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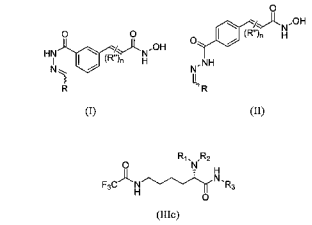
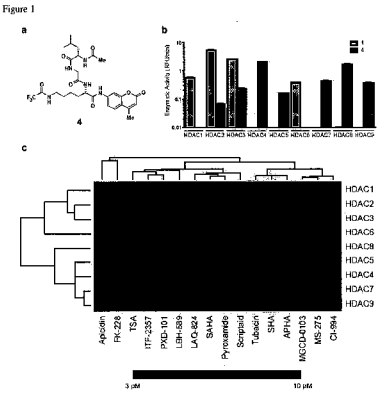
(54) Title: CLASS- AND ISOFORM-SPECIFIC HDAC INHIBITORS AND USES THEREOF



(I) (II)

(IIIc)



(57) Abstract: HDAC inhibitors of the general formula (I) and (II) and pharmaceutically acceptable salts thereof, as described herein, are useful as inhibitors of histone deacetylases or other deacetylases, and thus are useful for the treatment of various diseases and disorders associated with acetylase/deacetylase activity as described herein (e.g., cancer). In certain embodiments, the compounds of the invention selectively target either a class or isoform of the HDAC family. Another aspect of the invention provides an assay for determining the inhibitory effect of a test compound on an HDAC protein comprising: incubating the HDAC protein with a substrate of general formula (IIIc) in the presence of a test compound; and determining the activity of the HDAC protein.

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Class- and Isoform-Specific HDAC Inhibitors and Uses Thereof

Related Applications

The present application claims priority under 35 U.S.C. § 119(e) to U.S. provisional patent application, USSN 61/233,035, filed August 11, 2009, which is incorporated herein by reference.

Background of the Invention

The identification of small organic molecules that affect specific biological functions has the potential to greatly impact both biology and medicine. Such molecules are useful not only as therapeutic agents and as probes of biological function. In but one example from the emerging field of chemical genetics, in which small molecules are used to alter the function of biological molecules to which they bind, these molecules have been useful at elucidating signal transduction pathways by acting as chemical protein knockouts, thereby causing a loss of protein function (Schreiber *et al., J. Am. Chem. Soc.*, **1990**, *112*, 5583; Mitchison, *Chem. and Biol.*, **1994**, *1*, 3). Additionally, due to the interaction of these small molecules with particular biological targets and their ability to affect specific biological function, they may also serve as candidates or leads for the development of new therapeutic agents. For example, natural products, which are small molecules obtained from nature, clearly have played an important role in advances in the fields of biology, chemistry, and medicine, serving as pharmaceutical leads, drugs (Newman *et al.*, *Nat. Prod. Rep.* **2000**, *17*, 215-234), and powerful tools for studying cell biology (Schreiber, S.L. *Chem. and Eng. News* **1992** (October 26), 22-32).

One biological target of recent interest is histone deacetylase (see, for example, a discussion of the use of inhibitors of historic deacetylases in the treatment of cancer: Marks *et al. Nature Reviews Cancer* **2001**, *1*,194; Johnstone *et al. Nature Reviews Drug Discovery* **2002**, *1,* 287). Post-translational modification of proteins (*e.g.*, histones, transcription factors, tubulin) through the acetylation and deacetylation of lysine residues has a critical role in regulating their biological function. HDACs are zinc hydrolases that modulate gene expression through deacetylation of the N-acetyl-lysine residues of histone proteins and other transcriptional regulators (Hassig *et al. Curr. Opin. Chem. Biol.* **1997**, *1*, 300-308). The function of other proteins such as tubulin is also thought to be regulated by their acetylation state. HDACs participate in cellular pathways that control cell shape and differentiation, and

an HDAC inhibitor has been shown effective in treating an otherwise recalcitrant cancer (Warrell *et al. J. Natl. Cancer Inst.* **1998**, *90*,1621-1625). Eleven human HDACs, which use zinc as a cofactor, have been characterized (Taunton *et al. Science* **1996**, *272*, 408-411; Yang *et al. J. Biol. Chem.* **1997**, *272*, 28001-28007; Grozinger *et al. Proc. Natl. Acad. Scl. U.S.A.* **1999**, *96*, 4868-4873; Kao *et al. Genes Dev.* **2000**, *14*, 55-66; Hu *et al. J. Biol. Chem.* **2000**, *275*, 15254-15264; Mon *et al. Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10572-10577; Venter *et al.* Science **2001**, *291*, 1304-1351). These members fall into three related classes (Class I, II, and IV) (Gregoretti *et al., J. Mol. Biol.* **2004**, *338*, 17-31). Class I HDACs include HDAC1, HDAC2, and HDAC3. Class II includes HDAC4, HDAC5, HDAC6, HDAC7, HADC9, and HDAC10. Class II is further subdivided into Class IIa, which includes HDAC4, HDAC5, HDAC7, and HDAC9, and Class IIb, which includes HDAC6 and HDAC10. Class IV includes HDAC11. An additional Class of HDACs has been identified which use NAD as a cofactor. These have been termed Class III deacetylases, also known as the sirtuin deacetylases (SIRT1-7).

Class IIa enzymes (HDAC4, 5, 7, and 9) have been shown to have important regulatory functions in the body. To provide a few examples: HDAC9 has been recently shown to have important regulatory function in regulatory T cells, and that HDAC9 inhibitors seem highly desirable for the treatment of transplant patients as well as the treatment of autoimmune diseases (Tao *et al. Nat. Med.* **2007**, *13*, 1299-1307). HDAC7 inhibitors have been proposed for the treatment of life-threatening vascular diseases (Miano *et al. Nat. Med.* **2006**, *12*, 997-998), and HDAC5 inhibitors for the treatment of drug addiction (Nestler *et al. Neuron* **2007**, *56*, 517-529).

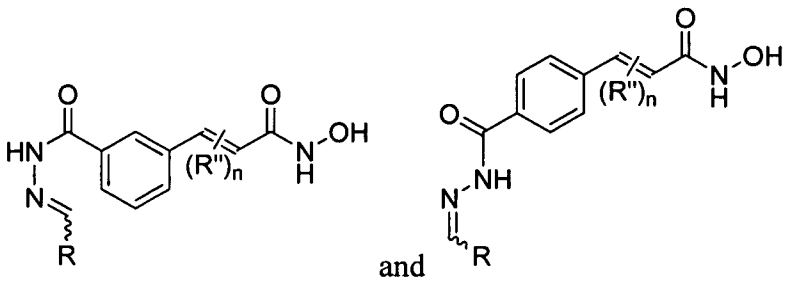
Based on this understanding of known HDACs, efforts are currently focused on developing novel HDAC inhibitors that are isoform- or class-specific inhibitors. Such specificity may allow for the development of pharmaceutical agents for the treatment of HDAC-associated diseases, with greater potency and/or decreased unwanted side effects based on greater on-target activity.

Summary of the Invention

To date, no small molecules have been reported that selectively target either a class or individual member of the HDAC family (on the other hand, ortholog-selective HDAC inhibitors have been reported: (a) Meinke *et al. J. Med. Chem.* **2000**, *14*, 4919-4922; (b) Meinke *et al Carr. Med. Chem.* **2001**, *8*, 211-235). Furthermore, no compound is known which inhibits all HDACs (*i.e.*, no or minimal selectivity).

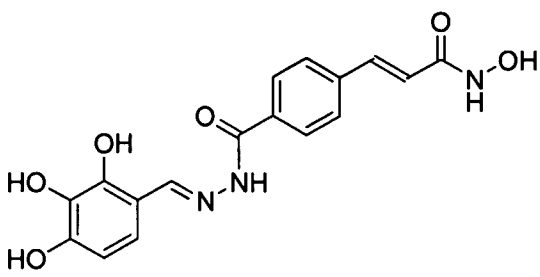
The present invention provides novel cinnamic hydroxymate deacetylase inhibitors and methods of preparing and using these compounds. A phylogenetic analysis of Class I and II HDACs as targets of a comprehensive, structurally diverse panel of inhibitors revealed unexpected isoform selectivity even among inhibitors widely perceived as non-selective. These data informed the design of a focused library of cinnamic hydroxymates, which allowed the identification of a truly non-selective HDAC inhibitor as well as selective HDAC inhibitors. In particular, cinnamic hydroxymates have been discovered that selectively inhibit Class IIa HDACs (HDAC4, 5, 7, and 9). These novel HDAC inhibitors are useful as research tools as well as for the treatment of various HDAC-associated diseases, including, but not limited to, proliferative diseases, such as cancer; autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.*, Huntington's disease); vascular diseases, such as restenosis; musculoskeletal diseases; cardiovascular diseases, such stroke and myocardial infarction; pulmonary diseases; and gastric diseases.

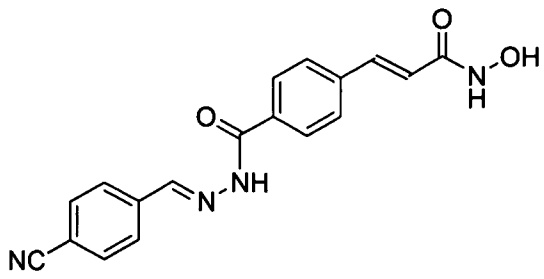
In one aspect, the present invention provides novel cinnamic hydroxymate compounds of the general formula (I) and (II):

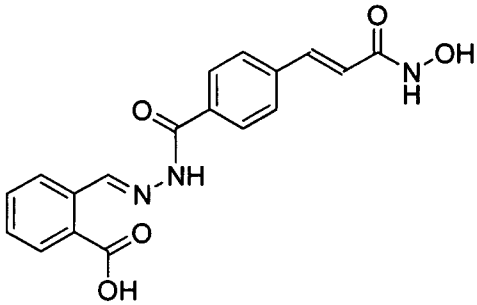


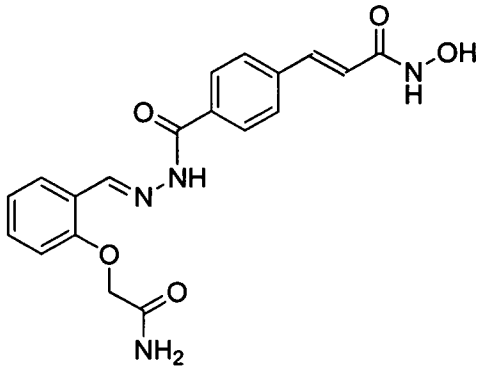
(I) and (II)

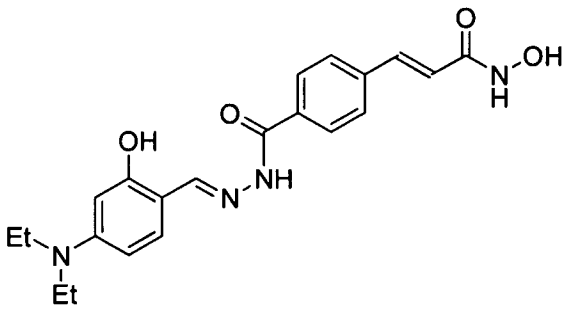
and pharmaceutically acceptable salts thereof, as described herein. The compounds are useful as inhibitors of histone deacetylases or other deacetylases (*e.g.*, tubulin deacetylase), and thus are useful for the treatment of various diseases and disorders associated with acetylase/deacetylase activity as described herein. The inventive compounds are additionally useful as tools to probe biological function. Exemplary inventive HDAC inhibitors with a 1,4-substitution pattern about the phenyl ring include compounds of the formula:

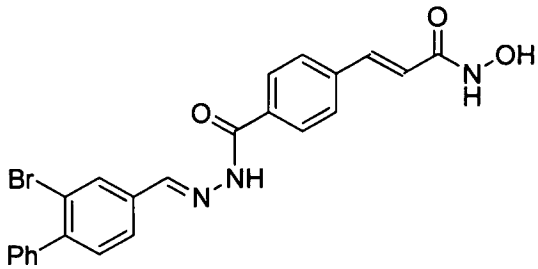


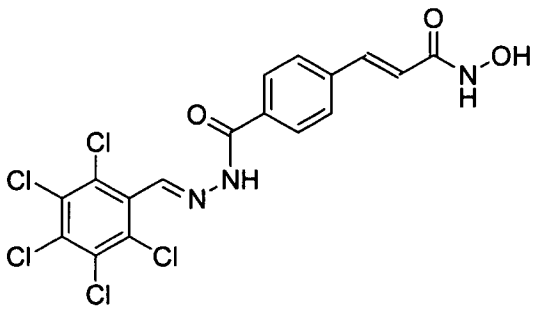




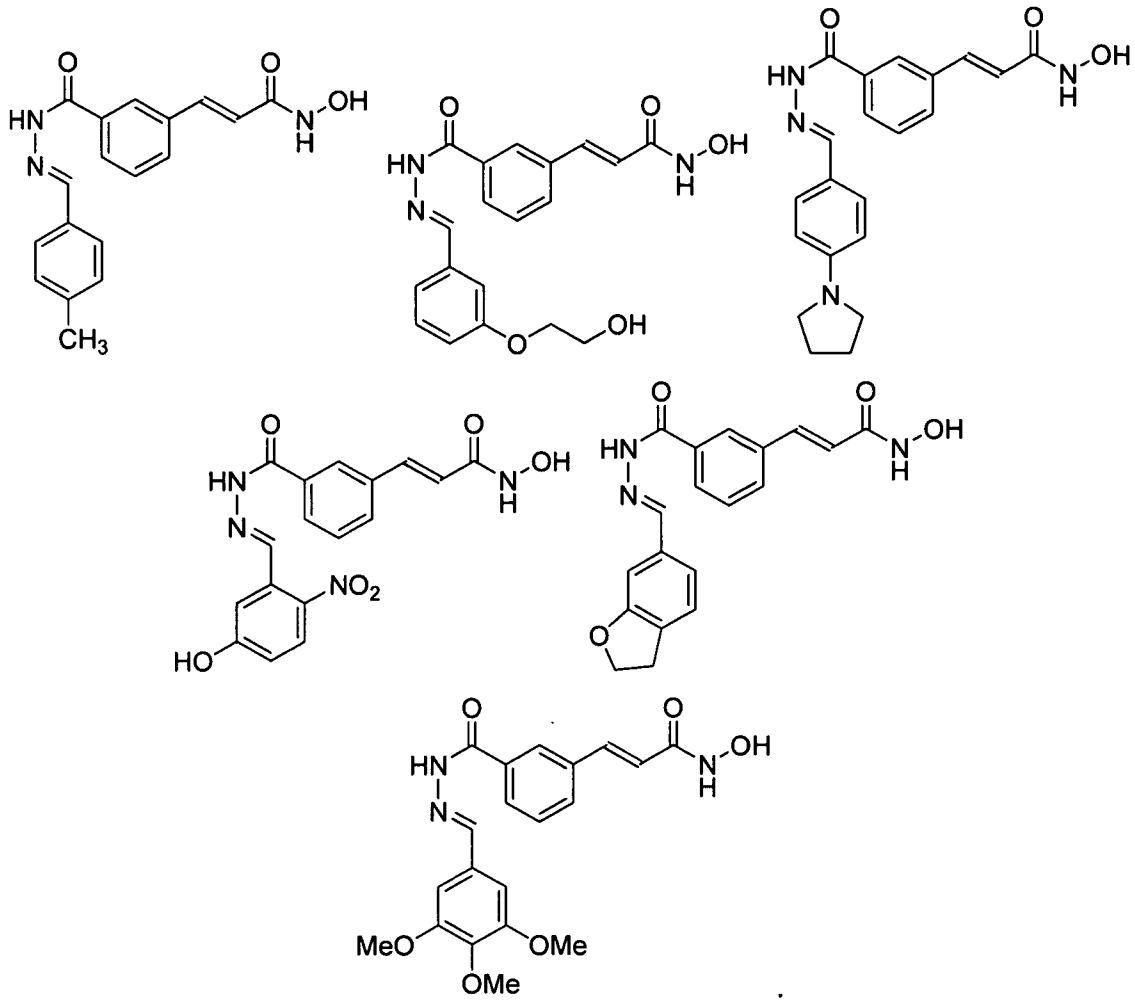








Other exemplary HDAC inhibitors with a 1,3-substitution pattern include compounds of the formula:



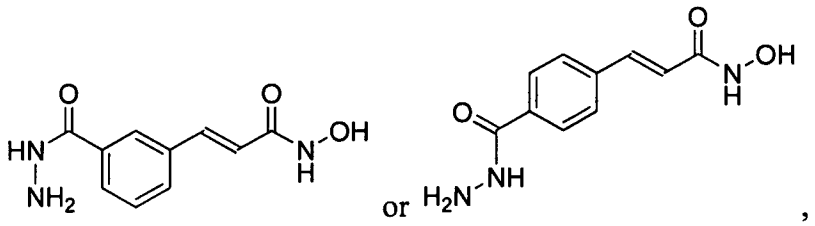
In one aspect, the present invention provides methods for inhibiting histone deacetylase activity or other deacetylase activity in a patient or a biological sample, comprising administering to said patient, or contacting said biological sample, with an effective inhibitory amount of a compound of the invention. In certain embodiments, the compound specifically inhibits a particular HDAC isoform (*e.g.*, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11) or Class of HDACs (*e.g.*, Class I, II, or IV). In certain embodiments, the compound specifically inhibits Class IIa HDACs. In still another aspect, the present invention provides methods for treating diseases or disorders involving histone deacetylase activity, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of the invention. In certain embodiments, the disease can be proliferative diseases, such as cancer; autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.* Huntington's disease); vascular diseases, such as

restenosis; musculoskeletal diseases; cardiovascular diseases, such as stroke; pulmonary diseases; and gastric diseases. Diseases associated with Class IIa enzymes include autoimmune diseases, transplant rejection, vascular diseases, and drug addiction; therefore, Class IIa-specific HDAC inhibitors may be particularly useful in treating such diseases.

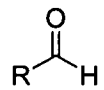
The compounds may be administered to a subject by any method known in the art. In certain embodiments, the compounds are administered paranterally or orally. The compounds may also be administered topically. The invention also provides pharmaceutical compositions comprising a therapeutically effective amount of an inventive compounds and a pharmaceutically acceptable excipient.

In certain aspect, the present invention provides a kit comprising at least one container having an inventive cinnamic hydroxymate compound or pharmaceutical composition thereof, and instructions for use. In other aspect of the invention the container comprises multiple dosage units of an inventive pharmaceutical composition. For example, the kit may include a whole treatment regimen of the inventive compound.

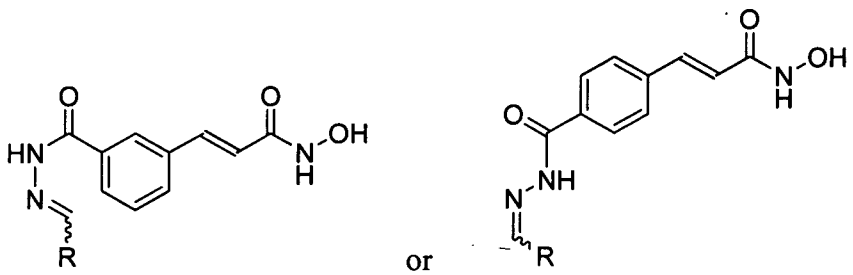
In another aspect, the present invention provides methods of preparing compounds of the invention. The method comprises reacting a hydrazine of the general formula:



or protected form thereof, with an aldehyde of formula :



under suitable conditions to yield a compound of the general formulae (I) or (II):



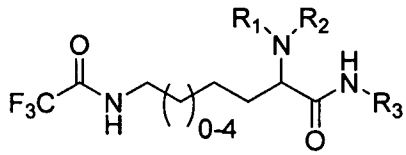
(I) (II).

Libraries of inventive cinnamic hydroxymates can be prepared by varying either one or both of the starting materials. In certain embodiments, the library is generated by varying

the aldehyde. In other embodiments, the library is generated by varying the hydrazine. For example, the double bond of the cinnamic hydroxymate may be substituted.

In certain aspect, the present invention provides an assay to determine the inhibitory effect of a test compound on an HDAC protein. The assay comprises incubating the HDAC protein with a substrate of general formula (III) in the presence of a test compound; and determining the activity of the HDAC protein.

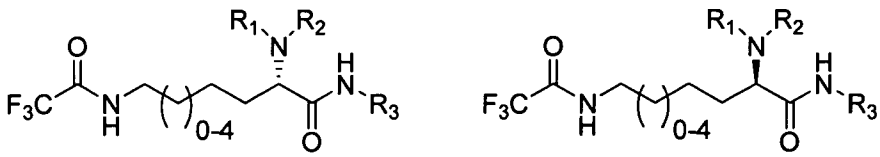
formula (III)



(III)

wherein R_1, R_2, and R_3 are each independently hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR^B$; $-C(=O)R^B$; $-CO_2R^B$; $-C(=O)N(R^B)_2$; $-SR^B$; $-SOR^B$; $-SO_2R^B$; $-N(R^B)_2$; $-NHC(O)R^B$; or $-C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino; dialkylamino; heteroaryloxy; heteroarylthioxy; an amino acid; a peptide; a protecting group; or a tag; or salt thereof; in the presence of a test compound; and determining the activity of the HDAC protein.

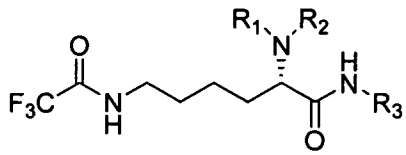
In certain embodiments, general formula (III) is



or

(IIIa) (IIIb) .

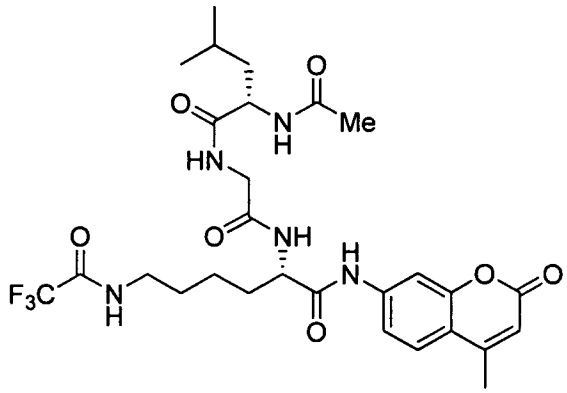
In another embodiments, formula (IIIa) is formula (IIIc):



(IIIc)

to be used as a substrate in the assay. With these inventive compounds, Class IIa HDACs exhibit markedly faster kinetics further reducing requisite enzyme concentration and allowing a high-throughput, precise profiling of HDACi against all Class IIa enzymes.

Exemplary inventive compounds of formula (IIIc) include:



Definitions

Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group," has used herein, it is meant that a particular functional moiety, *e.g.*, C, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In certain embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen, and carbon protecting groups may be utilized. Exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended

to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in *Protective Groups in Organic Synthesis*, Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference. Furthermore, a variety of carbon protecting groups are described in Myers, A.; Kung, D.W.; Zhong, B.; Movassaghi, M.; Kwon, S. *J. Am. Chem. Soc.* **1999**, *121*, 8401-8402, the entire contents of which are hereby incorporated by reference.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example, of HDAC-associated diseases (*e.g.*, cancer). The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes described herein.

The term "acyl", as used herein, refers to a carbonyl-containing functionality, *e.g.*, -C(=O)R, wherein R is an aliphatic, alycyclic, heteroaliphatic, heterocyclic, aryl, heteroaryl, (aliphatic)aryl, (heteroaliphatic)aryl, heteroaliphatic(aryl), or heteroaliphatic(heteroaryl) moiety, whereby each of the aliphatic, heteroaliphatic, aryl, or heteroaryl moieties is substituted or unsubstituted, or is a substituted (*e.g.*, hydrogen or aliphatic, heteroaliphatic,

aryl, or heteroaryl moieties) oxygen or nitrogen containing functionality (*e.g.*, forming a carboxylic acid, ester, or amide functionality).

The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched) or branched aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, and alkynyl moieties. Thus, as used herein, the term "alkyl" includes straight and branched alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl", and the like.

Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl", and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

In certain embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 14 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties, and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl, and the like.

The term "alicyclic", as used herein, refers to compounds which combine the properties of aliphatic and cyclic compounds and include but are not limited to cyclic, or polycyclic aliphatic hydrocarbons and bridged cycloalkyl compounds, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "alicyclic" is intended herein to include, but is not limited to, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties, which are optionally substituted with one or more functional groups. Illustrative alicyclic groups thus include, but are not limited to, for

example, cyclopropyl, -CH_2-cyclopropyl, cyclobutyl, -CH_2-cyclopentyl, cyclopentyl, -CH_2-cyclopentyl, cyclohexyl, -CH_2.cyclohexyl, cyclohexenylethyl, cyclohexanylethyl, norborbyl moieties, and the like, which may bear one or more substituents.

The term "alkoxy" or "alkyloxyl" or "thioalkyl", as used herein, refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy, and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

The term "alkylamino" refers to a group having the structure –NHR' wherein R' is alkyl, as defined herein. The term "aminoalkyl" refers to a group having the structure NH_2R'-, wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl contains 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino, n-propylamino, and the like.

Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to, aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br, -I; -OH; -NO_2; -CN; -CF_3; -CH_2CF_3; -$CHCl_2$; -CH_2OH; -CH_2CH_2OH; -CH_2NH_2; -$CH_2SO_2CH_3$; -$C(O)R_x$; -$CO_2(R_x)$; -$CON(R_x)_2$; -$OC(O)R_x$; -OCO_2R_x; -$OCON(R_x)_2$; -$N(R_x)_2$; -$S(O)_2R_x$; -$NR_x(CO)R_x$; wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alycyclic, heteroaliphatic, heterocyclic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be

substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

In general, the term "aromatic moiety", as used herein, refers to a stable mono- or polycyclic, unsaturated moiety having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. In certain embodiments, the term "aromatic moiety" refers to a planar ring having p-orbitals perpendicular to the plane of the ring at each ring atom and satisfying the Huckel rule where the number of pi electrons in the ring is (4n+2), wherein n is an integer. A mono- or polycyclic, unsaturated moiety that does not satisfy one or all of these criteria for aromaticity is defined herein as "non-aromatic," and is encompassed by the term "alicyclic."

In general, the term "heteroaromatic moiety", as used herein, refers to a stable mono- or polycyclic, unsaturated moiety having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted; and comprising at least one heteroatom selected from O, S, and N within the ring (*i.e.*, in place of a ring carbon atom). In certain embodiments, the term "heteroaromatic moiety" refers to a planar ring comprising at least on heteroatom, having p-orbitals perpendicular to the plane of the ring at each ring atom, and satisfying the Huckel rule where the number of pi electrons in the ring is (4n+2), wherein n is an integer.
It will also be appreciated that aromatic and heteroaromatic moieties, as defined herein may be attached via an alkyl or heteroalkyl moiety and thus also include -(alkyl)aromatic, -(heteroalkyl)aromatic, -(heteroalkyl)heteroaromatic, and -(heteroalkyl)heteroaromatic moieties. Thus, as used herein, the phrases "aromatic or heteroaromatic moieties" and "aromatic, heteroaromatic, -(alkyl)aromatic, -(heteroalkyl)aromatic, -(heteroalkyl)heteroaromatic, and -(heteroalkyl)heteroaromatic" are interchangeable. Substituents include, but are not limited to, any of the previously mentioned substituents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound.

The term "aryl", as used herein, does not differ significantly from the common meaning of the term in the art, and refers to an unsaturated cyclic moiety comprising at least one aromatic ring. In certain embodiments, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like.

The term "heteroaryl", as used herein, does not differ significantly from the common meaning of the term in the art, and refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O, and N; zero, one, or two ring atoms

are additional heteroatoms independently selected from S, O, and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one or more of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br, -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; and $-NR_x(CO)R_x$; wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aromatic, heteroaromatic, aryl, heteroaryl, -(alkyl)aryl or (alkyl)heteroaryl substituents described above and herein may be substituted or unsubstituted. Additionally, it will be appreciated, that any two adjacent groups taken together may represent a 4, 5, 6, or 7-membered substituted or unsubstituted alicyclic or heterocyclic moiety. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like, which, as in the case of aliphatic, alicyclic, heteroaliphatic or heterocyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br, -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; -

$CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$; wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aromatic, heteroaromatic, aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The term "heteroaliphatic", as used herein, refers to aliphatic moieties in which one or more carbon atoms in the main chain have been substituted with a heteroatom. Thus, a heteroaliphatic group refers to an aliphatic chain which contains one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be linear or branched, and saturated or unsaturated. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to, aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; -F; -Cl; -Br, -I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; and $-NR_x(CO)R_x$; wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aromatic, heteroaromatic, aryl or heteroaryl substituents described herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The term "heterocycloalkyl", "heterocycle" or "heterocyclic", as used herein, refers to compounds which combine the properties of heteroaliphatic and cyclic compounds and include, but are not limited to, saturated and unsaturated mono- or polycyclic cyclic ring systems having 5-16 atoms wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally be oxidized), wherein

the ring systems are optionally substituted with one or more functional groups, as defined herein. In certain embodiments, the term "heterocycloalkyl", "heterocycle" or "heterocyclic" refers to a non-aromatic 5-, 6-, or 7- membered ring or a polycyclic group wherein at least one ring atom is a heteroatom selected from O, S, and N (wherein the nitrogen and sulfur heteroatoms may be optionally be oxidized), including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 2 double bonds, each 6-membered ring has 0 to 2 double bonds and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative heterocycles include, but are not limited to, heterocycles such as furanyl, thiofuranyl, pyranyl, pyrrolyl, thienyl, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolyl, oxazolidinyl, isooxazolyl, isoxazolidinyl, dioxazolyl, thiadiazolyl, oxadiazolyl, tetrazolyl, triazolyl, thiatriazolyl, oxatriazolyl, thiadiazolyl, oxadiazolyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, dithiazolyl, dithiazolidinyl, tetrahydrofuryl, and benzofused derivatives thereof. In certain embodiments, a "substituted heterocycle, or heterocycloalkyl or heterocyclic" group is utilized and as used herein, refers to a heterocycle, or heterocycloalkyl or heterocyclic group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy; heteroaryloxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; NO_2; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$; wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic; heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl, or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aromatic, heteroaromatic, aryl, or heteroaryl substituents described herein may be substituted or unsubstituted. Additional examples or generally applicable substituents are illustrated by the specific embodiments described herein.

Additionally, it will be appreciated that any of the alicyclic or heterocyclic moieties described herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments described herein.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine, and iodine.

The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like. In certain embodiments, the alkyl group is perhalogenated (*e.g.*, perfluorinated).

The term "amino", as used herein, refers to a primary ($-NH_2$), secondary ($-NHR_x$), tertiary ($-NR_xR_y$), or quaternary ($-N^+R_xR_yR_z$) amine, where R_x, R_y, and R_z, are independently an aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic or heteroaromatic moiety, as defined herein. Examples of amino groups include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, iso-propylamino, piperidino, trimethylamino, and propylamino.

The term "alkylidene", as used herein, refers to a substituted or unsubstituted, linear or branched saturated divalent radical of carbon and hydrogen atoms, having from one to n carbon atoms and having a free valence at both ends of the radical. The alkylidene moiety may be substituted.

The term "alkenylidene", as used herein, refers to a substituted or unsubstituted, linear or branched unsaturated divalent radical of carbon and hydrogen atoms, having from two to n carbon atoms and having a free valence at both ends of the radical, and wherein the unsaturation is present only as double bonds and wherein a double bond can exist between the first carbon of the chain and the rest of the molecule. The alkenylidene moiety may be substituted.

The term "alkynylidene", as used herein, refers to a substituted or unsubstituted, linear or branched unsaturated divalent radical of carbon and hydrogen atoms, having from two to n carbon atoms, having a free valence "-" at both ends of the radical, and wherein the unsaturation is present only as triple bonds and wherein a triple bond can exist between the first carbon of the chain and the rest of the molecule. The alkynylidene moiety may be substituted.

The term "carbamate", as used herein, refers to any carbamate derivative known to one of ordinary skill in the art. Examples of carbamates include t-Boc, Fmoc, benzyloxy-

carbonyl, alloc, methyl carbamate, ethyl carbamate, 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, Tbfmoc, Climoc, Bimoc, DBD-Tmoc, Bsmoc, Troc, Teoc, 2-phenylethyl carbamate, Adpoc, 2-chloroethyl carbamate, 1,1-dimethyl-2-haloethyl carbamate, DB-t-BOC, TCBOC, Bpoc, t-Bumeoc, Pyoc, Bnpeoc, *N*-(2-pivaloylamino)-1,1-dimethylethyl carbamate, NpSSPeoc. In certain embodiments, carbamates are used as nitrogen protecting groups.

Unless otherwise indicated, as used herein, the terms "alkyl", "alkenyl", "alkynyl", "heteroalkyl", "heteroalkenyl", "heteroalkynyl", "alkylidene", "alkynylidene", -(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)heteroaryl, and the like encompass substituted and unsubstituted, and linear and branched groups. Similarly, the terms "aliphatic", "heteroaliphatic", and the like encompass substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms "cycloalkyl", "heterocycle", "heterocyclic", and the like encompass substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms "cycloalkenyl", "cycloalkynyl", "heterocycloalkenyl", "heterocycloalkynyl", "aromatic", "heteroaromatic, "aryl", "heteroaryl", and the like encompass both substituted and unsubstituted groups.

The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety, which is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester, which is cleaved in vivo to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. The biological activity of pro-drugs may also be altered by appending a functionality onto the compound, which may be catalyzed by an enzyme. Also, included are oxidation and reduction reactions, including enzyme-catalyzed oxidation and reduction reactions. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives are discussed in more detail herein.

"Compound": The term "compound" or "chemical compound" as used herein can include organometallic compounds, organic compounds, metals, transitional metal

complexes, and small molecules. In certain embodiments, polynucleotides are excluded from the definition of compounds. In other embodiments, polynucleotides and peptides are excluded from the definition of compounds. In certain embodiments, the term compound refers to small molecules (*e.g.*, preferably, non-peptidic and non-oligomeric) and excludes peptides, polynucleotides, transition metal complexes, metals, and organometallic compounds.

"Small Molecule": As used herein, the term "small molecule" refers to a non-peptidic, non-oligomeric organic compound, either synthesized in the laboratory or found in nature. A small molecule is typically characterized in that it contains several carbon-carbon bonds, and has a molecular weight of less than 2000 g/mol, preferably less than 1500 g/mol, although this characterization is not intended to be limiting for the purposes of the present invention. Examples of "small molecules" that occur in nature include, but are not limited to, taxol, dynemicity and rapamycin, Examples of "small molecules" that are synthesized in the laboratory include, but are not limited to, compounds described in Tan *et al.*, ("Stereoselective Synthesis of over Two Million Compounds Having Structural Features Both Reminiscent of Natural Products and Compatible with Miniaturized Cell-Based Assays" *J. Am. Chem. Soc.* **1998**, *120*, 8565; incorporated herein by reference).

"HDAC": The term "HDAC" or "HDACs" refers to histone deacetylase(s).

"Acetylase activity": The term "acetylase activity" refers to the regulation of a cellular process by modulating protein structure and/or function by the removal of an acetyl group.

"Biological sample": As used herein the term "biological sample" includes, without limitation, cell cultures, or extracts thereof; biopsied material obtained from an animal (*e.g.*, mammal) or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof. For example, the term "biological sample" refers to any solid or fluid sample obtained from, excreted by or secreted by any living organism, including single-celled micro-organisms (such as bacteria and yeasts) and multicellular organisms (such as plants and animals, for instance a vertebrate or a mammal, and in particular a healthy or apparently healthy human subject or a human patient affected by a condition or disease to be diagnosed or investigated). The biological sample can be in any form, including a solid material such as a tissue, cells, a cell pellet, a cell extract, cell homogenates, or cell fractions; or a biopsy, or a biological fluid. The biological fluid may be obtained from any site (*e.g.*, blood, saliva (or a mouth wash containing buccal cells), tears, plasma, serum, urine, bile, cerebrospinal fluid, amniotic fluid, peritoneal fluid, and pleural fluid, or cells therefrom,

aqueous or vitreous humor, or any bodily secretion), a transudate, an exudate (*e.g.*, fluid obtained from an abscess or any other site of infection or inflammation), or fluid obtained from a joint (*e.g.*, a normal joint or a joint affected by disease such as rheumatoid arthritis, osteoarthritis, gout or septic arthritis). The biological sample can be obtained from any organ or tissue (including a biopsy or autopsy specimen) or may comprise cells (whether primary cells or cultured cells) or medium conditioned by any cell, tissue, or organ. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. Biological samples also include mixtures of biological molecules including proteins, lipids, carbohydrates, and nucleic acids generated by partial or complete fractionation of cell or tissue homogenates. Although the sample is preferably taken from a human subject, biological samples may be from any animal, plant, bacteria, virus, yeast, *etc.*

"Animal": The term animal, as used herein, refers to humans as well as non-human animals, at any stage of development, including, for example, mammals, birds, reptiles, amphibians, fish, worms, and single cells. In certain exemplary embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). An animal may be a transgenic animal or a clone.

"Pharmaceutically acceptable salt": As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts of amines, carboxylic acids, and other types of compounds, are well known in the art. For example, Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* **1977**, *6,* 1-19, incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of a compound of the invention, or separately by reacting a free base or free acid function with a suitable reagent, as described generally below. For example, a free base can be reacted with a suitable acid. Furthermore, where the compound of the invention carries an acidic moiety, suitable pharmaceutically acceptable salts thereof may, include metal salts such as alkali metal salts, *e.g.* sodium or potassium salts; and alkaline earth metal salts, *e.g.* calcium or magnesium salts. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid; or by using other methods used in the art such as ion exchange.

Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

Brief Description of the Drawings

Figure 1 illustrates chemical phylogenetic analysis of HDACs identifying unexpected selectivity of HDAC inhibitors. (a) Chemical structure of substrate 4. (b) Comparative enzymatic activity of HDAC1-9 with tripeptide substrate 1 and trifluoro acetyl-lysine tripeptide substrate 4, studied at equivalent substrate concentration (10 μM). Substrate 4 allows miniaturized, kinetic study of HDAC4, 5, 7, 8 and 9. (c) Hierarchical clustering of HDACs and a focused library of structurally-diverse HDAC inhibitor tool and investigational compounds weighted by inhibitory potency (K_i).

Figure 2 shows chemical structures of various HDAC inhibitors used for biochemical profiling.

Figure 3 illustrates the synthesis and testing of an HDAC-biased chemical library and identification of a non-selective HDAC inhibitor. (a) Library design of meta- and para-substituted hydroxamic acid HDAC inhibitors, utilizing parallel condensation of aldehydes, efficiently samples chemical diversity at the capping feature. (b) Biochemical profiling data for the para-substituted sub-library (n=160 compounds), presented in dose-response format for inhibition of HDAC5. Structural variation in the capping feature was observed to confer a broad range of potency, as illustrated with the most (IC_{50}= 18 nM) and least (IC_{50} = 55 μM) potent small molecules tested. (c) Comparative biochemical profiling of meta- (red) and para-substituted (blue) sub-libraries for relative inhibition of HDAC2 and HDAC3. The complete library was studied and is displayed at a range of concentrations (0.03, 0.3, 3.0

and 30.0 μM). Compounds of this structural class do not discriminate between HDAC2 and HDAC3. (d) Comparative biochemical profiling of meta- (red) and para-substituted (blue) sub-libraries for relative inhibition of HDAC5 and HDAC7. The complete library was studied and is displayed at a range of concentrations (0.03, 0.3, 3.0 and 30.0 pM). Para-substituted cinnamic hydroxamic acids exhibit increased potency for HDAC5, relative to meta-substituted regioisomers. (e-g) Specificity profiles of (e) MS275, (f) SAHA and (g) pandacostat overlaying molecular phylogeny (Fabian *et. al. Nat. Biotechnol.* **2005**, *23*, 329-336) HDAC dendrograms are adapted from *Figure 6*. Circles are proportionate in size to K on a logarithmic scale, as shown. (h) Chemical structure of pandacostat.

Figure 4 illustrates comparative profiling of HDAC substrates identifying preferences distinct from molecular phylogenetic class assignments. (a) Chemical structure of substrates 1 and 3. (b) Comparative activity of HDAC1, 2, 3 and 6 for Boc-protected acetyl-lysine substrate 3 and tripeptide acetyl-lysine substrate 1, studied at equivalent substrate concentrations (10 μM). Substrate 1 is the preferred substrate for these Class I and IIb enzymes. (c) Chemical structure of substrates 2 and 4. (d) Comparative activity of HDAC4, 5, 7, 8 and 9 for Boc-protected trifluoro acetyl-lysine substrate 2 and tripeptide trifluoro acetyl-lysine substrate 4, studied at equivalent substrate concentrations (10 μM). Substrate 4 is the preferred substrate for these Class I and IIa enzymes. The robust activity of HDAC8 for trifluoro acetyl lysine-based substrates resonates with published observations from the Schwienhorst laboratory, who have innovated HDAC assay design and substrate preference determination. (Minucci *et al. Nat. Rev. Cancer* **2006**, *6*, 38-51; Lee *et al. Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 284-295.)

Figure 5 illustrates the determination of K_M for substrate 4. (a-e) Michaelis-Menten Plots for substrate 4 and human, recombinant HDACs (as labeled) in a miniaturized, kinetic trypsin-coupled assay. (f) Table of K_M values derived. Also provided are concentrations of enzymes required for the miniaturized HDAC assay, afforded by substrate 4. The reduction in enzyme used per well enables reagent-efficient compound annotation as well as high-throughput screening.

Figure 6 illustrates the phylogenetic analysis of human HDAC1-9. Amino acid sequences for each human histone deacetylase were retrieved from the National Centers for Biotechnology Information, and aligned using MAFFT as described above. (a) A neighbor-joining method with bootstrap resampling was utilized to compute evolutionary distance data

for all conserved sites (Saitou, N. *et. al Mol. Biol. Evol.* **1987**, *4*, 406-425). Amino acid replacement was performed using the maximum-likelihood approach of Whelan and Goldman (Whelan, S. *et. al. Mol. Biol. Evol.* **2001**, *18*, 691-699). Analyses were performed using the online research portal of Dr. Katoh (http://align.bmr.kyushu-u.ac.jp/mafft/software/). (b) Phylogenetic tree reconstruction was performed on MAFFT aligned sequence using reported rapid bootstrapping and rapid maximum likelihood search algorithms (Randomized Axelerated Maximum Likelihood (RAxML) (Stamatakis, A., Hoover, P. & Rougemont, J. A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* **57**, 758-71 (2008)); Cyberinfrastructure for Phylogenetic Research online portal (http://www.phylo.org/). (a,b) Phylogenetic trees were generated using Molecular Evolutionary Genetics Analysis software (Kumar, S., Nei, M., Dudley, J. & Tamura, K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform.* **9**, 299-306 (2008)). Notably, both methods of phylogenetic analysis produced identically branched trees.

Figure 7 shows the examination of zinc chelation by HDAC inhibitors in published, crystallographic data. (a) Trichostatin A bound to HDAC8 (1T64) (Somoza, J. R. *et. al. Structure* **2004**, 1325-1334). (b) SAHA bound to a bacterial Class II histone deacetylase homologue (1ZZ1) (Nielsen, T. K. *et. al. J. Mol. Biol.* **2005**, *354*, 107-120). (c) HDAC4 in complex with hydroxamate based inhibitors (2VQM) (Bottomley, M. J. *et. al. J. Biol. Chem.* **2008**, *283*, 26694-26704). (d) HDAC7 in complex with TSA (3C10) (Schuetz, A. *J. Biol. Chem. et. al.* **2008**, , *283*, 11355-11363). All data were obtained from the Protein Data Bank (Research Collaboratory for Structural Bioinformatics) and images were created in PyMOL Molecular Viewer (DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, Palo Alto, CA, USA.).

Figure 8 illustrates the comparative biochemical profiling of (a) trichostatin A (TSA), (b) SAHA, and (c) pandacostat, for inhibition of HDAC1-9. Compounds were arrayed in 384-well plate format as library stock solutions at 10 mM top concentration. Dilution series (3-fold) were created by hand micropipette. Compounds were studied for inhibition of HDACs following robotic pin transfer and a brief pre-incubation period. Dose-response data are presented for each compound. Data comprise the mean of three replicates. Curves were fit by logistic regression using Graph Pad Prism. These data confirm the unexpected selectivity of TSA and SAHA; they also confirm the markedly improved selectivity of pandacostat.

Figure 9 shows the biochemical inhibition of HDAC1-9 by pandacostat. (a) Visualization of biochemical inhibition of individual HDAC isoforms by pandacostat. (b) Summary of pandacostat K_i values for HDAC1-9 presented with standard deviation (Spotfire DecisionSite).

Figure 10 illustrates the biochemical inhibition of HDAC1-9.

Figure 11 shows the MAFFT alignment of HDAC1-9.

Figure 12 illustrates the IC_{50} or percentage inhibition of pandacostat (Mlg-1-164) against sirtuins.

Figure 13 shows the raw data for the effect of pandacostat (Mlg-1-164) on Sirtuin1 activity.

Figure 14 illustrates the effects of pandacostat (Mlg-1-164) on Sirtuin 1 activity .

Figure 15 shows the raw data for the effect of pandacostat (Mlg-1-164) on Sirtuin2 activity.

Figure 16 illustrates the effects of pandacostat (Mlg-1-164) on Sirtuin 2 activity.

Figure 17 represents the raw data for the effect of pendacostat (Mlg-1-164) on Sirtuin3 activity.

Figure 18 illustrates the effects of pandacostat (Mlg-1-164) on Sirtuin 3 activity.

Figure 19A-D illustrates the inhibitory effects of four exemplary inventive cinnamic hydroxamates at various concentrations on HDAC4 (yellow), HDAC5 (green), HDAC7 (blue), and HDAC9 (purple).

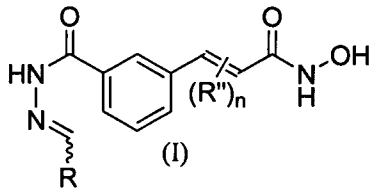
Detailed Description of the Invention

As discussed above, there remains a need for the development of novel histone deacetylase inhibitors, particularly ones that are class- or isoform-specific. The present invention provides novel compounds of general formula (I) and general formula (II), and methods for the synthesis thereof, which compounds are useful as inhibitors of histone deacetylases, and thus are useful for the treatment of diseases or disorders associated with deacetylase activity. In certain embodiments, the inventive compounds are useful in the treatment of proliferative diseases, such as cancer; autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.* Huntington's disease); vascular diseases, such as restenosis; musculoskeletal diseases; cardiovascular diseases, such as stroke; pulmonary diseases; and gastric diseases. In particular, the inventive compounds are cinnamic hydroxymates. In certain embodiments, the compounds are class-specific. In certain embodiments, the

compounds are isoform-specific. In certain embodiments, the compounds of the invention are Class I HDAC inhibitors. In other embodiments, the compounds are Class IIa HDAC inhibitors. In still other embodiments, the compounds are Class IIb HDAC inhibitors. In certain embodiments, the compounds are Class III HDAC inhibitors. In certain embodiments, the compounds are Class IV HDAC inhibitors.

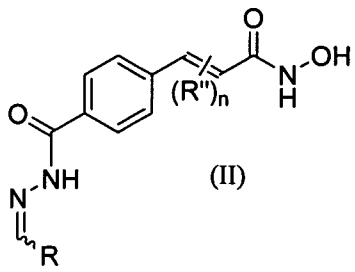
Compounds of the Invention

Compounds of this invention include those, as set forth above and described herein, and are illustrated in part by the various classes, subgenera, and species disclosed elsewhere herein. In general, the present invention provides cinnamic hydroxymates compounds having the general formula (I) or (II):



(I)

or



(II)

wherein

R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl-moiety or a substituted or unsubstituted heteroaryl moiety;

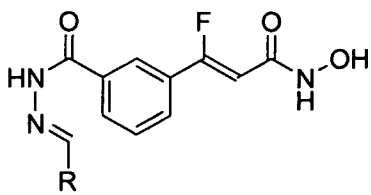
each occurrence of R″ is independently hydrogen, halogen, or C_{1-6} alkyl;

n is 0, 1, or 2; and pharmaceutically acceptable salts thereof.

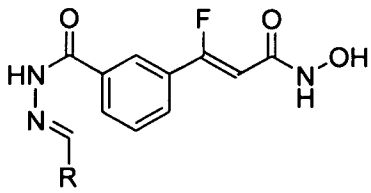
In certain embodiments, n is 0. In certain embodiments, n is 1. In certain embodiments, n is 2.

In certain embodiments, R″ is halogen. In certain embodiments, R″ is fluorine. In certain embodiments, R″ is C_{1-6} alkyl. In certain embodiments, R″ is methyl. In certain embodiments, R″ is ethyl.

In certain embodiments, the compound is of formula:



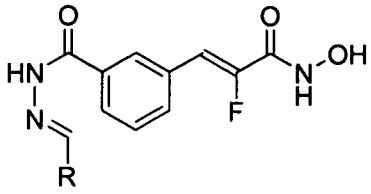
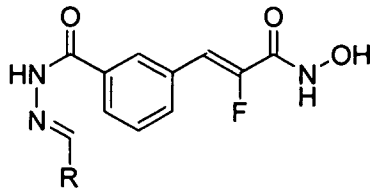
or



,

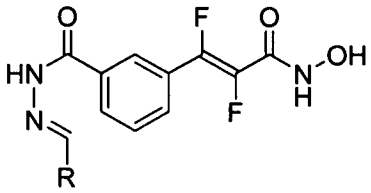
wherein R is as described herein.

In certain embodiments, the compound is of formula:


or

,

wherein R is as described herein.

In certain embodiments, the compound is of formula: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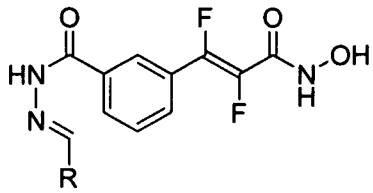
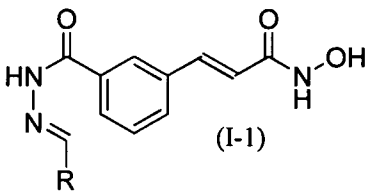
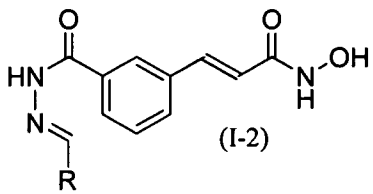

,

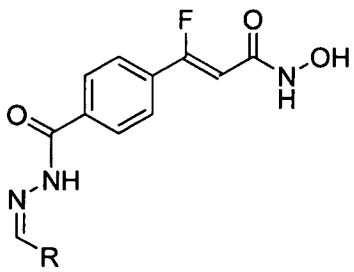
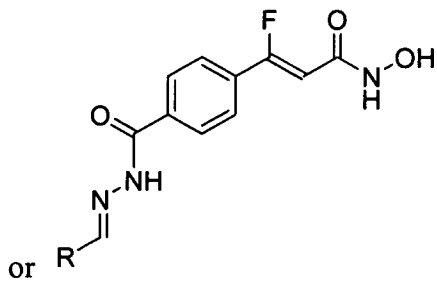
wherein R is as described herein.

In certain embodiments, the compound is of formula (I-1) or (I-2):


(I-1)
or

(I-2)
,

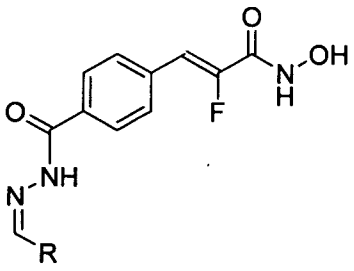
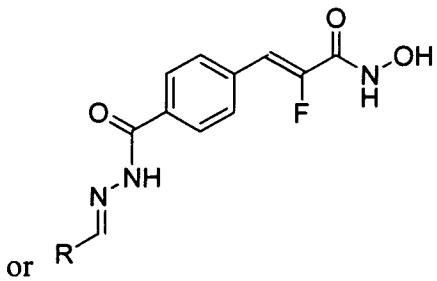
wherein R is as described herein.

In other embodiments, the compound is of formula:


or

,

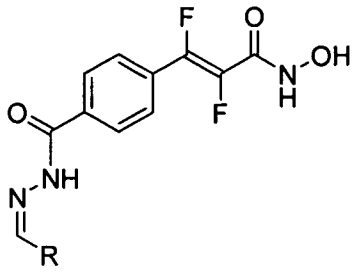
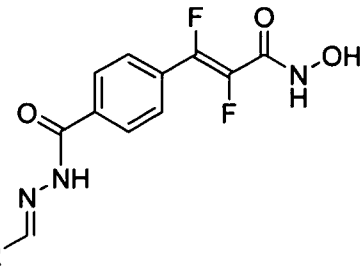
wherein R is as described herein.

In other embodiments, the compound is of formula:


or

,

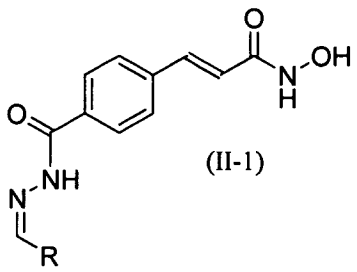
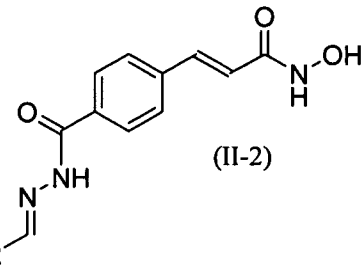
wherein R is as described herein.

In other embodiments, the compound is of formula:

wherein R is as described herein.

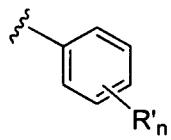
In other embodiments, the compound is of formula (II-1) or (II-2):


(II-1)

(II-2)

wherein R is as described herein.

In certain embodiments, R is unsubstituted or substituted aryl. In further embodiments, R is unsubstituted or substituted phenyl, bicyclic aryl, tricyclic aryl, or polyclic aryl.

In certain embodiments, R is of the formula:

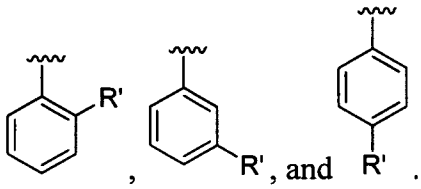


wherein

n is an integer 0-5, inclusive;

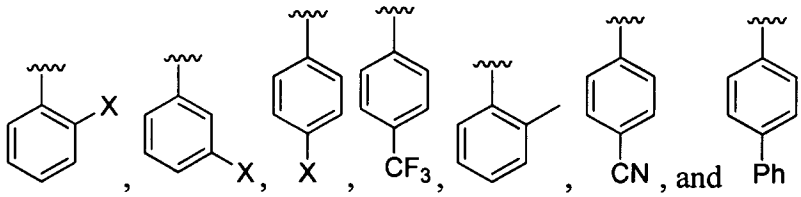
each occurrence of R' is independently hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; OR^B; $-C(=O)R^B$; $-CO_2R^B$; $-C(=O)N(R^B)_2$; -CN; -SCN; $-SR^B$; $-SOR^B$; $-SO_2R^B$; $-NO_2$; $-N(R^B)_2$; $-NHC(O)R^B$; or $-C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino; dialkylamino; heteroaryloxy; or heteroarylthioxy. In certain embodiments, n is 0. In other embodiments, n is 1, 2, 3, 4 or 5.

