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## Supplementary information

# Accelerated rational PROTAC design via deep learning and molecular simulations

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# Supplementary Information - Accelerated rational PROTAC design via deep learning and molecular simulations

Shuangjia, Zheng,[1,2,#] Youhai Tan,[1,#] Zhenyu Wang,[2] Chengtao Li,[2] Zhiqing Zhang,[2] Xu Sang,[2] Hongming Chen,[3] Yuedong Yang[1,*]

[1] *School of Data and Computer Science, Sun Yat-sen University, Guangzhou 510006, China*

[2] *Galixir technologies, No.48A Zhichun Road, Haidian District, Beijing 100083, China*

[3]*Center of Cell Lineage and Atlas, Bioland Laboratory (Guangzhou Regenerative Medicine and Health—Guangdong Laboratory), Guangzhou 510530, China*

** Correspondence: yangyd25@mail.sysu.edu.cn*

[#]*These authors contribute equally to this work.*

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Chemical Synthesis and Analytical Data

**Property filters of ZINC data set**

We referred to the statistics of PROTACs in Ermondi et al.[1] and developed the following filtering rules:

**For terminal fragments:**

200 ≤ molecular weight (MW) ≤ 750 and
10 ≤ the number of carbon atoms (nC) ≤ 40 and
1 ≤ the number of aromatic rings (NAR) ≤ 4 and
0 ≤ the number of donor atoms for H-bonds (HBD) ≤ 7 and
1 ≤ the number of acceptor atoms for H-bonds (HBA) ≤ 13 and
2 ≤ Kier's flexibility index (PHI) ≤ 13 and
15 ≤ the topological polar surface area (TPSA) ≤ 200;

The warhead-like and E3 ligand-like compounds were selected from terminal fragments following the below criteria:
200 ≤ molecular weight (MW) ≤ 1100 and
8 ≤ the number of carbon atoms (nC) ≤ 55 and
0 ≤ the number of aromatic rings (NAR) ≤ 8 and
0 ≤ the number of donor atoms for H-bonds (HBD) ≤ 10 and
1 ≤ the number of acceptor atoms for H-bonds (HBA) ≤ 22 and
1 ≤ Kier's flexibility index (PHI) ≤ 24 and
5 ≤ the topological polar surface area (TPSA) ≤ 290.

**For linkers:**

10 ≤ molecular weight (MW) ≤ 600 and
1 ≤ the number of carbon atoms (nC) ≤ 25 and
0 ≤ the number of aromatic rings (NAR) ≤ 2 and
0 ≤ the number of donor atoms for H-bonds (HBD) ≤ 7 and
0 ≤ the number of acceptor atoms for H-bonds (HBA) ≤ 15 and
0 ≤ Kier's flexibility index (PHI) ≤ 35 and
0 ≤ the topological polar surface area (TPSA) ≤ 180.

**Hyperparameters of PROTAC-RL**

1. **Proformer:** We built the Proformer model with the Transformer architecture included 8 heads and its dimension of hidden layer was set to 256.

We first trained Proformer model in quasi-PROTAC training set 300,000 steps and each step contained 4096 tokens. After the pre-training, we fine-tuned the model on PROTAC training set 7,000 steps and the number of tokens is the same with pre-training. Adam was used as optimizer in both training process above and its parameters beta1 and beta2 were set to 0.9 and 0.998 respectively. The initial learning rate of Adam was set as 2.0.

2. **Reinforcement Learning:** Proformer was further trained with reinforcement learning and specific scoring functions when giving warhead and E3 ligand. In our examples, we launched 2,000 steps in reinforcement learning and each iteration

generated 100 compounds. In PK task, alpha of scoring function was normally set to 8. We used a fixed learning rate 1 x $10^{-5}$ in this process.

**Detailed implementation of hierarchical deep ensemble predictors**

**Deep ensemble predictors.** For post-generation screening, we trained an ensemble classifier to predict the potential of degradation activity. The training data were extracted from PROTAC-DB as well, and details are showed below:

1. Check whether PROTAC has DC50 data. If found, set the value < 1,000nM as active and >= 1,000 nM inactive.
2. For those who are lack of DC50, find the degradation percentage of POI. If found, set the value > 70% as active and <= 70% inactive.
3. For those who are lack of data of degradation, search their IC50. If found, set the value < 1000 nM as active and >= 1,000 nM inactive.
4. For those who are lack of data showed above, set to inactive.

As a result, we got 830 active PROTACs and 3,144 inactive PROTACs (one PROTAC may be active or inactive to several targets). For each compound-POI record, the 2048 Morgan Fingerprints and target protein constitute features were calculated as input features.

To avoid the impact of overfitting, we introduced bagging ensemble. Models comprise of popular Extreme gradient boosting (XGBoost) classifier, Random Forest (RF) classifier, Support Vector Machine (SVM) classifier and neural network (NN) classifier. XGBoost models were built in XGBoost package and set number of estimators as 30, max depth as 7, learning rate as 0.3, min_child_weight=1, gamma=0; RF models were built in Scikit-learn package and set number of estimators, max depth as 30, max depth as 10; SVM models were built in Scikit-learn package and set C as 0.1 and Gamma as 1e-5; NN models were built in PyTorch, and set only one hidden layer including 64 neurons.

We used a three-fold cross-validation strategy and reported the mean of AUC by ensemble the above models. When making a prediction, the outputs of each classifier were averaged as the final degradation score for the input molecule.

**Hierarchical selection.** Due to the small training sample of PROTAC degradation predictor, the ranking ability and generalization ability of the model are not strong enough. Therefore, we do not rely entirely on model scoring, but design a hierarchical selection protocol based on the size of clusters as follow:

- In cluster 0, which includes 574 compounds, we randomly selected 6 candidates from Top 100 possible compounds given by our deep learning predictor;
- In cluster 1, which includes 258 compounds, we randomly selected 3 candidates from Top 50 possible compounds given by our deep learning predictor;
- For clusters whose size is greater than or equal to 100 and less than 200, we randomly selected 2 candidates from Top 30 possible compounds given by our deep learning predictor;

- For clusters whose size is greater than or equal to 50 and less than 100, we randomly selected 1 candidate from Top 20 possible compounds given by our deep learning predictor;
- For clusters whose size is greater than or equal to 10 and less than 50, we randomly selected 1 candidate from Top 10 possible compounds given by our deep learning predictor;
- For clusters whose size is greater than or equal to 3 and less than 10, we randomly selected 1 candidate;
- For clusters whose size is less than 3, we dropped;

All the predicted possibility of candidates must be larger than 0.5. As a result, only 51 candidates from 41 clusters were remained after hierarchical selection.

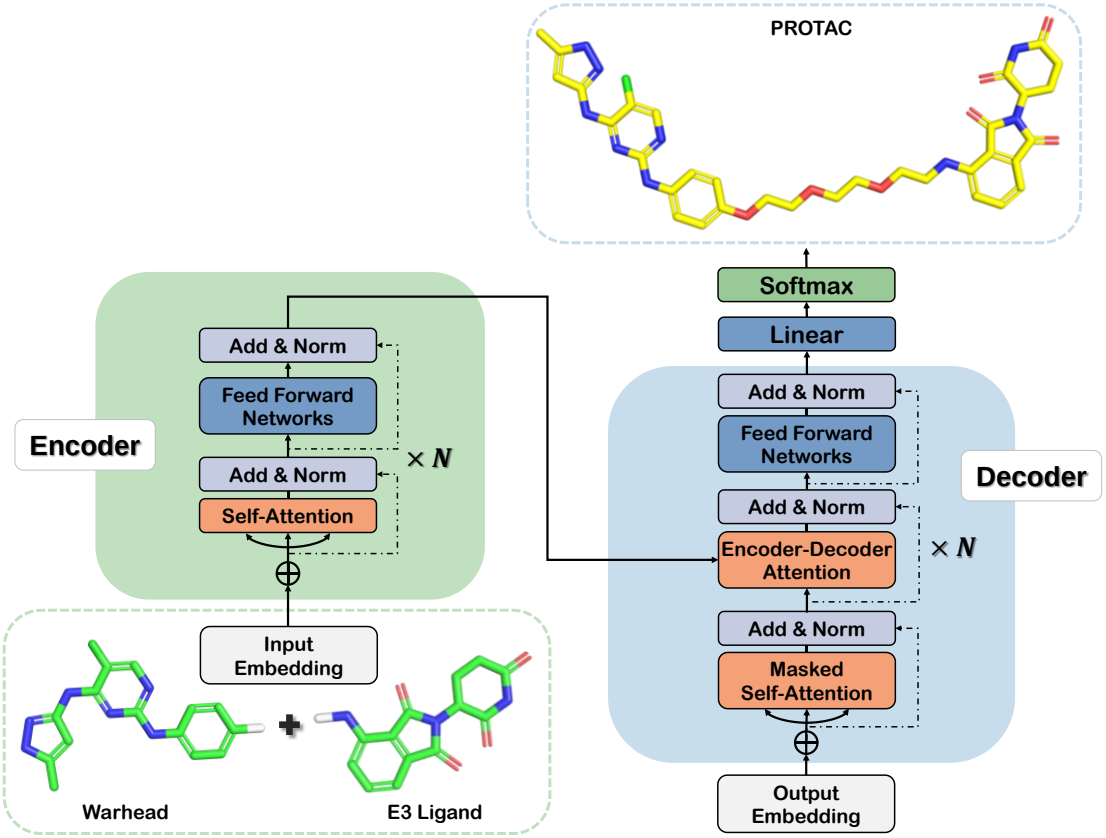



Fig S1. The architecture of Proformer.

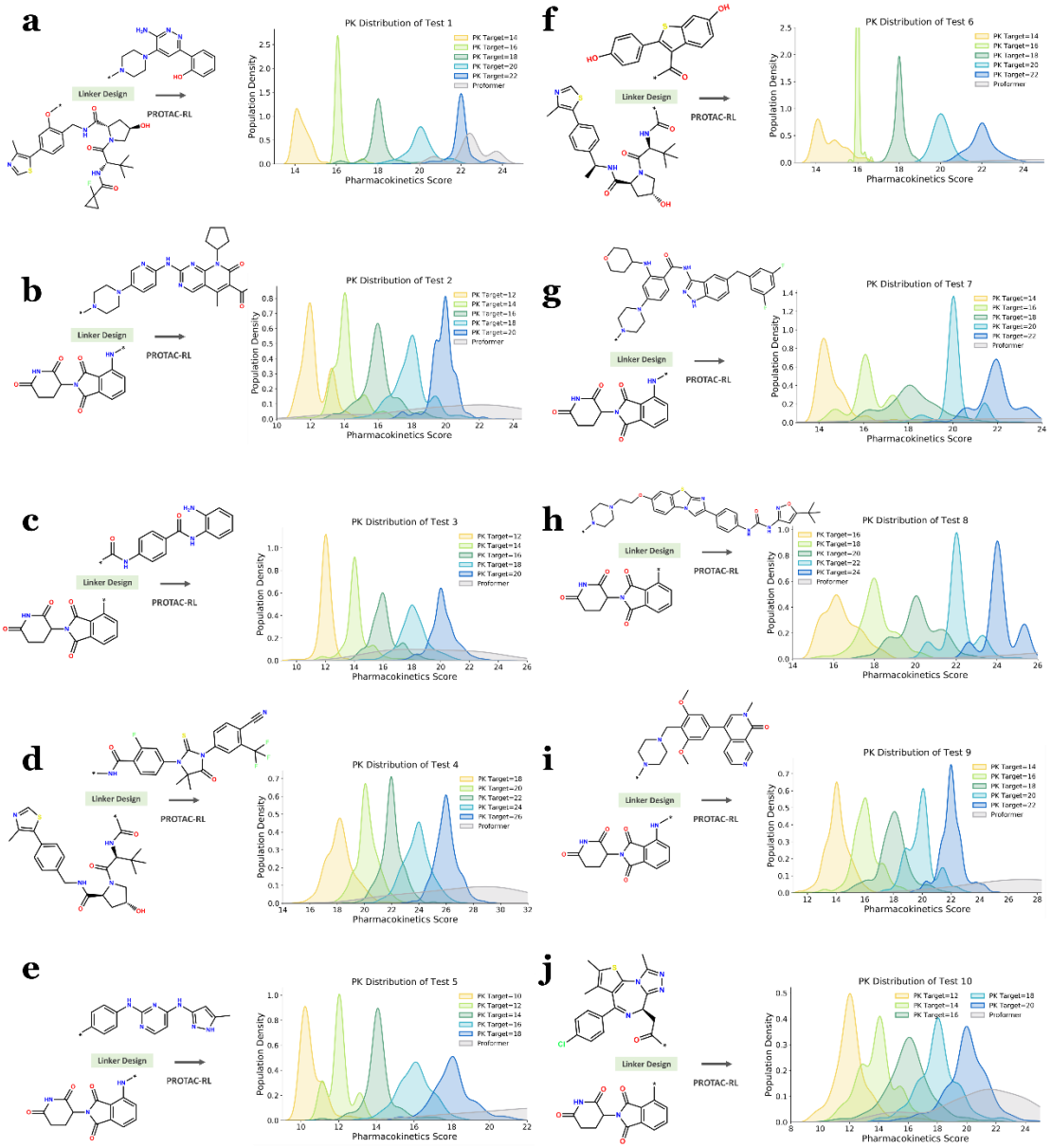



Fig S2. a~h) PK score distributions of PROTACs generated from 10 randomly selected test pairs of warhead and E3 ligand. Structures sampled from Agents towards different targeted PK score for conditoinal generation.

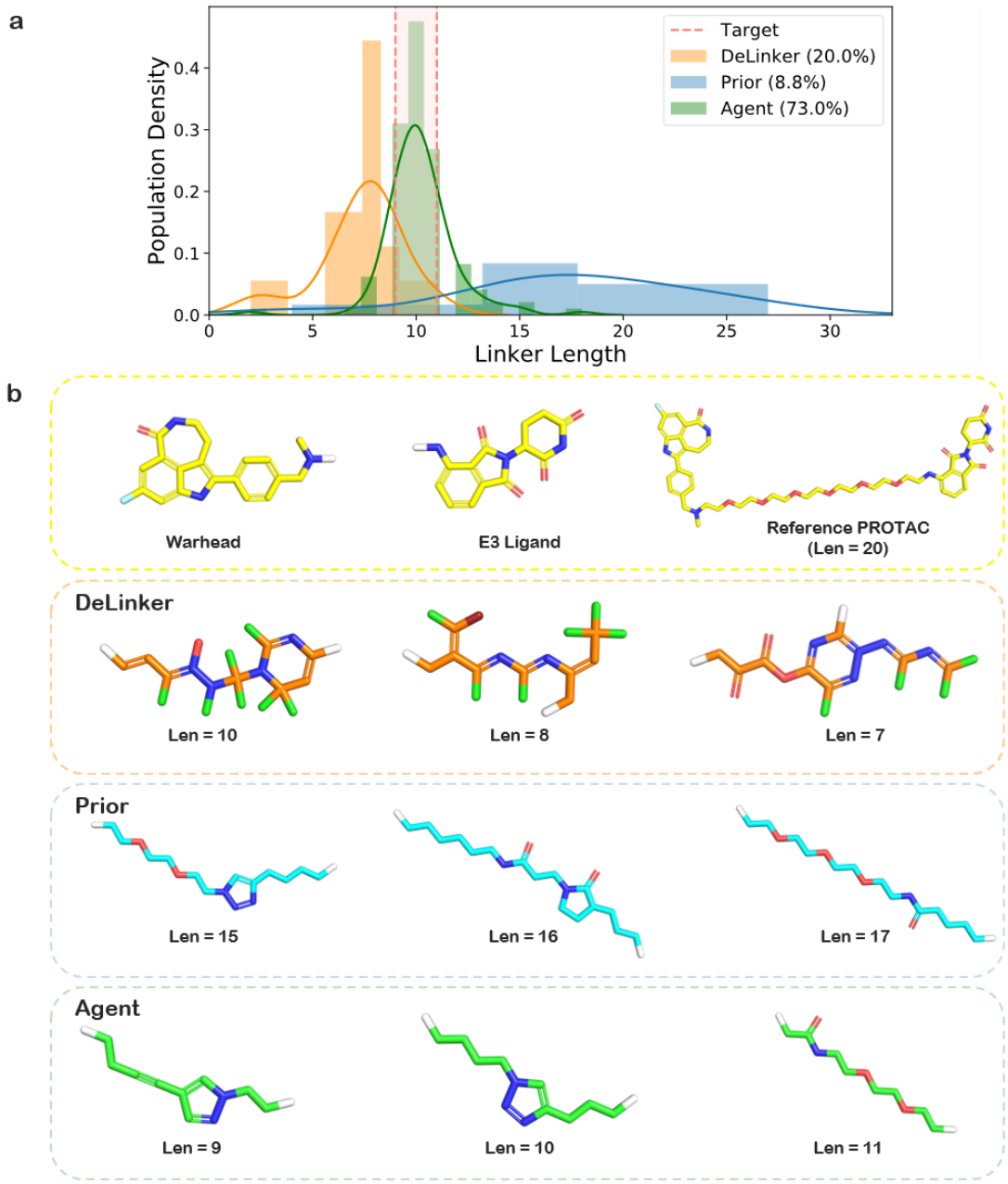



Fig S3. The performance comparison of DeLinker, Prior and Agent in linker length task. (a) The linker length distribution of generations. Blue and green represent DeLinker, Prior and Agent respectively. Red dashed line with transparently red span represents success region. Percentage after each model in legend is success rate. (b) Examples of generation. Reference PROTAC with its warhead and E3 ligand is shown in the yellow frame, and some example compounds of generations of DeLinker, Prior and Agent are in orange, blue and green frame respectively. Len represents the length of the linker.

