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(71) Applicant *(for all designated States except US)*: **THE J. DAVID GLADSTONE INSTITUTES** [US/US]; 1650 Owens Street, San Francisco, CA 94158 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GAN, Li** [US/US]; 1700 Easton Drive, Burlingame, CA 94010 (US).

(74) Agent: **BORDEN, Paula A.**; Bozicevic, Field & Francis, 1900 University Avenue, Suite 200, East Palo Alto, CA 94303 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING TAU LEVELS

(57) Abstract: Methods and agents for reducing a level of an acetylated Tau polypeptide in a cell are provided. Methods for treating a tauopathy in an individual are also provided. Also provided is a method for diagnosing a cognitive impairment disorder in an individual. Methods for identifying an agent suitable for treating a tauopathy are also provided.

METHODS AND COMPOSITIONS FOR MODULATING TAU LEVELS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/258,822, filed November 6, 2009, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Neurodegenerative diseases represent a heterogeneous group of genetic and acquired neurological disorders that result in severe and progressive cognitive and motor impairment with on-set during mid- to late-life. The most common cause of dementia is Alzheimer's disease. In less than 5% of the cases Alzheimer's disease genetic factors are involved, the rest of the cases arc sporadic.

[0003] Tau protein is expressed in central nervous system and plays a critical role in the neuronal architecture by stabilizing intracellular microtubule network. Impairment of the physiological role of the tau protein either by truncation, hyperphosphorylation or by disturbing the balance between the six naturally occurring tau isoforms leads to the formation of neurofibrillary tangles (NFT), dystrophic neurites and neuropil threads. These structures represent ultrastructural hallmarks of Alzheimer's Disease (AD). The major protein subunit of these structures is microtubule associated protein Tau. The amount of NFT found in autopsies of AD patients correlates with clinical symptoms including intellectual decline. Therefore, Tau protein plays a critical role in AD pathology. The recent discovery of co-segregation of specific mutations in the Tau gene with the disease frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) has confirmed that certain abnormalities in the Tau protein can be a primary cause of neurodegeneration and dementia in affected individuals.

[0004] There is a need in the art for methods of treating tauopathies.

Literature

[0005] U.S. Patent Publication No. 2009/0117543; U.S. Patent Publication No. 2008/0194803; U.S. Patent Publication No. 2006/0025337.

SUMMARY OF THE INVENTION

[0006] Methods and agents for reducing a level of an acetylated Tau polypeptide in a cell are provided. Methods for treating a tauopathy in an individual are also provided. Also provided is a method for diagnosing a cognitive impairment disorder in an individual. Methods for identifying an agent suitable for treating a tauopathy are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Figures 1A-G depicts *in vitro* and *in vivo* acetylation of Tau. Figure 1B depicts SEQ ID NO:1.

[0008] Figures 2A-E depict acetylation of Tau by p300 acetyltransferase.

[0009] Figures 3A-I depict deacetylation of Tau by SIRT1, SIRT2, and HDAC6 in *in vitro* cell culture.

[0010] Figures 4A-F depict SIRT1-mediated reduction of Tau acetylation in *in vitro* neurons and *in vivo*. Figure 4D depicts SEQ ID NO: 52.

[0011] Figures 5A-C depict SIRT1 interaction with Tau.

[0012] Figures 6A-K depict the effect of acetylation on Tau turnover and Tau ubiquitination.

[0013] Figures 7A-D depict elevated Tau acetylation under pathological conditions.

[0014] Figures 8A-D depict the effect of reduction of Tau acetylation on p-Tau.

[0015] Figures 9A-D depict an amino acid sequence alignment of human Tau isoform amino acid sequences.

[0016] Figure 10 presents an amino acid sequence alignment of rat, mouse, and human Tau (isoform 2) amino acid sequences.

DEFINITIONS

[0017] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, e.g., in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, e.g., causing regression of the disease, e.g., to completely or partially remove symptoms of the disease.

[0018] The term "effective amount" or "therapeutically effective amount" means a dosage sufficient to provide for treatment for the disease state being treated or to otherwise provide the desired effect (e.g., induction of an effective immune response, reduction of chronic immune hyperactivity, etc.). The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., weight, age, etc.), the disease, and the treatment being effected.

[0019] The terms "individual," "host," "subject," and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines, lagomorphs, non-human

primates, humans, etc. In some embodiments, an individual is a human. In some embodiments, an individual is a rodent (e.g., a mouse, a rat, etc.) or a lagomorph.

[0020] A "pharmaceutically acceptable excipient," "pharmaceutically acceptable diluent," "pharmaceutically acceptable carrier," and "pharmaceutically acceptable adjuvant" means an excipient, diluent, carrier, and adjuvant that are useful in preparing a pharmaceutical composition that are generally safe, non-toxic and neither biologically nor otherwise undesirable, and include an excipient, diluent, carrier, and adjuvant that are acceptable for veterinary use as well as human pharmaceutical use. "A pharmaceutically acceptable excipient, diluent, carrier and adjuvant" as used in the specification and claims includes one and more than one such excipient, diluent, carrier, and adjuvant.

[0021] As used herein, a "pharmaceutical composition" is meant to encompass a composition suitable for administration to a subject, such as a mammal, e.g., a human. In general a "pharmaceutical composition" is sterile, and is free of contaminants that are capable of eliciting an undesirable response within the subject (e.g., the compound(s) in the pharmaceutical composition is pharmaceutical grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intratracheal and the like. In some embodiments the composition is suitable for administration by a transdermal route, using a penetration enhancer other than dimethylsulfoxide (DMSO). In other embodiments, the pharmaceutical compositions are suitable for administration by a route other than transdermal administration. A pharmaceutical composition will in some embodiments include a compound and a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutically acceptable excipient is other than DMSO.

[0022] As used herein, "pharmaceutically acceptable derivatives" of a compound of the invention include salts, esters, enol ethers, enol esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs thereof. Such derivatives may be readily prepared by those of skill in this art using known methods for such derivatization. The compounds produced may be administered to animals or humans without substantial toxic effects and are either pharmaceutically active or are prodrugs.

[0023] A "pharmaceutically acceptable salt" of a compound means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-

hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

[0024] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0025] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0027] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a Tau polypeptide" includes a plurality of Tau polypeptides and reference to "the agent" includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as

antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0028] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0029] Methods and agents for reducing a level of an acetylated Tau polypeptide in a cell are provided. Methods for treating a tauopathy in an individual are also provided. Also provided is a method for diagnosing a cognitive impairment disorder in an individual. Methods for identifying an agent suitable for treating a tauopathy are also provided.

[0030] The following observations were made: 1) Tau is acetylated; 2) acetylation of Tau is increased in early-stage Alzheimer's Disease (e.g., mild cognitive impairment) patients; 3) acetylation of Tau precedes phosphorylation of Tau in disease; 4) Tau is deacetylated by a histone deacetylase (e.g., SIRT1, SIRT2, HDAC6); and 5) Tau is acetylated by a histone acetyltransferase (e.g., p300). Agents that inhibit acetylation of Tau, and agents that deacetylated acetylated Tau, are suitable for reducing the level of acetylated Tau in a cell that produces Tau, e.g., in a neuron or a glial cell. Such agents are useful for treating a tauopathy in an individual.

[0031] The level of acetylated Tau can provide a diagnostic measure for a cognitive impairment disorder, and can serve as a marker for response to treatment for a tauopathy. Antibodies specific for acetylated Tau are thus useful in various diagnostic assays, which are provided.

[0032] Identification of candidate agents for use in the treatment of a tauopathy can be carried out by identifying an agent that increases deacetylation of acetylated Tau, or by identifying an agent that inhibits acetylation of Tau. The present disclosure thus provides methods for identifying an agent suitable for treating a tauopathy.

METHODS OF REDUCING THE LEVEL OF ACETYLATED TAU POLYPEPTIDE IN A NEURONAL CELL

[0033] The present disclosure provides a method for reducing the level of an acetylated Tau (Ac-Tau) polypeptide in a cell (e.g., a cell that normally produces Tau, e.g., a neuron or a glial cell). The method generally involves contacting a cell (e.g., a neuronal cell or a glial cell) with an agent that reduces the level of Ac-Tau polypeptide in the cell, e.g., an agent that increases the activity of a polypeptide that deacetylates an Ac-Tau polypeptide in the cell; an

agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in the cell; etc. The present disclosure provides a method for treating a tauopathy in an individual. The method comprising administering to an individual in need thereof an effective amount of an agent that reduces the level of Ac-Tau in a cell (e.g., a neuronal cell or a glial cell) in the individual.

[0034] Tau amino acid sequences are known in the art. See, e.g., the amino acid sequences found under the GenBank accession numbers in parentheses in the following: Human Tau transcript variant 1 mRNA (NM_016835.3) and isoform 1 protein (NP_058519.2); human Tau transcript variant 2 mRNA (NM_005910.4) and isoform 2 protein (NP_005901.2); human Tau transcript variant 3 mRNA (NM_016834.3) and isoform 3 protein (NP_058518.1); human Tau transcript variant 4 mRNA (NM_016841.3) and isoform 4 protein (NP_058525.1); human Tau transcript variant 5 mRNA (NM_001123067.2) and isoform 5 protein (NP_001116539.1); and human Tau transcript variant 6 mRNA (NM_001123066.2) and isoform 6 protein (NP_001116538.1).

[0035] Exemplary Tau amino acid sequences are depicted in Figures 9A-D (SEQ ID NOs:1-6, respectively), where the sequences in Figures 9A-D are: *Homo sapiens* Tau isoform 2 (GenBank Accession No. NP_005901; SEQ ID NO:1); *Homo sapiens* Tau isoform 3 (GenBank Accession No. NP_058518; SEQ ID NO:2); *Homo sapiens* Tau isoform 4 (GenBank Accession No. NP_058525; SEQ ID NO:3); *Homo sapiens* Tau isoform 5 (GenBank Accession No. NP_001116539; SEQ ID NO:4); *Homo sapiens* Tau isoform 1 (GenBank Accession No. NP_058519; SEQ ID NO:5); and *Homo sapiens* Tau isoform 6 (GenBank Accession No. NP_001116538; SEQ ID NO:6). The amino acid sequences set forth in SEQ ID NOs:1-6 are aligned in Figures 9A-D.

[0036] Figure 10 depicts an amino acid sequence alignment of human Tau isoform 1 (SEQ ID NO:1), rat Tau (SEQ ID NO:7), and mouse Tau (SEQ ID NO:8).

[0037] A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of about 350 amino acids of any one of the amino acid sequences set forth in SEQ ID NOs:1-6. A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to 383 amino acids of the amino acid sequence set forth in SEQ ID NO:2 (*Homo sapiens* Tau isoform 3). A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 412 amino

acids of the amino acid sequence set forth in SEQ ID NO:4 (*Homo sapiens* Tau isoform 5). A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, or from about 400 amino acids to about 441 amino acids, of the amino acid sequence set forth in SEQ ID NO:1 (*Homo sapiens* Tau isoform 2). A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 758 amino acids, of the amino acid sequence set forth in SEQ ID NO:5 (*Homo sapiens* Tau isoform 1). A Tau polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 776 amino acids, of the amino acid sequence set forth in SEQ ID NO:6 (*Homo sapiens* Tau isoform 6).

[0038] Figure 1B provides an amino acid sequence of a Tau isoform 2 polypeptide (SEQ ID NO:1). Tubulin binding regions of the amino acid sequence set forth in SEQ ID NO:1 and shown in Figure 1B include amino acids 243-274, amino acids 275-305, and amino acids 337-368. Corresponding tubulin binding regions in other Tau polypeptides can be readily determined experimentally, or by examining the amino acid sequence alignment presented in Figures 12A-D.

[0039] Possible phosphorylation sites of a Tau isoform 2 polypeptide (e.g., as depicted in Figure 1B and as set forth in SEQ ID NO:1) include the following amino acids: 46, 50, 69, 111, 123, 153, 175, 181, 195, 198, 199, 202, 205, 208, 210, 212, 214, 217, 231, 235, 237, 238, 258, 262, 293, 305, 320, 324, 352, 356, 373, 394, 396, 400, 404, 409, 412, 413, 416, and 422. For example, one or more of serine residues 46, 199, 202, 235, 262, 396, 404, and 422 and/or one or more of threonine residues 50, 69, 111, 153, 175, 181, 205, 212, 217, and 231 of a Tau polypeptide can be phosphorylated. Corresponding phosphorylation sites in other Tau polypeptides can be readily determined experimentally, or by examining the amino acid sequence alignment presented in Figures 9A-D and Figure 10.

[0040] A Tau polypeptide can have a length of from about 350 amino acids to about 780 amino acids, e.g., from about 350 amino acids to about 385 amino acids, from about 385 amino acids to about 415 amino acids, from about 415 amino acids to about 445 amino acids, from about 445 amino acids to about 760 amino acids, or from about 760 amino acids to about 780 amino acids. In some embodiments, a Tau polypeptide has a length of 352 amino acids, 383 amino acids, 412 amino acids, 441 amino acids, 758 amino acids, or 776 amino acids.

[0041] A number of Lysine (Lys) residues on a Tau polypeptide can be acetylated. For example, a Tau isoform 2 can be acetylated at one or more amino acids, including but not limited to, Lys-163, Lys-174, Lys-180, Lys-190, Lys-267, Lys-274, Lys-281, Lys-369, and Lys-385 (e.g., of the amino acid sequence depicted in Figure 1B and as set forth in SEQ ID NO:1). Corresponding acetylation sites in other Tau polypeptides can be readily determined experimentally (e.g., as described in the Examples), or by examining the amino acid sequence alignment presented in Figures 9A-D. For example, as shown in Figures 9A-D, Lys-163 of Tau isoform 2 corresponds to amino acid 105 of Tau isoform 3, amino acid 105 of Tau isoform 4, amino acid 134 of Tau isoform 5, amino acid 480 of Tau isoform 6, and amino acid 580 of Tau isoform 1.

[0042] In some embodiments, an acetylated Tau polypeptide is acetylated at two, three, four, five, six, seven, eight, or nine of Lys-163, Lys-174, Lys-180, Lys-190, Lys-267, Lys-274, Lys-281, Lys-369, and Lys-385 of a Tau isoform 2 polypeptide or corresponding lysine residues in a different Tau isoform. In some embodiments, an acetylated Tau polypeptide comprises acetylated Lys-163, acetylated Lys-174, and acetylated Lys-190 of a Tau isoform 2 polypeptide or corresponding lysine residues in a different Tau isoform. In some embodiments, an acetylated Tau polypeptide comprises acetylated Lys-163, acetylated Lys-174, acetylated Lys-180, acetylated Lys-190, acetylated Lys-267, acetylated Lys 274, acetylated Lys-281, acetylated Lys-369, and acetylated Lys-385 of a Tau isoform 2 polypeptide or corresponding lysine residues in a different Tau isoform.

[0043] An agent that decreases the level of acetylated Tau polypeptide in a cell that normally produces Tau (e.g., a neuronal cell; a glial cell) includes an agent that reduces the level of acetylated Tau polypeptide in the cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the level of acetylated Tau polypeptide in the cell in the absence of the agent.

[0044] A decrease in the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) can result in a decrease in the level of phosphorylated Tau and/or a decrease in the level of total Tau. Thus, in some

embodiments, an agent that decreases the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) also decreases the level of phosphorylated Tau polypeptide in the cell. An agent that decreases the level of acetylated Tau polypeptide in a cell that normally produces Tau (e.g., a neuronal cell; a glial cell) in some embodiments reduces the level of phosphorylated Tau in the cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the level of phosphorylated Tau in the cell in the absence of the agent.

[0045] A decrease in the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) can result in a decrease in total Tau levels. Thus, in some embodiments, an agent that decreases the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) also decreases the level of total Tau polypeptide in the cell. An agent that decreases the level of acetylated Tau polypeptide in a cell that normally produces Tau (e.g., a neuronal cell; a glial cell) in some embodiments reduces the level of total Tau in the cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the level of total Tau in the cell in the absence of the agent.

[0046] A decrease in the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) can result in an increase in a biological activity of a Tau polypeptide. Thus, in some embodiments, an agent that decreases the level of acetylated Tau polypeptide in a cell (e.g., in a cell that normally produces Tau, such as a neuron or a glial cell) also increases a biological activity of Tau polypeptide in the cell. An agent that decreases the level of acetylated Tau polypeptide in a cell that normally produces Tau (e.g., a neuronal cell; a glial cell) in some embodiments increases the level of active Tau polypeptide in the cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 5-fold, at least about 7-fold, at least about 10-fold, or more than 10-fold, compared to the level of active Tau polypeptide in the cell in the absence of the agent. Tau biological activity includes, e.g., stabilization of microtubules.

Agents that increase Tau deacetylation

[0047] Agents that increase Tau deacetylation include agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide. Polypeptides that deacetylate an

acetylated Tau polypeptide include, e.g., a histone deacetylase, SIRT1, SIRT2, HDAC6, etc. Agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide include, e.g., agents that increase the activity of one or more of SIRT1, SIRT2, and HDAC6. Agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide also include agents that increase the protein levels of such a polypeptide, e.g., a nucleic acid comprising a nucleotide sequence encoding SIRT1, SIRT2, HDAC6, etc.

SIRT1

[0048] SIRT1 (also known as (silent mating type information regulation 2 homolog) 1 (S. cerevisiae)) gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and are grouped into four classes. The protein encoded by this gene is included in class I of the sirtuin family. Alternative splicing results in multiple transcript variants. Transcript variant 1 (NM_012238.4) represents the longer transcript and encodes the longer isoform a (NP_036370.2). Transcript variant 2 (NM_001142498.1) encodes isoform b (NP_001135970.1).

[0049] A SIRT1 polypeptide includes a polypeptide that deacetylates an acetylated Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell), and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 450 amino acids, or from about 450 amino acids to about 555 amino acids, of the amino acid sequence set forth in SEQ ID NO:9 (GenBank AAH12499; *Homo sapiens* SIRT1). A SIRT1 polypeptide includes a polypeptide that deacetylates an acetylated Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell), and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 747 amino acids, of the amino acid sequence set forth in SEQ ID NO:10 (GenBank NP_036370; *Homo sapiens* SIRT1 isoform a). A SIRT1 polypeptide includes a polypeptide that deacetylates an acetylated Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell), and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, or from about

400 amino acids to about 452 amino acids, of the amino acid sequence set forth in SEQ ID NO:11 (GenBank NP_001135970; *Homo sapiens* SIRT1 isoform b).

SIRT1 activators

[0050] A number of SIRT1 activators are known in the art. A suitable SIRT1 activator can increase the enzymatic activity of a SIRT1 polypeptide (e.g., the enzymatic activity of a SIRT1 polypeptide in deacetylating an acetylated Tau polypeptide in a neuron or a glial cell) by at least about 25%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, or more than 20-fold. In some embodiments, the SIRT1 activator is a SIRT1-selective activator.

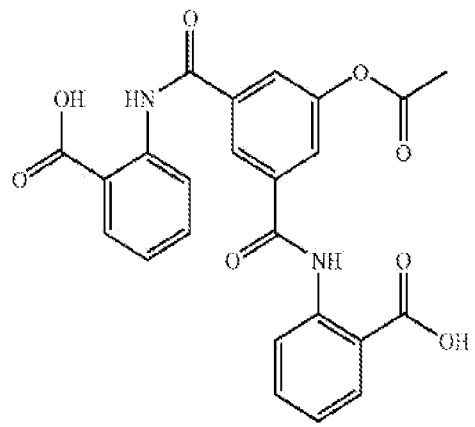
[0051] A suitable SIRT1 activator can increase SIRT1 enzymatic activity at an EC_{50} (half maximal effective concentration) of from about 1 nM to about 1 mM, e.g., from about 1 nM to about 10 nM, from about 10 nM to about 15 nM, from about 15 nM to about 25 nM, from about 25 nM to about 50 nM, from about 50 nM to about 75 nM, from about 75 nM to about 100 nM, from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 450 nM, from about 450 nM to about 500 nM, from about 500 nM to about 750 nM, from about 750 nM to about 1 μM, from about 1 μM to about 10 μM, from about 10 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, from about 75 μM to about 100 μM, from about 100 μM to about 250 μM, from about 250 μM to about 500 μM, or from about 500 μM to about 1 mM.

[0052] Examples of SIRT1 activators that are suitable for use in a subject method include, but are not limited to, resveratrol ((*E*)-5-(*p*-Hydroxystyryl)resorcinol (*E*)-5-(4-hydroxystyryl)benzene-1,3-diol); or 3,5,4'-trihydroxy-trans-stilbene); butein (3,4,2',4'-tetrahydroxychalcone); piceatannol (3,5,3',4'-tetrahydroxy-trans-stilbene); isoliquiritigenin (4,2',4'-trihydroxychalcone); fisetin (3,7,3',4'-tetrahydroxyflavone); quercetin (3,5,7,3',4'-pentahydroxyflavone); a SIRT1 activator as described in U.S. Patent No. 7,345,178; a SIRT1 activator as described in U.S. Patent Publication No. 2008/02555382; and a SIRT1 activator as described in U.S. Patent Publication No. 2009/0012080. Pharmaceutically acceptable salts of any of the foregoing SIRT1 activators are also suitable for use in a subject method.

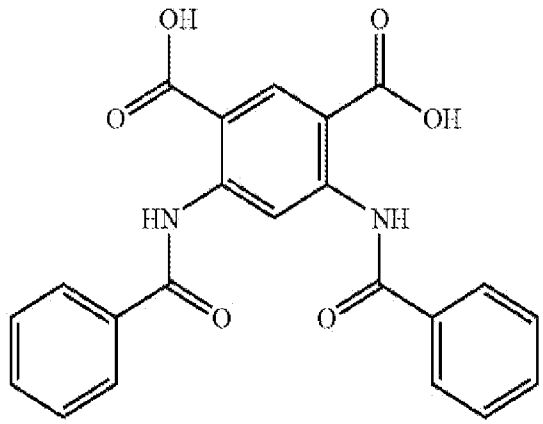
[0053] For example, a suitable SIRT1 activator is a compound of any one of Formulas I-XXVIII as described in U.S. Patent No. 7,345,178, where substituents are as described in U.S. Patent No. 7,345,178, or a pharmaceutically acceptable salt of a compound of any one of Formulas I-XXVIII as described in U.S. Patent No. 7,345,178, provided that the compound activates SIRT1 activity. For example, a suitable SIRT1 activator includes a compound shown in Table 4 of U.S. Patent No. 7,345,178.

[0054] For example, suitable SIRT1 activators are shown in Table 1, below.

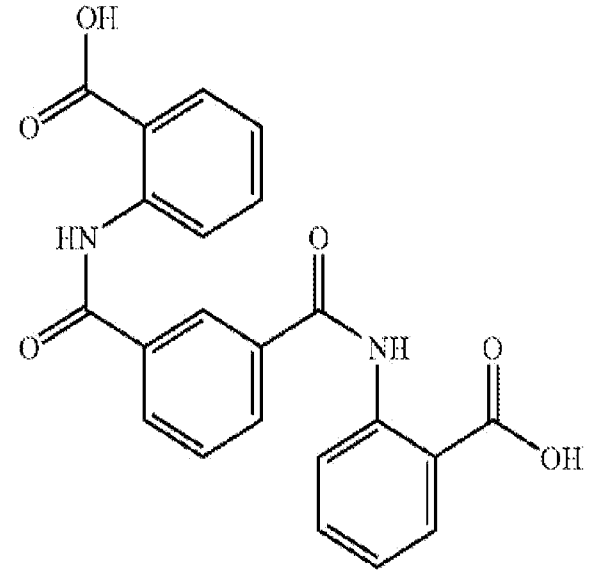
Table 1



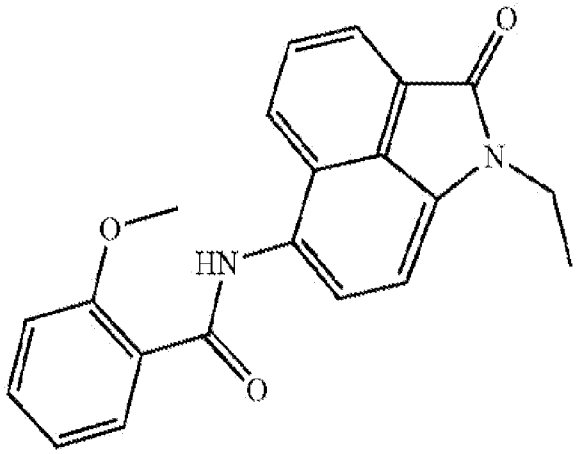
Compound 1



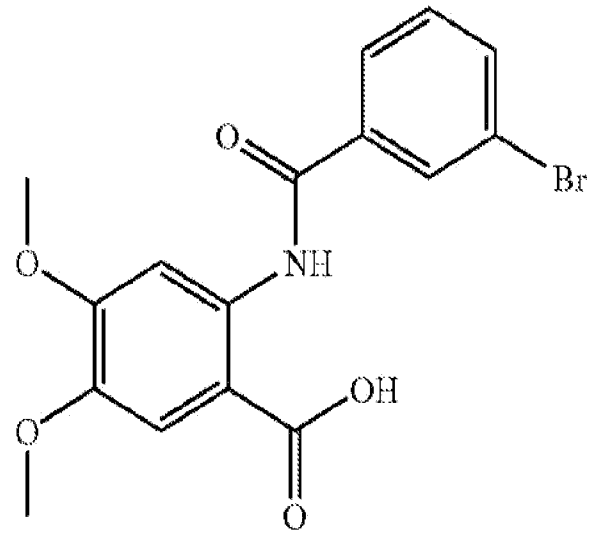
Compound 2



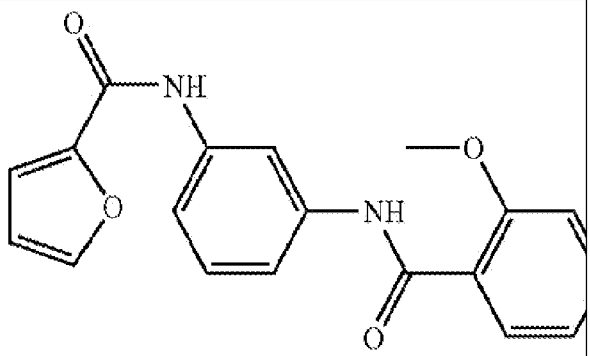
Compound 3



Compound 4



Compound 5



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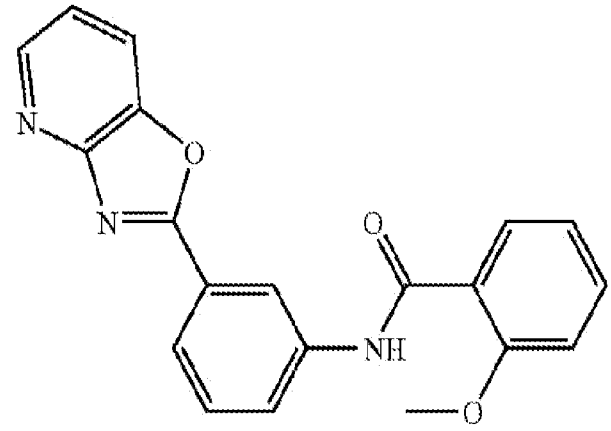
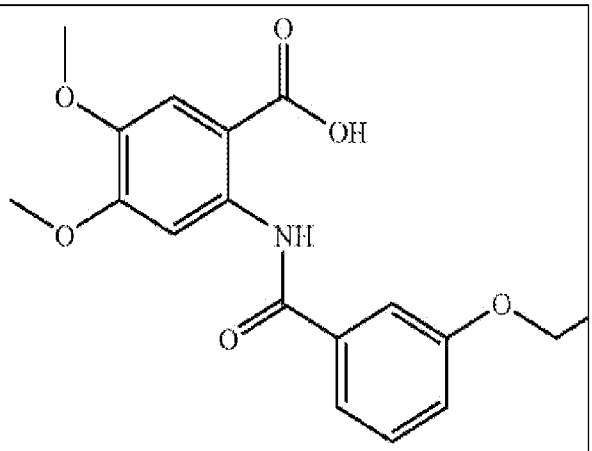
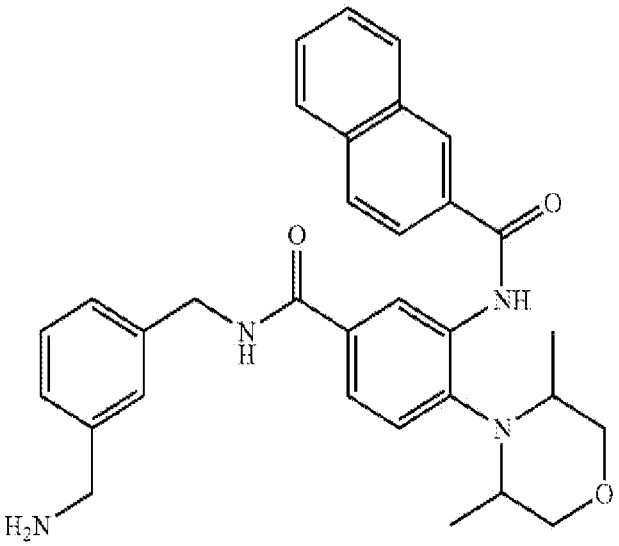


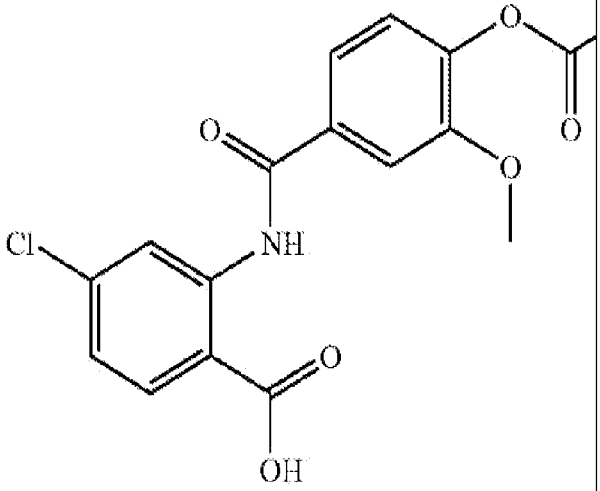
Compound 7



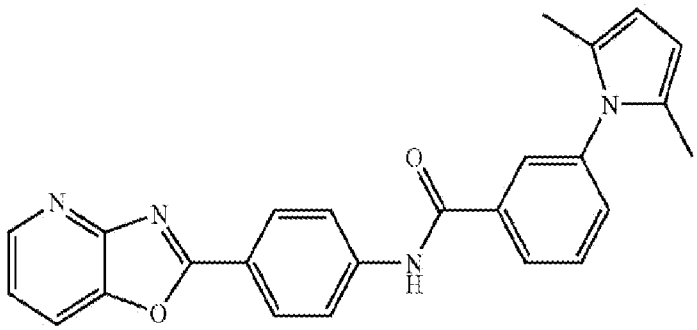
Compound 8



Compound 9



Compound 10



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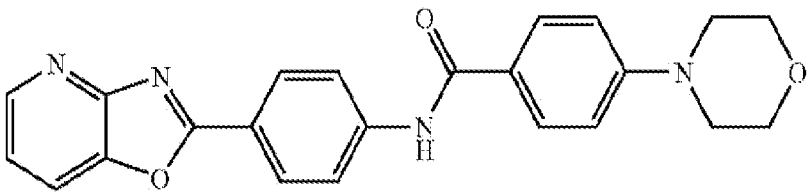

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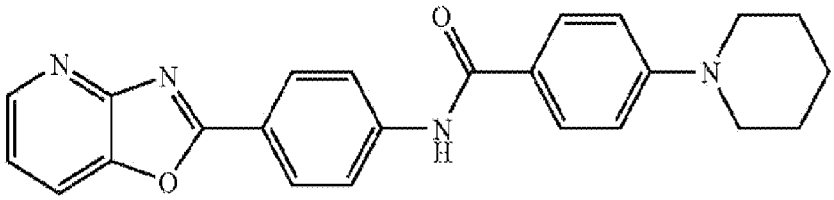

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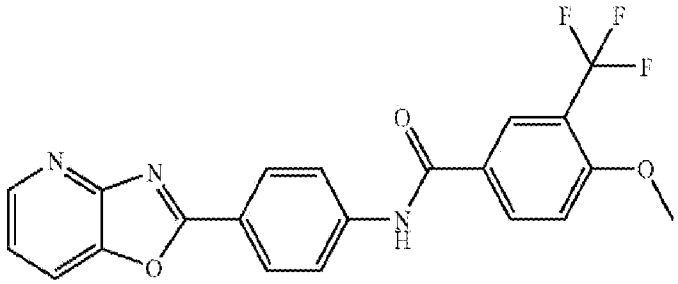

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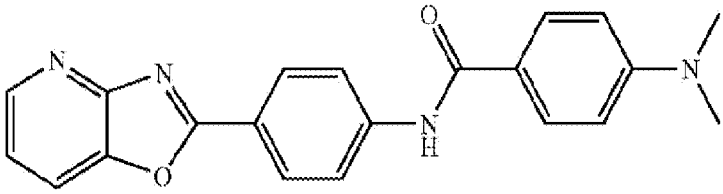

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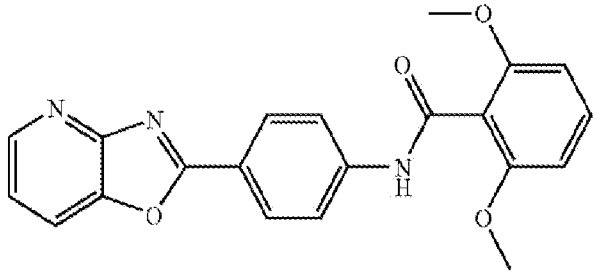

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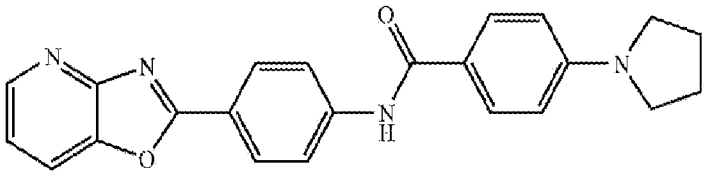

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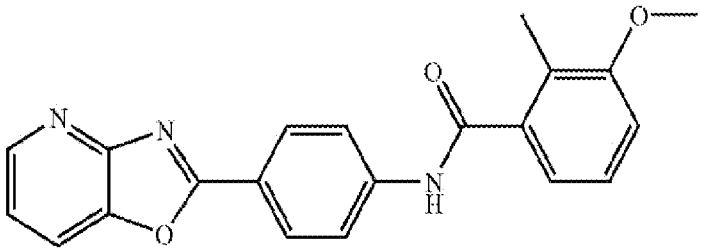

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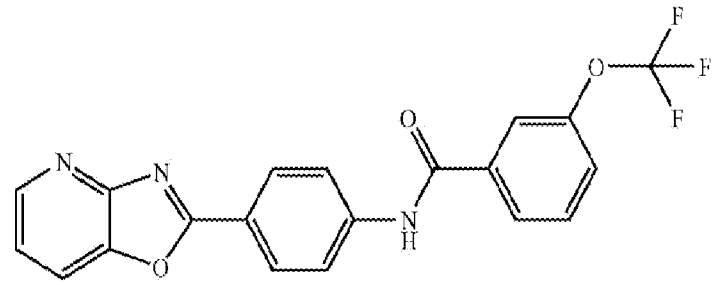

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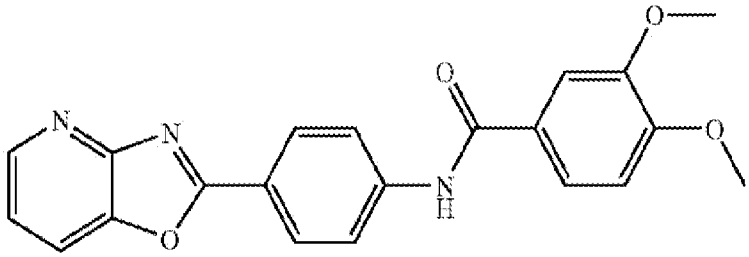

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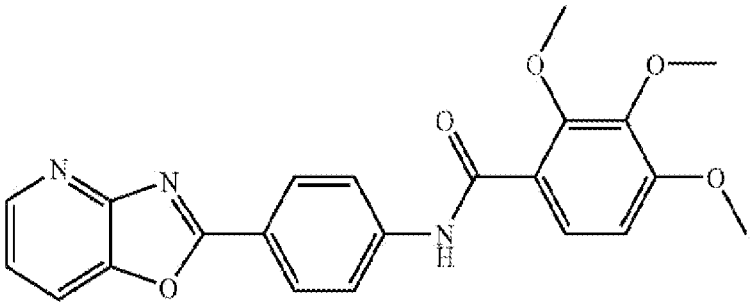

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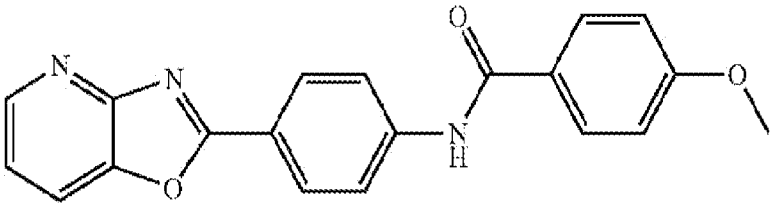

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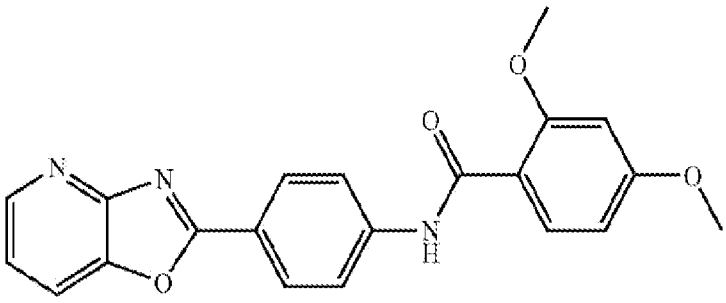

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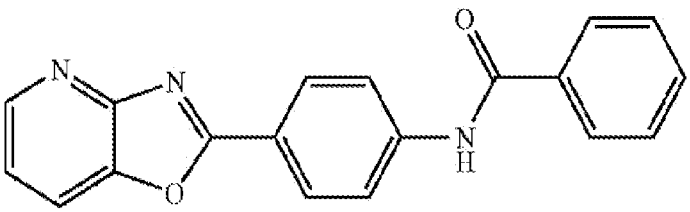

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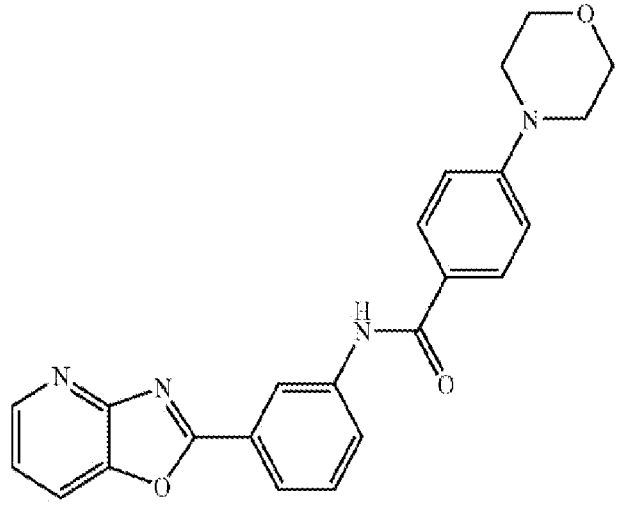
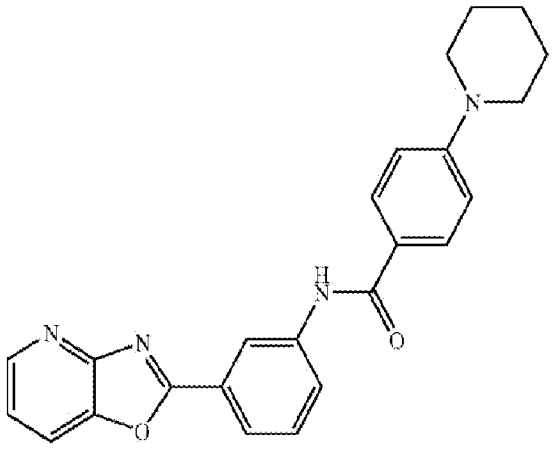
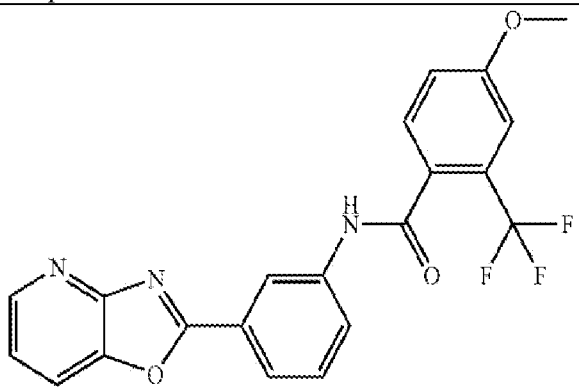
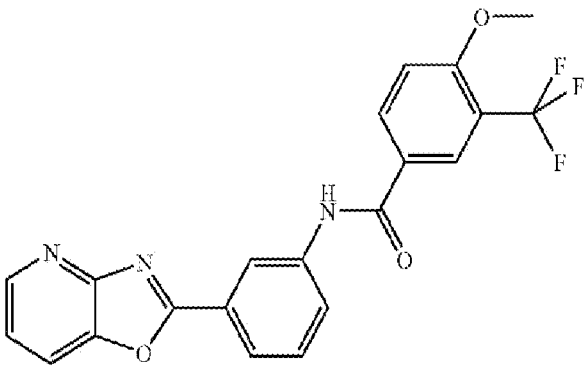
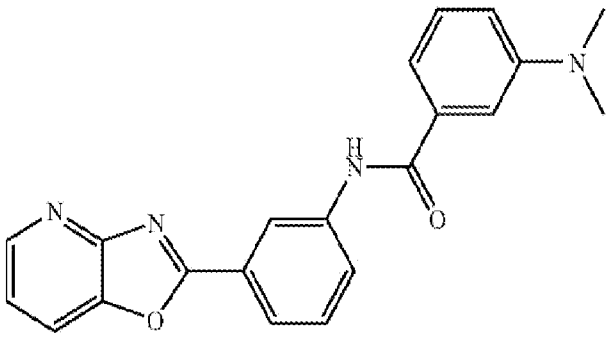
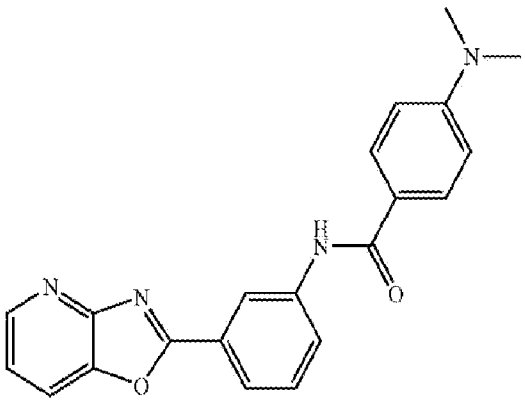
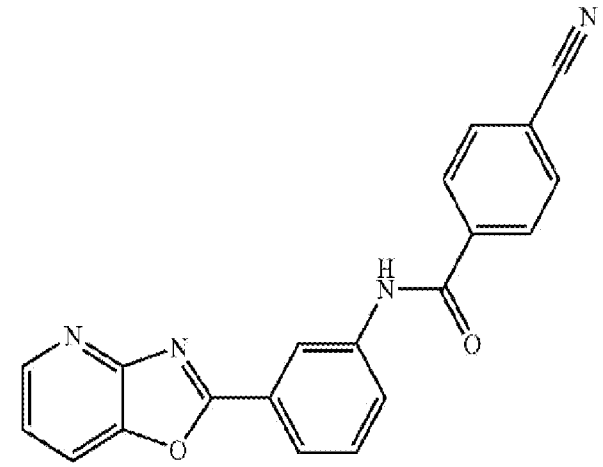
 Compound 38	 Compound 39
 Compound 40	 Compound 41
 Compound 42	 Compound 43



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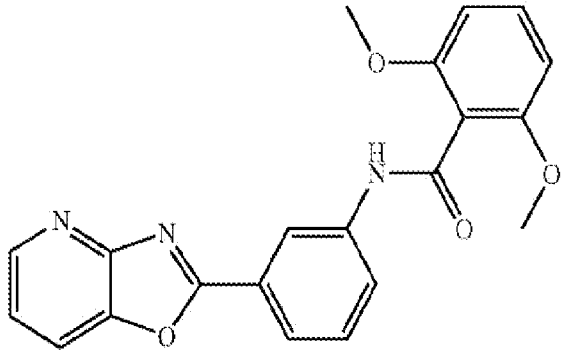

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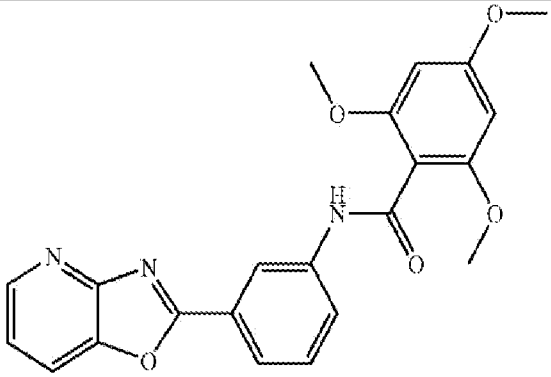

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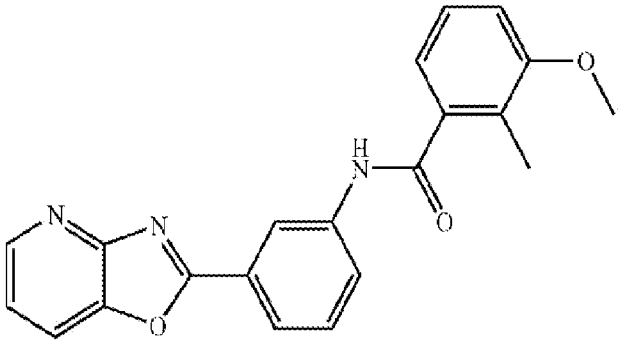

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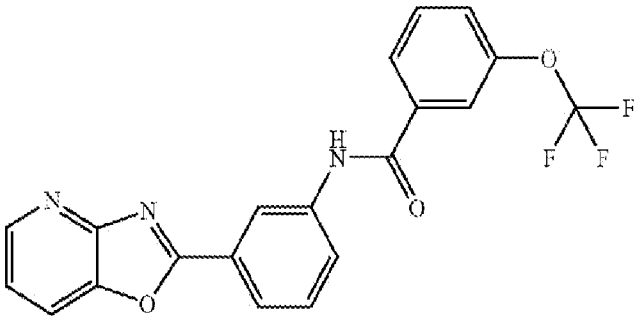
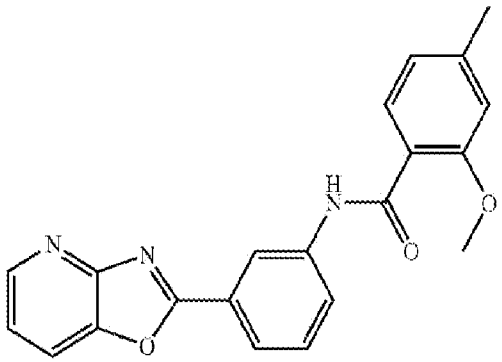
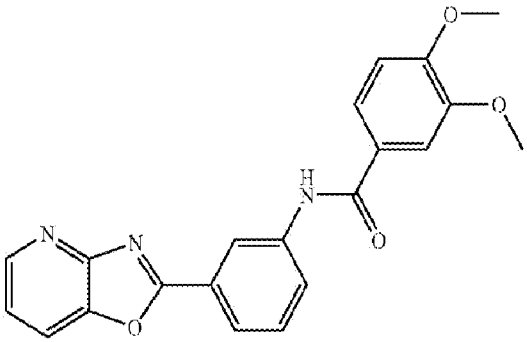


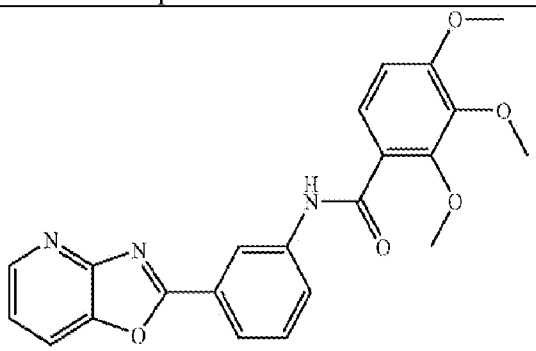
Compound 50



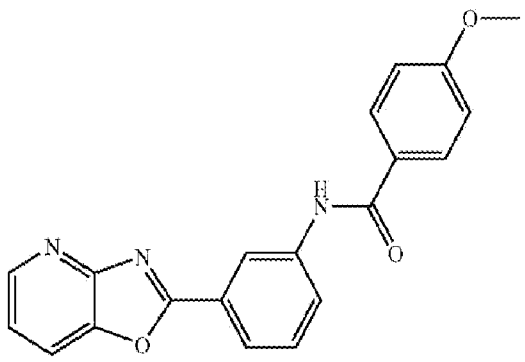
Compound 51



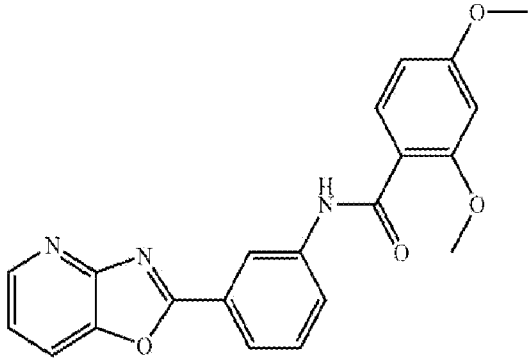
Compound 52



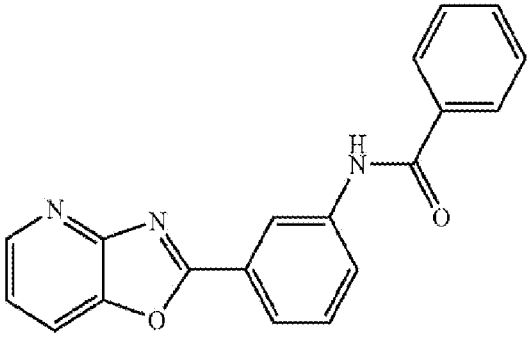
Compound 53



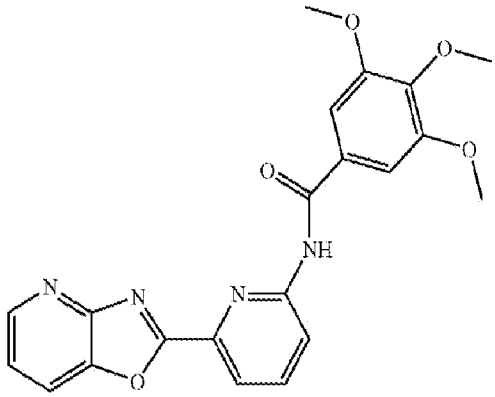
Compound 54



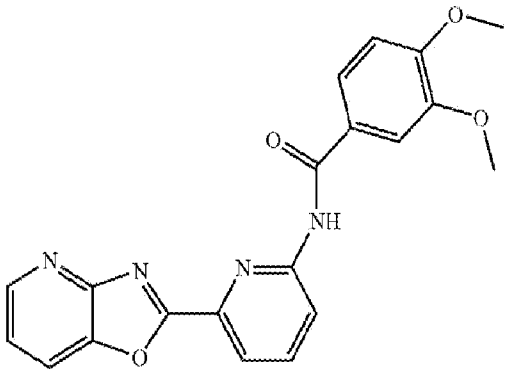
Compound 55



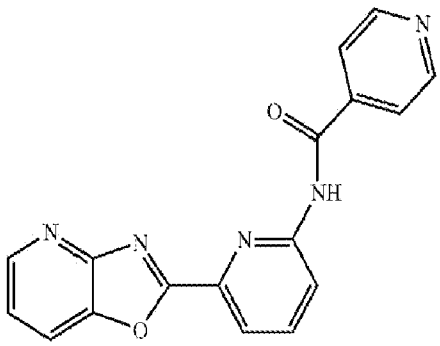
Compound 56



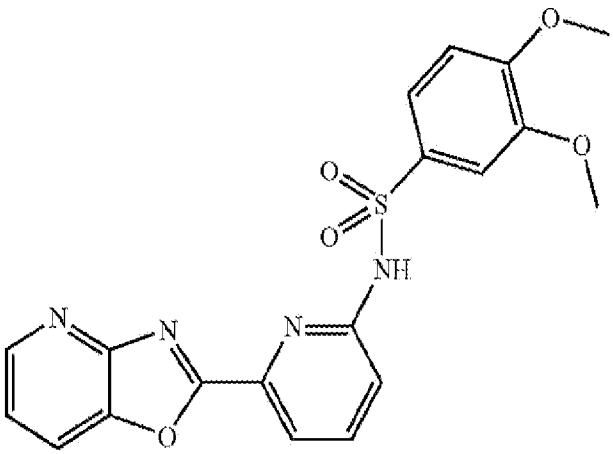
Compound 57



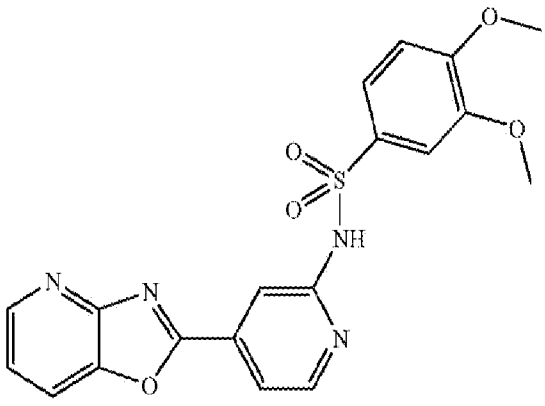
Compound 58



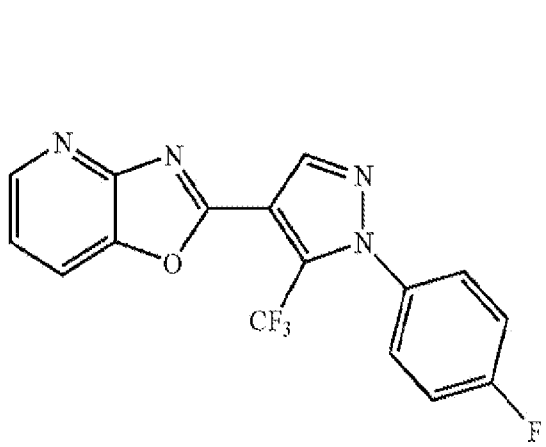
Compound 59



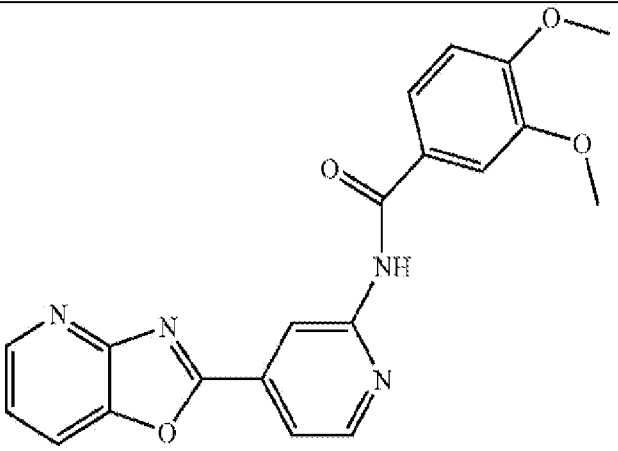
Compound 60



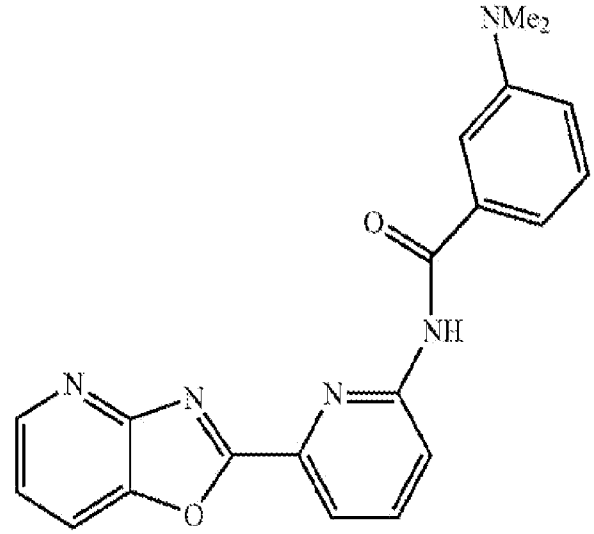
Compound 61



Compound 62



Compound 63



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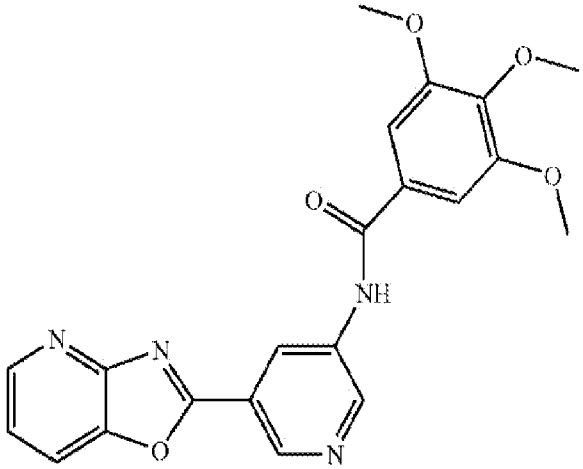

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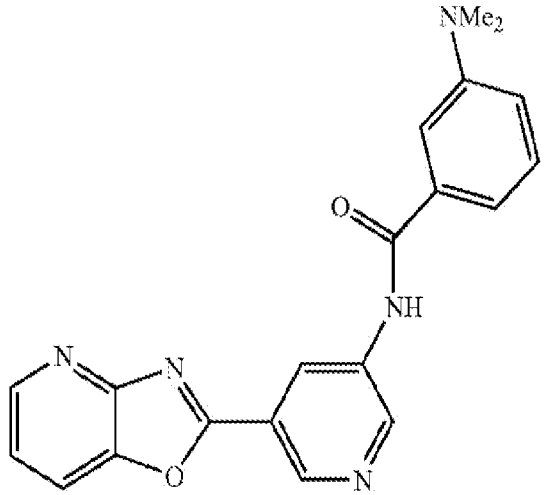
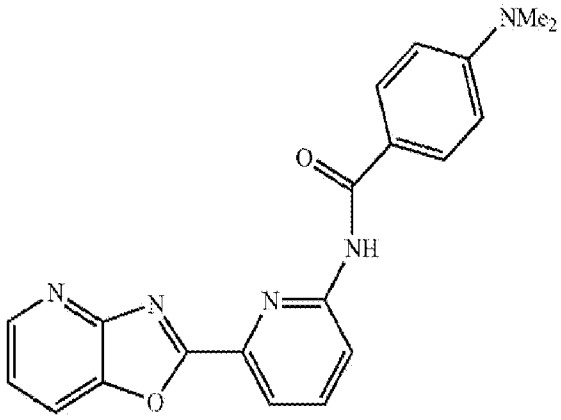
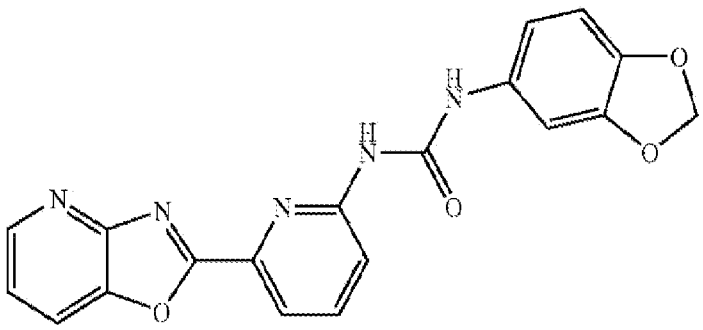


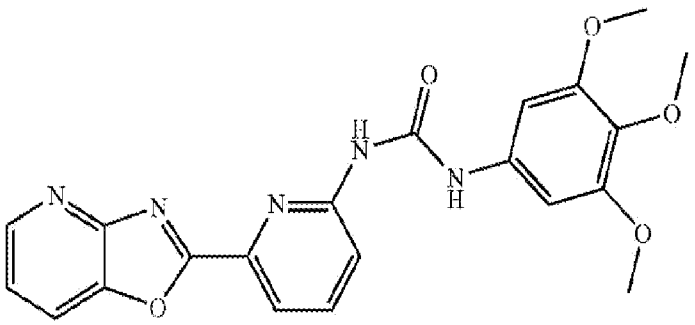
Compound 68



Compound 70



Compound 71



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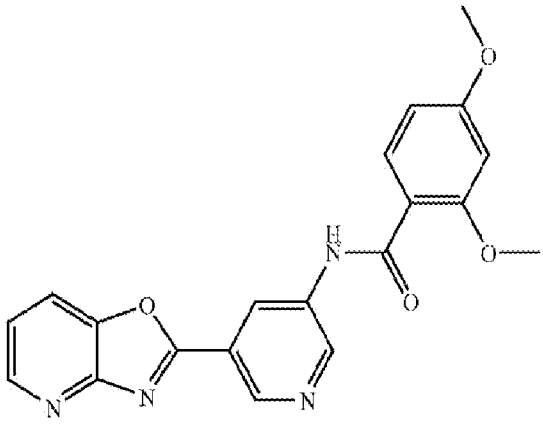

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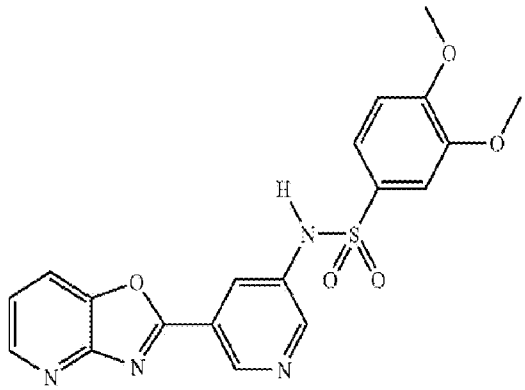

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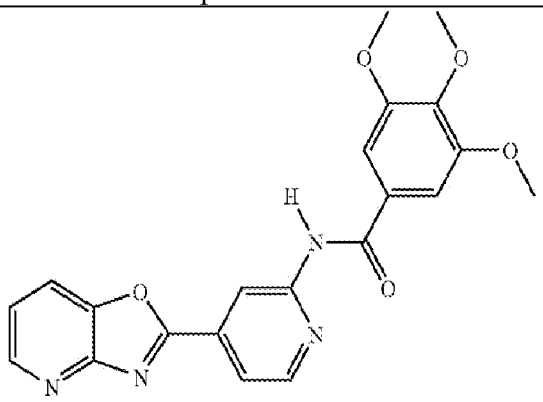

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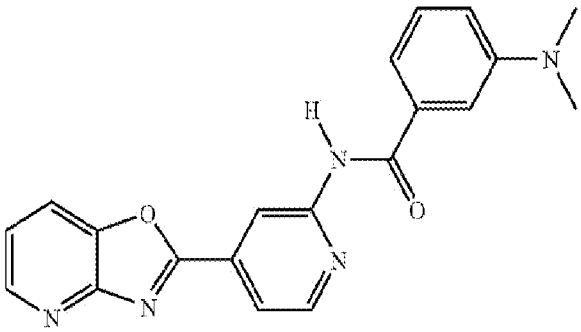

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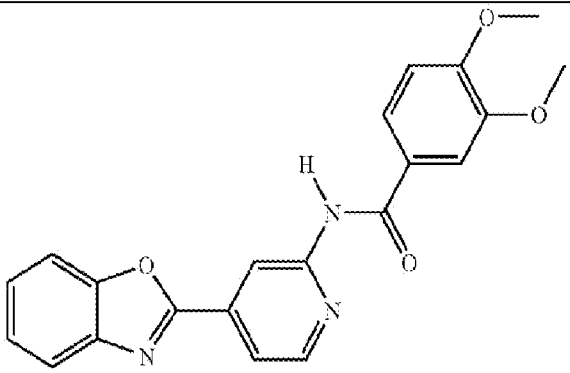

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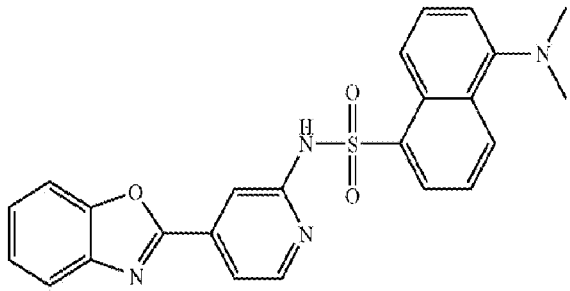

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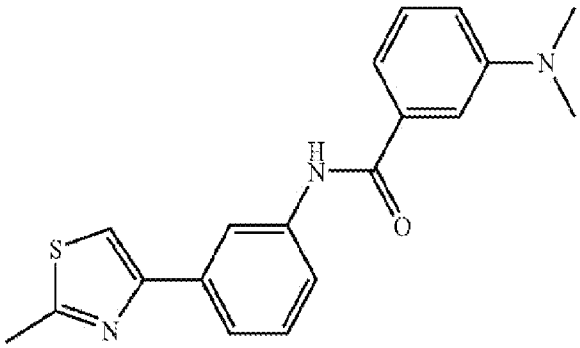

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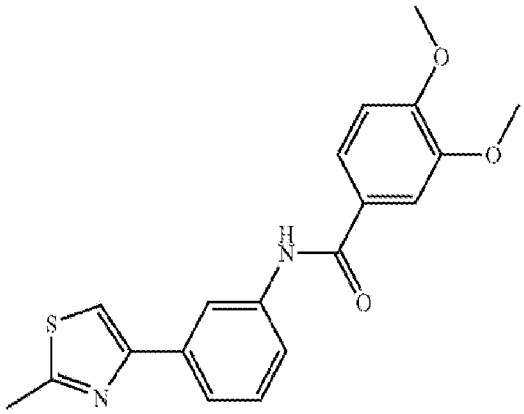

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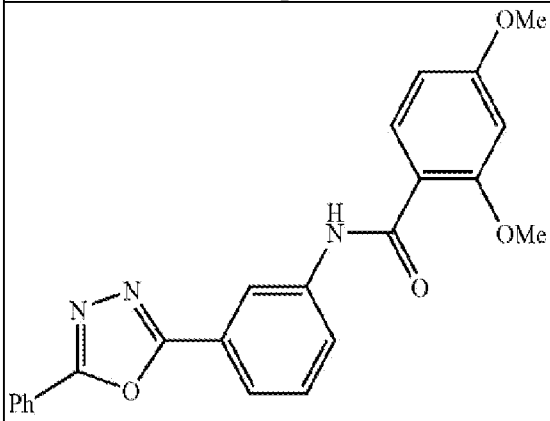

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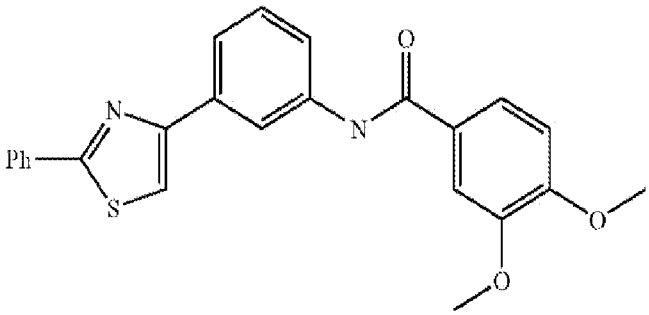

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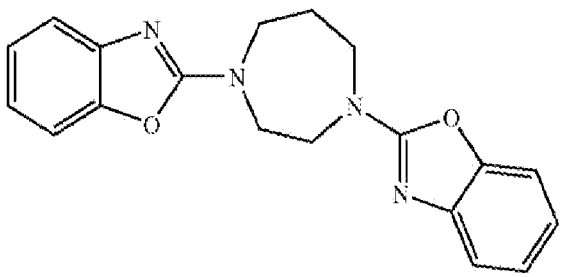

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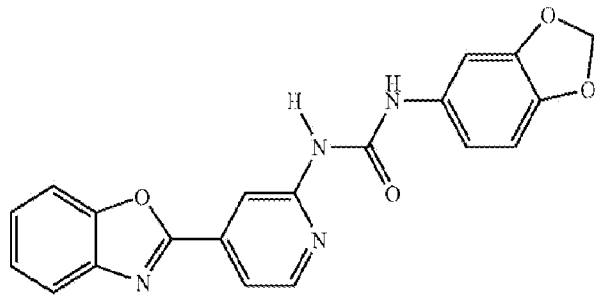

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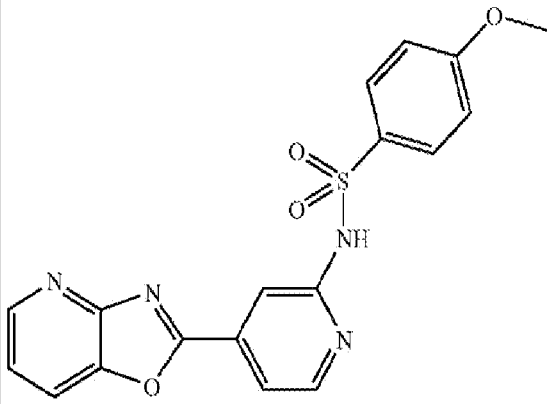

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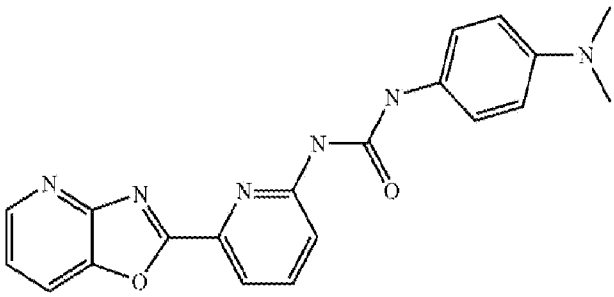

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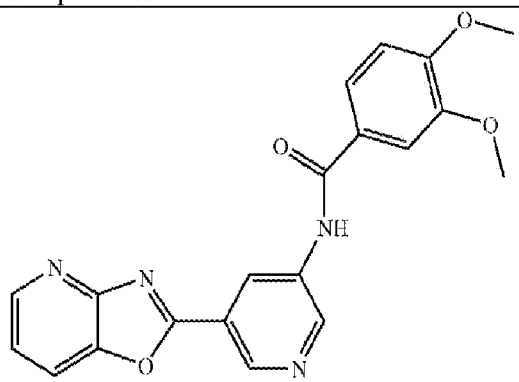

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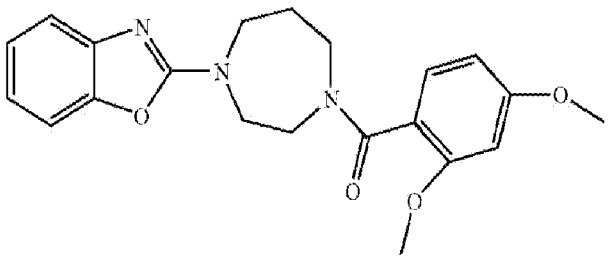

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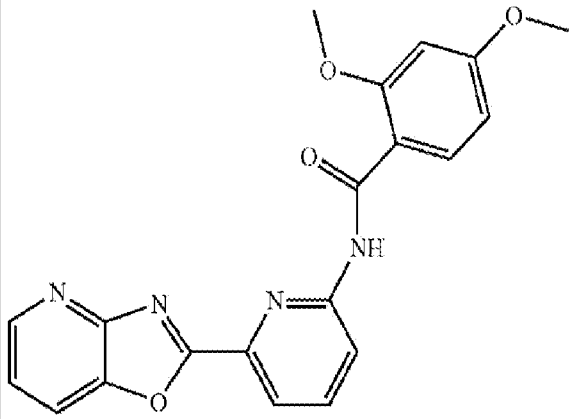

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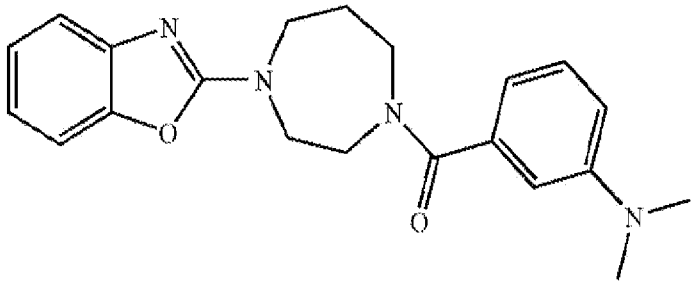

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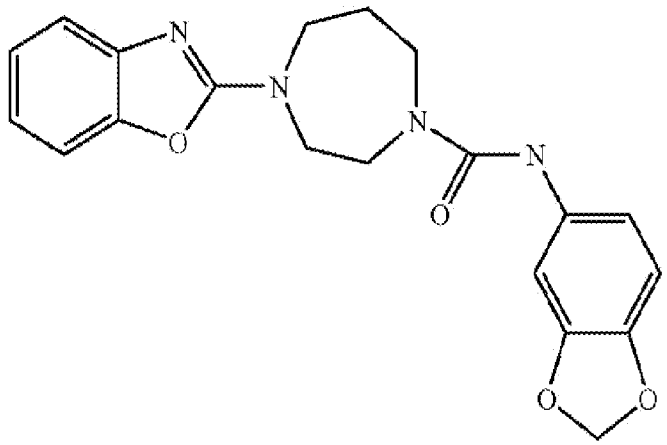

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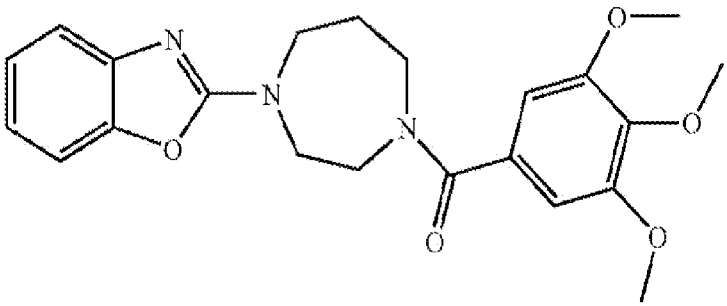

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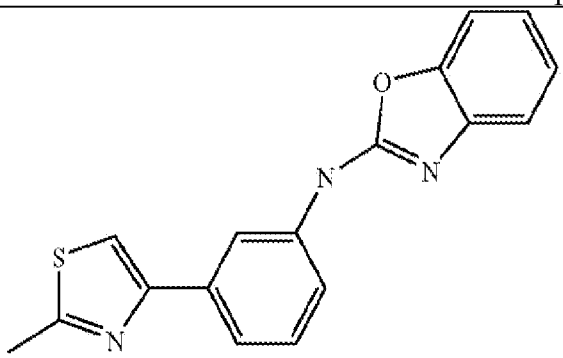

