_{0-3}$-C_1-C_3 alkoxy, $C(O)NRa^5L$, OL, NRa^5L, or L;

Ra^2 is H, C_1-C_6 alkyl, $(CH_2)_{0-3}$-heterocyclyl, $(CH_2)_{0-3}$-phenyl, or L, wherein the heterocyclyl comprises one saturated 5- or 6-membered ring and 1-2 heteroatoms selected from N, O, and S and is optionally substituted with C_1-C_3 alkyl, L, or C(O)L, and wherein the phenyl is optionally substituted with C_1-C_3 alkyl, CN, halogen, OH, C_1-C_3 alkoxy, or L;

nn2 is 0, 1, 2, or 3;

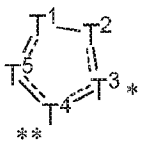
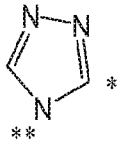
each Ra^3 is independently C_1-C_3 alkyl, $(CH_2)_{0-3}$-CN, $(CH_2)_{0-3}$-halogen, L, or $C(O)NRa^5L$;

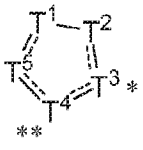
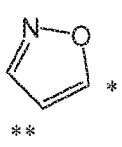
Ra^4 is C_1-C_3 alkyl;

Ra^5 is H or C_1-C_3 alkyl; and

L is a Linker,

provided that the compound of Formula TL-I is substituted with only one L.

In certain embodiments,  is  .

In certain embodiments,  is  .

In certain embodiments, A^1 is S.

In certain embodiments, A^1 is C=C.

In certain embodiments, A^2 is NRa^5. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, Ra^5 is methyl.

In certain embodiments, A^2 is O.

In certain embodiments, nn1 is 0.

In certain embodiments, nn1 is 1.

In certain embodiments, nn1 is 2.

In certain embodiments, at least one Ra^1 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, at least one Ra^1 is methyl. In further embodiments, two Ra^1 are methyl.

In certain embodiments, at least one Ra^1 is CN, (CH_2)-CN, $(CH_2)_2$-CN, or $(CH_2)_3$-CN. In further embodiments, at least one Ra^1 is (CH_2)-CN.

In certain embodiments, at least one Ra^1 is halogen (*e.g.*, F, Cl, or Br), (CH_2)-halogen, $(CH_2)_2$-halogen, or $(CH_2)_3$-halogen. In further embodiments, at least one Ra^1 is Cl, (CH_2)-Cl, $(CH_2)_2$-Cl, or $(CH_2)_3$-Cl.

In certain embodiments, at least one Ra^1 is OH, (CH_2)-OH, $(CH_2)_2$-OH, or $(CH_2)_3$-OH.

In certain embodiments, at least one Ra^1 is C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy), (CH_2)-C_1-C_3 alkoxy, $(CH_2)_2$-C_1-C_3 alkoxy, or $(CH_2)_3$-C_1-C_3 alkoxy. In certain embodiments, at least one Ra^1 is methoxy.

In certain embodiments, one Ra^1 is $C(O)NRa^5L$. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, one Ra^1 is OL.

In certain embodiments, one Ra^1 is NRa^5L. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In other embodiments, Ra^5 is methyl.

In certain embodiments, one Ra^1 is L.

In certain embodiments, Ra^2 is H.

In certain embodiments, Ra^2 is straight-chain C_1-C_6 or branched C_3-C_6 alkyl (*e.g.*, methyl, ethyl, propyl, i-propyl, butyl, i-butyl, t-butyl, pentyl, or hexyl). In further embodiments, Ra^2 is methyl, ethyl, or t-butyl.

In certain embodiments, Ra^2 is heterocyclyl, (CH_2)-heterocyclyl, $(CH_2)_2$-heterocyclyl, or $(CH_2)_3$-heterocyclyl. In further embodiments, Ra^2 is $(CH_2)_3$-heterocyclyl. In further embodiments, the heterocyclyl is selected from pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, piperidinyl, piperazinyl, hexahydropyrimidinyl, morpholinyl, and thiomorpholinyl. In further embodiments, the heterocyclyl is piperazinyl.

In certain embodiments, the heterocyclyl is substituted with C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, the heterocyclyl is substituted with C(O)L.

In certain embodiments, the heterocyclyl is substituted with L.

In certain embodiments, Ra^2 is phenyl, (CH_2)-phenyl, $(CH_2)_2$-phenyl, or $(CH_2)_3$-phenyl. In further embodiments, Ra^2 is phenyl.

In certain embodiments, the phenyl is substituted with C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In certain embodiments, the phenyl is substituted with CN. In certain embodiments, the phenyl is substituted with halogen (*e.g.*, F, Cl, or Br). In certain embodiments, the phenyl is substituted with OH. In certain embodiments, the phenyl is substituted with C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy).

In certain embodiments, the phenyl is substituted with L.

In certain embodiments, Ra^2 is L.

In certain embodiments, nn2 is 0.

In certain embodiments, nn2 is 1.

In certain embodiments, nn2 is 2.

In certain embodiments, nn2 is 3.

In certain embodiments, at least one Ra^3 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, at least one Ra^3 is methyl.

In certain embodiments, at least one Ra^3 is CN, (CH_2)-CN, $(CH_2)_2$-CN, or $(CH_2)_3$-CN. In further embodiments, at least one Ra^3 is CN.

In certain embodiments, at least one Ra^3 is halogen (*e.g.*, F, Cl, or Br), (CH_2)-halogen, $(CH_2)_2$-halogen, or $(CH_2)_3$-halogen. In further embodiments, at least one Ra^3 is Cl, (CH_2)-Cl, $(CH_2)_2$-Cl, or $(CH_2)_3$-Cl. In further embodiments, at least one Ra^3 is Cl.

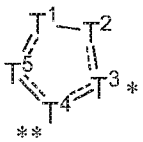
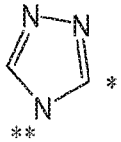
In certain embodiments, one Ra^3 is L.

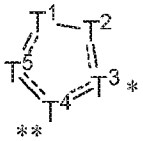
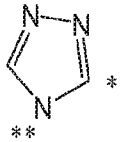
In certain embodiments, one Ra^3 is $C(O)NRa^5L$. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

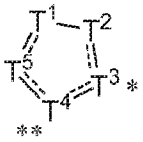
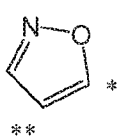
In certain embodiments, Ra^4 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, Ra^4 is methyl.

In certain embodiments, Ra^5 is H.

In certain embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, Ra^5 is methyl.

In certain embodiments,  is  , and A^1 is S.

In certain embodiments,  is  , and A^1 is C=C.

In certain embodiments,  is  , and A^1 is C=C.

In certain embodiments, A^2 is NH, and Ra^2 is $(CH_2)_{0-3}$-heterocyclyl. In further embodiments, Ra^2 is $(CH_2)_3$-heterocyclyl. In further embodiments, the heterocyclyl is piperazinyl. In further embodiments, the heterocyclyl is substituted with C_1-C_3 alkyl, L, or C(O)L.

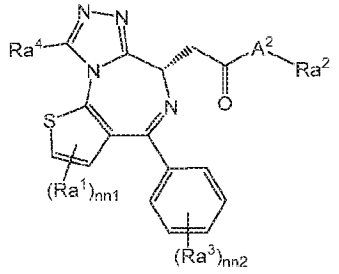
In certain embodiments, A^2 is NH, and Ra^2 is $(CH_2)_{0-3}$-phenyl. In further embodiments, Ra^2 is phenyl. In further embodiments, the phenyl is substituted with OH or L.

In certain embodiments, A^2 is NH, and Ra^2 is L.

In certain embodiments, A^2 is NH, and Ra^2 is H or C_1-C_6 alkyl. In further embodiments, Ra^2 is C_1-C_4 alkyl.

In certain embodiments, A^2 is O, and Ra^2 is H or C_1-C_6 alkyl. In further embodiments, Ra^2 is C_1-C_4 alkyl.

In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I1:

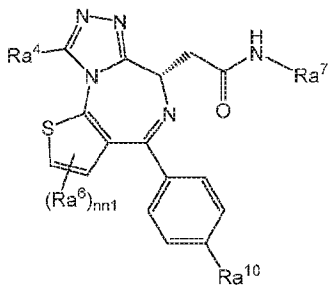


(TL-I1),

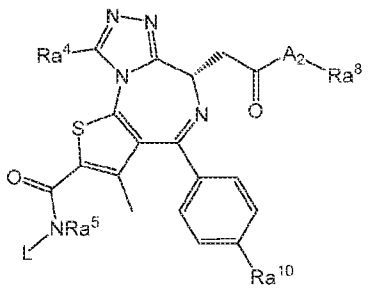
or a pharmaceutically acceptable salt thereof, wherein A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 are each as defined above in Formula TL-I.

Each of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 may be selected from the moieties described above in Formula TL-I. Each of the moieties defined for one of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, can be combined with any of the moieties defined for the others of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, as described above in Formula TL-I.

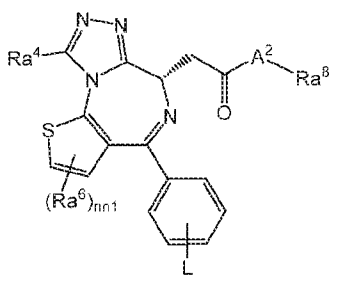
In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I1a – TL-I1d:



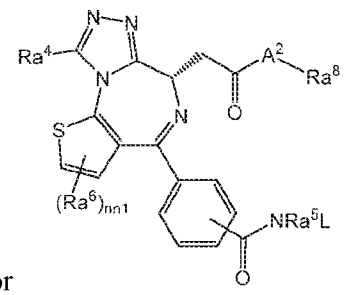
(TL-I1a),



(TL-I1b),



(TL-I1c), or



(TL-I1d),

or a pharmaceutically acceptable salt thereof, wherein:

each Ra^6 is independently C_1-C_3 alkyl, $(CH_2)_{0-3}$-CN, $(CH_2)_{0-3}$-halogen, $(CH_2)_{0-3}$-OH, or $(CH_2)_{0-3}$-C_1-C_3 alkoxy;

Ra^7 is $(CH_2)_{0-3}$-heterocyclyl, $(CH_2)_{0-3}$-phenyl, or L, wherein the heterocyclyl comprises one saturated 5- or 6-membered ring and 1-2 heteroatoms selected from N, O, and S and is substituted with L or C(O)L, and wherein the phenyl is substituted with L;

Ra^8 is H, C_1-C_6 alkyl, $(CH_2)_{0-3}$-heterocyclyl, or $(CH_2)_{0-3}$-phenyl, wherein the heterocyclyl comprises one saturated 5- or 6-membered ring and 1-2 heteroatoms selected from N, O, and S and is optionally substituted with C_1-C_3 alkyl, and wherein the phenyl is optionally substituted with C_1-C_3 alkyl, CN, halogen, OH, or C_1-C_3 alkoxy;

Ra^{10} is C_1-C_3 alkyl, $(CH_2)_{0-3}$-CN, or $(CH_2)_{0-3}$-halogen; and

A^2, Ra^4, Ra^5, nn1, and L are each as defined above in Formula TL-I.

In certain embodiments, nn1 is 0.

In certain embodiments, nn1 is 1.

In certain embodiments, nn1 is 2.

In certain embodiments, at least one Ra^6 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, at least one Ra^6 is methyl. In further embodiments, two Ra^6 are methyl.

In certain embodiments, at least one Ra^6 is CN, (CH_2)-CN, $(CH_2)_2$-CN, or $(CH_2)_3$-CN. In further embodiments, at least one Ra^6 is (CH_2)-CN.

In certain embodiments, at least one Ra^6 is halogen (*e.g.*, F, Cl, or Br), (CH_2)-halogen, $(CH_2)_2$-halogen, or $(CH_2)_3$-halogen. In further embodiments, at least one Ra^6 is Cl, (CH_2)-Cl, $(CH_2)_2$-Cl, or $(CH_2)_3$-Cl.

In certain embodiments, at least one Ra^6 is OH, (CH_2)-OH, $(CH_2)_2$-OH, or $(CH_2)_3$-OH.

In certain embodiments, at least one Ra^6 is C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy), (CH_2)-C_1-C_3 alkoxy, $(CH_2)_2$-C_1-C_3 alkoxy, or $(CH_2)_3$-C_1-C_3 alkoxy. In certain embodiments, at least one Ra^6 is methoxy.

In certain embodiments, Ra^7 is heterocyclyl, (CH_2)-heterocyclyl, $(CH_2)_2$-heterocyclyl, or $(CH_2)_3$-heterocyclyl. In further embodiments, Ra^7 is $(CH_2)_3$-heterocyclyl. In further embodiments, the heterocyclyl is selected from pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxazolidinyl,

isoxazolidinyl, thiazolidinyl, isothiazolidinyl, piperidinyl, piperazinyl, hexahydropyrimidinyl, morpholinyl, and thiomorpholinyl. In further embodiments, the heterocyclyl is piperazinyl.

In certain embodiments, the heterocyclyl is substituted with C(O)L.

In certain embodiments, the heterocyclyl is substituted with L.

In certain embodiments, Ra^7 is phenyl, (CH_2)-phenyl, $(CH_2)_2$-phenyl, or $(CH_2)_3$-phenyl. In further embodiments, Ra^7 is phenyl.

In certain embodiments, Ra^7 is L.

In certain embodiments, Ra^8 is H.

In certain embodiments, Ra^8 is straight-chain C_1-C_6 or branched C_3-C_6 alkyl (*e.g.*, methyl, ethyl, propyl, i-propyl, butyl, i-butyl, t-butyl, pentyl, or hexyl). In further embodiments, Ra^8 is methyl, ethyl, or t-butyl.

In certain embodiments, Ra^8 is heterocyclyl, (CH_2)-heterocyclyl, $(CH_2)_2$-heterocyclyl, or $(CH_2)_3$-heterocyclyl. In further embodiments, Ra^8 is $(CH_2)_3$-heterocyclyl. In further embodiments, the heterocyclyl is selected from pyrrolidinyl, pyrazolidinyl, imidazolidinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, piperidinyl, piperazinyl, hexahydropyrimidinyl, morpholinyl, and thiomorpholinyl. In further embodiments, the heterocyclyl is piperazinyl.

In certain embodiments, the heterocyclyl is substituted with C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, Ra^8 is phenyl, (CH_2)-phenyl, $(CH_2)_2$-phenyl, or $(CH_2)_3$-phenyl. In further embodiments, Ra^8 is phenyl.

In certain embodiments, the phenyl is substituted with C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In certain embodiments, the phenyl is substituted with CN. In certain embodiments, the phenyl is substituted with halogen (*e.g.*, F, Cl, or Br). In certain embodiments, the phenyl is substituted with OH. In certain embodiments, the phenyl is substituted with C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy).

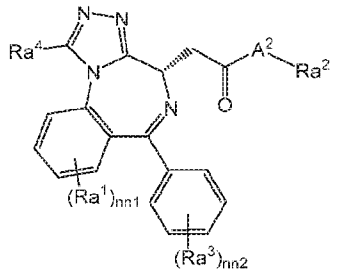
In certain embodiments, Ra^{10} is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, Ra^{10} is CN, (CH_2)-CN, $(CH_2)_2$-CN, or $(CH_2)_3$-CN.

In certain embodiments, Ra^{10} is halogen (*e.g.*, F, Cl, or Br), (CH_2)-halogen, $(CH_2)_2$-halogen, or $(CH_2)_3$-halogen. In further embodiments, Ra^{10} is Cl, (CH_2)-Cl, $(CH_2)_2$-Cl, or $(CH_2)_3$-Cl. In further embodiments, Ra^{10} is Cl.

Each of A^2, Ra^4, Ra^5, and nn1 may be selected from the moieties described above in Formula TL-I. Each of the moieties defined for one of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^{10}, and nn1, can be combined with any of the moieties defined for the others of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^{10}, and nn1, as described above and in Formula TL-I.

In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I2:

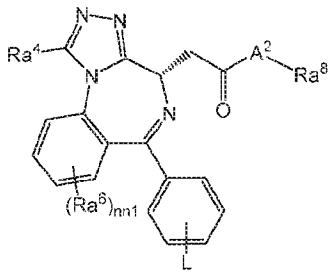


(TL-I2),

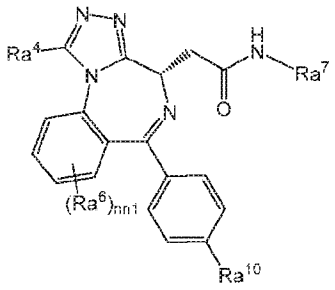
or a pharmaceutically acceptable salt thereof, wherein A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 are each as defined above in Formula TL-I.

Each of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 may be selected from the moieties described above in Formula TL-I. Each of the moieties defined for one of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, can be combined with any of the moieties defined for the others of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, as described above in Formula TL-I.

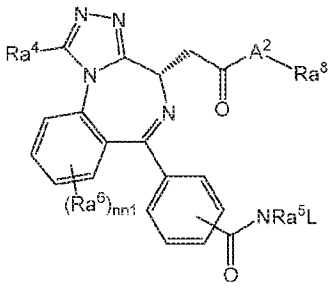
In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I2a – TL-I2c:



(TL-I2a),



(TL-I2b), or

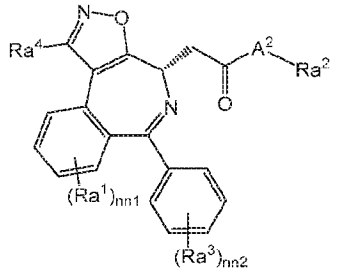


(TL-I2c),

or a pharmaceutically acceptable salt thereof, wherein A^2, Ra^4, Ra^5, nn1, and L are each as defined above in Formula TL-I, and Ra^6, Ra^7, Ra^8, and Ra^{10} are each as defined above in Formula TL-I1a – TL-I1d.

Each of A^2, Ra^4, Ra^5, and nn1 may be selected from the moieties described above in Formula TL-I, and each of Ra^6, Ra^7, Ra^8, and Ra^{10} may be selected from the moieties described above in Formula TL-I1a – TL-I1d. Each of the moieties defined for one of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^{10}, and nn1, can be combined with any of the moieties defined for the others of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^{10}, and nn1, as described above in Formula TL-I and TL-I1a – TL-I1d.

In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I3:

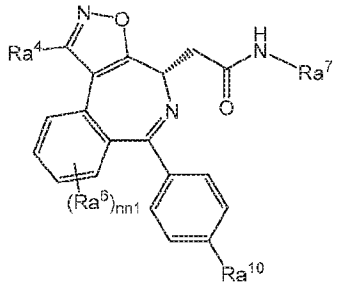


(TL-I3),

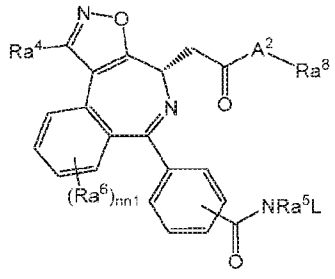
or a pharmaceutically acceptable salt thereof.

A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 are each as defined above in Formula TL-I. Each of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2 may be selected from the moieties described above in Formula TL-I. Each of the moieties defined for one of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, can be combined with any of the moieties defined for the others of A^2, Ra^1, Ra^2, Ra^3, Ra^4, Ra^5, nn1, and nn2, as described above in Formula TL-I.

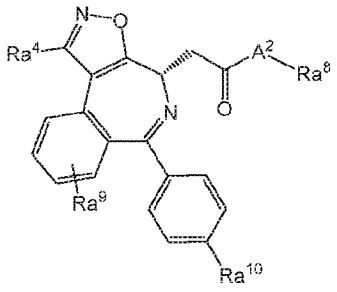
In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-I3a – TL-I3c:



(TL-I3a),



(TL-I3b), or



(TL-I3c),

or a pharmaceutically acceptable salt thereof, wherein:

Ra^9 is C(O)NRa^5L, OL, NRa^5L, or L;

A^2, Ra^4, Ra^5, nn1, and L are each as defined above in Formula TL-I; and

Ra^6, Ra^7, Ra^8, and Ra^{10} are each as defined above in Formula TL-I1a – TL-I1d.

In certain embodiments, Ra^9 is C(O)NRa^5L. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

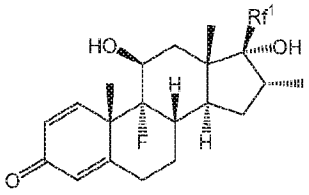
In certain embodiments, Ra^9 is OL.

In certain embodiments, Ra^9 is NRa^5L. In further embodiments, Ra^5 is H. In other embodiments, Ra^5 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In other embodiments, Ra_5 is methyl.

In certain embodiments, Ra^9 is L.

Each of A^2, Ra^4, Ra^5, and nn1 may be selected from the moieties described above in Formula TL-I, and each of Ra^6, Ra^7, Ra^8, and Ra^{10} may be selected from the moieties described above in Formula TL-I1a – TL-I1d. Each of the moieties defined for one of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^9, Ra^{10}, and nn1, can be combined with any of the moieties defined for the others of A^2, Ra^4, Ra^5, Ra^6, Ra^7, Ra^8, Ra^9, Ra^{10}, and nn1, as described above and in Formula TL-I and TL-I1a – TL-I1d.

In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-VI:



(TL-VI),

or a pharmaceutically acceptable salt thereof, wherein:

Rf^1 is $C(O)NRf^2L$, OL, NRf^2L, or L;

Rf^2 is independently H or C_1-C_3 alkyl; and

L is a Linker.

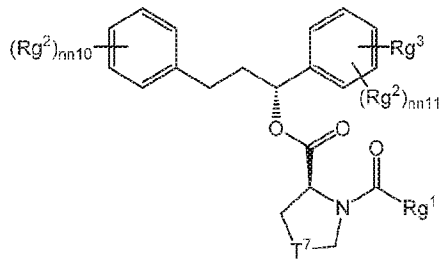
In certain embodiments, Rf^1 is $C(O)NRf^2L$. In further embodiments, Rf^2 is H. In other embodiments, Rf^2 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, Rf^1 is OL.

In certain embodiments, Rf^1 is NRe^4L. In further embodiments, Rf^2 is H. In other embodiments, Rf^2 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In other embodiments, Rf^2 is methyl.

In certain embodiments, Rf^1 is L.

In certain embodiments, a dTAG Targeting Ligand is a compound of Formula TL-VII:



(TL-VII),

or a pharmaceutically acceptable salt thereof, wherein:

T^7 is CH_2 or CH_2CH_2;

Rg^1 is $C(O)Rg^5$ or $(CH_2)_{1-3}Rg^6$;

nn10 is 0, 1, 2, or 3;

nn11 is 0, 1, 2, or 3;

each Rg^2 is independently C_1-C_3 alkyl, C_1-C_3 alkoxy, CN, or halogen;

Rg^3 is $C(O)NRg^4L$, OL, NRg^4L, L, O-$(CH_2)_{1-3}$-$C(O)NRg^4L$, or $NHC(O)$-$(CH_2)_{1-3}$-$C(O)NRg^4L$;

Rg^4 is H or C_1-C_3 alkyl;

Rg^5 is C_1-C_6 alkyl;

Rg^6 is phenyl optionally substituted with C_1-C_3 alkyl, C_1-C_3 alkoxy, CN, or halogen; and

L is a Linker.

In certain embodiments, T^7 is CH_2.

In certain embodiments, T^7 is CH_2CH_2.

In certain embodiments, Rg^1 is $C(O)Rg^5$.

In certain embodiments, Rg^1 is (CH_2)-Rg^6, $(CH_2)_2$-Rg^6, or $(CH_2)_3$-Rg^6.

In certain embodiments, Rg^5 is straight-chain C_1-C_6 or branched C_3-C_6 alkyl (*e.g.*, methyl, ethyl, propyl, i-propyl, butyl, i-butyl, t-butyl, pentyl, or hexyl).

In certain embodiments, Rg^6 is unsubstituted phenyl.

In certain embodiments, Rg^6 is phenyl substituted with one, two, three, or more substituents independently selected from C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl), C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy), CN, and halogen (*e.g.*, F, Cl, or Br).

In certain embodiments, nn10 is 0.

In certain embodiments, nn10 is 1.

In certain embodiments, nn10 is 2.

In certain embodiments, nn10 is 3.

In certain embodiments, nn11 is 0.

In certain embodiments, nn11 is 1.

In certain embodiments, nn11 is 2.

In certain embodiments, nn11 is 3.

In certain embodiments, at least one Rg^2 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In further embodiments, at least one Rg^2 is methyl.

In certain embodiments, at least one Rg^2 is C_1-C_3 alkoxy (*e.g.*, methoxy, ethoxy, or propoxy). In further embodiments, at least one Rg^2 is methoxy.

In certain embodiments, at least one Rg^2 is CN.

In certain embodiments, at least one Rg^2 is halogen (*e.g.*, F, Cl, or Br).

In certain embodiments, Rg^3 is $C(O)NRg^4L$. In further embodiments, Rg^4 is H. In other embodiments, Rg^4 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, Rg^3 is OL.

In certain embodiments, Rg^3 is NRg^4L. In further embodiments, Rg^4 is H. In other embodiments, Rg^4 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl). In other embodiments, Rg^4 is methyl.

In certain embodiments, Rg^3 is L.

In certain embodiments, Rg^3 is O-(CH_2)-C(O)NRg^4L, O-$(CH_2)_2$-C(O)NRg^4L, or O-$(CH_2)_3$-C(O)NRg^4L. In further embodiments, Rg^3 is O-(CH_2)-C(O)NRg^4L. In further embodiments, Rg^4 is H. In other embodiments, Rg^4 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, Rg^3 is NHC(O)-(CH_2)-C(O)NRg^4L, NHC(O)-$(CH_2)_2$-C(O)NRg^4L, or NHC(O)-$(CH_2)_3$-C(O)NRg^4L. In further embodiments, Rg^3 is NHC(O)-(CH_2)-C(O)NRg^4L, NHC(O)-$(CH_2)_2$-C(O)NRg^4L. In further embodiments, Rg^3 is NHC(O)-$(CH_2)_2$-C(O)NRg^4L. In further embodiments, Rg^4 is H. In other embodiments, Rg^4 is C_1-C_3 alkyl (*e.g.*, methyl, ethyl, propyl, or i-propyl).

In certain embodiments, the dTAG Targeting Ligand is selected from the structures of Figure 28, wherein R is the point at which the Linker is attached.

In certain embodiments, the dTAG Targeting Ligands or targets are chosen based on existence (known dTAG binding moieties) and ability to develop potent and selective ligands with functional positions that can accommodate a Linker. Some embodiments relate to dTAG Targeting Ligands with less selectivity, which may benefit from degradation coupled with proteomics as a measure of compound selectivity or target ID.

Some embodiments of the present application relate to degradation or loss of 30% to 100% of the CAR. Certain embodiments relate to the loss of 50-100% of the CAR. Other embodiments relate to the loss of 75-95% of the CAR.

Non-limiting examples of heterobifunctional compounds for use in the present invention include those of Figures 29, 30, 31, and 32.

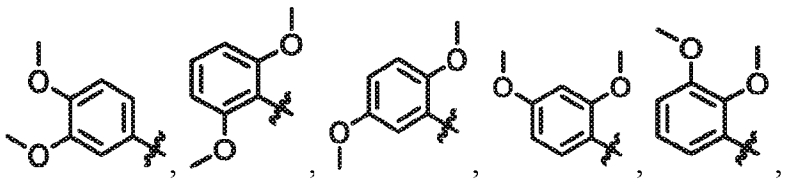
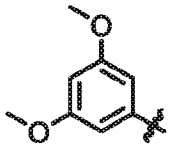
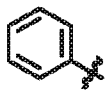
Figure 29, provides specific heterobifunctional compounds for use in the present invention.

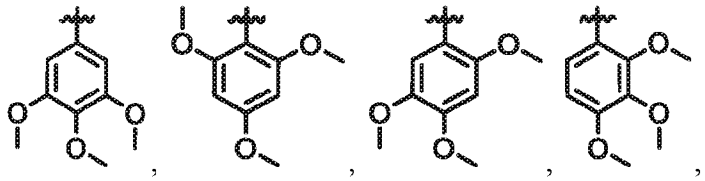
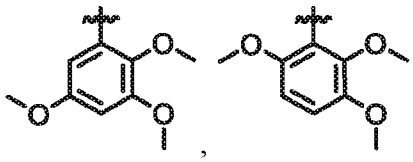
Figure 30, provides specific heterobifunctional compounds for use in the present invention, wherein X in the above structures is a halogen chosen from F, Cl, Br, and I.

Figure 31, provides specific heterobifunctional compounds for use in the present invention.

Figure 32, provides heterobifunctional compounds for use in the present invention,

wherein:

R^{AR1} is selected from:  ,  , and  ; and

R^{AR2} is selected from:  ,  , and  .

Additional compounds for use in the present invention include the structures of Figure 33.

Some of the foregoing heterobifunctional compounds include one or more asymmetric centers, and thus can exist in various isomeric forms, *e.g.*, stereoisomers and/or diastereomers. Thus, compounds and pharmaceutical compositions thereof may be in the form of an individual enantiomer, diastereomer, or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the application are enantiopure compounds. In certain other embodiments, mixtures of stereoisomers or diastereomers are provided.

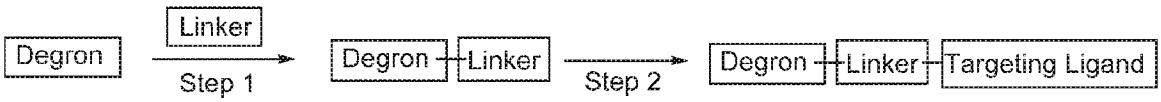
Furthermore, certain heterobifunctional compounds, as described herein may have one or more double bonds that can exist as either the Z or E isomer, unless otherwise indicated. The application additionally encompasses the compounds as individual isomers substantially free of other isomers and alternatively, as mixtures of various isomers, *e.g.*, racemic mixtures of stereoisomers. In addition to the above-mentioned compounds per se, this application also encompasses pharmaceutically acceptable derivatives of these heterobifunctional compounds and compositions comprising one or more compounds of the application and one or more pharmaceutically acceptable excipients or additives.

Heterobifunctional compounds of the application may be prepared by crystallization of the compound under different conditions and may exist as one or a combination of polymorphs of the compound forming part of this application. For example, different polymorphs may be identified and/or prepared using different solvents, or different mixtures of solvents for recrystallization; by performing crystallizations at different temperatures; or by using various modes of cooling, ranging from very fast to very slow cooling during crystallizations. Polymorphs may also be obtained by heating or melting the compound followed by gradual or fast cooling. The presence of polymorphs may be determined by solid probe NMR spectroscopy, IR spectroscopy, differential scanning calorimetry, powder X-ray diffractogram and/or other techniques. Thus, the present application encompasses heterobifunctional compounds, their derivatives, their tautomeric forms, their stereoisomers, their polymorphs, their pharmaceutically acceptable salts their pharmaceutically acceptable solvates and pharmaceutically acceptable compositions containing them.

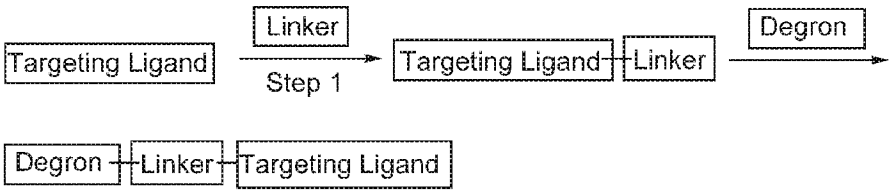
General Synthesis of the Heterobifunctional Compounds

The heterobifunctional compounds described herein can be prepared by methods known by those skilled in the art. In one non-limiting example the disclosed heterobifunctional compounds can be made by the following schemes.

Scheme 1



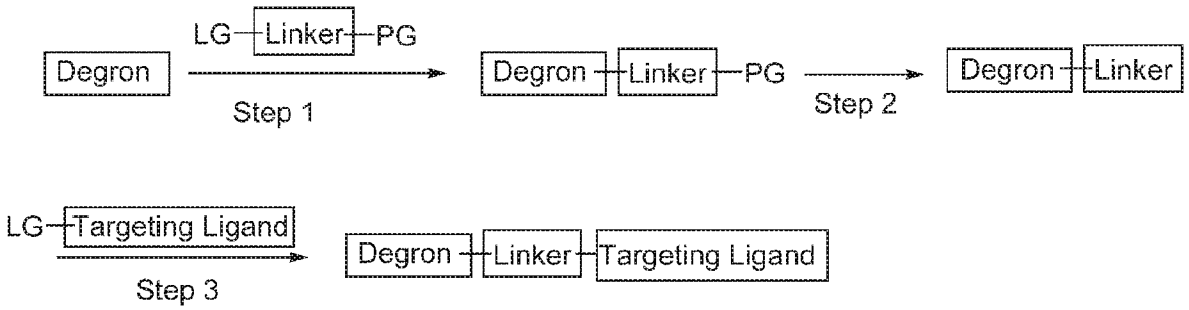
Scheme 2



As shown in Scheme 1 heterobifunctional compounds for use in the present invention can be prepared by chemically combining a Degron and a Linker followed by subsequent addition of a dTAG Targeting Ligand. Similarly, in Scheme 2 heterobifunctional compounds for use in the

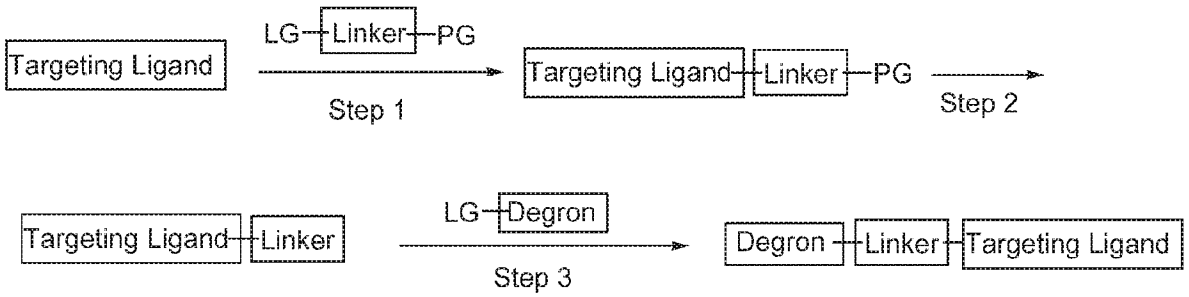
present invention are prepared by chemically combing a dTAG Targeting Ligand and Linker first, followed by subsequent addition of a Degron. As illustrated in the above and following schemes, heterobifunctional compounds for use in the present invention can readily be synthesized by one skilled in the art in a variety of methods and chemical reactions.

Scheme 3



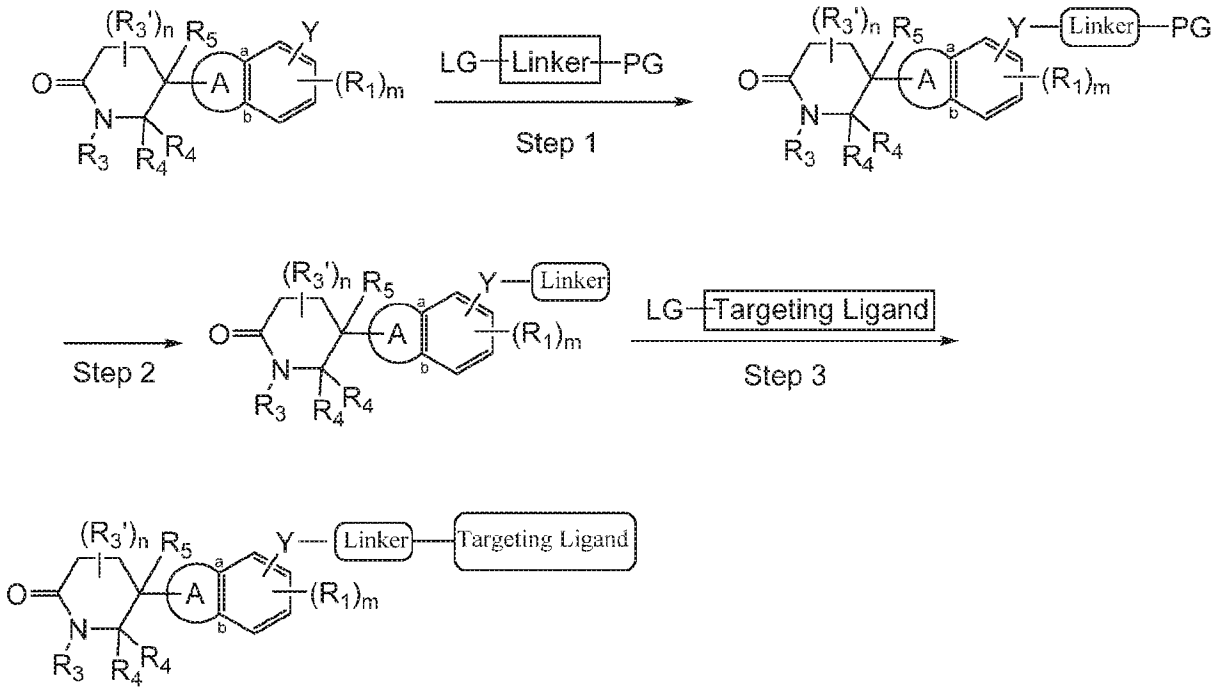
Scheme 3: In Step 1, a nucleophilic Degron displaces a leaving group on the Linker to make a Degron Linker fragment. In Step 2, the protecting group is removed by methods known in the art to free a nucleophilic site on the Linker. In Step 3, the nucleophilic Degron Linker fragment displaces a leaving group on the dTAG Targeting Ligand to form a compound for use in the present invention. In an alternative embodiment Step 1 and/or Step 2 is accomplished by a coupling reaction instead of a nucleophilic attack.

Scheme 4

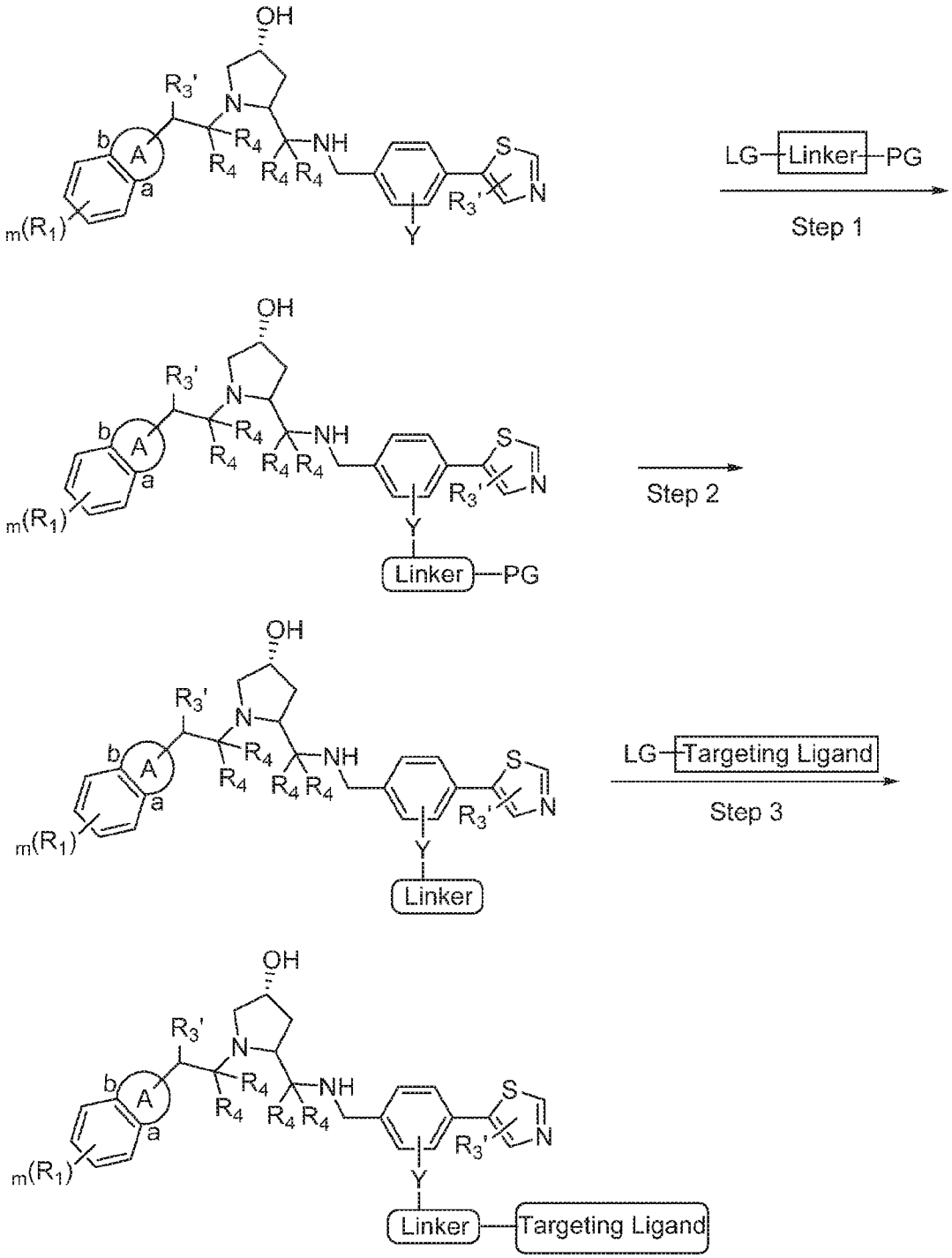


Scheme 4: In Step 1, a nucleophilic dTAG Targeting Ligand displaces a leaving group on the Linker to make a dTAG Targeting Ligand Linker fragment. In Step 2, the protecting group is removed by methods known in the art to free a nucleophilic site on the Linker. In Step 3, the nucleophilic dTAG Targeting Ligand Linker fragment displaces a leaving group on the Degron to form a compound for use in the present invention. In an alternative embodiment Step 1 and/or Step 2 is accomplished by a coupling reaction instead of a nucleophilic attack.

Scheme 5


(R3')n
R5
Y
A
(R1)m
O
N
R3
R4
R4
a
b
LG
Linker
PG
Step 1
Step 2
Step 3
Targeting Ligand

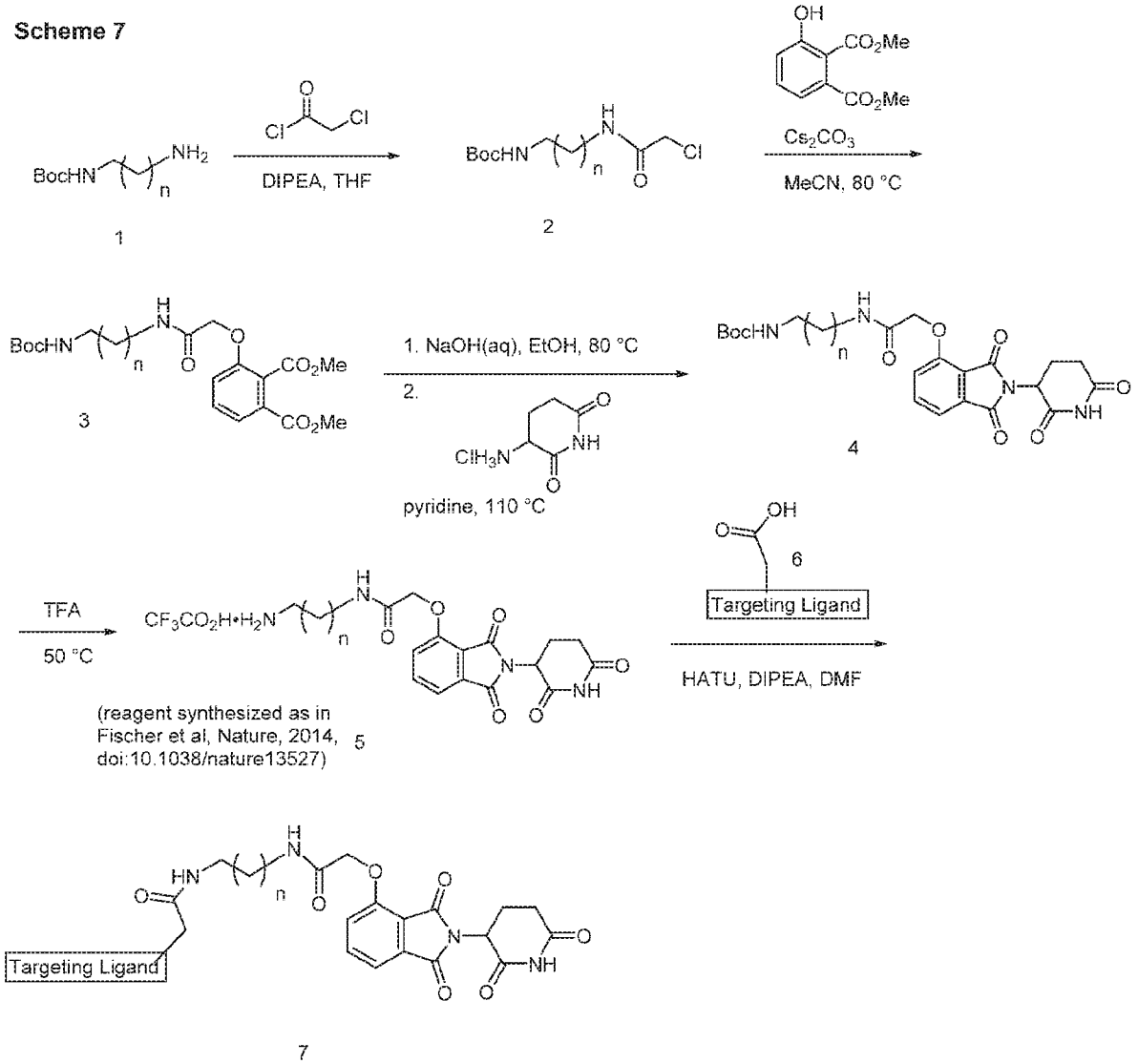
Scheme 6



Scheme 5 and Scheme 6: In Step 1, a nucleophilic Degron displaces a leaving group on the Linker to make a Degron Linker fragment. In Step 2, the protecting group is removed by methods

known in the art to free a nucleophilic site on the Linker. In Step 3, the nucleophilic Degron Linker fragment displaces a leaving group on the dTAG Targeting Ligand to form a compound of Formula I or Formula II. In an alternative embodiment Step 1 and/or Step 2 is accomplished by a coupling reaction instead of a nucleophilic attack.

Scheme 7



a) reacting *tert*-Butyl (2-aminoethyl)carbamate or its analog (*e.g.*, n = 1-20) (**1**) or its analog (*e.g.*, n = 1-20) with chloroacetyl chloride under suitable conditions to generate *tert*-butyl (2-(2-chloroacetamido)ethyl)carbamate or its analog (*e.g.*, n = 1-20) (**2**);

b) reacting *tert*-butyl (2-(2-chloroacetamido)ethyl)carbamate or its analog (**2**) with dimethyl 3-hydroxyphthalate under suitable conditions to provide dimethyl 3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phthalate or its analog (**3**);

c) reacting dimethyl 3-(2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phthalate or its analog (**3**) with strong base, followed by 3-aminopiperidine-2,6-dione hydrochloride to generate tert-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethyl)carbamate or its analog (**4**);

d) deprotecting compound (**4**) to provide diaminoethyl-acetyl-O-thalidomide trifluoroacetate or its analog (**5**)

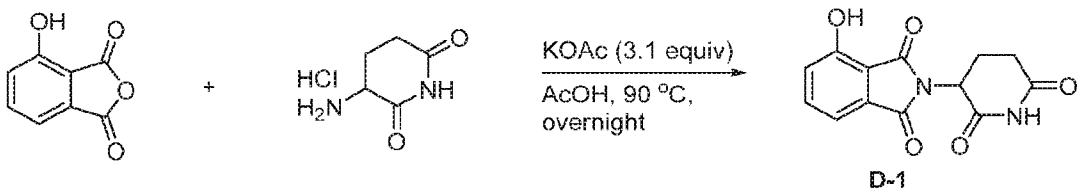
e) reacting compound (**5**) with an acid derivative of a dTAG Targeting Ligand (compound (**6**)) under suitable conditions to yield a bifunctional compound (**7**).

In certain embodiments, the methods described above are carried out in solution phase. In certain other embodiments, the methods described above are carried out on a solid phase. In certain embodiments, the synthetic method is amenable to high-throughput techniques or to techniques commonly used in combinatorial chemistry.

Representative Synthesis of the Heterobifunctional Compounds

Unless otherwise indicated, starting materials are either commercially available or readily accessible through laboratory synthesis by anyone reasonably familiar with the art. Described generally below, are procedures and general guidance for the synthesis of compounds as described generally and in subclasses and species herein.

Synthetic Example 1': Synthesis of IMiD derivatives and Degrons



D-1

General procedure I: IMiD condensation

2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (D-1)

In a 20 mL glass vial, a mixture of 3-hydroxyphthalic anhydride (500 mg, 3.05 mmol, 1 equiv), potassium acetate (927 mg, 9.44 mmol, 3.1 equiv) and 3-aminopiperidine-2,6-dione

hydrochloride (552 mg, 3.35 mmol, 1.1 equiv) in acetic acid (10.2 mL, 0.3 M) was heated to 90 °C overnight. The black reaction mixture was cooled to room temperature and diluted to 20 mL with water, and subsequently cooled on ice for 30 min. The resulting slurry was transferred to a 50 mL Falcon tube, which was centrifuged at 3500 rpm for 5 min. The supernatant was discarded and the black solid was transferred to a 250 mL RBF with methanol and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH (9:1)) to afford the title compound as a white solid (619 mg, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.07 (s, 1H), 7.65 (dd, J = 8.4, 6.8 Hz, 1H), 7.31 (d, J = 6.8 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 5.06 (dd, J = 12.8, 5.4 Hz, 1H), 2.94 – 2.82 (m, 1H), 2.64 – 2.43 (m, 2H), 2.08 – 1.97 (m, 1H); MS (ESI) calcd for $C_{13}H_{11}N_2O_5$ $[M+H]^+$ 275.07, found 275.26.

2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (D-10)

General procedure I was followed using 3-nitrophthalic anhydride (300 mg, 1.55 mmol, 1 equiv), potassium acetate (473 mg, 4.82 mmol, 3.1 equiv) and 3-aminopiperidine-2,6-dione hydrochloride (281 mg, 1.71 mmol, 1.1 equiv) to afford the title compound as a light yellow solid (280 mg, 59%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (9:1)). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 8.35 (d, J = 8.1 Hz, 1H), 8.24 (d, J = 7.5 Hz, 1H), 8.14 – 8.10 (m, 1H), 5.20 (dd, J = 12.9, 5.5 Hz, 1H), 2.93 – 2.84 (m, 1H), 2.64 – 2.45 (m, 2H), 2.11 – 2.04 (m, 1H); MS (ESI) calcd for $C_{13}H_{10}N_3O_6$ $[M+H]^+$ 304.06, found 304.19.

2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline-1,3-dione (D-2)

General procedure I was followed using 4-nitrophthalic anhydride (300 mg, 1.55 mmol), potassium acetate (473 mg, 4.82 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (281 mg, 1.71 mmol) to afford the title compound as a white solid (409 mg, 87%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (30:1)). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (s, 1H), 8.68 (dd, J = 8.1, 1.9 Hz, 1H), 8.56 (d, J = 1.9 Hz, 1H), 8.19 (d, J = 8.1 Hz, 1H), 5.24 (dd, J = 12.9, 5.4 Hz, 1H), 2.90 (ddd, J = 17.2, 13.9, 5.5 Hz, 1H), 2.69 – 2.48 (m, 2H), 2.14 – 2.05 (m, 1H); MS (ESI) calcd for $C_{13}H_{10}N_3O_6$ $[M+H]^+$ 304.06, found 304.19.

2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-6)

General procedure I was followed using phthalic anhydride (155 mg, 1.05 mmol), potassium acetate (318 mg, 3.24 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (189 mg, 1.15 mmol) to afford the title compound as a white solid (235 mg, 87%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (15:1)). ^1H NMR (500 MHz, DMSO-d_6) δ 11.13 (s, 1H), 8.00 – 7.76 (m, 4H), 5.16 (dd, J = 12.8, 5.4 Hz, 1H), 2.89 (ddd, J = 16.8, 13.7, 5.4 Hz, 1H), 2.65 – 2.42 (m, 2H), 2.12 – 1.99 (m, 1H); MS (ESI) calcd for $C_{13}H_{11}N_2O_4$ $[M+H]^+$ 259.07, found 259.23.

2-(2,5-dioxopyrrolidin-3-yl)isoindoline-1,3-dione (D-7)

General procedure I was followed using phthalic anhydride (90 mg, 0.608 mmol), potassium acetate (185 mg, 1.88 mmol) and 3-aminopyrrolidine-2,5-dione hydrochloride (101 mg, 0.668 mmol) to afford the title compound as a white solid (95 mg, 64%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (14:1)). MS (ESI) calcd for $C_{12}H_9N_2O_4$ $[M+H]^+$ 245.06, found 245.26.

2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxylic acid (D-13)

General procedure I was followed using 1,2,4-benzenetricarboxylic anhydride (200 mg, 1.04 mmol), potassium acetate (317 mg, 3.23 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (188 mg, 1.15 mmol) to afford the title compound as a white solid (178 mg, 57%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (9:1)). MS (ESI) calcd for $C_{14}H_{11}N_2O_6$ $[M+H]^+$ 303.06, found 303.24.

2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (D-14)

General procedure I was followed using 3-fluorophthalic anhydride (200 mg, 1.20 mmol), potassium acetate (366 mg, 3.73 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (218 mg, 1.32 mmol) to afford the title compound as a white solid (288 mg, 86%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (50:1)). ^1H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.96 (ddd, J = 8.3, 7.3, 4.5 Hz, 1H), 7.82 – 7.71 (m, 2H), 5.17 (dd, J = 13.0, 5.4 Hz, 1H), 2.90 (ddd, J = 17.1, 13.9, 5.4 Hz, 1H), 2.65 – 2.47 (m, 2H), 2.10 – 2.04 (m, 1H), MS (ESI) calcd for $C_{13}H_{10}FN_2O_4$ $[M+H]^+$ 277.06, found 277.25.

2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline-1,3-dione (D-19)

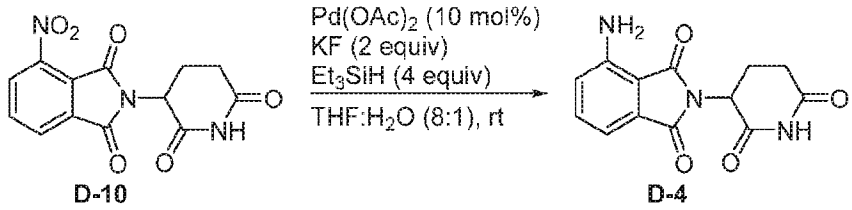
General procedure I was followed using 3-methylphthalic anhydride (150 mg, 0.925 mmol), potassium acetate (281 mg, 2.87 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (167 mg, 1.02 mmol) to afford the title compound as a white solid (168 mg, 67%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (15:1)). MS (ESI) calcd for $C_{14}H_{13}N_2O_4$ $[M+H]^+$ 273.09, found 273.24.

2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (D-24)

General procedure I was followed using 4-fluorophthalic anhydride (200 mg, 1.20 mmol), potassium acetate (366 mg, 3.73 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (218 mg, 1.32 mmol) to afford the title compound as a white solid (254 mg, 76%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (15:1)). MS (ESI) calcd for $C_{13}H_{10}FN_2O_4$ $[M+H]^+$ 277.06, found 277.24.

2-(2,6-dioxopiperidin-4-yl)isoindoline-1,3-dione (D-43)

General procedure I was followed using phthalic anhydride (60 mg, 0.311 mmol), potassium acetate (95 mg, 0.963 mmol) and 4-aminopiperidine-2,6-dione hydrochloride (56 mg, 0.342 mmol) to afford the title compound as a white solid (40 mg, 43%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (9:1)). MS (ESI) calcd for $C_{13}H_{11}N_2O_4$ $[M+H]^+$ 259.07, found 259.18.



D-10 D-4

General procedure II: Reduction of aromatic nitro groups

4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-4)

A solution of 2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (173 mg, 0.854 mmol), $Pd(OAc)_2$ (12.8 mg, 0.0854 mmol, 10 mol%) and potassium fluoride (66 mg, 1.71 mmol, 2 equiv) in THF:water (8:1) (5.7 mL, 0.1 M) was stirred at room temperature. Triethylsilane (365

μL, 3.41 mmol, 4 equiv) was added slowly, and the resulting black solution was stirred at room temperature for 1 hour. The reaction mixture was filtered through a pad of celite, which was washed excessively with ethyl acetate. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH (7:1)) to afford the title compound as a yellow powder (72 mg, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.08 (s, 1H), 7.47 (dd, J = 8.5, 7.0 Hz, 1H), 7.06 – 6.95 (m, 1H), 6.59 – 6.44 (m, 1H), 5.04 (dd, J = 12.7, 5.4 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.64 – 2.45 (m, 2H), 2.05 – 1.98 (m, 1H); MS (ESI) calcd for $C_{13}H_{11}N_3O_4$ $[M+H]^+$ 274.08, found 274.23.

2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline-1,3-dione (D-8)

General procedure II was followed using 2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline-1,3-dione (100 mg, 0.330 mmol), $Pd(OAc)_2$ (7.4 mg, 0.033 mmol), potassium fluoride (38 mg, 0.660 mmol) and triethylsilane (211 μL, 1.32 mmol to afford the title compound as a yellow solid (33 mg, 37%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (9:1)). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.05 (s, 1H), 7.52 (d, J = 8.2 Hz, 1H), 6.94 (d, J = 2.0 Hz, 1H), 6.83 (dd, J = 8.2, 2.0 Hz, 1H), 6.55 (s, 2H), 5.01 (dd, J = 12.8, 5.4 Hz, 1H), 2.86 (ddd, J = 16.9, 13.9, 5.5 Hz, 1H), 2.68 – 2.43 (m, 2H), 2.03 – 1.93 (m, 1H); MS (ESI) calcd for $C_{13}H_{12}N_3O_4$ $[M+H]^+$ 274.08, found 274.59.

4-amino-2-(1-benzyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-12)

General procedure II was followed using 2-(1-benzyl-2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (48 mg, 0.122 mmol), $Pd(OAc)_2$ (2.7 mg, 0.0122 mmol), potassium fluoride (14 mg, 0.244 mmol) and triethylsilane (78 μL, 0.488 mmol to afford the title compound as a yellow solid (7 mg, 16%) following purification by flash column chromatography on silica gel (0 to 100% EtOAc in hexanes). MS (ESI) calcd for $C_{20}H_{18}N_3O_4$ $[M+H]^+$ 364.13, found 364.34.

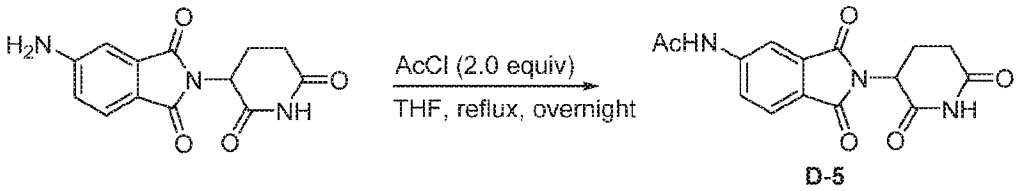
3-(5-amino-2-methyl-4-oxoquinazolin-3(4H)-yl)piperidine-2,6-dione (D-17)

General procedure II was followed using 3-(2-methyl-5-nitro-4-oxoquinazolin-3(4H)-yl)piperidine-2,6-dione (21 mg, 0.0664 mmol), $Pd(OAc)_2$ (1.5 mg, 0.0066 mmol), potassium fluoride (7.7 mg, 0.133 mmol) and triethylsilane (42 μL, 0.266 mmol to afford the title compound

as a white solid (7 mg, 37%) following purification by preparative HPLC. MS (ESI) calcd for $C_{14}H_{15}N_4O_3$ $[M+H]^+$ 287.11, found 287.30.

3-(7-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (D-41)

General procedure II was followed using 3-(7-nitro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (11 mg, 0.038 mmol), $Pd(OAc)_2$ (0.9 mg, 0.0038 mmol), potassium fluoride (4.4 mg, 0.076 mmol) and triethylsilane (24 μL, 0.152 mmol to afford the title compound as a yellow solid (2 mg, 21%) following purification by flash column chromatography on silica gel (0 to 10% MeOH in CH_2Cl_2). MS (ESI) calcd for $C_{13}H_{14}N_3O_3$ $[M+H]^+$ 260.10, found 260.52.



D-5

General procedure III: Acylation of anilines

***N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)acetamide (D-5)**

In a 4 mL glass vial, a mixture of 5-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (30 mg, 0.110 mmol, 1 equiv) and acetyl chloride (26 μL, 0.220 mmol, 2 equiv) in THF (1.8 mL, 0.1 M) was heated to reflux overnight. The reaction mixture was filtered, and the filter cake was washed with Et_2O to give the title compound as a white solid (27 mg, 47%), that was used without further purification. ^1H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 10.63 (s, 1H), 8.24 (d, J = 1.5 Hz, 1H), 7.91 – 7.83 (m, 2H), 5.11 (dd, J = 12.8, 5.4 Hz, 1H), 2.88 (ddd, J = 17.0, 13.8, 5.4 Hz, 1H), 2.63 – 2.46 (m, 2H), 2.13 (s, 3H), 2.09 – 2.00 (m, 1H); MS (ESI) calcd for $C_{15}H_{14}N_3O_5$ $[M+H]^+$ 316.09, found 316.23.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (D-3)**

General procedure III was followed using 4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (50 mg, 0.183 mmol) and acetyl chloride (26 μL, 0.366 mmol) to afford the title compound as a white solid (10 mg, 17%). ^1H NMR (500 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.73 (s, 1H), 8.44 (d, J = 8.4 Hz, 1H), 7.83 (dd, J = 8.4, 7.3 Hz, 1H), 7.62 (d, J = 7.2 Hz, 1H), 5.14 (dd, J = 12.9, 5.4 Hz, 1H), 2.90 (ddd, J = 17.1, 13.9, 5.4 Hz, 1H), 2.66 – 2.45 (m, 2H), 2.19 (s, 3H), 2.14 – 2.00 (m, 1H); MS (ESI) calcd for $C_{15}H_{14}N_3O_5$ $[M+H]^+$ 316.09, found 316.27.

2-chloro-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)acetamide (D-32)

General procedure III was followed using 5-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10 mg, 0.0366 mmol) and chloroacetyl chloride (6 μL, 0.0732 mmol) to afford the title compound as a white solid (7.1 mg, 55%). MS (ESI) calcd for $C_{15}H_{13}ClN_3O_5$ $[M+H]^+$ 350.05, found 350.23.

2-chloro-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (D-34)

General procedure III was followed using 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (20 mg, 0.0771 mmol) and chloroacetyl chloride (12 μL, 0.154 mmol) to afford the title compound as a white solid (14.9 mg, 56%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (s, 1H), 10.20 (s, 1H), 7.81 (dd, J = 7.7, 1.3 Hz, 1H), 7.65 – 7.47 (m, 2H), 5.16 (dd, J = 13.3, 5.1 Hz, 1H), 4.45 – 4.34 (m, 2H), 4.33 (s, 2H), 3.00 – 2.85 (m, 1H), 2.68 – 2.56 (m, 1H), 2.41 – 2.28 (m, 1H), 2.09 – 1.97 (m, 1H); MS (ESI) calcd for $C_{15}H_{15}ClN_3O_4$ $[M+H]^+$ 336.07, found 336.31.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acrylamide (D-35)**

General procedure III was followed using 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (20 mg, 0.0771 mmol) and acryloyl chloride (13 μL, 0.154 mmol) to afford the title compound as a white solid (18 mg, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.77 (s, 1H), 14.81 (s, 1H), 12.65 (dd, J = 7.4, 1.6 Hz, 1H), 12.37 – 12.18 (m, 2H), 11.28 (dd, J = 17.0, 10.2 Hz, 1H), 11.06 (dd, J = 17.0, 1.9 Hz, 1H), 10.57 (dd, J = 10.2, 1.9 Hz, 1H), 9.91 (dd, J = 13.3, 5.1 Hz, 1H), 9.24 – 9.05 (m, 2H), 7.67 (ddd, J = 17.2, 13.7, 5.5 Hz, 1H), 7.36 (dt, J = 17.3, 3.8 Hz, 1H), 7.20 – 7.03 (m, 1H), 6.83 – 6.72 (m, 1H); MS (ESI) calcd for $C_{16}H_{16}N_3O_4$ $[M+H]^+$ 314.11, found 314.24.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)acrylamide (D-36)**

General procedure III was followed using 5-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10 mg, 0.0366 mmol) and acryloyl chloride (6 μL, 0.0732 mmol) to afford the title compound as a white solid (8.8 mg, 73%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.12 (s, 1H), 10.83 (s, 1H), 8.33 (d, J = 1.8 Hz, 1H), 7.99 (dd, J = 8.2, 1.9 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 6.48 (dd, J = 17.0, 10.1 Hz, 1H), 6.36 (dd, J = 17.0, 1.9 Hz, 1H), 5.88 (dd, J = 10.0, 1.9 Hz, 1H), 5.13 (dd,

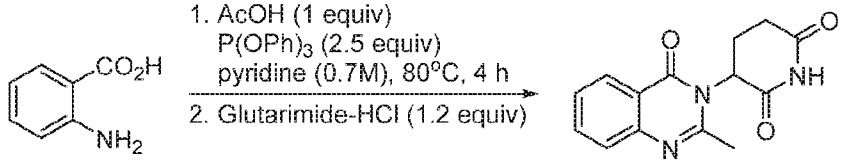
J = 12.8, 5.5 Hz, 1H), 2.95 – 2.84 (m, 1H), 2.67 – 2.46 (m, 2H), 2.09 – 2.01 (m, 1H); MS (ESI) calcd for $C_{16}H_{14}N_3O_5$ $[M+H]^+$ 328.09, found 328.23.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (D-37)**

General procedure III was followed using 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (20 mg, 0.0771 mmol) and acetyl chloride (11 μL, 0.154 mmol) to afford the title compound as a white solid (17 mg, 71%). MS (ESI) calcd for $C_{15}H_{16}N_3O_4$ $[M+H]^+$ 302.11, found 301.99.

***N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)cyclopropanecarboxamide (D-38)**

General procedure III was followed using 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (20 mg, 0.0771 mmol) and cyclopropanecarbonyl chloride (14 μL, 0.154 mmol) to afford the title compound as a white solid (19 mg, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.06 (s, 1H), 7.84 (dd, J = 7.2, 1.9 Hz, 1H), 7.66 – 7.38 (m, 2H), 5.14 (dd, J = 13.3, 5.1 Hz, 1H), 4.52 – 4.30 (m, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.64 – 2.54 (m, 1H), 2.45 – 2.27 (m, 1H), 2.08 – 1.95 (m, 1H), 1.93 – 1.83 (m, 1H), 0.90 – 0.75 (m, 4H); MS (ESI) calcd for $C_{17}H_{18}N_3O_4$ $[M+H]^+$ 328.13, found 328.00.



D-9

General procedure IV: Quinazolinone condensation

3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)piperidine-2,6-dione (D-9)

In a 20 mL glass vial, anthranilic acid (100 mg, 0.729 mmol, 1 equiv), acetic acid (42 μL, 0.729 mmol, 1 equiv) and $P(OPh)_3$ (479 μL, 1.82 mmol, 2.5 equiv) in pyridine (1.0 uL, 0.7 M) was heated to 90 °C. After 4 hours, the reaction mixture was cooled to room temperature and 3-aminopiperidine-2,6-dione hydrochloride (144 mg, 0.875 mmol, 1.2 equiv) was added. The reaction mixture was reheated to 90 °C for 1.5 h, whereupon it was stirred at room temperature overnight. The reaction mixture was taken up in EtOAc (15 mL) and water (15 mL). The organic layer was washed with brine (2x25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (0-5% MeOH in CH_2Cl_2) to afford the title compound as a white solid (79 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H),

8.03 (dd, J = 7.9, 1.5 Hz, 1H), 7.82 (ddd, J = 8.5, 7.1, 1.6 Hz, 1H), 7.62 (dd, J = 8.3, 1.1 Hz, 1H), 7.50 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 5.27 (dd, J = 11.5, 5.7 Hz, 1H), 2.92 – 2.78 (m, 1H), 2.73 – 2.56 (m, 5H), 2.26 – 2.06 (m, 1H); MS (ESI) calcd for $C_{14}H_{14}N_3O_3$ $[M+H]^+$ 272.10, found 272.33.

3-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)pyrrolidine-2,5-dione (D-11)

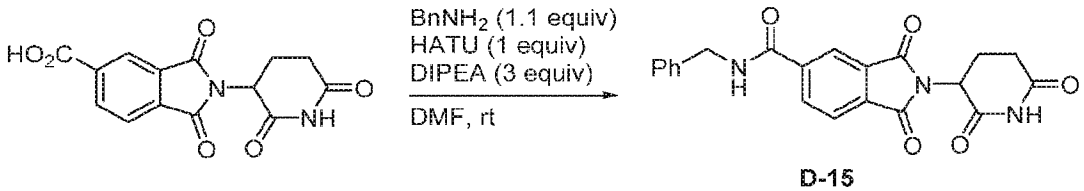
General procedure IV was followed using anthranilic acid (200 mg, 1.46 mmol), acetic acid (84 μL, 1.46 mmol), $P(OPh)_3$ (959 μL, 3.65 mmol) and 3-aminopyrrolidine-2,5-dione hydrochloride (263 mg, 1.75 mmol) to afford the title compound as a white solid (25 mg, 7%) following purification by flash column chromatography on silica gel (CH_2Cl_2:MeOH (15:1)). MS (ESI) calcd for $C_{13}H_{12}N_3O_3$ $[M+H]^+$ 258.09, found 258.22.

3-(5-fluoro-2-methyl-4-oxoquinazolin-3(4*H*)-yl)piperidine-2,6-dione (D-66)

General procedure IV was followed using 6-fluoro anthranilic acid (100 mg, 0.645 mmol), acetic acid (37 μL, 0.644 mmol), $P(OPh)_3$ (424 μL, 1.61 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (127 mg, 0.774 mmol) to afford the title compound as a white solid (70 mg, 38%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 7.84 – 7.76 (m, 1H), 7.44 (dd, J = 8.2, 1.0 Hz, 1H), 7.25 (ddd, J = 11.1, 8.2, 1.0 Hz, 1H), 5.24 (dd, J = 11.3, 5.7 Hz, 1H), 2.90 – 2.75 (m, 1H), 2.62 (s, 3H), 2.61 – 2.56 (m, 2H), 2.20 – 2.12 (m, 1H); MS (ESI) calcd for $C_{14}H_{13}FN_3O_3$ $[M+H]^+$ 290.09, found 290.27.

3-(2-methyl-5-nitro-4-oxoquinazolin-3(4*H*)-yl)piperidine-2,6-dione (D-67)

General procedure IV was followed using 6-nitroanthranilic acid (100 mg, 0.549 mmol), acetic acid (31 μL, 0.549 mmol), $P(OPh)_3$ (361 μL, 1.37 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (108 mg, 0.659 mmol) to afford the title compound as a white solid (29 mg, 17%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). MS (ESI) calcd for $C_{14}H_{13}N_4O_5$ $[M+H]^+$ 317.09, found 317.58.

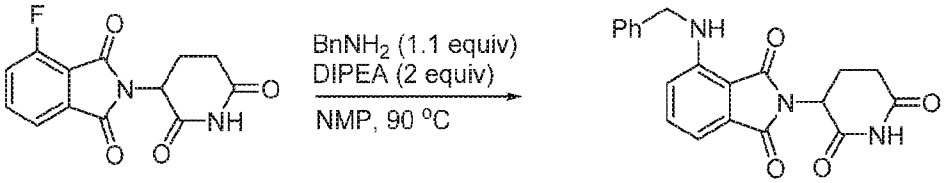


D-15

General procedure V: Amide coupling

***N*-benzyl-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamide (D-15)**

In a 4 mL glass vial, 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxylic acid (10 mg, 0.033 mmol, 1 equiv), HATU (13 mg, 0.033 mmol, 1 equiv), DIPEA (17 μL, 0.099 mmol, 3 equiv) and benzyl amine (4 μL, 0.036 mmol, 1.1 equiv) in DMF (331 μL, 0.1 M) was stirred at room temperature overnight. The reaction mixture was diluted with MeOH to 4 mL, filtered and then purified by preparative HPLC to afford the title compound as a white solid (6 mg, 46%). MS (ESI) calcd for $C_{21}H_{18}N_3O_5$ $[M+H]^+$ 392.12, found 392.33.



D-16

General procedure VI: Nucleophilic aromatic substitution

4-(benzylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-16)

In a 4 mL glass vial, 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (10 mg, 0.036 mmol, 1 equiv), benzyl amine (4.4 μL, 0.040 mmol, 1.1 equiv) and DIPEA (13 μL, 0.072 mmol, 2 equiv) in NMP (362 μL, 0.1 M) was heated to 90 °C overnight. The reaction mixture was cooled to room temperature and taken up in EtOAc (15 mL). The organic layer was washed with $NaHCO_3$ (aq) (15 mL), water (15 mL) and brine (3x15 mL), and subsequently dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (0-100% EtOAc in hexanes) to afford the title compound as a yellow film (5 mg, 38%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 7.44 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.40 – 7.25 (m, 5H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.71 (t, *J* = 5.9 Hz, 1H), 4.93 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.51 (d, *J* = 5.9 Hz, 2H), 2.93 – 2.66 (m, 3H), 2.21 – 2.07 (m, 1H); MS (ESI) calcd for $C_{20}H_{18}N_3O_4$ $[M+H]^+$ 364.13, found 364.31.

2-(2,6-dioxopiperidin-3-yl)-4-(isopropylamino)isoindoline-1,3-dione (D-18)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), isopropylamine (10 μL, 0.119 mmol) and DIPEA (21 μL, 0.119 mmol) to afford the title compound as a yellow film (11 mg, 32%) following purification by flash

column chromatography on silica gel (0-100 % EtOAc in hexanes). MS (ESI) calcd for $C_{16}H_{18}N_3O_4$ $[M+H]^+$ 316.13, found 316.65.

4-(diethylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-21)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), diethylamine (11 μL, 0.130 mmol) and DIPEA (32 μL, 0.181 mmol) to afford the title compound as a yellow film (28 mg, 97%) following purification by flash column chromatography on silica gel (0-100 % EtOAc in hexanes). MS (ESI) calcd for $C_{17}H_{20}N_3O_4$ $[M+H]^+$ 330.14, found 330.62.

5-(benzylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-25)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), benzyl amine (13 μL, 0.119 mmol) and DIPEA (38 μL, 0.217 mmol) to afford the title compound as a yellow film (6 mg, 15%) following purification by flash column chromatography on silica gel (0-100 % EtOAc in hexanes). MS (ESI) calcd for $C_{20}H_{18}N_3O_4$ $[M+H]^+$ 364.13, found 364.34.

2-(2,6-dioxopiperidin-3-yl)-5-(isopropylamino)isoindoline-1,3-dione (D-26)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), isopropyl amine (11 μL, 0.130 mmol) and DIPEA (38 μL, 0.217 mmol) to afford the title compound as a yellow film (6 mg, 17%) following purification by flash column chromatography on silica gel (0-100 % EtOAc in hexanes). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.00 (s, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 2.1 Hz, 1H), 6.64 (dd, *J* = 8.3, 2.2 Hz, 1H), 4.86 (dd, *J* = 12.3, 5.4 Hz, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 2.86 – 2.58 (m, 3H), 2.12 – 2.01 (m, 1H), 1.26 – 1.15 (m, 6H); MS (ESI) calcd for $C_{16}H_{18}N_3O_4$ $[M+H]^+$ 316.13, found 316.30.

5-(diethylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-27)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), diethylamine (14 μL, 0.130 mmol) and DIPEA (38 μL, 0.217 mmol) to afford the title compound as a yellow film (6 mg, 31%) following purification by flash column chromatography on silica gel (0-100 % EtOAc in hexanes). ^{1}H NMR (500 MHz,

Chloroform-*d*) δ 8.08 (s, 1H), 7.57 (d, *J* = 8.6 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.4 Hz, 1H), 4.90 – 4.80 (m, 1H), 3.40 (q, *J* = 7.1 Hz, 4H), 2.89 – 2.61 (m, 3H), 2.11 – 2.01 (m, 1H), 1.16 (t, *J* = 7.1 Hz, 6H); MS (ESI) calcd for $C_{17}H_{20}N_3O_4$ $[M+H]^+$ 330.14, found 330.69.

2-(2,6-dioxopiperidin-3-yl)-5-((furan-2-ylmethyl)amino)isoindoline-1,3-dione (D-28)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (50 mg, 0.181 mmol), furfurylamine (18 μL, 0.199 mmol) and DIPEA (63 μL, 0.362 mmol) to afford the title compound as a yellow film (8 mg, 13%) following purification by flash column chromatography on silica gel (0-5 % MeOH in CH_2Cl_2). MS (ESI) calcd for $C_{18}H_{16}N_3O_4$ $[M+H]^+$ 354.11, found 354.25.

***tert*-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate (D-29)**

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (50 mg, 0.181 mmol), 1-Boc-ethylendiamine (32 mg, 0.199 mmol) and DIPEA (63 μL, 0.362 mmol) to afford the title compound as a yellow film (31 mg, 41%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (bs, 1H), 7.50 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.39 (t, *J* = 6.1 Hz, 1H), 4.96 – 4.87 (m, 1H), 4.83 (bs, 1H), 3.50 – 3.41 (m, 2H), 3.41 – 3.35 (m, 2H), 2.92 – 2.66 (m, 3H), 2.16 – 2.09 (m, 1H), 1.45 (s, 9H); MS (ESI) calcd for $C_{20}H_{25}N_4O_6$ $[M+H]^+$ 417.18, found 417.58.

***tert*-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethyl)carbamate (D-30)**

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (50 mg, 0.181 mmol), 1-Boc-ethylendiamine (32 mg, 0.199 mmol) and DIPEA (63 μL, 0.362 mmol) to afford the title compound as a yellow film (22 mg, 29%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). MS (ESI) calcd for $C_{20}H_{25}N_4O_6$ $[M+H]^+$ 417.18, found 417.32.

2-(2,6-dioxopiperidin-3-yl)-4-((furan-2-ylmethyl)amino)isoindoline-1,3-dione (D-31)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (19.5 mg, 0.0706 mmol), furfurylamine (7 μL, 0.078 mmol) and DIPEA (25 μL, 0.141 mmol) to afford the title compound as a yellow film (19 mg, 76%) following purification by flash column chromatography on silica gel (0-2.5 % MeOH in CH_2Cl_2). MS (ESI) calcd for $C_{18}H_{16}N_3O_4$ $[M+H]^+$ 354.11, found 354.27.

3-(5-(benzylamino)-2-methyl-4-oxoquinazolin-3(4*H*)-yl)piperidine-2,6-dione (D-39)

With the exception that the reaction mixture was heated to 170°C instead of 90 °C, general procedure VI was followed using 3-(5-fluoro-2-methyl-4-oxoquinazolin-3(4H)-yl)piperidine-2,6-dione (30 mg, 0.104 mmol), benzylamine (13 μL, 0.114 mmol) and DIPEA (36 μL, 0.207 mmol) to afford the title compound as a white solid (15 mg, 38%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.73 (t, *J* = 5.7 Hz, 1H), 8.39 (s, 1H), 7.41 (t, *J* = 8.1 Hz, 1H), 7.39 – 7.19 (m, 5H), 6.77 (d, *J* = 7.7 Hz, 1H), 6.41 (d, *J* = 8.3 Hz, 1H), 4.67 (dd, *J* = 11.5, 5.9 Hz, 1H), 4.43 (d, *J* = 5.7 Hz, 2H), 3.03 – 2.79 (m, 2H), 2.72 – 2.61 (m, 1H), 2.60 (s, 3H), 2.15 – 2.07 (m, 1H); MS (ESI) calcd for $C_{21}H_{21}N_4O_3$ $[M+H]^+$ 377.16, found 377.02.

3-(5-(isopropylamino)-2-methyl-4-oxoquinazolin-3(4H)-yl)piperidine-2,6-dione (D-40)

With the exception that the reaction mixture was heated to 170°C instead of 90 °C, general procedure VI was followed using 3-(5-fluoro-2-methyl-4-oxoquinazolin-3(4H)-yl)piperidine-2,6-dione (30 mg, 0.104 mmol), isopropylamine (10 μL, 0.114 mmol) and DIPEA (36 μL, 0.207 mmol) to afford the title compound as a white solid (5 mg, 15%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.31 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 7.50 – 7.37 (m, 1H), 6.70 (dd, *J* = 7.9, 0.9 Hz, 1H), 6.47 (d, *J* = 8.4 Hz, 1H), 4.65 (dd, *J* = 11.4, 5.9 Hz, 1H), 3.69 – 3.56 (m, 1H), 3.03 – 2.80 (m, 3H), 2.58 (s, 3H), 2.14 – 2.03 (m, 1H), 1.27 (d, *J* = 2.7 Hz, 3H), 1.26 (d, *J* = 2.7 Hz, 3H); MS (ESI) calcd for $C_{17}H_{21}N_4O_3$ $[M+H]^+$ 329.16, found 329.97.

2-(2,6-dioxopiperidin-3-yl)-4-((2-hydroxyethyl)amino)isoindoline-1,3-dione (D-68)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (30 mg, 0.109 mmol), aminoethanol (7 μL, 0.119 mmol) and DIPEA (38 μL, 0.217 mmol) to afford the title compound as a yellow film (6 mg, 18%) following purification by flash column chromatography on silica gel (0-5 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.26 (s, 1H), 7.50 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 4.97 – 4.85 (m, 1H), 3.94 – 3.79 (m, 2H), 3.47 (q, *J* = 5.5 Hz, 2H), 3.03 – 2.68 (m, 3H), 2.19 – 2.04 (m, 1H); MS (ESI) calcd for $C_{15}H_{16}N_3O_5$ $[M+H]^+$ 318.11, found 318.22.

4-(cyclopropylamino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D47)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (20 mg, 0.0724 mmol), cyclopropylamine (6 μL, 0.080 mmol) and DIPEA (25 μL, 0.141 mmol) to afford the title compound as a yellow film (16 mg, 70%) following purification by flash column chromatography on silica gel (0-5 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.05 (s, 1H), 7.53 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.33 – 7.21 (m, 1H), 7.15 (dd, *J* = 7.1, 0.7 Hz, 1H), 6.44 (bs, 1H), 4.95 – 4.85 (m, 1H), 2.98 – 2.66 (m, 3H), 2.62 – 2.50 (m, 1H), 2.19 – 2.06 (m, 1H), 0.92 – 0.78 (m, 2H), 0.67 – 0.56 (m, 2H); MS (ESI) calcd for $C_{16}H_{16}N_3O_4$ $[M+H]^+$ 314.11, found 314.54.

4-((2-(1*H*-indol-3-yl)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-48)

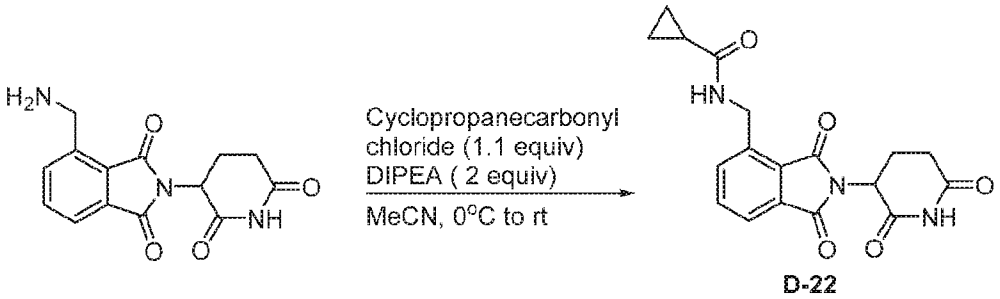
General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (20 mg, 0.0724 mmol), tryptamine (13 mg, 0.080 mmol) and DIPEA (25 μL, 0.144 mmol) to afford the title compound as a yellow film (10 mg, 33%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.14 (s, 1H), 8.11 (s, 1H), 7.65 – 7.55 (m, 1H), 7.45 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.37 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.21 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.16 – 7.04 (m, 3H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.34 (t, *J* = 5.6 Hz, 1H), 4.89 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.59 (td, *J* = 6.8, 5.5 Hz, 2H), 3.19 – 3.03 (m, 2H), 2.93 – 2.64 (m, 3H), 2.14 – 2.04 (m, 1H); MS (ESI) calcd for $C_{23}H_{21}N_4O_4$ $[M+H]^+$ 417.16, found 417.26.

2-(2,6-dioxopiperidin-3-yl)-4-((4-hydroxyphenethyl)amino)isoindoline-1,3-dione (D-49)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (20 mg, 0.0724 mmol), tyramine (11 mg, 0.080 mmol) and DIPEA (25 μL, 0.144 mmol) to afford the title compound as a yellow film (15 mg, 54%) following purification by flash column chromatography on silica gel (0-5 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.20 (s, 1H), 7.51 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.17 – 7.08 (m, 2H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.85 – 6.72 (m, 2H), 4.95 – 4.90 (m, 1H), 3.52 – 3.46 (m, 2H), 2.97 – 2.87 (m, 2H), 2.86 – 2.72 (m, 2H), 2.21 – 2.09 (m, 1H); MS (ESI) calcd for $C_{21}H_{20}N_3O_5$ $[M+H]^+$ 394.14, found 394.25.

4-((2-(1H-imidazol-2-yl)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-50)

General procedure VI was followed using 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (20 mg, 0.0724 mmol), histamine (15 mg, 0.080 mmol) and DIPEA (25 μL, 0.144 mmol) to afford the title compound as a yellow film (5 mg, 19%) following purification by flash column chromatography on silica gel (0-10 % MeOH in CH_2Cl_2). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.19 (s, 1H), 7.61 (d, *J* = 1.2 Hz, 1H), 7.47 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.07 (d, *J* = 6.9 Hz, 1H), 6.96 – 6.83 (m, 2H), 6.39 (t, *J* = 5.7 Hz, 1H), 4.97 – 4.79 (m, 1H), 3.59 (q, *J* = 6.5 Hz, 2H), 2.95 (t, *J* = 6.6 Hz, 2H), 2.92 – 2.62 (m, 2H), 2.16 – 2.04 (m, 1H); MS (ESI) calcd for $C_{18}H_{18}N_5O_4$ $[M+H]^+$ 368.14, found 368.47.



D-22

General procedure VII: Acylation of primary amines

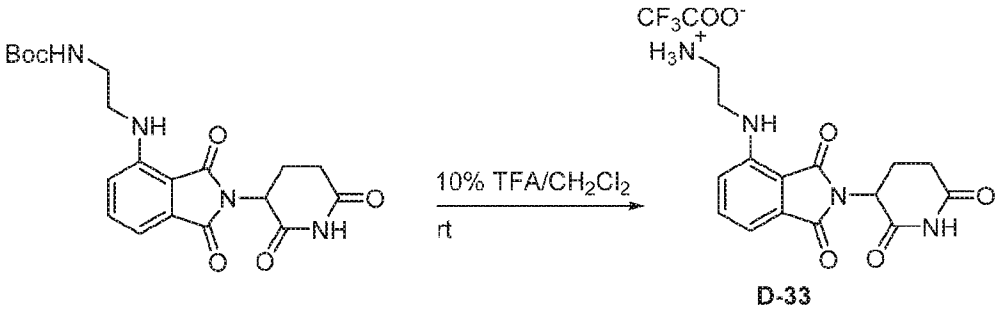
***N*-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)methyl)cyclopropanecarboxamide (D-22)**

In a 4 mL glass vial, 4-(aminomethyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (25 mg, 0.087 mmol, 1 equiv) and DIPEA (30 μL, 0.174 mmol, 2 equiv) in MeCN (250 μL, 0.35 M) was cooled to 0 °C. Cyclopropanecarbonyl chloride (8.7 μL, 0.096 mmol) was added slowly

and the reaction mixture was stirred at room temperature overnight. The product was isolated by filtration to afford the title compound as a white solid (4.8 mg, 15%), that was used without further purification. MS (ESI) calcd for $C_{18}H_{18}N_3O_5$ $[M+H]^+$ 356.12, found 356.32.

N-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)methyl)acetamide (D-23)

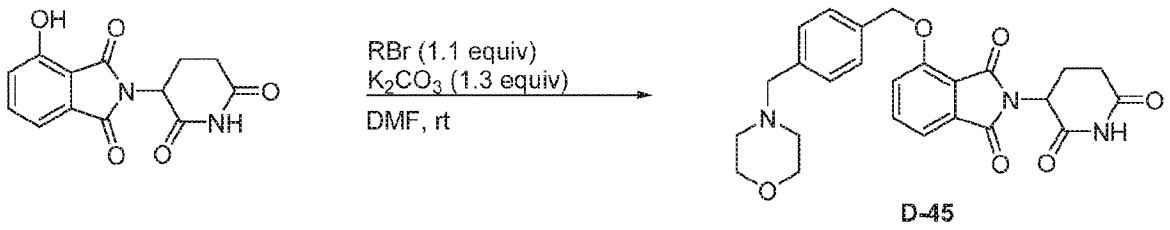
General procedure VII was followed using 4-(aminomethyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (25 mg, 0.087 mmol), DIPEA (30 μL, 0.174 mmol) and acetyl chloride (7 μL, 0.096 mmol) to afford the title compound as a white solid (4.5 mg, 16%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.13 (s, 1H), 8.47 (t, J = 6.0 Hz, 1H), 7.88 – 7.76 (m, 2H), 7.70 (dt, J = 7.3, 1.1 Hz, 1H), 5.15 (dd, J = 12.7, 5.4 Hz, 1H), 4.69 (d, J = 6.0 Hz, 2H), 2.90 (ddd, J = 16.8, 13.8, 5.4 Hz, 1H), 2.64 – 2.44 (m, 2H), 2.15 – 2.01 (m, 1H), 1.92 (s, 3H); MS (ESI) calcd for $C_{16}H_{16}N_3O_5$ $[M+H]^+$ 330.11, found 330.05.



D-33

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethan-1-aminium 2,2,2-trifluoroacetate (D-33)

A stirred solution of *tert*-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate (205 mg, 0.492 mmol, 1 equiv) in dichloromethane (2.25 mL) was added trifluoroacetic acid (0.250 mL). The reaction mixture was stirred at room temperature for 4 h, whereupon the volatiles were removed *in vacuo*. The title compound was obtained as a yellow solid (226 mg, >95%), that was used without further purification. ^{1}H NMR (500 MHz, MeOD) δ 7.64 (d, J = 1.4 Hz, 1H), 7.27 – 7.05 (m, 2H), 5.10 (dd, J = 12.5, 5.5 Hz, 1H), 3.70 (t, J = 6.0 Hz, 2H), 3.50 – 3.42 (m, 2H), 3.22 (t, J = 6.0 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.80 – 2.69 (m, 2H), 2.17 – 2.10 (m, 1H); MS (ESI) calcd for $C_{15}H_{17}N_4O_4$ $[M+H]^+$ 317.12, found 317.53.



D-45

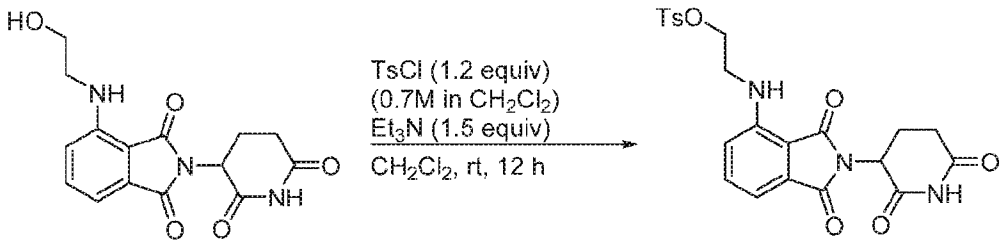
General procedure VIII: Phenol alkylation

2-(2,6-dioxopiperidin-3-yl)-4-((4-(morpholinomethyl)benzyl)oxy)isoindoline-1,3-dione (D-45)

In a 4 mL glass vial, 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (30 mg, 0.109 mmol, 1 equiv) and K_2CO_3 (15 mg, 0.109 mmol, 1 equiv) in DMF (365 μL, 0.3 M) was stirred at room temperature. 4-(4-(bromomethyl)benzyl)morpholine (30 mg, 0.109 mmol, 1 equiv) in DMF (200 μL) was added and the reaction mixture was stirred at room temperature for 4 days. The reaction mixture was taken up in water (15 mL) and EtOAc (15 mL), and the organic layer was washed with brine (3x15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (0 to 10% MeOH in CH_2Cl_2) to afford the title compound as a white solid (20 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.82 (dd, J = 8.5, 7.2 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.50 – 7.42 (m, 3H), 7.35 (d, J = 8.1 Hz, 2H), 5.35 (s, 2H), 5.09 (dd, J = 12.8, 5.5 Hz, 1H), 3.64 – 3.51 (m, 4H), 3.46 (s, 2H), 2.88 (ddd, J = 17.0, 14.1, 5.4 Hz, 1H), 2.63 – 2.47 (m, 2H), 2.38 – 2.31 (m, 4H), 2.07 – 1.99 (m, 1H); MS (ESI) calcd for $C_{25}H_{26}N_3O_6$ $[M+H]^+$ 464.18, found 464.00.

4-(benzyloxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-46)

General procedure VIII was followed using 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (30 mg, 0.109 mmol), K_2CO_3 (15 mg, 0.109 mmol) and benzyl bromide (8 μL, 0109 mmol) to afford the title compound as a white solid (8 mg, 20%) after purification by flash column chromatography on silica gel (0 to 10% MeOH in CH_2Cl_2). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.83 (dd, J = 8.5, 7.3 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.53 – 7.50 (m, 2H), 7.47 (d, J = 7.2 Hz, 1H), 7.45 – 7.39 (m, 2H), 7.38 – 7.32 (m, 1H), 5.38 (s, 2H), 5.09 (dd, J = 12.8, 5.5 Hz, 1H), 2.88 (ddd, J = 16.9, 13.8, 5.5 Hz, 1H), 2.64 – 2.46 (m, 2H), 2.07 – 1.99 (m, 1H); MS (ESI) calcd for $C_{20}H_{17}N_2O_5$ $[M+H]^+$ 365.11, found 365.21.



D-44

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl 4-methylbenzene-sulfonate (D-44)

In a 4 mL glass vial, 2-(2,6-dioxopiperidin-3-yl)-4-((2-hydroxyethyl)amino)isoindoline-1,3-dione (7 mg, 0.0221 mmol, 1 equiv) and Et_3N (3 μL, 0.033 mmol, 1.5 equiv) in CH_2Cl_2 (200 μL) was stirred at room temperature. Tosyl chloride (6 mg, 0.026 mmol, 1.2 equiv) in CH_2Cl_2 (100 μL) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (0-10% MeOH in CH_2Cl_2) to afford the title compound as a white solid (4 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.64 – 7.59 (m, 2H), 7.46 (dd, J = 8.6, 7.1 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.04 – 6.93 (m, 2H), 6.58 (t, J = 6.4 Hz, 1H), 5.09 (dd, J = 12.7, 5.4 Hz, 1H), 4.15 (t, J = 5.1 Hz, 2H), 3.65 – 3.52 (m, 2H), 2.97 – 2.83 (m, 1H), 2.67 – 2.46 (m, 2H), 2.27 (s, 3H), 2.12 – 2.02 (m, 1H); MS (ESI) calcd for $C_{22}H_{22}N_3O_7S$ $[M+H]^+$ 472.12, found 472.39.

(R)-4-hydroxy-2-(3-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-52)

Hydroxyisobenzofuran-1,3-dione (147.08 mg, 0.896 mmol, 1 eq) was added to (*R*)-3-amino-3-methylpiperidine-2,6-dione hydrochloric acid (127.32 mg, 0.896 mmol, 1 eq). Pyridine (3.584 ml, 0.25 M) was then added to the mixture and it was stirred at 110 °C for 17 hours. The mixture was diluted with methanol and was condensed under reduced pressure. The crude material was purified by column chromatography (ISCO, 24 g silica column, 0 to 10% MeOH/DCM 25 minute gradient) to give a white oil (110.9 mg, 42.63 % yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.61 (dd, J = 8.4, 7.2 Hz, 1H), 7.27 – 7.14 (m, 2H), 2.73 – 2.63 (m, 1H), 2.57 – 2.51 (m, 1H), 2.04 – 1.97 (m, 1H), 1.86 (s, 3H).

LCMS 289 (M+H).

(S)-4-hydroxy-2-(3-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-53)

4-hydroxyisobenzofuran-1,3-dione (148.99 mg, 0.907 mmol, 1 eq) was added to (*S*)-3-amino-3-methylpiperidine-2,6-dione hydrochloric acid (128.97 mg, 0.907 mmol, 1 eq). Pyridine (3.628 ml, 0.25 M) was then added to the mixture and it was stirred at 110 °C for 17 hours. The mixture was diluted with methanol and was condensed under reduced pressure. The crude material was purified by column chromatography (ISCO, 24 g silica column, 0 to 10% MeOH/DCM 25 minute gradient) to give a white oil (150 mg, 57.4 % yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.62 (dd, J = 8.4, 7.2 Hz, 1H), 7.27 – 7.16 (m, 2H), 2.75 – 2.62 (m, 1H), 2.55 (dd, J = 14.0, 4.3 Hz, 1H), 2.05 – 1.96 (m, 1H), 1.86 (s, 3H). **LCMS** 289 (M+H).

(S)-2-((2-(3-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (D-55)

TFA (0.63 ml, 0.1 M) was added to *tert*-butyl (*S*)-2-((2-(3-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (25.4 mg, 0.063 mmol, 1 eq) and the mixture was stirred at 50 °C for an hour. The mixture was then diluted with methanol and condensed under reduced pressure to give a white powder (20.5 mg, 93.9% yield) that was carried forward without further purification. **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.81 – 7.75 (m, 1H), 7.50 (d, J = 7.3 Hz, 1H), 7.45 (d, J = 8.6 Hz, 2H), 7.43 – 7.37 (m, 3H), 5.09 (dd, J = 12.8, 5.5 Hz, 1H), 4.76 (s, 2H), 4.63 (dd, J = 9.1, 5.2 Hz, 1H), 3.66 – 3.55 (m, 30H), 3.51 – 3.41 (m, 5H), 2.90 – 2.83 (m, 1H), 2.79 – 2.71 (m, 2H), 2.69 (s, 3H), 2.43 (s, 3H), 2.14 (ddt, J = 10.5, 5.5, 3.2 Hz, 1H), 1.69 (s, 3H). **LCMS** 347 (M+H).

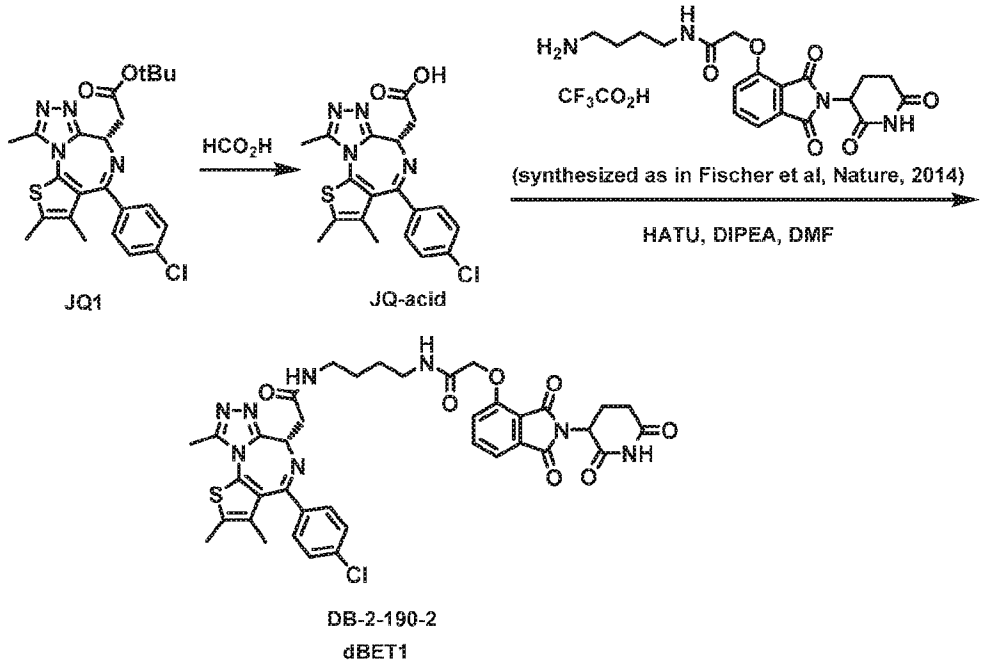
(R)-2-((2-(3-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (D-54)

TFA (1.78 ml, 0.1 M) was added to *tert*-butyl (*R*)-2-((2-(3-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (71.3 mg, 0.178 mmol, 1 eq) and the mixture was stirred at 50 °C for an hour. The mixture was then diluted with methanol and condensed under reduced pressure to give a white powder (47.2 mg, 76.63% yield) that was carried forward without further purification. **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.72 (ddd, J = 8.5, 7.3, 5.0 Hz, 1H), 7.46 – 7.42 (m, 1H), 7.30 (dd, J = 8.6, 4.5 Hz, 1H), 4.94 (d, J = 5.3 Hz, 2H), 2.81 – 2.56 (m, 2H), 2.24 – 2.07 (m, 1H), 2.00 (s, 2H), 0.90 (t, J = 6.5 Hz, 2H). **LCMS** 347 (M+H).

4,7-dichloro-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (D-51)

4,7-dichloroisobenzofuran-1,3-dione (434.6 mg, 2.002 mmol, 1 eq) was added to 3-aminopiperidine-2,6-dione hydrochloric acid (362.6 mg, 2.203 mmol, 1.1 eq). Potassium acetate (609.07 mg, 6.206 mmol, 3.1 eq) and acetic acid (6.67 ml, 0.3 M) were then added to the mixture and it was stirred at 90 °C for 18 hours. The mixture was cooled down to room temperature, diluted with DI water and centrifuged for 5 minutes. The precipitate was diluted with methanol and was condensed under reduced pressure. The crude material was purified by column chromatography (ISCO, 12 g silica column, 0 to 10% MeOH/DCM 25 minute gradient) to give a white powder (160.4 mg, 24.5 % yield). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$) δ 11.15 (s, 1H), 7.91 (s, 2H), 5.17 (dd, *J* = 12.9, 5.4 Hz, 1H), 2.88 (ddd, *J* = 17.2, 13.9, 5.4 Hz, 1H), 2.68 – 2.54 (m, 1H), 2.05 (ddd, *J* = 10.5, 5.4, 2.7 Hz, 1H). **LCMS** 328 (M+H).

Synthetic Example 1: Synthesis of dBET1



(1) Synthesis of JQ-acid

JQ1 (1.0 g, 2.19 mmol, 1 eq) was dissolved in formic acid (11 mL, 0.2 M) at room temperature and stirred for 75 hours. The mixture was concentrated under reduced pressure to give a yellow solid (0.99 g, quant yield) that was used without purification. ^{1}H NMR (400 MHz,

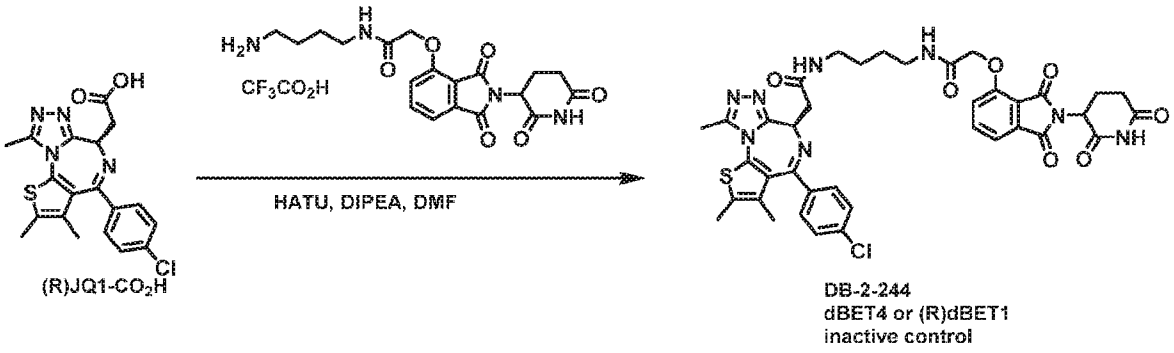
Methanol-d_4) δ 7.50 – 7.36 (m, 4H), 4.59 (t, J = 7.1 Hz, 1H), 3.51 (d, J = 7.1 Hz, 2H), 2.70 (s, 3H), 2.45 (s, 3H), 1.71 (s, 3H). LCMS 401.33 (M+H).

N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamidetrifluoroacetate was synthesized according to the previously published procedure (Fischer et al., *Nature* 512 (2014):49).

(2) Synthesis of dBET1

JQ-acid (11.3 mg, 0.0281 mmol, 1 eq) and N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (14.5 mg, 0.0281 mmol, 1 eq) were dissolved in DMF (0.28 mL, 0.1 M) at room temperature. DIPEA (14.7 microliters, 0.0843 mmol, 3 eq) and HATU (10.7 mg, 0.0281 mmol, 1 eq) were then added and the mixture was stirred for 19 hours. The mixture was then purified by preparative HPLC to give dBET1 as a yellow solid (15.90 mg, 0.0202 mmol, 72%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 (dd, J = 8.3, 7.5 Hz, 1H), 7.49 (d, J = 7.3 Hz, 1H), 7.47 – 7.37 (m, 5H), 5.07 (dd, J = 12.5, 5.4 Hz, 1H), 4.74 (s, 2H), 4.69 (dd, J = 8.7, 5.5 Hz, 1H), 3.43 – 3.32 (m, 3H), 3.29 – 3.25 (m, 2H), 2.87 – 2.62 (m, 7H), 2.43 (s, 3H), 2.13 – 2.04 (m, 1H), 1.72 – 1.58 (m, 7H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.41, 172.33, 171.27, 171.25, 169.87, 168.22, 167.76, 166.73, 166.70, 156.26, 138.40, 138.23, 137.44, 134.83, 133.92, 133.40, 132.30, 132.28, 131.97, 131.50, 129.87, 121.85, 119.31, 118.00, 69.53, 54.90, 50.54, 40.09, 39.83, 38.40, 32.12, 27.74, 27.65, 23.61, 14.42, 12.97, 11.57. LCMS 785.44 (M+H).

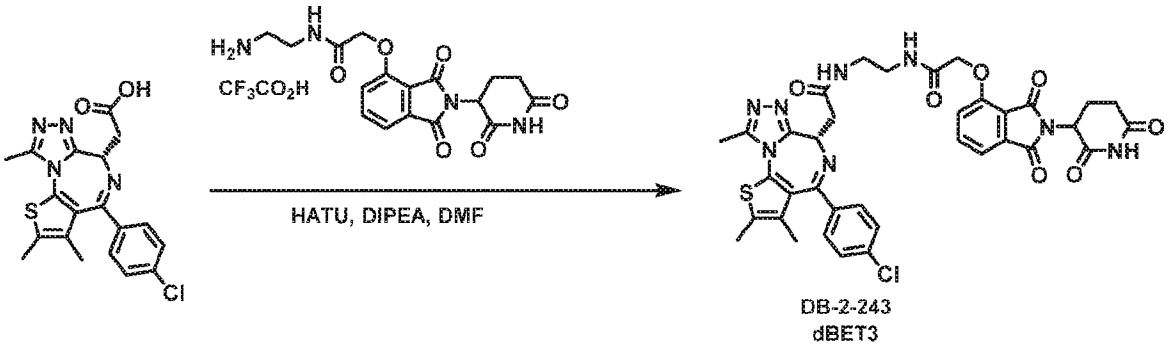
Synthetic Example 2: Synthesis of dBET4



A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.438 mL, 0.0438 mmol 1.2 eq) was added to (R)-JQ-acid (prepared from (R)-JQ1 in an analogous method to JQ-acid) (14.63 mg, 0.0365 mmol, 1 eq) at room temperature. DIPEA (19.1 microliters, 0.1095 mmol, 3 eq) and HATU

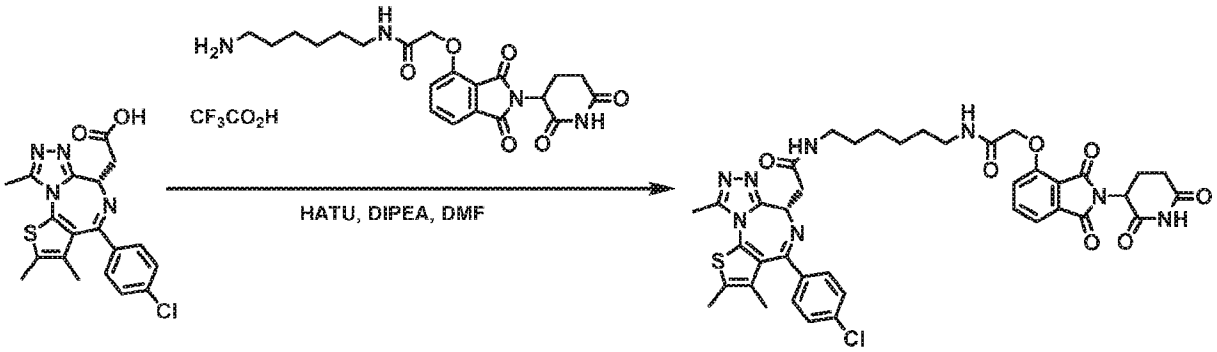
(15.3 mg, 0.0402 mmol, 1.1 eq) were added and the mixture was stirred for 24 hours, then diluted with MeOH and concentrated under reduced pressure. The crude material was purified by preparative HPLC to give a yellow solid (20.64 mg, 0.0263 mmol, 72%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (dd, J = 8.4, 7.4 Hz, 1H), 7.51 (d, J = 7.3 Hz, 1H), 7.47 – 7.39 (m, 5H), 5.11 – 5.06 (m, 1H), 4.75 (s, 2H), 4.68 (dd, J = 8.8, 5.5 Hz, 1H), 3.47 – 3.31 (m, 5H), 2.83 – 2.65 (m, 7H), 2.44 (s, 3H), 2.13 – 2.06 (m, 1H), 1.68 (s, 3H), 1.67 – 1.60 (m, 4H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.43, 172.40, 171.29, 169.92, 168.24, 167.82, 166.71, 156.31, 153.14, 138.38, 138.24, 137.54, 134.88, 133.86, 133.44, 132.29, 132.00, 131.49, 129.88, 122.46, 121.90, 119.38, 118.02, 69.59, 54.96, 50.55, 40.09, 39.84, 38.45, 32.14, 27.75, 27.65, 23.62, 14.41, 12.96, 11.56. MS 785.48 (M+H).

Synthetic Example 3: Synthesis of dBET3



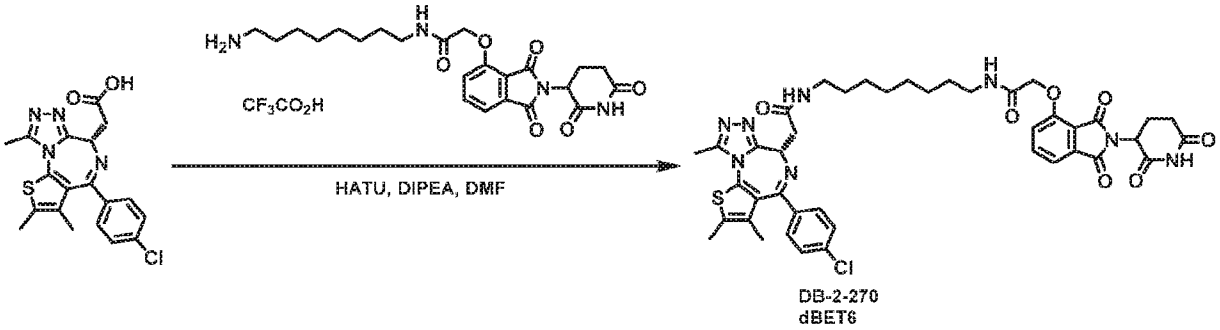
A 0.1 M solution of *N*-(2-aminoethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.475 mL, 0.0475 mmol, 1.2 eq) was added to JQ-acid (15.86 mg, 0.0396 mmol, 1 eq) at room temperature. DIPEA (20.7 microliters, 0.1188 mmol, 3 eq) and HATU (16.5 mg, 0.0435 mmol, 1.1 eq) were then added and the mixture was stirred for 24 hours, then purified by preparative HPLC to give a yellow solid (22.14 mg, 0.0292 mmol, 74%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.82 – 7.75 (m, 1H), 7.52 – 7.32 (m, 6H), 5.04 (dd, J = 11.6, 5.5 Hz, 1H), 4.76 (d, J = 3.2 Hz, 2H), 4.66 (d, J = 6.6 Hz, 1H), 3.58 – 3.35 (m, 6H), 2.78 – 2.58 (m, 6H), 2.48 – 2.41 (m, 3H), 2.11 – 2.02 (m, 1H), 1.70 (d, J = 11.8 Hz, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.38, 171.26, 171.19, 170.26, 168.86, 168.21, 167.76, 166.72, 156.27, 153.14, 138.44, 138.36, 138.19, 134.87, 133.71, 132.31, 131.57, 131.51, 129.90, 129.86, 121.81, 119.36, 117.95, 69.48, 54.83, 50.52, 40.09, 39.76, 38.30, 32.09, 23.63, 14.40, 11.61. LCMS 757.41 (M+H).

