Title: TREATMENT OF MITOCHONDRIAL DISEASES

Abstract: The invention relates the method of treatment or amelioration of mitochondrial disorders such as Alzheimer’s disease, Parkinson’s disease, Friedreich’s ataxia (FRDA), cerebellar ataxias, Leber’s hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, stroke (MELAS), Myoclonic Epilepsy with Ragged Red Fibers (MERRF), amyotrophic lateral sclerosis (ALS), motor neuron diseases, Huntington’s disease, macular degeneration, and epilepsy, with chroman derivatives of Formula I or Formula II as described herein.
TREATMENT OF MITOCHONDRIAL DISEASES

Background Information

The present invention relates to a method of treatment, amelioration, or prevention of mitochondrial diseases with certain pharmaceutical formulations containing chroman derivatives.

Mitochondrial dysfunction leads to impaired calcium buffering, generation of free radicals that may participate in intracellular and extracellular processes, changes in mitochondrial permeability and oxidative damage which is observed in several neurodegenerative diseases. Some disorders involved in mitochondrial dysfunction affecting cellular processes include but are not limited to as Alzheimer's disease, Parkinson's disease, Friedreich's ataxia and other ataxias, Leber's hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, stroke (MELAS), Myoclonic Epilepsy with Ragged Red Fibers (MERFF), amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, Huntington's disease, macular degeneration, and epilepsy.

Friedreich's ataxia ("FRDA") is 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.

Mitochondrial myopathy, encephalopathy, lactacidosis, stroke ("MELAS") is a progressive neurodegenerative disorder. The typical presentation of patients with MELAS syndrome includes features that comprise the name of the disorder such as mitochondrial encephalomyopathy, lactic
acidosis, and stroke-like episodes. Other features, such as diabetes mellitus and hearing loss, clearly are part of the disorder. MELAS is characterized by stroke-like episodes and a mitochondrial myopathy.


Parkinson's disease ("PD") is 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.

Amyotrophic lateral sclerosis ("ALS"), also known as Lou Gehrig's disease or Charcot syndrome, is a progressive motor neuron disease characterized by both upper and lower motor neuron damage. The disease is characterized by degeneration of motor neurons in the cortex, brainstem and spinal cord (Principles of Internal Medicine, 1991 McGraw-Hill, Inc., New York; Tandan et al. Ann. Neurol., 18:271-280, 419-431, 1985). Generally, the onset is between the third and sixth decade, typically in the sixth decade. Symptoms may include tripping, stumbling and falling, loss of muscle control and strength in hands and arms, difficulty speaking, swallowing and/or breathing, chronic fatigue, and muscle twitching and/or cramping. Symptoms of upper motor neuron damage include stiffness (spasticity), muscle twitching (fasciculations), and muscle shaking (clonus). Symptoms of lower motor neuron damage include muscle weakness and muscle shrinking (atrophy).


The use of antioxidants targeted to mitochondria shown to be effective at slowing disease progression has been reported by Jauslin, ML in FASEB Journal, express article 10.1096/fj.03-0240fje.
Therapeutic benefit of administering γ-tocopherol derivatives and metabolites as antioxidants and nitrogen oxide scavengers which treat high blood pressure, thromboembolic diseases, cardiovascular disease, cancer, natriuretic disease, formation of neuropathological lesion and reduced immune system response are disclosed in US Patents 6,555,575; 6,242,479; 6,150,402; and 6,410,589. The use of certain chroman derivatives in cosmetic and dermatological preparations is disclosed in US 2002/0127252. Beneficial effects of Vitamin E in the progression of a number of major degenerative diseases of the nervous system is examined in Fryer, *Nutritional Neuroscience*, (1998) Vol. 1, 327-351.

The present invention addresses the desire to provide new therapies for conditions characterized by mitochondrial dysfunction. The present invention particularly addresses the method of treating or ameliorating the symptoms of Friedreich's ataxia, a condition affecting young children, for which there is no effective cure or treatment. Most children afflicted by this debilitating condition, Friedreich's ataxia, die in or before early adulthood.

**SUMMARY OF THE INVENTION**

It has surprisingly been found that certain compounds 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 mitochondrial dysfunction caused by MPP⁺ / MPTP (1-methyl-4-phenylpyridinium / 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine) or of oxidative stress caused by beta amyloid or high glutamate. These compounds show protection in the experimental model using FRDA fibroblasts and may be used for the treatment of Friedreich's disease and other ataxias, Leber's hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, stroke (MELAS), Myoclonic Epilepsy with Ragged Red Fibers (MERRF), macular degeneration, Down's syndrome, Creutzfeldt-Jakob syndrome.

In a first aspect, the present invention relates to the method of treating or ameliorating a subject suffering from a mitochondrial disorder comprising administering to said subject a therapeutically effective amount of a compound of Formula I or Formula II

![Formula I](image)

wherein:

- $A - B -$ is $-CH_2-CH_2-; -CH=CH- or -CH_2-O-$;
- $n$ is 0;
- $R^1$ is C$_{1-4}$ alkyl;
R^2 is C_{1-20} alkyl or C_{2-20} alkenyl, halogen, cyano, or
A and R^2 together with the atoms to which they are attached may form a ring;
R^3 is
- hydrogen;
- halogen;
- aralkyl, optionally substituted with one or more substituents selected from alkyl, haloalkyl, hydroxy, alkoxy, halogen, oxo, cyano, amino, \textit{SO}_2NR_2 or -C(O)OR;
- heteroaralkyl optionally substituted with one or more substituents selected from alkyl, haloalkyl, hydroxy, alkoxy, halogen, oxo, cyano, amino, \textit{SO}_2NR_2 or -C(O)OR;
- C_{1-6} alkyl;
- C_{2-20} alkenyl;
- \{C_{(CR_2)_{1-3}S(O)_{2-2}CR_2}_{1-3}C(O)O\}R;
- \{C_{(CR_2)_{1-3}O\}R^a;}
- \{C_{(CR_2)_{1-3}NR_2^{b}}R^c}
- -C(O)R^d; or
- nitro;
R^4 is hydrogen; optionally substituted C_{1-6} alkyl; C_{2-12} alkenyl; hydroxyalkyl; acyl; glucoside; phosphoryl; phosphorylxyalkyl; carboxyalkylcarbonyl; or aminoalkylcarbonyl;
R^5 and R^6 are independently of each other hydrogen, halogen, haloalkyl, nitro, acyl, C_{1-6} alkyl or C_{2-12} alkenyl; or
R^5 and R^6 taken together with the carbon to which they are attached jointly complete a 5-6 membered aliphatic, unsaturated or aromatic ring, optionally substituted with C_{1-6} alkyl, C_{1-6} alkoxy, hydroxy, carboxy, carboxyalkyl, alkoxyalkyl, alkoxyalkylalkyl, aminocarbonyl, aminocarbonylalkyl, or hydroxyalkyl;
R^7 is hydrogen or C_{1-6} alkyl;
R^6 is hydrogen; optionally substituted C_{1-6} alkyl; optionally substituted C_{2-12} alkenyl; optionally substituted aryl; optionally substituted cycloalkyl; or optionally substituted saturated, partially unsaturated or unsaturated heterocyclyl;
R^6 and R^7 are independently of each other hydrogen; C_{1-6} alkyl; hydroxyalkyl; aminoalkyl; optionally substituted aryl; optionally substituted benzyl; or optionally substituted heterocyclyl; or R^6 and R^7 taken together with the atom to which they are attached form a 5 to 8 membered aromatic, saturated or unsaturated ring, optionally incorporating one additional atom chosen from N, O, or S and optionally substituted with a substituent selected from the group consisting of lower alkyl, halo, cyano, alkylthio, lower alkoxy, oxo, phenyl, benzyl and carboxy;
with the proviso that the compound is not alpha-tocopherol;
or
wherein:
G is O, S, SO, SO₂, a secondary or tertiary amine group, a phosphate group, a phosphoester group, or an unsubstituted or substituted methylene group,
R⁷ and R⁸ independently are H, OH, alkyl, aryl, alkenyl, alkynyl, aromatic, ether, ester, unsubstituted or substituted amine, amide, halogen or unsubstituted or substituted sulfonyl, or jointly complete a 5- or 6-member aliphatic or aromatic ring,
R⁹ and R¹⁰ independently are H, OH, alkyl, aryl, alkenyl, alkynyl, aromatic, ether, ester, unsubstituted or substituted amine, amide, halogen or unsubstituted or substituted sulfonyl, or jointly complete a 5- or 6-member aliphatic, aromatic or heterocyclic ring,
R¹¹ is H, OH, alkyl, aryl, alkenyl, alkynyl, aromatic, ester or unsubstituted or substituted amine,
R¹² is COOH, COOR, CONH₂, CONHR, CONNR, R¹⁴, NH₂, NHR, NR³R¹⁴, or a carboxylate salt,
R¹³ and R¹⁴ independently are unsubstituted or substituted alkyl, aryl, alkaryl, aralkyl, alkenyl or alkynyl,
p is 0 to 3, and
m is 0 to 5;
or
single stereoisomers, mixtures of stereoisomers, or pharmaceutically acceptable salts thereof.

