

PRODRUGS AND CONJUGATES OF 2,4-DINITROPHENOL, AND COMPOSITIONS AND METHODS THEREOF

Priority Claims and Related Patent Applications

[0001] This application claims the benefit of priority to Chinese Application No. 202311324983.X, filed October 12, 2023, and to U.S. Provisional Application Serial No. 63/544,113, filed October 13, 2023, the entire content of each of which is incorporated herein by reference.

Technical Field of the Invention

[0002] The invention generally relates to novel compounds and therapeutic uses thereof. More particularly, the invention provides novel 2,4-dinitrophenol (DNP) derivatives, prodrugs and conjugates 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, dyslipidemia, liver diseases, heart or renal failure, neurodegenerative diseases and aging related diseases, including sarcopenia, osteoporosis, *etc.*)

Background of the Invention

[0003] Metabolic syndrome, represented by type 2 diabetes, obesity, dyslipidemia, hypertension, and metabolic dysfunction-associated fatty liver disease (MAFLD), 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.) The likely causes of these conditions include excessive lipid accumulation within the respective organs, along with chronic inflammation caused by excessive reactive oxygen species (ROS) production overtime. 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.

[0004] 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 or 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 clinical 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).

[0005] In addition to its utility in anti-obesity through elevated energy expenditure, in particular, through increased lipid metabolism, mitochondria uncouplers, in general, can be viewed as in-direct activators for AMPK, one of the master regulators for energy homeostasis, which critically impacts on maintaining/building muscle mass and function (Narkar, *et al.* **2008** *Cell*, 134, 405; Fan, *et al.* **2017**, *Cell Metabolism*, 25, 242). Additionally, DNP has also been shown to reduce excessive ROS productions, prevention of mitochondria Ca^{2+} overload and induction of brain derived neurotrophic factor (BDNF), which may bring benefits for many neurodegenerative diseases. (Kishimoto, *et al.* **2020** *Neurobiology of Aging* 85, 123.)

[0006] In summary, 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, neurodegenerative and aging related diseases and disorders, *e.g.*, sarcopenia or osteoporosis.

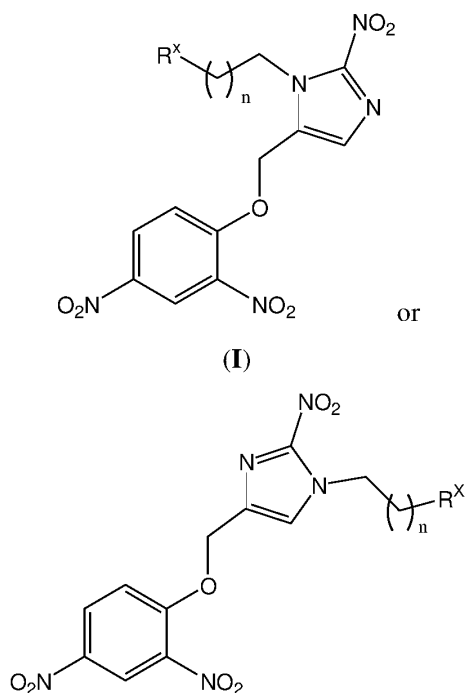
Summary of the Invention

[0007] 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 administration, these compounds release DNP with significantly lowered plasma C_{max} over AUC ratios, as compared to DNP itself, resulting in improved safety windows.

[0008] Additionally, the present invention provides novel conjugate compounds that affect or are involved in two or more mechanisms of action, *e.g.*, mitochondria uncoupling with a mechanism that reduces the production of MGO (*e.g.*, a Nrf2 activator) and/or an anti-inflammatory mechanism.

[0009] Thus, compounds of the invention can therefore be used to treat various metabolic diseases, neurodegenerative diseases, aging related diseases, liver disease, and/or cardiovascular disease.

[0010] In one aspect, the invention generally relates to a compound having structural formula (I) or (II):



(II)

or a pharmaceutically acceptable form or an isotope derivative thereof,

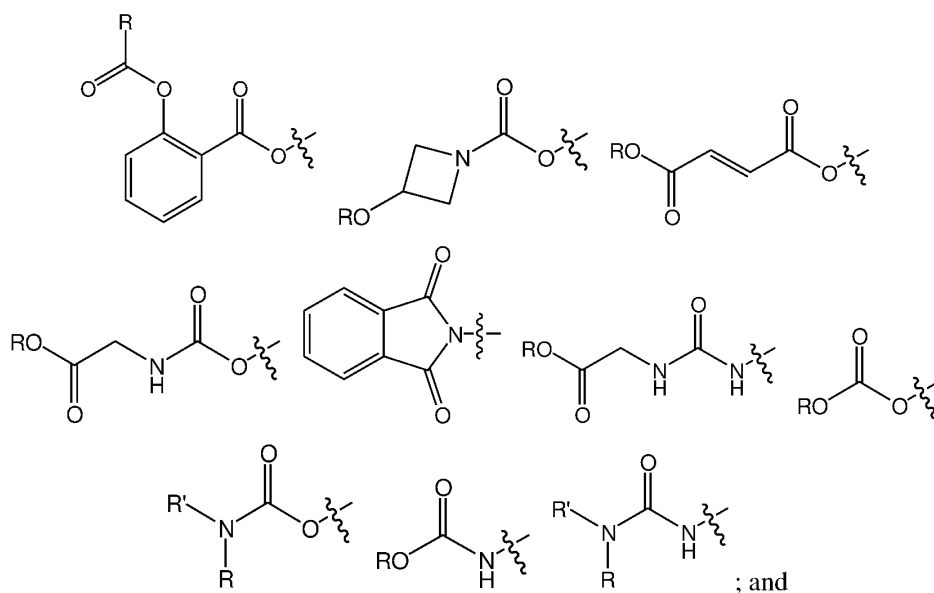
wherein

n is 1, 2, 3 or 4; and

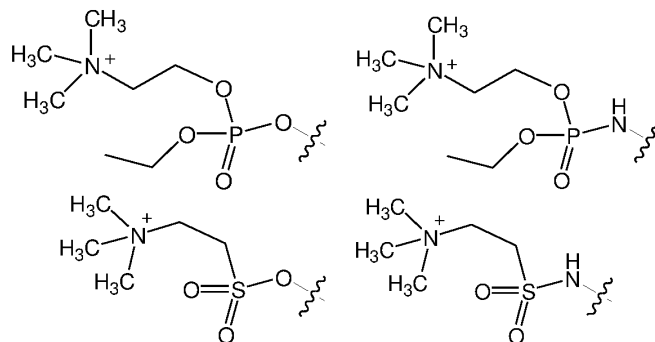
R^X is R^{X1} , R^{X2} or R^{X3} , wherein

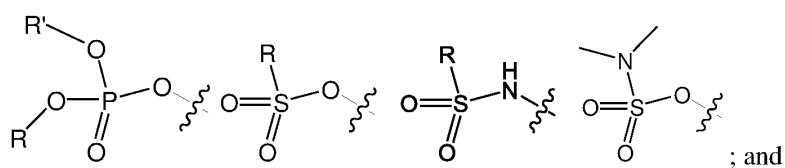
R^{X1} is a group selected from: CH₂C(=O)OR³, CH₂C(=O)SR³, C(=O)NR⁴R⁵, OR⁶, SR⁶, NR⁷R⁸, NR⁹C(=O)R¹⁰, OC(=O)R¹¹, SC(=O)R¹², OC(=O)OR¹³, SC(=O)OR¹³, NR⁹C(=O)OR¹³, OC(=O)NR¹⁴R¹⁵, SC(=O)NR¹⁴R¹⁵, NR⁹C(=O)NR¹⁴R¹⁵, OC(=O)SR³, SC(=O)SR³, NR⁹C(=O)SR³, halo, CN, H, and C₁₋₆ alkyl; and

R^{X2} is a group selected from:



R^{X3} is a group selected from:





each of R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} :

is independently selected from H and C_{1-6} alkyl, 3- to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A , or

R^4 and R^5 , R^7 and R^8 , or R^{14} and R^{15} , 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 ;

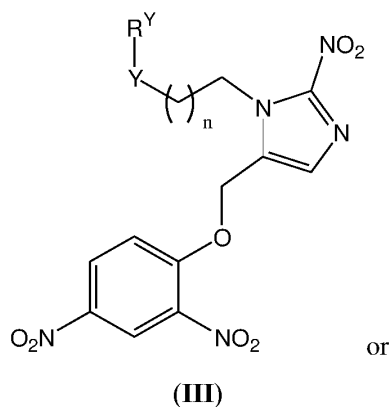
each R^A is independently selected from the group consisting of: deuterium D, halo, R, OR and NRR' ; and

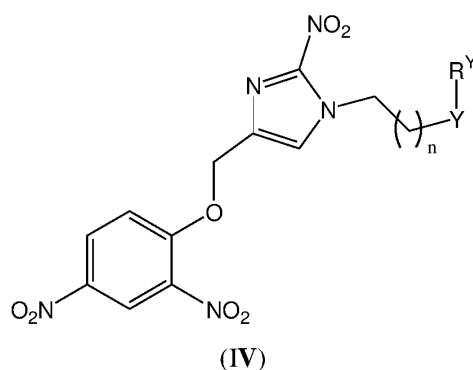
each of R and R'

is independently H or C_{1-6} alkyl, optionally substituted with 1-4 groups selected from D, halo, C_{1-6} alkoxy and amino; or

R and R' together with the N atom they are bonded to, respectively, form a 3- to 6-membered heterocyclic ring, optionally substituted with 1-4 groups selected from D, halo, C_{1-6} alkyl or alkoxy, and amino.

[0011] In another aspect, the invention generally relates to a compound having structural formula (III) or (IV):





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

n is 1, 2, 3 or 4;

Y is selected from a single bond, NR, S, CRR' and O;

R^Y is a group selected from: H, halo, CN, R, C(=O)OR, C(=O)NRR', C(=O)R and C(=O)SR; and

each of R and R' is independently H or C₁₋₆ alkyl, or R and R', together with the N atom they are bonded to, form a 3- to 6-membered heterocyclic ring, wherein the alkyl or heterocyclic ring is optionally substituted with 1-4 groups selected from D, halo, C₁₋₆ alkyl or alkoxy, and amino.

[0012] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising a compound disclosed herein.

[0013] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of a compound disclosed herein.

[0014] 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.

[0015] 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.

[0016] 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.

[0017] 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

[0018] **FIG. 1.** Exemplary data on mean plasma concentration 2,4-DNP after an IV dose of 1 mg/kg and a PO dose of 5mg/kg, respectively, in male C57BL/6 mice (N=3/group).

[0019] **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).

[0020] **FIG. 3.** 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, respectively, in male SD Rats (N=3/group).

[0021] **FIG. 4.** Exemplary data on mean plasma concentration of Compound 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).

[0022] **FIG. 5.** Exemplary data on mean plasma concentration of Compound 14 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).

[0023] **FIG. 6.** Exemplary data on mean plasma concentration of Compound 1 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).

[0024] **FIG. 7.** Exemplary data on SD rats rectal temperature following a single oral gavage of 2,4-DNP.

[0025] **FIG. 8.** Exemplary data on SD rats survival acute curve following a single oral gavage of 2,4-DNP.

[0026] **FIG. 9.** Exemplary data on SD rats rectal temperature following a single oral gavage of 2,4-DNP or Compound 1, respectively.

[0027] **FIG. 10.** Exemplary data on Body weight change after Compound 1 treatment.

[0028] **FIG. 11.** Exemplary data on fasting blood glucose levels after Compound 1 treatment.

[0029] **FIG. 12.** Exemplary data on fasting blood insulin levels after Compound 1 treatment.

[0030] **FIG. 13a.** Exemplary data on plasma TC levels after Compound 1 treatment.

[0031] **FIG. 13b.** Exemplary data on plasma TG levels after Compound 1 treatment.

[0032] **FIG. 13c.** Exemplary data on plasma HDL levels after Compound 1 treatment.

- [0033] **FIG. 13d.** Exemplary data on plasma LDL levels after Compound **1** treatment.
- [0034] **FIG. 13e.** Exemplary data on plasma MDA levels after Compound **1** treatment.
- [0035] **FIG. 14a.** Exemplary data on liver TC levels after Compound **1** treatment.
- [0036] **FIG. 14b.** Exemplary data on liver TG levels after Compound **1** treatment.
- [0037] **FIG. 15a.** Exemplary data on plasma ALT levels after Compound **1** treatment.
- [0038] **FIG. 15b.** Exemplary data on plasma AST levels after Compound **1** treatment.
- [0039] **FIG. 16a.** Exemplary data on NAS score after Compound **1** treatment.
- [0040] **FIG. 16b.** Exemplary data on steatosis score after Compound **1** treatment.
- [0041] **FIG. 16c.** Exemplary data on inflammation score after Compound **1** treatment.
- [0042] **FIG. 16d.** Exemplary data on hepatocytes ballooning score after Compound **1** treatment.
- [0043] **FIG. 17a.** Exemplary data on running distance after Compound **1** treatment.
- [0044] **FIG. 17b.** Exemplary data on running time after Compound **1** treatment.
- [0045] **FIG. 18.** Exemplary data on total muscle index after Compound **1** treatment.
- [0046] **FIG. 19.** Exemplary data on Type I muscle fibers changes after Compound **1** treatment.
- [0047] **FIG. 20.** Exemplary data on p-AMPK α /AMPK α protein ratio in left quadriceps muscle after Compound **1** treatment.
- [0048] **FIG. 21.** Exemplary data on PDK4 gene expression in left quadriceps muscle after Compound **1** treatment.

Definitions

[0049] 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.

[0050] The following terms, unless indicated otherwise according to the context wherein the terms are found, are intended to have the following meanings.

[0051] 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.

[0052] Any compositions or methods disclosed herein can be combined with one or more of any of the other compositions and methods provided herein.

[0053] 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.

[0054] 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.

[0055] 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)-.

[0056] 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).

[0057] 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.

[0058] As used herein, "at least" a specific value is understood to be that value and all values greater than that value.

[0059] 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.

[0060] 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.

[0061] As used herein, the term “hydrate” means a compound which further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

[0062] 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.

[0063] In certain embodiments, a “pharmaceutically acceptable form” includes, but is not limited to, pharmaceutically acceptable salts, esters and isotopically labeled derivatives thereof.

[0064] In certain embodiments, a “pharmaceutically acceptable form” includes, but is not limited to, pharmaceutically acceptable isomers and stereoisomers and isotopically labeled derivatives thereof.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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 intermolecular forces.

[0072] 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).

[0073] 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.

[0074] 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.

[0075] 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.

[0076] As used herein, the terms "alk" or "alkyl" refer to straight or branched chain 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.

[0077] As used herein, the term "alkoxy" refers to an -O-alkyl radical.

[0078] 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.

[0079] 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.

[0080] As used herein, the term "halo" or "halogen" refers to any radical of fluorine, chlorine, bromine or iodine.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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

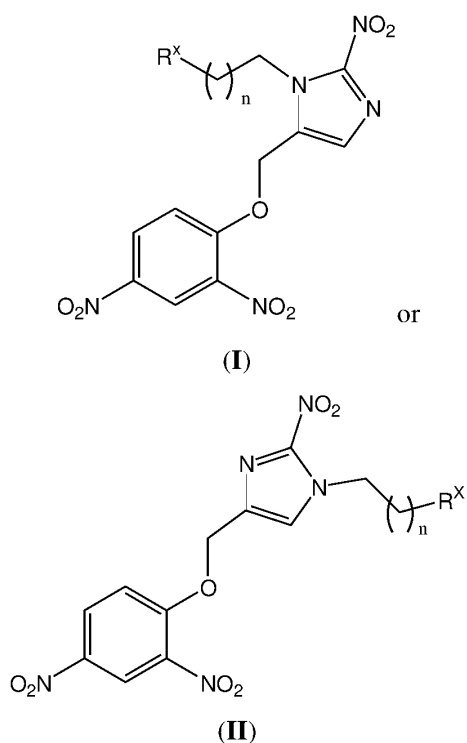
[0086] The invention provides novel DNP derivatives, prodrugs and conjugates thereof, as modulators of mitochondrial activities. The invention also provides pharmaceutical compositions comprising a compound of the invention and methods thereof for treating various diseases and disorders associated with or related to mitochondrial dysfunctions (*e.g.*, obesity, diabetes, insulin resistance, liver diseases, neurodegenerative diseases, heart or renal failure).