In certain embodiments, n is 1. In other embodiments R is selected from the group consisting of:

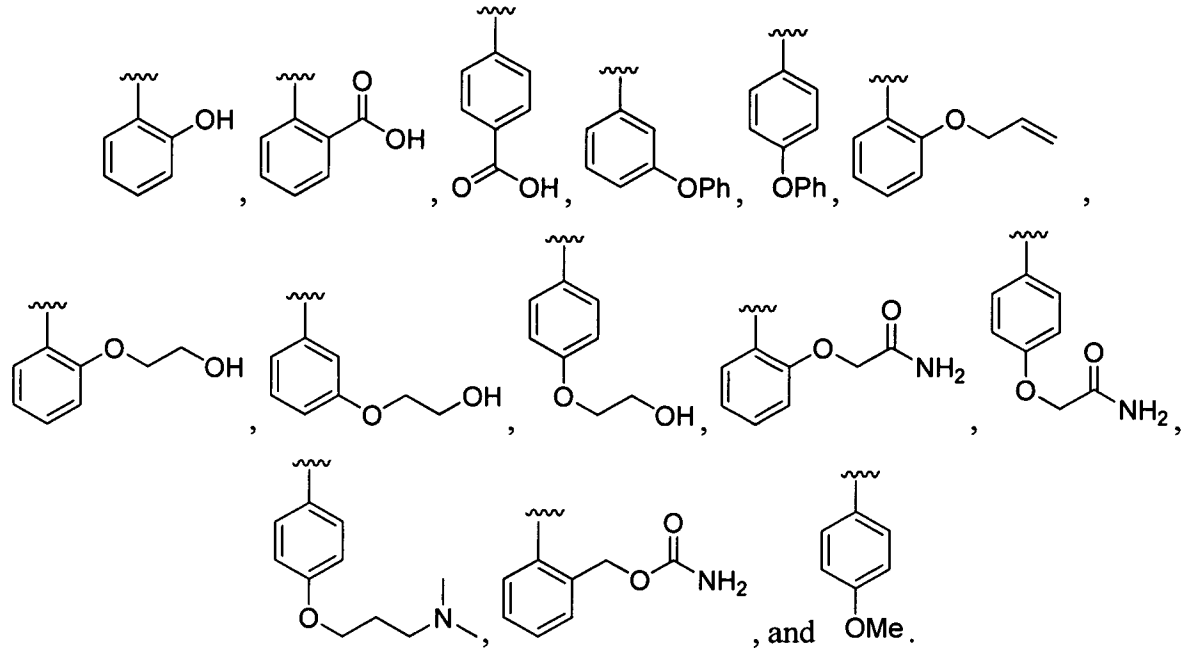


In certain embodiments, R' is halogen. In certain embodiments, R' is fluorine. In other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In still other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In further embodiments, R' is substituted or unsubstituted, branched or unbranched acyl. In certain embodiments, R' is substituted or unsubstituted, branched or unbranched aryl. In other embodiments, R' is substituted or unsubstituted, branched or unbranched heteroaryl. In still other embodiments, R' is OR^B. In further embodiments, R' is $-C(=O)R^B$. In certain embodiments, R' is $-CO_2R^B$. In other embodiments, R' is $-C(=O)N(R^B)_2$. In still other embodiments, R' is –CN. In further embodiments, R' is –SCN. In certain embodiments, R' is $-SR^B$. In other embodiments, R' is $-SOR^B$. In still other embodiments, R' is $-SO_2R^B$. In further embodiments, R' is $-NO_2$. In certain embodiments, R' is $-N(R^B)_2$. In other embodiments, R' is $-NHC(O)R^B$. In still other embodiments, R' is or $-C(R^B)_3$. In other embodiments, R' is hydroxyl.

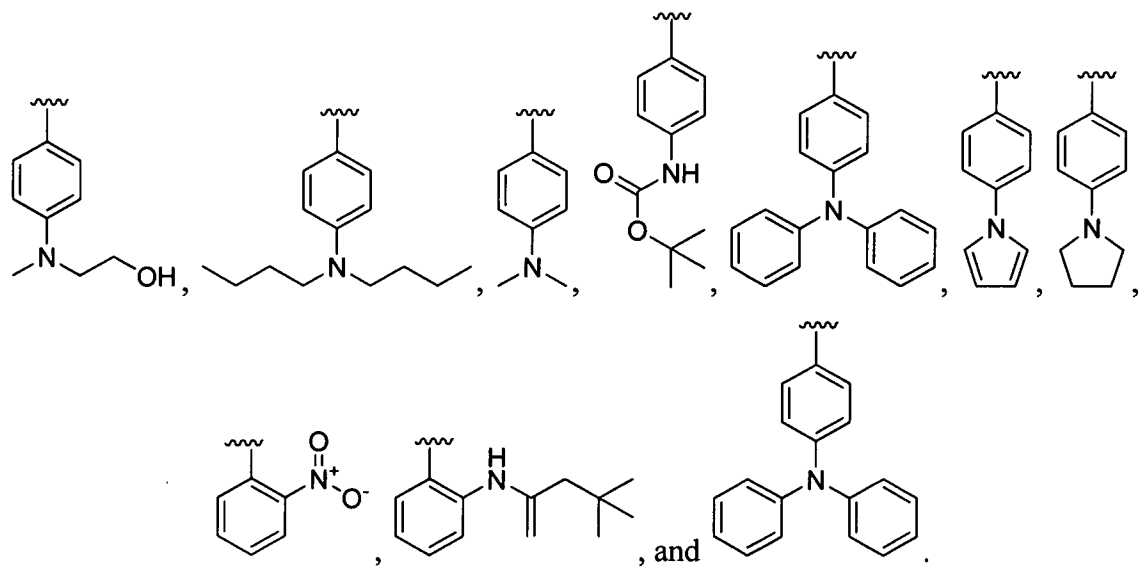
In certain embodiments R is selected from the group consisting of:



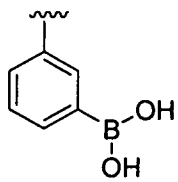
wherein X is halogen. In other embodiments, R is selected from a group consisting of:



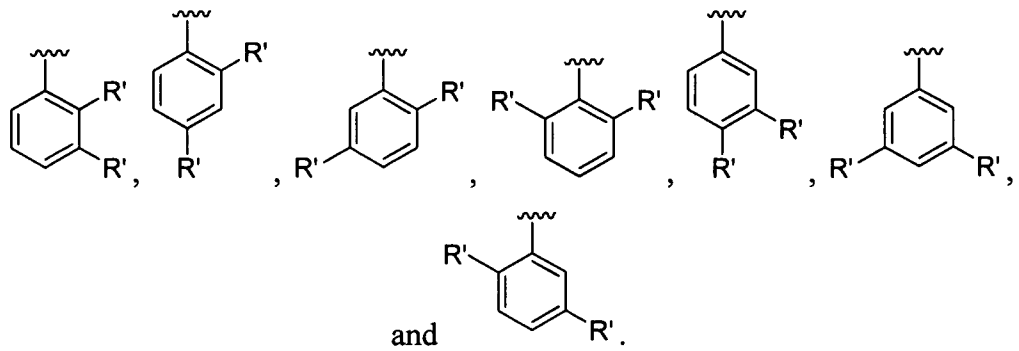
In further embodiments, R is selected from a group consisting of:



In certain embodiments, R is of the formula:



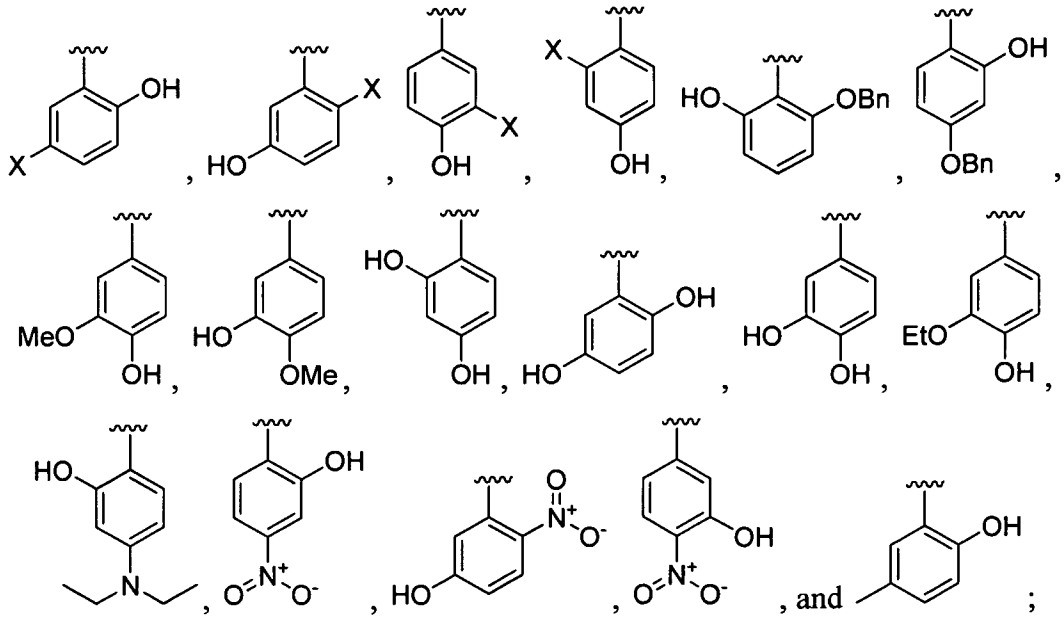
In other embodiments, n is 2 . In certain embodiments, R is selected from a group consisting of:



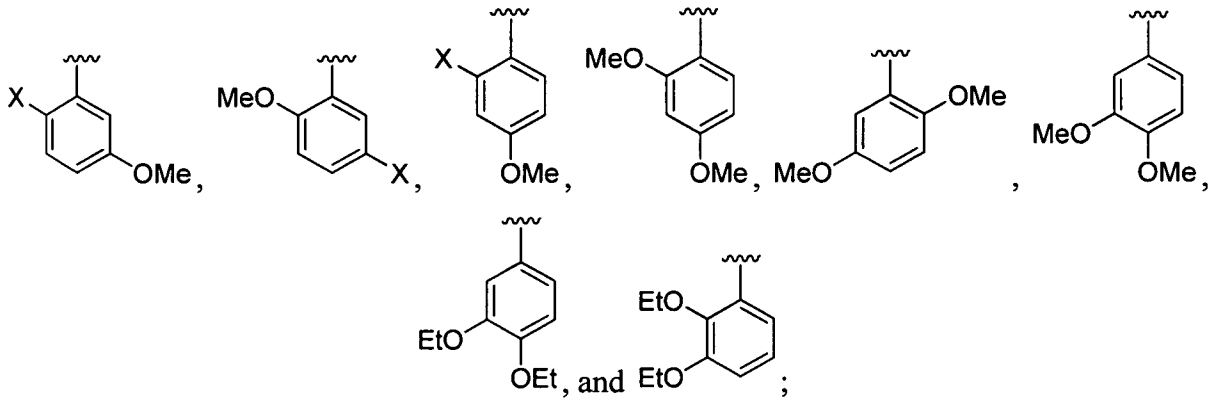
In certain embodiments, the R' groups are the same. In other embodiments, the R' groups are different. In further embodiments, two R' groups are taken together to form a ring. In certain embodiments, two R' groups are taken together to form a carbocyclic ring. In other embodiments, two R' groups are taken together to form a heterocyclic ring. In further embodiments, two R' groups are taken together to form an aromatic ring. In certain embodiments, two R' groups are taken together to form an aryl ring. In other embodiments, two R' groups are taken together to form a heteroaryl ring.

In certain embodiments, R' is halogen. In certain embodiments, R' is fluorine. In other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In still other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In further embodiments, R' is substituted or unsubstituted, branched or unbranched acyl. In certain embodiments, R' is substituted or unsubstituted, branched or unbranched aryl. In other embodiments, R' is substituted or unsubstituted, branched or unbranched heteroaryl. In still other embodiments, R' is $-OR^B$. In further embodiments, R' is $-C(=O)R^B$. In certain embodiments, R' is $-CO_2R^B$. In other embodiments, R' is $-C(=O)N(R^B)_2$. In still other embodiments, R' is –CN. In further embodiments, R' is –SCN. In certain embodiments, R' is $-SR^B$. In other embodiments, R' is $-SOR^B$. In still other embodiments, R' is $-SO_2R^B$. In further embodiments, R' is $-NO_2$. In certain embodiments, R' is $-N(R^B)_2$. In other embodiments, R' is $-NHC(O)R^B$. In still other embodiments, R' is or $-C(R^B)_3$. In further embodiments, R' is hydroxyl.

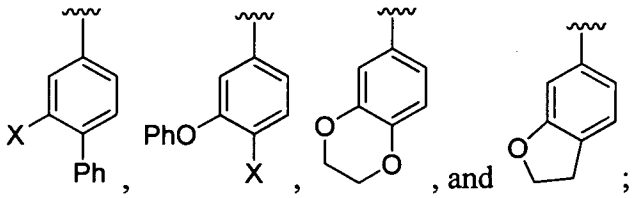
In certain embodiments, R is selected from the group consisting of:



wherein X is halogen. In other embodiments, R is selected from the group consisting of:

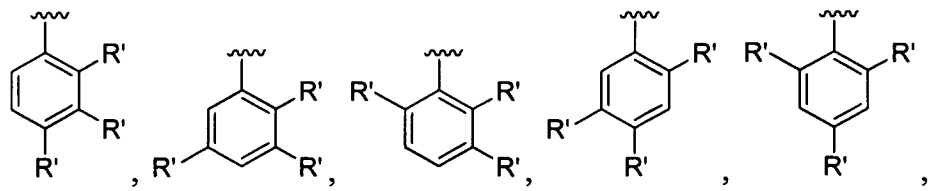


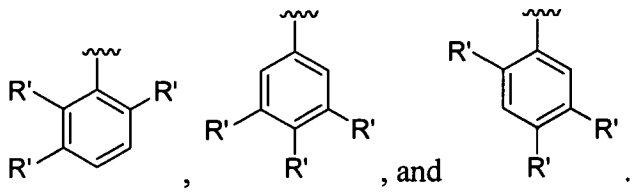
wherein X is halogen. In still other embodiments, R is selected from the group consisting of:



wherein X is halogen.

In other embodiments, n is 3. In certain embodiments, R is selected from the group consisting of:

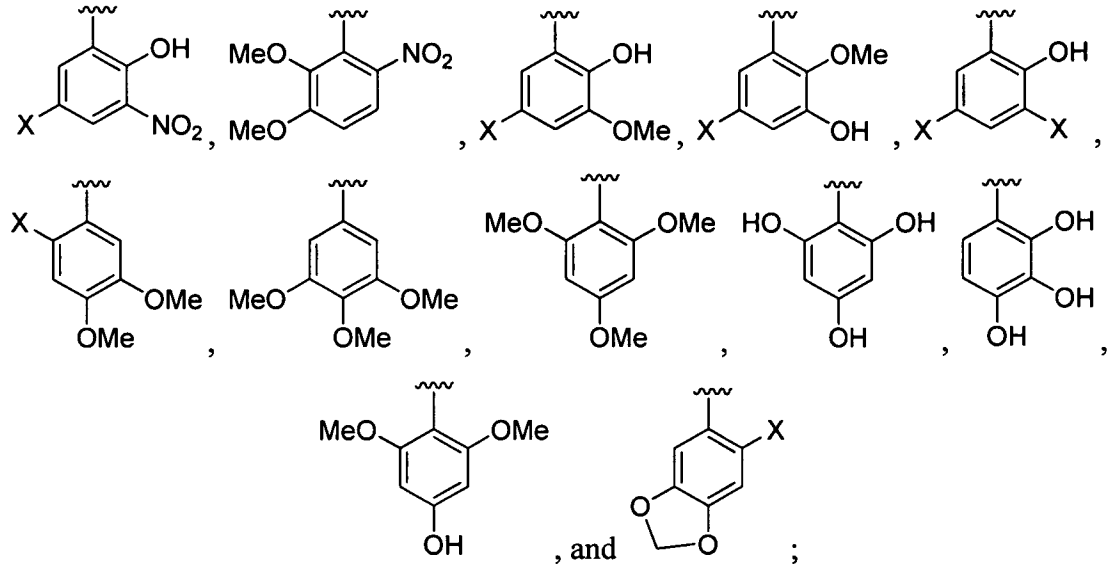




In certain embodiments, the R' groups are the same. In other embodiments, the R' groups are different. In further embodiments, two R' groups are taken together to form a cyclic structure. In certain embodiments, two R' groups are taken together to form a carbocyclic ring. In other embodiments, two R' groups are taken together to form a heterocyclic ring. In further embodiments, two R' groups are taken together to form an aromatic ring. In certain embodiments, two R' groups are taken together to form an aryl ring. In other embodiments, two R' groups are taken together to form a heteroaryl ring.

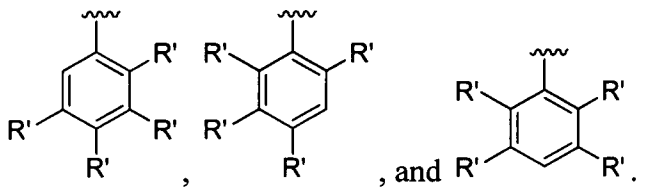
In certain embodiments, R' is halogen. In certain embodiments, R' is fluorine. In other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In still other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In further embodiments, R' is substituted or unsubstituted, branched or unbranched acyl. In certain embodiments, R' is substituted or unsubstituted, branched or unbranched aryl. In other embodiments, R' is substituted or unsubstituted, branched or unbranched heteroaryl. In still other embodiments, R' is $-OR^B$. In further embodiments, R' is $-C(=O)R^B$. In certain embodiments, R' is $-CO_2R^B$. In other embodiments, R' is $-C(=O)N(R^B)_2$. In still other embodiments, R' is –CN. In further embodiments, R' is –SCN. In certain embodiments, R' is $-SR^B$. In other embodiments, R' is $-SOR^B$. In still other embodiments, R' is $-SO_2R^B$. In further embodiments, R' is $-NO_2$. In certain embodiments, R' is $-N(R^B)_2$. In other embodiments, R' is $-NHC(O)R^B$. In still other embodiments, R' is or $-C(R^B)_3$. In further embodiments, R' is hydroxyl.

In certain embodiments, R is selected from the group consisting of:



wherein X is halogen.

In other embodiments n is 4. In certain embodiments, R is selected from the group consisting of:

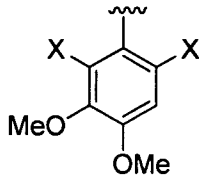


In certain embodiments, the R' groups are the same. In other embodiments, the R' groups are different. In further embodiments, two R' groups are taken together to form a ring. In certain embodiments, two R' groups are taken together to form a carbocyclic ring. In other embodiments, two R' groups are taken together to form a heterocyclic ring. In further embodiments, two R' groups are taken together to form an aromatic ring. In certain embodiments, two R' groups are taken together to form n aryl ring. In other embodiments, two R' groups are taken together to form heteroaryl ring.

In certain embodiments, R' is halogen. In certain embodiments, R' is fluorine. In other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In still other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In further embodiments, R' is substituted or unsubstituted, branched or unbranched acyl. In certain embodiments, R' is substituted or unsubstituted, branched or unbranched aryl. In other embodiments, R' is substituted or unsubstituted, branched or unbranched heteroaryl. In still other embodiments, R' is $-OR^B$. In further embodiments, R' is $-C(=O)R^B$. In certain embodiments, R' is $-CO_2R^B$. In other embodiments, R' is $-C(=O)N(R^B)_2$. In still other embodiments, R' is –CN. In further

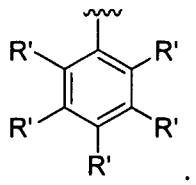
embodiments, R' is –SCN. In certain embodiments, R' is -SR^B. In other embodiments, R' is -SOR^B. In still other embodiments, R' is -SO_2R^B. In further embodiments, R' is -NO_2. In certain embodiments, R' is -$N(R^B)_2$. In other embodiments, R' is -$NHC(O)R^B$. In still other embodiments, R' is or –$C(R^B)_3$. In further embodiments, R' is hydroxyl.

In certain embodiments, R is of the formula:



wherein X is halogen.

In other embodiments n is 5. In certain embodiments, R is of the formula:



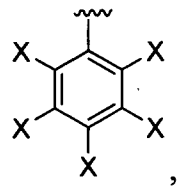
.

In certain embodiments, the R' groups are the same. In other embodiments, the R' groups are different. In further embodiments, two R' groups are taken together to form a cyclic structure. In certain embodiments, two R' groups are taken together to form a carbocyclic ring. In other embodiments, two R' groups are taken together to form a heterocyclic ring. In further embodiments, two R' groups are taken together to form an aromatic ring. In certain embodiments, two R' groups are taken together to form an aryl ring. In other embodiments, two R' groups are taken together to form a heteroaryl ring.

In certain embodiments, R' is halogen. In certain embodiments, R' is fluorine. In other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In still other embodiments, R' is cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic. In further embodiments, R' is substituted or unsubstituted, branched or unbranched acyl. In certain embodiments, R' is substituted or unsubstituted, branched or unbranched aryl. In other embodiments, R' is substituted or unsubstituted, branched or unbranched heteroaryl. In still other embodiments, R' is -OR^B. In further embodiments, R' is -$C(=O)R^B$. In certain embodiments, R' is -CO_2R^B. In other embodiments, R' is -$C(=O)N(R^B)_2$. In still other embodiments, R' is –CN. In further embodiments, R' is –SCN. In certain embodiments, R' is -SR^B. In other embodiments, R' is -SOR^B. In still other embodiments, R' is -SO_2R^B. In further embodiments, R' is -NO_2. In

certain embodiments, R' is $-N(R^B)_2$. In other embodiments, R' is $-NHC(O)R^B$. In still other embodiments, R' is or $-C(R^B)_3$. In further embodiments, R' is hydroxyl.

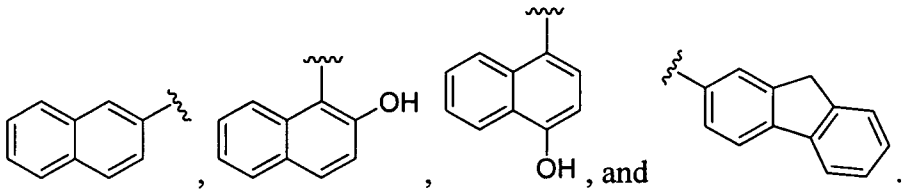
In other embodiments, R is of the formula:



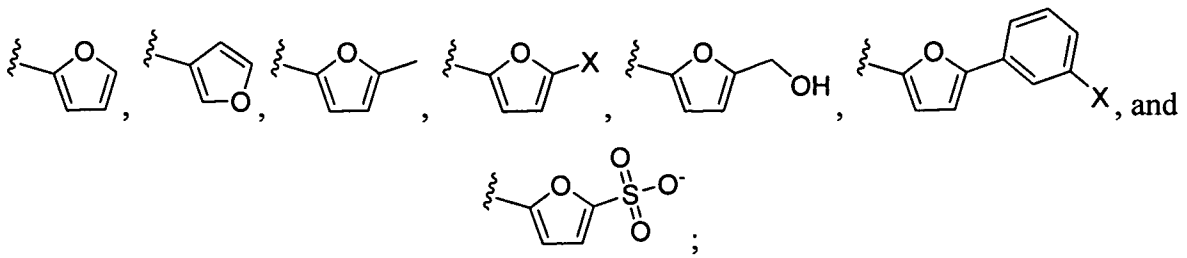
wherein X is halogen.

In certain embodiments, R is a monocyclic substituted or unsubstituted aryl moiety. In other embodiments, R is a bicyclic substituted or unsubstituted aryl moiety. In still other embodiments, R is a polycyclic substituted or unsubstituted aryl moiety. In certain embodiments, R is a polycyclic substituted or unsubstituted aryl moiety. In further embodiments, R is substituted or unsubstituted phenyl, naphthyl, tetrahydronaphthyl, indanyl, or indenyl moiety. In certain embodiments, R is substituted phenyl.

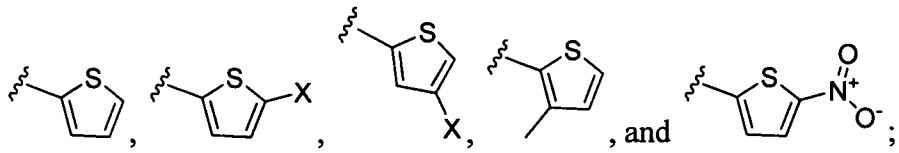
In certain embodiment, R is selected from the group consisting of:



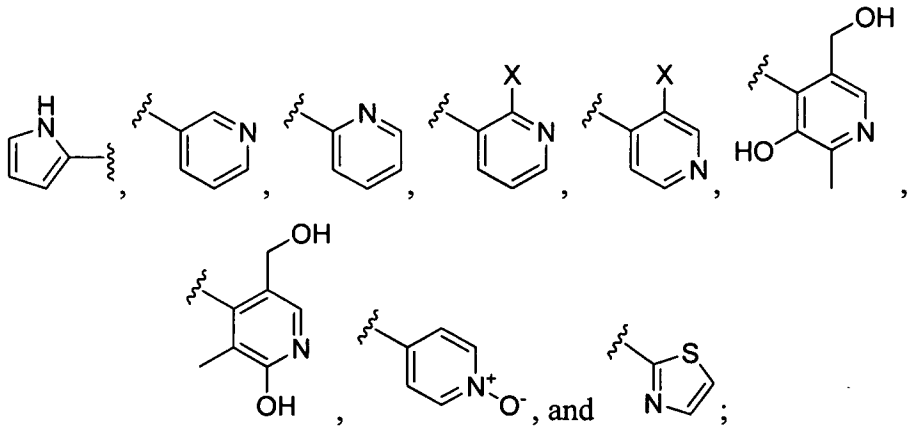
In certain embodiments, R is a monocyclic substituted or unsubstituted heteroaryl moiety. In certain embodiments, R is a substituted or unsubstituted furanyl, thiofuranyl, pyranyl, pyrrolyl, thienyl, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolyl, oxazolidinyl, isooxazolyI, isoxazolidinyl, dioxazolyl, thiadiazolyl, oxadiazolyl, tetrazolyl, triazolyl, thiatriazolyl, oxatriazolyl, thiadiazolyl, oxadiazolyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, dithiazolyl, dithiazolidinyl, tetrahydrofuryl, and benzofused derivatives thereof. In other embodiments, R is a substituted or unsubstituted heteroaryl moiety. In certain embodiments, R is a substituted or unsubstituted furanyl moiety. In certain embodiments, R is a substituted or unsubstituted thiophenyl moiety. In certain embodiments, R is a substituted or unsubstituted pyridinyl moiety. In yet other embodiments, R is selected from the group consisting of:



wherein X is halogen. In still other embodiments, R is selected from the group consisting of:

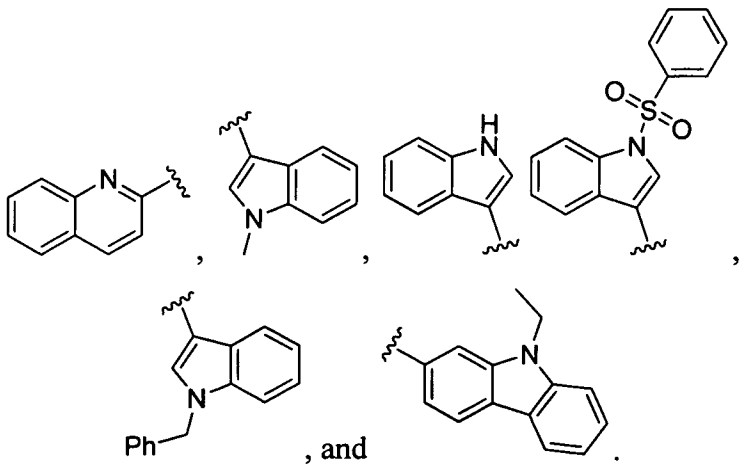


wherein X is halogen. In further embodiments, R is selected from the group consisting of:

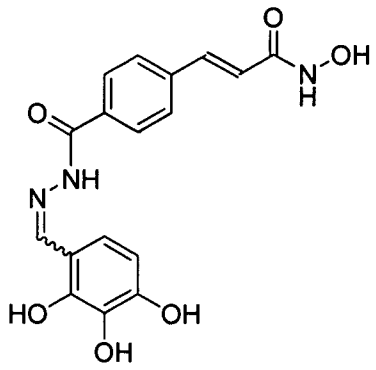


wherein X is halogen.

In other embodiments, R is a bicyclic substituted or unsubstituted heteroaryl moiety. In still other embodiments, R is a polycyclic substituted or unsubstituted heteroaryl moiety. In certain embodiments, R is a polycyclic substituted or unsubstituted heteroaryl moiety. In further embodiments, R is selected from the group consisting of:



In a specific embodiment, the compound is

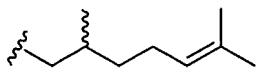


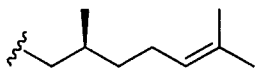
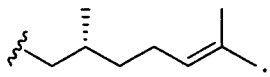
(Pandacostat).

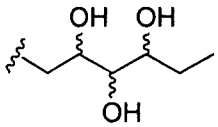
In certain embodiments, R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety. In other embodiments, R is a cyclic or acyclic, substituted or unsubstituted C_{1-12} alkyl.

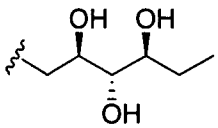
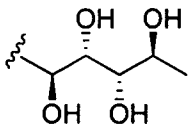
In yet other embodiments, R is a cyclic or acyclic, substituted or unsubstituted C_{1-6} alkyl. In still other embodiments, R is methyl, ethyl, propyl, butyl, pentyl, or hexyl. In certain embodiments R is an aliphatic chain containing at least one stereocenter. In other embodiments, R is a heteroaliphatic chain containing at least one stereocenter.

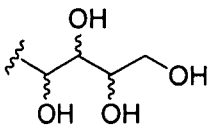
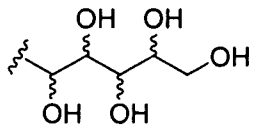
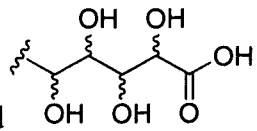
In certain embodiments, R is a substituted or unsubstituted, branched or unbranched alkenyl.

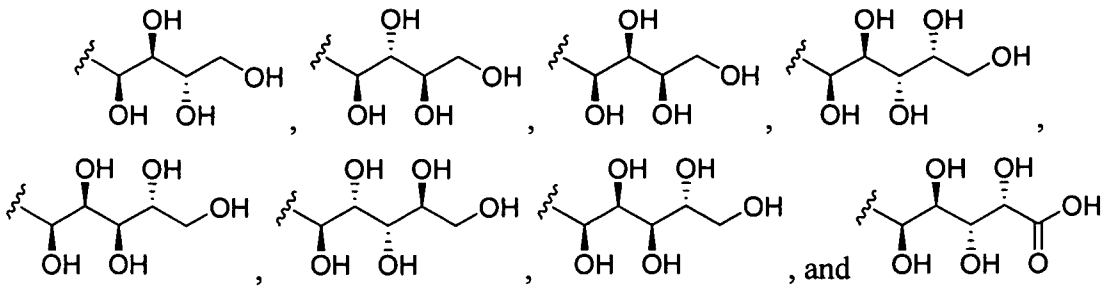
In certain embodiments, R is  . In other embodiments, R is selected from a group consisting of:

 , and  .

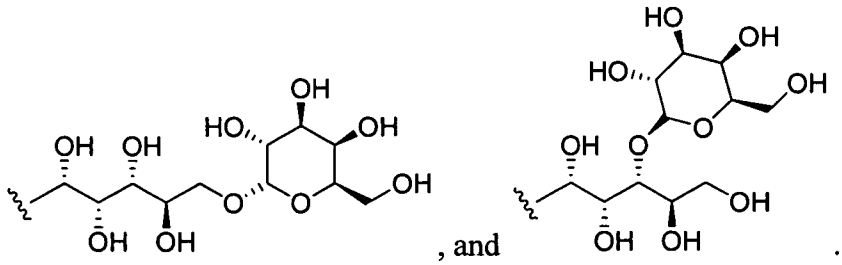
In certain embodiments, R is a hydroxyl substituted alkyl. In other embodiments, R is  . In still other embodiments, R is selected from the group consisting of:

 , and  . In certain embodiments R is selected from the group consisting of:

 ,  , and  . In other embodiments R is selected from the group consisting of:

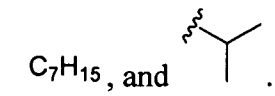


In certain embodiments R comprises glucose. In other embodiments, R is selected from a group consisting of:

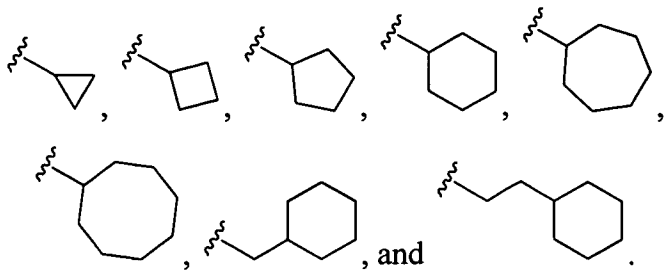


In certain embodiments, R is an aliphatic alkyl. In other embodiments, R is a unsubstituted C_{1-12} alkyl. In still other embodiments, R is a substituted C_{1-12} alkyl. In other embodiments, R is a unsubstituted C_{1-6} alkyl. In still other embodiments, R is a substituted C_{1-6} alkyl. In other embodiments, R is a branched C_{1-12} alkyl. In still other embodiments, R is a unbranched C_{1-12} alkyl. In other embodiments, R is a branched C_{1-6} alkyl . In still other embodiments, R is a unbranched C_{1-6} alkyl. In certain embodiments, R is methyl. In certain embodiments, R is ethyl. In certain embodiments, R is n-propyl. In certain embodiments, R is iso-propyl. In certain embodiments, R is iso-butyl. In certain embodiments, R is n-butyl. In certain embodiments, R is tert-butyl. In certain embodiments, R is pentyl. In certain embodiments, R is hexyl. In certain embodiments, R is heptyl. In certain embodiments, R is octyl. In certain embodiments, R is nonyl. In certain embodiments, R is decyl. In certain embodiments, R is undecyl. In certain embodiments, R is dodecyl.

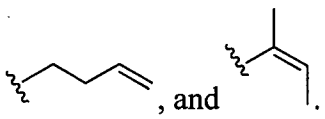
In certain embodiments, R is selected from the group consisting of:



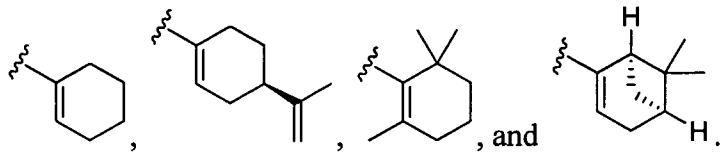
In certain embodiments, R is a substituted or unsubstituted cyclic alkyl. In other embodiments, R is a substituted or unsubstituted carbocyclic alkyl. In other embodiments, R is a substituted or unsubstituted C_{3-20} carbocyclic alkyl. In still other embodiments, R is a substituted or unsubstituted C_{3-20} carbocyclic alkyl. In certain embodiments, R is selected from the group consisting of:



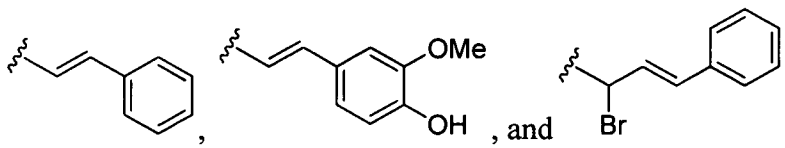
In certain embodiments, R is a substituted or unsubstituted, branched or unbranched alkylenyl. In other embodiments, R is selected from the group consisting of:



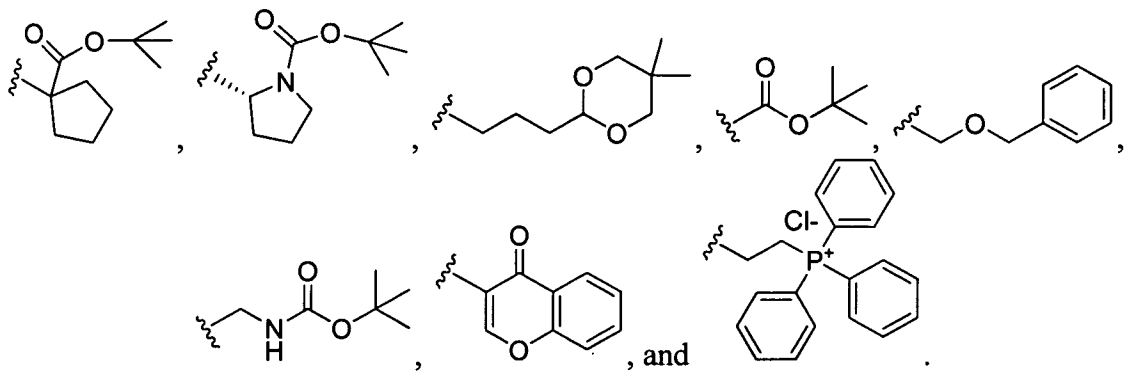
In yet other embodiments, R is selected from the group consisting of:



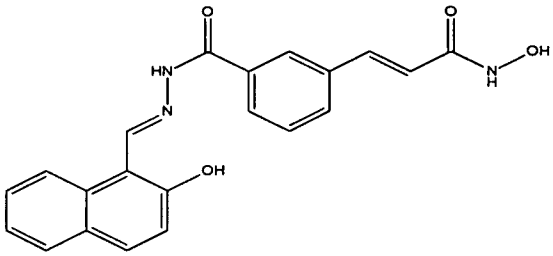
In still other embodiments, R is selected from the group consisting of:

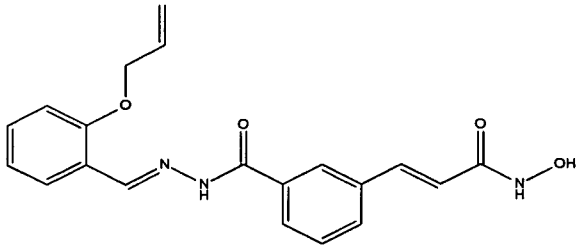


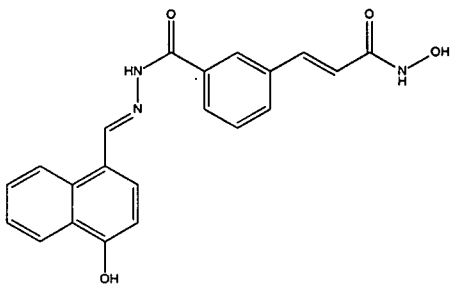
In certain embodiments, R is selected from the group consisting of:

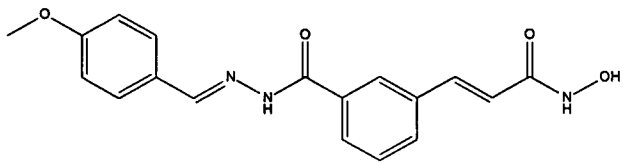


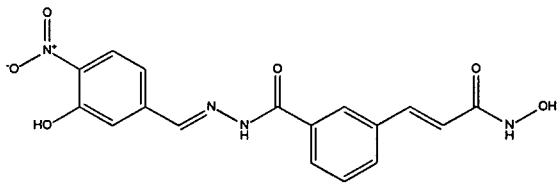
In specific embodiments, the compound is of one of forumulae:

