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(54) Title: BENZOFURAN DERIVATIVES

(57) Abrégé/Abstract:
Certain benzofuran derivatives are useful in the treatment of certain ischemic or inflammatory conditions, as well as neuroinflammation, neurodegeneration or degenerative diseases in which mitochondrial dysfunction leads to tissue degeneration. They are also useful in the manufacture of pharmaceutical formulations for the treatment of such conditions.

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(54) Title: BENZOFURAN DERIVATIVES

(57) Abstract: Certain benzofuran derivatives are useful in the treatment of certain ischemic or inflammatory conditions, as well as neuroinflammation, neurodegeneration or degenerative diseases in which mitochondrial dysfunction leads to tissue degeneration. They are also useful in the manufacture of pharmaceutical formulations for the treatment of such conditions.

BENZOFURAN DERIVATIVES

Field of the Invention

The present invention is directed to methods for treating neuroinflammation, neurodegeneration, or degenerative diseases in which mitochondrial dysfunction leads to tissue degeneration.

The invention relates to certain novel compounds having cytoprotective and neuroprotective activity, and particularly to a series of benzofuran derivatives. The invention is directed to formulations and methods for treating stroke, myocardial infarction and chronic heart failure, as well as other oxidative stress-related conditions that are typically responsive to cellular enzyme modulation. The invention is also directed to a method of treating inflammation by reducing C-reactive protein (CRP).

Background Information

The invention is concerned with cytoprotective and neuroprotective compounds, which are benzofuran derivatives, said derivatives including steroisomers, mixtures of stereoisomers and therapeutically acceptable salts thereof.

The present invention is concerned with agents providing protection against mitochondrial dysfunction, neuroinflammation, or neurodegeneration. Compositions of the invention are active in certain experimental models that predict efficacy in degenerative diseases in which mitochondrial dysfunction leads to tissue degeneration either directly or indirectly, and in diseases involving neuroinflammation and neurodegeneration. Some of the diseases include, but are not limited to Alzheimer's disease, drug-induced Parkinsonism or Parkinson's disease, Friedreich's ataxia (FRDA) and other ataxias, Leber's hereditary optic neuropathy, epilepsy, and myodegenerative disorders such as MELAS (mitochondrial encephalophaty, lactic acidosis and stroke) and MERRF (myoclonic epilepsy ragged red fiber syndrome).

Compositions of the invention are active in certain experimental models that predict efficacy in, for example, certain ischemic or inflammatory conditions, including but not limited to stroke, myocardial infarction, congestive heart failure, and skin disorders characterized by

inflammation or oxidative damage. The invention is therefore related to the use of the cytoprotective derivatives in such conditions.

2,3-Dihydro-5-oxy-4,6,7-trimethyl-2-optionally substituted alkyl benzofurans have been disclosed as antioxidizing pharmaceutical products having anti-ischemic properties in US 5,114,966. Hydroxamine derivatives of 2,3-dihydrobenzofuran carboxy acids have been disclosed in US 5,480,645. 2,3-Dihydrofuran derivatives useful in preventing and treating neovascularization have been disclosed in US 5,719,167 and US 5,798,356. 5-Hydroxybenzofurans have been disclosed for the treatment of a pathological cell proliferative disease in US 5,674,876. A method of inhibiting mammalian leukotriene biosynthesis with 6-hydroxybenzofurans has been disclosed in US 4,714,711.

While various agents have heretofore been provided for such conditions, it has, however, remained desired to provide new therapies for conditions characterized by oxidative stress, and particularly, for providing protection in the event of cerebral ischemia and inflammation; especially desired are agents that are effective even if first administered after a significant period of time (e.g., about 5 or more hours) following an ischemic or oxidative insult.

Furthermore, there is a need for compounds, compositions, and methods that limit or prevent damage to organelles, cells and tissues initiated by various consequences of mitochondrial dysfunction, neuroinflammation or neurodegeneration. Such agents would be suitable for the treatment of degenerative diseases particularly mitochondria associated diseases. The present invention fulfills these needs.

SUMMARY OF THE INVENTION

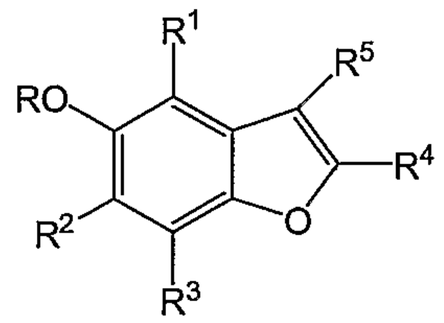
One aspect of the present invention is concerned with certain compounds that are effective in the treatment of diseases associated with mitochondrial dysfunction, as well as neuroinflammatory or neurodegenerative diseases, including but not limited to Alzheimer's disease, diabetes mellitus, drug-induced Parkinsonism or Parkinson's disease, neuronal and cardiac ischemia, Huntington's disease, Friedreich's ataxia and other cerebellar ataxias, Leber's hereditary optic neuropathy, schizophrenia, MELAS, MERRF, and other myodegenerative disorders, comprising administering to a subject in need of treatment an effective amount of a compound having the structure of Formula I or Formula II, as described herein.

It has surprisingly been found that certain compounds of this invention limit or prevent damage to organelles, cells and tissues caused by mitochondrial dysfunction, oxidative stress, or neuroinflammation as demonstrated by providing protection in standard experimental models of mitochondria dysfunction caused by MPP^+ (1-methyl-4-phenylpyridinium) or oxidative stress caused by beta-amyloid or high glutamate.

Certain compounds are particularly active in restoring or preserving metabolic integrity in oxidatively competent cells that have been subjected to oxygen deprivation. Such compounds, predominantly benzofuran derivatives are useful in the manufacture of pharmaceutical compositions for treating a number of conditions characterized by oxidative stress, and particularly, in providing protection in the event of cerebral ischemia, ultraviolet exposure, or

inflammation, even when administered a significant time interval after an ischemic or oxidative insult. In particular, the compositions of the present invention are useful in the treatment of stroke, as demonstrated by providing neuroprotection in a standard experimental model of focal cerebral ischemia. They are also useful in the treatment of myocardial ischemia (myocardial infarction), as well as other indications characterized by oxidative stress and/or inflammation, including, but not limited to, diabetes, renal disease, pre-menstrual syndrome, asthma, cardiopulmonary inflammatory disorders, chronic heart failure, rheumatoid arthritis, muscle fatigue, intermittent claudication, and for the preservation of allograft tissue for transplantation. Particularly with regard to dermatological conditions, the compounds, formulations and methods of the present invention are useful in regulating skin condition, regulating the signs of skin aging, and in treating a number of conditions, including, but not limited to preventing and protecting skin tissue against age-related damage or damage resulting from insults such as harmful (UV) radiation, stress and fatigue.

The present invention concerns the compounds represented by the Formula I:



Formula I

wherein:

R^1 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^2 and R^3 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^4 is: hydrogen, optionally substituted aryl, (optionally substituted(C_1-C_6)-alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^5 is: hydrogen, (C_1-C_6)-alkyl, (C_2-C_{10})-alkenyl, (optionally substituted alkoxy)carbonyl, carboxy, (optionally substituted amino)carbonyl, or optionally substituted aryl;

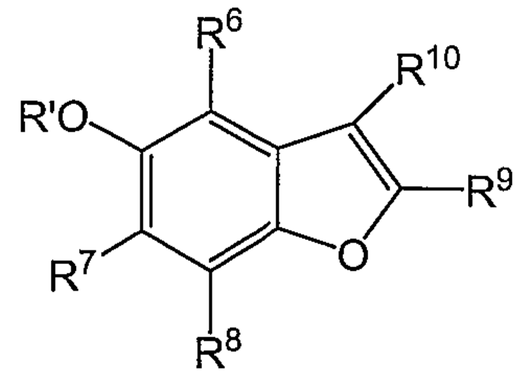
provided that one of R^4 or R^5 is hydrogen, and that when R^4 is hydrogen R^5 is not hydrogen, and when R^5 is hydrogen R^4 is not hydrogen; and

R is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R and R^1 with the atoms to which they are attached form an optionally substituted ring;

and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

The present invention also concerns the compounds represented by the Formula II:



Formula II

wherein:

R^6 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^7 and R^8 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^9 is: optionally substituted aryl, (optionally substituted(C_1-C_6)- alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^{10} is: (C_1-C_6)-alkyl, or (C_2-C_{10})-alkenyl, and amino;

R' is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R' and R^6 with the atoms to which they are attached form an optionally substituted ring; and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

A preferred embodiment of this invention concerns the compounds of Formula I where R^2 and R^3 are (C_1-C_6)-alkyl, preferably methyl, and within that subset those compounds of Formula I wherein R is hydrogen.

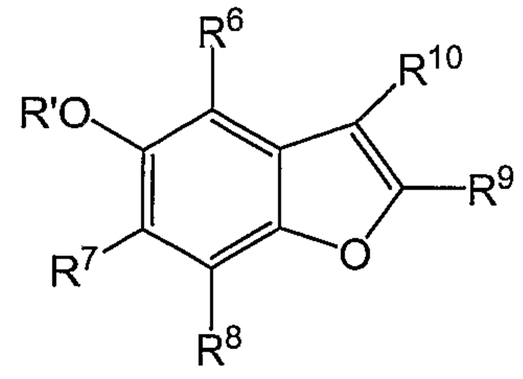
In another embodiment, the invention concerns the compounds of Formula I wherein R^2 and R^3 are (C_1-C_6)-alkyl, preferably methyl, R is hydrogen, R^5 is hydrogen, and R^4 is optionally substituted aryl, (optionally substituted alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted alkoxy)carbonyl, (optionally substituted alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)alkyl, especially wherein said aryl is unsubstituted phenyl or substituted phenyl with one or more substitutents selected from alkyl, alkoxy, hydroxy, (optionally substituted alkoxy)carbonyl, nitro, halo, and cyano.

In another embodiment, the invention concerns the compounds of Formula I wherein R^2 and R^3 are (C_1-C_6)-alkyl, preferably methyl, R is hydrogen, R^4 is hydrogen, and R^5 is optionally substituted aryl, preferably unsubstituted phenyl or phenyl substituted with one or more substitutents selected from alkyl, alkoxy, hydroxy, (optionally substituted alkoxy)carbonyl, nitro,

halo, and cyano.

In another embodiment, the invention concerns the compounds of Formula I wherein R and R^1 form a ring, preferably R and R^1 form a furan ring substituted with an unsubstituted phenyl or with a phenyl substituted with one or more substitutents selected from alkyl, alkenyl, hydroxy, alkoxy, nitro, cyano, carboxy, carboxyester, haloalkyl, and halo.

In another embodiment the invention concerns the novel compounds of Formula II:



Formula II

wherein:

R^6 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^7 and R^8 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^9 is: optionally substituted aryl, (optionally substituted(C_1-C_6)- alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^{10} is: (C_1-C_6)-alkyl, or (C_2-C_{10})-alkenyl; and

R' is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R' and R^6 with the atoms to which they are attached form an optionally substituted ring; and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof, with the proviso that the compound is not (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone or 3-amino-5-hydroxy-4,6,7-trimethyl-benzofuran-2-carboxylic acid ethyl ester.

In another embodiment the invention concerns the compounds of Formula II wherein R^7 and R^8 are (C_1-C_6)-alkyl and R^6 is hydrogen, in another embodiment R^{10} is (C_1-C_6)-alkyl and R' is hydrogen, in another embodiment R^9 is optionally substituted aryl carbonyl, preferably phenylcarbonyl wherein the phenyl group may be unsubstituted or substituted with one or more substituents selected from alkyl, alkenyl, alkoxy, hydroxy, hydroxyalkyl, haloalkyl, (optionally substituted alkoxy)carbonyl, carboxy, nitro, halo, and cyano. In another embodiment the invention concerns the compounds of Formula II wherein R^{10} is amino, R^9 is substituted (C_1-C_6)-alkoxycarbonyl and in another embodiment the compound is 3-amino-5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester.

Certain embodiments of the invention provide novel and preferred combinations of the

substituent groups pendant from the formulae of the different inventions.

In another aspect, the invention relates to pharmaceutical and/or cosmetic compositions containing a therapeutically effective amount of a compound of any of Formula I or Formula II, or a pharmaceutically acceptable salt thereof, admixed with at least one pharmaceutically acceptable excipient. Particularly preferred are those pharmaceutical or cosmetic compositions wherein a compound of Formula I or Formula II is selected from the Preferred Compounds.

Another aspect of the present invention concerns methods of treatment for a mammal suffering from a condition characterized by oxidative stress by administering a therapeutically effective amount of a compound represented by the Formula I or Formula II, including single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

In one embodiment, the invention relates to a method of treatment of a cardiovascular, cerebrovascular, neurologic, inflammatory, autoimmune, and/or dermatologic condition.

In another embodiment the invention relates to a condition selected from stroke, cerebral ischemia, myocardial infarction, chronic heart failure, retinal ischemia, post-surgical cognitive dysfunctions, peripheral neuropathy, spinal cord injury, head injury and surgical trauma. In another embodiment the condition involves inflammatory or autoimmune components.

In another embodiment the condition relates to a method of treatment diseases involving mitochondrial dysfunction, particularly with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Friedreich's ataxia and other cerebellar ataxias, Leber's hereditary optic neuropathy, and epilepsy with a compound of Formula I or of Formula II., particularly with (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone or 3-amino-5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester; or a compound of Formula I wherein R^1 is halogen, R^4 is (optionally substituted(C_1-C_6)- alkyl)carbonyl and R^5 is hydrogen; or a compound of Formula II wherein R^7, R^8 and R^{10} are (C_1-C_6)-alkyl, and R^9 is phenylcarbonyl, wherein said phenyl group is substituted with one or more substituents selected from alkyl, alkenyl, alkoxy, hydroxy, hydroxyalkyl, haloalkyl, (optionally substituted alkoxy)carbonyl, carboxy, nitro, halo, and cyano, or a compound of Formula II wherein $R^7$and R^8 are alkyl, R^{10} is amino, and R^9 is (C_1-C_6)-alkoxycarbonyl.

In still another embodiment, the invention relates to a method of treating stroke and other oxidative stress-related conditions that are responsive to cellular enzyme modulation such as cerebral ischemia, myocardial infarction, chronic heart failure, and exposure to UV radiation in a mammal, by administering to a mammal in need of such treatment a therapeutically effective amount of a compound of any of Formula I or Formula II, or a pharmaceutically acceptable salt thereof.

In still another aspect, the invention relates to a method of reducing levels of C-reactive protein (CRP) associated with inflammation, including but not limited to cardiovascular inflammatory condition, respiratory inflammatory condition, sepsis, diabetes, muscle fatigue, systemic lupus erythematosis (SLE), end stage renal disease (ERSD), periodontal disease, and inflammatory skin conditions.

Particularly preferred are those methods of treatment and uses in the manufacture of

pharmaceutical and/or cosmetic compositions thereof, wherein a compound of Formula I or Formula II is selected from the Preferred Compounds.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

The term "optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, “optionally substituted alkyl” means either “alkyl” or “substituted alkyl,” as defined below. It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical and/or synthetically non-feasible.

The term "acyl" refers to the groups -C(O)-H, -C(O)-(optionally substituted alkyl), -C(O)-(optionally substituted cycloalkyl), -C(O)-(optionally substituted alkenyl), -C(O)-(optionally substituted cycloalkenyl), -C(O)-(optionally substituted aryl), -C(O)-(optionally substituted heteroaryl) and -C(O)-(optionally substituted heterocyclyl).

The term “alkenyl” refers to a monoradical branched or unbranched, unsaturated or polyunsaturated hydrocarbon chain, having from about 2 to 20 carbon atoms, more preferably about 2 to 10 carbon atoms. This term is exemplified by groups such as ethenyl, but-2-enyl, 3-methyl-but-2-enyl (also referred to as “prenyl”), octa-2,6-dienyl, 3,7-dimethyl-octa-2,6-dienyl (also referred to as “geranyl”), and the like.

The term “substituted alkenyl” refers to an alkenyl group in which 1 or more (up to about 5, preferably up to about 3) hydrogen atoms is replaced by a substituent, for example: hydroxy, alkoxy, carboxy, cyano, halogen or nitro.

The term "alkoxy" refers to the groups -O-alkyl, -O-alkenyl, -O-cycloalkyl, -O-cycloalkenyl, and -O-alkynyl. Preferred alkoxy groups are -O-alkyl and -O-alkenyl and include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, 3,7-dimethyl-octa-2,6-dienyloxy and the like.

The term "substituted alkoxy" refers to the groups -O-(substituted alkyl), -O-(substituted alkenyl), -O-(substituted cycloalkyl), -O-(substituted cycloalkenyl), -O-(substituted alkynyl) and -O-(optionally substituted alkylene)-alkoxy. One preferred substituted alkoxy group is “polyalkoxy” or -O-(substituted alkylene)-alkoxy, and includes groups such as $-OCH_2OCH_3$, —$OCH_2CH_2OCH_3$, and (or PEG) groups such as $-O(CH_2CH_2O)_xCH_3$ and $-O(CH_2CH_2O)_xH$ where x is an integer of about 2-20, preferably about 2-10, and more preferably about 2-5.

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from about 1 to 20 carbon atoms, more preferably about 1 to 10 carbon

atoms, and even more preferably about 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, n-hexyl, n-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group in which 1 or more (up to about 5, preferably up to about 3) hydrogen atoms is replaced by a substituent independently selected from the group: =O, =S, acyl, acyloxy, optionally substituted alkoxy, optionally substituted amino, azido, carboxyl, (optionally substituted alkoxy)carbonyl, (optionally substituted amino)carbonyl, cyano, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, heterocyclyl, carboxy, halogen, hydroxyl, nitro, cyano, sulfanyl, sulfinyl, and sulfony, or two substituents with the carbon to which they are attached may form a ring. One of the preferred optional substituents for alkyl is hydroxy, exemplified by hydroxyalkyl groups, such as 2-hydroxyethyl, 3-hydroxypropyl, 3-hydroxybutyl, 4-hydroxybutyl, and the like; dihydroxyalkyl groups (glycols), such as 2,3-dihydroxypropyl, 3,4-dihydroxybutyl, 2,4-dihydroxybutyl, and the like; and those compounds known as polyethylene glycols, polypropylene glycols and polybutylene glycols, and the like. A preferred "substituted alkyl" wherein the substitutents form a ring is 6-hydroxy-3-methyl-[1,3]oxazinan-6-yl.

The term "Alzheimer's Disease" ("AD") refers to a progressive disease of the human central nervous system. It is manifested by dementia in the elderly, by disorientation, loss of memory, difficulty with language, calculation, or visual-spatial skills, and by psychiatric manifestations. It is associated with degenerating neurons in several regions of the brain. Alzheimer's Disease is reviewed by Price, D. L. et al. (*Clin. Neuropharm.* 14:S9-S14 (1991)); Pollwein, P. et al. (*Nucl. Acids Res.* 20:63-68 (1992)); Regland, B. et al. (*Med. Hypoth.* 38:11-19 (1992)) and Johnson, S. A. (In: *Review of Biological Research in Aging*, Vol. 4., Rothstein, M. (Ed.), Wiley-Liss, NY, 163-170 (1990)).

The term "amino" refers to the group $-NH_2$,-NHR, or -NRR where each R is independently selected from the group: optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted cycloalkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted heterocyclyl, acyl, optionally substituted alkoxy, carboxy and alkoxycarbonyl, and wherein RR form with the nitrogen to which they are attached a cyclic amine optionally incorporating one or more additional heteroatoms selected from O, N and S.

The term "aryl" refers to an aromatic cyclic hydrocarbon group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "substituted aryl" refers to an aryl group as defined above, which unless otherwise constrained by the definition for the aryl substituent, is substituted with from 1 to 5 substituents, and preferably 1 to 3 substituents, independently selected from the group consisting of: =O, =S, acyl, acyloxy, optionally substituted alkenyl, optionally substituted alkoxy, optionally substituted alkyl (such as tri-halomethyl), optionally substituted alkynyl, optionally substituted amino, optionally substituted aryl, optionally substituted aryloxy, azido, carboxyl, (optionally

substituted alkoxy)carbonyl, (optionally substituted amino)carbonyl, cyano, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, halogen, optionally substituted heteroaryl, optionally substituted heteroaryloxy, optionally substituted heterocyclyl, optionally substituted heterocyclooxy, hydroxyl, nitro, sulfanyl, sulfinyl, and sulfonyl. Preferred aryl substituents include optionally substituted alkenyl, alkyl, alkoxy, substituted amino, halo, hydroxy, alkoxycarbonyl, carboxy, cyano, nitro, phosphoryl.