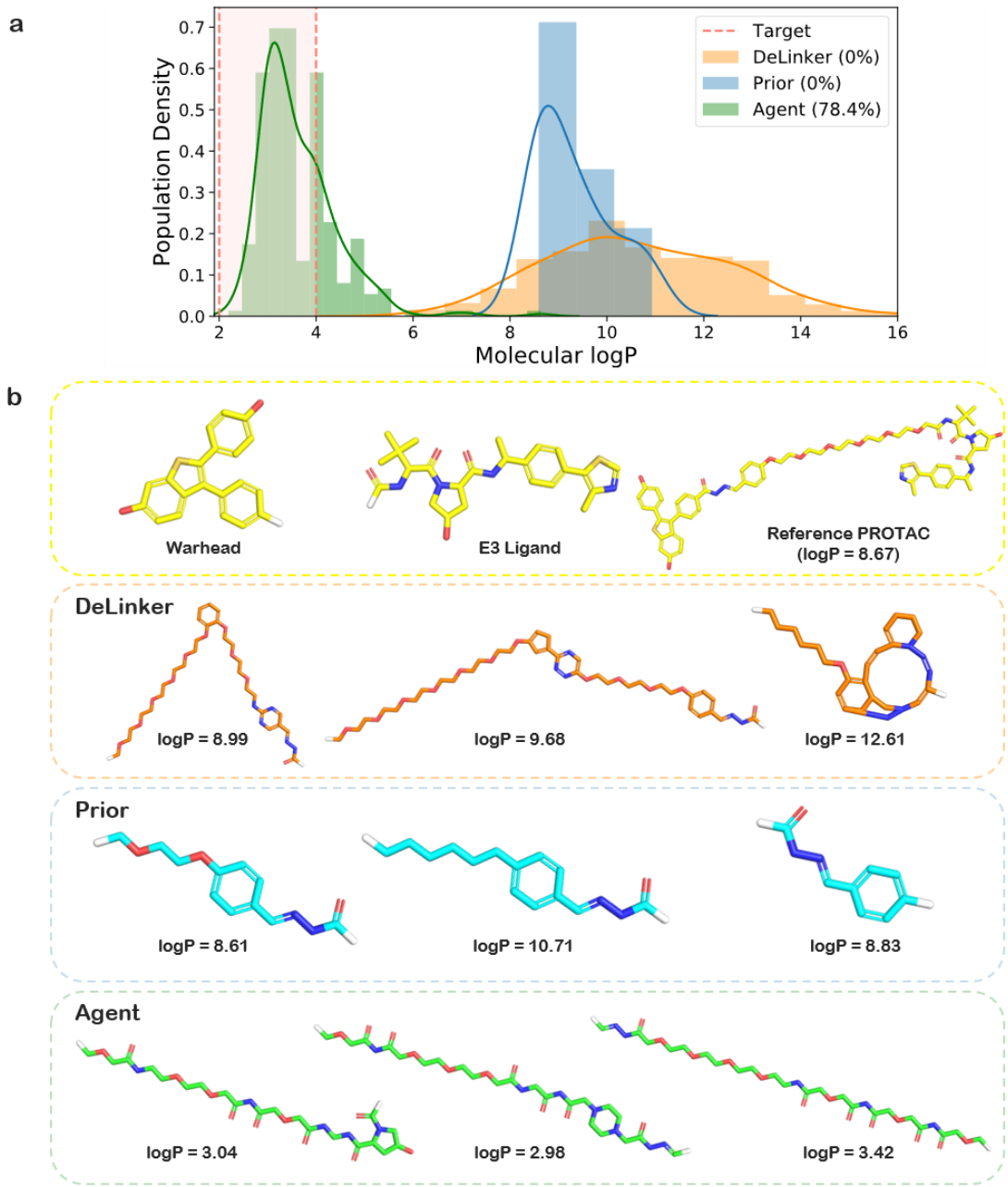



Fig S4. The performance comparison of DeLinker, Prior and Agent in logP task. (a) The logP distribution of generations. Orange, blue and green represent DeLinker, Prior and Agent respectively. Red dashed line with transparently red span represents success region. Percentage after each model in legend is success rate. (b) Examples of generation. Reference PROTAC with its warhead and E3 ligand are shown in yellow frame, and some example compounds of generations of DeLinker, Prior and Agent are in orange, blue and green frame respectively. logP represents the logP of whole PROTAC.

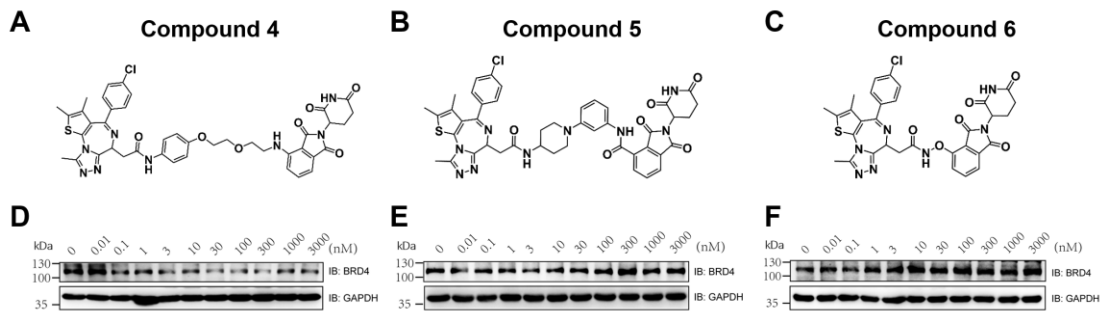



Fig S5. A~C) Compound structures for compound 4~6. D~E) Immunoblot for BRD4, and Actin following 3 hour drug incubation. Experiments were repeated at least once and similar results were obtained. Source data for this figure are on pages 39–41.

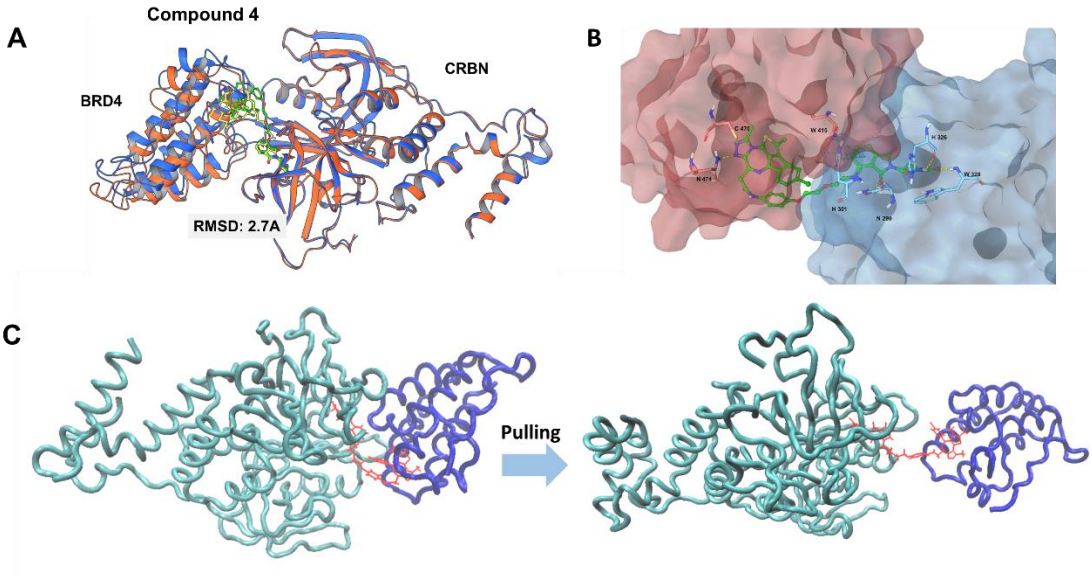



Fig S6. A) The overlap of compound 4 complex and reference dBET6 crystal structure 6BOY. B) Ternary complex and interaction mode of compound 4. C)The starting and finishing structural state of the PROTAC complexes during Pulling analysis.

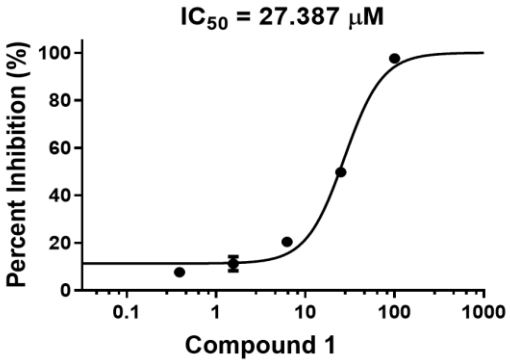



Fig S7. Inhibitory effect of compound 1 on hERG channels. Plotted as mean ± SEM for n = 3 replicates.

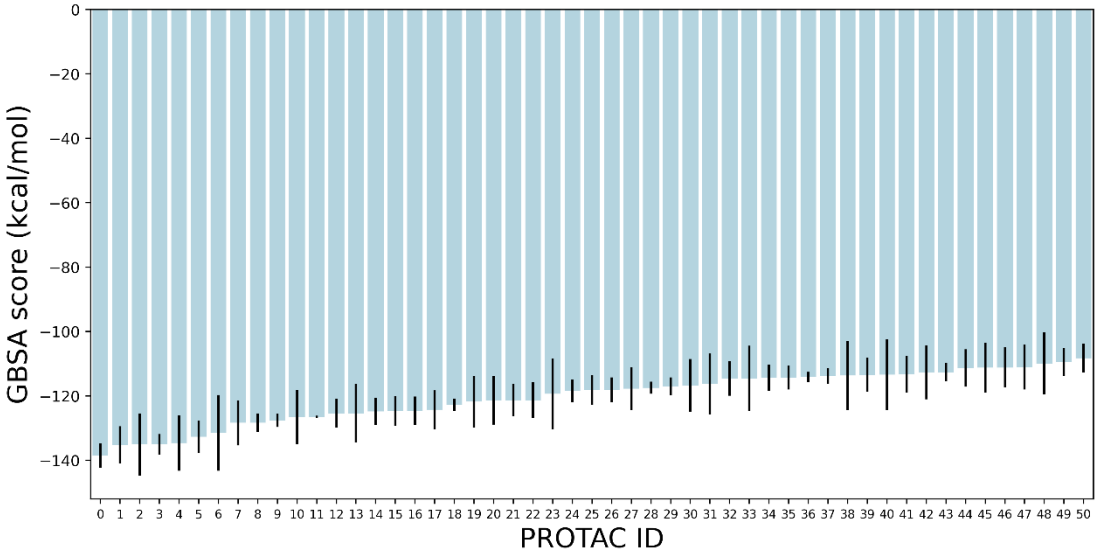



Figure S8. Bar plot of GBSA score for candidate PROTACs. The average results of three independent runs are reported. Error bars indicate the standard deviations.

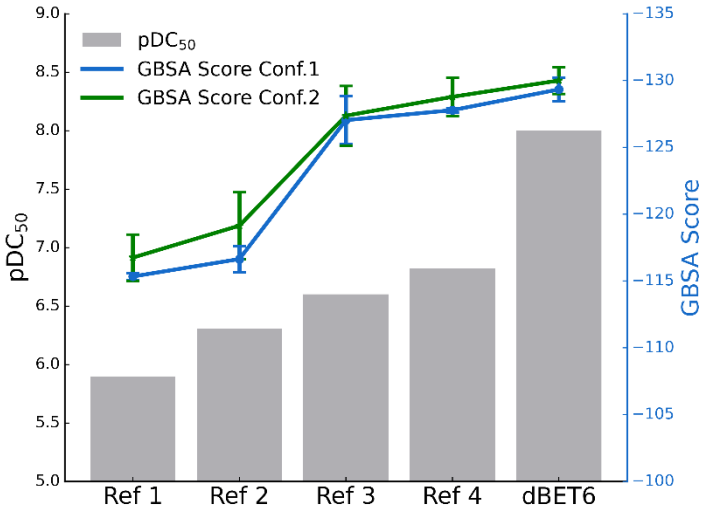



Figure S9. GBSA score comparisons for the top and second highest scoring conformations. The average results of three independent runs are reported. Error bars indicate the standard deviations.

