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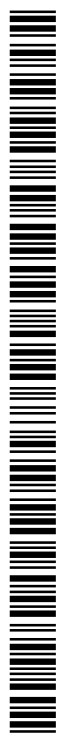
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(54) Title: COMPOUNDS, COMPOSITIONS, AND METHODS FOR REDUCING OXIDATIVE STRESS IN CARDIOMYOCYTES

(57) Abstract: The invention provides compounds, compositions, and methods for reducing oxidative stress in cardiomyocytes. In certain embodiments, the compound or composition comprises a selective class IIa histone deacetylase (HDAC) inhibitor, or a salt or solvate thereof.



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TITLE OF THE INVENTION

Compounds, Compositions, and Methods For Reducing Oxidative Stress in Cardiomyocytes

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/398,254, filed September 22, 2016, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

10 Oxidative stress has been shown to be involved in the development of many disease states, including cancer, neurodegenerative disease, transplantation, end stage renal disease, and atherosclerosis/heart failure. Oxidative stress injury occurs when there is an increased production of oxidizing species simultaneously with a reduction in antioxidant defenses, resulting in the manifestation of reactive oxygen species (ROS), which regulate signaling
15 molecules and transcription factors involved in hypertrophy and cell death. ROS can be produced by various cellular various sources, such as mitochondrial leakage and NAD(P)H oxidases, and proceed to oxidize signaling molecules and transcription factors. Reperfusion of a previously ischemic tissue is a prominent disease pathway in the development of a large amount of ROS. This overwhelms the cellular defense system and subsequently damages
20 normal cellular functions that can ultimately lead to death. In particular, hydrogen peroxide (H₂O₂), the most abundant form of the ROS produced during ischemia/reperfusion, plays an important role by inducing pro-inflammatory cytokine release and apoptosis, which further potentiate tissue damage.

 Oxidative stress plays a major role in cardiac dysfunction leading to a variety of
25 ailments including heart failure, thereby resulting in the need of major surgery. Cardiac hypertrophy, defined by the enlargement of ventricular mass, is initially adaptive against hemodynamic overloads, such as high blood pressure. However, the long-term presence of hypertrophy often leads to heart failure, possibly because of increased cell death.

 There are natural cellular processes that regulate the levels of ROS in the body. One
30 such system is controlled by the transcription factor Nuclear Factor (erythroid-derived 2)-Like 2, also known as NFE2L2 or NRF2. NRF2 is a basic leucine zipper protein that regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation. Under normal or unstressed conditions, NRF2 stays in the cytoplasm and is degraded quickly by a cluster of proteins. Under oxidative stress, NRF2

is not degraded, but instead travels to the nucleus, where it binds to a DNA promoter and initiates transcription of anti-oxidative genes and their proteins, which reduce levels of ROS in cells and tissues. As a natural regulator of ROS, NRF2 activation could be beneficial for treating cardiac hypertrophy and other cardiac indications, such as but not limited to post-myocardial infarction remodeling of the heart and fibrosis, as well as promoting regeneration.

There is thus a need in the art to identify novel compositions and treatments that can be used to treat cardiac conditions that are associated with oxidative stress, such as but not limited to cardiac hypertrophy, post-myocardial infarction remodeling of the heart, and fibrosis. The present invention addresses and meets these needs.

BRIEF SUMMARY OF THE INVENTION

The invention provides a method of reducing oxidative stress in a cardiomyocyte. The invention further provides a method of promoting cardiac tissue regeneration, or treating or preventing post-myocardial infarction remodeling of the heart or fibrosis, in a subject.

In certain embodiments, the method comprises contacting a cardiomyocyte with a selective class IIa histone deacetylase (HDAC) inhibitor, or a salt or solvate thereof. In other embodiments, the method comprises administering to the subject a therapeutically effective amount of a selective class IIa histone deacetylase (HDAC) inhibitor, or a salt or solvate thereof, to the subject.

In other embodiments, the inhibitor has an inhibitory selectivity for one or more class IIa HDACs over one or more non-class IIa HDACs of about 3, about 10, about 30, about 100, about 300, about 1,000, about 3,000, about 10,000, or greater than about 10,000. In yet other embodiments, the inhibitor is TMP195 (N-(2-methyl-2-(2-phenyloxazol-4-yl)propyl)-3-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzamide), or a salt, solvate, isomer, derivative, and/or analogue thereof. In yet other embodiments, the inhibitor lacks a thiol (-SH) group and/or a disulfide (-S-S-) group. In yet other embodiments, the inhibitor stimulates NRF2 activity in the cardiomyocyte. In yet other embodiments, the inhibitor stimulates NRF2 activity in a cardiomyocyte of the subject. In yet other embodiments, the cardiomyocyte *in vivo* in a subject. In yet other embodiments, the contacting prevents, ameliorates, or treats post-myocardial infarction remodeling of the heart or fibrosis in the subject. In yet other embodiments, cardiac tissue is at least partially regenerated in the subject. In yet other embodiments, the inhibitor is administered to the subject by at least one route selected from the group consisting of nasal, inhalational, topical, oral, buccal, rectal, pleural, peritoneal, vaginal, intramuscular, subcutaneous, transdermal, epidural, intratracheal, otic, intraocular,

intrathecal and intravenous. In yet other embodiments, the inhibitor is formulated in a pharmaceutically acceptable composition further comprising one or more pharmaceutically acceptable carriers. In yet other embodiments, the subject is further administered at least one additional agent for promoting cardiac tissue regeneration, or treating or preventing post-myocardial infarction remodeling of the heart or fibrosis. In yet other embodiments, the subject is a mammal. In yet other embodiments, the mammal is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of specific embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, specific embodiments are shown in the drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIG. 1 illustrates the chemical structure of TMP195, also known as N-(2-methyl-2-(2-phenyloxazol-4-yl)propyl)-3-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzamide.

FIG. 2 illustrates certain HDAC classes. TMP195 was found to inhibit only class IIa HDACs.

FIG. 3 illustrates certain protein domains for a class IIa HDAC. Class IIa HDACs have no measurable histone deacetylase activity.

FIG. 4 illustrates the finding that the HDAC domain of class IIa HDACs acts as a redox sensor, at least in part due to the depicted disulfide bond.

FIG. 5 comprises a set of graphs illustrating that TMP195 is a highly selective inhibitor of class IIa HDACs.

FIG. 6 illustrates the finding that TMP195 stimulates NRF2 target gene expression in cultured cardiac myocytes. Neonatal rat ventricular myocytes (NRVMs) were treated with 3 μ M TMP195 or DMSO vehicle for 48 hours. RNA was harvested and analyzed by RNA-seq. Bioinformatic analysis revealed that TMP195 altered expression (red – upregulated; green – downregulated) of about 1000 genes. Among the most highly upregulated genes in TMP195-treated cells were those regulated by the NRF2 gene.

FIG. 7 illustrates a non-limiting NRF2-KEAP-1 antioxidant pathway.

FIG. 8 comprises a set of graphs illustrating that a class IIa HDAC inhibitor stimulates NRF2 target gene expression in cardiomyocytes. Neonatal rat ventricular myocytes (NRVMs) were treated with DMSO vehicle, TMP195, or the positive control, AI-1 (4-Chloro-1,2-dihydro-1-methyl-2-oxo-3-quinolinecarboxylic acid ethyl ester, also known as

Ethyl 4-chloro-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate; AI-1 promotes NRF2 activation via the covalent modification of Kelch-like ECH-associated protein 1 (Keap1), a negative regulator of NRF2.). RNA was harvested and analyzed by qPCR. The NRF2 target genes Heme Oxygenase-1 and Sulfiredoxin-1 were up-regulated by both compounds.

5 FIG. 9 comprises a set of graphs illustrating the finding that TMP195 is a specific activator of NRF2 in cardiomyocytes. Neonatal rat cardiac fibroblasts or HEK293 cells were treated with DMSO vehicle, TMP195, or the positive control, AI-1. RNA was harvested and analyzed by qPCR. TMP failed to induce the NRF2 target gene Heme Oxygenase-1 in fibroblasts. The data suggests the TMP195 selectively activates NRF2 in cardiomyocytes.