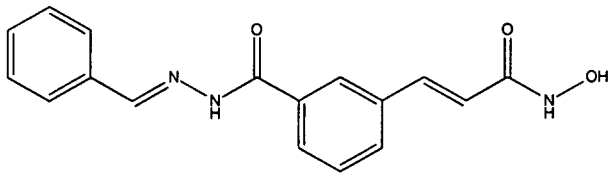


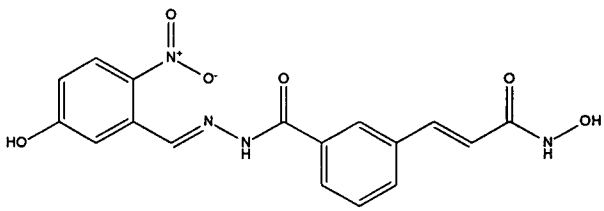


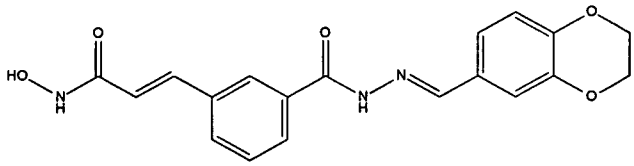


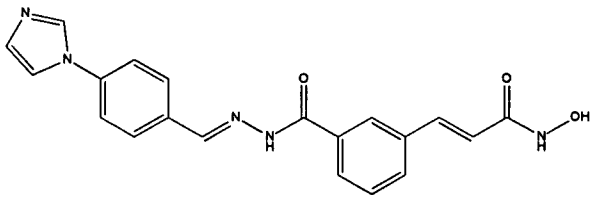


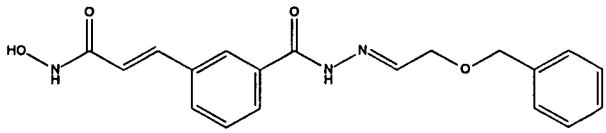


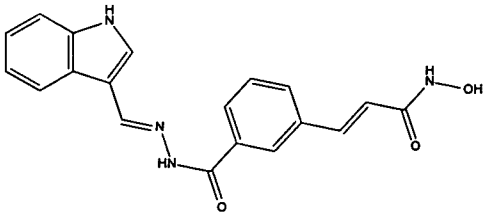


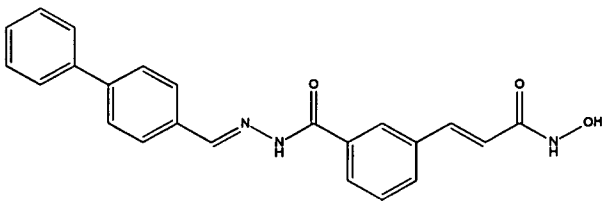


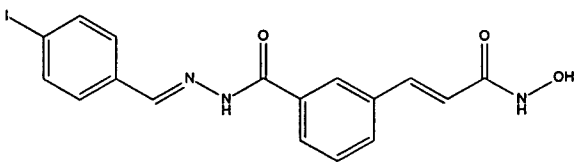


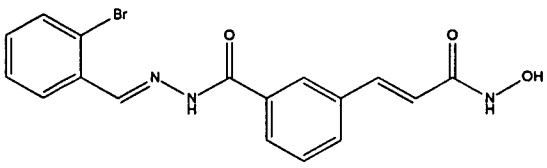


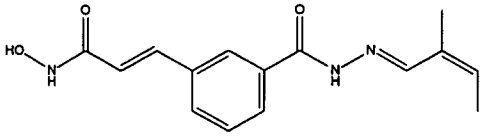


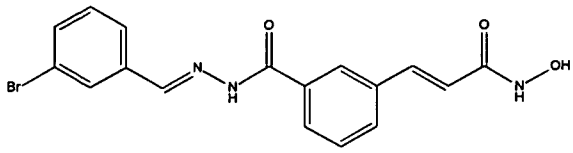


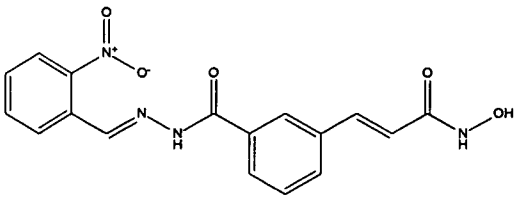


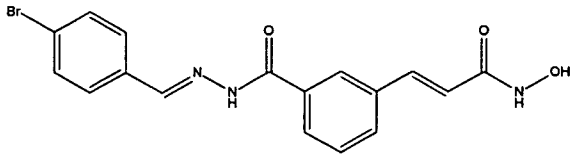


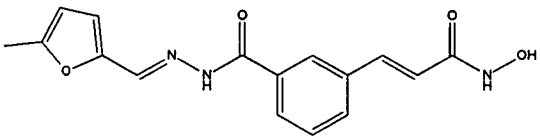


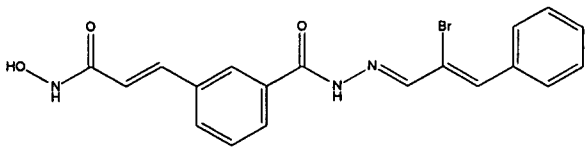


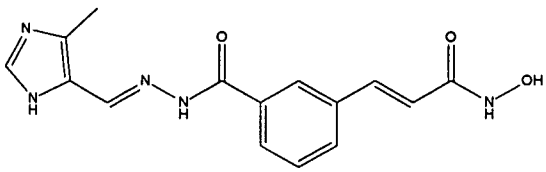


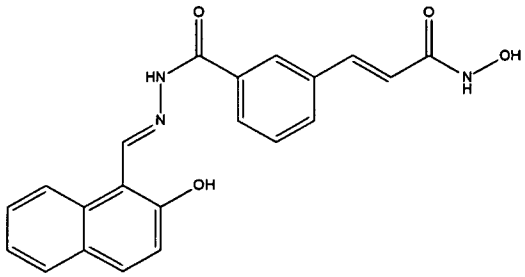


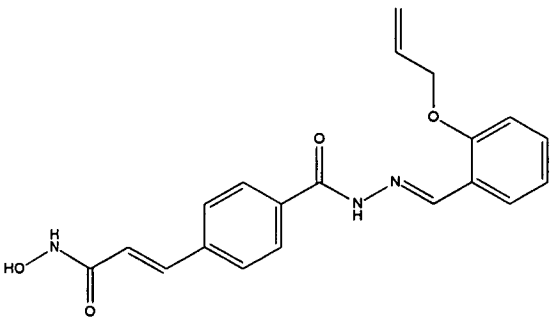


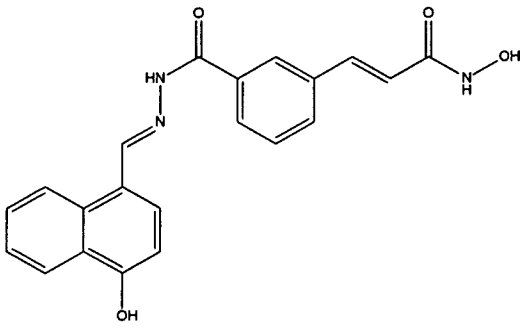


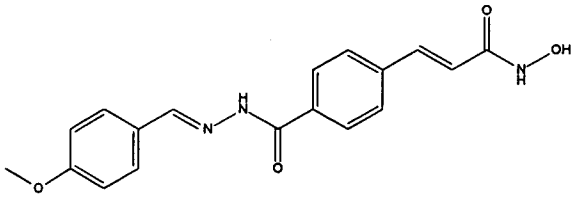


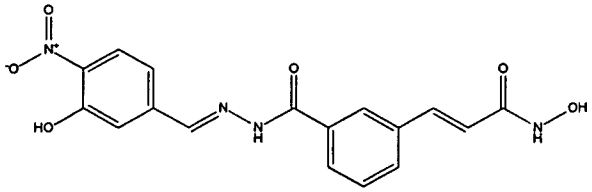


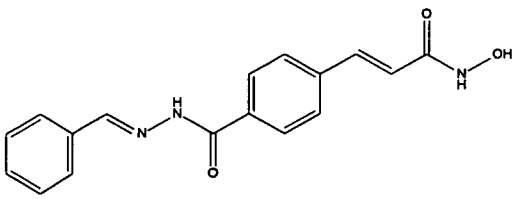


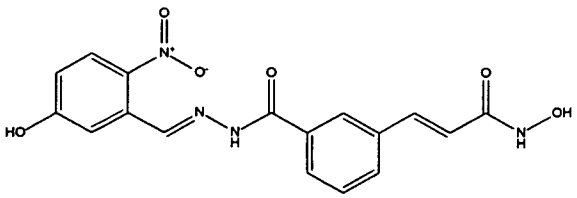


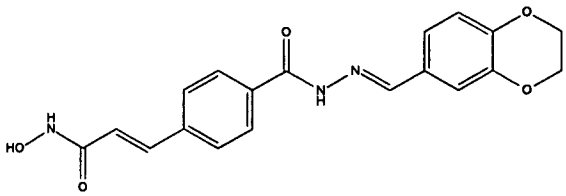


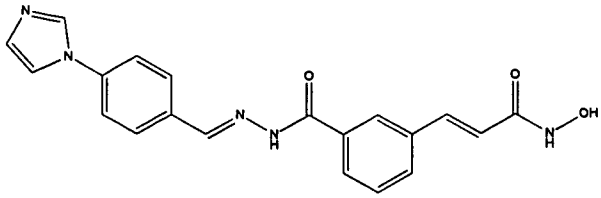


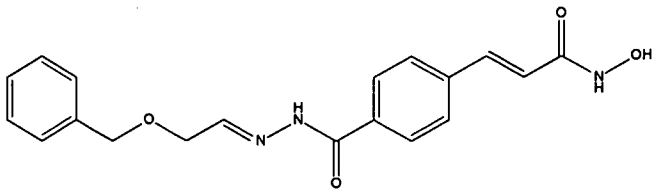


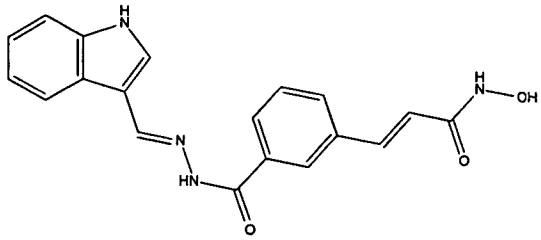


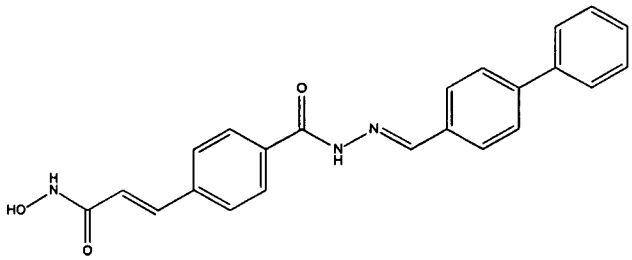


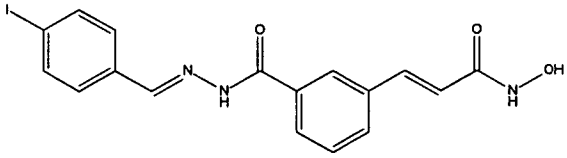


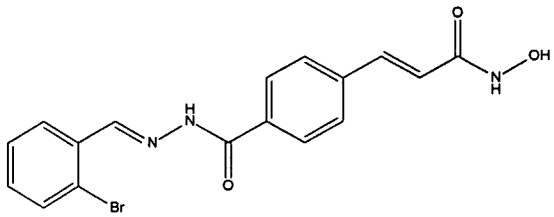


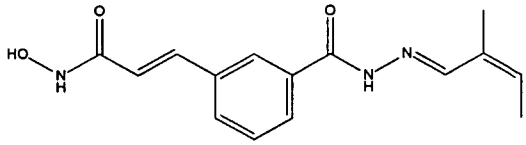


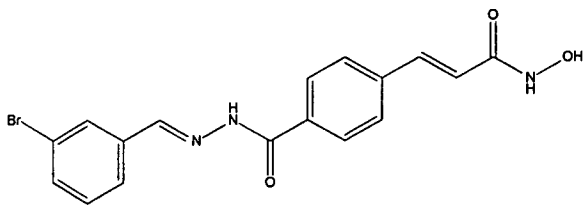


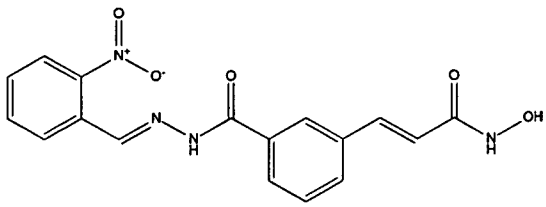


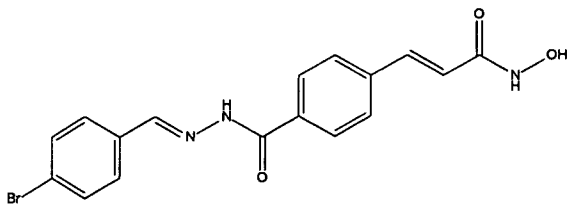


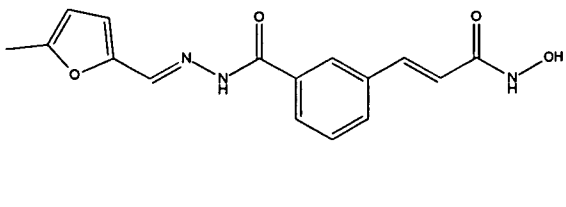


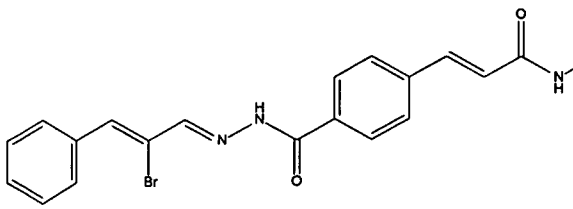


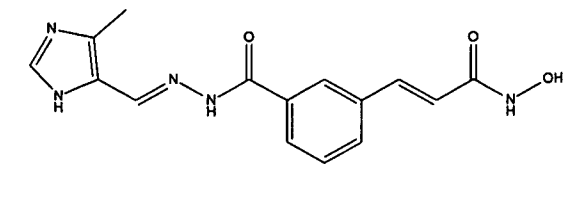


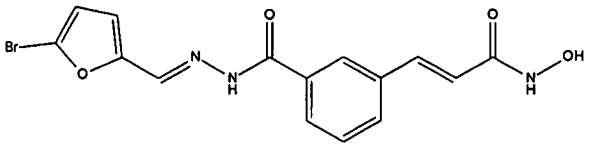


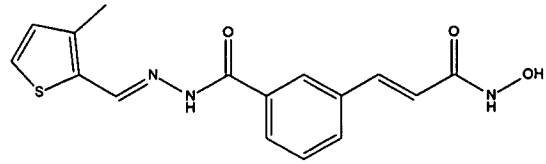


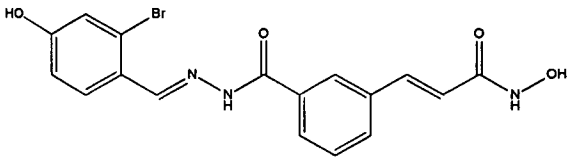


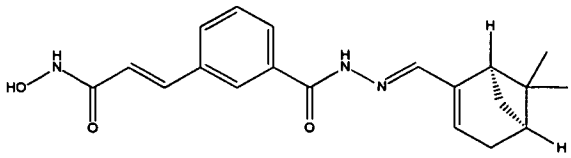


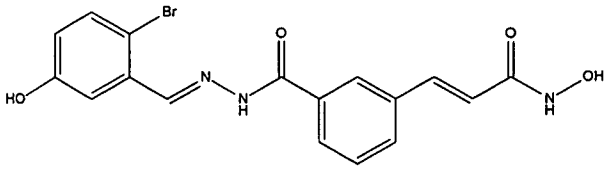


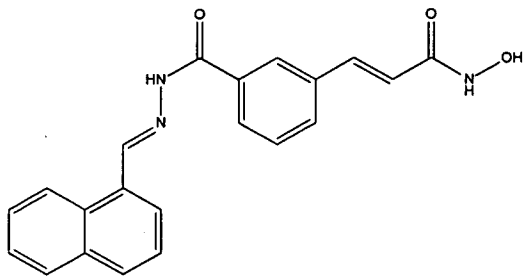


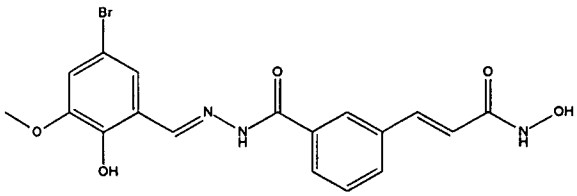


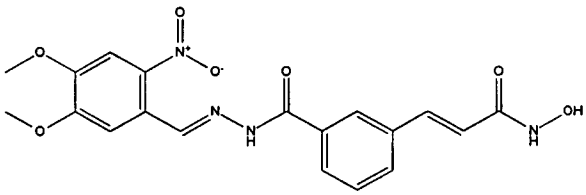


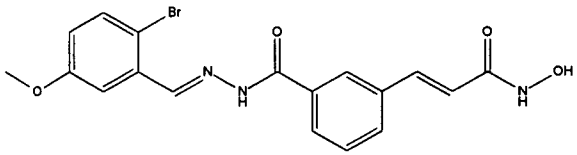


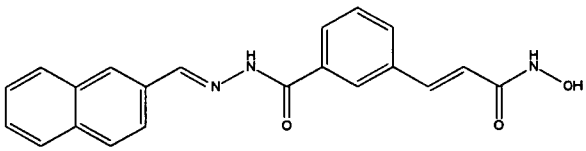


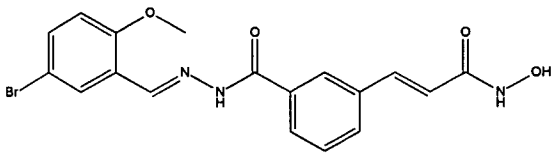


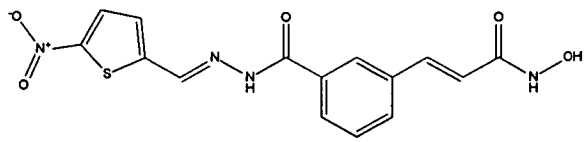


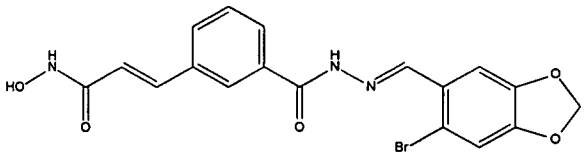


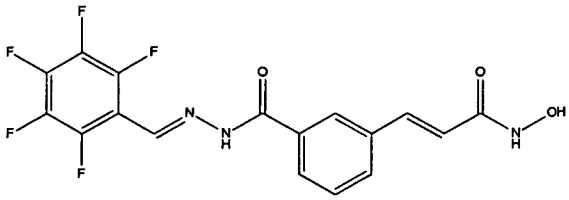


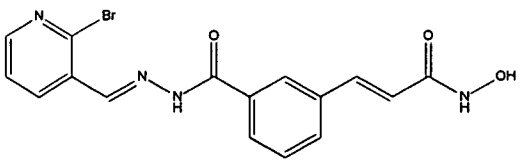


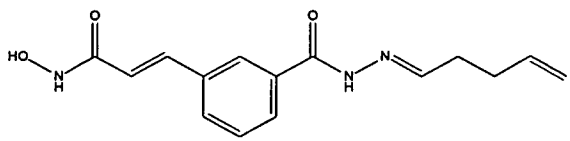


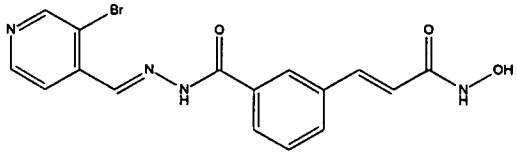


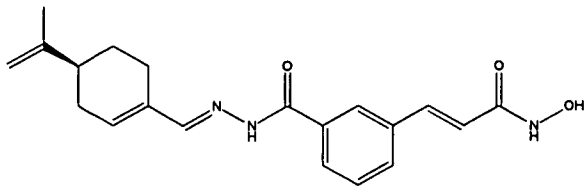


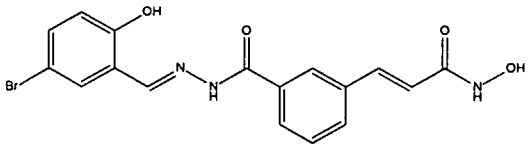


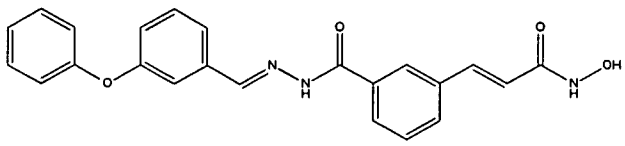


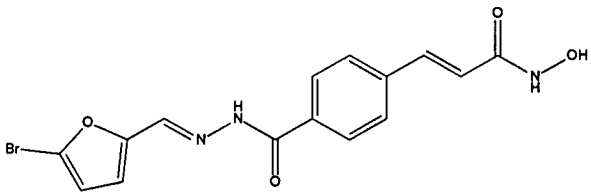


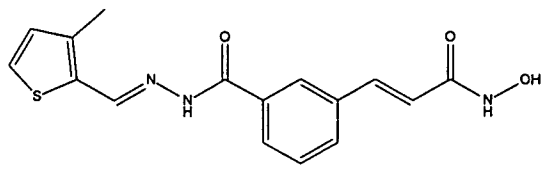


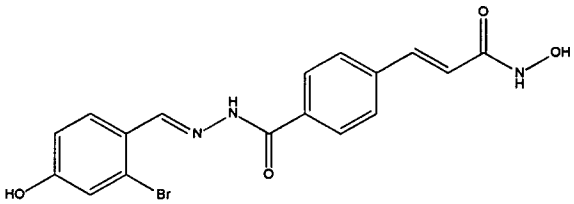


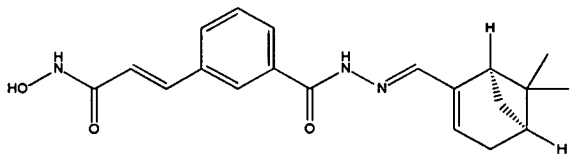


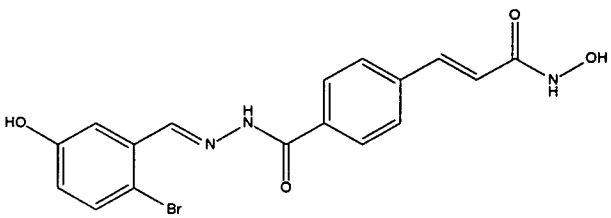


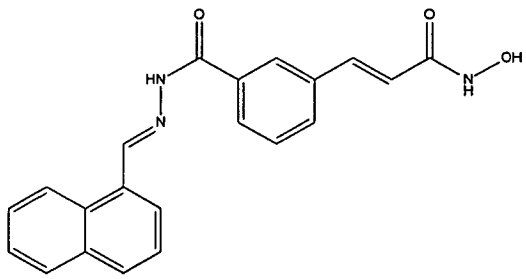


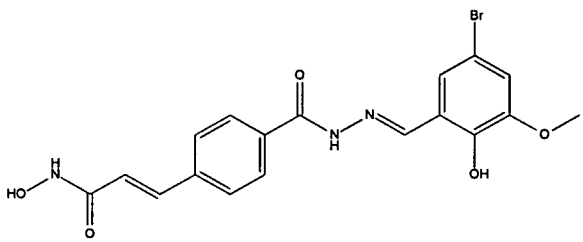


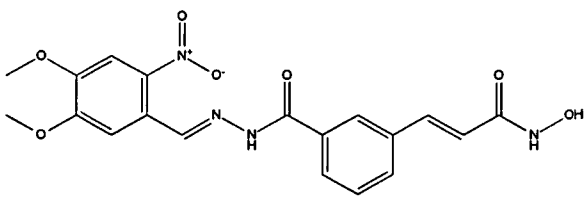


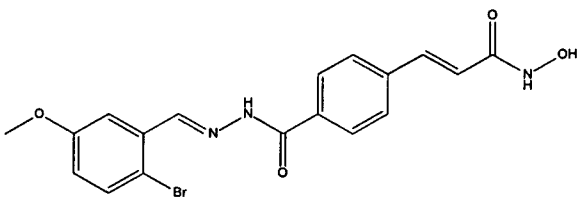


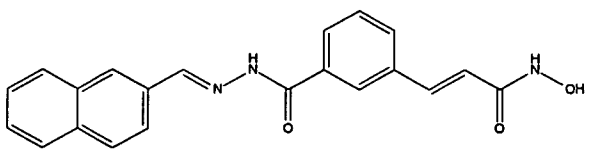


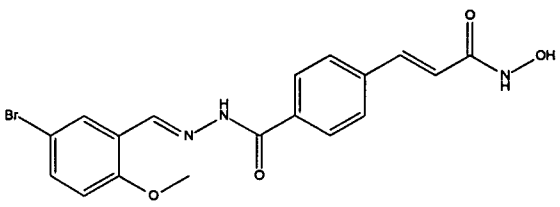


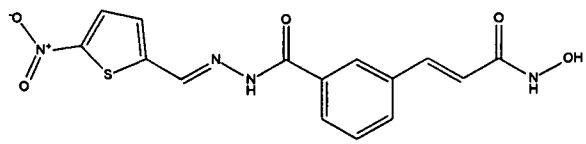


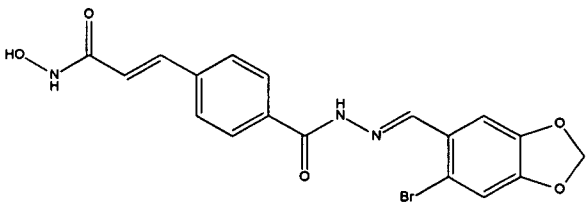


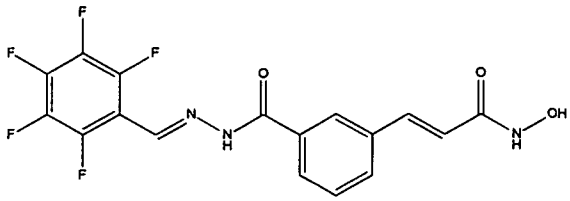


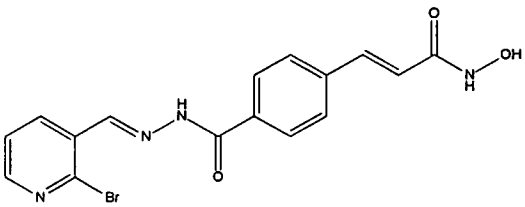


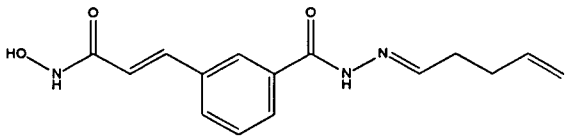


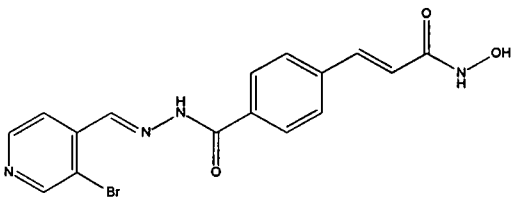


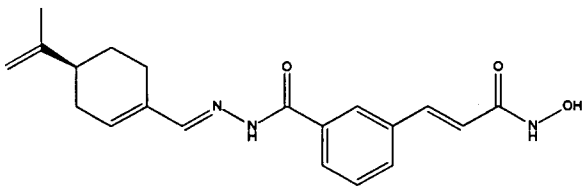


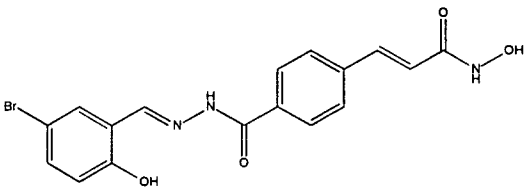


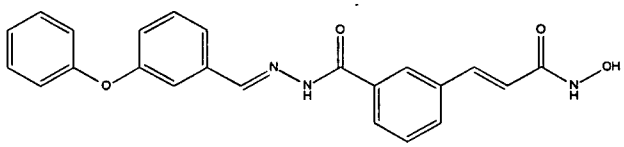


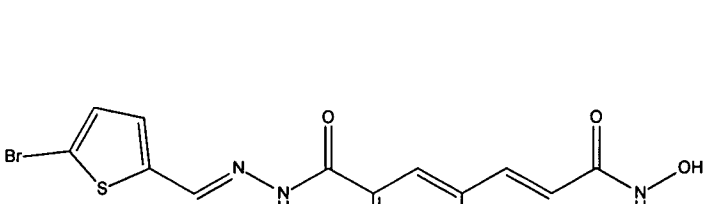


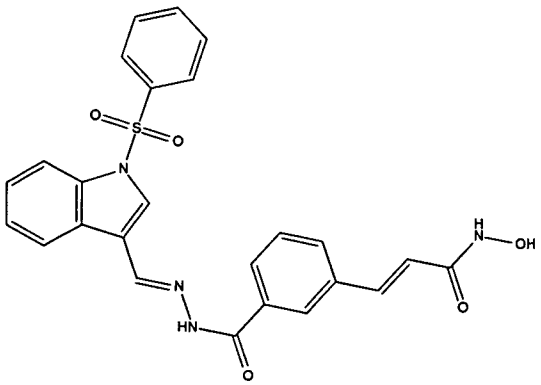


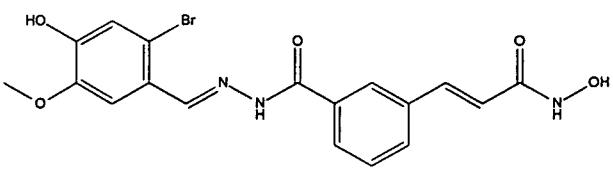


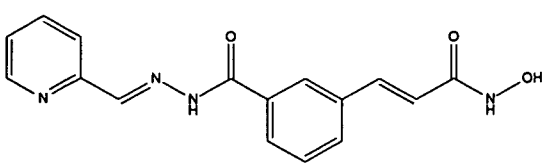


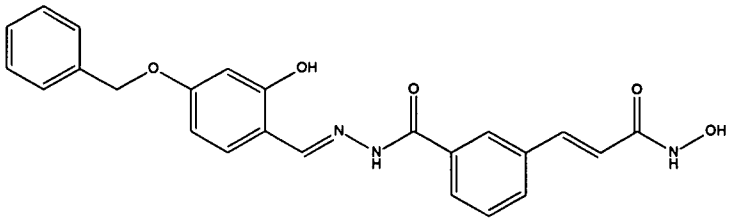


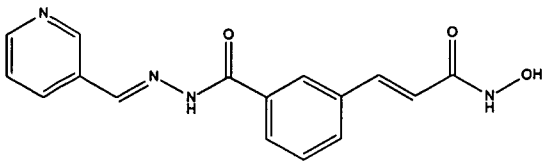


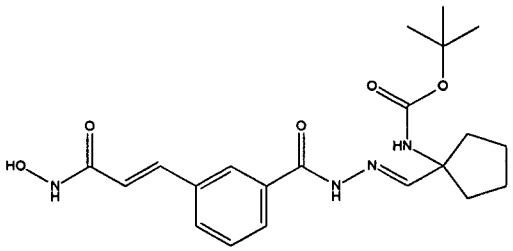


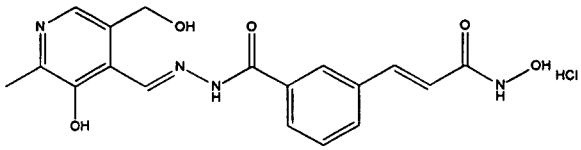


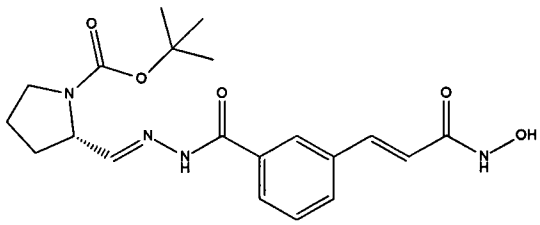


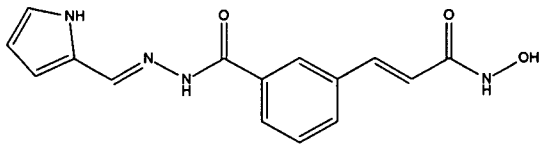


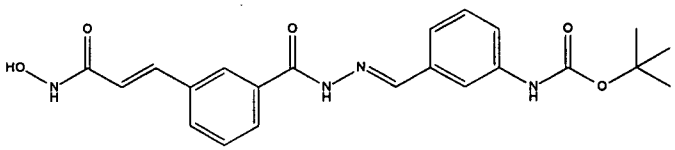


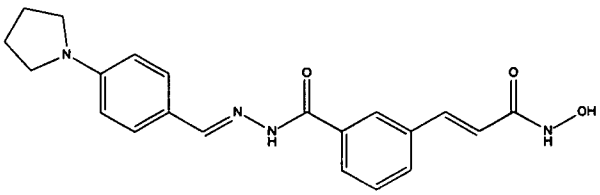


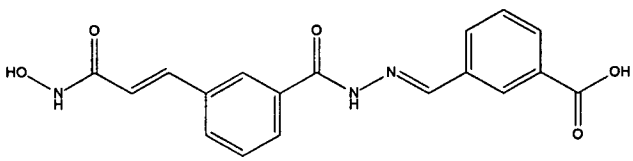


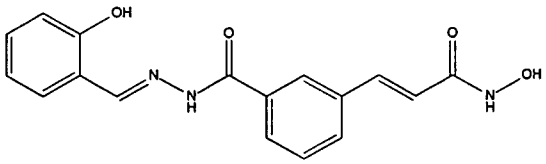


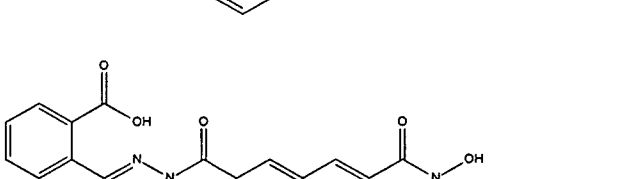


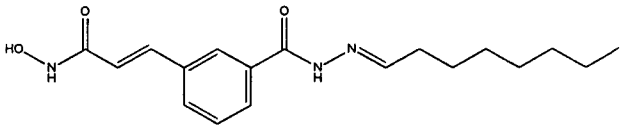


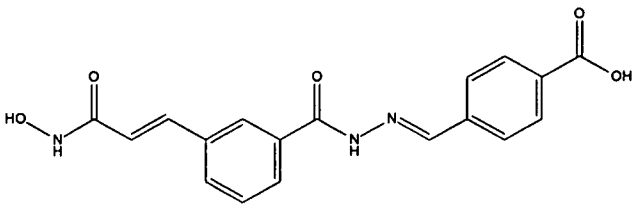


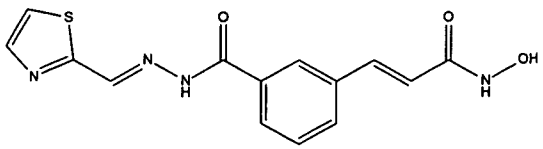












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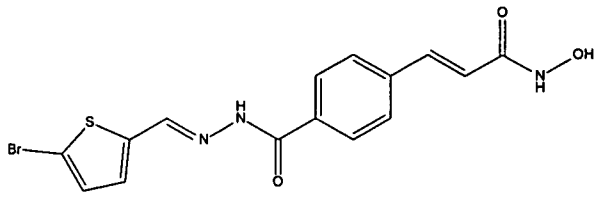
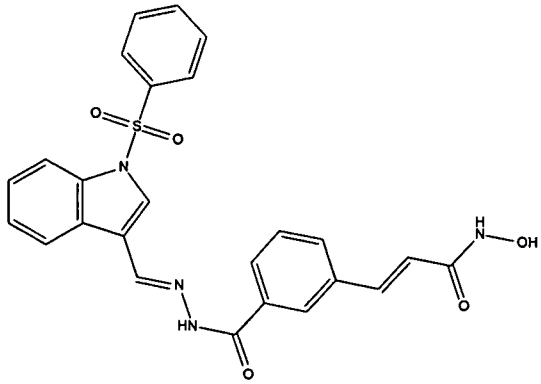
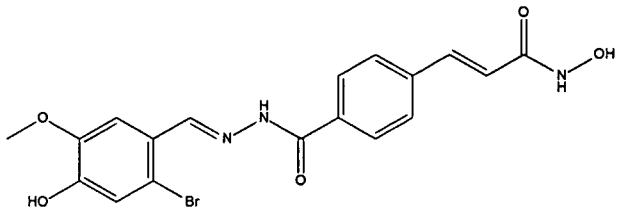
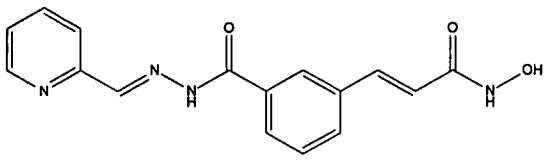
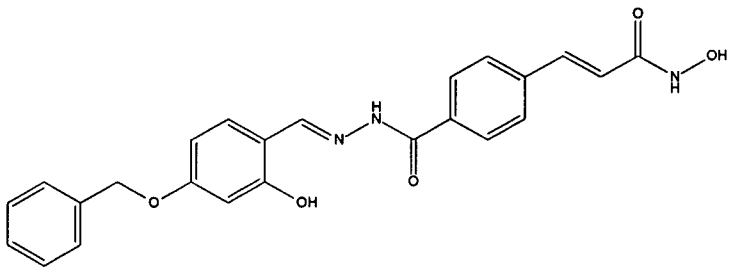
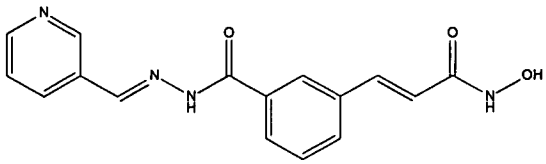