[0087] Recent studies showed that dicarbonyl stress, a situation where dicarbonyl metabolites, such as methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone accumulate due to increased production and/or decreased detoxification, to be associated with metabolic and age-related diseases, both of which are characterized by a pro-inflammatory and pro-oxidant state. (Nigro, *et al.* **2019** *Cells* 8, 749.) For example, MGO, the most reactive one among them, is produced due to aberrant glucose/fructose metabolisms. Not only it significantly promotes reactive oxygen species (ROS) production and depletion of glutathione in cells, it also forms advanced glycation end products (AGE), resulting in the loss of normal function of proteins, mitochondrial dysfunction and cell aging or death. (Seo, *et al.* **2014** *Toxicology Res.* 30, 193; Alejandra **2017** *Adv. Nutr.* 8, 54; Nigro, *et al.* **2019** *Cells* 8, 749.)

[0088] Without wishing to be bound by the theory, certain compounds of the invention involve two or more mechanisms of action that combine mitochondria uncoupling with a mechanism for

reducing production of MGO (*e.g.*, a Nrf2 activator) and/or an anti-inflammatory mechanism has significant implications in treating metabolic diseases, neuro-degenerative diseases and aging related diseases.

[0089] In one aspect, the invention generally relates to a compound having structural formula (I) or (II):



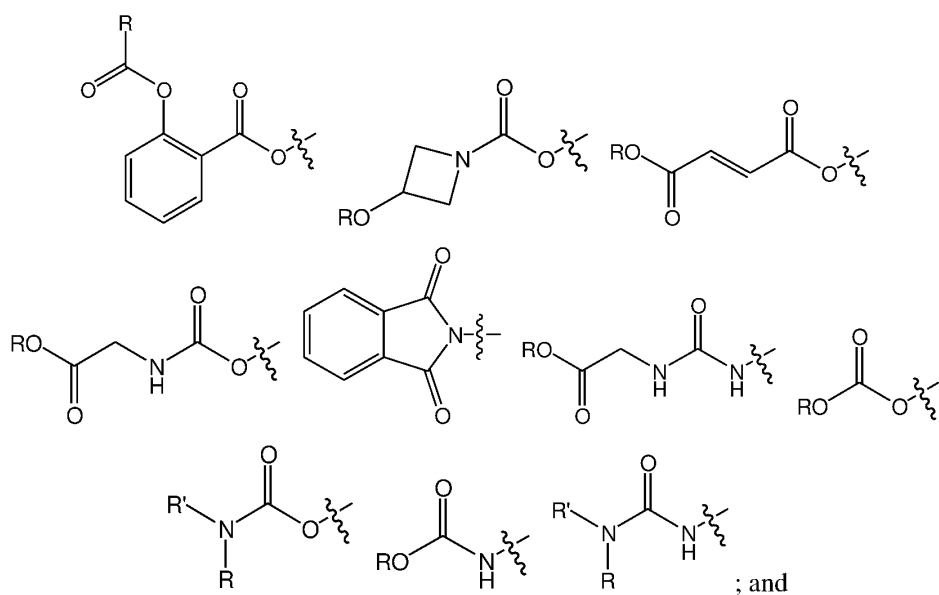
or a pharmaceutically acceptable form or an isotope derivative thereof,
wherein

n is 1, 2, 3 or 4; and

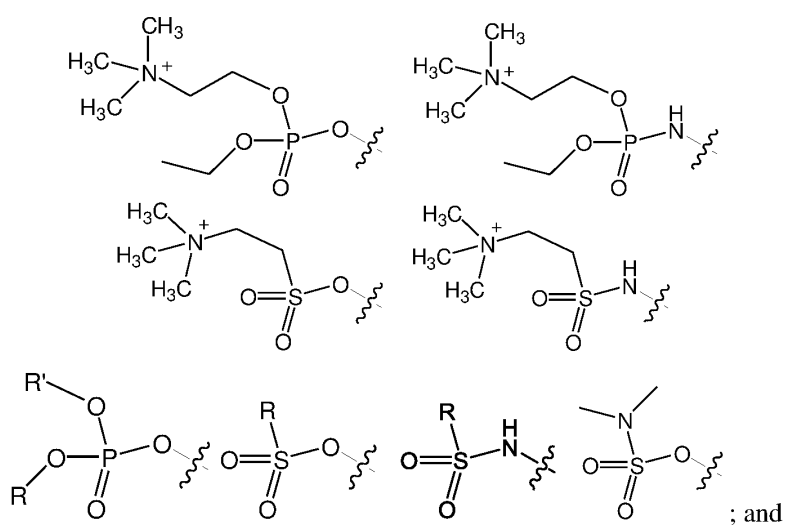
R^X is R^{X1} , R^{X2} or R^{X3} , wherein

R^{X1} is a group selected from: $CH_2C(=O)OR^3$, $CH_2C(=O)SR^3$, $C(=O)NR^4R^5$, OR^6 , SR^6 , NR^7R^8 , $NR^9C(=O)R^{10}$, $OC(=O)R^{11}$, $SC(=O)R^{12}$, $OC(=O)OR^{13}$, $SC(=O)OR^{13}$, $NR^9C(=O)OR^{13}$, $OC(=O)NR^{14}R^{15}$, $SC(=O)NR^{14}R^{15}$, $NR^9C(=O)NR^{14}R^{15}$, $OC(=O)SR^3$, $SC(=O)SR^3$, $NR^9C(=O)SR^3$, halo, CN, H, and C_{1-6} alkyl; and

R^{X2} is a group selected from:



R^{X3} is a group selected from:



each of R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} .

is independently selected from H and C_{1-6} alkyl, 3- to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A , or

R^4 and R^5 , R^7 and R^8 , or R^{14} and R^{15} , 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, OR and

NRR'; and

each of R and R'

is independently H or C₁₋₆ alkyl, optionally substituted with 1-4 groups selected from D, halo, C₁₋₆ alkoxy and amino; or

R and R' together with the N atom they are bonded to, respectively, form a 3- to 6-membered heterocyclic ring, optionally substituted with 1-4 groups selected from D, halo, C₁₋₆ alkyl or alkoxy, and amino.

[0090] In certain embodiments, a compound of the invention has the formula (I).

[0091] In certain embodiments, a compound of the invention has the formula (II).

[0092] In certain embodiments of formula (I) or (II), R^X is R^{X1}.

[0093] In certain embodiments, R^{X1} is C(=O)OR³ or OC(=O)R¹¹.

[0094] In certain embodiments, R^{X1} is C(=O)NR⁴R⁵ or NR⁹C(=O)R¹⁰.

[0095] In certain embodiments, R^{X1} is OC(=O)OR¹³ or OC(=O)NR¹⁴R¹⁵.

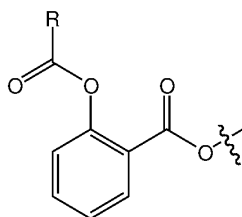
[0096] In certain embodiments, R^{X1} is OR⁶.

[0097] In certain embodiments, R^{X1} is NR⁷R⁸.

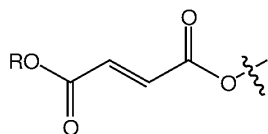
[0098] In certain embodiments, R^{X1} is halo, CN or C₁₋₃ alkyl.

[0099] In certain embodiments of formula (I) or (II), R^X is R^{X2}.

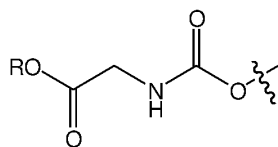
[0100] In certain embodiments, R^{X2} is:



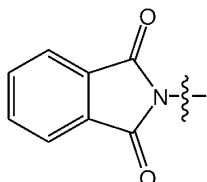
[0101] In certain embodiments, R^{X2} is:



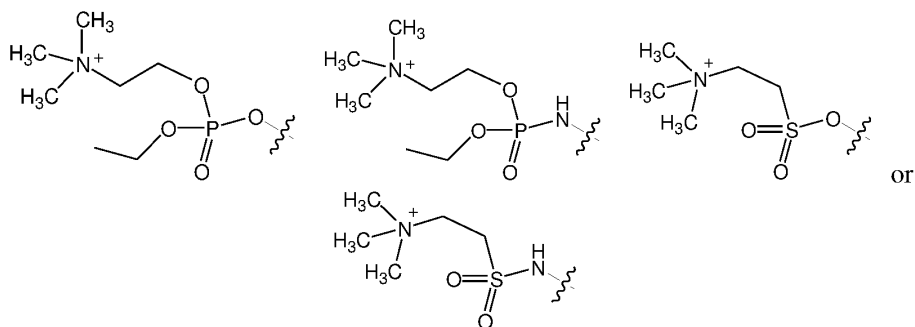
[0102] In certain embodiments, R^{X2} is:



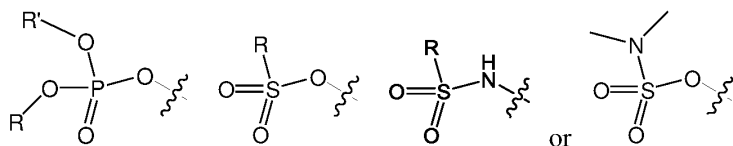
[00103] In certain embodiments, R^{X2} is:



[00104] In certain embodiments, R^{X3} is:



[00105] In certain embodiments, R^{X3} is:

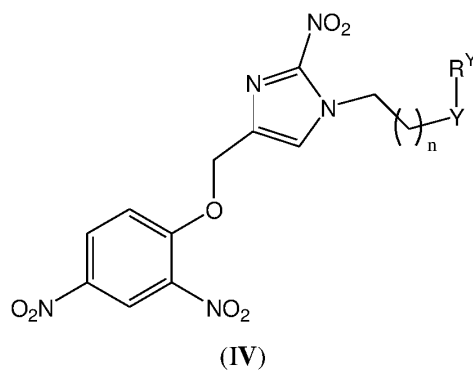
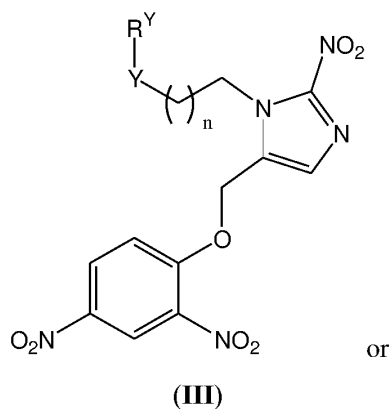


[00106] In certain embodiments of formula (I) or (II), n is not 1.

[00107] In certain embodiments of formula (I) or (II), R^X is R^{X3} .

[00108] In certain embodiments of formula (I) or (II), each of R and R' , if present, is independently H or C_{1-3} alkyl.

[00109] In another aspect, the invention generally relates to a compound having structural formula (III) or (IV):



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

n is 1, 2, 3 or 4;

Y is selected from a single bond, NR , S , CRR' and O ;

R^Y is a group selected from: H , halo, CN , R , $C(=O)OR$, $C(=O)NRR'$, $C(=O)R$ and $C(=O)SR$; and

each of R and R' is independently H or C_{1-6} alkyl, or R and R' , together with the N atom they are bonded to, form a 3- to 6-membered heterocyclic ring, wherein the alkyl or heterocyclic ring is optionally substituted with 1-4 groups selected from D , halo, C_{1-6} alkyl or alkoxy, and amino.

[00110] In certain embodiments, a compound of the invention has the formula (III).

[00111] In certain embodiments, a compound of the invention has the formula (IV).

[00112] In certain embodiments of formula (III) or (IV), Y is O . In certain embodiments, R^Y is selected from R , $C(=O)OR$, $C(=O)NRR'$, $C(=O)R$, and $C(=O)SR$.

[00113] In certain embodiments of formula (III) or (IV), Y is S. In certain embodiments, R^Y is selected from R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.

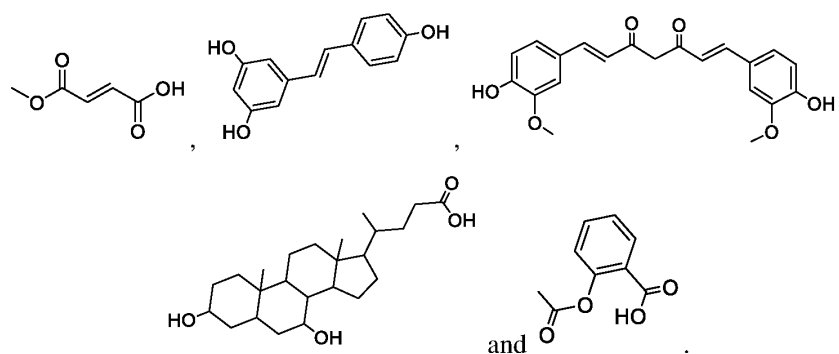
[00114] In certain embodiments of formula (III) or (IV), Y is NR. In certain embodiments, R^Y is selected from R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.

[00115] In certain embodiments of formula (III) or (IV), Y is CRR'. In certain embodiments, R^Y is selected from halo, CN, R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.

[00116] In certain embodiments of formula (III) or (IV), Y is a single bond.

[00117] In yet another aspect, the invention generally relates to a conjugate compound formed by a compound disclosed herein and a biologically active moiety characterized by an anti-ROS and/or anti-inflammatory activity.

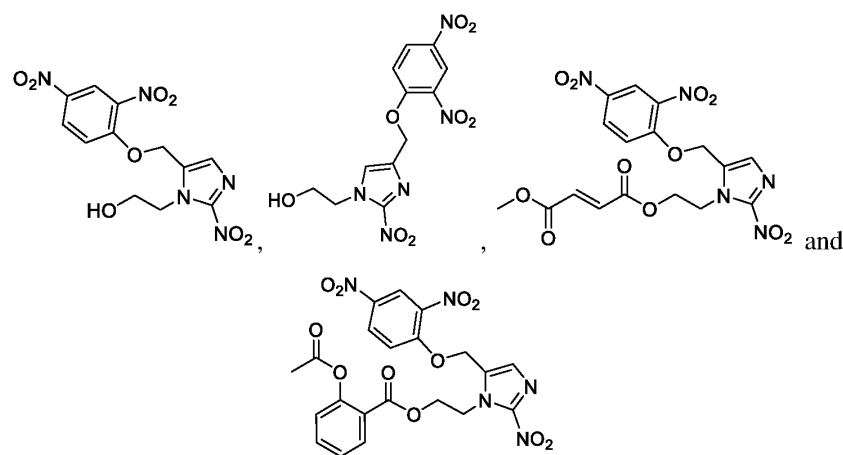
[00118] In certain embodiments of the conjugate compound, the biologically active moiety is selected from, but not limited to the following:



[00119] Exemplary compounds of the invention include those listed in **Table 1A**.

[00120] Exemplary compounds of the invention include those listed in **Table 1B**.

[00121] In certain embodiments, the conjugate compound is selected from the following:



[00122] Compounds of the invention include those having one or more deuterium atoms in place of one or more hydrogen atoms.

[00123] In another aspect, the invention generally relates to a pharmaceutical composition comprising a compound disclosed herein.

[00124] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of a compound disclosed herein.

[00125] In certain embodiments, the unit dosage form is a tablet.

[00126] In certain embodiments, the unit dosage form is a capsule.

[00127] 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.

[00128] In certain embodiments, the disease or disorder is associated with mitochondria function of a subject.

[00129] In certain embodiments, the disease or disorder is associated with one or more defects in mitochondrial function of a subject.

[00130] In certain embodiments, the disease or disorder is metabolic disease, neurodegenerative diseases, aging-related diseases, liver disease, cardiovascular disease, or a related disease or disorder.

[00131] 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), and nonalcoholic Steatohepatitis (NASH), or a related disease or disorder.

[00132] 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.

[00133] In certain embodiments, administration is via oral administration.

[00134] 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.

[00135] In yet another aspect, the invention generally relates to use of a compound disclosed herein for treating a disease or disorder.

[00136] In certain embodiments of the use, the disease or disorder is associated with one or more defect in mitochondrial function.

[00137] In certain embodiments of the use, the disease or disorder is metabolic disease, neurodegenerative diseases, aging-related diseases, liver disease, cardiovascular disease, or a related disease or disorder.

[00138] In certain embodiments of the use, the disease or disorder is 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, nonalcoholic fatty liver disease (NAFLD), nonalcoholic Steatohepatitis (NASH), or a related disease or disorder.

[00139] 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.*, R¹, R², 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.

[00140] 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).

[00141] Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds.

[00142] 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.

[00143] 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.

[00144] 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.

[00145] 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.

[00146] 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 ("a pharmaceutical 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.

[00147] 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 carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block

polymers, polyethylene glycol and wool fat.

[00148] 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).

[00149] 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.

[00150] 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.

[00151] 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.

[00152] 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.

[00153] 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.

[00154] 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.

[00155] 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.

[00156] 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.

[00157] 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.

[00158] 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.

[00159] 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.

[00160] 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.

[00161] 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).