Table S1. Performance comparison of different models on PROTAC test set.

| **Model** | DeLinker | SyntaLinker | DeLinker -retraining | SyntaLinker -retraining | Proformer |
|---|---|---|---|---|---|
| Recovery (%) | 0.6 ±0.6 | 0.6 ±0 | 4.8 ±1.0 | 10.4 ±0.4 | 43.0 ±1.8 |
| Uniqueness (%) | 95.8 ±1.0 | 100 ±0 | 95.8 ±0.5 | 84.7 ±1.0 | 88.3 ±0.6 |
| Novelty (%) | 98.7 ±0.4 | 100 ±0 | 91.7 ±0.3 | 32.1 ±0.5 | 42.1 ±0.5 |
| Validity (%) | 88.8 ±0.9 | 0.6 ±0 | 90.8 ±0.8 | 56.7 ±0.6 | 78.5 ±1.1 |

Table S2. Score overview of synthesized PROTACs.

| Name | linker length | PK score | Degradation activity score | Rosetta score | RMSD to reference | GBSA score (mean) |
|---|---|---|---|---|---|---|
| Compound 1 | 13 | 18.031 | 0.663 | -627.912 | 11.415 | -138.543 |
| Compound 2 | 12 | 20.041 | 0.651 | -628.767 | 11.505 | -135.065 |
| Compound 3 | 10 | 14.884 | 0.656 | -639.861 | 12.308 | -125.455 |
| Compound 4 | 12 | 20.737 | 0.614 | -659.190 | 2.266 | -117.016 |
| Compound 5 | 10 | 15.628 | 0.643 | -640.473 | 11.620 | -126.477 |
| Compound 6 | 2 | 10.701 | 0.549 | -656.030 | 12.149 | -116.740 |

Table S3. Cell viability assay results summary for compounds 1, 2 and 3.

| Compound | Min Inhibition% | Max Inhibition% | Rel IC50 (uM) | Abs IC50 (uM) | Top Concentration (uM) |
|---|---|---|---|---|---|
| Compound1 | 8.999 | 104.644 | 0.116 | 0.103 | 30.000 |
| Compound2 | -6.748 | 89.653 | 5.610 | 6.049 | 30.000 |
| Compound3 | -0.734 | 64.072 | 21.733 | 18.934 | 30.000 |

Table S4. Raw data of inhibitory effects of compound 1 on hERG channels.

| Compound ID | Concentration (μM) | % of hERG inhibition | | Average % of hERG inhibition | SD | IC50 (μM) |
|---|---|---|---|---|---|---|
| | | Cell 1 | Cell 2 | | | |
| Compound 1 | 0.39 | 8.82 | 6.52 | 7.67 | 1.62 | 27.387 |
| | 1.56 | 13.33 | 9.10 | 11.22 | 2.99 | |
| | 6.25 | 19.78 | 21.14 | 20.46 | 0.96 | |
| | 25.00 | 50.66 | 48.88 | 49.77 | 1.26 | |
| | 100.00 | 96.81 | 98.33 | 97.57 | 1.08 | |

Table S5. Pharmacokinetic results summary for compounds 1 (n=3) and dBET6 (n=3).

| Pharmacokinetics of Compound 1 (ng/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Compound 1 | | | | | | | |
| IP1 | | | | | | | |
| **Time (h)** | **M01** | **M02** | **M03** | **Mean** | | **SD** | **CV (%)** |
| **0.0830** | 43.4 | 38.9 | 84.6 | 55.6 | ± | 25.2 | 45.3 |
| **0.250** | 207 | 96.1 | 211 | 171 | ± | 65.2 | 38.1 |
| **0.500** | 116 | 142 | 134 | 131 | ± | 13.3 | 10.2 |
| **1.00** | 96.8 | 163 | 126 | 129 | ± | 33.2 | 25.8 |
| **2.00** | 31.6 | 54.3 | 43.4 | 43.1 | ± | 11.4 | 26.3 |
| **4.00** | 12.7 | 18.8 | 17.9 | 16.5 | ± | 3.29 | 20.0 |
| **8.00** | 4.23 | 8.42 | 6.16 | 6.27 | ± | 2.10 | 33.4 |
| **24.0** | BQL | BQL | BQL | ND | ± | ND | ND |
| **PK Parameters** | **M01** | **M02** | **M03** | **Mean** | | **SD** | **CV (%)** |
| **Rsq_adj** | 0.963 | 0.858 | 0.963 | -- | ± | -- | -- |
| **No. points used for $T_{1/2}$** | 3.00 | 3.00 | 3.00 | 3.00 | ± | -- | -- |
| **$C_{max}$ (ng/mL)** | 207 | 163 | 211 | 194 | ± | 26.6 | 13.8 |
| **$T_{max}$ (h)** | 0.250 | 1.00 | 0.250 | 0.500 | ± | 0.433 | 86.6 |
| **$T_{1/2}$ (h)** | 2.12 | 2.35 | 2.19 | 2.22 | ± | 0.118 | 5.31 |
| **$T_{last}$ (h)** | 8.00 | 8.00 | 8.00 | 8.00 | ± | -- | -- |
| **$AUC_{0\text{-}last}$ (ng.h/mL)** | 246 | 336 | 315 | 299 | ± | 47.1 | 15.7 |
| **$AUC_{0\text{-}inf}$ (ng.h/mL)** | 259 | 365 | 334 | 319 | ± | 54.5 | 17.1 |
| **$MRT_{0\text{-}last}$ (h)** | 1.76 | 2.07 | 1.88 | 1.90 | ± | 0.156 | 8.21 |
| **$MRT_{0\text{-}inf}$ (h)** | 2.23 | 2.80 | 2.42 | 2.48 | ± | 0.290 | 11.7 |
| **$AUC_{Extra}$ (%)** | 5.01 | 7.82 | 5.82 | 6.22 | ± | 1.45 | 23.3 |
| **$AUMC_{Extra}$ (%)** | 24.9 | 31.8 | 26.8 | 27.8 | ± | 3.56 | 12.8 |

| Pharmacokinetics of dBET6 (ng/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|
| dBET | | | | | | | |
| IP1 | | | | | | | |
| **Time (h)** | **M01** | **M02** | **M03** | **Mean** | | **SD** | **CV (%)** |
| **0.0830** | 37.0 | 24.1 | 58.5 | 39.9 | ± | 17.4 | 43.6 |
| **0.250** | 203 | 81.2 | 194 | 159 | ± | 67.9 | 42.6 |
| **0.500** | 86.2 | 104 | 112 | 101 | ± | 13.2 | 13.1 |
| **1.00** | 102 | 130 | 87.1 | 106 | ± | 21.8 | 20.5 |
| **2.00** | 11.6 | 23.6 | 18.6 | 17.9 | ± | 6.03 | 33.6 |
| **4.00** | BQL | BQL | BQL | ND | ± | ND | ND |
| **8.00** | BQL | BQL | BQL | ND | ± | ND | ND |
| **24.0** | BQL | BQL | BQL | ND | ± | ND | ND |
| **PK Parameters** | **M01** | **M02** | **M03** | **Mean** | | **SD** | **CV (%)** |
| **Rsq_adj** | 0.692 | ND | 0.918 | -- | ± | -- | -- |
| **No. points used for $T_{1/2}$** | 3.00 | 0.00 | 3.00 | ND | ± | -- | -- |
| **$C_{max}$ (ng/mL)** | 203 | 130 | 194 | 176 | ± | 39.8 | 22.7 |
| **$T_{max}$ (h)** | 0.250 | 1.00 | 0.250 | 0.500 | ± | 0.433 | 86.6 |
| **$T_{1/2}$ (h)** | 0.476 | ND | 0.556 | 0.516 | ± | ND | ND |
| **$T_{last}$ (h)** | 2.00 | 2.00 | 2.00 | 2.00 | ± | -- | -- |
| **$AUC_{0\text{-}last}$ (ng.h/mL)** | 144 | 154 | 155 | 151 | ± | 6.08 | 4.03 |
| **$AUC_{0\text{-}inf}$ (ng.h/mL)** | 152 | ND | 170 | 161 | ± | ND | ND |
| **$MRT_{0\text{-}last}$ (h)** | 0.747 | 0.920 | 0.749 | 0.805 | ± | 0.0993 | 12.3 |
| **$MRT_{0\text{-}inf}$ (h)** | 0.849 | ND | 0.930 | 0.890 | ± | ND | ND |
| **$AUC_{Extra}$ (%)** | 5.23 | ND | 8.80 | 7.02 | ± | ND | ND |
| **$AUMC_{Extra}$ (%)** | 16.6 | ND | 26.5 | 21.6 | ± | ND | ND |

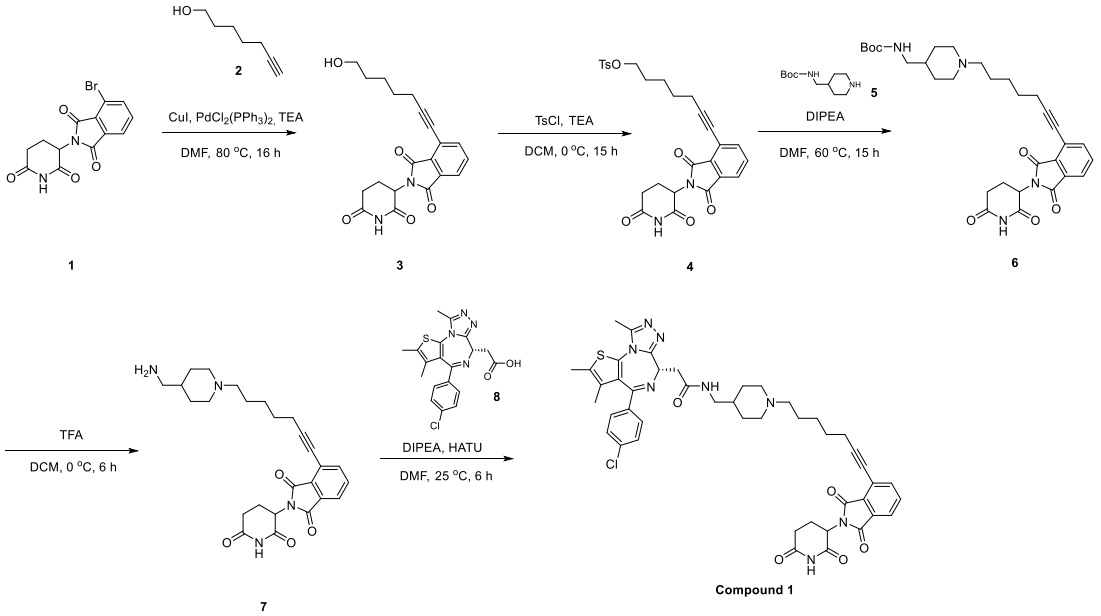
Table S6. Summary of properties of compound 1

| MW | logS | logD | wlogP*/clogP* | BBB permeant* | hERG $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| 847.44 | 1.42 | 3.27 | 4.26/5.42 | No | 27.39 |
| $IC_{50}$(nM) | $C_{max}$ (ng/ML) | $T_{max}$ (h) | $T_{1/2}$ (h) | $T_{last}$ (h) | $AUC_{0\text{-}inf}$ (ng*h/mL) |
| 116 | 194 | 0.25-1.00 | 2.22 | 8.0 | 319 |

*denote *in silico* calculation by Swissadme[2].

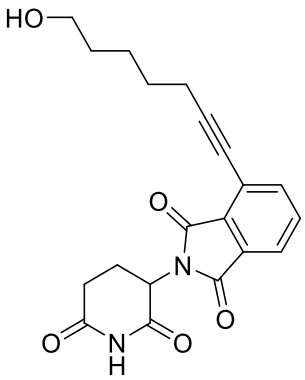
## Chemical Synthesis and Analytical Data

### Chemical Synthesis of compound 1




Scheme 1. Synthetic route to **compound 1**

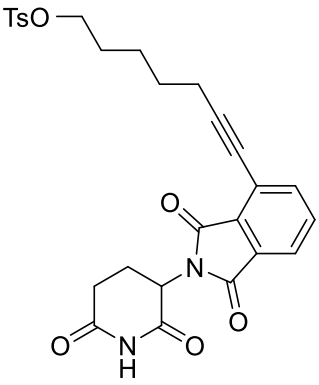
### 2-(2,6-dioxopiperidin-3-yl)-4-(7-hydroxyhept-1-yn-1-yl)isoindole-1,3-dione



**3**

To a mixture of hept-6-yn-1-ol (0.37 g, 3.3 mmol, 1.1 *eq*), 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (1.0 g, 3.0 mmol, 1.0 *eq*), Copper(I) iodide (0.060 g, 0.3 mmol, 0.10 *eq*), Bis(triphenylphosphine)palladium(II) chloride (0.11 g, 0.15 mmol, 0.05 *eq*) in DMF (10 mL) was added triethylamine (0.30 g, 3.0 mmol, 1.0 *eq*) degassed and purged with $N_2$ for 3 times, and then the mixture was stirred at 80 ℃ for 16 h under $N_2$ . LCMS showed desired MS. The mixture was washed with saturated brine (5 mL×3) and extracted with EA (30 mL×2). The combined organic layers were dried over $Na_2SO_4$. The residue was purified by flash silica column chromatography (0~3% MeOH/DCM) to afford 2-(2,6-dioxopiperidin-3-yl)-4-(7-hydroxyhept-1-yn-1-yl)isoindole-1,3-dione (0.38 g, 0.90 mmol, 30% yield) as a yellow solid. LCMS: Retention time: 1.174 min (0.1% $NH_3/H_2O$), $[M+H]^+$ calcd. for $C_{20}H_{20}N_2O_5$ 369.4; found 369.1.

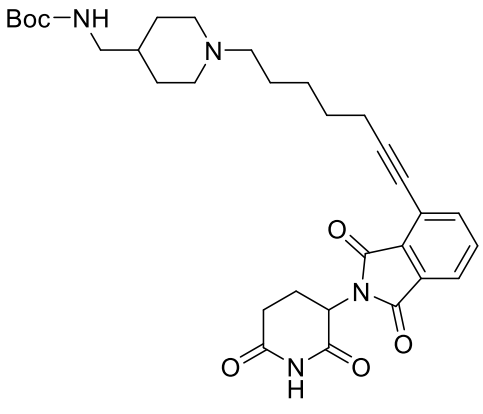
**7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl 4-methylbenzenesulfonate**




**4**

A round-bottom flask containing a mixture of 2-(2,6-dioxopiperidin-3-yl)-4-(7-hydroxyhept-1-yn-1-yl)isoindole-1,3-dione (0.27 g, 0.73 mmol, 1.0 *eq*), 4-Dimethylaminopyridine (44.77 mg, 0.37 mmol, 0.50 *eq*) and 1-chloro-4-methyl-1-sulfonylbenzene (0.17 g, 0.88 mmol, 1.2 *eq*) in DCM (3.0 mL) was placed in ice bath and reacted at 0 ℃ for 15 h. LCMS showed desired MS. The mixture was washed with water (5 mL) and extracted with EA (20 mL × 2). The combined organic layers were dried over $Na_2SO_4$. The residue was purified by flash silica column chromatography (0~60% EA/PE) to afford 7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl 4-methylbenzenesulfonate (0.18 g, 0.23 mmol, 31% yield) as a yellow solid. LCMS: Retention time: 1.59 min (0.1% $NH_3/H_2O$), $[M+H]^+$ calcd.for $C_{27}H_{26}N_2O_7S$ 523.1; found 523.1.

**tert-butyl [(1-{7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl}piperidin-4-yl)methyl]amino formate**

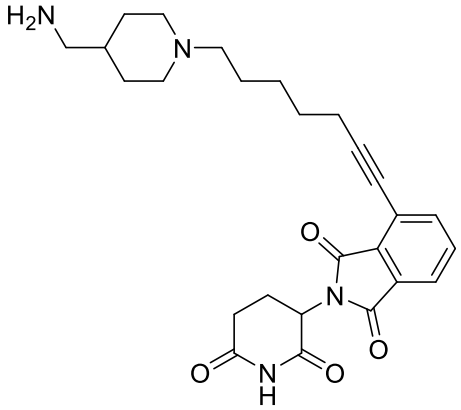



**6**

To a mixture of 7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl 4-methylbenzenesulfonate (0.18 g, 0.34 mmol, 1.0 *eq*), tert-butyl (piperidin-4-ylmethyl)amino formate (0.15 g, 0.68 mmol, 2.0 *eq*) and sodium iodide (0.025 g, 0.17 mmol, 0.50 *eq*) in DMF (1.0 mL) was added triethylamine (0.13 g, 1.02 mmol, 3.0

*eq*). The mixture was stirred at 60 ℃ for 15 h under $N_2$ atmosphere. LCMS showed desired MS. The mixture was washed with saturated brine (5 mL × 2) and extracted with EA (10 mL × 2). The combined organic layers were dried over $Na_2SO_4$. The residue was purified by flash silica column chromatography (0~3% MeOH/DCM) to afford tert-butyl [(1-{7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl}piperidin-4-yl)methyl]amino formate (0.070 g, 0.11 mmol, 34% yield) as a yellow solid. LCMS: Retention time: 1.68 min (0.1% $NH_3.H_2O$), $[M+H]^+$ calcd. for $C_{31}H_{40}N_4O_6$ 565.3; found 565.3.

