



US 20060074124A1

(19) **United States**(12) **Patent Application Publication**
Napper et al.(10) **Pub. No.: US 2006/0074124 A1**(43) **Pub. Date: Apr. 6, 2006**(54) **METHODS OF TREATING A DISORDER**(76) Inventors: **Andrew Napper**, Salem, MA (US);
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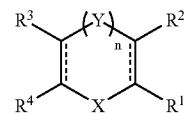
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FISH & RICHARDSON PC**P.O. BOX 1022****MINNEAPOLIS, MN 55440-1022 (US)**(21) Appl. No.: **11/077,664**(22) Filed: **Mar. 11, 2005****Related U.S. Application Data**(63) Continuation-in-part of application No. 10/940,269,
filed on Sep. 13, 2004.(60) Provisional application No. 60/502,811, filed on Sep.
12, 2003. Provisional application No. 60/531,443,
filed on Dec. 19, 2003. Provisional application No.
60/560,509, filed on Apr. 7, 2004.**Publication Classification**(51) **Int. Cl.****A61K 31/403** (2006.01)**C07D 209/82** (2006.01)(52) **U.S. Cl.** **514/411; 548/444**

(57)

ABSTRACT

Compound of formula (I)



(I)

and methods of treating disorders by administering a com-
pound of formula (I) are described herein. Examples of
disorders include neoplastic disorders, fat-cell related dis-
orders, neurodegenerative disorders, and metabolic disor-
ders.

Compound Number	SirT1	SirT2	SirT3	HDAC	NaDase	Selectivity	
						SirT2/ SirT1	SirT3/ SirT1
8	0.038	3.96	27.6	>50	>50	104	726
4	0.357	5.99	>50	>50	>50	17	>140
5	0.639	14.44	>50	>50	>50	23	>78
15	>50	>50					
16	>50	>50					

FIG. 1

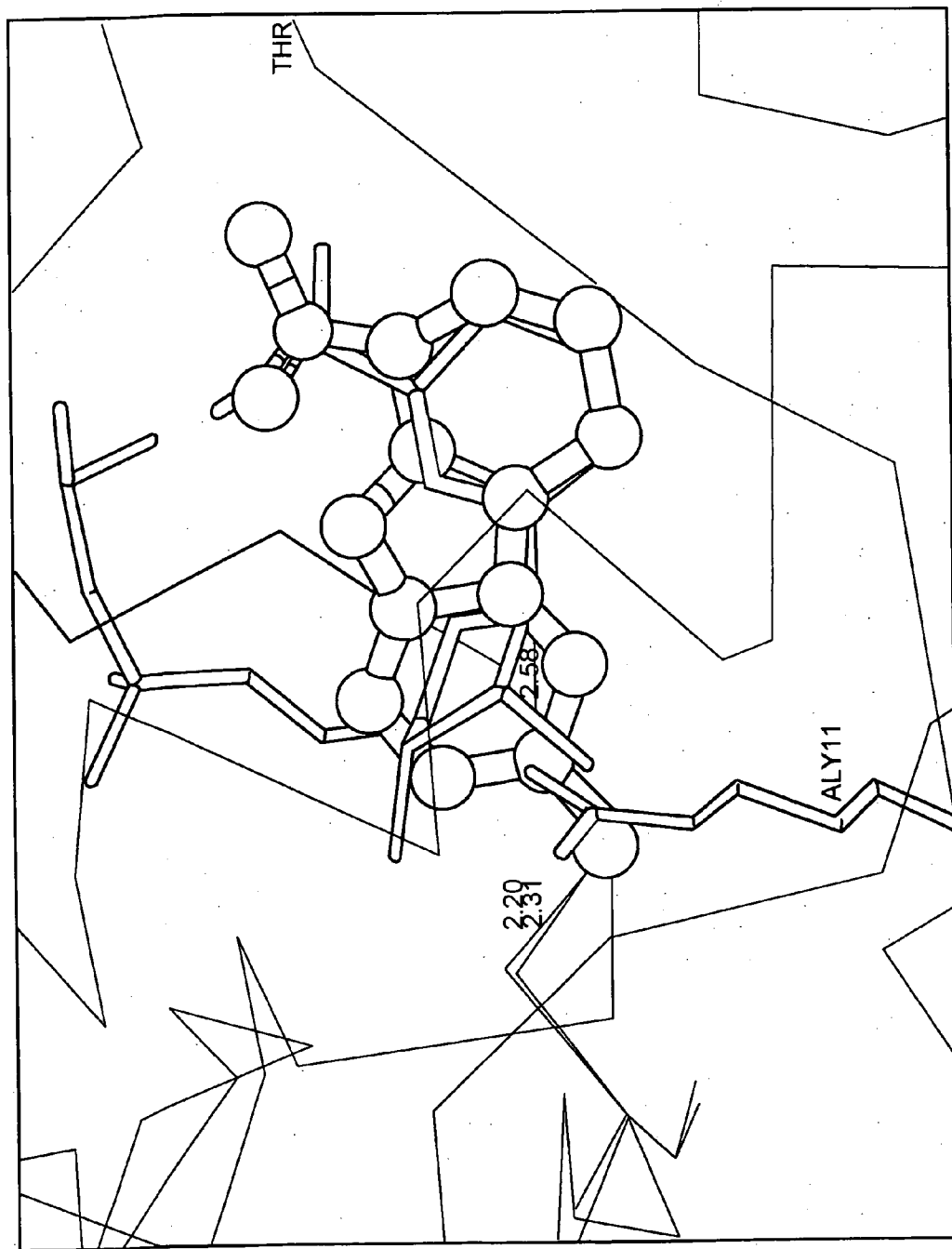
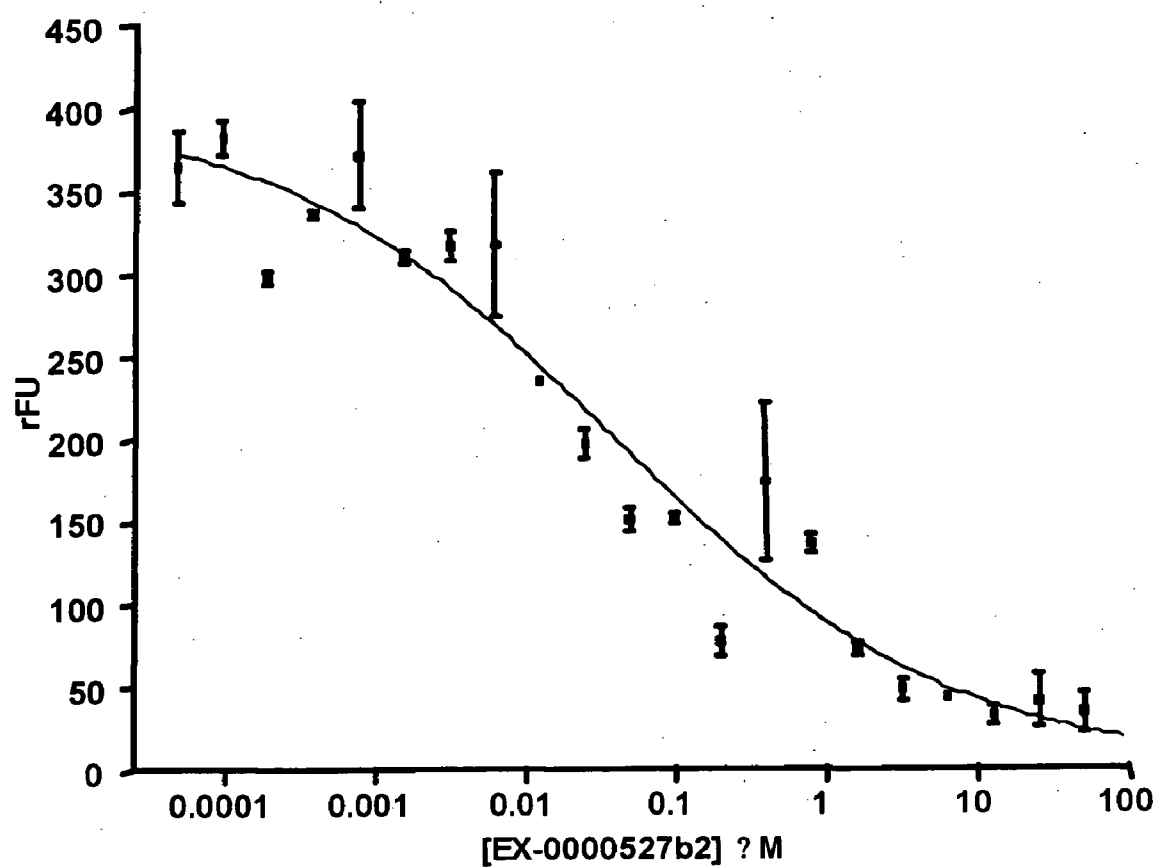


FIG. 2

Compound 8 inhibition of
Pulldown derived GST:SirT1



Data Set-B	
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.0
TOP	399.9
LOGEC50	-1.412
HILLSLOPE	-0.3895
EC50	0.03870

FIG. 3A

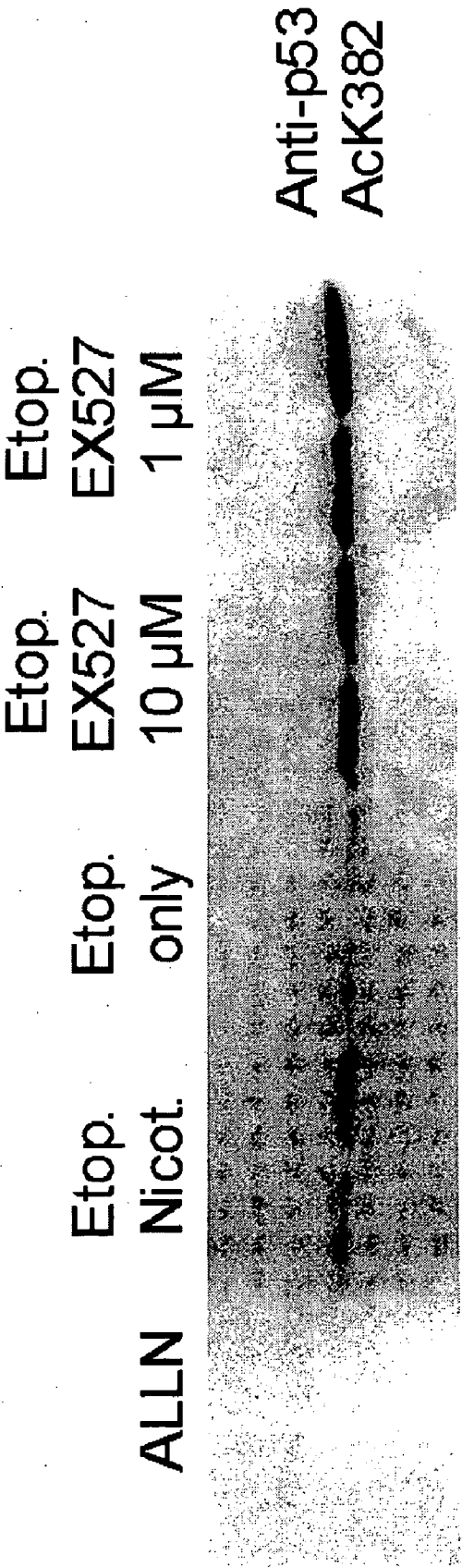


FIG. 3B

Effects of 1.0 μ M 527 and enantiomers on p53 acetylation in the presence of etoposide

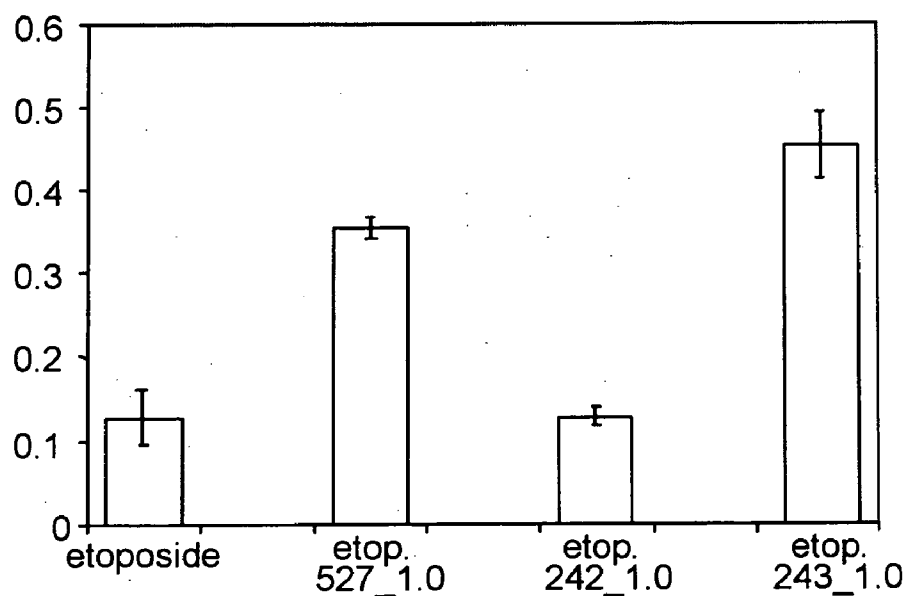
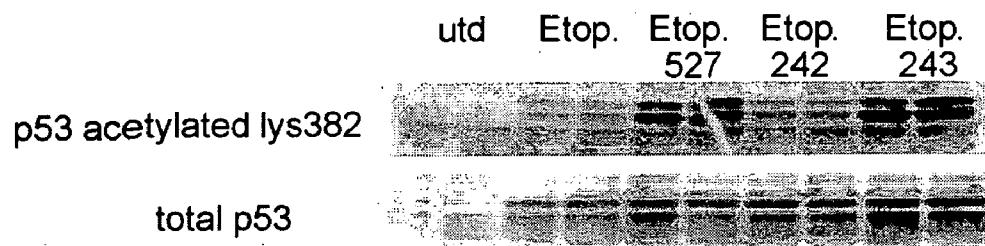


FIG. 4

1-21-04 Acetylation of p53 in response to active and inactive SIRT1 inhibitors

NCI-H460 cells were treated for 6 hours with 20μ M etoposide , 25 nM TSA, And 1μM of SIRT1 inhibitor compounds. Acetylated p53 was visualized by Western blot.

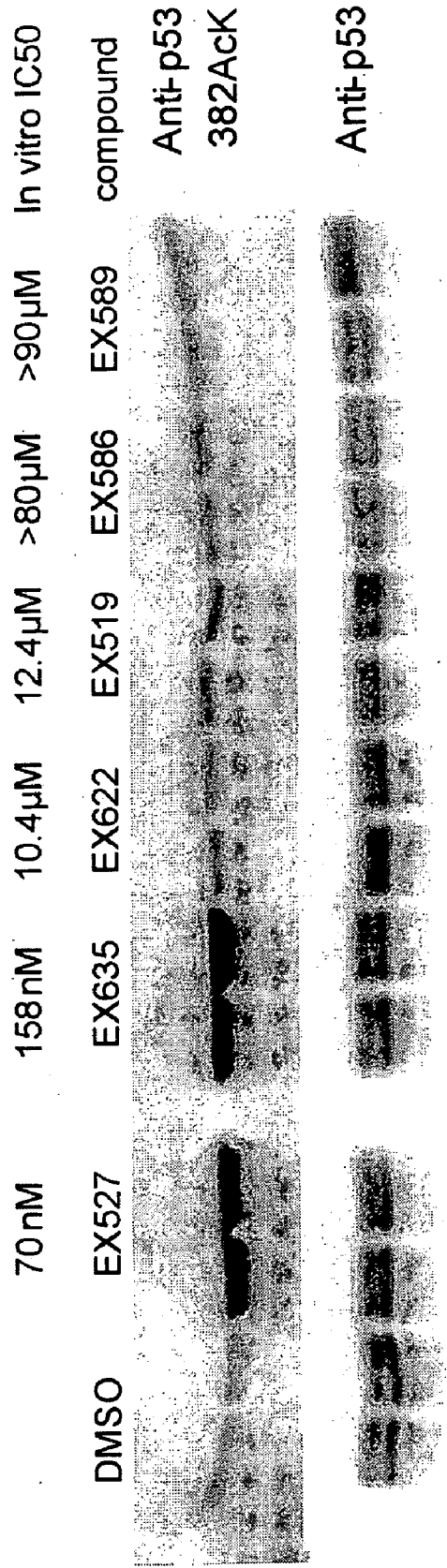


FIG. 5

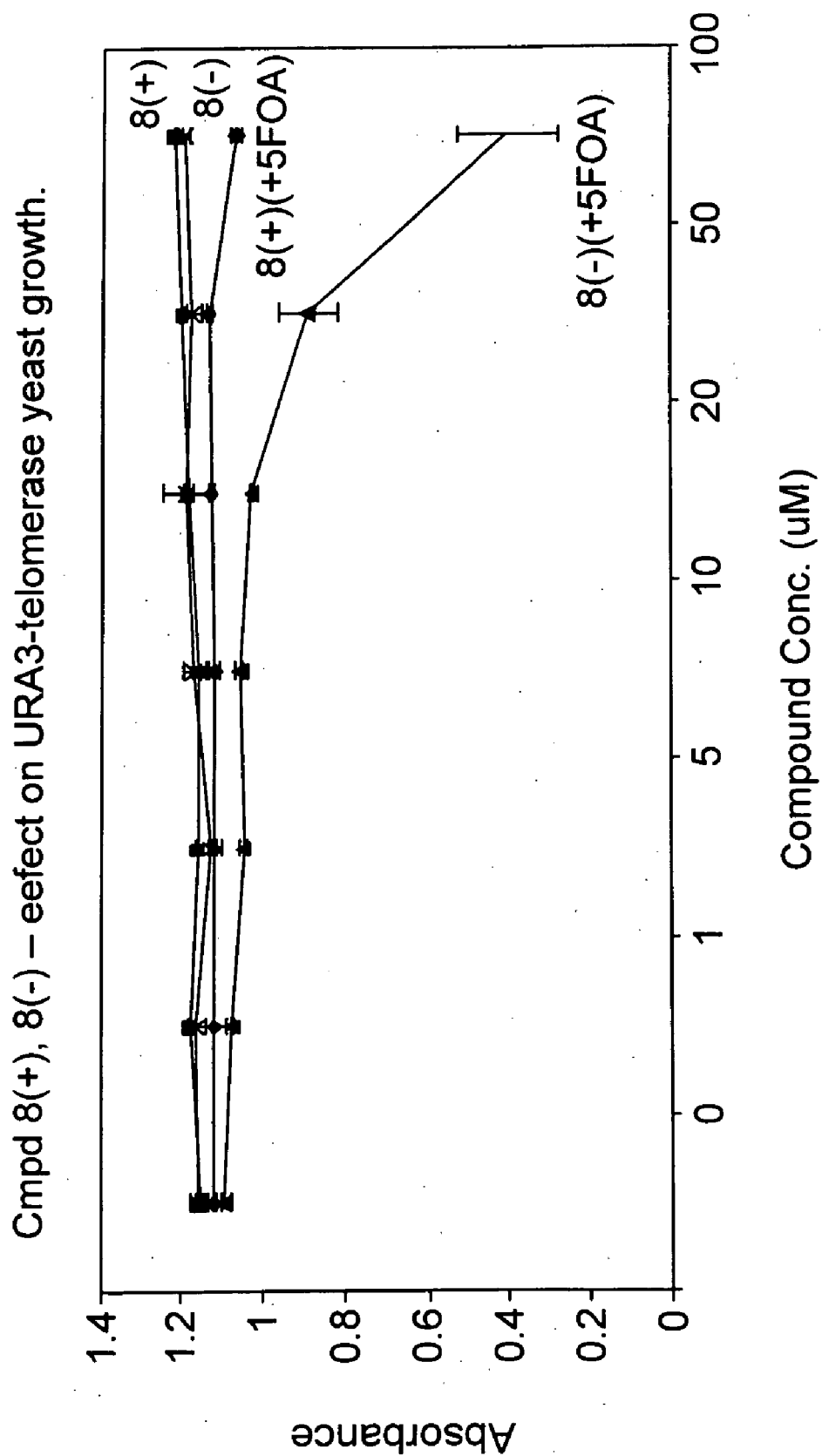


FIG. 6

Acetylation of p53 by EX 527 in MCF - 7 and U2 OS cells.

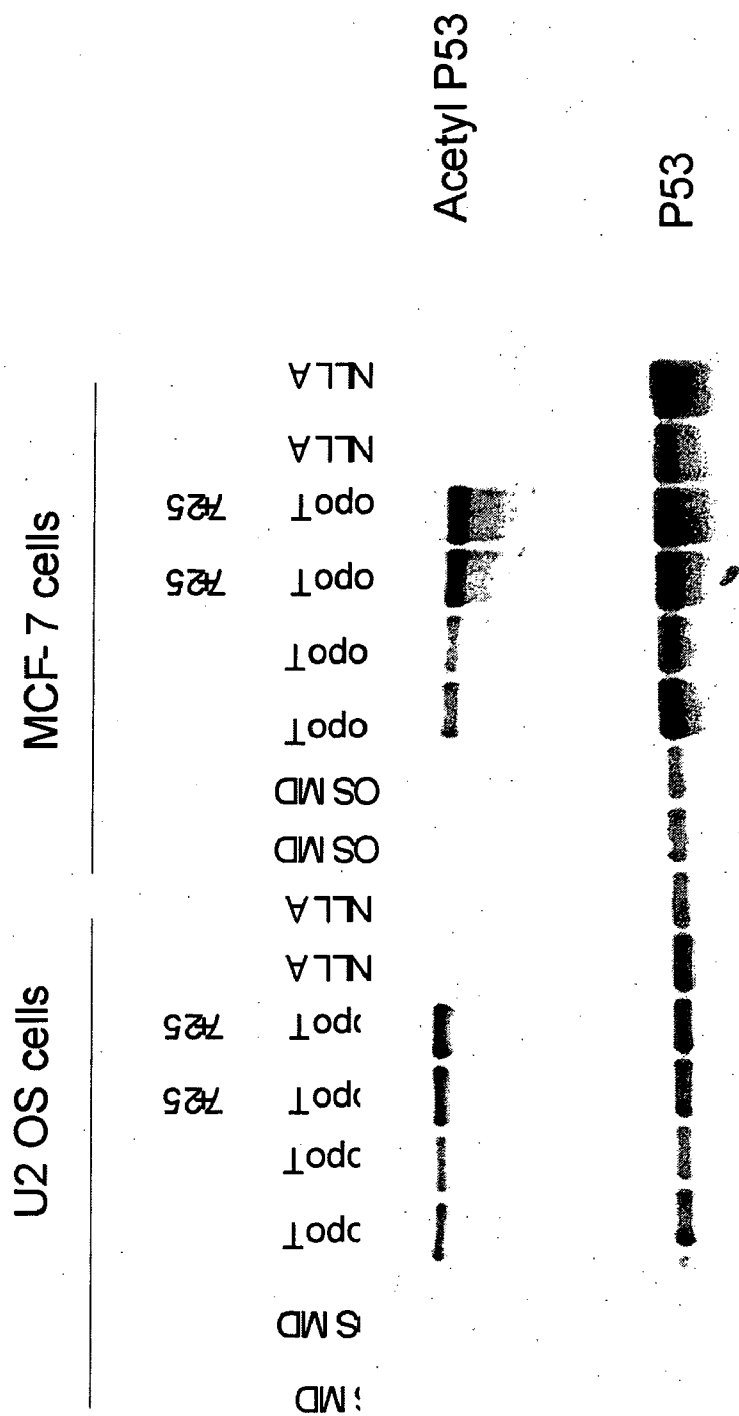


FIG. 7

FIG. 7

12-18-03 Viability of NCI-H460
cells 48 hours after addition of
etoposide and either EX527 or
nicotinamide

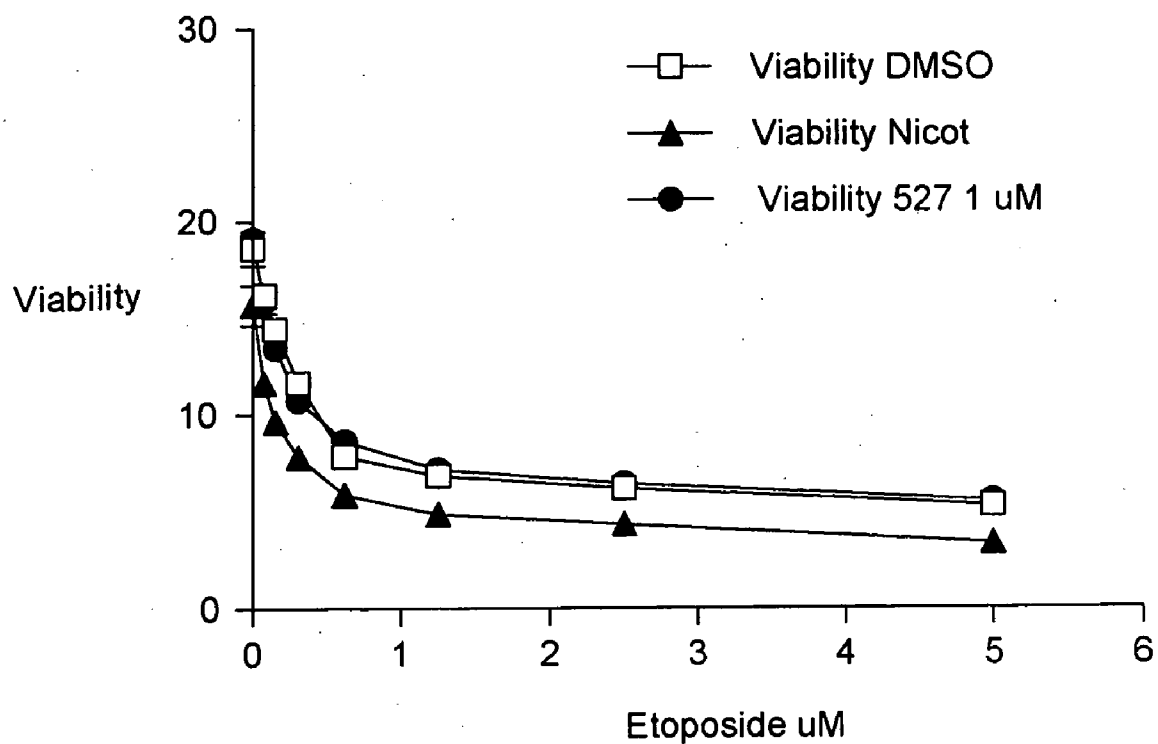


FIG. 8

No Effect of 527 on Etoposide Toxicity: Pre-treatment,
concurrent treatment or post - treatment