10 FIG. 10 is a non-limiting scheme illustrating that class IIa HDAC catalytic activity controls cardiomyocyte antioxidant gene expression.

 FIG. 11A comprises gel images obtained by treating NRVMs with vehicle control (-), TMP195, or AI-1 for the indicated times. Both TMP195 and AI-1 treatment led to increased NRF2 protein expression, albeit with distinct kinetics.

15 FIG. 11B comprises gel images illustrating the finding that induction of NRF2 protein by TMP195 correlated with reduced expression of its negative regulator, KEAP1. For these studies, NRVMs were treated with DMSO (0.1% final concentration), 3 μ M TMP195 or 10 μ M AI-1, which served as a positive control. Total cell lysates were harvested. Protein levels of NRF2 and KEAP1 were detected by immunoblotting.

20

DETAILED DESCRIPTION OF THE INVENTION

 This invention provides compounds, compositions, and methods for stimulating NRF2 activity in a cardiomyocyte. In certain embodiments, selective class IIa histone deacetylase inhibitors are useful to stimulate NRF2 activity in a cardiomyocyte. In other
25 embodiments, the inhibitor useful within the invention has an inhibitory selectivity for one or more class IIa HDACs over one or more non-class IIa HDACs of at least about 3, about 10, about 30, about 100, about 300, about 1,000, about 3,000, about 10,000, or greater than about 10,000. In yet other embodiments, the inhibitor is TMP195, or a salt, solvate, isomer, derivative, and/or analogue thereof.

30 Non-limiting examples of class IIa HDACs include HDAC4, HDAC5, HDAC7, and HDAC9. Non-limiting examples of non-class IIa HDACs include: class I (HDAC1, HDAC2, HDAC3, HDAC8), class IIb (HDAC6, HDAC10), class III (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7), and class IV (HDAC11).

NRF2 protects cells and multiple tissues by coordinately upregulating ARE-related detoxification and antioxidant genes and molecules required for the defense system. NRF2-activation suppresses oxidative stress and inflammation, and has been shown to be neuroprotective. Accordingly, therapeutic strategies that increase NRF2 biological activity or expression can be used to treat or prevent diseases, disorders, or conditions related to oxidative stress, including inflammatory disorders, and neurodegenerative disorders.

In certain aspects, the disease, disorder, or condition is related to oxidative stress, for example, a pulmonary inflammatory condition, pulmonary fibrosis, asthma, chronic obstructive pulmonary disease (COPD), emphysema, sepsis, septic shock, meningitis, encephalitis, hemorrhage, ischemic injury, cerebral ischemia, heart ischemia, a cognitive deficit, and a neurodegenerative disorder.

In certain embodiments, the present invention provides compounds and methods for treating or preventing a disease, disorder or condition associated with an NRF2-regulated pathway, including those associated with an autoimmune disease, comorbidity associated with diabetes, such as retinopathy and nephropathy, bone marrow transplant for leukemia and related cancers, bone marrow deficiencies, inborn errors of metabolism, and other immune disorders, oxidative stress, respiratory infection, ischemia, neurodegenerative disorders, radiation injury, chemotherapy injury, neutropenia caused by chemotherapy, autoimmunity, and congenital neutropenic disorders, and for restoring a corticosteroid responsiveness.

In certain embodiments, the present invention provides a method for treating a disease, disorder, or condition associated with oxidative stress. Mammals having reduced levels of NRF2 are particularly susceptible to tissue damage associated with oxidative stress, including pulmonary inflammatory conditions, sepsis, and neuronal cell death associated with ischemic injury. NRF2 provides protection against oxidative stress and reduces neuronal cell death associated with ischemic injury. Accordingly, agents that increase the expression or biological activity of NRF2 are useful for preventing and treating diseases or disorders associated with increased levels of oxidative stress or reduced levels of antioxidants, including pulmonary inflammatory conditions, pulmonary fibrosis, asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), emphysema, sepsis, septic shock, ischemic injury, including cerebral ischemia and heart ischemia, cognitive deficits, and neurodegenerative disorders.

In certain embodiments, the invention provides a method of reducing oxidative stress in a cardiomyocyte. In other embodiments, the method comprises contacting a cardiomyocyte with a selective class IIa HDAC inhibitor, such as but not limited to TMP195,

or a salt or solvate thereof. In yet other embodiments, a selective class IIa HDAC inhibitor can be used to activate NRF2 and reduce oxidative stress in a cardiomyocyte. In yet other embodiments, such activation can be used to block post-myocardial infarction remodeling of the heart and fibrosis, as well as promote regeneration.

5 In certain embodiments, the invention provides a method of activating NRF2 in a cardiomyocyte. In other embodiments, the method comprises contacting a cardiomyocyte with a selective class IIa HDAC inhibitor, such as but not limited to TMP195, or a salt or solvate thereof. In yet other embodiments, a selective class II HDAC inhibitor upregulates expression of certain NRF2 target gene expression, such as but not limited to heme
10 oxygenase-1 and sulfiredoxin-1, both of which are endogenous antioxidants.

 In certain embodiments, the compound is formulated in a pharmaceutically acceptable composition further comprising one or more pharmaceutically acceptable carriers.

 In certain embodiments, the subject is further administered at least one additional agent for treating, ameliorating, and/or preventing any of the diseases or disorders
15 contemplated herein.

 In certain embodiments, the subject is a mammal. In other embodiments, the mammal is a human.

 In certain embodiments, the compound is administered to the subject by at least one route selected from the group consisting of nasal, inhalational, topical, oral, buccal, rectal,
20 pleural, peritoneal, vaginal, intramuscular, subcutaneous, transdermal, epidural, intratracheal, otic, intraocular, intrathecal and intravenous.

 The invention further includes a kit comprising at least one compound contemplated in the invention, such as a selective class IIa HDAC inhibitor, an applicator, and an instructional material for use thereof.

25 In certain embodiments, the instructional material included in the kit comprises instructions for treating, ameliorating, and/or preventing any of the diseases or disorders contemplated herein. In other embodiments, the kit further comprises at least one additional agent for treating, ameliorating, and/or preventing any of the diseases or disorders contemplated herein.

30

Definitions

 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein

can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

5 As used herein, the articles “a” and “an” are used to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

 As used herein, “about,” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more
10 preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

 As used herein, the term “AI-1” refers to 4-Chloro-1,2-dihydro-1-methyl-2-oxo-3-quinolinecarboxylic acid ethyl ester, also known as Ethyl 4-chloro-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate.

15 A disease or disorder is “alleviated” if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

 In one aspect, the terms “co-administered” and “co-administration” as relating to a subject refer to administering to the subject a compound of the invention, or a derivative,
20 solvate, salt or prodrug salt thereof, along with a compound that may also treat the disorders or diseases contemplated within the invention. In one embodiment, the co-administered compounds are administered separately, or in any kind of combination as part of a single therapeutic approach. The co-administered compound may be formulated in any kind of combinations as mixtures of solids and liquids under a variety of solid, gel, and liquid
25 formulations, and as a solution.

 As used herein, the term “composition” or “pharmaceutical composition” refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in
30 the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, nasal, pulmonary and topical administration.

 A “disease” as used herein is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

A “disorder” as used herein in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

5 As used herein, the terms “effective amount,” “pharmaceutically effective amount” and “therapeutically effective amount” refer to a nontoxic but sufficient amount of an agent to provide the desired biological result. That result may be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of
10 ordinary skill in the art using routine experimentation.

 “Instructional material,” as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of the composition and/or compound of the invention in a kit. The instructional material of the kit may, for example, be affixed to a container that contains the compound
15 and/or composition of the invention or be shipped together with a container that contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively. Delivery of the instructional material may be, for example, by physical delivery of the publication or other medium of expression
20 communicating the usefulness of the kit, or may alternatively be achieved by electronic transmission, for example by means of a computer, such as by electronic mail, or download from a website.