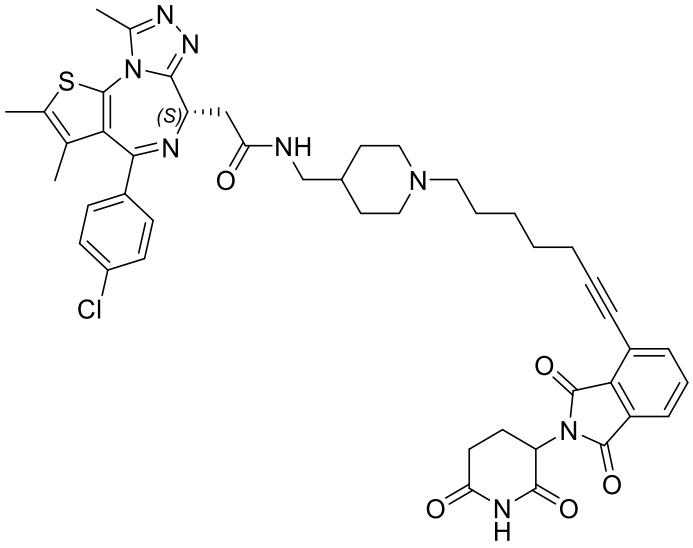
**4-{7-[4-(aminomethyl)piperidin-1-yl]hept-1-yn-1-yl}-2-(2,6-dioxopiperidin-3-yl)isoindole-1, 3-dione**



**7**

To a solution of tert-butyl [(1-{7-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]hept-6-yn-1-yl}piperidin-4-yl)methyl]amino formate (0.07 g, 0.12 mmol, 1 *eq*) in DCM (1 mL) placed in ice bath was added TFA (0.2 mL), The mixture was stirred at 0 ℃ for 6 h. LCMS showed desired MS. The mixture was washed with MTBE (2 mL*5) and evaporated to afford 4-{7-[4-(aminomethyl)piperidin-1-yl]hept-1-yn-1-yl}-2-(2,6-dioxopiperidin-3-yl) isoindole-1,3-dione (0.053 g, 0.11 mmol, 91% yield) as a green solid, which was used for the next step without further purification.. LCMS: Retention time: 1.18 min (0.1%TFA), $[M+H]^+$ calcd.for $C_{31}H_{40}N_4O_6$ 465.2; found 465.2.

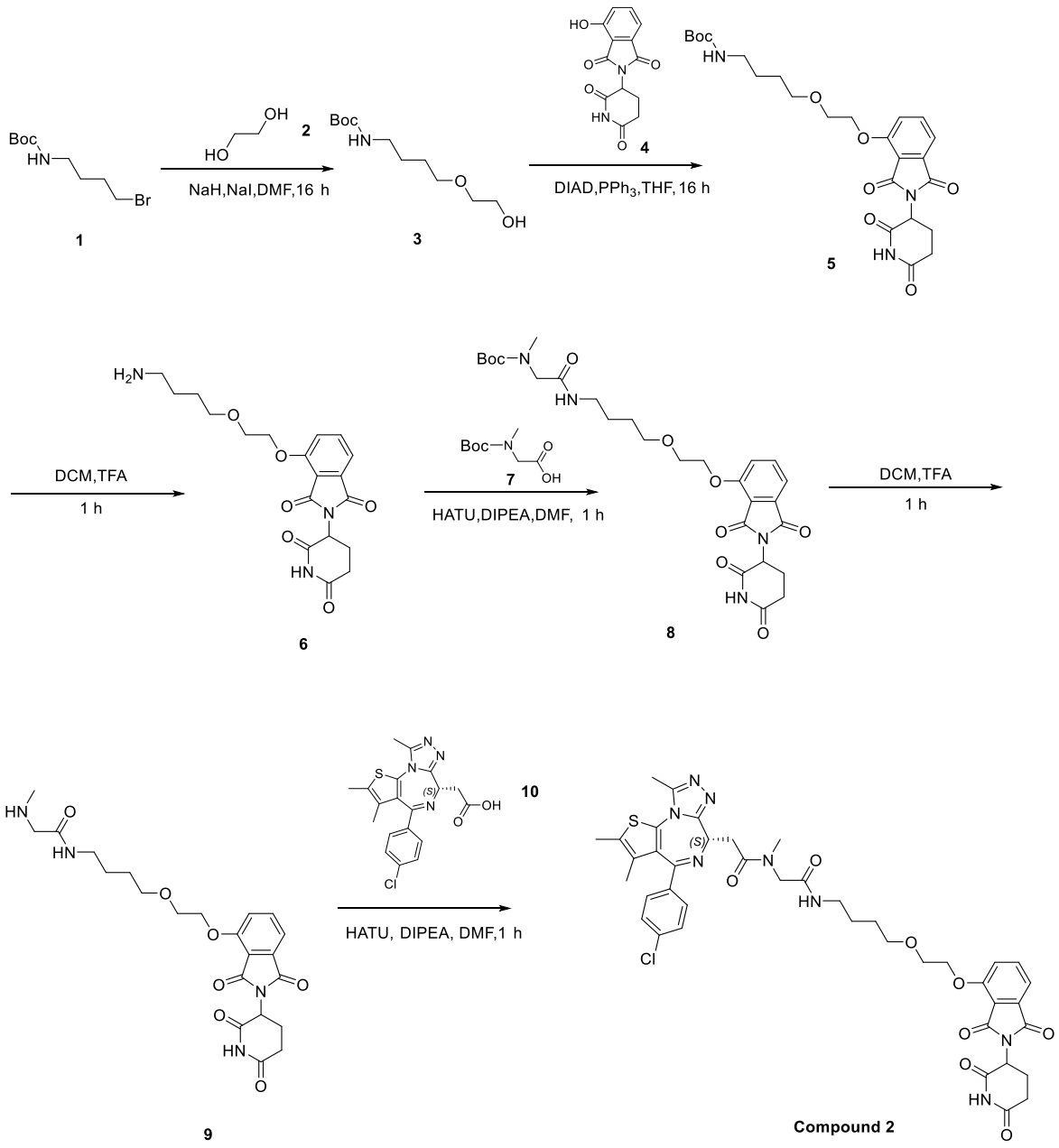
**2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-((1-(7-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hept-6-yn-1-yl)piperidin-4-yl)methyl)acetamide**



**Compound 1**

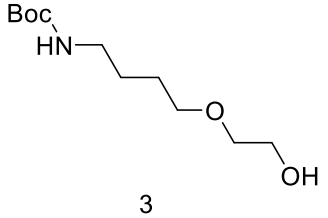
To a solution of (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (0.046 g, 0.11 mmol, 1.0 *eq*) in DMF (1.0 mL) was added 2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (0.065 g, 0.17 mmol, 1.5 *eq*) and stirred at 0 ℃ for 5 min. Then, Ethyldiisopropylamine (0.044 g, 0.34 mmol, 3.0 *eq*), 4-{7-[4-(aminomethyl)piperidin-1-yl]hept-1-yn-1-yl}-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (0.053 g, 0.11 mmol, 1.0 *eq*) was added and continuously stirred at room temperature for 6 h. LCMS showed desired MS. The mixture was washed with water (5.0 mL) and extracted with EA (20 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by prep-HPLC (Gemini 5um C18 150*21.2mm, mobile phase: [water (0.05% TFA v/v)-ACN]) and lyophilized to get 2-((S)-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-((1-(7-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hept-6-yn-1-yl)piperidin-4-yl)methyl)acetamide (13.5 mg, 0.015 mmol, 13.4% yield, 96% purity) as a white solid. HPLC (column: Gemini 5um C18 150*21.2mm, water (0.05% $NH_3H_2O$)-ACN): Retention time: 3.429 min. 1H-NMR (400 MHz, DMSO-$d_6$) δ 11.10 (d, *J* = 12.4 Hz, 1H), 8.16 (s, 1H), 7.81 (dd, *J* = 13.6, 7.4 Hz, 3H), 7.45 (dd, *J* = 23.6, 8.2 Hz, 4H), 5.13 (dd, *J* = 12.6, 5.2 Hz, 1H), 4.58 – 4.37 (m, 1H), 3.26 (d, *J* = 8.8 Hz, 3H), 3.17 (dd, *J* = 15.0, 5.4 Hz, 4H), 3.06 – 2.95 (m, 3H), 2.90 (d, *J* = 11.4 Hz, 2H), 2.59 (s, 3H), 2.41 (s, 3H), 2.35 (s, 2H), 2.06 (d, *J* = 10.6 Hz, 1H), 1.94 (t, *J* = 11.2 Hz, 2H), 1.64 (d, *J* = 19.2 Hz, 7H), 1.48 (s, 4H), 1.16 (s, 2H). LCMS: Retention time: 0.930 min (0.1% TFA), $[M+H]^+$ calcd. for $C_{45}H_{47}ClN_8O_5S$ 847.3; found 847.3.

**Chemical Synthesis of Compound 2**




Scheme 1. Synthetic route to **Compound 2**

**Tert-butyl (4-(2-hydroxyethoxy)butyl)carbamate**

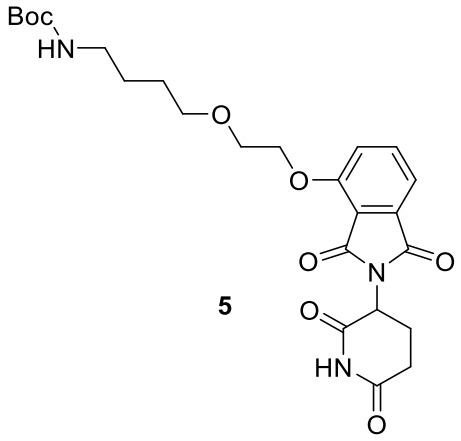



3

To a solution of Ethane-1,2-diol (107 mg, 1.73 mmol, 1.1 *eq*) in DMF (5 ml) was added NaH (63.2 mg, 1.58 mmol, 1.0 *eq*) at 0°C and stirred for 30 min, then tert-butyl (4-bromobutyl)carbamate (400

mg, 1.58 mmol, 1.0 *eq*) and NaI (118 mg, 0.79 mmol, 0.5 *eq*) was added the mixture and stirred at 25 ℃ for 16 h. The product was detected by LCMS. The mixture was quenched with $H_2O$ (1 mL), then extracted with EA (10 ml *2), the combined organic layers were washed with brine (10 mL*2), dried over $Na_2SO_4$, filtrated, and concentrated under vacuum. The residue was purified by flash silica column chromatography (0~50% EA/PE) to give tert-butyl (4-(2-hydroxyethoxy)butyl)carbamate (200 mg, 0.68 mmol, 43.22% yield) as a light-yellow oil. LCMS: Retention time: 0.999 min, $[M+H]^+$ calcd.for C11H23NO4 234.0; found 234.1.

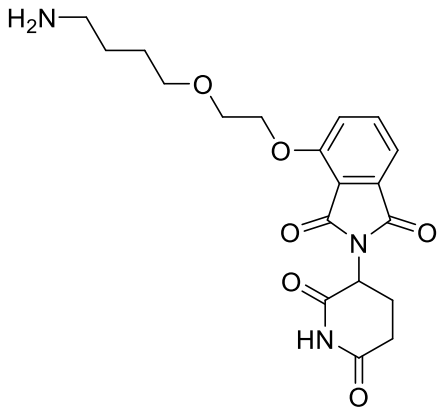
**tert-butyl (4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)carbamate**




**5**

To a solution of $PPh_3$ (302 mg, 1.15 mmol,1.5 *eq*) in THF (10 ml) was added DIAD (186 mg,0.92 mmol, 1.2 *eq*) dropwise at 0 ℃ and stirred for 20 min, then tert-butyl (4-(2-hydroxyethoxy)butyl)carbamate (180 mg, 0.76 mmol, 1.0 *eq*) and 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (210 mg, 0.76 mmol, 1.0 *eq*) was added the mixture and stirred at 25 ℃ for 16 h. The mixture was quenched with $H_2O$ (5 ml) at 0 ℃, then extracted with EA (10 mL *2). The combined organic layers were washed with brine (10 mL*2), dried over $Na_2SO_4$, filtrated, and concentrated under vacuum. The residue was purified by flash silica column chromatography (0~5% MeOH/DCM) to give tert-butyl (4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)carbamate (110 mg, 0.19 mmol, 24.81% yield) as a colorless oil. LCMS: Retention time: 1.246 min, $[M+H]^+$ calcd.for C24H31N3O8 490.2; found 490.2

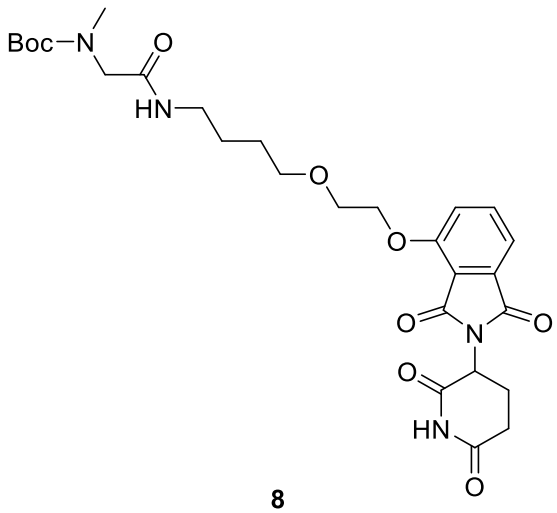
**4-(2-(4-aminobutoxy)ethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione**



**6**

To a solution of tert-butyl (4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)carbamate (110 mg, 0.22 mmol,1.0 *eq*) in DCM (5 ml) was added TFA (1 ml) drop-wise at 0 ℃ and the mixture was stirred at 25 ℃ for 1 h. The product was detected by LCMS. The mixture was concentrated under vacuum to give 4-(2-(4-aminobutoxy)ethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (110 mg, 0.28 mmol, 100% yield) as a light yellow oil, which was used for the next step without further purification. LCMS: Retention time: 0.620 min, $[M+H]^+$ calcd.for C19H23N3O6 390.2; found 390.2.

**tert-butyl (2-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)amino)-2-oxoethyl)(methyl)carbamate**

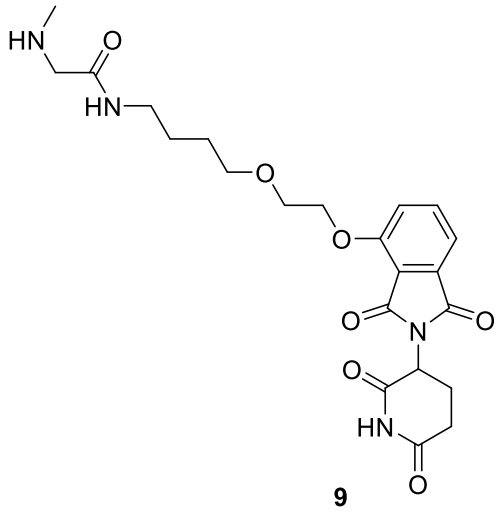


**8**

To a solution of 4-(2-(4-aminobutoxy)ethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (110 mg, 0.28 mmol, 1.0 *eq*), N-(tert-butoxycarbonyl)-N-methylglycine (54 mg, 0.28 mmol, 1.0 *eq*) and DIPEA (109 mg, 0.84 mmol, 3.0 *eq*) in DMF (5 ml) was added HATU (193 mg, 0.50 mmol, 1.8 *eq*) at 0 ℃ and the mixture was stirred at 25 ℃ for 1 h. The product was detected by LCMS. The mixture was extracted with EA (10 mL *2), the combined organic layers were washed with brine (10 mL*2), dried over $Na_2SO_4$, filtrated, and concentrated under vacuum. The residue was purified by flash silica column chromatography (0~5% MeOH/DCM) to give tert-butyl (2-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)amino)-2-

oxoethyl)(methyl)carbamate (100 mg, 0.16 mmol, 59.89% yield) as a colorless oil. LCMS: Retention time: 1.144 min, $[M+H]^+$ calcd.for C27H36N4O9 561.2; found 561.2

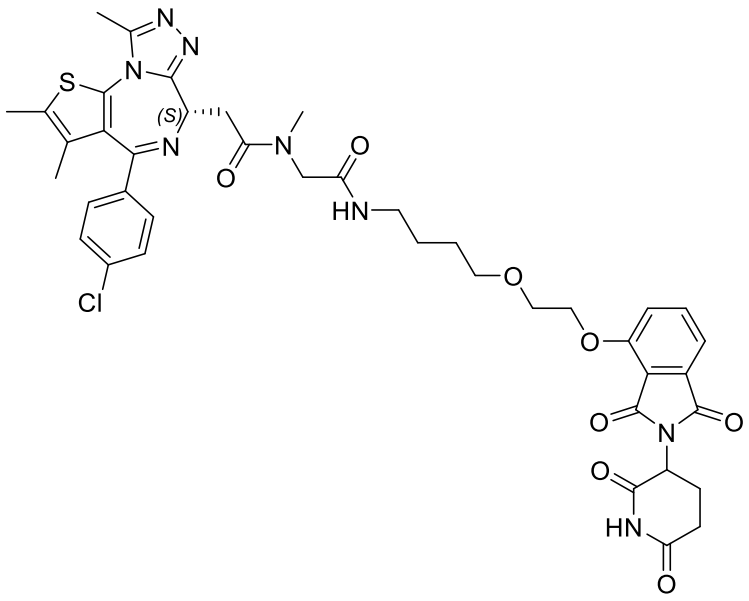
**N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)-2-(methylamino)acetamide**



**9**

To a solution of tert-butyl (2-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)amino)-2-oxoethyl)(methyl)carbamate (100 mg, 0.17 mmol, 1.0 *eq*) in DCM (5 mL) was added TFA (1 mL) dropwise at 0 ℃ and the mixture was stirred at 25 ℃ for 1 h. The product was detected by LCMS. The mixture was concentrated under vacuum to afford N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)-2-(methylamino)acetamide (100 mg, 0.17 mmol, 100% yield) as a colorless oil, which was used for the next step without further purification. LCMS: Retention time: 0.643 min, $[M+H]^+$ calcd.for C22H28N4O7 461.2; found 461.2.