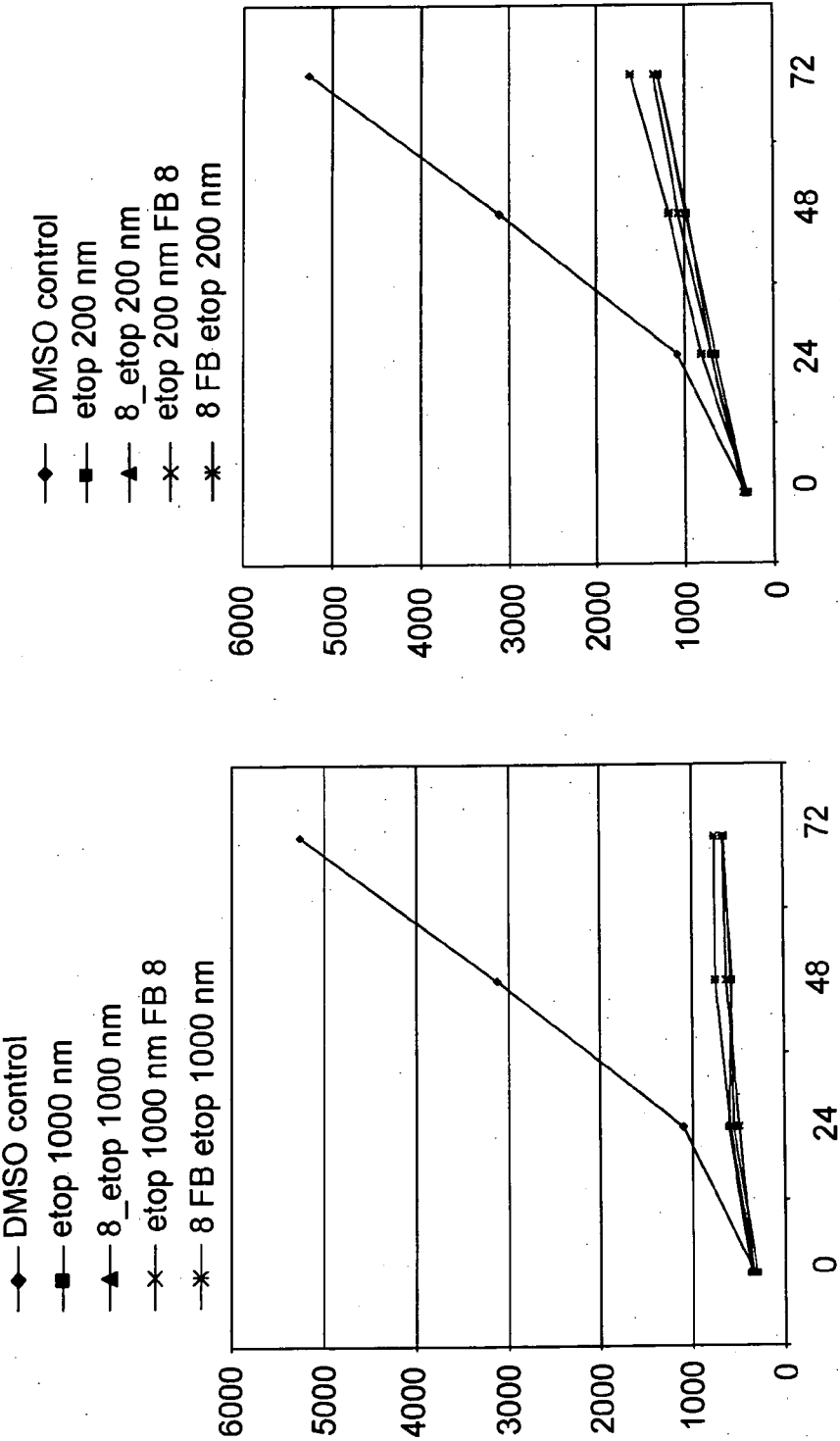


FIG. 9A

No Effect of 527 on Etoposide Toxicity: Pre-treatment,
concurrent treatment or post - treatment

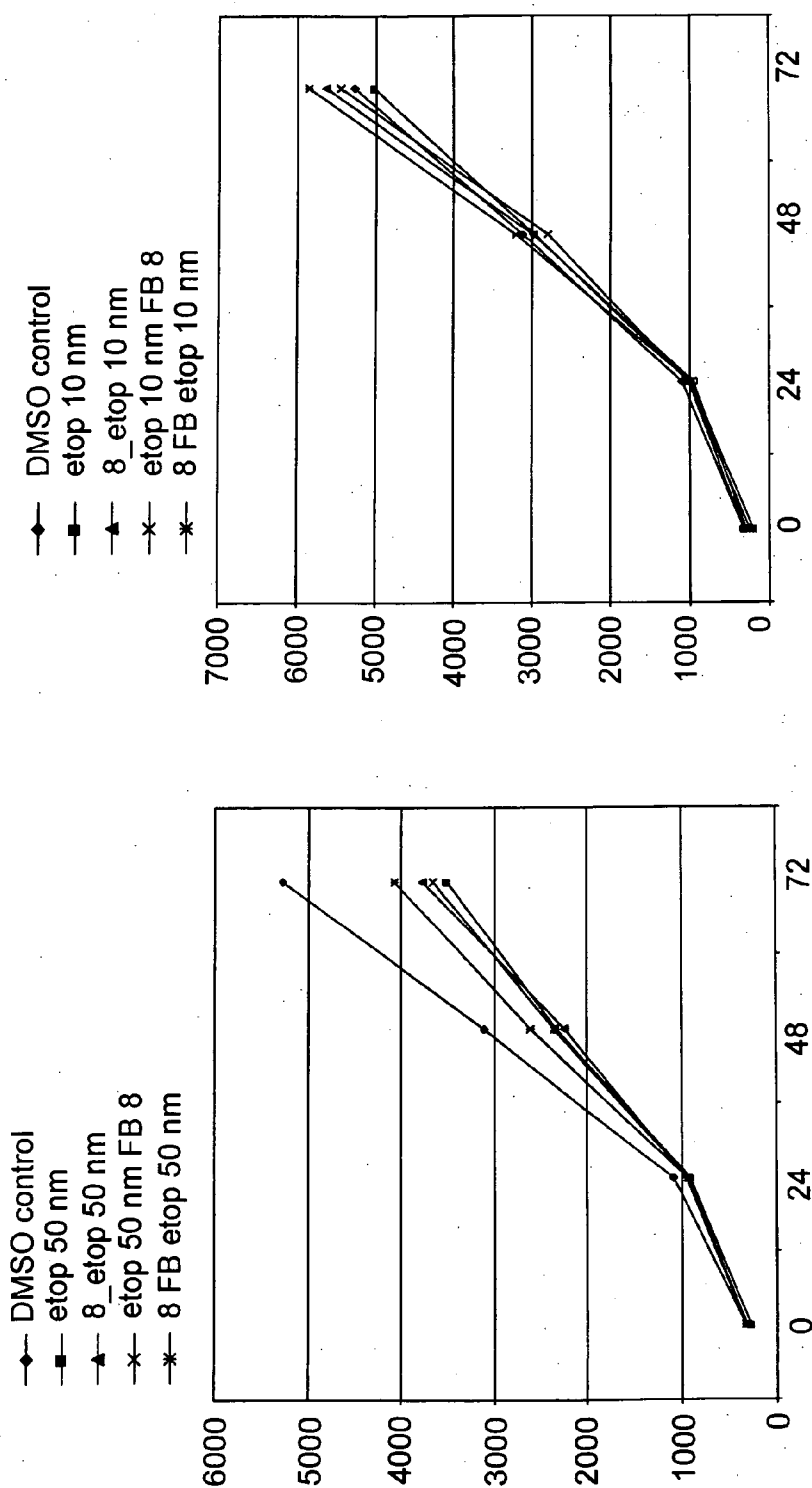


FIG. 9B

No Effect of 527 on Etoposide Toxicity: Pre-treatment,
concurrent treatment or post - treatment

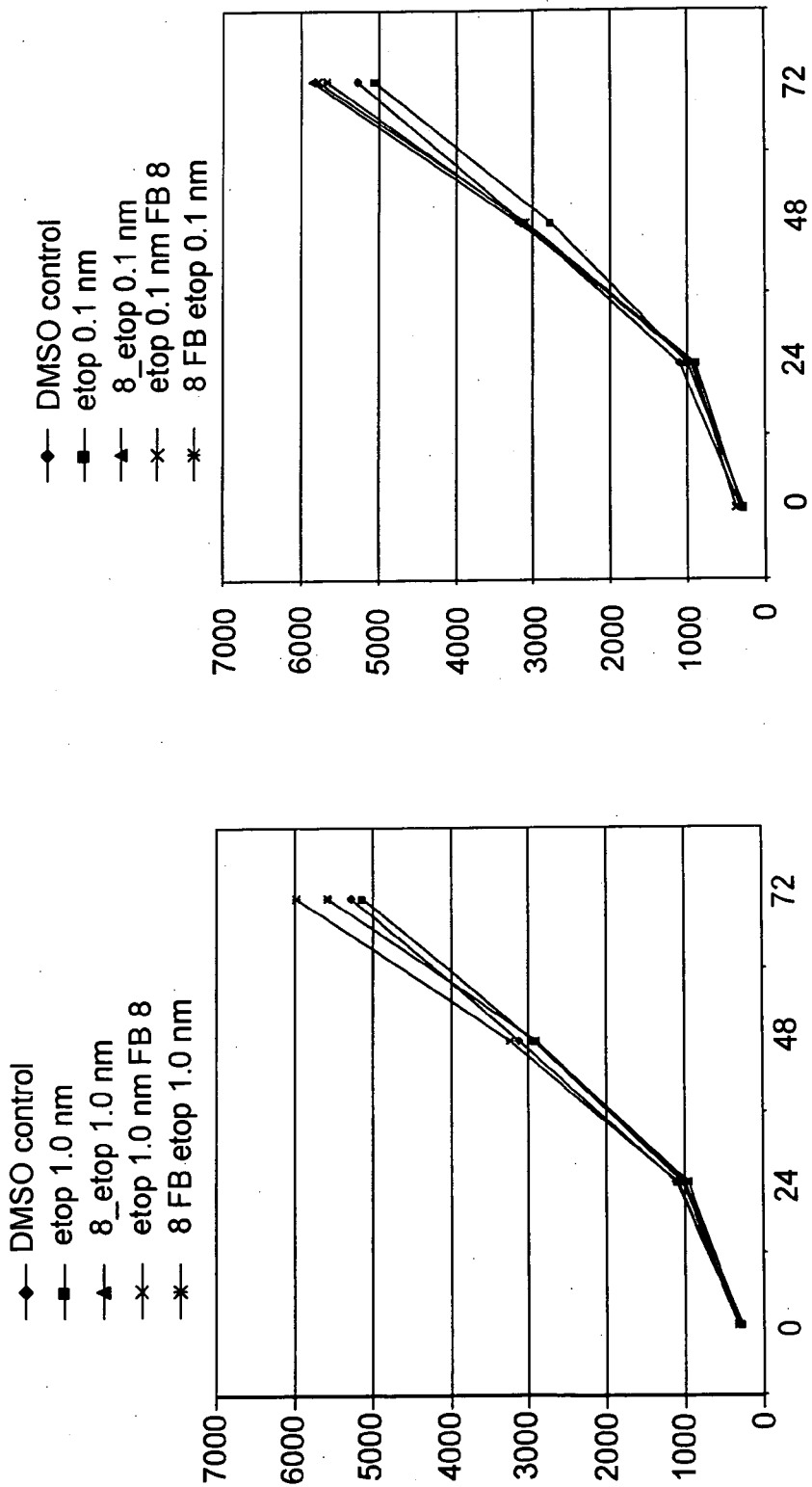


FIG. 9C

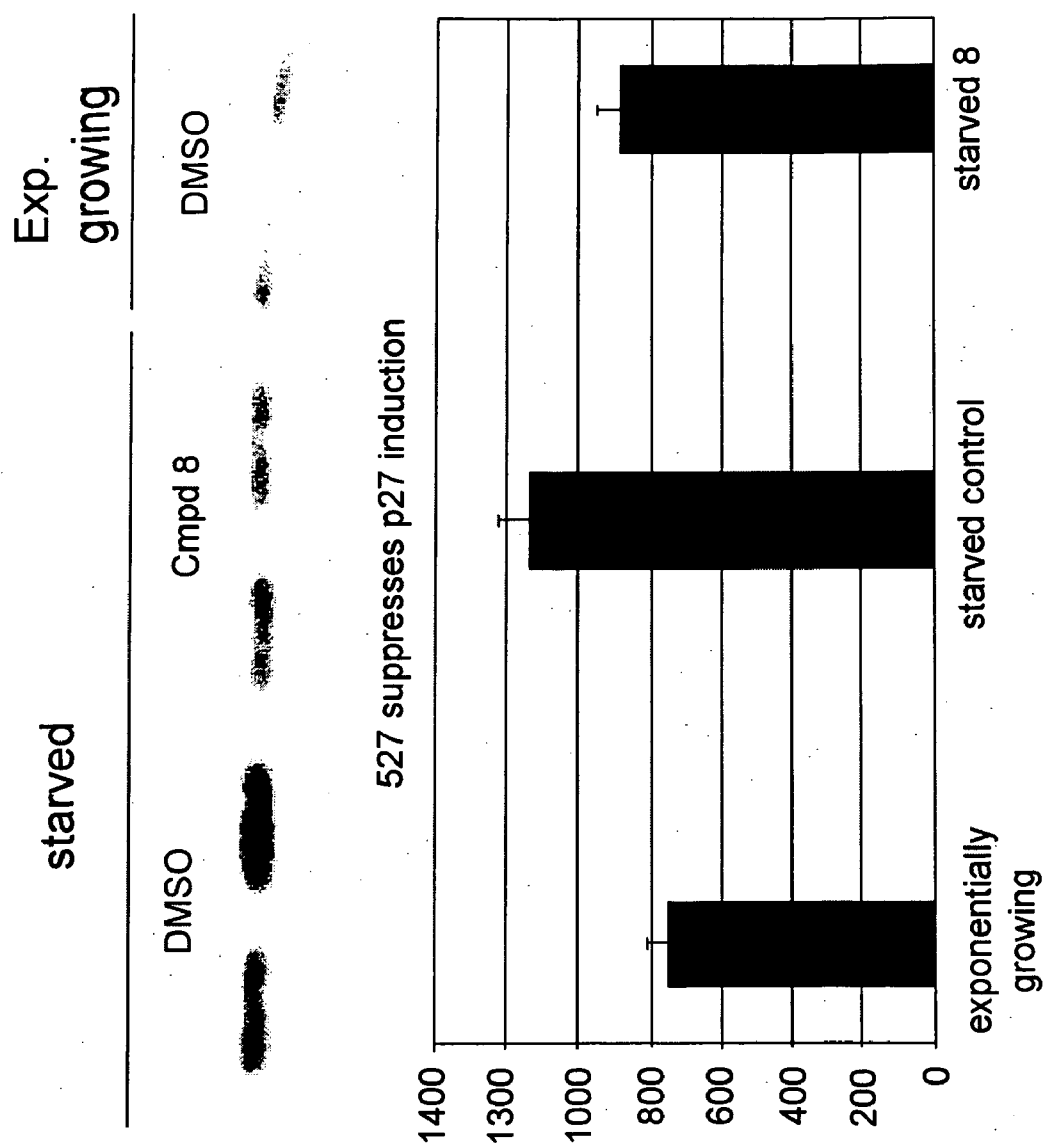


FIG. 10

METHODS OF TREATING A DISORDER

CLAIM OF PRIORITY

[0001] This application claims priority under 35 USC § 119(e) to U.S. Patent Application Ser. No. 60/502,811, filed on Sep. 12, 2003, U.S. Patent Application Ser. No. 60/531,443, filed on Dec. 19, 2003, and U.S. patent application Ser. No. 60/560,509, filed on Apr. 7, 2004, and is a continuation-in-part of U.S. patent application Ser. No. 10/940,269, filed Sep. 13, 2004, the entire contents of each of which are hereby incorporated by reference.

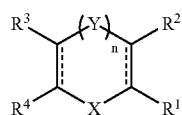
BACKGROUND

[0002] The Sir2 protein is a deacetylase which uses NAD as a cofactor (Imai et al., 2000; Moazed, 2001; Smith et al., 2000; Tanner et al., 2000; Tanny and Moazed, 2001). Unlike other deacetylases, many of which are involved in gene silencing, Sir2 is insensitive to histone deacetylase inhibitors like trichostatin A (TSA) (Imai et al., 2000; Landry et al., 2000a; Smith et al., 2000).

SUMMARY

[0003] The invention relates to substituted heterocyclic compounds, compositions comprising the compounds, and methods of using the compounds and compound compositions. The compounds and compositions comprising them are useful for treating disease or disease symptoms, including those mediated by sirtuin, e.g., SIRT1, mediated deacetylation.

[0004] In one aspect, this invention relates to a method for treating or preventing a disorder in a subject, e.g., a disorder described herein. The method includes administering to the subject an effective amount of a compound having a formula (I):



(I)

[0005] wherein,

[0006] R^1 and R^2 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkyl, C_5 - C_{10} heterocyclyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, C_6 - C_{10} aryl, or C_5 - C_{10} heteroaryl, each of which may be optionally substituted with 1-5 R^5 ; or R^1 is H, S-alkyl, or S-aryl, and R^2 is amidoalkyl wherein the nitrogen is substituted with alkyl, aryl, or arylalkyl, each of which is optionally further substituted with alkyl, halo, hydroxy, or alkoxy;

[0007] R^3 and R^4 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkyl, C_5 - C_{10} heterocyclyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, C_6 - C_{10} aryl, or C_5 - C_{10} heteroaryl, each of which may be optionally substituted with 1-5 R^6 ;

[0008] each of R^5 and R^6 is, independently, halo, hydroxy, C_1 - C_{10} alkyl, C_1 - C_6 haloalkyl, C_1 - C_{10} alkoxy, C_1 - C_6 haloalkoxy, C_6 - C_{10} aryl, C_5 - C_{10} heteroaryl,

C_7 - C_{12} aralkyl, C_7 - C_{12} heteroaralkyl, C_3 - C_8 heterocyclyl, C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, carboxy, carboxylate, cyano, nitro, amino, C_1 - C_6 alkyl amino, C_1 - C_6 dialkyl amino, mercapto, SO_3H , sulfate, $S(O)NH_2$, $S(O)_2NH_2$, phosphate, C_1 - C_4 alkylendioxy, oxo, acyl, aminocarbonyl, C_1 - C_6 alkyl aminocarbonyl, C_1 - C_6 dialkyl aminocarbonyl, C_1 - C_{10} alkoxycarbonyl, C_1 - C_{10} thioalkoxycarbonyl, hydrazinocarbonyl, C_1 - C_6 alkyl hydrazinocarbonyl, C_1 - C_6 dialkyl hydrazinocarbonyl, hydroxyaminocarbonyl; alkoxyaminocarbonyl; or one of R^5 or R^6 and R^7 form a cyclic moiety containing 4-6 carbons, 1-3 nitrogens, 0-2 oxygens and 0-2 sulfurs, which may be optionally substituted with oxo or C_1 - C_6 alkyl;

[0009] X is NR^7 , O, or S; Y is NR^7 , O or S;

[0010] - - - represent optional double bonds;

[0011] each of R^7 and R^7 is, independently, hydrogen, C_1 - C_6 alkyl, C_7 - C_{12} arylalkyl, C_7 - C_{12} heteroarylalkyl; or R^7 and one of R^5 or R^6 form a cyclic moiety containing 4-6 carbons, 1-3 nitrogens, 0-2 oxygens and 0-2 sulfurs, which may be optionally substituted with oxo or C_1 - C_6 alkyl; and n is 0 or 1.

[0012] Embodiments can include one or more of the following.

[0013] In certain embodiments, n can be 1.

[0014] X can be NR^7 and Y can be NR^7 . R^7 and R^7 can each be, e.g., hydrogen or CH_3 . One of R^7 and R^7 can be hydrogen and the other can be CH_3 .

[0015] R^1 and R^2 can form C_5 - C_{10} cycloalkenyl.

[0016] R^1 and R^2 can form C_6 - C_{10} aryl.

[0017] R^1 and R^2 can form C_5 - C_{10} cycloalkenyl, which may be substituted with R^5 , and R^3 and R^4 can form C_6 - C_{10} aryl, which may be substituted with R^6 .

[0018] In certain embodiments, the cycloalkenyl double bond can be between the carbon attached to R^1 and the carbon attached to R^2 . C_5 - C_{10} cycloalkenyl, e.g., C_6 or C_7 cycloalkenyl, can be substituted with R^5 and C_6 - C_{10} aryl can be substituted with R^6 .

[0019] R^6 can be halo (e.g., chloro or bromo), C_1 - C_6 alkyl (e.g., CH_3), C_1 - C_6 haloalkyl (e.g., CF_3) or C_1 - C_6 haloalkoxy (e.g., OCF_3). R^5 can be for example, C_1 - C_6 alkyl substituted with a substituent such as an amino substituent, or aminocarbonyl (for example a substituted aminocarbonyl, substituted with substituents such as an aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aminocarbonyl, alkylaminocarbonyl, alkoxycarbonyl or other substituents. In each instances, the substituents can be further substituted with other substituents).

[0020] n can be 0.

[0021] R^1 and R^2 can form C_5 - C_{10} cycloalkenyl.

[0022] R^1 and R^2 can form C_6 - C_{10} aryl.

[0023] X can be NR^7 , and R^7 can be, e.g., hydrogen or CH_3 .

[0024] R^1 and R^2 can form C_5 - C_{10} cycloalkenyl, which may be substituted with R^5 , and R^3 and R^4 can form C_6 - C_{10} aryl, which may be substituted with R^6 .

[0025] In certain embodiments, the cycloalkenyl double bond can be between the carbon attached to R¹ and the carbon attached to R². C₅-C₁₀ cycloalkenyl, e.g., C₆ or C₇ cycloalkenyl, can be substituted with R⁵ and C₆-C₁₀ aryl can be substituted with R⁶.

[0026] R⁶ can be halo (e.g., chloro), C₁-C₆ alkyl (e.g., CH₃), C₁-C₆ haloalkyl (e.g., CF₃) or C₁-C₆ haloalkoxy (e.g., OCF₃). R⁵ can be aminocarbonyl.

[0027] n can be 0.

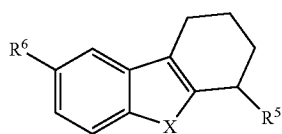
[0028] R¹ and R² can form C₅-C₁₀ cycloalkenyl.

[0029] R¹ and R² can form C₆-C₁₀ aryl.

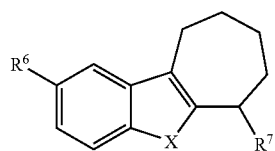
[0030] X can be NR⁷, and R⁷ can be, e.g., hydrogen or CH₃.

[0031] R¹ and R² can form C₅-C₁₀ cycloalkenyl, which may be substituted with R⁵, and R³ and R⁴ can form C₆-C₁₀ aryl, which may be substituted with R⁶.

[0032] In certain embodiments, the cycloalkenyl double bond can be between the carbon attached to R¹ and the carbon attached to R². C₅-C₁₀ cycloalkenyl, e.g., C₆ or C₇ cycloalkenyl, can be substituted with R⁵ and C₆-C₁₀ aryl can be substituted with R⁶. These compounds may have formula (II) or formula (III):

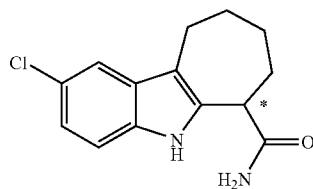


(II)

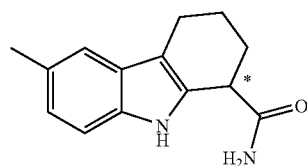


(III)

[0033] R⁶ can be halo (e.g., chloro or bromo), C₁-C₆ alkyl (e.g., CH₃), C₁-C₆ haloalkyl (e.g., CF₃) or C₁-C₆ haloalkoxy (e.g., OCF₃). R⁵ can be aminocarbonyl. The compound may be a compound selected from **FIG. 1** or compounds (IV), (V), (VI), or (VII).

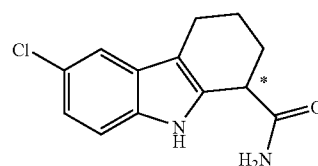


(IV)

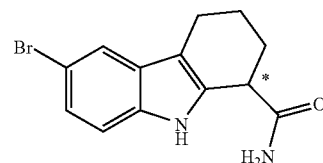


(V)

-continued



(VI)



(VII)

[0034] In one instance, the compound can be a compound of formula (VI) having a high enantiomeric excess of a single isomer, wherein the optical rotation of the predominant isomer is negative, for example, $[\alpha]_D^{25} -14.1$ (c=0.33, DCM) or, for example, $[\alpha]_D^{25} -41.2^\circ$ (c 0.96, CH₃OH). In a second instance, the compound can be a compound of formula (IV) having a high enantiomeric excess of a single isomer, wherein the optical rotation of the predominant isomer is negative. In some instances, a compound of formula (IV), (V), or (VII) is administered having a high enantiomeric excess of a single isomer, where the predominant isomer has the same absolute configuration as the negative isomer of the compound of formula (VI) as corresponds to the asterisk carbon shown above.

[0035] The compound can preferentially inhibit SIRT1 relative to a non-SIRT1 sirtuin, e.g., at least a 1.5, 2, 5, or 10 fold preference. The compound can have a K_i for SIRT1 that is less than 500, 100, 50, or 40 nM.

[0036] In some instances, the compound reduces the activity of a FOXO transcription factor such as FoxO1 or FoxO3.

[0037] The amount can be effective to ameliorate at least one symptom of the disorder.

[0038] The disease or disorder can be, e.g., an age-associated disorder, a geriatric disorder, a disorder having an age-associated susceptibility factor, a neoplastic disorder, a non-neoplastic disorder, a neurological disorder, a cardiovascular disorder, a metabolic disorder, a dermatological disorder, or a dermatological tissue condition. In one embodiment, the disease or disorder can be a neurodegenerative disease or disorder in which the neurodegenerative disorder can be mediated at least in part by polyglutamine aggregation, e.g., Huntington's disease, Spinalbulbar Muscular Atrophy (SBMA or Kennedy's Disease) Dentatorubropallidoluysian Atrophy (DRPLA), Spinocerebellar Ataxia 1 (SCA1), Spinocerebellar Ataxia 2 (SCA2), Machado-Joseph Disease (MJD; SCA3), Spinocerebellar Ataxia 6 (SCA6), Spinocerebellar Ataxia 7 (SCA7), and Spinocerebellar Ataxia 12 (SCA12). The neurodegenerative disorder can be Parkinson's or Alzheimer's.