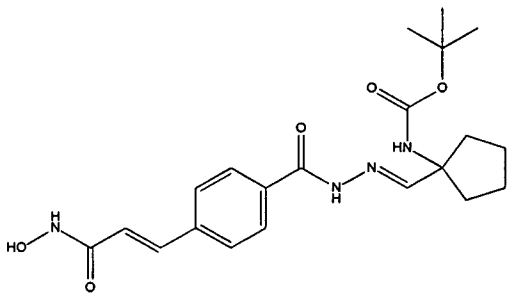


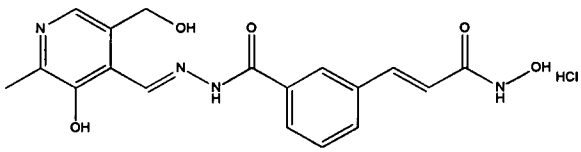


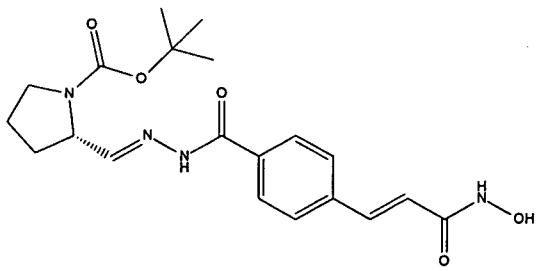


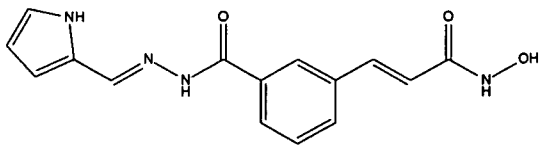


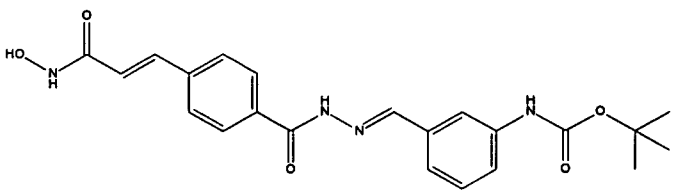


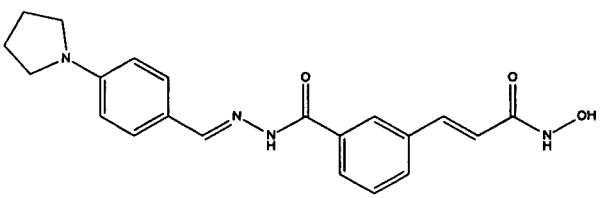


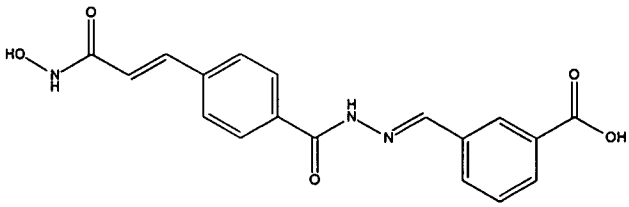


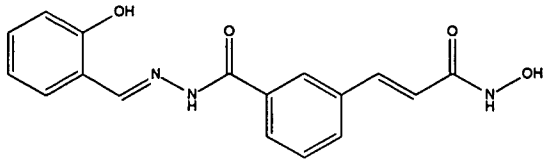


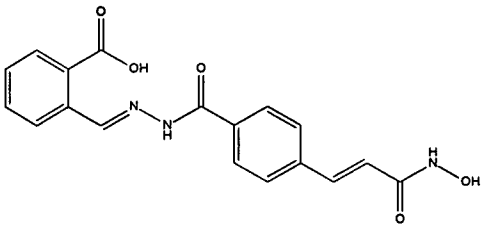


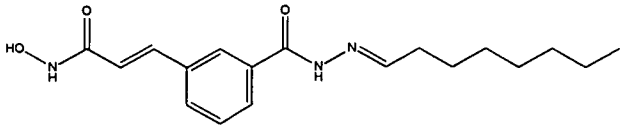


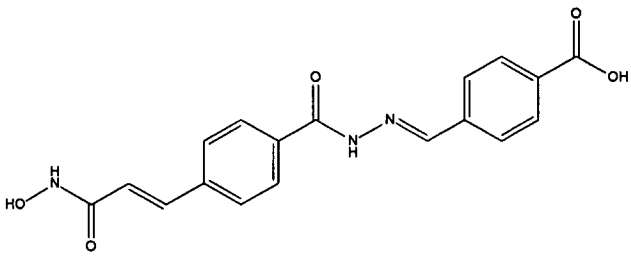


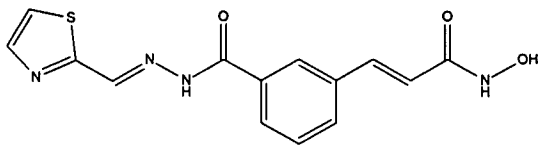


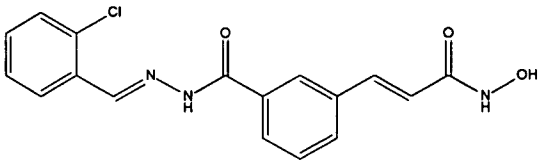


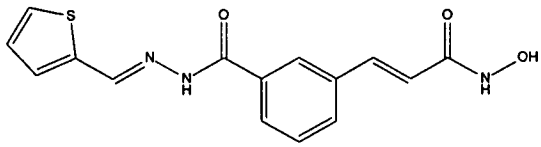


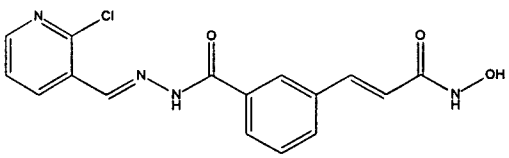


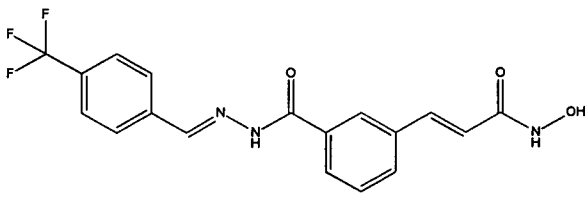


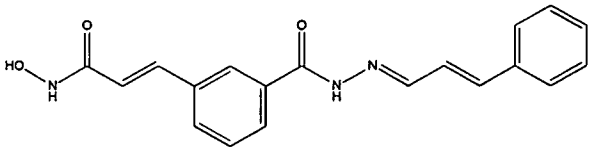


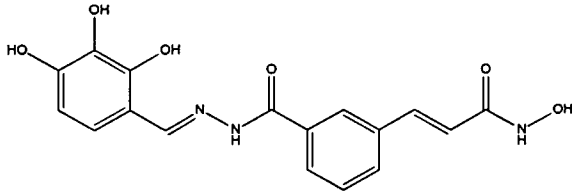


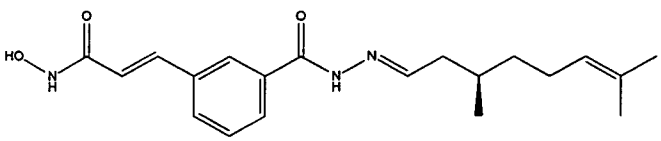


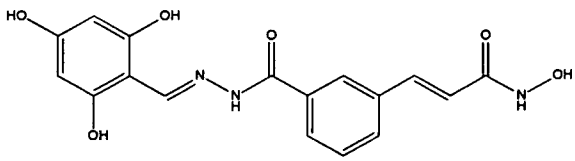


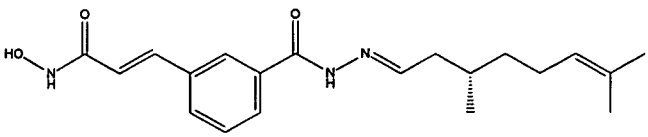


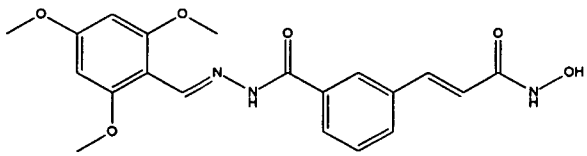


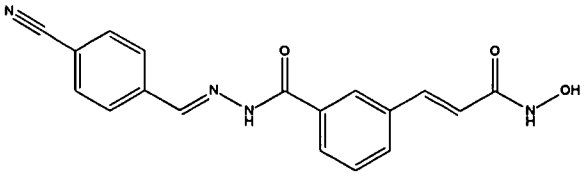


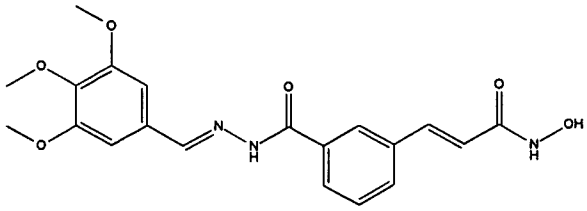


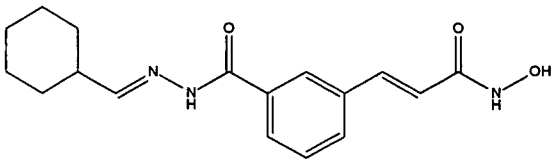


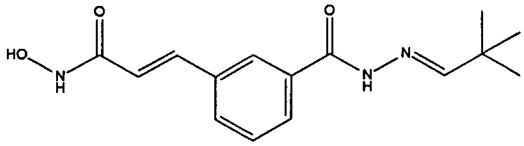


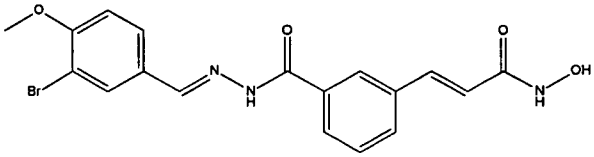


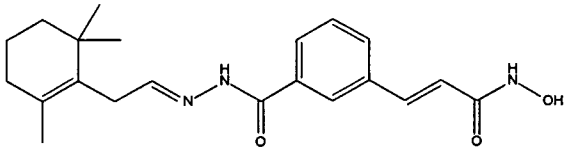


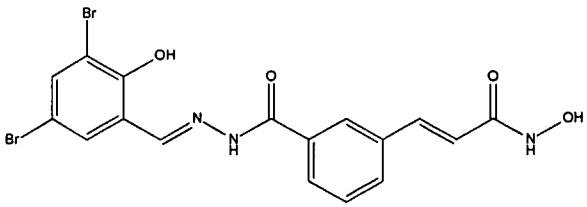


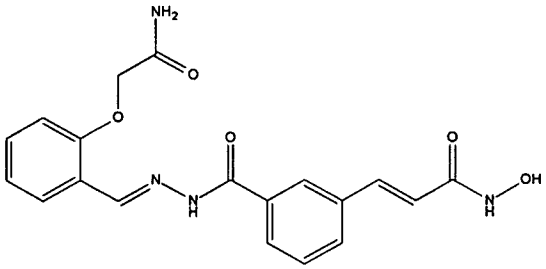


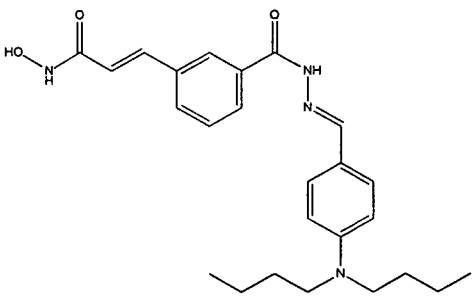


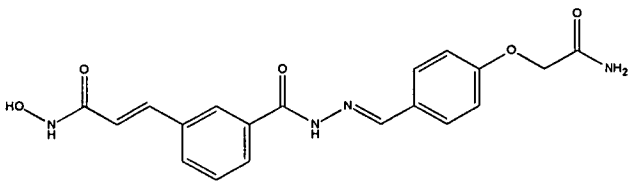


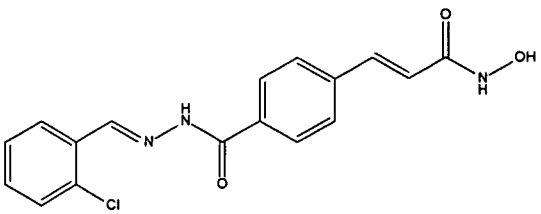


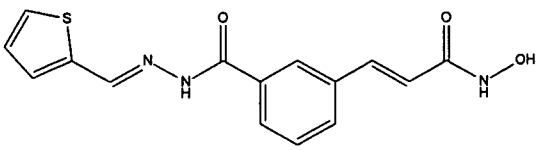


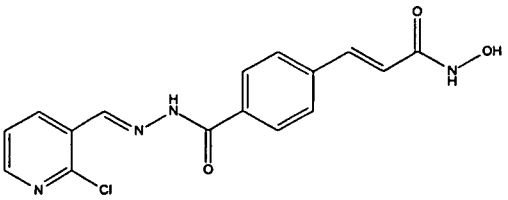


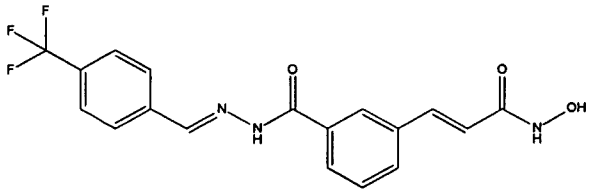


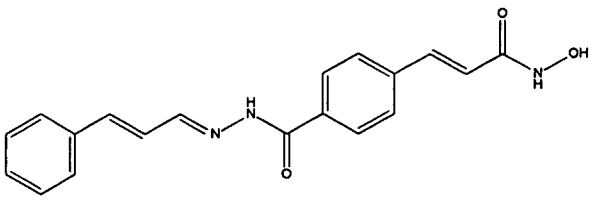


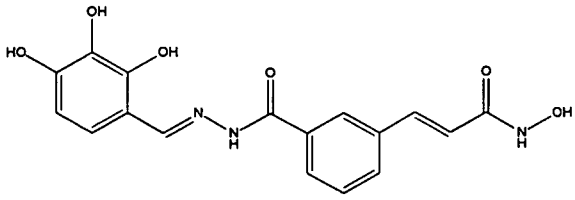


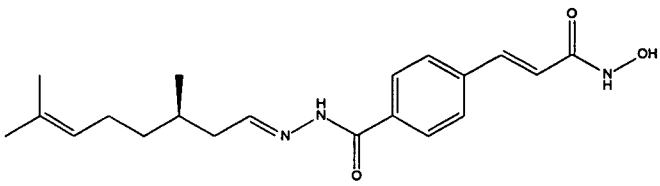


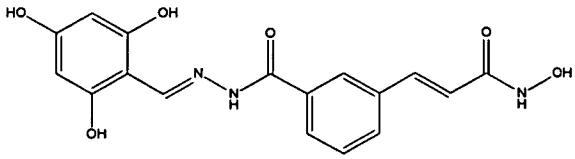


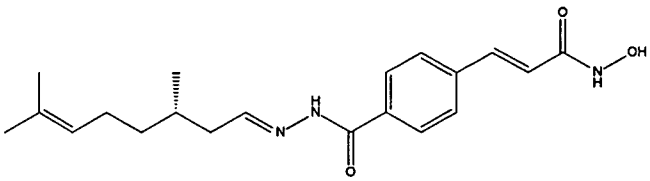


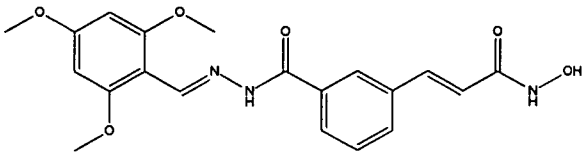


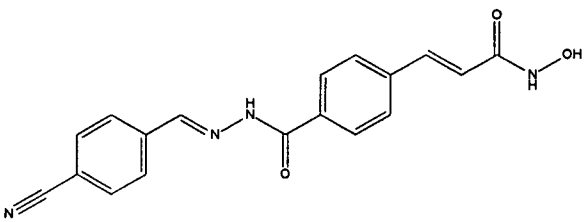


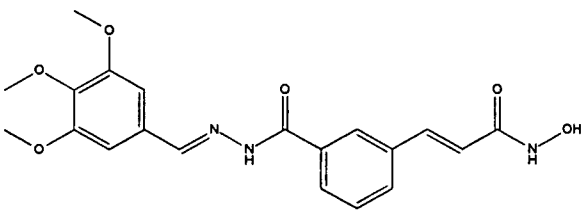


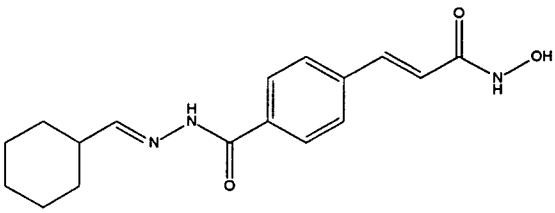


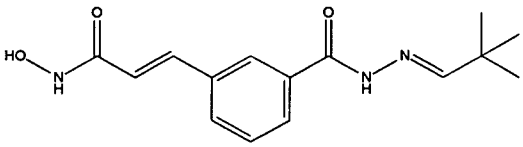


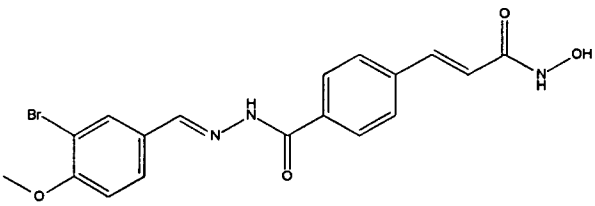


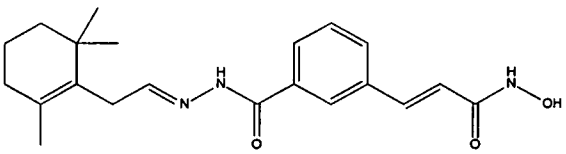


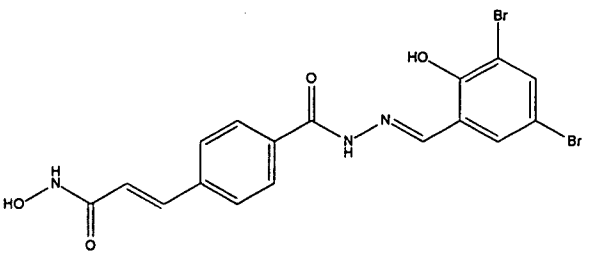


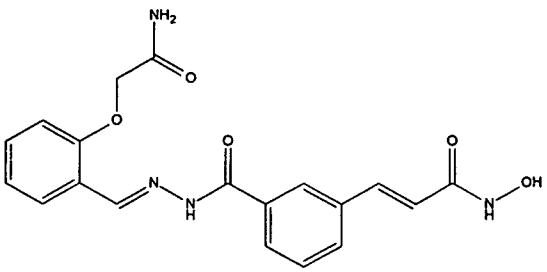


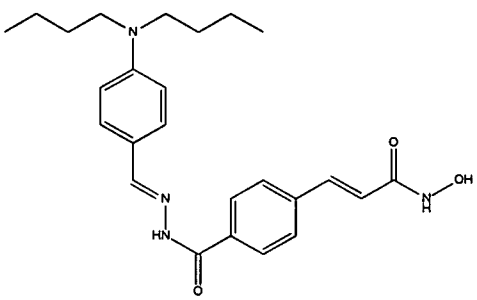


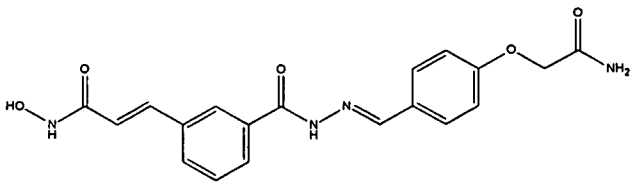


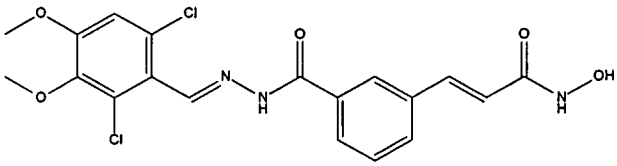


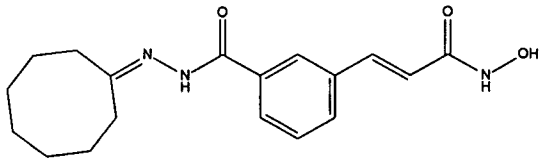


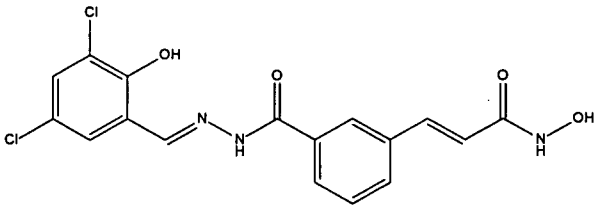


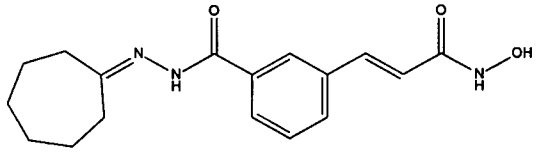


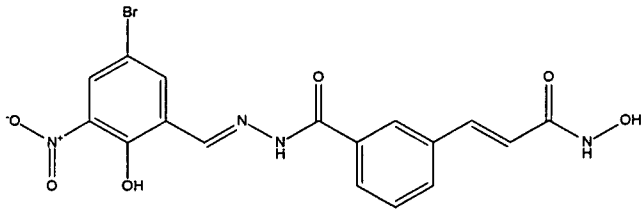


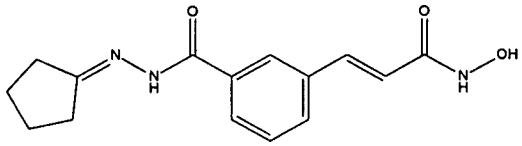


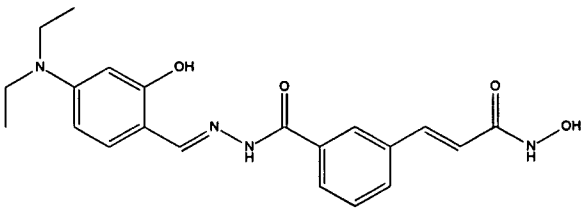


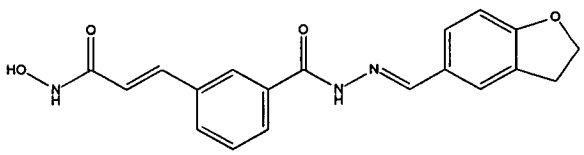


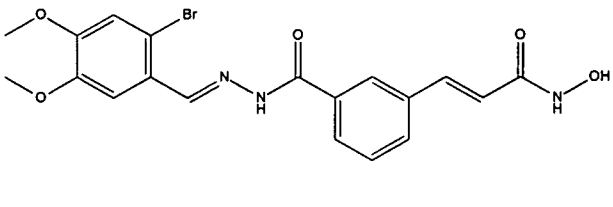


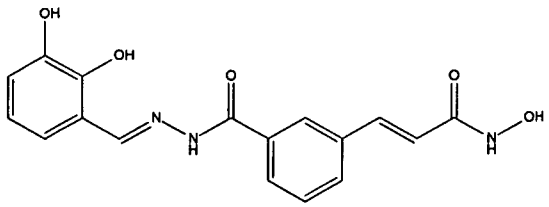


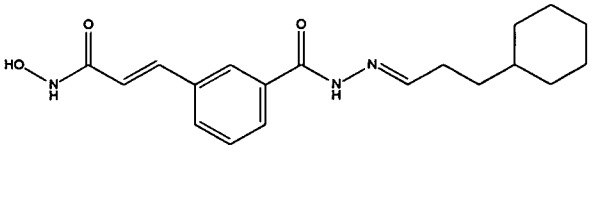


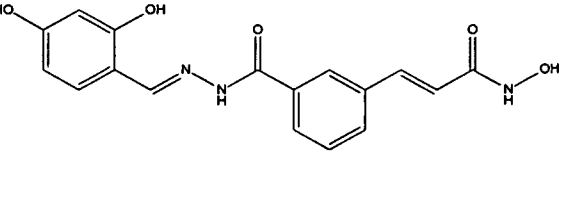


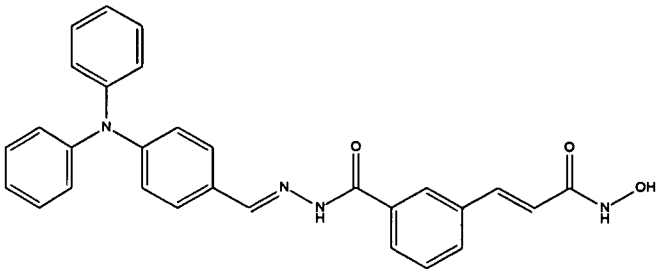


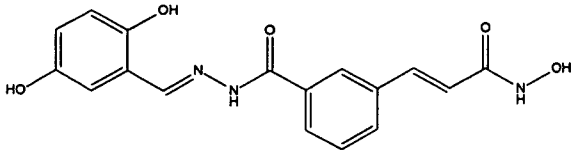


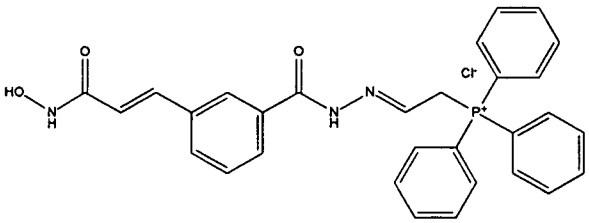


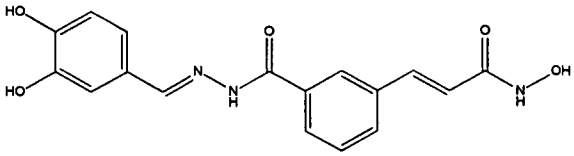


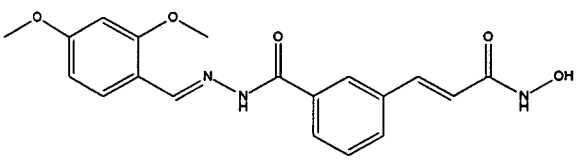


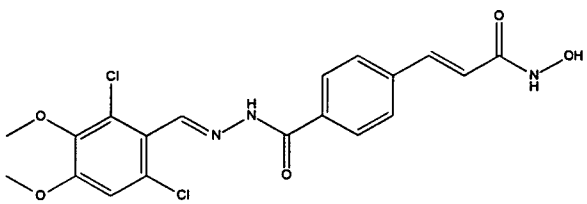


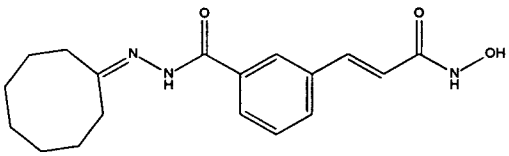


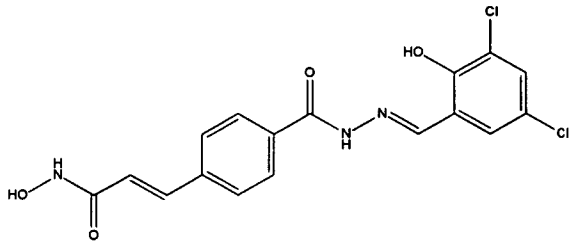


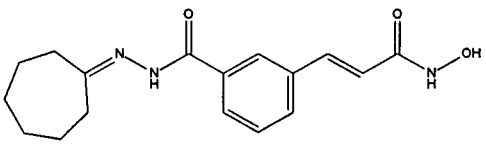


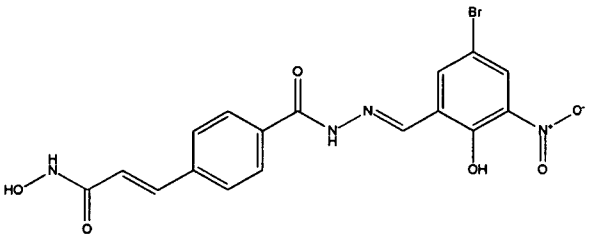


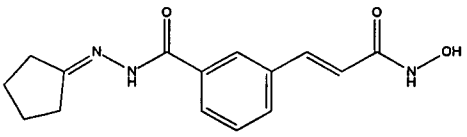


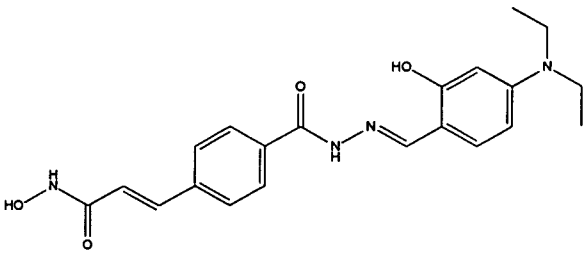


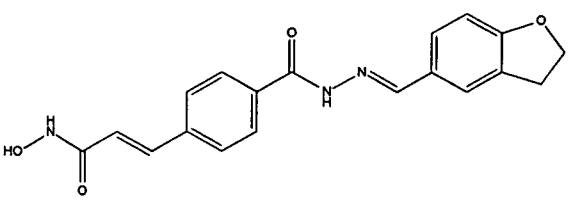


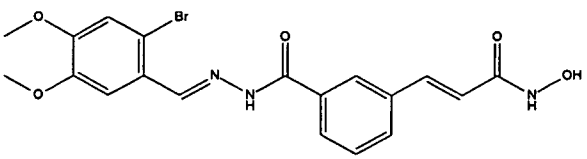


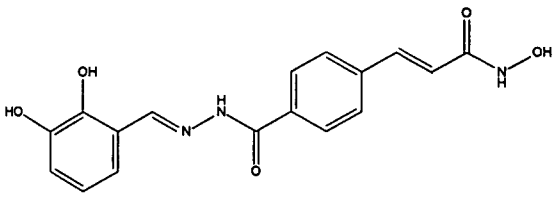


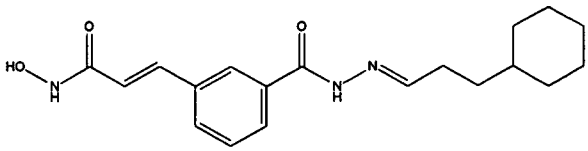


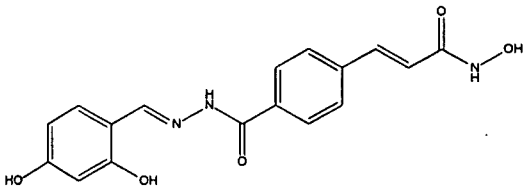


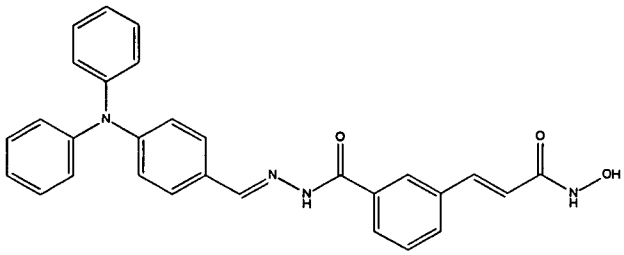


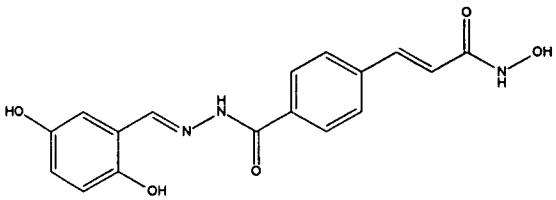


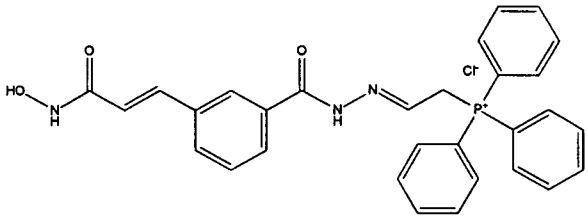


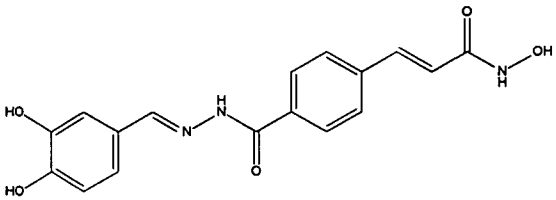


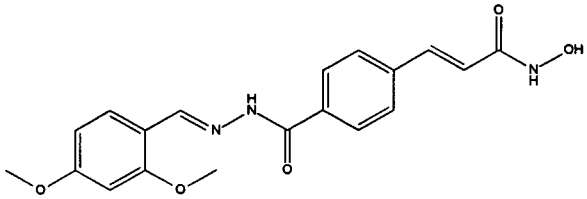


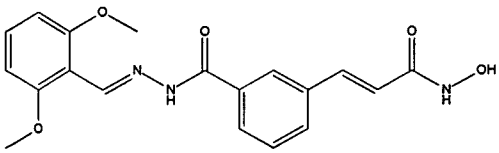


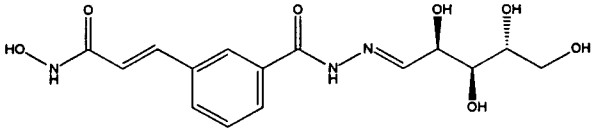


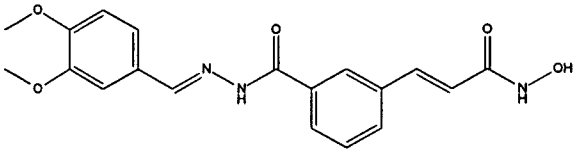


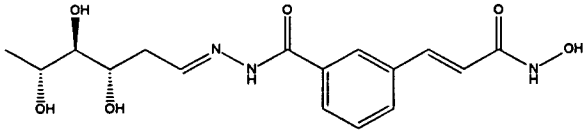


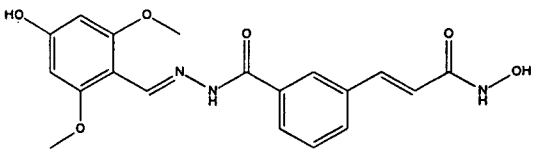


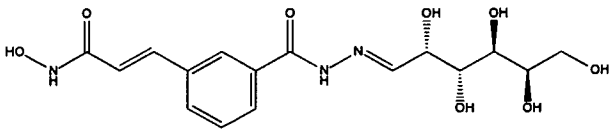


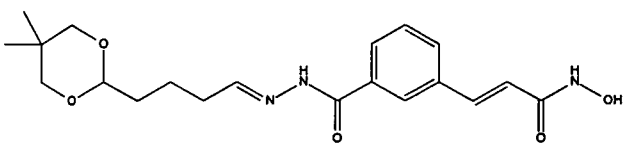


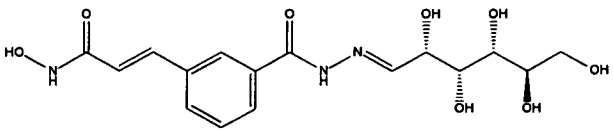


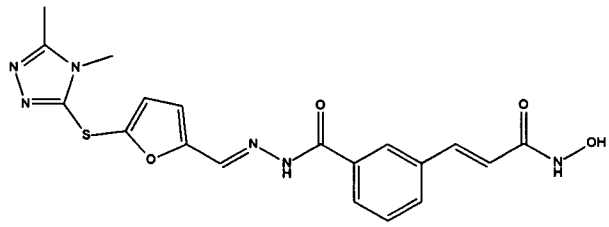


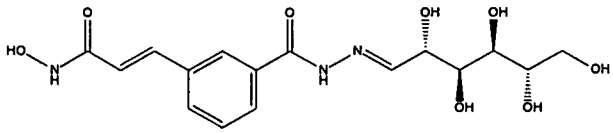


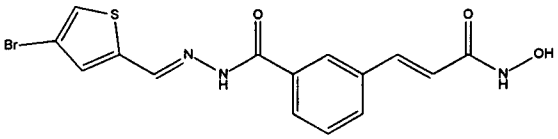


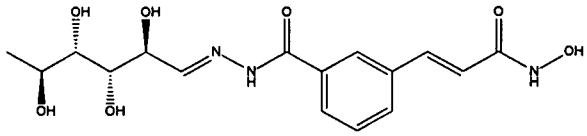


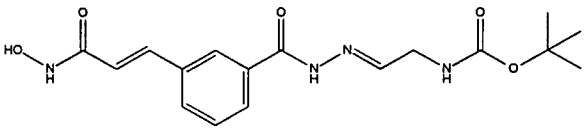


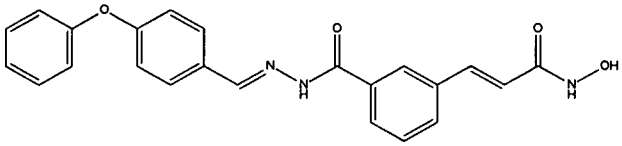


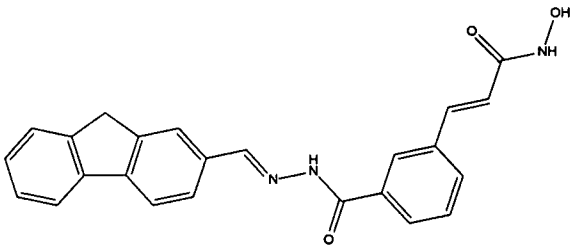


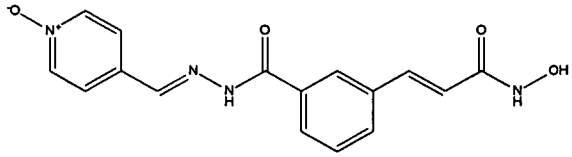


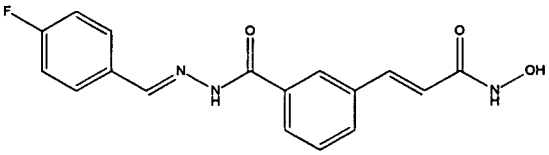


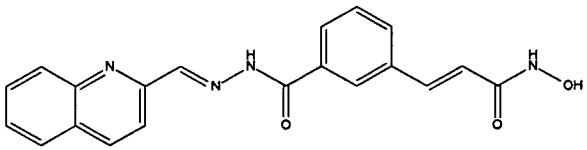


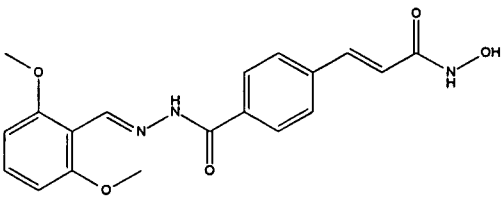


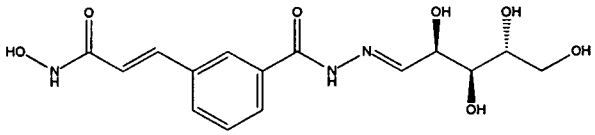


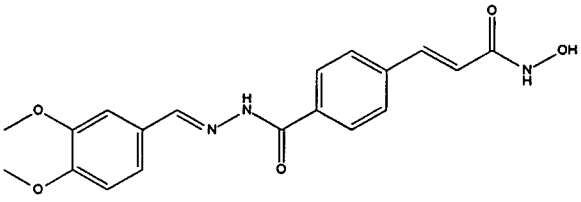


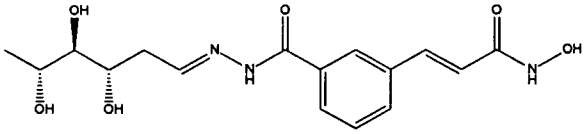


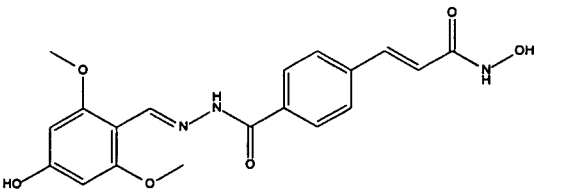


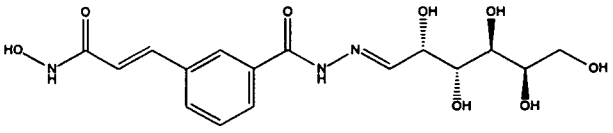


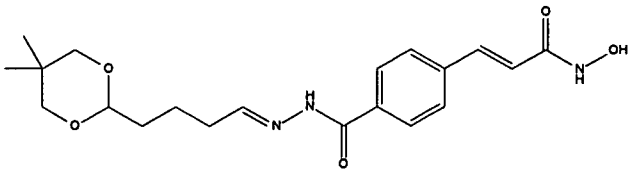
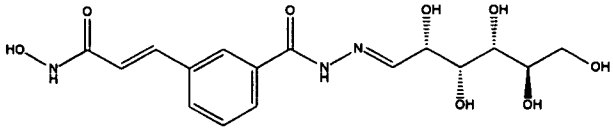
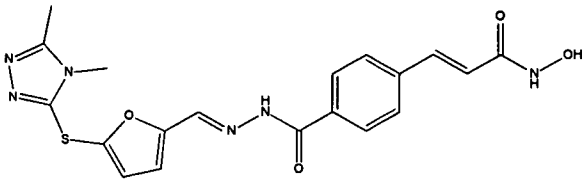
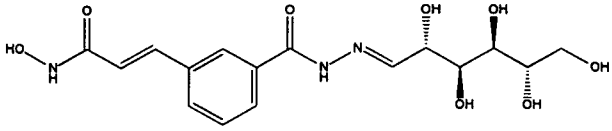
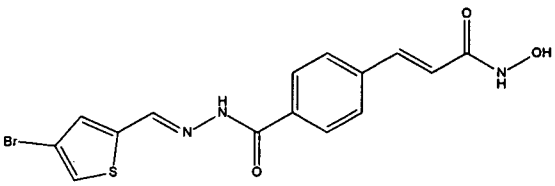
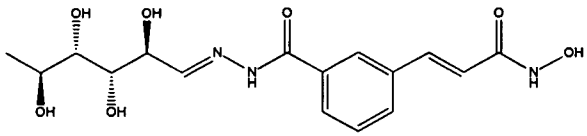
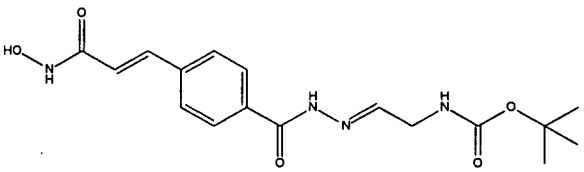
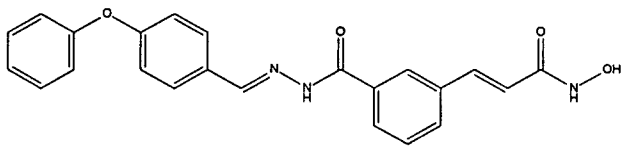
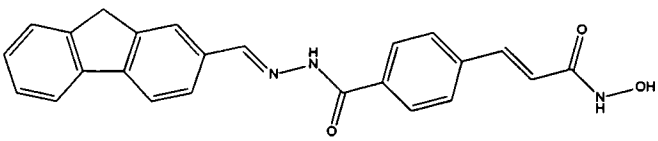
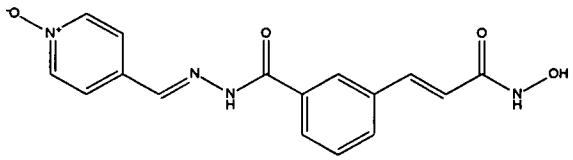


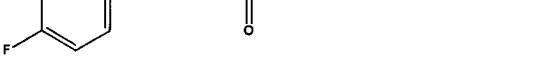


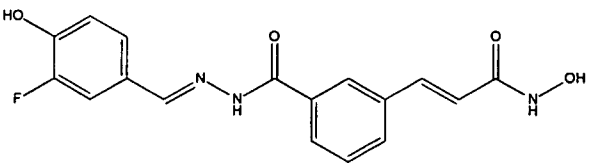
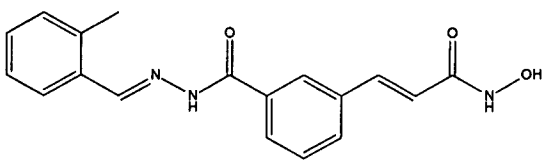
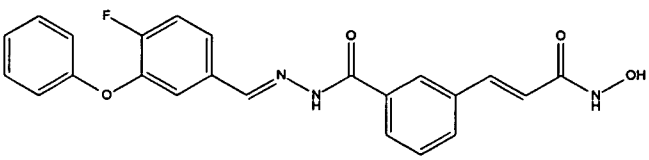
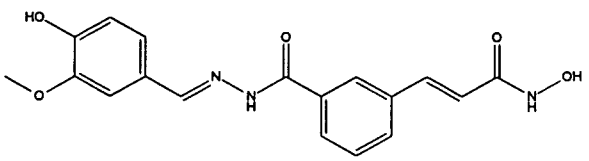
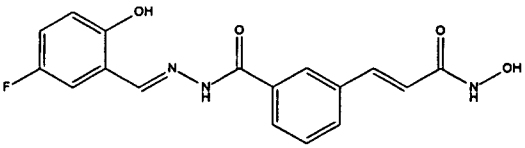
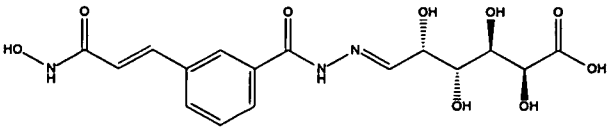


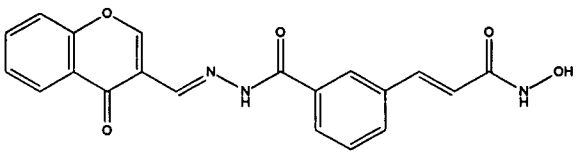


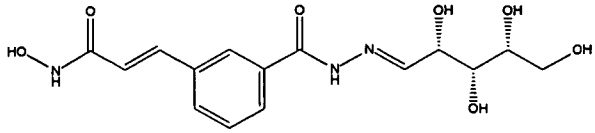


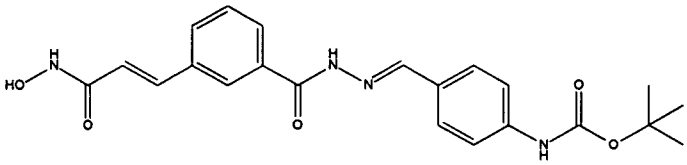











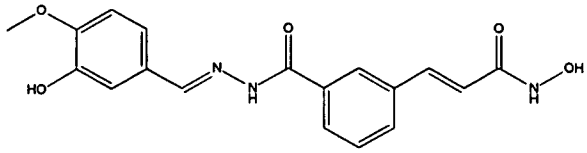


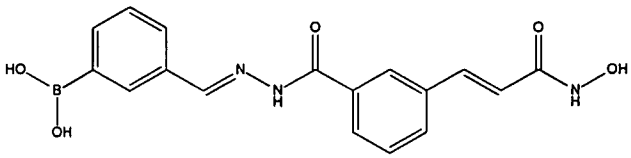







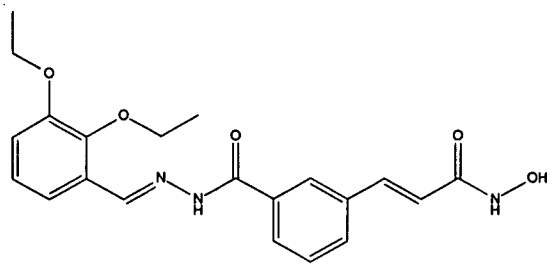


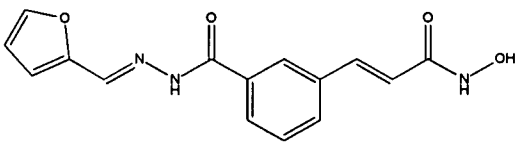


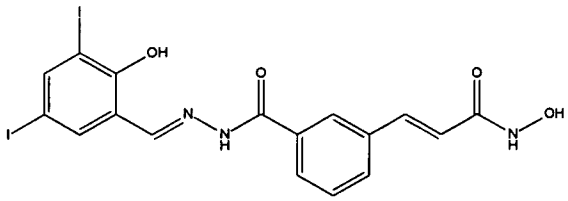


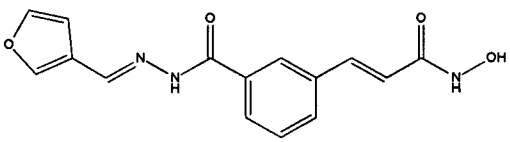


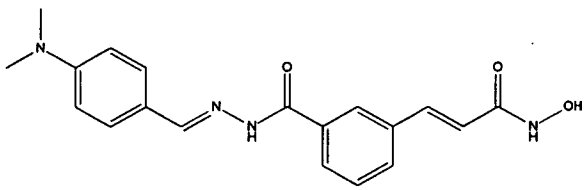


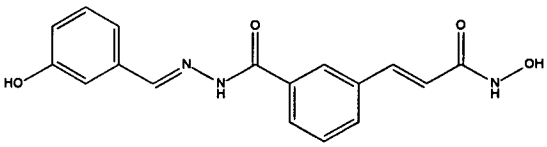


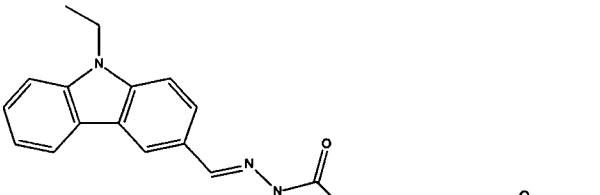


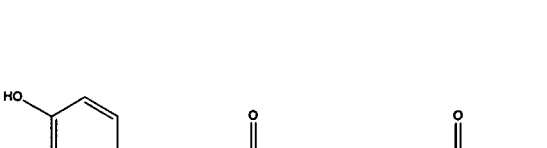


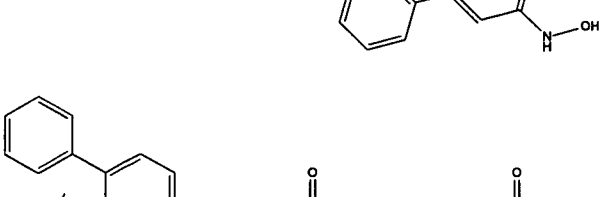


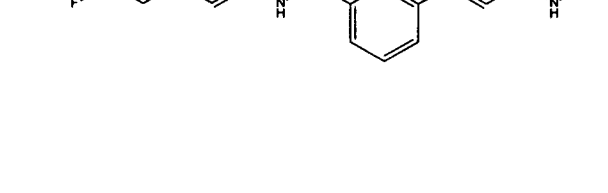


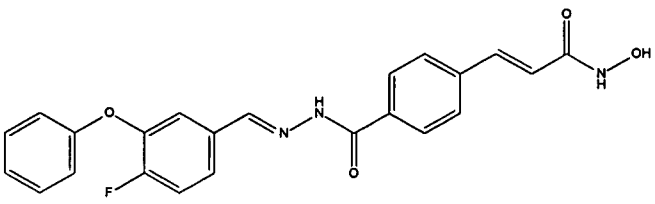


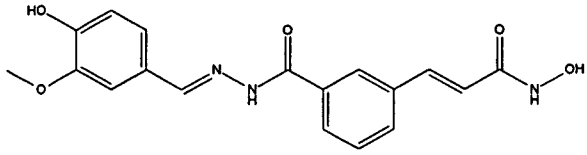


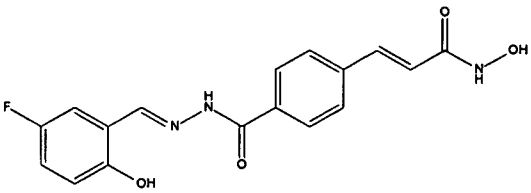


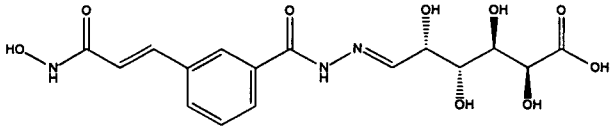


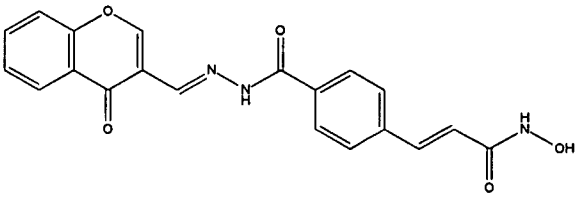


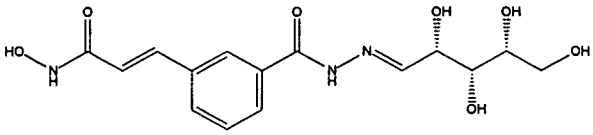


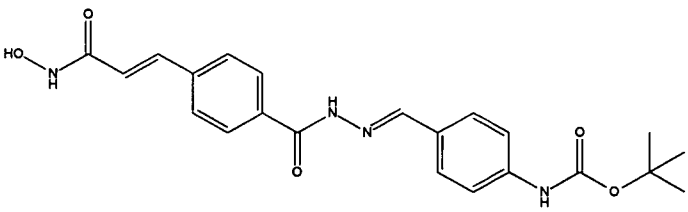


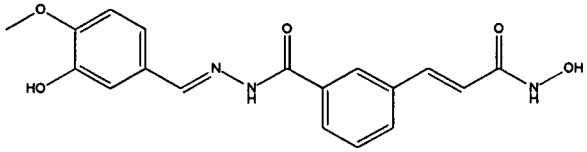


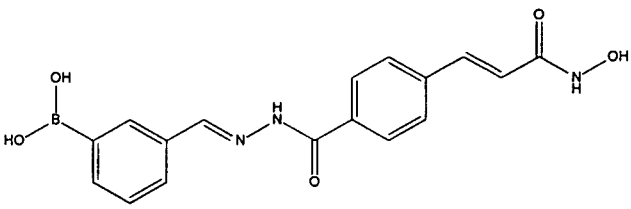


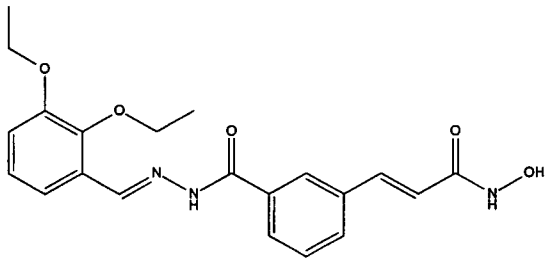


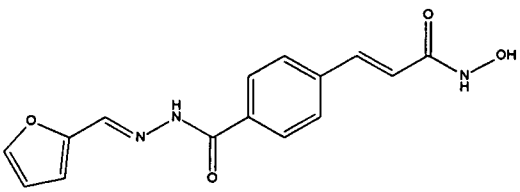


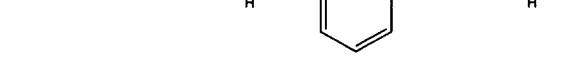


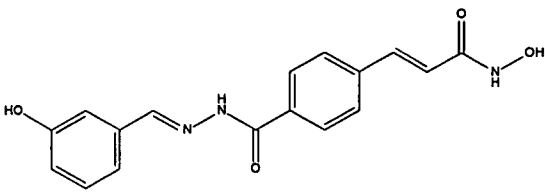


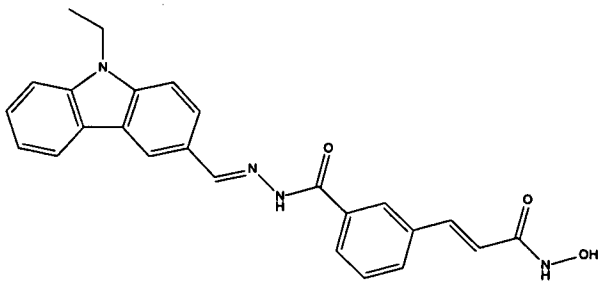


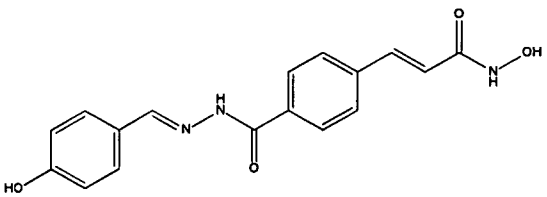


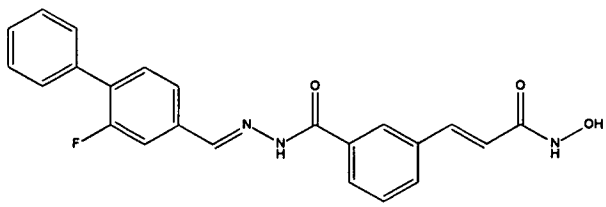


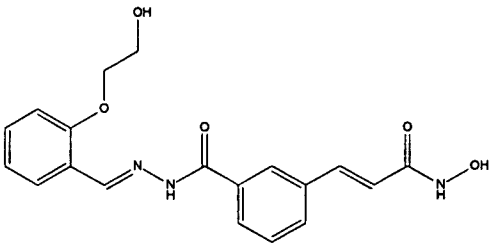


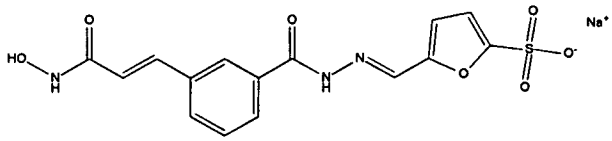


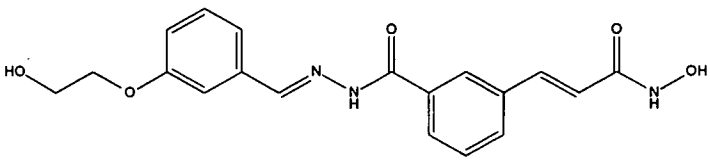


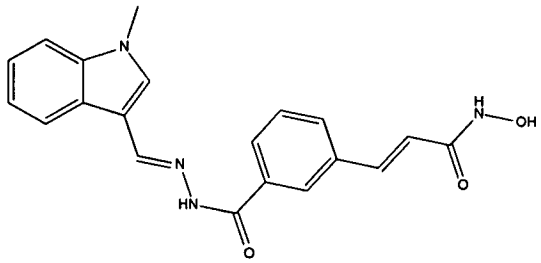


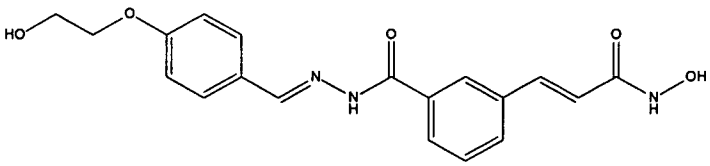


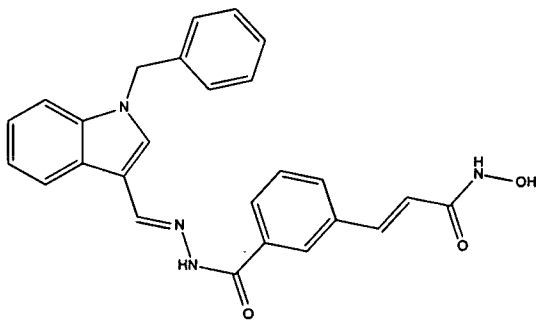


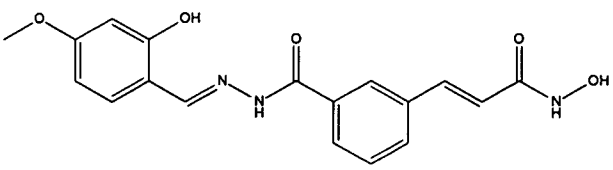


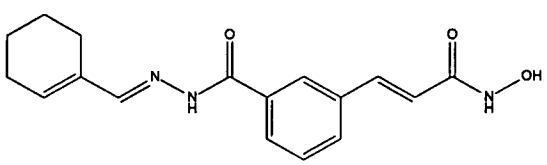


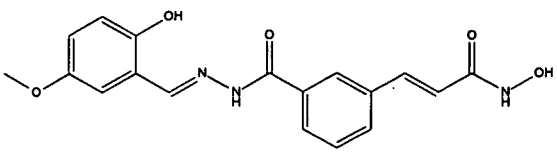


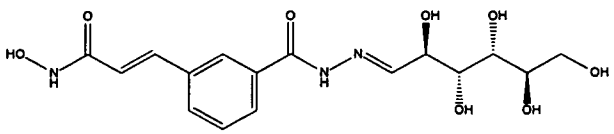


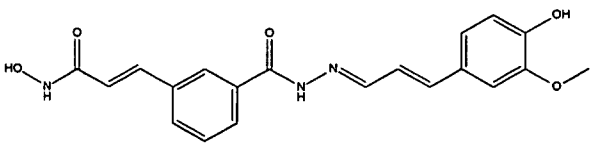


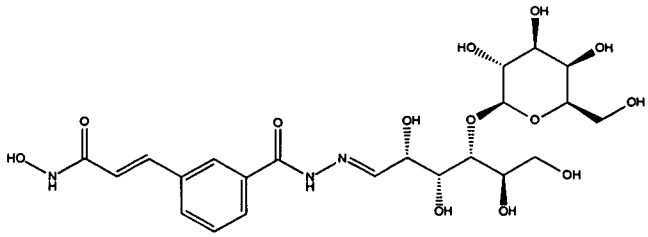


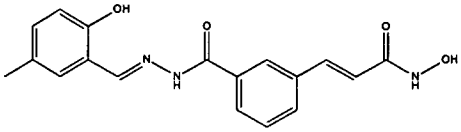


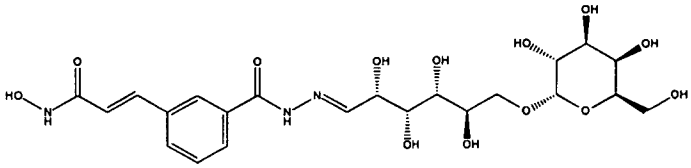


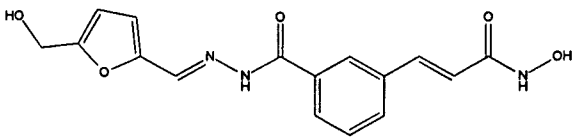


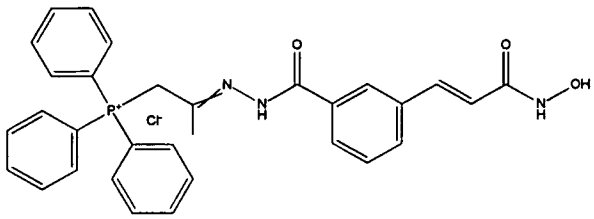


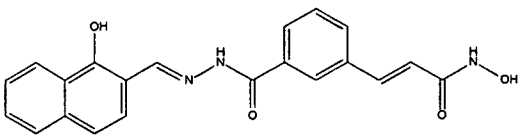


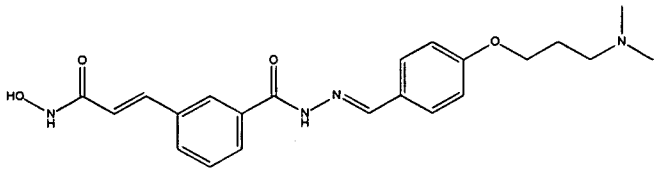


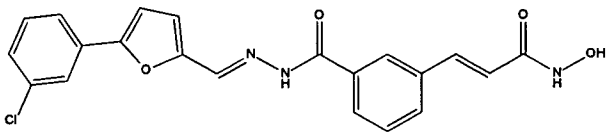


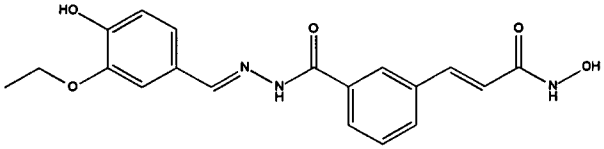


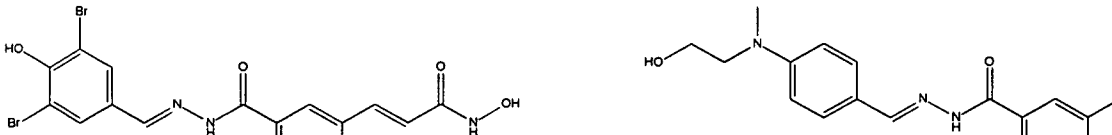


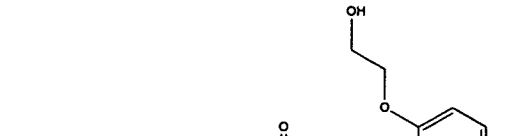


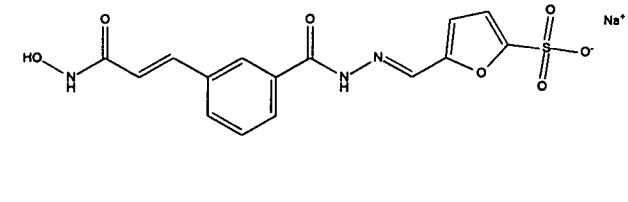


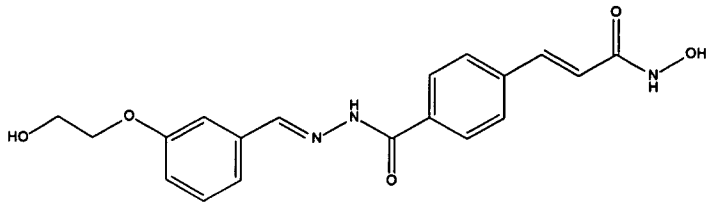


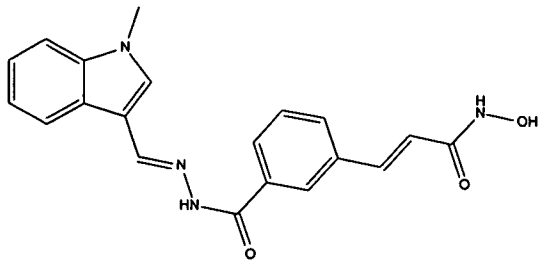


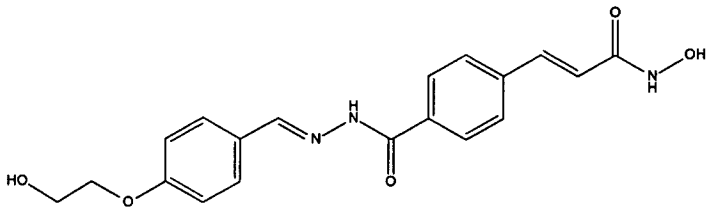


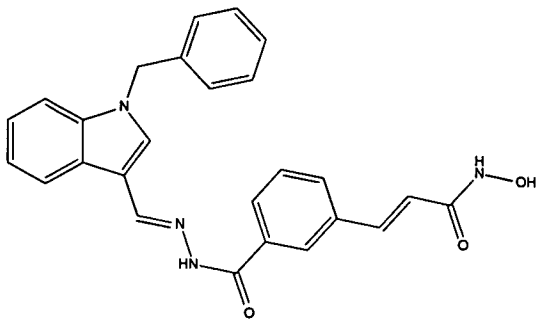


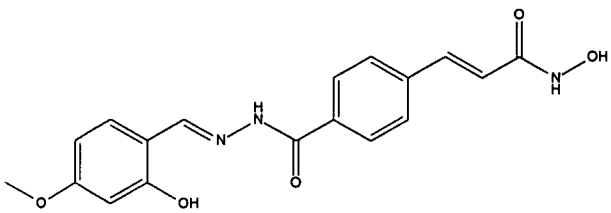


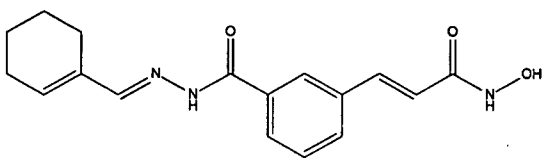


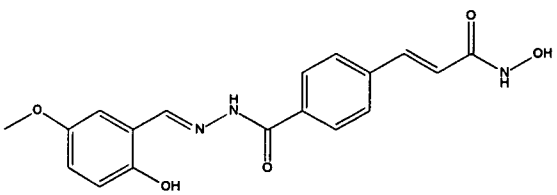


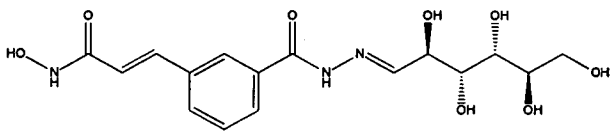


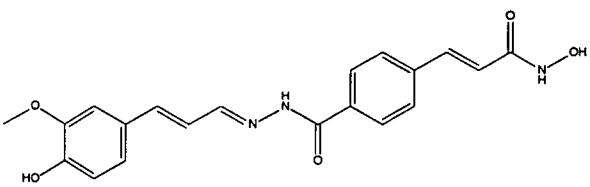


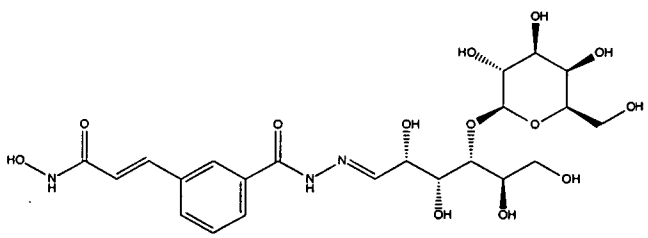


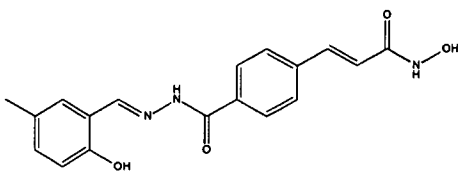


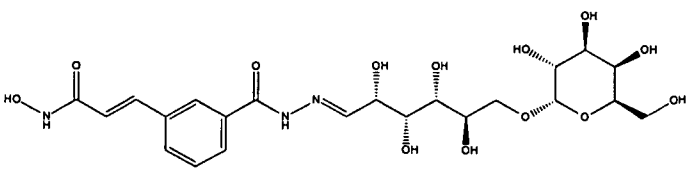


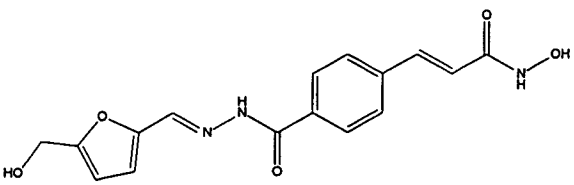


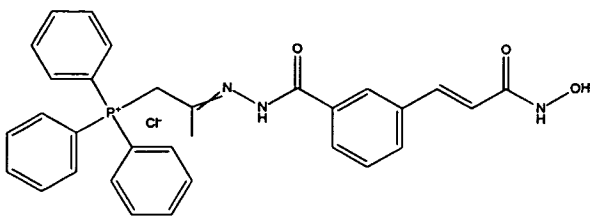


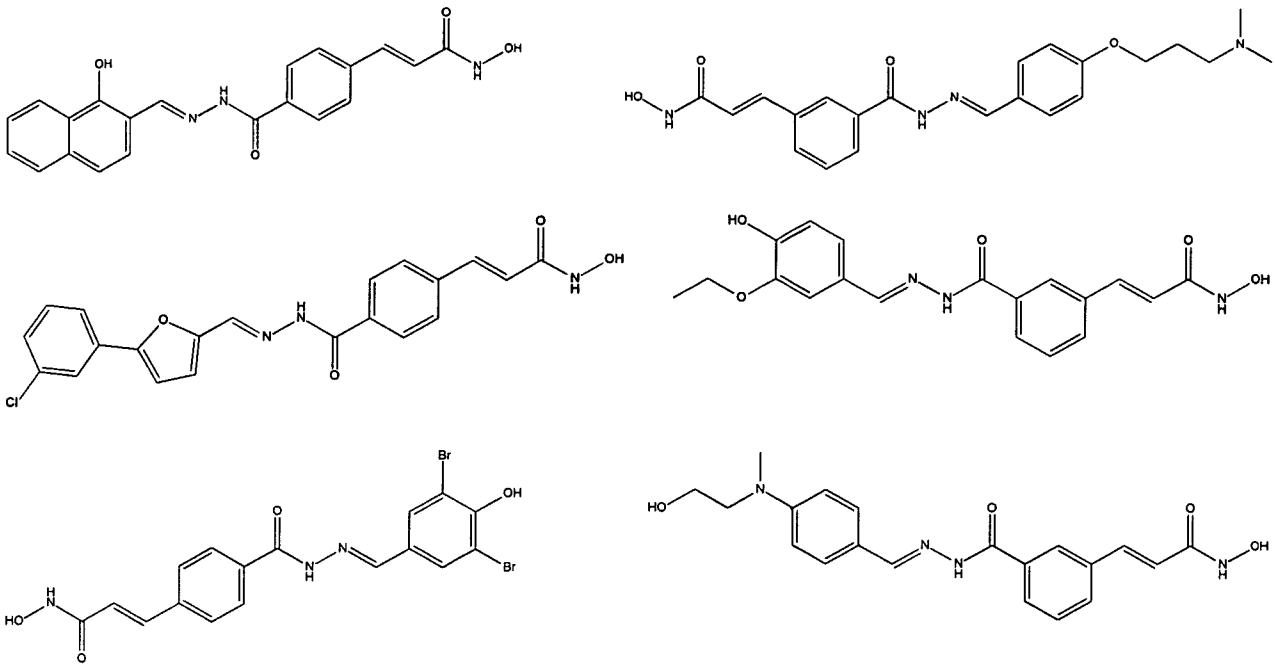












Pharmaceutical compositions

The present invention provides novel compounds useful in the treatment of diseases or disorders associated with HDAC activity. The compounds are useful in the treatment of diseases or condition that benefit from inhibition of deacetylation activity (*e.g.*, HDAC inhibition). In certain embodiments, the inventive cinnamic hydroxymates are useful in the treatment of proliferative diseases, such as cancer (*e.g.*, cutaneous T-cell lymphoma, peripheral T-cell lymphoma) or benign proliferative diseases; autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.* Huntington's disease); vascular diseases, such as restenosis; musculoskeletal diseases; cardiovascular diseases, such as stroke; pulmonary diseases; gastric diseases; and infectious diseases. Class- or isoform-specific HDAC inhibitors may be particularly useful in the treatment of disease or disorders associated with aberrant HDAC activity from a particular Class or isoform. For example, Class IIa HDAC inhibitors may be useful in the treatment of autoimmune or allergic diseases, cardiovascular diseases, or neurodegenerative diseases since Class IIa HDACs have been suggested to play a role in immune tolerance, cardiac remodeling, and neuronal death.

Accordingly, in another aspect of the present invention, pharmaceutical compositions are provided, which comprise any one of the compounds described herein (or a prodrug, pharmaceutically acceptable salt or other pharmaceutically acceptable derivative thereof) and optionally a pharmaceutically acceptable excipient. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents.

Alternatively, a compound of this invention may be administered to a patient in need thereof in combination with the administration of one or more other therapeutic agents. For example, in the treatment of cancer, an additional therapeutic agents for conjoint administration or inclusion in a pharmaceutical composition with a compound of this invention may be an approved chemotherapeutic agent.

It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a pro-drug or other adduct or derivative of a compound of this invention which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

As described above, the pharmaceutical compositions of the present invention optionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, antioxidants, solid binders, lubricants, and the like, as suited to the particular dosage form desired. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, PA, 1980) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable excipients include, but are not limited to, sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil, sesame oil; olive oil; corn oil and soybean oil; glycols; such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar, buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening,

flavoring and perfuming agents, preservatives, and antioxidants can also be present in the composition, according to the judgment of the formulator.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, com, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the

drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include (poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol, or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcelhdose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monosteamte, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols, and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols, and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The present invention encompasses pharmaceutically acceptable topical formulations of inventive compounds. The term "pharmaceutically acceptable topical formulation", as used herein, means any formulation which is pharmaceutically acceptable for intradermal administration of a compound of the invention by application of the formulation to the epidermis. In certain embodiments of the invention, the topical formulation comprises a excipient system. Pharmaceutically effective excipients include, but are not limited to, solvents (*e.g.*, alcohols, poly alcohols, water), creams, lotions, ointments, oils, plasters, liposomes, powders, emulsions, microemulsions, and buffered solutions (*e.g.*, hypotonic or buffered saline) or any other excipient known in the art for topically administering pharmaceuticals. A more complete listing of art-known carvers is provided by reference texts that are standard in the art, for example, *Remington's Pharmaceutical Sciences*, 16th Edition, 1980 and 17th Edition, 1985, both published by Mack Publishing Company, Easton, Pennsylvania, the disclosures of which are incorporated herein by reference in their entireties. In certain other embodiments, the topical formulations of the invention may comprise excipients. Any pharmaceutically acceptable excipient known in the art may be used to prepare the inventive pharmaceutically acceptable topical formulations. Examples of excipients that can be included in the topical formulations of the invention include, but are not limited to, preservatives, antioxidants, moisturizers, emollients, buffering agents, solubilizing agents, other penetration agents, skin protectants, surfactants, and propellants, and/or additional therapeutic agents used in combination to the inventive compound. Suitable preservatives include, but are not limited to, alcohols, quaternary amines, organic

acids, parabens, and phenols. Suitable antioxidants include, but are not limited to, ascorbic acid and its esters, sodium bisulfite, butylated hydroxytoluene, butylated hydroxyarrisole, tocopherols, and chelating agents like EDTA and citric acid. Suitable moisturizers include, but are not limited to, glycerine, sorbitol, polyethylene glycols, urea, and propylene glycol. Suitable buffering agents for use with the invention include, but are not limited to, citric, hydrochloric, and lactic acid buffers. Suitable solubilizing agents include, but are not limited to, quaternary ammonium chlorides, cyclodextrins, benzyl benzoate, lecithin, and polysorbates. Suitable skin protectants that can be used in the topical formulations of the invention include, but are not limited to, vitamin E oil, allatoin, dimethicone, glycerin, petrolatum, and zinc oxide.

In certain embodiments, the pharmaceutically acceptable topical formulations of the invention comprise at least a compound of the invention and a penetration enhancing agent. The choice of topical formulation will depend or several factors, including the condition to be treated, the physicochemical characteristics of the inventive compound and other excipients present, their stability in the formulation, available manufacturing equipment, and costs constraints. As used herein the term "penetration enhancing agent" means an agent capable of transporting a pharmacologically active compound through the stratum coreum and into the epidermis or dermis, preferably, with little or no systemic absorption. A wide variety of compounds have been evaluated as to their effectiveness in enhancing the rate of penetration of drugs through the skin. See, for example, *Percutaneous Penetration Enhancers*, Maibach H. I. and Smith H. E. (eds.), CRC Press, Inc., Boca Raton, Fla. (1995), which surveys the use and testing of various skin penetration enhancers, and Buyuktimkin *et al.*, *Chemical Means of Transdermal Drug Permeation Enhancement in Transdermal and Topical Drug Delivery Systems*, Gosh T. K., Pfister W. R., Yum S. I. (Eds.), Interpharm Press Inc., Buffalo Grove, Ill. (1997). In certain exemplary embodiments, penetration agents for use with the invention include, but are not limited to, triglycerides (*e.g.*, soybean oil), aloe compositions (*e.g.*, aloe-vera gel), ethyl alcohol, isopropyl alcohol, octolyphenylpolyethylene glycol, oleic acid, polyethylene glycol 400, propylene glycol, N-decylmethylsulfoxide, fatty acid esters (*e.g.*, isopropyl myristate, methyl laurate, glycerol monooleate, and propylene glycol monooleate), and N-methyl pyrrolidone.

In certain embodiments, the compositions may be in the form of ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. In certain exemplary embodiments, formulations of the compositions according to the invention are creams, which may further contain saturated or unsaturated fatty acids such as stearic acid, palmitic acid,

oleic acid, palmito-oleic acid, cetyl or oleyl alcohols, stearic acid being particularly preferred. Creams of the invention may also contain a non-ionic surfactant, for example, polyoxy-40-stearate. In certain embodiments, the active component is admixed under sterile conditions with a pharmaceutically acceptable excipient and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are made by dissolving or dispensing the compound in the proper medium. As discussed above, penetration enhancing agents can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix (*e.g.*, PLGA) or gel.

It will also be appreciated that the compounds and pharmaceutical compositions of the present invention can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another immunomodulatory agent or anticancer agent), or they may achieve different effects (*e.g.*, control of any adverse effects).

For example, other therapies or anticancer agents that may be used in combination with the inventive compounds of the present invention for cancer therapy include surgery, radiotherapy (in but a few examples, γ-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioactive isotopes, to name a few), endocrine therapy, biologic response modifiers (interferon, interleukins, and tumor necrosis factor (TNF) to name a few), hyperthermia and cryotherapy, agents to attenuate any adverse effects (*e.g.*, antiemetics), and other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimetabolites (Methotrexate), purine antagonists and pyrimidine antagonists (6-Mercaptopurine, 5-Fluorouracil, Cytarabile, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel), podophyllotoxins (Etoposide, Irinotecan,

Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin), nitrosoureas (Carmustine, Lomustine), inorganic ion (Cisplatin, Carboplatin), enzymes (Asparaginase), and hormones (Tamoxifen, Leuprelide, Flutamide, and Megestrol), to name a few. For a more comprehensive discussion of updated cancer therapies see, *The Merck Manual*, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference. See also the National Cancer Institute (CNI) website (www.nci.nih.gov) and the Food and Drug Administration (FDA) website for a list of the FDA approved oncology drugs (www.fda.gov/cder/cancer/draglis&ame).

In certain embodiments, the pharmaceutical compositions of the present invention further comprise one or more additional therapeutically active ingredients (*e.g.*, chemotherapeutic and/or palliative). For purposes of the invention, the term "palliative" refer, to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medication and anti-sickness drugs. In addition, chemotherapy, radiotherapy and surgery can all be used palliatively (that is, to reduce symptoms without going for cure; *e.g.*, for shrinking tumors and reducing pressure, bleeding, pain and other symptoms of cancer).

Additionally, the present invention provides pharmaceutically acceptable derivatives of the inventive compounds, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents.

It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a prodrug or other adduct or derivative of a compound of this invention which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

Another aspect of the invention relates to a kit for conveniently and effectively carrying out the methods in accordance with the present invention. In general, the pharmaceutical pack or kit comprises one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Such kits are especially suited for the topical delivery of the inventive compounds. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the

manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Pharmaceutical Uses and Methods of Treatment

In general, methods of using the compounds of the present invention comprise administering to a subject in need thereof a therapeutically effective amount of a compound of the present invention. The compounds of the invention are generally inhibitors of deacetyalse activity. As discussed above, the compounds of the invention are typically inhibitors of histone deacetylases and, as such, are useful in the treatment of disorders modulated by histone deacetylases. Diseases associated with a particular HDAC Class or isoform may be treated by an inventive compound that specifically inhibits that particular Class or isoform. Other deacetylases such as tubulin deacetylases may also be inhibited by the inventive compounds.

In certain embodiments, compounds of the invention are useful in the treatment of proliferative diseases (*e.g.*, cancer, benign neoplasms, inflammatory disease, autoimmune diseases). In other embodiments, the inventive compounds are useful in the treatment of autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.* Huntington's disease); vascular diseases, such as restenosis; musculoskeletal diseases; cardiovascular diseases, such as stroke; pulmonary diseases; gastric diseases; and infectious diseases.

In another aspect of the invention, methods for the treatment of cancer are provided comprising administering a therapeutically effective amount of an inventive compound, as described herein, to a subject in need thereof. In certain embodiments, a method for the treatment of cancer is provided comprising administering a therapeutically effective amount of an inventive compound, or a pharmaceutical composition comprising an inventive compound to a subject in need thereof, in such amounts and for such time as is necessary to achieve the desired result. In certain embodiments, the inventive compound is administered parenterally. In certain embodiments, the inventive compound is administered intravenously. In certain embodiments, the inventive compound is administered topically. In certain embodiments of the present invention, a "therapeutically effective amount" of the inventive compound or pharmaceutical composition is that amount effective for killing or inhibiting the growth of tumor cells. The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for killing or inhibiting the growth of tumor cells. Thus, the expression "amount

effective to kill or inhibit the growth of tumor cells," as used herein, refers to a sufficient amount of agent to kill or inhibit the growth of tumor cells. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular anticancer agent, its mode of administration, and the like.

In certain embodiments, the method involves the administration of a therapeutically effective amount of the compound or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or animal) in need of it. In certain embodiments, the inventive compounds as useful for the treatment of cancer (including, but not limited to, glioblastoma, retinoblastoma, breast cancer, cervical cancer, colon and rectal cancer, leukemia, lymphoma, lung cancer (including, but not limited to small cell lung cancer), melanoma and/or skin cancer, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer and gastric cancer, bladder cancer, uterine cancer, kidney cancer, testicular cancer, stomach cancer, brain cancer, liver cancer, or esophageal cancer).

