

Description

Title of Invention: DERIVATIVES AND PRODRUGS OF 2, 4-DINITROPHENOL, AND COMPOSITIONS AND METHODS THEREOF

[0001] Priority Claims and Related Patent Applications

[0002] This application claims the benefit of priority to Chinese Application No. 202311013356.4, filed August 10, 2023, and to U.S. Provisional Application Serial Nos. 63/532, 665, filed August 14, 2023, and 63/536, 051, filed August 31, 2023, the entire content of each of which is incorporated herein by reference.

Technical Field of the Invention

[0003] The invention generally relates to novel compounds and therapeutic uses thereof. More particularly, the invention provides novel 2, 4-dinitrophenol (DNP) derivatives and prodrugs thereof, as modulators of mitochondrial activities. The invention also provides pharmaceutical compositions comprising a compound of the invention and methods thereof for treating various diseases and disorders associated with or related to mitochondria or mitochondrial dysfunctions (e.g., obesity, diabetes, insulin resistance, liver diseases, heart or renal failure, neurodegenerative or aging related diseases) .

Background of the Invention

[0004] Metabolic syndrome, represented by type 2 diabetes, obesity, dyslipidemia, hypertension, metabolic dysfunction-associated fatty liver disease (MAFLD) and metabolic dysfunction-associated steatohepatitis (MASH) , is a cluster of diseases and disorders characterized by hepatic and peripheral insulin resistance. (Fabbrini, et al. 2009 PNAS 106, 15430; Petersen, et al. 2018 Physiology Reviews 98, 2133.) Likely cause of these conditions includes excessive lipid accumulation within the respective organs, along with chronic inflammation caused by excessive reactive oxygen species (ROS) production. Over the past decade, despite significant progress made in drug discovery efforts from the perspective of reducing energy-intake (e.g., Glp-1R agonists, SGLT2 inhibitors) , the progress in exploring energy-out has not been as satisfactory.

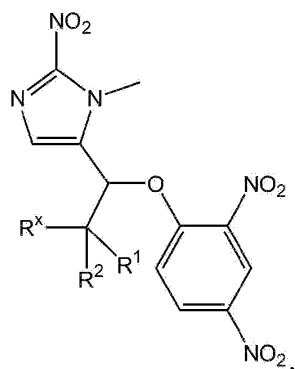
[0005] Mitochondria uncoupling, an endogenous energy-dissipating process, where the ATP synthesis is dissociated at the end of the electron transport chain due to “proton leak” , occurs in all eukaryotic cells and accounts for 20~30%of the basal metabolic rate depending on the tissue type. (Geisler 2019 Cells 8, 280.) Over the years, in addition to the endogenous uncoupling proteins, e.g., UCP-1, UCP-2, and UCP-3, many chemical uncouplers have been discovered and studied, among which DNP is the

best known. (Chen, et al. 2021 Metabolism Clinical and Experimental 117, 154724; Goedeke, et al. 2021 Molecular Metabolism 46, 101178.) DNP was used in the 1930s for weight loss in well over 100,000 people; however, its dose related toxicities, such as rash, cataract or hyperthermia related death, limited its use and eventually led to its ban by the FDA in 1938. Since then, much effort has been made to explore different approaches to increase the therapeutic windows for DNP. Examples of such effort included a liver-targeted approach, a formulation with slow release of DNP, and a prodrug that lowered the $C_{\max}$ /AUC ratio significantly as compared to DNP itself. (Perry, et al. 2013 Cell Metabolism 18, 740; Perry, et al. 2015 Science 347, 1253) ; WO 2018/129258A1) .

- [0006] In addition to its utility in anti-obesity through elevated energy expenditure with lipid and/or glucose metabolism, mitochondria uncoupling with DNP has also been shown to reduce excessive ROS productions and induction of brain derived neurotrophic factor (BDNF) , which may bring benefits for many neurodegenerative or aging related diseases. (Kishimoto, et al. 2020 Neurobiology of Aging 85, 123.)
- [0007] There is an urgent need for novel therapeutic agents as modulators of mitochondria functions, in particular, novel mitochondria uncouplers that are useful in treating metabolic syndrome, neuro-degenerative or aging related diseases and disorders.

Summary of the Invention

- [0008] The invention is based in part on novel DNP derivatives and prodrugs, pharmaceutical compositions thereof, and methods of their preparation and use as mitochondria uncouplers in treating or reducing various diseases or disorders. More particularly, the present invention provides novel compounds and their pharmaceutically acceptable salts thereof, that act as prodrugs for DNP. Upon oral administration, these compounds release DNP with significantly lowered plasma $C_{\max}$ over AUC ratios, as compared to DNP itself, resulting in increased safety window. Compounds of the invention are useful for regulating mitochondria functions and activities, including accelerated metabolism for glucose and lipid. Compounds of the invention can therefore be used to treat metabolic diseases, such as type 2 diabetes, hypertriglyceridemia, NAFLD, NASH, obesity and other neurodegenerative diseases.
- [0009] In one aspect, the invention generally relates to a compound having structural formula (I) :



(I)

- [0010] or a pharmaceutically acceptable form or an isotope derivative thereof, wherein
- [0011] each of R¹ and R² is independently a C₁₋₆ alkyl, or R¹ and R², together with the carbon atom they are bonded to, form a 3-to 8-membered (e.g., 3-, 4-, 5-, 6-, 7-, 8-membered) carbocyclic or heterocyclic ring, wherein the C₁₋₆ alkyl and the 3-to 8-membered carbocyclic or heterocyclic ring are optionally substituted with 1-6 R^A; and
- [0012] R^X is L-R^{X1}, L-R^{X2} or L-R^{X3}, wherein
- [0013] L is a single bond or (CH₂)_n, wherein n is 1, 2 or 3;
- [0014] R^{X1} is a group selected from: C(=O)OR³, C(=O)NR⁴R⁵, OR⁶, NR⁷R⁸, NR⁹C(=O)R¹⁰, OC(=O)R¹¹, halo and CN;
- [0015] R^{X2} is a 5- or 6-membered monocyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-4 R^B; and
- [0016] R^{X3} is an 8- to 10-membered bicyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-6 R^B;
- [0017] each of R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ and R¹¹
- [0018] is independently selected from H, C₁₋₆ alkyl, 3- to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A, or
- [0019] R⁴ and R⁵ or R⁷ and R⁸, together with the N atom they are bonded to, respectively, form a 3- to 8-membered heterocyclic ring, optionally substituted with 1-6 R^A;
- [0020] each R^A is independently selected from the group consisting of: D, halo, R and OR;
- [0021] each R^B is independently selected from the group consisting of: D, halo, CN, R, OR, NRR', C(=O)OR, C(=O)NRR', NRC(=O)R', OC(=O)R, SO₄R, and OC(=O)CHCHC(=O)OR'; and
- [0022] each R and R' is independently H or C₁₋₃ alkyl.
- [0023] In another aspect, the invention generally relates to a pharmaceutical composition comprising a compound disclosed herein.

- [0024] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of a compound disclosed herein.
- [0025] In yet another aspect, the invention generally relates to a method for treating or reducing a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound disclosed herein.
- [0026] In yet another aspect, the invention generally relates to a method for reducing toxicity or side effects in treating mitochondria-related disorders or conditions comprising administering to a subject in need thereof a therapeutically effective amount of a compound disclosed herein.
- [0027] In yet another aspect, the invention generally relates to use of a compound disclosed herein, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating a disease or disorder.
- [0028] In yet another aspect, the invention generally relates to use of a compound disclosed herein for treating a disease or disorder.

Brief Description of the Drawings

- [0029] FIG. 1. Exemplary data on mean plasma concentration 2, 4-DNP after an IV dose of 1 mg/kg and a PO dose of 5 mg/kg in male C57BL/6 mice (N=3/group) .
- [0030] FIG. 2. Exemplary data on mean plasma concentration of compound 1 and its metabolite 2, 4-DNP after IV (1 mg/kg) or PO (5 mg/kg) dosing, respectively, in male C57BL/6 mice (N=3/group) .
- [0031] FIG. 3. Exemplary data on mean plasma concentration of compound 7 and its metabolite 2, 4-DNP after IV (1 mg/kg) or PO (5 mg/kg) dosing, respectively, in male C57BL/6 mice (N=3/group) .
- [0032] FIG. 4. Exemplary data on mean plasma concentration 2, 4-DNP after an IV (1 mg/kg) or a PO (5 mg/kg) dosing in male SD Rats (N=3/group) .
- [0033] FIG. 5. Exemplary data on mean plasma concentration of 1 and its metabolite 2, 4-DNP after an IV dose (1 mg/kg) or a PO dose (5 mg/kg) , respectively, in male SD rats (N=3/group) .
- [0034] FIG. 6. Exemplary data on mean plasma concentration of 7 and its metabolite 2, 4-DNP after an IV dose (1 mg/kg) or a PO dose (5 mg/kg) , respectively, in male SD rats (N=3/group) .
- [0035] FIG. 7. Exemplary data on mean plasma concentration of 7 and its metabolite 2, 4-DNP after an IV dose (1 mg/kg) or a PO dose (5 mg/kg) , respectively, in male Beagle dogs (N=3/group) .
- [0036] FIG. 8. Exemplary data on SD rats rectal temperature following a single oral gavage of 2,4-DNP.

- [0037] FIG. 9. Exemplary data on SD rats survival acutely curve following a single oral gavage of 2, 4-DNP.
- [0038] FIG. 10. Exemplary data on SD rats rectal temperature following a single oral gavage of 2, 4-DNP or compound 1, respectively.
- [0039] FIG. 11. Exemplary data on the seconds from falling off the rotarod of C57BL/6J mice before 6-OHDA injection and compound 7 treatment. Data were shown as Mean $\pm$ SEM, analyzed by one-way ANOVA, n=10.
- [0040] FIG. 12. Exemplary data on the seconds from falling off the rotarod at 5 weeks post 6-OHDA injection and compound 7 treatment. Data were shown as Mean $\pm$ SEM, analyzed by one-way ANOVA, n=10, *p<0.05, ***p<0.001, ****p<0.0001 vs Model.
- [0041] FIG. 13. Exemplary data on the peak grip strength at 5 weeks post 6-OHDA injection and compound 7 treatment. Data were shown as Mean $\pm$ SEM, analyzed by one-way ANOVA, n=10, *p<0.05, ***p<0.001, ****p<0.0001 vs Model.
- [0042] FIG. 14. Exemplary data on the number of TH+ cells in SN at 5 weeks post 6-OHDA injection and compound 7 treatment. Data were shown as Mean $\pm$ SEM, analyzed by one-way ANOVA, n=4, *p<0.05, ***p<0.001, ****p<0.0001 vs Model.
- [0043] FIG. 15. Exemplary data on the density of TH+ fibers in Str at 5 weeks post 6-OHDA injection and compound 7 treatment. Data were shown as Mean $\pm$ SEM, analyzed by one-way ANOVA, n=4, *p<0.05, ***p<0.001, ****p<0.0001 vs Model.
- [0044] Definitions
- [0045] 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. General principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 2006.
- [0046] The following terms, unless indicated otherwise according to the context wherein the terms are found, are intended to have the following meanings.
- [0047] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 16 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.
- [0048] Any compositions or methods disclosed herein can be combined with one or more of any of the other compositions and methods provided herein.
- [0049] The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

- [0050] Definitions of specific functional groups and chemical terms are described in more detail below. When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example, "C₁₋₆ alkyl" is intended to encompass, C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅, and C₅₋₆ alkyl.
- [0051] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., -C(=O)-O-is equivalent to -O-C(=O)-.
- [0052] Structures of compounds of the invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds that are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions (e.g., aqueous, neutral, and several known physiological conditions).
- [0053] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.
- [0054] As used herein, "at least" a specific value is understood to be that value and all values greater than that value.
- [0055] As used herein, the terms "comprises," "comprising," or "having" when used to define compositions and methods, are intended to mean that the compositions and methods include the recited elements, but do not exclude other elements. The term "consisting essentially of", when used to define compositions and methods, shall mean that the compositions and methods include the recited elements and exclude other elements of any essential significance to the compositions and methods. For example, "consisting essentially of" refers to administration of the pharmacologically active agents expressly recited and excludes pharmacologically active agents not expressly recited. The term consisting essentially of does not exclude pharmacologically inactive or inert agents, e.g., pharmaceutically acceptable excipients, carriers or diluents. The term "consisting of", when used to define compositions and methods, shall mean excluding trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.
- [0056] As used herein, the terms "disease" and "disorder" are used interchangeably and refer to any condition that damages or interferes with the normal function of a cell, tissue, or organ.

- [0057] As used herein, the term "hydrate" means a compound which further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent inter-molecular forces.
- [0058] As used herein, the term "pharmaceutically acceptable" refers to being suitable for use in contact with the tissues of humans and other mammals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable form" of a disclosed compound includes, but is not limited to, pharmaceutically acceptable salts, esters, hydrates, solvates, polymorphs, isomers and isotopically labeled derivatives thereof.
- [0059] In certain embodiments, a "pharmaceutically acceptable form" includes, but is not limited to, pharmaceutically acceptable salts, esters and isotopically labeled derivatives thereof.
- [0060] In certain embodiments, a "pharmaceutically acceptable form" includes, but is not limited to, pharmaceutically acceptable isomers and stereoisomers and isotopically labeled derivatives thereof.
- [0061] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66: 1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, organic acids from which salts can be derived include, for

example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, lactic acid, trifluoroacetic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

[0062] The salts can be prepared in situ during the isolation and purification of the disclosed compounds, or separately, such as by reacting the free base or free acid of a parent compound with a suitable base or acid, respectively. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and N^+ (C_{1-4} alkyl)₄ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines, including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, such as isopropyl amine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt can be chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

[0063] In certain embodiments, the pharmaceutically acceptable form is a "solvate" (e.g., a hydrate). As used herein, the term "solvate" refers to compounds that further include a stoichiometric or non-stoichiometric amount of solvent bound by non-covalent intermolecular forces. The solvate can be of a disclosed compound or a pharmaceutically acceptable salt thereof. Where the solvent is water, the solvate is a "hydrate". Pharmaceutically acceptable solvates and hydrates are complexes that, for example, can include 1 to about 100, or 1 to about 10, or 1 to about 2, about 3 or about 4, solvent or water molecules. It will be understood that the term "compound" as used herein encompasses the compound and solvates of the compound, as well as mixtures thereof.

[0064] As used herein, the term "prodrug" (or "pro-drug") refers to compounds that are transformed in vivo to yield a disclosed compound or a pharmaceutically acceptable form of the compound. A prodrug can be inactive when administered to a subject, but is converted in vivo to an active compound, for example, by hydrolysis (e.g., hydrolysis in blood). In certain cases, a prodrug has improved physical and/or delivery properties over the parent compound. Prodrugs can increase the bioavailability of the compound when administered to a subject (e.g., by permitting enhanced absorption into the blood following oral administration) or which enhance delivery to a biological compartment of interest (e.g., the brain or lymphatic system) relative to the parent

compound. Exemplary prodrugs include derivatives of a disclosed compound with enhanced aqueous solubility or active transport through the gut membrane, relative to the parent compound.

[0065] As used herein, the term “pharmaceutically acceptable excipient, carrier, or diluent” refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent 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 and not injurious to the patient. Some examples of materials which can 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; 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. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0066] As used herein, the term “polymorph” means solid crystalline forms of a compound or complex thereof which may be characterized by physical means such as, for instance, X-ray powder diffraction patterns or infrared spectroscopy. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (e.g., to heat, light or moisture), compressibility and density (important in formulation and product manufacturing), hygroscopicity, solubility, and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing.

For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it.