[0039] The disease or disorder can be associated with or mediated at least in part by a sirtuin, e.g., the disease or disorder can be associated with or mediated at least in part by sirtuin-mediated deacetylation, e.g., excessive sirtuin activity or excessive levels of deacetylated p53, FoxO1, or FoxO3. The sirtuin can be SIRT1, e.g., human SIRT1.

[0040] The disease or disorder can be cancer. The amount can be, e.g., effective to reduce cancer or tumor cell mass, risk of metastasis, or rate of tumor cell growth. The amount can be effective to modulate (e.g., increase) apoptosis.

[0041] The disease or disorder can be a metabolic disease, such as metabolic syndrome or diabetes (e.g., type I diabetes or type II diabetes). The amount can be, for example, effective to increase insulin sensitivity, increase insulin secretion, or otherwise or lower levels of glucose. In some instances, the disease or disorder is related to a metabolic disease, such as cardiac disorder related diabetes.

[0042] The disease or disorder can be a fat related disorder such as obesity or dislipidemia or hyperlipidemia. The amount can be, for example, effective to reduce weight in a subject or to prevent weight gain in a subject.

[0043] The disease or disorder can be a neurological disorder such as Alzheimer's disease or Parkinson's disease. The amount can be, for example, effective to reduce one or more symptoms of the neurological disorder.

[0044] The method can include administering the compound more than once, e.g., repeatedly administering the compound. The compound can be administered in one or more boluses or continuous. The compound can be administered from without (e.g., by injection, ingestion, inhalation, etc), or from within, e.g., by an implanted device.

[0045] The method can include administering the compound locally.

[0046] The amount can be effective to increase acetylation of a sirtuin substrate (e.g., a nuclear protein, e.g., a histone or a transcription factor, e.g., p53, FoxO1, or FoxO3) in at least some cells of the subject.

[0047] The subject can be a mammal, e.g., a human.

[0048] The subject can be identified as being in need of such treatment or prevention.

[0049] The method further can further include identifying a subject in need of such treatment, e.g., by evaluating sirtuin activity in a cell of the subject, evaluating nucleotide identity in a nucleic acid of the subject that encodes a sirtuin, evaluating the subject for neoplastic cells or a neoplastic growth (e.g., a tumor), evaluating the genetic composition or expression of genes in a cell of the subject, e.g., a tumor biopsy.

[0050] The method can further include monitoring the subject, e.g., imaging the subject, evaluating tumor size in the subject, evaluating sirtuin activity in a cell of the subject, evaluating the subject for side effects, e.g., renal function.

[0051] In another aspect, this invention relates to a method of inhibiting sirtuin-mediated deacetylation of a substrate, such as a FoxO transcription factor. The method includes contacting a sirtuin with a compound of formula (I). The inhibiting can occur in vitro, in cell-free medium, in cell culture, or in an organism, e.g., a mammal, preferably a human.

[0052] In a further aspect, this invention relates to a method for evaluating a plurality of compounds, the method includes: a) providing library of compound that comprises a plurality of compounds, each having a formula (I); and b) for each of a plurality of compounds from the library, i) con-

tacting the compound to a sirtuin test protein that comprises a functional deacetylase domain of a sirtuin; and ii) evaluating interaction between the compound and the sirtuin test protein in the presence of the compound.

[0053] Embodiments can include one or more of the following.

[0054] In one embodiment, evaluating the interaction between the compound and the sirtuin test protein includes evaluating enzymatic activity of the sirtuin test protein.

[0055] In one embodiment, evaluating the interaction between the compound and the sirtuin test protein includes evaluating a binding interaction between the compound and the sirtuin test protein

[0056] The method can further include selecting, based on results of the evaluating, a compound that modulates deacetylase activity for a substrate. The substrate can be an acetylated lysine amino acid, an acetylated transcription factor (e.g., p53, FoxO1, or FoxO3) or an acetylated peptide thereof, an acetylated histone or an acetylated peptide thereof.

[0057] The method may also further include selecting, based on results of the evaluating, a compound that modulates sirtuin deacetylase activity of a substrate.

[0058] The method may also further include selecting, based on results of the evaluating, a compound that modulates the sirtuin.

[0059] In one aspect, this invention relates to a conjugate that includes: a targeting agent and a compound, wherein the targeting agent and the compound are covalently linked, and the compound has a formula (I).

[0060] Embodiments can include one or more of the following.

[0061] The targeting agent can be an antibody, e.g., specific for a cell surface protein, e.g., a cancer-specific antigen.

[0062] The targeting agent can be a synthetic peptide.

[0063] The targeting agent can be a domain of a naturally occurring protein.

[0064] In another aspect, this invention relates to a kit which includes: a compound described herein, and instructions for use for treating a disease described herein. The kit may further include a printed material comprising a rendering of the structure of the name of the compound.

[0065] In another aspect, this invention relates to a method of analyzing or designing structures, the method includes: providing a computer-generated image or structure (preferably a three dimensional image or structure) for a compound described herein, e.g., a compound of formula I, providing a computer-generated image or structure (preferably a three dimensional image or structure) for a second compound, e.g., another compound described herein, (e.g., a compound of formula I, NAD) or a target, e.g., e.g., a sirtuin (e.g., a human sirtuin, e.g., SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7), or an off-target molecule, e.g., a sirtuin other than SIRT1, e.g., SIRT2 or SIRT3, or non-sirtuin histone deacetylase; and comparing the structure of the first and second compound, e.g., comparing the structure, e.g., a parameter related to bond angle, inter-or intra-molecular

distance, position of an atom or moiety; e.g., a first or second generation compound—the predicted ability of compound to interact or inhibit a target or off-target molecule.

[0066] In a preferred embodiment, the structure is further evaluated *in vitro*, *in vivo*, or *in silico* with target or off-target molecule.

[0067] In a further aspect, this invention relates to a database, which includes: information about or identifying the structure, information about activity of the structure, e.g., *in vitro*, *in vivo* or *in silico*, e.g., at least 5, 10, 50, or 100 records.

[0068] In one aspect, this invention relates to a database, which includes a plurality of records, each record having: a) information about or identifying a compound that has a structure described herein, e.g., a structure of formula I; and b) information about a parameter of a patient, the parameter relating to a neoplastic disorder or a neurodegenerative disorder, e.g. a patient parameter.

[0069] In one aspect, this invention relates to a method of evaluating a compound, the method includes: providing a first compound that has a structure of formula I, or a data record having information about the structure; providing a second compound that has a structure of formula I or not having formula I, or a data record having information about the structure; evaluating a first compound and the second compound, e.g., *in vivo*, *in vitro*, or *in silico*; and comparing the ability of a second compound to interact, e.g., inhibit a sirtuin, e.g., SIRT1, with a first compound, thereby evaluating ability of the second compound to interact with SIRT1.

[0070] In other aspects, the invention relates to a composition comprising a compound of any of the formulae herein, and a pharmaceutically acceptable carrier. The composition may contain an additional therapeutic agent, e.g., an anti-tumor agent or a neurodegenerative disease agent. Also within the scope of this invention is the use of such a composition for the manufacture of a medicament for the just-mentioned use.

[0071] In another aspect, the invention is a method for treating or preventing a disease characterized by unwanted cell proliferation, e.g., cancer, e.g., a p53 dependent cancer or a p53 independent cancer, in a subject. The method includes administering a SIRT1 antagonist. For example, the SIRT1 antagonist can be one or more of: antisense of SIRT1, RNAi, an antibody, an intrabody, and other compounds identified by a method described herein, e.g., compounds that induce apoptosis in a SIRT1 expressing cell.

[0072] In a preferred embodiment, the method includes administering a SIRT1 antagonist in combination with one or more therapeutic agents, e.g., a therapeutic agent or agent for treating unwanted cell proliferation. The therapeutic agents include, for example, one or more of a chemotherapeutic agent, a radioisotope, and a cytotoxin. Examples of chemotherapeutic agents include taxol, cytochalasin B, gramicidin D, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, busulfan, cisplatin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, chlorambucil, gemcitabine, actinomycin, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids and analogs or homologs thereof. Additional therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thiogua-

nine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids). Radioisotopes can include alpha, beta and/or gamma emitters. Examples of radioisotopes include ^{212}Bi , ^{213}Bi , ^{131}I , ^{211}At , ^{186}Re , ^{90}Y and ^{117}Lu .

[0073] The SIRT1 antagonist and the therapeutic agents can be administered simultaneously or sequentially.

[0074] Also within the scope of this invention is a packaged product. The packaged product includes a container, one of the aforementioned compounds in the container, and a legend (e.g., a label or insert) associated with the container and indicating administration of the compound for treating a disorder described herein (e.g., cancer or neurodegenerative disorders), diseases, or disease symptoms, including any of those delineated herein.

[0075] The subject can be a mammal, preferably a human. The subject can also be a non-human subject, e.g., an animal model. In certain embodiments the method can further include identifying a subject. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g., opinion) or objective (e.g., measurable by a test or diagnostic method).

[0076] The term “mammal” includes organisms, which include mice, rats, cows, sheep, pigs, rabbits, goats, and horses, monkeys, dogs, cats, and preferably humans.

[0077] The term “treating” or “treated” refers to administering a compound described herein to a subject with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a disease, e.g., an infection, the symptoms of the disease or the predisposition toward the disease.

[0078] An effective amount of the compound described above may range from about 0.1 mg/Kg to about 500 mg/Kg, alternatively from about 1 to about 50 mg/Kg. Effective doses will also vary depending on route of administration, as well as the possibility of co-usage with other agents.

[0079] The term “halo” or “halogen” refers to any radical of fluorine, chlorine, bromine or iodine.

[0080] The term “alkyl” refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, $\text{C}_1\text{-C}_{12}$ alkyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it. The term “haloalkyl” refers to an alkyl in which one or more hydrogen atoms are replaced by halo, and includes alkyl moieties in which all hydrogens have been replaced by halo (e.g., perfluoroalkyl). The terms “arylalkyl” or “aralkyl” refer to an alkyl moiety in which an alkyl hydrogen atom is replaced by an aryl group. Aralkyl includes groups in which more than one hydrogen atom has been replaced by an aryl group. Examples of “arylalkyl” or

“aralkyl” include benzyl, 2-phenylethyl, 3-phenylpropyl, 9-fluorenyl, benzhydryl, and trityl groups.

[0081] The term “alkylene” refers to a divalent alkyl, e.g., $-\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$, and $-\text{CH}_2\text{CH}_2\text{CH}_2-$.

[0082] The term “alkenyl” refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and having one or more double bonds. Examples of alkenyl groups include, but are not limited to, allyl, propenyl, 2-butenyl, 3-hexenyl and 3-octenyl groups. One of the double bond carbons may optionally be the point of attachment of the alkenyl substituent. The term “alkynyl” refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and characterized in having one or more triple bonds. Examples of alkynyl groups include, but are not limited to, ethynyl, propargyl, and 3-hexynyl. One of the triple bond carbons may optionally be the point of attachment of the alkynyl substituent.

[0083] The terms “alkylamino” and “dialkylamino” refer to $-\text{NH}(\text{alkyl})$ and $-\text{NH}(\text{alkyl})_2$ radicals respectively. The term “aralkylamino” refers to a $-\text{NH}(\text{aralkyl})$ radical. The term alkylaminoalkyl refers to a $(\text{alkyl})\text{NH-alkyl-radical}$; the term dialkylaminoalkyl refers to a $(\text{alkyl})_2\text{N-alkyl-radical}$. The term “alkoxy” refers to an $-\text{O-alkyl}$ radical. The term “mercapto” refers to an SH radical. The term “thioalkoxy” refers to an $-\text{S-alkyl}$ radical. The term thioaryloxy refers to an $-\text{S-aryl}$ radical.

[0084] The term “aryl” refers to an aromatic monocyclic, bicyclic, or tricyclic hydrocarbon ring system, wherein any ring atom capable of substitution can be substituted (e.g., by one or more substituents). Examples of aryl moieties include, but are not limited to, phenyl, naphthyl, and anthracenyl.

[0085] The term “cycloalkyl” as employed herein includes saturated cyclic, bicyclic, tricyclic, or polycyclic hydrocarbon groups having 3 to 12 carbons. Any ring atom can be substituted (e.g., by one or more substituents). The cycloalkyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of cycloalkyl moieties include, but are not limited to, cyclopropyl, cyclohexyl, methylcyclohexyl, adamantyl, and norbornyl.

[0086] The term “heterocyclyl” refers to a nonaromatic 3-10 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). The heteroatom may optionally be the point of attachment of the heterocyclyl substituent. Any ring atom can be substituted (e.g., by one or more substituents). The heterocyclyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of heterocyclyl include, but are not limited to, tetrahydrofuran, tetrahydropyranyl, piperidyl, morpholino, pyrrolinyl, pyrimidinyl, quinolinyl, and pyrrolidinyl.

[0087] The term “cycloalkenyl” refers to partially unsaturated, nonaromatic, cyclic, bicyclic, tricyclic, or polycyclic hydrocarbon groups having 5 to 12 carbons, preferably 5 to 8 carbons. The unsaturated carbon may optionally be the point of attachment of the cycloalkenyl substituent. Any ring

atom can be substituted (e.g., by one or more substituents). The cycloalkenyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of cycloalkenyl moieties include, but are not limited to, cyclohexenyl, cyclohexadienyl, or norbornenyl.

[0088] The term “heterocycloalkenyl” refers to a partially saturated, nonaromatic 5-10 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). The unsaturated carbon or the heteroatom may optionally be the point of attachment of the heterocycloalkenyl substituent. Any ring atom can be substituted (e.g., by one or more substituents). The heterocycloalkenyl groups can contain fused rings. Fused rings are rings that share a common carbon atom. Examples of heterocycloalkenyl include but are not limited to tetrahydropyridyl and dihydropyranyl.

[0089] The term “heteroaryl” refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). Any ring atom can be substituted (e.g., by one or more substituents).

[0090] The term “oxo” refers to an oxygen atom, which forms a carbonyl when attached to carbon, an N-oxide when attached to nitrogen, and a sulfoxide or sulfone when attached to sulfur.

[0091] The term “acyl” refers to an alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, or heteroarylcarbonyl substituent, any of which may be further substituted (e.g., by one or more substituents).

[0092] The terms “aminocarbonyl,” alkoxycarbonyl,” hydrazinocarbonyl, and hydroxyaminocarbonyl refer to the radicals $-\text{C}(\text{O})\text{NH}_2$, $-\text{C}(\text{O})\text{O}(\text{alkyl})$, $-\text{C}(\text{O})\text{NH}_2\text{NH}_2$, and $-\text{C}(\text{O})\text{NH}_2\text{NH}_2$, respectively.

[0093] The term “amido” refers to a $-\text{NHC}(\text{O})-$ radical, wherein N is the point of attachment.

[0094] The term “substituents” refers to a group “substituted” on an alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, heterocycloalkenyl, cycloalkenyl, aryl, or heteroaryl group at any atom of that group. Any atom can be substituted. Suitable substituents include, without limitation, alkyl (e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12 straight or branched chain alkyl), cycloalkyl, haloalkyl (e.g., perfluoroalkyl such as CF_3), aryl, heteroaryl, aralkyl, heteroaralkyl, heterocyclyl, alkenyl, alkynyl, cycloalkenyl, heterocycloalkenyl, alkoxy, haloalkoxy (e.g., perfluoroalkoxy such as OCF_3), halo, hydroxy, carboxy, carboxylate, cyano, nitro, amino, alkyl amino, SO_3H , sulfate, phosphate, methylenedioxy ($-\text{O}-\text{CH}_2-\text{O}-$ wherein oxygens are attached to vicinal atoms), ethylenedioxy, oxo, thioxo (e.g., $\text{C}=\text{S}$), imino (alkyl, aryl, aralkyl), $\text{S}(\text{O})_n\text{alkyl}$ (where n is 0-2), $\text{S}(\text{O})_n\text{aryl}$ (where n is 0-2), $\text{S}(\text{O})_n\text{heteroaryl}$ (where n is 0-2), $\text{S}(\text{O})_n\text{heterocyclyl}$ (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, aryl, heteroaryl,

and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, aryl, heteroaryl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof). In one aspect, the substituents on a group are independently any one single, or any subset of the aforementioned substituents. In another aspect, a substituent may itself be substituted with any one of the above substituents.

[0095] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

[0096] All references cited herein, whether in print, electronic, computer readable storage media or other form, are expressly incorporated by reference in their entirety, including but not limited to, abstracts, articles, journals, publications, texts, treatises, internet web sites, databases, patents, patent applications and patent publications. U.S. Ser. No. 60/502,811, filed Sep. 12, 2003, is also incorporated by reference in its entirety.

DESCRIPTION OF DRAWINGS

[0097] FIG. 1 is a table of representative compounds and data.

[0098] FIG. 2 is a computer-generated model showing one possible orientation of compound 8 bound in the active site of SIRT.

[0099] FIG. 3a is a graph depicting the inhibition of mammalia SirT1 by compound 8.

[0100] FIG. 3b is a Western blot of NCI-H460 cells treated with etoposide only or etoposide and compound 8.

[0101] FIG. 4 is a bar graph depicting that enantiomer 8(-) of compound 8 leads to an increase in p53 acetylation.

[0102] FIG. 5 is a Western blot depicting that compounds which inhibit SirT catalytic activity also effect p53 acetylation.

[0103] FIG. 6 is a graph depicting that enantiomer 8(-) of compound 8 preferentially inhibits yeast sir2 relative to enantiomer 8(+).

[0104] FIG. 7 is a gel assay depicting the effectiveness of compound 8 for inhibiting SirT1 in U2OS cells and MCF-7 cells.

[0105] FIG. 8 is a graph depicting the effect of compound 8 on cell survival after DNA damage.

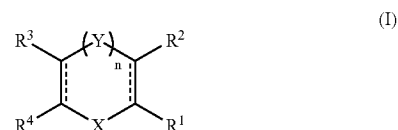
[0106] FIG. 9 are graphs depicting the effect of compound 8 on cell survival of NCI-H460 cells.

[0107] FIG. 10 is a bar graph depicting that compound 8 leads to abrogation of serum starvation-mediated upregulation of the cell cycle inhibitor p27.

DETAILED DESCRIPTION

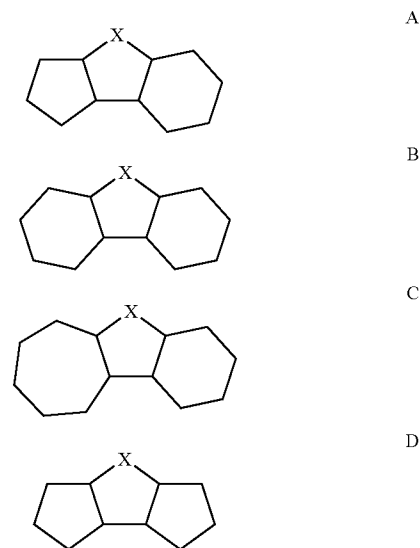
[0108] Structure of Compounds Compounds that can be used in practicing the invention have a general formula (I) and contain a substituted five or six membered ring core containing one or two, respectively, oxygen, nitrogen, or

sulfur atoms as a constituent atom of the ring, e.g., X and Y in formula (I) below.

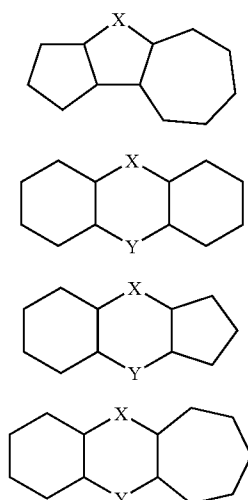


[0109] Any ring carbon atom can be substituted. For example, R¹, R², R³, and R⁴ may include without limitation substituted or unsubstituted alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, heterocycloalkenyl, cycloalkenyl, aryl, heteroaryl, etc. The five or six membered ring core may be saturated, i.e. containing no double bonds, or partially or fully saturated, i.e. one or two double bonds respectively. When n=0, "X" may be oxygen, sulfur, or nitrogen, e.g., NR⁷. The substituent R⁷ can be without limitation hydrogen, alkyl, e.g., C₁, C₂, C₃, C₄ alkyl, SO₂(aryl), acyl, or the ring nitrogen may form part of a carbamate, or urea group. When n=1, X can be NR⁷, O, or S; and Y can be NR⁷, O or S. X and Y can be any combination of heteroatoms, e.g., N,N,N,O, N, S, etc.

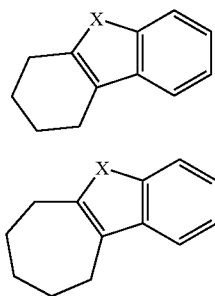
[0110] A preferred subset of compounds of formula (I) includes those having one, or preferably, two rings that are fused to the five or six membered ring core, e.g., R¹ and R², together with the carbons to which they are attached, and/or R³ and R⁴, together with the carbons to which they are attached, can form, e.g., C₅-C₁₀ cycloalkyl (e.g., C₅, C₆, or C₇), C₅-C₁₀ heterocyclyl (e.g., C₅, C₆, or C₇), C₅-C₁₀ cycloalkenyl (e.g., C₅, C₆, or C₇), C₅-C₁₀ heterocycloalkenyl (e.g., C₅, C₆, or C₇), C₆-C₁₀ aryl (e.g., C₆, C₈ or C₁₀), or C₆-C₁₀ heteroaryl (e.g., C₅ or C₆). Fused ring combinations may include without limitation one or more of the following:



-continued

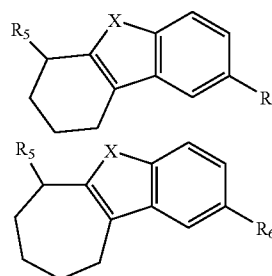


Preferred combinations include B, e.g. having C₆ aryl and C₆ cycloalkenyl (B1), and C, e.g. having C₆ aryl and C₇ cycloalkenyl (C1):



[0111] Each of these fused ring systems may be optionally substituted with substituents, which may include without limitation halo, hydroxy, C₁-C₁₀ alkyl (C1,C2,C3,C4,C5,C6,C7,C8,C9,C10), C₁-C₆ haloalkyl (C1,C2,C3,C4,C5,C6), C₁-C₁₀ alkoxy (C1,C2,C3,C4,C5,C6,C7,C8,C9,C10), C₁-C₆ haloalkoxy (C1,C2,C3,C4,C5,C6), C₆-C₁₀ aryl (C6,C7,C8,C9,C10), C₅-C₁₀ heteroaryl (C5,C6,C7,C8,C9,C10), C₇-C₁₂ aralkyl (C7,C8,C9,C10,C11,C12), C₇-C₁₂ heteroaralkyl (C7,C8,C9,C10,C11,C12), C₃-C₈ heterocyclyl (C3,C4,C5,C6,C7,C8), C₂-C₁₂ alkenyl (C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12), C₂-C₁₂ alkynyl (C2,C3,C4,C5,C6,C7,C8,C9,C10,C11,C12), C₅-C₁₀ cycloalkenyl (C5,C6,C7,C8,C9,C10), C₅-C₁₀ heterocycloalkenyl (C5,C6,C7,C8,C9,C10), carboxy, carboxylate, cyano, nitro, amino, C₁-C₆ alkyl amino (C1,C2,C3,C4,C5,C6), C₁-C₆ dialkyl amino (C1,C2,C3,C4,C5,C6), mercapto, SO₃H, sulfate, S(O)NH₂, S(O)₂NH₂, phosphate, C₁-C₄ alkylendioxy (C1,C2,C3,C4), oxo, acyl, aminocarbonyl, C₁-C₆ alkyl aminocarbonyl (C1,C2,C3,C4,C5,C6), C₁-C₆ dialkyl aminocarbonyl (C1,C2,C3,C4,C5,C6), C₁-C₁₀ alkoxycarbonyl (C1,C2,C3,C4,C5,C6,C7,C8,C9,C10), C₁-C₁₀ thioalkoxycarbonyl (C1,C2,C3,C4,C5,C6,C7,C8,C9,C10), hydrazinocarbonyl, C₁-C₆ alkyl

hydrazinocarbonyl (C1,C2,C3,C4,C5,C6), C₁-C₆ dialkyl hydrazinocarbonyl (C1,C2,C3,C4,C5,C6), hydroxyaminocarbonyl, etc. Preferred substituents include halo (e.g., fluoro, chloro, bromo), C₁-C₁₀ alkyl (e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10), C₁-C₆ haloalkyl (e.g., C1, C2, C3, C4, C5, C6, e.g., CF₃), C₁-C₆ haloalkoxy (e.g., C1, C2, C3, C4, C5, C6, e.g., OCF₃), or aminocarbonyl. The substitution pattern on the two fused rings may be selected as desired, e.g., one ring may be substituted and the other is not, or both rings may be substituted with 1-5 substituents (1,2,3,4,5 substituents). The number of substituents on each ring may be the same or different. Preferred substitution patterns are shown below:



[0112] In certain embodiments, when n is 0 and X is NR⁷, the nitrogen substituent R⁷ can form a cyclic structure with one of the fused rings containing, e.g., 4-6 carbons, 1-3 nitrogens, 0-2 oxygens and 0-2 sulfurs. This cyclic structure may optionally be substituted with oxo or C₁-C₆ alkyl.

[0113] Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject).