[00162] 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.

[00163] 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.

[00164] 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.

[00165] 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.

[00166] 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.

[00167] 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 a 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.

[00168] 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.

[00169] 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.

[00170] 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.

[00171] 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.

[00172] 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.

[00173] 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.

[00174] 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.

[00175] 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.

[00176] 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.

[00177] The following examples are meant to be illustrative of the practice of the invention and not limiting in any way.

Examples

Abbreviations

Abbreviation	Name
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
DIAD	Diisopropyl azodicarboxylate

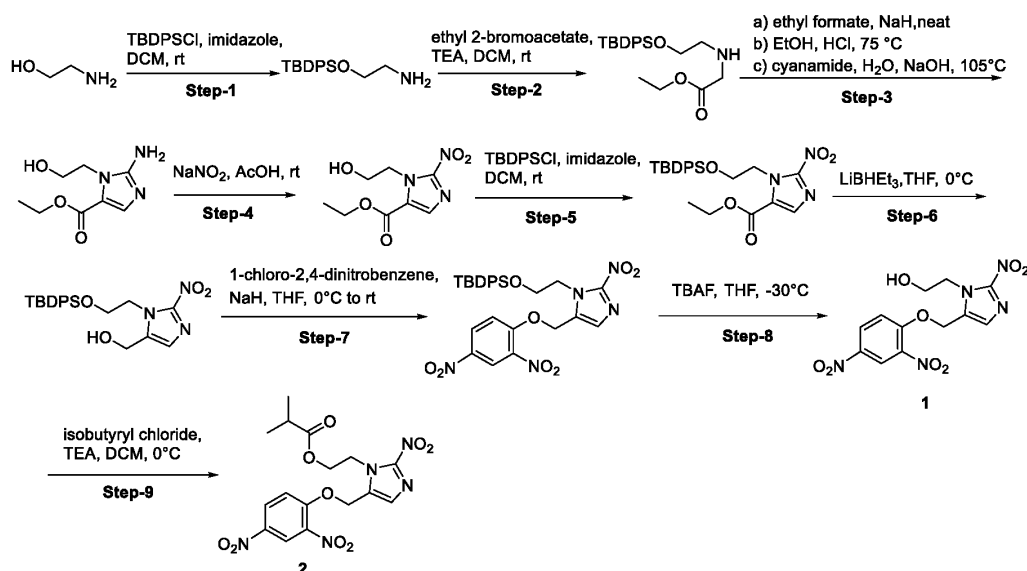
DIO	Diet Induced Obesity
DIPEA/DIEA	N,N-Diisopropylethylamine
EDTA	Ethylenediaminetetraacetic acid
EtOAc or EA	Ethyl acetate
equiv. or eq.	Equivalent
Et	Ethyl
ESI	Electron spray ionization
FW	Formula weight
HATU	Hexafluorophosphate azabenzotriazole tetramethyluronium
HOAc or AcOH	Acetic acid
HOBt	Hydroxybenzotriazole
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
NBS	N-Bromosuccinimide
NMI	N-methylimidazole
NMR	Nuclear magnetic resonance
PK	Pharmacokinetics
PO	Per Oral (by mouth)
rt	Room temperature
SM	Starting material
TBDPSCI	tert-Butyl(chloro)diphenylsilane
TCFH	N,N,N',N'-tetramethylchloroformamidinium hexafluorophosphate
TEA	Triethylamine
T _{max}	Time to maximum plasma concentration

t _{1/2}	Terminal half-life
TG	Triglyceride
TosCl	4-Toluenesulfonyl chloride
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
NAS	Non-alcoholic fatty liver disease Activity Score
AMPK	5'-Adenosine MonoPhosphate activated Protein Kinase
PDK4	Pyruvate Dehydrogenase Kinase 4
MDA	Malondialdehyde

Chemistry Methods

[00178] 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-*d*₄, 3.31 ppm (¹H), 49.00 ppm (¹³C); DMSO-*d*₆, 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 = broad, m = multiplet, abq = ab quartet), number of protons, and coupling constants. Mass spectral data were measured using Agilent 1260 and 6120MSD LC-MS. All compounds submitted for biological testing were confirmed to be ≥ 95% pure by Shimadzu LC-2030C 3D analytical HPLC. Synthetic methods, spectral data, and MS for novel compounds are described in detail below.

Synthesis Scheme 1



[00179] **Step-1: 2-((tert-butyldiphenylsilyl)oxy)ethan-1-amine.** To a mixture of 2-aminoethanol (40 g, 0.65 mol) in DCM (400 mL) was added imidazole (89 g, 1.31 mol) and TBDPSCl (198 g, 0.72 mol) at 25 °C. The mixture was stirred at 25 °C for 16 hrs. The reaction mixture was poured into water (1000 mL) and extracted with DCM (500 mL x 3). The combined organic layer was washed with brine (500 mL), dried over Na₂SO₄ and concentrated in vacuum to give the desired product (150 g, yield 77%) as yellow oil: MS (ESI), m/z: calcd for C₅H₅N₃O₃ Exact Mass: 299.2 found *t_R* = 1.726 min. [M+H]⁺ = 300.1; ¹H NMR (400 MHz, CD₃Cl) δ 7.68-7.66 (m, 4H), 7.45-7.36 (m, 6H), 3.67 (t, *J* = 5.2 Hz, 2H), 2.80 (t, *J* = 5.2 Hz, 2H), 1.06 (s, 9H).

[00180] **Step-2: Ethyl 2-((tert-butyldiphenylsilyl)oxy)ethylglycidate.** To a solution of 2-((tert-butyldiphenylsilyl)oxy)ethan-1-amine (320 g, 1.07 mol) in DCM (3200 mL) was added ethyl 2-bromoacetate (178.4 g, 1.07 mol) and TEA (324.4 g, 3.21 mol) at 25 °C. The reaction mixture was stirred at 25 °C for 16 hrs. The reaction mixture was concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=10/1-3/1) to give the product (300 g, yield 73%) as yellow oil: MS (ESI), m/z: calcd for C₅H₅N₃O₃ Exact Mass: 385.2 found *t_R* = 1.544 min. [M+H]⁺ = 386.1.

[00181] **Step-3: Ethyl 2-amino-1-(2-hydroxyethyl)-1H-imidazole-5-carboxylate.** To a solution of ethyl 2-((tert-butyldiphenylsilyl)oxy)ethylglycidate (100 g, 0.26 mol) in ethyl formate (500 mL) was added NaH (36.3 g, 0.91 mol) at 25 °C. The mixture was stirred at 25 °C

for 16 hrs. The reaction mixture was concentrated in vacuum to give the desired product (100 g, crude) as yellow oil, which was used for the next step directly without further purification.

[00182] To a solution of the compound (100 g, crude) described in the previous step in EtOH (400 mL) was added con. HCl (320 mL) at 25 °C. The mixture was stirred at 75 °C for 2 hrs. The reaction solution was cooled to room temperature and concentrated. The residue was adjusted pH to 3~4 with aq. NaOH (2 N), and the mixture was used for the next step directly.

[00183] Added water (1000 mL) to the solution made in the previous step, and then cyanamide (17.3 g, 0.41 mol) was added at 25 °C. The mixture was stirred at 105 °C for 2 hrs. The reaction solution was cooled to room temperature, adjusted pH=8-9 with solid Na₂CO₃ and extracted with EA (500 mL x 3). The combined organic layer was washed with brine (500 mL), dried over Na₂SO₄ and concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=20/1-1/1) to give the desired compound (15 g, yield 31% over 3 steps) as a yellow solid: MS (ESI), m/z: calcd for C₅H₅N₃O₃ Exact Mass: 199.1 found t_R = 0.948 min. [M+H]⁺ = 200.1; ¹H NMR (400 MHz, CD₃Cl) δ 7.26 (s, 1H), 4.96 (s, 2H), 4.26-4.20 (m, 4H), 3.97 (t, J = 4.4Hz, 2H), 1.32 (t, J = 7.2 Hz, 2H).

[00184] **Step-4: Ethyl 1-(2-hydroxyethyl)-2-nitro-1H-imidazole-5-carboxylate.** To a solution of Ethyl 2-amino-1-(2-hydroxyethyl)-1H-imidazole-5-carboxylate (18.4 g, 0.092 mol) in CH₃COOH (190 mL) and H₂O (190 mL) was added NaNO₂ (38.1g, 0.55 mol) at 10 °C. The mixture was stirred at 25 °C for 16 hrs. The reaction mixture was poured into water (500 mL) and extracted with EA (500 mL x 3). The combined organic layer was washed with brine (500 mL), dried over Na₂SO₄ and concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=10/1-3/1) to give desired compound (10 g, yield 47%) as a yellow solid: MS (ESI), m/z: calcd for C₅H₅N₃O₃ Exact Mass: 229.1 found t_R = 1.385 min. [M+H]⁺ = 230.0; ¹H NMR (400 MHz, CD₃Cl) δ 7.76 (s, 1H), 5.09 (t, J = 5.2 Hz, 2H), 4.40 (q, J = 7.2 Hz, 2H), 4.01 (t, J = 5.2 Hz, 2H), 1.41 (t, J = 6.8 Hz, 2H).

[00185] **Step-5: Ethyl 1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2-nitro-1H-imidazole-5-carboxylate.** To a mixture of ethyl 1-(2-hydroxyethyl)-2-nitro-1H-imidazole-5-carboxylate (12 g, 0.052 mol) in DCM (120 mL) was added TBDPSCl (15.8 g, 0.058 mol) and imidazole (7.1 g, 0.10 mol) at 25 °C. The mixture was stirred at 25 °C for 16 hrs. The reaction mixture was poured into water (200 mL) and extracted with DCM (100 mL x 3). The combined organic layer was washed with brine (200 mL), dried over Na₂SO₄ and concentrated in vacuum to give the crude

product. The crude product was purified by flash column (PE/EA=10/1-3/1) to give the product (20 g, yield 82%) as a yellow solid: ^1H NMR (400 MHz, CD_3Cl) δ 7.73 (s, 1H), 7.44-7.40 (m, 6H), 7.38-7.32 (m, 4H), 5.20 (t, $J = 4.8$ Hz, 2H), 4.31 (q, $J = 7.2$ Hz, 2H), 3.86 (t, $J = 4.8$ Hz, 2H), 1.36 t, $J = 7.2$ Hz, 2H), 0.93 (s, 9H).

[00186] **Step-6: (1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2-nitro-1H-imidazol-5-yl)methanol.** To a mixture of ethyl 1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2-nitro-1H-imidazole-5-carboxylate (20 g, 0.043 mol) in THF (200 mL) was added LiBHET_3 (0.13 L, 0.13 mol, 1 mmol/L in THF) at 0 °C. The mixture was stirred at 0 °C for 2 hrs. The reaction mixture was poured into ammonium chloride aqueous solution (200 mL) and extracted with EA (200 mL x 3). The combined organic layer was washed with brine (300 mL), dried over Na_2SO_4 and concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=10/1-3/1) to give the product (8.6 g, yield 47%) as a yellow solid: MS (ESI), m/z : calcd for $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_3$ Exact Mass: 425.2 found $t_R = 2.304$ min. $[\text{M}+\text{H}]^+ = 426.1$; ^1H NMR (400 MHz, CD_3Cl) δ 7.46-7.41 (m, 6H), 7.38-7.34 (m, 4H), 7.17 (s, 1H), 4.76 (s, 2H), 4.68 (t, $J = 4.8$ Hz, 2H), 3.97 (t, $J = 4.8$ Hz, 2H), 0.98 (s, 9H).

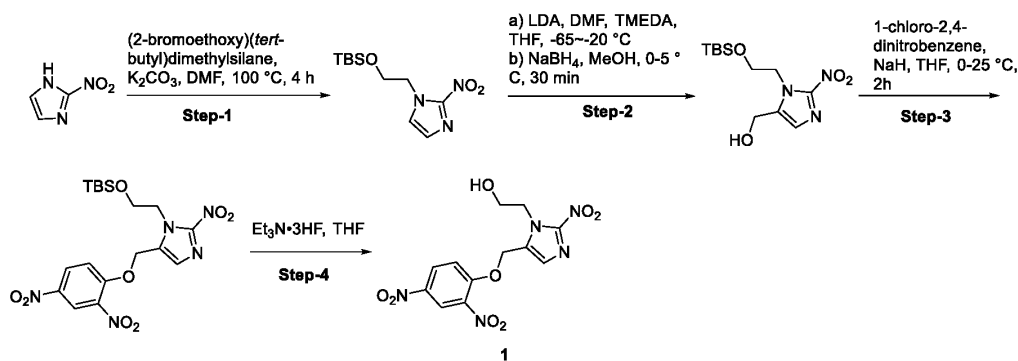
[00187] **Step-7: 1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole.** To a solution of (1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2-nitro-1H-imidazol-5-yl)methanol (8.6 g, 0.020 mol) in THF (90 mL) was added NaH (1.2 g, 0.030 mol) at 0 °C and stirred for 0.5 hr. And then 1-chloro-2,4-dinitrobenzene (6.1 g, 0.03 mol) was added. The mixture was stirred at 25 °C for 2 hrs. The reaction mixture was poured into water (200 mL) and extracted with DCM (100 mL x 3). The combined organic layer was washed with brine (200 mL), dried over Na_2SO_4 and concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=10/1-3/1) to give the product (8 g, yield 67%) as a yellow solid; ^1H NMR (400 MHz, CD_3Cl) δ 8.76 (d, $J = 2.8$ Hz, 1H), 8.40 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.46-7.40 (m, 4H), 7.39-7.34 (m, 7H), 7.08 (d, $J = 9.2$ Hz, 1H), 5.43 (s, 2H), 4.76-4.72 (m, 2H), 4.08 (t, $J = 4.8$ Hz, 1H), 1.00 (s, 9H).

[00188] **Step-8: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-ol (1).** To a solution of 1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole (3 g, 0.0051 mol) in THF (30 mL) was added TBAF (10 mL, 0.010 mol, 1 mmol/L in THF) at -30 °C. The mixture was stirred at -30 °C for 2 hrs. The reaction mixture was poured into saturated aqueous solution of ammonium chloride (100 mL) and extracted with EA

(100 mL x 3). The crude product was purified by flash column (PE/EA=10/1-3/1) to give Compound **1** (16 g, yield 56%) as a yellow solid: MS (ESI), m/z : calcd for $C_5H_5N_3O_3$ Exact Mass: 353.1 found $t_R = 1.757$ min. $[M+H]^+ = 354.1$; 1H NMR (400 MHz, $DMSO-d_6$) δ 8.80 (d, $J = 2.8$ Hz, 1H), 8.57 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.82 (d, $J = 9.2$ Hz, 1H), 7.41 (s, 1H), 5.66 (s, 2H), 5.13 (t, $J = 5.2$ Hz, 1H), 4.51 (t, $J = 5.2$ Hz, 2H), 3.71 (q, $J = 5.2$ Hz, 2H).

[00189] **Setp-9: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl isobutyrate (2)**. To a solution of Compound **1** (90 mg, 0.25 mmol) and TEA (77 mg, 0.76 mmol) in DCM (3 mL) was added isobutyryl chloride (41 mg, 0.38 mmol) in DCM (0.5 mL) at 0 °C under N_2 . The reaction mixture was stirred under N_2 at 0 °C for 1 hour. The reaction was quenched by addition water (10 mL) and extracted with DCM (3 x 5mL). 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 **2** (54 mg, yield 49 %) as a yellow solid: MS (ESI), m/z : calcd for $C_{16}H_{17}N_5O_9$ Exact Mass: 423.103 found $t_R = 1.928$ min. $[M+H]^+ = 424.0$; 1H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, $J = 2.7$ Hz, 1H), 8.56 – 8.49 (m, 1H), 7.38 (d, $J = 9.2$ Hz, 1H), 7.33 (s, 1H), 5.44 (s, 2H), 4.86 (t, $J = 5.7$ Hz, 2H), 4.47 (t, $J = 5.7$ Hz, 2H), 2.53 – 2.40 (m, 1H), 1.09 (d, $J = 7.0$ Hz, 6H).