 The terms “patient,” “subject” or “individual” are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods
25 described herein. In a non-limiting embodiment, the patient, subject or individual is a human.

 As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any
30 of the components of the composition in which it is contained.

 As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the

invention within or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As used herein, “pharmaceutically acceptable carrier” also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the invention, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions.

The “pharmaceutically acceptable carrier” may further include a pharmaceutically acceptable salt, prodrug, solvate or derivative of the compound useful within the invention. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington’s Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As used herein, the language “pharmaceutically acceptable salt” refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids, organic acids, solvates, hydrates, or clathrates thereof.

The term “prevent,” “preventing” or “prevention,” as used herein, means avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences.

As used herein, by “oxidative stress” is meant cellular damage or a molecular alteration in response to a reactive oxygen species. Oxidative Stress describes the level of oxidative damage caused by reactive oxygen species (ROS) in a cell, tissue, or organ. ROS (*e.g.*, free radicals, reactive anions) are generated in endogenous metabolic reactions.

- 5 Exogenous sources of reactive oxygen species include exposure to cigarette smoke and environmental pollutants. Reactions between free radicals and cellular components result in the alteration of macromolecules, such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Oxidative stress results when the formation of free radicals exceeds antioxidant activity. By “disease or disorder related to oxidative stress” is meant any
- 10 pathology characterized by an increase in oxidative stress. Oxidative stress is implicated in a variety of disease states, including Alzheimer's disease, Parkinson's disease, inflammatory diseases, neurodegenerative diseases, heart disease, HIV disease, chronic fatigue syndrome, hepatitis, cancer, autoimmune diseases, and aging. Methods of treating a disease, disorder, or condition associated with oxidative stress are disclosed in International PCT Patent
- 15 Application Publication No. WO2007/005879, which is incorporated herein by reference in its entirety.

A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

- As used herein, the term “TMP195” refers to N-(2-methyl-2-(2-phenyloxazol-4-yl)propyl)-3-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzamide, or a salt or solvate thereof.
- 20

- As used herein, the term “treatment” or “treating” is defined as the application or administration of a therapeutic agent, *i.e.*, a compound of the invention (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a condition contemplated herein, a symptom of a condition
- 25 contemplated herein or the potential to develop a condition contemplated herein, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect a condition contemplated herein, the symptoms of a condition contemplated herein or the potential to develop a condition contemplated herein. Such treatments may be specifically
- 30 tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

By the term “specifically bind” or “specifically binds,” as used herein, is meant that a first molecule preferentially binds to a second molecule (*e.g.*, a particular receptor or enzyme), but does not necessarily bind only to that second molecule.

Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.1, 5.3, 5.5, and 6. This applies regardless of the breadth of the range.

Combination Therapies

In certain embodiments, the compounds contemplated within the invention are useful within the methods of the invention in combination with at least one additional agent useful for regulating ROS levels in a cardiomyocyte. This additional compound may comprise compounds identified herein or compounds, *e.g.*, commercially available compounds, known to treat, prevent or reduce the symptoms of any disease or disorder contemplated herein.

A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid- $E_{\max}$ equation (Holford & Scheiner, 1981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22:27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Administration/Dosage/Formulations

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after the onset of a disease or disorder contemplated in the invention. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder contemplated in the invention. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect
5 may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound to treat a disease or disorder contemplated in the invention. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies
10 of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

15 Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The therapeutically effective amount or dose of a compound of the present invention
20 depends on the age, sex and weight of the patient, the current medical condition of the patient and the progression of a disease or disorder contemplated in the invention.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of
25 the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

A suitable dose of a compound of the present invention may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for
30 example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

Compounds of the invention for administration may be in the range of from about 1 µg to about 10,000 mg, about 20 µg to about 9,500 mg, about 40 µg to about 9,000 mg, about 75 µg to about 8,500 mg, about 150 µg to about 7,500 mg, about 200 µg to about 7,000 mg, about 3050 µg to about 6,000 mg, about 500 µg to about 5,000 mg, about 750 µg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments there between.

10 In some embodiments, the dose of a compound of the invention is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second
15 compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about
20 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In one embodiment, the compositions of the invention are administered to the patient in dosages that range from one to five times per day or more. In another embodiment, the
25 compositions of the invention are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention varies from individual to individual depending on many factors including, but not limited to, age, disease
30 or disorder to be treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physical taking all other factors about the patient into account.

It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on

5 Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on.

In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the inhibitor of the invention is optionally given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday optionally
10 varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday includes from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%,
15 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced, as a function of the disease or disorder, to a level at which the improved disease is retained. In one embodiment, patients require intermittent treatment on a long-term
20 basis upon any recurrence of symptoms and/or infection.

The compounds for use in the method of the invention may be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosage for patients undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in
25 association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Toxicity and therapeutic efficacy of such therapeutic regimens are optionally
30 determined in cell cultures or experimental animals, including, but not limited to, the determination of the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD₅₀ and ED₅₀. The data obtained from cell culture assays and animal studies are optionally used in

formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The dosage optionally varies within this range depending upon the dosage form employed and the route of administration utilized.

5 In one embodiment, the compositions of the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier.

10 The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many
15 cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition.

20 In one embodiment, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of a disease or disorder contemplated in the invention.

25 Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for any suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, analgesic agents.

30 Routes of administration of any of the compositions of the invention include nasal, inhalational, topical, oral, buccal, rectal, pleural, peritoneal, vaginal, intramuscular, subcutaneous, transdermal, epidural, intratracheal, otic, intraocular, intrathecal and intravenous route administration.

Suitable compositions and dosage forms include, for example, dispersions, suspensions, solutions, syrups, granules, beads, powders, pellets, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

Oral Administration

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients which are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and lubricating agents such as magnesium stearate. The tablets may be uncoated or they may be coated by known techniques for elegance or to delay the release of the active ingredients. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert diluent.

For oral administration, the compounds of the invention may be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, polyvinylpyrrolidone, hydroxypropylcellulose or hydroxypropylmethylcellulose); fillers (*e.g.*, cornstarch, lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrates (*e.g.*, sodium starch glycollate); or wetting agents (*e.g.*, sodium lauryl sulphate). If desired, the tablets may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (*e.g.*, OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400). Liquid preparation for oral administration may be in the form of solutions, syrups or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.*, methyl or propyl p-hydroxy benzoates or sorbic acid).

Granulating techniques are well known in the pharmaceutical art for modifying starting powders or other particulate materials of an active ingredient. The powders are typically mixed with a binder material into larger permanent free-flowing agglomerates or granules referred to as a “granulation.” For example, solvent-using “wet” granulation processes are generally characterized in that the powders are combined with a binder material and moistened with water or an organic solvent under conditions resulting in the formation of a wet granulated mass from which the solvent must then be evaporated.

Melt granulation generally consists in the use of materials that are solid or semi-solid at room temperature (*i.e.* having a relatively low softening or melting point range) to promote granulation of powdered or other materials, essentially in the absence of added water or other liquid solvents. The low melting solids, when heated to a temperature in the melting point range, liquefy to act as a binder or granulating medium. The liquefied solid spreads itself over the surface of powdered materials with which it is contacted, and on cooling, forms a solid granulated mass in which the initial materials are bound together. The resulting melt granulation may then be provided to a tablet press or be encapsulated for preparing the oral dosage form. Melt granulation improves the dissolution rate and bioavailability of an active (*i.e.* drug) by forming a solid dispersion or solid solution.

U.S. Patent No. 5,169,645 discloses directly compressible wax-containing granules having improved flow properties. The granules are obtained when waxes are admixed in the melt with certain flow improving additives, followed by cooling and granulation of the admixture. In certain embodiments, only the wax itself melts in the melt combination of the wax(es) and additives(s), and in other cases both the wax(es) and the additives(s) will melt.