The term “carbonyl” refers to the di-radical “-C(=O)-“, which is also illustrated as “-C(O)-“.

The term "(optionally substituted alkoxy)carbonyl" refers to the groups: -C(O)O-(optionally substituted alkyl), -C(O)O-(optionally substituted cycloalkyl), -C(O)O-(optionally substituted alkenyl), -C(O)O-(optionally substituted alkynyl), -C(O)O-(optionally substituted aryl), -C(O)O-(optionally substituted heteroaryl), and -C(O)O-(optionally substituted heterocyclyl). These moieties are also referred to as esters.

The term "(optionally substituted amino)carbonyl" refers to the group -C(O)-(optionally substituted amino). This moiety is also referred to as a primary, secondary or tertiary carboxamide, and the “substituted amino” can be a cyclic amine.

The term “carboxy” or “carboxyl” refers to the moiety “-C(O)OH”, which is also illustrated as “-COOH”.

The term “compound of Formula I” or “compound of Formula II”, is intended to encompass the derivatives of the invention as disclosed, and/or the pharmaceutically acceptable salts of such compounds. In addition, the compounds employed in this invention include the individual stereochemical isomers (arising from the selection of substituent groups) and mixtures of isomers.

The term “CRP” or “C-reactive protein” refers to a biochemical marker of inflammation. The presence of elevated levels of CRP has been shown to be associated with various inflammatory conditions such as for example, cardiovascular diseases or disorders, including atrial fibrillation, unstable angina, coronary artery disease, peripheral artery disease, cardiac allograft vasculopathy (CAVD); mastitis; preeclampsia; inflammatory bowel conditions; stroke; tissue infarction; lumbosciatic; estrogen/progestin hormone replacement therapy (HRT); infection (bacterial, viral and protozoan); bacterial meningitis; trauma; surgery; biomaterial implants; smoking; obesity; neurodegenerative diseases such as, Alzheimers; infectious disease, such as, for example, myocarditis, cardiomyopathy, acute endocarditis, pericarditis; atherosclerosis; Systemic Inflammatory Response Syndrome (SIRS)/sepsis; adult respiratory distress syndrome (ARDS); asthma; rheumatoid arthritis, osteoarthritis, systemic lupus erythematosis; Airway hyper-responsiveness (AHR); bronchial hyper-reactivity; Chronic Obstructive Pulmonary disease (COPD); Congestive Heart Failure (CHF); inflammatory complications of diabetes mellitus type I and type II; metabolic syndrome; end stage renal disease (ESRD), pre-menstrual syndrome (PMS) or muscle fatigue or inflammation; multiple organ dysfunction syndrome (MODS); airway hyper-responsiveness (AHR); bronchial hyper-reactivity; aging; acute allergic reactions; gingivitis and dermal conditions. CRP has been reported as a marker for systemic inflammation

Spanheimer (2001, *Postgrad. Med.* 109 (4) 26) and Ridkler et al (2000, *N.E.J.M.* 342 (12) 836-43).

The term "cycloalkyl" refers to the monovalent saturated radical consisting of one or more rings, which can optionally be susbstituted with hydroxy, cyano, lower alkyl, lower alkoxy, thioalkyl, halo, haloalkyl, hydroxyalkyl, nitro, alkoxycarbonyl, amino, alkylamino, dialkylamino, aminocarbonyl, carbonylamino, aminosulfonyl, sulfonylamino or sulfonyl, unless otherwise indicated. Examples of cycloalkyl radicals include but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, etc.

The term "epilepsy" refers to any neurological condition that makes people susceptible to seizures. A seizure is a change in sensation, awareness, or behavior brought about by a brief electrical disturbance in the brain. Seizures vary from a momentary disruption of the senses, to short periods of unconsciousness or staring spells, to convulsions. Some people have just one type of seizure. Others have more than one type. Although they look different, all seizures are caused by the same thing: a sudden change in how the cells of the brain send electrical signals to each other.

The term "Friedreich's ataxia" refers to an autosomal recessive multi-system degenerative disorder that results in progressive damage to the nervous system and causes symptoms ranging from muscle weakness and speech problems to heart disease. Ataxia results from the degeneration of nerve tissue in the spinal cord and of nerves that control muscle movement in the arms and legs. Symptoms usually begin between the ages of 5 and 15 but can appear as early as 18 months or as late as 30 years of age. The first symptom is usually difficulty in walking. The ataxia gradually worsens and slowly spreads to the arms and then the trunk. Foot deformities such as clubfoot, flexion (involuntary bending) of the toes, hammer toes, or foot inversion (turning in) may be early signs. Rapid, rhythmic, involuntary movements of the eyeball are common. Most people with Friedreich's ataxia develop scoliosis (a curving of the spine to one side), which, if severe, may impair breathing. Other symptoms include chest pain, shortness of breath, and heart palpitations.

The term "halo" or "halogen" refers to fluoro, chloro, bromo, and iodo.

The term "heteroaryl" refers to an aromatic cyclic hydrocarbon group having about 1 to 40 (preferably from about 3 to 15) carbon atoms and about 1 to 10 hetero atoms (preferably about 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen) within at least one ring. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, [2,2']bipyridinyl, pyrrolyl and furyl.

The terms "heterocycle", "heterocyclic", "heterocyclo", and "heterocyclyl" refer to a monoradical, saturated or unsaturated, non-aromatic cyclic hydrocarbon group having about 1 to 40 (preferably from about 3 to 15) carbon atoms and about 1 to 10 hetero atoms (preferably about 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen) within the ring. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, 1,3-oxazinane, and the like.

The terms "substituted heterocycle", "substituted heterocyclic", "substituted heterocyclyl" and "substituted heterocyclo" refer to a heterocyclyc group as defined above, which unless otherwise constrained by the definition for the heterocycle, is substituted with from 1 to 5 substituents, and preferably 1 to 3 substituents, independently selected from the group consisting of: =O, =S, acyl, acyloxy, optionally substituted alkenyl, optionally substituted alkoxy, optionally substituted alkyl (such as tri-halomethyl), optionally substituted alkynyl, optionally substituted amino, optionally substituted aryl, optionally substituted aryloxy, azido, carboxyl, (optionally substituted alkoxy)carbonyl, (optionally substituted amino)carbonyl, cyano, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, halogen, optionally substituted heteroaryl, optionally substituted heteroaryloxy, optionally substituted heterocyclyl, optionally substituted heterocyclooxy, hydroxyl, nitro, sulfanyl, sulfinyl, and sulfonyl.

The term "heterocycloalkyl" refers to the moiety "-alkylene-heterocycle"each having the meaning as defined herein.

The term "substituted heterocycloalkyl" refers to the moiety "-(optionally substituted aklylene)- (optionally substituted heterocycle)", each having the meaning as defined herein.

The term "hydroxy (optionally substituted) alkyl" refers to the group –(optionally substituted alkyl)-OH, wherein –(optionally substituted alkyl)- is an unsubstituted or a substituted alkyl group as described herein. This term is exemplified by the groups 2-hydroxymethyl, 1-hydroxy-2-morpholin-4-yl-ethyl, and 6-hydroxy-3-methyl-[1,3]oxazinan-6-yl.

The term "inflammation", "inflammatory conditions", or "inflammation conditions" includes but is not limited to muscle fatigue, osteoarthritis, rheumatoid arthritis, inflammatory bowel syndrome or disorder, skin inflammation, such as atopic dermatitis, contact dermatitis, allergic dermatitis, xerosis, eczema, rosacea, seborrhea, psoriasis, atherosclerosis, thermal and radiation burns, acne, oily skin, wrinkles, excessive cellulite, excessive pore size, intrinsic skin aging, photo aging, photo damage, harmful UV damage, keratinization abnormalities, irritation including retinoid induced irritation, hirsutism, alopecia, dyspigmentation, inflammation due to wounds, scarring or stretch marks, loss of elasticity, skin atrophy and gingivitis.

The term "ischemia" refers to deficiency of blood to an organ or tissue due to functional constriction or actual obstruction of a blood vessel. Cerebral ischemia, also known as stroke, usually results from the interruption or reduction of blood and oxygen to the blood vessels of the brain; more rarely this may be the result of an hemorrhage. Signs of stroke include paralysis, slurred speech, general confusion, impairment of gait, cortical sensory loss over toes, foot and leg, and urinary incontinence, to name just a few. Many types of heart disease including cardiac arrhythmias or diseases due to cardiac structural abnormalities may produce cerebral emboli. Atrial fibrillation from any cause, including rheumatic valvular disease, may result in emboli being produced which can migrate into the arteries of the brain. Emboli formation and migration can occur as a result of atherosclerotic cardiovascular disease and myocardial infarction. Emboli formation is also a definite risk for intracardiac surgery and prosthetic valve replacement. Heart bypass surgery and angioplasty can result in the formation of microemboli which can migrate into the arteries of the brain and cause a series of occlusions in a number of arteries, resulting in

mental impairment. Cerebral embolism is also the principal complication in the transplant of artificial hearts. Furthermore, the overall risk of stroke after any type of general surgery is 0.2 to 1 percent. The vegetations of acute and subacute bacterial endocarditis can give rise to emboli which can occlude a major intracranial artery. Populations at risk of ischemia include but are not limited to patients scheduled for coronary arterial bypass graft surgery (CABG), patients at risk for postoperative complications, patients with subarachnoid hemorrhage (SAH), patients with a first or second ischemic stroke, patients with acute ischemic stroke, patients undergoing cardiopulmonary resuscitation (CPR), patients with temporary lobectomy, patients with dominant hemisphere resection, patients receiving prophylactic brain radiation, patients with closed head trauma with neurological loss, patients with microvascular multi-infarct dementia, patients with homozygous and heterozygous MELAS (Mitochondrial myopathy, encephalopathy, lactacidosis, stroke); patients with atherosclerotic or progressive supranuclear palsy disease, patients with symptomatic and asymptomatic Huntington's disease, patients with neonatal asphyxia, patients with meningitis or encephalitis, patients with post herpetic neuropathy, patients with intermittent claudication, patients with spinal cord injury, patients with Huntington's disease, Amyotrophic Lateral Sclerosis (ALS) or Friedreich's ataxia, patients with diabetic neuropathy or patients with a disease associated with a hypercoagulable state secondary to systemic disease, carcinoma, vasoconstriction (including reversible cerebral vasoconstriction, e.g. migraine , trauma, idiopathy), or venous conditions (including dehydration, pulmonary embolism, pericranial infection, postpartum and postoperative states and system cancer).

The term "mitochondrial associated diseases" refers to degenerative diseases that may be caused by or associated with either direct or indirect alterations in mitochondrial dysfunctions. These include Alzheimer's disease, diabetes mellitus, Parkinson's disease, neuronal and cardiac ischemia, Huntington's disease, spinal bulbar muscular atrophy, Macado-Joseph disease, Dentatorubral-Pallidoluysian Atrophy (DRPLA), Friedreich's ataxia (FRDA) and other spinocerebellar ataxias, dystonia, Leber's hereditary optic neuropathy (LHON), schizophrenia, MELAS and MERFF.

The term "neurodegeneration" refers to a progressive death and disappearance of a population of nerve cells occurring in a manner characteristic of a particular disease state and leading to brain or other neuronal damage. It is the process of cell destruction resulting from primary destructive events, or secondary delayed and progressive destructive mechanisms that are invoked by cells due to the occurrence of the primary destructive event. Primary destructive events include disease processes or physical injury or insult, including stroke, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, epilepsy, cerebral ischemia, and physical trauma such as crush or compression injury in the CNS, including a crush or compression injury of the brain, spinal cord, nerves or retina, or any acute injury or insult. Secondary destructive mechanisms include any mechanism that leads to the generation and release of neurotoxic molecules, including apoptosis, depletion of cellular energy stores because of changes in mitochondrial membrane permeability, release or failure to reuptake excessive glutamate, reperfusion injury, and activity of cytokines and inflammation.

The term "neurodegenerative condition" refers to a primary destructive event or secondary destructive mechanism resulting in neurodegeneration as defined herein.

The term "neuroprotection" refers to a therapeutic strategy for slowing or preventing the irreversible loss of neurons due to neurodegeneration after a primary destructive event, whether the neurodegeneration loss is due to disease mechanisms associated with the primary destructive event or to secondary destructive mechanisms.

The term “Parkinson's disease” (“PD”) refers to a neurodegenerative disease especially affecting the dopaminergic neurons of the substantia nigra--pars compacta--and its nigrostriatal projections in nearly one million Americans. The symptomatic manifestations are motor disorders such as tremor, muscular rigidity, bradykinesia, poor balance and walking problems. Secondary symptoms include depression, sleep disturbances, dizziness, stooped posture, constipation, dementia, and problems with speech, breathing, swallowing, and sexual function symptoms. Due to their mimicry of effects of Parkinson's Disease, treatment of animals with methamphetamine or MPTP has been used to generate models for Parkinson's Disease. These animal models have been used to evaluate the efficacy of various therapies for Parkinson's Disease.

The terms "pharmaceutically acceptable carrier" or “pharmaceutically acceptable excipient” include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto. Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl,

substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

The term “phosphoryl” refers to the group -P(O)(OR”)$_2$, where R” is independently selected from hydrogen or alkyl and aryl, which group is sometimes also referred to as “phosphono” or as a “phosphate” or “phosphonic acid.”

The term "therapeutically effective amount" refers to that amount of a compound of this invention that is sufficient to effect treatment, as defined below, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the particular compound chosen, the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which can readily be determined by one of ordinary skill in the art.

The term "treatment" or "treating" means any treatment of a disease or disorder in a mammal, including:

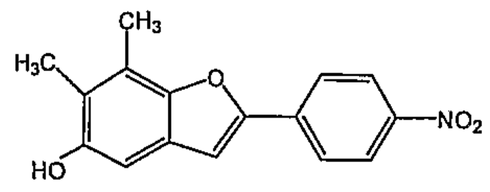
- preventing or protecting against the disease or disorder, that is, causing the clinical symptoms not to develop;
- inhibiting the disease or disorder, that is, arresting or suppressing the development of clinical symptoms; and/or
- relieving the disease or disorder that is causing the regression of clinical symptoms.

It will be understood by those skilled in the art that in human medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, as used herein the term "prophylaxis" is intended as an element of "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis."

Nomenclature

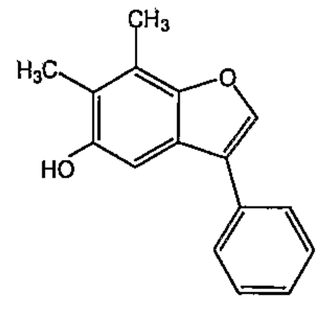
In general the nomenclature used in this Application is based on Autonom™ v.2.1, a Beilstein Institute computerized system for the generation of IUPAC systematic nomenclature.

The compounds of the present invention are named and numbered as described below.



Formula Ia

Formula Ia represents the compound according to Formula I where R, R^1 and R^5 are hydrogen, R^2 and R^3 are methyl, and R^4 is p-nitro-phenyl, and can be named 6,7-dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol.



Formula Ib

Formula Ib represents the compound according to Formula I where R, R^1, and R^4 are hydrogen, R^2 and R^3 are methyl, and R^5 is phenyl, and can be named 6,7-dimethyl-3-phenyl-benzofuran-5-ol.

Synthesis of the Compounds of the Invention

Synthetic Reaction Parameters

The terms "solvent", "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith. Solvents employed in synthesis of the compounds of the invention include, for example, methanol (“MeOH”), acetone, water, acetonitrile, 1,4-dioxane, dimethylformamide ("DMF"), benzene, toluene, xylene, tetrahydrofuran ("THF"), chloroform, methylene chloride (or dichloromethane, (“DCM”)), diethyl ether, pyridine and the like, as well as mixtures thereof. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert organic solvents.

The term "q.s." means adding a quantity sufficient to achieve a stated function, e.g., to bring a solution to the desired volume (i.e., 100%).

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure within a temperature range from -10°C to 110°C (preferably from 0°C to 40°C; most preferably at "room" or "ambient" temperature, e.g., 20°C). Further, unless otherwise specified, the reaction times and conditions are intended to be approximate, e.g., taking place at about atmospheric pressure within a temperature range of about -10°C to about 110°C (preferably from about 0°C to about 40°C; most preferably at about "room" or "ambient" temperature, e.g., approximately 20°C) over a period of about 1 to about 10 hours (preferably about 5 hours). Parameters given in the Examples are intended to be specific, not approximate.

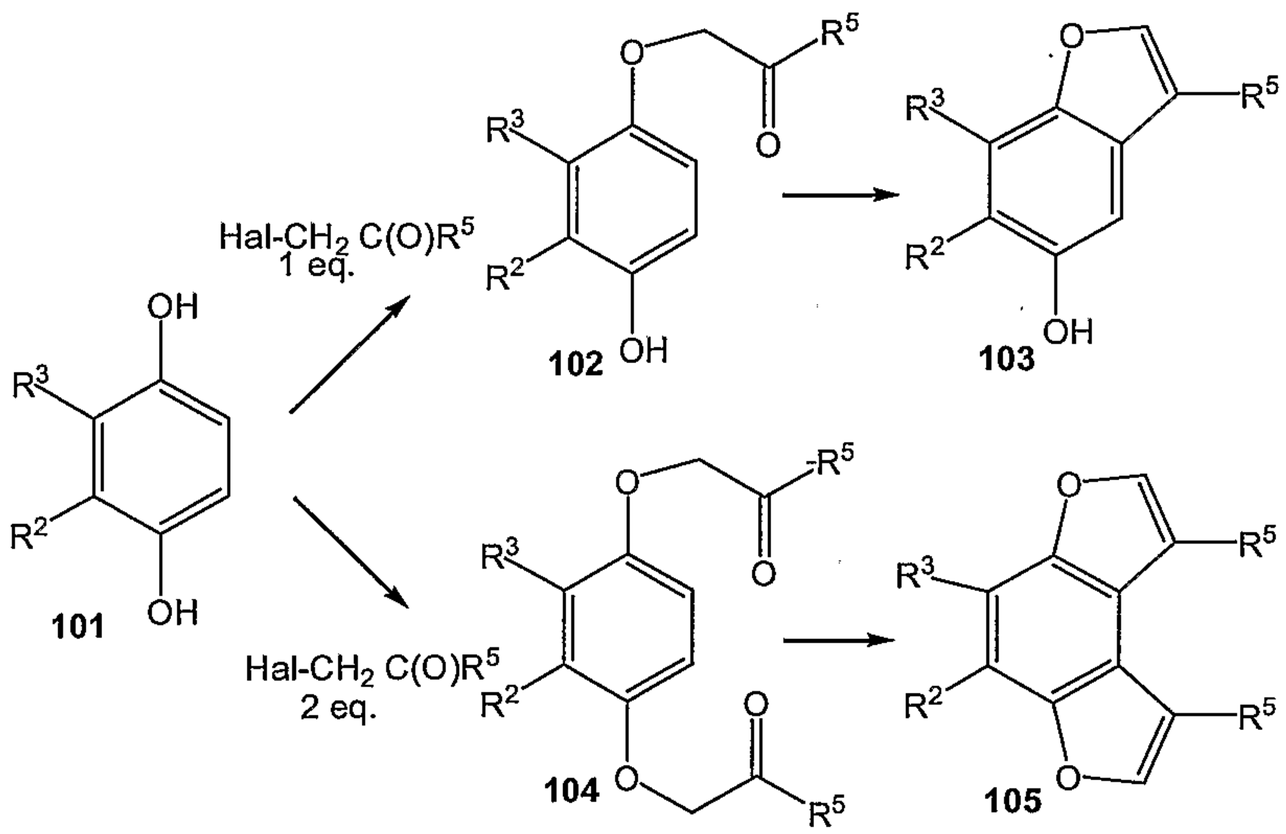
Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples

hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

Starting Materials

The starting compounds , e.g., 2,3-dimethylhydroquinone, are commercially available, e.g. from Aldrich Chemical Company, Milwaukee, WI, or may be readily prepared by those skilled in the art using commonly employed methodology.