Synthetic Example 4: Synthesis of dBET5



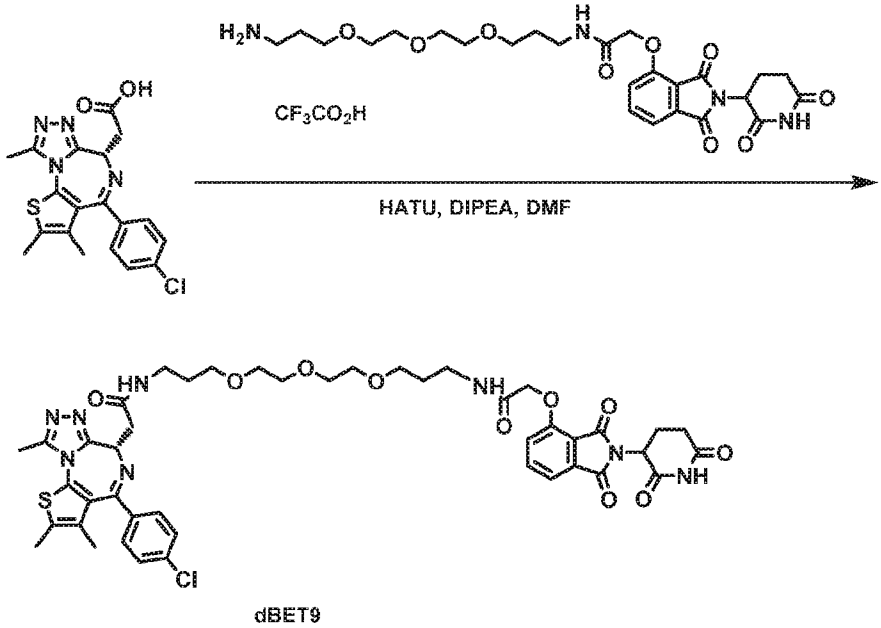
A 0.1M solution of *N*-(6-aminohexyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.247 mL, 0.0247 mmol, 1 eq) was added to JQ-acid (9.9 mg, 0.0247 mmol, 1 eq) at room temperature. DIPEA (12.9 microliters, 0.0741 mmol, 3 eq) and HATU (9.4 mg, 0.0247 mmol, 1 eq) were then added. the mixture was stirred for 21 hours, then diluted with MeOH and concentrated under reduced pressure. The crude material was purified by preparative HPLC to give a yellow solid (13.56 mg, 0.0167 mmol, 67%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.82 – 7.78 (m, 1H), 7.53 (dd, J = 7.3, 2.0 Hz, 1H), 7.49 – 7.37 (m, 5H), 5.10 (dt, J = 12.4, 5.3 Hz, 1H), 4.76 (s, 2H), 4.70 (dd, J = 8.7, 5.5 Hz, 1H), 3.42 – 3.33 (m, 2H), 3.25 (dt, J = 12.3, 6.0 Hz, 3H), 2.87 – 2.67 (m, 7H), 2.48 – 2.42 (m, 3H), 2.14 – 2.09 (m, 1H), 1.69 (d, J = 4.8 Hz, 3H), 1.58 (s, 4H), 1.42 (d, J = 5.2 Hz, 4H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.51, 171.31, 171.26, 169.82, 168.27, 168.26, 167.75, 156.26, 150.46, 138.20, 134.92, 133.92, 133.47, 132.34, 132.01, 131.52, 129.88, 121.69, 119.34, 117.95, 111.42, 69.39, 54.97, 50.56, 40.39, 40.00, 38.40, 32.15, 30.46, 30.16, 27.58, 27.48, 23.64, 14.41, 12.96, 11.55. LCMS 813.38.

Synthetic Example 5: Synthesis of dBET6



A 0.1M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.191 mL, 0.0191 mmol, 1 eq) was added to JQ-acid (7.66 mg, 0.0191 mmol, 1 eq) at room temperature. DIPEA (10 microliters, 0.0574 mmol, 3 eq) and HATU (7.3 mg, 0.0191 mmol, 1 eq) were added and the mixture was stirred for 22 hours, diluted with MeOH, and concentrated under reduced pressure. The crude material was purified by preparative HPLC to give a cream colored solid. (8.53 mg, 0.0101 mmol, 53%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.80 (dd, J = 8.4, 7.4 Hz, 1H), 7.53 (d, J = 7.4 Hz, 1H), 7.49 – 7.36 (m, 5H), 5.10 (dt, J = 12.3, 5.3 Hz, 1H), 4.75 (s, 2H), 4.69 (dd, J = 8.8, 5.3 Hz, 1H), 3.42 (dd, J = 15.0, 8.9 Hz, 1H), 3.30 – 3.18 (m, 4H), 2.90 – 2.64 (m, 7H), 2.45 (s, 3H), 2.13 (dtt, J = 10.8, 5.2, 2.6 Hz, 1H), 1.71 (d, J = 4.4 Hz, 3H), 1.56 (d, J = 6.2 Hz, 4H), 1.33 (d, J = 17.1 Hz, 8H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.50, 172.38, 171.30, 169.81, 168.28, 167.74, 166.64, 156.25, 138.38, 138.20, 137.55, 134.92, 133.88, 133.42, 132.27, 132.02, 131.50, 129.85, 121.66, 119.30, 117.95, 69.37, 55.01, 50.58, 40.51, 40.12, 38.44, 32.18, 30.46, 30.33, 30.27, 30.21, 27.91, 27.81, 23.63, 14.42, 12.96, 11.55. LCMS 841.64 (M+H).

Synthetic Example 6: Synthesis of dBET9

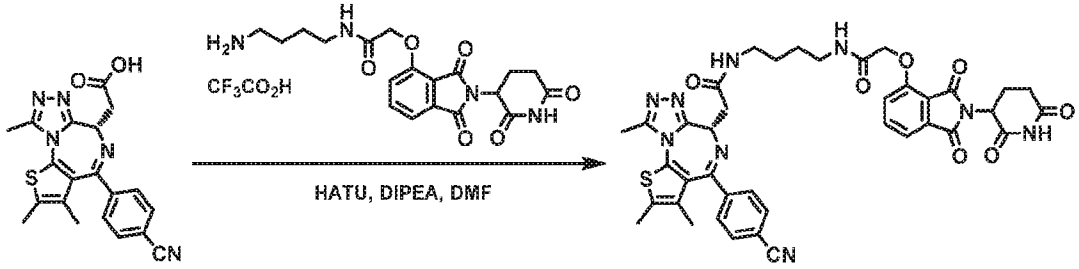


dBET9

A 0.1M solution of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.321 mL, 0.0321 mmol, 1 eq) was added to JQ-acid (12.87 mg, 0.0321 mmol, 1 eq) at room temperature. DIPEA (16.8 microliters, 0.0963 mmol, 3 eq) and HATU (12.2 mg, 0.0321 mmol, 1 eq) were added and the mixture was stirred for 24 hours, diluted with MeOH, and concentrated under reduced pressure. The crude material was purified by preparative HPLC to give a yellow oil. (16.11 mg, 0.0176 mmol, 55%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (dd, J = 8.4, 7.4 Hz, 1H), 7.52 (d, J = 7.2 Hz, 1H), 7.49 – 7.36 (m, 5H), 5.10 (dd, J = 12.5, 5.5 Hz, 1H), 4.78 – 4.67 (m, 3H), 3.64 – 3.52 (m, 11H), 3.48 – 3.32 (m, 6H), 2.94 – 2.64 (m, 7H), 2.52 – 2.43 (m, 3H), 2.18 – 2.08 (m, 1H), 1.81 (p, J = 6.3 Hz, 4H), 1.73 – 1.67 (m, 3H). LCMS 918.45 (M+H).

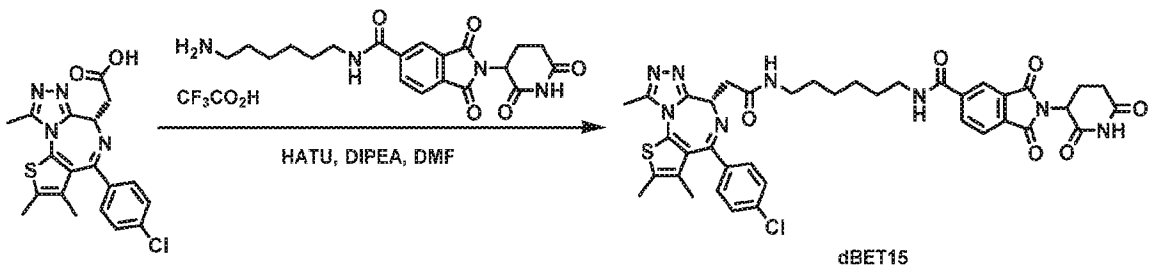
Synthetic Example 7: Synthesis of dBET17



A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.281 mL, 0.0281 mmol 1 eq) was added to (*S*)-2-(4-(4-cyanophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetic acid (11 mg, 0.0281 mmol, 1 eq) at room temperature. DIPEA (14.7 microliters, 0.0843 mmol, 3 eq) and HATU (10.7 mg, 0.0281 mmol, 1 eq) were added and the mixture was stirred for 24 hours, diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (ISCO, 4 g silica column 0-10%MeOH/DCM) gave a white solid (14.12 mg, 0.0182 mmol, 65%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.82 – 7.72 (m, 3H), 7.61 (dd, J = 8.5, 2.0 Hz, 2H), 7.51 (d, J = 7.9 Hz, 1H), 7.44 – 7.40 (m, 1H), 5.11 – 5.05 (m, 1H), 4.76 (s, 2H), 4.66 (dd, J = 9.0, 5.1 Hz, 1H), 3.48 – 3.32 (m, 4H), 3.30 – 3.23 (m, 1H), 2.87 – 2.61 (m, 7H), 2.43 (s, 3H), 2.10 (dt, J = 10.7, 5.2 Hz, 1H), 1.70 – 1.59 (m, 7H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.42, 172.65, 171.27, 169.92, 168.25, 167.80, 165.88, 156.31, 143.55, 138.24, 134.88, 133.92, 133.50, 133.39, 131.72, 131.46, 130.55, 121.93, 119.39, 119.21, 118.02, 115.17, 69.59, 55.50, 50.55, 40.10, 39.83, 38.86, 32.11, 27.78, 27.67, 23.62, 14.41, 12.91, 11.64. LCMS 776.39 (M+H).

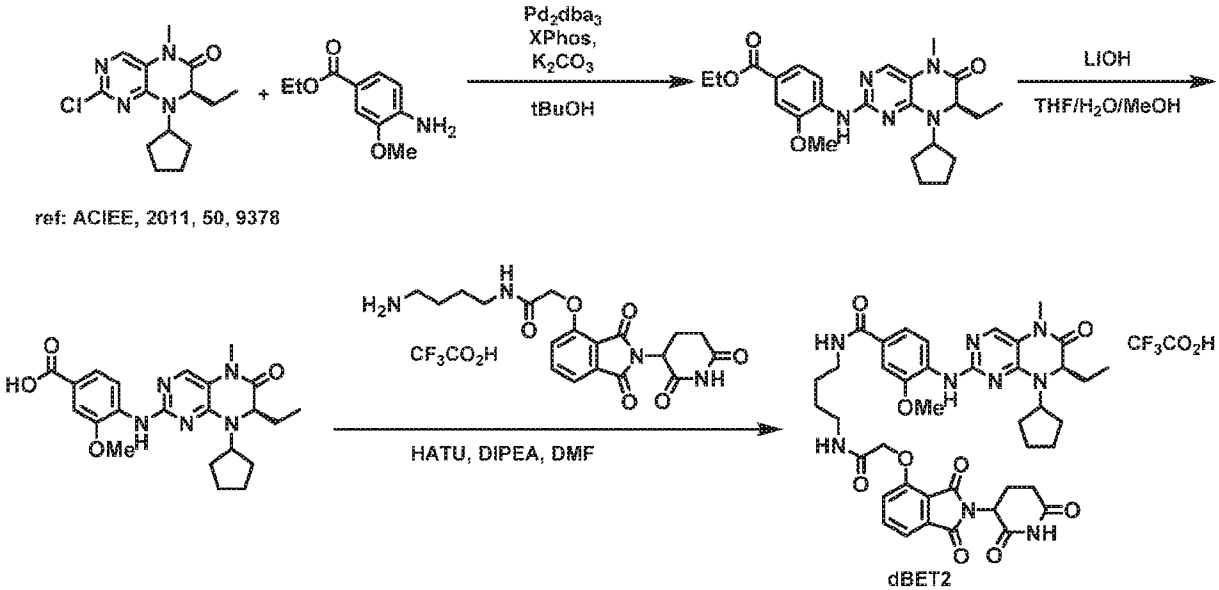
Synthetic Example 8: Synthesis of dBET15



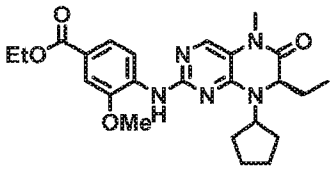
N-(6-aminohexyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamide trifluoroacetate (13.29 mg, 0.258 mmol, 1 eq) and JQ-acid (10.3 mg, 0.0258 mmol, 1 eq) were dissolved in DMF (0.26 mL). DIPEA (13.5 microliters, 0.0775 mmol, 3 eq) was added, followed by HATU (9.8 mg, 0.0258 mmol, 1 eq) and the mixture was stirred at room temperature. After 24 hours, the material was diluted with DCM and purified by column chromatography (ISCO, 0-15%MeOH/DCM) followed by preparative HPLC to give a pale yellow solid (11.44 mg, 0.0146 mmol 57%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.29 – 8.23 (m, 2H), 7.93 (dd, J = 8.1, 4.2 Hz, 1H), 7.50 – 7.34 (m, 4H), 5.17 – 5.11 (m, 1H), 4.75 – 4.69 (m, 1H), 3.53 – 3.32 (m, 6H), 3.25 (dd, J = 13.8, 6.7 Hz, 1H), 2.90 – 2.67 (m, 6H), 2.49 – 2.38 (m, 3H), 2.18 – 2.10 (m, 1H), 1.64 (d, J = 22.4 Hz, 6H), 1.47 (s, 4H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.48, 171.17, 168.05, 168.03, 167.99, 167.70, 166.63, 141.81, 138.40, 137.47, 135.09, 134.77, 134.74, 133.96, 133.94, 133.38, 132.24, 132.05, 131.44, 129.85, 124.57, 123.12, 123.09, 54.98, 50.78, 40.88, 40.08, 38.37, 32.13, 30.40, 30.23, 27.34, 27.26, 23.58, 14.40, 12.96, 11.54. LCMS 783.43 (M+H).

Synthetic Example 9: Synthesis of dBET2

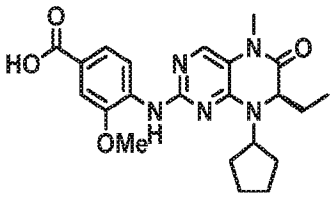


(1) Synthesis of (*R*)-ethyl 4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoate



(*R*)-2-chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one (44.2 mg, 0.15 mmol, 1 eq), ethyl 4-amino-3-methoxybenzoate (35.1 mg, 0.18 mmol, 1.2 eq), Pd_2dba_3 (6.9 mg, 0.0075 mmol, 5 mol %), XPhos (10.7 mg, 0.0225 mmol, 15 mol %) and potassium carbonate (82.9 mg, 0.60 mmol, 4 eq) were dissolved in tBuOH (1.5 mL, 0.1 M) and heated to 100 °C. After 21 hours, the mixture was cooled to room temperature, filtered through celite, washed with DCM and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-100% EtOAc/hexanes over an 18 minute gradient) gave a yellow oil (52.3 mg, 0.115 mmol, 77%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 8.5 Hz, 1H), 7.69 (td, *J* = 6.2, 2.9 Hz, 2H), 7.54 (d, *J* = 1.8 Hz, 1H), 4.52 (t, *J* = 7.9 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 4.23 (dd, *J* = 7.9, 3.7 Hz, 1H), 3.97 (s, 3H), 3.33 (s, 3H), 2.20 – 2.12 (m, 1H), 2.03 – 1.97 (m, 1H), 1.86 (ddd, *J* = 13.9, 7.6, 3.6 Hz, 4H), 1.78 – 1.65 (m, 4H), 1.40 (t, *J* = 7.1 Hz, 3H), 0.88 (t, *J* = 7.5 Hz, 3H). LCMS 454.32 (M+H).

(2) Synthesis of (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoic acid



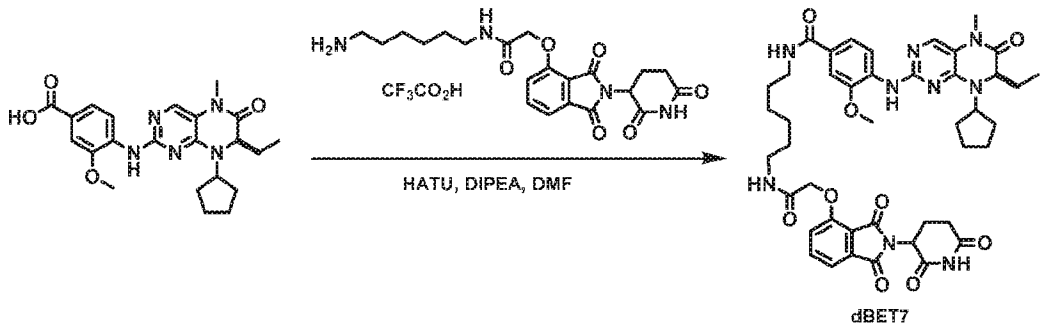
(*R*)-ethyl 4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoate (73.8 mg, 0.163 mmol, 1 eq) and LiOH (11.7 mg, 0.489 mmol, 3 eq) were dissolved in MeOH (0.82 mL) THF (1.63 mL) and water (0.82 mL). After 20 hours, an additional 0.82 mL of water was added and the mixture was stirred for an additional 24 hours before being purified by preparative HPLC to give a cream colored solid (53 mg, 0.125 mmol, 76%). ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 7.97 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.64 – 7.59 (m, 2H), 4.38 (dd, *J* = 7.0, 3.2 Hz, 1H), 4.36 – 4.29 (m, 1H), 3.94 (s, 3H), 3.30 (s, 3H), 2.13 – 1.98 (m, 2H), 1.95 – 1.87 (m, 2H), 1.87 – 1.76 (m, 2H), 1.73 – 1.57 (m, 4H), 0.86 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 168.67, 163.72, 153.59, 150.74, 150.60, 130.95, 127.88,

125.97, 123.14, 121.68, 116.75, 112.35, 61.76, 61.66, 56.31, 29.40, 29.00, 28.68, 28.21, 23.57, 23.41, 8.69. LCMS 426.45 (M+H).

(3) Synthesis of dBET2

A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.183 mL, 0.0183 mmol 1.2 eq) was added to (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoic acid (6.48 mg, 0.0152 mmol, 1 eq) at room temperature. DIPEA (7.9 microliters, 0.0456 mmol, 3 eq) and HATU (6.4 mg, 0.0168 mmol, 1.1 eq) were added and the mixture was stirred for 23 hours, before being purified by preparative HPLC to give a yellow solid (9.44 mg, 0.0102 mmol, 67%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.77 (m, 2H), 7.58 (d, J = 1.8 Hz, 2H), 7.53 – 7.46 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 5.11 – 5.05 (m, 1H), 4.76 (s, 2H), 4.48 (dd, J = 6.5, 3.1 Hz, 1H), 4.33 – 4.24 (m, 1H), 3.95 (s, 3H), 3.49 – 3.35 (m, 4H), 2.97 (d, J = 10.5 Hz, 3H), 2.89 – 2.65 (m, 5H), 2.17 – 1.99 (m, 4H), 1.89 (dd, J = 14.5, 7.3 Hz, 2H), 1.69 – 1.54 (m, 6H), 1.36 (dt, J = 7.6, 3.9 Hz, 1H), 0.85 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 176.52, 174.48, 173.05, 171.34, 169.99, 168.91, 168.25, 167.80, 164.58, 156.34, 154.48, 153.10, 150.63, 138.22, 134.89, 133.96, 129.53, 123.93, 121.87, 120.78, 119.36, 117.99, 111.54, 69.55, 63.29, 63.10, 56.68, 50.55, 40.71, 39.86, 32.15, 29.43, 29.26, 28.73, 28.63, 27.81, 27.77, 24.25, 23.63, 8.47. LCMS 810.58 (M+H).

Synthetic Example 10: Synthesis of dBET7

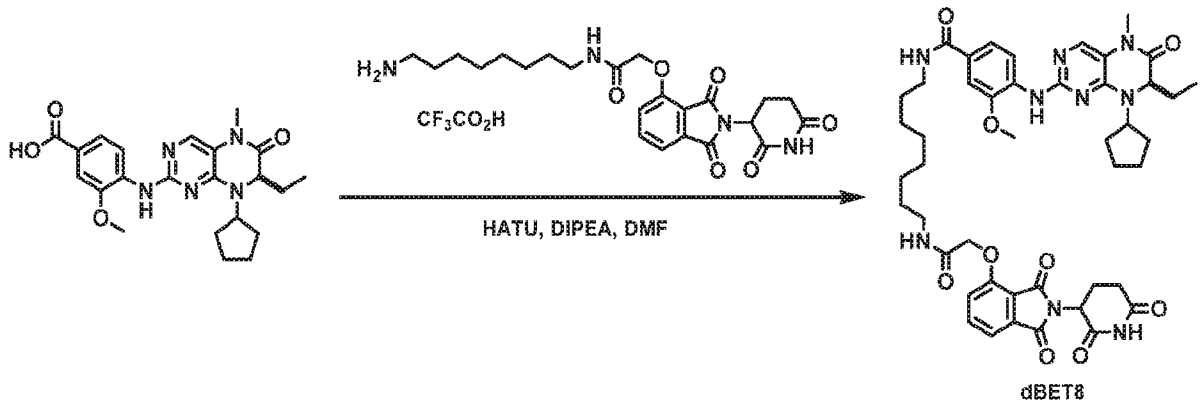


A 0.1 M solution *N*-(6-aminohexyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.186 mL, 0.0186 mmol 1 eq) was added to (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoic acid (7.9 mg, 0.0186 mmol, 1 eq) at room temperature. DIPEA (9.7 microliters,

0.0557 mmol, 3 eq) and HATU (7.1 mg, 0.0186 mmol, 1 eq) were added and the mixture was stirred for 19 hours, before being purified by preparative HPLC to give the desired trifluoracetate salt as a yellow solid(13.62 mg, 0.0143 mmol, 77%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.80 (t, J = 8.3 Hz, 2H), 7.61 – 7.57 (m, 2H), 7.55 – 7.49 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.75 (s, 2H), 4.48 (dd, J = 6.5, 3.2 Hz, 1H), 4.33 – 4.24 (m, 1H), 3.97 (s, 3H), 3.40 (t, J = 7.1 Hz, 2H), 3.34 (d, J = 6.7 Hz, 2H), 3.30 (s, 3H), 2.98 (d, J = 8.5 Hz, 1H), 2.89 – 2.82 (m, 1H), 2.79 – 2.63 (m, 3H), 2.17 – 2.00 (m, 4H), 1.91 (dt, J = 14.4, 7.1 Hz, 3H), 1.61 (dt, J = 13.4, 6.6 Hz, 7H), 1.47 – 1.41 (m, 3H), 0.86 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cd3od) δ 174.54, 171.37, 169.84, 168.84, 168.27, 167.74, 164.59, 156.26, 154.47, 153.18, 150.69, 138.19, 134.91, 134.05, 129.47, 124.78, 124.01, 121.65, 120.77, 119.29, 117.92, 117.86, 111.55, 69.34, 63.31, 63.13, 56.67, 50.53, 40.97, 39.96, 32.16, 30.42, 30.19, 29.42, 29.26, 28.72, 28.62, 27.65, 27.46, 24.26, 23.65, 8.47. LCMS 838.60 (M+H).

Synthetic Example 11: Synthesis of dBET8



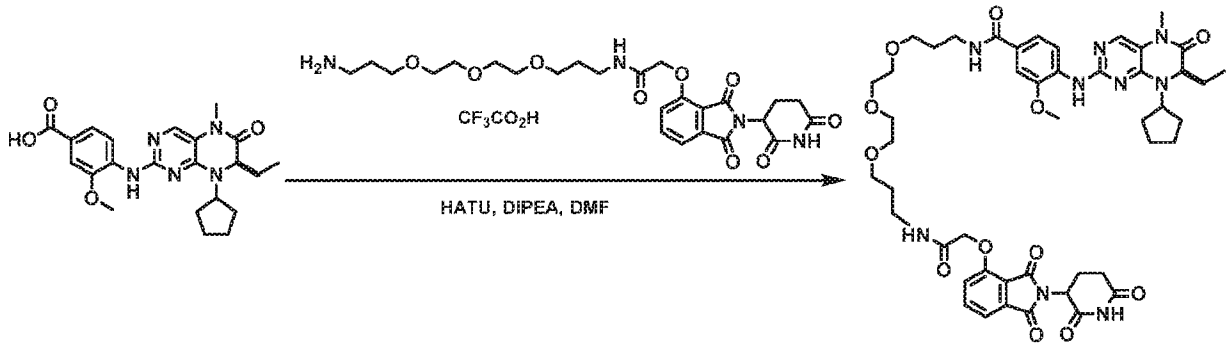
dBET8

A 0.1 M solution *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.186 mL, 0.0186 mmol 1 eq) was added to (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoic acid (7.9 mg, 0.0186 mmol, 1 eq) at room temperature. DIPEA (9.7 microliters, 0.0557 mmol, 3 eq) and HATU (7.1 mg, 0.0186 mmol, 1 eq) were added and the mixture was stirred for 16 hours, before being purified by preparative HPLC to give the desired trifluoracetate salt as an off-white solid(7.15 mg, 0.007296 mmol, 39%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.83 – 7.77 (m, 2H), 7.61 – 7.56 (m, 2H), 7.55 – 7.50 (m, 2H), 7.42 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.75 (s, 2H), 4.49 (dd, J = 6.6, 3.3 Hz,

1H), 4.33 – 4.24 (m, 1H), 3.97 (s, 3H), 3.39 (t, J = 7.1 Hz, 2H), 3.34 – 3.32 (m, 2H), 3.30 (s, 3H), 3.01 – 2.83 (m, 2H), 2.82 – 2.65 (m, 3H), 2.17 – 2.01 (m, 4H), 1.91 (dt, J = 14.2, 7.4 Hz, 1H), 1.68 – 1.54 (m, 7H), 1.37 (s, 7H), 0.86 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.52, 171.35, 169.81, 168.85, 168.28, 167.74, 164.58, 156.27, 154.47, 153.89, 150.64, 138.19, 134.93, 134.18, 129.52, 129.41, 124.91, 123.83, 121.67, 120.76, 119.31, 117.95, 117.89, 111.57, 69.37, 63.37, 63.17, 56.67, 50.58, 41.12, 40.12, 32.19, 30.43, 30.28, 30.22, 30.19, 29.40, 29.25, 28.71, 28.62, 27.94, 27.75, 24.29, 23.65, 8.46. LCMS 866.56 (M+H).

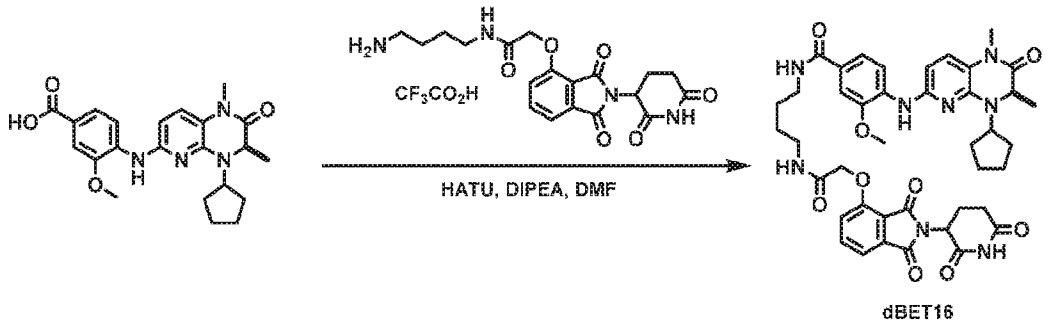
Synthetic Example 12: Synthesis of dBET10



A 0.1 M solution *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.172 mL, 0.0172 mmol 1 eq) was added to (*R*)-4-((8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl)amino)-3-methoxybenzoic acid (7.3 mg, 0.0172 mmol, 1 eq) at room temperature. DIPEA (9.0 microliters, 0.0515 mmol, 3 eq) and HATU (6.5 mg, 0.0172 mmol, 1 eq) were added and the mixture was stirred for 23 hours, before being purified by preparative HPLC to give the desired trifluoracetate salt as an off-white oil (10.7 mg, 0.0101 mmol, 59%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (d, J = 8.3 Hz, 1H), 7.75 (dd, J = 8.4, 7.4 Hz, 1H), 7.56 – 7.51 (m, 2H), 7.49 – 7.44 (m, 2H), 7.36 (d, J = 8.4 Hz, 1H), 5.08 (dd, J = 12.4, 5.4 Hz, 1H), 4.69 (s, 2H), 4.44 (dd, J = 6.7, 3.2 Hz, 1H), 4.30 – 4.21 (m, 1H), 3.92 (s, 3H), 3.59 – 3.42 (m, 12H), 3.35 (t, J = 6.7 Hz, 2H), 3.25 (s, 3H), 2.95 – 2.64 (m, 5H), 2.13 – 1.95 (m, 4H), 1.91 – 1.71 (m, 7H), 1.65 – 1.48 (m, 4H), 0.81 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.50, 171.35, 169.83, 168.77, 168.25, 167.68, 164.57, 156.26, 154.47, 153.05, 150.59, 138.19, 134.92, 133.89, 129.53, 124.57, 123.98, 121.72, 120.75, 119.26, 117.95, 117.86, 111.54, 71.51, 71.46, 71.28, 71.20,

70.18, 69.65, 69.41, 63.27, 63.07, 56.71, 50.57, 38.84, 37.59, 32.17, 30.41, 30.32, 29.46, 29.26, 28.73, 28.64, 24.27, 23.65, 8.49. LCMS 942.62 (M+H).

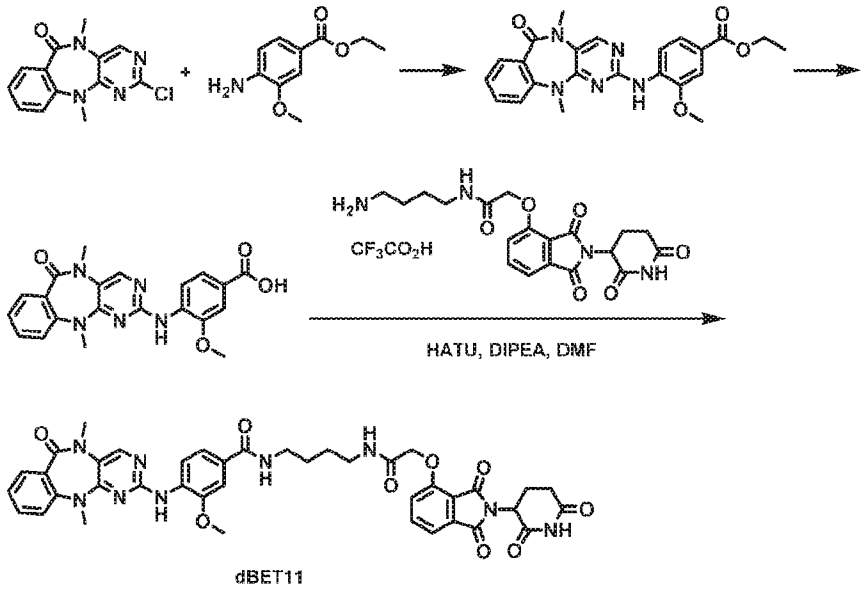
Synthetic Example 13: Synthesis of dBET16



A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.402 mL, 0.0402 mmol 1 eq) was added (*R*)-4-((4-cyclopentyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl)amino)-3-methoxybenzoic acid (16.55 mg, 0.0402 mmol, 1 eq) at room temperature. DIPEA (21 microliters, 0.1206 mmol, 3 eq) and HATU (15.3 mg, 0.0402 mmol, 1 eq) were added and the mixture was stirred for 21 hours, before being purified by preparative HPLC, followed by column chromatography (ISCO, 12 g NH2-silica column, 0-15% MeOH/DCM, 20 min gradient) to give HPLC to give a brown solid (10.63 mg, 0.0134 mmol, 33%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.22 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 8.4, 7.4 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.49 (d, J = 7.4 Hz, 2H), 7.46 – 7.39 (m, 2H), 6.98 (d, J = 8.8 Hz, 1H), 5.97 – 5.87 (m, 1H), 5.06 (dd, J = 12.6, 5.4 Hz, 1H), 4.76 (s, 2H), 3.98 (s, 3H), 3.61 (s, 2H), 3.44 – 3.36 (m, 4H), 2.92 (s, 1H), 2.78 (dd, J = 14.3, 5.2 Hz, 1H), 2.68 (ddd, J = 17.7, 8.2, 4.5 Hz, 2H), 2.36 – 2.26 (m, 2H), 2.10 – 1.90 (m, 5H), 1.76 – 1.62 (m, 6H), 1.31 (d, J = 16.0 Hz, 4H). LCMS 795.38 (M+H).

Synthetic Example 14: Synthesis of dBET11



(1) Synthesis of ethyl 4-((5,11-dimethyl-6-oxo-6,11-dihydro-5H-benzo[e]pyrimido[5,4-b][1,4]diazepin-2-yl)amino)-3-methoxybenzoate

2-chloro-5,11-dimethyl-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-6(11*H*)-one(82.4 mg, 0.30 mmol, 1 eq), ethyl 4-amino-3-methoxybenzoate (70.3 mg, 0.36 mmol, 1.2 eq) Pd_2dba_3 (13.7 mg, 0.015 mmol, 5 mol%), XPhos (21.5 mg, 0.045 mmol, 15 mol%) and potassium carbonate (166 mg, 1.2 mmol, 4 eq) were dissolved in tBuOH (3.0 mL) and heated to 100 °C. After 17 hours, the mixture was cooled room temperature and filtered through celite. The mixture was purified by column chromatography (ISCO, 12 g silica column, 0-100% EtOAc/hexanes, 19 min gradient) to give an off white solid (64.3 mg, 0.148 mmol, 49%).

^{1}H NMR (400 MHz, 50% $cd_3od/cdcl_3$) δ 8.51 (d, J = 8.5 Hz, 1H), 8.17 (s, 1H), 7.73 (ddd, J = 18.7, 8.1, 1.7 Hz, 2H), 7.52 (d, J = 1.8 Hz, 1H), 7.46 – 7.41 (m, 1H), 7.15 – 7.10 (m, 2H), 4.34 (q, J = 7.1 Hz, 4H), 3.95 (s, 3H), 3.47 (s, 3H), 3.43 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, 50% $cd_3od/cdcl_3$) δ 169.28, 167.39, 164.29, 155.64, 151.75, 149.73, 147.45, 146.22, 133.88, 133.18, 132.37, 126.44, 124.29, 123.70, 123.36, 122.26, 120.58, 118.05, 116.83, 110.82, 61.34, 56.20, 38.62, 36.25, 14.51. LCMS 434.33 (M+H).

(2) Synthesis of 4-((5,11-dimethyl-6-oxo-6,11-dihydro-5H-benzo[e]pyrimido[5,4-b][1,4]diazepin-2-yl)amino)-3-methoxybenzoic acid

Ethyl 4-((5,11-dimethyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxybenzoate (108.9 mg, 0.251 mmol, 1 eq) and LiOH (18 mg) were dissolved in THF (2.5 mL) and water (1.25 mL). After 24 hours, MeOH (0.63 mL) was added to improved solubility) and stirred for an additional 24 hours before being diluted with MeOH and purified by preparative HPLC to give a light yellow solid (41.31 mg).

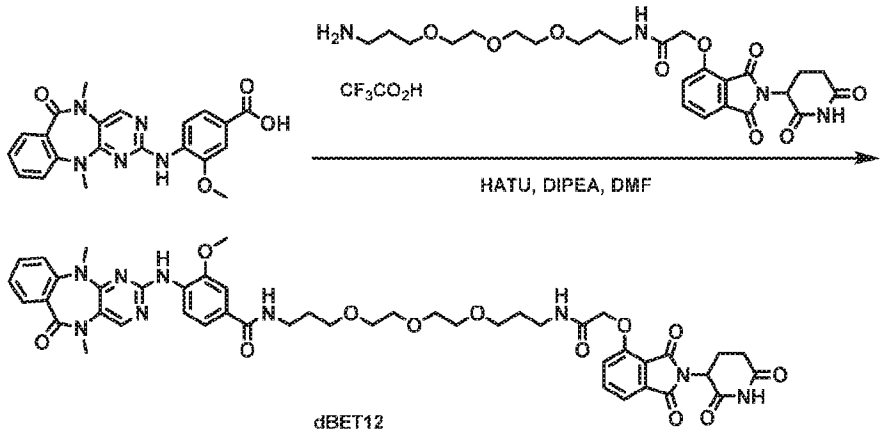
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.51 (d, J = 8.5 Hz, 1H), 8.22 (s, 1H), 7.73 (ddd, J = 11.8, 8.1, 1.7 Hz, 2H), 7.57 (d, J = 1.8 Hz, 1H), 7.49 – 7.44 (m, 1H), 7.19 – 7.11 (m, 2H), 3.97 (s, 3H), 3.48 (s, 3H), 3.45 (s, 3H). LCMS 406.32 (M+H).

(3) Synthesis of dBET11

A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.190 mL, 0.0190 mmol 1 eq) was added to 4-((5,11-dimethyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxybenzoic acid(7.71 mg, 0.0190 mmol, 1 eq) at room temperature. DIPEA (9.9 microliters, 0.0571 mmol, 3 eq) and HATU (7.2 mg, 0.0190 mmol, 1 eq) were added and the mixture was stirred for 22 hours, before being purified by preparative HPLC to give HPLC to give the desired trifluoracetate salt as a cream colored solid (6.72 mg, 0.00744 mmol, 39%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.46 (d, J = 8.3 Hz, 1H), 8.21 (s, 1H), 7.79 – 7.73 (m, 2H), 7.52 (d, J = 7.1 Hz, 1H), 7.50 – 7.43 (m, 3H), 7.33 (d, J = 8.2 Hz, 1H), 7.15 (dd, J = 7.7, 5.9 Hz, 2H), 4.98 (dd, J = 12.0, 5.5 Hz, 1H), 4.69 (s, 2H), 3.97 (s, 3H), 3.49 (s, 3H), 3.46 – 3.34 (m, 7H), 2.81 – 2.67 (m, 3H), 2.13 – 2.08 (m, 1H), 1.69 (dt, J = 6.6, 3.5 Hz, 4H). ^{13}C NMR (100 MHz, cd$_3$od) δ 173.40, 170.10, 169.68, 169.00, 168.85, 167.60, 167.15, 164.77, 156.01, 155.42, 151.83, 150.03, 148.21, 137.82, 134.12, 133.48, 132.58, 132.52, 128.11, 126.72, 124.54, 122.33, 121.06, 120.63, 118.77, 118.38, 117.94, 117.62, 109.67, 68.90, 56.33, 49.96, 40.16, 39.48, 38.72, 36.34, 31.82, 27.24, 23.16. LCMS 790.48 (M+H).

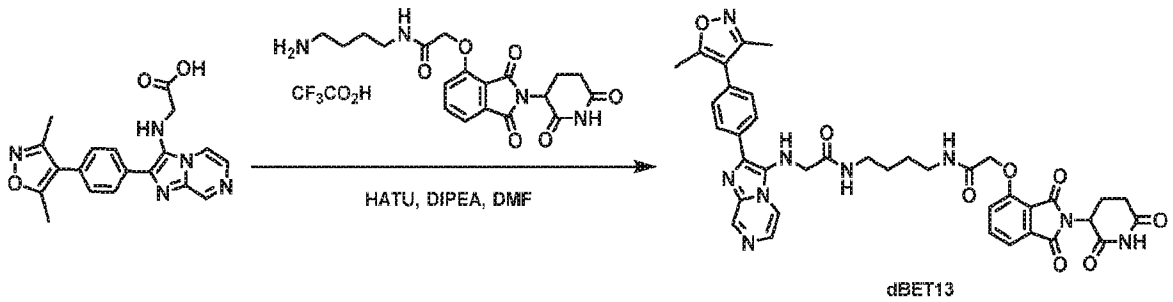
Synthetic Example 15: Synthesis of dBET12



A 0.1 M solution *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.186 mL, 0.0186 mmol 1 eq) was added to 4-((5,11-dimethyl-6-oxo-6,11-dihydro-5*H*-benzo[*e*]pyrimido[5,4-*b*][1,4]diazepin-2-yl)amino)-3-methoxybenzoic acid(7.53 mg, 0.0186 mmol, 1 eq) at room temperature. DIPEA (9.7 microliters, 0.0557 mmol, 3 eq) and HATU (7.1 mg, 0.0186 mmol, 1 eq) were added and the mixture was stirred for 22 hours, before being purified by preparative HPLC to give HPLC to give the desired trifluoracetate salt as a cream colored solid (7.50 mg, 0.00724 mmol, 39%).

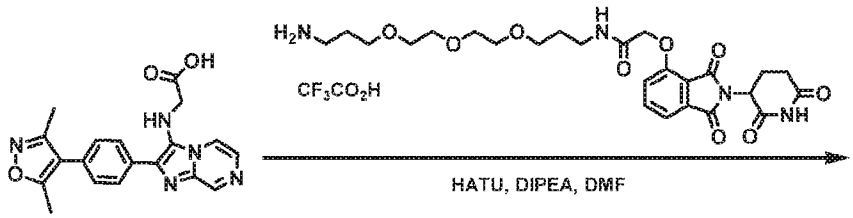
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.46 (d, J = 8.9 Hz, 1H), 8.21 (s, 1H), 7.73 (dd, J = 15.2, 7.8 Hz, 2H), 7.50 – 7.42 (m, 3H), 7.28 (d, J = 8.5 Hz, 1H), 7.15 (t, J = 7.7 Hz, 2H), 5.01 (dd, J = 11.8, 5.8 Hz, 1H), 4.68 (s, 2H), 3.97 (s, 3H), 3.67 – 3.58 (m, 7H), 3.58 – 3.43 (m, 10H), 3.39 (t, J = 6.8 Hz, 2H), 3.35 (s, 2H), 2.97 (s, 1H), 2.84 – 2.70 (m, 3H), 2.16 – 2.07 (m, 1H), 1.93 – 1.76 (m, 4H). LCMS 922.57 (M+H).

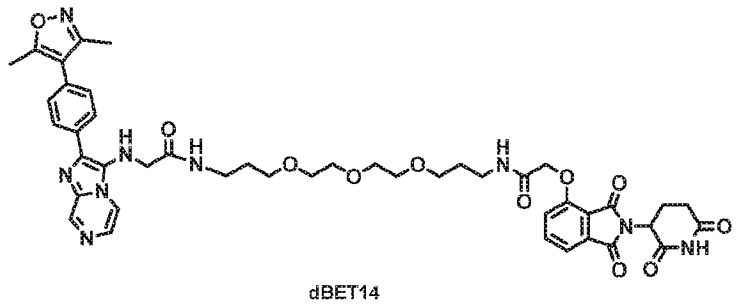
Synthetic Example 16: Synthesis of dBET13



A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.501 mL, 0.0501 mmol 1 eq) was added to 2-((2-(4-(3,5-dimethylisoxazol-4-yl)phenyl)imidazo[1,2-*a*]pyrazin-3-yl)amino)acetic acid (synthesized as in McKeown et al, J. Med. Chem, 2014, 57, 9019) (18.22 mg, 0.0501 mmol, 1 eq) at room temperature. DIPEA (26.3 microliters, 0.150 mmol, 3 eq) and HATU (19.0 mg, 0.0501 mmol, 1 eq) were added and the mixture was stirred for 21 hours, before being purified by preparative HPLC to give HPLC to give the desired trifluoracetate salt as a dark yellow oil (29.66 mg, 0.0344 mmol, 69%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 9.09 (s, 1H), 8.65 (d, J = 5.2 Hz, 1H), 8.14 – 8.06 (m, 2H), 7.94 – 7.88 (m, 1H), 7.80 – 7.74 (m, 1H), 7.59 – 7.47 (m, 3H), 7.40 (dd, J = 8.4, 4.7 Hz, 1H), 5.11 – 5.06 (m, 1H), 4.72 (d, J = 9.8 Hz, 2H), 3.90 (s, 2H), 3.25 – 3.22 (m, 1H), 3.12 (t, J = 6.4 Hz, 1H), 2.96 (s, 2H), 2.89 – 2.79 (m, 1H), 2.76 – 2.62 (m, 2H), 2.48 – 2.42 (m, 3H), 2.29 (s, 3H), 2.10 (ddq, J = 10.2, 5.3, 2.7 Hz, 1H), 1.49 – 1.45 (m, 2H), 1.37 (dd, J = 6.7, 3.6 Hz, 2H). **^{13}C NMR** (100 MHz, cd$_3$od) δ 174.45, 171.98, 171.35, 169.88, 168.17, 167.85, 167.40, 159.88, 156.28, 141.82, 138.26, 135.85, 134.82, 133.09, 132.06, 130.75, 129.67, 122.07, 121.94, 119.30, 118.98, 118.06, 117.24, 69.56, 50.56, 40.05, 39.73, 32.13, 27.53, 23.62, 18.71, 17.28, 11.64, 10.85. **LCMS** 748.49 (M+H).

Synthetic Example 17: Synthesis of dBET14





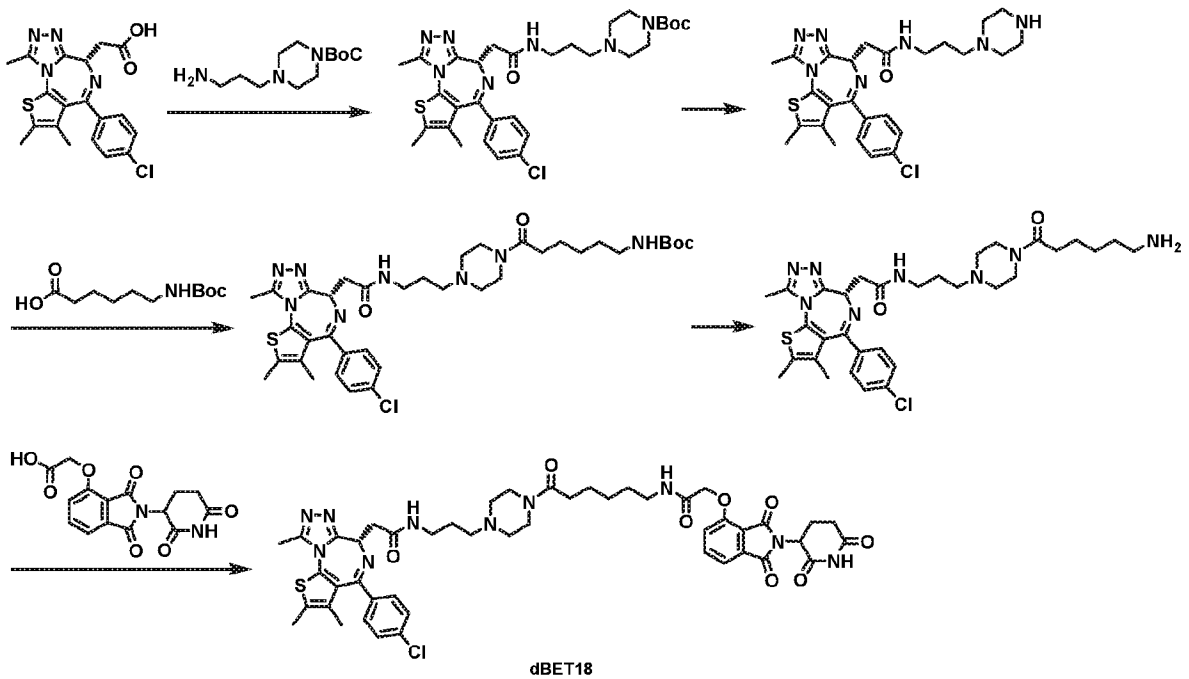
dBET14

A 0.1 M solution *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.510 mL,

0.0510 mmol 1 eq) was added to 2-((2-(4-(3,5-dimethylisoxazol-4-yl)phenyl)imidazo[1,2-*a*]pyrazin-3-yl)amino)acetic acid (synthesized as in McKeown et al, J. Med. Chem, 2014, 57, 9019) (18.52 mg, 0.0510 mmol, 1 eq) at room temperature. DIPEA (26.6 microliters, 0.153 mmol, 3 eq) and HATU (19.4 mg, 0.0510 mmol, 1 eq) were added and the mixture was stirred for 22 hours, before being purified by preparative HPLC to give HPLC to give the desired trifluoracetate salt as a dark yellow oil (32.63 mg, 0.0328 mmol, 64%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 9.09 (s, 1H), 8.66 (d, J = 5.4 Hz, 1H), 8.17 – 8.08 (m, 2H), 7.92 (d, J = 5.6 Hz, 1H), 7.77 (dd, J = 8.4, 7.4 Hz, 1H), 7.60 – 7.47 (m, 3H), 7.39 (d, J = 8.4 Hz, 1H), 5.09 (dd, J = 12.4, 5.5 Hz, 1H), 4.71 (s, 2H), 3.91 (s, 2H), 3.62 – 3.46 (m, 10H), 3.38 (dt, J = 16.0, 6.4 Hz, 3H), 3.18 (t, J = 6.8 Hz, 2H), 2.97 (s, 1H), 2.89 – 2.81 (m, 1H), 2.78 – 2.66 (m, 2H), 2.47 (s, 3H), 2.31 (s, 3H), 2.16 – 2.08 (m, 1H), 1.79 (dt, J = 12.8, 6.5 Hz, 2H), 1.64 (t, J = 6.3 Hz, 2H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.48, 171.88, 171.34, 169.80, 168.22, 167.69, 167.42, 159.87, 156.24, 141.87, 138.21, 135.89, 134.88, 133.13, 132.04, 130.76, 129.67, 122.08, 121.69, 119.20, 117.94, 117.23, 71.44, 71.22, 71.10, 69.92, 69.62, 69.38, 50.57, 49.64, 38.11, 37.55, 32.16, 30.30, 30.20, 23.63, 11.67, 10.88. LCMS 880.46 (M+H).

Synthetic Example 18: Synthesis of dBET18



(1) Synthesis of (S)-tert-butyl 4-(3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)propyl)piperazine-1-carboxylate

JQ-acid (176.6 mg, 0.441 mmol, 1 eq) was dissolved in DMF (4.4 mL) at room temperature. HATU (176 mg, 0.463 mmol, 1.05 eq) was added, followed by DIPEA (0.23 mL), 1.32 mmol, 3 eq). After 10 minutes, *tert*-butyl 4-(3-aminopropyl)piperazine-1-carboxylate (118 mg, 0.485 mmol, 1.1 eq) was added as a solution in DMF (0.44 mL). After 24 hours, the mixture was diluted with half saturated sodium bicarbonate and extracted twice with DCM and once with EtOAc. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (ISCO, 24 g silica column, 0-15% MeOH/DCM, 23 minute gradient) gave a yellow oil (325.5 mg, quant yield)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67 (t, *J* = 5.3 Hz, 1H), 7.41 – 7.28 (m, 4H), 4.58 (dd, *J* = 7.5, 5.9 Hz, 1H), 3.52 – 3.23 (m, 8H), 2.63 (s, 9H), 2.37 (s, 3H), 1.80 – 1.69 (m, 2H), 1.64 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 171.41, 164.35, 155.62, 154.45, 150.20, 136.92, 136.64, 132.19, 131.14, 130.98, 130.42, 129.98, 128.80, 80.24, 56.11, 54.32, 52.70, 38.96, 37.85, 28.42, 25.17, 14.43, 13.16, 11.82. LCMS 626.36 (M+H).

(2) Synthesis of (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(3-(piperazin-1-yl)propyl)acetamide

(*S*)-*tert*-butyl 4-(3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)propyl)piperazine-1-carboxylate (325.5 mg) was dissolved in DCM (5 mL) and MeOH (0.5 mL). A solution of 4M HCl in dioxane (1 mL) was added and the mixture was stirred for 16 hours, then concentrated under a stream of nitrogen to give a yellow solid (231.8 mg) which was used without further purification.

^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 7.64 – 7.53 (m, 4H), 5.05 (t, *J* = 7.1 Hz, 1H), 3.81 – 3.66 (m, 6H), 3.62 – 3.33 (m, 9H), 3.30 (p, *J* = 1.6 Hz, 1H), 2.94 (s, 3H), 2.51 (s, 3H), 2.09 (dq, *J* = 11.8, 6.1 Hz, 2H), 1.72 (s, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 171.78, 169.38, 155.83, 154.03, 152.14, 140.55, 136.33, 134.58, 134.53, 133.33, 132.73, 130.89, 130.38, 56.07, 53.54, 41.96, 37.22, 36.23, 25.11, 14.48, 13.14, 11.68. LCMS 526.29 (M+H).

(3) Synthesis of (S)-tert-butyl (6-(4-(3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)propyl)piperazin-1-yl)-6-oxohexyl)carbamate

(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(3-(piperazin-1-yl)propyl)acetamide (62.1 mg) and 6-((*tert*-butoxycarbonyl)amino)hexanoic acid (24.0 mg, 0.1037 mmol, 1 eq) were dissolved in DMF (1 mL). DIPEA (72.2 microliters, 0.4147 mmol, 4 eq) was added, followed by HATU (39.4 mg, 0.1037 mmol, 1 eq) and the mixture was stirred for 25 hours. The mixture was diluted with half saturated sodium bicarbonate and extracted three times with DCM. The combined organic layer was dried over sodium sulfate, filtered and condensed. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 15 minute gradient) gave a yellow oil (71.75 mg, 0.0970 mmol, 94%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 (s, 1H), 7.43 – 7.28 (m, 4H), 4.63 (s, 1H), 4.61 – 4.56 (m, 1H), 3.82 – 3.21 (m, 10H), 3.11 – 3.01 (m, 2H), 2.61 (d, *J* = 24.3 Hz, 9H), 2.38 (s, 3H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.73 (dq, *J* = 13.8, 7.4 Hz, 2H), 1.63 – 1.55 (m, 2H), 1.53 – 1.24 (m, 14H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 171.63, 171.11, 164.34, 156.17, 155.66, 150.21, 136.96, 136.72, 132.25, 131.14, 131.01, 130.47, 130.00, 128.85, 79.11, 56.42, 54.46, 53.06, 52.82, 45.04, 41.02, 40.47, 39.29, 38.33, 33.00, 29.90, 28.54, 26.60, 25.29, 24.86, 14.47, 13.20, 11.86. LCMS 739.37 (M+H).

(4) Synthesis of (S)-N-(3-(4-(6-aminohexanoyl)piperazin-1-yl)propyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide

(*S*)-*tert*-butyl (6-(4-(3-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)propyl)piperazin-1-yl)-6-oxohexyl)carbamate (71.75 mg, 0.0970 mmol, 1 eq) was dissolved in DCM (2 mL) and MeOH (0.2 mL). A solution of 4M HCl in dioxane (0.49 mL) was added and the mixture was stirred for 2 hours, then concentrated under a stream of nitrogen, followed by vacuum to give a yellow foam (59.8 mg, 0.0840 mmol, 87%).

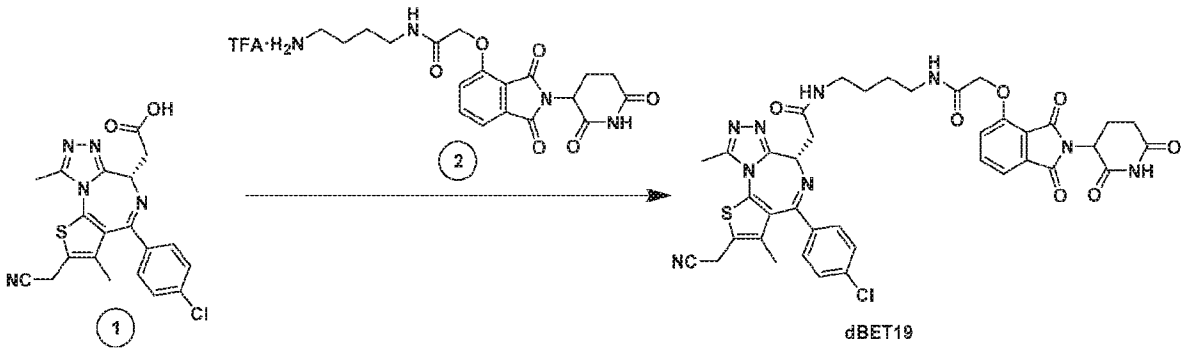
^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 7.68 – 7.53 (m, 4H), 5.04 (d, *J* = 6.6 Hz, 1H), 4.66 (d, *J* = 13.6 Hz, 1H), 4.23 (d, *J* = 13.6 Hz, 1H), 3.63 – 3.34 (m, 7H), 3.29 – 3.00 (m, 5H), 2.95 (d, *J* = 6.0 Hz, 5H), 2.51 (d, *J* = 9.2 Hz, 5H), 2.08 (s, 2H), 1.77 – 1.62 (m, 7H), 1.45 (dt, *J* = 15.3, 8.6 Hz, 2H). ^{13}C NMR (100 MHz, cd$_3$od) δ 173.77, 171.84, 169.35, 155.85, 153.99, 140.56, 136.40, 134.58, 133.35, 132.70, 130.39, 55.83, 53.57, 52.92, 52.70, 43.57, 40.55, 39.67, 37.33, 36.25, 33.17, 28.26, 26.94, 25.33, 25.26, 14.49, 13.15, 11.65. LCMS 639.35 (M+H).

(5) Synthesis of dBET18

(*S*)-*N*-(3-(4-(6-aminohexanoyl)piperazin-1-yl)propyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide dihydrochloride (20.0 mg, 0.0281 mmol, 1 eq) and 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (9.32 mg, 0.0281 mmol, 1 eq) were dissolved in DMF (0.281 mL). DIPEA (19.6 microliters, 0.1124 mmol, 4 eq) was added, followed by HATU (10.7 mg, 0.0281 mmol, 1 eq). After 24 hours, the mixture was diluted with MeOH and purified by preparative HPLC to give the desired trifluoracetate salt.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.83 – 7.79 (m, 1H), 7.54 (d, J = 7.1 Hz, 1H), 7.45 (q, J = 8.8 Hz, 5H), 5.12 (dd, J = 12.5, 5.4 Hz, 1H), 4.76 (s, 2H), 4.68 (t, J = 7.3 Hz, 1H), 3.59 – 3.32 (m, 8H), 3.28 – 3.18 (m, 4H), 2.87 (ddd, J = 19.0, 14.7, 5.3 Hz, 2H), 2.80 – 2.65 (m, 6H), 2.44 (d, J = 6.8 Hz, 5H), 2.33 – 2.25 (m, 1H), 2.14 (dd, J = 9.8, 4.9 Hz, 1H), 2.06 – 1.89 (m, 3H), 1.70 (s, 3H), 1.61 (dq, J = 14.4, 7.3, 6.9 Hz, 4H), 1.45 – 1.37 (m, 2H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.52, 173.97, 173.69, 171.44, 169.88, 168.26, 167.83, 166.72, 156.36, 138.28, 137.84, 134.89, 133.52, 132.12, 131.83, 131.38, 129.89, 121.87, 119.32, 118.01, 69.52, 55.64, 55.03, 52.79, 50.58, 43.69, 39.77, 38.57, 36.89, 33.47, 32.16, 29.93, 27.34, 25.76, 25.45, 23.63, 14.39, 12.94, 11.66. LCMS 953.43 (M+H).

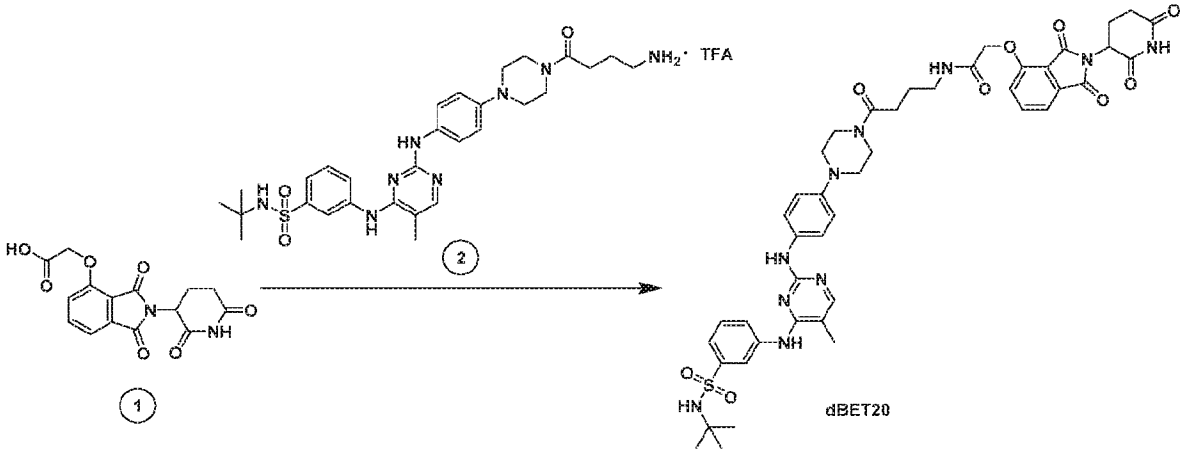
Synthetic Example 19: Synthesis of dBET19



A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (235 microliters, 0.0235 mmol, 1 eq) was added to (*S*)-2-(4-(4-chlorophenyl)-2-(cyanomethyl)-3,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetic acid (10 mg, 0.0235 mmol, 1 eq) at room temperature. DIPEA (12.3 microliters, 0.0704 mmol, 3 eq) and HATU (8.9 mg, 0.0235 mmol, 1

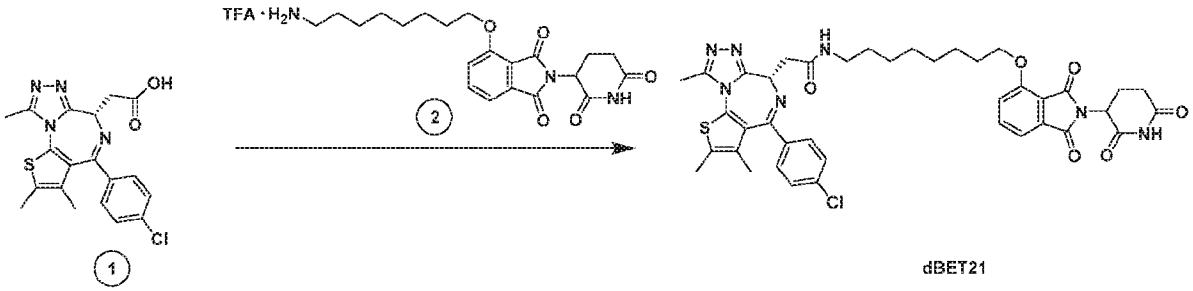
eq) were added and the mixture was stirred for 18.5 hours. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (12.96 mg, 0.0160 mmol, 68%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 7.80 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.55 – 7.37 (m, 6H), 5.14 – 5.06 (m, 1H), 4.77 (d, *J* = 1.5 Hz, 2H), 4.64 (dd, *J* = 8.0, 5.6 Hz, 1H), 3.45 – 3.32 (m, 5H), 3.29 – 3.21 (m, 2H), 2.83 – 2.66 (m, 6H), 2.58 (s, 3H), 2.14 – 2.06 (m, 1H), 1.71 – 1.57 (m, 4H). **LCMS** 810.30, M+H).

Synthetic Example 20: Synthesis of dBET20



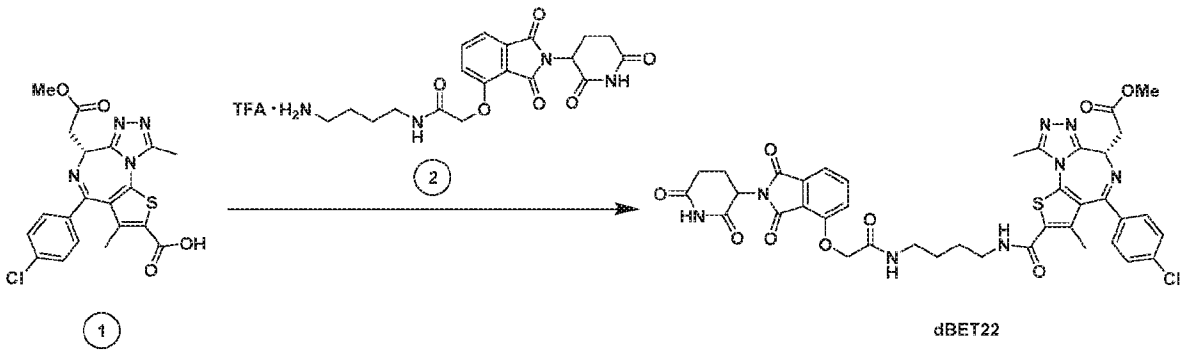
3-((2-((4-(4-(4-aminobutanoyl)piperazin-1-yl)phenyl)amino)-5-methylpyrimidin-4-yl)amino)-*N*-(*tert*-butyl)benzenesulfonamide trifluoroacetate (7.41 mg, 0.0107 mmol, 1 eq) and 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (3.6 mg, 0.0107 mmol, 1 eq) were dissolved in DMF (214 microliters, 0.05M) at room temperature. DIPEA (5.6 microliters, 0.0321 mmol, 3 eq) and HATU (4.1 mg, 0.0107 mmol, 1 eq) were added. After 22.5 hours, the mixture was diluted with MeOH and purified by preparative HPLC to give the desired product as a brown residue (6.27 mg, 0.00701 mmol, 65%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.06 (s, 1H), 7.84 – 7.75 (m, 3H), 7.65 (s, 1H), 7.55 (t, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.25 – 7.20 (m, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 5.11 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.78 (s, 2H), 3.79 – 3.66 (m, 4H), 3.40 (t, *J* = 6.6 Hz, 2H), 3.24 – 3.13 (m, 4H), 2.82 – 2.68 (m, 3H), 2.52 (t, *J* = 7.4 Hz, 2H), 2.24 – 2.19 (m, 3H), 2.12 (dd, *J* = 10.2, 5.1 Hz, 1H), 1.92 (dd, *J* = 13.4, 6.4 Hz, 2H), 1.18 (s, 9H). **LCMS** 895.63 (M+H).

Synthetic Example 21: Synthesis of dBET21



A 0.1 M solution of 4-((10-aminodecyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione trifluoroacetate in DMF (232 microliters, 0.0232 mmol, 1 eq) was added to JQ-acid (9.3 mg, 0.0232 mmol, 1 eq) at room temperature. DIPEA (12.1 microliters, 0.0696 mmol, 3 eq) and HATU (8.8 mg, 0.0232 mmol, 1 eq) were added and the mixture was stirred for 18 hours. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by preparative HPLC followed by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an off-white residue (1.84 mg, 0.00235 mmol, 10%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.77 – 7.73 (m, 1H), 7.50 – 7.33 (m, 6H), 5.09 (dd, J = 12.5, 5.5 Hz, 1H), 4.62 (s, 1H), 4.21 (t, J = 6.4 Hz, 2H), 3.36 (s, 2H), 2.87 – 2.67 (m, 6H), 2.44 (s, 3H), 1.88 – 1.82 (m, 2H), 1.70 (s, 3H), 1.58 (s, 4H), 1.29 (s, 8H). **LCMS** 784.51 (M+H).

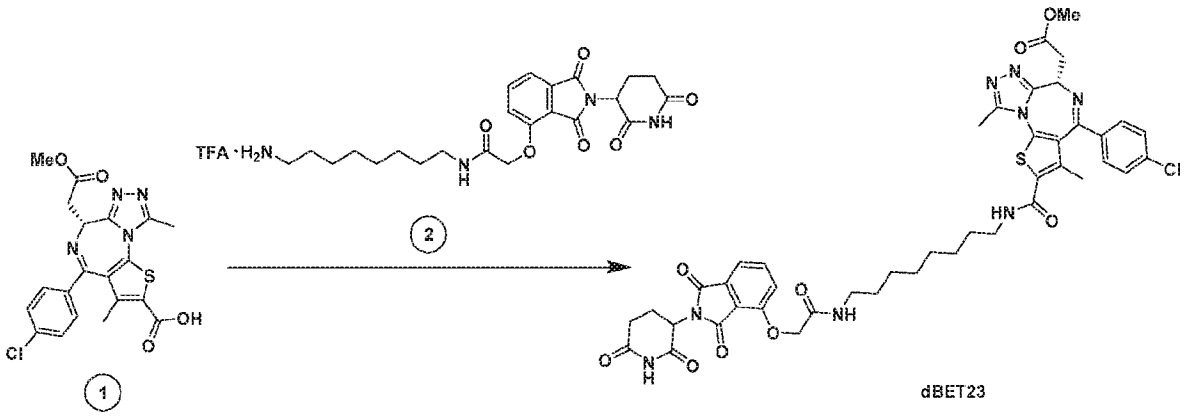
Synthetic Example 22: Synthesis of dBET22



A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (247 microliters, 0.0247 mmol, 1 eq)

was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-3,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (10.98 mg, 0.0247 mmol, 1 eq) at room temperature. DIPEA (12.9 microliters, 0.0740 mmol, 3 eq) and HATU (9.4 mg, 0.0247 mmol, 1 eq) were added. The mixture was then stirred for 21 hours, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (9.79 mg, 0.0118 mmol, 48%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.80 (dd, J = 8.4, 7.4 Hz, 1H), 7.51 (dd, J = 7.1, 1.5 Hz, 1H), 7.48 – 7.34 (m, 5H), 5.11 (ddd, J = 12.4, 5.4, 3.5 Hz, 1H), 4.76 (s, 2H), 4.69 (td, J = 7.2, 1.4 Hz, 1H), 3.76 (s, 3H), 3.55 (d, J = 7.2 Hz, 2H), 3.48 – 3.33 (m, 4H), 2.93 – 2.82 (m, 1H), 2.78 – 2.64 (m, 5H), 2.14 – 2.07 (m, 1H), 1.96 (d, J = 0.9 Hz, 3H), 1.66 (s, 4H). **LCMS** 829.39 (M+H).

Synthetic Example 23: Synthesis of dBET23



A 0.1 M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (220 microliters, 0.0220 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-3,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (9.87 mg, 0.0220 mmol, 1 eq) at room temperature. DIPEA (11.5 microliters, 0.0660 mmol, 3 eq) and HATU (8.4 mg, 0.0220 mmol, 1 eq) were added. The mixture was then stirred for 21 hours, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a

white solid (8.84 mg, 0.00998 mmol, 45%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.81 (dd, J = 8.4, 7.4 Hz, 1H), 7.53 (d, J = 7.3 Hz, 1H), 7.50 – 7.39 (m, 5H), 5.12 (dd, J = 12.6, 5.4 Hz, 1H), 4.75 (s, 2H), 4.68 (t, J = 7.2 Hz, 1H), 3.76 (s, 3H), 3.54 (d, J = 7.2 Hz, 2H), 3.39 – 3.32 (m, 3H), 3.29 (s, 1H), 2.90 – 2.83 (m, 1H), 2.79 – 2.68 (m, 5H), 2.14 (dd, J = 8.9, 3.7 Hz, 1H), 1.99 (s, 3H), 1.65 – 1.53 (m, 4H), 1.36 (d, J = 6.5 Hz, 8H). **LCMS** 885.47 (M+H).

Synthetic Example 24: Synthesis of dBET24

Step 1: Synthesis of tert-butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)ethyl)carbamate

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (200 mg, 0.602 mmol, 1 eq) was dissolved in DMF (6.0 mL, 0.1M). HATU (228.9 mg, 0.602 mmol, 1 eq), DIPEA (0.315 mL, 1.81 mmol, 3 eq) and *N*-Boc-2,2′-(ethylenedioxy)diethylamine (0.143 mL, 0.602 mmol, 1 eq) were added sequentially. After 6 hours, additional HATU (114 mg, 0.30 mmol, 0.5 eq) were added to ensure completeness of reaction. After an additional 24 hours, the mixture was diluted with EtOAc, and washed with saturated sodium bicarbonate, water and twice with brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 12 g silica column, 0-15% MeOH/DCM, 15 minute gradient) gave the desired product as a yellow oil (0.25 g, 0.44 mmol, 74%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.82 – 7.75 (m, 1H), 7.51 (d, J = 7.4 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 12.4, 5.5 Hz, 1H), 4.76 (s, 2H), 3.66 – 3.58 (m, 6H), 3.53 – 3.45 (m, 4H), 3.19 (t, J = 5.6 Hz, 2H), 2.95 – 2.83 (m, 1H), 2.80 – 2.67 (m, 2H), 2.19 – 2.12 (m, 1H), 1.41 (s, 9H). **LCMS** 563.34 (M+H).

Step 2: Synthesis of *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate

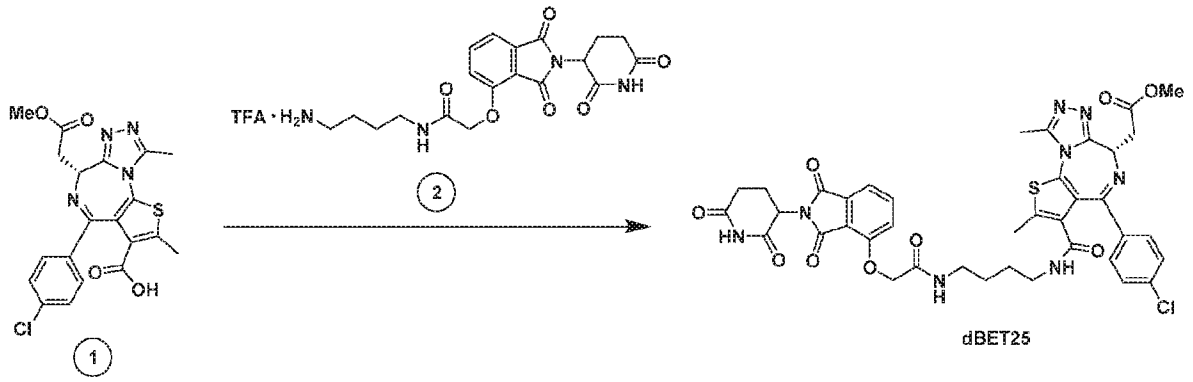
tert-butyl (2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethoxy)ethoxy)ethyl)carbamate (0.25 g, 0.44 mmol, 1 eq) was dissolved in TFA (4.5 mL) and heated to 50 °C. After 3 hours, the mixture was cooled to room temperature, diluted with MeOH, and concentrated under reduced pressure. Purification by preparative HPLC gave the desired product as a tan solid (0.197 g, 0.342 mmol, 77%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.81 (ddd, J = 8.4, 7.4, 1.1 Hz, 1H), 7.55 – 7.50 (m, 1H), 7.43 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 12.7, 5.5 Hz, 1H), 4.78 (s, 2H), 3.74 – 3.66 (m, 6H), 3.64 (t, J = 5.4 Hz, 2H), 3.52 (t, J = 5.3 Hz,

2H), 3.14 – 3.08 (m, 2H), 2.89 (ddd, *J* = 17.5, 13.9, 5.2 Hz, 1H), 2.80 – 2.66 (m, 2H), 2.16 (dtd, *J* = 13.0, 5.7, 2.7 Hz, 1H). **LCMS** 463.36 (M+H).

Step 2: Synthesis of dBET24

A 0.1 M solution of *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.324 mL, 0.0324 mmol, 1 eq) was added to JQ-acid (13.0 mg, 0.324 mmol, 1 eq). DIPEA 16.9 microliters, 0.0972 mmol, 3 eq) and HATU (12.3 mg, 0.0324 mmol, 1 eq) were then added and the mixture was stirred for 18 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an off-white solid (20.0 mg, 0.0236 mmol, 73%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.77 – 7.72 (m, 1H), 7.49 (d, *J* = 7.4 Hz, 1H), 7.45 – 7.35 (m, 5H), 5.09 (ddd, *J* = 12.3, 5.4, 3.7 Hz, 1H), 4.76 (s, 2H), 4.60 (dd, *J* = 8.9, 5.3 Hz, 1H), 3.68 – 3.62 (m, 6H), 3.59 (t, *J* = 5.6 Hz, 2H), 3.54 – 3.48 (m, 2H), 3.47 – 3.35 (m, 4H), 2.84 (ddd, *J* = 19.4, 9.9, 4.6 Hz, 1H), 2.77 – 2.69 (m, 2H), 2.68 (d, *J* = 1.8 Hz, 3H), 2.43 (s, 3H), 2.12 (dt, *J* = 9.8, 5.3 Hz, 1H), 1.68 (s, 3H). **LCMS** 845.39 (M+H).

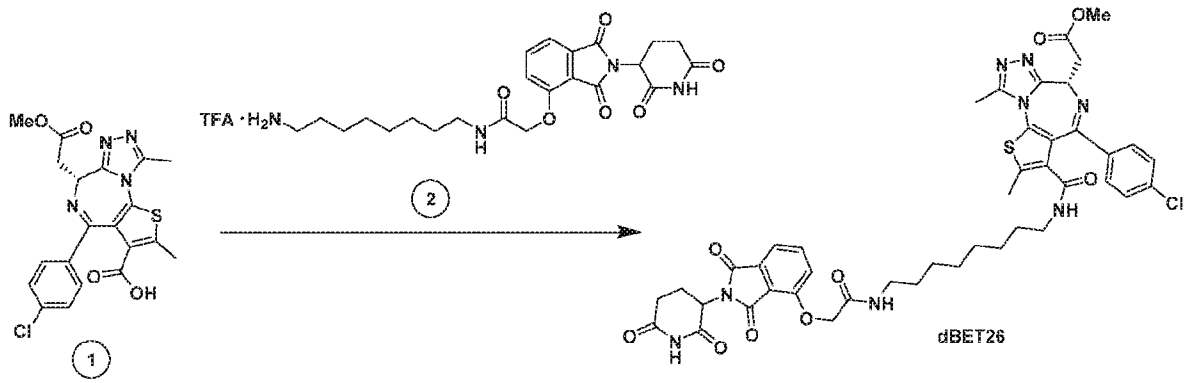
Synthetic Example 25: Synthesis of dBET25



A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (183 microliters, 0.0183 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-2,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-3-carboxylic acid (8.16 mg, 0.0183 mmol, 1 eq) at room temperature. DIPEA (9.6 microliters, 0.0550 mmol, 3 eq) and HATU (7.0 mg, 0.0183 mmol, 1 eq) were added. The mixture was then stirred for 23 hours, then diluted with EtOAc and washed with

saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a yellow solid (4.39 mg, 0.00529 mmol, 29%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.82 (dd, J = 8.4, 7.4 Hz, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.43 – 7.31 (m, 4H), 5.16 – 5.10 (m, 1H), 4.77 (d, J = 1.5 Hz, 2H), 4.56 (s, 1H), 3.74 (d, J = 1.8 Hz, 3H), 3.66 – 3.60 (m, 1H), 3.50 (dd, J = 16.5, 7.3 Hz, 1H), 3.37 – 3.32 (m, 1H), 3.28 (s, 3H), 2.85 (t, J = 7.2 Hz, 2H), 2.75 (d, J = 7.8 Hz, 1H), 2.71 (d, J = 0.9 Hz, 3H), 2.59 (d, J = 1.0 Hz, 3H), 2.18 – 2.10 (m, 1H), 1.36 – 1.24 (m, 4H). **LCMS** 829.38 (M+H).

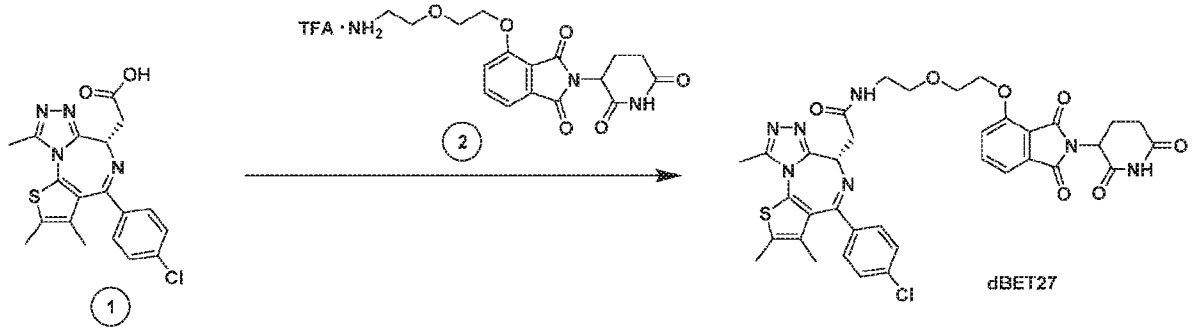
Synthetic Example 26: Synthesis of dBET26



A 0.1 M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (186 microliters, 0.0186 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-2,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-3-carboxylic acid (8.26 mg, 0.0186 mmol, 1 eq) at room temperature. DIPEA (9.7 microliters, 0.0557 mmol, 3 eq) and HATU (7.1 mg, 0.0186 mmol, 1 eq) were added. The mixture was then stirred for 23 hours, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a cream colored solid (6.34 mg, 0.00716 mmol, 38%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.83 – 7.78 (m, 1H), 7.53 (dd, J = 7.3, 2.2 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.32 (dd, J = 8.5, 1.3 Hz, 2H), 5.16 – 5.08 (m, 1H), 4.76 (s, 2H), 4.56 (s, 1H), 3.75 (s, 3H), 3.66 (dd, J = 15.9, 8.7 Hz, 1H), 3.50 (dd, J

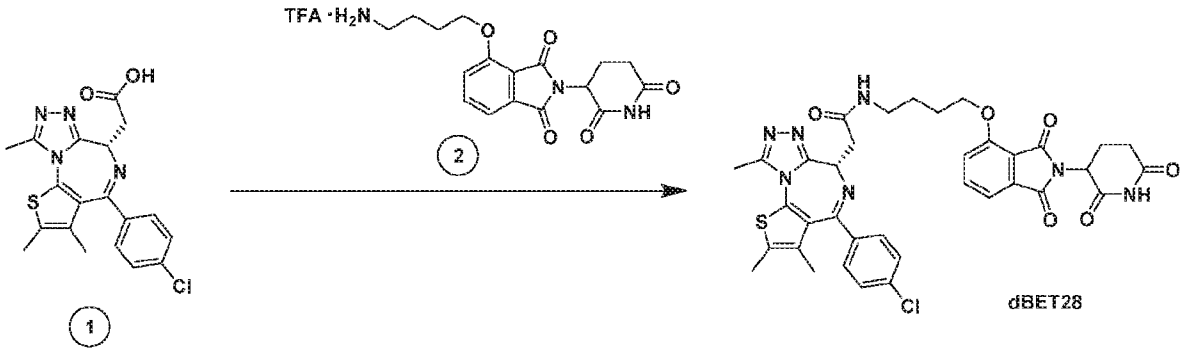
= 16.9, 6.9 Hz, 1H), 3.32 (d, *J* = 2.8 Hz, 4H), 2.84 – 2.74 (m, 3H), 2.70 (d, *J* = 1.1 Hz, 3H), 2.66 – 2.54 (m, 3H), 2.14 (d, *J* = 5.3 Hz, 1H), 1.62 – 1.22 (m, 12H). **LCMS** 885.48 (M+H).

Synthetic Example 27: Synthesis of dBET27



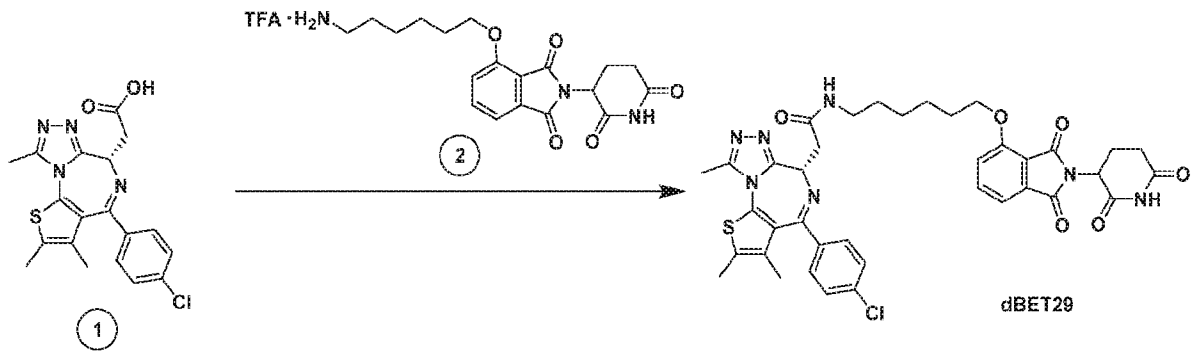
A 0.1 M solution of 4-(2-(2-aminoethoxy)ethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione trifluoroacetate in DMF (257 microliters, 0.0257 mmol, 1 eq) was added to JQ-acid (10.3 mg, 0.0257 mmol, 1 eq). DIPEA (13.4 microliters, 0.0771 mmol, 3 eq) and HATU (9.8 mg, 0.0257 mmol, 1 eq) were then added and the mixture was stirred for 18 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (14.53 mg, 0.0195 mmol, 76%). **^{1}H NMR** (400 MHz, Methanol-*d*$_4$) δ 7.75 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H), 7.47 – 7.30 (m, 6H), 5.00 (ddd, *J* = 25.4, 12.2, 5.2 Hz, 1H), 4.61 (td, *J* = 9.4, 5.0 Hz, 1H), 4.36 (q, *J* = 4.8 Hz, 2H), 3.96 – 3.89 (m, 2H), 3.74 (q, *J* = 5.6 Hz, 2H), 3.53 – 3.41 (m, 3H), 3.30 – 3.24 (m, 1H), 2.78 – 2.53 (m, 6H), 2.41 (d, *J* = 3.9 Hz, 3H), 2.09 – 1.98 (m, 1H), 1.67 (d, *J* = 5.0 Hz, 3H).

Synthetic Example 28: Synthesis of dBET28



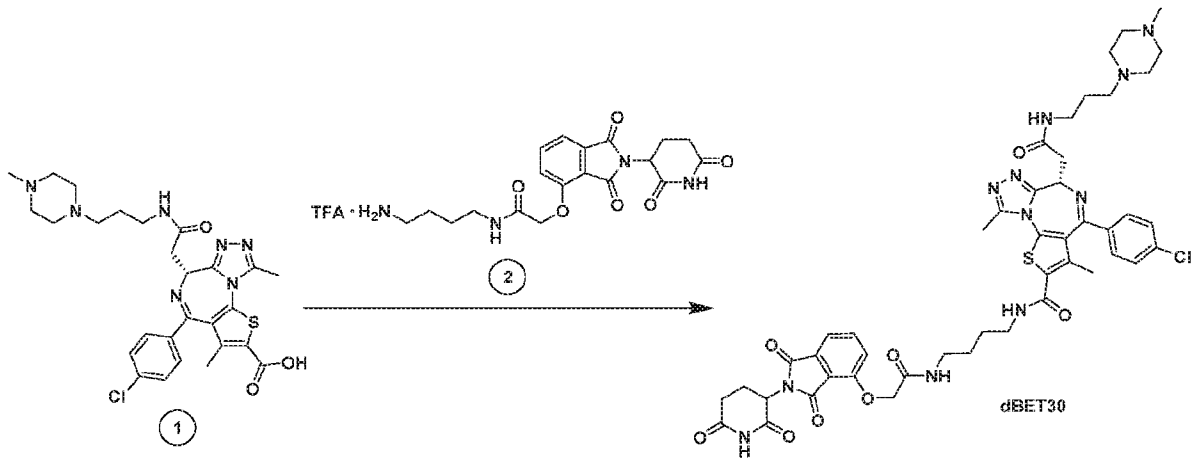
A 0.1 M solution of 4-(4-aminobutoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione trifluoroacetate in DMF (202 microliters, 0.0202 mmol, 1 eq) was added to JQ-acid (8.1 mg, 0.0202 mmol, 1 eq). DIPEA (10.6 microliters, 0.0606 mmol, 3 eq) and HATU (7.7 mg, 0.0202 mmol, 1 eq) were then added and the mixture was stirred for 18.5 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a cream colored solid (10.46 mg, 0.0144 mmol, 71%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.76 (t, J = 7.5 Hz, 1H), 7.43 (td, J = 6.5, 2.5 Hz, 4H), 7.34 (t, J = 8.8 Hz, 2H), 5.08 – 4.98 (m, 1H), 4.64 (td, J = 9.1, 5.0 Hz, 1H), 4.26 (t, J = 5.3 Hz, 2H), 3.57 – 3.32 (m, 4H), 2.84 – 2.59 (m, 6H), 2.45 – 2.37 (m, 3H), 2.08 – 2.01 (m, 1H), 2.00 – 1.91 (m, 2H), 1.82 (dq, J = 13.8, 6.9 Hz, 2H), 1.68 (d, J = 11.7 Hz, 3H). **LCMS** 728.38 (M+H).

Synthetic Example 29: Synthesis of dBET29



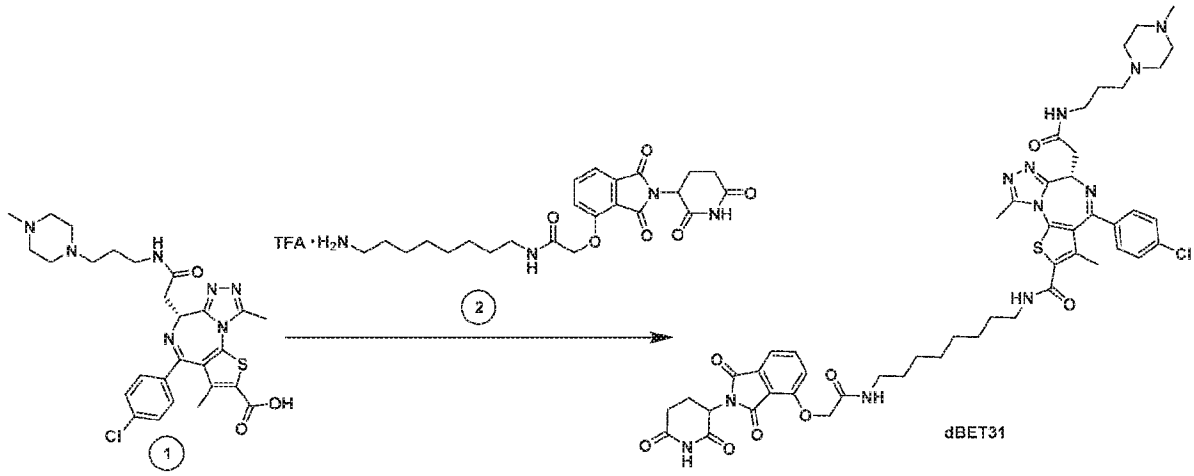
A 0.1 M solution of 4-((6-aminohexyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione in DMF (205 microliters, 0.0205 mmol, 1 eq) was added to JQ-acid (8.2 mg, 0.0205 mmol, 1 eq). DIPEA (10.7 microliters, 0.0614 mmol, 3 eq) and HATU (7.8 mg, 0.0205 mmol, 1 eq) were then added and the mixture was stirred for 19 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (8.04 mg, 0.0106 mmol, 52%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.75 – 7.71 (m, 1H), 7.51 – 7.34 (m, 6H), 5.07 (ddd, J = 12.1, 5.4, 2.4 Hz, 1H), 4.62 (dd, J = 9.0, 5.2 Hz, 1H), 4.22 (t, J = 6.4 Hz, 2H), 3.44 – 3.32 (m, 2H), 3.29 – 3.21 (m, 2H), 2.88 – 2.65 (m, 6H), 2.43 (s, 3H), 2.13 – 2.06 (m, 1H), 1.86 (dt, J = 13.9, 6.7 Hz, 2H), 1.68 (s, 3H), 1.59 (dq, J = 14.2, 7.0 Hz, 4H), 1.54 – 1.45 (m, 2H). **LCMS** 756.40 (M+H).

Synthetic Example 30: Synthesis of dBET30



A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (163 microliters, 0.0163 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-3,9-dimethyl-6-(2-((3-(4-methylpiperazin-1-yl)propyl)amino)-2-oxoethyl)-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (9.31 mg, 0.0163 mmol, 1 eq) at room temperature. DIPEA (8.5 microliters, 0.0490 mmol, 3 eq) and HATU (6.2 mg, 0.0163 mmol, 1 eq) were added. The mixture was then stirred for 23.5 hours, then purified by prepartive HPLC togive the desired product as a yellow oil (11.48 mg, 0.0107 mmol, 66%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.82 – 7.78 (m, 1H), 7.54 – 7.35 (m, 6H), 5.09 (td, J = 12.7, 5.4 Hz, 1H), 4.77 – 4.70 (m, 3H), 3.56 – 3.31 (m, 12H), 3.23 (dd, J = 8.0, 6.0 Hz, 3H), 3.05 (d, J = 3.2 Hz, 2H), 2.93 – 2.81 (m, 5H), 2.78 – 2.63 (m, 5H), 2.15 – 2.05 (m, 2H), 1.96 – 1.86 (m, 4H), 1.68 (s, 4H). **LCMS** 954.55 (M+H).

Synthetic Example 31: Synthesis of dBET31

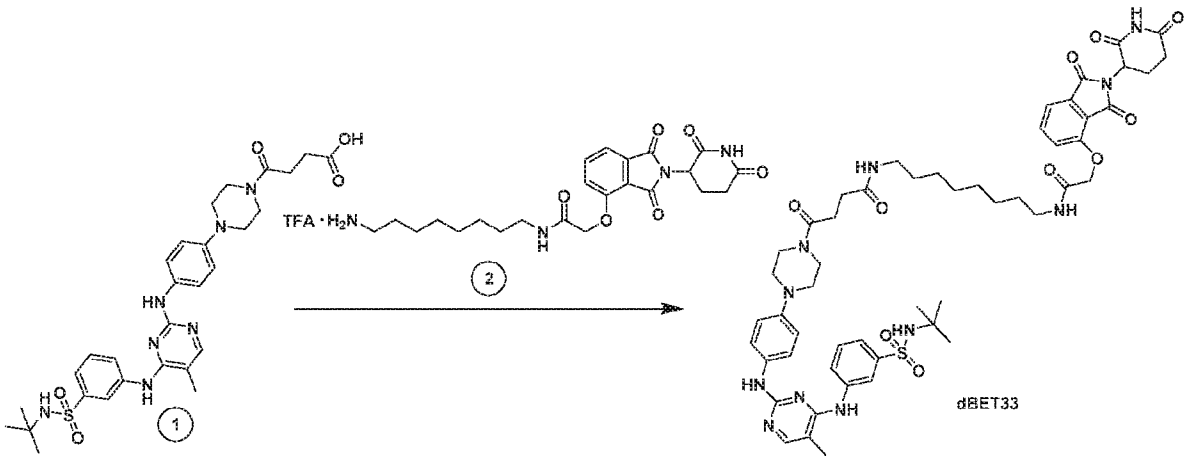


A 0.1 M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (153 microliters, 0.0153 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-3,9-dimethyl-6-(2-((3-(4-methylpiperazin-1-yl)propyl)amino)-2-oxoethyl)-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (8.7 mg, 0.0153 mmol, 1 eq) at room temperature. DIPEA (7.9 microliters, 0.0458 mmol, 3 eq) and HATU (5.8 mg, 0.0153 mmol, 1 eq) were added. The mixture was then stirred for 25 hours, then purified by prepartive HPLC togive the desired product as a nice brown (not like poop brown, kind of like brick) oil (9.52 mg, 0.00847 mmol, 55%). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.81 (dd, J = 8.4, 7.4 Hz, 1H), 7.59 – 7.40 (m, 6H), 5.12 (dd, J = 12.5, 5.4 Hz, 1H), 4.75 (s, 2H), 4.71 (t, J = 7.4 Hz, 1H), 3.53 – 3.34 (m, 8H), 3.29 – 3.11 (m, 6H), 3.03 – 2.61 (m, 13H), 2.15 (s, 1H), 2.01 – 1.84 (m, 5H), 1.59 (s, 4H), 1.37 (s, 8H). **LCMS** 1010.62 (M+H).

Synthetic Example 32: Synthesis of dBET32

A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (180 microliters, 0.0180 mmol, 1 eq) was added to 4-(4-(4-((4-((3-(*N*-(*tert*-butyl)sulfamoyl)phenyl)amino)-5-methylpyrimidin-2-yl)amino)phenyl)piperazin-1-yl)-4-oxobutanoic acid (10.7 mg, 0.0180 mmol, 1 eq) at room temperature. DIPEA (9.4 microliters, 0.0539 mmol, 3 eq) and HATU (6.8 mg, 0.0180 mmol, 1 eq) were added and the mixture was stirred for 19 hours. The mixture was then diluted with methanol and purified by preparative HPLC to give the desired product as a brown oil (4.40 mg, 0.00449 mmol, 25%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.08 (d, J = 13.6 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.63 (s, 1H), 7.57 – 7.51 (m, 2H), 7.41 (d, J = 8.4 Hz, 1H), 7.22 (td, J = 6.7, 2.2 Hz, 2H), 7.03 – 6.97 (m, 2H), 5.14 (dd, J = 12.5, 5.5 Hz, 1H), 4.76 (d, J = 16.8 Hz, 2H), 3.72 (dt, J = 10.0, 5.2 Hz, 4H), 3.34 – 3.33 (m, 1H), 3.23 – 3.12 (m, 5H), 2.97 (dd, J = 8.8, 4.0 Hz, 3H), 2.80 – 2.69 (m, 4H), 2.64 (dd, J = 7.6, 5.5 Hz, 1H), 2.50 (t, J = 6.8 Hz, 1H), 2.22 (dd, J = 2.4, 0.9 Hz, 3H), 2.17 – 2.11 (m, 1H), 1.67 – 1.52 (m, 4H), 1.18 (d, J = 0.8 Hz, 9H). **LCMS** 980.64 (M+H).

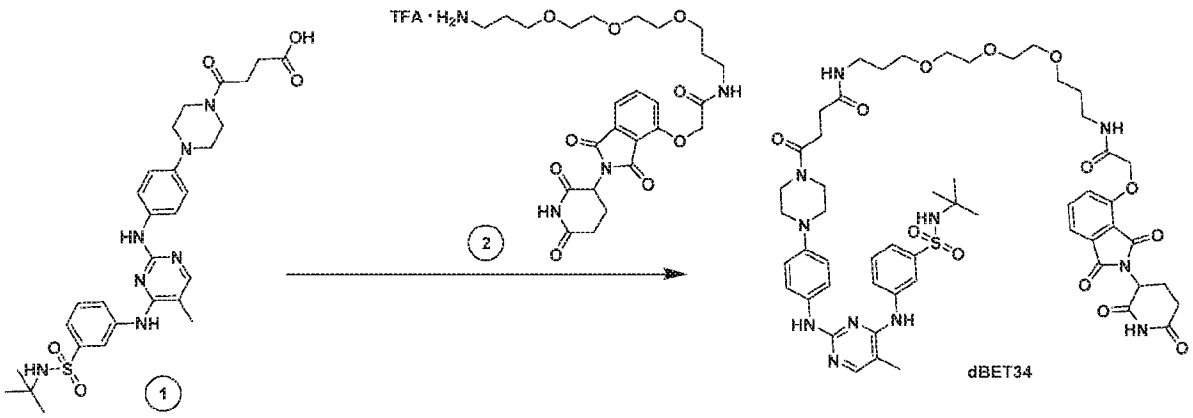
Synthetic Example 33: Synthesis of dBET33



A 0.1 M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (188 microliters, 0.0188 mmol, 1 eq) was added to 4-(4-(4-((4-((3-(*N*-(*tert*-butyl)sulfamoyl)phenyl)amino)-5-methylpyrimidin-2-yl)amino)phenyl)piperazin-1-yl)-4-oxobutanoic acid (10.8 mg, 0.0188 mmol, 1 eq) at room temperature. DIPEA (9.8 microliters, 0.0564 mmol, 3 eq) and HATU (7.1 mg, 0.0188 mmol, 1 eq) were added and the mixture was stirred for 23 hours. The mixture was then diluted with methanol

and purified by preparative HPLC to give the desired product as a brown residue (7.41 mg, 0.00715 mmol, 38%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.06 (s, 1H), 7.80 (ddd, J = 10.5, 7.6, 3.2 Hz, 3H), 7.65 (d, J = 4.5 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.41 (dd, J = 8.4, 2.9 Hz, 1H), 7.25 (td, J = 6.7, 2.9 Hz, 2H), 7.02 (t, J = 8.0 Hz, 2H), 5.16 – 5.09 (m, 1H), 4.75 (d, J = 9.5 Hz, 2H), 3.76 (dq, J = 16.0, 5.3 Hz, 4H), 3.29 – 3.12 (m, 7H), 3.00 – 2.67 (m, 7H), 2.51 (t, J = 6.8 Hz, 1H), 2.22 (d, J = 3.1 Hz, 3H), 2.13 (dtd, J = 10.4, 5.7, 3.1 Hz, 1H), 1.59 – 1.52 (m, 2H), 1.51 – 1.43 (m, 2H), 1.32 (t, J = 16.6 Hz, 8H), 1.18 (d, J = 1.3 Hz, 9H). **LCMS** 1036.69 (M+H).

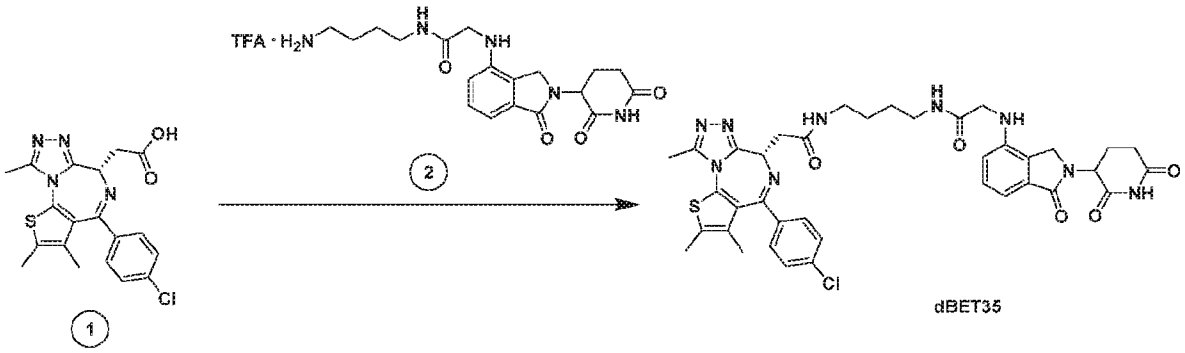
Synthetic Example 34: Synthesis of dBET34



A 0.1 M solution of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (173 microliters, 0.0173 mmol, 1 eq) was added to 4-(4-(4-((4-((3-(*N*-(*tert*-butyl)sulfamoyl)phenyl)amino)-5-methylpyrimidin-2-yl)amino)phenyl)piperazin-1-yl)-4-oxobutanoic acid (10.3 mg, 0.0173 mmol, 1 eq) at room temperature. DIPEA (9.0 microliters, 0.0519 mmol, 3 eq) and HATU (6.6 mg, 0.0173 mmol, 1 eq) were added and the mixture was stirred for 25 hours. The mixture was then diluted with methanol and purified by preparative HPLC to give the desired product as a brown residue (7.99 mg, 0.00718 mmol, 42%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.06 (s, 1H), 7.83 – 7.76 (m, 3H), 7.65 (s, 1H), 7.58 – 7.50 (m, 2H), 7.43 (dd, J = 17.7, 8.4 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.02 (t, J = 8.0 Hz, 2H), 5.13 (dt, J = 12.7, 5.2 Hz, 1H), 4.76 (d, J = 12.4 Hz, 2H), 3.73 (q, J = 6.3 Hz, 4H), 3.63 – 3.49 (m, 10H), 3.41 (q, J = 6.6 Hz, 2H), 3.27 – 3.15 (m, 5H), 3.01 – 2.81 (m, 4H), 2.79 – 2.63 (m, 5H), 2.50 (t, J = 6.8 Hz, 1H), 2.22

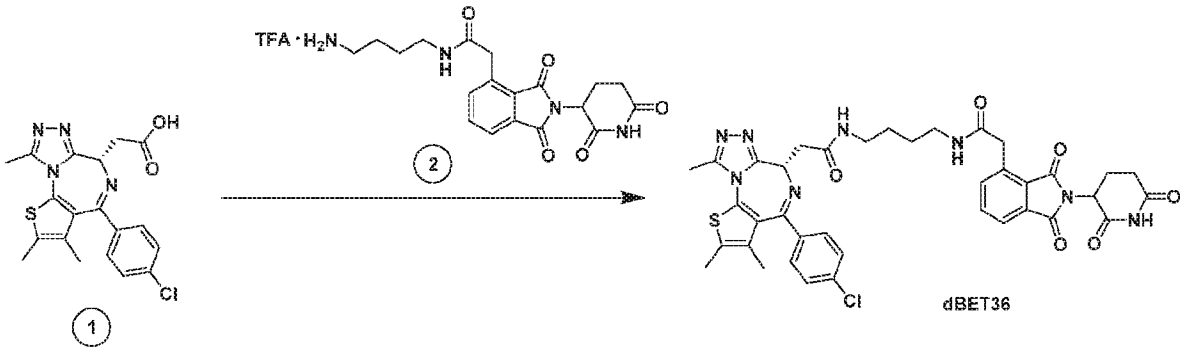
(d, J = 2.3 Hz, 3H), 2.17 – 2.11 (m, 1H), 1.88 – 1.70 (m, 4H), 1.18 (d, J = 1.2 Hz, 9H). **LCMS** 1112.74 (M+H).

Synthetic Example 35: Synthesis of dBET35



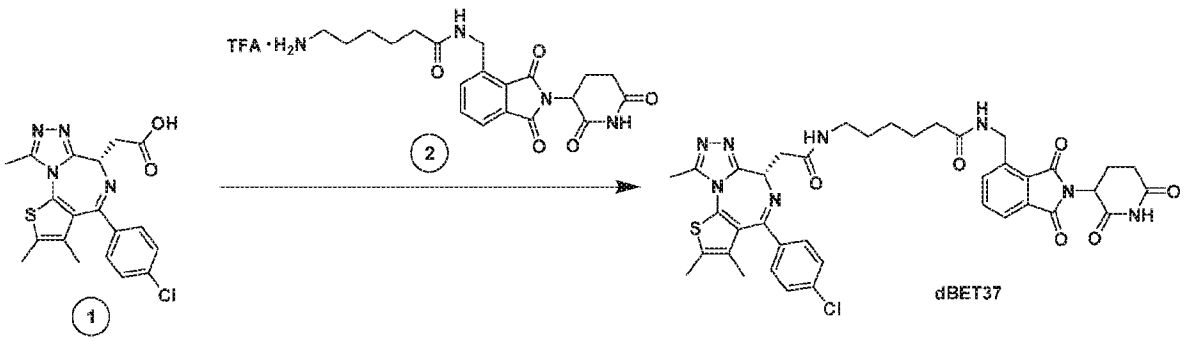
A 0.1 M solution of *N*-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)acetamide trifluoroacetate in DMF (185 microliters, 0.0185 mmol, 1 eq) was added to JQ-acid (7.4 mg, 0.0185 mmol, 1 eq). DIPEA (9.6 microliters, 0.0554 mmol, 3 eq) and HATU (7.0 mg, 0.0185 mmol, 1 eq) were then added and the mixture was stirred for 17 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (2.71 mg, 0.00351 mmol, 19%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.48 – 7.37 (m, 4H), 7.34 (t, J = 7.8 Hz, 1H), 7.14 (dd, J = 7.4, 2.4 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 5.14 (td, J = 13.5, 5.2 Hz, 1H), 4.66 – 4.60 (m, 1H), 4.59 (d, J = 8.3 Hz, 2H), 4.43 – 4.31 (m, 2H), 3.88 (s, 2H), 3.25 (dd, J = 14.8, 7.1 Hz, 4H), 2.94 – 2.72 (m, 3H), 2.68 (d, J = 4.9 Hz, 3H), 2.49 – 2.40 (m, 4H), 2.21 – 2.12 (m, 1H), 1.68 (s, 3H), 1.53 (s, 4H). **LCMS** 770.51 (M+H).

Synthetic Example 36: Synthesis of dBET36



A 0.1 M solution of *N*-(4-aminobutyl)-2-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide trifluoroacetate in DMF (222 microliters, 0.0222 mmol, 1 eq) was added to JQ-acid (8.9 mg, 0.0222 mmol, 1 eq). DIPEA (11.6 microliters, 0.0666 mmol, 3 eq) and HATU (8.4 mg, 0.0222 mmol, 1 eq) were then added and the mixture was stirred for 17.5 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (12.42 mg, 0.0156 mmol, 70%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.80 – 7.74 (m, 2H), 7.68 (d, J = 6.8 Hz, 1H), 7.42 (q, J = 8.7 Hz, 4H), 5.11 (dt, J = 12.3, 4.6 Hz, 1H), 4.63 (dd, J = 8.8, 5.5 Hz, 1H), 4.10 – 4.00 (m, 2H), 3.39 (ddd, J = 14.9, 8.8, 2.5 Hz, 1H), 3.30 – 3.21 (m, 5H), 2.88 – 2.76 (m, 1H), 2.74 – 2.65 (m, 5H), 2.44 (s, 3H), 2.15 – 2.08 (m, 1H), 1.69 (s, 3H), 1.63 – 1.55 (m, 4H). **LCMS** 769.49 (M+H).

Synthetic Example 37: Synthesis of dBET37



A 0.1 M solution of 6-amino-N-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)methyl)hexanamide trifluoroacetate in DMF (195 microliters, 0.0195 mmol, 1 eq) was added to JQ-acid (7.8 mg, 0.0195 mmol, 1 eq). DIPEA (10.2 microliters, 0.0584 mmol, 3 eq) and HATU (7.4 mg, 0.0195 mmol, 1 eq) were then added and the mixture was stirred for 18 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (11.83 mg, 0.0151 mmol, 77%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.78 – 7.74 (m, 2H), 7.71 (dd, J = 5.3, 3.5 Hz, 1H), 7.42 (q, J = 8.5 Hz, 4H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.82 (s, 2H), 4.63 (dd, J = 8.8, 5.5 Hz, 1H), 3.40 (ddd, J = 15.0, 8.8, 1.6 Hz, 1H), 3.30 – 3.21 (m, 3H), 2.86 (ddd, J = 18.4, 14.6, 4.8 Hz, 1H), 2.74 (ddd, J = 13.8, 10.1, 2.8 Hz, 2H), 2.69 (s, 3H), 2.44 (s, 3H), 2.30 (t, J = 7.4 Hz, 2H), 2.13 (dtd, J = 12.9, 4.9, 2.3 Hz, 1H), 1.74 – 1.64 (m, 5H), 1.59 (p, J = 7.0 Hz, 2H), 1.46 – 1.38 (m, 2H). **LCMS** 783.47 (M+H).

Synthetic Example 38: Synthesis of dBET38

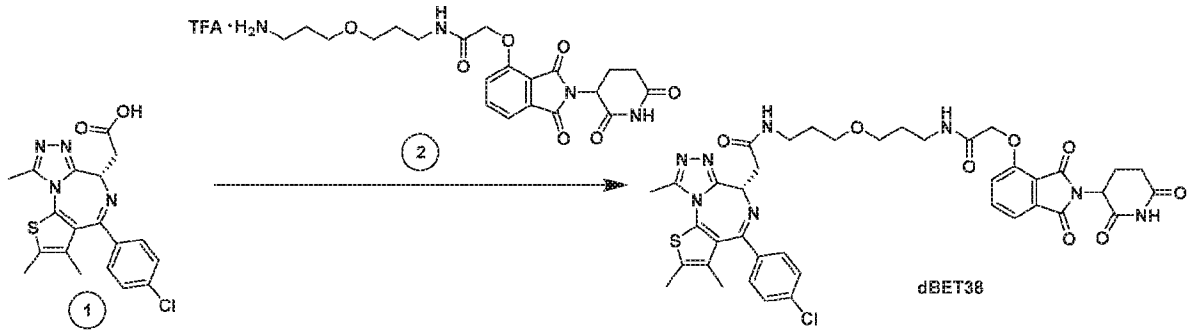
Step 1: Synthesis of *tert-* butyl (3-(3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)propoxy)propyl)carbamate

tert-butyl (3-(3-aminopropoxy)propyl)carbamate (134.5 mg, 0.579 mmol, 1 eq) was dissolved in DMF (5.79 ml, 0.05 M) then added to 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (192.38 mg, 0.579 mmol, 1eq). DIPEA (0.28 ml, 1.74 mmol, 3 eq) and HATU (153.61 mg, 0.579 mmol, 1 eq) were added and the mixture was stirred for 18 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water then brine. The organic layer was dried over sodium sulfate, filtered and condensed to give a yellow oil (157.1 mg). The crude material was purified by column chromatography (ISCO, 12 g silica column, 0 to 15% MeOH/DCM 25 minute gradient) to give a yellow oil (121.3 mg, 0.222 mmol, 38.27 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (dd, J = 8.4, 7.4 Hz, 1H), 7.50 (d, J = 7.3 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 12.4, 5.5 Hz, 1H), 4.75 (s, 2H), 3.53 – 3.37 (m, 6H), 3.14 – 3.07 (m, 2H), 2.94 – 2.88 (m, 1H), 2.79 – 2.68 (m, 2H), 2.16 (ddd, J = 12.8, 6.6, 2.7 Hz, 1H), 1.81 (p, J = 6.4 Hz, 2H), 1.73 – 1.65 (m, 2H), 1.40 (s, 9H). LCMS 547.6 (M+H).

Step 2: Synthesis of N-(3-(3-aminopropoxy)propyl)-2-((2-(2,6-dioxopuperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate salt

TFA (2.22ml, 0.1 M) was added to *tert*- butyl (3-(3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)propoxy)propyl)carbamate (121.3 mg, 0.222 mmol, 1 eq) and the mixture was stirred at 50° C for 2 hours. The mixture was then dissolved in MeOH and concentrated under reduced pressure to give a brown oil (114.1 mg) that was carried forward without further purification. **^{1}H NMR** (400 MHz, Methanol-d_4) δ 7.81 – 7.74 (m, 1H), 7.50 (d, J = 7.3 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 5.12 (dd, J = 12.7, 5.5 Hz, 1H), 4.76 (s, 2H), 3.57 – 3.52 (m, 2H), 3.48 (t, J = 5.9 Hz, 2H), 3.40 (t, J = 6.6 Hz, 2H), 3.06 (t, J = 6.5 Hz, 2H), 2.87 (ddd, J = 14.1, 10.1, 7.0 Hz, 1H), 2.79 – 2.65 (m, 2H), 2.15 (dtd, J = 12.8, 5.5, 2.6 Hz, 1H), 1.92 (dt, J = 11.7, 5.9 Hz, 2H), 1.81 (p, J = 6.3 Hz, 2H). **LCMS** 447.2 (M+H).