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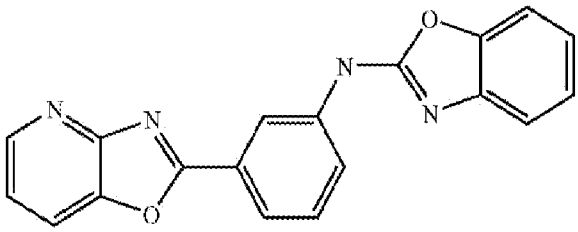

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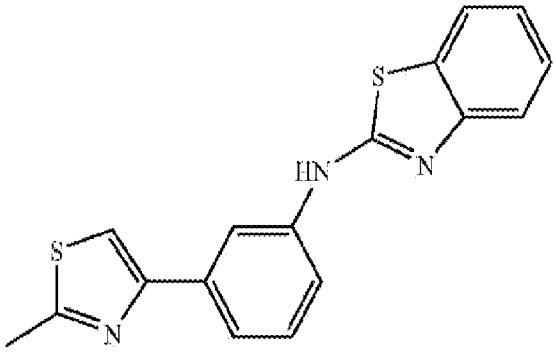

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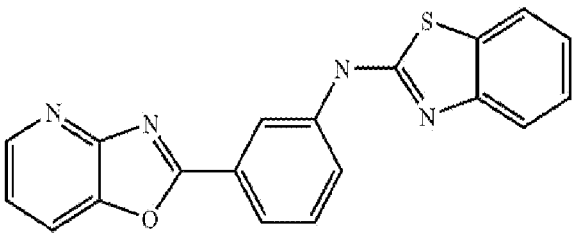

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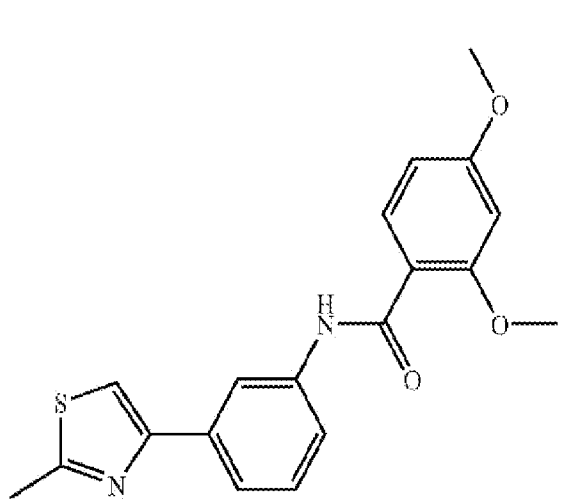

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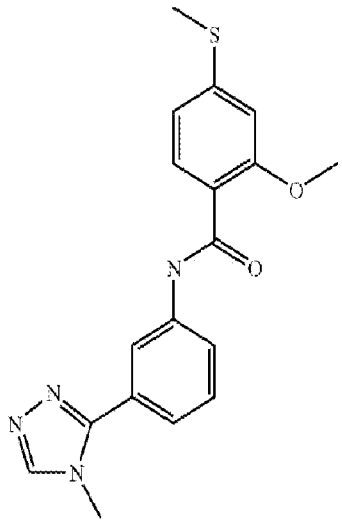

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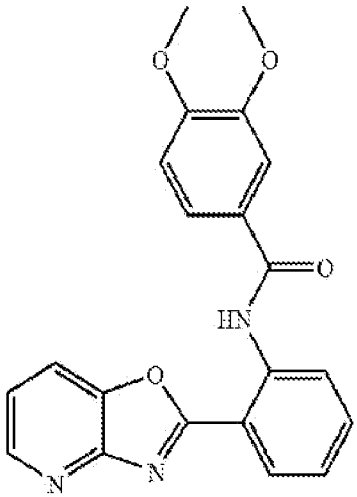

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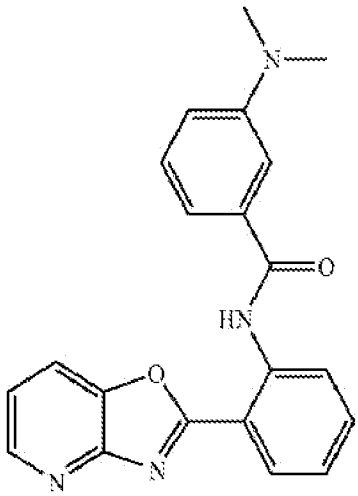

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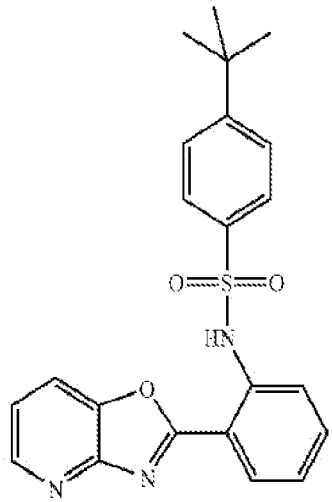

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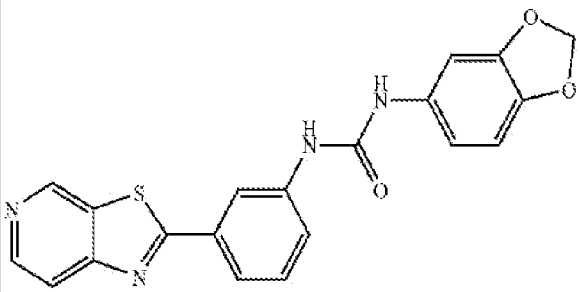

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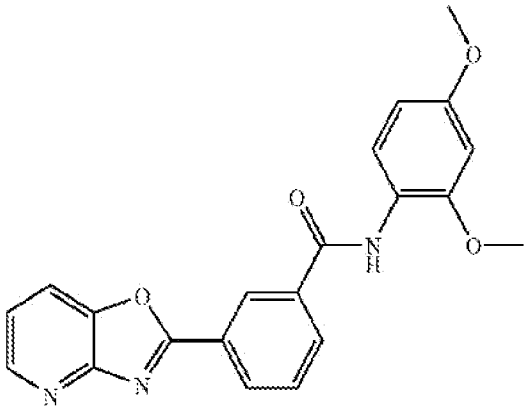

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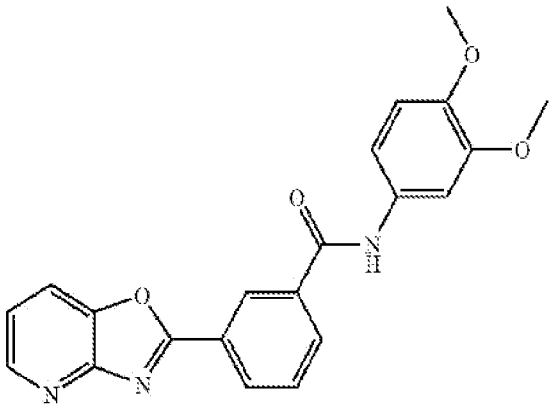

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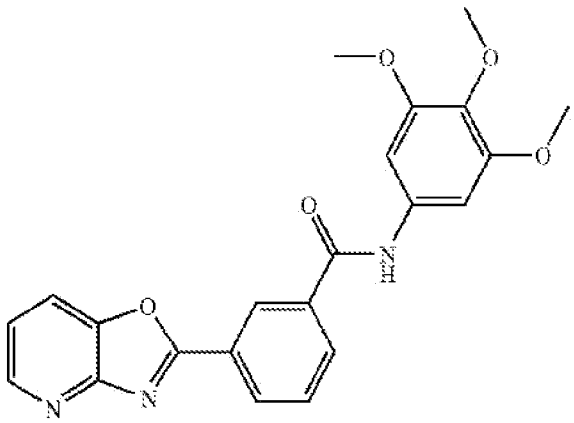

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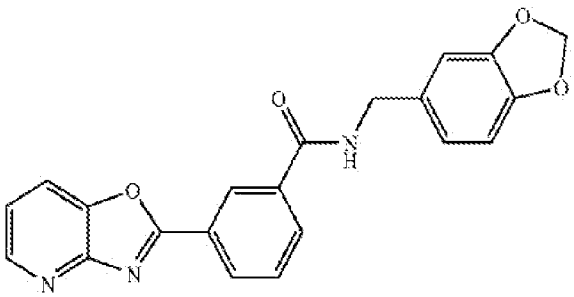

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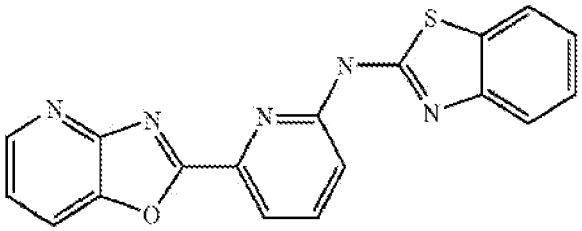

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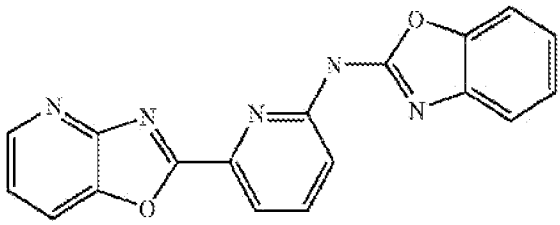

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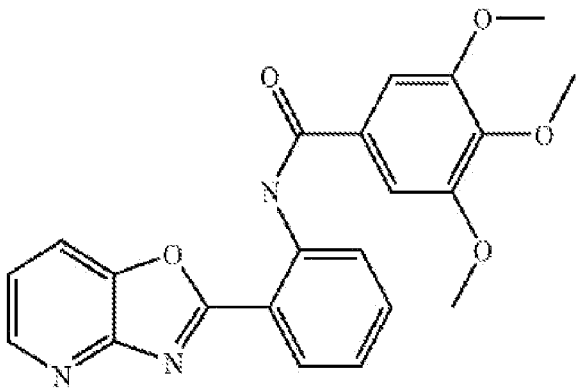

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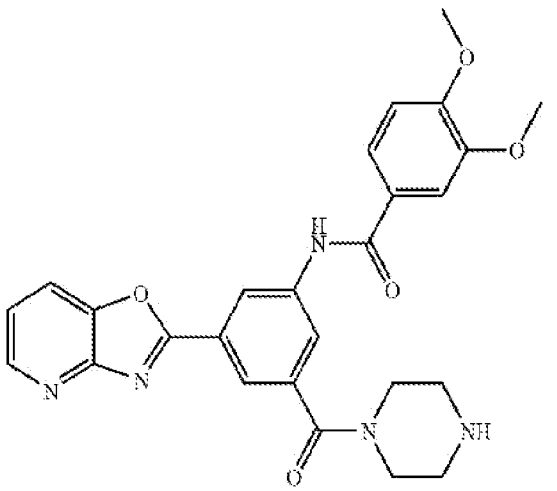

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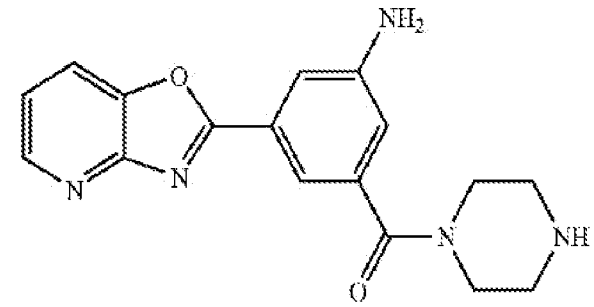

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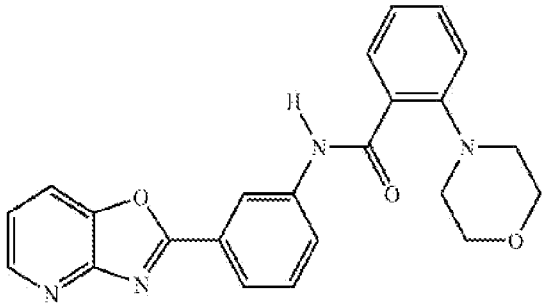

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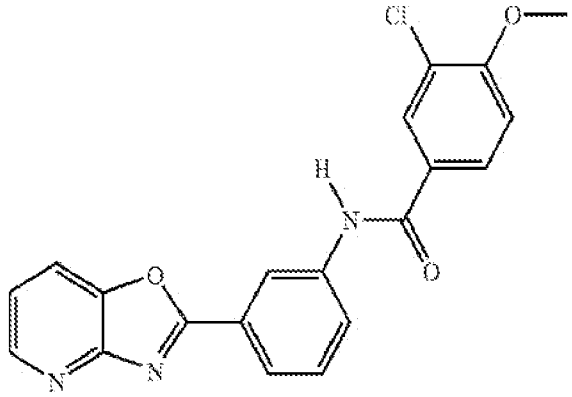

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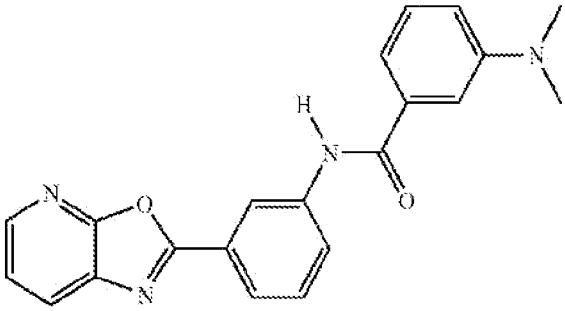

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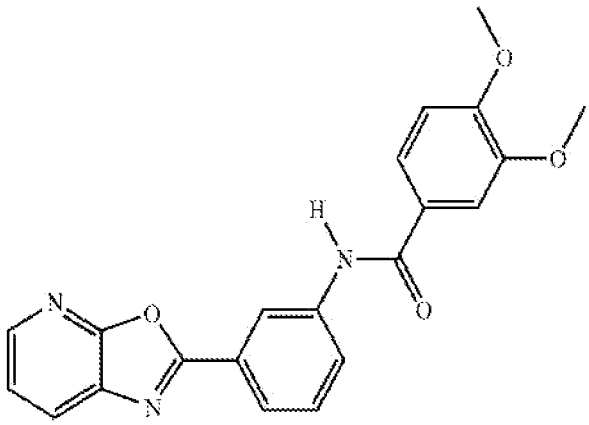

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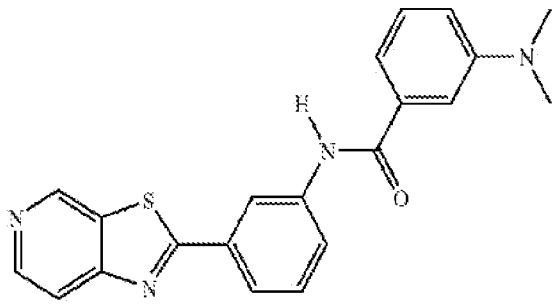

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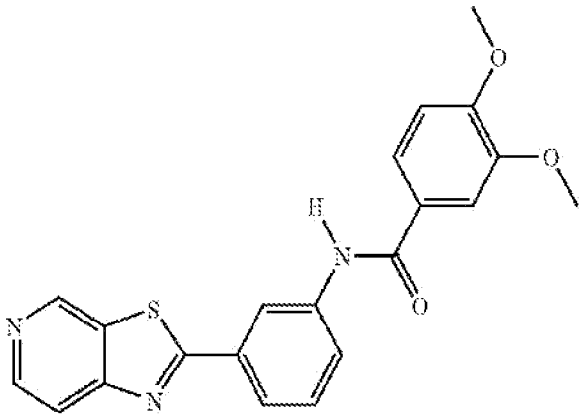

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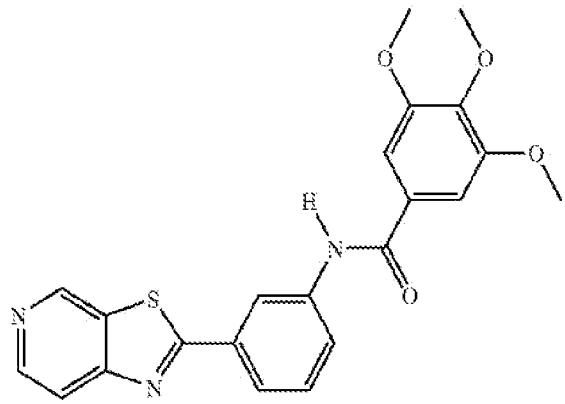

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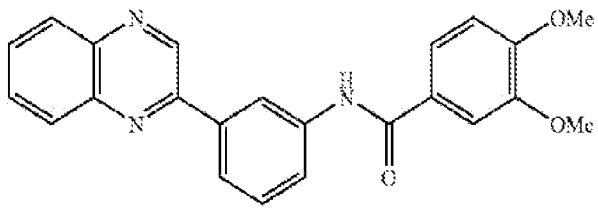

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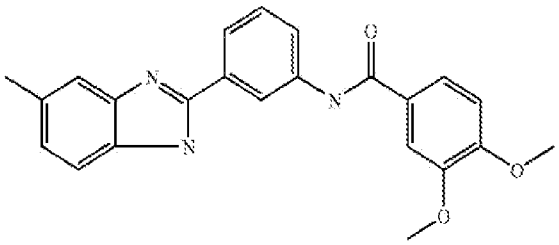

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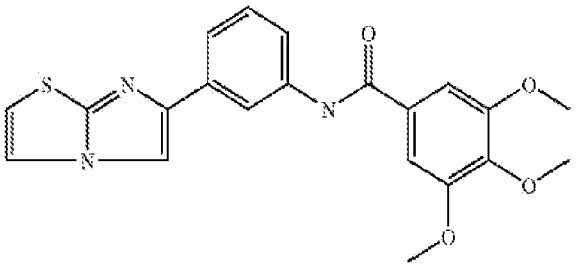

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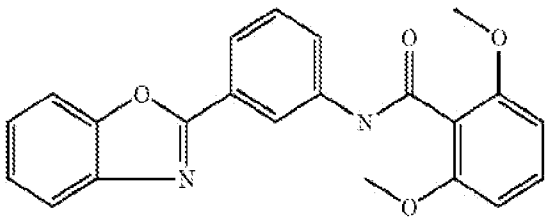

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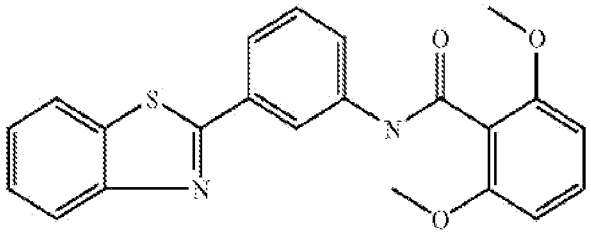

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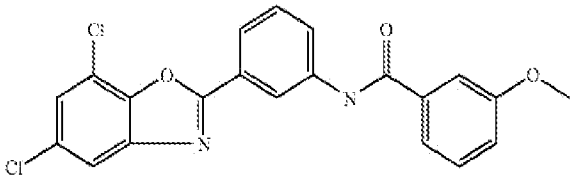

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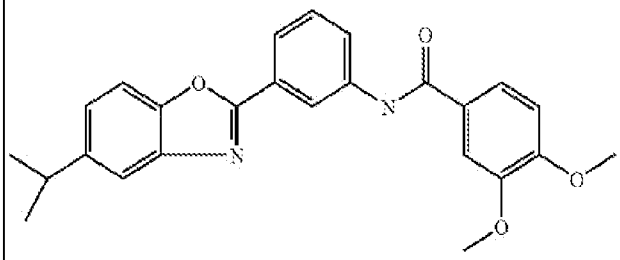

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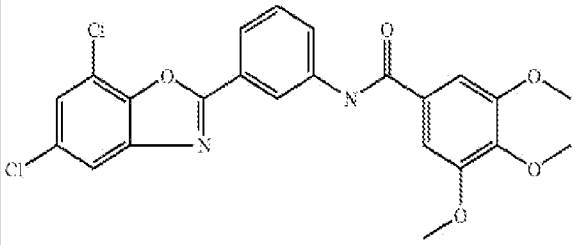

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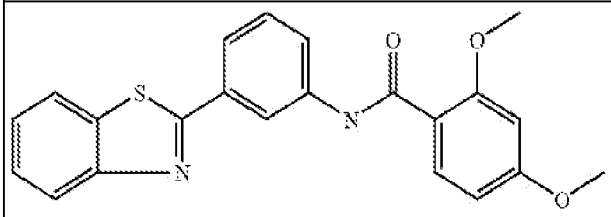

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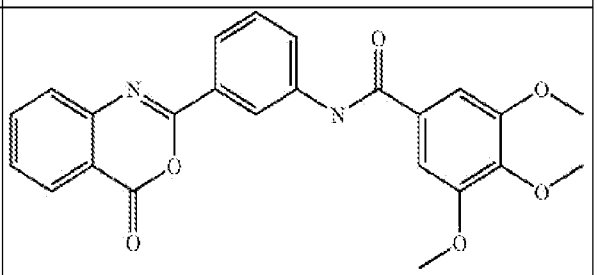

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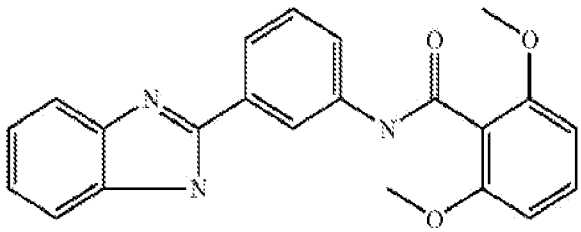

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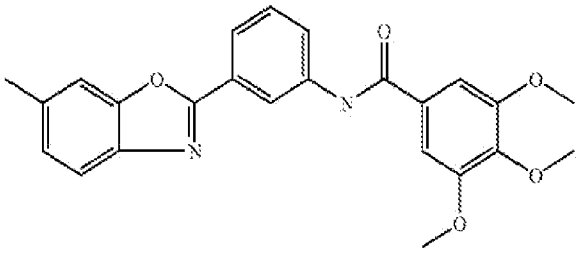

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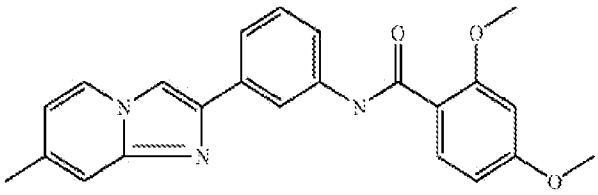

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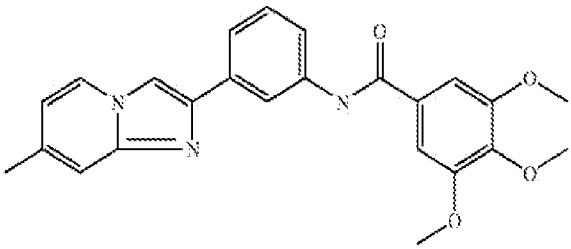

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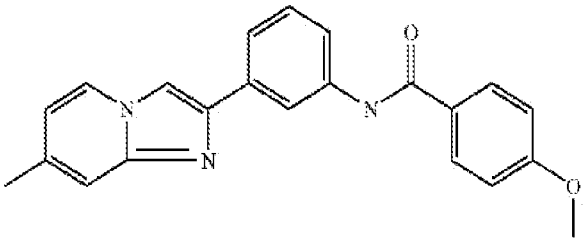

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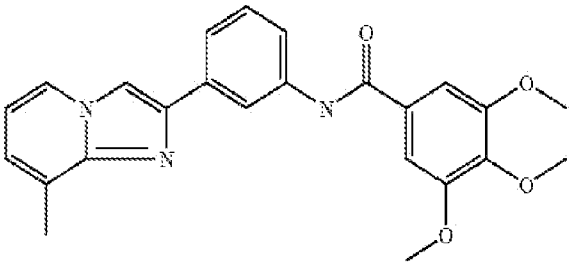

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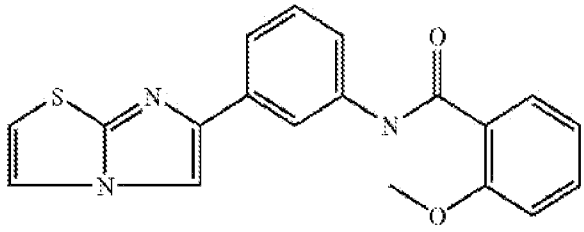

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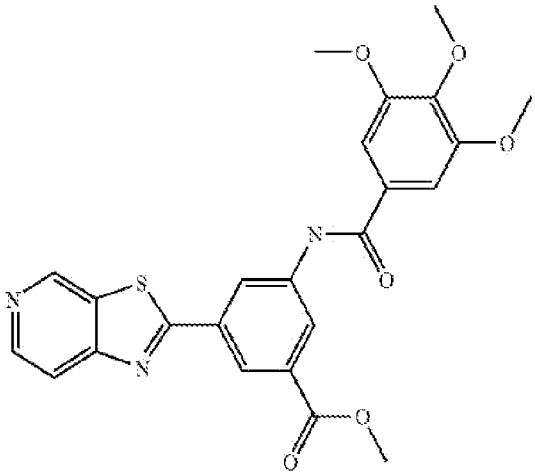

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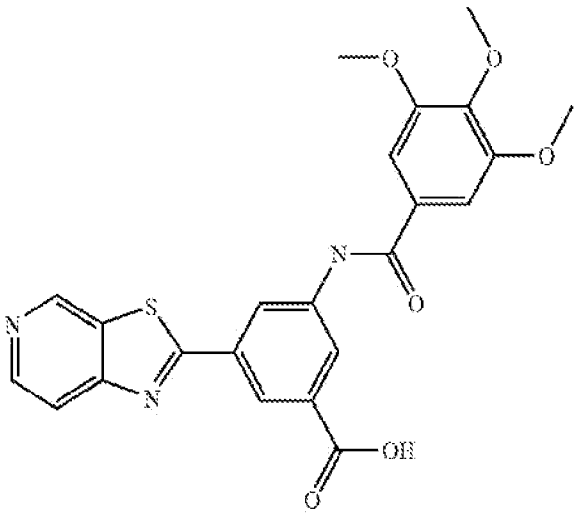

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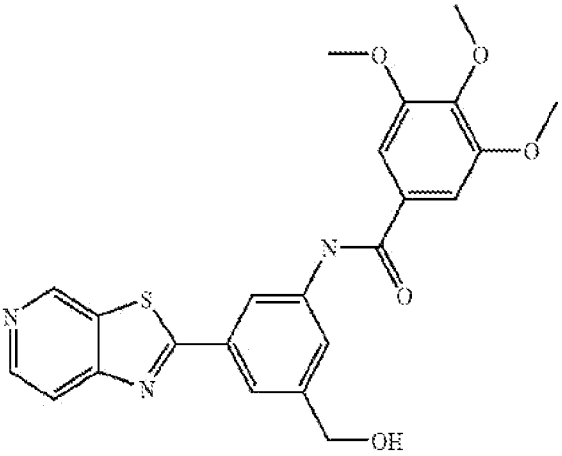

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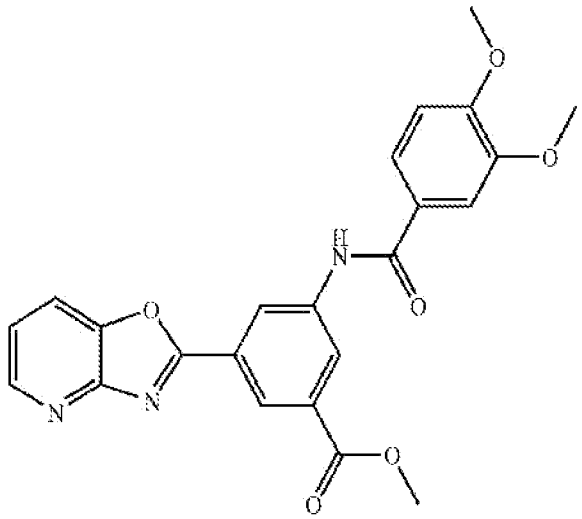

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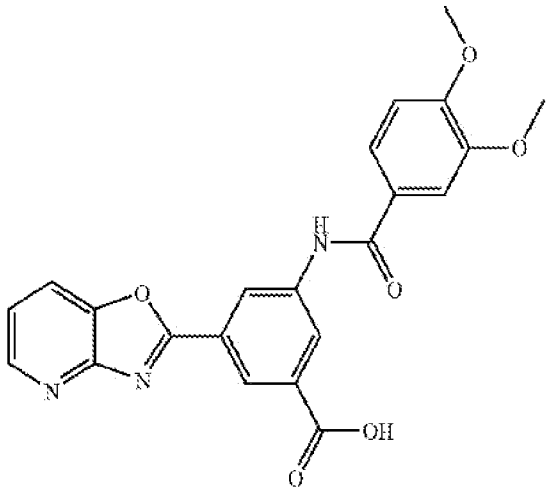

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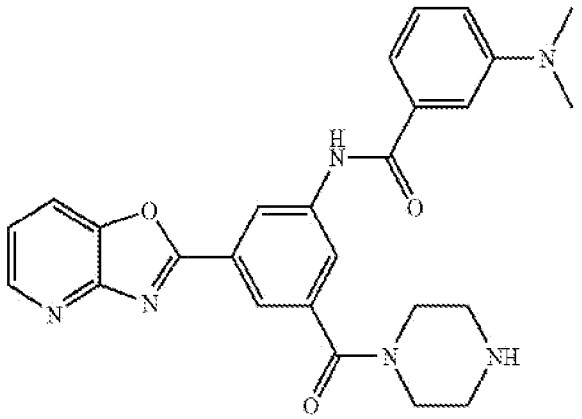

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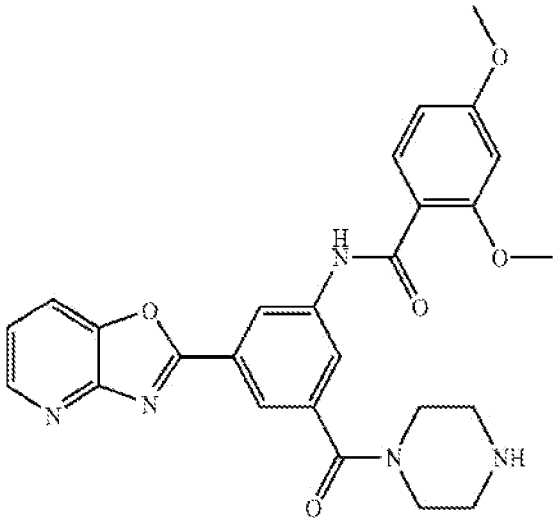

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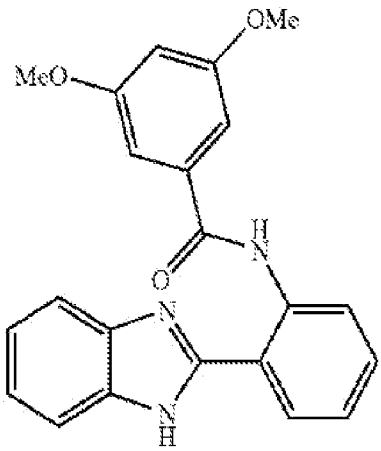

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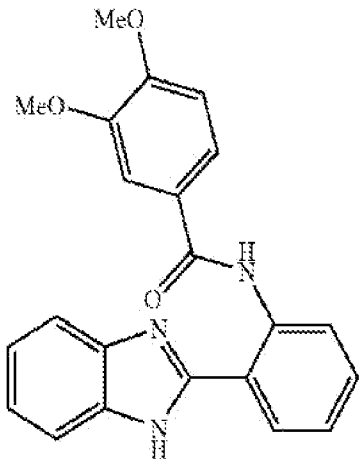

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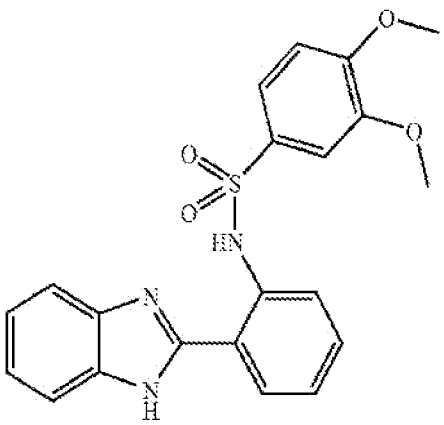
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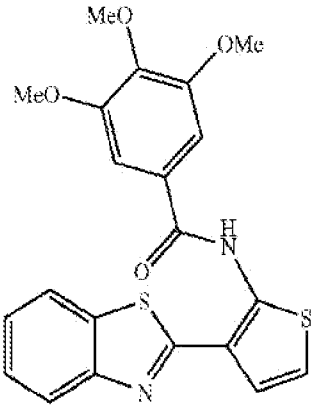
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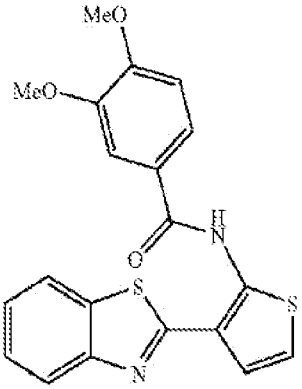
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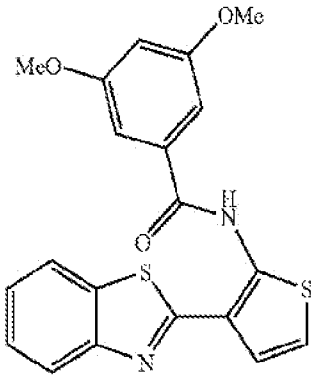
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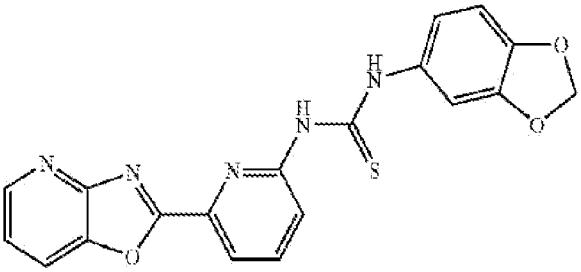
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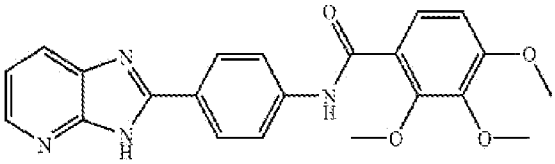
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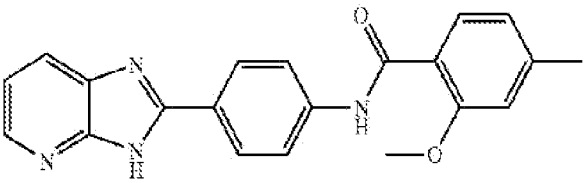
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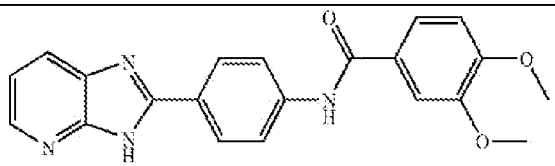
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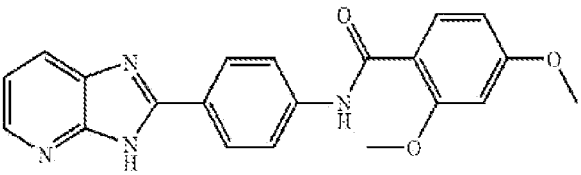
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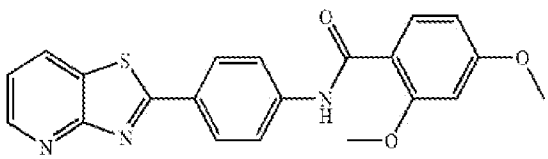
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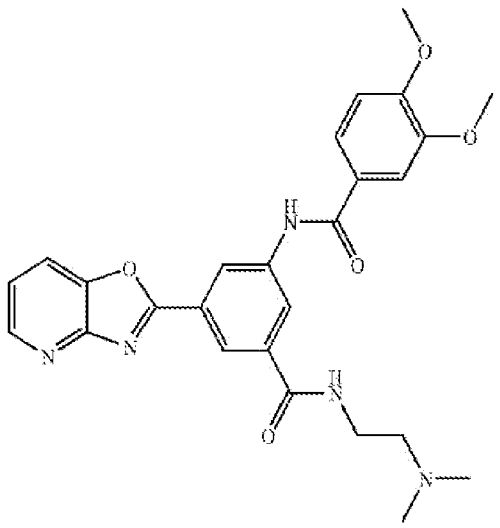

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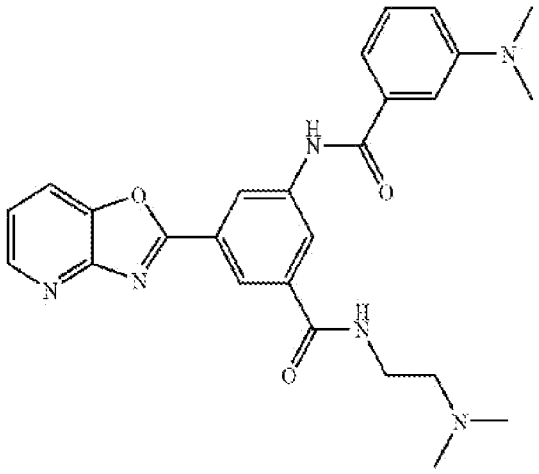

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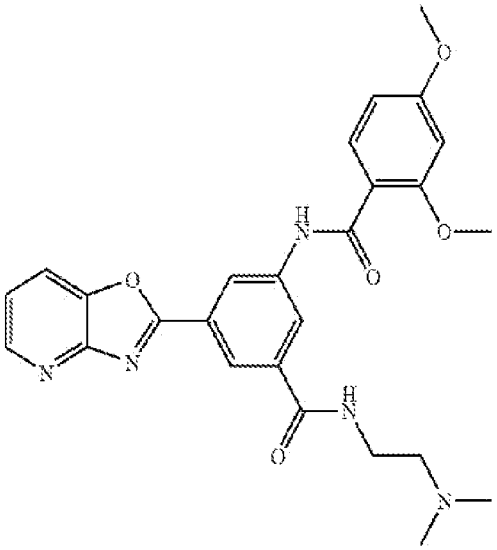

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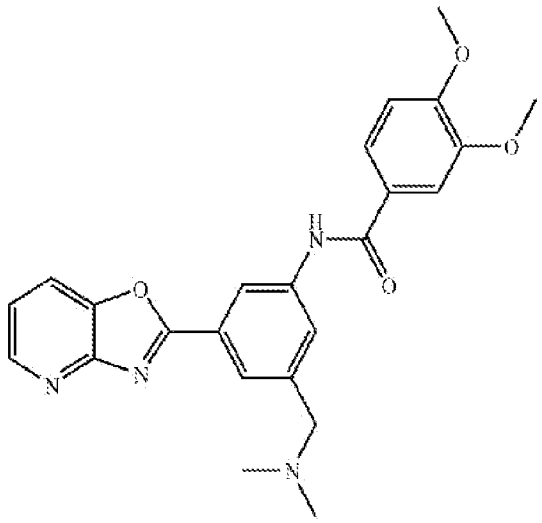

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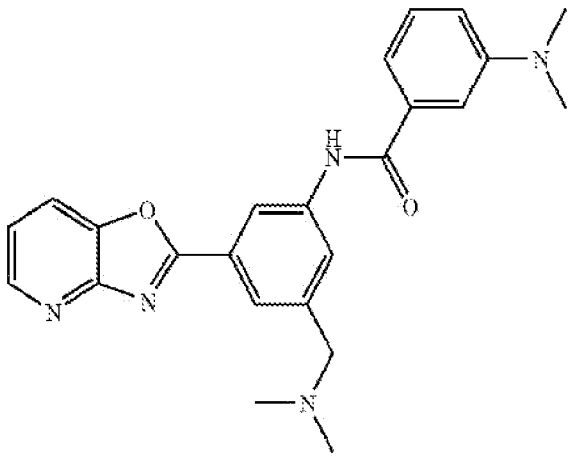

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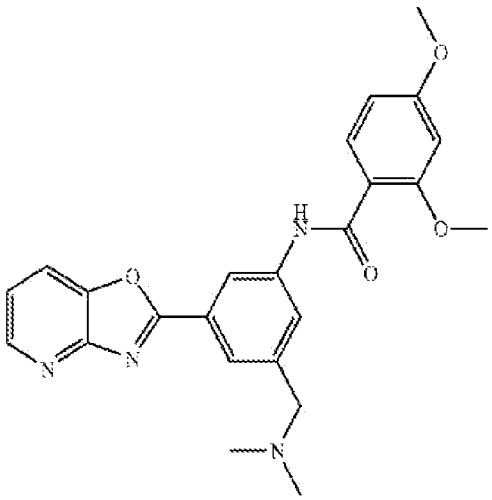

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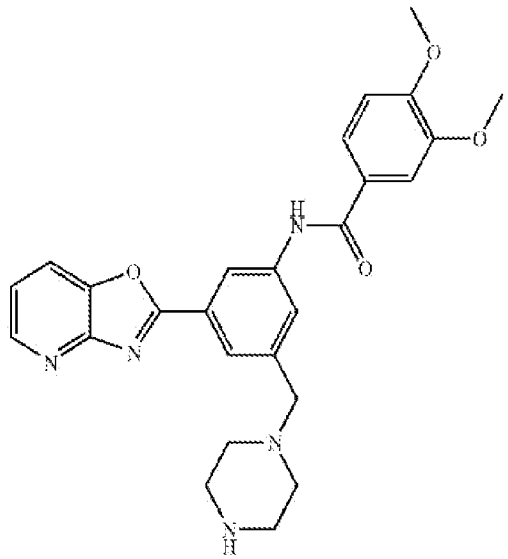

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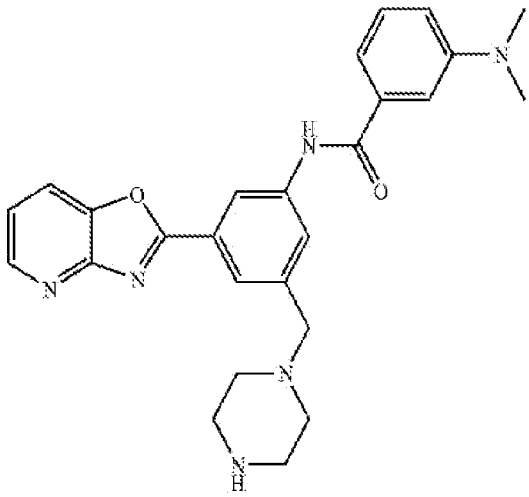

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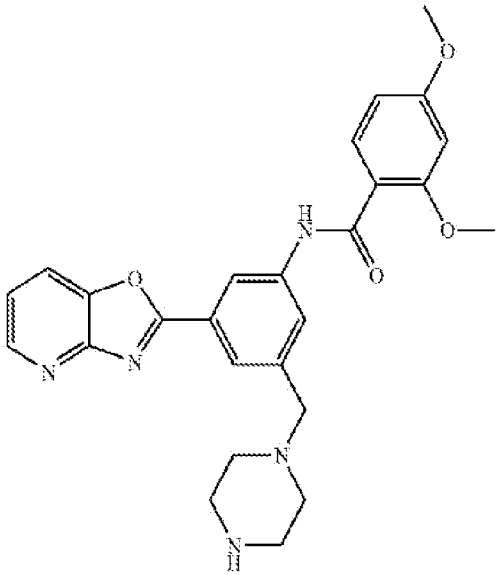

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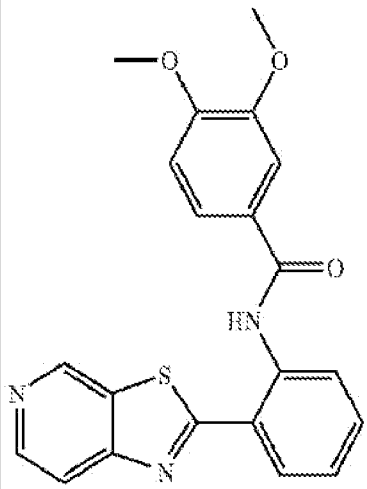

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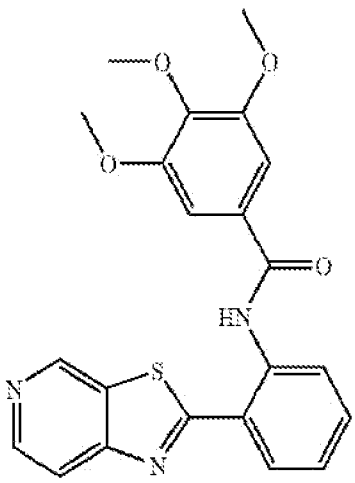

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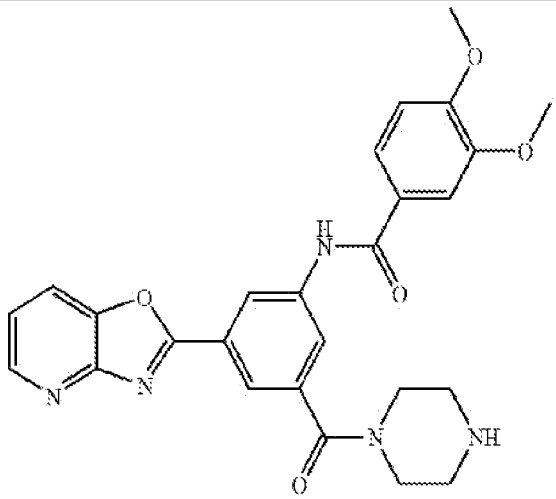

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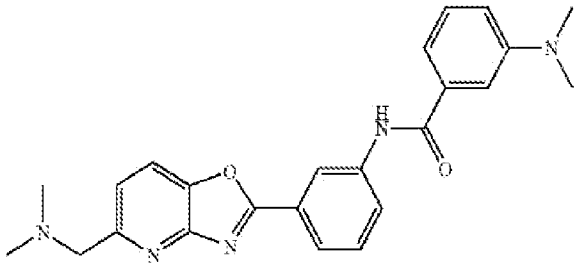

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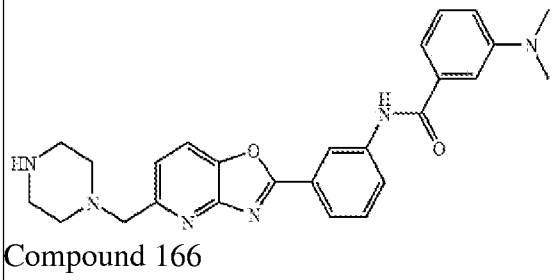

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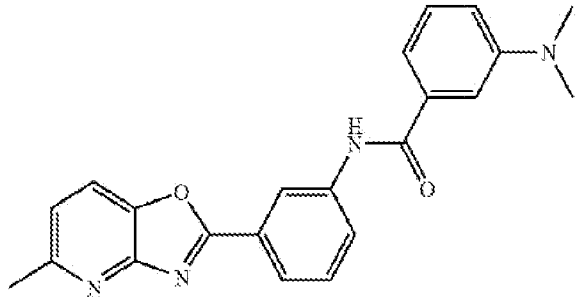

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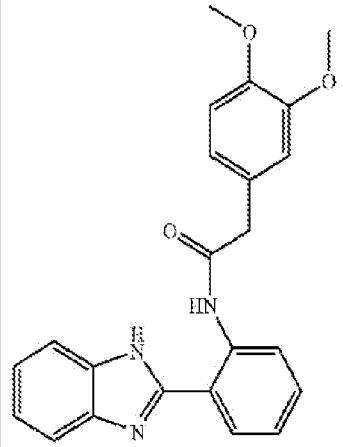

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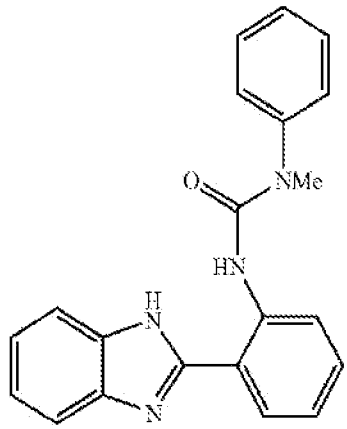

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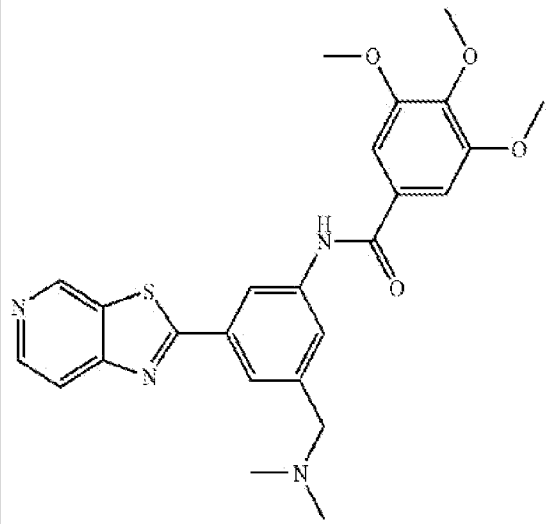

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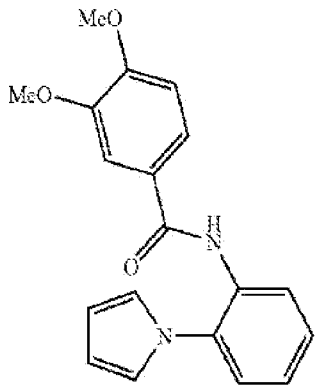

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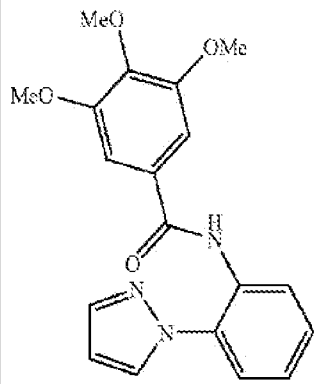

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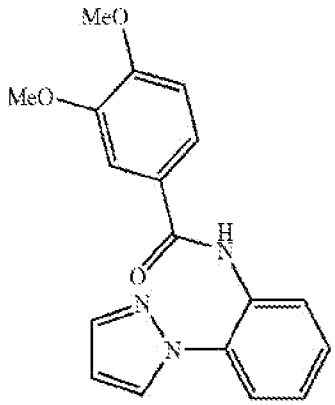

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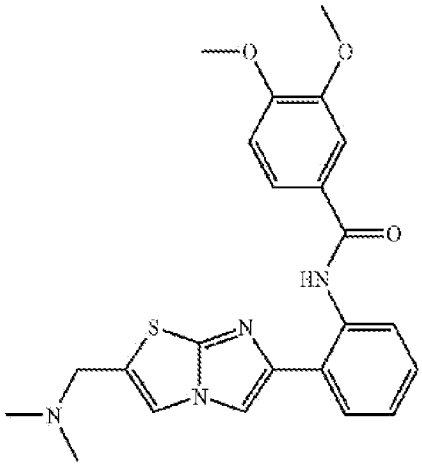

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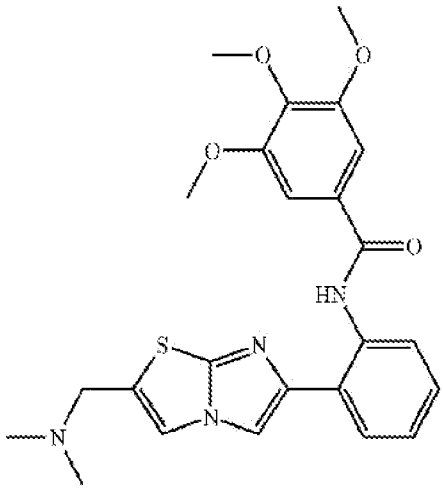

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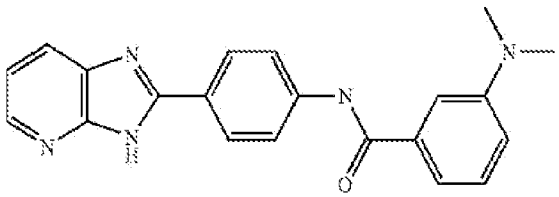

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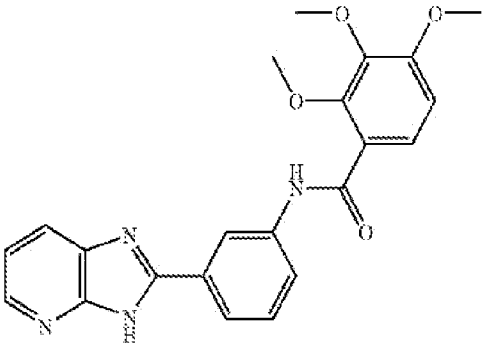

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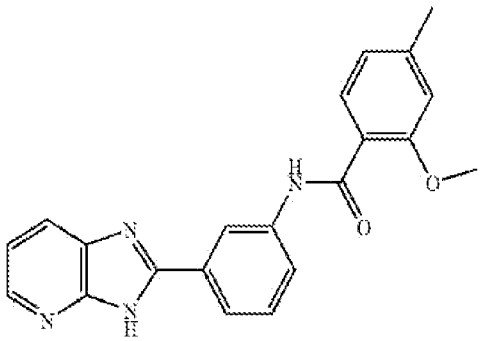

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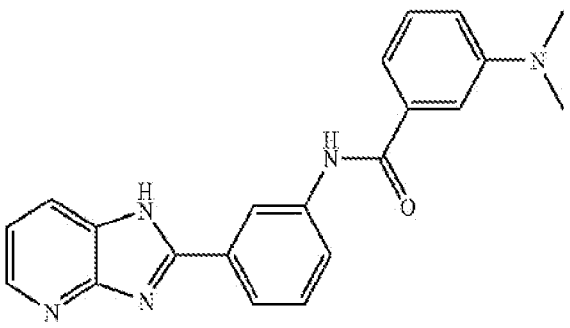

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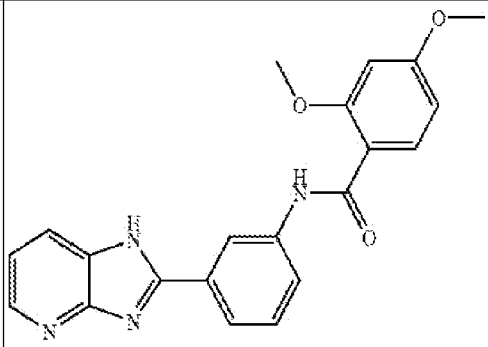

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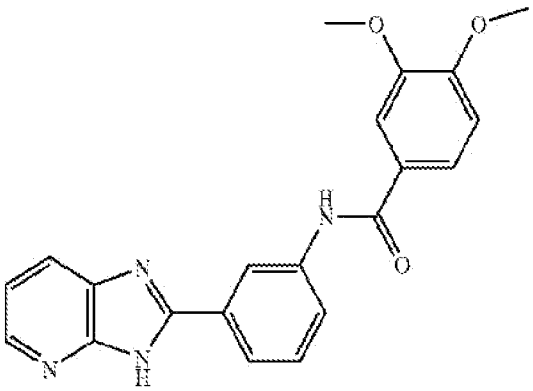

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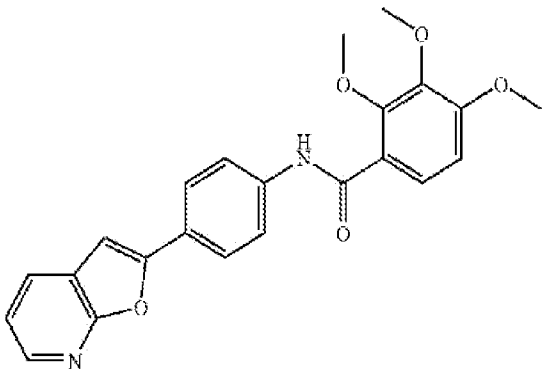

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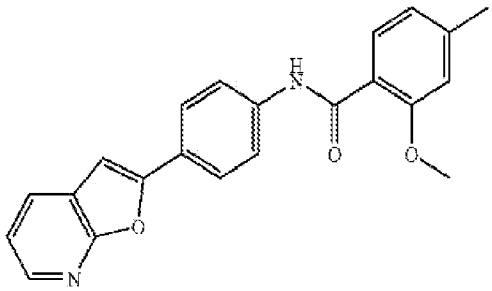

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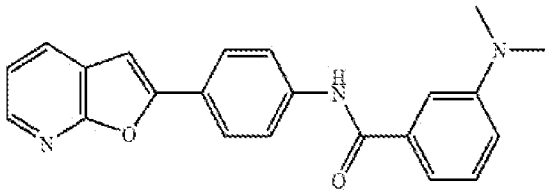

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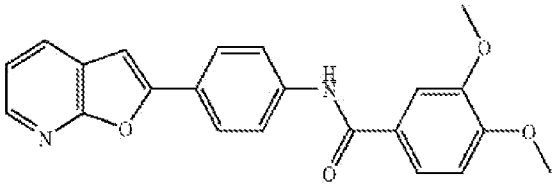

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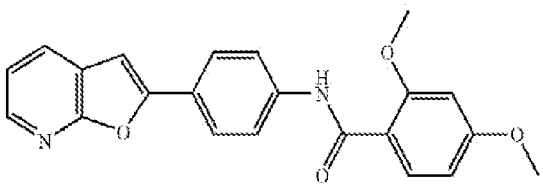

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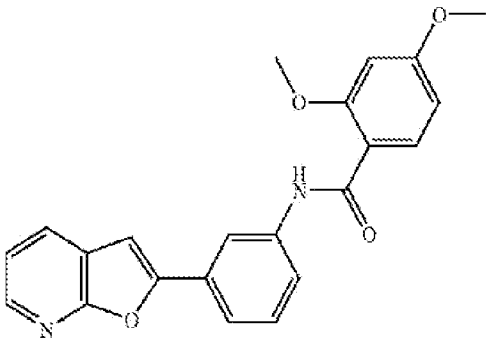

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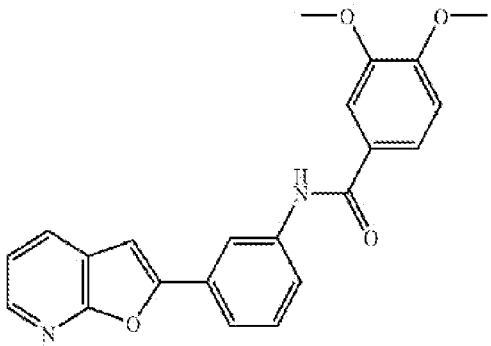

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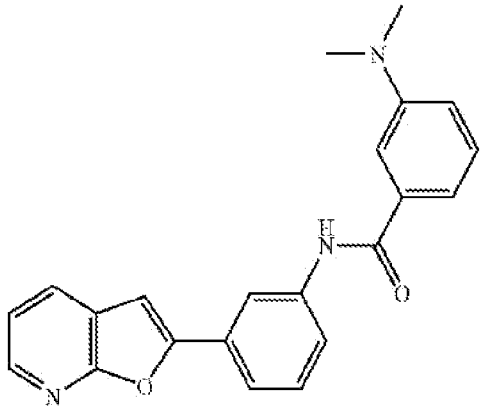

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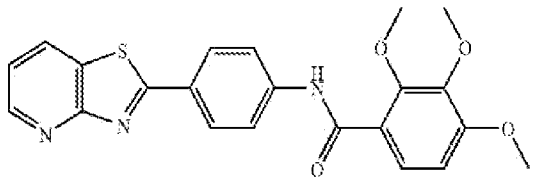

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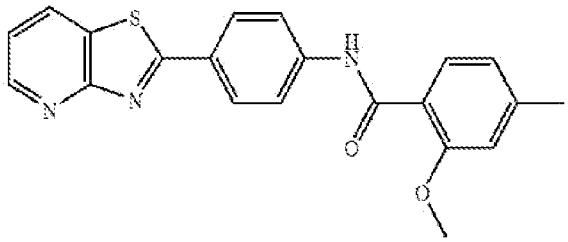

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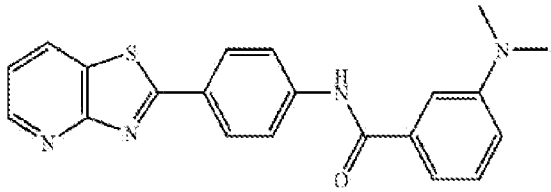

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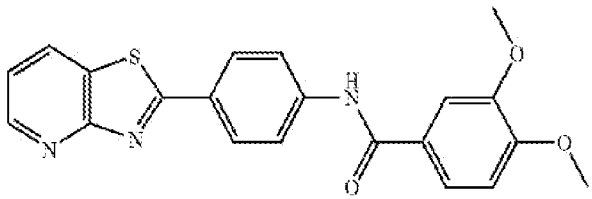

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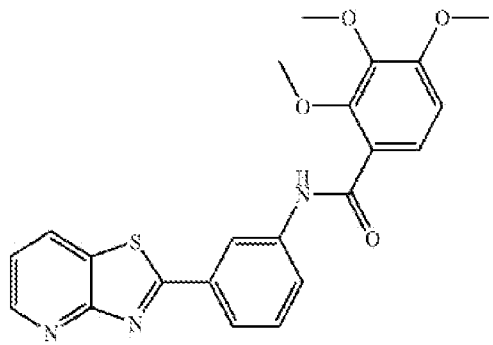

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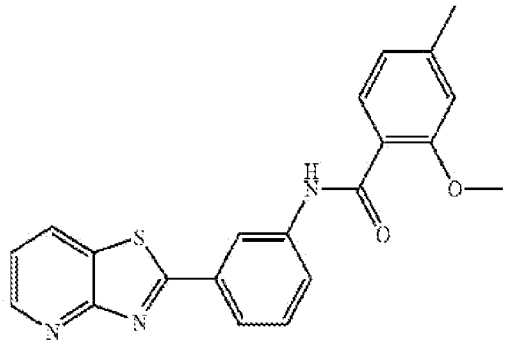

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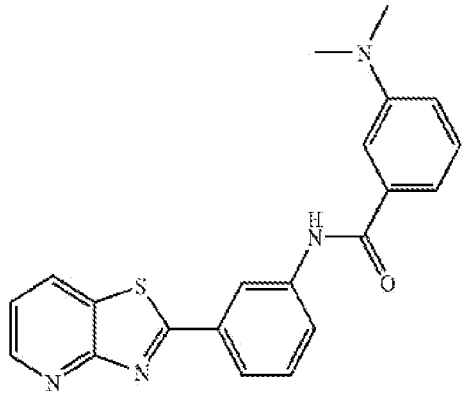

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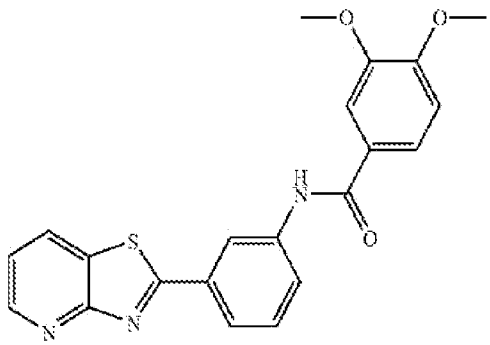

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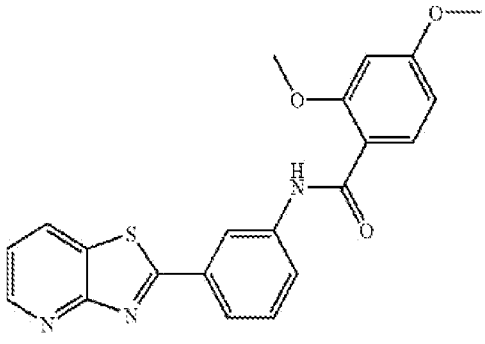

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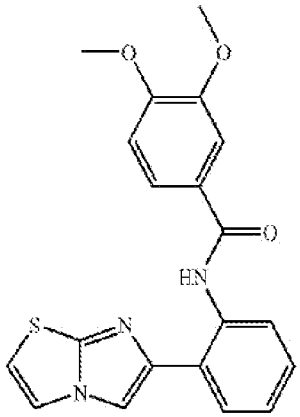

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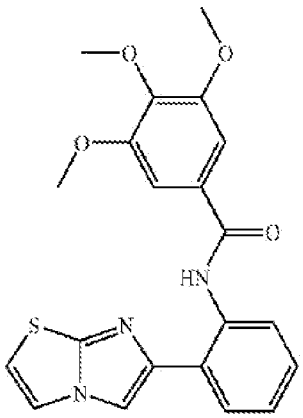

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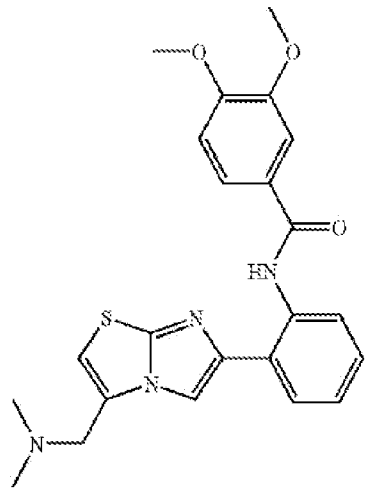

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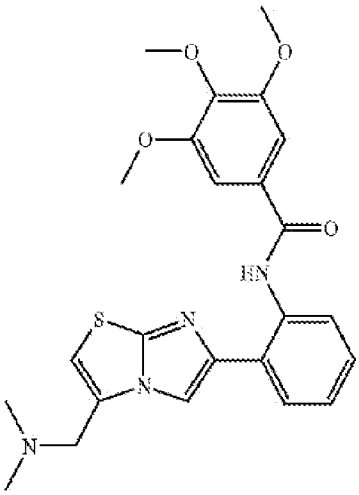

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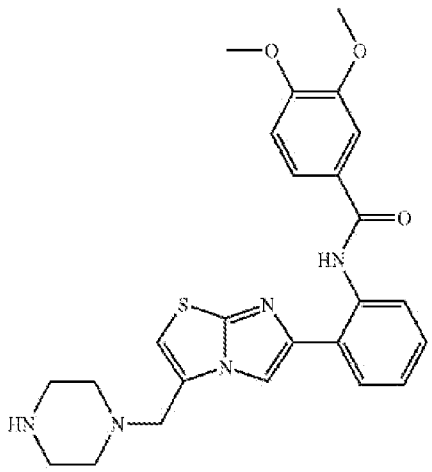

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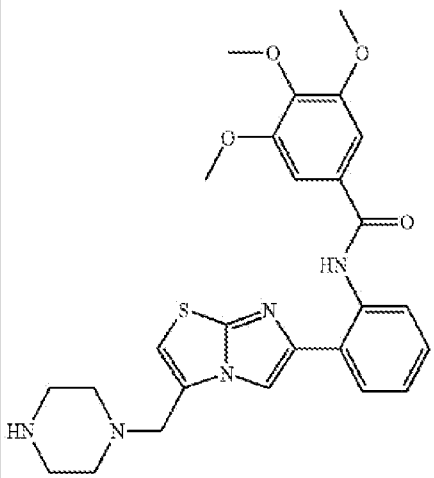

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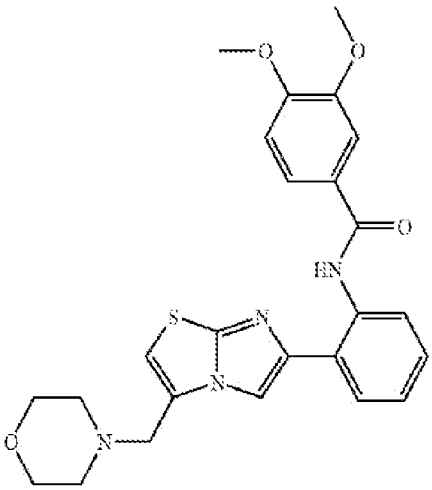

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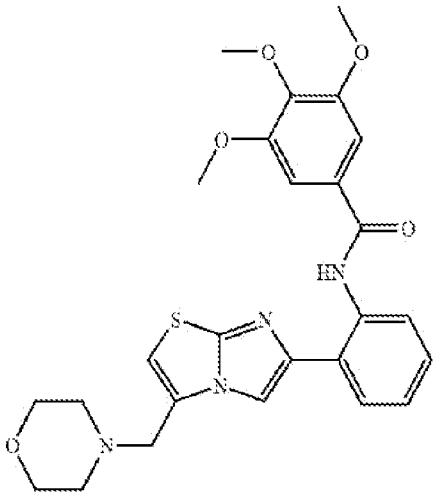

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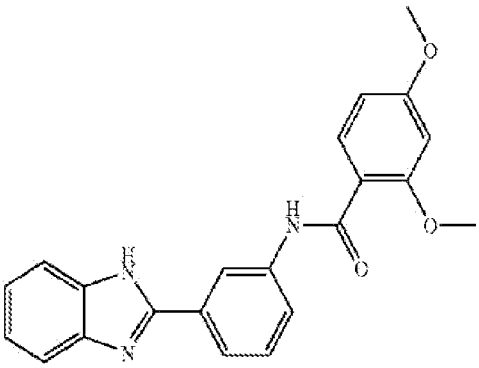

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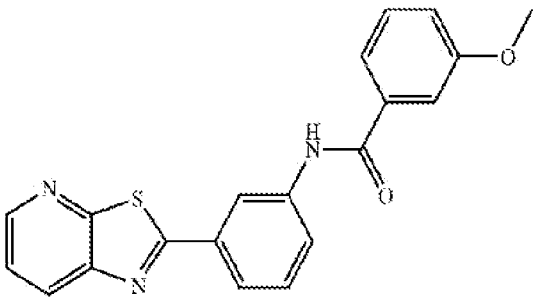

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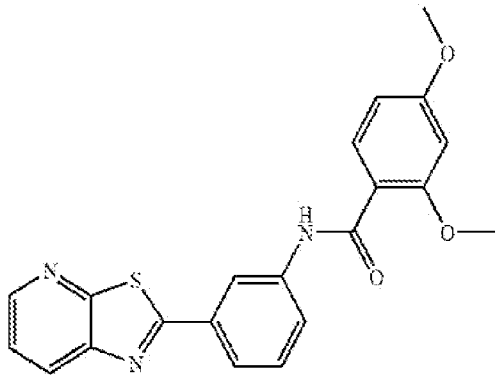
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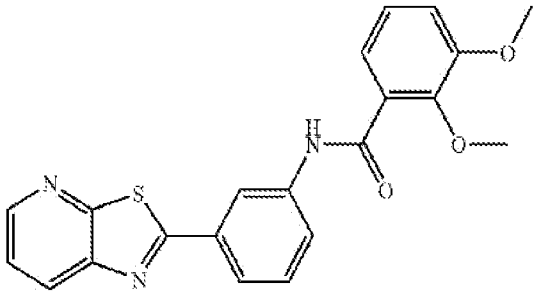
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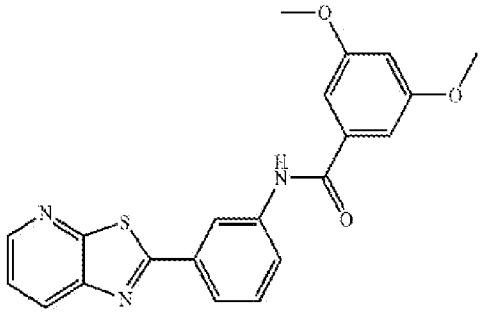
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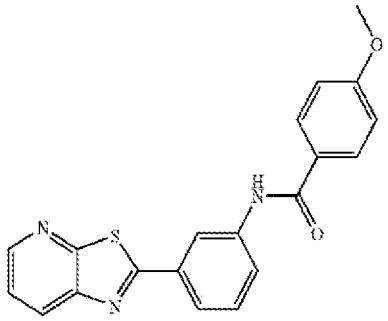
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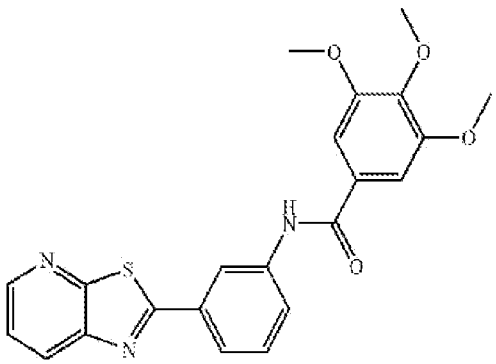
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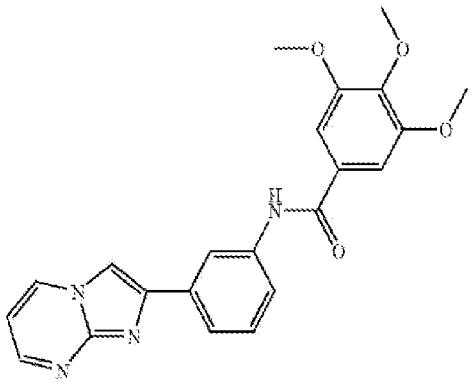
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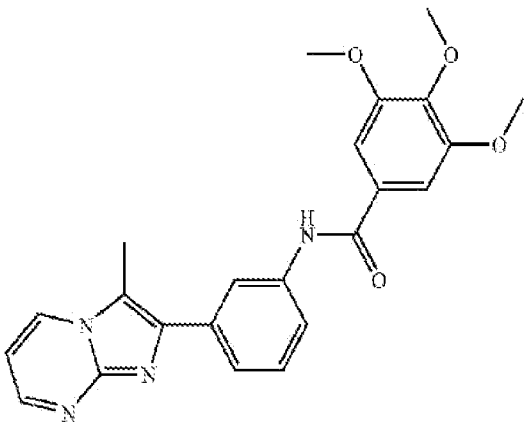
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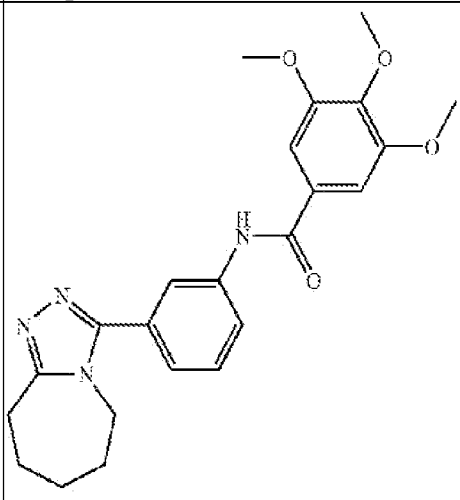
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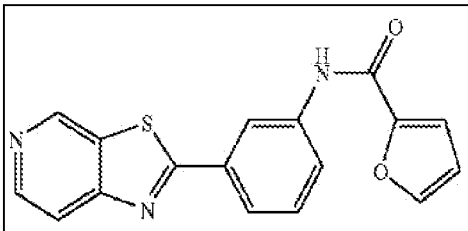
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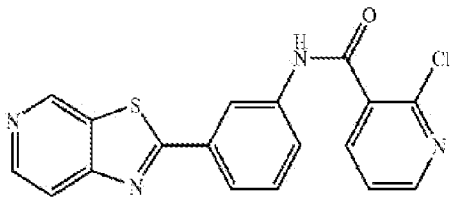
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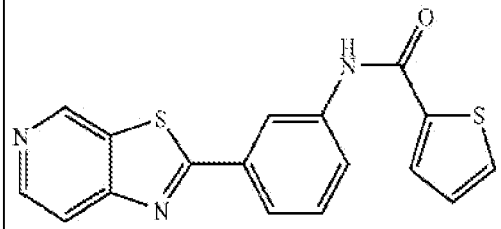
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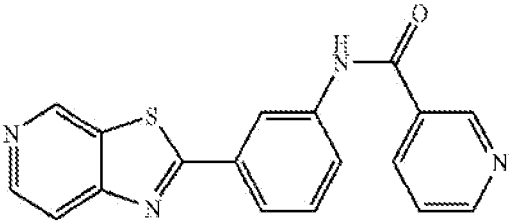
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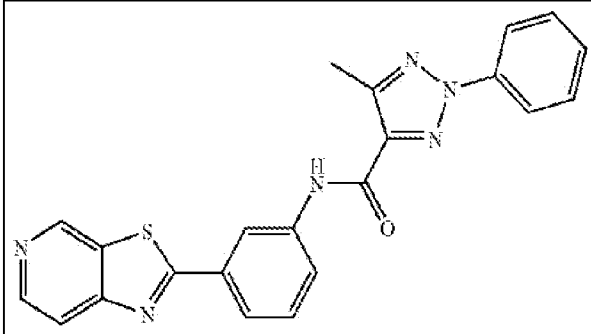
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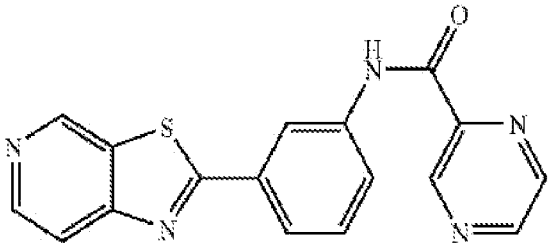
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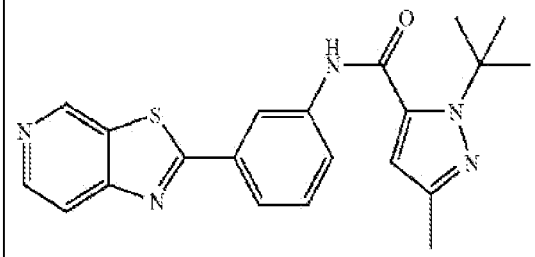
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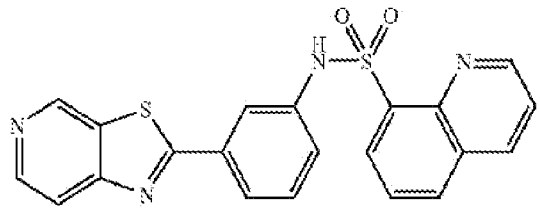
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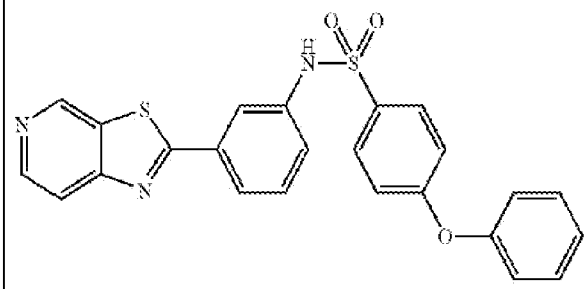
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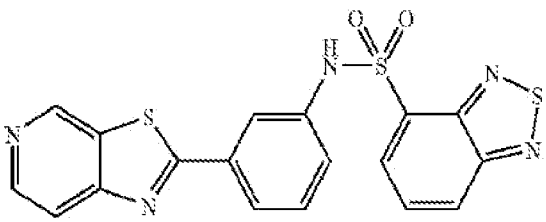
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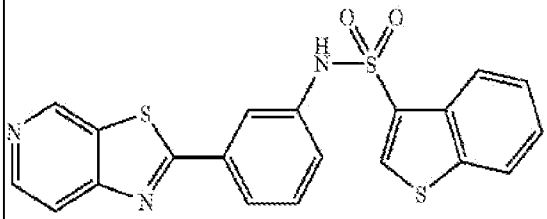
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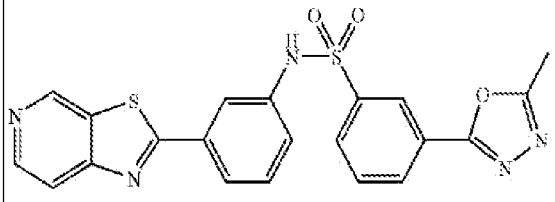
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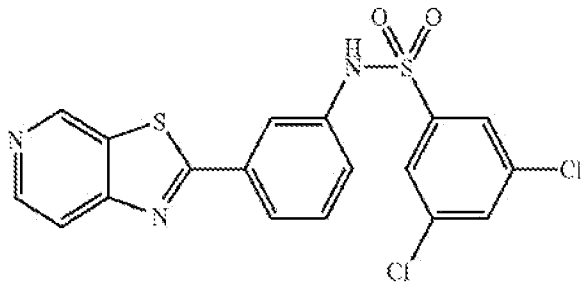