Synthesis Scheme 2



[00190] **Step-1: 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-2-nitro-1H-imidazole**. To a mixture of 2-nitro-1H-imidazole (275 g, 2.43 mol) in DMF (4 L) was added 2-(tert-butyldimethylsilyloxy)-1-bromoethane (871.6 g, 3.65 mol) and K_2CO_3 (673 g, 4.86 mol). The resulting mixture was stirred at 100 °C for 12 h. The reaction mixture was cooled to room temperature. DMF was removed under reduced pressure. The residue was partitioned between water and EtOAc. The combined organic layers were washed with water and brine, dried over

Na₂SO₄ and concentrated. The crude product was triturated in petroleum ether (1000 mL) and filtered to afford the titled compound (627 g, yield 95%) as pale yellow solid: MS (ESI), m/z: calcd for C₁₁H₂₁N₃O₃Si, Exact Mass:271.1, found [M+H]⁺ = 272.0; ¹H NMR (400 MHz, CDCl₃, 7.32ppm): δ 7.20 (d, *J* = 0.8 Hz, 1H), 7.18 (d, *J* = 0.8 Hz, 1H), 4.60 (t, *J* = 5.0 Hz, 2H), 4.00 (t, *J* = 5.0 Hz, 2H), 0.88 (s, 9H), 0.00 (s, 6H); Total H count from HNMR data: 21.

[00191] **Step-2: (1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-2-nitro-1H-imidazol-5-yl)methanol.** To a stirred solution of 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-2-nitro-1H-imidazole (25 g, 92.1 mmol) and TMEDA (16 g, 138.2 mmol) in THF (400 mL) was added LDA dropwise (69 mL, 138.2 mol, 2M in THF) at -70 °C ~-65 °C under nitrogen atmosphere. The resulting dark solution was stirred at -70 °C ~-65 °C for 30 min. DMF (10.1 g, 138.2 mmol) was added dropwise to the above mixture. The resulting mixture was stirred at -70 °C ~-65 °C for 2h. The reaction mixture was poured into saturated aqueous NH₄Cl solution. The organic layer was collected. THF was removed under reduced pressure. The residue was partitioned between water and EtOAc. The EtOAc layer was collected. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na₂SO₄ and concentrated. The residue was purified through silica gel flash column chromatography (PE/EtOAc= 10/1→3/1) to afford crude compound as pale yellow solid (8.5 g, a mixture of start material and product).

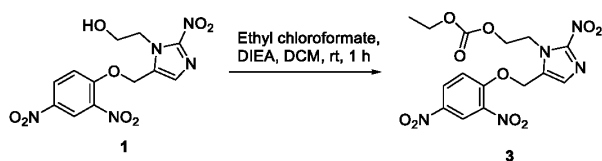
[00192] To a mixture of the crude product (8.5 g from previous step) in methanol (100 mL) was added NaBH₄ in portions at 15 °C. TLC monitored the reaction until the starting material disappeared. The reaction mixture was concentrated. The residue was partitioned between water and EtOAc. The EtOAc layer was collected. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na₂SO₄ and concentrated. The residue was purified through silica gel flash column chromatography (PE/EtOAc= 8/1→1/1) to afford the titled compound as pale yellow solid (3 g, yield 10.8% over two steps): MS (ESI), m/z: calcd for C₁₂H₂₃N₃O₄Si, Exact Mass:301.2, found [M+H]⁺ = 302.0; ¹H NMR (400 MHz, CDCl₃, 7.28ppm): δ 7.17 (s, 1H), 4.73 (d, *J* = 5.6 Hz, 2H), 4.67 (t, *J* = 4.8 Hz, 2H), 4.08 (t, *J* = 4.8 Hz, 2H), 3.26 (t, *J* = 5.8 Hz, 1H), 0.83 (s, 9H), 0.00 (s, 6H); Total H count from HNMR data: 23.

[00193] **Step-3: 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole.** To a mixture of (1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-2-nitro-1H-

imidazol-5-yl)methanol (31 g, 103 mmol) and 1-chloro-2,4-dinitrobenzene (31.4 g, 154 mmol) in DMF (300 mL) at 25 °C was added Cs₂CO₃ (50.3 g, 154 mmol). The resulting mixture was stirred at 25 °C for 12 h. The reaction mixture was partitioned between water and EtOAc. The EtOAc layer was collected. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na₂SO₄ and concentrated. The residue was purified through silica gel flash column chromatography to afford a crude product of the titled compound. The crude product was recrystallized to afford pure product (22.4 g, yield 47%) as a pale yellow solid. The mother liquid could be purified through column (PE/EtOAc= 8/1→1/1) to afford more desired intermediate: MS (ESI), m/z: calcd for C₁₈H₂₅N₅O₈Si, Exact Mass:467.2, found [M+H]⁺=468.1; ¹H NMR (400 MHz, CDCl₃, 7.26 ppm) δ 8.77 (d, *J* = 2.4 Hz, 1H), 8.50-8.47 (dd, *J* = 2.8 Hz, 5.2 Hz, 1H), 7.33 (d, *J* = 9.2 Hz, 1H), 7.30 (s, 1H), 5.48 (s, 2H), 4.71 (t, *J* = 4.6 Hz, 2H), 4.01 (t, *J* = 4.6 Hz, 2H), 0.79 (s, 9H), -0.06 (s, 6H); Total H count from HNMR data: 25.

[00194] **Step-4: 2-(5-[(2,4-dinitrophenyl)oxy]methyl)-2-nitroimidazol-1-yl)ethan-1-ol (1).** To a solution of 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole (26.5 g, 56.7 mmol) in THF (250 mL) at 15 °C was added triethylamine trihydrofluoride (45.7 g, 283.5 mmol). The resulting mixture was stirred at room temperature for 4 h. THF was removed under reduced pressure. The residue was triturated with EtOAc. The precipitate was collected through filtration, washed with EtOAc, and dried to afford first batch of product (13 g). The filtrate was concentrated. The residue was suspended in water and adjusted to pH 8 with NaHCO₃. The precipitate was collected through filtration, washed with water, and dried to afford product (4.0 g) as a pale yellow solid (total yield 85%). (The reaction was set in a glass three-necked flask.): MS (ESI), m/z: calcd for C₁₂H₁₁N₅O₈, Exact Mass:353.1, found [M+H]⁺=353.8; ¹H NMR (400 MHz, DMSO-d₆, 2.50ppm): δ 8.08 (d, *J* = 2.8 Hz, 1H), 8.59-8.56 (dd, *J* = 2.8 Hz, 5.2 Hz, 1H), 7.84 (d, *J* = 9.2 Hz, 1H), 7.41 (s, 1H), 5.67 (s, 2H), 5.13 (t, *J* = 5.0 Hz, 1H), 4.52 (t, *J* = 5.2 Hz, 2H), 3.73-3.69 (m, 2H); Total H count from HNMR data: 11.

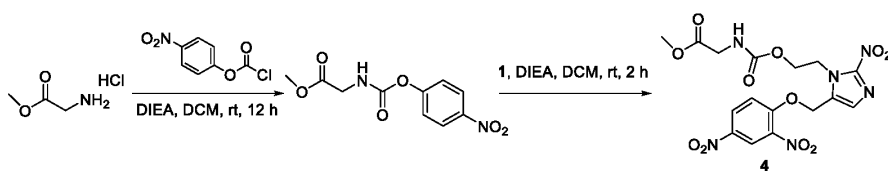
Synthesis Scheme 3



Step-1: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl ethyl carbonate

(3). To a solution of Compound **1** (110 mg, 0.31 mmol) and DIEA (120 mg, 0.93 mmol) in DCM (3 mL) stirred at 0 °C was added a solution of ethyl chloroformate (335 mg, 3.1 mmol) in DCM (1 mL) dropwise. The reaction mixture was stirred at room temperature for 1 hour. LCMS showed SM was consumed and DP was detected. The reaction mixture was quenched by H₂O (50 mL), extracted by DCM (50 mL x 3). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 5%) to give product **3** (68 mg, yield 56%) as a yellow solid: MS(ESI), m/z: calcd for C₁₅H₁₅N₅O₁₀ Exact Mass: 425.08 found t_R = 1.256 min. [M+H]⁺ = 426.00; ¹H NMR (400 MHz, CDCl₃) δ 8.72 (d, *J* = 2.4 Hz, 1H), 8.44 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.31 (d, *J* = 9.2 Hz, 1H), 7.24 (s, 1H), 5.39 (s, 2H), 4.80 (t, *J* = 5.0 Hz, 2H), 4.49 (t, *J* = 5.0 Hz, 2H), 4.07 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H).

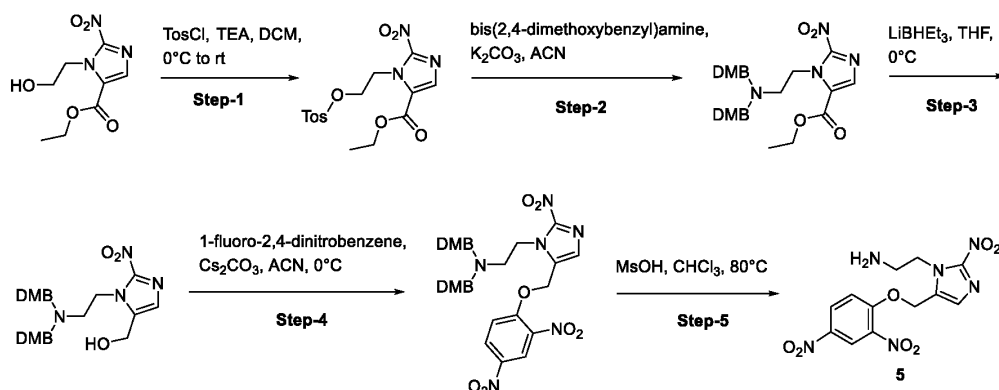
Synthesis Scheme 4



Step-1: Methyl ((4-nitrophenoxy)carbonyl)glycinate To a solution of 4-nitrophenyl carbonochloridate (1.93 g, 9.6 mmol) and DIEA (3.10 g, 24.0 mmol) in DCM (20 mL) stirred at 0 °C was added methyl glycinate hydrochloride (1.0 g, 8.0 mmol) in DCM (10 mL) dropwise. The reaction mixture was stirred at room temperature for 12 hours. LCMS showed SM was consumed and DP was detected. The reaction mixture was quenched by H₂O (50 mL), extracted by DCM (50 mL x 3). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by FCC (12 g, silica gel, EA in PE = 30%) to give methyl ((4-nitrophenoxy)carbonyl)glycinate (300 mg, 15% yield) as a white solid.

Step-2: Synthesis of methyl ((2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethoxy)carbonyl)glycinate (4). To a solution of Compound 1 (150 mg, 0.42 mmol) and DIEA (163 mg, 1.26 mmol) in DCM (5 mL) stirred at 0 °C was added a solution of methyl ((4-nitrophenoxy)carbonyl)glycinate (127 mg, 0.50 mmol) in DCM (3 mL) dropwise. The reaction mixture was stirred at room temperature for 2 hours. LCMS showed SM was consumed and DP was detected. The reaction mixture was diluted by H₂O (50 mL), extracted by DCM (50 mL x 3). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 5%) to give product 4 (24 mg, yield 12%) as a yellow solid: MS(ESI), m/z: calcd for C₁₆H₁₆N₆O₁₁ Exact Mass: 468.09 found t_R = 1.135 min. [M+H]⁺ = 469.00; ¹H NMR (400 MHz, MeOD) δ 8.79 (d, *J* = 2.8 Hz, 1H), 8.55 (dd, *J* = 9.6, 9.2 Hz, 1H), 7.74 (d, *J* = 9.2 Hz, 1H), 7.37 (s, 1H), 5.62 (s, 2H), 4.84 – 4.81 (m, 2H), 4.54 (t, *J* = 5.0 Hz, 2H), 3.76 (s, 2H), 3.70 (s, 3H).

Synthesis Scheme 5



[00198] **Step-1: Ethyl 2-nitro-1-(2-(tosyloxy)ethyl)-1H-imidazole-5-carboxylate.** To a solution of ethyl 1-(2-hydroxyethyl)-2-nitro-1H-imidazole-5-carboxylate (1.00 g, 4.37 mmol) in DCM (20 mL) was added TEA (1.32 g, 13.1 mmol) and TosCl (1.00 g, 5.25 mmol) at 0 °C. The mixture was stirred at 25 °C for 16 hrs. The reaction mixture was poured into water (50 mL) and extracted with DCM (10 mL x 3). The combined organic layer was washed with brine (50 mL), dried over Na₂SO₄ and concentrated in vacuum to give the crude product (740 mg, yield 44%) as yellow solid which was used directly for the next step without further purification: MS (ESI), m/z: calcd for C₁₅H₁₇N₃O₇S Exact Mass: 383.08 found t_R = 2.314 min. [M+H]⁺ = 384.1.

[00199] **Step-2: Ethyl 1-(2-(bis(2,4-dimethoxybenzyl)amino)ethyl)-2-nitro-1H-imidazole-5-carboxylate.** To a solution of ethyl 2-nitro-1-(2-(tosyloxy)ethyl)-1H-imidazole-5-carboxylate (800 mg, 2.09 mmol) in ACN (16 mL) was added bis(2,4-dimethoxybenzyl)amine (795 mg, 2.51 mmol) and K_2CO_3 (865 mg, 6.27 mmol) at 25 °C. The reaction mixture was then stirred at 60 °C for 16 hrs. The reaction mixture was cooled to room temperature and filtered; the filtrate was concentrated in vacuum to give the crude product. The crude product was purified by flash column (PE/EA=10/1-1/1) to give desired compound (650 mg, yield 59%) as a yellow solid: MS (ESI), m/z: calcd for $C_{26}H_{32}N_4O_8$ Exact Mass: 528.22 found t_R = 1.560 min. $[M+H]^+ = 529.3$.

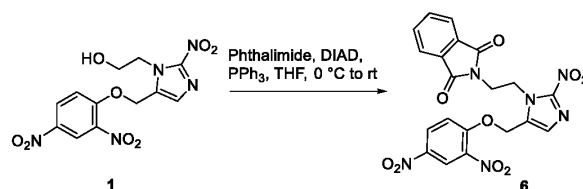
[00200] **Step-3: (1-(2-(bis(2,4-dimethoxybenzyl)amino)ethyl)-2-nitro-1H-imidazol-5-yl)methanol.** To a solution of ethyl 1-(2-(bis(2,4-dimethoxybenzyl)amino)ethyl)-2-nitro-1H-imidazole-5-carboxylate (600 mg, 1.14 mmol) in THF (12 mL) was added 1M solution of $LiBHET_3$ (3.41 mL, 3.41 mmol) in THF dropwise at 0 °C. The mixture was stirred at 0 °C for 2 hrs. The reaction mixture was quenched with sat. NH_4Cl solution (20 mL) and extracted with EA (10 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash column (DCM/MeOH=100/1-30/1) to give the product (330 mg, yield 60%) as yellow solid: MS (ESI), m/z: calcd for $C_{24}H_{30}N_4O_7$ Exact Mass: 486.21 found t_R = 1.408 min. $[M+H]^+ = 487.2$; 1H NMR (400 MHz, $CDCl_3$) δ 7.07-7.05 (m, 2H), 6.89 (s, 1H), 6.33-6.31 (m, 4H), 4.35-4.30 (m, 2H), 4.05-3.95 (m, 2H), 3.75-3.70 (m, 12H), 3.69-3.62 (m, 4H), 2.95-2.85 (m, 2H).

[00201] **Step-4: N,N-bis(2,4-dimethoxybenzyl)-2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-amine.** To a solution of (1-(2-(bis(2,4-dimethoxybenzyl)amino)ethyl)-2-nitro-1H-imidazol-5-yl)methanol (50 mg, 0.10 mmol) in ACN (1 mL) was added Cs_2CO_3 (33.5 mg, 0.10 mmol) and 1-fluoro-2,4-dinitrobenzene (19.2 mg, 0.10 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 hrs. Another 6 batches were carried out in the same procedure. The reaction mixture was combined and filtered. The filtrate was concentrated in vacuum to give the crude product. Purification by *Prep*-TLC (PE/EA=1/1) to give desired product (315 mg, yield 67%) as a yellow solid: MS (ESI), m/z: calcd for $C_{30}H_{32}N_6O_{11}$ Exact Mass: 652.21 found t_R = 1.206, 1.365 min. $[M+H]^+ = 653.2$.

[00202] **Step-5: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-amine (5).** To a solution of N,N-bis(2,4-dimethoxybenzyl)-2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-amine (50 mg, 0.08 mmol) in $CHCl_3$ (0.5 mL) was added $MsOH$

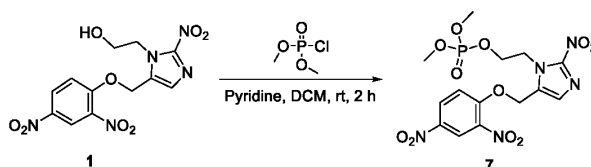
(0.1 mL). The mixture was stirred at 80 °C for 16 hrs. Another 5 batches were carried out at the same protocol. The reaction mixture was combined and diluted with water (6 mL) and extracted with DCM (5 mL x 3). The aqueous layer was purified by *Prep*-HPLC (Columns: sunfire 5um 19-150mm; Mobile phase: CH₃CN/H₂O (0.1%TFA); Gradient: 10-60 %, CH₃CN, 7 min; flow rate: 500 μL/min) to give compound **5** (20 mg, yield 12%) as a brown solid: MS (ESI), m/z: calcd for C₁₂H₁₂N₆O₇ Exact Mass: 352.08 found *t_R* = 1.187 min. [M+H]⁺ = 353.0; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.84 (d, *J* = 2.8 Hz, 1H), 8.62 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.01 (brs, 3H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.49 (s, 1H), 5.62 (s, 2H), 4.65 (t, *J* = 6.8 Hz, 2H), 3.32-3.31 (m, 2H).