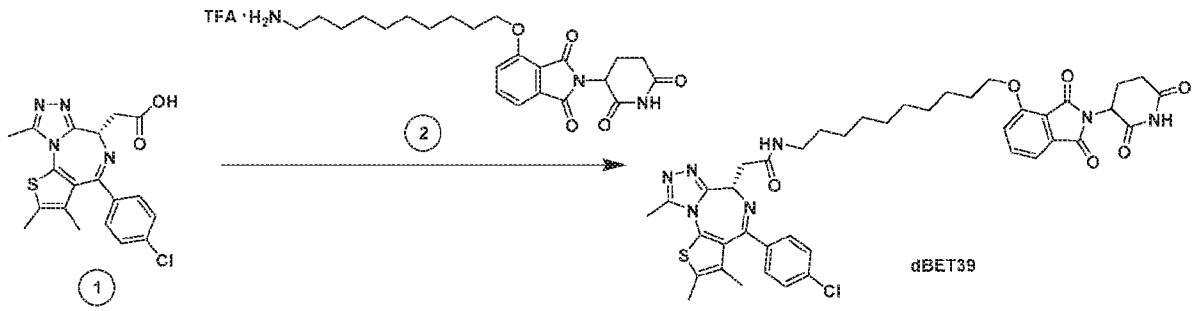
Step 3: Synthesis of dBET38



A 0.1 M solution of *N*-(3-(3-aminopropoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.215 mL, 0.0215 mmol, 1 eq) was added to JQ-acid (8.6 mg, 0.0215 mmol, 1 eq) at room temperature. DIPEA (11.2 microliters, 0.0644 mmol, 3 eq) and HATU (8.2 mg, 0.0215 mmol, 1 eq) were added. After 19 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 25 minute gradient) gave the desired product as a cream colored solid (10.6 mg, 0.0127 mmol, 59%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.79 – 7.74 (m, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.46 – 7.36 (m, 5H), 5.11 (ddd, J = 12.4, 5.5, 1.7 Hz, 1H), 4.73 (s, 2H), 4.62 (ddd, J = 8.7, 5.4, 1.4 Hz, 1H), 3.50 (q, J = 6.3 Hz, 4H), 3.43 (t, J = 6.5 Hz, 2H), 3.41 – 3.32 (m, 3H), 3.29

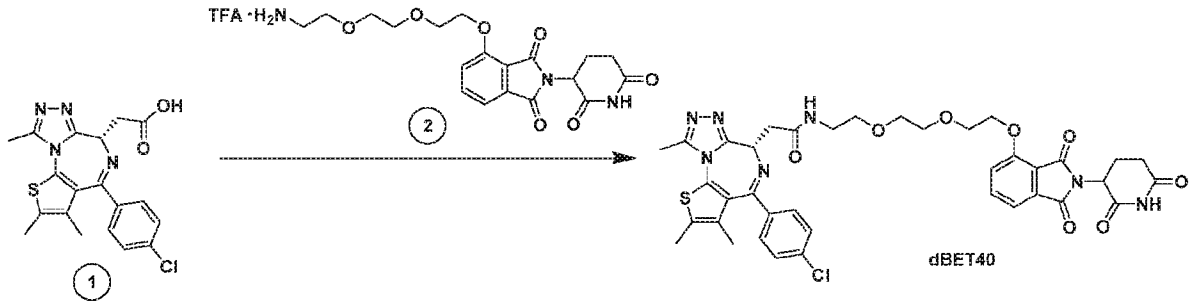
– 3.24 (m, 1H), 2.85 (ddd, J = 18.3, 14.6, 4.2 Hz, 1H), 2.77 – 2.65 (m, 5H), 2.43 (s, 3H), 2.17 – 2.09 (m, 1H), 1.80 (h, J = 6.4 Hz, 4H), 1.68 (s, 3H). **LCMS** 829.32 (M+H).

Synthetic Example 39: Synthesis of dBET39



A 0.1 M solution of 4-((10-aminodecyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione trifluoroacetate in DMF (0.212 mL, 0.0212 mmol, 1 eq) was added to JQ-acid (8.5 mg, 0.0212 mmol, 1 eq) at room temperature. DIPEA (11.1 microliters, 0.0636 mmol, 3 eq) and HATU (8.1 mg, 0.0212 mmol, 1 eq) were added. After 19 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-15% MeOH/DCM, 25 minute gradient) and preparative HPLC gave the desired product (0.39 mg, 0.00048 mmol, 2.3%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.77 – 7.73 (m, 1H), 7.56 – 7.31 (m, 6H), 5.11 – 5.06 (m, 1H), 4.62 (dd, J = 9.2, 5.0 Hz, 1H), 4.58 (s, 2H), 4.21 (t, J = 6.3 Hz, 2H), 3.42 – 3.38 (m, 1H), 3.24 – 3.20 (m, 1H), 2.90 – 2.68 (m, 6H), 2.45 (d, J = 6.7 Hz, 3H), 2.11 (s, 1H), 1.83 (dd, J = 14.7, 6.6 Hz, 2H), 1.70 (s, 3H), 1.61 – 1.49 (m, 4H), 1.32 (d, J = 23.2 Hz, 10H). **LCMS** 812.60 (M+H).

Synthetic Example 40: Synthesis of dBET40



A 0.1 M solution of 4-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-2-(2,6-dioxopiperidin-3-

yl)isoindoline-1,3-dione trifluoroacetate in DMF (0.242 mL, 0.0242 mmol, 1 eq) was added to JQ-acid (9.7 mg, 0.0242 mmol, 1 eq) at room temperature. DIPEA (12.6 microliters, 0.0726 mmol, 3 eq) and HATU (9.2 mg, 0.0242 mmol, 1 eq) were added. After 22 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) and preparative HPLC gave the desired product as a brown oil (4.74 mg, 0.00601mmol, 25%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.77 – 7.67 (m, 1H), 7.52 – 7.36 (m, 5H), 5.09 – 5.03 (m, 1H), 4.64 (d, J = 4.8 Hz, 1H), 4.40 – 4.32 (m, 2H), 3.97 – 3.88 (m, 2H), 3.81 – 3.74 (m, 2H), 3.69 – 3.60 (m, 5H), 3.55 – 3.38 (m, 4H), 2.89 – 2.54 (m, 6H), 2.45 (d, J = 5.9 Hz, 3H), 2.11 (s, 1H), 1.70 (d, J = 8.6 Hz, 3H). **LCMS** 788.42 (M+H).

Synthetic Example 41: Synthesis of dBET41

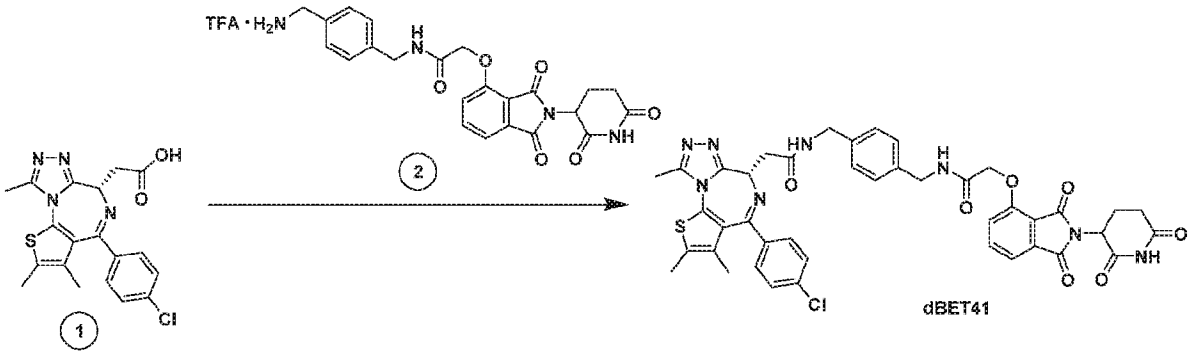
Step 1: Synthesis of *tert*-butyl (4-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)methyl)benzyl)carbamate

tert-butyl (4-(aminomethyl)benzyl)carbamate (183.14 mg, 0.755 mmol, 1eq) was dissolved in DMF (15.1 ml, 0.05 M) and added to 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (250.90 mg, 0.755 mmol, 1 eq). DIPEA (0.374 ml, 2.265 mmol, 3 eq) and HATU (296.67 mg, 0.755 mmol, 1 eq) were added and the mixture was stirred for 20 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water then brine. The organic layer was dried over sodium sulfate, filtered and condensed to give a light brown oil. The crude material was purified by column chromatography (ISCO, 12 g silica column, 0 to 15% MeOH/DCM 25 minute gradient) to give a light brown oil (373.1 mg, 0.678 mmol, 89.8 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 11.10 (s, 2H), 8.48 (t, J = 5.8 Hz, 1H), 7.80 (dd, J = 8.4, 7.3 Hz, 1H), 7.49 (d, J = 7.2 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.26 – 7.08 (m, 4H), 5.11 (dd, J = 12.9, 5.4 Hz, 1H), 4.86 (s, 2H), 4.33 (d, J = 3.9 Hz, 2H), 4.09 (d, J = 5.3 Hz, 2H), 2.65 – 2.51 (m, 3H), 2.07 – 1.99 (m, 1H), 1.38 (s, 9H). **LCMS** 551.5 (M+H).

Step 2: Synthesis of N-(4-(aminomethyl)benzyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoracetate salt

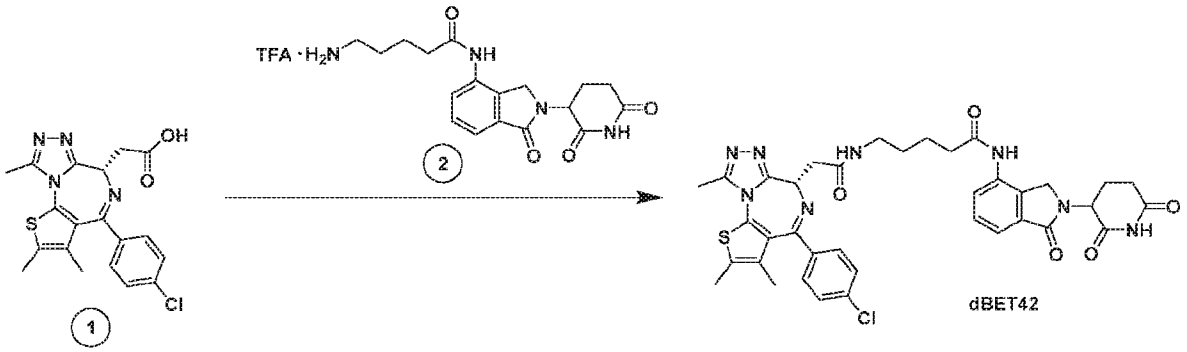
TFA (6.77 ml, 0.1 M) was added to *tert*-butyl (4-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)methyl)benzyl)carbamate (373.1 mg, 0.677 mmol, 1 eq) and the mixture was stirred at 50° C for 1.5 hours. The mixture was then dissolved in MeOH and concentrated under reduced pressure to give a brown oil (270.29 mg) that was carried forward without further purification. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 8.55 (t, J = 6.2 Hz, 1H), 8.07 (s, 3H), 7.81 (dd, J = 8.5, 7.3 Hz, 1H), 7.51 (d, J = 7.2 Hz, 1H), 7.40 (dd, J = 14.9, 8.3 Hz, 3H), 7.31 (d, J = 8.2 Hz, 2H), 5.11 (dd, J = 12.9, 5.4 Hz, 1H), 4.87 (s, 2H), 4.37 (d, J = 6.1 Hz, 2H), 4.01 (q, J = 5.8 Hz, 2H), 2.66 – 2.51 (m, 3H), 2.07 – 1.99 (m, 1H). **LCMS** 451.3 (M+H).

Step 3: Synthesis of dBET41



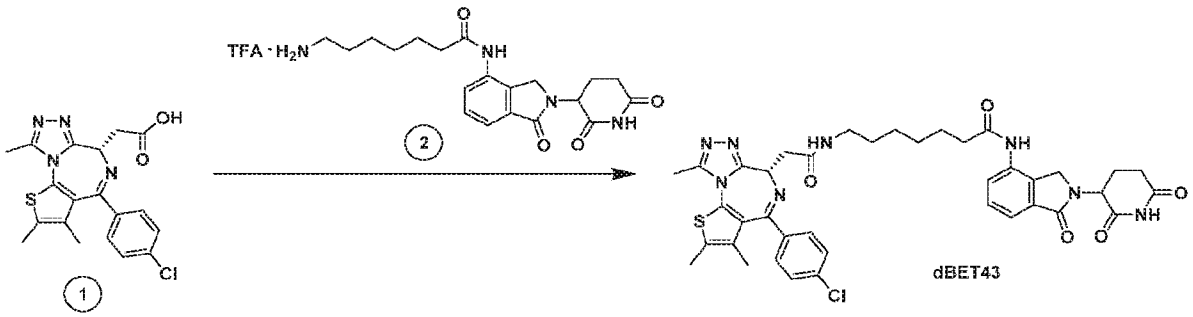
A 0.1 M solution of *N*-(4-(aminomethyl)benzyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (0.237 mL, 0.0237 mmol, 1 eq) was added to JQ-acid (9.5 mg, 0.0237 mmol, 1 eq) at room temperature. After 23 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a cream colored solid (11.8 mg, 0.0142 mmol, 60%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.80 – 7.75 (m, 1H), 7.51 (dd, J = 7.3, 1.5 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 2.2 Hz, 4H), 7.34 – 7.28 (m, 4H), 5.10 – 5.00 (m, 1H), 4.82 (s, 2H), 4.67 – 4.64 (m, 1H), 4.61 – 4.42 (m, 4H), 4.34 (dd, J = 14.9, 12.8 Hz, 1H), 3.49 (ddd, J = 14.8, 9.5, 5.2 Hz, 1H), 2.83 – 2.75 (m, 1H), 2.73 – 2.61 (m, 5H), 2.44 – 2.39 (m, 3H), 2.06 (ddq, J = 9.8, 4.7, 2.6 Hz, 1H), 1.66 (d, J = 4.2 Hz, 3H). **LCMS** 832.92 (M+H).

Synthetic Example 42: Synthesis of dBET42



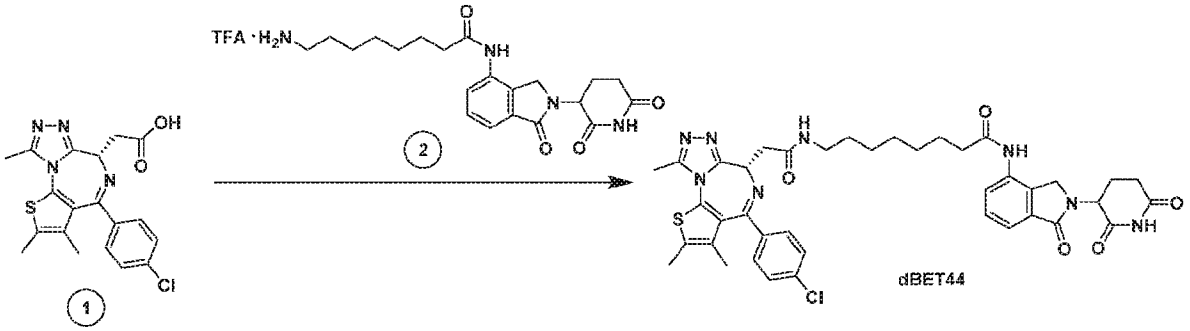
A 0.1 M solution of 5-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pentanamide trifluoroacetate in DMF (222 microliters, 0.0222 mmol, 1 eq) was added to JQ-acid (8.9 mg, 0.0222 mmol, 1 eq). DIPEA (11.6 microliters, 0.0666 mmol, 3 eq) and HATU (8.4 mg, 0.0222 mmol, 1 eq) were then added and the mixture was stirred for 24 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a white solid (12.23 mg, 0.0165 mmol, 74%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.76 – 7.71 (m, 1H), 7.66 – 7.62 (m, 1H), 7.51 (td, J = 7.8, 2.5 Hz, 1H), 7.45 – 7.35 (m, 4H), 5.11 (ddd, J = 13.2, 11.3, 5.2 Hz, 1H), 4.63 (ddd, J = 8.8, 5.7, 3.2 Hz, 1H), 4.47 (s, 2H), 3.45 – 3.32 (m, 3H), 3.30 – 3.27 (m, 1H), 2.90 – 2.80 (m, 1H), 2.73 – 2.63 (m, 4H), 2.49 (t, J = 7.4 Hz, 2H), 2.46 – 2.38 (m, 4H), 2.11 (ddtd, J = 12.8, 10.5, 5.3, 2.3 Hz, 1H), 1.84 – 1.75 (m, 2H), 1.66 (dd, J = 16.2, 7.6 Hz, 5H). **LCMS** 741.46 (M+H).

Synthetic Example 43: Synthesis of dBET43



A 0.1 M solution of 7-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)heptanamide trifluoroacetate in DMF (227 microliters, 0.0227 mmol, 1 eq) was added to JQ-acid (9.1 mg, 0.0227 mmol, 1 eq). DIPEA (11.9 microliters, 0.0681 mmol, 3 eq) and HATU (8.6 mg, 0.0227 mmol, 1 eq) were then added and the mixture was stirred for 25.5 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an off-white solid (12.58 mg, 0.0164 mmol, 72%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.71 (d, J = 7.9 Hz, 1H), 7.64 (d, J = 7.4 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.46 – 7.38 (m, 4H), 5.14 (ddd, J = 13.3, 5.2, 2.2 Hz, 1H), 4.62 (ddd, J = 8.6, 5.6, 2.1 Hz, 1H), 4.49 – 4.45 (m, 2H), 3.39 (ddd, J = 14.9, 8.7, 1.3 Hz, 1H), 3.30 – 3.24 (m, 3H), 2.93 – 2.83 (m, 1H), 2.79 – 2.65 (m, 4H), 2.50 – 2.40 (m, 6H), 2.16 (ddq, J = 9.9, 5.2, 2.6 Hz, 1H), 1.78 – 1.70 (m, 2H), 1.68 (d, J = 2.1 Hz, 3H), 1.63 – 1.57 (m, 2H), 1.50 – 1.42 (m, 4H). **LCMS** 769.55 (M+H).

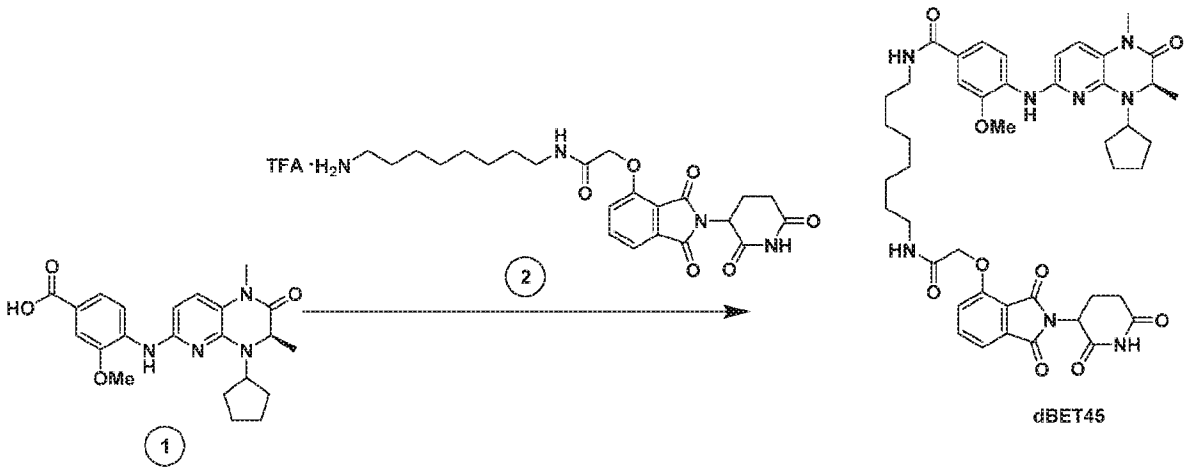
Synthetic Example 44: Synthesis of dBET44



A 0.1 M solution of 8-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)octanamide trifluoroacetate in DMF (217 microliters, 0.0217 mmol, 1 eq) was added to JQ-acid (8.7 mg, 0.0217 mmol, 1 eq). DIPEA (11.3 microliters, 0.0651 mmol, 3 eq) and HATU (8.3 mg, 0.0217 mmol, 1 eq) were then added and the mixture was stirred for 20.5 hours at room temperature. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an cream colored solid (14.28 mg, 0.0182 mmol, 84%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.72 – 7.68 (m, 1H),

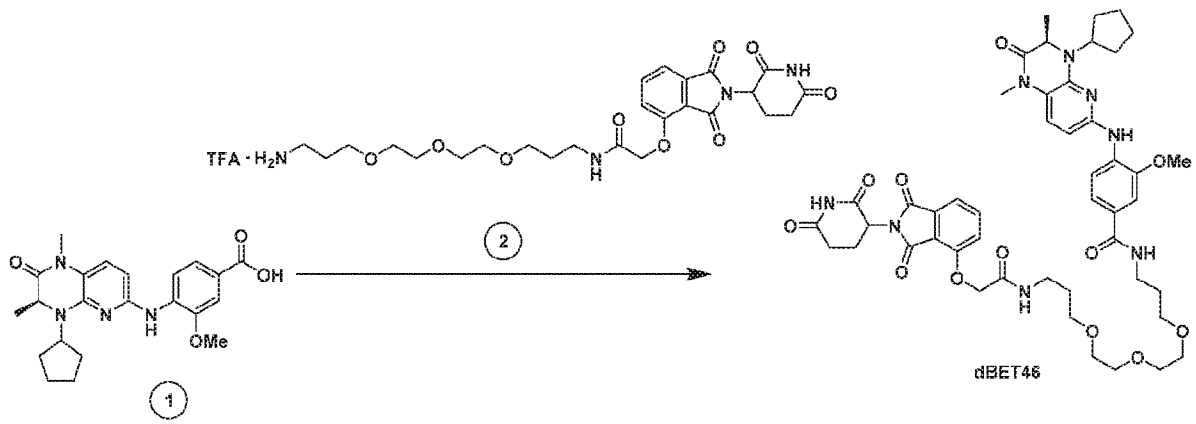
7.64 (d, J = 7.5 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.46 – 7.39 (m, 4H), 5.14 (dt, J = 13.3, 5.0 Hz, 1H), 4.62 (dd, J = 8.8, 5.4 Hz, 1H), 4.48 – 4.44 (m, 2H), 3.40 (ddd, J = 14.9, 8.8, 0.9 Hz, 1H), 3.26 (dt, J = 13.2, 6.9 Hz, 3H), 2.88 (ddd, J = 18.7, 13.5, 5.4 Hz, 1H), 2.75 (dddd, J = 17.6, 7.1, 4.5, 2.4 Hz, 1H), 2.68 (d, J = 2.2 Hz, 3H), 2.49 – 2.39 (m, 6H), 2.17 (ddt, J = 9.8, 5.3, 2.3 Hz, 1H), 1.76 – 1.70 (m, 2H), 1.70 – 1.67 (m, 3H), 1.61 – 1.54 (m, 2H), 1.42 (s, 6H). **LCMS** 783.53 (M+H).

Synthetic Example 45: Synthesis of dBET45



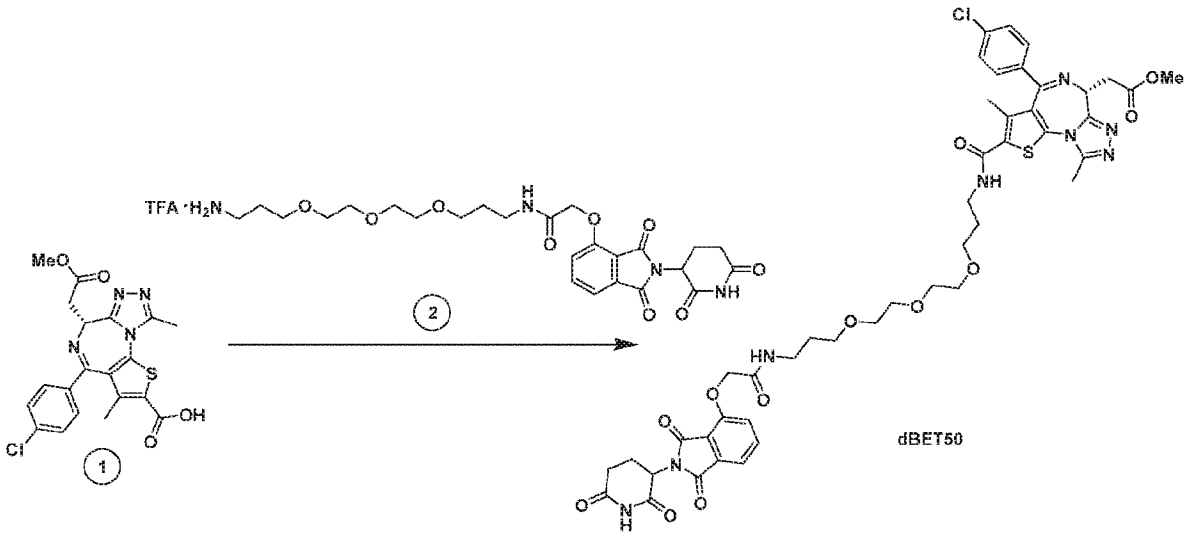
A 0.1 M solution of *N*-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (268 microliters, 0.0268 mmol, 1 eq) was added to (*R*)-4-((4-cyclopentyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl)amino)-3-methoxybenzoic acid (11.0 mg, 0.0268 mmol, 1 eq) at room temperature. DIPEA (14.0 microliters, 0.0804 mmol, 3 eq) and HATU (10.2 mg, 0.0268 mmol, 1 eq) were then added and the mixture was stirred for 18.5 hours. The mixture was then diluted with methanol and purified by preparative HPLC to give the desired product as a dark brown solid (10.44 mg, 0.0108 mmol, 40%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.38 (d, J = 8.4 Hz, 1H), 7.80 – 7.75 (m, 1H), 7.55 – 7.48 (m, 1H), 7.48 – 7.35 (m, 3H), 7.27 (d, J = 8.3 Hz, 1H), 6.45 (d, J = 8.2 Hz, 1H), 5.12 (dd, J = 12.5, 5.5 Hz, 1H), 4.72 (d, J = 5.1 Hz, 2H), 4.53 (s, 1H), 4.28 (d, J = 6.8 Hz, 1H), 3.98 (d, J = 4.1 Hz, 3H), 3.48 – 3.33 (m, 4H), 2.90 – 2.82 (m, 1H), 2.80 – 2.69 (m, 2H), 2.18 – 2.01 (m, 4H), 1.88 – 1.52 (m, 10H), 1.34 (d, J = 42.9 Hz, 10H), 1.17 (d, J = 6.8 Hz, 3H). **LCMS** 851.67 (M+H).

Synthetic Example 46: Synthesis of dBET46



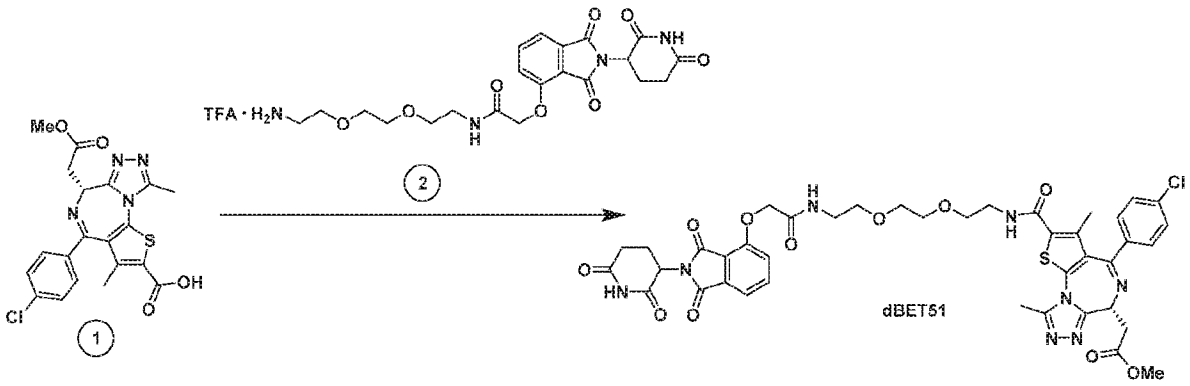
A 0.1 M solution of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (256 microliters, 0.0256 mmol, 1 eq) was added to (*R*)-4-((4-cyclopentyl-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazin-6-yl)amino)-3-methoxybenzoic acid (10.5 mg, 0.0256 mmol, 1 eq) at room temperature. DIPEA (13.4 microliters, 0.0767 mmol, 3 eq) and HATU (9.7 mg, 0.0256 mmol, 1 eq) were then added and the mixture was stirred for 20 hours. The mixture was then diluted with methanol and purified by preparative HPLC to give the desired product as a dark brown solid (13.69 mg, 0.0132 mmol, 51%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.28 – 8.24 (m, 1H), 7.74 – 7.71 (m, 1H), 7.49 (dd, J = 7.3, 3.7 Hz, 1H), 7.39 – 7.34 (m, 2H), 7.28 – 7.25 (m, 1H), 7.14 – 7.10 (m, 1H), 6.34 (d, J = 8.3 Hz, 1H), 5.01 – 4.97 (m, 1H), 4.62 (s, 2H), 4.25 (q, J = 6.7 Hz, 1H), 3.95 (d, J = 5.4 Hz, 3H), 3.60 (ddd, J = 9.0, 6.1, 1.6 Hz, 8H), 3.53 – 3.46 (m, 6H), 3.40 – 3.37 (m, 2H), 2.78 (td, J = 11.1, 6.6 Hz, 3H), 2.16 – 2.00 (m, 4H), 1.84 (ddt, J = 33.5, 13.0, 6.4 Hz, 7H), 1.75 – 1.60 (m, 6H), 1.17 (d, J = 6.8 Hz, 3H). **LCMS** 927.74 (M+H).

Synthetic Example 47: Synthesis of dBET50



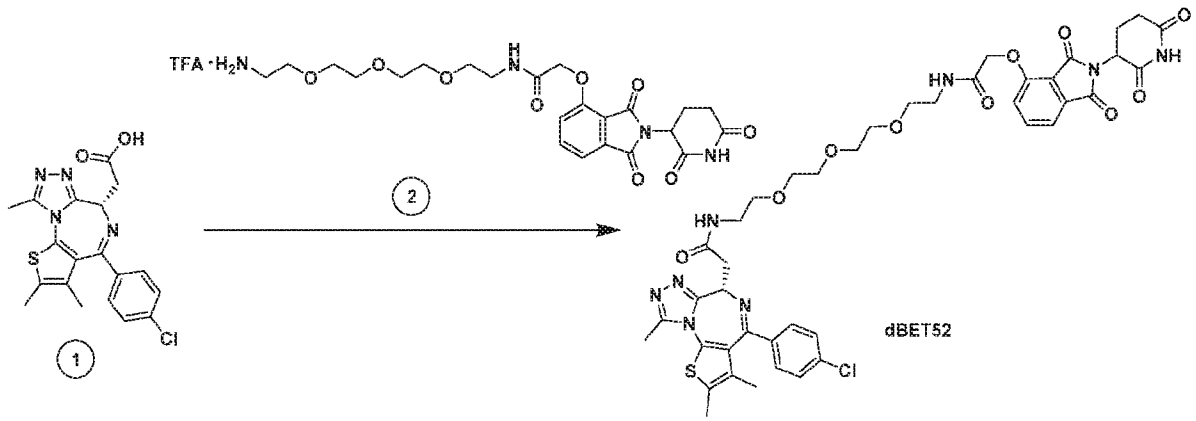
A 0.1 M solution of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.0200 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-3,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (8.9 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. The mixture was then stirred for 17 hours, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a cream colored solid (9.31 mg, 0.00968 mmol, 48%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.82 – 7.78 (m, 1H), 7.52 (dd, J = 7.1, 1.6 Hz, 1H), 7.49 – 7.40 (m, 5H), 5.10 (ddd, J = 12.8, 5.5, 2.9 Hz, 1H), 4.74 (s, 2H), 4.67 (t, J = 7.1 Hz, 1H), 3.76 (s, 3H), 3.62 – 3.50 (m, 14H), 3.49 – 3.43 (m, 2H), 3.40 (q, J = 6.5 Hz, 2H), 2.87 (ddd, J = 17.6, 14.0, 5.3 Hz, 1H), 2.79 – 2.67 (m, 5H), 2.12 (ddq, J = 10.3, 5.4, 2.9 Hz, 1H), 2.00 (s, 3H), 1.86 (q, J = 6.3 Hz, 2H), 1.80 (p, J = 6.4 Hz, 2H). **LCMS** 961.67 (M+H).

Synthetic Example 48: Synthesis of dBET51



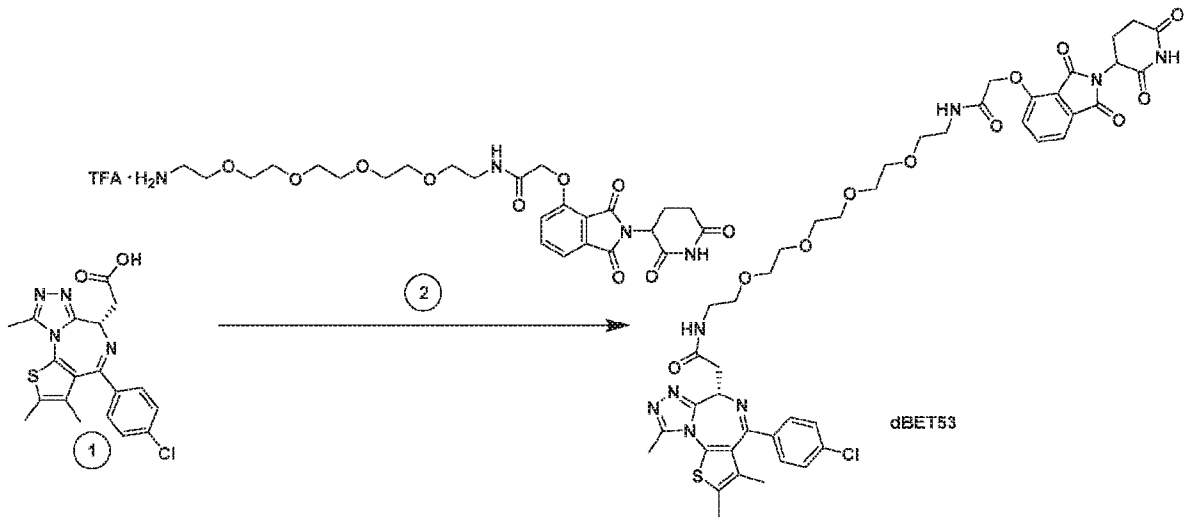
A 0.1 M solution of *N*-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.0200 mmol, 1 eq) was added to (*S*)-4-(4-chlorophenyl)-6-(2-methoxy-2-oxoethyl)-3,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-2-carboxylic acid (8.9 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. The mixture was then stirred for 17 hours, then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an off-white solid (8.38 mg, 0.00942 mmol, 47%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.80 (dd, J = 8.4, 7.4 Hz, 1H), 7.52 (dd, J = 7.2, 1.3 Hz, 1H), 7.48 – 7.38 (m, 5H), 5.08 (ddd, J = 12.7, 5.5, 1.6 Hz, 1H), 4.74 (d, J = 2.7 Hz, 2H), 4.66 (t, J = 7.1 Hz, 1H), 3.75 (d, J = 3.0 Hz, 3H), 3.65 (t, J = 4.1 Hz, 6H), 3.59 (t, J = 5.3 Hz, 2H), 3.57 – 3.49 (m, 4H), 3.49 – 3.40 (m, 2H), 2.93 – 2.84 (m, 1H), 2.78 – 2.64 (m, 5H), 2.15 – 2.09 (m, 1H), 2.00 (d, J = 0.9 Hz, 3H). **LCMS** 889.58 (M+H).

Synthetic Example 49: Synthesis of dBET52



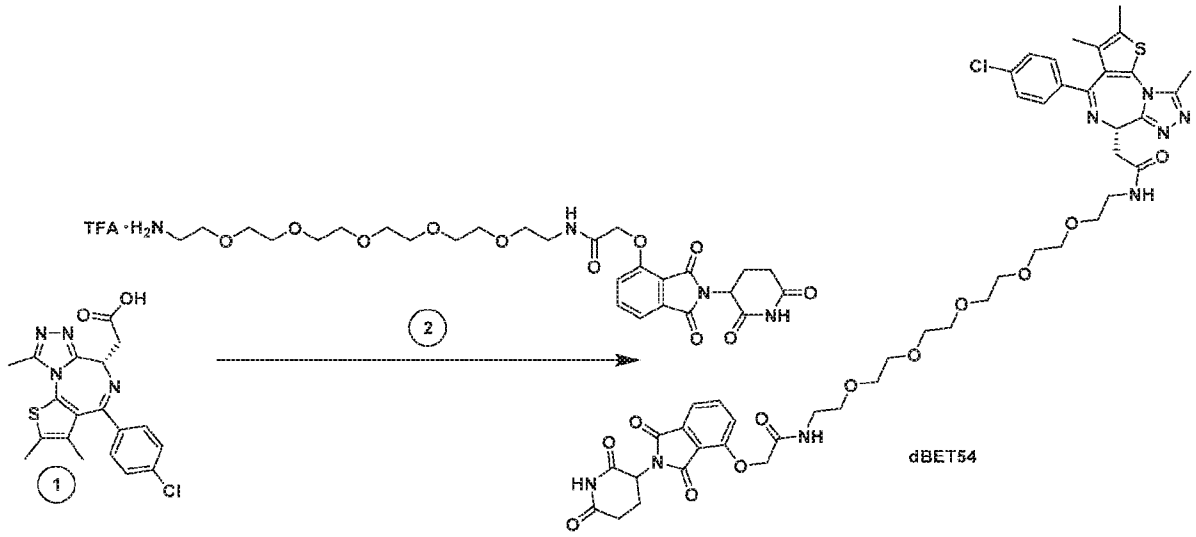
A 0.1 M solution of *N*-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.020 mmol, 1 eq) was added to JQ-acid (8.0 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. After 17.5 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as a colorless residue (9.12 mg, 0.01025 mmol, 51%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.77 (t, J = 7.9 Hz, 1H), 7.50 (dd, J = 7.3, 1.5 Hz, 1H), 7.47 – 7.36 (m, 5H), 5.09 (ddd, J = 13.0, 7.6, 5.5 Hz, 1H), 4.76 (s, 2H), 4.62 (dd, J = 9.1, 5.1 Hz, 1H), 3.62 (ddt, J = 17.3, 11.2, 6.5 Hz, 12H), 3.52 – 3.41 (m, 5H), 3.28 (d, J = 5.1 Hz, 1H), 2.90 – 2.81 (m, 1H), 2.79 – 2.66 (m, 5H), 2.44 (s, 3H), 2.16 – 2.09 (m, 1H), 1.69 (s, 3H). **LCMS** 889.38 (M+H).

Synthetic Example 50: Synthesis of dBET53



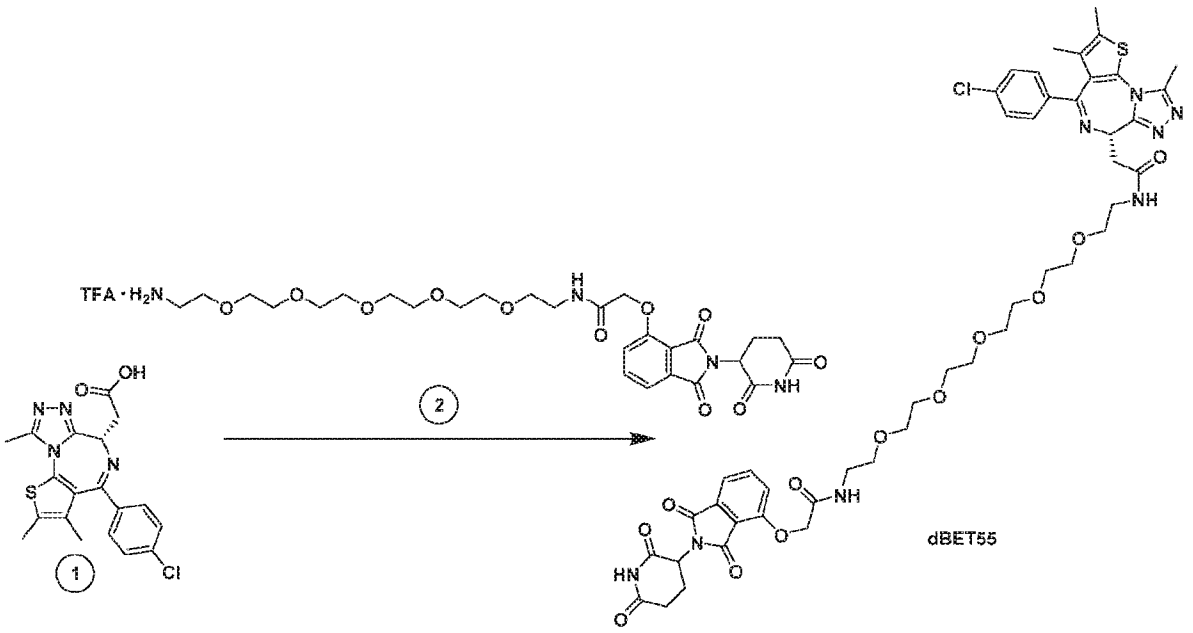
A 0.1 M solution of *N*-(14-amino-3,6,9,12-tetraoxatetradecyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.020 mmol, 1 eq) was added to JQ-acid (8.0 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. After 17.5 hours, additional HATU (7.6 mg) and DIPEA (10.5 microliters were added) and the mixture was stirred for an additional 5 hours. The mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product (3.66 mg). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.79 (dd, J = 8.4, 7.4 Hz, 1H), 7.51 (d, J = 7.3 Hz, 1H), 7.45 (d, J = 7.7 Hz, 2H), 7.43 – 7.36 (m, 3H), 5.08 (ddd, J = 12.7, 5.5, 2.2 Hz, 1H), 4.78 – 4.74 (m, 2H), 4.62 (dd, J = 9.1, 5.1 Hz, 1H), 3.70 – 3.51 (m, 16H), 3.50 – 3.41 (m, 5H), 3.27 (dd, J = 5.1, 2.3 Hz, 1H), 2.87 (ddt, J = 18.2, 9.5, 4.9 Hz, 1H), 2.78 – 2.66 (m, 5H), 2.44 (s, 3H), 2.16 – 2.09 (m, 1H), 1.69 (s, 3H). **LCMS** 933.43 (M+H).

Synthetic Example 51: Synthesis of dBET54



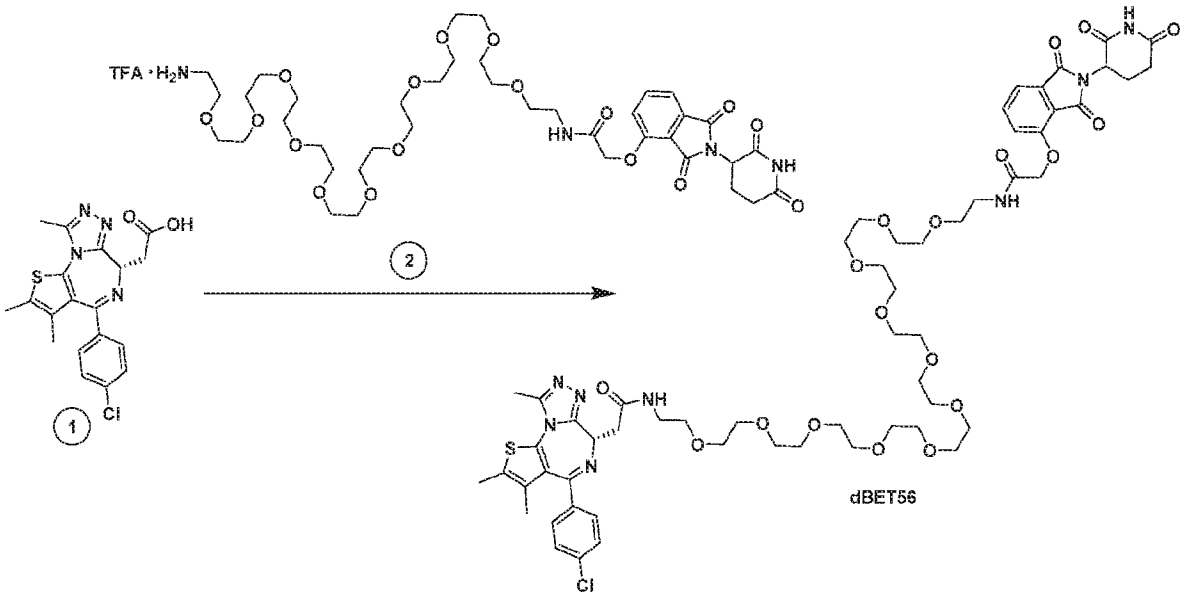
A 0.1 M solution of *N*-(17-amino-3,6,9,12,15-pentaoxaheptadecyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.020 mmol, 1 eq) was added to JQ-acid (8.0 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. After 16 hours the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product (6.27 mg, 0.00641 mmol, 32%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.81 – 7.76 (m, 1H), 7.51 (d, J = 7.1 Hz, 1H), 7.47 – 7.38 (m, 5H), 5.09 (dd, J = 12.6, 5.5 Hz, 1H), 4.77 (s, 2H), 4.62 (dd, J = 8.8, 5.0 Hz, 1H), 3.67 – 3.55 (m, 20H), 3.46 (ddd, J = 20.1, 10.2, 4.7 Hz, 5H), 3.28 (d, J = 5.1 Hz, 1H), 2.91 – 2.83 (m, 1H), 2.78 – 2.68 (m, 5H), 2.44 (s, 3H), 2.16 – 2.10 (m, 1H), 1.72 – 1.66 (m, 3H). **LCMS** 977.50 (M+H).

Synthetic Example 52: Synthesis of dBET55



A 0.1 M solution of *N*-(29-amino-3,6,9,12,15,18,21,24,27-nonaoxanonacosyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.020 mmol, 1 eq) was added to JQ-acid (8.0 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. After 18 hours the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product (10.55 mg, 0.00914 mmol, 46%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.82 (dd, J = 8.4, 7.4 Hz, 1H), 7.55 (d, J = 7.0 Hz, 1H), 7.49 – 7.41 (m, 5H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.80 (s, 2H), 4.65 (dd, J = 9.1, 5.1 Hz, 1H), 3.68 – 3.58 (m, 36H), 3.53 – 3.44 (m, 5H), 2.94 – 2.86 (m, 1H), 2.81 – 2.70 (m, 5H), 2.46 (s, 3H), 2.19 – 2.13 (m, 1H), 1.74 – 1.69 (m, 3H). **LCMS** 1153.59 (M+H).

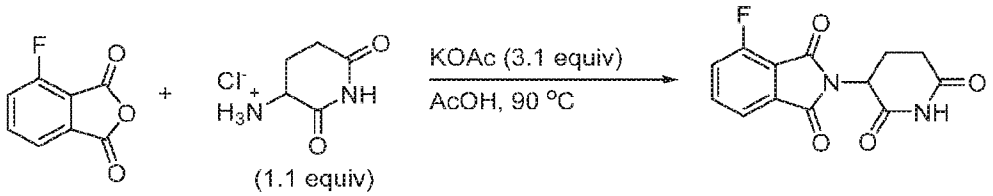
Synthetic Example 53: Synthesis of dBET56



A 0.1 M solution of *N*-(35-amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate in DMF (200 microliters, 0.020 mmol, 1 eq) was added to JQ-acid (8.0 mg, 0.020 mmol, 1 eq) at room temperature. DIPEA (10.5 microliters, 0.060 mmol, 3 eq) and HATU (7.6 mg, 0.020 mmol, 1 eq) were added. After 20 hours the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g silica column, 0-10% MeOH/DCM, 25 minute gradient) gave the desired product as an oily residue (9.03 mg, 0.00727 mmol, 36%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 7.81 (dd, J = 8.4, 7.4 Hz, 1H), 7.53 (d, J = 7.1 Hz, 1H), 7.50 – 7.40 (m, 5H), 5.11 (dd, J = 12.6, 5.5 Hz, 1H), 4.78 (s, 2H), 4.68 (dd, J = 8.6, 5.0 Hz, 1H), 3.69 – 3.56 (m, 44H), 3.52 – 3.43 (m, 5H), 3.34 (dd, J = 7.9, 3.5 Hz, 1H), 2.88 (ddd, J = 18.0, 14.0, 5.2 Hz, 1H), 2.79 – 2.68 (m, 5H), 2.46 (s, 3H), 2.17 – 2.12 (m, 1H), 1.71 (s, 3H). **LCMS** 1241.60 (M+H).

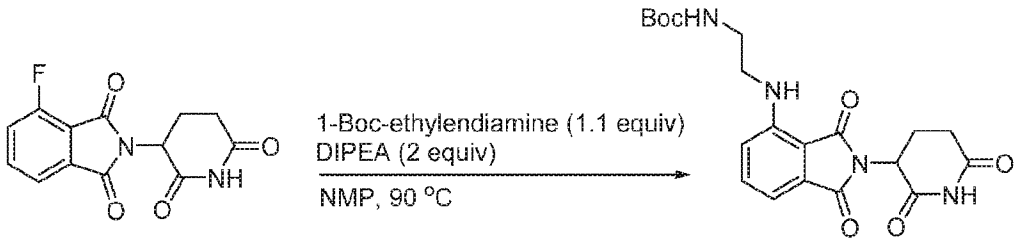
Synthetic Example 54: Synthesis of dBET57

Step 1: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione



A solution of 4-fluoroisobenzofuran-1,3-dione (200 mg, 1.20 mmol, 1 equiv) in AcOH (4.0 mL, 0.3 M) was added 2,6-dioxopiperidin-3-amine hydrochloride (218 mg, 1.32 mmol, 1.1 equiv) and potassium acetate (366 mg, 3.73 mmol, 3.1 equiv). The reaction mixture was heated to 90 °C overnight, whereupon it was diluted with water to 20 mL and cooled on ice for 30 min. The resulting slurry was filtered, and the black solid was purified by flash column chromatography on silica gel (2% MeOH in CH_2Cl_2, R_f = 0.3) to afford the title compound as a white solid (288 mg, 86%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.96 (ddd, J = 8.3, 7.3, 4.5 Hz, 1H), 7.82 – 7.71 (m, 2H), 5.17 (dd, J = 13.0, 5.4 Hz, 1H), 2.90 (ddd, J = 17.1, 13.9, 5.4 Hz, 1H), 2.65 – 2.47 (m, 2H), 2.10 – 2.04 (m, 1H), MS (ESI) cald for $C_{13}H_{10}FN_2O_4$ $[M+H]^+$ 277.06, found 277.25.

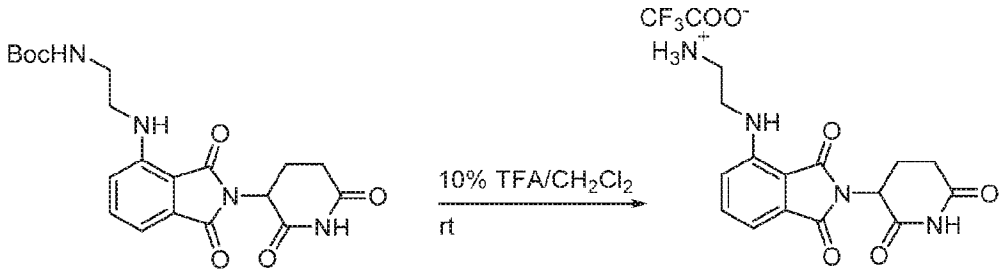
Step 2: Synthesis of *tert*-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate



A stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (174 mg, 0.630 mmol, 1 equiv) in DMF (6.3 mL, 0.1 M) was added DIPEA (220 µL, 1.26 mmol, 2 equiv) and 1-Boc-ethylendiamine (110 µL, 0.693 mmol, 1.1 equiv). The reaction mixture was heated to 90 °C overnight, whereupon it was cooled to room temperature and taken up in EtOAc (30 mL) and water (30 mL). The organic layer was washed with brine (3x20 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (0→10% MeOH in CH_2Cl_2) to give the title compound as a yellow solid (205 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (bs, 1H), 7.50 (dd, J = 8.5, 7.1 Hz, 1H), 7.12 (d, J = 7.1 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 6.39 (t, J = 6.1 Hz, 1H), 4.96 – 4.87 (m, 1H), 4.83 (bs, 1H), 3.50 – 3.41 (m,

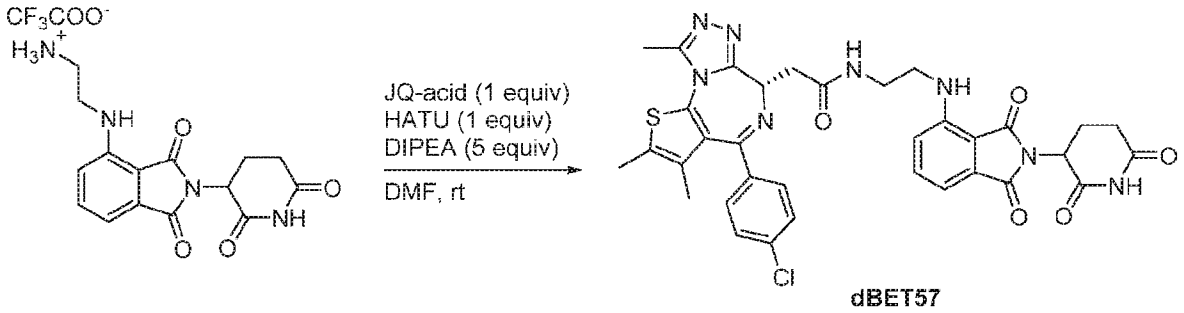
2H), 3.41 – 3.35 (m, 2H), 2.92 – 2.66 (m, 3H), 2.16 – 2.09 (m, 1H), 1.45 (s, 9H); MS (ESI) cald for $C_{20}H_{25}N_4O_6$ $[M+H]^+$ 417.18, found 417.58.

Step 3: Synthesis of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethan-1-aminium 2,2,2-trifluoroacetate



A stirred solution of *tert*-butyl (2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)carbamate (205 mg, 0.492 mmol, 1 equiv) in dichloromethane (2.25 mL) was added trifluoroacetic acid (0.250 mL). The reaction mixture was stirred at room temperature for 4 h, whereupon the volatiles were removed *in vacuo*. The title compound was obtained as a yellow solid (226 mg, >95%), that was used without further purification. ^{1}H NMR (500 MHz, MeOD) δ 7.64 (d, J = 1.4 Hz, 1H), 7.27 – 7.05 (m, 2H), 5.10 (dd, J = 12.5, 5.5 Hz, 1H), 3.70 (t, J = 6.0 Hz, 2H), 3.50 – 3.42 (m, 2H), 3.22 (t, J = 6.0 Hz, 1H), 2.93 – 2.85 (m, 1H), 2.80 – 2.69 (m, 2H), 2.17 – 2.10 (m, 1H); MS (ESI) cald for $C_{15}H_{17}N_4O_4$ $[M+H]^+$ 317.12, found 317.53.

Step 2: Synthesis of dBET57

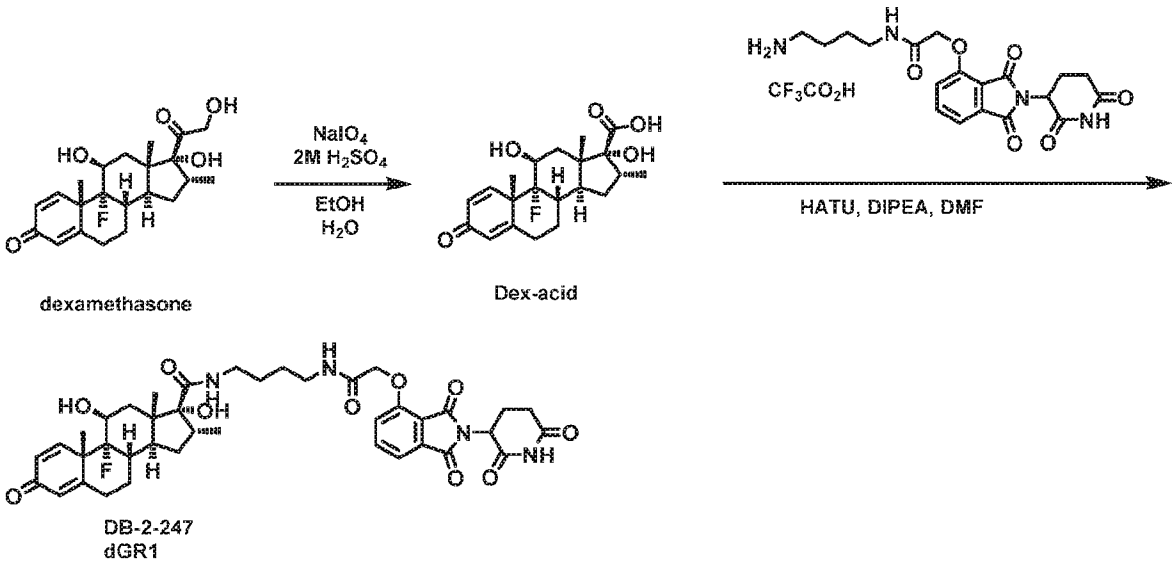


dBET57

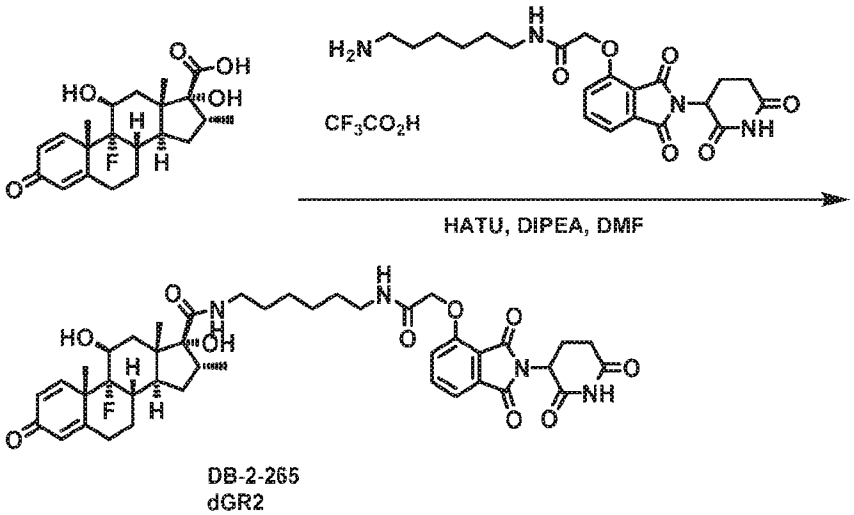
JQ-acid (8.0 mg, 0.0200 mmol, 1 eq) and 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethan-1-aminium 2,2,2-trifluoroacetate (8.6 mg, 0.0200 mmol, 1 equiv) were dissolved in DMF (0.200 mL, 0.1 M) at room temperature. DIPEA (17.4 μL, 0.100 mmol, 5 equiv) and HATU (7.59 mg, 0.0200 mmol, 1 equiv) were then added and the mixture was stirred at room temperature overnight. The reaction mixture was taken up in EtOAc (15 mL), and washed with satd. $NaHCO_3$ (aq) (15 mL), water (15 mL) and brine (3x15 mL). The organic layer

was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (0→10% MeOH in CH_2Cl_2, R_f = 0.3 (10% MeOH in CH_2Cl_2)) to give the title compound as a bright yellow solid (11.2 mg, 80%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.49 (bs, 0.6H), 8.39 (bs, 0.4H), 7.51 – 7.43 (m, 1H), 7.38 (d, J = 7.8 Hz, 2H), 7.29 (dd, J = 8.8, 1.7 Hz, 2H), 7.07 (dd, J = 7.1, 4.9 Hz, 1H), 6.97 (dd, J = 8.6, 4.9 Hz, 1H), 6.48 (t, J = 5.9 Hz, 1H), 6.40 (t, J = 5.8 Hz, 0.6H), 4.91 – 4.82 (m, 0.4H), 4.65 – 4.60 (m, 1H), 3.62 – 3.38 (m, 6H), 2.87 – 2.64 (m, 3H), 2.63 (s, 3H), 2.40 (s, 6H), 2.12 – 2.04 (m, 1H), 1.67 (s, 3H), rotamers; MS (ESI) calcd for $C_{34}H_{32}ClN_8O_5S$ $[M+H]^+$ 700.19, found 700.34.

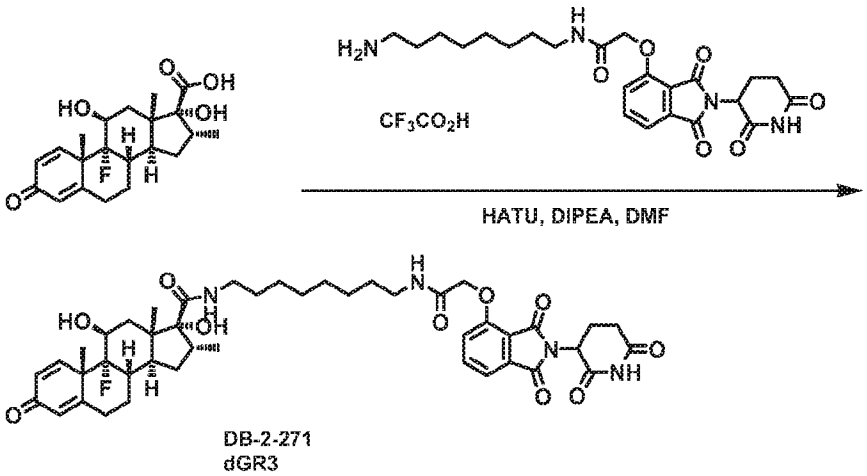
Synthetic Example 55: Synthesis of dGR1



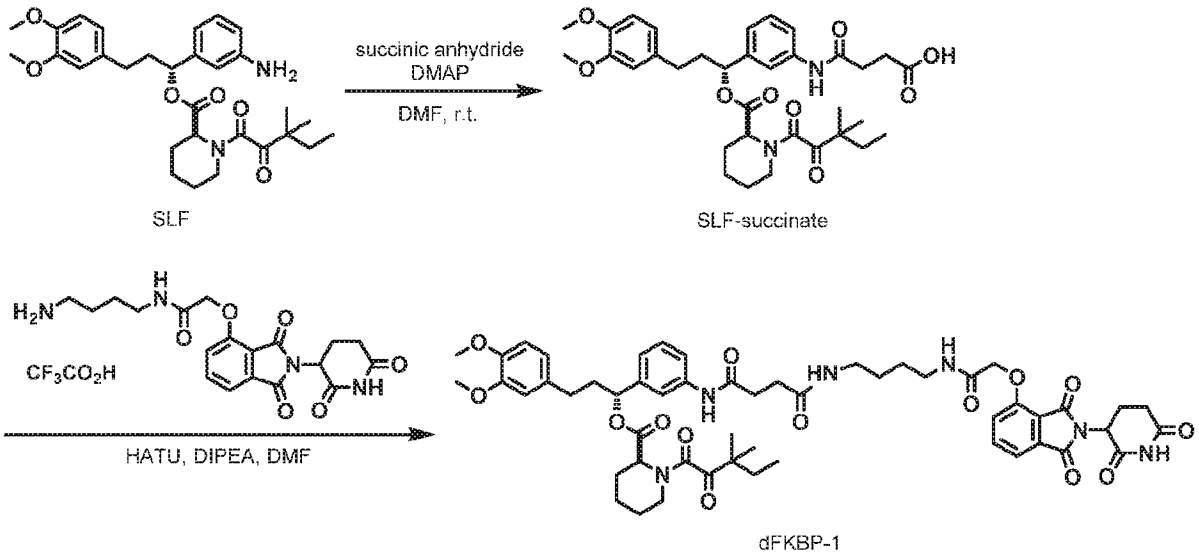
Synthetic Example 56: Synthesis of dGR2:



Synthetic Example 57: Synthesis of dGR3:



Synthetic Example 58: Synthesis of dFKBP-1



(1) Synthesis of SLF-succinate

SLF (25 mg, 2.5 mL of a 10 mg/mL solution in MeOAc, 0.0477 mmol, 1 eq) was combined with DMF (0.48 mL, 0.1 M) and succinic anhydride (7.2 mg, 0.0715 mmol, 1.5 eq) and stirred at room temperature for 24 hours. Low conversion was observed and the mixture was placed under a stream of N_2 to remove the MeOAc. An additional 0.48 mL of DMF was added, along with an additional 7.2 mg succinic anhydride and DMAP (5.8 mg, 0.0477 mmol, 1 eq). The mixture was then stirred for an additional 24 hours before being purified by preparative HPLC to give SLF-succinate as a yellow oil (24.06 mg, 0.0385 mmol, 81%).

^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.62 (d, *J* = 10.7 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.26 (td, *J* = 7.9, 2.7 Hz, 1H), 7.07 – 6.97 (m, 1H), 6.80 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.74 – 6.66 (m, 2H), 5.73 (dd, *J* = 8.1, 5.5 Hz, 1H), 5.23 (d, *J* = 4.8 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.39 – 3.29 (m, 4H), 3.21 (td, *J* = 13.2, 3.0 Hz, 1H), 2.68 – 2.50 (m, 5H), 2.37 – 2.19 (m, 2H), 2.12 – 2.02 (m, 1H), 1.79 – 1.61 (m, 4H), 1.49 – 1.30 (m, 2H), 1.27 – 1.05 (m, 6H), 0.82 (dt, *J* = 41.2, 7.5 Hz, 3H). LCMS 624.72 (M+H).

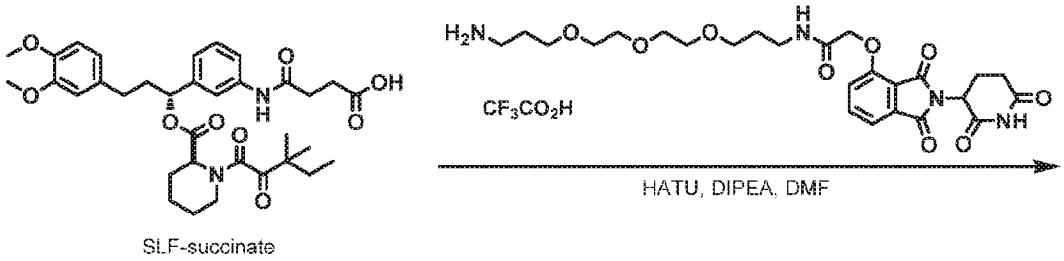
(2) Synthesis of dFKBP-1

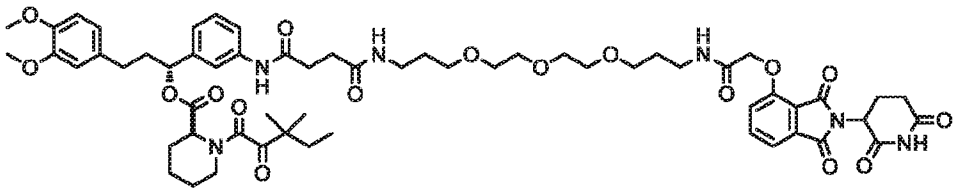
N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (9.9 mg, 0.0192 mmol, 1 eq) was added to SLFsuccinate (11.98 mg, 0.0192 mmol, 1 eq) as a solution in 0.192 mL DMF (0.1 M). DIPEA (10.0 microliters, 0.0575 mmol, 3 eq) was added, followed by HATU (7.3 mg, 0.0192 mmol, 1 eq). The mixture was stirred

for 17 hours, then diluted with MeOH and purified by preparative HPLC to give dFKBP-1 (7.7 mg, 0.00763 mmol, 40%) as a yellow solid.

^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.81 (s, 1H), 7.77 – 7.70 (m, 1H), 7.55 – 7.49 (m, 2H), 7.26 (dd, *J* = 8.0, 5.3 Hz, 2H), 7.05 – 6.99 (m, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.66 (d, *J* = 6.8 Hz, 2H), 5.77 – 5.72 (m, 1H), 5.24 (d, *J* = 4.8 Hz, 1H), 4.99 (dd, *J* = 12.3, 5.7 Hz, 1H), 4.68 – 4.59 (m, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.32 (dt, *J* = 3.3, 1.6 Hz, 4H), 3.26 – 3.14 (m, 3H), 2.79 (dd, *J* = 18.9, 10.2 Hz, 3H), 2.64 – 2.48 (m, 5H), 2.34 (d, *J* = 14.4 Hz, 1H), 2.22 (d, *J* = 9.2 Hz, 1H), 2.14 – 2.02 (m, 2H), 1.78 – 1.49 (m, 9H), 1.43 – 1.30 (m, 2H), 1.20 – 1.04 (m, 6H), 0.90 – 0.76 (m, 3H). 13C NMR (100 MHz, cd3od) δ 208.51, 173.27, 172.64, 171.63, 169.93, 169.51, 168.04, 167.69, 167.09, 166.71, 154.92, 149.05, 147.48, 140.76, 138.89, 137.48, 133.91, 133.67, 129.36, 122.19, 120.61, 120.54, 119.82, 118.41, 118.12, 117.79, 112.12, 111.76, 68.54, 56.10, 55.98, 51.67, 46.94, 44.57, 39.32, 39.01, 38.23, 32.64, 31.55, 31.43, 26.68, 26.64, 25.08, 23.52, 23.21, 22.85, 21.27, 8.76. LCMS 1009.66 (M+H).

Synthetic Example 59: Synthesis of dFKBP-2





dFKBP-2

(1) Synthesis of tert-butyl (1-chloro-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate

tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (1.0 g, 3.12 mmol, 1 eq) was dissolved in THF (31 mL, 0.1 M). DIPEA (0.543 mL, 3.12 mmol, 1 eq) was added and the solution was cooled to 0 °C. Chloroacetyl chloride (0.273 mL, 3.43 mmol, 1.1 eq) was added and the mixture was warmed slowly to room temperature. After 24 hours, the mixture was diluted

with EtOAc and washed with saturated sodium bicarbonate, water then brine. The organic layer was dried over sodium sulfate, filtered and condensed to give a yellow oil (1.416 g) that was carried forward without further purification.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 (s, 1H), 5.00 (s, 1H), 3.98 – 3.89 (m, 2H), 3.54 (dddt, *J* = 17.0, 11.2, 5.9, 2.2 Hz, 10H), 3.47 – 3.40 (m, 2H), 3.37 – 3.31 (m, 2H), 3.17 – 3.07 (m, 2H), 1.79 – 1.70 (m, 2H), 1.67 (p, *J* = 6.1 Hz, 2H), 1.35 (s, 9H). ^{13}C NMR (100 MHz, cdcl3) δ 165.83, 155.97, 78.75, 70.49, 70.47, 70.38, 70.30, 70.14, 69.48, 42.61, 38.62, 38.44, 29.62, 28.59, 28.40. LCMS 397.37 (M+H).

(2) Synthesis of dimethyl 3-((2,2-dimethyl-4,20-dioxo-3,9,12,15-tetraoxa-5,19-diazahenicosan-21-yl)oxy)phthalate

tert-butyl (1-chloro-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (1.41 g, 3.12 mmol, 1 eq) was dissolved in MeCN (32 mL, 0.1 M). Dimethyl 3-hydroxyphthalate (0.721 g, 3.43 mmol, 1.1 eq) and cesium carbonate (2.80 g, 8.58 mmol, 2.75 eq) were added. The flask was fitted with a reflux condenser and heated to 80 °C for 19 hours. The mixture was cooled to room temperature and diluted water and extracted once with chloroform and twice with EtOAc. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography (ISCO, 24 g silica column, 0-15% MeOH/DCM 22 minute gradient) to give a yellow oil (1.5892 g, 2.78 mmol, 89% over two steps).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.00 (t, *J* = 5.3 Hz, 1H), 5.06 (s, 1H), 4.46 (s, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.47 (ddd, *J* = 14.9, 5.5, 2.8 Hz, 8H), 3.39 (dt, *J* = 9.4, 6.0 Hz, 4H), 3.29 (q, *J* = 6.5 Hz, 2H), 3.09 (d, *J* = 6.0 Hz, 2H), 1.70 (p, *J* = 6.5 Hz, 2H), 1.63 (p, *J* = 6.3 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, cdcl3) δ 167.68, 167.36, 165.45, 155.93, 154.41, 130.87, 129.60, 125.01, 123.20, 117.06, 78.60, 70.40, 70.17, 70.06, 69.39, 68.67, 68.25, 52.77, 52.57, 38.38, 36.58, 29.55, 29.20, 28.34. LCMS 571.47 (M+H).

(3) Synthesis of N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate

Dimethyl 3-((2,2-dimethyl-4,20-dioxo-3,9,12,15-tetraoxa-5,19-diazahenicosan-21-yl)oxy)phthalate (1.589 g, 2.78 mmol, 1 eq) was dissolved in EtOH (14 mL, 0.2 M). Aqueous 3M NaOH (2.8 mL, 8.34 mmol, 3 eq) was added and the mixture was heated to 80 °C for 22 hours.

The mixture was then cooled to room temperature, diluted with 50 mL DCM and 20 mL 0.5 M HCl. The layers were separated and the organic layer was washed with 25 mL water. The aqueous layers were combined and extracted three times with 50 mL chloroform. The combined organic layers were dried over sodium sulfate, filtered and condensed to give 1.53 g of material that was carried forward without further purification. LCMS 553.44.

The resultant material (1.53 g) and 3-aminopiperidine-2,6-dione hydrochloride (0.480 g, 2.92 mmol, 1 eq) were dissolved in pyridine (11.7 mL, 0.25 M) and heated to 110 °C for 17 hours. The mixture was cooled to room temperature and concentrated under reduced pressure to give crude *tert*-butyl (1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate as a black sludge (3.1491 g) that was carried forward without further purification. LCMS 635.47.

The crude *tert*-butyl (1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (3.15 g) was dissolved in TFA (20 mL) and heated to 50 °C for 2.5 hours. The mixture was cooled to room temperature, diluted with MeOH and concentrated under reduced pressure. The material was purified by preparative HPLC to give *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (1.2438 g, 1.9598 mmol, 71% over 3 steps) as a dark red oil.

^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.77 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.49 (d, *J* = 7.3 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 5.12 (dd, *J* =12.8, 5.5 Hz, 1H), 4.75 (s, 2H), 3.68 – 3.51 (m, 12H), 3.40 (t, *J* = 6.8 Hz, 2H), 3.10 (t, *J* = 6.4 Hz, 2H), 2.94 – 2.68 (m, 3H), 2.16 (dtd, *J* = 12.6, 5.4, 2.5 Hz, 1H), 1.92 (p, *J* = 6.1 Hz, 2H), 1.86 – 1.77 (m, 2H). ^{13}C NMR (100 MHz, cd3od) δ 173.17, 169.97, 168.48, 166.87, 166.30, 154.82, 136.89, 133.41, 120.29, 117.67, 116.58, 69.96, 69.68, 69.60, 68.87, 68.12, 67.92, 49.19, 38.62, 36.14, 30.80, 28.92, 26.63, 22.22. LCMS 536.41 (M+H).

(4) Synthesis of dFKBP-2

N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate(12.5 mg, 0.0193 mmol, 1 eq) was added to SLF-succinate (12.08 mg, 0.0193 mmol, 1 eq) as a solution in 0.193 mL in DMF (0.1 M). DIPEA (10.1 microliters, 0.0580 mmol, 3 eq) and HATU (7.3 mg, 0.0193 mmol, 1 eq) were added and the mixture was stirred for 19 hours. The mixture was then diluted with MeOH and purified by preparative HPLC to give dFKBP-2 (9.34 mg, 0.00818 mmol, 42%) as a yellow oil.

^{1}H NMR (400 MHz, 50% MeOD/Chloroform-*d*) δ 7.76 – 7.70 (m, 1H), 7.58 – 7.45 (m, 3H), 7.26 (t, *J* = 8.2 Hz, 2H), 7.05 – 6.98 (m, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 6.71 – 6.63 (m, 2H), 5.73 (dd, *J* = 8.1, 5.6 Hz, 1H), 5.23 (d, *J* = 5.4 Hz, 1H), 5.03 – 4.95 (m, 1H), 4.64 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.62 – 3.52 (m, 8H), 3.47 (t, *J* = 6.1 Hz, 2H), 3.44 – 3.33 (m, 3H), 3.27 – 3.14 (m, 3H), 2.84 – 2.70 (m, 3H), 2.64 – 2.47 (m, 6H), 2.34 (d, *J* = 14.1 Hz, 1H), 2.24 (dd, *J* = 14.3, 9.3 Hz, 2H), 2.13 – 2.00 (m, 2H), 1.83 (p, *J* = 6.3 Hz, 2H), 1.67 (dtd, *J* = 38.4, 16.8, 14.8, 7.0 Hz, 7H), 1.51 – 1.26 (m, 3H), 1.22 – 1.05 (m, 6H), 0.80 (dt, *J* = 39.8, 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 208.64, 173.39, 173.01, 171.76, 170.11, 169.62, 168.24, 167.92, 167.36, 166.69, 155.02, 149.23, 147.66, 140.94, 139.18, 137.57, 134.09, 133.91, 129.49, 122.32, 120.75, 120.52, 119.93, 118.42, 117.75, 112.33, 111.98, 70.77, 70.51, 70.40, 69.45, 69.04, 68.48, 56.20, 56.10, 51.88, 47.09, 44.78, 38.40, 37.48, 36.91, 32.80, 32.71, 31.70, 31.59, 31.55, 29.53, 29.30, 26.77, 25.22, 23.63, 23.33, 22.98, 21.43. LCMS 1141.71 (M+H).

Synthetic Example 60: Synthesis of dFKBP-3

A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (0.233 mL, 0.0233 mmol, 1 eq) was added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-(3,3-dimethyl-2-oxopentanoyl)pyrrolidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (13.3 mg, 0.0233 mmol, 1 eq). DIPEA (12.2 microliters, 0.0700 mmol, 3 eq) was added, followed by HATU (8.9 mg, 0.0233 mmol, 1 eq). The mixture was stirred for 23 hours, then diluted with MeOH and purified by preparative HPLC to give a white solid (10.72 mg mg, 0.0112 mmol, 48%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 – 7.74 (m, 1H), 7.52 (d, *J* = 7.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.26 (t, *J* = 8.1 Hz, 1H), 6.97 – 6.90 (m, 2H), 6.89 – 6.84 (m, 1H), 6.79 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.73 – 6.64 (m, 2H), 5.73 – 5.65 (m, 1H), 5.07 – 4.99 (m, 1H), 4.67 (s, 2H), 4.57 – 4.51 (m, 1H), 4.48 (dd, *J* = 5.7, 2.5 Hz, 2H), 3.82 (d, *J* = 1.9 Hz, 3H), 3.80 (s, 3H), 3.66 – 3.39 (m, 3H), 2.88 – 2.48 (m, 6H), 2.42 – 1.87 (m, 9H), 1.73 – 1.51 (m, 6H), 1.19 – 0.92 (m, 6H), 0.75 (dt, *J* = 56.7, 7.5 Hz, 3H). LCMS 954.52 (M+H).

Example 61: Synthesis of dFKBP-4

A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (0.182 mL, 0.0182 mmol, 1 eq) was added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (10.6 mg, 0.0182 mmol, 1 eq). DIPEA (9.5 microliters, 0.0545 mmol, 3 eq) was added, followed by HATU (6.9 mg, 0.0182 mmol, 1 eq). The mixture was stirred for 26 hours, then diluted with MeOH and purified by preparative HPLC to give a white solid (9.74 mg, 0.01006 mmol, 55%).

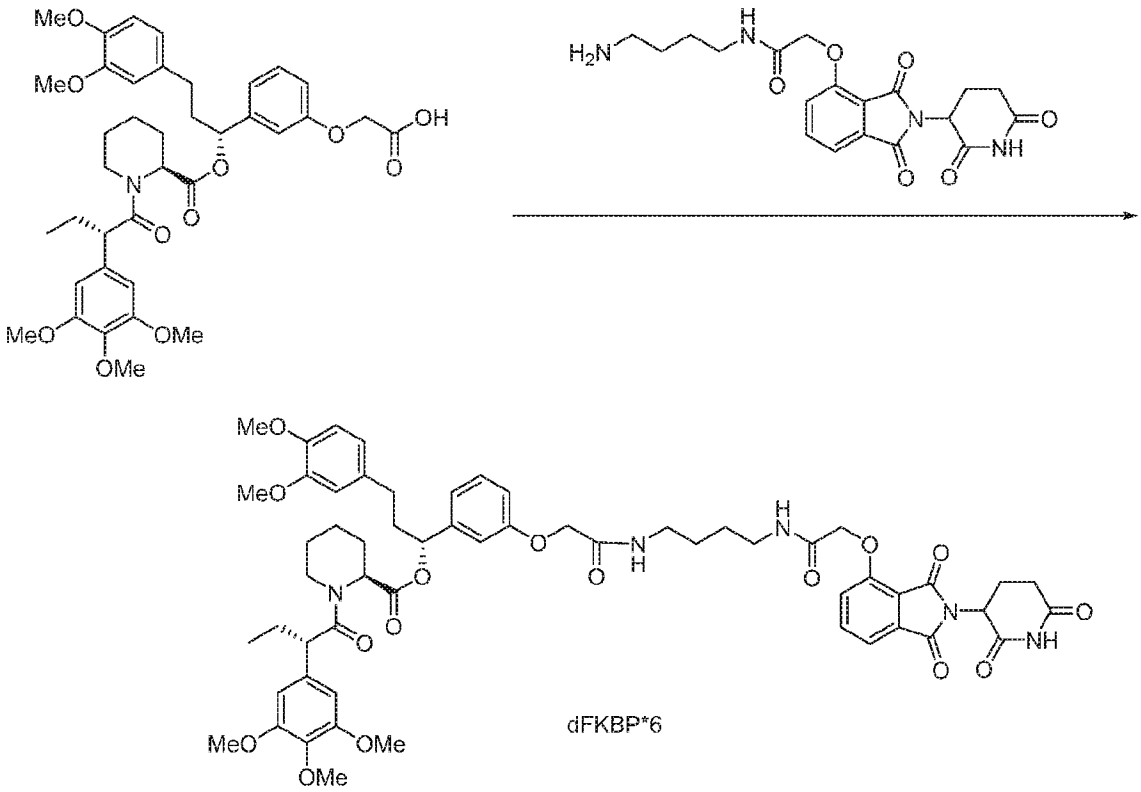
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 (dd, J = 8.3, 7.4 Hz, 1H), 7.53 (d, J = 2.3 Hz, 1H), 7.33 – 7.25 (m, 2H), 7.00 – 6.84 (m, 3H), 6.79 (dd, J = 8.1, 2.5 Hz, 1H), 6.72 – 6.65 (m, 2H), 5.75 – 5.70 (m, 1H), 5.23 (d, J = 4.9 Hz, 1H), 5.05 – 4.96 (m, 1H), 4.66 (s, 2H), 4.46 (s, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.39 – 3.32 (m, 4H), 3.20 – 3.12 (m, 1H), 2.82 – 2.69 (m, 3H), 2.62 – 2.49 (m, 2H), 2.37 – 2.00 (m, 5H), 1.78 – 1.30 (m, 11H), 1.24 – 1.08 (m, 6H), 0.81 (dt, J = 32.9, 7.5 Hz, 3H). LCMS 968.55 (M+H).

Synthetic Example 62: Synthesis of dFKBP-5

A 0.1 M solution of N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (0.205 mL, 0.0205 mmol, 1 eq) was added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-(2-phenylacetyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (11.8 mg, 0.0205 mmol, 1 eq). DIPEA (10.7 microliters, 0.0615 mmol, 3 eq) was added, followed by HATU (7.8 mg, 0.0205 mmol, 1 eq). The mixture was stirred for 29 hours, then diluted with MeOH and purified by preparative HPLC to give a white solid (10.62 mg, 0.01106 mmol, 54%).

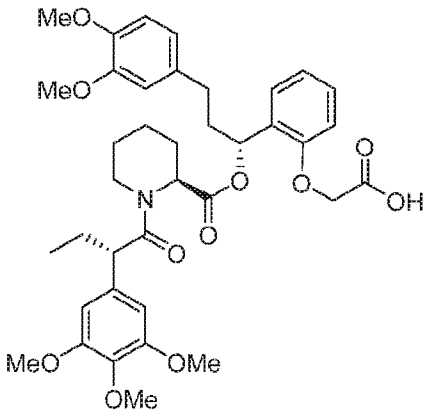
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 – 7.72 (m, 1H), 7.52 (s, 1H), 7.31 – 7.11 (m, 7H), 6.92 – 6.77 (m, 4H), 6.68 – 6.62 (m, 2H), 5.70 – 5.64 (m, 1H), 5.38 (d, J = 3.8 Hz, 1H), 4.99 (d, J = 4.6 Hz, 1H), 4.65 (s, 2H), 4.45 – 4.39 (m, 2H), 3.80 (dd, J = 6.7, 2.4 Hz, 8H), 3.13 – 3.03 (m, 1H), 2.83 – 2.68 (m, 3H), 2.63 – 2.45 (m, 3H), 2.34 – 1.93 (m, 6H), 1.71 – 1.52 (m, 7H), 1.34 – 1.20 (m, 3H). LCMS 960.54 (M+H).

Synthetic Example 63: Synthesis of dFKBP-6

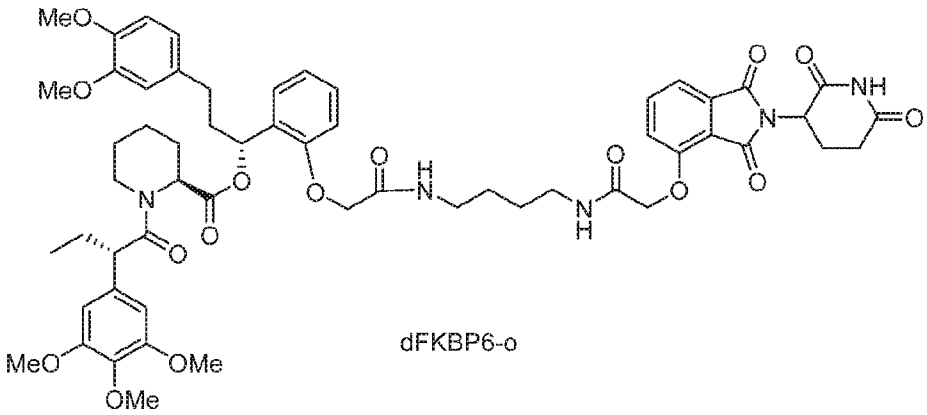


N-(4-aminobutyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate(11.9 mg, 0.0231 mmol, 1 eq) is added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-((*S*)-2-(3,4,5-trimethoxyphenyl)butanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (16.0 mg, 0.0231 mmol, 1 eq) as a solution in 0.231 mL DMF (0.1 M). DIPEA (12.1 microliters, 0.0692 mmol, 3 eq) and HATU (8.8 mg, 0.0231 mmol, 1 eq) are added and the mixture is stirred for 21 hours. The mixture is diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material is purified by column chromatography.

The above synthetic scheme can also be used to provide the analogous *ortho* or *para* bonding configuration in the dFKBP structures herein by choice of starting material, as illustrated below. Any of these positional isomers can be used in the present invention to degrade FKBP. For example:

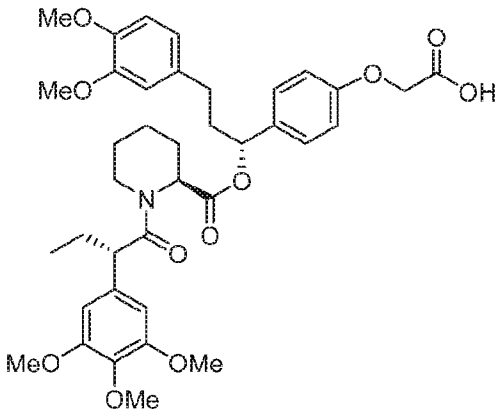


will produce

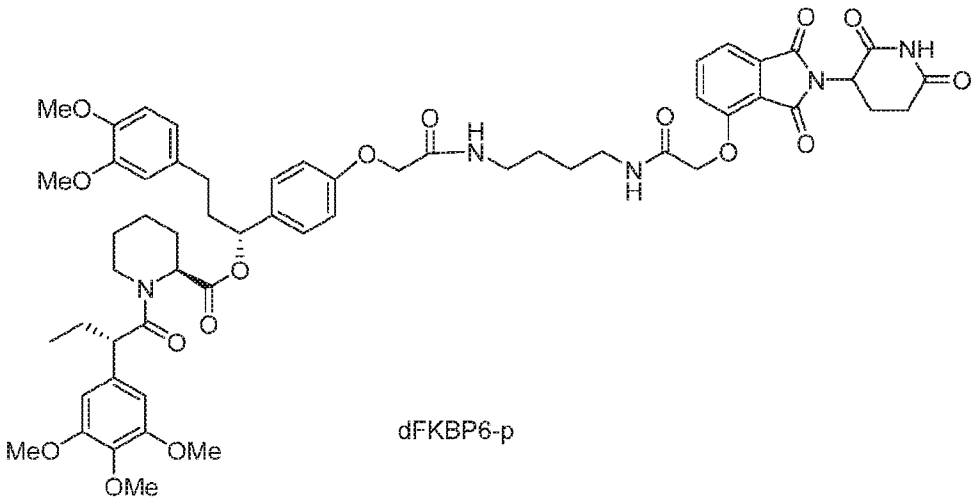


dFKBP6-o

Similarly use of:

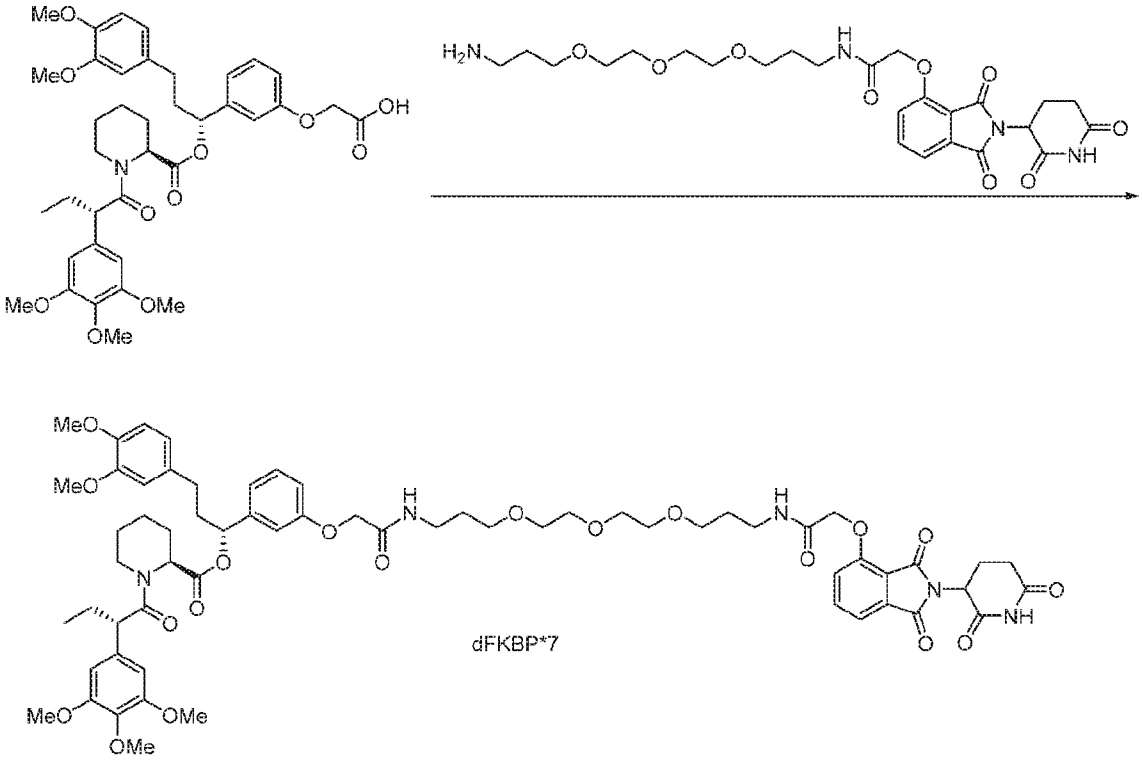


will produce



dFKBP6-p

Synthetic Example 64: Synthesis of dFKBP-7

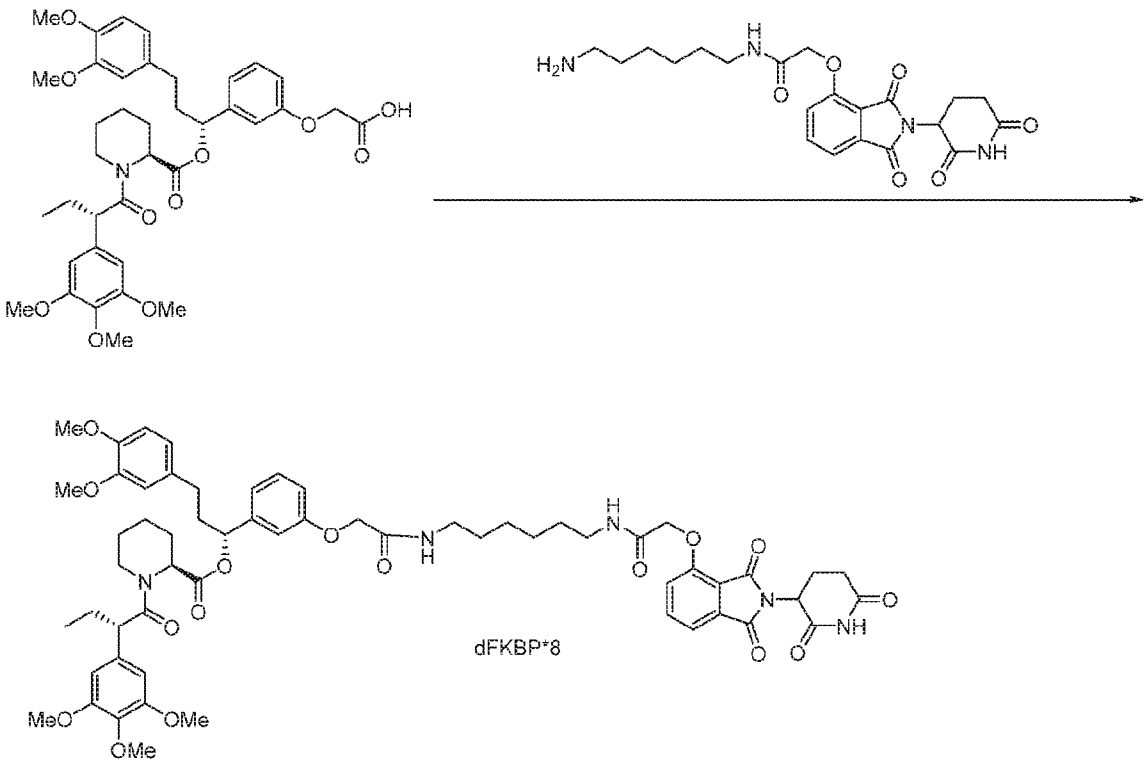


dFKBP*7

N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoracetate (12.3 mg, 0.0189 mmol, 1 eq) is added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-((*S*)-2-(3,4,5-trimethoxyphenyl)butanoyl) piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (13.1 mg, 0.0189 mmol, 1 eq) as a solution in 0.189 mL

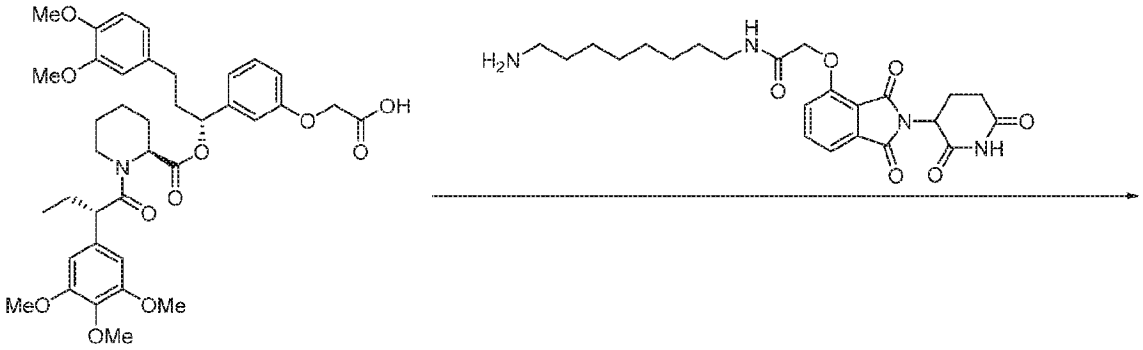
DMF (0.1 M). DIPEA (9.9 microliters, 0.0566 mmol, 3 eq) and HATU (7.2 mg, 0.0189 mmol, 1 eq) are added and the mixture is stirred for 17 hours. The mixture is diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material is purified by column chromatography.

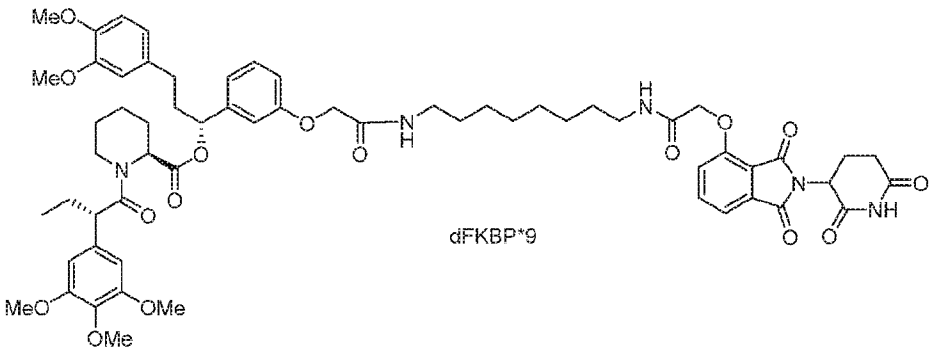
Synthetic Example 65: Synthesis of dFKBP-8



N-(6-aminohexyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoracetate (12.7 mg, 0.0233 mmol, 1.3 eq) is added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-((*S*)-2-(3,4,5-trimethoxyphenyl)butanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (12.4 mg, 0.0179 mmol, 1 eq) as a solution in 0.233 mL DMF (0.1 M). DIPEA (9.3 microliters, 0.0537 mmol, 3 eq) and HATU (6.8 mg, 0.0179 mmol, 1 eq) are added and the mixture is stirred for 22 hours. The mixture is diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material is purified by column chromatography.

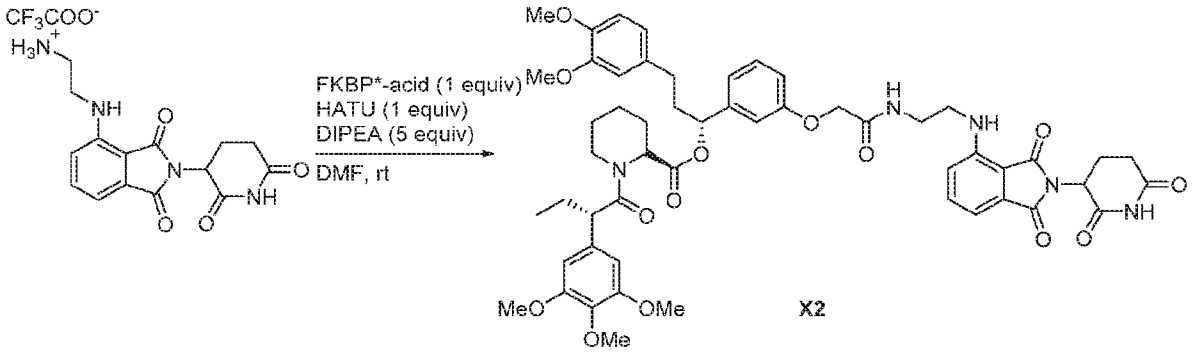
Synthetic Example 66: Synthesis of dFKBP-9





N-(8-aminooctyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (10.4 mg, 0.0181 mmol, 1 eq) is added to 2-(3-((*R*)-3-(3,4-dimethoxyphenyl)-1-(((*S*)-1-((*S*)-2-(3,4,5-trimethoxyphenyl)butanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid (12.5 mg, 0.0181 mmol, 1 eq) as a solution in 0.181 mL DMF (0.1 M). DIPEA (9.5 microliters, 0.0543 mmol, 3 eq) and HATU (6.9 mg, 0.0181 mmol, 1 eq) are added and the mixture is stirred for 22 hours. The mixture is diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material is purified by column chromatography.

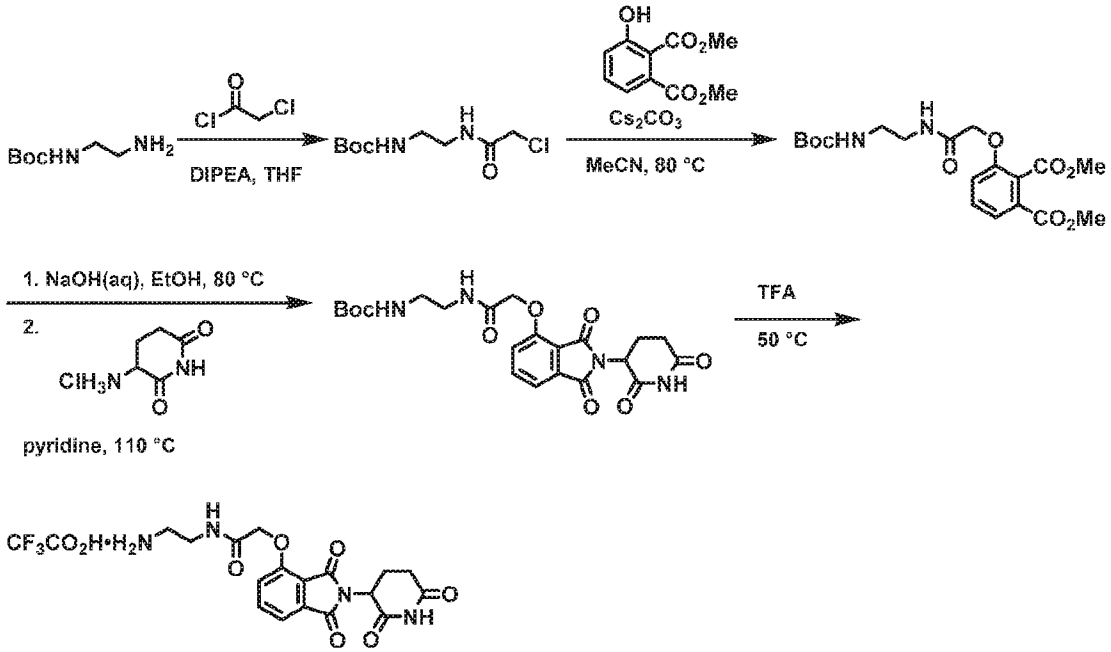
Synthetic Example 67: Synthesis of dFKBP



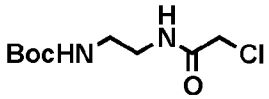
X2

FKBP*-acid (14.0 mg, 0.0202 mmol, 1 eq) and 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethan-1-aminium 2,2,2-trifluoroacetate (8.7 mg, 0.0202 mmol, 1 equiv) are dissolved in DMF (0.202 mL, 0.1 M) at room temperature. DIPEA (17.6 □L, 0.101 mmol, 5 equiv) and HATU (7.6 mg, 0.0200 mmol, 1 equiv) are then added and the mixture is stirred at room temperature overnight. The reaction mixture is taken up in EtOAc (15 mL), and washed with satd. $NaHCO_3$ (aq) (15 mL), water (15 mL) and brine (3x15 mL). The organic layer is dried over Na_2SO_4 and concentrated *in vacuo*. The crude material is purified by column chromatography.

Synthetic Example 68: Synthesis of diaminoethyl-acetyl-O-thalidomide trifluoroacetate

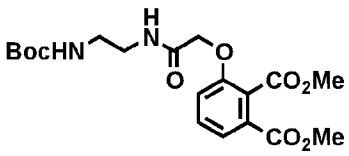


(1) Synthesis of *tert*-Butyl (2-(2-chloroacetamido)ethyl)carbamate



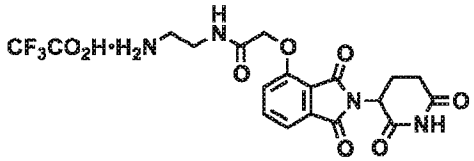
tert-butyl (2-aminoethyl)carbamate (0.40 mL, 2.5 mmol, 1 eq) was dissolved in THF (25 mL, 0.1 M) and DIPEA (0.44 mL, 2.5 mmol, 1 eq) at 0 °C. Chloroacetyl chloride (0.21 mL, 2.75 mmol, 1.1 eq) was added and the mixture was allowed to warm to room temperature. After 22 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure to give a white solid (0.66 g, quantitative yield) that carried forward to the next step without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.16 (s, 1H), 4.83 (s, 1H), 4.04 (s, 2H), 3.42 (q, *J* = 5.4 Hz, 2H), 3.32 (q, *J* = 5.6 Hz, 2H), 1.45 (s, 9H). LCMS 237.30 (M+H).

(2) Synthesis of dimethyl 3-(2-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phthalate



tert-butyl (2-(2-chloroacetamido)ethyl)carbamate (0.66 g, 1 eq) was dissolved in MeCN (17 mL, 0.15 M). Dimethyl 3-hydroxyphthalate (0.578 g, 2.75 mmol, 1.1 eq) and cesium carbonate (2.24 g, 6.88 mmol, 2.75 eq) were then added. The flask was fitted with a reflux condenser and heated to 80 °C for 32 hours. The mixture was then cooled to room temperature, diluted with EtOAc and washed three times with water. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4g silica column, 0-15% MeOH/DCM over a 15 minute gradient) gave a yellow solid (0.394 g, 0.960 mmol, 38% over 2 steps). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 – 7.56 (m, 1H), 7.50 – 7.41 (m, 1H), 7.27 (s, 1H), 7.11 (dd, *J* = 8.4, 4.1 Hz, 2H), 5.17 (s, 1H), 4.57 (d, *J* = 6.3 Hz, 2H), 3.94 (s, 2H), 3.88 (s, 2H), 3.40 (p, *J* = 5.8 Hz, 4H), 3.32 – 3.19 (m, 4H), 1.39 (d, *J* = 5.7 Hz, 13H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 168.37, 168.23, 165.73, 156.13, 154.71, 131.24, 130.09, 124.85, 123.49, 117.24, 79.42, 68.48, 53.22, 52.83, 40.43, 39.54, 28.44. LCMS 411.45 (M+H).

(3) Synthesis of diaminoethyl-acetyl-O-thalidomide trifluoroacetate

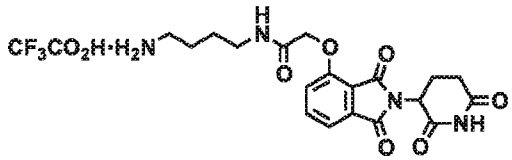


Dimethyl 3-(2-((2-((*tert*-butoxycarbonyl)amino)ethyl)amino)-2-oxoethoxy)phthalate (0.39 g, 0.970 mmol, 1 eq) was dissolved in EtOH (9.7 mL, 0.1 M). Aqueous 3M NaOH (0.97 mL, 2.91 mmol, 3 eq) was added and the mixture was heated to 80 °C for 3 hours. The mixture was cooled to room temperature, diluted with 50 mL DCM, 5 mL 1 M HCl and 20 mL water. The layers were separated and the organic layer was washed with 20 mL water. The combined aqueous layers were then extracted 3 times with 50 mL chloroform. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to give a yellow solid (0.226 g) that was carried forward without further purification. LCMS 383.36.

The resultant yellow solid (0.226 g) and 3-aminopiperidine-2,6-dione hydrochloride (0.102 g, 0.6197 mmol, 1 eq) were dissolved in pyridine (6.2 mL, 0.1 M) and heated to 110 °C for 16 hours. The mixture was cooled to room temperature and concentrated under reduced pressure to give *tert*-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethyl)carbamate as a poorly soluble black tar (0.663 g) which was carried forward without purification (due to poor solubility). LCMS 475.42 (M+H).

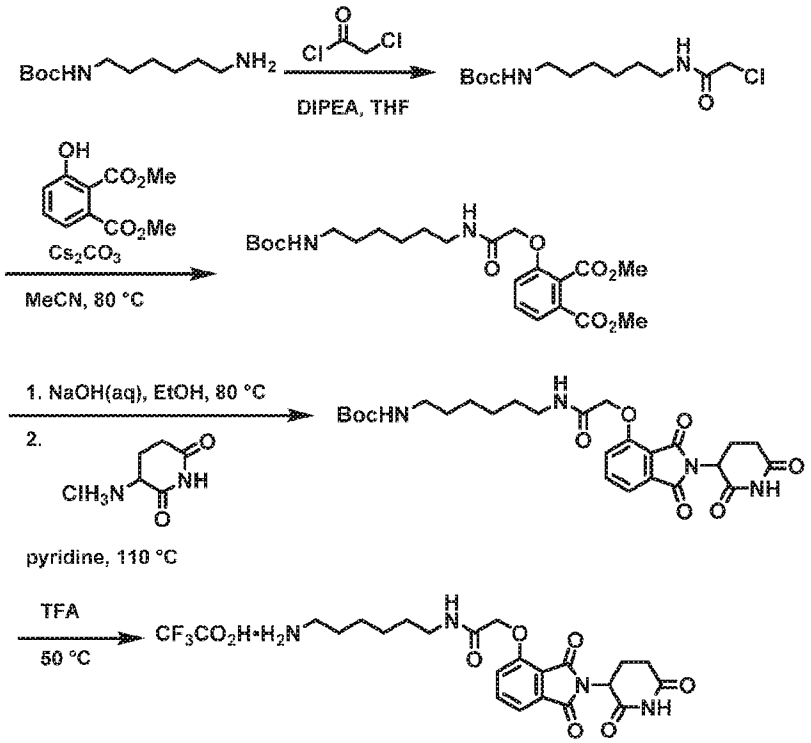
The crude *tert*-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)ethyl)carbamate was dissolved in TFA (10 mL) and heated to 50 °C for 3.5 hours, then concentrated under reduced pressure. Purification by preparative HPLC gave a red oil (176.7 mg, 0.362 mmol, 37% over 3 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.85 – 7.76 (m, 1H), 7.57 – 7.50 (m, 1H), 7.48 – 7.41 (m, 1H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.81 (s, 2H), 3.62 (td, J = 5.6, 1.8 Hz, 2H), 3.14 (t, J = 5.8 Hz, 2H), 2.97 (s, 1H), 2.80 – 2.66 (m, 2H), 2.15 (dddd, J = 10.1, 8.0, 5.8, 2.8 Hz, 1H). ^{13}C NMR (100 MHz, cd$_3$od) δ 173.09, 170.00, 169.99, 166.78, 166.62, 154.93, 136.88, 133.46, 120.71, 117.93, 116.77, 68.29, 49.17, 39.37, 38.60, 30.73, 22.19. LCMS 375.30 (M+H for free base).

Synthetic Example 69: Synthesis of diaminobutyl-acetyl-O-thalidomide trifluoroacetate

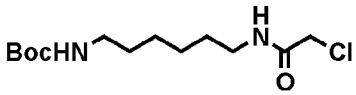


Diaminobutyl-acetyl-O-thalidomide trifluoroacetate was prepared according to the procedure in Fischer *et al. Nature*, **2014**, *512*, 49–53.

Synthetic Example 70: Synthesis of diaminohexyl-acetyl-O-thalidomide trifluoroacetate

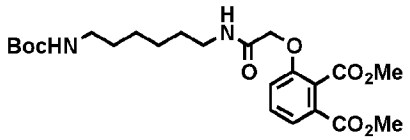


(1) Synthesis of *tert*-butyl (6-(2-chloroacetamido)hexyl)carbamate



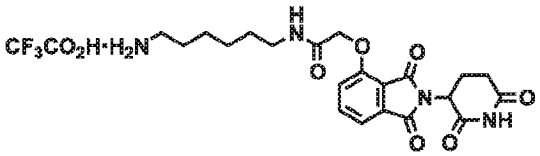
tert-butyl (6-aminohexyl)carbamate (0.224 mL, 1.0 mmol, 1 eq) was dissolved in THF (10 mL, 0.1 M). DIPEA (0.17 mL, 1.0 mmol, 1 eq) was added and the mixture was cooled to 0 °C. Chloroacetyl chloride (88 microliters, 1.1 mmol, 1.1 eq) was added and the mixture was warmed to room temperature and stirred for 18 hours. The mixture was then diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give a white solid (0.2691 g, 0.919 mmol, 92%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.60 (s, 1H), 4.51 (s, 1H), 4.05 (s, 2H), 3.30 (q, *J* = 6.9 Hz, 2H), 3.11 (d, *J* = 6.7 Hz, 2H), 1.57 – 1.46 (m, 4H), 1.44 (s, 9H), 1.38 – 1.32 (m, 4H). LCMS 293.39 (M+H).

(2) Synthesis of dimethyl 3-(2-((6-((*tert*-butoxycarbonyl)amino)hexyl)amino)-2-oxoethoxy)phthalate



tert-butyl (6-(2-chloroacetamido)hexyl)carbamate (0.2691 g, 0.919 mmol, 1 eq) was dissolved in MeCN (9.2 mL, 0.1 M). Dimethyl 3-hydroxyphthalate (0.212 g, 1.01 mmol, 1.1 eq) and cesium carbonate (0.823 g, 2.53 mmol, 2.75 eq) were added. The flask was fitted with a reflux condenser and heated to 80 °C for 14 hours. The mixture was cooled to room temperature and diluted with EtOAc, washed three times with water and back extracted once with EtOAc. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography (ISCO, 12 g silica column, 0-15% MeOH/DCM 15 minute gradient) to give a yellow oil (0.304 g, 0.651 mmol, 71%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 – 7.58 (m, 1H), 7.44 (td, *J* = 8.2, 1.6 Hz, 1H), 7.15 – 7.08 (m, 1H), 6.96 (s, 1H), 4.56 (s, 2H), 3.92 (t, *J* = 1.6 Hz, 3H), 3.88 (t, *J* = 1.6 Hz, 3H), 3.27 (q, *J* = 6.9 Hz, 2H), 3.10 – 3.00 (m, 2H), 1.41 (s, 13H), 1.33 – 1.22 (m, 4H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 167.97, 167.37, 165.58, 155.95, 154.37, 130.97, 129.74, 124.94, 123.26, 116.81, 78.96, 68.04, 52.89, 52.87, 52.69, 52.67, 40.41, 38.96, 29.88, 29.13, 28.39, 26.33, 26.30. LCMS 467.49.