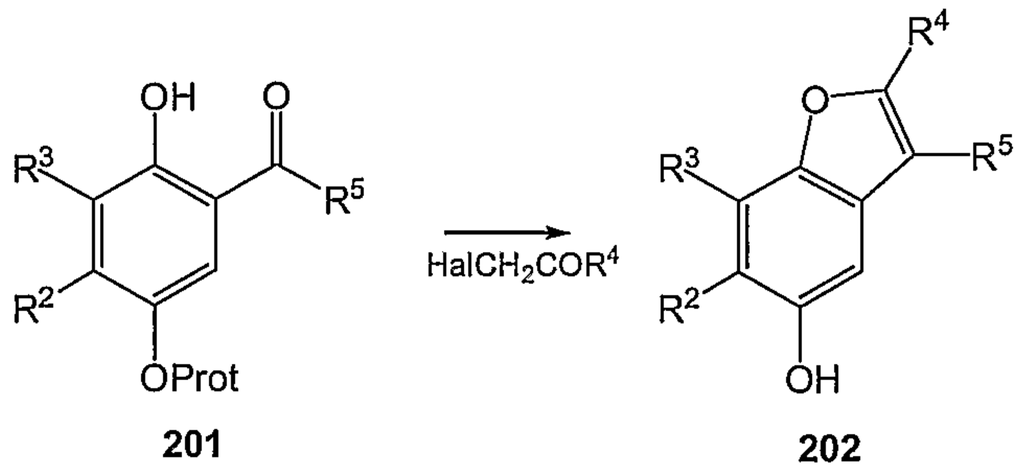
Reaction Scheme 1



Referring to Reaction Scheme 1, a 2,3- substituted hydroquinone of Formula 101 is treated with one equivalent of a 2-halo-1-substituted-ethanone, preferably 2-bromo-1-substituted-ethanone, most preferably 2-bromo-1-substituted phenyl ethanone, in the presence of a base such as sodium carbonate, potassium carbonate or cesium carbonate in a solvent such as acetone, THF, or DMF, to give 2-(4-hydroxy-2,3-substituted-phenoxy)-1-substituted-ethanone of Formula 102. Compound of Formula 102 can subsequently be treated with an acid such as polyphosphoric acid, hydrochloric acid, or sulfuric acid in an inert solvent such as toluene or xylene, to cyclise into a benzofuran compound of Formula 103.

Similarly treating a 2,3-substituted hydroquinone of Formula 102 with 2 or more equivalents of a 2-halo-1-substituted ethanone in the presence of a base, followed by cyclization in the presence of an acid, can give a compound of Formula 105.

Reaction Scheme 2



Referring to Scheme 2, a compound of Formula 201 wherein "Prot" is a hydroxy protecting group, preferably a tetrahydropyranyl group, when reacted with a 2-halo-1-substituted ethanone, preferably a 2-bromo-1-substituted ethanone in the presence of a base such as sodium carbonate, potassium carbonate, or cesium carbonate in a solvent such as acetone, THF, or DMF, followed by cyclization in the presence of an acid as described in reaction scheme 1, can give a compound of Formula 202.

Preferred Compounds

The following combinations and permutations of substituent groups (sub-grouped, respectively, in increasing order of preference) define compounds that are preferred as compositions of matter and compounds for use in the methods and pharmaceutical and cosmetic compositions according to the invention.

- The compounds of any of Formula I where R^2 and R^3 are optionally substituted alkyl, particularly those wherein R^2 and R^3 are methyl.
    - Preferably those where R^1 is hydrogen or halogen
        - Especially those where R is hydrogen.
- The compounds of any of Formula I where R^1 is hydrogen or halo
    - Preferably those where R^1 is hydrogen
- The compounds of any of Formula I where R is hydrogen, alkyl, acyl, phosphoryl or polyalkoxy, preferably hydrogen, and R^1 is hydrogen
    1. Particularly those wherein R^2 and R^3 are methyl
        - Especially those of Formula I where R^5 is optionally substituted aryl, wherein the substitutents are chosen from alkyl, alkoxy, hydroxy, (optionally substituted alkoxy)carbonyl, nitro, halo, and cyano
            - Particularly those where R^5 is optionally substituted phenyl.
            - Particularly those where R^5 is optionally substituted phenyl and R^4 is hydrogen
        - Especially those of Formula I where R^4 is optionally substituted aryl
            - Particularly those where R^4 is optionally substituted phenyl
            - Preferably those where R^4 is para-substituted phenyl, wherein the substitutents are chosen from alkyl, alkoxy, hydroxy, (optionally substituted alkoxy)carbonyl, nitro, halo, and cyano
                - Preferably those where R^4 is 4-nitrophenyl, 4-cyanophenyl and R^5 is hydrogen.
        - Especially those of Formula I where R^4 is formyl, (optionally substituted alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl
            - Particularly those where R^4 is (optionally substituted aryl)carbonyl
            - Particularly those where R^4 is (optionally substituted alkyl)carbonyl
            - Particularly those where R^4 is alkylcarbonyl optionally substituted with

halogen, hydroxy or heterocyclyl, especially substituted with morpholin-1-yl

- Particularly those where R^4 is formyl
  - Preferably those where R^4 is selected from formyl, phenylcarbonyl, bromoacetyl, morpholin-1-yl-acetyl, and acetyl; and R^5 is hydrogen
- Especially those wherein R^4 is (optionally substituted alkoxy)carbonyl, (optionally substituted alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, or hydroxy(optionally substituted)alkyl;
- Particularly those where R^4 is (optionally substituted alkoxy)carbonyl, preferably those where the alkoxy is polyalkoxy, or (optionally substituted alkenyloxy) carbonyl, preferably those where the alkenyloxy is geranyloxy.
- Particularly those where R^4 is (optionally substituted amino)carbonyl, preferably those wherein the amino is substituted with optionally substituted alkyl, especially hydroxyalkyl, or wherein the amino is a cyclic amine.
  - Preferably those where R^4 is morpholin-1-yl-carbonyl; bis-(2-hydroxy-ethyl)-amide, 2-hydroxy-ethyl-amide, carboxylic acid; carboxylic acid methyl ester; carboxylic acid 3,7-dimethyl-octa-2,6-dienyl ester; [2-(2-methoxy-ethoxy)-ethoxy]ethyl ester; and R^5 is hydrogen.
- Especially those where R^4 are hydroxy (optionally substituted) alkyl
  - Particularly where R^4 is hydroxymethyl, 1-hydroxy-2-morpholiny-4-yl ethyl or 6-hydroxy-3-methyl-[1,3]oxazinan-6-yl.

- The compounds of any of Formula I wherein R and R^1 with the atoms to which they are attached form an optionally substituted ring
  - Especially those wherein R and R^1 with the atoms to which they are attached form a furan ring substituted with an unsubstituted phenyl ring or with a substituted phenyl ring, wherein one or more substituents are independently selected from alkyl, alkenyl, halo alkyl, hydroxy, alkoxy, carboxy, ester, haloalkyl, and halo.

The compounds preferred for use in the invention include the following, as well as their stereoisomers, salts, and mixtures thereof (as appropriate):

(5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone;

(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-morpholin-4-yl-methanone;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

Acetic acid 2-(2-bromo-acetyl)-6,7-dimethyl-benzofuran-5-yl ester;

2-(1-Hydroxy-2-morpholin-4-yl-ethyl)-6,7-dimethyl-benzofuran-5-ol;

Acetic acid 6,7-dimethyl-2-(2-morpholiin-1-yl-acetyl)-benzofuran-5-yl ester;

Acetic acid 2-(6-hydroxy-3-methyl-[1,3]oxazinan-6-yl)-6,7-dimethyl-benzofuran-5-yl ester;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone;

1-(4-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;
2-Hydroxymethyl-6,7-dimethyl-benzofuran-5-ol;
6,7-Dimethyl-3-phenyl-benzofuran-5-ol;
6,7-Dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carbaldehyde;
4-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-benzonitrile;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 3,7-dimethyl-octa-2,6-dienyl ester;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid bis-(2-hydroxy-ethyl)-amide;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester;
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid (2-hydroxy-ethyl)-amide;
3-(4-Methoxy-phenyl)-6,7-dimethyl-benzofuran-5-ol;
3-(4-Chloro-phenyl)-6,7-dimethyl-benzofuran-5-ol;
3-(4-Fluoro-phenyl)-6,7-dimethyl-benzofuran-5-ol;
4,5-Dimethyl-1,8-diphenyl-benzo[1,2-b;4,3-b']difuran;
1,8-Bis-(4-fluoro-phenyl)-4,5-dimethyl-benzo[1,2-b;4,3-b']difuran;
1,8-Bis-(4-methoxy-phenyl)-4,5-dimethyl-benzo[1,2-b;4,3-b']difuran; and.
3-Amino-5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester.

Utility, Testing and Administration

General Utility

Compounds, compositions, formulations and methods of the present invention are useful for the treatment of disorders characterized by defective mitochondrial activity. In particular compounds of the present invention can be used in the treatment of diseases such as Alzheimer's disease, drug-induced Parkinsonism or Parkinson's disease, Friedreich's ataxia and other ataxias, Leber's hereditary optic neuropathy, and epilepsy.

Compounds, compositions/formulations and methods of the present invention are useful in treating a number of disorders, particularly those characterized by oxidative stress and/or inflammation. In particular, compounds of the present invention can be used in the treatment of cerebral ischemia ("stroke"), myocardial ischemia (myocardial infarction and other forms of heart disease), diabetes, renal disease, pre-menstrual syndrome, asthma, cardiopulmonary inflammatory disorders, chronic heart failure, rheumatoid arthritis, muscle fatigue, intermittent claudication and for the preservation of allograft tissue for transplantation.

The compounds of the present invention have also shown use in reducing C-reactive protein (CRP) associated with inflammation and /or inflammatory conditions including cardiovascular diseases or disorders, such as atrial fibrillation, unstable angina, coronary artery disease, peripheral artery disease, cardiac allograft vasculopathy (CAVD), mastitis, preeclampsia, inflammatory bowel conditions, stroke, tissue infarction, lumbosciatic, estrogen/progestin hormone replacement therapy (HRT); infection (bacterial, viral and protozoan), bacterial meningitis, trauma,

surgery, biomaterial implants, smoking, obesity, neurodegenerative diseases such as Alzheimer's, infectious disease such as for example myocarditis, cardiomyopathy, acute endocarditis or pericarditis, atherosclerosis, systemic inflammatory response (SIRS)/sepsis, adult respirator distress syndrome (ARDS), asthma, rheumatoid arthritis, osteoarthritis, systemic lupus erythematosis, airway hyperresponsiveness (AHR), bronchial hyper-reactivity, chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), inflammatory complications of diabetes mellitus type I and II, metabolic syndrome, end stage renal disease (ESRD), pre-menstrual syndrome (PMS) or muscle fatigue or inflammation, multiple organ dysfunction syndrome (MODS), airway hyper-responsiveness (AHR), bronchia hyper-reactivity, aging, acute allergic reactions, periodontal disease, such as gingivitis and dermal conditions including inflammatory skin conditions.

Testing

This section describes how compositions incorporating compositions of the present invention are selected, using *in vitro* and/or *in vivo* animal models, for example, and used as therapeutic interventions in three exemplary indications, i.e., stroke, chronic heart failure and myocardial infarction.

The effectiveness of a compound of this invention as a mitochondria protecting agent or neuroprotecting agent may be determined by assays such as the MPP+ Cell Death Assay, the MPTP assay or the Amyloid Assay.

Insults to the brain that disrupt its blood supply, as in ischemia, or its oxygen supply, as in hypoxia (low oxygen) or anoxia (no oxygen), rapidly cause neuronal imbalance leading to cell death (Flynn, C.J., *et al.*, 1989, *in* G. Siegel *et al., (Eds), Basic Neurochemistry,* Raven Press, NY). Investigations into the cellular and molecular mechanisms that lead to neuronal damage and inflammation associated with various types of brain ischemia can be carried out using *in vitro* model systems, such as primary cell cultures, that retain the metabolic characteristics of neurons *in vivo*. The use of such cell-based models has led to advances in identification of biochemical mechanisms leading to neuronal death in conditions such as anoxia, hypoglycemia, excitotoxicity, and exposure to reactive oxygen species. Neuronal cell lines such as the pheochromocytoma cell line, PC12, are also useful models for studying the effects of oxidative stress on the structure and function of neuron-specific proteins that are expressed in the cell lines. As many neuronal cell lines do not express all the properties of genuine neurons, primary neuronal cultures are now widely used as *in vitro* models in which to discern the processes that occur in intact brain.

In vitro models of ischemia approximate oxygen and glucose deprivation that mimic *in vivo* conditions, for example, by placing neuronal cultures into large anaerobic or hypoxic chambers and exchanging culture medium with de-oxygenated and defined ionic composition media. The toxic overstimulation of neuronal glutamate receptors, especially N-methyl-D-aspartate (NMDA) receptors, contributes to hypoxic-ischemic neuronal injury (Choi, D.M., 1988, *Neuron* 1: 623-634), ischemic induction of reactive oxygen species (ROS) (Watson, B.D., *et al.,*1988, Ann NY Acad Sci., 59: 269-281), excessive calcium influx (Grotta, J.C., 1988, *Stroke* 19: 447-454), arachidonic acid increase (Siesjo, B.K., 1981, *J. Cereb. Blood Flow Metab.* 1: 155-186)

and DNA damage (MacManus, J.P., *et al., 1993, Neurosci. Lett.,* 164: 89-92), each causing a cascade of neurodegeneration.

Primary embryonic hippocampal neuronal cells are widely recognized as useful in models of neuronal function. The hippocampus is a source of a relatively homogenous population of neurons with well-characterized properties typical of central nervous system (CNS) neurons in general. Pyramidal neurons, the principal cell type in the hippocampus, have been estimated to account for 85% to 90% of the total neuronal population (Banker and Goslin, 1998, *Culturing Nerve Cells, 2nd edition*. The MIT Press, Cambridge, Massachusetts). The hippocampus also exhibits a remarkable capacity for activity-dependent changes in synaptic function, such as long-term potentiation (Hawkins RD, Kandel ER, Siegelbaum SA. (1993) Learning to modulate transmitter release: themes and variations in synaptic plasticity [review], *Ann. Rev Neurosci.* 16:625-665.).

In experiments carried out in support of the present invention according to methods detailed in the Examples, anoxia/ischemia was induced in primary cultures of hippocampal neuronal cells, and compounds were tested for their ability to prevent cell death. Compounds found to have activity in such *in vitro* assays are then further tested in one or more animal models of cerebral ischemia ("stroke"), such as the middle cerebral artery occlusion (MCAO) model in rats.

Briefly, primary cultures of hippocampal neurons are used to test compounds for activity in neuronal protection. Hippocampal cultures are typically prepared from 18- to 19-day fetal rats. At this age, the generation of pyramidal neurons, which begins in the rat at about E15, is essentially complete. The brain tissue at this stage is relatively easy to dissociate, the meninges are removed readily, and the number of glial cells still is relatively modest (Park LC, Calingasan NY, Uchida K, Zhang H, Gibson GE. Metabolic impairment elicits brain cell type-selective changes in oxidative stress and cell death in culture. *J Neurochem* 74(1):114-124, (2000)).

In order to evaluate the activity of compounds of the present invention, a test compound is assessed for its ability to protect cells against one or more standard stressors, including hypoxia, as detailed in the Examples. In general, desirable therapeutic compound candidates are effective in this model at concentrations less than about 1 mM and even more preferably, less than about 100 μM. By effective, it is meant that such compounds protect at least 20%, preferably 30%, more preferably 40% and even more preferably 50% or more of the cells tested from stressor-induced death. By way of example, compounds that are effective in providing protection over a concentration a range of about 1 to 1000 μM would be expected to provide neuroprotection *in vivo*. Since precise values may vary depending upon the specific conditions under which the neuroprotective cell assay is carried out, it is the intent of the present disclosure to provide the foregoing criteria as guidance in the form of a benchmark against which to compare subsequently tested compounds, rather than to provide absolute concentrations at which the compounds of the present invention are considered to be effective. Typically, compounds that are found to be neuroprotective in such *in vitro* cell systems are then further tested in an *in vivo*

animal model of neuroprotection, such as the rat middle cerebral artery occlusion model described below, or other appropriate models such as are well known in the art.

Cerebral ischemic insults are modeled in animals by occluding vessels to, or within, the cranium (Molinari, G.F., 1986, *in* H.J.M. Barnett, *et al.,* (Eds) *Stroke: Pathophysiology, Diagnosis and Management,* Vol. 1, Churchill Livingstone, NY). The rat middle cerebral artery occlusion (MCAO) model is one of the most widely used techniques to induce transient focal cerebral ischemia approximating cerebral ischemic damage in humans, e.g., those who suffer from a stroke. The middle cerebral artery used as the ischemic trigger in this model is the most affected vessel in human stroke. The model also entails a period of reperfusion, which typically occurs in human stroke victims. MCAO involving a two-hour occlusion has been found to produce the maximum size of cortical infarction obtainable without increased mortality at twenty-four hours.

Briefly, a nylon filament is implanted into the right carotid artery of the rat. To effect occlusion, the rat is anesthetized, and the filament is advanced into the internal carotid artery 18-20 mm from the point of bifurcation of internal and external arteries and a suture is tightly ligated around the filament for a period of two hours. Two hours post occlusion, animals are re-anesthetized, and the filament is removed, to allow reperfusion for the remainder of the experiment. Test drugs can be administered any time during this process – before, during or after occlusion, and can be administered by one or more of a variety of means, including but not limited to intracerebroventricular (ICV) infusion, intravenous (IV) infusion, intraperitoneal (IP) administration, as well as enteral administration (e.g., gavage). Animals are maintained normothermic during the experiment, as described in the Examples. At a pre-determined time following occlusion and reperfusion, animals are sacrificed and their brains are removed and processed for assessment of damage as measured by infarct volume. In general, compounds are considered to have activity in this model, if they provide a significant reduction in total infarct volume at a dose that is less than about 10 mg/kg, preferably less than 1 mg/kg, more preferably less than 100 μg/kg and even more preferably less than about 1 μg/kg, when administered ICV or IV. By significant reduction of total infarct volume is meant a reduction of at least 20%, preferably at least 30%, more preferably at least 40%, and even more preferably about 50%, compared to control values.

Further validation of efficacy in neuroprotection can be assessed in functional tests, such as the grip strength test or the rotorod test. Animals treated with compounds that show neuroprotection maintain their pre-MCAO grip strength values after MCAO, as compared to untreated animals, which showed a significant reduction in grip strength, indicating loss of sensorimotor function. Likewise, animals treated with compounds that show neuroprotection also maintained their pre-MCAO rotorod activity scores after MCAO, as compared to untreated animals, which showed a significant reduction in rotorod scores, indicating loss of sensorimotor function at higher brain levels.

Similarly, primary cultures of myocytes can be used to test compounds *in vitro* for ability to provide protection against heart damage, resulting for example from myocardial ischemia or congestive heart failure. Preparation of myocardiocytes from neonatal rats is described in the

Examples. Such cells are typically used to study molecular models of myocardial ischemia (Webster, KA, Discher, DJ & Bishopric, NH. *J. Mol. Cell Cardiol.* **27**:453-458 (1995); Camilleri, L, Moins, N, Papon, J, Maublant, J, Bailly, P, de Riberolles, C & Veyre, A. *Cell Biol. & Toxicol.* **13**:435-444 (1997); Bielawska, AE, Shapiro, JP, Jiang, L, Melkonyan, HS, Piot, C, Wolfe, CL, Tomei, LD, Hannun, YA & Umansky, SR. *Am. J. Pathol.* **151**:1257-1263 (1997)) and are therefore accepted as indicative of myoprotective activity. Exemplary stressor assays for this purpose are provided in the Examples. For example, cardiomyocytes in culture exhibit contractile ("beating") activity; each cardiomyocyte contraction is associated with a rise in intracellular calcium termed a "calcium transient". These calcium transients can be measured using Fluo-4, a fluorescent dye which exhibits large fluorescence intensity increases upon the binding of calcium. This assay is cell-based and tests the ability of potential cytoprotectant molecules to guard against ischemic damage and allow the cells to maintain their contractile function.

Further validation of compounds can be carried out in a whole organ assay, such as the isolated heart (Langendorff) model of cardiac function. Similarly, compounds can be further validated in additional animal models of disease (e.g., diabetes, renal failure, asthma, muscle fatigue, inflammation), such as are well known in the art.