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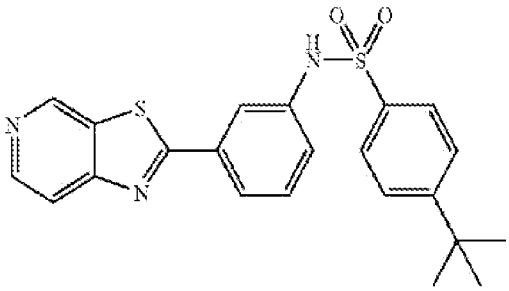

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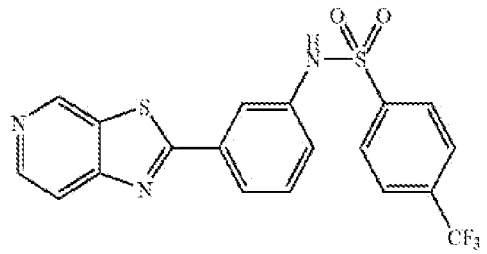

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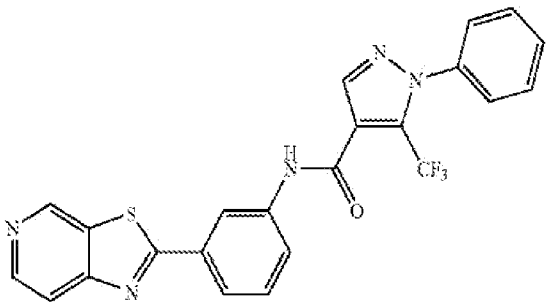

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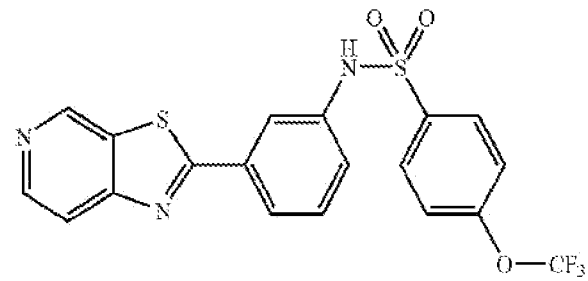

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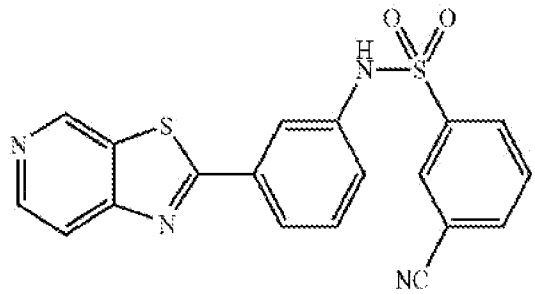

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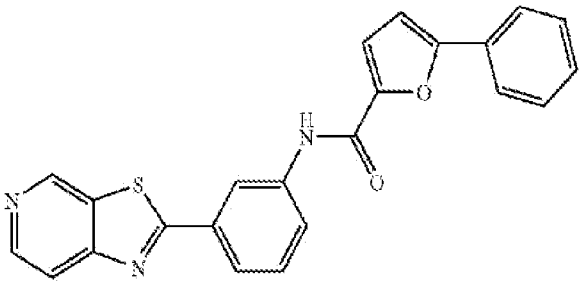

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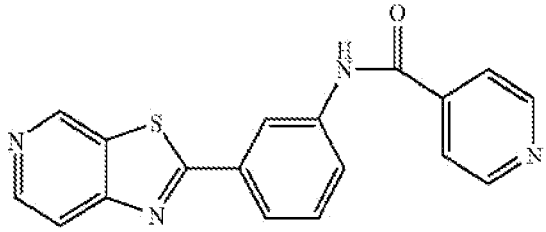

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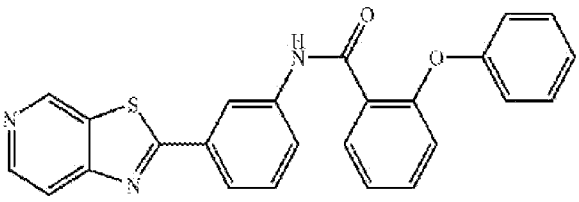

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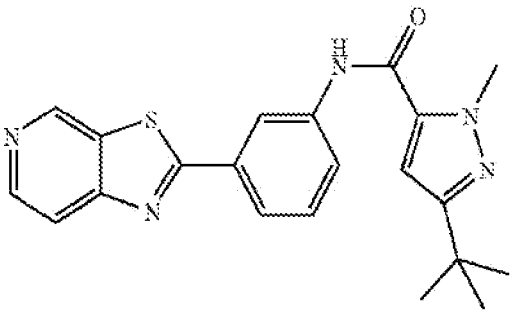

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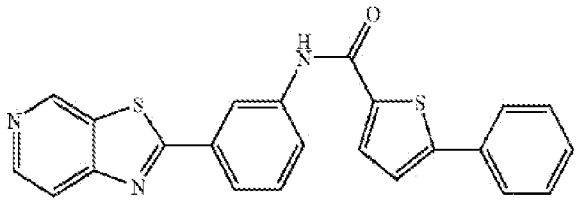
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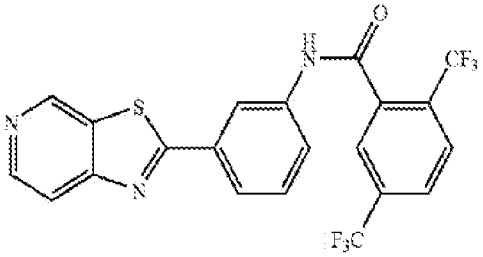
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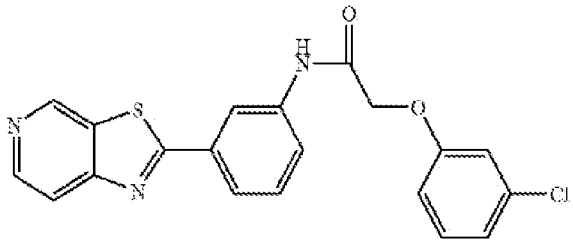
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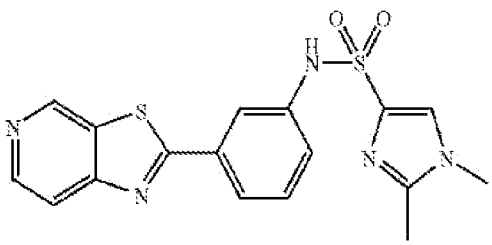
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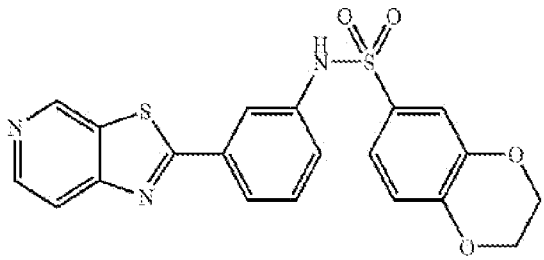
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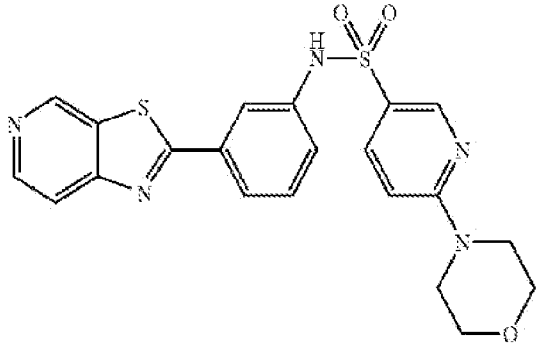
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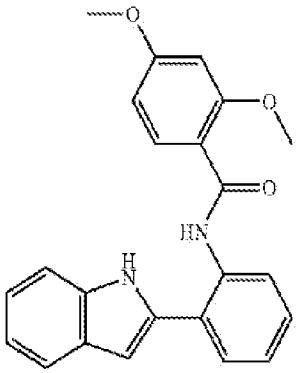
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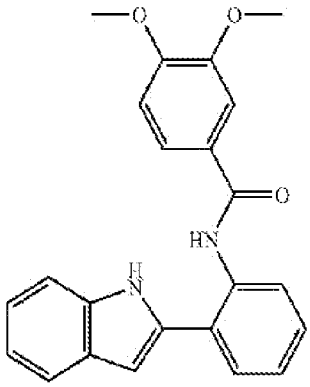
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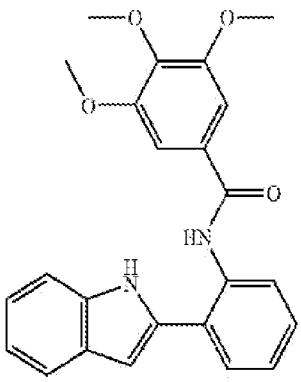
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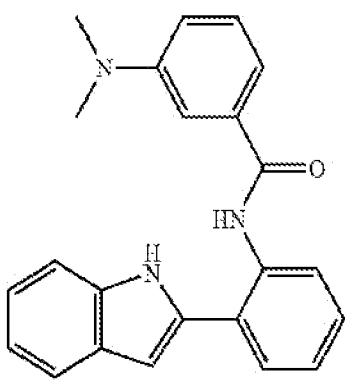
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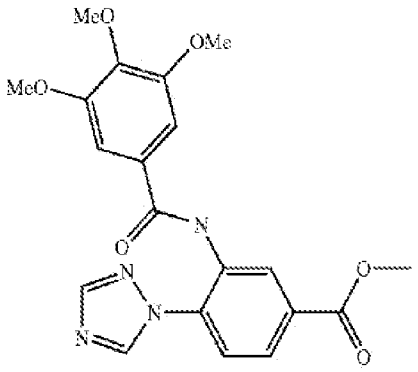

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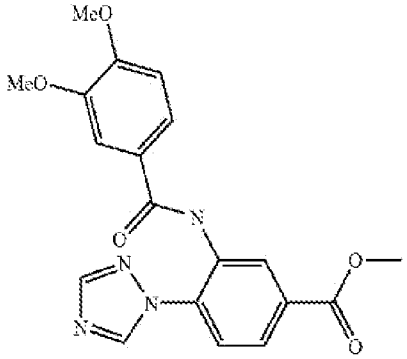

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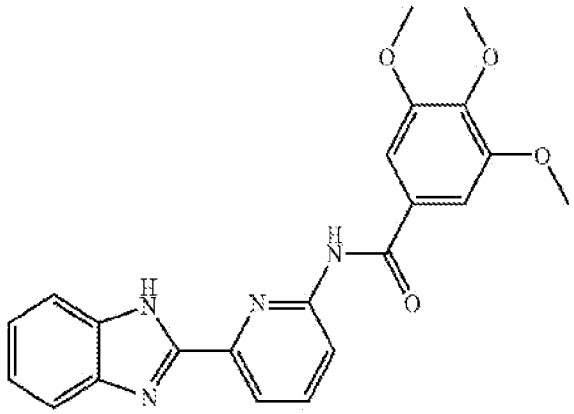

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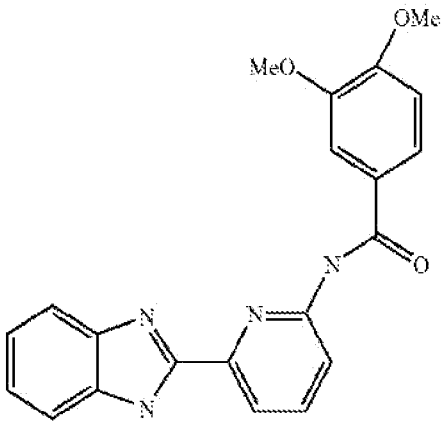

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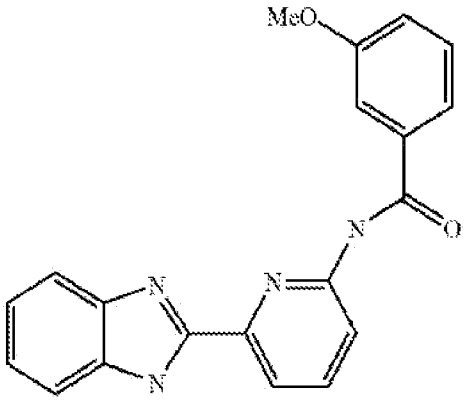

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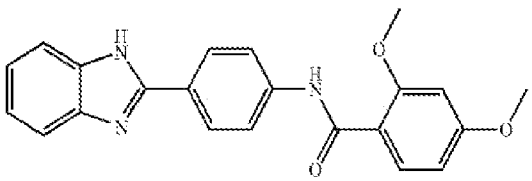

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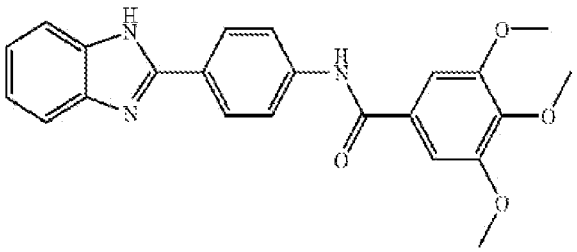

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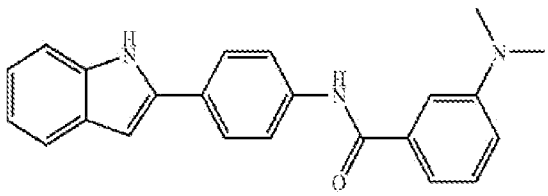

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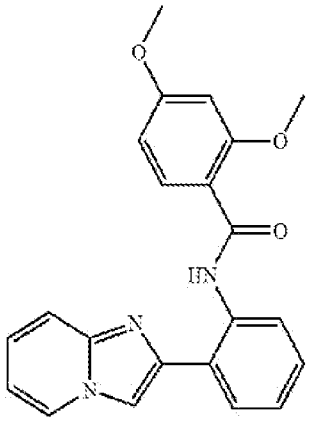
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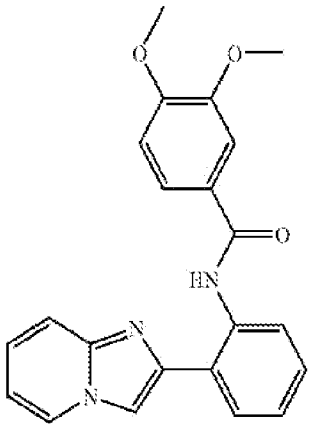
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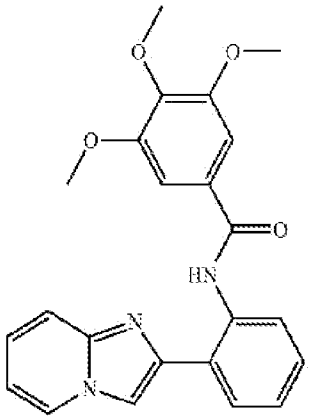
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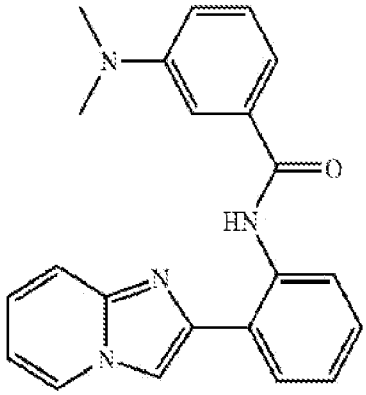
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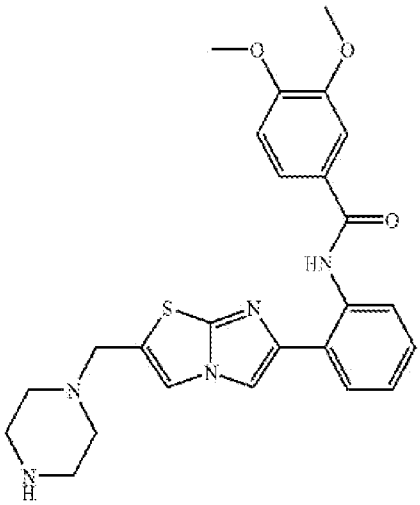
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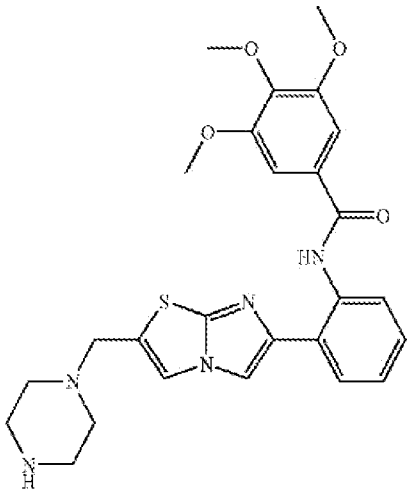
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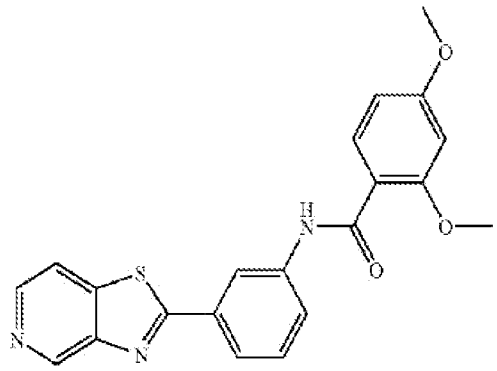

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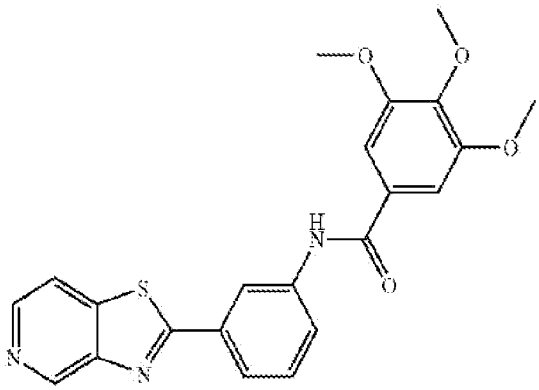

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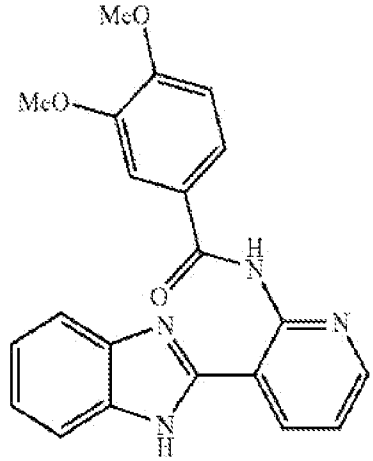

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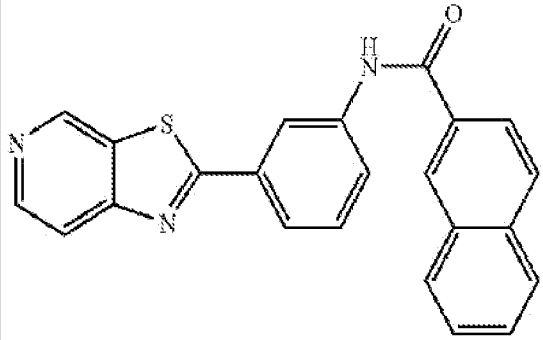

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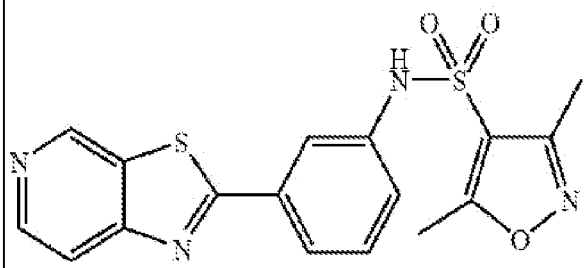

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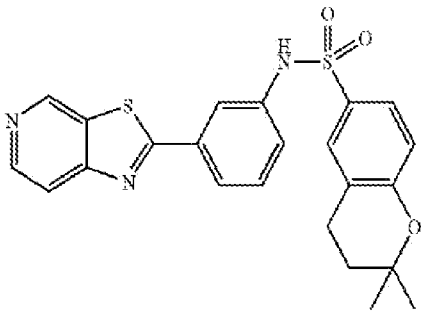

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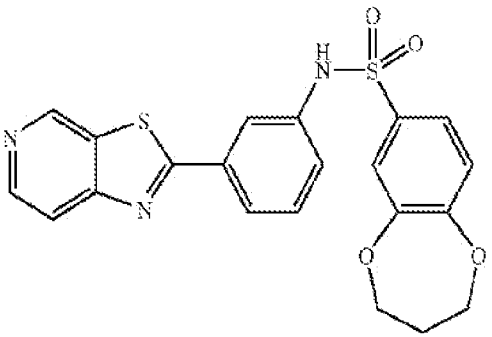

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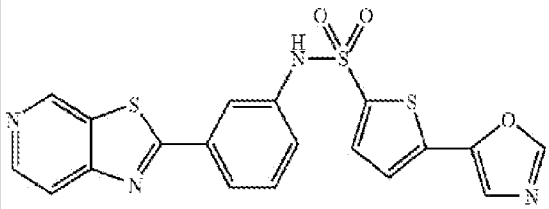

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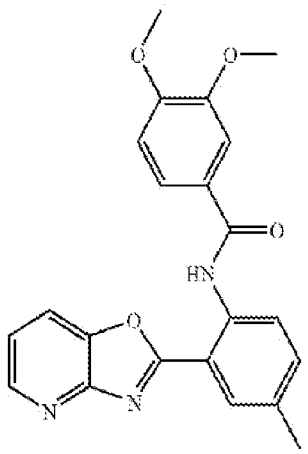

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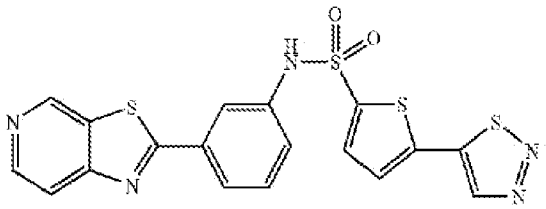

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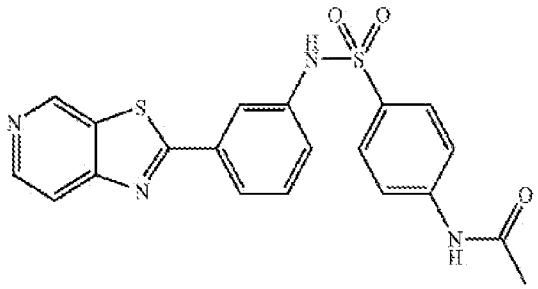

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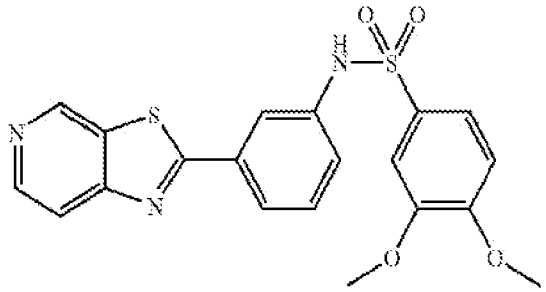

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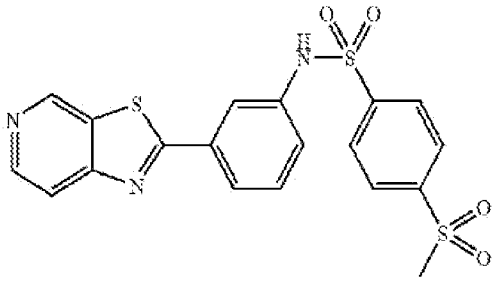

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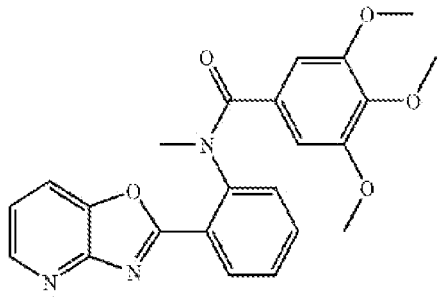

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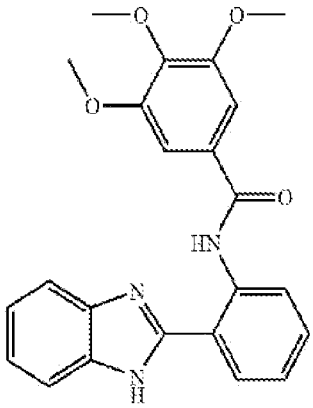

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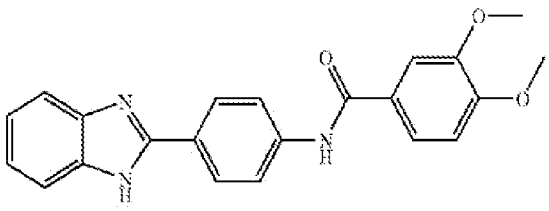

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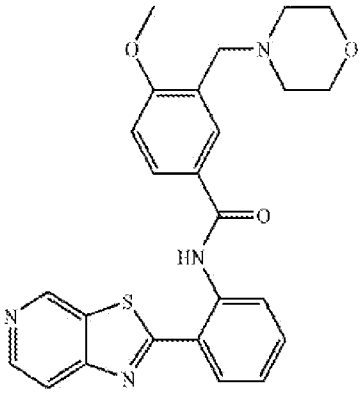

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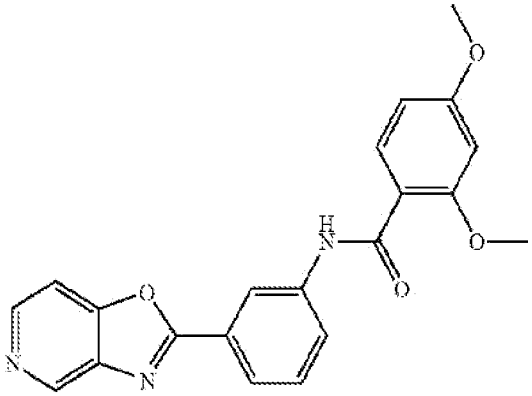

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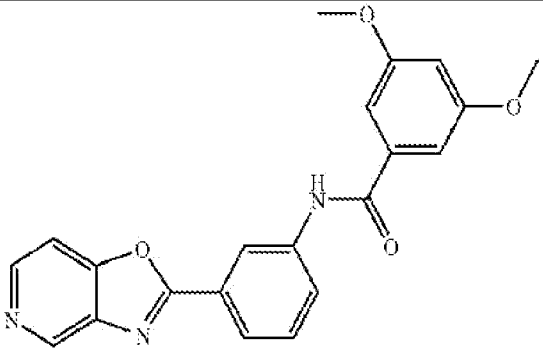

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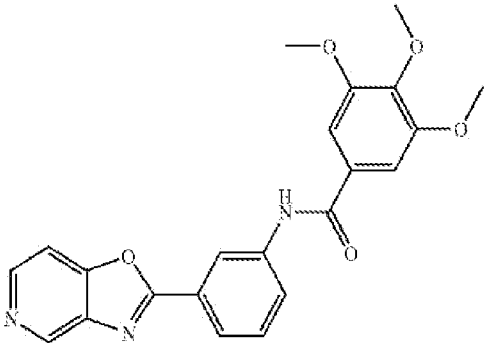

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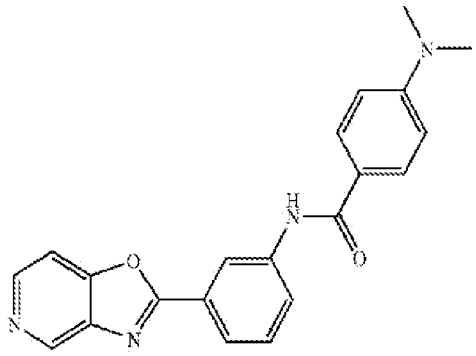

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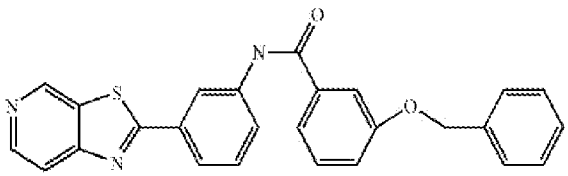

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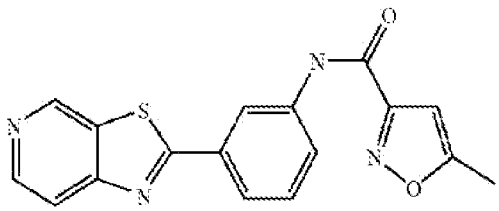

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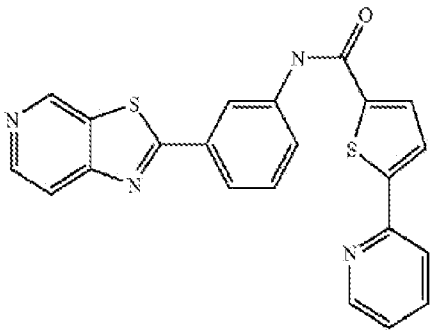

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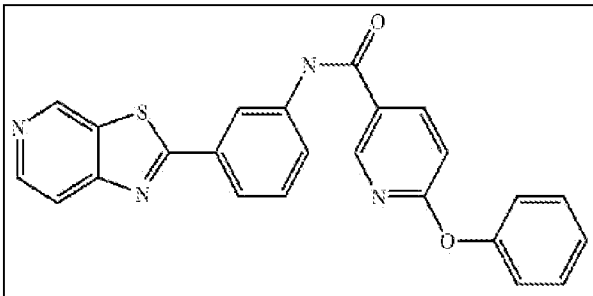
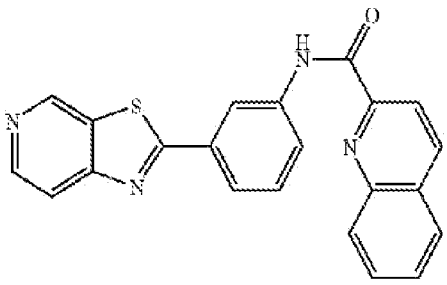
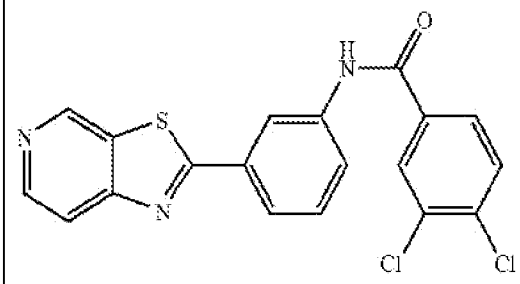
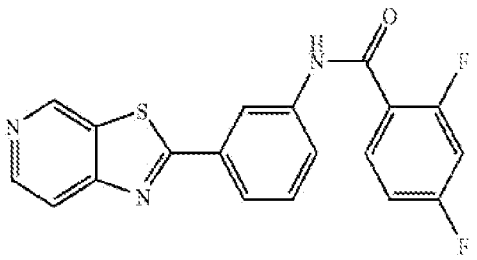
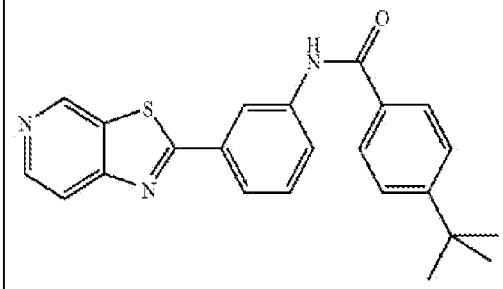
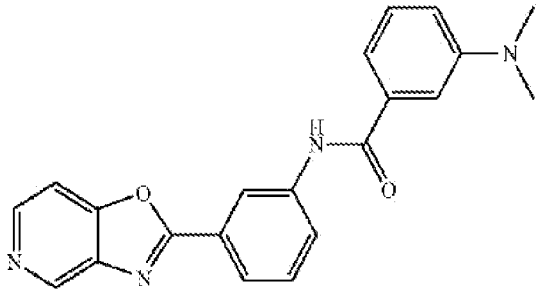
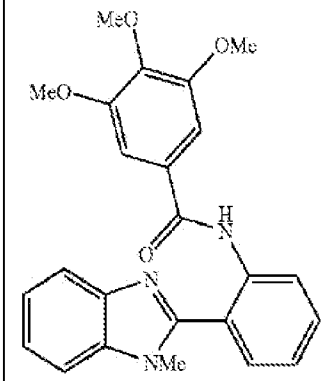
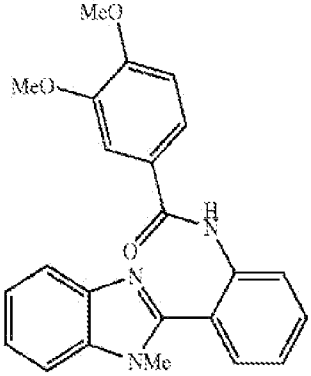
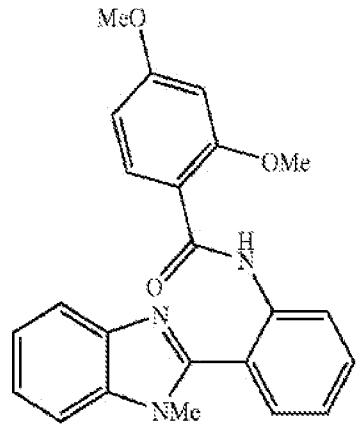
 Compound 306	 Compound 307
 Compound 308	 Compound 309
 Compound 310	 Compound 311
 Compound 313	 Compound 314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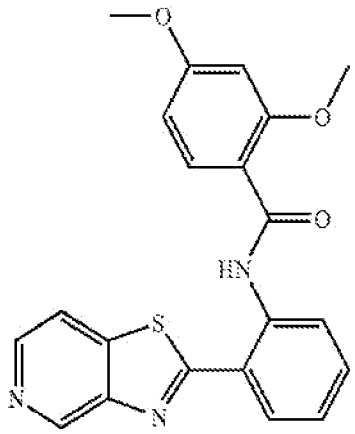
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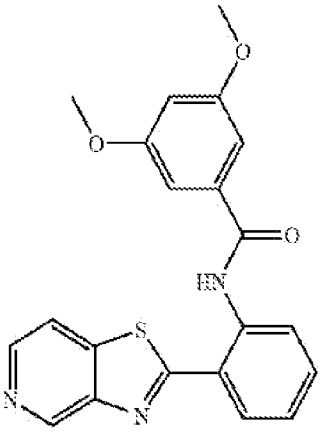
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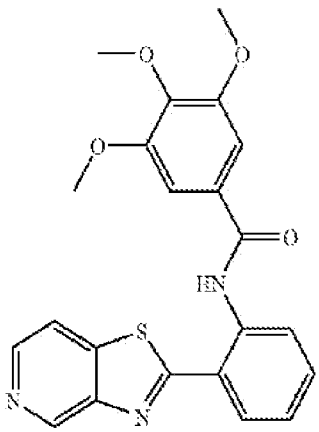
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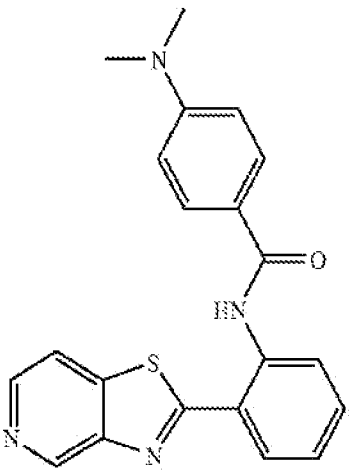
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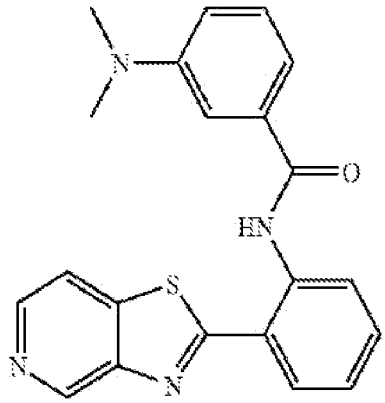
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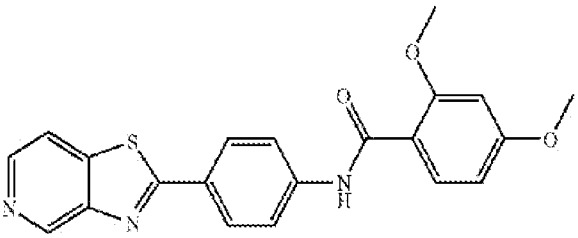
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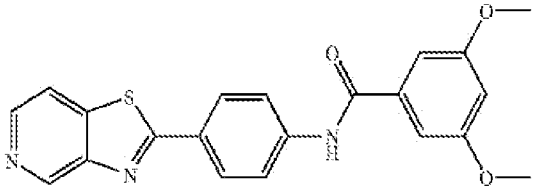
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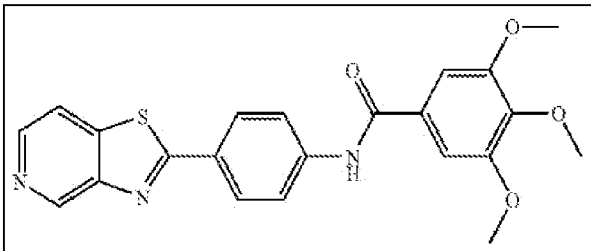

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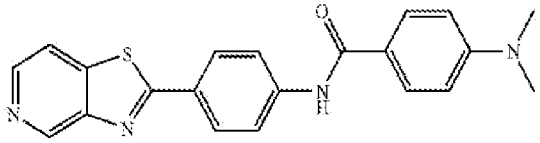

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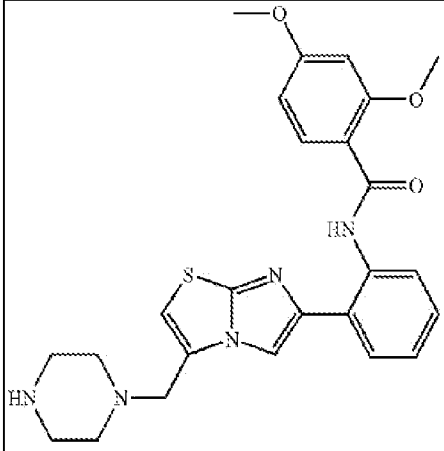

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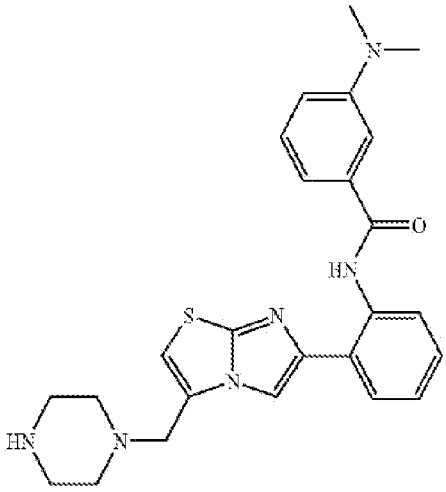

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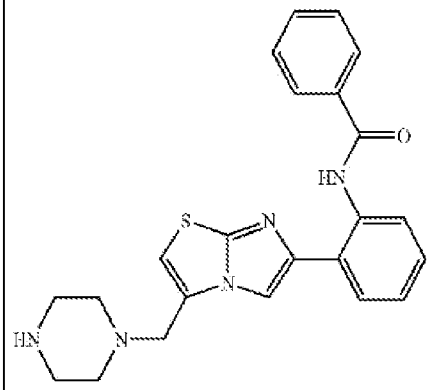

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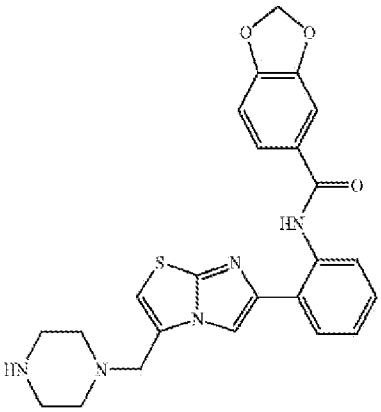

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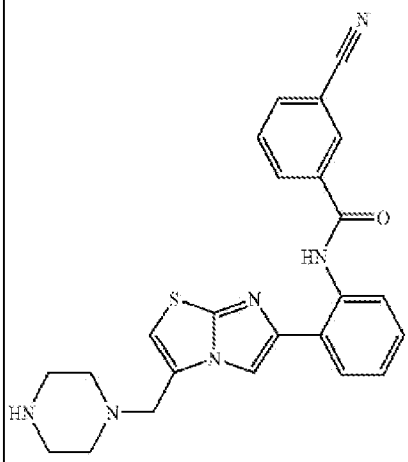

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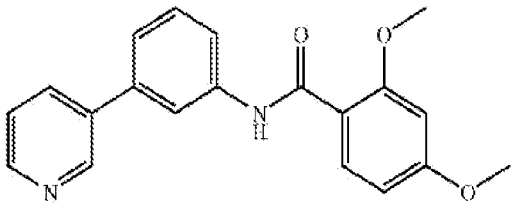

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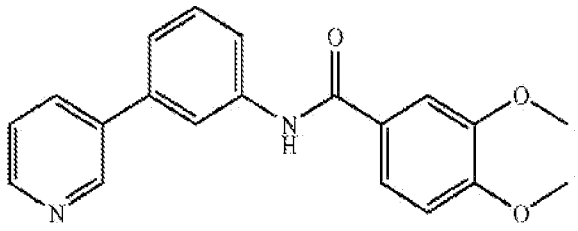

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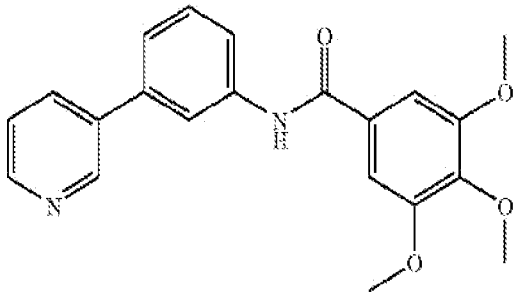

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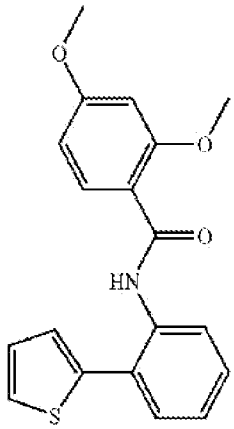

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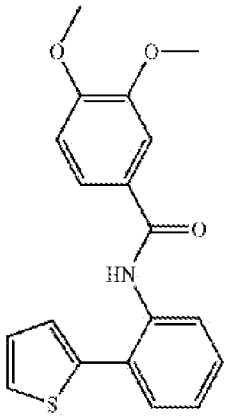

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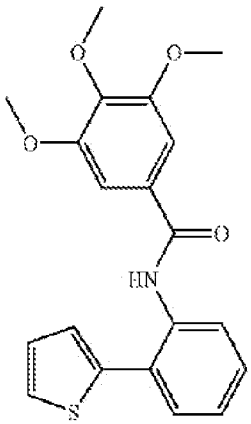

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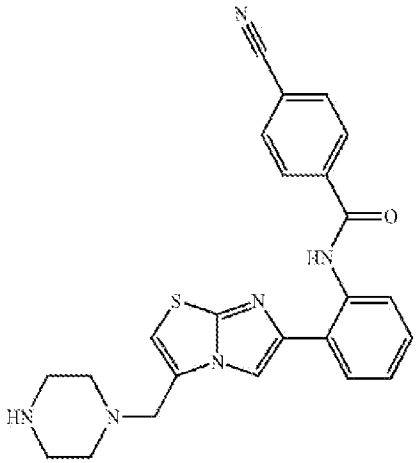

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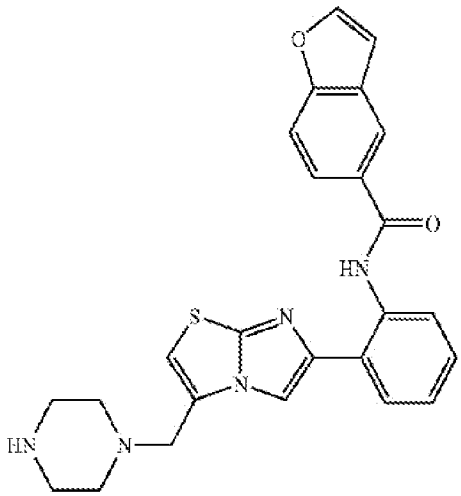

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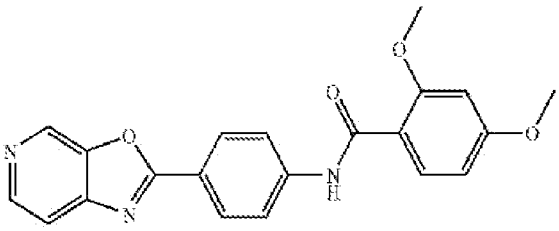

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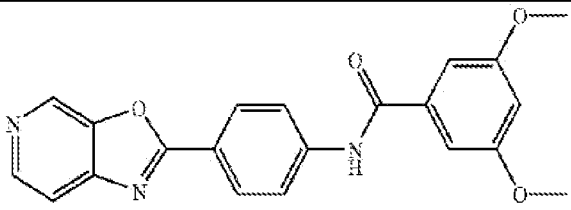

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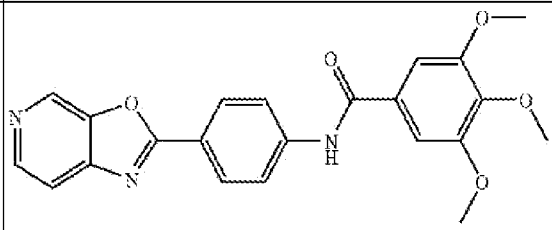

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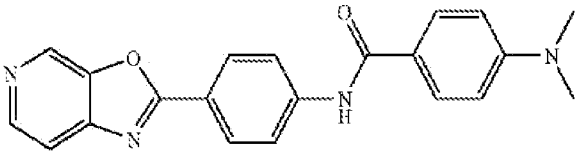

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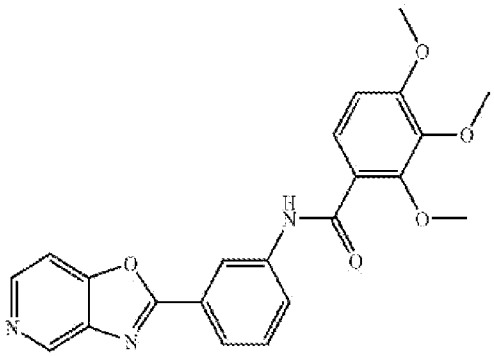

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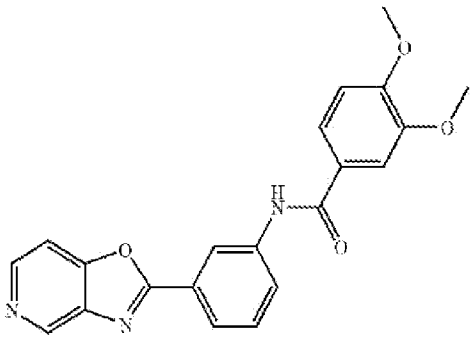

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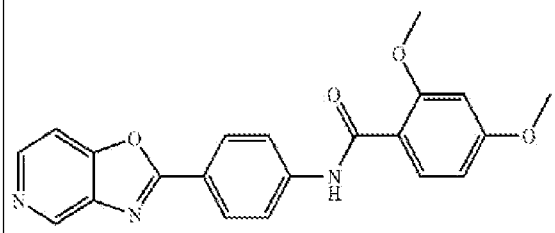

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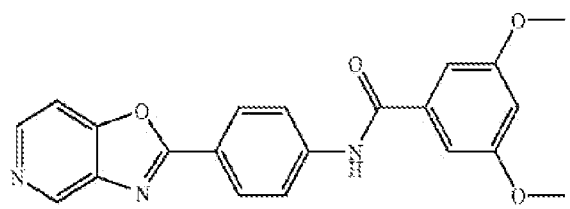
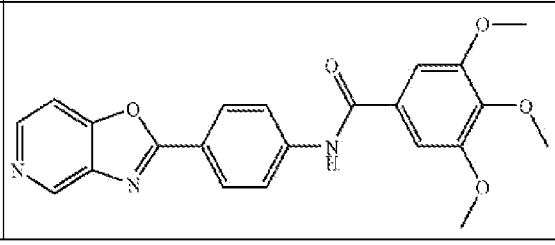
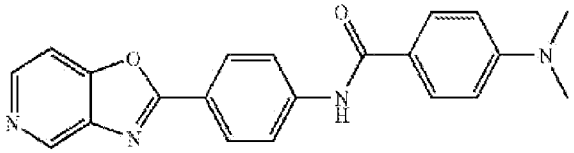
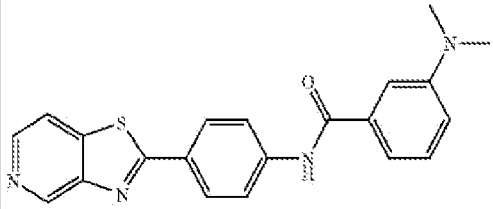
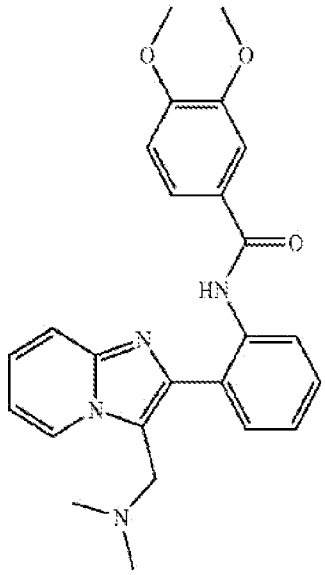
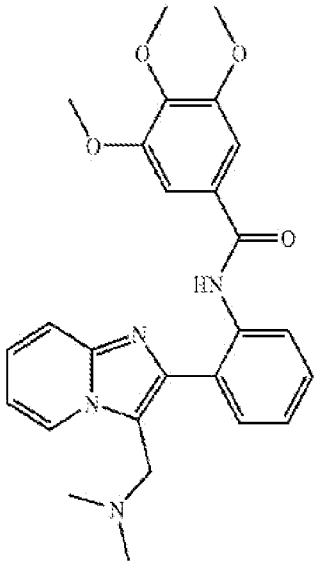
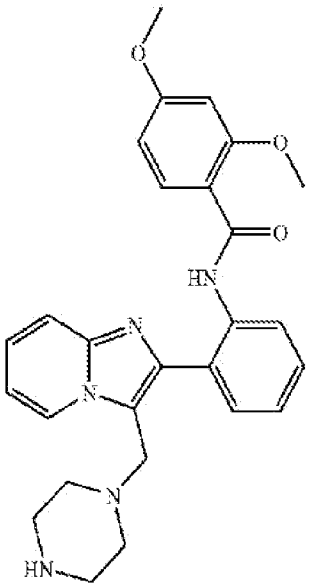
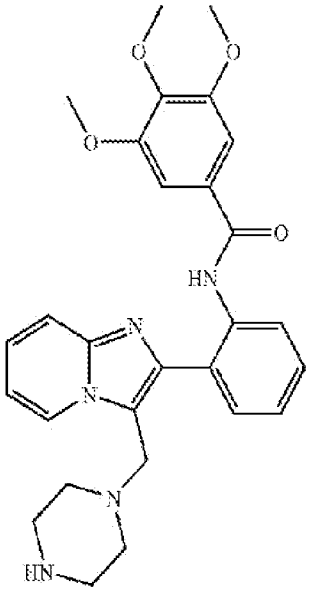
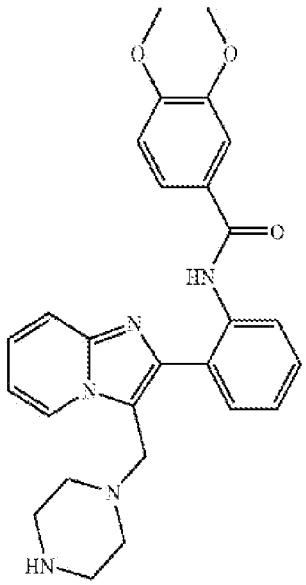




Compound 346	Compound 347
 Compound 348	 Compound 349
 Compound 350	 Compound 351
 Compound 359	 Compound 362



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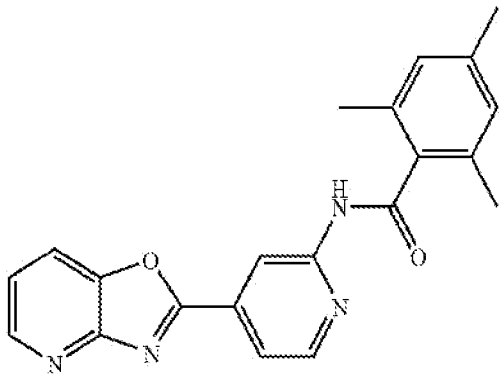

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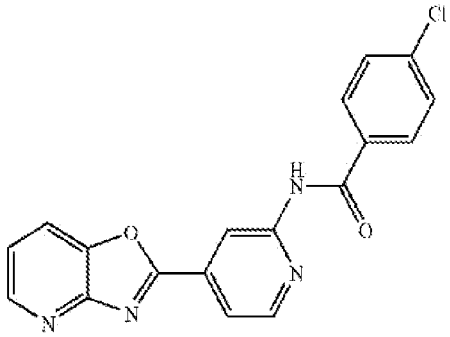

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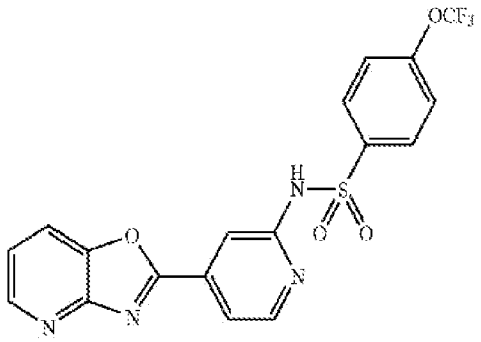

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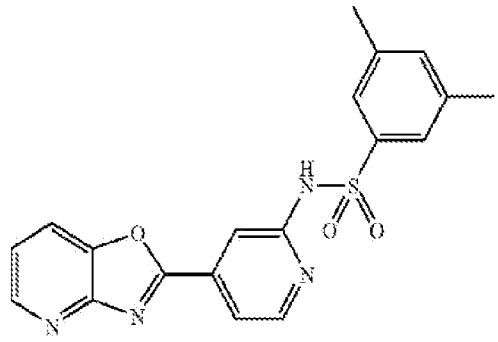

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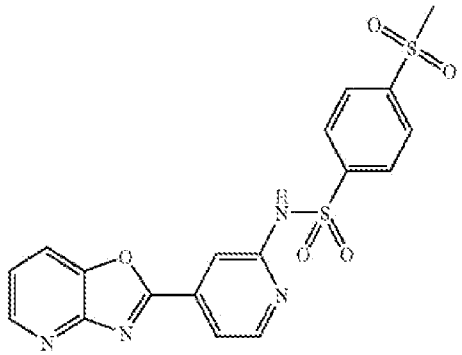

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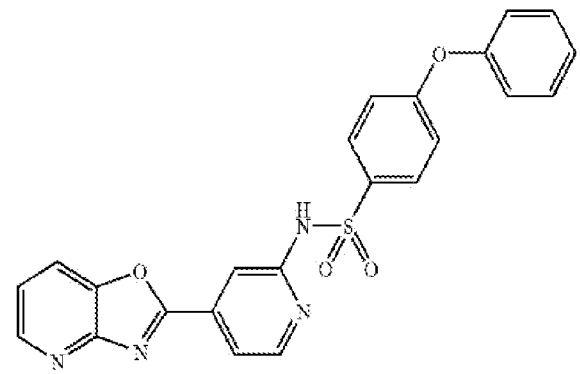
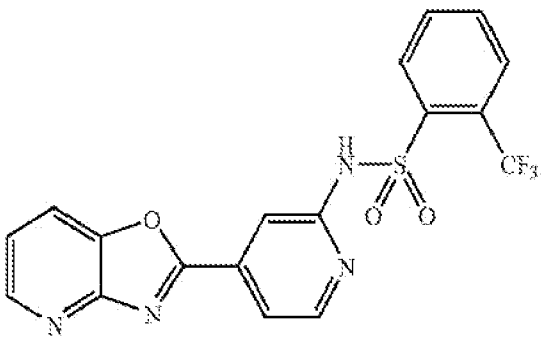
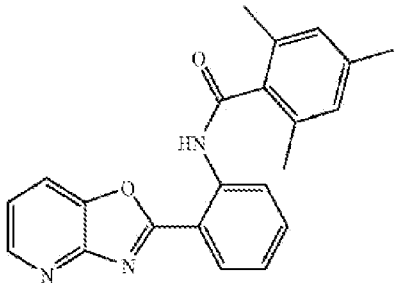
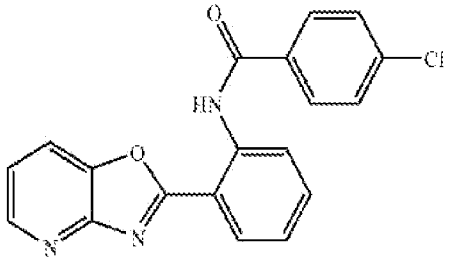
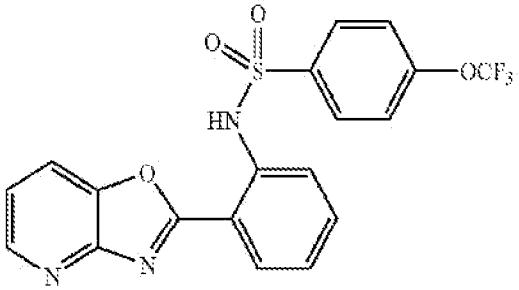
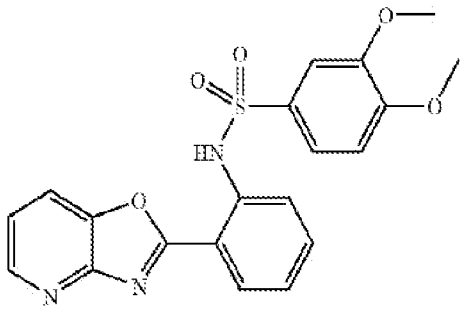
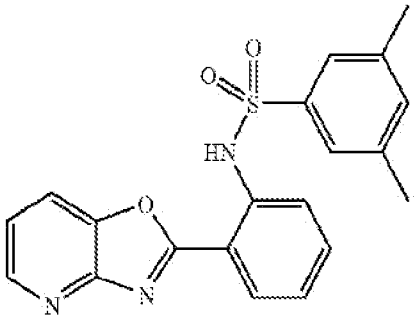
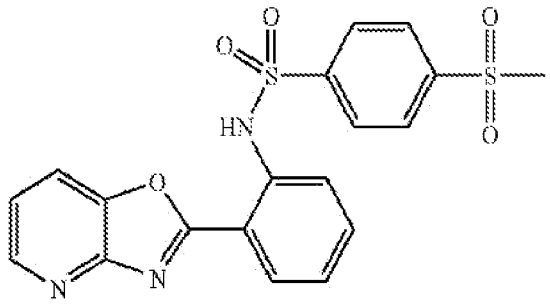
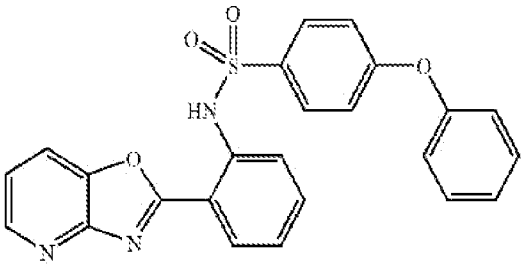
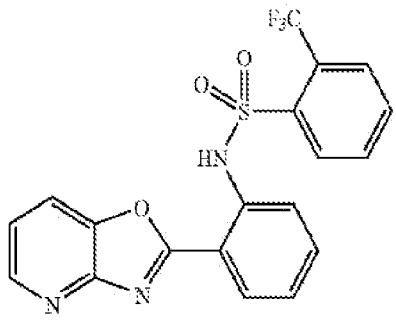
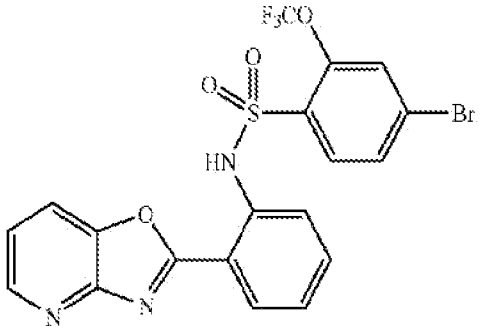
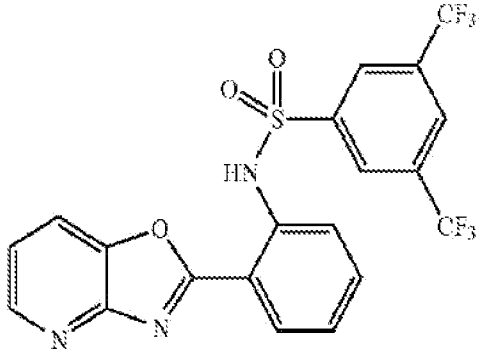




Compound 374

Compound 373	
 Compound 375	 Compound 376
 Compound 377	 Compound 378
 Compound 379	 Compound 380
 Compound 381	 Compound 382
 Compound 383	

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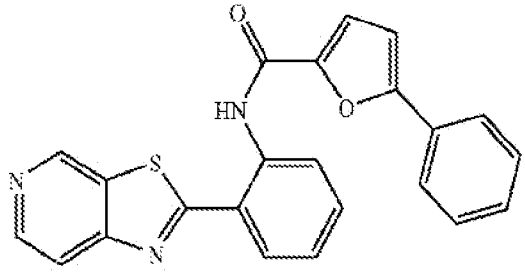
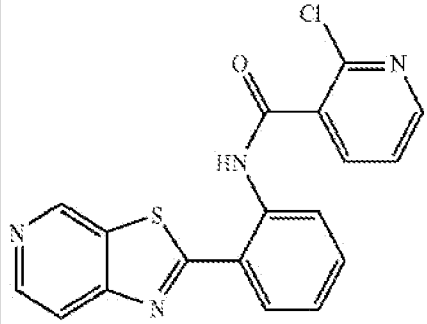
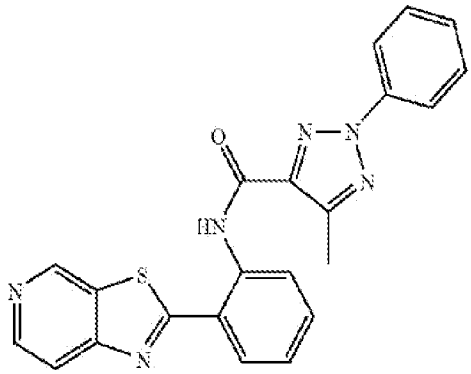
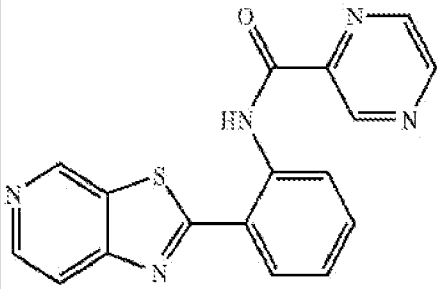
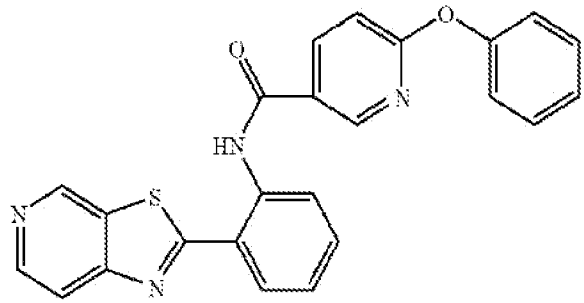
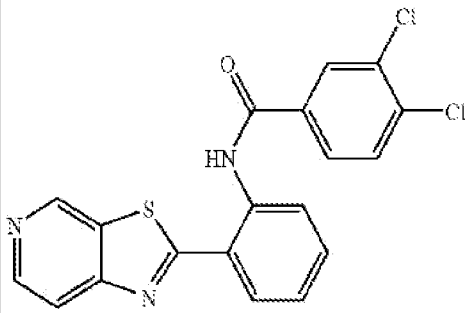
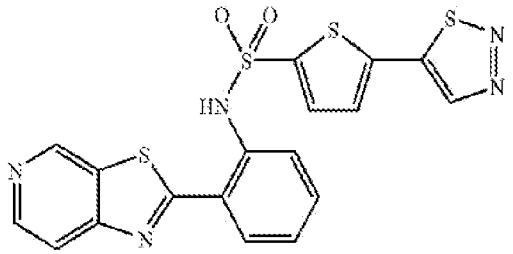
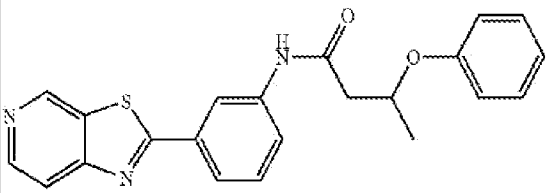
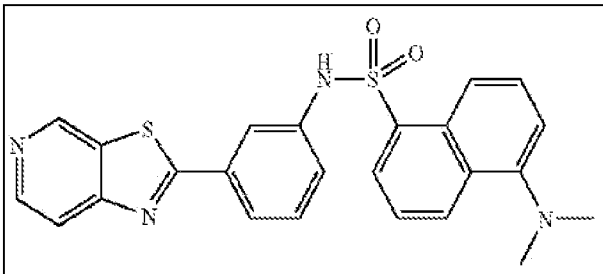
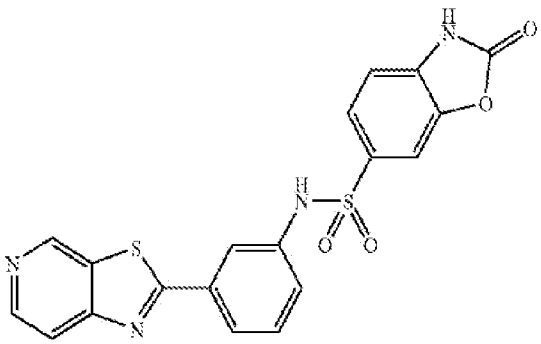
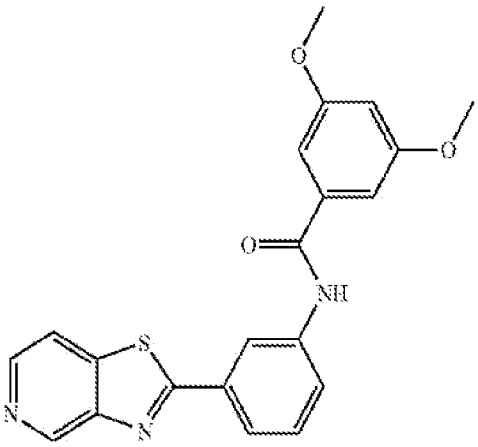
 Compound 385	 Compound 387
 Compound 390	 Compound 391
 Compound 392	 Compound 393
 Compound 394	 Compound 396



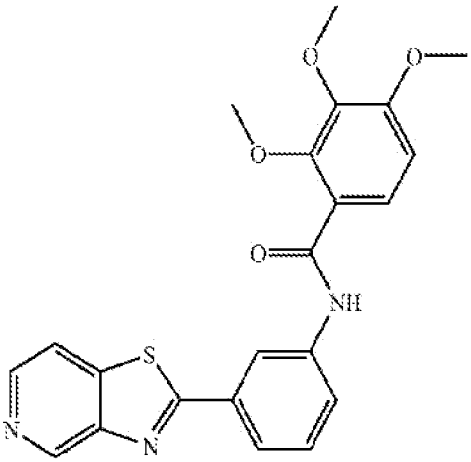
Compound 398



Compound 399



Compound 400



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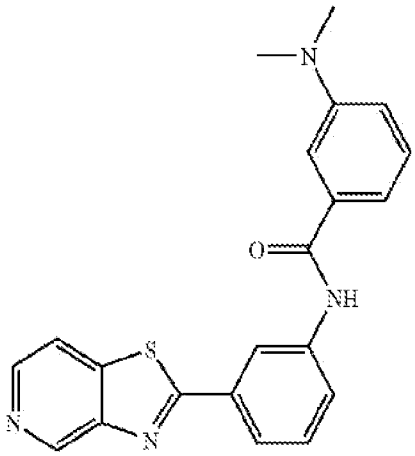

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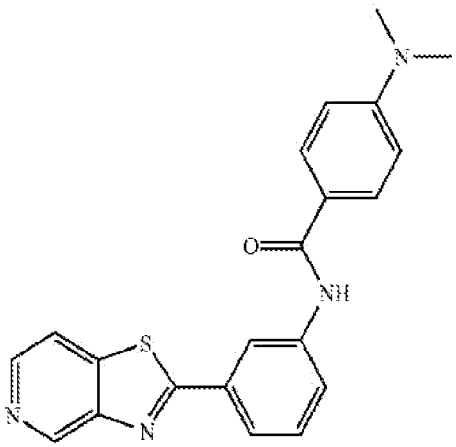