Synthesis Scheme 6



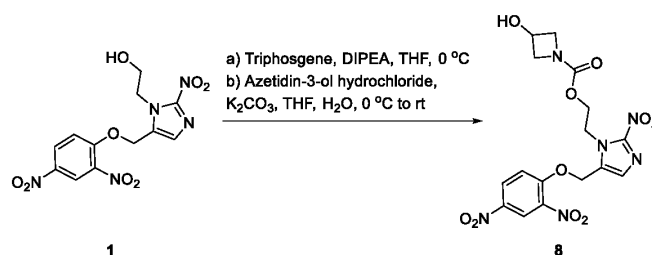
[00203] **Step-1: 2-(2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-ylethyl)isoindoline-1,3-dione (6).** To a mixture of Compound **1** (100 mg, 0.28 mmol) in THF (3 mL) was added phthalimide (62 mg, 0.42 mmol), PPh₃ (111 mg, 0.42 mmol) and DIAD (172 mg, 0.85 mmol) at 0°C. The mixture was stirred at 25°C for 16 hrs. The reaction mixture was poured into water (10 mL) and extracted with EA (20 mL x 3). The combined organic layers were washed with brine (30 mL), dried over Na₂SO₄ and concentrated in vacuum to give the crude product. The crude product was purified by prep-TLC (PE/EA=1/1) to give the product **6** (60 mg, yield 44%) as a yellow solid: MS (ESI), m/z: calcd for C₂₀H₁₄N₆O₉ Exact Mass: 482.1 found RT = 1.985 min. [M+H]⁺ = 483.1; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.77 (d, *J* = 2.8 Hz, 1H), 8.60 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.88-7.79 (m, 5H), 7.45 (s, 1H), 5.62 (s, 2H), 4.71 (t, *J* = 6.4 Hz, 2H), 4.08 (t, *J* = 6.0 Hz, 2H).

Synthesis Scheme 7



[00204] **Step-1: 2-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dimethyl phosphate (7).** To a solution of Compound **1** (110 mg, 0.311 mmol) in DCM/pyridine (4 mL/2 mL) was added dimethyl chlorophosphonate (135 mg, 0.934 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 2 hours. After the reaction was completed, the mixture was diluted with DCM (10 mL) and washed with H₂O (10 mL). The collected organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (4 g, silica gel, MeOH in DCM = 5%) to give the product **7** (34 mg, yield 23%) as a yellow solid: MS(ESI), m/z: calcd for C₁₄H₁₆N₅O₁₁P Exact Mass: 461.06 found *t_R* = 1.105 min. [M+H]⁺ = 461.90; ¹H NMR (400 MHz, CDCl₃) δ 8.79 (d, *J* = 2.8 Hz, 1H), 8.51 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.33 (s, 1H), 5.47 (s, 2H), 4.87 (t, *J* = 4.8 Hz, 2H), 4.46 (dt, *J* = 9.6, 5.2 Hz, 2H), 3.69 (s, 3H), 3.66 (s, 3H).

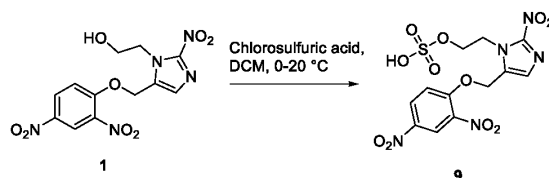
Synthesis Scheme 8



[00205] **Step-1: 2-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 3-hydroxyazetidine-1-carboxylate (8).** To a solution of **1** (100 mg, 0.28 mmol) and DIPEA (92 mg, 0.71 mmol) in THF (5 mL) was added ditrichloromethyl dicarbonate (58 mg, 0.17 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. Then a solution of azetidin-3-ol hydrochloride (62 mg, 0.57 mmol) and K₂CO₃ (200 mg, 1.40 mmol) in THF/H₂O (5 mL/5 mL) was added. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 1 hour. After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was purified by FCC (12 g silica gel, MeOH in DCM = 5%), followed by prep-HPLC (C18 column, MeCN in H₂O (0.1% FA)) to give the **9** (78 mg, yield 61%) as a white solid: MS(ESI), m/z: calcd for C₁₆H₁₆N₆O₁₀ Exact Mass: 452.09 found *t_R* = 1.058 min. [M+H]⁺ = 453.0; ¹H NMR (400 MHz, *d*₆-DMSO) δ 8.81 (d, *J* = 2.8 Hz, 1H), 8.60 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.44 (s, 1H), 5.69 (d, *J* = 6.4 Hz, 1H), 5.63 (s,

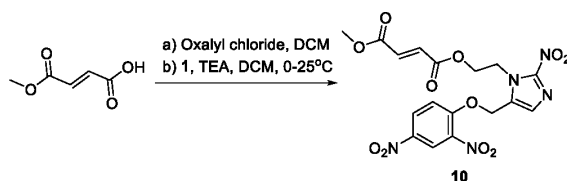
2H), 4.74 (t, $J = 5.2$ Hz, 2H), 4.42 – 4.34 (m, 1H), 4.29 (t, $J = 5.2$ Hz, 2H), 4.01 – 3.90 (m, 2H), 3.62 – 3.49 (m, 2H).

Synthesis Scheme 9



[00206] **Step-1: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl hydrogen sulfate (9).** To a solution of Compound **1** (100 mg, 0.28 mmol) in DCM (2 mL) was added chlorosulfuric acid (330 mg, 2.83 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 minutes and at 20 °C for another 1.5 hours. After the reaction was completed, the mixture was concentrated under reduced pressure to remove DCM. The residue was purified by Prep-HPLC (C18 column, ACN – H₂O (0.1% NH₃·H₂O)) to give the product **9** (35 mg, yield 27%, ammonium salt) as a white solid: MS(ESI), m/z : calcd for C₁₂H₁₁N₅O₁₁S Exact Mass: 433.02 found $t_R = 1.002$ min. $[M-H]^+ = 432$. ¹H NMR (400 MHz, *d*₆-DMSO) δ 8.79 (d, $J = 2.8$ Hz, 1H), 8.54 (dd, $J = 9.6, 2.8$ Hz, 1H), 7.79 (d, $J = 9.6$ Hz, 1H), 7.35 (s, 1H), 7.04 (br s, 4H), 5.73 (s, 2H), 4.65 (t, $J = 4.8$ Hz, 2H), 4.13 (t, $J = 4.8$ Hz, 2H).

Synthesis Scheme 10

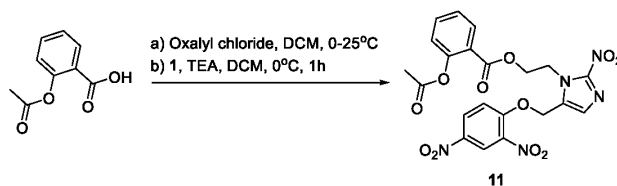


[00207] **Step-1: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl methyl fumarate (10).** To a solution of monomethyl fumarate (22 mg, 0.17 mmol) in DCM (2 mL) was added oxalyl chloride (28.7 mg, 0.23 mmol) at 0 °C. The reaction mixture was stirred under N₂ at 25 °C for 3 hours. The reaction mixture was evaporated in vacuo to give acyl chloride.

[00208] A solution of Compound **1** (40 mg, 0.11 mmol) and TEA (45.7 mg, 0.45 mmol) in DCM (2 mL) was added acyl chloride in DCM (2 mL) at 0 °C. The reaction mixture was stirred under N₂ at 25 °C for 2 hours. The reaction was quenched with additional water (10 mL) and extracted with DCM (3 x 5 mL). The combined organic layer was washed with brine (5 mL),

dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by pre-TLC (DCM/MeOH=15/1) to give product **10** (19 mg, yield 36%) as an off-white solid: MS (ESI), m/z : calcd for $C_{17}H_{15}N_5O_{11}$ Exact Mass: 465.077 found $t_R = 1.806$ min. $[M+H]^+ = 466.0$; 1H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, $J = 2.7$ Hz, 1H), 8.55 – 8.50 (m, 1H), 7.38 (d, $J = 9.2$ Hz, 1H), 7.34 (s, 1H), 6.84 – 6.67 (m, 2H), 5.41 (s, 2H), 4.91 (t, $J = 5.5$ Hz, 2H), 4.64 (t, $J = 5.5$ Hz, 2H), 3.82 (s, 3H).

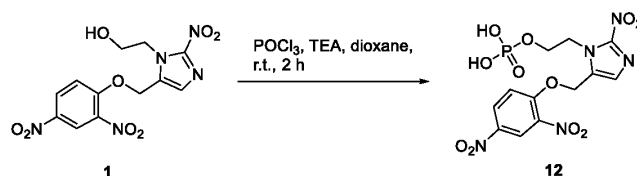
Synthesis Scheme 11



[00209] **Step-1: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 2-acetoxybenzoate (11).** To a solution of acetylsalicylic acid (1 g, 5.6 mmol) in DCM (20 mL) was added oxalyl chloride (1.42 g, 11.2 mmol) at 0°C. The reaction mixture was stirred under N_2 at 25°C for 3 hours. The reaction mixture was evaporated in vacuo to give the acyl chloride as a brown solid.

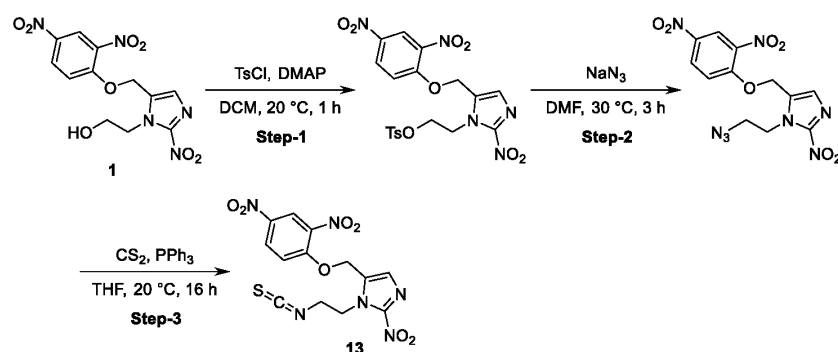
[00210] To a solution of Compound **1** (90 mg, 0.25 mmol) and TEA (77.4 mg, 0.76 mmol) in DCM (4 mL) was added acyl chloride (152 mg, 0.76 mmol) in DCM (1 mL) at 0°C. The reaction mixture was stirred under N_2 at 0°C for 1 hour. The reaction was quenched by addition water (10 mL) and extracted with DCM (3 x 5 mL). The combined organic layer was washed with brine (5 mL), dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by pre-TLC (DCM/MeOH=15/1) to give product **11** (90 mg, yield 64%) as an off-white solid: MS (ESI), m/z : calcd for $C_{21}H_{17}N_5O_{11}$ Exact Mass: 515.092 found $t_R = 2.415$ min. $[M+H]^+ = 516.0$; 1H NMR (400 MHz, $DMSO-d_6$) δ 8.78 (d, $J = 2.8$ Hz, 1H), 8.58 – 8.53 (m, 1H), 7.78 – 7.73 (m, 2H), 7.69 – 7.62 (m, 1H), 7.45 (s, 1H), 7.38 – 7.33 (m, 1H), 7.22 – 7.18 (m, 1H), 5.66 (s, 2H), 4.86 (t, $J = 5.5$ Hz, 2H), 4.63 (t, $J = 5.5$ Hz, 2H), 2.15 (s, 3H).

Synthesis Scheme 12



[00211] **Step-1: Synthesis of 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dihydrogen phosphate (12).** To a solution of **1** (100 mg, 0.028 mmol) and POCl_3 (22 mg, 0.141 mmol) in dioxane (4 mL) was added TEA (6 mg, 0.057 mmol) dropwise 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 H_2O (10 mL), extracted with EA (10 mL x 3). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by prep-HPLC to give **12** (60 mg, yield 47%) as a yellow solid: MS(ESI), m/z : calcd for $\text{C}_{12}\text{H}_{12}\text{N}_5\text{O}_{11}\text{P}$ Exact Mass: 433.03 found $t_R = 1.050$ min. $[\text{M}+\text{H}]^+ = 433.85$; ^1H NMR (400 MHz, d_6 -DMSO) δ 8.76 (d, $J = 2.8$ Hz, 1H), 8.51 (dd, $J = 9.2, 2.8$ Hz, 1H), 7.96 (d, $J = 9.2$ Hz, 1H), 7.29 (s, 1H), 5.82 (s, 2H), 4.58 (t, $J = 4.8$ Hz, 2H), 4.02 (m, 2H).

Synthesis Scheme 13



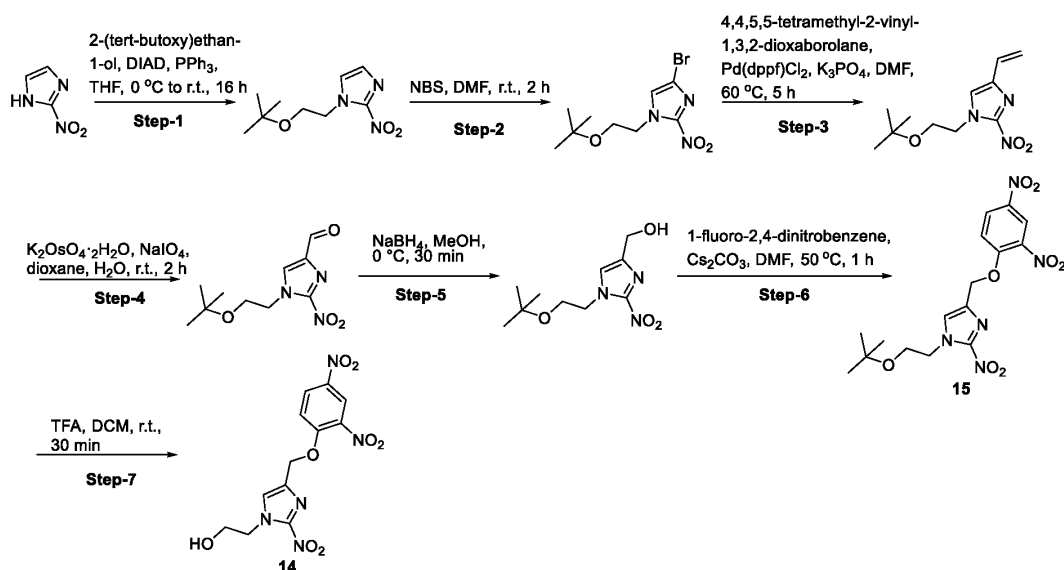
[00212] **Step-1: 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate.** To a solution of **1** (260 mg, 2.12 mmol) and DMAP (260 mg, 2.12 mmol) in DCM (10 mL) was added TsCl (175 mg, 0.92 mmol) at 20 °C. Then the reaction mixture was stirred at 20 °C for 1 hour. After the reaction was completed, the mixture was concentrated in vacuo to obtain a residue. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 0~5%) to give 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate (260 mg, 0.51 mmol, yield 24%) as a brown solid: MS(ESI), m/z : calcd for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_{10}\text{S}$ Exact Mass: 507.07 found $t_R = 1.365$ min. $[\text{M}+\text{H}]^+ = 508$; ^1H NMR

(400 MHz, CDCl₃) δ 8.80 (d, J = 2.8 Hz, 1H), 8.53 (dd, J = 9.2, 2.8 Hz, 1H), 7.62–7.57 (m, 2H), 7.43 (d, J = 9.2 Hz, 1H), 7.34–7.30 (m, 3H), 5.50 (s, 2H), 4.87 (t, J = 4.6 Hz, 2H), 4.43 (t, J = 4.6 Hz, 2H).