- [0067] As used herein, the term “solvate” means a compound which further includes a stoichiometric or non-stoichiometric amount of solvent such as water, acetone, ethanol, methanol, dichloromethane, 2-propanol, or the like, bound by non-covalent inter-molecular forces.
- [0068] As used herein, the term “stable compounds” refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., formulation into therapeutic products, intermediates for use in production of therapeutic compounds, isolatable or storable intermediate compounds, treating a disease or disorder responsive to therapeutic agents) .
- [0069] As used herein, the term “stereoisomer” refers to both enantiomers and diastereomers. As used herein, the term “substantially free of other stereoisomers” means less than 25% of other stereoisomers, preferably less than 10% of other stereoisomers, more preferably less than 5% of other stereoisomers and most preferably less than 2% of other stereoisomers, or less than “X” % of other stereoisomers (wherein X is a number between 0 and 100, inclusive) are present. Methods of obtaining or synthesizing diastereomers are well known in the art and may be applied as practicable to final compounds or to starting material or intermediates. Other embodiments are those wherein the compound is an isolated compound. The term “at least X% enantiomerically enriched” as used herein means that at least X% of the compound is a single enantiomeric form, wherein X is a number between 0 and 100, inclusive.
- [0070] As used herein, the terms “treating” or “reducing” a disease or disorder refers to a method of reducing, delaying or ameliorating such a condition before or after it has occurred. Treatment may be directed at one or more effects or symptoms of a disease and/or the underlying pathology. The treatment can be any reduction and can be, but is not limited to, the complete ablation of the disease or the symptoms of the disease. Treating or treatment thus refers to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving or stabilizing a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters, for example, the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. As compared with an equivalent untreated control, such reduction

or degree of amelioration may be at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, or 100% as measured by any standard technique.

- [0071] As used herein, the term “subject” refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms “subject” and “patient” are used interchangeably herein in reference to a human subject.
- [0072] As used herein, the terms “alk” or “alkyl” refer to straight, branched or cyclic hydrocarbon groups having 1 to 12 carbon atoms, preferably 1 to 8 carbon atoms, containing no unsaturation. The expression “lower alkyl” refers to alkyl groups of 1 to 4 carbon atoms (inclusive). Whenever it appears herein, a numerical range such as “1 to 10” refers to each integer in the given range; e.g., “1 to 10 carbon atoms” means that the alkyl group can consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 10 carbon atoms, although the present definition also covers the occurrence of the term “alkyl” where no numerical range is designated. In some embodiments, “alkyl” can be a C₁₋₆ alkyl group. In some embodiments, “alkyl” can be a C₁₋₃ alkyl group.
- [0073] As used herein, the term “alkoxy” refers to an -O-alkyl radical.
- [0074] As used herein, the terms “carbocycle”, “carbocyclic” and “carbocyclyl” each refers to a monocyclic or polycyclic radical that contains only carbon as ring atoms, and can be saturated or partially unsaturated. Fully saturated carbocyclic is termed cycloalkyl. Partially unsaturated cycloalkyl groups can be termed “cycloalkenyl” if the carbocycle contains at least one double bond, or “cycloalkynyl” if the carbocycle contains at least one triple bond. Unless stated otherwise in the specification, the term is intended to include both substituted and unsubstituted carbocyclic groups. The term “carbocyclic” also includes bridged and spiro-fused cyclic structures containing no hetero ring atoms. The term also includes monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of ring atoms) groups. Polycyclic groups include bicycles, tricycles, tetracycles, and the like. Unless stated otherwise in the specification, a carbocyclic group can be optionally substituted by one or more substituents.
- [0075] As used herein, the term “cycloalkyl” as employed herein refers to a cyclic alkyl group and includes saturated and partially unsaturated cyclic, respectively, hydrocarbon groups having 3 to 12 carbons, preferably 3 to 8 carbons.
- [0076] As used herein, the terms “aromatic” or “aryl” refer to a radical with 6 to 14 ring atoms (e.g., C₆₋₁₄ aromatic or C₆₋₁₄ aryl) that has at least one ring having a conjugated pi electron system which is carbocyclic (e.g., phenyl, fluorenyl, naphthyl, and anthracene). An aryl group may be, for example, 6 membered monocyclic, 10 membered bicyclic or 14 membered tricyclic ring systems, each with 6 to 14 carbon atoms.

- [0077] As used herein, the term “halo” or “halogen” refers to any radical of fluorine, chlorine, bromine or iodine.
- [0078] As used herein, the term “heteroaryl” or, alternatively, “heteroaromatic” refers to a radical of a 5-18 membered monocyclic or polycyclic (e.g., bicyclic, tricyclic, tetracyclic and the like) aromatic ring system (e.g., having 6, 10 or 14 π electrons shared in a cyclic array) having ring carbon atoms and 1-6 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, phosphorous and sulfur (“5-18 membered heteroaryl”). Heteroaryl polycyclic ring systems can include one or more heteroatoms in one or both rings. Whenever it appears herein, a numerical range such as “5 to 18” refers to each integer in the given range; e.g., “5 to 18 ring atoms” means that the heteroaryl group can consist of 5 ring atoms, 6 ring atoms, etc., up to and including 18 ring atoms. In some instances, a heteroaryl can have 5 to 14 ring atoms. In some embodiments, the heteroaryl has, for example, bivalent radicals derived from univalent heteroaryl radicals whose names end in “-yl” by removal of one hydrogen atom from the atom with the free valence are named by adding “-ene” to the name of the corresponding univalent radical, e.g., a pyridyl group with two points of attachment is a pyridylene. The term “heteroaryl”, for example, may refer to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group of 5 to 12 ring atoms containing one, two, three or four ring heteroatoms selected from N, O, or S, the remaining ring atoms being C, and, in addition, having a completely conjugated pi-electron system, wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, quinazoline, isoquinoline, purine and carbazole.
- [0079] As used herein, the terms “heterocycle”, “heterocyclic” or “heterocyclyl” refer to fully saturated or partially unsaturated cyclic groups, for example, 3 to 7 membered monocyclic, 7 to 12 membered bicyclic, or 10 to 15 membered tricyclic ring systems, which have at least one heteroatom in at least one ring, wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3 or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom of the ring or ring system.
- [0080] As used herein, the term “substituents” refers to a group “substituted” on any functional group delineated herein, e.g., alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, or heteroaryl group at any atom of that group. Suitable

substituents include, without limitation halogen, CN, NO₂, OR¹⁵, SR¹⁵, S(O)₂OR¹⁵, NR¹⁵R¹⁶, C₁-C₂ perfluoroalkyl, C₁-C₂ perfluoroalkoxy, 1, 2-methylenedioxy, C(O)OR¹⁵, C(O)NR¹⁵R¹⁶, OC(O)NR¹⁵R¹⁶, NR¹⁵C(O)NR¹⁵R¹⁶, C(NR¹⁶)NR¹⁵R¹⁶, NR¹⁵C(NR¹⁶)NR¹⁵R¹⁶, S(O)₂NR¹⁵R¹⁶, R¹⁷, C(O)R¹⁷, NR¹⁵C(O)R¹⁷, S(O)R¹⁷, S(O)₂R¹⁷, R¹⁶, oxo, C(O)R¹⁶, C(O)(CH₂)_nOH, (CH₂)_nOR¹⁵, (CH₂)_nC(O)NR¹⁵R¹⁶, NR¹⁵S(O)₂R¹⁷, where n is independently 0-6 inclusive. Each R¹⁵ is independently hydrogen, C₁-C₄ alkyl or C₃-C₆ cycloalkyl. Each R¹⁶ is independently hydrogen, alkenyl, alkynyl, C₃-C₆ cycloalkyl, aryl, heterocyclyl, heteroaryl, C₁-C₄ alkyl or C₁-C₄ alkyl substituted with C₃-C₆ cycloalkyl, aryl, heterocyclyl or heteroaryl. Each R¹⁷ is independently C₃-C₆ cycloalkyl, aryl, heterocyclyl, heteroaryl, C₁-C₄ alkyl or C₁-C₄ alkyl substituted with C₃-C₆ cycloalkyl, aryl, heterocyclyl or heteroaryl. Each C₃-C₆ cycloalkyl, aryl, heterocyclyl, heteroaryl and C₁-C₄ alkyl in each R¹⁵, R¹⁶ and R¹⁷ can optionally be substituted with halogen, CN, C₁-C₄ alkyl, OH, C₁-C₄ alkoxy, NH₂, C₁-C₄ alkylamino, C₁-C₄ dialkylamino, C₁-C₂ perfluoroalkyl, C₁-C₂ perfluoroalkoxy, or 1, 2-methylenedioxy.

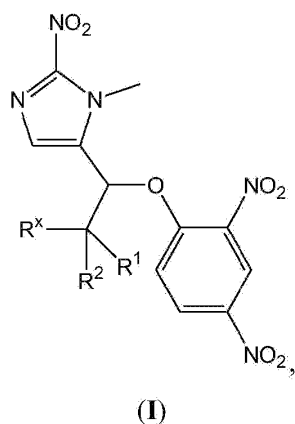
[0081] The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

[0082] 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 be represented in multiple tautomeric forms, in such instances, the invention expressly includes all tautomeric forms of the compounds described herein. 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.

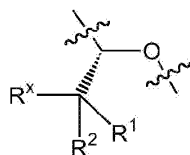
Detailed Description of the Invention

[0083] The invention provides novel DNP derivatives and prodrugs thereof, as modulators of mitochondrial activities. The invention also provides pharmaceutical compositions comprising a compound of the invention and methods thereof for treating various diseases and disorders associated with or related to mitochondrial dysfunctions (e.g., obesity, diabetes, insulin resistance, liver diseases, heart or renal failure, neurodegenerative diseases or aging related diseases).

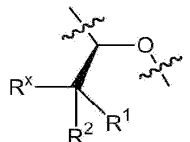
[0084] In one aspect, the invention generally relates to a compound having structural formula (I) :



- [0085] or a pharmaceutically acceptable form or an isotope derivative thereof, wherein
- [0086] each of R^1 and R^2 is independently a C_{1-6} alkyl, or R^1 and R^2 , together with the carbon atom they are bonded to, form a 3-to 8-membered (e.g., 3-, 4-, 5-, 6-, 7-, 8-membered) carbocyclic or heterocyclic ring, wherein the C_{1-6} alkyl and the 3-to 8-membered carbocyclic or heterocyclic ring are optionally substituted with 1-6 R^A ; and
- [0087] R^X is $L-R^{X1}$, $L-R^{X2}$ or $L-R^{X3}$, wherein
- [0088] L is a single bond or $(CH_2)_n$, wherein n is 1, 2 or 3;
- [0089] R^{X1} is a group selected from: $C(=O)OR^3$, $C(=O)NR^4R^5$, OR^6 , NR^7R^8 , $NR^9C(=O)R^{10}$, $OC(=O)R^{11}$, halo and CN;
- [0090] R^{X2} is a 5- or 6-membered monocyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-4 R^B ; and
- [0091] R^{X3} is an 8-to 10-membered bicyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-6 R^B ;
- [0092] each of R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} and R^{11}
- [0093] is independently selected from H, C_{1-6} alkyl, 3-to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A , or
- [0094] R^4 and R^5 or R^7 and R^8 , together with the N atom they are bonded to, respectively, form a 3-to 8-membered heterocyclic ring, optionally substituted with 1-6 R^A ;
- [0095] each R^A is independently selected from the group consisting of: D, halo, R and OR;
- [0096] each R^B is independently selected from the group consisting of: D, halo, CN, R, OR, NRR' , $C(=O)OR$, $C(=O)NRR'$, $NRC(=O)R'$, $OC(=O)R$, SO_4R and $OC(=O)CHCHC(=O)OR'$; and
- [0097] each R and R' is independently H or C_{1-3} alkyl.
- [0098] In certain embodiments of (I), the compound exhibits a chirality as shown below:



[0099] In certain embodiments of (I), the compound exhibits a chirality as shown below:



[0100] In certain embodiments, each of R^1 and R^2 is independently a C_{1-3} alkyl (e.g., methyl, ethyl, propyl, isopropyl), unsubstituted or substituted (e.g., with D, F, Cl or CH_3).

[0101] In certain embodiments, each of R^1 and R^2 is methyl.

[0102] In certain embodiments, R^1 and R^2 , together with the carbon atom they are bonded to, form a 3- to 6-membered (e.g., 3-, 4-, 5-, 6-membered) carbocyclic ring. In certain embodiments, R^1 and R^2 , together with the carbon atom they are bonded to, form a cyclopropyl group.

[0103] In certain embodiments, R^1 and R^2 , together with the carbon atom they are bonded to, form a 3- to 6-membered (e.g., 3-, 4-, 5-, 6-membered) heterocyclic ring, wherein the heterocyclic ring has 1, 2 or 3 heteroatoms selected from N, O and S.

[0104] In certain embodiments of (I), R^x is $L-R^{X1}$.

[0105] In certain embodiments, R^{X1} is $C(=O)OR^3$.

[0106] In certain embodiments, R^3 is H, C_{1-3} alkyl (e.g., methyl, ethyl, propyl, isopropyl), unsubstituted or substituted (e.g., with D, F, Cl, CH_3 , OH or OCH_3).

[0107] In certain embodiments, R^{X1} is $C(=O)NR^4R^5$.

[0108] In certain embodiments, R^4 , R^5 are independently selected from H, C_{1-3} alkyl (e.g., methyl, ethyl, propyl, isopropyl), unsubstituted or substituted (e.g., with D, F, Cl, CH_3 , OH or OCH_3).

[0109] In certain embodiments, R^4 and R^5 together with the N atom they are bonded to form a 3- to 6-membered (e.g., 3-, 4-, 5-, 6-membered) heterocyclic ring. In certain embodiments, R^4 and R^5 , together with the N atom they are bonded to, form an azetidine or a morpholinyl group.

[0110] In certain embodiments of (I), R^x is $L-R^{X2}$.

[0111] In certain embodiments, R^{X2} is 5-membered heteroaryl group, optionally substituted with 1-4 R^B . In certain embodiments, R^{X2} is oxadiazolyl group.

[0112] In certain embodiments, R^{X2} is 6-membered aryl or heteroaryl group, optionally substituted with 1-4 R^B .

- [0113] In certain embodiments of (I), R^X is $L-R^{X3}$.
- [0114] In certain embodiments, R^{X3} is a 9-membered bicyclic aryl or heteroaryl group, optionally substituted with 1-6 R^B . In certain embodiments, R^{X3} is benzimidazolyl group.
- [0115] In certain embodiments, R^{X3} is a 10-membered bicyclic aryl or heteroaryl group, optionally substituted with 1-6 R^B .
- [0116] In certain embodiments of (I), L is a single bond.
- [0117] In certain embodiments of (I), L is $(CH_2)_n$, wherein n is 1, 2 or 3. In certain embodiments of (I), n is 1 and L is CH_2 .
- [0118] In certain embodiments, the compound is selected from Table 1.
- [0119] Compounds of the invention include those having one or more deuterium atoms in place of one or more hydrogen atoms.
- [0120] In another aspect, the invention generally relates to a pharmaceutical composition comprising a compound disclosed herein.
- [0121] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of a compound disclosed herein.
- [0122] In certain embodiments, the unit dosage form is a tablet.
- [0123] In certain embodiments, the unit dosage form is a capsule.
- [0124] In yet another aspect, the invention generally relates to a method for treating or reducing a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound disclosed herein.
- [0125] In certain embodiments, the disease or disorder is associated with mitochondria function of a subject.
- [0126] In certain embodiments, the disease or disorder is associated with one or more defects in mitochondrial function of a subject.
- [0127] In certain embodiments, the disease or disorder is selected from metabolic disease, liver disease, cardiovascular disease, or a related disease or disorder.
- [0128] In certain embodiments, the disease or disorder is obesity, excess body fat, diabetes, insulin resistance or intolerance, high blood pressure, dyslipidemia, heart or renal failure, atherosclerosis, hypertriglyceridemia, acquired lipodystrophy, inherited lipodystrophy, partial lipodystrophy, metabolic syndrome, Rett's syndrome, metabolic syndrome associated with aging, metabolic diseases associated with increased reactive oxygen species (ROS), Friedreich's ataxia, Nonalcoholic fatty liver disease (NAFLD), Nonalcoholic Steatohepatitis (NASH), or a related disease or disorder.
- [0129] In yet another aspect, the invention generally relates to a method for reducing toxicity or side effects in treating mitochondria-related disorders or conditions comprising administering to a subject in need thereof a therapeutically effective amount of a compound disclosed herein.