This section describes how compositions incorporating compositions of the present invention are selected, using *in vitro* and *in vivo* animal models and used as therapeutic interventions in dermatological indications. A number of cell screening assays for mediators of inflammatory response are well known in the art. Such mediators include but are not limited to inflammatory cytokines, interleukin-1 beta, and tumor necrosis factor alpha (TNF-alpha). Other molecules have been reported for use as markers of inflammation, including for example C-reactive protein (CRP), certain adhesion molecules, and chemical mediators such as leukotriene, thromboxane and isoprostane.

In vitro evaluation of anti-inflammatory activity can be determined by well-characterized assays such as the E-selectin (ELAM) production assay or the CRP assay, and *in vivo* evaluation can be determined by the carrageenan-induced paw edema assay, as exemplified in Examples. The ELAM assay measures activity of test compounds in reducing expression of ELAM in activated endothelial cells. Briefly, endothelial cells are created by adding known activators such as lipopolysaccharides, TNF, or IL-1.beta., alone or in some combination. Activated cells produce ELAM, which can be measured using, for example, an E-selecting monoclonal antibody-based ELISA assay. In studies carried out in support of the present invention, ELAM production was decreased. In *vivo* evaluation of anti-inflammatory activity as described in Examples can be determined by well-characterized assay. (Gabor, M. *Mouse Ear Inflammation Models and their Pharmacological Appications*, 2000). Carrageenan-Induced Paw Edema is a model of inflammation, which causes time-dependent edema formation following carrageenan administration into the intraplantar surface of a rat paw. The application of arachidonic acid (AA) to the ears of mice produces immediate vasodilatation and erythema, followed by the abrupt development of edema, which is maximal at 40 to 60 min. The onset of edema coincides with the extravasations of protein and leukocytes. After one hour the edema wanes rapidly and the

inflammatory cells leave the tissue so that at 6 hours the ears have returned to near normal. These assays, respectively, measure a test compounds' ability to treat these inflammatory processes via systemic and topical routes of administration.

MPTP/MPP^+-induced neurodegeneration of dopaminergic neurons is a well-characterized model that is widely used to understand the pathogenesis of Parkinson's disease. The compounds were tested for their ability to protect against MPTP/MPP^+ induced neuronal death *in vitro* and *in vivo* as shown in the following examples.

In vitro evaluation of protection against mitochondrial dysfunction is carried out using substantia nigra-derived dopaminergic progenitor cell line (described in Son JH, Chun et al. *J Neurosci*, **19**: 10-20 (1999)) exposed to 1-methyl-4-phenylpyridinium (MPP^+).

In vivo evaluation is carried out using mice that have been treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin. MPTP is metabolized into 1-methyl-4-phenylpyridinium (MPP^+) which then selectively kills dopaminergic neurons thereby inducing Parkinsonism. The striking pathologic and clinical similarities between idiopathic Parkinson's disease and MPTP-induced Parkinsonism suggest that the two disorders share common pathogenic mechanism.

A cellular assay using FRDA-patient derived fibroblasts (as described by Jauslin, ML et al, *Human Molecular Genetics* **11**; 3055-3063 (2002)); is used to determine the cell protecting effect of the test compounds by analyzing survival of skin fibroblasts taken from FRDA patients and unaffected normal donors under conditions of partial GSH depletion. Exposure of FRDA fibroblasts to BSO (L-buthionine (*S,R*)-sulfoximine) under conditions of restricted selenium and depletion of cellular glutathione (GSH) causes severe plasma membrane damage leading to cell death. Preincubation with the test compounds before the addition of BSO is used to determine if they can protect FRDA cells from BSO-mediated cell death. Cellular GSH content is also directly measured to exclude the possibility that the compounds may simply prevent BSO-mediated GSH depletion.

Protection against redox stress can be further evaluated in cell culture using either high glutamate induced oxidative stress in dopaminergic cell lines or beta-amyloid induced oxidative stress in hippocampal neurons. Using these assays the potency and efficacy of test articles against redox injury and cell death can be established in a high throughput manner as described in the Examples.

Administration

The compounds of this invention are administered at a therapeutically effective dosage, e.g., a dosage sufficient to provide treatment for the disease states previously described. Administration of the compounds of the invention or the pharmaceutically acceptable salts thereof can be via any of the accepted modes of administration for agents that serve similar utilities.

While human dosage levels have yet to be optimized for the compounds of the invention, generally, a daily dose is from about 0.01 to 2.0 mg/kg of body weight, preferably about 0.1 to 1.5 mg/kg of body weight, and most preferably about 0.3 to 1.0 mg/kg of body weight. Thus, for

administration to a 70 kg person, the dosage range would be about 0.7 to 140 mg per day, preferably about 7.0 to 105 mg per day, and most preferably about 21 to 70 mg per day. The amount of active compound administered will, of course, be dependent on the subject and disease state being treated, the severity of the affliction, the manner and schedule of administration and the judgment of the prescribing physician.

In employing the compounds of this invention for treatment of the above conditions, any pharmaceutically acceptable mode of administration can be used. The compounds of this invention can be administered either alone or in combination with other pharmaceutically acceptable excipients, including solid, semi-solid, liquid or aerosol dosage forms, such as, for example, tablets, capsules, powders, liquids, suspensions, suppositories, aerosols or the like. The compounds of this invention can also be administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, pills, transdermal (including electrotransport) patches, and the like, for the prolonged administration of the compound at a predetermined rate, preferably in unit dosage forms suitable for single administration of precise dosages. The compositions will typically include a conventional pharmaceutical carrier or excipient and a compound of this invention or a pharmaceutically acceptable salt thereof. In addition, these compositions may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, and the like, including, but not limited to anticoagulants, blood clot dissolvers, permeability enhancers and slow release formulations.

Generally, depending on the intended mode of administration, the pharmaceutically acceptable composition will contain about 0.1% to 90%, preferably about 0.5% to 50%, by weight of a compound or salt of formula I, the remainder being suitable pharmaceutical excipients, carriers, etc.

One preferred manner of administration for the conditions detailed above is oral, using a convenient daily dosage regimen which can be adjusted according to the degree of affliction. For such oral administration, a pharmaceutically acceptable, non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium crosscarmellose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations and the like.

Preferably the compositions will take the form of a pill or tablet and thus the composition will contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, or the like; a lubricant such as magnesium stearate or the like; and a binder such as starch, gum acacia, polyvinylpyrrolidine, gelatin, cellulose and derivatives thereof, and the like.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances

such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, sodium acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 15th Edition, 1975. The composition or formulation to be administered will, in any event, contain a quantity of the active compound in an amount effective to alleviate the symptoms of the subject being treated.

Dosage forms or compositions containing active ingredient in the range of 0.005% to 95% with the balance made up from non-toxic carrier may be prepared.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, talcum, cellulose derivatives, sodium crosscarmellose, glucose, sucrose, magnesium carbonate, sodium saccharin, talcum and the like. Such compositions take the form of solutions, suspensions, tablets, capsules, powders, sustained release formulations and the like. Such compositions may contain 0.01%-95% active ingredient, preferably 0.1-50%.

For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is preferably encapsulated in a gelatin capsule. Such diester solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patents Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, e.g. in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g. water, to be easily measured for administration.

Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the active compound or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g. propylene carbonate) and the like, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells.

Other useful formulations include those set forth in U.S. Patents Nos. Re. 28,819 and 4,358,603.

The formulation can be administered in a single unit dosage form for continuous treatment or in a single unit dosage form *ad libitum* when relief of symptoms is specifically required. For example, the formulation may be administered as a bolus or as a continuous intravenous infusion after onset of symptoms of stroke, myocardial infarction or chronic heart failure.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, solubility enhancers, and the like, such as for example, sodium

acetate, sorbitan monolaurate, triethanolamine oleate, cyclodextrins, etc.

A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.01% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably the composition will comprise 0.2-2% of the active agent in solution.

Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients can also be administered.

Formulations of the active compound or a salt may also be administered to the respiratory tract as an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation have diameters of less than 50 microns, preferably less than 10 microns.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

General Characterization Methods

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker DTX 300 spectrometer using, in most cases, tetramethyl silane (TMS) as the internal reference. Mass spectra were obtained on an Agilent 1100 LC/MSD instrument using either electrospray ionization (positive or negative mode) (ESI) or atmospheric pressure chemical ionization (positive or negative mode) (APCI).

Example 1

Determination of Activity Utilizing-Neuronal Cell Stress Assay

A. Isolation and Culture of Primary Hippocampal Neuronal Cells.

Materials

- Neurobasal/B27i: Neurobasal medium (Life Technologies, Rockville, MD) with 1x B27 supplement (Life Technologies), 0.5 μM L-glutamine, 25 μM L-glutamic acid, and 1 x Penicillin/Streptomycin.
- Hank's Basic Salt Solution (HBSS, Ca/Mg-free) was prepared by preparing 1X Hanks CMF (Gibco) supplemented with HEPES (10 mM, pH 7.3), sodium bicarbonate (0.35%), 1X Penicillin/Streptomycin, and 1 mM pyruvate.
- Poly-D-lysine (Sigma, St. Louis, MO), 50 μg/ml solution filtered through 0.2 μm filter tubes.
- Sigmacote (Sigma, St. Louis, MO).
- Plastic Culture Flasks (T75 cm^2) or 12-well cell culture plates treated with Poly-D-Lysine (Sigma, St. Louis, MO).

Preparation of Primary Hippocampal Neuronal Cells

A pregnant female mouse (E18-E19) was euthanized with CO_2 prior to removal of the uterus, which was then placed in a sterile plastic petri dish. The embryos were removed from the sac, and the embryonic brains were removed and immersed in cold (4°C) Buffered Salt Solution (HBSS; Ca/Mg free; Life Technologies) in a small petri dish. Hippocampi were then removed from the brains under a dissecting microscope and were placed on a paraffin-covered dish. The meninges were stripped away and the dissected hippocampi were collected in a small petri dish in HBSS. The hippocampi were transferred to a 15-ml centrifuge tube (normally 10-12 brains) filled with HBSS. The tube containing the brains was centrifuged at 1000 rpm for 2 min in a tabletop centrifuge. The supernatant was removed, 2 ml of HBSS was added to the hippocampi in the tube, and the resulting suspension was triturated 2 times each with long-tipped siliconized glass pipettes having progressively smaller apertures, starting with a pipette with a standard size

opening (approximately 1.0 mm diameter), following with one having an aperture of half standard size (approximately 0.5 mm diameter), then with one having an aperture about one-half that size (0.25 mm diameter). The suspension was then centrifuged again at 1000 rpm for 2 min in a tabletop centrifuge, the supernatant was discarded, and 2 ml of Neurobasal/B27i (with antibiotics) was added to the tube. The trituration procedure described above was then repeated on this suspension.

The density of cells was determined on a small aliquot of cells using standard counting procedures and correcting for cell viability by trypan blue stain exclusion. Using this procedure, the expected yield is 3×10^5 - 6×10^5 cells/brain. Cells were then added to PDL-coated 24-well plates, flasks or MetTek dishes in Neurobasal/B27I at a density of about 1.5×10^6 cells (T75 flask) or about 70,000 cells/well of a 24-well plate. Plated cells were incubated at 37 degrees in an atmosphere of 5 % CO_2/ 95 % O_2. Media was renewed after 3-4 days by replacing half of it with fresh Neurobasal/B27m medium, containing 5 µM cytosine arabinoside (AraC). Seven to eight days from the initial culture, the media was renewed again, by removing one-half or it and replacing with an equal amount of fresh Neurobasal/B27m medium (without Ara-C).

B. Hippocampal Anoxia-Reoxygenation Cell Death Assay.

This assay was used to induce ischemia by anoxia-reoxygenation in cultured hippocampal neuronal cells. Test compounds were added to assess potency and efficacy against ischemia-induced neuronal cell injury and cell death.

Materials.

- Neurobasal media, NoG neurobasal media, B27 supplement and B27 Supplement minus AO were obtained from Invitrogen Life Technologies.
- Neurobasal/B27 medium was prepared with 2X B27 minus AO supplement, 0.5 mM L-glutamine and 0.25X penicillin/streptomycin.
- Cell Tracker Green was obtained from Molecular Probes and a fresh 5µM solution was prepared from 10 mM stock just before use.
- LoG-Neurobasal contains NoG neurobasal medium plus 1 mM glucose, 0.5 mM L-glutamine, 0.25X Penicillin/Streptomycin, and 10mM Hepes (pH 7.4).
- Primary hippocampal neuronal cells were prepared according to the methods described above and were cultured in poly-D-lysine coated 24-well plates for 10-11 days prior to use.

Deoxygenated LoG-Neurobasal medium (100 ml) was prepared by pre-equilibrating the medium in a T150 cm^2 flask in a hypoxic chamber overnight. Following pre-incubation under hypoxic conditions, the LoG-Neurobasal media was lightly bubbled with 100% N_2 for 30 min to completely deoxygenate the media. An additional 20 ml LoG-Neurobasal was pre-equilibrated in a T75 cm^2 flask and was incubated in a normal incubator (5% CO_2) overnight. Reoxygenated medium was prepared by placing Neurobasa/B27 media overnight in the culture incubator (5% CO_2/95% O_2).

10-11 Days after plating the hippocampal neurons, existing culture medium (Neurobasal/B27m) was removed from the cells by aspiration. Cells were washed once with 600

µl/well (24-well culture plates) of glucose free-BSS. Neurons were replenished with deoxygenated LoG-Neurobasal (400 µl per well for each well of a 24-well plate). Test compounds were added directly to each well (usually 3 concentrations of the compound plus positive control, each in triplicate). Most test compounds were dissolved in 100% DMSO; however, concentrations were adjusted such that the final concentration of DMSO in the cell media never exceeded 0.5%. Plates containing cells with test compounds were placed in a hypoxic chamber for 4-5 hr with plate lids ajar. For normoxia controls, pre-equilibrated normoxic LoG-Neurobasal medium was added to each well of cells, and the plate was replaced in the normal culture incubator for4- 5 hr. After 4-5 hr of hypoxia, the existing media was carefully aspirated off, and 400 µL of new, reoxygenated (pre-equilibrated) Neurobasal/B27 was added to each well. The same test compounds (in the same the concentrations) were added back into the corresponding wells. Plates were placed in the cell culture incubator (5% CO_2/95% O_2) and reoxygenated for 20-24 hr. After reoxygenation for 20-24 hr, live neurons were quantitated using the cell tracker green fluorescence method, described below.

To test for cell viability, existing culture medium was aspirated from each well of the 24 well plates, and neurons were washed once with 1 mL of HBSS (pH 7.4, pre-warmed to 30-37°C). To each well was added 500 µL of 5 µM Cell Tracker Green fluorescent dye dissolved in HBSS. Plates were placed in the dark at room temperature for 15 minutes, then were washed with 1 mL of HBSS. 500µL of HBSS was then added to each well, and fluorescent cells were counted using a fluorescent microscope. Significantly increased cell viability compared to control cells is indicative of a protective compound.

Certain compounds of the present invention such as:

3-(4-Fluoro-phenyl)-6,7-dimethyl-benzofuran-5-ol;

3-(4-Methoxy-phenyl)-6,7-dimethyl-benzofuran-5-ol

3-(4-Chloro-phenyl)-6,7-dimethyl-benzofuran-5-ol

when tested as described above provided protection against stressor-induced cell death in at least about 20% of the cells tested, at concentrations ranging from about 4 to 100 µM.

Example 2

Myocyte Calcium-Contractility Assay.

A. Isolation and Culture of Primary Neonate Myocytes.

Materials

- 10X Heart Dissection Solution (HDS) contains the following components (g/l) in tissue grade water:_NaCl, 68; HEPES, 47.6; NaH_2PO_4, 2; Glucose, 10; KCl, 4; $MgSO_4$, 1, pH adjusted to 7.4. Prior to filter sterilization of diluted (1XHDS) solution, 10 mg phenol red was added to each 500 milliliters of medium.
- Transferrin and Bovine Insulin were obtained from Life Technologies, and resuspended at a concentration of 4 mg/ml in tissue culture grade water.
- DMEM-F12 – DMEM/F12, powder, 1:1 containing glutamine and pyridoxine hydrochloride was purchased from Life Technologies. To one liter equivalent of the powder was added

2.43g of sodium bicarbonate and 10 ml of 100X Penicillin/Streptomycin in 950ml of tissue culture grade water with stirring. The pH was adjusted to 7.2 with 1M HCl and volume was adjusted to 1 liter. The solution was filter sterilized then 2.5 ml of 4mg/ml Transferrin, 250µl 4mg/ml Insulin and 30.7 mg of bromodeoxyuridine were added.

- DMEM-F12 was also prepared 4% FBS for pre-coating the tissue culture plates and initial suspension of the cardiomyocyte pellet.
- Collagenase solution– 49mg of collagenase was resuspended in 120 ml 1x HDS.

Preparation of Primary Neonatal Myocyte Cultures

Tissue culture ware was pre-coated with DMEM-F12-4%FBS by incubating 50µl per well of a 96-well plate and 0.25ml per 12-well plate at 37°C.

Two-day old rat pups were removed from their mothers and placed in a sterile container. Pups were dipped quickly into 70% alcohol, then decapitated and the body was placed in an empty sterile tissue culture dish. An incision was made starting at the neck and progressing towards the belly, cutting through the sternum. The heart was removed and placed in a tissue culture dishes containing 1x HDS. The atria were trimmed, and the remaining ventricles were placed into a separate tissue culture dish containing 1x HDS, where they were sectioned into 3-4 pieces each. Ventricles were then transferred to a sterile 250ml glass flask and the 1x HDS was removed. Twenty milliliters of pre-warmed collagenase solution were added to the ventricles, followed by incubation at 37°C with shaking. After 30 minutes, the collagenase solution was removed and replaced with 20ml fresh pre-warmed collagenase. Incubation was continued for an additional 30 minutes. At the end of the incubation, any tissue chunks were allowed to settle prior to removing the collagenase (containing the isolated cardiomyocytes) from the disrupted tissue pieces. The isolated myocytes were added to a 50ml Falcon tube containing 2ml Fetal Bovine Serum (FBS). The remaining tissue pieces were subjected to a second digestion by adding 20ml fresh pre-warmed collagenase and incubating as above for 30 minutes. This second digest was then centrifuged at 1000 rpm for 10 minutes (tabletop centrifuge). The resulting supernatant was discarded, and the cell pellet was suspended with 4ml FBS. The resulting cell suspension was placed in the incubator at 37°C. This step was repeated several additional times to harvest additional material.

Percoll gradients were prepared by adding 2.5ml of 10x HDS to 22.5ml of Percoll (Life Technologies) with mixing (Percoll Stock). Top Gradient solution (11ml Percoll Stock and 14ml 1x HDS) and Bottom Gradient solution (13ml Percoll Stock and 7ml 1x HDS) were prepared. Four milliliters of the Top Gradient solution were transferred into 6 x 15ml sterile Falcon tubes. Three milliliters of the Bottom Gradient solution were placed in each tube by inserting a serological pipette to the bottom of the tube and slowly adding the liquid.

All the digests (5) were pooled in one 50ml Falcon tube and centrifuged on a tabletop centrifuge at 1000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in 12ml of 1x HDS. Two milliliters of the cell suspension was added to the top of each gradient. The gradient tubes were then centrifuged at 3000 rpm for 30 minutes without

braking in a Beckman Allegra 6 centrifuge (GH 3.8A rotor). Following centrifugation, the cells segregated into two sharp bands at the two interfaces. The lower band of the two bands was enriched for cardiomyocytes; there was also a cardiomyocyte pellet at the bottom of the tube. The upper band was enriched for fibroblasts and other non-cardiomyocytes. The upper portion of the gradient was aspirated down to just above the cardiomyocyte layer. The cardiomyocyte layer was then carefully removed along with the pellet, and the two fractions were pooled in a sterile 50ml Falcon tube, along with corresponding fractions from additional gradient tube; then 1x HDS was added to a total volume of about 50ml. The tube was centrifuged at 1000 rpm for 7 minutes. The supernatant was discarded and resuspended in 25ml 1x HDS. A further 25ml of 1x HDS was added and the centrifugation step was repeated. The cell pellet was resuspended carefully but thoroughly in 40-50 of DMEMF12-4% FBS.