[00213] **Step 2: 1-(2-azidoethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole.** A mixture of 2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 4-methylbenzenesulfonate (100 mg, 0.197 mmol) and NaN₃ (26 mg, 0.394 mmol) in DMF (3 mL) was stirred at 30 °C for 3 hours. After the reaction was completed, the mixture was diluted with water (50 mL) and extracted with EtOAc (30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo to obtain a residue. The residue was purified by FCC (4 g, silica gel, MeOH in DCM = 0 ~ 10%) to give product (22 mg, 0.058 mmol, yield 29%) as a yellow solid: MS(ESI), m/z : calcd for C₁₂H₁₀N₈O₇ Exact Mass: 378.07 found t_R = 1.183 min. $[M+H]^+$ = 379; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.81 (d, J = 2.8 Hz, 1H), 8.59 (dd, J = 9.2, 2.8 Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 7.46 (s, 1H), 5.68 (s, 2H), 4.63 (t, J = 5.8 Hz, 2H), 3.85 (t, J = 5.8 Hz, 2H).

[00214] **Step-3: 5-((2,4-dinitrophenoxy)methyl)-1-(2-isothiocyanatoethyl)-2-nitro-1H-imidazole (13).** To a mixture of 1-(2-azidoethyl)-5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole (42 mg, 0.11 mmol) in THF (3 mL) were added triphenylphosphane (43 mg, 0.166 mmol) and methanedithione (237 mg, 3.1 mmol) at 20 °C. Then the reaction mixture was stirred at 20 °C for 16 hours. After the reaction was completed, the reaction mixture was concentrated in vacuo to obtain a residue. The residue was purified by FCC (12 g, silica gel, MeOH in DCM = 5%), followed by Prep-HPLC (C18, MeCN/H₂O (containing 0.1% FA)) to give product **13** (11 mg, 0.028 mmol, yield 25%) as a yellow solid: MS(ESI), m/z : calcd for C₁₃H₁₀N₆O₇S Exact Mass: 394.03 found t_R = 1.326 min. $[M+H]^+$ = 395; ¹H NMR (400 MHz, CDCl₃): δ 8.80 (d, J = 2.8 Hz, 1H), 8.54 (dd, J = 9.2, 2.8 Hz, 1H), 7.42 (d, J = 9.2 Hz, 1H), 7.39 (s, 1H), 5.48 (s, 2H), 4.83 (t, J = 5.2 Hz, 2H), 4.17 (t, J = 5.2 Hz, 2H).

Synthesis Scheme 14



[00215] **Step-1: 1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole.** To a mixture of 2-nitro-1*H*-imidazole (1.0 g, 8.8 mmol), 2-(*tert*-butoxy)ethan-1-ol (1.35 g, 11.4 mmol) and PPh₃ (6.92 g, 0.0264 mol) in THF (40 mL) was added DIAD (5.34 g, 0.0264mmol) at 0 °C under N₂. The reaction mixture was stirred at room temperature for 16 hours. After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was purified by FCC (MeOH in DCM = 1%) to give the product (1.3 g, 6.10 mmol, yield 69%) as a brown oil: MS(ESI), *m/z*: calcd for C₉H₁₅N₃O₃ Exact Mass: 213.11 found *t_R* = 1.118 min. [M+H]⁺ = 214.15.

[00216] **Step 2: 4-bromo-1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole.** To a solution of 1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole (1.0 g, 4.7 mmol) in DMF (10 mL) was added NBS (1.3 g, 7.0 mmol) at room temperature. The reaction mixture was stirred at this temperature for 2 hours. After the reaction was completed, the reaction was quenched with water (50 mL) and extracted with EtOAc (30 mL × 2). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain a residue. The residue was purified by FCC (25 g silica gel, EtOAc in PE = 10%) to give the product (350 mg, 1.2 mmol, yield 26%) as a colorless oil: MS(ESI), *m/z*: calcd for C₉H₁₄BrN₃O₃ Exact Mass: 291.02 found *t_R* = 1.330 min. [M+H]⁺ = 292.05; ¹H NMR (400 MHz, CDCl₃): δ 7.18 (s, 1H), 4.55 (t, *J* = 4.8 Hz, 2H), 3.68 (t, *J* = 4.8 Hz, 2H), 1.09 (s, 9H).

[00217] **Step-3: 1-(2-(*tert*-butoxy)ethyl)-2-nitro-4-vinyl-1*H*-imidazole.** A mixture of 4-bromo-1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole (320 mg, 1.10 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (253 mg, 1.64 mmol), K₃PO₄ (696 mg, 3.29 mmol) and Pd(dppf)Cl₂ (80 mg, 0.11 mmol) in DMF (5 mL) was stirred and heated at 60 °C under N₂ for 5 hours. After the reaction was completed, the reaction was quenched with water (30 mL) and extracted with MTBE (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 FCC (25 g silica gel, EtOAc in PE = 20%) to give the product (150 mg, 0.63 mmol, 57% yield) as a yellow oil: MS(ESI), m/z: calcd for C₁₁H₁₇N₃O₃ Exact Mass: 239.13 found t_R = 1.278 min. [M+H]⁺ = 240.20; ¹H NMR (400 MHz, CDCl₃): δ 7.14 (s, 1H), 6.57 (dd, *J* = 17.6, 10.8 Hz, 1H), 5.96 (dd, *J* = 17.6, 1.2 Hz, 1H), 5.33 (dd, *J* = 10.8, 1.2 Hz, 1H), 4.52 (t, *J* = 4.8 Hz, 2H), 3.68 (t, *J* = 4.8 Hz, 2H), 1.08 (s, 9H).

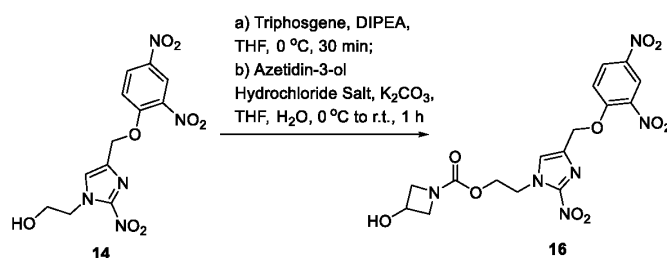
[00218] **Step-4: 1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole-4-carbaldehyde.** To a solution of 1-(2-(*tert*-butoxy)ethyl)-2-nitro-4-vinyl-1*H*-imidazole (150 mg, 0.63 mmol) and NaIO₄ (270 mg, 1.25 mmol) in 1,4-dioxane/H₂O (3 mL/3 mL) was added K₂OsO₄·2H₂O (11.5 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred at room temperature for 2 hours. After the reaction was completed, the reaction was quenched with water (30 mL) and extracted with EtOAc (30 mL × 2). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain the product (85 mg, crude) as a grey solid: MS(ESI), m/z: calcd for C₁₀H₁₅N₃O₄ Exact Mass: 241.11 found t_R = 1.121 min. [M+H]⁺ = 242.15; ¹H NMR (400 MHz, CDCl₃): δ 9.90 (s, 1H), 7.79 (s, 1H), 4.62 (t, *J* = 4.8 Hz, 2H), 3.70 (t, *J* = 4.8 Hz, 2H), 1.08 (s, 9H).

[00219] **Step-5: (1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazol-4-yl)methanol.** To a solution of 1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazole-4-carbaldehyde (85 mg, 0.35 mmol) in MeOH (2 mL) was added NaBH₄ (20 mg, 0.53 mmol) at 0 °C. Then the reaction mixture was stirred at 0 °C for 30 minutes. After the reaction was completed, the reaction was quenched with Sat.NH₄Cl (30 mL) and extracted with EtOAc (20 mL × 2). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to obtain the product (42 mg, crude) as a colorless oil: MS(ESI), m/z: calcd for C₁₀H₁₇N₃O₄ Exact Mass: 243.12 found t_R = 0.973 min. [M+H]⁺ = 244.15.

[00220] **Step-6: 1-(2-(*tert*-butoxy)ethyl)-4-((2,4-dinitrophenoxy)methyl)-2-nitro-1*H*-imidazole (15).** To a solution of (1-(2-(*tert*-butoxy)ethyl)-2-nitro-1*H*-imidazol-4-yl)methanol (42 mg, 0.17 mmol) and 1-fluoro-2,4-dinitrobenzene (37 mg, 0.20 mmol) in DMF (1 mL) was added Cs₂CO₃ (140 mg, 0.43 mmol) at room temperature. Then the reaction mixture was stirred and heated at 50 °C for 1 hour. After the reaction was completed, the reaction was quenched with Sat.NH₄Cl (20 mL) and extracted with EtOAc (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 FCC (silica gel, MeOH in DCM = 0~5%) to give **15** (64 mg, 0.156 mmol) as a brown oil: MS(ESI), *m/z*: calcd for C₁₆H₁₉N₅O₈ Exact Mass: 409.12 found *t_R* = 1.455 min. [M+H]⁺ = 410; ¹H NMR (400 MHz, CDCl₃): δ 8.76 (d, *J* = 2.8 Hz, 1H), 8.45 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.44 (d, *J* = 9.2 Hz, 1H), 7.39 (s, 1H), 5.36 (s, 2H), 4.58 (t, *J* = 5.2 Hz, 2H), 3.68 (t, *J* = 5.2 Hz, 2H), 1.04 (s, 9H).

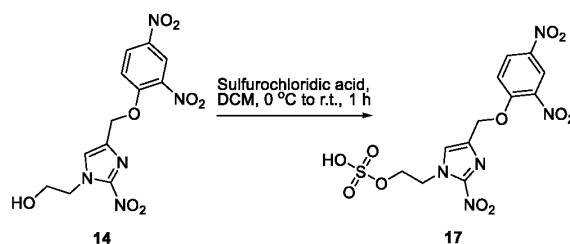
[00221] **Step-7: 2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1*H*-imidazol-1-yl)ethan-1-ol (14).** To a solution of **15** (65 mg, 0.16 mmol) in DCM (2 mL) was added TFA (1 mL) dropwise at room temperature. Then the reaction mixture was stirred at this temperature for 30 minutes. After the reaction was completed, the reaction mixture was diluted with DCM (30 mL) and washed with water (25 mL × 2). The organic layer was dried over Na₂SO₄ and concentrated under reduced to obtain a residue. The residue was purified by FCC (12 g silica gel, MeOH in DCM = 0~5%) and prep-HPLC (C18, MeCN/H₂O (0.1% FA)) to give **14** (32.49 mg) as a yellow solid. MS(ESI), *m/z*: calcd for C₁₂H₁₁N₅O₈, Exact Mass: 353.06, found *t_R* = 1.239 min, [M+H]⁺ = 354.05; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.78 (d, *J* = 2.8 Hz, 1H), 8.54 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.80 (d, *J* = 9.2 Hz, 1H), 7.76 (s, 1H), 5.41 (s, 2H), 5.03 (br s, 1H), 4.46 (t, *J* = 5.2 Hz, 2H), 3.76–3.69 (m, 2H).

Synthesis Scheme 15



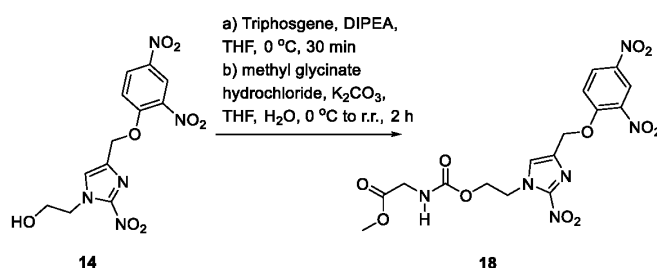
[00223] **Step-1: 2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 3-hydroxyazetidine-1-carboxylate (16).** To a mixture of **14** (100 mg, 0.28 mmol) and DIPEA (146 mg, 1.13 mmol) in THF (2 mL) was added triphosgene (50 mg, 0.17 mmol) at 0 °C under N₂. The reaction mixture was stirred at 0 °C for 30 minutes. Then a mixture of azetidin-3-ol hydrochloride (62 mg, 0.57 mmol) and K₂CO₃ (195 mg, 1.42 mmol) in THF/H₂O (2 mL/2 mL) was added dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 30 minutes and at room temperature for another 30 minutes. After the reaction was completed, the reaction was quenched with water (30 mL) and extracted with EtOAc (30 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% FA)) to give **16** (64 mg, yield 51%) as a pale-yellow solid: MS(ESI), m/z: calcd for C₁₆H₁₆N₆O₁₀ Exact Mass: 452.09 found t_R = 1.072 min. [M+H]⁺ = 453; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.78 (d, *J* = 2.8 Hz, 1H), 8.54 (dd, *J* = 9.6, 2.8 Hz, 1H), 7.84–7.72 (m, 2H), 5.66 (d, *J* = 6.0 Hz, 1H), 5.43 (s, 2H), 4.73–4.55 (m, 2H), 4.43–4.24 (m, 3H), 4.01–3.89 (m, 2H), 3.61–3.48 (m, 2H).

Synthesis Scheme 16



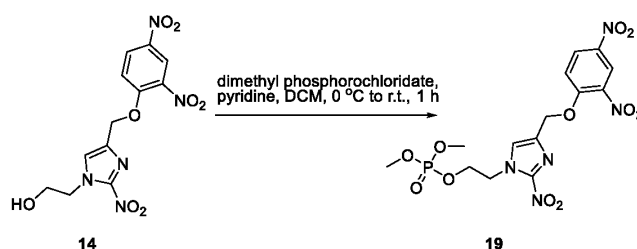
[00225] **Step-1: 2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl hydrogen sulfate (17).** To a solution of **14** (100 mg, 0.28 mmol) in DCM (1 mL) was added sulfurochloridic acid (660 mg, 5.66 mmol) at 0 °C. Then the reaction mixture was stirred at room temperature for 1 hour. After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (C18, MeCN/H₂O (0.1% NH₃·H₂O)) to give **17** (16 mg, NH₃ salt) as a pale-yellow solid: MS(ESI), m/z: calcd for C₁₂H₁₁N₅O₁₁S Exact Mass: 433.02 found t_R = 1.029 min. [M-H]⁻ = 432; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.76 (d, *J* = 2.8 Hz, 1H), 8.53 (dd, *J* = 9.6, 2.8 Hz, 1H), 7.79 (m, 2H), 7.07 (br s, 4H), 5.42 (s, 2H), 4.61 (t, *J* = 5.2 Hz, 2H), 4.10 (t, *J* = 5.2 Hz, 2H).

Synthesis Scheme 17



[00227] **Step-1: methyl ((2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethoxy) carbonyl)glycinate (18).** To a mixture of **14** (100 mg, 0.28 mmol) and DIPEA (146 mg, 1.13 mmol) in THF (2 mL) was added Triphosgene (50 mg, 0.17 mmol) at 0 °C under N₂. The reaction mixture was stirred at 0 °C for 30 minutes. Then a mixture of methyl glycinate hydrochloride (70 mg, 0.57 mmol) and K₂CO₃ (195 mg, 1.42 mmol) in THF/H₂O (2 mL/2 mL) was added dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 1 hour and at room temperature for another 1 hour. After the reaction was completed, the mixture diluted with water (50 mL) and extracted with EtOAc (30 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% FA)) to give **18** (71 mg, 0.15 mmol, yield 54%) as a pale-yellow solid: MS(ESI), m/z: calcd for C₁₆H₁₆N₆O₁₁ Exact Mass: 468.09 found t_R = min. [M+H]⁺ = 469; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.78 (d, *J* = 3.2 Hz, 1H), 8.54 (dd, *J* = 9.2, 3.2 Hz, 1H), 7.80–7.74 (m, 2H), 7.61 (t, *J* = 6.0 Hz, 1H), 5.40 (s, 2H), 4.65 (t, *J* = 4.8 Hz, 2H), 4.38 (t, *J* = 4.8 Hz, 2H), 3.69 (d, *J* = 6.0 Hz, 2H), 3.62 (s, 3H).

Synthesis Scheme 18

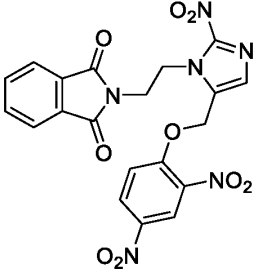
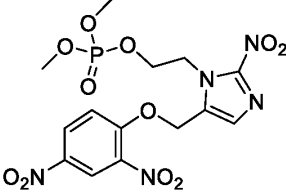
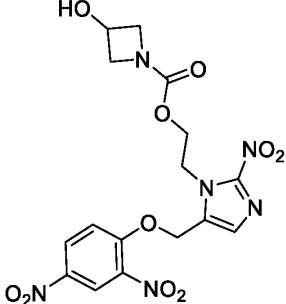
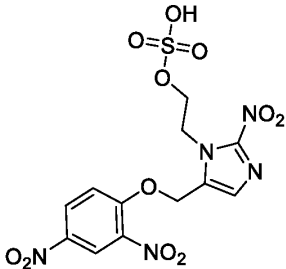
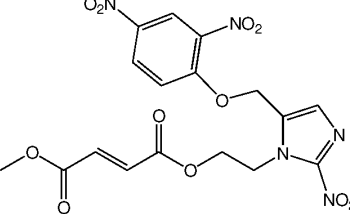


[00229] **Step 1: 2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dimethyl phosphate (19).** To a solution of **14** (100 mg, 0.28 mmol) in DCM (2 mL) were added pyridine (1 mL) and dimethyl phosphorochloridate (123 mg, 0.85 mmol) at 0 °C. Then the reaction mixture was stirred at 0 °C for 0.5 hours and at room temperature for another 0.5 hours.