- [0130] In certain embodiments, administration is via oral administration.
- [0131] In yet another aspect, the invention generally relates to use of a compound disclosed herein, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating a disease or disorder.
- [0132] In yet another aspect, the invention generally relates to use of a compound disclosed herein for treating a disease or disorder.
- [0133] In certain embodiments, a compound disclosed herein is used to treat a disease or disorder associated with mitochondria function.
- [0134] In certain embodiments, a compound disclosed herein is used to treat a disease or disorder associated with one or more defect in mitochondrial function.
- [0135] In certain embodiments, a compound disclosed herein is used to treat a disease or disorder selected from the group consisting of obesity, excess body fat, diabetes, insulin resistance or intolerance, high blood pressure, dyslipidemia, heart or renal failure, , atherosclerosis, hypertriglyceridemia, acquired lipodystrophy, inherited lipodystrophy, partial lipodystrophy, metabolic syndrome, Rett's syndrome, metabolic syndrome associated with aging, metabolic diseases associated with increased reactive oxygen species (ROS) , Friedreich's ataxia, Parkinson diseases, ALS, NAFLD or NASH, or a related disease or disorder.
- [0136] The specific approaches and compounds disclosed herein are not intended to be limiting. The chemical structures in the schemes herein depict variables that are hereby defined commensurately with chemical group definitions (moieties, atoms, etc.) of the corresponding position in the compound formulae herein, whether identified by the same variable name (e.g., R^1 , R^2 , R, R' , X, etc.) or not. The suitability of a chemical group in a compound structure for use in synthesis of another compound structure is within the knowledge of one of ordinary skill in the art. Additional methods of synthesizing compounds of the formulae herein and their synthetic precursors, including those within routes not explicitly shown in schemes herein, are within the means of chemists of ordinary skill in the art. Methods for optimizing reaction conditions, if necessary, minimizing competing by-products, are known in the art. The methods described herein may also additionally include steps, either before or after the steps described specifically herein, to add or remove suitable protecting groups in order to ultimately allow synthesis of the compounds herein. In addition, 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 applicable compounds are known in the art and include, for example, those described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989) ; T.W. Greene and

P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 3rd Ed., John Wiley and Sons (1999) ; 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.

- [0137] The methods delineated herein contemplate converting compounds of one formula to compounds of another formula. The process of converting refers to one or more chemical transformations, which can be performed in situ, or with isolation of intermediate compounds. The transformations can include reacting the starting compounds or intermediates with additional reagents using techniques and protocols known in the art, including those in the references cited herein. Intermediates can be used with or without purification (e.g., filtration, distillation, sublimation, crystallization, trituration, solid phase extraction, and chromatography) .
- [0138] Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds.
- [0139] Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis-and trans-isomers, atropisomers, R-and S-enantiomers, diastereomers, (D) -isomers, (L) -isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.
- [0140] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50: 50, 60: 40, 70: 30, 80: 20, 90: 10, 95: 5, 96: 4, 97: 3, 98: 2, 99: 1, or 100: 0 isomer ratios are contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.
- [0141] If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic methods well known in the art, and subsequent recovery of the pure enantiomers.

- [0142] Solvates and polymorphs of the compounds of the invention are also contemplated herein. Solvates of the compounds of the present invention include, for example, hydrates.
- [0143] The invention also provides compositions comprising an effective amount of a compound of any of the formulae herein, or a pharmaceutically acceptable salt, solvate, hydrate, or polymorph, if applicable, of said compound; and an acceptable carrier. Preferably, a composition of this invention is formulated for pharmaceutical use ("apharmaceutical composition"), wherein the carrier is a pharmaceutically acceptable carrier. The carrier (s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and, in the case of a pharmaceutically acceptable carrier, not deleterious to the recipient thereof in amounts typically used in medicaments.
- [0144] 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, 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 carboxymethyl-cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.
- [0145] The pharmaceutical compositions of the invention include those suitable for oral, rectal, nasal, topical (including buccal and sublingual) , vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. In certain embodiments, the compound of the formulae herein is administered transdermally (e.g., using a transdermal patch) . Other formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. See, for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA (17th ed. 1985) .
- [0146] Such preparative methods include the step of bringing into association with the molecule to be administered ingredients such as the carrier that constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers, liposomes or finely divided solid carriers or both, and then if necessary, shaping the product.
- [0147] In certain preferred embodiments, the compound is administered orally. Compositions of the present invention suitable for oral administration may be presented

as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, or packed in liposomes and as a bolus, etc. Soft gelatin capsules can be useful for containing such suspensions, which may beneficially increase the rate of compound absorption.

- [0148] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets optionally may be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein. Methods of formulating such slow or controlled release compositions of pharmaceutically active ingredients, such as those herein and other compounds known in the art, are known in the art and described in several issued US Patents, some of which include, but are not limited to, US Patent Nos. 4,369,172; and 4,842,866, and references cited therein. Coatings can be used for delivery of compounds to the intestine (see, e.g., U.S. Patent Nos. 6,638,534, 5,217,720, and 6,569,457, 6,461,631, 6,528,080, 6,800,663, and references cited therein). A useful formulation for the compounds of this invention is the form of enteric pellets of which the enteric layer comprises hydroxypropylmethylcellulose acetate succinate.
- [0149] In the case of tablets for oral use, carriers that 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 cornstarch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.
- [0150] Compositions suitable for topical administration include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia.
- [0151] Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented

in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

[0152] Such injection solutions may be in the form, for example, of 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.

[0153] The pharmaceutical compositions of this invention may 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.

[0154] 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.

[0155] Topical administration of the pharmaceutical compositions of this invention is especially 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. 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 and iontophoretic administration are also included in this invention.

- [0156] In another embodiment, a composition of the present invention further comprises a second therapeutic agent. The second therapeutic agent includes any compound or therapeutic agent known to have or that demonstrates advantageous properties when administered with a compound of any of the formulae herein.
- [0157] Such agents are described in detail in the art. Preferably, the second therapeutic agent is an agent useful in the treatment or prevention of metabolic diseases or disorders.
- [0158] In another embodiment, the invention provides separate dosage forms of a compound of this invention and a second therapeutic agent that are associated with one another. The term “associated with one another” as used herein means that the separate dosage forms are packaged together or otherwise attached to one another such that it is readily apparent that the separate dosage forms are intended to be sold and administered together (within less than 24 hours of one another, consecutively or simultaneously) .
- [0159] In the pharmaceutical compositions of the invention, the compound of the present invention is present in an effective amount. As used herein, the term “effective amount” refers to an amount which, when administered in a proper dosing regimen, is sufficient to reduce or ameliorate the severity, duration or progression of the disorder being treated, prevent the advancement of the disorder being treated, cause the regression of the disorder being treated, or enhance or improve the prophylactic or therapeutic effect (s) of another therapy.
- [0160] The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described in Freireich, et al. 1966 Cancer Chemother Rep 50: 219. Body surface area may be approximately determined from height and weight of the patient. (See, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardley, N. Y., 1970, 537.) An effective amount of a compound of this invention can range from about 0.001 mg/kg to about 500 mg/kg, more preferably 0.01 mg/kg to about 50 mg/kg, more preferably 0.1 mg/kg to about 2.5 mg/kg. Effective doses will also vary, as recognized by those skilled in the art, depending on the diseases treated, the severity of the disease, the route of administration, the sex, age and general health condition of the patient, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents and the judgment of the treating physician.

- [0161] For pharmaceutical compositions that comprise a second therapeutic agent, an effective amount of the second therapeutic agent is between about 20% and 100% of the dosage normally utilized in a monotherapy regime using just that agent. Preferably, an effective amount is between about 70% and 100% of the normal monotherapeutic dose. The normal monotherapeutic dosages of these second therapeutic agents are well known in the art. (See, e.g., Wells, et al., eds. 2000 Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn.; PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. 2000, each of which references are entirely incorporated herein by reference.
- [0162] The invention also provides a method of treating a subject suffering from or susceptible to a disease or disorder or symptom thereof (e.g., those delineated herein) comprising the step of administering to said subject an effective amount of a compound or a composition of this invention. Some diseases are well known in the art and are also disclosed herein.
- [0163] In certain embodiments, the methods disclosed herein are suitable for treating diseases or disorders that are age-related including common neurodegenerative diseases, such as AD, PD, and HD.
- [0164] The term “co-administered” as used herein means that the second therapeutic agent may be administered together with a compound of this invention as part of a single dosage form (such as a composition of this invention comprising a compound of the invention and an second therapeutic agent as described above) or as separate, multiple dosage forms. Alternatively, the additional agent may be administered prior to, consecutively with, or following the administration of a compound of this invention. In such combination therapy treatment, both the compounds of this invention and the second therapeutic agent (s) are administered by conventional methods. The administration of a composition of this invention comprising both a compound of the invention and a second therapeutic agent to a subject does not preclude the separate administration of that same therapeutic agent, any other second therapeutic agent or any compound of this invention to said subject at another time during a course of treatment.
- [0165] Effective amounts of these second therapeutic agents are well known to those skilled in the art and guidance for dosing may be found in patents and published patent applications referenced herein, as well as in Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000) ; PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000) , and other medical texts. However, it is well within the skilled artisan’s purview to determine the second therapeutic agent’s optimal effective-amount range.

- [0166] In one embodiment of the invention where a second therapeutic agent is administered to a subject, the effective amount of the compound of this invention is less than its effective amount would be where the second therapeutic agent is not administered. In another embodiment, the effective amount of the second therapeutic agent is less than its effective amount would be where the compound of this invention is not administered. In this way, undesired side effects associated with high doses of either agent may be minimized. Other potential advantages (including without limitation improved dosing regimens and/or reduced drug cost) will be apparent to those of skill in the art.
- [0167] In yet another aspect, the invention provides the use of a compound of any of the formulae herein alone or together with one or more of the above-described second therapeutic agents in the manufacture of a medicament, either as a single composition or as separate dosage forms, for treatment or prevention in a subject of a disease, disorder or symptom set forth above. Another aspect of the invention is a compound of the formulae herein for use in the treatment or prevention in a subject of a disease, disorder or symptom thereof delineated herein.
- [0168] In other aspects, the methods herein include those further comprising monitoring subject response to the treatment administrations. Such monitoring may include periodic sampling of subject tissue, fluids, specimens, cells, proteins, chemical markers, genetic materials, etc. as markers or indicators of the treatment regimen. In other methods, the subject is prescreened or identified as in need of such treatment by assessment for a relevant marker or indicator of suitability for such treatment.
- [0169] In one embodiment, the invention provides a method of monitoring treatment progress. The method includes the step of determining a level of diagnostic marker (Marker) (e.g., any target or cell type delineated herein modulated by a compound herein) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof delineated herein, in which the subject has been administered a therapeutic amount of a compound herein sufficient to treat the disease or symptoms thereof. The level of Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level of Marker in the subject is determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of disease or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment according to this invention; this pre-treatment level of Marker can then be compared to the level of Marker in the subject after the treatment commences, to determine the efficacy of the treatment.

- [0170] In certain method embodiments, a level of Marker or Marker activity in a subject is determined at least once. Comparison of Marker levels, e.g., to another measurement of Marker level obtained previously or subsequently from the same patient, another patient, or a normal subject, may be useful in determining whether therapy according to the invention is having the desired effect, and thereby permitting adjustment of dosage levels as appropriate. Determination of Marker levels may be performed using any suitable sampling/expression assay method known in the art or described herein. Preferably, a tissue or fluid sample is first removed from a subject. Examples of suitable samples include blood, urine, tissue, mouth or cheek cells, and hair samples containing roots. Other suitable samples would be known to the person skilled in the art. Determination of protein levels and/or mRNA levels (e.g., Marker levels) in the sample can be performed using any suitable technique known in the art, including, but not limited to, enzyme immunoassay, ELISA, radiolabeling/assay techniques, blotting/chemiluminescence methods, real-time PCR, and the like.
- [0171] The present invention also provides kits for use to treat diseases, disorders, or symptoms thereof, including those delineated herein. These kits comprise: a) a pharmaceutical composition comprising a compound of any of the formula herein or a salt thereof; or a prodrug, or a salt of a prodrug thereof; or a hydrate, solvate, or polymorph thereof, wherein said pharmaceutical composition is in a container; and b) instructions describing a method of using the pharmaceutical composition to treat the disease, disorder, or symptoms thereof, including those delineated herein.
- [0172] The container may be any vessel or other sealed or sealable apparatus that can hold said pharmaceutical composition. Examples include bottles, divided or multi-chambered holders or bottles, wherein each division or chamber comprises a single dose of said composition, a divided foil packet wherein each division comprises a single dose of said composition, or a dispenser that dispenses single doses of said composition. The container can be in any conventional shape or form as known in the art which is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container) , or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle, which is in turn contained within a box. Preferably, the container is a blister pack.
- [0173] The kit may additionally comprise information and/or instructions for the physician, pharmacist or subject. Such memory aids include numbers printed on each chamber or

division containing a dosage that corresponds with the days of the regimen which the tablets or capsules so specified should be ingested, or days of the week printed on each chamber or division, or a card which contains the same type of information.

[0174] The following examples are meant to be illustrative of the practice of the invention and not limiting in any way.