(3) Synthesis of diaminohexyl-acetyl-O-thalidomide trifluoroacetate

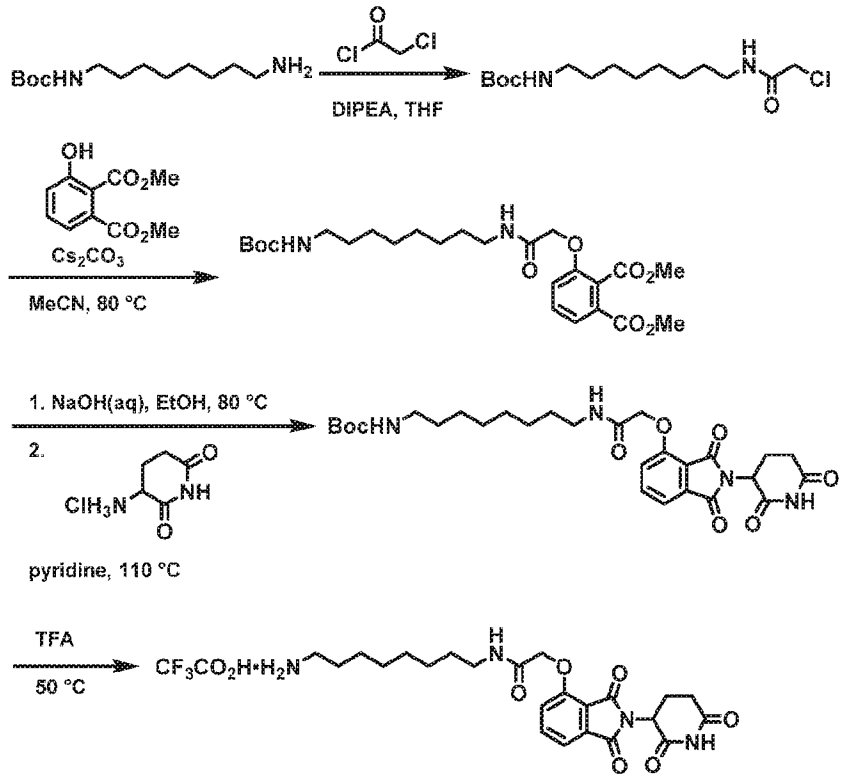


Dimethyl 3-(2-((6-((*tert*-butoxycarbonyl)amino)hexyl)amino)-2-oxoethoxy)phthalate (0.304 g, 0.651 mmol, 1 eq) was dissolved in EtOH (6.5 mL, 0.1 M). Aqueous 3M NaOH (0.65 mL, 1.953 mmol, 3 eq) was added and the mixture was heated to 80 °C for 18 hours. The mixture was cooled to room temperature and diluted with 50 mL DCM and 10 mL 0.5 M HCl. The layers were separated and the organic layer was washed with 20 mL water. The combined aqueous layers were then extracted 3 times with chloroform. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to give a yellow foam (0.290 g) that was carried forward without further purification. LCMS 439.47.

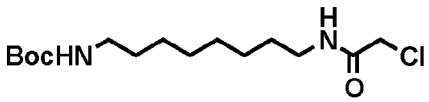
The resultant yellow solid (0.290 g) and 3-aminopiperidine-2,6-dione hydrochloride (0.113 g, 0.69 mmol, 1 eq) were dissolved in pyridine (6.9 mL, 0.1 M) and heated to 110 °C for 17 hours. The mixture was cooled to room temperature and concentrated under reduced pressure to give *tert*-butyl (6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexyl)carbamate as a black solid (0.4216 g) which was carried forward without purification (due to poor solubility). LCMS 531.41 (M+H).

The crude *tert*-butyl (6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)hexyl)carbamate (0.4216 g) was dissolved in TFA (10 mL) and heated to 50 °C for 2 hours. The mixture was concentrated under reduced pressure, then concentrated under reduced pressure. Purification by preparative HPLC gave a brown solid (379.2 mg). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (dd, J = 8.4, 7.4 Hz, 1H), 7.52 (d, J = 7.2 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 5.13 (dd, J = 12.6, 5.5 Hz, 1H), 4.75 (s, 2H), 3.32 (t, J = 7.6 Hz, 2H), 2.96 – 2.89 (m, 2H), 2.89 – 2.65 (m, 3H), 2.16 (ddt, J = 10.4, 5.4, 2.9 Hz, 1H), 1.63 (dp, J = 20.6, 7.1 Hz, 4H), 1.51 – 1.34 (m, 4H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.57, 171.42, 169.90, 168.24, 167.79, 156.23, 138.23, 134.87, 121.69, 119.22, 117.98, 69.36, 50.53, 40.64, 39.91, 32.14, 30.01, 28.44, 27.23, 26.96, 23.63. LCMS 431.37 (M+H).

Synthetic Example 71: Synthesis of diaminooctyl-acetyl-O-thalidomide trifluoroacetate



(1) Synthesis of *tert*-Butyl (8-(2-chloroacetamido)octyl)carbamate

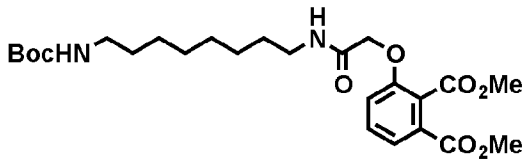


Octane-1,8-diamine (1.65 g, 11.45 mmol, 5 eq) was dissolved in chloroform (50 mL). A solution of di-tert-butyl dicarbonate (0.54 g, 2.291 mmol, 1 eq) in chloroform (10 mL) was added slowly at room temperature and stirred for 16 hours before being concentrated under reduced pressure. The solid material was resuspended in a mixture of DCM, MeOH, EtOAc and 0.5 N NH_3 (MeOH), filtered through celite and concentrated under reduced pressure. Purification by column chromatography (ISCO, 12 g NH2-silica column, 0-15% MeOH/DCM over a 15 minute gradient) gave a mixture (1.75 g) of the desired product and starting material which was carried forward without further purification.

This mixture was dissolved in THF (72 mL) and DIPEA (1.25 mL, 7.16 mmol) and cooled to 0 °C. Chloroacetyl chloride (0.63 mL, 7.88 mmol) was added and the mixture was allowed to warm to room temperature. After 16 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water and brine. The resultant mixture was purified by column

chromatography (ISCO, dry load onto silica, 24 g column, 0-100% EtOAc/hexanes, over a 21 minute gradient) to give a white solid (0.56 g, 1.745 mmol, 76% over 2 steps). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.55 (s, 1H), 4.48 (s, 1H), 4.05 (s, 2H), 3.30 (q, *J* = 6.9 Hz, 2H), 3.10 (d, *J* = 6.2 Hz, 2H), 1.44 (s, 12H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 165.86, 156.14, 77.36, 42.86, 40.73, 40.00, 30.18, 29.44, 29.26, 28.59, 26.86, 26.82. LCMS 321.34 (M+H).

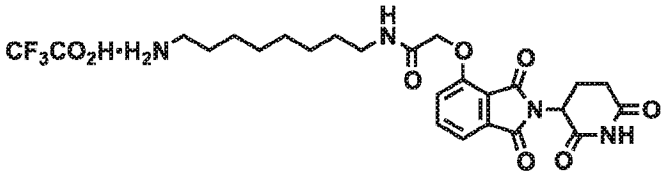
(2) Synthesis of dimethyl 3-(2-((8-((*tert*-butoxycarbonyl)amino)octyl)amino)-2-oxoethoxy)phthalate



tert-butyl (8-(2-chloroacetamido)octyl)carbamate (0.468 g, 1.46 mmol, 1 eq) was dissolved in MeCN (15 mL, 0.1 M). Dimethyl 3-hydroxyphthalate (0.337 g, 1.60 mmol, 1.1 eq) and cesium carbonate (1.308 g, 4.02 mmol, 2.75 eq) were added. The flask was fitted with a reflux condenser and heated to 80 °C for 18 hours. The mixture was cooled to room temperature and diluted water and extracted once with chloroform and twice with EtOAc. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure.

The crude material was purified by column chromatography (ISCO, 24 g silica column, 0-15% MeOH/DCM 20 minute gradient) to give a yellow oil (0.434 g, 0.878 mmol, 60%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 (dd, *J* = 7.9, 0.8 Hz, 1H), 7.40 (t, *J* = 8.1 Hz, 1H), 7.07 (dd, *J* = 8.4, 0.7 Hz, 1H), 6.89 (t, *J* = 5.3 Hz, 1H), 4.63 (s, 1H), 4.52 (s, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 3.22 (q, *J* = 6.9 Hz, 2H), 3.01 (q, *J* = 6.4 Hz, 2H), 1.36 (s, 12H), 1.20 (s, 9H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 167.89, 167.29, 165.54, 155.97, 154.38, 130.95, 129.69, 124.96, 123.23, 116.86, 78.82, 68.05, 52.83, 52.82, 52.66, 52.64, 40.54, 39.06, 29.97, 29.19, 29.10, 29.06, 28.40, 26.66, 26.61. LCMS 495.42 (M+H).

(3) Synthesis of diaminooctyl-acetyl-O-thalidomide trifluoroacetate



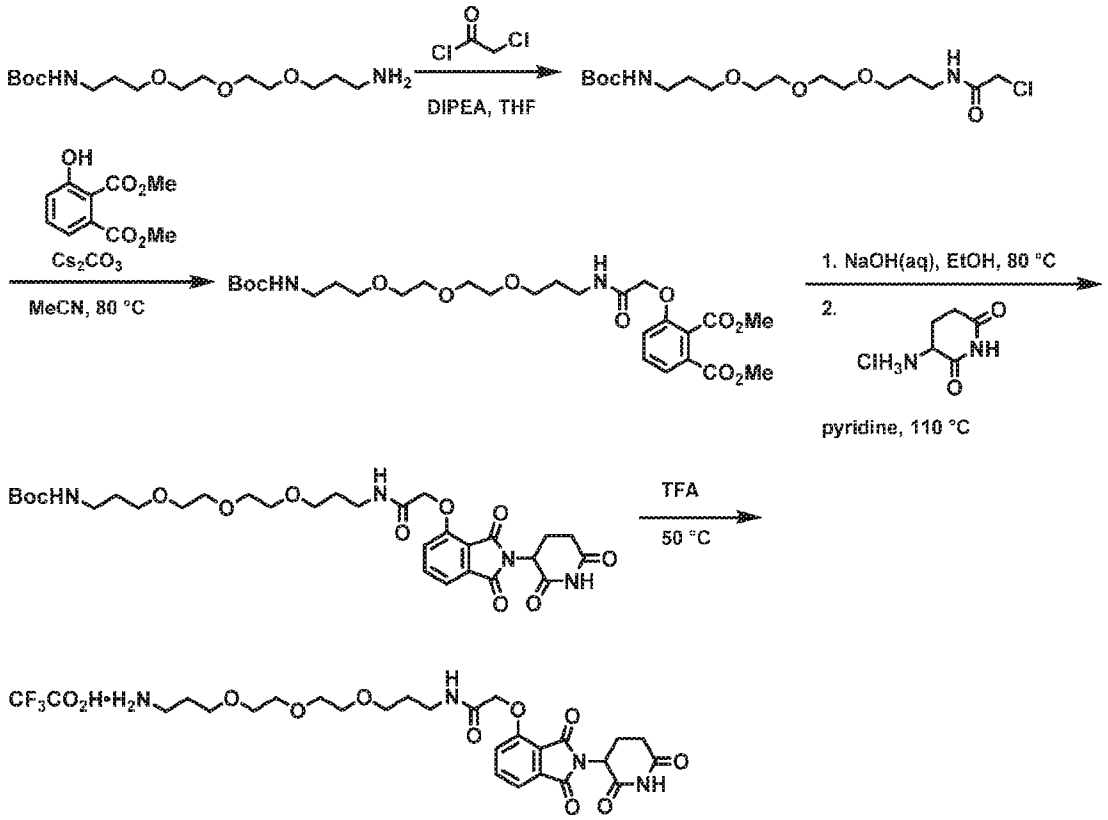
Dimethyl 3-(2-((8-((*tert*-butoxycarbonyl)amino)octyl)amino)-2-oxoethoxy)phthalate (0.434 g, 0.878 mmol, 1 eq) was dissolved in EtOH (8.8 mL, 0.1 M) Aqueous 3M NaOH (0.88

mL, 2.63 mmol, 3 eq) was added and the mixture was heated to 80 °C for 24 hours. The mixture was cooled to room temperature and diluted with 50 mL DCM and 10 mL 0.5 M HCl. The layers were separated and the organic layer was washed with 20 mL water. The combined aqueous layers were then extracted 3 times with chloroform. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to give a yellow solid (0.329 g) that was carried forward without further purification. LCMS 467.41.

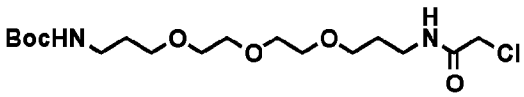
The resultant yellow solid (0.329 g) and 3-aminopiperidine-2,6-dione hydrochloride (0.121 g, 0.734 mmol, 1 eq) were dissolved in pyridine (7.3 mL, 0.1 M) and heated to 110 °C for 20 hours. The mixture was cooled to room temperature and concentrated under reduced pressure to give *tert*-butyl (8-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido) octyl) carbamate as a black tar (0.293 g) which was carried forward without purification (due to poor solubility). LCMS 559.45 (M+H).

The crude *tert*-butyl (8-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)octyl)carbamate (0.293 g) was dissolved in TFA (10 mL) and heated to 50 °C for 4 hours. The mixture was concentrated under reduced pressure, then concentrated under reduced pressure. Purification by preparative HPLC gave a brown residue (114.69 mg, 23% over 3 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.78 (m, 1H), 7.54 (d, J = 7.3 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 5.13 (dd, J = 12.5, 5.5 Hz, 1H), 4.76 (s, 2H), 3.32 (d, J = 4.1 Hz, 1H), 3.30 (d, J = 3.3 Hz, 1H), 2.94 – 2.84 (m, 3H), 2.80 – 2.70 (m, 2H), 2.19 – 2.12 (m, 1H), 1.67 – 1.55 (m, 4H), 1.40 – 1.34 (m, 8H). ^{13}C NMR (100 MHz, cd$_3$od) δ 174.57, 171.37, 169.85, 168.26, 167.78, 156.26, 138.22, 134.91, 121.70, 119.28, 117.97, 69.37, 50.57, 40.76, 40.08, 32.17, 30.19, 30.05, 30.01, 28.52, 27.68, 27.33, 23.63. LCMS 459.41 (M+H).

Synthetic Example 72: Synthesis of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate



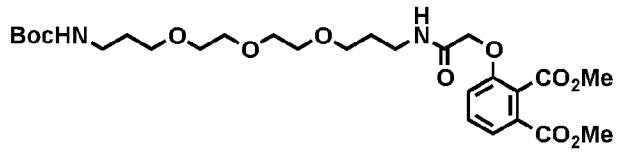
(1) Synthesis of *tert*-butyl (1-chloro-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate



tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (1.0 g, 3.12 mmol, 1 eq) was dissolved in THF (31 mL, 0.1 M). DIPEA (0.543 mL, 3.12 mmol, 1 eq) was added and the solution was cooled to 0 °C. Chloroacetyl chloride (0.273 mL, 3.43 mmol, 1.1 eq) was added and the mixture was warmed slowly to room temperature. After 24 hours, the mixture was diluted with EtOAc and washed with saturated sodium bicarbonate, water then brine. The organic layer was dried over sodium sulfate, filtered and condensed to give a yellow oil (1.416 g) that was carried forward without further purification. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 (s, 1H), 5.00 (s, 1H), 3.98 – 3.89 (m, 2H), 3.54 (dddt, *J* = 17.0, 11.2, 5.9, 2.2 Hz, 10H), 3.47 – 3.40 (m, 2H), 3.37 – 3.31 (m, 2H), 3.17 – 3.07 (m, 2H), 1.79 – 1.70 (m, 2H), 1.67 (p, *J* = 6.1 Hz, 2H), 1.35 (s, 9H).

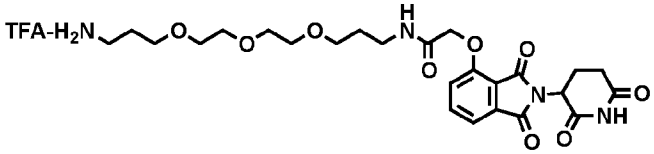
^{13}C NMR (100 MHz, cdcl$_3$) δ 165.83, 155.97, 78.75, 70.49, 70.47, 70.38, 70.30, 70.14, 69.48, 42.61, 38.62, 38.44, 29.62, 28.59, 28.40. LCMS 397.37 (M+H).

(2) Synthesis of dimethyl 3-((2,2-dimethyl-4,20-dioxo-3,9,12,15-tetraoxa-5,19-diazahenicosan-21-yl)oxy)phthalate



tert-butyl (1-chloro-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (1.41 g, 3.12 mmol, 1 eq) was dissolved in MeCN (32 mL, 0.1 M). Dimethyl 3-hydroxyphthalate (0.721 g, 3.43 mmol, 1.1 eq) and cesium carbonate (2.80 g, 8.58 mmol, 2.75 eq) were added. The flask was fitted with a reflux condenser and heated to 80 °C for 19 hours. The mixture was cooled to room temperature and diluted water and extracted once with chloroform and twice with EtOAc. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography (ISCO, 24 g silica column, 0-15% MeOH/DCM 22 minute gradient) to give a yellow oil (1.5892 g, 2.78 mmol, 89% over two steps). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.35 (t, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 7.00 (t, *J* = 5.3 Hz, 1H), 5.06 (s, 1H), 4.46 (s, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.47 (ddd, *J* = 14.9, 5.5, 2.8 Hz, 8H), 3.39 (dt, *J* = 9.4, 6.0 Hz, 4H), 3.29 (q, *J* = 6.5 Hz, 2H), 3.09 (d, *J* = 6.0 Hz, 2H), 1.70 (p, *J* = 6.5 Hz, 2H), 1.63 (p, *J* = 6.3 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 167.68, 167.36, 165.45, 155.93, 154.41, 130.87, 129.60, 125.01, 123.20, 117.06, 78.60, 70.40, 70.17, 70.06, 69.39, 68.67, 68.25, 52.77, 52.57, 38.38, 36.58, 29.55, 29.20, 28.34. LCMS 571.47 (M+H).

(3) Synthesis of *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate



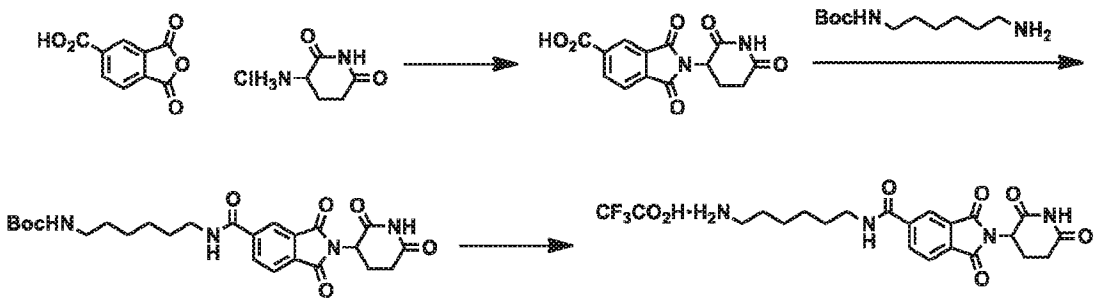
dimethyl 3-((2,2-dimethyl-4,20-dioxo-3,9,12,15-tetraoxa-5,19-diazahenicosan-21-yl)oxy)phthalate (1.589 g, 2.78 mmol, 1 eq) was dissolved in EtOH (14 mL, 0.2 M). Aqueous 3M NaOH (2.8 mL, 8.34 mmol, 3 eq) was added and the mixture was heated to 80 °C for 22 hours. The mixture was then cooled to room temperature, diluted with 50 mL DCM and 20 mL 0.5 M

HCl. The layers were separated and the organic layer was washed with 25 mL water. The aqueous layers were combined and extracted three times with 50 mL chloroform. The combined organic layers were dried over sodium sulfate, filtered and condensed to give 1.53 g of material that was carried forward without further purification. LCMS 553.44.

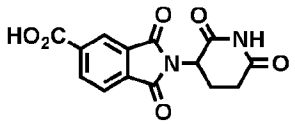
The resultant material (1.53 g) and 3-aminopiperidine-2,6-dione hydrochloride (0.480 g, 2.92 mmol, 1 eq) were dissolved in pyridine (11.7 mL, 0.25 M) and heated to 110 °C for 17 hours. The mixture was cooled to room temperature and concentrated under reduced pressure to give crude *tert*-butyl (1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate as a black sludge (3.1491 g) that was carried forward without further purification. LCMS 635.47.

The crude *tert*-butyl (1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (3.15 g) was dissolved in TFA (20 mL) and heated to 50 °C for 2.5 hours. The mixture was cooled to room temperature, diluted with MeOH and concentrated under reduced pressure. The material was purified by preparative HPLC to give *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide trifluoroacetate (1.2438 g, 1.9598 mmol, 71% over 3 steps) as a dark red oil. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 (dd, J = 8.3, 7.5 Hz, 1H), 7.49 (d, J = 7.3 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 5.12 (dd, J = 12.8, 5.5 Hz, 1H), 4.75 (s, 2H), 3.68 – 3.51 (m, 12H), 3.40 (t, J = 6.8 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.94 – 2.68 (m, 3H), 2.16 (dtd, J = 12.6, 5.4, 2.5 Hz, 1H), 1.92 (p, J = 6.1 Hz, 2H), 1.86 – 1.77 (m, 2H). ^{13}C NMR (100 MHz, cd$_3$od) δ 173.17, 169.97, 168.48, 166.87, 166.30, 154.82, 136.89, 133.41, 120.29, 117.67, 116.58, 69.96, 69.68, 69.60, 68.87, 68.12, 67.92, 49.19, 38.62, 36.14, 30.80, 28.92, 26.63, 22.22. LCMS 536.41 (M+H).

Synthetic Example 73: Synthesis of *N*-(6-aminohexyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamide

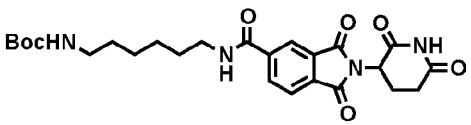


(1) Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxylic acid



1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylic acid (0.192 g, 1 mmol, 1 eq) and 3-aminopiperidine-2,6-dione hydrochloride (0.165 g, 1 mmol, 1 eq) were dissolved in DMF (2.5 mL) and acetic acid (5 mL) and heated to 80 °C for 24 hours. The mixture was then concentrated under reduced pressure and diluted with EtOH, from which a precipitate slowly formed. The precipitate was washed twice with EtOH to give a white solid (84.8 mg, 0.28 mmol, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.74 (s, 1H), 11.12 (s, 1H), 8.39 (dd, J = 7.8, 1.4 Hz, 1H), 8.26 (s, 1H), 8.04 (d, J = 7.8 Hz, 1H), 5.18 (dd, J = 12.8, 5.4 Hz, 1H), 2.93 – 2.88 (m, 1H), 2.84 (d, J = 4.7 Hz, 0H), 2.66 – 2.50 (m, 2H), 2.12 – 1.99 (m, 1H). LCMS 303.19 (M+H).

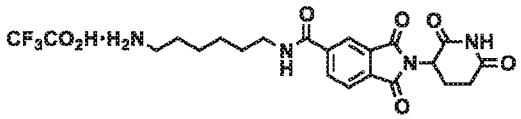
(2) Synthesis of *tert*-butyl (6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamido)hexyl)carbamate



2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxylic acid (22.7 mg, 0.0751 mmol, 1 eq) and HATU (31.4 mg, 0.0826 mmol, 1.1 eq) were dissolved in DMF (0.75 mL). After 5 minutes, DIPA (39.2 microliters, 0.225 mmol, 3 eq) was added. After an additional 5 minutes, *tert*-butyl (6-aminohexyl)carbamate (19.5 mg, 0.0901 mmol, 1.2 eq) was added as a solution in DMF (0.75 mL). The mixture was stirred for 20 hours, then diluted with EtOAc. The organic layer was washed three times with brine, dried over sodium sulfate and concentrated under reduced pressure. Purification by column chromatography (ISCO, 4 g column, 0-10%MeOH/DCM, 25

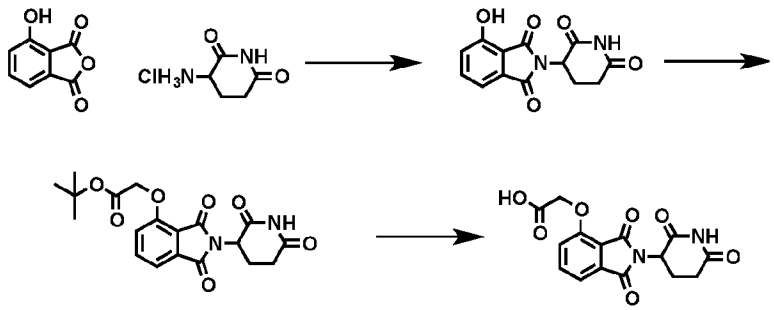
minute gradient) to give a yellow oil (17.18 mg, 0.03432 mmol, 46%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (d, *J* = 6.2 Hz, 2H), 8.16 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 6.91 (s, 1H), 5.00 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.58 (s, 1H), 3.47 (q, *J* = 6.7 Hz, 2H), 3.14 (q, *J* = 8.5, 7.3 Hz, 2H), 2.97 – 2.69 (m, 3H), 2.17 (ddd, *J* = 10.4, 4.8, 2.6 Hz, 1H), 1.65 (p, *J* = 6.9 Hz, 2H), 1.53 – 1.32 (m, 15H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 174.69, 170.77, 167.86, 166.67, 165.27, 156.49, 141.06, 133.95, 133.71, 132.13, 124.21, 122.27, 77.36, 49.71, 39.75, 31.54, 30.27, 29.22, 28.57, 25.70, 25.37, 22.73. LCMS 501.28 (M+H).

(3) Synthesis of *N*-(6-aminohexyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamide

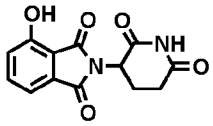


tert-butyl (6-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-5-carboxamido)hexyl)carbamate (17.18 mg, 0.343 mmol, 1 eq) was dissolved in TFA (1 mL) and heated to 50 °C for 2 hours. The mixture was concentrated under reduced pressure to give a yellow oil (13.29 mg) which was deemed sufficiently pure without further purification. ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ 8.27 (dd, *J* = 9.3, 1.3 Hz, 2H), 7.99 (d, *J* = 7.6 Hz, 1H), 5.18 (dd, *J* = 12.5, 5.4 Hz, 1H), 3.48 – 3.40 (m, 2H), 2.96 – 2.84 (m, 3H), 2.76 (ddd, *J* = 17.7, 8.1, 3.7 Hz, 2H), 2.20 – 2.12 (m, 1H), 1.75 – 1.63 (m, 4H), 1.53 – 1.43 (m, 4H). LCMS 401.31 (M+H).

Synthetic Example 74: Synthesis of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid

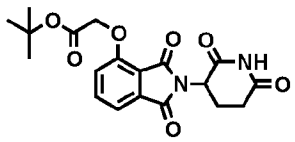


(1) Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione



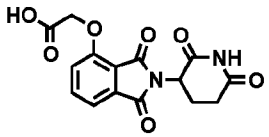
4-hydroxyisobenzofuran-1,3-dione (0.773 g, 4.71 mmol, 1 eq) and 3-aminopiperidine-2,6-dione hydrochloride (0.775 g, 4.71 mmol, 1 eq) were dissolved in pyridine (19 mL) and heated to 110 °C for 16 hours. The mixture was concentrated under reduced pressure and purified by column chromatography (ISCO, 12 g silica column, 0-10% MeOH/DCM, 25 minute gradient) to give an off white solid (1.14 g, 4.16 mmol, 88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 11.07 (s, 1H), 7.65 (dd, J = 8.3, 7.3 Hz, 1H), 7.31 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 5.07 (dd, J = 12.8, 5.4 Hz, 1H), 2.88 (ddd, J = 17.7, 14.2, 5.4 Hz, 1H), 2.63 – 2.50 (m, 2H), 2.11 – 1.95 (m, 1H). LCMS 275.11 (M+H).

(2) Synthesis of *tert*-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate



2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (218.8 mg, 0.798 mmol, 1 eq) was dissolved in DMF (8 mL). Potassium carbonate (165.9 mg, 1.20 mmol, 1.5 eq) was added, followed by tert-butyl bromoacetate (118 microliters, 0.798 mmol, 1 eq) and the mixture was stirred at room temperature for 3 hours. The mixture was diluted with EtOAc and washed once with water and twice with brine. Purification by column chromatography (ISCO, 12 g silica column, 0-100% EtOAc/hex, 17 minute gradient) gave a white solid (0.26 g, 0.669 mmol, 84%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.74 (s, 1H), 7.61 (dd, J = 8.4, 7.3 Hz, 1H), 7.46 – 7.41 (m, 1H), 7.06 (d, J = 8.3 Hz, 1H), 4.98 – 4.92 (m, 1H), 4.74 (s, 2H), 2.83 – 2.69 (m, 3H), 2.12 – 2.04 (m, 1H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 171.58, 168.37, 166.96, 166.87, 165.49, 155.45, 136.27, 133.89, 119.78, 117.55, 116.83, 83.05, 66.52, 49.20, 31.37, 28.03, 22.55. LCMS 411.23 (M+Na).

(3) Synthesis of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid



tert-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (47.5 mg, 0.122 mmol, 1 eq) was dissolved in TFA (1.3 mL) at room temperature. After 3 hours, the mixture was diluted with DCM and concentrated under reduced pressure to yield a white solid (42.27 mg), which was deemed sufficiently pure without further purification. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.76 (dd, J = 8.5, 7.3 Hz, 1H), 7.50 (d, J = 7.3 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 5.11 (dd, J = 12.5, 5.5 Hz, 1H), 4.96 (s, 2H), 2.87 (ddd, J = 17.8, 14.2, 5.0 Hz, 1H), 2.80 – 2.65 (m, 2H), 2.18 – 2.09 (m, 1H). LCMS 333.15 (M+H).

Heterobifunctional Compound Pharmaceutical Compositions

In another aspect of the present application, pharmaceutical compositions are provided, which comprise any one of the heterobifunctional compounds described herein (or a prodrug, pharmaceutically acceptable salt or other pharmaceutically acceptable derivative thereof), and optionally comprise a pharmaceutically acceptable carrier. According to the present application, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a pro-drug or other adduct or derivative of a compound of this application which upon administration to a patient in need is capable of providing, directly or indirectly, a heterobifunctional compound as otherwise described herein, or a metabolite or residue thereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts of amines, carboxylic acids, and other types of compounds, are well known in the art. For example, S. M. Berge, et al. describe pharmaceutically acceptable salts in detail in *J Pharmaceutical Sciences* 66 (1977):1-19, incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the heterobifunctional compounds of the application, or separately by reacting a free base or free acid function with a suitable reagent, as described generally below. For

example, a free base function can be reacted with a suitable acid. Furthermore, where the heterobifunctional compounds of the application carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may, include metal salts such as alkali metal salts, *e.g.* sodium or potassium salts; and alkaline earth metal salts, *e.g.* calcium or magnesium salts. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

Additionally, as used herein, the term "pharmaceutically acceptable ester" refers to esters that hydrolyze in vivo and include those that break down readily in the human body to leave the parent heterobifunctional compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

Furthermore, the term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the heterobifunctional compounds of the present application which are, within the scope of sound medical judgment, suitable for use in contact with the issues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a

reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the application. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formula, for example by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, *Pro-drugs as Novel Delivery Systems*, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., *Bioreversible Carriers in Drug Design*, American Pharmaceutical Association and Pergamon Press, (1987), both of which are incorporated herein by reference.

As described above, the pharmaceutical heterobifunctional compound compositions of the present application additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., (1980)) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the application, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this application. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil, sesame oil; olive oil; corn oil and soybean oil; glycols; such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other

biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this application with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner.

Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled

gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The active heterobifunctional compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active heterobifunctional compound may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The present application encompasses pharmaceutically acceptable topical formulations of inventive compounds. The term "pharmaceutically acceptable topical formulation", as used herein, means any formulation which is pharmaceutically acceptable for intradermal administration of a compound of the application by application of the formulation to the epidermis. In certain embodiments of the application, the topical formulation comprises a carrier system. Pharmaceutically effective carriers include, but are not limited to, solvents (*e.g.*, alcohols, poly alcohols, water), creams, lotions, ointments, oils, plasters, liposomes, powders, emulsions, microemulsions, and buffered solutions (*e.g.*, hypotonic or buffered saline) or any other carrier known in the art for topically administering pharmaceuticals. A more complete listing of art-known carriers is provided by reference texts that are standard in the art, for example, *Remington's Pharmaceutical Sciences*, 16th Edition, (1980) and 17th Edition, (1985), both published by Mack Publishing Company, Easton, Pa., the disclosures of which are incorporated herein by reference in their entireties. In certain other embodiments, the topical formulations of the application may comprise excipients. Any pharmaceutically acceptable excipient known in the art may be used to prepare the inventive pharmaceutically acceptable topical formulations. Examples of excipients that can be included in the topical formulations of the application include, but are not limited to, preservatives, antioxidants, moisturizers, emollients, buffering agents, solubilizing agents, other

penetration agents, skin protectants, surfactants, and propellants, and/or additional therapeutic agents used in combination to the inventive compound. Suitable preservatives include, but are not limited to, alcohols, quaternary amines, organic acids, parabens, and phenols. Suitable antioxidants include, but are not limited to, ascorbic acid and its esters, sodium bisulfite, butylated hydroxytoluene, butylated hydroxyanisole, tocopherols, and chelating agents like EDTA and citric acid. Suitable moisturizers include, but are not limited to, glycerine, sorbitol, polyethylene glycols, urea, and propylene glycol. Suitable buffering agents for use with the application include, but are not limited to, citric, hydrochloric, and lactic acid buffers. Suitable solubilizing agents include, but are not limited to, quaternary ammonium chlorides, cyclodextrins, benzyl benzoate, lecithin, and polysorbates. Suitable skin protectants that can be used in the topical formulations of the application include, but are not limited to, vitamin E oil, allatoin, dimethicone, glycerin, petrolatum, and zinc oxide.

In certain embodiments, the pharmaceutically acceptable topical formulations of the application comprise at least a compound of the application and a penetration enhancing agent. The choice of topical formulation will depend or several factors, including the condition to be treated, the physicochemical characteristics of the inventive compound and other excipients present, their stability in the formulation, available manufacturing equipment, and costs constraints. As used herein the term "penetration enhancing agent" means an agent capable of transporting a pharmacologically active compound through the stratum corneum and into the epidermis or dermis, preferably, with little or no systemic absorption. A wide variety of compounds have been evaluated as to their effectiveness in enhancing the rate of penetration of drugs through the skin. See, for example, Maibach H. I. and Smith H. E. (eds.), *Percutaneous Penetration Enhancers*, CRC Press, Inc., Boca Raton, Fla. (1995), which surveys the use and testing of various skin penetration enhancers, and Buyuktimkin et al., *Chemical Means of Transdermal Drug Permeation Enhancement in Transdermal and Topical Drug Delivery Systems*, Gosh T. K., Pfister W. R., Yum S. I. (eds.), Interpharm Press Inc., Buffalo Grove, Ill. (1997). In certain exemplary embodiments, penetration agents for use with the application include, but are not limited to, triglycerides (*e.g.*, soybean oil), aloe compositions (*e.g.*, aloe-vera gel), ethyl alcohol, isopropyl alcohol, octolyphenylpolyethylene glycol, oleic acid, polyethylene glycol 400, propylene glycol, N-decylmethylsulfoxide, fatty acid esters (*e.g.*, isopropyl myristate, methyl laurate, glycerol monooleate, and propylene glycol monooleate), and N-methylpyrrolidone.

In certain embodiments, the compositions may be in the form of ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. In certain exemplary embodiments, formulations of the compositions according to the application are creams, which may further contain saturated or unsaturated fatty acids such as stearic acid, palmitic acid, oleic acid, palmito-oleic acid, cetyl or oleyl alcohols, and stearic acid are useful. Creams of the application may also contain a non-ionic surfactant, for example, polyoxy-40-stearate. In certain embodiments, the active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this application. Additionally, the present application contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are made by dissolving or dispensing the compound in the proper medium. As discussed above, penetration enhancing agents can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

It will also be appreciated that certain heterobifunctional compounds of present application can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present application, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a prodrug or other adduct or derivative of a compound of this application which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

In one embodiment the heterobifunctional compound as any one of the pharmaceutical compositions described above, is administered to a host in need thereof to stop expression of a protein of interest by action on a synthetic endogenous protein-dTAG hybrid protein. Alternatively, the heterobifunctional compound as any one of the pharmaceutical compositions described above, is administered to a host in need thereof to start expression of a protein of interest by action on a synthetic endogenous protein-dTAG hybrid protein.

EXAMPLES

Examples are further provided of exemplary engineering of endogenous protein-dTAG hybrid proteins having a dTAG capable of being bound by or binding to a heterobifunctional compound, which, when exposed to the heterobifunctional compound is degraded by the ubiquitin proteasomal pathway (UPP). The examples are exemplary only and are not intended to be limited, instead serving as illustrations of a method of modulating the expression of a protein-of-interest through specific degradation of the target with a heterobifunctional compound targeting the endogenous protein-dTAG hybrid protein.

Example 1: Proprotein convertase subtilisin/kexin type 9 (PCSK9)-dTAG

To further describe the targeting of endogenous proteins of interest for degradation through the use of a dTAG as contemplated herein, the targeting of an exemplary protein of interest, the gene product of PCSK9, for insertion of a nucleic acid encoding a dTAG is illustrated.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme that controls cholesterol homeostasis. PCSK9 regulates the expression of low density lipoprotein (LDL) receptor in the liver. LDLR binds to, and internalizes free LDL cholesterol from the blood, effectively reducing cholesterol levels. When PCSK9 is deregulated, the enzyme binds and degrades LDLR, thus increasing free blood cholesterol resulting in hypercholesterolemia. Inhibition, or degradation of PCSK9 would restore LDLC expression and effectively reduce free blood cholesterol in the liver. Since increased levels of free LDL are associated with an increased risk of cardiac disease, efforts to reduce PCKS9 expression or activity are of great interest to the community.

To engineer the endogenous protein-dTAG hybrid protein, a homologous donor construct is cloned that includes a left homology region (portion of intron 1), dTAG nucleic acid sequence (derived from the dTAG FKBP* - SEQ. ID. NO.: 2) cloned in frame with exon 1 of PCSK9, and a right homology region (portion of intron 2). The dTAG peptide is cloned in frame with a 2X glycine linker. To initiate homologous recombination, a CRISPR sgRNA is designed to target the coding sequence PCSK9 in exon 1. CAS9 expression induces a double strand break which is repaired by homologous recombination repair using the donor construct as template. The end result is a gene locus with dTAG nucleic acid cloned in frame with exon 1 of PCSK9.

As derived, the resultant nucleic acid sequence including the in frame dTAG nucleic acid

insert results in the following genomic nucleic acid sequence, wherein lower case letters indicate intronic sequences of the PCSK9 genomic sequence, capital, underlined sequences indicate the sgRNA target (GAGGGAGATTTGACACACACAGG) (SEQ. ID. NO.: 45), *ATG* indicates the transcriptional start site of the PCSK9 protein or PCSK9-dTAG hybrid, capital letters indicate the exon coding sequence of the PCSK9 protein, and capital, italicized letters indicate the in frame insertion of the FKBP* derived dTAG nucleic acid with a 2X glycine linker (***GGGGGG***) (SEQ. ID. NO.: 46). An illustration representing the exemplified HR strategy is provided for in Figure 2.

Targeted PCSK9 Genomic Locus (SEQ. ID. NO.: 47)

gtgtggggctgcctccccgagcttccatctgccgctggggccacaccccaggcccagggatgggaccccacagtggtcacatcatcttgc
agcagaacccaggtacagctcctggagcagatggtggtcccaagcacgggtgggaccagaaaggactctcacctgggctaactcagctg
cagcctcagttccctcctcacacacgacgaggaacatggactggaagcctgcccagcaggccttctgctcgatgtgcgttgtgtggcttacg
tccagggagggaagcagcctctgtgctgtcttctagataagcctgtattccccgggctgtctgccaatgtatccagttgtcccgtcagcctgg
aagctctgagggaaaaccttgggctgcttcctgagcacctgtatcccctgcagccagcccggggcctctgctaggagcagactgagcatg
gcttatgggcctggcaccatctggcctctgcccaccttgctggccttgtcttgtgtctgccccttcgacattccatagcccagctcaatatctagt
ggttcctctagggtggcgagcactgtttggtctccagatgtcttcaggtcggagctcacagcgctctcagccacccctttcccagtgtagcacc
gggcacatggtagatgcctattgatgagtgaaagctcctaacacactcagagagcaaggactccgcctcatcccacagcctgggaggaga
ggcagactgccaaggacctgctcagcatgctacagaagaaaccaaagtgcccacgggactgatcagtggagcttcctgccgagactgga
ggccttagggcagggtagacagtgtgtgtgcaggctggggactcacagttcggactgtgcccagacctactagcatagtgggtgggtggg
aggatgcgggactgggggccgaccttgcctgaaattcatgtgggatctcagagcagccactgaattgctctgtagggggctaaatagtggc
ccccacagatacacacacccagacagagcctgtgagccagaccttatttggagaaaaggtctttgtagatgtaattaagcatctcaagatgg
catcatctggattatgcggtgggctgtaagtcctgtgatgtgtcttt*ATG*AGAGAAAGGCAGAGGGAGATTTGACA
CACACAGGAGGGGCCACGTGGAGACAGAGGTGGAGATTGGAGAAATGTGGCCACA
AGCCAGGGAACACCAGCAGCCACCAGAAGCCGGAAGACGTGAGGCAGGGTTCTTCC
CAGAGCCTTCGCTGCTGAGTCTGGGAATTTGTGACCGAAGCCATAAGAAGTGGGTA
CACGCCCTGAGCCTCCCACACTTGCTCACCTGTCCTGAGATGAGAATCTCTACTCTG
CAGCATATTTGGAGGATCACTGCGGGGGCCACAGAGGTGCTGTTCAGATGGCACTTC
AGAAGACTCAGGAGACCCTGGGGCAGGAGCAGTTTGACTGACAGCCCAGAGGGCTG
CCCTCTGATTCCACCTGAGGCCCTGCTTTTCCTGGCTGCAGGGGTTCCAGGGCCAGG
CCATTTCCGCTGGCGCAGGACTCTGCTAGCAGCAACCTGCCTGAAGTCTTCCTTTGG
CCTGGCTGAGAGTTTCTGAGACCTGCGCTGGAGCGGAGGTGCTTCCTTCCTTGCTTC
CTTTCTTCCTCTCTCCCTTCTCCATCCAGCAGGCTGGACCTGCCTGGCATCTGTGAGC
TCTCCCTACTTTCTCCTATACCCTAACCTTTGTCCTGCATGGGCGACTCCCCCAGTGA
GTCTCTTGCAGCTTTTACCCCAGTGCCTGCTTCTTGGAGAATCCAAACTGATCCAGTT
AGGGATGATAAAGTGTAGGGTAGGCGCTCGGTGACTGTTTTCTCTGAGGTTGTGACT
CGTGTGAGGCAGAAGCAGTCCCCGTGAGCCCTCCTGGTATCTTGTGGAGTGGAGAA

CGCTTGGACCTGGAGCCAGGAGGCCCAGACATACATCCTGTCCGAGCTGCAGCTTCC
TGTCTCTAAAATGAGCCGGCCAGCGCAGGTGGCCAGACATCACTGTTATTCTCCTTT
GAGTCTTTAAATCTTGTTGTCTTTCTTGCAGACTCGGTGAGCTGTGAAAGGCTATAAT
AGGGGCTTTATTTTACACTTTGATACTATTTTTTGAACATTCATATTATTGTTAGATA
TTGATATTCATATGAAGGAGCAGGATGACTTGGGTCCTTCTTGGCAGTAGCATTGCC
AGCTGATGGCCTTGGACAGTTACCTGCCCTCTCTAGGCCTCCCTTTCCTTGTCTATGA
AATACATTATAGAATAGGATGTAGTGTGTGAGGATTTTTTGGAGGTTAAACGAGTGA
ATATATTTAAGGCGCTTTCACCAGTGCCTGGGATGTGCTCTGTAGTTTCTGTGTGTTA
ACTATAAGGTTGACTTTATGCTCATTCCCTCCTCTCCCACAAATGtcgccttggaaagacggagg
cagcctggtggaggtgtatctcctagacaccagcatacagagtgaccaccgggaaatcgagggcagggtcatggtcaccgacttcgaga
atgtgcccgaggaggacgggacccgcttccacagacaggtaagcacggccgtctgatgggagggctgcctctgcccatatccccatcct
ggaggtgggtggggactgccaccccagagcgttgcagctgtactcctgggttgcacccccccccagctgtcactgtcccctccctgccatca
gttgtgggaagggcgttcatccatccagccacctgctgatttgttatagggtggaggggggggtctttctcatgtggtccttgtgttcgtcgagc
aggccagcaagtgtgacagtcatggcacccacctggcaggggtggtcagcggccgggatgccggcgtggccaagggtgccagcatgc
gcagcctgcgcgtgctcaactgccaagggaagggcacggttagcggcaccctcataggtaagtgatggccccagacgctggtctctctcc
atctggacctggcctgggaggtggcttgggctgggcccagggagagctaatgtctcctaaccaagaatgctgtggcagcctctgccgcag
agccagagaaccagagtgccaaggctggcagggttcccagtggccacgagtgcagatgaagaaacccaggccccaagagggtcatgc
aggtagcccagggagttcagccttgaccctgggtcaatgacctttccacagttccacactgctccccttttaaaatccggtgatgtctttatgtct
tttgttatgttatcttcaatgtggagggactcgaggtgatctaagcaaacttttctatcttctgcttgcatacctctgagaccaggggactcactc
acttgcatgactgggccctgcaggtcacactggccaggcagatgtggtggaggaactggcagaggacttttctagactgtgactacattta
gtccacccagcggccccctatgaagtccagttgagaactaggactctgggggccggtggacagagaagag.

Resultant PCSK9-dTAG Hybrid (SEQ. ID. NO.: 48)

gtgtggggctgcctccccgagcttccatctgccgctggggccacaccccaggcccagggatgggaccccacagtggtcacatcatcttgc
agcagaacccaggtacagctcctggagcagatggtggtcccaagcacgggtgggaccagaaaggactctcacctgggctaactcagctg
cagcctcagttccctcctcacacacgacgaggaacatggactggaagcctgcccagcaggccttctgctcgatgtgcgttgtgtggcttacg
tccagggagggaagcagcctctgtgctgtcttctagataagcctgtattccccgggctgtctgccaatgtatccagttgtcccgtcagcctgg
aagctctgagggaaaaccttgggctgcttcctgagcacctgtatcccctgcagccagcccggggcctctgctaggagcagactgagcatg
gcttatgggcctggcaccatctggcctctgcccaccttgctggccttgtcttgtgtctgccccttcgacattccatagcccagctcaatatctagt
ggttcctctagggtggcgagcactgtttggtctccagatgtcttcaggtcggagctcacagcgctctcagccacccctttcccagtgtagcacc
gggcacatggtagatgcctattgatgagtgaaagctcctaacacactcagagagcaaggactccgcctcatcccacagcctgggaggaga
ggcagactgccaaggacctgctcagcatgctacagaagaaaccaaagtgcccacgggactgatcagtggagcttcctgccgagactgga
ggccttagggcagggtagacagtgtgtgtgcaggctggggactcacagttcggactgtgcccagacctactagcatagtgggtgggtggg
aggatgcgggactgggggccgaccttgcctgaaattcatgtgggatctcagagcagccactgaattgctctgtagggggctaaatagtggc
ccccacagatacacacacccagacagagcctgtgagccagaccttatttggagaaaaggtctttgtagatgtaattaagcatctcaagatgg
catcatctggattatgcggtgggctgtaagtcctgtgatgtgtctttATG*GGAGTGCAGGTGGAAACCATCTCCCCA*
GGAGACGGGCGCACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCACTACACCGGGA
TGCTTGAAGATGGAAAGAAAGTTGATTCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTAT
GCTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTG
GGTCAGAGAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCA

*GGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTG**GGGGGG***
AGAGAAAGGCAGAGGGAGATTTGACACACACAGGAGGGGCCACGTGGAGACAGAG
GTGGAGATTGGAGAAATGTGGCCACAAGCCAGGGAACACCAGCAGCCACCAGAAG
CCGGAAGACGTGAGGCAGGGTTCTTCCCAGAGCCTTCGCTGCTGAGTCTGGGAATTT
GTGACCGAAGCCATAAGAAGTGGGTACACGCCCTGAGCCTCCCACACTTGCTCACCT
GTCCTGAGATGAGAATCTCTACTCTGCAGCATATTTGGAGGATCACTGCGGGGGCCA
CAGAGGTGCTGTTCAGATGGCACTTCAGAAGACTCAGGAGACCCTGGGGCAGGAGC
AGTTTGACTGACAGCCCAGAGGGCTGCCCTCTGATTCCACCTGAGGCCCTGCTTTTC
CTGGCTGCAGGGGTTCCAGGGCCAGGCCATTTCCGCTGGCGCAGGACTCTGCTAGCA
GCAACCTGCCTGAAGTCTTCCTTTGGCCTGGCTGAGAGTTTCTGAGACCTGCGCTGG
AGCGGAGGTGCTTCCTTCCTTGCTTCCTTTCTTCCTCTCTCCCTTCTCCATCCAGCAG
GCTGGACCTGCCTGGCATCTGTGAGCTCTCCCTACTTTCTCCTATACCCTAACCTTTG
TCCTGCATGGGCGACTCCCCCAGTGAGTCTCTTGCAGCTTTTACCCCAGTGCCTGCTT
CTTGGAGAATCCAAACTGATCCAGTTAGGGATGATAAAGTGTAGGGTAGGCGCTCG
GTGACTGTTTTCTCTGAGGTTGTGACTCGTGTGAGGCAGAAGCAGTCCCCGTGAGCC
CTCCTGGTATCTTGTGGAGTGGAGAACGCTTGGACCTGGAGCCAGGAGGCCCAGAC
ATACATCCTGTCCGAGCTGCAGCTTCCTGTCTCTAAAATGAGCCGGCCAGCGCAGGT
GGCCAGACATCACTGTTATTCTCCTTTGAGTCTTTAAATCTTGTTGTCTTTCTTGCAG
ACTCGGTGAGCTGTGAAAGGCTATAATAGGGGCTTTATTTTACACTTTGATACTATTT
TTTGAACATTCATATTATTGTTAGATATTGATATTCATATGAAGGAGCAGGATGACT
TGGGTCCTTCTTGGCAGTAGCATTGCCAGCTGATGGCCTTGGACAGTTACCTGCCCT
CTCTAGGCCTCCCTTTCCTTGTCTATGAAATACATTATAGAATAGGATGTAGTGTGTG
AGGATTTTTTGGAGGTTAAACGAGTGAATATATTTAAGGCGCTTTCACCAGTGCCTG
GGATGTGCTCTGTAGTTTCTGTGTGTTAACTATAAGGTTGACTTTATGCTCATTCCCT
CCTCTCCCACAAATGtcgccttggaaagacggaggcagcctggtggaggtgtatctcctagacaccagcatacagagtgac
caccgggaaatcgagggcagggtcatggtcaccgacttcgagaatgtgcccgaggaggacgggacccgcttccacagacaggtaagca
cggccgtctgatgggagggctgcctctgcccatatccccatcctggaggtgggtggggactgccaccccagagcgttgcagctgtactcct
gggttgcacccccccccagctgtcactgtcccctccctgccatcagttgtgggaagggcgttcatccatccagccacctgctgatttgttatag
ggtggaggggggggtctttctcatgtggtccttgtgttcgtcgagcaggccagcaagtgtgacagtcatggcacccacctggcaggggtggt
cagcggccgggatgccggcgtggccaagggtgccagcatgcgcagcctgcgcgtgctcaactgccaagggaagggcacggttagcgg
caccctcataggtaagtgatggccccagacgctggtctctctccatctggacctggcctgggaggtggcttgggctgggcccagggagag
ctaatgtctcctaaccaagaatgctgtggcagcctctgccgcagagccagagaaccagagtgccaaggctggcagggttcccagtggcca
cgagtgcagatgaagaaacccaggccccaagagggtcatgcaggtagcccagggagttcagccttgaccctgggtcaatgacctttccac
agttccacactgctccccttttaaaatccggtgatgtctttatgtcttttgttatgttatcttcaatgtggagggactcgaggtgatctaagcaaactt
tttctatcttctgcttgcatacctctgagaccaggggactcactcacttgcatgactgggccctgcaggtcacactggccaggcagatgtggtg
gaggaactggcagaggacttttttctagactgtgactacatttagtccacccagcggcccccctatgaagtccagttgagaactaggactctgg
gggccggtggacagagaagag.

Example 2: β-catenin (CTNNB1)-dTAG

To further describe the targeting of endogenous proteins of interest for degradation through the use of a dTAG as contemplated herein, the targeting of an exemplary protein of interest, β-catenin (CTNNB1), for dTAG insertion is illustrated.

β-catenin is encoded by the CTNNB1 gene. β-catenin regulates both cell-cell adhesion and gene transcription as a downstream effector of the WNT signaling pathway. Under normal conditions, β-catenin function and expression is mediated by phosphorylation and ubiquitin mediated destruction via the βTrCP E3 ligase. Normally, β-catenin is regulated upon binding to a repressive complex, which includes, axin, GSK3β, and APC. Upon WNT stimulation, axin is sequestered to frizzled receptors, thus releasing β-catenin from the destruction complex. The protein then translocates to the nucleus to bind TCF/LEF to activate transcriptional programs. Upon release of Wnt ligands, free beta-catenin is phosphorylated by GSK3β and degraded through binding and ubiquitination by βTrCRP E3 ligase.

The Wnt/β-catenin pathway is frequently mutated in human cancers, with β-catenin mutations being observed in nearly 25% of hepatocellular carcinoma. Recurrent mutations are found within the βTrCP binding site, conferring stability to the oncogenic transcriptional regulator. While a bonafide oncology target, historical small molecule programs have failed as β-catenin is a relatively flat protein with few known ligands that bind with high affinity. These data suggested β-catenin as an exemplary gene to target for conditional degradation.

To engineer the endogenous protein-dTAG hybrid protein, a homologous donor construct is cloned that includes a left homology region (portion of intron 1), dTAG nucleic acid sequence (derived from the dTAG FKBP* - SEQ. ID. NO.: 2) cloned in frame with a short exon 1 of CTNNB1, intron 2, exon2, and a right homology region (portion of intron 3). The dTAG nucleic acid sequence is cloned in frame with a 2X glycine linker.

To initiate homologous recombination, a CRISPR sgRNA is designed to target the coding sequence β-catenin in exon 2. CAS9 expression induces a double strand break which is repaired by homologous recombination repair using the donor construct as template. The end result is a gene locus with a dTAG nucleic acid sequence cloned in frame with exon 1 of CTNNB1.

As derived, the resultant nucleic acid sequence including the in frame dTAG nucleic acid insert results in the following genomic nucleic acid sequence, wherein lower case letters indicate intronic sequences of the CTNNB1 genomic sequence, capital, underlined sequences indicate the

sgRNA target (TACCACAGCTCCTTCTCTGAGTGG) (SEQ. ID. NO.: 49), *ATG* indicates the transcriptional start site of the CTNNB1 protein (βcatenin) or β-catenin (CTNBB1)-dTAG hybrid, capital letters indicate the exon coding sequence of the β-catenin protein, and capital, italicized letters indicate the in frame insertion of the FKBP* derived dTAG nucleic acid with a 2X glycine linker (***GGGGGG***) (SEQ. ID. NO.: 46). An illustration representing the exemplified HR strategy is provided for in Figure 3.

CTNNB1 Genomic Locus (SEQ. ID. NO.: 50)

aaataatttttgatggcactatatcagaaaacaacttgttaaagaaaatgtggagtttttaaaatcccactgtacctctgttatccaaaggggatct
gtgaatttttctgtgaaaggttaaaaaaggagagacctttaggaattcagagagcagctgatttttgaatagtgttttcccctccctggcttttatta
ttacaactctgtgctttttcatcaccatcctgaatatctataattaatatttatactattaataaaaagacatttttggtaaggaggagttttcactgaa
gttcagcagtgatggagctgtggttgaggtgtctggaggagaccatgaggtctgcgtttcactaacctggtaaaagaggatatgggtttttttt
gtgggtgtaatagtgacatttaacaggtatcccagtgacttaggagtattaatcaagctaaatttaaatcctaatgacttttgattaacttttttttagg
gtatttgaagtataccatacaactgttttgaaaatccagcgtggaca*ATG*GCTACTCAAGgtttgtgtcattaaatctttagttactga
attggggctctgcttcgttgccattaagccagtctggctgagatccccctgctttcctctctccctgcttacttgtcaggctacctttttgctccatttt
ctgctcactcctcctaatggcttggtgaaatagcaaacaagccaccagcaggaatctagtctggatgactgcttctggagcctggatgcagta
ccattcttccactgattcagtgagtaactgttaggtggttccctaagggattaggtatttcatcactgagctaaccctggctatcattctgcttttctt
ggctgtctttcagatttgactttatttctaaaaatatttcaatgggtcatatcacagattctttttttttaaattaaagtaacatttccaatctactaatgct
aatactgtttcgtatttatagCTGATTTGATGGAGTTGGACATGGCCATGGAACCAGACAGAAAAG
CGGCTGTTAGTCACTGGCAGCAACAGTCTTACCTGGACTCTGGAATCCATTCTGGTG
CCACTACCACAGCTCCTTCTCTGAGTGGTAAAGGCAATCCTGAGGAAGAGGATGTG
GATACCTCCCAAGTCCTGTATGAGTGGGAACAGGGATTTTCTCAGTCCTTCACTCAA
GAACAAGTAGCTGgtaagagtattatttttcattgccttactgaaagtcagaatgcagttttgagaactaaaaagttagtgtataata
gtttaaataaaatgttgtggtgaagaaaagagagtaatagcaatgtcacttttaccatttaggatagcaaatacttaggtaaatgctgaactgtg
gatagtgagtgttgaattaacctttccagATATTGATGGACAGTATGCAATGACTCGAGCTCAGAGGG
TACGAGCTGCTATGTTCCCTGAGACATTAGATGAGGGCATGCAGATCCCATCTACAC
AGTTTGATGCTGCTCATCCCACTAATGTCCAGCGTTTGGCTGAACCATCACAGATGC
TGAAACATGCAGTTGTAAACTTGATTAACTATCAAGATGATGCAGAACTTGCCACAC
GTGCAATCCCTGAACTGACA

Resultant CTNNB1-dTAG Hybrid (SEQ. ID. NO.: 51)

aaataatttttgatggcactatatcagaaaacaacttgttaaagaaaatgtggagtttttaaaatcccactgtacctctgttatccaaaggggatct
gtgaatttttctgtgaaaggttaaaaaaggagagacctttaggaattcagagagcagctgatttttgaatagtgttttcccctccctggcttttatta
ttacaactctgtgctttttcatcaccatcctgaatatctataattaatatttatactattaataaaaagacatttttggtaaggaggagttttcactgaa
gttcagcagtgatggagctgtggttgaggtgtctggaggagaccatgaggtctgcgtttcactaacctggtaaaagaggatatgggtttttttt
gtgggtgtaatagtgacatttaacaggtatcccagtgacttaggagtattaatcaagctaaatttaaatcctaatgacttttgattaacttttttttagg
gtatttgaagtataccatacaactgttttgaaaatccagcgtggaca*ATGGGAGTGCAGGTGGAAACCATCTCCCCA*
GGAGACGGGCGCACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCACTACACCGGGA

TGCTTGAAGATGGAAAGAAAGTTGATTCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTAT
GCTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTG
GGTCAGAGAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCA
*GGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACT**GGGGGGG***
ATGGCTACTCAAGgtttgtgtcattaaatctttagttactgaattggggctctgcttcgttgccattaagccagtctggctgagatccc
cctgctttcctctctccctgcttacttgtcaggctacctttgctccattttctgctcactcctcctaatggcttggtgaaatagcaaacaagccacc
agcaggaatctagtctggatgactgcttctggagcctggatgcagtaccattcttccactgattcagtgagtaactgttaggtggttccctaagg
gattaggtatttcatcactgagctaaccctggctatcattctgcttttcttggctgtctttcagatttgactttatttctaaaaatatttcaatgggtcat
atcacagattctttttttttaaattaaagtaacatttcaatctactaatgctaatactgtttcgtatttatagcCTGATTTGATGGAGT
TGGACATGGCCATGGAACCAGACAGAAAAGCGGCTGTTAGTCACTGGCAGCAACAG
TCTTACCTGGACTCTGGAATCCATTCTGGTGCCAC<u>TACCACAGCTCCTTCTCTGAGTG</u>
<u>G</u>TAAAGGCAATCCTGAGGAAGAGGATGTGGATACCTCCCAAGTCCTGTATGAGTGG
GAACAGGGATTTTCTCAGTCCTTCACTCAAGAACAAGTAGCTGgtaagagtattatttttcattgcctt
actgaaagtcagaatgcagttttgagaactaaaaagttagtgtataatagtttaaataaaatgttgtggtgaagaaaagagagtaatagcaatgt
cacttttaccatttaggatagcaaatacttaggtaaatgctgaactgtggatagtgagtgttgaattaacctttccagATATTGATGG
ACAGTATGCAATGACTCGAGCTCAGAGGGTACGAGCTGCTATGTTCCCTGAGACATT
AGATGAGGGCATGCAGATCCCATCTACACAGTTTGATGCTGCTCATCCCACTAATGT
CCAGCGTTTGGCTGAACCATCACAGATGCTGAAACATGCAGTTGTAAACTTGATTAA
CTATCAAGATGATGCAGAACTTGCCACACGTGCAATCCCTGAACTGACA

In any of the below examples either dFKBP13-o and dFKBP 13-p or dFKBP7-o and dFKBP7-p can be used.

Example 3:

Figure 4 illustrates an example to confirm selective degradation of FKBP*-fused proteins with dFKBP7.

The dTAG is predicated on the selectivity of FKBP* specific ligands over endogenous, wild type FKBP. In 293T cells expressing wild type FKBP12 or FKBP*, dFKBP7 induces targeted degradation only in FKBP* expressing cells. An immunoblot of cells treated with heterobifunctional compounds described in the present invention was performed. 293FT cells (CRBN-WT or CRBN-/-) expressing either HA-tagged FKBP12WT or FKBP* were treated with indicated concentrations of dFKBP7 for 4 hours. CRBN-dependent degradation of FKBP* and not FKBPWT confirms selective activity of dFKBP7 for mutant FKBP*.

Example 4:

Figures 5A-B illustrate an example of profiling of a panel of dFKBP heterobifunctional compounds to measure differential degradation activity.

In an effort to identify potent and selective dFKPB heterobifunctional compounds, high throughput measurements of targeted FKBP* degradation were measured by surrogate levels of luciferase. Here, FKBP* is exogenously expressed as a multicistronic transcript with two types of luciferase: nano luciferase (NLuc) and firefly luciferase (FLuc) that allow for cell normalized quantification of FKBP* protein levels. Degradation of FKBP* is measured as a signal ration (Nluc/Fluc) in wild type (Figure 4A) or CRBN -/- (Figure 4B) 293FT cells treated with indicated concentrations of dFKBPs for 4 hours. A decrease in the signal ratio indicates FKBP* (Nluc) degradation and molecules that effectively degrade FKBP* in a cereblon dependent manner are observed (ex. dFKBP7).

Example 5:

Figure 6 illustrates an example of selective degradation of FKBP*-fused proteins with dFKBP7 and dFKBP13, bifunctional molecules described in the present invention.

In 293T cells expressing wild type FKBP12 or FKBP*, treatment with dFKBP7 and dFKBP13 induces targeted degradation only in FKBP* expressing cells. Isogenic 293FT cells (CRBN-WT or CRBN-/-) were engineered to expressed either FKBP12WT or FKBP*. Cells were treated with 100nM of either dFKBP7 or dFKBP13 for 4 hours before lysates were prepared for western immunoblot analysis. CRBN-dependent degradation of FKBP* and not FKBP12WT or endogenous FKBP12 confirms selectivity of dFKBP7 and dFKBP13 for mutant FKBP*.

Example 6:

Figure 7 illustrates and example of dose-dependent degradation of HA-tagged FKBP12* with a bifunctional molecule dFKBP13.

In an effort to define the optimal concentration of dFKB13 heterobifunctional compound to induce degradation of FKBP*, degradation was measured upon treatment with increasing concentrations of dFKBP13. Isogenic 293FT cells (CRBN-WT or CRBN-/-) were engineered to expressed HA-tagged FKBP*. Cells were treated with the indicated dose of dFKBP13 for 4 hours

before lysates were prepared for western immunoblot analysis. These data confirm dose- and CRBN-dependent degradation of HA-tagged FKBP* by dFKBP13.

Example 7:

Figure 8 illustrates the kinetic control of dFKBP13-dependent degradation of HA-tagged FKBP*.

To evaluate the kinetic control of targeted degradation FKBP*, dFKBP13 was administered by increased duration. 293FT cells (CRBN-WT) were engineered to express HA-tagged FKBP*. Cells were treated with 100nM dFKBP13 for the indicated times. Cells were harvested and protein lysates immunoblotted to measure the kinetics of HA-tagged FKBP* degradation induced by dFKBP13.

Example 8:

Figure 9 illustrates and example to confirm CRBN- and proteasome-dependent degradation of FKBP* by the bifunctional molecule dFKBP13.

293FT cells (CRBN-WT) were engineered to express FKBP*. Cells were pretreated with 1uM Carfilzomib (proteasome inhibitor), 0.5uM MLN4924 (neddylation inhibitor), and 10uM Lenalidomide (CRBN binding ligand) for two hours prior to a 4 hour treatment with dFKBP13. Lysates were prepared and western immunoblot analysis performed. Degradation of HA-tagged FKBP* by dFKBP13 was rescued by the proteasome inhibitor Carfilzomib, establishing a requirement for proteasome function. Pre-treatment with the NAE1 inhibitor MLN4924 rescued HA-tagged FKBP* establishing dependence on CRL activity, as expected for cullin-based ubiquitin ligases that require neddylation for processive E3 ligase activity. Pre-treatment with excess Lenalidomide abolished dFKBP13-dependent FKBP* degradation, confirming the requirement of CRBN engagement for degradation.

Example 9:

Figures 10A-B confirms targeted degradation of proteins of interest when fused to dTAG.

To test the general utility of the dTAG technology across several protein types, the indicated proteins fused to the dTAG in MV4;11 leukemia cells were expressed. Upon treatment

with the indicated dFKBP bifunctional molecules (dFKBP7 and dFKBP13), targeted protein degradation was observed as measured by western blot. Cells were treated for 16 hours with indicated concentrations of FKBP* selective heterobifunctional compounds and degradation was observed with nanomolar concentrations.

Example 10:

Figure 11 illustrates an example confirming degradation of N-terminal dTAG-KRAS.

In N-terminal dTAG-KRAS, dFKBP7 treatment resulted in potent degradation as well as a downstream decrease in p-AKT signal suggesting the biological relevance of overexpressed endogenous protein–dTAG hybrid proteins. Cells were treated with 500nM dFKBP7 for the indicated time. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). Overexpression of dTAG KRAS resulted in the activation of the relevant downstream signaling pathways as an observed increase in p-AKT signal as measured by western blot.

Example 11:

Figure 12 illustrates the profiling of dFKBP heterobifunctional compounds to induce degradation of dTAG-KRAS.

In an effort to identify the best performing dFKBP molecule, dTAG-KRAS degradation was profiled across a series of dFKBP molecules. Western blotting of NIH3T3 cells expressing dTAG-KRASG12V were treated with 1 μM of the indicated dFKBP heterobifunctional compounds for 24 hours. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP9, dFKBP12, and dFKBP13 induce potent degradation of FKBP*-KRAS and inhibition of downstream signaling.

Example 12:

Figure 13 illustrates an example confirming targeted degradation of dTAG-KRAS with dFKBP13.

The dFKBP13 heterobifunctional compound potently degrades dTAG-KRAS at nanomolar concentrations. Western blotting of NIH3T3 cells expressing FKBP* fused to the N-terminus of KRAS treated with the indicated concentrations of dFKBP13 for 24 hours. Cells were harvested

and immunoblotted to measure degradation of FKBP*-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP13 induces potent degradation of FKBP*-KRAS and inhibits downstream signaling potently with an IC50 >100nM.

Example 13:

Figure 14 illustrates an example of the kinetic control of targeted degradation of dTAG-KRAS with dFKBP13.

To evaluate the kinetic control of targeted degradation of dTAG-KRAS, dFKBP13 was administered by increased duration. Western blotting of NIH3T3 cells expressing FKBP* fused to the N-terminus of KRAS treated with 1μM dFKBP13 for the indicated time. Cells were harvested and immunoblotted to measure degradation of FKBP*-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP13 induces potent degradation of FKBP*-KRAS and inhibition of downstream signaling as early as 1 hour post treatment.

Example 14:

Figure 15 illustrates and example to confirm CRBN- and proteasome-dependent degradation of dTAG-KRASG12V by the heterobifunctional compound dFKBP13.

NIH3T3 cells (CRBN-WT) were engineered to express dTAG-KRASG12V. Cells were pretreated with 1uM Carfilzomib (proteasome inhibitor), 0.5uM MLN4924 (neddylation inhibitor), and 10uM Lenalidomide (CRBN binding ligand) for two hours prior to a 4 hour treatment with dFKBP13. Lysates were prepared and western immunoblot analysis performed. Degradation of dTAG-KRASG12V by dFKBP13 was rescued by the proteasome inhibitor Carfilzomib, establishing a requirement for proteasome function. Pre-treatment with the NAE1 inhibitor MLN4924 rescued dTAG-KRASG12V expression establishing dependence on CRL activity, as expected for cullin-based ubiquitin ligases that require neddylation for processive E3 ligase activity. Pre-treatment with excess Lenalidomide abolished dFKBP13-dependent dTAG-KRASG12V degradation, confirming the requirement of CRBN engagement for degradation.

Example 15:

Figure 16 illustrates an example confirming targeted degradation of oncogenic dTAG-KRAS alleles with dFKBP13.

The dFKBP13 heterobifunctional compound potently degrades dTAG-KRAS mutant alleles. NIH3T3 cells were engineered to express KRAS alleles either WT or mutant forms of amino acid glycine 12 (G12C, G12D, and G12V). Western blotting of NIH3T3 cells expressing dTAG fused to the N-terminus of KRAS alleles were treated with 1uM of dFKBP13 for 24 hours. Cells were harvested and immunoblotted to measure degradation of dTAG-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP13 induces potent degradation of WT and mutant KRAS alleles and potently inhibits downstream signaling.

Example 16:

Figure 17 illustrates an example confirming targeted degradation of oncogenic dTAG-KRAS alleles with dFKBP13.

The dFKBP13 heterobifunctional compound potently degrades dTAG-KRAS mutant alleles. NIH3T3 cells were engineered to express either WT or mutant KRAS alleles (G13D, Q61L, and Q61R). Western blotting of NIH3T3 cells expressing dTAG fused to the N-terminus of KRAS alleles were treated with 1uM of dFKBP13 for 24 hours. Cells were harvested and immunoblotted to measure degradation of dTAG-KRAS and downstream surrogates of KRAS signaling (e.g. pMEK and pAKT). The data suggest that dFKBP13 induces potent degradation of WT and mutant KRAS alleles and potently inhibits downstream signaling.

Example 17:

Figures 18A-D illustrates an experiment performed to confirm phenotypical changes induced upon degradation of dTAG-KRAS.

Morphological changes were observed in NIH3T3 cells upon overexpression of dTAG-KRAS as shown by phase contrast imaging. Upon treatment with dFKBP13 for 24 hours, cells morphologically revert back to the wild type (DMSO control) state.

Example 18:

Figures 19A-D illustrates the phenotypic consequence of dTAG-KRAS degradation on the viability of NIH3T3 cells.

The ATPlite 1-step luminescence assay measures cell proliferation and cytotoxicity in cells based on the production of light caused by the reaction of ATP with added luciferase and D-luciferin. A decrease in signal indicates a reduction in cell number. To evaluate the effect of dFKBP13 on proliferation in NIH3T3 cells expressing dTAG-KRAS, viability was assessed by surrogate measurements of ATP levels. Cells were treated with the indicated concentrations of dFKBPs for 72 hours and cell viability was measured using an ATPlite assay.

Example 19

Figure 20 illustrates the phenotypic consequence of dTAG-KRAS degradation on the cell cycle profile of NIH3T3 cells.

NIH3T3 cells were engineered to express dTAG-KRASG12V. NIH3T3 cells expressing dTAG-KRASG12V were treated with dFKBP7 and dFKBP13 for 48 hours to induce targeted dTAG-KRASG12V degradation. Fixed cells were stained with propidium iodide and cell cycle analysis was performed. Treatment with both dFKBP7 and dFKBP13 resulted in diminished S-phase entry, in agreement with the biological role of endogenous KRASG12V in driving S-phase entry. These data are consistent with the observed effect on dTAG-KRASG12V degradation on cell viability.

Example 20: Delivery of CRISPR-CAS9 and homologous donor vectors to the liver

Targeted gene therapy can be accomplished using both viral and non-viral approaches such as adeno-associated or lentivirus, or lipid-based formulations. For example, a single bicistronic vector system is used to deliver sgRNA targeting either PCKS9 or CTNNB1 with CAS9 being expressed from a neighboring promoter. Both the CRISPR vector and donor homology plasmid are encapsulated in Poly (beta-amino esters) (PBAEs) cationic polymers that provide the added specificity of cancer cell targeting vs. normal hepatocytes. PBAE nanoparticles are also biodegradable, or degrade by hydrolysis, thus releasing plasmid DNAs in the cytoplasm of tumor cells upon internalization. PBAE-encapsulated plasmid DNAs will be delivery locally via intrahepatic artery administration and systemically via intravenous injection. Upon successful

recombination, and following administration of a heterobifunctional compound, local core biopsies would be taken to confirm degradation of either the PCKS9 gene product or the CTNNB1 gene product.

This specification has been described with reference to embodiments of the invention. However, one of ordinary skill in the art appreciates that various modifications and changes can be made without departing from the scope of the invention as set forth in the claims below. Accordingly, the specification is to be regarded in an illustrative rather than a restrictive sense, and all such modifications are intended to be included within the scope of invention.

CLAIMS

We Claim:

1. A transformed cell comprising:

a genomically integrated nucleic acid sequence encoding a heterobifunctional compound targeting protein capable of being bound by a heterobifunctional compound;

wherein the dTAG comprises an amino acid sequence derived from EGFR, BCR-ABL, ALK, JAK2, BRAF, Src, LRRK2, PDGFRα, or RET;

wherein the nucleic acid sequence encoding the dTAG is integrated genomically in-frame in a 5' or 3' orientation with a nucleic acid sequence of a gene encoding an endogenous protein;

wherein expression of the gene encoding an endogenous protein produces an endogenous protein-dTAG hybrid protein;

wherein the heterobifunctional compound is capable of binding to a) the endogenous protein-dTAG hybrid protein through the dTAG and b) a ubiquitin ligase in a manner that brings the endogenous protein-dTAG hybrid protein into proximity of the ubiquitin ligase;

wherein the endogenous protein-dTAG hybrid protein is ubiquitinated and then degraded by a proteasome.

2. The transformed cell of claim 1, wherein the cell is a human cell.
3. The transformed cell of claim 2, wherein the human cell is a liver cell.
4. The transformed cell of any one of claims 1 to 3, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence from a non-endogenous protein.
5. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence selected from SEQ. ID. NO.: 53-63.
6. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from EGFR.
7. The transformed cell of claim 6, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 53.
8. The transformed cell of claim 6, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 54.

9. The transformed cell of claim 6, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 55.
10. The transformed cell of claim 6, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 56.
11. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BCR-ABL.
12. The transformed cell of claim 11, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 57.
13. The transformed cell of claim 11, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 58.
14. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from ALK.
15. The transformed cell of claim 14, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 59.
16. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from JAK2.
17. The transformed cell of claim 16, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 60.
18. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BRAF.
19. The transformed cell of claim 18, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 61.
20. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from Src.
21. The transformed cell of claim 20, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 62.
22. The transformed cell of claim 20, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 63.
23. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from LKKR2.

24. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from PDGFRα.
25. The transformed cell of any one of claims 1 to 4, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from RET.
26. The transformed cell of any one of claims 1 to 25, wherein the nucleic acid sequence encoding the heterobifunctional compound targeting protein is inserted in frame with a gene encoding an endogenous protein associated with a disease that is a result of a gain of function mutation, amplification or increased expression, a monogenetic disease, a proteopathy, or a combination thereof.
27. The transformed cell of any one of claims 1 to 26, further comprising a nucleic acid sequence encoding a CRISPR RNA-guided endonuclease.
28. The transformed cell of claim 27, wherein the CRISPR RNA-guided endonuclease is selected from Cas1, Cas IB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cpf1.
29. The transformed cell of claim 28, wherein the nucleic acid encodes a Cas9 endonuclease comprised of an amino acid sequence of SEQ. ID. NO.: 52.
30. The transformed cell of any one of claims 1 to 29, wherein the heterobifunctional compound targeting protein does not substantially interfere with the function of the endogenously expressed protein.
31. A method of modulating gene expression in a subject comprising:

administering to the subject an effective amount of a heterobifunctional compound;

wherein the subject has one or more transformed cells that have been transformed with a nucleic acid sequence encoding a heterobifunctional compound targeting protein (dTAG);

wherein the dTAG comprises an amino acid sequence derived from EGFR, BCR-ABL, ALK, JAK2, BRAF, Src, LRRK2, PDGFRα, or RET;

wherein the nucleic acid sequence encoding the dTAG is integrated genomically in-frame in a 5' or 3' orientation with a nucleic acid sequence of an endogenous protein associated with a disease;

wherein the insertion of the nucleic acid sequence encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid protein upon expression;

wherein the heterobifunctional compound binds to a) the endogenous protein-dTAG hybrid protein through the dTAG and b) a ubiquitin ligase in a manner that brings the endogenous protein-dTAG hybrid protein into proximity of the ubiquitin ligase, wherein the endogenous protein-dTAG hybrid protein is ubiquitinated and then degraded by a proteasome.

32. The method of claim 31, wherein the cell is a human cell.
33. The method of claim 32, wherein the human cell is a liver cell.
34. The method of any one of claims 31 to 33, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence from a non-endogenous protein.
35. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence selected from SEQ. ID. NO.: 53-63.
36. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from EGFR.
37. The method claim 36, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 53.
38. The method of claim 36, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 54.
39. The method of claim 36, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 55.
40. The method of claim 36, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 56.
41. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BCR-ABL.
42. The method of claim 41, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 57.
43. The method of claim 41, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 58.
44. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from ALK.

45. The method of claim 44, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 59.
46. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from JAK2.
47. The method of claim 46, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 60.
48. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BRAF.
49. The method of claim 48, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 61.
50. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from Src.
51. The method of claim 50, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 62.
52. The method of claim 50, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 63.
53. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from LKKR2.
54. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from PDGFRα.
55. The method of any one of claims 31 to 34, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from RET.
56. The method of any one of claims 31 to 55, wherein the nucleic acid sequence encoding the heterobifunctional compound targeting protein is inserted in frame with a gene encoding an endogenous protein associated with a disease that is a result of a gain of function mutation, amplification or increased expression, a monogenetic disease, a proteopathy, or a combination thereof.
57. The method of any one of claims 31 to 56, further comprising a nucleic acid sequence encoding a CRISPR RNA-guided endonuclease.
58. The method of claim 57, wherein the CRISPR RNA-guided endonuclease is selected from Cas1, Cas IB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Csy1, Csy2, Csy3,

Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cpf1.

59. The method of claim 58, wherein the nucleic acid encodes a Cas9 endonuclease comprised of an amino acid sequence of SEQ. ID. NO.: 52.

60. The method of any one of claims 31 to 59, wherein the heterobifunctional compound targeting protein does not substantially interfere with the function of the endogenously expressed protein.

61. A method of reducing gene overexpression in a subject comprising:

administering to the subject an effective amount of a heterobifunctional compound;

wherein the subject has one or more transformed cells that have been transformed with a nucleic acid sequence encoding a heterobifunctional compound targeting protein (dTAG);

wherein the dTAG comprises an amino acid sequence derived from EGFR, BCR-ABL, ALK, JAK2, BRAF, Src, LRRK2, PDGFRα, or RET;

wherein the nucleic acid sequence encoding the dTAG is integrated genomically in-frame in a 5' or 3' orientation with a nucleic acid sequence of an endogenous protein associated with a disease due to overexpression of a protein;

wherein the insertion of the nucleic acid sequence encoding the dTAG into the genomic sequence results in an endogenous protein-dTAG hybrid protein upon expression;

wherein the heterobifunctional compound binds to a) the endogenous protein-dTAG hybrid protein through the dTAG and b) a ubiquitin ligase in a manner that brings the endogenous protein-dTAG hybrid protein into proximity of the ubiquitin ligase, wherein the endogenous protein-dTAG hybrid protein is ubiquitinated and then degraded by a proteasome.

62. The method of claim 61, wherein the cell is a human cell.

63. The method of claim 62, wherein the human cell is a liver cell.

64. The method of any one of claims 61 to 63, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence from a non-endogenous protein.

65. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein comprises an amino acid sequence selected from SEQ. ID. NO.: 53-63.

66. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from EGFR.
67. The method claim 66, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 53.
68. The method of claim 66, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 54.
69. The method of claim 66, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 55.
70. The method of claim 66, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 56.
71. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BCR-ABL.
72. The method of claim 71, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 57.
73. The method of claim 71, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 58.
74. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from ALK.
75. The method of claim 74, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 59.
76. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from JAK2.
77. The method of claim 76, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 60.
78. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from BRAF.
79. The method of claim 78, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 61.
80. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from Src.

81. The method of claim 80, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 62.
82. The method of claim 80, wherein the heterobifunctional compound targeting protein is an amino acid sequence of SEQ. ID. NO.: 63.
83. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from LKKR2.
84. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from PDGFRα.
85. The method of any one of claims 61 to 64, wherein the heterobifunctional compound targeting protein is an amino acid sequence derived from RET.
86. The method of any one of claims 61 to 85, wherein the nucleic acid sequence encoding the heterobifunctional compound targeting protein is inserted in frame with a gene encoding an endogenous protein associated with a disease that is a result of a gain of function mutation, amplification or increased expression, a monogenetic disease, a proteopathy, or a combination thereof.
87. The method of any one of claims 31 to 56, further comprising a nucleic acid sequence encoding a CRISPR RNA-guided endonuclease.
88. The method of claim 87, wherein the CRISPR RNA-guided endonuclease is selected from Cas1, Cas IB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cpf1.
89. The method of claim 88, wherein the nucleic acid encodes a Cas9 endonuclease comprised of an amino acid sequence of SEQ. ID. NO.: 52.
90. The method of any one of claims 61 to 89, wherein the heterobifunctional compound targeting protein does not substantially interfere with the function of the endogenously expressed protein.

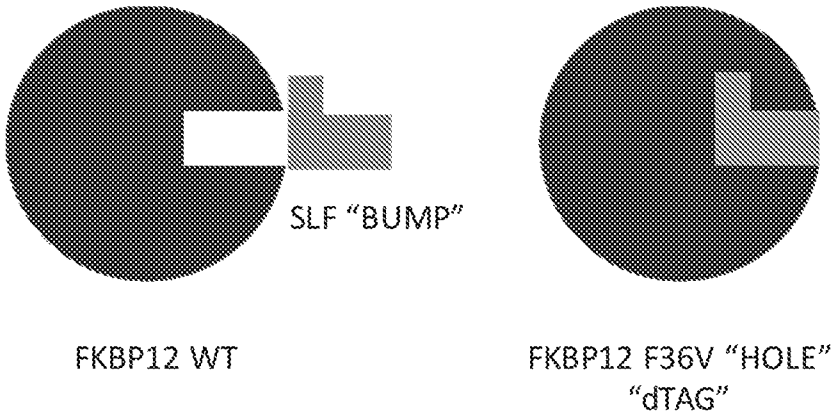


FIG. 1

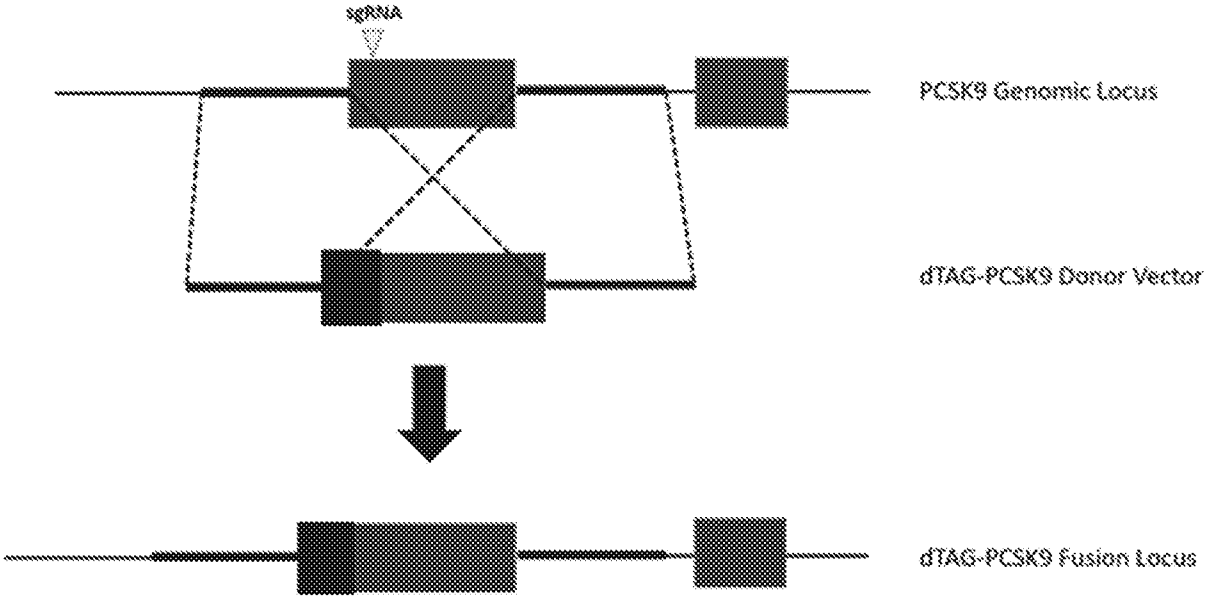

sgRNA
PCSK9 Genomic Locus
dTAG-PCSK9 Donor Vector
dTAG-PCSK9 Fusion Locus

FIG. 2

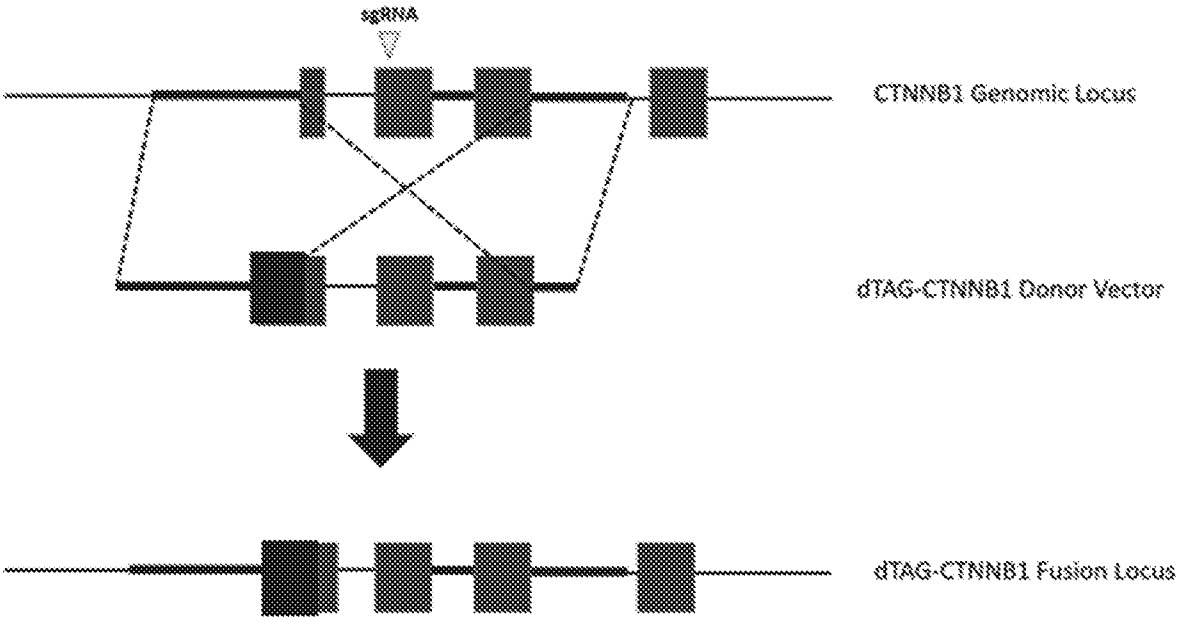

sgRNA
CTNNB1 Genomic Locus
dTAG-CTNNB1 Donor Vector
dTAG-CTNNB1 Fusion Locus

FIG. 3

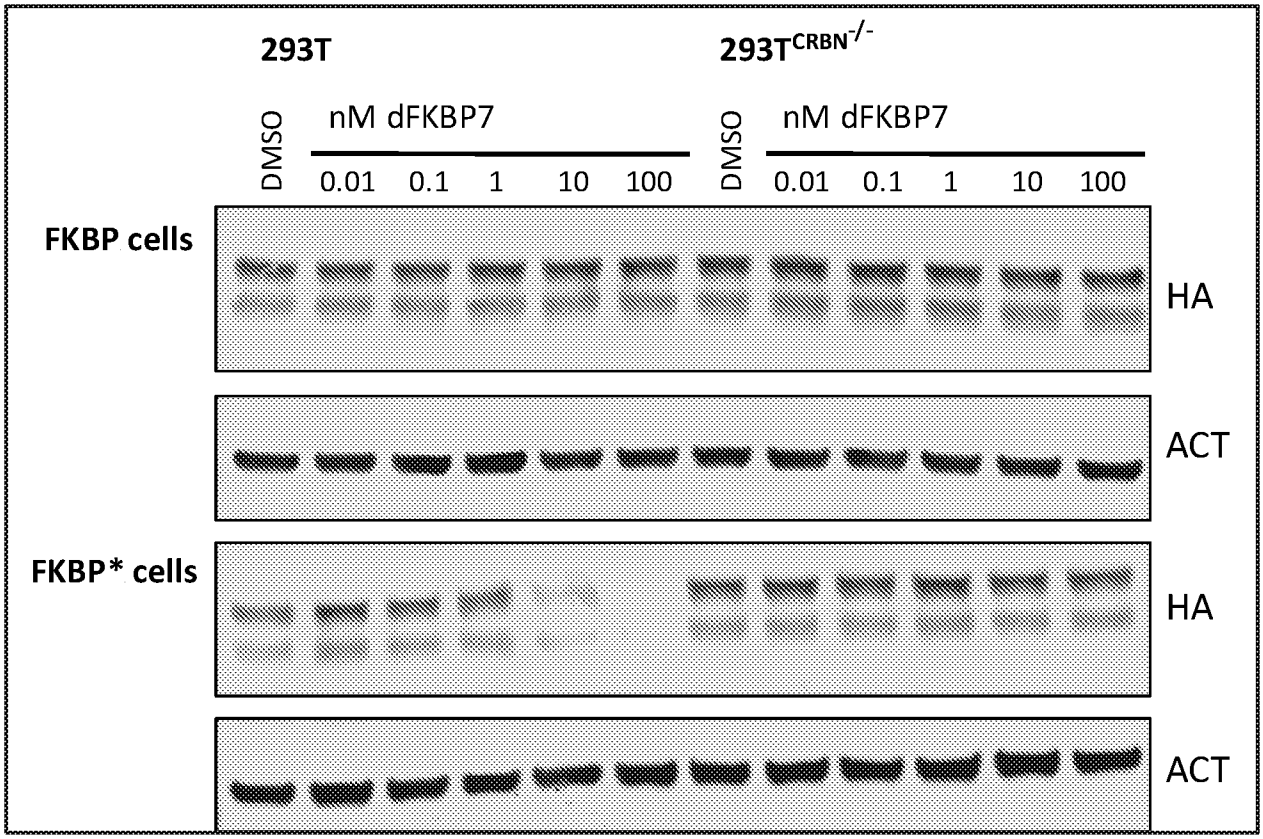

293T
293T^CRBN-/-
DMSO
nM dFKBP7
0.01
0.1
1
10
100
DMSO
nM dFKBP7
0.01
0.1
1
10
100
FKBP cells
HA
ACT
FKBP* cells
HA
ACT

FIG. 4

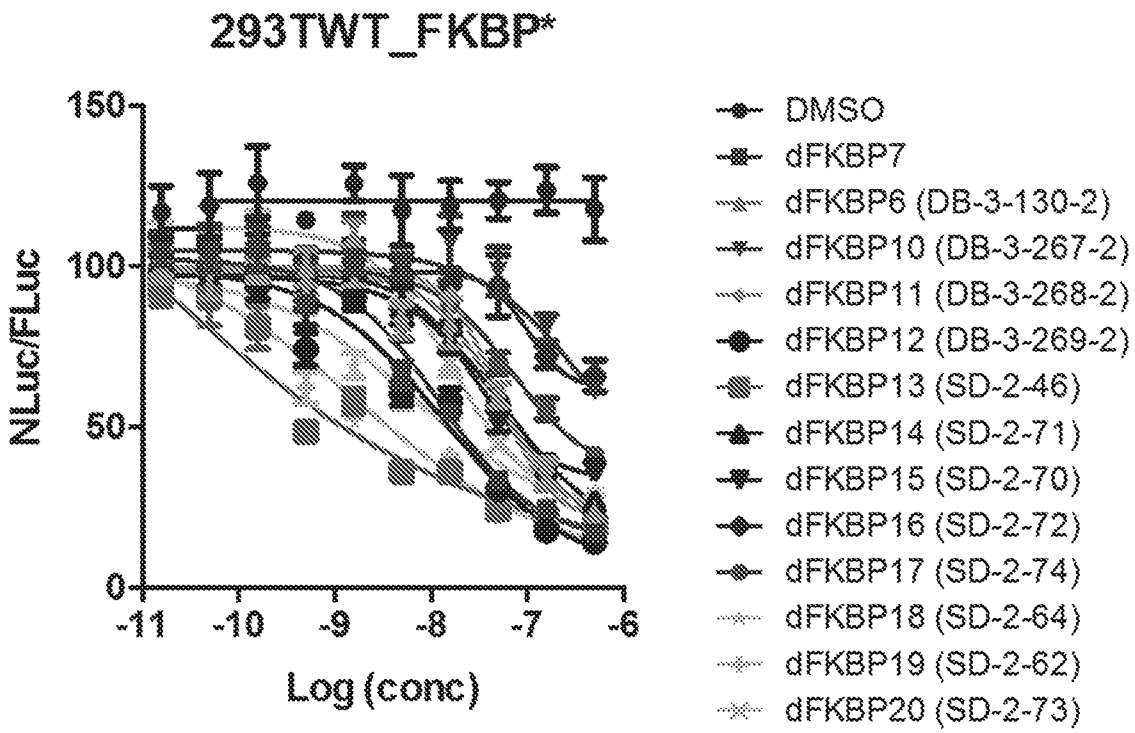

293TWT_FKBP*
NLuc/FLuc
150
100
50
0
-11
-10
-9
-8
-7
-6
Log (conc)
DMSO
dFKBP7
dFKBP6 (DB-3-130-2)
dFKBP10 (DB-3-267-2)
dFKBP11 (DB-3-268-2)
dFKBP12 (DB-3-269-2)
dFKBP13 (SD-2-46)
dFKBP14 (SD-2-71)
dFKBP15 (SD-2-70)
dFKBP16 (SD-2-72)
dFKBP17 (SD-2-74)
dFKBP18 (SD-2-64)
dFKBP19 (SD-2-62)
dFKBP20 (SD-2-73)

FIG. 5A

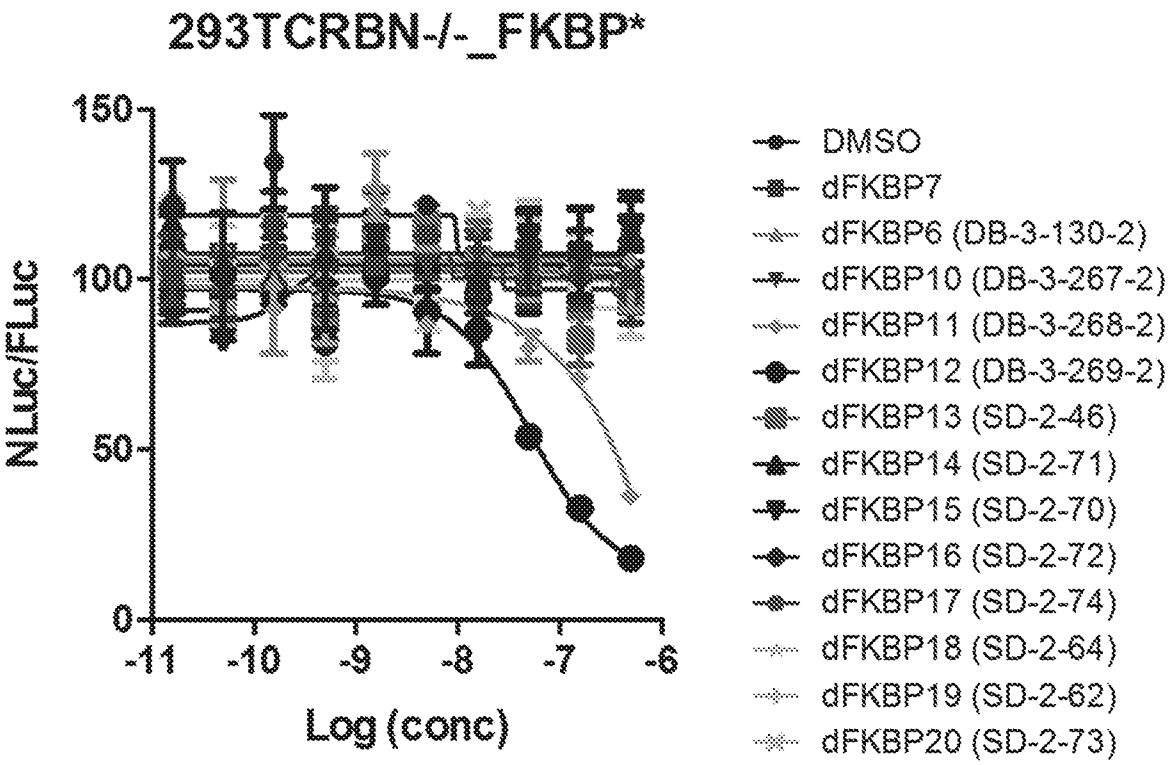

293TCRBN-/-_FKBP*
NLuc/FLuc
150
100
50
0
-11
-10
-9
-8
-7
-6
Log (conc)
DMSO
dFKBP7
dFKBP6 (DB-3-130-2)
dFKBP10 (DB-3-267-2)
dFKBP11 (DB-3-268-2)
dFKBP12 (DB-3-269-2)
dFKBP13 (SD-2-46)
dFKBP14 (SD-2-71)
dFKBP15 (SD-2-70)
dFKBP16 (SD-2-72)
dFKBP17 (SD-2-74)
dFKBP18 (SD-2-64)
dFKBP19 (SD-2-62)
dFKBP20 (SD-2-73)

FIG. 5B

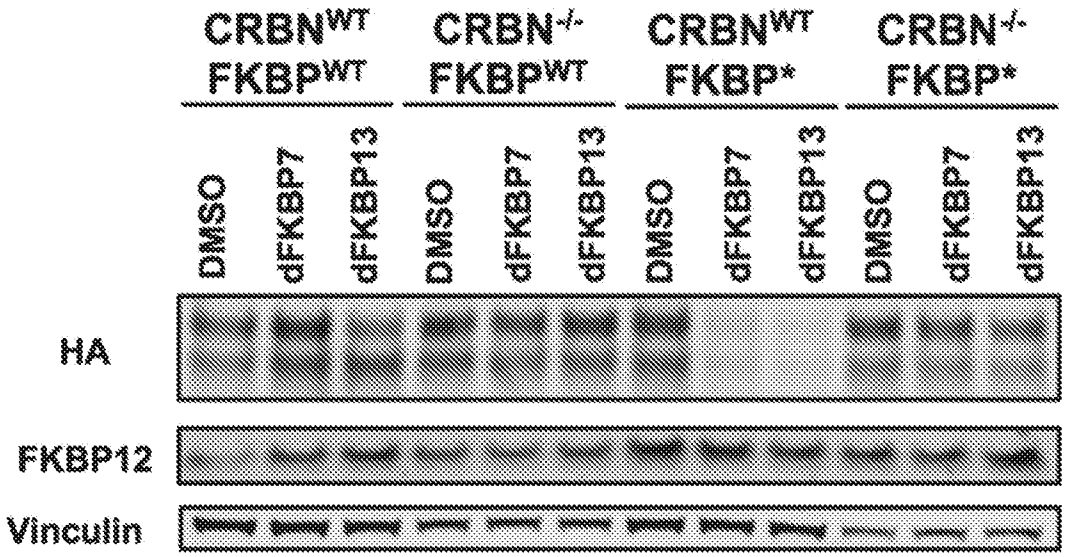

CRBN^WT FKBP^WT
CRBN^-/- FKBP^WT
CRBN^WT FKBP*
CRBN^-/- FKBP*
DMSO
dFKBP7
dFKBP13
HA
FKBP12
Vinculin

FIG. 6

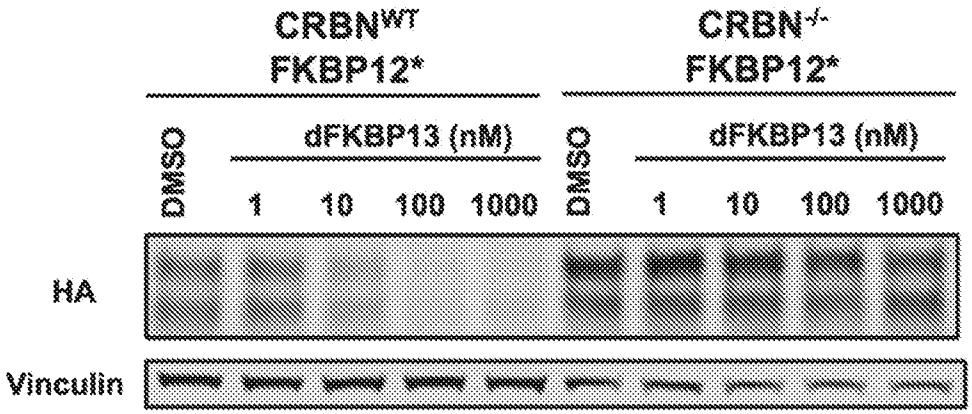

CRBN^WT
FKBP12*
CRBN^-/-
FKBP12*
DMSO
dFKBP13 (nM)
1
10
100
1000
DMSO
dFKBP13 (nM)
1
10
100
1000
HA
Vinculin

FIG. 7

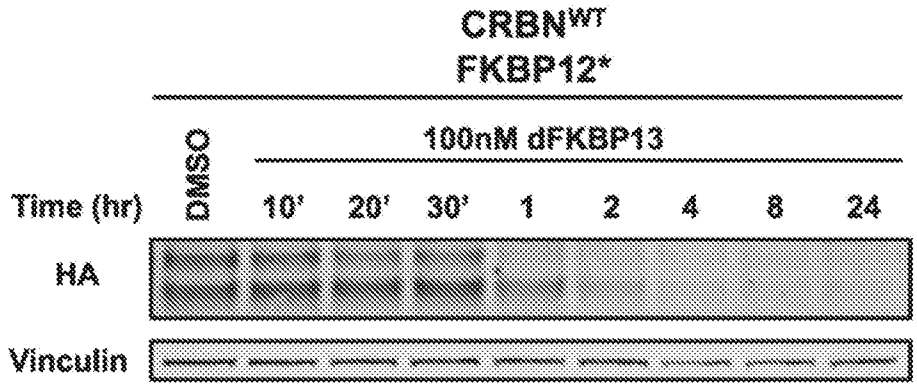

CRBN^WT
FKBP12*
DMSO
100nM dFKBP13
Time (hr)
10'
20'
30'
1
2
4
8
24
HA
Vinculin

FIG. 8

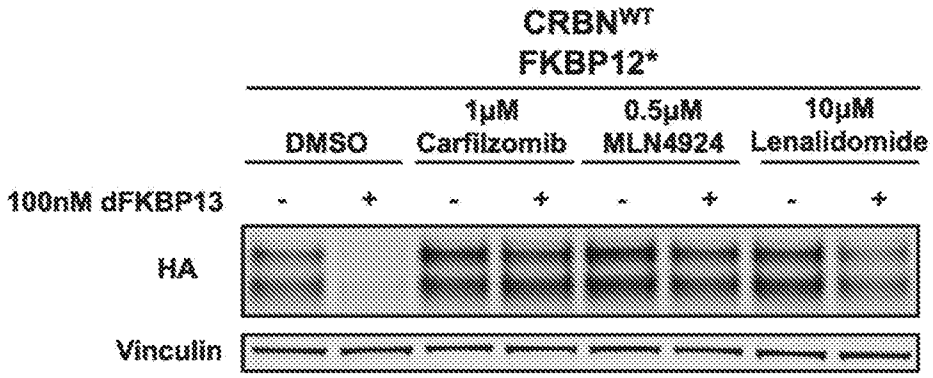

CRBN^WT
FKBP12*
DMSO
1μM Carfilzomib
0.5μM MLN4924
10μM Lenalidomide
100nM dFKBP13
- + - + - + - +
HA
Vinculin

FIG. 9

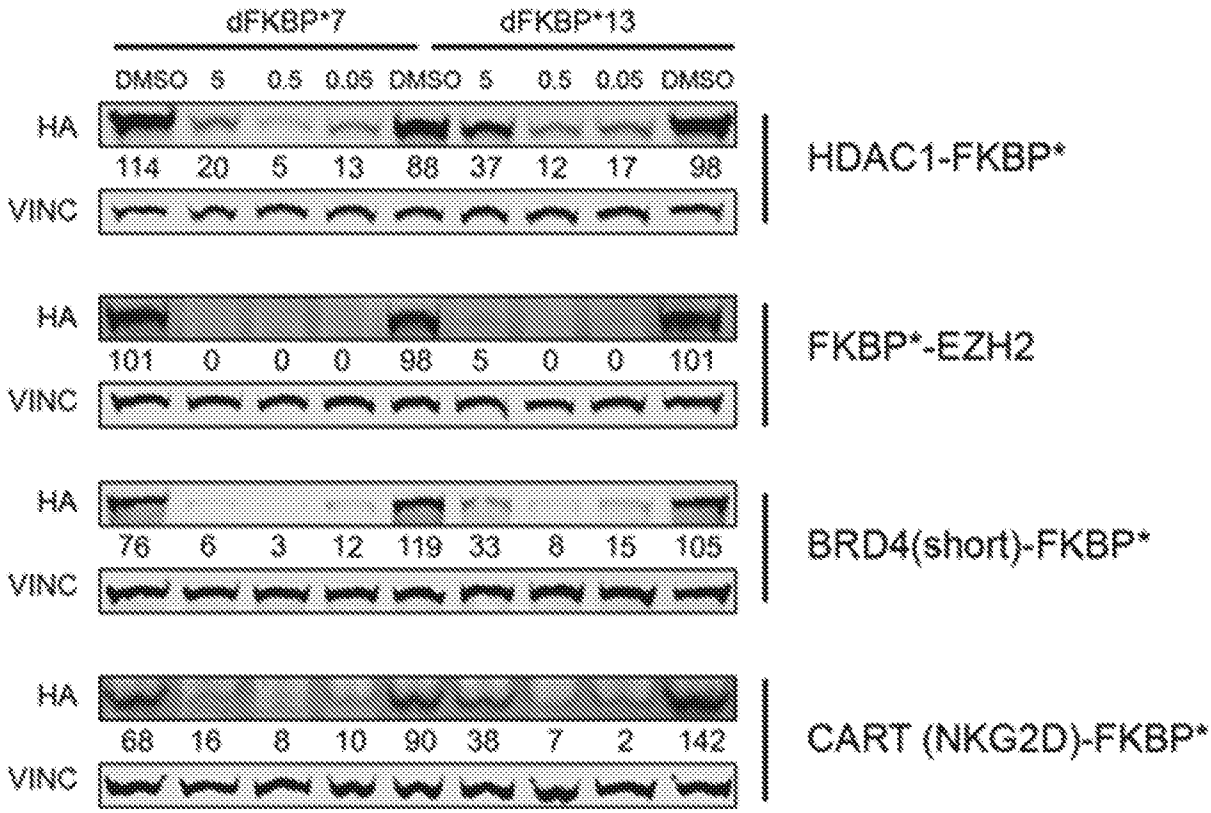

dFKBP*7
dFKBP*13
DMSO 5 0.5 0.05 DMSO 5 0.5 0.05 DMSO
HA
114 20 5 13 88 37 12 17 98
VINC
HDAC1-FKBP*
HA
101 0 0 0 98 5 0 0 101
VINC
FKBP*-EZH2
HA
76 6 3 12 119 33 8 15 105
VINC
BRD4(short)-FKBP*
HA
68 16 8 10 90 38 7 2 142
VINC
CART (NKG2D)-FKBP*

FIG. 10A

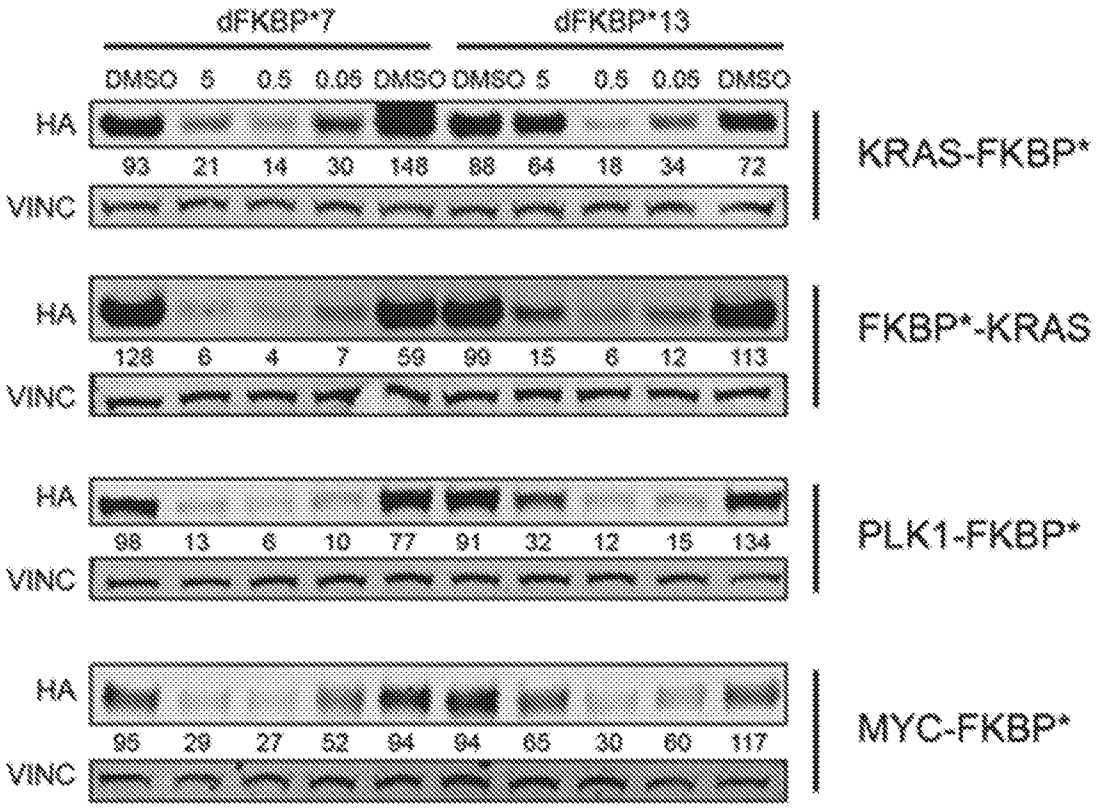

dFKBP*7
dFKBP*13
DMSO 5 0.5 0.05 DMSO DMSO 5 0.5 0.05 DMSO
HA
93 21 14 30 148 88 84 18 34 72
VINC
KRAS-FKBP*
HA
128 6 4 7 59 99 15 6 12 113
VINC
FKBP*-KRAS
HA
98 13 6 10 77 91 32 12 15 134
VINC
PLK1-FKBP*
HA
95 29 27 52 94 94 65 30 60 117
VINC
MYC-FKBP*