[0114] Exemplary compounds include those depicted in Table 1 below*:

TABLE 1

Exemplary compounds		
Compound number	Chemical name	Ave. SirT1 p53-382 IC50 (μM)
1	7-Chloro-1,2,3,4-tetrahydro-cyclopenta[b]indole-3-carboxylic acid amide	A
2	2,3,4,9-Tetrahydro-1H-b-carboline-3-carboxylic acid amide	C
3	6-Bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid amide	B
4	6-Methyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	A
5	2,3,4,9-Tetrahydro-1H-carbazole-1-carboxylic acid amide	B
6	2-Chloro-5,6,7,8,9,10-hexahydro-cyclohepta[b]indole-6-carboxylic acid amide	A

TABLE 1-continued

Exemplary compounds		
Compound number	Chemical name	Ave. SirT1 p53-382 IC50 (μM)
7	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid hydroxyamide	C
8	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	A
9	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid amide	C
10	1,2,3,4-Tetrahydro-cyclopenta[b]indole-3-carboxylic acid amide	B
11	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid (5-chloro-pyridin-2-yl)-amide	B
12	1,6-Dimethyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	C
13	6-Trifluoromethoxy-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid amide	C
14	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid diethylamide	D
15	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid carbamoylmethyl-amide	D
16	8-Carbamoyl-6,7,8,9-tetrahydro-5H-carbazole-1-carboxylic acid	D
17	6-Methyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid	D
18	8-Carbamoyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
19	[(6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carbonyl)-amino]-acetic acid ethyl ester	D
20	9-Benzyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	D
21	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid methyl ester	D
22	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid	D
23	C-(6-Methyl-2,3,4,9-tetrahydro-1H-carbazol-1-yl)-methylamine	D
24	6,9-Dimethyl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	D
25	7-Methyl-1,2,3,4-tetrahydro-cyclopenta[b]indole-3-carboxylic acid amide	D
26	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethylamide	D
27	2-(1-Benzyl-3-methylsulfanyl-1H-indol-2-yl)-N-p-tolyl-acetamide	D
28	N-Benzyl-2-(1-methyl-3-phenylsulfanyl-1H-indol-2-yl)-acetamide	D
29	N-(4-Chloro-phenyl)-2-(1-methyl-3-phenylsulfanyl-1H-indol-2-yl)-acetamide	D
30	N-(3-Hydroxy-propyl)-2-(1-methyl-3-phenylsulfanyl-1H-indol-2-yl)-acetamide	D
31	2-(1-Benzyl-3-phenylsulfanyl-1H-indol-2-yl)-N-(3-hydroxy-propyl)-acetamide	D
32	2-(1-Benzyl-3-methylsulfanyl-1H-indol-2-yl)-N-(4-methoxy-phenyl)-acetamide	D
33	2-(1-Benzyl-1H-indol-2-yl)-N-(4-methoxy-phenyl)-acetamide	D
34	2-(1-Methyl-3-methylsulfanyl-1H-indol-2-yl)-N-p-tolyl-acetamide	D
35	2-(1-Benzyl-3-methylsulfanyl-1H-indol-2-yl)-N-(2-chloro-phenyl)-acetamide	D
36	2-(1,5-Dimethyl-3-methylsulfanyl-1H-indol-2-yl)-N-(2-hydroxy-ethyl)-acetamide	D
37	(6-Chloro-2,3,4,9-tetrahydro-1H-carbazol-1-yl)-[4-(furan-2-carbonyl)-piperazin-1-yl]-methanone	D
38	2-(1-Benzyl-1H-indol-2-yl)-N-(2-chloro-phenyl)-acetamide	D
39	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D

TABLE 1-continued

Exemplary compounds		
Compound number	Chemical name	Ave. SirT1 p53-382 IC50 (μM)
40	6-Chloro-9-methyl-2,3,4,9-tetrahydro-1H-carbazole-4-carboxylic acid ethyl ester	D
41	5,7-Dichloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
42	7-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
43	5,7-Dichloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid	D
44	6-Chloro-9-methyl-2,3,4,9-tetrahydro-1H-carbazole-4-carboxylic acid	D
45	6-Chloro-9-methyl-2,3,4,9-tetrahydro-1H-carbazole-4-carboxylic acid amide	D
46	6-Morpholin-4-yl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
47	6-Morpholin-4-yl-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	D
48	6-Bromo-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
49	6-Fluoro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid ethyl ester	D
50	3-Carbamoyl-1,3,4,9-tetrahydro-b-carboline-2-carboxylic acid tert-butyl ester	D
51	6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid (1-phenyl-ethyl)-amide	D
52	7,8-Difluoro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide	D
53	6-bromo-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid	D
54	6-hydroxy-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid	C
55	6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxamide	B
56	6-chloro-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxamide	C
57	6-bromo-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxamide	D
58	2-acetyl-6-chloro-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-1-carboxamide	C

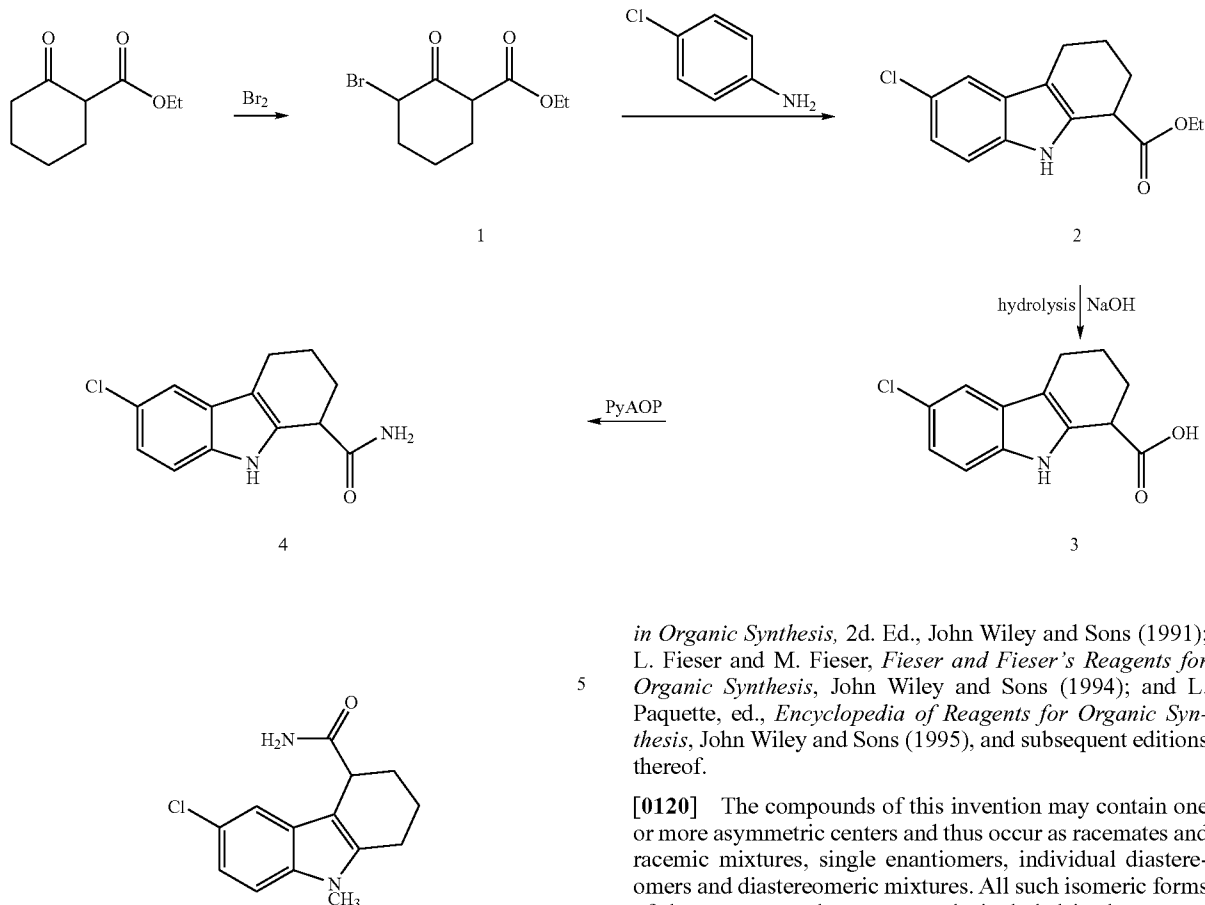
* Compounds having activity designated with an A have an IC₅₀ of less than 1.0 μM. Compounds having activity designated with a B have an IC₅₀ between 1.0 μM and 10.0 μM. Compounds having activity designated with a C have an IC₅₀ greater than 10.0 μM. Compounds designated with a D were not tested in this assay.

[0115] Compounds that can be useful in practicing this invention can be identified through both in vitro (cell and non-cell based) and in vivo methods. A description of these methods is described in the Examples.

[0116] Synthesis of Compounds

[0117] The compounds described herein can be obtained from commercial sources (e.g., Asinex, Moscow, Russia; Bionet, Camelford, England; ChemDiv, San Diego, Calif.; Comgenex, Budapest, Hungary; Enamine, Kiev, Ukraine; IF Lab, Ukraine; Interbioscreen, Moscow, Russia; Maybridge, Tintagel, UK; Specs, The Netherlands; Timtec, Newark, DE; Vitas-M Lab, Moscow, Russia) or synthesized by conventional methods as shown below using commercially available starting materials and reagents. For example, exemplary compound 4 can be synthesized as shown in Scheme 1 below.

Scheme 1



[0118] Brominated β -keto ester 1 can be condensed with 4-chloroaniline followed by cyclization can afford indole 2. Ester saponification can afford acid 3. Finally amination with PyAOP can yield the amide 4. Other methods are known in the art, see, e.g., U.S. Pat. No. 3,859,304, U.S. Pat. No. 3,769,298, *J. Am. Chem. Soc.* 1974, 74, 5495. The synthesis above can be extended to other anilines, e.g., 3,5-dichloroaniline, 3-chloroaniline, and 4-bromoaniline. Regioisomeric products, e.g., 5, may be obtained using N-substituted anilines, e.g., 4-chloro-N-methylaniline.

[0119] The compounds described herein can be separated from a reaction mixture and further purified by a method such as column chromatography, high-pressure liquid chromatography, or recrystallization. As can be appreciated by the skilled artisan, further methods of synthesizing the compounds of the formulae herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T. W. Greene and P. G. M. Wuts, *Protective Groups*

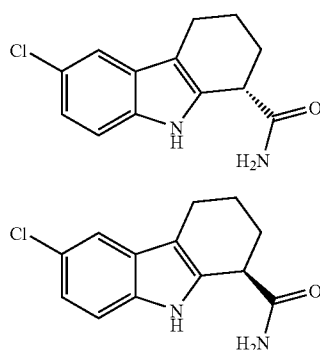
in *Organic Synthesis*, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and subsequent editions thereof.

[0120] The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention. The compounds of this invention may also contain linkages (e.g., carbon-carbon bonds) or substituents that can restrict bond rotation, e.g. restriction resulting from the presence of a ring or double bond. Accordingly, all cis/trans and E/Z isomers are expressly included in the present invention. The compounds of this invention may also be represented in multiple tautomeric forms, in such instances, the invention expressly includes all tautomeric forms of the compounds described herein, even though only a single tautomeric form may be represented (e.g., alkylation of a ring system may result in alkylation at multiple sites, the invention expressly includes all such reaction products). All such isomeric forms of such compounds are expressly included in the present invention. All crystal forms of the compounds described herein are expressly included in the present invention.

[0121] Techniques useful for the separation of isomers, e.g., stereoisomers are within skill of the art and are described in Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*, Wiley Interscience, NY, 1994. For example compound 3 or 4 can be resolved to a high enantiomeric excess (e.g., 60%, 70%, 80%, 85%, 90%, 95%, 99% or greater) via formation of diastereomeric salts, e.g. with a chiral base, e.g., (+) or (−) α -methylbenzylamine, or via high performance liquid chromatography using a chiral column. In some embodiments, the crude

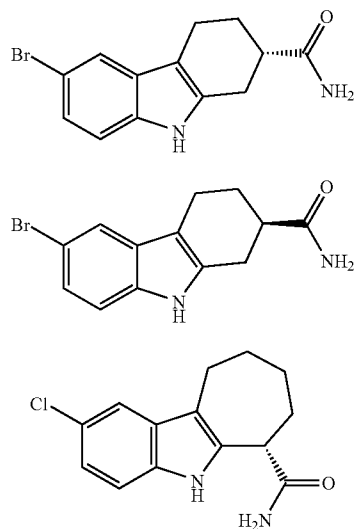
product 4, is purified directly on a chiral column to provide enantiomerically enriched compound.

[0122] For purposes of illustration, enantiomers of compound 4 are shown below.

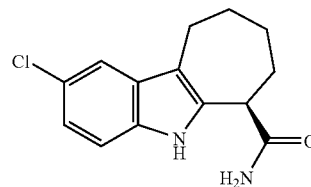


In some instances, the compounds disclosed herein are administered where one isomer (e.g., the R isomer or S isomer) is present in high enantiomeric excess. In general, the isomer of compound 4 having a negative optical rotation, e.g., -14.1 ($c=0.33$, DCM) or $[\alpha]_D^{25} -41.18^\circ$ (c 0.960, CH₃OH) has greater activity against the SirT1 enzyme than the enantiomer that has a positive optical rotation of $+32.8$ ($c=0.38$, DCM) or $[\alpha]_D^{25} +22.72^\circ$ (c 0.910, CH₃OH). Accordingly, in some instances, it is beneficial to administer to a subject a compound 4 having a high enantiomeric excess of the isomer having a negative optical rotation to treat a disease.

[0123] While the enantiomers of compound 4 provide one example of a stereoisomer, other stereoisomers are also envisioned, for example as depicted in compounds 6 and 7 below.

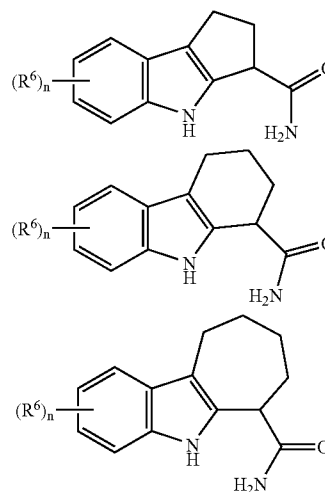


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As with the compound of formula 4, in some instances it is beneficial to administer to a subject an isomer of compounds 6 or 7 that has a greater affinity for SirT1 than its enantiomer. For example, in some instances, it is beneficial to administer a compound 7, enriched with the (–) optical rotamer, wherein the amide (or other substituent) has the same configuration as the negative isomer of compound 4.

[0124] In some instances, it is beneficial to administer a compound having the one of the following structures where the stereochemical structure of the amide (or other substituent) corresponds to the amide in compound 4 having a negative optical rotation.



(n is an integer from 0 to 4.)

[0125] The compounds of this invention include the compounds themselves, as well as their salts and their prodrugs, if applicable. A salt, for example, can be formed between an anion and a positively charged substituent (e.g., amino) on a compound described herein. Suitable anions include chloride, bromide, iodide, sulfate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, and acetate. Likewise, a salt can also be formed between a cation and a negatively charged substituent (e.g., carboxylate) on a compound described herein. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. Examples of prodrugs include esters and other pharmaceutically acceptable derivatives, which, upon administration to a subject, are capable of providing active compounds.

[0126] The compounds of this invention may be modified by appending appropriate functionalities to enhance selected

biological properties, e.g., targeting to a particular tissue. Such modifications are known in the art and include those which increase biological penetration into a given biological compartment (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

[0127] In an alternate embodiment, the compounds described herein may be used as platforms or scaffolds that may be utilized in combinatorial chemistry techniques for preparation of derivatives and/or chemical libraries of compounds. Such derivatives and libraries of compounds have biological activity and are useful for identifying and designing compounds possessing a particular activity. Combinatorial techniques suitable for utilizing the compounds described herein are known in the art as exemplified by Obrecht, D. and Villalgró, J. M., *Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries*, Pergamon-Elsevier Science Limited (1998), and include those such as the “split and pool” or “parallel” synthesis techniques, solid-phase and solution-phase techniques, and encoding techniques (see, for example, Czarnik, A. W., *Curr. Opin. Chem. Bio.*, (1997) 1, 60. Thus, one embodiment relates to a method of using the compounds described herein for generating derivatives or chemical libraries comprising: 1) providing a body comprising a plurality of wells; 2) providing one or more compounds identified by methods described herein in each well; 3) providing an additional one or more chemicals in each well; 4) isolating the resulting one or more products from each well. An alternate embodiment relates to a method of using the compounds described herein for generating derivatives or chemical libraries comprising: 1) providing one or more compounds described herein attached to a solid support; 2) treating the one or more compounds identified by methods described herein attached to a solid support with one or more additional chemicals; 3) isolating the resulting one or more products from the solid support. In the methods described above, “tags” or identifier or labeling moieties may be attached to and/or detached from the compounds described herein or their derivatives, to facilitate tracking, identification or isolation of the desired products or their intermediates. Such moieties are known in the art. The chemicals used in the aforementioned methods may include, for example, solvents, reagents, catalysts, protecting group and deprotecting group reagents and the like. Examples of such chemicals are those that appear in the various synthetic and protecting group chemistry texts and treatises referenced herein.

Sirtuins

[0128] Sirtuins are members of the Silent Information Regulator (SIR) family of genes. Sirtuins are proteins that include a SIR2 domain as defined as amino acid sequences that are scored as hits in the Pfam family “SIR2”—PF02146. This family is referenced in the INTERPRO database as INTERPRO description (entry IPR003000). To identify the presence of a “SIR2” domain in a protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against the Pfam database of HMMs (e.g., the Pfam database, release 9) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). The SIR2 domain is indexed in Pfam as PF02146 and in INTERPRO as INTERPRO description

(entry IPR003000). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in “The Pfam Protein Families Database” Bateman A, Birney E, Cerruti L, Durbin R, Eddy S R, Griffiths-Jones S, Howe K L, Marshall M, Sonnhammer E L (2002) *Nucleic Acids Research* 30(1):276-280 and Sonnhammer et al. (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al. (1990) *Meth. Enzymol.* 183:146-159; Gribskov et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh et al. (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz et al. (1993) *Protein Sci.* 2:305-314.

[0129] The proteins encoded by members of the SIR2 gene family may show high sequence conservation in a 250 amino acid core domain. A well-characterized gene in this family is *S. cerevisiae* SIR2, which is involved in silencing HM loci that contain information specifying yeast mating type, telomere position effects and cell aging (Guarente, 1999; Kaerberlein et al., 1999; Shore, 2000). The yeast Sir2 protein belongs to a family of histone deacetylases (reviewed in Guarente, 2000; Shore, 2000). The Sir2 protein is a deacetylase which can use NAD as a cofactor (Imai et al., 2000; Moazed, 2001; Smith et al., 2000; Tanner et al., 2000; Tanny and Moazed, 2001). Unlike other deacetylases, many of which are involved in gene silencing, Sir2 is relatively insensitive to histone deacetylase inhibitors like trichostatin A (TSA) (Imai et al., 2000; Landry et al., 2000a; Smith et al., 2000). Mammalian Sir2 homologs, such as SIRT1, have NAD-dependent deacetylase activity (Imai et al., 2000; Smith et al., 2000).

[0130] Exemplary mammalian sirtuins include SIRT1, SIRT2, and SIRT3, e.g., human SIRT1, SIRT2, and SIRT3. A compound described herein may inhibit one or more activities of a mammalian sirtuin, e.g., SIRT1, SIRT2, or SIRT3, e.g., with a K_i of less than 500, 200, 100, 50, or 40 nM. For example, the compound may inhibit deacetylase activity, e.g., with respect to a natural or artificial substrate, e.g., a substrate described herein, e.g., as follows.

[0131] Natural substrates for SIRT1 include histones, p53, and FoxO transcription factors such as FoxO1 and FoxO3. SIRT1 proteins bind to a number of other proteins, referred to as “SIRT1 binding partners.” For example, SIRT1 binds to p53 and plays a role in the p53 pathway, e.g., K370, K371, K372, K381, and/or K382 of p53 or a peptide that include one or more of these lysines. For example, the peptide can be between 5 and 15 amino acids in length. SIRT1 proteins can also deacetylate histones. For example, SIRT1 can deacetylate lysines 9 or 14 of histone H3 or small peptides that include one or more of these lysines. Histone deacetylation alters local chromatin structure and consequently can regulate the transcription of a gene in that vicinity. Many of the SIRT1 binding partners are transcription factors, e.g., proteins that recognize specific DNA sites. For example, SIRT1 deacetylates and downregulates forkhead proteins (i.e., FoxO proteins). Interaction between SIRT1 and SIRT1 binding partners can deliver SIRT1 to specific regions of a

genome and can result in a local manifestation of substrates, e.g., histones and transcription factors localized to the specific region.

[0132] Natural substrates for SIRT2 include tubulin, e.g., alpha-tubulin. See, e.g., North et al. Mol Cell. 2003 February; 11(2):437-44. Exemplary substrates include a peptide that includes lysine 40 of alpha-tubulin.

[0133] Still other exemplary sirtuin substrates include cytochrome c and acetylated peptides thereof.

[0134] The terms "SIRT1 protein" and "SIRT1 polypeptide" are used interchangeably herein and refer a polypeptide that is at least 25% identical to the 250 amino acid conserved SIRT1 catalytic domain, amino acid residues 258 to 451 of SEQ ID NO:1. SEQ ID NO:1 depicts the amino acid sequence of human SIRT1. In preferred embodiments, a SIRT1 polypeptide can be at least 30, 40, 50, 60, 70, 80, 85, 90, 95, 99% homologous to SEQ ID NO:1 or to the amino acid sequence between amino acid residues 258 and 451 of SEQ ID NO:1. In other embodiments, the SIRT1 polypeptide can be a fragment, e.g., a fragment of SIRT1 capable of one or more of: deacetylating a substrate in the presence of NAD and/or a NAD analog and capable of binding a target protein, e.g., a transcription factor. Such functions can be evaluated, e.g., by the methods described herein. In other embodiments, the SIRT1 polypeptide can be a "full length" SIRT1 polypeptide. The term "full length" as used herein refers to a polypeptide that has at least the length of a naturally-occurring SIRT1 polypeptide (or other protein described herein). A "full length" SIRT1 polypeptide or a fragment thereof can also include other sequences, e.g., a purification tag, or other attached compounds, e.g., an attached fluorophore, or cofactor. The term "SIRT1 polypeptides" can also include sequences or variants that include one or more substitutions, e.g., between one and ten substitutions, with respect to a naturally occurring Sir2 family member. A "SIRT1 activity" refers to one or more activity of SIRT1, e.g., deacetylation of a substrate (e.g., an amino acid, a peptide, or a protein), e.g., transcription factors (e.g., p53) or histone proteins, (e.g., in the presence of a cofactor such as NAD and/or an NAD analog) and binding to a target, e.g., a target protein, e.g., a transcription factor.

[0135] As used herein, a "biologically active portion" or a "functional domain" of a protein includes a fragment of a protein of interest which participates in an interaction, e.g., an intramolecular or an inter-molecular interaction, e.g., a binding or catalytic interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction (e.g., the interaction can be transient and a covalent bond is formed or broken). An inter-molecular interaction can be between the protein and another protein, between the protein and another compound, or between a first molecule and a second molecule of the protein (e.g., a dimerization interaction). Biologically active portions/functional domains of a protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the protein which include fewer amino acids than the full length, natural protein, and exhibit at least one activity of the natural protein. Biological active portions/functional domains can be identified by a variety of techniques including truncation analysis, site-directed mutagenesis, and proteolysis. Mutants or proteolytic fragments can be assayed for activity by an appro-

priate biochemical or biological (e.g., genetic) assay. In some embodiments, a functional domain is independently folded. Typically, biologically active portions comprise a domain or motif with at least one activity of a protein, e.g., SIRT1. An exemplary domain is the SIRT1 core catalytic domain. A biologically active portion/functional domain of a protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions/functional domain of a protein can be used as targets for developing agents which modulate SIRT1.

[0136] The following are exemplary SIR sequences:

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>sp |Q96EB6| SIR1_HUMAN NAD-dependent deacetylase
sirtuin 1 (EC 3.5.1.-) (hSIRT1) (hSIR2)
(SIR2-like protein 1) - Homo sapiens (Human).
(SEQ ID NO:1)
MADEAALALQPGGSPSAAGADREAASSPAGEPLRKRPRRDGPGGLERSPG
PGGAAPEREVPAAARGCPGAAAAALWREAEAEAAAAGGQEQAQATAAAGE
GDNGPGLQGPSREPLADNLYDEDDDEGEDEEEAAAAAIGYRDNLFLGD
EITITNGFHSCEDEEDRASHASSSDWTTPRPRIGPYTFVQOHLMIGTDPRT
ILKDLLPETIPPELDDMTLWQIVINILSEPPKRRKRKINTIEDAVKLL
QECKKIIVLTGAGVSVSCGIPDFRSRDGIYARLAVDFPDLDPQAMFDIE
YFRKDPDPFFFAKEIYPGQFQPSLCHKFIALSDKEGLLRNYTQNDITL
EQVAGIQRIIQCHGSFATASCLICKYKVDCEAVRGDIFNQVVRPCRPCA
DEPLAIMKPEIVFFGENLPEQFHRAMKYDKDEVDLLIVIGSSLKVRPVAL
IPSSIPHEVPQILINREPLPHLHFDVELGDCDVIINELCHRLGGEYAKL
CCNPVKLSEITEKPPRTQKELAYLSELPPTPLHVSDESSSPERTSPDSS
VIVTLLDQAAKSNDDLVDVSESKGCMEEKPQEVQTSRNVESIAEQMENPDL
KNVGSSTGEKNERTSVAGTVRKCWPNRVAKEQISRRLDGNQYLFPPNRY
IFHGAEVYSDSEDDVLSSSSCGNSDSGTCQSPSLEEPMEDESEIEEFYN
GLEDEPDVPERAGGAGFGTDGDDQEAINEAISVKQEVVTDMNYPNSKS
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>sp |Q8IXJ6| SIR2_HUMAN NAD-dependent deacetylase
sirtuin 2 (EC 3.5.1.-) (SIR2-like)
(SIR2-like protein 2) - Homo sapiens (Human)
(SEQ ID NO:2)
MAEPDPSPHLETLQAGKVQEAQDSDSDEGGAAGGEADMFLRNLFSQTL
LGSQKERLLDELTEGVARYMQSERCRVICLVGAGISTSAGIPDFRSP
TGLYDNLEKYHLPPYEAIFEISYFKKHPEPPFALAKELYPGQKPTICHY
FMRLLDKGLLLRCYTQNDITLERIAGLEQEDLVEAHGTFYTSCHVSASC
RHEYPLSWMKEKIFSEVTPKCEDCQSLVDPDVFVFGESLPARFFSCMQSD
FLKVDLLVMGTSLSQVQPFASLISKAPLSTPRLINKEKAGQSDPFLGMI
MGLGGMGDFDSKKAYRDVAWLGECDQGCIALAELLGWKKELEDLVREHA
SIDAQSGAGVNPSTASAPKSPPPAKDEARTTEREKPKQ
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>sp |Q9NTG7| SIR3_HUMAN NAD-dependent deacetylase
sirtuin 3, mitochondrial precursor (EC 3.5.1.-)
(SIR2-like protein 3) (hSIRT3) - Homo sapiens
(Human).
(SEQ ID NO:3)
MAFWGWPAALRLWGRVVERVEAGGVGFQACGRLVLGGRDDVSAGL
RGSHGARGEPLDPAFLQRPPEVPRAFRRQPRAAAPSFSSISKGRR
SISFVSGASSVVGSGSSDKGLSLQDVAEILRAPACQVVMVVGAGIST
PSGIPDFRSPGSGLYSNLQYDLPYEAIFELEFFHNPFPFTLAKELY
PGNYKPNVTHYFLRLLHDKGLLLRLYTQNDIGLERVSGIPASKLVEAHGT
FASATCTVCQRFPGEDIRADVMADRVPRCPVCTGVVKPDIVFVGEPLPQ
RFLHVVDFPMADLLLILGTSLEVEFPASLTAEVRSVPRLLINRDLVGP
LAWHPRSRDVAQLGDVVHGVESLVELLWGTEEMRDLVQRETGLKDGPDK
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>sp |Q9Y6E7| SIR4_HUMAN NAD-dependent deacetylase
sirtuin 4 (EC 3.5.1.-) (SIR2-like protein 4) -
Homo sapiens (Human).
(SEQ ID NO:4)
MKMSFALTFRSAKGRWIANPSQPCSKASIGLFPASPPDPEKVKELQRF
ITLSKRLVMTGAGISTESGIPDYRSEKVGLYARTDRRPIQHGDVRSAP
IRQRYWARNFVWGPQFSHQPNPAHWALSTWEKLGKLYLVLTQNVNLT
KAGSRRLTELHGCMRDLVCLDCGEQTPRGVLQERFQVLNPTWSAEAHGLA
PDGDVFLSEEQVRSFQVPTCVQCGHKLKPDVVFVGTVPNDKVDVHHRV
KEADSLLVGSSSLQVYSYGRFILTAWKKLPATILNIGPTRSDDLACLKL
NSRCGELLPLIDPC
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>sp |Q9NXA8| SIR5_HUMAN NAD-dependent deacetylase
sirtuin 5 (EC 3.5.1.-) (SIR2-like protein 5) -
Homo sapiens (Human).
                               (SEQ ID NO:5)
MRPLQIVPSRLISQLYCLGKPPASTRNQICLKMARPSSSMADFRKFFAKA
KHVITISGAGVSAESGVPTFRGAGGYWRKWAQDLATPLAFANPSRVWE
FYHYRREVMGSKPEPNAGHRAIAECETRLGKQGRVVITQNIDELHRRKAG
TKNLLIEHGSFLKTRCTSCGVVAENYKSPICPALSGKGAPEPGTQDASIP
VEKLPRCEEAGCGLLRPHVWVFGENLDPAILEEVDRELAHCDLCLVVGVT
SSVVYPAAMFAPQVAARGVPVAFENETETTPATNRFHFQGPCCTTLPEA
LACHENETVS