[0175] Examples

[0176] Abbreviations

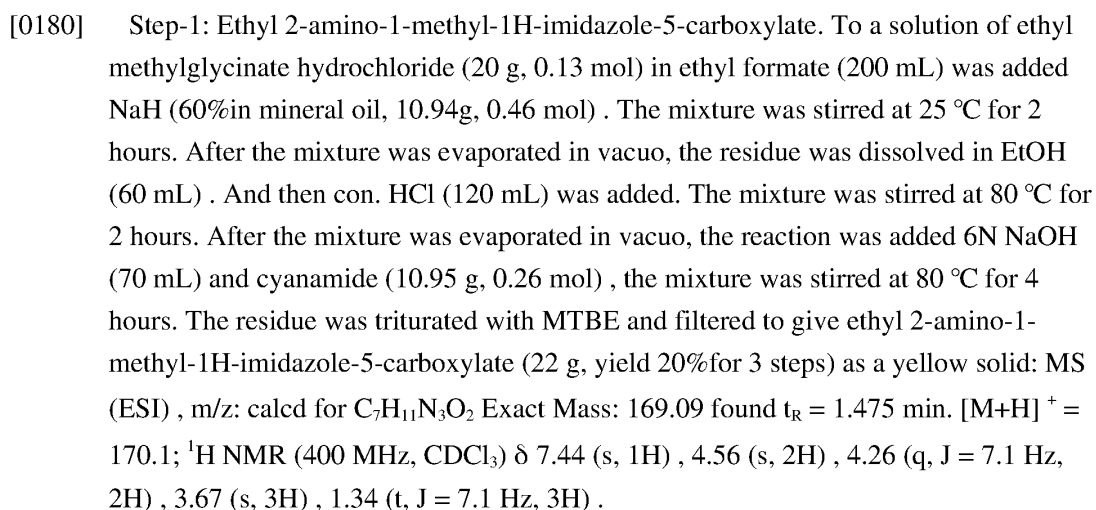
Abbreviation	Name
ACN	Acetonitrile
aq.	Aqueous solution
Ar	Argon
AUC _{Inf}	Area under concentration-time curve from time 0 to infinity
AUC _{last}	Area under concentration-time curve from time 0 to the last quantifiable concentration
Boc	tert-Butyloxycarbonyl
CL	Clearance
C _{max}	Maximum plasma concentration
DCM	Dichloromethane
DMAP	4,4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess–Martin periodinane
DIPEA	N,N-Diisopropylethylamine
EDTA	Ethylenediaminetetraacetic acid
EtOAc or EA	Ethyl acetate
equiv. or eq.	Equivalent
Et	Ethyl
ESI	Electron spray ionization
FW	Formula Weight
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
HOAc or AcOH	Acetic acid

HOBt	Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IV or iv	Intravenous
MS	Mass spectrometry
m/z	Ratio of mass to charge
Me	Methyl
MeCN	Acetonitrile
MTBE	Methyl tert-butyl ether
NMR	Nuclear magnetic resonance
NMI	<i>N</i> -methylimidazole
PK	Pharmacokinetics
PO	Per Oral (by mouth)
rt	Room temperature
SM	Starting material
TCFH	<i>N,N,N',N'</i> -tetramethylchloroformamidinium hexafluorophosphate
TEA	Triethanolamine
T _{max}	Time to maximum plasma concentration
T _{1/2}	Terminal half-life
TH	Tyrosine Hydroxylase

[0177] Chemistry Methods

[0178] All chemicals were purchased from commercial suppliers and used without further purification. Unless otherwise specified, reactions were performed under an inert atmosphere of argon and monitored by thin-layer chromatography (TLC) and/or LCMS. All reagents were purchased from commercial suppliers and used as provided. Synthetic intermediates and final compounds were purified using Biotage Isolera Prime 3.2 chromatography system on 230-400 mesh silica gel or GILSON GX-281 prep-HPLC. ¹H and ¹³C NMR spectra were obtained using Bruker Ascend 400 spectrometer at 400 MHz and 100 MHz, respectively. NMR chemical shifts were described in δ (ppm) using residual solvent peaks as standard (Chloroform-d, 7.26 ppm (¹H) , 77.16 ppm (¹³C) ; Methanol-d₄, 3.31 ppm (¹H) , 49.00 ppm (¹³C) ; DMSO-d₆, 2.50 ppm (¹H) , 39.52 ppm (¹³C)) . Data were reported in a format as follows: chemical shift, multiplicity (s=singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br

[0179] Synthesis Scheme 1



- [0181] Step-2: Ethyl 1-methyl-2-nitro-1H-imidazole-5-carboxylate. To a solution of NaNO_2 (53.82 g, 0.78 mol) in $\text{AcOH}/\text{H}_2\text{O}=1/1$ (600 mL) was added ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate (22 g, 0.13 mol) at 0 °C. The mixture was stirred at 25 °C for 2 hours and then adjusted pH to 8 with saturated aqueous solution of potassium carbonate (600 mL) and extracted with DCM (3 x 400 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by silica gel column, eluted with $\text{PE}/\text{EA}=50/1 \sim 20/1$ to give ethyl 1-methyl-2-nitro-1H-imidazole-5-carboxylate (8 g, yield 41%) as an off-white solid: MS (ESI), m/z : calcd for $\text{C}_7\text{H}_9\text{N}_3\text{O}_4$ Exact Mass: 199.06 found $t_R = 1.446$ min. $[\text{M}+\text{H}]^+ = 200.1$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.79 (s, 1H), 4.34 (q, $J = 7.1$ Hz, 2H), 4.18 (s, 3H), 1.32 (t, $J = 7.1$ Hz, 3H).
- [0182] Step-3: (1-methyl-2-nitro-1H-imidazol-5-yl) methanol. To a solution of 1-methyl-2-nitro-1H-imidazole-5-carboxylate (10 g, 0.05 mol) in $\text{THF}/\text{MeOH}=8/1$ (100 mL) stirred at 0°C was added NaBH_4 (3.8 g, 0.1 mol). The reaction mixture was stirred at 25°C for 2 hours. The mixture was poured into water (100 mL) and extracted with EA (6 x 50 mL). The mixture was concentrated in vacuo. The residue was triturated with DCM and filtered to give (1-methyl-2-nitro-1H-imidazol-5-yl) methanol (5 g, yield 63%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_5\text{H}_7\text{N}_3\text{O}_3$ Exact Mass: 157.05 found $t_R = 1.254$ min. $[\text{M}+\text{H}]^+ = 158.0$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.11 (s, 1H), 5.49 (t, $J = 5.4$ Hz, 1H), 4.54 (d, $J = 5.3$ Hz, 2H), 3.92 (s, 3H).
- [0183] Step-4: 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde. To a solution of (1-methyl-2-nitro-1H-imidazol-5-yl) methanol (10 g, 63.6 mmol) in DCM (100 mL) was added DMP (29.7 g, 70.0 mmol). The mixture was stirred at 25°C for 1 hour. The mixture was poured into aq. $\text{Na}_2\text{S}_2\text{O}_3$ saturation (100 mL) and extracted with DCM (2 x 100 mL). The combine organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by silica gel column, eluted with $\text{PE}/\text{EA}=10/1 \sim 8/1$ to give 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde (8.5 g, yield 86%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_5\text{H}_5\text{N}_3\text{O}_3$ Exact Mass: 155.0 found $RT = 1.341$ min. $[\text{M}+\text{H}]^+ = 155.7$; ^1H NMR (400 MHz, CDCl_3) δ 9.93 (s, 1H), 7.82 (s, 1H), 4.36 (s, 1H).
- [0184] Step-5: Methyl 3-hydroxy-2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate. To a solution of methyl 2-bromo-2-methylpropanoate (19.0 g, 104.8 mmol) in THF (200 mL) stirred under nitrogen at -78°C was added $n\text{-BuLi}$ (41.9 mL, 104.8 mmol, 2.5 mol/L). The mixture was stirred at -78°C for 30 mins. Then 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde (12.5 g, 80.6 mmol) was added and stirred another 5 h. The mixture was poured into saturated aqueous solution of ammonium chloride (200 mL) and extracted with EA (2 x 200 mL). The combine

organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by silica gel column, eluted with PE/EA=8/1 $\sim$ 5/1 to give methyl 3-hydroxy-2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (8.0 g, yield 35%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_5$ Exact Mass: 257.1 found RT = 1.809 min. $[\text{M}+\text{H}]^+ = 257.8$; ^1H NMR (400 MHz, DCCl_3) δ 7.27 (s, 1H), 4.70 (d, $J = 8.0$ Hz, 1H), 4.07 (s, 3H), 3.78 (s, 3H), 3.70 (d, $J = 8.4$ Hz, 1H), 1.38 (s, 3H), 1.32 (s, 3H).

[0185] Step-6: Methyl 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (1). To a solution of methyl 3-hydroxy-2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (8.0 g, 31.1 mmol) in THF (100 mL) was added NaH (60%, 1.6 g, 40.4 mmol) at 0°C . The mixture was stirred at 0°C for 30 mins. Then 1-chloro-2, 4-dinitrobenzene (7.4 g, 40.4 mmol) was added and stirred at 25°C for 4 h. The mixture was adjusted PH=5-6 with aq. HCl (0.5 mol/L) and extracted with EA (2 x 100 mL). The combine organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by silica gel column, eluted with PE/EA=2/1 $\sim$ 1/1 to give compound 1 (5.0 g, yield 38%) as a yellow solid.

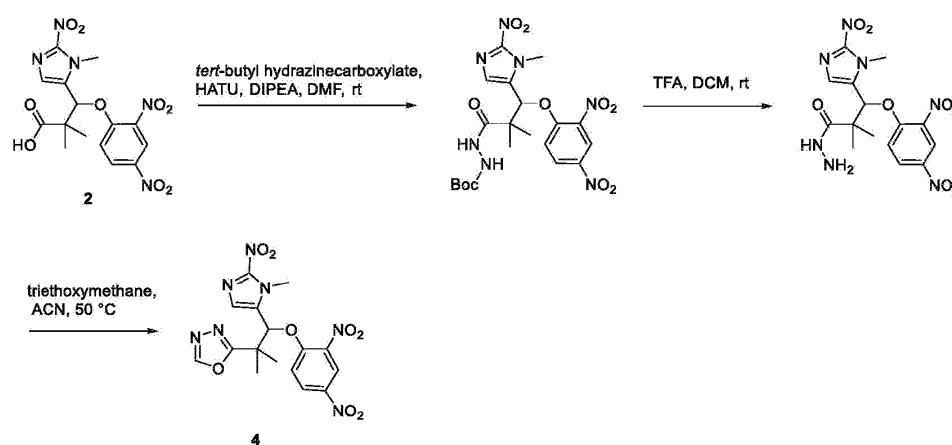
[0186] MS (ESI), m/z : calcd for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_9$ Exact Mass: 423.1 found RT = 3.080 min. $[\text{M}+\text{H}]^+ = 424.1$; ^1H NMR (400 MHz, CDCl_3) δ 8.75 (d, $J = 2.8$ Hz, 1H), 8.36 (dd, $J = 9.2, 2.4$ Hz, 1H), 7.26 (d, $J = 5.2$ Hz, 1H), 6.98 (d, $J = 9.2$ Hz, 1H), 5.96 (s, 1H), 4.16 (s, 3H), 3.73 (s, 3H), 1.50 (s, 3H), 1.28 (s, 3H).

[0187] Step-7: 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoic acid (2). To a solution of 1 (500 mg, 1.18 mmol) in THF/ H_2O =1/1 (10 mL) stirred at 0°C was added lithium hydroxide (33.94 mg, 1.42 mmol). The reaction mixture was stirred at 25°C for 20 hours. The mixture was adjusted pH to 3 with 1N HCl (40 mL), then extracted with EtOAc (40 mL*3). The combined organic layer was dried over anhydrous Na_2SO_4 . The residue was purified via Prep-HPLC (Columns: Sunfire 5 μm 19-150 mm; Mobile phase: ACN/ H_2O (0.1% $\text{NH}_3\text{H}_2\text{O}$); Gradient: 10 $\sim$ 60%, ACN, 7 min; flow rate: 20 mL/min) to give compound 2. A total of seven batches of 1 were de-esterified as described above and then combined to give compound 2 (1.9 g, yield 48%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_{15}\text{H}_{15}\text{N}_5\text{O}_9$ Exact Mass: 409.09 found $t_R = 3.316$ min. $[\text{M}+\text{H}]^+ = 410.0$; ^1H NMR (400 MHz, CD_3OD) δ 8.74 (d, $J = 2.8$ Hz, 1H), 8.39 (dd, $J = 9.3, 2.8$ Hz, 1H), 7.30 (d, $J = 9.3$ Hz, 1H), 7.20 (s, 1H), 6.21 (s, 1H), 4.18 (s, 3H), 1.45 (s, 3H), 1.29 (s, 3H).

[0188] Step-8: 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) -1-morpholinopropan-1-one (3). To a solution of compound 2 (200 mg, 0.489 mmol), HOBt (79.2 mg, 0.586 mmol) and EDCI (112 mg, 0.586 mmol) in DCM

(3 mL) was stirred under nitrogen at 25 °C was added DIPEA (189 mg, 1.47 mmol) and morpholine (63.9 mg, 0.733 mmol). The reaction mixture was stirred at 25 °C for 3 hours. The mixture was washed with water (5 mL) and brine (5 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo, which was purified by Prep-TLC (DCM/MeOH=15/1) to give Compound 3 (111 mg, yield 99%) as an off-white solid: MS (ESI), *m/z*: calcd for C₁₉H₂₂N₆O₉ Exact Mass: 478.14 found *t_R* = 2.062 min. [M + H]⁺ = 479.3; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.74 (d, *J* = 2.8 Hz, 1H), 8.37 (dd, *J* = 9.3, 2.9 Hz, 1H), 7.38 (d, *J* = 9.4 Hz, 1H), 7.18 (s, 1H), 6.30 (s, 1H), 4.11 (s, 3H), 3.54 (d, *J* = 4.0 Hz, 8H), 1.51 (s, 3H), 1.31 (s, 3H).

[0189] Synthesis Scheme 2



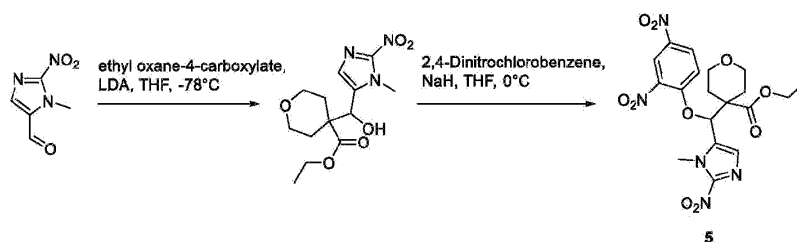
[0190] Step-1: Tert-butyl 2-(3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoyl)hydrazine-1-carboxylate. To a solution of Compound 2 (150 mg, 0.36 mmol) in DMF (5 mL) was added HATU (167.2 mg, 0.44 mmol) and DIPEA (94.7 mg, 0.73 mmol). The mixture was stirred at 25 °C for 30 min. Then tert-butyl carbazate (58.57 mg, 0.44 mmol) was added. The mixture was stirred at 25 °C for 4 hours, and then poured into water (20 mL), extracted with EtOAc (30 mL*3). The combine organic layer was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo, which was purified by silica gel column, eluted with DCM/MeOH=50/1 ~ 20/1 to give the desired compound (140 mg, yield 73%) as a yellow solid: MS (ESI), *m/z*: calcd for C₂₀H₂₅N₇O₁₀ Exact Mass: 523.17 found *t_R* = 1.733 min. [M+H]⁺ = 524.3.

[0191] Step-2: 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanehydrazide. To a solution of tert-butyl 2-(3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoyl)hydrazine-1-carboxylate (140 mg, 0.27 mmol) in DCM (5 mL) was added TFA (2.5 mL) at 0 °C. The mixture was stirred at 30 °C for 2 hours. The mixture was concentrated in vacuo to give

desired compound (110 mg, yield 97%) as a yellow solid: MS (ESI) , m/z: calcd for $C_{15}H_{17}N_7O_8$ Exact Mass: 423.11 found $t_R = 1.437$ min. $[M+H]^+ = 424.2$; 1H NMR (400 MHz, $DMSO-d_6$) δ 9.18 (s, 1H) , 8.75 (d, $J = 2.8$ Hz, 1H) , 8.36 (dd, $J = 9.3, 2.8$ Hz, 1H) , 7.35 (d, $J = 9.4$ Hz, 1H) , 7.10 (s, 1H) , 6.25 (s, 1H) , 4.07 (s, 3H) , 1.33 (s, 3H) , 1.16 (s, 3H) .

[0192] Step-3: 2- (1- (2, 4-dinitrophenoxy) -2-methyl-1- (1-methyl-2-nitro-1H-imidazol-5-yl) propan-2-yl) -1, 3, 4-oxadiazole (4) . To a solution of 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl) propanehydrazide (100 mg, 0.26 mmol) in ACN (5 mL) was added triethoxymethane (116 g, 0.78 mmol) at 30 °C. The mixture was stirred under N_2 atmosphere at 50 °C for 6 hours. The mixture was poured into water (5 mL) and extracted with EtOAc (3 x 20 mL) . The organic layer was washed with water (10 mL) and brine (5 mL) , dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by Prep-TLC (DCM/MeOH=20/1) to give compound 4 (73 mg, yield 64%) as a yellow solid: MS (ESI) , m/z: calcd for $C_{16}H_{15}N_7O_8$ Exact Mass: 433.1 found $t_R = 1.355$ min. $[M+H]^+ = 434.1$; 1H NMR (400 MHz, $DMSO-d_6$) δ 9.26 (s, 1H) , 8.75 (d, $J = 2.8$ Hz, 1H) , 8.36 (dd, $J = 9.3, 2.8$ Hz, 1H) , 7.30 (d, $J = 9.4$ Hz, 1H) , 6.89 (s, 1H) , 6.41 (s, 1H) , 3.99 (s, 3H) , 1.54 (d, $J = 9.5$ Hz, 6H) .

