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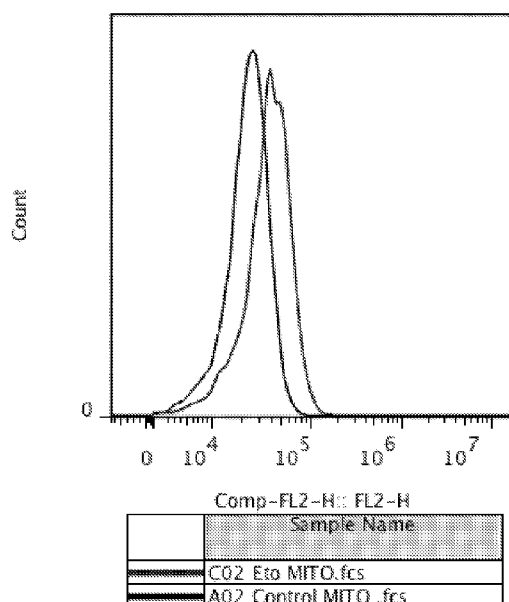
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(54) Title: METHODS OF TREATING A SUBJECT HAVING BEEN EXPOSED TO A CATASTROPHIC EVENT

(57) Abstract: The invention relates to methods for treating a subject by using an autophagy inhibitor to inhibit the development of cancer in response to a catastrophic triggering event.

Figure 1





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METHODS OF TREATING A SUBJECT HAVING BEEN EXPOSED TO A CATASTROPHIC EVENT

RELATED APPLICATION

5 This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application serial number US 61/470,450, entitled "METHODS OF TREATING A SUBJECT HAVING BEEN EXPOSED TO A CATASTROPHIC EVENT" filed on March 31, 2011, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

10 The invention relates to methods for treating a subject having been associated with or exposed to a catastrophic event. The methods involve the use of autophagy inhibitors and fatty acid metabolism inhibitors for treating the subject.

BACKGROUND OF INVENTION

15 A new model of cancer development, referred to as chromothripsis, has been described (Stephens et al, Cell 144, 27-40 (2011)). In contrast to the traditional model of cancer development that suggest cancer develops through a series of phases and results from a series of assaults, the chromothripsis model proposes that a single catastrophic event causes massive genomic rearrangement that can promote the development of
20 cancer. The study by Stephens et al., involved a detailed analytical study of genomic remodeling in cancer cells. The authors concluded, based on their analysis, that at least 2-3% of all cancers and 25% of bone cancers have genomic remodeling that likely arises from a single catastrophic event. The model suggests that the catastrophic event causes tens to hundreds of DNA breaks, most of which result in a cell that is marked to undergo
25 apoptosis, but in a few cells the genomic remodeling produces a cell that has a selective advantage. For instance the mutated cell may selectively activate cancer causing genes or disrupt tumor suppressor genes, It is those cells which go on to develop a tumor, often many years later.

SUMMARY OF INVENTION

30 The invention in some aspects is a method for treating a subject by administering to a subject that has been exposed to a catastrophic trigger an autophagy inhibitor, with

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the proviso that the subject be not otherwise in need of treatment with an autophagy inhibitor and wherein the subject has not been diagnosed with cancer.

In other aspects the invention is a method for treating a subject by administering to a subject that has been exposed to a catastrophic trigger selected from the group
5 consisting of radiation, electromagnetic waves, severe burns, severe oxidative stress, severe hypoxia, and severe sunburn an acute dosage regimen of an autophagy inhibitor. In some embodiments the autophagy inhibitor is balifomycin A.

According to other aspects the invention is a method for treating a subject by administering to a subject that has been exposed to radiation an autophagy inhibitor.

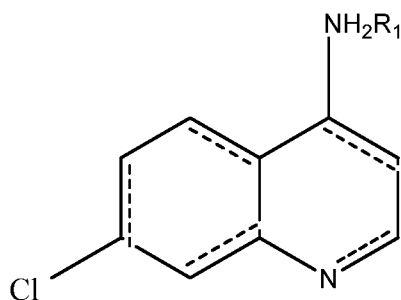
10 The catastrophic trigger may be, for example, radiation, electromagnetic waves, severe burns, severe oxidative stress, severe hypoxia, and/or severe sunburn.

In some embodiments the method further involves identifying the subject as one who has been exposed to radiation, electromagnetic waves, severe burns, severe oxidative stress, severe hypoxia, or severe sunburn.