FIG. 10B

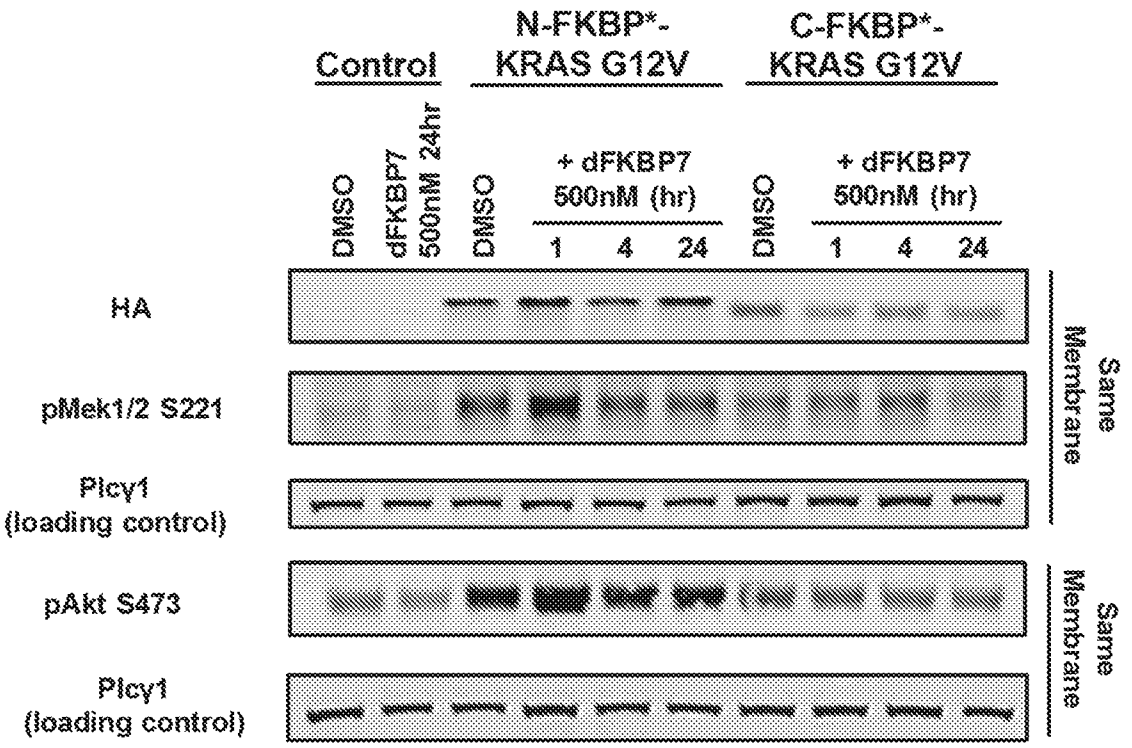

Control
N-FKBP*-
KRAS G12V
C-FKBP*-
KRAS G12V
DMSO
dFKBP7
500nM 24hr
DMSO
+ dFKBP7
500nM (hr)
1
4
24
DMSO
+ dFKBP7
500nM (hr)
1
4
24
HA
pMek1/2 S221
Plcγ1
(loading control)
pAkt S473
Plcγ1
(loading control)
Same
Membrane
Same
Membrane

FIG. 11

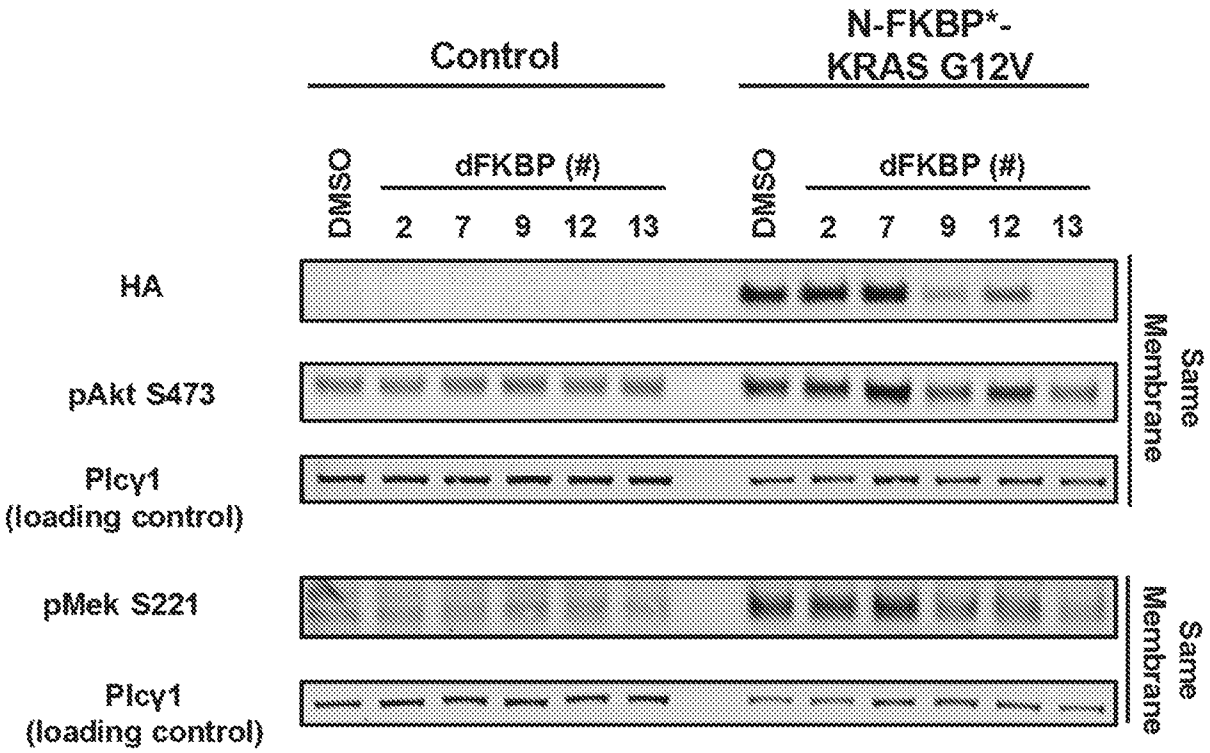

Control
N-FKBP*-
KRAS G12V
DMSO
dFKBP (#)
2 7 9 12 13
DMSO
dFKBP (#)
2 7 9 12 13
HA
pAkt S473
Plcγ1
(loading control)
Same
Membrane
pMek S221
Plcγ1
(loading control)
Same
Membrane

FIG. 12

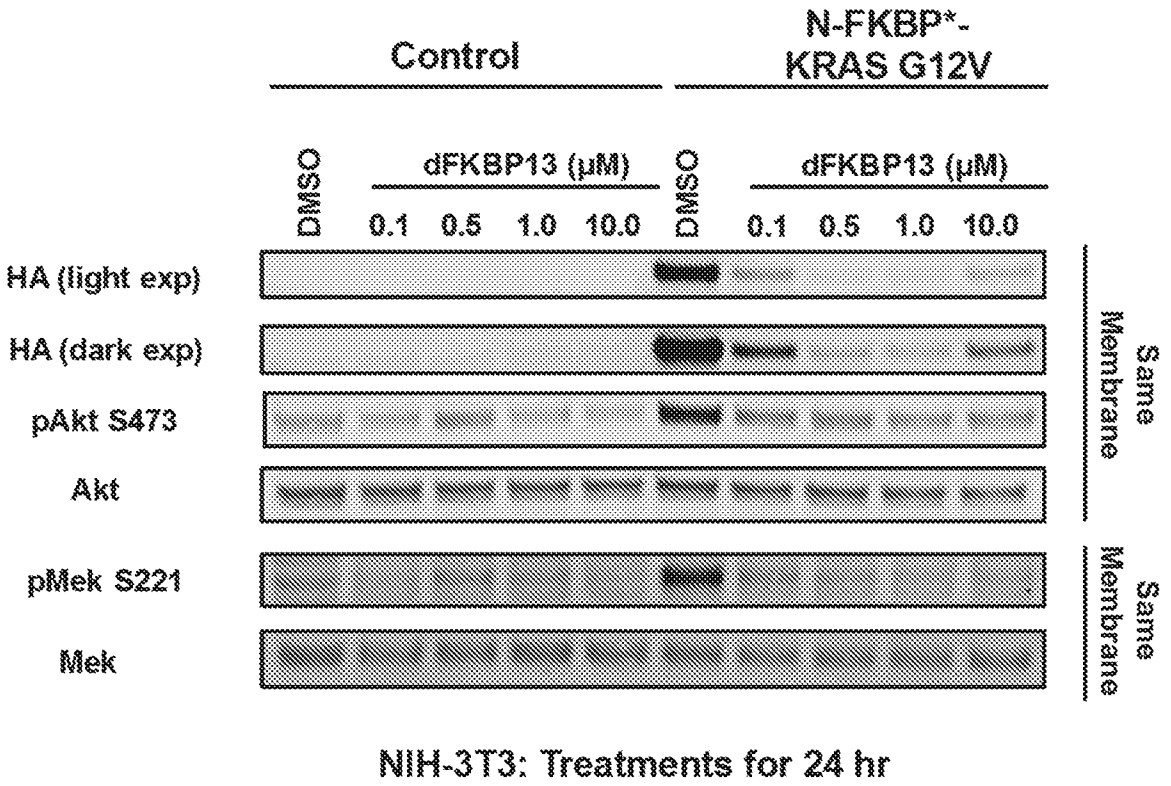

Control
N-FKBP*-
KRAS G12V
DMSO
dFKBP13 (μM)
0.1 0.5 1.0 10.0
DMSO
dFKBP13 (μM)
0.1 0.5 1.0 10.0
HA (light exp)
HA (dark exp)
pAkt S473
Akt
pMek S221
Mek
Same Membrane
Same Membrane
NIH-3T3: Treatments for 24 hr

FIG. 13

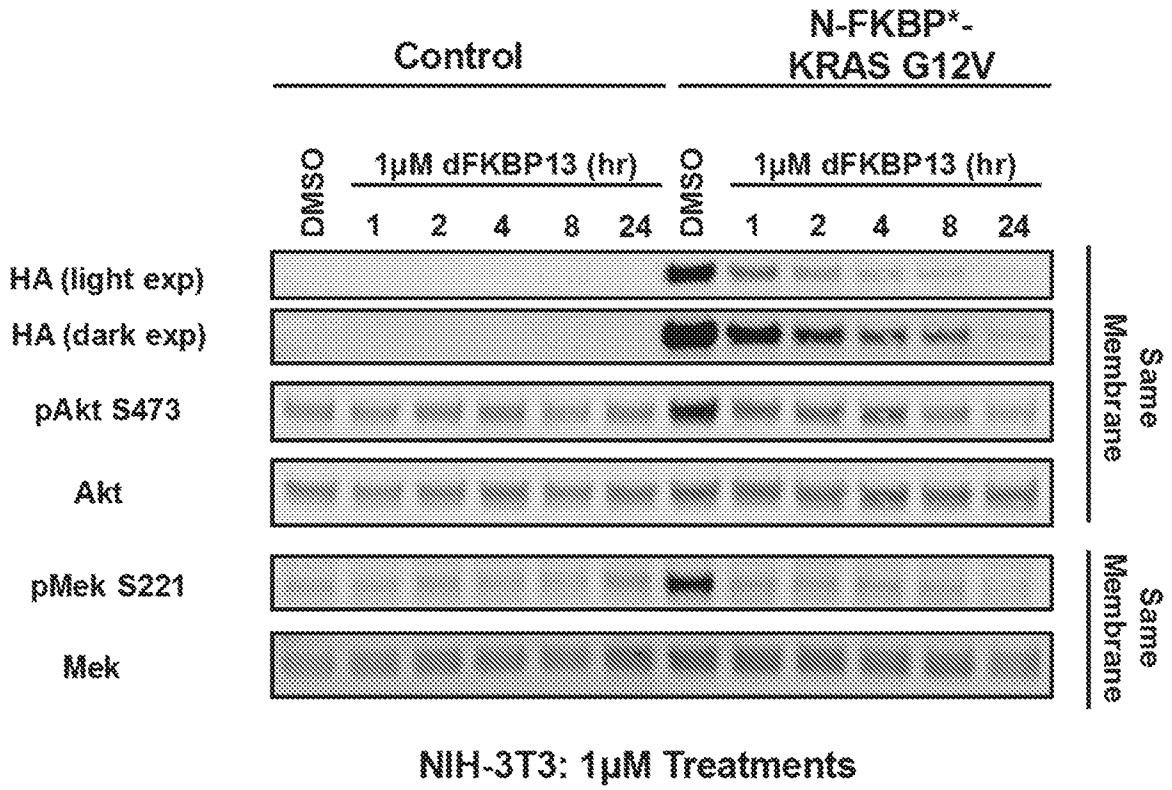

Control
N-FKBP*-
KRAS G12V
DMSO
1μM dFKBP13 (hr)
1 2 4 8 24
DMSO
1μM dFKBP13 (hr)
1 2 4 8 24
HA (light exp)
HA (dark exp)
pAkt S473
Akt
pMek S221
Mek
Same Membrane
Same Membrane
NIH-3T3: 1μM Treatments

FIG. 14

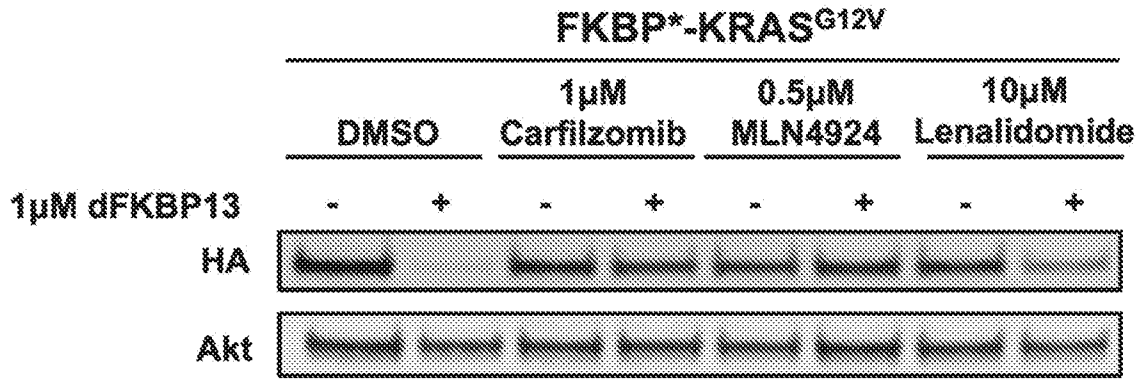

FKBP*-KRAS^{G12V}
DMSO
1μM Carfilzomib
0.5μM MLN4924
10μM Lenalidomide
1μM dFKBP13
- + - + - + - +
HA
Akt

FIG. 15

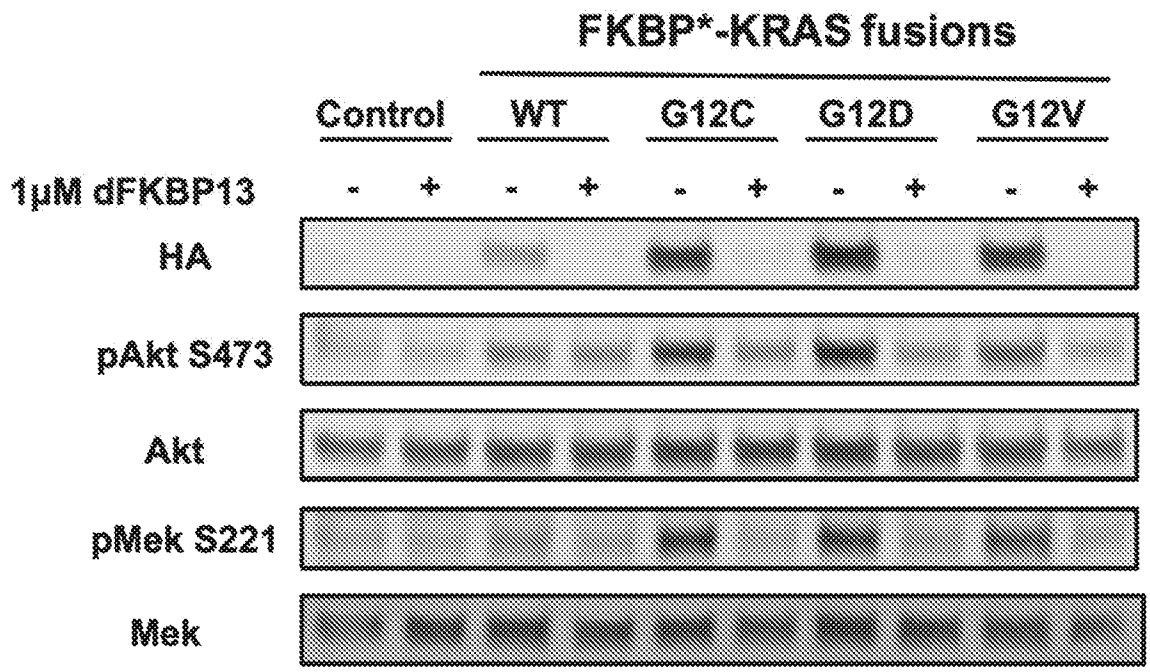

FKBP*-KRAS fusions
Control
WT
G12C
G12D
G12V
1μM dFKBP13
- + - + - + - + - +
HA
pAkt S473
Akt
pMek S221
Mek

FIG. 16

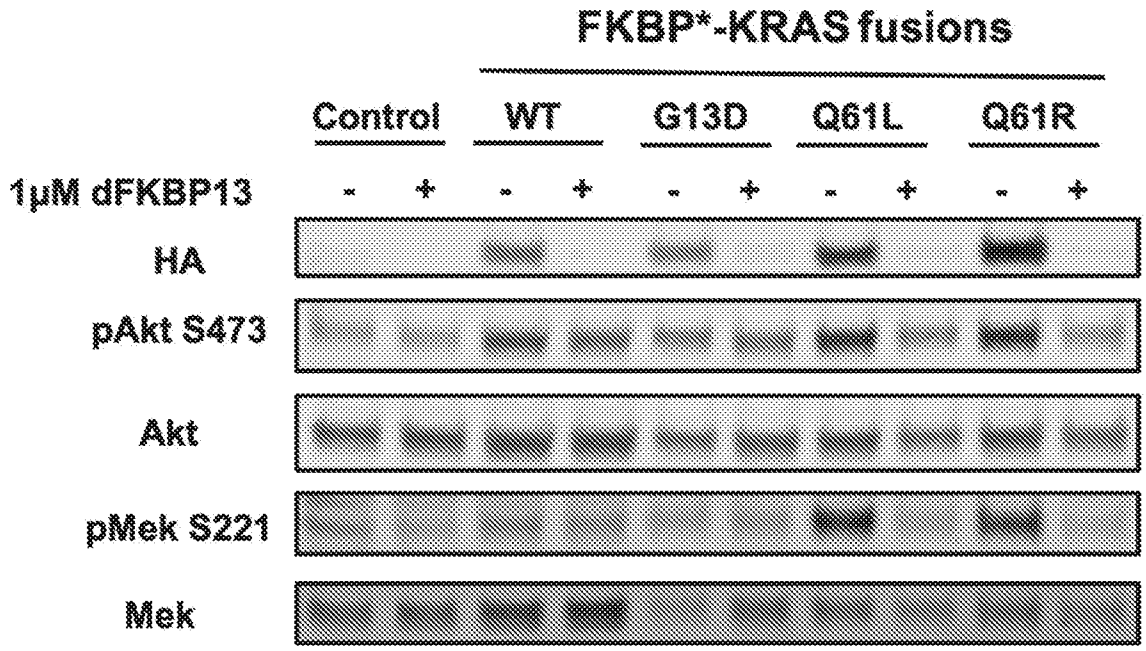

FKBP*-KRAS fusions
Control
WT
G13D
Q61L
Q61R
1μM dFKBP13
- + - + - + - + - +
HA
pAkt S473
Akt
pMek S221
Mek

FIG. 17

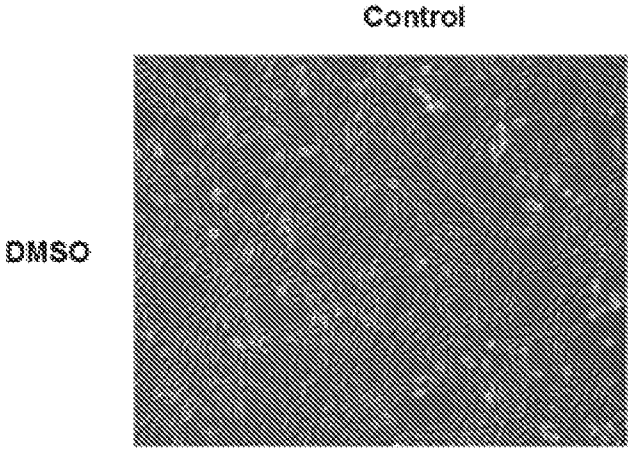


FIG. 18A

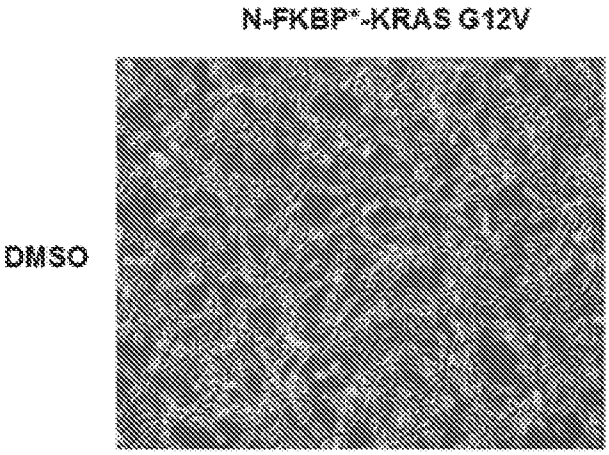


FIG. 18B

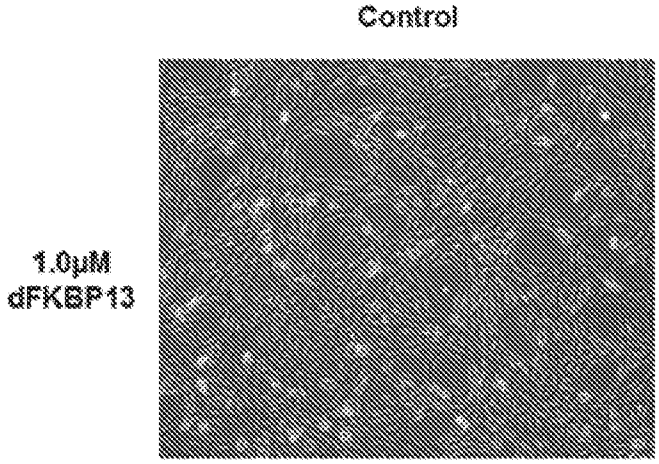


FIG. 18C

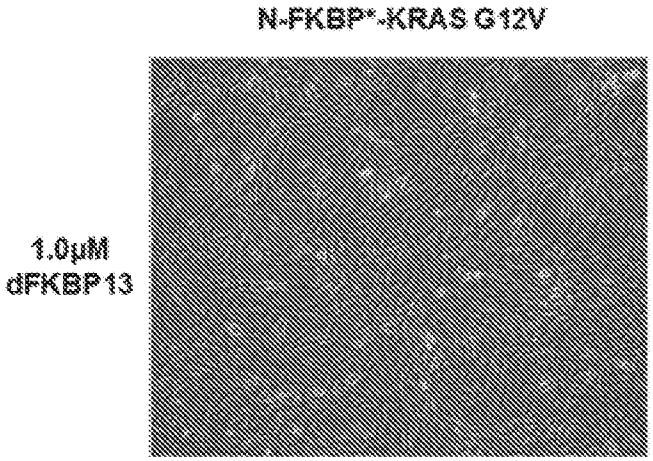


FIG. 18D

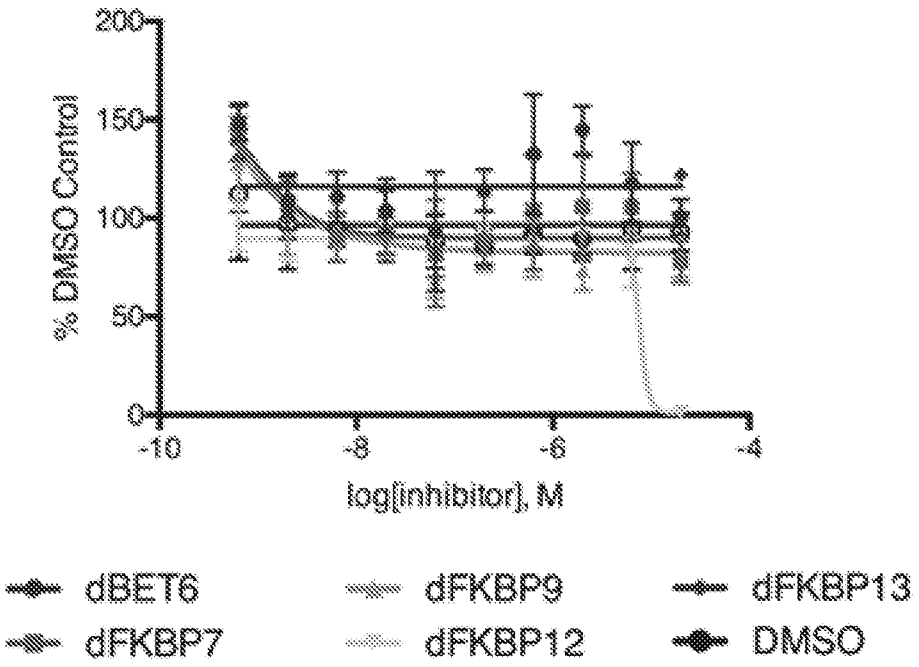


FIG. 19A

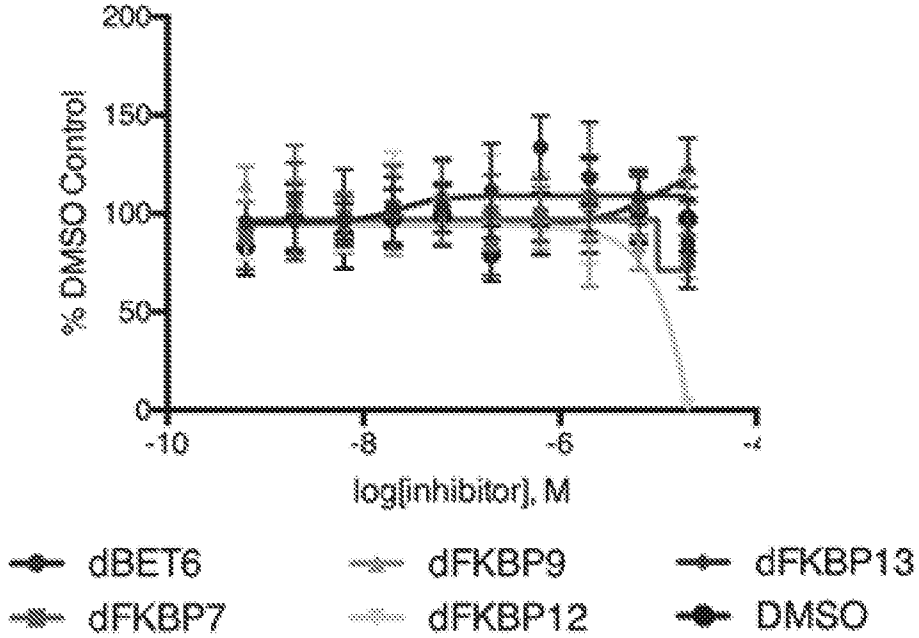


FIG. 19B

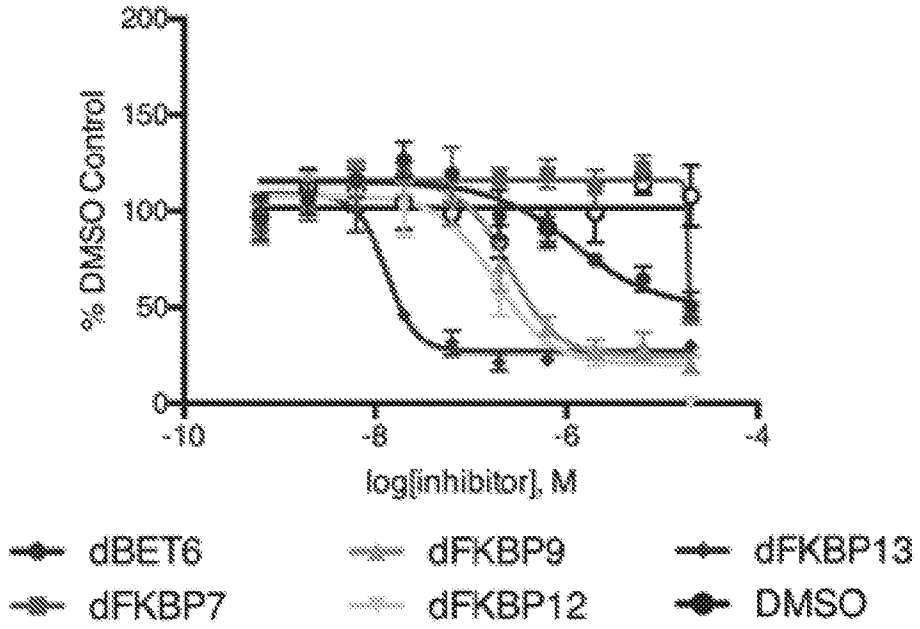


FIG. 19C

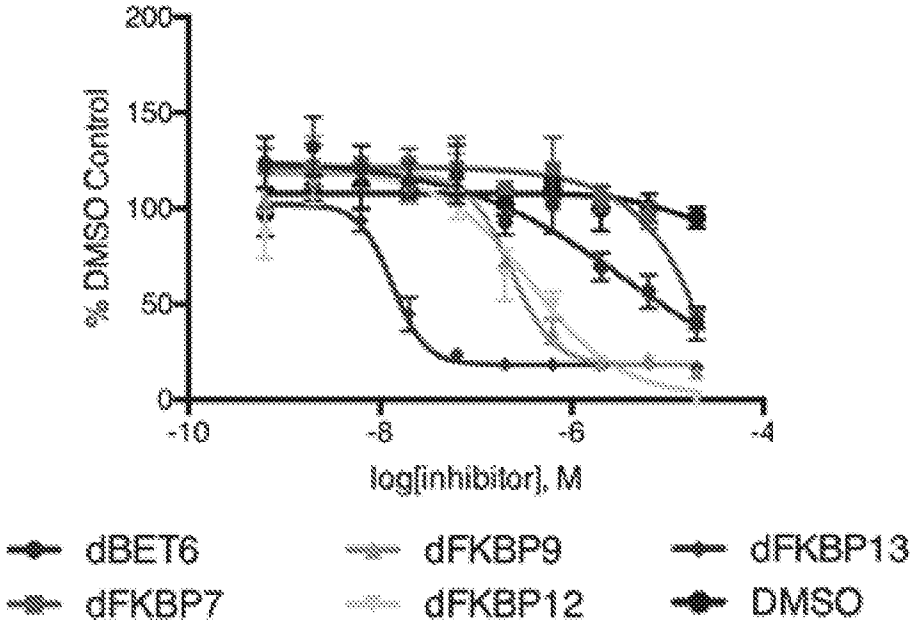


FIG. 19D

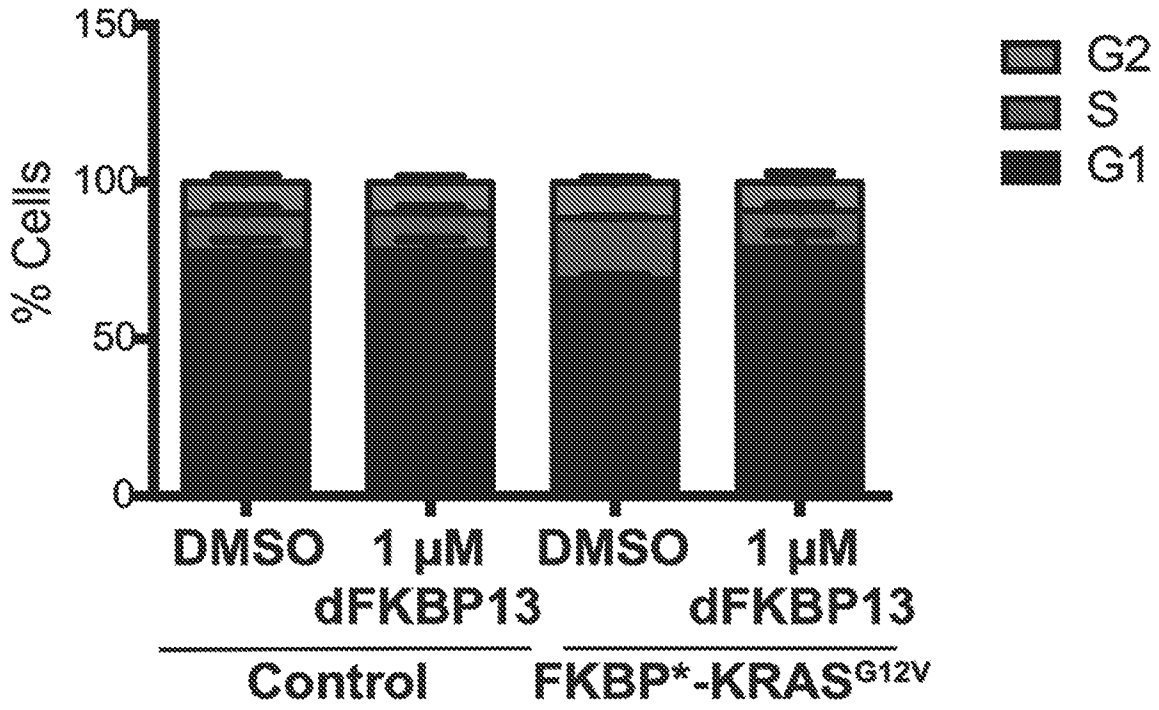

150
100
50
0
% Cells
G2
S
G1
DMSO
1 µM dFKBP13
DMSO
1 µM dFKBP13
Control
FKBP*-KRASG12V

FIG. 20

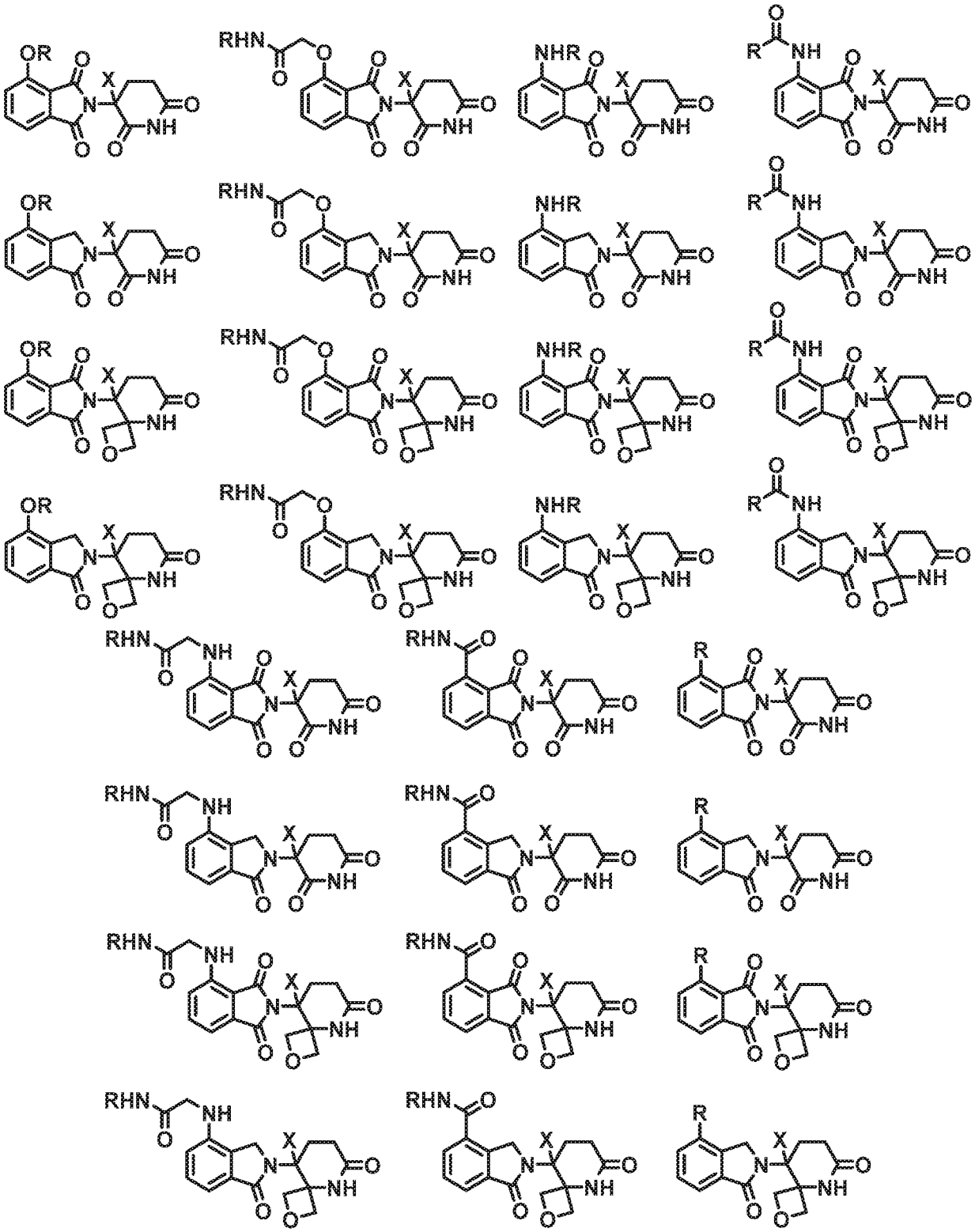


FIG. 21A

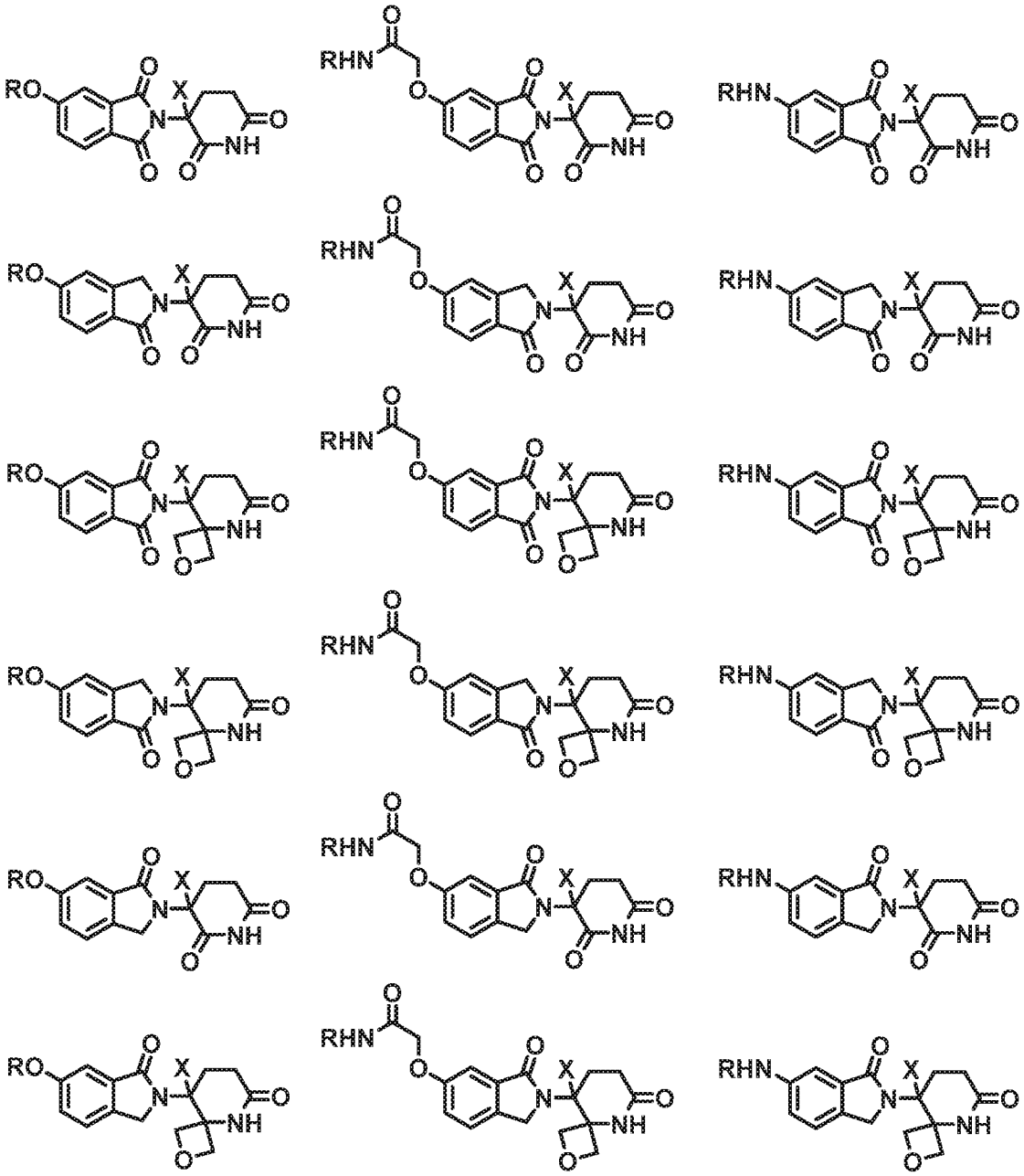


FIG. 21B

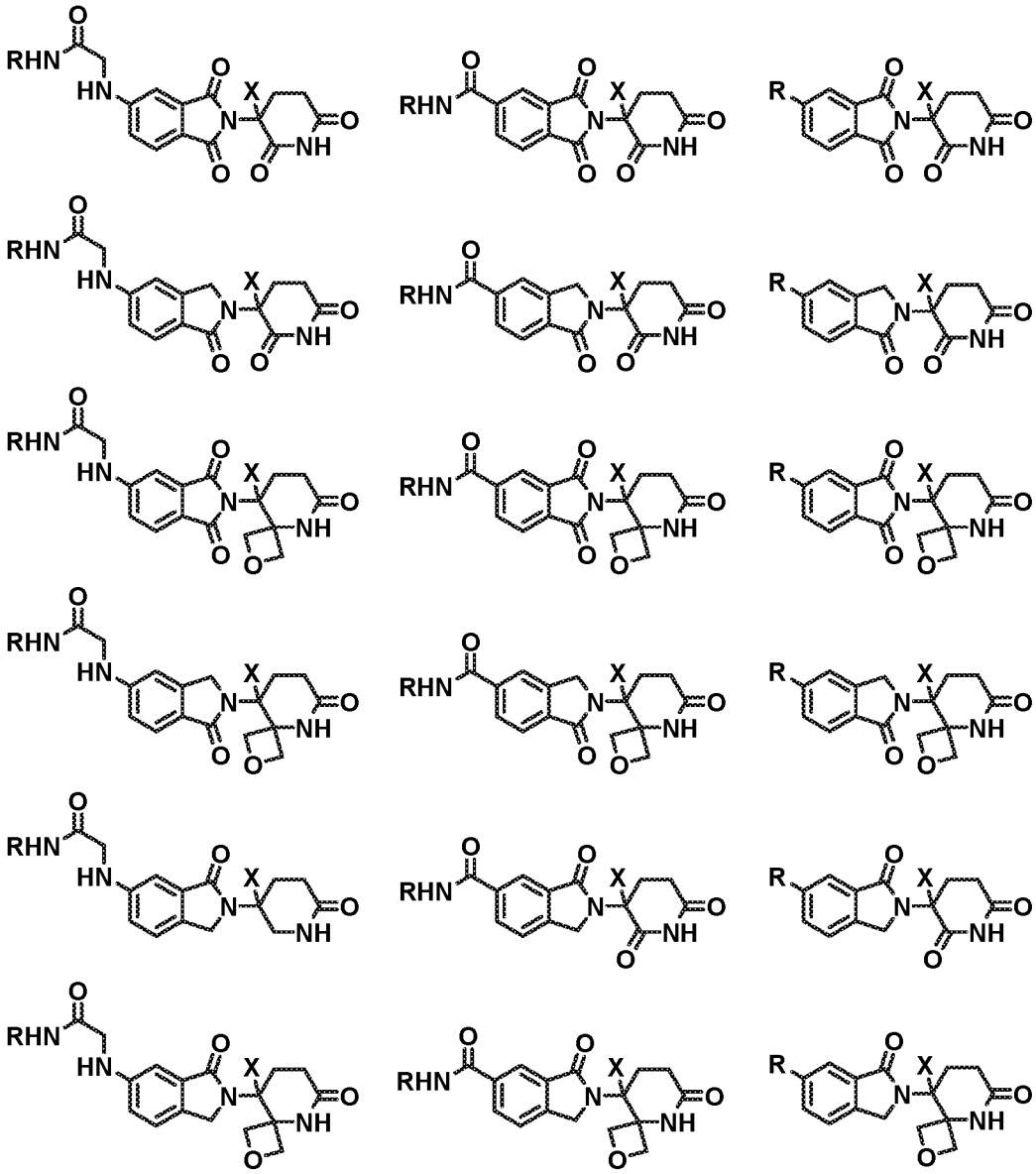


FIG. 21C

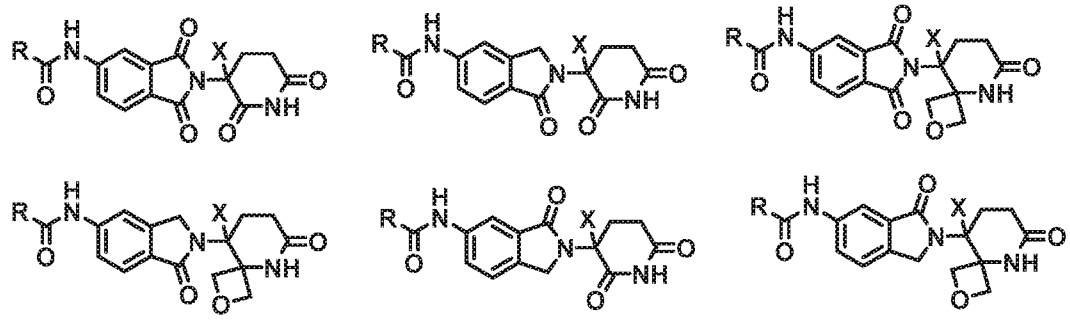


FIG. 21D

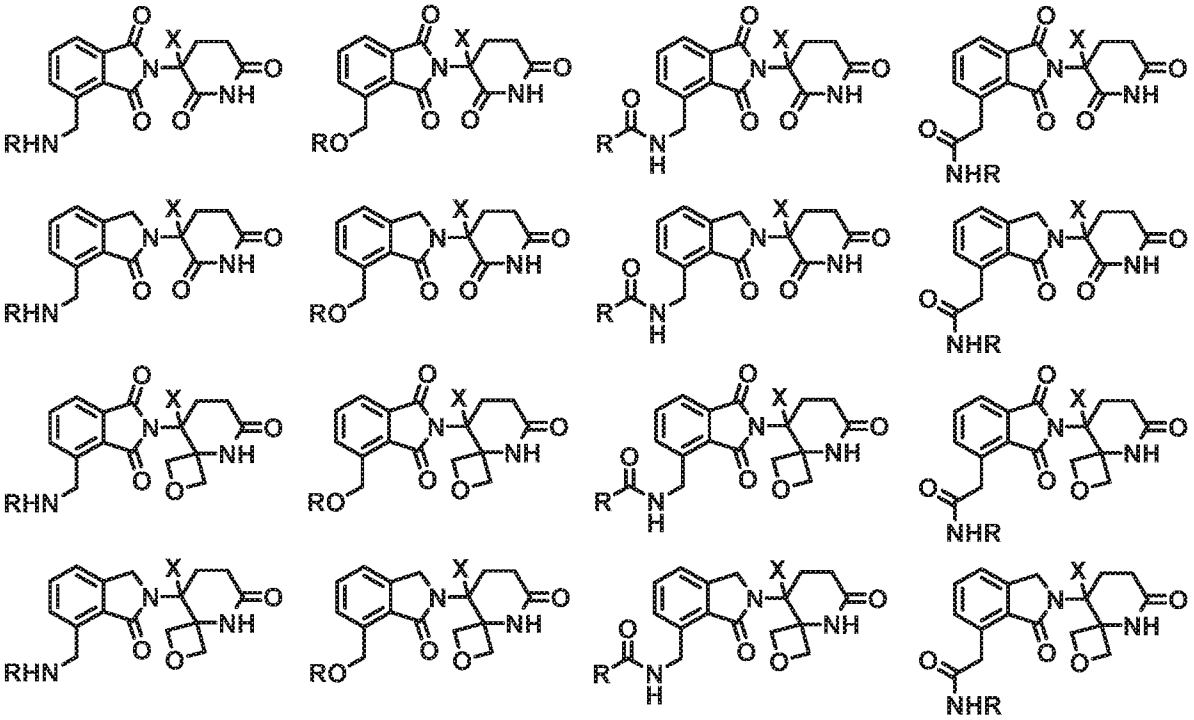


FIG. 21E

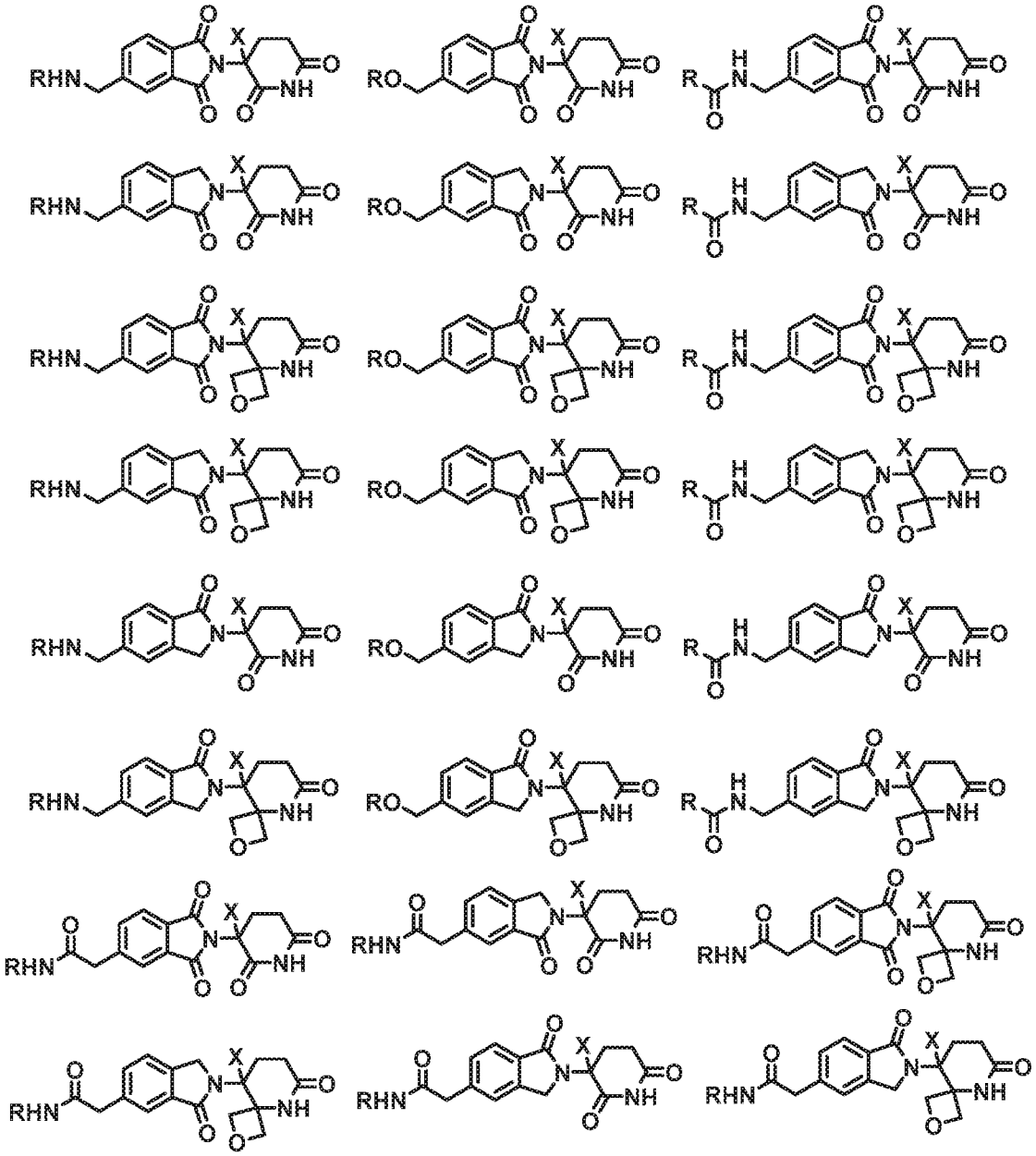


FIG. 21F

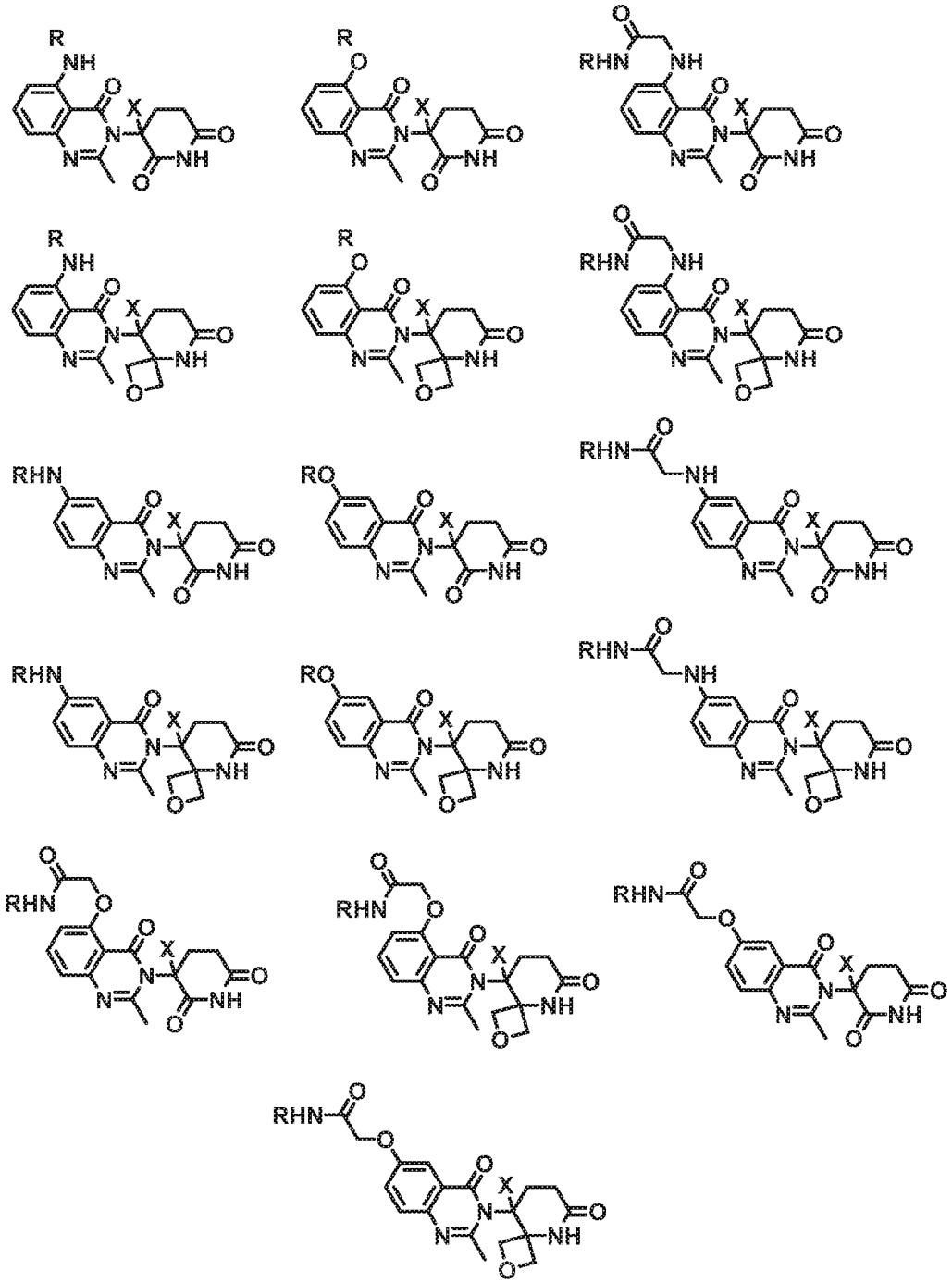


FIG. 21G

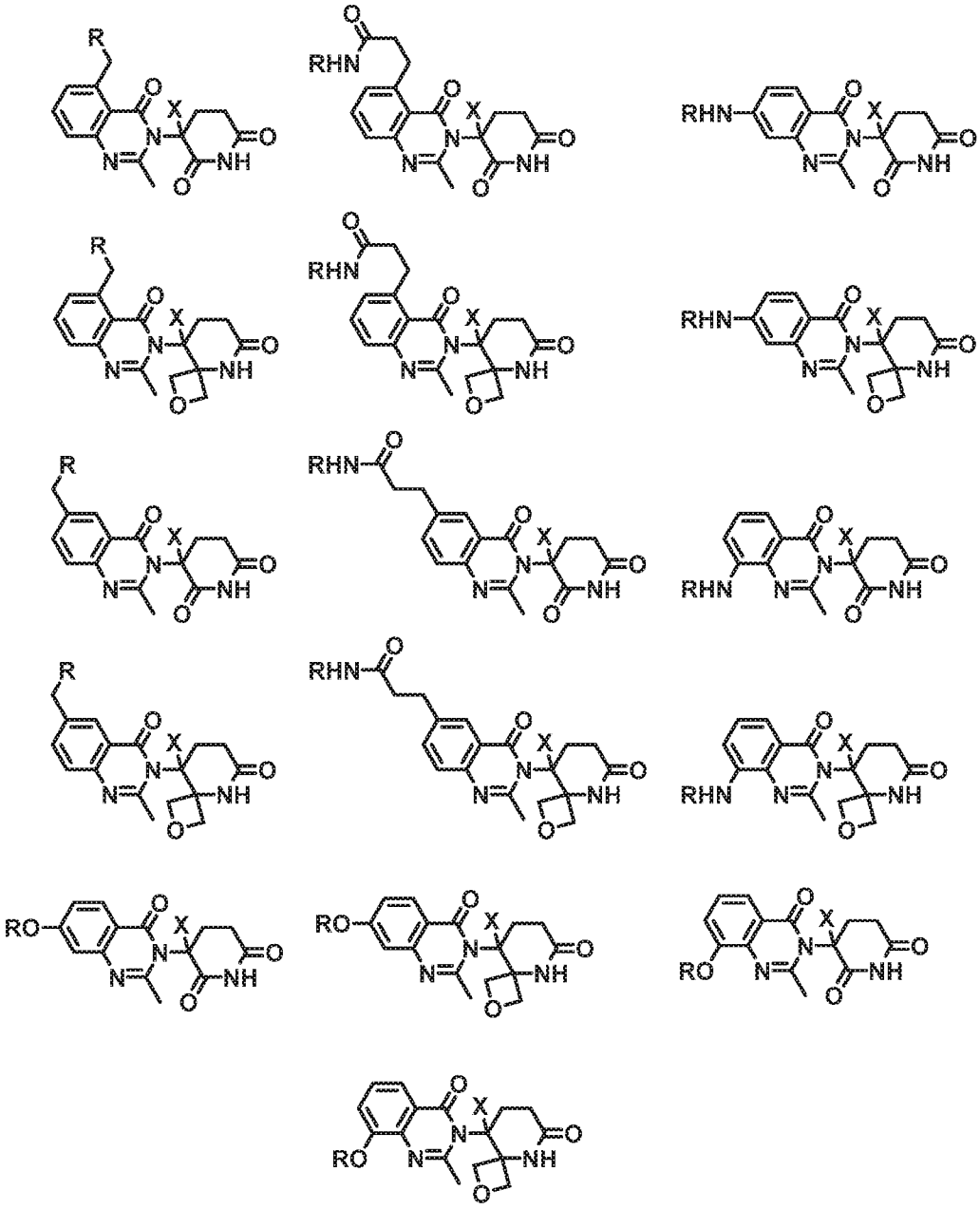


FIG. 21H

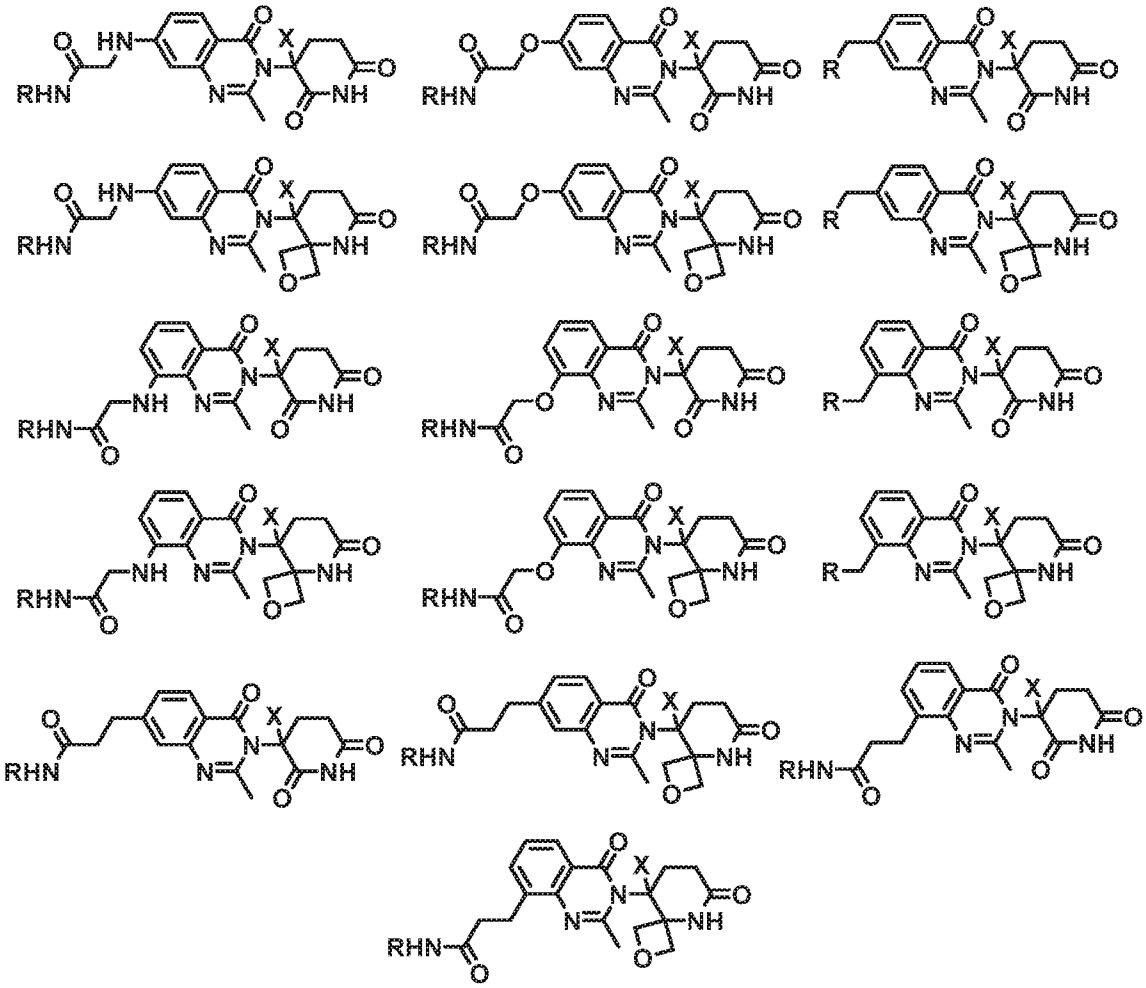


FIG. 21I

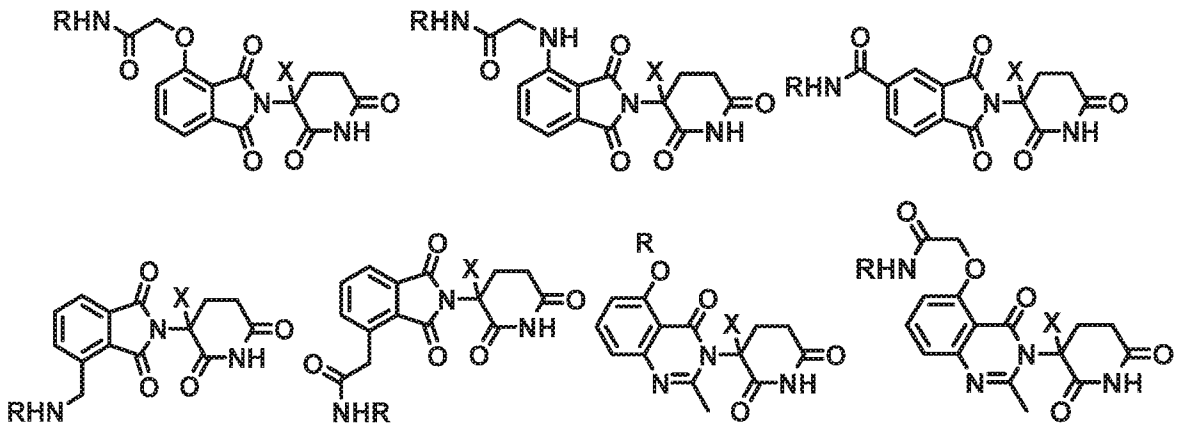


FIG. 22

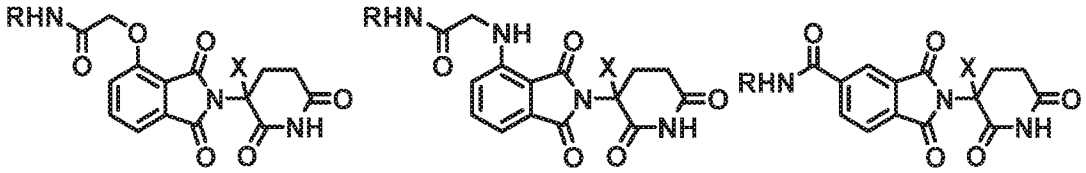


FIG. 23

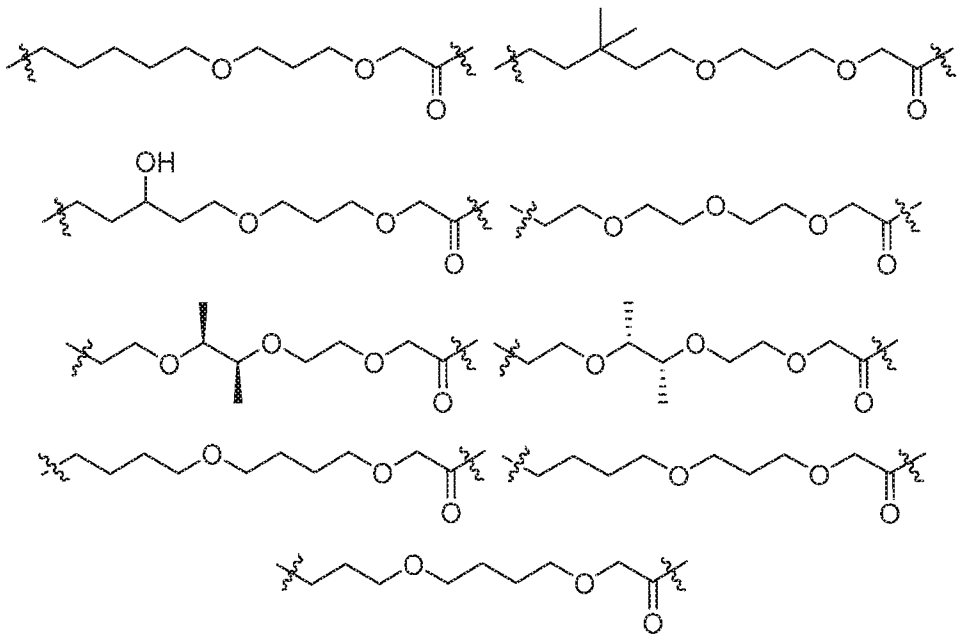


FIG. 24

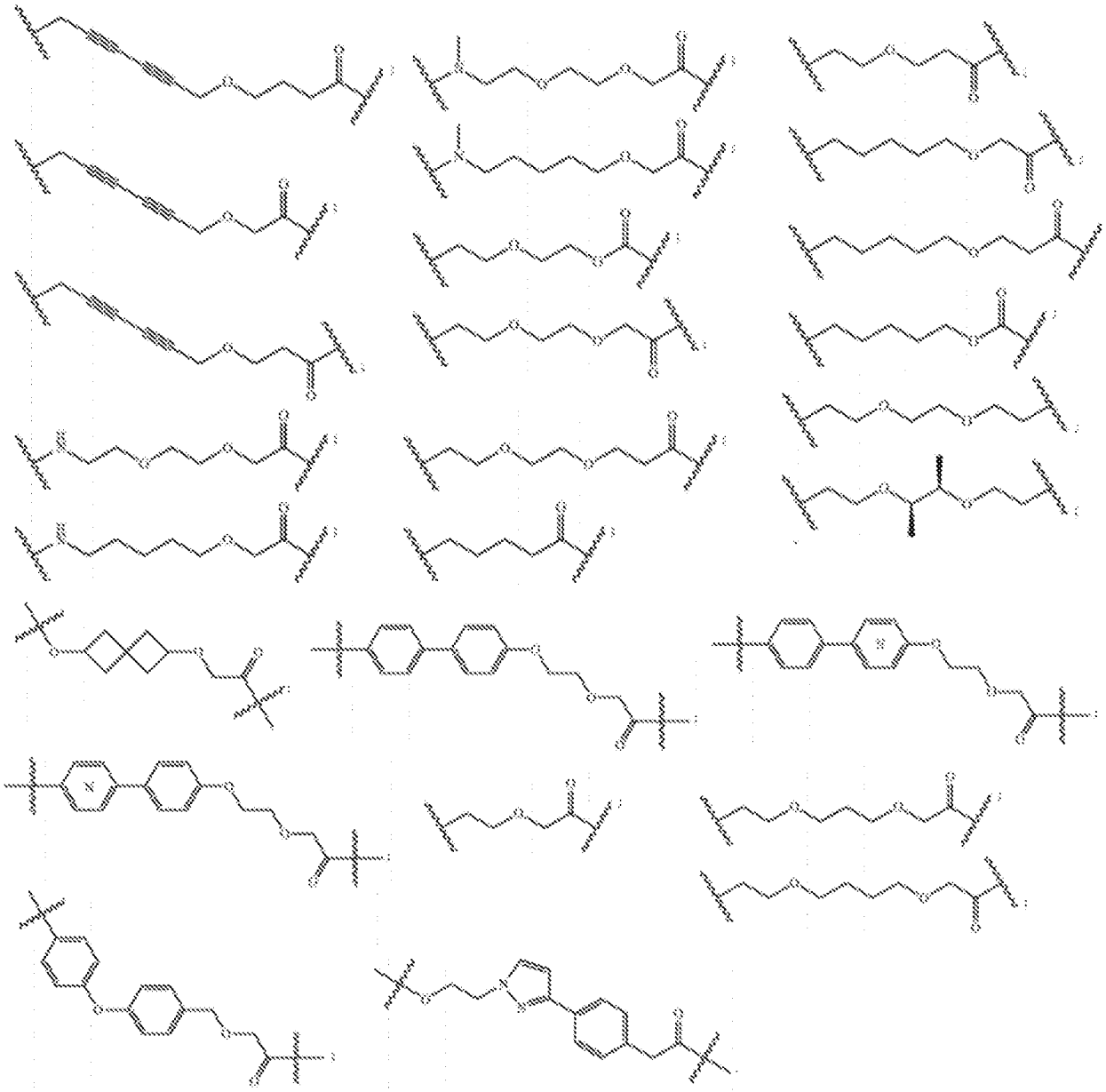


FIG. 25

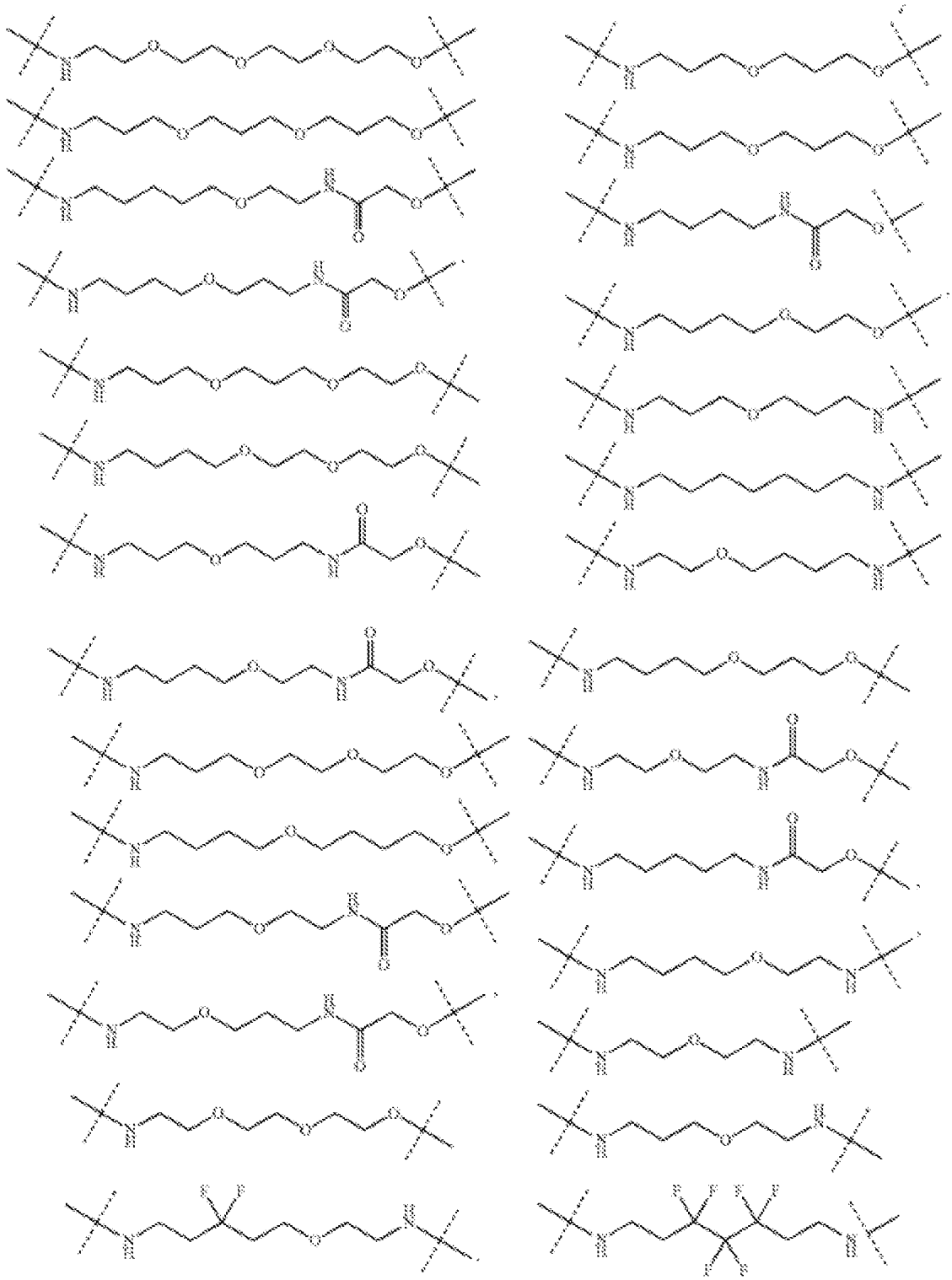


FIG. 26

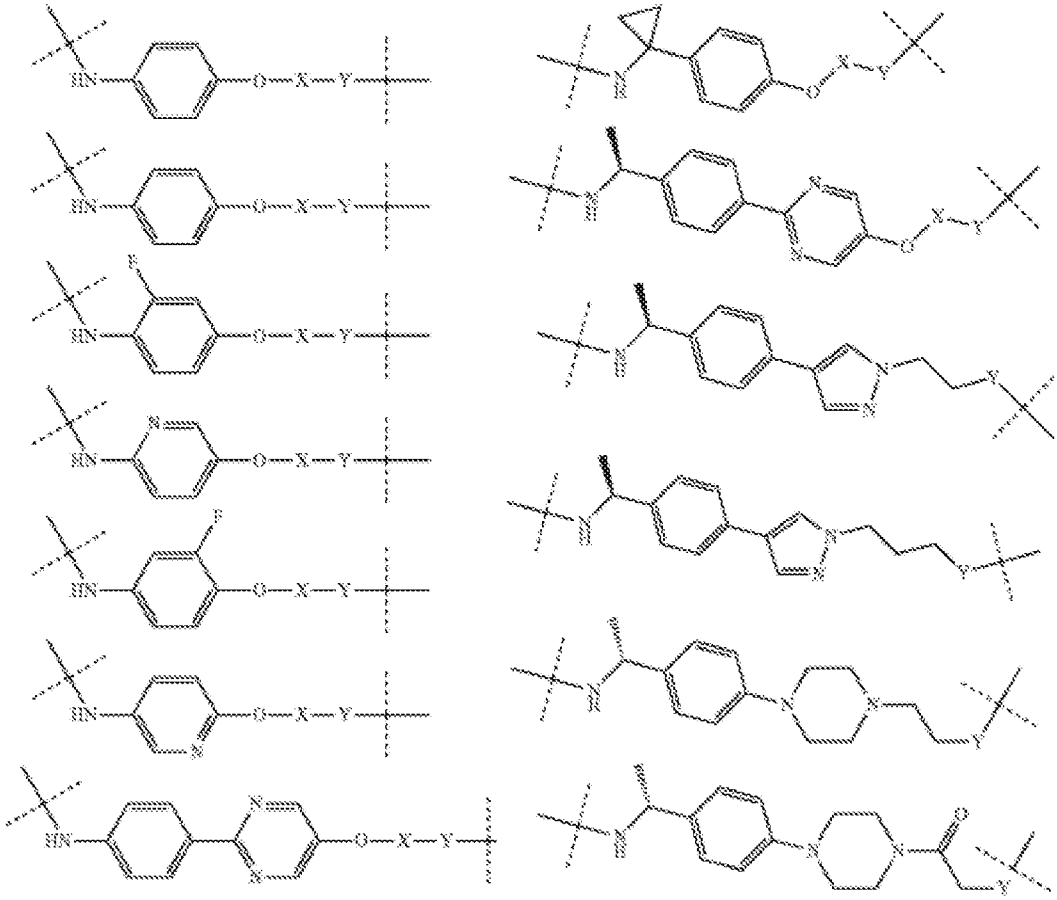


FIG. 27

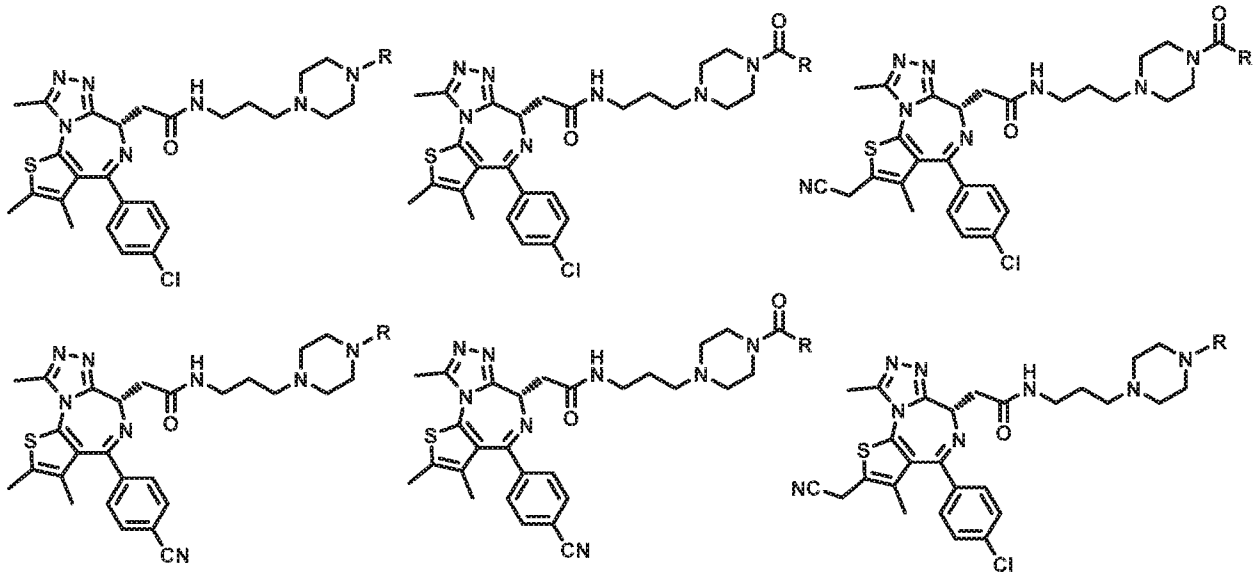


FIG. 28A

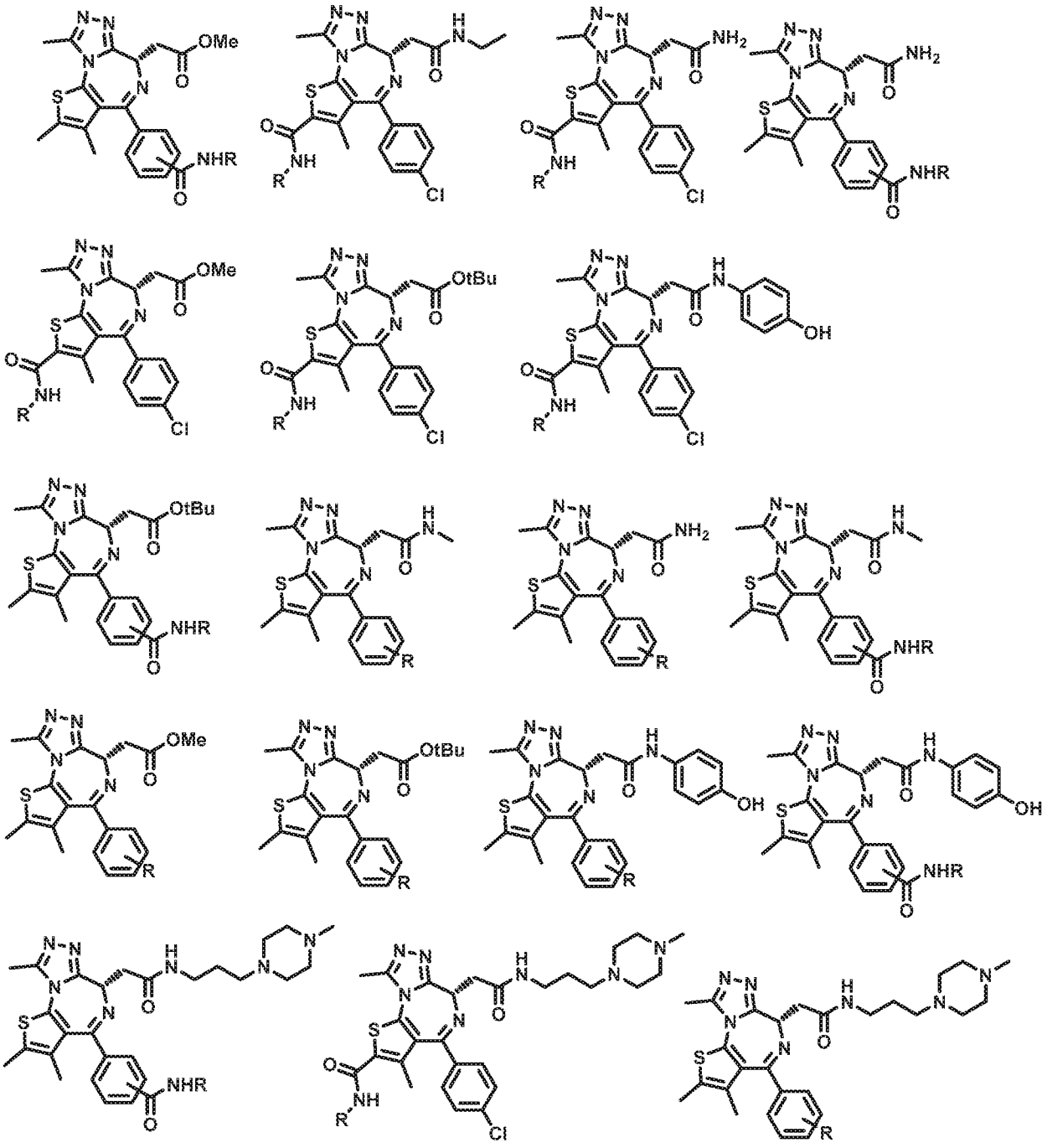


FIG 28B

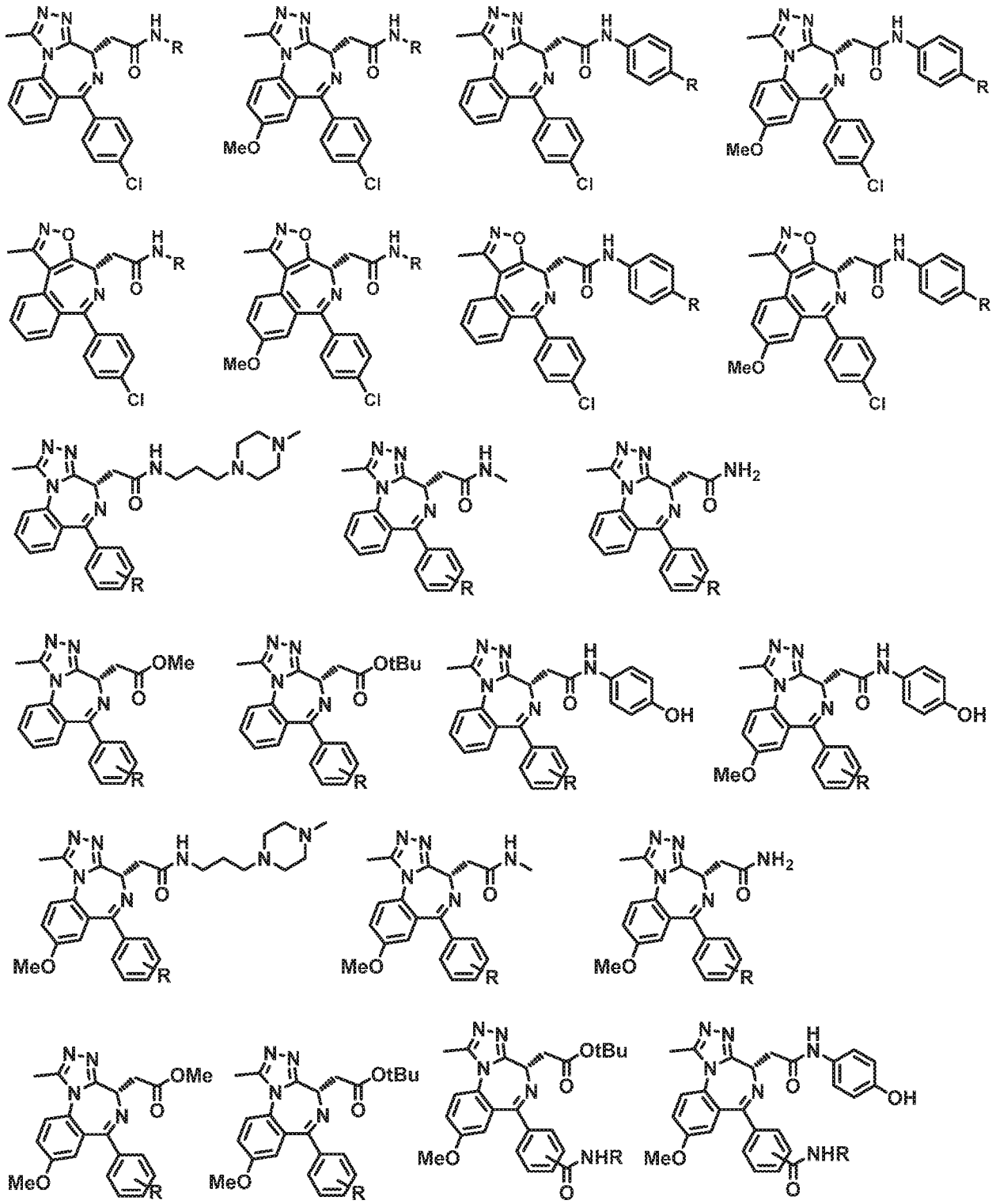


FIG. 28C

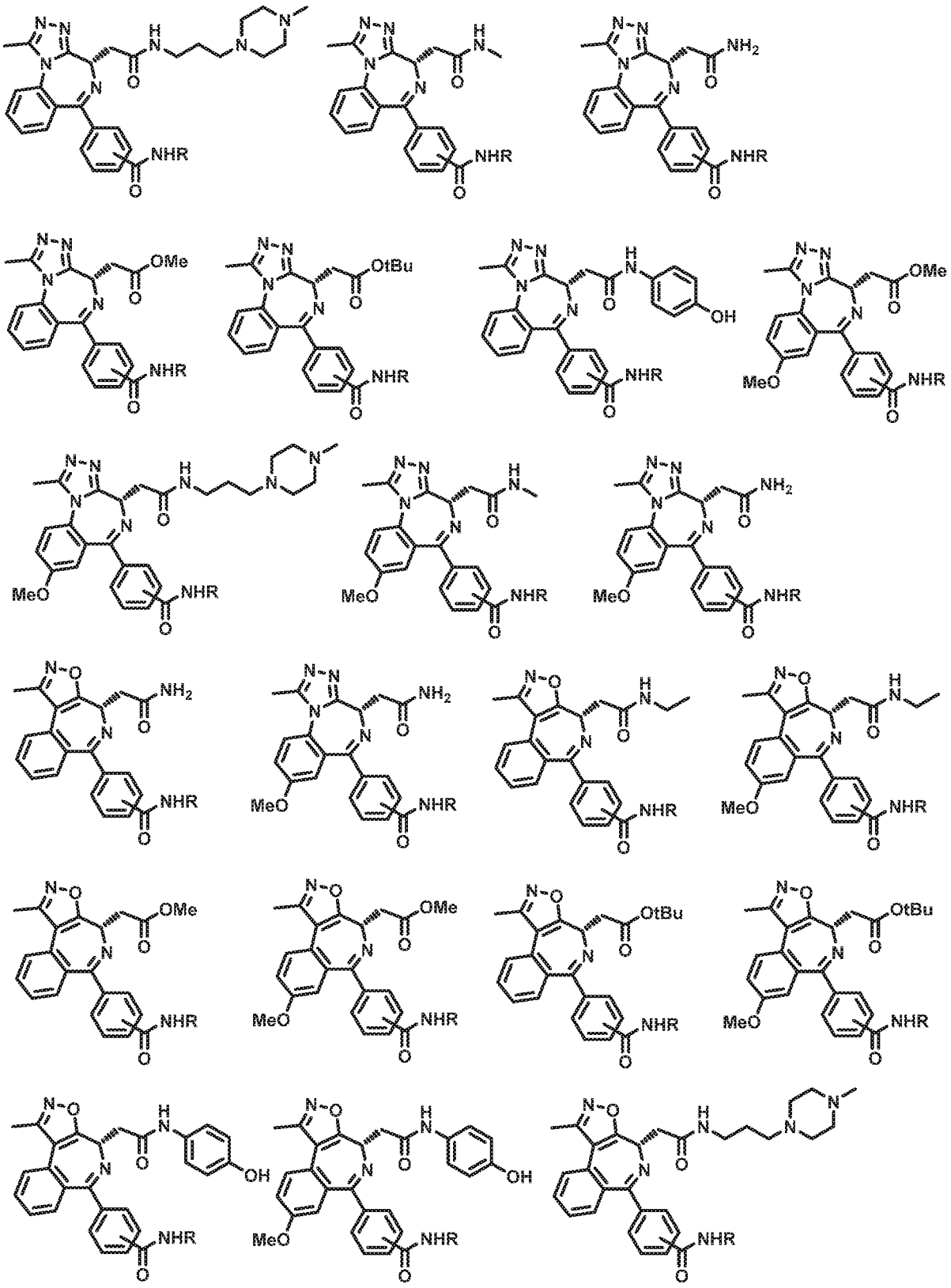


FIG. 28D

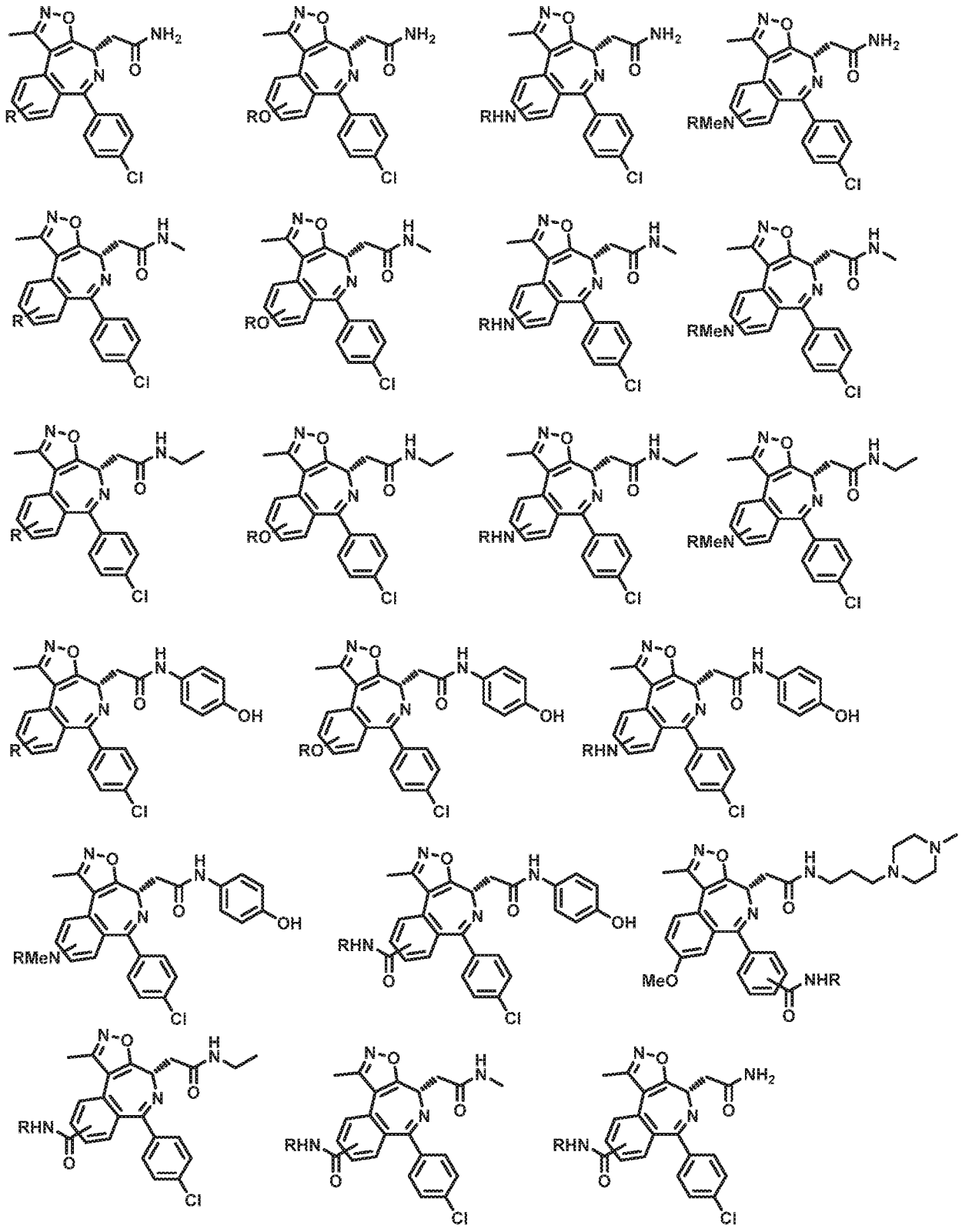


FIG. 28E

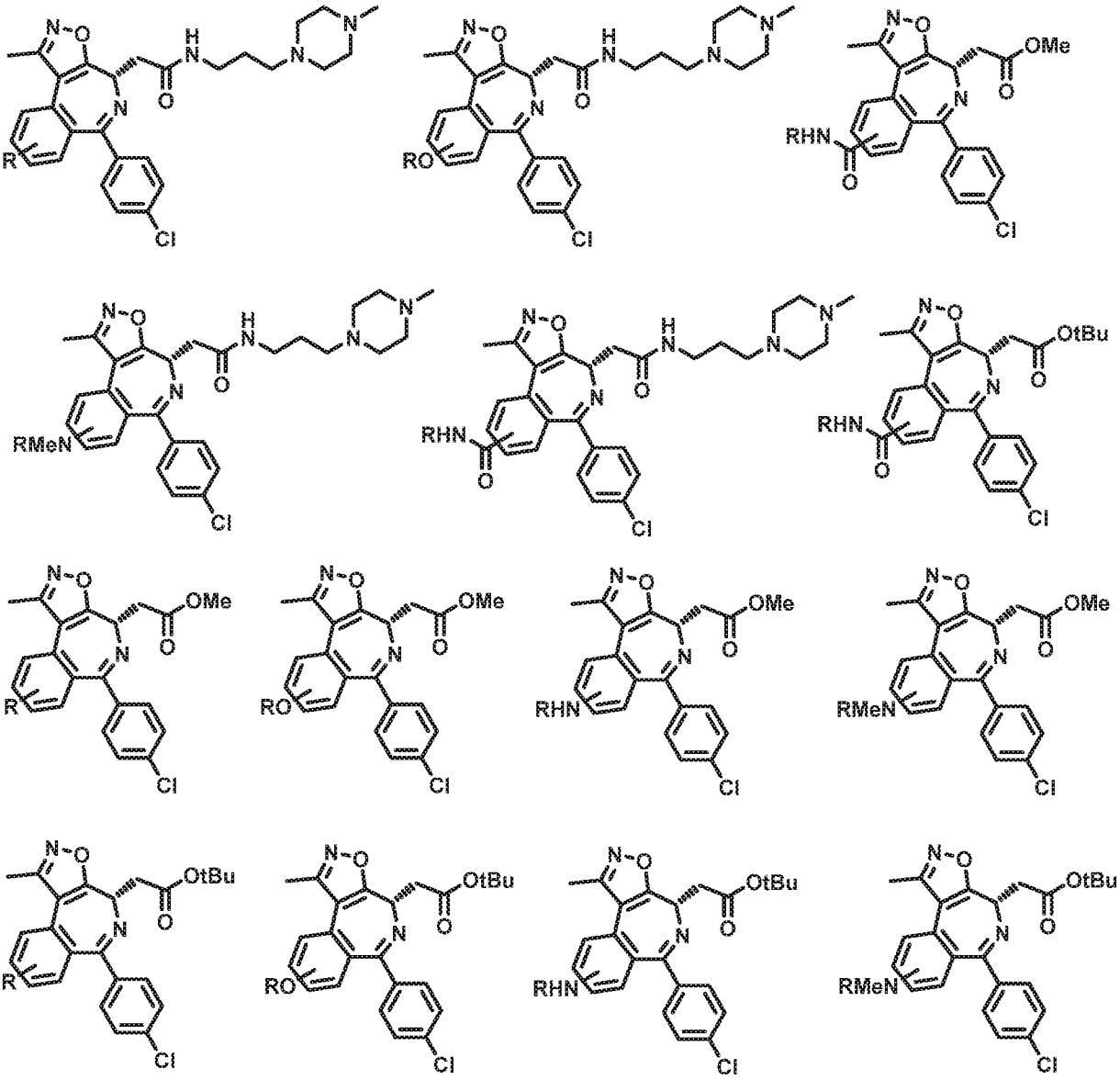


FIG. 28F

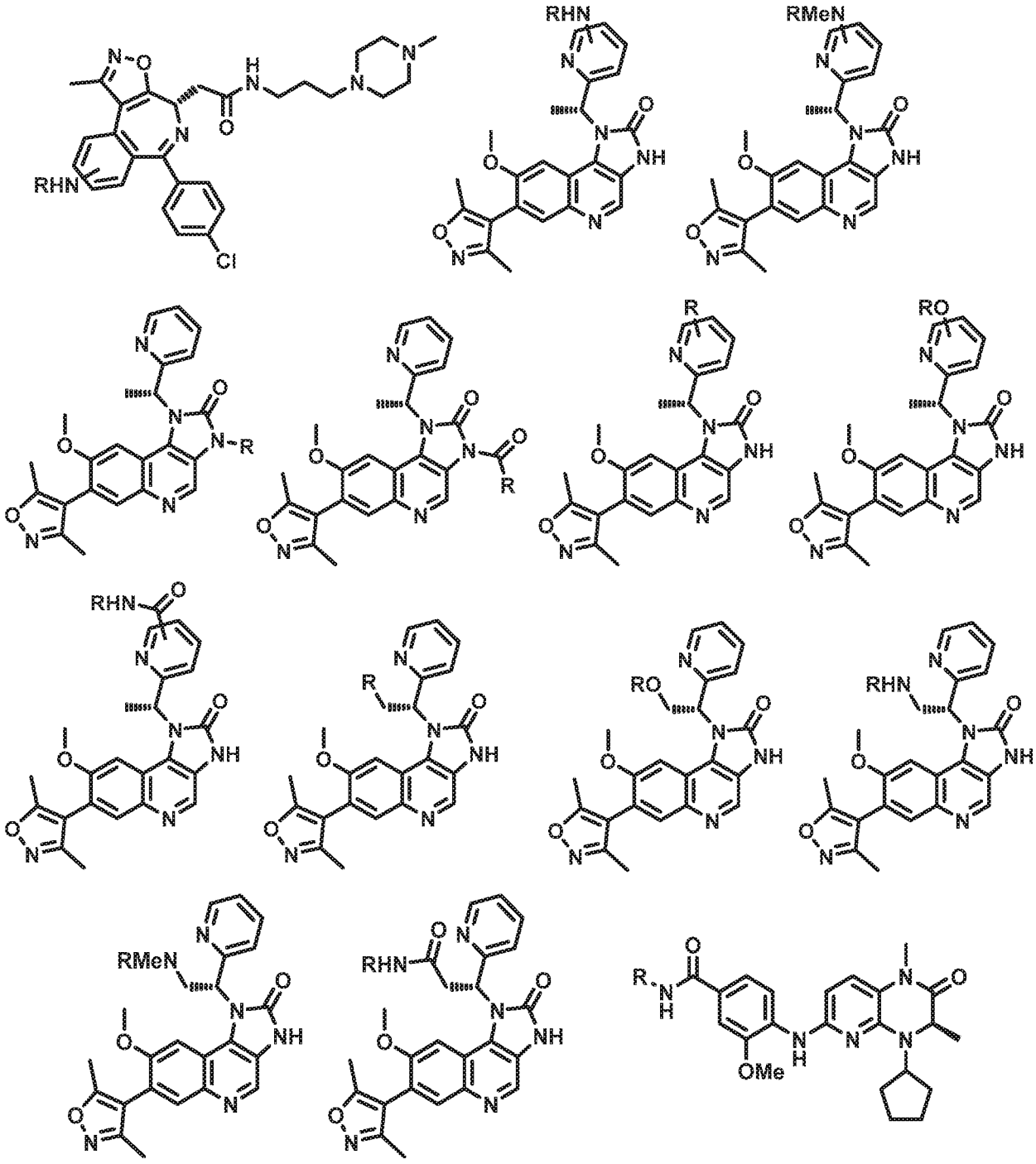


FIG. 28G

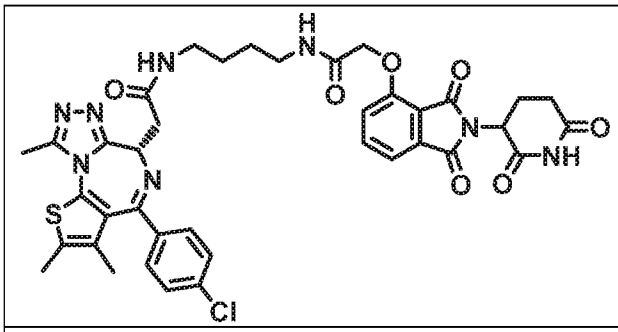
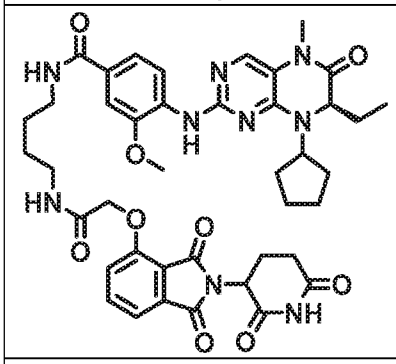
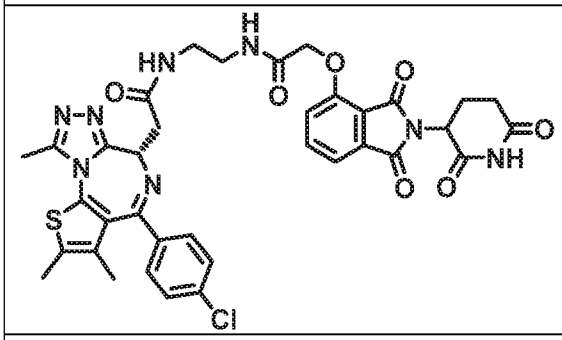
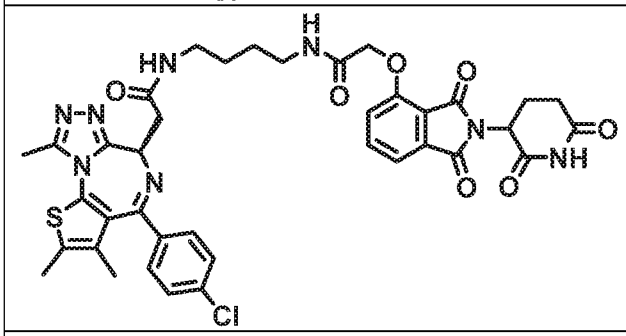
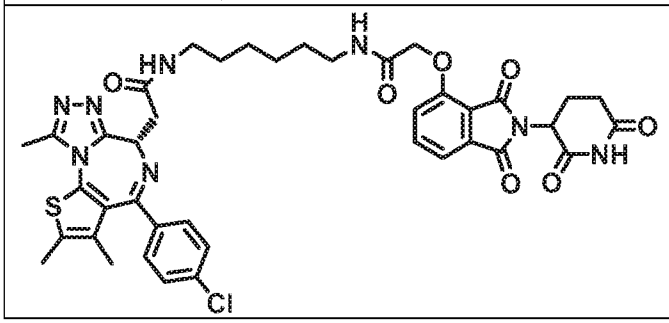
	dBET1
	dBET2
	dBET3
	dBET4
	dBET5

FIG. 29A

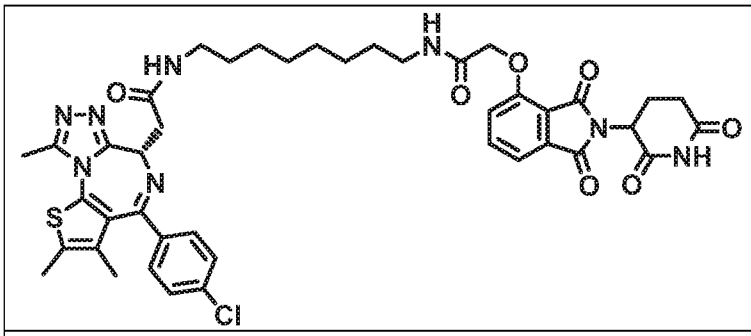
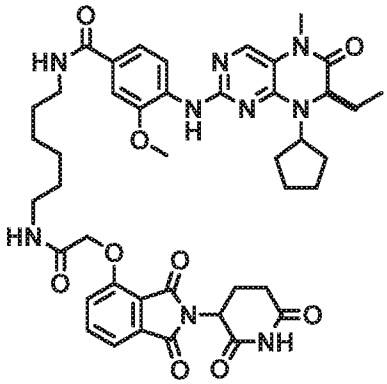
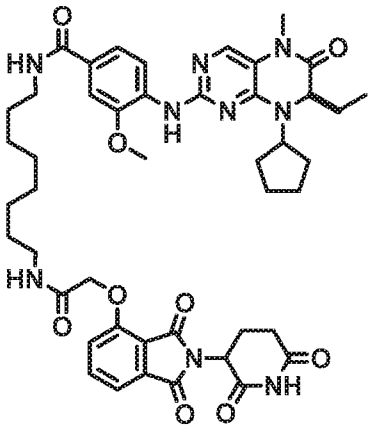
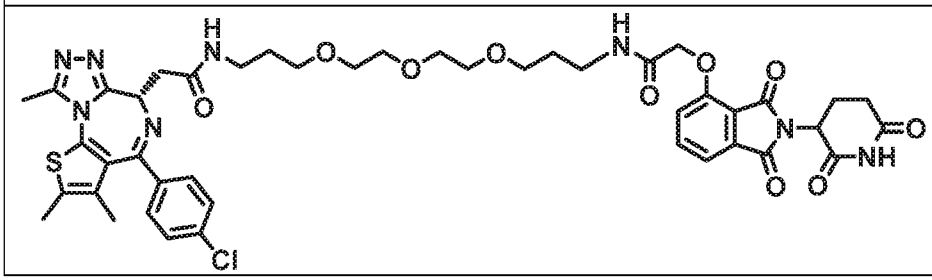
	dBET6
	dBET7
	dBET8
	dBET9

FIG. 29B

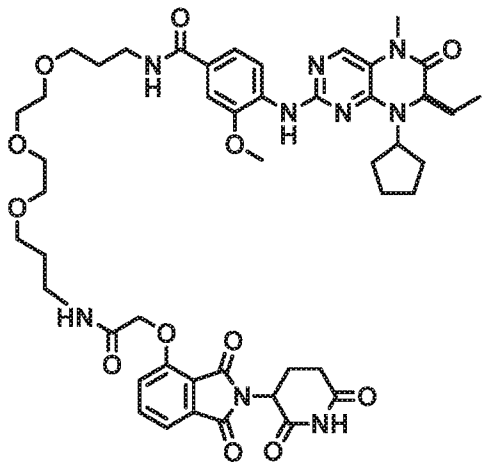
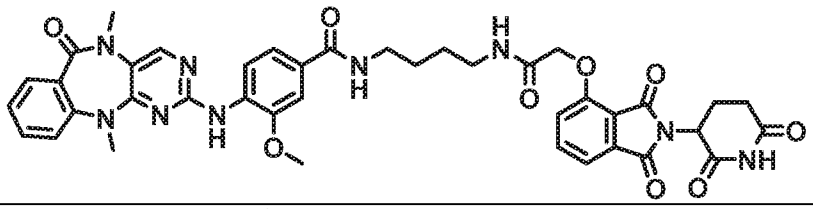
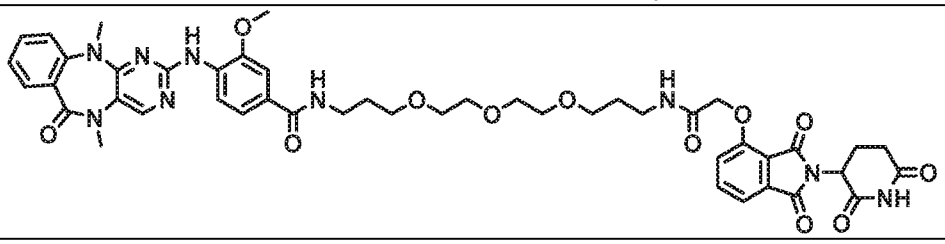
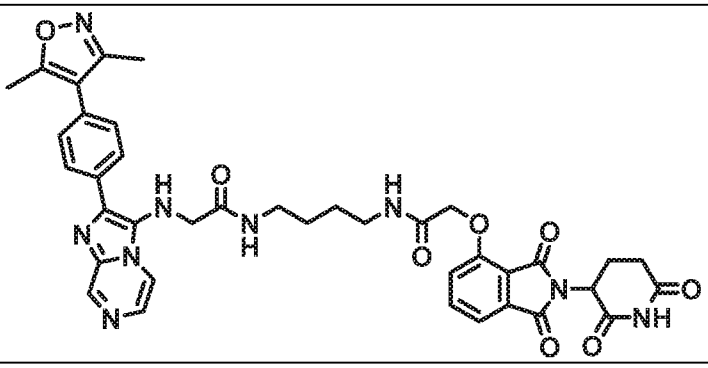
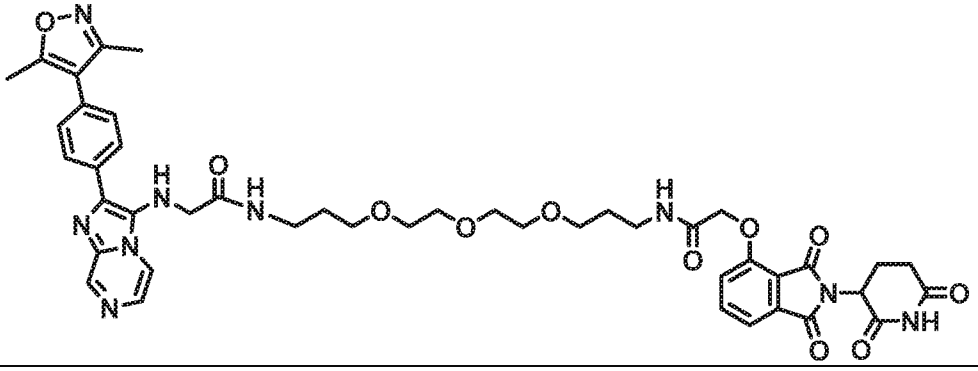
	dBET10
	dBET11
	dBET12
	dBET13
	dBET14