A small aliquot of the cell suspension was counted in a hemocytometer. The DMEM/F12-FBS coating medium was aspirated from the tissue culture dishes. The cardiomyocytes were added to the dishes at a plating density of $7.5x10^4$ / well per 96-well in 200µL and 1.5×10^5 /well per 12-well in 3ml. The cultures were incubated at 37°C with 5% CO_2 overnight. The original medium was removed, and add fresh DMEM/F12-5% FBS was added to each culture, prior to incubation at 37°C with 5% CO_2 for a further 48 hours, before use.

B. Contractility Assay

Materials

- Complete DMEM-F12: DMEM/F12, powder, 1:1 containing glutamine and pyridoxine hydrochloride was purchased from Life Technologies (Invitrogen Life Technologies, Carlsbad, CA). Powder sufficient to prepare one liter of buffer and 2.43g of sodium bicarbonate was mixed into 950ml of tissue culture grade water. The pH was adjusted to 7.2 with 1M HCl and the remaining water was added to make 1 liter. Following filter sterilization, 10ml of 100X Penicillin/Streptomycin, 2.5ml of 4mg/ml Transferrin, 250µl 4mg/ml Insulin and 30.7 mg of bromodeoxyuridine were added, and the mixture was incubated at 37°C prior to use.
- 1 mM glucose in DMEM was made from DMEM without L-glutamine, without glucose, without sodium pyruvate, purchased from Life Technologies.
- 20µM Fluo-4: Cell permanent AM ester of Fluo-4 was obtained from Molecular Probes (Eugene, OR) as a dry powder to be stored at -20°C. This fluorescent dye is light sensitive and should be made up fresh at 1mM in DMSO prior to use to prevent light degradation.
- 10mM $CaCl_2$ solution was made fresh each day in 1x HBSS and incubated at 37°C prior to use.

Neonatal cardiomyocytes were isolated as described above. The cardiomyocytes were plated in 96-well format (black clear-bottomed plates) at a density of 7.5×10^4 per well and grown for 2 days in the presence of 5% FBS prior to use in the assay.

Physiological ischemia was simulated by placing the cardiomyocytes in an anaerobic chamber (0% O_2, 85% N_2, 5% CO_2 & 10% H_2) in DMEM containing 1mM glucose. Positive control cells are treated with DMEM-F12 containing 25mM Glucose, which protects against the anoxia.

The test compounds were made up in DMEM-1mM glucose in 96 deep-well mother plates and appropriately diluted for use in the assay. The media was removed from the cells and replaced with 200µl of either DMEM-F12 or 1mM DMEM with or without test compounds. The plates were then placed inside the 37°C incubator in the anaerobic chamber and incubated for 16 hours. The plates were then removed and reoxygenated by the addition of DMEM-F12. The DMEM with or without test compounds is carefully removed from the cells and replaced with pre-warmed DMEM-F12 containing 5% FBS. Since the anoxic treatment may damage and/or kill the cells, causing them to dislodge from the bottom of the wells gentle aspiration of media is required at this step. The cells were then placed in a normal incubator at 37°C and incubated for two hours to allow the cells to reoxygenate.

A working solution of 20 µM Fluo-4 was added to pre-warmed 1xHBSS. The cells were loaded with Fluo-4 by first removing media from the cells and replacing with 100 µl of 20 µM Fluo-4. Unloaded control cells were treated in parallel with 1xHBSS alone. All cells were then incubated at 37°C for 30 minutes. Before fluorescence measurements were made, the cells were washed in indicator-free medium (HBSS) to remove any dye that is non-specifically associated with the cell surface. Cells were then incubated for an additional 20 minutes at room temperature. Basal Fluo-4 fluorescence was measured using the 485nm excitation and 538nm emission filter pair on a microplate flourometer (Fluorskan™, Thermo Labsystems Oy, Helsinki, Finland). Each well was read for 160ms to obtain a baseline reading, then stimulated to contract by addition of 10mM $CaCl_2$. Following incubation at 37°C for 30 minutes, a stimulated fluorescence was taken after 90 minutes.

Compounds of the present invention such as

(5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone;

Acetic acid 6,7-dimethyl-2-(2-morpholin-1-yl-acetyl)-benzofuran-5-yl ester;

2-(1-Hydroxy-2-morpholin-4-yl-ethyl)-6,7-dimethyl-benzofuran-5-ol;

Acetic acid 2-(2-bromo-acetyl)-6,7-dimethyl-benzofuran-5-yl ester;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone;

1-(4-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

4-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-benzonitrile;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carbaldehyde;

2-Hydroxymethyl-6,7-dimethyl-benzofuran-5-ol;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid bis-(2-hydroxy-ethyl)-amide;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester; and
5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid bis-(2-hydroxy-ethyl)-amide;
when tested as described above show a protection of at least 20% and the presence of calcium transients in amounts indicative of ability guard against ischemic damage and allow the cells to maintain their contractile function.

Example 3

Rat Middle Cerebral Artery Occlusion (MCAO) Model of Cerebral Ischemia

A. Animal Preparation

Male Wistar rats (Harlan, IN) weighing 300-350g are commonly used in these experiments. Animals are allowed free access to water and commercial rodent diet under standard laboratory conditions. Room temperature is maintained at 20-23 °C and room illumination is on a 12/12-hour light/dark cycle. Animals are acclimatized to the laboratory environment 5 to 7 days prior to the study, and fasted (with free access to water) overnight before surgery.

B. Middle Cerebral Artery Occlusion (MCAO)

Anesthesia is maintained by inhalation of 3.0% isoflurane (Aerrane, Front Dodge, IA) in 0.8% oxygen. The animal's neck was shaved and sterilized before operation. Body temperatures were controlled and maintained at 37.5°C +/-1 degree via external heating and cooling devices. To lower the body temperature, animals are placed in a cooling chamber, which uses ice to cool circulating air. Throughout the study the body temperature is recorded using a temperature transponder (BMDS Inc., Seaford, DL) implanted subcutaneously at the time of MCAO between the rat shoulder blades that allows the user to read the body temperature via a pocket scanner (BMDS Inc., Seaford, DL). The body temperature may also be taken by inserting the temperature probe into the animal's rectum. Body temperature is recorded every hour for 6 hours post-occlusion; however, body temperatures were taken more frequently so that they could be maintained at the normothermic temperature.

Animals were subjected to two hours MCAO using a modified intraluminal filament technique, as follows: A midline incision on the ventral part of the neck is made to expose external and internal carotid arteries. The right external and common carotid arteries are ligated by a suture (silk 5/0, Carlisle Laboratories, Farmers Branch, TX) and the right internal artery is temporarily ligated using a microvascular clip (Fine Science Tool Inc., Foster City, CA). A small incision was made in the common carotid artery. A nylon filament, its tip rounded by heating, is prepared from a fishing line (Stren Fishing Lines, Wilmington, DE) and is inserted from the right common carotid artery. The filament is advanced into the internal carotid artery 18-20 mm from the point of bifurcation of internal and external arteries and a suture is tightly ligated around the filament. Two hours post occlusion, animals are re-anesthetized to allow reperfusion for the remaining of the experiment by removal of the filament.

C. Drug Administration

Test compounds may be administered by any of a number of routes, such as those described below. Compounds can be administered before, during or after occlusion, as appropriate to the protocol.

a) Intracerebroventricular (ICV) Infusion

The anesthetized animal is placed on a stereotaxic apparatus (Harvard Apparatus, S. Natick, MA). Anesthesia is maintained by inhalation of 3.0% isoflurane (Aerrane, Front Dodge, IA) in 0.8% oxygen throughout the entire procedure. The scalp is shaved and sterilized prior to surgery. A midline sagittal incision about 3 cm long is made slightly behind the eyes to expose the skull. The skull is scraped with a rounded end spatula to remove periosteal connective tissue. A bur hole is placed 1.5mm lateral, 1 mm posterior to the left of the bregma to mark the left lateral ventricle. A brain infusion cannula (ALZET Co., Palo Alto, CA) is inserted 4 mm deep into the hole. The desired depth is adjusted by attaching spacers to the cannula. The cannula attached to a 4-cm silastic catheter (Helix Medical Inc., Carpinteria, CA) fixed in place with dental cement (Ketac-cement, Norristown, PA). The catheter is either attached to a primed osmotic pump placed subcutaneously between the shoulder blades for permanent infusion or to a syringe for a short infusion.

b) Intravenous (IV) Osmotic Pump Implantation into the jugular vein

Anesthesia is maintained by inhalation of 3.0% isoflurane (Aerrane, Front Dodge, IA) in 0.8% oxygen throughout the entire procedure. The animal's neck will be shaved and sterilized before operation. A midline incision is made on the ventral part of the neck to exposes the jugular vein. The vein is isolated and ligated with a suture (silk 5/0, Carlisle Laboratories, Farmers Branch, TX) rostral to the point of the incision and a microvascular clip (Fine Science Tool Inc., Foster City, CA) close to the heart. A small incision is made between two ligations. A 2-cm silastic catheter (Helix Medical Inc.) attached to a PE-60 tube (Becton. Dickinson and Co. Sparks, MD) connected to an ALZET (ALZET CO. Palo Alto, CA) pump is introduced and advanced 2 mm into the jugular vein toward the heart. The microvascular clip is removed and the catheter is secured in place with a suture (silk 5/0, Carlisle Laboratories, Farmers Branch, TX). The pump is placed into a pocket made subcutaneously between the shoulder blades, allowing the catheter to reach over neck to the jugular vein with sufficient slack to permit free movement of neck and head.

c) IV infusion via femoral vein

Anesthesia is maintained by inhalation of 3.0% isoflurane (Aerrane, Front Dodge, IA) in 0.8% oxygen throughout the entire procedure. The exterior site of the right femoral vein is shaved and sterilized prior to surgery. A 3-cm incision is made in the right groin region and the femoral vein is isolated. A small incision is made on the femoral vein temporarily ligated with a microvascular clip to introduce and advance a polyethylene (PE-50) catheter (Becton Dickinson and Co. Sparks, MD). The catheter is secured in place with suture (silk 5/0, Carlisle Laboratories, Farmers Branch, TX). The other end of the catheter is attached to a syringe filled with the heparinized saline for the bolus injection. Using a hemostat, a pocket is made subcutaneously on the back of the animal so the PE catheter can be brought up to the exteriorization point at the nape of the neck for either a bolus injection or a continuous injection by an osmotic pump.

d) Intraperitoneal (IP) Injection

An awake rat is held in a standard hand hold position, a 23 3/4G needle is injected into the lower right quarter of the abdomen pass the peritoneum, slightly off the midline. To avoid organ injection, the plunger of the syringe is slightly pulled back. If no fluid is withdrawn, the content of the syringe is delivered into the abdominal cavity.

D. Behavioral Assessment

One hour after MCAO, the animal was gently held by its tail and observed for forelimb flexion. Then the animal is placed on the floor to be observed for walking pattern; only the animals that score 3 on Bederson grading system (Table 1) are included in the study.

Table 1

Bederson Grading System for Neurological Evaluation

Neurological deficit	**Grading**	**Behavioral observation**
Normal	grade 0:	No observable deficit
Moderate	grade 1:	forelimb flexion
Severe	grade 2:	forelimb flexion, decreased resistance to lateral push
Extreme	grade 3:	forelimb flexion, decreased resistance to lateral push, circle to paretic side

E. Evaluation of Ischemic Damage

Twenty-four hours post-MCAO, or longer, in some experiments, animals were sacrificed by CO_2 asphyxiation (dry ice). The brain was quickly removed from the skull, using standard procedures, rinsed in chilled saline solution, and placed on a rat brain tissue slicer (ASI instrument, MI). Seven 2-mm thick coronal slices are cut from each brain using razor blades. The slices were immersed in 0.9% saline containing 1.0% 2,3,5-triphenyltetrazolume chloride (TTC) (Sigma Chemical Co., St. Louis, MO) and incubated in a 37°C water bath for 30 minutes.

After staining, each 2-mm slice is photographed with a TMC-7 camera (JH Technologies, Ca) which is directly connected to a desktop PC to capture and save the image of each brain slice. This image is used for the measurements of the regions of interest using a computer-based image processing system (Metamorph).

To measure each area, the region of interest is selected using a freehand selection tool, the area is automatically computed by selecting the measure command. The measurements for primary regions of interest are right hemisphere, left hemisphere, total infarct, subcortical infarct, total penumbra and subcortical penumbra. After all regions of interest are measured for all seven slices of the brain, they are sorted by slice number and the corresponding regions of interest using an Excell macro called statistic final. This macro also calculates the cortical penumbra, cortical infarct and total ischemic damage for each slice; the corresponding areas of each rat brain will be added together to produce a single measurement for each area. Since the ipsilateral hemisphere is swollen following MCAO, edema volume is calculated and reported as the volumetric differences between the right and left hemispheres of each brain slice. Using the % of hemispheric swelling all the volumes will be corrected for the edema. The volume of the damage is determined using the calculations below for each rat's brain.

Measurement	Equation	Corrected Value(s)
Cortical Penumbra (C.P.)	Total Penumbra- Subcortical Penumbra	Total Penumbra ($T.P._{corr}$) = (T.P. x %H.S./100) $C.P._{corr.}$= C.P. - (C.P. x %H.S./100) $S.P._{corr.}$ = S.P. - (S.P. x %H.S./100)
Cortical Infarct	Total Infarct- Subcortical Infarct	$T.I._{corr.}$ = T.I. - (T.I. x %H.S./100) $S.I._{corr.}$ = S.I. - (S.I. x %H.S./100) $C.I._{corr.}$ = C.I. - (C.I. x %H.S./100)
Total Ischemic Damage (T.I.D.)	Total Penumbra + Total Infarct	$T.I.D._{corr}$= T.I.D.-(T.I.D.x%H.S./100)
Total Volume (mm^3)	Each value is multiplied by 2 (the thickness of the tissue).	
Edema Volume	The volumetric differences between the sum of right and left hemispheres determines the edema volume.	
% Hemispheric swelling (H.S.)	Edema x 100/left hemisphere	

F. Statistical Analysis

Sample size is chosen to achieve a 90% probability of significant results. The measurements, which represented the same region of interest in seven slices of each rat's brain are added together to yield a single measurement for total infarct, subcortical infarct, cortical infarct, total penumbra, subcortical penumbra, cortical penumbra, total ischemic damage and edema in each animal. Group data are presented as means +/- SEM. Differences at the level of $p<0.05$ are considered statistically significant. Between groups comparison of each region of interest are carried out by unpaired student t test (between two groups) or one way ANOVA followed by post hoc Bonferroni's multiple comparisons or by the nonparametric Dunnett's test (between control and the drug treated groups).

Compounds of the present invention can be tested as described above.

Example 4

FRDA Fibroblast Assay for Protection from Oxidative Stress

A. Cell culture and reagents

Primary fibroblasts were derived from donors with a molecular diagnosis of FRDA and control donors with no mitochondrial disease. Cell lines C1 and F1 were provided by the Swiss Network on Friedreich Ataxia Research, line F3 was provided by Hopital Necker, Paris (France), and lines F2, C2 and C3 were obtained from Coriell Cell Repositories (Camden, NJ, USA; catalog nos GM04078, GM 08402 and GM08399, respectively). All cell types were diagnosed at the molecular level for intronic GAA triplet repeat length using a PCR-based method, according to

methods known in the art. FRDA-cell types had ~400-450 repeats (F2 line) or more (F1 and F3), whereas control cell lines displayed PCR products of normal length. The cells were seeded in microtiter plates at a density of 4000 cells per 100µl in growth medium consisting of 25% (v/v) M199 EBS and 64% (v/v) MEM EBS without phenol red (Bioconcept, Allschwil, Switzerland) supplemented with 10% (v/v) fetal calf serum (PAA Laboratories, Linz, Austria), 100 U/ml penicillin, 100 µg/ml streptomycin (PAA Laboratories, Linz, Austria), 10µg/ml insulin (Sigma, Buchs, Switzerland), 10 ng/ml EGF (Sigma, Buchs, Switzerland), 10 ng/ml bFGF (PreproTech, Rocky Hill, NJ, USA) and 2mM glutamine (Sigma, Buchs, Switzerland). The cells were incubated in the presence of the various test compounds for 24 h before addition of BSO (L-buthionine (*S,R*)-sulfoximine).

B. Cell viability measurements

Cell viability was measured after the first signs of toxicity appeared in the BSO-treated controls (typically after 16-48h). The cells were stained for 60 min at room temperature in PBS with 1.2µm calceinAM and 4µm ethidium homodimer (Live/Dead assay, Molecular Probes, Eugene, OR, USA). Fluorescence intensity was measured with a Gemini Spectramax XS spectrofluorimeter (Molecular Devices, Sunnyvale, CA, USA) using excitation and emission wavelengths of 485 and 525 nm, respectively. Live cell imaging was performed with a Zeiss Axiovert 135 M fluorescence microscope equipped with a cooled CCD camera (Sensicam, PCO Computer Optics, Kelheim, Germany). Image acquisition was performed with the ImagePro Plus software (Media Cybermetics, Silver Spring, MD, USA).

C. Glutathione content

Cellular GSH content was measured to exclude the possibility that the compounds simply prevented BSO-mediated GSH depletion. Cells were removed from 100 mm culture dishes by trypsinization, washed twice with PBS, snap-frozen in 100 µl PBS and lysed in PBS supplemented with a protease inhibitor cocktail (Complete, Roche Diagnostics, Rotkreuz, Switzerland) by four freeze-thaw cycles. Total protein content was measured with the BioRad protein assay (BioRad, Hercules, CA, USA). Reduced glutathione content was determined essentially as described by Kamencic et al. (2000) with a final monochlorobimane (mCIB, Molecular Probes, Eugene, OR, USA) concentration of 25µM. The GSH-mCIB adduct fluorescence was measured with a Gemini spectrofluorimeter using excitation and emission wavelengths of 380 and 470 nm, respectively.

D. Glutathione peroxidase assay

Cell extracts obtained for total GSH measurements were adjusted to a final protein concentration of 1.85 mg/ml. Enzymatic activity was measured with the Glutathione peroxidase cellular activity assay kit (Sigma, St Louis, MO, USA) according to manufacturer's instructions with minor modifications. Enzymatic activity was measured with 40 µg total protein extract in a final reaction volume of 100 µl monitoring NADPH consumption as a decrease in NADPH

fluorescence. GPX enzymatic activities were determined by measuring the maximum velocity of the NADPH consumption using tert-butyl hydroperoxide as substrate and purified bovine erythrocytes GPX (Sigma, St Louis, MO, USA) as standard. All measurements were done in triplicate in 96-well plates with a Gemini spectrofluorimeter using excitation and emission wavelengths of 340 and 445 nm, respectively.

E. Data and statistics

In experiments carried out in support of the present invention, certain compounds such as 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester; 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 3,7-dimethyl-octa-2,6-dienyl ester; 2-Hydroxymethyl-6,7-dimethyl-benzofuran-5-ol; (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone ; and 3-amino-5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester significantly reduced cell death in FRDA fibroblasts compared to untreated FRDA fibroblasts with an EC_{50} of between about 0.8μM and 27μM.

Example 5

Evaluations of Sensorimotor Behavior

A. Fore and Hindlimb Grip Strength Test in Rats

Animals with cerebral infarction induced by transient or permanent unilateral occlusion of the middle cerebral artery (MCA) and sham-operated rats are tested for grip strength, a standard model of neuromuscular function and sensorimotor integration, using a Computerized Grip Strength Meter for Rats (Dual Stand Model, Columbus Instruments, Columbus, OH).

Animals are moved into the testing room for 30 minutes before testing. Prior to testing, each gauge is calibrated with a set of known weights and the apparatus is adjusted for the size of animal, according to manufacturer's instructions. The forelimb measurements are carried out with the meter in the tension peak mode to freeze the reading as the subject is pulled away from the grip bar. The hindlimb measurements are carried out with the meter in the compression peak mode to freeze the reading as the subject's hindlimbs are pulled over the bar toward the meter. Each animal is hand-held by the investigator while pulled past the grip bars, using a consistent technique, leaving the fore and hind limbs free to grasp the grip bars.

Testing is carried out on postoperative day 2 and repeated, in a blind-randomized fashion, twice weekly for a defined interval. Typically, three successive readings are taken for each animal with an intertrial interval long enough to record the data and zero both meters for the next trail.

B. Rota-Rod Test in Rats

Apparatus: Rota-Rod Treadmill for Rats (7750 Accelerating Model, from UGO BASILE, COMERIO-ITALY).