[0193] Synthesis Scheme 3

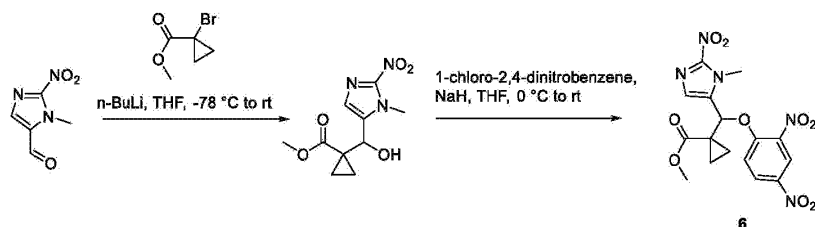


[0194] Step-1: Ethyl 4- (hydroxy (1-methyl-2-nitro-1H-imidazol-5-yl) methyl) tetrahydro-2H-pyran-4-carboxylate. To a solution of ethyl oxane-4-carboxylate (1.27 g, 8.06 mmol) in THF (5 mL) was added LDA (4 mL, 8.06 mmol, 2 mmol/L) under N_2 at -78°C. The mixture was stirred for 0.5 hours under N_2 at -78°C. The reaction mixture was added to a solution of 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde (500 mg, 3.22 mmol) in THF (1 mL) under N_2 at -78°C. The mixture was stirred for 2 hours under N_2 at 25°C. The mixture was quenched by saturated NH_4Cl solution (10 mL) and extracted with EA (3 x 5 mL) . The combine organic layer was washed with brine (10 mL) , dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by prep-TLC with PE/EA=1/1 to give ethyl 4- (hydroxy (1-methyl-2-nitro-1H-imidazol-5-yl) methyl) tetrahydro-2H-pyran-4-carboxylate (200 mg, yield 20%) as a yellow solid: MS (ESI) , m/z: calcd for $C_{13}H_{19}N_3O_6$ Exact Mass: 313.1 found $t_R = 1.514$ min. $[M+H]^+$

$^+ = 314.1$; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.08 (s, 1H), 6.18 (d, $J = 5.5$ Hz, 1H), 4.81 (d, $J = 5.5$ Hz, 1H), 4.11 (td, $J = 7.1, 5.9$ Hz, 2H), 3.80 (dd, $J = 11.7, 3.7$ Hz, 3H), 3.29–3.18 (m, 2H), 3.30–3.20 (m, 2H), 1.99 (d, $J = 13.2$ Hz, 1H), 1.88 (d, $J = 13.4$ Hz, 1H), 1.73 (td, $J = 12.8, 4.7$ Hz, 1H), 1.61 (td, $J = 13.0, 4.8$ Hz, 1H), 1.14 (t, $J = 7.1$ Hz, 3H).

[0195] Step-2: Ethyl 4-((2,4-dinitrophenoxy)(1-methyl-2-nitro-1H-imidazol-5-yl)methyl) tetrahydro-2H-pyran-4-carboxylate (5). To a solution of ethyl 4-(hydroxy(1-methyl-2-nitro-1H-imidazol-5-yl)methyl) tetrahydro-2H-pyran-4-carboxylate (75 mg, 0.23 mmol) in THF (1 mL) was added NaH (10 mg, 0.250 mmol, 60% in oil) at 0°C under N_2 . After 0.5 hours, the reaction was added 1-chloro-2,4-dinitrobenzene (70 mg, 0.34 mmol) at 0°C under N_2 . The mixture was stirred for 2 hours at 0°C under N_2 . The mixture was quenched by saturated NH_4Cl solution (20 mL) and extracted with EA (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo, which was purified by prep-TLC with PE/EA=1/1 to give 5 (90 mg, yield 27%) as an off-white solid: MS (ESI), m/z : calcd for $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}_{10}$ Exact Mass: 479.13 found RT = 2.386 min. $[\text{M}+\text{H}]^+ = 480.1$; ^1H NMR (400 MHz, CDCl_3) δ 8.75 (d, $J = 2.7$ Hz, 1H), 8.35 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.23 (s, 1H), 6.92 (d, $J = 9.3$ Hz, 1H), 5.67 (s, 1H), 4.24 (qd, $J = 7.1, 2.3$ Hz, 2H), 4.10 (s, 3H), 4.01–3.91 (m, 2H), 3.60 (td, $J = 11.9, 1.6$ Hz, 1H), 3.45–3.36 (m, 1H), 2.27 (d, $J = 13.4$ Hz, 1H), 2.11 (d, $J = 11.2$ Hz, 1H), 1.86 (td, $J = 12.7, 5.2$ Hz, 1H), 1.70 (td, $J = 12.5, 4.7$ Hz, 1H), 1.26 (t, $J = 7.1$ Hz, 3H).

[0196] Synthetic Scheme 4

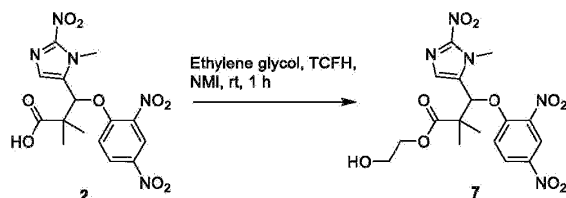


[0197] Step-1: Methyl 1-(hydroxy(1-methyl-2-nitro-1H-imidazol-5-yl)methyl) cyclopropane-1-carboxylate. A solution of methyl 1-bromocyclopropane-1-carboxylate (807 mg, 4.51 mmol) in THF (10 mL) was cooled to -78°C in a dry ice-acetone bath under nitrogen. To the stirred solution was added $n\text{-BuLi}$ (3.61 mL, 9.02 mmol, 2.5 mol/L in THF). After 1 hour, a solution of 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde (700 mg, 4.51 mmol) in THF (2 mL) was added at -78°C . The mixture was stirred for 1 hour and then allowed to warm to 25°C . The reaction was quenched by saturated aqueous ammonium chloride (10 mL) and then extracted with EtOAc (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over

anhydrous sodium sulfate, filtered and evaporated to obtain crude product. The crude product was purified by prep-TLC (EA/PE=1/1) to afford the product (110 mg, yield 10 %) as yellow oil: MS (ESI) , m/z: calcd for $C_{10}H_{13}N_3O_5$ Exact Mass: 255.09 found $t_R = 1.181$ min. $[M+H]^+ = 256.2$; 1H NMR (400 MHz, $DMSO-d_6$) δ 7.03 (s, 1H) , 5.81 (d, J = 6.7 Hz, 1H) , 5.21 (d, J = 6.7 Hz, 1H) , 3.96 (s, 3H) , 1.25 – 1.10 (m, 2H) , 1.12 – 0.93 (m, 2H) .

[0198] Step-2: Methyl 1- ((2, 4-dinitrophenoxy) (1-methyl-2-nitro-1H-imidazol-5-yl) methyl) cyclopropane-1-carboxylate (6) . To a solution of methyl 1- (hydroxy (1-methyl-2-nitro-1H-imidazol-5-yl) methyl) cyclopropane-1-carboxylate (20 mg, 0.0784 mmol) in THF (2 mL) stirred at 0°C was added sodium hydride (3.45 mg, 0.862 mmol, 60% in oil) and 1-chloro-2,4-dinitrobenzene (17.1 mg, 0.0784 mmol) . The reaction mixture was stirred at 25°C for 1 h. The reaction was quenched by saturated aqueous ammonium chloride (5 mL) and then extracted with EtOAc (3 x 5 mL) . The combined organic layer was washed with brine (5 mL) , dried over anhydrous sodium sulfate, filtered and evaporated to obtain crude product. The crude product was purified by prep-HPLC (Column: XBridge-1, 5 μ m, 19-150 mm; detector: 254 nm; mobile phase: ACN/ H_2O (0.1% NH_3 . H_2O in H_2O) ; gradient: 20-53/9 min, 95-95/2 min, ACN in H_2O ; retention time: 8.7 min) to afford compound 6 (14.2 mg, yield 7%) as a yellow solid: MS (ESI) , m/z: calcd for $C_{16}H_{15}N_5O_9$ Exact Mass: 421.09 found $t_R = 2.343$ min. $[M+H]^+ = 422.1$; 1H NMR (400 MHz, $DMSO-d_6$) δ 8.78 (d, J = 2.6 Hz, 1H) , 8.41 (dd, J = 9.3, 2.6 Hz, 1H) , 7.62 (d, J = 9.4 Hz, 1H) , 7.11 (s, 1H) , 6.73 (s, 1H) , 4.00 (s, 3H) , 3.62 (s, 4H) , 1.29 – 1.07 (m, 2.5H) , 0.93 – 0.91 (m, 1.5H) .

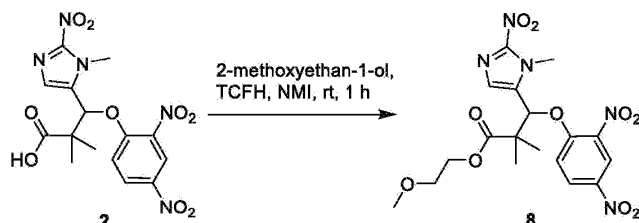
[0199] Synthesis Scheme 5



[0200] 2-Hydroxyethyl 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (7) . A mixture of compound 2 (120 mg, 0.29 mmol) , TCFH (164 mg, 0.58 mmol) and NMI (120 mg, 1.46 mmol) in ethylene glycol (1819 mg, 29.32 mmol) was stirred at rt for 1 hour. LCMS showed SM was consumed and DP was detected. The reaction mixture was diluted with DCM (120 mL) and washed with 1 N HCl (50 mL) . The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 5%) to give product 7 (81 mg, yield 62%) as a yellow solid: MS (ESI) , m/z: calcd for $C_{17}H_{19}N_5O_{10}$ Exact Mass: 453.11 found $t_R = 1.155$ min.

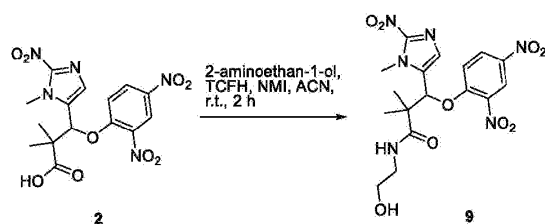
$[M+H]^+ = 454.10$; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.76 (d, $J = 2.8$ Hz, 1H), 8.36 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.27 (s, 1H), 6.98 (d, $J = 9.2$ Hz, 1H), 5.97 (s, 1H), 4.31–4.21 (m, 2H), 4.16 (s, 3H), 3.85–3.76 (m, 2H), 1.68 (br s, 1H), 1.52 (s, 3H), 1.30 (s, 3H).

[0201] Synthesis Scheme 6



[0202] 2-methoxyethyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate (8). A mixture of 2 (110 mg, 0.27 mmol), TCFH (151 mg, 0.54 mmol) and NMI (66 mg, 0.81 mmol) in 2-methoxyethan-1-ol (2044 mg, 26.89 mmol) was stirred at rt for 1 hour. LCMS showed SM was consumed and DP was detected. The reaction mixture was diluted with DCM (120 mL) and washed with 1 N HCl (50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 5%) to give product 8 (39 mg, yield 31%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_{10}$ Exact Mass: 467.13 found $t_R = 1.283$ min. $[M+H]^+ = 468.00$; $^1\text{H NMR}$ (400 MHz, MeOD) δ 8.75 (d, $J = 2.8$ Hz, 1H), 8.39 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.27 (d, $J = 9.2$ Hz, 1H), 7.21 (s, 1H), 6.23 (s, 1H), 4.31–4.26 (m, 1H), 4.21–4.15 (m, 4H), 3.55–3.45 (m, 2H), 3.20 (s, 3H), 1.45 (s, 3H), 1.31 (s, 3H).

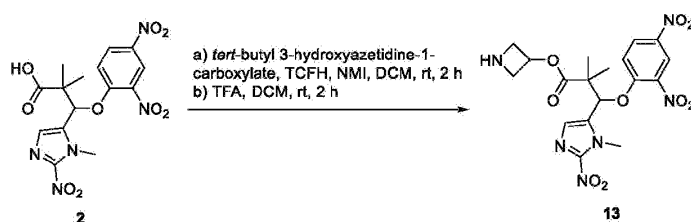
[0203] Synthesis Scheme 7



[0204] 3-(2,4-dinitrophenoxy)-N-(2-hydroxyethyl)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanamide (9). A mixture of compound 2 (65 mg, 0.16 mmol), 2-aminoethan-1-ol (11.6 mg, 0.19 mmol), TCFH (89 mg, 0.32 mmol) and NMI (52 mg, 0.64 mmol) in ACN (2 mL) was stirred at room temperature for 2 hours. After the reaction was completed, the reaction mixture was diluted with 1 N HCl (20 mL) and extracted with EtOAc (20 mL $\times$ 2). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (C18, MeCN/ H_2O (0.1%FA)) to give product 9 (39 mg,

yield 54%) as a white solid: MS (ESI) , m/z : calcd for $C_{17}H_{20}N_6O_9$ Exact Mass: 452.13 found $t_R = 1.051$ min. $[M+H]^+ = 453.0$; 1H NMR (400 MHz, DMSO- d_6) : δ 8.75 (d, $J = 2.8$ Hz, 1H) , 8.36 (dd, $J = 9.2, 2.8$ Hz, 1H) , 7.73 (t, $J = 5.6$ Hz, 1H) , 7.32 (d, $J = 9.2$ Hz, 1H) , 7.12 (s, 1H) , 6.23 (s, 1H) , 4.07 (s, 3H) , 3.35–3.24 (m, 2H) , 3.17–3.04 (m, 2H) , 1.32 (s, 3H) , 1.17 (s, 3H) .

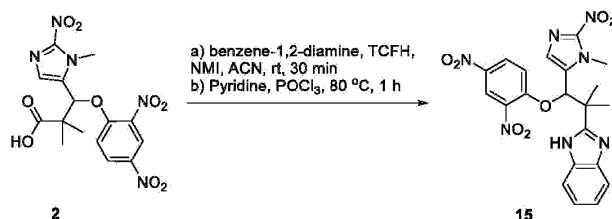
[0205] Synthesis Scheme 8



[0206] Azetidin-3-yl 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (13) . To a solution of compound 2 (110 mg, 0.27 mmol) , TCFH (151 mg, 0.54 mmol) and NMI (47 mg, 0.54 mmol) in DCM (3 mL) was added tert-butyl 3-hydroxyazetidine-1-carboxylate (56 mg, 0.32 mmol) . The reaction mixture was stirred at room temperature for 2 hours. After the reaction was completed, the reaction was quenched by H_2O (50 mL) and extracted by DCM (10 mL x 3) . The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (12 g, silica gel, DCM in $CH_3OH = 5\%$) to give the intermediate

[0207] A solution of intermediate generated in previous step in TFA/DCM (1 mL/3 mL) was stirred at room temperature for 2 hours. After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was freeze-dried to give product 13 (84 mg, yield 67%for 2 steps) as a yellow solid: MS (ESI) , m/z : calcd for $C_{18}H_{20}N_6O_9$ Exact Mass: 464.13 found $t_R = 0.844$ min. $[M+H]^+ = 465.00$; 1H NMR (400 MHz, MeOD) δ 8.76 (d, $J = 2.8$ Hz, 1H) , 8.40 (dd, $J = 9.2, 2.4$ Hz, 1H) , 7.24–7.19 (m, 2H) , 6.18 (s, 1H) , 5.35 (m, 1H) , 4.46–4.38 (m, 2H) , 4.18 (s, 3H) , 4.16–4.09 (m, 2H) , 1.49 (s, 3H) , 1.36 (s, 3H) .