FIG. 29C

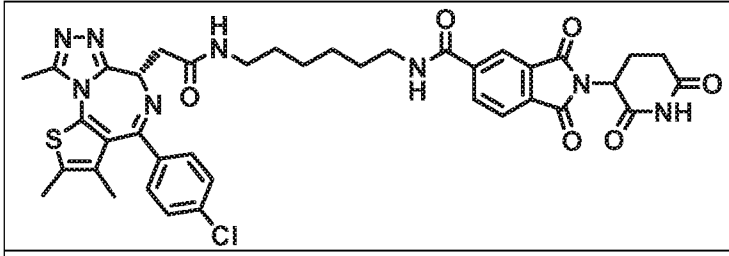
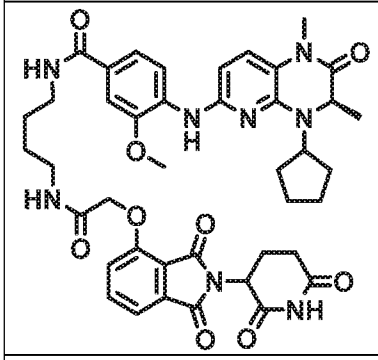
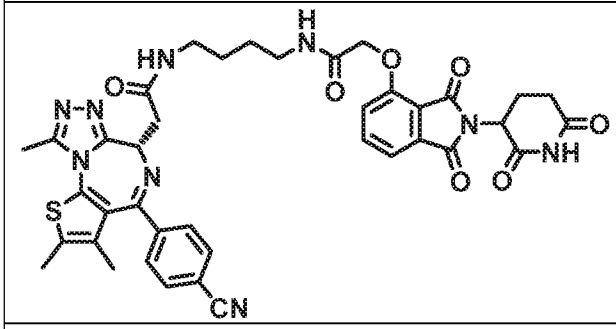
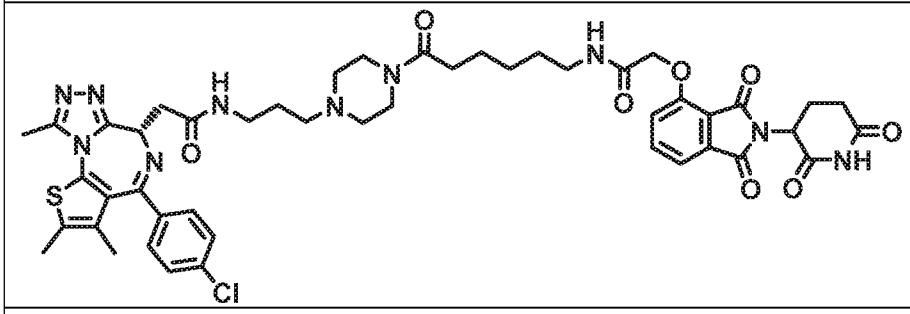
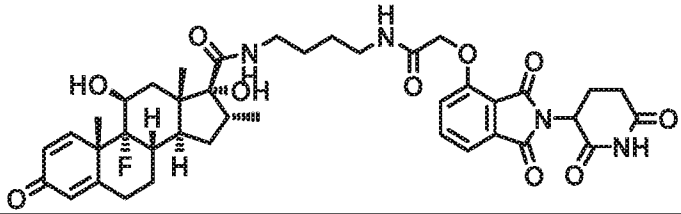
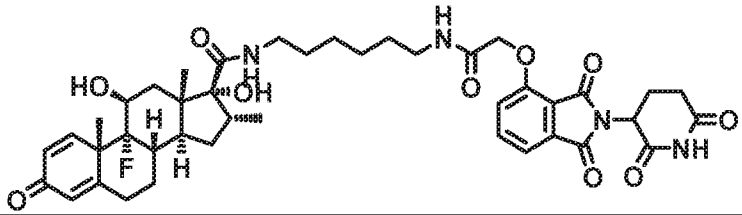
	dBET15
	dBET16
	dBET17
	dBET18
	dGR1
	dGR2

FIG. 29D

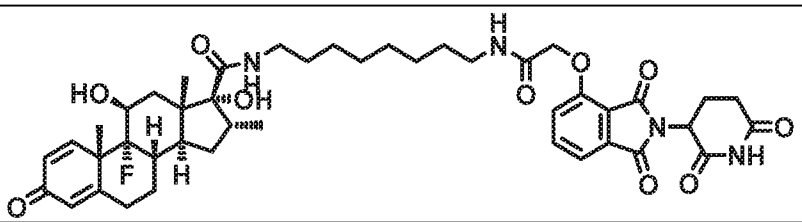
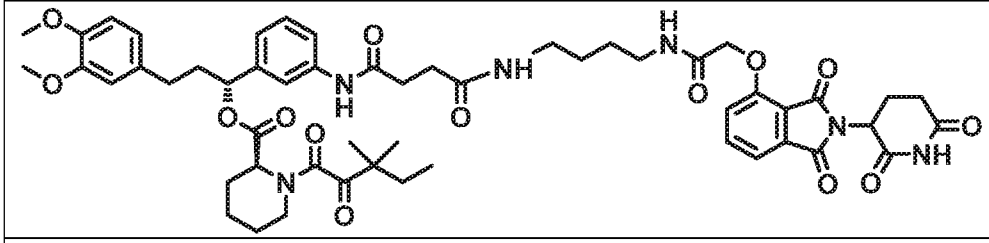
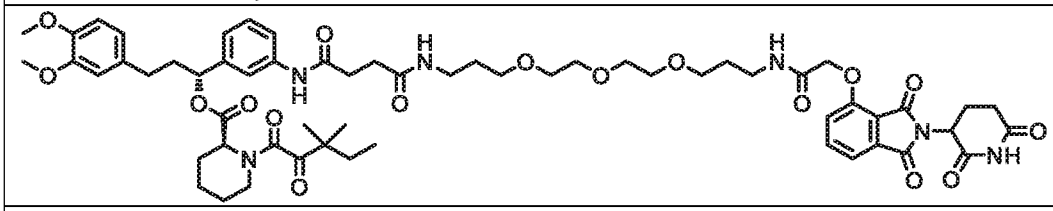
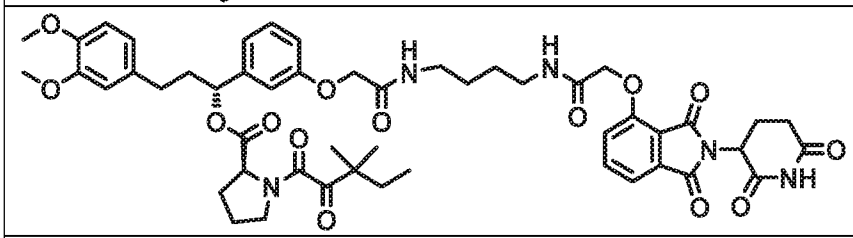
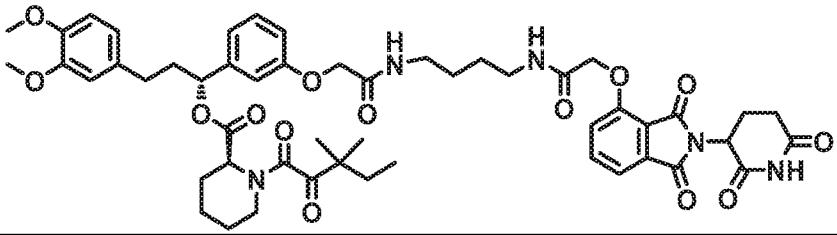
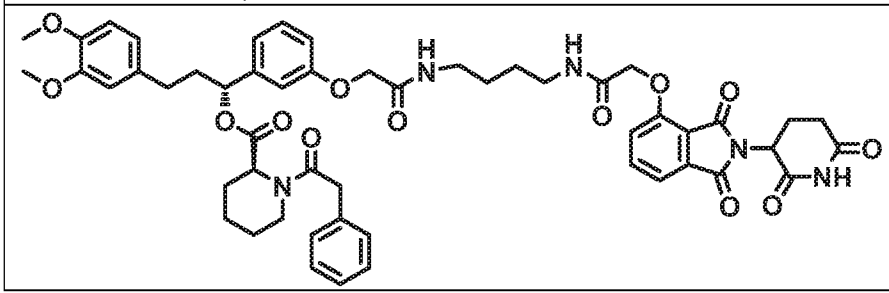
	dGR3
	dFKBP-1
	dFKBP-2
	dFKBP-3
	dFKBP-4
	dFKBP-5

FIG. 29E

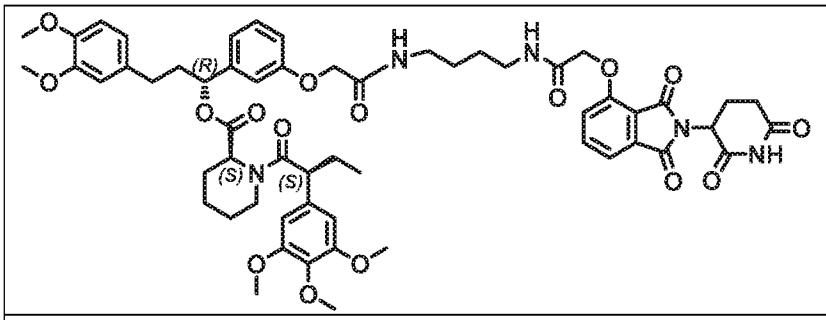
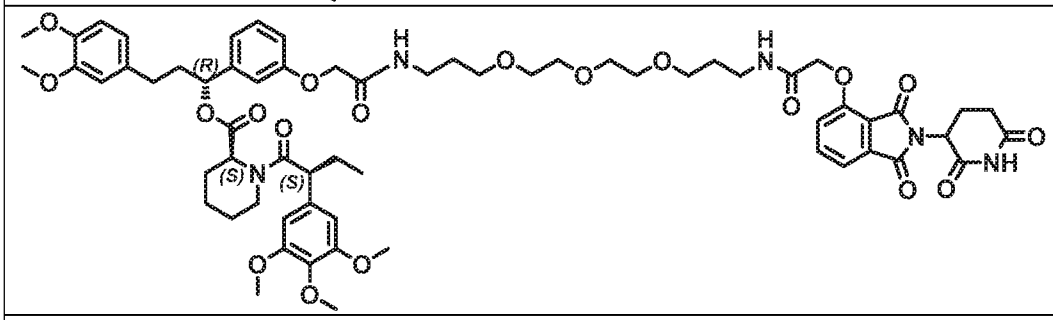
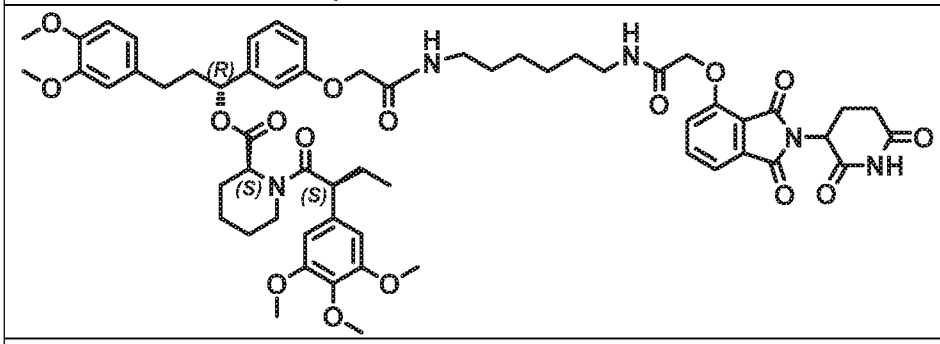
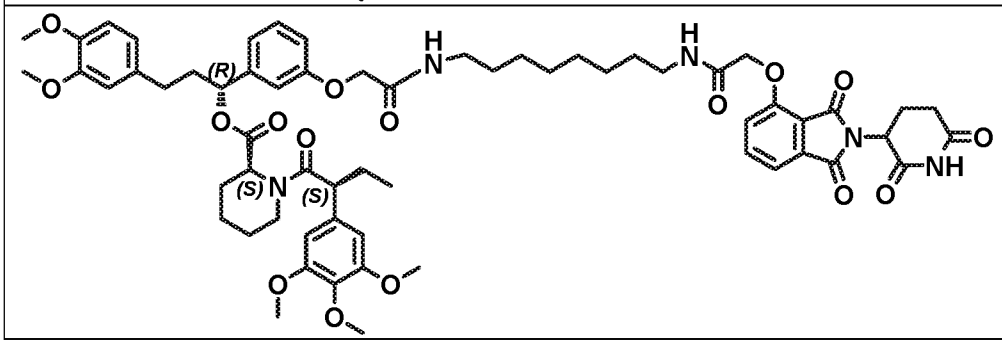
	dFKBP-6
	dFKBP-7
	dFKBP-8
	dFKBP-9

FIG. 29F

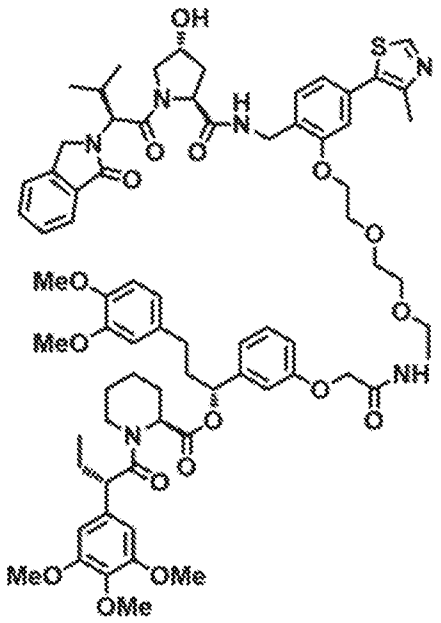
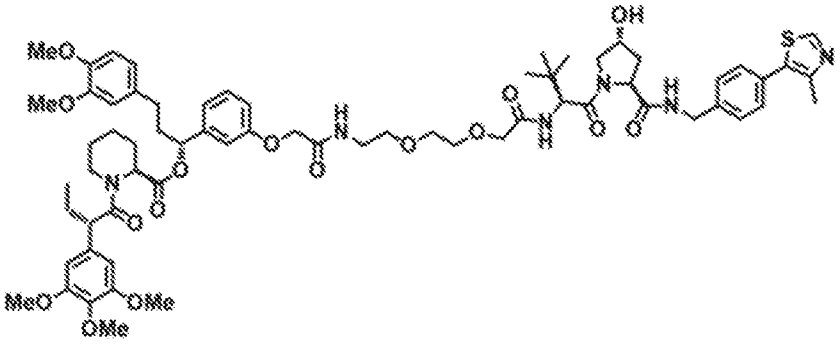
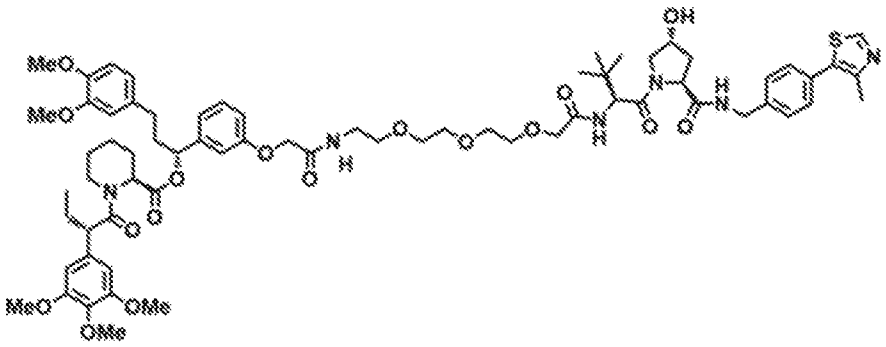
	dFKBP-10
	dFKBP-11
	dFKBP-12

FIG. 29G

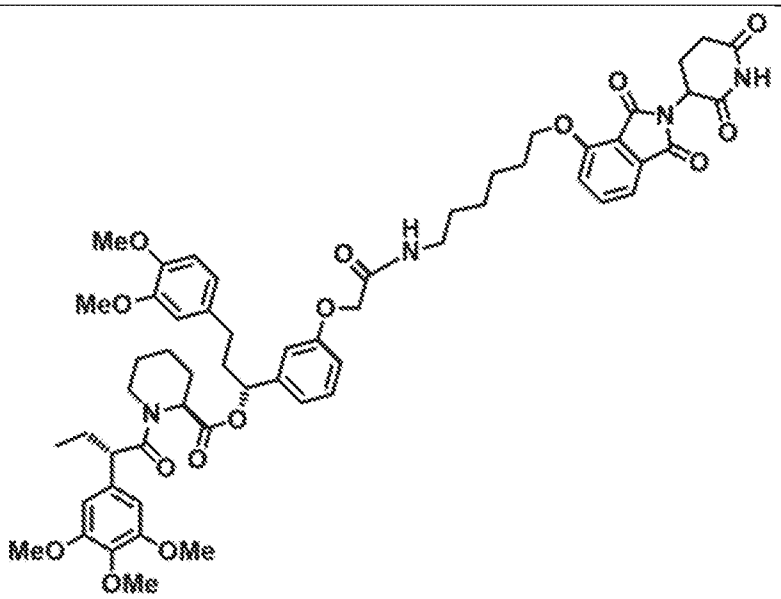
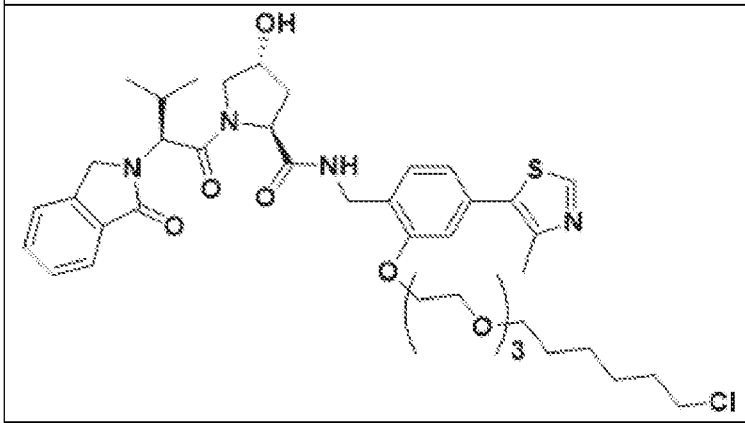
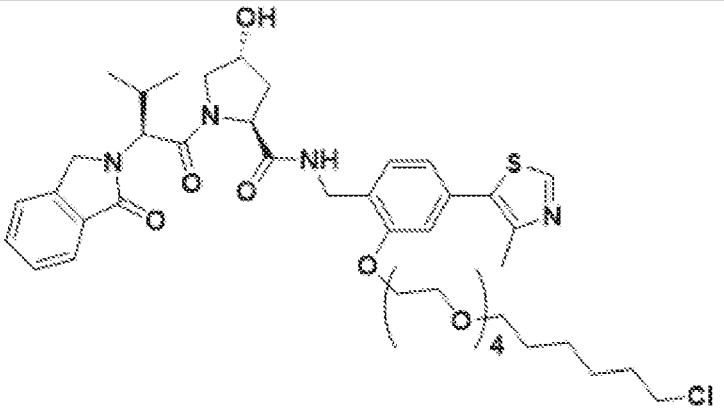
	dFKBP-13
	dHalo-1
	dHalo-2

FIG. 29H

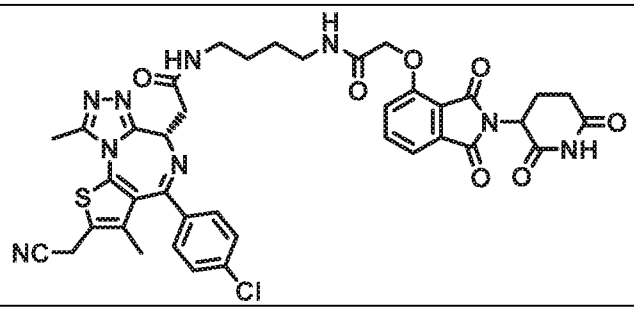
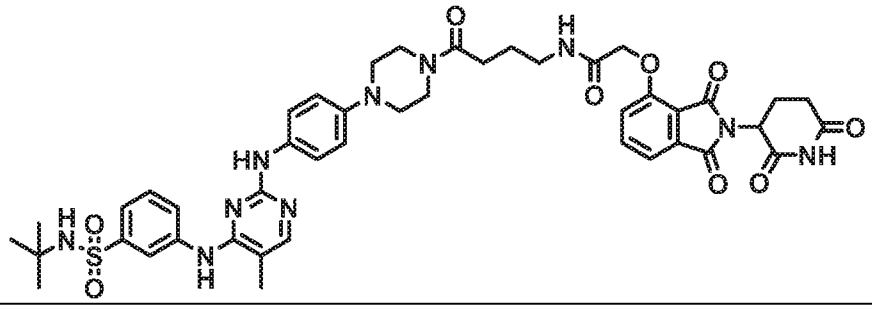
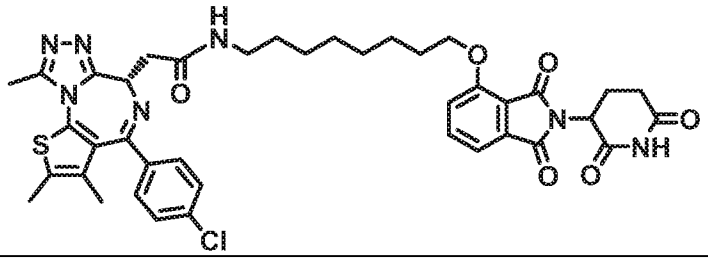
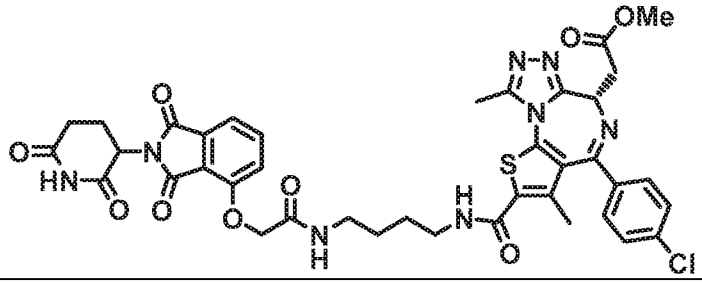
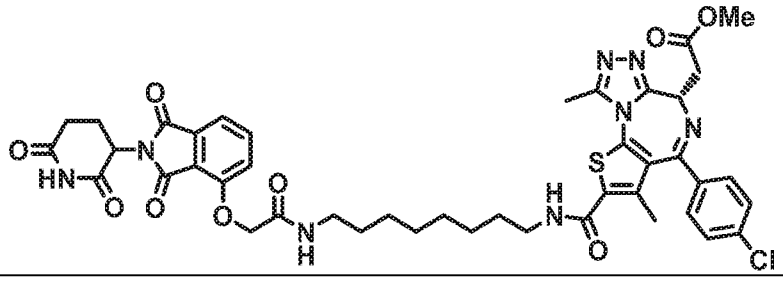
Cmpd. No.	Structure
dBET19	
dBET20	
dBET21	
dBET22	
dBET23	

FIG. 30A

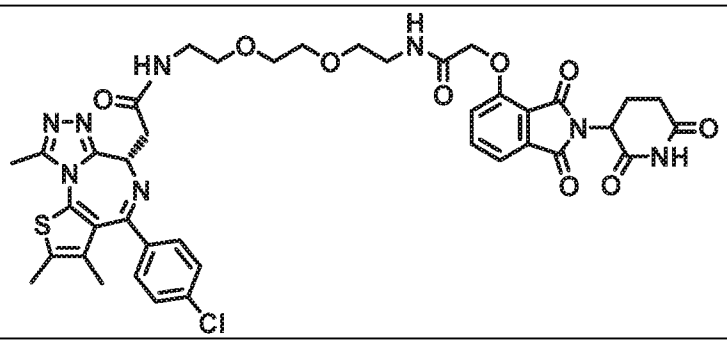
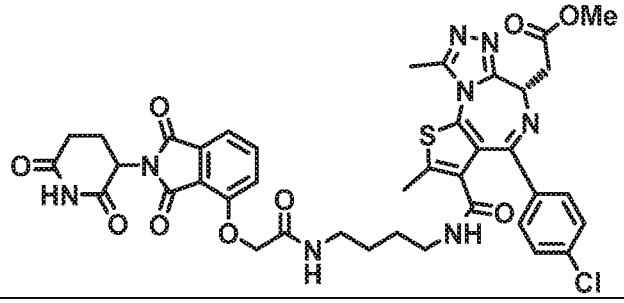
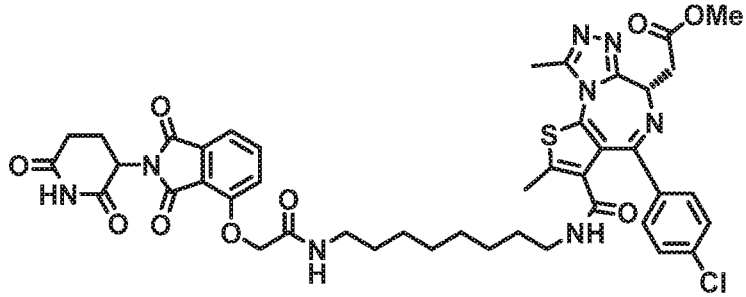
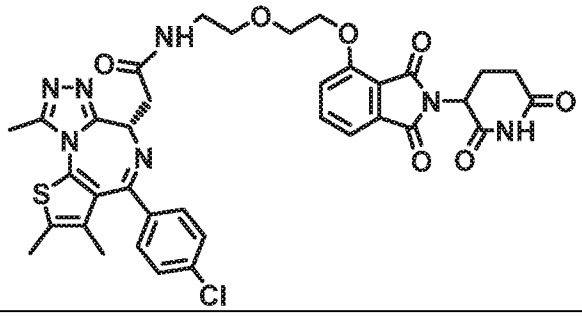
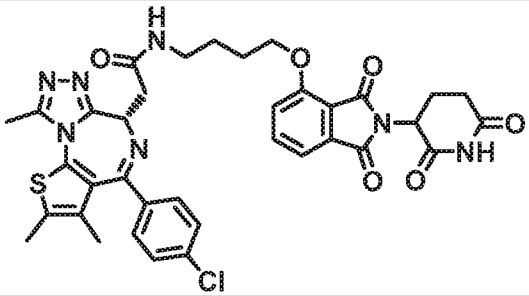
dBET24	
dBET25	
dBET26	
dBET27	
dBET28	

FIG. 30B

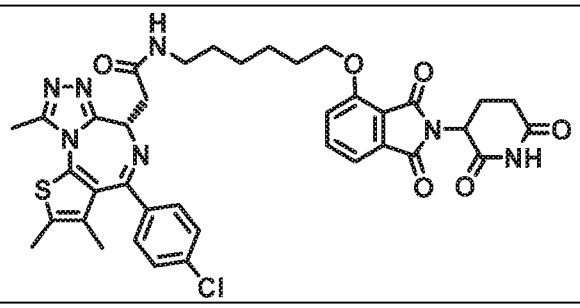
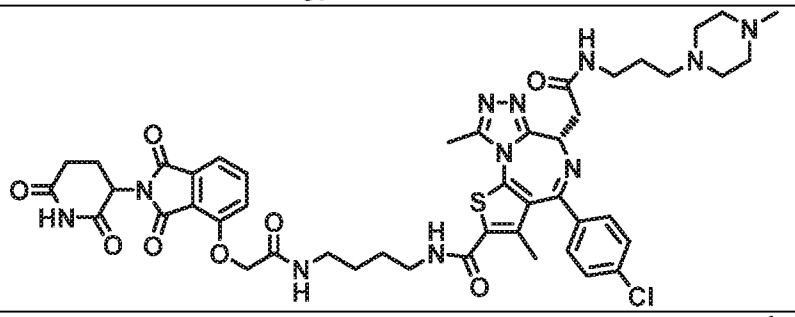
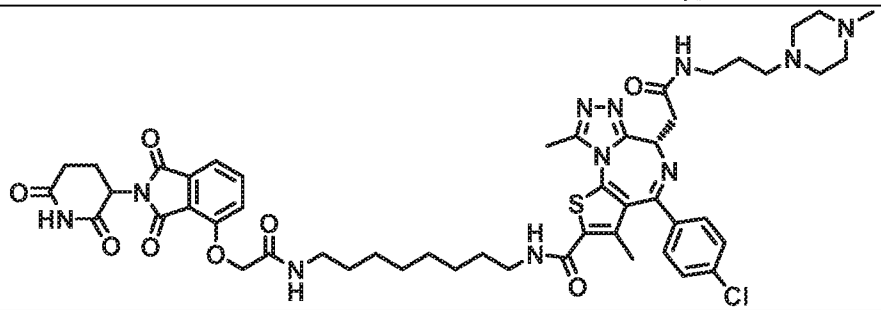
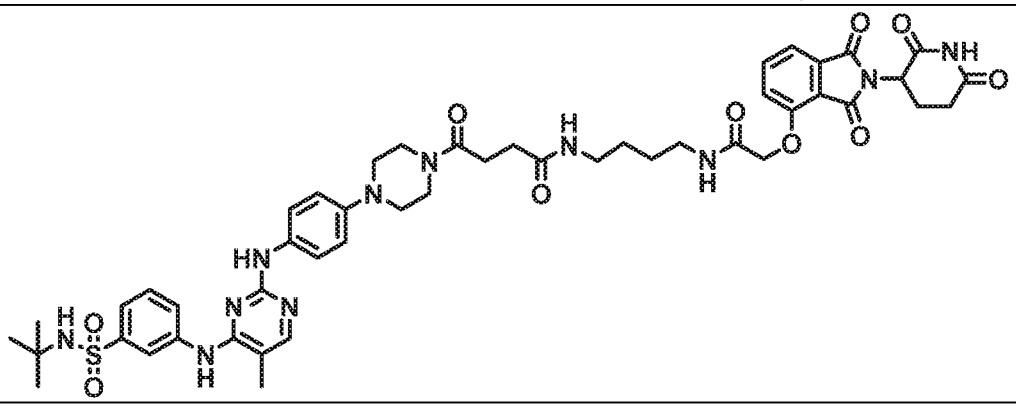
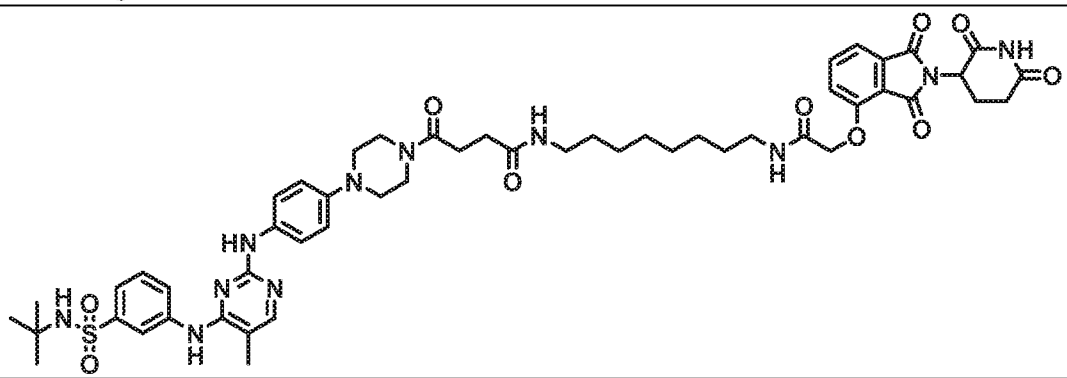
dBET29	
dBET30	
dBET31	
dBET32	
dBET33	

FIG. 30C

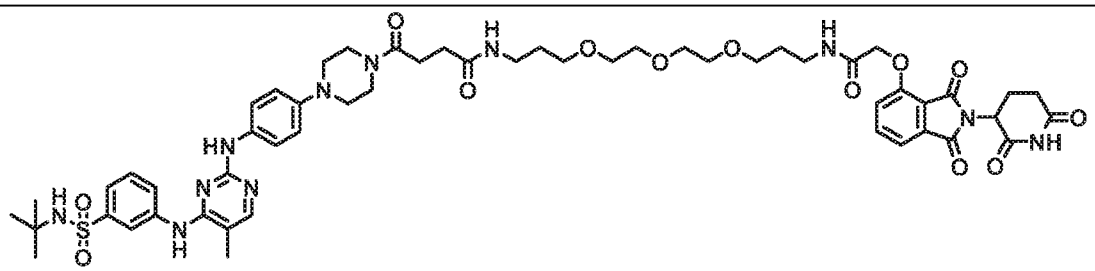
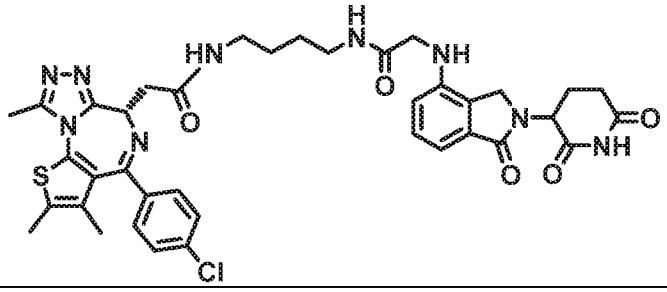
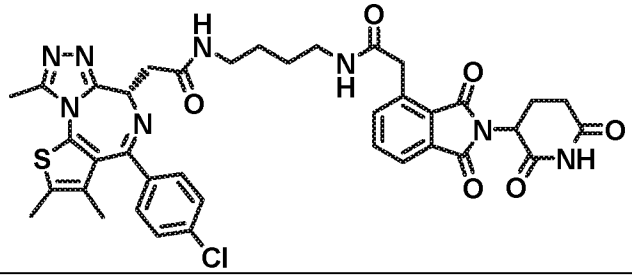
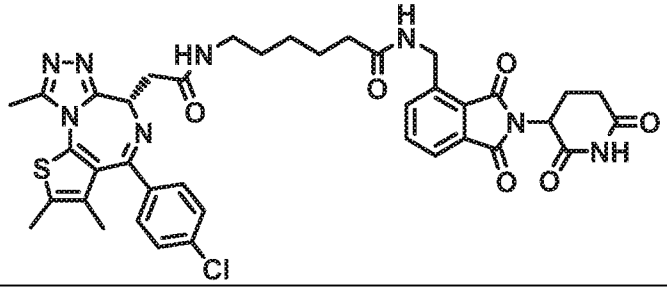
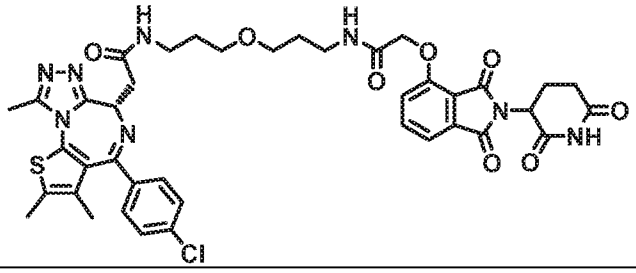
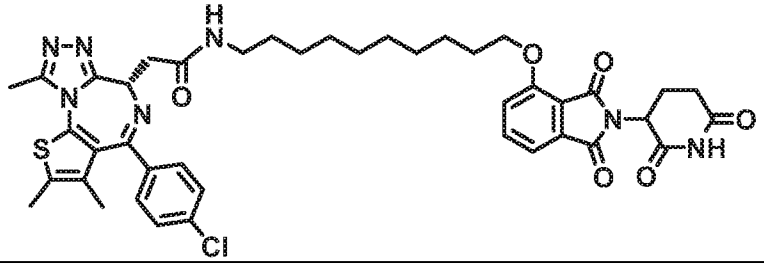
dBET34	
dBET35	
dBET36	
dBET37	
dBET38	
dBET39	

FIG. 30D

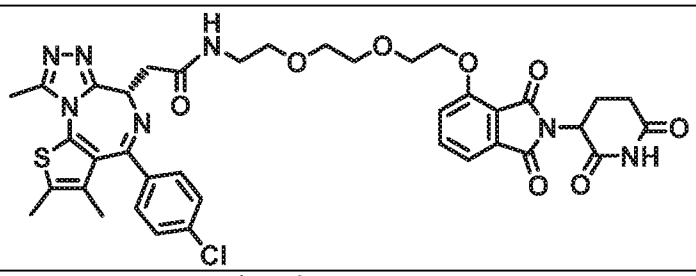
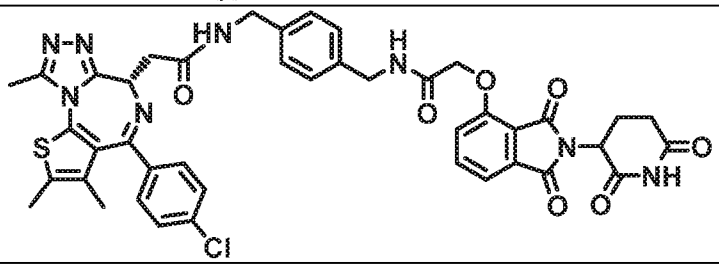
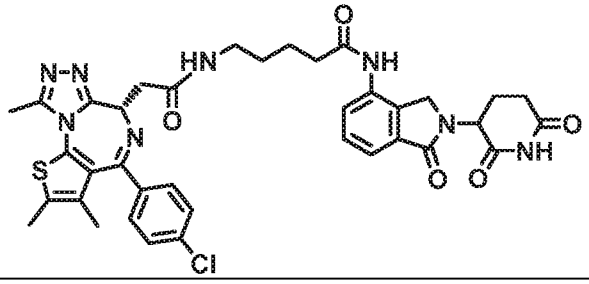
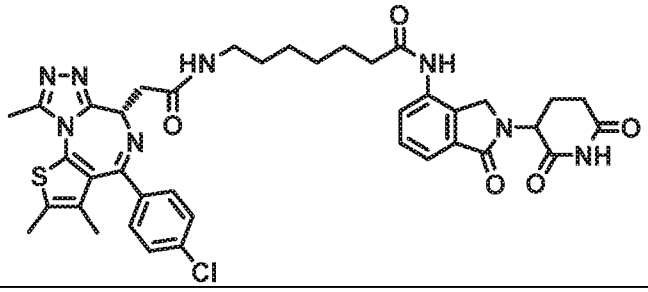
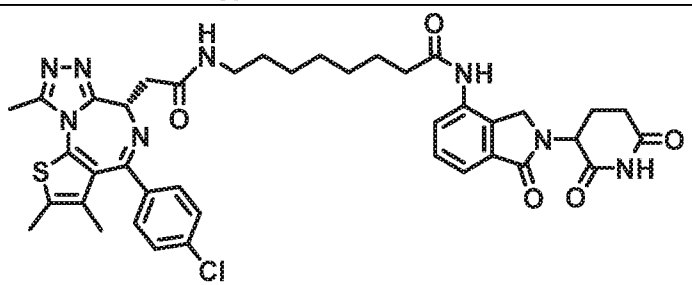
dBET40	
dBET41	
dBET42	
dBET43	
dBET44	

FIG. 30E

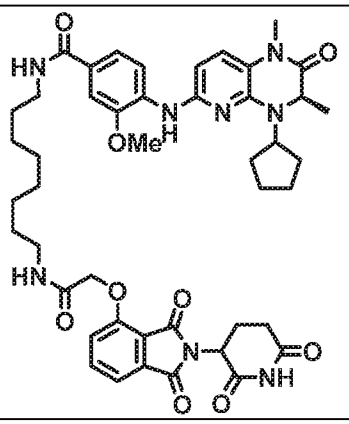
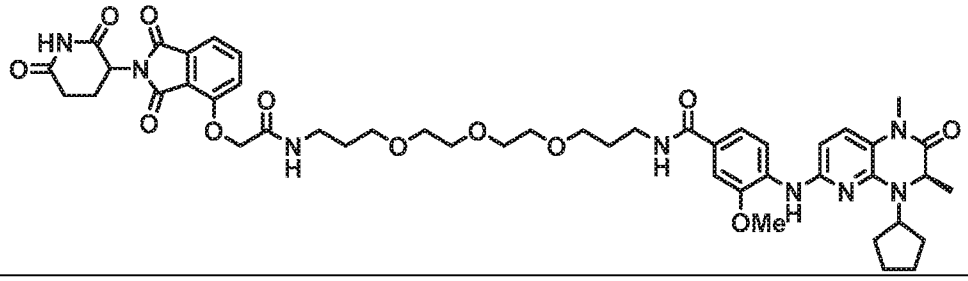
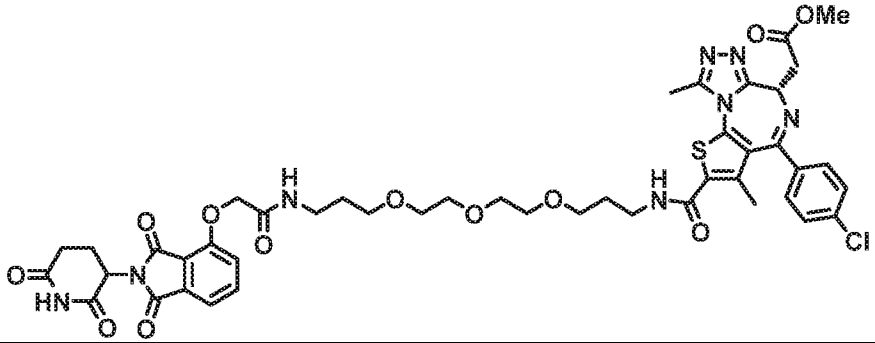
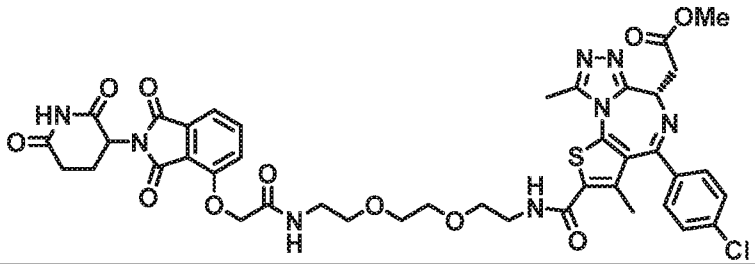
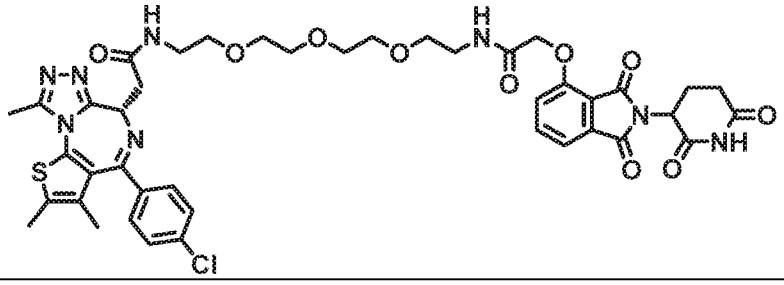
dBET45	
dBET46	
dBET50	
dBET51	
dBET52	

FIG. 30F

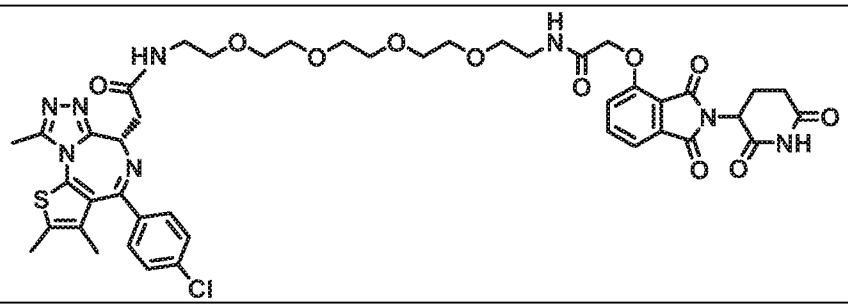
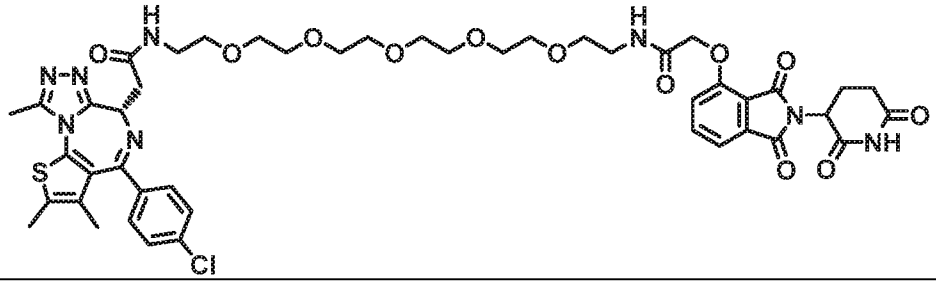
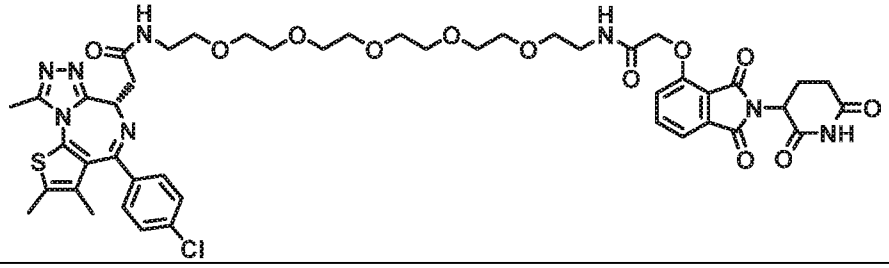
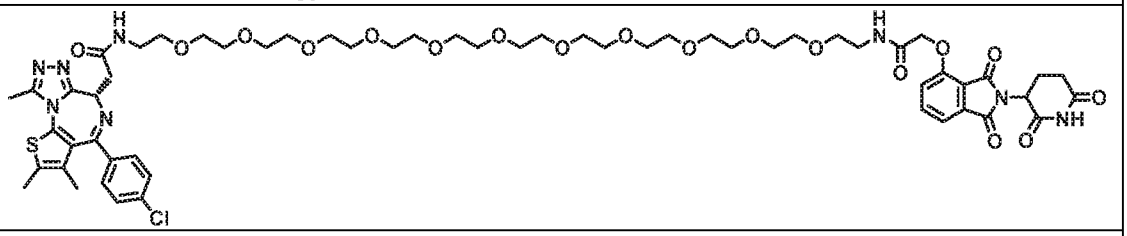
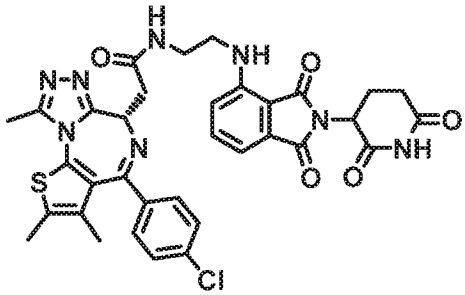
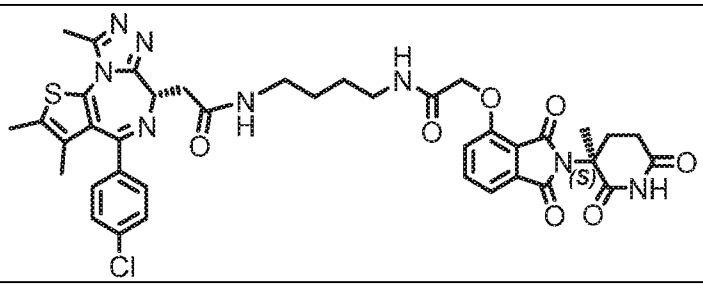
dBET53	
dBET54	
dBET55	
dBET56	
dBET57	
dBET58	

FIG. 30G

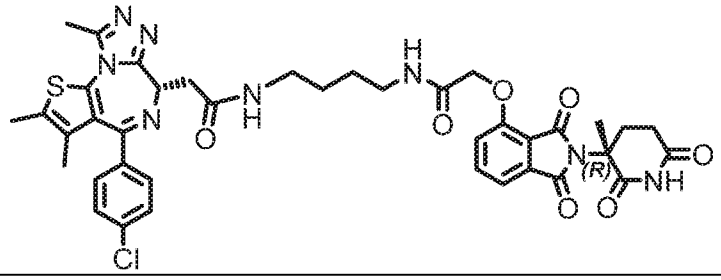
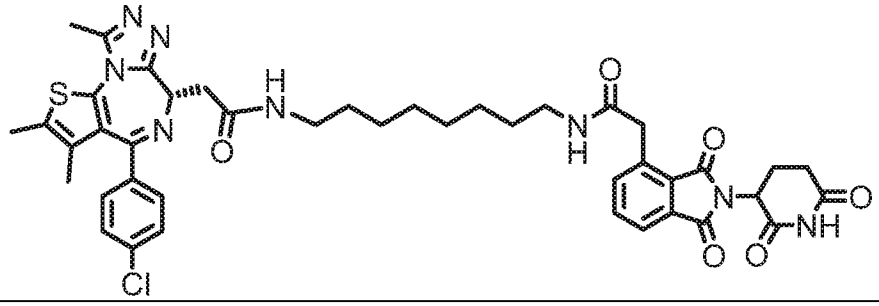
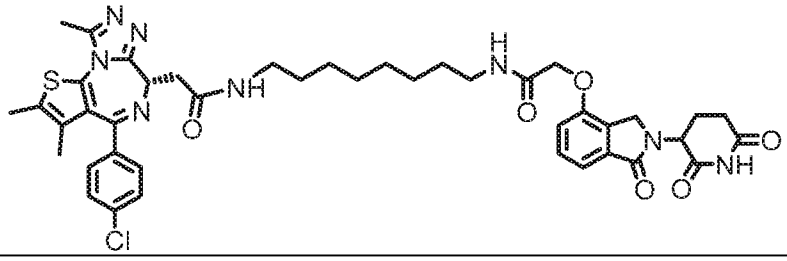
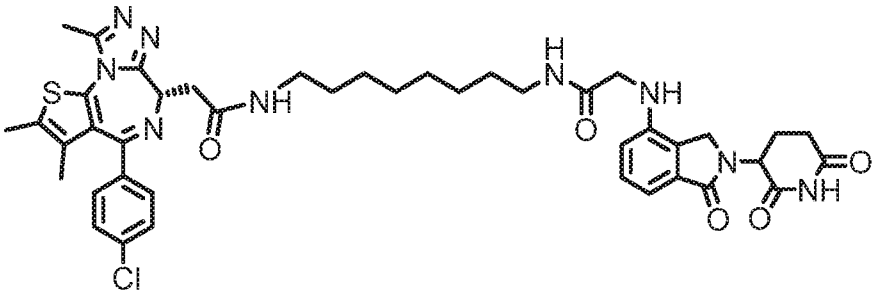
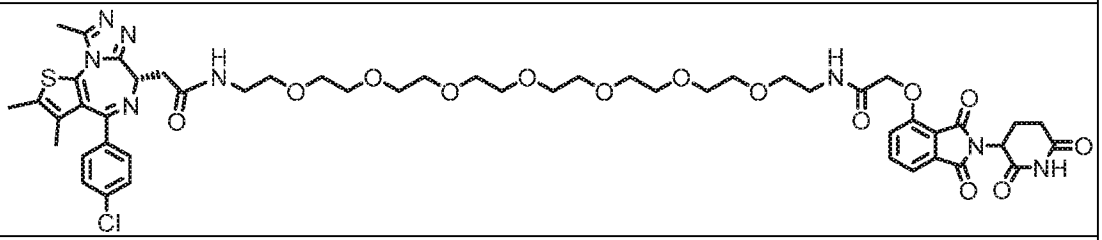
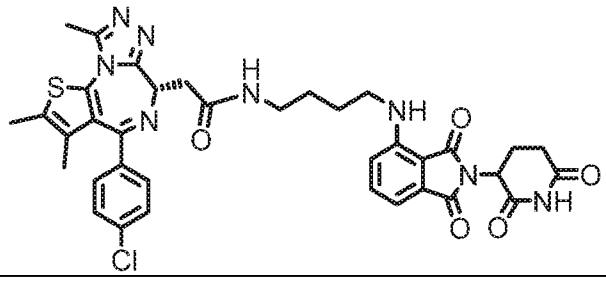
dBET59	
dBET60	
dBET61	
dBET62	
dBET63	
dBET64	

FIG. 30H

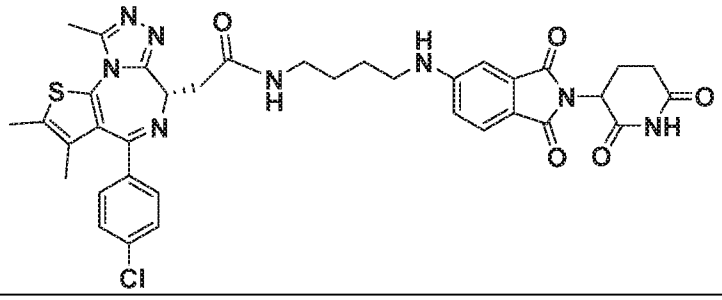
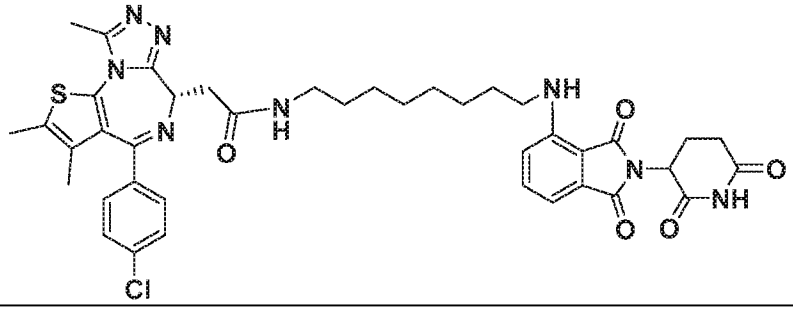
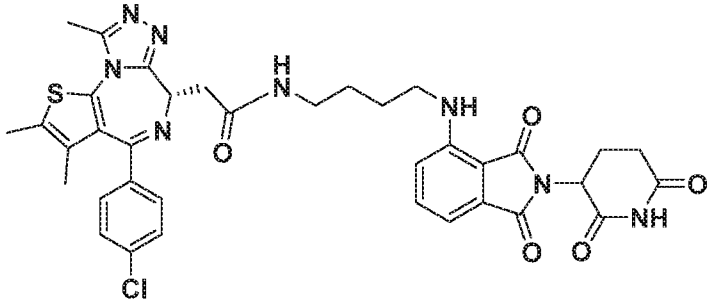
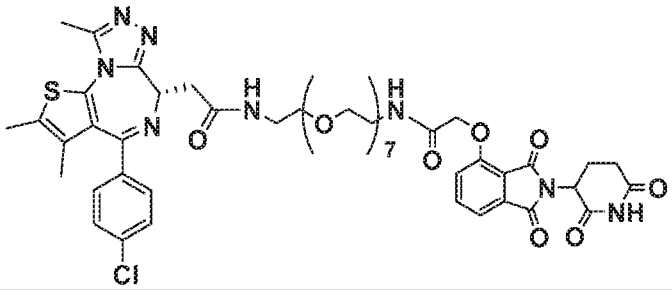
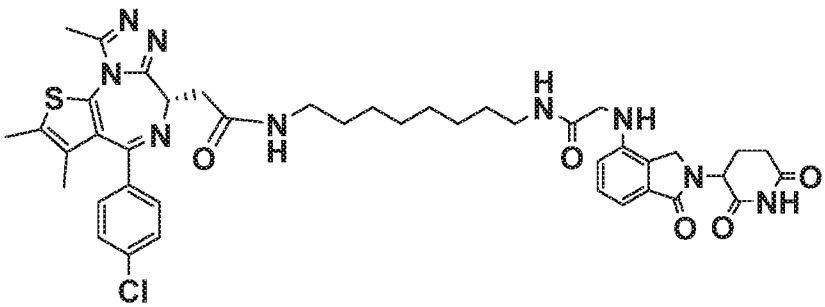
dBET100	
dBET101	
dBET102	
dBET103	
dBET104	

FIG. 30I

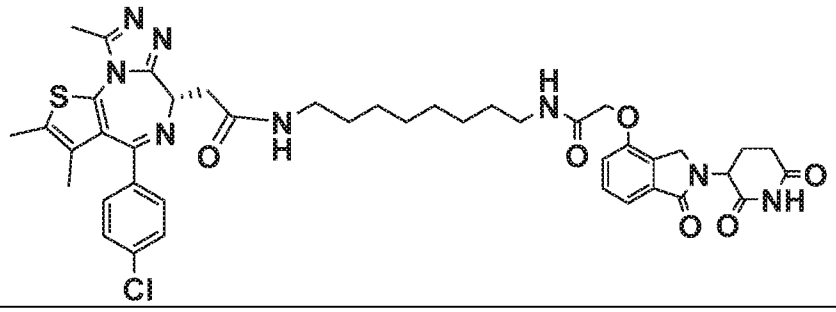
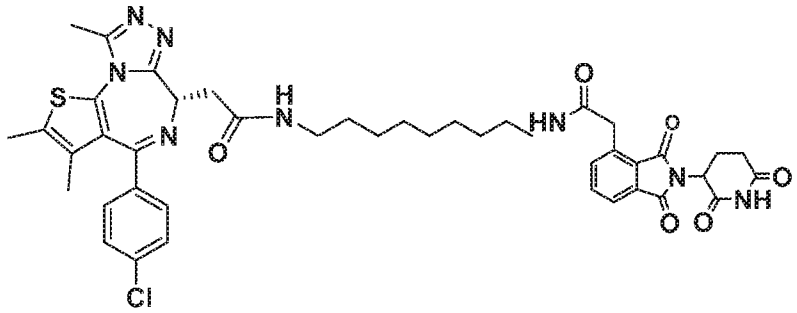
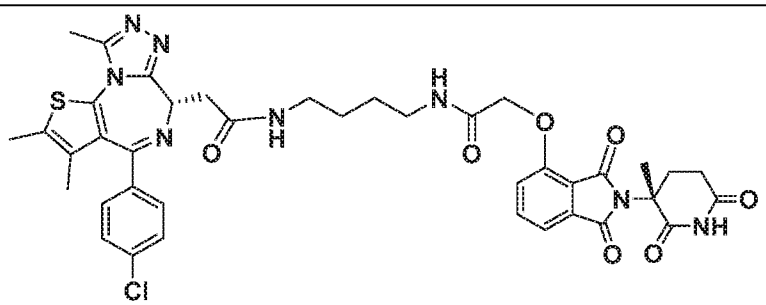
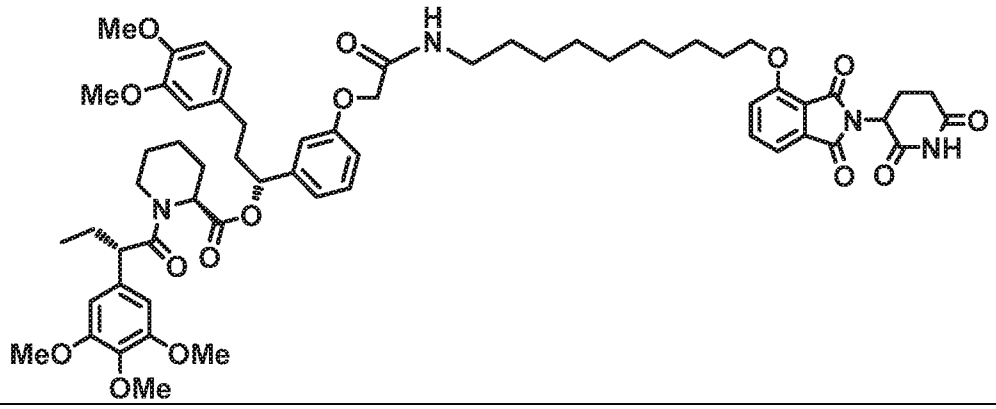
dBET105	
dBET106	
dBET107	
dFKBP-14	

FIG. 30J

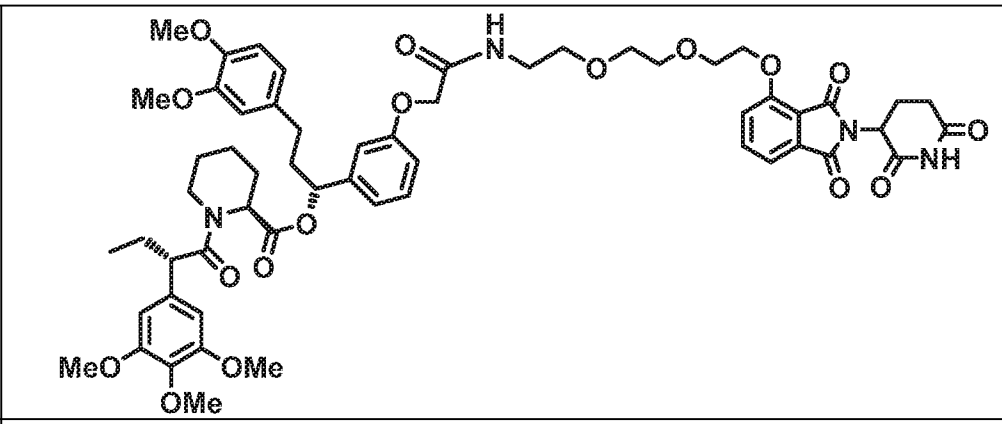
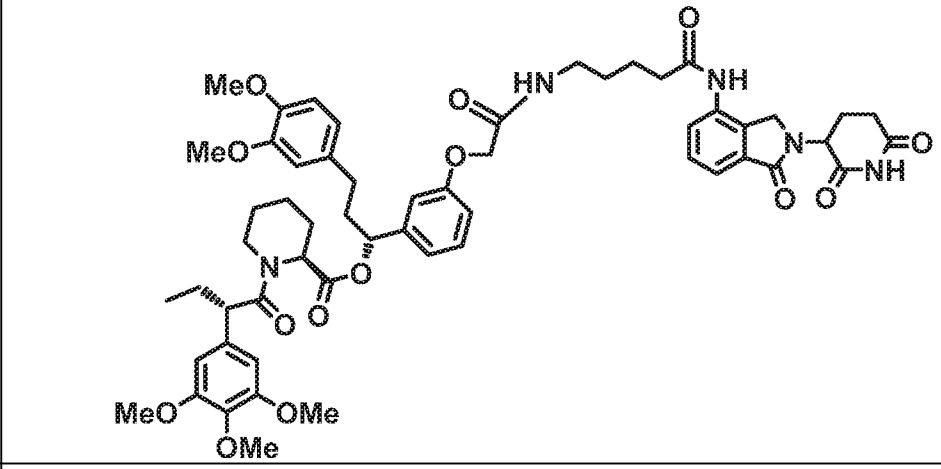
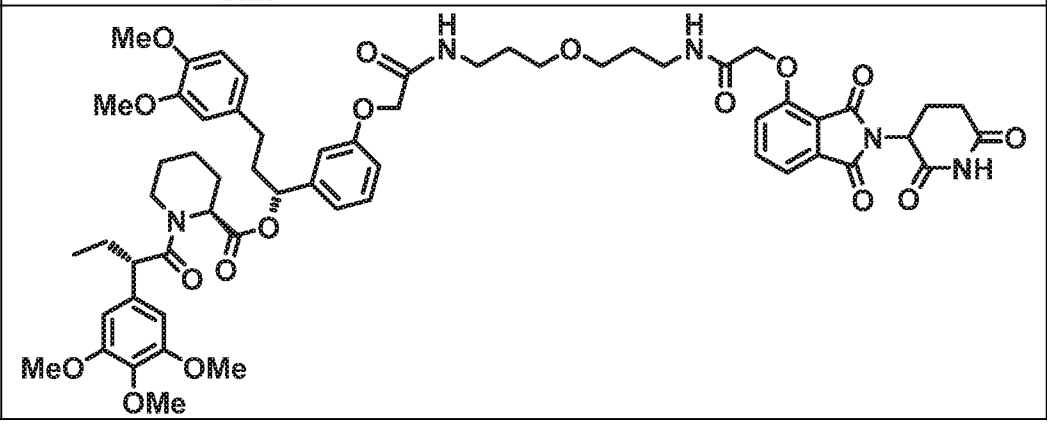
dFKBP-15	
dFKBP-16	
dFKBP-17	

FIG. 30K

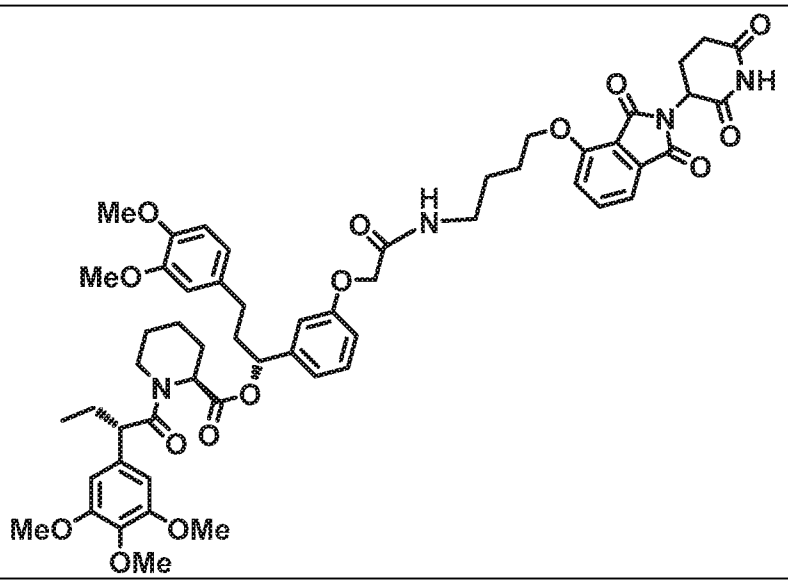
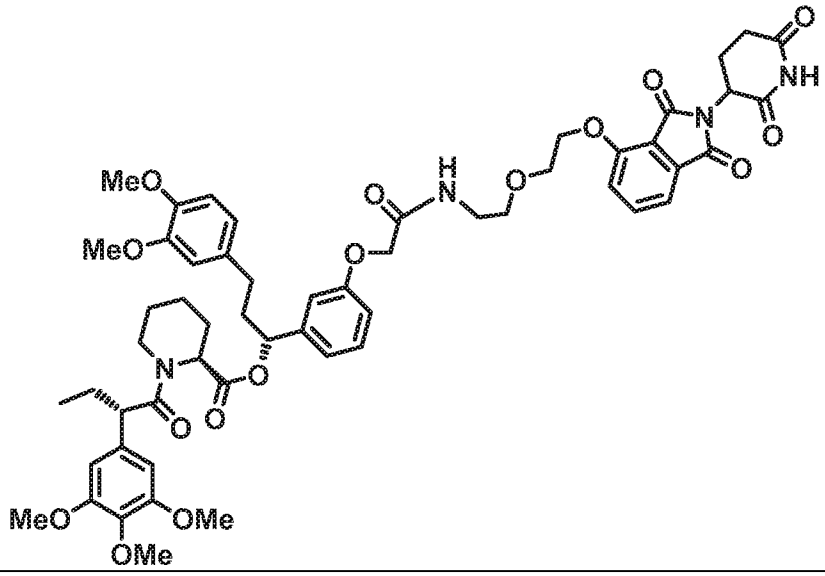
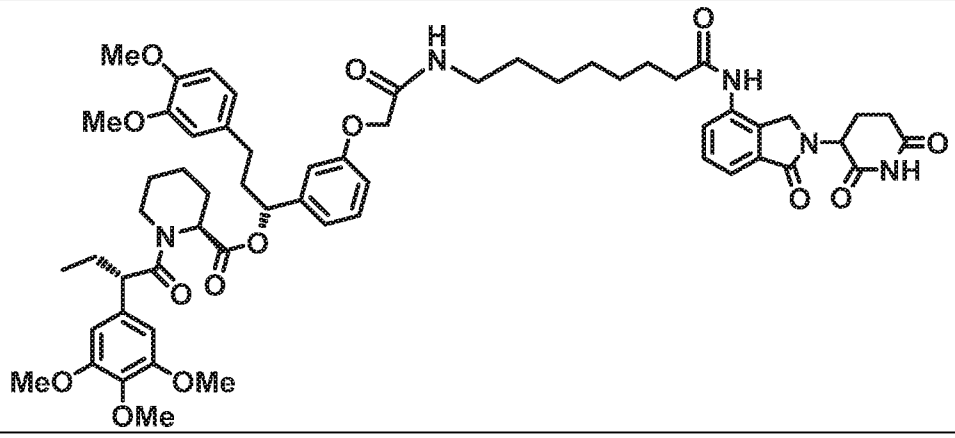
dFKBP-18	
dFKBP-19	
dFKBP-20	

FIG. 30L

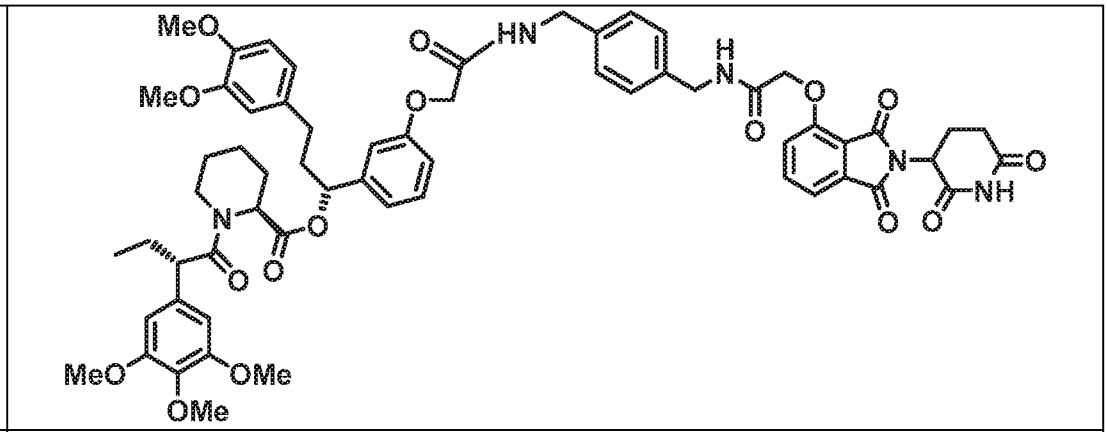
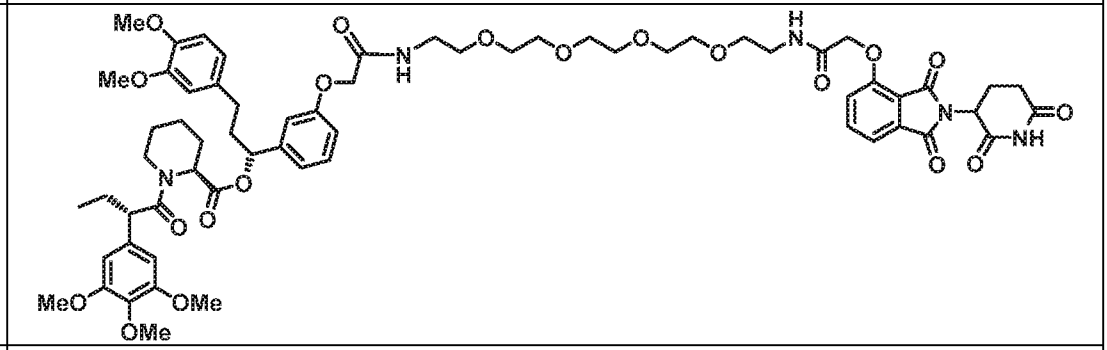
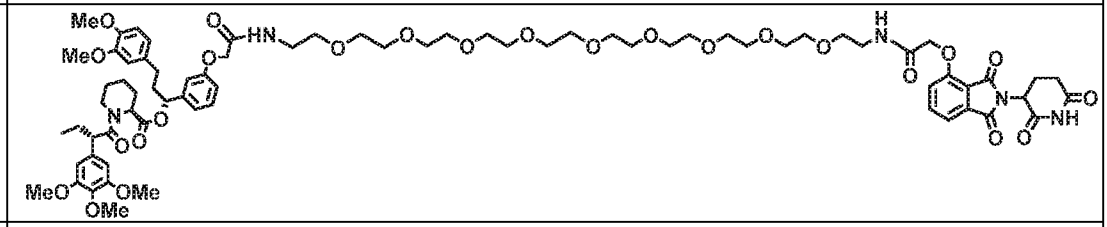
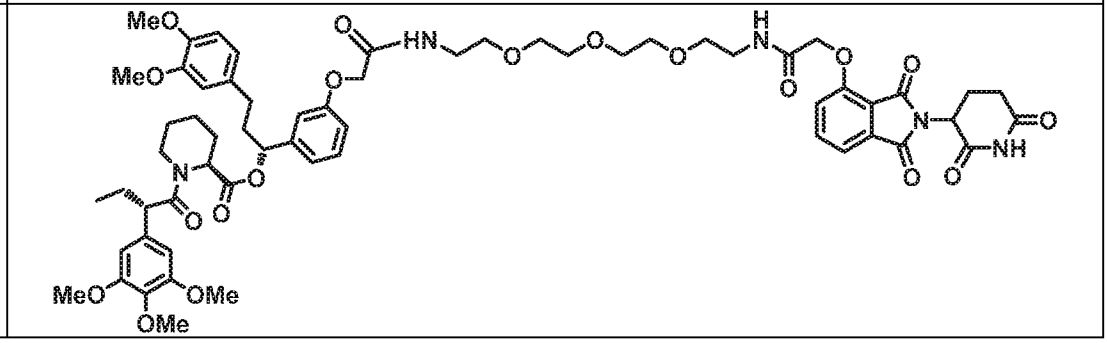
dFKBP-21	
dFKBP-24	
dFKBP-25	
dFKBP-26	

FIG. 30M

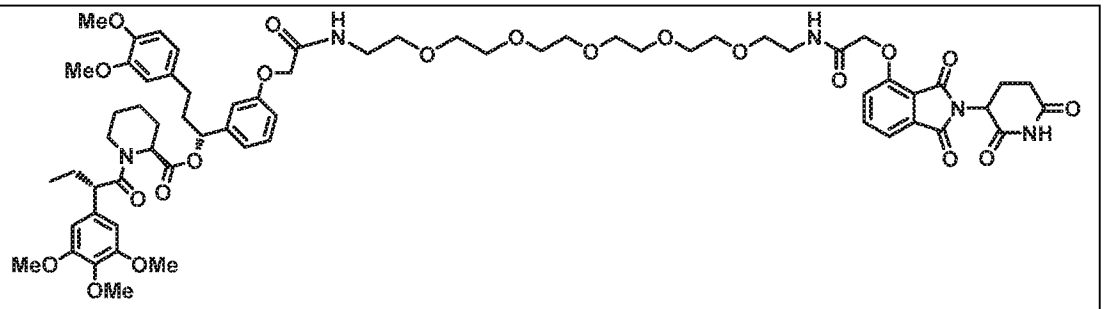
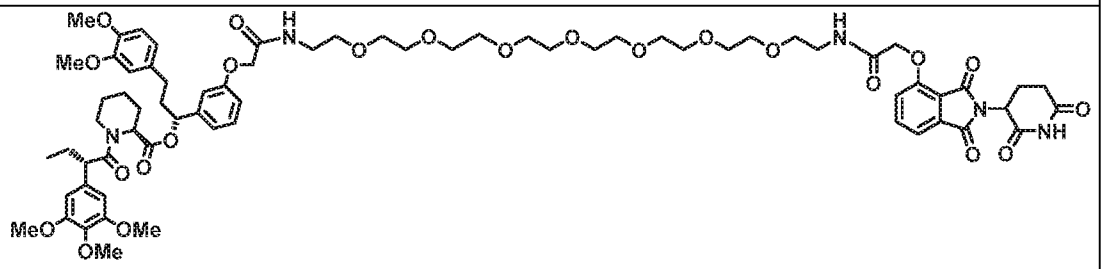
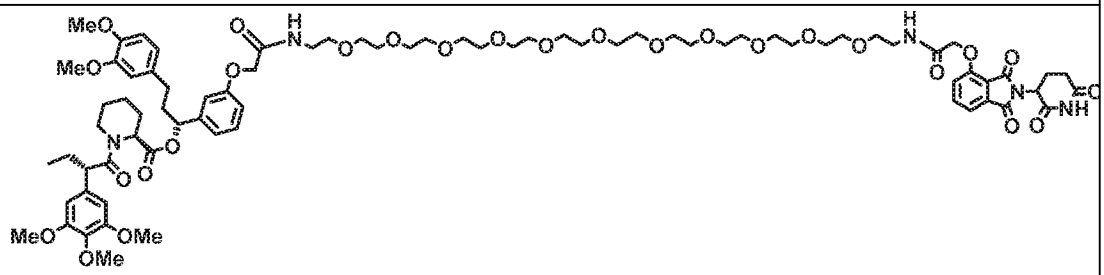
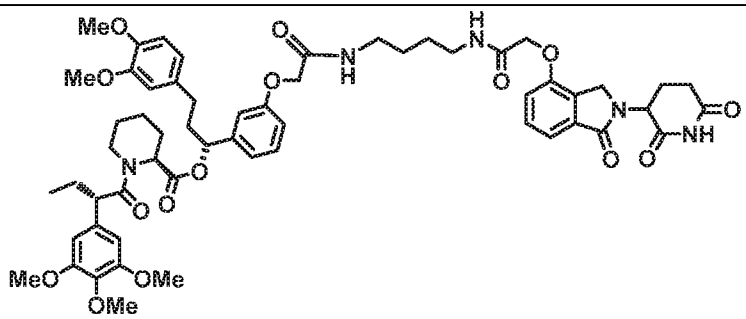
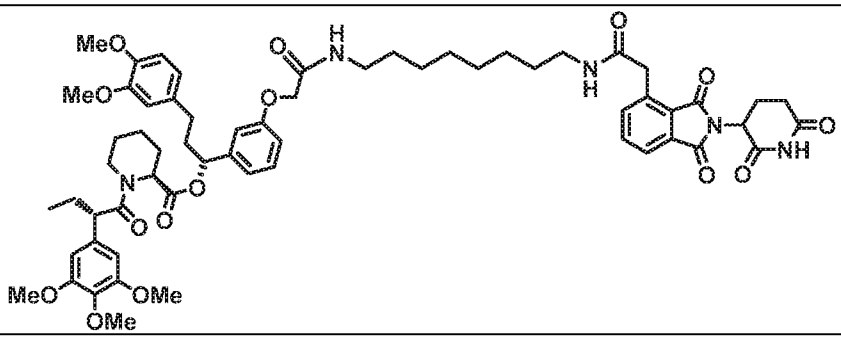
dFKBP-27	
dFKBP-28	
dFKBP-29	
dFKBP-30	
dFKBP-31	

FIG. 30N

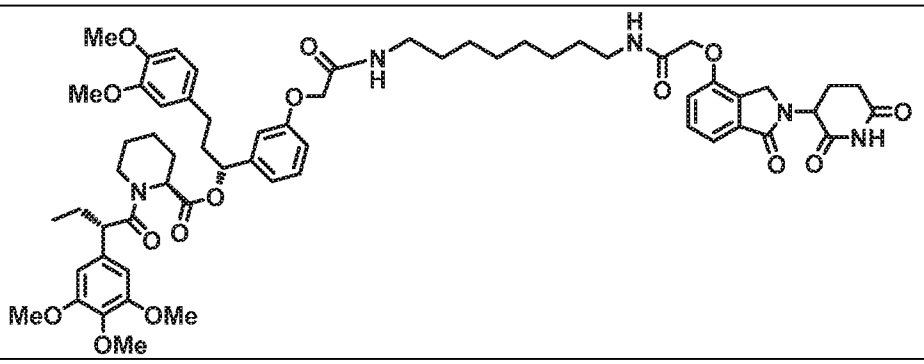
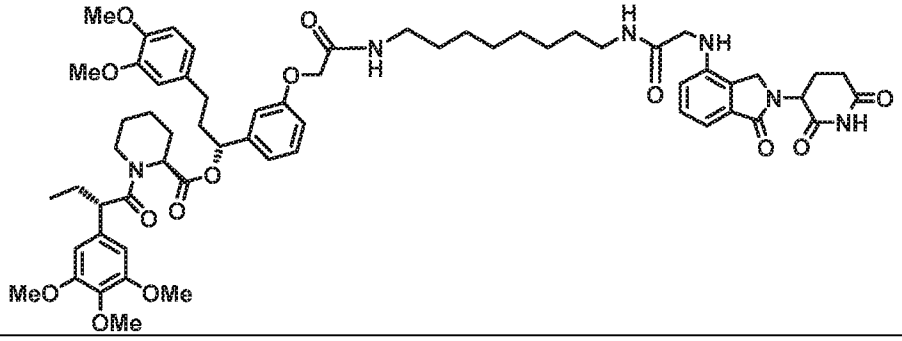
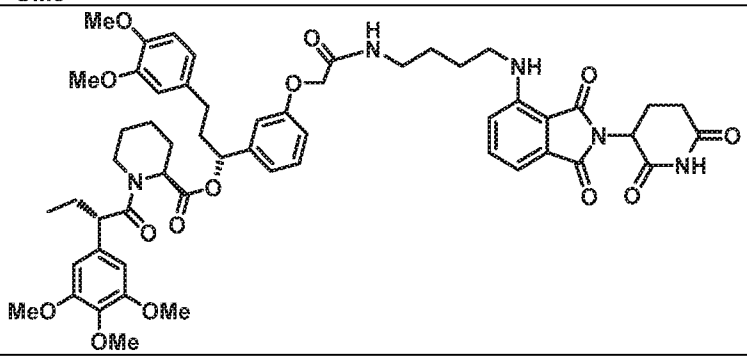
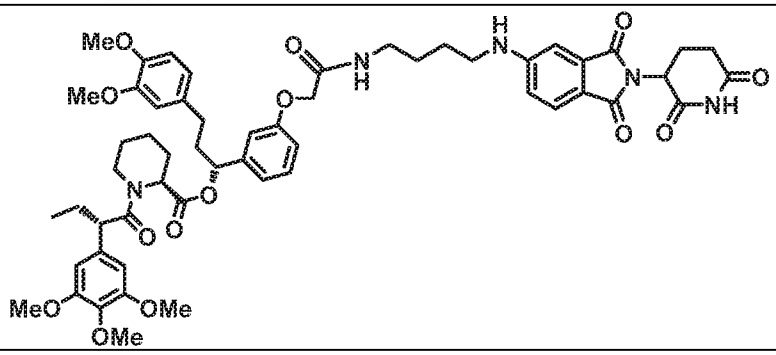
dFKBP-32	
dFKBP-33	
dFKBP-34	
dKFBP-35	

FIG. 30O

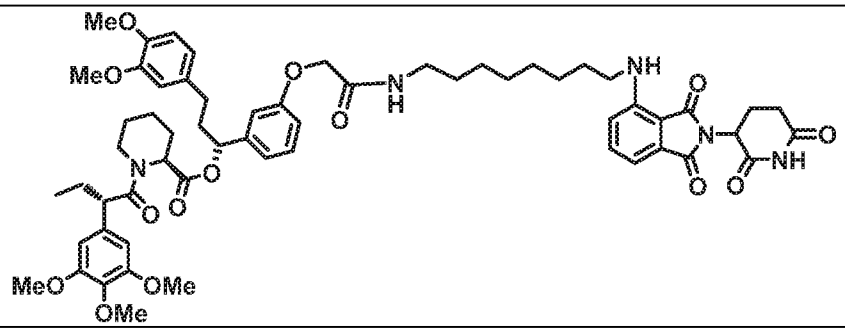
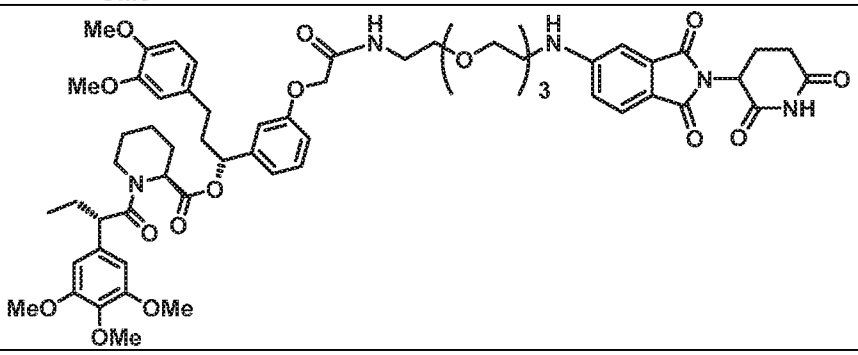
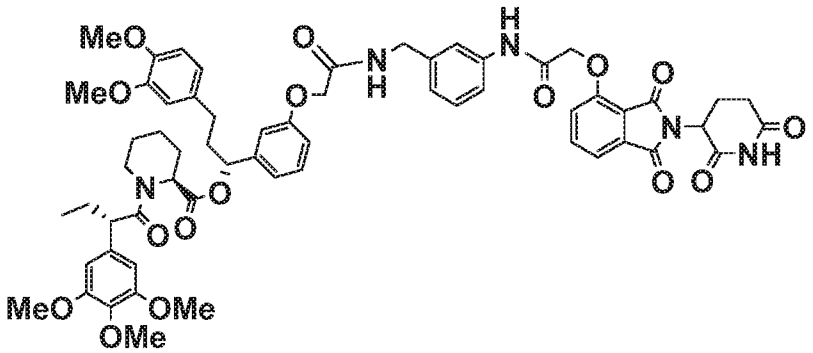
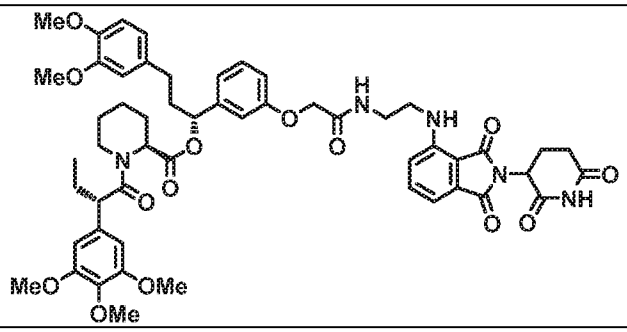
dFKBP-36	
dFKBP-37	
dFKBP-38	
dFKBP100	

FIG. 30P

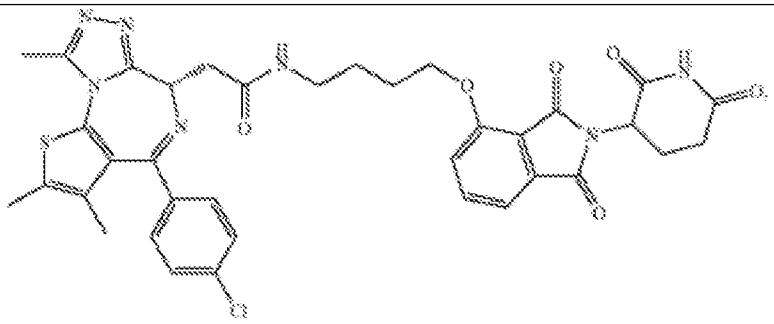
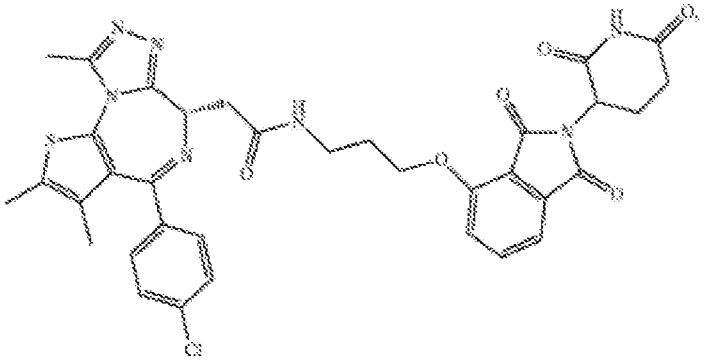
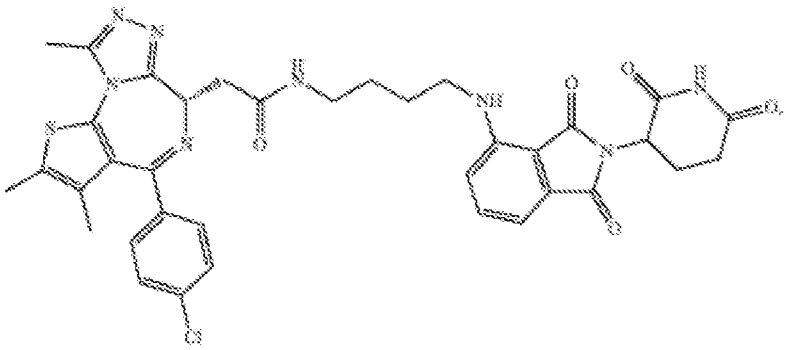
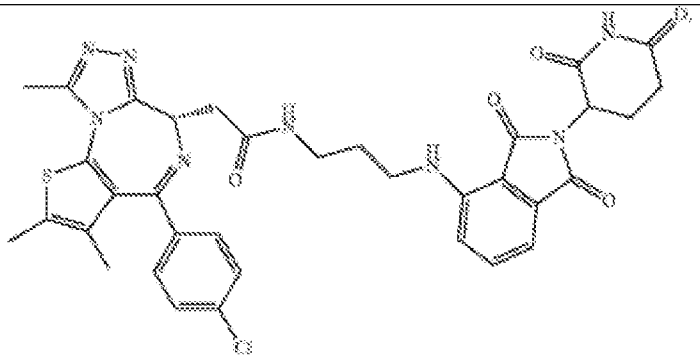
Cmpd. No.	Structure
dBET200	
dBET201	
dBET202	
dBET203	

FIG. 31A

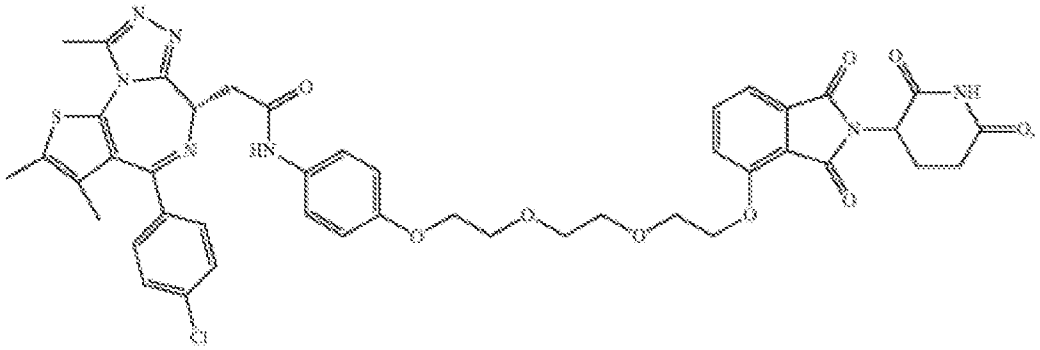
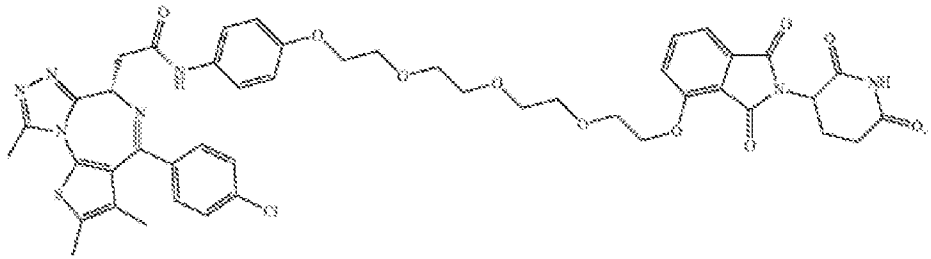
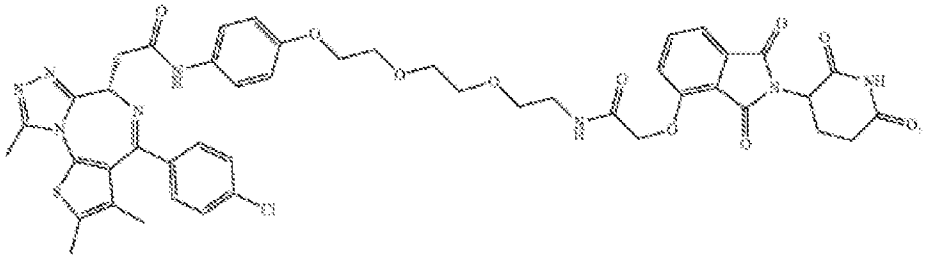
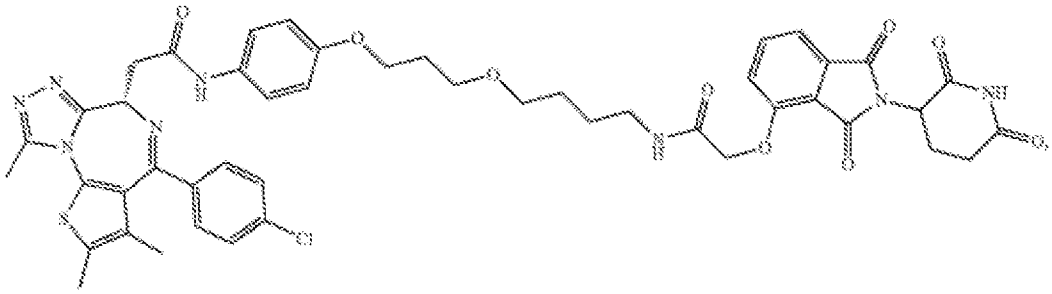
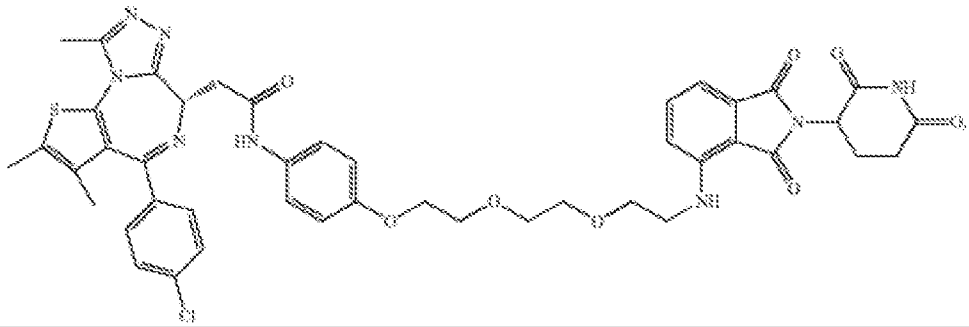
dBET204	
dBET205	
dBET206	
dBET207	
dBET208	

FIG. 31B

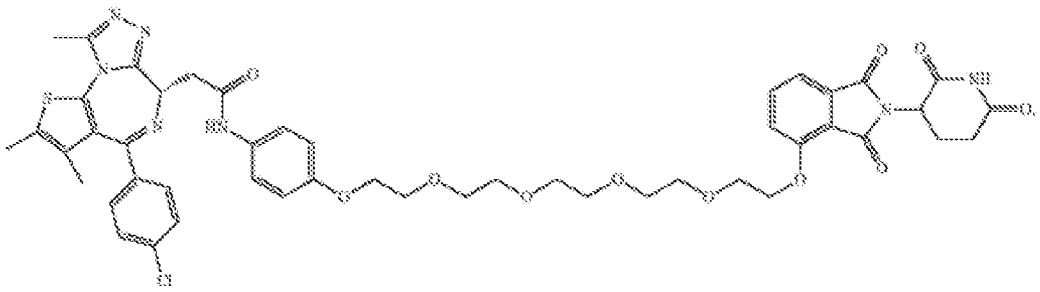
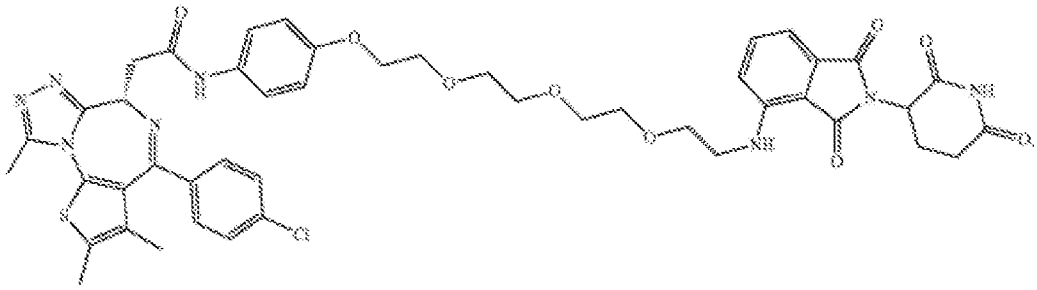
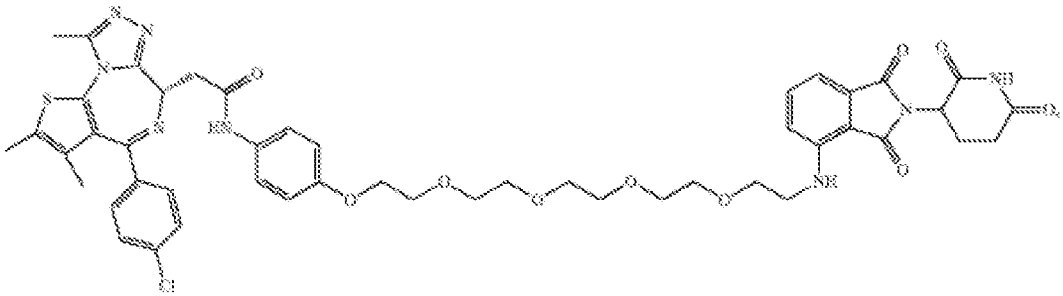
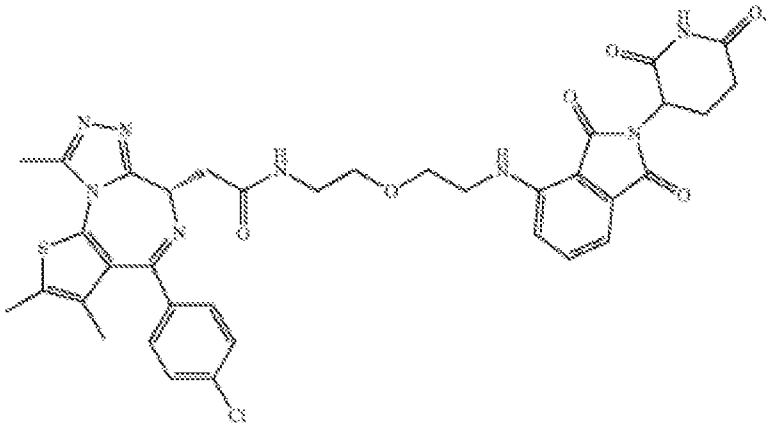
dBET209	
dBET210	
dBET211	
dBET212	

FIG. 31C

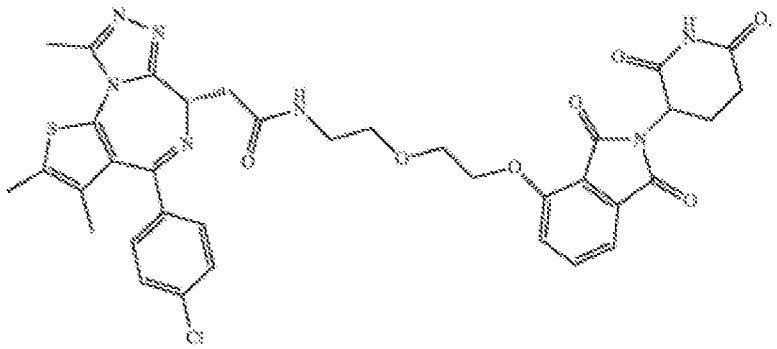
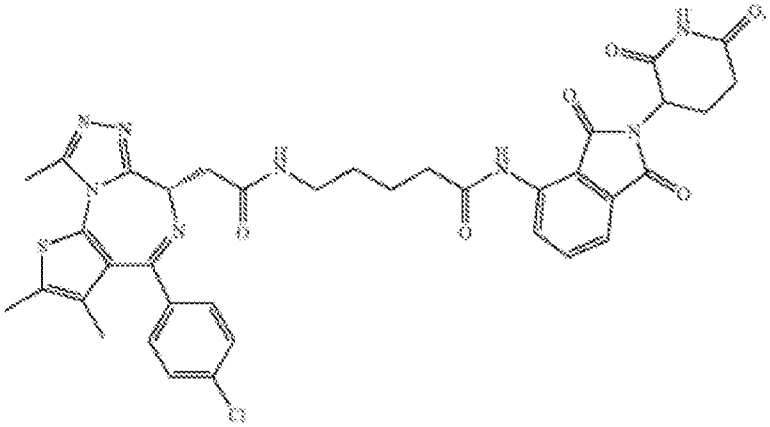
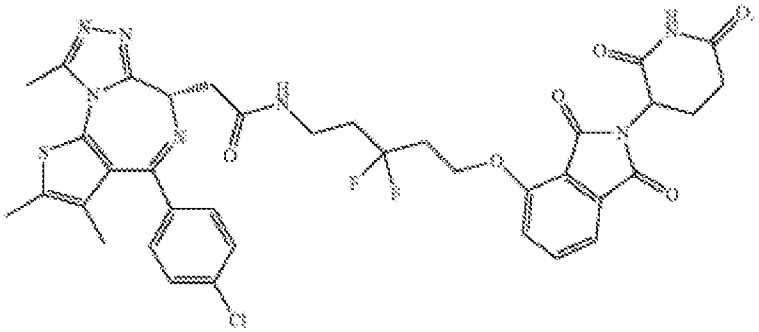
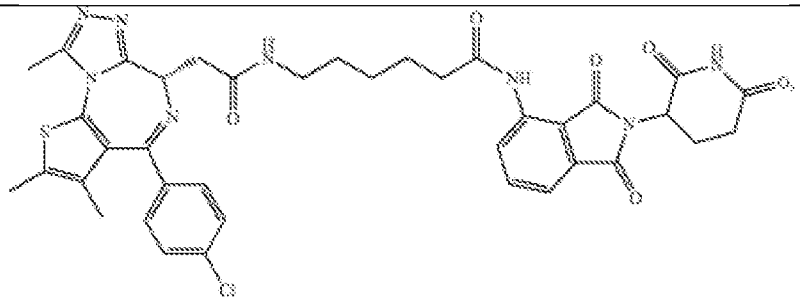
dBET213	
dBET214	
dBET215	
dBET216	

FIG. 31D

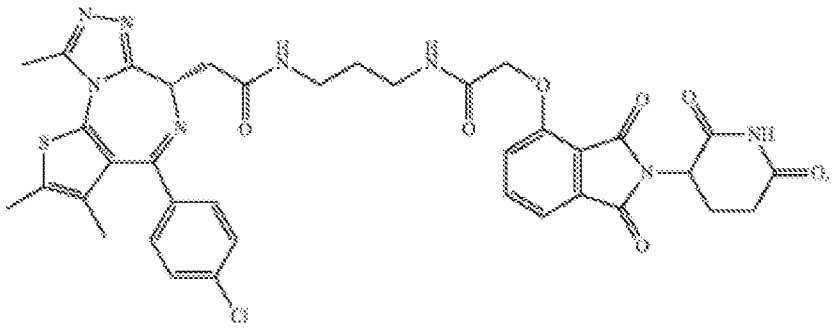
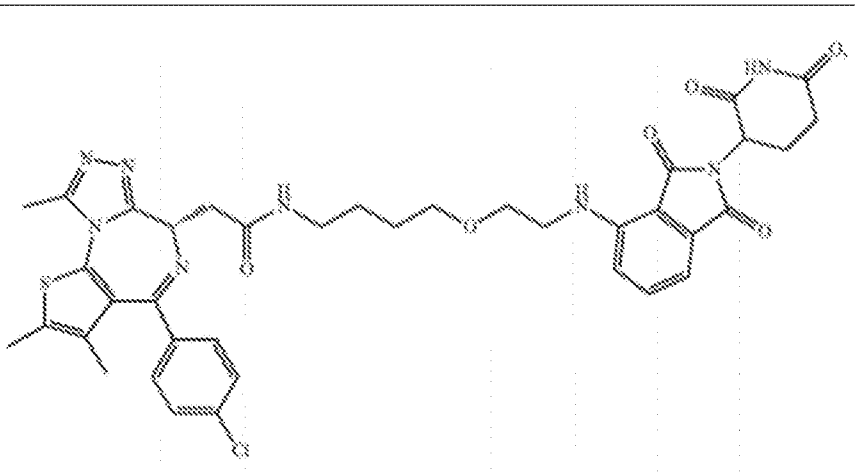
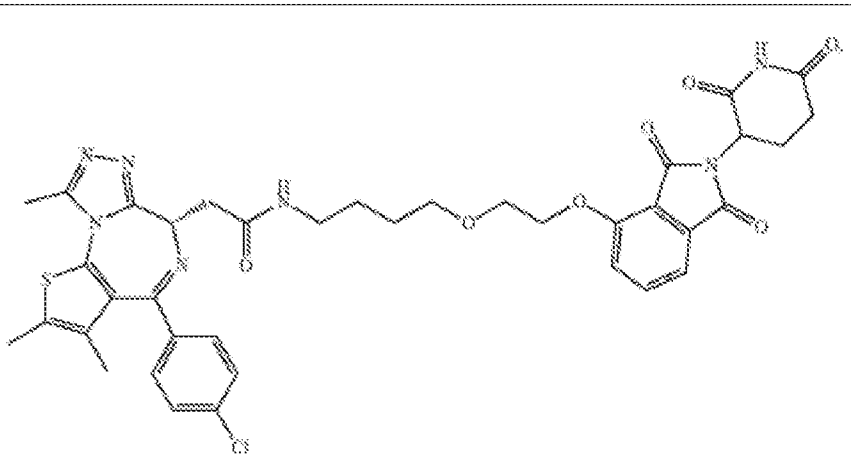
dBET217	
dBET218	
dBET219	

FIG. 31E

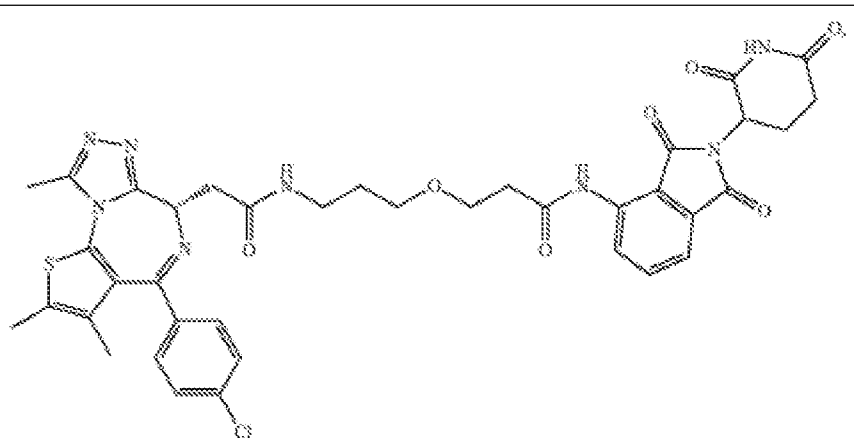
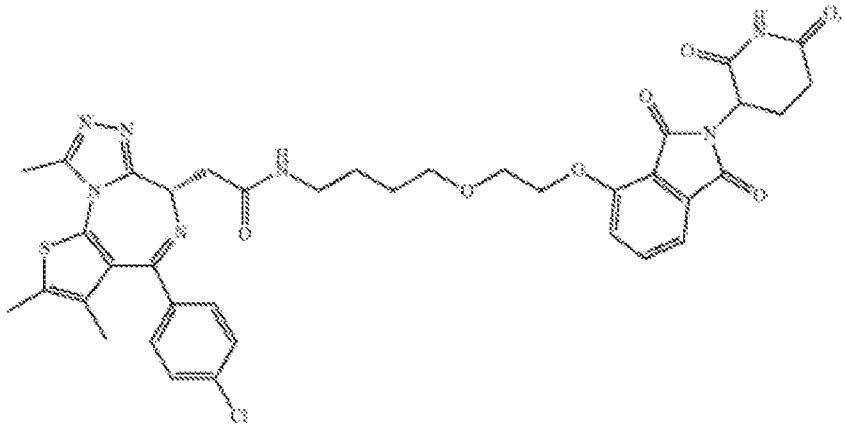
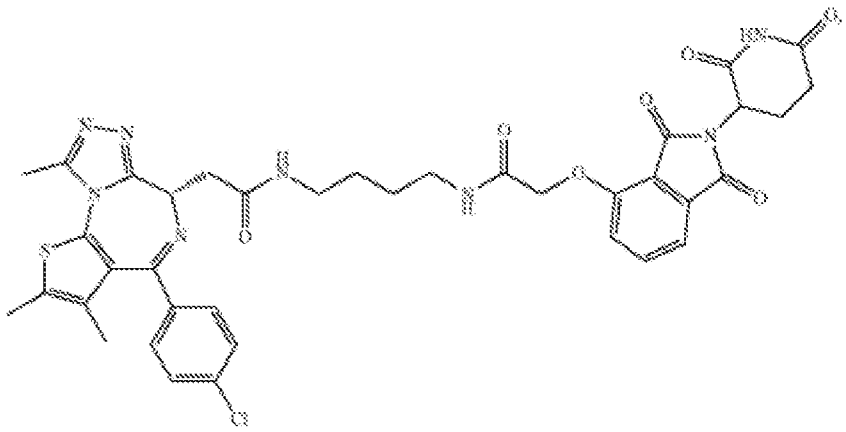
dBET220	
dBET221	
dBET222	

FIG. 31F

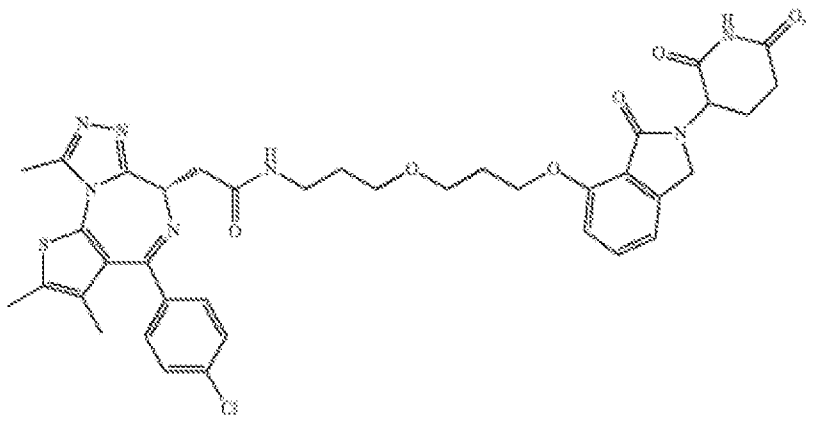
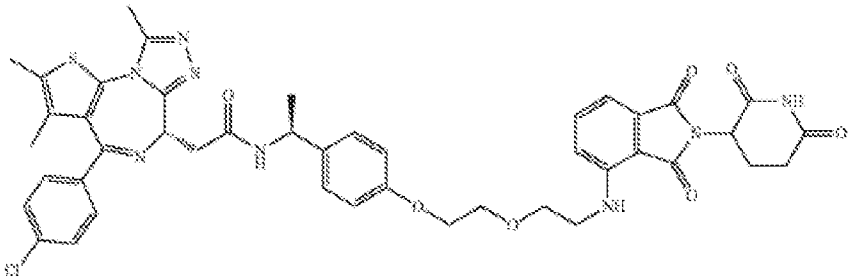
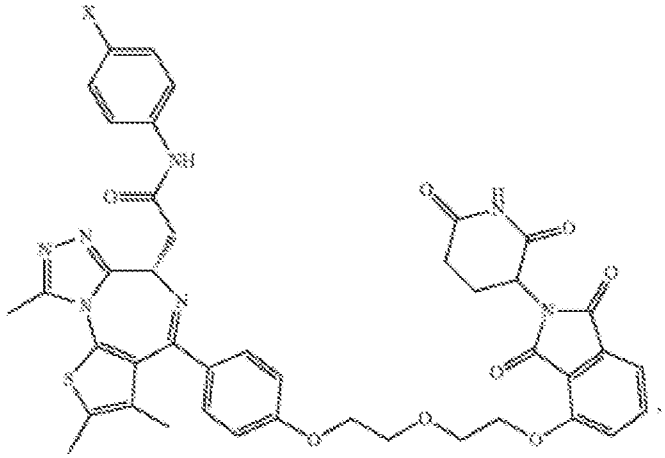
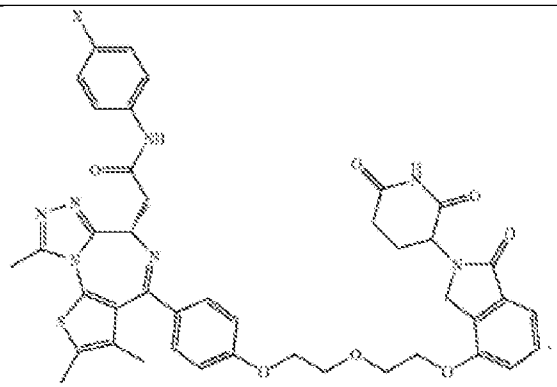
dBET223	
dBET224	
dBET225	
dBET226	

FIG. 31G

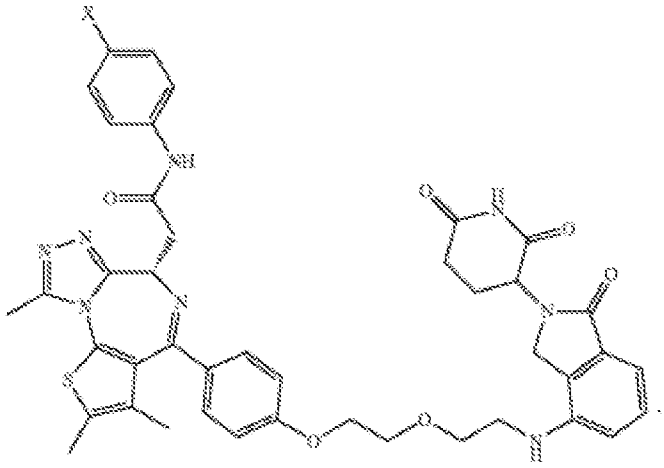
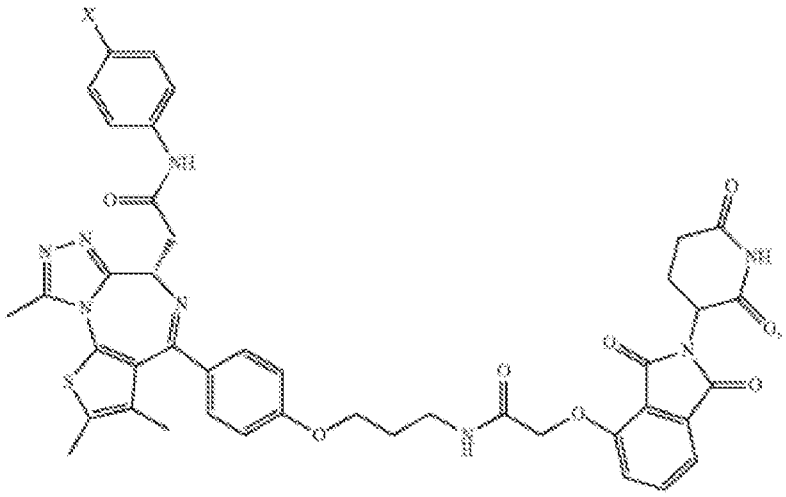
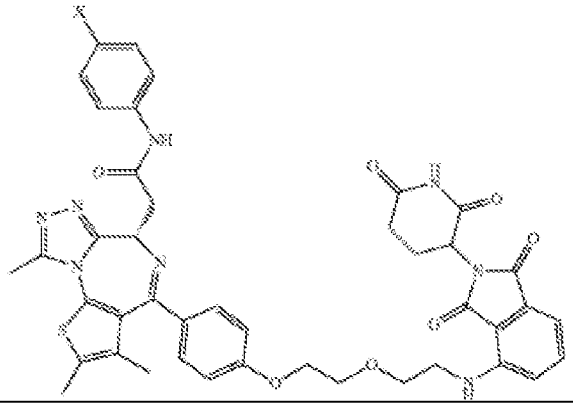
dBET227	
dBET228	
dBET229	