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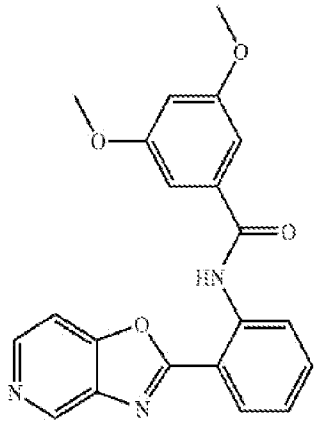
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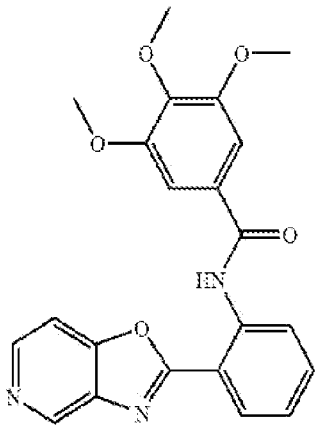
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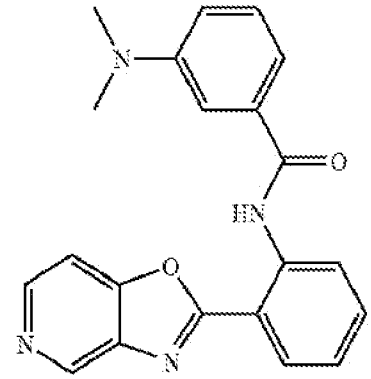
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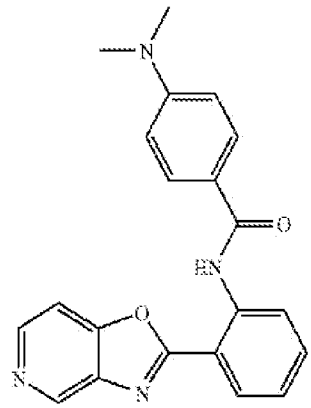
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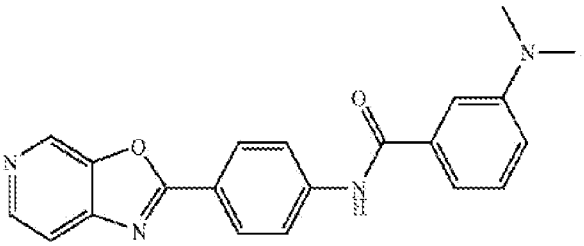
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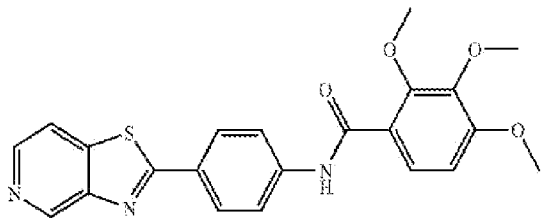
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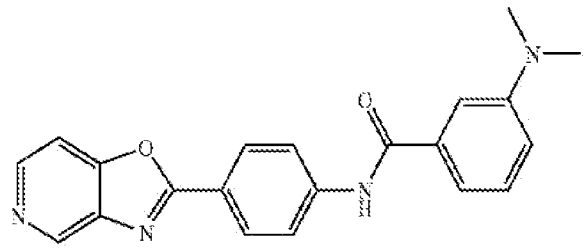
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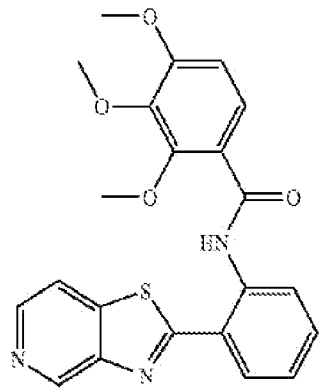
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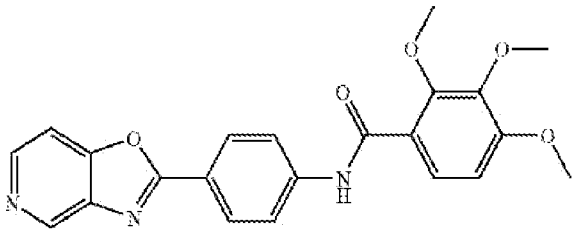
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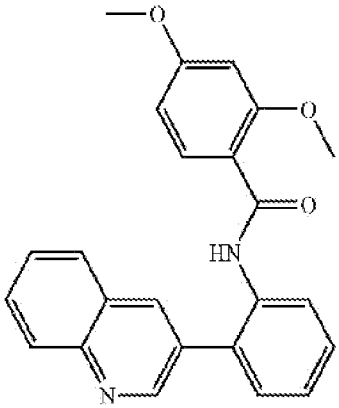
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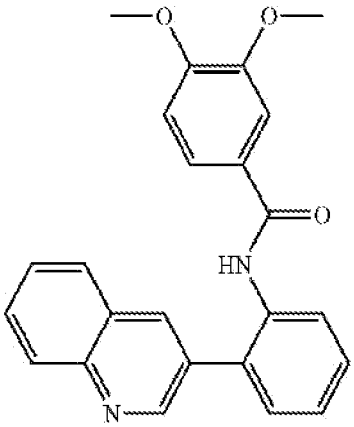
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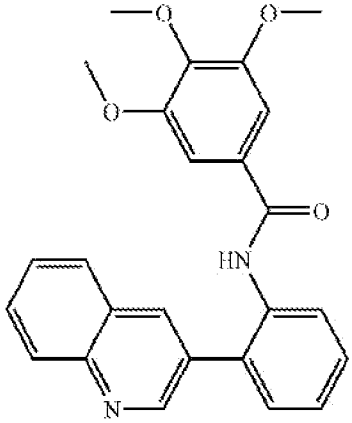
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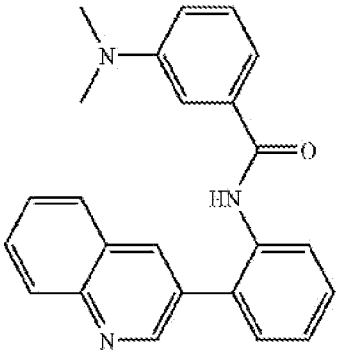
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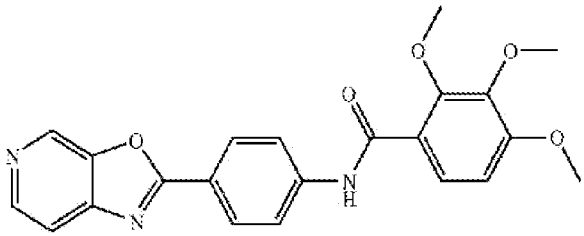
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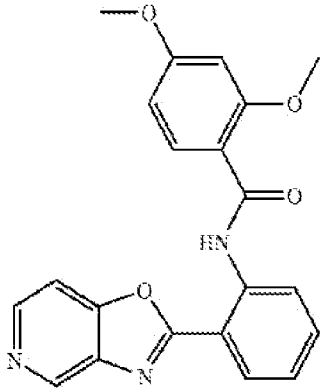
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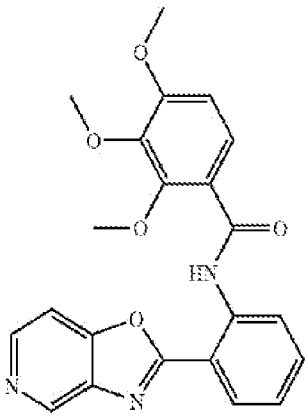
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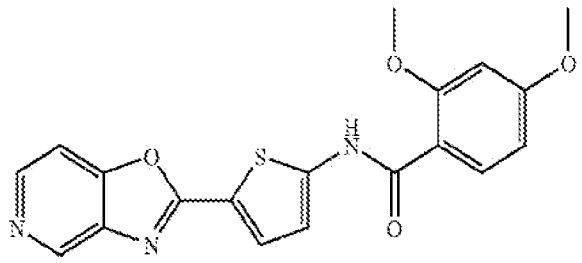
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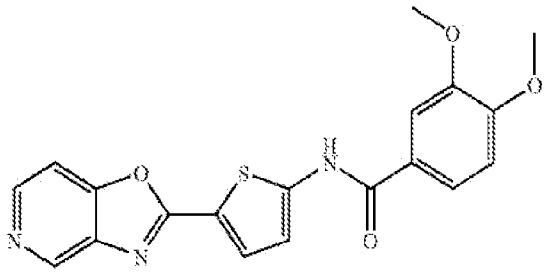
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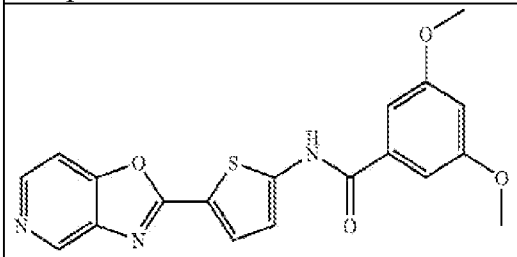
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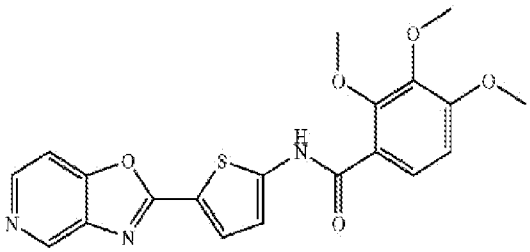
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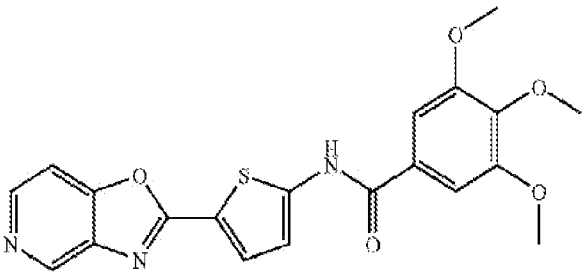
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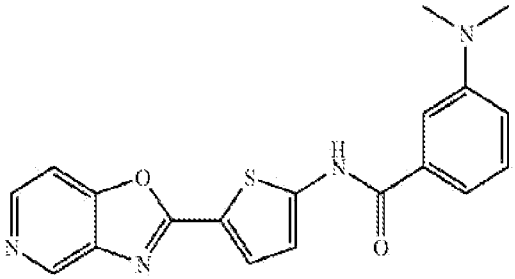
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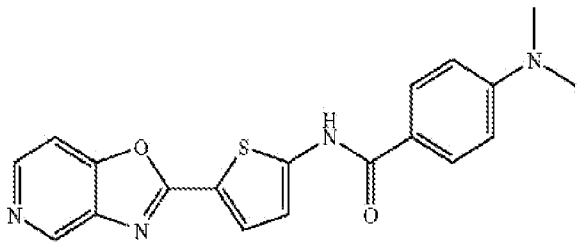
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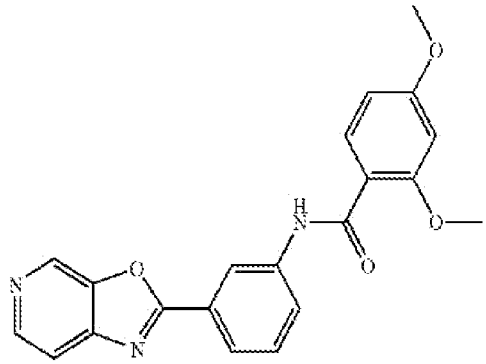
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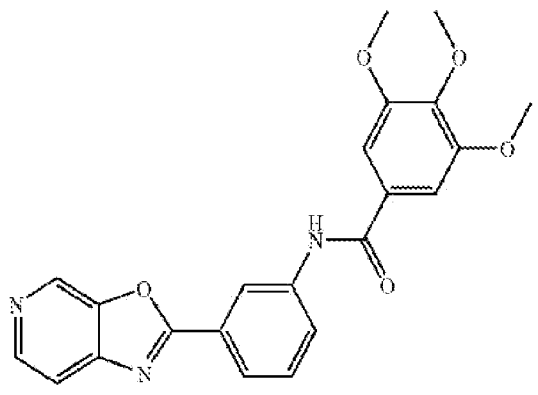
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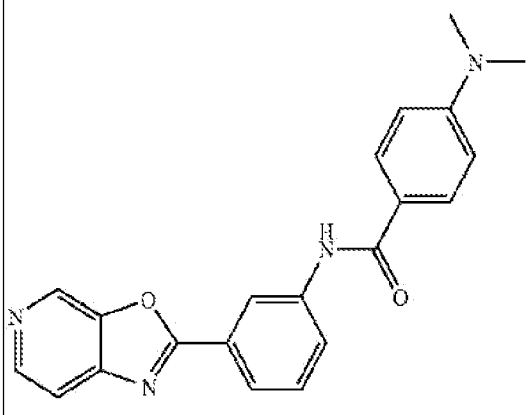
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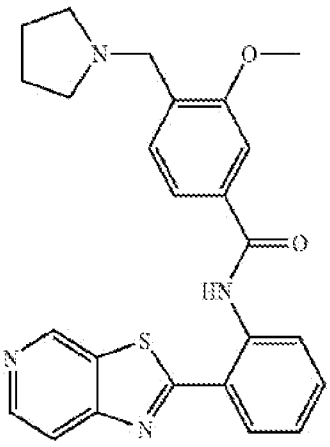

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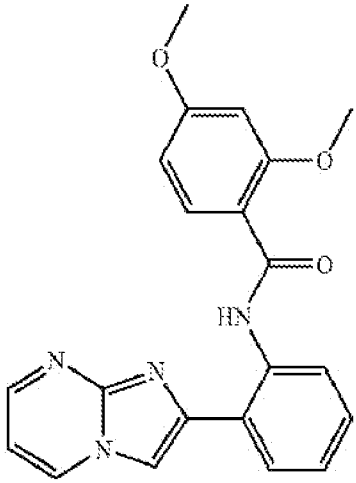

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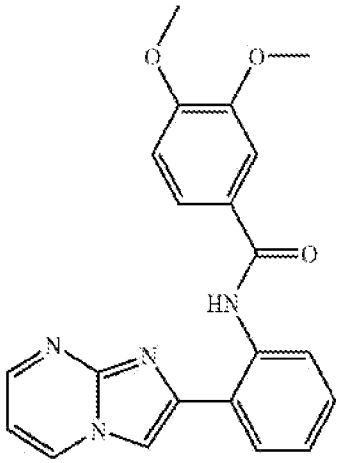

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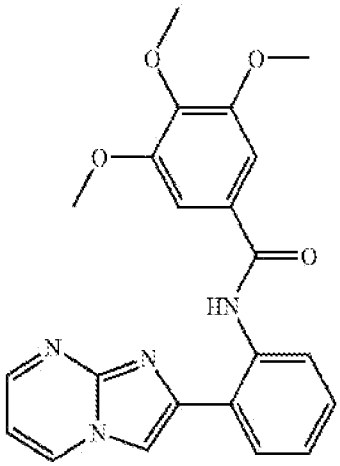

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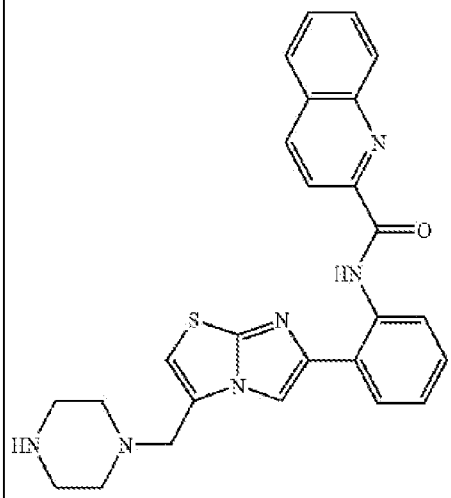

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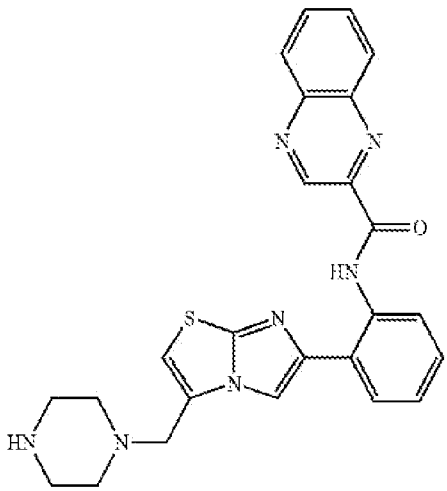

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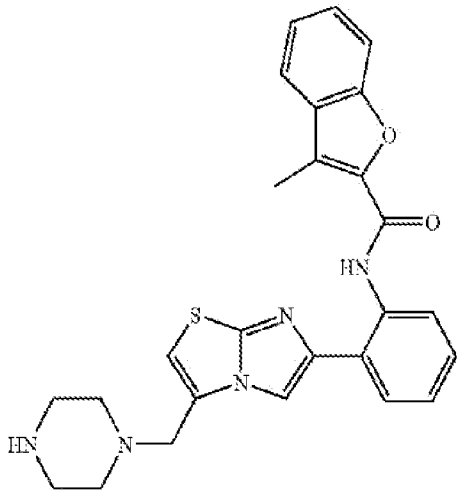

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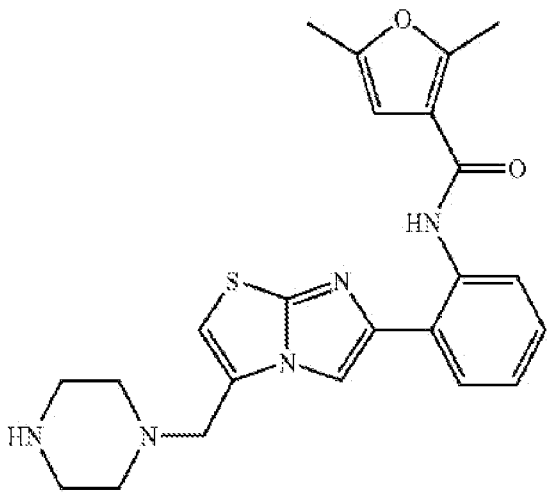

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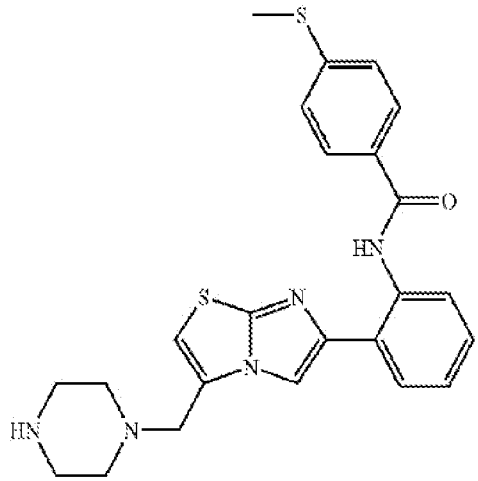

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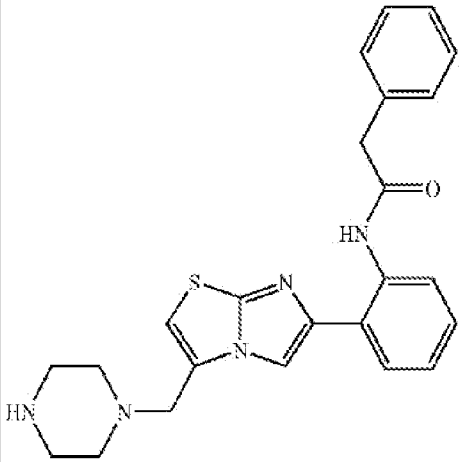

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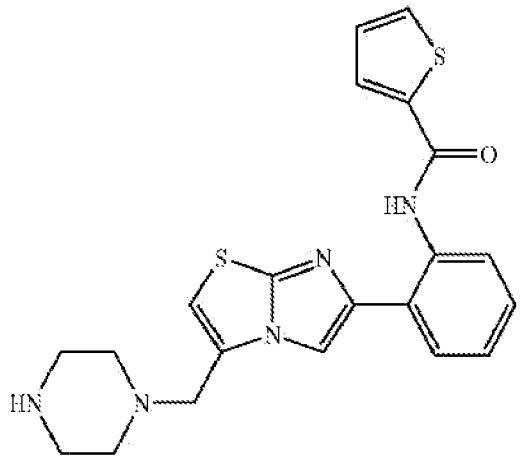

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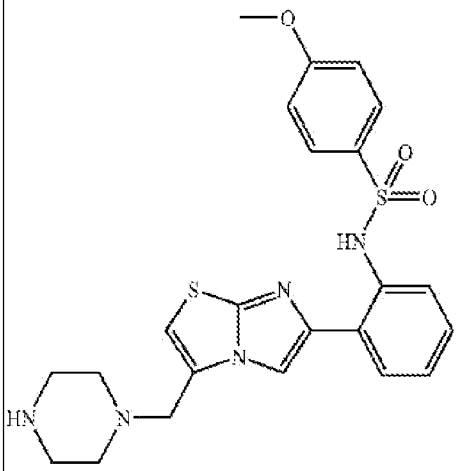

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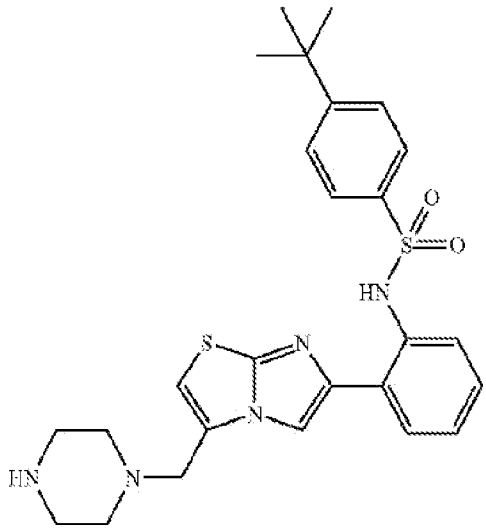

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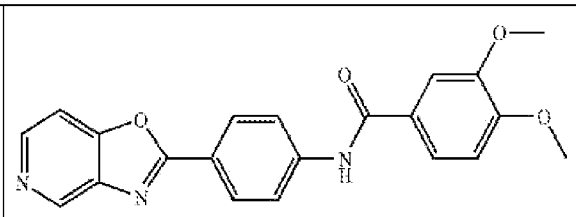

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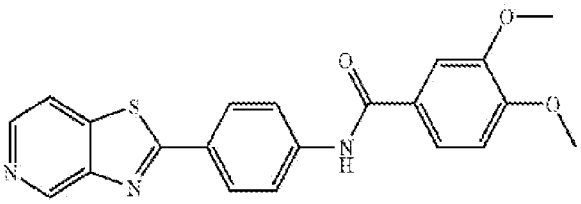

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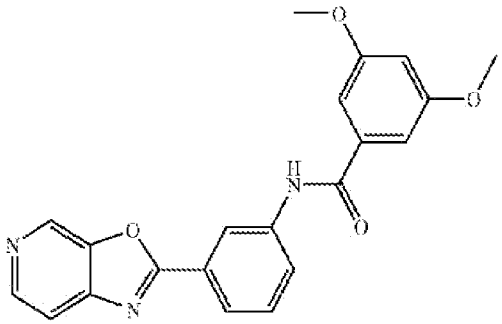

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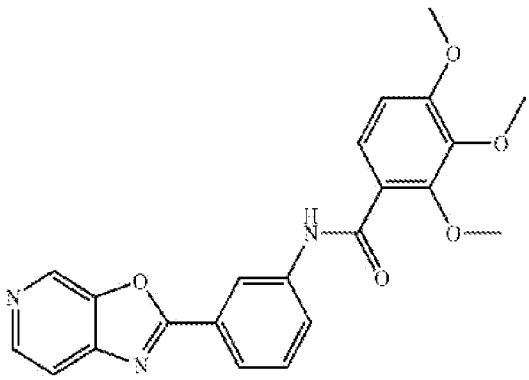

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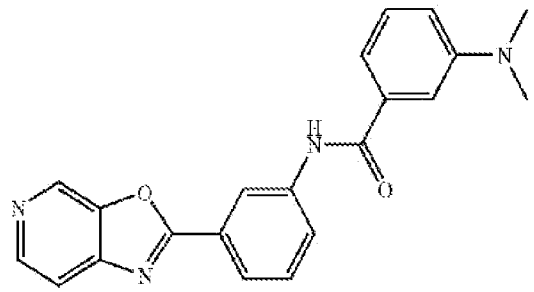

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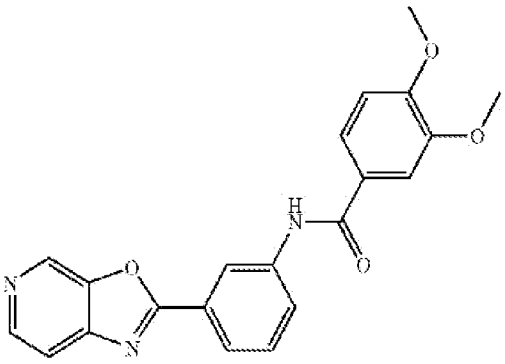

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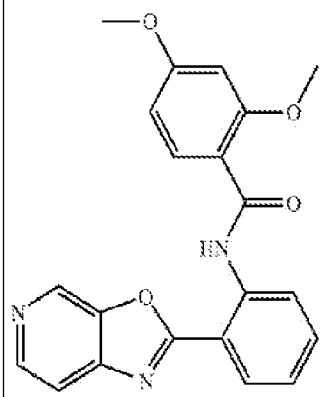

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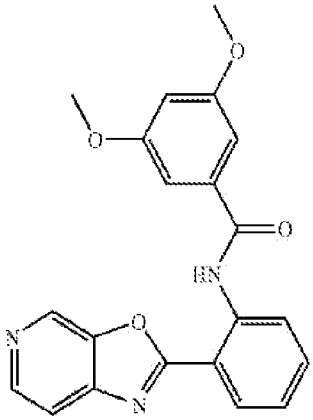

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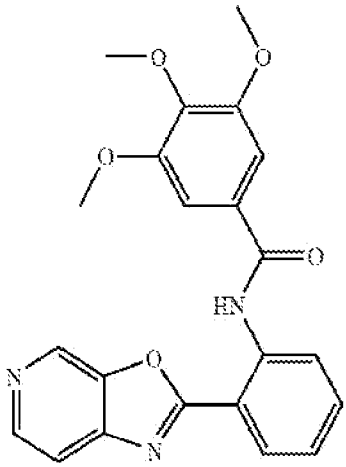

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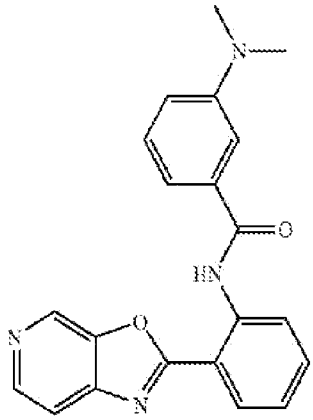

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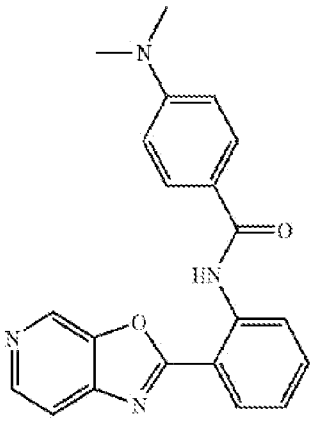

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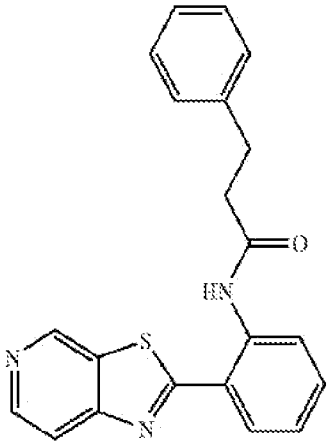

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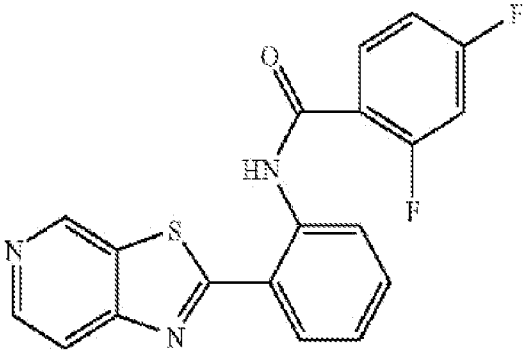

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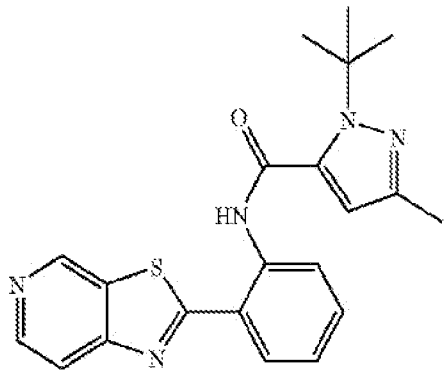
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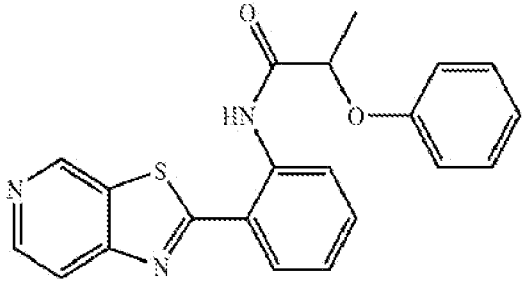
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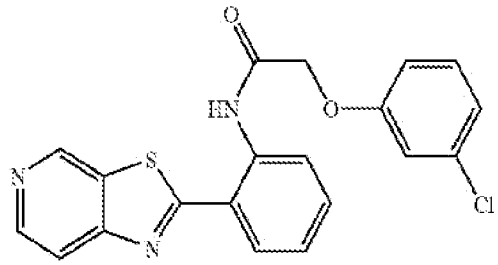
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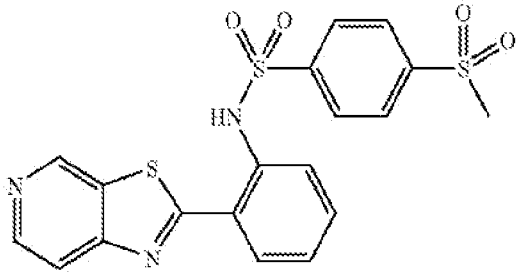
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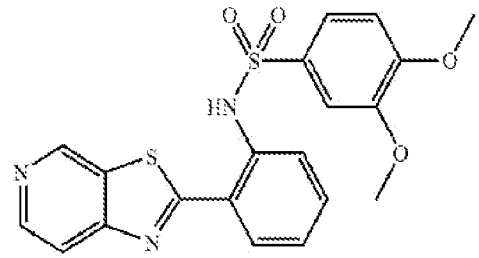
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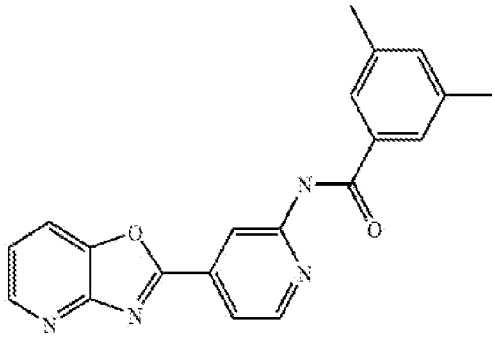
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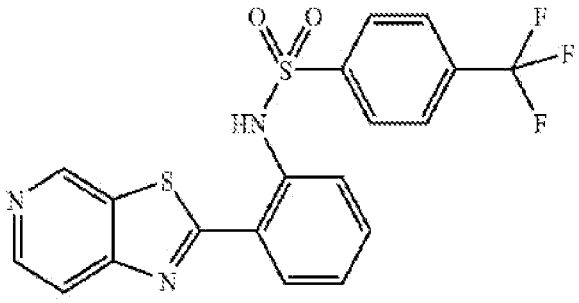
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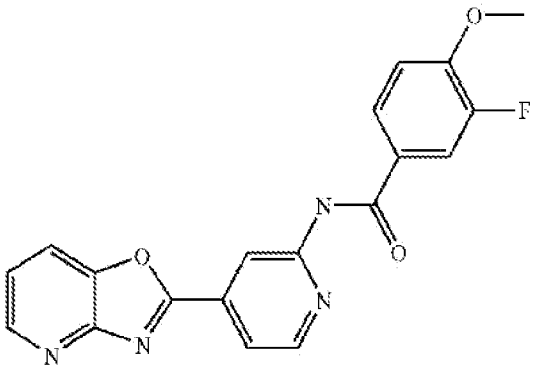
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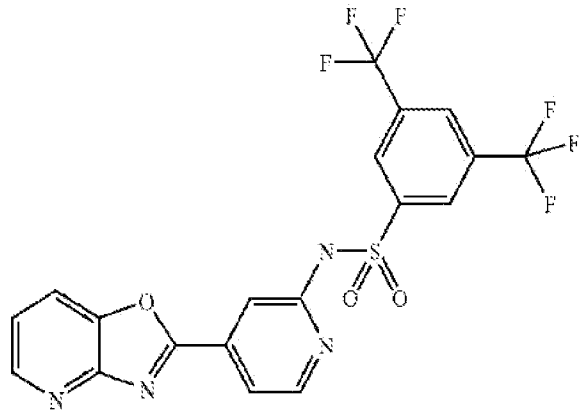

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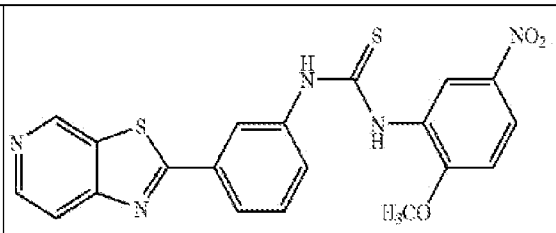

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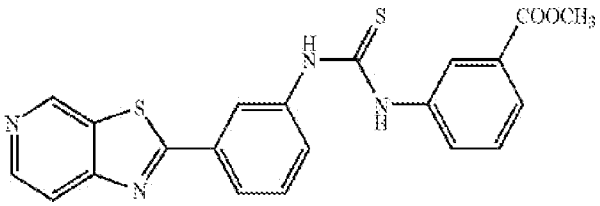

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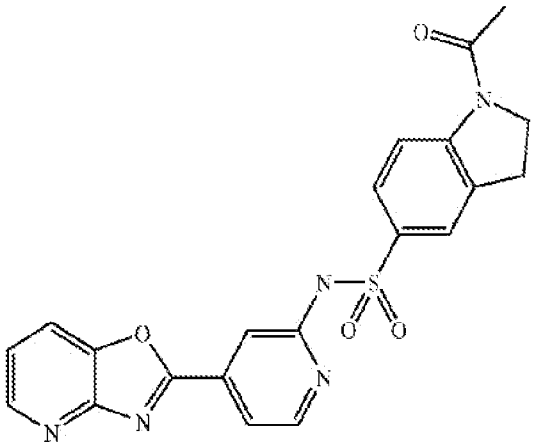

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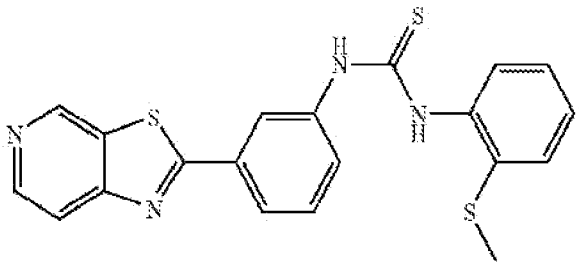

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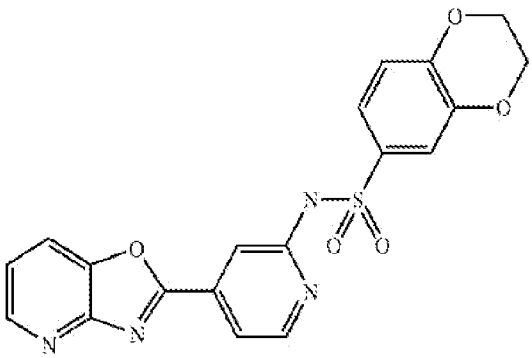

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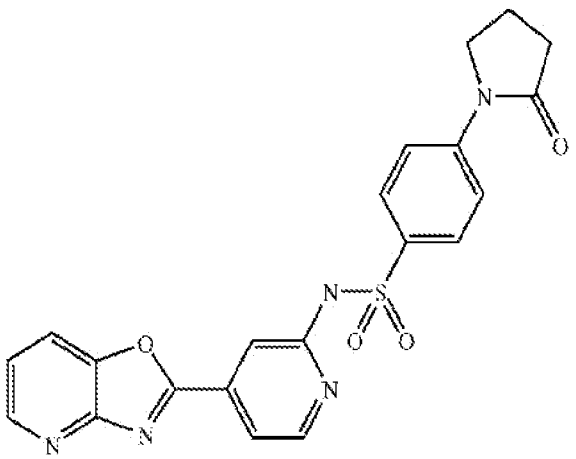
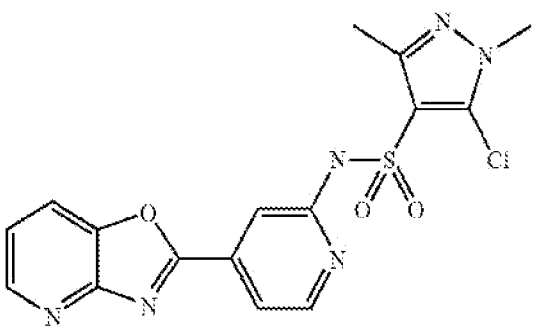
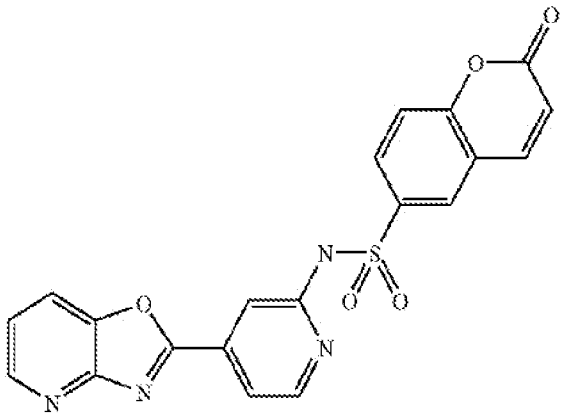
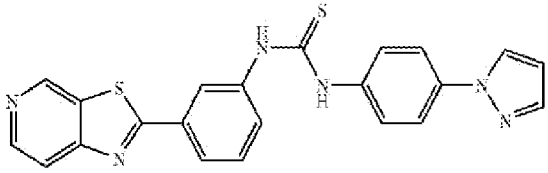
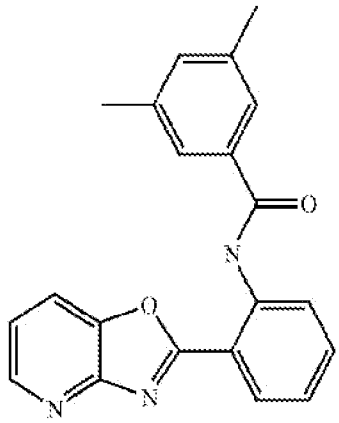
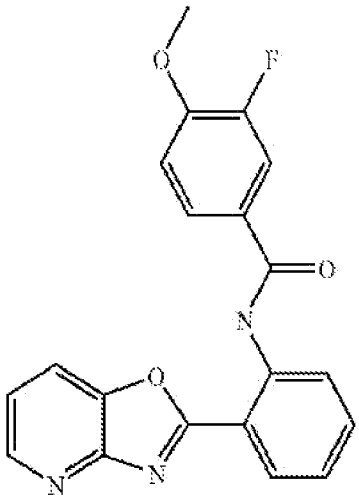
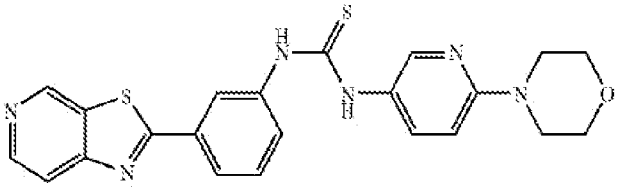
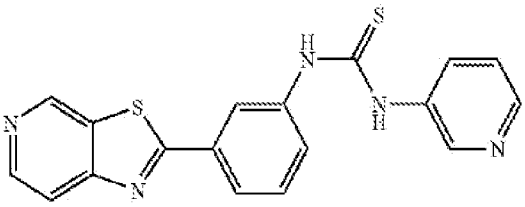
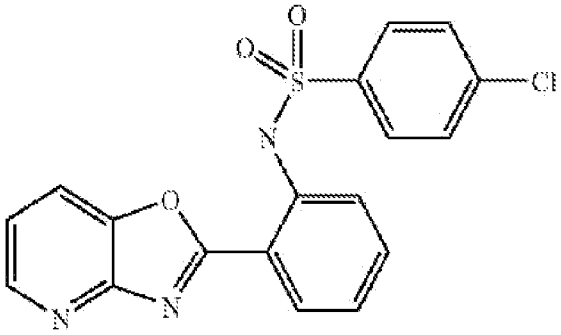
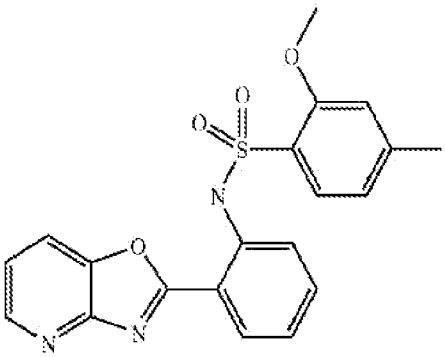
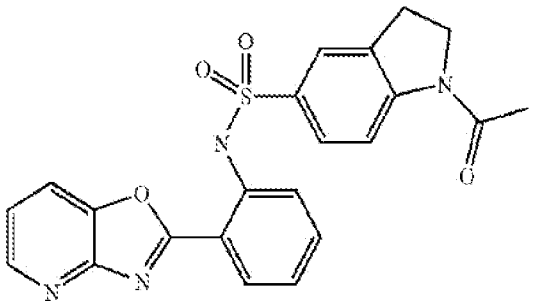




Compound 478

Compound 477	
 Compound 479	 Compound 481
 Compound 482	 Compound 483
 Compound 484	 Compound 485
 Compound 486	 Compound 487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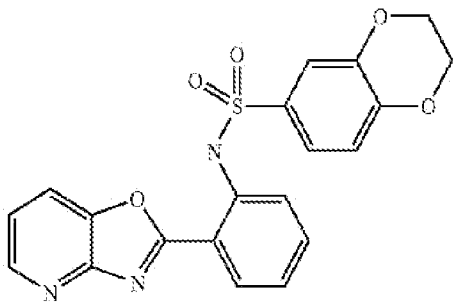
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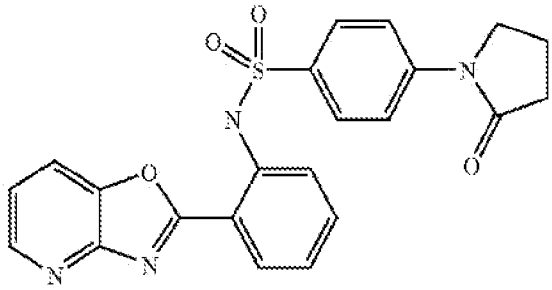
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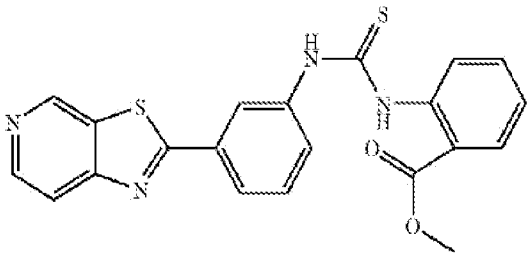
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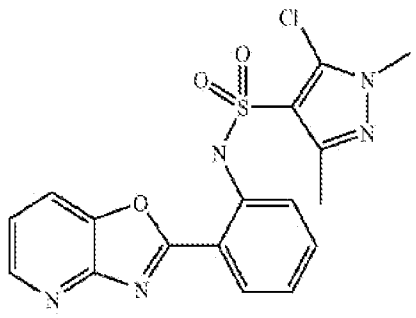
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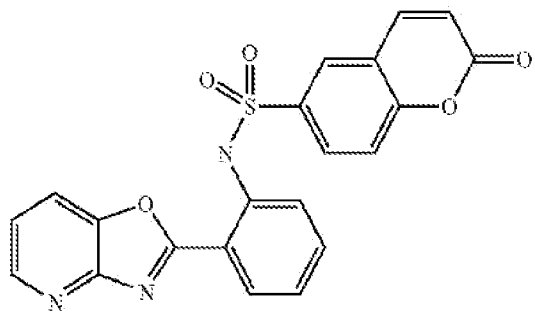
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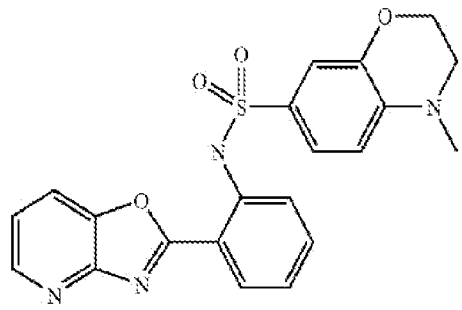
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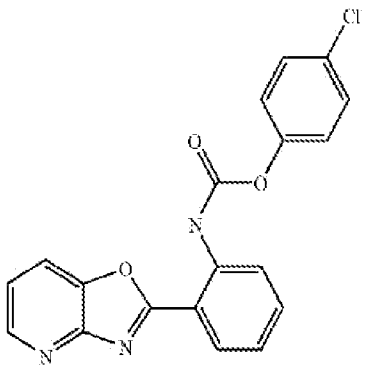
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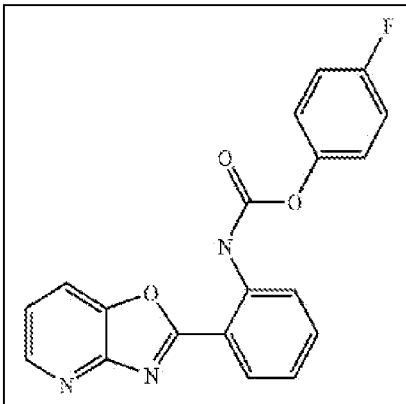
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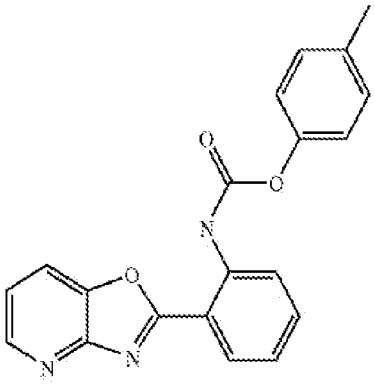
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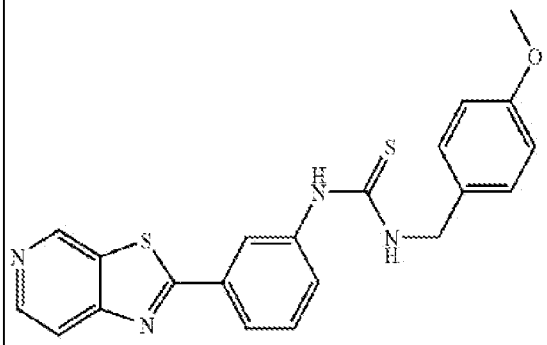
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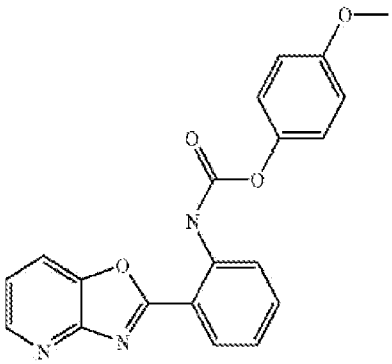
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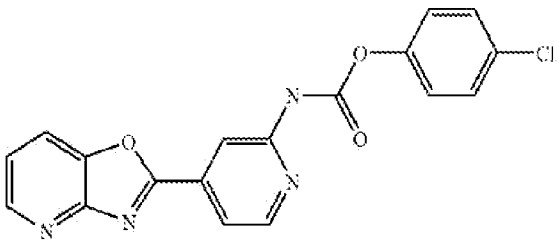
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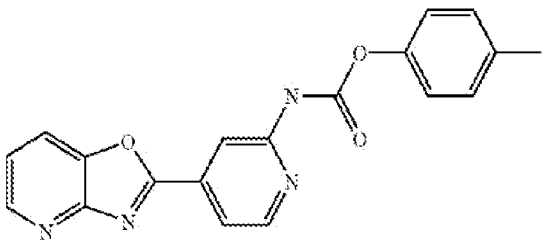
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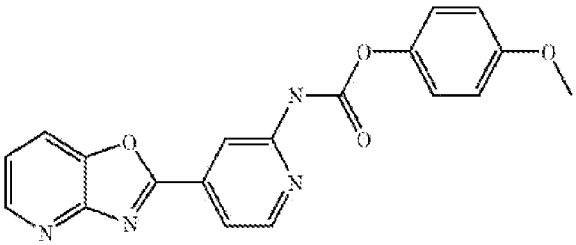
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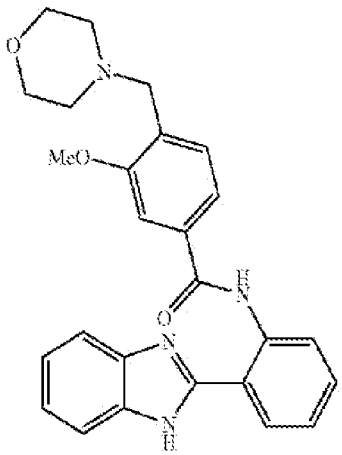
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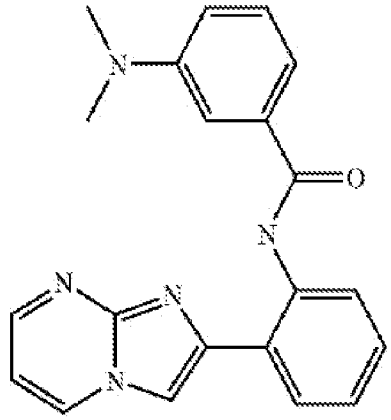

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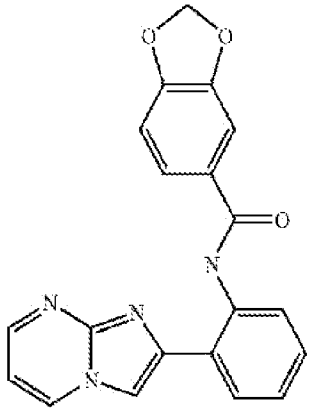

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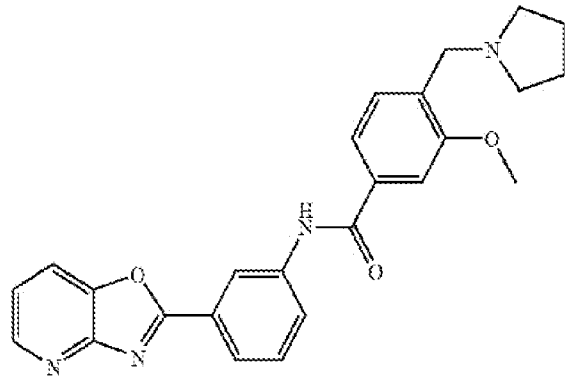

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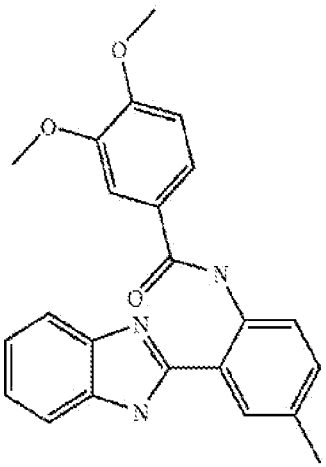

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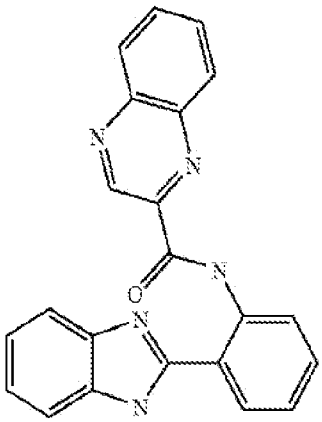

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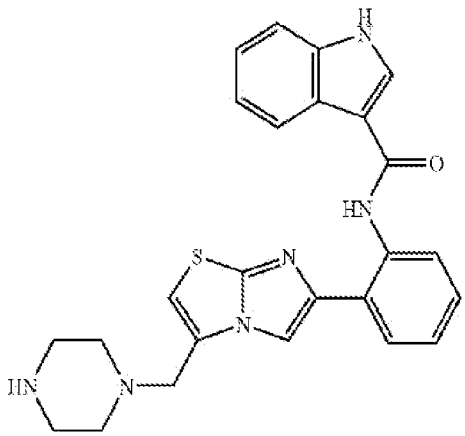

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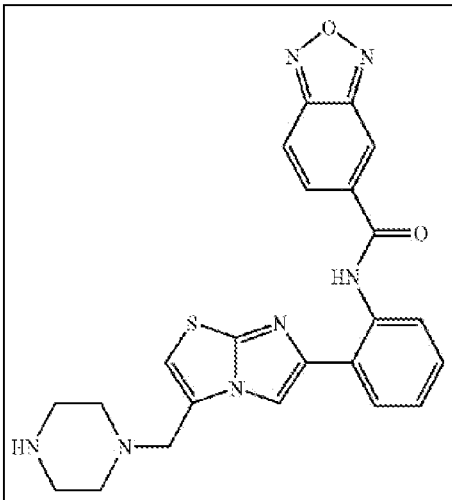

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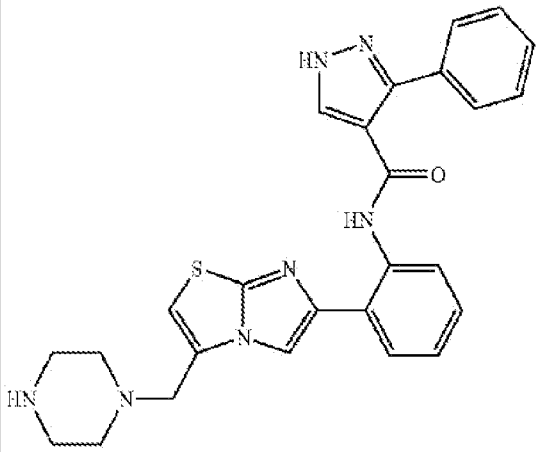

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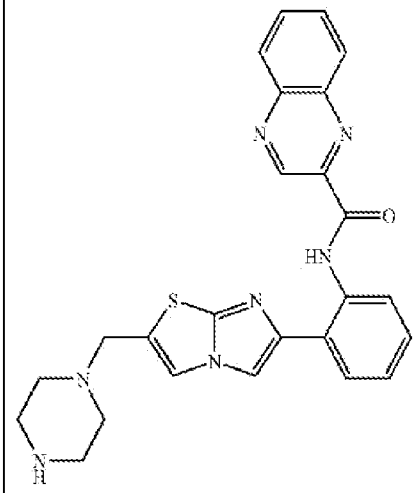

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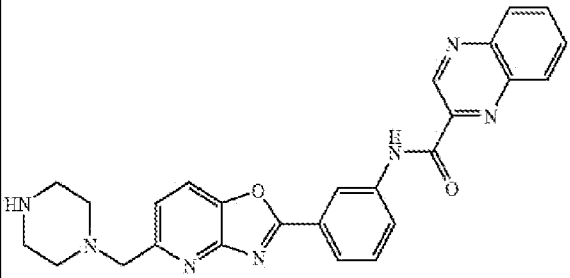

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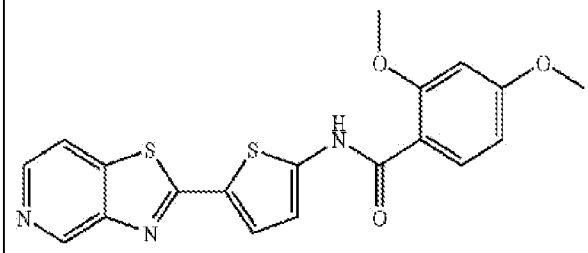

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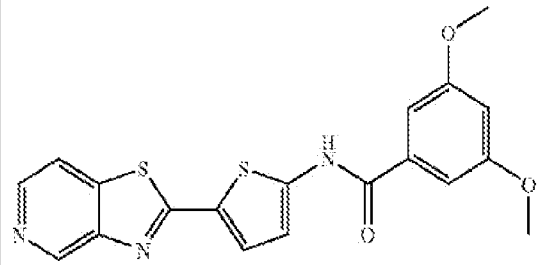

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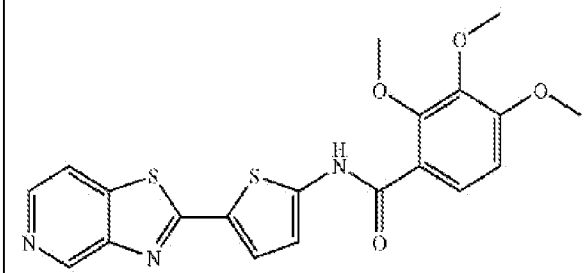

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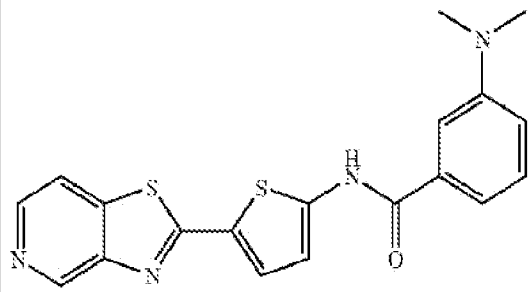

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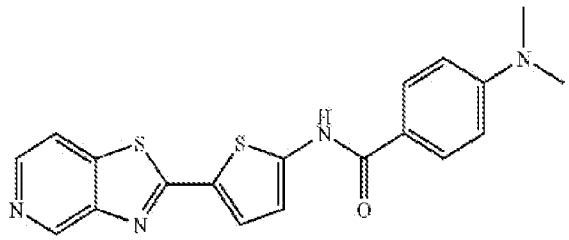

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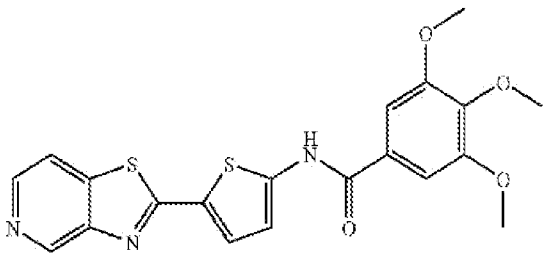

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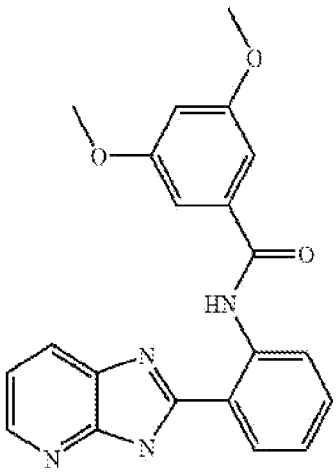

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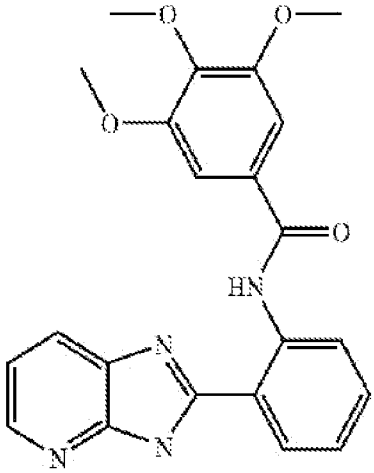

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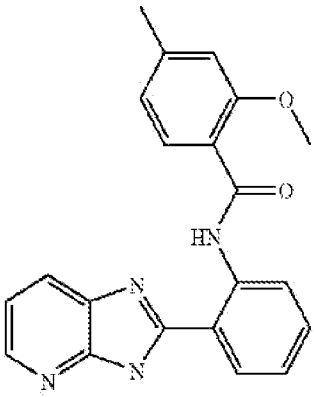

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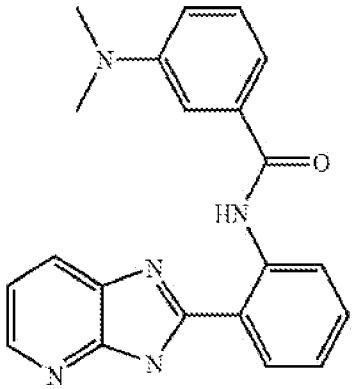

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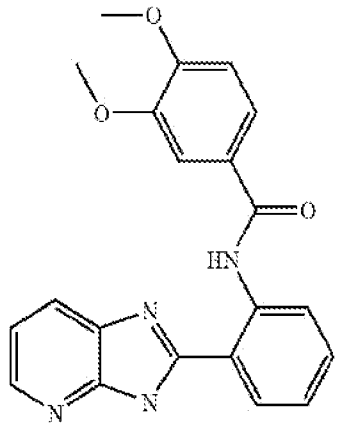

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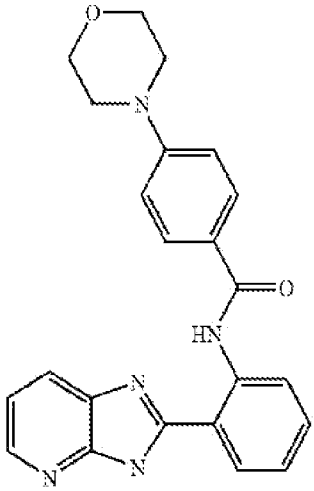

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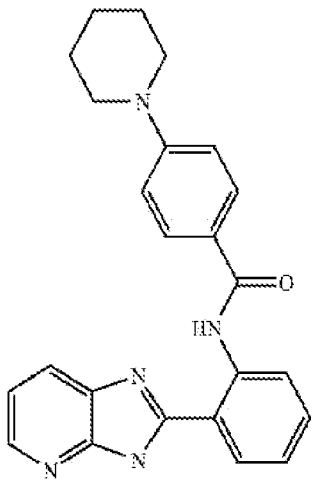
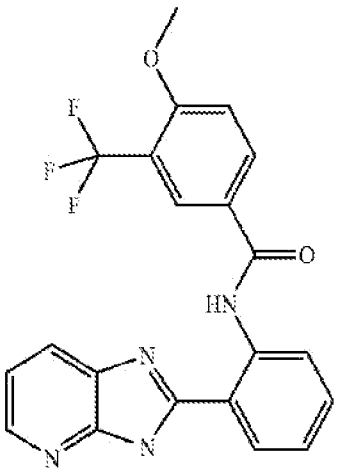
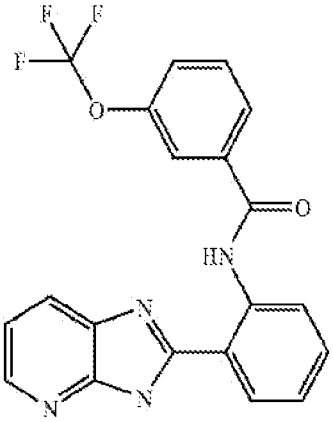
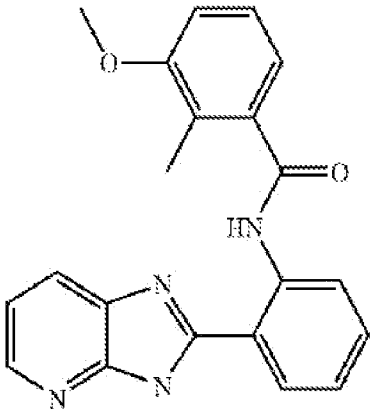
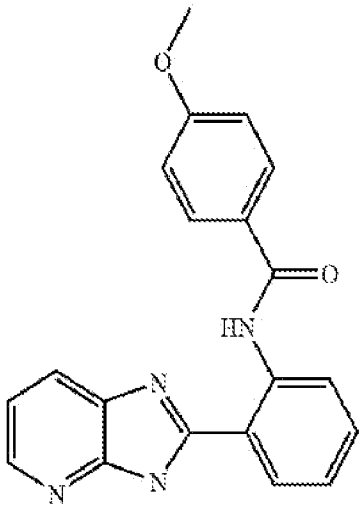
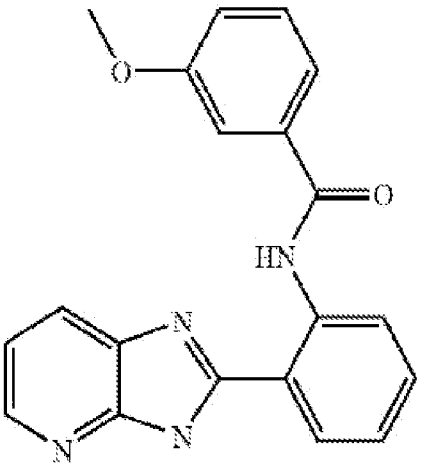
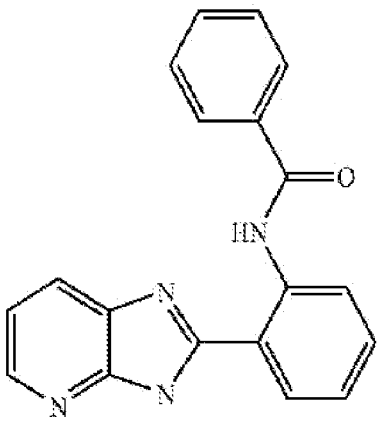
 Compound 527	 Compound 528
 Compound 529	 Compound 530
 Compound 531	 Compound 532



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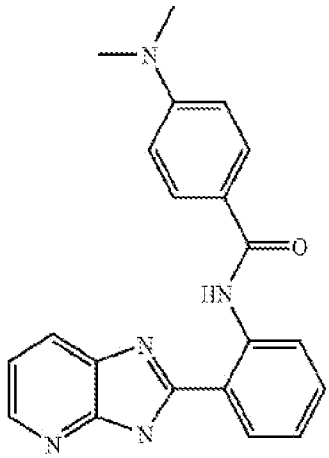

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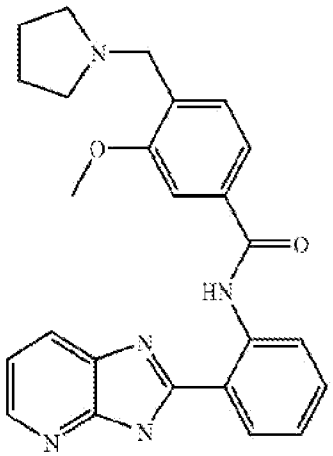

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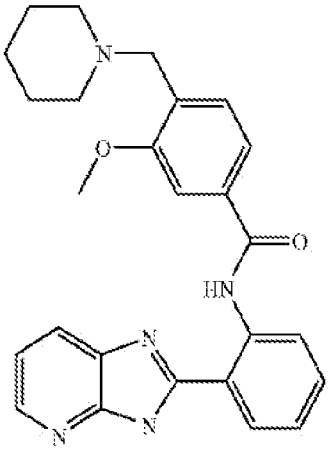

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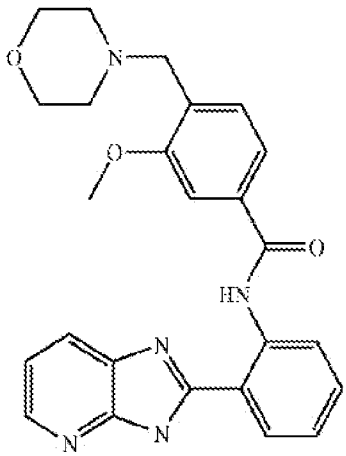

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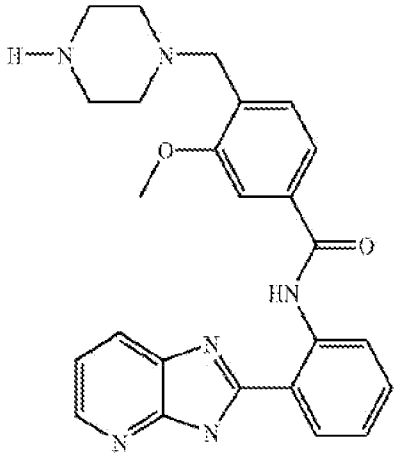

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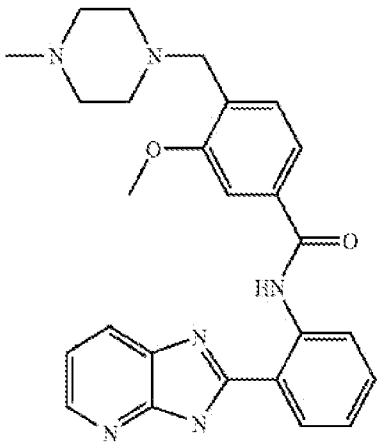

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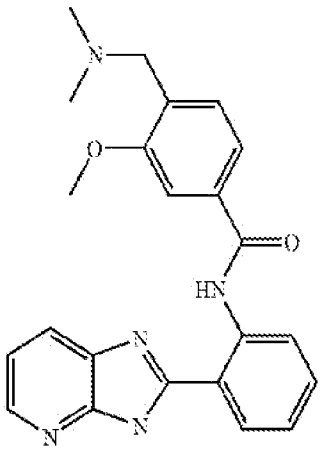

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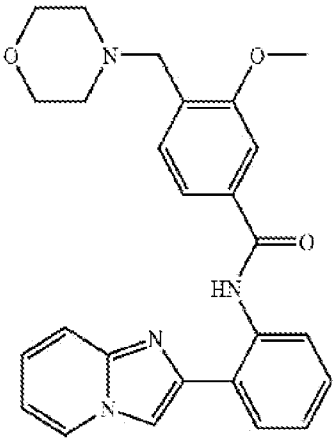

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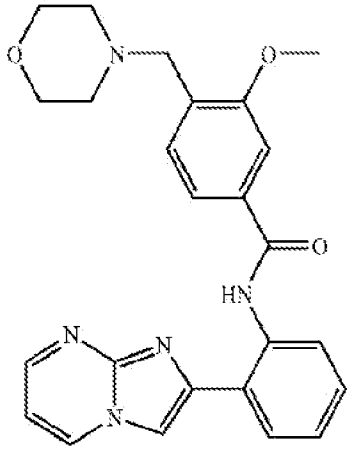

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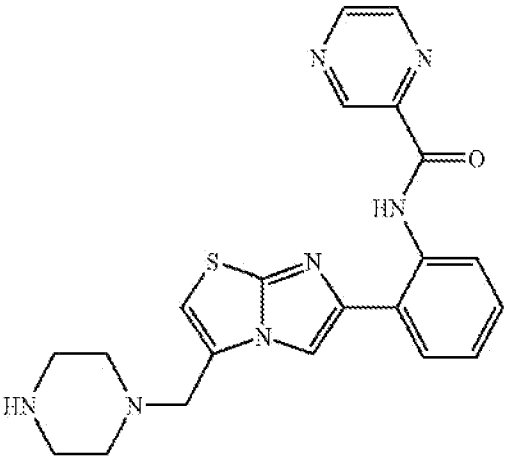

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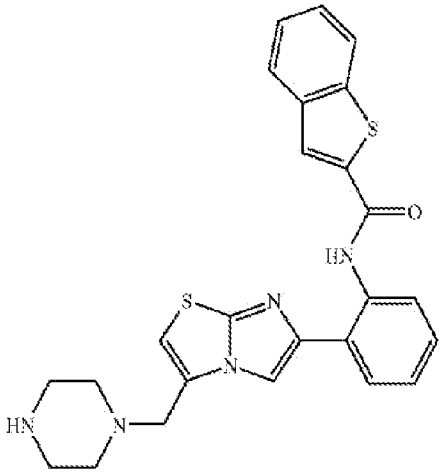

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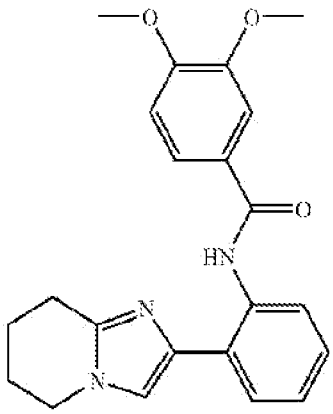
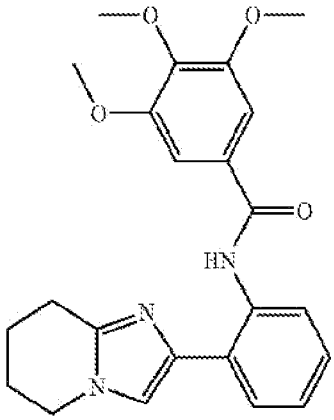
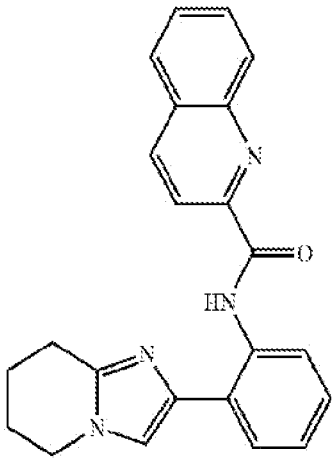
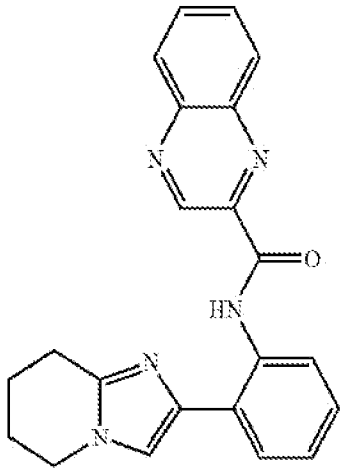
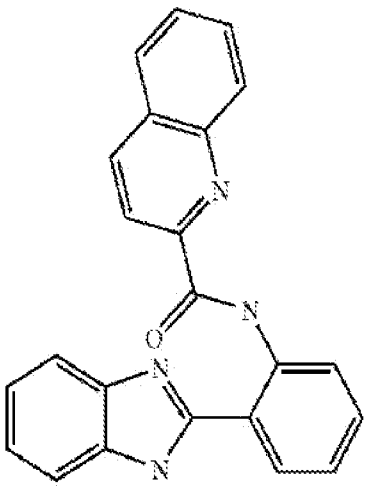
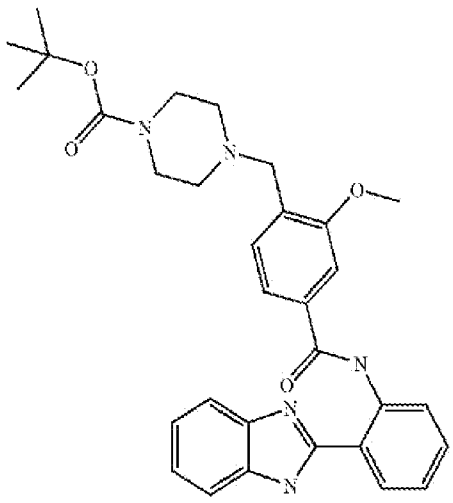
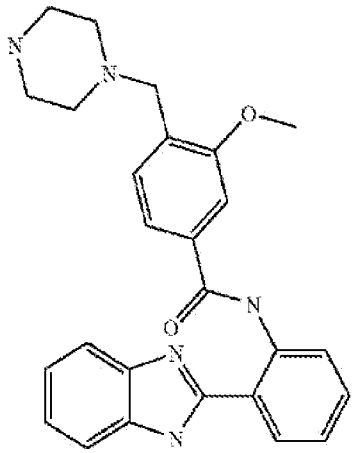
 Compound 545	 Compound 546
 Compound 547	 Compound 548
 Compound 556	 Compound 557