15 Preferably the methods involves preventing tumor formation by blocking the process of autophagy.

The autophagy inhibitor may be administered by any schedule and route of administration. In some embodiments the autophagy inhibitor is administered once a week. In other embodiments it is administered once every two weeks. In some
20 embodiments the autophagy inhibitor therapy is initiated within a year of exposure to the catastrophic trigger. The autophagy inhibitor may be administered chronically or acutely.

In some embodiments the autophagy inhibitor is a 4-aminoquinoline. 4-aminoquinolines include compounds having the following structure:



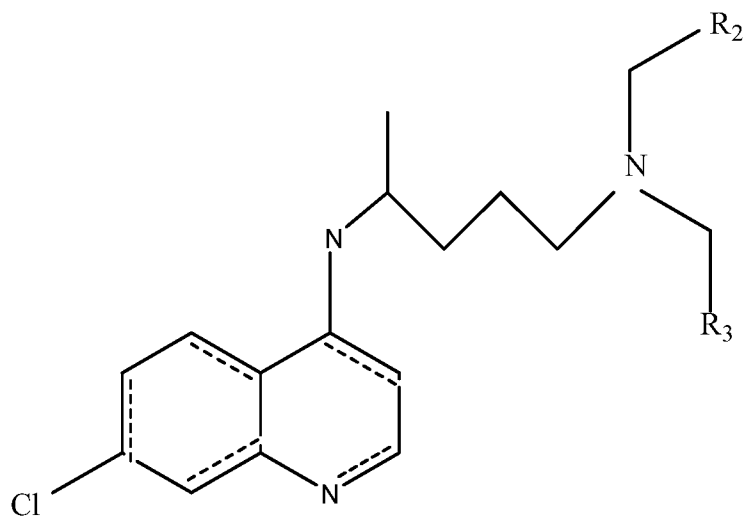
or a pharmaceutically acceptable salt or prodrug thereof, wherein R₁ is defined herein;

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each instance of the dotted line independently represents a single bond or a double bond which can be in the *cis* or *trans* configuration; and

R₁ is 1 or 2 hydrogens, alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

5 In other embodiments the 4-aminoquinoline has the following structure:



or a pharmaceutically acceptable salt or prodrug thereof, wherein R₂ and R₃ are defined herein;

10 each instance of the dotted line independently represents a single bond or a double bond which can be in the *cis* or *trans* configuration;

R₂ and R₃ is independently a hydroxalkyl, an alkyl, alkyloxy, alkylcarboxy, alkylene or alkenylene having from one to six carbon atoms.

Examples of 4-aminoquinolines useful according to the invention include but are not limited to chloroquine compounds including chloroquine and 2-hydroxychloroquine, aminoquinoline derivatives, amodiaquine, mondesethylchloroquine, quinoline phosphate, 3-methyladenine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate cAMP levels, adenosine, N6-mercaptopurine riboside, wortmannin, vinblastine and chloroquine phosphate or mixtures thereof.

20

In some embodiments the chloroquine compound is selected from the group consisting of chloroquine phosphate; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline

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(desmethylchloroquine); 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline (hydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino]-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 7-chloro-2-(o-chlorostyryl)-4-[4-diethylamino-1-methylbutyl]aminoquinoline phosphate; 3,4-dihydro-1(2H)-quinolinecarboxyaldehyde; 1,r-pentamethylenediquinoleinium diiodide; and 8-quinolinol sulfate, enantiomers thereof, as well as suitable pharmaceutical salts thereof.

In other embodiments the aminoquinoline derivative is selected from the group consisting of (S)-N₂-(7-Chloro-quinolin-4-yl) Ni, N, -dimethyl-propane- 1,2-diamine; (R)- N₂-(7-chloroquinolin-4-yl)- Ni, Ni-dimethyl-propane-1,- 2-diamine; Ni-(7-chloro-quinolin-4-yl)-2, N₂, N₂-trimethyl-propane-1,2-diamine; N₃-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1,3-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-piperidin-3-yl)-amine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-pyrrolidin-3-yl)-amine; (RS)-N₂-(7-Chloroquinolin-4-yl)- Ni, Nidimethyl-propane-1,-2-diamine; (RS)-N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1,- 2-diamine; (S)- N₂-(7-chloro-quinolin-4-yl)- N₁, N₁-diethyl-propane- 1,2-diamine; (R)- N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1,2- diamine; (RS)-7-chloro-quinolin-4-yl)-(1-methyl-2-pyrrolidin-1-yl-ethyl)-amine; N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-dimethyl-ethane-1,- 2-diamine; N₂-(7-chloro-quinolin-4-yl) Ni, Ni-diethylethane-1,2-diamine; N₃-(7-chloro-quinolin-4-yl) Ni, N₁-dimethyl-propane-1,3-diamine; (R) Ni-(7-chloroquinolin-4-yl) N₂, N₂-dimethyl-propane-

