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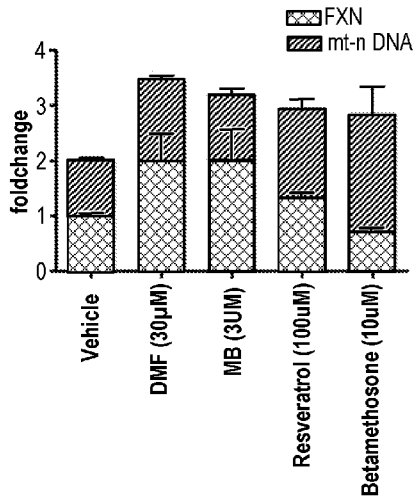
(57) Abstract: The present invention provides methods for promoting or increasing mitochondrial biogenesis in a neural cell. In some embodiments, the method comprises contacting the neural cell or administering a compound of Formula (I) described herein, a compound of Formula (II) described herein, resveratrol, betamethasone, an analog thereof, and/or a pharmaceutically acceptable salt thereof. Methods for preventing or treating disorders associated with insufficient mitochondrial function in neural cells are also provided.

FIG. 9C



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METHODS FOR PROMOTING MITOCHONDRIAL BIOGENESIS IN NEURAL CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 62/617,968, filed January 16, 2018, and U.S. Provisional Application No. 62/627,127, filed February 6, 2018, the disclosures of which are herein incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] The mitochondrion is an intracellular organelle that is the major contributor of cellular energy production by way of the mitochondrial electron transport chain (ETC) and ATP synthase activity. The ETC is comprised of four mitochondrial complexes: NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), coenzyme Q-cytochrome c reductase (complex III), and cytochrome c oxidase (complex IV). Together, these complexes produce a proton gradient across the mitochondrial inner membrane that drives ATP synthase, thereby converting ADP to ATP.

[0003] Many neurological disorders, including several degenerative diseases, result from insufficient mitochondrial mass or function in neural cells. One therapeutic strategy for the prevention and treatment of these disorders is to increase mitochondrial biogenesis in neural cells, the idea being that a small defect in function might be ameliorated by increased mitochondrial mass or function overall. Currently, there is no FDA-approved drug for the prevention or treatment of mitochondrial disease. As such, there is a need for new therapies that can promote mitochondrial biogenesis in neural cells. The present invention satisfies this need, and provides related advantages as well.

BRIEF SUMMARY OF THE INVENTION

[0004] In a first aspect, the present invention provides a method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, wherein the method comprises contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I), a compound of

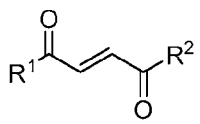
Formula (II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof.

[0005] In a second aspect, the present invention provides a method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, wherein the method comprises administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof.

[0006] In a third aspect, the present invention provides a method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, wherein the method comprises contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof.

[0007] In a fourth aspect, the present invention provides a method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, wherein the method comprises administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof.

[0008] In some embodiments, Formula (I) has the following structure:



(I).

[0009] In some embodiments, R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy. In some embodiments, E is an electron-withdrawing group. In some embodiments, n is 0-3.

[0010] In some embodiments, E is selected from the group consisting of $–NO_2$, $-N(R^7{}_2)$, $-N(R^7{}_3)^+$, $-NH_3^+$, $-SO_3H$, $-SO_3R^8$, $-S(O_2)R^8$ (sulfone), $-S(O)R^8$ (sulfoxide), $-S(O_2)NH_2$ (sulfonamide), $-SO_2NHR^8$, $-SO_2NR^8{}_2$, $-PO(OR^8)_2$, $-PO_3H_2$, $-PO(NR^8{}_2)_2$, 2-pyridinyl, 3-

pyridinyl, 4-pyridinyl, pyrazolyl, indazolyl, imidazolyl, thiazolyl, benzothiazolyl, oxazolyl, benzimidazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, triazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinazolinyl, pyrimidinyl, a 5- or 6-membered heteroaryl with a C-N double bond optionally fused to a 5- or 6-membered heteroaryl, pyridinyl N-oxide , C≡N, -CX_3, -C(O)X, -COOH, -$COOR^8$, -$C(O)R^8$, -$C(O)NH_2$, -$C(O)NHR^8$, -$C(O)NR^8{}_2$, -C(O)H, -$P(O)(OR^8)OR^9$, and X.

[0011] In some embodiments, X is a halogen. In some embodiments, R^7, R^8, and R^9 are each independently selected from the group consisting of hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

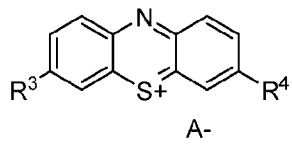
[0012] In some embodiments, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a fumarate ester. In particular embodiments, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of monomethyl fumarate (MMF), monomethyl maleate, monoethyl fumarate, monoethyl maleate, monobutyl fumarate, monobutyl maleate, monooctyl fumarate, monooctyl maleate, mono (phenylmethyl) fumarate, mono (phenylmethyl) maleate, mono (2-hydroxypropyl) fumarate, mono (2-hydroxypropyl) maleate, mono (2-ethylhexyl) fumarate, mono (2-ethylhexyl) maleate, dimethyl fumarate (DMF), dimethyl maleate, diethyl fumarate, diethyl maleate, dipropyl fumarate, dipropyl maleate, diisopropyl fumarate, diisopropyl maleate, dibutyl fumarate, dibutyl maleate, diisobutyl fumarate, diisobutyl maleate, diheptyl fumarate, diheptyl maleate, bis(2-ethylhexyl) fumarate, bis(2-ethylhexyl) maleate, (-)-dimethyl fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) maleate, bis(2-trifluoroethyl) fumarate, bis(2-trifluoroethyl) maleate, and a combination thereof. In some instances, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises dimethyl fumarate (DMF).

[0013] In some embodiments, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is supplied as a prodrug. In some instances, the prodrug is selected from the group consisting of O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, O,O'-(3-

(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, and a combination thereof.

[0014] In some embodiments, the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 2,000 mg per day. In particular embodiments, the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of about 120 mg, about 240 mg, about 360 mg, about 480 mg, about 600 mg, or about 720 mg per day. In some embodiments, the compound of Formula (I) is DMF, and the therapeutically effective amount comprises a dose of between about 10 and about 160 mg/kg of body weight per day. In some instances, the therapeutically effective amount comprises a dose of between about 40 and about 160 mg/kg of body weight per day.

[0015] In some embodiments, Formula (II) has the following structure:



(II).

[0016] In some embodiments, R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  . In some embodiments, R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$. In some embodiments, n' is 1-6. In some embodiments, Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

[0017] In some embodiments, the halogen is independently selected from the group consisting of F, Cl, Br, and I.

[0018] In some embodiments, A- is a counterion. In some embodiments, A- is an anion, a dianion, or a trianion. In particular embodiments, A- is selected from the group consisting of Cl^-, Br^-, I^-, F^-, NO_3^-, $CH_3SO_3^-$, HSO_4^-, $CHCO_2^-$ SO_4^{2-}, HPO_4^{2-}, and PO_4^{3-}.

[0019] In some embodiments, the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene

blue, leuco-methylene blue, acetyl-methylene blue, leuco-methylonium bis(hydromethanesulfonate) (LMTM), diaminophenothiazine, 2-chlorophenothiazine, phenothiazine, toluidine blue, tolonium chloride, toluidine blue O, seleno toluidine blue, methylene green, chlorpromazine, sulphoxide chlorpromazine, sulphone chlorpromazine, chlordiethazine promethazine, thioproperazine, prochlorperazine, pipotiazine, dimetotiazine, propericiazine, metazionic acid, oxomemazine neutral red, iminostilbene, imipramine, and a combination thereof. In particular embodiments, the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, tolonium chloride, leuco-methylonium bis(hydromethanesulfonate) (LMTM), and a combination thereof.

[0020] In some embodiments, the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 300 mg per day. In particular embodiments, the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of about 75 mg or about 125 mg, contacted with the cell or administered twice per day.

[0021] In some embodiments, the therapeutically effective amount of resveratrol comprises a dose of between about 0.5 grams and about 10 grams per day. In particular embodiments, the therapeutically effective amount of resveratrol comprises a dose of between about 1 gram and about 5 grams per day. In some embodiments, the therapeutically effective amount of betamethasone comprises a dose of between about 1 mg and about 30 mg per day.

[0022] In some embodiments, contacting the neural cell with the active agent or administering the active agent promotes and/or increases mitochondrial biogenesis in the neural cell. In some embodiments, promoting and/or increasing mitochondrial biogenesis in the neural cell comprises increasing mitochondrial mass and/or copy number in the neural cell.

[0023] In some embodiments, contacting the neural cell with two or more different active agents or administering two or more different active agents produces a synergistic promotion and/or increase in mitochondrial biogenesis in the neural cell compared to when the active agents are contacted or administered alone. In some embodiments, promoting and/or increasing mitochondrial biogenesis in the neural cell increases mitochondrial gene expression in the neural cell. In particular embodiments, promoting and/or increasing

mitochondrial biogenesis in the neural cell increases frataxin expression and/or activity in the neural cell.

[0024] In some embodiments, the insufficient mitochondrial function in the neural cell is associated with insufficient mitochondrial mass and/or mitochondrial copy number in the neural cell.

[0025] In some embodiments, the disorder is a neurodegenerative disease. In some embodiments, the disorder is associated with decreased frataxin expression and/or activity in the neural cell. In particular embodiments, the disorder is selected from the group consisting of Friedreich's ataxia; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic epilepsy with ragged red fibers (MERRF); Leber's hereditary optic neuropathy (LHON); neuropathy, ataxia, and retinitis pigmentosa (NARP); maternally inherited Leigh syndrome (MILS); multiple sclerosis (MS); and a combination thereof.

[0026] In some embodiments, the subject is not exhibiting any signs or symptoms of the disorder. In other embodiments, the subject is exhibiting one or more signs or symptoms of the disorder. In particular embodiments, promoting and/or increasing mitochondrial biogenesis in the neural cell prevents, delays, reduces, mitigates, ameliorates, and/or inhibits one or more signs or symptoms associated with the disorder.

[0027] In some embodiments, the active agent comprises a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, the active agent comprises a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, the active agent comprises resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, the active agent comprises betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0028] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof.

[0029] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0030] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0031] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0032] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0033] In some embodiments, the active agent comprises a combination of resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0034] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0035] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0036] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0037] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0038] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of

Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

[0039] In some embodiments, two or more different active agents are contacted with the neural cell or administered concomitantly. In other embodiments, two or more different active agents are contacted with the neural cell or administered sequentially.

[0040] In some embodiments, the method further comprises contacting the neural cell with a delivery-enhancing agent or administering to the subject a delivery-enhancing agent. In some embodiments, the delivery-enhancing agent is selected from the group consisting of a cyclodextrin, a hepatitis E virus-like particle, an inactivated yeast, an inactivated bacterium, polyvinyl acetate (PVA), an inulin or an ester thereof, and a combination thereof.

[0041] In some embodiments, the cyclodextrin is selected from the group consisting of an α-cyclodextrin, a β-cyclodextrin, a γ-cyclodextrin, a salt thereof, a derivative thereof, and a combination thereof. In particular embodiments, the β-cyclodextrin is selected from the group consisting of a hydroxypropyl-β-cyclodextrin, an endotoxin controlled β-cyclodextrin sulfobutyl ether, a β-cyclodextrin sulfobutyl ether, a sodium salt thereof, an anionic derivative thereof, and a combination thereof. In some instances, the cyclodextrin is selected from the group consisting of a hepta-substituted sulfobutyl-ether-β-cyclodextrin mixture, betadex-sulfobutyl-ether-β-cyclodextrin sodium salt, and a combination thereof. In some embodiments, the cyclodextrin is contacted with the neural cell or administered at a concentration of between about 1 mg/mL and about 300 mg/mL.

[0042] In some embodiments, the method further comprises contacting the neural cell with a pharmaceutically acceptable carrier or administering to the subject a pharmaceutically acceptable carrier.

[0043] In some embodiments, a sample is obtained from the subject before and/or after the active agent is contacted with the neural cell or administered to the subject. In particular embodiments, the sample comprises blood, tissue, or a combination thereof. In some embodiments, the tissue sample comprises neural tissue. In some embodiments, the tissue sample comprises normal or abnormal tissue. In particular embodiments, the sample is an abnormal blood and/or tissue sample that comprises a lower mitochondrial mass and/or number compared to a normal blood and/or tissue sample.

[0044] In some embodiments, the presence or level of a biomarker is determined in the sample. In particular embodiments, the biomarker comprises frataxin, COX4, or a combination thereof. In some embodiments, the presence or level of the biomarker in the sample is compared to a reference value. In particular embodiments, the reference value is determined from a sample obtained from the subject, a different subject, or a population of subjects.

[0045] Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] FIG. 1 shows that dimethyl fumarate (DMF) increases the expression of mitochondrial subunits.

[0047] FIGS. 2A and 2B show that DMF increases mitochondrial gene expression in retinal cells in a mouse model of Parkinson's disease and optic neuropathy (Ndufs4 KO). FIG. 2A shows the expression of mitochondrial gene *ND2*. FIG. 2B shows the expression of mitochondrial gene *ND6*. * denotes $p < 0.05$ as determined using Student's T-test.

[0048] FIG. 3 shows the effects of DMF on mitochondrial respiration.

[0049] FIG. 4 shows the results of visual cliff tests in wild-type and *Ndufs4* KO mice that were treated with vehicle control or DMF.

[0050] FIG. 5 shows tyrosine hydroxylase (TH) levels in wild-type and *Ndufs4* KO mice that were treated with vehicle control or DMF (10 mg/kg) by i.p. injection for 14 days.

[0051] FIG. 6 shows that methylene blue and its analog tolonium chloride dose-dependently protected Friedreich's ataxia (FA) patient cells from death.

[0052] FIG. 7 shows Western blot results assaying frataxin expression in FA patient lymphoblasts that were treated with methylene blue (MB).

[0053] FIG. 8 shows that MB induced frataxin expression in the brains of a FA mouse model.

[0054] FIGS. 9A-9C show that DMF, MB, resveratrol, and betamethasone increased frataxin expression and mitochondrial DNA copy number in FA patient-derived fibroblasts. FIG. 9A shows frataxin expression. FIG. 9B shows mitochondrial DNA copy number. FIG.

9C combines the data shown in FIGS. 9A and 9B. Asterisks denote statistically significant differences compared to vehicle control.

[0055] FIGS. 10A-10C show the dose-dependent effects of DMF, MB, resveratrol, and betamethasone on frataxin expression and mitochondrial DNA copy number in FA patient-derived fibroblasts. FIG. 10A shows frataxin expression. FIG. 10B shows mitochondrial DNA copy number. FIG. 10C combines the data shown in FIGS. 10A and 10B.

[0056] FIGS. 11A-11C show the effects of DMF, resveratrol, and betamethasone, alone and in combination, on frataxin expression and mitochondrial DNA copy number in FA patient-derived fibroblasts. FIG. 11A shows frataxin expression. FIG. 11B shows mitochondrial DNA copy number. FIG. 11C combines the data shown in FIGS. 11A and 11B.

[0057] FIG. 12 shows the effects of monomethyl fumarate (MMF) on frataxin expression.

[0058] FIG. 13 shows the effects of MMF on heme oxygenase-1 (*HO-1*) expression.

[0059] FIGS. 14A and 14B show the structures of fumarate prodrugs. FIG. 14A shows O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate. FIG. 14B shows O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate.

[0060] FIGS. 15A and 15B show the structures of methylene blue analogs. FIG. 15A shows methylonium chloride (MTC). FIG. 15B shows leuco-methylthioninium bis(hydromethanesulfonate) (LMTM).

[0061] FIGS. 16A and 16B show changes in frataxin (Fxn) and COX4 expression in response to different doses of dimethyl fumarate (DMF). Western blot images are shown at the top of each panel and average fold-change in expression (normalized to vehicle-only control) at each dose is shown in the bar graphs at the bottom of each panel. FIG. 16A shows expression in whole brain. FIG. 16B shows expression in cerebellum.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0062] Dimethyl fumarate (DMF) is a methyl ester of fumaric acid with known anti-inflammatory properties. The inventors have previously discovered that DMF dose-dependently increases frataxin, a mitochondrial protein, the deficiency of which ultimately

causes the disease Friedreich's ataxia (FA). Furthermore, the inventors have previously discovered that methylene blue and its analogs promote Nrf2 and mitochondrial protein and frataxin induction, dose-dependently, and that there is additivity between DMF and MB in the context of frataxin induction. However, frataxin induction can occur in the absence of an increase in mitochondrial biogenesis.

[0063] The present invention is based, in part, on the discovery that DMF, MB (and its analogs), resveratrol, and betamethasone can promote mitochondrial biogenesis and/or increase frataxin expression. The invention is also based, in part, on the discovery that these effects can be potentiated by a combination of drugs.

II. Definitions

[0064] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0065] An "effective amount" or "therapeutically effective amount" includes an amount or quantity effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0066] The term "subject" typically includes humans, but can also include other animals such as, *e.g.*, other primates, rodents, canines, felines, equines, ovines, porcines, and the like.

[0067] The terms "administering" and "administration" include oral administration, topical contact, administration as a suppository, intravenous, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal (*e.g.*, inhalation, nasal mist or drops), or subcutaneous administration, or the implantation of a slow-release device, *e.g.*, a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.*, buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* One skilled in the art will know of additional methods for administering a therapeutically effective amount of an active agent described herein for preventing or relieving one or more symptoms associated with the presence or activity of a disease associated with insufficient mitochondrial function in a neural cell. By "co-administer" it is meant that an active agent is

administered at the same time, just prior to, or just after the administration of a second active agent.

[0068] The terms "treating" and "treated" refer to any indications of success in the treatment or amelioration of a pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the pathology or condition more tolerable to the subject, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, or improving a subject's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters, including the results of a physical examination, histopathological examination (*e.g.*, analysis of biopsied tissue), laboratory analysis of urine, saliva, tissue sample (*e.g.*, obtained from a biopsy), serum, plasma, or blood, or imaging.

[0069] The term "alkyl," by itself or as part of another substituent, refers to a straight or branched, saturated, aliphatic radical having the number of carbon atoms indicated. Alkyl can include any number of carbons, such as C_{1-2}, C_{1-3}, C_{1-4}, C_{1-5}, C_{1-6}, C_{1-7}, C_{1-8}, C_{1-9}, C_{1-10}, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{3-4}, C_{3-5}, C_{3-6}, C_{4-5}, C_{4-6} and C_{5-6}. For example, C_{1-6} alkyl includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, hexyl, etc. Alkyl can also refer to alkyl groups having up to 20 carbons atoms, such as, but not limited to heptyl, octyl, nonyl, decyl, etc. Alkyls can be substituted or unsubstituted. "Substituted alkyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy. Alkyl groups can also be cyclized to form a ring structure ("cycloalkyl").

[0070] The term "alkenyl" refers to a straight chain or branched hydrocarbon having at least 2 carbon atoms and at least one double bond. Alkenyl can include any number of carbons, such as C_2, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{2-7}, C_{2-8}, C_{2-9}, C_{2-10}, C_3, C_{3-4}, C_{3-5}, C_{3-6}, C_4, C_{4-5}, C_{4-6}, C_5, C_{5-6}, and C_6. Alkenyl groups can have any suitable number of double bonds, including, but not limited to, 1, 2, 3, 4, 5 or more. Examples of alkenyl groups include, but are not limited to, vinyl (ethenyl), propenyl, isopropenyl, 1-butenyl, 2-butenyl, isobutenyl, butadienyl, 1-pentenyl, 2-pentenyl, isopentenyl, 1,3-pentadienyl, 1,4-pentadienyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,5-hexadienyl, 2,4-hexadienyl, or 1,3,5-hexatrienyl. Alkenyl groups can be substituted or unsubstituted. "Substituted alkenyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy.

[0071] The term "alkynyl" refers to either a straight chain or branched hydrocarbon having at least 2 carbon atoms and at least one triple bond. Alkynyl can include any number of carbons, such as C_2, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{2-7}, C_{2-8}, C_{2-9}, C_{2-10}, C_3, C_{3-4}, C_{3-5}, C_{3-6}, C_4, C_{4-5}, C_{4-6}, C_5, C_{5-6}, and C_6. Examples of alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butynyl, 2-butynyl, isobutynyl, sec-butynyl, butadiynyl, 1-pentynyl, 2-pentynyl, isopentynyl, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatriynyl. Alkynyl groups can be substituted or unsubstituted. "Substituted alkynyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy.

[0072] The term "heteroalkyl," by itself or as part of another substituent, refers to an alkyl group of any suitable length and having from 1 to 3 heteroatoms such as N, O and S. For example, heteroalkyl can include ethers, thioethers and alkyl-amines. Additional heteroatoms including, but not limited to, B, Al, Si and P, can also be useful. The heteroatoms can be oxidized to form moieties such as -S(O)- and $-S(O)_2-$. The heteroatom portion of the heteroalkyl can replace a hydrogen of the alkyl group to form a hydroxy, thio, or amino group. Alternatively, the heteroatom portion can be the connecting atom, or be inserted between two carbon atoms. Heteroalkyls can be substituted or unsubstituted. Heteroalkyls can also be cyclized to form a ring structure ("heterocycloalkyl").

[0073] The term "aryl," by itself or as part of another substituent, refers to an aromatic ring system having any suitable number of ring atoms and any suitable number of rings. Aryl groups can include any suitable number of ring atoms, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 ring atoms, as well as from 6 to 10, 6 to 12, or 6 to 14 ring members. Aryl groups can be monocyclic, fused to form bicyclic (*e.g.*, benzocyclohexyl) or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. Other aryl groups include benzyl, having a methylene linking group. Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl. Some other aryl groups have 6 ring members, such as phenyl. Aryl groups can be substituted or unsubstituted. "Substituted aryl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy.

[0074] The term "alkoxy," by itself or as part of another substituent, refers to a group having the formula -OR, wherein R is alkyl.

[0075] The terms "halo" and "halogen," by themselves or as part of another substituent, refer to a fluorine, chlorine, bromine, or iodine atom.

[0076] The term "amino" refers to a moiety $-NR_3$, wherein each R group is H or alkyl. An amino moiety can be ionized to form the corresponding ammonium cation.

[0077] The term "hydroxy" refers to the moiety –OH.

[0078] The term "cyano" refers to a carbon atom triple-bonded to a nitrogen atom (*i.e.*, the moiety –C≡N).

[0079] The term "carboxy" refers to the moiety –C(O)OH. A carboxy moiety can be ionized to form the corresponding carboxylate anion.

[0080] The term "amido" refers to a moiety –NRC(O)R or $-C(O)NR_2$, wherein each R group is H or alkyl.

[0081] The terms "stereoisomer" and "stereoisomers" refer to compounds that have the same atomic connectivity but different atomic arrangement in space. Stereoisomers include *cis*- and *trans*- isomers, *E* and *Z* isomers, enantiomers, and diastereomers. Compounds described herein, or their pharmaceutically acceptable salts, can contain one or more asymmetric centers and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that can be defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or, as (D)- or (L)- for amino acids. The present invention is meant to include all such possible isomers, as well as their racemic and optically pure forms. Optically active (+) and (-), (*R*)- and (*S*)-, or (D)- and (L)- isomers can be prepared using chiral synthons, chiral reagents, or resolved using conventional techniques, such as by: formation of diastereoisomeric salts or complexes which can be separated, for example, by crystallization; via formation of diastereoisomeric derivatives which can be separated, for example, by crystallization, selective reaction of one enantiomer with an enantiomer-specific reagent, for example, enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where a desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step

may be required to liberate the desired enantiomeric form. Alternatively, a specific enantiomer can be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts, or solvents, or by converting one enantiomer to the other by asymmetric transformation. For a mixture of enantiomers, enriched in a particular enantiomer, the major component enantiomer can be further enriched (with concomitant loss in yield) by recrystallization.

[0082] When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers

[0083] The term "tautomer" refers to alternate forms of a molecule that differ only in electronic bonding of atoms and/or in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a -N=C(H)-H- ring atom arrangement, such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. A person of ordinary skill in the art would recognize that other tautomeric ring atom arrangements are possible and contemplated herein.

[0084] The term "pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate, and the like. Pharmaceutically acceptable acid addition salts are those salts that retain the biological effectiveness of the free bases while formed by acid partners that are not biologically or otherwise undesirable, *e.g.*, inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, as well as organic acids such as acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic 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. Pharmaceutically acceptable base addition salts include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts, and the like.

[0085] Exemplary salts are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins, and the like. Exemplary organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline, and caffeine. (*see, e.g.*, S. M. Berge, *et al.*, "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977; 66:1-19 which is incorporated herein by reference for all purposes).

[0086] The term "prodrug" refers to compounds that are transformed *in vivo* to yield the parent or active compound, for example, by hydrolysis in the gut or enzymatic conversion in blood. Common examples include, but are not limited to, ester and amide forms of a compound having an active form bearing a carboxylic acid moiety. Examples of pharmaceutically acceptable esters of the compounds of this invention include, but are not limited to, alkyl esters (for example, with between about one and about six carbons) where the alkyl group is a straight or branched chain. Acceptable esters also include cycloalkyl esters and arylalkyl esters such as, but not limited to, benzyl. Examples of pharmaceutically acceptable amides of the compounds of this invention include, but are not limited to, primary amides, and secondary and tertiary alkyl amides (for example, with between about one and about six carbons). Amides and esters of the compounds of the present invention can be prepared according to conventional methods. A thorough discussion of prodrugs is provided in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference for all purposes.

[0087] The terms "frataxin," "FA," "X25," "CyaY" "FARR," "MGC57199," and "FXN" interchangeably refer to nucleic acids and polypeptide polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 90% amino acid sequence identity, for example, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or greater amino acid sequence identity, preferably over a region of at least

about 25, 50, 100, 200, 300, 400, or more amino acids, or over the full-length, to an amino acid sequence encoded by a frataxin gene or a frataxin nucleic acid (*see, e.g.*, GenBank Accession Nos. NM_000144.4 (isoform 1); NM_181425.2 (isoform 2); NM_001 161706.1 (isoform 3)) or to an amino acid sequence of a frataxin polypeptide (*see, e.g.* GenBank Accession Nos. NP_000135.2 (isoform 1); NP_852090.1 (isoform 2); NP_001155178.1 (isoform 3)); (2) bind to antibodies, *e.g.*, polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of a frataxin polypeptide (*e.g.*, frataxin polypeptides described herein); or an amino acid sequence encoded by a frataxin gene or a frataxin nucleic acid (e.g., a frataxin gene or a frataxin polynucleotides described herein), and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to an anti-sense strand corresponding to a nucleic acid sequence encoding a frataxin protein, and conservatively modified variants thereof; or (4) have a nucleic acid sequence that has greater than about 90%, preferably greater than about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, 2000 or more nucleotides, or over the full-length, to a frataxin nucleic acid (e.g., frataxin polynucleotides, as described herein, and frataxin polynucleotides that encode frataxin polypeptides, as described herein).