FIG. 31H

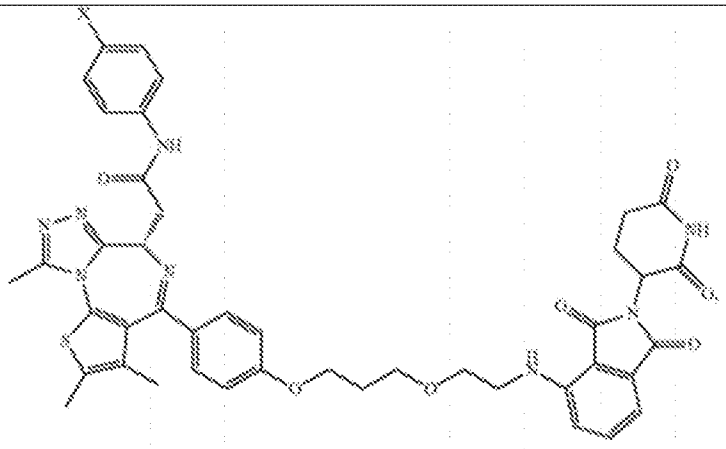
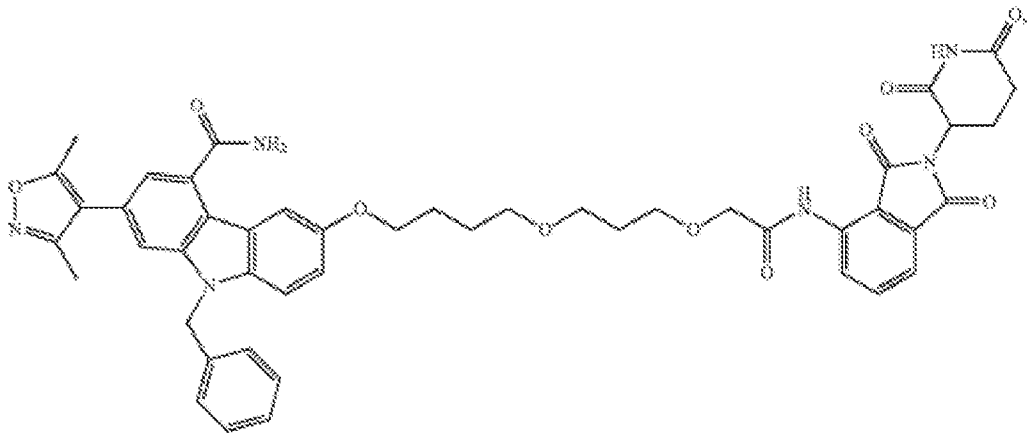
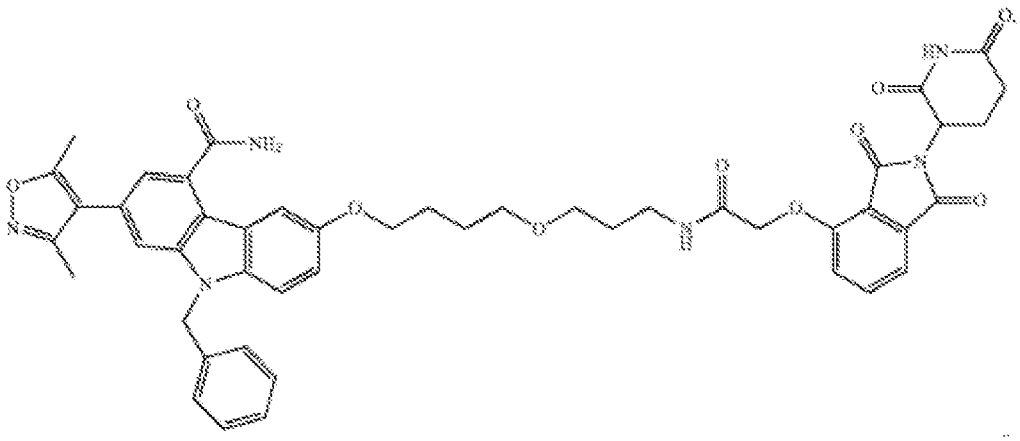
dBET230	
dBET231	
dBET232	

FIG. 31I

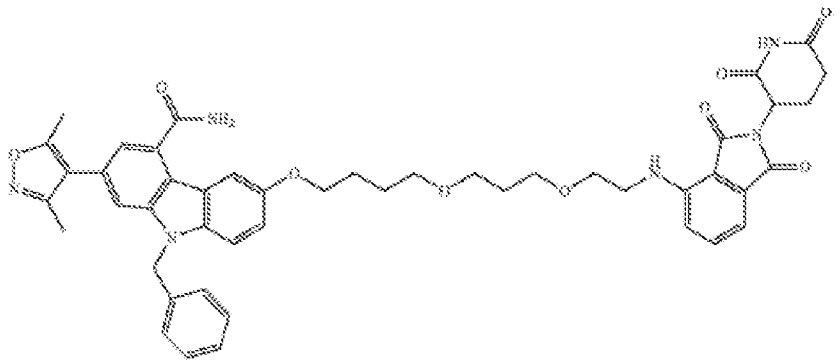
dBET233	

FIG. 31J

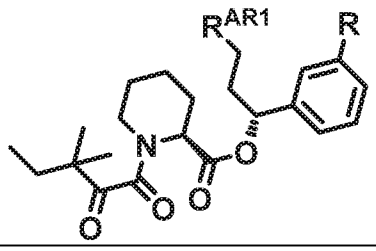
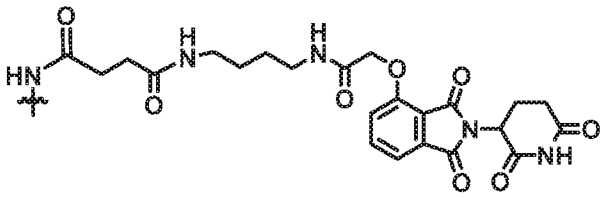
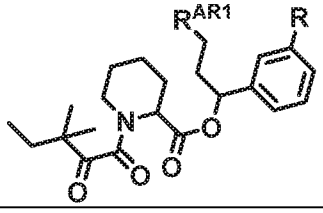
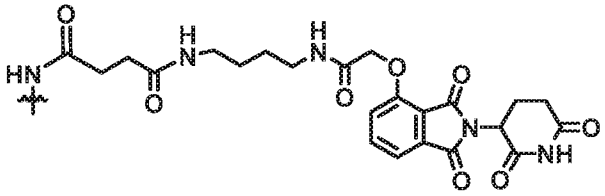
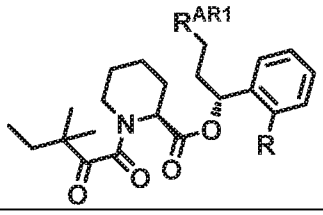
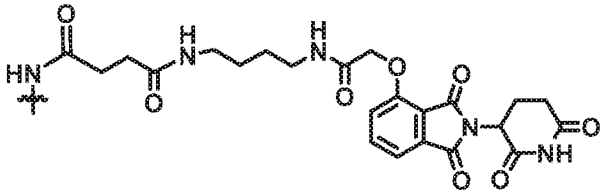
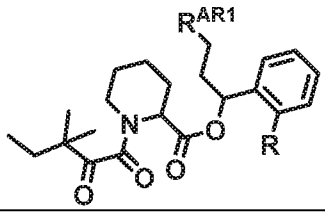
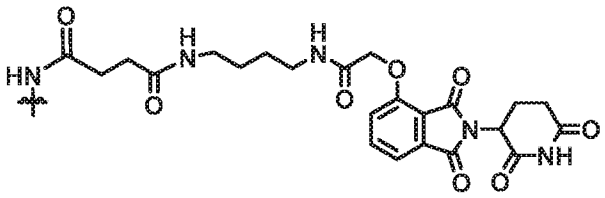
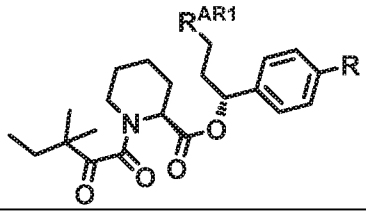
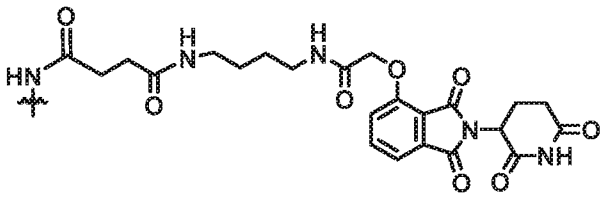
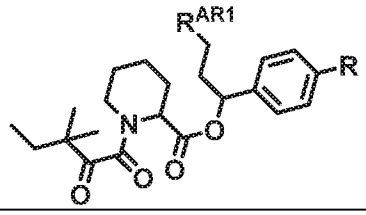
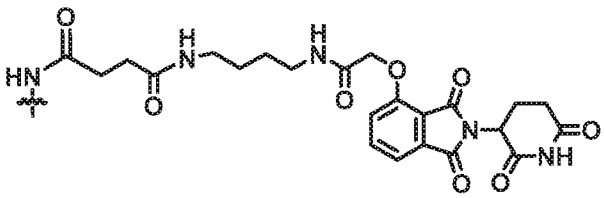
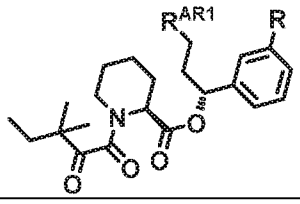
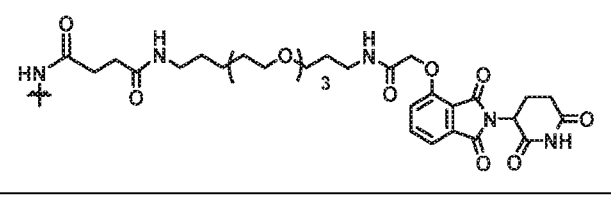
Cmpd ID	Structures	R
dFKBP-1-I-m		
dFKBP-1-I-m″		
dFKBP-1-I-o		
dFKBP-1-I-o″		
dFKBP-1-I-p		
dFKBP-1-I-p″		
dFKBP-2-I-m		

FIG. 32A

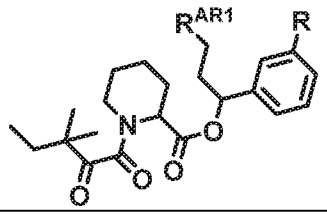
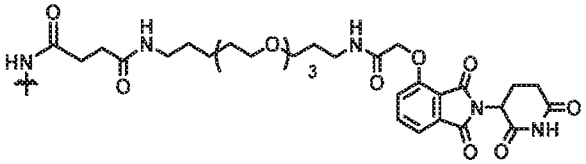
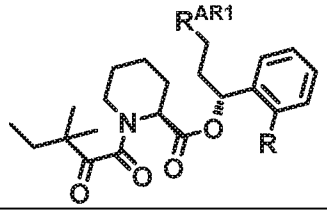
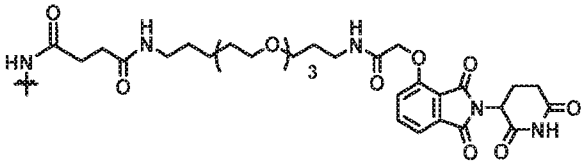
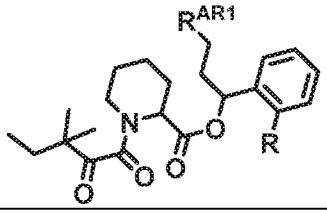
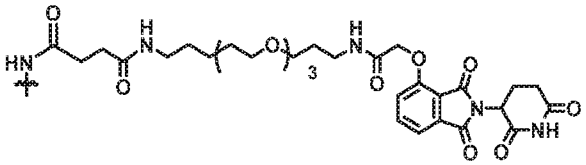
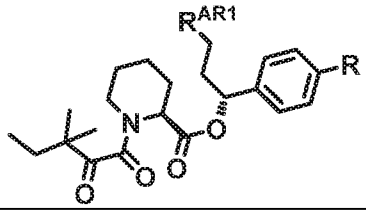
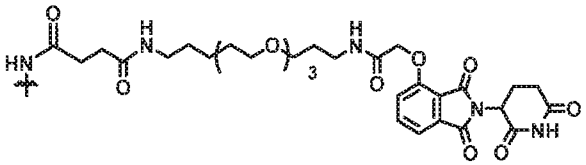
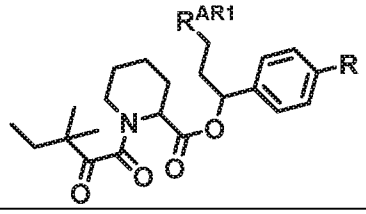
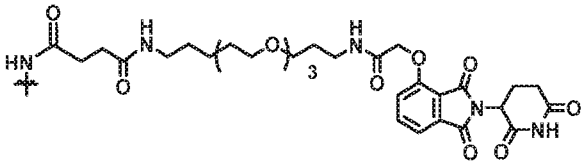
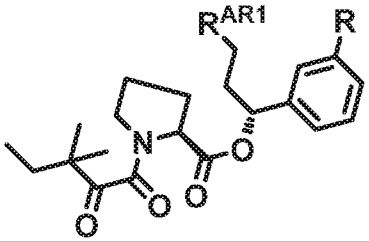
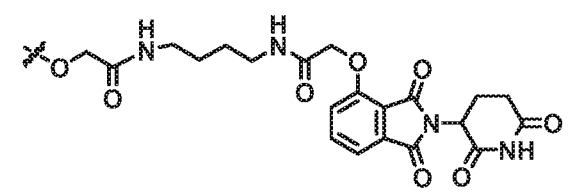
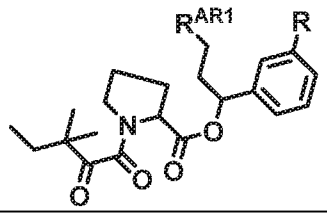
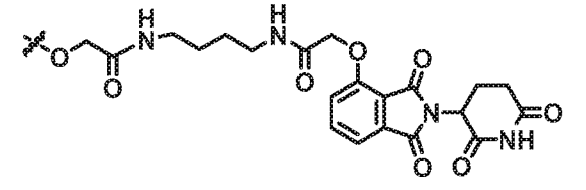
dFKBP-2-I-m″		
dFKBP-2-I-o		
dFKBP-2-I-o″		
dFKBP-2-I-p		
dFKBP-2-I-p″		
dFKBP-3-I-m		
dFKBP-3-I-m″		

FIG. 32B

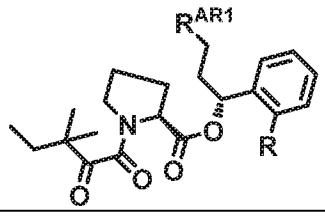
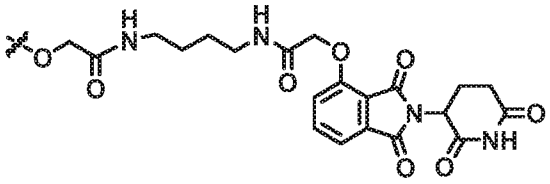
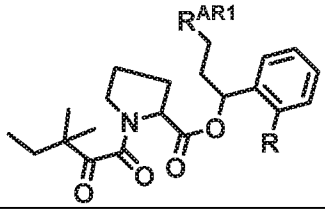
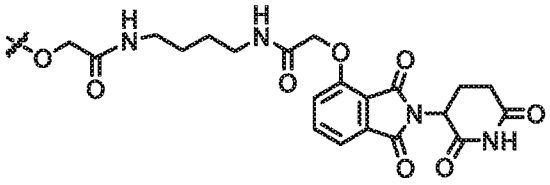
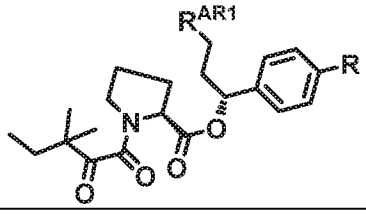
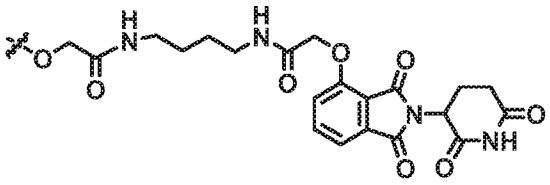
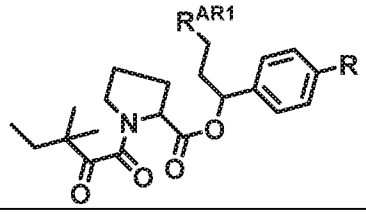
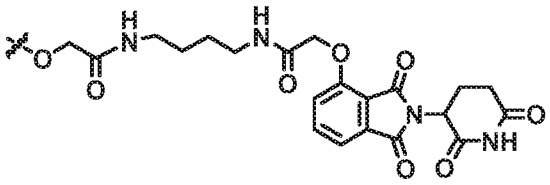
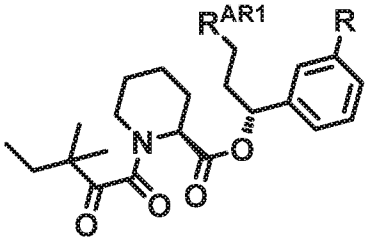
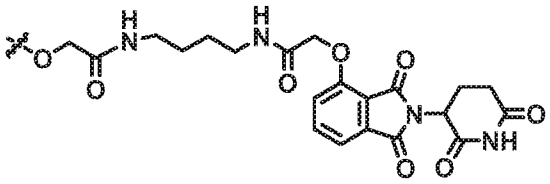
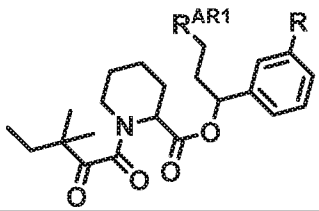
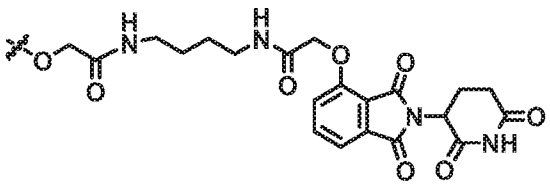
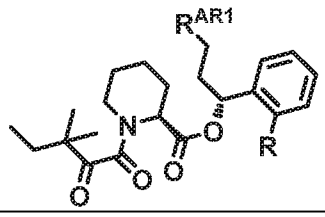
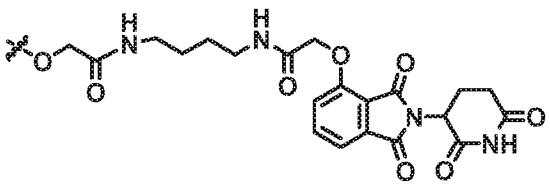
dFKBP-3-I-o		
dFKBP-3-I-o″		
dFKBP-3-I-p		
dFKBP-3-I-p″		
dFKBP-4-I-m		
dFKBP-4-I-m″		
dFKBP-4-I-o		

FIG. 32C

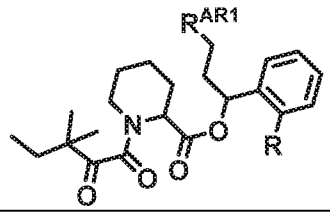
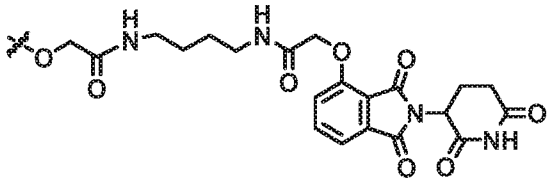
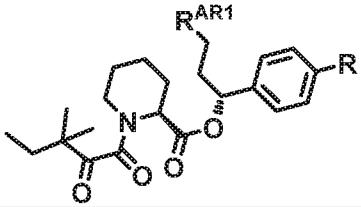
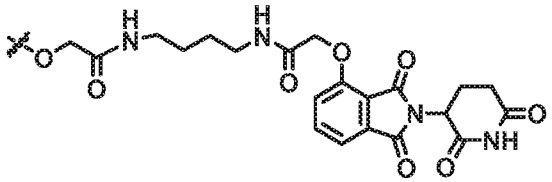
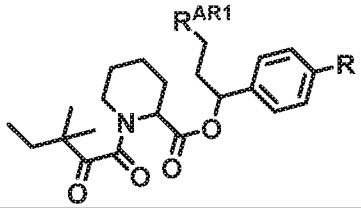
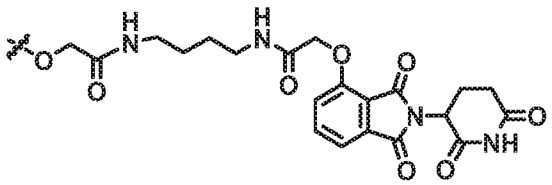
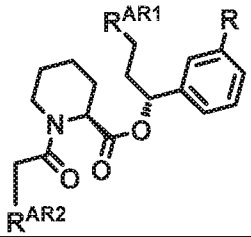
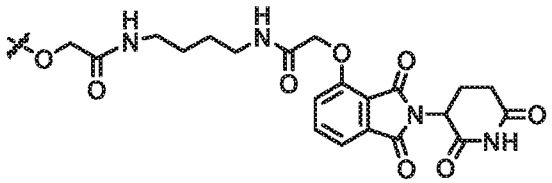
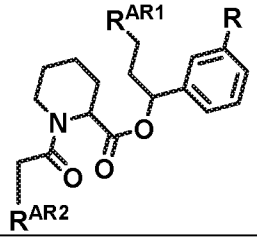
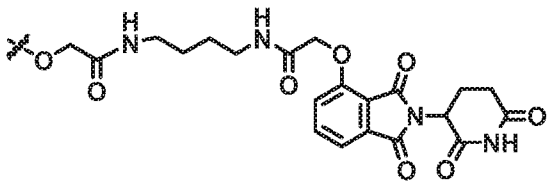
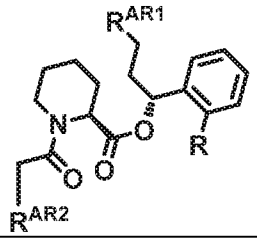
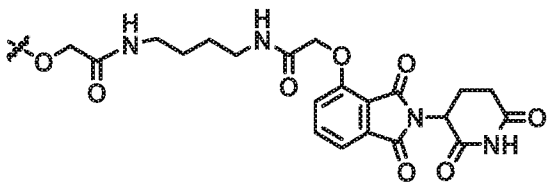
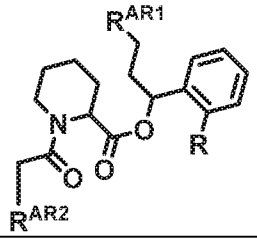
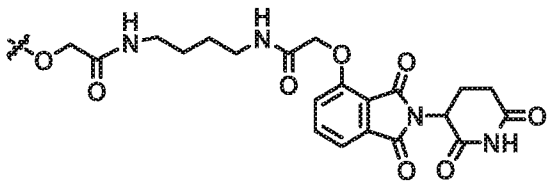
dFKBP-4-I-o″		
dFKBP-4-I-p		
dFKBP-4-I-p″		
dFKBP-5-I-m		
dFKBP-5-I-m″		
dFKBP-5-I-o		
dFKBP-5-I-o″		

FIG. 32D

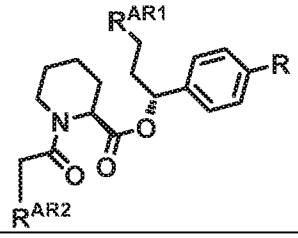
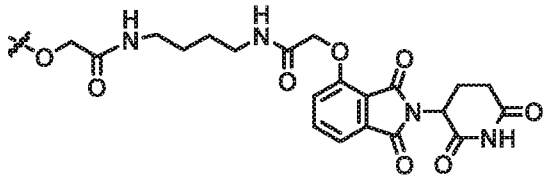
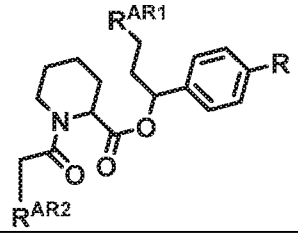
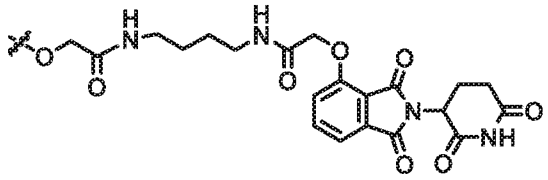
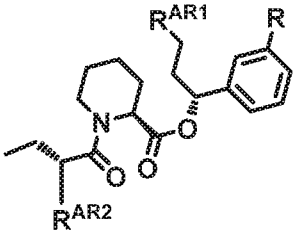
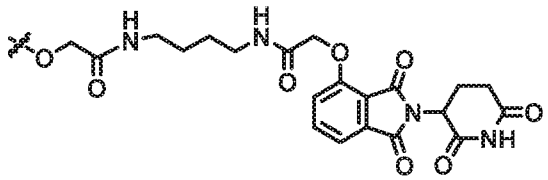
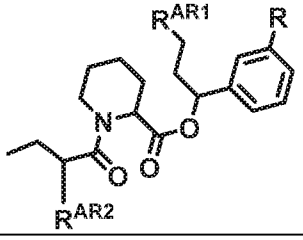
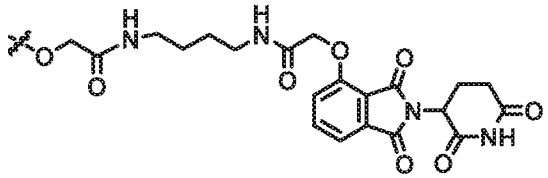
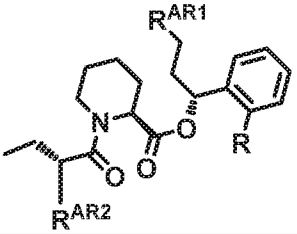
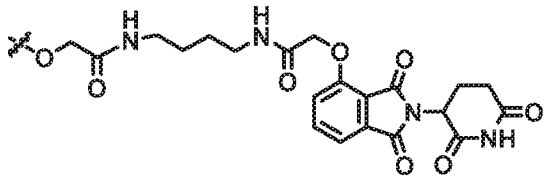
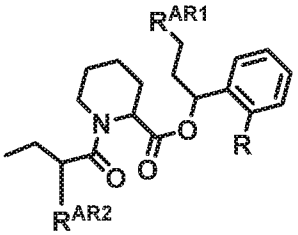
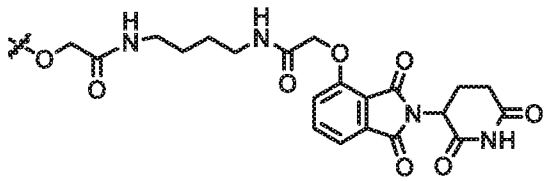
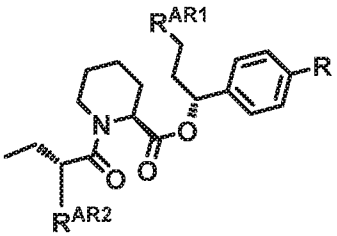
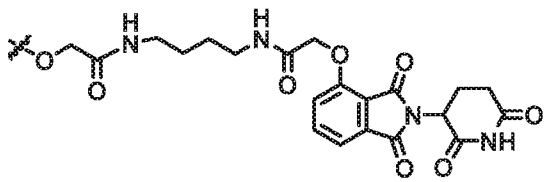
dFKBP-5-I-p		
dFKBP-5-I-p″		
dFKBP-6-I-m		
dFKBP-6-I-m″		
dFKBP-6-I-o		
dFKBP-6-I-o″		
dFKBP-6-I-p		

FIG. 53E

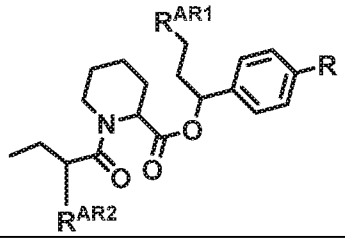
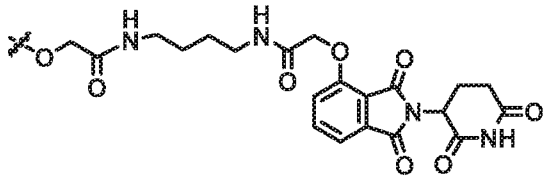
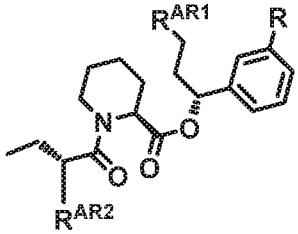
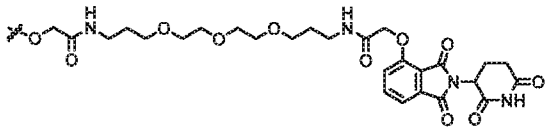
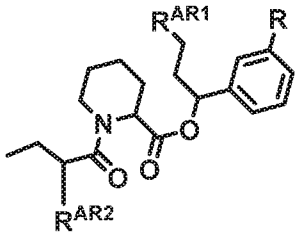
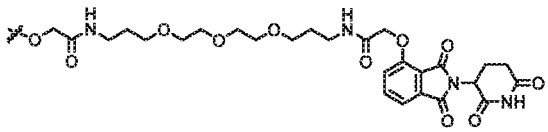
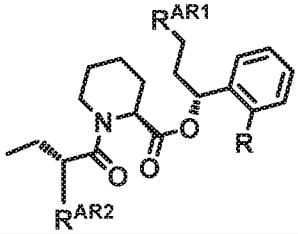
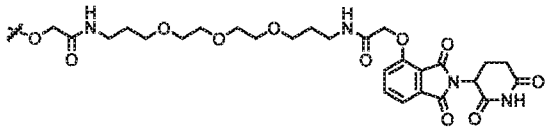
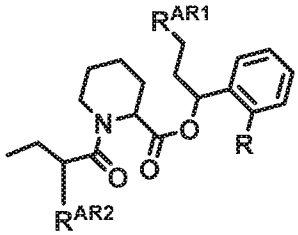
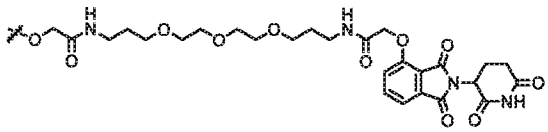
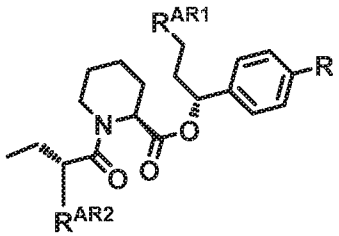
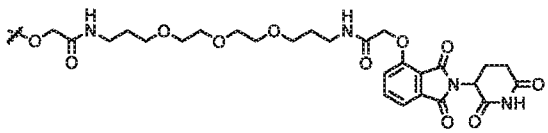
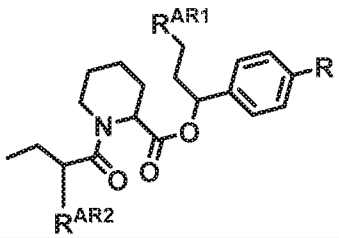
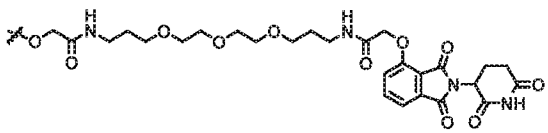
dFKBP-6-I-p″		
dFKBP-7-I-m		
dFKBP-7-I-m″		
dFKBP-7-I-o		
dFKBP-7-I-o″		
dFKBP-7-I-p		
dFKBP-7-I-p″		

FIG. 32F

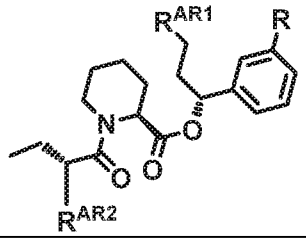
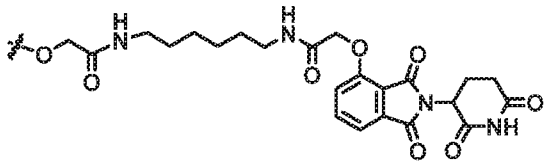
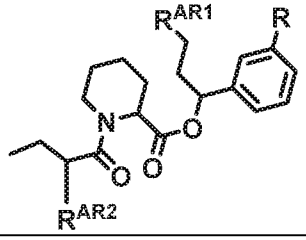
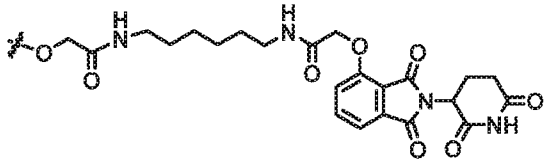
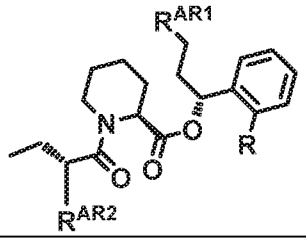
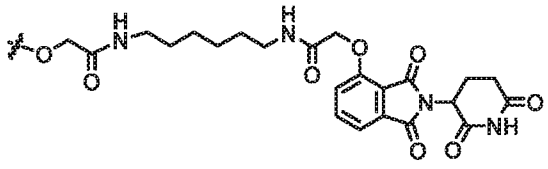
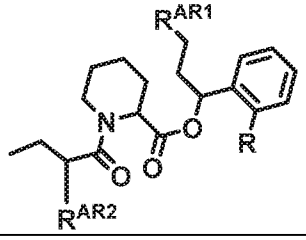
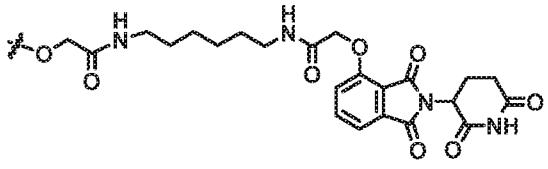
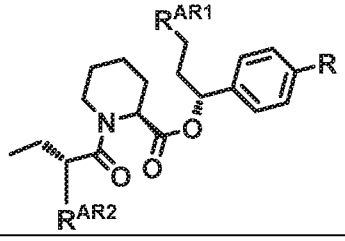
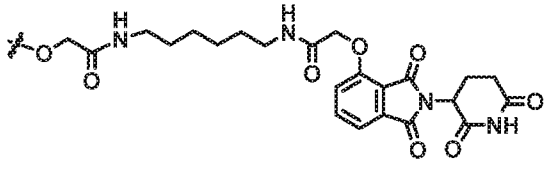
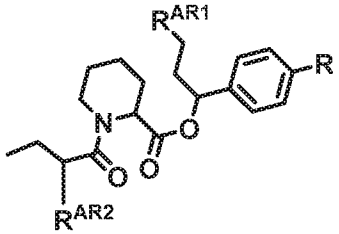
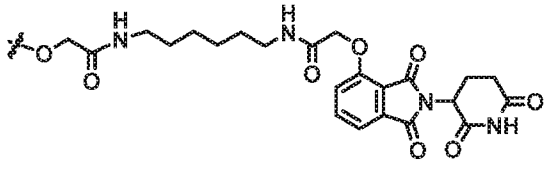
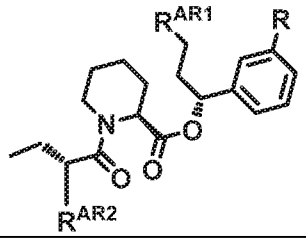
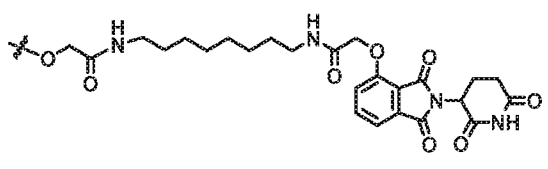
dFKBP-8-I-m		
dFKBP-8-I-m″		
dFKBP-8-I-o		
dFKBP-8-I-o″		
dFKBP-8-I-p		
dFKBP-8-I-p″		
dFKBP-9-I-m		

FIG. 32G

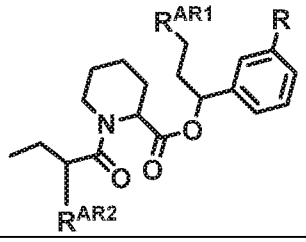
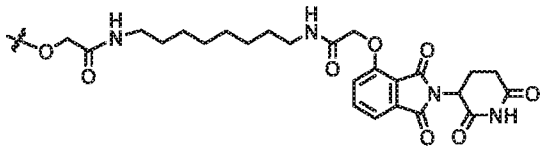
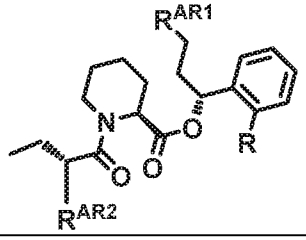
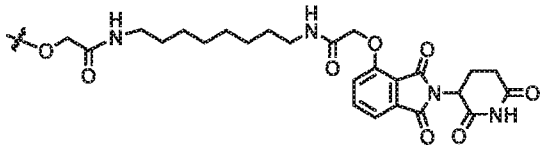
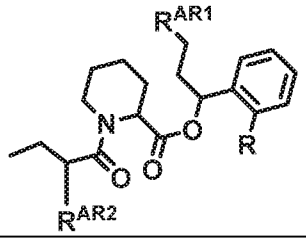
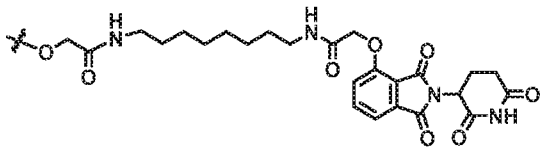
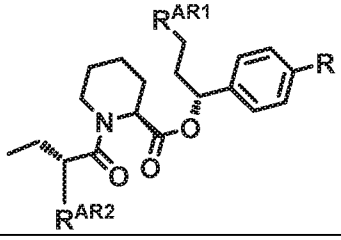
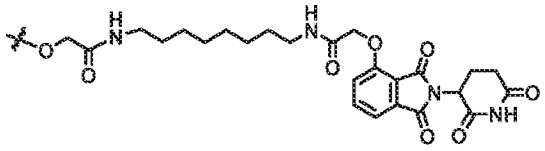
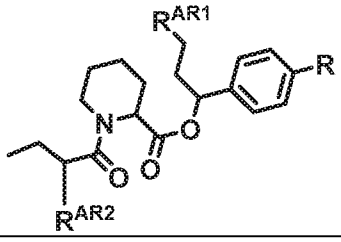
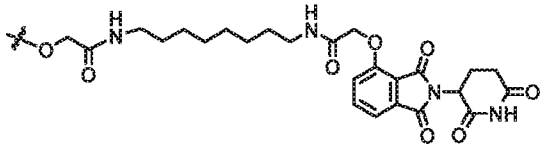
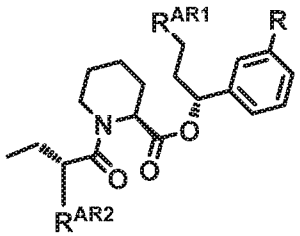
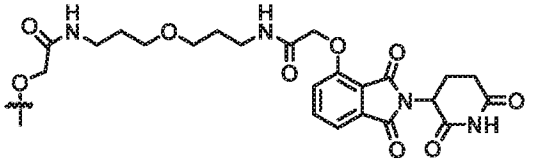
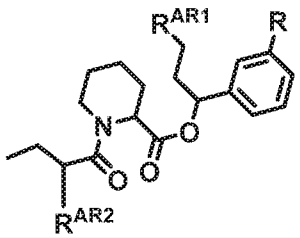
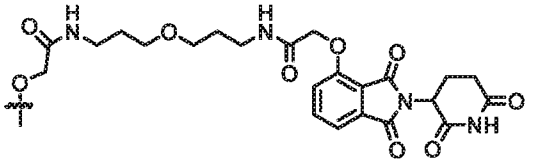
dFKBP-9-I-m″		
dFKBP-9-I-o		
dFKBP-9-I-o″		
dFKBP-9-I-p		
dFKBP-9-I-p″		
dFKBP-17-I-m		
dFKBP-17-I-m″		

FIG. 32H

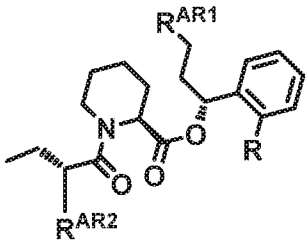
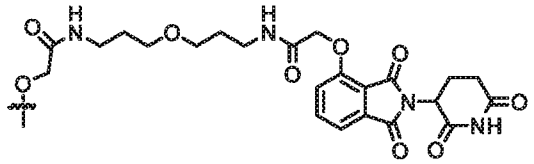
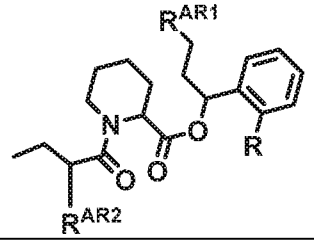
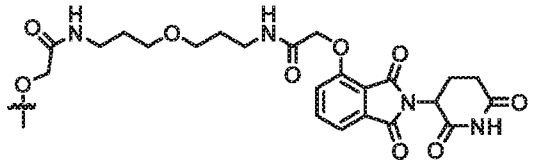
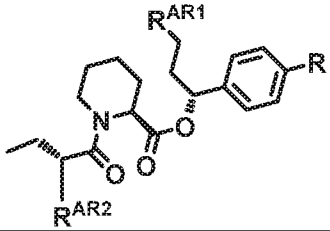
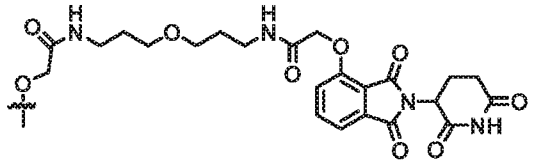
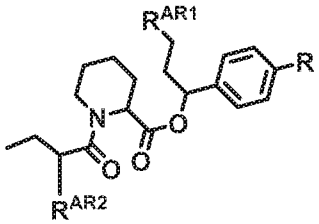
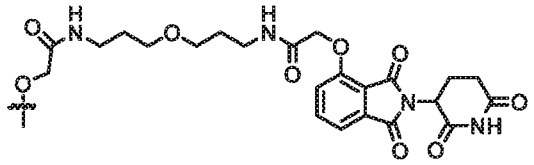
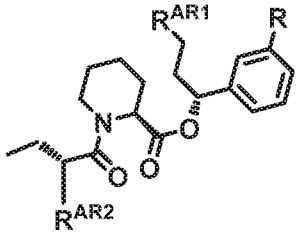
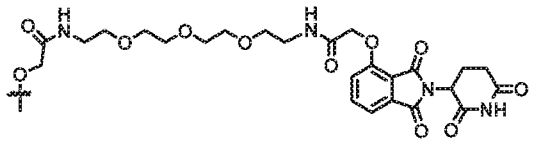
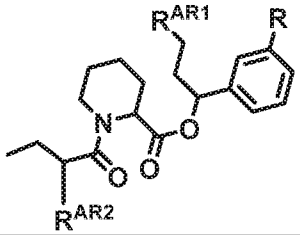
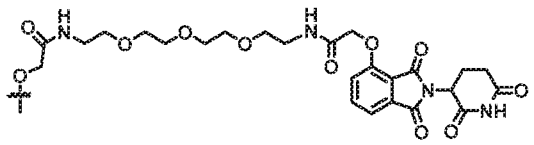
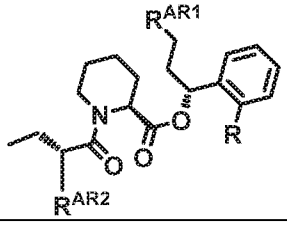
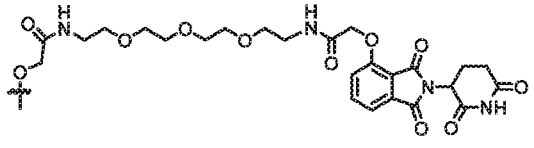
dFKBP-17-I-o		
dFKBP-17-I-o″		
dFKBP-17-I-p		
dFKBP-17-I-p″		
dFKBP-26-I-m		
dFKBP-26-I-m″		
dFKBP-26-I-o		

FIG. 32I

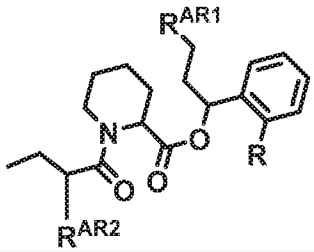
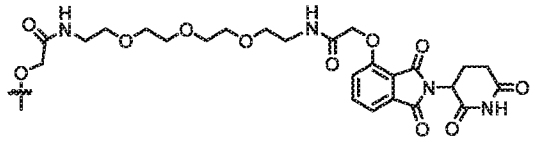
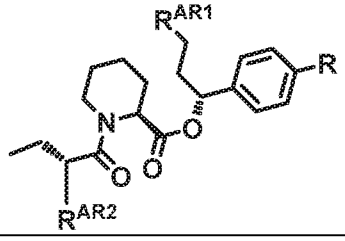
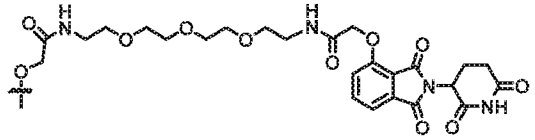
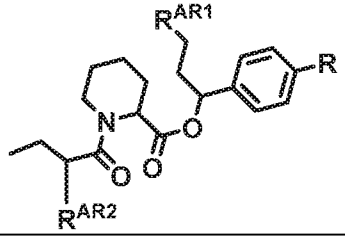
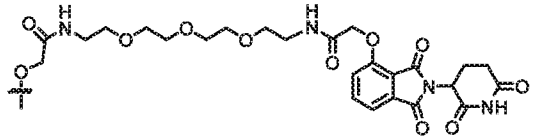
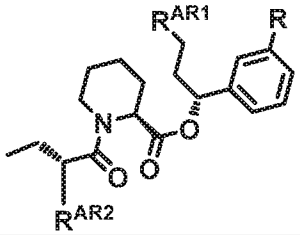
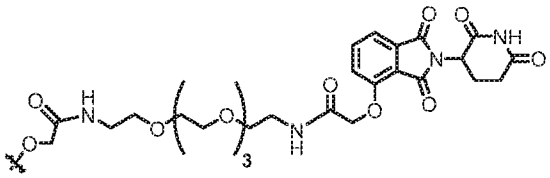
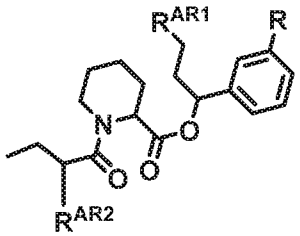
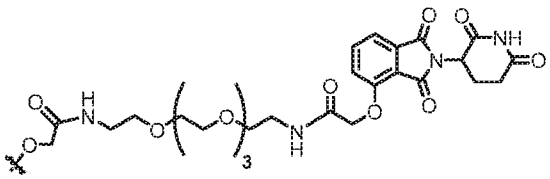
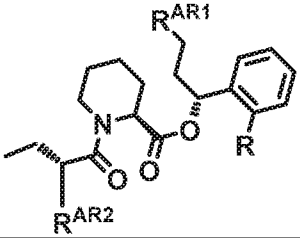
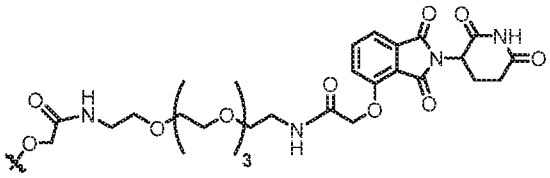
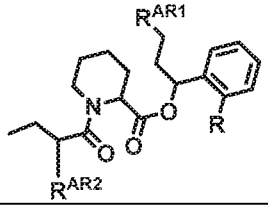
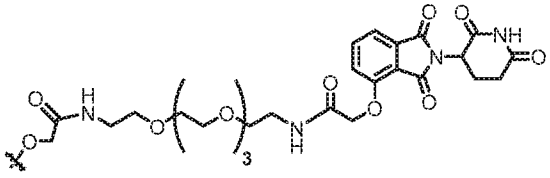
dFKBP-26-I-o″		
dFKBP-26-I-p		
dFKBP-26-I-p″		
dFKBP-24-I-m		
dFKBP-24-I-m″		
dFKBP-24-I-o		
dFKBP-24-I-o″		

FIG. 32J

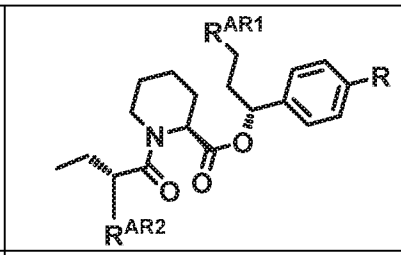
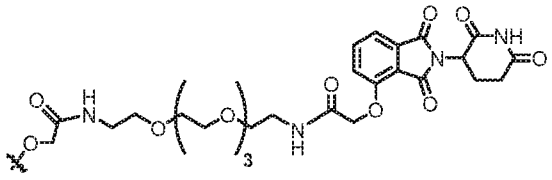
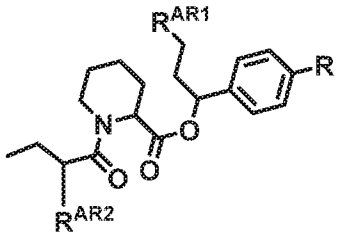
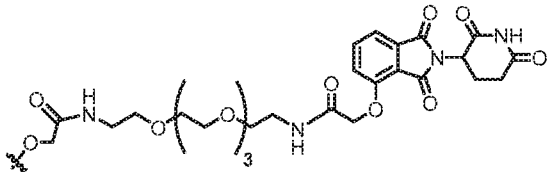
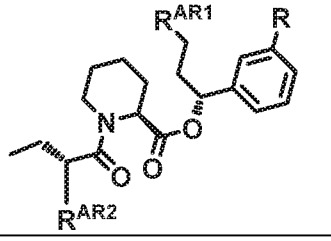
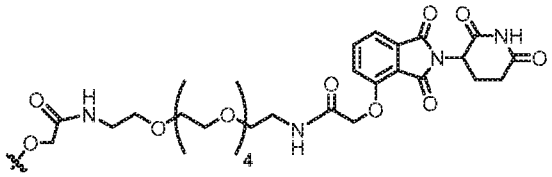
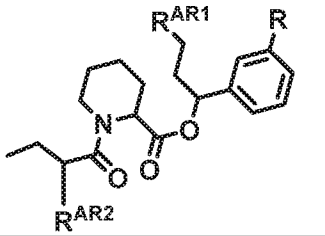
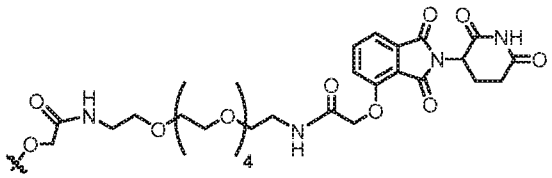
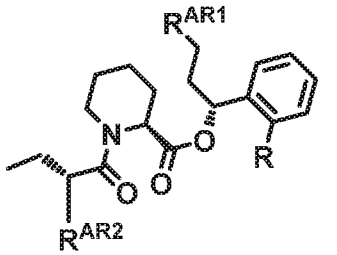
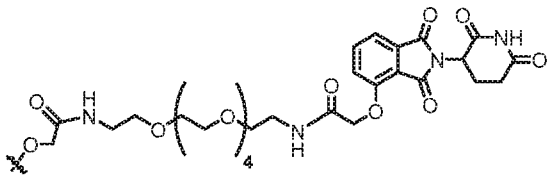
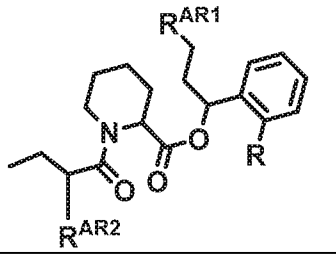
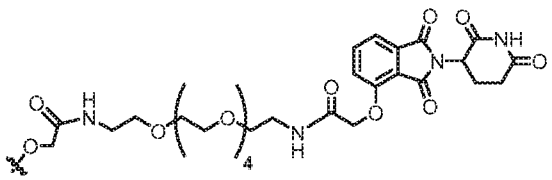
dFKBP-24-I-p		
dFKBP-24-I-p″		
dFKBP-27-I-m		
dFKBP-27-I-m″		
dFKBP-27-I-o		
dFKBP-27-I-o″		

FIG. 32K

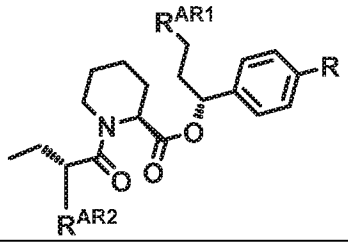
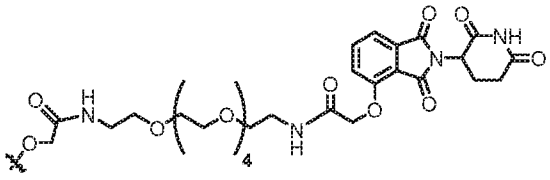
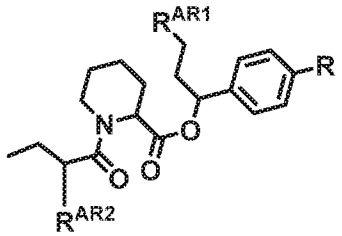
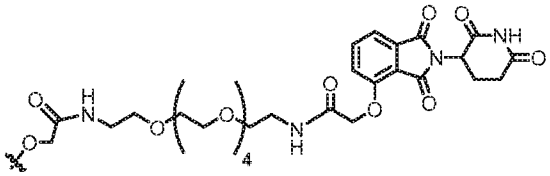
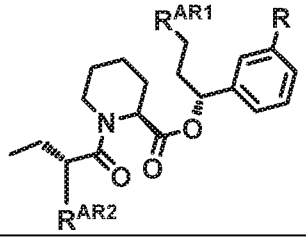
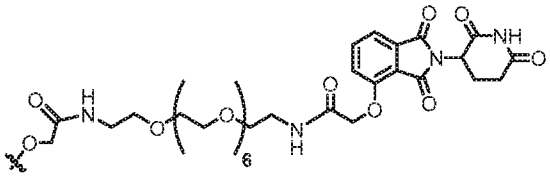
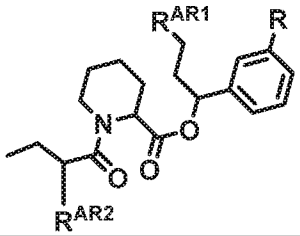
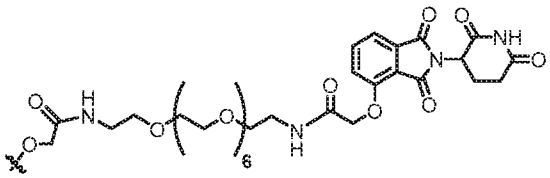
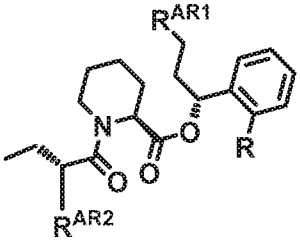
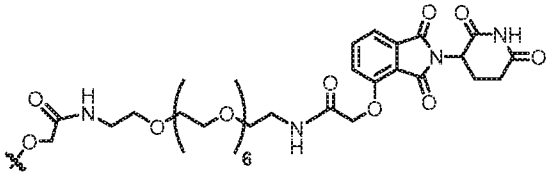
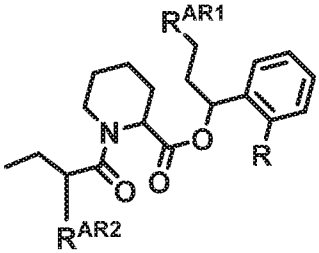
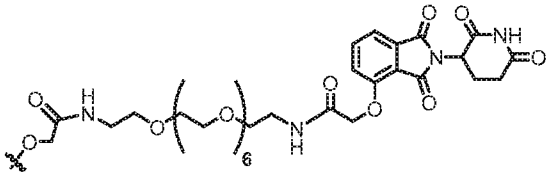
dFKBP-27-I-p		
dFKBP-27-I-p″		
dFKBP-28-I-m		
dFKBP-28-I-m″		
dFKBP-28-I-o		
dFKBP-28-I-o″		

FIG. 32L

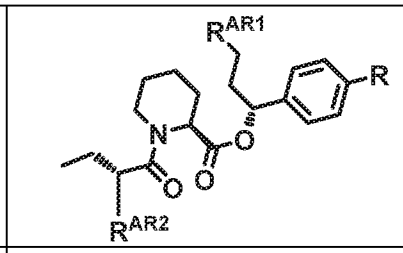
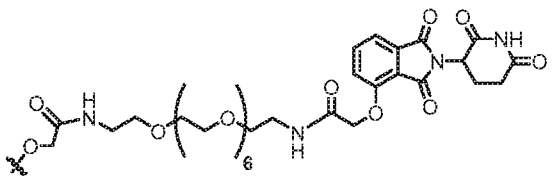
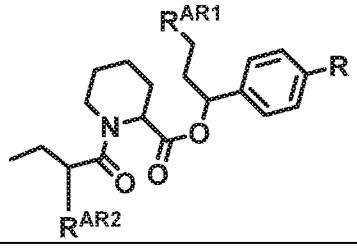
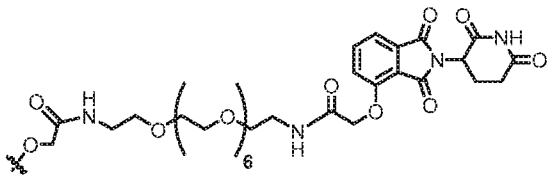
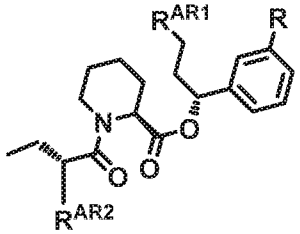
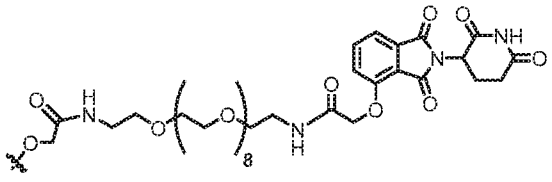
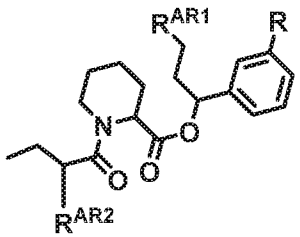
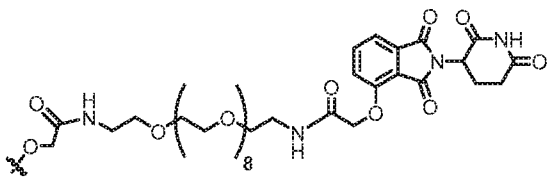
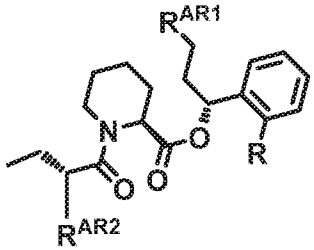
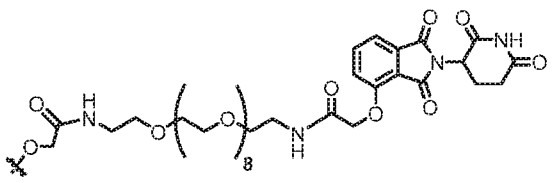
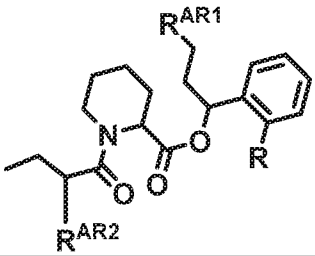
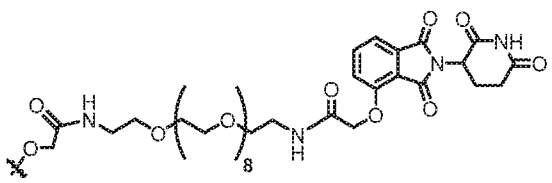
dFKBP-28-I-p		
dFKBP-28-I-p″		
dFKBP-25-I-m		
dFKBP-25-I-m″		
dFKBP-25-I-o		
dFKBP-25-I-o″		

FIG. 32M

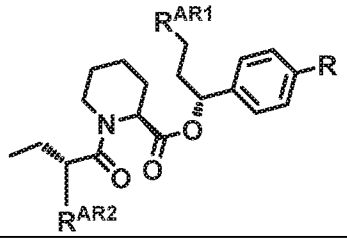
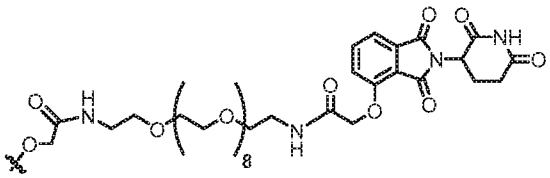
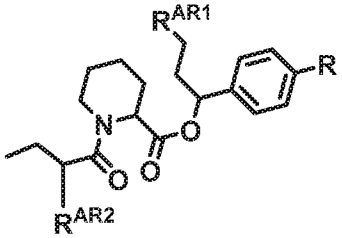
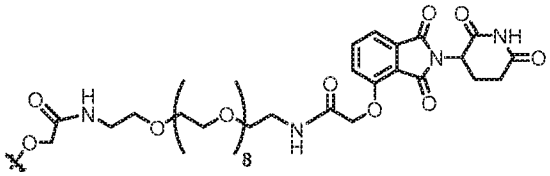
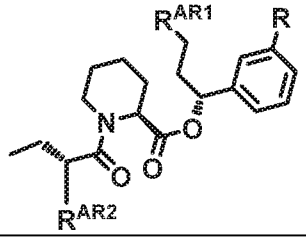
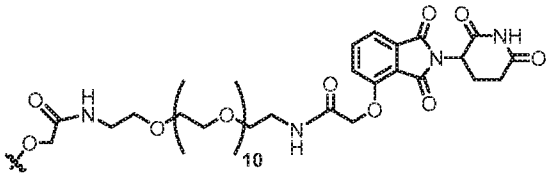
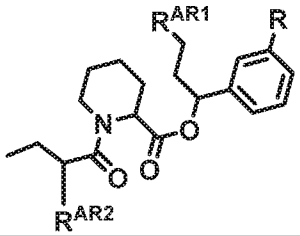
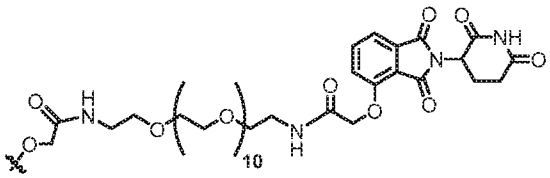
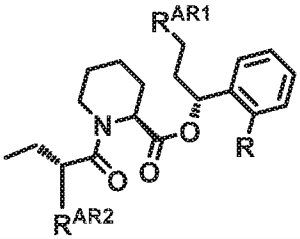
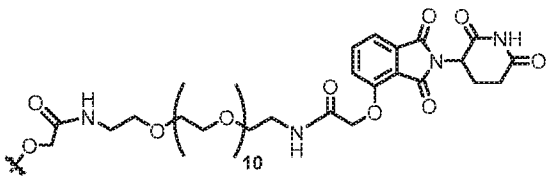
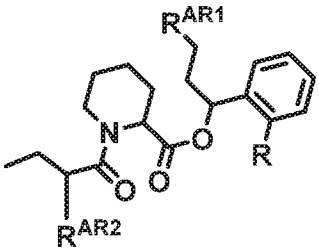
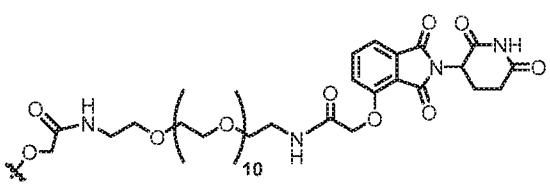
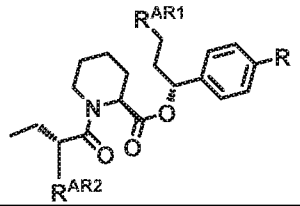
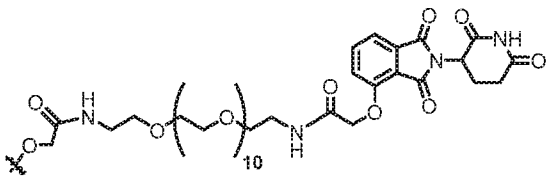
dFKBP-25-I-p		
dFKBP-25-I-p″		
dFKBP-29-I-m		
dFKBP-29-I-m″		
dFKBP-29-I-o		
dFKBP-29-I-o″		
dFKBP-29-I-p		

FIG. 32N

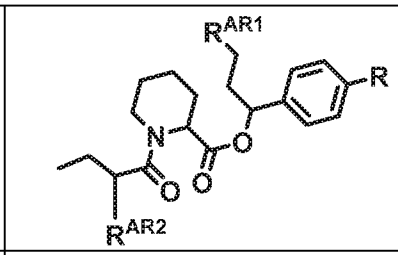
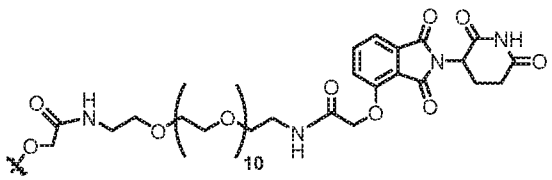
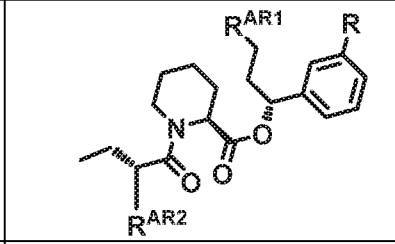
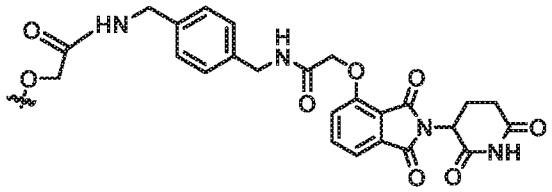
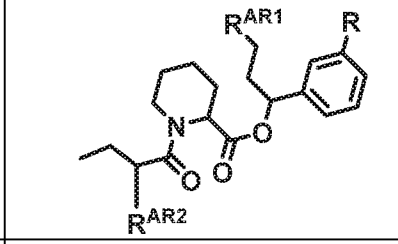
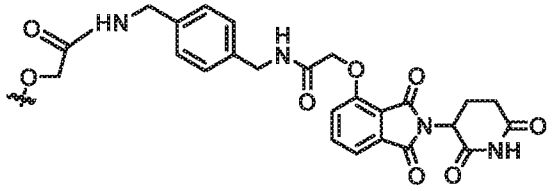
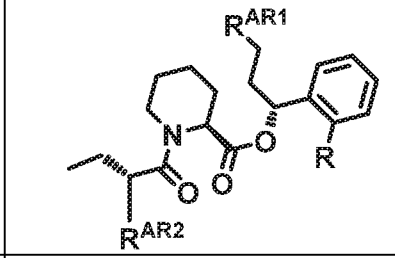
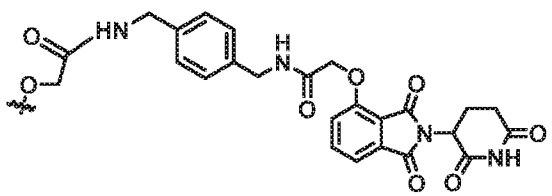
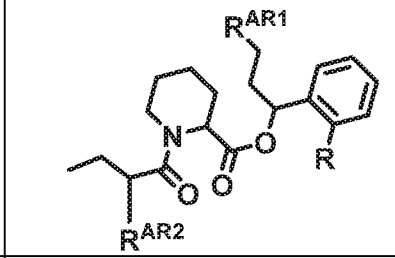
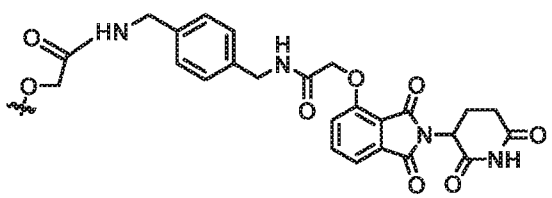
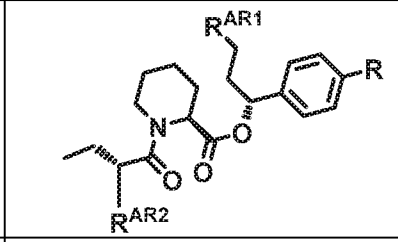
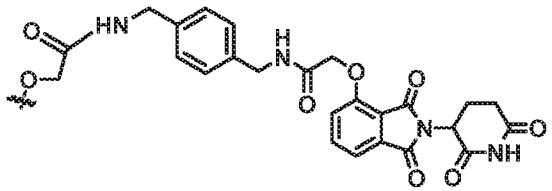
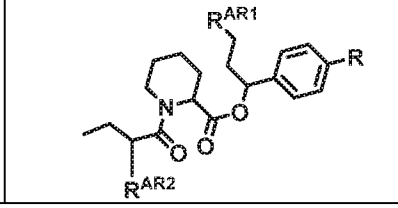
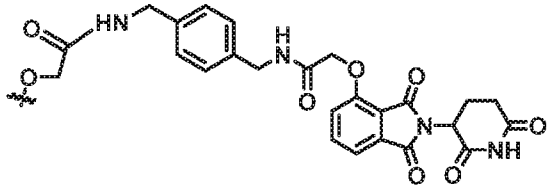
dFKBP-29-I-p″		
dFKBP-21-I-m		
dFKBP-21-I-m″		
dFKBP-21-I-o		
dFKBP-21-I-o″		
dFKBP-21-I-p		
dFKBP-21-I-p″		

FIG. 32O

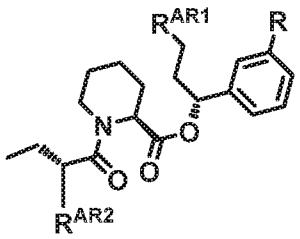
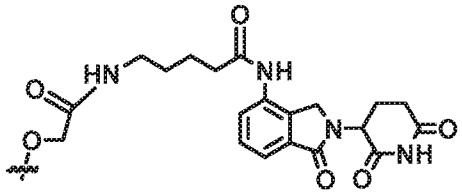
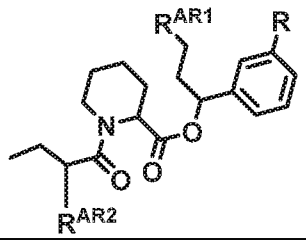
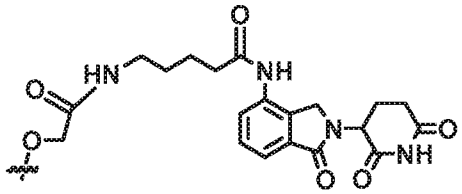
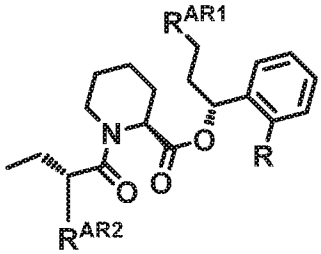
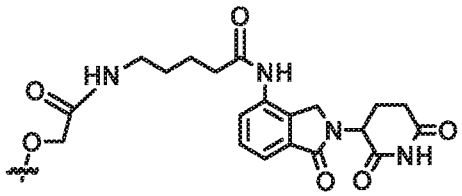
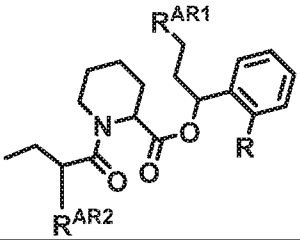
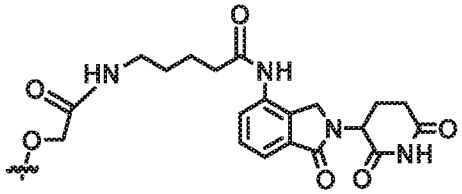
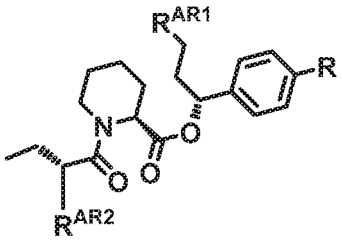
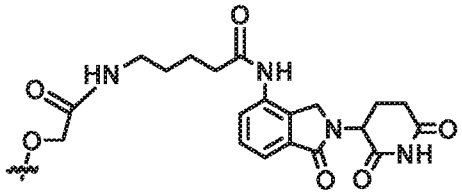
dFKBP-16-I-m		
dFKBP-16-I-m″		
dFKBP-16-I-o		
dFKBP-16-I-o″		
dFKBP-16-I-p		

FIG. 32P

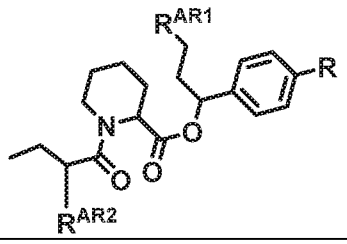
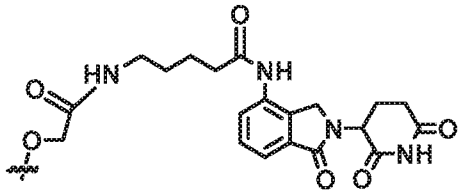
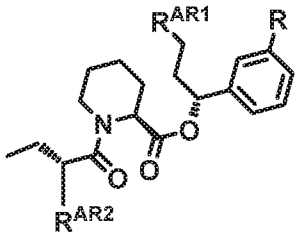
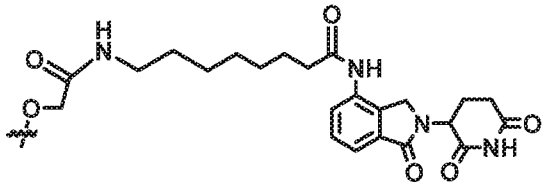
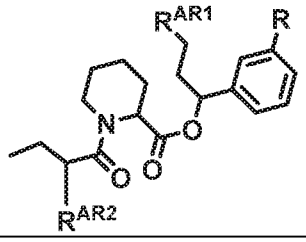
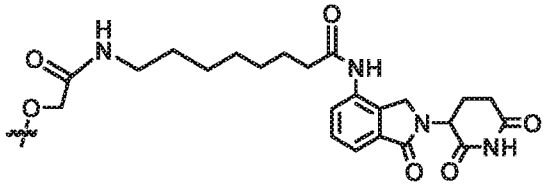
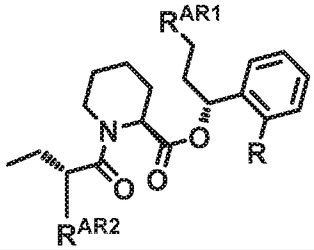
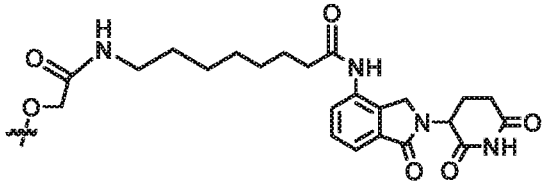
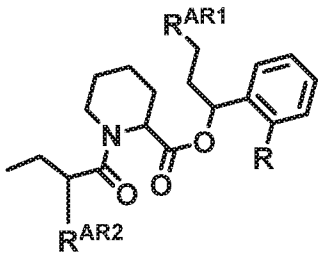
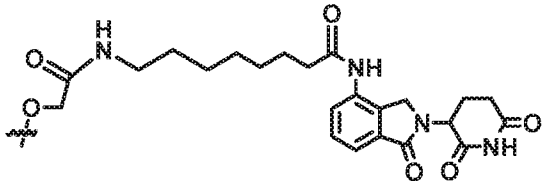
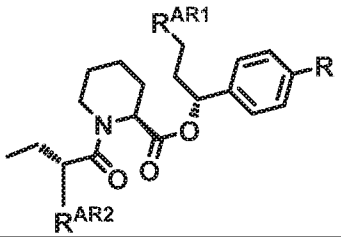
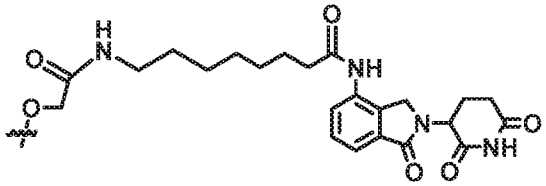
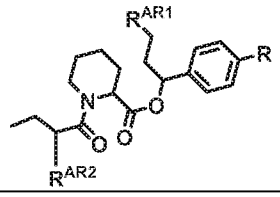
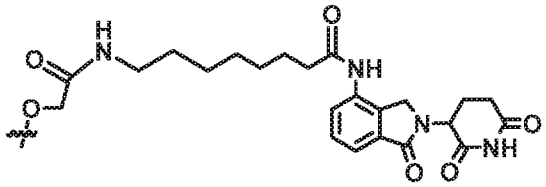
dFKBP-16-I-p″		
dFKBP-20-I-m		
dFKBP-20-I-m″		
dFKBP-20-I-o		
dFKBP-20-I-o″		
dFKBP-20-I-p		
dFKBP-20-I-p″		

FIG. 32Q

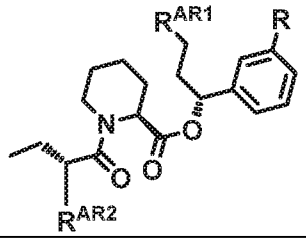
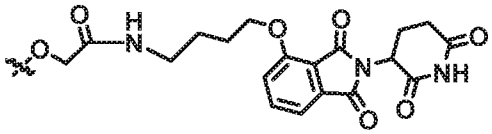
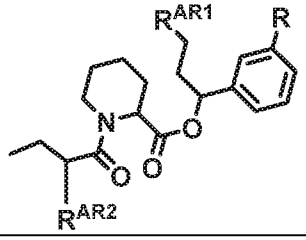
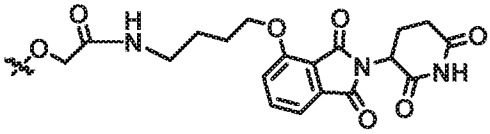
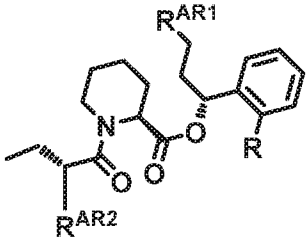
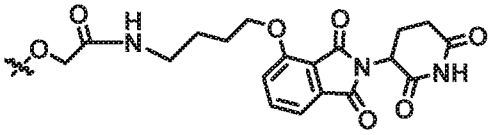
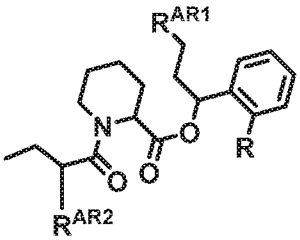
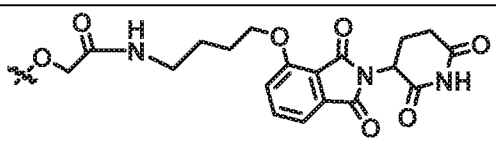
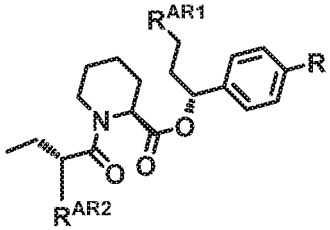
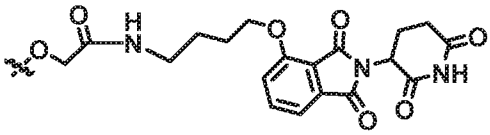
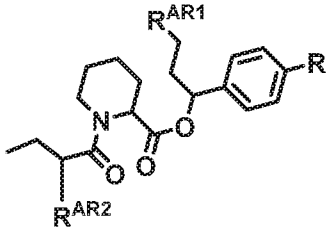
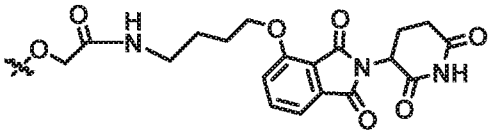
dFKBP-18-I-m		
dFKBP-18-I-m″		
dFKBP-18-I-o		
dFKBP-18-I-o″		
dFKBP-18-I-p		
dFKBP-18-I-p″		

FIG. 32R

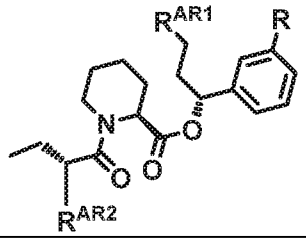
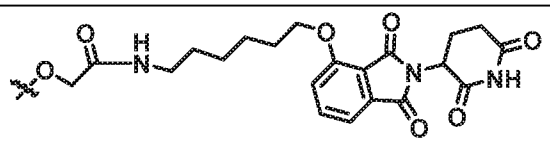
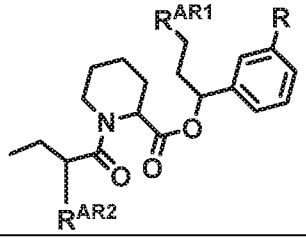
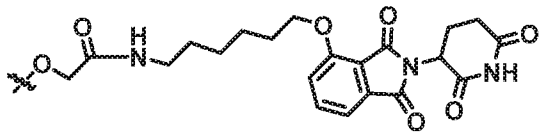
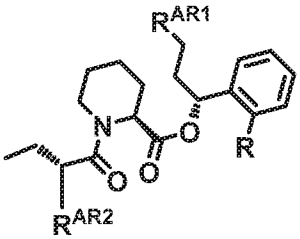
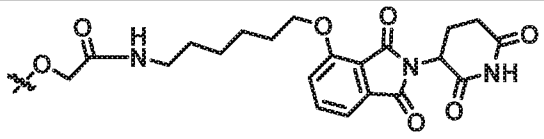
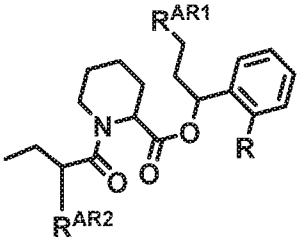
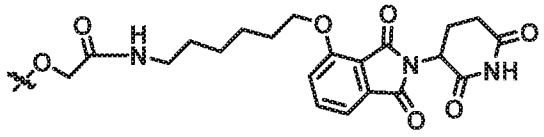
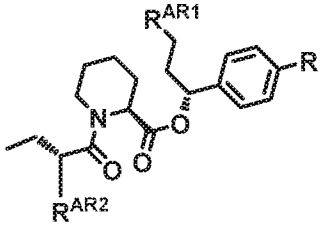
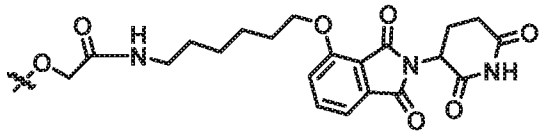
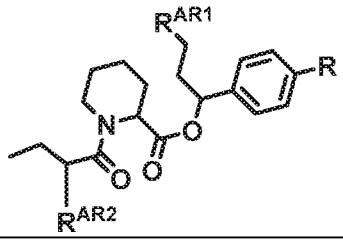
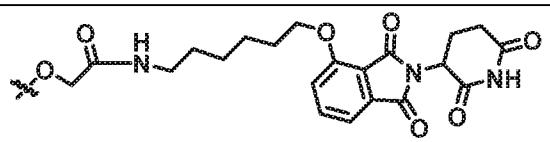
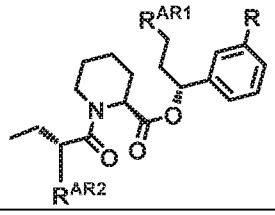
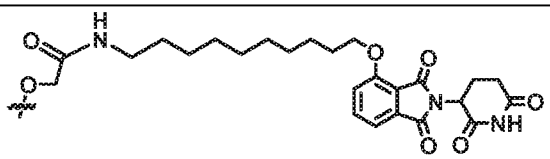
dFKBP-13-I-m		
dFKBP-13-I-m″		
dFKBP-13-I-o		
dFKBP-13-I-o″		
dFKBP-13-I-p		
dFKBP-13-I-p″		
dFKBP-14-I-m		

FIG. 32S

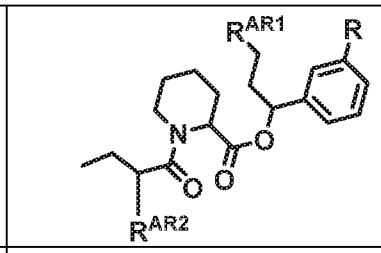
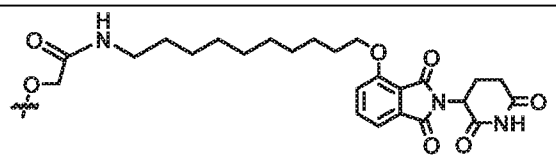
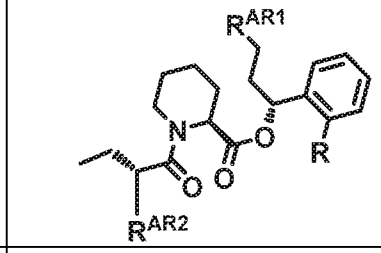
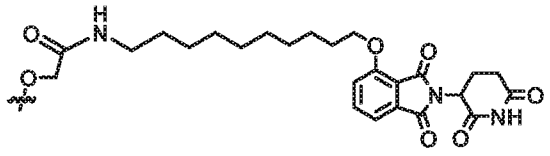
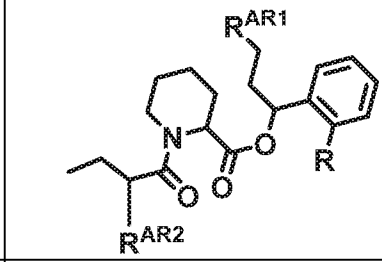
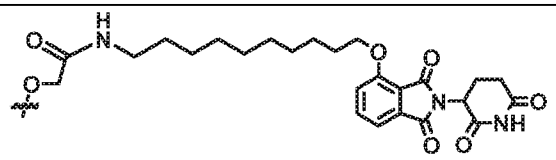
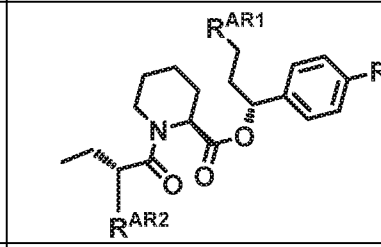
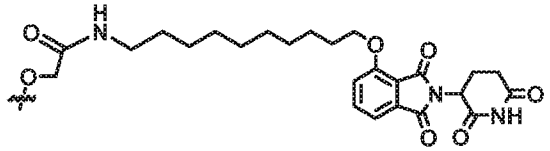
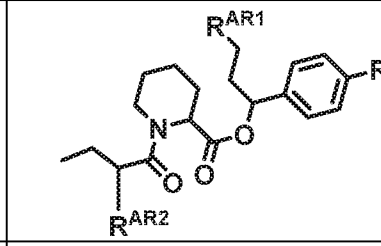
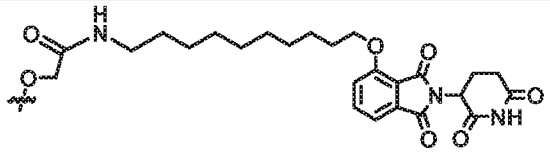
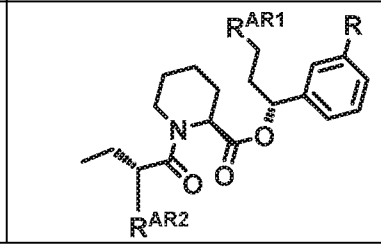
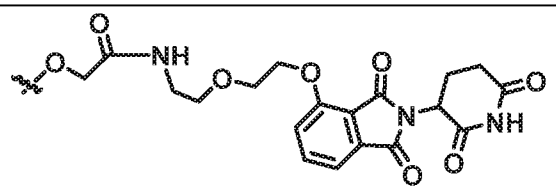
dFKBP-14-I-m″		
dFKBP-14-I-o		
dFKBP-14-I-o″		
dFKBP-14-I-p		
dFKBP-14-I-p″		
dFKBP-19-I-m		

FIG. 32T

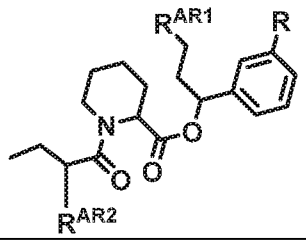
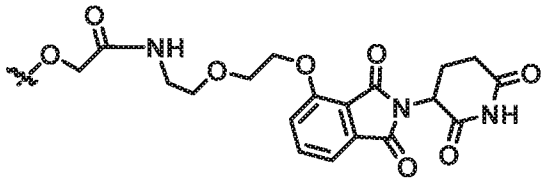
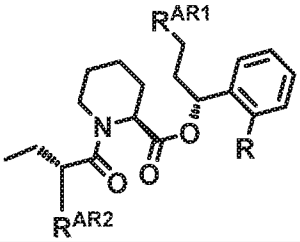
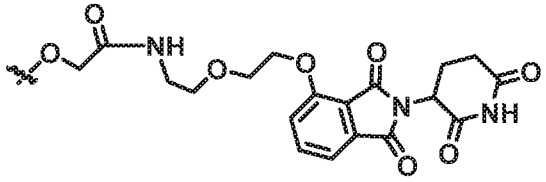
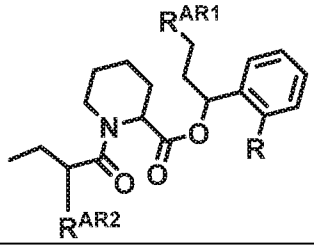
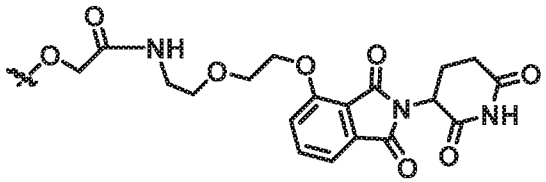
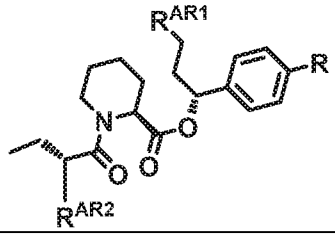
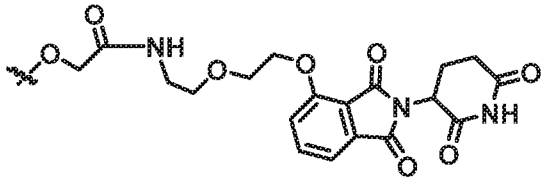
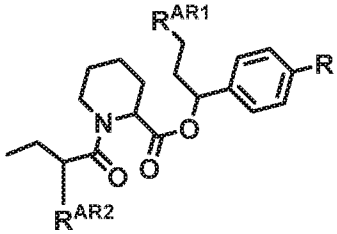
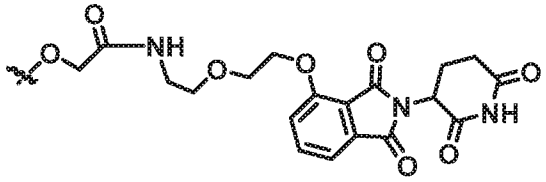
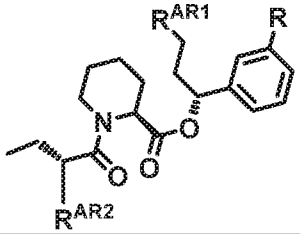
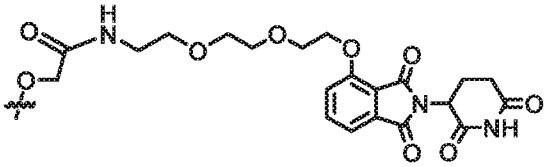
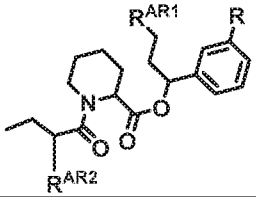
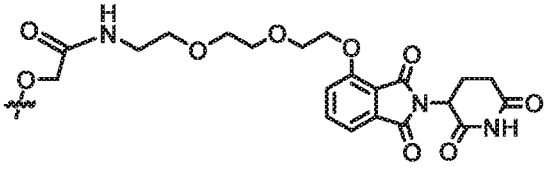
dFKBP-19-I-m″		
dFKBP-19-I-o		
dFKBP-19-I-o″		
dFKBP-19-I-p		
dFKBP-19-I-p″		
dFKBP-15-I-m		
dFKBP-15-I-m″		

FIG. 32U

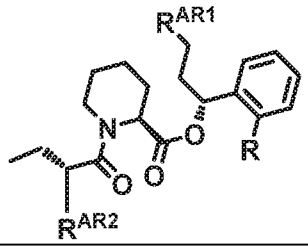
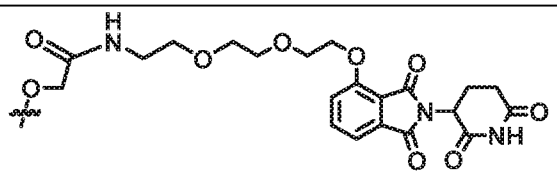
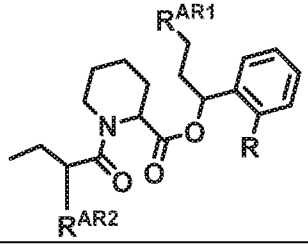
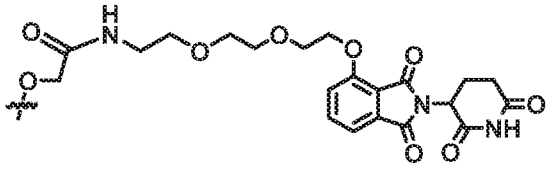
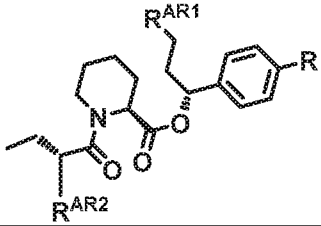
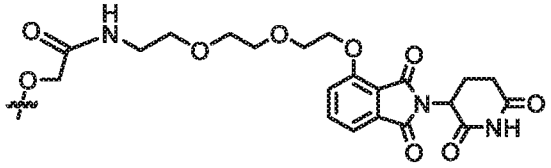
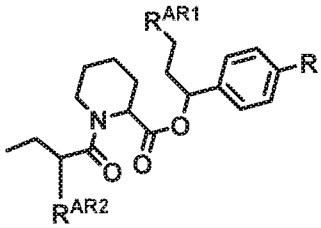
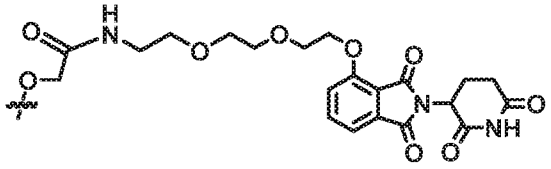
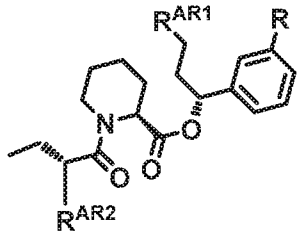
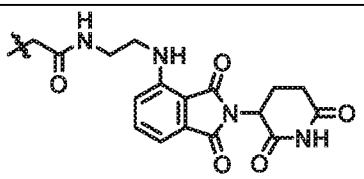
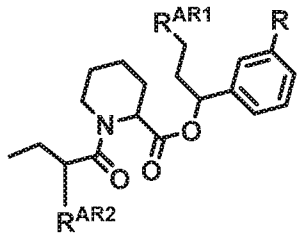
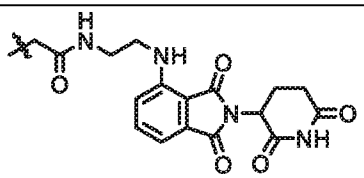
dFKBP-15-I-o		
dFKBP-15-I-o″		
dFKBP-15-I-p		
dFKBP-15-I-p″		
dFKBP-A-m		
dFKBP-A-m″		

FIG. 32V

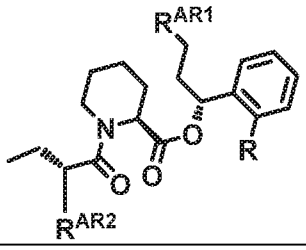
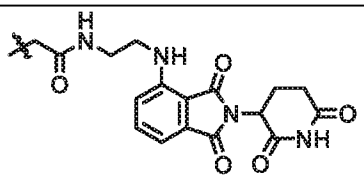
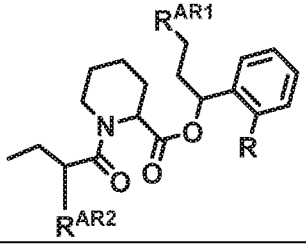
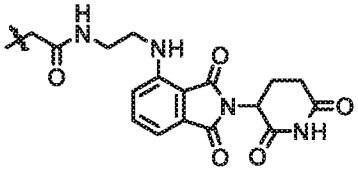
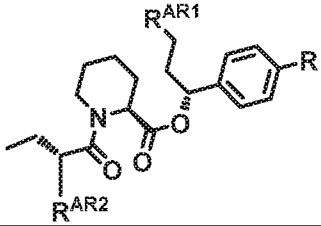
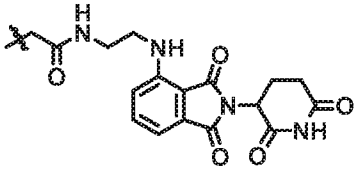
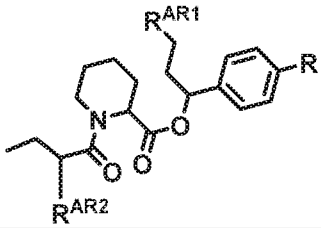
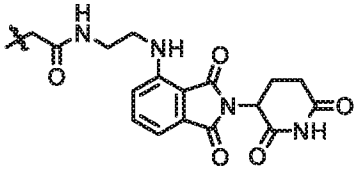
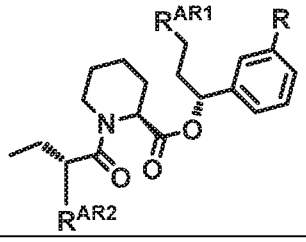
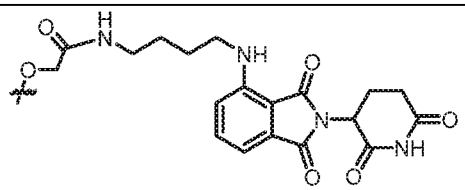
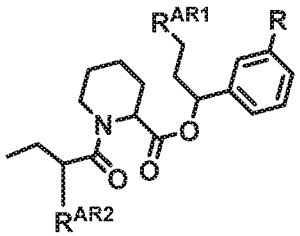
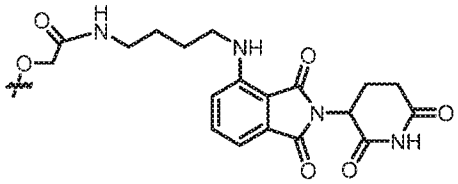
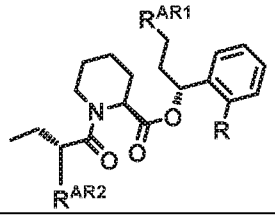
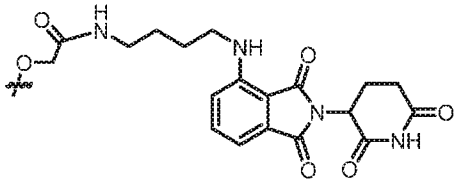
dFKBP-A-o		
dFKBP-A-o″		
dFKBP-A-p		
dFKBP-A-p″		
dFKBP-34-I-m		
dFKBP-34-I-m″		
dFKBP-34-I-o		

FIG. 32W

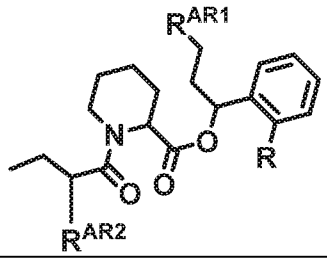
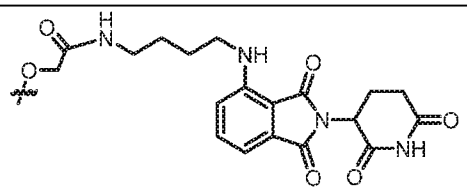
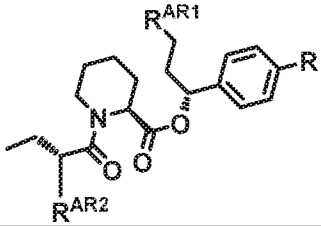
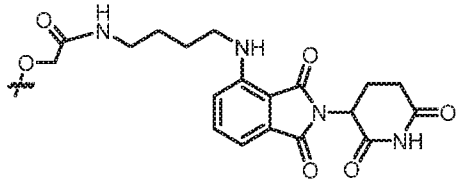
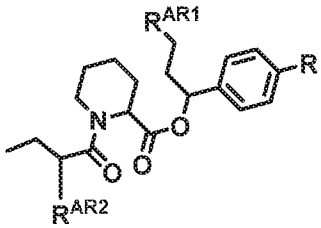
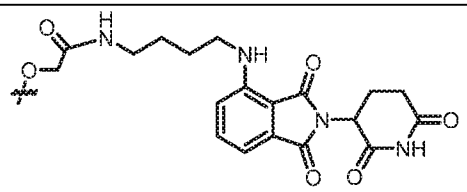
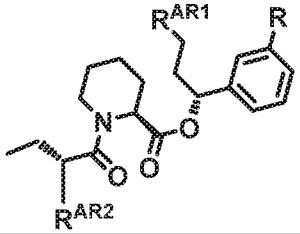
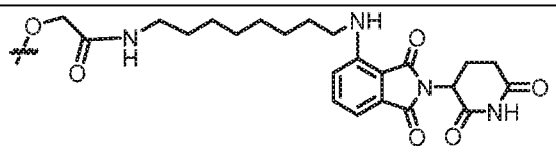
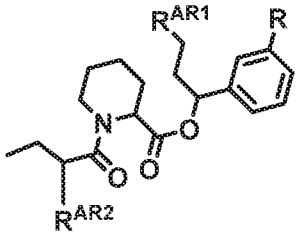
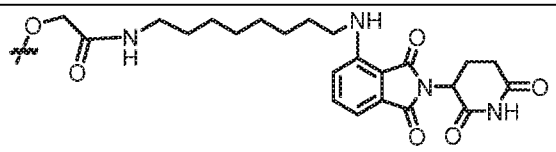
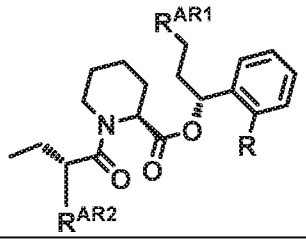
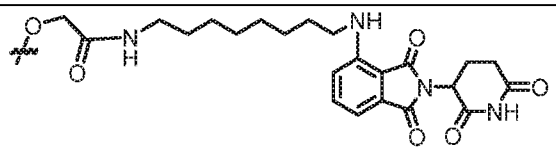
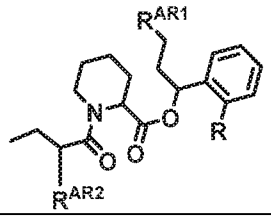
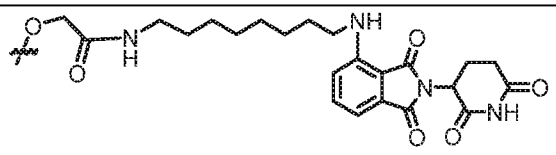
dFKBP-34-I-o″		
dFKBP-34-I-p		
dFKBP-34-I-p″		
dFKBP-36-I-m		
dFKBP-36-I-m″		
dFKBP-36-I-o		
dFKBP-36-I-o″		

FIG. 32X

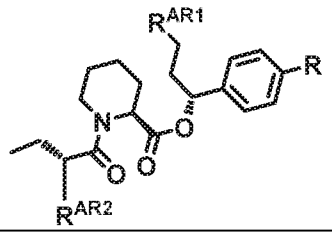
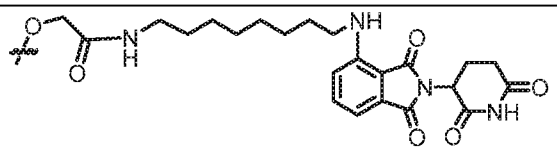
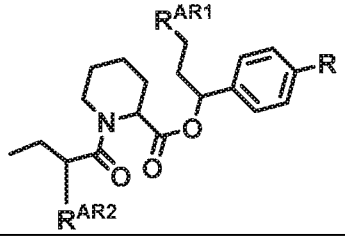
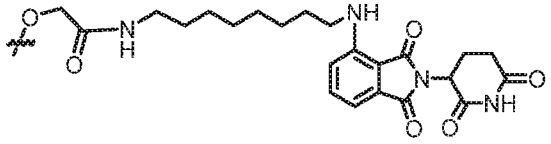
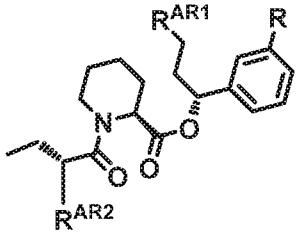
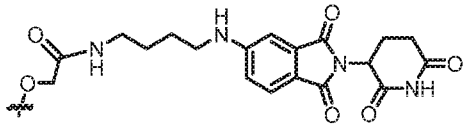
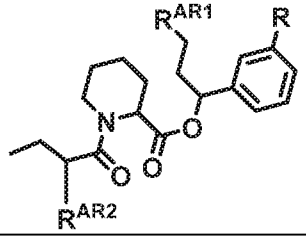
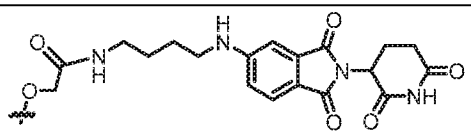
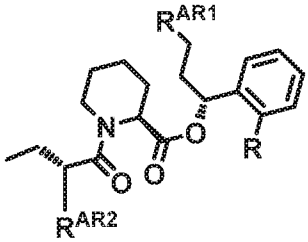
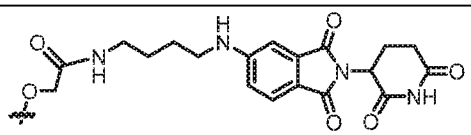
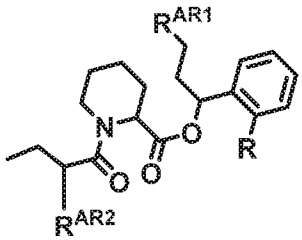
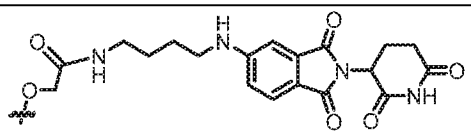
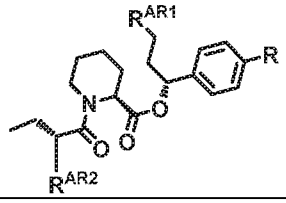
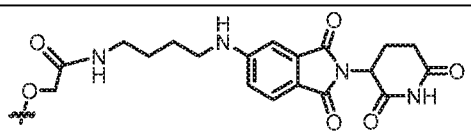
dFKBP-36-I-p		
dFKBP-36-I-p″		
dFKBP-35-I-m		
dFKBP-35-I-m″		
dFKBP-35-I-o		
dFKBP-35-I-o″		
dFKBP-35-I-p		

FIG. 32Y

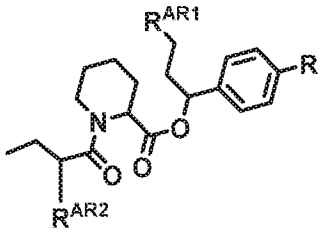
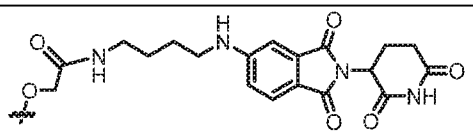
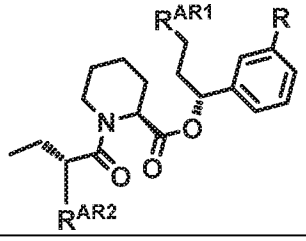
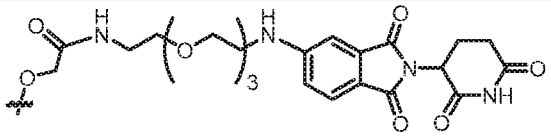
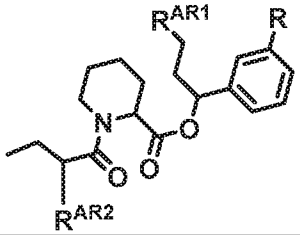
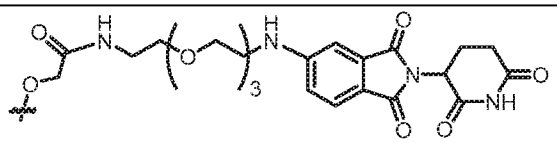
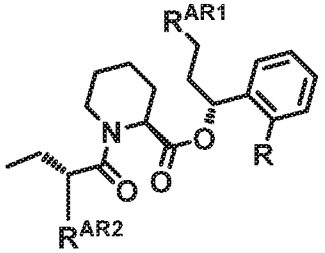
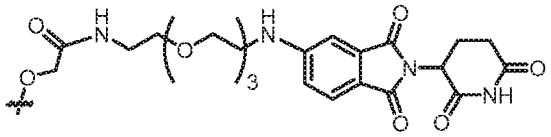
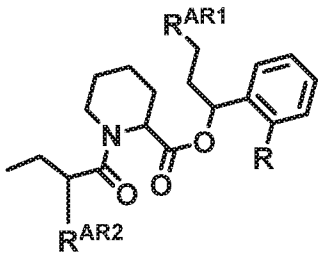
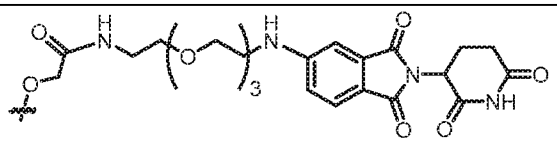
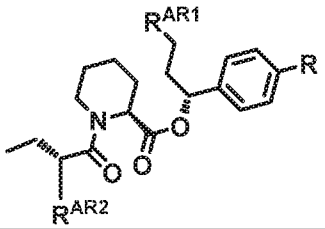
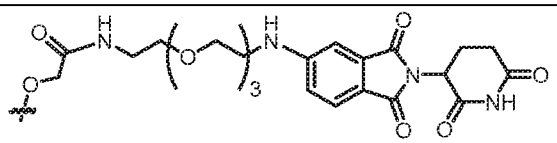
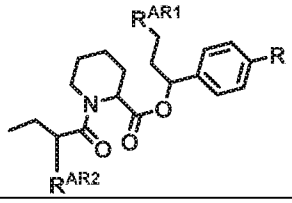
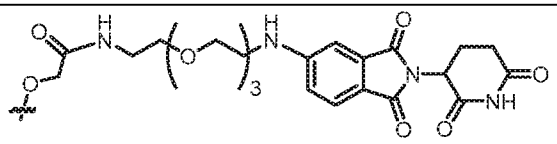
dFKBP-35-I-p″		
dFKBP-37-I-m		
dFKBP-37-I-m″		
dFKBP-37-I-o		
dFKBP-37-I-o″		
dFKBP-37-I-p		
dFKBP-37-I-p″		

FIG. 32Z

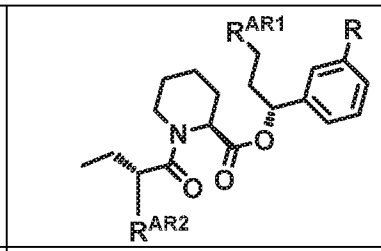
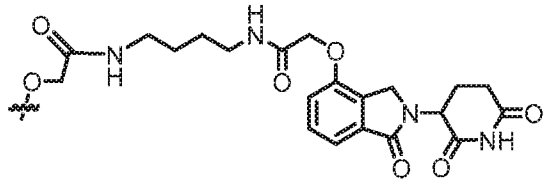
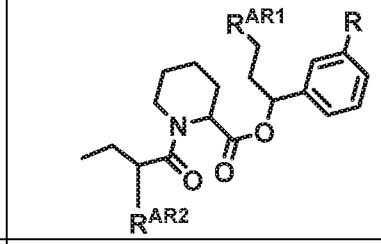
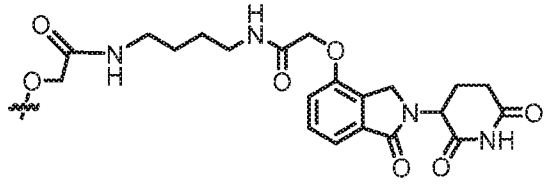
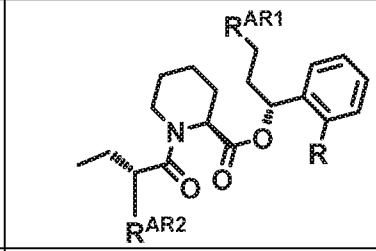
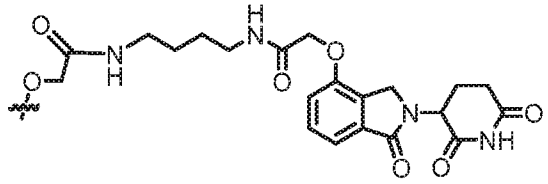
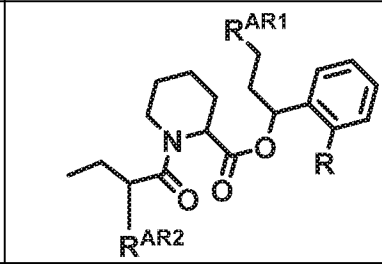
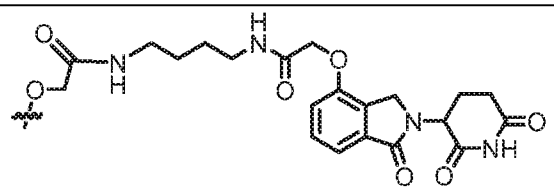
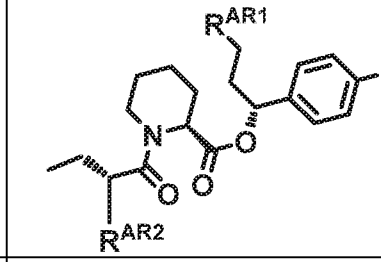
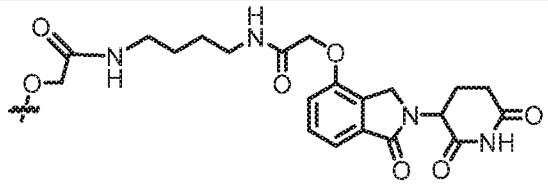
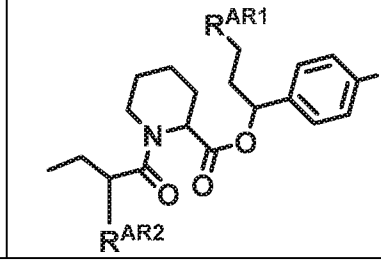
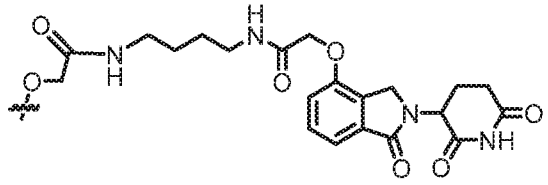
dFKBP-30-I-m		
dFKBP-30-I-m″		
dFKBP-30-I-o		
dFKBP-30-I-o″		
dFKBP-30-I-p		
dFKBP-30-I-p″		