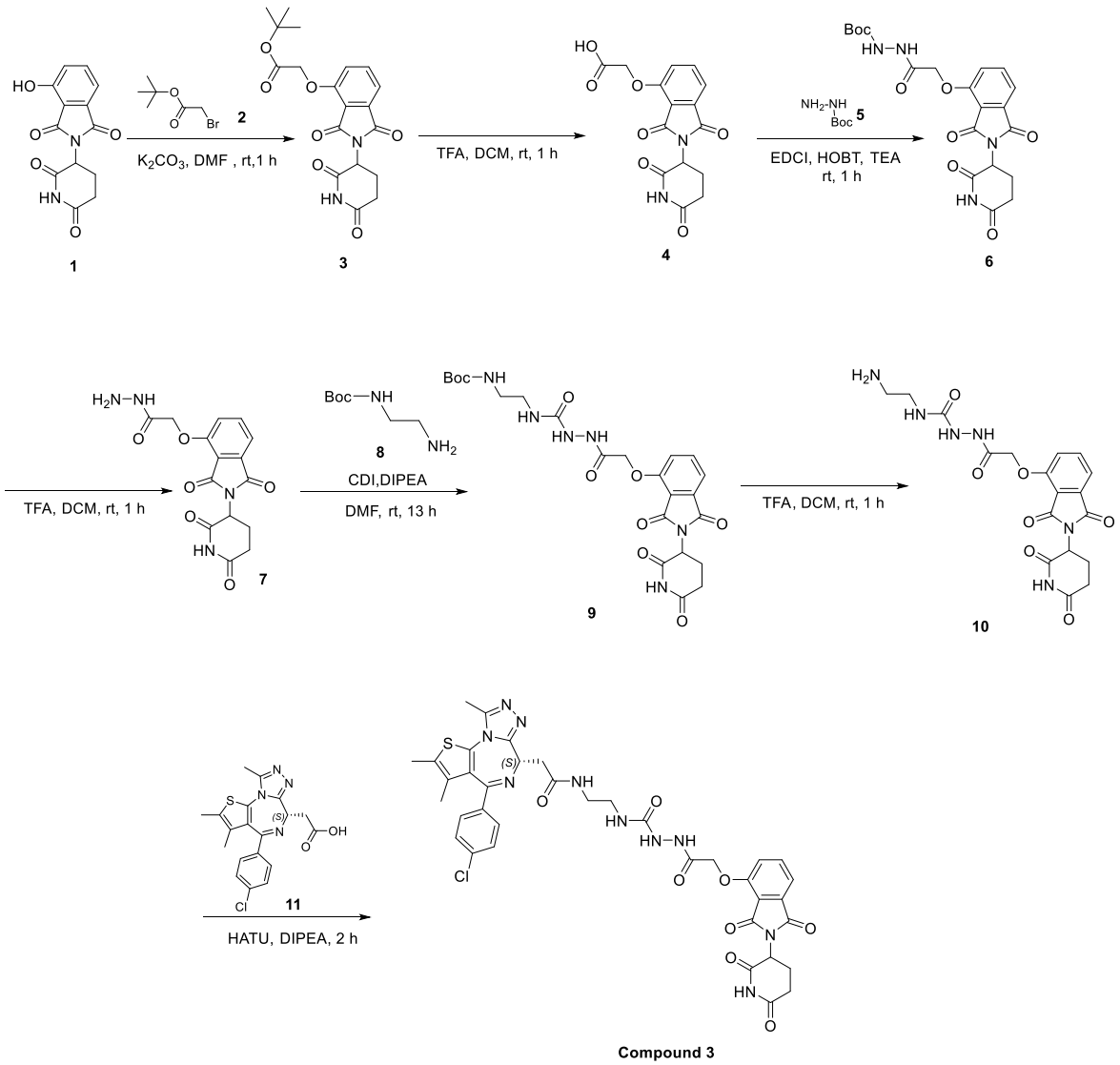
**(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid**



**Compound 2**

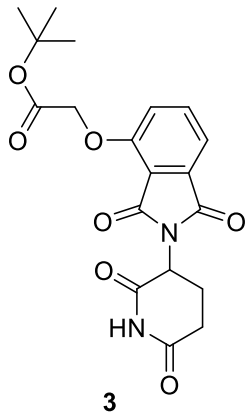
To a solution of N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)butyl)-2-(methylamino)acetamide (100 mg, 0.21 mmol, 1.0 *eq*), **compound 10** (87.0 mg,0.21 mmol, 1.0 *eq*) and DIPEA (84.2 mg, 0.65 mmol, 3.0 *eq*) in DMF (5 ml) was added HATU (148 mg, 0.39 mmol, 1.8 *eq*) at 0 ℃ and the mixture was stirred at 25 ℃ for 1 h. The product was detected by LCMS. The mixture was extracted with EA (10 ml *2), the combined organic layers were washed with brine (10 mL*2), dried over $Na_2SO_4$, filtrated and concentrated in vacuum to get the residue. The crude product was purified by (column: Gemini 5um C18 150*21.2mm, water (0.05% $NH_3H_2O$)-ACN) and lyophilized to get (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)acetic acid (47 mg, 0.05 mmol, 25.41% yield, 99.89% purity, 0.42 FA salt) as a white solid. HPLC: Retention time: 3.021 min. $^1$H-NMR (400MHz, DMSO-d6) δ 11.09 (s, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.74-7.38 (m, 7H), 5.08 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.55 (td, *J* = 6.8, 2.8 Hz, 1H), 4.33 (s, 2H), 4.17 (q, *J* = 17.6 Hz, 1H), 3.91 (d, *J* = 3.0 Hz, 1H), 3.78-3.70 (m, 2H), 3.70-3.36 (m, 4H), 3.18 (s, 3H), 3.09-3.01 (m, 1H), 2.82 (s, 3H), 2.59 (d, *J* = 1.4 Hz, 5H), 2.41 (d, *J* = 4.7 Hz, 3H), 2.10-1.94 (m, 1H), 1.63 (d, *J* = 2.6 Hz, 3H), 1.56-1.39 (m, 4H). LCMS: Retention time: 1.093 min, $[M+H]^+$ calcd. for C41H43ClN8O8S 843.4; found 843.4.

**Chemical Synthesis of Compound 3**




Scheme 1. Synthetic route to **Compound 3**

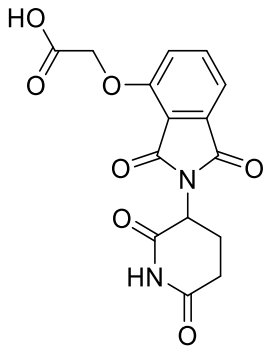
**tert-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate**



To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindole-1,3-dione (2.0 g, 0.0073 mol, 1.0 *eq*) in DMF (100 mL) was added tert-butyl 2-bromoacetate (1.71 g, 0.0088 mol, 1.2 *eq*) at 0 ℃, and

then the mixture was stirred at room temperature for 2 h. LCMS showed desired MS. The mixture was extracted with DCM (100 mL × 3) and washed with water (500 mL × 3). The combined organic layers were washed with brine (100 mL × 2), dried over $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by flash silica column chromatography (50-70% EA/PE) to tert-butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetate (1.8 g, 0.0030 mol, 41.10% yield) was obtained as a yellow solid. $^1$H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 7.95-7.78 (m, 1H), 7.49-7.37 (m, 2H), 4.97-4.95 (m, 3H), 2.51-248 (m, 4H), 1.43 (s, 9H).

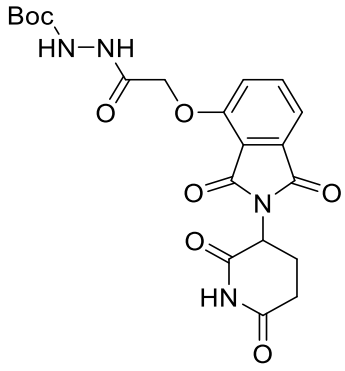
**2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid**



**4**

To a solution of tert-butyl 2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy}acetate (1.8 g, 0.0046mol, 1.0 *eq*) in DCM (10.0 ml) stirred under $N_2$ at 0 ℃ was added TFA (10 ml). The reaction mixture was stirred at room temperature for 1 h. LCMS showed desired MS. All the solvent was removed under vacuum to afford 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (1.5 g, 0.0036 mmol, 84.00% yield) as a yellow solid, which was used for the next step without further purification. LCMS: Retention time: 0.950 min, $[M+H]^+$ calcd. for $C_{15}H_{12}N_2O_7$ 333.1; found 333.1.

**tert-butyl 2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxylate**

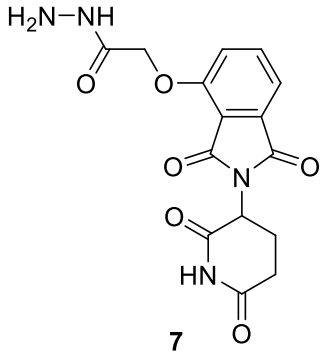


**6**

To a solution of {[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy}acetic acid (1.5 g, 0.0045 mmol,1.0 *eq*) in DMF (15 mL) was added HATU (2.57 g, 0.0067 mmol,1.5 *eq*). The mixture was stirred at 25 ℃ for 0.25 h. Then tert-butyl hydrazinyl formate (0.72 g, 0.0054 mmol,1.2 *eq*), and TEA (2.28 g, 0.0225 mmol,5.0 *eq*) was added into the solution at room temperature. The mixture

was stirred at room temperature for 1 h. LCMS showed desired MS. The mixture was washed with water (100 mL) and extracted with DCM (100 mL*3). The combined organic layers were washed with brine (30 mL*2), dried over $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by silica column chromatography (0~1% MeOH/DCM) to afford tert-butyl 2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxylate(600 mg, 11.90 mmol, 79.73% yield) as a white solid, which was used for the next step without further purification. $^1$H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 9.83 (s, 1H), 9.54 (d, *J* = 47.7 Hz, 1H), 8.91 (s, 1H), 7.80 (dd, *J* = 14.8, 7.2 Hz, 1H), 7.55-7.47 (m, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 5.23-5.02 (m, 1H), 4.91 (d, *J* = 24.8 Hz, 2H), 2.64-2.53 (m, 2H), 2.10-2.00 (m, 1H), 1.40 (s, 9H). LCMS: Retention time: 0.800 min, $[M+Na]^+$ calcd. for $C_{20}H_{22}N_4O_8$ 469.1; found 469.1.

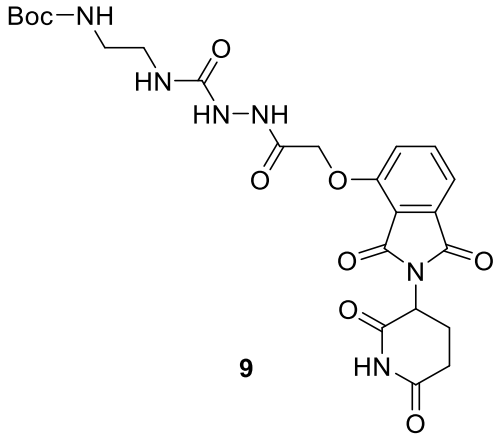
**2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetohydrazide**



**7**

To a solution of tert-butyl 2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxylate (600 mg, 1.341mmol,1.0 *eq*) in DCM (6.0 ml) stirred under $N_2$ at 0℃ was added TFA(2 mL). The reaction mixture was stirred at room temperature for 1 h. LCMS showed desired MS. All the solvent was removed under vacuum to afford 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (420 mg, 1.09 mmol, 90.00% yield) as a white solid, which was used for the next step without further purification. LCMS: Retention time: 0.792 min, $[M+H]^+$ calcd. for $C_{15}H_{14}N_4O_6$ 347.1; found 347.1.

**tert-butyl(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamido)ethyl)carbamate**

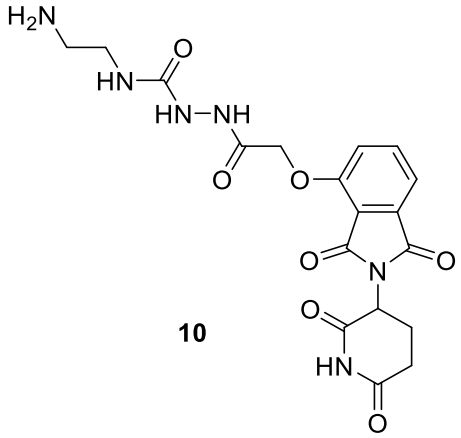


**9**

To a solution of tert-butyl (2-aminoethyl)carbamate (93.11 mg, 0.58 mmol,1.0 *eq*) in DMF (4 mL) was added CDI (23.41 mg, 0.58 mmol,1.0 *eq*) and DIPEA (74.64 mg, 0.58mmol,1.0 *eq*). The

mixture was stirred at 0 ℃ for 1 h. 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (200 mg, 0.58mmol,1.0 *eq*) and DIPEA (186.62 mg, 1.44mmol,2.5*eq*) was added at room temperature. The mixture was stirred at 50 ℃ for 12 h. LCMS showed desired MS. The mixture was washed with water (100 mL) and extracted with DCM (100 mL*3). The combined organic layers were washed with brine (30 mL*2), dried over $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by silica column chromatography (0~1% MeOH/DCM) to afford tert-butyl(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamido)ethyl)carbamate (200 mg, 0.34 mmol, 58.42% yield) as a white solid. $^1$H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 9.72 (s, 1H), 8.04 (d, *J* = 53.8 Hz, 1H), 7.85 – 7.64 (m, 1H), 7.55 – 7.17 (m, 2H), 6.82 (dd, *J* = 26.9, 17.9 Hz, 2H), 6.57 (s, 1H), 5.11 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.87 (s, 2H), 3.16 – 3.00 (m, 2H), 2.91 (ddd, *J* = 17.0, 12.8, 5.6 Hz, 2H), 2.73 – 2.54 (m, 2H), 2.05 (dd, *J* = 12.8, 7.7 Hz, 1H), 1.37 (d, *J* = 3.9 Hz, 9H). LCMS: Retention time: 0.767 min, [M+H]+ calcd. for C23H28N6O9 533.2; found 533.2.