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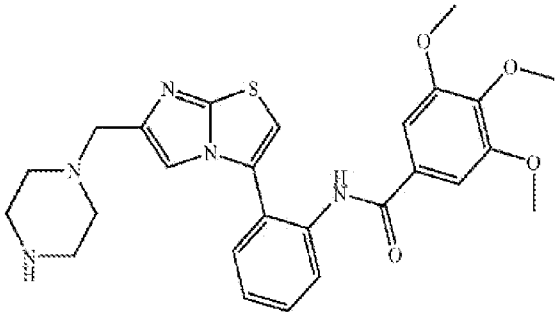

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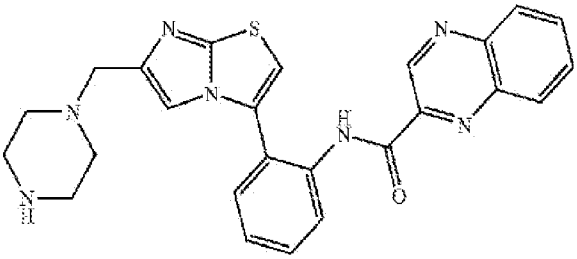

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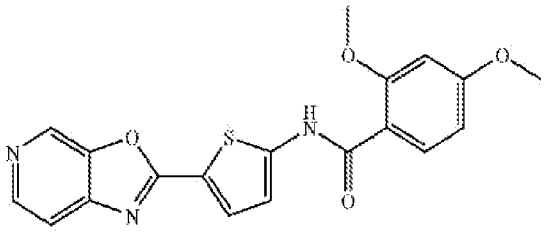

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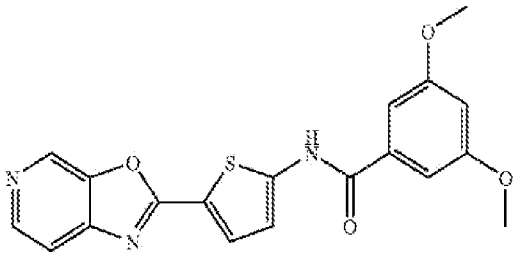

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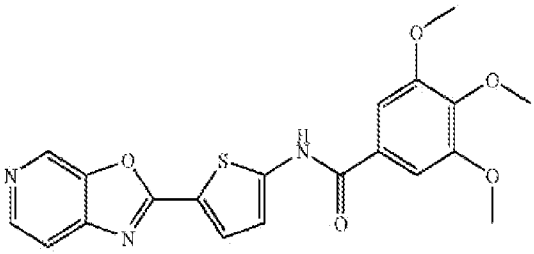

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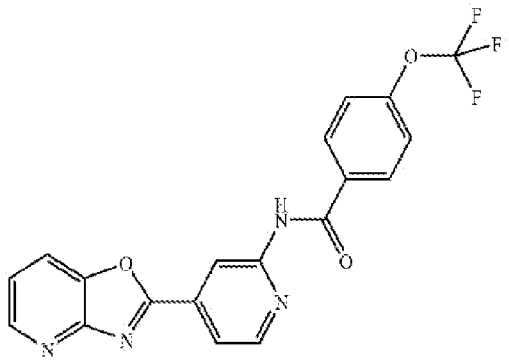

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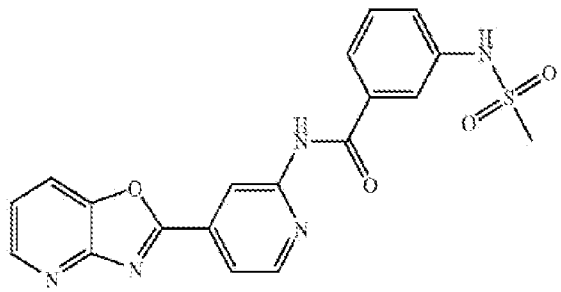

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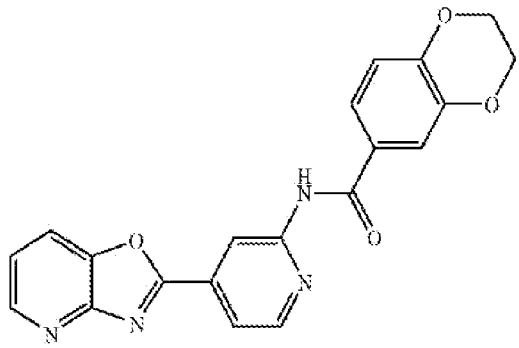

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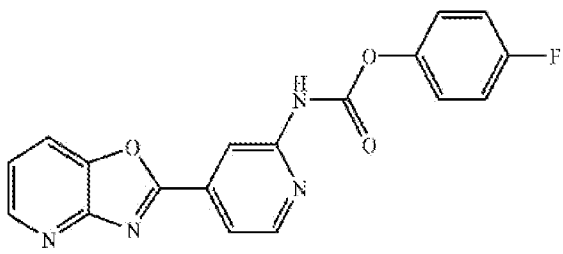

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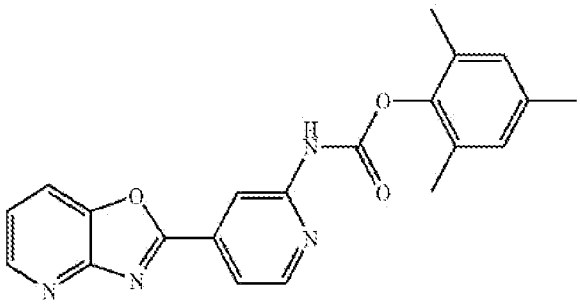

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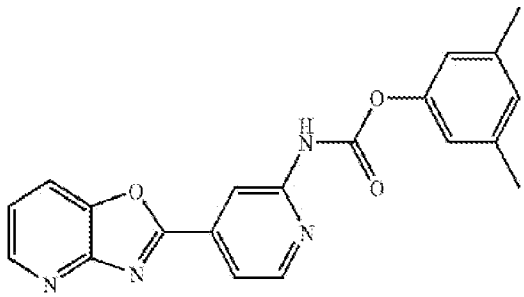

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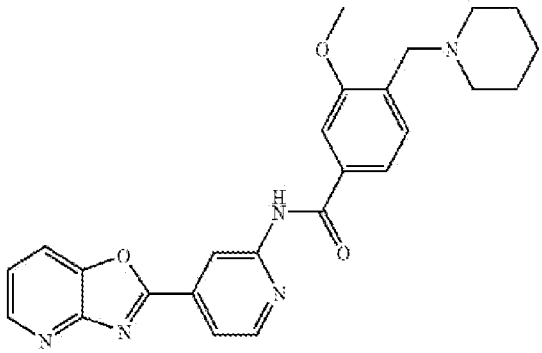

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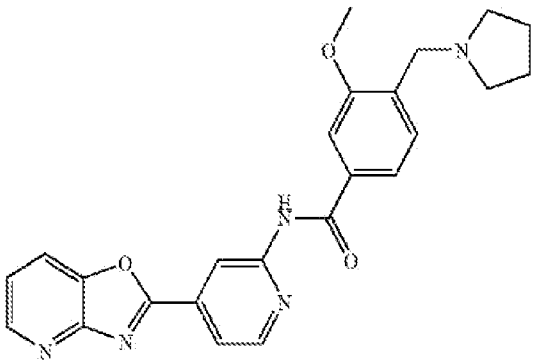

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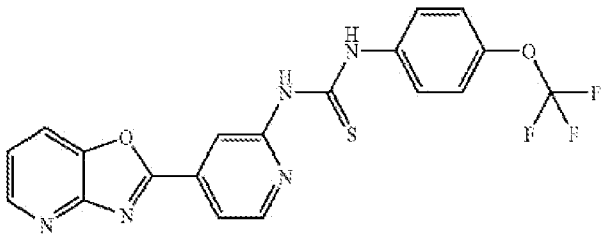

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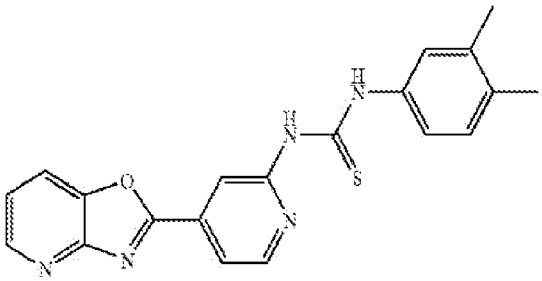

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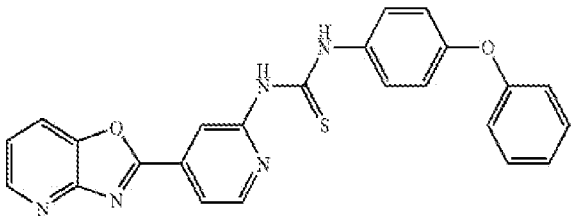

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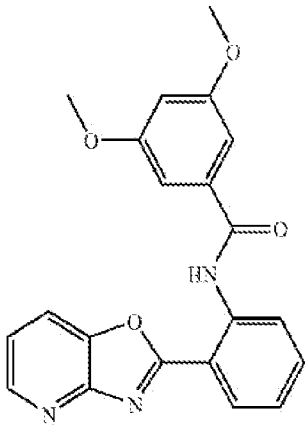

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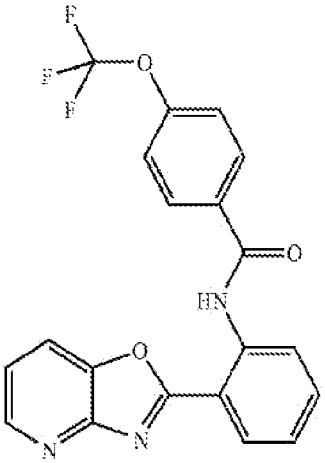

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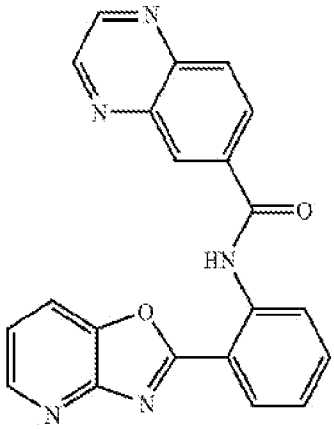

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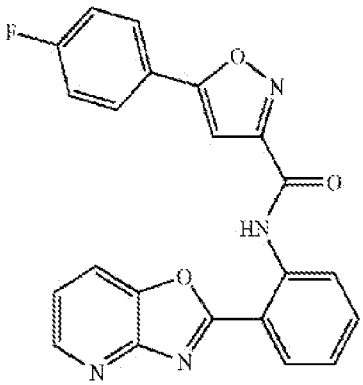

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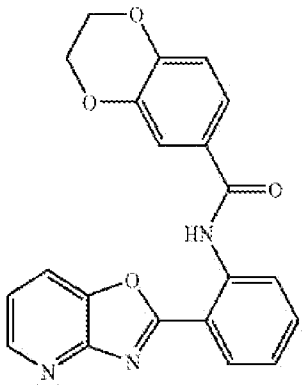

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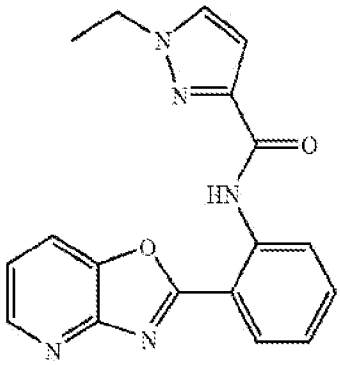

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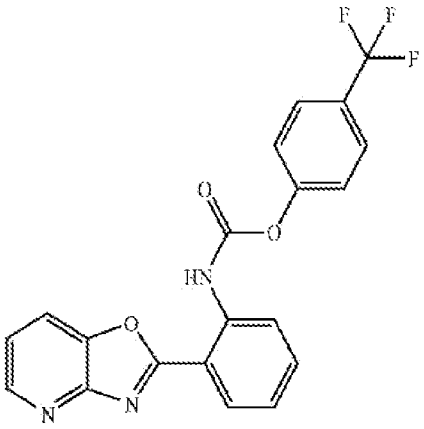

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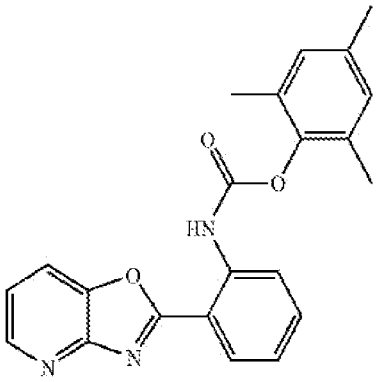
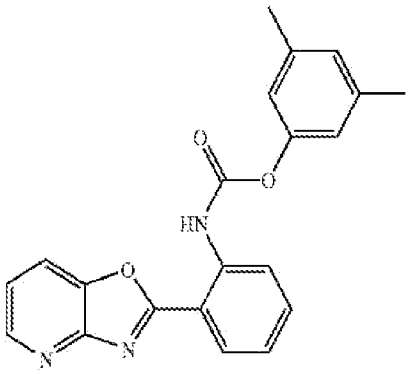
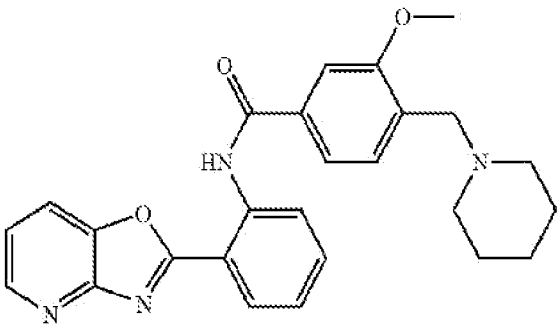
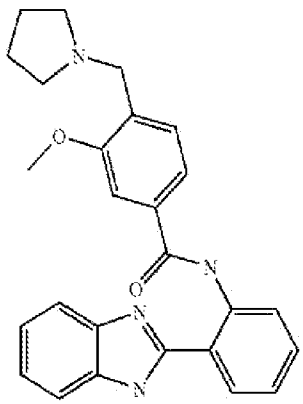
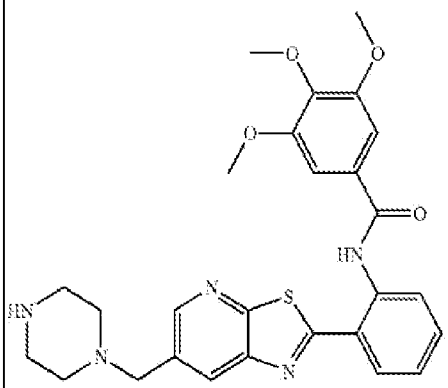
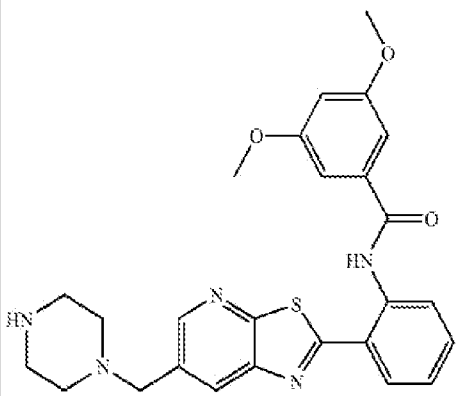
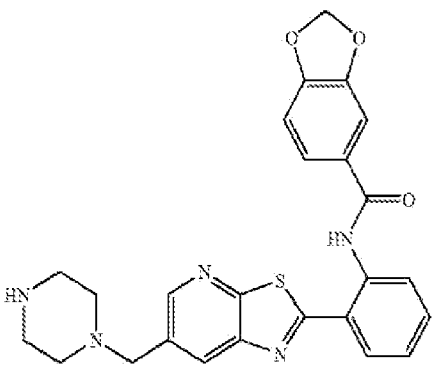
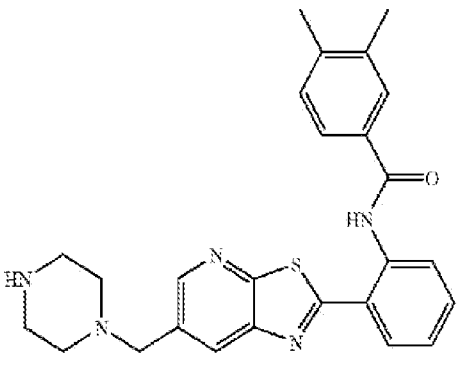
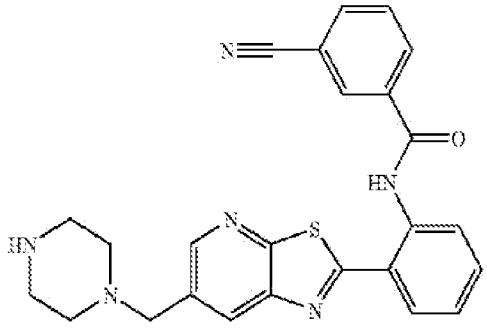
 Compound 583	 Compound 584
 Compound 585	 Compound 587
 Compound 588	 Compound 589
 Compound 590	 Compound 591



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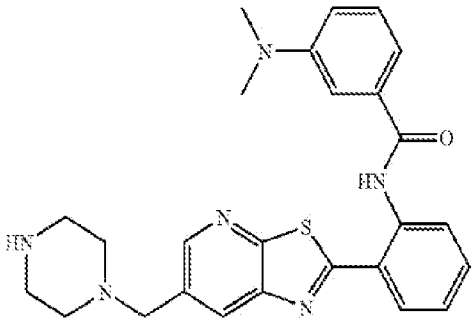

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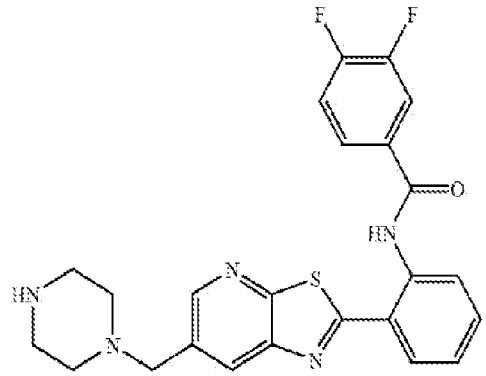
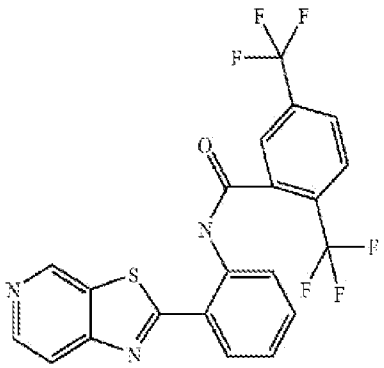


Compound 594



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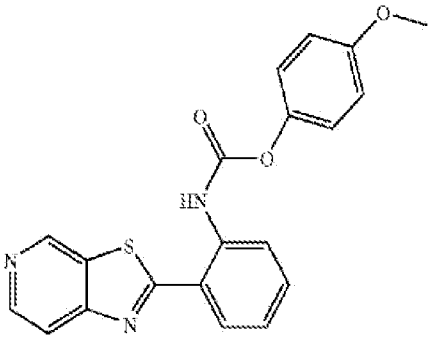

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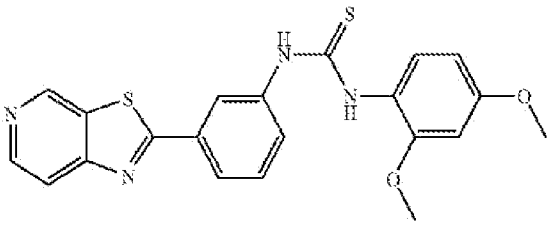

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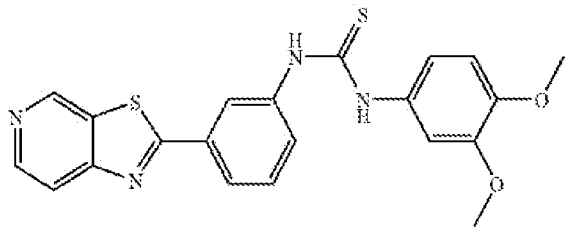

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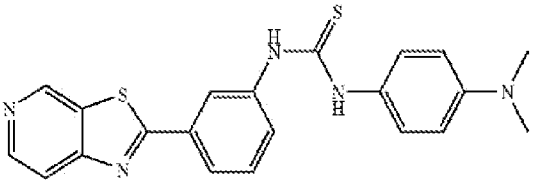

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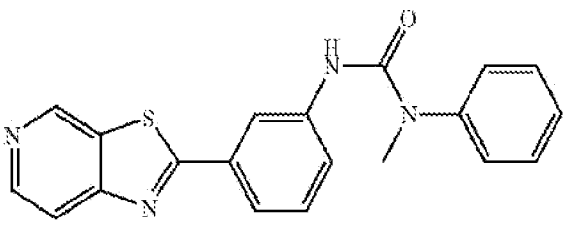

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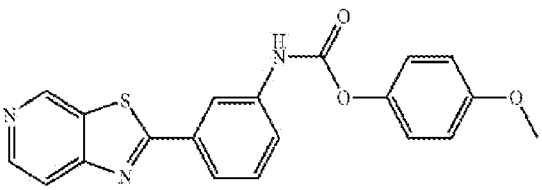

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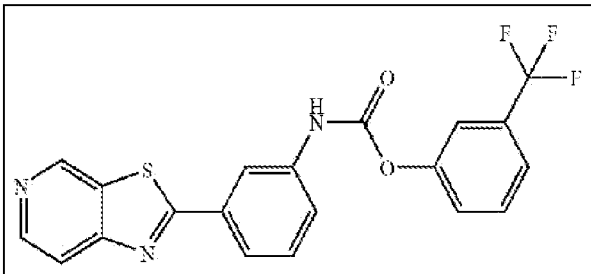
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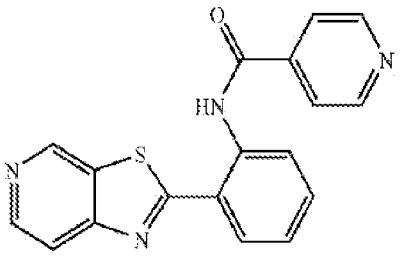
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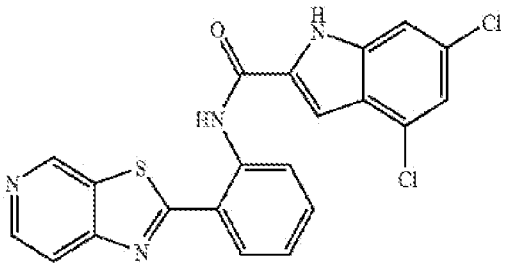
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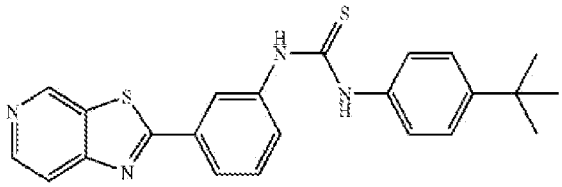
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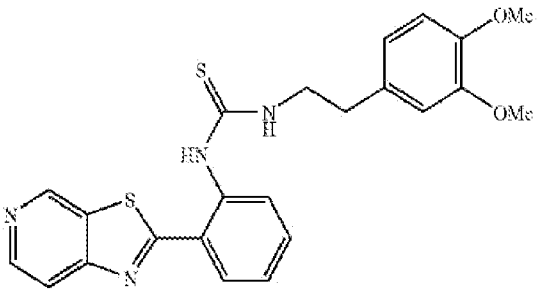
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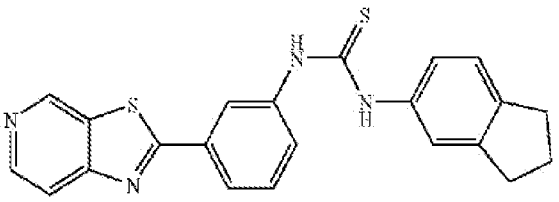
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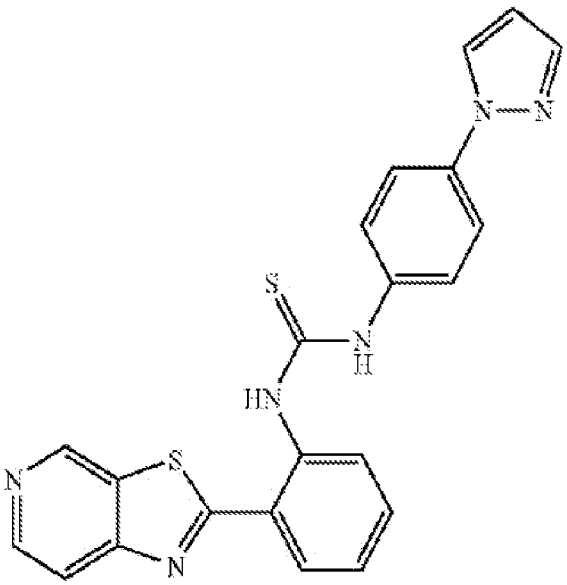
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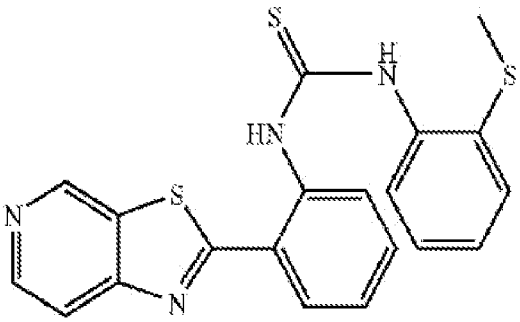
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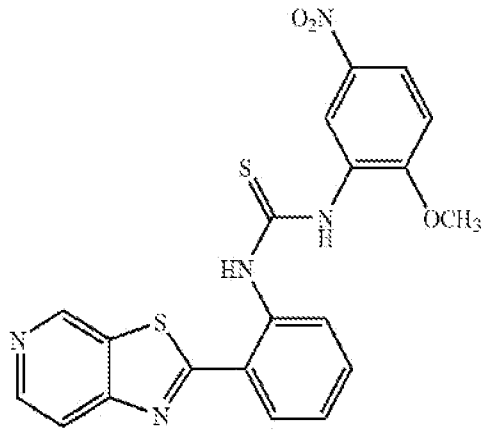

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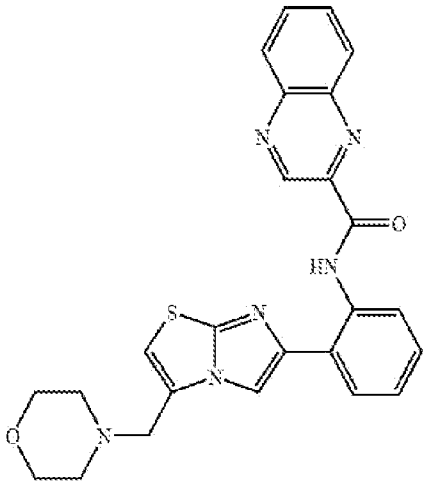

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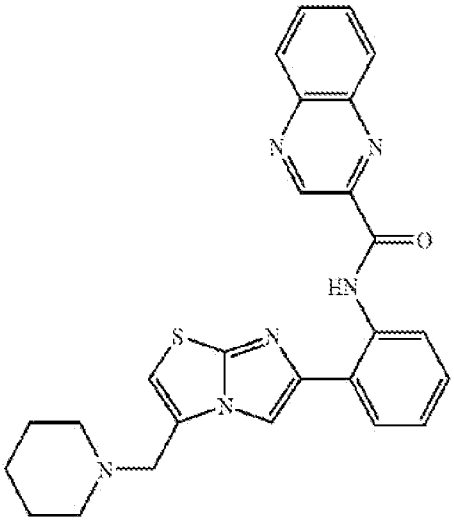

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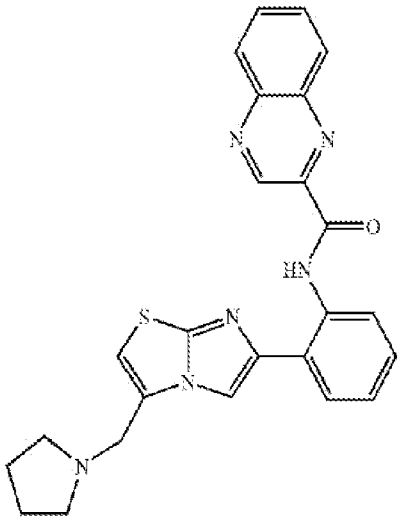

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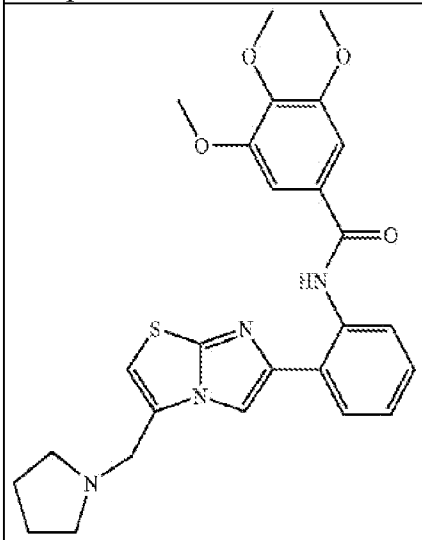

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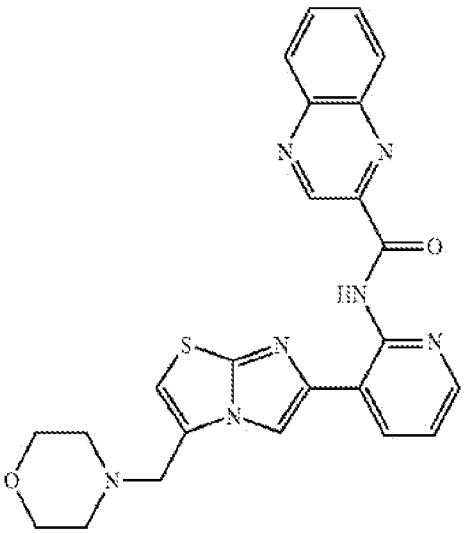

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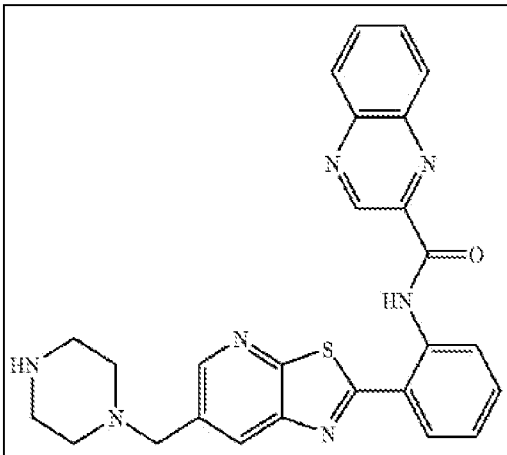

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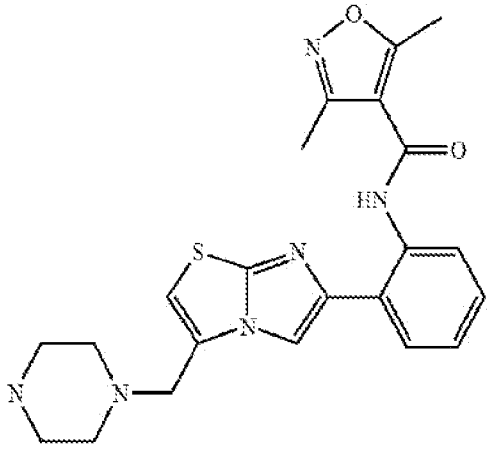

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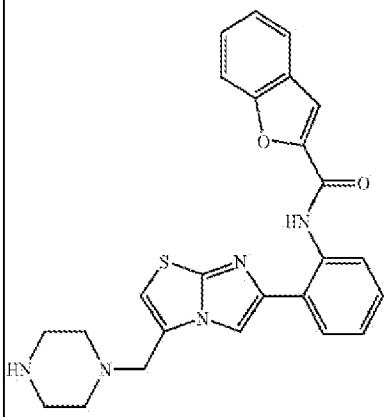

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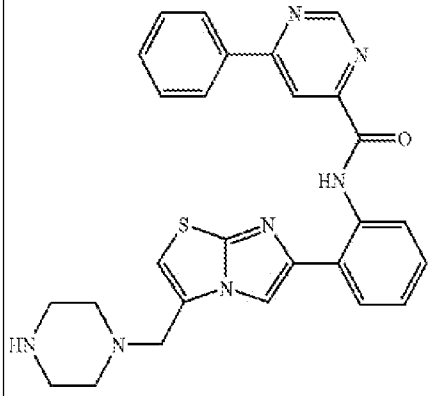

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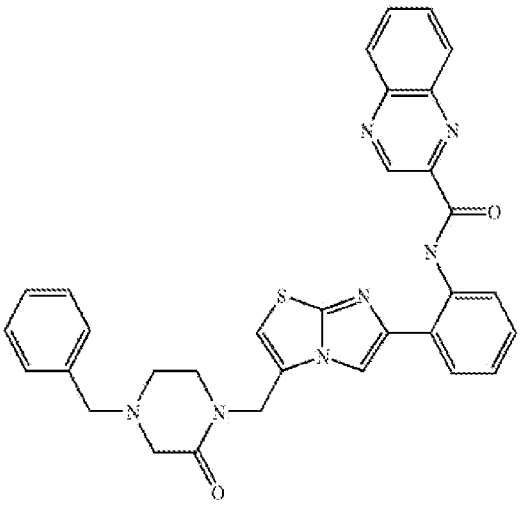

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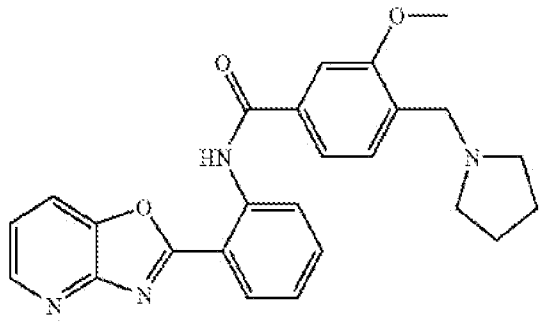

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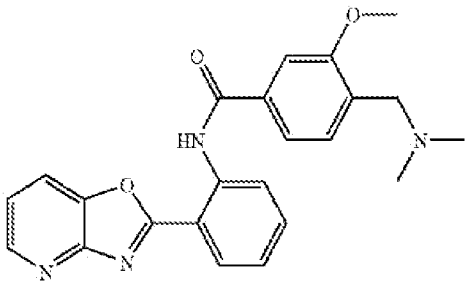

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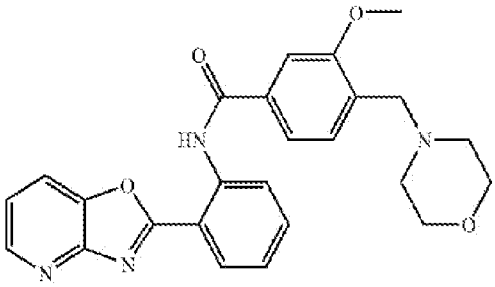

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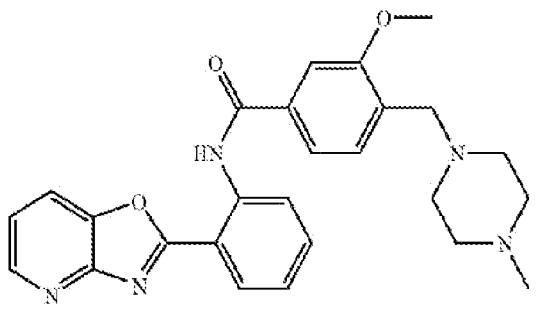
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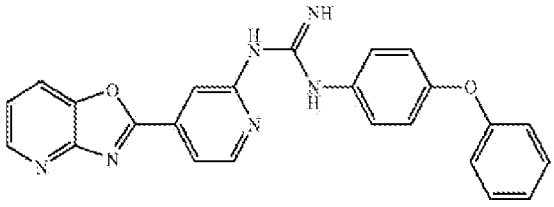
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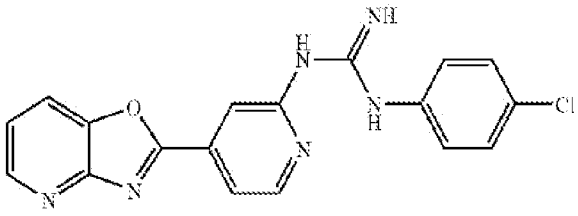
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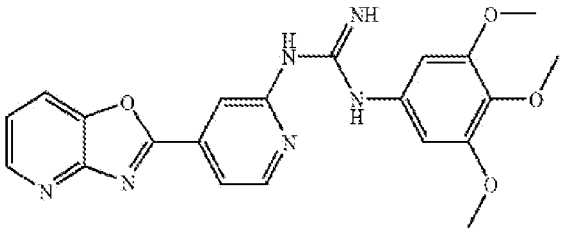
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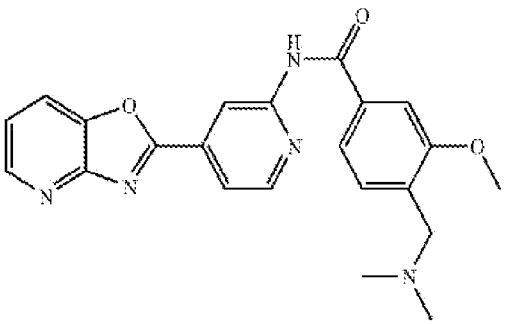
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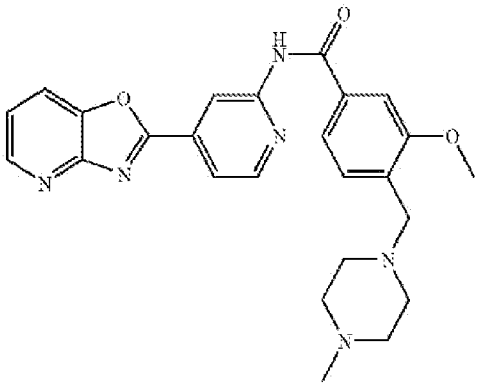
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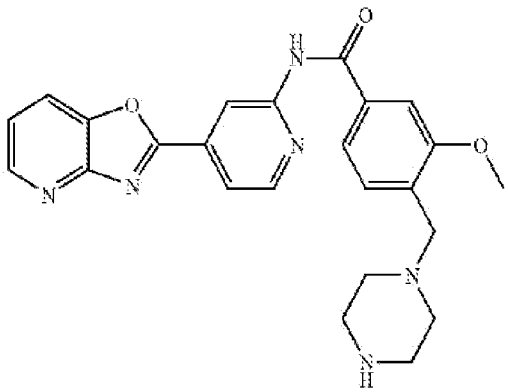
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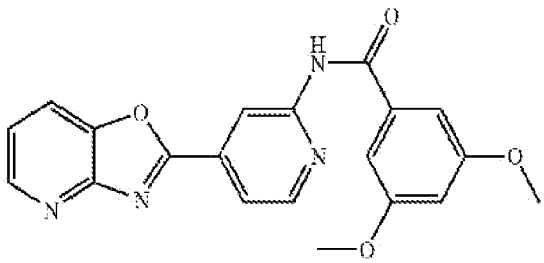
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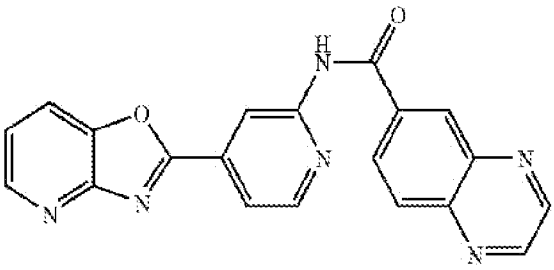
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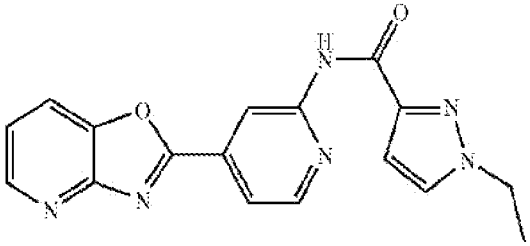
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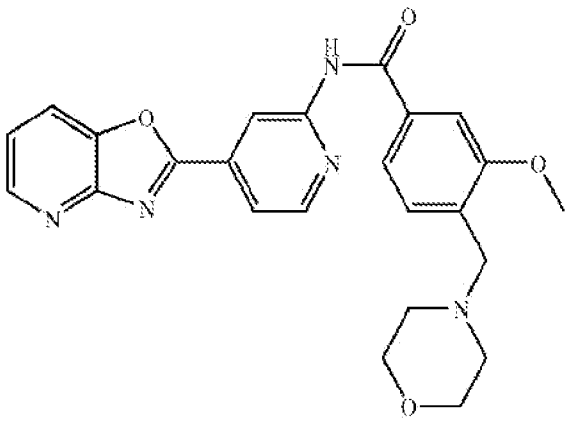

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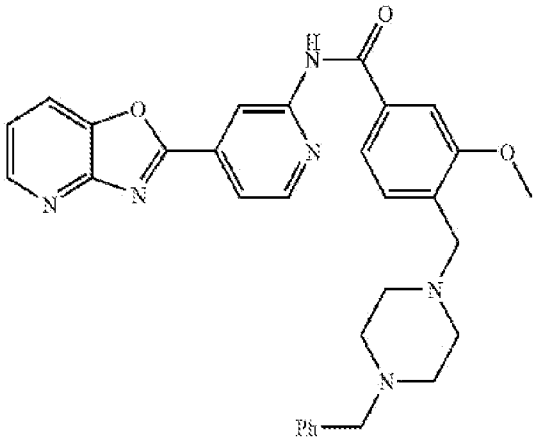

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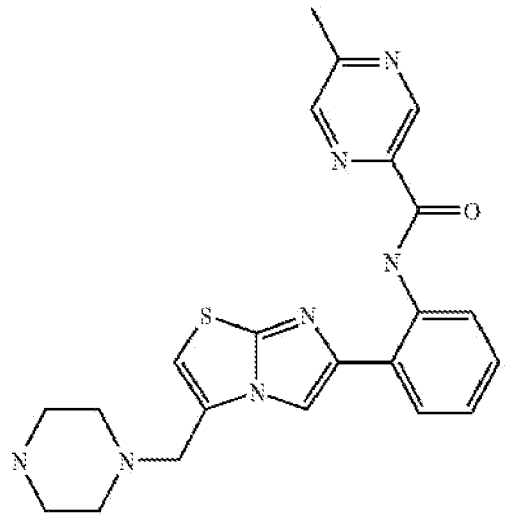

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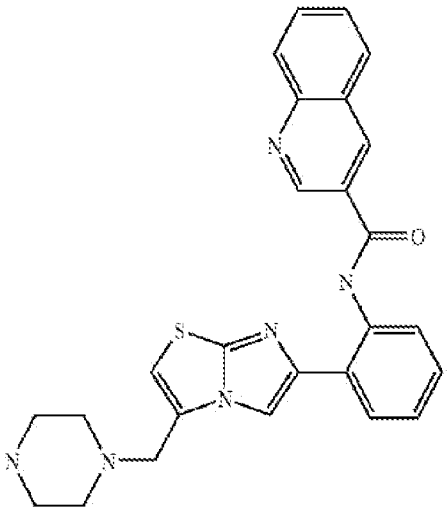

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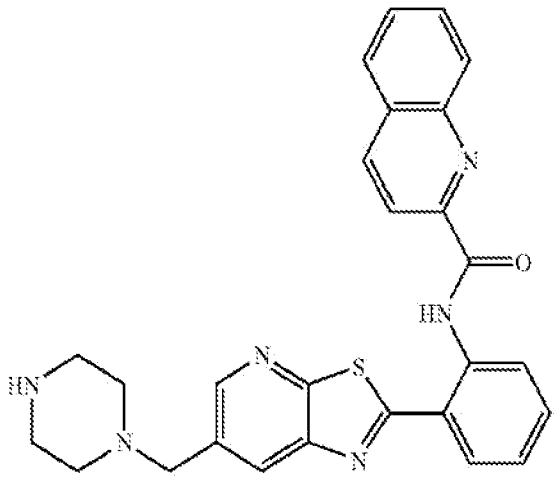

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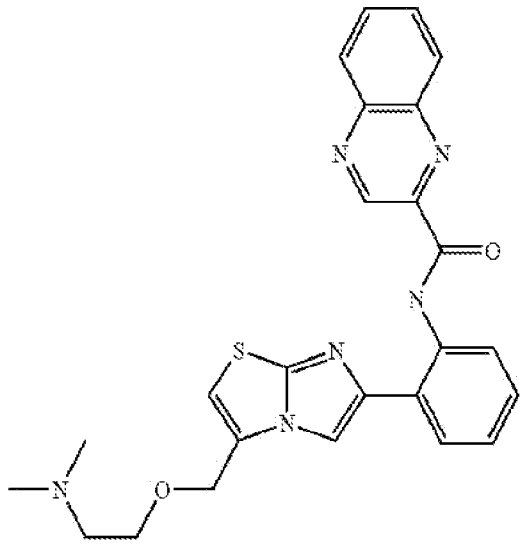

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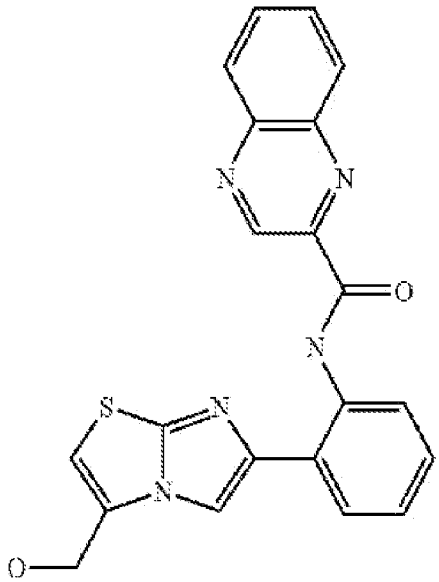

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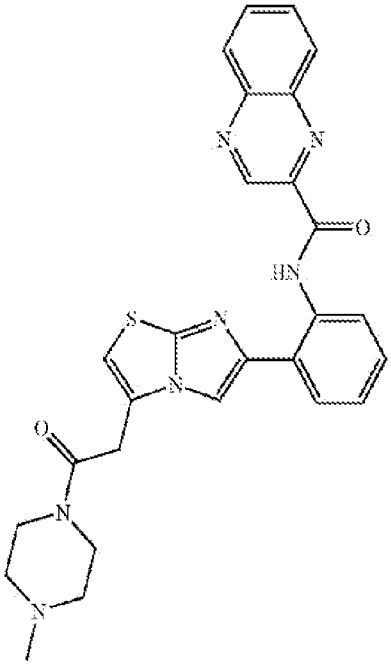

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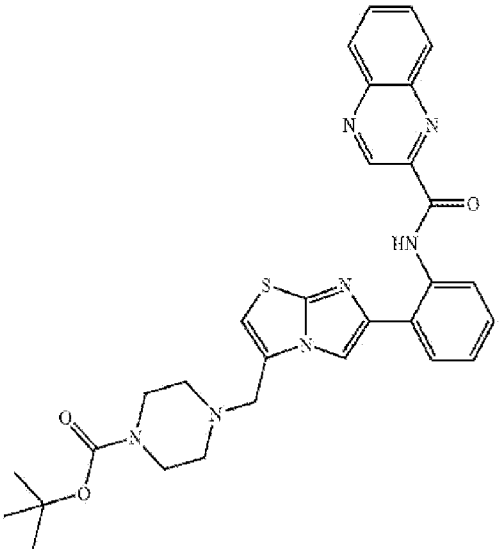

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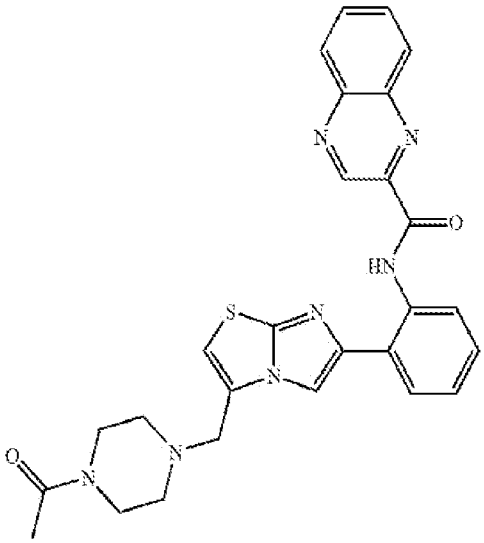

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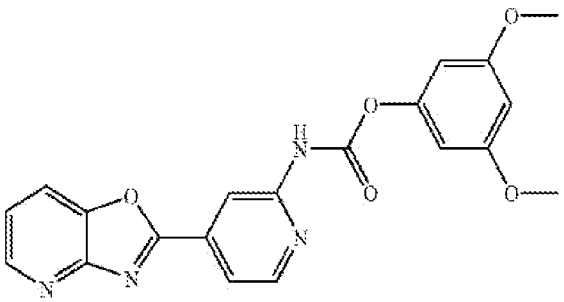

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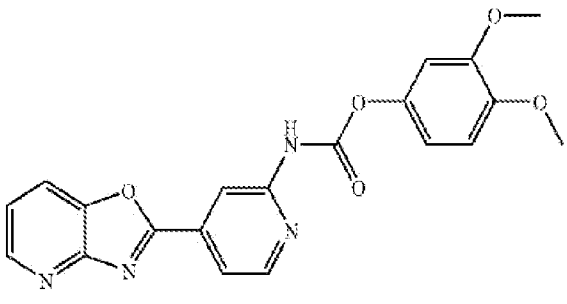

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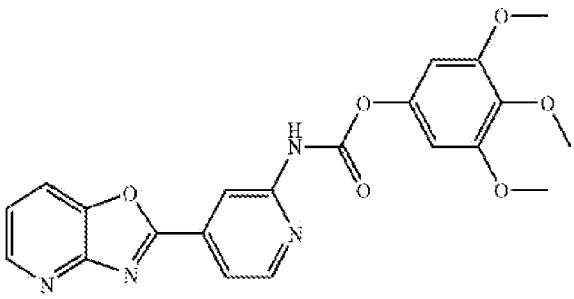
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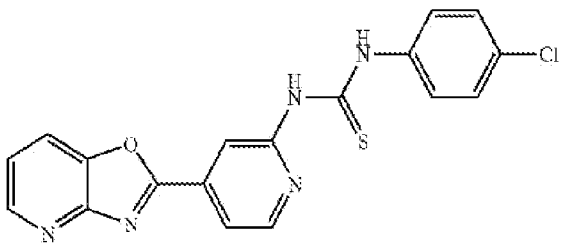
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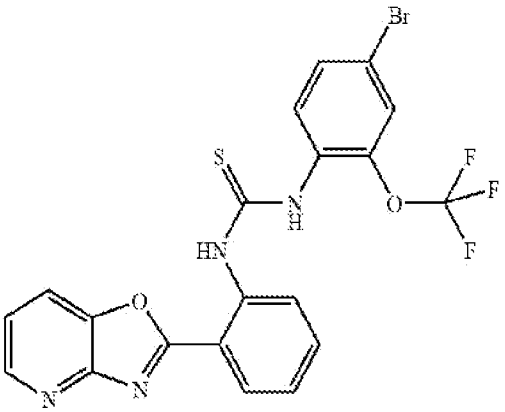
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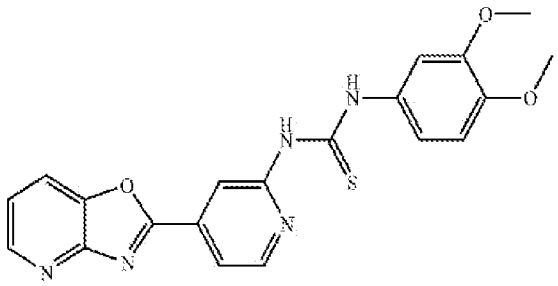
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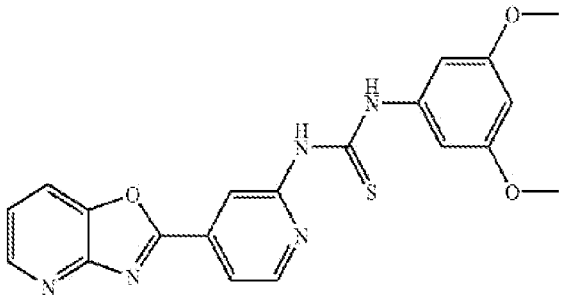
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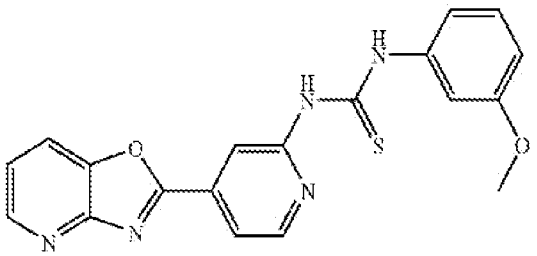
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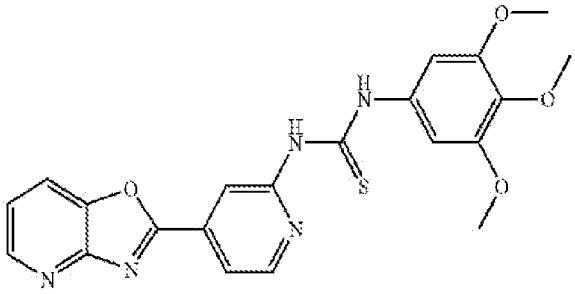
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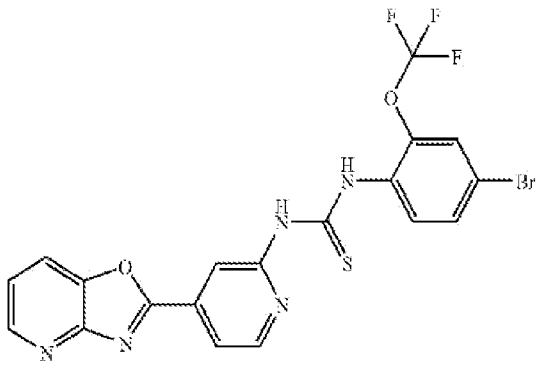
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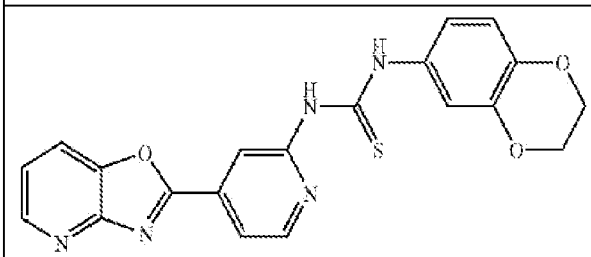
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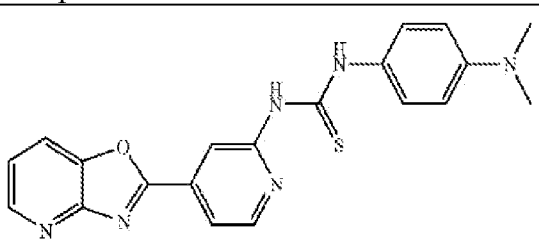
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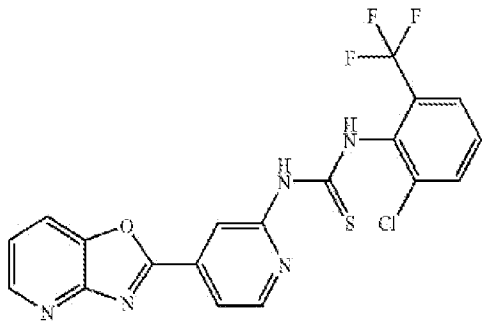

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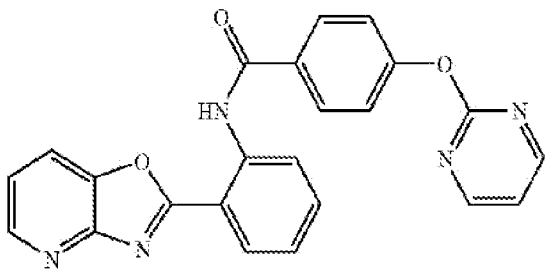

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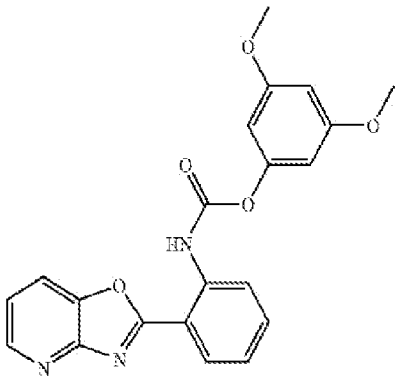

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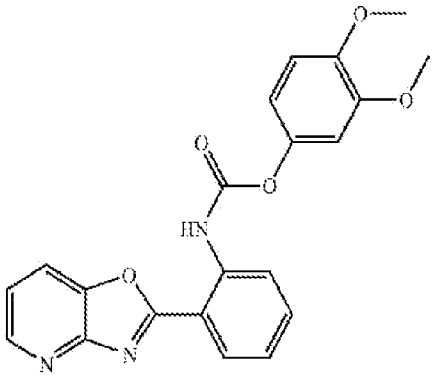

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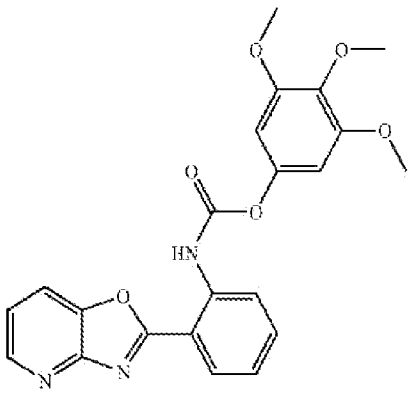

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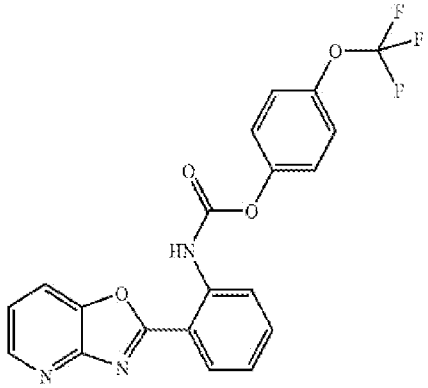

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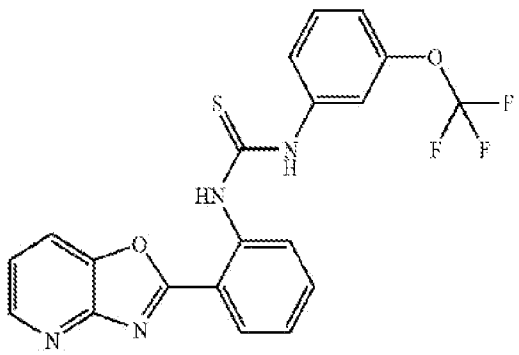

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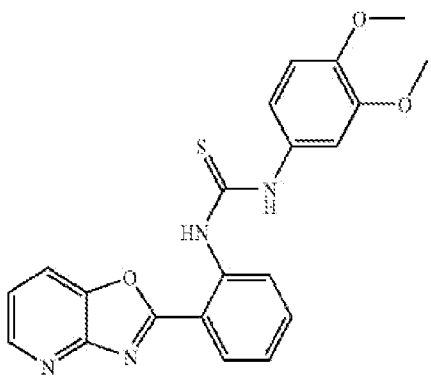

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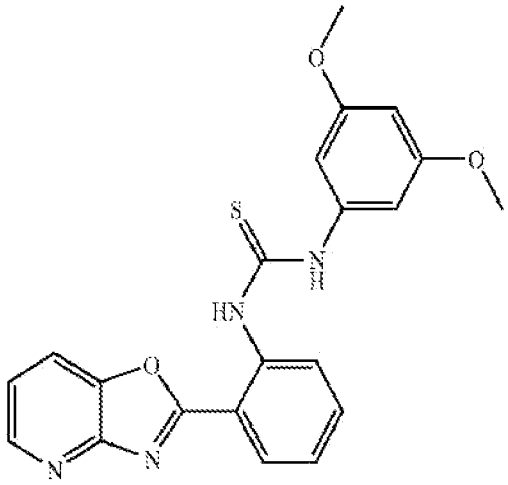

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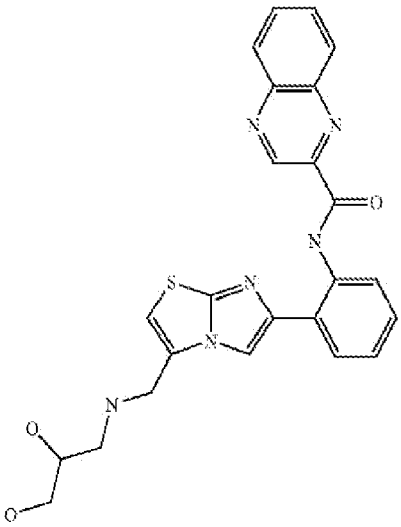

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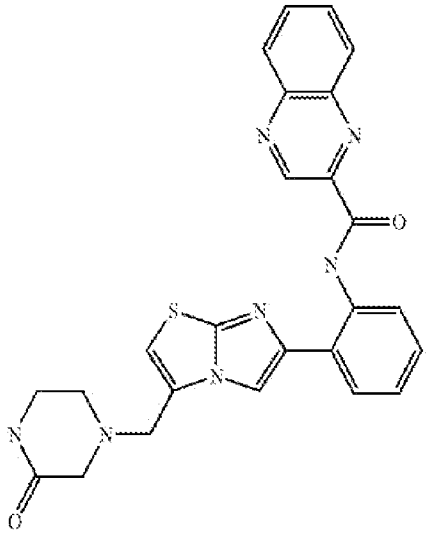

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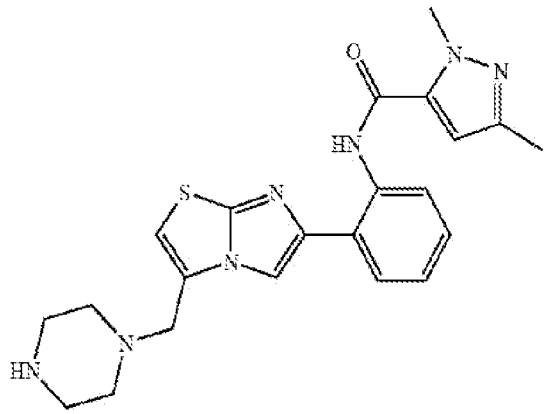

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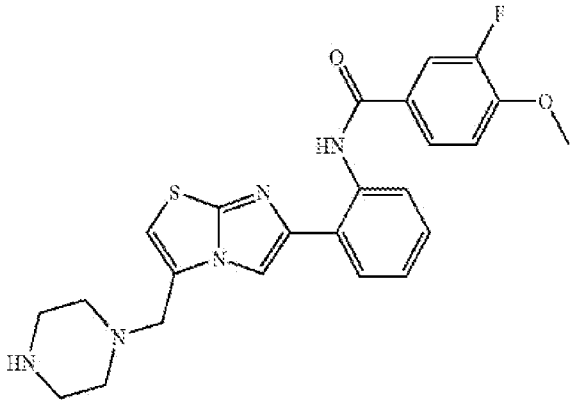

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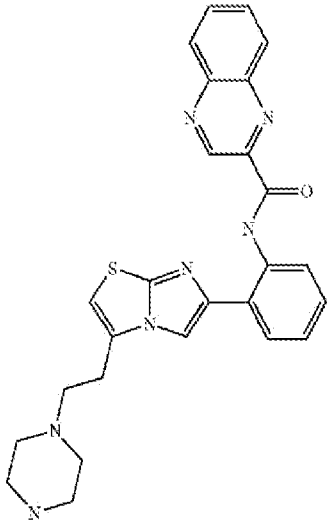

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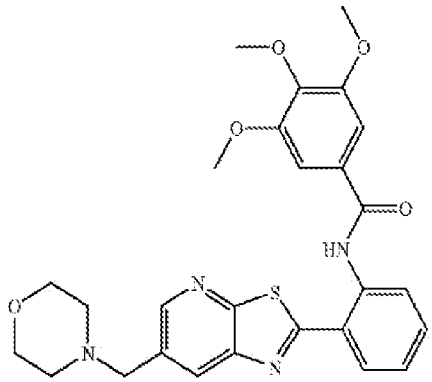
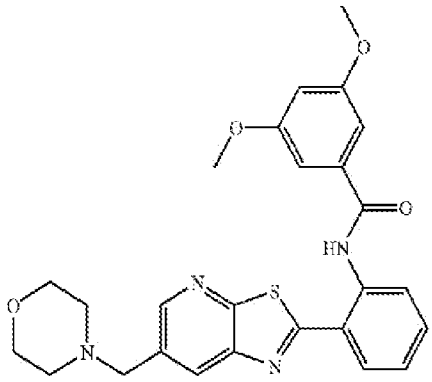
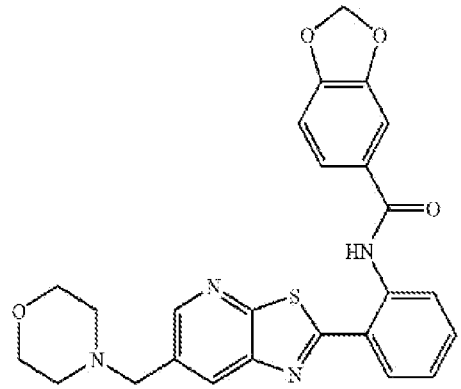
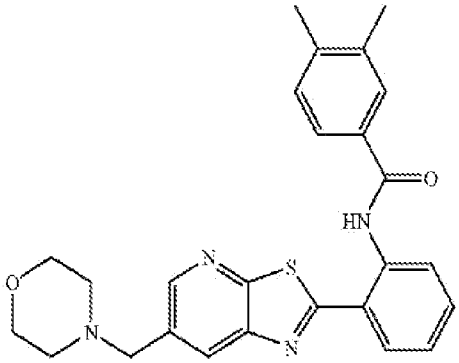
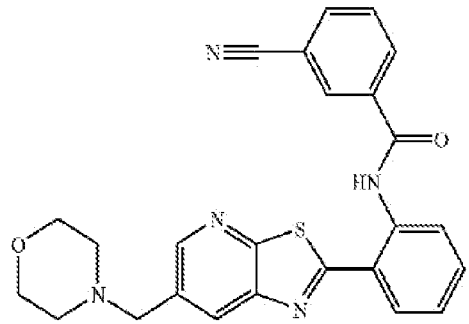
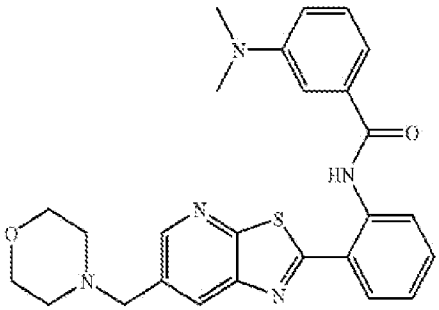
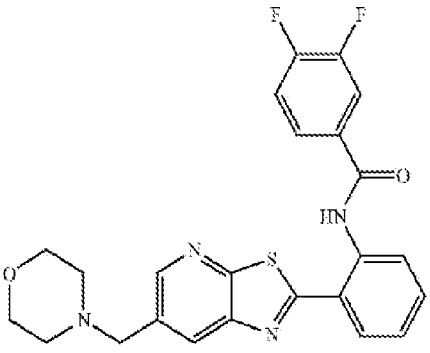
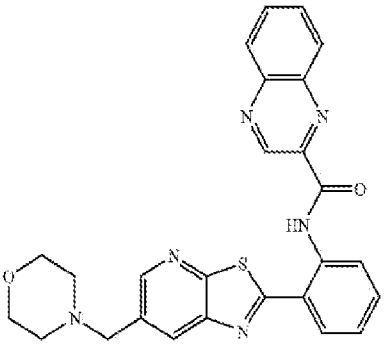
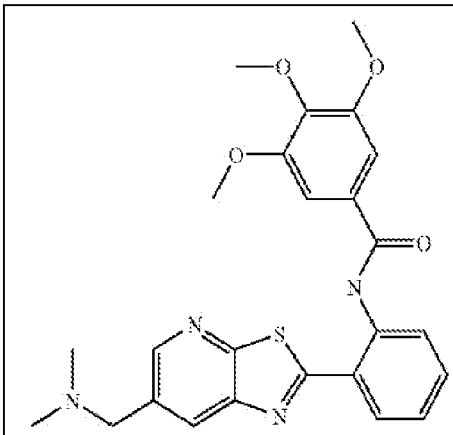
 Compound 681	 Compound 682
 Compound 683	 Compound 684
 Compound 685	 Compound 686
 Compound 687	 Compound 688



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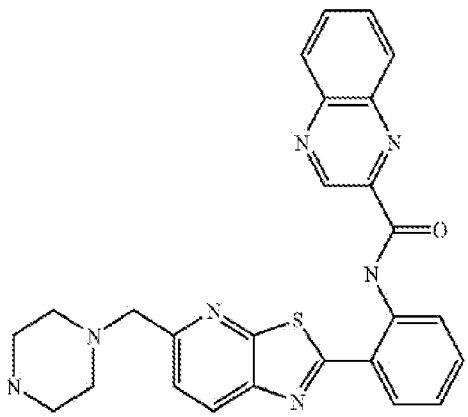

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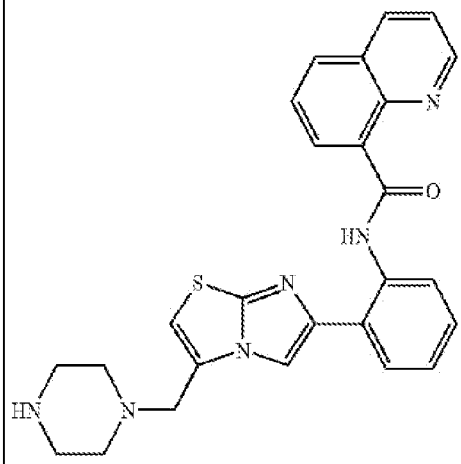

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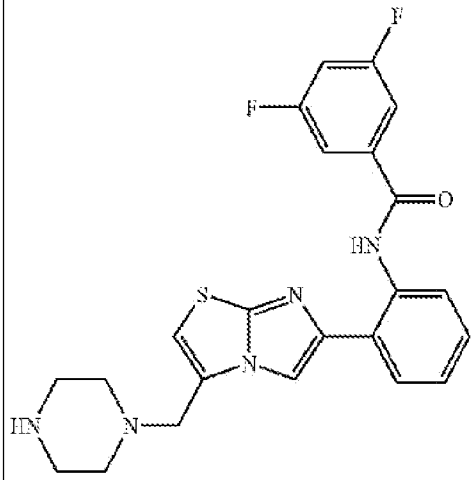

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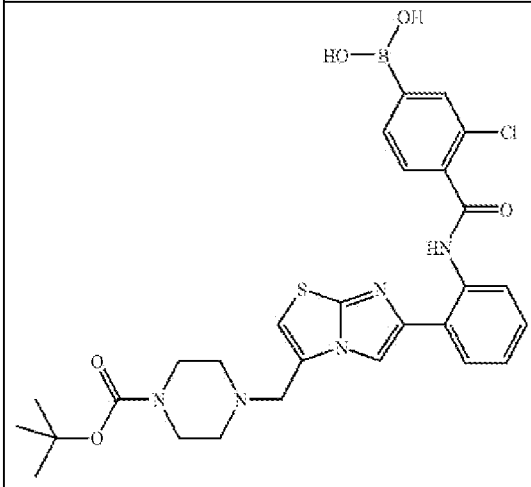

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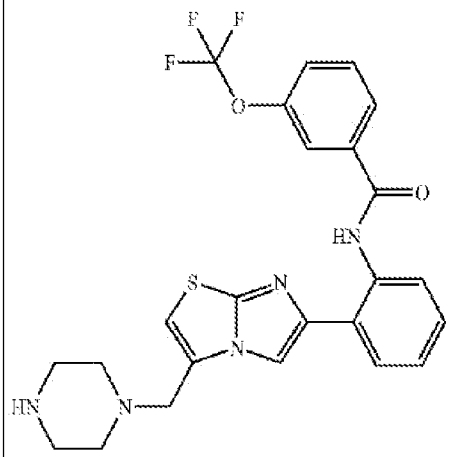

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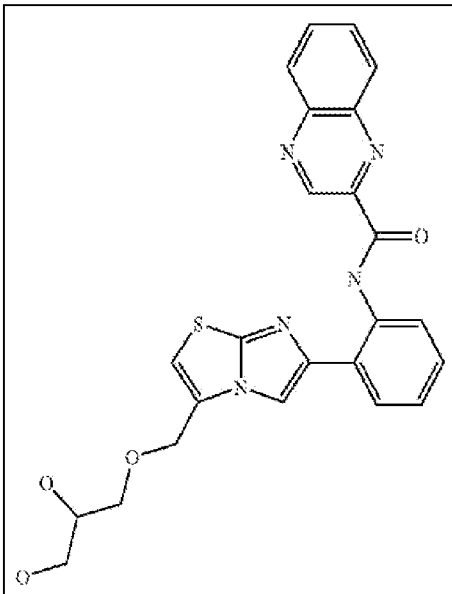

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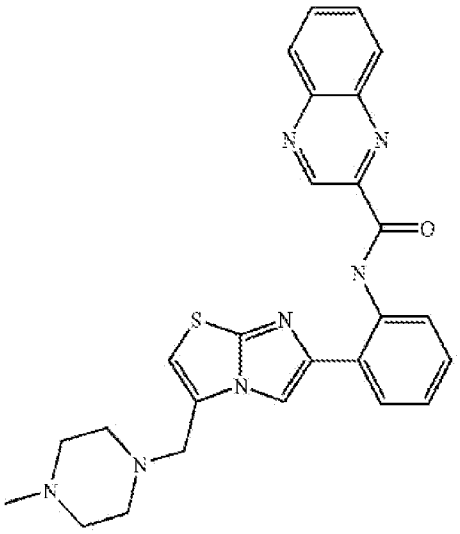

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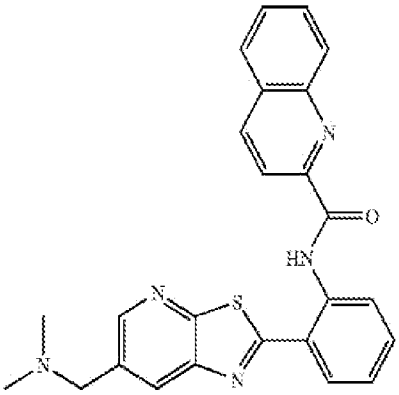

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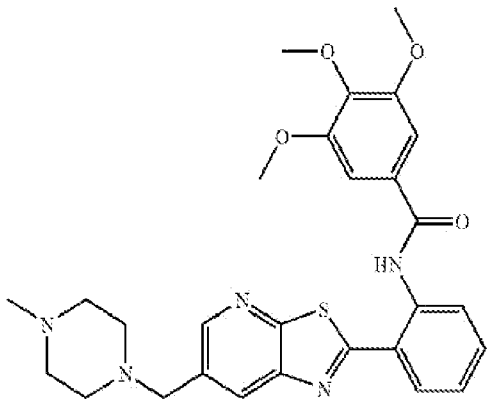