[0088] The terms "Friedreich's ataxia," "FA," and "FRDA" interchangeably refer to an autosomal recessive congenital ataxia caused by a mutation in gene *FXN* (formerly known as *X25*) that codes for frataxin, located on chromosome 9. The genetic basis for FRDA involves GAA trinucleotide repeats in an intron region of the gene encoding frataxin. This segment is normally repeated from about 5 to about 33 times within the FXN gene. In people with Friedreich ataxia, the GAA segment is typically repeated from about 66 to more than about 1,000 times. People with GAA segments repeated fewer than about 300 times tend to have a later appearance of symptoms (commonly, after age 25) than those with larger GAA trinucleotide repeats. The presence of these repeats results in reduced transcription and expression of the gene. Frataxin is involved in regulation of mitochondrial iron content. The mutation in the FXN gene causes progressive damage to the nervous system, resulting in symptoms ranging from gait disturbance to speech problems; it can also lead to heart disease and diabetes. The ataxia of Friedreich's ataxia results from the degeneration of nerve tissue in the spinal cord, in particular sensory neurons essential (through connections with the cerebellum) for directing muscle movement of the arms and legs. The spinal cord becomes thinner and nerve cells lose some of their myelin sheath (the insulating covering on some

nerve cells that helps conduct nerve impulses). A subject with FRDA may exhibit one or more of the following symptoms: muscle weakness in the arms and legs, loss of coordination, vision impairment, hearing impairment, slurred speech, curvature of the spine (scoliosis), high plantar arches (pes cavus deformity of the foot), carbohydrate intolerance, diabetes mellitus, heart disorders (*e.g.*, atrial fibrillation, tachycardia, and hypertrophic cardiomyopathy). A subject with FRDA may further exhibit involuntary and/or rapid eye movements, loss of deep tendon reflexes, loss of extensor plantar responses, loss of vibratory and proprioceptive sensation, cardiomegaly, symmetrical hypertrophy, heart murmurs, and heart conduction defects. Pathological analysis may reveal sclerosis and degeneration of dorsal root ganglia, spinocerebellar tracts, lateral corticospinal tracts, and posterior columns.

[0089] The term "synergy" or "synergistic effect" refers to an effect produced by two or more compounds (*e.g.*, a compound of Formula (I), a compound of Formula (II), resveratrol, and/or betamethasone) that is greater than the effect produced by a sum of the effects of the individual compounds (*i.e.*, an effect that is greater than an additive effect). Several methods are available for determining whether a combination of drugs produces a synergistic effect. As a non-limiting example, the Highest Single Agent approach simply reflects that the fact that the resulting effect of a combination of drugs (E_{AB}) is greater than the effects of the individual drugs (E_A and E_B). A combination index (CI) can be calculated according to the formula:

$$CI = \frac{\max(E_A, E_B)}{E_{AB}}.$$

[0090] As another non-limiting example, according to the Response Additivity Approach, a synergistic drug combination effect occurs when the E_{AB} is greater than the expected additive effects of the individual drugs (E_A and E_B). Here, the CI is calculated using the formula:

$$CI = \frac{E_A + E_B}{E_{AB}}.$$

[0091] As yet another non-limiting example, the Bliss Independence model is based on the principle that drug effects are the outcomes of probabilistic processes, and makes the assumption that drugs act independently such that they do not interfere with each other (*i.e.*, different sites of action). However, the model also assumes that each drug contributes to the production of a common result. According to this method, the observed combination effect is expressed as a probability ($0 \leq E_{AB} \leq 1$) and is compared to the expected additive effect expressed as

$$E_A + E_B (1-E_A) = E_A + E_B - E_AE_B,$$

where $0 \leq E_A \leq 1$ and $0 \leq E_B \leq 1$. The CI for this method is calculated using the formula:

$$CI = \frac{E_A+E_B-E_AE_B}{E_{AB}}.$$

[0092] Methods of identifying synergistic effects are further discussed in Foucquier J. and Guedj M. *Pharmacology Research & Perspectives* (2015) (3)3:e00149, incorporated herein by reference in its entirety for all purposes.

III. Detailed Description of the Embodiments

A. Methods of Use

[0093] In one aspect, the present invention provides a method for promoting and/or increasing mitochondrial biogenesis in a neural cell (*e.g.*, in a subject). In some embodiments, the method comprises contacting the neural cell with a therapeutically effective amount of an active agent described herein (*e.g.*, a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, an analog thereof, a therapeutically acceptable salt thereof, a prodrug thereof, or a combination thereof). In other embodiments, the method comprises administering to a subject a therapeutically effective amount of an active agent described herein. In some embodiments, contacting the neural cell is via an *in vivo* method. In other embodiments, contacting the neural cell is via an *in vitro* method.

[0094] In another aspect, the present invention provides a method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell (*e.g.* in a subject). In some embodiments, the method comprises contacting the neural cell with a therapeutically effective amount of an active agent described herein (*e.g.*, a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, an analog thereof, a therapeutically acceptable salt thereof, a prodrug thereof, or a combination thereof). In other embodiments, the method comprises administering to a subject a therapeutically effective amount of an active agent described herein. In some embodiments, contacting the neural cell is via an *in vivo* method. In other embodiments, contacting the neural cell is via an *in vitro* method.

[0095] In some embodiments, the active agent comprises a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof. In some embodiments, the active agent comprises a compound of Formula (II), an analog thereof, a

pharmaceutically acceptable salt thereof, or a prodrug thereof. In some embodiments, the active agent comprises resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof. In some embodiments, the active agent comprises betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0096] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0097] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0098] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0099] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0100] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0101] In some embodiments, the active agent comprises a combination of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0102] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug

thereof; a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; and resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0103] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0104] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0105] In some embodiments, the active agent comprises a combination of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0106] In some embodiments, the active agent comprises a combination of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof; and betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[0107] The neural cell can be any suitable neural cell. Non-limiting examples of neural cells include neurons and glial cells (*e.g.*, oligodendrocytes, astrocytes, ependymal cells, Schwann cells, microglia, and satellite cells). The neural cell can be located, for example, in any region or structure within the brain, within the spinal cord or another structure associated with the central nervous system, or within structures associated with the peripheral nervous system (*e.g.*, motor or sensory nerves).

[0108] In some embodiments, contacting a neural cell with an active agent described herein or administering an active agent described herein (*e.g.*, to a subject in need thereof) promotes and/or increases mitochondrial biogenesis (*e.g.*, in a neural cell). In some embodiments, promoting and/or increasing mitochondrial biogenesis (*e.g.*, in a neural cell) comprises increasing mitochondrial mass (*e.g.*, in the neural cell). In other embodiments, promoting and/or increasing mitochondrial biogenesis comprises increasing mitochondrial copy number (*e.g.*, in the neural cell). In particular embodiments, promoting and/or increasing mitochondrial biogenesis comprises increasing mitochondrial mass and/or copy number (*e.g.*, in the neural cell). In some embodiments, promoting and/or increasing mitochondrial biogenesis results in increased mitochondrial function, *e.g.*, in the neural cell (*e.g.*, increased ATP production, increased activity of a mitochondrial complex, and/or increased oxygen consumption rate), increased neural cell survival, and/or decreased neural cell death. In some embodiments, promoting and/or increasing mitochondrial biogenesis (*e.g.*, in a neural cell) prevents, delays, reduces, mitigates, ameliorates, and/or inhibits one or more signs or symptoms associated with a disorder (*e.g.*, a disorder associated with or caused by insufficient function (*e.g.*, in a neural cell)).

[0109] In some embodiments, promoting and/or increasing mitochondrial biogenesis (*e.g.*, in a neural cell) is associated with an increase in mitochondrial gene expression and/or activity (*e.g.*, in the neural cell). For example, mitochondrial gene transcription and/or translation can be increased. In particular embodiments, the mitochondrial gene is selected from the group consisting of frataxin, ND2, ND6, HO-1, and a combination thereof. In particular embodiments, frataxin expression and/or activity (*e.g.*, in the neural cell) is increased.

[0110] In some embodiments, mitochondrial gene expression and/or activity is increased by at least about 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, 4-fold, 4.1-fold, 4.2-fold, 4.3-fold, 4.4-fold, 4.5-fold, 4.6-fold, 4.7-fold, 4.8-fold, 4.9-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, or 50-fold, or more.

[0111] In some embodiments, promoting and/or increasing mitochondrial biogenesis increases the expression and/or activity of a gene and/or protein in the Nrf2 pathway. Nuclear factor (erythroid-derived 2)-like 2, also known as Nrf2, is a transcription factor that in humans is encoded by the *NFE2L2* gene. Under normal conditions, Nrf2 is tethered in the cytoplasm by another protein called Kelch like-ECH- associated protein 1 (Keapl). Keapl acts as a substrate adaptor protein for Cullin 3-based ubiquitination, which results in the proteasomal degradation of Nrf2. Oxidative stress or electrophilic stress disrupts critical cysteine residues in Keapl, resulting in a disruption of the Keapl-Cul3 ubiquitination system and a build-up of Nrf2 in the cytoplasm. Unbound Nrf2 is then able to translocate into the nucleus, where it heterodimerizes with a small Maf protein and binds to an Antioxidant Response Element (ARE) in the upstream promoter region of many anti-oxidative genes to initiate transcription of many cytoprotective proteins. These include NAD(P)H quinone oxidoreductase, glutamate-cysteine ligase, heme oxygenase-1 (HMOXl, H0-1), the glutathione S-transferase (GST) family, the UDP- glucuronosyltransferase (UGT) family, thioredoxin reductase and multidrug resistance- associated proteins.

[0112] In some embodiments, use of two or more different active agents produces a synergistic effect. For example, contacting a neural cell with or administering 2, 3, 4, or more different active agents can produce, in some embodiments, a synergistic effect (*e.g.,* a promotion and/or increase in mitochondrial biogenesis, or another therapeutic effect), *e.g.,* in a neural cell, compared to the effect that is produced when the active agents are contacted or administered alone.

[0113] The term "disorder associated with insufficient mitochondrial function" refers to any disorder, disease, or condition (*e.g.,* in a neural cell) that is caused by, either directly or indirectly, a particular cell, tissue, or organ having inadequate mitochondrial function to maintain normal physiology, function, and/or structure. In some embodiments, the disorder is associated with or caused by insufficient mitochondrial mass and/or copy number (*e.g.,* in a neural cell), *i.e.,* there is insufficient mitochondrial mass or copy number to allow a cell, tissue or organ to maintain normal physiology, function, and/or structure. In some embodiments, the disorder is associated with or caused by insufficient mitochondrial biogenesis (*e.g.,* in a neural cell). In particular embodiments, the disorder is associated with or caused by decreased expression and/or activity of a mitochondrial gene (*e.g.,* in a neural cell). In some embodiments, the disorder is associated with or caused by decreased frataxin expression and/or activity (*e.g.,* in a neural cell), *e.g.,* Freidreich's ataxia.

[0114] In some embodiments, the disorder is a neurodegenerative disease. In other embodiments, the disorder is associated with or caused by suboptimal expression or activity of one or more genes and/or proteins within the Nrf2 pathway.

[0115] Non-limiting examples of disorders, diseases, or conditions suitable for methods of the present invention include neurodegenerative diseases (*e.g.*, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS)), stroke, and conditions characterized by neurodegeneration and/or neuroinflammation, *i.e.*, conditions in which either or both of those processes leads to a failure of a subject's nervous system to function normally. The loss of normal function may be located in either or both of the central nervous system (*e.g.*, the brain, spinal cord) and the peripheral nervous system. Examples of such conditions include adrenal leukodystrophy (ALD), alcoholism, Alexander's disease, Alper's disease, ataxia telangiectasia, Batten disease (also known as Spielmeyer-Vogt-SjcSgren-Batten disease), bovine spongiform encephalopathy (BSE), Canavan disease, cerebral palsy, Cockayne syndrome, corticobasal degeneration, Creutzfeldt- Jakob disease, familial fatal insomnia, frontotemporal lobar degeneration, HIV-associated dementia, Kennedy's disease, Krabbe's disease, Lewy body dementia, neuroborreliosis, Machado-Joseph disease (Spinocerebellar ataxia type 3), multiple system atrophy, narcolepsy, Niemann Pick disease, Pelizaeus- Merzbacher Disease, Pick's disease, primary lateral sclerosis, prion diseases, progressive supranuclear palsy, Refsum's disease, Sandhoff disease, Schilder's disease, subacute combined degeneration of spinal cord secondary to pernicious anemia, spinocerebellar ataxia, spinal muscular atrophy, Steele-Richardson-Olszewski disease, Tabes dorsalis, toxic encephalopathy, progressive external ophthalmoplegia (PEO), Leigh's Syndrome, MNGIE (Myopathy and external ophthalmoplegia; Neuropathy; Gastro-Intestinal; Encephalopathy), Kearns-Sayre Syndrome (KSS), hereditary spastic paraparesis, mitochondrial myopathy, Friedreich's ataxia; MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibers), LHON (Leber's hereditary optic neuropathy) NARP (neuropathy, ataxia, and retinitis pigmentosa), MILS (maternally inherited Leigh syndrome), and a combination thereof.

[0116] In particular embodiments, the disorder that is prevented or treated according to methods of the present invention is selected from the group consisting of Friedreich's ataxia; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic

epilepsy with ragged red fibers (MERRF); Leber's hereditary optic neuropathy (LHON); neuropathy, ataxia, and retinitis pigmentosa (NARP); maternally inherited Leigh syndrome (MILS); multiple sclerosis (MS); and a combination thereof.

[0117] In some embodiments, the subject is not exhibiting any signs or symptoms of the disorder. In other embodiments, the subject is exhibiting one or more signs or symptoms of the disorder.

[0118] When methods of the present invention are used to prevent or treat Friedreich's ataxia (FA), the subject may be homozygous for a mutation (*e.g.,* a GAA expansion or point mutation) that inhibits or reduces the expression levels of frataxin. For subjects homozygous for a mutation in the frataxin gene that results in insufficient expression levels of the frataxin polypeptide, the risk of developing symptoms of FA generally increases with age. Accordingly, in asymptomatic subjects homozygous for a mutation in the frataxin gene that results in insufficient expression levels of the frataxin polypeptide, in certain embodiments, prophylactic application is contemplated for subjects over 5 years of age, *e.g.*, in subjects over about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years of age. Subjects with late or very late onset of disease can also be treated.

[0119] The present methods are especially useful for individuals who do have a known genetic risk of Friedreich's ataxia, whether they are asymptomatic or showing symptoms of disease. Such individuals include those having relatives who have experienced FA (*e.g.,* a parent, a grandparent, or a sibling), and those whose risk is determined by analysis of genetic and/or biochemical markers. Genetic markers of risk toward Friedreich's ataxia include mutations in the frataxin gene, which in humans is located on chromosome 9, in various embodiments mapped to an intron at 9ql3-q21.

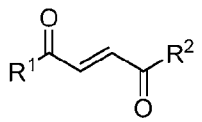
[0120] In some embodiments, the subject is exhibiting symptoms of FA, for example, muscle weakness in the arms and/or legs, loss of coordination, loss of deep tendon reflexes, loss of extensor plantar responses, loss of vibratory and proprioceptive sensation, vision impairment, involuntary and/or rapid eye movements, hearing impairment, slurred speech, curvature of the spine (scoliosis), high plantar arches (pes cavus deformity of the foot), carbohydrate intolerance, diabetes mellitus, and/or heart disorders (*e.g.*, atrial fibrillation, tachycardia, hypertrophic cardiomyopathy, cardiomegaly, symmetrical hypertrophy, heart murmurs, and/or heart conduction defects).

B. Active Agents

[0121] Active agents that find use in the present methods are effective in promoting and/or increasing mitochondrial biogenesis in a neural cell (*e.g.*, in a subject). The active agents are also effective for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell (*e.g.*, in a subject). In particular embodiments, the active agents are useful for preventing, reducing, delaying, or inhibiting one or more signs or symptoms of a disorder associated with insufficient mitochondrial function in a neural cell. In other embodiments, the disorder is Friedreich's ataxia. In various embodiments, agents that find use directly or indirectly (*e.g.*, via the Nrf2 pathway) induce or increase expression of frataxin polypeptide from the frataxin gene, increase mitochondrial function in the cells of a subject with a disorder associated with insufficient mitochondrial function (*e.g.*, Friedreich's ataxia), and/or increase cell viability and/or prevent cell death in a subject with a disorder associated with insufficient mitochondrial function (*e.g.*, Friedreich's ataxia).

[0122] In some embodiments, the active agents are selected from the group consisting of a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, prodrugs thereof, and combinations thereof. The terms "compound of Formula (I)," "Compound of Formula (II)," "resveratrol," and betamethasone also encompass all useful stereoisomers and tautomers. Particularly useful compounds of Formula (I) include, but at not limited to, dimethyl fumarate (DMF), monomethyl fumarate (MMF), and the fumarate prodrugs O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate and O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, as well as combinations thereof. Particularly useful compounds of Formula (II) include, but are not limited to, methylene blue, tolonium chloride, leuco-methylonium bis(hydromethanesulfonate) (LMTM), and combinations thereof.

[0123] In some embodiments, the active agent comprises a compound of Formula (I):



(I).

In particular embodiments, analogs of compounds of Formula (I) are used. In other embodiments, pharmaceutically acceptable salt of compounds of Formula (I) are used. In some embodiments, a prodrug of Formula (I) is used. In some embodiments, a compound of

Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof of, and/or a prodrug thereof is used.

[0124] In some embodiments, R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy. In some embodiments, E is an electron-withdrawing group. In some embodiments, n is 0-3.

[0125] In some embodiments, E is selected from the group consisting of $–NO_2$, $-N(R^7{}_2)$, $-N(R^7{}_3)^+$, $-NH_3^+$, $-SO_3H$, $-SO_3R^8$, $-S(O_2)R^8$ (sulfone), $-S(O)R^8$ (sulfoxide), $-S(O_2)NH_2$ (sulfonamide), $-SO_2NHR^8$, $-SO_2NR^8{}_2$, $-PO(OR^8)_2$, $-PO_3H_2$, $-PO(NR^8{}_2)_2$, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, pyrazolyl, indazolyl, imidazolyl, thiazolyl, benzothiazolyl, oxazolyl, benzimidazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, triazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinazolinyl, pyrimidinyl, a 5- or 6-membered heteroaryl with a C-N double bond optionally fused to a 5- or 6-membered heteroaryl, pyridinyl N-oxide , C≡N, $-CX_3$, -C(O)X, -COOH, $-COOR^8$, $-C(O)R^8$, $-C(O)NH_2$, $-C(O)NHR^8$, $-C(O)NR^8{}_2$, -C(O)H, $-P(O)(OR^8)OR^9$, and X.

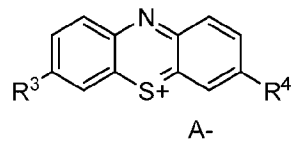
[0126] In some embodiments, X is a halogen. In some embodiments, R^7, R^8, and R^9 are each independently selected from the group consisting of hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

[0127] In some embodiments, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a fumarate ester. In particular embodiments, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of monomethyl fumarate (MMF), monomethyl maleate, monoethyl fumarate, monoethyl maleate, monobutyl fumarate, monobutyl maleate, monooctyl fumarate, monooctyl maleate, mono (phenylmethyl) fumarate, mono (phenylmethyl) maleate, mono (2-hydroxypropyl) fumarate, mono (2-hydroxypropyl) maleate, mono (2-ethylhexyl) fumarate, mono (2-ethylhexyl) maleate, dimethyl fumarate (DMF), dimethyl maleate, diethyl fumarate, diethyl maleate, dipropyl fumarate, dipropyl maleate, diisopropyl fumarate, diisopropyl maleate, dibutyl fumarate, dibutyl maleate, diisobutyl fumarate, diisobutyl maleate, diheptyl fumarate, diheptyl maleate, bis(2-ethylhexyl) fumarate, bis(2-ethylhexyl) maleate, (-)-dimethyl fumarate, (-)-bis((S)-l-

(ethoxycarbonyl)ethyl) fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) maleate, bis(2-trifluoroethyl) fumarate, bis(2-trifluoroethyl) maleate, and a combination thereof. In some instances, the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises dimethyl fumarate (DMF).

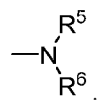
[0128] Compounds of Formula (I), analogs thereof, and/or pharmaceutically acceptable salts thereof can be supplied (*e.g.*, administered to a subject or a patient in need thereof) as a prodrug. Non-limiting examples of suitable prodrugs include O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, the structures of which are shown in FIGS. 14A and 14B, respectively, and a combination thereof.

[0129] In some embodiments, the active agent comprises a compound of Formula (II):



(II).

In particular embodiments, analogs of compounds of Formula (II) are used. In other embodiments, pharmaceutically acceptable salt of compounds of Formula (II) are used. In some embodiments, a prodrug of Formula (II) is used. In some embodiments, a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof of, and/or a prodrug thereof is used.

[0130] In some embodiments, R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  . In some embodiments, R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$. In some embodiments, n' is 1-6. In some embodiments, Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

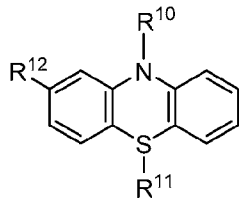
[0131] In some embodiments, the halogen is independently selected from the group consisting of F, Cl, Br, and I.

[0132] In some embodiments, A- is a counterion. In some embodiments, A- is an anion, a dianion, or a trianion. In particular embodiments, A- is selected from the group consisting of Cl^-, Br^-, I^-, F^-, NO_3^-, $CH_3SO_3^-$, HSO_4^-, $CHCO_2^-$ SO_4^{2-}, HPO_4^{2-}, and PO_4^{3-}.

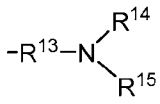
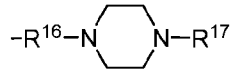
[0133] In some embodiments, the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, leuco-methylene blue, acetyl-methylene blue, leuco-methylonium bis(hydromethanesulfonate) (LMTM), diaminophenothiazine, 2-chlorophenothiazine, phenothiazine, toluidine blue, tolonium chloride, toluidine blue O, seleno toluidine blue, methylene green, chlorpromazine, sulphoxide chlorpromazine, sulphone chlorpromazine, chlordiethazine promethazine, thioproperazine, prochlorperazine, pipotiazine, dimetotiazine, propericiazine, metazionic acid, oxomemazine neutral red, iminostilbene, imipramine, and a combination thereof. In particular embodiments, the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, tolonium chloride, leuco-methylonium bis(hydromethanesulfonate) (LMTM), and a combination thereof.

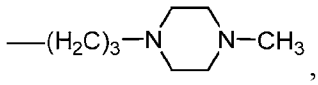
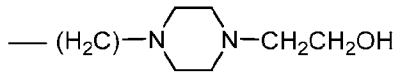
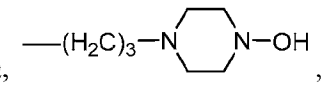
[0134] The methylene blue analog LMTM is also known as TRx0237, LMTX, and tau aggregation inhibitor (TAI) and is available, for example, from TauRx Therapeutics, Ltd. The structures of the methylene blue analogs methylonium chloride (MTC) and LMTM are shown in FIGS. 15A and 15B, respectively.

[0135] In some embodiments, an analog of methylene blue is a compound of Formula (IV):

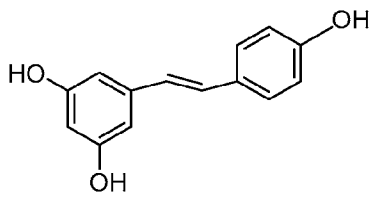


(IV).

In some embodiments, the S atom is neutral. In other embodiments, the S atom is positively charged. In some embodiments, R^{10} is  or  . In some embodiments, R^{14}, R^{15}, and R^{17} are each independently selected from hydrogen, substituted or unsubstituted alkyl, -OH, and R^{18}-OH. R^{13}, R^{16}, and R^{18} are each independently selected from substituted or unsubstituted alkylene.

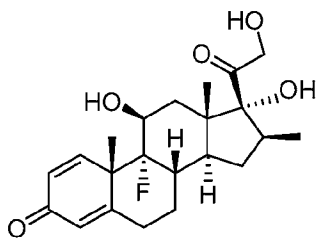
[0136] For example, R^{10} can be $-CH_2N(CH_3)_2$, $-CH_2CH(CH_3)CH_2N(CH_3)_2$, $-CH_2C(CH_3)_2CH_2N(CH_3)_2$, $-CH_2CH(CH_3)CH_2N(C_2H_5)_2$, $-CH_2CH(CH_3)N(C_2H_5)_2$, $-(CH_2)_2N(C_2H_5)_2$, $-(CH_2)_3N(CH_3)_2$, $-CH_2CH(CH_3)N(CH_3)_2$, $-CH_2CH(CH_3)CH_2N(CH_3)_2$, $-CH_2C(CH_3)_2CH_2N(CH_3)_2$, $-CH_2CH(CH_3)CH_2N(C_2H_5)_2$, $-CH_2CH(CH_3)N(C_2H_5)_2$, $-(CH_2)_2N(C_2H_5)_2$,  ,  , $-CH_2CH(CH_3)N(CH_3)_2$,  , $-CH_3$, $-(CH_2)_3N(CH_3)_2$, $(CH_2)_3N(CH_3)_2$, or $-CH_2CH(CH_3)CH_2N(CH_3)_2$. R^{11} can be present or absent. If present, R^{11} can be –OH or =O. R^{12} can be hydrogen, halogen (*e.g.*, F, Cl, BR, or I), $-CN$, $-CF_3$, $-CH_2CO_2H$, or $-SO_2N(CH_3)_2$.

[0137] In some embodiments, the active agent comprises resveratrol (3,5,4′-trihydroxystilbene), which has the following structure:



The term "resveratrol" includes both the *cis*- (*Z*) and *trans*- (*E*) (shown above) isomers. Resveratrol is a stilbenoid and a phytoalexin that is produced by several different types of plants in response to injury or attack by foreign organisms. Resveratrol is found, for example, in the skins of grapes, blueberries, raspberries, and mulberries. Analogs and/or pharmaceutically acceptable salts of resveratrol or its analogs are also contemplated for use in the present invention.

[0138] In some embodiments, the active ingredient comprises betamethasone, which has the following structure:



Betamethasone is a steroid that is commonly administered orally, topically, and by intramuscular injection, although any suitable route of administration is contemplated for use

in the present invention. This compound is also known as Alphatrex®, Beta-Val®, Betaderm, ® Betatrex®, Celestone®, Dermabet®, Diprolene®, Diprosone®, Luxiq®, Sernivo®, Uticort®, Valisone®, and Valnac®. Analogs and/or pharmaceutically acceptable salts of betamethasone or its analogs are also contemplated for use in the present invention.