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1, 2-diamine; (S) Ni-(7-chloro-quinoline-4-yl) N₂, N₂-dimethyl-propane-1,2-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-pyrrolidin-2-yl-methyl)-amine; Ni .1 -(7-Chloro-quinolin-4-yl) N₂-(3-chloro-benzyl)-2-methyl-propane-1, 2-diamine; N)-(7-chloro-quinolin-4-yl) N₂-(benzyl)-2-methyl-propane-1,2-diamine; Nr(7-chloro-quinolin-4-yl)-N₂-(2-hydroxy-3-methoxy-benzyl)-2-methyl-propane-1,2-diamine; Ni-(7-chloro-quinolin-4-yl)- N₂-(2-hydroxy-5-methoxy-benzyl)-2-methyl-propane- 1,2-diamine; and Ni-(7-chloro-quinolin-4-yl)- N₂-(4-hydroxy-3-methoxy-benzyl)-2-methyl-propane-1,2-diamine; (1 S,2S)-Ni-(7-chloro-quinolin-4-yl)- N₂-(benzyl)-cyclohexane-1,- 2-diamine; (1S,2S)- Ni-(7-chloro-quinolin-4-yl)- N₂-(4-chlorobenzyl)-cyclohexane- 1,2-diamine; (1S,2S)- Ni-(7-chloro-quinolin-4-yl)- N₂-(4-dimethylamino-benzyl)-cyclohexane-1,2-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(3-chloro-benzyl)-cyclohexane-1,4-diamine; cis- Nj-(7-chloro-quinolin-4-yl)- N₄-(2-hydroxy-4-methoxy-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(3,5-dimethoxy-benzyl)-cyclohexane-1,4-diamine; cis- N]- (7-chloro-quinolin-4-yl)- N₄-(4-methylsulphanyl-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(4-diethylamino-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(biphenyl-4-yl)methyl-cyclohexane-1,4-diamine; trans- Ni-(7-chloro-quinolin-4-yl)- N₄-[2-(3,5-dimethoxy-phenyl)~ ethyl]-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)-N₄-(4-methoxy-benzyl)-cyclohexane-1,4-diamine; trans- Ni-(7-chloro-quinolin-4-yl)- N₄-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; and trans- N₁-(7-chloro-quinolin-4-yl)-N₄-(2,6-difluoro-benzyl)-cyclohexane- 1,4-diamine.

The invention in other aspects is a method treating a subject by administering to a subject that has been exposed to a catastrophic trigger a dichloroacetate compound, with the proviso that the subject be not otherwise in need of treatment with a dichloroacetate compound and wherein the subject has not been diagnosed with cancer. In some embodiments the dichloroacetate compound is sodium dichloroacetate.

A method for treating a subject by administering to a subject that has been exposed to a catastrophic trigger a fatty acid metabolism inhibitor is provided. The subject be not otherwise in need of treatment with a fatty acid metabolism inhibitor and the subject has not been diagnosed with cancer. In some embodiments the fatty acid

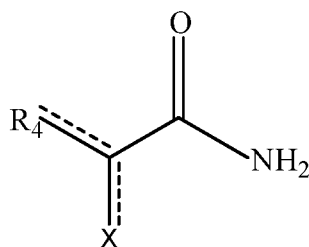
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metabolism inhibitor is an inhibitor of fatty acid oxidation, a fatty acid transporter inhibitor, a reductase inhibitor, or an isomerase inhibitor within the fatty acid metabolism pathway.

The inhibitor of fatty acid metabolism may be an inhibitory nucleic acid. The
 5 inhibitory nucleic acid may be, for instance, specific for an enzyme selected from the group consisting of 2,4-dienoyl-CoA reductase, 2,4-dienoyl-CoA isomerase, and butyryl dehydrogenase.

In other embodiments the inhibitor of fatty acid metabolism is oxamate. The
 oxamate may be, for instance an alkyl oxamate such as, ethyl oxamate or sodium
 10 oxamate.