Procedure: Animals with cerebral infarction induced by transient or permanent unilateral occlusion of the middle cerebral artery (MCA) and sham-operated rats are tested in this study, using a Rota-Rod Treadmill for Rats (7750 Accelerating Model, UGO Basile, Comerio, Italy). The

animals are moved into the testing room 30 minutes before testing. Every rat receives 2-3 training runs of 1-2 minutes at intervals of 2-3 hours before testing.

The cylinder on the apparatus is set in motion before placing the rats in position. The motor is set at a constant selected speed in 7700 on RESET mode, and the rats are placed, one by one, in their sections.

Testing is carried out on postoperative day 2 and repeated, in a blind-randomized fashion, twice weekly for a defined interval. Typically, three successive readings are taken for each animal with an intertrial interval long enough to record the data and zero both meters for the next trail.

Example 6

High Glutamate-Induced Oxidative Stress Assay

This procedure was used to induce high glutamate oxidative stress (HGOS) in a dopaminergic neuronal cell line. Using this assay the potency and efficacy of test articles against HGOS neuronal cell injury and cell death was established in a high throughput manner.

Materials

- Dopaminergic neuronal cell line
- DMEM-No Glucose (Life Technologies Cat # 11966-025)
- L-glutamine (Life Technologies Cat # 25030-081)
- L-glutamic acid, monosodium salt (Sigma Cat # G5889)
- D-glucose (Sigma Cat # G-6151)
- 10x HBSS buffer(pH 7.4) (950ml Pyrogen-free water, 2.44g/L MgCl2.6H20, 3.73g/L KCl, 59.58g/L Hepes, 58.44g/L NaCl, 1.36g/L KH2PO4, 1.91g/L CaCl2 .2H2O and pH to 4.5 with HCl)
- Cell Tracker Green fluorescent dye (Molecular Probes, Cat # 2925). Prepare a 5µM solution in pre-warmed HBSS just prior to use.
- Sterile 96-well plates precoated with poly-D-lysine (Corning Catalog # 3665)
- 96-well deep well mother plate, DyNA Block 1000 (VWR Catalog # 40002-008)

Neuronal Cells

The cells were seeded into 96-well plates at a density of 2000 per well and left to grow for 72 hours in a 33°C incubator with 5% CO_2 in air atmosphere. The passage number of the cells for each assay experiment were no later than p11 in order to minimize experimental variation.

Compound Preparation in Deep-well Mother Plates

VWRBrand DyNA Block 1000, deep well mother plates (VWR Cat. # 40002-008) were used for the preparation of the test compounds.

All compounds were dissolved in DMEM-No Glu containing 1mM glucose, 30 mM glutamate and 1x Pen/Strep. DMEM-No Glu with 1mM glucose and 1x P/S was used as the

negative control, DMEM-No Glucose with 1mM glucose, 100 M glutamate was used as a positive control and 100μM Glutathione was added to the positive control as a standard. All of the procedures for this involving the making and dilution of compounds were performed using aseptic conditions and with minimal light.

Cell Preparation

The plates were removed from the incubator and examined under the microscope for morphological appearance and density. Using an aseptic technique and an 8-channel aspirator the media was carefully removed from the cells and replaced with 200μl of 1x HBSS. This was done as quickly as possible to prevent the cells drying out. The plates were then placed in the humidified 37°C incubators of the Biomek 2000 Side Loader. Four plates were washed at a time so as to minimize the time that the cells were sitting in 1x HBSS prior to addition of the compound test solution.

Experimental Setup

The Beckman Biomek workstations were used to load the compounds and controls from the mother plates onto the cell plates that were prewashed with HBSS under sterile conditions. The plates were incubated in the upper HTS incubator at 37°C in 5% CO_2 for exactly 16 hrs. The following day, using the Beckman Biomek workstations, the plates were removed from the incubator. Using Cell Tracker Addition, the compounds were removed from the plates, washed once with 200μM of pre-warmed 1x HBSS and then 100μL of 5μM Cell Tracker Green was added to each well. The plates were incubated at 37°C for 30 min to allow the dye to enter the cell and be cleaved by the esterases. After washing the cells twice with prewarmed 1x HBSS, the plates were read with the 485 excitation; 538 emission filter pair on a Fluoroskan.

Certain compounds of the present invention such as:

6,7-dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol;

4-(5-H\hydroxy-6,7-dimethyl-benzofuran-2-yl)-benzonitrile;

6,7-dimethyl-3-phenyl-benzofuran-5-ol;

2-hydroxymethyl-6,7-dimethyl-benzofuran-5-ol;

2-bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone; and

(5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone;

were considered to be active when they exhibited protection against HGOS cell injury and cell death at an EC_{50} of less than 30 μM, preferably less than 10 μM.

Example 7

MPP^+ Cell Death Assay

Media Composition

RF media: DMEM-No glucose, glucose (29.1mM), L-glutamine (1.4mM), 10% heat-inactivated FBS, and 1x penicillin/streptomycin (P/S)

Wash media: DMEM-No glucose and 1x P/S

Low serum media: DMEM-No glucose, glucose (29.1mM), L-glutamine (1.4mM), 0.5% FBS, and 1x P/S

Assay Media: DMEM-No glucose, L-glutamine (1.4mM), 0.5%FBS, and 1x P/S

Experimental procedure

The substantia nigra-derived dopaminergic progenitor cell line was seeded in poly-D-lysine-coated 24-well plates at a density of 4500 cells per well in RF media. The cells were left to attach for 16 hours in a 33°C incubator (5% CO_2) after which time they were washed once with 500µL wash media and then differentiated into a neuronal phenotype by incubating in low serum media for 24 hours in a 39°C incubator (5% CO_2).

After 24 hours the low serum medium was aspirated from the cells and the monolayer was washed once with 500µL wash media. Test articles were diluted to 2-fold the desired testing concentration in assay media and 250µL was added to the cells. From a 10mM stock, a working solution of 140µM 1-methyl-4-phenylpyridinium (MPP^+) (Sigma, St. Louis, MO) was made in assay media and 250µL of this working solution was also added to the cells. The final volume in each well was 500µL and the final concentration of MPP^+ was 70µM. As a negative control, cells were incubated with 500µL assay media with no additions.

Cells were incubated in a 39°C incubator (5% CO_2) for 24 hours. After this time, the number of live neurons remaining in each well was determined using a fluorescent vital cell stain, Cell Tracker Green (Molecular Probes, Eugene, OR). Assay media was aspirated from the cells and 400µL of 2.5 µM Cell Tracker Green was added to each well. Cells were placed in a 37°C incubator for 5 minutes after which time the cell stain was aspirated off and 500µL of HBSS (Invitrogen Life Technologies, Carlsbad, CA) was added to each well. The number of live cells in each well was then quantitated using an automated fluorescent microscope/imaging system (Universal Imaging, Downingtown PA).

Results:

Certain compounds of the present invention when tested as described above provided protection in at least 30% of the cells tested at concentrations ranging from about 0.1 to 10µM. 5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)phenyl-methanone provided protection in 78% of the cells at a concentration of 2µM, and 1-(3-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone provided in 65% of the cells at a concentration of 100µM.

Example 8

Beta-Amyloid Cell Death Assay

Media Composition

Neurobasal/B27i: Neurobasal medium plus 1x B27 supplement, 0.5mM L-glutamine, 25µM L-glutamic acid, and 0.5x Penicillin/Streptomycin

Neurobasal/B27m: Neurobasal medium plus 1x B27 supplement and 0.5mM L-glutamine

BSS (Ca/Mg free): HBSS (calcium/magnesium free) plus 10mM Hepes (pH 7.25), 1x Penicillin/Streptomycin, and 1mM Sodium Pyruvate

Glucose-free BSS_0: 143.6 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 10 mg/l phenol red, 0.25x Penicillin/Streptomycin, and 10mM Hepes (pH 7.4)

Papain Quench solution: Neurobasal medium plus 1x B27 supplement, 1 x Penicillin/Streptomycin and 0.5 mg/ml DNase1

Assay media: Neurobasal medium plus 1x B27 (minus AO) supplement, 0.5mM L-glutamine, and 0.25x Penicillin/Streptomycin.

Experimental procedure

Hippocampal cell culture

Hippocampal neurons were isolated from E18 rat embryos as follows. Embryos were decapitated and the heads immersed in cold BSS (Ca/Mg free). Using a dissecting microscope the hippocampi were dissected out and placed in cold BSS (Ca/Mg free). The isolated hippocampi were then centrifuged at 1000 rpm for 2 min, the BSS aspirated off and 2ml of 2mg/ml Papain in Neurobasal media added per 10 embryos. After mixing on a rotational shaker for 10 min at 37°C, 5ml Papain Quench solution was added. Cells were then centrifuged at 1000 rpm for 2 min, the supernatant was aspirated and 2ml of Neurobasal/B27i was added. The cells were triturated 6 times with siliconized pipettes (decreasing bore size) after which an additional 5ml Neurobasal/B27i was added. The cell suspension was then centrifuged at 1000 rpm for 2 min, the supernatant was aspirated and 2ml of Neurobasal/B27i was added. Cells were titurated again as described above and the volume of Neurobasal/B27i was adjusted to 1ml/embryo. Cells were then counted and seeded at a density of 75,000 cells per well in a poly-D-lysine coated 24-well plate. After four days media was removed from the cells and replaced with Neurobasal/B27m media + 5µM Ara-C (cytosine arabinoside). Seven days after isolation the media was removed again and replaced with fresh Neurobasal/B27m media. Ten days after isolation the hippocampal cultures were used in the assay described below.

Preparation of oligomeric beta-amyloid (Aβ) peptide

Aggregation of Aβ(1-42) (American Peptide) into oligomers was carried out according to the method of Dahlgren et al, (2002) *J Biol Chem* 277: 32046-32053. The Aβ peptide was dissolved to 1mM in hexafluoroisopropanol (HFIP) and aliquoted into sterile microcentrifuge tubes. The HFIP was removed under vacuum and the peptide film stored at -20 °C. The day before the assay, the peptide film was resuspended in dry DMSO to a concentration of 5mM. Ham's F-12 media was then added to bring the peptide to a final concentration of 100µM, and this solution was incubated at 4 °C for 24 hours to allow formation of oligomers.

Treatment of hippocampal neurons with oligomeric Aβ

The existing growth medium was aspirated from the hippocampal cultures and the monolayer was washed once with 500µl glucose free-BSS_0. Test articles were diluted to 2-fold the desired testing concentration in assay media and 250µL was added to the cells. From the 100µM oligomeric beta-amyloid peptide solution described above, a working solution of 6µM was

made in assay media. 250µL of this working solution was also added to the cells. The final volume for each well was 500µL and the final concentration of Aβ peptide was 3µM. As a negative control, cells were incubated with 500µL assay media with no additions.

Cells were incubated in a 39°C incubator (5% CO_2) for 24 hours. After this time, the number of live neurons remaining in each well was determined using a fluorescent vital cell stain, Cell Tracker Green (Molecular Probes, Eugene, OR). Assay media was aspirated from the cells and 400µL of 2.5 µM Cell Tracker Green was added to each well. Cells were placed in a 37°C incubator for 5 minutes after which time the cell stain was aspirated off and 500µl of HBSS (Invitrogen, Life Technologies, Carlsbad, CA) was added to each well. The number of live cells in each well was then quantitated using an automated fluorescent microscope/imaging system (Universal Imaging, Downingtown PA

Results:

Certain compounds of the present invention when tested as described above were active in this assay. 5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)phenyl-methanone showed 28% protection at 25µM.

Example 9

***In vitro* cellular inflammation assay:**

A. ***Human Hep3B Cells – CRP assay.*** Hep3B Cell Line is obtained from the American Type Culture Collection (ATCC Catalog No. HB-8064). The Hep3B cell line was derived from liver tissue of an 8-year-old Black male. The cells are epithelial in morphology and produce tumors in nude mice. The cells produce α -fetoprotein, hepatitis B surface antigen, albumin, α-2-macroglobulin, α -1-antitrypsin, transferrin, plasminogen, complement C3 and β-lipoprotein (Knowles BB, et al., Science, 1980, 209:497-499). This cell line has been widely used to study hepatocyte cytokine and acute phase protein release (e.g., Damtew B, et al.,1993, J Immunol 150:4001-4007).

HEP3B cells are grown in Minimum Essential Medium (MEM; GIBCO) supplemented with 10% Fetal Bovine Serum (FBS; Hyclone), 1x Penicillin/Streptomycin (GIBCO, Cat #. 15140-122) and 0.1mM non-essential amino acids (GIBCO, Catalog No. 11140-050). Cells are thawed and transferred to warm medium according to standard methods known in the art.

Cells are incubated in flasks at 37°C with 5% CO_2 in an air atmosphere incubator. HEP3B growth media is changed every 2 days until the cells reach 70-80% confluence (approx. 3-4 days). For assay, the cells are transferred to 96-well plates, seeded at 5000 cells per well in culture media, and left to grow for 7 days in a 37°C incubator (air supplemented with 5% CO_2). Media is replaced daily until assay.

Test compounds are diluted into "Stimulus Buffer" (MEM medium containing 0.1 mM non-essential amino acids, 1X penicillin/streptomycin, 10% FBS with 10 ng/ml IL-1β, 20 ng/ml IL-6 and 1 µM dexamethasone. Media is removed from the cells and is replaced with 200 µl of test

dilution. Cells are returned to the incubator for three days at 37˚C. CRP ELISA is then performed on supernatant from the cells, as described below.

Costar EIA/RIA plates are coated with rabbit anti-human CRP (DAKO) diluted 1:4000 in carbonate buffer (100 µl/well) for 45 minutes at 37°C. Plates are then washed 5x with CRP washing buffer (50 mM Tris-HCl, 0.3M NaCl, 0.5 Ml Tween-20, pH 8.0) using an automatic plate washer. Plates may be dried, covered and refrigerated until use. Supernatant (100 µl) is removed from each well of the test plates and added to the corresponding well of a precoated ELISA plate.

100 µl HRP-conjugated rabbit anti-human CRP (DAKO) diluted 1:500 (in CRP wash buffer) is added to each well, followed by incubation for 30 minutes at 37°C. Plates are washed 5x with CRP washing buffer using the automatic plate washer. 200µl of 3,3',5,5'-Tetramethyl Benzidine (TMB) liquid Substrate System (Sigma, St. Louis, MO) is added to each well, followed by incubation in the dark for 15 minutes at room temperature. Finally, 50 µl of 1M H_2SO_4 is added to each well and absorbance at 450 nm is immediately measured in a microtiter spectrophotometer.

CRP measured as above is normalized to cell count per well, using a cell viability assay, such as the Cell Tracker Green assay. To do this, the remainder of the medium is from the cell test plates, cells are washed with 200 µl of pre-warmed 1x Hanks Basic Salt Solution (HBSS; GIBCO), and 100 µL of 5µM Cell Tracker Green (Molecular Probes, Eugene, OR) is added to each well. Plates are then incubated at 37°C for 30 minutes. Cells are then washed twice with prewarmed 1x HBSS. Plates are immediately read using a Fluoroskan® flourometer with a 485 excitation/538 emission filter pair.

In a CRP assay such as the one disclosed herein, compounds such as:

6,7-Dimethyl-3-phenyl-benzofuran-5-ol;

1-(4-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone;

Acetic acid 2-(6-hydroxy-3-methyl-[1,3]oxazinan-6-yl)-6,7-dimethyl-benzofuran-5-yl ester;

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

3-(4-Methoxy-phenyl)-6,7-dimethyl-benzofuran-5-ol;

4,5-Dimethyl-1,8-diphenyl-benzo[1,2-b;4,3-b']difuran; and

1,8-Bis-(4-fluoro-phenyl)-4,5-dimethyl-benzo[1,2-b;4,3-b']difuran;

at an EC_{50} of between about 10µM to 40 µM were effective at reducing CRP levels.

B. *Human endothelial Cells—ELAM assy*

Endothelial-Leukocyte Adhesion Molecule (ELAM), also known as E-selectin, is expressed on the surface of endothelial cells. In this assay, lipopolysaccharide (LPS) and IL-1β are used to stimulate the expression of ELAM; test agents are tested for their abilities to reduce this expression, in accordance with studies showing that reduction of leukocyte adhesion to endothelial cell surface is associated with decreased cellular damage (e.g., Takada, M., Et al., Transplantation 64: 1520-25, 1997; Steinberg, J.B., et al., J. Heart Lung Trans. 13:306-313, 1994).

Endothelial cells may be selected from any of a number of sources and cultured according to methods known in the art; including, for example, coronary artery endothelial cells, human brain microvascular endothelial cells (HBMEC; Hess, D.C., et al., Neurosci. Lett. 213(1): 37-40, 1996), or lung endothelial cells. Cells are conveniently cultured in 96-well plates. Cells are stimulated by adding a solution to each well containing 10 µg/ml LPS and 100 pg/ml IL-1β for 6 hours in the presence of test agent (specific concentrations and time may be adjusted depending on the cell type). Treatment buffer is removed and replaced with pre-warmed Fixing Solution® (100 µl/well) for 25 minutes at room temperature. Cells are then washed 3X, then incubated with Blocking Buffer (PBS + 2% FBS) for 25 minutes at room temperature. Blocking Buffer containing Monoclonal E-Selectin Antibody (1:750, Sigma Catalog #S-9555) is added to each well. Plates are sealed and stored at 4˚ overnight. Plates are washed 4X with 160 µL Blocking Buffer per well. Second Antibody-HRP diluted 1:5000 in Blocking Buffer is then added (100 µL/well), and plates are incubated at room temperature (protected from light) for two hours. Plates are then washed 4X with Blocking Buffer before addition of 100 µL of ABTS Substrate solution at room temperature (Zymed, Catalog #00-2024). Wells are allowed to develop for 35 minutes, before measurement at 402 nm in a Fluoroskan® Reader with shake program for 10 seconds. Positive results are recorded as a decrease in ELAM concentration in tested wells, as compared to control wells.

In an ELAM assay, such as the one described herein, certain compounds of the present invention at EC_{50} in a range of 10-1000 µM, and in particular compounds such as;

2-bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone;

6,7-dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol;

6,7-dimethyl-3-phenyl-benzofuran-5-ol;

5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 3,7-dimethyl-octa-2,6-dienyl ester;

1-(5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone;

3-(4-Fluoro-phenyl)-6,7-dimethyl-benzofuran-5-ol;

3-(4-Methoxy-phenyl)-6,7-dimethyl-benzofuran-5-ol;

3-(4-Chloro-phenyl)-6,7-dimethyl-benzofuran-5-ol;and

5-Hydroxy-6,7-dimethyl-3-(4-nitro-phenyl)-benzofuran-2-carboxylic acid methyl ester

were able to reduce the expression of ELAM at EC_{50} of between 10µM and 50µM.

C. Mouse Microglial Cells-Interleukin-1.beta Assay

Materials And Equipment

A. Materials For Cell Preparation And Experiment

- Mouse microgial cell line
- DMEM High Glucose media (Gibco Catalog # 11965-092)
- FBS (Hyclone Catalog # SH30070.03)
- 100x Penicillin/Streptomycin (Gibco Catalog # 15140-122).
- LPS (Sigma Catalog # L2537)
- Interferon-gamma (Sigma Catalog # I4777)
- Cell Tracker Green (Molecular Probes Catalog # C2925)

- HBSS buffer (950ml Pyrogen-free water, 2.44g/L MgCl2.6H20, 3.73g/L KCl, 59.58g/L Hepes, 58.44g/L NaCl, 1.36g/L KH2PO4, 1.91g/L CaCl2 .2H2O and pH to 4.5 with HCl)
- Sterile 96-well plates precoated with poly-D-lysine (Corning Catalog # 3665)
- 96-well deep well mother plate, DyNA Block 1000 (VWR Catalog # 40002-008)

B. Materials For Il-1beta Elisa

- Mouse IL-1beta Duo Set (R & D Systems Catalog # DY401)
- Substrate Solution (R & D Systems Catalog # DY 999)
- Bovine Serum Albumin fraction V (BSA V) (Sigma Catalog # A4503)
- 96-well Costar EIA high binding plates (VWR Catalog # 29442-302)
- Plate seal (VWR Catalog # 29442-310)
- PBS (Irvine Scientific Catalog # 9240)
- Cell Culture Grade Water (Irvine Scientific Catalog # 9312)
- Tween 20 (Sigma Catalog # P 1379)
- Sucrose (Sigma Catalog # S7903)
- Sodium Azide (Sigma Catalog # S 8032)
- H_2SO_4 5N (VWR Catalog # JT 5691-2)

EXPERIMENTAL PREPARATION AND PROCEDURE:

Mouse IL-1beta Elisa:

Solutions:

Wash Buffer: PBS 1L + 500µl Tween 20 (final 0.05%) pH 7.2 - 7.4.