In a preferred embodiment the method of treating or ameliorating a subject suffering from a mitochondrial disorder comprises administering to said subject a therapeutically effective amount of a compound of Formula I; in another preferred embodiment the method comprises administering a compound of Formula I wherein R³ is hydrogen, C₁₋₆ alkyl, or C₂₋₂₀ alkenyl and in another embodiment the method comprises administering a compound of Formula I wherein R⁵ and R⁶ are C₁₋₄ alkyl or halogen, preferably wherein R⁵ and R⁶ are C₁₋₄ alkyl or halogen and R¹ is C₁₋₆ alkyl and R² is C₁₋₂₀ alkyl.
In another embodiment, the method comprises administering a compound of Formula I wherein R¹ is C₁₋₈ alkyl and R₂ is C₁₋₆ alkyl or C₁₋₈ alkenyl, and in yet another embodiment the method comprises administering a compound of Formula I wherein R⁸ and R⁹ taken together with the carbon to which they are attached form a 5-6 membered carbocyclic ring, which may be optionally substituted with C₁₋₆ alkyl, C₁₋₆ alkoxy, hydroxy, carboxy, carboxyalkyl, alkoxyalkyl, alkoxyalkyl, aminocarbonyl, aminocarboxyalkyl, or hydroxyalkyl.

In another preferred embodiment the method of treating or ameliorating a subject suffering from a mitochondrial disorder comprises administering to said subject a therapeutically effective amount of a
compound of Formula II; and in another preferred embodiment the method comprises administering a compound of Formula II wherein R\(^{12}\) is –COOH or –COOR\(^{13}\) and R\(^{13}\) is C\(_{1-4}\) alkyl.

In a preferred embodiment, the invention relates to a method of treating or ameliorating a subject suffering from disorders including but not limited to a disease involving mitochondrial disorders selected from Alzheimer's disease, Parkinson's disease, Friedreich's ataxia (FRDA), cerebellar ataxias, Leber's hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactacidosis, stroke (MELAS), Myoclonic Epilepsy with Ragged Red Fibers (MERFF), amyotrophic lateral sclerosis (ALS), motor neuron diseases, Huntington's disease, macular degeneration, and epilepsy by administering to said subject a therapeutically effective amount of a compound of Formula I or Formula II or a pharmaceutically acceptable salt thereof. In a more preferred embodiment the invention relates to a method of treating or ameliorating a subject suffering from Friedreich's ataxia, MELAS, or MERFF by administering to said subject a therapeutically effective amount of a compound of Formula I or Formula II.

In another preferred embodiment the treatment comprises protecting or ameliorating a subject in need of protection of Friedreich's ataxia comprising administering a therapeutically effective amount of a tocopherol selected from beta-tocopherol, delta-tocopherol, gamma-tocopherol, alpha-tocotrienol, beta-tocotrienol, delta-tocotrienol, or gamma-tocotrienol.

Particularly preferred are those methods of treatment and uses in the manufacture of pharmaceutical compositions therefor, wherein the compound of Formula I or Formula II is selected from the preferred compounds, and especially from the compounds selected from:

- 2,2,7,8-Tetramethyl-2H-chromen-6-ol;
- 3-(6-Hydroxy-2-methyl-3,4-dihydro-2H-benzo[h]chromen-2-yl)-propionic acid;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 2,7,8-Trimethyl-6-morpholin-4-ylmethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
- 5-(4-Benzyl-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
- 3-(8-Dihydroxy-2-methyl-chroman-2-yl)-propionic acid;
- 5-[3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propyl]-thiazolidine-2,4-dione;
- 2-Hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 3-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylsulfanyl)-2-methyl-propionic acid;
- 2-Hydroxymethyl-6-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)-tetrahydro-pyran-3,4,5-triol;
- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
- 3-(6-Hydroxy-2-methyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-[8-(2-Methoxycarbonyl-ethyl)-3,5,6,8-tetramethyl-1,2,3,8,9,10-hexahydro-pyano[3,2-f]chromen-3-yl]-propionic acid methyl ester;
3-[8-(2-Carboxy-ethyl)-3,5,6,8-tetramethyl-1,2,3,8,9,10-hexahydro-pyran0[3,2-f]chromen-3-yl]-propionic acid;
3-(6-Hydroxy-2-methyl-chroman-2-yl)-propionic acid;
3-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid;
3-(2,5,7,8-Tetramethyl-chroman-2-yl)-propionic acid;
3-(6-Hydroxy-2,7,8-trimethyl-5-nitro-chroman-2-yl)-propionic acid;
3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
3-[6-Hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid;
3-(6-Hydroxy-2,8-dimethyl-chroman-2-yl)-propionic acid;
2,2,7,8-Tetramethyl-chroman-6-ol;
2,2,7,8-Tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
2,2,7,8-Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylsulfanyl)-acetic acid methyl ester;
2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
2-(3-Chloro-propyl)-2,7,8-trimethyl-chroman-6-ol;
2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
1-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-pyrrolidine-2-carboxylic acid methyl ester;
2,2,5,8-Tetramethyl-chroman-6-ol;
7-Bromo-2,2,5,8-tetramethyl-chroman-6-ol;
2,2,7,8-Tetramethyl-5-(3-methyl-penty)-chroman-6-ol;
2,2,5,8-Tetramethyl-7-(3-methyl-butyl)-chroman-6-ol;
5-Isopropoxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
5-Hexyloxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
5-tert-Butyloxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
3-(5-Bromo-6-hydroxy-2-methyl-3,4-dihydro-2H-benzo[h]chromen-2-yl)-propionic acid
2-(2-Hydroxy-ethyl)-2,7,8-trimethyl-chroman-6-ol;
Disodium salt of Phosphoric acid mono-[2,2,7,8-tetramethyl-5-(3-methyl-butyl)-chroman-6-
yloxyethyl] ester;
2-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-propane-1,3-diol;
6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carbonitrile;
2,2,5,7,8-Pentamethyl-2H-chromen-6-ol;
2-Ethynyl-2,5,7,8-tetramethyl-chroman-6-ol;
10-Methoxy-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromen-6-ol;
5-(2-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-morpholin-4-yl-methanone;
5-(1-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
5-Hydroxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
2,2,5,7-Tetramethyl-8-nitro-chroman-6-ol;
6-Hydroxy-2,2,5,7-tetramethyl-chroman-8-carbaldehyde;
2,2,7,8-Tetramethyl-5-nitro-chroman-6-ol;
2,2,5,7-Tetramethyl-8-(3-methyl-but-2-enyl)-chroman-6-ol;
8-Fluoro-2,2,5,7-tetramethyl-chroman-6-ol;
2,2,5,7-Tetramethyl-8-(3-methyl-butyl-chroman-6-ol;
5-Methoxy-2,2,7,8-tetramethyl-chroman-6-ol;
8-tert-Butyl-2,2,5-trimethyl-chromanol-6-ol;
2,2,5,7-Tetramethyl-8-trifluoromethyl-chroman-6-ol;
Acetic acid 2,2,5-trimethyl-3,4-dihydro-2H-benzo[h]chromen-6-yl ester;
7-tert-Butyl-2-(3-chloro-propyl)-2-methyl-chroman-6-ol;
2-(3-Chloro-propyl)-8-isopropyl-2,5-dimethyl-chroman-6-ol;
5-Chloro-2-(3-chloro-propyl)-2,8-dimethyl-chroman-6-ol;
8-Chloro-2-(3-chloro-propyl)-2,5-dimethyl-chroman-6-ol;
3-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid methyl ester;
2,5,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol;
2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol;
2,8-Dimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
2,5,7,8-Tetramethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triaryl)-chroman-6-ol;
2,5,8-trimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triaryl)-chroman-6-ol;
2,7,8-trimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triaryl)-chroman-6-ol;
2,8-dimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triaryl)-chroman-6-ol;
or single stereoisomers, mixtures of stereoisomers, or pharmaceutically acceptable salts thereof.

Certain embodiments of the invention provide novel and preferred combinations of substituent
groups pendant from the formulae of the different inventions

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 physically 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), and -C(O)-(optionally substituted heterocyclyl).

The term "alkenyl" refers to a monoradical branched or unbranched, unsaturated or polunsaturated 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 "alkoxy" refers to the groups -O-alkyl, -O-alkenyl, -O-cycloalkyl, -O-cycloalkenyl, and -O-alkynyl. Preferred alkoxy groups are -O-alkyl 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, and the like.

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. The term "alkyl" also means a combination of linear or branched and cyclic saturated hydrocarbon radical consisting solely of carbon and hydrogen 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 "lower alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain of 1 to 6 atoms.

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 (wherein the amino group may be a cyclic amine), azido, carboxyl, (optionally substituted alkoxy)carbonyl, (optionally substituted amino)carbonyl, cyano, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, halogen, hydroxyl, nitro, sulfamoyl, sulfanyl, sulfinyl, sulfonyl, and sulfonic acid. One of the preferred optional substituents for alkyl is hydroxy, exemplified by hydroxalkyl groups, such as 2-hydroxyethyl, 3-hydroxypropyl, 3-hydroxybutyl, 4-hydroxybutyl, and the like; dihydroxalkyl groups (glycols), such as 2,3-dihydroxypropyl, 3,4-dihydroxybutyl, 2,4-dihydroxybutyl, and the like; aminoalkyl groups such as dimethyl aminoalkyl, piperidinylalkyl, morpholinylalkyl, and those compounds known as polyethylene glycols, polypropylene glycols and polybutylene glycols, and the like. Another preferred optional substituent for alkyl is sulfanyl exemplified by allylsulfanyl, carboxypropylsulfanyl, 2-methyl-propionylpyrrolidine-2-carboxylic acid, 5-methyl-1-H-benzimidazol-2-yl-sulfanyl, sulfoxidemethylsulfanyl, 4,6-dimethyl-...
pyrimidin-2-ylsulfanyl, 4-carboxy-benzyl-sulfanyl, isobutylsulfanyl, and the like. Other preferred optional substituents for alkyl are \(-\text{N-hydroxyureidyl, -N-hydroxythiureidyl or -N-hydroxyacetamide.}\)

The term "amino" refers to the group \(-\text{NH}_2\) as well as to the groups \(-\text{NHR or -NRR}\) 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 heterocyclyl, acyl, optionally substituted alkoxy, carboxy and alkoxy carbonyl, and where \(-\text{NRR}\) may be a cyclic amine.