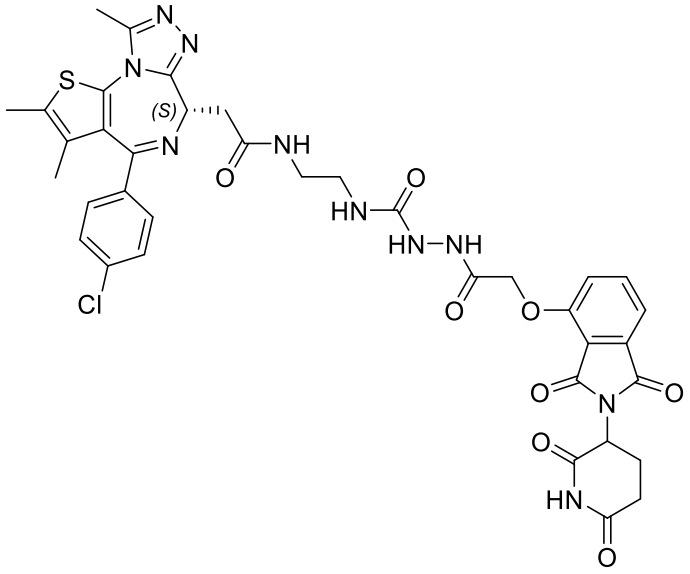
**N-(2-aminoethyl)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamide**



**10**

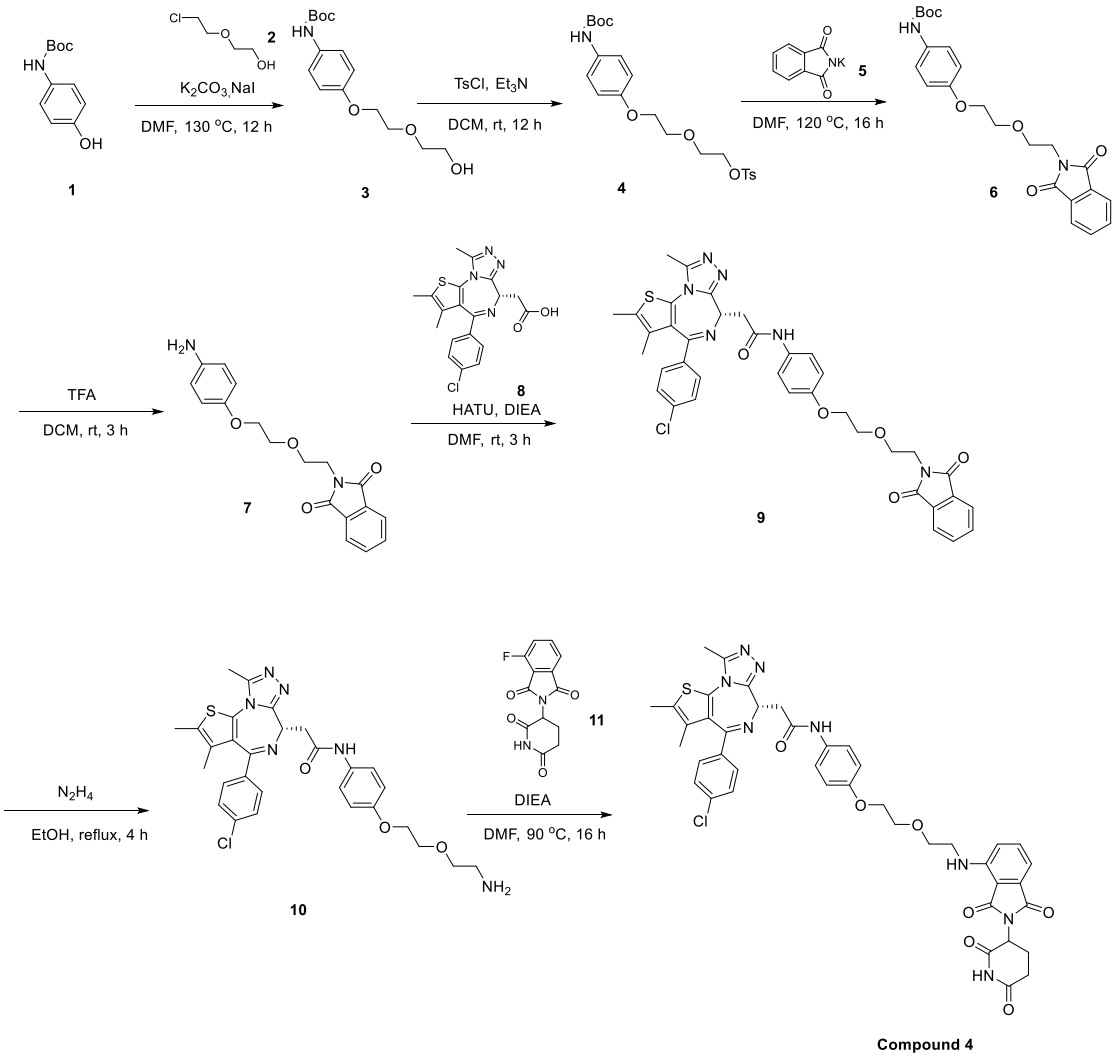
To a solution of tert-butyl(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamido)ethyl)carbamate (200 mg, 0.38mmol,1.0 *eq*) in DCM (1.5 ml) stirred under $N_2$ at 0℃ was added TFA (0.5 mL). The reaction mixture was stirred at 25 ºC for 1 h. LCMS showed desired MS. All the solvent was removed under vacuum to afford N-(2-aminoethyl)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamide (100 mg, 0.21 mmol, 55.51% yield) as a white solid, which was used for the next step without further purification. LCMS: Retention time: 0.500 min, $[M+H]^+$ calcd. for $C_{18}H_{20}N_6O_7$ 433.2; found 433.2.

**N-(2-(2-((S)-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)ethyl)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamide**



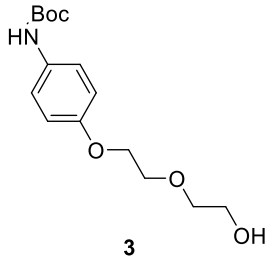
To a solution of N-(2-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)ethyl)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamide (100 mg, 0.23 mmol, 1.0 *eq*) in DMF (2.0 mL) was added HATU (131.92 mg, 0.35 mmol, 1.5 *eq*), the mixture was stirred at 25 ℃ for 0.25 h. Then the mixture was added (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)acetic acid (111.27 mg, 0.28 mmol, 1.2 *eq*), and DIPEA (89.68 mg, 0.69 mmol, 3.0 *eq*) at room temperature. The mixture was stirred at 25 ℃ for 2 h. LCMS showed desired MS. The mixture was washed with water (100 mL) and extracted with DCM (100 mL × 3). The combined organic layers were washed with brine (30 mL × 2), dried over $Na_2SO_4$, filtered, and concentrated in vacuum to the crude product. The crude product was purified by prep-HPLC (column: Gemini 5um C18 150*21.2mm, water (0.05% $NH_3H_2O$)-ACN) and lyophilized to get the product. N-(2-(2-((S)-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)ethyl)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetyl)hydrazine-1-carboxamide (18.7 mg, 0.023 mmol, 9.81% yield, 99.696% purity) was obtained as a pale yellow solid. HPLC: Retention time: 2.647 min. $^1$H NMR (400 MHz, MeOD) δ 7.74 (dd, *J* = 15.8, 7.6 Hz, 1H), 7.56 – 7.37 (m, 6H), 5.10 (ddd, *J* = 5.3 Hz, 1H), 4.91 (s, 2H), 4.70 (t, J = 7.3 Hz, 1H), 3.52 – 3.43 (m, 1H), 3.41 (d, *J* = 7.9 Hz, 2H), 2.90 – 2.79 (m, 1H), 2.75 (d, *J* = 2.3 Hz, 1H), 2.71 (d, *J* = 2.5 Hz, 4H), 2.42 (d, *J* = 20.6 Hz, 3H), 2.19 – 2.06 (m, 1H), 1.69 (d, *J* = 12.6 Hz, 3H), 1.39 (dd, *J* = 6.7, 3.2 Hz, 1H). LCMS: Retention time: 1.100 min, $[M+H]^+$ calcd. for $C_{37}H_{35}ClN_{10}O_8S$ 815.1; found 815.1.

## Chemical Synthesis of Compound 4




Scheme 1. Synthetic route to **compound 4**

## tert-butyl {4-[2-(2-hydroxyethoxy)ethoxy]phenyl}amino formate

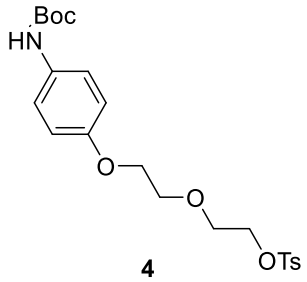



3

A round-bottom flask containing a mixture of 2-(2-chloroethoxy)ethanol (5.0 g, 0.040 mol, 1.0 *eq*), tert-butyl (4-hydroxyphenyl)amino formate (8.4 g, 0.040 mol, 1.0 *eq*), Potassium carbonate (8.31 g, 0.060 mol, 1.5 *eq*) and NaI (6.0 g, 0.040 mol, 1.0 *eq*) in DMF (50 mL) was placed in oil bath and heated to 130 °C for 12 h. LCMS showed desired MS. The mixture was treated with water (100 mL) and extracted with EA (100 mL ×3). The combined organic layers were washed with brine (1 L), dried over $Na_2SO_4$. The residue was purified by flash silica gel chromatography (0~50% EA/PE) to obtain

tert-butyl {4-[2-(2-hydroxyethoxy)ethoxy]phenyl}amino formate (2.17 g, 6.9 mol, 17.21% yield) as a brown solid. LCMS: Retention time: 1.110 min, $[M+H]^+$ calcd.for $C_{15}H_{23}NO_5$ 298.2; found 298.1.

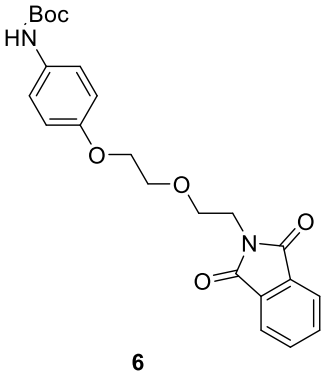
**tert-butyl {4-[2-(2-{[(4-methylbenzene)sulfonyl]oxy}ethoxy)ethoxy]phenyl}amino formate**




**4**

A round-bottom flask containing a mixture of tert-butyl {4-[2-(2-hydroxyethoxy)ethoxy]phenyl}amino formate (2.00 g, 6.7 mol, 1.0 *eq*) and $Et_3N$ (1.36 g, 0.013 mol, 1.5 *eq*] in DCM (20 mL) was immersed in an ice bath for 30 min. The addition of TsCl (1.52 g, 8.0 mmol, 1.1 *eq*) was followed to the reaction. Upon completion of the addition, the flask was removed from the ice bath and stirred at room temperature for 16 h. The mixture was treated with water (100 mL) and extracted with DCM (50 mL × 3). The combined organic layers were washed with brine (200 mL), dried over $Na_2SO_4$. The residue was purified by flash silica gel chromatography (0~30% EA/PE) to obtain tert-butyl {4-[2-(2-{[(4-methylbenzene)sulfonyl]oxy}ethoxy)ethoxy]phenyl}amino formate (2.1 g, 0.0043 mol, 64.18% yield) as a yellow oil. LCMS: Retention time: 1.609 min, $[M+H]^+$ calcd.for $C_{22}H_{29}NO_7S$ 452.2; found 452.1.

**tert-butyl (4-{2-[2-(1,3-dioxoisoindol-2-yl)ethoxy]ethoxy}phenyl)amino formate**

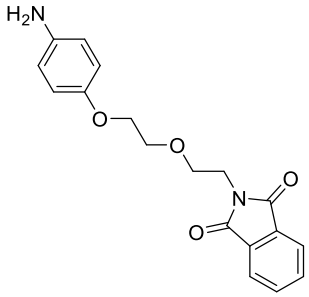



**6**

A round-bottom flask containing a mixture of tert-butyl {4-[2-(2-{[(4-methylbenzene)sulfonyl]oxy}ethoxy)ethoxy]phenyl}amino formate (2.00 g, 0.0044 mol, 1.0 *eq*) and 2-potassioisoindole-1,3-dione (1.20 g, 6.6 mmol, 1.5 *eq*) in DMF (20 mL) was placed in oil bath heated to 120 $^oC$ under $N_2$ for 16 h. LCMS showed desired

MS. The mixture was treated with water (50 mL) and extracted with EA (30 mL × 3). The combined organic layers were washed with brine (100 mL), dried over $Na_2SO_4$. The residue was purified by flash silica gel chromatography (0~30% EA/PE) to obtain tert-butyl (4-{2-[2-(1,3-dioxoisoindol-2-yl)ethoxy]ethoxy}phenyl)amino formate (1.30 g, 2.90 mmol, 65.91% yield) as a white solid. LCMS: Retention time: 1.504 min, $[M+H]^+$ calcd.for $C_{23}H_{26}N_2O_6$ 427.2; found 427.1.

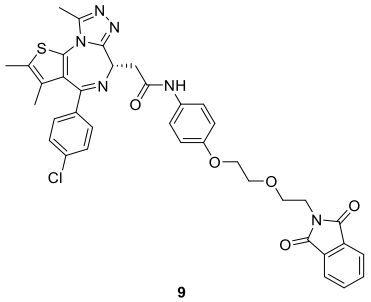
**2-{2-[2-(4-aminophenoxy)ethoxy]ethyl}isoindole-1,3-dione**



7

To a mixture of tert-butyl (4-{2-[2-(1,3-dioxoisoindol-2-yl)ethoxy]ethoxy}phenyl)amino formate (1.30 g, 3.0 mmol) in DCM (20 mL) was added TFA (5mL). The mixture was continuously stirred at room temperature for 3 h. LCMS showed desired MS. The reaction mixture was concentrated and lyophilized. The crude product (778 mg, 2.4 mmol, 79.51% yield) was obtained as a yellow solid without further purification. LCMS: Retention time: 0.692 min, $[M+H]^+$ calcd.for C18H18N2O4 327.1; found 327.2.

**(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-(4-(2-(2-(1,3-dioxoisoindolin-2-yl)ethoxy)ethoxy)phenyl)acetamide**

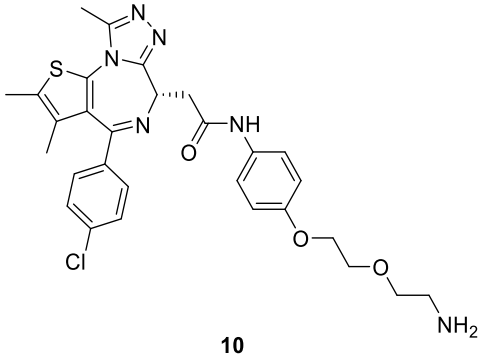


9

To a solution 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)acetic acid (337 mg, 0.84 mmol, 1.0 *eq*) in DMF (9 mL) was added HATU (352 mg, 0.92 mmol, 1.1 *eq*). The reaction mixture was stirred at room temperature for 0.5 h. A Mixture of 2-{2-[2-(4-aminophenoxy)ethoxy]ethyl}isoindole-1,3-dione (275 mg, 0.8427 mmol, 1.0 *eq*) in 1 mL DMF ) and DIEA (217 mg, 1.68 mmol, 2.0 *eq*) was added to the solution. The reaction mixture was stirred at room temperature for 3 h. LCMS showed desired MS. The mixture was treated with water (50 mL) and extracted with EA (30 mL × 3). The

combined organic layers were washed with brine (100 mL), dried over $Na_2SO_4$. The residue was purified by flash silica gel chromatography (0~10% MeOH/DCM) to obtain **(**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-(4-(2-(2-(1,3-dioxoisoindolin-2-yl) ethoxy)ethoxy)phenyl)acetamide (247 mg, 0.33 mmol, 96.82% yield) as a yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.68 (d, *J* = 8.8 Hz, 2H), 4.64 (t, *J* = 6.8 Hz, 1H), 3.97–3.90 (m, 1H), 3.85 (t, *J* = 5.6 Hz, 1H), 3.75 (d, *J* = 5.4 Hz, 2H), 3.64 (dd, *J* = 14.5, 7.6 Hz, 1H), 3.45 (d, *J* = 20.7 Hz, 1H), 2.63 (s, 2H), 2.34 (s, 2H), 1.60 (s, 2H). LCMS: Retention time: 1.300 min, $[M+H]^+$ calcd.for $C_{37}H_{33}ClN_6O_5S$ 709.2; found 709.3.