>sp |Q8N6T7| SIR6_HUMAN NAD-dependent deacetylase
sirtuin 6 (EC 3.5.1.-) (SIR2-like protein 6) -
Homo sapiens (Human).
                               (SEQ ID NO:6)
MSVNYAAGLSPYADKKGKGLPEIFDPPEELERKVVWELARLVQSSSVFVH
TGAGISTASGIPDFRGPHGVWMEERGLAPKFDITTFESARPTQTHMALVQ
LEVRGLRLFLVSNQVNDGLHVRSGFPRDKLAELHGNMFVEECAKCTQYVR
DTVVGTMGLKATGRCLTVAKARGLRACRGELRDTILDWEDSLPDRDLALA
DEASRNADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHA
DLRIHGYVDEVMTRLMKHLGLEIPAWDGPVLERALPPLPRPPTPKLEPK
EESPTRINGSIPAGPKQEPCAQHNGSEFASPKRERPTSPAPHRPFPKRVKA
KAVPS

>sp |Q9NRC8| SIR7_HUMAN NAD-dependent deacetylase
sirtuin 7 (EC 3.5.1.-) (SIR2-like protein 7) -
Homo sapiens (Human).
                               (SEQ ID NO:7)
MAAGGLSRSEKAAEVRRLREEQQRELRQVSRILRKAASAEAGRL
LAESADLVTELQGRSRREGLKRRQEEVCDDEELRGKVRSLASAVRNAK
YLVVYTGAGISTAASIPDYRGPNVWTLQKGRSVSAADLSEAEPTLTHM
SITRLHEQKLVQHVVSQNCGLHRLSGLPRTAISELHGNMYIEVCTSCVP
NREYVRFVDVTERTALHRHQTGRCHKCGTQLRDTIVHFGERGTLGQPLN
WEAATEAASRADTILCLGSSLKVLKYPRLWCMTKPPSRPKLYIVNLQW
TPKDDWAALKHKGKDDVMRLMAELGLEIPAYSRWQDPIFSLATPLRAG
EEGSHSRKSLCRSREEAPPGRGAPLSAPILGGWFGRGCTKTRKRRKKVT

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[0137] Exemplary compounds described herein may inhibit activity of SIRT1 or a functional domain thereof by at least 10, 20, 25, 30, 50, 80, or 90%, with respect to a natural or artificial substrate described herein. For example, the compounds may have a K_i of less than 500, 200, 100, or 50 nM.

[0138] A compound described herein may also modulate a complex between a sirtuin and a transcription factor, e.g., increase or decrease complex formation, deformation, and/or stability. Exemplary sirtuin-TF complexes include Sir2-PCAF, Sir2-MyoD, Sir2-PCAF-MyoD, Sir2-p53, Sir2-FoxO1, and Sir2-FoxO3. A compound described herein may also modulate expression of a Sir2 regulated gene, e.g., a gene described in Table 1 of Fulco et al. (2003) *Mol. Cell* 12:51-62.

In Vitro Assays

[0139] In some embodiments, interaction with, e.g., binding of, SIRT1 can be assayed in vitro. The reaction mixture can include a SIRT1 co-factor such as NAD and/or a NAD analog.

[0140] In other embodiments, the reaction mixture can include a SIRT1 binding partner, e.g., a transcription factor, e.g., p53 or a transcription factor other than p53 such as FoxO1 or FoxO3, and compounds can be screened, e.g., in an in vitro assay, to evaluate the ability of a test compound to modulate interaction between SIRT1 and a SIRT1 binding partner, e.g., a transcription factor. This type of assay can be accomplished, for example, by coupling one of the components, with a radioisotope or enzymatic label such that

binding of the labeled component to the other can be determined by detecting the labeled compound in a complex. A component can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, a component can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Competition assays can also be used to evaluate a physical interaction between a test compound and a target.

[0141] Cell-free assays involve preparing a reaction mixture of the target protein (e.g., SIRT1) and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

[0142] The interaction between two molecules can also be detected, e.g., using a fluorescence assay in which at least one molecule is fluorescently labeled. One example of such an assay includes fluorescence energy transfer (FET or FRET for fluorescence resonance energy transfer) (see, for example, Lakowicz et al., U.S. Pat. No. 5,631,169; Stavrianopoulos, et al., U.S. Pat. No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. A FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

[0143] Another example of a fluorescence assay is fluorescence polarization (FP). For FP, only one component needs to be labeled. A binding interaction is detected by a change in molecular size of the labeled component. The size change alters the tumbling rate of the component in solution and is detected as a change in FP. See, e.g., Nasir et al. (1999) *Comb Chem HTS* 2:177-190; Jameson et al. (1995) *Methods Enzymol* 246:283; Seethala et al. (1998) *Anal Biochem* 255:257. Fluorescence polarization can be monitored in multiwell plates, e.g., using the Tecan Polarion™ reader. See, e.g., Parker et al. (2000) *Journal of Biomolecular Screening* 5:77-88; and Shoeman, et al. (1999) 38, 16802-16809.

[0144] In another embodiment, determining the ability of the SIRT1 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al. (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIA-core). Changes in the mass at the binding surface (indicative

of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

[0145] In one embodiment, SIRT1 is anchored onto a solid phase. The SIRT1/test compound complexes anchored on the solid phase can be detected at the end of the reaction, e.g., the binding reaction. For example, SIRT1 can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

[0146] It may be desirable to immobilize either the SIRT1 or an anti-SIRT1 antibody to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a SIRT1 protein, or interaction of a SIRT1 protein with a second component in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/SIRT1 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or SIRT1 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of SIRT1 binding or activity determined using standard techniques.

[0147] Other techniques for immobilizing either a SIRT1 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated SIRT1 protein or target molecules can be prepared from biotin-NHS(N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

[0148] In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, e.g., using a labeled antibody

specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

[0149] In one embodiment, this assay is performed utilizing antibodies reactive with a SIRT1 protein or target molecules but which do not interfere with binding of the SIRT1 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or the SIRT1 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the SIRT1 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the SIRT1 protein or target molecule.

[0150] Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A. P., (1993) *Trends Biochem Sci* 18:284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. (1999) *Current Protocols in Molecular Biology*, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N. H., (1998) *J Mol Recognit* 11:141-8; Hage, D. S., and Tweed, S.A. (1997) *J Chromatogr B Biomed Sci Appl.* 699:499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

[0151] In a preferred embodiment, the assay includes contacting the SIRT1 protein or biologically active portion thereof with a known compound which binds a SIRT1 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a SIRT1 protein, wherein determining the ability of the test compound to interact with the SIRT1 protein includes determining the ability of the test compound to preferentially bind to the SIRT1 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

[0152] An exemplary assay method includes a 1536 well format of the SIRT1 enzymatic assay that is based on the commercial "Fluor-de-Lys" assay principle by Biomol, which is fluorogenic (www.biomol.com/store/Product_Data_PDFs/ak500.pdf). In this assay, deacetylation of the e-amino function of a lysyl residue is coupled to a fluorogenic "development step that is dependent on the unblocked e-amino functionality and generates fluorescent aminomethylcoumarin. Fluorescence can be read on a commercial macroscopic reader.

Additional Assays

[0153] A compound or library of compounds described herein can also be evaluated using one of the following model systems for a disease or disorder, or other known models of a disease or disorder described herein.

[0154] Models for evaluating the effect of a test compound on muscle atrophy include, e.g., 1) rat medial gastrocnemius muscle mass loss resulting from denervation, e.g., by severing the right sciatic nerve at mid-thigh; 2) rat medial gastrocnemius muscle mass loss resulting from immobilization, e.g., by fixed the right ankle joint at 90 degrees of flexion; 3) rat medial gastrocnemius muscle mass loss resulting from hindlimb suspension; (see, e.g., U.S. 2003-0129686); 4) skeletal muscle atrophy resulting from treatment with the cachectic cytokine, interleukin-1 (IL-1) (R. N. Cooney, S. R. Kimball, T. C. Vary, Shock 7, 1-16 (1997)); and 5) skeletal muscle atrophy resulting from treatment with the glucocorticoid, dexamethasone (A. L. Goldberg, J Biol Chem 244, 3223-9 (1969)). Models 1, 2, and 3 induce muscle atrophy by altering the neural activity and/or external load a muscle experiences to various degrees. Models 4 and 5 induce atrophy without directly affecting those parameters MS (experimental autoimmune encephalomyelitis (EAE)), e.g., as described by Goverman et al., Cell. 72:551-60 (1993), and primate models as reviewed by Brok et al., Immunol. Rev., 183:173-85 (2001).

[0155] Exemplary animal models for AMD (age-related macular degeneration) include: laser-induced mouse model simulating exudative (wet) macular degeneration Bora et al., Proc. Natl. Acad. Sci. USA., 100:2679-84 (2003); a transgenic mouse expressing a mutated form of cathepsin D resulting in features associated with the "geographic atrophy" form of AMD (Rakoczy et al., Am. J. Pathol., 161:1515-24 (2002)); and a transgenic mouse overexpressing VEGF in the retinal pigment epithelium resulting in CNV. Schwesinger et al., Am. J. Pathol. 158:1161-72 (2001).

[0156] Exemplary animal models of Parkinson's disease include primates rendered parkinsonian by treatment with the dopaminergic neurotoxin 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP) (see, e.g., US Appl 20030055231 and Wichmann et al., Ann. N.Y. Acad. Sci., 991:199-213 (2003); 6-hydroxydopamine-lesioned rats (e.g., Lab. Anim. Sci.,49:363-71 (1999)); and transgenic invertebrate models (e.g., Lakso et al., J. Neurochem., 86:165-72 (2003) and Link, Mech. Ageing Dev., 122:1639-49 (2001)).

[0157] Exemplary molecular models of Type II diabetes include: a transgenic mouse having defective Nkx-2.2 or Nkx-6.1; (U.S. Pat. No. 6,127,598); Zucker Diabetic Fatty fa/fa (ZDF) rat. (U.S. Pat. No. 6,569,832); and Rhesus monkeys, which spontaneously develop obesity and subsequently frequently progress to overt type 2 diabetes (Hotta et al., Diabetes, 50:1126-33 (2001); and a transgenic mouse with a dominant-negative IGF-I receptor (KR-IGF-R) having Type 2 diabetes-like insulin resistance.

[0158] Exemplary animal and cellular models for neuropathy include: vincristine induced sensory-motor neuropathy in mice (U.S. Pat. No. 5,420,112) or rabbits (Ogawa et al., Neurotoxicology, 21:501-11 (2000)); a streptozotocin (STZ)-diabetic rat for study of autonomic neuropathy (Schmidt et al., Am. J. Pathol., 163:21-8 (2003)); and a progressive motor neuropathy (pmn) mouse (Martin et al., Genomics, 75:9-16 (2001)).

[0159] Structure-Activity Relationships and Structure-Based Design. It is also possible to use structure-activity relationships (SAR) and structure-based design principles to

produce a compound that interact with a sirtuin, e.g., antagonizes or agonizes a sirtuin. SARs provide information about the activity of related compounds in at least one relevant assay. Correlations are made between structural features of a compound of interest and an activity. For example, it may be possible by evaluating SARs for a family of compounds related to a compound described herein to identify one or more structural features required for the agonist's activity. A library of compounds can then be chemically produced that vary these features. In another example, a single compound that is predicted to interact is produced and evaluated in vitro or in vivo.

[0160] Structure-based design can include determining a structural model of the physical interaction of a functional domain of a sirtuin and a compound. The structural model can indicate how the compound can be engineered, e.g., to improve interaction or reduce unfavorable interactions. The compound's interaction with the sirtuin can be identified, e.g., by solution of a crystal structure, NMR, or computer-based modeling, e.g., docking methods. See, e.g., Ewing et al. J Comput Aided Mol Des. 2001 May; 15(5):411-28.

[0161] Both the SAR and the structure-based design approach, as well as other methods, can be used to identify a pharmacophore. A pharmacophore is defined as a distinct three dimensional (3D) arrangement of chemical groups. The selection of such groups may be favorable for biological activity. Since a pharmaceutically active molecule must interact with one or more molecular structures within the body of the subject in order to be effective, and the desired functional properties of the molecule are derived from these interactions, each active compound must contain a distinct arrangement of chemical groups which enable this interaction to occur. The chemical groups, commonly termed descriptor centers, can be represented by (a) an atom or group of atoms; (b) pseudo-atoms, for example a center of a ring, or the center of mass of a molecule; (c) vectors, for example atomic pairs, electron lone pair directions, or the normal to a plane. Once formulated a pharmacophore can be used to search a database of chemical compound, e.g., for those having a structure compatible with the pharmacophore. See, for example, U.S. Pat. No. 6,343,257; Y. C. Martin, 3D Database Searching in Drug Design, J. Med. Chem. 35, 2145(1992); and A. C. Good and J. S. Mason, Three Dimensional Structure Database Searches, Reviews in Comp. Chem. 7, 67(1996). Database search queries are based not only on chemical property information but also on precise geometric information.

[0162] Computer-based approaches can use database searching to find matching templates; Y. C. Martin, Database searching in drug design, J. Medicinal Chemistry, vol. 35, pp 2145-54 (1992), which is herein incorporated by reference. Existing methods for searching 2-D and 3-D databases of compounds are applicable. Lederle of American Cyanamid (Pearl River, N.Y.) has pioneered molecular shape-searching, 3D searching and trend-vectors of databases. Commercial vendors and other research groups also provide searching capabilities (MACSS-3D, Molecular Design Ltd. (San Leandro, Calif.); CAVEAT, Lauri, G et al., University of California (Berkeley, Calif.); CHEM-X, Chemical Design, Inc. (Mahwah, N.J.)). Software for these searches can be used to analyze databases of potential drug compounds indexed by their significant chemical and geometric structure (e.g., the Standard Drugs File (Derwent Publications

Ltd., London, England), the Bielstein database (Bielstein Information, Frankfurt, Germany or Chicago), and the Chemical Registry database (CAS, Columbus, Ohio)).

[0163] Once a compound is identified that matches the pharmacophore, it can be tested for activity in vitro, in vivo, or in silico, e.g., for binding to a sirtuin or domain thereof.

[0164] In one embodiment, a compound that is an agonist or a candidate agonist, e.g., a compound described in Nature. 2003 Sep. 11; 425(6954):191-196 can be modified to identify an antagonist, e.g., using the method described herein. For example, a library of related compounds can be prepared and the library can be screened in an assay described herein.

[0165] Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)₄⁺ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Salt forms of the compounds of any of the formulae herein can be amino acid salts of carboxy groups (e.g. L-arginine, -lysine, -histidine salts).

[0166] The compounds of the formulae described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a dosage ranging from about 0.5 to about 100 mg/kg of body weight, alternatively dosages between 1 mg and 1000 mg/dose, every 4 to 120 hours, or according to the requirements of the particular drug. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

[0167] Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for

any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

[0168] Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

[0169] The compositions delineated herein include the compounds of the formulae delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of disease or disease symptoms, including those described herein.

[0170] The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

[0171] Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- α -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α -, β -, and γ -cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl- β -cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

[0172] The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intra-

muscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

[0173] The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic 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 mannitol, water, Ringer's solution 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 may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0174] The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

[0175] The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

[0176] Topical administration of the pharmaceutical compositions of this invention is useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of

the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

[0177] The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

[0178] A composition having the compound of the formulae herein and an additional agent (e.g., a therapeutic agent) can be administered using an implantable device. Implantable devices and related technology are known in the art and are useful as delivery systems where a continuous, or timed-release delivery of compounds or compositions delineated herein is desired. Additionally, the implantable device delivery system is useful for targeting specific points of compound or composition delivery (e.g., localized sites, organs). Negrin et al., *Biomaterials*, 22(6):563 (2001). Timed-release technology involving alternate delivery methods can also be used in this invention. For example, timed-release formulations based on polymer technologies, sustained-release techniques and encapsulation techniques (e.g., polymeric, liposomal) can also be used for delivery of the compounds and compositions delineated herein.

[0179] Also within the invention is a patch to deliver active chemotherapeutic combinations herein. A patch includes a material layer (e.g., polymeric, cloth, gauze, bandage) and the compound of the formulae herein as delineated herein. One side of the material layer can have a protective layer adhered to it to resist passage of the compounds or compositions. The patch can additionally include an adhesive to hold the patch in place on a subject. An adhesive is a composition, including those of either natural or synthetic origin, that when contacted with the skin of a subject, temporarily adheres to the skin. It can be water resistant. The adhesive can be placed on the patch to hold it in contact with the skin of the subject for an extended period of time. The adhesive can be made of a tackiness, or adhesive strength, such that it holds the device in place subject to incidental contact, however, upon an affirmative act (e.g., ripping, peeling, or other intentional removal) the adhesive gives way to the external pressure placed on the device or the adhesive itself, and allows for breaking of the adhesion contact. The adhesive can be pressure sensitive, that is, it can allow for positioning of the adhesive (and the device to be adhered to the skin) against the skin by the application of pressure (e.g., pushing, rubbing) on the adhesive or device.

[0180] When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

Neoplastic Disorders

[0181] The compounds of the invention can be used in the treatment of cancer. As used herein, the terms “cancer”, “hyperproliferative”, “malignant”, and “neoplastic” are used interchangeably, and refer to those cells an abnormal state or condition characterized by rapid proliferation or neoplasm. The terms include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. “Pathologic hyperproliferative” cells occur in disease states characterized by malignant tumor growth.

[0182] The common medical meaning of the term “neoplasia” refers to “new cell growth” that results as a loss of responsiveness to normal growth controls, e.g. to neoplastic cell growth. A “hyperplasia” refers to cells undergoing an abnormally high rate of growth. However, as used herein, the terms neoplasia and hyperplasia can be used interchangeably, as their context will reveal, referring generally to cells experiencing abnormal cell growth rates. Neoplasias and hyperplasias include “tumors,” which may be benign, premalignant or malignant.

[0183] Examples of cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx, prostate, ovary as well as adenocarcinomas which include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and so forth. Metastatic lesions of the aforementioned cancers can also be treated or prevented using a compound described herein.

[0184] The subject method can be useful in treating malignancies of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract, prostate, ovary, pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Exemplary solid tumors that can be treated include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing’s tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squa-

mous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms’ tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0185] The term “carcinoma” is recognized by those skilled in the art and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

[0186] The term “sarcoma” is recognized by those skilled in the art and refers to malignant tumors of mesenchymal derivation.

[0187] The subject method can also be used to inhibit the proliferation of hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. For instance, the invention contemplates the treatment of various myeloid disorders including, but not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97). Lymphoid malignancies which may be treated by the subject method include, but are not limited to acute lymphoblastic leukemia (ALL), which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom’s macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to, non-Hodgkin’s lymphoma and variants thereof, peripheral T-cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL) and Hodgkin’s disease.

Alzheimer’s Disease

[0188] Alzheimer’s Disease (AD) is a complex neurodegenerative disease that results in the irreversible loss of neurons and is an example of a neurodegenerative disease that has symptoms caused at least in part by protein aggregation. A compound described herein can be used to ameliorate at least one symptom of a subject that has AD.

[0189] Clinical hallmarks of Alzheimer’s Disease include progressive impairment in memory, judgment, orientation to physical surroundings, and language. Neuropathological hallmarks of AD include region-specific neuronal loss, amy-

loid plaques, and neurofibrillary tangles. Amyloid plaques are extracellular plaques containing the β amyloid peptide (also known as A β , or A β 42), which is a cleavage product of the β -amyloid precursor protein (also known as APP). Neurofibrillary tangles are insoluble intracellular aggregates composed of filaments of the abnormally hyperphosphorylated microtubule-associated protein, tau. Amyloid plaques and neurofibrillary tangles may contribute to secondary events that lead to neuronal loss by apoptosis (Clark and Karlawish, *Ann. Intern. Med.* 138(5):400-410 (2003)). For example, β -amyloid induces caspase-2-dependent apoptosis in cultured neurons (Troy et al. *J. Neurosci.* 20(4):1386-1392). The deposition of plaques in vivo may trigger apoptosis of proximal neurons in a similar manner.

[0190] Mutations in genes encoding APP, presenilin-1, and presenilin-2 have been implicated in early-onset AD (Lendon et al. *JAMA* 227:825 (1997)). Mutations in these proteins have been shown to enhance proteolytic processing of APP via an intracellular pathway that produces A β . Aberrant regulation of A β processing may be central to the formation of amyloid plaques and the consequent neuronal damage associated with plaques.

[0191] A variety of criteria, including genetic, biochemical, physiological, and cognitive criteria, can be used to evaluate AD in a subject. Symptoms and diagnosis of AD are known to medical practitioners. Some exemplary symptoms and markers of AD are presented below. Information about these indications and other indications known to be associated with AD can be used as an "AD-related parameter." An AD-related parameter can include qualitative or quantitative information. An example of quantitative information is a numerical value of one or more dimensions, e.g., a concentration of a protein or a tomographic map. Qualitative information can include an assessment, e.g., a physician's comments or a binary ("yes"/"no") and so forth. An AD-related parameter includes information that indicates that the subject is not diagnosed with AD or does not have a particular indication of AD, e.g., a cognitive test result that is not typical of AD or a genetic APOE polymorphism not associated with AD.

[0192] Progressive cognitive impairment is a hallmark of AD. This impairment can present as decline in memory, judgment, decision making, orientation to physical surroundings, and language (Nussbaum and Ellis, *New Eng. J. Med.* 348(14):1356-1364 (2003)). Exclusion of other forms of dementia can assist in making a diagnosis of AD.

[0193] Neuronal death leads to progressive cerebral atrophy in AD patients. Imaging techniques (e.g., magnetic resonance imaging, or computed tomography) can be used to detect AD-associated lesions in the brain and/or brain atrophy.

[0194] AD patients may exhibit biochemical abnormalities that result from the pathology of the disease. For example, levels of tau protein in the cerebrospinal fluid is elevated in AD patients (Andreasen, N. et al. *Arch Neurol.* 58:349-350 (2001)). Levels of amyloid beta 42 (A β 42) peptide can be reduced in CSF of AD patients (Galasko, D., et al. *Arch. Neurol.* 55:937-945 (1998)). Levels of A β 42 can be increased in the plasma of AD patients (Ertekin-Taner, N., et al. *Science* 290:2303-2304 (2000)). Techniques to detect biochemical abnormalities in a sample from a subject include cellular, immunological, and other biological meth-

ods known in the art. For general guidance, see, e.g., techniques described in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory, N.Y. (2001), Ausubel et al., *Current Protocols in Molecular Biology* (Greene Publishing Associates and Wiley Interscience, N.Y. (1989), (Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), and updated editions thereof.

[0195] For example, antibodies, other immunoglobulins, and other specific binding ligands can be used to detect a biomolecule, e.g., a protein or other antigen associated with AD. For example, one or more specific antibodies can be used to probe a sample. Various formats are possible, e.g., ELISAs, fluorescence-based assays, Western blots, and protein arrays. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt et al. (2000). *Nature Biotech.* 18, 989-994; Lueking et al. (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S. L. (2000). *Science* 289, 1760-1763; and WO 99/51773A1. Proteins can also be analyzed using mass spectroscopy, chromatography, electrophoresis, enzyme interaction or using probes that detect post-translational modification (e.g., a phosphorylation, ubiquitination, glycosylation, methylation, or acetylation).

[0196] Nucleic acid expression can be detected in cells from a subject, e.g., removed by surgery, extraction, post-mortem or other sampling (e.g., blood, CSF). Expression of one or more genes can be evaluated, e.g., by hybridization based techniques, e.g., Northern analysis, RT-PCR, SAGE, and nucleic acid arrays. Nucleic acid arrays are useful for profiling multiple mRNA species in a sample. A nucleic acid array can be generated by various methods, e.g., by photolithographic methods (see, e.g., U.S. Pat. Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Pat. No. 5,384,261), pin-based methods (e.g., as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

[0197] Metabolites that are associated with AD can be detected by a variety of means, including enzyme-coupled assays, using labeled precursors, and nuclear magnetic resonance (NMR). For example, NMR can be used to determine the relative concentrations of phosphate-based compounds in a sample, e.g., creatine levels. Other metabolic parameters such as redox state, ion concentration (e.g., Ca²⁺) (e.g., using ion-sensitive dyes), and membrane potential can also be detected (e.g., using patch-clamp technology).

[0198] Information about an AD-associated marker can be recorded and/or stored in a computer-readable format. Typically the information is linked to a reference about the subject and also is associated (directly or indirectly) with information about the identity of one or more nucleotides in a gene that encodes a sirtuin in the subject.