Blocking Buffer: 500ml PBS + 5g BSA V (1%) + 25g Sucrose (5%) + 0.25g Sodium Azide (0.05%).

Reagent Diluent: 500ml PBS + 5g BSA V (1%) pH 7.2 - 7.4 and filter sterilize through 0.2µm.

Stop Solution: Make 2N sulfuric acid by adding 10ml 5N H_2SO_4 to 15ml of dd H_2O.

Duo Set Preparations:

1. The IL-1.beta. capture antibody was reconstituted in 1ml of PBS to give a final concentration of 720µg/ml, and the working concentration was 4µg/ml. For coating one 96-well plate (at 100µl/well) 56µl of the 720µg/ml stock was diluted into 10 ml of PBS.

2. The IL-1.beta.standards were reconstituted in 0.5ml of Reagent Diluent (70ng/ml).. For a high standard of 1ng/ml (2wells at 100µl each + enough for series dilution) 7.1µl of the 70ng/ml standard were diluted into 0.5ml of Reagent Diluent

3. The IL-1.beta. detection antibody was reconstituted in 1ml of Reagent Diluent to give a final concentration of 18µg/ml and the working concentration is 100ng/ml. For one 96-well plate (at 100µl/well) 56µl of the 18µg/ml stock was diluted into 10 ml of Reagent Diluent.

IL-1.beta ELISA Procedure:

Plate Preparation:

1. The Costar EIA Hi-binding plate was coated with capture antibody at 4µg/ml. 56µl of 720µg/ml stock was taken for one plate and added to 10 ml of PBS. Each well was coated with 100µl, and the plate was sealed and incubated overnight at room temperature.

2. Each well was aspirated and washed 3x with Wash Buffer. Each well was filled to the

top, dispensed, and any remaining buffer was removed by inverting the plate and gently blotting against clean paper towels.

3. Non-specific binding sites were blocked by adding 300µl of Blocking Buffer to each well, and after sealing, incubating for at least 1hour at room temperature.

4. After washing the plate was now ready for the samples.

Assay Procedure:

5. 100µl of either standard or sample were added in each well of the capture-coated and pre-blocked plate. The plate was sealed and incubated for 2 hours at room temperature, followed with washing as in step 2.

6. 100µl of the detection antibody (100ng/ml) were added to each well. For one 96-well plate 56µl of the 18µg/ml stock were diluted into 10 ml of Reagent Diluent.

7. The plate was sealed and incubated at room temperature for 2 hours, followed with washing as in step 2.

8. 100µl of the working dilution of Streptavidin-HRP was added, and the plate was sealed and incubated in the dark for 20 minutes at room temperature, followed with washing as in Step 2.

9. The fresh Substrate Solution was prepared by mixing Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) in a 1:1 ratio. 100µl of this Substrate Solution mixture was added to each well and the plate was incubated in the dark for 20 minutes at room temperature.

10. 50µl of Stop Solution was added to each well, mixing was ensured by gently tapping.

11. Each plate was read with the Spectramax once at 450nm. If wavelength correction is available set to 540 or 570nm.

In an IL-1 assay such as the one disclosed herein, compounds such as:

6,7-Dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol;

6,7-Dimethyl-3-phenyl-benzofuran-5-ol;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid (2-hydroxy-ethyl)-amide;

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester; and

(5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone.

at an EC_{50} of between 0.5µM to about 40 µM were effective at reducing IL-1β levels.

Example 10

In vivo cellular inflammation assay

These assays measure the ability of test compounds to prevent or reduce inflammation secondary to oxazolone or arachidonic acid.

A. *Arachidonic acid.* Albino male CD-1 mice, 7-9 weeks old were used in this test. A 20% (w/v) arachidonic acid solution in acetone is prepared. Twenty microliters of the arachidonic acid solution is applied to the dorsal left ear of the mouse. Immediately thereafter, test compounds (20 µL in 70% ethanol/30% propylene glycol) are applied to the left ear. The untreated right ears served as control. Mice are sacrificed by CO_2 inhalation, one hour after treatment. The left and

right ears are removed and 7 mm punch biopsies taken from each. The punch biopsies are weighed, and the differences calculated.

B. *Oxazolone.* CD-1 mice are induced by applying 3% oxazolone (Sigma) (30 mg/ml prepared in corn oil:acetone) to the shaved abdomen. Five days later, the mice are challenged with 2% oxazolone (20 mg/ml) in acetone on the left ear (right ear was untreated control). One hour after challenge, test compounds are applied to the left ear in 70% ethanol/30% propylene glycol. Animals are sacrificed 24 hours later and 7 mm ear punches are removed. The ear punches are placed on a balance scale, and the difference between the untreated and treated ears is determined. Percent inhibition is calculated by comparing the means of each group to the vehicle group. (Hydrocortisone serves as a positive control in this test.).

Compounds of the present invention can be tested for their ability to reduce inflammation in this model.

Example 11

6,7-Dimethyl-3-phenyl-benzofuran-5-ol

Step 1:

A mixture of 2,3-dimethyl-1,4-dihydroquinone (1.08 g, 7.826 mmol), 2-bromoacetophenone (1.45 g, 7.29 mmol), and potassium carbonate (1.78 g, 12.90 mmol) in acetone (30 mL) was stirred at room temperature for 3 h. The mixture was then poured into water resulting in the formation of a precipitate. The precipitate was washed with water and hexane, and dried to yield a mixture of mono and bis products. Purification by silica gel column eluting with 30% EtAOc in hexane, 2-(4-Hydroxy-2,3-dimethyl-phenoxy)-1-phenyl-ethanone (0.65 g) and 2-[2,3-Dimethyl-4-(2-oxo-2-phenyl-ethoxy)-phenoxy]-1-phenyl-ethanone (0.5 g).

Step 2:

A mixture of 2-(4-hydroxy-2,3-dimethyl-phenoxy)-1-phenyl-ethanone (1.2 g, 4.70 mmol) and polyphosphoric acid (ca. 100 mg) in xylene (10 mL) was stirred at 150 ^{o}C for 5 h. The mixture was poured into water, extracted with ethylacetate, washed and dried to give 6,7-dimethyl-3-phenyl-benzofuran-5-ol (560 mg) as a light brown solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.71 (s, 1H), 7.60-7.20 (m, 5H), 7.06 (s, 1H), 4.76 (s, 1H, OH), 2.47, 2.29 (2s, 6H) ppm. ^{13}C NMR (CDCl3, 75 MHz) δ: 150.43, 150.30, 141.15, 132.58, 129.00, 127.32, 123.48, 122.13, 121.31, 120.59, 102.25, 12.31, 11.95 ppm. MS (m/z): 239 ($M+H^+$).

Similarly by following the procedure described above but replacing bromoacetophenone with the appropriate substituted bromoacetophenones the following compounds were obtained:

- 3-(4-Fluoro-phenyl)-6,7-dimethyl-benzofuran-5-ol; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.65 (m, 1H), 7.50 (m, 2H), 7.10-6.95 (m, 3H), 4.90 (s, 1H, OH), 2.44, 2.31 (2s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 163.79, 160.53, 150.37, 150.30, 140.97, 128.90, 128.79, 128.56, 128.52, 123.44, 121.41, 121.24, 120.78, 116.06, 115.78, 102.04, 12.30, 11.96 ppm. ^{19}F NMR ($CDCl_3$, 300 MHz, TFA as reference) δ: -115.31 ppm. MS (m/z): 257 ($M+H^+$).
- 3-(4-Methoxy-phenyl)-6,7-dimethyl-benzofuran-5-ol ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.64 (s, 1H), 7.50 (dd, J = 2.1, 6.7 Hz, 2H, 2H), 7.00 (m, 3H), 4.83 (s, 1H, OH), 3.84 (s, 3H), 2.46, 2.28 (2s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 158.92, 150.33, 150.23, 140.52, 128.47,

125.02, 123.69, 121.70, 121.25, 120.49, 114.45, 102.20, 55.47, 12.30, 11.95 ppm. MS (m/z): 269 ($M+H^+$).

- 3-(4-Chloro-phenyl)-6,7-dimethyl-benzofuran-5-ol. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.72 (s, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H), 7.02 (s, 1H), 4.86 (s, 1H, OH), 2.47, 2.30 (2s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 150.41, 141.25, 133.04, 131.06, 129.17, 128.49, 123.17, 121.45, 121.12, 120.79, 101.99, 12.30, 11.94 ppm. MS (m/z): 273, 275 ($M+H^+$).

Example 12

4,5-Dimethyl-1,8-diphenyl-benzo[1,2-b;4,3-b']difuran

A mixture of 2-[2,3-Dimethyl-4-(2-oxo-2-phenyl-ethoxy)-phenoxy]-1-phenyl-ethanone (480 mg, 1.28 mmol) synthesized as described in Example 11, an excess of bromoacetophenone, and polyphosphoric acid (PPA, 100 mg) in xylene (10 mL) was stirred at 150 °C for 10 h. The mixture was then poured into water, extracted with EtOAc, the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column eluting with 10-20% EtOAc in hexane to give 190 mg of 4,5-dDimethyl-1,8-diphenyl-benzo[1,2-b;4,3-b']difuran (250 mg). ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.60 (s, 2H), 7.05 (m, 4H), 6.90 (m, 2H), 6.85 (m, 4H), 2.60 (s, 6H) ppm. ^{13C} NMR ($CDCl_3$, 75 MHz) δ: 152.57, 141.48, 133.19, 128.42, 127.52, 126.87, 124.05, 117.40, 116.29, 11.80 ppm. MS (m/z): 339 ($M+H^+$).

Similarly by following the procedure of Example 12, the following compounds were obtained:

- 1,8-Bis-(4-fluoro-phenyl)-4,5-dimethyl-benzo[1,2-b;4,3-b']difuran; . ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.56 (s, 2H), 7.05 (m, 4H), 6.60 (m, 4H), 2.60 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 163.91, 160.66, 152.51, 141.44, 130.18, 129.16, 129.12, 122.77, 117.59, 116.27, 114.65, 114.36, 11.78 ppm. ^{19}F NMR ($CDCl_3$, 300 MHz, TFA as reference) δ: -116.57 ppm. MS (m/z): 375 (MH^+).
- 1,8-Bis-(4-methoxy-phenyl)-4,5-dimethyl-benzo[1,2-b;4,3-b']difuran). ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.56 (s, 2H), 6.95 (d, J = 8.7 Hz, 4H), 6.90 (m, 2H), 6.43 (d, J = 8.7 Hz, 4H), 3.75 (s, 6H), 2.60 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 158.49, 152.39, 141.07, 129.78, 125.51, 123.5, 117.24, 116.74, 112.98, 54.94, 11.78 ppm. MS (m/z): 399 ($M+H^+$).

Example 13

(5-Hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone

Step 1;

To a suspension of 2,3-dimethyl-1,4-dihydroquinone (11.58 g) and triethylamine (25 mL) in dichloromethane (100 mL) was slowly added acetic anhydride (16 mL). Then the mixture was stirred at room temperature for 2h until the solid was dissolved. The mixture was washed with water, dried over MgSO4, and concentrated to dryness to give a solid. The solid was washed with hexane and ether to give 15.40 g of the 1,4 diacetate derivative of the starting material.

Step 2:

A suspension of the diacetate from Step 1 (5.08 g) in boron trifluoride acetic acid complex (15 mL) was stirred at 110 °C for 1 h. After cooling, the mixture was poured into ice, and the mixture was extracted with dichloromethane. The organic layer was washed with water and dried. After evaporation, the residue was recrystallized from EtOAc-hexane to give 4.37 g of 1-(2-hydroxy-5-acetoxy-3,4-dimethyl-phenyl)-ethanone.

Step 3:

The phenylethanone of Step 2 (500 mg) was dissolved in MeOH (20 mL), and potassium carbonate (1 eq.) was added followed by water (1 mL). The mixture was stirred at room temperature for 1 h, and then was poured into water. The solution was acidified with HCl and a precipitate was formed. The precipitate was collected and air dried to give about 350 mg of 1-(2,5-dihydroxy-3,4-dimethyl-phenyl)-ethanone.

Step 4:

1-(2,5-Dihydroxy-3,4-dimethyl-phenyl)-ethanone of Step 3 (275 mg) was dissolved in dichloromethane (20 mL) and 3,4-dihydro-2*H*-pyran (0.2 mL) was added followed by pyridinium p-toluenesulfonate (PPTS) (30 mg). The mixture was stirred at room temperature for 4 h. The mixture was dried over $MgSO_4$ and purified on silica gel column eluting with 30% EtOAc in hexane to give 397 mg of 1-[2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yloxy)-phenyl]-ethanone as a yellow solid.

Step 5

A mixture of the tetrahydropyran ether from Step 4 (250 mg), 2-bromo-1-phenyl-ethanone (250 mg), and potassium carbonate (300 mg) in DMF was stirred at room temperature for 3h. The mixture was poured into water and extracted with ethyl acetate; the organic layer was dried and evaporated. Purification on a silica gel column eluting with 30% EtOAc in hexane gave 260 mg of a colorless solid which was then dissolved in DMF followed by the addition of cesium carbonate (2 eq.). The mixture was stirred overnight at room temperature, then poured into water and extracted with ethylacetate, the organic layer was dried and evaporated. The crude product was dissolved in MeOH with dil. HCl and stirred for 1 h. It was then poured into water and extracted with ethylacetate; the organic layer was washed, dried and evaporated. Purification on a silica gel column eluting with 30% EtOAc in hexane gave (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone (187 mg). ^{1}H NMR (CDCl3 with MeOH-d4, 300 MHz)δ: 8.10(m, 2H), 7.50(m, 3H), 6.88(s, 1H), 2.55, 2.43, 2.30 (3s, 9H) ppm. ^{13}C NMR ($CDCl_3$ with MeOH-d4, 75 MHz) δ:186.58, 152.26, 149.41, 148.23, 138.47, 132.81, 130.08, 128.61, 128.38, 127.37, 126.55, 121.64, 101.68, 101.63, 12.72, 12.51, 10.53 ppm. MS: 281 ($M+H^+$).

Example 14

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone

A mixture of 1-[6,7-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzofuran-2-yl]-ethanone (1.1 g) (prepared from 2,5-dihydroxy-3,4-dimethyl-benzaldehyde following the procedure of Steps 3, 4

and 5 of Example 13) and conc. HCl (10 drops) in MeOH (20 mL) was stirred at room temperature for 2h. Water was poured into the mixture and left to precipitate overnight in the refrigerator. The precipitate was collected, washed with hexane and dried to give 1-(5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone as a light brown solid (490 mg). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.38(s, 1H), 6.89 (s, 1H), 4.88 (1H, OH), 2.60, 2.50, 2.31 (3s, 9H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 192.76, 155.45, 155.13, 153.72, 130.29, 127.27, 124.75, 117.44, 106.18, 29.30, 15.56, 15.28 ppm. MS (m/z): 205 ($M+H^+$).

Example 15

1-(3-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone

To a solution of 1-[6,7-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzofuran-2-yl]-ethanone (200 mg) in chloroform (20 mL) was added bromine (120 mg). After 1 h, the solution was washed with water and dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column eluting with 10% hexane in DCM to give 1-(3-Bromo-5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone, as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.38(s, 1H), 5.55 (s, 1H, OH), 2.60, 2.50, 2.31 (3s, 9H) ppm. MS (m/z): 283, 285 ($M+H^+$).

Example 16

2-Bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone

1-(5-Acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone (83 mg) (prepared from 1-(5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-ethanone from Example 14 with acetanhydride and pyridine), was dissolved in chloroform (10 mL). Bromine (50 mg) was added and the solution was stirred at 70 °C for 10 min. When the color of bromine disapeared, the mixture was evaporated to dryness. The residue was purified by silica gel column eluting with 30% EtOAc in hexane to give 85 mg of 2-bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.58 (s, 1H), 7.26 (s, 1H), 4.43(s, 2H), 2.51, 2.37, 2.21(3s, 9H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 182.55, 170.13, 153.71, 150.74, 146.81, 131.21, 124.51, 122.92, 115.15, 113.28, 30.69, 21.27, 13.54, 12.80 ppm.

Example 17

Acetic acid 6,7-dimethyl-2-(2-morpholin-4-yl-acetyl)-benzofuran-5-yl ester

A solution of 2-bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone (47 mg) from Example 16, morpholine (15 mg), and potassium carbonate (25 mg) in acetone (10mL) was stirred at room temperature for 30 min. It was then evaporated to dryness, and the residue was purified by silica gel column eluting with 5% MeOH in DCM to give 43 mg of acetic acid 6,7-dimethyl-2-(2-morpholin-4-yl-acetyl)-benzofuran-5-yl ester as an oil. Conversion into the HCl salt gave a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.60 (s, 1H), 7.20 (s, 1H), 3.78(s+t, 6H), 2.64(m, 4H), 2.50(s, 3H), 2.40(s, 3H), 2.20(s, 3H) ppm. ^{13}C NMR (CD_3OD, 75 MHz) (as HCl salt) δ: 180.94, 170.31, 153.65, 150.33, 147.19, 131.94, 124.33, 122.34, 116.04, 113.59, 63.74, 60.89, 53.28, 19.64, 12.18, 12.22 ppm. M/S 332 ($M+H^+$).

Example 18

1-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone

A solution of acetic acid 6,7-dimethyl-2-(2-morpholin-4-yl-acetyl)-benzofuran-5-yl ester (23 mg), prepared as in Example 17, in MeOH (10 mL) was stirred while sodium bicarbonate (10 mg) was added. Then the mixture was stirred at RT overnight, followed by evaporation to dryness. The residue was purified by silica gel column, eluting with 5% MeOH in dichloromethane to give 11 mg of 1-(5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-2-morpholin-4-yl-ethanone as an oil. Conversion into HCl salt gave a yellow solid. ^{1}H NMR (CD_3OD, 300 MHz) δ:: 7.60 (s, 1H), 7.20 (s, 1H), 3.78(m, 6H), 2.64(m, 4H), 2.50(s, 3H), 2.40(s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz)δ: 187.98, 170.20, 153.31, 152.29, 146.58, 130.44, 124.53, 122.80, 114.36, 113.15, 64.69, 53.79, 21.28, 13.47, 12.86 ppm. M/S 290 ($M+H^+$).

Example 19

2-(1-Hydroxy-2-morpholin-4-yl-ethyl)-6,7-dimethyl-benzofuran-5-ol

A solution of acetic acid 6,7-dimethyl-2-(2-morpholin-4-yl-acetyl)-benzofuran-5-yl ester (35 mg), prepared as in Example 17, in MeOH (10 mL) was stirred while NaBH4 (40 mg) was added, and the mixture was stirred at room temperature for an additional 4 h. The mixture was poured into water, and extracted with ethylacetate. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column eluting with 5% MeOH in DCM to give 21 mg of 2-(1-hydroxy-2-morpholin-4-yl-ethyl)-6,7-dimethyl-benzofuran-5-ol as an oil. Conversion into HCl salt gave a yellow solid. ^{1}H NMR (CD_3OD, 300 MHz) δ: 6.77 (s, 1H), 6.67 (s, 1H), 5.29 (dd, J = 10.4, 3.3 Hz, 1H), 4.04(m, 2H), 3.85(q, 2H), 3.70-3.50 (m, 4H), 3.30(m, 2H), 2.40(s, 3H), 2.20(s, 3H) ppm. ^{13}C NMR (CD_3OD, 75 MHz) (as HCl salt) δ: 154.72, 151.88, 149.40, 125.02, 121.67, 120.21, 104.51, 102.50, 63.77, 63.69, 61.70, 60.19, 53.82, 51.28, 11.24, 11.10 ppm. M/S 292 ($M+H^+$).