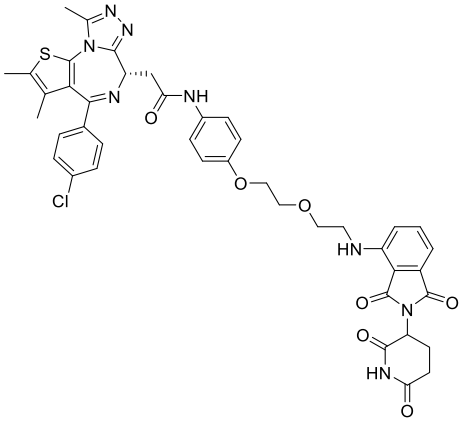
**(S)-N-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide**




**10**

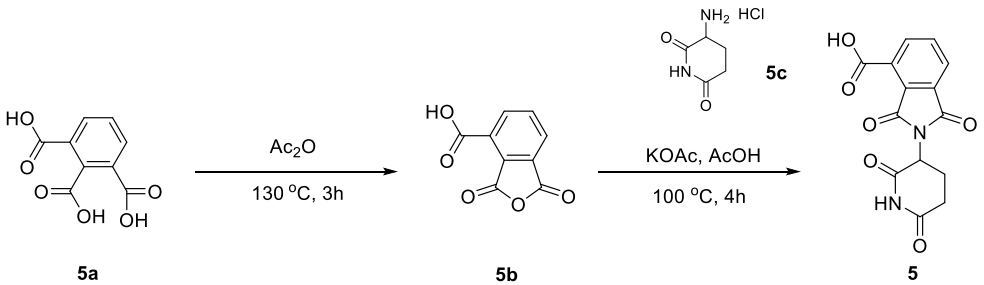
To a mixture of 2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo [8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]-N-(4-{2-[2-(1,3-dioxoisoindol-2-yl)ethoxy] ethoxy} phenyl)acetamide (247 mg, 0.35 mmol, 1.0 *eq*) in EtOH (5 mL) was added $N_2H_4$-$H_2O$ (10 mL). The mixture was placed in oil bath heated to 85 $^o$C for 4 h under $N_2$. LCMS showed desired MS. The mixture was treated with water (50 mL) and extracted with EA (30 mL × 3). The combined organic layers were washed with brine (100 mL), dried over $Na_2SO_4$, filtered and concentrated. The crude product (S)-N-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-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 (200 mg, 0.34 mmol, 99.12% yield) was obtained without purification.
LCMS: Retention time: 0.957 min, $[M+H]^+$ calcd.for $C_{29}H_{31}ClN_6O_3S$ 579.2; found 579.1.

**2-((S)-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-(4-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)phenyl)acetamide**

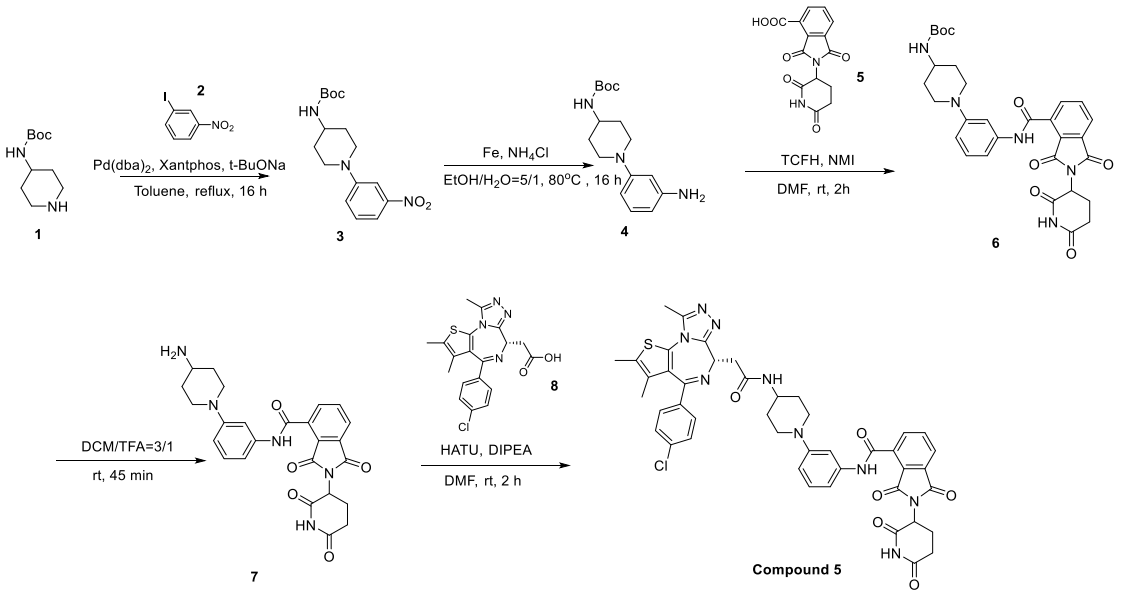


A round-bottom flask containing a mixture of (S)-N-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamide (100 mg, 0.1727 mmol, 1.0 *eq*), 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindole-1,3-dione (57 mg, 0.21 mmol, 1.2 *eq*) and DIEA (44 mg, 0.34 mmol, 2.0 *eq*) in DMF (3 mL) was placed in oil bath heated to 90 $^{o}$C for 16 h under $N_2$. LCMS showed desired MS. The mixture was treated with water (50 mL) and extracted with EA (30 mL × 3). The combined organic layers were washed with brine (100 mL), dried over $Na_2SO_4$, filtered and concentrated. The residue was purified by Prep-HPLC (column: Gemini 5um C18 150*21.2mm, water (0.05% $NH_3.H_2O$)-ACN) and lyophilized to get the product 2-((S)-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-(4-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)phenyl)acetamide (40 mg, 0.047 mmol, 27.62% yield, 99.708% purity) was obtained as a yellow solid. HPLC: Retention time: 4.072 min. $^{1}$H NMR (400 MHz, MeOD) δ 7.51 (t, *J* = 8.2 Hz, 1H), 7.46-7.40 (m, 6H), 5.15-5.12 (m, 1H), 5.05 (s, 2H), 4.72-4.69 (m, 1H), 3.52-3.47 (m ,3H), 2.83-2.70 (m, 6H), 2.44 (s, 3H), 2.13-2.10 (m, 1H), 1.68 (s, 3H), 1.40-1.37 (m, 1H). LCMS: Retention time: 1.252 min, $[M+H]^{+}$ calcd. for $C_{42}H_{39}ClN_8O_7S$ 835.2; found 835.4.

Chemical Synthesis of compound **5**

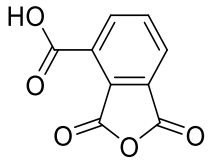



Scheme 1. Synthetic route to compound 5




Scheme 2. Synthetic route to **compound 5**

**1,3-dioxo-1,3-dihydroisobenzofuran-4-carboxylic acid**

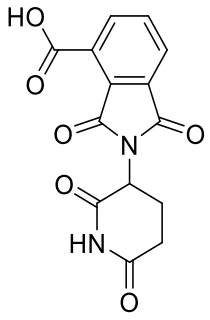


**5b**

A round-bottom flask containing a mixture of benzene-1,2,3-tricarboxylic acid (1.0 g, 4.8 mmol) and $Ac_2O$ (6 mL) was placed in oil bath heated to 130 ℃ for 3 h. The solvent was evaporated, and 1 mL EA was added to dissolve the mixture. The PE (100 mL) was added for precipitation. The filter cake was washed by PE (10 ml) for twice to obtain 1,3-dioxo-1,3-dihydroisobenzofuran-4-carboxylic acid (0.74 g, 3.8 mmol, 79.17 % yield) as a pale yellow solid.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 7.2 Hz, 1H), 8.30 (d, *J* = 7.2 Hz, 1H), 8.13 (t, *J* = 7.8 Hz, 1H).

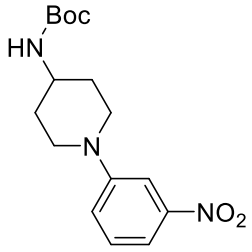
**2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxylic acid**



**5**

A round-bottom flask containing a mixture of 3-aminopiperidine-2,6-dione hydrochloride (210 mg, 1.6 mmol, 1.0 *eq*), 1,3-dioxo-2-benzofuran-4-carboxylic acid (300 mg, 1.6 mmol, 1.0 *eq*) and AcOK (490 mg, 4.9 mmol, 3.0 *eq*) was placed in oil bath heated to 100 ℃ for 4 h, then cool to room temperature. The mixture was filtrated and wash by PE 10 ml for twice to obtain 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxylic acid (210 mg, 0.69 mmol, 43.12 % yield) as a black solid.
$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.75 (s, 1H), 11.14 (s, 1H), 8.05-7.92 (m, 3H), 5.18-5.14 (m, 1H), 2.94-2.85 (m, 1H), 2.63-2.54 (m, 2H), 2.10-2.05 (m, 1H).

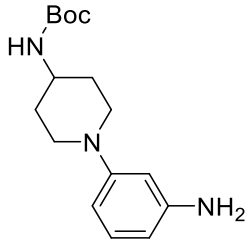
**Tert-butyl (1-(3-nitrophenyl)piperidin-4-yl)carbamate**



**3**

To a solution of 1-iodo-3-nitrobenzene (500 mg, 2 mmol, 1.0 *eq*), tert-butyl piperidin-4-ylamino formate (520 mg, 2.6 mmol, 1.3 *eq*) in toluene (6 mL) was added Xantphos (120 mg, 0.2 mmol, 0.1 *eq*), *t*-BuONa (260 mg, 2.7 mmol, 1.4 *eq*) and $Pd_2(dba)_3$ (30 mg, 0.2 mmol, 0.1 *eq*). The mixture was stirred at 110℃ for 16 h under $N_2$. The solvent was concentrated under vacuum. The crude product was purified by flash silica column chromatography (0~25% EA/PE) to afford compound Tert-butyl (1-(3-nitrophenyl)piperidin-4-yl)carbamate (0.17 g, 0.53 mmol, 26.50% yield) as a yellow solid.
$^1$H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 8.03 Hz, 1H), 7.26 (d, *J* = 6.8 Hz, 1H), 3.72 (d, *J* = 13.0 Hz, 2H), 2.98 (t, *J* = 12.2 Hz, 2H), 2.11 (d, *J* = 13.2 Hz, 2H), 1.46 (s, 9H).

**Tert-butyl (1-(3-aminophenyl)piperidin-4-yl)carbamate**

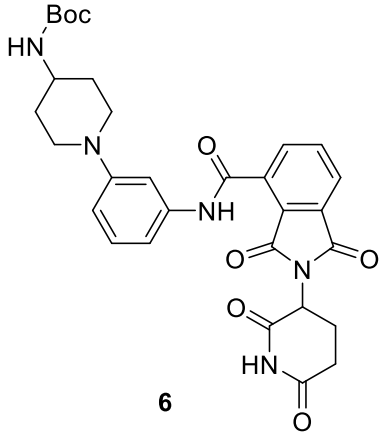



**4**

A round-bottom flask containing a mixture of tert-butyl [1-(3-nitrophenyl)piperidin-4-yl]amino formate (60 mg, 0.19 mmol, *1.0* eq), Fe (52 mg, 0.93 mmol, 4.9 *eq*) and $NH_4Cl$ (40 mg, 0.93 mmol, 4.9 *eq*) was placed in oil bath heated to 80 ℃ for 16 h. The solvent was concentrated under vacuum. The crude product was purified by flash silica column chromatography (0~2% MeOH/DCM) to afford tert-butyl (1-(3-aminophenyl)piperidin-4-yl)carbamate (50 mg, 0.17 mmol, 89.41% yield) as a brown solid.

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.05 (t, *J* = 8.0 Hz, 1H), 6.40 (d, *J* = 8.2 Hz, 1H), 6.36 (s, 1H), 6.25 (d, *J* = 7.8 Hz, 1H), 3.57(d, *J* = 13.0 Hz, 2H), 2.85 (t, *J* = 11.8 Hz, 2H), 2.05-2.02 (m, 2H), 1.62 (d, *J* = 8.4 Hz, 2H), 1.45 (s, 9H).

**Tert-butyl (1-(3-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamido)phenyl)piperidin-4-yl)carbamate**



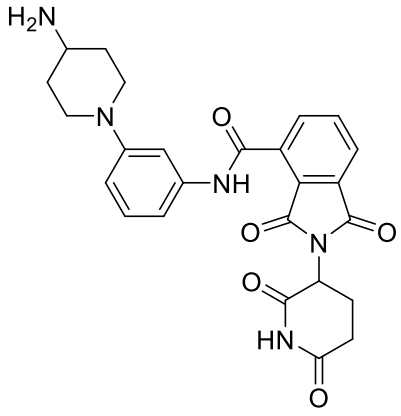

**6**

A round-bottom flask containing a mixture of 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindole-4-carboxylic acid (28 mg, 0.09 mmol, 1.0 *eq*), (1-(3-aminophenyl)piperidin-4-yl)amino tert-butyl formate (25 mg, 0.085 mmol, 0.95 *eq*), TCFH (28 mg, 0.10 mmol, 1.1 *eq)* and NMI (24 mg, 0.30 mmol, 3.3 *eq*) was dissolved in 5 mL DMF and stirred under room temperature for 2 h. The mixture was treated with 100 mL water and extracted with EA. The combined organic layers were concentrated in vacuo. The crude product was purified by flash silica column chromatography (0~50 % EA/PE) to afford tert-butyl (1-(3-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamido)phenyl)piperidin-4-yl)carbamate (17 mg, 0.03 mmol, 33.33% yield) as a yellow solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.14 (s, 1H), 10.54 (s, 1H), 8.04 (t, *J* = 7.6 Hz, 2H), 7.98 (t, *J* = 7.4 Hz, 1H), 7.34 (s, 1H), 7.17 (t, *J* = 8.2 Hz, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.32 (d, *J* = 4.8 Hz, 1H), 5.21-5.16

(m, 1H), 3.61(d, *J* = 12.8 Hz, 2H), 2.03-1.97 (m, 3H), 1.80 (d, *J* = 12.0 Hz, 2H), 1.49-1.39 (m, 14H).