C. Delivery-Enhancing Agents

[0139] In some embodiments, a method of the present invention further comprises contacting a cell (*e.g.*, a neural cell) with a delivery-enhancing agent or administering (*e.g.*, to a subject in need thereof) a delivery-enhancing agent. In some embodiments, a delivery-enhancing agent is an agent or compound that increases the functionality of one or more active agents and/or increases the bioavailability or effectiveness of one or more active agents (*e.g.*, to a neural cell). A delivery-enhancing agent can, for example, increase retention of one or more active agents (*e.g.*, in a subject following administration), stabilize one or more active agents, or decrease the required dose of one or more active agents, *i.e.*, such that a lower dose of an active agent needs to be contacted with a cell (*e.g.*, a neural cell) or administered (*e.g.*, to a subject in need thereof) compared to when the delivery-enhancing agent is not used. In some embodiments, a delivery-enhancing agent increases the concentration (*e.g.*, peak concentration, mean concentration, or median concentration) of one or more active agents (*e.g.*, in a blood sample or tissue sample). In particular embodiments, delivery-enhancing agents are used when active agents are administered to a subject, in order to stabilize the active agents and/or protect them from digestion in the gut, thereby increasing bioavailability.

[0140] Non-limiting examples of suitable delivery-enhancing agents include cyclodextrins, hepatitis E virus-like particles, inactivated yeast, inactivated bacteria, polyvinyl acetate (PVA), inulins and/or esters thereof, and combinations thereof.

[0141] In some embodiments, the delivery-enhancing agent comprises a cyclodextrin. Cyclodextrins, which are a family of compounds that comprise cyclic oligosaccharides, can take the form of alpha-cyclodextrins (having a 6-membered ring), beta-cyclodextrins (having a 7-membered ring), or gamma cyclodextrins (having an 8-membered ring). Cyclodextrins can increase the aqueous solubility of compounds and can increase bioavailability and stability. Polycationic amphiphilic cyclodextrins enhance the interaction of compounds with cell membranes.

[0142] In some embodiments, a cyclodextrin used in methods of the present invention is selected from the group consisting of an α-cyclodextrin, a β-cyclodextrin, a γ-cyclodextrin, a salt thereof, a derivative thereof, and a combination thereof. In some instances, the β-cyclodextrin is selected from the group consisting of a hydroxypropyl-β-cyclodextrin, an endotoxin controlled β-cyclodextrin sulfobutyl ether, a β-cyclodextrin sulfobutyl ether, a sodium salt thereof, an anionic derivative thereof, and a combination thereof.

[0143] Non-limiting examples of particularly useful cyclodextrins include hepta-substituted sulfobutyl-ether-β-cyclodextrin mixtures (*e.g.*, Captisol®), betadex-sulfobutyl-ether-β-cyclodextrin sodium salts (*e.g.*, Dexolve™), and combinations thereof. Cyclodextrins such as Captisol® are useful for, among other things, improving the solubility, stability, or bioavailability of active agents for administration, as well as decreasing volatility, irritation, smell, or taste of active agents. Dexolve™ is available, for example, from CycloLab.

[0144] In some embodiments, a cyclodextrin is contacted with a cell (*e.g.*, a neural cell) or administered (*e.g.*, to a subject in need thereof) at a concentration between about 1 mg/mL and about 300 mg/mL (*e.g.*, about 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17 mg/mL, 18 mg/mL, 19 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, 50 mg/mL, 55 mg/mL, 60 mg/mL, 65 mg/mL, 70 mg/mL, 75 mg/mL, 80 mg/mL, 85 mg/mL, 90 mg/mL, 95 mg/mL, 100 mg/mL, 105 mg/mL, 110 mg/mL, 115 mg/mL, 120 mg/mL, 125 mg/mL, 130 mg/mL, 135 mg/mL, 140 mg/mL, 145 mg/mL, 150 mg/mL, 155 mg/mL, 160 mg/mL, 165 mg/mL, 170 mg/mL, 175 mg/mL, 180 mg/mL, 185 mg/mL, 190 mg/mL, 195 mg/mL, 200 mg/mL, 205 mg/mL, 210 mg/mL, 215 mg/mL, 220 mg/mL, 225 mg/mL, 230 mg/mL, 235 mg/mL, 240 mg/mL, 245 mg/mL, 250 mg/mL, 255 mg/mL, 260 mg/mL, 265 mg/mL, 270 mg/mL, 275 mg/mL, 280 mg/mL, 285 mg/mL, 290 mg/mL, 295 mg/mL, or 300 mg/mL).

[0145] In some embodiments, a delivery-enhancing agent comprises a hepatitis E virus-like particle (HEV-VLP), which can serve as non-infectious drug delivery agents. HEV-VLPs are further described in US. Patent Nos. 8,906,862, 8,906,863, and 9,637,524 and U.S. Patent Application Publication No. US 2017/0107261, hereby incorporated by reference in their entirety for all purposes.

[0146] In some embodiments, a delivery-enhancing agent comprises inactivated bacteria or yeast. Encapsulating active agents described herein into inactivated bacteria and yeast is

especially useful for oral administration, as the active agents can be delivered to the gut and protected from digestion. This method is further described in PCT Application Publication No. WO 2016/069740, hereby incorporated by reference in its entirety for all purposes.

[0147] In some embodiments, the delivery-enhancing agent comprises polyvinyl acetate (PVA), which is a synthetic resin having the formula $(C_4H_6O_2)_n$ and is formed by the polymerization of vinyl acetate. PVA allows two or more compounds (*e.g.*, active agents described herein), including those having differences in solubility (*e.g.*, aqueous solubility), to be packaged together (*e.g.*, in one tablet). In some instances, a compound of Formula (I), a compound of Formula (II), resveratrol, betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, and/or a prodrug thereof are packaged together into PVA.

[0148] In some embodiments, the delivery-enhancing agent comprises an inulin. Inulins are a class of naturally occurring polysaccharides that belong to a class of dietary fibers known as fructans. In humans, inulins are indigestible, whereas bacterial fermentation can lead to the generation of butyrate and propionate from inulins. Because of their resistance to acids and human digestive enzymes, inulins are useful for oral drug delivery. Suitable inulin esters include, but are not limited to inulin butyrate esters, inulin propionate esters, and a combination thereof.

[0149] In some embodiments, the delivery-enhancing agent is present in an amount that is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%,. 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% by weight. In some embodiments, the delivery-enhancing agent is between about 1%-10%, 1%-20%, 1%-30%, 1%-40%, 1%-50%, 1%-60%, 1%-70%, 1%-80%, 1%-90%, 1%-99%, 10%-20%, 10%-30%, 10%-40%, 10%-50%, 10%-60%, 10%-70, 10%-80, 10%-90, 10%-99%, 20%-30%, 20%-40%, 20%-50%, 20%-60%, 20%-70%, 20%-80%, 20%-90%, 20%-99%, 30%-40%, 30%-50%, 30%-60%, 30%-70%, 30%-80%, 30%-90%, 30%-99%, 40%-50%, 40%-60%, 40%-70%, 40%-80%, 40%-90%, 40%-99%, 50%-60%, 50%-70%, 50%-80%, 50%-90%, 50%-99%, 60%-70%, 60%-80%, 60%-90%, 60%-99%, 70%-80%, 70%-90%, 70%-99%, 80%-90%, 80%-99%, or 90%-99% by weight.

[0150] In some embodiments, the delivery-enhancing agent is present in an amount that is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%,. 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% by volume. In some embodiments, the delivery-enhancing agent is between about 1%-10%, 1%-20%, 1%-30%, 1%-40%, 1%-50%, 1%-60%, 1%-70%, 1%-80%, 1%-90%, 1%-99%, 10%-20%, 10%-30%, 10%-40%, 10%-50%, 10%-60%, 10%-70, 10%-80, 10%-90, 10%-99%, 20%-30%, 20%-40%, 20%-50%, 20%-60%, 20%-70%, 20%-80%, 20%-90%, 20%-99%, 30%-40%, 30%-50%, 30%-60%, 30%-70%, 30%-80%, 30%-90%, 30%-99%, 40%-50%, 40%-60%, 40%-70%, 40%-80%, 40%-90%, 40%-99%, 50%-60%, 50%-70%, 50%-80%, 50%-90%, 50%-99%, 60%-70%, 60%-80%, 60%-90%, 60%-100%, 70%-80%, 70%-90%, 70%-99%, 80%-90%, 80%-99%, or 90%-99% by volume.

D. Dosage

[0151] In some embodiments, a daily dose of an active agent for use in methods of the present invention comprises between about 1 mg and about 10,000 mg (*e.g.*, about 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, 400 mg, 410 mg, 420 mg, 430 mg, 440 mg, 450 mg, 460 mg, 470 mg, 480 mg, 490 mg, 500 mg, 510 mg, 520 mg, 530 mg, 540 mg, 550 mg, 560 mg, 570 mg, 580 mg, 590 mg, 600 mg, 610 mg, 620 mg, 630 mg, 640 mg, 650 mg, 660 mg, 670 mg, 680 mg, 690 mg, 700 mg, 710 mg, 720 mg, 730 mg, 740 mg, 750 mg, 760 mg, 770 mg, 780 mg, 790 mg, 800 mg, 810 mg, 820 mg, 830 mg, 840 mg, 850 mg, 860 mg, 870 mg, 880 mg, 890 mg, 900 mg, 910 mg, 920 mg, 930 mg, 940 mg, 950 mg, 960 mg, 970 mg, 980 mg, 990 mg, 1,000 mg, 1,500 mg, 2,000 mg, 2,500 mg, 3,000 mg, 3,500 mg, 4,000 mg, 4,500 mg, 5,000 mg, 5,500 mg, 6,000 mg, 6,500 mg, 7,000 mg, 7,500 mg, 8,000 mg, 8,500 mg, 9,000 mg, 9,500 mg, or 10,000 mg) per day.

[0152] The dosage of active agents administered can be dependent on the subject's body weight, age, individual condition, surface area or volume of the area to be treated and on the form of administration. The size of the dose can also be determined by the existence, nature, and extent of any adverse effects that accompany the administration of a particular compound in a particular subject. Typically, a dosage of the active compounds of the present invention is a dosage that is sufficient to achieve the desired effect. Optimal dosing schedules can be calculated from measurements of agent accumulation in the body of a subject. In general, dosage may be given once or more daily, weekly, or monthly. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

[0153] In some embodiments, the therapeutic agent is administered one or more times a day, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times a day.

[0154] In some embodiments, the therapeutic agent is administered for about 1 to about 31 days, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days. In some embodiments, the therapeutic agent is administered for at least 1 day. In other embodiments, the therapeutic agent is administered for one or more weeks, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more weeks. In yet other embodiments, the therapeutic agent is administered for one or more months, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months.

[0155] To achieve the desired therapeutic effect, compounds or agents may be administered for multiple days at the therapeutically effective daily dose. Thus, therapeutically effective administration of compounds to treat a pertinent condition or disease described herein in a subject may require periodic (*e.g.*, daily or twice daily) administration that continues for a period ranging from three days to two weeks or longer. While consecutive daily doses are a possible route to achieve a therapeutically effective dose, a therapeutically beneficial effect can also be achieved if the agents are not administered daily. For example, one can administer the agents every day, every other day, or, if higher dose ranges are employed and tolerated by the subject, twice a week.

[0156] Optimum dosages, toxicity, and therapeutic efficacy of such compounds or agents may vary depending on the relative potency of individual compounds or agents and can be determined by standard pharmaceutical procedures in experimental animals, for example, by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and

therapeutic effects is the therapeutic index and can be expressed as the ratio, LD_{50}/ED_{50}. Agents that exhibit large therapeutic indices are preferred. While agents that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue to minimize potential damage to normal cells and, thereby, reduce side effects.

[0157] The data obtained from, for example, animal studies can be used to formulate a dosage range for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration.

[0158] A dose can be formulated in animal models to achieve a concentration range that includes the IC_{50} (the concentration of the agent that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in blood or a tissue sample can be measured, for example, by high performance liquid chromatography (HPLC). In general, the dose equivalent of agents is from about 1 ng/kg to about 200 mg/kg for a typical subject.

[0159] The dosage of a pharmaceutical composition of the present invention can be monitored and adjusted throughout treatment, depending on severity of symptoms, frequency of recurrence, and/or the physiological response to the therapeutic regimen. Those of skill in the art commonly engage in such adjustments in therapeutic regimens.

[0160] When more than one active agent is used in methods of the present invention, the active agents may be used (*e.g.*, contacted with a cell such as a neural cell, or administered to a subject) sequentially and/or concomitantly. Delivery or dministration may be by the same or different route, or together in the same pharmaceutical formulation. In some embodiments, two or more active agents are delivered or administered by one route, and additional active agent(s) are delivered or administered by one or more different other routes.

[0161] In some embodiments, a therapeutically effective amount of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 1 mg and about 2,000 mg (*e.g.*, about 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg,

130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, 400 mg, 410 mg, 420 mg, 430 mg, 440 mg, 450 mg, 460 mg, 470 mg, 480 mg, 490 mg, 500 mg, 510 mg, 520 mg, 530 mg, 540 mg, 550 mg, 560 mg, 570 mg, 580 mg, 590 mg, 600 mg, 610 mg, 620 mg, 630 mg, 640 mg, 650 mg, 660 mg, 670 mg, 680 mg, 690 mg, 700 mg, 710 mg, 720 mg, 730 mg, 740 mg, 750 mg, 760 mg, 770 mg, 780 mg, 790 mg, 800 mg, 810 mg, 820 mg, 830 mg, 840 mg, 850 mg, 860 mg, 870 mg, 880 mg, 890 mg, 900 mg, 910 mg, 920 mg, 930 mg, 940 mg, 950 mg, 960 mg, 970 mg, 980 mg, 990 mg, 1,000 mg, 1,500 mg, or 2,000 mg) per day. In particular embodiments, a therapeutically effective amount of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose between about 100 mg and about 800 mg (*e.g.*, about 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, 400 mg, 410 mg, 420 mg, 430 mg, 440 mg, 450 mg, 460 mg, 470 mg, 480 mg, 490 mg, 500 mg, 510 mg, 520 mg, 530 mg, 540 mg, 550 mg, 560 mg, 570 mg, 580 mg, 590 mg, 600 mg, 610 mg, 620 mg, 630 mg, 640 mg, 650 mg, 660 mg, 670 mg, 680 mg, 690 mg, 700 mg, 710 mg, 720 mg, 730 mg, 740 mg, 750 mg, 760 mg, 770 mg, 780 mg, 790 mg, or 800 mg). In some instances, a therapeutically effective amount of a compound of Formula (I), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of about 120 mg, about 240 mg, about 360 mg, about 480 mg, about 600 mg, or about 720 mg per day.

[0162] In some embodiments, a therapeutically effective amount of a compound of Formula (I) (*e.g.*, DMF), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 10 and about 200 mg/kg of body weight per day (*e.g.*, about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg/kg of body weight per day). In some instances, the therapeutically effective amount comprises a dose of between about 40 and about 160 mg/kg of body weight per day (*e.g.*, about 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, or 160 mg/kg of body weight per day).

[0163] In some embodiments, a therapeutically effective amount of a compound of Formula (I) (*e.g.*, DMF), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 20 and about 200, between about 20 and

about 180, between about 20 and about 160, between about 20 and about 140, between about 20 and about 120, between about 20 and about 100, between about 20 and about 80, between about 20 and about 60, between about 20 and about 40, between about 40 and about 200, between about 40 and about 180, between about 40 and about 160, between about 40 and about 140, between about 40 and about 120, between about 40 and about 100, between about 40 and about 80, between about 40 and about 60, between about 60 and about 200, between about 60 and about 180, between about 60 and about 160, between about 60 and about 140, between about 60 and about 120, between about 60 and about 100, between about 60 and about 80, between about 80 and about 200, between about 80 and about 180, between about 80 and about 160, between about 80 and about 140, between about 80 and about 120, between about 80 and about 100, between about 100 and about 200, between about 100 and about 180, between about 100 and about 160, between about 100 and about 140, between about 100 and about 120, between about 120 and about 200, between about 120 and about 180, between about 120 and about 160, between about 120 and about 140, between about 140 and about 200, between about 140 and about 180, between about 140 and about 160, between about 160 and about 200, or between about 160 and about 180 mg/kg of body weight per day.

[0164] In some embodiments, a therapeutically effective amount of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 1 mg and about 300 mg (*e.g.*, about 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, or 300 mg) per day. In particular embodiments, a therapeutically effective amount of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 50 mg and about 150 mg (*e.g.*, about 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, 130 mg, 135 mg, 140 mg, 145 mg, or 150 mg), delivered, contacted with a cell (*e.g.*, a neural cell), or administered (*e.g.*, to a subject in need thereof) twice per day. In some instances, a therapeutically effective amount of a compound of Formula (II), an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of about 75 mg or about 125 mg, delivered, contacted with a cell, or administered twice per day.

[0165] In some embodiments, a therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 0.5 gram and about 10 grams (*e.g.*, about 0.5 gram, 1 gram, 1.5 grams, 2 grams, 2.5 grams, 3 grams, 3.5 grams, 4 grams, 4.5 grams, 5 grams, 5.5 grams, 6 grams, 6.5 grams, 7 grams, 7.5 grams, 8 grams, 8.5 grams, 9 grams, 9.5 grams, or 10 grams) per day. In particular embodiments, a therpeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 1 gram and about 5 grams (*e.g.*, about 1 gram, 1.5 grams, 2 grams, 2.5 grams, 3 grams, 3.5 grams, 4 grams, 4.5 grams, or 5 grams) per day.

[0166] In some embodiments, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent that is between about 0.1 μM and about 10 μM (*e.g.*, about 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1 μM, 1.1 μM, 1.2 μM, 1.3 μM, 1.4 μM, 1.5 μM, 1.6 μM, 1.7 μM, 1.8 μM, 1.9 μM, 2 μM, 2.1 μM, 2.2 μM, 2.3 μM, 2.4 μM, 2.5 μM, 2.6 μM, 2.7 μM, 2.8 μM, 2.9 μM, 3 μM, 3.1 μM, 3.2 μM, 3.3 μM, 3.4 μM, 3.5 μM, 3.6 μM, 3.7 μM, 3.8 μM, 3.9 μM, 4 μM, 4.1 μM, 4.2 μM, 4.3 μM, 4.4 μM, 4.5 μM, 4.6 μM, 4.7 μM, 4.8 μM, 4.9 μM, 5 μM, 5.1 μM, 5.2 μM, 5.3 μM, 5.4v, 5.5 μM, 5.6 μM, 5.7 μM, 5.8 μM, 5.9 μM, 6 μM, 6.1 μM, 6.2 μM, 6.3 μM, 6.4 μM, 6.5 μM, 6.6 μM, 6.7 μM, 6.8 μM, 6.9 μM, 7 μM, 7.1 μM, 7.2 μM, 7.3 μM, 7.4 μM, 7.5 μM, 7.6 μM, 7.7 μM, 7.8 μM, 7.9 μM, 8 μM, 8.1 μM, 8.2 μM, 8.3 μM, 8.4 μM, 8.5 μM, 8.6 μM, 8.7 μM, 8.8 μM, 8.9 μM, 9 μM, 9.1 μM, 9.2 μM, 9.3 μM, 9.4 μM, 9.5 μM, 9.6 μM, 9.7 μM, 9.8 μM, 9.9 μM, or 10 μM), *e.g.*, in a blood sample.

[0167] In particular embodiments, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent that is about 0.5-10 μM, about 1-10 μM, about 1.5-10 μM, about 2-10 μM, about 0.5-9 μM, about 1-9 μM, about 1.5-9 μM, about 2-9 μM, about 0.5-8 μM, about 1-8 μM, about 1.5-8 μM, about 2-8 μM, about 0.5-7 μM, about 1-7 μM, about 1.5-7 μM, about 2-7 μM, about 0.5-6 μM, about 1-6 μM, about 1.5-6 μM, about 2-6 μM, about 0.5-5 μM, about 1-5 μM, about 1.5-5 μM, about 2-5 μM, about 0.5-4 μM, about 1-4 μM, about 1.5-4 μM, about 2-4 μM, about 0.5-3 μM, about 1-3 μM, about 1.5-3 μM, about 2-3 μM, or about 2-2.5 μM, *e.g.*, in a blood sample. In some instances, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent that is about 2.4 μM.

[0168] In some embodiments, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent that is between about 1 ng/mL and about 5,000 ng/mL (*e.g.*, about 1 ng/mL, 2 ng/mL, 3 ng/mL, 4 ng/mL, 5 ng/mL, 6 ng/mL, 7 ng/mL, 8 ng/mL, 9 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL, 50 ng/mL, 60 ng/mL, 70 ng/mL, 80 ng/mL, 90 ng/mL, 100 ng/mL, 110 ng/mL, 120 ng/mL, 130 ng/mL, 140 ng/mL, 150 ng/mL, 160 ng/mL, 170 ng/mL, 180 ng/mL, 190 ng/mL, 200 ng/mL, 210 ng/mL, 220 ng/mL, 230 ng/mL, 240 ng/mL, 250 ng/mL, 260 ng/mL, 270 ng/mL, 280 ng/mL, 290 ng/mL, 300 ng/mL, 310 ng/mL, 320 ng/mL, 330 ng/mL, 340 ng/mL, 350 ng/mL, 360 ng/mL, 370 ng/mL, 380 ng/mL, 390 ng/mL, 400 ng/mL, 410 ng/mL, 420 ng/mL, 430 ng/mL, 440 ng/mL, 450 ng/mL, 460 ng/mL, 470 ng/mL, 480 ng/mL, 490 ng/mL, 500 ng/mL, 550 ng/mL, 600 ng/mL, 650 ng/mL, 700 ng/mL, 750 ng/mL, 800 ng/mL, 850 ng/mL, 900 ng/mL, 1,000 ng/mL, 1,100 ng/mL, 1,200 ng/mL, 1,300 ng/mL, 1,400 ng/mL, 1,500 ng/mL, 1,600 ng/mL, 1,700 ng/mL, 1,800 ng/mL, 1,900 ng/mL, 2,000 ng/mL, 2,100 ng/mL, 2,200 ng/mL, 2,300 ng/mL, 2,400 ng/mL, 2,500 ng/mL, 2,600 ng/mL, 2,700 ng/mL, 2,800 ng/mL, 2,900 ng/mL, 3,000 ng/mL, 3,100 ng/mL, 3,200 ng/mL, 3,300 ng/mL, 3,400 ng/mL, 3,500 ng/mL, 3,600 ng/mL, 3,700 ng/mL, 3,800 ng/mL, 3,900 ng/mL, 4,000 ng/mL, 4,100 ng/mL, 4,200 ng/mL, 4,300 ng/mL, 4,400 ng/mL, 4,500 ng/mL, 4,600 ng/mL, 4,700 ng/mL, 4,800 ng/mL, 4,900 ng/mL, or 5,000 ng/mL), *e.g.*, in a blood sample.

[0169] In particular embodiments, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent of about 1-4,000 ng/mL, about 1-3,000 ng/mL, about 1-2,000 ng/mL, about 1-1,000 ng/mL, about 1-900 ng/mL, about 1-800 ng/mL, about 1-700 ng/mL, about 1-600 ng/mL, about 1-500 ng/mL, about 1-400 ng/mL, about 100-1,000 ng/mL, about 100-900 ng/mL, about 100-800 ng/mL, about 100-700 ng/mL, about 100-600 ng/mL, about 100-500 ng/mL, about 100-400 ng/mL, about 200-1,000 ng/mL, about 200-900 ng/mL, about 200-800 ng/mL, about 200-700 ng/mL, about 200-600 ng/mL, about 200-500 ng/mL, about 200-400 ng/mL, about 250-350 ng/mL, about 260-340 ng/mL, about 270-330 ng/mL, about 280-320 ng/mL, or about 290-310 ng/mL, *e.g.*, in a blood sample. In some instances, the therapeutically effective amount of resveratrol, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof achieves a concentration of the active agent of about 300 ng/mL.

[0170] In some embodiments, a therapeutically effective amount of betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of between about 1 mg and about 30 mg (*e.g.*, about 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, or 30 mg) per day. In some embodiments, a therapeutically effective amount of betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof comprises a dose of less than about 1 mg per day. In some instances, when a range or doses is suitable for methods of the present invention, doses in the lower end of the range are used. In particular instances, a dose of betamethasone, an analog thereof, a pharmaceutically acceptable salt thereof, or a prodrug thereof is adjusted to provide optimal frataxin expression (*e.g.*, in a neural cell).

E. Formulations

[0171] The one or more active agents, analogs thereof, pharmaceutically acceptable salts thereof, and/or prodrugs thereof described herein can be administered orally, parenterally (*e.g.*, intravenously (IV), intramuscularly (IM), depo-IM, subcutaneously (SQ), and depo-SQ), sublingually, intranasally (*e.g.*, inhalation, nasal mist or drops), intrathecally, topically, transmucosally, bucally, sublingually, ionophoretically, or rectally.

[0172] Compositions are provided that contain therapeutically effective amounts of the one or more active agents. The compounds are preferably formulated into suitable pharmaceutical preparations such as tablets, capsules, or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration.

[0173] The one or more active agents, analogs thereof, and/or prodrugs thereof described herein can be administered in the "native" form or, if desired, in the form of salts, esters, amides, prodrugs, derivatives, and the like, provided the salt, ester, amide, prodrug or derivative is suitable pharmacologically, *i.e.*, effective in the present method(s). Salts, esters, amides, prodrugs and other derivatives of the active agents can be prepared using standard procedures described, for example, by March (1992) Advanced Organic Chemistry; Reactions, Mechanisms and Structure, 4th Ed. N.Y. Wiley-Interscience, which is incorporated in its entirety for all purposes. Prodrugs of the agents readily undergo chemical changes under physiological conditions to provide the agents of the present invention. Conversion usually occurs after administration to a subject (*e.g.*, a patient).

[0174] Methods of formulating such derivatives are known. For example, the disulfide salts of a number of delivery agents are described in PCT application publication number WO 2000/059863, which is incorporated herein by reference in its entirety for all purposes. Similarly, acid salts of agents can be prepared from the free base using conventional methodology that typically involves reaction with a suitable acid. Generally, the base form of the drug is dissolved in a polar organic solvent such as methanol or ethanol and the acid is added thereto. The resulting salt either precipitates or can be brought out of solution by addition of a less polar solvent. Suitable acids for preparing acid addition salts include, but are not limited to both organic acids, *e.g.*, acetic acid, carboxylic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, suberic acid, lactic acid, benzene sulfonic acid, *p*-tolylsulfonic acid, arginine, glucuronic acid, galactunoric acid phthalic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid isobutyric, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, *e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like (*see, e.g.*, Berge *et al., J. Pharm. Sci.* (1977) 66, 1-19, which is incorporated in its entirety for all purposes).

[0175] For the preparation of salt forms of basic drugs, the pKa of the counterion is preferably at least about 2 pH lower than the pKa of the drug. Similarly, for the preparation of salt forms of acidic drugs, the pKa of the counterion is preferably at least about 2 pH higher than the pKa of the drug. This permits the counterion to bring the solution's pH to a level lower than the pH_{max} to reach the salt plateau, at which the solubility of the salt prevails over the solubility of the free acid or base. The generalized rule of difference in pKa units of the ionizable group in the active pharmaceutical ingredient (API) and in the acid or base is meant to make the proton transfer energetically favorable. When the pKa of the API and counterion are not significantly different, a solid complex may form but may rapidly disproportionate (*i.e.*, break down into the individual entities of drug and counterion) in an aqueous environment.