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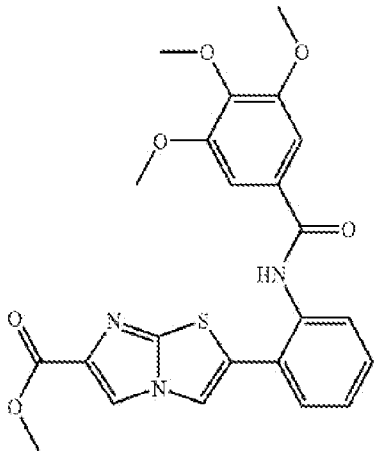

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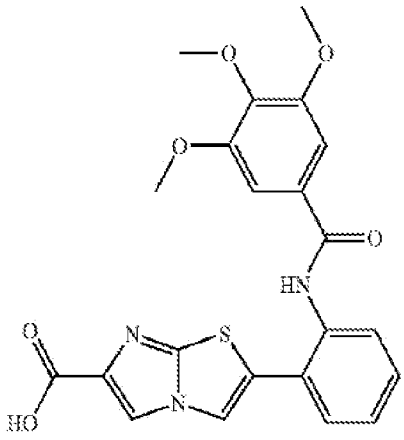

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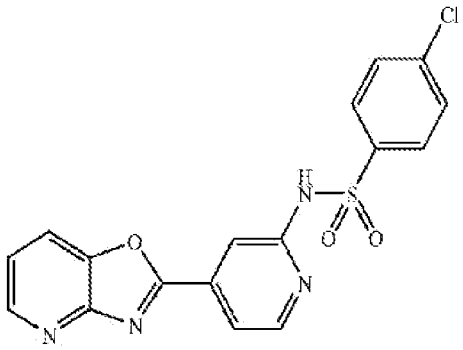

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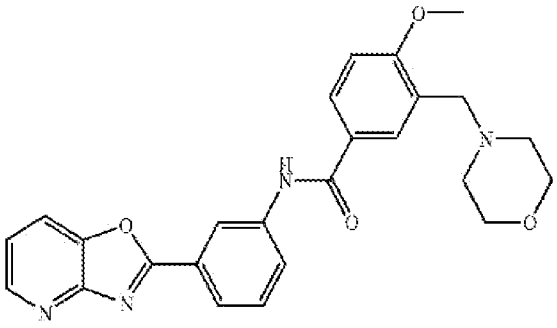

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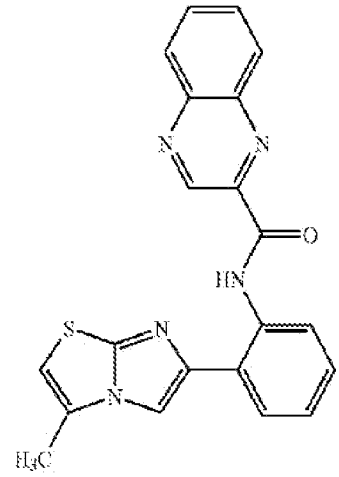

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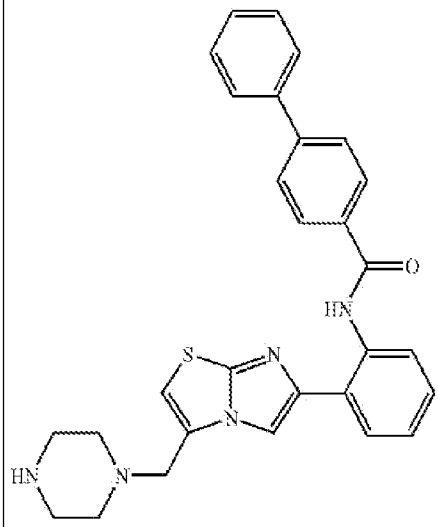

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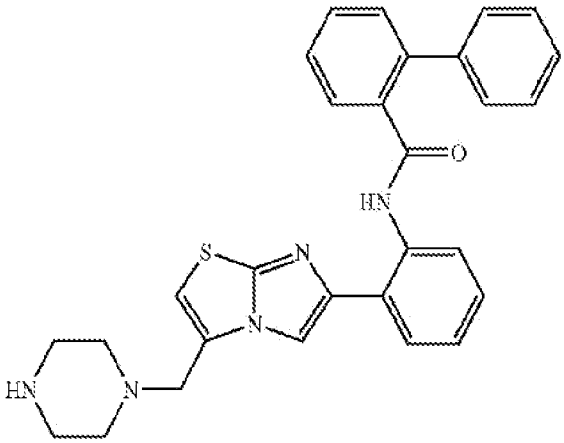

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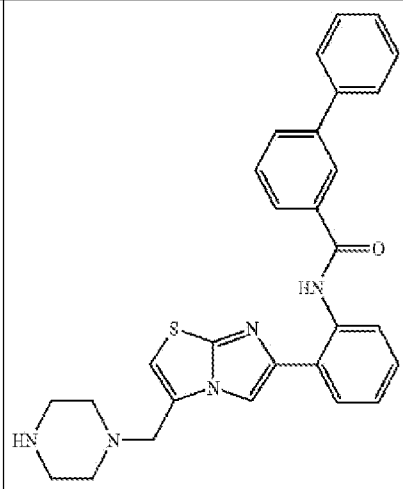

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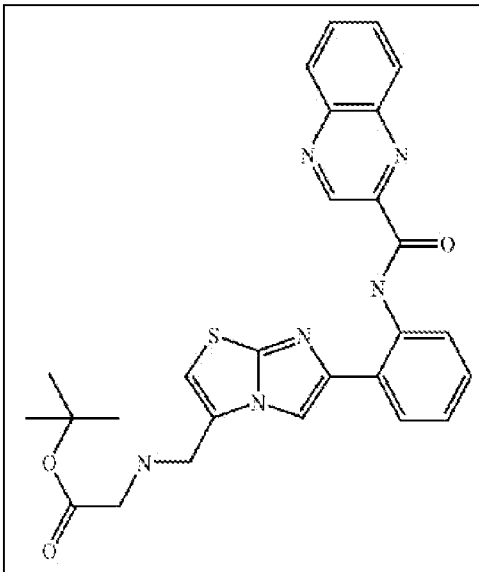

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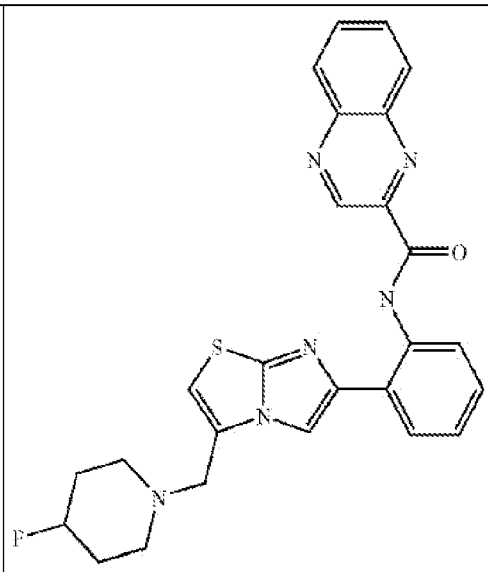

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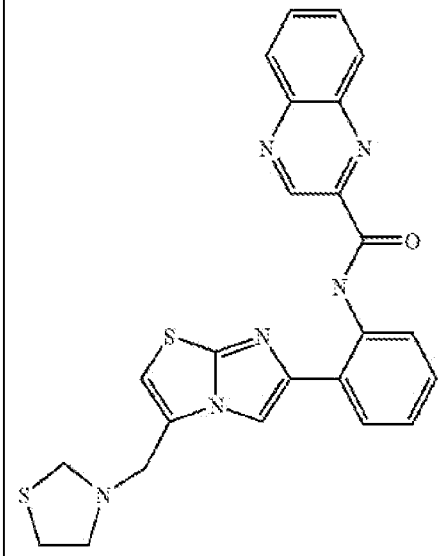

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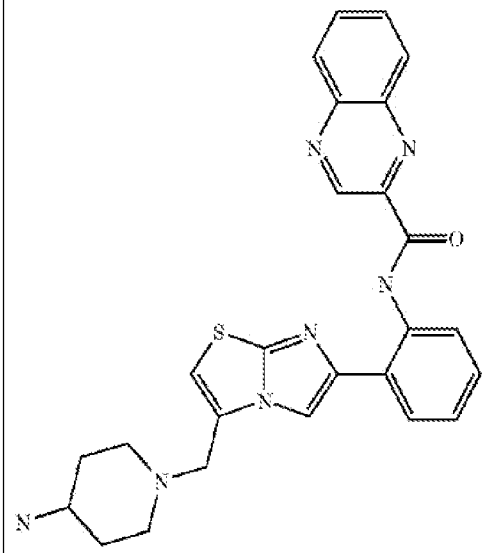

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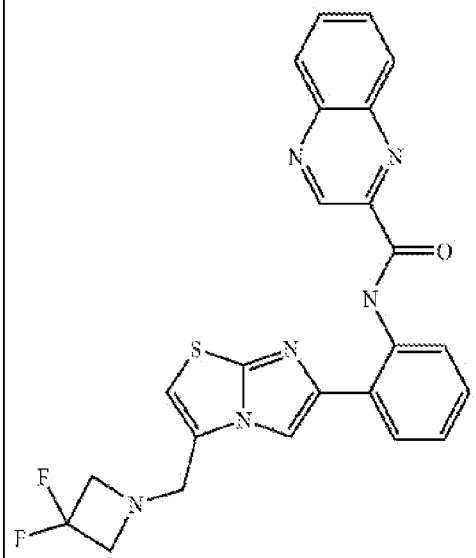

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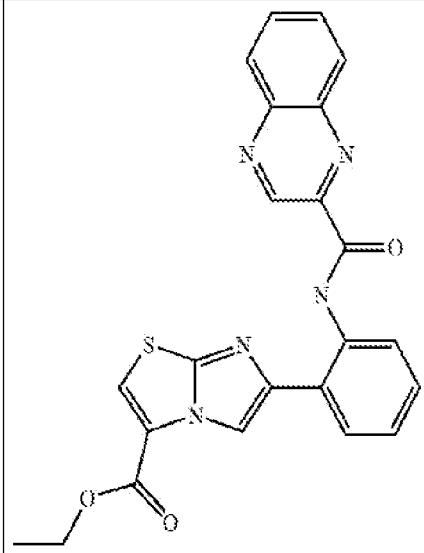

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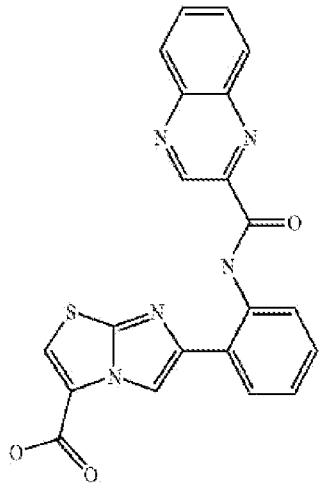

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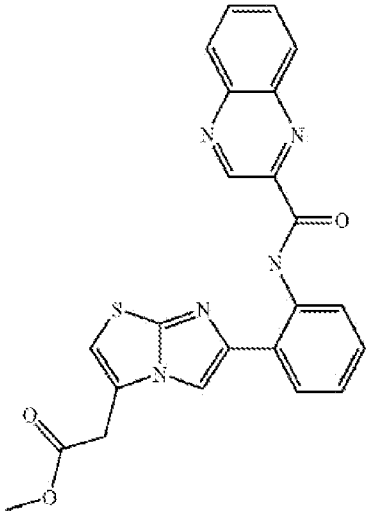

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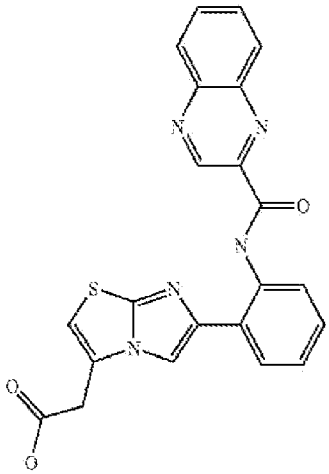

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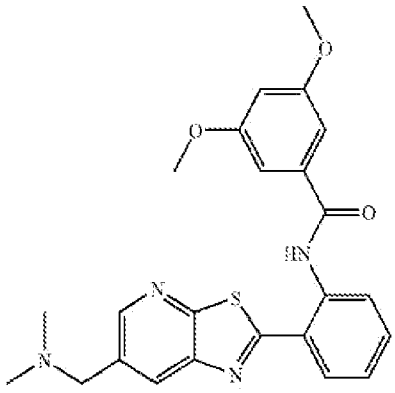

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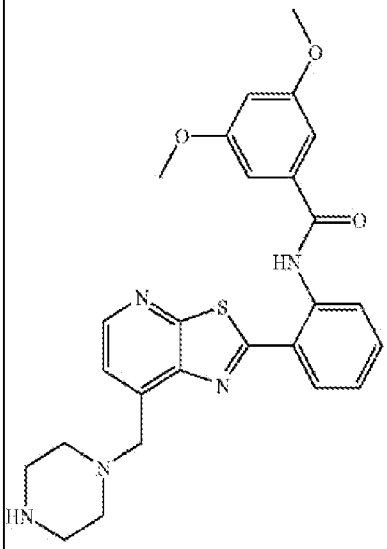

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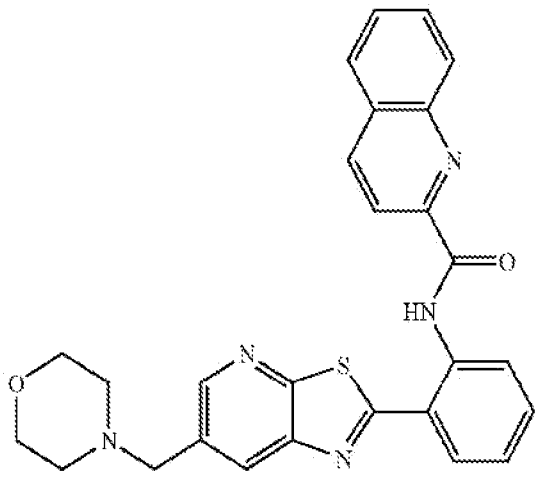

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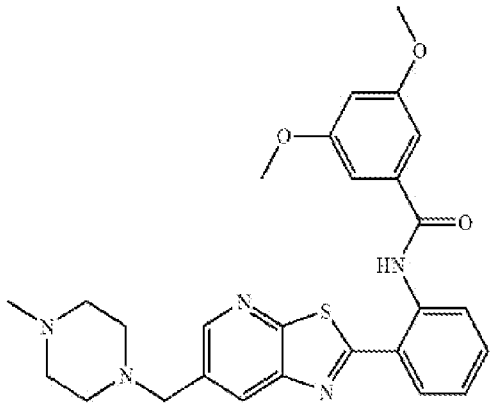
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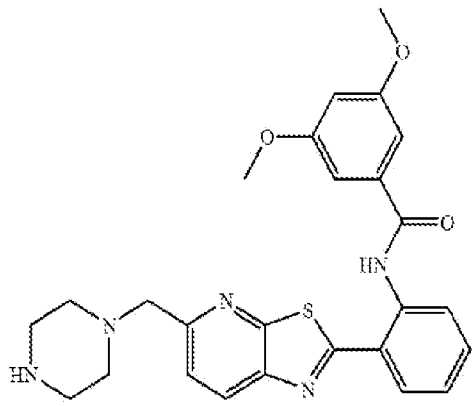
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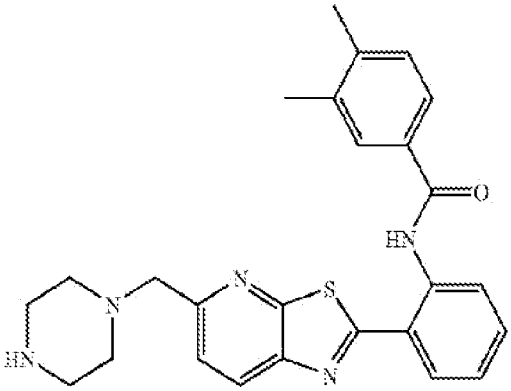
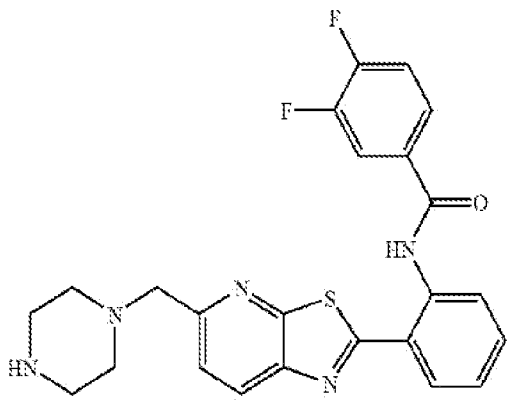
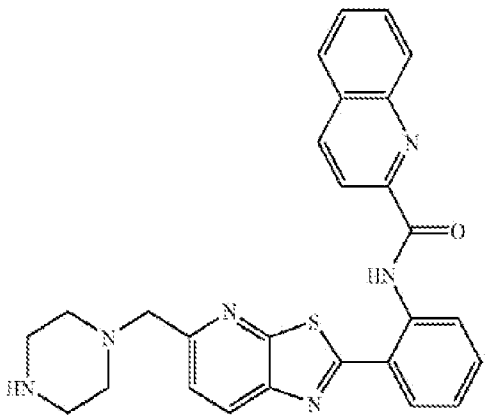
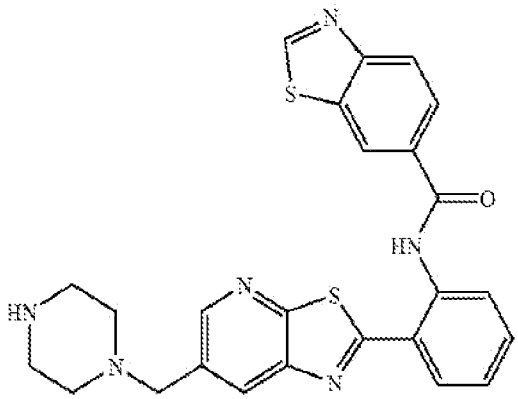
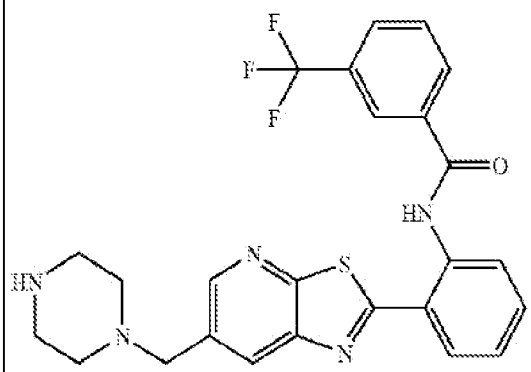
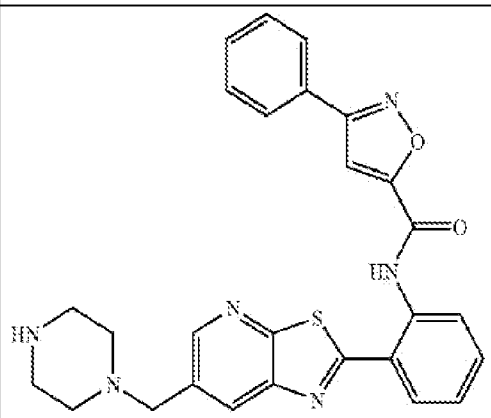

Compound 727

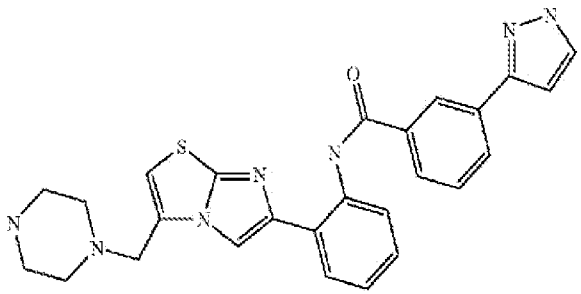

Compound 728


Compound 729

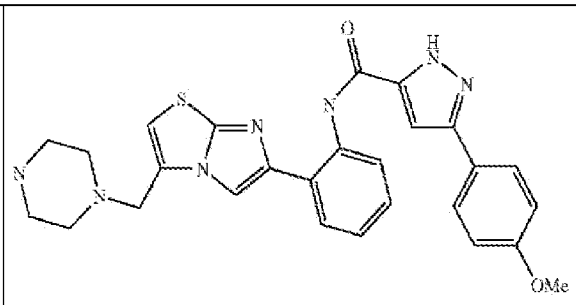

Compound 730


Compound 731

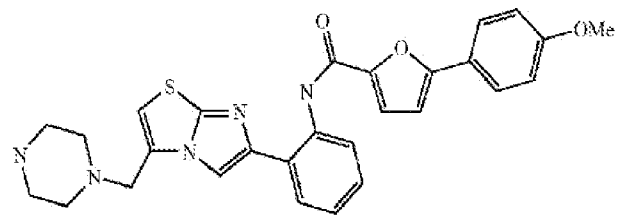

Compound 732



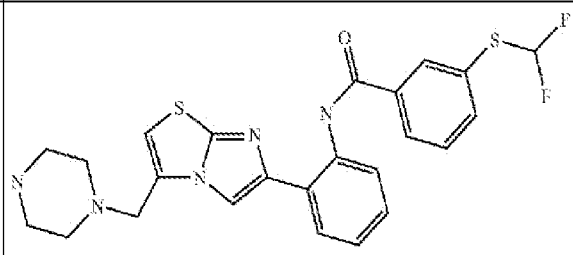
Compound 733



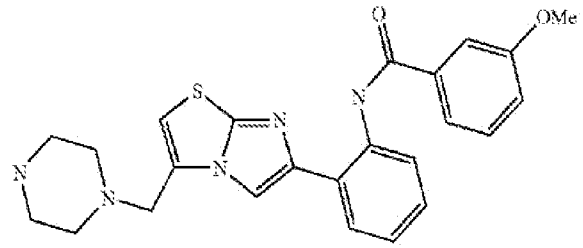
Compound 735



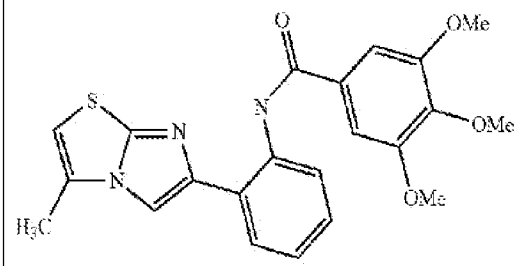
Compound 736



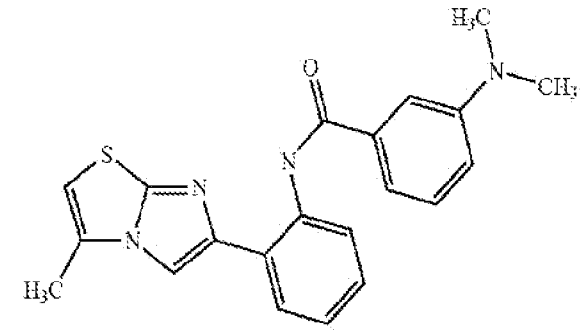
Compound 737



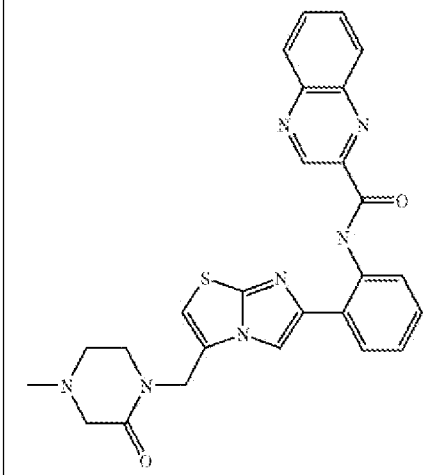
Compound 738



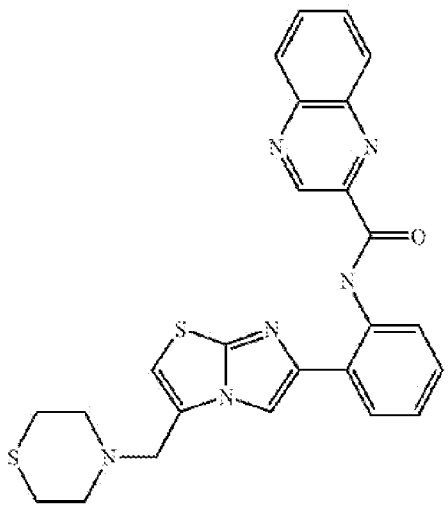
Compound 739



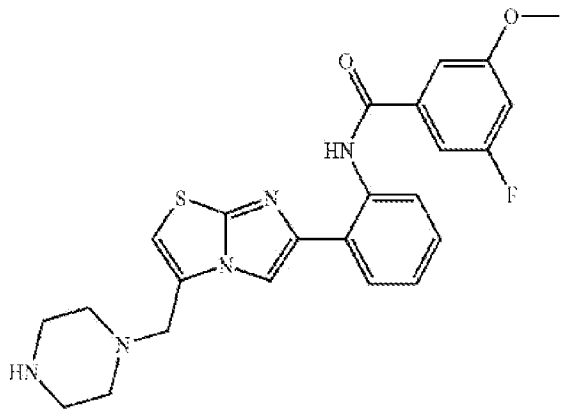
Compound 740



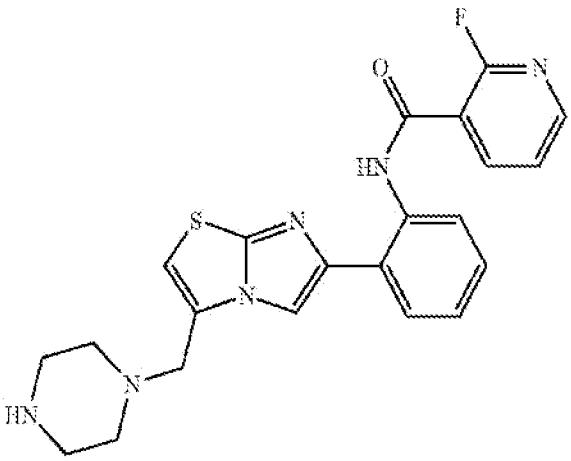
Compound 741



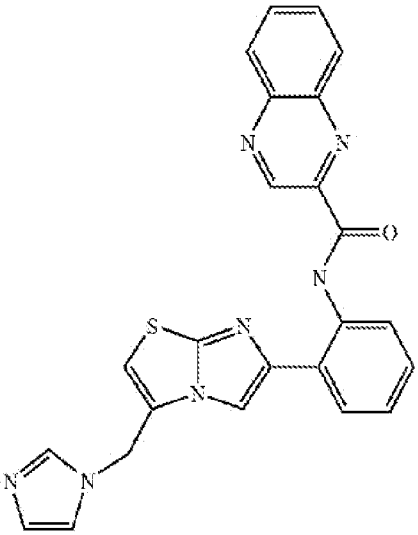
Compound 742



Compound 743

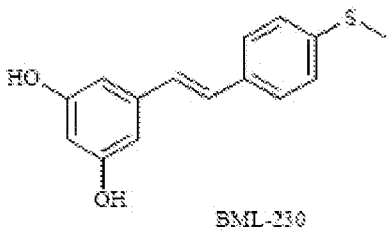


Compound 744

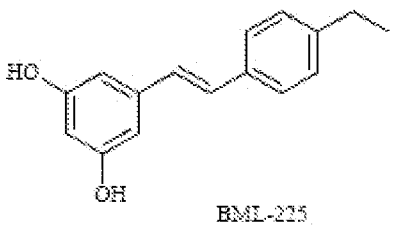


Compound 745

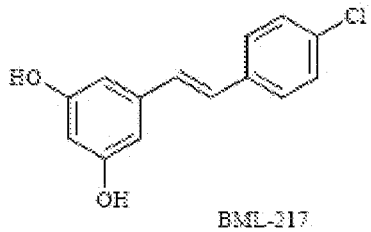
[0055] Non-limiting examples of suitable SIRT1 activators include, e.g.,



BML-230 ;

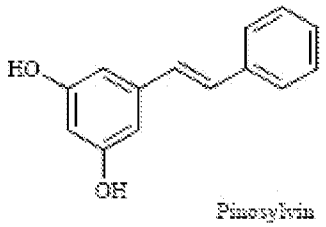


BML-225 ;



BML-217

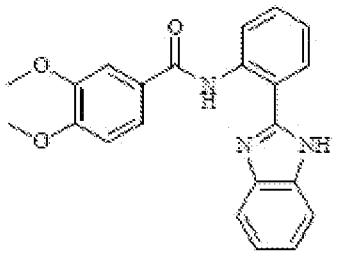
; and



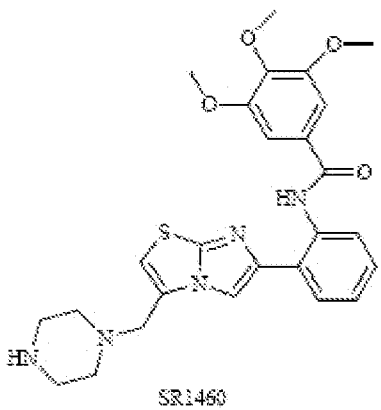
Pinosylvin

.

[0056] Other suitable SIRT1 activators include, e.g.,

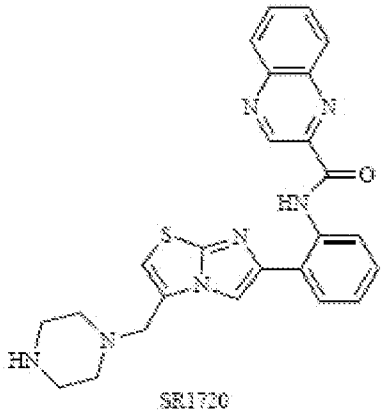


;

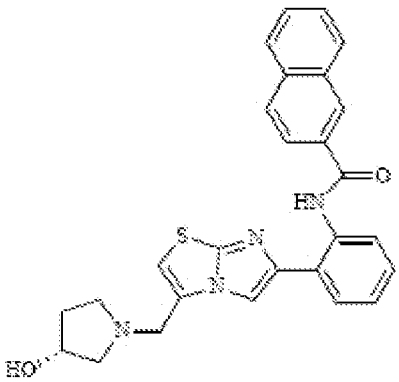


SR1460

;



SRT1720 ; and

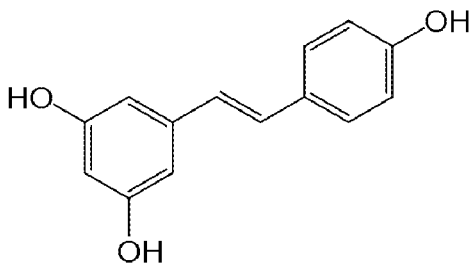


SRT-2183

[0057] SRT1720, SRT1460, and SRT2183 are selective SIRT1 activators. See, e.g., Milne et al. (2007) *Nature* 450:712.

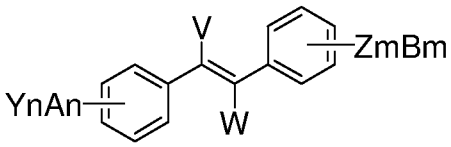
[0058] Also suitable for use are SIRT1 activators that are quinoxaline compounds. Suitable quinoxaline SIRT1 activators include, e.g., 3-benzenesulfonyl-1-(4-fluoro-phenyl)-1H-pyrrolo[2,3-b]quinoxalin-2ylamine; 2-amino-1-(2-ethyl-phenyl)-1H-pyrrolo[2,3-b]quinoxaline-3-carboxylic acid (tetrahydro-furan-2-ylmethyl)-amine; 2-amino-1-(3-methoxy-propyl)-1H-pyrrolo[2,3-b]quinoxaline-3-carboxylic acid cyclopentylamide. See, e.g., Nayagam et al. (2006) *J. Biolmolec. Screening* 11:959.

[0059] Other suitable SIRT1 activators include, e.g., stilbene compounds, e.g., ester analogs of resveratrol, e.g., as described in U.S. Patent Publication No. 2008/0255382. For example, suitable SIRT1 activators include, e.g., ester analogs of 3,5,4'-trihydroxy-trans-stilbene.



(resveratrol)

[0060] Ester analogs include compounds of the formula:



,

[0061] where each Y and each Z is independently –O (ethers), -O-C=O; -C=O-O (esters); -O-C=O-O (carbonates); -O-C=O-NH; -O=O-NR; -NH-C=O-O; -NR-C=O-O (carbamates); -NH-C=P; -NR-C=O; -C=O-NH; -C=O-NR (primary and secondary amides)-NH; -NR (primary and secondary amines); -N (heterocyclic rings); -S (thiol ethers); and halogen;

[0062] where each n and each m is independently 1, 2, 3, 4, or 5;

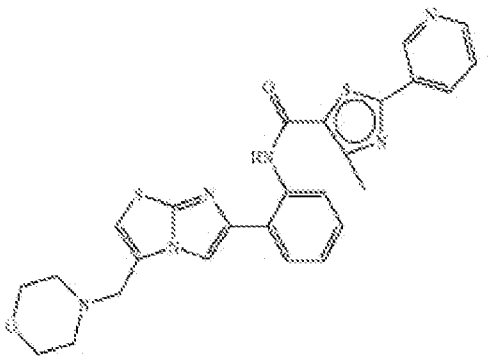
[0063] where each A and each B is independently H, R, or absent;

[0064] where each V and each W is independently H, straight or branched alkyl of from 1 to 6 carbon atoms, cycloalkyl of from 3 to 8 carbon atoms, alkoxy, phenyl, benzyl, or halogen,

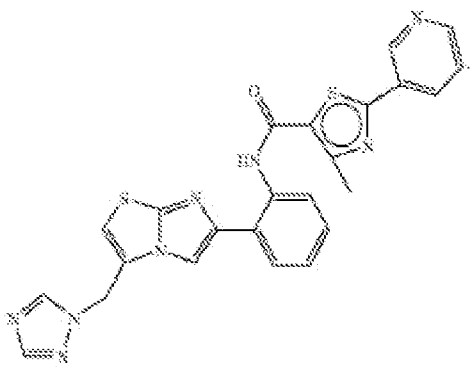
[0065] and where R is an alkyl with at least one carbon atom, an aryl, or an aralkyl.

[0066] Suitable SIRT1 activators include, e.g., 4'-acetoxy-3,5-bis(methoxymethoxy)stilbene; 4'-acetoxy-3,5-dihydroxystilbene; 3,5-diacetoxy-4'-chloroacetoxy stilbene; 3,5-diacetoxy-4'-hydroxy stilbene; 3,4'-diacetoxy-5-hydroxystilbene; 3-acetoxy-4'5-dihydroxystilbene; and 3,4,5'-triacetoxystilbene.

[0067] Suitable SIRT1 activators include compounds of any one of Formulas I-VI as described in U.S. Patent Publication No. 2009/0012080. For example, a suitable SIRT1 activator is a compound of the formula:



; or



.

[0068] For example, a suitable SIRT1 activator is 4-methyl-N-(2(3-morpholinomethyl)imidazol[2,1-b]thiazol-6-yl)phenyl)-2-(pyridin-3-yl)thiazol-5-carboxamide, or a pharmaceutically acceptable salt thereof.

[0069] In certain cases, an agent that decreases Ac-Tau levels is not a SIRT1 activator.

HDAC6

[0070] Histone deacetylases (HDAC) (EC number 3.5.1) are a class of enzymes that remove acetyl groups from an s-N-acetyl lysine amino acid on a histone. HDAC6 (mRNA GenBank accession no.: NM_006044.2, protein GenBank accession no.: NP_006035.2) is a cytoplasmic, microtubule-associated enzyme. HDAC6 deacetylates tubulin, Hsp90, and cortactin, and forms complexes with other partner proteins.

[0071] As described herein, HDAC6 deacetylates Tau. Overexpression of HDAC6 decreases the levels of Ac-Tau. Agents that increase the activity of HDAC6, for example, a nucleic acid comprising a nucleotide sequence encoding HDAC6, are suitable for use in a subject method. Non-limiting examples of suitable HDAC activators include, e.g., theophylline (3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione); theophylline analogs; and the like.

SIRT2

[0072] As described herein, SIRT2 deacetylates Tau. Agents that increase the activity of SIRT2, e.g., a nucleic acid comprising a nucleotide sequence encoding SIRT2, are suitable for use in a subject method.

[0073] Nucleotide sequences encoding SIRT2 polypeptides are known in the art. See, e.g., GenBank Accession No. NM_012237 (*Homo sapiens*); GenBank Accession No. NM_022432 (*Mus musculus*); NM_001008368.1 (*Rattus norvegicus*); and GenBank Accession No. XM_001168375.1 (*Pan troglodytes*).

Agents that inhibit acetylation of Tau

[0074] Agents that inhibit Tau acetylation include agents that inhibit the activity of a polypeptide that acetylates a Tau polypeptide. Polypeptides that acetylate a Tau polypeptide include an acetyltransferase, e.g., a histone acetyltransferase, e.g., p300. Agents that inhibit the activity of a polypeptide that acetylates a Tau polypeptide include agents that inhibit the activity of p300. In certain cases, the agent specifically inhibits the activity of p300 in acetylating Tau, e.g., the agent does not substantially inhibit any other acetyltransferase such as serotonin N-acetyltransferase, or a histone actyltransferase such as pCAF, GCN5 (e.g., GenBank Accession No. AAC50641), Rtt109, Sas, and MOZ.

p300

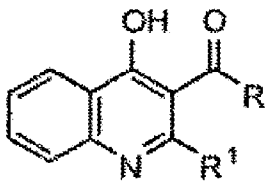
[0075] P300 is also known as E1A binding protein p300 (EP300). p300 (mRNA, GenBank accession no. NM_001429.3; protein, GenBank accession no. NP_001420.2) functions as histone acetyltransferase. As shown in the Examples section, p300 also acetylates Tau.

[0076] A p300 polypeptide includes a polypeptide that acetylates Tau, and that comprises an amino acid sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1800 amino acids to about 2000 amino acids, from about 2000 amino acids to about 2200 amino acids, or from about 2200 amino acids to about 2414 amino acids, of the amino acid sequence set forth in SEQ ID NO:12.

p300 inhibitors

[0077] As described herein, p300 acetylates Tau and increases Tau stability, resulting in increase in the steady state levels of Tau. Agents that inhibit the activity of p300 that are suitable for use in a subject method include, but are not limited to, isothiazolone-based histone acetyltransferase (HAT) inhibitors; Lys-CoA; a Lys-CoA derivative comprising a peptide of from about 5 amino acids to about 20 amino acids in length covalently linked to the lysine; Curcumin; anacardic acid; a polyprenylated benzophenone known as garcinol; a p300 specific siRNA; a compound as described in U.S. Patent No. 6,369,030; a p300 inhibitor as described in U.S. Patent Publication No. 2009/0076155; a 4-hydroxyquinoline compound as described in Mai et al. (2009) *Bioorg. Med. Chem. Lett.* 19:1132; etc. The structure of Lys-CoA is shown as Compound 1 in Zheng et al. (2005) *J. Am. Chem. Soc.* 127:17182 (see below). In some embodiments, a suitable p300 inhibitor is a selective p300 inhibitor. In some embodiments, a suitable p300 inhibitor is cell-permeable. Cell-permeable, selective p300 inhibitors that are suitable for use include those described in Zheng et al. (2005) *J. Am. Chem. Soc.* 127:17182.

[0078] For example, suitable p300 inhibitors include Compounds 1-8, where the parent formula is:



[0079] where

[0080] in Compound 1, R = OC_2H_5 and $R^1 = CH_3$;

[0081] in Compound 2, R = OH and $R^1 = CH_3$;

[0082] in Compound 3, R = OC_2H_5 and $R^1 = C_6H_{11}$;

[0083] in Compound 4, R = OC_2H_5 and $R^1 = C_{10}H_{21}$;

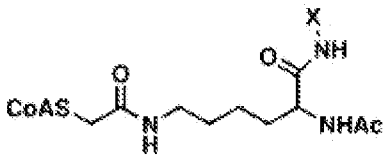
[0084] in Compound 5, R = OH and $R^1 = C_{10}H_{21}$;

[0085] in Compound 6, R = OC_2H_5 and $R^1 = C_{15}H_{31}$; and

[0086] in Compound 7, R = OH and $R^1 = C_{15}H_{31}$.

[0087] See, e.g., Mai et al. (2009) *Bioorg. Med. Chem. Lett.* 19:1132.

[0088] For example, suitable p300 inhibitors include Compounds 2-6, as shown below, of the formula:



,

[0089] where

[0090] in Compound 1 (Lys-CoA), X = H;

[0091] in Compound 2, X = YGRKKRRQRRR-CO_2H (SEQ ID NO:13);

[0092] in Compound 3, X = YGRKKRRQRRRGYK-NH_2 (SEQ ID NO:14);

[0093] in Compound 4, X = Ahx-R-Ahx-RR-Ahx-RR-Ahx-RR-Ahx-K-NH_2 (SEQ ID NO:15);

[0094] in Compound 5, X = GRRRRRRRRRRGK-NH_2 (SEQ ID NO:16); and

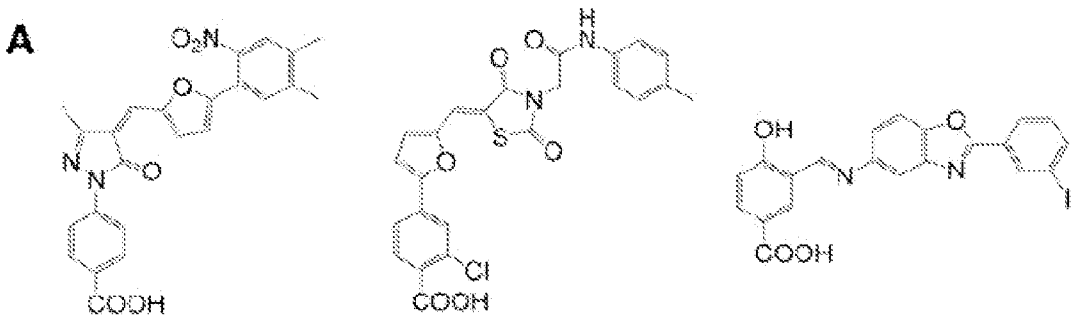
[0095] in Compound 6, X = Ahx-RRRRRRRRRR-NH_2 (SEQ ID NO:17);

[0096] and where Ahx is 6-aminohexanoic acid.

[0097] A number of p300 inhibitors are known in the art. A suitable p300 inhibitor can decrease the enzymatic activity of a p300 polypeptide (e.g., the activity of the p300 polypeptide in acetylating a Tau polypeptide) by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the activity of the p300 polypeptide in the absence of the inhibitor.

[0098] A suitable p300 inhibitor can inhibit p300 enzymatic activity at an IC_{50} (half maximal inhibitory concentration) of from about 1 nM to about 1 mM, e.g., from about 1 nM to about 10 nM, from about 10 nM to about 15 nM, from about 15 nM to about 25 nM, from about 25 nM to about 50 nM, from about 50 nM to about 75 nM, from about 75 nM to about 100 nM, from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 450 nM, from about 450 nM to about 500 nM, from about 500 nM to about 750 nM, from about 750 nM to about 1 μM, from about 1 μM to about 10 μM, from about 10 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, from about 75 μM to about 100 μM, from about 100 μM to about 250 μM, from about 250 μM to about 500 μM, or from about 500 μM to about 1 mM.

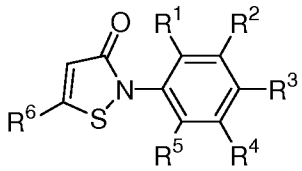
[0099] Suitable p300 inhibitors include compounds such as C646, C375, and C146. The structures of C646, C375, and C146 are shown below.



[00100] Suitable p300 inhibitors include N-alkyl- and N-aryl-substituted isothiazolones; such compounds have been identified as inhibitors of p300 (35-90% inhibition at 35 μmol/L) (Stimson et al. (October 1, 2005) Mol Cancer Ther 4:1521). These N-substituted isothiazolones-based compounds are shown in Tables 2 and 3.

Table 2:

Parent Structure:

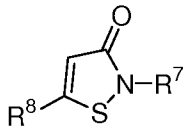


Compound	R^1	R^2	R^3	R^4	R^5	R^6
1	NO_2	H	H	H	H	H
2	H	NO_2	H	H	H	H
3	H	H	NO_2	H	H	H
4	H	H	NO_2	H	H	Cl
5	H	NO_2	Cl	H	H	H
6	OMe	H	H	H	H	H
7	H	H	OMe	H	H	H
8	Cl	H	H	H	H	H
9	H	Cl	H	H	H	H
10	H	H	Cl	H	H	H
11	Cl	H	H	Cl	H	H
12	Cl	H	H	Cl	H	Cl
13	Cl	H	Cl	H	H	H
14	Cl	Cl	H	H	H	H
15	CH_3	H	H	H	H	H
16	H	CH_3	H	H	H	H
17	H	H	CH_3	H	H	H
18	CO_2Et	H	H	H	H	H
19	H	CO_2Et	H	H	H	H
20	H	H	CO_2Et	H	H	H
21	CF_3	H	H	H	H	H

22	H	CF_3	H	H	H	H
23	H	CF_3	H	H	H	Cl
24	H	CF_3	F	H	H	H
25	H	CF_3	F	H	H	Cl
26	H	Cl	CH_3	H	H	H
27	H	OPh	H	H	H	H

Table 3:

Parent Structure:



Compound	R^7	R^8
28	iPr	H
29	cyclopropyl	H
30	benzyl	H
31	Et	H
32	Et	Cl
33	decyl	H
34	CH_2CH_2OPh	H
35	CH_2CH_2OPh	Cl

CBP

[00101] In some embodiments, a subject method involves use of a CREB-binding protein (CBP) inhibitor. In some embodiments, a p300 inhibitor also inhibits a CBP polypeptide.

[00102] CBP polypeptides are known in the art. For example, GenBank Accession No. NP_004371.2 provides an amino acid sequence of *Homo sapiens* CBP; GenBank Accession No. XP_523285.2 provides an amino acid sequence of *Pan troglodytes* CBP; GenBank Accession No. XP_001095225.1 provides an amino acid sequence of *Macaca mulatta* CBP; GenBank Accession No. NP_596872.3 provides an amino acid sequence of *Rattus norvegicus* CBP; and GenBank Accession No. NP_001020603.1 provides an amino acid sequence of *Mus musculus* CBP. The amino acid sequence set forth in GenBank Accession No. NP_004371.2 is provided herewith as SEQ ID NO:53.

Methods of treating a tauopathy

[00103] The present disclosure provides a method for treating a tauopathy in an individual. The method comprising administering to an individual in need thereof an effective amount of an agent that reduces the level of acetylated Tau in the cell, e.g., an agent that inhibits the acetyltransferase activity of an acetyltransferase that acetylates a Tau polypeptide, an agent that increases the deacetylase activity of a deacetylase that deacetylates an acetylated Tau polypeptide, etc.

[00104] Tauopathies are neurodegenerative diseases that are characterized, at least in part, by pathological aggregation of Tau protein, e.g., in neurofibrillary tangles. Examples of tauopathies include frontotemporal dementia, Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, Down syndrome, dementia pugilistica, inclusion-body myositis, and frontotemporal lobar degeneration, also known as Pick's disease. Exemplary tauopathies include: diseases showing coexistence of tau and amyloid pathologies, e.g., Alzheimer's disease, Creutzfeldt-Jakob disease, dementia pugilistica, Down's syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion body myositis, and prion protein cerebral amyloid angiopathy; diseases without distinct amyloid pathology, e.g., amyotrophic lateral sclerosis/Parkinsonism-dementia complex, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with Parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease type C, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, subacute sclerosing panencephalitis, and tangle-predominant Alzheimer's disease.

[00105] In some embodiments, a subject method involves administration of an agent that inhibits acetylation of a Tau polypeptide. As explained above, agents that reduce Tau acetylation include agents that inhibit the activity of a polypeptide that acetylates a Tau polypeptide. Polypeptides that acetylate a Tau polypeptide include histone acetyltransferases, e.g., p300. Several agents that inhibit the activity of p300 are disclosed above, for example in Table 2.

[00106] In some embodiments, a subject method involves administration of an agent that increases deacetylation of an Ac-Tau polypeptide. As discussed above, agents that increase Tau deacetylation include agents that increase the activity of a polypeptide that deacetylates an Ac-Tau polypeptide. Polypeptides that deacetylate Tau include, e.g., SIRT1, SIRT2, HDAC 6, etc. Agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide include, e.g., agents that increase the activity of SIRT1. Several activators of SIRT1 are provided above, for example in Table 1. Agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide include, e.g., agents that increase the activity of SIRT2. Agents that increase the activity of a polypeptide that deacetylates an acetylated Tau polypeptide include, e.g., agents that increase the activity of HDAC6.

[00107] In some embodiments, a subject method involves administration of an agent that inhibits acetylation of a Tau polypeptide and an agent that increases deacetylation of an Ac-Tau polypeptide. In addition to the individual administration of an agent(s) that increases Ac-Tau polypeptide deacetylation and of an agent(s) that decreases Tau polypeptide acetylation, a combination therapy may be used in treating a tauopathy. For example, a combination of a

SIRT1 activator (or a SIRT2 activator or an HDAC6 activator) and a p300 (or CBP) inhibitor may be used in a combination therapy for treating a tauopathy. A combination therapy is in some embodiments more effective in treating a tauopathy than administering a Tau-deacetylase polypeptide or an inhibitor of a Tau-acetyltransferase in monotherapy. Agents that inhibit acetylation of a Tau polypeptide and/or agents that increase deacetylation of Ac-Tau polypeptide are referred to herein as "active agents" or "active agent." In some embodiments, an agent that inhibits acetylation of a Tau polypeptide and an agent that increases deacetylation of an Ac-Tau polypeptide, when administered in combination therapy, provide for a synergistic effect.

[00108] A subject method for treating a tauopathy in an individual generally involves administering an effective amount of an agent that inhibits acetylation of a Tau polypeptide in a neuronal cell and/or an agent that increases deacetylation of an Ac-Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell) in the individual. In some embodiments, a subject method involves monotherapy, e.g., administration of an effective amount of a single active agent, e.g., an agent that inhibits acetylation of a Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell). In some embodiments, a subject method involves monotherapy, e.g., administration of an effective amount of a single active agent, e.g., an agent that increases deacetylation of an Ac-Tau polypeptide in a cell that produces Tau (e.g., a neuronal cell and/or a glial cell). In some embodiments, a subject method involves a combination therapy, e.g., administration of an agent that inhibits acetylation of a Tau polypeptide in a cell (e.g., a neuron; a glial cell) and an agent that increases deacetylation of an Ac-Tau polypeptide in a cell (e.g., a neuron; a glial cell) in combined effective amounts.

[00109] An effective amount of an active agent is an amount that is effective to ameliorate at least one symptom of a tauopathy, e.g., to alleviate an adverse symptom and/or to increase a normal function that was impaired as a result of the tauopathy. For example, in some embodiments, an effective amount of an active agent is an amount that is effective to reduce the number of neurofibrillary lesions in the brain of an individual having a tauopathy. Where a subject method involves combination therapy, combined effective amounts of the active agents are amounts that, in combination, are effective to reduce the number of neurofibrillary lesions in the brain of an individual having a tauopathy. In some embodiments, an effective amount of an active agent is an amount that is effective to increase a cognitive function in the individual. Where a subject method involves combination therapy, combined effective amounts of the active agents are amounts that, in combination, are effective to increase a cognitive function in the individual.

Formulations, dosages, and routes of administration

[00110] An active agent (e.g., an agent that inhibits acetylation of a Tau polypeptide; an agent that increases deacetylation of Ac-Tau polypeptide) can be incorporated into a variety of formulations for therapeutic administration. More particularly, an active agent can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as, powders, granules, solutions, injections, inhalants, gels, hydrogels, microspheres, etc. As such, administration of an active agent can be achieved in various ways, including local, such as delivery into the affected tissue, oral, catheter mediated, intrathecal, buccal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc., administration. The active agent may be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation.

[00111] In some embodiments, an active agent(s) is formulated to cross the blood brain barrier (BBB). One strategy for drug delivery through the blood brain barrier (BBB) entails disruption of the BBB, either by osmotic means such as mannitol or leukotrienes, or biochemically by the use of vasoactive substances such as bradykinin. A BBB disrupting agent can be co-administered with an active agent when the compositions are administered by intravascular injection. Other strategies to go through the BBB may entail the use of endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and active efflux transporters such as p-glycoprotein. Active transport moieties may also be conjugated to an active agent for use in the methods disclosed herein to facilitate transport across the epithelial wall of the blood vessel. Alternatively, drug delivery behind the BBB is by intrathecal delivery of therapeutics directly to the cranium, as through an Ommaya reservoir.

[00112] Pharmaceutical compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers of diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, buffered water, physiological saline, phosphate buffered saline (PBS), Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation can include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

[00113] Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see. Langer, Science 249:1527-1533 (1990).

[00114] The pharmaceutical compositions can be administered for prophylactic and/or therapeutic treatments. Toxicity and therapeutic efficacy of the active agent can be determined according to standard pharmaceutical procedures in cell cultures and/or experimental animals, including, for example, determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred.

[00115] The data obtained from cell culture and/or animal studies can be used in formulating a range of dosages for humans. The dosage of the active agent typically lines within a range of circulating concentrations that include the ED50 with low toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

[00116] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (e.g., at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions intended for *in vivo* use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under Good Manufacturing Practice (GMP) conditions.

[00117] The effective amount of an active agent(s) to be given to a particular patient will depend on a variety of factors, several of which will be different from patient to patient. A competent clinician will be able to determine an effective amount of an active agent to administer to a patient to treat a tauopathy. Utilizing LD50 animal data, and other information available for the inhibitor, a clinician can determine the maximum safe dose for an individual, depending on the route of administration. For instance, an intravenously administered dose may be more than an intrathecally administered dose, given the greater body of fluid into which the therapeutic composition is being administered. Similarly, compositions which are rapidly cleared from the body may be administered at higher doses, or in repeated doses, in order to maintain a therapeutic concentration. Utilizing ordinary skill,

the competent clinician will be able to optimize the dosage of a particular therapeutic in the course of routine clinical trials.

Formulations

[00118] In carrying out a subject treatment method (e.g., reducing the level of acetylated Tau polypeptide in a cell (e.g., a neuron; a glial cell) in an individual; treating a tauopathy), an active agent(s) may be administered to the host using any convenient means capable of resulting in the desired physiological effect (e.g., reduction in the level of acetylated Tau polypeptide in a neuronal cell and/or a glial cell in an individual; increase in cognitive function; reduction in neurofibrillary lesions; reduction in adverse effect of a tauopathy; etc.). Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, an active agent can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[00119] In pharmaceutical dosage forms, an active agent(s) can be administered in the form of its (their) pharmaceutically acceptable salts, or the active agent may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[00120] For oral preparations, an active agent can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[00121] An active agent can be formulated into preparations for injection by dissolving, suspending or emulsifying the active agent in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00122] An active agent can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

[00123] Furthermore, an active agent can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. An active agent can be

administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[00124] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more active agents. Similarly, unit dosage forms for injection or intravenous administration may comprise the active agent(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

[00125] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of an active agent calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a unit dosage form of an active agent depend on the particular active agent employed and the effect to be achieved, and the pharmacodynamics associated with each active agent in the host.

[00126] Other modes of administration will also find use with the subject invention. For instance, an active agent can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), e.g., about 1% to about 2%.

[00127] Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of an active agent by the nasal mucosa.

[00128] An active agent can be administered as injectables. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active agent encapsulated in liposome vehicles.

[00129] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be

administered will, in any event, contain a quantity of the active agent adequate to achieve the desired state in the subject being treated.

[00130] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Oral formulations

[00131] In some embodiments, an active agent is formulated for oral delivery to an individual in need of such an agent.

[00132] For oral delivery, a formulation comprising an active agent will in some embodiments include an enteric-soluble coating material. Suitable enteric-soluble coating material include hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methyl cellulose phthalate (HPMCP), cellulose acetate phthalate (CAP), polyvinyl phthalic acetate (PVPA), Eudragit™, and shellac.

[00133] As one non-limiting example of a suitable oral formulation, an active agent is formulated with one or more pharmaceutical excipients and coated with an enteric coating, as described in U.S. Patent No. 6,346,269. For example, a solution comprising an active agent and a stabilizer is coated onto a core comprising pharmaceutically acceptable excipients, to form an active agent-coated core; a sub-coating layer is applied to the active agent-coated core, which is then coated with an enteric coating layer. The core generally includes pharmaceutically inactive components such as lactose, a starch, mannitol, sodium carboxymethyl cellulose, sodium starch glycolate, sodium chloride, potassium chloride, pigments, salts of alginic acid, talc, titanium dioxide, stearic acid, stearate, micro-crystalline cellulose, glycerin, polyethylene glycol, triethyl citrate, tributyl citrate, propanyl triacetate, dibasic calcium phosphate, tribasic sodium phosphate, calcium sulfate, cyclodextrin, and castor oil. Suitable solvents for an active agent include aqueous solvents. Suitable stabilizers include alkali-metals and alkaline earth metals, bases of phosphates and organic acid salts and organic amines. The sub-coating layer comprises one or more of an adhesive, a plasticizer, and an anti-tackiness agent. Suitable anti-tackiness agents include talc, stearic acid, stearate, sodium stearyl fumarate, glyceryl behenate, kaolin and aerosil. Suitable adhesives include polyvinyl pyrrolidone (PVP), gelatin, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), vinyl acetate (VA), polyvinyl alcohol (PVA), methyl cellulose (MC), ethyl cellulose (EC), hydroxypropyl methyl cellulose phthalate (HPMCP), cellulose acetate phthalates (CAP), xanthan gum, alginic acid, salts of alginic acid, Eudragit™, copolymer of methyl acrylic acid/methyl methacrylate with polyvinyl acetate phthalate (PVAP). Suitable plasticizers include glycerin, polyethylene glycol, triethyl citrate, tributyl citrate, propanyl triacetate and castor oil.

Suitable enteric-soluble coating material include hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methyl cellulose phthalate(HPMCP), cellulose acetate phthalate (CAP), polyvinyl phthalic acetate (PVPA), Eudragit™ and shellac.

[00134] Suitable oral formulations also include an active agent, formulated with any of the following: microgranules (see, e.g., U.S. Patent No. 6,458,398); biodegradable macromers (see, e.g., U.S. Patent No. 6,703,037); biodegradable hydrogels (see, e.g., Graham and McNeill (1989) *Biomaterials* 5:27-36); biodegradable particulate vectors (see, e.g., U.S. Patent No. 5,736,371); bioabsorbable lactone polymers (see, e.g., U.S. Patent No. 5,631,015); slow release protein polymers (see, e.g., U.S. Patent No. 6,699,504; Pelias Technologies, Inc.); a poly(lactide-co-glycolide/polyethylene glycol block copolymer (see, e.g., U.S. Patent No. 6,630,155; Atrix Laboratories, Inc.); a composition comprising a biocompatible polymer and particles of metal cation-stabilized agent dispersed within the polymer (see, e.g., U.S. Patent No. 6,379,701; Alkermes Controlled Therapeutics, Inc.); and microspheres (see, e.g., U.S. Patent No. 6,303,148; Octoplus, B.V.).

[00135] Suitable oral formulations also include an active agent formulated with any of the following: a carrier such as Emisphere® (Emisphere Technologies, Inc.); TIMERx, a hydrophilic matrix combining xanthan and locust bean gums which, in the presence of dextrose, form a strong binder gel in water (Penwest); Geminex™ (Penwest); Procise™ (GlaxoSmithKline); SAVIT™ (Mistral Pharma Inc.); RingCap™ (Alza Corp.); Smartrix® (Smartrix Technologies, Inc.); SQZgel™ (MacroMed, Inc.); Geomatrix™ (Skye Pharma, Inc.); Oros® Tri-layer (Alza Corporation); and the like.

[00136] Also suitable for use are formulations such as those described in U.S. Patent No. 6,296,842 (Alkermes Controlled Therapeutics, Inc.); U.S. Patent No. 6,187,330 (Scios, Inc.); and the like.

[00137] Also suitable for use herein are formulations comprising an intestinal absorption enhancing agent. Suitable intestinal absorption enhancers include, but are not limited to, calcium chelators (e.g., citrate, ethylenediamine tetracetic acid); surfactants (e.g., sodium dodecyl sulfate, bile salts, palmitoylcarnitine, and sodium salts of fatty acids); toxins (e.g., zonula occludens toxin); and the like.

Controlled release formulations

[00138] In some embodiments, an active agent is formulated in a controlled release formulation.

[00139] Controlled release formulations suitable for use can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present disclosure: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release,

repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

[00140] The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems.

[00141] Physical systems include, but are not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

[00142] Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in Agis F. Kydonieus, Controlled Release Technologies: Methods, Theory and Applications, 1980 (CRC Press, Inc.).

[00143] There are a number of controlled release drug formulations that are developed for oral administration. These include, but are not limited to, osmotic pressure-controlled gastrointestinal delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gastric fluid-resistant intestine targeted controlled-release gastrointestinal delivery devices; gel diffusion-controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs. Additional information regarding controlled release drug delivery systems may be found in Yie W. Chien, Novel Drug Delivery Systems, 1992 (Marcel Dekker, Inc.). Some of these formulations will now be discussed in more detail.

[00144] Enteric coatings are applied to tablets to prevent the release of drugs in the stomach either to reduce the risk of unpleasant side effects or to maintain the stability of the drug

which might otherwise be subject to degradation of expose to the gastric environment. Most polymers that are used for this purpose are polyacids that function by virtue or the fact that their solubility in aqueous medium is pH-dependent, and they require conditions with a pH higher than normally encountered in the stomach.

[00145] One exemplary type of oral controlled release structure is enteric coating of a solid or liquid dosage form. The enteric coatings are designed to disintegrate in intestinal fluid for ready absorption. Delay of absorption of the active agent that is incorporated into a formulation with an enteric coating is dependent on the rate of transfer through the gastrointestinal tract, and so the rate of gastric emptying is an important factor. Some investigators have reported that a multiple-unit type dosage form, such as granules, may be superior to a single-unit type. Therefore, in one exemplary embodiment, an active agent may be contained in an enterically coated multiple-unit dosage form. In an exemplary embodiment, an active agent dosage form is prepared by spray-coating granules of an active agent-enteric coating agent solid dispersion on an inert core material. These granules can result in prolonged absorption of the drug with good bioavailability.

[00146] Typical enteric coating agents include, but are not limited to, hydroxypropylmethylcellulose phthalate, methacryclic acid-methacrylic acid ester copolymer, polyvinyl acetate-phthalate and cellulose acetate phthalate. Akihiko Hasegawa, Application of solid dispersions of Nifedipine with enteric coating agent to prepare a sustained-release dosage form, Chem. Pharm. Bull. **33**: 1615-1619 (1985). Various enteric coating materials may be selected on the basis of testing to achieve an enteric coated dosage form designed *ab initio* to have an optimal combination of dissolution time, coating thicknesses and diametral crushing strength. S.C. Porter et al., The Properties of Enteric Tablet Coatings Made From Polyvinyl Acetate-phthalate and Cellulose acetate Phthalate, J. Pharm. Pharmacol. **22**:42p (1970).

[00147] Another type of useful oral controlled release structure is a solid dispersion. A solid dispersion may be defined as a dispersion of one or more active ingredients in an inert carrier or matrix in the solid state prepared by the melting (fusion), solvent, or melting-solvent method. Akihiko Hasegawa, Super Saturation Mechanism of Drugs from Solid Dispersions with Enteric Coating Agents, Chem. Pharm. Bull. **36**: 4941-4950 (1998). The solid dispersions may be also called solid-state dispersions. The term "coprecipitates" may also be used to refer to those preparations obtained by the solvent methods.

[00148] The selection of the carrier may have an influence on the dissolution characteristics of the dispersed active agent because the dissolution rate of a component from a surface may be affected by other components in a multiple component mixture. For example, a water-soluble carrier may result in a fast release of the active agent from the matrix, or a poorly soluble or insoluble carrier may lead to a slower release of the active agent from the matrix.

The solubility of the active agent may also be increased owing to some interaction with the carriers.

[00149] Examples of carriers useful in solid dispersions include, but are not limited to, water-soluble polymers such as polyethylene glycol, polyvinylpyrrolidone, and hydroxypropylmethyl – cellulose. Alternative carriers include phosphatidylcholine. Phosphatidylcholine is an amphoteric but water-insoluble lipid, which may improve the solubility of otherwise insoluble active agents in an amorphous state in phosphatidylcholine solid dispersions.

[00150] Other carriers include polyoxyethylene hydrogenated castor oil. Poorly water-soluble active agents may be included in a solid dispersion system with an enteric polymer such as hydroxypropylmethylcellulose phthalate and carboxymethylethylcellulose, and a non-enteric polymer, hydroxypropylmethylcellulose. Another solid dispersion dosage form includes incorporation of the active agent with ethyl cellulose and stearic acid in different ratios.

[00151] There are various methods commonly known for preparing solid dispersions. These include, but are not limited to, the melting method, the solvent method and the melting-solvent method.

[00152] Another controlled release dosage form is a complex between an ion exchange resin and an active agent. Ion exchange resin-drug complexes have been used to formulate sustained-release products of acidic and basic drugs. In one exemplary embodiment, a polymeric film coating is provided to the ion exchange resin-drug complex particles, making drug release from these particles diffusion controlled. See Y. Raghunathan et al., Sustained-released drug delivery system I: Coded ion-exchange resin systems for phenylpropanolamine and other drugs, J. Pharm. Sciences 70: 379-384 (1981).

[00153] Injectable microspheres are another controlled release dosage form. Injectable micro spheres may be prepared by non-aqueous phase separation techniques, and spray-drying techniques. Microspheres may be prepared using polylactic acid or copoly(lactic/glycolic acid). Shigeyuki Takada, Utilization of an Amorphous Form of a Water-Soluble GPIIb/IIIa Antagonist for Controlled Release From Biodegradable Micro spheres, Pharm. Res. **14**:1146-1150 (1997), and ethyl cellulose, Yoshiyuki Koida, Studies on Dissolution Mechanism of Drugs from Ethyl Cellulose Microcapsules, Chem. Pharm. Bull. **35**:1538-1545 (1987).

[00154] Other controlled release technologies that may be used include, but are not limited to, SODAS (Spheroidal Oral Drug Absorption System), INDAS (Insoluble Drug Absorption System), IPDAS (Intestinal Protective Drug Absorption System), MODAS (Multiporous Oral Drug Absorption System), EFVAS (Effervescent Drug Absorption System), PRODAS (Programmable Oral Drug Absorption System), and DUREDAS (Dual Release Drug Absorption System) available from Elan Pharmaceutical Technologies. SODAS are multi particulate dosage forms utilizing controlled release beads. INDAS are a family of drug

delivery technologies designed to increase the solubility of poorly soluble drugs. IPDAS are multi particulate tablet formation utilizing a combination of high density controlled release beads and an immediate release granulate. MODAS are controlled release single unit dosage forms. Each tablet consists of an inner core surrounded by a semipermeable multiparous membrane that controls the rate of drug release. EFVAS is an effervescent drug absorption system. PRODAS is a family of multi particulate formulations utilizing combinations of immediate release and controlled release mini-tablets. DUREDAS is a bilayer tablet formulation providing dual release rates within the one dosage form. Although these dosage forms are known to one of skill, certain of these dosage forms will now be discussed in more detail.

[00155] INDAS was developed specifically to improve the solubility and absorption characteristics of poorly water soluble drugs. Solubility and, in particular, dissolution within the fluids of the gastrointestinal tract is a key factor in determining the overall oral bioavailability of poorly water soluble drug. By enhancing solubility, one can increase the overall bioavailability of a drug with resulting reductions in dosage.

[00156] IPDAS is a multi-particulate tablet technology that may enhance the gastrointestinal tolerability of potential irritant and ulcerogenic drugs. Intestinal protection is facilitated by the multi-particulate nature of the IPDAS formulation which promotes dispersion of an irritant lipoate throughout the gastrointestinal tract. Controlled release characteristics of the individual beads may avoid high concentration of active agent being both released locally and absorbed systemically. The combination of both approaches serves to minimize the potential harm of the active agent with resultant benefits to patients.

[00157] IPDAS is composed of numerous high density controlled release beads. Each bead may be manufactured by a two step process that involves the initial production of a micromatrix with embedded active agent and the subsequent coating of this micromatrix with polymer solutions that form a rate-limiting semipermeable membrane *in vivo*. Once an IPDAS tablet is ingested, it may disintegrate and liberate the beads in the stomach. These beads may subsequently pass into the duodenum and along the gastrointestinal tract, e.g., in a controlled and gradual manner, independent of the feeding state. Release of the active agent occurs by diffusion process through the micromatrix and subsequently through the pores in the rate controlling semipermeable membrane. The release rate from the IPDAS tablet may be customized to deliver a drug-specific absorption profile associated with optimized clinical benefit. Should a fast onset of activity be necessary, an immediate release granulate may be included in the tablet. The tablet may be broken prior to administration, without substantially compromising drug release, if a reduced dose is required for individual titration.

[00158] MODAS is a drug delivery system that may be used to control the absorption of water soluble agents. Physically MODAS is a non-disintegrating table formulation that

manipulates drug release by a process of rate limiting diffusion by a semipermeable membrane formed *in vivo*. The diffusion process essentially dictates the rate of presentation of drug to the gastrointestinal fluids, such that the uptake into the body is controlled. Because of the minimal use of excipients, MODAS can readily accommodate small dosage size forms. Each MODAS tablet begins as a core containing active drug plus excipients. This core is coated with a solution of insoluble polymers and soluble excipients. Once the tablet is ingested, the fluid of the gastrointestinal tract may dissolve the soluble excipients in the outer coating leaving substantially the insoluble polymer. What results is a network of tiny, narrow channels connecting fluid from the gastrointestinal tract to the inner drug core of water soluble drug. This fluid passes through these channels, into the core, dissolving the drug, and the resultant solution of drug may diffuse out in a controlled manner. This may permit both controlled dissolution and absorption. An advantage of this system is that the drug releasing pores of the tablet are distributed over substantially the entire surface of the tablet. This facilitates uniform drug absorption reduces aggressive unidirectional drug delivery. MODAS represents a very flexible dosage form in that both the inner core and the outer semipermeable membrane may be altered to suit the individual delivery requirements of a drug. In particular, the addition of excipients to the inner core may help to produce a microenvironment within the tablet that facilitates more predictable release and absorption rates. The addition of an immediate release outer coating may allow for development of combination products.

[00159] Additionally, PRODAS may be used to deliver an active agent. PRODAS is a multi particulate drug delivery technology based on the production of controlled release mini tablets in the size range of 1.5 to 4 mm in diameter. The PRODAS technology is a hybrid of multi particulate and hydrophilic matrix tablet approaches, and may incorporate, in one dosage form, the benefits of both these drug delivery systems.

[00160] In its most basic form, PRODAS involves the direct compression of an immediate release granulate to produce individual mini tablets that contain an active agent. These mini tablets are subsequently incorporated into hard gels and capsules that represent the final dosage form. A more beneficial use of this technology is in the production of controlled release formulations. In this case, the incorporation of various polymer combinations within the granulate may delay the release rate of drugs from each of the individual mini tablets. These mini tablets may subsequently be coated with controlled release polymer solutions to provide additional delayed release properties. The additional coating may be necessary in the case of highly water soluble drugs or drugs that are perhaps gastroirritants where release can be delayed until the formulation reaches more distal regions of the gastrointestinal tract. One value of PRODAS technology lies in the inherent flexibility to formulation whereby combinations of mini tablets, each with different release rates, are incorporated into one

dosage form. As well as potentially permitting controlled absorption over a specific period, this also may permit targeted delivery of drug to specific sites of absorption throughout the gastrointestinal tract. Combination products also may be possible using mini tablets formulated with different active ingredients.

[00161] DUREDAS is a bilayer tableting technology that may be used to formulate an active agent. DUREDAS was developed to provide for two different release rates, or dual release of a drug from one dosage form. The term bilayer refers to two separate direct compression events that take place during the tableting process. In an exemplary embodiment, an immediate release granulate is first compressed, being followed by the addition of a controlled release element which is then compressed onto this initial tablet. This may give rise to the characteristic bilayer seen in the final dosage form.

[00162] The controlled release properties may be provided by a combination of hydrophilic polymers. In certain cases, a rapid release of an active agent may be desirable in order to facilitate a fast onset of therapeutic affect. Hence one layer of the tablet may be formulated as an immediate release granulate. By contrast, the second layer of the tablet may release the drug in a controlled manner, e.g., through the use of hydrophilic polymers. This controlled release may result from a combination of diffusion and erosion through the hydrophilic polymer matrix.

[00163] A further extension of DUREDAS technology is the production of controlled release combination dosage forms. In this instance, two different active agents may be incorporated into the bilayer tablet and the release of drug from each layer controlled to maximize therapeutic affect of the combination.

[00164] An active agent can be incorporated into any one of the aforementioned controlled released dosage forms, or other conventional dosage forms. The amount of active agent contained in each dose can be adjusted, to meet the needs of the individual patient, and the indication. One of skill in the art and reading this disclosure will readily recognize how to adjust the level of active agent and the release rates in a controlled release formulation, in order to optimize delivery of an active agent and its bioavailability.

Inhalational formulations

[00165] An active agent will in some embodiments be administered to a patient by means of a pharmaceutical delivery system for the inhalation route. An active agent may be formulated in a form suitable for administration by inhalation. The inhalational route of administration provides the advantage that the inhaled drug can bypass the blood-brain barrier. The pharmaceutical delivery system is one that is suitable for respiratory therapy by delivery of an active agent to mucosal linings of the bronchi. An active agent can be delivered by a system that depends on the power of a compressed gas to expel the active agent from a container. An aerosol or pressurized package can be employed for this purpose.

[00166] As used herein, the term "aerosol" is used in its conventional sense as referring to very fine liquid or solid particles carries by a propellant gas under pressure to a site of therapeutic application. When a pharmaceutical aerosol is employed in this invention, the aerosol contains an active agent, which can be dissolved, suspended, or emulsified in a mixture of a fluid carrier and a propellant. The aerosol can be in the form of a solution, suspension, emulsion, powder, or semi-solid preparation. Aerosols employed in the present invention are intended for administration as fine, solid particles or as liquid mists via the respiratory tract of a patient. Various types of propellants known to one of skill in the art can be utilized. Suitable propellants include, but are not limited to, hydrocarbons or other suitable gas. In the case of the pressurized aerosol, the dosage unit may be determined by providing a value to deliver a metered amount.

[00167] An active agent can also be formulated for delivery with a nebulizer, which is an instrument that generates very fine liquid particles of substantially uniform size in a gas. For example, a liquid containing an active agent is dispersed as droplets. The small droplets can be carried by a current of air through an outlet tube of the nebulizer. The resulting mist penetrates into the respiratory tract of the patient.