Example 20

2-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-4-methyl-morpholin-2-ol

A mixture of 2-bromo-1-(5-acetoxy-6,7-dimethyl-benzofuran-2-yl)-ethanone (30 mg), methylaminoethanol (7 mg), and potassium carbonate (20 mg) in acetone (10 mL) was stirred at RT for 1h. The mixture was poured into water and extracted with ethylacetate. The organic layer was dried and evaporated followed by purification by silica gel column chromatography eluting with 5% MeOH in DCM to give 20 mg of 2-(5-hydroxy-6,7-dimethyl-benzofuran-2-yl)-4-methyl-morpholin-2-ol as an oil, and was converted into the HCl salt. The NMR showed an equilibrium of the hemiketal and ketone form. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.51, 7.26 (2s, combined, 1H), 7.06, 6.87 (2s, combined 1H), 4.2-3.7 (m, 2H), 3.2-2.8 (m, 4H), 2.5-2.0 (m, 12 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 189.26, 170.43, 170.18, 156.79, 152.53, 145.71, 125.79, 125.39,124.46, 121.73, 113.88, 113.14, 111.52, 104.28, 92.48, 63.55, 62.62, 61.11, 59.68, 54.75, 46.37, 21.30, 13.48, 13.02, 12.90 ppm. M/S 320 ($M+H^+$).

Example 21

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester

Step 1:

2,5-Dihydroxy-3,4-dimethyl-benzaldehyde (2.0 g) was dissolved in dichloromethane (50 mL) and 3,4-dihydro-2*H*-pyran (1.5 g) was added followed by addition of p-toluenesulfonic acid monohydrate (200 mg). The solution was stirred at room temperature for 1 hour and quenched by adding sodium bicarbonate solution (1 mL). Then the dichloromethane solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column eluting with hexane and ethyl acetate (8:2) to give 1.5g of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzaldehyde product.

Step 2

A mixture of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzaldehyde (150 mg) from Step 1, methyl bromoacetate (180 mg) and potassium carbonate (200 mg) in DMF (10 mL) was stirred at room temperature for 2 hours. Then the solution was poured into water and extracted with ethylacetate. The ethyl acetate was washed with water and brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column eluting with hexane and ethyl acetate (9:1) to give 100 mg of [6-formyl-2,3-dimethyl-4-(tetrahydro-pyran-2-yloxy)-phenoxy]-acetic acid methyl ester, which was then dissolved in DMF (15 mL). To this solution was added cesium carbonate (200 mg) and the mixture was stirred at room temperature overnight. Then the solution was poured into water and extracted with ethylacetate. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated to give 6,7-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzofuran-2-carboxylic acid methyl ester. The tetrahydropyranyl ether was dissolved in methanol, p-toluenesulfonic acid monohydrate (20 mg) was added, and the solution was stirred at room temperature for an additional 30 min. After the addition of a small amount of $NaHCO_3$, the methanol was evaporated. The residue was purified by silica gel column chromatography, eluting with hexane and ethyl acetate (7:3) to give 30 mg of 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.39 (s, 1H), 6.87 (s, 1H), 4.75 (s, 1H), 3.95 (s, 3H), 2.50, 2.26 (2s, 6H) ppm.

Example 22

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester

A mixture of acid (50 mg), 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethanol (100 µL) , DCC (120 mg), DMAP (50 mg) in DCM (20 mL) was stirred at RT for overnight. The mixture was poured into water and extracted with ethylacetate. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatopraphy eluting with 40%EtOAc in hexane to give the tetrahydropyranyl ether of 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester (40 mg). This intermediate was dissolved in MeOH, diluted HCl was added, and the mixture was

stirred for 30 min. The mixture was poured into water and extracted with ethylacetate. The organic layer was washed with water and brine, dried over MgSO4 and concentrated. Purification by silica gel column eluting with 2% MeOH in DCM gave 30 mg of 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl ester. ^{1}HNMR ($CDCl_3$, 300 MHz) δ: 7.27(s, 1H), 6.84(s, 1H), 6.30(br., 1H) 4.50(m, 2H), 3.87(m, 2H), 3.71-3.50(m, 8H), 3.35(s, 3H), 2.42(s, 3H), 2.26(s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 75 MHz)δ: 159.33, 150.91, 150.11, 143.97, 124.82, 123.33, 121.14, 102.70, 71.04, 70.23, 70.15, 70.11, 68.76, 63.74, 58.56, 11.86, 11.81 ppm. MS: 375.1 ($M+H^+$).

Similarly by following the procedure described above but replacing 2-[2-(2-methoxy-ethoxy)-ethoxy]-ethanol with the appropriate alcohol the following compounds were prepared:

Geraniol gave 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid 3,7-dimethyl-octa-2,6-dienyl ester; ^{1}HNMR ($CDCl_3$, 300MHz) δ: 7.54(s, 1H), 7.07(s, 1H), 5.48(m, 1H), 5.36(s, 1H), 5.09(m, 1H), 4.87(m, 2H), 2.49(s, 3H), 2. 28(s, 3H), 2.08(m, 4H), 1.80, 1.60, 1.39 (3s, 9H) ppm. ^{13}CNMR ($CDCl_3$, 75MHz) δ: 159.67, 150.50, 150.28, 144.67, 142.55, 131.49, 124.38, 123.53, 123.27, 121.39, 117.48, 113.59, 102.87, 61.79, 39.14, 25.83, 25.26, 17.28, 16.19, 11.89, 11.94 ppm. MS: 343($M+H^+$).

2-Aminoethanol gave 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid (2-hydroxy-ethyl)-amide; 1HNMR(DMSO-d6, 300MHz) δ: 9.3 (br., 1H), 8.42(s, 1H), 7.25(s, 1H), 6.83(s, 1H), 4.77(br., 1H) 3.47(m, 4H), 2.37(s, 3H), 2.11(s, 3H) ppm. ^{13}CNR(DMSO-d6, 75 MHz) δ: 158.90, 151.77, 148.24, 147.89, 123.98, 123.77, 120.63, 109.69, 102.62, 59.44, 12.20, 11.95 ppm. MS 250 (M+H+).

2-(2-Hydroxy-ethylamino)-ethanol gave 5-hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid bis-(2-hydroxy-ethyl)-amide. ^{1}HNMR (CD_3OD, 300 MHz) δ: 7.26(s, 1H), 6.83(1H), 3.90-3.70(m, 8H), 2.40(s, 3H), 2.23(s, 3H)ppm. ^{13}CNMR (CD_3OD, 75MHz) δ: 161.71, 151.76, 148.60, 147.35, 123.77, 123.25, 119.93, 112.09, 101.73, 60.29, 58.78, 51.76, 50.27, 10.49, 10.43ppm. MS 294 ($M+H^+$).

Morpholine gave (5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-morpholin-4-yl-methanone; ^{1}HNMR (DMSO-d_6-D_2O, 300 MHz) δ: 7.22(s, 1H), 5.88(1H), 3.65(m, 8H), 2.34(s, 3H), 2.15(s, 3H) ppm. MS 276 ($M+H^+$).

Example 23

2-Hydroxymethyl-6,7-dimethyl-benzofuran-5-ol

5-Hydroxy-6,7-dimethyl-benzofuran-2-carboxylic acid methyl ester (231mg) prepared as in Example 2, was dissolved in THF and lithium aluminum hydride (93 mg) was added. The mixture was stirred at room temperature for 4 h. Then the solution was poured into water and extracted with ethylacetate. The ethyl acetate was washed with water and brine, dried over $MgSO_4$ and concentrated. Purification by silica gel column eluting with 50% EtOAc in hexane gave 89 mg of 2-Hydroxymethyl-6,7-dimethyl-benzofuran-5-ol. ^{1}HNMR (CD_3OD, 300MHz) δ: 6.75, 1H), 6.47(s, 1H), 4.61(s, 2H), 2.37(s, 3H), 2.20(s, 3H) ppm. ^{13}CNMR (CD_3OD, 75 MHz) δ: 155.87, 150.51, 148.68, 124.69, 119.93, 119.20, 103.01, 101.62, 56.32, 10.43, 10.29 ppm. MS: 175(M-

OH)$^+$.

Example 24

6,7-Dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol

A mixture of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzaldehyde (200 mg) prepared as in Example 21, p-nitrobenzyl bromide (200 mg) and potassium carbonate (220 mg) in DMF (10 mL) was stirred overnight at room temperature. Then the solution was poured into water and extracted with ethylacetate. The ethylacetate was washed with water and brine and dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column eluting with hexane: ethylacetate (8:2) to give 242 mg of the tetrahydropyranyl ether of 6,7-Dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol. A mixture of the ether (240 mg) and NaOMe (17 mg) in DMF (10 mL) was stirred at 70 °C for 2 h. Then the solution was poured into water and extracted with ethylacetate. The organic layer was washed with water and brine, dried over MgSO4 and concentrated. The residue was dissolved in MeOH and p-toluenesulfonic acid monohydrate (20 mg) was added and the solution was stirred at room temperature for 1.5h. After the addition of a small amount of $NaHCO_3$, the methanol was evaporated, and the residue was purified by silica gel column chromatography eluting with hexane and ethyl acetate (7:3) to give 170 mg of 6,7-dimethyl-2-(4-nitro-phenyl)-benzofuran-5-ol. 1H NMR ($CDCl_3$-DMSO-d_6, 300 MHz) δ: 9.16 (s, 1H), 8.28 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 8.1 Hz, 2H), 7.46 (s, 1H), 6.87 (s, 1H), 2.44, 2.18 (2s, 6H) ppm. ^{13}C NMR (CDCl3-DMSO-d6, 75 MHz) δ: 152.87, 152.57, 149.68, 147.15, 137.19, 126.16, 125.64, 125.07, 123.56, 120.66, 107.03, 103.13, 12.92, 12.84 ppm.

Similarly following the procedure described above, the following compound was prepared: 4-(5-Hydroxy-6,7-dimethyl-benzofuran-2-yl)-benzonitrile; ^1H NMR (DMSO-d_6, 300 MHz) δ: 9.24 (s, 1H), 8.00 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.47 (s, 1H), 6.87 (s, 1H), 2.41, 2.18 (2s, 6H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 152.92, 152.82, 149.36, 135.22, 133.75, 126.19, 125.50, 123.17, 120.75, 119.72, 110.80, 106.30, 103.10, 12.95, 12.84 ppm .

Example 25

5-Hydroxy-6,7-dimethyl-benzofuran-2-carbaldehyde

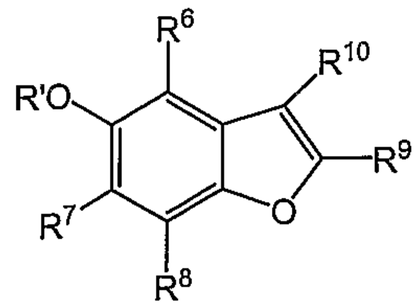
A mixture of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yloxy)-benzaldehyde (300 mg), bromoacetaldhyde dimethyl acetal (300 mg) and potassium carbonate (330 mg) in DMF (10 mL) was stirred at 140 °C for 2 h. Then the solution was poured into water and extracted with ethylacetate. The organic layer was washed with water and brine, dried over MgSO4 and concentrated. The residue was purified by silica gel column chromatography eluting with hexane:ethylacetate (8:2) to give 102 mg of the tetrahydropyranyl ether of 5-hydroxy-6,7-dimethyl-benzofuran-2-carbaldehyde . A solution of this intermediate (100 mg) in acetic acid (15 mL) was refluxed for 2 h. Then acetic acid was evaporated and the residue was purified on silica gel column chromatography eluting with 30% ethyl acetate in hexane to give 70 mg of 5-hydroxy-6,7-dimethyl-benzofuran-2-carbaldehyde. Further purification by silica gel eluting with

dichloromethane and ethyl acetate (9:1) gave a pure product (36 mg). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 9.75 (s, 1H), 9.55 (s, 1H), 7.79 (s, 1H), 7.00 (s, 1H), 2.39 (s, 3H), 2.20 (s, 3H) ppm. ^{13}C NMR (DMSO-d6, 75 MHz) δ: 181.06, 181.02, 153.46, 152.89, 150.56, 128.00, 124.47, 121.72, 120.79, 104.14, 13.34, 12.84 ppm.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto. All patents and publications cited above are hereby incorporated by reference.

We claim:

1. A compound represented by Formula II:



Formula II

wherein:

R^6 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^7 and R^8 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^9 is: optionally substituted aryl, (optionally substituted(C_1-C_6)- alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^{10} is: (C_1-C_6)-alkyl, (C_2-C_{10})-alkenyl, or amino; and

R' is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R' and R^6 with the atoms to which they are attached form an optionally substituted ring;

with the proviso that the compound is not (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone or 3-amino-5-hydroxy-4,6,7-trimethyl-benzofuran-2-carboxylic acid ethyl ester; and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

2. The compound of Claim 1, wherein R^7 and R^8 are (C_1-C_6)-alkyl and R^6 is hydrogen.

3. The compound of Claim 2, wherein R^{10} is (C_1-C_6)-alkyl, and R' is hydrogen.

4. The compound of Claim 1, wherein R^9 is phenylcarbonyl and wherein said phenyl group is unsubstituted or substituted with one or more substituents selected from alkyl, alkenyl, alkoxy, hydroxy, hydroxyalkyl, haloalkyl, (optionally substituted alkoxy)carbonyl, carboxy, nitro, halo, and cyano.

5. The compound of Claim 2, wherein R^{10} is amino and R^9 is (C_1-C_6)-alkoxycarbonyl.

6. A pharmaceutical composition comprising a compound of Claim 1 admixed with an acceptable excipient.

7. A method of treatment for a mammal suffering from a condition characterized by oxidative stress, comprising administering a therapeutically effective amount of a compound of Claim 1.

8. The method of treatment for a mammal suffering from a condition characterized by oxidative stress, comprising administering a therapeutically effective amount of a pharmaceutical composition of Claim 6.

9. The method of Claim 7, wherein the condition is selected from stroke, cerebral ischemia, retinal ischemia, myocardial infarction, chronic heart failure, post-surgical cognitive dysfunctions, peripheral neuropathy, spinal cord injury, head injury, and surgical trauma.

10. The method of Claim 7, wherein the condition involves inflammatory or autoimmune components.

11. The method of Claim 10, wherein the inflammatory condition is a dermatologic condition.

12. A method of treatment for a mammal suffering from a condition characterized by mitochondrial dysfunction or neurodegeneration, comprising administering a therapeutically effective amount of a compound of Claim 1.

13. The method of Claim 12, wherein the condition is selected from Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, cerebellar ataxias, Leber's hereditary optic neuropathy, epilepsy, and myodegenerative disorders.

14. The method of Claim 13, wherein the condition is epilepsy.

15. The method of Claim 13, wherein the condition is Parkinson's disease.

16. The method of Claim 13, wherein the condition is Friedreich's ataxia.

17. A method of protecting cellular mitochondrial function against a toxic insult with compounds of Claim 1.

18. The method of claim 17, wherein said cellular mitochondrial function is in a neuronal cell.

19. The method of claim 18, wherein said neuronal cell is dopaminergic cell.

20. The method of claim 19, wherein said dopaminergic cells are in the neurons of the substantia nigra-pars compacta.

21. A pharmaceutical composition comprising (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone admixed with an acceptable excipient.

22. A method of treatment for a mammal suffering from a condition characterized by oxidative stress, comprising administering a therapeutically effective amount of (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone.

23. The method of treatment for a mammal suffering from a condition characterized by oxidative stress, comprising administering a therapeutically effective amount of a pharmaceutical composition of Claim 21.

24. The method of Claim 22, wherein the condition is selected from stroke, cerebral ischemia, retinal ischemia, myocardial infarction, chronic heart failure, post-surgical cognitive dysfunctions, peripheral neuropathy, spinal cord injury, head injury, and surgical trauma.

25. The method of Claim 22, wherein the condition involves inflammatory or autoimmune components.

26. The method of Claim 25, wherein the inflammatory condition is a dermatologic condition.

27. A method of treatment for a mammal suffering from a condition characterized by mitochondrial dysfunction or neurodegeneration, comprising administering a therapeutically effective amount of (5-hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone.

28. The method of Claim 27, wherein the condition is selected from Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, cerebellar ataxias, Leber's hereditary optic neuropathy, epilepsy, and myodegenerative disorders.

29. The method of Claim 28, wherein the condition is epilepsy.

30. The method of Claim 28, wherein the condition is Parkinson's disease.

31. The method of Claim 28, wherein the condition is Friedreich's ataxia.

32. A method of protecting cellular mitochondrial function against a toxic insult with (5-

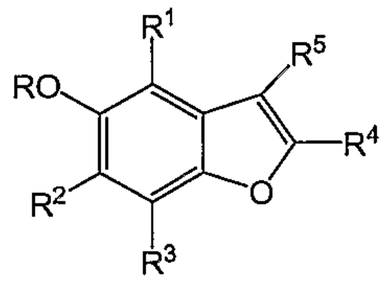
hydroxy-3,6,7-trimethyl-benzofuran-2-yl)-phenyl-methanone.

33. The method of claim 32, wherein said cellular mitochondrial function is in a neuronal cell.

34. The method of claim 33, wherein said neuronal cell is dopaminergic cell.

35. The method of claim 34, wherein said dopaminergic cells are in the neurons of the substantia nigra-pars compacta.

36. A method of treatment for a mammal suffering from a condition characterized by mitochondrial dysfunction or neurodegeneration, comprising administering a therapeutically effective amount of a compound of Formula I:



Formula I

wherein:

R^1 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^2 and R^3 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^4 is: hydrogen, optionally substituted aryl, (optionally substituted(C_1-C_6)-alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^5 is: hydrogen, (C_1-C_6)-alkyl, (C_2-C_{10})-alkenyl, (optionally substituted alkoxy)carbonyl, carboxy, (optionally substituted amino)carbonyl, or optionally substituted aryl;

provided that one of R^4 or R^5 is hydrogen, and that when R^4 is hydrogen R^5 is not hydrogen, and when R^5 is hydrogen R^4 is not hydrogen; and

R is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R and R^1 with the atoms to which they are attached form an optionally substituted ring; and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

37. The method of Claim 36, wherein R^1 is halogen, R^4 is (optionally substituted(C_1-C_6)-alkyl)carbonyl and R^5 is hydrogen.

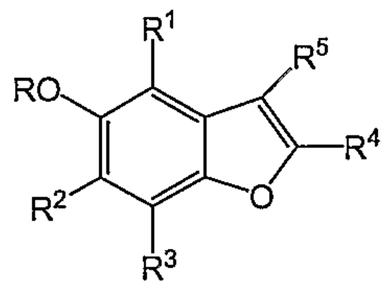
38. The method of Claim 36, wherein the condition is selected from Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, cerebellar ataxias, Leber's hereditary optic neuropathy, epilepsy, and myodegenerative disorders.

39. The method of Claim 38, wherein the condition is epilepsy.

40. The method of Claim 38, wherein the condition is Parkinson's disease.

41. The method of Claim 38, wherein the condition is Friedreich's ataxia.

42. A method of protecting cellular mitochondrial function against a toxic insult with compounds of Formula I:



Formula I

wherein:

R^1 is: hydrogen, optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, optionally substituted aryl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, or halogen;

R^2 and R^3 are independently selected from optionally substituted (C_1-C_6)-alkyl, optionally substituted (C_2-C_{10})-alkenyl, or optionally substituted (C_3-C_8)-cycloalkyl;

R^4 is: hydrogen, optionally substituted aryl, (optionally substituted(C_1-C_6)-alkyl)carbonyl, (optionally substituted aryl)carbonyl, (optionally substituted heterocyclyl)carbonyl, (optionally substituted heterocyclylalkyl)carbonyl, (optionally substituted (C_1-C_6)-alkoxy)carbonyl, (optionally substituted (C_2-C_{10})-alkenyloxy)carbonyl, (optionally substituted amino)carbonyl, carboxy, formyl, or hydroxy(optionally substituted)(C_1-C_6)-alkyl;

R^5 is: hydrogen, (C_1-C_6)-alkyl, (C_2-C_{10})-alkenyl, (optionally substituted alkoxy)carbonyl, carboxy, (optionally substituted amino)carbonyl, or optionally substituted aryl;

provided that one of R^4 or R^5 is hydrogen, and that when R^4 is hydrogen R^5 is not hydrogen, and when R^5 is hydrogen R^4 is not hydrogen; and

R is: hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkylcarbonyl, phosphoryl, or polyalkoxy; or

R and R^1 with the atoms to which they are attached form an optionally substituted ring;

and single stereoisomers, mixtures of stereoisomers, and the pharmaceutically acceptable salts thereof.

43. The method of claim 42, wherein said cellular mitochondrial function is in a neuronal cell.

44. The method of claim 43, wherein said neuronal cell is dopaminergic cell.

45. The method of claim 44, wherein said dopaminergic cells are in the neurons of the substantia nigra-pars compacta.