[0176] Preferably, the counterion is a pharmaceutically acceptable counterion. Suitable anionic salt forms include, but are not limited to, acetate, benzoate, besylate, benzylate, bitartrate, bromide, carbonate, chloride, citrate, edetate, edisylate, estolate, fumarate, gluceptate, gluconate, hydrobromide, hydrochloride, iodide, lactate, lactobionate, malate, maleate, mandelate, mesylate, methyl bromide, methyl sulfate, mucate, napsylate, nitrate, pamoate (embonate), phosphate and diphosphate, salicylate and disalicylate, stearate,

succinate, sulfate, tartrate, tosylate, triethiodide, valerate, and the like. Suitable cationic salt forms include, but are not limited, to aluminum, benzathine, calcium, ethylene diamine, lysine, magnesium, meglumine, potassium, procaine, sodium, tromethamine, zinc, and the like.

[0177] In various embodiments, preparation of esters typically involves functionalization of hydroxyl and/or carboxyl groups that are present within the molecular structure of the active agent. In certain embodiments, the esters are typically acyl-substituted derivatives of free alcohol groups, *i.e.*, moieties that are derived from carboxylic acids of the formula RCOOH where R is alkyl, and in some instances is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures.

[0178] Amides can also be prepared using techniques described in the pertinent literature. For example, amides may be prepared from esters, using suitable amine reactants, or they may be prepared from an anhydride or an acid chloride by reaction with ammonia or a lower alkyl amine.

[0179] To prepare compositions for use in methods of the present invention, the one or more active agents is mixed with a suitable pharmaceutically acceptable carrier. Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion, or the like. Liposomal suspensions may also be suitable as pharmaceutically acceptable carriers. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for lessening or ameliorating at least one symptom of the disease, disorder, or condition treated and may be empirically determined.

[0180] Pharmaceutical carriers or vehicles suitable for use in methods of the present invention include any such carriers known to be suitable for the particular mode of administration. In addition, the active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, or have another action. The compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients.

[0181] Where the compounds exhibit insufficient solubility, methods for solubilizing may be used. Such methods include, but are not limited to, using cosolvents such as dimethylsulfoxide (DMSO), using surfactants such as Tween™, and dissolution in aqueous

sodium bicarbonate. Derivatives of the compounds, such as salts or prodrugs, may also be used in formulating effective pharmaceutical compositions.

[0182] The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in known *in vitro* and *in vivo* model systems for the treated disorder. A therapeutically or prophylactically effective dose can be determined by first administering a low dose, and then incrementally increasing until a dose is reached that achieves the desired effect with minimal or no undesired side effects.

[0183] In various embodiments, one or more active agents, analogs thereof, pharmaceutically acceptable salts thereof, and/or prodrugs thereof described herein can be enclosed in multiple or single dose containers. The enclosed compounds and compositions can be provided in kits, for example, including component parts that can be assembled for use. For example, an active agent or composition for use in methods of the present invention can be in lyophilized form and a suitable diluent may be provided as separated components for combination prior to use. A kit may include an active agent or composition and a second therapeutic agent for co-administration. The inhibitor and second therapeutic agent may be provided as separate component parts. A kit may include a plurality of containers, each container holding one or more unit dose of the one or more active agents. The containers can be adapted for the desired mode of administration, including, but not limited to tablets, gel capsules, sustained-release capsules, and the like for oral administration; depot products, pre-filled syringes, ampules, vials, and the like for parenteral administration; and patches, medipads, creams, and the like for topical or transdermal administration.

[0184] The concentration and/or amount of active agent(s) in compositions for use in methods of the present invention will depend on absorption, inactivation, and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

[0185] The active agent(s) may be administered at once, or may be divided into a number of smaller doses (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or more doses) to be administered at intervals of time. The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. Concentrations and dosage values may also vary with the severity

of the condition to be alleviated. For any particular subject, specific dosage regimens can be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed methods.

[0186] If oral administration is desired, the active agent(s) or compositions can be provided in a formulation that protects it from the acidic environment of the stomach. For example, the active agent(s) or compositions can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active agent(s) in the intestine. The active agent(s) or compositions may also be formulated in combination with an antacid or other such ingredient.

[0187] Oral compositions generally include an inert diluent or an edible carrier and may be compressed into tablets or enclosed in gelatin capsules. For the purpose of oral therapeutic administration, the active compound or compounds can be incorporated with excipients and used in the form of tablets, capsules, or troches. Pharmaceutically compatible binding agents and adjuvant materials can be included as part of the composition.

[0188] In various embodiments, the tablets, pills, capsules, troches, and the like can contain any of the following ingredients or compounds of a similar nature: a binder such as, but not limited to, gum tragacanth, acacia, corn starch, or gelatin; an excipient such as microcrystalline cellulose, starch, or lactose; a disintegrating agent such as, but not limited to, alginic acid and corn starch; a lubricant such as, but not limited to, magnesium stearate; a gildant, such as, but not limited to, colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; and a flavoring agent such as peppermint, methyl salicylate, or fruit flavoring.

[0189] When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials, which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, medicated chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings, and flavors.

[0190] The active materials can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action.

[0191] Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent such as water for injection, saline solution, fixed oil, a naturally occurring vegetable oil such as sesame oil, coconut oil, peanut oil, cottonseed oil, and the like, or a synthetic fatty vehicle such as ethyl oleate, and the like, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antimicrobial agents such as benzyl alcohol and methyl parabens; antioxidants such as ascorbic acid and sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates, and phosphates; and agents for the adjustment of tonicity such as sodium chloride and dextrose. Parenteral preparations can be enclosed in ampules, disposable syringes, or multiple dose vials made of glass, plastic, or other suitable material. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

[0192] Suitable carriers for intravenous administration include physiological saline, phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents such as glucose, polyethylene glycol, polypropyleneglycol, and mixtures thereof. Liposomal suspensions including tissue-targeted liposomes may also be suitable as pharmaceutically acceptable carriers.

F. Monitoring Efficacy

[0193] In particular embodiments, a sample (*e.g.*, test sample) is obtained from the subject. The test sample can be obtained before and/or after the active agent(s) are contacted with a cell (*e.g.*, a neural cell) or are administered (*e.g.*, to the subject). Non-limiting examples of suitable samples include blood, serum, plasma, cerebrospinal fluid, tissue or cells (*e.g.*, neural tissue or neural cells), saliva, and urine. In some instances, the sample comprises normal tissue or cells. In other instances, the sample comprises abnormal tissue or cells. The sample can also be made up of a combination of normal and abnormal tissue or cells. In some embodiments, the sample comprises abnormal blood and/or tissue (*e.g.* abnormal neural tissue) that comprises a lower mitochondrial mass and/or number compared to a normal blood and/or tissue sample.

[0194] In some embodiments, a reference sample is obtained. The reference sample can be obtained, for example, from the subject and can comprise normal tissue. The reference

sample can be also be obtained from a different subject and/or a population of subjects. In some instances, the reference sample is obtained from the subject, a different subject, or a population of subjects before and/or after the active agent(s) are contacted with a cell (*e.g.*, neural cell) or are administered (*e.g.*, to the subject), and comprises normal tissue. However, in some instances the reference sample comprises abnormal tissue and is obtained from the subject and/or from a different subject or a population of subjects.

[0195] In some embodiments, the level of one or more biomarkers is determined in the test sample and/or reference sample. In some embodiments, the biomarker is expressed only in mitochondria, although it need not be so. Non-limiting examples of suitable biomarkers include frataxin, COX4, TFAM, ND2, ND6, and HO-1. The term "COX4" refers to cytochrome c oxidase subunit 4, which is the last enzyme in the respiratory electron transport chain and is expressed exclusively in mitochondria. Isoform 1 of COX4 is encoded by the *COX4I1* gene in humans, whereas isoform 2 is encoded by the *COX4I2* gene. The term "TFAM" refers to mitochondrial transcription factor A, abbreviated as TFAM or mtTFA, which is a protein that is encoded by the *TFAM* gene in humans and serves as a marker of mitochondrial biogenesis and mitochondrial gene transcription. "ND2" and "ND6" are subunits of mitochondrial complex 1 and are encoded by the *MT-ND2* and *MT-ND6* genes, respectively. In particular embodiments, the one or biomarkers comprise frataxin. "HO-1" refers to heme oxygenase-1, one of three isoforms of heme oxygenase and is encoded by the *HMOX1* gene in humans. Heme oxygenases catalyze the degradation of heme, producing biliverdin. As non-limiting example, HO-1 can be used as a marker of Nrf2 activity (*e.g.*, a marker of transcription or activity of genes downstream from Nrf2 in the Nrf2 pathway).

[0196] Typically, the level of the one or more biomarkers in one or more test samples is compared to the level of the one or more biomarkers in one or more reference samples. Depending on the biomarker, and increase or a decrease relative to a normal control or reference sample can be indicative of the presence or increased risk of a disorder associated with insufficient mitochondrial function. As a non-limiting example, levels of one or biomarkers in test samples taken before and after the active agent(s) are contacted with a cell (*e.g.*, neural cell) or are administered (*e.g.*, to the subject) are compared to the level of the one or more biomarkers in a reference sample that is either normal blood or tissue obtained from the subject, or normal blood tissue that is obtained from a different subject or a population of subjects. In some instances, the level of frataxin in a test sample obtained from the subject before the active agent(s) are contacted with a cell or are administered to the subject is lower

than the level of frataxin in the reference sample. In other instances, the level of frataxin in a test sample obtained from the subject after contact or administration of the active agent(s) is increased relative to the level of frataxin in a test sample obtained prior to contact or administration. Alternatively, as another non-limiting example, the difference in frataxin level between a sample obtained from the subject after contact or administration of active agent(s) and a reference sample is smaller than a difference between the frataxin level in a sample obtained from the subject prior to contact or administration of active agent(s) and the reference sample (*i.e.*, administration results in an increase in frataxin in the test sample such that the difference between the level measured in the test sample and the level measured in the reference sample is diminished or eliminated).

[0197] The differences between the reference sample or value and the test sample need only be sufficient to be detected. In some embodiments, a decreased level of a biomarker (*e.g.*, frataxin) in the test sample, and hence the presence or increased risk of a disorder associated with insufficient mitochondrial function, is determined when the biomarker levels are at least, *e.g.*, about 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, or 50-fold lower in comparison to a negative control. In other embodiments, an increased level of a biomarker in the test sample, and hence the presence or increase risk of a disorder associated with insufficient mitochondrial function, is determined when the biomarker levels are at least, *e.g.*, about 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, or 50-fold higher in comparison to a negative control.

[0198] The biomarker levels can be detected using any method known in the art, including the use of antibodies specific for the biomarkers. Exemplary methods include, without limitation, PCR, Western Blot, dot blot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, FACS analysis, electrochemiluminescence, and multiplex bead assays (*e.g.*, using Luminex or fluorescent microbeads). In some instances, nucleic acid sequencing is employed.

[0199] In certain embodiments, the presence of decreased or increased levels of one or more biomarkers is indicated by a detectable signal (*e.g.*, a blot, fluorescence, chemiluminescence, color, radioactivity) in an immunoassay or PCR reaction (*e.g.*,

quantitative PCR). This detectable signal can be compared to the signal from a control sample or to a threshold value. In some embodiments, a decreased presence is detected, and the presence or increased risk of a disorder associated with insufficient mitochondrial function is indicated, when the detectable signal of biomarker(s) in the test sample are at least, *e.g.*, about 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, or 50-fold lower in comparison to the signal of antibodies in the reference sample or the predetermined threshold value. In other embodiments, an increased presence is detected, and the presence or increased risk of a disorder associated with insufficient mitochondrial function is indicated, when the detectable signal of biomarker(s) in the test sample is at least, *e.g.*, about 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, or 50-fold greater in comparison to the signal of antibodies in the reference sample or the predetermined threshold value.

[0200] In some embodiments, the results of the biomarker level determinations are recorded in a tangible medium. For example, the results of diagnostic assays (*e.g.*, the observation of the presence or decreased or increased presence of one or more biomarkers) and the diagnosis of whether or not there is an increased risk or the presence of a disorder associated with insufficient mitochondrial function can be recorded, *e.g.*, on paper or on electronic media (*e.g.*, audio tape, a computer disk, a CD, a flash drive, *etc.*).

[0201] In other embodiments, the methods further comprise the step of providing the diagnosis to the patient (*i.e.*, the subject) and/or the results of treatment.

IV. Examples

[0202] The present invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1. Dimethyl fumarate and methylene blue promote mitochondrial biogenesis and frataxin induction.

DMF promotes mitochondrial biogenesis and mitochondrial subunit synthesis

[0203] DMF, when used alone, can promote mitochondrial biogenesis. In previous experiments performed by the inventors using human fibroblast cell lines, after a 48-hour treatment with DMF at doses of 3, 10 and 30 μM in concentration, the cells exhibited increases in a mitochondrial biogenesis marker, TFAM. Additionally, dose-dependent increases were observed in the expression of multiple subunits of the four mitochondrial complexes and ATP synthase involved in the electron transport chain to produce ATP. The mitochondrial complex 1 subunits are ND2 and ND6, the mitochondrial complex 2 subunits are SDHA and SDHB, the mitochondrial complex 3 subunits are mt-CYB and CYC1, the mitochondrial complex 4 subunits are mt-CO1 and mt-CO2, and the ATP synthase subunits are ATP5B and mt-ATP6. The expression levels of these subunits were measured using qRT-PCR in healthy human-derived fibroblasts that were treated with DMF using concentrations of 3, 10, or 30 μM for 48 hours. As shown in FIG. 1, expression of the subunits was significantly increased when a 30 μM concentration of DMF was used, and the expression of many of the subunits was significantly increased even when a concentration of 10 μM was used. These results are consistent with observations that mitochondrial copy number per nucleus in human fibroblasts dose-dependently increased following 48 hours of DMF exposure, from 140% to 175% relative to vehicle treatment at the 10 μM and 30 μM concentrations, respectively.

DMF increases mitochondrial copy number in the eye

[0204] Mitochondrial gene expression was measured in retinas of a mouse model of Parkinson's disease and optic neuropathy (Ndufs4 KO). Briefly, wild-type and Ndufs4 KO mice were administered vehicle only or DMF by intraperitoneal (i.p.) injection for 14 days at a dose of 10 mg/kg. qRT-PCR was used to assay expression of mitochondrial genes *ND2* and *ND6*. As shown in FIG. 2, the dosed mice exhibited increased expression of *ND2* and *ND6* in their retinas.

DMF increases mitochondrial functionality

[0205] Mitochondrial functionality was measured by the rate at which mitochondria consume oxygen (OCR) in healthy human fibroblast cells using an Agilent Seahorse XF

analyzer. Cells were treated with DMF for 48 hours using a concentration of 3, 10, or 30 μM. FIG. 3 shows that DMF dose-dependently increased basal mitochondrial respiration in segment A and dose-dependently increased maximal mitochondrial respiration in segment B, and that these increases were significant relative to vehicle-treated cells.

DMF rescues vision loss in a mouse model of mitochondrial disease

[0206] In addition to increasing mitochondrial functionality in cells (FIG. 3), and increasing mitochondrial number and expression in animals (FIG. 2), DMF also increased functional vision in the Ndufs4 KO mouse, which is phenotypically blind starting at about P30. When treated with DMF at a dose of 10 mg/kg i.p for 2 weeks, the Ndufs4 KO's vision was preserved to within wild-type levels. The visual cliff test evaluates a subject's ability to detect a simulated cliff. As shown in FIG. 4, visual cliff testing of wild type and Ndufs4 KO mice treated with DMF demonstrated that visual function was intact, whereas Ndufs4 KO mice that were not treated or treated with vehicle only displayed no indication of a functioning visual system.

DMF rescues dopaminergic neurons from death in a mouse mitochondrial disease model

[0207] Tyrosine Hydroxylase (TH) is a marker of dopaminergic neuron health in the substantia nigra and striatum, two areas of the brain that degenerate in Parkinson's disease (PD). PD patients have a 20 to 80 percent decrease in TH staining in the substantia nigra and striatum. In an animal model of PD, the Ndufs4 KO mouse, a decrease was observed in TH [FIG. 5 WT vehicle vs. KO vehicle]. Conversely, Ndufs4 KO mice treated with 10 mg/kg DMF by i.p. injection for 14 days experienced a 20 to 30 percent increase in TH expression, which is a biomarker of dopaminergic neuron health. Following treatment, striatum was dissected to extract protein for the determination of TH expression by Western blot. Thus, through its ability to increase mitochondrial number, DMF increased the TH level, which is protective in multiple models of PD.

Methylene blue also protects FA cells from death

[0208] Methylene blue (MB) (FIG. 6A) and its analog tolonium chloride (FIG. 6B) dose-dependently protected Friedreich's ataxia (FA) patient cells from death (FIG. 6). Briefly, FA patient-derived fibroblasts were incubated with MB or tolonium chloride for 24 hours. Post-treatment, the cells were incubated with 125 μM diamide for 14 to 18 hours. Cell viability was then measured using a Calcein AM viability assay kit (Invitrogen).

Methylene Blue dose-dependently induces frataxin in FA patient lymphoblasts, and is a more potent inducer than DMF

[0209] In cell-western blots were carried out to identify the dose-dependence of MB's ability to increase frataxin protein in FA patient cells (FIG. 7), and MB was active at a low concentration range. Similarly, as described further in FIG. 10 and Example 2 below, the dose-dependence of frataxin mRNA induction by MB and DMF was investigated. Both MB and DMF were able to induce in the low micromolar range, with MB being about 3 times more effective on a molar basis. Lastly, as discussed further in Example 2 below, the ability of MB to induce mitochondrial biogenesis was investigated and was even more potent than DMF on a molar basis.

Methylene Blue induces frataxin in a mouse model of FA with deficient frataxin

[0210] The *in vivo* effect of MB was investigated by dosing MB at 10 mg/kg for 7 days i.p in a YG8 mouse model of FA (FIG. 8). Post-treatment, protein was extracted from the cerebellum and frataxin expression was measured by Western blot. MB produced a significant elevation, over vehicle control dosing, of frataxin expression in cerebellum, the tissue that is frataxin-deficient in FA and that degenerates in FA. DMF produced a similar rise in frataxin in brain tissue.

[0211] In summary, DMF and MB increase frataxin expression at the protein and transcriptional level, in human cell and mouse models of Friedreich's ataxia. The sole cause of Friedreich's ataxia is a reduction of frataxin, so increasing frataxin levels will ameliorate the signs and symptoms of this disease. Also, DMF and MB increase mitochondrial number, and mitochondrial gene expression. In mitochondrial disease it has recently been shown that a simple numerical increase in the number of mitochondria mitigates pathophysiological outcomes in multiple animal models. With regard to function in cells and animals, DMF rescued vision loss in a mouse model of mitochondrial blindness, and preserved neurons.

Methods

Fibroblast cell culture and drug treatment

[0212] For healthy human fibroblast experiments, the healthy human fibroblast cell line AG09429 (Coriell Institute, Camden, NJ, USA) was maintained at 37 degrees Celsius in a humidified atmosphere with 5% CO_2. DMEM (Corning, Inc., Corning, NY, USA) supplemented with 10% fetal bovine serum (JRScientific, Woodland, CA, USA) and 1x

penicillin-streptomycin solution (Corning, Inc., Corning, NY, USA) was used as growth media. Media was changed every two days. The human fibroblasts were plated in a 12-well format at $0.1x10^6$ cells per well. The cells were incubated with 0.1% DMSO as vehicle control or 3–30 mM of dimethyl fumarate (Sigma- Aldrich, St. Louis, MO, USA) dissolved in DMSO. Total RNA and DNA were extracted following a 48-hour incubation period.

DNA and RNA extraction

[0213] Total DNA was extracted from human fibroblasts using a DNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA), respectively, following manufacturer's instructions. DNA was quantified using a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

[0214] Total RNA was extracted from B-lymphoblast cells using an RNeasy Plus Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. RNA quantity and quality were measured using a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Quantitative RT-PCR

[0215] cDNA was synthesized from mRNA using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) per the manufacturer's instructions in a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). A SensiFAST SYBR No-ROX Kit (Bioline, Taunton, MA, USA) was used to perform qPCR on the synthesized cDNA in a Roche Lightcycler 480 (Roche Diagnostics, Indianapolis, IN, USA). The second derivative of the amplification curve was used to determine the cycle threshold, and the data were analyzed by a delta CT calculation.

Measurement of oxygen consumption in DMF-treated fibroblasts by Seahorse XF Analyzer

[0216] Fibroblast cell lines were seeded at a density of 60,000 cells/well in 200 µL of culture medium in a 24-well Seahorse tissue culture plate (Seahorse Biosciences, Billerica, MA, USA). Following a 24-hour incubation, the media was replaced and incubated with 200 µL of 0.1% DMSO or 3 µM, 10 µM, or 30 µM dimethyl fumarate in 0.1% DMSO. Prior to reading the oxygen consumption, the medium was changed to unbuffered DMEM without phenol red (Corning, Inc., Corning, NY, USA), 10% fetal bovine serum (JR-Scientific, Woodland, CA, USA), 200 mM glutamax, 100 mM sodium pyruvate, 25 mM glucose (Invitrogen, Waltham, MA, USA), and was adjusted to pH 7.4. Cells were pre-equilibrated

for 20 minutes; oxygen consumption rate (OCR) and proton production rate (PPR) were recorded with the Seahorse XF-24 after sequential addition of oligomycin (1 μg/ml), FCCP (10 μM), and a combination of antimycin A (1 μM) and rotenone (1 μM) (Sigma-Aldrich, St. Louis, MO, USA). Total protein in each well was measured and protein concentration was used to normalize the readings.

Data analysis

[0217] Data analysis was carried out with GraphPad Prism 5.0 statistics software (GraphPad Software, La Jolla, CA, USA). A list of analyses includes two-way ANOVA with Bonferroni post-hoc multiple comparison test and one-way ANOVA with Newman–Keuls post-hoc multiple comparison test.

Example 2. Dimethyl fumarate, methylene blue, resveratrol, and betamethasone promote frataxin induction and mitochondrial biogenesis.

[0218] This example describes a series of experiments that were performed in order to assess the effects of dimethyl fumarate (DMF), methylene blue (MB), resveratrol, and betamethasone, alone and in combination, on frataxin expression and mitochondrial biogenesis.

[0219] FIG. 9 shows the effects of DMF, MB, resveratrol, and betamethasone on frataxin expression and mitochondrial copy number in Friedreich's ataxia (FA) patient-derived fibroblasts (*i.e.*, GM4078 cells with 541 and 420 GAA repeats). The concentrations of DMF, MB, resveratrol, and betamethasone were 30 μM, 3 μM, 100 μM, and 10 μM, respectively. Cells were contacted with drug for 48 hours. 3 replicates were used for each group. The drug concentrations were selected based on published literature relating to their safety profiles and their ability to pass through the blood-brain barrier. After drug treatment, RNA and DNA were extracted to assay frataxin expression and mitochondrial DNA copy number, respectively, by qRT-PCR.

[0220] As shown in FIG. 9A, DMF, MB, and resveratrol increased frataxin expression. As shown in FIG. 9B, DMF, resveratrol, and betamethasone increased mitochondrial DNA copy number. Combined data are shown in FIG. 9C.

[0221] FIG. 10 shows the dose-dependence of the ability of DMF, MB, resveratrol, and betamethasone to modulate frataxin expression and mitochondrial DNA copy number (FIGS. 10A and 10B, respectively). For DMF, concentrations of 3 μM, 10 μM, and 30 μM were

used. For MB, concentrations of 1 μM, 3 μM, and 10 μM were used. For resveratrol, concentrations of 10 μM, 30 μM, and 100 μM were used. For betamethasone, concentrations of 1 μM, 3 μM, and 10 μM were used. DMF, MB, and resveratrol increased frataxin expression in a dose-dependent manner and DMF and resveratrol increased mitochondrial DNA copy number in a dose-dependent manner.

[0222] FIG. 11 shows the effects of combining multiple drugs on frataxin expression and mitochondrial copy number. The effects of 10 μM DMF, 10 μM resveratrol, and 1 μM betamethasone, when used alone, were compared to combinations of the three drugs. As shown in FIG. 11A, all three combinations produced a greater increase in frataxin expression than any drug alone. Also, all three combinations produced a greater increase in mitochondrial DNA copy number than any drug along (FIG. 11B). These results complement previous data showing that MB potentiates the ability of DMF to increase frataxin expression.

Example 3. Monomethyl fumarate promote frataxin induction and Nrf2-dependent activity.

[0223] This example describes experiments that examined the effects of fumarates on frataxin expression, as well as that of Nrf2-responsive genes.

[0224] FIG. 12 shows the effects of monomethyl fumarate (MMF) on frataxin expression. Friedreich's ataxia (FA) lymphoblast cells (*i.e.*, GM-14518 cells with approximately 900 GAA repeats) were treated with increasing doses of MMF for 24 hours. DMF was used as a positive control. RNA was extracted after drug treatment and expression of frataxin (*FXN*) was measured using qRT-PCR.

[0225] FIG. 13 shows the effects of MMF on heme oxygenase-1 (*HO-1*) expression. HO-1 is a marker of Nrf2 activity. FA lymphoblast cells (*i.e.*, GM-14518 cells with approximately 900 GAA repeats) were treated with increasing doses of MMF for 24 hours. DMF was used as a positive control. RNA was extracted after drug treatment and expression of *HO-1* was measured using qRT-PCR.

Example 4. Dimethyl fumarate dose-dependently increases mitochondrial copy number and gene expression.

[0226] This example shows that dimethyl fumarate (DMF) increases the expression of frataxin and COX4, both of which are exclusively expressed in mitochondria, in a dose-dependent manner.

[0227] DMF was administered to C57Bl6 mice in increasing doses. 4-month-old C57Bl6 mice were single-caged and divided into 6 groups. Each group had 5 animals and an equal ratio of males to females. DMF doses were prepared by dissolving DMF in PBS with the minimum amount of required ethanol. Animals were administered PBS+ethanol (vehicle-only control) or DMF once per day by intraperitoneal (IP) injection according to the indicated dose (as shown in FIG. 16) for 13 days. Animals were sacrificed 2 hours after the last dose and organs were collected and snap frozen in liquid nitrogen before being stored at -80 °C for later experiments.

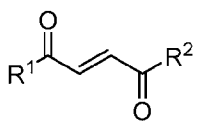
[0228] Western blots were performed to measure protein expression. For Western blots, small pieces of organs were cut from the frozen stock and were broken down in lysis buffer. Proteins were then extracted. Proteins extracts were electrophoresed and then Western blotted using an anti-frataxin (Fxn) antibody or an anti-COX4 antibody. Both frataxin and COX4 are exclusively expressed in mitochondria.

[0229] As shown in FIGS. 16A and 16B, a dose-dependent increase in both frataxin and COX4 expression was observed in the neural cells, demonstrating that DMF dose-dependently increases mitochondrial copy number and gene expression.