In certain embodiments, the inventive anticancer agents are useful in the treatment of cancers and other proliferative disorders, including, but not limited to breast cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer, melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer, to name a few. In certain embodiments, the inventive anticancer agents are active against leukemia cells and melanoma cells, and thus are useful for the treatment of leukemias (*e.g.*, myeloid, lymphocytic, myelocytic and lymphoblastic leukemias) and malignant melanomas. In still other embodiments, the inventive anticancer agents are active against solid tumors. In certain embodiments, the inventive compounds also find use in the prevention of restenosis of blood vessels subject to traumas such as angioplasty and stenting. For example, it is contemplated that the compounds of the invention will be useful as a coating for implanted medical devices, such as tubings, shunts, catheters, artificial implants, pins, electrical implants such as pacemakers, and especially for arterial or venous stents, including balloon-expandable stents. In certain embodiments inventive compounds may be bound to an implantable medical device, or alternatively, may be passively adsorbed to the surface of the implantable device. In certain other embodiments, the inventive compounds may be formulated to be contained within, or, adapted to release by a surgical or medical device or implant, such as, for example, stents, sutures, indwelling catheters, prosthesis, and the like. For example, drugs having antiproliferative and anti-inflammatory activities have been

evaluated as stent coatings, and have shown promise in preventing retenosis (See, for example, Presbitero *et al.*, "Drug eluting stents do they make the difference?", *Minerva Cardioangiol.*, 2002, 50(5):431-442; Ruygrok *et al.*, "Rapamycin in cardiovascular medicine", *Intern. Med. J.*, 2003, 33(3):103-109; and Marx *et al.*, "Bench to bedside: the development of rapamycin and its application to stent restenosis", *Circulation*, 2001, 104(8):852-855, each of these references is incorporated herein by reference in its entirety). Accordingly, without wishing to be bound to any particular theory, Applicant proposes that inventive compounds having antiproliferative effects can be used as stent coatings and/or in stent drug delivery devices, inter alia for the prevention of restenosis or reduction of restenosis rate. Suitable coatings and the general preparation of coated implantable devices are described in US Patents 6,099,562; 5,886,026; and 5,304,121; each of which is incorporated herein by reference. The coatings are typically biocompatible polymeric materials such as a hydrogel polymer, polymethyldisiloxane, polycaprolactone, polyethylene glycol, polylactic acid, ethylene vinyl acetate, and mixtures thereof. The coatings may optionally be further covered by a suitable topcoat of fluorosilicone, polysaccarides, polyethylene glycol, phospholipids or combinations thereof to impart controlled release characteristics in the composition. A variety of compositions and methods related to stem coating and/or local stent drug delivery for preventing restenosis are known in the art (see, for example, U.S. Patent Nos.: 6,517,889; 6,273,913; 6,258,121; 6,251,136; 6,248,127; 6,231,600; 6,203,551; 6,153,252; 6,071,305; 5,891,507; 5,837,313 and published U.S. patent application No.: US200110027340, each of which is incorporated herein by reference in its entirety). For example, stents may be coated with polymer-drug conjugates by dipping the stent in polymer-drug solution or spraying the stent with such a solution. In certain embodiment, suitable materials for the implantable device include biocompatible and nontoxic materials, and maybe chosen from the metals such as nickel-titanium alloys, steel, or biocompatible polymers, hydrogels, polyurethanes, polyethylenes, ethylenevinyl acetate copolymers, *etc.* In certain embodiments, the inventive compound is coated onto a stent for insertion into an artery or vein following balloon angioplasty.

The compounds of this invention or pharmaceutically acceptable compositions thereof may also be incorporated into compositions for coating implantable medical devices, such as prostheses, artificial valves, vascular grafts, stents and catheters. Accordingly, the present invention, in another aspect, includes a composition for coating an implantable device comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a excipient suitable for coating said implantable device. In still

another aspect, the present invention includes an implantable device coated with a composition comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a excipient suitable for coating said implantable device.

Within other aspects of the present invention, methods are provided for expanding the lumen of a body passageway, comprising inserting a stent into the passageway, the stent having a generally tubular structure, the surface of the structure being coated with (or otherwise adapted to release) an inventive compound or composition, such that the passageway is expanded. In certain embodiments, the lumen of a body passageway is expanded in order to eliminate a biliary, gastrointestinal, esophageal, tracheal/bronchial, urethral, and/or vascular obstruction.

Methods for eliminating biliary, gastrointestinal, esophageal, tracheal/bronchial, urethral and/or vascular obstructions using stents are known in the art. The skilled practitioner will know how to adapt these methods in practicing the present invention. For example, guidance can be found in US. Patent Application Publication No.: 2003/0004209 in paragraphs [0146]-[0155], which paragraphs are hereby incorporated herein by reference.

Another aspect of the invention relates to a method for inhibiting the growth of multidrug resistant cells in a biological sample or a patient, which method comprises administering to the patient, or contacting said biological sample with a compound of formula I or II, or a composition comprising said compound.

Additionally, the present invention provides pharmaceutically acceptable derivatives of the inventive compounds, and methods of treating a subject using such compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents.

Another aspect of the invention relates to a method of treating or lessening the severity of a disease or condition associated with a proliferation disorder in a patient, said method comprising a step of administering to said patient, a compound of formula I or II, or a composition comprising said compound.

The compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of therapeutic agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any

particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, mute of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts (see, for example, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Tenth Edition, A. Gilman, J.Hardman and L. Limbird, eds., McGraw-Bill Press, 155-173, 2001, which is incorporated herein by reference in its entirety).

Another aspect of the invention relates to a method for inhibiting histone deacetylase activity in a biological sample or a patient, which method comprises administering to the patient, or contacting said biological sample with an inventive compound or a composition comprising said compound.

Furthermore, after formulation with an appropriate pharmaceutically acceptable excipient in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, creams or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered at dosage levels of about 0.001 mg/kg to about 50 mg/kg, from about 0.01 mg/kg to about 25 mg/kg, or from about 0.1 mg/kg to about 10 mg/kg of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect. It will also be appreciated that dosages smaller than 0.001 mg/kg or greater than 50 mg/kg (for example 50-100 mg/kg) can be administered to a subject. In certain embodiments, compounds are administered orally or parenterally.

Uses

The present invention provides novel compounds useful in the treatment of diseases or disorders associated with HDAC activity. The compounds are useful in the treatment of diseases or condition that benefit from inhibition of deacetylation activity (*e.g.*, HDAC inhibition). In particular, the compounds are useful in treating diseases that benefit from inhibiting a particular HDAC isoform or class of HADCs. In certain embodiments, the compounds are useful in treating a disease that benefits from inhibiting Class IIa HDACs. In certain embodiments, the inventive cinnamic hydroxymates are useful in the treatment of

cellular proliferative diseases, such as cancer *(e.g.*, cutaneous T-cell lymphoma) or benign proliferative diseases; autoimmune diseases; allergic and inflammatory diseases; diseases of the central nervous system (CNS), such as neurodegenerative diseases (*e.g.* Huntington's disease); vascular diseases, such as restenosis; musculoskeletal diseases; cardiovascular diseases; stroke; pulmonary diseases; gastric diseases; and infectious diseases.

In certain embodiments, the compounds of the present invention are useful as inhibitors of histone deacetylases and thus are useful as antiproliferative agents, and thus may be useful in the treatment of cancer, by effecting tumor cell death or inhibiting the growth of tumor cells. In certain exemplary embodiments, the inventive compounds are useful in the treatment of cancers and other proliferative disorders, including, but not limited to breast cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer, melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer, to name a few. In certain embodiments, the inventive anticancer agents are active against leukemia cells and melanoma cells, and thus are useful for the treatment of leukemias (*e.g.*, myeloid, lymphocytic, myelocytic and lymphoblastic leukemias) and malignant melanomas. In certain embodiments, the inventive compounds are active against cutaneous T-cell lymphoma. Additionally, as described hereein, the inventive compounds may also be useful in the treatment of protozoal infections. Additionally, as described herein, the inventive compounds may also be useful in the treatment of autoimmune or inflammatory diseases. Furthermore, as described herein, the inventive compounds may also be useful in the treatment of neurodegenerative diseases. As described herein, the inventive compounds may also be useful in the treatment of cardiovascular diseases. In certain exemplary embodiments, the compounds of the invention are useful for disorders resulting from protein deacetylation activity or reduced protein acetylation In certain exemplary embodiments, the compounds of the invention are useful for disorders resulting from histone deacetylation activity or reduced histone acetylation.

Uses according to the present invention, the inventive compounds may be assayed in any of the available assays known in the art for identifying compounds having antiprotozoal, HDAC inhibitory, hair growth, androgen signaling inhibitory, estrogen signaling inhibitory, and/or antiproliferative activity. For example, the assay may be cellular or non-cellular, *in vivo* or *in vitro*, high- or low-throughput format, *etc.*

Thus, in one aspect, compounds of this invention which are of particular interest include those which:

• exhibit HDAC inhibitory activity;

• exhibit HDAC Class I inhibitory activity (*e.g.*, HDAC1, HDAC2, HDAC3, HDAC8);

• exhibit HDAC Class II inhibitory activity (*e.g.*, HDAC4, HDAC5, HDAC6, HDAC7, HDAC9a, HDAC9b, HDRP/HDAC9c, HDAC10);

• exhibit HDAC Class IIa inhibitory activity (*e.g.*, HDAC4, HDAC5, HDAC7, HDAC9a, HDAC9b, HDRP/HDAC9c);

• exhibit HDAC Class IIb inhibitory activity (*e.g.*, HDAC6, HDAC10);

• exhibit HDAC Class III inhibitory activity;

• exhibit HDAC Class IV inhibitory activity (*e.g.*, HDAC11);

• exhibit Sirtuin inhibitory activity (*e.g.*, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7)

• exhibit the ability to inhibit HDAC1 (Genbank Accession No. NP_004955, incorporated herein by reference);

• exhibit the ability to inhibit HDAC2 (Genbank Accession No. NP_001518, incorporated herein by reference);

• exhibit the ability to inhibit HDAC3 (Genbank Accession No. 015739, incorporated herein by reference);

• exhibit the ability to inhibit HDAC4 (Genbank Accession No. AAD29046, incorporated herein by reference);

• exhibit the ability to inhibit HDAC5 (Genbank Accession No. NP_005465, incorporated herein by reference);

• exhibit the ability to inhibit HDAC6 (Genbank Accession No. NP_006035, incorporated herein by reference);

• exhibit the ability to inhibit HDAC7 (Genbank Accession No. AAP63491, incorporated herein by reference);

• exhibit the ability to inhibit HDAC8 (Genbank Accession No. AAF73428, NM_018486, AF245664, AF230097, each of which is incorporated herein by reference);

• exhibit the ability to inhibit HDAC9 (Genbank Accession No. NM_178425, NM_178423, NM_058176, NM 014707, BC111735, NM 058177, each of which is incorporated herein by reference)

• exhibit the ability to inhibit HDAC10 (Genbank Accession No. NM 032019, incorporated herein by reference)

• exhibit the ability to inhibit HDAC11 (Geabank Accession No. B0009676, incorporated herein by reference);

• exhibit the ability to inhibit SIRT1 (Genbank Accession No. NM 003173, NM 001098202, NM 006497, BC 012499, GL 000099, CM000261, each of which is incorporated herein by reference);

• exhibit the ability to inhibit SIRT2 (Genbank Accession No. NM 030593, NM 012237, CM000270, AC 000151, NM 033331, CU678487, AK290716, each of which is incorporated herein by reference);

• exhibit the ability to inhibit SIRT3 (Genbank Accession No. CM000262, NC 000011, AC 000143, NW 001838015, AC 000054, each of which incorporated herein by reference);

• exhibit the ability to inhibit SIRT4 (Genbank Accession No. AM270988, CM000263, NT 166525, NC 000012, NT 009775, AC 000144, each of which is incorporated herein by reference);

• exhibit the ability to inhibit SIRT5 (Genbank Accession No. AM270990, AM270988, CM000257, CM000663, GL000052, GL000006, each of which is incorporated herein by reference);

• exhibit the ability to inhibit SIRT6 (Genbank Accession No. CM000270, NC 000019, NW 001838477, AC 000151, incorporated herein by reference);

• exhibit the ability to inhibit SIRT7 (Genbank Accession No. NC 000017, NT 010663, AC 000149, NW 001838459, each of which is incorporated herein by reference);

• exhibit the ability to inhibit tubulin deacetylation (TDAC);

• exhibit the ability to inhibit the deacetylation of other acetylated proteins;

• exhibit cytotoxic or growth inhibitory effect on cancer cell lines maintained *in vitro* or in animal studies using a scientifically acceptable cancer cell xenograft model; and/or

• exhibit a therapeutic profile (*e.g.*, optimum safety and curative effect) that is superior to existing chemotherapeutic agents.

In certain embodiments, the compound's specificity against Class IIa HDACs relative to Class I's inhibition is 1:10. In other embodiments, said specificity is 1:50. In yet other embodiments, said specificity is 1:100. In certain embodiments, said specificity is 1:500. In other embodiments, said specificity is 1:1000.

In certain embodiments, the compound's specificity against Class IIa HDACs relative to Class IIb's inhibition is 1:10. In other embodiments, said specificity is 1:50. In yet other embodiments, said specificity is 1:100. In certain embodiments, said specificity is 1:500. In other embodiments, said specificity is 1:1000.

In certain embodiments, the compound's specificity against Class IIa HDACs relative to Class IV's inhibition is 1:10. In other embodiments, said specificity is 1:50. In yet other embodiments, said specificity is 1:100. In certain embodiments, said specificity is 1:500. In other embodiments, said specificity is 1:1000.

In certain embodiments, the compound's specificity against either HDAC4, 5, 7, 9 relative to either HDAC1, 2, 3, 6, or 8 is 1:10. In certain embodiments, the compound's specificity against either HDAC4, 5, 7, 9 relative to either HDAC1, 2, 3, 6, or 8 is 1:50. In certain embodiments, the compound's specificity against either HDAC4, 5, 7, 9 relative to either HDAC1, 2, 3, 6, or 8 is 1:100. In other embodiments, said specificity is 1:500. In yet other embodiments, said specificity is 1:1000.

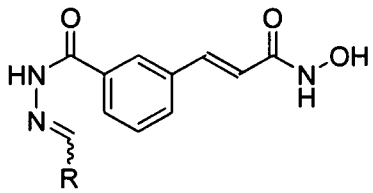
As detailed in the exemplification herein, in assays to determine the ability of compounds to inhibit HDAC activity certain inventive compounds may exhibit IC_{50} values $\leq$ 100 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 50 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 40 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 30 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 20 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$10 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 7.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 5 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 2.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 1 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 0.75 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 0.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 0.25 μM. In certain embodiments, inventive compounds exhibit IC_{50} values $\leq$ 0.1 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 75 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 50 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 25 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 10 nM. In other embodiments, exemplary compounds exhibited IC_{50} values $\leq$ 7.5 nM. In other embodiments, exemplary compounds exhibited IC_{50} values $\leq$ 5 nM.

In assays to determine the ability of compounds to inhibit cancer cell growth certain inventive compounds may exhibit IC_{50} values $\leq$ 100 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 50 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values $\leq$ 40 μM. In certain other embodiments, inventive

compounds exhibit IC_{50} values ≤ 30 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 20 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 10 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 7.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 5 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 2.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 1 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 0.75 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 0.5 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 0.25 μM. In certain embodiments, inventive compounds exhibit IC_{50} values ≤ 0.1 μM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 75 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 50 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 25 nM. In certain other embodiments, inventive compounds exhibit IC_{50} values ≤ 10 nM. In other embodiments, exemplary compounds exhibited IC_{50} values ≤ 7.5 nM. In other embodiments, exemplary compounds exhibited IC_{50} values ≤ 5 nM.

Methods of Synthesis

The invention also provides methods for preparing the inventive compounds. In one aspect of the invention, a method for synthesizing a compound of formula (I) is provided

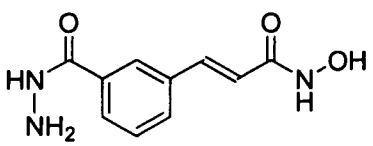


(I)

wherein R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl-moiety or a substituted or unsubstituted heteroaryl moiety;

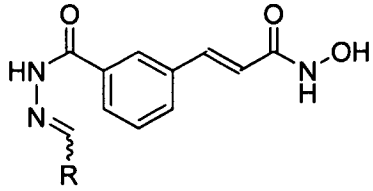
the method comprising :

reacting hydrazine of formula:



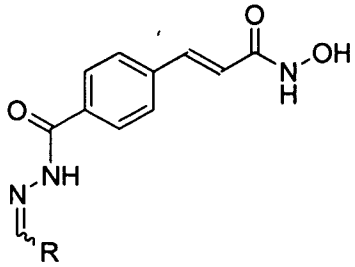
or a protected form thereof with an aldehyde of formula :

under suitable conditions to yield a compound of formula (I):



(I).

In another aspect of the invention, a method for synthesizing a compound of formula (II) is provided:

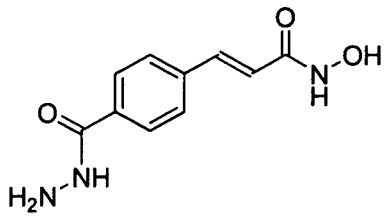


(II)

wherein R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl-moiety or a substituted or unsubstituted heteroaryl moiety;

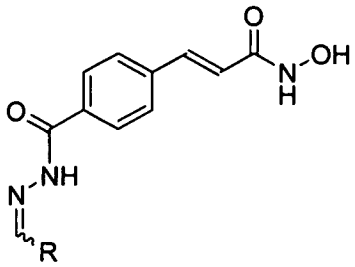
the method comprising :

reacting hydrazine of formula:



or protected form thereof with an aldehyde of formula :

under suitable conditions to yield a compound of formula (II):



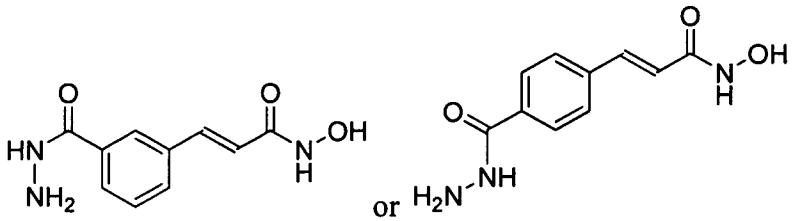
(II).

In certain embodiments, the methods described above are carried out in solution phase. In certain other embodiments, the methods described above are carried out on a solid phase. In certain embodiments, the synthetic method is amenable to high-throughput techniques or to techniques commonly used in combinatorial chemistry.

As would be appreciated by one of skill in the art, the suitable reaction conditions include, temperature, solvent, reaction time, concentration, *etc.* In certain embodiments, the solvent is a polar solvent. In other embodiments, the solvent is a non-nucleophilic solvent. In still other embodiments, the solvent is a polar aprotic solvent. In further embodiments, the solvent is DMF, dioxane, HMPT (hexamethylphosphorotriamide), THF, or Et_2O. In a specific embodiment, the solvent is DMSO.

In certain embodiments, the aldehyde is in a solution of 0.01-0.5 M. In other embodiments, the aldehyde is in solution of 0.1-0.25 M. In other embodiments, the aldehyde is in a solution of 0.2 M. In a specific embodiment, the aldehyde is in DMSO at a concentration of 0.2 M.

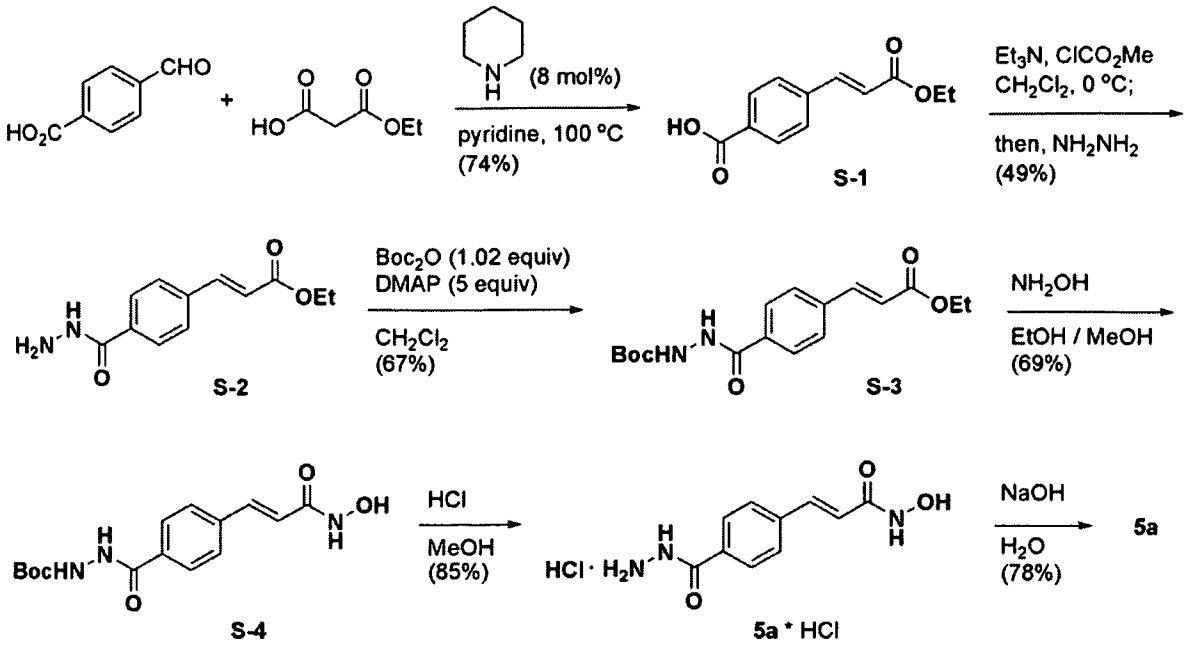
In certain embodiments, the hydrazine of general formula:



is in a solution of 0.01-1 M. In other embodiments, the hydrazine is in solution of 0.1-1 M. In other embodiments, the hydrazine is in a solution of 0.1-0.5 M. In yet other embodiments, the hydrazine is in a solution of 0.01-0.1 M. In a specific embodiment, the hydrazine is in DMSO at a concentration of 0.01 M.

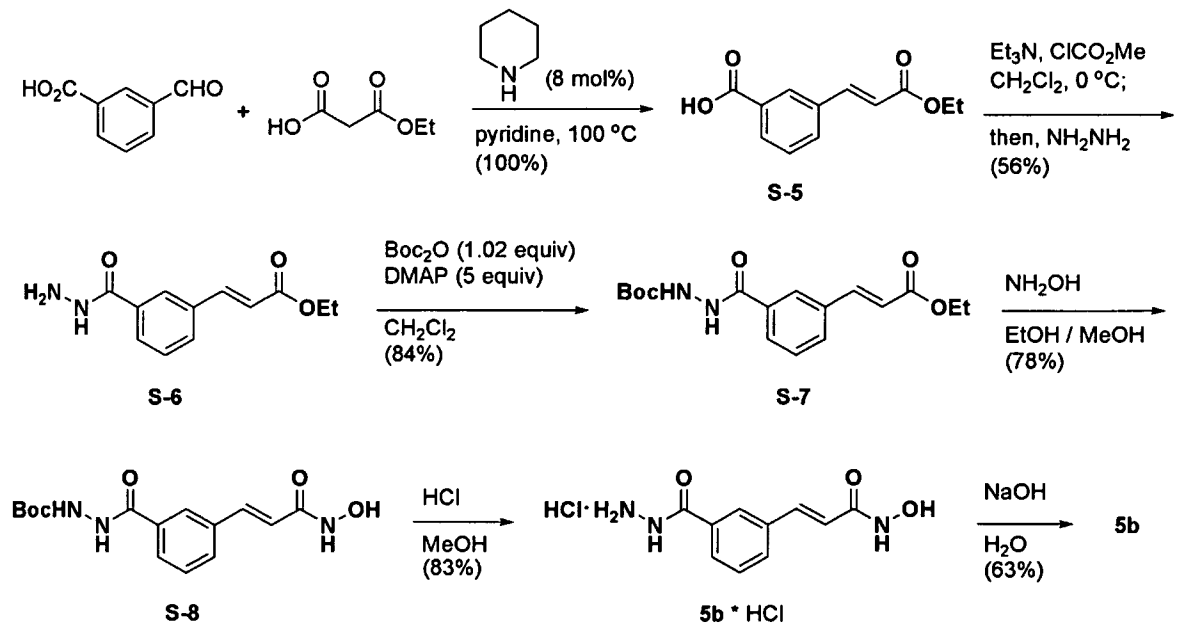
In certain embodiments, the starting material are synthesized. In other embodiments, the starting materials are purchased from a commercial source. The starting materials may be protected before reacting them. In certain embodiments, the hydrazine is synthesized as illustrated in Scheme 1.

Scheme 1



In certain embodiments, the hydrazine is synthesized as illustrated in Scheme 2.

Scheme 2



As will be appreciated by one of skill in the art, various changes to the synthetic schemes above may be made without departing from the scope of the invention.

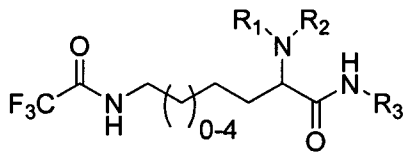
In certain embodiments, the reaction mixture of the hydrazine and the aldehyde is heated. In other embodiments, the reaction temperature is 50-120 °C. In yet other embodiments, the reaction temperature is 50-60 °C. In still other embodiments, the reaction

temperature is 60-70 °C. In certain embodiments, the reaction temperature is 70-80 °C. In other embodiments, the reaction temperature is 80-90 °C. In yet other embodiments, the reaction temperature is 90-100 °C. In still other embodiments, the reaction temperature is 100-110 °C. In certain embodiments, the reaction temperature is 110-120 °C. In a specific embodiment, the reaction temperature is 70 °C.

HDAC Assay

The invention also provides an assay to determine the inhibitory effect of a test compound on an HDAC protein. To overcome low catalytic turnover of assays for Class IIa HDAC, a new tripeptide substrate **4** (as shown in Figure 1), which features a relatively labile and sterically demanding trifluoroacetyl group that is readily hydrolyzed by the catalytically less avid Class IIa HDACs (Figure 1a,b) was prepared. With substrate **4**, Class IIa HDACs exhibit markedly faster kinetics further reducing requisite enzyme concentration (0.002-0.03 ng/μL; Supplementary Fig 2,3) and allowing a high-throughput, precise profiling of HDACi against all Class IIa enzymes (Figure 2).

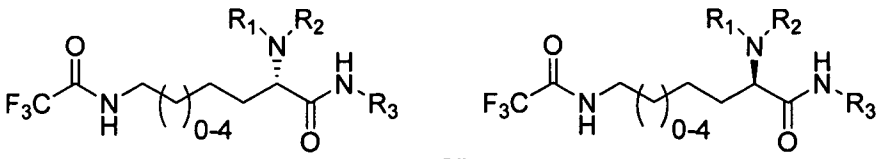
The assay comprises the steps of incubating an HDAC protein with a substrate of general formula (III)



(III)

wherein R_1, R_2, and R_3 are each independently hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR^B$; $-C(=O)R^B$; $-CO_2R^B$; $-C(=O)N(R^B)_2$; $-SR^B$; $-SOR^B$; $-SO_2R^B$; $-N(R^B)_2$; $-NHC(O)R^B$; or $-C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino; dialkylamino; heteroaryloxy; heteroarylthioxy; an amino acid; a peptide; a protecting group; or a tag; or salt thereof; in the presence of a test compound; and determining the activity of the HDAC protein.

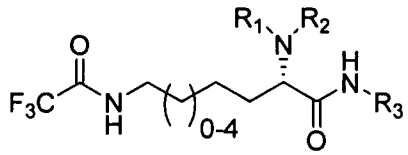
In certain embodiments, general formula (III) is



or

(IIIa) (IIIb) .

In other embodiments, general formula (IIIa) is



(IIIc) .

In certain embodiments, the step of determining the activity of the HDAC protein comprises monitoring the release of a tag from the substrate. In other embodiments, the step of determining the activity of the HDAC protein comprises monitoring the release of the tag from the substrate by an esterase or a protease. Preferably the tag released from the substrated is detectable by a chemical, cpectrophotometric, or physical means. In further embodiments, the protease is a serine protease. In still further embodiments, the serine protease is trypsin.

In certain embodiments, the tag is selected from the group consisting of a fluorescent tag, a bioluminescent tag, a chemoluminescent tag, a photoluminescent tag, a radioluminescent tag, and a thermoluminescent tag. In other embodiments, the tag is selected from the group consisting og: an epitope tag, an isotope tag, a radioactive tag, and a radiolabeled tag. In further embodiments, the tag is a spin label. In other embodiments, the tag comprises coumarin. In still other embodiments, the tag comprises a coumarin derivateive. In further embodiments, the tag is selected from the group consisting of: brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, or derivatives thereof. In specific embodiments, the tag comprises 7-amino-4-methylcoumarin.

In certain embodiments, the tag is removed by a chemical process. In other embodiments, the tag is removed by an enzymatic process. In still other embodiments, the tag is removed by a mechanical process.

In certain embodiments, the HDAC protein is a recombinant, full length HDAC protein. In other embodiments, the HDAC protein is a purified HDAC protein. In still other embodiments, the HDAC protein is a crude HDAC protein. In further embodiments, the HDAC protein is purified from natural sources. In other embodiments, the HDAC protein is a

modified form of an HDAC protein. In other embodiments, the HDAC protein is a mutant form of an HDAC protein. In other embodiments, the HDAC protein is a truncated form of an HDAC protein. In still other embodiments, the HDAC protein is a truncated form of an HDAC protein which includes at least an active site.

In certain embodiments, the assay is carried out at a concentration of the substrate greater than the substrate K_m. In other embodiments, the assay is carried out at a concentration of the substrate approximately equivalent to the substrate K_m.

In certain embodiments, the HDAC protein is a Class I HDAC. In other embodiments, the HDAC protein is a Class II HDAC. In certain embodiments, the HDAC protein is a Class IIa HDAC. In certain embodiments, the HDAC protein is a Class IIb HDAC protein. In still other embodiments, the HDAC protein is a Class III HDAC. In further embodiments, the HDAC protein is a Class IV HDAC. In certain embodiments, the HDAC protein is a sirtuin. In other embodiments, the HDAC protein is a protein with deacetylase activity.

In certain embodiments, the HDAC protein is HDAC1. In other embodiments, the HDAC protein is HDAC2. In specific embodiments, the HDAC protein is a sirtuin. In still other embodiments, the HDAC protein is HDAC3. In further embodiments, the HDAC protein is HDAC4. In certain embodiments, the HDAC protein is HDAC5. In other embodiments, the HDAC protein is HDAC6. In still other embodiments, the HDAC protein is HDAC7. In further embodiments, the HDAC protein is HDAC8. In further embodiments, the HDAC protein is HDAC9. In certain embodiments, the HDAC protein is HDAC10. In other embodiments, the HDAC protein is HDAC11.

The inventive assay is suitable for high-throughput screening, and multiple assy may be run in parallel. This aspect of the assay allows for the screening of many test compounds at multiple concentrations at once using more than one HDAC protein. In certain embodiments, multiple assays are run in parallel. In other embodiments, at least 10 assays are run in parallel. In still other embodiments, at least 50 assays are run in parallel. In further embodiments, at least 100 assays are run in parallel. In certain embodiments, at least 500 assays are run in parallel. In other embodiments, at least 1000 assays are run in parallel.

In certain embodiments, the assay is performed at approximately room temperature. In other embodiments, the assay is performed at approximately 25 °C. In still other embodiments, the assay is performed at approximately 37 °C. In further embodiments, the assay is performed at approximately 20-40 °C. In certain embodiments, the assay is performed below 25 °C. In other embodiments, the assay is performed above 25 °C. In still

other embodiments, the assay is performed at approximately 10-15 °C. In further other embodiments, the assay is performed at approximately 15-20 °C. In certain embodiments, the assay is performed at approximately 20-25 °C. In other embodiments, the assay is performed at approximately 25-30 °C. In still other embodiments, the assay is performed at approximately 30-35 °C. In further embodiments, the assay is performed at approximately 35-40 °C. In certain embodiments, the assay is performed at approximately 40-45 °C. In other embodiments, the assay is performed at approximately 45-50 °C. In still other embodiments, the assay is performed at approximately 50-60 °C. In further embodiments, the assay is performed above 60 °C. In ceratin embodiments, the assay is performed at any temperature at which an HDAC enzyme functions. In other embodiments, the assay is performed at a temperature optimum for an HDAC enzyme to function.

In certain embodiments, the assay is performed for approximately 30 seconds to 12 hours. In other embodiments, the assay is performed for approximately 30 seconds to 5 minutes hours. In still other embodiments, the assay is performed for approximately 5 minutes to 15 minutes. In further embodiments, the assay is performed for approximately 15 minutes to 30 minutes. In certain embodiments, the assay is performed for approximately 30 minutes to 1 hour. In other embodiments, the assay is performed for approximately 1 hour to 3 hours. In still other embodiments, the assay is performed for approximately 3 hours to 6 hours. In further embodiments, the assay is performed for approximately 6 hours to 9 hours. In certain embodiments, the assay is performed for approximately 9 hours to 12 hours. In certain embodiments, the assay is performed for less than 3 hours. In certain embodiments, the assay is performed for approximately 3 hours. In certain embodiments, the assay is performed for less than 12 hours. In other embodiments, the assay is performed for greater than 12 hours.

In certain embodiments, the assay is performed in water. In other embodiments, the assay is performed in an organic solvent. In still other embodiments, the assay in performed in a buffer. In certain embodiments, the buffer is an assay buffer. In other embodiments, the assay buffer comprises HEPES, KCl, Tween-20, BSA, and TCEP. In further embodiments, the assay buffer is 50 nM HEPES, 100 mM KCl, 0.001% Tween-20, 0.05% BSA, 200 μM TCEP, pH 7.4. In certain embodiments, the assay is performed at approximately pH 5.0-6.0. In certain embodiments, the assay is performed at approximately pH 5.0-6.0. In other embodiments, the assay is performed at approximately pH 6.0-6.5. In still other embodiments, the assay is performed at approximately pH 6.5-7.0. In further embodiments, the assay is performed at approximately pH 7.0-7.5. In certain embodiments, the assay is

performed at approximately pH 7.4. In other embodiments, the assay is performed at approximately pH 7.5-8.0. In still other embodiments, the assay is performed at approximately pH 8.0-9.0. In certain embodiments, the assay is performed at a pH optimum for an HDAC enzyme to function.

In certain embodiments, the concentration of the substrate is l-100 μM. In futher embodiments, the concentration of the substrate is 1-20 μM. In other embodiments, the concentration of the substrate is 1-5 μM. In still other embodiments, the concentration of the substrate is 5-10 μM. In yet other embodiments, the concentration of the substrate is 10-15 μM. In further embodiments, the concentration of the substrate is 15-20 μM. In other embodiments, the concentration of the substrate is 10-20 μM. In further embodiments, the concentration of the substrate is 20-30 μM. In certain embodiments, the concentration of the substrate is 30-40 μM In other embodiments, the concentration of the substrate is 40-50 μM. In still other embodiments, the concentration of the substrate is 50-60 μM. In further embodiments, the concentration of the substrate is 60-70 μM. In certain embodiments, the concentration of the substrate is 70-80 μM. In other embodiments, the concentration of the substrate is 80-90 μM. In still other embodiments, the concentration of the substrate is 90-100 μM. In certain embodiments, the concentration of the substrate is less than 20 μM. In other embodiments, the concentration of the substrate is greater than 20 μM.

In certain embodiments, the concentration of the HDAC protein is less than 1 ng/μL. In other embodiments, the concentration of the HDAC protein is greater than 1 ng/μL. In certain embodiments, the concentration of the HDAC protein is less than 5 ng/μL. In other embodiments, the concentration of the HDAC protein is greater than 5 ng/μL. I n certain embodiments, the concentration of the HDAC protein is 0.01-5 ng/μL. In other embodiments, the concentration of the HDAC protein is 0.01-0.05 ng/μL. In still other embodiments, the concentration of the HDAC protein is 0.05-0.1 ng/μL. In further embodiments, the concentration of the HDAC protein is 0.1-0.5 ng/μL. In certain embodiments, the concentration of the HDAC protein is 0.5-5 ng/μL.

In certain embodiments, the concentration of HDAC1 is approximately 1 ng/μL. In other embodiments, the concentration of HDAC1 is approximately 2 ng/μL. In still other embodiments, the concentration of HDAC1 is approximately 3 ng/μL. In further embodiments, the concentration of HDAC1 is approximately 4 ng/μL.

In certain embodiments, the concentration of HDAC2 is approximately 0.5 ng/μL. In other embodiments, the concentration of HDAC2 is approximately 0.75 ng/μL. In still other embodiments, the concentration of HDAC2 is approximately 1 ng/μL. In further

embodiments, the concentration of HDAC2 is approximately 1.25ng/μL. In other embodiments, the concentration HDAC2 is approximately 1.5ng/μL.

In certain embodiments, the concentration of HDAC3 is approximately 0.1 ng/μL. In other embodiments, the concentration of HDAC3 is approximately 0.15 ng/μL. In still other embodiments, the concentration of HDAC3 is approximately 0.2 ng/μL. In further embodiments, the concentration of HDAC3 is approximately 0.25ng/μL.

In certain embodiments, the concentration of HDAC4 is approximately 0.001 ng/μL. In other embodiments, the concentration of HDAC4 is approximately 0.0015 ng/μL. In still other embodiments, the concentration of HDAC4 is approximately 0.002 ng/μL. In further embodiments, the concentration of HDAC4 is approximately 0.0025ng/μL.

In certain embodiments, the concentration of HDAC5 is approximately 0.02 ng/μL. In other embodiments, the concentration of HDAC5 is approximately 0.025 ng/μL. In still other embodiments, the concentration of HDAC5 is approximately 0.03 ng/μL. In further embodiments, the concentration of HDAC5 is approximately 0.033 ng/μL. In certain embodiments, the concentration of HDAC5 is approximately 0.04 ng/μL.

In certain embodiments, the concentration of HDAC6 is approximately 0.75 ng/μL. In other embodiments, the concentration of HDAC6 is approximately 1.0 ng/μL. In still other embodiments, the concentration of HDAC6 is approximately 1.3 ng/μL. In further embodiments, the concentration of HDAC6 is approximately 1.75 ng/μL. In certain embodiments, the concentration of HDAC6 is approximately 2 ng/μL.

In certain embodiments, the concentration of HDAC7 is approximately 0.001 ng/μL. In other embodiments, the concentration of HDAC7 is approximately 0.002 ng/μL. In still other embodiments, the concentration of HDAC7 is approximately 0.003 ng/μL. In further embodiments, the concentration of HDAC7 is approximately 0.004 ng/μL. In certain embodiments, the concentration of HDAC7 is approximately 0.005 ng/μL.

In certain embodiments, the concentration of HDAC8 is approximately 0.02 ng/μL. In other embodiments, the concentration of HDAC8 is approximately 0.025 ng/μL. In still other embodiments, the concentration of HDAC8 is approximately 0.03 ng/μL. In further embodiments, the concentration of HDAC8 is approximately 0.035 ng/μL. In certain embodiments, the concentration of HDAC8 is approximately 0.04 ng/μL.

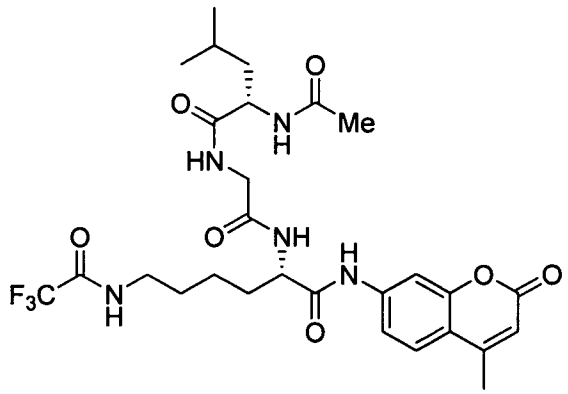
In certain embodiments, the concentration of HDAC9 is approximately 0.02 ng/μL. In other embodiments, the concentration of HDAC9 is approximately 0.025 ng/μL. In still other embodiments, the concentration of HDAC9 is approximately 0.03 ng/μL. In further

embodiments, the concentration of HDAC9 is approximately 0.035 ng/μL. In certain embodiments, the concentration of HDAC9 is approximately 0.04 ng/μL.

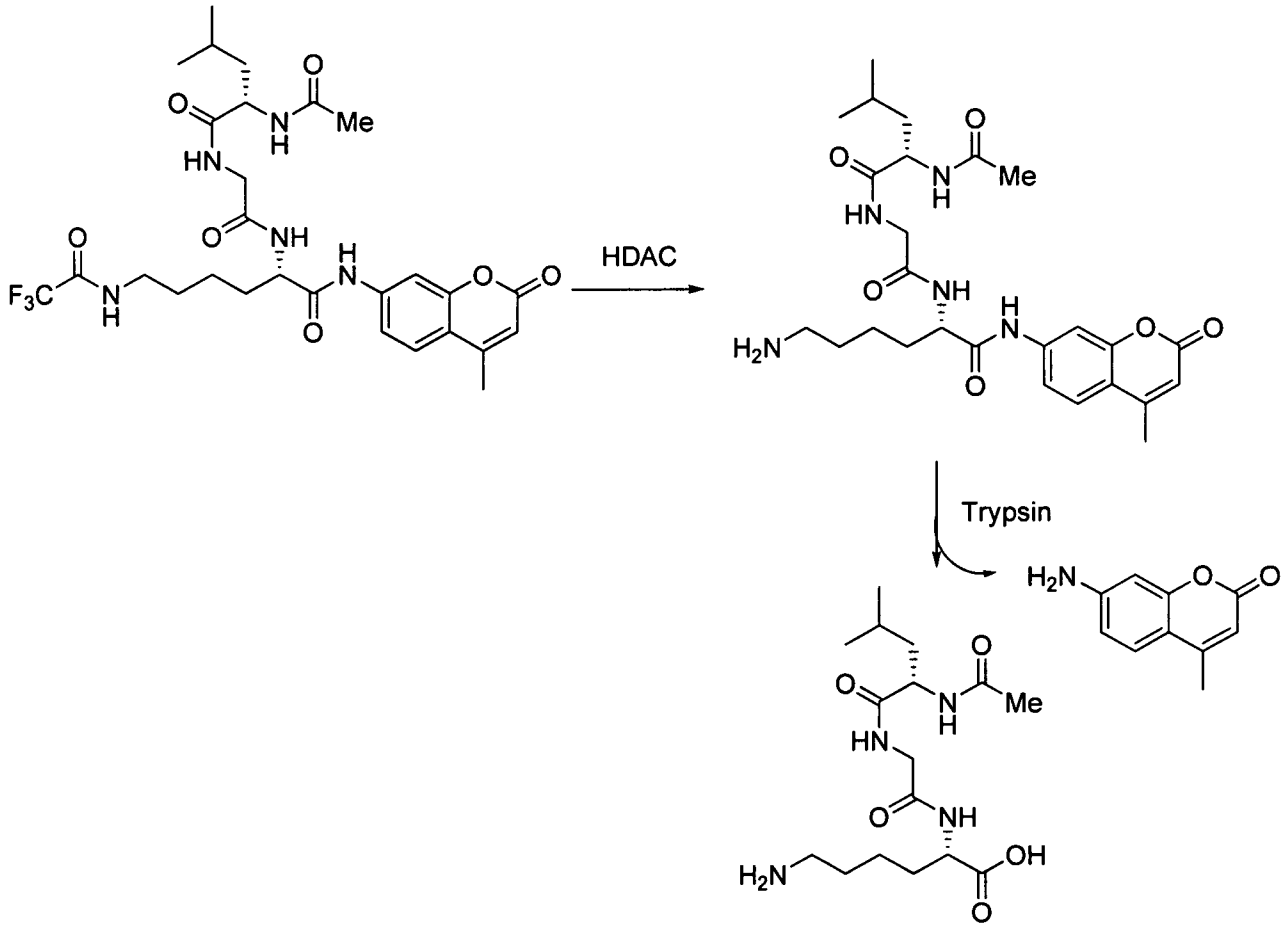
In certain embodiments, the concentration of the Sirtuin is approximately 100 to 1500 ng/μL. In other embodiments, the concentration of the Sirtuin is approximately 100-250 ng/μL. In still other embodiments, the concentration of the Sirtuin is approximately 250-500 ng/μL. In further embodiments, the concentration of the Sirtuin is approximately 500-750 ng/μL. In certain embodiments, the concentration of the Sirtuin is approximately 750-1000 ng/μL. In other embodiments, the concentration of the Sirtuin is approximately 1000-1250 ng/μL. In still other embodiments, the concentration of the Sirtuin is approximately 1250-1500 ng/μL. In further embodiments, the concentration of the Sirtuin is approximately 150 ng/μL.

In certain embodiments, the assay is performed at the same concentration per test compound. In other embodiments, the assay is performed at multiple concentrations per test compound.

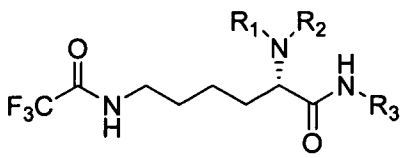
In another aspect, the invention provides an assay for determining the inhibitory effect of a test compound on an HDAC protein comprising: incubating the HDAC protein with a substrate of formula:



in the presence of a test compound; and determining the activity of the HDAC protein by monitoring the release of 7-amino-4-methylcoumarin after cleavage by trypsin. The assay is represented the scheme below.



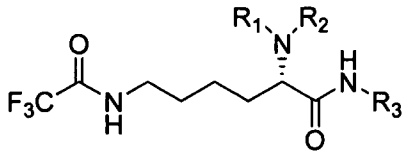
In yet another aspect, the invention provides an assay for determining the binding affinity of a test compound for an HDAC protein comprising incubating HDAC protein with a compound of general formula (IIIc)



(IIIc)

wherein R_1, R_2 and R_3 are as described herein, and determining binding of the test compound to the HDAC protein.

In another aspect of the invention, compounds of general formula (III) are used in the assay



(IIIc)

wherein R_1, R_2 and R_3 are as described herein.

In certain embodiments, R_1, R_2 and R_3 are each independently hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; OR^B; -$C(=O)R^B$; -CO_2R^B; -$C(=O)N(R^B)_2$; -SR^B; -SOR^B; -SO_2R^B; -$N(R^B)_2$; -$NHC(O)R^B$; or –$C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino; dialkylamino; heteroaryloxy; heteroarylthioxy; an amino acid; a peptide; a protecting group; or a tag; or pharmaceutically acceptable salt thereof. In further embodiments, neither R_1 nor R_2 is Boc. In certain embodiments, at least one of R_1, R_2, or R_3 is a tag. In other embodiments, R_1 or R_2 is a tag. In still other embodiments, R_1 and R_2 are tags. In certain embodiments, R_3 is a tag.

In certain embodiments, at least one of R_1, R_2, and R_3 is an amino acid. In other embodiments, R_1 is an amino acid. In still other embodiments, R_2 is an amino acid. In further embodiments, R_1 is an amino acid. In certain embodiments, R_2 is an amino acid. In other embodiments, R_3 is an amino acid.

In certain embodiments, at least one of R_1, R_2, and R_3 is a natural amino acid. In other embodiments, R_1 is a natural amino acid. In still other embodiments, R_2 is a natural amino acid. In further embodiments, R_1 is a natural amino acid. In certain embodiments, R_2 is a natural amino acid. In other embodiments, R_3 is a natural amino acid.

In certain embodiments, at least one of R_1, R_2, and R_3 is an unnatural amino acid. In other embodiments, R_1 is an unnatural amino acid. In still other embodiments, R_2 is an unnatural amino acid. In further embodiments, R_1 is an unnatural amino acid. In certain embodiments, R_2 is an unnatural amino acid. In other embodiments, R_3 is an unnatural amino acid.

In certain embodiments, at least R_1 or R_2 is hydrogen. In other embodiments, R_1 and R_2 are hydrogens. In further embodiments, R_1, R_2, and R_3 are hydrogens.

In certain embodiments, at least R_1 or R_2 is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In other embodiments, at least R_1 or R_2 is a C_{1-6} alkyl group. In still other embodiments, at least R_1 or R_2 is a C_{1-4} alkyl group. In certain embodiments, R_3 is cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic. In other embodiments, R_3 is a C_{1-6} alkyl group. In still other embodiments, R_3 is a C_{1-4} alkyl group.

In certain embodiments, at least one of R_1, R_2, and R_3 is a peptide. In other embodiments, R_1 is a peptide. In still other embodiments, R_2 is a peptide. In further embodiments, R_1 is a peptide. In certain embodiments, R_2 is a peptide. In other embodiments, R_3 is a peptide. In other embodiments, R_1, R_2, and R_3 are peptides.

In certain embodiments, at least one of R_1, R_2, and R_3 is a two-mer peptide. In other embodiments, R_1 is a two-mer peptide. In still other embodiments, R_2 is a two-mer peptide. In further embodiments, R_1 is a two-mer peptide. In certain embodiments, R_2 is a two-mer peptide. In other embodiments, R_3 is a two-mer peptide. In other embodiments, R_1, R_2, and R_3 are two-mer peptides.

In certain embodiments, at least one of R_1, R_2, and R_3 is a three-mer peptide. In other embodiments, R_1 is a three-mer peptide. In still other embodiments, R_2 is a three-mer peptide. In further embodiments, R_1 is a three-mer peptide. In certain embodiments, R_2 is a three-mer peptide. In other embodiments, R_3 is a three-mer peptide. In other embodiments, R_1, R_2, and R_3 are three-mer peptides.

In certain embodiments, at least one of R_1, R_2, and R_3 is a four-mer peptide. In other embodiments, R_1 is a four-mer peptide. In still other embodiments, R_2 is a four-mer peptide. In further embodiments, R_1 is a four-mer peptide. In certain embodiments, R_2 is a four-mer peptide. In other embodiments, R_3 is a four-mer peptide. In other embodiments, R_1, R_2, and R_3 are four-mer peptides.

In certain embodiments, one of R_1 or R_2 is a two-mer peptide; and the other of R_1 and R_2 is hydrogen or cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag. In other embodiments, one of R_1 or R_2 is a two-mer peptide; and the other of R_1 and R_2 is hydrogen.

In certain embodiments, one of R_1 or R_2 is a three-mer peptide; and the other of R_1 and R_2 is hydrogen or cyclic or acyclic, substituted or unsubstituted, branched or unbranched

aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag. In other embodiments, one of R_1 or R_2 is a three-mer peptide; and the other of R_1 and R_2 is hydrogen.

In certain embodiments, one of R_1 or R_2 is a four-mer peptide; and the other of R_1 and R_2 is hydrogen or cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag. In other embodiments, one of R_1 or R_2 is a four-mer peptide; and the other of R_1 and R_2 is hydrogen.

In certain embodiments, one of R_1 or R_2 is a nitrogen protecting group. In other embodiments, R_1 and R_2 are nitrogen protecting groups. In certain embodiments, R_1 is selected from the group consisting of t-Boc, Fmoc, benzyloxy-carbonyl, and alloc. In further embodiments, R_1 and R_2 are selected from the group consisting of t-Boc, Fmoc, benzyloxy-carbonyl, and alloc. In still further embodiments, R_1 and/or R_2 lithographic protecting group. In certain embodiments, at least R_1, R_2 , or R_3 is a tag. In other embodiments, R_1 is a tag. In further embodiments, R_3 is a tag. In certain embodiments, neither R_1, R_2 , nor R_3 is a tag.

In certain embodiments, the tag is selected from the group consisting of a fluorescent tag, a bioluminescent tag, a chemoluminescent tag, a photoluminescent tag, a radioluminescent tag, and a thermoluminescent tag. In other embodiments, the tag is selected from the group consisting og: an epitope tag, an isotope tag, a radioactive tag, and a radiolabeled tag. In further embodiments, the tag is a spin label.

In certain embodiments, at least R_1, R_2 , or R_3 comprises a chromophore. In other embodiments, R_1 comprises a chromophore. In still other embodiments, R_3 comprises a chromophore. In certain embodiments, at least R_1, R_2 , or R_3 comprises a fluorochrome. In other embodiments, R_1 comprises a fluorochrome. In still other embodiments, R_3 comprises a fluorochrome. In certain embodiments, at least R_1, R_2 , or R_3 comprises a ferromagnetic substance. In other embodiments, R_1 comprises a ferromagnetic substance. In still other embodiments, R_3 comprises a ferromagnetic substance.

In certain embodiments, at least R_1, R_2 , or R_3 comprises coumarin. In other embodiments, R_1 comprises coumarin. In still other embodiments, R_3 comprises coumarin. In

further embodiments, R_1, and R_3 comprises coumarin. In other embodiments, R_1, R_2, and R_3 comprises coumarin.

In certain embodiments, at least R_1, R_2 , or R_3 is selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof. In other embodiments, R_1 is selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof. In still other embodiments, R_3 is selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof. In further embodiments, R_1, and R_3 are selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof. In other embodiments, R_1, R_2 , and R_3 are selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof.

In certain embodiments, at least R_1, R_2 , or R_3 is 7-amino-4-methylcoumarin. In other embodiments, R_1 is 7-amino-4-methylcoumarin. In still other embodiments, R_3 is 7-amino-4-methylcoumarin. In further embodiments, R_1, and R_3 are 7-amino-4-methylcoumarin.

In certain embodiments, at least R_1, R_2 , or R_3 is ethidium bromide. In other embodiments, R_1 is ethidium bromide. In still other embodiments, R_3 is ethidium bromide. In further embodiments, R_1, and R_3 are ethidium bromide. In certain embodiments, at least R_1, R_2 , or R_3 is fluorescein. In other embodiments, R_1 is fluorescein. In still other embodiments, R_3 is fluorescein. In further embodiments, R_1, and R_3 are fluorescein.

In certain embodiments, at least R_1, R_2 , or R_3 can be cleaved by an enzyme. In other embodiments, R_1 can be cleaved by an enzyme. In still other embodiments, R_3 can be cleaved by an enzyme. In further embodiments, R_1, and R_3 can be cleaved by an enzyme. In other embodiments, R_1, R_2 , and R_3 can be cleaved by an enzyme. In certain embodiments, the enzyme is an esterase. In other embodiments, the enzyme is a protease. In further embodiments, the enzyme is trypsin.

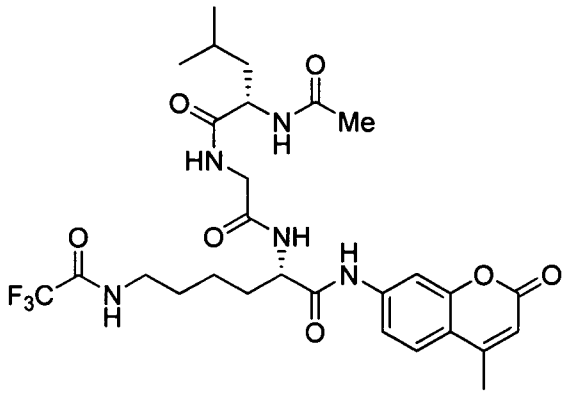
In certain embodiments, the tag is toxic to the cell once cleaved. In other embodiments, the tag is not toxic to the cell once cleaved.

In certain embodiments, at least R_1, R_2 , or R_3 can be cleaved by an enzyme. In other embodiments, R_1 can be cleaved by an enzyme. In still other embodiments, R_3 can be cleaved by an enzyme. In further embodiments, R_1, and R_3 can be cleaved by an enzyme. In other embodiments, R_1, R_2 , and R_3 can be cleaved by an enzyme. In certain embodiments, the

enzyme is an esterase. In other embodiments, the enzyme is a protease. In further embodiments, the enzyme is trypsin.

In certain embodiments, at least R_1, R_2 , or R_3 comprises the peptide sequence Leu-Gly. In other embodiments R_1, comprises the peptide sequence Leu-Gly. In still other embodiments, R_3 comprises the peptide sequence Leu-Gly.

In certain embodiments, the compound is



Equivalents

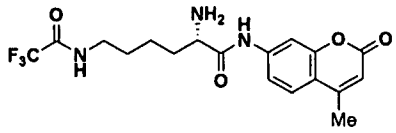
The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. It should further be appreciated that, unless otherwise indicated, the entire contents of each of the references cited herein are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

These and other aspects of the present invention will be further appreciated upon consideration of the following Examples, which are intended to illustrate certain particular embodiments of the invention but are not intended to limit its scope, as defined by the claims.