[0208] Synthesis Scheme 9

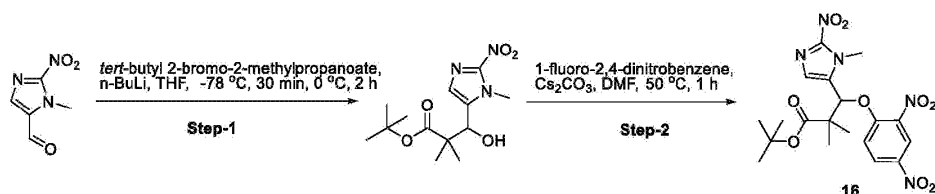


[0209] 2- (1- (2, 4-dinitrophenoxy) -2-methyl-1- (1-methyl-2-nitro-1H-imidazol-5-yl) propan-2-yl) -1H-benzo [d] imidazole (15) . A mixture of compound 2 (120 mg,

0.293 mmol), benzene-1, 2-diamine (35 mg, 0.322 mmol), N, N, N', N'-Tetramethylchloroformamidinium-hexafluorophosphate (TCFH, 206 mg, 0.733 mmol) and 1-methylimidazole (NMI, 96 mg, 1.173 mmol) in ACN (5 mL) was stirred at room temperature for 30 minutes. After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was purified by FCC (25 g silica gel, MeOH in DCM = 5%) to give the intermediate as a brown oil.

[0210] To a solution of the intermediate in pyridine (3 mL) was added phosphoryl trichloride (80 mg, 0.52 mmol) at room temperature under N₂. The reaction mixture was stirred and heated at 80 °C for 1 hour under N₂. After the reaction was completed, the mixture was diluted with EtOAc (30 mL) and washed with 0.5 N HCl (30 mL × 2). The collected organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (12 g silica gel, MeOH in DCM = 0~5%) to give the product 15 (30 mg, yield 21% for 2 steps) as a yellow solid: MS (ESI), m/z: calcd for C₂₁H₁₉N₇O₇ Exact Mass: 481.13 found t_R = 1.066 min. [M+H]⁺ = 482.0; ¹H NMR (400 MHz, d₆-DMSO) δ 12.20 (br s, 1H), 8.71 (d, J = 2.8 Hz, 1H), 8.34 (dd, J = 9.2, 2.8 Hz, 1H), 7.63–7.42 (m, 2H), 7.39 (d, J = 9.2 Hz, 1H), 7.20–7.12 (m, 2H), 7.06 (s, 1H), 6.44 (s, 1H), 3.76 (s, 3H), 1.67 (s, 3H), 1.51 (s, 3H).

[0211] Synthesis Scheme 10

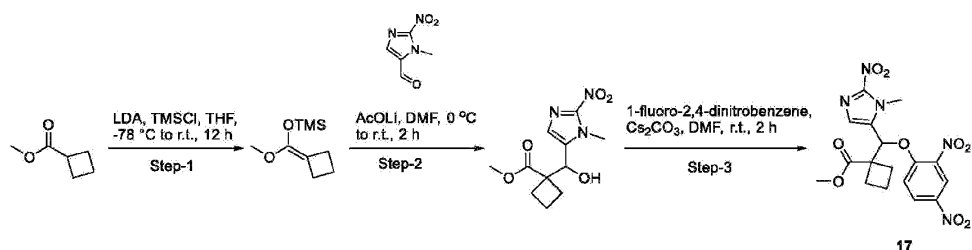


[0212] Step 1: tert-butyl 3-hydroxy-2, 2-dimethyl-3- (3-methyl-2-nitroimidazol-4-yl) propanoate. To a solution of tert-butyl 2-bromo-2-methylpropanoate (11.21 g, 0.05 mol) in THF (100 mL) stirred under nitrogen at -78 °C was added n-BuLi (3.22 g, 0.05 mmol) dropwise. The reaction mixture was stirred at -78 °C for 30 minutes. Then a solution of 3-methyl-2-nitroimidazole-4-carbaldehyde (6.0 g, 0.04 mol) in THF (20 mL) was added dropwise. The reaction mixture was stirred at 0 °C for another 2 hours. The reaction was quenched with NH₄Cl (30 mL) and diluted with EtOAc (30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (80 g, silica gel, PE/EA = 60%) to give the Compound 3 (5.0 g, 43% yield) as a yellow solid.

[0213] Step 2: tert-butyl 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (3-methyl-2-nitroimidazol-4-yl) propanoate (16). A solution of tert-butyl 3-hydroxy-2, 2-dimethyl-3- (3-methyl-2-nitroimidazol-4-yl) propanoate (5 g, 0.017 mol), 1-fluoro-2, 4-dini-

trobenzene (3.7 g, 0.020 mol) and Cs_2CO_3 (9.15 g, 0.028 mol) in DMF (20 mL) was stirred and heated at 50 °C for 1 hour. The reaction mixture was diluted with H_2O (100 mL), extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (80 g, silica gel, PE/EA = 60%) to give the product 16 (6.2 g, 80% yield) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_9$, Exact Mass: 465.15 found $[\text{M}+\text{H}]^+ = 466.1$.

[0214] Synthesis Scheme 11



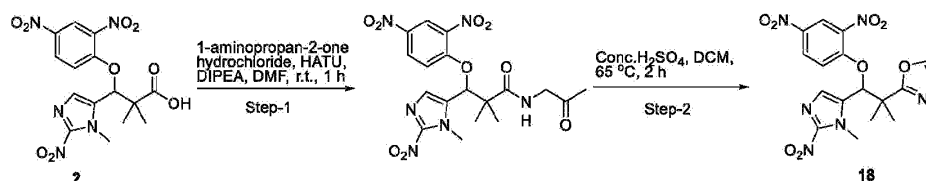
[0215] Step-1: (cyclobutylidene (methoxy) methoxy) trimethylsilane. To a solution of methyl cyclobutanecarboxylate (4.0 g, 35 mmol) in THF (12 mL) was added Lithium diisopropylamide (LDA, 22 mL, 44 mmol, 2 M in THF) at -78 °C under N_2 . The reaction mixture was stirred at -78 °C for 1 hour. Then a solution of chlorotrimethylsilane (TMSCl, 4.75 g, 43.7 mmol) in THF (4 mL) was added. The reaction mixture was stirred at -78 °C for 1 hour and stirred at room temperature for another 10 hours. After the reaction was completed, the reaction mixture was diluted with PE (100 mL) and washed with water (100 mL). The organic layer was collected, dried over Na_2SO_4 and concentrated under reduced pressure to obtain the product (5.5 g, yield 84%) as a brown oil: ^1H NMR (400 MHz, CDCl_3): δ 3.36 (s, 3H), 2.58–2.50 (m, 2H), 2.41–2.36 (m, 2H), 1.76–1.68 (m, 2H), 0.11 (s, 9H).

[0216] Step-2: methyl 1- (hydroxy (1-methyl-2-nitro-1H-imidazol-5-yl) methyl) cyclobutane-1-carboxylate. To a mixture of 1-methyl-2-nitro-1H-imidazole-5-carbaldehyde (110 mg, 0.71 mmol) and AcOLi (14 mg, 0.213 mmol) in DMF (2 mL) was added (cyclobutylidene (methoxy) methoxy) trimethylsilane (528 mg, 2.84 mmol) at 0 °C under N_2 . The reaction mixture was stirred at 0 °C for 1 hour and at room temperature for 1 hour. After the reaction was completed, the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (30 mL x 2). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (4 g silica gel, EtOAc in PE = 0~50%) to give the product (18 mg, yield 9%) as a brown oil: MS (ESI), m/z : calcd for $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_5$ Exact Mass: 269.10 found $t_R = 0.932$ min. $[\text{M}+\text{H}]^+ = 270.10$; ^1H NMR (400 MHz, CDCl_3): δ 6.68 (s, 1H), 4.87 (d, $J = 10.4$ Hz, 1H), 4.12 (s, 3H), 3.95 (d, J

= 10.4 Hz, 1H), 3.81 (s, 3H), 2.77–2.68 (m, 1H), 2.58–2.47 (m, 2H), 2.18–2.12 (m, 1H), 2.05–1.96 (m, 2H).

[0217] Step 3: methyl 1-((2, 4-dinitrophenoxy)(1-methyl-2-nitro-1H-imidazol-5-yl)methyl)cyclobutane-1-carboxylate (17). To a mixture of methyl 1-(hydroxy(1-methyl-2-nitro-1H-imidazol-5-yl)methyl)cyclobutane-1-carboxylate (18 mg, 0.07 mmol) and 1-fluoro-2, 4-dinitrobenzene (16.2 mg, 0.087 mmol) in DMF (1 mL) was added Cs_2CO_3 (33 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 2 hours. After the reaction was completed, the reaction mixture was diluted with Sat. NaCl (50 mL) and extracted with EtOAc (20 mL $\times$ 2). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (4 g silica gel, MeOH in DCM = 0 ~ 5%), followed by prep-HPLC (C18, MeCN/ H_2O (0.1%FA)) to obtain product 17 (6 mg, yield 20%) as a white solid: MS (ESI), m/z : calcd for $\text{C}_{17}\text{H}_{17}\text{N}_5\text{O}_9$ Exact Mass: 435.10 found $t_R = 1.333$ min. $[\text{M}+\text{H}]^+ = 436$; ^1H NMR (400 MHz, CDCl_3): δ 8.77 (d, $J = 2.4$ Hz, 1H), 8.39 (dd, $J = 9.2, 2.4$ Hz, 1H), 7.26 (s, 1H), 7.04 (d, $J = 9.2$ Hz, 1H), 5.87 (s, 1H), 4.10 (s, 3H), 3.77 (s, 3H), 2.60–2.41 (m, 4H), 2.23–2.11 (m, 1H), 1.94–1.84 (m, 1H).

[0218] Synthesis Scheme 11

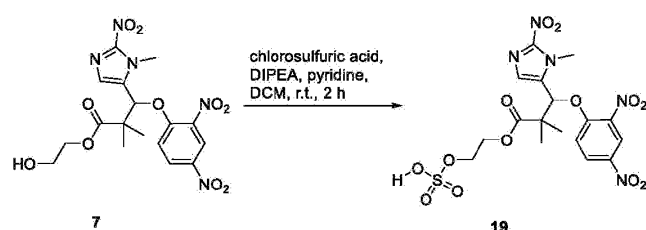


[0219] Step-1: 3-(2, 4-dinitrophenoxy)-2, 2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)-N-(2-oxopropyl)propanamide. A mixture of compound 2 (250 mg, 0.61 mmol), 1-aminopropan-2-one hydrochloride (53 mg, 0.73 mmol), HATU (278 mg, 0.73 mmol) and DIPEA (275 mg, 2.14 mmol) in DMF (5 mL) was stirred at room temperature for 1 hour. After the reaction was completed, the reaction mixture was diluted with EtOAc (40 mL) and washed with water (50 mL $\times$ 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (12 g silica gel, MeOH in DCM = 5%) to give the product (220 mg, yield 78%) as a brown oil: MS (ESI), m/z : calcd for $\text{C}_{18}\text{H}_{20}\text{N}_6\text{O}_9$ Exact Mass: 464.13 found $t_R = 1.150$ min. $[\text{M}+\text{H}]^+ = 465$.

[0220] Step-2: 2-(1-(2, 4-dinitrophenoxy)-2-methyl-1-(1-methyl-2-nitro-1H-imidazol-5-yl)propan-2-yl)-5-methyloxazole (18). To a stirred solution of 3-(2, 4-dinitrophenoxy)-2, 2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)-N-(2-oxopropyl)propanamide (140 mg, 0.61 mmol) in DCM (2 mL) was added Conc. H_2SO_4 (2 mL)

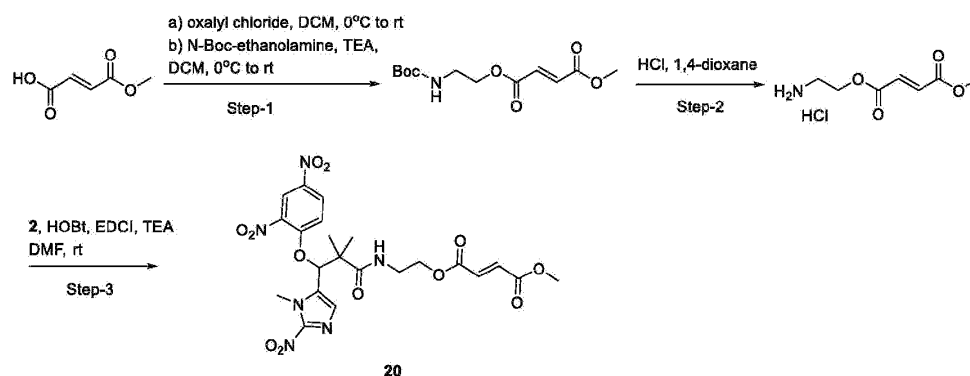
at room temperature. Then the reaction mixture was stirred and heated at 65 °C for 2 hours. After the reaction was completed, the reaction mixture was poured into water (50 mL) and extracted with DCM (30 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (C18, MeCN/H₂O (0.1%FA)) to give compound 18 (35 mg, yield 26%) as a white solid: MS (ESI), m/z: calcd for C₁₈H₁₈N₆O₈ Exact Mass: 446.12 found t_R = 1.346 min, [M+H]⁺ = 447; ¹H NMR (400 MHz, CDCl₃): δ 8.74 (d, J = 2.8 Hz, 1H), 8.34 (dd, J = 9.2, 2.8 Hz, 1H), 7.19 (s, 1H), 6.97 (d, J = 9.2 Hz, 1H), 6.64 (s, 1H), 5.89 (s, 1H), 3.94 (s, 3H), 2.28 (s, 3H), 1.65 (s, 3H), 1.51 (s, 3H).

[0221] Synthesis Scheme 12



[0222] 2- (sulfoxy) ethyl 3- (2, 4-dinitrophenoxy) -2, 2-dimethyl-3- (1-methyl-2-nitro-1H-imidazol-5-yl) propanoate (19). To a solution of compound 7 (90 mg, 0.20 mmol) in DCM (2 mL) was added a solution of DIPEA (256 mg, 1.98 mmol) and pyridine (156 mg, 1.98 mmol) in DCM (1 mL) at 0 °C. Then a solution of chlorosulfuric acid (231 mg, 1.98 mmol) in DCM (1 mL) was added at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. After the reaction was completed, the reaction mixture was diluted with water (20 mL) and extracted with DCM (20 mL × 2). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (C18, MeCN/H₂O (0.1%NH₃·H₂O)) to give product 19 (52 mg, yield 49%) as a yellow solid: MS(ESI), m/z: calcd for C₁₇H₁₉N₅O₁₃S Exact Mass: 533.07 found t_R = 1.092 min. [M-H]⁻ = 532; ¹H NMR (400 MHz, DMSO-d₆): δ 8.74 (d, J = 2.8 Hz, 1H), 8.36 (dd, J = 9.2, 2.8 Hz, 1H), 7.48 (d, J = 9.2 Hz, 1H), 7.23–6.94 (m, 5H), 6.36 (s, 1H), 4.21–4.11 (m, 2H), 4.09 (s, 3H), 3.93–3.81 (m, 2H), 1.34 (s, 3H), 1.22 (s, 3H).

[0223] Synthesis Scheme 13



[0224] Step-1: 2-((tert-butoxycarbonyl)amino)ethyl methyl fumarate. To a solution of (E)-4-methoxy-4-oxobut-2-enoic acid (1 g, 7.7 mmol) in DCM (10 mL) was added oxalyl chloride (1.47 g, 11.6 mmol) at 0 °C. The reaction mixture was stirred under N₂ at 25 °C for 3 hours. The reaction mixture was evaporated in vacuo to give crude product methyl (E)-4-chloro-4-oxobut-2-enoate (1 g, yield 88 %) as a brown solid.