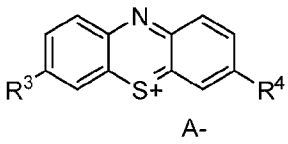
V. Exemplary Embodiments

[0230] Exemplary embodiments provided in accordance with the presently disclosed subject matter include, but are not limited to, the claims and the following embodiments:

1. A method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, the method comprising contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



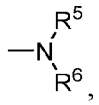
(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

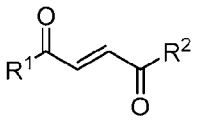
wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

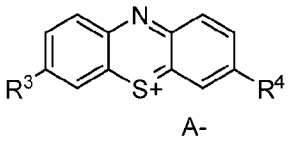
wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $–OCOCH_3$.

2. A method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, the method comprising administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



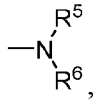
(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

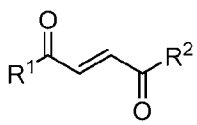
3. The method of embodiment 1 or 2, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell comprises increasing mitochondrial mass and/or copy number in the neural cell.

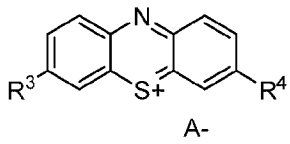
4. The method of any one of embodiments 1 to 3, wherein contacting the neural cell with two or more different active agents or administering two or more different active agents produces a synergistic promotion and/or increase in mitochondrial biogenesis in the neural cell compared to when the active agents are contacted or administered alone.

5. The method of any one of embodiments 1 to 4, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases mitochondrial gene expression in the neural cell.

6. The method of any one of embodiments 1 to 5, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases frataxin expression and/or activity in the neural cell.

7. A method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, the method comprising contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):

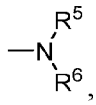
 (I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

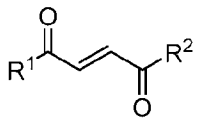
wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

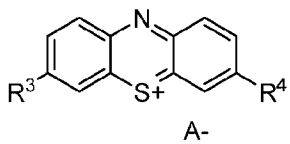
wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $–OCOCH_3$.

8. A method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, the method comprising administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



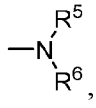
(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

9. The method of embodiment 7 or 8, wherein the insufficient mitochondrial function in the neural cell is associated with insufficient mitochondrial mass and/or mitochondrial copy number in the neural cell.

10. The method of any one of embodiments 7 to 9, wherein the disorder is a neurodegenerative disease.

11. The method of any one of embodiments 7 to 10, wherein the disorder is associated with decreased frataxin expression and/or activity in the neural cell.

12. The method of any one of embodiments 7 to 11, wherein the disorder is selected from the group consisting of Friedreich's ataxia; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic epilepsy with ragged red fibers (MERRF); Leber's hereditary optic neuropathy (LHON); neuropathy, ataxia, and retinitis pigmentosa (NARP); maternally inherited Leigh syndrome (MILS); multiple sclerosis (MS); and a combination thereof.

13. The method of any one of embodiments 7 to 12, wherein the subject is not exhibiting any signs or symptoms of the disorder.

14. The method of any one of embodiments 7 to 12, wherein the subject is exhibiting one or more signs or symptoms of the disorder.

15. The method of any one of embodiments 7 to 14, wherein contacting the neural cell with the active agent or administering the active agent promotes and/or increases mitochondrial biogenesis in the neural cell.

16. The method of embodiment 15, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell comprises increasing mitochondrial mass and/or copy number in the neural cell.

17. The method of embodiment 15 or 16, wherein contacting the neural cell with two or more different active agents or administering two or more different active agents produces a synergistic promotion and/or increase in mitochondrial biogenesis in the neural cell compared to when the active agents are contacted or administered alone.

18. The method of any one of embodiments 15 to 17, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases mitochondrial gene expression in the neural cell.

19. The method of any one of embodiments 15 to 18, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases frataxin expression and/or activity in the neural cell.

20. The method of any one of embodiments 15 to 19, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell prevents, delays, reduces, mitigates, ameliorates, and/or inhibits one or more signs or symptoms associated with the disorder.

21. The method of any one of embodiments 1 to 20, wherein the active agent comprises a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof.

22. The method of any one of embodiments 1 to 20, wherein the active agent comprises a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof.

23. The method of any one of embodiments 1 to 20, wherein the active agent comprises resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

24. The method of any one of embodiments 1 to 20, wherein the active agent comprises betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

25. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof.

26. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically

acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

27. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

28. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

29. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

30. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

31. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

32. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

33. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

34. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

35. The method of any one of embodiments 1 to 20, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

36. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, or 35, wherein E is selected from the group consisting of $-NO_2$, $-N(R^7{}_2)$, $-N(R^7{}_3)^+$, $-NH_3^+$, $-SO_3H$, $-SO_3R^8$, $-S(O_2)R^8$ (sulfone), $-S(O)R^8$ (sulfoxide), $-S(O_2)NH_2$ (sulfonamide), $-SO_2NHR^8$, $-SO_2NR^8{}_2$, $-PO(OR^8)_2$, $-PO_3H_2$, $-PO(NR^8{}_2)_2$, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, pyrazolyl, indazolyl, imidazolyl, thiazolyl, benzothiazolyl, oxazolyl, benzimidazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, triazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinazolinyl, pyrimidinyl, a 5- or 6-membered heteroaryl with a C-N double bond optionally fused to a 5- or 6-membered heteroaryl, pyridinyl N-oxide , $C\equiv N$, $-CX_3$, -C(O)X, -COOH, $-COOR^8$, $-C(O)R^8$, $-C(O)NH_2$, $-C(O)NHR^8$, $-C(O)NR^8{}_2$, -C(O)H, $-P(O)(OR^8)OR^9$, and X,

wherein X is a halogen and R^7, R^8, and R^9 are each independently selected from the group consisting of hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

37. The method of any one of embodiments 1 to 20, 22, 25, 28, 29, 31, 32, 34, or 35, wherein A- is an anion, a dianion, or a trianion.

38. The method of embodiment 37, wherein A- is selected from the group consisting of Cl^-, Br^-, I^-, F^-, NO_3^-, $CH_3SO_3^-$, HSO_4^-, $CHCO_2^-$ SO_4^{2-}, HPO_4^{2-}, and PO_4^{3-}.

39. The method of any one of embodiments 1 to 22, 25 to 29, or 31 to 38, wherein the halogen is independently selected from the group consisting of F, Cl, Br, and I.

40. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, 35, 36, or 39, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a fumarate ester.

41. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, 35, 36, 39, or 40, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of monomethyl fumarate (MMF), monomethyl maleate, monoethyl fumarate, monoethyl maleate, monobutyl fumarate, monobutyl maleate, monooctyl fumarate, monooctyl maleate, mono (phenylmethyl) fumarate, mono (phenylmethyl) maleate, mono (2-hydroxypropyl) fumarate, mono (2-hydroxypropyl) maleate, mono (2-ethylhexyl) fumarate, mono (2-ethylhexyl) maleate, dimethyl fumarate (DMF), dimethyl maleate, diethyl fumarate, diethyl maleate, dipropyl fumarate, dipropyl maleate, diisopropyl fumarate, diisopropyl maleate, dibutyl fumarate, dibutyl maleate, diisobutyl fumarate, diisobutyl maleate, diheptyl fumarate, diheptyl maleate, bis(2-ethylhexyl) fumarate, bis(2-ethylhexyl) maleate, (-)-dimethyl fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) maleate, bis(2-trifluoroethyl) fumarate, bis(2-trifluoroethyl) maleate, and a combination thereof.

42. The method of embodiment 41, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises dimethyl fumarate (DMF).

43. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, 35, 36, or 39 to 42, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is provided as a prodrug.

44. The method of embodiment 43, wherein the prodrug is selected from the group consisting of O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, and a combination thereof.

45. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, 35, 36, or 39 to 44, wherein the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 2,000 mg per day.

46. The method of embodiment 45, wherein the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof

comprises a dose of about 120 mg, about 240 mg, about 360 mg, about 480 mg, about 600 mg, or about 720 mg per day.

47. The method of any one of embodiments 1 to 21, 25 to 27, 31 to 33, 35, 36, or 39 to 44, wherein the compound of Formula (I) is DMF, and the therapeutically effective amount comprises a dose of between about 10 and about 160 mg/kg of body weight per day.

48. The method of embodiment 47, wherein the therapeutically effective amount comprises a dose of between about 40 and about 160 mg/kg of body weight per day.

49. The method of any one of embodiments 1 to 20, 22, 25, 28, 29, 31, 32, 34, 35, or 37 to 39, wherein the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, leuco-methylene blue, acetyl-methylene blue, leuco-methylonium bis(hydromethanesulfonate) (LMTM), diaminophenothiazine, 2-chlorophenothiazine, phenothiazine, toluidine blue, tolonium chloride, toluidine blue O, seleno toluidine blue, methylene green, chlorpromazine, sulphoxide chlorpromazine, sulphone chlorpromazine, chlordiethazine promethazine, thioproperazine, prochlorperazine, pipotiazine, dimetotiazine, propericiazine, metazionic acid, oxomemazine neutral red, iminostilbene, imipramine, and a combination thereof.

50. The method of embodiment 49, wherein the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, tolonium chloride, leuco-methylonium bis(hydromethanesulfonate) (LMTM), and a combination thereof.

51. The method of any one of embodiments 1 to 20, 22, 25, 28, 29, 31, 32, 34, 35, 37 to 39, 49, or 50, wherein the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 300 mg per day.

52. The method of embodiment 51, wherein the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of about 75 mg or about 125 mg, contacted with the cell or administered twice per day.

53. The method of any one of embodiments 1 to 20, 23, 26, 28, 30, 31, or 33 to 35, wherein the therapeutically effective amount of resveratrol comprises a dose of between about 0.5 grams and about 10 grams per day.

54. The method of embodiment 53, wherein the therapeutically effective amount of resveratrol comprises a dose of between about 1 gram and about 5 grams per day.

55. The method of any one of embodiments 1 to 20, 24, 27, 29, 30, or 32 to 35, wherein the therapeutically effective amount of betamethasone comprises a dose of between about 1 mg and about 30 mg per day.

56. The method of any one of embodiments 1 to 55, wherein two or more different active agents are contacted with the neural cell or administered concomitantly.

57. The method of any one of embodiments 1 to 55, wherein two or more different active agents are contacted with the neural cell or administered sequentially.

58. The method of any one of embodiments 1 to 57, wherein the method further comprises contacting the neural cell with a delivery-enhancing agent or administering to the subject a delivery-enhancing agent.

59. The method of embodiment 58, wherein the delivery-enhancing agent is selected from the group consisting of a cyclodextrin, a hepatitis E virus-like particle, an inactivated yeast, an inactivated bacterium, polyvinyl acetate (PVA), an inulin or an ester thereof, and a combination thereof.

60. The method of embodiment 59, wherein the cyclodextrin is selected from the group consisting of an α-cyclodextrin, a β-cyclodextrin, a γ-cyclodextrin, a salt thereof, a derivative thereof, and a combination thereof.

61. The method of embodiment 60, wherein the β-cyclodextrin is selected from the group consisting of a hydroxypropyl-β-cyclodextrin, an endotoxin controlled β-cyclodextrin sulfobutyl ether, a β-cyclodextrin sulfobutyl ether, a sodium salt thereof, an anionic derivative thereof, and a combination thereof.

62. The method of any one of embodiments 59 to 61, wherein the cyclodextrin is selected from the group consisting of a hepta-substituted sulfobutyl-ether-β-cyclodextrin mixture, betadex-sulfobutyl-ether-β-cyclodextrin sodium salt, and a combination thereof.

63. The method of any one of embodiments 59 to 62, wherein the cyclodextrin is contacted with the neural cell or administered at a concentration of between about 1 mg/mL and about 300 mg/mL.

64. The method of any one of embodiments 1 to 63, wherein the method further comprises contacting the neural cell with a pharmaceutically acceptable carrier or administering to the subject a pharmaceutically acceptable carrier.

65. The method of any one of embodiments 1 to 64, wherein a sample is obtained from the subject before and/or after the active agent is contacted with the neural cell or administered to the subject.

66. The method of embodiment 65, wherein the sample comprises blood, tissue, or a combination thereof.

67. The method of embodiment 66, wherein the tissue sample comprises neural tissue.

68. The method of embodiment 66 or 67, wherein the tissue sample comprises normal or abnormal tissue.

69. The method of embodiment 66 or 67, wherein the sample is an abnormal blood and/or tissue sample that comprises a lower mitochondrial mass and/or number compared to a normal blood and/or tissue sample.

70. The method of any one of embodiments 65 to 69, wherein the presence or level of a biomarker is determined in the sample.

71. The method of embodiment 70, wherein the biomarker comprises frataxin, COX4, or a combination thereof.

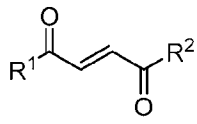
72. The method of embodiment 70 or 71, wherein the presence or level of the biomarker in the sample is compared to a reference value.

73. The method of embodiment 72, wherein the reference value is determined from a sample obtained from the subject, a different subject, or a population of subjects.

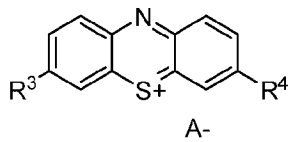
[0231] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, patent applications, and accession numbers cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, the method comprising contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

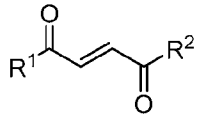
wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

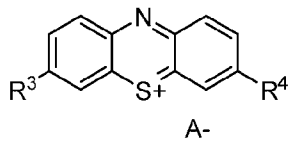
wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

2. A method for promoting and/or increasing mitochondrial biogenesis in a neural cell in a subject, the method comprising administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

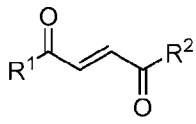
3. The method of claim 1 or 2, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell comprises increasing mitochondrial mass and/or copy number in the neural cell.

4. The method of claim 1 or 2, wherein contacting the neural cell with two or more different active agents or administering two or more different active agents produces a synergistic promotion and/or increase in mitochondrial biogenesis in the neural cell compared to when the active agents are contacted or administered alone.

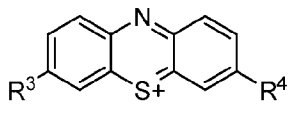
5. The method of claim 1 or 2, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases mitochondrial gene expression in the neural cell.

6. The method of claim 1 or 2, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases frataxin expression and/or activity in the neural cell.

7. A method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, the method comprising contacting the neural cell with a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

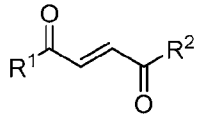
wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

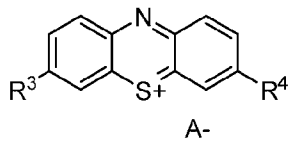
wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

8. A method for preventing or treating a disorder associated with insufficient mitochondrial function in a neural cell in a subject, the method comprising administering to the subject a therapeutically effective amount of an active agent selected from the group consisting of a compound of Formula (I):



(I), a compound of Formula (II):



(II), resveratrol, betamethasone, analogs thereof, pharmaceutically acceptable salts thereof, and combinations thereof;

wherein R^1 and R^2 are each independently selected from the group consisting of –E, $-CH_{3-n}E_n$, -OH, -O, and branched or unbranched C_{1-8} alkoxy, provided that at least one of R^1 and R^2 is C_{1-8} alkoxy,

wherein E is an electron-withdrawing group and n is 0-3; and

wherein A- is a counterion and R^3 and R^4 are each independently selected from the group consisting of hydrogen, halogen, -CN, -OH, $-NH_2$, -COOH, $-CF_3$, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-OCOCH_3$, and  ,

wherein R^5 and R^6 are each independently selected from the group consisting of hydrogen, $-OCOCH_3$, and linear or branched $C_{n'}H_{2n'}Y$, wherein n' is 1-6 and Y is selected from the group consisting of hydrogen, halogen, -OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, -CN, and $-OCOCH_3$.

9. The method of claim 7 or 8, wherein the insufficient mitochondrial function in the neural cell is associated with insufficient mitochondrial mass and/or mitochondrial copy number in the neural cell.

10. The method of claim 7 or 8, wherein the disorder is a neurodegenerative disease.

11. The method of claim 7 or 8, wherein the disorder is associated with decreased frataxin expression and/or activity in the neural cell.

12. The method of claim 7 or 8, wherein the disorder is selected from the group consisting of Friedreich's ataxia; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic epilepsy with ragged red fibers (MERRF); Leber's hereditary optic neuropathy (LHON); neuropathy, ataxia, and retinitis pigmentosa (NARP);

maternally inherited Leigh syndrome (MILS); multiple sclerosis (MS); and a combination thereof.

13. The method of claim 7 or 8, wherein the subject is not exhibiting any signs or symptoms of the disorder.

14. The method of claim 7 or 8, wherein the subject is exhibiting one or more signs or symptoms of the disorder.

15. The method of claim 7 or 8, wherein contacting the neural cell with the active agent or administering the active agent promotes and/or increases mitochondrial biogenesis in the neural cell.

16. The method of claim 15, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell comprises increasing mitochondrial mass and/or copy number in the neural cell.

17. The method of claim 15, wherein contacting the neural cell with two or more different active agents or administering two or more different active agents produces a synergistic promotion and/or increase in mitochondrial biogenesis in the neural cell compared to when the active agents are contacted or administered alone.

18. The method of claim 15, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases mitochondrial gene expression in the neural cell.

19. The method of claim 15, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell increases frataxin expression and/or activity in the neural cell.

20. The method of claim 15, wherein promoting and/or increasing mitochondrial biogenesis in the neural cell prevents, delays, reduces, mitigates, ameliorates, and/or inhibits one or more signs or symptoms associated with the disorder.

21. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof.

22. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof.

23. The method of claim 1, 2, 7, or 8, wherein the active agent comprises resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

24. The method of claim 1, 2, 7, or 8, wherein the active agent comprises betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

25. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof.

26. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

27. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

28. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

29. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

30. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

31. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof.

32. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

33. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

34. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

35. The method of claim 1, 2, 7, or 8, wherein the active agent comprises a combination of a compound of Formula (I), an analog thereof, or a pharmaceutically acceptable salt thereof; a compound of Formula (II), an analog thereof, or a pharmaceutically acceptable salt thereof; resveratrol, an analog thereof, or a pharmaceutically acceptable salt thereof; and betamethasone, an analog thereof, or a pharmaceutically acceptable salt thereof.

36. The method of claim 1, 2, 7, or 8, wherein E is selected from the group consisting of $-NO_2$, $-N(R^7{}_2)$, $-N(R^7{}_3)^+$, $-NH_3^+$, $-SO_3H$, $-SO_3R^8$, $-S(O_2)R^8$ (sulfone), $-S(O)R^8$ (sulfoxide), $-S(O_2)NH_2$ (sulfonamide), $-SO_2NHR^8$, $-SO_2NR^8{}_2$, $-PO(OR^8)_2$, $-PO_3H_2$, -

$PO(NR^8_2)_2$, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, pyrazolyl, indazolyl, imidazolyl, thiazolyl, benzothiazolyl, oxazolyl, benzimidazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, triazolyl, benzotriazolyl, quinolinyl, isoquinolinyl, quinazolinyl, pyrimidinyl, a 5- or 6-membered heteroaryl with a C-N double bond optionally fused to a 5- or 6-membered heteroaryl, pyridinyl N-oxide , C≡N, $-CX_3$, -C(O)X, -COOH, $-COOR^8$, $-C(O)R^8$, $-C(O)NH_2$, $-C(O)NHR^8$, $-C(O)NR^8_2$, -C(O)H, $-P(O)(OR^8)OR^9$, and X,

wherein X is a halogen and R^7, R^8, and R^9 are each independently selected from the group consisting of hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

37. The method of claim 1, 2, 7, or 8, wherein A- is an anion, a dianion, or a trianion.

38. The method of claim 37, wherein A- is selected from the group consisting of Cl^-, Br^-, I^-, F^-, NO_3^-, $CH_3SO_3^-$, HSO_4^-, $CHCO_2^-$ SO_4^{2-}, HPO_4^{2-}, and PO_4^{3-}.

39. The method of claim 1, 2, 7, or 8, wherein the halogen is independently selected from the group consisting of F, Cl, Br, and I.

40. The method of claim 1, 2, 7, or 8, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a fumarate ester.

41. The method of claim 1, 2, 7, or 8, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of monomethyl fumarate (MMF), monomethyl maleate, monoethyl fumarate, monoethyl maleate, monobutyl fumarate, monobutyl maleate, monooctyl fumarate, monooctyl maleate, mono (phenylmethyl) fumarate, mono (phenylmethyl) maleate, mono (2-hydroxypropyl) fumarate, mono (2-hydroxypropyl) maleate, mono (2-ethylhexyl) fumarate, mono (2-ethylhexyl) maleate, dimethyl fumarate (DMF), dimethyl maleate, diethyl fumarate, diethyl maleate, dipropyl fumarate, dipropyl maleate, diisopropyl fumarate, diisopropyl maleate, dibutyl fumarate, dibutyl maleate, diisobutyl fumarate, diisobutyl maleate, diheptyl fumarate, diheptyl maleate, bis(2-ethylhexyl) fumarate, bis(2-ethylhexyl) maleate, (-)-dimethyl fumarate, (-)-bis((S)-l-(ethoxycarbonyl)ethyl) fumarate, (-)-bis((S)-l-

(ethoxycarbonyl)ethyl) maleate, bis(2-trifluoroethyl) fumarate, bis(2-trifluoroethyl) maleate, and a combination thereof.

42. The method of claim 41, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises dimethyl fumarate (DMF).

43. The method of claim 1, 2, 7, or 8, wherein the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof is provided as a prodrug.

44. The method of claim 43, wherein the prodrug is selected from the group consisting of O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate, and a combination thereof.

45. The method of claim 1, 2, 7, or 8, wherein the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 2,000 mg per day.

46. The method of claim 45, wherein the therapeutically effective amount of the compound of Formula (I), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of about 120 mg, about 240 mg, about 360 mg, about 480 mg, about 600 mg, or about 720 mg per day.

47. The method of claim 1, 2, 7, or 8, wherein the compound of Formula (I) is DMF, and the therapeutically effective amount comprises a dose of between about 10 and about 160 mg/kg of body weight per day.

48. The method of claim 47, wherein the therapeutically effective amount comprises a dose of between about 40 and about 160 mg/kg of body weight per day.

49. The method of claim 1, 2, 7, or 8, wherein the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, leuco-methylene blue, acetyl-methylene blue, leuco-methylonium bis(hydromethanesulfonate) (LMTM), diaminophenothiazine, 2-chlorophenothiazine, phenothiazine, toluidine blue, tolonium chloride, toluidine blue O, seleno toluidine blue, methylene green, chlorpromazine, sulphoxide chlorpromazine,

sulphone chlorpromazine, chlordiethazine promethazine, thioproperazine, prochlorperazine, pipotiazine, dimetotiazine, propericiazine, metazionic acid, oxomemazine neutral red, iminostilbene, imipramine, and a combination thereof.

50. The method of claim 49, wherein the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof is selected from the group consisting of methylene blue, tolonium chloride, leuco-methylonium bis(hydromethanesulfonate) (LMTM), and a combination thereof.

51. The method of claim 1, 2, 7, or 8, wherein the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of between about 1 mg and about 300 mg per day.

52. The method of claim 51, wherein the therapeutically effective amount of the compound of Formula (II), analog thereof, or pharmaceutically acceptable salt thereof comprises a dose of about 75 mg or about 125 mg, contacted with the cell or administered twice per day.

53. The method of claim 1, 2, 7, or 8, wherein the therapeutically effective amount of resveratrol comprises a dose of between about 0.5 grams and about 10 grams per day.

54. The method of claim 53, wherein the therapeutically effective amount of resveratrol comprises a dose of between about 1 gram and about 5 grams per day.

55. The method of claim 1, 2, 7, or 8, wherein the therapeutically effective amount of betamethasone comprises a dose of between about 1 mg and about 30 mg per day.

56. The method of claim 1, 2, 7, or 8, wherein two or more different active agents are contacted with the neural cell or administered concomitantly.

57. The method of claim 1, 2, 7, or 8, wherein two or more different active agents are contacted with the neural cell or administered sequentially.

58. The method of claim 1, 2, 7, or 8, wherein the method further comprises contacting the neural cell with a delivery-enhancing agent or administering to the subject a delivery-enhancing agent.

59. The method of claim 58, wherein the delivery-enhancing agent is selected from the group consisting of a cyclodextrin, a hepatitis E virus-like particle, an inactivated yeast, an inactivated bacterium, polyvinyl acetate (PVA), an inulin or an ester thereof, and a combination thereof.

60. The method of claim 59, wherein the cyclodextrin is selected from the group consisting of an α-cyclodextrin, a β-cyclodextrin, a γ-cyclodextrin, a salt thereof, a derivative thereof, and a combination thereof.

61. The method of claim 60, wherein the β-cyclodextrin is selected from the group consisting of a hydroxypropyl-β-cyclodextrin, an endotoxin controlled β-cyclodextrin sulfobutyl ether, a β-cyclodextrin sulfobutyl ether, a sodium salt thereof, an anionic derivative thereof, and a combination thereof.

62. The method of claim 59, wherein the cyclodextrin is selected from the group consisting of a hepta-substituted sulfobutyl-ether-β-cyclodextrin mixture, betadex-sulfobutyl-ether-β-cyclodextrin sodium salt, and a combination thereof.

63. The method of claim 59, wherein the cyclodextrin is contacted with the neural cell or administered at a concentration of between about 1 mg/mL and about 300 mg/mL.

64. The method of claim 1, 2, 7, or 8, wherein the method further comprises contacting the neural cell with a pharmaceutically acceptable carrier or administering to the subject a pharmaceutically acceptable carrier.

65. The method of claim 1, 2, 7, or 8, wherein a sample is obtained from the subject before and/or after the active agent is contacted with the neural cell or administered to the subject.

66. The method of claim 65, wherein the sample comprises blood, tissue, or a combination thereof.

67. The method of claim 66, wherein the tissue sample comprises neural tissue.

68. The method of claim 66, wherein the tissue sample comprises normal or abnormal tissue.

69. The method of claim 66, wherein the sample is an abnormal blood and/or tissue sample that comprises a lower mitochondrial mass and/or number compared to a normal blood and/or tissue sample.