[0199] In one embodiment, a non-human animal model of AD (e.g., a mouse model) is used, e.g., to evaluate a compound or a therapeutic regimen, e.g., of a compound described herein. For example, U.S. Pat. No. 6,509,515 describes one such model animal which is naturally able to be used with learning and memory tests. The animal expresses an amyloid precursor protein (APP) sequence at a level in brain tissues such that the animal develops a

progressive neurologic disorder within a short period of time from birth, generally within a year from birth, preferably within 2 to 6 months, from birth. The APP protein sequence is introduced into the animal, or an ancestor of the animal, at an embryonic stage, preferably the one cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage. The zygote or embryo is then developed to term in a pseudo-pregnant foster female. The amyloid precursor protein genes are introduced into an animal embryo so as to be chromosomally incorporated in a state which results in super-endogenous expression of the amyloid precursor protein and the development of a progressive neurologic disease in the cortico-limbic areas of the brain, areas of the brain which are prominently affected in progressive neurologic disease states such as AD. The gliosis and clinical manifestations in affected transgenic animals model neurologic disease. The progressive aspects of the neurologic disease are characterized by diminished exploratory and/or locomotor behavior and diminished 2-deoxyglucose uptake/utilization and hypertrophic gliosis in the cortico-limbic regions of the brain. Further, the changes that are seen are similar to those that are seen in some aging animals. Other animal models are also described in U.S. Pat. Nos. 5,387,742; 5,877,399; 6,358,752; and 6,187,992.

Parkinson's Disease

[0200] Parkinson's disease includes neurodegeneration of dopaminergic neurons in the substantia nigra resulting in the degeneration of the nigrostriatal dopamine system that regulates motor function. This pathology, in turn, leads to motor dysfunctions. (see, e.g., and Lotharius et al., *Nat. Rev. Neurosci.*, 3:932-42 (2002).) Exemplary motor symptoms include: akinesia, stooped posture, gait difficulty, postural instability, catalepsy, muscle rigidity, and tremor. Exemplary non-motor symptoms include: depression, lack of motivation, passivity, dementia and gastrointestinal dysfunction (see, e.g., Fahn, *Ann. N.Y. Acad. Sci.*, 991:1-14 (2003) and Pfeiffer, *Lancet Neurol.*, 2:107-16 (2003)) Parkinson's has been observed in 0.5 to 1 percent of persons 65 to 69 years of age and 1 to 3 percent among persons 80 years of age and older. (see, e.g., Nussbaum et al., *N. Engl. J. Med.*, 348:1356-64 (2003)).

[0201] A compound described herein can be used to ameliorate at least one symptom of a subject that has Parkinson's disease.

[0202] Molecular markers of Parkinson's disease include reduction in aromatic L-amino acid decarboxylase (AADC). (see, e.g., US Appl 20020172664); loss of dopamine content in the nigrostriatal neurons (see, e.g., Fahn, *Ann. N.Y. Acad. Sci.*, 991:1-14 (2003) and Lotharius et al., *Nat. Rev. Neurosci.*, 3:932-42 (2002)). In some familial cases, PD is linked to mutations in single genes encoding alpha-synuclein and parkin (an E3 ubiquitin ligase) proteins. (e.g., Riess et al., *J. Neurol.* 250 Suppl 1:13-10 (2003) and Nussbaum et al., *N. Engl. J. Med.*, 348:1356-64 (2003)). A missense mutation in a neuron-specific C-terminal ubiquitin hydrolase gene is also associated with Parkinson's. (e.g., Nussbaum et al., *N. Engl. J. Med.*, 348:1356-64 (2003))

[0203] A compound or library of compounds described herein can be evaluated in a non-human animal model of Parkinson's disease. Exemplary animal models of Parkinson's disease include primates rendered parkinsonian by treatment with the dopaminergic neurotoxin 1-methyl-4

phenyl 1,2,3,6-tetrahydropyridine (MPTP) (see, e.g., US Appl 20030055231 and Wichmann et al., *Ann. N.Y. Acad. Sci.*, 991:199-213 (2003); 6-hydroxydopamine-lesioned rats (e.g., *Lab. Anim. Sci.*, 49:363-71 (1999)); and transgenic invertebrate models (e.g., Lakso et al., *J. Neurochem.*, 86:165-72 (2003) and Link, *Mech. Ageing Dev.*, 122:1639-49 (2001)).

Evaluating Polyglutamine Aggregation

[0204] A variety of cell free assays, cell based assays, and organismal assays are available for evaluating polyglutamine aggregation, e.g., Huntingtin polyglutamine aggregation. Some examples are described, e.g., in U.S. 2003-0109476.

[0205] Assays (e.g., cell free, cell-based, or organismal) can include a reporter protein that includes a polyglutamine repeat region which has at least 35 polyglutamines. The reporter protein can be easily detectable, e.g., by fluorescence. For example, the protein is conjugated to a fluorophore, for example, fluorescein isothiocyanate (FITC), allophycocyanin (APC), R-phycoerythrin (PE), peridinin chlorophyll protein (PerCP), Texas Red, Cy3, Cy5, Cy7, or a fluorescence resonance energy tandem fluorophore such as PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, PE-Cy7, PE-Texas Red, and APC-Cy7. In another example the protein is "intrinsically fluorescent" in that it has a chromophore is entirely encoded by its amino acid sequence and can fluoresce without requirement for cofactor or substrate. For example, the protein can include a green fluorescent protein (GFP)-like chromophore. As used herein, "GFP-like chromophore" means an intrinsically fluorescent protein moiety comprising an 11-stranded β -barrel with a central α -helix, the central α -helix having a conjugated π -resonance system that includes two aromatic ring systems and the bridge between them.

[0206] The GFP-like chromophore can be selected from GFP-like chromophores found in naturally occurring proteins, such as *A. victoria* GFP (GenBank accession number AAA27721), *Renilla reniformis* GFP, FP583 (GenBank accession no. AF168419) (DsRed), FP593 (AF272711), FP483 (AF168420), FP484 (AF168424), FP595 (AF246709), FP486 (AF168421), FP538 (AF168423), and FP506 (AF168422), and need include only so much of the native protein as is needed to retain the chromophore's intrinsic fluorescence. Methods for determining the minimal domain required for fluorescence are known in the art. Li et al., *J. Biol. Chem.* 272:28545-28549 (1997).

[0207] Alternatively, the GFP-like chromophore can be selected from GFP-like chromophores modified from those found in nature. Typically, such modifications are made to improve recombinant production in heterologous expression systems (with or without change in protein sequence), to alter the excitation and/or emission spectra of the native protein, to facilitate purification, to facilitate or as a consequence of cloning, or are a fortuitous consequence of research investigation. The methods for engineering such modified GFP-like chromophores and testing them for fluorescence activity, both alone and as part of protein fusions, are well-known in the art. A variety of such modified chromophores are now commercially available and can readily be used in the fusion proteins of the present invention. For example, EGFP ("enhanced GFP"), Cormack et al., *Gene* 173:33-38 (1996); U.S. Pat. Nos. 6,090,919 and 5,804,

387, is a red-shifted, human codon-optimized variant of GFP that has been engineered for brighter fluorescence, higher expression in mammalian cells, and for an excitation spectrum optimized for use in flow cytometers. EGFP can usefully contribute a GFP-like chromophore to the fusion proteins that further include a polyglutamine region. A variety of EGFP vectors, both plasmid and viral, are available commercially (Clontech Labs, Palo Alto, Calif., USA). Still other engineered GFP proteins are known. See, e.g., Heim et al., *Curr. Biol.* 6:178-182 (1996); Cormack et al., *Gene* 173:33-38 (1996), BFP2, EYFP ("enhanced yellow fluorescent protein"), EBFP, Ormo et al., *Science* 273:1392-1395 (1996), Heikal et al., *Proc. Natl. Acad. Sci. USA* 97:11996-12001 (2000). ECFP ("enhanced cyan fluorescent protein") (Clontech Labs, Palo Alto, Calif., USA). The GFP-like chromophore can also be drawn from other modified GFPs, including those described in U.S. Pat. Nos. 6,124,128; 6,096,865; 6,090,919; 6,066,476; 6,054,321; 6,027,881; 5,968,750; 5,874,304; 5,804,387; 5,777,079; 5,741,668; and 5,625,048.

[0208] In one embodiment, a reporter protein that includes a polyglutamine repeat region which has at least 35 polyglutamines is used in a cell-based assay.

[0209] In one example, PC12 neuronal cell lines that have a construct engineered to express a protein encoded by HD gene exon 1 containing alternating, repeating codons fused to an enhanced GFP (green fluorescent protein) gene can be used. See, e.g., Boado et al. *J. Pharmacol. and Experimental Therapeutics* 295(1): 239-243 (2000) and Kazantsev et al. *Proc. Natl. Acad. Sci. USA* 96: 11404-09 (1999). Expression of this gene leads to the appearance of green fluorescence co-localized to the site of protein aggregates. The HD gene exon 1-GFP fusion gene is under the control of an inducible promoter regulated by muristerone. A particular construct has approximately 46 glutamine repeats (encoded by either CAA or CAG). Other constructs have, for example, 103 glutamine repeats. PC12 cells are grown in DMEM, 5% Horse serum (heat inactivated), 2.5% FBS and 1% Pen-Strep, and maintained in low amounts on Zeocin and G418. The cells are plated in 24-well plates coated with poly-L-lysine coverslips, at a density of $5 \cdot 10^5$ cells/ml in media without any selection. Muristerone is added after the overnight incubation to induce the expression of HD gene exon 1-GFP. The cells can be contacted with a test compound, e.g., before or after plating and before or after induction. The data can be acquired on a Zeiss inverted 100M Axioskop equipped with a Zeiss 510 LSM confocal microscope and a Coherent Krypton Argon laser and a Helium Neon laser. Samples can be loaded into Lab-Tek II chambered coverglass system for improved imaging. The number of Huntingtin-GFP aggregations within the field of view of the objective is counted in independent experiments (e.g., at least three or seven independent experiments).

[0210] Other exemplary means for evaluating samples include a high throughput apparatus, such as the Amersham Biosciences IN Cell Analysis System and Cellomics™ ArrayScan HCS System which permit the subcellular location and concentration of fluorescently tagged moieties to be detected and quantified, both statically and kinetically. See also, U.S. Pat. No. 5,989,835.

[0211] Other exemplary mammalian cell lines include: a CHO cell line and a 293 cell line. For example, CHO cells

with integrated copies of HD gene exon 1 with approximately 103Q repeats fused to GFP as a fusion construct encoding HD gene exon 1 Q103-GFP produce a visible GFP aggregation at the nuclear membrane, detectable by microscopy, whereas CHO cells with integrated copies of fusion constructs encoding HD gene exon 1 Q24-GFP in CHO cells do not produce a visible GFP aggregation at the nuclear membrane. In another example, 293 cells with integrated copies of the HD gene exon 1 containing 84 CAG repeats are used.

[0212] A number of animal model system for Huntington's disease are available. See, e.g., Brouillet, *Functional Neurology* 15(4): 239-251 (2000); Ona et al. *Nature* 399: 263-267 (1999), Bates et al. *Hum Mol Genet.* 6(10):1633-7 (1997); Hansson et al. *J. of Neurochemistry* 78: 694-703; and Rubinstein, D.C., *Trends in Genetics*, Vol. 18, No. 4, pp. 202-209 (a review on various animal and non-human models of HD).

[0213] In one embodiment, the animal is a transgenic mouse that can express (in at least one cell) a human Huntingtin protein, a portion thereof, or fusion protein comprising human Huntingtin protein, or a portion thereof, with, for example, at least 36 glutamines (e.g., encoded by CAG repeats (alternatively, any number of the CAG repeats may be CAA) in the CAG repeat segment of exon 1 encoding the polyglutamine tract).

[0214] An example of such a transgenic mouse strain is the R6/2 line (Mangiarini et al. *Cell* 87: 493-506 (1996)). The R6/2 mice are transgenic Huntington's disease mice, which over-express exon one of the human HD gene (under the control of the endogenous promoter). The exon 1 of the R6/2 human HD gene has an expanded CAG/polyglutamine repeat lengths (150 CAG repeats on average). These mice develop a progressive, ultimately fatal neurological disease with many features of human Huntington's disease. Abnormal aggregates, constituted in part by the N-terminal part of Huntingtin (encoded by HD exon 1), are observed in R6/2 mice, both in the cytoplasm and nuclei of cells (Davies et al. *Cell* 90: 537-548 (1997)). For example, the human Huntingtin protein in the transgenic animal is encoded by a gene that includes at least 55 CAG repeats and more preferably about 150 CAG repeats.

[0215] These transgenic animals can develop a Huntington's disease-like phenotype. These transgenic mice are characterized by reduced weight gain, reduced lifespan and motor impairment characterized by abnormal gait, resting tremor, hindlimb clasping and hyperactivity from 8 to 10 weeks after birth (for example the R6/2 strain; see Mangiarini et al. *Cell* 87: 493-506 (1996)). The phenotype worsens progressively toward hypokinesia. The brains of these transgenic mice also demonstrate neurochemical and histological abnormalities, such as changes in neurotransmitter receptors (glutamate, dopaminergic), decreased concentration of N-acetylaspartate (a marker of neuronal integrity) and reduced striatum and brain size. Accordingly, evaluating can include assessing parameters related to neurotransmitter levels, neurotransmitter receptor levels, brain size and striatum size. In addition, abnormal aggregates containing the transgenic part of or full-length human Huntingtin protein are present in the brain tissue of these animals (e.g., the R6/2 transgenic mouse strain). See, e.g., Mangiarini et al. *Cell* 87: 493-506 (1996), Davies et al. *Cell* 90: 537-548 (1997),

Brouillet, Functional Neurology 15(4): 239-251 (2000) and Cha et al. Proc. Natl. Acad. Sci. USA 95: 6480-6485 (1998).

[0216] To test the effect of the test compound, e.g., a compound described herein or present in a library described herein, in an animal model, different concentrations of test compound are administered to the transgenic animal, for example by injecting the test compound into circulation of the animal. In one embodiment, a Huntington's disease-like symptom is evaluated in the animal. For example, the progression of the Huntington's disease-like symptoms, e.g. as described above for the mouse model, is then monitored to determine whether treatment with the test compound results in reduction or delay of symptoms. In another embodiment, disaggregation of the Huntingtin protein aggregates in these animals is monitored. The animal can then be sacrificed and brain slices are obtained. The brain slices are then analyzed for the presence of aggregates containing the transgenic human Huntingtin protein, a portion thereof, or a fusion protein comprising human Huntingtin protein, or a portion thereof. This analysis can include, for example, staining the slices of brain tissue with anti-Huntingtin antibody and adding a secondary antibody conjugated with FITC which recognizes the anti-Huntingtin's antibody (for example, the anti-Huntingtin antibody is mouse anti-human antibody and the secondary antibody is specific for human antibody) and visualizing the protein aggregates by fluorescent microscopy. Alternatively, the anti-Huntingtin antibody can be directly conjugated with FITC. The levels of Huntingtin's protein aggregates are then visualized by fluorescent microscopy.

[0217] A *Drosophila melanogaster* model system for Huntington's disease is also available. See, e.g., Steffan et al., Nature, 413: 739-743 (2001) and Marsh et al., Human Molecular Genetics 9: 13-25 (2000). For example, a transgenic *Drosophila* can be engineered to express human Huntingtin protein, a portion thereof (such as exon 1), or fusion protein comprising human Huntingtin protein, or a portion thereof, with, for example, a polyglutamine region that includes at least 36 glutamines (e.g., encoded by CAG repeats (preferably 51 repeats or more) (alternatively, any number of the CAG repeats may be CAA)) The polyglutamine region can be encoded by the CAG repeat segment of exon 1 encoding the poly Q tract. These transgenic flies can also be engineered to express human Huntingtin protein, a portion thereof (such as exon 1), or fusion protein comprising human Huntingtin protein, or a portion thereof, in neurons, e.g., in the *Drosophila* eye.

[0218] The test compound (e.g., different concentrations of the test compound) or a compound described herein can be administered to the transgenic *Drosophila*, for example, by applying the pharmaceutical compositions that include the compound into the animal or feeding the compound as part of food. Administration of the compound can occur at various stages of the *Drosophila* life cycle. The animal can be monitored to determine whether treatment with the compound results in reduction or delay of Huntington's disease-like symptoms, disaggregation of the Huntingtin protein aggregates, or reduced lethality and/or degeneration of photoreceptor neurons are monitored.

[0219] Neurodegeneration due to expression of human Huntingtin protein, a portion thereof (such as exon 1), or fusion protein comprising human Huntingtin protein, or a

portion thereof, is readily observed in the fly compound eye, which is composed of a regular trapezoidal arrangement of seven visible rhabdomeres (subcellular light-gathering structures) produced by the photoreceptor neurons of each *Drosophila ommatidium*. Expression of human Huntingtin protein, a portion thereof (such as exon 1), or fusion protein comprising human Huntingtin protein, or a portion thereof, leads to a progressive loss of rhabdomeres. Thus, an animal to which a test compound is administered can be evaluated for neuronal degeneration.

[0220] Morely et al. (2002) Proc. Nat. Acad. USA Vol. 99:10417 describes a *C. elegans* system for evaluating Huntington's disease related protein aggregation.

Evaluating Huntington's Disease

[0221] A compound described herein can be used to ameliorate at least one symptom of Huntington's disease in a subject.

[0222] A variety of methods are available to evaluate and/or monitor Huntington's disease. A variety of clinical symptoms and indicia for the disease are known. Huntington's disease causes a movement disorder, psychiatric difficulties and cognitive changes. The degree, age of onset, and manifestation of these symptoms can vary. The movement disorder can include quick, random, dance-like movements called chorea.

[0223] One method for evaluating Huntington's disease uses the Unified Huntington's disease Rating Scale (UNDRS). It is also possible to use individual tests alone or in combination to evaluate if at least one symptom of Huntington's disease is ameliorated. The UNDRS is described in *Movement Disorders* (vol. 11:136-142, 1996) and Marder et al. *Neurology* (54:452-458, 2000). The UNDRS quantifies the severity of Huntington's Disease. It is divided into multiple subsections: motor, cognitive, behavioral, functional. In one embodiment, a single subsection is used to evaluate a subject. These scores can be calculated by summing the various questions of each section. Some sections (such as chorea and dystonia) can include grading each extremity, face, bucco-oral-ligual, and trunk separately.

[0224] Exemplary motor evaluations include: ocular pursuit, saccade initiation, saccade velocity, dysarthria, tongue protrusion, finger tap ability, pronate/supinate, a fist-hand-palm sequence, rigidity of arms, bradykinesia, maximal dystonia (trunk, upper and lower extremities), maximal chorea (e.g., trunk, face, upper and lower extremities), gait, tandem walking, and retropulsion. An exemplary treatment can cause a change in the Total Motor Score 4 (TMS-4), a subscale of the UHDRS, e.g., over a one-year period.

Diabetes

[0225] The invention provides methods of treating and preventing diabetes. Examples of diabetes include insulin dependent diabetes mellitus and non-insulin dependent diabetes. For example the method includes administering to a patient having diabetes or at risk of diabetes a compound described herein. In some instances, a patient can be identified as being at risk of developing diabetes by having impaired glucose tolerance (IGT), or fasting hyperglycemia.

[0226] For example, a compound described herein can be administered to a subject in a therapeutically effective

amount to decrease gluconeogenesis, improve glycemic control (i.e., lower fasting blood glucose), or normalize insulin sensitivity. The compound can be administered to a subject suffering from diabetes or obesity.

[0227] Insulin dependent diabetes mellitus (Type 1 diabetes) is an autoimmune disease, where insulinitis leads to the destruction of pancreatic β -cells. At the time of clinical onset of type 1 diabetes mellitus, significant number of insulin producing β cells are destroyed and only 15% to 40% are still capable of insulin production (McCulloch et al. (1991) *Diabetes* 40:673-679). β -cell failure results in a life long dependence on daily insulin injections and exposure to the acute and late complication of the disease.

[0228] Type 2 diabetes mellitus is a metabolic disease of impaired glucose homeostasis characterized by hyperglycemia, or high blood sugar, as a result of defective insulin action which manifests as insulin resistance, defective insulin secretion, or both. A patient with Type 2 diabetes mellitus has abnormal carbohydrate, lipid, and protein metabolism associated with insulin resistance and/or impaired insulin secretion. The disease leads to pancreatic β cell destruction and eventually absolute insulin deficiency. Without insulin, high glucose levels remain in the blood. The long term effects of high blood glucose include blindness, renal failure, and poor blood circulation to these areas, which can lead to foot and ankle amputations. Early detection is critical in preventing patients from reaching this severity. The majority of patients with diabetes have the non-insulin dependent form of diabetes, currently referred to as Type 2 diabetes mellitus.

[0229] The invention also includes methods of treating disorders related to or resulting from diabetes, for example end organ damage, diabetic gastroparesis, diabetic neuropathy, cardiac dysrhythmia, etc.

[0230] Exemplary molecular models of Type II diabetes include: a transgenic mouse having defective Nkx-2.2 or Nkx-6.1; (U.S. Pat. No. 6,127,598); Zucker Diabetic Fatty fa/fa (ZDF) rat. (U.S. Pat. No. 6,569,832); and Rhesus monkeys, which spontaneously develop obesity and subsequently frequently progress to overt type 2 diabetes (Hotta et al., *Diabetes*, 50:1126-33 (2001)); and a transgenic mouse with a dominant-negative IGF-I receptor (KR-IGF-R) having Type 2 diabetes-like insulin resistance.

Metabolic Syndrome

[0231] The invention provides a method of treating metabolic syndrome, including administering to a subject an effective amount of a compound described herein.

[0232] The metabolic syndrome (e.g., Syndrome X) is characterized by a group of metabolic risk factors in one person. They include: central obesity (excessive fat tissue in and around the abdomen), atherogenic dyslipidemia (blood fat disorders—mainly high triglycerides and low HDL cholesterol—that foster plaque buildups in artery walls); insulin resistance or glucose intolerance (the body can't properly use insulin or blood sugar); prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor [-1] in the blood); raised blood pressure (i.e., hypertension) (130/85 mmHg or higher); and proinflammatory state (e.g., elevated high-sensitivity C-reactive protein in the blood).

[0233] The underlying causes of this syndrome are overweight/obesity, physical inactivity and genetic factors.

People with metabolic syndrome are at increased risk of coronary heart disease, other diseases related to plaque buildups in artery walls (e.g., stroke and peripheral vascular disease) and type 2 diabetes. Metabolic syndrome is closely associated with a generalized metabolic disorder called insulin resistance, in which the body can't use insulin efficiently.

Fat-Cell Related Disorders

[0234] The invention provides a method of enhancing adipogenesis comprising administering to a subject a compound described herein. For example, the subject can be underweight, have reduced fat content, or require additional fat cells, either locally (e.g., at a topical location such as the skin of the face) or systemically

[0235] The compounds may also be used to modulate a fat cell, e.g., an adipocyte, e.g., differentiation of the adipocyte. For example, a compound described herein can be administered in an amount effective to prevent fat accumulation in a normal or a pathological state. Disorders relating to adipocytes include obesity. "Obesity" refers to a condition in which a subject has a body mass index of greater than or equal to 30. "Over-weight" refers to a condition in which a subject has a body mass index of greater or equal to 25.0. The body mass index and other definitions are according to the "NIH Clinical Guidelines on the Identification and Evaluation, and Treatment of Overweight and Obesity in Adults" (1998). In particular, obesity can lead to type II diabetes in successive phases. Clinically, these phases can be characterized as normal glucose tolerance, impaired glucose tolerance, hyperinsulinemic diabetes, and hypoinsulinemic diabetes. Such a progressive impairment of glucose storage correlates with a rise in basal glycemia.

[0236] Examples of other fat-cell related disorders include) dislipidemia, and hyperlipidemia (including high triglycerides, high LDL, high fatty acid levels).

[0237] Exemplary models for the treatment of obesity include two primary animal model systems: 1) diet-induced obesity (DIO) caused by feeding rodents ~60% fat content of caloric intake. Animals treated for up to 12-16 weeks on this type of diet gain substantial body weight (>50% increase), accumulate excessive fat mass, become hyperglycemic, hyperinsulinemic and insulin resistant. In this model compounds can be tested prior to the initiation of the diet or at any time during development of obesity. 2) db/db mutant mice (leptin receptor spontaneous mutant). These animals exhibit a similar phenotype as the DIO animals only more severe with regard to various readouts. Animals can be treated similar to the DIO model. As a surrogate readout of SirT1 inhibitor activity, sister animals can be sacrificed along the treatment regimen and assessed biochemically for increased acetylation status of FoxO1 proteins in various tissues, such as liver, muscle and white adipose tissue.

[0238] Compound described herein can be used to treat AMD. Macular degeneration includes a variety of diseases characterized by a progressive loss of central vision associated with abnormalities of Bruch's membrane and the retinal pigment epithelium. (see, e.g., US Appl 20030138798). AMD occurs in 1.2% of the population between 52 and 64 years of age and 20% of patients over the age of 75. (see, e.g., US Appl 20030087889) Macular degeneration occurs in two forms, "atrophic" ("non-exuda-

tive" or "dry" form) and "exudative" ("wet" form). A less common form of AMD is "atrophic AMD," which is due to dead RPE cells. (U.S. Application 20030093064).

[0239] Symptoms of AMD include: straight lines in the field of vision appear wavy; type in books, magazines and newspapers appears blurry; and dark or empty spaces block the center of vision. (see, e.g., US Appl 20030065020)

[0240] Exemplary molecular markers that can be used to evaluate an AMD status include: the nucleic acid sequence of a gene encoding FBN1 or the amino acid sequence of the FBN1 protein: 345Arg>Trp and 362 Arg>Gln; (see, e.g., US Appl 20030138798); increases in the pigment A2E, N-retinyl-N-retinylidene ethanolamine, ultimately leading to release of cytochrome c into the cytoplasm (US Appl 20030050283); auto-antibodies against various macular degeneration-associated molecules including fibulin-3, vitronectin, β -crystallin A2, β -crystallin A3, β -crystallin A4, β -crystallin S, calreticulin, 14-3-3 protein epsilon, serotransferrin, albumin, keratin, pyruvate carboxylase, or villin 2 (see, e.g., U.S. Appl 20030017501); abnormal activity or level of complement pathway molecules including clusterin, C6 or C5b-9 complex (see, e.g., US Appl 20020015957); and accumulation of the pigment lipofuscin in lysosomes of retinal pigment epithelial (RPE) cells (Suter et al., *J Biol. Chem.* 275:39625-30 (2000)).

Tissue Repair

[0241] A compound described herein may also be used to modulate tissue repair or tissue state. Exemplary implementations for tissue repair include wound healing, burns, ulcers (e.g., ulcers in a diabetic, e.g., diabetic foot ulcers), surgical wounds, sores, and abrasions. The method can decrease at least one symptom of the tissue. For example, the method includes administering (e.g., locally or systemically) an effective amount of a compound described herein.

[0242] A compound may be used for a dermatological disease or disorder.

Skeletal Muscle Atrophy

[0243] Muscle atrophy includes numerous neuromuscular, metabolic, immunological and neurological disorders and diseases as well as starvation, nutritional deficiency, metabolic stress, diabetes, aging, muscular dystrophy, or myopathy. Muscle atrophy occurs during the aging process. Muscle atrophy also results from reduced use or disuse of the muscle. Symptoms include a decline in skeletal muscle tissue mass. In human males, muscle mass declines by one-third between the ages of 50 and 80.