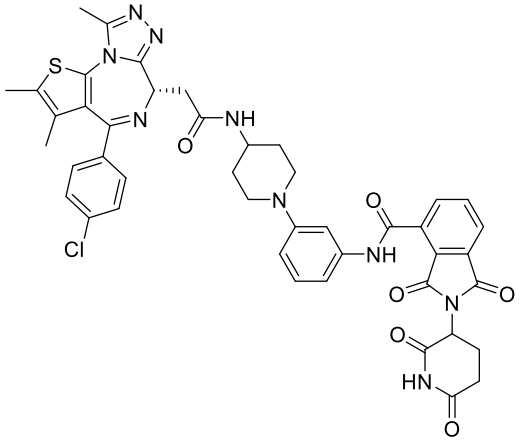
**N-(3-(4-aminopiperidin-1-yl)phenyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamide**



**7**

To a solution of tert-butyl (1-{3-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindole-4-amido]phenyl}piperidin-4-yl)amino formate (17 mg, 0.029 mmol) in 2 mL DCM was added 1 mL TFA dropwise. The reaction mixture was stirred at room temperature for 45min. Solvent was evaporated after reaction to afford N-(3-(4-aminopiperidin-1-yl)phenyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamide (14 mg) as a brown solid, without further purification yield: 95%. LCMS: Rt = 0.669 min, $[M+H]^+$ calculated for $C_{25}H_{25}N_5O_5$: 476.2, Found: 476.2

**N-(3-(4-(2-((S)-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)piperidin-1-yl)phenyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamide**

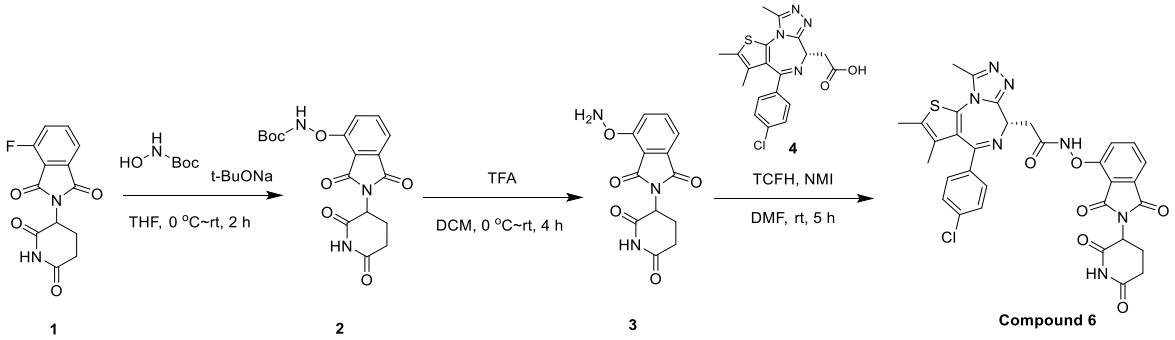


DIPEA (15 mg, 0.12 mmol, 4.0 *eq*) were added successively to a solution of N-(3-(4-aminopiperidin-1-yl)phenyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamide (15 mg, 0.03 mmol, 1.0 *eq*), (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (12 mg, 0.03 mmol, 1.0 *eq*) and N,N,N’,N’-HATU (15.4 mg, 0.03 mmol, 1.0 *eq*) in 2 mL DMF. The reaction

mixture was stirred at room temperature for 2 h. The mixture was treated with 100 mL water and extracted with EA. The combined organic layers were concentrated in vacuo. The crude product was purified by flash silica column chromatography (0~5 % MeOH/DCM) to afford N-(3-(4-(2-((S)-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)piperidin-1-yl)phenyl)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carboxamide (13.8 mg, 0.014 mmol, 46.66 % yield, 99.109% purity) as a yellow solid.

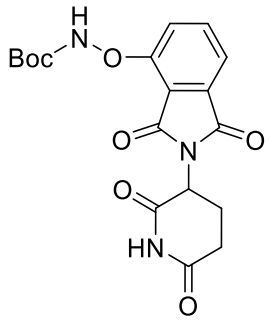
$^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.14 (s, 1H), 10.57 (s, 1H), 8.23 (d, *J* = 7.8 Hz, 2H), 8.07-8.03 (m, 2H), 7.98 (t, *J* = 7.4 Hz 1H), 7.50 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 3H), 7.19 (t, *J* = 8.2 Hz, 1H), 7.06 (d, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 5.21-5.17 (m, 1H), 4.53-4.49 (m, 1H), 3.83-3.81(m, 1H), 3.66 (t, *J* = 11.6 Hz, 2H), 3.26 (t, *J* = 7.6 Hz, 1H), 3.19-3.14 (m, 1H), 2.94-2.81 (m, 3H), 2.63-2.56 (m, 4H), 2.41(s, 3H), 2.08-2.05 (m, 1H), 1.91-1.81 (m, 2H), 1.62-1.54 (m, 5H). HPLC: Retention time: 2.846 min. LCMS: R.t = 0.986 min, $[M+H]^+$ calculated for $C_{44}H_{40}ClN_9O_6S$: 858.2, Found: 858.2.

**Chemical Synthesis of Compound 6**



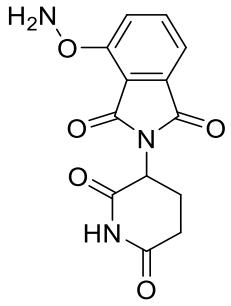

Scheme 1. Synthetic route to **Compound 6**

**tert-butyl ((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)carbamate**



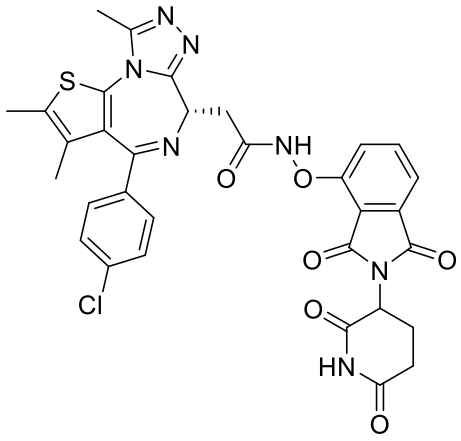
To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindole-1,3-dione (550 mg, 2.0 mmol, 1.0 *eq*) in THF (8 mL) was slowly added *t*-BuONa (230 mg, 2.4 mmol, 1.2 *eq*) under ice bath. The mixture was stirred at 0 ℃ for 30 min. Then tert-butyl hydroxyamino formate (535 mg, 4.0 mmol, 2.0 *eq*) was added to the mixture and stirred at rt for 2 h. LCMS showed desired MS. The mixture was quenched by saturated ammonium chloride solution and extracted with EA (30 mL*3). The combined organic layers were washed with brine (50 mL*2), dried over $Na_2SO_4$, filtered and concentrated in vacuum. The residue was purified by flash silica column chromatography (0~ 8 % MeOH/DCM) to afford tert-butyl ((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)carbamate (360 mg, 0.92 mmol, 46.30 % yield) as a white solid.
1H NMR (400 MHz, DMSO) δ 11.20 (s, 1H), 11.12 (s, 1H), 7.84 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.59 – 7.53 (m, 2H), 5.12 (dd, *J* = 12.8, 5.4 Hz, 1H), 2.89 (ddd, *J* = 16.6, 12.8, 5.4 Hz, 1H), 2.56 (dd, J = 16, 12.8 Hz, 2H), 2.12–2.01 (m, 1H), 1.45 (s, 9H).
LCMS: Retention time: 0.938 min, $[M+H\text{-}Boc]^+$ calcd. for C18H19N3O7 290.1; found 290.1

**4-(aminooxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione**



To a solution of tert-butyl {[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy}amino formate (320 mg, 0.82 mmol) in dry DCM (5 mL) was added TFA (1 mL) at 0 ºC. The reaction mixture was stirred at rt for 4 h. LCMS showed desired MS. The mixture was concentrated in vacuum to afford 4-(aminooxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (270 mg), which was used into next step without further purification.
LCMS: Retention time: 0.655 min, $[M+H]^+$ calcd. for C13H11N3O5 290.1; found 290.1.

**2-((S)-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-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide**



To a solution of TCFH (84 mg, 0.30 mmol, 1.2 *eq*) and NMI (72 mg, 0.87 mmol, 3.5 *eq*) in DMF (3 mL) was added (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)acetic acid (100 mg, 0.25 mmol, 1.0 *eq*) and stirred at rt for 10 min. Then 4-(aminooxy)-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione(87 mg, 0.30 mmol, 1.2 *eq*) was added, and the reaction mixture was continuously stirred at rt for 5 h. LCMS showed desired MS. The mixture was treated with water (40 mL) and extracted with EA (30 mL*3). The combined organic layers were washed with brine (20 mL), dried over $Na_2SO_4$, filtered, and concentrated in vacuum. The crude product was purified by prep-HPLC (column: Gemini 5um C18 150*21.2mm, water (0.05% $NH_3H_2O$)-ACN) to afford 2-((S)-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-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (30 mg, 0.045 mmol, 17.84 % yield ) as a white solid.

1H NMR (400 MHz, DMSO) δ 12.61 (s, 1H), 11.13 (s, 1H), 7.86-7.77 (m, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.59-7.51 (m, 3H), 7.47 (d, *J* = 8.4 Hz, 2H), 5.13 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.58 (t, *J* = 7.2 Hz, 1H), 3.40 (d, *J* = 7.2 Hz, 2H), 3.30 (s, 1H), 2.96- 2.84 (m, 1H), 2.62 (s, 3H), 2.60- 2.54 (m, 1H), 2.41 (s, 3H), 2.10-2.01 (m, 1H), 1.63 (s, 3H).

LCMS: Retention time: 1.051 min, $[M+H]^+$ calcd. for C32H26ClN7O6S 672.1; found 672.1.

## References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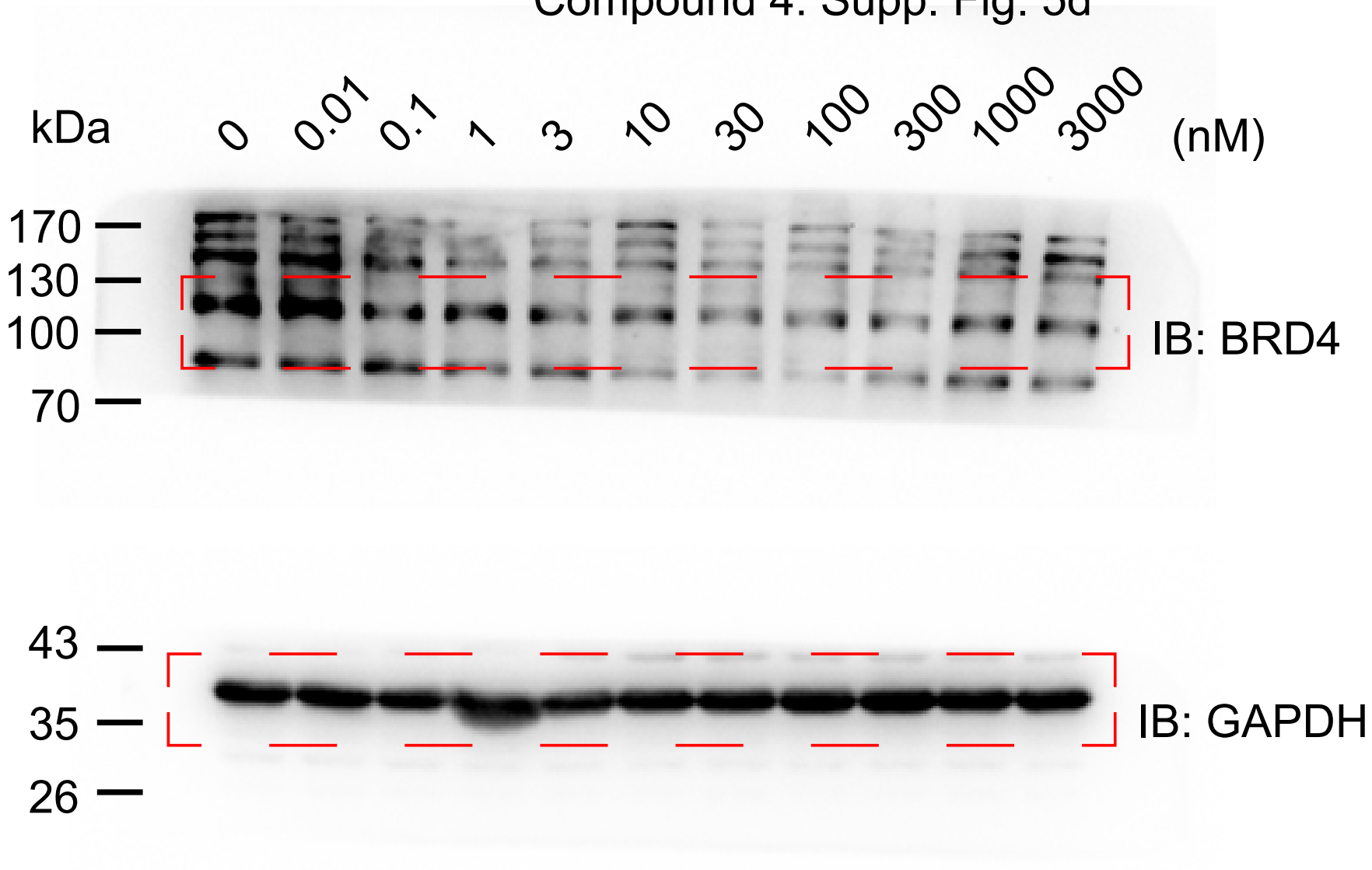
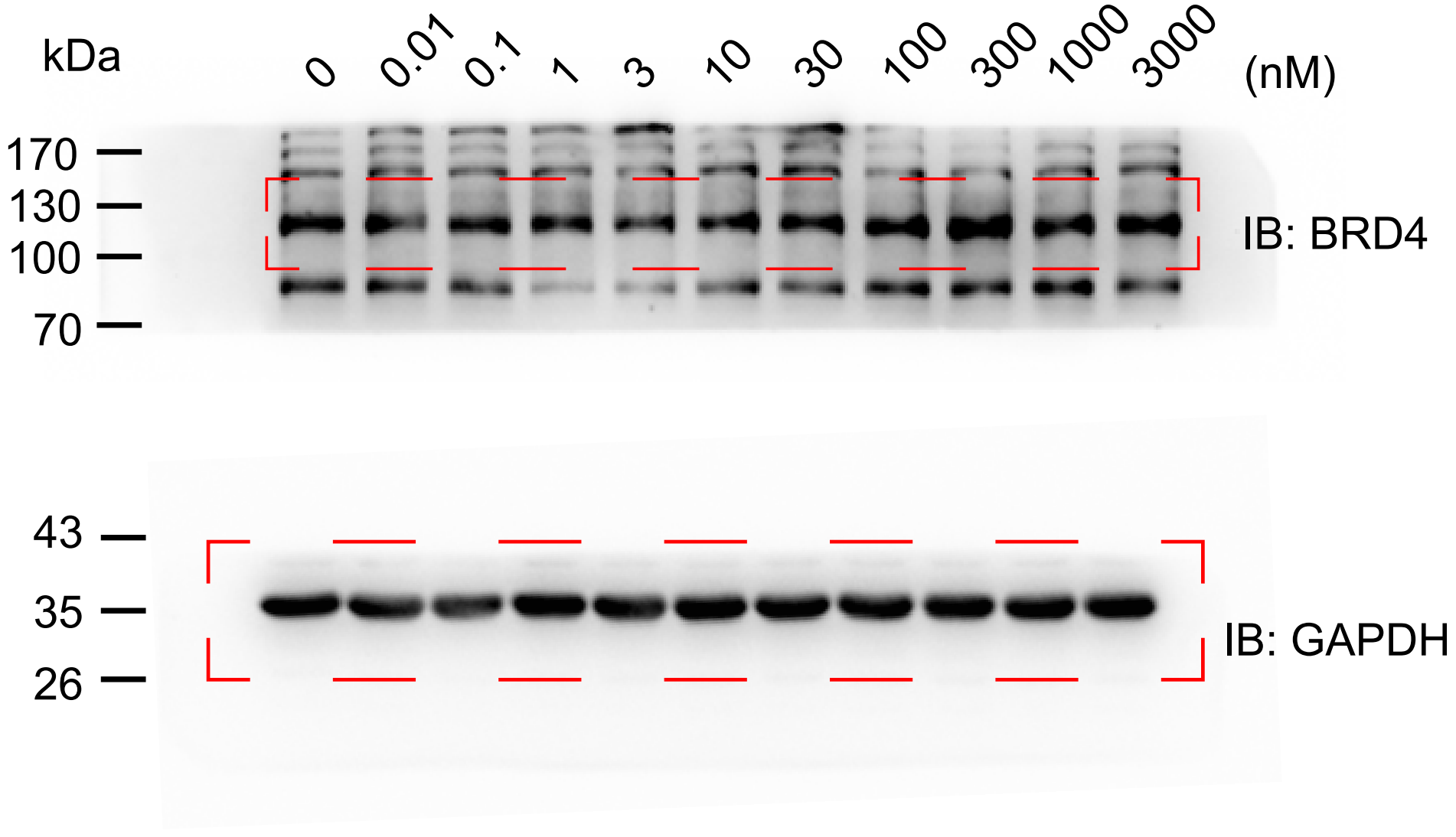
2 Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* **7**, 42717, doi:10.1038/srep42717 (2017).

Compound 4: Supp. Fig. 5d



Compound 5: Supp. Fig. 5e



Compound 6: Supp. Fig. 5f