70. The method of claim 65, wherein the presence or level of a biomarker is determined in the sample.

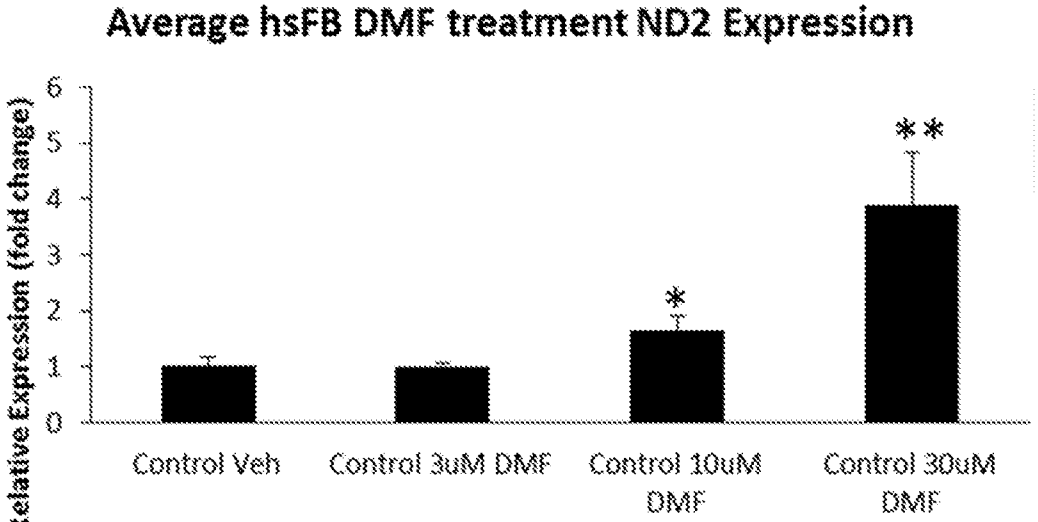
71. The method of claim 70, wherein the biomarker comprises frataxin, COX4, or a combination thereof.

72. The method of claim 70, wherein the presence or level of the biomarker in the sample is compared to a reference value.

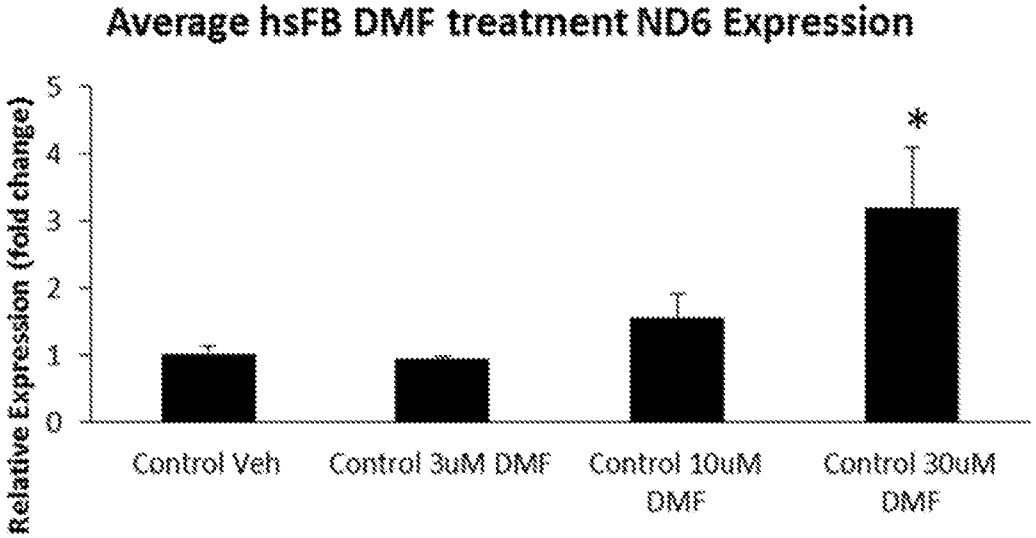
73. The method of claim 72, wherein the reference value is determined from a sample obtained from the subject, a different subject, or a population of subjects.

FIG. 1

Complex 1



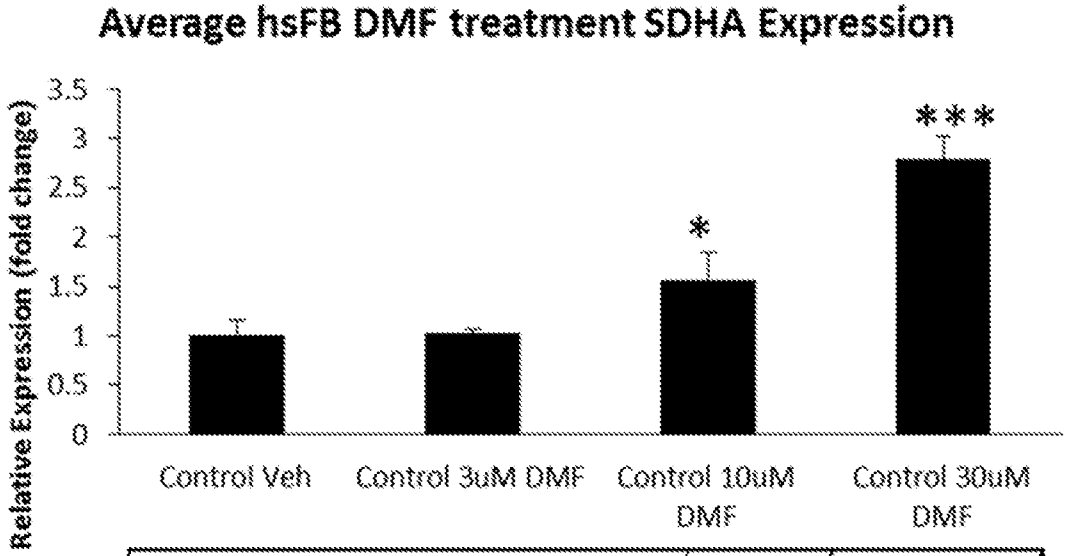
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.9755	NO
Control Veh-Control 10uM DMF	0.0285	YES
Control Veh-Control 30uM DMF	0.0068	YES



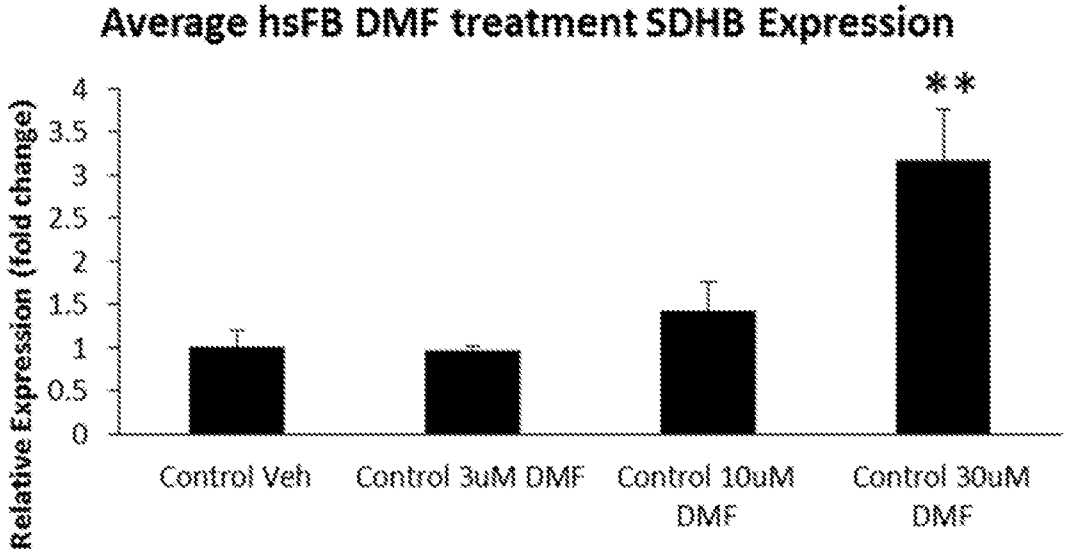
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.3827	NO
Control Veh-Control 10uM DMF	0.0568	NO
Control Veh-Control 30uM DMF	0.0132	YES

FIG. 1 (cont'd)

Complex 2



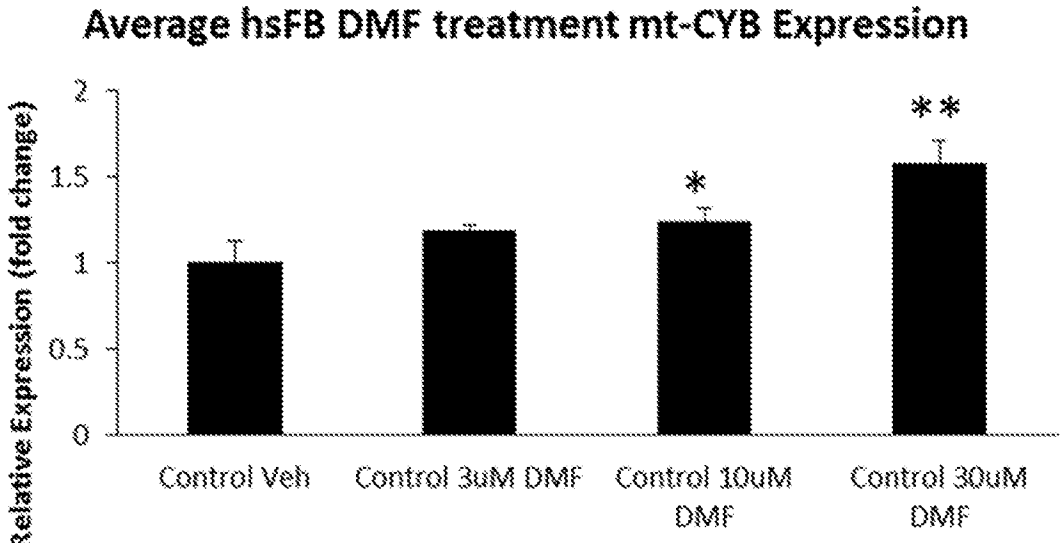
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.9186	NO
Control Veh-Control 10uM DMF	0.0449	YES
Control Veh-Control 30uM DMF	0.0004	YES



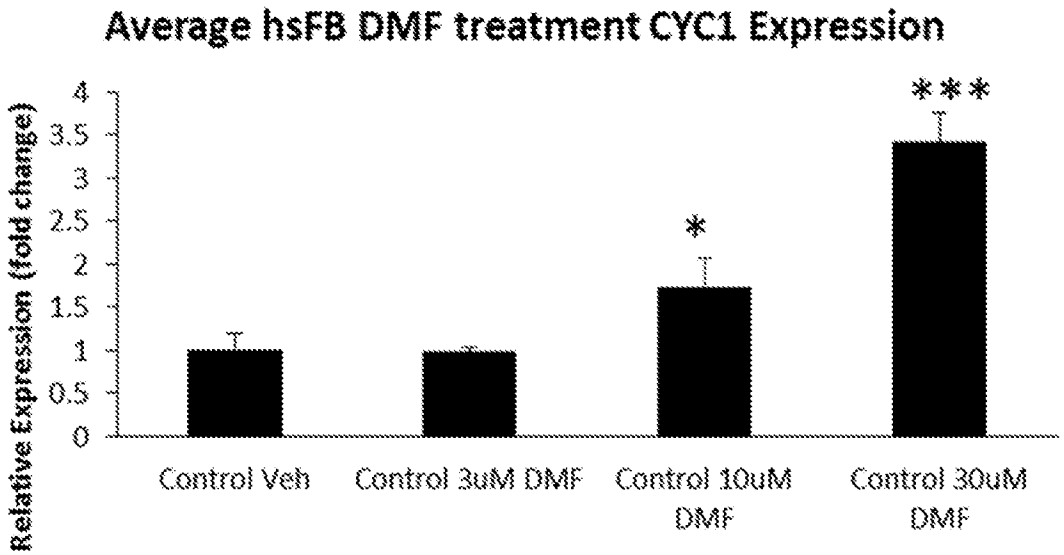
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.6849	NO
Control Veh-Control 10uM DMF	0.1183	NO
Control Veh-Control 30uM DMF	0.0041	YES

FIG. 1 (cont'd)

Complex 3



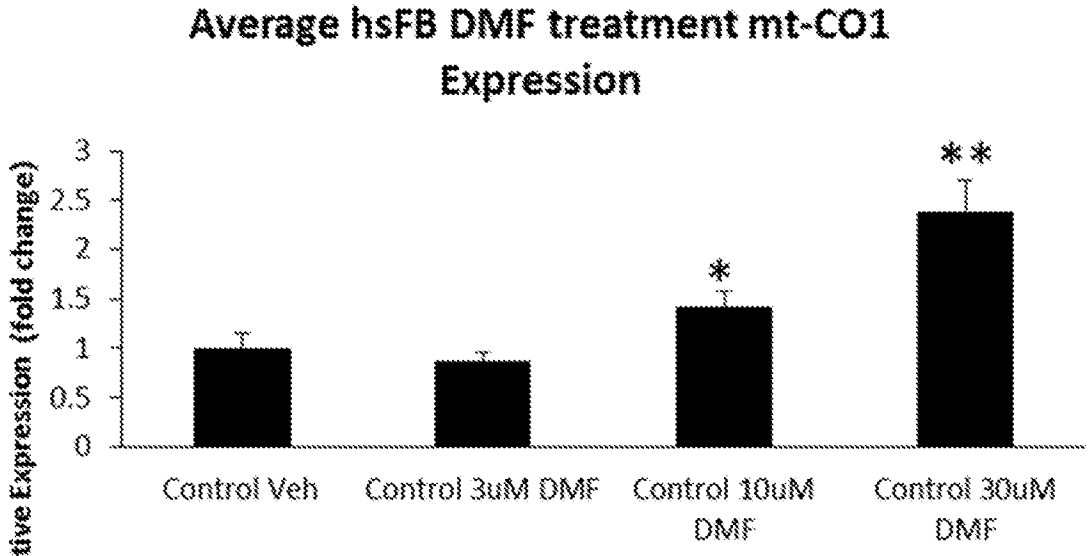
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.0735	NO
Control Veh-Control 10uM DMF	0.0467	YES
Control Veh-Control 30uM DMF	0.0047	YES



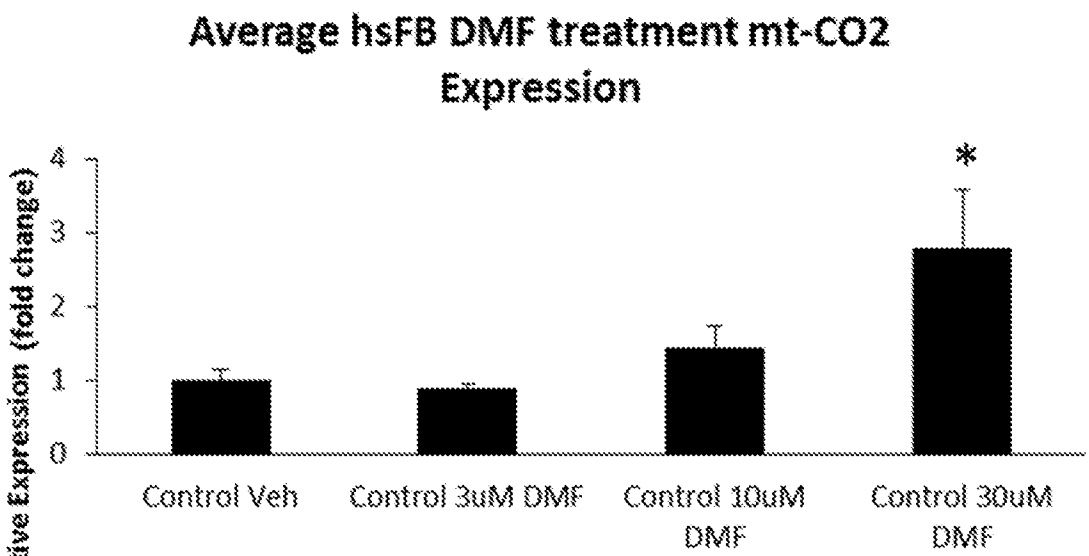
Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.8405	NO
Control Veh-Control 10uM DMF	0.0267	YES
Control Veh-Control 30uM DMF	0.0004	YES

FIG. 1 (cont'd)

Complex 4



Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.2321	NO
Control Veh-Control 10uM DMF	0.0286	YES
Control Veh-Control 30uM DMF	0.0023	YES

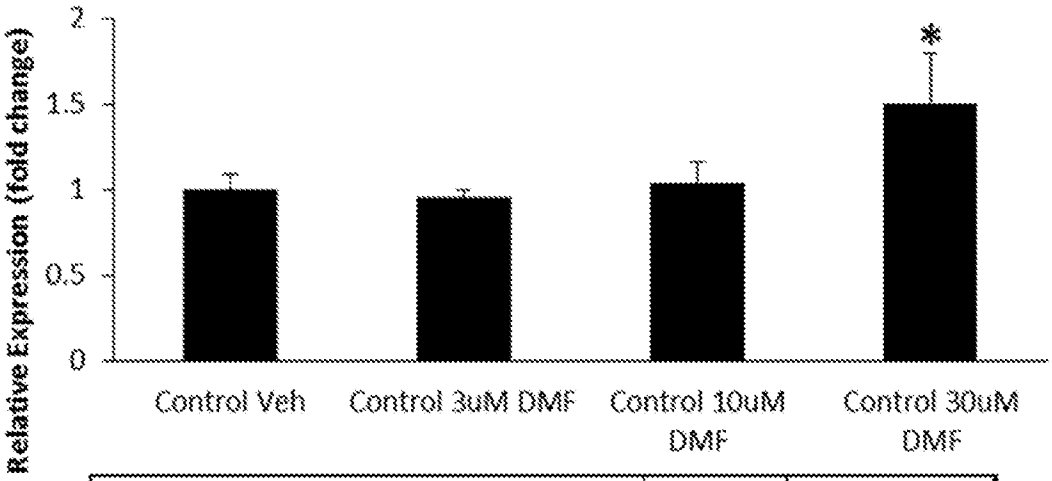


Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.2615	NO
Control Veh-Control 10uM DMF	0.0911	NO
Control Veh-Control 30uM DMF	0.0180	YES

FIG. 1 (cont'd)

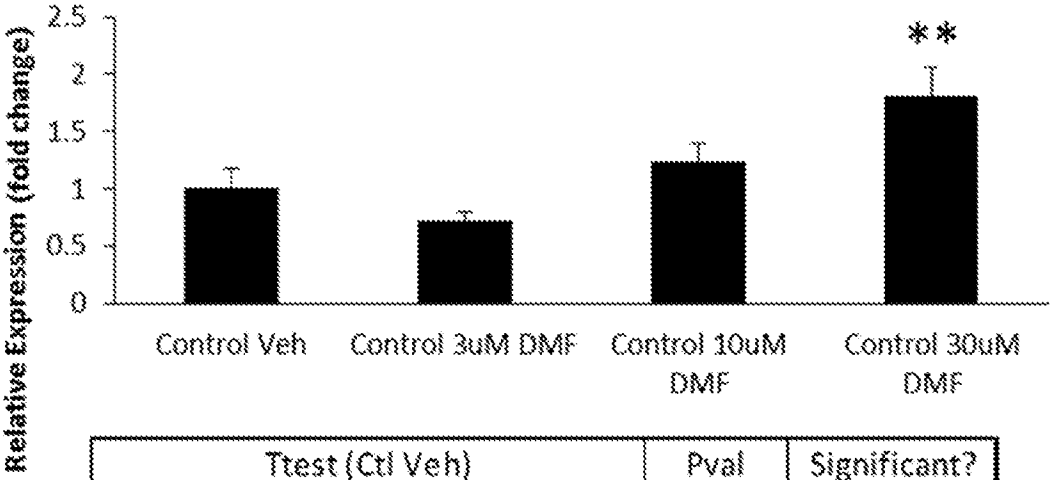
ATP synthase

Average hsFB DMF treatment ATP5B expression



Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.4532	NO
Control Veh-Control 10uM DMF	0.7007	NO
Control Veh-Control 30uM DMF	0.0469	YES

Average hsFB DMF treatment mt-ATP6 expression



Ttest (Ctl Veh)	Pval	Significant?
Control Veh-Control 3uM DMF	0.0533	NO
Control Veh-Control 10uM DMF	0.1597	NO
Control Veh-Control 30uM DMF	0.0092	YES

FIG. 2A

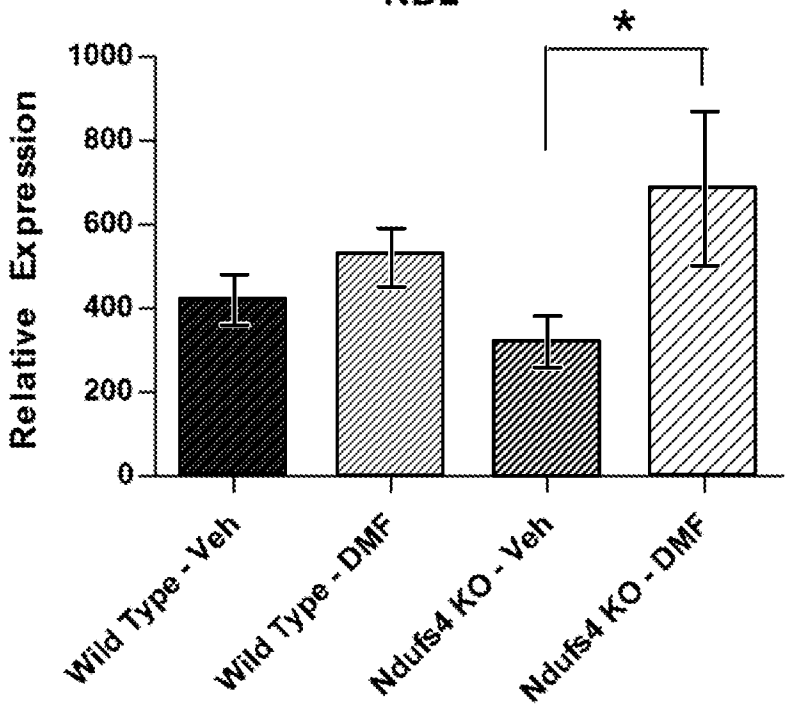

ND2
*
Relative Expression
1000
800
600
400
200
0
Wild Type - Veh
Wild Type - DMF
Ndufs4 KO - Veh
Ndufs4 KO - DMF

FIG. 2B

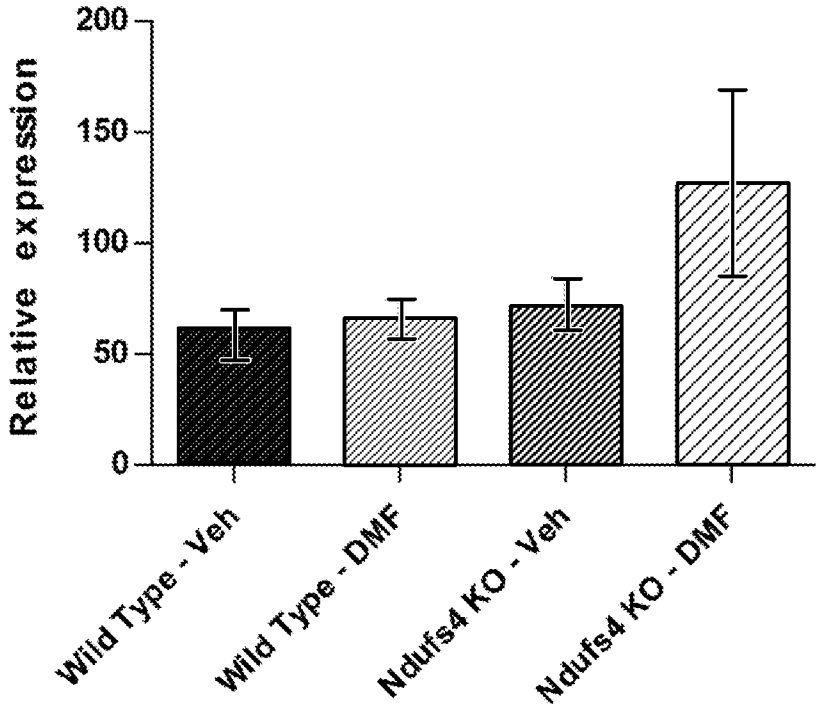
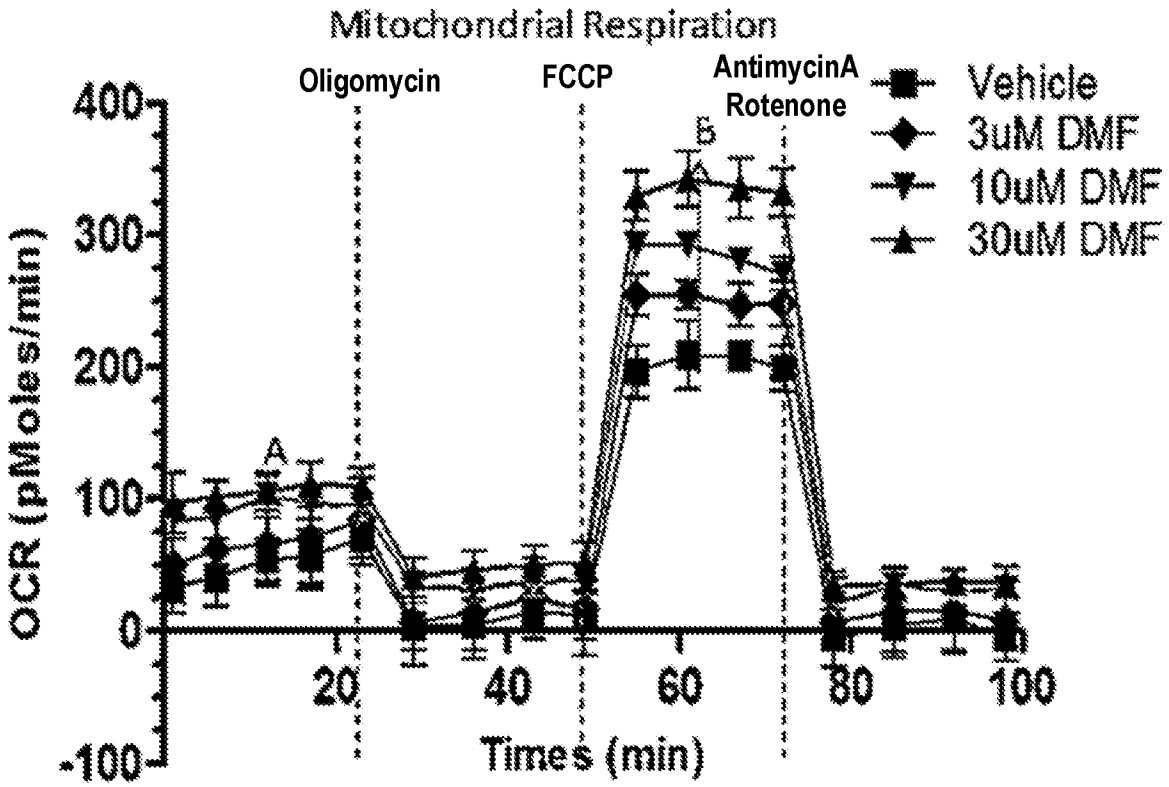

ND6
Relative expression
200
150
100
50
0
Wild Type - Veh
Wild Type - DMF
Ndufs4 KO - Veh
Ndufs4 KO - DMF

FIG. 3



Max Mito Resp (FCCP)	Veh	3uM DMF	10uM DMF	30uM DMF
Average	204	252	274	329
Drug/Veh	1	1.235	1.348	1.616
t-test to vehicle (p value)		0.00451	0.00183	0.00028