The term "amino acid" or "natural amino acid" refers to any of the twenty (20) common amino acids as generally accepted in the peptide art.

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

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: hydroxy, thiol, 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 arloxy, azido, carboxyl, (optionally substituted alkoxy)carbonyl, (optionally substituted amino)carbonyl, cyano, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, halogen, optionally substituted heterocyclyl, optionally substituted heterocycloxy, hydroxyl, nitro, sulfanyl, sulfanyl, sulfanyl, and sulfonic acid. Preferred aryl substituents include alkyl, alkenyl, alkoxy, halo, cyano, nitro, trihalomethyl, carboxy, amino, amido, sulfonamido, and sulfanyl.

The term "carbonyl" refers to the di-radical \(-\text{C(=O)\text{-}}\), which is also illustrated as \(-\text{C(O)=}\).

The term "(optionally substituted amino)carbonyl" refers to the group \(-\text{C(O)\text{-}}\) (optionally substituted amino). This moiety is also referred to as a primary, secondary or tertiary carboxamide.

The term "carboxy" or "carboxyl" refers to the moiety \(-\text{C(O)OH}\), which is also illustrated as \(-\text{COOH}\).

The term "cycloalkyl" refers to non-aromatic cyclic hydrocarbon groups of having about 3 to 40 (preferably about 4 to 15) carbon atoms having a single ring or multiple condensed or bridged rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like. The term "cycloalkyl" additionally encompasses spiro systems wherein the cycloalkyl ring has a carbon ring atom in common with another ring.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.
The terms "heterocycle", "heterocyclic", "heterocyclo", and "heterocycl" refer to a monovalent, saturated, partially unsaturated or unsaturated (aromatic), carbocyclic radical having one or more rings incorporating one, two, three or four heteroatoms within the ring (chosen from nitrogen, oxygen, and/or sulfur). Preferred heterocycles include morpholine, piperidine, piperazine, thiazole, thiazolidine, isothiazole, oxazole, isoxazole, pyrazole, pyrazolidine, pyrazoline, imidazole, imidazolidine, benzothiazole, pyridine, pyrazine, pyrimidine, pyridazine, pyrrole, pyrrolidone, quinoline, quinazoline, purine, carbazole, benzimidazole, pyrimidine, thiophene, benzothiophene, pyran, tetrahydropyran, benzopyran, furan, tetrahydrofuran, indole, indoline, indazole, xanthene, thioxanthene, acridine, quinuclidine, and the like.

The terms "substituted heterocycle", "substituted heterocyclic", "substituted heterocyclo" and "substituted heterocycl" refer to a heterocycle 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: hydroxy, thiol, 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 heterocyclo, optionally substituted heterocycloxy, hydroxyl, nitro, sulfanyl, sulfinyl, and sulfonic acid. Preferred substituted heterocycles include thiazolidine-2,4-dione and 3-methyl-6-oxo-4,5-dihydro-1H-pyrrozol.

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 alkylene)-(optionally substituted heterocycle)", each having the meaning as defined herein.

The term "mitochondrial diseases or disorders" of which hundreds of varieties have been identified -- can cause a complex variety of symptoms. These include muscle weakness, muscle cramps, seizures, food reflux, learning disabilities, deafness, short stature, paralysis of eye muscles, diabetes, cardiac problems and stroke-like episodes, to name a few. The symptoms can range in severity from life-threatening to almost unnoticeable, sometimes taking both extremes in members of the same family. Because some people have specific subsets of these symptoms, clinical researchers have grouped those that occur together into "syndromes," producing a bewildering array of descriptive acronyms such as MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) or MERFF (myoclonus epilepsy with ragged red fibers). This term also includes disorders such as Kearns-Sayre syndrome (KSS), Leigh's syndrome, maternally inherited Leigh's syndrome (MILS), Myasthenic myopathy (MNGIE), Neuropathy, ataxia and retinitis pigmentosa (NARP), Friedreich's ataxia (FRDA), amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, Huntington's disease,
macular degeneration, epilepsy, Alzheimer's, Leber's hereditary optic neuropathy (LHON), Progressive external ophthalmpoplegia (PEO), and Pearson syndrome.

The term "neurodegenerative disorders" refers to disorders characterized by a loss of neurons and may or may not include an inflammatory process. Neurodegenerative disorders include stroke, head trauma, cerebral hypoxia, spinal cord injury, senile dementia, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, cerebral amyloid angiopathy, HIV-related dementia, Parkinson's disease, Huntington's disease, prion diseases, myasthenia gravis, Down's syndrome, Creutzfeldt-Jakob disease, Friedrich's ataxia (FRDA), Fergusson and Critchley's ataxia and other ataxias, Leber's hereditary optic neuropathy diabetic neuropathy (LHON), neuropathic pain, encephalitis, meningitis, and Duchenne's muscular dystrophy.

The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes 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, 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, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic group.
Specific examples of suitable amines include, by way of example only, isopropylamine, trimethylamine, diethyl amine, tri(iso-propyl) amine, tri(n-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like.

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 "seizure" is used herein as meaning the physical affliction characterized by transient clouding of consciousness, generally associated with a disturbance in the electrical activity of the cortex of the brain. Illustrative of such seizures are those associated with petit mal epilepsy.

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 "tocopherol" means any of a family of molecules (including both tocopherols and tocotrienols and derivatives thereof) which are characterized by a chroman-6-ol ring structure and a side chain at the 2-position. Tocopherols possess a 4',8',12'-trimethyltridecyl phytyl side chain and the tocotrienols differ by the presence of double bonds at 3',7',11" positions of the side chain. The chemical names for the tocopherols are:

- beta-tocopherol: 2,5,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol;
- gamma-tocopherol: 2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol;
- delta-tocopherol: 2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-chroman-6-ol;
- alpha-tocotrienol: 2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triynyl)-chroman-6-ol
- beta-tocotrienol: 2,5,8-trimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triynyl)-chroman-6-ol;
- gamma tocotrienol: 2,7,8-trimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triynyl)-chroman-6-ol;
- delta tocotrienol: 2,8-dimethyl-2-(4,8,12-trimethyl-trideca-3,7,11-triynyl)-chroman-6-ol.

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;
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- 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 was generated using or with the help of version 2.2 of the AUTONOM™ naming package within the ChemOffice® version 7.0.3 suite of programs by CambridgeSoft Corp (Cambridge, MA).

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, tetrahydrofuran ("THF"), chloroform, methylene chloride (also named dichloromethane ("DCM"), diethyl ether, ethyl acetate ("EtOAc"), 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 0°C to 110°C (preferably from 0°C to 25°C; most preferably at "room" or "ambient" temperature ("RT"), 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 0°C to about 110°C (preferably from about 0°C to about 25°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).

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 herein below. However, other equivalent separation or isolation procedures can also be used.

Some compounds of the present invention are available commercially or can be synthesized as known in the art. Some exemplary syntheses of compounds of this invention are also described in Examples.

Preferred Compounds

The compounds of Formula I or Formula II encompass the chroman derivatives of the invention as disclosed, and/or the pharmaceutically acceptable salts of such compounds. In addition, the compounds of this invention include the individual stereochemical isomers and mixtures thereof, arising from the selection of substituent groups. 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.

Preferred for the compounds, pharmaceutical formulations, methods of manufacture and use of the present invention are the following combinations and permutations of substituent groups of Formula I and Formula II.

Utility, Testing and Administration

General Utility

Compound, 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 degenerative diseases of the brain (Wernicke-Korsakoff disease, Kreuzfeld-Jakob disease (KJD), Hallervorden-Spatz disease, Schilder's disease, Alzheimer's disease, senile dementia, Down's syndrome in middle age, Abercrombie's disease, Prion diseases, Zellweger syndrome, Alper's Syndrome), spinocerebellar degenerations (spinal ataxia, cerebellar cortical degenerations, Friedreich's ataxia (FRDA) and other ataxias), multiple system degenerations (Menzel, Dejerine-Thomas, Shy-Drager, and Machado Joseph), systemic disorders (Refsum disease, ataxia telangiectasia), epilepsy, mitochondrial disorders (MELAS, MERFF, KSS, Leigh's, MILS, MNGIE, NARP, PEO, Pearson), demyelinating core disorders (multiple sclerosis, acute transverse myelitis), muscular atrophies (amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), infantile spinal muscular atrophy, Huntington's disease, spinobulbar atrophy (SBA), juvenile spinal muscular atrophy, myasthenia gravis and other motor neuron diseases), movement disorder (drug-induced Parkinsonism or Parkinson's disease), retinopathy (Leber's hereditary optic neuropathy, age-related macular degeneration (AMD), cataracts), cerebral ischemia ("stroke" most often caused by
thrombosis, vasoconstriction and embolism), myocardial ischemia (including chronic stable angina, angina pectoris, unstable angina and Prinzmetal's angina, silent ischemia, reinfarction, reocclusion, restenosis, myocardial infarction and other forms of heart disease), diabetes, renal disease, pre-menstrual syndrome (PMS), asthma, cardiopulmonary inflammatory disorders, chronic heart failure, rheumatoid arthritis, muscle fatigue, irritable bowel syndrome, inflammatory bowel disease, intermittent claudication and for the preservation of allograft tissue for transplantation. Certain compounds of the present invention are also useful in treating conditions falling with the group of dermatologic conditions, in particular prevention and protecting skin tissue against age-related damage or damage resulting from insults such as harmful ultraviolet (UV) radiation, stress and fatigue, and in the treatment of contact dermatitis, skin irritation, skin pigmentation, psoriasis, or acne.