Examples

Example 1

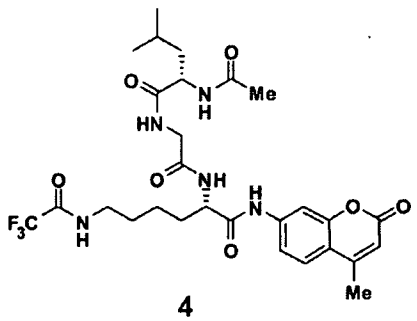
(*S*)-2-amino-N-(4-methyl-2-oxo-2H-chromen-7-yl)-6-(2,2,2-trifluoroacetamido)hexanamide (ε-trifluoroacetyl-L-lysine-AMC hydrochloride)



To a solution of Boc-ε-trifluoroacetyl-L-lysine-AMC (4.6 g, 9.2 mmol) in dry dichloromethane at 0 °C was added 5 mL of a 4 M solution of HCl / dioxane (Lahm, A. *et. al. Proc. Natl. Acad. Sci. U.S.A.* **2007**,*104*, 17335-17340). The reaction mixture was warmed to room temperature and stirred over night. The solvent was evaporated under reduced pressure to afford the desired product in quantitative yield (4.0 g) and excellent purity as white powder, which was used without further purification. ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H), 9.47 (s, 1H), 8.47 (s, 3H), 7.95 – 7.80 (m, 1H), 7.76 (d, J = 8.8, 1H), 7.58 (d, J = 8.1, 1H), 6.30 (s, 1H), 4.12 (s, 1H), 3.17 (d, J = 5.5, 2H), 2.41 (d, J = 6.7, 3H), 1.88 (s, 2H), 1.61 – 1.46 (m, 2H), 1.38 (d, J = 6.4, 2H); ^{13}C NMR (101 MHz, DMSO) δ 168.33, 159.93, 156.15 (q, J = 35.8), 153.54, 153.09, 141.46, 126.11, 115.64, 115.59 (q, J = 289 Hz), 115.47, 112.69, 106.07, 52.93, 38.79, 30.60, 27.75, 21.45, 18.04.

Example 2

(*S*)-2-(2-((*S*)-2-acetamido-4-methylpentanamido)acetamido)-*N*-(4-methyl-2-oxo-2H-chromen-7-yl)-6-(2,2,2-trifluoroacetamido)hexanamide (**4**)



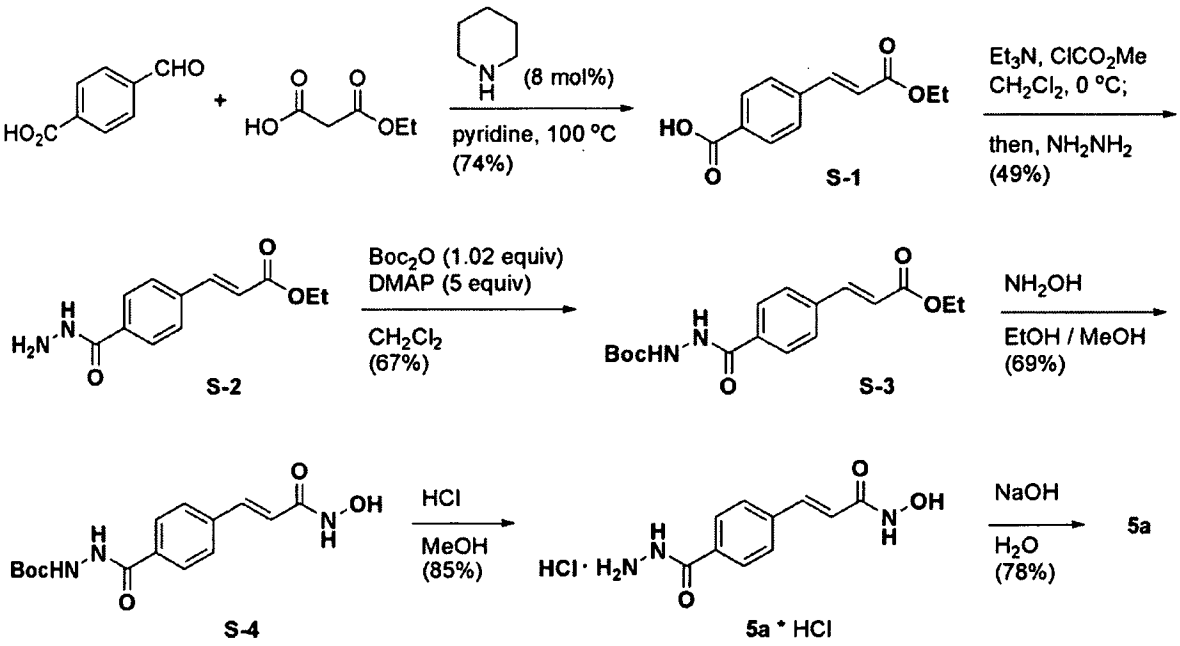
4

ε-trifluoroacetyl-L-lysine-AMC hydrochloride (1.35 g, 3.10 mmol) was added to a solution of N,N-diisopropylethylamine (2.5 mL) and Ac-Leu-Gly-OH (805 mg, 3.50 mmol) in 100mL anhydrous dichloromethane followed by PyBop (1.8 g, 3.5 mmol) in dichloromethane (5 mL). After stirring over night at room temperature the reaction mixture was diluted with dichloromethane (200 mL) and washed with dilute HCl and then saturated aqueous sodium bicarbonate solution. The organic layer was dried over sodium sulfate and the solvent was

removed under reduced pressure. The crude product was purified on silica gel (dichloromethane, MeOH 10:1) to yield the desired product as off-white solid (1.57 g, 83%). ^{1}H NMR (400 MHz, DMSO) δ 10.39 (s, 1H), 9.42 (t, J = 5.6, 1H), 8.35 (t, J = 5.8, 1H), 8.11 (d, J = 7.3, 1H), 8.05 (dd, J = 7.9, 4.0, 1H), 7.79 (d, J = 2.0, 1H), 7.71 (d, J = 8.7, 1H), 7.51 (dd, J = 8.7, 2.0, 1H), 6.26 (d, J = 1.2, 1H), 4.46 – 4.28 (m, 1H), 4.22 (dd, J = 15.0, 7.3, 1H), 3.85 – 3.63 (m, 2H), 3.16 (dd, J = 13.1, 6.8, 2H), 2.39 (d, J = 1.1, 3H), 1.86 (s, 3H), 1.81 – 1.19 (m, 9H), 0.85 (dd, J = 17.5, 6.5, 6H); ^{13}C NMR (101 MHz, DMSO) (mix of confomers) δ 172.93, 172.31, 171.38, 169.75, 169.13, 169.07, 166.34, 160.04, 156.10 (q, J = 36Hz), 153.65, 153.10, 142.13, 125.95, 115.98 (q, J=288Hz), 115.30, 115.16, 105.76, 53.53, 51.49, 50.88, 45.57, 44.89, 42.03, 41.24, 40.96, 40.52, 31.30, 27.98, 25.63, 24.18, 23.74, 23.09, 22.95, 22.72, 22.52, 22.49, 21.64, 21.55, 18.02.

Example 3

Synthesis of hydrazine **5a**.



Example 4

(*E*)-4-(3-ethoxy-3-oxoprop-1-enyl)benzoic acid (**S-1**)

To a flask was added 4-formylbenzoic acid (1.5 g, 10 mmol), 3-ethoxy-3-oxopropanoic acid (2.0 g, 15 mmol), piperidine (0.08 mL, 0.81 mmol), and pyridine (4 mL) at room temperature. The reaction mixture was heated to 100 °C for 18 h under a steady flow of nitrogen gas, cooled to room temperature, and poured into 2 M aqueous HCl (100 mL). The

resulting mixture was cooled to 0 °C and filtered. The filter cake was washed with acetonitrile (2 x 10 mL) and dried *in vacuo*. Cinnamyl ester S-1 (1.63 g, 74%) was isolated as a white solid and carried on to hydrazine formation without further purification.

Example 5

(*E*)-ethyl 3-(4-(hydrazinecarbonyl)phenyl)acrylate (**S-2**)

To a solution of **S-1** (0.44 g, 2.0 mmol) in dichloromethane (10 mL) was added triethylamine (0.36 mL, 2.0 mmol) and methyl chloroformate (0.19 mL, 2.0 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C before hydrazine (0.30 mL, 6.0 mmol) was added. The resulting solution was stirred for an additional 2 h at 0 °C. Saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixtures and the resulting biphasic solution was stirred for 30 min at room temperature. The organic layer was separated, dried, and the solvent removed via rotary evaporation. The resulting residue was purified by flash chromatography on silica (eluting with EtOAc) to yield compound **S-2** (0.23 g, 49%) as a white solid.

Example 6

(*E*)-*tert*-butyl 2-(4-(3-ethoxy-3-oxoprop-1-enyl)benzoyl)hydrazinecarboxylate (**S-3**)

To a solution of hydrazine **S-2** (6.00 g, 25.6 mmol) in dichloromethane (300 mL) was added Boc anhdyride (5.40 g, 26.2 mmol) and DMAP (12.5 g, 103 mmol). The mixture was stirred at room temperature for 3 h, concentrated, and loaded directly on to silica. Flash chromatography, eluting with 1:1 EtOAc / petroleum ether, yielded **S-3** (5.76 g, 67.3%).

Example 7

(*E*)-*tert*-butyl 2-(4-(3-(hydroxyamino)-3-oxoprop-1-enyl)benzoyl)hydrazinecarboxylate (**S-4**)

To a solution of **S-3** (5.76 g, 17.2 mmol) in methanol (300 mL) was added a solution of hydroxylamine hydrochloride (11.9 g, 171 mmol) in 1 M NaOH/ethanol (341 mL). The reaction mixture was stirred for 18 h and concentrated. The residue was dissolved in water to yield a colorless homogenous solution, which was neutralized to pH 7 by the addition of aqueous 1 M HCl. The resulting suspension was extracted with ethyl acetate. The combined organic extracts were dried and concentrated via rotary evaporation. Crude **S-4** was loaded on to silica and purified via flash chromatography, eluting with ethyl acetate, to yield **S-4** (3.80 g, 68.8%).

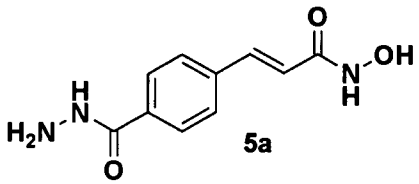
Example 8

(*E*)-3-(4-(hydrazinecarbonyl)phenyl)-*N*-hydroxyacrylamide hydrochloride (**5a**·HCl)

Boc protected hydrazine **S-4** (3.50 g, 10.9 mmol) was dissolved in 6 M HCl/methanol (20 mL) and stirred at ambient temperature for 1 h, while a white precipitate formed. The reaction mixture was filtered to yield the title compound as a white solid (2.38 g, 84.9%).

Example 9

(*E*)-3-(4-(hydrazinecarbonyl)phenyl)-*N*-hydroxyacrylamide (**5a**)

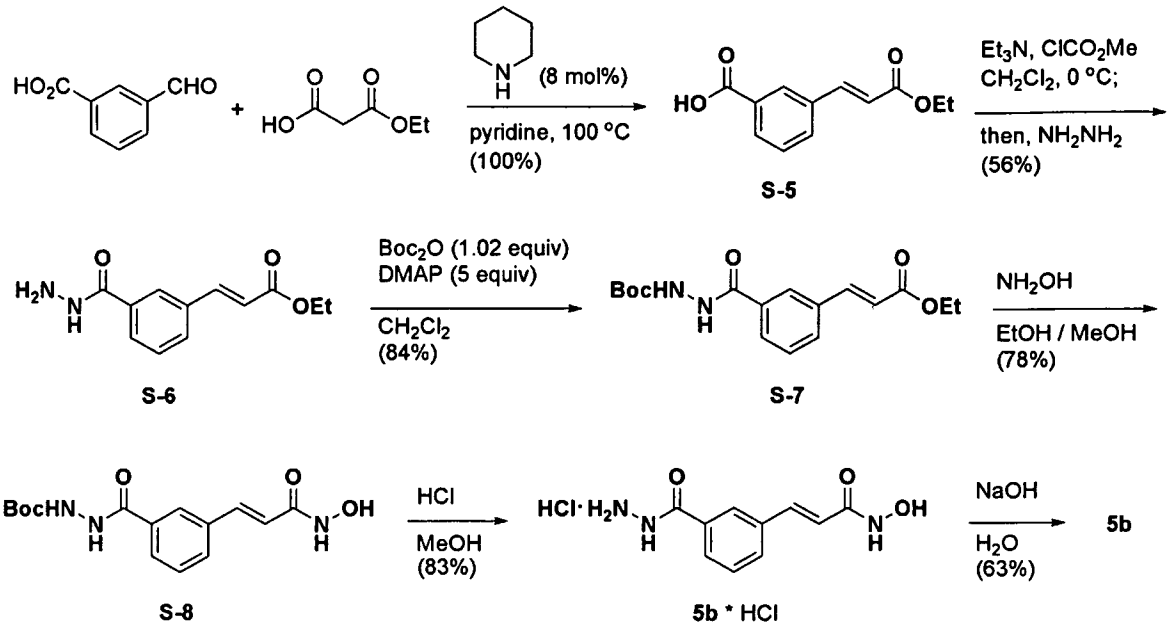


5a

A solution of 1 M aqueous NaOH was added dropwise to a suspension of **5a**·HCl (1.8 g, 7.0 mmol) in water (200 mL) until the pH reached 11. The colorless, homogeneous solution was neutralized with dilute aqueous HCl. The resulting precipitate was isolated via filtration and dried in vacuo to yield 5a (1.2 g, 78%) as a gray solid. ^{1}H NMR (500 MHz, DMSO) δ 10.85 (s, 1H), 9.84 (s, 1H), 9.12 (s, 1H), 7.85 (d, J = 7.8, 2H), 7.63 (d, J = 7.8, 2H), 7.49 (d, J = 15.8, 1H), 6.55 (d, J = 15.8, 1H), 4.72 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 165.92, 163.13, 138.08, 138.03, 134.44, 128.22, 128.07, 121.36.

Example 10

Synthesis of hydrazine **5b**.



Example 11

(*E*)-3-(3-ethoxy-3-oxoprop-1-enyl)benzoic acid (**S-5**)

To a flask was added 3-formylbenzoic acid (1.5 g, 10 mmol), 3-ethoxy-3-oxopropanoic acid (2.0 g, 15 mmol), piperidine (0.08 mL, 0.81 mmol), and pyridine (4 mL) at room temperature. The reaction mixture was heated to 100 °C for 18 h under a steady flow of nitrogen gas, cooled to room temperature, and poured into 2 M aqueous HCl (100 mL). The resulting mixture was cooled to 0 °C and filtered. The filter cake washed with acetonitrile (2 x 10 mL), and dried *in vacuo*. Cinnamyl ester **S-5** (2.20 g, 100%) was isolated as a white solid and carried on to hydrazine formation without further purification.

Example 12

(*E*)-ethyl 3-(4-(hydrazinecarbonyl)phenyl)acrylate (**S-6**)

To a solution of **S-5** (0.44 g, 2.0 mmol) in dichloromethane (10 mL) was added triethylamine (0.36 mL, 2.0 mmol) and methyl chloroformate (0.19 mL, 2.0 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C before hydrazine (0.30 mL, 6.0 mmol) was added. The resulting solution was stirred for an additional 2 h at 0 °C. Saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and the resulting biphasic soluton was stirred for 30 min at room temperature. The organic layer was separated, dried, and the solvent removed

via rotary evaporation. The resulting residue was purified by flash chromatography on silica (eluting with EtOAc) to yield compound **S-6** (0.26 g, 56%) as a white solid.

Example 13

(*E*)-*tert*-butyl 2-(4-(3-ethoxy-3-oxoprop-1-enyl)benzoyl)hydrazinecarboxylate (**S-7**)

To a solution of hydrazine **S-6** (6.00 g, 25.6 mmol) in dichloromethane (200 mL) was added Boc anhdyride (5.40 g, 26.2 mmol) and DMAP (12.5 g, 103 mmol). The mixture was stirred at room temperature for 3 h. The mixture was concentrated and loaded directly on to silica to yield **S-7** (7.2 g, 84%) following flash chromatography (eluting with 1:1 EtOAc/petroleum ether).

Example 14

(*E*)-*tert*-butyl 2-(4-(3-(hydroxyamino)-3-oxoprop-1-enyl)benzoyl)hydrazinecarboxylate (**S-8**)

To a solution of **S-7** (7.0 g, 20.8 mmol) in methanol (300 mL) was added a solution of hydroxylamine hydrochloride (14.5 g, 208 mmol) in 1 M NaOH in ethanol 420 mL). The reaction mixture was stirred for 18 h and then concentrated. The residue was dissolved in water to yield a colorless homogenous solution, which was neutralized to pH 7 by the addition of aqueous 1 M HCl. The resulting suspension was extracted with ethyl acetate. The combined organic extracts were dried and concentrated via rotary evaporation. Crude **S-8** was loaded on to silica and purified via flash chromatography, eluting with ethyl acetate, to yield **S-8** (5.2 g, 78%).

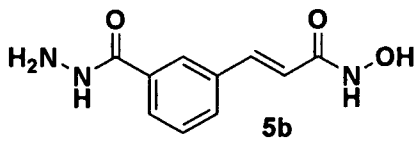
Example 15

(*E*)-3-(4-(hydrazinecarbonyl)phenyl)-*N*-hydroxyacrylamide hydrochloride (**5b**·HCl)

Boc protected hydrazine **S-8** (4.50 g, 14.0 mmol) was dissolved in 6 M HCl/methanol (30 mL) and stirred at ambient temperature for 1 h, while a white precipitate formed. The reaction mixture was filtered to yield the title compound as a white solid (3.0 g, 83%).

Example 16

(*E*)-3-(3-(hydrazinecarbonyl)phenyl)-*N*-hydroxyacrylamide (**5b**)

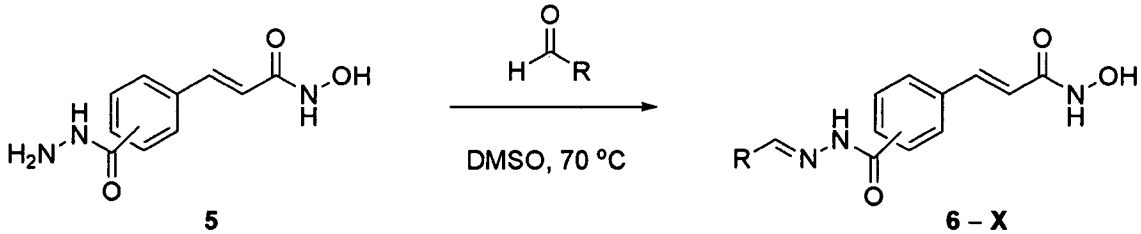


5b

A solution of 1 N aqueous NaOH was added dropwise to a suspension of **5b**·HCl (2.0 g, 7.8 mmol) in water (100 mL) until the pH reached 7. A precipitate formed and was isolated via filtration and dried in vacuo to yield **5b** (1.1 g, 63%) as a gray solid. ^{1}H NMR (500 MHz, DMSO) δ 10.81 (s, 1H), 9.85 (s, 1H), 9.08 (s, 1H), 8.02 (s, 1H), 7.80 (d, J = 7.5, 1H), 7.69 (d, J = 7.4, 1H), 7.63 – 7.36 (m, 2H), 6.55 (d, J = 15.8, 1H), 4.58 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 166.12, 163.22, 138.37, 135.63, 134.60, 131.04, 129.72, 128.45, 126.28, 120.75.

Example 17

Library synthesis

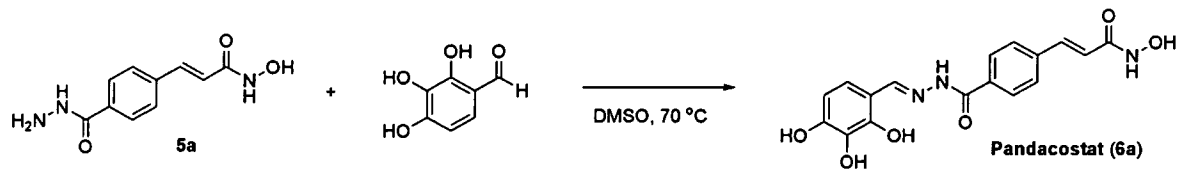


5 **6 – X**

Each well of a 96-well microtiter plate was charged with 10 μL of a distinct, commercially-available aldehyde (0.2 M in DMSO) and 190 μL of a stock solution of the appropriate isomer of **5** in DMSO (0.0105 M). The plate was heated at 70 °C for 36 h. LCMS analysis confirmed that a sampling of acyl hydrazone products were analytically pure (>95%). This stock plate of *m*- and *p*-substituted cinnamyl acyl hydrazones was used in screening, as described.

Example 18

(*E*)-*N*-hydroxy-3-(4-((*E*)-2-(2,3,4-trihydroxybenzylidene) hydrazinecarbonyl) phenyl) acrylamide (**6a**)



Compound **6a** was resynthesized and purified to be re-subjected to the biochemical assay to confirm the results from the initial library screen. To a 4 dram vial charged with 2,3,4-trihydroxybenzaldehyde (25.9 mg, 0.168 mmol) was added 420 μL of a 200 mM solution of hydrazine **5a** (0.084 mmol) in DMSO. The solution was heated on a rotating heating block at 70 °C for 16 h. Reaction progress was monitored via LCMS. Following purification by reverse phase preparatory LCMS (44 mL/min, CH_3CN/H_2O with 1% formic acid, 5 min

gradient), **6a** (7 mg) was isolated as a yellow powder (98% pure, by analytical LCMS). ^{1}H NMR (300 MHz, DMSO) δ 12.01 (s, 1H), 11.51 (s, 1H), 10.84 (s, 1H), 9.49 (s, 1H), 9.13 (s, 1H), 8.54 (s, 1H), 8.48 (s, 1H), 7.96 (d, J = 8.3, 2H), 7.73 (d, J = 8.2, 2H), 7.53 (d, J = 16.2, 1H), 6.80 (d, J = 8.6, 1H), 6.59 (d, J = 15.9, 1H), 6.40 (d, J = 8.4, 1H); *m/z* (ES–) 356 ([M-H]).

Example 19

The broad study of histone deacetylases in chemistry, biology and medicine relies on tool compounds to derive mechanistic insights. A phylogenetic analysis of Class I and II HDACs as targets of a comprehensive, structurally diverse panel of inhibitors revealed unexpected isoform selectivity even among compounds widely perceived as non-selective. These data informed the design of a focused library of cinnamic hydroxamates, which allowed the identification of a truly non-selective HDAC inhibitor.

Histone deacetylases (HDACs) regulate diverse cellular processes by modulating protein structure and function. Lysine acetylation is reversibly mediated by HDACs and acetyl transferases, establishing a dynamic post-translational modification state of broad relevance to cell signaling and state. As components of chromatin modifying enzyme complexes, HDACs target the amino-terminal tails of histone proteins affecting chromatin conformation and gene-specific transcription (Minucci, S. *et al.*, *Nat. Rev. Cancer* **2006**, *6*, 38-51; Lee, K. K. *et al.*, *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 284-295). Recent research has identified a significant number of non-histone protein substrates, extending the mechanistic relevance and research interest in HDACs well beyond the field of chromatin biology.

The common classification of human deacetylases is based on molecular phylogenetic analysis of primary structure, subsequently grouped based on homology to yeast enzymes LIT. This approach yields four distinct classes that vary in size and function. Class I (HDAC1, 2, 3 and 8), Class IIa (HDAC4, 5, 7 and 9), Class IIb (HDAC6 and 10) and Class IV (HDAC11) HDACs contain predicted zinc-dependent deacetylase domains (de Ruijter, A. J. *et al. Biochem. J.* **2003**, *370*, 737-749). The Class III proteins form a structurally and mechanistically distinct Class of NAD+dependent hydrolases (Sirtuins; Sirt1-7) (Smith, B. C. *et al. Chem. Biol.* **2008**, *15*, 1002-1013). Studies of human deacetylases have benefitted from the availability of small-molecule HDAC inhibitors (HDACi), most of which as a group obey a common "cap-linker-chelator" pharmacophore model (Sternson, S. M. *et al. Org. Lett.* **2001**, *3*, 4239-4242). The remarkable demonstration of pro-differentiation and anti-

proliferative effects in cancer model systems prompted the further development of these tool compounds into investigational agents for therapeutic use in humans. One pharmaceutical HDACi has been approved for use in humans (SAHA; Zolinza© (vorinostat) Merck Research Laboratories) and more than ten additional compounds are in advanced clinical testing (Bolden, I. E. *et al. Nat. Rev. Drug. Discov.* **2006**, *5*, 769-784). As such, there is considerable interest in HDACi as tool compounds for cellular biology and as therapeutic agents for the treatment of cancer, inflammatory conditions and infectious diseases.

Widely maintained is the perception that many of the currently used small-molecule inhibitors are non-selective (Bolden, I. E. *et al. Nat. Rev. Drug. Discov.* **2006**, *5*, 769-784). Recent research has revealed unique aspects of Class IIa HDAC biochemistry, which calls into question the accuracy of prior homogeneous assays for reporting target potency (Jones, P. *et al. Bioorg. Med. Chem. Lett.* **2008**, *18*, 1814-1819). This is problematic as the mechanistic understanding of Class IIa HDACs is expanding, enhanced by the availability of genetic probes of protein function such as silencing reagents and knock-out mice (Zhou, X. *et al. Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1056-1061; Parra, M. *et al. J. Biol. Chem.* **2005**, *280*, 13762-13770; Mottet, D. *et al. Circ. Res.* **2007**, *101*, 1237-1246; Renthal, W. *et al. Neuron* **2007**, *56*, 517-529; Tsankova, N. M. *et al. Nat. Neurosci.*, **2006**, *9*, 519-525; Bolger, T. A. *et al. J. Neurosci.* **2005**, *25*, 9544-9553; Cohen, T. J. *et al. J. Biol. Chem.* **2007**, 282, 33752-33759). Key regulatory roles have been suggested in immune tolerance, cardiac remodeling and neuronal death. We therefore endeavored to derive a more complete knowledge of isoform-specific potency and to instruct a more thoughtful use of these compounds as chemical probes of discrete HDAC targets in both the research and clinical setting.

We have synthesized and assembled a panel of structurally-diverse small-molecule HDACi that resemble most of the relevant literature reported tool compounds and pharmacologically developed clinical candidates (Figure 2). Recently, we have optimized a miniaturized kinetic assay for biochemical profiling of HDAC1, 2, 3, 6 and 8 (Bowers, A. *et al. J. Am. Chem. Soc.* **2008**, *130*, 11219-11222). However, implementation of this assay for Class IIa HDACs proved challenging due to the low catalytic turnover of the acetylated tripeptide substrate (**1**) as well as a Class IIa-specific substrate reported by Jones *et.al.* (**2**), both of which require a prohibitively significant amount of enzyme (Jones, P. *et al. Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1814-1819; Riester, D. *et al. Biochem. Biophys. Res. Commun.* **2004**, *324*, 1116-1123). During assay development, we observed diminished turnover by Class I HDACs of Boc-protected substrate **3** compared to tripeptide substrate **1** (Figure 4)

(Riester, D. *et al. Biochem. Biophys. Res. Commun.* **2004**, *324*, 1116-1123). We therefore devised a new tripeptide substrate **4**, which features as **2** the relatively labile and sterically more demanding trifluoroacetyl group that is readily hydrolyzed by the catalytically less avid Class IIa HDACs (Fig 1a,b). With substrate **4**, Class IIa HDACs exhibit markedly faster kinetics further reducing requisite enzyme concentration (0.002-0.03 ng/μL; Figures 4 and 5) and allowing a high-throughput, precise profiling of HDACi against all Class IIa enzymes (Figure 2).

Using statistical methods validated for assessing evolutionary relatedness, we constructed a chemical genetic phylogeny of deacetylases derived from these kinetic data (Fig 1c). This approach was selected to prompt inferences into biochemical, pharmacologic and structural relationships. The analysis revealed a number of unexpected findings. First and foremost, the Class IIa enzymes are not targeted by most HDACi at pharmacologically-relevant concentrations. None of the inhibitors tested demonstrated a preference for Class IIa enzymes. In fact, significant inhibitory activity was only observed several orders of magnitude above the *Ki* for Class I/IIb enzymes. Consequently, none of the inhibitors tested is suitable for use as a tool compound to inhibit Class IIa function in settings where Class I/IIb enzymes are functionally present (i.e. in cells).

Interrogating the bidirectional hierarchical clustering of small molecules and proteins, remarkable chemotype-deacetylase relationships emerge. Driving the striking alignment of HDACi are principally the linker-chelator features, as most clearly observed with the benzamides (ortho-aminoanilides MS-275, CI-994 and MGDC-0103). In the second dimension, a provocative correlation was observed when comparing this chemical phylogeny to the molecular phylogeny of HDACII-9 (Figure 6). HDACs with known, high sequence and predicted structural identity exhibit relatedness in both analyses. Yet pharmacology defies phylogeny for HDAC6 and HDAC8, between which Class assignments are reversed. Here, the inhibitor sensitivity emulates the substrate preferences, as for all deacetylases studied (Figure 1b), rendering **4** also the preferred reagent for biochemical studies of HDAC8.

The inability of orthoaminoanilides to inhibit Class IIa HDACs was not surprising based on prior studies of HDAC6 and HDAC8, which suggested extraordinary selectivity for HDAC1, 2 and 3. However, the overall lack of potency of hydroxamic acid-based inhibitors was highly unexpected. We interpret this observation based on the available crystal structures HDAC4 (2VQM) and HDAC7 (3COZ, 3C10) bound to hydroxamate inhibitors. None of the ligand-protein complexes shows the expected bidentate chelation geometry of the central Zinc cation, as observed in the structures of ligand-bound human HDAC8 (1T64, 1T69) and

bacterial homologs (*e.g.* 1ZZ7). According to calculations by Wiest and Helquist, the tight bidentate complexation is a result of the deprotonation of the hydroxamic acid upon ligand binding (Wang, D. *et al. J. Org. Chem.* **2007**, *72*, 5446-5549). The observed geometry in the published structures, however, is more in line with weaker monodentate binding mode of the neutral form of the hydroxamic acid (Figure 7) (Wang, D. *et al. J. Org. Chem.* **2007**, *72*, 5446-5549). Common to all Class IIa HDACs is the substitution of a tyrosine residue in the active site, which is conserved in Class I enzymes, as a histidine. Arrowsmith (HDAC7) and Gallinari and Jones (HDAC4) have shown that the mutation of the respective histidine to tyrosine markedly increases the biochemical activity of both enzymes (Schuetz, A. *et al. J. Biol. Chem.* **2008**, *283*, 11355-11363; Bottomley, M. J. *et al. J. Biol. Chem.* **2008**, *283*, 26694-26704; Lahm, A. *et al. Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*,17335-17340). Interestingly, in the Class I structures, this tyrosine forms a hydrogen bond to the hydroxamic acid carbonyl, which will increase binding affinity through hydrogen bond formation and as we speculate, sufficiently lower the pKa of the bound chelator facilitating deprotonation and consequently tighter binding. Consistent with this model is the 100-fold increased affinity observed with the hydroxamate LAQ-824 for the H976Y HDAC4 gain of function mutant (Jones, P. *et al. Bioorg. Med. Chem. Lett.* **2008**, *18*, 1814-1819; Schuetz, A. *et al. J. Biol. Chem.* **2008**, *283*, 11355-11363; Bottomley, M. J. *et al. J. Biol. Chem.* **2008**, *283*, 26694-26704). These observations may explain, in part, the differential potency of hydroxamate-based HDAC inhibitors and provide useful guidance for Class IIa-selective inhibitor design.

The new knowledge that HDACi are, indeed, much more selective than previously appreciated incited an interest to discover a truly non-selective inhibitor. Such a tool compound would have great utility to the research community. As suggested by the chemical phylogenetics, the central clustering of cinnamic hydroxamates suggests this pharmacophore as most leveraged for non-selectivity. We and others have observed dramatic contributions to ligand potency and selectivity by the structure and conformation of HDACi capping features (Bowers, A. A. *et al., J. Am. Chem. Soc.* **2009**; Wong, J. C. *et al. Chem. Biol.* **2004**, *11*, 1279-1291). Thus, we endeavored to expand a library of capped cinnamic hydroxamic acids, based on a high-throughput, parallel synthesis scheme we have used previously with success in targeting individual HDACs (Vegas, A. J. *et al., Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 7960-7964; Patel, V. *et al. J. Med. Chem.* **2009**). This approach involves the clean and efficient condensation of a hydrazide-based linker-chelator feature with a diverse collection of aldehydes to readily explore the chemical space of the capping group. Meta- and para-

substituted hydrazide-functionalized cinnamic hydroxamic acids were prepared and condensed with a set of 160 aliphatic and aromatic aldehydes to yield a HDAC-biased library of 320 compounds (Figure 3a). The entire library was profiled against Class I and IIa HDACs in dose-ranging format to provide a richly annotated data set.

The capping feature was confirmed to confer a dramatic effect on target potency, as shown in Figure 3b. Pair-wise comparison of potency for individual deacetylases revealed a substantial impact of linker substitution and geometry on target selectivity, particularly evident between HDAC6 and other Class IIa enzymes (Figure 3c,d). Based on these profiling data, we selected four compounds with high potency against Class IIa HDACs relative to Class I inhibition. These compounds were resynthesized on 30 mg scale, purified by reversed phase HPLC and assayed in dense dose-response format for the accurate determination of potency and selectivity. One compound was identified, which uniformly inhibited all profiled HDAC isoforms, in contrast to control compounds MS-275, SAHA and trichostatin A (Figure 3e,f,g; Figures 8 and 9). We term this compound pandacostat (Figure 3h).

We present, for the first time, the kinetic study of the biochemically active HDACs and a comprehensive library of tool and pharmaceutical deacetylase inhibitors. These data are derived from robust assays and a novel substrate, which allow for the rapid and efficient study of Class IIa HDACs. Our studies have revealed the unexpected selectivity of previously perceived "non-selective" HDAC inhibitors. From literature-reported crystallographic data and *ab initio* calculations, we provide a rationale for the diminished potency that will guide future ligand development for Class IIa HDACs. Recognizing the broad, potential utility of a non-selective HDACi, we synthesized a library of Class IIa-biased inhibitors and identified the first pan-HDACi reported, to date. In studying the chemical phylogenetics of HDACs, we demonstrate how a focused, structurally-diverse library of small molecules can be used for the functional classification of a protein family.

Example 20

Biochemical HDAC assay

The inhibitory effect of compounds on HDAC1-9 function was determined *in vitro* using an optimized homogenous assay performed in 384-well plate format. In this assay, recombinant, full-length HDAC protein (HDAC1 3.33 ng/μL, HDAC2 1 ng/μL, HDAC3/NCOR2 0.17 ng/μL, HDAC4 0.0016 ng/μL, HDAC5 0.033 ng/μL, HDAC6 1.3 ng/μL, HDAC7 0.0033 ng/μL, HDAC8 0.033 ng/μL, HDAC9 0.033 ng/μL; BPS Biosciences) is incubated with a commercially-available fluorophore conjugated substrate at

a concentration equivalent to the substrate K_m (1.6 μM for HDAC1, 3 μM for HDAC2, 6 μM for HDAC3 and 16 mM for HDAC6; concentrations of 4 for HDAC4, 5, 7, 8, 9 are provided Figure 5f). Reactions are performed in assay buffer (50 mM HEPES, 100 mM KCl, 0.001% Tween-20, 0.05% BSA, 200 μM TCEP, pH 7.4) and followed for fluorigenic release of 7-amino-4-methylcoumarin from substrate upon deacetylase and trypsin enzymatic activity. Fluorescence measurements are obtained approximately every five minutes using a multilabel plate reader and plate-stacker (Envision; Perkin-Elmer). Data are analyzed on a plate-by-plate basis for the linear range of fluorescence over time. The first derivative of data obtained from the plate capture corresponding to the mid-linear range is imported into analytical software and annotated with well identity and compound concentration (Spotfire DecisionSite). Replicate experimental data from incubations with inhibitor are normalized to control, solvent-only wells.

Example 21

Statistical methods

Biochemical inhibition of HDAC enzymes by small-molecule inhibitors is measured as described in Example 20. Data are analyzed by logistic regression with determination of IC50 and standard deviation (Spotfire DecisionSite and GraphPad Prism). Calculation of Ki is determined using a derivation of the standard formula Ki = [Inhibitor]/((VON;)*(1+S/Km))-[Substrate]/Km)-1. Multiple sequence alignment of human HDAC1-9 by Multiple Alignment were performed using Fast Fourier Transform (MAFFT). Amino acid sequences for each human histone deacetylase were retrieved from the National Centers for Biotechnology Information (HDAC1 Accession No. Q13547; HDAC2 Accession No. Q92769; HDAC3 Accession No. 015379; HDAC4 Accession No. P56524; HDAC5 Accession No. Q9UQL6; HDAC6 Accession No. Q9UBN7; HDAC7 Accession No. Q8WUI4; HDAC8 Accession No. Q9BY41; HDAC9 Accession No. Q9BY41). Alignments were generated using MAFFT version 6 (online portal; http://align.bmr.kyushu-u.ac.jp/maff /softwareo, as described). Phylogenetic analysis was performed as described in Figure 6. In brief, first a neighbor- joining method with bootstrap resampling was utilized to compute evolutionary distance data for all conserved sites (Saitou *et al. Mol. Biol. Evol.* **1987**, *4*, 406-425). Amino acid replacement was performed using the maximum likelihood approach of Whelan and Goldman (Whelan *et al. Mol. Biol. Evol.* **2001**, *18*, 691-699). Analyses were performed using the online research portal of Dr. Katch (http://align.bmr.kyushu-u.ac.jp/mafft/software/). Phylogenetic tree reconstruction was performed on MAFFT aligned

sequence using reported rapid bootstrapping and rapid maximum likelihood search algorithms (Randomized Axelerated Maximum Likelihood (RAxML) 5; Cyberinfrastructure for Phylogenetic Research online portal; hftp://www.phylo.org/). Phylogenetic trees were generated using Molecular Evolutionary Genetics Analysis software 6. Bidirectional hierarchical clustering was performed on biochemical profiling data (Ki) for each HDAC1-9 by generating a pairwise distance matrix using the unweighted pair group method with arithmetic mean and a Euclidean distance similarity measure (Spotfire DecisionSite).

Example 22

The purpose of the study is to determine the effects of pandacostat on the enzymatic activities of recombinant human Sirtuins using *in vitro* enzymatic assays.

Compound	Compound Supplied	Dissolving Solvent	Stock Concentration	Test Range (μM)	Intermediate Dilution
Pandacostat	Solution		10 mM	0.003-100	10% DMSO in HDAC Assay Buffer

A series of compound dilutions (10 fold higher than final concentrations) are made in 10% DMSO in HDAC assay buffer. 5μl of each dilution is added to 50μl of the reaction mixture so that the final concentration of DMSO is 1% in all of reactions.

Enzymes and Substrates

Assay	Enzyme (ng) /Reaction	Substrate
Sirtuin1	200	10μM HDAC Substrate 1
Sirtuin2	1,500	10μM HDAC Substrate 1
Sirtuin3	1,000	10μM HDAC Substrate 1

All of the enzymatic reactions were conducted in duplicate at room temperature for 3 hours in a 50 μl mixture containing HDAC assay buffer, 5 μg BSA, 100 μM NAD^+, 10 μM HDAC substrate 1, a sirtuin enzyme, and the test compound.

After enzymatic reactions, 50μl of 2 x HDAC Developer was added to each well and the plate was incubated at room temperature for an additional 20 minutes. Fluorescence intensity was measured at an excitation of 360 nm and an emission of 460 nm using a BioTek Synergy™ 2 microplate reader.

Sirtuin activity assays were performed in duplicates at each concentration. The fluorescent intensity data were analyzed using the computer software, Graphpad Prism. In the absence of the compound, the fluorescent intensity (F_t) in each data set was defined as 100% activity. In the absence of the sirtuin, the fluorescent intensity (F_b) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: %activity = $(F-F_b)/(F_t-F_b)$, where F= the fluorescent intensity in the presence of the compound, F_b= the fluorescent intensity in the absence of the sirtuin, and F_t = the fluorescent intensity in the absence of the compound.

The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y=B+(T-B)/1+10^{((LogEC50-X)\times Hill\ Slope)}$, where Y=percent activity, B=minimum percent activity, T=maximum percent activity, X= logarithm of compound and Hill Slope=slope factor or Hill coefficient. The IC_{50} value was determined by the concentration causing a half-maximal percent activity.

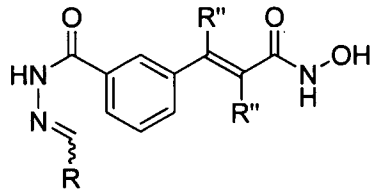
The IC_{50} values of the compound against sirtuins are summarized in Figure 12. If the IC_{50} value is not available, the % inhibition of the compound at the highest testing concentration was calculated.

The effects of pandacostat on the individual Sirtuin activity are summarized in Figures 13-18. Depicted in Figure 16 about 62% inhibition of Sirtuin2 activity is observed at 100 μM pendactostat, while in Figure 18 about 57% inhibition of Sirtuin3 activity is observed at 100 μM of pendacostat.

Claims

What is claimed is:

1. A compound of the formula (I):



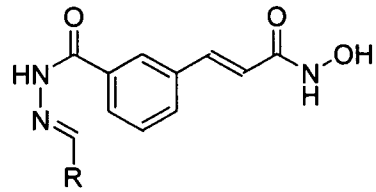
(I)

wherein

R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl moiety; or a substituted or unsubstituted heteroaryl moiety;

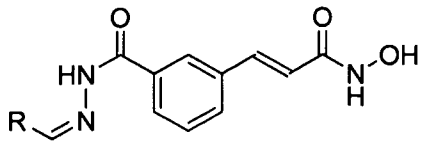
each occurrence of R″ is independently hydrogen, halogen, or C_{1-6}alkyl; and pharmaceutically acceptable salts thereof.

2. The compound of claim 1, wherein formula (I) is formula (I-1):



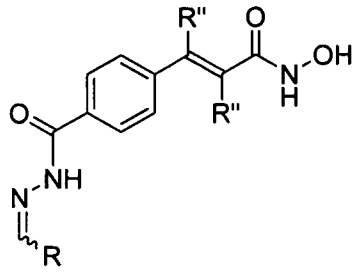
(I-1).

3. The compound of claim 1, wherein formula (I) is formula (I-2):



(I-2).

4. A compound of the formula (II):



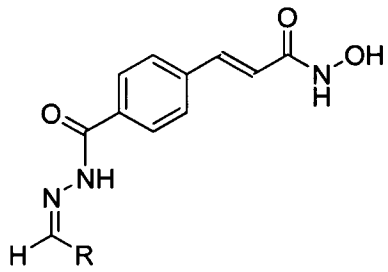
(II)

wherein

R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl moiety; or a substituted or unsubstituted heteroaryl moiety;

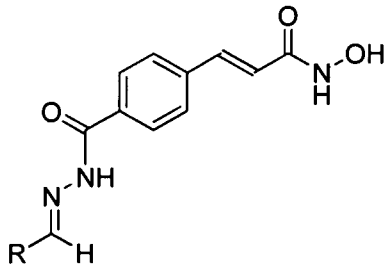
each occurrence of R″ is independently hydrogen, halogen, or C_{1-6}alkyl; and pharmaceutically acceptable salts thereof.

5. The compound of claim 4, wherein formula (II) is formula (II-2):



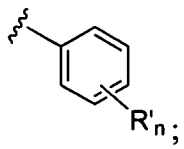
(II-1).

6. The compound of claim 4, wherein formula (II) is formula (II-2):



(II-2).

7. The compound of any one of claims 1-6, wherein R is



wherein

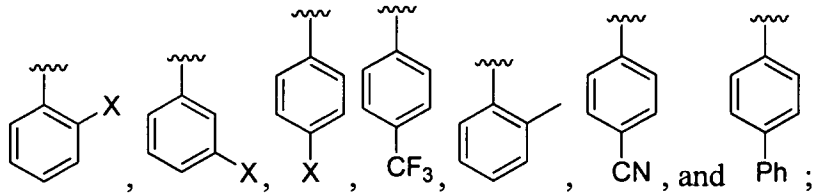
n is an integer 0-5, inclusive;

each occurrence of R' is independently hydrogen; halogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; OR^B; $-C(=O)R^B$; $-CO_2R^B$; $-C(=O)N(R^B)_2$; -CN; -SCN; $-SR^B$; $-SOR^B$; $-SO_2R^B$; $-NO_2$; $-N(R^B)_2$; $-NHC(O)R^B$; or $-C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino; dialkylamino; heteroaryloxy; or heteroarylthioxy.

8. The compound of claim 7, wherein n is 0.

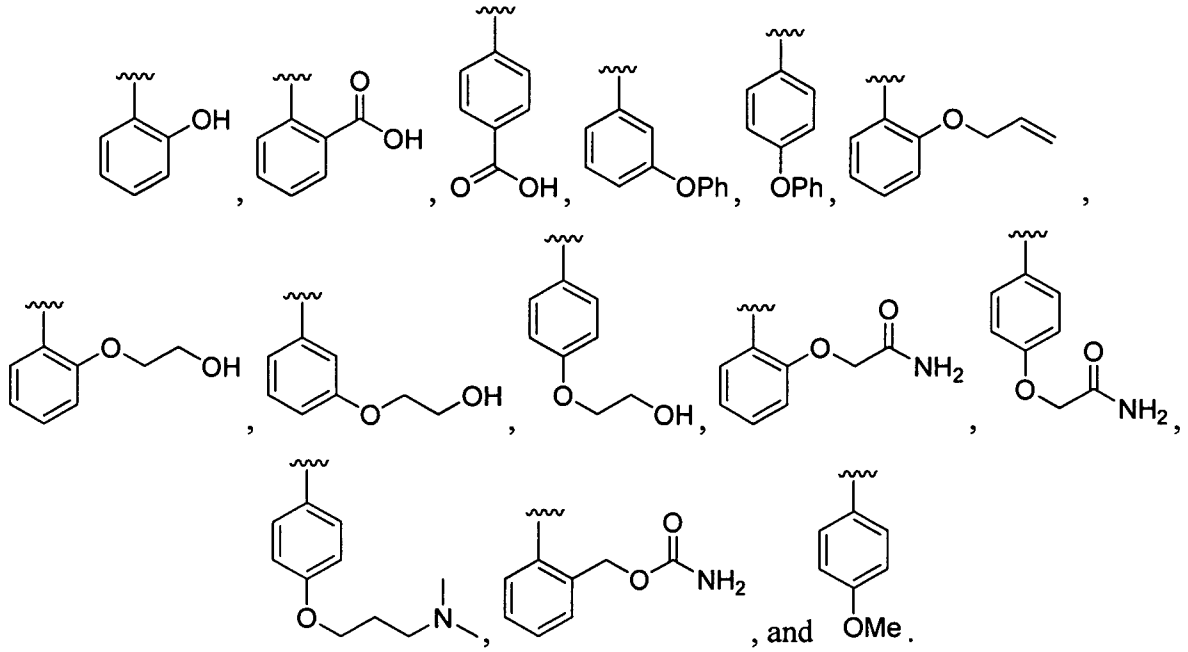
9. The compound of claim 7, wherein n is 1.

10. The compound of claim 7, wherein R is selected from a group consisting of:

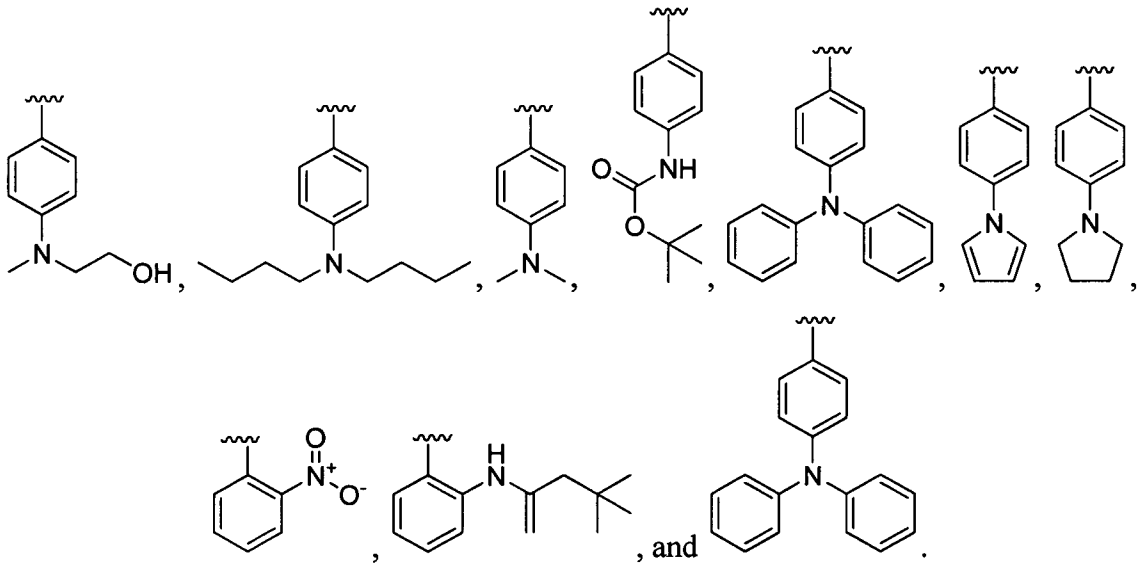


wherein X is halogen.

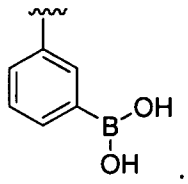
11. The compound of claim 7, wherein R is selected from a group consisting of:



12. The compound of claim 7, wherein R is selected from a group consisting of:

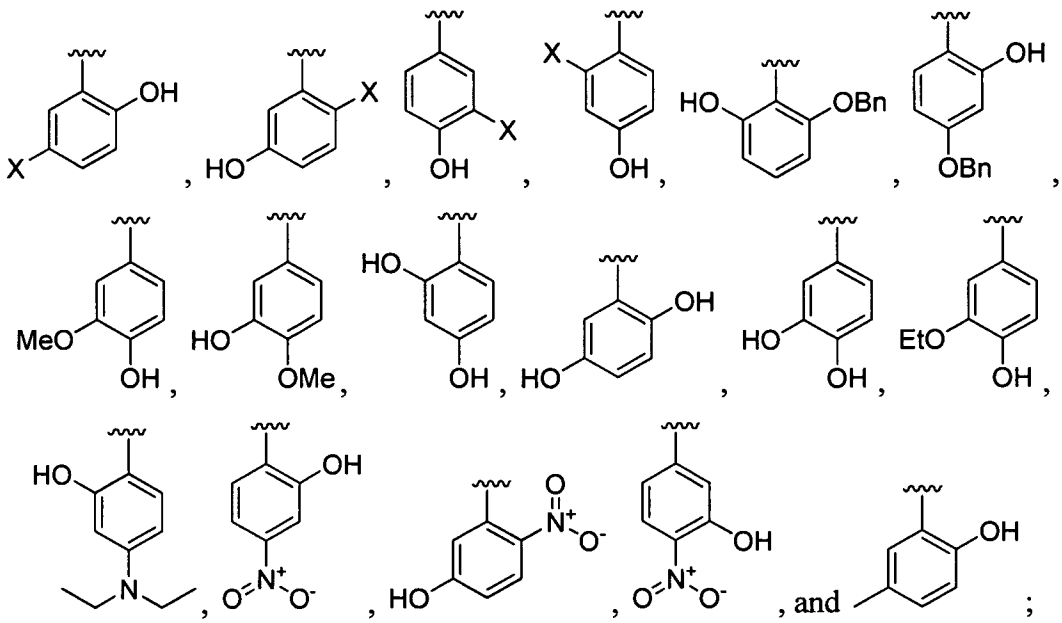


13. The compound of claim 7, wherein R is:



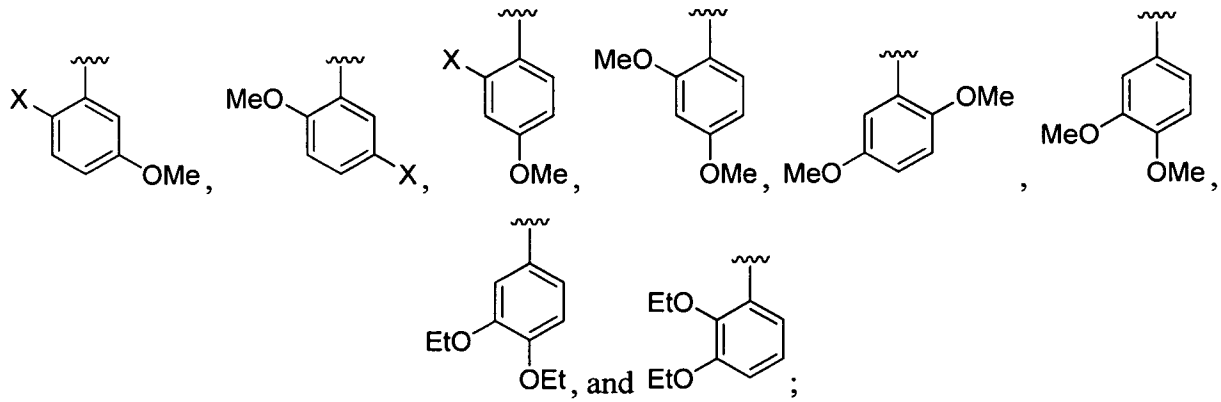
14. The compound of claim 6, wherein n is 2.

15. The compound of claim 14, wherein R is selected from a group consisting of:



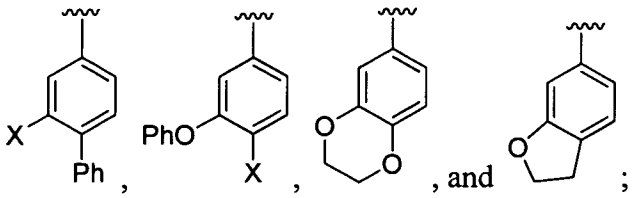
wherein X is halogen.

16. The compound of claim 14, wherein R is selected from a group consisting of:



wherein X is halogen.