FIG. 32AA

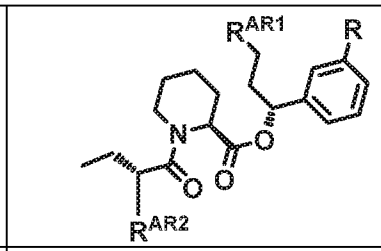
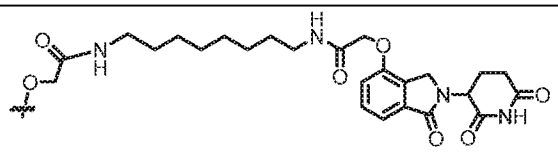
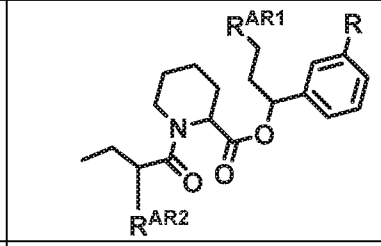
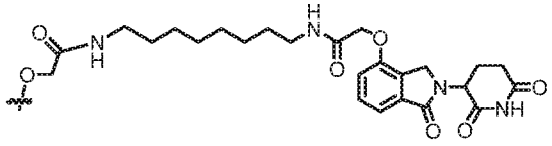
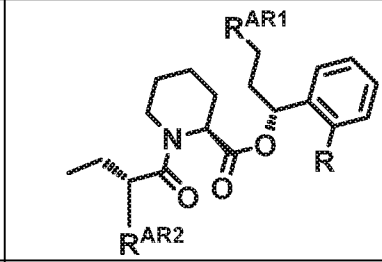
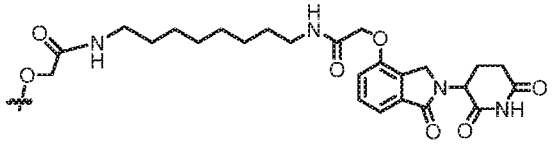
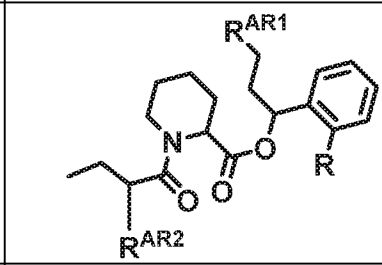
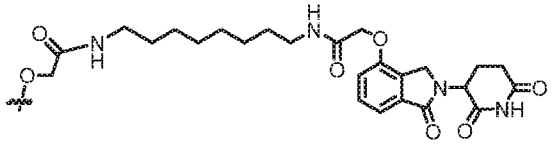
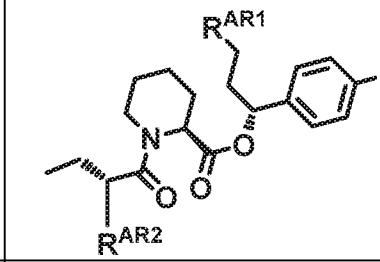
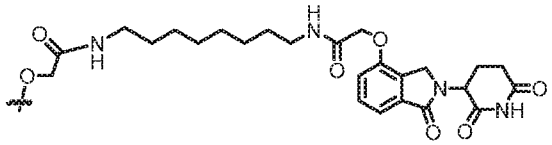
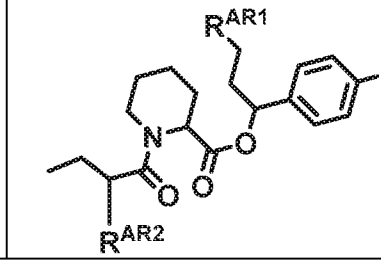
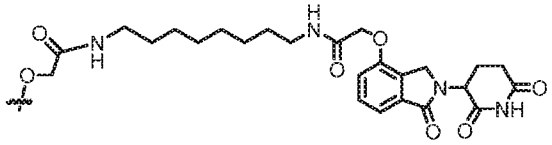
dFKBP-32-I-m		
dFKBP-32-I-m″		
dFKBP-32-I-o		
dFKBP-32-I-o″		
dFKBP-32-I-p		
dFKBP-32-I-p″		

FIG. 32BB

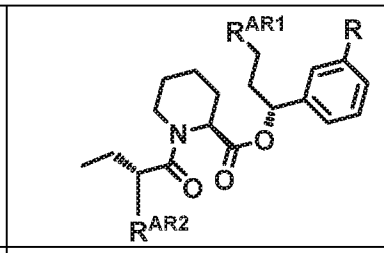
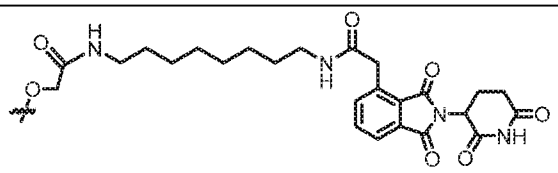
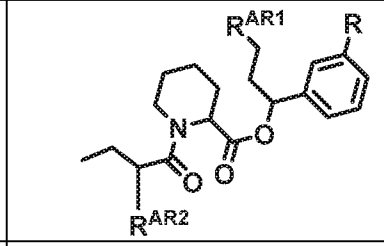
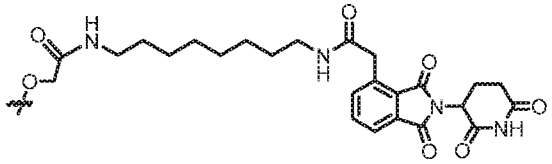
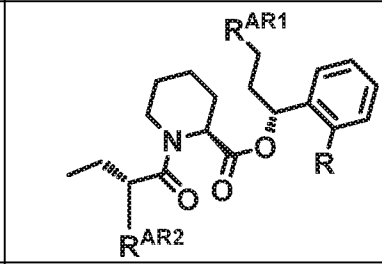
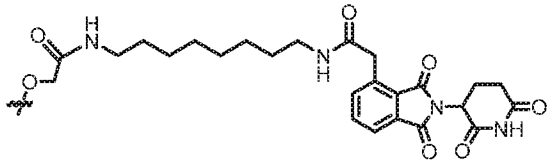
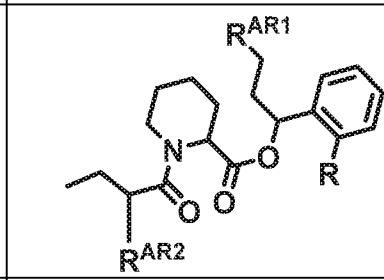
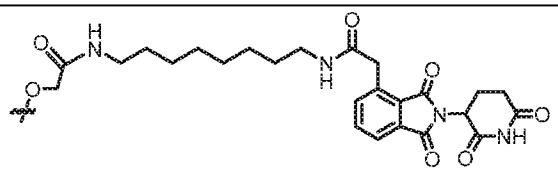
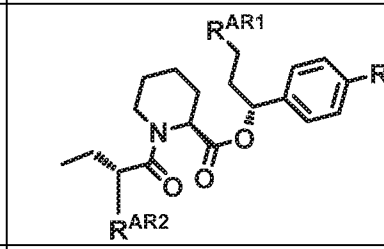
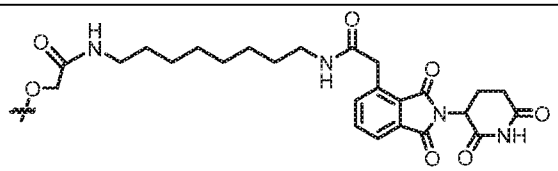
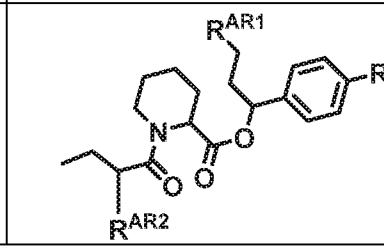
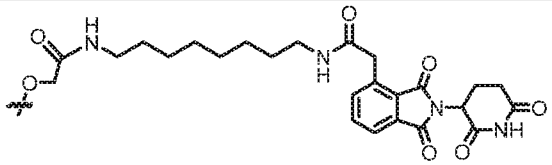
dFKBP-31-I-m		
dFKBP-31-I-m″		
dFKBP-31-I-o		
dFKBP-31-I-o″		
dFKBP-31-I-p		
dFKBP-31-I-p″		

FIG. 32CC

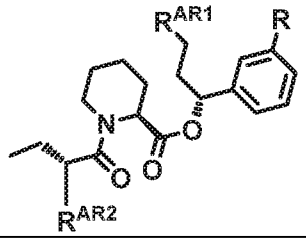
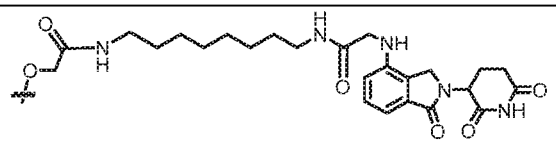
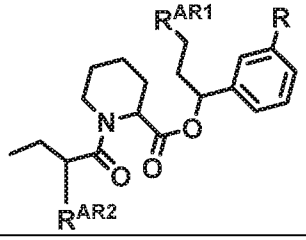
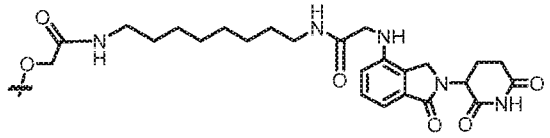
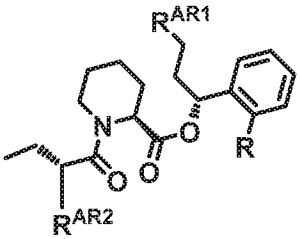
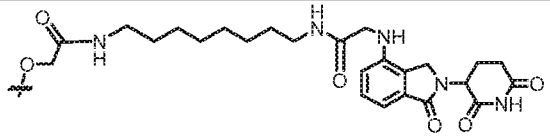
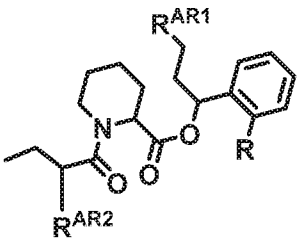
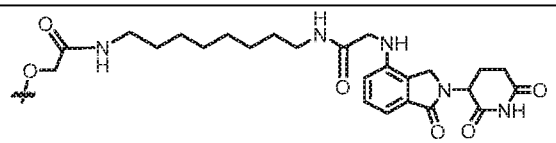
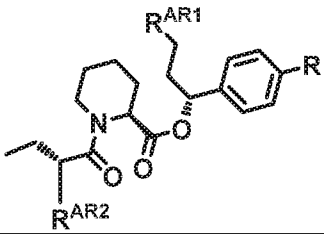
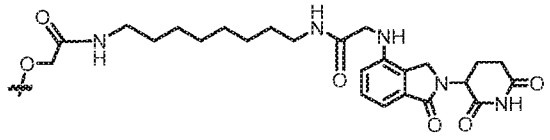
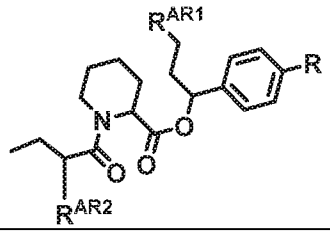
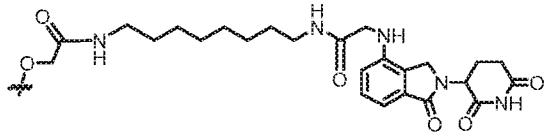
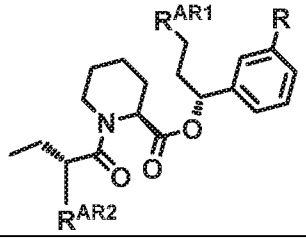
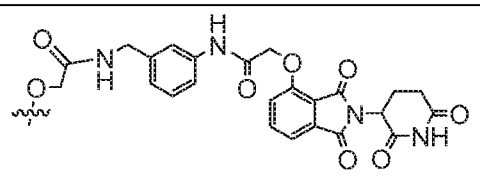
dFKBP-33-I-m		
dFKBP-33-I-m″		
dFKBP-33-I-o		
dFKBP-33-I-o″		
dFKBP-33-I-p		
dFKBP-33-I-p″		
dFKBP-38-I-m		

FIG. 32DD

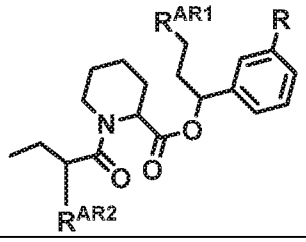
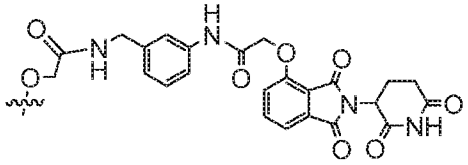
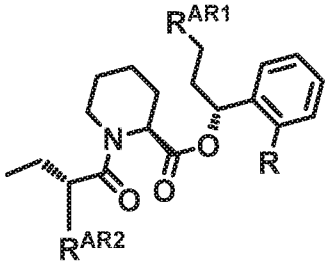
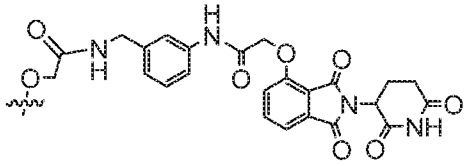
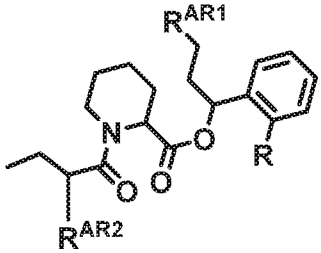
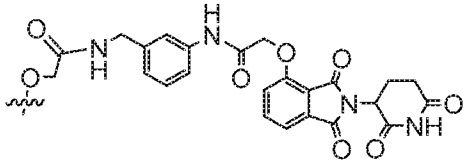
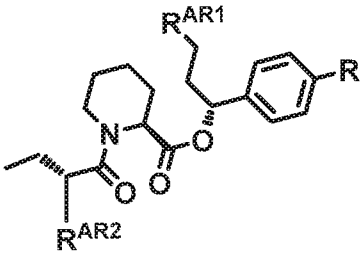
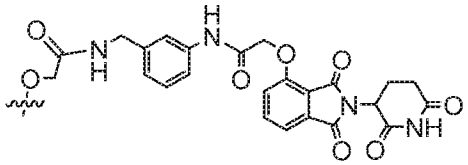
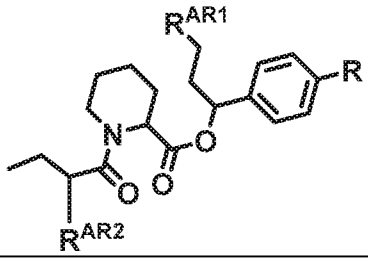
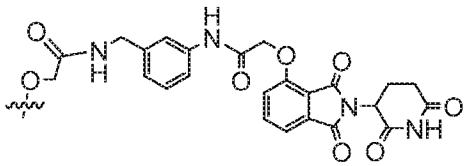
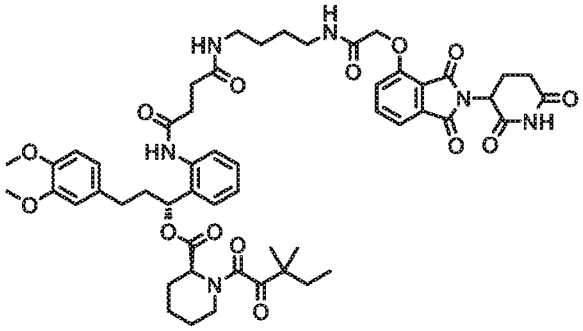
dFKBP-38-I-m″		
dFKBP-38-I-o		
dFKBP-38-I-o″		
dFKBP-38-I-p		
dFKBP-38-I-p″		

FIG. 32EE



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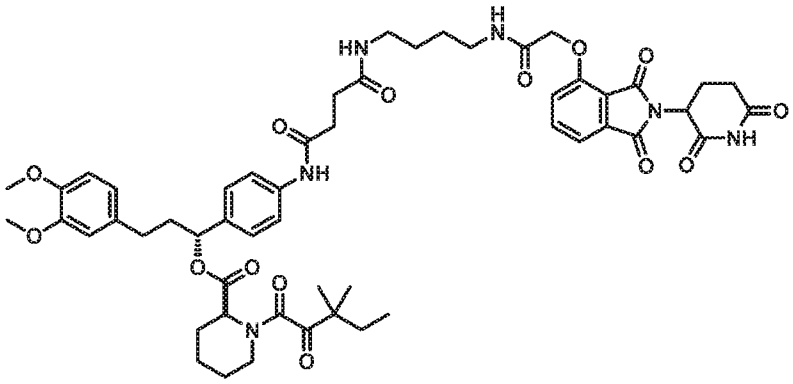
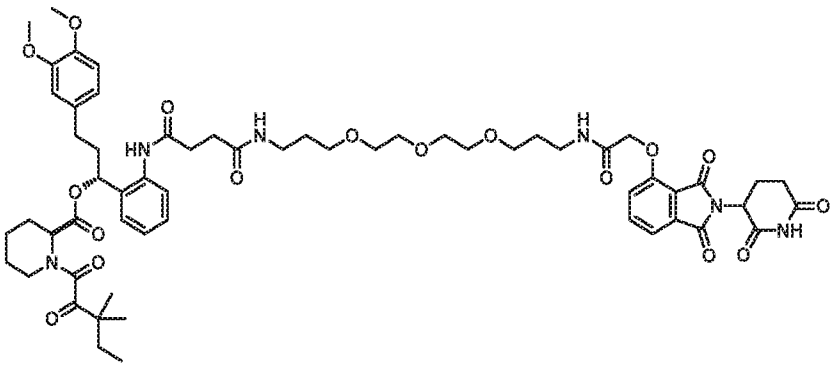
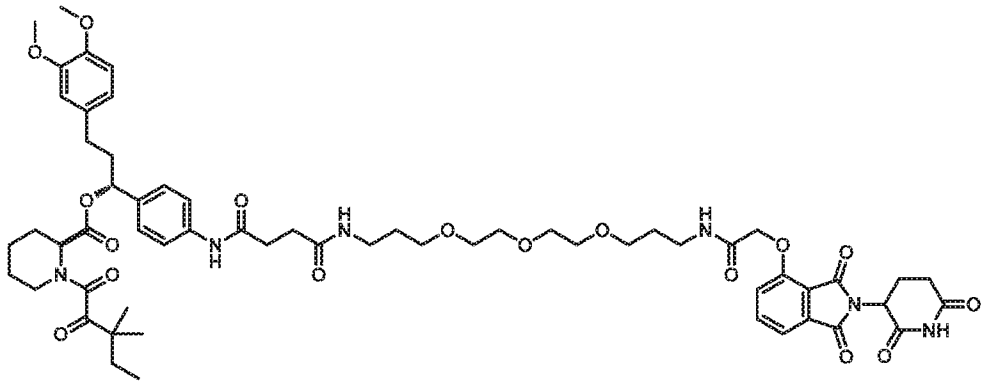


dFKBP-1-*p*

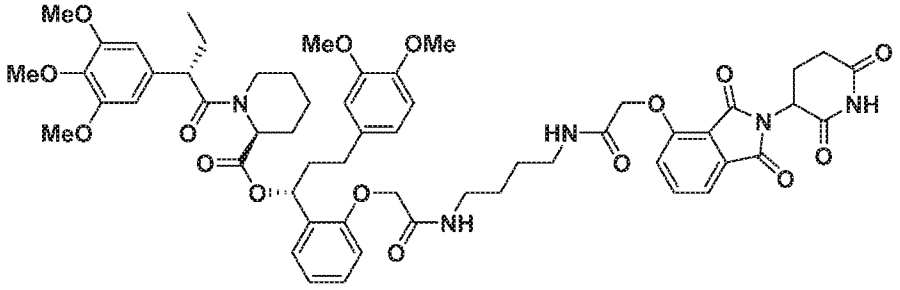


dFKBP-2-*o*

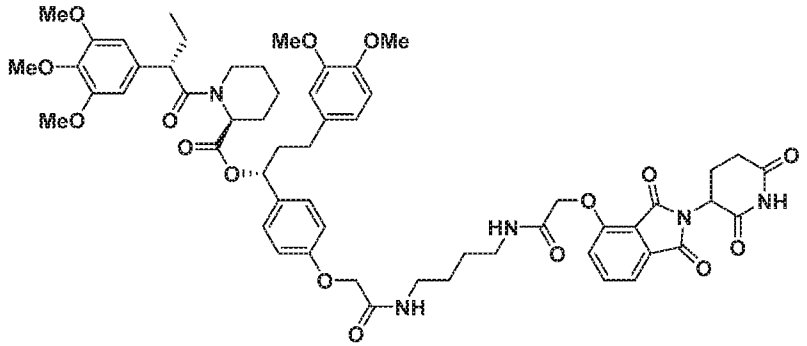
FIG. 33A



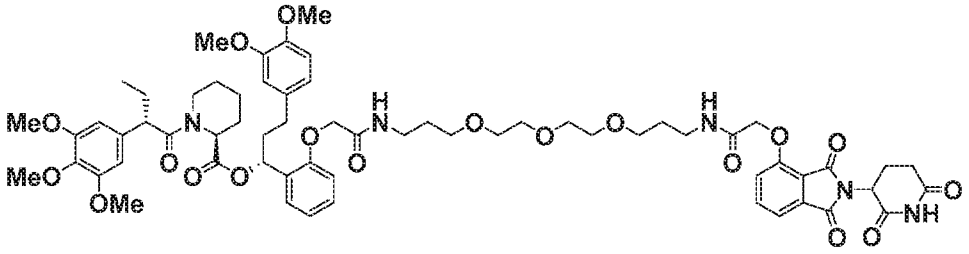
dFKBP-2-*p*



dFKBP*6-*o*

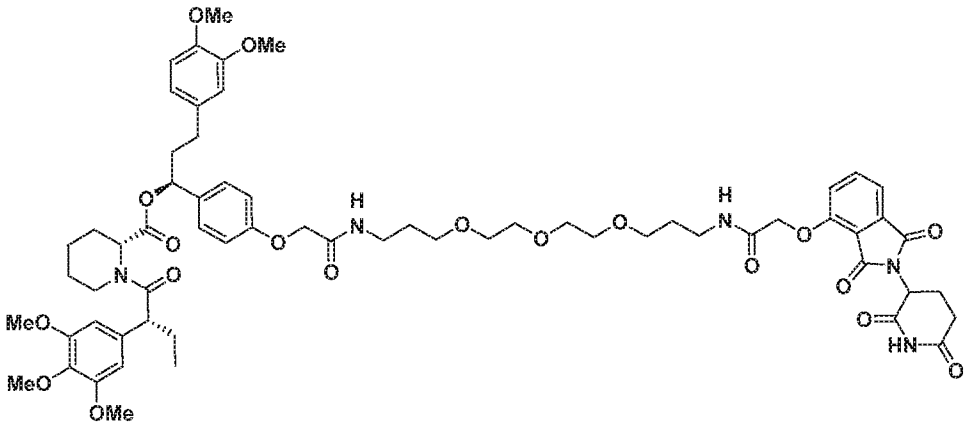


dFKBP*6-*p*

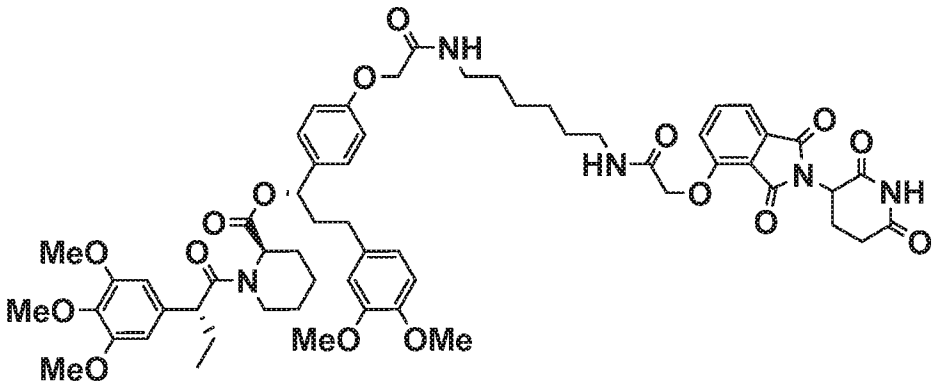


dFKBP*7-*o*

FIG. 33B



dFKBP*7-*p*



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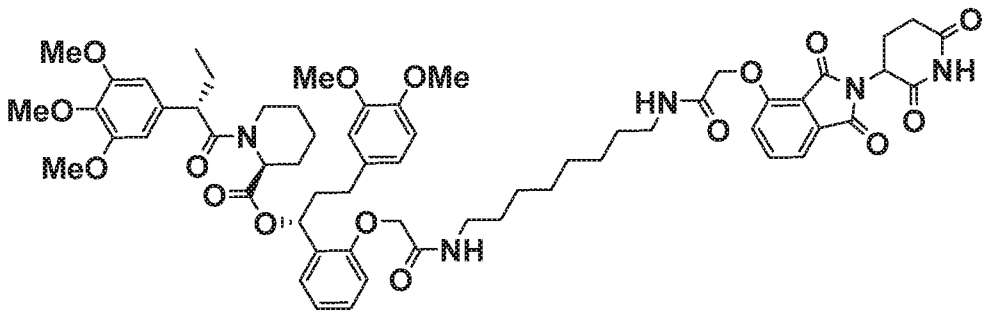
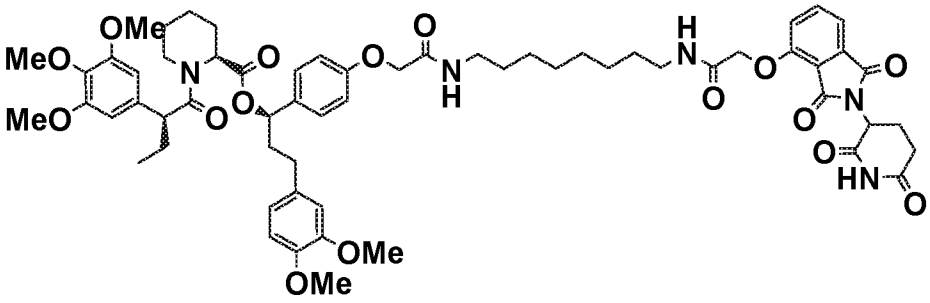
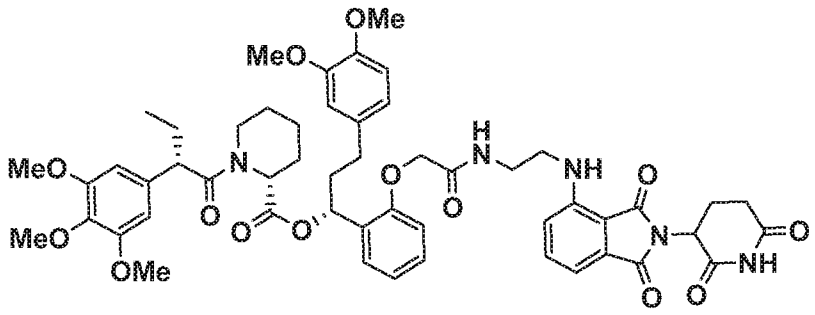


dFKBP*9-*o*

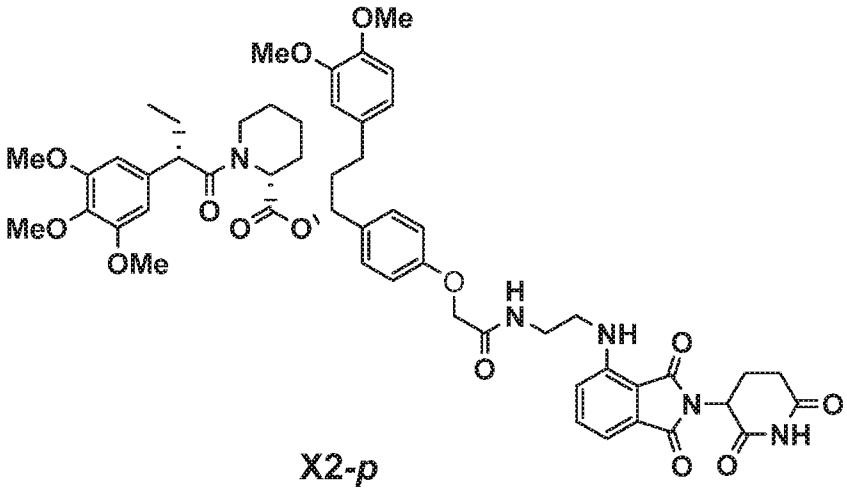
FIG. 33C



dFKBP*9-*p*

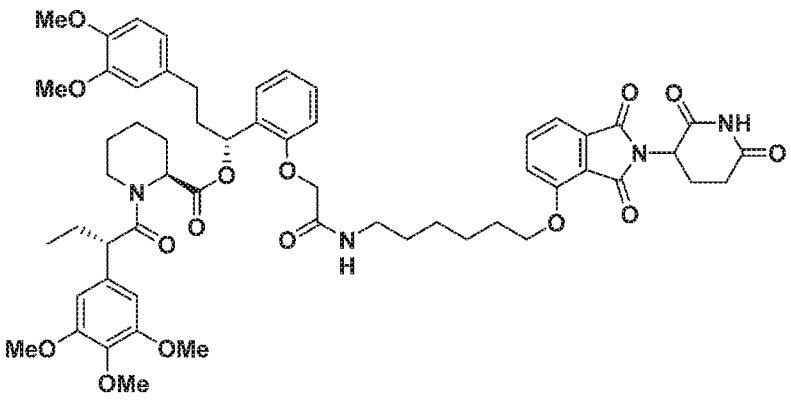


X2-*o*

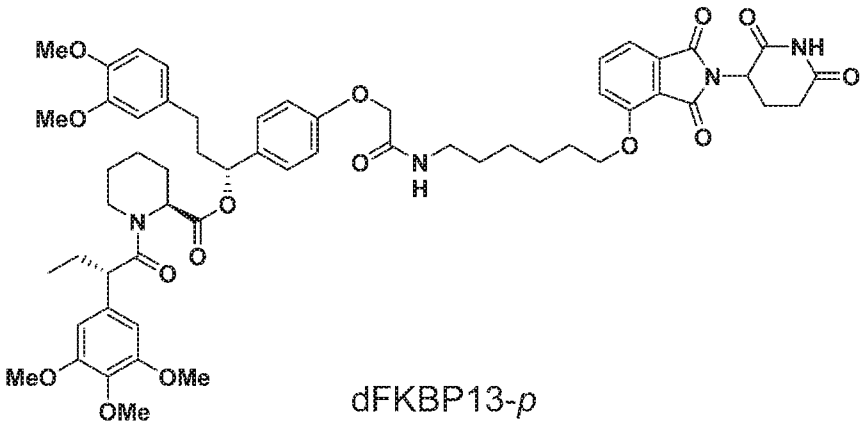


X2-*p*

FIG. 33D



dFKBP13-*o*



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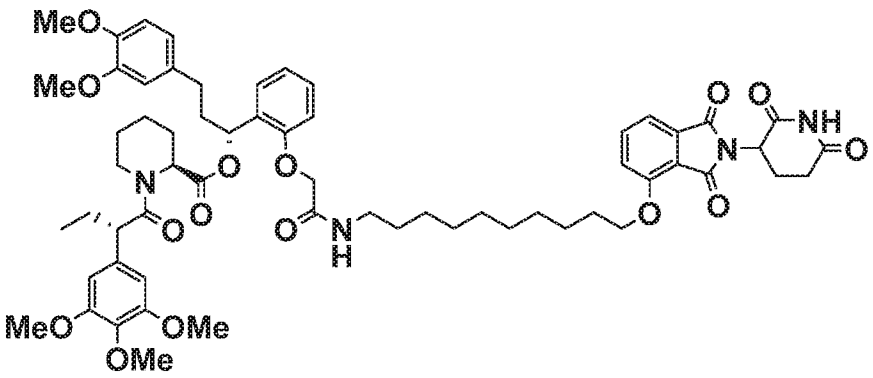
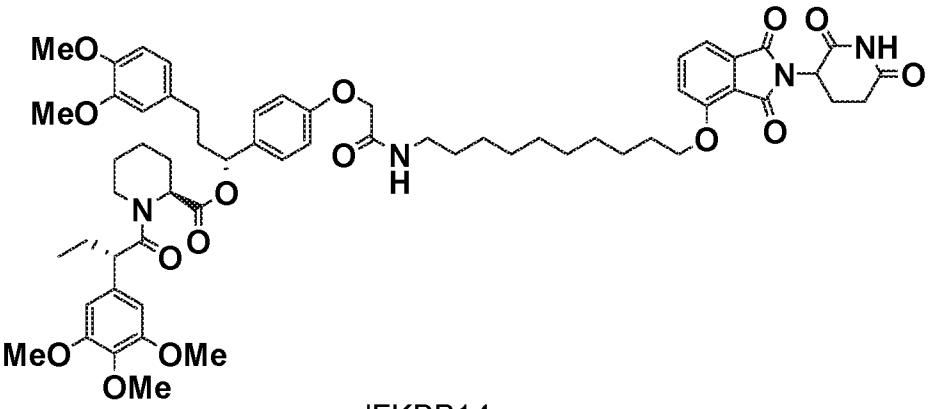


dFKBP14-*o*

FIG. 33E



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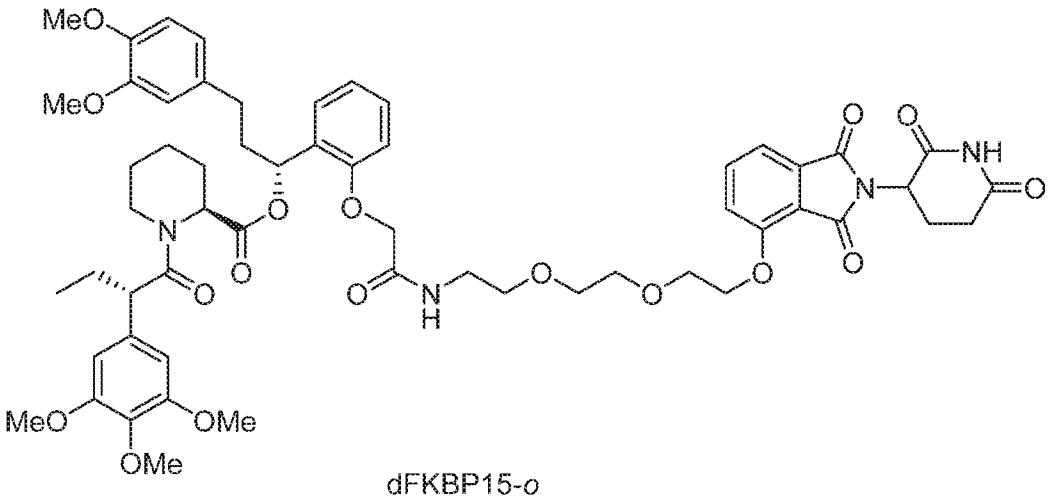

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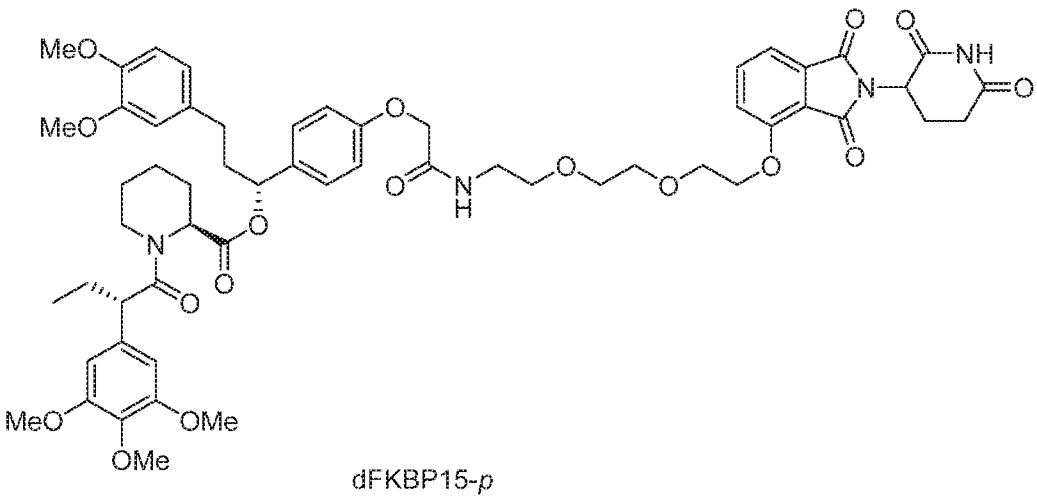
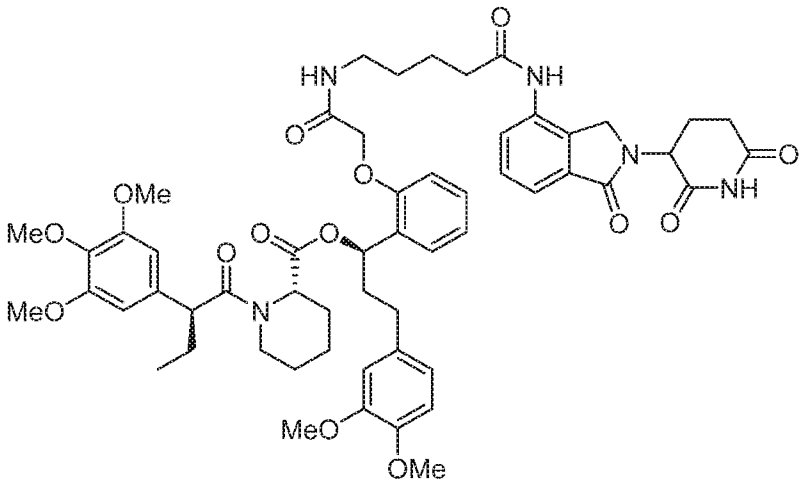
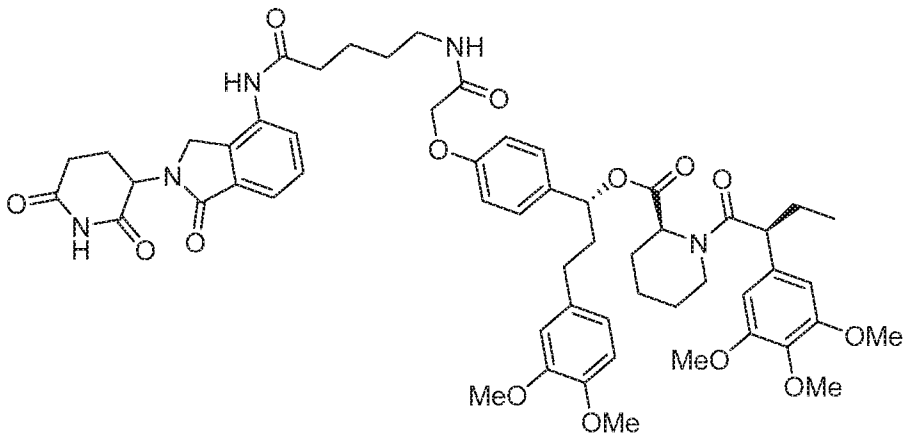


dFKBP15-*p*

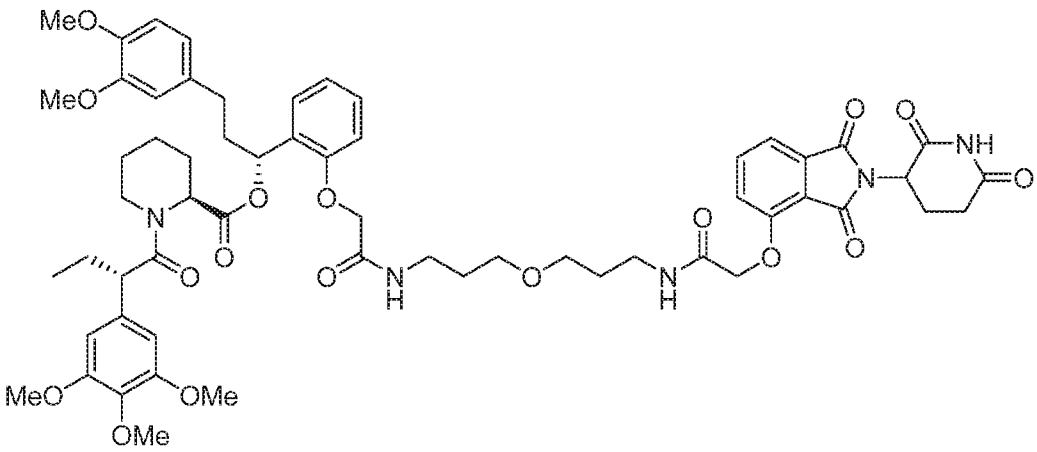
FIG. 33F



dFKBP16-o

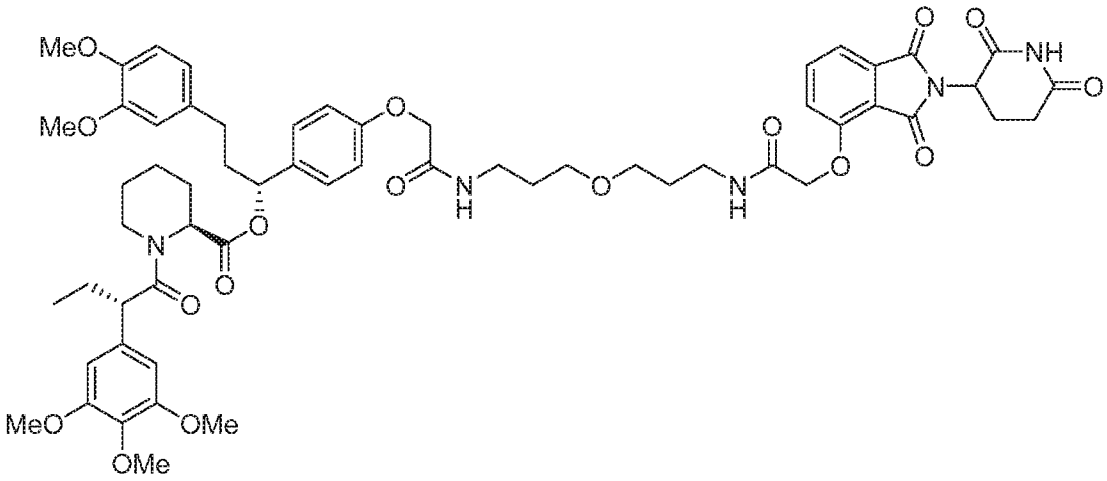


dFKBP16-p



dFKBP17-o

FIG. 33G



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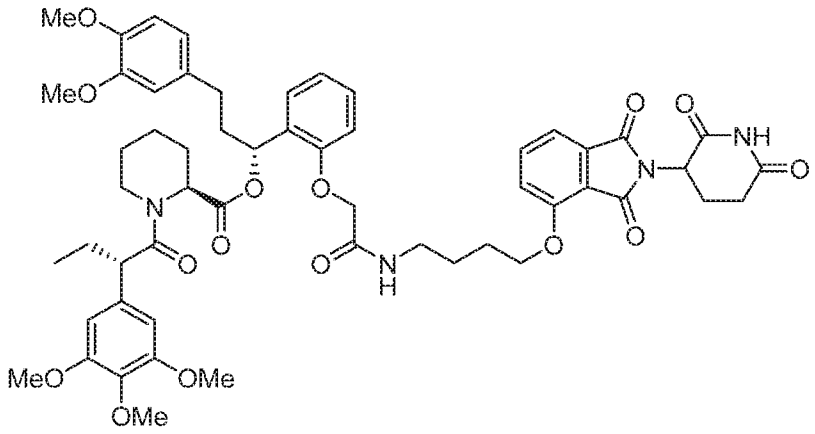

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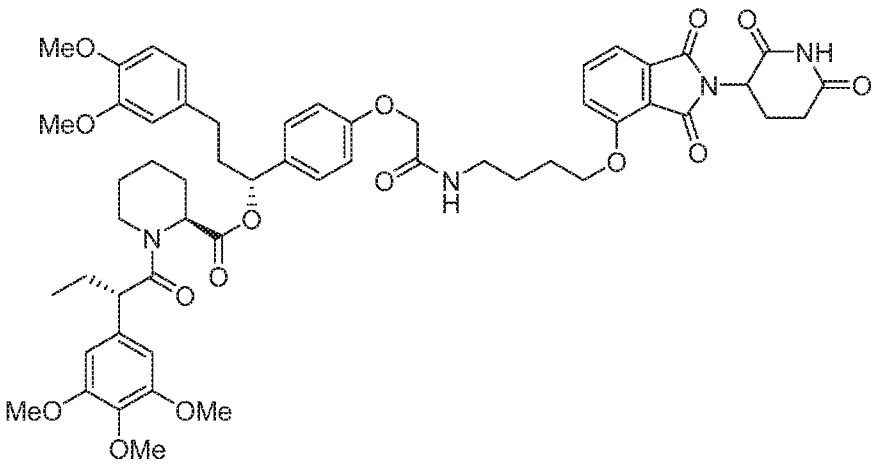
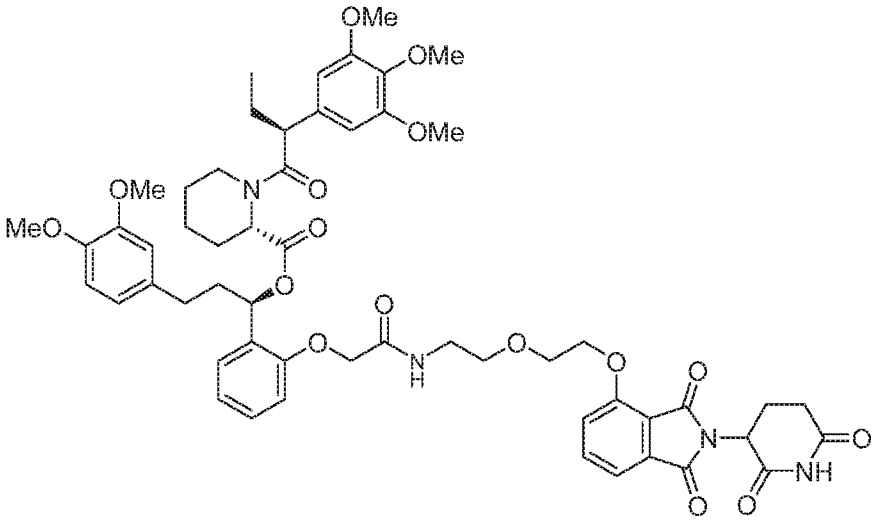
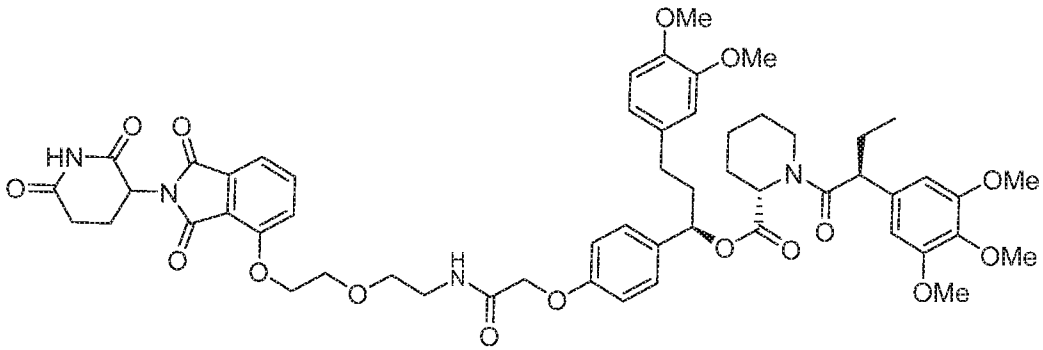


dFKBP18-p

FIG. 33H



dFKBP19-o



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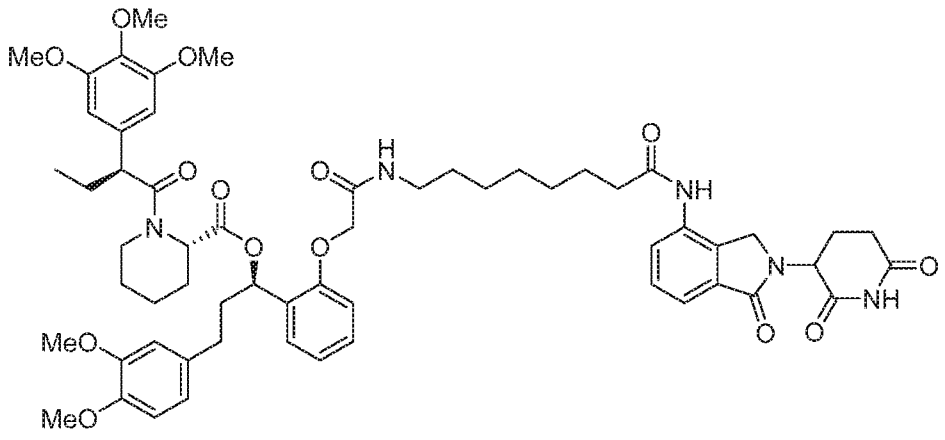
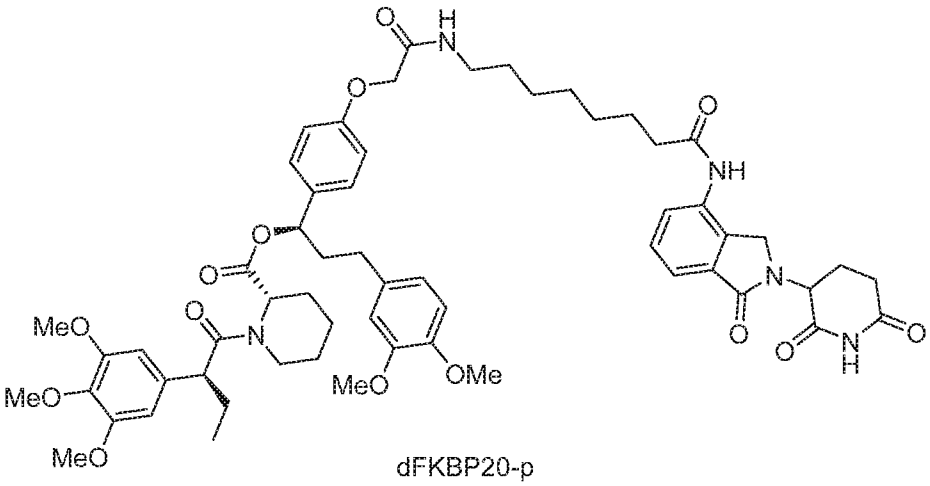


dFKBP20-o

FIG. 33I



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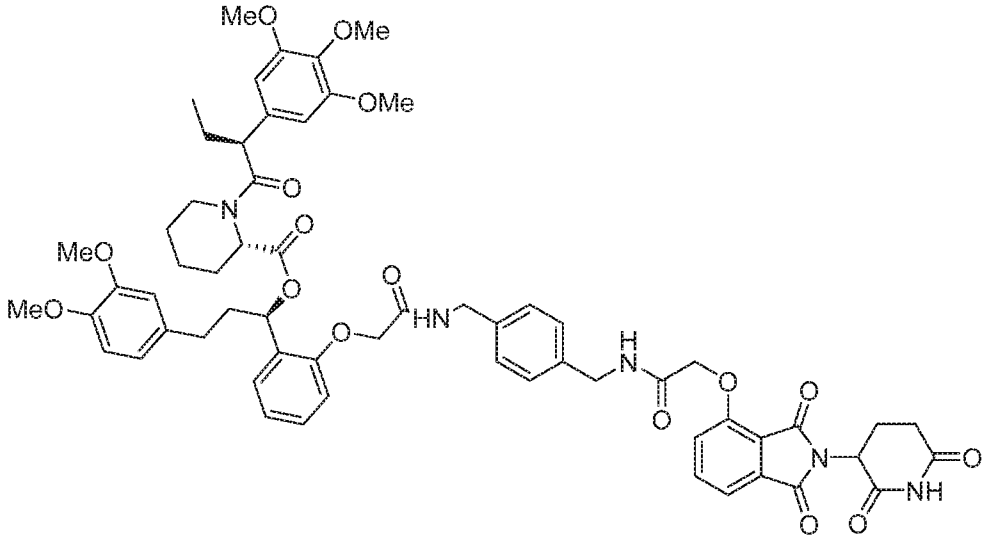

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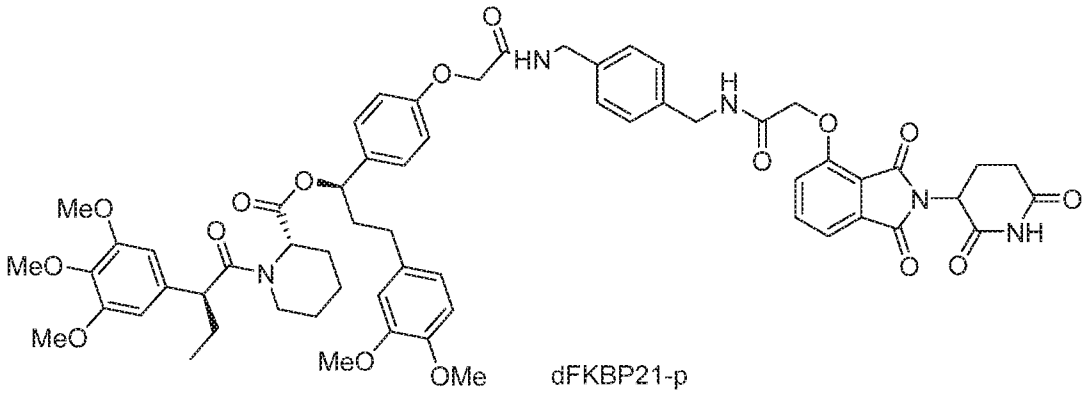
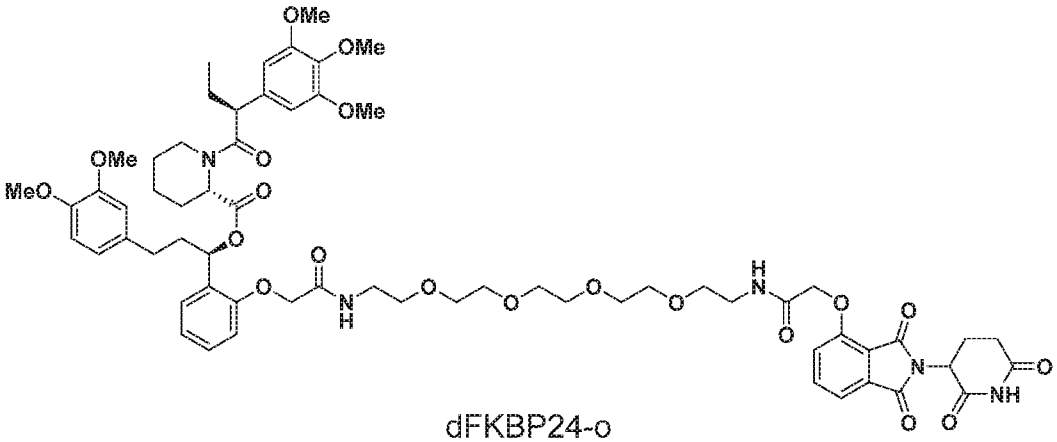
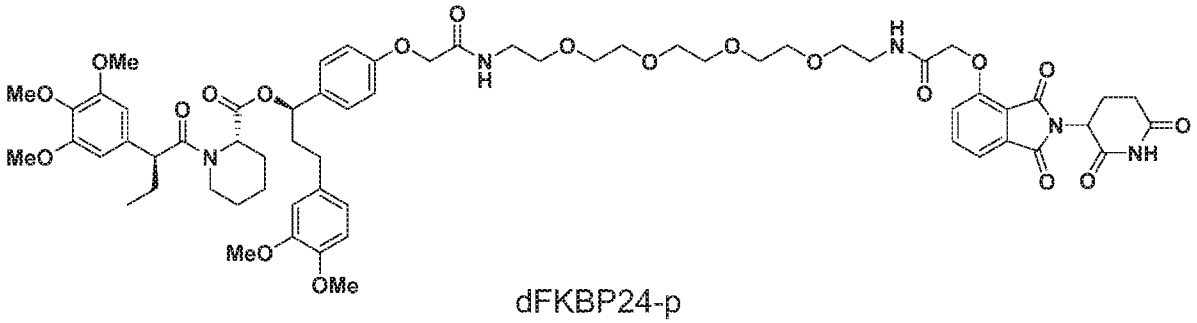


dFKBP21-p

FIG. 33J



dFKBP24-o



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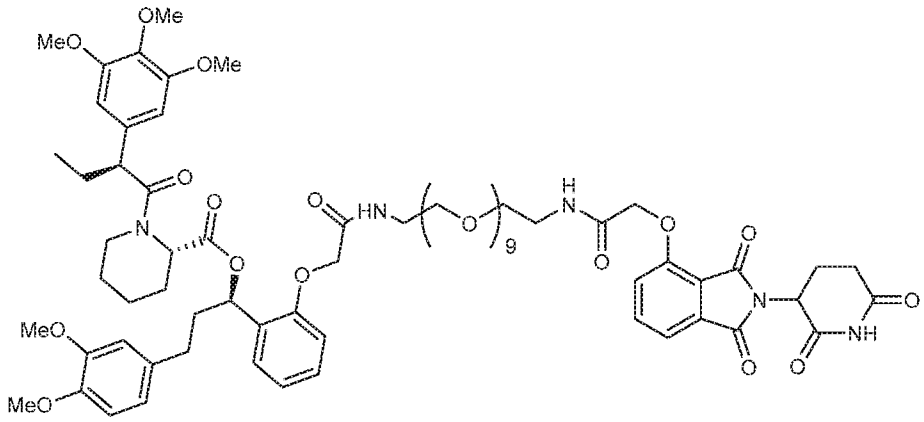
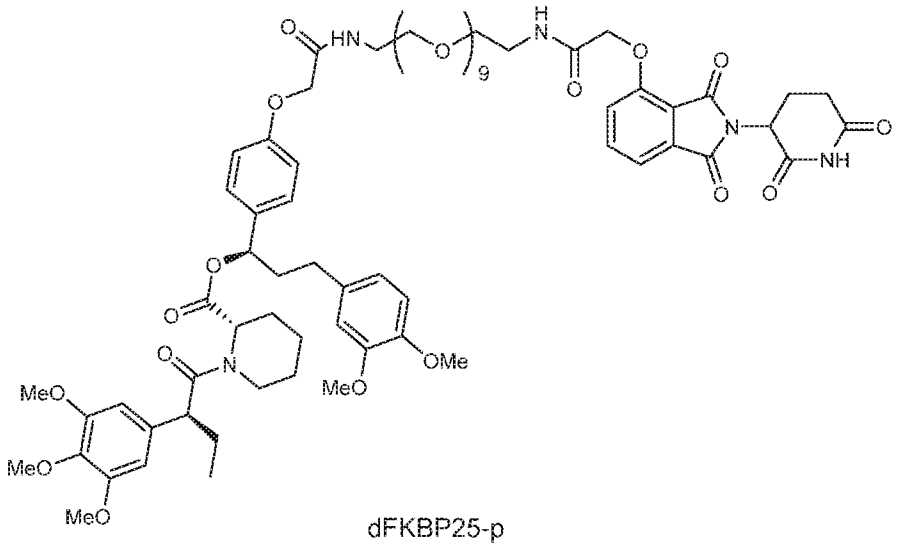


dFKBP25-o

FIG. 33K



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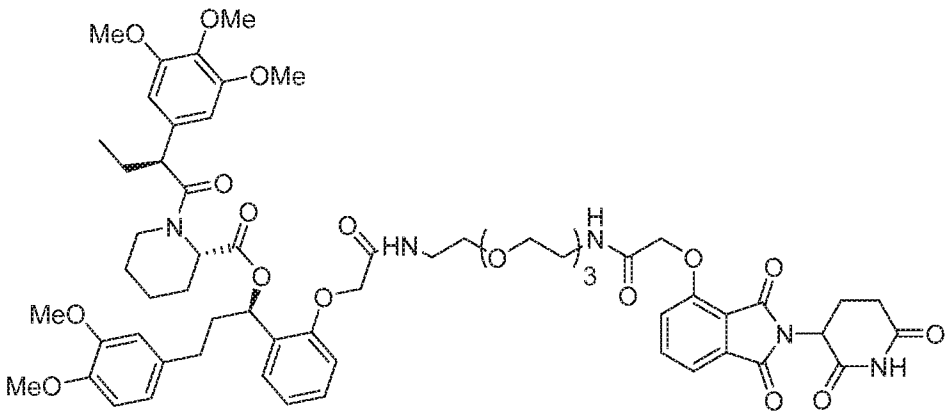

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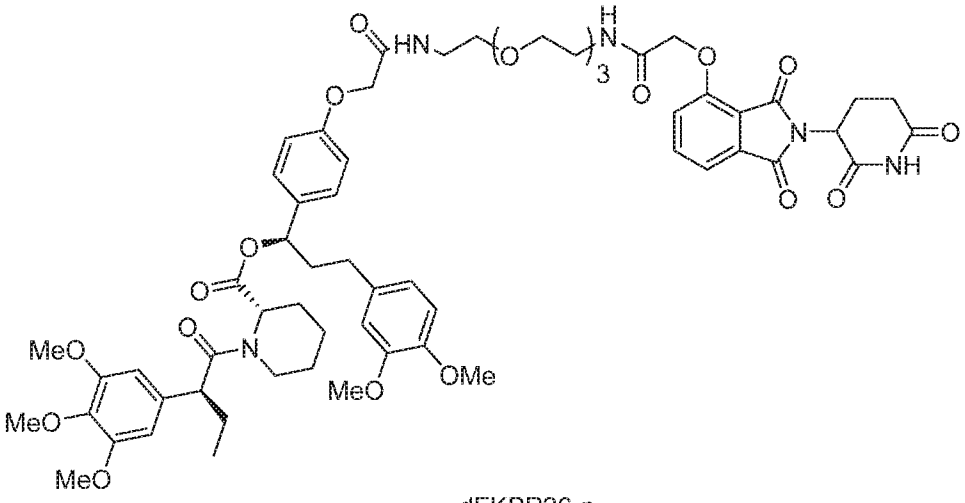

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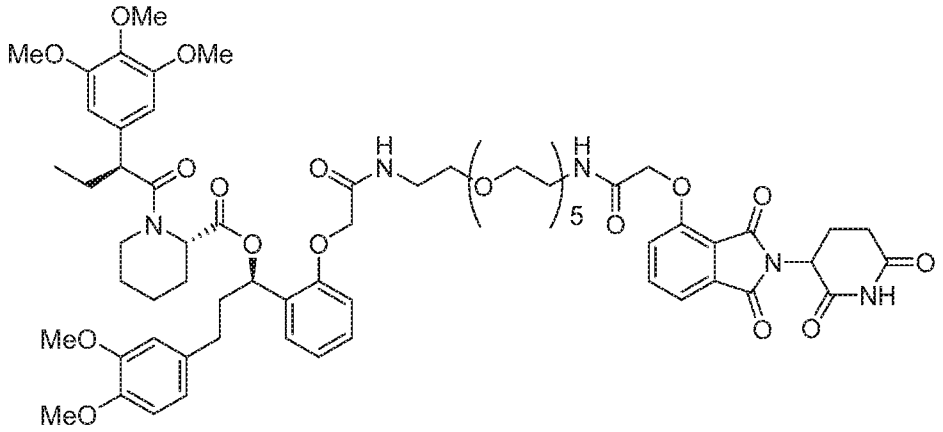
FIG. 33L



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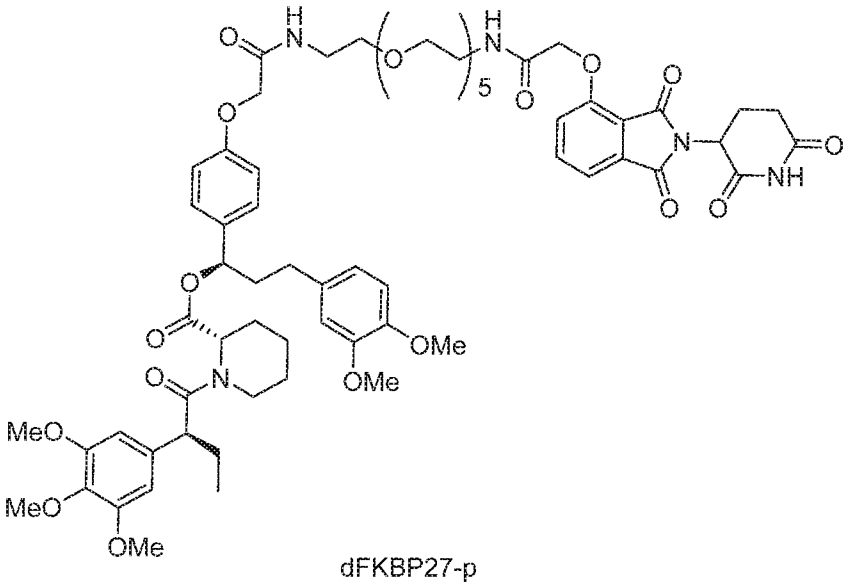
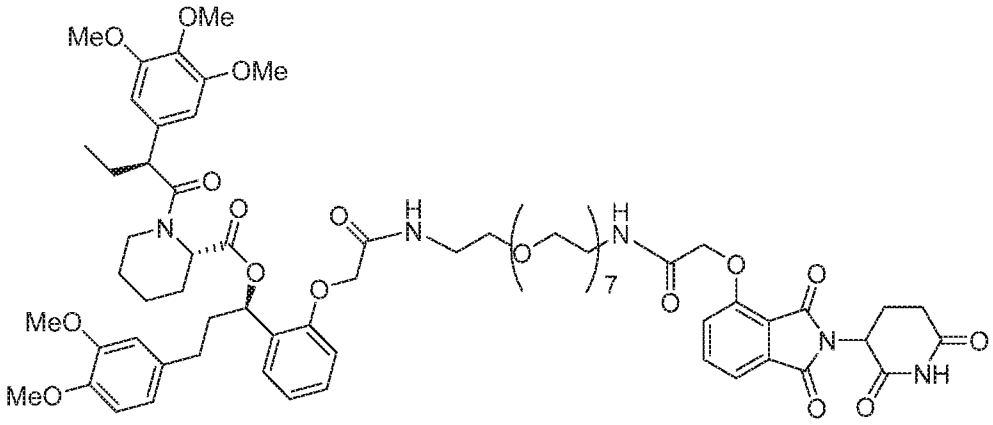


dFKBP27-p



dFKBP28-o

FIG. 33M

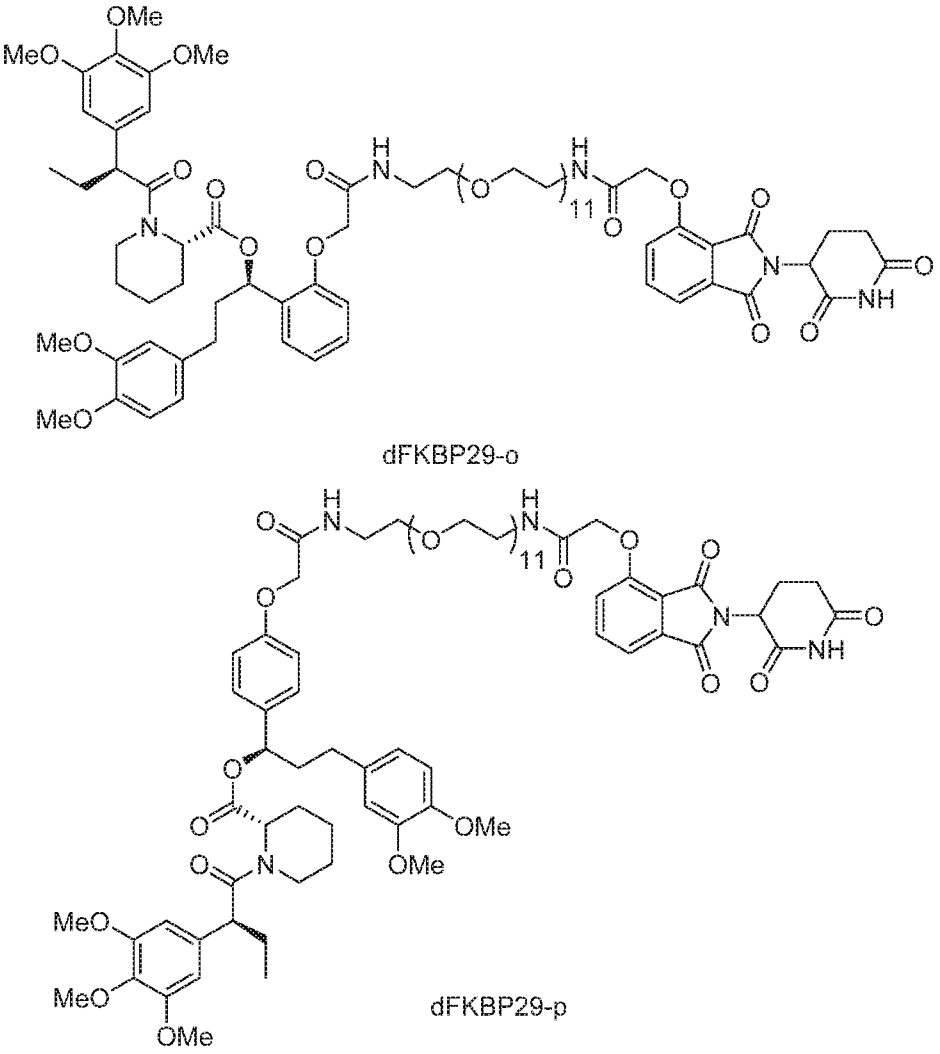
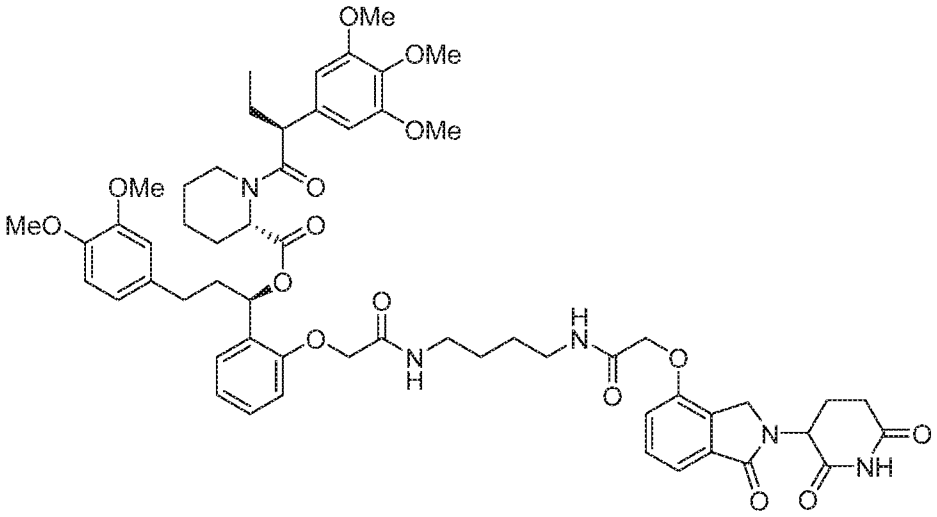
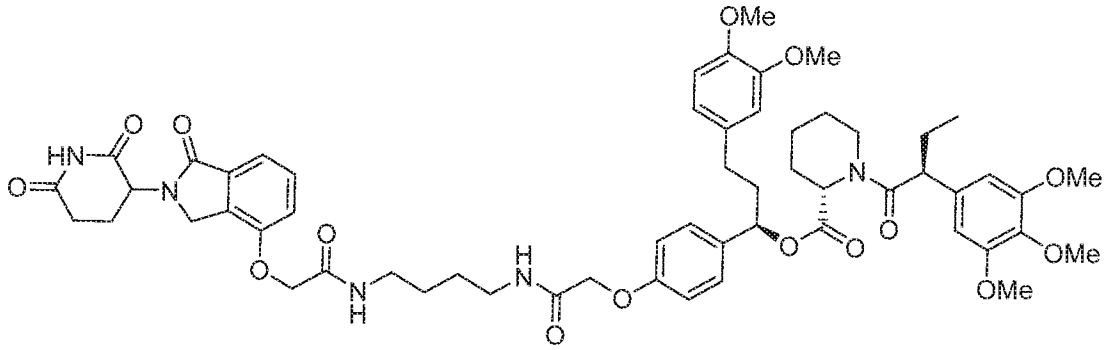


FIG. 33N



dFKBP30-o



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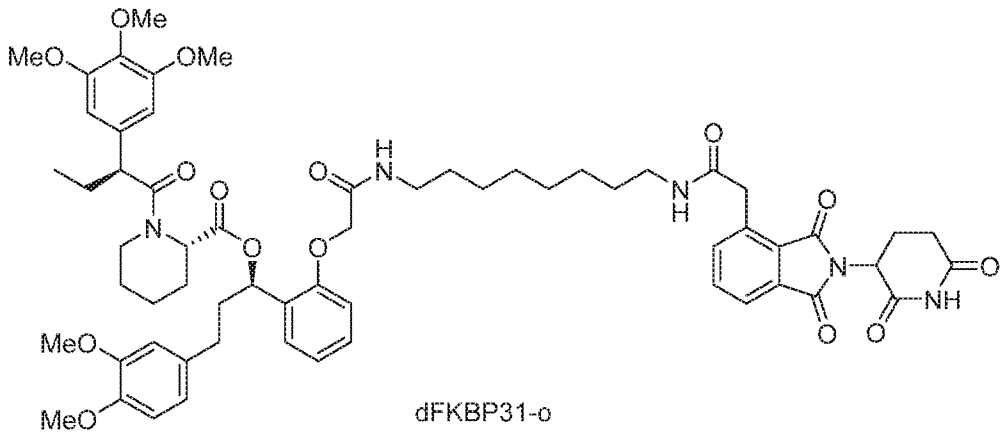
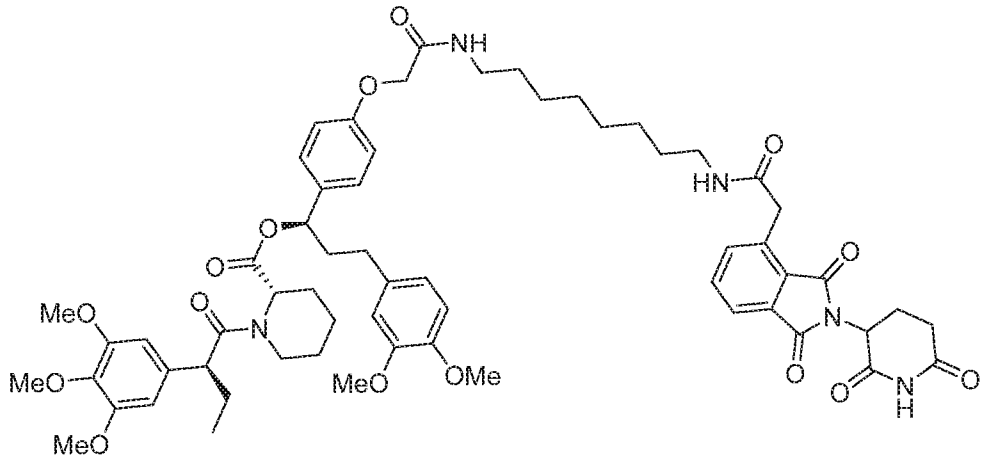
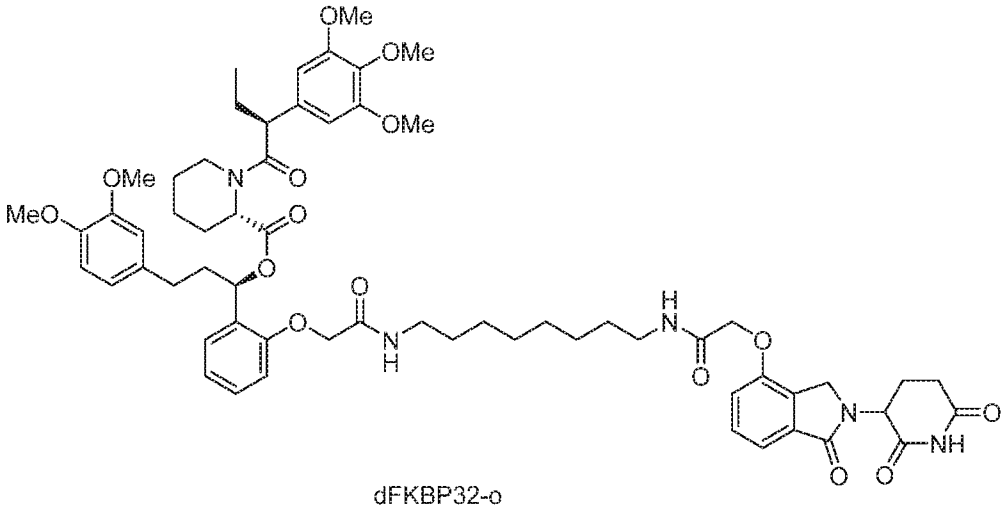


dFKBP31-o

FIG. 33O



dFKBP31-p



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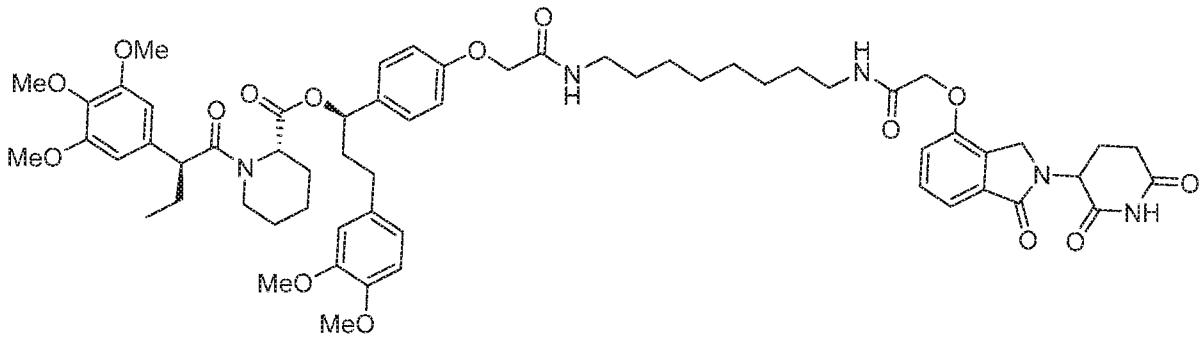
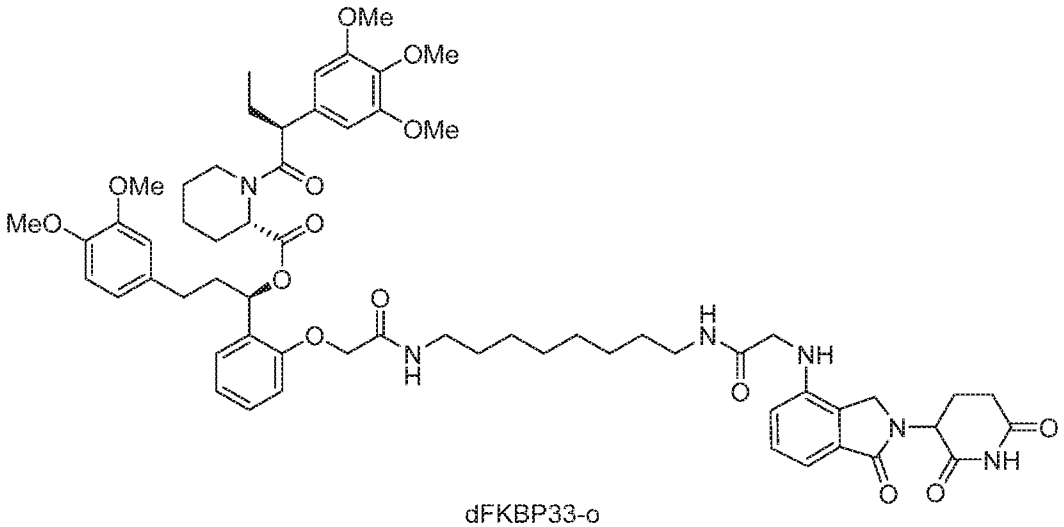
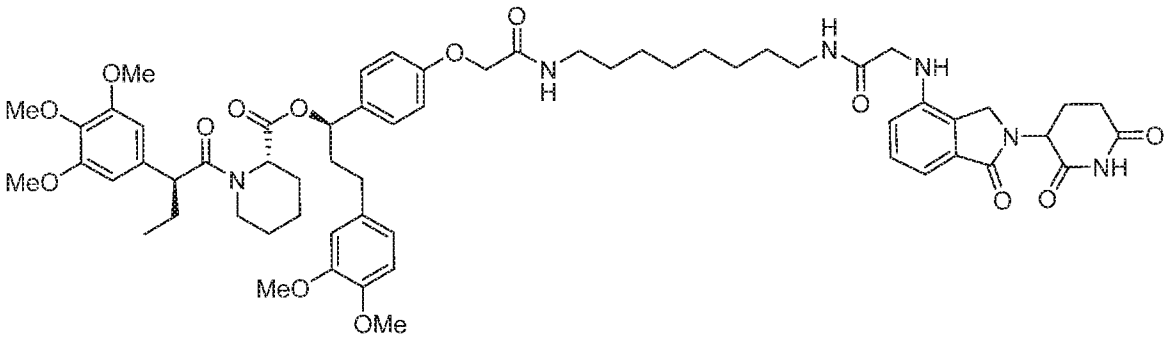


dFKBP32-p

FIG. 33P



dFKBP33-o



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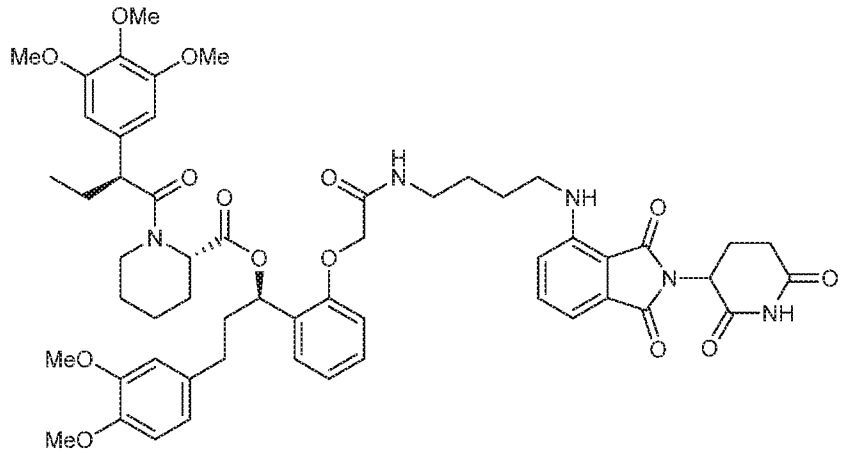
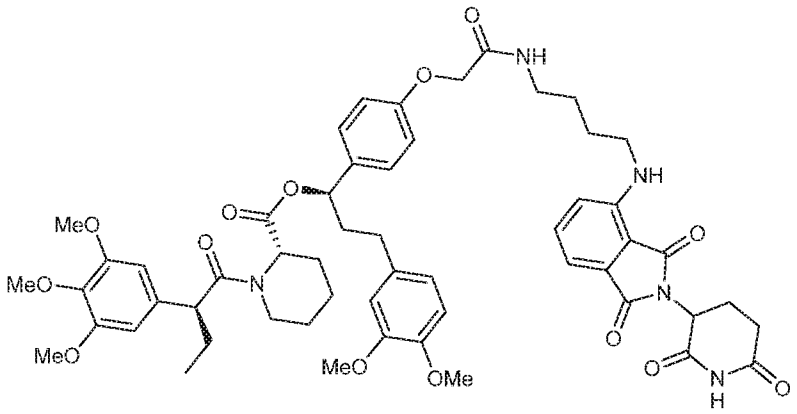


dFKBP34-o

FIG. 33Q



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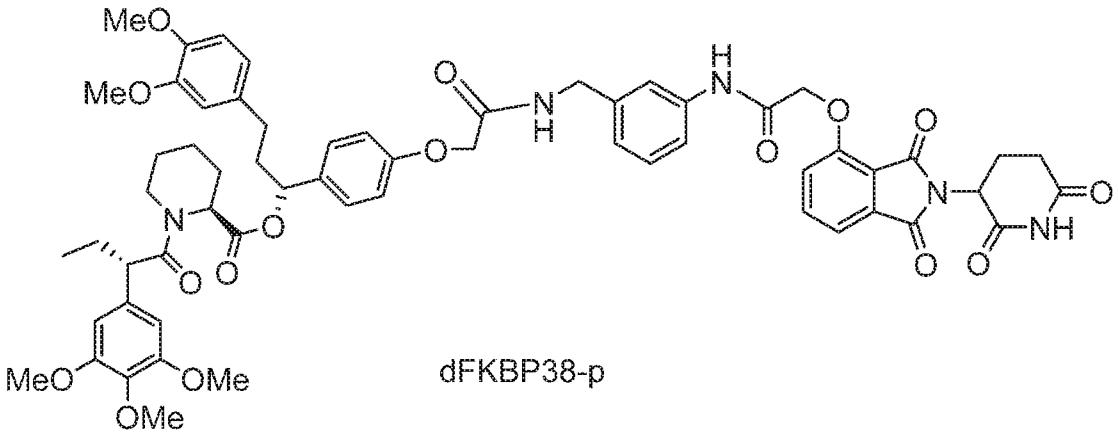
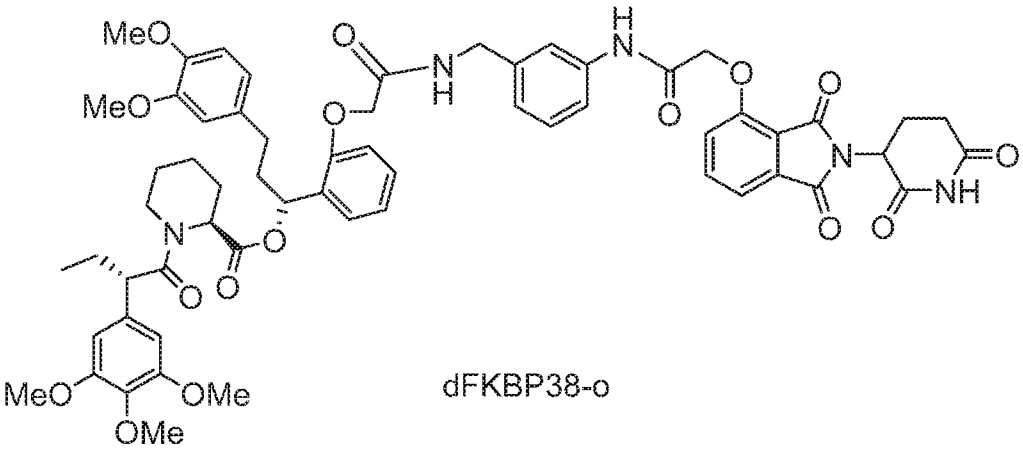
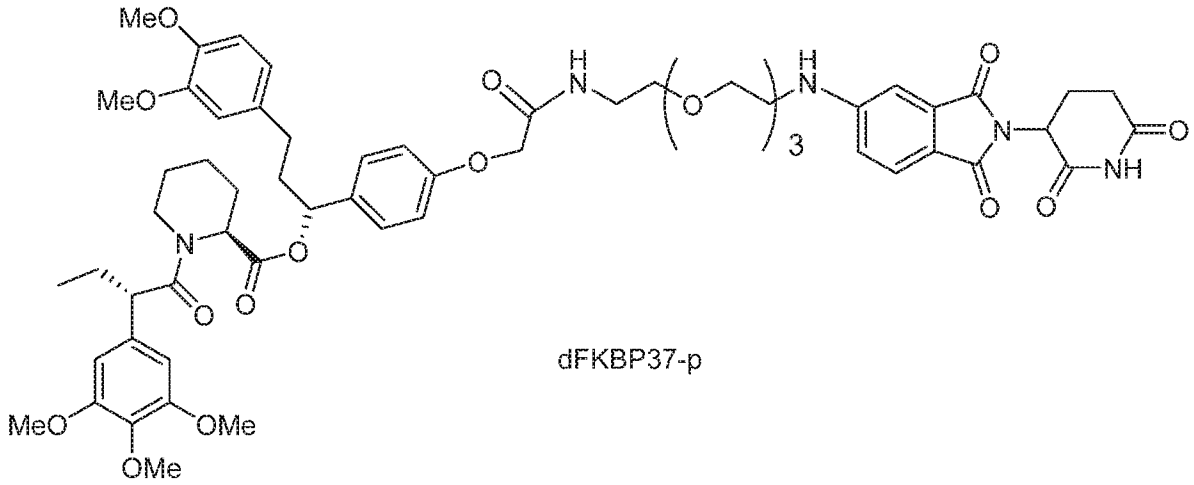


dFKBP38-p

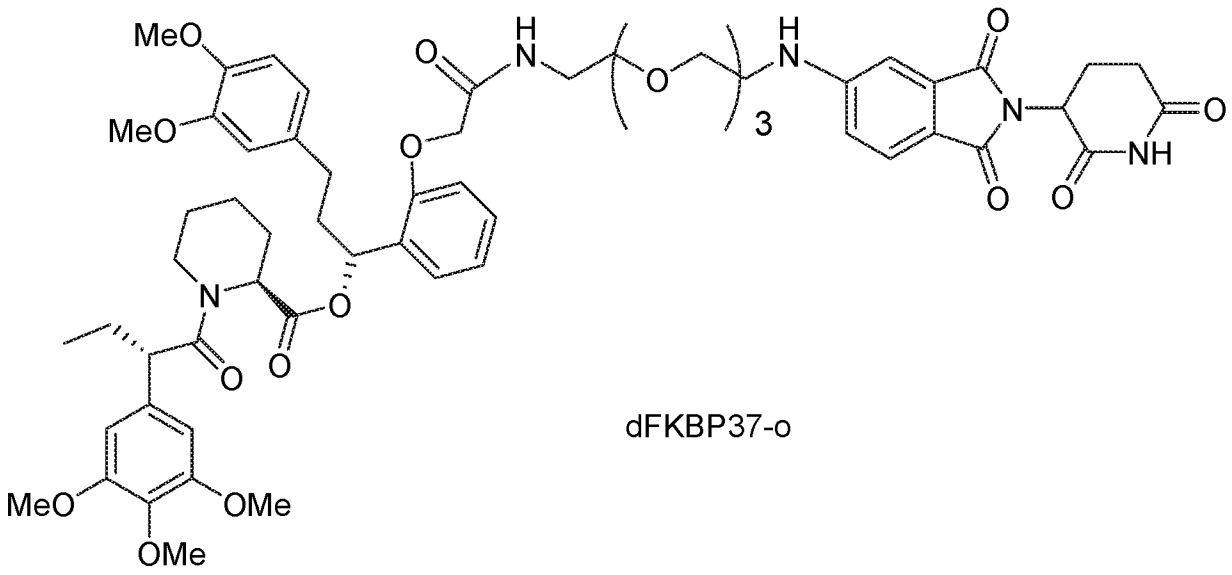


dFKBP38-o

FIG. 33R



dFKBP37-p



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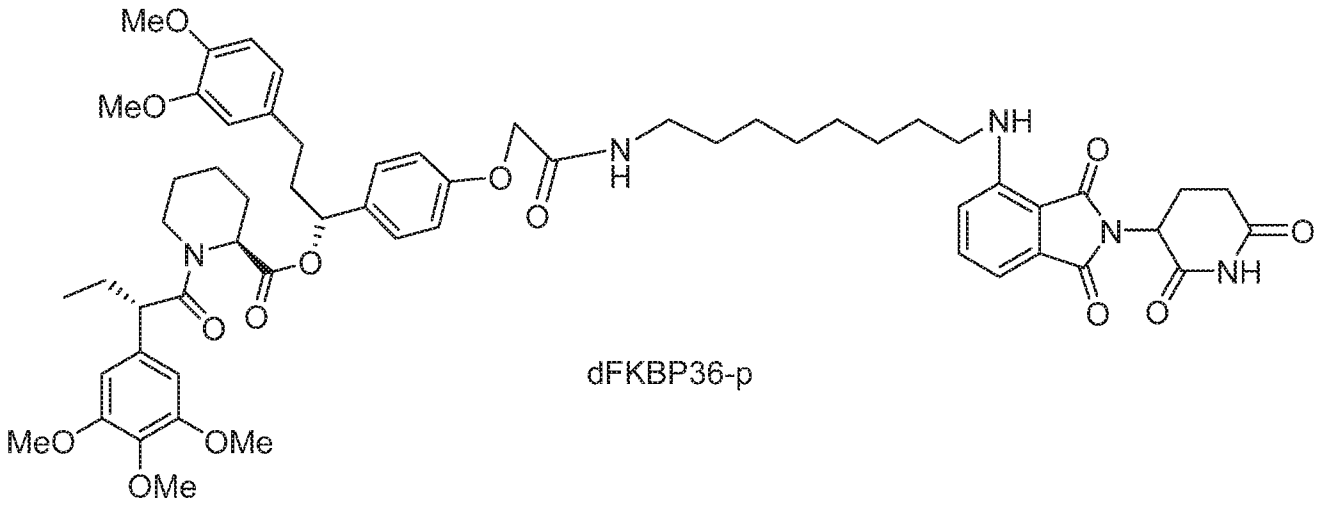
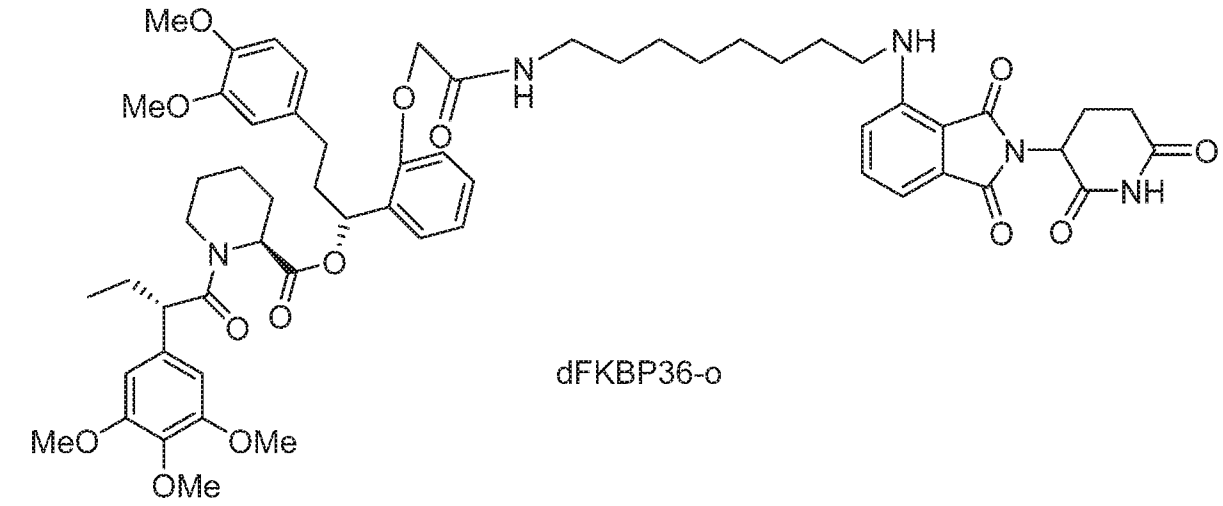


dFKBP36-p

FIG. 33S



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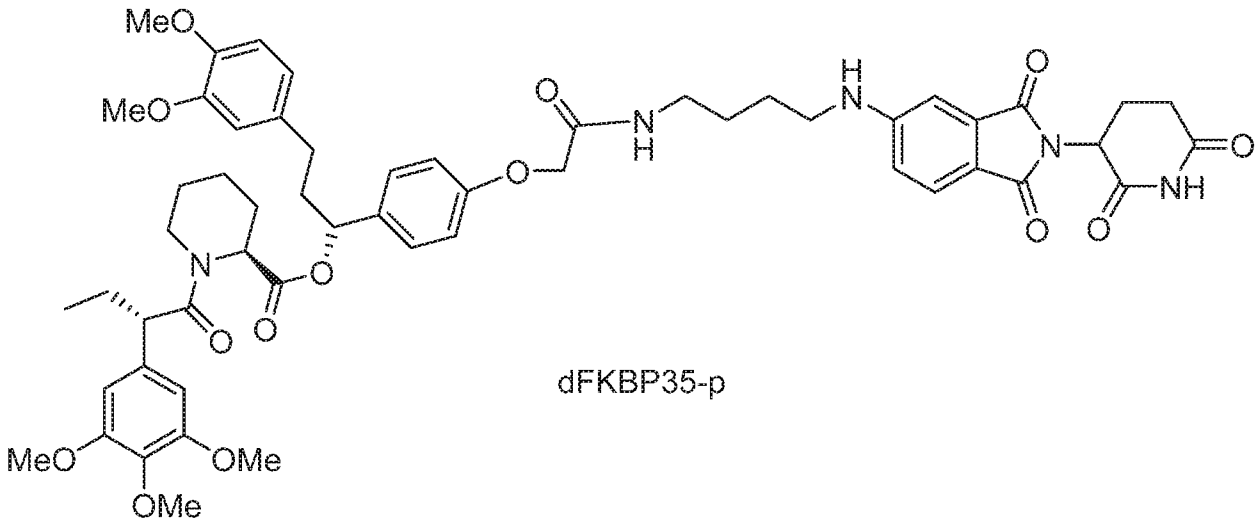
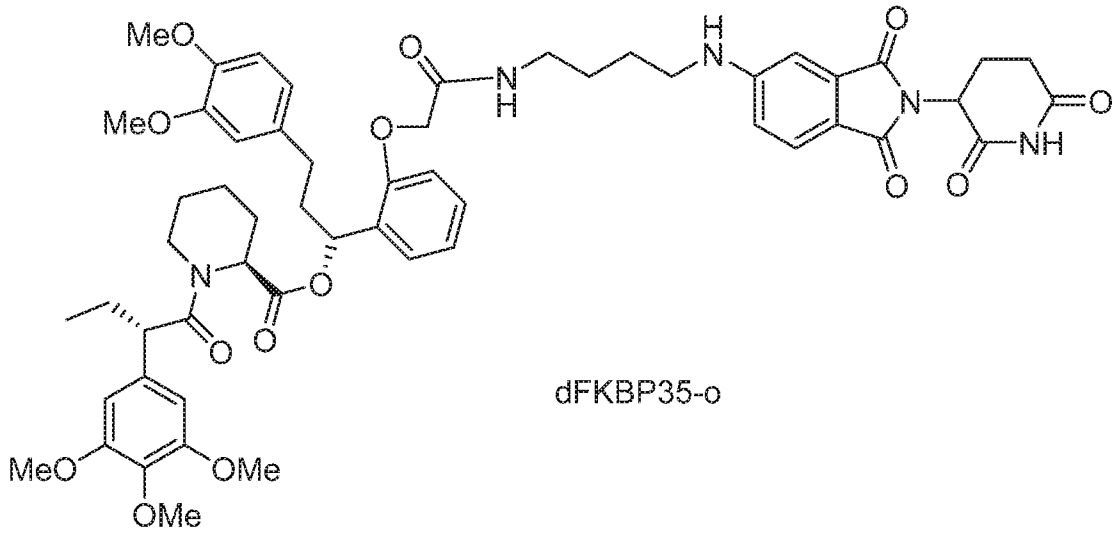
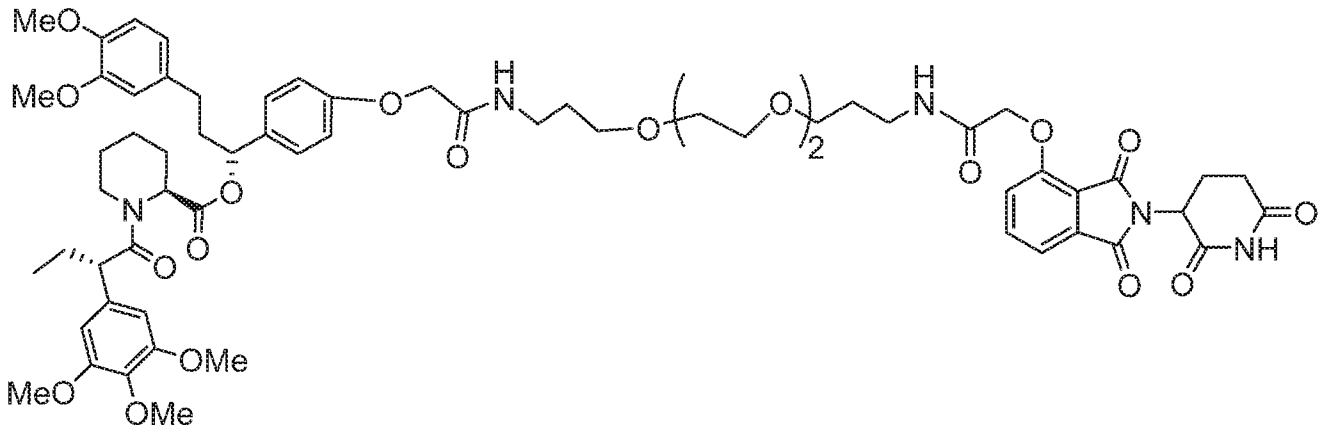


dFKBP35-p



dFKBP35-o

FIG. 33T



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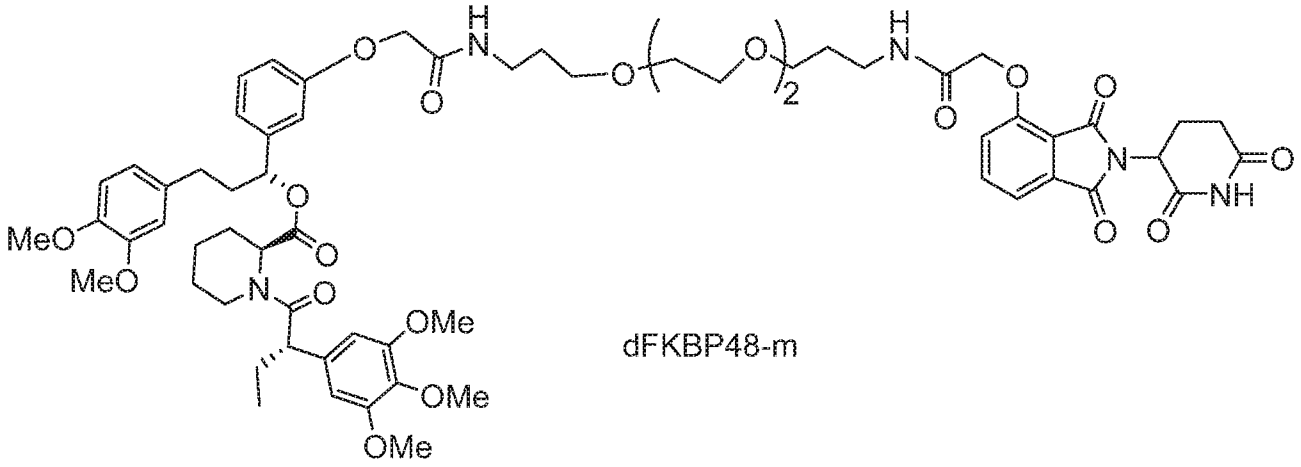
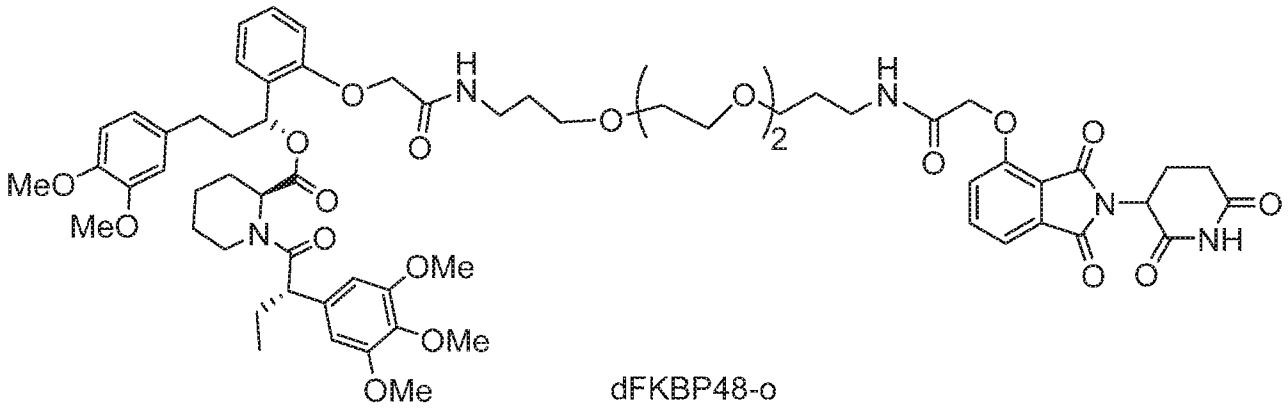
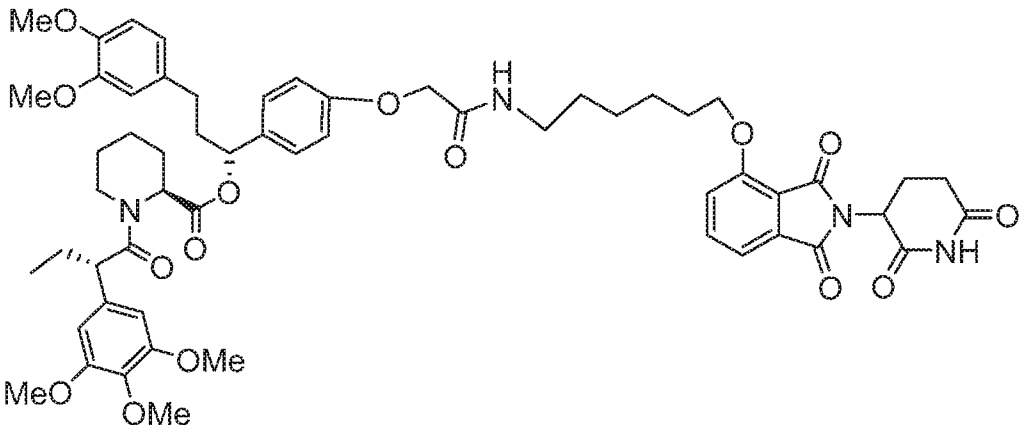


dFKBP48-m

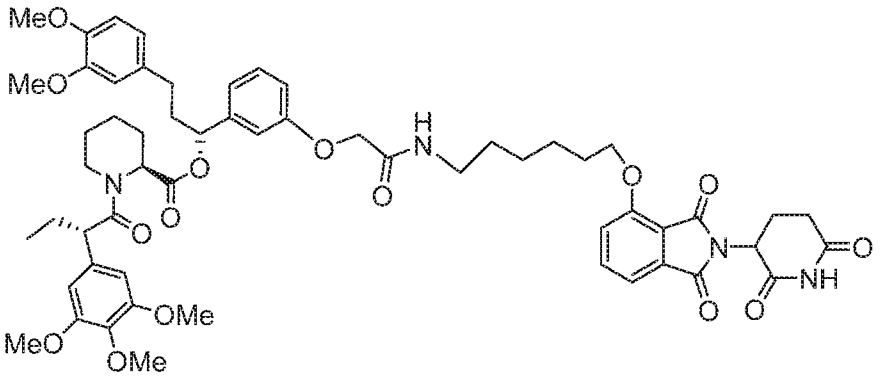


dFKBP48-o

FIG. 33U



dFKBP49-p



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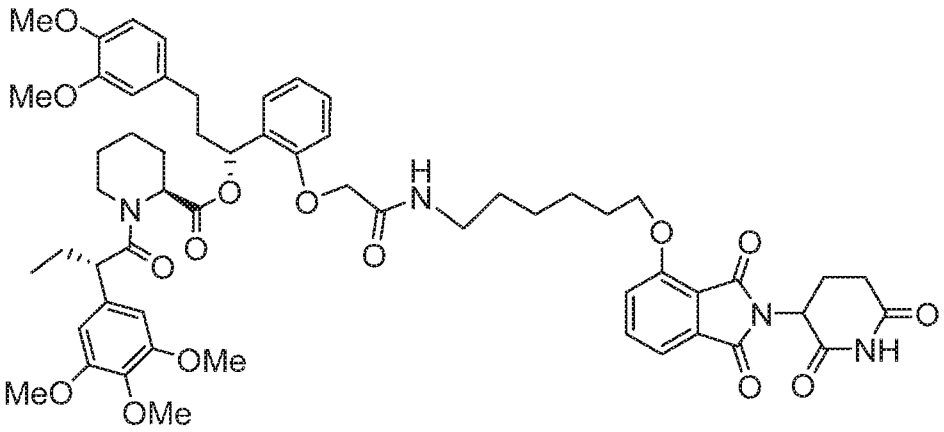
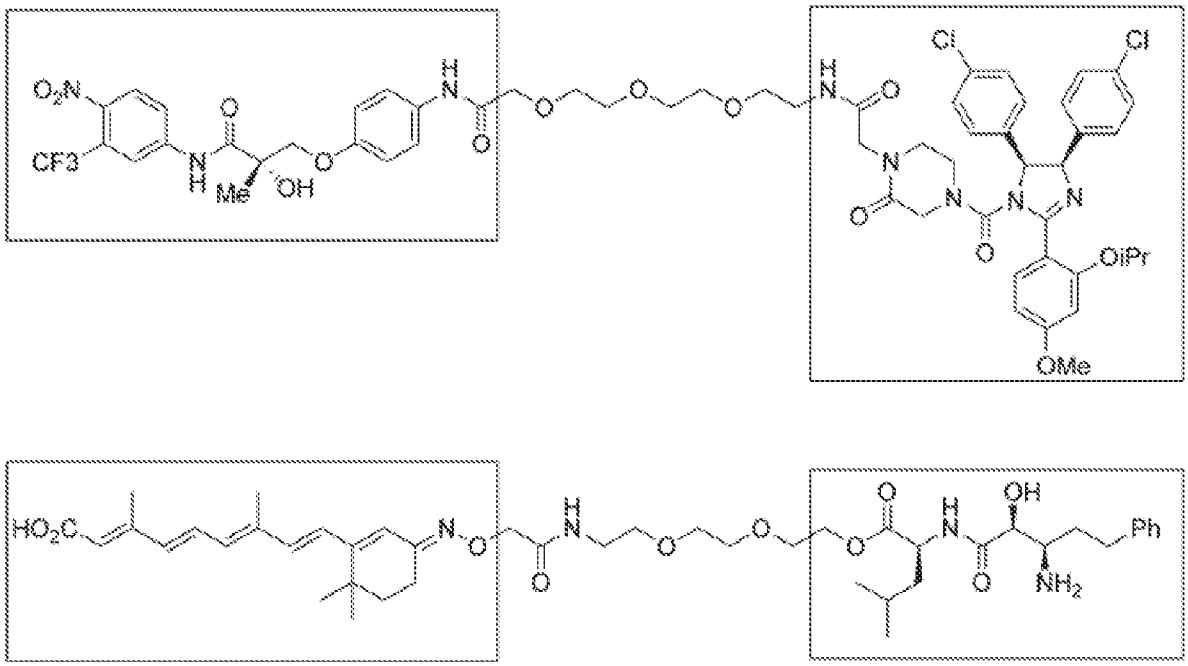


dFKBP49-o

FIG. 33V


O2N
CF3
H
N
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OH
O
H
N
O
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O
O
H
N
O
N
N
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O
O
Cl
Cl
OiPr
OMe
HO2C
N
O
H
N
O
O
O
O
O
H
N
OH
O
NH2
Ph

FIG. 33W

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/17468

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/17, 35/15, 35/12, 38/16; C12N 9/64, 5/10, 5/071; A61P 35/00, 35/04 (2018.01)

CPC - A61K 38/16, 38/177, 35/17, 35/15, 35/12; C12N 9/64, 5/10, 5/067; A61P 35/00, 35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0235730 A1 (DANA-FARBER CANCER INSTITUTE, INC.) 18 August 2016; paragraphs [0010], [0017], [0027], [0049], [0052], [0188]-[0191], [0528], [0529], [0736], [0762]; claims 1, 2.	1-3, 4/1-3, 31-33, 34/31-33, 61-63, 64/61-63
Y	US 2004/0072319 A1 (NASH, P. et al.) 15 April 2004; paragraphs [0002], [0006], [0046], [0048], [0056], [0131]-[0133], [0137], [0167]-[0168], [0236], [0237].	1-3, 4/1-3, 31-33, 34/31-33, 61-63, 64/61-63
Y	LI, H. et al. TGF-β Induces Degradation of PTHrP Through Ubiquitin-Proteasome System in Hepatocellular Carcinoma. Journal of Cancer; 05 April 2015, Vol. 6, pages 511-518, doi: 10.7150/jca.10830; abstract; page 512, second column, third paragraph; page 513, second column, fifth paragraph-page 514, first column, second paragraph.	3, 4/3, 33, 34/33, 63, 64/63
P, X	WO 2017/024319 A1 (DANA-Farber Cancer Institute, INC.) 9 February 2017; entire document.	1-3, 4/1-3, 31-33, 34/31-33, 61-63, 64/61-63

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2018 (27.03.2018)	05 APR 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/17468

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos..
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-30, 35-60, 65-90
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.