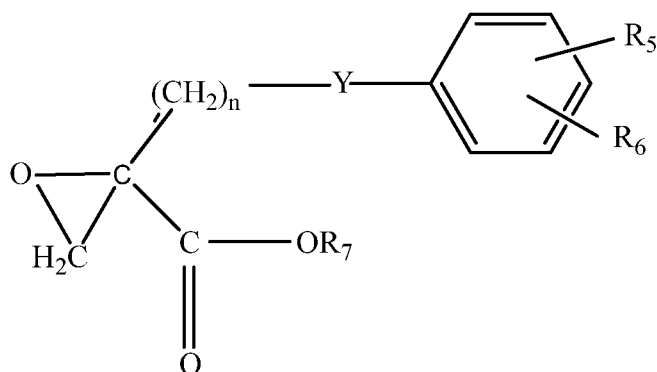
In yet another embodiment, the inhibitor of fatty acid metabolism is a compound having the following structure:



15 or a pharmaceutically acceptable salt or prodrug thereof, wherein R_4 is defined herein; wherein the dashed line is a double bond at one of the indicated positions and a single bond in the other; wherein R_4 is $\text{O}-\text{C}-\text{CH}_3$, $-\text{ONa}$, $-\text{OH}$, $-\text{O}-(\text{CH}_2)_3-\text{CH}_3$, $-\text{CH}_2-\text{C}(\text{O})-\text{C}(\text{O})-\text{O}-\text{R}_8$ or $-\text{CH}=\text{C}(\text{OH})-\text{C}(\text{O})-\text{O}-\text{R}_8$, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocyclyl, heterocycloalkyl, substituted alkyl, substituted cycloalkyl or
 20 substituted aryl, substituted aralkyl, substituted heteroaryl, substituted heteroaralkyl, substituted heterocyclyl, substituted heterocycloalkyl; wherein X is: $=\text{O}$, $=\text{N}-\text{O}-\text{R}_2$; and wherein R_2 is independently selected from hydrogen, H_2 , alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

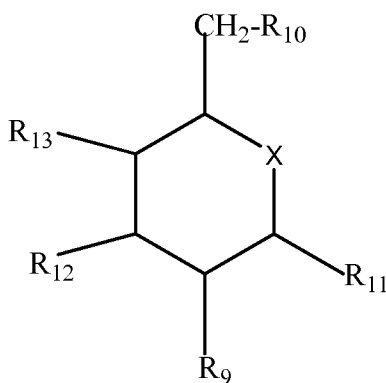
The method involves the use of a fatty acid metabolism inhibitor that is an
 25 oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof in some embodiments. The subject may not have an indication otherwise indicated for treatment with the compound. In some embodiments the oxirane carboxylic acid compound has the formula:

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or a pharmaceutically acceptable salt or prodrug thereof, wherein R_5 , R_6 and R_7 are herein; wherein R_5 represents a hydrogen atom, a halogen atom, a 1-4C alkyl group, a 1-4C alkoxy group, a nitro group or a trifluoromethyl group, R_6 has one of the meanings of R_5 , R_7 represents a hydrogen atom or a 1-4C alkyl group, Y represents the grouping $—O—(CH_2)_m—$, m is 0 or a whole number from 1 to 4, and n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8. R_5 in some embodiments is a halogen atom, R_6 is a hydrogen atom, m is 0, and n is 6. In other embodiments R_7 is an ethyl group. The oxirane carboxylic acid compound is etomoxir in some embodiments.

The methods may also involve the administration of a glycolytic inhibitor to the subject. Glycolytic inhibitors include, for instance, a 2-deoxyglucose compound, such as 2-deoxyglucose compounds having the formula:



or a pharmaceutically acceptable salt or prodrug thereof, wherein R_9 , R_{10} , R_{11} , R_{12} , and R_{13} are herein; wherein X represents an O or S atom; R_9 represents a hydrogen atom or a halogen atom; R_{10} represents a hydroxyl group, a halogen atom, a thiol group, or $CO-R_6$; R_{11} , R_{12} , and R_{13} each represent a hydroxyl group, a halogen atom, or $CO-R_{14}$, R_{14} represents an alkyl group of from 1 to 20 carbon atoms, and at least two of R_{11} ,

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R₁₂, and R₁₃ are hydroxyl groups. In one embodiment the 2-deoxyglucose compound is 2-deoxy-D-glucose.

In some aspects the invention involves the methods described herein wherein the active component is more than one of the active agents described herein. For instance, in some methods a combination of two or more of autophagy inhibitors, fatty acid metabolism inhibitors, DCA, or glycolysis inhibitors are used. The combination of agents may be administered at the same time in the same or different formulations or at different times. For instance, the different compounds may be administered to the same subject in cycles to reduce any side effects.