[00168] A powder composition containing an active agent, with or without a lubricant, carrier, or propellant, can be administered to a mammal in need of therapy. This embodiment can be carried out with a conventional device for administering a powder pharmaceutical composition by inhalation. For example, a powder mixture of the active agent and a suitable powder base such as lactose or starch may be presented in unit dosage form in for example capsular or cartridges, *e.g.* gelatin, or blister packs, from which the powder may be administered with the aid of an inhaler.

[00169] There are several different types of inhalation methodologies which can be employed in connection with the present disclosure. An active agent can be formulated in basically three different types of formulations for inhalation. First, an active agent can be formulated with low boiling point propellants. Such formulations are generally administered by conventional meter dose inhalers (MDI's). However, conventional MDI's can be modified so as to increase the ability to obtain repeatable dosing by utilizing technology which measures the inspiratory volume and flow rate of the patient as discussed within U.S. Patents 5,404,871 and 5,542,410.

[00170] Alternatively, an active agent can be formulated in aqueous or ethanolic solutions and delivered by conventional nebulizers. In some embodiments, such solution formulations are aerosolized using devices and systems such as disclosed within U.S. Patent 5,497,763; 5,544,646; 5,718,222; and 5,660,166.

[00171] An active agent can be formulated into dry powder formulations. Such formulations can be administered by simply inhaling the dry powder formulation after creating an aerosol

mist of the powder. Technology for carrying such out is described within U.S. Patent 5,775,320 issued July 7, 1998 and U.S. Patent 5,740,794 issued April 21, 1998.

Dosages

[00172] Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dosage range is one which provides up to about 1 μg to about 1,000 μg or about 10,000 μg of an active agent and can be administered in a single dose. Alternatively, a target dosage of an active agent can be considered to be about in the range of about 0.1-1000 μM, about 0.5-500 μM, about 1-100 μM, or about 5-50μM in a sample of host blood drawn within the first 24-48 hours after administration of the agent.

[00173] Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Routes of administration

[00174] An active agent is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00175] Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. The active agent can be administered in a single dose or in multiple doses. In some embodiments, the active agent is administered orally. In other specific embodiments, the active agent is administered via an inhalational route. In some embodiments, the active agent is administered intranasally.

[00176] The active agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the present disclosure include, but are not necessarily limited to, enteral, parenteral, and inhalational routes.

[00177] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[00178] The active agent can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[00179] Methods of administration of the active agent through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more.

[00180] By treatment is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom, associated with the pathological condition being treated, such as a tauopathy. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

[00181] A variety of subjects (wherein the term "subject" is used interchangeably herein with the terms "individual," "host," and "patient") are treatable according to the subject methods. Generally such subjects are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In many embodiments, the subject will be a human.

SUBJECTS SUITABLE FOR TREATMENT

[00182] Individuals suitable for treatment with a subject treatment method include individuals who have been diagnosed with a tauopathy; individuals who have a tauopathy and who have been treated with an agent other than an agent discussed herein and who have either failed to respond to such treatment or who initially responded and subsequently relapsed.

DIAGNOSTIC METHODS

[00183] The present disclosure provides a method of diagnosing a cognitive impairment disorder in an individual. The method generally involves detecting a level of acetylated Tau polypeptide in a biological sample obtained from an individual. A level of acetylated Tau polypeptide that is higher than a normal control level indicates that the individual has a cognitive impairment disorder. The present disclosure also provides a method of determining the risk that an individual will develop a cognitive impairment disorder. The method

generally involves detecting a level of acetylated Tau polypeptide in a biological sample obtained from an individual. A level of acetylated Tau polypeptide that is higher than a normal control level indicates that the individual is at greater risk than the general population of developing a cognitive impairment disorder.

[00184] A level of acetylated Tau polypeptide in a biological sample (e.g., a biological sample that comprises a neuronal cell and/or a glial cell; a biological sample derived from a neuronal cell and/or a glial cell (e.g., a neuronal cell lysate; a glial cell lysate); etc.) that is at least about 10% higher, at least about 15% higher at least about 20% higher, at least about 25% higher, at least about 50% higher, at least about 2-fold higher, at least about 5-fold higher, at least about 10-fold higher, or more than 10-fold higher, than a normal control level, indicates that the individual from whom the biological sample was obtained has or is at greater risk than the general population of having, a cognitive impairment disorder.

[00185] Mild cognitive impairment (MCI, also known as incipient dementia, or isolated memory impairment) is a diagnosis given to individuals who have cognitive impairments beyond that expected for their age and education, but that do not interfere significantly with their daily activities. MCI is considered to be the boundary or transitional stage between normal aging and dementia. Although MCI can present with a variety of symptoms, when memory loss is the predominant symptom it is termed "amnestic MCI" and is frequently seen as a risk factor for Alzheimer's disease. Studies suggest that individuals with amnestic MCI tend to progress to probable Alzheimer's disease at a rate of approximately 10% to 15% per year. Additionally, when individuals have impairments in domains other than memory it is classified as non-amnestic single- or multiple-domain MCI and these individuals are believed to be more likely to convert to other dementias (e.g., dementia with Lewy bodies).

[00186] Since MCI is a risk factor for Alzheimer's disease, diagnosis of MCI leads to diagnosis of early-stage Alzheimer's disease. The diagnosis of MCI requires considerable clinical judgment, and a comprehensive clinical assessment including clinical observation, neuroimaging, blood tests and neuropsychological. A level of Ac-Tau polypeptide that is higher than a normal control level indicates that the individual has a cognitive impairment disorder. Thus, measurement of Ac-Tau polypeptide may be used to detect and diagnose MCI and tauopathies, such as, Alzheimer's disease.

[00187] The level of Ac-Tau polypeptide may be determined by using a reagent specific for Ac-Tau, such as, an antibody that recognizes the acetylated form of Tau but not the non acetylated-Tau polypeptide (e.g., Anti-Ac-Tau: Ab708 described below). Ac-Tau specific antibodies may be generated using a standard immunization protocol. For example, a Tau peptide acetylated at one or more lysine residues (e.g., Lys-163, and/or, Lys-174, and/or Lys-190 of Tau isoform 2) may be used to immunize an appropriate host animal (e.g., rabbit, goat, sheep, etc.). At about 10 days after the second booster immunization, antibody titers

may be determined using ELISA. Usually two booster immunizations are sufficient for obtaining high antibody titers.

[00188] A number of biological samples may be used to detect Ac-Tau levels. For example, cerebrospinal fluid, plasma, serum, blood, urine, brain biopsy sample, may be used to determine the Ac-Tau levels of an individual. The obtained sample may be supplemented with an enzyme inhibitor at the time of or after the collection of the sample in order to prevent the change of a Tau protein (fragmentation, dephosphorylation, deacetylation, etc.) or the coagulation of the blood in the sample. Enzyme inhibitors that can be utilized include: phosphatase inhibitors, such as, EDTA, EGTA, okadaic acid, pyrophosphoric acid, phosphate, sodium fluoride, β-glycerophosphoric acid, and cyclosporine A; and protease inhibitors such as aprotinin, antipain, pepstatin, leupeptin, EDTA, EGTA, PMSF (phenylmethanesulfonyl fluoride), and TLCK (tosyl lysine chloromethyl ketone). The samples may be obtained and tested fresh or the samples may be stored before determining Ac-Tau levels. The samples may be stored at, for example, at 4°C or lower, for example, at -20°C or lower.

[00189] A corresponding biological sample from an age matched non-demented individual, e.g., an individual with normal cognitive abilities, is used as a control. In addition, Ac-Tau levels may be normalized with reference to a normalization control, for example, a protein that is known to be present at comparable levels between patients with MCI and normal individuals, such as, GAPDH (glyceraldehyde-3-phosphate dehydrogenase), HPRT1 (hypoxanthine phosphoribosyltransferase-1).

[00190] Ac-Tau levels may be measured using anti-Ac-Tau antibody, for example, in an immunoassay, such as, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), protein blot (Western blot) assay, and the like. In exemplary embodiments, sandwich ELISA may be used. If Ac-Tau protein is measured by the sandwich ELISA method or the like, an antibody used in combination with the antibody specific to a Ac-Tau protein is an antibody that recognizes a tau protein regardless of kind of isoforms and acetylation status (hereinafter, which is also referred to as a "nonspecific anti-tau protein antibody") is used. Specific examples of the non-specific anti-tau protein antibody include anti-tau protein monoclonal antibodies HT7 (that binds to amino acid numbers 159-163 of a Tau protein) and BT2 (that binds to amino acid numbers 193-198 of a Tau protein) commercially available from Innogenetics.

SCREENING METHODS

[00191] The present disclosure provides a method of identifying a candidate agent suitable for use in treating a tauopathy. A subject screening method is generally an *in vitro* method, and can be carried out in a cell *in vitro*, or in an *in vitro* cell-free assay system.

[00192] In some cases, the method involves: a) contacting a sample with a test agent, where the sample comprises: i) an enzyme that deacetylates acetylated Tau; and ii) an acetylated Tau polypeptide; and b) determining the effect of the test agent on the degree of acetylation of the Tau polypeptide. A test agent that increases deacetylation of the Tau polypeptide is a candidate agent for treating a tauopathy. The method can be carried out *in vitro* in a cell-based assay, e.g., using a cell that produces an enzyme that deacetylates acetylated Tau and an acetylated Tau polypeptide, where the method involves contacting the cell with a test agent. The method can be carried out *in vitro* in a cell-free assay system, e.g., where the enzyme that deacetylates acetylated Tau and the acetylated Tau polypeptide are contacted with a test agent in a cell-free system.

[00193] In other cases, the method involves: a) contacting a sample with a test agent, where the sample comprises: i) an enzyme that acetylates a Tau polypeptide; and ii) a non-acetylated Tau polypeptide; and b) determining the effect of the agent on the degree of acetylation of the Tau polypeptide. A test agent that inhibits acetylation of the Tau polypeptide is a candidate agent for treating a tauopathy. The method can be carried out *in vitro* in a cell-based assay, e.g., using a cell that produces an enzyme that acetylates a Tau polypeptide and a non-acetylated Tau polypeptide, where the method involves contacting the cell with a test agent. The method can be carried out *in vitro* in a cell-free assay system, e.g., where the enzyme that acetylates Tau and the non-acetylated Tau polypeptide are contacted with a test agent in a cell-free system.

[00194] A subject screening method generally includes appropriate controls, e.g., a control sample that lacks the test agent. Generally a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[00195] A variety of other reagents may be included in the screening assay. These include reagents such as salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The components of the assay mixture are added in any order that provides for the requisite binding or other activity. Incubations are performed at any suitable temperature, typically between 4°C and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hour will be sufficient.

[00196] As used herein, the term "determining" refers to both quantitative and qualitative determinations and as such, the term "determining" is used interchangeably herein with "assaying," "measuring," and the like.

[00197] The terms “candidate agent,” “test agent,” "agent", "substance" and "compound" are used interchangeably herein. Candidate agents encompass numerous chemical classes, including synthetic, semi-synthetic, and naturally occurring inorganic or organic molecules. Candidate agents include those found in large libraries of synthetic or natural compounds. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ComGenex (South San Francisco, CA), and MicroSource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, Wis.) and can also be used. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from Pan Labs (Bothell, WA) or are readily producible.

[00198] Candidate agents may be small organic or inorganic compounds having a molecular weight of more than 50 daltons and less than about 2,500 daltons. Candidate agents may comprise functional groups necessary for structural interaction with proteins, e.g., hydrogen bonding, and may include at least an amine, carbonyl, hydroxyl or carboxyl group, and may contain at least two of the functional chemical groups. The candidate agents may comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00199] A test agent can be a small molecule. The test molecules may be individual small molecules of choice or in some cases, the small molecule test agents to be screened come from a combinatorial library, i.e., a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks." For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. Indeed, theoretically, the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds. See, e.g., Gallop et al., (1994), J. Med. Chem., 37(9), 1233-1251. Preparation and screening of combinatorial chemical libraries are well known in the art. Combinatorial chemical libraries include, but are not limited to: diversomers such as hydantoins, benzodiazepines, and dipeptides, as described in, e.g., Hobbs et al., (1993), Proc. Natl. Acad. Sci. U.S.A., 90:6909-6913; analogous organic syntheses of small compound libraries, as described in Chen et al., (1994), J. Amer. Chem. Soc., 116:2661-2662; Oligocarbamates, as described in Cho, et al., (1993), Science, 261:1303-1305; peptidyl phosphonates, as described in Campbell et al., (1994), J.

Org. Chem., 59: 658-660; and small organic molecule libraries containing, e.g., thiazolidinones and metathiazanones (U.S. Pat. No. 5,549,974), pyrrolidines (U.S. Pat. Nos. 5,525,735 and 5,519,134), benzodiazepines (U.S. Pat. No. 5,288,514).

[00200] Numerous combinatorial libraries are commercially available from, e.g., ComGenex (Princeton, N.J.); Asinex (Moscow, Russia); Tripos, Inc. (St. Louis, Mo.); ChemStar, Ltd. (Moscow, Russia); 3D Pharmaceuticals (Exton, Pa.); and Martek Biosciences (Columbia, MD).

Cell-based *in vitro* assay

[00201] As noted above, in some embodiments, a subject screening method is a cell-based *in vitro* assay. As noted above, in some embodiments, a subject screening method involves contacting a cell that produces a deacetylase, and an acetylated Tau polypeptide, with a test agent; and determining the effect of the test agent on the level of Ac-Tau polypeptide in the cell. As noted above, in some embodiments, a subject screening method involves contacting a cell that produces an enzyme that acetylates a Tau polypeptide, and a non-acetylated Tau polypeptide, with a test agent; and determining the effect of the test agent on the level of Ac-Tau polypeptide in the cell.

[00202] A test agent that reduces the level of acetylated Tau in the cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than 80%, compared to the level of acetylated Tau polypeptide in the cell in the absence of the test agent, is considered a candidate agent for treating a tauopathy.

[00203] A number of different types of cells can be used, for example, mouse embryonic fibroblasts (MEFs); primary neuronal cultures, such as cortical neurons; a neuronal cell line (e.g., an immortalized neuronal cell line); and the like. In certain cases, the screening is carried out using a primary neuronal culture, where the neuron is obtained from a human donor, a non-human primate, a rodent, etc. In alternative embodiments, the neuron is obtained by differentiation of an embryonic stem cell, or a neuronal cell line, such as, PC12, or a neuroblastoma cell line, etc.

[00204] Suitable cell lines include, but are not limited to, a human glioma cell line, e.g., SVGp12 (ATCC CRL-8621), CCF-STTG1 (ATCC CRL-1718), SW 1088 (ATCC HTB-12), SW 1783 (ATCC HTB-13), LLN-18 (ATCC CRL-2610), LNZTA3WT4 (ATCC CRL-11543), LNZTA3WT11 (ATCC CRL-11544), U-138 MG (ATCC HTB-16), U-87 MG (ATCC HTB-14), H4 (ATCC HTB-148), and LN-229 (ATCC CRL-2611); a human medulloblastoma-derived cell line, e.g., D342 Med (ATCC HTB-187), Daoy (ATCC HTB-186), D283 Med (ATCC HTB-185); a human tumor-derived neuronal-like cell, e.g., PFSK-1 (ATCC CRL-2060), SK-N-DZ (ATCCCRL-2149), SK-N-AS (ATCC CRL-2137), SK-N-FI (ATCC CRL-2142), IMR-32 (ATCC CCL-127), etc.; a mouse neuronal cell line, e.g.,

BC3H1 (ATCC CRL-1443), EOC1 (ATCC CRL-2467), C8-D30 (ATCC CRL-2534), C8-S (ATCC CRL-2535), Neuro-2a (ATCC CCL-131), NB41A3 (ATCC CCL-147), SW10 (ATCC CRL-2766), NG108-15 (ATCC HB-12317); a rat neuronal cell line, e.g., PC-12 (ATCC CRL-1721), CTX TNA2 (ATCC CRL-2006), C6 (ATCC CCL-107), F98 (ATCC CRL-2397), RG2 (ATCC CRL-2433), B35 (ATCC CRL-2754), R3 (ATCC CRL-2764), SCP (ATCC CRL-1700), OA1 (ATCC CRL-6538).

[00205] One or more of the deacetylase, acetyltransferase, and Tau (acetylated or non-acetylated) can be endogenous to the cells utilized in the screen. In some embodiments, one or more of the deacetylase, acetyltransferase, and Tau (acetylated or non-acetylated) is provided exogenously, for example, by genetically modifying a host cell with a nucleic acid (e.g., an expression construct(s)) comprising a nucleotide sequence(s) encoding one or more of the polypeptides; the genetically modified host cell is contacted with a test agent. When provided exogenously, the deacetylase can be a deacetylase known to deacetylate Ac-Tau and the acetyltransferase can be an acetyltransferase known to acetylate non-acetylated Tau to generate Ac-Tau.

[00206] In certain embodiments, the acetyltransferase is a p300 polypeptide (mRNA GenBank accession no.: NM_001429.3. protein GenBank accession no.: NP_001420.2). In certain cases, a nucleic acid (e.g., an expression construct) comprising a nucleotide sequence that encodes a p300 polypeptide is introduced into a host cell, where the nucleotide sequence encodes a p300 polypeptide that acetylates a Tau polypeptide and comprises an amino acid sequence that has at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1800 amino acids to about 2000 amino acids, from about 2000 amino acids to about 2200 amino acids, or from about 2200 amino acids to about 2414 amino acids, of the amino acid sequence set forth in SEQ ID NO:12. In these embodiments, the host cell is genetically modified with the p300 polypeptide-encoding expression construct.

[00207] In certain cases, a nucleic acid (e.g., an expression construct) comprising a nucleotide sequence that encodes a deacetylase is introduced into a host cell, where the nucleotide sequence encodes a SIRT1 polypeptide that i) deacetylates an acetylated Tau polypeptide and ii) comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 450 amino acids, or from about 450 amino acids to about 555 amino acids, of the amino acid sequence set forth in SEQ ID NO:9 (GenBank AAH12499; *Homo sapiens* SIRT1); or where the nucleotide sequence encodes a SIRT1 polypeptide that i) deacetylates an acetylated Tau polypeptide and ii) comprises an amino acid sequence

having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 747 amino acids, of the amino acid sequence set forth in SEQ ID NO:10 (GenBank NP_036370; *Homo sapiens* SIRT1 isoform a); or where the nucleotide sequence encodes a SIRT1 polypeptide that i) deacetylates an acetylated Tau polypeptide and ii) comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, or from about 400 amino acids to about 452 amino acids, of the amino acid sequence set forth in SEQ ID NO:11 (GenBank NP_001135970; *Homo sapiens* SIRT1 isoform b). In these embodiments, the host cell is genetically modified with the SIRT1 polypeptide-encoding expression construct.

[00208] In certain cases, a nucleic acid (e.g., an expression construct) comprising a nucleotide sequence that encodes a Tau polypeptide is introduced into the cell, where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of about 350 amino acids of any one of the amino acid sequences set forth in SEQ ID NOs:1-6; or where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to 383 amino acids of the amino acid sequence set forth in SEQ ID NO:2 (*Homo sapiens* Tau isoform 3); or where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 412 amino acids of the amino acid sequence set forth in SEQ ID NO:4 (*Homo sapiens* Tau isoform 5); or where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, or from about 400 amino acids to about 441 amino acids, of the amino acid sequence set forth in SEQ ID NO:1 (*Homo sapiens* Tau isoform 2); or where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least

about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 758 amino acids, of the amino acid sequence set forth in SEQ ID NO:5 (*Homo sapiens* Tau isoform 1); or where the nucleotide sequence encodes a Tau polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 776 amino acids, of the amino acid sequence set forth in SEQ ID NO:6 (*Homo sapiens* Tau isoform 6).

[00209] A nucleotide sequence encoding, for example, an acetyltransferase (e.g., a p300 polypeptide), and/or a Tau polypeptide, and/or a deacetylase (e.g., a SIRT1 polypeptide) can be introduced into a suitable expression vector. The expression vector is introduced into a suitable host cell. Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of polynucleotide sequences. Transcription cassettes may be prepared comprising a transcription initiation region, a nucleotide sequence encoding a polypeptide (e.g., an acetyltransferase (e.g., a p300 polypeptide), a Tau polypeptide, or a deacetylase (e.g., a SIRT1 polypeptide)), and a transcriptional termination region. The transcription cassettes may be introduced into a variety of vectors, e.g. plasmid; retrovirus, e.g. lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

[00210] The Tau polypeptide can be a fusion protein, e.g., a polypeptide comprising Tau and a fusion partner. Suitable fusion partners include peptides and polypeptides that confer enhanced stability *in vivo* (e.g., enhanced serum half-life); provide ease of purification, e.g., $(His)_n$, e.g., 6His, and the like; provide for secretion of the fusion protein from a cell; provide an epitope tag, e.g., glutathione-S-transferase (GST), hemagglutinin (HA; e.g., CYPYDVPDYA; SEQ ID NO:18), FLAG (e.g., DYKDDDDK; SEQ ID NO:19), c-myc (e.g., CEQKLISEEDL; SEQ ID NO:20), and the like; provide a detectable signal, e.g., an enzyme that generates a detectable product (e.g., β-galactosidase, luciferase), or a protein that is itself detectable, e.g., a green fluorescent protein, etc.; provides for multimerization, e.g., a multimerization domain such as an Fc portion of an immunoglobulin; and the like.

[00211] The various manipulations to generate an expression construct may be carried out in vitro or may be performed in an appropriate host cell, e.g. *Escherichia coli*. After each manipulation, the resulting construct may be cloned, the vector isolated, and the DNA screened or sequenced to ensure the correctness of the construct. The sequence may be screened by restriction analysis, sequencing, or the like.

[00212] Determining the effect of a test agent is generally carried out by determining the level of acetylated Tau polypeptide in the cell. Ac-Tau levels can be measured using anti-Ac-Tau antibody specific for Ac-Tau ("anti-Ac-Tau antibody"), for example, in an immunoassay, such as, ELISA, RIA, protein blot (Western blot) assay, and the like. The anti-Ac-Tau antibody can comprise a detectable label, and binding of the anti-Ac-Tau to acetylated Tau can be determined by detecting the label. Alternatively, binding of the anti-Ac-Tau antibody to acetylated Tau can be detected using a detectably labeled secondary antibody that binds to the anti-Ac-Tau antibody. The cell can be cultured in a liquid culture medium that includes a radiolabelled acetyl donor compound, such that any acetylated Tau produced by the cell comprises a radiolabelled acetyl group, where the level of acetylated Tau is carried out by detecting radiolabelled acetylated Tau. The level of Ac-Tau can be measured in an intact cell, or in a cell lysate, or using Ac-Tau isolated from the cell.

Cell-free *in vitro* assay

[00213] As noted above, in some embodiments, a subject screening method is carried out in an *in vitro* cell-free assay system. As noted above, in some embodiments, a subject screening method involves contacting i) an enzyme that deacetylates acetylated Tau; and ii) an acetylated Tau polypeptide with a test agent in a cell-free system. As noted above, in some embodiments, a subject screening method involves contacting i) an enzyme that acetylates a Tau polypeptide; and ii) a non-acetylated Tau polypeptide with a test agent in a cell-free system.

[00214] The polypeptides used (e.g., an enzyme that deacetylates acetylated Tau and an acetylated Tau polypeptide; or an enzyme that acetylates a Tau polypeptide and a non-acetylated Tau polypeptide) can be purified, e.g., where the polypeptides are at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% pure, e.g., free of other macromolecules and/or contaminants. The polypeptides can be produced recombinantly, then purified; the polypeptides can be purified from a naturally-occurring source of the polypeptides; or the polypeptides can be synthesized (e.g., using a cell-free chemical synthesis method).

[00215] A test agent that reduces the level of acetylated Tau by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more than

80%, compared to the level of acetylated Tau polypeptide in the absence of the test agent, is considered a candidate agent for treating a tauopathy.

[00216] A suitable enzyme that deacetylates acetylated Tau is as described above. For example, a suitable enzyme is a polypeptide that deacetylates an acetylated Tau polypeptide in a neuronal cell, and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 450 amino acids, or from about 450 amino acids to about 555 amino acids, of the amino acid sequence set forth in SEQ ID NO:9 (GenBank AAH12499; *Homo sapiens* SIRT1); or is a polypeptide that deacetylates an acetylated Tau polypeptide in a neuronal cell, and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 747 amino acids, of the amino acid sequence set forth in SEQ ID NO:10 (GenBank NP_036370; *Homo sapiens* SIRT1 isoform a); or is a polypeptide that deacetylates an acetylated Tau polypeptide in a neuronal cell, and that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, or from about 400 amino acids to about 452 amino acids, of the amino acid sequence set forth in SEQ ID NO:11 (GenBank NP_001135970; *Homo sapiens* SIRT1 isoform b).

[00217] A suitable enzyme that acetylates a Tau polypeptide includes an acetyltransferase that acetylates Tau and that comprises an amino acid sequence at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1800 amino acids to about 2000 amino acids, from about 2000 amino acids to about 2200 amino acids, or from about 2200 amino acids to about 2414 amino acids, of the amino acid sequence set forth in SEQ ID NO:12.

[00218] A suitable Tau polypeptide includes a polypeptide that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of about 350 amino acids of any one of the amino acid sequences set forth in SEQ ID NOs:1-6; or that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to

a contiguous stretch of from about 350 amino acids to 383 amino acids of the amino acid sequence set forth in SEQ ID NO:2 (*Homo sapiens* Tau isoform 3); or that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 412 amino acids of the amino acid sequence set forth in SEQ ID NO:4 (*Homo sapiens* Tau isoform 5); or that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, or from about 400 amino acids to about 441 amino acids, of the amino acid sequence set forth in SEQ ID NO:1 (*Homo sapiens* Tau isoform 2); or that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 758 amino acids, of the amino acid sequence set forth in SEQ ID NO:5 (*Homo sapiens* Tau isoform 1); or that comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, from about 500 amino acids to about 600 amino acids, from about 600 amino acids to about 700 amino acids, or from about 700 amino acids to about 776 amino acids, of the amino acid sequence set forth in SEQ ID NO:6 (*Homo sapiens* Tau isoform 6).

[00219] Acetylated Tau polypeptide can be produced recombinantly, e.g., in a neuronal cell line that produces a Tau polypeptide and an enzyme that acetylates a Tau polypeptide. Acetylated Tau polypeptide can also be produced by chemically acetylating a non-acetylated Tau polypeptide *in vitro*.

[00220] Determining the effect of a test agent is generally carried out by determining the level of acetylated Tau polypeptide. Ac-Tau levels can be measured using anti-Ac-Tau antibody specific for Ac-Tau ("anti-Ac-Tau antibody"), for example, in an immunoassay, such as, ELISA, RIA, protein blot (Western blot) assay, and the like. The anti-Ac-Tau antibody can comprise a detectable label, and binding of the anti-Ac-Tau to acetylated Tau can be determined by detecting the label. Alternatively, binding of the anti-Ac-Tau antibody to acetylated Tau can be detected using a detectably labeled secondary antibody that binds to the anti-Ac-Tau antibody. In some embodiments, the acetylated Tau polypeptide is produced

by a cell that is cultured in a liquid medium comprising a radiolabelled acetyl donor, such that the acetylated Tau produced by the cell comprises one, two, three, or more radiolabelled acetyl groups. The determining step can in these cases be carried out by determining the amount of radioactively labeled Tau polypeptide.

In vivo screening

[00221] A candidate agent identified by an *in vitro* screening assay as described above can be tested for its ability to decrease Ac-Tau polypeptide levels in a neuron and/or a glial cell *in vivo*. Alternatively, one can assess test agents for those that decrease Ac-Tau polypeptide levels *in vivo*.

[00222] A non-human model for cognitive impairment can be used to screen test agents to identify candidate agents that decrease the level of Ac-Tau polypeptides. Exemplary non-human models include transgenic animal models for Alzheimer's disease; transgenic animal models overexpressing human Tau, transgenic animal models showing cognitive impairment and/or tau-positive neurofibrillary tangles. A number of such non-human animal models are known in the art. A transgenic non-human animal model refers to a non-human animal (e.g., a rodent) which contain a transgene which is involved in human neurodegenerative diseases, such as tauopathies, which present with dementia, such as Alzheimer's disease, and includes the following exemplary transgenic non-human animals: LID mice (Yakar S, et al, 1999, Proc Natl Acad Sci USA 96; 7324-7329). transgenic animals carriers of mutations in presenilins and beta amyloid (Hock B J, Jr., Lamb B T ,2001, Trends Genet 17: S7-12), animals carriers of other mutations and alterations (US20030229907, Transgenic non-human mammals with progressive neurologic disease; US20030145343, Transgenic animals expressing human p25; US20030131364, Method for producing transgenic animal models with modulated phenotype and animals produced therefrom; US20030101467, Transgenic animal model for Alzheimer's disease; US200030093822, Transgenic animal model of neurodegenerative disorders; U.S. Pat. No. 6,717.031, Method for selecting a transgenic mouse model of Alzheimer's disease; U.S. Pat. No. 6,593,512. Transgenic mouse expressing human tau gene; U.S. Pat. No. 6,563.015, Transgenic mice expressing mutant human APP and forming congo red staining plaques; U.S. Pat. No. 6.455,757, Transgenic mice expressing human APP and TGF-beta demonstrate cerebrovascular amyloid deposits; U.S. Pat. No. 6.452,065, Transgenic mouse expressing non-native wild-type and familial Alzheimer's Disease mutant presenilin 1 protein on native presenilin 1 null background; W003053136, Triple transgenic model of Alzheimer disease: WO03046172. In general, any animal model for tauopathy that has a higher than normal level of Ac-Tau and/or Tau may be used in an in vivo screening assay to identify compounds that lower level of Tau and/or Ac-Tau.

[00223] In addition to determining the levels of Ac-Tau present in the non-human animal model, these non-human animal models can also be used to assess the efficacy of a candidate agent identified via a subject *in vitro* screening method(s). Efficacy of a candidate agent may tested in a animal model for MCI, for example, by assessing improvement in symptoms associated with tauopathy, such as, cognition.

EXAMPLES

[00224] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); wt, wild type; and the like.

Example 1: Acetylation of Tau inhibits its degradation and contributes to tauopathy

EXPERIMENTAL PROCEDURES

Chemicals and Reagents

[00225] C646 was from Chembridge (San Diego, CA). C37 (inactive analog of C646) was synthesized. EX527 (Tocris Bioscience, Ellisville, MO), resveratrol (EMD Chemicals, Gibbstown, NJ), MG-132 (Sigma, St. Louis, MO), cycloheximide (Sigma), Aβ42 peptide (rPeptide, Bogart, GA), and recombinant tau (rPeptide) were purchased. Aβ42 oligomers were prepared as described. Chen et al. (2005) *J. Biol. Chem.* 280:40364.

Primary Antibodies

[00226] Two rabbit polyclonal anti-ac-tau antisera were generated against two acetylated peptides of tau (Abgent, San Diego, CA). PHF1 antibody was a gift. Other antibodies were obtained and used at the indicated concentrations: Tau 5 (1:5000; Abcam, Cambridge, MA), anti-p300 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti-GAPDH (1:10000; Sigma), anti-tubulin (1:10000; Sigma), anti-FLAG (1:2000; Sigma), AT8 for p-tau (1:500; Thermo Fisher Scientific, Rockford, IL), anti-Sir2 (1:2000; Millipore, Billerica, MA), anti-hemagglutinin (anti-HA) (1:1000; Cell Signaling Technology, Danvers, MA). Secondary

antibodies: peroxidase-conjugated goat anti-rabbit and anti-mouse IgGs (1:2000; GE Healthcare, Piscataway, NJ).

Expression Plasmids

[00227] For expression in HEK293T cells, cDNAs encoding hTauwt, hTau2KR (K174R, K180R), hTau3KR (K163R, K174R, K180 R), p300, SIRT1, H363Y (SIRT1), SIRT2, HDAC5, HDAC6, and HA-ubiquitin were cloned into pcDNA3.1 vector (Invitrogen). For protein expression in bacteria, hTauwt cDNA was cloned into pGEX4T-1 vector for GST-fusion protein expression. For expression in primary neurons, cDNAs encoding hTauwt, hTau3KR, hTauP301, and cre recombinase were cloned into lentiviral FUGW vectors.

Mice

[00228] All procedures involving animals were in compliance with the policies of the Animal Care and Use Committee at the University of California, San Francisco. PS19 mice were obtained from Jackson Laboratory (Bar Harbor, ME). The hT-PAC-N line was a gift. SIRT-null and *SIRT1*$^{F/F}$ mice were provided by Fred Alt (Harvard Medical School).

Cell Cultures and Transient Transfections

[00229] HEK293T cells and MEFs were grown at 37°C in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. For overexpression, transfections were performed with Lipofectamine 2000 (Invitrogen). For siRNA oligonucleotide transfection, HEK 293T cells were seeded at $1x10^5$ cells/well on 12-well culture plates. After 12 h, cells were transfected with 10 nM ON-TARGETplus SMARTpool siRNA (Thermo Scientific-Dharmacon, Chicago, IL) with Lipofectamine RNAiMAX transfection reagent (Invitrogen), according to the manufacturer's protocol. SIRT1 siRNA (L-094699-01), SIRT2 siRNA (L-004826-00), HDAC6 siRNA (L-003499-00), p300 siRNA (L-003486-00), and PCAF siRNA (L-005055-00) were used to target specific cellular genes; siControl Non-Targeting siRNA#1 (Dharmacon) was used as a negative control. About 48 h after siRNA transfection, plasmid pcDNA3.1-hTauwt was transfected into the same culture plates. Cells were harvested 24 h later for real-time RT-PCR or western blot analyses.

Primary Neuronal Cultures and Lentiviral Infections

[00230] Primary cultures were established from cortices of Sprague-Dawley rat pups (Charles River Laboratories) or *SIRT1*$^{F/F}$ mice on postnatal day 0 or 1. Purified cells were plated at 160,000 cells/ml in Neurobasal medium supplemented with B27 (Invitrogen) on poly-ornithine coated plates. All treatments were performed at 7–13 DIV in Neurobasal medium supplemented with N2 (Invitrogen) unless noted otherwise.

[00231] Lentivirus was generated, purified, and used for infection as described. Chen et al. (2005) *supra*. Recombinant lentivirus was produced by co-transfection of the shuttle vector (FUGW), two helper plasmids, delta8.9 packaging vector, and VSV-G envelope vector into

293T cells and purified by ultracentrifugation. Viral titers were measured by p24 enzyme-linked immunosorbent assays at the Gladstone-UCSF Laboratory of Clinical Virology.

Homogenization of Cells and Tissues and Western Blot Analyses

[00232] Cells or human or mouse brain tissues were lysed in RIPA buffer containing protease inhibitor cocktail (Sigma), 1 mM phenylmethyl sulfonyl fluoride (PMSF) (Sigma), phosphatase inhibitor cocktail (Roche), and HDAC inhibitors, including 5 mM nicotinamide (Sigma) and 1 μM trichostatin A (Sigma). After sonication, lysates from human or mouse brain tissues were centrifuged at 170,000 *g* at 4°C for 15 min and at 18,000 *g* at 4°C for 10 min. Supernatants were collected and analyzed by western blot. Bands in immunoblots were visualized by enhanced chemiluminescence (Pierce) and quantified by densitometry and Quantity One 4.0 software (Bio-Rad, Hercules, CA).

***In Vitro* Acetylation Assays**

[00233] The reactions were performed as described. Pagans et al. (2005) *PLoS Biol* 3:e41. Briefly, 1 μg of human recombinant tau, 2 nM of acetyl CoA (Sigma), and 1 μl of purified GST-p300 in acetylation buffer (50 mM HEPES, pH 8.0, 10% glycerol, 1 mM dithiothreitol (DTT), and 10 mM Na butyrate) were incubated for 30 min at 30°C with constant shaking. Reactions were stopped by adding 2X LDS sampling buffer (Invitrogen), followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analyses.

MALDI-TOF Analyses

[00234] Samples from in vitro acetylation reactions were run on SDS-polyacrylamide gels and stained with Coomassie Blue. The band at approximately 65 kDa was cut out and sent to Stanford Mass Spectrometry Laboratory for analyses. In-gel digestion was done with Promega MS grade trypsin overnight. Before digestion, the gel slices were cut into approximately 1 mm x 1 mm cubes, reduced with 5 mM dithiothreitol (DTT) and alkylated with acrylamide. Peptides were extracted and dried down using a speed-vac before reconstitution and analysis.

[00235] Nano reversed-phase high performance liquid chromatography (HPLC) was done with an Eksigent 2D nanoLC (Eksigent, Dublin, CA) with buffer A consisting of 0.1 % formic acid in water and buffer B consisting of 0.1% formic acid in acetonitrile. A fused silica column self packed with Duragel C18 (Peeke, Redwood City, CA) matrix was used with a linear gradient from 5% B to 40% B over 80 min at a flow rate of 450 nl/min. The nanoHPLC was interfaced with an Advion Nanomate (Ithaca, NY) for nano-electrospray ionization into the mass spectrometer. The mass spectrometer was a LCQ Deca XP Plus (Thermo Scientific), which was set in data dependent acquisition mode to perform MS/MS on the top three most intense ions with a dynamic exclusion setting of two. The DTA files

were extracted from the raw data and systematically searched with Mascot. At least two peptides with a probability >95% were needed for the assignment of a protein.

***In Vitro* Deacetylation Assays**

[00236] The reactions were modified from established procedures. Pagans et al. (2005) *supra*. HEK293T cells were transfected with human FLAG-tagged SIRT1 plasmid or mock plasmid with Lipofectamine 2000 (Invitrogen). After 24 h, cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 0.5 % NP-40, 150 mM NaCl, and protease inhibitor cocktails). After centrifugation at 13,000 rpm at 4°C for 10 min, equal amounts of supernatant proteins were immunoprecipitated with FLAG M2 agarose beads (Sigma) for 3 h at 4°C. Immuoprecipitated beads were washed twice in lysis buffer and once in deacetylation buffer (50 mM Tris-HCl, pH 9.0, 4 mM $MgCl_2$, and 0.2 mM DTT) and incubated with *in vitro* ac-tau in deacetylation buffer at 30°C for 3 h with constant shaking. Reactions were stopped by adding 2X LDS sampling buffer (Invitrogen) and analyzed by western blot.

***In Vivo* Ubiquitination Assays**

[00237] Procedures were modified from a published study. Oh et al. (2009) *Nat Cell Biol* 11:295. HEK293T cells were transfected with expression vectors encoding FLAG-tagged human tau and HA-ubiquitin with or without Myc-SIRT1 (wildtype or H363Y mutant). After 2 h of incubation, cells were treated with EX527, resveratrol, or dimethylsulfoxide (DMSO) in Dulbecco's modified Eagle's medium and incubated for 20 h. MG-132 (20 μM) was added and incubated for 4 h. Cells were lysed in ubiquitination buffer (20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.2% Triton X-100, 150 mM NaCl, and protease inhibitor cocktail). Supernatant proteins were immunoprecipitated with FLAG M2 agarose beads (Sigma) for 3 h at 4°C. Reactions were washed at least three times with ubiquitination buffer and analyzed by SDS-PAGE and western blot with anti-HA antibody (Cell Signaling Technology).

Purification of GST Fusion Proteins and Interaction Assays

[00238] Full-length cDNA encoding human tau was subcloned into pGEX-4T-1 bacterial expression vector (Sigma) and transformed in the BL21 (DE3) strain. After induction with 100 μM isopropyl β-D-1-thiogalactopyranoside, bacterial cells were harvested and sonicated in phosphate-buffered saline with 1 mM EDTA, 0.5% Triton X-100, and protease inhibitor cocktail (Sigma). Glutathione-S-transferase (GST)-tagged human tau or GST protein was purified with glutathione-agarose beads (GenScript).

[00239] In GST pull-down assays, bead-bound forms of purified GST-tau were incubated with lysates from HEK293T cells that were not transfected (for interaction with endogenous SIRT1) or transfected with FLAG-tagged human SIRT1. Beads were washed at least three times with lysis buffer containing Triton X-100 or Nonidet-40 and analyzed by SDS-PAGE and western blot with anti-SIRT1 antibody (Millipore) or anti-FLAG antibody (Sigma).

[00240] In coimmunoprecipitation assays, HEK293T cells were transfected with pcDNA3.1-hTau-FLAG. Triton X-100-solubilized lysates were incubated with FLAG M2 agarose beads for 3 h at 4°C. Beads were washed at least three times with lysis buffer containing Triton X-100 (0.5%) and analyzed by SDS-PAGE and western blot with anti-SIRT1 antibody (Millipore) or anti-FLAG antibody (Sigma).

Characterization of C646, a Selective p300 Inhibitor

[00241] C646 was identified as one of the putative inhibitors of p300 by a computational docking screen. Bowers et al. (2010) *Chem Biol* 17:471. A convenient spectrophotometric assay was performed to validate it as a p300 inhibitor (Kim et al. (2000) *Anal Biochem* 280:308), followed by a series of secondary assays. In the coupled spectrophotometric assay, the acetyltransferase reaction product CoASH becomes a substrate for alphaketoglutarate dehydrogenase, which converts NAD to NADH, resulting in an increase of UV absorbance at 340 nm. Kim et al. (2000), *supra.* A radioactive p300 HAT assay was subsequently performed to directly measure the IC_{50} of C646. The specific inhibition of p300 versus other acetyltransferases by C646 was further examined. These acetyltransferases included serotonin N-acetyltransferase, and the HATs pCAF, GCN5, Rtt109, Sas, and MOZ.

Data analyses

[00242] Statistical analyses were conducted with Graphpad Prism. Differences among multiple (≥3) means with one variable were evaluated by one-way ANOVA and the Tukey-Kramer *posthoc* test. Differences between two means were assessed with the paired or unpaired two-tailed t test. $P < 0.05$ was considered significant.

RESULTS

Tau Is Acetylated in Vitro and in Vivo

[00243] To demonstrate that tau is acetylated, recombinant tau was incubated with recombinant acetyltransferase p300 or pCAF (p300/CBP-associated factor) with ^{14}C-acetyl-coenzyme A. Incubation with p300, not pCAF, led to tau acetylation, while both p300 and pCAF were active in transferring acetyl groups to histones as expected (Figure 1A). Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) spectrometry identified multiple lysines that were acetylated by p300 *in vitro*. A total of 23 putatively acetylated lysines were detected out of 383 residues (~86.8% coverage; Table 4) throughout the tau sequence (2N4R, 441 amino acids). A few putative acetylated lysines were in the N- and C-terminal regions; 13 were in microtubule-binding domains (Figure 1B and Table 4). Putative acetylated N-terminal lysines (e.g., lysines 163, 174, and 180) appeared to be acetylated in all mass spectrometry (MS) analyses. Those in the microtubule-binding domains appeared to be acetylated in a subset of MS analyses, suggesting variable acetylation at these sites *in vitro* (Table 4).

Table 4

Peptide position (Tau441)	Peptide sequence	SEQQUEST XCorr Score	Modifications identified by spectrum	Acetylation observed in peptides
2–24	AEPRQEFEVMEDHAGTYGLGDRK (SEQ ID NO:21)	4.20		none
24–44	KDQGGYTMHQDQEGDTDAGLK (SEQ ID NO:22)	4.09	Oxidation (+16)	none
45–67	ESPLQTPTEDGSEEPGSETSDAK (SEQ ID NO:23)	4.90		none
68–87	STPTAEDVTAPLVDEGAPGK (SEQ ID NO:24)	3.17		none
144–155	GADG**K**T**K**IATPR (SEQ ID NO:25)	2.98	Acetyl (+42)	all
156–170	GAAPPGQ**K**GQANATR (SEQ ID NO:26)	3.02	Acetyl (+42)	all
171–190	IPA**K**TPPAPKTPPSSGEPPK (SEQ ID NO:27)	3.16	Acetyl (+42)	all
175–194	TPPAP**K**TPPSSGEPPKSGDR (SEQ ID NO:28)	2.59	Acetyl (+42)	all
181–194	TPPSSGEPPKSGDR (SEQ ID NO:29)	3.60		none
212–225	TPSLPTPPTREPKK (SEQ ID NO:30)	3.76		none
243–259	LQTAPVPMPDL**K***NV**K***SK (SEQ ID NO:31)	3.49	Acetyl (+42)	some
255–267	NV**K***S**K***IGSTENLK (SEQ ID NO:32)	2.95	Acetyl (+42)	some
258–267	SKIGSTENL**K*** (SEQ ID NO:33)	2.61	Acetyl (+42)	some
260–280	IGSTENLKHQPGGG**K**VQIIN**K*** (SEQ ID NO:34)	6.28	Acetyl (+42)	some
268–280	HQPGGG**K**VQIINK (SEQ ID NO:35)	3.39	Acetyl (+42)	some
275–290	VQIIN**K*K***LDLSNVQSK (SEQ ID NO:36)	3.86	Acetyl (+42)	some
281–294	KLDLSNVQS**K***CGSK (SEQ ID NO:37)	3.46	Acetyl (+42)	some
282–294	LDLSNVQS**K***CGSK (SEQ ID NO:38)	2.85	Acetyl (+42)	some
295–317	DNI**K***HVPGGGSVQIVYKPVDLSK (SEQ ID NO:39)	6.74	Acetyl (+42)	some
299–321	HVPGGGSVQIVYKPVDLS**K***VTSK	5.63	Acetyl (+42)	some

Peptide position (Tau441)	Peptide sequence	SEQQUEST XCorr Score	Modifications identified by spectrum	Acetylation observed in peptides
	(SEQ ID NO:40)			
308–321	IVYKPVDLSKVTS**K*** (SEQ ID NO:41)	3.42	Acetyl (+42)	some
318–340	VTS**K***CGSLGNIHHKPGGGQVEVK (SEQ ID NO:42)	5.32	Acetyl (+42)	some
350–370	VQS**K***IGSLDNITHVPGGGN**K***K (SEQ ID NO:43)	5.08	Acetyl (+42)	some
354–370	IGSLDNITHVPGGGN**K***K (SEQ ID NO:44)	4.13	Acetyl (+42)	some
354–375	IGSLDNITHVPGGGN**K*K***IETHK (SEQ ID NO:45)	6.50	Acetyl (+42)	some
370–379	KIETH**K***LTFR (SEQ ID NO:46)	3.64	Acetyl (+42)	some
380–395	ENA**KA**KTDHGAEIVYK (SEQ ID NO:47)	4.58	Acetyl (+42)	all
384–406	A**K**TDHGAEIVY**K***SPVVSGDTSPR (SEQ ID NO:48)	3.63	Acetyl (+42)	all (AK), some (YK)
386–406	TDHGAEIVY**K***SPVVSGDTSPR (SEQ ID NO:49)	4.91	Acetyl (+42)	some
407–438	HLSNVSSTGSIDMVDSPQLATLADEVSASLAK (SEQ ID NO:50)	5.50	Oxidation (+16),	none
419–438	MVDSPQLATLADEVSASLAK (SEQ ID NO:51)	4.19	Oxidation (+16), not acetylated	none

[00244] Table 4 depicts putative modification of human tau peptides in the presence of p300. Only peptides with lysines are shown. Total coverage: 383/441 (86.8%) from three independent analyses. The putative acetylated lysines are bolded and underlined. Shown are the highest SEQUEST XCorr scores for each peptide among multiple observations. The cut-off of the XCorr score is 2.5. Lysines are also observed to be non-acetylated.

[00245] To examine tau acetylation *in vivo*, a polyclonal antibody (anti-ac-tau, Ab708) was generated using a synthetic tau peptide (amino acids 160–182 for 2N4R tau isoform; underlined in Figure 10) containing acetylated lysines at positions 163 and 174 and 180. A control antibody was generated (anti-tau, Ab707) using the same peptide with nonacetylated lysines. To test the specificity of Ab708 against ac-tau, recombinant human tau (441; 2N4R isoform) was incubated with glutathione S-transferase (GST) alone or GST-p300. Immunoblotting with Ab708 detected strong tau signals after incubation with GST-p300, but not with GST alone (Figure 1C). In contrast, Ab707 or Tau 5 antibody detected similar levels of total tau (t-tau) with either GST or GST-p300 (Figure 1C). Thus, Ab708 specifically recognizes tau acetylated by p300 under cell-free conditions. In HEK293T cells transfected with tau, overexpression of p300 markedly elevated the levels of ac-tau detected with Ab708 while the increase in the levels of t-tau was modest, suggesting that Ab708 preferentially recognizes p300-induced ac-tau in cultured cells (Figure 1D). Mutation of lysines 163, 174 and 180 (Tau3KR) reduced ac-tau levels relative to t-tau levels in HEK293T cells (Figure 1E). A smaller yet still significant reduction was also observed when two lysines were mutated in Tau2KR(K174R/K180R) (Figure 1E). These findings suggest that Ab708 recognizes human tau acetylated at positions 163, 174 or 180 and possibly other acetylated lysines on tau, but not nonacetylated tau.

[00246] To detect ac-tau *in vivo*, western blots were performed with brain lysates from transgenic mice expressing human tau cDNA (1N4R) with P301S mutation (P19) (Yoshiyama et al. (2007) *Neuron* 53:337) or from transgenic mice expressing the entire human wildtype *MAPT* (hT-PAC-N) with 0N3R and 0N4R as the two predominant tau isoforms (McMillan et al. (2008) *J Comp Neurol* 511:788). Human and mouse tau differ at three positions in the region used to generate Ab708 and Ab707 (Figure 10). Ab708 detected specific signals in lysates from P19 and hT-PAC-N mice, but not those from nontransgenic (NTG) littermates (Figure 1F). These findings suggest that Ab708 recognizes various isoforms of human ac-tau, but not mouse ac-tau. The control antibody Ab707, which recognizes human t-tau, does not recognize mouse tau either. Endogenous tau in NTG mice was detected with Tau 5 antibody (Figure 1F).

[00247] Rat tau is more similar to human tau than mouse tau in the region used to generate Ab708 (Figure 10). Ab708 detected endogenous ac-tau in rat primary cortical neurons (Figure

1G). Levels of ac-tau/t-tau gradually increased as neurons matured from 5–12 days in vitro (DIV), suggesting that tau acetylation is regulated developmentally (Figure 1G). However, the isoforms of rat tau detected by Ab708 remain to be defined.

[00248] **Figures 1A-G. Tau is Acetylated *in Vitro* and *in Vivo*.** (A) Acetylation of h-tau (2N4R) by p300 but not pCAF under cell-free conditions, as shown by autoradiography. (B) MALDI-TOF spectrometry identified ac-lysines on h-tau by p300 *in vitro*. *Red*: lysines (K) with acetyl group. *Underlined*: sequence covered by MS analysis. *Blue box*: microtubule-binding domains. (C–E) Ab708 specifically recognizes ac-tau. (C) Ab708 only recognized recombinant tau acetylated by GST-p300, not nonacetylated tau with GST alone. Similar levels of t-tau were detected with Ab707 and Tau 5 antibody. (D) Overexpressing p300 markedly enhanced ac-tau, detected with Ab708, in HEK293T cells. Levels of t-tau, detected with Tau 5, were similar with or without p300. Blots are representative of >5 experiments. (E) Putatively acetylated lysine sites recognized by Ab708. Ac-tau/t-tau levels in HEK293T cells expressing wildtype tau were set as 1. n = 4. *, P=0.012; ** P=0.003; ***, P=0.0003 (one-way ANOVA with Tukey-Kramer *posthoc* analysis). (F) Ab708 recognizes human ac-tau in brains of PS19 or hT-PAC-N transgenic mice, not in non-transgenic (NTG) littermates. Human t-tau was detected with Ab707 antibody; human and mouse t-tau was detected with Tau 5 antibody. See Figure 10 for the sequence similarity among human, mouse and rat tau. (G) Levels of Ab708-positive ac-tau were elevated in primary rat neurons as they matured in culture (DIV=5–12). n=2–7 from 2–3 independent experiments. ***, P<0.001 (DIV5 vs. DIV8 or DIV12); **, P<0.01 (DIV5 vs. DIV9–11). Values are means ± SEM (E, G).

Acetylation of Tau by p300 Acetyltransferase

[00249] To determine the role of endogenous p300 or pCAF in tau acetylation, HEK293T cells expressing human tau cDNA (2N4R) were transfected with siRNAs targeting p300 or pCAF (Figure 2A) and the effects on ac-tau or t-tau were assessed. Inhibiting p300 significantly reduced levels of ac-tau, but not t-tau (Figure 2B, 2C). In contrast, inhibiting pCAF had no effects (Figure 2B, 2C). These findings are consistent with the results of *in vitro* studies (Figure 1A). Next, primary neurons were treated with C646, a pyrazolone-containing small-molecule inhibitor of p300 with a Ki of 400 nM. Bowers et al. (2010) *supra.*. Under cell-free conditions, C646 at 10 μM inhibits p300 in a highly selective manner (86% inhibition vs. <10% for the six other acetyltransferases). Bowers et al. (2010), *supra*. Inhibition of p300 with C646 (20 μM) drastically reduced levels of ac-tau in primary neurons within 8 h. The levels of t-tau remained unchanged (Figure 2D). p300 is a transcriptional coactivator. Goodman and Smolik (2000) *Genes Dev* 14:1553. However, C646 treatment for 8 h did not suppress tau transcripts as quantified with real-time reverse transcription-polymerase chain reaction (RT-PCR). Thus short-

term (8 h) inhibition of p300 deacetylates tau without affecting t-tau levels. Extended treatment with C646 for 20 h lowered the levels of ac-tau relative to the t-tau (ac-tau/t-tau), but also those of t-tau (Figure 2E).

[00250] **Figures 2A-E. Tau Is Acetylated by p300 Acetyltransferase.** (A–C) Inhibiting p300, not pCAF, reduced ac-tau in HEK293T cells. (A) Inhibition of p300 or pCAF expression by siRNA transfections. Levels of p300/GAPDH or pCAF/GAPDH in control siRNA-transfected cells were set as 1. ***, P=0.0006 (p300 vs. control) or P<0.0001 (pCAF vs. control). (B) Representative western blots (from three experiments) showing levels of p300 or pCAF, ac-tau, t-tau, and GAPDH in cells transfected with control siRNA (CTRL) or siRNA targeting p300 or pCAF. (C) Inhibition of p300, not pCAF, reduced ac-tau levels. Levels of ac-tau/GAPDH or t-tau/GAPDH in control siRNA-transfected cells were set as 1. n=5–6. **, P=0.008 (paired t test). (D) Inhibiting p300 acutely with C646 (20 μM for 8 h) eliminated ac-tau without affecting t-tau levels in primary rat cortical neurons. *Left:* Representative western blot from three experiments. *Right:* Ac-tau/t-tau levels in vehicle-treated cells were set as 1. n=3. ***, P=0.0001 (unpaired t test). (E) Extended treatment with C646 (20 μM for 20 h) lowered t-tau in primary cortical neuron. Blots are representative of two experiments. Values are means ± SEM (A, C–E).

Deacetylation of Tau by SIRT1 in Cultures

[00251] To investigate the enzymes that deacetylate tau, an expression vector encoding FLAG-tagged SIRT1, SIRT2, HDAC5, or HDAC6 was transfected into HEK293T cells expressing human tau. All HDACs were expressed at high levels (Figure 3A). Although expressed at lower levels than SIRT1 and SIRT2, HDAC6 eliminated tubulin acetylation (Hubbert et al. (2002) *Nature* 417:455), suggesting sufficient expression (Figure 3B). Overexpression of SIRT1 reduced levels of Ab708-positive ac-tau. SIRT2 and HDAC6 overexpression also lowered ac-tau, although to lesser extents (Figure 3B, 3C). Levels of t-tau were also reduced in cells overexpressing SIRT1 and HDAC6. Nevertheless, the ac-tau/t-tau ratio was significantly reduced by SIRT1 overexpression (Figure 3D). The modest reduction in ac-tau/t-tau induced by HDAC6 or SIRT2 overexpression was not statistically significant (Figure 3D).

[00252] To examine the effects of endogenous HDACs on ac-tau, expression of SIRT1, SIRT2, or HDAC6 was inhibited with siRNAs (Figure 3E). Relative to control siRNA, target siRNAs significantly reduced levels of SIRT1, SIRT2, and HDAC6. Despite modest inhibition, HDAC6 increased ac-tubulin levels (Figure 3F). However, only inhibition of SIRT1 increased ac-tau levels, suggesting the involvement of endogenous SIRT1 in deacetylating tau (Figure 3G). Consistent with the observation that SIRT1 overexpression reduced t-tau, SIRT1 inhibition led to a trend of increase in t-tau (Figure 3G). Nevertheless, inhibition of SIRT1, not SIRT2 or

HDAC6, significantly elevated levels of ac-tau relative to t-tau (ac-tau/t-tau) (Figure 3H). These results provided direct support that SIRT1 is involved in tau deacetylation. Our findings so far do not support a prominent role of SIRT2 or HDAC6, which both deacetylate tubulin (Hubbert et al. (2002) *supra*; North et al. (2003) *Mol. Cell* 11:437), in tau deacetylation. However, their involvement cannot be ruled out since only partial silencing of SIRT2 or HDAC6 was achieved with siRNA transfections.

[00253] To further investigate the role of SIRT1 in tau deacetylation, low-passage mouse embryonic fibroblasts (MEF) with (*SIRT1*$^{+/+}$) or without SIRT1 (*SIRT1*$^{-/-}$) were transfected with human tau cDNA. Deleting SIRT1 significantly raised ac-tau levels. The increase in t-tau did not reach statistical significance, suggesting SIRT1 deacetylates tau in MEFs. In HEK293T cells, when lysines 163, 174, and 180 were mutated to arginines (Tau3KR), levels of ac-tau were significantly reduced (Figure 3I). SIRT1 overexpression reduced ac-tau in TauWT cells, but the reduction was much attenuated in Tau3KR cells (Figure 3I). These results implicate SIRT1 in deacetylating lysines 163, 174, and 180. However, SIRT1 reduced ac-tau to lower levels in Tau3KR cells than in TauWT cells, indicating that SIRT1 could deacetylate additional lysine residues besides those at positions 163, 174, and 180 (Figure 3I).

[00254] **Figures 3A-I. SIRT1 Deacetylates Tau in Culture.** (A–D) SIRT1 overexpression lowered ac-tau levels in HEK293T cells. (A) Western blot showing expression of FLAG-tagged SIRT1, SIRT2, HDAC5, or HDAC6 with an anti-FLAG antibody. Blots are representative of 2–3 experiments. (B) Western blot showing levels of ac-tau, t-tau, tubulin, and ac-tubulin in cells overexpressing SIRT1, SIRT2, HDAC5, or HDAC6. Blots are representative of 2–3 experiments. (C) Overexpression of SIRT1, SIRT2 or HDAC6 significantly reduced levels of ac-tau/GAPDH. Levels of t-tau were also reduced by SIRT1 or HDAC6 overexpression. n=9–18 from 6–10 independent experiments. ***, $P < 0.001$ (Mock vs. SIRT1 or Mock vs. HDAC6); **, $P < 0.01$ (Mock vs. SIRT2) (two-way ANOVA and Bonferroni *posthoc* test). (D) Overexpression of SIRT1 significantly reduced ac-tau/t-tau. n=9–18 from 6–10 independent experiments, ***, $P<0.001$ (Mock vs. SIRT1) (one-way ANOVA and Tukey-Kramer *posthoc* test). (E–H) Inhibition of SIRT1 elevated ac-tau in HEK293T cells. (E) Inhibition of SIRT1, SIRT2, or HDAC6 expression mediated by siRNA transfections. n=4–6 from 2–3 experiments. **, P =0.0015; ***, P=0.0001 (SIRT2 vs. control) or P=0.001 (HDAC6 vs. control) (paired t test). (F) Western blot showing levels of ac-tau, t-tau, tubulin, and ac-tubulin in cells transfected with control siRNA or siRNA targeting SIRT1, SIRT2, or HDAC6. Blots are representative of 2–3 experiments. (G–H) Inhibition of SIRT1, significantly elevated levels of ac-tau/GAPDH (G) or ac-tau/t-tau (H). n=4–6 from 2–3 experiments. *, $P<0.05$ (paired t test). Levels of deacetylase/GAPDH (E), ac-tau or t-tau/GAPDH (G), and ac-tau/t-tau (H) in control siRNA-

transfected cells were set as 1. (I) Deacetylation of Tau3KR by SIRT1. *Left:* Representative western blot showing levels of ac-tau, t-tau, FLAG-tagged SIRT1, and GAPDH. *Right:* Ac-tau/t-tau levels in mock-transfected cells expressing wildtype tau were set as 1. n=10–20 from 4–10 independent experiments. ***, $P<0.001$; ns, not significant (one-way ANOVA and Tukey-Kramer *posthoc* analysis). Values are means ± SEM (C–E, G–I).

SIRT1 Reduces Tau Acetylation in Primary Neurons and in Vivo

[00255] In primary neurons, ac-tau/t-tau increased as the neurons matured (Figure 1G) but levels of full-length SIRT1 decreased (Figure 4A). Consistent with the notion that SIRT1 deactylates tau, levels of SIRT1 negatively correlated with levels of ac-tau/t-tau in primary neurons during development (Figure 4B). To investigate if SIRT1 negatively regulates tau acetylation in neurons, SIRT1 was deleted in neurons by infecting neurons from SIRT1 conditional knockout mice ($SIRT1^{F/F}$) (Chua et al. (2005) *Cell Metab* 2:67) with a lentiviral vector expressing cre recombinase (Lenti-cre) (Figure 4C). Controls were infection with an empty vector (Lenti-con). $SIRT1^{F/F}$ neurons were also infected with a lentiviral vector expressing human tau. Deleting SIRT1 significantly elevated levels of acetylated human tau relative to t-tau (Figure 4C), indicating that SIRT1 deacetylates tau in neurons.

[00256] To examine the effects of SIRT1 deletion on the acetylation of mouse tau *in vivo*, another ac-tau-specific antibody targeting the microtubule-binding region (264–287), which is 100% conserved between mouse and human (Figure 4D), was developed. Recombinant tau was incubated with p300 to induce acetylation. Like Ab708, antibody 9AB recognized recombinant tau acetylated by GST-p300, but not tau incubated with GST alone, suggesting that 9AB does not cross-react with non-ac-tau. In HEK293T cells, overexpression of p300 markedly elevated levels of ac-tau detected with 9AB, but only modestly those of t-tau. Thus, 9AB also preferentially recognizes p300-induced ac-tau in cultured cells (Figure 4E). In mouse brains, 9AB detected low levels of ac-tau, which was absent in $tau^{-/-}$ mice. To delete SIRT1, $SIRT1^{+/-}$ mice were crossed on an outbred background, which partially rescued the embryonic lethality of SIRT1-null mice on the inbred background. Chen et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:10794. Deleting SIRT1 significantly enhanced levels of ac-tau in the brain, providing direct evidence that SIRT1 deacetylates tau *in vivo* (Figure 4F).

[00257] **Figures 4A-F. SIRT1 Reduces Tau Acetylation in Neurons and *in Vivo*** (A) Western blot showing expression of SIRT1 in primary cortical neurons during maturation in culture (DIV5–11). Blots are representative of 2–3 independent cultures. (B) Levels of endogenous ac-tau relative to t-tau correlated negatively with levels of SIRT1 in primary rat neuronal cultures (DIV5–9). Levels of SIRT1 or ac-tau/t-tau at DIV=5 were set as 1. n=20 independent measurements. $P=0.0007$, Pearson correlation coefficient $r^2=0.4791$. (C) Deleting SIRT1 in

neurons elevated levels of ac-tau relative to t-tau. Neurons cultured from *SIRT1*$^{F/F}$ mice were infected with control virus or virus expressing cre recombinase (Lenti-cre). Both cultures were also infected with a lentiviral vector expressing h-tau. n=8. *P*=0.001, (unpaired t test). (D) Acetyl-specific antibody (9AB) recognized tau acetylated by GST-p300, but not non-ac-tau. Also shown is the sequence of the antigen used to generate 9AB. (E) Overexpressing p300 enhanced 9AB-positive ac-tau in HEK293T cells. Levels of t-tau, detected with Tau 5, were similar with or without p300 overexpression. Blots are representative of three independent experiments. (F) Deletion of SIRT1 elevated ac-tau relative to t-tau in the brain. *Left*: Representative western blots showing levels of ac-tau, t-tau, and GAPDH. *Right:* Levels of ac-tau/t-tau in *SIRT1*$^{+/+}$, *SIRT1*$^{+/-}$, and *SIRT1*$^{-/-}$ brains. n=3–6 mice/genotype. *, *P*=0.02 (*SIRT1*$^{+/-}$ vs. *SIRT1*$^{-/-}$) (one-way ANOVA and Tukey-Kramer *posthoc* test). Values are means ± SEM (C, F).

SIRT1 Interacts with Tau Directly

[00258] Although mainly localized in the nucleus, SIRT1 can be shuttled to the cytoplasm. To determine if SIRT1 directly deacetylates tau, *in vitro* deacetylation assays were performed. Recombinant tau acetylated by p300 was incubated with SIRT1 immunoprecipitated from SIRT1-overexpressing HEK293T cells. Ac-tau/t-tau levels were significantly lower in the presence of immunoprecipitated SIRT1 (Figure 5A). To confirm that SIRT1 interacts with tau directly *in vivo*, GST pull-down assays were performed. Bead-bound GST-tau, not GST alone, interacted with FLAG-SIRT1 expressed in HEK293T cells or endogenous SIRT1 in nontransfected cells (Figure 5B). Moreover, in coimmunoprecipitation assays, after immunoprecipitation with an anti-FLAG antibody, endogenous SIRT1 was detected with an anti-SIRT1 antibody, and tau was detected with a pan-tau antibody (Tau 5) in HEK293T cells expressing FLAG-tagged tau (Figure 5C).

[00259] **Figures 5A-C. SIRT1 Interacts with Tau.** (A) SIRT1 directly deacetylated ac-tau *in vitro*. Ac-tau/t-tau levels in the absence of immunoprecipitated SIRT1 were set as 1. Ac-tau was detected with Ab708 antibody. n=5 from two experiments. **, *P*=0.0063 (unpaired t test). (B) GST pull-down assays. GST-tau protein (lanes 7–9) or GST alone (lanes 4–6) was incubated with lysates of cells transfected with FLAG-tagged SIRT1 or of nontransfected cells. Lanes 1, 4 and 7: 0.1% Triton X-100; lanes 2, 5, and 8: 0.5% Triton X-100; lanes 3, 6, and 9: 0.5% NP-40. Data shown are representative of two experiments. The lower band is likely to represent the cleavage product of SIRT1 observed previously. Ohsawa and Miura (2006) *FEBS Lett* 580:5875. (C) Coimmunoprecipitation assays. HEK293T cells were transfected with a plasmid encoding FLAG-tagged human tau. Cell lysates were collected 24 h later, immunoprecipitated

with an anti-FLAG antibody, and immunoblotted with Tau 5 or an anti-SIRT1 antibody. Lanes 1–2: input; lane 3: no primary antibody; lane 4: anti-FLAG antibody. Values are means ± SEM (A).

SIRT1 Deficiency Increases Tau Acetylation and Suppresses Degradation of p-Tau

[00260] As a class III lysine deacetylase, SIRT1 supports and promotes longevity in diverse organisms. Besides regulating endocrine and behavioral responses to caloric restriction, SIRT1 has been strongly implicated in neurodegenerative diseases. Gan and Mucke (2008) *Neuron* 58:10. In AD brains, SIRT1 levels are significantly reduced, and the reduction appears to correlate with tau accumulation and aggregation. Thus, deficient SIRT1 activity may contribute to tauopathy. In primary neurons, inhibiting SIRT1 with a specific inhibitor EX527 (Napper et al. (2005) *J Med Chem* 48:8045) markedly increased ac-tau, AT8-positive p-tau, as well as t-tau (Figure 6A). Levels of ac-tau or p-tau relative to t-tau were significantly increased with EX527 treatments (Figure 6A). Thus, the increase in ac-tau induced by SIRT1 deficiency is accompanied by accumulation of pathogenic p-tau in primary neurons. In mouse brains, deleting SIRT1, which elevated ac-tau, also increased AT8-positive p-tau (Figure 6B).

[00261] How might elevated tau acetylation lead to higher levels of p-tau? Acetylation of lysines can preclude its ubiquitination and stabilize proteins that are normally degraded by the UPS, including p53, Runx3, β-catenin, and other regulatory factors. Since tau is ubiquitinated and the degradation of tau, especially p-tau, involves the proteasome-mediated pathway, it was hypothesized that acetylation precludes tau ubiquitination and suppresses its degradation.

[00262] To test this hypothesis, the involvement of acetylated lysines in regulating protein turnover was assessed. The turnover rates of human wildtype tau (hTauwt) and human tau3KR (hTau3KR) were compared. Primary cortical neurons were infected with Lenti-hTauwt and Lenti-hTau3KR and treated with CHX (Figure 6C). Infection of Lenti-hTau3KR resulted in much weaker Ab708-positive signal than that of Lenti-hTauwt, providing further support that Ab708 recognizes acetylation of lysines 163, 174, and 180. Mutating these three lysines to arginines significantly increased the half-life of tau, possibly by permanently blocking ubiquitination at the three sites (Figure 6D). These results support the notion that the acetylated lysines can be ubiquitinated.

[00263] To directly test if enhancing acetylation can block ubiquitination, HEK293T cells were transfected with expression plasmids encoding tau and hemagglutinin (HA)-tagged ubiquitin and then treated with EX527 to inhibit SIRT1 and with MG132 to block the proteasome-mediated degradation. Ubiquitinated tau was immunoprecipitated with an anti-FLAG antibody and detected with an anti-HA antibody. EX527 prevented polyubiquitination of tau in a dose-

dependent manner, indicating that tau ubiquitination is suppressed by enhanced acetylation (Figure 6E). EX527 also elevated ac-tau levels as expected (Figure 6F). In contrast, SIRT1-mediated deacetylation appears to enhance tau ubquitination. Treatment with resveratrol, which may be indirectly involved in activating SIRT1, significantly increased tau ubiquitination in cells transfected with wildtype SIRT1, but not those with H363Y mutant.

[00264] It was then directly examined whether enhancing acetylation of tau slows the turnover of endogenous tau. Primary neurons were treated with EX527 to enhance tau acetylation and with cycloheximide (CHX) to inhibit translation of new proteins. Endogenous rat tau in primary neurons had a half-life of around 5 h. Inhibiting SIRT1 with EX527 slowed tau turnover and increased the half-life of t-tau in a dose-dependent manner (Figure 6G, 6H). Consistent with this notion, ac-tau appears to be degraded slower than that of t-tau. In primary neurons, CHX markedly reduced t-tau levels after 5 h, whereas ac-tau levels were only slightly reduced after 8 h. Moreover, inhibition of SIRT1 with 10 μM of EX527 blocked the turnover of ac-tau, leading its accumulation (Figure 6I, 6J). Higher dose of EX527 (50 μM) resulted in more pronounced accumulation of ac-tau (Figure 6I). Treatment with EX527 also blocked the degradation of AT8-positive p-tau in a dose-dependent manner (Figure 6K).

[00265] **Figures 6A-K. Acetylation Slows Tau Turnover by Inhibiting Its Ubiquitination.** (A) Inhibiting SIRT1 with EX527 (50 μM) elevated ac-tau and p-tau in rat primary neurons (DIV=10). *Left:* Representative western blots. p-tau was detected with AT8. *Right*: Levels of ac-tau/t-tau or p-tau/t-tau in vehicle-treated cells were set as 1. n=6 independent treatments. ***, $P<0.001$; *, $P<0.05$ (paired t test). (B) Deletion of SIRT1 elevated AT8-positive p-tau in the brain. n=3–4 mice/genotype. *, $P<0.05$ ($SIRT1^{+/+}$ vs. $SIRT1^{-/-}$) (one-way ANOVA and Tukey-Kramer *posthoc* test). (C and D) Tau3KR was more stable than wildtype tau in primary neurons. Cells were infected with Lenti-hTauwt or Lenti-hTau3KR and treated with CHX for 8–32 h 4 days after infection (DIV=9). (C) Representative western blot of 2 experiments showing ac-tau, t-tau, and GAPDH. (D) The turnover of t-tau was slower in cells expressing Tau3KR. t-tau/GAPDH levels in cells harvested at time 0 were set as 1. n=3–5 from 2 experiments. *, $P=0.04$ (8 h), $P=0.015$ (24 h); ***, $P<0.0001$ (32 h) (unpaired t test for each time-point). (E and F) SIRT1 inhibitor EX527 (1–50 μM) suppressed tau ubiquitination and elevated ac-tau in a dose-dependent manner. Blots are representative of 3 experiments. (G–K) The SIRT1 inhibitor EX527 increases the half-life of tau in rat primary neurons (DIV=8) in a dose-dependent manner. Neurons were treated with CHX for 0–8 h in the presence or absence of EX527 (10–50 μM). Representative western blots of 3 experiments showing the turnover of t-tau (G), ac-tau (I), or p-tau (K) in neurons with or without EX527. (H and J) The turnover of t-tau (H) or ac-tau (J) was

markedly slowed by treatment of EX527. Levels of t-tau/tubulin or ac-tau/tubulin in cells harvested at time 0 were set as 1. n=3. **, $P<0.01$; ***, $P<0.001$ (two-way ANOVA, EX527-treated vs. vehicle-treated). Values are means ± SEM (A–B, D, G, I).

Elevation of Tau Acetylation in Pathological Conditions

[00266] Since degradation of tau was slowed by its acetylation, it was hypothesized that acetylation is a critical early event that contributes to accumulation of p-tau that is normally degraded via the proteasome-mediated pathway. In primary neurons, treatment with low levels of amyloid β (Aβ) oligomers, a key pathogen in AD, increased levels of ac-tau in a dose-dependent manner (Figure 7A). Higher levels of ac-tau were observed in primary neurons expressing human tau carrying FTD-linked mutation (hTauP301L) than those expressing similar levels of hTauwt (Figure 7B). These findings suggest that tau acetylation is elevated by stress, such as Aβ accumulation, or FTD-linked mutations.

[00267] Tau acetylation in the frontal cortex of patients with various degrees of tau pathology was examined. Braak and Braak (1991) *Neuropathol. Berl.* 82:239. Patients at Braak stages 1–2 or 3–4 had significantly higher levels of ac-tau in the soluble fraction of the brain lysates than patients at Braak stage 0 (Figure 7C). Ab708 can recognize various human tau isoforms in transgenic mice overexpressing human tau (Figure 1D). However, unlike Tau 5, which detects all isoforms, Ab708 appears to detect some isoforms preferentially in AD brains (Figure 7C). Hyperphosphorylated tau detected with PHF-1 (Figure 1D) or AT8 was observed only in patients at stages 5–6, consistent with lack of significant NFTs in the frontal cortex of patients at earlier Braak stages (Braak and Braak (1991) *supra*). Thus, these findings support the notion that enhanced tau acetylation precedes hyperphosphorylation of tau and NFT formation. However, in patients at Braak stages 5–6, especially those at stage 6, with NFTs in the frontal cortex, levels of ac-tau were slightly lower than patients at mild to moderate stages. This end-stage reduction might be explained by severe loss of neurons, or sequestration of ac-tau in the NFTs, thus remaining in the insoluble fractions of the lysates.