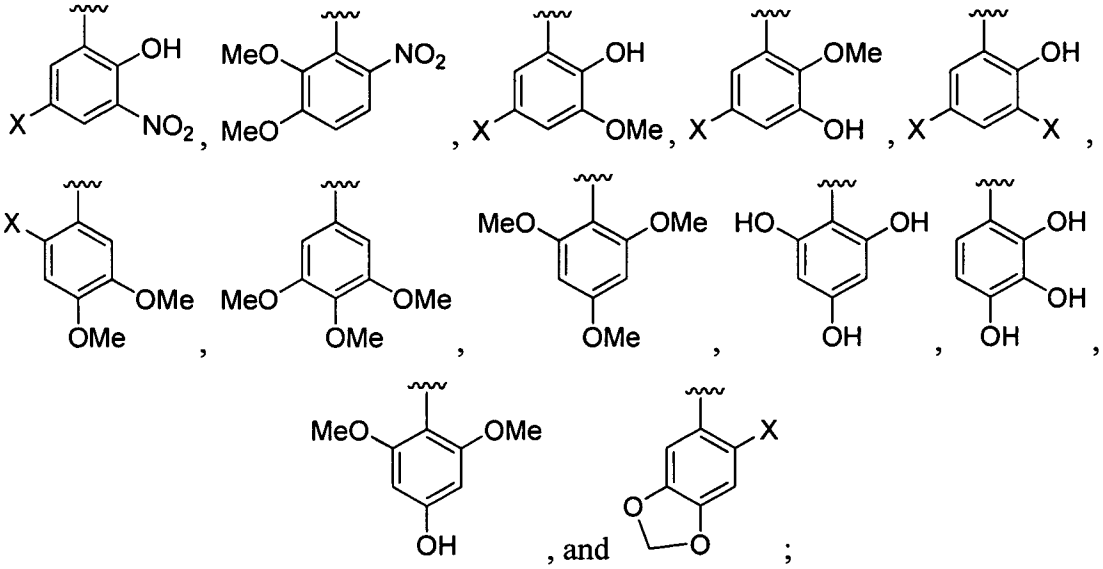
17. The compound of claim 14, wherein R is selected from a group consisting of:



wherein X is halogen.

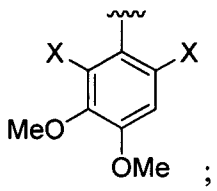
18. The compound of claim 6, wherein n is 3, 4, or 5.

19. The compound of claim 18, wherein R is selected from the group consisting of:



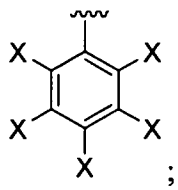
wherein X is halogen.

20. The compound of claim 18, wherein R is:



wherein X is halogen.

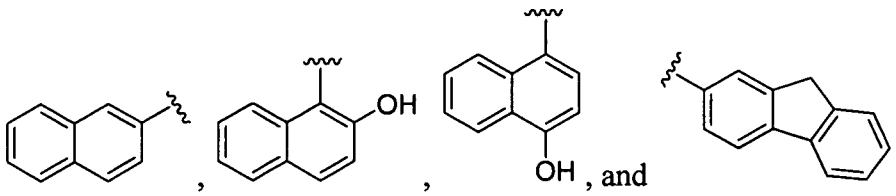
21. The compound of claim 18, wherein R is



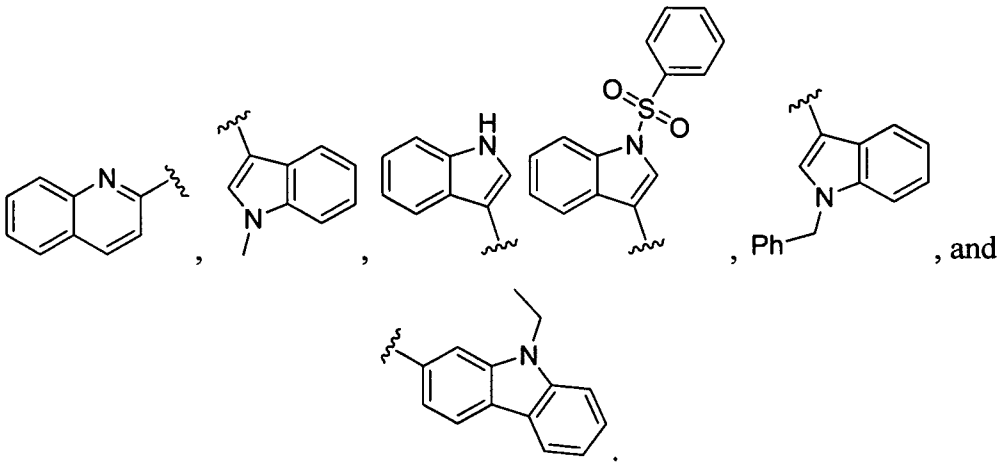
wherein X is halogen.

22. The compound of any one of claims 1-6, wherein R is selected from a polycyclic substituted or unsubsituted aryl or heteroaryl moiety.

23. The compound of claim 22, wherein R is selected from the group consisting of:

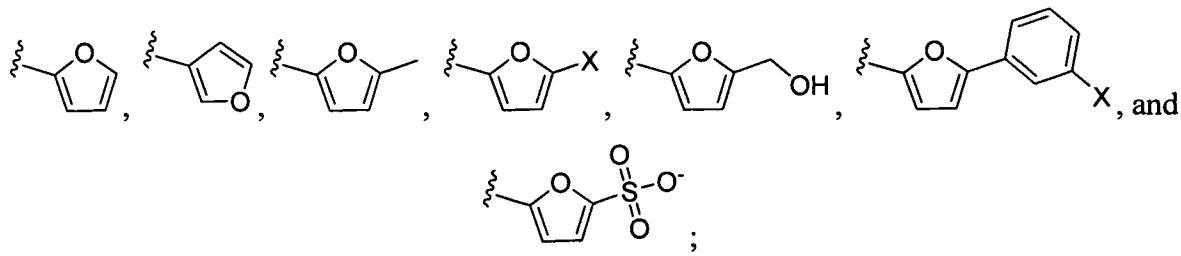


24. The compound of claim 22, wherein R is selected from the group consisting of:



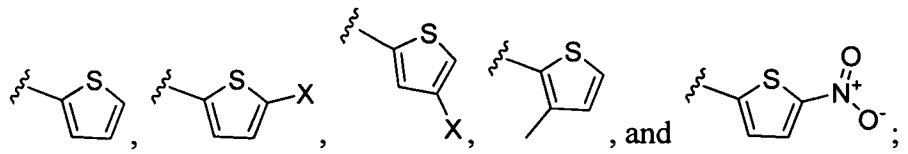
25. The compound of any one of claims 1-6, wherein R is a substituted or unsubsituted heteroaryl moiety.

26. The compound of claim 25, wherein R is selected from the group consisting of:



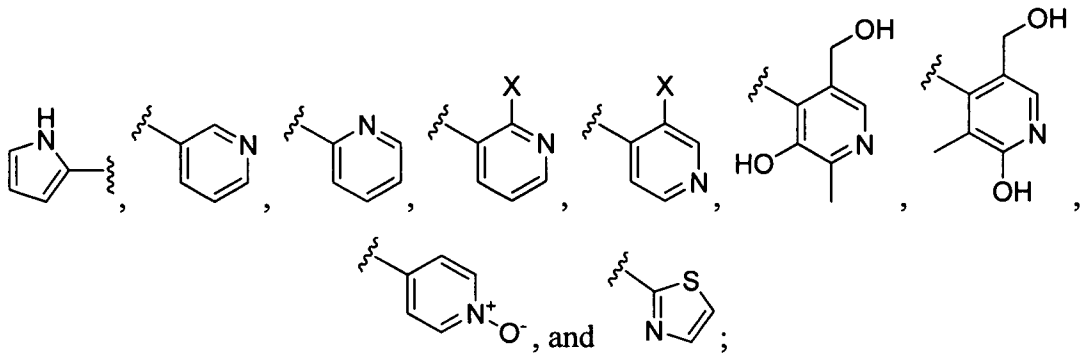
wherein X is halogen.

27. The compound of claim 25, wherein R is selected from the group consisting of:



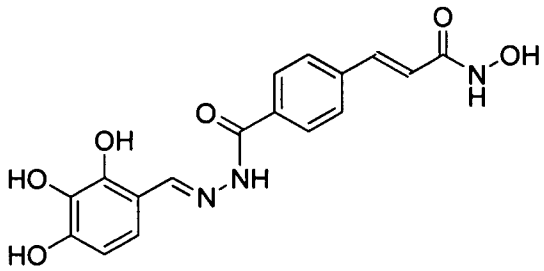
wherein X is halogen.

28. The compound of claim 25, wherein R is selected from the group consisting of:



wherein X is halogen.

29. The compound of claim 25, wherein the compound is



30. The compound of any one of claims 1-6, wherein R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety.

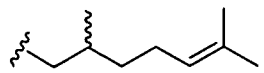
31. The compound of claim 30, wherein R is a cyclic or acyclic, substituted or unsubstituted C_{1-12} alkyl.

32. The compound of claim 30, wherein R is a cyclic or acyclic, substituted or unsubsituted C_{1-6} alkyl.

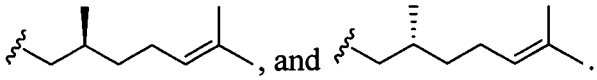
33. The compound of any of claims 30-32, wherein R is an aliphatic chain containing at least one stereocenter.

34. The compound of any of claims 1-6, wherein R is a heteroaliphatic chain containing at least one stereocenter.

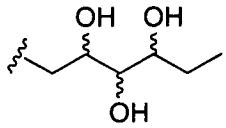
35. The compound of claim 34, wherein R is:



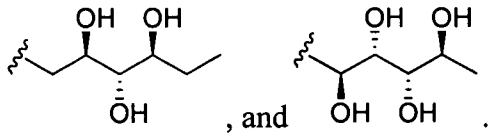
36. The compound of claim 34, wherein R is selected from a group consisting of:



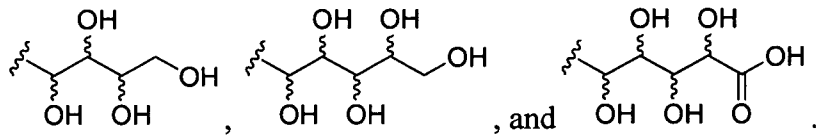
37. The compound of claim 34, wherein R is:



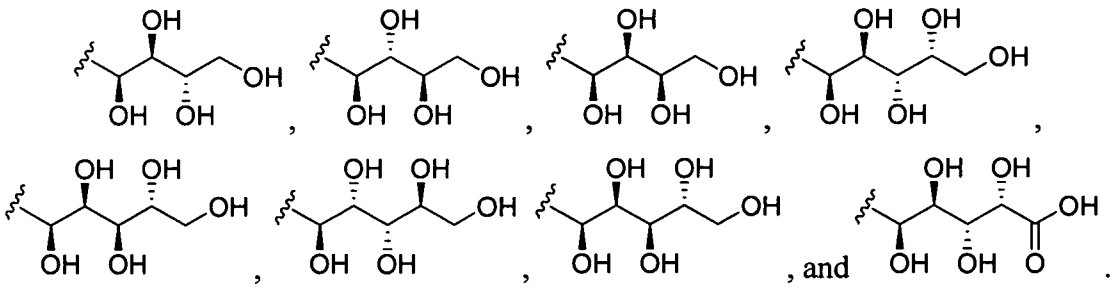
38. The compound of claim 34, wherein R is selected from the group consisting of:



39. The compound of claim 35, wherein R is selected from the group consisting of:

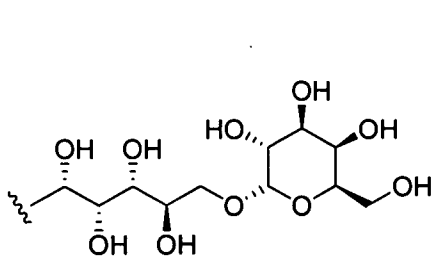
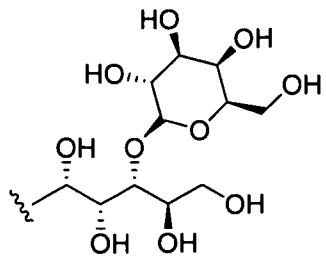


40. The compound of claim 35, wherein R is selected from the group consisting of:



41. The compound of any one of claims 1-6, wherein R comprises glucose.

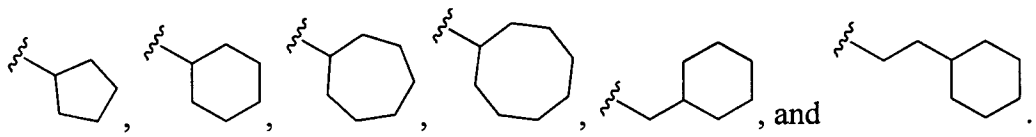
42. The compound of any one of claim 1-6, wherein R is selected from a group consisting of:


, and

.

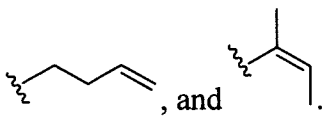
43. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:

C_7H_{15}, and  .

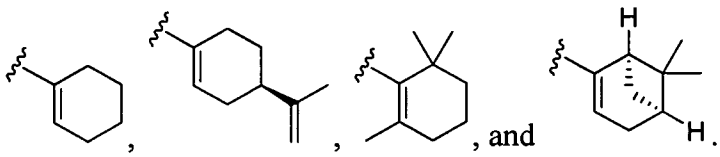
44. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:



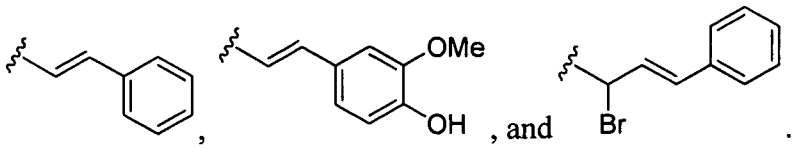
45. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:



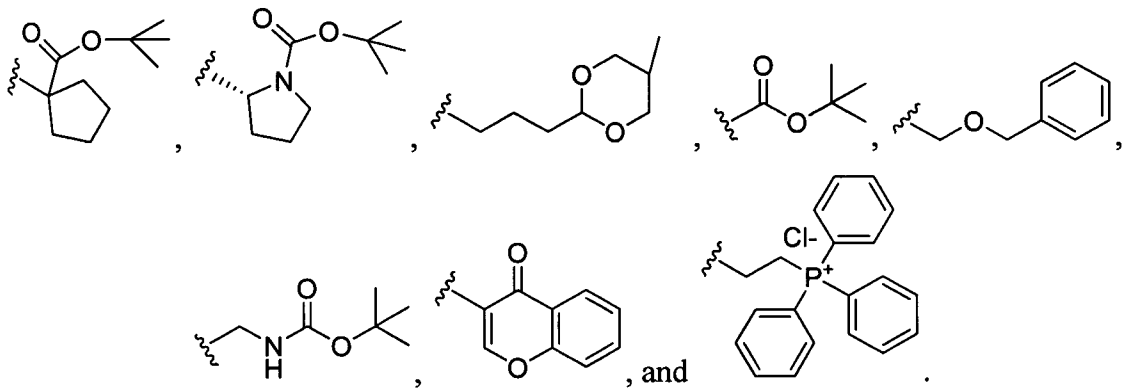
46. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:



47. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:

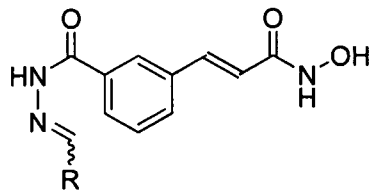


48. The compound of any one of claims 1-6, wherein R is selected from the group consisting of:



49. A pharmaceutical composition comprising a therapeutically effective amount of a compound of one of the claims 1-48 and a pharmaceutically acceptable excipient.

50. A method for synthesizing a compound of formula (I):

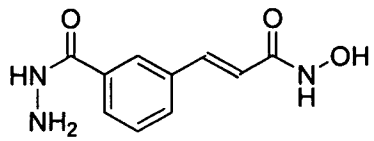


(I)

wherein R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl-moiety or a substituted or unsubstituted heteroaryl moiety;

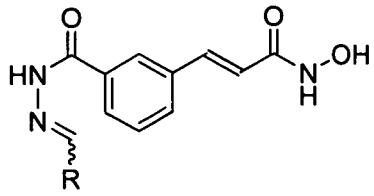
the method comprising :

reacting a hydrazine of formula:



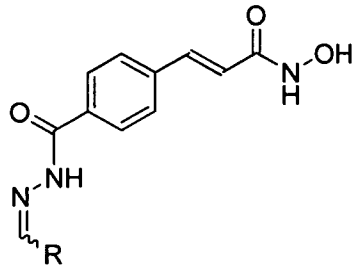
with an aldehyde of formula :

under suitable conditions to yield a compound of formula (I):



(I).

51. A method for synthesizing a compound of formula (II):

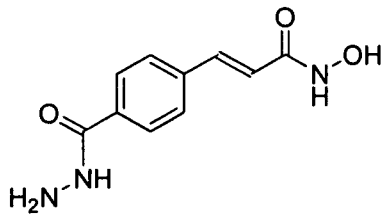


(II)

wherein R is a cyclic or acyclic, substituted or unsubstituted aliphatic moiety; a cyclic or acyclic, substituted or unsubstituted heteroaliphatic moiety; a substituted or unsubstituted aryl-moiety or a substituted or unsubstituted heteroaryl moiety;

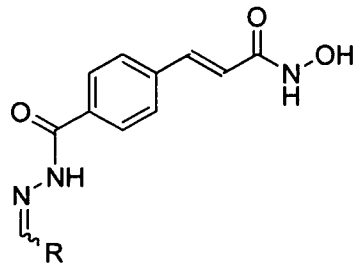
the method comprising :

reacting a hydrazine of formula:



with an aldehyde of formula :

under suitable conditions to yield a compound of formula (II):



(II).

52. A method of inhibiting histone deacetylase, the method comprising steps of:

contacting a histone deacetylase with an effective amount of a compound of one of the claims 1-48.

53. The method of claim 52, wherein the histone deacetylase is purified.

54. The method of claim 52, wherein the histone deacetylase is in a cell.

55. The method of any one of claims 52-54, wherein the histone deacetylase is a Class IIa HDAC.

56. A method of treating a subject with a proliferative disorder, the method comprising step of:

administering a therapeutically effective amount of a compound of one of claims 1-48.

57. The method of claim 56, wherein the subject is a mammal.

58. The method of claim 56, wherein the subject is human.

59. The method of claim 56-58, wherein the proliferative disorder is cancer.

60. The method of claim 56-58, wherein the proliferative disorder is an inflammatory disease.

61. The method of claim 52-60, wherein the step of administering comprises administering the compound parentarally.

62. The method of claim 52-60, wherein the step of administering comprises administering the compound intraveneously.

63. The method of claim 52-60, wherein the step of administering comprises administering the compound orally.

64. The method of claim 52-60, wherein the step of administering comprises administering the compound topically.

65. A method of treating a subject with a cardiovascular disease, the method comprising step of:

administering a therapeutically effective amount of a compound of one of claims 1-48.

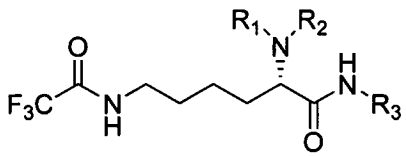
66. A method of treating a subject with an autoimmune or inflammatory disease, the method comprising step of:

administering a therapeutically effective amount of a compound of one of claims 1-48.

67. A method of treating a subject with a neurodegenerative disease, the method comprising step of:

administering a therapeutically effective amount of a compound of one of claims 1-48.

68. A compound of general formula (IIIc):



(IIIc)

wherein

R_1, R_2, and R_3 are each independently hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; $-OR^B$; $-C(=O)R^B$; $-CO_2R^B$; $-C(=O)N(R^B)_2$; $-SR^B$; $-SOR^B$; $-SO_2R^B$; $-N(R^B)_2$; $-NHC(O)R^B$; or $-C(R^B)_3$; wherein each occurrence of R^B is independently hydrogen; halogen; a protecting group; aliphatic; heteroaliphatic; acyl; aryl moiety; heteroaryl; hydroxyl; aloxy; aryloxy; alkylthioxy; arylthioxy; amino; alkylamino;

dialkylamino; heteroaryloxy; heteroarylthioxy; an amino acid; a peptide; a protecting group; or a tag; or salt thereof.

69. The compound of claim 68, wherein at least one of R_1, R_2, or R_3 is a tag.

70. The compound of claim 68, wherein R_3 is a tag.

71. The compound of claim 68, with the proviso that neither R_1 nor R_2 is Boc.

72. The compound of claim 68, wherein R_1 is an amino acid.

73. The compound of claim 68, wherein R_1 is a natural amino acid.

74. The compound of claim 68, wherein R_1 is a unnatural amino acid.

75. The compound of claim 68, wherein R_1 is a C_{1-6} alkyl group.

76. The compound of claim 68, wherein R_1 is a C_{1-4} alkyl group.

77. The compound of claim 68, wherein R_1 is H.

78. The compound of claim 68, wherein R_1 is a peptide.

79. The compound of claim 68, wherein R_1 is two-mer peptide, three-mer peptide, or four-mer peptide.

80. The compound of claim 68, wherein one of R_1 or R_2 is a two-mer peptide; and the other of R_1 and R_2 is hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag.

81. The compound of claim 68, wherein one of R_1 or R_2 is a three-mer peptide; and the other of R_1 and R_2 is hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag.

82. The compound of claim 68, wherein one of R_1 or R_2 is a four-mer peptide; and the other of R_1 and R_2 is hydrogen; cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; substituted or unsubstituted, branched or unbranched acyl; substituted or unsubstituted, branched or unbranched aryl; substituted or unsubstituted, branched or unbranched heteroaryl; an amino acid; a peptide; a protecting group; or a tag.

83. The compound of claim 68, wherein R_3 is an amino acid.

84. The compound of claim 68, wherein R_3 is a natural amino acid.

85. The compound of claim 68, wherein R_3 is a unnatural amino acid.

86. The compound of claim 68, wherein R_3 is a C_{1-6} alkyl group.

87. The compound of claim 68, wherein R_3 is a C_{1-4} alkyl group.

88. The compound of claim 68, wherein R_3 is H.

89. The compound of claim 68, wherein R_3 is a peptide.

90. The compound of claim 68, wherein R_3 is two-mer peptide, three-mer peptide, or four-mer peptide.

91. The compound of claim 68, wherein R_3 is a two-mer peptide.

92. The compound of claim 68, wherein R_3 is a three-mer peptide.

93. The compound of claim 68, wherein R_3 is a four-mer peptide.

94. The compound of claim 68, wherein one or both of R_1 and R_2 is a nitrogen protecting group.

95. The compound of claim 94, wherein the nitrogen protecting group is carbamate.

96. The compound of claim 94, wherein the nitrogen protecting group is a lithographic protecting group.

97. The compound of claim 68, wherein R_1 or R_2 is a tag.

98. The compound of claim 68, wherein R_3 comprises a fluorescent tag.

99. The compound of claim 68, wherein R_3 comprises coumarin.

100. The compound of claim 68, wherein R_3 comprises a derivative of coumarin.

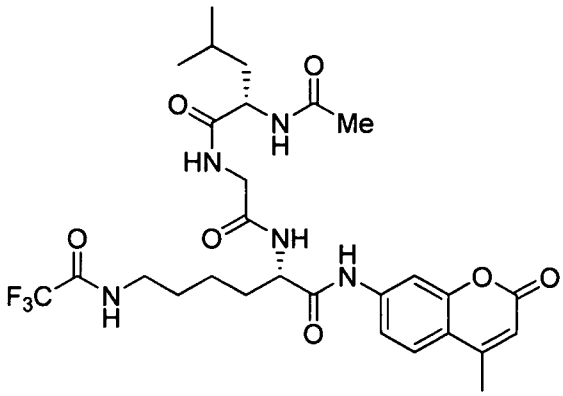
101. The assay of claim 128, wherein the tag is selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, and derivatives thereof.

102. The compound of claim 68, wherein the tag is 7-amino-4-methylcoumarin.

103. The compound of claim 68, wherein R_3 is ethidium bromide.

104. The compound of claim 68, wherein R_3 is fluorescein.

105. The compound of claim 68, wherein the compound is:



106. The compound of claim 68, wherein R_3 is a luminescent tag.

107. The compound of claim 68, wherein R_3 is a bioluminescent tag.

108. The compound of claim 68, wherein R_3 is a chemoluminescent tag.

109. The compound of claim 68, wherein R_3 is a photoluminescent tag.

110. The compound of claim 68, wherein R_3 is a radioluminescent tag.

111. The compound of claim 68, wherein R_3 is a thermoluminescent tag.

112. The compound of claim 68, wherein R_3 is an epitope tag.

113. The compound of claim 68, wherein R_3 is an isotope tag.

114. The compound of claim 68, wherein R_3 is a radioactive tag.

115. The compound of claim 68, wherein R_3 is a radiolabeled tag.

116. The compound of claim 68, wherein R_3 is a spin label.

117. The compound of claim 68, wherein R_3 comprises a chromophore.

118. The compound of claim 68, wherein R_3 comprises a fluorochrome.

119. The compound of claim 68, wherein R_3 comprises a ferromagnetic substance.

120. The compound of claim 70, wherein the tag of R_3 is cleaved by an enzyme.

121. The compound of claim 120, wherein the enzyme is esterase or protease.

122. The compound of claim 120, wherein the enzyme is trypsin.

123. The compound of claim 120, wherein the tag is toxic to the cell once cleaved.

124. An assay for determining the inhibitory effect of a test compound on an HDAC protein comprising: incubating the HDAC protein with a substrate of claim 68-125 in the presence of a test compound; and determining the activity of the HDAC protein.

125. The assay of claim 120, wherein the step of determining the activity of the HDAC protein comprises monitoring the release of a tag from the substrate.

126. The assay of claim 121, wherein the step of determining the activity of the HDAC protein comprises monitoring the release of the tag from the substrate by trypsin.

127. The assay of claim 121, wherein the tag is a fluorescent tag.

128. The assay of claim 121, wherein the tag comprises coumarin.

129. The assay of claim 121, wherein the tag comprises a derivative of coumarin.

130. The assay of claim 121, wherein the tag is selected from a group consisting of brodifacoum, bromadiolone, coumafuryl, difenacoum, auraptene, ensaculin, phenprocoumon, warfarin, or derivatives thereof.

131. The assay of claim 121, wherein the tag comprises 7-amino-4-methylcoumarin.

132. The assay of claim 120, wherein the HDAC protein is a recombinant, full length HDAC protein.

133. The assay of claim 120, wherein the assay is carried out at a concentration of the substrate greater than the substrate K_m.

134. The assay of claim 120, wherein the assay is carried out at a concentration of the substrate approximately equivalent to the substrate K_m.

135. The assay of claim 120, wherein the HDAC protein is a Class I HDAC.

136. The assay of claim 120, wherein the HDAC protein is a Class II HDAC.

137. The assay of claim 120, wherein the HDAC protein is a Class III HDAC.

138. The assay of claim 120, wherein the HDAC protein is a Class IV HDAC.

139. The assay of claim 120, wherein the HDAC protein is HDAC1.

140. The assay of claim 120, wherein the HDAC protein is HDAC2.

141. The assay of claim 120, wherein HDAC protein is a sirtuin.

142. The assay of claim 120, wherein the HDAC protein is HDAC3.

143. The assay of claim 120, wherein the HDAC protein is HDAC4.

144. The assay of claim 120, wherein the HDAC protein is HDAC5.

145. The assay of claim 120, wherein the HDAC protein is HDAC6.

146. The assay of claim 120, wherein the HDAC protein is HDAC7.

147. The assay of claim 120, wherein the HDAC protein is HDAC8.

148. The assay of claim 120, wherein the HDAC protein is HDAC9.

149. The assay of claim 120, wherein the HDAC protein is HDAC10.

150. The assay of claim 120, wherein the HDAC protein is HDAC11.

151. The assay of claim 120, wherein multiple assays are run in parallel.

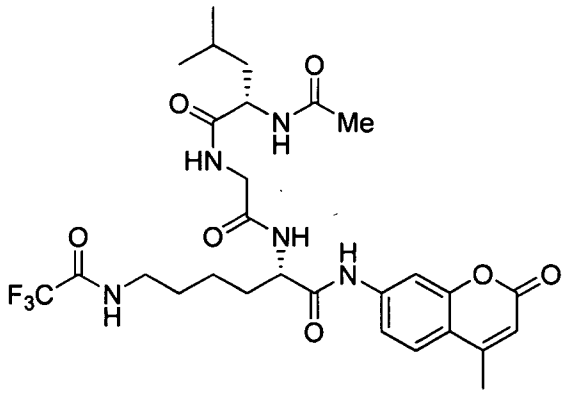
152. The assay of claim 120, wherein at least 50 assays are run in parallel.

153. The assay of claim 120, wherein at least 100 assays are run in parallel.

154. The assay of claim 120, wherein at least 500 assays are run in parallel.

155. The assay of claim 120, wherein at least 1000 assays are run in parallel.

156. The assay of claim 120, wherein the substrate is



157. The assay of claim 121, wherein the tag is removal by a chemical process or an enzymatic process.

158. The assay of claim 120, wherein the assay is performed at approximately room temperature.

159. The assay of claim 120, wherein the assay is performed at approximately 25 °C.

160. The assay of claim 120, wherein the assay is performed at approximately 37 °C.

161. The assay of claim 120, wherein the assay is performed at approximately 20-40 °C.

162. The assay of claim 120, wherein the assay is performed for approximately 3 hours.

163. The assay of claim 120, wherein the assay is performed for greater than approximately 3 hours.

164. The assay of claim 120, wherein the assay is performed for less than 3 hours.

165. The assay of claim 120, wherein the assay is run in an assay buffer.

166. The assay of claim 165, wherein the assay buffer comprises HEPES, KCl, Tween-20, BSA, and TCEP.

167. The assay of claim 166, wherein the assay buffer is 50 nM HEPES, 100 mM KCl, 0.001% Tween-20, 0.05% BSA, 200 μM TCEP, pH 7.4.

168. The assay of claim 120, wherein the concentration of the substrate is 1-20 μM.

169. The assay of claim 120, wherein the concentration of the substrate is 5-10 μM.

170. The assay of claim 120, wherein the concentration of the substrate is 10-15 μM.

171. The assay of claim 120, wherein the concentration of the substrate is 15-20 μM.

172. The assay of claim 120, wherein the concentration of the HDAC protein is 0.01-5 ng/μL.

173. The assay of claim 120, wherein the concentration of the HDAC protein is 0.01-0.05 ng/μL.

174. The assay of claim 120, wherein the concentration of the HDAC protein is 0.05-0.1 ng/μL.

175. The assay of claim 120, wherein the concentration of the HDAC protein is 0.1-0.5 ng/μL.

176. The assay of claim 120, wherein the concentration of the HDAC protein is 0.5-5 ng/μL.

177. The assay of claim 120, wherein the concentration of HDAC1 is approximately 3 ng/μL.

178. The assay of claim 120, wherein the concentration of HDAC2 is approximately 1 ng/μL.

179. The assay of claim 120, wherein the concentration of HDAC3 is approximately 0.2 ng/μL.

180. The assay of claim 120, wherein the concentration of HDAC4 is approximately 0.002 ng/μL.

181. The assay of claim 120, wherein the concentration of HDAC5 is 0.033 ng/μL.

182. The assay of claim 120, wherein the concentration of HDAC6 is 1.3 ng/μL.

183. The assay of claim 120, wherein the concentration of HDAC7 is approximately 0.003 ng/μL.

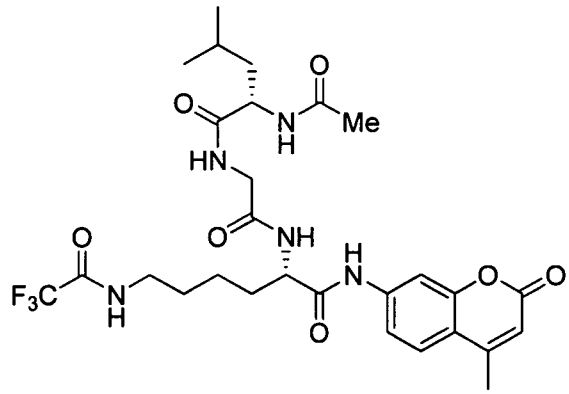
184. The assay of claim 120, wherein the concentration of HDAC8 is approximately 0.03 ng/μL.

185. The assay of claim 120, wherein the concentration of HDAC9 is approximately 0.03 ng/μL.

186. The assay of claim 120, wherein the assay is performed at multiple concentrations per test compound.

187. The assay of claim 139, wherein the R_1 of the substrate comprise the peptide sequence Gly-Leu.

188. An assay for determining the inhibitory effect of a test compound on an HDAC protein comprising incubating the HDAC protein with a substrate of formula:



in the presence of a test compound; and determining the activity of the HDAC protein by monitoring the release of 7-amino-4-methylcoumarin after cleavage by trypsin.

189. An assay for determining the binding affinity of a test compound for an HDAC protein comprising incubating HDAC protein with a compound of claim 68-123; and determining binding of the test compound to the HDAC protein.

Figure 1

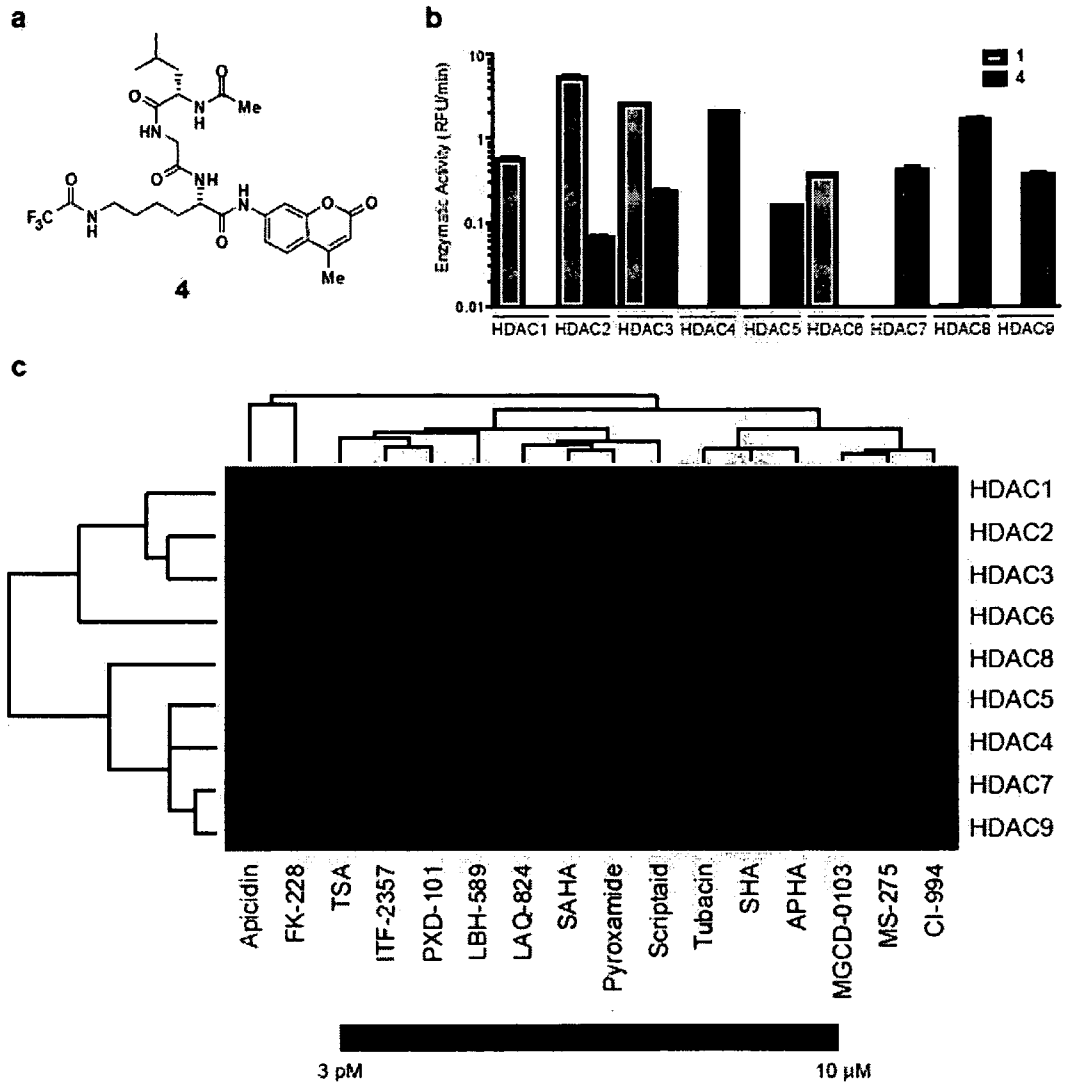

a
4
b
Enzymatic Activity (RFU/min)
10
1
0.1
0.01
1
4
HDAC1 HDAC2 HDAC3 HDAC4 HDAC5 HDAC6 HDAC7 HDAC8 HDAC9
c
HDAC1
HDAC2
HDAC3
HDAC6
HDAC8
HDAC5
HDAC4
HDAC7
HDAC9
Apicidin
FK-228
TSA
ITF-2357
PXD-101
LBH-589
LAQ-824
SAHA
Pyroxamide
Scriptaid
Tubacin
SHA
APHA
MGCD-0103
MS-275
CI-994
3 pM
10 μM

Figure 2

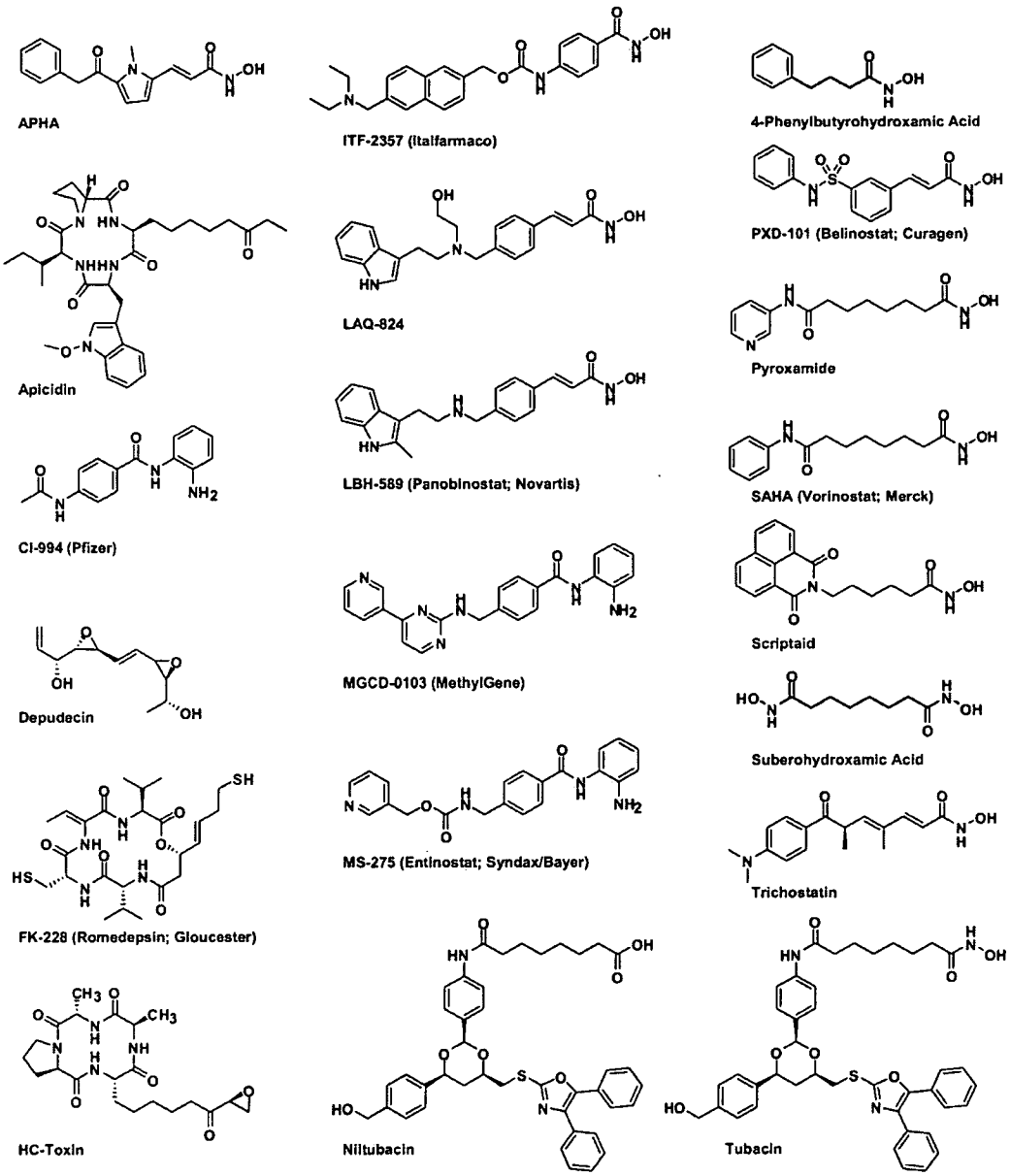

APHA
ITF-2357 (Italfarmaco)
4-Phenylbutyrohydroxamic Acid
PXD-101 (Belinostat; Curagen)
LAQ-824
Pyroxamide
Apicidin
LBH-589 (Panobinostat; Novartis)
SAHA (Vorinostat; Merck)
CI-994 (Pfizer)
MGCD-0103 (MethylGene)
Scriptaid
Suberohydroxamic Acid
Depudecin
MS-275 (Entinostat; Syndax/Bayer)
Trichostatin
FK-228 (Romedepsin; Gloucester)
HC-Toxin
Niltubacin
Tubacin

Figure 3

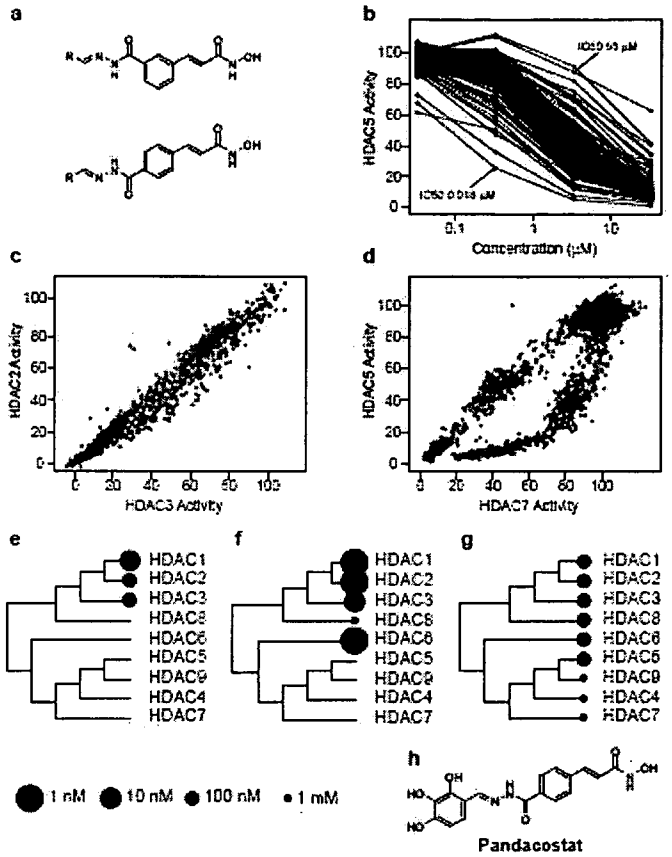

a
b
HDAC5 Activity
Concentration (μM)
c
HDAC3 Activity
HDAC3 Activity
d
HDAC5 Activity
HDAC7 Activity
e
f
g
HDAC1
HDAC2
HDAC3
HDAC8
HDAC6
HDAC5
HDAC9
HDAC4
HDAC7
1 nM
10 nM
100 nM
1 mM
h
Pandacostat

Figure 4

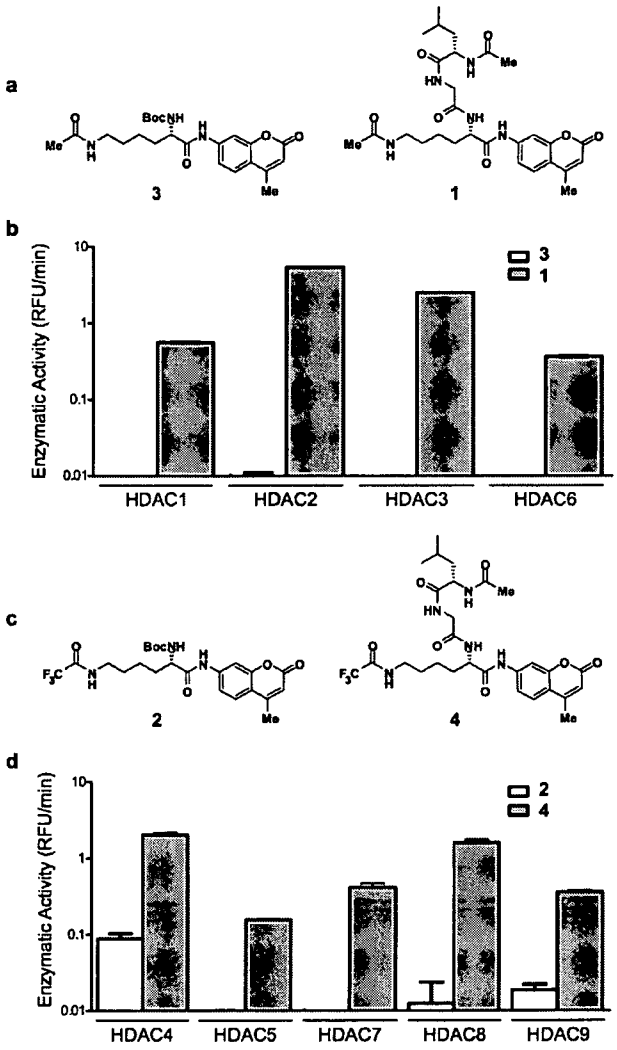
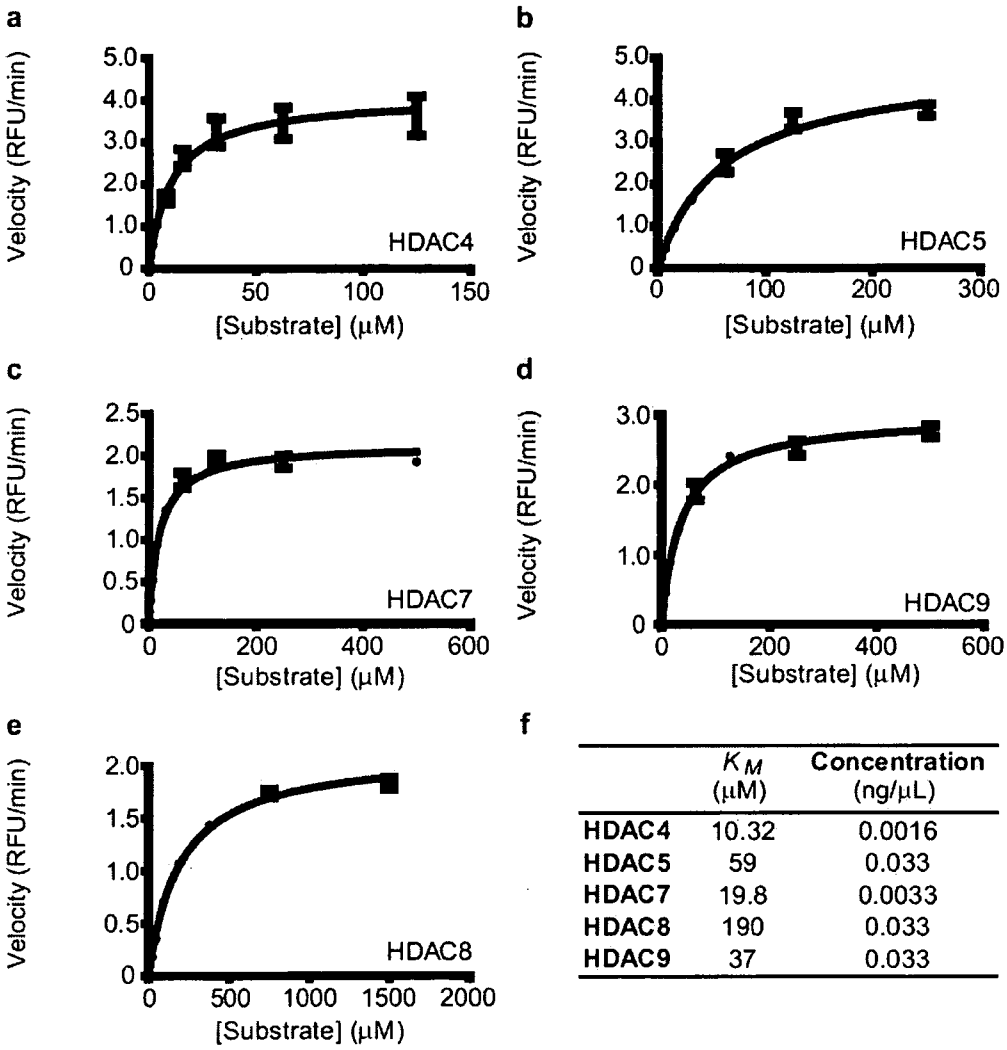

a
3
1
b
Enzymatic Activity (RFU/min)
10
1
0.1
0.01
3
1
HDAC1
HDAC2
HDAC3
HDAC6
c
2
4
d
Enzymatic Activity (RFU/min)
10
1
0.1
0.01
2
4
HDAC4
HDAC5
HDAC7
HDAC8
HDAC9

Figure 5



f

	K_M (μM)	Concentration (ng/μL)
HDAC4	10.32	0.0016
HDAC5	59	0.033
HDAC7	19.8	0.0033
HDAC8	190	0.033
HDAC9	37	0.033