In other aspects the invention includes compositions of one or more of autophagy inhibitors, fatty acid metabolism inhibitors, DCA, or glycolysis inhibitors and a protease inhibitor.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of “including,” “comprising,” or “having,” “containing,” “involving,” and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

BRIEF DESCRIPTION OF DRAWINGS

The figures are illustrative only and are not required for enablement of the invention disclosed herein.

Figure 1 is a graph showing the results of flow cytometry analysis. The data demonstrate that a fatty acid inhibitor (etomoxir) increases mitochondrial membrane potential and promotes cell death in endothelial cells.

Figure 2 is a graph showing the results of flow cytometry analysis. The data demonstrate that Bcl-XL protein lowers mitochondrial membrane potential and protects the cell from apoptosis.

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Figure 3 is a graph showing the results of flow cytometry analysis. The data demonstrate removal of all glucose from endothelial cell medium increases mitochondrial membrane potential, thus promoting cell death.

Figure 4 is a graph showing the results of flow cytometry analysis. The data demonstrate that Bcl-XL alone or mixed with etomoxir or GFM (glucose free medium) results in lower mitochondrial membrane potential and protects the cell from apoptosis, ordinarily induced by etomoxir or GFM.

Figure 5 is a bar graph showing the results of flow cytometry analysis. The data demonstrate that endothelial cells depend on both fatty acid oxidation (as inhibited by etomoxir) and glutaminolysis (as indicated by inhibition with DON).

Figure 6: HTB-77 cells were treated with Chloroquine, Etomoxir, or in combination. Following treatment cells were stained for B7.H1 and HLA-DR (6a). In addition, cell viability was determined by hemacytometer counts using trypan blue exclusion (6b).

Figure 7: Daudi cells were treated with Chloroquine, Etomoxir, or in combination. Following treatment cells were stained for B7.H1 and CLIP (7a). In addition, cell viability was determined by hemacytometer counts using trypan blue exclusion (7b).

DETAILED DESCRIPTION

Chromothripsis (described in Stephens et al, Cell 144, 27-40 (2011)) is a new model that proposes that a single catastrophic event causes massive genomic rearrangement that can promote the development of cancer. It has been discovered herein that the development of cancer can be inhibited by treating a subject who has been exposed to a catastrophic trigger that is capable of producing a mutated early cancer cell, referred to herein as a precancerous cell or tissue or cancer stem cell. The cell which survives the catastrophic trigger with genomic mutations that select for its survival is dependent on autophagy. The invention, thus, calls for the administration of autophagy inhibitors to subjects that have experienced a catastrophic trigger or event and are susceptible to genomically remodeled cells which are not susceptible to apoptosis and may develop into cancer cells.

A catastrophic event or trigger as used herein refers to an external or internal event that has the ability to induce dsDNA breaks in a chromosome of a subject. These

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events include but are not limited to exposure to radiation, such as ionizing radiation, exposure to electromagnetic waves, the development of severe burns, oxidative stress, hypoxia, or sunburn on the body. The exposure to radiation may be exposure at any level that is capable of causing DNA damage in a cell of the body. This includes low-level radiation exposure. In some embodiments the exposure level to radiation or electromagnetic waves in the methods of the invention is a minimum of a threshold value exceeding safe exposure levels. Threshold levels for safe exposure to radiation and electromagnetic waves are well known in the art. For Example, a book called Ionizing radiation: protection and dosimetry by Guy Paic, Chapter 2, and in particular pages 20-24 describe safety threshold levels of radiation. Thermal radiation values of greater than 5 kW/m², for example, can result in severe burns and are considered a threshold level of thermal radiation. In some embodiments the exposure level to radiation or electromagnetic waves in the methods of the invention is at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 times greater than a determined threshold value of safe exposure levels.

Other catastrophic triggers include the development of severe burns, oxidative stress, hypoxia, or sunburn. The term “severe” as used herein in this context refers to a level that is considered to be above a threshold safety level, such that the DNA of cells could be damaged. For instance the minimal erythema dose (MED) is used to identify the minimal dose of sunlight that can be tolerated before observing harmful effects on the skin.

The invention in some aspects is a method for treating a subject by administering to a subject that has been exposed to a catastrophic trigger an autophagy inhibitor, with the proviso that the subject be not otherwise in need of treatment with an autophagy inhibitor and wherein the subject has not been diagnosed with cancer.