The present invention also includes a multi-layer tablet comprising a layer providing for the delayed release of one or more compounds of the invention, and a further layer providing for the immediate release of a medication for treatment of a brain-related disease or disorder. Using a wax/pH-sensitive polymer mix, a gastric insoluble composition may be obtained in which the active ingredient is entrapped, ensuring its delayed release.

Parenteral Administration

For parenteral administration, the compounds of the invention may be formulated for injection or infusion, for example, intravenous, intramuscular or subcutaneous injection or infusion, or for administration in a bolus dose and/or continuous infusion. Suspensions, solutions or emulsions in an oily or aqueous vehicle, optionally containing other formulatory agents such as suspending, stabilizing and/or dispersing agents may be used.

Additional Administration Forms

Additional dosage forms of this invention include dosage forms as described in U.S. Patents Nos. 6,340,475; 6,488,962; 6,451,808; 5,972,389; 5,582,837; and 5,007,790.

5 Additional dosage forms of this invention also include dosage forms as described in U.S. Patent Applications Nos. 20030147952; 20030104062; 20030104053; 20030044466; 20030039688; and 20020051820. Additional dosage forms of this invention also include dosage forms as described in PCT Applications Nos. WO 03/35041; WO 03/35040; WO 03/35029; WO 03/35177; WO 03/35039; WO 02/96404; WO 02/32416; WO 01/97783; WO 10 01/56544; WO 01/32217; WO 98/55107; WO 98/11879; WO 97/47285; WO 93/18755; and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

In one embodiment, the formulations of the present invention may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, 15 delayed release and pulsatile release formulations.

The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over 20 an extended time period. The period of time may be as long as a month or more and should be a release which is longer than the same amount of agent administered in bolus form.

For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material that provides sustained release properties to the compounds. As such, the compounds for use the method of the invention may be administered in the form of 25 microparticles, for example, by injection or in the form of wafers or discs by implantation.

In one embodiment of the invention, the compounds of the invention are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

The term delayed release is used herein in its conventional sense to refer to a drug 30 formulation that provides for an initial release of the drug after some delay following drug administration and that may, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

5 The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

10 As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Those skilled in the art will recognize, or be able to ascertain using no more than
15 routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures,
20 atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the
25 scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set
30 forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way

be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

FIG. 5 comprises a set of graphs illustrating that TMP195 is a highly selective inhibitor of class IIa HDACs.

5 FIG. 6 illustrates the finding that TMP195 stimulates NRF2 target gene expression in cultured cardiac myocytes. Neonatal rat ventricular myocytes (NRVMs) were treated with 3 μ M TMP195 or DMSO vehicle for 48 hours. RNA was harvested and analyzed by RNA-seq. Bioinformatic analysis revealed that TMP195 altered expression (red – upregulated; green – downregulated) of about 1000 genes. Among the most highly upregulated genes in TMP195-
10 treated cells were those regulated by the NRF2 gene.

FIG. 8 comprises a set of graphs illustrating that a class IIa HDAC inhibitor stimulates NRF2 target gene expression in cardiomyocytes. Neonatal rat ventricular myocytes (NRVMs) were treated with DMSO vehicle, TMP195, or the positive control, AI-1 (4-Chloro-1,2-dihydro-1-methyl-2-oxo-3-quinolinecarboxylic acid ethyl ester, also known as
15 Ethyl 4-chloro-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylate; AI-1 promotes NRF2 activation via the covalent modification of Kelch-like ECH-associated protein 1 (Keap1), a negative regulator of NRF2.). RNA was harvested and analyzed by qPCR. The NRF2 target genes Heme Oxygenase-1 and Sulfiredoxin-1 were up-regulated by both compounds.

FIG. 9 comprises a set of graphs illustrating the finding that TMP195 is a specific
20 activator of NRF2 in cardiomyocytes. Neonatal rat cardiac fibroblasts or HEK293 cells were treated with DMSO vehicle, TMP195, or the positive control, AI-1. RNA was harvested and analyzed by qPCR. TMP failed to induce the NRF2 target gene Heme Oxygenase-1 in fibroblasts. The data suggests the TMP195 selectively activates NRF2 in cardiomyocytes.

FIG. 11A comprises gel images obtained by treating NRVMs with vehicle control (-),
25 TMP195, or AI-1 for the indicated times. Both TMP195 and AI-1 treatment led to increased NRF2 protein expression, albeit with distinct kinetics.

FIG. 11B comprises gel images illustrating the finding that induction of NRF2 protein by TMP195 correlated with reduced expression of its negative regulator, KEAP1. For these studies, NRVMs were treated with DMSO (0.1% final concentration), 3 μ M TMP195 or 10
30 μ M AI-1, which served as a positive control. Total cell lysates were harvested. Protein levels of NRF2 and KEAP1 were detected by immunoblotting.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent
5 variations.

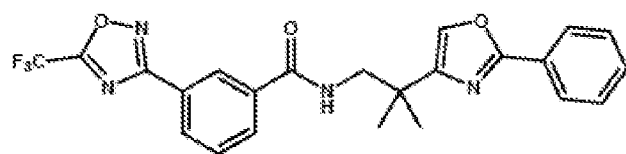
CLAIMS

What is claimed:

1. A method of reducing oxidative stress in a cardiomyocyte, the method comprising contacting a cardiomyocyte with a selective class IIa histone deacetylase (HDAC) inhibitor, or a salt or solvate thereof.
2. The method of claim 1, wherein the inhibitor has an inhibitory selectivity for one or more class IIa HDACs over one or more non-class IIa HDACs of about 3, about 10, about 30, about 100, about 300, about 1,000, about 3,000, about 10,000, or greater than about 10,000.
3. The method of any one of claims 1-2, wherein the inhibitor is TMP195 (N-(2-methyl-2-(2-phenyloxazol-4-yl)propyl)-3-(5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl)benzamide), or a salt or solvate thereof.
4. The method of any one of claims 1-3, wherein the inhibitor stimulates NRF2 activity in the cardiomyocyte.
5. The method of any one of claims 1-4, wherein the cardiomyocyte is *in vivo* in a subject.
6. The method of claim 5, wherein the contacting prevents, ameliorates, or treats post-myocardial infarction remodeling of the heart or fibrosis in the subject.
7. The method of any one of claims 5-6, wherein cardiac tissue is at least partially regenerated in the subject.
8. A method of promoting cardiac tissue regeneration, or treating or preventing post-myocardial infarction remodeling of the heart or fibrosis, in a subject, the method comprising administering to the subject a therapeutically effective amount of a selective class IIa histone deacetylase (HDAC) inhibitor, or a salt or solvate thereof, to the subject.
9. The method of claim 8, wherein the inhibitor has an inhibitory selectivity for one or more class IIa HDACs over one or more non-class IIa HDACs of at least about 3, about 10, about 30, about 100, about 300, about 1,000, about 3,000, about 10,000, or greater than about 10,000.

10. The method of any one of claims 8-9, wherein the inhibitor is TMP195, or a salt or solvate thereof.
11. The method of any one of claims 8-10, wherein the inhibitor stimulates NRF2 activity in a cardiomyocyte of the subject.
12. The method of any one of claims 8-11, wherein the inhibitor is administered to the subject by at least one route selected from the group consisting of nasal, inhalational, topical, oral, buccal, rectal, pleural, peritoneal, vaginal, intramuscular, subcutaneous, transdermal, epidural, intratracheal, otic, intraocular, intrathecal and intravenous.
13. The method of any one of claims 8-12, wherein the inhibitor is formulated in a pharmaceutically acceptable composition further comprising one or more pharmaceutically acceptable carriers.
14. The method of any one of claims 8-13, wherein the subject is further administered at least one additional agent for promoting cardiac tissue regeneration, or treating or preventing post-myocardial infarction remodeling of the heart or fibrosis.
15. The method of any one of claims 8-14, wherein the subject is a mammal.
16. The method of any one of claims 8-15, wherein the mammal is a human.