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 the exemplary indications, i.e., stroke, epilepsy, Parkinson's disease, Friedrich's ataxia, MELAS, macular degeneration, ALS, and Alzheimer's disease.

MPTP/MPP⁺-induced neurodegeneration of dopaminergic neurons is a well characterized model which is therefore widely used to understand the pathogenesis of Parkinson's disease. The compounds were tested on 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 as described in Son JH, et al JW. (1999) *J Neurosci*, 19: 10-20, 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 by astrocytes into 1-methyl-4-phenylpyridinium (MPP⁺), a substrate for the dopamine transporter which then selectively inhibits complex 1 of the mitochondrial electron transport chain. This results in depletion of ATP, the production of reactive oxygen species and, consequently cell death. In a number of species, including humans, non-primates and rodents, MPTP produces an irreversible and severe parkinsonian syndrome which includes virtually all the clinical features of the disease. 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 dermal 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)-sulfirximine) under conditions of restricted selenium causes depletion of cellular
glutathione (GSH) and 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.

Protection against redox stress can be further evaluated in cell culture using high glutamate induced oxidative stress (HGOS) in mouse dopaminergic cell lines. The cytotoxic effect of glutamate is not due to excitotoxicity, as this cell line is devoid of inotropic glutamate receptors. Rather, the glutamate-induced toxicity of dopaminergic cells is associated with an inhibition of cystine transport which subsequently leads to depletion of intracellular glutathione (GSH) levels (Murphy T. H., et al. Neuron 2, 1547 -1558, 1989), activation of neuronal 12-lipoxygenase (Li, Y. et al., Neuron 19,453 -463, 1997), increased ROS production (Tan S. et al., J. Cell Biol. 141, 1423 -1432, 1998) and elevated intracellular Ca^{2+} (Li, Y. et al., see supra). Some molecules were measured for their ability to protect such cells against glutamate-induced stress and the assay is detailed in Examples.

In addition IL-1 elevation has been associated with many neurodegenerative diseases. There is increasing evidence for a role of IL-1 in Alzheimer’s Disease (AD) (Mrak RE et al. Neurobiol Aging 22(6):903-908, 2001). Elevated levels of IL-1β have been shown to surround amyloid plaques in the disease and recent genetic studies have indicated that a polymorphism in IL-1α is linked to an increased risk of AD (3-6 fold increase) (Griffin WS et al., J Leukoc Biol 72(2):233-238, 2002). This polymorphism has also been correlated with rate of cognitive decline in AD patients (Murphy GM et al., Neurology, 56(11)1595-1597, 2001). The risk of AD is increased even further when the polymorphism in IL-1.α is found in combination with another polymorphism in IL-1β (see Griffin WS, supra), providing convincing evidence that these cytokines play an important role in the pathology of the disease.

This assay measures the release of IL-1β from a mouse microglial cell line following an inflammatory challenge with LPS and interferon-gamma. The ability of test articles to inhibit microglial cell activation and IL-1β release is determined by co-incubation of the test article with the inflammatory challenge. Cytokine release is measured using a mouse IL-1β ELISA and cell toxicity is determined using Cell Tracker Green (a fluorescent dye that measures cell viability).

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.
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.

Administration

The compounds of this invention are administered at a therapeutically effective dosage, e.g., a dosage sufficient to provide treatment or amelioration 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 10.0 mg/kg of body weight, preferably about 0.1 to 5.0 mg/kg of body weight, and most preferably about 0.3 to 1.0 mg/kg of body weight. 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 or amelioration 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 Formulae I or II, 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, polyvinylpyrrolidone, 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.

Another preferred manner of administration is the topical administration. "Topical administration" refers to application of the present compositions by spreading, spraying, etc. onto the surface of the skin. The typical amount applied may vary from about 0.1 mg of composition per square centimeter of skin to about 25 mg of composition per square centimeter of skin. The compounds of the present invention may be formulated for topical administration to the epidermis as ointments, creams or lotions or as transdermal patch. Formulations suitable for topical administration in the mouth include lozenges, pastilles and mouthwashes.

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

As reported in the following examples, 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

Beta-Amyloid Cell Death Assay

Media Composition

**Neurobasal/B27**: 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**: 143.6 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, 1 mM Na₂HPO₄, 26.2 mM NaHCO₃, 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/B27 was added. The cells were triturated 6 times with siliconized pipettes (decreasing bore size) after which an additional 5ml Neurobasal/B27 was added. The cell suspension was then
centrifuged at 1000 rpm for 2 min, the supernatant was aspirated and 2ml of Neurobasal/B27a was added. Cells were triturated again as described above and the volume of Neurobasal/B27a 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 Co, Sunnyvale, CA) into oligomers was carried out according to the method of Dahlgren et al, (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J Biol Chem 277: 32046-32053. The Aβ peptide was dissolved to 1mM in hexafluorisopropanol (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-BSS0. 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% CO₂) 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).

Certain compounds of the present invention such as

- 2,2,7,8-Tetramethyl-chroman-6-ol; and
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;

when tested as described above exhibited assay between 10% and 40% protection in the β amyloid cell assay.
Example 2
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

A 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% CO₂) 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% CO₂).

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% CO₂) 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 such as

- 2,2,7,8-Tetramethyl-chroman-6-ol;
- 4-[1-(Carboxymethyl-carbamoyl)-2-mercapto-ethylcarbamoyl]-2-[3-(6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionylamin]-butyric acid;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 2,5-Dimethyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol;
Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
5,6,9-Trimethyl-8-oxa-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6-trien-4-ol;
(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylsulfanyl)-acetic acid methyl ester;
2-Methyl-2-thiophen-2-yl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol;
2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol; and
2,2-Dimethyl-5-(3-methyl-but-2-enyl)-3,4,7,10-tetrahydro--7,10-ethano-2H-benzo[h]chromen-6-ol
when tested as described above provided protection in at least 30%, preferably in at least 50% of
the cells tested at concentrations ranging from 1 to 25μM.

Example 3
MPTP Animal Model

Male C57/BL6 mice (Harlan, IN), weight 25-30g, were used in all studies. MPTP-HCl (Sigma) was
administered i.p. according to one of the following protocols. The maximum volume which was given
per injection is 200μL. In all studies, animals were euthanized with carbon dioxide and, brains were
removed for subsequent determination of dopamine depletion where appropriate.

Subacute model
Animals received 25mg/kg of MPTP once a day for 5 consecutive days. The end point was 2 days after
the final dose.

Acute model
Animals received 4 x 20mg/kg of MPTP at 2 hour intervals. The end point was at either 7 or 14 days.

Subchronic model
Animals received 2 x 40mg/kg of MPTP with this repeated 16 hours later. The end point was at either 14
or 28 days.

Chronic model
Animals received 25mg/kg of MPTP, given twice weekly for 5 weeks. The end point was either 1, 3 or 24
weeks after the final dose.

Neurobehavioral outcome measures

Compound efficacy was examined with the use of neurobehavioral models. These models allowed the
determination of a given compound’s ability to reverse the motor deficits seen with MPTP
treatment.

All animals received pre-training for the individual model and a baseline reading was obtained
one day prior to the commencement of MPTP treatment.
Open field test: The open field test measures spontaneous activity. Deficits can be observed with hippocampal and basal ganglia lesions, and with hindlimb dysfunction. It is sensitive to moderate dopamine (DA) depletion.

The open field test was carried out in a clear, acrylic open box 60cm x 60cm. The base of the box was marked into a 5 x 5 grid of 12cm x12cm squares. Animals were individually placed in the box and allowed to roam free. The number of squares an animal crossed in a 90 second time period was recorded. In order to be scored, the animal must either have had all four limbs within a given square or, all four limbs must have left a given square.

Tactile adhesion model: The tactile adhesion model measures an animal’s ability to complete a complex sensorimotor task. It is sensitive to moderate to severe DA depletion.

A tactile stimulus (0.5 x 0.5 cm square of “sticky tape”) was applied to each side of the animal’s face and the time taken to remove it is recorded. The following measurements were recorded:

a. Latency to contact left side tape  
b. Latency to contact right side tape  
c. Order of side contacted (left vs. right)  
d. Latency to remove left side tape  
e. Latency to remove right side tape

Pole test: The pole test evaluates motor co-ordination.

A rough surfaced metal pole (diameter 8mm, height 50cm) protruding from a cage filled with animal bedding was used for this test. Animals were placed head upwards at the top of the pole. They were required to turn and descend face downwards and the time taken for this was recorded (latency to reach bedding). Timing was started when the individual animal gripped the top of the pole and was stopped when all four limbs contacted the bedding.

Compound administration

All test compounds were administered ip. The maximum volume which was given per injection was 200µL. Compounds were dosed up to twice daily, two days prior and 7 days post MPTP treatment.

Data analysis

A baseline reading was taken for each animal one day prior to MPTP treatment. All subsequent readings were normalized to the individual animal’s baseline. Values were expressed as a percent baseline.

Data was percent baseline and expressed as mean ± std dev:

Summary

Compound and vehicle given for 9 days in total, 2 days prior to MPTP and 7 days after MPTP treatment. L-DOPA given 1 hour prior to the pole test, each day.