A subject is exposed to a catastrophic trigger if the subject has had a proximate relationship with the catastrophic trigger. For instance, if the subject has been within physical proximity of a nuclear radiation leak such that the subject has greater than normal levels of radiation in the body, then the subject has a proximate relationship with the catastrophic trigger. Likewise a subject has had a proximate relationship with the catastrophic trigger when the subject has had a severe sunburn.

The subject may be administered an acute or chronic dose of the autophagy inhibitor. In general, when a catastrophic trigger is severe or high dose, it may be

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desirable to treat the subject with an acute therapeutic regimen that involves a higher dosage, a stronger drug that may have more side effects, and/or frequent administration regimen than would be given when the catastrophic trigger is less severe. For example under conditions of direct bodily exposure to liquid nuclear radiation a choice
5 therapeutic regimen might involve a course of acute treatment, followed by a less aggressive chronic treatment regimen. A chronic treatment regimen may last anywhere from 6 months to an entire lifetime. The purpose of the treatment is to prevent the development of cancer from the damaged-autophagy dependent cells. Thus, it may be desirable to continue the chronic treatment for as long as the subject lives, especially in
10 the instance that a safe compound such as chloroquine or hydroxychloroquine is used.

In some but not all embodiments of the invention the methods include the proviso that the subject be not otherwise in need of treatment with an autophagy inhibitor. Thus, in this embodiment if a subject were already being treated with chloroquine to treat malarial infection or autoimmune disease such a subject would be excluded from that
15 particular claimed method.

In some but not all embodiments of the invention the methods do not include subjects that have already been diagnosed with cancer. Thus, in this embodiment if a subject were already diagnosed with cancer such a subject would be excluded from that particular claimed method.

20 The chromothripsis model indicates that at least 2-3% of all cancers and 25% of bone cancers have genomic remodeling that likely arises from a single catastrophic event. Thus, the methods of the invention are useful for inhibiting the development of all cancer types. Cancers in general are neoplasms, malignant tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth such that it would be
25 considered cancerous. The cancer may be a primary or metastatic cancer. Cancers include, but are not limited to, gastrointestinal cancers, biliary tract cancer; bladder cancer; brain cancer including glioblastomas and medulloblastomas; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous
30 leukemia; multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas including Hodgkin's disease and lymphocytic

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lymphomas; mesothelioma; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Kaposi's sarcoma, basocellular cancer, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma, teratomas, choriocarcinomas; stromal tumors and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms' tumor.

10 According to one set of embodiments, the cells are exposed to an autophagy inhibitor. An "autophagy modulator," as used herein, is a lysosomotropic agent, meaning that it accumulates preferentially in the lysosomes of cells in the body and blocks pathways involved in break down of cellular components. An autophagy inhibitor, as used herein, is any compound which blocks the collection or metabolism of lipids in the lysosome. The inhibitor is effective for killing cells by inhibiting autophagy in cells that depend on autophagy to survive. While no one knows exactly the mechanism by which autophagy inhibitors function, it may well be through the inhibition of the acidic hydrolases (enzymes in the lysosomes) that are necessary to break down proteins, lipids, etc. for processing and removal by increasing the pH to decrease the necessary acidity for the enzymes to work.

20 In some embodiments, the autophagy inhibitor is selected from the group consisting of: chloroquine compounds, 3-methyladenine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate cAMP levels, adenosine, N6-mercaptopurine riboside, wortmannin, and vinblastine.

30 The autophagy inhibitor is preferably a chloroquine compound. Chloroquine is a synthetically manufactured drug containing a quinoline nucleus (The Merck Index, p. 2220, 1996). The chloroquine compounds useful according to the invention include chloroquine analogs and derivatives. A number of chloroquine analogs and derivatives are well known. For example, suitable compounds include but are not limited to chloroquine, chloroquine phosphate, hydroxychloroquine, chloroquine diphosphate,

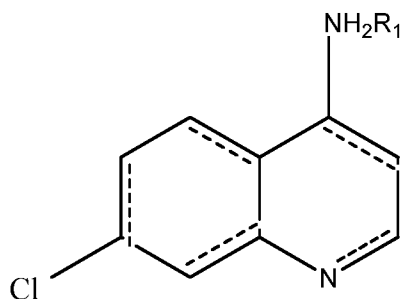
- 13 -

chloroquine sulphate, hydroxychloroquine sulphate, quinacrine, primaquine, mefloquine, halofantrine, lumefantrine and tafenoquine or enantiomers, derivatives, analogs, metabolites, pharmaceutically acceptable salts, and mixtures thereof.