FIG. 4

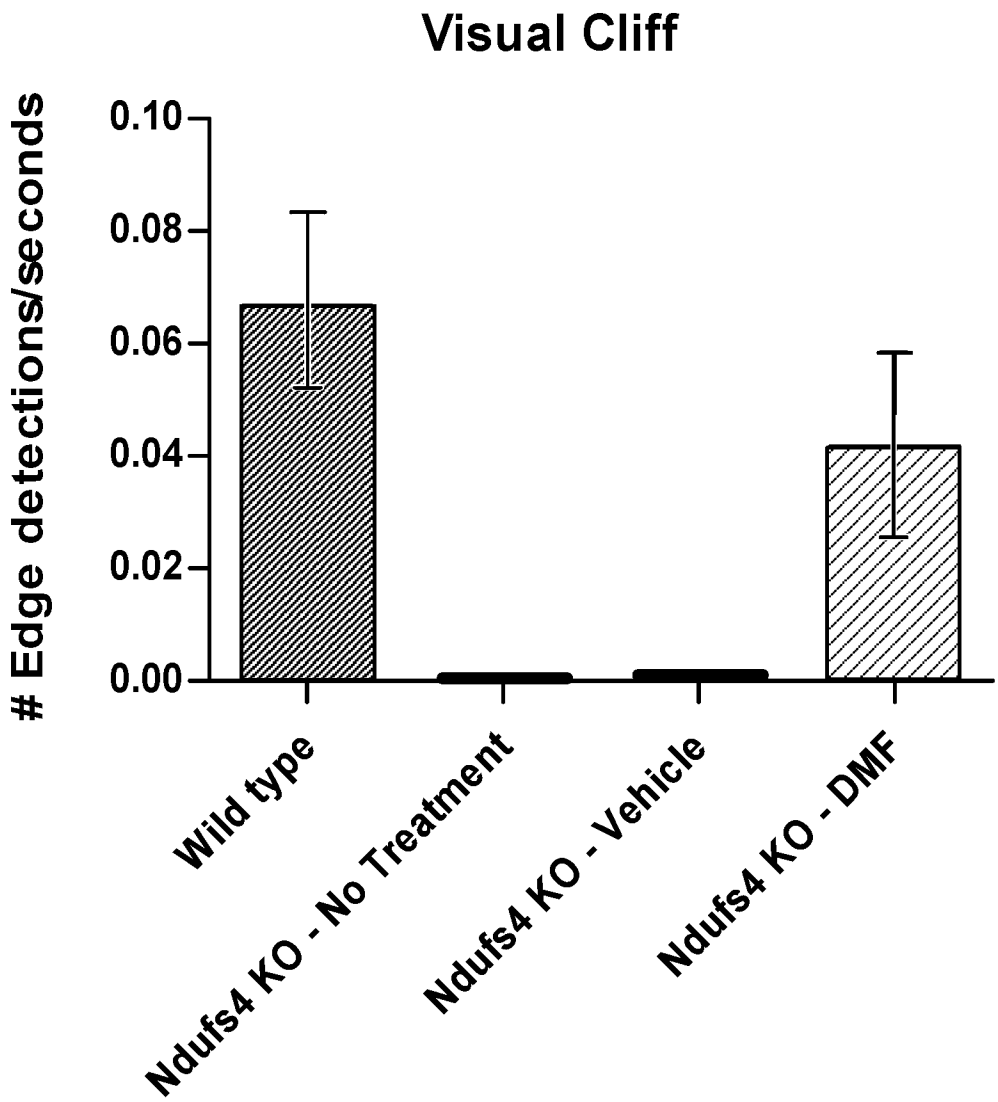

Visual Cliff
Edge detections/seconds
0.10
0.08
0.06
0.04
0.02
0.00
Wild type
Ndufs4 KO - No Treatment
Ndufs4 KO - Vehicle
Ndufs4 KO - DMF

FIG. 5

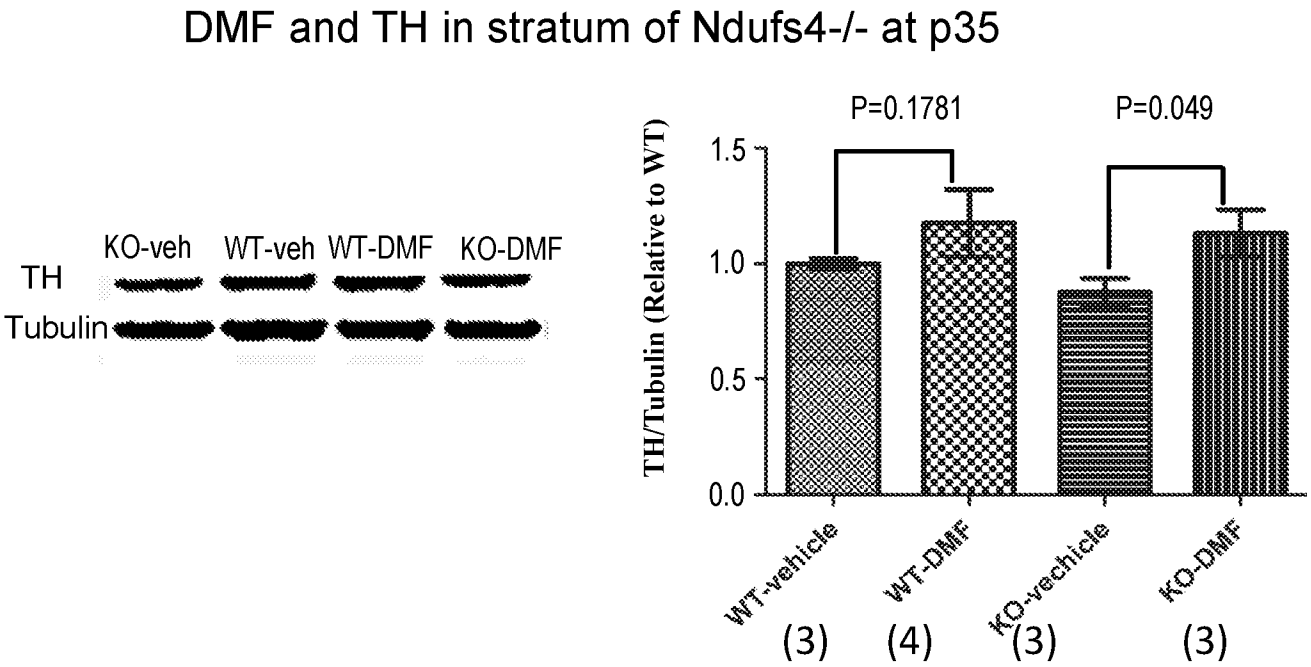

DMF and TH in stratum of Ndufs4-/- at p35
KO-veh
WT-veh
WT-DMF
KO-DMF
TH
Tubulin
P=0.1781
P=0.049
TH/Tubulin (Relative to WT)
1.5
1.0
0.5
0.0
WT-vehicle
WT-DMF
KO-vechicle
KO-DMF
(3)
(4)
(3)
(3)

FIG. 6A

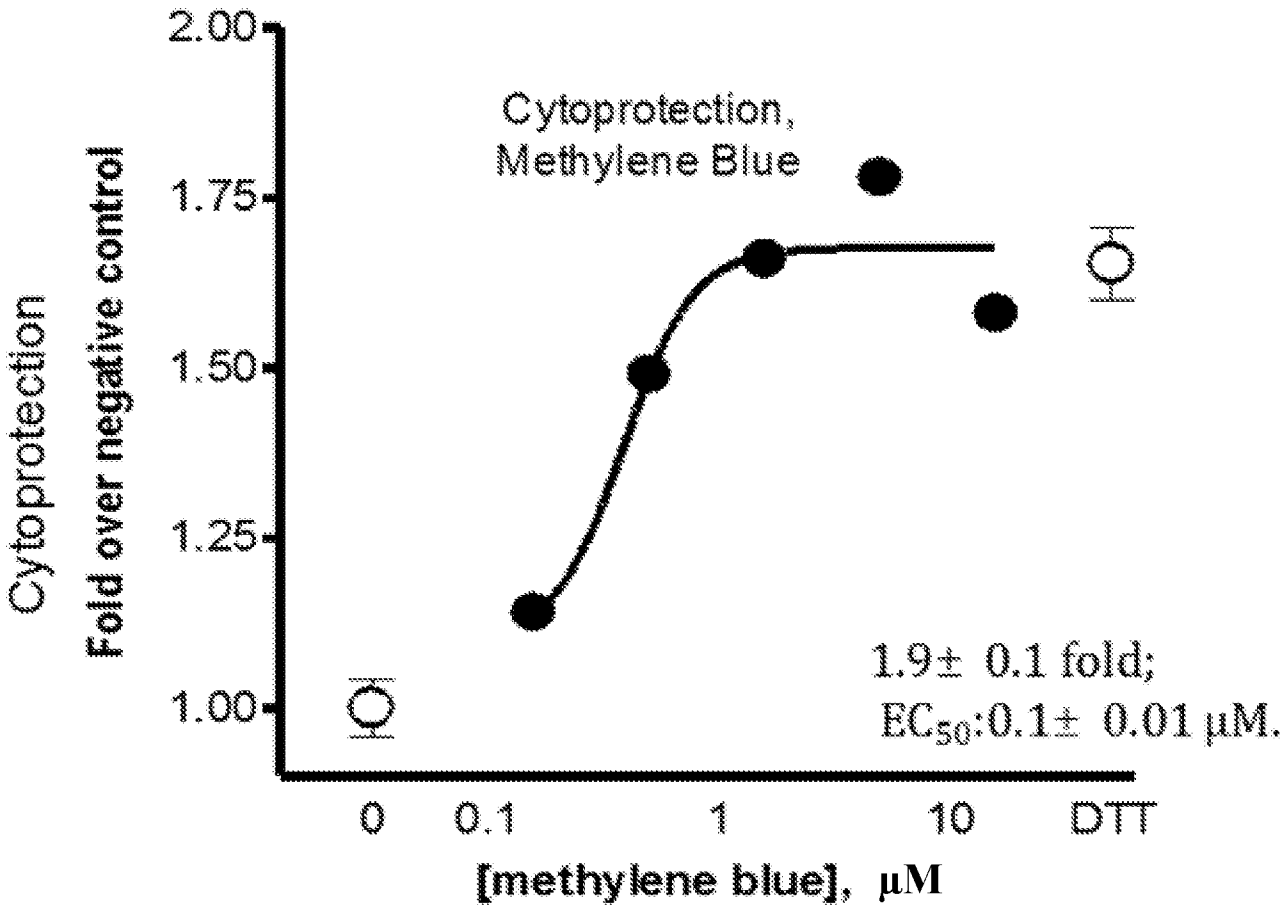
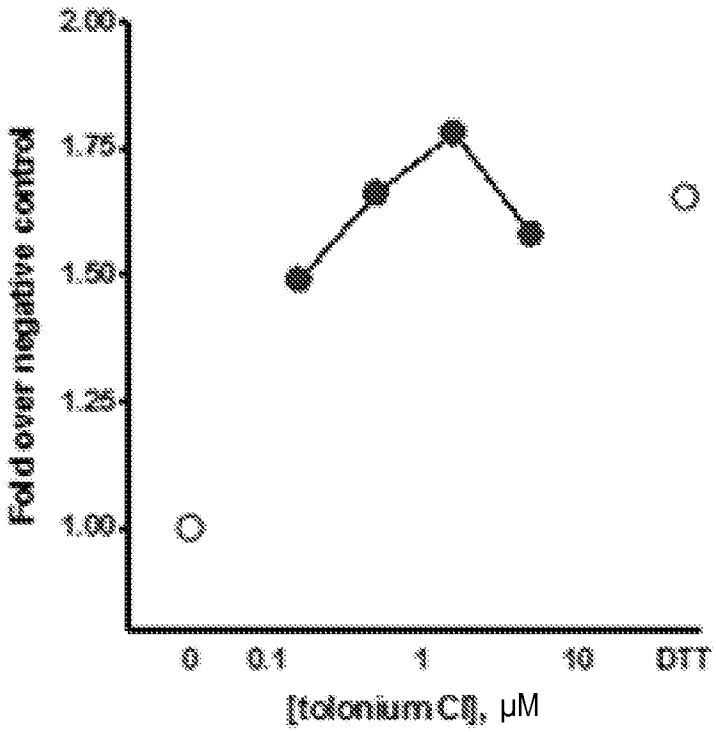

Cytoprotection
Fold over negative control
2.00
1.75
1.50
1.25
1.00
Cytoprotection,
Methylene Blue
1.9± 0.1 fold;
EC50:0.1± 0.01 µM.
0
0.1
1
10
DTT
[methylene blue], µM

FIG. 6B



MB analog tolonium also cytoprotects

FIG. 7

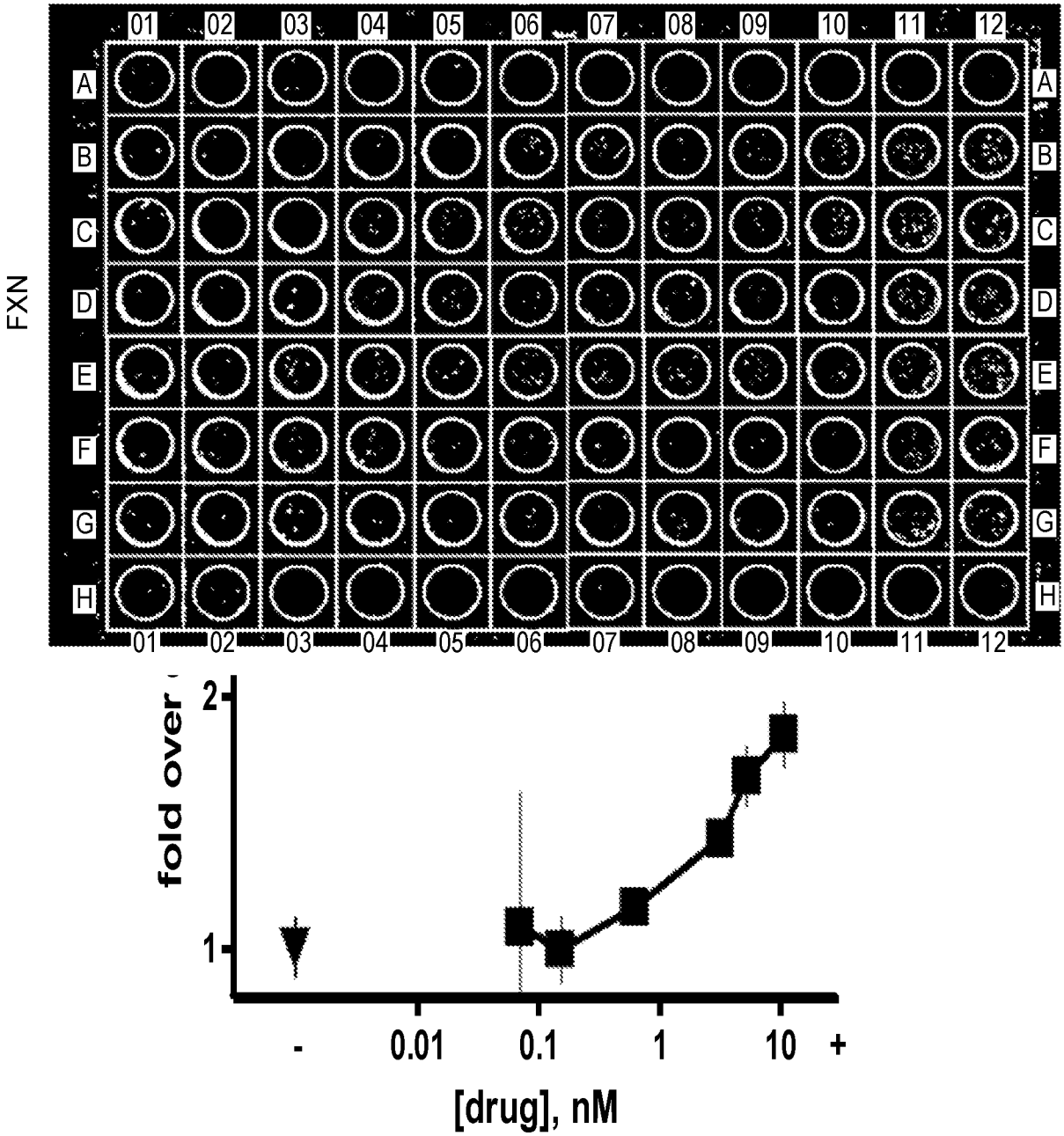

FXN
01 02 03 04 05 06 07 08 09 10 11 12
A B C D E F G H
fold over
2
1
-
0.01
0.1
1
10
+
[drug], nM

FIG. 8

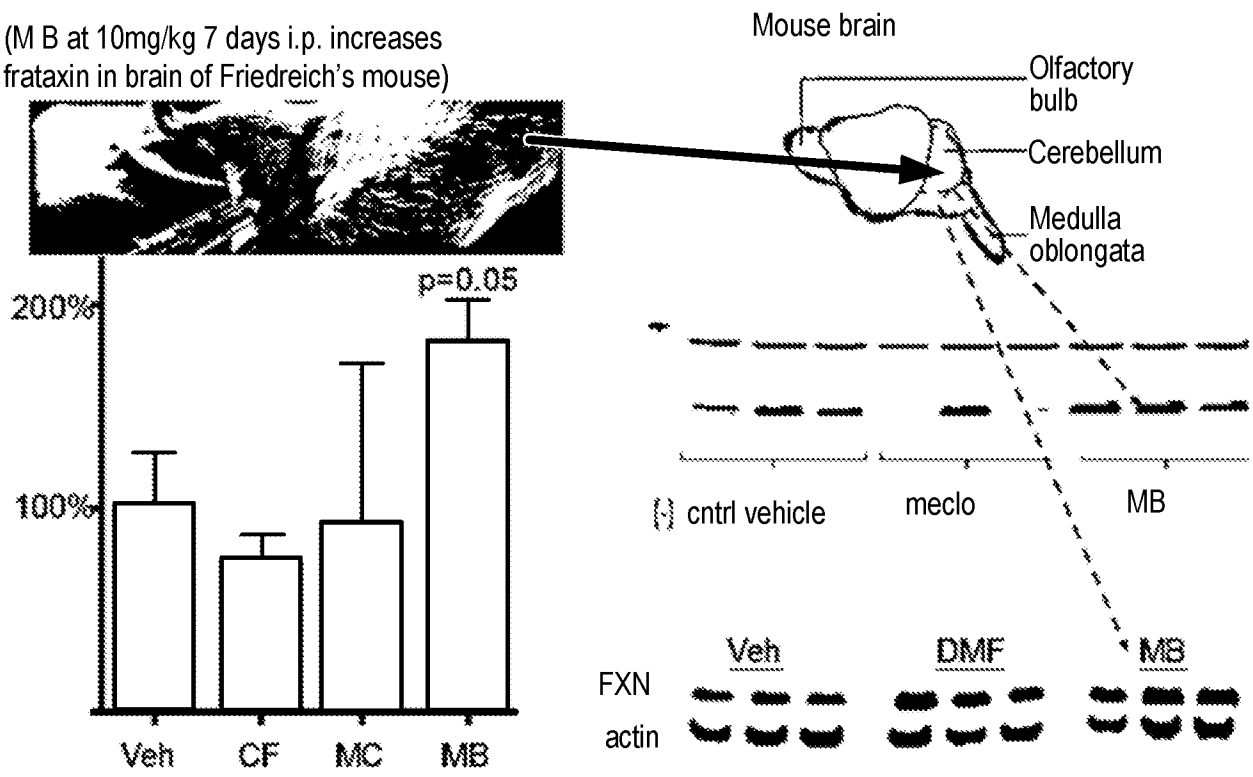

(M B at 10mg/kg 7 days i.p. increases frataxin in brain of Friedreich's mouse)
Mouse brain
Olfactory bulb
Cerebellum
Medulla oblongata
p=0.05
200%
100%
Veh
CF
MC
MB
(-) cntrl vehicle
meclo
MB
Veh
DMF
MB
FXN
actin