Results

Certain compounds of the present invention such as
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- 2,2,7,8-Tetramethyl-2H-chromen-6-ol;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-[6-Hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid;
- 3-(6-Hydroxy-2,8-dimethyl-chroman-2-yl)-propionic acid;
- 2,2,7,8-Tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
- (6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylsulfanyl)-acetic acid methyl ester.

at a dose of 30mg/kg/day significantly reduced the functional deficit produced by MPTP on each of days 1-3. Functional outcome was significantly better on days 1 and 2 than in vehicle treated animals and in both cases were comparable to that of the negative control group. Doses of 10mg/kg/day and 3mg/kg/day reduced the MPTP induced deficit on days 2 and 3 but this may not be significantly different from vehicle treated animals.

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. Lines F2, C2 and C3 were obtained from Coriell Cell Repositories (Camden, NJ, USA; catalog #’s GM04078, GM 08402 and GM08399, respectively). All cell types were diagnosed at the molecular level for intronic GAA triplet repeat length in the tratarixin gene using a PCR-based method, according to methods known in the art. FRDA-fibroblasts 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 1 mM 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 calcinAM 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.
C. Data and statistics

In experiments carried out in support of the present invention, certain compounds such as

- 2,2,7,8-Tetramethyl-2H-chromen-6-ol;
- 4-[1-(Carboxymethyl-carbamoyl)-2-mercapto-ethylcarbamoyl]-2-[3-(6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionylamino]-butyric acid);
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 2-Amino-4-{1-(carboxymethyl-carbamoyl)-2-[6-hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-5-ylmethylsulfanyl]-ethylcarbamoyl}-butyric acid;
- 5-[3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propyl]-thiazolidine-2,4-dione;
- Delta tocopherol;
- Delta tocotrienol;
- Gamma-tocopherol;
- Gamma-tocotrienol;
- 3-(6-Hydroxy-2,8-dimethyl-chroman-2-yl)-propionic acid;
- 2,2,7,8-Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol; and

reduced cell death in FRDA fibroblasts compared to untreated FRDA fibroblasts with an EC50 of between 0.01μM and 6μM.

Example 5

High Glutamate-Induced Oxidative Stress Assay (HGOS)

This procedure was used to induce high glutamate-induced 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 lines
- 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 MgCl2.6H2O, 3.73g/L KCl, 59.58g/L Heps, 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% CO2 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% CO2 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-Hydroxy-2,2,5,7-tetramethyl-chroman-8-carbaldehyde;
• 2,2,5,7-Tetramethyl-8-nitro-chroman-6-ol;
• 5-(1-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
• 5-Hydroxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
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- 5-(2-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
- 10-Methoxy-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromen-6-ol;
- 2-Ethynyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 2-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)-N-(3-trifluoromethyl-phenyl)-acetamide;
- (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-piperazin-1-yl-methanone;
- 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxamidine;
- N-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethyl)-N-methyl-acetamide;
- 2,2,5,7,8-Pentamethyl-2H-chromen-6-ol;
- 2-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-propane-1,3-diol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
- 2,2,5,8-Tetramethyl-chroman-6-ol;
- 2-(3-Chloro-propyl)-2,7,8-trimethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
- (6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyisulfanyl)-acetic acid methyl ester;
- 5,6,9-Trimethyl-8-oxa-tricyclo[7.3.1.03,7]trideca-2,4,6-trien-4-ol;
- 2,2,7,8-Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
- 2-Hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 5-[3(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propyl]-thiazolidine-2,4-dione;
- 5-(4-Benzyl-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2-methyl-3,4-dihydro-2H-benzo[h]chroman-2-yl)-propionic acid; and
- 2,2,7,8-Tetramethyl-chroman-6-ol;

were considered to be active when they exhibited protection against HGOS cell injury and cell death with an EC<sub>50</sub> in a range of 5μM or less.

**Example 6**

2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol

![Structure of 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol](image)

**Step 1:**

To a solution of 4-chloro-butanone-2 (2.65 g, 25 mmol) in 25 ml of THF at -20°C was added dropwise a solution of 1M vinyl magnesium bromide (40 ml, 40 mmol). After the completion of addition the mixture was stirred for one more hours at room temperature. To the mixture was added 10 ml of water at 0°C with stirring. The mixture was dried over MgSO<sub>4</sub>. After the removal of the solvent, the residue was
purified via flash column purification with 1:3 ethyl acetate and hexane as elute to afford 800 mg of 5-chloro-3-methyl-pent-1-en-3-ol. $^1$H-NMR (300MHz, CDCl$_3$) δ (ppm): 5.86-5.90 (dd, 1H); 5.29 (d, 1H), 5.13 (d, 1H), 3.50-3.59 (t, 3H); 1.87-1.83 2.18 (m, 2H); 1.63-1.69 (m, 2H), 1.31 (s, 3H)

**Step 2:**
A mixture of 2,3-dimethyl-dihydroquinone (794mmol), 1.0ml (3.97mmol) of BF$_3$Et$_2$O and 25 ml of dioxane was heated up to 110°C under a nitrogen atmosphere. A solution of 5-chloro-3-methyl-pent-1-en-3-ol (1.12g, 8.6 mmol) in 24 ml of dioxane was added slowly to the mixture. The addition was completed after two hours and the mixture was continued to reflux for an additional 3 hours. After cooling down to room temperature, the mixture was poured to water, extracted with ethyl acetate, washed with water and dried over MgSO$_4$. After the removal of solvents, the residue was mixed with methanol / HCl and let stirred overnight at room temperature. After the removal of the solvents the residue was purified via flash column chromatography (1:3 EtOAc/Hex) twice to give 2-(2-chloro-ethyl)-2,7,8-trimethylchroman-6-ol $^1$H-NMR (300MHz, CDCl$_3$) δ (ppm): 6.39 (s, 1H), 4.26 (s, 1H), 3.80-3.85 (m, 2H), 2.85-2.70 (m, 2H), 2.16 (s, 3H), 2.12 (s, 3H), 2.20-2.05 (m, 2H), 1.85-1.75 (m, 2H), 1.31 (s, 3H). MS: 255 (M + H$^+$, 100%)

**Example 7**

2,2,7,8-Tetramethyl-4H-benzo[1,3]dioxin-6-ol

![Structure](image)

**Step 1**
To a mixture of 2,3-dimethylhydroquinone (1.38 g, 10 mmol), K$_2$CO$_3$ (2.76g, 20 mmol), potassium iodide (0.83 g, 5 mmol) in 50 mL dry acetone was added benzyl bromide (1.88 g, 11 mmol). The resulting suspension was vigorously stirred for 48 at RT. The solid was filtered off and the liquid was concentrated. The residue was chromatographed to afford the benzyl derivative, 4-benzylxylo-3-methyl-phenol, as a light brown solid (1.15 g). $^1$H-NMR (300 MHz, CDCl$_3$) δ (ppm): 7.49-7.35 (m, 5 H), 6.70 (d, J = 8.7, 1 H), 6.61 (d, J = 8.7, 1 H), 5.03 (s, 2 H), 4.43 (s, 1 H), 2.26 (s, 3 H), 2.22 (s, 3 H); MS (ESI) m/z: 229 (M+H$^+$, 100%).

**Step 2:**
To 684 mg (3 mmol) of 4-benzylxylo-3-methyl-phenol in 10 mL toluene and 1.5 mL DME (dimethoxyethane) in a sealable tube was added paraformaldehyde (1.8 g, 60 mmol). The tube was flushed with argon and sealed. It was heated to 130°C for 48h under stirring. After cooling to room temperature, the solid was filtered off and washed with 1:1 hexane/EtOAc and the liquid was concentrated. The residue was chromatographed to afford 4-benzylxylo-6-hydroxymethyl-2,3-dimethyl-phenol, as a light brown solid (640 mg). $^1$H-NMR (300 MHz, CDCl$_3$/CD$_2$OD) δ (ppm): 7.43-7.29 (m, 5 H), 6.55 (s, 1 H), 4.95 (s, 2 H), 4.70 (s, 2 H), 2.18 (s, 3 H), 2.16 (s, 3 H); MS (ESI) m/z: 241 (M-OH$^-$, 100%).
Step 3:
A solution of 4-benzyloxy-6-hydroxymethyl-2,3-dimethyl-phenol (86 mg, 0.33 mmol) in dimethoxypropane (10 mL) in the presence of toluene sulfonic acid (7 mg) was stirred at RT for 15 h. It was added 30 mg of anion-exchange resin and stirring was continued for 20 more min. The resin was then filtered off and the solution was concentrated. The crude product was purified by chromatography on a silicagel column to afford 6-benzyloxy-2,2,7,8-tetramethyl-4H-benzo[1,3]dioxine as a white sticky solid (86 mg). $^1$H-NMR (CDCl$_3$, 300 MHz) δ (ppm): 7.51-7.35 (m, 5 H), 6.45 (s, 1 H), 5.02 (s, 2 H), 4.85 (s, 2 H), 2.26 (s, 3 H), 2.19 (s, 3 H), 1.59 (s, 6 H); $^{13}$C-NMR δ (ppm): 150.6, 143.3, 137.8, 128.5, 127.8, 126.3, 125.8, 115.9, 105.4, 99.1, 70.9, 61.1, 24.9, 12.2, 11.5.