[0244] Some molecular features of muscle atrophy include the upregulation of ubiquitin ligases, and the loss of myofibrillar proteins (Furuno et al., *J. Biol. Chem.*, 265:8550-8557, 1990). The breakdown of these proteins can be followed, e.g., by measuring 3-methyl-histidine production, which is a specific constituent of actin, and in certain muscles of myosin (Goodman, *Biochem. J.* 241:121-12, 1987 and Lowell, et al., *Metabolism*, 35:1121-112, 1986; Stein and Schluter, *Am. J. Physiol. Endocrinol. Metab.* 272: E688-E696, 1997). Release of creatine kinase (a cell damage marker) (Jackson, et al., *Neurology*, 41: 101-104, 1991) can also be indicative.

Multiple Sclerosis

[0245] Multiple sclerosis (MS) is a neuromuscular disease characterized by focal inflammatory and autoimmune degeneration of cerebral white matter. White matter becomes inflamed, and inflammation is followed by destruction of myelin (forming "lesions" which are marked by an infiltration of numerous immune cells, especially T-cell lymphocytes and macrophages. MS can cause a slowing or complete block of nerve impulse transmission and, thus, diminished or lost bodily function. A patient who has MS may have one of a variety of grade of MS (e.g., relapsing-remitting MS, primary progressive MS, secondary progressive, and Marburg's variant MS).

[0246] Symptoms can include vision problems such as blurred or double vision, red-green color distortion, or even blindness in one eye, muscle weakness in the extremities, coordination and balance problems, muscle spasticity, muscle fatigue, paresthesias, fleeting abnormal sensory feelings such as numbness, prickling, or "pins and needles" sensations, and in the worst cases, partial or complete paralysis. About half of the people suffering from MS also experience cognitive impairments, such as for example, poor concentration, attention, memory and/or judgment. (see, e.g., U.S. 2003-0130357 and 2003-0092089)

[0247] Molecular markers of MS include a number of genetic factors, e.g., Caucasian haplotype DRB*1501-DQA1*0102-DQB1*0602 (US Appl 20030113752), a point mutation in the protein tyrosine phosphatase receptor-type C. (US Appl 20030113752), absence of wild-type SARG-1-protein, presence of mutated SARG-1-protein, or absence or mutation in the nucleic acids encoding wild-type SARG-1. (see, e.g., US Appl 20030113752) and protein indicators, e.g., Myelin Basic Protein auto-antibody in cerebrospinal fluid. (see, e.g., US Appl 20030092089)

[0248] Cellular and animal models of MS include transgenic mouse model for chronic MS (experimental autoimmune encephalomyelitis (EAE)), e.g., as described by Goverman et al., *Cell* 72:551-60 (1993), and primate models as reviewed by Brok et al., *Immunol. Rev.*, 183:173-85 (2001).

Amyotrophic Lateral Sclerosis (ALS; Lou Gehrig's Disease)

[0249] A compound described herein can be used to modulate ALS. ALS refers to a class of disorders that comprise upper and lower motor neurons. The incidence of ALS increases substantially in older adults. These disorders are characterized by major pathological abnormalities include selective and progressive degeneration of the lower motor neurons in the spinal cord and the upper motor neurons in the cerebral cortex resulting in motor neuron death, which causes the muscles under their control to weaken and waste away leading to paralysis. Examples of ALS disorders include classical ALS (typically affecting both lower and upper motor neurons), Primary Lateral Sclerosis (PLS, typically affecting only the upper motor neurons), Progressive Bulbar Palsy (PBP or Bulbar Onset, a version of ALS that typically begins with difficulties swallowing, chewing and speaking), Progressive Muscular Atrophy (PMA, typically affecting only the lower motor neurons) or familial ALS (a genetic version of ALS), or a combination of these conditions. (see, e.g., US Appl 20020198236 and US Appl 20030130357).

[0250] The ALS status of an individual may be evaluated by neurological examination or other means, such as MRI, FVC, MUNE etc. (see, e.g., US Appl 20030130357). Symptoms include muscle weakness in the hands, arms, legs; swallowing or breathing difficulty; twitching (fasciculation) and cramping of muscles; and reduced use of the limbs. The invention includes administering an agent that modulates the IGF-1/GH axis in an amount effective to relieve one or more ALS symptoms, e.g., in an individual having, at risk to,

[0251] Methods for evaluating ALS status of an individual can include evaluating the “excitatory amino acid transporter type 2” (EAAT2) protein or gene, the Copper-Zinc Superoxide Dismutase (SOD1) protein or gene, mitochondrial Complex I activity, levels of polyamines, such as putrescine, spermine and spermidine, ornithine decarboxylase activity, and a gene that encodes a putative GTPase regulator (see Nat. Genet., 29(2): 166-73 (2001)).

[0252] Cells and animals for evaluating the effect of a compound on ALS status include a mouse which has an altered SOD gene, e.g., a SOD1-G93A transgenic mouse which carries a variable number of copies of the human G93A SOD mutation driven by the endogenous promoter, a SOD1-G37R transgenic mouse (Wong et al., Neuron, 14(6):1105-16 (1995)); SOD1-G85R transgenic mouse (Bruijn et al., Neuron, 18(2):327-38 (1997)); *C. elegans* strains expressing mutant human SOD1 (Oeda et al., Hum Mol Genet., 10:2013-23 (2001)); and a *Drosophila* expressing mutations in Cu/Zn superoxide dismutase (SOD). (Phillips et al., Proc. Natl. Acad. Sci. U.S.A., 92:8574-78 (1995) and McCabe, Proc. Natl. Acad. Sci. U.S.A., 92:8533-34 (1995)).

Neuropathy

[0253] A compound described herein can be used to modulate a neuropathy. A neuropathy can include a central and/or peripheral nerve dysfunction caused by systemic disease, hereditary condition or toxic agent affecting motor, sensory, sensorimotor or autonomic nerves. (see, e.g., US App 20030013771).

[0254] Symptoms can vary depending upon the cause of the nerve damage and the particular types of nerves affected. For example, symptoms of motor neuropathy include clumsiness in performing physical tasks or as muscular weakness, exhaustion after minor exertion, difficulty in standing or walking and attenuation or absence of a neuromuscular reflex. (US App 20030013771) symptoms of autonomic neuropathy include constipation, cardiac irregularities and attenuation of the postural hypotensive reflex. (US App 20030013771), symptoms of sensory neuropathy include pain and numbness; tingling in the hands, legs or feet; and extreme sensitivity to touch, and symptoms of retinopathy include blurred vision, sudden loss of vision, black spots, and flashing lights.

[0255] Guillain-Barré syndrome is a type of motor neuropathy that usually occurs two to three weeks after a flu-like disease or other infection. Symptoms include ascending weakness wherein weakness begins in the lower extremities and ascends to the upper extremities. An elevation of the protein level in the spinal fluid without an increase in the number of white cells also results. (US Appl 20030083242)

Disorders

[0256] Additional disorders for which the compounds described herein may be useful and definitions therefore include the following:

[0257] An “age-associated disorder” or “age-related disorder” is a disease or disorder whose incidence is at least 1.5 fold higher among human individuals greater than 60 years of age relative to human individuals between the ages of 30-40, at the time of filing of this application and in a selected population of greater than 100,000 individuals. A preferred population is a United States population. A population can be restricted by gender and/or ethnicity.

[0258] A “geriatric disorder” is a disease or disorder whose incidence, at the time of filing of this application and in a selected population of greater than 100,000 individuals, is at least 70% among human individuals that are greater than 70 years of age. In one embodiment, the geriatric disorder is a disorder other than cancer or a cardio-pulmonary disorder. A preferred population is a United States population. A population can be restricted by gender and/or ethnicity.

[0259] A disorder having an “age-associated susceptibility factor” refers to a disease or disorder whose causation is mediated by an externality, but whose severity or symptoms are substantially increased in human individuals over the age of 60 relative to human individuals between the ages of 30-40, at the time of filing of this application and in the United States population. For example, pneumonia is caused by pathogens, but the severity of the disease is greater in humans over the age of 60 relative to human individuals between the ages of 30-40.

[0260] A “neoplastic disorder” is a disease or disorder characterized by cells that have the capacity for autonomous growth or replication, e.g., an abnormal state or condition characterized by proliferative cell growth. An “age-associated neoplastic disorder” is a neoplastic disorder that is also an age-associated disorder.

[0261] A “non-neoplastic disorder” is a disease or disorder that is not characterized by cells that have the capacity for autonomous growth or replication. An “age-associated non-neoplastic disorder” is a non-neoplastic disorder that is also an age-associated disorder.

[0262] A “neurological disorder” is a disease or disorder characterized by an abnormality or malfunction of neuronal cells or neuronal support cells (e.g., glia or muscle). The disease or disorder can affect the central and/or peripheral nervous system. Exemplary neurological disorders include neuropathies, skeletal muscle atrophy, and neurodegenerative diseases, e.g., a neurodegenerative disease caused at least in part by polyglutamine aggregation or a neurodegenerative disease other than one caused at least in part by polyglutamine aggregation. Exemplary neurodegenerative diseases include: Alzheimer’s, Amyotrophic Lateral Sclerosis (ALS), and Parkinson’s disease. An “age-associated neurological disorder” is a neurological disorder that is also an age-associated disorder.

[0263] A “cardiovascular disorder” is a disease or disorder characterized by an abnormality or malfunction of the cardiovascular system, e.g., heart, lung, or blood vessels. Exemplary cardiovascular disorders include: cardiac dys-

rhythmias, chronic congestive heart failure, ischemic stroke, coronary artery disease, elevated blood pressure (i.e., hypertension), and cardiomyopathy. An “age-associated cardiovascular disorder” is a cardiovascular disorder that is also an age-associated disorder.

[0264] A “metabolic disorder” is a disease or disorder characterized by an abnormality or malfunction of metabolism. One category of metabolic disorders are disorders of glucose or insulin metabolism. An “age-associated metabolic disorder” is a metabolic disorder that is also an age-associated disorder.

[0265] A “dermatological disorder” is a disease or disorder characterized by an abnormality or malfunction of the skin. A “dermatological tissue condition” refers to the skin and any underlying tissue (e.g., support tissue) which contributes to the skin's function and/or appearance, e.g., cosmetic appearance.

[0266] Exemplary diseases and disorders that are relevant to certain implementations include: cancer (e.g., breast cancer, colorectal cancer, CCL, CML, prostate cancer); skeletal muscle atrophy; adult-onset diabetes; diabetic nephropathy, neuropathy (e.g., sensory neuropathy, autonomic neuropathy, motor neuropathy, retinopathy); obesity; bone resorption; age-related macular degeneration, ALS, Alzheimer's, Bell's Palsy, atherosclerosis, cardiovascular disorders (e.g., cardiac dysrhythmias, chronic congestive heart failure, ischemic stroke, coronary artery disease, high blood pressure (i.e., hypertension), and cardiomyopathy), chronic renal failure, type 2 diabetes, ulceration, cataract, presbiopia, glomerulonephritis, Guillan-Barre syndrome, hemorrhagic stroke, short-term and long-term memory loss, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, SLE, Crohn's disease, osteoarthritis, Parkinson's disease, pneumonia, and urinary incontinence. In addition, many neurodegenerative disorders and disorders associated with protein aggregation (e.g., other than polyglutamine aggregation) or protein misfolding can also be age-related. Symptoms and diagnosis of diseases are well known to medical practitioners. The compositions may also be administered to individuals being treated by other means for such diseases, for example, individuals being treated with a chemotherapeutic (e.g., and having neutropenia, atrophy, cachexia, nephropathy, neuropathy) or an elective surgery.

Kits

[0267] A compound described herein described herein can be provided in a kit. The kit includes (a) a compound described herein, e.g., a composition that includes a compound described herein, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of a compound described herein for the methods described herein.

[0268] The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to methods for administering the compound.

[0269] In one embodiment, the informational material can include instructions to administer a compound described herein in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or

mode of administration (e.g., a dose, dosage form, or mode of administration described herein). In another embodiment, the informational material can include instructions to administer a compound described herein to a suitable subject, e.g., a human, e.g., a human having or at risk for a disorder described herein.

[0270] The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in printed matter, e.g., a printed text, drawing, and/or photograph, e.g., a label or printed sheet. However, the informational material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. In another embodiment, the informational material of the kit is contact information, e.g., a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about a compound described herein and/or its use in the methods described herein. Of course, the informational material can also be provided in any combination of formats.

[0271] In addition to a compound described herein, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer, a preservative, a flavoring agent (e.g., a bitter antagonist or a sweetener), a fragrance or other cosmetic ingredient, and/or a second agent for treating a condition or disorder described herein. Alternatively, the other ingredients can be included in the kit, but in different compositions or containers than a compound described herein. In such embodiments, the kit can include instructions for admixing a compound described herein and the other ingredients, or for using a compound described herein together with the other ingredients.

[0272] A compound described herein can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that a compound described herein be substantially pure and/or sterile. When a compound described herein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When a compound described herein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

[0273] The kit can include one or more containers for the composition containing a compound described herein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of a compound described herein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of a compound described herein. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

[0274] The kit optionally includes a device suitable for administration of the composition, e.g., a syringe, inhalant, pipette, forceps, measured spoon, dropper (e.g., eye drop-

per), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In a preferred embodiment, the device is a medical implant device, e.g., packaged for surgical insertion.

Genetic Information

[0275] SIRT1 genetic information can be obtained, e.g., by evaluating genetic material (e.g., DNA or RNA) from a subject (e.g., as described below). Genetic information refers to any indication about nucleic acid sequence content

at one or more nucleotides. Genetic information can include, for example, an indication about the presence or absence of a particular polymorphism, e.g., one or more nucleotide variations. Exemplary polymorphisms include a single nucleotide polymorphism (SNP), a restriction site or restriction fragment length, an insertion, an inversion, a deletion, a repeat (e.g., trinucleotide repeat, a retroviral repeat), and so forth.

[0276] Exemplary SIRT1 SNPs are listed in Table 2.

TABLE 2

Exemplary SIRT1 SNPs							
start	stop	dbSNP rs#	local loci	transID	avg.het	s.e.het	
69520160	69520160	rs730821				0	
69520607	69520607	rs3084650				0	
69530733	69530733	rs4746715				0	
69531621	69531621	rs4745944				0	
69535743	69535743	rs3758391	SIRT1:locus;		0.267438	0.153425	
69536360	69536360	rs3740051	SIRT1:locus;		0.424806	0.114325	
69536618	69536618	rs932658	SIRT1:locus;			0	
69536736	69536736	rs3740053	SIRT1:locus;			0	
69536742	69536742	rs2394443	SIRT1:locus;			0	
69539733	69539733	rs932657	SIRT1:intron;			0	
69540006	69540006	rs737477	SIRT1:intron;		0.118187	0.201473	
69540390	69540390	rs911738	SIRT1:intron;			0	
69540762	69540762	rs4351720	SIRT1:intron;			0	
69540970	69540970	rs2236318	SIRT1:intron;		0.222189	0.135429	
69541621	69541621	rs2236319	SIRT1:intron;		0.455538	0.102018	
69544136	69544136	rs768471	SIRT1:intron;		0	0.01	
69547213	69547213	rs1885472	SIRT1:intron;			0	
69549191	69549191	rs2894057	SIRT1:intron;			0	
69551326	69551326	rs4746717	SIRT1:intron;			0	
69557788	69557788	rs2224573	SIRT1:intron;			0	
69558999	69558999	rs2273773	SIRT1;	NM_012238;	0.430062	0.135492	
69559302	69559302	rs3818292	SIRT1:intron;		0.456782	0.10598	
69564725	69564725	rs1063111	SIRT1;	NM_012238;		0	
69564728	69564728	rs1063112	SIRT1;	NM_012238;		0	
69564741	69564741	rs1063113	SIRT1;	NM_012238;		0	
69564744	69564744	rs1063114	SIRT1;	NM_012238;		0	
69565400	69565400	rs3818291	SIRT1:intron;		0.179039	0.132983	
69566230	69566237	rs5785840	SIRT1:intron;			0	
69566318	69566318	rs2394444	SIRT1:intron;			0	
69567559	69567559	rs1467568	SIRT1:intron;			0	

TABLE 2-continued

Exemplary SIRT1 SNPs							
start	stop	dbSNP	rs#	local loci	transID	avg.het	s.e.het
69567728	69567728	rs1966188	SIRT1:intron;			0	
69568961	69568961	rs2394445	SIRT1;		NM_012238:UTR;	0	
69568962	69568962	rs2394446	SIRT1;		NM_012238:UTR;	0	
69569231	69569231	rs4746720	SIRT1;		NM_012238:UTR;	0	
69569461	69569461	rs752578	SIRT1;		NM_012238:UTR;	0	
69570479	69570479	rs2234975	SIRT1;		NM_012238:UTR;	0	
69570580	69570580	rs1022764	SIRT1:locus;			0	
69570983	69570983	rs1570290	SIRT1:locus;			0.0392	0.167405
69572334	69572334	rs2025162				0	
69573968	69573968	rs4141919	DKFZP564G092: locus;			0	
69574252	69574252	rs14819	DKFZP564G092: locus;			0	
69575032	69575032	rs14840	DKFZP564G092: locus;			0	

[0277] It is possible to digitally record or communicate genetic information in a variety of ways. Typical representations include one or more bits, or a text string. For example, a biallelic marker can be described using two bits. In one embodiment, the first bit indicates whether the first allele (e.g., the minor allele) is present, and the second bit indicates whether the other allele (e.g., the major allele) is present. For markers that are multi-allelic, e.g., where greater than two alleles are possible, additional bits can be used as well as other forms of encoding (e.g., binary, hexadecimal text, e.g., ASCII or Unicode, and so forth). In some embodiments, the genetic information describes a haplotype, e.g., a plurality of polymorphisms on the same chromosome. However, in many embodiments, the genetic information is unphased.

[0278] A decision about whether to administer a compound described herein can be made depending on the genetic information about SIRT1. For example, a method for administering a compound described herein can include evaluating nucleic acid from a subject to obtain genetic information about SIRT1 or another sirtuin, and administering a compound described herein.

Databases

[0279] The invention also features a database that associates information about or identifying one or more of the compounds described herein with a parameter about a patient, e.g., a patient being treated with a disorder herein. The parameter can be a general parameter, e.g., blood pressure, core body temperature, etc., or a parameter related to a specific disease or disorder, e.g., as described herein.

EXAMPLES

[0280] In all of the Examples below, compounds are referred to as they correspond to their designation in Table 1 (i.e., exemplified compounds).

Example 1

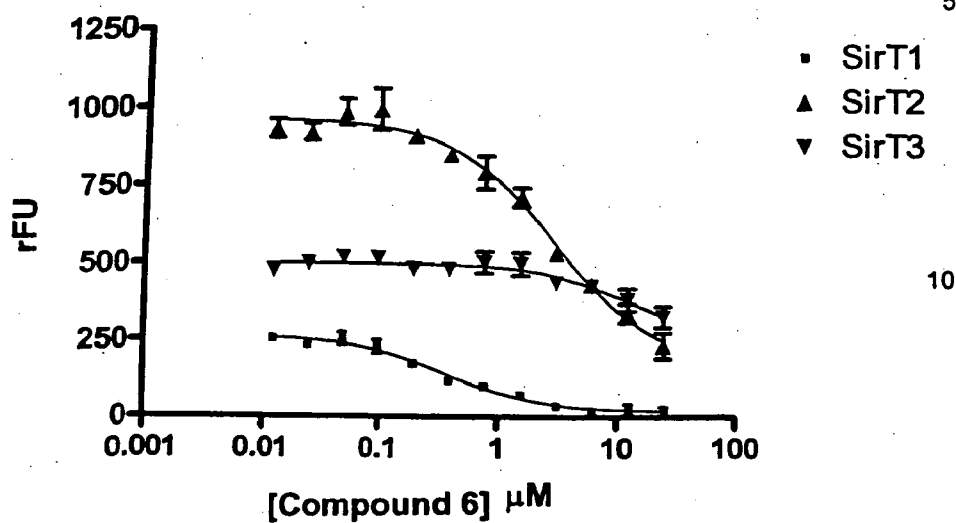
HeLa Apoptosis Assay

[0281] The following exemplary compounds were evaluated for their effect on a HeLa cell apoptosis assay using the Cell Death Detection ELISA plus kit from Roche Applied Science.

Compound	dose	average	SD
8	0	1.12	0.15
8	0.5	1.23	0.04
8	2.5	1.85	0.24
8	10	2.11	0.25
8	25	2.27	0.20
5	0	0.92	0.07
5	0.5	1.00	0.08
5	2.5	0.97	0.11
5	10	1.07	0.02
5	25	0.91	0.07
Resveratrol	0	0.73	0.08
Resveratrol	0.5	0.83	0.05
Resveratrol	2.5	0.84	0.02
Resveratrol	10	1.01	0.07
Resveratrol	25	0.56	0.08
DMSO	0	0.72	0.09
DMSO	0.5	0.79	0.12
DMSO	2.5	0.91	0.13
DMSO	10	0.76	0.09
DMSO	25	1.18	0.20

Example 2

Compound 6 inhibition of SirT enzymes with P53 Fluor de Lys Substrate



	SirT1	SirT2	SirT3
Sigmoidal dose-response (variable slope)			
Best-fit values			
BOTTOM	17.24	156.1	248.2
TOP	261.7	966.4	499.2
LOGEC50	-0.4290	0.4890	1.111
HILLSLOPE	-1.080	-0.9944	-1.146
EC50	0.3724	3.083	12.960



[0282] List of Reagents:

Name of Reagent	Supplied As	Source	Catalog Number	Storage
1 human SirT1	2.5 or 3.5 U/ul	Biomol	SE-239	-20 C.
2 Fluor de Lys Substrate	50 mM in DMSO	Biomol	KI-104	-20 C.
3 Fluor de Lys Developer	20× concentrate	Biomol	KI-105	-20 C.
4 NAD	solid	Sigma	N-1636	-20 C.
5 Nicotinamide	solid	Calbiochem	481907	RT
6 Trizma-HCl	solid	Sigma	T-5941	RT
7 Sodium Chloride	solid	Sigma	S-9888	RT
8 Magnesium Chloride	solid	Sigma	M-2393	RT
9 Potassium Chloride	solid	Sigma	P-3911	RT
10 Polyoxyethylene sorbitan monolaurate (Tween-20)	100%	Sigma	P-7949	RT
11 Fluor de Lys Deacetylated Standard	10 mM in DMSO	Biomol	KI-142	-20 C.

[0283] List of Equipment:

Tool Name	Tool Source	Catalog Number
1 Fluorescence Plate Reader Synergy HT	BIO-TEK	SIAFR
2 Matrix Impact2 16 Channel pipet	Apogent Discoveries	2069
3 37 C. Incubator	VWR	1540

[0284] List of Disposables:

Disposable	Source	Catalog Number
1 384 white low volume plates	Greiner/Bellco	4507-84075
2 Tips for matrix 16 chan pipet	Apogent Discoveries	7421
3 25 ml divided reagent reservoirs	Apogent Discoveries	8095
4 Plate Sealing Films	Apogent Discoveries	4418

[0285] Standard Reagent Formulations:

Prepared Reagent Name	Component Name	M.W.	Component Quantity (in water)	Final Component Concentration	Storage
1 Tris-HCl, pH 8.0	Trizma-HCl	157.6	157.6 g/L	1 M	RT
2 Sodium Chloride	HCl		to pH 8.0	pH 8.0	
3 Magnesium Chloride	NaCl	58.44	292 g/L	5 M	RT
4 Potassium Chloride	MgCl ₂	203.3	20.33 g/L	100 mM	RT
5 Polyoxyethylene sorbitan monolaurate	KCl	74.55	20.13 g/L	270 mM	RT
6 NAD	Tween-20		1 ml/10 ml	10%	RT
7 Nicotinamide	NAD	717	0.0717 g/ml	100 mM	-20 C.
8 Assay Buffer	Nicotinamide	122	0.0061 g/ml	50 mM	-20 C.
	Tris-HCl, pH 8.0		25 ml of 1 M stock/L	25 mM	4 C.
	NaCl		27.4 ml of 5 M stock/L	137 mM	
	KCl		10 ml of 270 mM stock/L	2.7 mM	
	MgCl ₂		10 ml of 100 mM stock/L	1 mM	
	Tween-20		5 ml of 10% stock/L	0.05%	
**Prepare working stocks below just before use			The following are prepared in assay buffer		
9 2x Substrates	Flour de Lys substrate		6 ul/ml	300 uM	ice
	NAD		20 ul of 100 mM stock/ml	2 mM	
10 Enzyme Mix	Biomol SirT1		**depends upon specific activity of lot.	0.125 U/ul (0.5 U/well)	ice
			Ex: 3.5 U/ul, 35.71 ul/ml		
11 Developer/stop reagent	20× developer concentrate		50 ul/ml	1× in assay buffer	ice
	nicotinamide		20 ul of 50 mM stock/ml	1 mM	

Example 3

[0286] In order to determine if the mammalian enzyme is inhibited by compound 8, 293T cells were transfected with a construct designed to express human SIRT1 fused to glutathione-S-transferase to allow for rapid purification from cell extracts. Following lysis cell extracts were incubated with glutathione-Sepharose beads followed by several washes in lysis buffer and a final wash in SIRT1 enzyme assay buffer. Beads with bound GST-SIRT1 were added to the Fleur-de-lys assay (Biomol) in the presence of a range of concentrations of compound 8. As can be seen in **FIG. 2a**, the EC_{50} value of compound 8 for mammalian SIRT1 is comparable to that obtained for the recombinant bacterially produced human enzyme.

[0287] As can be seen in **FIG. 2B**, compound 8 enters cells and increases p53 acetylation (at lysine 382) after etoposide treatment. In the experiment depicted in **FIG. 2B**, NCI-H460 cells were treated with 20 μ M etoposide (a DNA damaging agent) in the presence or absence of SIRT1 inhibitors, either compound 8 or nicotinamide and the amount of acetylated p53 (at lysine 382) was visualized by Western blot. Compound 8 is able to increase p53 acetylation significantly relative to DMSO alone and 1 μ M and 10 μ M is equally effective.

Example 4

[0288] Enantiomers of compound 8 were tested, where each enantiomer had a purity of greater than 90% enantiomeric excess, to determine if a single enantiomer was more potent than a mixture of enantiomers. NCI-H460 cells were treated for 6 hours with compounds 8(+) and 8(−) in the presence of 20 micromolar etoposide followed by lysis and immunoprecipitation of p53 using Ab-6 (Oncogene Science). Extracts were probed with an antibody that recognizes acetylated lysine 382 of p53 (Cell Signaling). **FIG. 3** demonstrates that there are active and inactive enantiomers of compound 8. Specifically the inactive enantiomer, compound 8(+), does not lead to increased acetylation of p53 in the presence of etoposide whereas compound 8(−) leads to a significant increase in acetylation and satbilization of p53 protein.