FIG. 1



TMP195

FIG. 2

HDAC Classes				
<u>I</u>	<u>IIa</u>	<u>IIb</u>	<u>III</u>	<u>IV</u>
HDAC1	HDAC4	HDAC6	SIRT1	HDAC11
HDAC2	HDAC5	HDAC10	SIRT2	
HDAC3	HDAC7		SIRT3	
HDAC8	HDAC9		SIRT4	
			SIRT5	
			SIRT6	
			SIRT7	

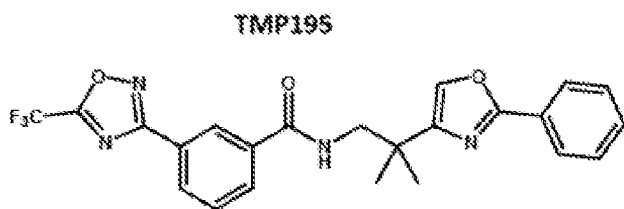


FIG. 3

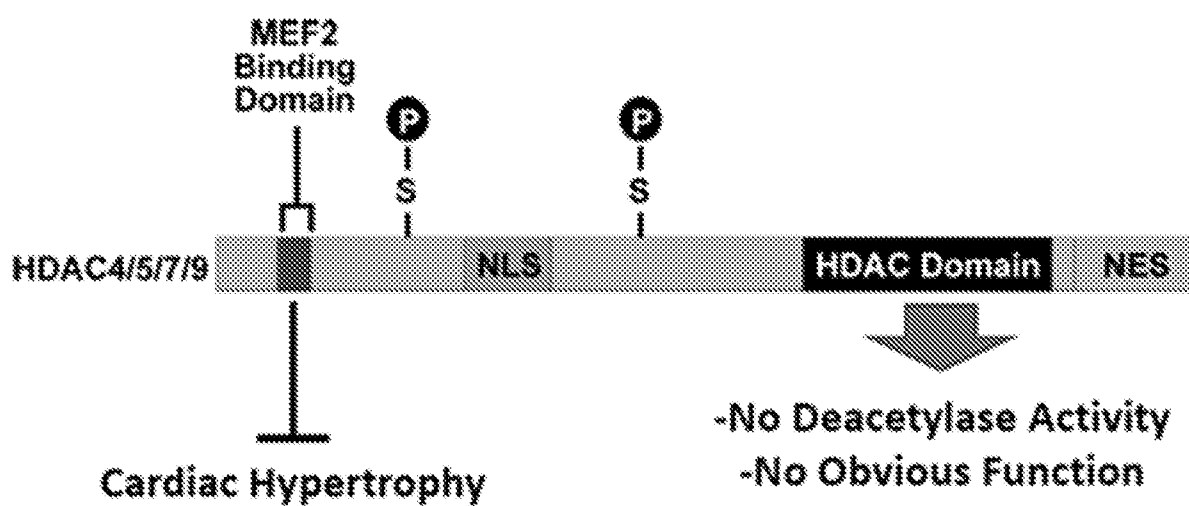


FIG. 4

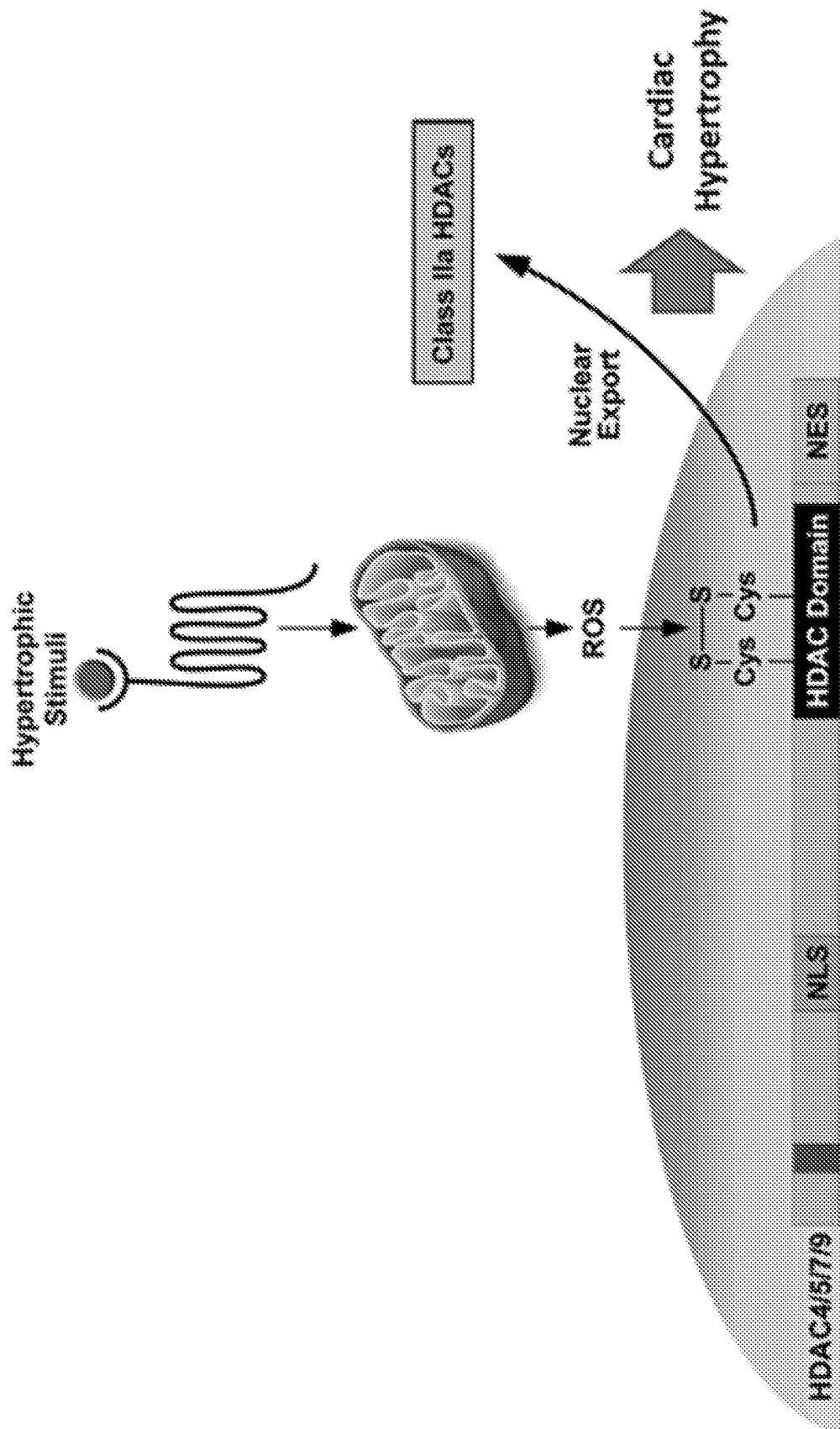
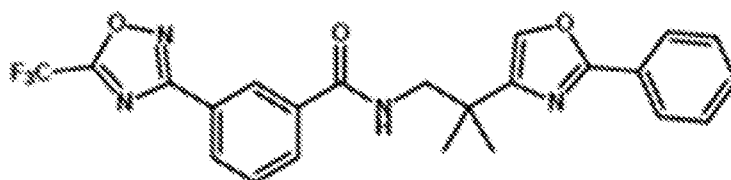
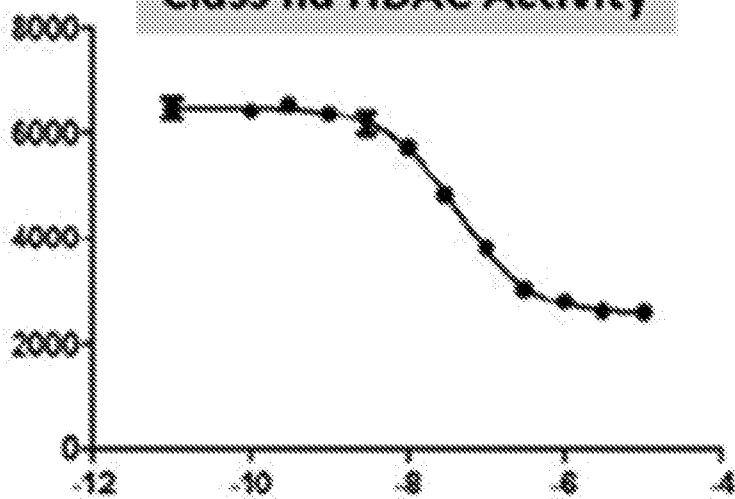