Chloroquine and hydroxychloroquine are generally racemic mixtures of (-)- and (+)-enantiomers. The (-)-enantiomers are also known as (R)-enantiomers (physical rotation) and 1-enantiomers (optical rotation). The (+)-enantiomers are also known as (S)-enantiomers (physical rotation) and r-enantiomers (optical rotation). Preferably, the (-)-enantiomer of chloroquine is used. The enantiomers of chloroquine and hydroxychloroquine can be prepared by procedures known to the art.

The compounds of the invention, such as, chloroquine may exhibit the phenomena of tautomerism, conformational isomerism, geometric isomerism, and/or optical isomerism. The invention covers any tautomeric, conformational isomeric, optical isomeric and/or geometric isomeric forms of the compounds described herein, as well as mixtures of these various different forms.

Thus in some embodiments the autophagy inhibitor useful in the invention is a 4-aminoquinoline. 4-aminoquinolines include compounds having the following structure:



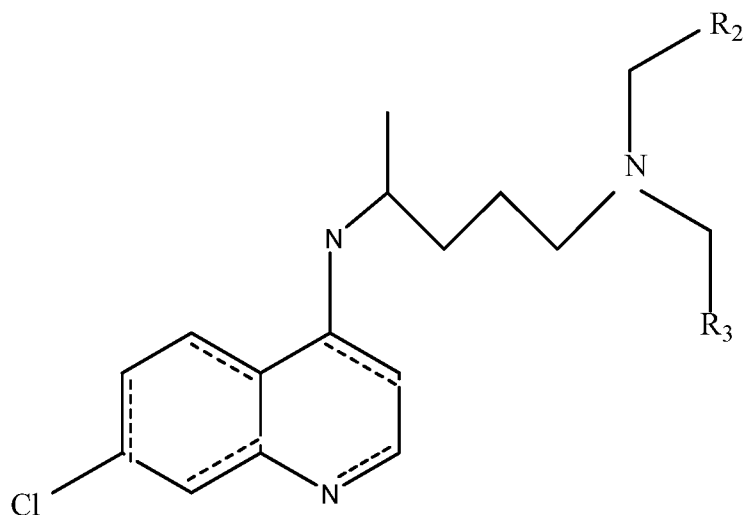
or a pharmaceutically acceptable salt or prodrug thereof, wherein R_1 is defined herein;

each instance of the dotted line independently represents a single bond or a double bond which can be in the *cis* or *trans* configuration; and

R_1 is 1 or 2 hydrogens, alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

In other embodiments the 4-aminoquinoline has the following structure:

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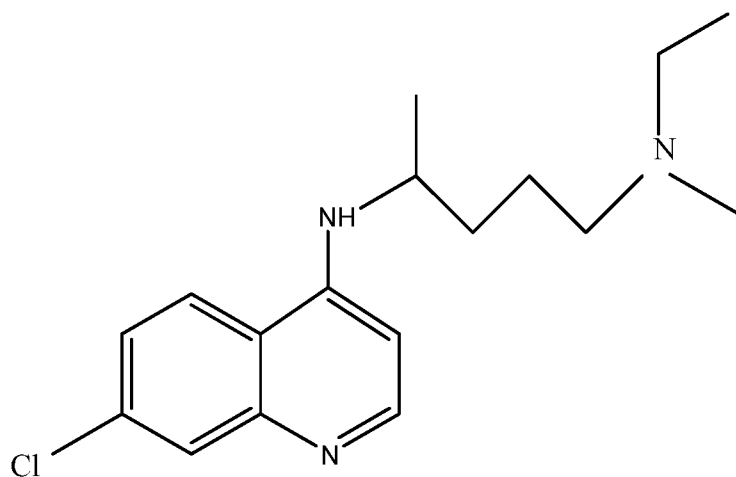
or a pharmaceutically acceptable salt or prodrug thereof, wherein R_2 and R_3 are defined herein;

each instance of the dotted line independently represents a single bond or a
 5 double bond which can be in the *cis* or *trans* configuration;

R_2 and R_3 is independently a hydroxalkyl, an alkyl, alkyloxy, alkylcarboxy, alkylene or alkenylene having from one to six carbon atoms.