[0225] To a solution of N-Boc-ethanolamine (1.31 g, 8.08 mmol) and TEA (1.63 g, 16.2 mmol) in DCM (10 mL) was added methyl (E)-4-chloro-4-oxobut-2-enoate (800 mg, 5.39 mmol) in DCM (5 mL) at 0 °C. The reaction mixture was stirred under N₂ at 0 °C for 1 hour. The reaction was quenched by addition water (50 mL) and extracted with DCM (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by silica gel column chromatography (PE/EA=30/1~10/1) to give 2-((tert-butoxycarbonyl)amino)ethyl methyl fumarate (400 mg, yield 27 %) as a white solid: MS (ESI), m/z: calcd for C₁₂H₁₉NO₆ Exact Mass: 273.121 found t_R = 1.815 min. [M+H-100]⁺ = 174.1; ¹H NMR (400 MHz, CDCl₃) δ 6.88 (s, 2H), 4.77 (br. s, 1H), 4.27 (t, J = 5.3 Hz, 2H), 3.82 (s, 3H), 3.45 (d, J = 5.1 Hz, 2H), 1.45 (s, 9H).

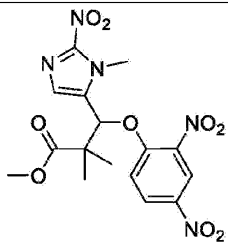
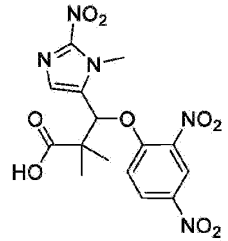
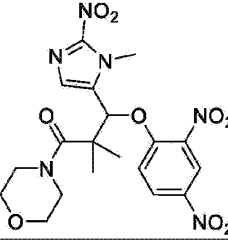
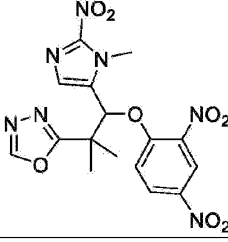
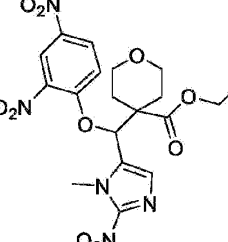
[0226] Step-2: 2-aminoethyl methyl fumarate hydrochloride. To a solution of 2-((tert-butoxycarbonyl)amino)ethyl methyl fumarate (450 mg, 1.64 mmol) in dioxane/HCl (9 mL). The reaction mixture was stirred at 25°C for 1 hour. The reaction mixture was evaporated in vacuo to give 2-aminoethyl methyl fumarate hydrochloride (270 mg, yield 64 %) as an off-white solid: MS (ESI), m/z: calcd for C₇H₁₁NO₄ Exact Mass: 173.069 found t_R = 0.353 min. [M+H]⁺ = 174.1; ¹H NMR (400 MHz, DMSO-d₆) δ 8.16 (br. s, 3H), 6.99 (d, J = 15.9 Hz, 1H), 6.77 (d, J = 15.9 Hz, 1H), 4.34 (t, J = 5.2 Hz, 2H), 3.77 (s, 3H), 3.15 (t, J = 5.2 Hz, 2H).

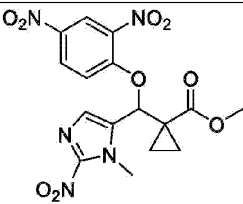
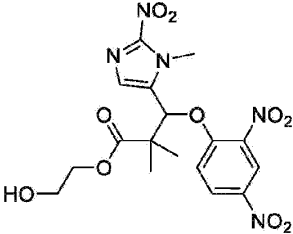
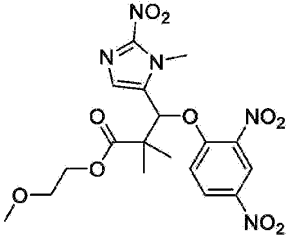
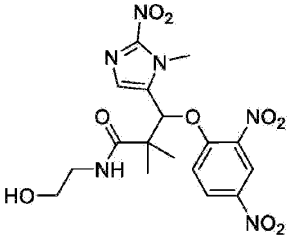
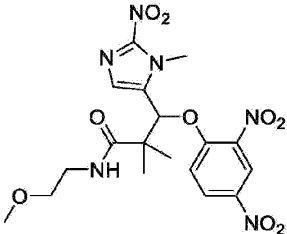
[0227] Step-3: 2-(3-(2-(4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanamido)ethyl methyl fumarate (20). To a solution of 2-aminoethyl methyl fumarate hydrochloride (156 mg, 0.64 mmol) and compound 2 (130 mg, 0.32 mmol) in DMF (3 mL) was added HOBT (51.5 mg, 0.38 mmol),

EDCI (73.1 mg, 0.38 mmol) and TEA (128 mg, 1.27 mmol) . The reaction mixture was stirred under N₂ at room temperature for 16 hours. The reaction was quenched by addition water (10 mL) and extracted with EA (3 x 3 mL) . The combined organic layer was washed with brine (5 mL) , dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by Prep-TLC (DCM/MeOH=20/1) to give product 20 (90 mg, yield 48 %) as pale yellow solid: MS (ESI) , m/z: calcd for C₂₂H₂₄N₆O₁₂ Exact Mass: 564.145 found t_R = 1.639 min and 1.699 min. [M+H]⁺ = 565.2; ¹H NMR (400 MHz, CDCl₃) δ 8.76 (t, J = 2.8 Hz, 1H) , 8.38 –8.32 (m, 1H) , 7.23 (d, J = 14.9 Hz, 1H) , 7.03 (d, J = 9.2 Hz, 0.4H) , 6.89 (t, J = 8.1 Hz, 0.6H) , 6.87 –6.75 (m, 2H) , 6.41 (br. s, 0.55H) , 6.19 (br. s, 0.45H) , 6.04 (d, J = 3.2 Hz, 1H) , 4.38 –4.26 (m, 1.6H) , 4.24 –4.14 (m, 3.4H) , 3.82 (d, J = 2.2 Hz, 3H) , 3.72 –3.48 (m, 2H) , 1.48 (d, J = 3.3 Hz, 3H) , 1.23 (d, J = 9.4 Hz, 3H) .

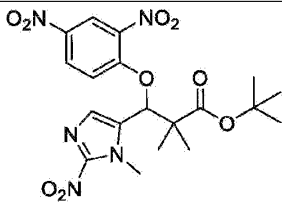
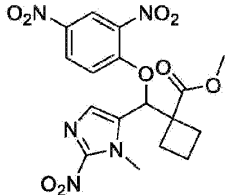
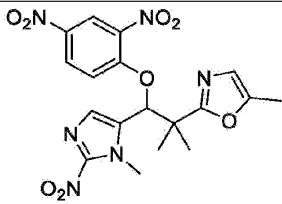
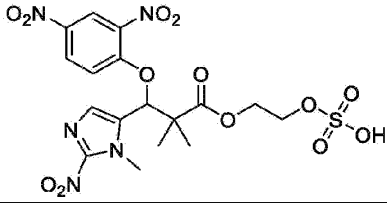
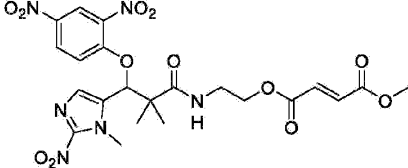
[0228] Table 1. Structures and Characterization of Exemplary Compounds

Example #	Structure	Chemical Name	LC-MS (M+H ⁺)
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1		methyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	424.1
2		3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoic acid	410.0
3		3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)-1-morpholinopropan-1-one	479.3
4		2-(1-(2,4-dinitrophenoxy)-2-methyl-1-(1-methyl-2-nitro-1H-imidazol-5-yl)propan-2-yl)-1,3,4-oxadiazole	434.1
5		ethyl 4-((2,4-dinitrophenoxy)(1-methyl-2-nitro-1H-imidazol-5-yl)methyl)tetrahydro-2H-pyran-4-carboxylate	480.1

6		methyl 1-((2,4-dinitrophenoxy)(1-methyl-2-nitro-1H-imidazol-5-yl)methyl)cyclopropane-1-carboxylate	422.1
7		2-hydroxyethyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	454.1
8		2-methoxyethyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	468.0
9		3-(2,4-dinitrophenoxy)-N-(2-hydroxyethyl)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanamide	453.0
10		3-(2,4-dinitrophenoxy)-N-(2-methoxyethyl)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanamide	

11		3-(2,4-dinitrophenoxy)-1-(3-hydroxyazetidin-1-yl)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propan-1-one	
12		3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)-1-(piperazin-1-yl)propan-1-one	
13		azetidin-3-yl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	465.0
14		piperidin-4-yl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	
15		2-(1-(2,4-dinitrophenoxy)-2-methyl-1-(1-methyl-2-nitro-1H-imidazol-5-yl)propan-2-yl)-1H-benzo[d]imidazole	482.0

16		tert-butyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	466.1
17		methyl 1-((2,4-dinitrophenoxy)(1-methyl-2-nitro-1H-imidazol-5-yl)methyl)cyclobutane-1-carboxylate	436.0
18		2-(1-(2,4-dinitrophenoxy)-2-methyl-1-(1-methyl-2-nitro-1H-imidazol-5-yl)propan-2-yl)-5-methyloxazole	447
19		2-(sulfooxy)ethyl 3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanoate	532
20		2-(3-(2,4-dinitrophenoxy)-2,2-dimethyl-3-(1-methyl-2-nitro-1H-imidazol-5-yl)propanamido)ethyl methyl fumarate	465.2

[0229] Study I. Pharmacokinetics Studies in Mice

[0230] General protocol

[0231] Male C57BL/6 mice (20~40 g, Zhejiang Vital River or SLAC) were randomly divided into groups (n = 3) to receive the intravenous (iv) dose (1 mg/kg, 5 mL/kg) or po. dose (5 mg/kg, 10 mL/kg) of the testing article. The formulations were prepared by adding the appropriate volume of the vehicle to the test article to achieve the desired concentration. For IV group, mice received a single IV bolus injection of the test article via tail vein. And for PO group, mice received oral gavage of test article. At least 30 μ L of blood was collected at each time point (at 2 min (IV only) , 5 min (PO only) , 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h after dosing) via saphenous or mandibular vein. The whole blood was immediately collected into an EP tube containing EDTA-K2 on wet ice and centrifuged at 3500g and at 4°C for 5 minutes to obtain the plasma within 30 minutes. Plasma samples were placed in sealed tubes on

dry ice, and then stored in -80°C freezer until analysis. The concentration of the analyte in the mouse plasma was quantified with a LC-MS/MS method based on multiple reaction monitoring (MRM) of fragment ions. The method consisted of two separate standard curves bracketing all other samples. Three levels of QCs (low, medium, and high) were used to ensure the reliability of the assay. Pharmacokinetic parameters were calculated with Phoenix WinNonlin software (version 8.3, Certara, Princeton, NJ) using non-compartmental analyses.

[0232] The pharmacokinetic of 2, 4-DNP following an IV (1 mg/kg) or a PO (5 mg/kg) dosing in male C57BL/6 mice, respectively, are shown in Table 2 and the mean plasma concentrations are shown in FIG. 1.

[0233] Table 2. Pharmacokinetic Study of 2, 4-DNP (Intravenous and Oral Administrations in Male C57BL/6 mice^a)

Compound	C0/Cmax (ng/mL)	AUC _{last} (h*ng/mL)	Cmax/AUC
2,4-DNP (iv) ^b	3777	7322	
2,4-DNP (po) ^c	11367	37907	0.30

[0234] ^a Mice PK parameter (mean, n = 3) . ^b Dosed IV (1 mg/kg) . ^c Dosed PO (5 mg/kg)

[0235] The results of pharmacokinetic study of compound 1 and its metabolite 2, 4-DNP after an IV (1 mg/kg) or a PO (5 mg/kg) dosing of 1 in male C57BL/6 mice, respectively, are shown in Table 3. The mean plasma concentrations of 1 and its metabolite 2, 4-DNP are shown in FIG. 2.

[0236] Table 3. Pharmacokinetic Study of Compound 1 (IV and PO administrations in Male C57BL/6 mice^a)

Compound	C0/Cmax (ng/mL)	AUC _{last} (h*ng/mL)	Cmax/AUC
1 (iv) ^b	974	476	
1_DNP (iv) ^d	742	2754	0.27
1 (p.o) ^c	567	2154	
1_DNP (p.o) ^d	2273	12406	0.18

- [0237] ^a Mice PK parameter (mean, n = 3) . ^b IV (1mg/kg) . ^c PO (5 mg/kg) . ^d2, 4-Dinitrophenol released from the parent compound
- [0238] These data indicated that compound 1 showed significantly lowered C_{max}/AUC ratios in mice as compared to 2, 4-DNP.
- [0239] The results of pharmacokinetic study of compound 7 and its metabolite 2, 4-DNP after an IV (1 mg/kg) or a PO (5 mg/kg) dosing of 7 in male C57BL/6 mice, respectively, are shown in Table 4. The mean plasma concentrations of 7 and its metabolite 2, 4-DNP are shown in FIG. 3.
- [0240] Table 4. Pharmacokinetic Study of Compound 7 (IV and PO administrations in Male C57BL/6 mice^a)

Compound	C ₀ /C _{max} (ng/mL)	AUC _{last} (h*ng/mL)	C _{max} /AUC
7 (iv) ^b	889	310	
7_DNP (iv) ^d	813	2239	0.36
7 (p.o) ^c	724	1098	
7_DNP (p.o) ^d	2717	10352	0.26

- [0241] ^a Mice PK parameter (mean, n = 3) . ^b IV (1mg/kg) . ^c PO (5 mg/kg) . ^d2, 4-Dinitrophenol released from the parent compound
- [0242] These data indicated that compound 7 showed significantly lowered C_{max}/AUC ratios in mice as compared to 2, 4-DNP.
- [0243] Study II. Pharmacokinetics Studies in Rats
- [0244] General protocol
- [0245] Male SD rats (170~300 g, Zhejiang Vital River) were randomly divided into groups (n = 3) to receive the intravenous (iv) dose (1 mg/kg, 5 mL/kg) or po. dose (5 mg/kg, 10 mL/kg) of the testing article. The formulations were prepared by adding the appropriate volume of the vehicle to the test article to achieve the desired concentration. For IV group, rats received a single intravenous (IV) bolus injection of the test article via tail vein. And for the PO group, animals received testing article via oral gavage. At least 65 μL of blood was collected at each time point (2 min (IV only) , 5 min (PO only) , 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h after dosing) via jugular vein. The whole blood was immediately collected into an EP tube containing EDTA-K2, kept on wet ice and centrifuged at 3500g at 4°C for 5 minutes to obtain the plasma within 30 minutes. Plasma samples were placed in sealed tubes on dry ice, and then stored

in -80°C freezer until analysis. The concentration of the analyte in rat plasma was quantified with a LC-MS/MS method based on multiple reaction monitoring (MRM) of fragment ions. The method consisted of two separate standard curves bracketing all other samples. Three levels of QCs (low, medium, and high) were used to ensure the reliability of the assay. Pharmacokinetic parameters were calculated with Phoenix WinNonlin software (version 8.3, Certara, Princeton, NJ) using non-compartmental analyses.

[0246] The results of pharmacokinetic study of 2, 4-DNP following IV (1 mg/kg) and PO (5 mg/kg) dosing in male SD rats are shown in Table 5 and the mean plasma concentrations are shown in FIG. 4.

[0247] Table 5. Pharmacokinetic Study of 2, 4-DNP (IV or PO administration in Male SD Rats^a)

Compound	C ₀ /C _{max} (ng/mL)	AUC _{last} (h*ng/mL)	C _{max} /AUC
2,4-DNP (iv) ^b	5596	15351	
2,4-DNP (po) ^c	15767	110659	0.14

[0248] ^a Rats PK parameter (mean, n = 3) . ^b Dosed IV (1 mg/kg) . ^c Dosed PO (5 mg/kg) .

[0249] The results of pharmacokinetic study of compound 1 and its metabolite 2, 4-DNP after an IV (1 mg/kg) or PO (5 mg/kg) dosing in male SD rats are shown in Table 6. The mean plasma concentrations of 1 and its metabolite 2, 4-DNP are shown in FIG. 5.