Step 4
To a solution of 6-benzyloxy-2,2,7,8-tetramethyl-4H-benzo[1,3]dioxine (86 mg, 0.29 mmol) in 10 mL EtOH was added Pd/C (15 mg, 10%). It was stirred in a hydrogen atmosphere for 1.5 h and filtered. The solution was concentrated and the crude product was purified by chromatography on silicagel to afford 2,2,7,8-tetramethyl-4H-benzo[1,3]dioxin-6-ol as a white solid (54 mg). $^1$H-NMR (CDCl$_3$, 300 MHz) δ (ppm): 6.28 (s, 1 H), 4.77 (s, 1 H), 4.76 (s, 2 H), 2.17 (s, 3 H), 2.13 (s, 1 H), 1.55 (s, 3 H), 1.54 (s, 3 H); $^{13}$C-NMR δ (ppm): 147.1, 142.9, 126.1, 122.7, 116.6, 107.5, 99.1, 60.9, 24.8, 11.9, 11.5.

Example 8
2,2,7,8-Tetramethyl-chroman-6-ol

Step 1
To a solution of 2,3-dimethylhydroquinone (2 g, 14.5 mmol) and BF$_3$-Et$_2$O (3.3 g, 23.2 mmol) in 30 mL of dioxane was slowly added a dilute solution of 2-methyl-but-3-en-2-ol (1.0 g, 11.6 mmol) in 10 mL of dioxane over a period of 30 min. Upon the completion of the alcohol addition, it was allowed to stir for 4 h. The reaction was quenched by pouring over ice (70 g) and the mixture was extracted with EtOAc (3x50 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated. The crude product was purified by chromatography (hexane/EtOAc =9:1) to afford 2,3-dimethyl-5-(3-methyl-but-2-enyl)-benzene-1,4-diol as a light brown oil (2 g). MS (ESI) m/z: 207 (M+H$^+$, 100%).

Step 2
A solution of 2,3-dimethyl-5-(3-methyl-but-2-enyl)-benzene-1,4-diol (2.54 g, 12.3 mmol) and BF$_3$-Et$_2$O (2.8 mL, 22.4 mmol) in 50 mL dioxane was heated to reflux for 5 h. It was cooled to RT and quenched onto ice (100 g). The mixture was extracted with EtOAc (3x60 mL) and dried over Na$_2$SO$_4$. The residue was purified by chromatography (Hexane/EtOAc=5:1) to afford 2,2,7,8-Tetramethyl-chroman-6-ol as a brown oil (1.5 g). $^1$H-NMR (300 MHz, CDCl$_3$) δ (ppm): 6.28 (s, 1 H), 5.30 (s, 1 H), 2.60 (t, J = 6.8, 2
Example 9

2,2,7,8-Tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol

To a solution of 2,2,7,8-tetramethyl-chroman-6-ol (305 mg, 1.39 mmol), prepared as described above, in 5 mL dry dioxane was added boron trifluoride (296 mg, 2.1 mmol). It was stirred for 3 min followed by dropwise addition of 2-methyl-but-3-en-2-ol solution (143 mg, 1.66 mmol, in 3 mL of dioxane). The reaction was allowed to stir for 5 h at RT before quenching on to ice (80 g). The mixture was extracted with DCM (3x50 mL) and the combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by chromatography (hexane) to afford 2,2,7,8-tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol as a light brown oil (229 mg). \(^1\)H-NMR (300 MHz, CDCl₃) δ (ppm): 5.16 (m, 1 H), 4.70 (s, 1 H), 3.34 (d, J = 6.8, 2.18 (s, 3 H), 2.14 (s, 3 H), 1.86 (s, 3 H), 1.81 (t, J = 13.8, 2 H), 1.77 (s, 3 H), 1.32 (s, 6 H); \(^1\)C-NMR δ (ppm): 145.7, 145.4, 134.1, 123.5, 122.2, 122.0, 121.8, 116.3, 72.5, 33.1, 26.7, 25.8, 20.8, 17.9, 12.1, 11.9; (ESI) m/z: 275 (M+H⁺, 100%).

Example 10

3-[6-Hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid

A solution of 3-(6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid (150 mg, 0.557 mmol) in 1M aq. NaOH (1.11 mL, 1.11 mmol) was cooled to 0 °C, and treated with prenyl bromide (0.064 mL, 0.557 mmol). Following stirring for 2 h at ambient temperature the reaction mixture was made slightly acidic with 0.5M HCl and shaken with EtOAc. The organic phase was evaporated yielding a brown residue which was subjected to column chromatography (SiO₂: hexane:EtOAc, 8:2 v/v) yielded 3-[6-hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid as a pale brown solid (50 mg). \(^1\)H-NMR (300 MHz, CDCl₃) δ (ppm): 5.15 (t, J = 7, 1H), 3.33 (d, J = 7, 2H), 2.73 (t, J = 7, 2H), 2.59 (t, J = 7, 2H), 2.17 (s, 3H), 2.12 (s, 3H), 1.86-2.10 (m, 2H), 1.85 (s, 3H), 1.80-1.85 (m, 2H), 1.76 (s, 3H), 1.26 (s, 3H). \(^1\)C-NMR (75 MHz, CDCl₃) δ (ppm): 180.2, 145.7, 145.2, 134.3, 123.7, 122.5, 122.0, 121.9, 116.2, 73.4, 34.4, 31.8, 28.7, 25.9, 25.6, 23.3, 20.4, 18.0, 12.2, 11.9. MS ESI-Pos m/z 333.2 (M+H⁺).
Example 11

2-Hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol

To a solution of 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (1.0 g, 4 mmol) in 50 mL dry THF at RT under argon atmosphere was added small portions of LiAlH₄ over a period of 30 min. The reaction was stirred for 2 h and quenched on to ice water (100 mL). It was extracted with EtOAc (3x50 mL) and the combined organic layers were washed with water (100 mL). The organic solution was dried over Na₂SO₄ and concentrated. The residue was chromatographed with hexane/EtOAc (3:1) and the desired compound 2-hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol was isolated as a cream-colored solid (655 mg). ¹H-NMR (300MHz, CDCl₃) δ (ppm): 4.90 (s, 1 H), 3.68 (m, 2 H), 2.70 (m, 2 H), 2.46 (m, 1 H), 2.20 (s, 3 H), 2.16 (s, 3 H), 2.15 (s, 3 H), 2.03 (m, 1 H), 1.76 (m, 1 H), 1.26 (s, 3 H); ¹³C-NMR δ (ppm): 145.2, 144.98, 122.6, 121.9, 119.3, 117.4, 75.2, 69.4, 27.9, 20.5, 20.4, 12.4, 12.0, 11.5; MS (ESI) m/z: 259 (M+Na⁺, 100%), 237 (M+H⁺, 45%).

Example 12

5-(4-Benzyl-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol

To a solution of bromo-tocopherol prepared as described above (1.00 g) in 25 ml of methylene chloride was added 1-benzylpiperazine (0.8 g) at room temperature. The solution was stirred at room temperature for 2 hours. More methylene chloride was added, washed with water, and dried over magnesium sulfate. Evaporation and chromatography (silica gel, methylene chloride) gave 250 mg of 5-(4-benzyl-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol. ¹H-NMR (300 MHz, CDCl₃) δ (ppm) 7.35-7.20 (m, 5H), 3.63 (s, 2H), 3.51 (s, 2H), 2.14 (s, 3H), 2.10 (s, 3H). MS (PAM-ES) m/z 605 (M+H⁺, 100%).

Similarly replacing benzylpiperazine with other amines the following compounds were produced:

2,7,8-trimethyl-5-piperazin-1-ylmethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol. ¹H-NMR (300 MHz, CDCl₃) δ (ppm) 3.62 (s, 2H), 2.14 (s, 3H), 2.10 (s, 3H). MS (PAM-ES) m/z 515 (M+H⁺, 100%).
2,7,8-trimethyl-5-morpholin-4-ylmethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$ (ppm) 3.74 (br. s, 4H), 3.64 (s, 2H), 2.65-2.50 (m, 6H), 2.14 (s, 3H), 2.10 (s, 3H).

2,7,8-trimethyl-5-pyrrolidin-1-ylmethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol; MS (ESI) m/z: 500 (M+H$^+$, 100%), and

2,7,8-trimethyl-5-piperidin-1-ylmethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol; MS (ESI) m/z: 514 (M+H$^+$, 100%)

**Example 13**

2,2,7,8-Tetramethyl-2H-chromen-6-ol

![Diagram](image)

**Step 1:**

A mixture of 2,3-dimethyl-1,4-dihydroquinone (5.0 g, mmol), acetone (80 mL), anhydrous potassium carbonate (18 g) and dimethyl sulfate (14 mL) was gently refluxed under nitrogen for 10 hours. The suspension was cooled to room temperature and poured into 300 mL of water. The precipitate was collected and washed with water, then dried in air to give 5.5 g of 1,4-dimethoxy-2,3-dimethyl-benzene as a purple solid.

**Step 2:**

A solution of 1,4-dimethoxy-2,3-dimethyl-benzene (1 g) in dichloromethane was stirred and cooled in an ice-water bath, and TiCl$_4$ (1.1 mL) was slowly added, followed by the addition of CHCl$_3$OCH$_3$ (0.58 g). The solution was stirred for another 15 min with ice-water bath, 30 min at RT, then 15 min at 35 °C. Then the red solution was poured into ice and extract with dichloromethane. Workup and purification by chromatography (silicagel column, eluting with EtOAc/hexane 1:4) gave 973 mg of 2,5-dimethoxy-3,4-dimethyl-benzaldehyde.