Figure 6

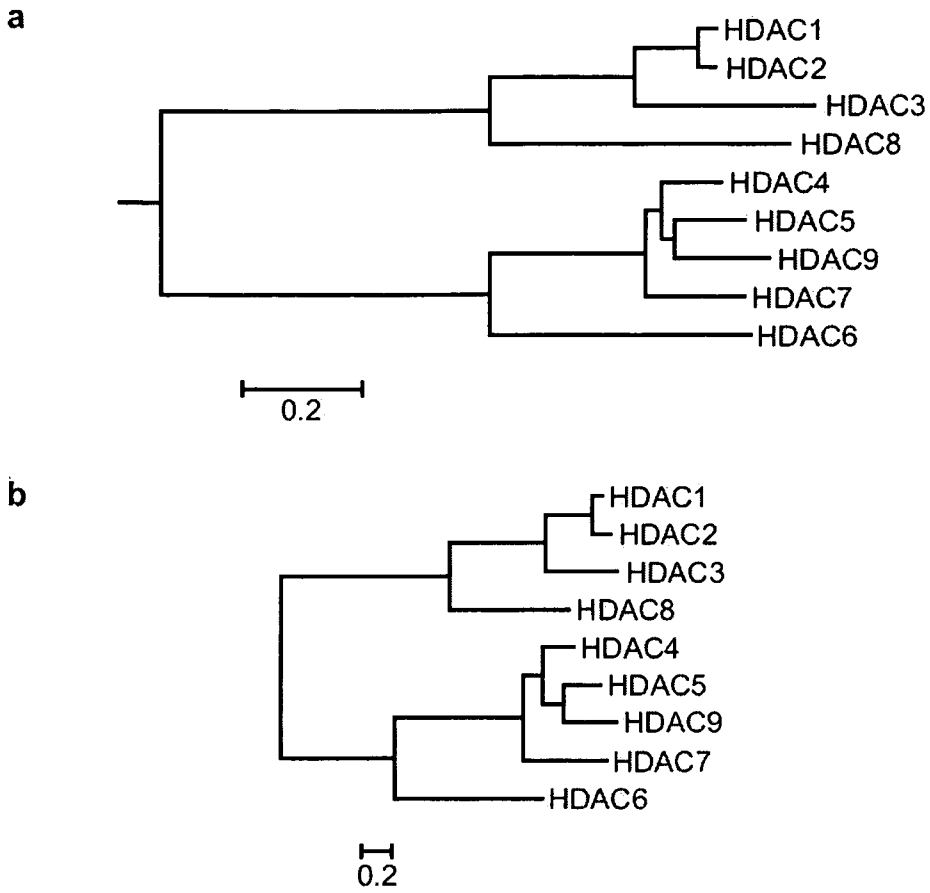

a
HDAC1
HDAC2
HDAC3
HDAC8
HDAC4
HDAC5
HDAC9
HDAC7
HDAC6
0.2
b
HDAC1
HDAC2
HDAC3
HDAC8
HDAC4
HDAC5
HDAC9
HDAC7
HDAC6
0.2

Figure 7

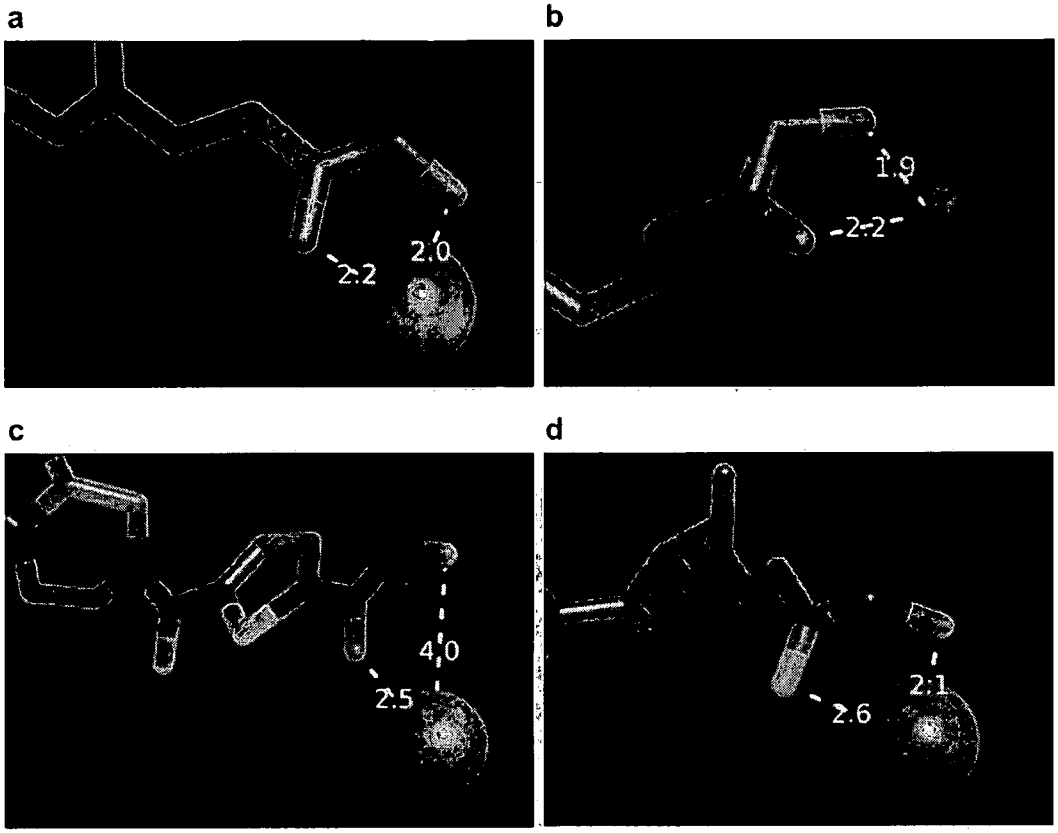


Figure 8

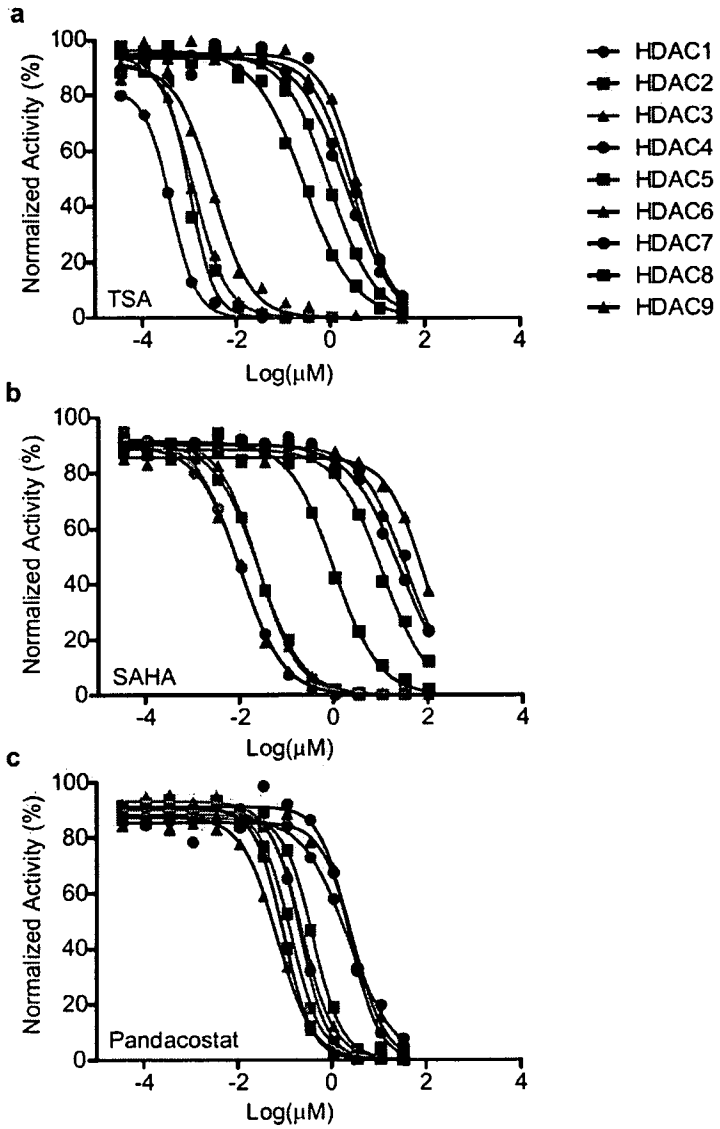
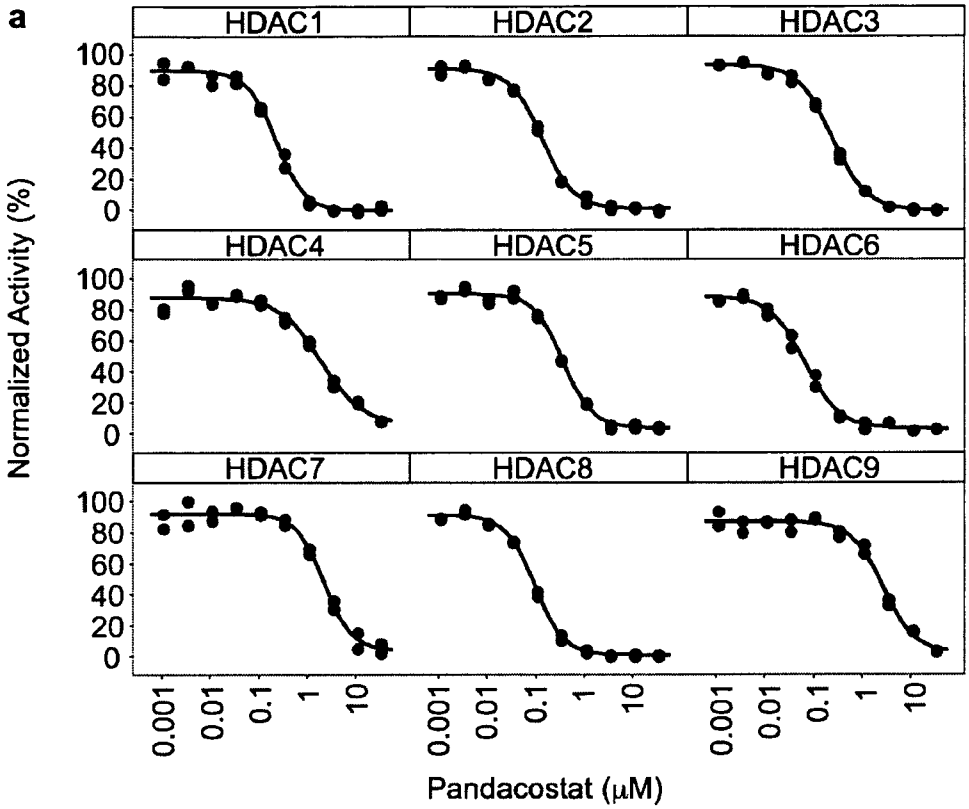

a
b
c
Normalized Activity (%)
Log(μM)
TSA
SAHA
Pandacostat
HDAC1
HDAC2
HDAC3
HDAC4
HDAC5
HDAC6
HDAC7
HDAC8
HDAC9

Figure 9

a



b

	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HDAC9
Pandacostat									
Ki	0.12	0.07	0.12	0.95	0.18	0.032	1.1	0.05	1.4
StdDev	0.01	0.0035	0.005	0.19	0.01	0.003	0.13	0.002	0.16

Figure 10

Compound	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HDAC9
APHA									
Ki	0.055	0.125	0.25	17.5	11.5	0.03	7	0.6	10
StdDev	0.004	0.01	0.02	1.2	0.6	0.004	0.9	0.04	1
Apicidin									
Ki	0.00004	0.00012	0.00026	-	-	-	-	0.049	-
StdDev	0.000004	0.000003	0.000005	-	-	-	-	0.02	-
CI-994									
Ki	0.05	0.19	0.55	-	-	-	-	-	-
StdDev	0.0045	0.015	0.035	-	-	-	-	-	-
Depudecin									
Ki	5.5	12.5	14.5	-	-	-	-	-	-
StdDev	0.4	0.65	0.95	-	-	-	-	-	-
FK-228									
Ki	0.0000015	0.000038	0.00015	0.0205	0.55	0.0095	1.25	0.00015	1.1
StdDev	0.0000001	0.000003	0.000025	0.0035	0.06	0.004	0.2	0.00003	0.22
HC-Toxin									
Ki	0.19	0.47	1.35	-	-	-	-	10.5	-
StdDev	0.02	0.06	0.115	-	-	-	-	1.4	-
ITF-2357									
Ki	0.002	0.003	0.003	1.05	0.6	0.0042	0.24	0.039	0.39
StdDev	0.0001	0.0001	0.0001	0.15	0.065	0.0002	0.025	0.001	0.05
LAQ-824									
Ki	0.00055	0.0014	0.0042	2.25	0.42	0.0095	9.5	0.34	9
StdDev	0.0005	0.00003	0.0001	0.3	0.04	0.00035	4.55	0.035	7.5
LBH-589									
Ki	0.001	0.00065	0.00011	0.55	0.08	0.0015	4.55	0.105	3.2
StdDev	0.0001	0.0001	0.00015	0.05	0.01	0.0005	0.315	0.02	0.2
MGCD-0103									
Ki	0.009	0.034	0.265	-	-	-	-	-	-
StdDev	0.001	0.002	0.015	-	-	-	-	-	-
MS-275									
Ki	0.022	0.065	0.36	-	-	-	-	-	-
StdDev	0.002	0.005	0.015	-	-	-	-	-	-
Niltubacin									
Ki	-	-	-	-	-	2.2	-	0.75	-
StdDev	-	-	-	-	-	0.38	-	0.07	-
4-PBHA									
Ki	0.295	0.43	1.65	-	16	0.15	-	1.85	-
StdDev	0.04	0.03	0.1	-	1.25	0.01	-	0.1	-
PXD-101									
Ki	0.00085	0.00085	0.0015	0.38	0.175	0.0016	0.075	0.025	0.25
StdDev	0.00005	0.00005	0.00005	0.06	0.02	0.00015	0.01	0.002	0.05
Pyroxamide									
Ki	0.0027	0.0036	0.008	-	4.75	0.0048	-	1	-
StdDev	0.00015	0.0002	0.00015	-	1.1	0.0003	-	0.11	-
SAHA									
Ki	0.0013	0.0016	0.005	-	3.6	0.0016	-	0.48	-
StdDev	0.00005	0.00005	0.0002	-	0.38	0.00005	-	0.02	-
Scriptaid									
Ki	0.0015	0.0022	0.0041	7.5	1	0.00025	2.25	0.105	8
StdDev	0.00005	0.00005	0.00005	0.75	0.1	0.0001	0.35	0.01	1
SuberoHA									
Ki	0.019	0.029	0.125	-	9.5	0.0145	-	0.95	-
StdDev	0.0035	0.0045	0.01	-	0.5	0.0015	-	0.1	-
Trichostatin									
Ki	0.0002	0.00065	0.0005	1.4	0.26	0.001	0.195	0.045	0.8
StdDev	0.000045	0.00005	0.0001	0.1	0.035	0.0001	0.02	0.015	0.1
Tubacin									
Ki	0.028	0.042	0.275	17	1.5	0.016	8.5	0.17	-
StdDev	0.004	0.0035	0.02	2.5	0.25	0.002	1.5	0.01	-

Figure 11

```
1          11         21         31         41         50
|          |          |          |          |          |
M--------- ---------- ---------- ---------- ----------     1 HD1
M--------- ---------- ---------- ---------- ----------     1 HD2
M--------- ---------- ---------- ---------- ----------     1 HD3
M--------- ---------- ---------- ---------- ----------     1 HD8
MSSQSHPDGL SGRDQPVELL NPARVNHMPS TVDVATALPL QVAPSAV---    47 HD4
MNSPNESDGM SGREPSLEIL PRTSLHSIPV TVEVKPVLP- RAMPSSMGGG    49 HD5
M--------- ---------- -----HSMIS SVDVKSEVPV GLEPIS----    22 HD9
M--------- ---------- ---------- ---------- ----------     1 HD7
M--------- ---------- ---------- ---------- ----------     1 HD6


---------- ---------- ---------- ---------- ----------     1 HD1
---------- ---------- ---------- ---------- ----------     1 HD2
---------- ---------- ---------- ---------- ----------     1 HD3
---------- ---------- ---------- ---------- ----------     1 HD8
------PMDL RLDHQFSLPV AEPALREQQL QQELLALKQK QQIQRQILIA    91 HD4
GGGSPSPVEL RGALVGSV-- -DPTLREQQL QQELLALKQQ QQLQKQLLFA    96 HD5
------PLDL RTDLRMMMPV VDPVVREKQL QQELLLIQQQ QQIQKQLLIA    66 HD9
--------DL RVGQRPPV-- ------EPPP EPTLLALQRP QRLHHHLFLA    35 HD7
---------- ---------- ---------- ---------- ----------     1 HD6


---------- ---------- ---------- ---------- ----------     1 HD1
---------- ---------- ---------- ---------- ----------     1 HD2
---------- ---------- ---------- ---------- ----------     1 HD3
---------- ---------- ---------- ---------- ----------     1 HD8
EFQRQHEQLS RQHEAQLHEH IKQQQEMLAM KHQQEL---- ----LEHQRK   133 HD4
EFQKQHDHLT RQHEVQLQKH LKQQQEMLAA KQQQEMLAAK RQQELEQQRQ   146 HD5
EFQKQHENLT RQHQAQLQEH IK---ELLAI KQQQEL---- ----LEKEQK   105 HD9
GLQQQRS--- ---------- ---------- ---------- ----VEPMR-    47 HD7
TSTGQDSTTT RQRRSRQNPQ SPPQDSSVTS KRNIKKGAVP RS--IPNLAE    49 HD6


---------- ---------- ---------- ---------- ----------     1 HD1
---------- ---------- ---------- ---------- ----------     1 HD2
---------- ---------- ---------- ---------- ----------     1 HD3
---------- ---------- ---------- ---------- ----------     1 HD8
LERHRQEQEL EKQHREQKLQ QLKNKEKGKE S---AVASTE VKMKLQEFVL   180 HD4
REQQRQE-EL EKQRLEQQLL ILRNKEKSKE S---AIASTE VKLRLQEFLL   192 HD5
LEQQRQEQEV ERHRREQQLP PLRGKDRGRE R---AVASTE VKQKLQEFLL   152 HD9
LSMDTPMPEL QVGPQEQELR QLLHKDKSKR S---AVASSV VKQKLAEVIL    94 HD7
VKKKGKMKKL GQAMEEDLIV GLQGMDLNLE AEALAGTGLV LDEQLNEFHC    99 HD6


---------- ---------- ---------- ---------- ----------     1 HD1
```

```
---------- ---------- ---------- ---------- ----------     1 HD2
---------- ---------- ---------- ---------- ----------     1 HD3
---------- ---------- ---------- ---------- ----------     1 HD8
NKK--KALAH RNLNHCISSD PRYWYGKTQH SSLDQSSPPQ S---GVSTSY   225 HD4
SKS--KEPTP GGLNHSLPQH PKCW--GAHH ASLDQSSPPQ SGPPGTPPSY   238 HD5
SKSATKDTPT NGKNHSVSRH PKLWYTAAHH TSLDQSSPPL S---GTSPSY   199 HD9
KKQ--QAALE RTVH---PNS PGIPYRTLEP LETEGATRSM L------SSF   133 HD7
LWD-D----- ---------- ---------- ---------- ----------   103 HD6

---------- ---------- ---------- ---------- ----------     1 HD1
---------- ---------- ---------- ---------- ----------     1 HD2
---------- ---------- ---------- ---------- ----------     1 HD3
---------- ---------- ---------- ---------- ----------     1 HD8
NHPVLG-MYD AKDDFPLRKT ASEPNLKLRS RLKQKVAERR SSPLLRRKDG   274 HD4
KLPLPG-PYD SRDDFPLRKT ASEPNLKVRS RLKQKVAERR SSPLLRRKDG   287 HD5
KYTLPG-AQD AKDDFPLRKT ASEPNLKVRS RLKQKVAERR SSPLLRRKDG   248 HD9
LPPVPSLPSD PPEHFPLRKT VSEPNLKLRY KPK-KSLERR KNPLLRKESA   182 HD7
---------- ---------- ---------- ---------- ----------   103 HD6

---------- ---------- ---------A QTQ-GTR--- ----------     8 HD1
---------- ---------- ---------A YSQGGGK--- ----------     9 HD2
---------- ---------- ---------A ---------- ----------     2 HD3
---------- ---------- ---------E EPEEPADSGQ S---------    13 HD8
PVVTALKKRP LDVT------ DSACSSAPGS GPSSPNNSSG SVSAENGIAP   318 HD4
TVISTFKKRA VEITGAGPGA SSVCNSAPGS GPSSPNSSHS TI-AENGFTG   336 HD5
NVVTSFKKRM FEVT-----E SSVSSSSPGS GPSSPNNGPT GSVTENE-TS   292 HD9
P--PSLRRRP AETLGD---S SPSSSSTPAS GCSSPNDSEH G---------   218 HD7
---------- ---------- ---------S FPEGPERLHA I---------   115 HD6

---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
AVPSIP--AE TSLAHRLVAR EGSAAPLPLY TSPSLPNITL GLPAT-----   361 HD4
SVPNIP--TE MLPQHRALPL DSSPNQFSLY TSPSLPNISL GLQATVTVTN   384 HD5
VLPPTPHAEQ MVSQQRILIH EDSMNLLSLY TSPSLPNITL GLPAV----P   338 HD9
--PNPILGSE ALLGQRLRLQ ETSVAPFALP TVSLLPAITL GLPA------   260 HD7
---KEQLIQE GLLDRCVSFQ ARFAEKEELM LVHSLEYIDL ----------   152 HD6

---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
---GPSAGTA GQQDTERLTL PALQQRLSLF PGTHLTPYLS TS--------   400 HD4
SHLTASPKLS TQQEAERQAL QSLRQ----- GGTLTGKFMS TSSIPGCLLG   429 HD5
SQLNASNSLK EKQKCE---T QTLRQ----- GVPLPGQYGG SIPASSSHPH   380 HD9
---------P ARADSDRRTH PTLGPRGPIL GSPHTPLFLP H---------   292 HD7
-----METTQ YMNEGELRVL ADTYDSVYLH PNSYSCACLA S---------   188 HD6
```

```
---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
-PLERDGGA- AHSPLLQHMV LLEQPPAQAP LVT--GLGAL PLHAQS-LVG   445 HD4
VALEGDGSPH GHASLLQHVL LLEQARQQST LI------AV PLHGQSPLVT   473 HD5
VTLEGKPPNS SHQALLQHLL LKEQMRQQKL LVA----GGV PLHPQSPLAT   426 HD9
-GLEPEAGG- TLPSRLQPIL LLDPSGSHAP LLTVPGLGPL PFHFAQSLMT   340 HD7
---------- GSVLRLVDAV LGAEIRNGMA IIRPPG---- -HHAQHSLMD   223 HD6
```

```
---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
A----DRVSP SI---HKLRQ HRPL------ ---GRTQSAP LPQNAQALQH   479 HD4
G----ERVAT SMRTVGKLPR HRPL------ ---SRTQSSP LPQSPQALQQ   510 HD5
K----ERISP GIRGTHKLPR HRPL------ ---NRTQSAP LPQS--TLAQ   461 HD9
T----ERLSG S-------GL HWPL------ ---SRTRSEP LPPSATA---   367 HD7
GYCMFNHVAV AARYAQQKHR IRRVLIVDWD VHHGQGTQFT FDQDPSVLYF   273 HD6
```

```
---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
LVIQQQHQQF LEKHKQQFQQ Q--------- ---------- ----------   500 HD4
LVMQQQHQQF LEKQK----Q Q--------- ---------- ----------   527 HD5
LVIQQQHQQF LEKQKQ--YQ Q--------- ---------- ----------   480 HD9
---------- ---------- ---------- ---------- ----------   367 HD7
SIHRYEQGRF WPHLKASNWS TTGFGQGQGY TINVPWNQVG MRDADYIAAF   323 HD6
```

```
---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
---------- -QLQMNKIIP KPSEPARQPE SHPEETEEEL REHQALLDE-   538 HD4
---------- -QLQLGKILT KTGELPRQPT THPEETEEEL TEQQEVLLG-   565 HD5
---------- -QIHMNKLLS KSIEQLKQPG SHLEEAEEEL QGDQAMQED-   518 HD9
---------- ---------- -----PPPPG PMQPRLEQLK THVQVIKRS-   391 HD7
LHVLLPVALE FQPQLVLVAA GFDALQGDPK GEMAATPAGF AQLTHLLMGL   373 HD6
```

```
---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
-PYLDRLPGQ KEAHAQAGVQ -VKQEPIESD EEEAEPPREV EPG------Q   580 HD4
-EGALTMPRE GSTESESTQE DLEEEDEEED GEEEEDCIQV KDEEGESGAE   614 HD5
-----RAPSS GN----STRS DSSACVDDTL G--QVGAVKV KEEPVDS--D   555 HD9
```

```
-----AKPSE KP----RLRQ IPSAEDLETD GGGPGQVV-- ---------D   421 HD7
AGGKLILSLE GGYNLRALAE GVSASLHTLL G--------- ---------D   405 HD6

---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
RQPSEQELLF RQ-------- -----QALLL EQQRIHQ--- --LRNYQASM   612 HD4
EGPDLEEPGA GY-------- -----KKLFS DAQPLQP--- --LQVYQAPL   646 HD5
EDAQIQEMES GE-------- -----QAAFM QQPFLEPTHT RALSVRQAPL   592 HD9
DGLEHRELGH GQPEARGPAP LQQHPQVLLW EQQRLAG--- --RLPRGSTG   466 HD7
PCPMLESPGA PC-------- ---------- --RSAQASVS CALEALEPFW   435 HD6

---------- ---------- ---------- ---------- ----------     8 HD1
---------- ---------- ---------- ---------- ----------     9 HD2
---------- ---------- ---------- ---------- ----------     2 HD3
---------- ---------- ---------- ---------- ----------    13 HD8
EAAGIPVSFG GHRPLSRAQS SPASATFPVS V--------- --QEPPTKPR   651 HD4
SLATVP---- -HQALGRTQS SPAAPGGMKS P--------- --PDQPVKHL   680 HD5
AAVGMD-GLE KHRLVSRTHS SPAASVLPHP A--------- --MDRPLQPG   630 HD9
DTVLLPLAQG GHRPLSRAQS SPAAPASLSA PEPASQARVL SSSETPARTL   516 HD7
EVLVRSTETV ERDNMEEDNV EESEEEGPWE P--------- --PVLPILTW   474 HD6

----RKVCYY YDGDVGNY-- -YYGQG-HPM KPHRIRMTHN LLLNYGLYRK    50 HD1
----KKVCYY YDGDIGNY-- -YYGQG-HPM KPHRIRMTHN LLLNYGLYRK    51 HD2
----KTVAYF YDPDVGNF-- -HYGAG-HPM KPHRLALTHS LVLHYGLYKK    44 HD3
----LVPVYI YSPEYVSM-- -CDSLA---K IPKRASMVHS LIEAYALHKQ    53 HD8
----FTTGLV YDTLMLKHQC TCGSSSSHPE HAGRIQSIWS RLQETGLRGK   697 HD4
----FTTGVV YDTFMLKHQC MCGNTHVHPE HAGRIQSIWS RLQETGLLSK   726 HD5
----SATGIA YDPLMLKHQC VCGNSTTHPE HAGRIQSIWS RLQETGLLNK   676 HD9
P---FTTGLI YDSVMLKHQC SCGDNSRHPE HAGRIQSIWS RLQERGLRSQ   563 HD7
PVLQSRTGLV YDQNMMNH-C NLWDSH-HPE VPQRILRIMC RLEELGLAGR   522 HD6

MEIYRPHKAN AEEMTKYHSD DYIKFLRSIR PDNMSEYSKQ ----------    90 HD1
MEIYRPHKAT AEEMTKYHSD EYIKFLRSIR PDNMSEYSKQ ----------    91 HD2
MIVFKPYQAS QHDMCRFHSE DYIDFLQRVS PTNMQGFTKS ----------    84 HD3
MRIVKPKVAS MEEMATFHTD AYLQHLQKVS QEGDDDHPDS ----------    93 HD8
CECIRGRKAT LEELQTVHSE AHT-LLYGTN PLNRQKLDSK KLLGSLA-SV   745 HD4
CERIRGRKAT LDEIQTVHSE YHT-LLYGTS PLNRQKLDSK KLLGPISQKM   775 HD5
CERIQGRKAS LEEIQLVHSE HHS-LLYGTN PLDGQKLDPR ILLGDDSQKF   725 HD9
CECLRGRKAS LEELQSVHSE RHV-LLYGTN PLSRLKLDNG KLAGLLAQRM   612 HD7
CLTLTPRPAT EAELLTCHSA EYVGHLRATE KMKTRELHRE ----------   562 HD6

---MQRFNVG EDCPVFDGLF ------EFCQ LSTGGSVASA VKLNKQ--QT   129 HD1
---MQRFNVG EDCPVFDGLF ------EFCQ LSTGGSVAGA VKLNRQ--QT   130 HD2
---LNAFNVG DDCPVFPGLF ------EFCS RYTGASLQGA TQLNNK--IC   123 HD3
---IE-YGLG YDCPATEGIF ------DYAA AIGGATITAA QCLIDG--MC   131 HD8
```

```
FVRLPCGGVG VD---SDTIW NEVHSAGAAR LAVGCVVELV FKVATGELKN   792 HD4
YAVLPCGGIG VD---SDTVW NEMHSSSAVR MAVGCLLELA FKVAAGELKN   822 HD5
FSSLPCGGLG VD---SDTIW NELHSSGAAR MAVGCVIELA SKVASGELKN   772 HD9
FVMLPCGGVG VD---TDTIW NELHSSNAAR WAAGSVTDLA FKVASRELKN   659 HD7
---------S SN---FD8IY ICPSTFACAQ LATGAACRLV EAVLSGEVLN   600 HD6


DIAVNWAGGL HHAKKSEASG FCYVNDIVLA ILELLK---Y HQRVLYIDID   176 HD1
DMAVNWAGGL HHAKKSEASG FCYVNDIVLA ILELLK---Y HQRVLYIDID   177 HD2
DIAINWAGGL HHAKKFEASG FCYVNDIVIG ILELLK---Y HPRVLYIDID   170 HD3
KVAINWSGGW HHAKKDEASG FCYLNDAVLG ILRLRR---K FERILYVDLD   178 HD8
GFAVVRPPG- HHAEESTPMG FCYFNSVAVA AKLLQQ-RLS VSKILIVDWD   840 HD4
GFAIIRPPG- HHAEESTAMG FCFFNSVAIT AKLLQQ-KLN VGKVLIVDWD   870 HD5
GFAVVRPPG- HHAEESTAMG FCFFNSVAIT AKYLRD-QLN ISKILIVDLD   820 HD9
GFAVVRPPG- HHADHSTAMG FCFFNSVAIA CRQLQQ-QSK ASKILIVDWD   707 HD7
GAAVVRPPG- HHAEQDAACG FCFFNSVAVA ARHAQTISGH ALRILIVDWD   649 HD6


IHHGDGVEEA FYTTDRVMTV SFHKYGE--Y FP--GTGDLR DIGAGKGKYY   222 HD1
IHHGDGVEEA FYTTDRVMTV SFHKYGE--Y FP--GTGDLR DIGAGKGKYY   223 HD2
IHHGDGVQEA FYLTDRVMTV SFHKYGNY-F FP--GTGDMY EVGAESGRYY   217 HD3
LHHGDGVEDA FSFTSKVMTV SLHKFSPG-F FP--GTGDVS DVGLGKGRYY   225 HD8
VHHGNGTQQA FYSDPSVLYM SLHRYDDGNF FP--GSGAPD EVGTGPGVGF   888 HD4
IHHGNGTQQA FYNDPSVLYI SLHRYDNGNF FP--GSGAPE EVGGGPGVGY   918 HD5
VHHGNGTQQA FYADPSILYI SLHRYDEGNF FP--GSGAPN EVGTGLGEGY   868 HD9
VHHGNGTQQT FYQDPSVLYI SLHRHDDGNF FP--GSGAVD EVGAGSGEGF   755 HD7
VHHGNGTQHM FEDDPSVLYV SLHRYDHGTF FPMGDEGASS QIGRAAGTGF   699 HD6


AVNYPLRDGI D----DESYE AIFKPVMSKV MEMFQPSAVV LQCGSDSLSG   268 HD1
AVNFPMRDGI D----DESYG QIFKPIISKV MEMYQPSAVV LQCGADSLSG   269 HD2
CLNVPLRDGI D----DQSYK HLFQPVINQV VDFYQPTCIV LQCGADSLGC   263 HD3
SVNVPIQDGI Q----DEKYY QICESVLKEV YQAFNPKAVV LQLGADTIAG   271 HD8
NVNMAFTGGL DPPMGDAEYL AAFRTVVMPI ASEFAPDVVL VSSGFDAVEG   938 HD4
NVNVAWTGGV DPPIGDVEYL TAFRTVVMPI AHEFSPDVVL VSAGFDAVEG   968 HD5
NINIAWTGGL DPPMGDVEYL EAFRTIVKPV AKEFDPDMVL VSAGFDALEG   918 HD9
NVNVAWAGGL DPPMGDPEYL AAFRIVVMPI AREFSPDLVL VSAGFDAAEG   805 HD7
TVNVAWNG-- -PRMGDADYL AAWHRLVLPI AYEFNPELVL VSAGFDAARG   746 HD6


--DRLGCFNL TIKGHAKCVE FVKSFNLPML MLG-GGGYTI RNVARCWTYE   315 HD1
--DRLGCFNL TVKGHAKCVE VVKTFNLPLL MLG-GGGYTI RNVARCWTYE   316 HD2
--DRLGCFNL SIRGHGECVE YVKSFNIPLL VLG-GGGYTV RNVARCWTYE   310 HD3
--DPMCSFNM TPVGIGKCLK YILQWQLATL ILG-GGGYNL ANTARCWTYL   318 HD8
HPTPLGGYNL SARCFGYLTK QLMGLAGGRI VLALEGGHDL TAICDASEAC   988 HD4
HLSPLGGYSV TARCFGHLTR QLMTLAGGRV VLALEGGHDL TAICDASEAC  1018 HD5
HTPPLGGYKV TAKCFGHLTK QLMTLADGRV VLALEGGHDL TAICDASEAC   968 HD9
HPAPLGGYHV SAKCFGYMTQ QLMNLAGGAV VLALEGGHDL TAICDASEAC   855 HD7
--DPLGGCQV SPEGYAHLTH LLMGLASGRI ILILEGGYNL TSISESMAAC   794 HD6


TAVALDTEIP NELPYNDYFE YFGPDFKLHI SPSN-MTNQN TNEYLEKIKQ   364 HD1
```

```
TAVALDCEIP NELPYNDYFE YFGPDFKLHI SPSN-MTNQN TPEYMEKIKQ   365 HD2
TSLLVEEAIS EELPYSEYFE YFAPDFTLHP DVSTRIENQN SRQYLDQIRQ   360 HD3
TGVILGKTLS SEIPDHEFFT AYGPDYVLEI TPSC-RPDRN EPHRIQQILN   367 HD8
VSALLGNELD PL-------- ---PEKVLQQ RPN--ANAVR SMEKVMEIHS  1025 HD4
VSALLSVELQ PL-------- ---DEAVLQQ KPN--INAVA TLEKVIEIQS  1055 HD5
VNALLGNELE PL-------- ---AEDILHQ SPN--MNAVI SLQKIIEIQS  1005 HD9
VAALLGNRVD PL-------- ---SEEGWKQ KPN--LNAIR SLEAVIRVHS   892 HD7
TRSLLG---D PP-------- ---PLLTLPR PPL--SGALA SITETIQVHR   828 HD6

RLFENLRML- ------PHAP GVQMQAIPED AIPEESGDE- --DED-----   399 HD1
RLFENLRML- ------PHAP GVQMQAIPED AVHEDSGDE- --DGE-----   400 HD2
TIFENLKML- ------NHAP SVQIHDVPAD LLTYDRTDEA DAEER-----   398 HD3
YIKGNLKHV- ---------- ---------- ---------- ----------   376 HD8
KYWRCLQRT- ------TSTA GRSLIEAQ-- ---------- TCENE-----  1051 HD4
KHWSCVQKF- ------AAGL GRSLREAQ-- ---------- AGETE-----  1081 HD5
M---SLKFS- ---------- ---------- ---------- ----------  1011 HD9
KYWGCMQRL- ------ASCP DSWVPRVP-- ---------- GADKE-----   918 HD7
RYWRSLRVMK VEDREGPSSS KLVTKKAPQP AKPRLAERMT TREKKVLEAG   878 HD6

---------- ---------- ---------- ---------- ----------   399 HD1
---------- ---------- ---------- ---------- ----------   400 HD2
---------- ---------- ---------- ---------- ----------   398 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
---------- ---------- ---------- ---------- ----------  1051 HD4
---------- ---------- ---------- ---------- ----------  1081 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
---------- ---------- ---------- ---------- ----------   918 HD7
MGKVTSASFG EESTPGQTNS ETAVVALTQD QPSEAATGGA TLAQTISEAA   928 HD6

---------- --------DP DKRISICSSD KRIACEEEFS DSEEEGEGGR   431 HD1
---------- --------DP DKRISIRASD KRIACDEEFS DSEDEGEGGR   432 HD2
---------- --------GP EENYSRPEAP NEFYDGDHDN DKESDVE---   427 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
---------- --------EA ETVTAMASLS VGVKPAEKR- ----------  1072 HD4
---------- --------EA ETVSAMALLS VGAEQAQAAA A---------  1104 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
---------- --------EV EAVTALASLS VGILAEDR-- ----------   938 HD7
IGGAMLGQTT SEEAVGGATP DQTTSEETVG GAILDQTTSE DAVGGATLGQ   978 HD6

KNSSNFKK-A KRVKTEDEKE KDPEEKKEVT EEEKTKE--- EKPEAKG---   474 HD1
RNVADHKKGA KKARIEEDKK ETEDKKTDVK EEDKSKDNSG EKTDTKG---   479 HD2
---------- ---------- ---------- ---------- ----------   427 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
---------- ---------- ---------- ---------- ---------P  1073 HD4
--REHSPR-- ---------- ---------- ---------- ---------P  1111 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
---------- ---------- ---------- ---------- ---------P   939 HD7
TTSEEAVGGA TLAQTTSEAA MEGATLDQTT SEEAPGGTEL IQTPLASSTD  1028 HD6
```

```
---------- ---------- ---------- ---------- ----------   474 HD1
---------- ---------- ---------- ---------- ----------   479 HD2
---------- ---------- ---------- ---------- ----------   427 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
DEEP------ ---------- ---------- ---------- ----------  1077 HD4
AEEP------ ---------- ---------- ---------- ----------  1115 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
SEQL------ ---------- ---------- ---------- ----------   943 HD7
HQTPPTSPVQ GTTPQISPST LIGSLRTLEL GSESQGASES QAPGEENLLG  1078 HD6


---------- ---------- ---------- ---------- ----------   474 HD1
---------- ---------- ---------- ---------- ----------   479 HD2
---------- ---------- ---------- ---------- ----------   427 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
---------- ---------- ---------- ---------- ----------  1077 HD4
---------- ---------- ---------- ---------- ----------  1115 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
---------- ---------- ---------- ---------- ----------   943 HD7
EAAGGQDMAD SMLMQGSRGL TDQAIFYAVT PLPWCPHLVA VCPIPAAGLD  1128 HD6


---------- ---------- ---------- ---------- ----------   474 HD1
---------- ---------- ---------- ---------- ----------   479 HD2
---------- ---------- ---------- ---------- ----------   427 HD3
---------- ---------- ---------- ---------- ----------   376 HD8
---------- ---------- ---------- ---------- ----------  1077 HD4
---------- ---------- ---------- ---------- ----------  1115 HD5
---------- ---------- ---------- ---------- ----------  1011 HD9
---------- ---------- ---------- ---------- ----------   943 HD7
VTQPCGDCGT IQENWVCLSC YQVYCGRYIN GHMLQHHGNS GHPLVLSYID  1178 HD6


---------- ---------- --------VK EE-VKLA                 482 HD1
---------- ---------- --------TK SEQLSNP                 488 HD2
---------- ---------- ---------- ------I                 428 HD3
---------- ---------- ---------- ------V                 377 HD8
---------- ---------- --------ME EEPPL--                1084 HD4
---------- ---------- --------ME QEPAL--                1122 HD5
---------- ---------- ---------- -------                1011 HD9
---------- ---------- --------VE EEEPMNL                 952 HD7
LSAWCYYCQA YVHHQALLDV KNIAHQNKFG EDMPHPH               1215 HD6
```

Figure 12

Sirtuins	IC50 (μM)
Sirtuin1	11μM
Sirtuin2	62% @ 100μM
Sirtuin3	57% @ 100μM

NI*=No significant inhibition.

Figure 13

Mlg1-164 (Log μM)	Sirtuin Activity (Fluorescent counts)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No CPD	39360	41754	96.57	103.43
-2.5	39283	40616	96.35	100.17
-2.0	40334	40824	99.36	100.77
-1.5	39364	40596	96.58	100.11
-1.0	40463	40516	99.73	99.88
-0.5	40124	43615	98.76	108.77
0.0	37172	36956	90.29	89.68
0.5	32809	34865	77.78	83.68
1.0	25113	24471	55.72	53.88
1.5	13789	13494	23.25	22.40
2.0	5151	5445	-1.52	-0.67
Background	5596	5764		

Figure 14

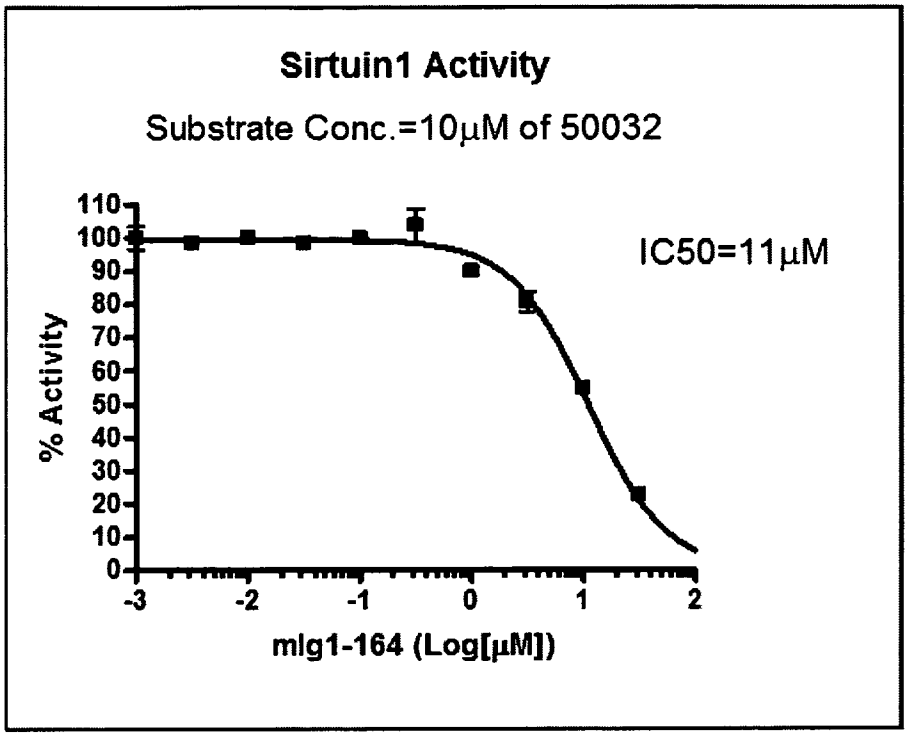
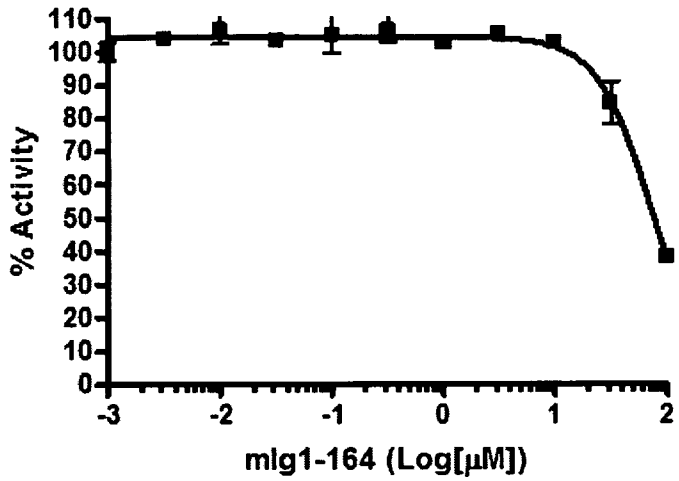

Sirtuin1 Activity
Substrate Conc.=10μM of 50032
IC50=11μM
% Activity
110
100
90
80
70
60
50
40
30
20
10
0
-3
-2
-1
0
1
2
mlg1-164 (Log[μM])

Figure 15

Mlg1-164 (Log μM)	Sirtuin Activity (Fluorescent counts)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No CPD	32166	33691	97.20	102.80
-2.5	33611	34391	102.50	105.36
-2.0	33516	35798	102.15	110.52
-1.5	33828	33735	103.30	102.96
-1.0	32870	35789	99.79	110.49
-0.5	35702	33689	110.17	102.79
0.0	33760	33579	103.05	102.39
0.5	34174	34760	104.57	106.72
1.0	34140	33300	104.44	101.36
1.5	27007	30460	78.29	90.95
2.0	16408	15678	39.43	36.75
Background	5622	5686		

Figure 16



About 62% inhibition of Sirtuin2 activity is observed at 100μM of mlg1-164.

Figure 17

Mlg1-164 (Log μM)	Sirtuin Activity (Fluorescent counts)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No CPD	40745	39611	101.64	98.36
-2.5	39788	40790	98.87	101.77
-2.0	41073	40152	102.59	99.92
-1.5	40841	41208	101.92	102.98
-1.0	41257	40890	103.13	102.06
-0.5	41362	40636	103.43	101.33
0.0	41295	40187	103.24	100.03
0.5	40881	39851	102.04	99.05
1.0	38565	38316	95.33	94.61
1.5	33722	32522	81.30	77.82
2.0	20343	20718	42.54	43.63
Background	5653	5660		

Figure 18

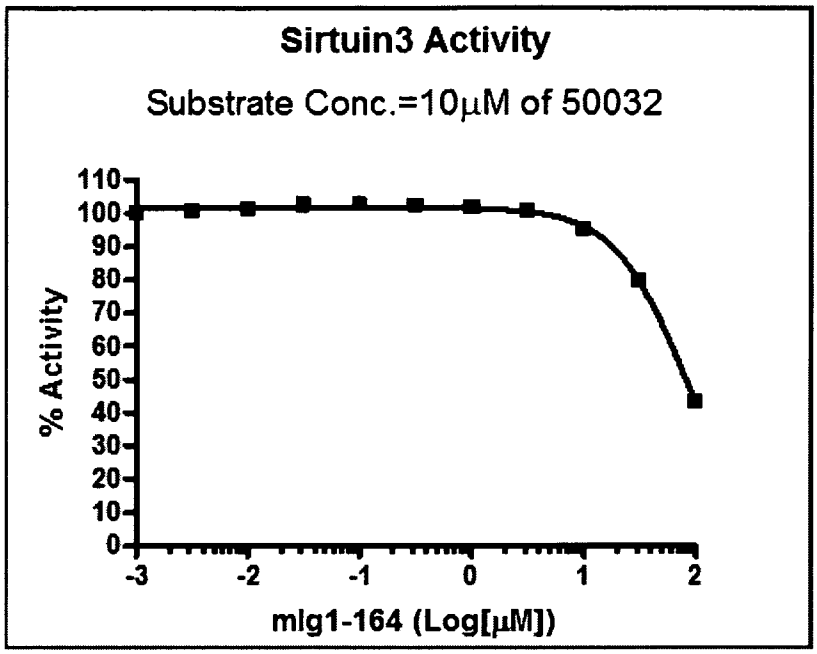

Sirtuin3 Activity
Substrate Conc.=10μM of 50032
% Activity
110
100
90
80
70
60
50
40
30
20
10
0
-3
-2
-1
0
1
2
mlg1-164 (Log[μM])

Figure 19A

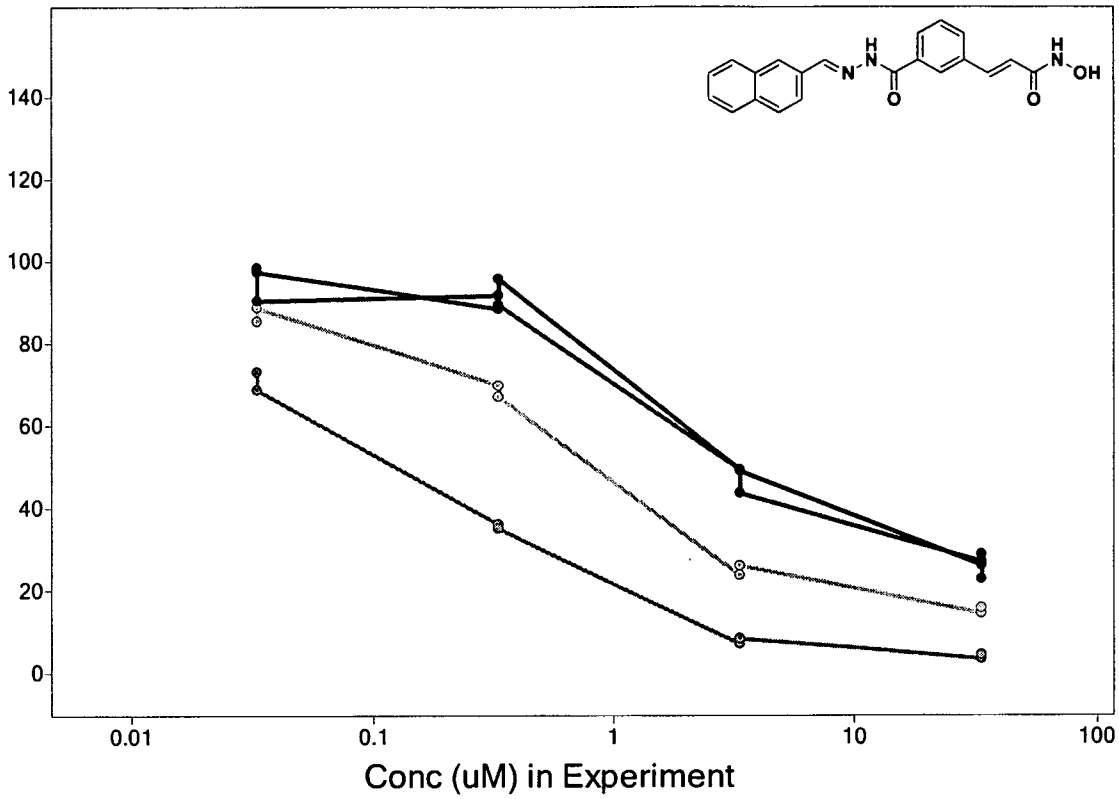

140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Conc (uM) in Experiment
H
N
N
O
H
N
OH
O

Figure 19B

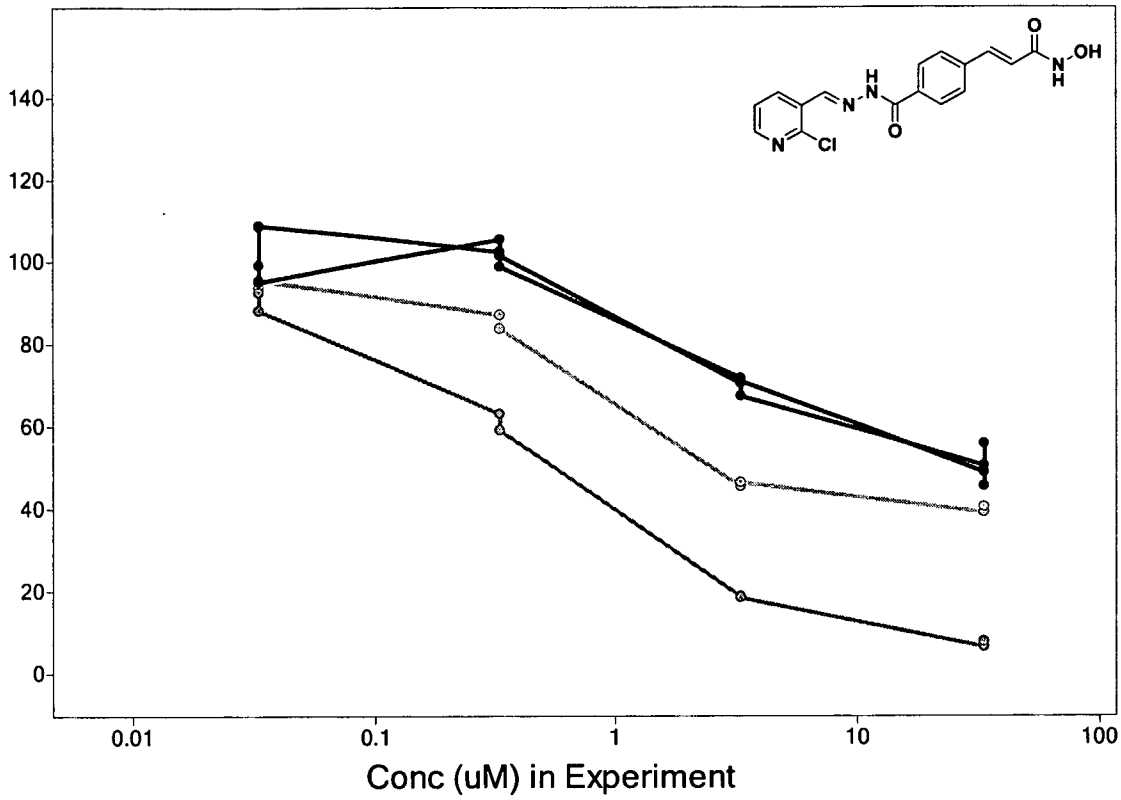

140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Conc (uM) in Experiment
O
N-OH
H
H
N
N
N
O
N
Cl

Figure 19C

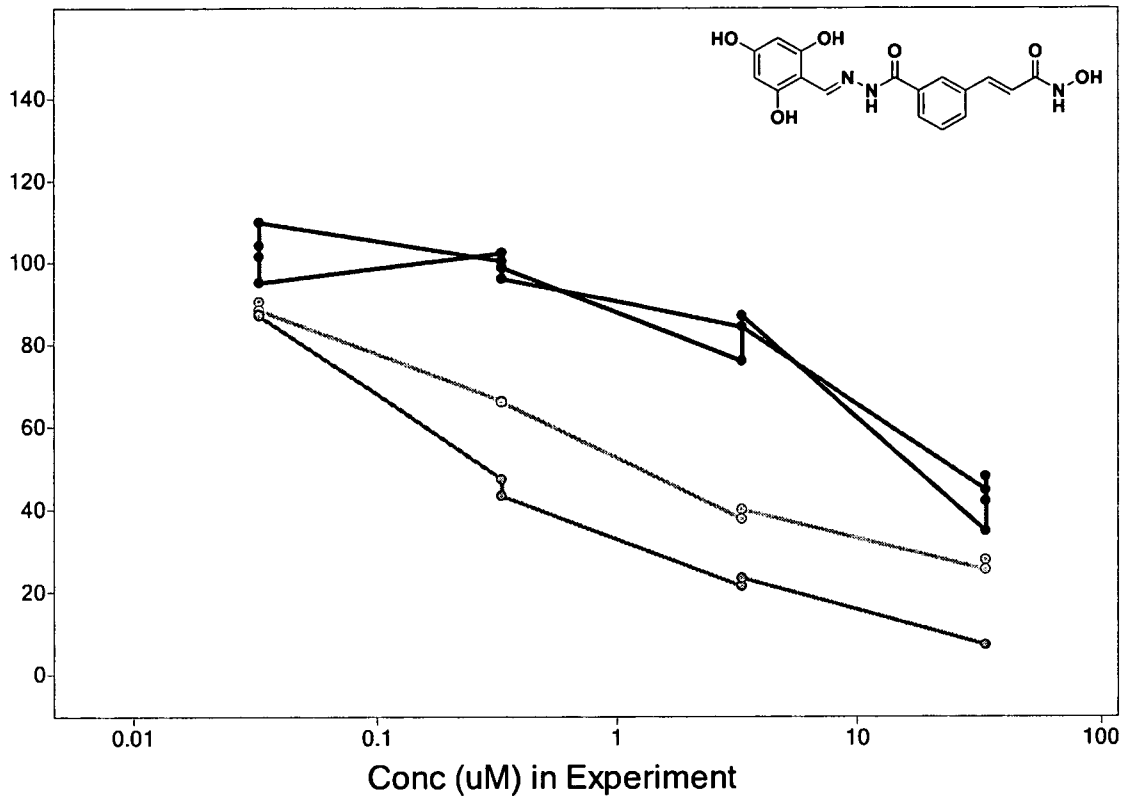
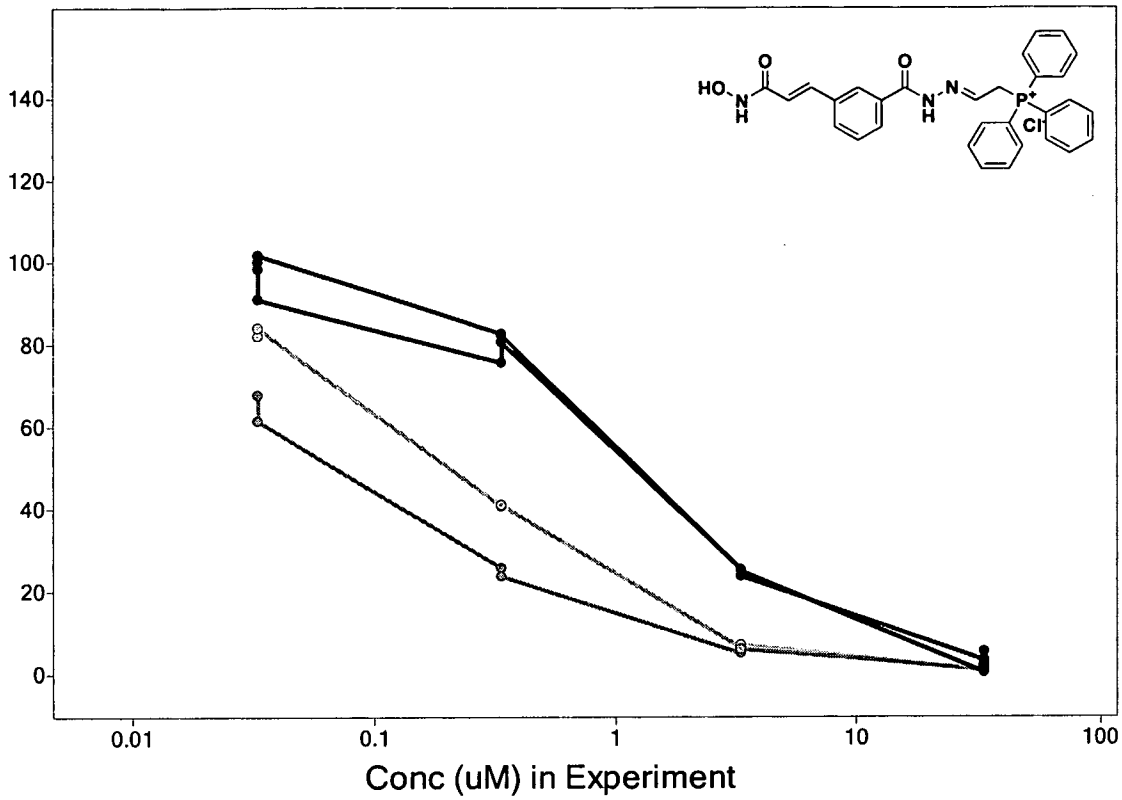

HO
OH
OH
N
N
H
O
O
N
H
OH
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Conc (uM) in Experiment

Figure 19D


140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Conc (uM) in Experiment
HO
N
H
O
O
N
H
N
P+
Cl