[0250] Table 6. Pharmacokinetic Study of compound 1 (IV (1 mg/kg) or a PO (5 mg/kg) administration in Male SD rats^a)

Compound	C ₀ /C _{max} (ng/mL)	AUC _{last} (h*ng/mL)	C _{max} /AUC
1 (iv) ^b	824	439	
1_DNP (iv) ^d	1002	7279	0.14
1 (po) ^c	215	577	
1_DNP (po) ^d	2083	28912	0.07

- [0251] ^a Rats PK parameter (mean, n = 3) . ^b Dosed IV (1 mg/kg) . ^c Dosed PO (5 mg/kg) .
^d2, 4-Dinitrophenol released from the parent compound 1
- [0252] The results indicated that compound 1 showed much lowered C_{max}/AUC ratios vs 2, 4-DNP in rats.
- [0253] The results of pharmacokinetic study of compound 7 and its metabolite 2, 4-DNP after an IV (1 mg/kg) or PO (5 mg/kg) dosing in male SD rats are shown in Table 7. The mean plasma concentrations of 7 and its metabolite 2, 4-DNP are shown in FIG. 6.
- [0254] Table 7. Pharmacokinetic Study of compound 7 (IV (1 mg/kg) or a PO (5 mg/kg) administration in Male SD rats^a)

Compound	C0/C _{max} (ng/mL)	AUC _{last} (h*ng/mL)	C _{max} /AUC
7 (iv) ^b	857	276	
7_DNP (iv) ^d	890	4211	0.21
7 (po) ^c	423	517	
7_DNP (po) ^d	2353	18170	0.13

- [0255] ^a Rats PK parameter (mean, n = 3) . ^b Dosed IV (1 mg/kg) . ^c Dosed PO (5 mg/kg) .
^d2, 4-Dinitrophenol released from the parent compound 7
- [0256] The results indicated that compound 7 showed much lowered C_{max}/AUC ratios vs 2, 4-DNP in rats.
- [0257] Study III. Pharmacokinetics Studies in Dogs
- [0258] General protocol
- [0259] Male Beagle dogs (7~10 kg, Beijing Marshall Biotechnology) were randomly divided into groups (n = 3) to receive the intravenous (iv) dose (1 mg/kg, 5 mL/kg) or po dose (5 mg/kg, 10 mL/kg) of the testing article. The formulations were prepared by adding the appropriate volume of the vehicle to the test article to achieve the desired concentration. For IV group, dogs received a single intravenous (IV) bolus injection of the test article via vein. And for PO group, dog received test article via oral gavage. At least 150 μL of blood was collected at each time point (5 min (IV only) , 10 min (PO only) , 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h (PO only) after dosing) . The whole blood was immediately collected into an EP tube containing EDTA-K2 on wet ice and centrifuged at 3500g at 4°C for 5 minutes to obtain the plasma within 30 minutes. Plasma samples were placed in sealed tubes on dry ice, and then stored in -80°C freezer until analysis. The concentration of the analyte in the rat plasma was quantified with a

LC-MS/MS method based on multiple reaction monitoring (MRM) of fragment ions. The method consisted of two separate standard curves bracketing all other samples. Three levels of QCs (low, medium, and high) were used to ensure the reliability of the assay. Pharmacokinetic parameters were calculated with Phoenix WinNonlin software (version 8.3, Certara, Princeton, NJ) using non-compartmental analyses.

[0260] The results of pharmacokinetic study of compound 7 and its metabolite 2, 4-DNP after an IV (1 mg/kg) or PO (5 mg/kg) dosing in male Beagle dogs are shown in Table 8. The mean plasma concentrations of 7 and its metabolite 2, 4-DNP are shown in FIG. 7.

[0261] Table 8. Pharmacokinetic Study of compound 7 (IV (1 mg/kg) or a PO (5 mg/kg) administration in male Beagle dogs^a)

Compound	C ₀ /C _{max} (ng/mL)	AUC _{last} (h*ng/mL)	C _{max} /AUC
7 (iv) ^b	879	762	
7_DNP (iv) ^d	639	5700	0.11
7 (po) ^c	849	1773	
7_DNP (po) ^d	1983	23585	0.084

[0262] ^a Dogs PK parameter (mean, n = 3) . ^b Dosed IV (1 mg/kg) . ^c Dosed PO (5 mg/kg) .

^d2, 4-Dinitrophenol released from the parent compound 7

[0263] The results indicated that compound 7 showed low C_{max}/AUC ratios.

[0264] Study IV. Rectal temperature measurement in rats

[0265] General protocol

[0266] Male SD Rats (200~250 g, Zhejiang Vital River) were randomly divided into groups (n = 8) to receive the oral gavage of various doses of desired compounds. The formulations were prepared by adding the appropriate volume of vehicle to the test article to achieve the desired concentration before the experiment. Rectal temperature was measured with a microprobe thermometer (Physitemp Instruments) at 0 min, 15 min, 30 min, 45 min, 60 min, 90 min and 120 min after dosing.

[0267] The results of SD rats' rectal temperature following a single oral gavage of 2, 4-DNP at 5 mg/kg, 25 mg/kg and 125 mg/kg, respectively, are shown in FIG. 8. The survival curves are shown in FIG. 9.

[0268] The results of SD rats' rectal temperature following a single oral gavage of 2, 4-DNP at 25 mg/kg, compound 1 at 50 mg/kg and 500 mg/kg, respectively, are shown in

FIG. 10. There are no significant differences in the temperature between vehicle and compound 1 treatment groups. No dose-dependent mortality was observed, either.

- [0269] Results disclosed herein support the conclusion that compound 1 did not cause body temperature increase, even at 500 mg/kg dose, as compared to the vehicle group, while 2, 4-DNP significantly increased body temperature at the dose of 25 mg/kg. The results are consistent with the lowered C_{max}/AUC ratio for compound 1 as compared to 2, 4-DNP, resulting in an improved safety profile.
- [0270] Study V. Compound protects dopaminergic neurons and improves behavior deficit in a mouse model of Parkinson's disease
- [0271] General protocol
- [0272] The merits of treating Parkinson's disease with compound 7 was evaluated in wild type mice with 6-hydroxydopamine hydrobromide (6-OHDA) injection. we examined the neuroprotective effect of varying compound 7 doses (2.5 mg/kg, 8 mg/kg or 16 mg/kg) against dopaminergic degeneration of nigral and striatal neurons induced by a single stereotaxic injection of 6-OHDA in the medial forebrain bundle (MFB) area of 8 weeks old male C57 BL/6 mice. Compound 7 was administered by daily oral gavage initiated at 3 days before 6-OHDA stereotaxic injection and continuing for 5 weeks thereafter. Dose volume was 10 mL/Kg. Mice were randomly assigned to one of five treatment groups (10 mice/group) . At 5 weeks post 6-OHDA injection, animals were injected with apomorphine and behavior changes were evaluated. The animals showed certain number of counterclockwise full-body rotations during 30 minutes indicating the success of PD model. Later motor performance was evaluated via rotarod test and grip strength measurements. Finally, animals were sacrificed, and brain samples were collected for further analysis.
- [0273] Tyrosine Hydroxylase (TH) immunoreactive neurons were quantified. FIG. 11 to FIG. 15 show the protective effect of compound 7 treatment against dopaminergic neuronal loss and behavior deficit.
- [0274] Compound 7 treatment ameliorated motor deficits in the 6-OHDA mouse PD model. As shown in FIG. 11, the performance of mice on rotarod was even and the mice were assigned to different treatment groups base on the baseline value of time on rotarod before 6-OHDA injection and compound treatment. The prolonged time on the rotarod after 5 weeks compound 7 treatment show a dose-dependent trend (FIG. 12) , which means that capacity of mice on rotarod from falling off was improved. And peak grip strength was significantly improved after compound 7 (2.5mpk, 8mpk and 16mpk) treatments (FIG. 13) .
- [0275] Additionally, tyrosine hydroxylase (TH) positive neurons were evaluated to see if compound treatment can ameliorate dopaminergic neuron loss in the 6-OHDA PD model. As shown in FIG. 14 and FIG. 15, 16mpk compound 7 was effective in

protecting dopaminergic neuronal loss in the substantia nigra and striatum after 6-OHDA injection. Significantly more TH immunoreactive neurons present in the substantia nigra of 16mpk compound 7 treated mice compared to the control mice. Besides, 16mpk compound 7 treatment preserved more striatal TH levels as compared to control group, suggesting the neuron protection effect of compound 7.

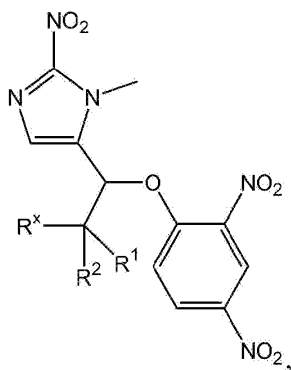
- [0276] Applicant's disclosure is described herein in preferred embodiments with reference to the Figures, in which like numbers represent the same or similar elements. Reference throughout this specification to "one embodiment," "an embodiment," or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment," "in an embodiment," and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.
- [0277] The described features, structures, or characteristics of Applicant's disclosure may be combined in any suitable manner in one or more embodiments. In the description, herein, numerous specific details are recited to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that Applicant's composition and/or method may be practiced without one or more of the specific details, or with other methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the disclosure.
- [0278] Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.
- [0279] Incorporation by Reference
- [0280] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.
- [0281] Equivalents
- [0282] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed,

various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

Claims

[Claim 1]

A compound having structural formula (I) :



(I)

or a pharmaceutically acceptable form or an isotope derivative thereof, wherein

each of R¹ and R² is independently a C₁₋₆ alkyl, or R¹ and R², together with the carbon atom they are bonded to, form a 3-to 8-membered carbocyclic or heterocyclic ring, wherein the C₁₋₆ alkyl and the 3-to 8-membered carbocyclic or heterocyclic ring are optionally substituted with 1-6 R^A; and

R^x is L-R^{X1}, L-R^{X2} or L-R^{X3}, wherein

L is a single bond or (CH₂)_n, wherein n is 1, 2 or 3;

R^{X1} is a group selected from: C(=O)OR³, C(=O)NR⁴R⁵, OR⁶, NR⁷R⁸, NR⁹C(=O)R¹⁰, OC(=O)R¹¹, halo and CN;

R^{X2} is a 5-or 6-membered monocyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-4 R^B; and

R^{X3} is an 8-to 10-membered bicyclic carbocyclic, heterocycle, aryl or heteroaryl group, optionally substituted with 1-6 R^B;

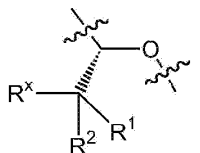
each of R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ and R¹¹

is independently selected from H and C₁₋₆ alkyl, 3-to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A, or R⁴ and R⁵ or R⁷ and R⁸, together with the N atom they are bonded to, respectively, form a 3-to 8-membered heterocyclic ring, optionally substituted with 1-6 R^A;

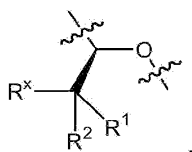
each R^A is independently selected from the group consisting of: D, halo, R and OR;

each R^B is independently selected from the group consisting of: D, halo, CN, R, OR, NRR' , $C(=O)OR$, $C(=O)NRR'$, $NRC(=O)R'$, $OC(=O)R$, SO_4R , and $OC(=O)CHCHC(=O)OR'$; and each R and R' is independently H or C_{1-3} alkyl.

[Claim 2] The compound of claim 1, having a chirality as shown below:



[Claim 3] The compound of claim 1, having a chirality as shown below:



[Claim 4] The compound of any one of claims 1-3, wherein each of R^1 and R^2 is independently a C_{1-3} alkyl.

[Claim 5] The compound of any one of claims 1-3, wherein each of R^1 and R^2 is methyl.

[Claim 6] The compound of any one of claims 1-3, wherein R^1 and R^2 , together with the carbon atom they are bonded to, form a 3-to 6-membered carbocyclic ring.

[Claim 7] The compound of claim 6, wherein the 3-to 6-membered carbocyclic ring is cyclopropyl.

[Claim 8] The compound of any one of claims 1-3, wherein R^1 and R^2 , together with the carbon atom they are bonded to, form a 3-to 6-membered heterocyclic ring.

[Claim 9] The compound of any one of claims 1-8, wherein R^x is $L-R^{X1}$.

[Claim 10] The compound of any one of claims 1-8, wherein R^x is $L-R^{X2}$.

[Claim 11] The compound of any one of claims 1-8, wherein R^x is $L-R^{X3}$.

[Claim 12] The compound of claim 9, wherein R^{X1} is $C(=O)OR^3$.

[Claim 13] The compound of claim 9, wherein R^{X1} is $C(=O)NR^4R^5$.

[Claim 14] The compound of claim 10, wherein R^{X2} is 5-membered heteroaryl group, optionally substituted with 1-4 R^B .

[Claim 15] The compound of claim 10, wherein R^{X2} is 6-membered aryl or heteroaryl group, optionally substituted with 1-4 R^B .

- [Claim 16] The compound of claim 11, wherein R^{X3} is a 9-membered bicyclic aryl or heteroaryl group, optionally substituted with 1-6 R^B .
- [Claim 17] The compound of claim 11, wherein R^{X3} is a 10-membered bicyclic aryl or heteroaryl group, optionally substituted with 1-6 R^B .
- [Claim 18] The compound of any one of claims 1-17, wherein L is a single bond.
- [Claim 19] The compound of any one of claims 1-17, wherein L is $(CH_2)_n$, wherein n is 1, 2 or 3.
- [Claim 20] The compound of claim 19, wherein n is 1.
- [Claim 21] A compound selected from Table 1.
- [Claim 22] The compound of any of claims 1-21, having one or more deuterium atoms in place of one or more hydrogen atoms.
- [Claim 23] The compound of claim 22, having one deuterium atom in place of one hydrogen atom.
- [Claim 24] A pharmaceutical composition comprising a compound according to any of claims 1-23.
- [Claim 25] A unit dosage form comprising a pharmaceutical composition of claim 24.
- [Claim 26] The unit dosage form of claim 25, being a tablet.
- [Claim 27] The unit dosage form of claim 25, being a capsule.
- [Claim 28] A method for treating or reducing a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of any one of claims 1-23.
- [Claim 29] The method of claim 28, wherein the disease or disorder is associated with one or more defect in mitochondrial function.
- [Claim 30] The method of claim 28, wherein the disease or disorder is obesity, diabetes, insulin resistance, liver disease, heart or renal failure, or a related disease or disorder.
- [Claim 31] The method of claim 28, wherein the disease or disorder is obesity, excess body fat, diabetes, insulin resistance or intolerance, high blood pressure, dyslipidemia, cardiovascular disease, atherosclerosis, hypertriglyceridemia, acquired lipodystrophy, inherited lipodystrophy, partial lipodystrophy, metabolic syndrome, Rett's syndrome, metabolic syndrome associated with aging, metabolic diseases associated with increased reactive oxygen species (ROS), Friedreich's ataxia, neurodegenerative diseases or liver disease, or a related disease or disorder.
- [Claim 32] A method for reducing toxicity or side effects in treating mitochondria-related disorders or conditions comprising administering

- to a subject in need thereof a therapeutically effective amount of a compound of any one of claims 1-23.
- [Claim 33] The method of any one of claims 28-32, wherein administration is via oral administration.
- [Claim 34] Use of a compound of any of claims 1-23, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating a disease or disorder.
- [Claim 35] Use of a compound of any of claims 1-23 for treating a disease or disorder.
- [Claim 36] The use of claim 34 or 35, wherein the disease or disorder is associated with one or more defect in mitochondrial function.
- [Claim 37] The use of claim 35, wherein the disease or disorder is selected from the group consisting of obesity, excess body fat, diabetes, insulin resistance or intolerance, high blood pressure, dyslipidemia, cardiovascular disease, atherosclerosis, hypertriglyceridemia, acquired lipodystrophy, inherited lipodystrophy, partial lipodystrophy, metabolic syndrome, Rett's syndrome, metabolic syndrome associated with aging, metabolic diseases associated with increased reactive oxygen species (ROS) , Friedreich's ataxia, and liver disease, or a related disease or disorder.