Example 5

[0289] In the results of the experiment below, which is depicted in **FIG. 4**, we show that a compound's ability to increase p53 acetylation correlates with its in vitro potency against SIRT1. A series of structurally similar compounds were added to cells at 1 μ M concentration. Only those compounds that inhibit SIRT1 with IC_{50} s below 1 μ M increased p53 acetylation, whereas compounds with IC_{50} s above 1 μ M did not.

Example 6

[0290] The experiment depicted in **FIG. 5** demonstrates that in a yeast silencing assay dependent on SIRT1 activity, the inactive enantiomer of compound 8, compound 8(+), has no effect on cell growth whereas the active enantiomer, 8(−), inhibits SIRT1 and allows for expression of URA3 which blocks growth in the presence of 5-fluorouracil. Strain SL8c (URA at the telomere) was used for yeast based assay to screen compounds. Cells were grown in −URA media to select de-silenced cells. The next day cells were diluted 1:20

into fresh YPD with 2% glucose then grow for 5 hrs. Cells were then diluted OD=0.01 in both SD and SD+0.1% 5FOA media. The compounds were then serially diluted into 10 μ l of SD or SD+0.1% 5FOA medium. 140 μ l of cells were pipetted into a 96 well plate and grown at 30 C for 18-24 hrs.

Example 7

[0291] Compound 8 inhibits the SIRT1 enzyme in additional cells. Cell lines U2OS and MCF7 cell lines were treated with compound 8 in the presence of 20 micromolar etoposide (TOPO) for 6 hours followed by lysis and immunoprecipitation with p53 Ab-6 conjugated to agarose beads. Samples were analyzed by SDS-PAGE and immunoblotted with an antibody that recognizes acetylated lysine 382 of p53. The results depicted in **FIG. 6** demonstrate that compound 8 is competent to inhibit SIRT1 in a variety of cell lines with similar effects on P53 acetylation.

Example 8

[0292] In order to assess whether the affects of compound 8 on p53 acetylation lead to changes in p53 function an experiment was performed to measure cell survival after DNA damage. NCI-H460 cells were damaged with varying concentrations of etoposide in the presence or absence of SIRT1 inhibitors. As depicted in **FIG. 7**, compound 8 by itself did not modulate p53 function significantly in this assay.

Example 9

[0293] Cells were plated at a density of 800 per well in 96 well cytostar plates in the presence of a range of etoposide concentrations and 1 micromolar compound 8. Thymidine incorporation was measured at 24 hours intervals. As depicted in **FIG. 9**, this experiment demonstrates that there is no synergy between etoposide and compound 8 on the growth characteristics of NCI-H460 cells under conditions in which compound was added concurrent to, prior to, and after treatment with etoposide.

Example 10

[0294] HEK293 cells were serum starved in the presence or absence of compound 8 for 24 hrs followed by lysis and immunoblotting analysis of p27 protein. As can be seen in **FIG. 9**, treatment of cells with compound 8 leads to abrogation of serum starvation-mediated upregulation of the cell cycle inhibitor p27. The proposed explanation for this result is that SIRT1-mediated deacetylation leads to inactivation of FOXO1-mediated transcription of p27 and the addition of compound 8 reverses this effect.

Example 11

[0295] Hela cells were transfected with GFP-hSIRT2 isoform 1 (green). At 36 hours post transfection 1 μ M of TSA and either DMSO or 50 μ M of compound 8 was added. The next morning cells were fixed, permeabilized, and stained for acetylated tubulin (red). In cells treated with DMSO there was very little acetylated tubulin in cells expressing SIRT2, in cells treated with compound 8 the tubulin is more highly acetylated indicating that the effect of SIRT2 was blocked.

[0296] It was also possible to observe the effect of the compounds using Western analysis. 293T cells were trans-

fectected with either eGFP (control) or with mouse SIRT2 Isoform 1 (mSIRT2). TSA was added to increase amount of acetylated tubulin and at the same time either DMSO or the compound listed below were added to 10 μ M.

[0297] Procedure Description:

[0298] Step Description

[0299] 1 Prepare amount of 2 \times Substrates necessary for the number of wells to be assayed.

[0300] 5 ul per well is needed

[0301] 2 Dispense 5 ul 2 \times substrates to test wells

[0302] 3 Dispense 1 ul of test compound to the test wells

[0303] Dispense 1 ul of compound solvent/diluent to the positive control wells

[0304] Dispense 1 ul of 1 mM nicotinamide to the 50% inhibition wells

[0305] Dispense 1 ul of 10 mM nicotinamide to the 100% inhibition wells

[0306] 4 Dispense 4 ul of assay buffer to negative control wells (no enzyme controls)

[0307] 5 Prepare amount of enzyme necessary for number of wells to assay. 4 ul enzyme mix needed per well

[0308] 6 Dispense 4 ul of enzyme mix to the test wells and positive control wells

[0309] 7 Cover and incubate at 37C for 45 minutes

[0310] 8 Less then 30 minutes before use, prepare amount of 1 \times developer/stop reagent for the number of wells being assayed

[0311] 9 Dispense 10 ul 1 \times developer/stop reagent to all wells

[0312] 10 Incubate at room temperature for at least 15 minutes

[0313] 11 Read in fluorescence plate reader, excitation=350-380 nm, emission=440-460

[0314] 12 Fluor de Lys in the substrate has an intrinsic fluorescence that needs to be subtracted as background before any calculations are to be done on the data. These values can be found in the negative control wells.

[0315] Appendix 1: Preparation of a Standard Curve Using Fluor de Lys Deacetylated Standard

[0316] 1 Determine the concentration range of deacetylated standard to use in conjunction with the above assay by making a 1 uM dilution of the standard. Mix 10 ul of the 1 uM dilution with 10 ul developer and read at the same wavelengths and sensitivity settings that the assay is read at. Use this estimate of AFU (arbitrary fluorescence units)/uM to determine the range of concentrations to test in the standard curve.

[0317] 2 Prepare, in assay buffer, a series of dilutions of the Fluor de Lys deacetylated standard that span the desired concentration range

[0318] 3 Pipet 10 ul assay buffer to the 'zero' wells

[0319] 4 Pipet 10 ul of the standard dilutions into wells

[0320] 5 Pipet 10 ul developer to the wells and incubate 15 minutes at RT

[0321] 6 Read plate at above wavelengths

[0322] 7 Plot fluorescence signal (y) versus concentration of the Fluor de Lys deacetylated standard (x) and determine the slope as AFU/uM

[0323] Protocol for Testing for Inhibitors of the Developer Reaction

[0324] 1 From the standard curve select concentration of deacetylated standard that gives a fluorescence signal equivalent to positive controls in assay (eg. 5 uM)

[0325] 2 Dispense 5 ul 2 \times deacetylated standard (eg. 10 uM)

[0326] 3 Dispense 1 ul compound, 4 ul assay buffer

[0327] 4 Dispense 10 ul developer

[0328] 5 Incubate at room temp 15 minutes (or equivalent time as in screen) and read at same settings as screen

Example 12

Synthesis of

2-chloro-5,6,7,8,9,10-hexahydrocyclohepta[b]indole-6

[0329] Preparation of methyl 3-bromo-2-oxocycloheptanecarboxylate: Methyl 2-oxo-1-cycloheptanecarboxylate (50 g, 294 mmole) was dissolved in carbon tetrachloride (200 mL) and chilled to 0° C. Bromine (46.8 g, 294 mmole) was added via addition funnel over ~30 minutes. The cooling bath was then removed and the reaction was allowed to warm to rt. The reaction was stirred under nitrogen at room temperature for 4 days. After 4 days the solution was poured into a separatory funnel containing water (1 L). The organic was washed with water and dried over sodium sulfate. Removal of the solvent in vacuo gave (72.4 g, 291 mmole, 99% crude yield). The material was carried on without further purification.

[0330] Preparation of methyl 2-chloro-5,6,7,8,9,10-hexahydrocyclohepta[b]indole-6-carboxylate: Methyl 3-bromo-2-oxocycloheptanecarboxylate (16.8 g, 67.4 mmol) and 4-chloroaniline (18.4 g, 98%, 2.13 eq, 143.6 mmol) were added to flask with thermometer, nitrogen inlet and mechanical stirring machine. As the temperature of the mixture passed 140° C. a relatively rapid exotherm and vigorous evolution of gas occurred. The reaction was cooled with water immediately. The reaction mixture was dissolved in DCM (200 mL). The material was transferred into a separatory funnel and washed with water (2 \times 50 mL), 3 HCl (3 \times 50 mL), water (2 \times 50 mL), brine, dried over Na₂SO₄, and the solvent was removed by vacuo. The crude residue was applied to a Biotage and eluted with 9/1 heptane/ethyl acetate to afford product 10 g (53%) as an off white solid, which was used for next reaction without further purification.

[0331] Preparation of 2-chloro-5,6,7,8,9,10-hexahydrocyclohepta[b]indole-6-carboxamide: Methyl 2-chloro-5,6,7,8,9,10-hexahydrocyclohepta[b]indole-6-carboxylate (10 g, 36 mmol) was dissolved in 7 N ammonia in methanol (350 mL) and transferred to Parr pressure reactor. The reaction vessel was purged briefly to displace any air with Nitrogen. The reaction was then heated to 90° C. for 48 h. The reaction was cooled to r.t. and the solvent removed in vacuo, the crude

residue was applied to a Biotage and eluted with grading (1/1 heptane/ethyl acetate to 0/1 heptane/ethyl acetate) to afford product as an off white foam, which was triturated with DCM to afford pure product 2 g (21%) as an off white solid.

[0332] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Other embodiments are in the claims.

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 85           90           95
Ala Ala Gly Glu Gly Asp Asn Gly Pro Gly Leu Gln Gly Pro Ser Arg
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Lys	Ser	Asn	Asp	Asp	Leu	Asp	Val	Ser	Glu	Ser	Lys	Gly	Cys	Met	Glu
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Gly Leu Glu Gln Glu Asp Leu Val Glu Ala His Gly Thr Phe Tyr Thr
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His Gly Cys Met Asp Arg Val Leu Cys Leu Asp Cys Gly Glu Gln Thr
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Met Ala Arg Pro Ser Ser Ser Met Ala Asp Phe Arg Lys Phe Phe Ala	35	40	45	
Lys Ala Lys His Ile Val Ile Ile Ser Gly Ala Gly Val Ser Ala Glu	50	55	60	
Ser Gly Val Pro Thr Phe Arg Gly Ala Gly Tyr Trp Arg Lys Trp	65	70	75	80
Gln Ala Gln Asp Leu Ala Thr Pro Leu Ala Phe Ala His Asn Pro Ser	85	90	95	
Arg Val Trp Glu Phe Tyr His Tyr Arg Arg Glu Val Met Gly Ser Lys	100	105	110	
Glu Pro Asn Ala Gly His Arg Ala Ile Ala Glu Cys Glu Thr Arg Leu	115	120	125	
Gly Lys Gln Gly Arg Arg Val Val Ile Thr Gln Asn Ile Asp Glu	130	135	140	
Leu His Arg Lys Ala Gly Thr Lys Asn Leu Leu Glu Ile His Gly Ser	145	150	155	160
Leu Phe Lys Thr Arg Cys Thr Ser Cys Gly Val Val Ala Glu Asn Tyr	165	170	175	
Lys Ser Pro Ile Cys Pro Ala Leu Ser Gly Lys Gly Ala Pro Glu Pro	180	185	190	
Gly Thr Gln Asp Ala Ser Ile Pro Val Glu Lys Leu Pro Arg Cys Glu	195	200	205	
Glu Ala Gly Cys Gly Gly Leu Leu Arg Pro His Val Val Trp Phe Gly	210	215	220	
Glu Asn Leu Asp Pro Ala Ile Leu Glu Glu Val Asp Arg Glu Leu Ala	225	230	235	240
His Cys Asp Leu Cys Leu Val Val Gly Thr Ser Ser Val Val Tyr Pro	245	250	255	
Ala Ala Met Phe Ala Pro Gln Val Ala Ala Arg Gly Val Pro Val Ala	260	265	270	

-continued

Glu Phe Asn Thr Glu Thr Thr Pro Ala Thr Asn Arg Phe Arg Phe His
275 280 285

Phe Gln Gly Pro Cys Gly Thr Thr Leu Pro Glu Ala Leu Ala Cys His
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Glu Asn Glu Thr Val Ser
305 310

<210> SEQ ID NO 6

<211> LENGTH: 355

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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Lys Cys Gly Leu Pro Glu Ile Phe Asp Pro Pro Glu Glu Leu Glu Arg
20 25 30

Lys Val Trp Glu Leu Ala Arg Leu Val Trp Gln Ser Ser Ser Val Val
35 40 45

Phe His Thr Gly Ala Gly Ile Ser Thr Ala Ser Gly Ile Pro Asp Phe
50 55 60

Arg Gly Pro His Gly Val Trp Thr Met Glu Glu Arg Gly Leu Ala Pro
65 70 75 80

Lys Phe Asp Thr Thr Phe Glu Ser Ala Arg Pro Thr Gln Thr His Met
85 90 95

Ala Leu Val Gln Leu Glu Arg Val Gly Leu Leu Arg Phe Leu Val Ser
100 105 110

Gln Asn Val Asp Gly Leu His Val Arg Ser Gly Phe Pro Arg Asp Lys
115 120 125

Leu Ala Glu Leu His Gly Asn Met Phe Val Glu Glu Cys Ala Lys Cys
130 135 140

Lys Thr Gln Tyr Val Arg Asp Thr Val Val Gly Thr Met Gly Leu Lys
145 150 155 160

Ala Thr Gly Arg Leu Cys Thr Val Ala Lys Ala Arg Gly Leu Arg Ala
165 170 175

Cys Arg Gly Glu Leu Arg Asp Thr Ile Leu Asp Trp Glu Asp Ser Leu
180 185 190

Pro Asp Arg Asp Leu Ala Leu Ala Asp Glu Ala Ser Arg Asn Ala Asp
195 200 205

Leu Ser Ile Thr Leu Gly Thr Ser Leu Gln Ile Arg Pro Ser Gly Asn
210 215 220

Leu Pro Leu Ala Thr Lys Arg Arg Gly Gly Arg Leu Val Ile Val Asn
225 230 235 240

Leu Gln Pro Thr Lys His Asp Arg His Ala Asp Leu Arg Ile His Gly
245 250 255

Tyr Val Asp Glu Val Met Thr Arg Leu Met Lys His Leu Gly Leu Glu
260 265 270

Ile Pro Ala Trp Asp Gly Pro Arg Val Leu Glu Arg Ala Leu Pro Pro
275 280 285

Leu Pro Arg Pro Pro Thr Pro Lys Leu Glu Pro Lys Glu Glu Ser Pro
290 295 300

Thr Arg Ile Asn Gly Ser Ile Pro Ala Gly Pro Lys Gln Glu Pro Cys
305 310 315 320

-continued

Ala Gln His Asn Gly Ser Glu Pro Ala Ser Pro Lys Arg Glu Arg Pro
 325 330 335

Thr Ser Pro Ala Pro His Arg Pro Pro Lys Arg Val Lys Ala Lys Ala
 340 345 350

Val Pro Ser
 355

<210> SEQ ID NO 7
 <211> LENGTH: 400
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Met Ala Ala Gly Gly Leu Ser Arg Ser Glu Arg Lys Ala Ala Glu Arg
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Val Arg Arg Leu Arg Glu Glu Gln Gln Arg Glu Arg Leu Arg Gln Val
 20 25 30

Ser Arg Ile Leu Arg Lys Ala Ala Ala Glu Arg Ser Ala Glu Glu Gly
 35 40 45

Arg Leu Leu Ala Glu Ser Ala Asp Leu Val Thr Glu Leu Gln Gly Arg
 50 55 60

Ser Arg Arg Arg Glu Gly Leu Lys Arg Arg Gln Glu Glu Val Cys Asp
 65 70 75 80

Asp Pro Glu Glu Leu Arg Gly Lys Val Arg Glu Leu Ala Ser Ala Val
 85 90 95

Arg Asn Ala Lys Tyr Leu Val Val Tyr Thr Gly Ala Gly Ile Ser Thr
 100 105 110

Ala Ala Ser Ile Pro Asp Tyr Arg Gly Pro Asn Gly Val Trp Thr Leu
 115 120 125

Leu Gln Lys Gly Arg Ser Val Ser Ala Ala Asp Leu Ser Glu Ala Glu
 130 135 140

Pro Thr Leu Thr His Met Ser Ile Thr Arg Leu His Glu Gln Lys Leu
 145 150 155 160

Val Gln His Val Val Ser Gln Asn Cys Asp Gly Leu His Leu Arg Ser
 165 170 175

Gly Leu Pro Arg Thr Ala Ile Ser Glu Leu His Gly Asn Met Tyr Ile
 180 185 190

Glu Val Cys Thr Ser Cys Val Pro Asn Arg Glu Tyr Val Arg Val Phe
 195 200 205

Asp Val Thr Glu Arg Thr Ala Leu His Arg His Gln Thr Gly Arg Thr
 210 215 220

Cys His Lys Cys Gly Thr Gln Leu Arg Asp Thr Ile Val His Phe Gly
 225 230 235 240

Glu Arg Gly Thr Leu Gly Gln Pro Leu Asn Trp Glu Ala Ala Thr Glu
 245 250 255

Ala Ala Ser Arg Ala Asp Thr Ile Leu Cys Leu Gly Ser Ser Leu Lys
 260 265 270

Val Leu Lys Lys Tyr Pro Arg Leu Trp Cys Met Thr Lys Pro Pro Ser
 275 280 285

Arg Arg Pro Lys Leu Tyr Ile Val Asn Leu Gln Trp Thr Pro Lys Asp
 290 295 300

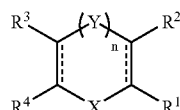
Asp Trp Ala Ala Leu Lys Leu His Gly Lys Cys Asp Asp Val Met Arg

-continued

305				310				315				320					
Leu	Leu	Met	Ala	Glu	Leu	Gly	Leu	Glu	Ile	Pro	Ala	Tyr	Ser	Arg	Trp		
				325				330				335					
Gln	Asp	Pro	Ile	Phe	Ser	Leu	Ala	Thr	Pro	Leu	Arg	Ala	Gly	Glu	Glu		
				340				345				350					
Gly	Ser	His	Ser	Arg	Lys	Ser	Leu	Cys	Arg	Ser	Arg	Glu	Glu	Ala	Pro		
				355				360				365					
Pro	Gly	Asp	Arg	Gly	Ala	Pro	Leu	Ser	Ser	Ala	Pro	Ile	Leu	Gly	Gly		
				370				375				380					
Trp	Phe	Gly	Arg	Gly	Cys	Thr	Lys	Arg	Thr	Lys	Arg	Lys	Lys	Val	Thr		
				385				390				395				400	

What is claimed is:

1. A method of treating or preventing a disorder, the method comprising administering to a subject an effective amount of a compound having a formula (I):



(I)

wherein,

R^1 and R^2 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkyl, C_5 - C_{10} heterocyclyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, C_6 - C_{10} aryl, or C_6 - C_{10} heteroaryl, each of which may be optionally substituted with 1-5 R^5 ; or R^1 is H, S-alkyl, or S-aryl, and R^2 is amidoalkyl wherein the nitrogen is substituted with alkyl, aryl, or arylalkyl, each of which is optionally further substituted with alkyl, halo, hydroxy, or alkoxy;

R^3 and R^4 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkyl, C_5 - C_{10} heterocyclyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, C_6 - C_{10} aryl, or C_6 - C_{10} heteroaryl, each of which are optionally substituted with 1-5 R^6 ;

each of R^5 and R^6 is, independently, halo, hydroxy, C_1 - C_{10} alkyl, C_1 - C_6 haloalkyl, C_1 - C_{10} alkoxy, C_1 - C_6 haloalkoxy, C_6 - C_{10} aryl, C_5 - C_{10} heteroaryl, C_7 - C_{12} aralkyl, C_7 - C_{12} heteroaralkyl, C_3 - C_8 heterocyclyl, C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, carboxy, carboxylate, cyano, nitro, amino, C_1 - C_6 alkyl amino, C_1 - C_6 dialkyl amino, mercapto, SO_3H , sulfate, $S(O)NH_2$, $S(O)_2NH_2$, phosphate, C_1 - C_4 alkylenedioxy, oxo, acyl, aminocarbonyl, C_1 - C_6 alkyl aminocarbonyl, C_1 - C_6 dialkyl aminocarbonyl, C_1 - C_{10} alkoxy carbonyl, C_1 - C_{10} thioalkoxy carbonyl, hydrazinocarbonyl, C_1 - C_6 alkyl hydrazinocarbonyl, C_1 - C_6 dialkyl hydrazinocarbonyl, hydroxyaminocarbonyl, alkoxyaminocarbonyl; or one of R^5 or R^6 and R^7 form a cyclic moiety containing 4-6 carbons, 1-3 nitrogens, 0-2 oxygens and 0-2 sulfurs, which are optionally substituted with oxo or C_1 - C_6 alkyl;

X is NR^7 , O, or S; Y is NR^7 , O or S;

- - - represent optional double bonds;

each of R^7 and $R^{7'}$ is, independently, hydrogen, C_1 - C_6 alkyl, C_7 - C_{12} arylalkyl, C_7 - C_{12} heteroarylalkyl; or R^7 and one of R^5 or R^6 form a cyclic moiety containing 4-6 carbons, 1-3 nitrogens, 0-2 oxygens and 0-2 sulfurs, which are optionally substituted with oxo or C_1 - C_6 alkyl; and

n is 0 or 1.

2. The method of claim 1, wherein R^1 and R^2 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkyl, C_5 - C_{10} heterocyclyl, C_5 - C_{10} cycloalkenyl, C_5 - C_{10} heterocycloalkenyl, C_6 - C_{10} aryl, or C_6 - C_{10} heteroaryl, each of which may be optionally substituted with 1-5 R^5 .

3. The method of claim 1, wherein R^1 and R^2 , together with the carbons to which they are attached, form C_5 - C_{10} cycloalkenyl.

4. The method of claim 3, wherein R^1 and R^2 are substituted with R^5 .

5. The method of claim 4, wherein R^5 is, C_1 - C_6 alkyl substituted with a substituent or amino carbonyl, substituted with a substituent.

6. The method of claim 5, wherein the substituent is an amino substituent, or aminocarbonyl.

7. The method of claim 1, wherein R^3 and R^4 , together with the carbons to which they are attached, form C_6 - C_{10} aryl.

8. The method of claim 5, wherein R^3 and R^4 are substituted with R^6 .

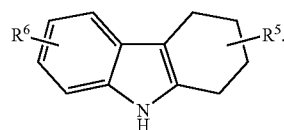
9. The method of claim 6 wherein R^6 is halo or C_1 - C_6 alkyl.

10. The method of claim 1, wherein n is 0.

11. The method of claim 1 wherein X is NR^7 .

12. The method of claim 1 wherein n is 0 and X is NR^7 .

13. The method of claim 1, having the formula (X) below:

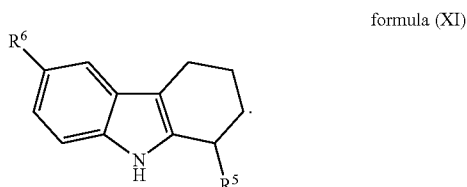


formula (X)

14. The method of claim 13, wherein R^6 is halo or C_1 - C_6 alkyl.

15. The method of claim 13, wherein R^5 is aminocarbonyl.

16. The method of claim 13, having the formula (XI) below:



17. The method of claim 16, wherein R^6 is halo or alkyl.

18. The method of claim 16, wherein R^5 is aminocarbonyl.

19. The method of claim 16, wherein R^6 is halo or alkyl and wherein R^5 is aminocarbonyl.

20. The method of claim 13 wherein the compound is 6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide.

21. The method of claim 20 wherein the compound comprises greater than a 60% enantiomeric excess of the enantiomer having an optical rotation of -14.1 ($c=0.33$ DCM).

22. The method of claim 21, wherein the compound comprises greater than a 90% enantiomeric excess of the enantiomer having an optical rotation of -14.1 ($c=0.33$ DCM).

23. The compound of claim 1, wherein the compound preferentially inhibits SirT1 relative to a non-SirT1 sirtuin.

24. The compound of claim 1, wherein the compound has at least a 5 fold preference for SirT1.

25. The compound of claim 1, wherein the compound has a K_i for SirT1 of less than about $1 \mu M$.

26. The method of claim 1 wherein the disorder is a neoplastic disorder.

27. The method of claim 26, wherein the neoplastic disorder is a cancer.

28. The method of claim 1 wherein the disorder is a neurodegenerative disorder.

29. The method of claim 28, wherein the neurodegenerative disorder is Alzheimer's Disease or Parkinson's disease.

30. The method of claim 1, wherein the disorder is a fat-cell related disorder.

31. The method of claim 30, wherein administration of the compound enhances adipogenesis in the subject.

32. The method of claim 1, wherein the disorder is diabetes.

33. The method of claim 32, wherein the subject has type I diabetes.

34. The method of claim 32, wherein the subject has type II diabetes.

35. The method of claim 1, wherein the subject is identified as being at risk of diabetes.

36. The method of claim 35, wherein the patient has been identified as being at risk of diabetes by having impaired glucose tolerance.

37. The method of claim 35, wherein the patient has been identified as being at risk of diabetes by having fasting hyperglycemia.

38. The method of claim 1, wherein the disorder is metabolic syndrome.

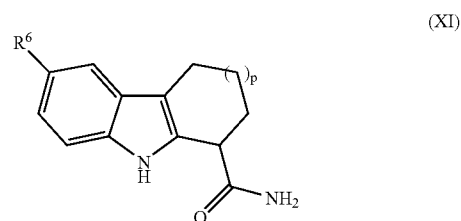
39. The method of claim 38, wherein the subject has atherogenic dyslipidemia.

40. The method of claim 38, wherein the subject is obese.

41. The method of claim 38, wherein the subject has insulin resistance or impaired glucose intolerance.

42. The method of claim 38, wherein the subject has hypertension.

43. A compound having formula (XI),



wherein R^6 is halo or C_1 - C_6 alkyl, and

p is 0, 1, or 2; and

wherein, the compound has the same relative stereochemistry as the stereoisomer of 6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide having an optical rotation of -14.1 ($c=0.33$ DCM).

44. The compound of claim 43, wherein R^6 is chloro or methyl.

45. The compound of claim 43, wherein p is 1.

46. The stereoisomer of 6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylic acid amide having an optical rotation of -14.1 ($c=0.33$ DCM).

* * * * *