FIG. 5

TMP195

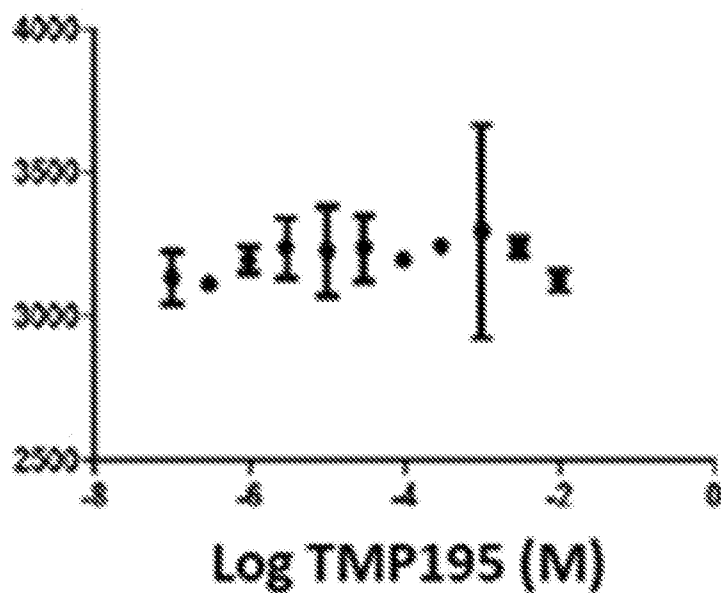


Class IIa HDAC Activity



Log TMP195 (M)

Class I HDAC Activity



Log TMP195 (M)