**Step 3:**

A solution of 2,5-dimethoxy-3,4-dimethyl-benzaldehyde (155 mg) in dichloromethane was stirred and cooled in an ice-water bath. BBr$_3$ (1M in CH$_2$Cl$_2$, 1.80 mL) was slowly added and the solution was stirred for another hour, then the red solution was poured into ice and extracted with dichloromethane. Workup and purification by chromatography (silicagel column, eluting with EtOAc/hexane 1:4) gave 110 mg of 2,5-dihydroxy-3,4-dimethyl-benzaldehyde.

**Step 4:**

To a solution of 2,5-dihydroxy-3,4-dimethyl-benzaldehyde (2.0 g) in dichloromethane (50 mL) was added 3,4-dihydro-2H-pyran (DHP)(1.5 g), followed by p-toluenesulfonic acid monohydrate (200 mg). The solution was stirred at RT for 1 hour and quenched by adding 1 mL of a sodium bicarbonate solution. Then dichloromethane was dried over MgSO$_4$ and concentrated. The residue was purified by silica gel
column chromatography eluting with hexane and ethyl acetate (8:2) to give 1.5 g of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yl)oxy-benzaldehyde.

**Step 5:**

A mixture of 2-hydroxy-3,4-dimethyl-5-(tetrahydro-pyran-2-yl)oxy-benzaldehyde (300 mg), methyl 3,3-dimethylacrylate (300 mg), and K₂CO₃ (300 mg) in 10 mL of DMF was stirred at 160 °C for 5 h. After letting it cool down to room temperature, it was worked up and purified by silica gel column chromatography eluting with 5% ethyl acetate in hexane, to give the THP-protected 2,2,7,8-tetramethyl-2H-chromen-6-ol intermediate. Removal of the protection group was accomplished as described above with toluenesulfonic acid in MeOH for 5 h. Purification by chromatography (silica gel column, eluting with 15% EtOAc in hexane) gave 83 mg of colorless solid of 2,2,7,8-tetramethyl-2H-chromen-6-ol. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 6.30 (s, 1H), 6.20 (d, J = 9.6 Hz, 1H), 5.58 (d, J = 9.6 Hz, 1H), 4.50 (s, 1H, OH), 2.13, 2.12 (2s, 6H), 1.39 (s, 6H) ppm. ¹³C NMR (CDCl₃, 75 MHz) δ: 147.02, 144.56, 130.88, 125.55, 123.59, 122.47, 119.03, 109.80, 75.49, 27.57, 12.18, 11.74. MS (m/z): 205 (M+H⁺).

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.
What is claimed is:

1. A method of treating or ameliorating a subject suffering from a mitochondrial disorder comprising administering to said subject a therapeutically effective amount of a compound of Formula I or Formula II:

![Chemical Structure](image)

Formula I

wherein:

- $\text{A-B-}$ is $\text{CH}_2\text{CH}_2\text{-}$; $\text{-CH=CH-}$ or $\text{-CH}_2\text{-OH}$;
- $n$ is 0;
- $R^1$ is C$_{1-4}$ alkyl;
- $R^2$ is C$_{1-20}$ alkyl or C$_{2-20}$ alkenyl, halogen, cyano, or R$^2$ and A together with the atoms to which they are attached form a ring;
- $R^3$ is
  - hydrogen;
  - halogen;
  - aralkyl, optionally substituted with one or more substituents selected from alkyl, haloalkyl, hydroxy, alkoxy, halogen, oxo, cyano, nitro, amino, -SO$_2$NR$_2$ or -C(O)OR;
  - heteroaralkyl optionally substituted with one or more substituents selected from alkyl, haloalkyl, hydroxy, alkoxy, halogen, oxo, cyano, nitro, amino, -SO$_2$NR$_2$ or -C(O)OR;
  - C$_{1-6}$ alkyl;
  - C$_{2-20}$ alkenyl;
  - -(CR$_2$)$_{1-3}$S(O)$_{0-2}$(CR$_2$)$_{1-3}$C(O)OR;
  - -(CR$_2$)$_{1-3}$OR$^a$;
  - -(CR$_2$)$_{1-3}$NR$^b$R$^c$
  - C(O)R$^d$; or
  - nitro;
- $R^4$ is hydrogen; optionally substituted C$_{1-6}$ alkyl; C$_{2-12}$ alkenyl; hydroxyalkyl; acyl; glucoside; phosphoryl; phosphoryloxyalkyl; carboxyalkylcarbonyl; or aminoalkylcarbonyl;
- $R^5$ and $R^6$ are independently of each other hydrogen, halogen, haloalkyl, nitro, acyl, C$_{1-8}$ alkyl or C$_{2-12}$ alkenyl; or $R^5$ and $R^6$ taken together with the carbon to which they are attached form a 5-6 membered aliphatic, unsaturated or aromatic ring, optionally substituted with C$_{1-6}$ alkyl, C$_{1-6}$
alkoxy, hydroxy, carboxy, carboxyalkyl, alkoxy-carbonyl, alkoxy-carbonylalkyl, aminocarbonyl, aminocarbonylalkyl, or hydroxyalkyl;

\( R \) is hydrogen or \( C_{1-6} \) alkyl;

\( R^a \) is hydrogen; optionally substituted \( C_{1-6} \) alkyl; optionally substituted \( C_{2-12} \) alkenyl; optionally substituted aryl; optionally substituted cycloalkyl; or optionally substituted saturated, partially unsaturated or unsaturated heterocyclyl;

\( R^b \) and \( R^c \) are independently of each other hydrogen; \( C_{1-6} \) alkyl; hydroxyalkyl; aminoalkyl; optionally substituted aryl; optionally substituted benzyl; or optionally substituted heterocyclyl; or \( R^b \) and \( R^c \)
taken together with the atom to which they are attached form a 5 to 8 membered aromatic, saturated or unsaturated ring, optionally incorporating one additional atom chosen from N, O, or S and optionally substituted with a substituent selected from the group consisting of lower alkyl, halo, cyano, alkylthio, lower alkoxy, oxo, phenyl, benzyl and carboxy;

with the proviso that the compound is not alpha-tocopherol;

or

![Formula II](image)

wherein:

\( G \) is -O-, -S-, -SO-, -SO₂-, a secondary or tertiary amine group, a phosphate group, a phosphoester group, or an unsubstituted or substituted methylene group,

\( R^7 \) and \( R^8 \) independently are hydrogen, hydroxy, alkyl, aryl, alkenyl, alkynyl, aromatic, ether, ester, unsubstituted or substituted amine, amide, halogen or unsubstituted or substituted sulfonyl, or jointly complete a 5- or 6-member aliphatic or aromatic ring,

\( R^9 \) and \( R^{10} \) independently are hydrogen, hydroxy, alkyl, aryl, alkenyl, alkynyl, aromatic, ether, ester, unsubstituted or substituted amine, amide, halogen or unsubstituted or substituted sulfonyl, or jointly complete a 5- or 6-member aliphatic, aromatic or heterocyclic ring,

\( R^{11} \) is hydrogen, hydroxy, alkyl, aryl, alkenyl, alkynyl, aromatic, ester or unsubstituted or substituted amine,

\( R^{12} \) is -COOH, -COOR \( R^{13} \), -CONH₂, -CONHR \( R^{13} \), -CONR \( R^{13} \) \( R^{14} \), -NH₂, -NHR \( R^{13} \), -NR \( R^{13} \) \( R^{14} \), or a carboxylate salt,

\( R^{13} \) and \( R^{14} \) independently are unsubstituted or substituted alkyl, aryl, alkaryl, aralkyl, alkenyl or alkynyl,

\( p \) is 0 to 3, and

\( m \) is 0 to 5;
or
single stereoisomers, mixtures of stereoisomers, or pharmaceutically acceptable salts thereof.

2. The method of Claim 1, comprising administering to said subject a therapeutically effective amount of a compound of Formula I.

3. The method of Claim 2, comprising administering the compound of Formula I wherein \( R^3 \) is hydrogen, \( C_{1-6} \) alkyl, or \( C_{2-20} \) alkenyl.

4. The method of Claim 2, comprising administering the compound of Formula I wherein \( R^5 \) and \( R^6 \) are independently of each other \( C_{1-4} \) alkyl or halogen.

5. The method of Claim 4, comprising administering the compound of Formula I wherein \( R^1 \) is \( C_{1-6} \) alkyl and \( R^2 \) is \( C_{1-6} \) alkyl.

6. The method of Claim 2, comprising administering the compound of Formula I wherein \( R^1 \) is \( C_{1-6} \) alkyl, and \( R^2 \) is \( C_{16} \) alkyl or \( C_{16} \) alkenyl.

7. The method of Claim 2, comprising administering the compound of Formula I wherein \( R^5 \) and \( R^6 \) taken together with the carbon to which they are attached form a 5-6 membered carbocyclic ring, optionally substituted with \( C_{1-6} \) alkyl, \( C_{1-6} \) alkoxy, hydroxy, carboxy, carboxyalkyl, alkoxy carbonyl, alkoxy carbonylalkyl, aminocarbonyl, aminocarbonylalkyl, or hydroxyalkyl.

8. The method of Claim 1, comprising administering to said subject a therapeutically effective amount of a compound of Formula II.

9. The method of Claim 8, comprising administering the compound of Formula II, wherein \( R^{12} \) is -COOH, or -COOR\(^{13} \) and \( R^{13} \) is \( C_{1-6} \) alkyl.