After the reaction was completed, the solvent was removed under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (C18, MeCN/H₂O (0.1% FA)) to give **19** (78 mg, yield 60%) as a pale-yellow solid: MS(ESI), m/z: calcd for C₁₄H₁₆N₅O₁₁P Exact Mass: 461.06 found *t_R* = 1.125 min. [M+H]⁺ = 462; ¹H NMR (400 MHz, CDCl₃): δ 8.75 (d, *J* = 3.2 Hz, 1H), 8.47 (dd, *J* = 9.2, 3.2 Hz, 1H), 7.51–7.38 (m, 2H), 5.36 (s, 2H), 4.77 (t, *J* = 4.8 Hz, 2H), 4.52–4.37 (m, 2H), 3.73 (d, *J* = 11.2 Hz, 6H).

Table 1A. Exemplary Compounds and Chemical Characterization

Compound No.	Structure	Chemical Name	LC-MS characterization (M+H ⁺)
1		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-ol	354.1
2		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl isobutyrate	424.0
3		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl ethyl carbonate	426.0
4		methyl ((2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethoxy)carbonyl)glycinate	469.0
5		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethan-1-amine	353.0

6		2-(2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl)isoindoline-1,3-dione	483.1
7		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dimethyl phosphate	461.9
8		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 3-hydroxyazetidine-1-carboxylate	453.0
9		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl hydrogen sulfate	432.2
10		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl methyl fumarate	466.0

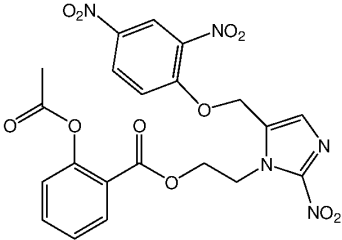
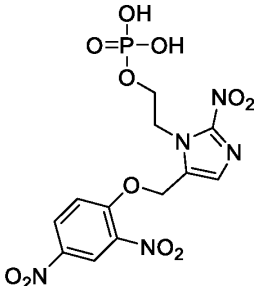
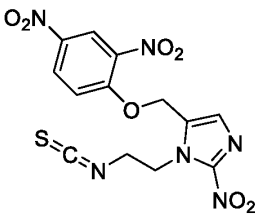
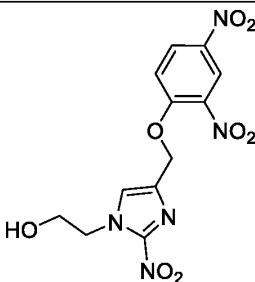
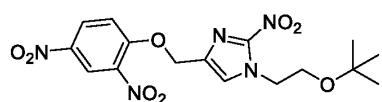
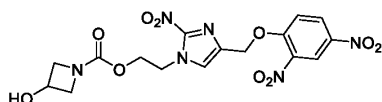
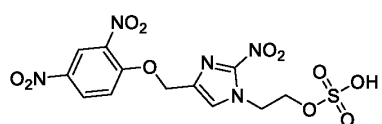
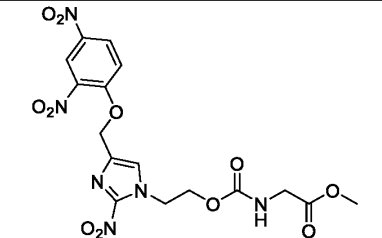
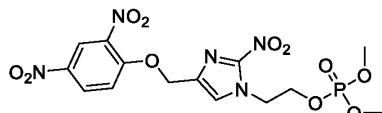
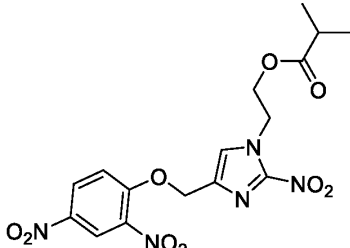
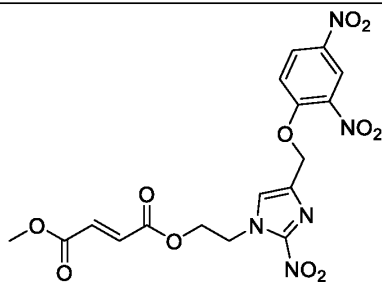
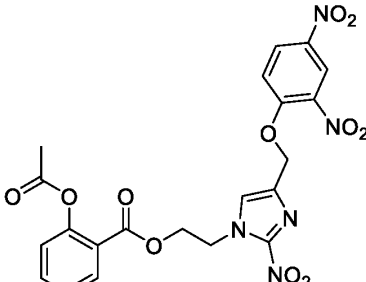
11		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 2-acetoxybenzoate	516.0
12		2-(5-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dihydrogen phosphate	433.9
13		5-((2,4-dinitrophenoxy)methyl)-1-(2-isothiocyanatoethyl)-2-nitro-1H-imidazole	395

Table 1B. Exemplary Compounds and Chemical Characterization

Compound No.	Structure	Chemical Name	LC-MS characterization (M+H ⁺)
14		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethanol	354.1
15		1-(2-(tert-butoxy)ethyl)-4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazole	410
16		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 3-hydroxyazetidine-1-carboxylate	453
17		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl hydrogen sulfate	432 [M-H] ⁻
18		methyl ((2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethoxy)carbonyl)glycinate	469
19		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl dimethyl phosphate	462

20		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl isobutyrate	424.0
21		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl methyl fumarate	466.0
22		2-(4-((2,4-dinitrophenoxy)methyl)-2-nitro-1H-imidazol-1-yl)ethyl 2-acetoxybenzoate	516.0

Study I. Pharmacokinetics Studies in Mice

General Protocol

[00230] Male C57BL/6 mice (20~30 g, supplied by 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 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 the PO group, mice received oral gavage of the test article. At least 30 μ L of blood was collected 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 collected into an EP tube containing EDTA-K2 and

immediately placed on wet ice, then centrifuged at 3500 g and at 4°C for 5 min to obtain the plasma within 30 min. 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.

[00231] The pharmacokinetic parameters of 2,4-DNP following iv injection at 1 mg/kg and oral gavage at 5 mg/kg in male C57BL/6 mice are shown in **Table 2** and the mean plasma concentrations are shown in **FIG. 1**.

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	0.52
2,4-DNP (po) ^c	11367	37907	0.30

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

[00232] The pharmacokinetic parameters of Compound **1** and its metabolite 2,4-DNP after an IV (1 mg/kg) or a P.O (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**.

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	1178	221	
1 _DNP (iv) ^d	1117	2604	0.42
1 (po) ^c	1182	894	
1 _DNP (po) ^d	3453	14798	0.23

^a Mice PK parameter (mean, n = 3). ^b IV 1 mg/kg. ^c PO 5 mg/kg. ^d 2,4-Dinitrophenol released from the parent compound.

Study II. Pharmacokinetics Studies in Rats

General Protocol

[00233] Male SD rats (170~300 g, supplied by Vital River) were randomly divided into groups (n = 3 or 4) 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 appropriate volume of the vehicle to the test article to achieve the desired concentration. For IV group, rats received a single IV bolus injection of the test article via tail vein. And for the PO group, rats received oral gavage of test article. At least 65 µL of blood was collected 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 jugular vein. The whole blood was immediately collected into an EP tube containing EDTA-K2 and placed on wet ice, then centrifuged at 3500g at 4°C for 5 min to obtain the plasma within 30 min. 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.

[00234] The pharmacokinetic parameters of 2,4-DNP following iv injection at 1 mg/kg and oral gavage at 5 mg/kg in male SD rats are shown in **Table 4** and the mean plasma concentrations are shown in **FIG. 3**.

Table 4. Pharmacokinetic Study of 2,4-DNP following Intravenous and Oral Administrations in Male SD Rats^a

Compound	C0/Cmax (ng/mL)	AUC _{last} (h*ng/mL)	Cmax/AUC
2,4-DNP (iv) ^b	5596	15351	0.36
2,4-DNP (po) ^c	15767	110659	0.14

^a Rats PK parameter (mean, n = 3). ^b Dosed intravenously at 1 mg/kg (iv). ^c Dosed orally at 5 mg/kg (po).

[00235] The pharmacokinetic parameters of Compound **1** and its metabolite 2,4-DNP after an IV (1 mg/kg) or P.O (5 mg/kg) dosing in male SD rats are shown in **Table 5**. The mean plasma concentrations of **1** and its metabolite 2,4-DNP are shown in **FIG. 4**.

Table 5. 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	1093	171	
1 _DNP (iv) ^d	1637	6070	0.27
1 (po) ^c	333	453	
1 _DNP (po) ^d	4103	40953	0.10

^a Rats PK parameter (mean, n = 3). ^b IV 1 mg/kg. ^c PO 5 mg/kg. ^d 2,4-Dinitrophenol released from the parent compound.

[00236] The pharmacokinetic parameters of Compound **14** and its metabolite 2,4-DNP after an IV (1 mg/kg) or P.O (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**.

Table 6. Pharmacokinetic Study of Compound **14** (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
14 (iv) ^b	1435	616	
14 _DNP (iv) ^d	734	2771	0.26
14 (po) ^c	860	1404	
14 _DNP (po) ^d	1130	10955	0.10

^a Rats PK parameter (mean, n = 3 or 4). ^b IV 1 mg/kg. ^c PO 5 mg/kg. ^d 2,4-Dinitrophenol released from the parent compound.

Study III. Pharmacokinetics Studies in Dogs

General Protocol

[00237] 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, 5mL/kg) or po. dose (5 mg/kg, 10mL/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 the IV group, dogs received a single IV bolus injection of the test article. For the PO group, dogs received test articles via oral gavage. At least 150 µL of blood/time point was collected at 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 collected into an EP tube containing EDTA-K2 and immediately placed on wet ice, then 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 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.

[00238] The pharmacokinetic parameters of Compound **1** and its metabolite 2,4-DNP after an IV (1 mg/kg) or P.O (5 mg/kg) dosing in male Beagle dogs are shown in **Table 7**. The mean plasma concentrations of **1** and its metabolite 2,4-DNP are shown in **FIG. 6**.

Table 7. Pharmacokinetic Study of Compound **1** (IV (1 mg/kg) or a PO (5 mg/kg) administration in Male Beagle Dogs^a)

Compound	Cmax (ng/mL)	AUC _{last} (h*ng/mL)	Cmax/AUC
1 (iv) ^b	481	356	
1 _DNP (iv) ^d	1147	7640	0.15
1 (po) ^c	833	978	
1 _DNP (po) ^d	6853	55327	0.12

^a Dogs PK parameter (mean, n = 3). ^b IV 1 mg/kg. ^c PO 5 mg/kg. ^d 2,4-Dinitrophenol released from the parent compound.

Study IV. Rectal temperature measurement in rats

General Protocol

[00239] Male SD Rats (200~250 g, supplied by 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 of the chemicals.

[00240] 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 are shown in **FIG. 7**. The survival curves are shown in **FIG. 8**.

[00241] 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. 9**. There are no significant differences in the temperature between vehicle and Compound **1** treatment groups. No dose-dependent mortality was observed, either.

[00242] 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 Cmax/AUC ratio for Compound **1** as compared to 2,4-DNP, resulting in an improved safety profile.

[00243]

Study V. In vivo pharmacology study for Compound **1** in DIO mice

General Protocol

[00244] For diet induced obesity (DIO) mice model development, C57BL/6j mice (provided by Jiangsu GemPharmatech biotechnology Co. Ltd.) were fed with high fat diet (HFD) for 17 weeks (D12492i, Rodent Diet with 60 kcal% Fat, Research diet) initiated at 6weeks old. Control mice were fed with standard control diet (SCD) during the study. DIO mice were randomized into three groups (Model, Compound **1** 15mg/kg and 50mg/kg) base on body weight and plasma lipid level. Day0 was defined as the first day of compound dosing. Compound **1** was administered once daily (q.d.) by oral gavage (p.o) for continuous six weeks. Dose volume was

10 ml/kg. Mice in model group and control group were dosed with vehicle (5% DMSO+10% Solutol HS-15+85% water). The test article formulation was freshly prepared for each dosing occasion. Mix well before using. Body weight and food intake were measured daily.

[00245] On day 39, animals were fasted overnight. Fasting glucose was measured at 2 hours post the compound treatment on day40. Then blood samples were collected via retro-orbital bleeding and collected into tubes pre-coated with EDTA-K2 for plasma preparation. Plasma samples were obtained by centrifugation at 4000 rpm for 10 minutes at 4°C and then stored at -80°C for fasting insulin measurement.

[00246] At the end of study (2 hours post the compound treatment) on Day43, animals were fasted overnight. Animals were euthanized with CO₂. Blood samples were collected through cardiac puncture, then collected into tubes pre-coated with EDTA-K2 for plasma preparation. Plasma was obtained by centrifugation at 4000 rpm for 10 minutes at 4°C and aliquoted. Plasma samples were stored at -80 °C for further analysis (lipids, liver function test et al). Soon after blood sample collection, all animals were sacrificed and perfused intracardially with ice-cold saline. Liver was collected and liver weight was measured. Right lobe of liver samples was quick frozen and stored at -80 °C for further analysis (liver lipids). Left lobe of liver samples were kept in tubes containing 10% neutral buffered formalin (NBF) and processed into formalin fixed paraffin-embedded (FFPE) block for further pathology staining and analysis. Data are presented as Mean ± SEM. Differences between the groups were analyzed with one-way ANOVA, Graphpad Prism. $p < 0.05$ was regarded as statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs G2 DIO Model.

[00247] As shown in **FIG.10**, Compared to the normal control group, DIO mice body weight significantly increased throughout the study period. Compared to the DIO mice model, treatment with Compound **1** (50 mg/kg, q.d.*6w) significantly slowed down the body weight gain from day 8 to the end of the study.

[00248] As shown in the **FIG.11** and **FIG.12**, compared to the normal control group, HFD feeding (DIO model group) significantly increased fasting glucose and fasting insulin level. Compared to the model group, Compound **1** (15mg/kg and 50mg/kg) treatment for six weeks significantly decreased fasting plasma glucose and insulin levels in a dose dependent manner.

[00249] For plasma lipid, as shown in **FIG.13a** and **FIG.13b**, compared to the normal control group, HFD feeding significantly increased levels of total cholesterol (TC), triglyceride (TG),

high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) in mice plasma. Compared to the model group, treatment with 15mg/kg of Compound **1** significantly decreased levels of TC and HDL in plasma, while treatment with 50 mg/kg of Compound **1** significantly decreased plasma TC, TG, HDL and LDL levels.

[00250] As shown in **FIG.13e**, compared to the normal control group, HFD feeding significantly increased plasma malondialdehyde (MDA) level, compared to the model group, treatment with 15mg/kg and 50mg/kg of Compound **1** significantly decreased plasma MDA level.

[00251] As shown in the **FIG.14a** and **FIG.14b**, compared to the model group, HFD feeding significantly increased mice liver weight and lipid level. Treatment with Compound **1** at 50mg/kg significantly decreased liver weight, liver TC and TG levels.

[00252] As shown in the **FIG.15a** and **FIG.15b**, compared to the normal control group, HFD feeding significantly increased mice plasma ALT and AST levels. Compared to the model group, treatment with Compound **1** at both 15mg/kg and 50mg/kg significantly decreased plasma ALT and AST levels.

[00253] Livers collected from the control group showed normal lobular architecture with central veins and radiating hepatic cords. HFD feeding resulted in severe pathological changes including steatosis, hepatocyte ballooning degeneration, inflammation in mice liver and significantly increased steatosis score, hepatocyte ballooning score, inflammation score and NAS score. Compared to the model group, treatment with Compound **1** at 15mg/kg and 50mg/kg (q.d.*6w) significantly decreased steatosis, inflammation and NAS scores, respectively (**FIG.16a-16c**). Treatment with Compound **1** at 50mg/kg significantly decreased hepatocyte ballooning score (**FIG.16d**).

[00254] Taken together, Compound **1** showed significant improvement on liver health via reducing liver fat accumulation, reducing hepatocytes ballooning, anti-inflammation and reversing hepatic steatosis. Compound **1** also showed systemic benefits as it decreased circulating lipid levels, improving insulin sensitivity and glucose homeostasis.