[00268] **Figures 7A-D. Tau Acetylation Is Elevated under Pathological Conditions.** (A) Tau acetylation was increased by low levels of Aβ oligomers in primary cortical neurons (DIV=11). n=5 from 3 experiments. **, $P=0.003$ (one-way ANOVA and Tukey-Kramer *posthoc* test). (B) Tau acetylation was associated with familial *MAPT* mutations in primary neurons (DIV=13). Ac-tau/t-tau levels in neurons infected with Lenti-hTauwt were set as 1. n=9 from three experiments. *, $P=0.013$ (unpaired t test). (C) Representative western blots showing levels of ac-tau, t-tau, and hyperphosphorylated tau in human brains (Bm-22, superior temporal gyrus) at different Braak stages (0–5). (D) Ac-tau levels were elevated in patients with mild (Braak stages 1–2) to

moderate (Braak stages 3–4) levels of tau pathology. n=8–18 cases/Braak range. *, $P<0.05$; **, $P<0.01$, one-way ANOVA Tukey-Kramer *posthoc* analyses. Values are means±SEM (A, B, D).

Reducing Tau Acetylation Eliminates p-Tau Induced by FTD-linked Mutation

[00269] To test the hypothesis that tau acetylation contributes to p-tau accumulation, it was determined if inhibiting tau acetylation eliminates p-tau and protects against tauopathy. Inhibiting p300 in primary neurons with the small molecule C646 eliminated ac-tau without affecting t-tau levels (Figure 8A). Strikingly, pathogenic tau phosphorylated at serine 202, detected with AT8 antibody, was also abolished within 2 h treatment with C646. Its inactive analog C37 had no effects (Figure 8B). These results suggest that deacetylation preferentially enhances degradation of p-tau, consistent with the observation that p-tau species are selectively degraded via the UPS pathway In primary neurons expressing hTauP301L, a cellular model of tauopathy, AT8-positive p-tau was also diminished by C646 treatment (Figure 8C). Reducing tau improves cognitive function in mouse models of AD and FTDP-17 (Roberson et al. (2007) *Science* 316:750; and Santacruz et al. (2005) *Science* 309:476) and protects against excitotoxicity (Roberson et al. (2007) *supra*). These results implicate modulating lysine acetylation as a new therapeutic strategy to reduce levels of tau, especially pathogenic forms of p-tau, in neurodegenerative tauopathies.

[00270] **Figures 8A-D. Reducing Tau Acetylation Eliminates p-Tau.** (A) C646 (20 μM) eliminated ac-tau and AT8-positive p-tau within 2 h in primary cortical neurons (DIV=9). Representative western blot of two experiments. (B) C646 (20 μM) eliminated p-tau. Levels of p-tau/GAPDH in non-treated cells were set as 1. n=4. ***, $P<0.0001$ (unpaired t test). (C) C646 (20 μM) eliminated AT8-positive p-tau in primary neurons expressing hTauP301L (DIV=12). *Left*, Representative western blot of two experiments. *Right*, Levels of p-tau/GAPDH in cells treated with control compound (C37) were set as 1. n=7. ***, $P=0.0001$ (unpaired t test). (D) Hypothetical model of how tau acetylation may contribute to tau-mediated neurodegeneration. Dashed lines and factors in grey indicate pathways not yet tested. Values are means±SEM (B–C).

[00271] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for reducing the level of an acetylated Tau polypeptide in a cell, the method comprising contacting the cell with an agent that increases the activity of a polypeptide that deacetylates a Tau polypeptide in the cell and/or an agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in the cell.

2. The method of claim 1, wherein the method comprises contacting the cell with an agent that increases the activity of a polypeptide that deacetylates a Tau polypeptide in the cell and an agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in the cell.

3. The method of claim 1, wherein the cell is a neuron.

4. The method of claim 1, wherein the cell is a glial cell.

5. The method of claim 1, wherein the agent is not a sirtuin activator.

6. The method of claim 1, wherein the agent is a p300 inhibitor or a CBP inhibitor.

7. The method of claim 1, wherein the agent is an activator of SIRT1, SIRT2, or HDAC6.

8. The method of claim 1, wherein said contacting reduces the level of phosphorylated Tau polypeptide in the cell.

9. The method of claim 1, wherein said contacting increases the level of active Tau polypeptide in the cell.

10. A method for treating a tauopathy in an individual, the method comprising administering to the individual an effective amount of an agent that reduces the level of acetylated Tau in a neuron or a glial cell in the individual.

11. The method of claim 10, wherein the administering comprises administering to the individual an agent that increases the activity of a polypeptide that deacetylates a Tau polypeptide in a

neuronal cell or a glial cell and an agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in a neuronal cell or a glial cell in combined effective amounts to treat the tauopathy.

12. The method of claim 10, wherein the tauopathy is frontotemporal dementia, Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, Down syndrome, dementia pugilistica, inclusion-body myositis, or frontotemporal lobar degeneration.

13. The method of claim 10, wherein the agent that reduces the level of acetylated Tau is a p300/CBP inhibitor.

14. The method of claim 10, wherein the agent that reduces the level of acetylated Tau is a SIRT1 activator.

15. The method of claim 10, wherein said administering reduces the level of phosphorylated Tau in a neuron or glial cell in the individual.

16. The method of claim 10, wherein said administering increases the level of active Tau in a neuron or glial cell in the individual.

17. A method of diagnosing a cognitive impairment disorder in an individual, the method comprising detecting a level of acetylated Tau polypeptide in a biological sample obtained from the individual, wherein a level of acetylated Tau polypeptide that is higher than a normal control level indicates that the individual has a cognitive impairment disorder.

18. The method of claim 17, wherein the biological sample is cerebrospinal fluid, blood, plasma, or serum.

19. A method of identifying a candidate agent for treating a tauopathy, the method comprising:

a) contacting a sample comprising an acetyltransferase and a Tau polypeptide with a test agent; and

b) determining the effect of the test agent on the acetylation of the Tau polypeptide, wherein a test agent that reduces acetylation of the Tau polypeptide is a candidate agent for treating a tauopathy.

20. A method of identifying a candidate agent for treating a tauopathy, the method comprising:

a) contacting a sample comprising a deacetylase and a Tau polypeptide with a test agent; and

b) determining the effect of the test agent on the acetylation of the Tau polypeptide, wherein a test agent that increases deacetylation of the Tau polypeptide is a candidate agent for treating a tauopathy.

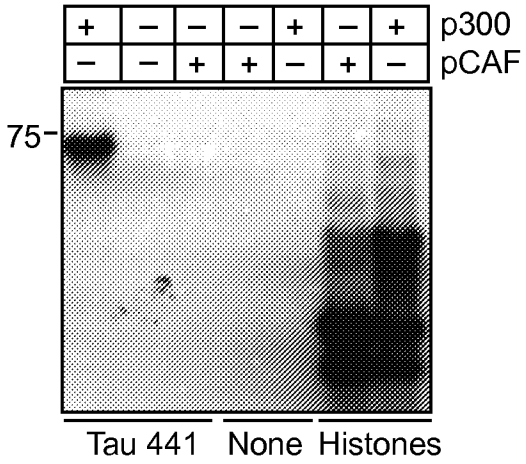


FIG. 1A

```
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 51 PTEDGSEEPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEG
101 TTAEEAGIGDTPSLEDEAAGHVTQARMVSKSKDGTGSDDKKAKGADGKTK
151 IATPRGAAPPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRSGYSSP
201 GSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPM
251 PDLKNVKSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHV
301 PGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRV
351 QSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVS
401 GDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
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FIG. 1B

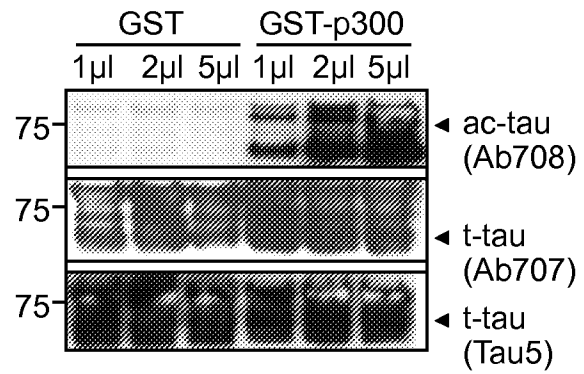

FIG. 1C

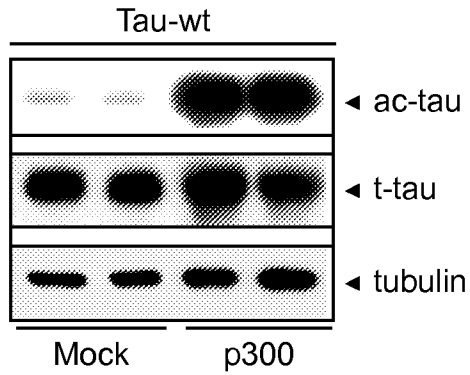

FIG. 1D

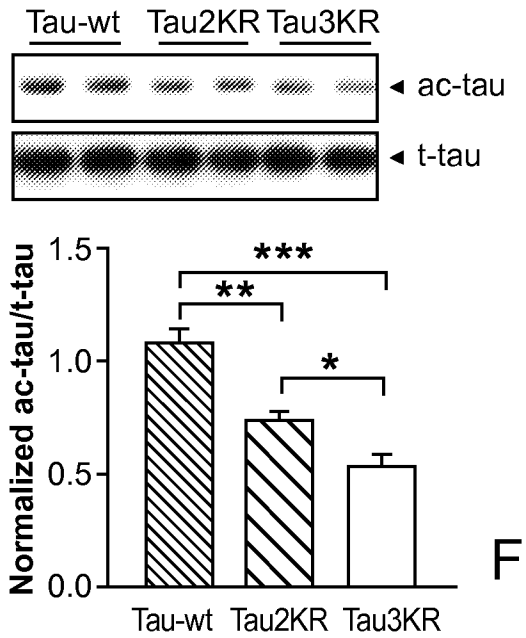

FIG. 1E

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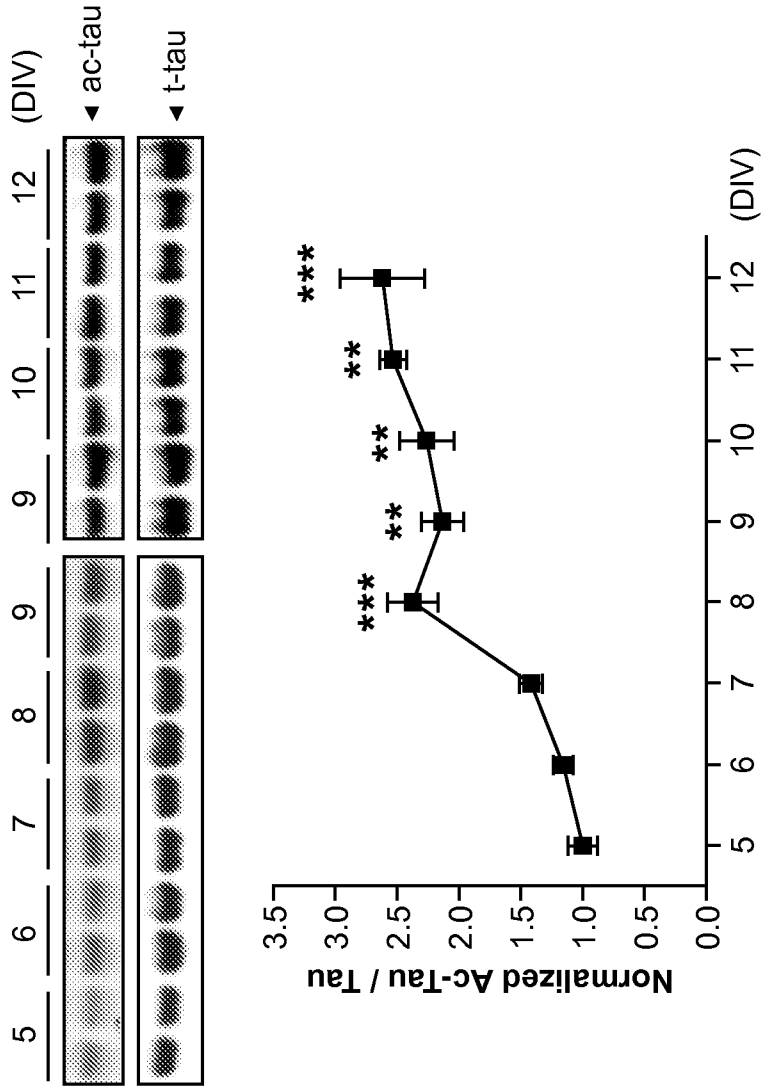

5
6
7
8
9
10
11
12
(DIV)
ac-tau
t-tau
Normalized Ac-Tau / Tau
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
**
**
**
***

FIG. 1G

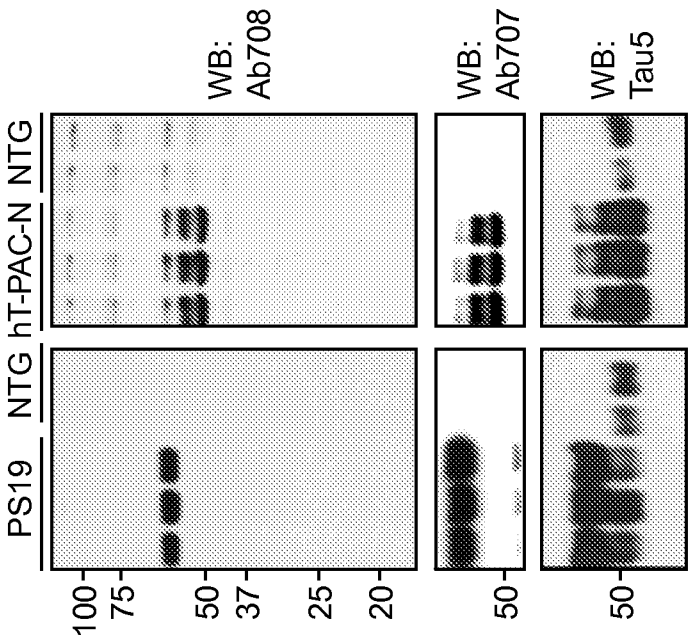

PS19
NTG
hT-PAC-N
NTG
WB:
Ab708
WB:
Ab707
WB:
Tau5
100
75
50
37
25
20
50
50

FIG. 1F

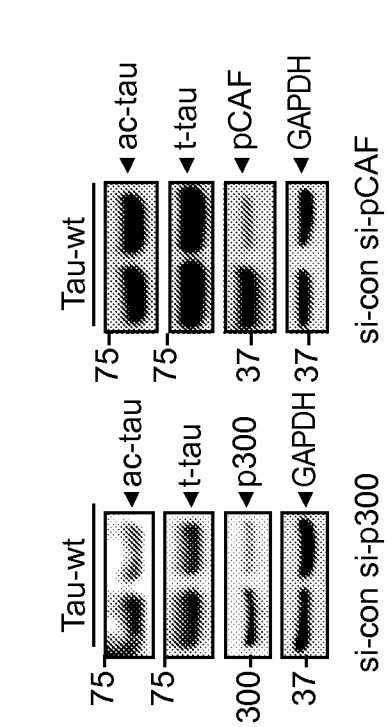


FIG. 2A

FIG. 2B

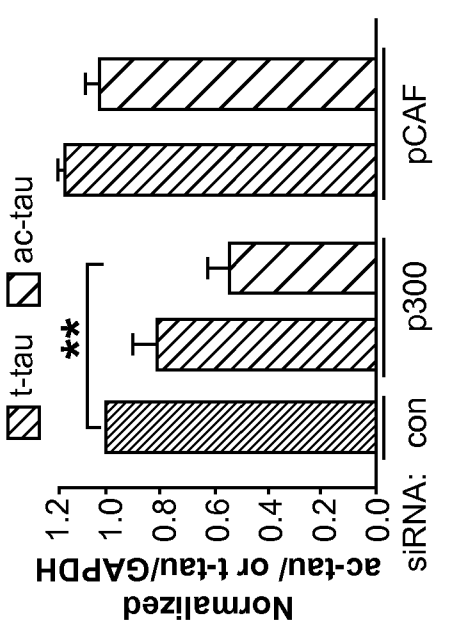
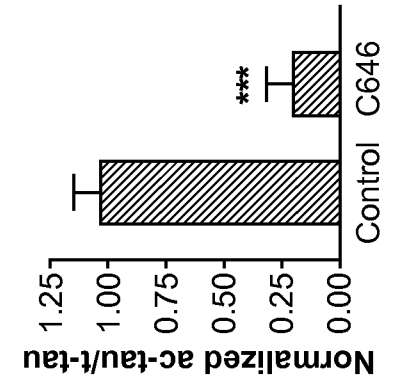
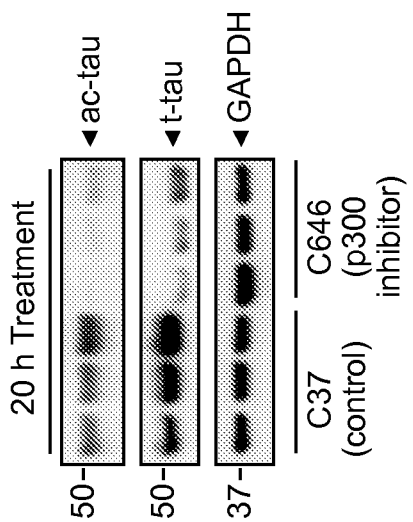
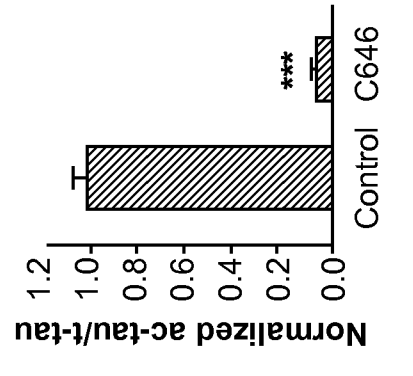


FIG. 2C







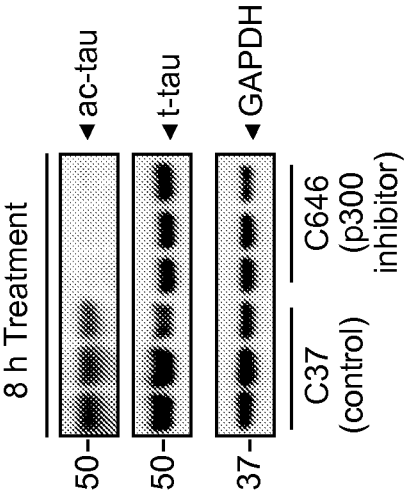


FIG. 2D

FIG. 2E

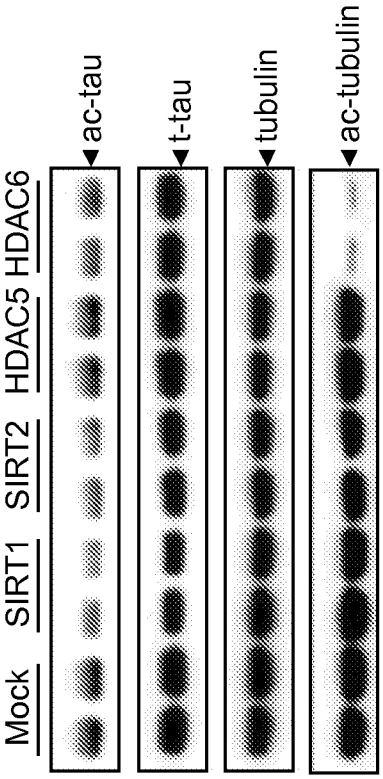


FIG. 3B

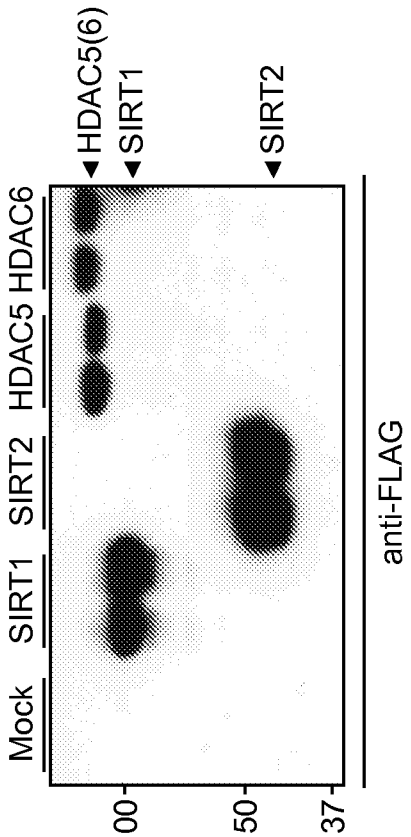


FIG. 3A

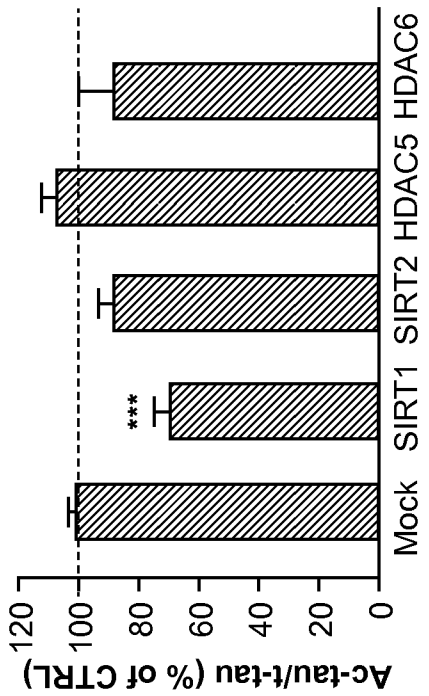


FIG. 3D

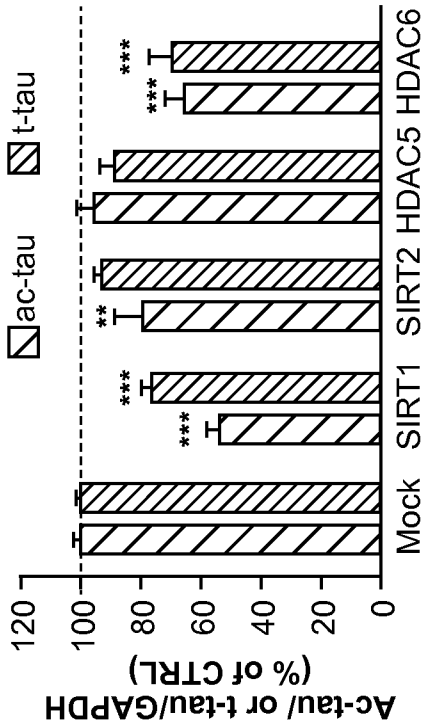


FIG. 3C

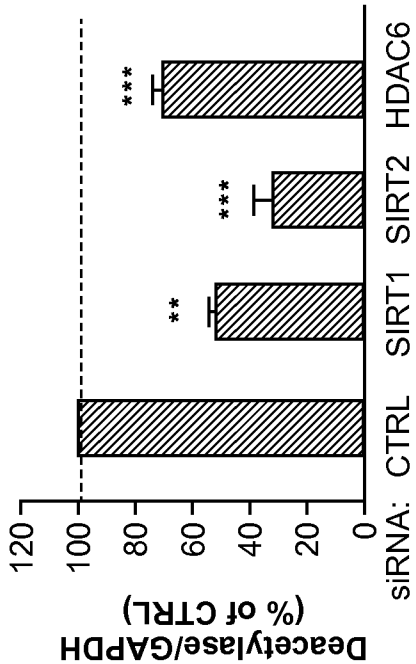


FIG. 3E

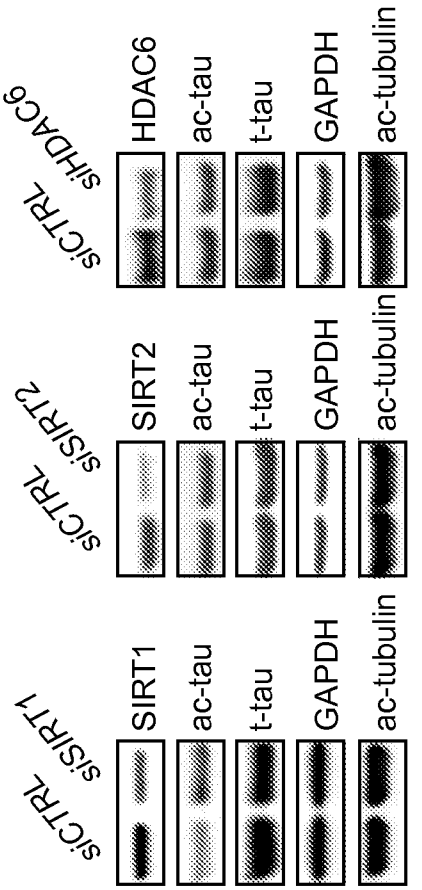


FIG. 3F

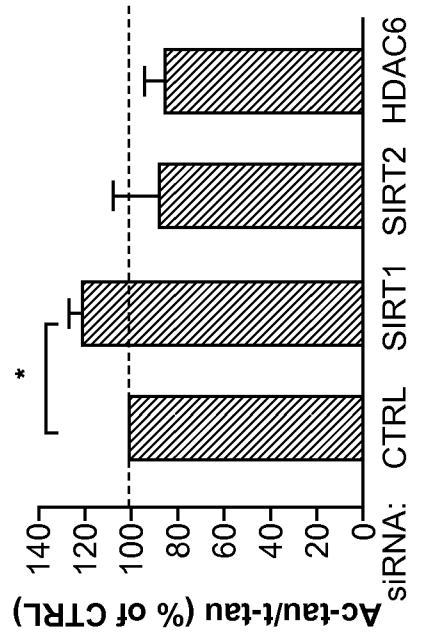


FIG. 3H

FIG. 3G

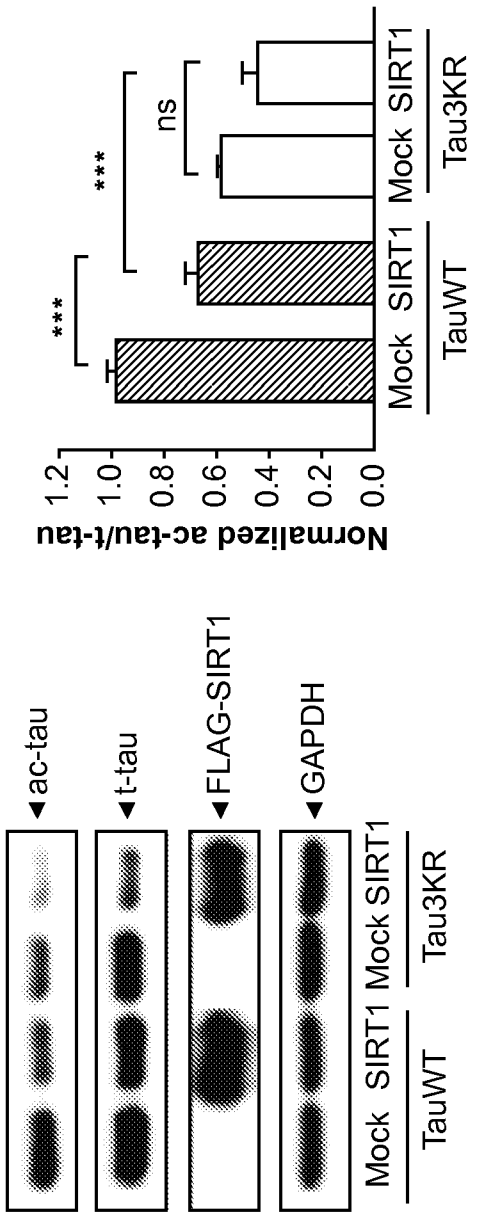

ac-tau
t-tau
FLAG-SIRT1
GAPDH
Mock SIRT1 Mock SIRT1
TauWT Tau3KR
Normalized ac-tau/t-tau
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
***
ns
Mock SIRT1 Mock SIRT1
TauWT Tau3KR

FIG. 3I

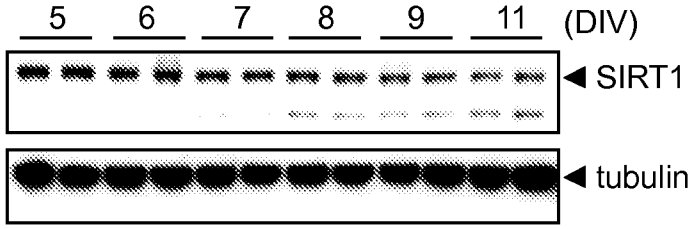


FIG. 4A

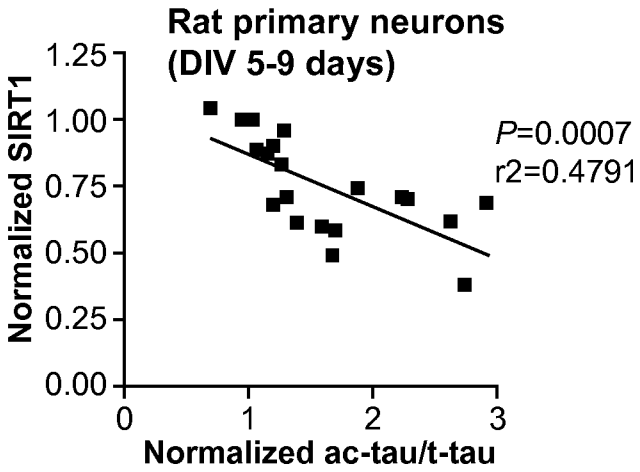


FIG. 4B

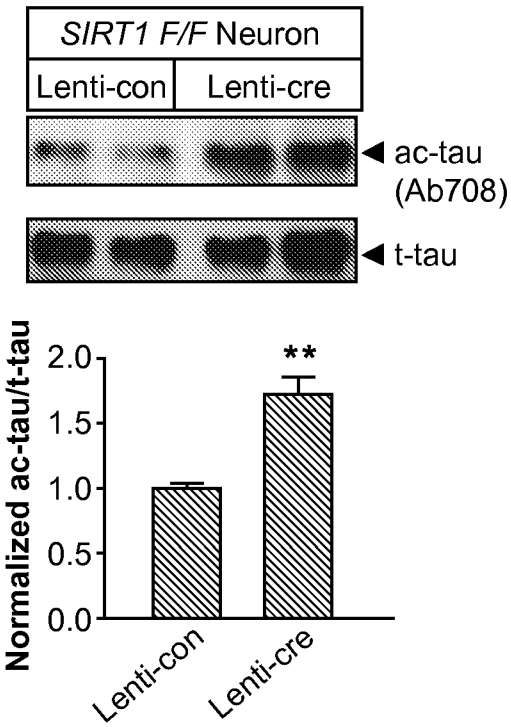


FIG. 4C

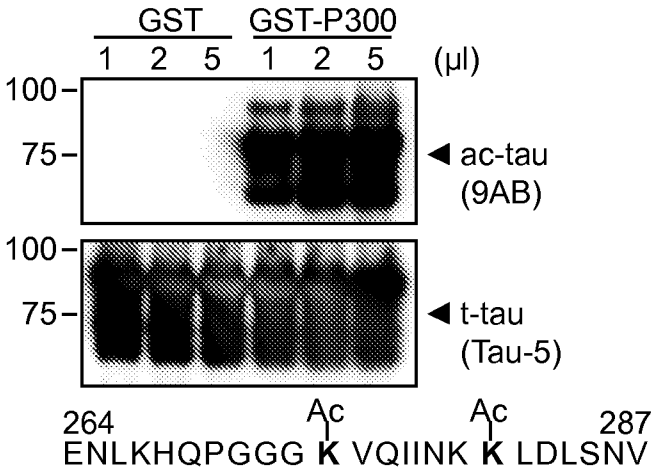


FIG. 4D

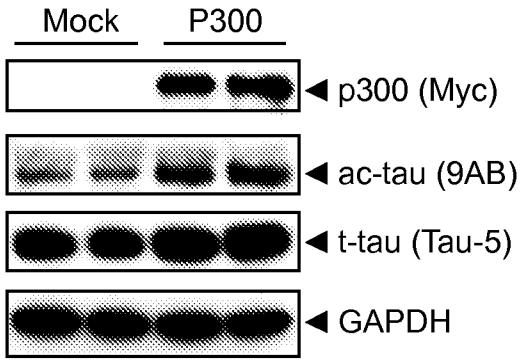


FIG. 4E

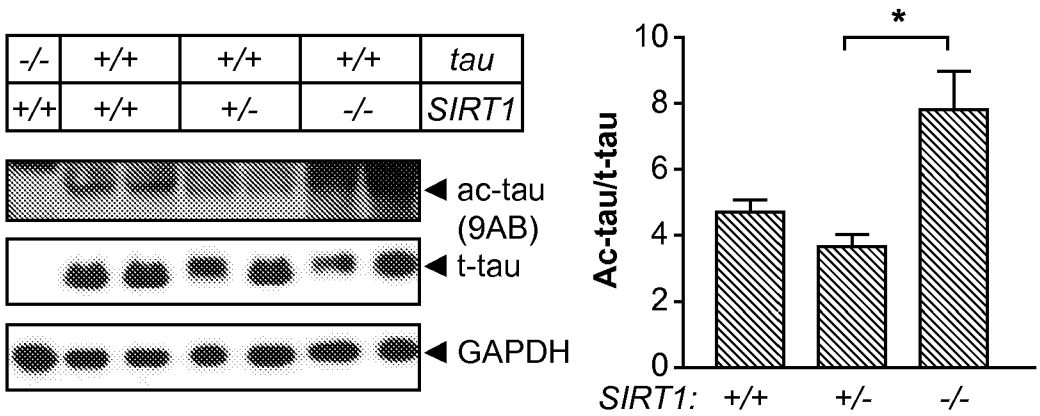


FIG. 4F

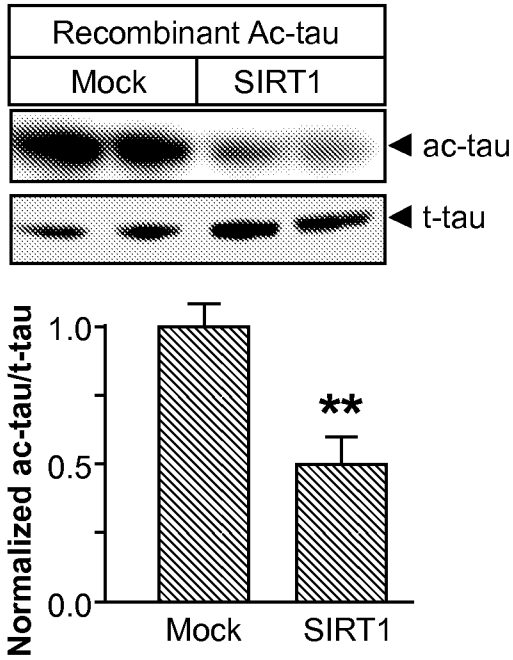


FIG. 5A

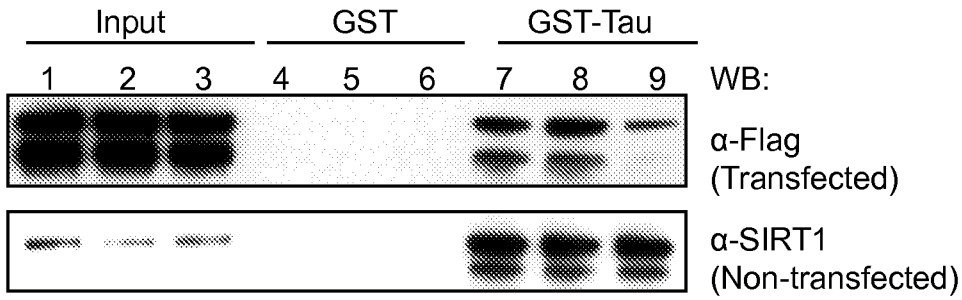


FIG. 5B

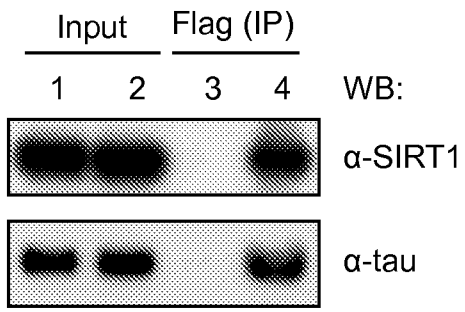


FIG. 5C

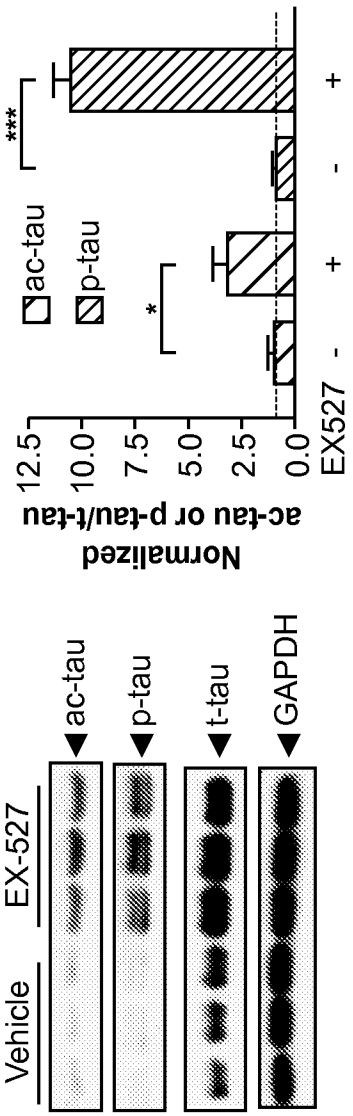

Vehicle
EX-527
ac-tau
p-tau
t-tau
GAPDH
Normalized
ac-tau or p-tau/t-tau
12.5
10.0
7.5
5.0
2.5
0.0
ac-tau
p-tau
*
***
EX527 - + - +

FIG. 6A

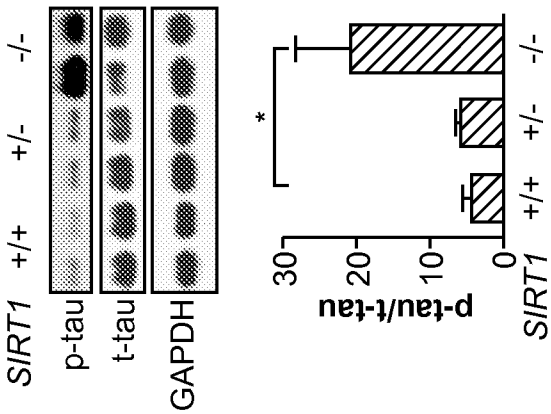

SIRT1 +/+ +/- -/-
p-tau
t-tau
GAPDH
*
p-tau/t-tau
30
20
10
0
SIRT1 +/+ +/- -/-

FIG. 6B

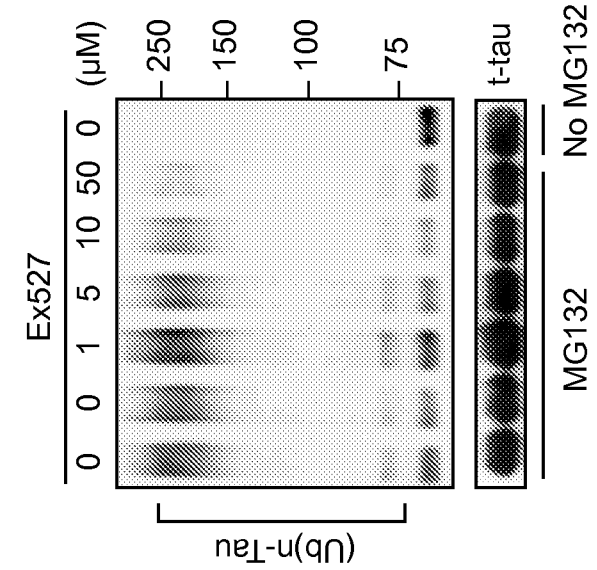


FIG. 6E

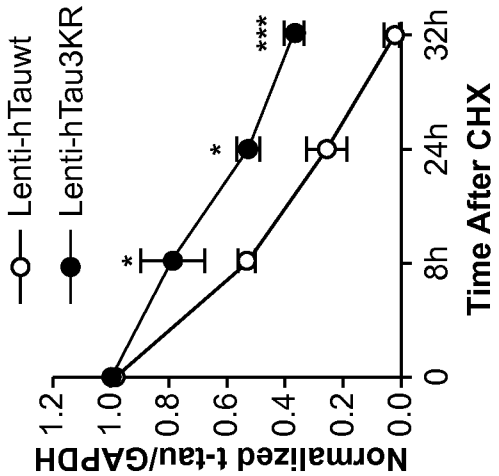


FIG. 6D

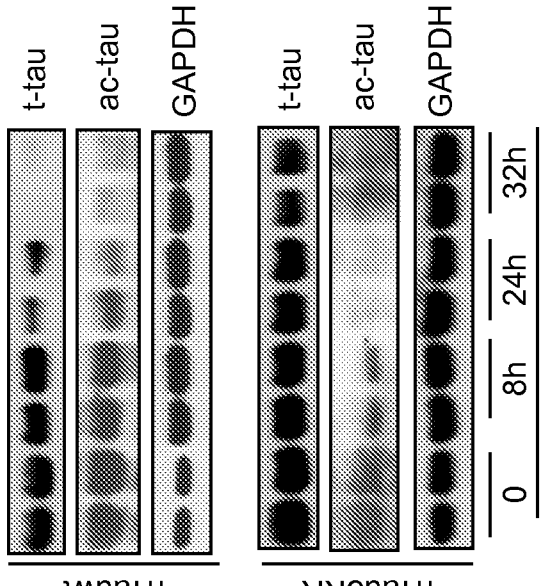


FIG. 6C

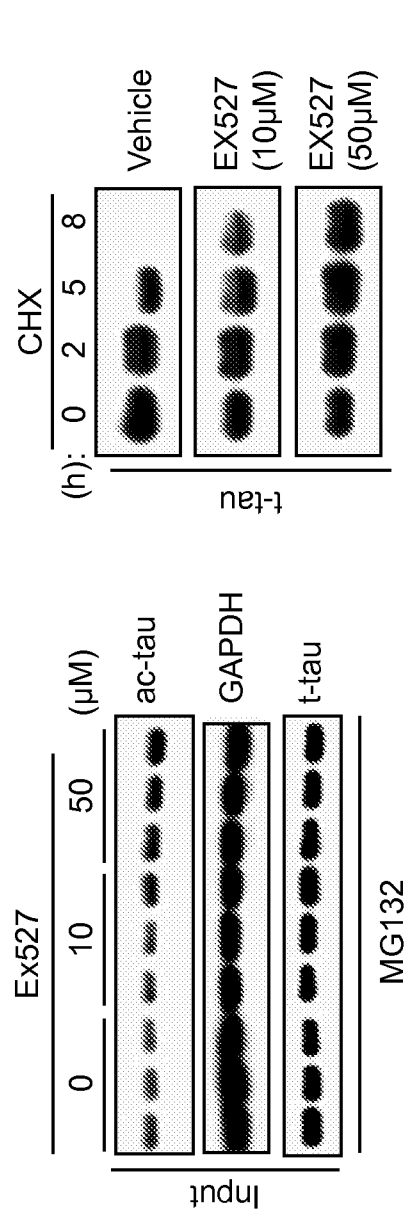


FIG. 6F

FIG. 6G

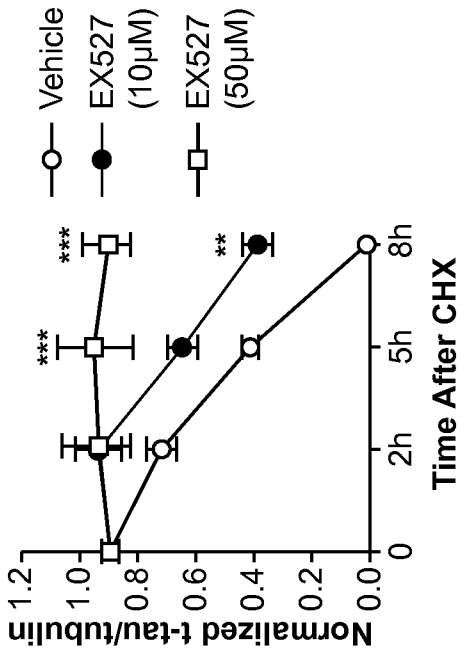


FIG. 6H

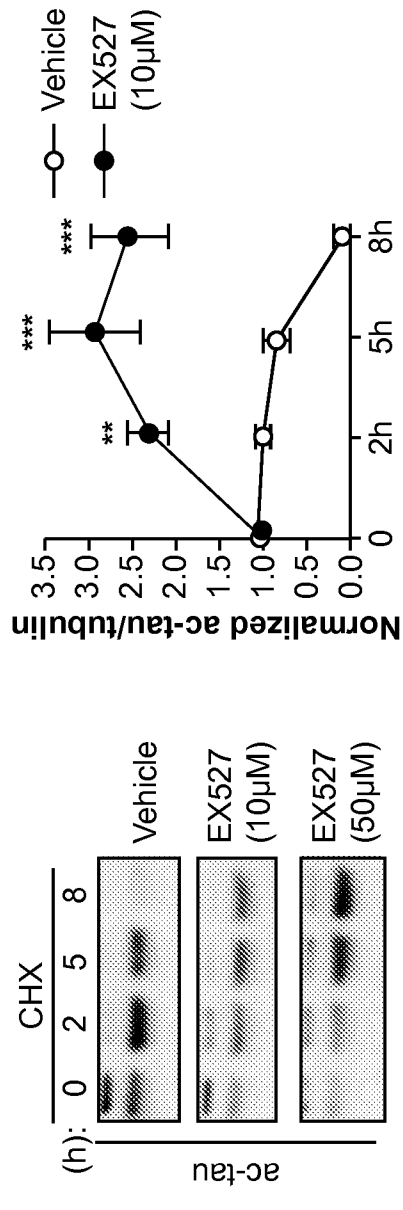


FIG. 6I

FIG. 6J

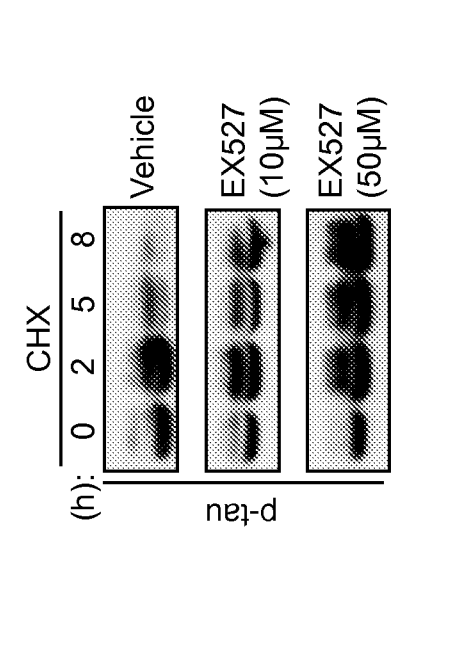


FIG. 6K

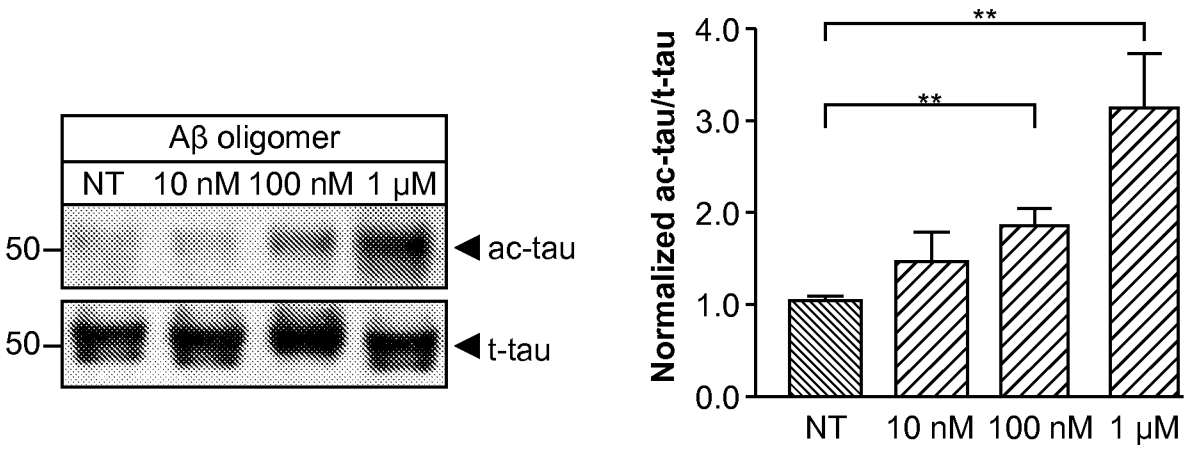


FIG. 7A

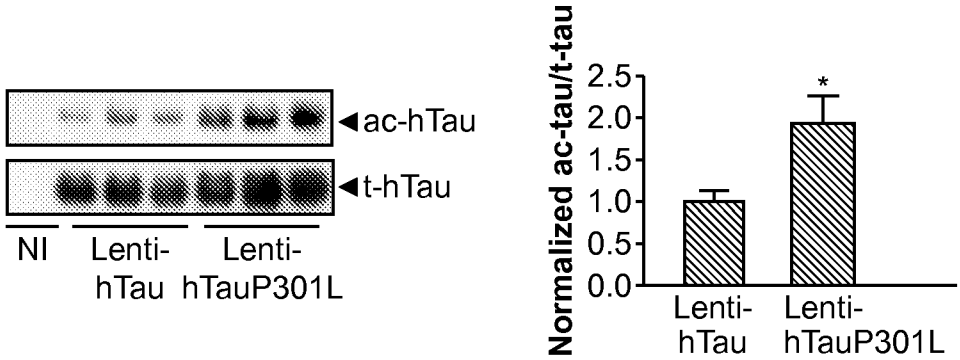


FIG. 7B

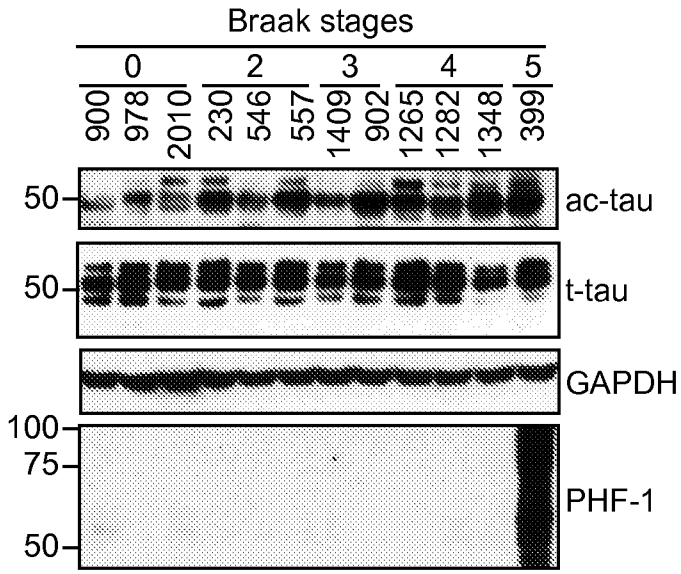


FIG. 7C

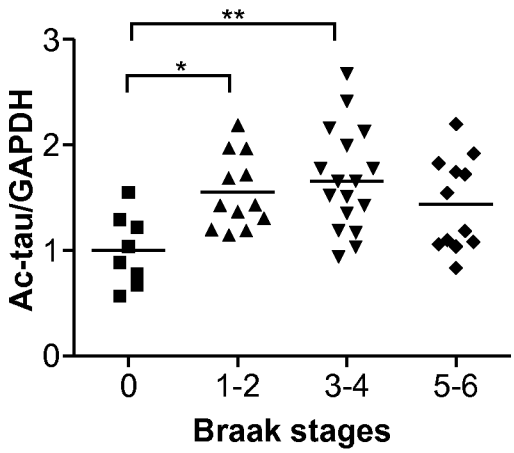


FIG. 7D

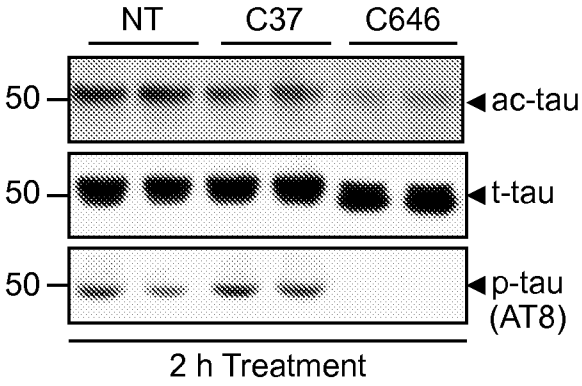


FIG. 8A

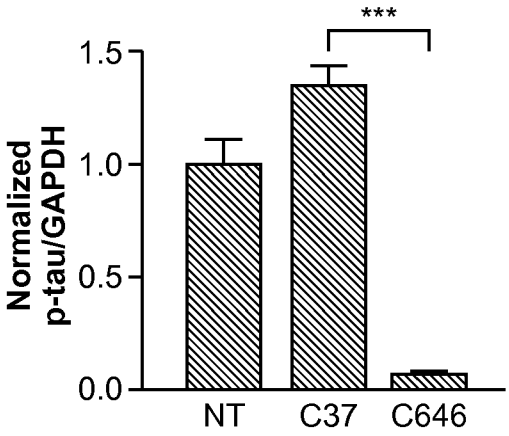


FIG. 8B

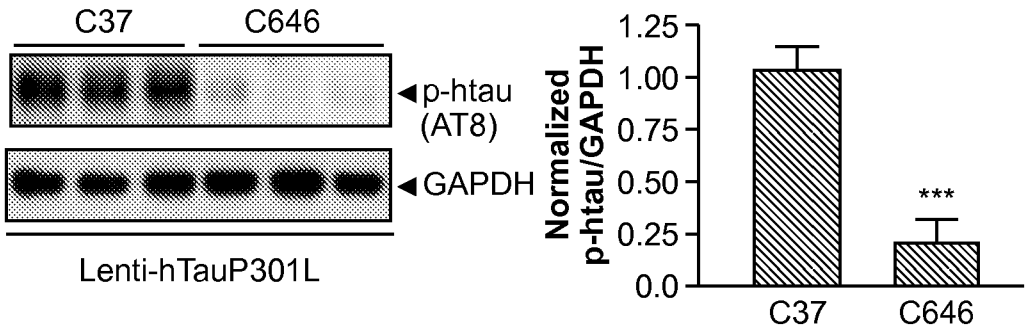


FIG. 8C

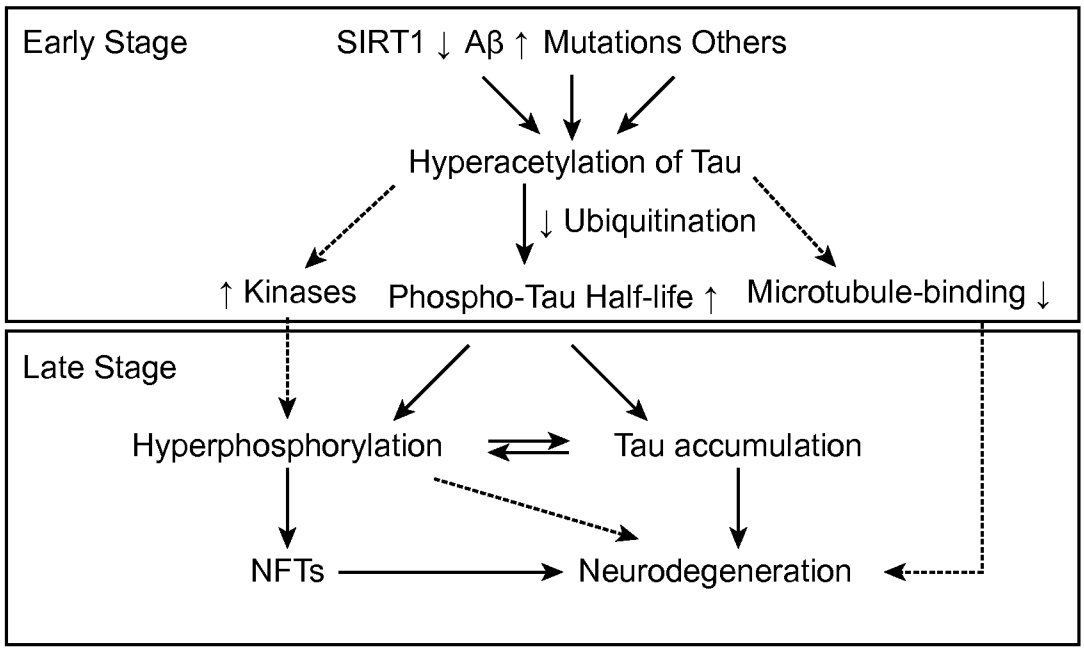


FIG. 8D

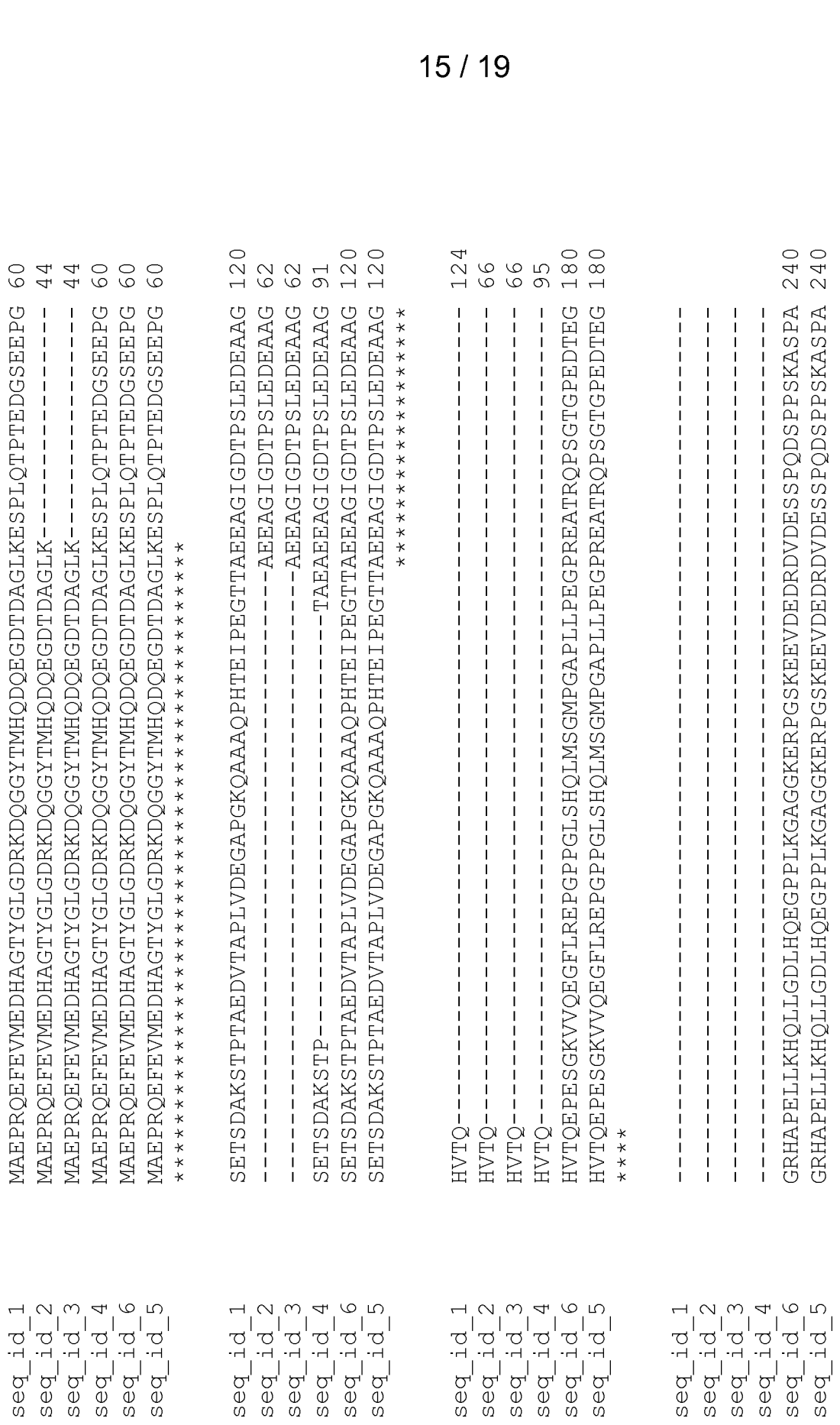

seq_id_1 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG 60
seq_id_2 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLK---------- 44
seq_id_3 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLK---------- 44
seq_id_4 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG 60
seq_id_6 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG 60
seq_id_5 MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG 60
****
seq_id_1 SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG 120
seq_id_2 ---------- AEEAGIGDTPSLEDEAAG 62
seq_id_3 ---------- AEEAGIGDTPSLEDEAAG 62
seq_id_4 SETSDAKSTP---------- TAEAEEAGIGDTPSLEDEAAG 91
seq_id_6 SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG 120
seq_id_5 SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG 120
****
seq_id_1 HVTQ---------- 124
seq_id_2 HVTQ---------- 66
seq_id_3 HVTQ---------- 66
seq_id_4 HVTQ---------- 95
seq_id_6 HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG 180
seq_id_5 HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG 180
****
seq_id_1 ----------
seq_id_2 ----------
seq_id_3 ----------
seq_id_4 ----------
seq_id_6 GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKASPA 240
seq_id_5 GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKASPA 240

FIG. 9A

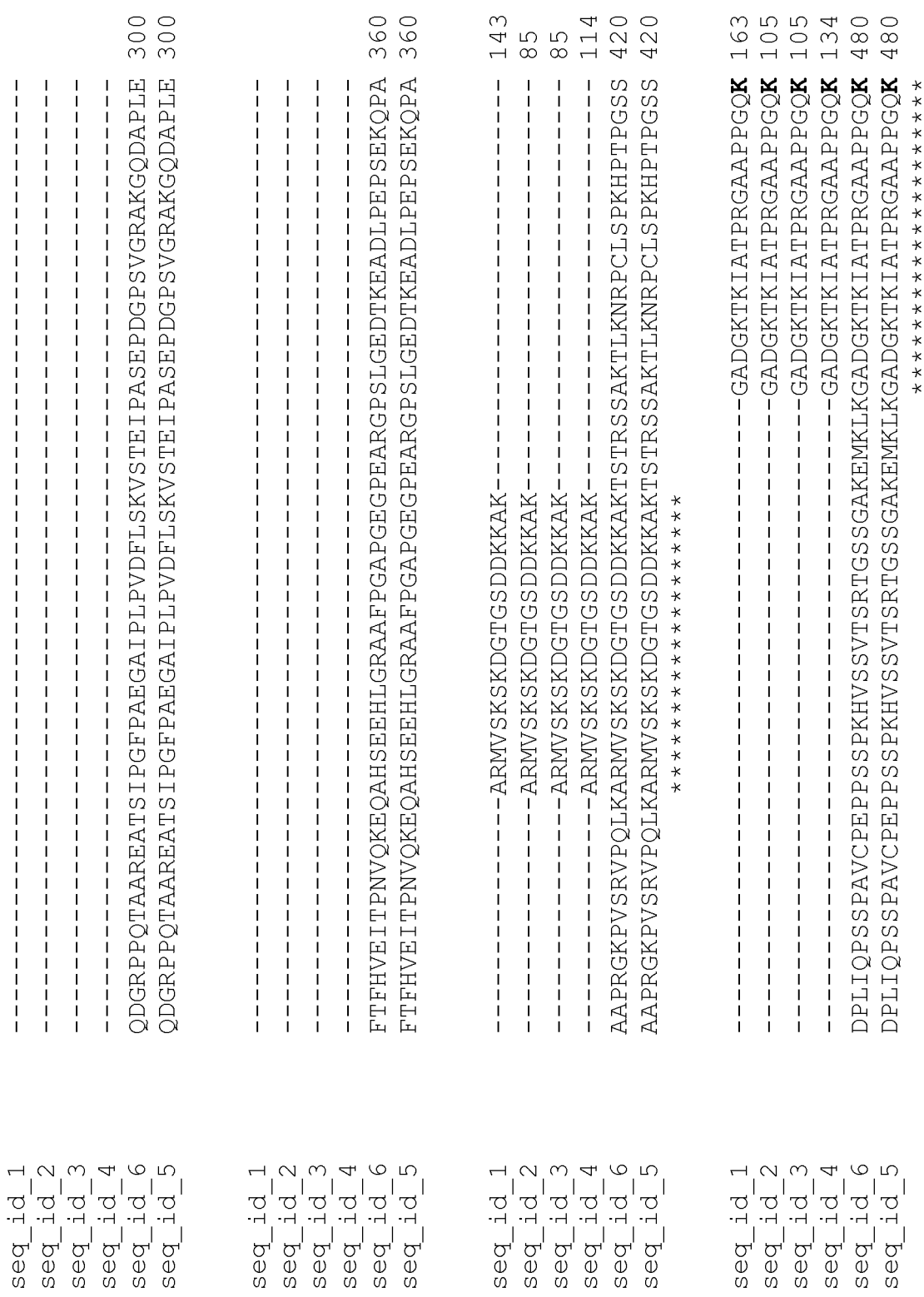

seq_id_1 ----------
seq_id_2 ----------
seq_id_3 ----------
seq_id_4 ----------
seq_id_6 QDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSVGRAKGQDAPLE 300
seq_id_5 QDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSVGRAKGQDAPLE 300
seq_id_1 ----------
seq_id_2 ----------
seq_id_3 ----------
seq_id_4 ----------
seq_id_6 FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA 360
seq_id_5 FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA 360
seq_id_1 ----------ARMVSKSKDGTGSDDKKAK---------- 143
seq_id_2 ----------ARMVSKSKDGTGSDDKKAK---------- 85
seq_id_3 ----------ARMVSKSKDGTGSDDKKAK---------- 85
seq_id_4 ----------ARMVSKSKDGTGSDDKKAK---------- 114
seq_id_6 AAPRGKPVSRVPQLKARMVSKSKDGTGSDDKKAKTSTRSSAKTLKNRPCLSPKHPTPGSS 420
seq_id_5 AAPRGKPVSRVPQLKARMVSKSKDGTGSDDKKAKTSTRSSAKTLKNRPCLSPKHPTPGSS 420
********** *********
seq_id_1 ----------GADGKTKIATPRGAAPPGQK 163
seq_id_2 ----------GADGKTKIATPRGAAPPGQK 105
seq_id_3 ----------GADGKTKIATPRGAAPPGQK 105
seq_id_4 ----------GADGKTKIATPRGAAPPGQK 134
seq_id_6 DPLIQPSSPAVCPEPPSSPKHVSSVTSRTGSSGAKEMKLKGADGKTKIATPRGAAPPGQK 480
seq_id_5 DPLIQPSSPAVCPEPPSSPKHVSSVTSRTGSSGAKEMKLKGADGKTKIATPRGAAPPGQK 480
********** **********

FIG. 9B

```
seq_id_1        GQANATRIPAKTPPAPKTPPSS-----------------GEPPKSGDRSGYSSPGSPGT 205
seq_id_2        GQANATRIPAKTPPAPKTPPSS-----------------GEPPKSGDRSGYSSPGSPGT 147
seq_id_3        GQANATRIPAKTPPAPKTPPSS-----------------GEPPKSGDRSGYSSPGSPGT 147
seq_id_4        GQANATRIPAKTPPAPKTPPSS-----------------GEPPKSGDRSGYSSPGSPGT 176
seq_id_6        GQANATRIPAKTPPAPKTPPSSATKQVQRRPPPAGPRSERGEPPKSGDRSGYSSPGSPGT 540
seq_id_5        GQANATRIPAKTPPAPKTPPSS-----------------GEPPKSGDRSGYSSPGSPGT 522
                **********************                 ********************

seq_id_1        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 265
seq_id_2        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 207
seq_id_3        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 207
seq_id_4        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 236
seq_id_6        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 600
seq_id_5        PGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTEN 582
                ************************************************************

seq_id_1        LKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSL 325
seq_id_2        LKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSL 267
seq_id_3        LKHQPGGGK-------------------------------VQIVYKPVDLSKVTSKCGSL 236
seq_id_4        LKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSL 296
seq_id_6        LKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSL 660
seq_id_5        LKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSL 642
                *********                               ********************

seq_id_1        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 385
seq_id_2        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 327
seq_id_3        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 296
seq_id_4        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 356
seq_id_6        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 720
seq_id_5        GNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAK 702
                ************************************************************
```

FIG. 9C

```
seq_id_1  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 441
seq_id_2  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 383
seq_id_3  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 352
seq_id_4  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 412
seq_id_6  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 776
seq_id_5  TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL 758
          ************************************************************
```

FIG. 9D

```
mouse           MADPRQEFDTMEDHAG-----------DYTLLQDQEGDMDHGLKESPPQPPADDGAEEPG 49
rat             MAEPRQEFDTMEDQAG-----------DYTMLQDQEGDMDHGLKESPPQPPADDGSEEPG 49
human           MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG 60
                **:*****:.***:**           .**: ****** * ****** *.*::**:****

mouse           SETSDAKSTPTAEDVTAPLVDERAPDKQAAAQPHTEIPEGITAEEAGIGDTPNQEDQAAG 109
rat             SETSDAKSTPTAEDVTAPLVEERAPDKQATAQSHTEIPEGTTAEEAGIGDTPNMEDQAAG 109
human           SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG 120
                ********************:* **.***:**.******* **********. **:***

mouse           HVTQARVA--SKDRTGNDEKKAKGADGKTGAKIATPRGAASPAQKGTSNATRIPAKTTPS 167
rat             HVTQARVAGVSKDRTGNDEKKAKGADGKTGAKIATPRGAATPGQKGTSNATRIPAKTTPS 169
human           HVTQARMVSKSKDGTGSDDKKAKGADGKT--KIATPRGAAPPGQKGQANATRIPAKTPPA 178
                ******:.  *** **.*:**********  *********.*.***.:*********.*:

mouse           PKTPPGSGEPPKSGERSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSA 227
rat             PKTPPGSGEPPKSGERSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSA 229
human           PKTPPSSGEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSS 238
                *****.********:*********************************************:

mouse           SKSRLQTAPVPMPDLKNVRSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIK 287
rat             SKSRLQTAPVPMPDLKNVRSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIK 289
human           AKSRLQTAPVPMPDLKNVKSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIK 298
                :*****************:****************************************

mouse           HVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLD 347
rat             HVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLD 349
human           HVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQSKIGSLD 358
                ************************************************************

mouse           NITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSID 407
rat             NITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSID 409
human           NITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSID 418
                ************************************************************

mouse           MVDSPQLATLADEVSASLAKQGL 430 (SEQ ID NO:8)
rat             MVDSPQLATLADEVSASLAKQGL 432 (SEQ ID NO:7)
human           MVDSPQLATLADEVSASLAKQGL 441 (SEQ ID NO:1)
                **********************
```

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/48989

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/04; C12N 15/11 (2010.01)
USPC - 536/24.5; 514/44A
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)
IPC(8) - C07H 21/04; C12N 15/11 (2010.01)
USPC - 536/24.5; 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/369, 514/375, 514/407, 514/102; 506/9; 548/183, 548/224, 548/365.7; 435/5, 435/6, 435/320.1, 435/235.1, 435/239, 435/325 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: Tau acetylation inhibitor, p300 acetyltransferase, deacetylate, acetylates, antagonist, inhibitor, SIRT1, SIRT2, HDAC6, activator, silent information regulator, Sir, tauopathy, treating, resveratrol, frontotemporal dementia, Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneratio

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/0025337 A1 (SINCLAIR et al.) 02 February 2006 (02.02.2006) para [0005], [0013], [0019], [1197], and [1225]	10, 12, 14-16 --- 2, 7, 11, 13
Y	BALASUBRAMANYAM et al. Curcumin, a Novel p300/CREB-binding Protein-specific Inhibitor of Acetyltransferase, Represses the Acetylation of Histone/Nonhistone Proteins and Histone Acetyltransferase-dependent Chromatin Transcription. J Biol Chem. 2004, 279(49):51163-71; Abstract; pg 51164, Fig 1B; and pg 51166, col 2, para 3	1-9
Y	GHOSH et al. Comparison of pathways controlling toxicity in the eye and brain in Drosophila models of human neurodegenerative diseases. Hum Mol Genet. 2004, 13(18):2011-2018; Abstract; pg 2012, col 2, top para; and pg 2013, col 2, para 3	1-9
Y	MORIMOTO et al. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. J Clin Invest. 2008, 118(3):868-78; Abstract; pg 868, col 2, top para; and pg 75, col 2, top para	11, 13
A	US 2008/0220449 A1 (VASAN et al.) 11 September 2008 (11.09.2008) para [0035]-[0036]	1-16
A	BIERNAT et al. The switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine- proline motifs upstream of the microtubule binding region. EMBO J. 1992, 11(4):1593 - 1597	1-16
X,P	MIN et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron. 23 September 2010, 67(6):953-66; Abstract; pg 954, col 2, last para; pg 956; and pg 958, Fig 3	1-9

☐ Further documents are listed in the continuation of Box C. ☐

* 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
19 January 2011 (19.01.2011)	08 FEB 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/48989

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.:
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I: claims 1-16, drawn to a method for reducing the level of an acetylated Tau polypeptide in a cell by contacting the cell with an agent that increases the activity of a polypeptide that deacetylates a Tau polypeptide in the cell and/or an agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in the cell.

Group II, claims 17-18, drawn to a method of diagnosing a cognitive impairment disorder in an individual, the method comprising detecting a level of acetylated Tau polypeptide in a biological sample obtained from the individual, wherein a level of acetylated Tau polypeptide that is higher than a normal control level indicates that the individual has a cognitive impairment disorder.

**********Continued in the extra sheet**********

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.:
Claims 1-16

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.

********** Supplemental Box **********

Continuation of: Box No. III (unity of invention is lacking)

Group III, claims 19-20, drawn to a method of identifying a candidate agent for treating a tauopathy.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.i because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I-II do not include the inventive concept of a method of identifying a candidate agent for treating a tauopathy, as required by Group III. Furthermore, the screening method is not a method of using the claimed p300/CBP inhibitor and/or an activator of SIRT1, SIRT2,or HDAC6. In the absence of any teaching as to the structure required for a compound to act as a p300/CBP inhibitor and/or an activator of SIRT1, SIRT2,or HDAC6, there is no single general concept that links the method to the claimed compounds. Thus, unity of invention is lacking (a priori).

The inventions of Groups I and III do not include the inventive concept of a method of diagnosing a cognitive impairment disorder in an individual by detecting a level of acetylated Tau polypeptide in a biological sample obtained from the individual, wherein a level of acetylated Tau polypeptide that is higher than a normal control level indicates that the individual has a cognitive impairment disorder, as required by Group II.

The inventions of Groups II-III do not include the inventive concept of a method for reducing the level of an acetylated Tau polypeptide in a cell by contacting the cell with an agent that increases the activity of a polypeptide that deacetylates a Tau polypeptide in the cell and/or an agent that decreases the activity of a polypeptide that acetylates a Tau polypeptide in the cell, as required by Group I.

None of these special technical features are common to the other groups, nor do they correspond to a special technical feature in the other groups. Thus, unity of invention is lacking.