10. The method of Claim 1, comprising administering a compound selected from:
- 8-Chloro-2-(3-chloro-propyl)-2,5-dimethyl-chroman-6-ol;
- 5-Chloro-2-(3-chloro-propyl)-2,8-dimethyl-chroman-6-ol;
- 7-tert-Butyl-2-(3-chloro-propyl)-2-methyl-chroman-6-ol;
- Acetic acid 2,2,5-trimethyl-3,4-dihydro-2H-benzo[h]chromen-6-yl ester;
- 2,2,5,7-Tetramethyl-8-trifluoromethyl-chroman-6-ol;
- 5-Methoxy-2,2,7,8-tetramethyl-chroman-6-ol;
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- 8-Fluoro-2,2,5,7-tetramethyl-chroman-6-ol;
- 2,2,5,7-Tetramethyl-8-(3-methyl-butyl-chroman-6-ol;
- 6-Hydroxy-2,2,5,7-tetramethyl-chroman-8-carbaldehyde;
- 2,2,5,7-Tetramethyl-8-nitro-chroman-6-ol;
- 5-(1-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
- 5-Hydroxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
- 5-(2-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
- 2-Ethynyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 2-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)-N-(3-trifluoromethyl-phenyl)-acetamide;
- (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-piperazin-1-yl-methanone;
- 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxamidine;
- N-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethyl)-N-methyl-acetamide;
- 2,2,5,7,8-Pentamethyl-2H-chromen-6-ol;
- 2-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-propane-1,3-diol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
- 2,2,5,8-Tetramethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
- 2-(3-Chloro-propyl)-2,7,8-trimethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
- 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
- (6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylsulfanyl)-acetic acid methyl ester;
- 5,6,9-Trimethyl-8-oxa-tricyclo[7.3.1.0²⁵⁻⁷]trideca-2,4,6-trien-4-ol;
- 2,2,7,8-Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
- 2,2,7,8-Tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
- 3-(6-Hydroxy-2,8-dimethyl-chroman-2-yl)-propionic acid;
- 2,2,7,8-Tetramethyl-chroman-6-ol;
- 3-[6-Hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
- 3-(6-Hydroxy-2,7,8-trimethyl-5-nitro-chroman-2-yl)-propionic acid;
- 3-(2,5,7,8-Tetramethyl-chroman-2-yl)-propionic acid;
- 3-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid;
- 3-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
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- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
- 2-Hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 5-[3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propyl]-thiazolidine-2,4-dione;
- 2-Amino-4-[1-(carboxymethyl-carbamoyl)-2-[6-hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-5-ylmethylsulfanyl]-ethylcarbamoyl]-butyric acid;
- 5-(4-Benzy1-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2-methyl-3,4-dihydro-2H-benzo[6]chroman-2-yl)-propionic acid;
- 4-[1-(Carboxymethyl-carbamoyl)-2-mercaptop-ethylcarbamoyl]-2-[3-(6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionylamino]-butyric acid);
- 2,2,7,8-Tetramethyl-chroman-6-ol;
- Delta tocopherol;
- Delta tocotrienol;
- Gamma-tocopherol;
- Gamma-tocotrienol; and
- Alpha tocotrienol;

or

single stereoisomers, mixtures of stereoisomers, or pharmaceutically acceptable salts thereof.

11. The method of Claim 1 or 10, comprising administering a therapeutically effective amount of a compound of Formula I or of Formula II to a subject suffering from a disease involving mitochondrial dysfunctions selected from Alzheimer's disease, Parkinson's disease, Friedreich's ataxia (FRDA), cerebellar ataxias, Leber's hereditary optic neuropathy (LHON), mitochondrial myopathy, encephalopathy, lactic acidosis, stroke (MELAS), Myoclonic Epilepsy with Ragged Red Fibers (MERFF), amyotrophic lateral sclerosis (ALS), motor neuron diseases, Huntington's disease, macular degeneration, and epilepsy.

12. The method of Claim 1, comprising administering a therapeutically effective amount of a compound of Formula I or of Formula II to a subject suffering from Friedreich's ataxia (FRDA), MELAS, or MERFF.

13. The method of Claim 12, comprising administering a therapeutically effective amount of a compound selected from:
- 8-Chloro-2-(3-chloro-propyl)-2,5-dimethyl-chroman-6-ol;
- 5-Chloro-2-(3-chloro-propyl)-2,8-dimethyl-chroman-6-ol;
- 7-tert-Butyl-2-(3-chloro-propyl)-2-methyl-chroman-6-ol;
• Acetic acid 2,2,5-trimethyl-3,4-dihydro-2H-benzo[h]chromen-6-yl ester;
• 2,2,5,7-Tetramethyl-8-trifluoromethyl-chroman-6-ol;
• 5-Methoxy-2,2,7,8-tetramethyl-chroman-6-ol;
• 8-Fluoro-2,2,5,7-tetramethyl-chroman-6-ol;
• 2,2,5,7-Tetramethyl-8-(3-methyl-butyl)-chroman-6-ol;
• 6-Hydroxy-2,2,5,7-tetramethyl-chroman-8-carbaldehyde;
• 2,2,5,7-Tetramethyl-8-nitro-chroman-6-ol;
• 5-(1-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
• 5-Hydroxymethyl-2,2,7,8-tetramethyl-chroman-6-ol;
• 5-(2-Hydroxy-ethyl)-2,2,7,8-tetramethyl-chroman-6-ol;
• 10-Methoxy-2,2-diethyl-3,4-dihydro-2H-benzo[h]chromen-6-ol;
• 2-Ethynyl-2,5,7,8-tetramethyl-chroman-6-ol;
• 2-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)-N-(3-trifluoromethyl-phenyl)-acetamide;
• (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-piperazin-1-yl-methanone;
• 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxamide;
• N-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-N-methyl-acetamide;
• 2,2,5,7,8-Pentamethyl-2H-chroman-6-ol;
• 2-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-propane-1,3-diol;
• 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
• 2,2,5,8-Tetramethyl-chroman-6-ol;
• 2-(2-Chloro-ethyl)-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
• 2-(3-Chloro-propyl)-2,7,8-trimethyl-chroman-6-ol;
• 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
• 2-(2-Chloro-ethyl)-2,7,8-trimethyl-chroman-6-ol;
• (6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethy)sulfanyl)-acetic acid methyl ester;
• 5,6,9-Trimethyl-8-oxa-tricyclo[7.3.1.02,7]trideca-2,4,6-trien-4-ol;
• 2,2,7,8-Tetramethyl-5-(3-methyl-butyl)-chroman-6-ol;
• 2,2,7,8-Tetramethyl-5-(3-methyl-but-2-enyl)-chroman-6-ol;
• 3-(6-Hydroxy-2,8-dimethyl-chroman-2-yl)-propionic acid;
• 2,2,7,8-Tetramethyl-chroman-6-ol;
• 3-[6-Hydroxy-2,7,8-trimethyl-5-(3-methyl-but-2-enyl)-chroman-2-yl]-propionic acid;
• 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
• 3-(6-Hydroxy-2,7,8-trimethyl-5-nitro-chroman-2-yl)-propionic acid;
• 3-(2,5,7,8-Tetramethyl-chroman-2-yl)-propionic acid;
• 3-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-propionic acid;
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- 3-(6-Hydroxy-2-methyl-chroman-2-yl)-propionic acid;
- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2-methyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid;
- 2-Hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-ol;
- 5-[3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propyl]-thiazolidine-2,4-dione;
- 2-Amino-4-[1-(carboxymethyl-carbamoyl)]-2-[6-hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-chroman-5-ylmethylsulfanyl]-ethylcarbamoyl]-butyric acid;
- 5-(4-Benzyl-piperazin-1-ylmethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol;
- 3-(5-Bromo-6-hydroxy-2,7,8-trimethyl-chroman-2-yl)-propionic acid methyl ester;
- 3-(6-Hydroxy-2-methyl-3,4-dihydro-2H-benzo[h]chroman-2-yl)-propionic acid;
- 4-[1-(Carboxymethyl-carbamoyl)]-2-mercaptop-ethylcarbamoyl]-2-[3-(6-hydroxy-2,7,8-trimethylchroman-2-yl)-propionylamino]-butyric acid);
- 2,2,7,8-Tetramethyl-chroman-6-ol;
- Delta tocopherol;
- Delta tocotrienol;
- Gamma-tocopherol;
- Gamma-tocotrienol; and
- Alpha tocotrienol;

or

single stereoisomers, mixtures of stereoisomers, or pharmaceutically acceptable salts thereof.

14. The method of Claim 12, comprising treating or ameliorating a subject in need of protection of Friedreich's ataxia comprising administering a therapeutically effective amount of a tocopherol selected from beta-tocopherol, delta-tocopherol, gamma-tocopherol, alpha-tocotrienol, beta-tocotrienol, delta-tocotrienol, and gamma-tocotrienol.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
   IPC(7): A61K 31/355
   US CL.: 514/458, 457, 456, 452
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
   Minimum documentation searched (classification system followed by classification symbols)
   U.S.: 514/458, 457, 456, 452
   Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
   NONE
   Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
   Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 98/34646 A2 (ATTERWILL et al.) 13 August 1998 (13 08 1998), see abstract and</td>
<td>1-14</td>
</tr>
<tr>
<td>Y</td>
<td>pages 6-9 and claims 1 and 21-24.</td>
<td>1-14</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search

Date of mailing of the international search report
13 JAN 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Dwayne C Jones
Telephone No. (571) 272-1600

Form PCT/ISA/210 (second sheet) (January 2004)
Continuation of B. FIELDS SEARCHED Item 3:
REGISTRY, HCAPLUS for inventor name search plus the following terms: mitochondrion, (disease? or disorder? or injur?)