Study VI. In vivo pharmacology study for Compound 1 in C57 BL/6 wild type mice

General Protocol

[00255] Wild type C57 BL/6 mice were randomized into two groups (control and Compound 1 treated at 30mg/kg) base on body weight. Compound 1 was administered once daily (q.d.) via oral gavage (p.o) for six weeks, continuously. Dose volume was 10 ml/kg. Mice in the control group were dosed with vehicle (5% DMSO+10% Solutol HS-15+85% water). The test article formulation was freshly prepared for each dosing occasion. Mix well before using. Mouse muscle endurance was evaluated by treadmill test. Each mouse was placed in individual treadmill lanes. Each lane was equipped with a shock grid that delivers a foot shock. Exhaustion was defined when mice were unable to avoid continuous 5 seconds electric shock time. Running distance (m), the total time (s) and shock times until exhaustion was recorded. At the end of the study, animals were euthanized with CO₂. All animals were sacrificed and perfused intracardially with ice-cold saline. Quadriceps muscle, gastrocnemius, tibialis anterior muscle, soleus, extensor digitorum longus were collected and muscle weight was measured.

[00256] The gastrocnemius was directly mounted in OCT compound and rapidly frozen in isopentane chilled with liquid nitrogen and stored at -80°C for pathology analysis. Frozen muscle cross sections were cut at 10 µm in thickness and stained with NADH-TR for Type I (dark) and Type II (light) myofibers analysis. The Type I muscle fibers and Type II muscle fibers were measured using “Nuclei Seg (HALO AI) and Nuclei Phenotyper (HALO AI)” module. Type I muscle fibers ratio was expressed as percentage of type I muscle fibers and all muscle fibers. Protein and RNA were extracted from quadriceps muscle for 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and pyruvate dehydrogenase kinase 4 (PDK4) analysis. Data are presented as Mean ± SEM. Differences between the groups were analyzed with the Student's t test, Graphpad Prism. p < 0.05 was regarded as statistically significant. *p < 0.05, **p < 0.01, ***p < 0.001 vs WT-Control group.

[00257] As shown in **FIG.17a** and **FIG.17b**, compared to the control group, treatment with Compound 1 (30mg/kg, p.o., q.d.) significantly increased the distance run and total time in treadmill test, indicating that Compound 1 significantly increased muscle endurance of mice.

[00258] As shown in **FIG.18** and **FIG.19**, compared to the control group, treatment with Compound 1 (30 mg/kg, p.o., q.d.) increased total skeletal muscle index and type I muscle ratio, suggesting that **Compound 1** treatment increased the ratios of lean muscle mass as well as type I muscle, resulting in improved endurance. Since type I muscle fibers utilize more oxidative metabolism for energy production, it's usually more fatigue-resistant.

[00259] Results also showed that treatment with Compound 1 activated AMPK expression and upregulated PDK4 expression (**FIG.20** and **FIG.21**), which are typically associated with increased fat oxidation during prolonged exercise.

[00260]

[00261] 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.

[00262] 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.

[00263] 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.

Incorporation by Reference

[00264] 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.

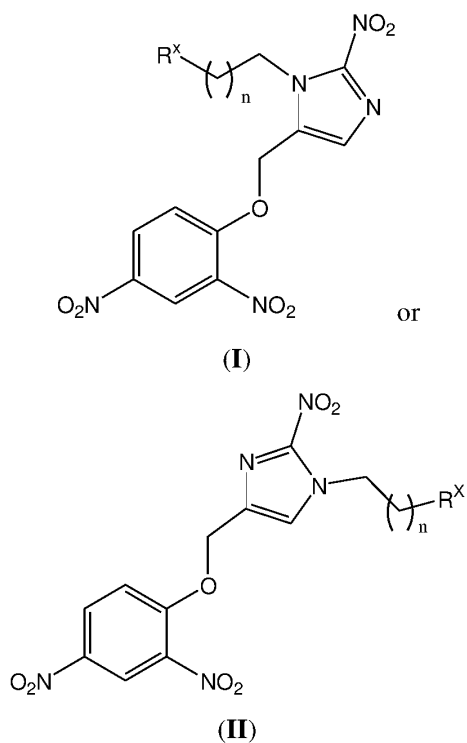
Equivalents

[00265] 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.

What is claimed is:

CLAIMS

1. A compound having structural formula (I) or (II):



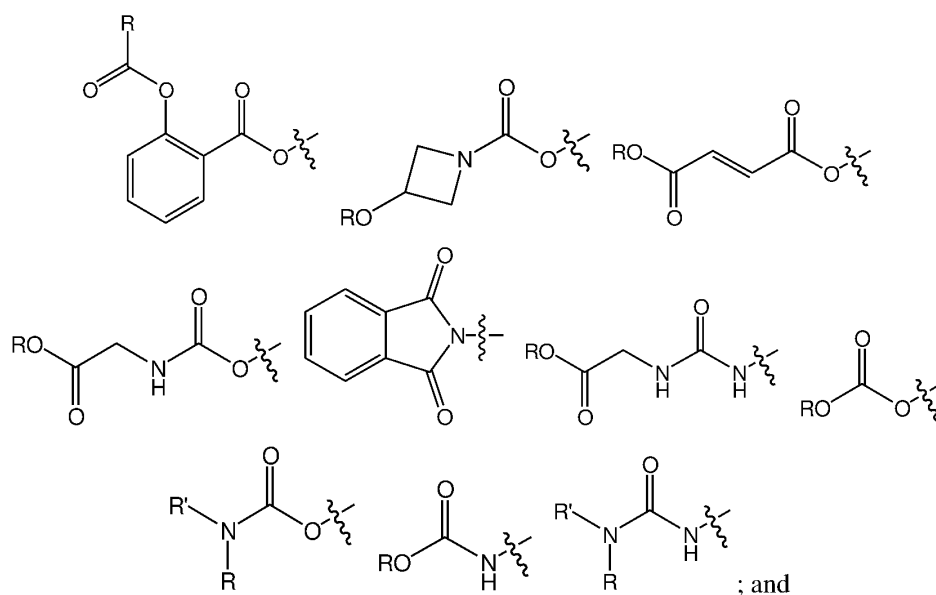
or a pharmaceutically acceptable form or an isotope derivative thereof,
wherein

n is 1, 2, 3 or 4; and

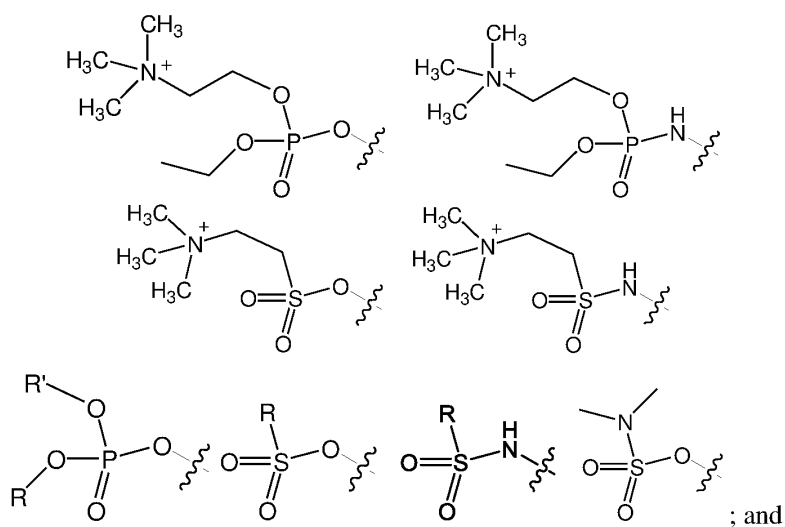
R^X is R^{X1} , R^{X2} or R^{X3} , wherein

R^{X1} is a group selected from: $CH_2C(=O)OR^3$, $CH_2C(=O)SR^3$, $C(=O)NR^4R^5$, OR^6 , SR^6 , NR^7R^8 , $NR^9C(=O)R^{10}$, $OC(=O)R^{11}$, $SC(=O)R^{12}$, $OC(=O)OR^{13}$, $SC(=O)OR^{13}$, $NR^9C(=O)OR^{13}$, $OC(=O)NR^{14}R^{15}$, $SC(=O)NR^{14}R^{15}$, $NR^9C(=O)NR^{14}R^{15}$, $OC(=O)SR^3$, $SC(=O)SR^3$, $NR^9C(=O)SR^3$, halo, CN, H, and C_{1-6} alkyl; and

R^{X2} is a group selected from:



R^{X3} is a group selected from:



each of R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} :

is independently selected from H and C_{1-6} alkyl, 3- to 8-membered carbocyclic or heterocyclic ring, optionally substituted with 1-6 R^A , or

R^4 and R^5 , R^7 and R^8 , or R^{14} and R^{15} , 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, OR and NRR' ; and

each of R and R'

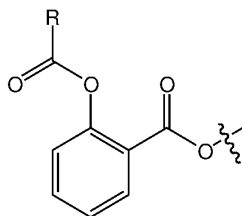
is independently H or C_{1-6} alkyl, optionally substituted with 1-4 groups selected from D, halo, C_{1-6} alkoxy and amino; or

R and R' together with the N atom they are bonded to, respectively, form a 3- to 6-membered heterocyclic ring, optionally substituted with 1-4 groups selected from D, halo, C_{1-6} alkyl or alkoxy, and amino.

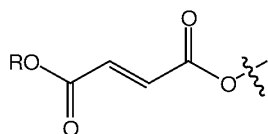
2. The compound of claim 1, having the structural formula (I).
3. The compound of claim 1, having the structural formula (II).
4. The compound of any one of claims 1-3, wherein R^X is R^{X1} .
5. The compound of claim 4, wherein R^{X1} is $CH_2C(=O)OR^3$ or $OC(=O)R^{11}$.
6. The compound of claim 4, wherein R^{X1} is $C(=O)NR^4R^5$ or $NR^9C(=O)R^{10}$.
7. The compound of claim 4, wherein R^{X1} is $CH_2C(=O)SR^3$ or $SC(=O)R^{12}$.
8. The compound of claim 4, wherein R^{X1} is $OC(=O)OR^{13}$, $SC(=O)OR^{13}$, $NR^9C(=O)OR^{13}$, $OC(=O)NR^{14}R^{15}$, $SC(=O)NR^{14}R^{15}$, $NR^9C(=O)NR^{14}R^{15}$, $OC(=O)SR^3$, $SC(=O)SR^3$ or $NR^9C(=O)SR^3$.
9. The compound of claim 4, wherein R^{X1} is OR^6 or SR^6 .
10. The compound of claim 4, wherein R^{X1} is NR^7R^8 .
11. The compound of claim 4, wherein R^{X1} is halo, CN or C_{1-3} alkyl.

12. The compound of any one of claims 1-3, wherein R^X is R^{X2} .

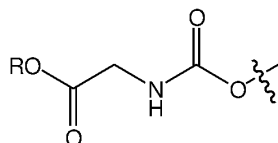
13. The compound of claim 12, wherein R^{X2} is:



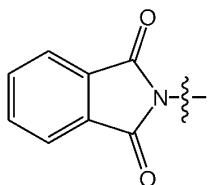
14. The compound of claim 12, wherein R^{X2} is:



15. The compound of claim 12, wherein R^{X2} is:



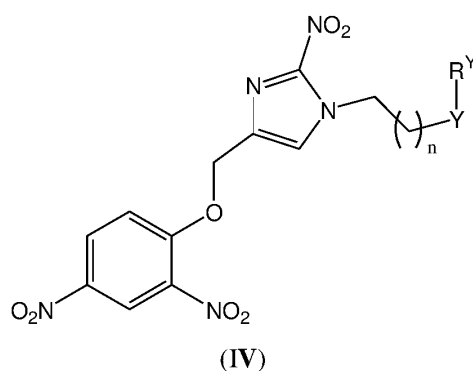
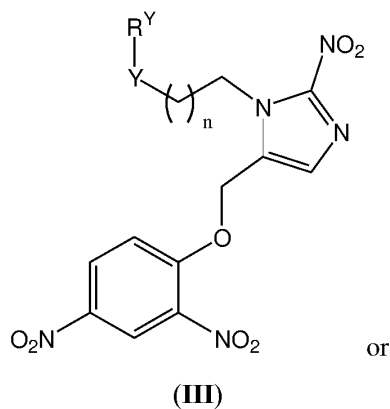
16. The compound of claim 12, wherein R^{X2} is:



17. The compound of any one of claims 1-3, wherein R^X is R^{X3} .

18. The compound of any one of claims 1-17, wherein each of R and R' , if present, is independently H or C_{1-3} alkyl.

19. A compound having structural formula (III) or (IV):



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

n is 1, 2, 3 or 4;

Y is selected from a single bond, NR , S , CRR' and O ;

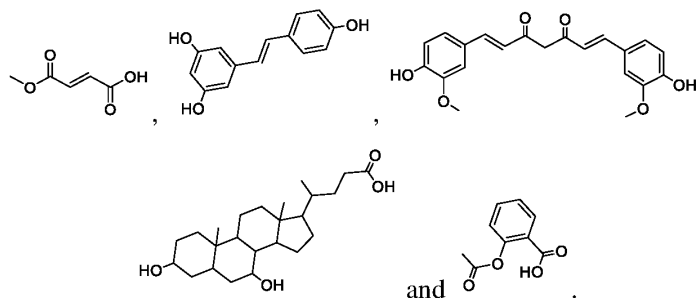
R^Y is a group selected from: H , halo, CN , R , C(=O)OR , $\text{C(=O)NRR}'$, C(=O)R and C(=O)SR ; and

each of R and R' is independently H or C_{1-6} alkyl, or R and R' , together with the N atom they are bonded to, form a 3- to 6-membered heterocyclic ring, wherein the alkyl or heterocyclic ring is optionally substituted with 1-4 groups selected from D , halo, C_{1-6} alkyl or alkoxy, and amino.

20. The compound of claim 19, having the structural formula (III).

21. The compound of claim 19, having the structural formula (IV).
22. The compound of any one of claims 19-21, wherein Y is O.
23. The compound of claim 22, wherein R^Y is selected from R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.
24. The compound of any one of claims 19-21, wherein Y is S.
25. The compound of claim 24, wherein R^Y is selected from R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.
26. The compound of any one of claims 19-21, wherein Y is NR.
27. The compound of claim 26, wherein R^Y is selected from R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.
28. The compound of any one of claims 19-21, wherein Y is CH₂.
29. The compound of claim 28, wherein R^Y is selected from halo, CN, R, C(=O)OR, C(=O)NRR', C(=O)R, and C(=O)SR.
30. The compound of any one of claims 19-21, wherein Y is a single bond.
31. A compound selected from **Table 1A**.
32. A compound selected from **Table 1B**.
33. A conjugate compound formed by a compound according to any one of claims 1-31 and a biologically active moiety characterized by an anti-ROS and/or anti-inflammatory activity.

34. The conjugate compound of claim 33, wherein the biologically active moiety is selected from:



35. The compound of any of claims 1-34, having one or more deuterium atoms in place of one or more hydrogen atoms.
36. The compound of claim 35, having one deuterium atom in place of one hydrogen atom.
37. A pharmaceutical composition comprising a compound according to any of claims 1-36.
38. A unit dosage form comprising a pharmaceutical composition of claim 37.
39. The unit dosage form of claim 38, being a tablet.
40. The unit dosage form of claim 38, being a capsule.
41. 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 1-36.
42. The method of claim 41, wherein the disease or disorder is associated with mitochondrial function of a subject.
43. The method of claim 41, wherein the disease or disorder is associated with one or more

defect in mitochondrial function.

44. The method of claim 41, wherein the disease or disorder is metabolic disease, neurodegenerative diseases, aging-related diseases, liver disease, cardiovascular disease, or a related disease or disorder.
45. The method of claim 41, wherein 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), and nonalcoholic Steatohepatitis (NASH), or a related disease or disorder.
46. 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 1-36.
47. The method of any one of claims 41-46, wherein administration is via oral administration.
48. Use of a compound of any of claims 1-36, and a pharmaceutically acceptable excipient, carrier, or diluent, in preparation of a medicament for treating a disease or disorder.
49. Use of a compound of any of claims 1-36 for treating a disease or disorder.
50. The use of claim 48 or 49, wherein the disease or disorder is associated with one or more defect in mitochondrial function.
51. The use of claim 48 or 49, wherein the disease or disorder is metabolic diseases, neurodegenerative diseases, aging-related diseases, liver disease, cardiovascular disease,

or a related disease or disorder.

52. The use of claim 48 or 49, 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, 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.