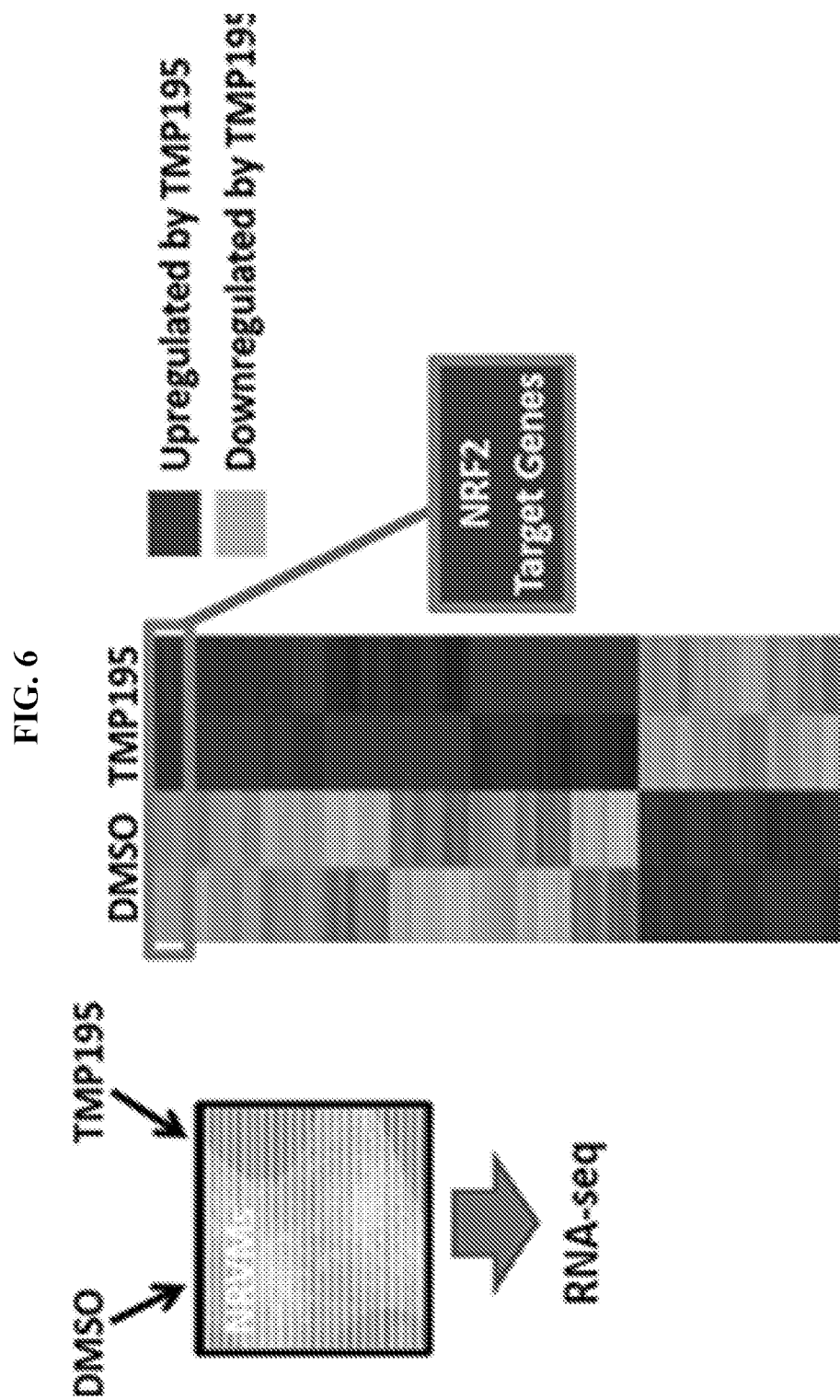


FIG. 7

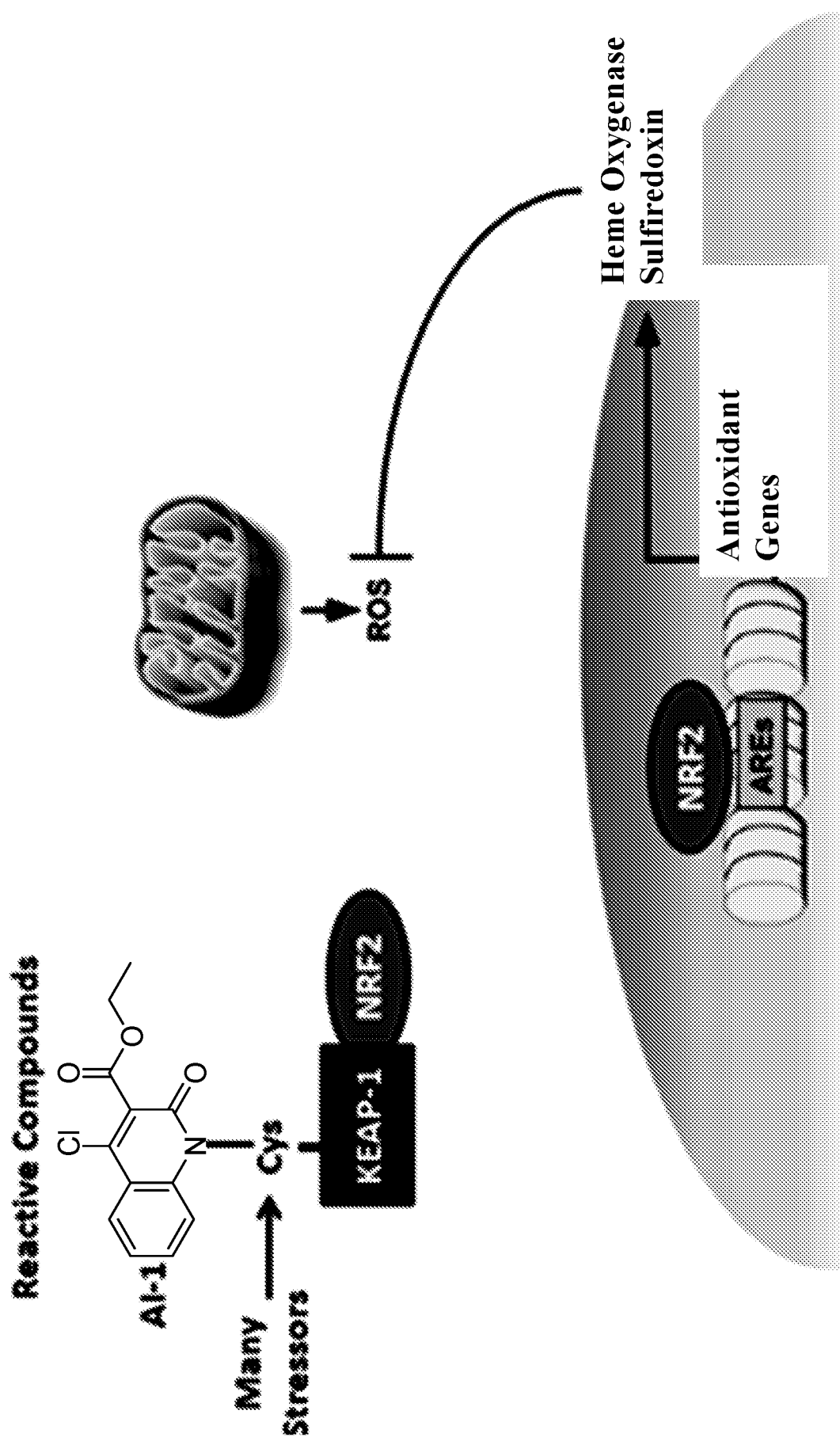


FIG. 8

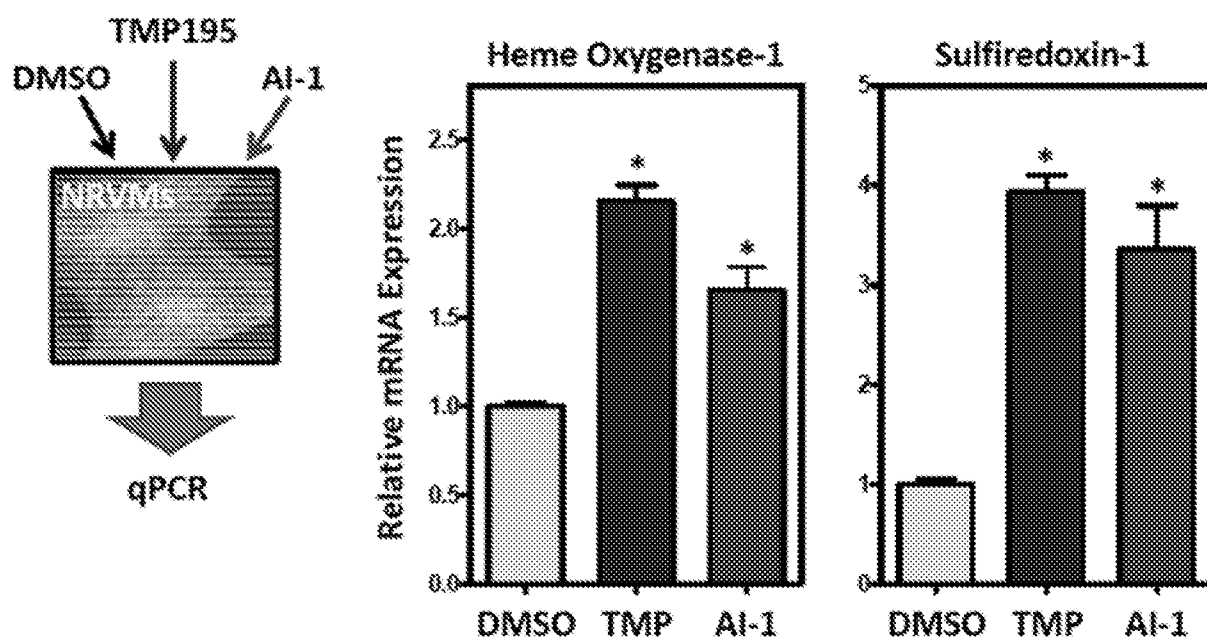


FIG. 9

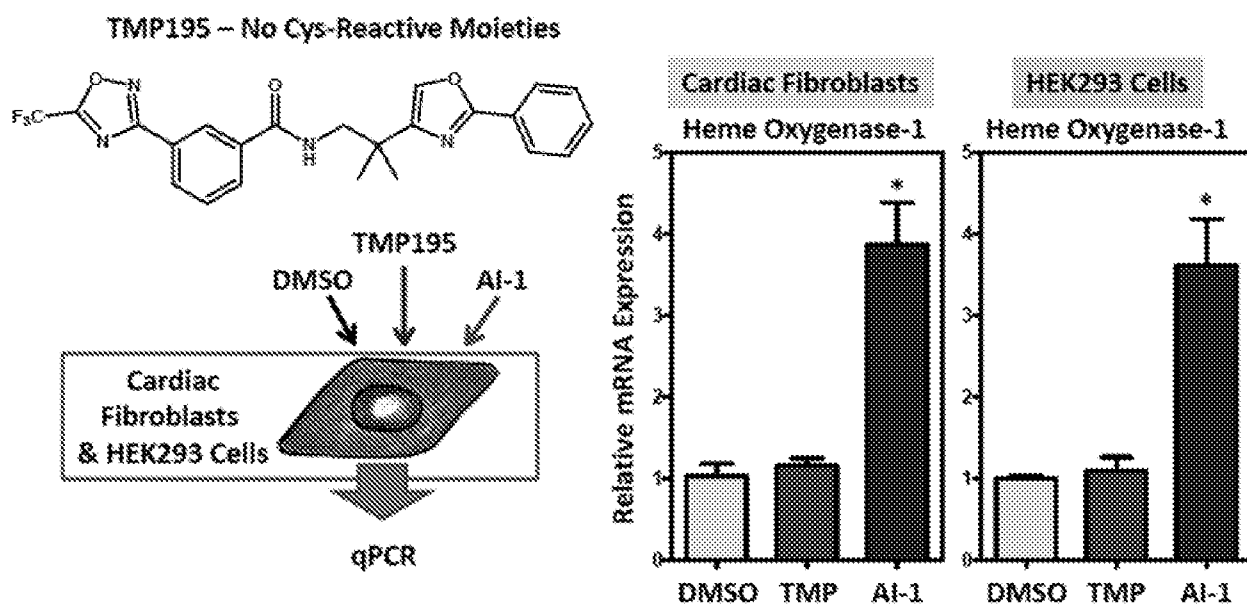


FIG. 10

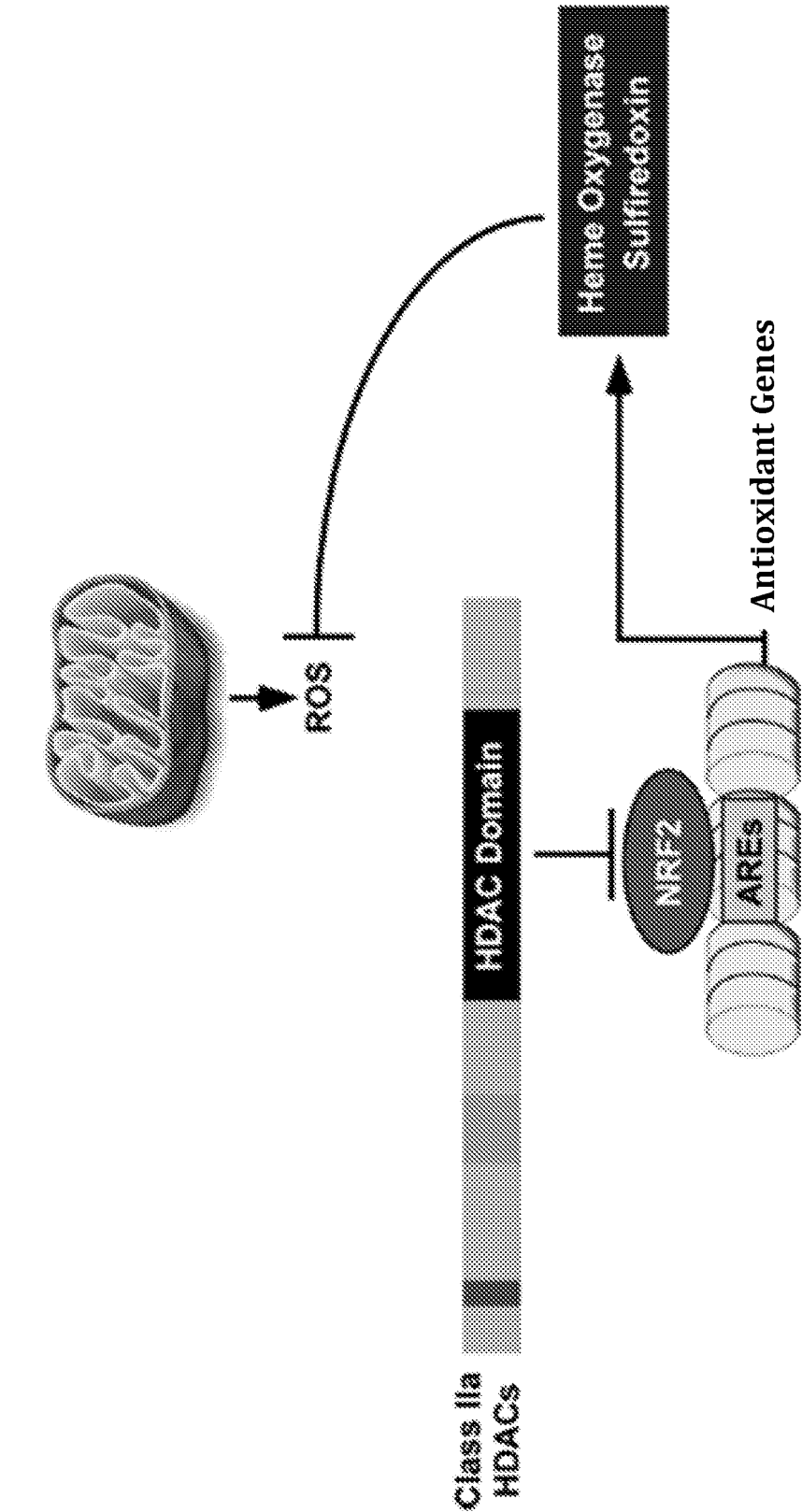


FIG. 11A

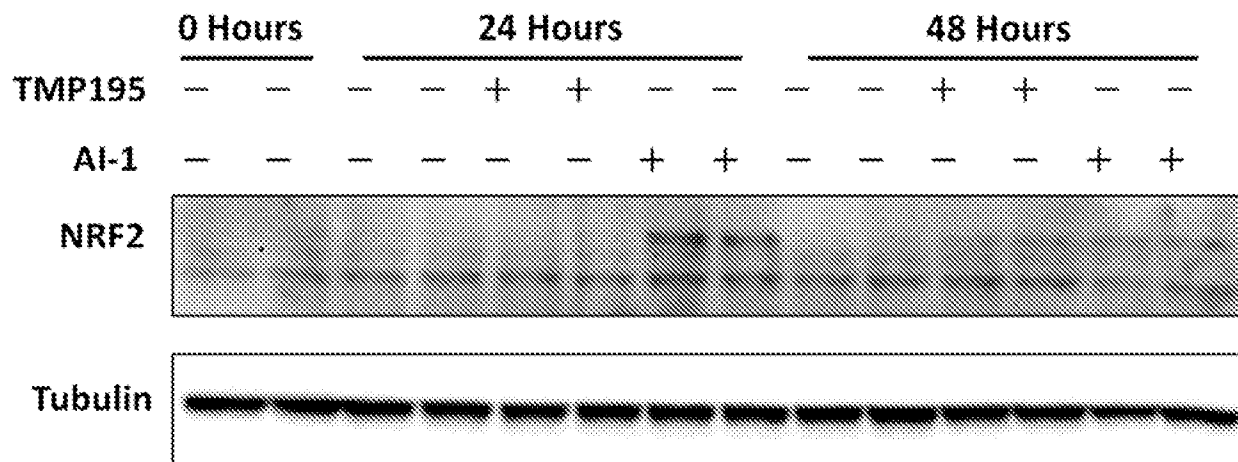
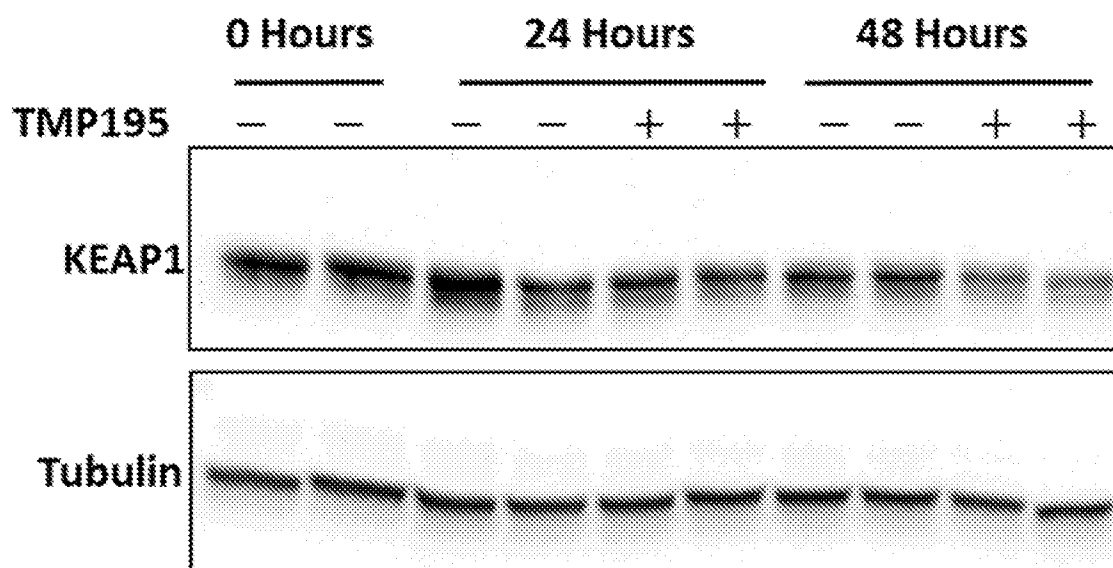


FIG. 11B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/53024

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-7 and 11-16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/53024

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 9/00; A61K 31/422; A61K 31/4245; C07D 413/02 (2017.01)

CPC - A61K 31/422; A61K 31/4245; C07D 271/06; C07D 263/02; C07D 413/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2009/0162329 A1 (ANVERSA et al.) 25 June 2009 (25.06.2009); para [0016], [0035], [0075], [0094]	8 ----- 9-10
Y	US 2016/0208013 A1 (TUFTS MEDICAL CENTER, INC.) 21 July 2016 (21.07.2016); para [0002], [0009], [0011], [0025]-[0026], [0044]	1-3
Y	MAI et al., "Class II (IIa)-Selective Histone Deacetylase Inhibitors. 1. Synthesis and Biological Evaluation of Novel (Aryloxopropenyl)pyrrolyl Hydroxyamides", Journal of Medicinal Chemistry, 2005, Vol.48, issue 9, pp. 3344-3353; retrieved from the Internet: <DOI: 10.1021/jm049002a>; see entire document, especially pg 3344, 3346-3348	1-3, 9-10
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☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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Date of the actual completion of the international search

10 November 2017

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