FIG. 9A

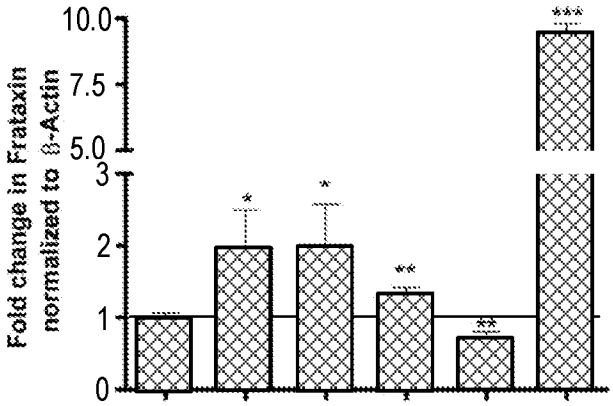


FIG. 9B

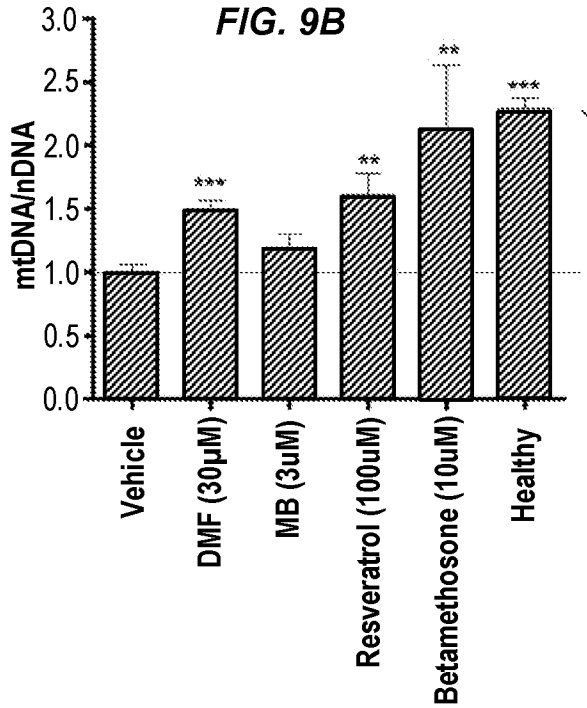


FIG. 9C

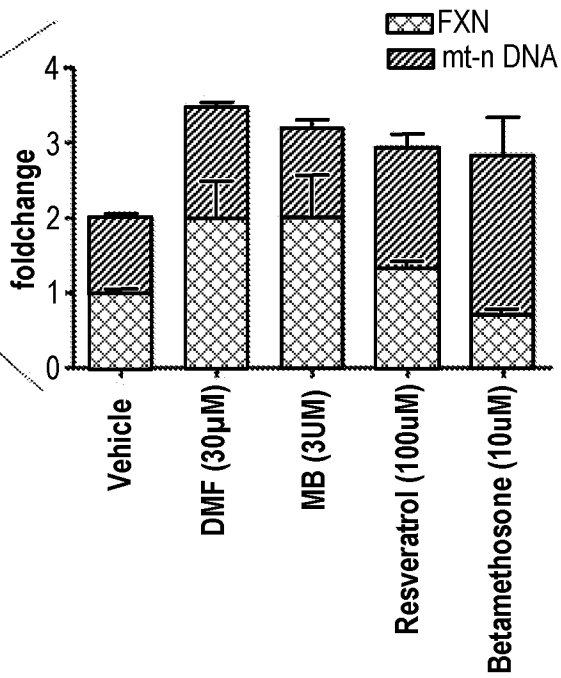


FIG. 10A

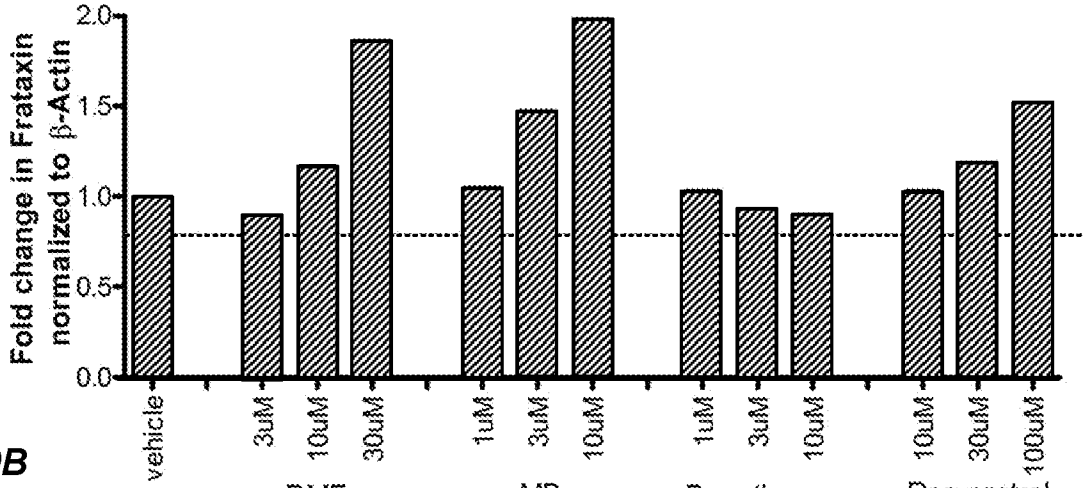


FIG. 10B

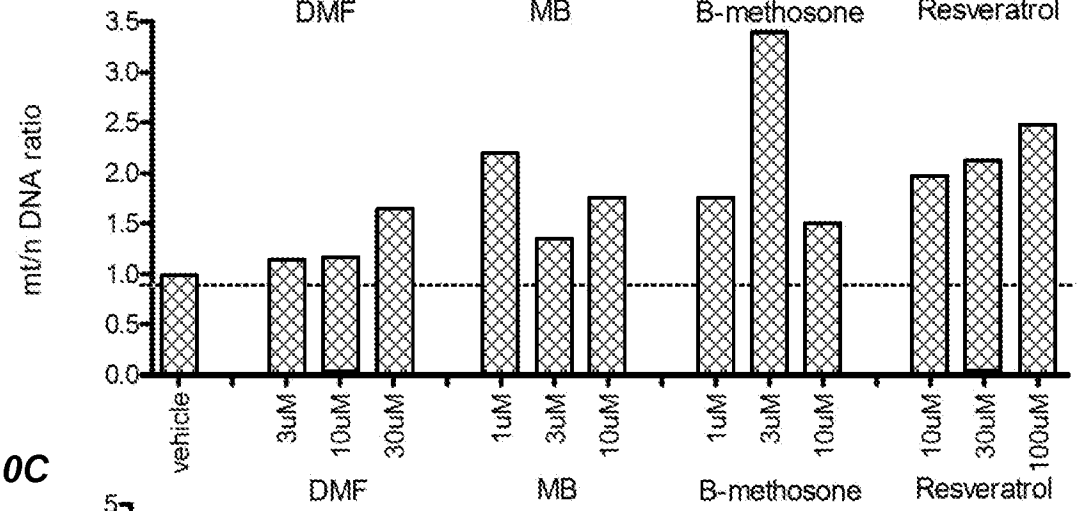


FIG. 10C

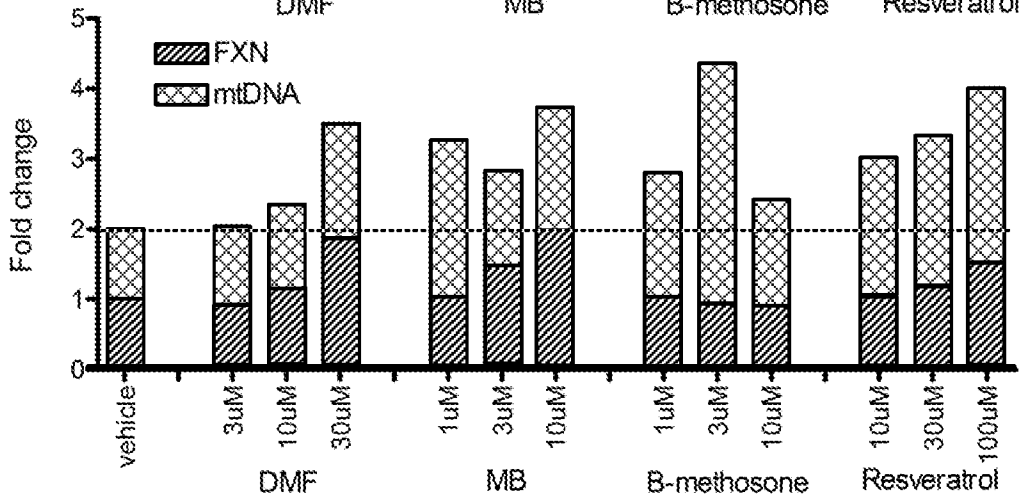


FIG. 11A

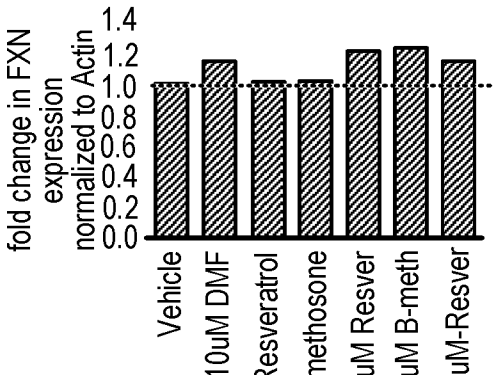


FIG. 11B

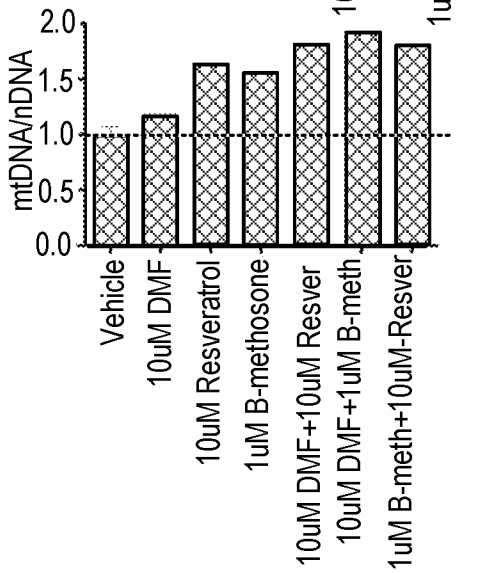


FIG. 11C

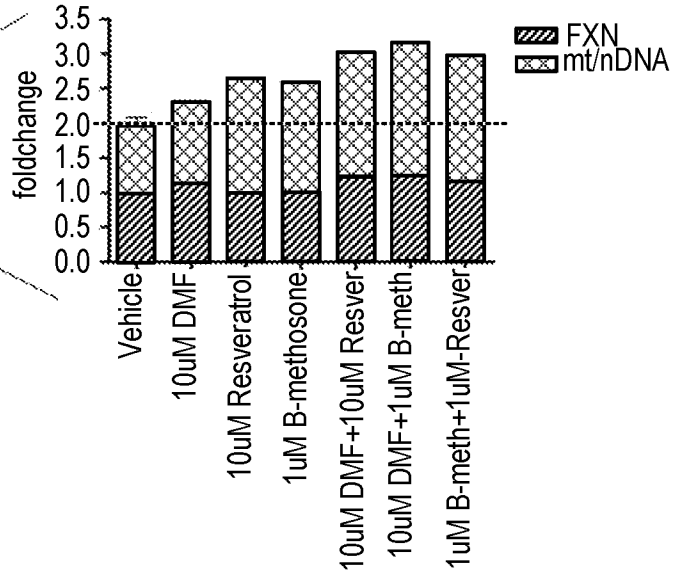


FIG. 12

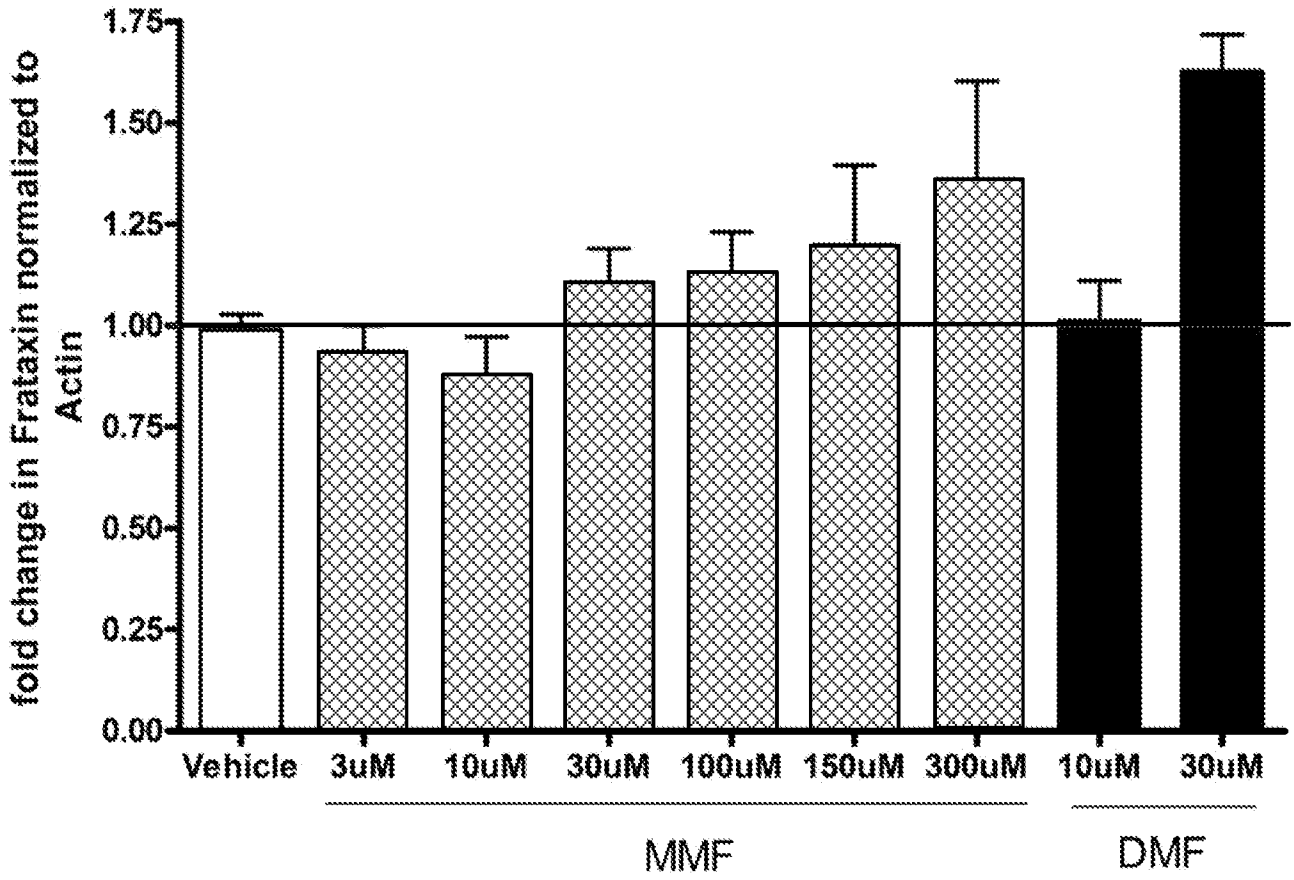

fold change in Frataxin normalized to Actin
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Vehicle
3uM
10uM
30uM
100uM
150uM
300uM
10uM
30uM
MMF
DMF

FIG. 13

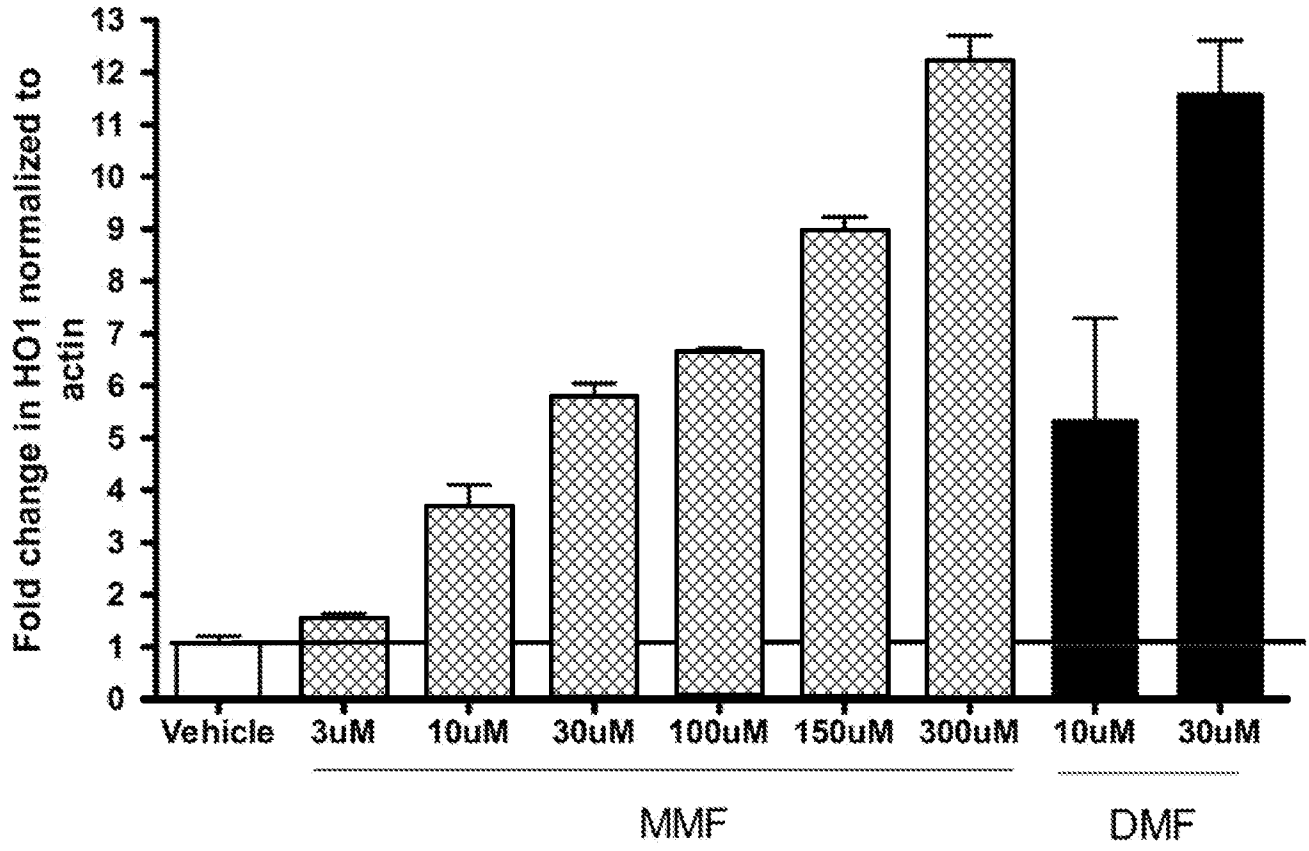
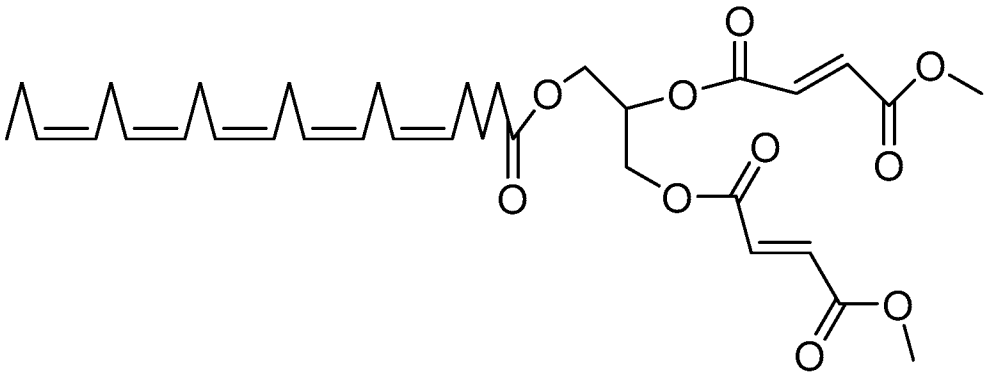
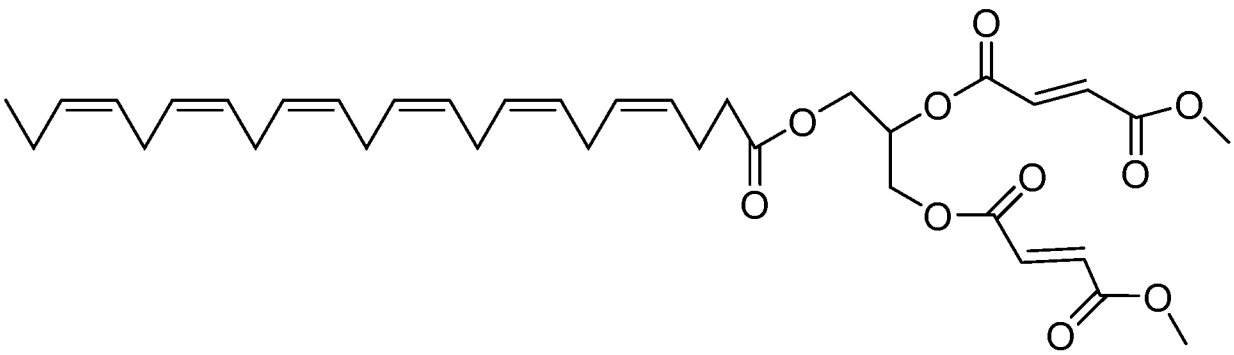

Fold change in HO1 normalized to actin
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Vehicle
3uM
10uM
30uM
100uM
150uM
300uM
10uM
30uM
MMF
DMF

FIG. 14A



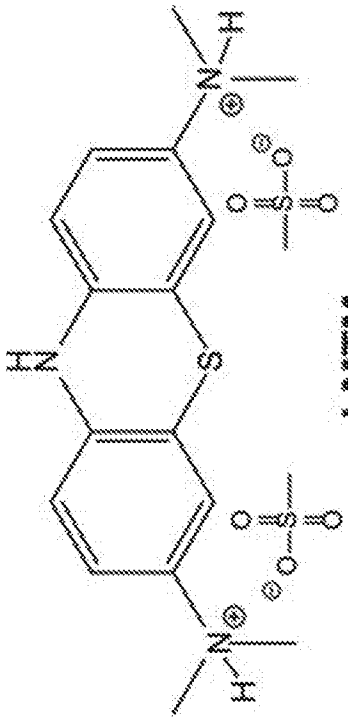
O,O'-(3-(((5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate

FIG. 14B



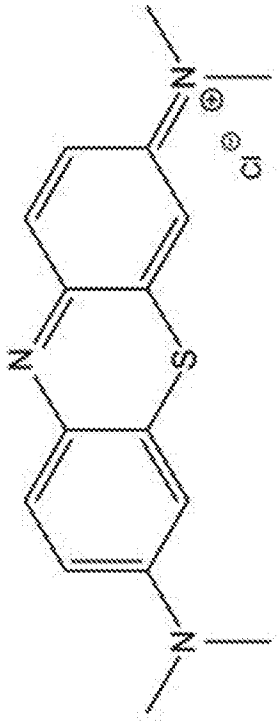
O,O'-(3-(((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoyl)oxy)propane-1,2-diyl)dimethyl difumarate

FIG. 15B



LMTM

FIG. 15A



MTC

FIG. 16A

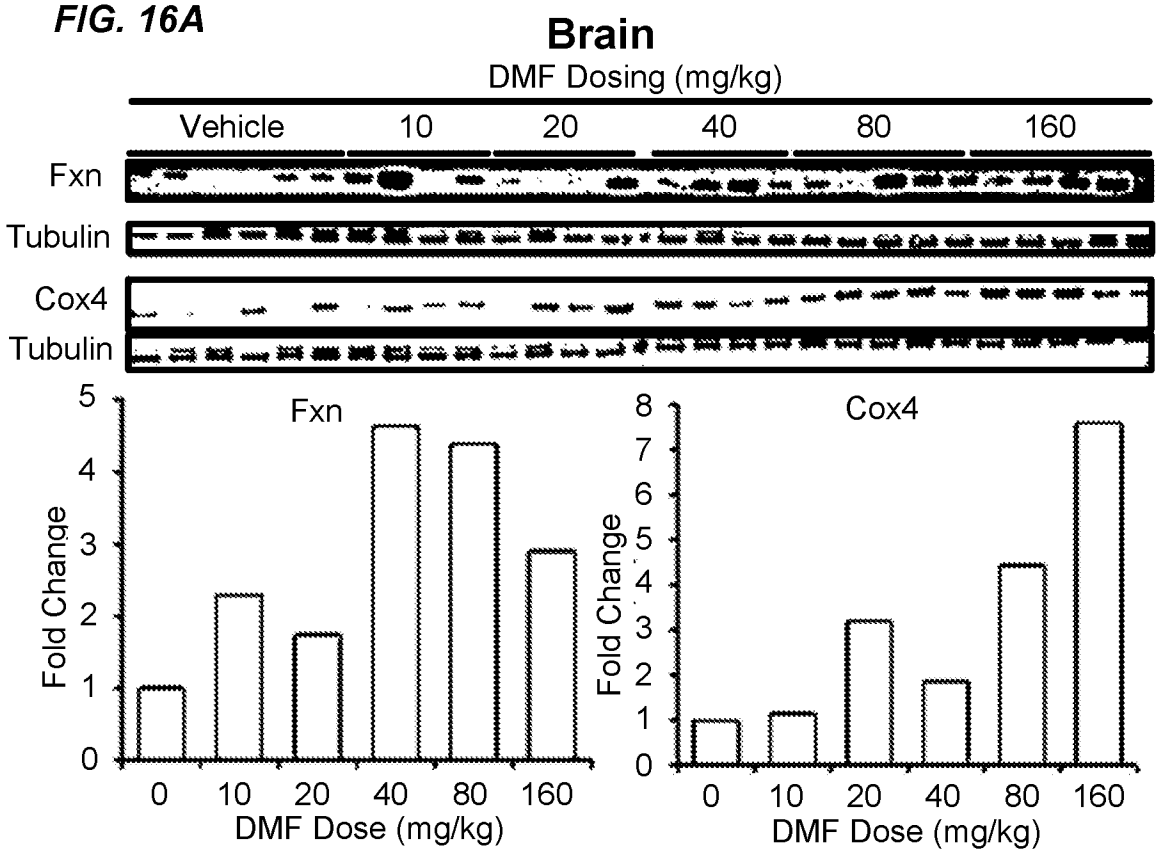
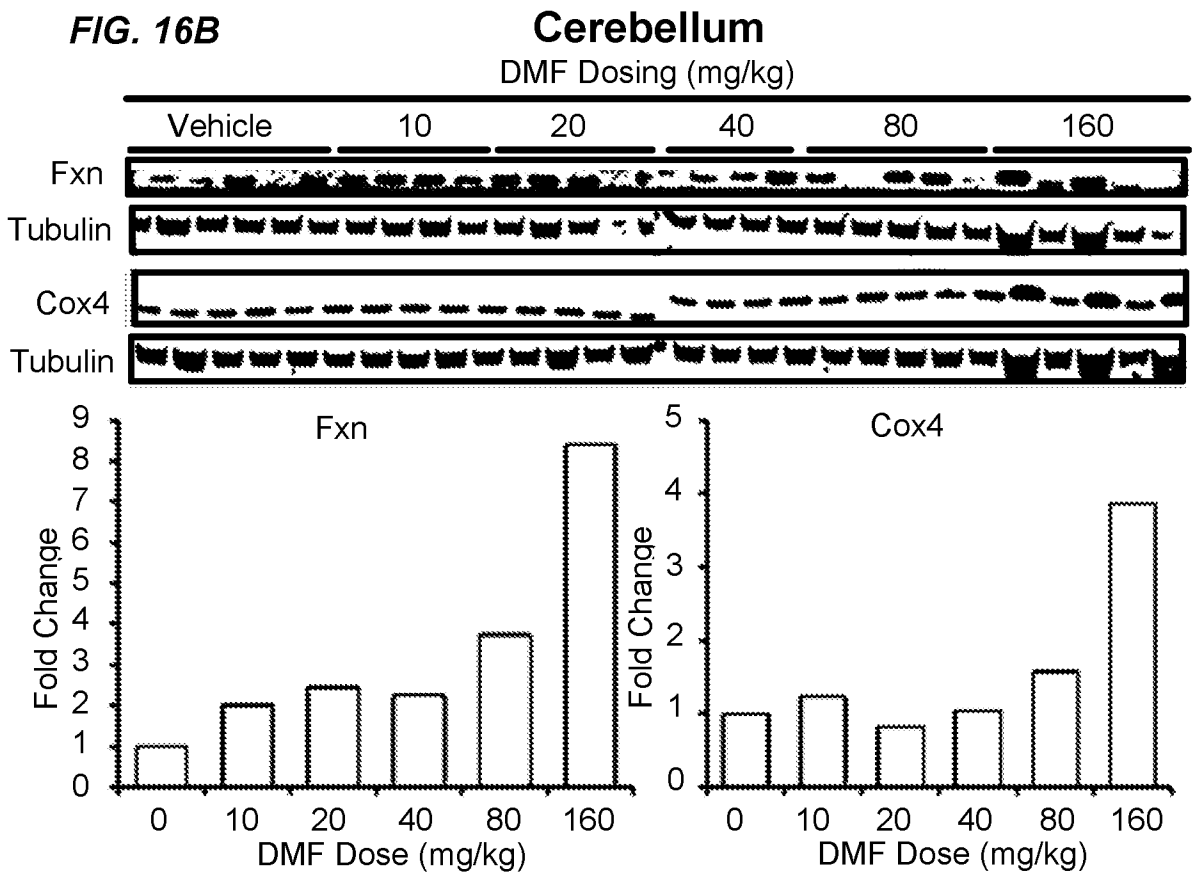


FIG. 16B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/13494

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 31/05; A61P 3/04 (2019.01)
CPC - A61K 31/05; A61P 3/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2 613 141 A1 (SIRTRIS PHARMACEUTICALS, INC., US) 18 January 2007; abstract; page 2, lines 30-32; page 4, lines 1-5; page 8, lines 10-25; page 135, lines 25-30; page 136, lines 25-30; page 141, lines 20-30; page 147, lines 1-5; page 151, lines 1-15; page 152, lines 5-25; page 153, lines 5-10; page 154, lines 30-32; page 155, lines 1-20; page 169, lines 10-15; page 174, lines 15-25; page 175, lines 1-10, 20-25; page 176, lines 25-30; page 193, lines 20-25; page 194, lines 20-30; page 199, lines 25-30; page 205, lines 25-30; page 217, lines 5-25; page 252, lines 30-32; page 253, lines 20-32; page 254, lines 5-10; page 256, lines 10-15; page 264, lines 5-10; page 265, lines 20-25; claim 60	1-2, 3/1-2, 4/1-2, 5/1-2, 6/1-2, 7-8, 9/7-8, 10/7-8, 11/7-8, 12/7-8, 13/7-8, 14/7-8, 15/7-8, 16/15/7-8, 17/15/7-8, 18/15/7-8, 19/15/7-8, 20/15/7-8, 21/1-2, 21/7-8, 22/1-2, 22/7-8, 23/1-2, 23/7-8, 24/1-2, 24/7-8, 25/1-2, 25/7-8, 26/1-2, 26/7-8, 27/1-2, 27/7-8, 28/1-2, 28/7-8, 29/1-2, 29/7-8, 30/1-2, 30/7-8, 31/1-2, 31/7-8, 32/1-2, 32/7-8, 33/1-2, 33/7-8, 34/1-2, 34/7-8, 35/1-2, 35/7-8, 37/1-2, 37/7-8, 38/37/1-2, 38/37/7-8, 39/1-2, 39/7-8, 40/1-2, 40/7-8, 41/1-2, 41/7-8, 42/41/1-2, 42/41/7-8, (continued on the next page)

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2019 (28.02.2019)	27 MAR 2019
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/13494

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
		45/1-2, 45/7-8, 47/1-2, 47/7-8, 48/47/1-2, 48/47/7-8, 49/1-2, 49/7-8, 51/1-2, 51/7-8, 52/51/1-2, 52/51/7-8, 53/1-2, 53/7-8, 54/53/1-2, 54/53/7-8, 56/1-2, 56/7-8, 57/1-2, 57/7-8, 58/1-2, 58/7-8, 59/58/1-2, 59/58/7-8, 60/59/58/1-2, 60/59/58/7-8, 61/60/59/58/1-2, 61/60/59/58/7-8, 63/59/58/1-2, 63/59/58/7-8, 64/1-2, 64/7-8, 65/1-2, 65/7-8, 66/65/1-2, 66/65/7-8, 67/66/65/1-2, 67/66/65/7-8, 68/66/65/1-2, 68/66/65/7-8, 69/66/65/1-2, 69/66/65/7-8, 70/65/1-2, 70/65/7-8, 71/70/65/1-2, 71/70/65/7-8, 72/70/65/1-2, 72/70/65/7-8, 73/72/70/65/1-2, 73/72/70/65/7-8
--- Y		--- 36/1-2, 36/7-8, 43/1-2, 43/7-8, 44/43/1-2, 44/43/7-8, 46/45/1-2, 46/45/7-8, 50/49/1-2, 50/49/7-8, 55/1-2, 55/7-8, 62/59/58/1-2, 62/59/58/7-8
Y	US 2014/0142095 A1 (CORTOPASSI, G et al) 22 May 2014; paragraphs [0074], [0084]-[0085], [0131]	36/1-2, 36/7-8, 46/45/1-2, 46/45/7-8, 50/49/1-2 and 50/49/7-8
Y	US 9,102,649 B1 (KANDULA) 11 August 2015; column 2, lines 10-20; column 18, lines 1-10; claim 1	43/1-2, 43/7-8, 44/43/1-2, 44/43/7-8
Y	MATSUMOTO, K et al. Recurrent Primary Central Nervous System Lymphoma Mimicking Neurodegenerative Disease An Autopsy Case Report. Neurologia Medico-Chirurgica (Tokio) Vol. 35, June 1995, pp. 360-363; page 360, column 2, paragraph 1	55/1-2, 55/7-8
Y	US 2016/0228576 A1 (ANTECIP BIOVENTURES II LLC) 11 August 2016; abstract; paragraph [0089]	62/59/58/1-2, 62/59/58/7-8