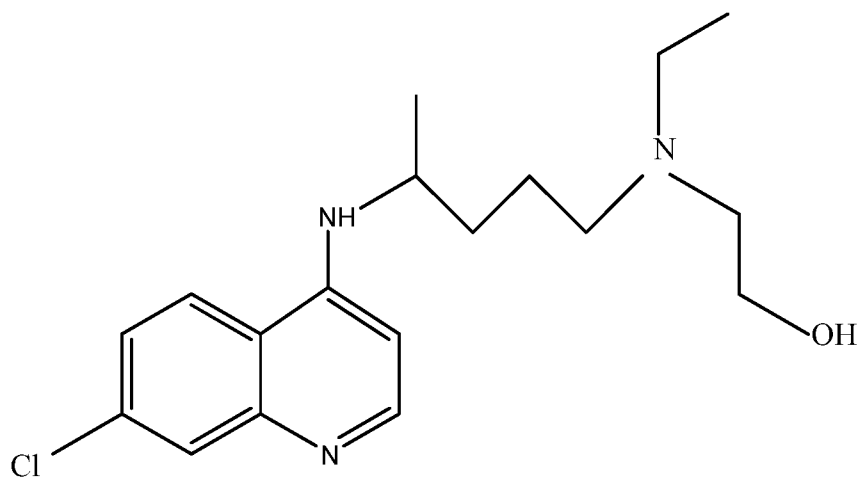
Examples of 4-aminoquinolines useful according to the invention include but are not limited to chloroquine, 2-hydroxychloroquine, amodiaquine,
 10 mondesethylchloroquine, quinoline phosphate, and chloroquine phosphate or mixtures thereof.

Choloroquine:



15 2-hydroxychloroquine

- 15 -



Other examples of preferred chloroquine compounds that can be used in the invention include chloroquine diphosphate and hydroxychloroquine (Plaquenil™).

The methods of the invention may also be achieved by metabolically disrupting fatty acids. Metabolic disruption of fatty acids can be achieved using inhibitors of fatty acid metabolism. A “fatty acid metabolism inhibitor,” as used herein, is a compound able to inhibit (e.g., prevent, or at least decrease or inhibit the activity by an order of magnitude or more) a reaction within the fatty acid metabolism pathway, such as an enzyme-catalyzed reaction within the pathway. The inhibitor may inhibit the enzyme, e.g., by binding to the enzyme or otherwise interfering with operation of the enzyme (for example, by blocking an active site or a docking site, altering the configuration of the enzyme, competing with an enzyme substrate for the active site of an enzyme, etc.), and/or by reacting with a coenzyme, cofactor, etc. necessary for the enzyme to react with a substrate. The fatty acid metabolism pathway is the pathway by which fatty acids are metabolized within a cell for energy (e.g., through the synthesis of ATP and the breakdown of fatty acids into simpler structures, such as CO₂, acyl groups, etc.) or to produce a carbohydrate source. For example inhibitors of fatty acid metabolism include inhibitors of gluconeogenesis, inhibitors of fatty acid oxidation, fatty acid transporter inhibitors, reductase inhibitors, and isomerase inhibitors within the fatty acid metabolism pathway.

An inhibitor of gluconeogenesis is a compound that prevents at least some and preferably a substantial amount of fatty acid conversion into carbohydrates such as glucose. For example, glycolytic inhibitors, oxamate and iodoacetate are inhibitors of gluconeogenesis.

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The fatty acid metabolism inhibitor in some embodiments is an inhibitor of fatty acid oxidation, a fatty acid transporter inhibitor, a reductase inhibitor, or an isomerase inhibitor within the fatty acid metabolism pathway. In one embodiment the reductase is 2,4-dienoyl-CoA reductase. In another embodiment the isomerase is 2,4-dienoyl-CoA isomerase. In yet other embodiments the inhibitor of fatty acid metabolism is an inhibitor of fatty acid oxidation and is any one or more of the following: oxirane carboxylic acid compound, such as etomoxir (2-(6-(4-chlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester), 2-(4-(3-chlorophenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(4-(3-trifluoromethylphenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(5(4-chlorophenoxy)-pentyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(3,4-dichlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(4-fluorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-phenoxyhexyl)-oxirane-2-carboxylic acid ethyl ester, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocamitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, beta-hydroxy butyrate, and a non-hydrolyzable analog of camitine or pharmacologically acceptable salts thereof.

The fatty acid metabolism pathway includes several enzymatic reactions, which use various enzymes such as reductases or isomerases. Specific examples of enzymes within the fatty acid metabolism pathway include 2,4-dienoyl-CoA reductase, 2,4-dienoyl-CoA isomerase, butyryl dehydrogenase, etc, as further discussed below. In one embodiment, the fatty acid metabolism inhibitor is an inhibitor able to inhibit a beta-oxidation reaction in the fatty acid metabolism pathway. In another embodiment, the inhibitor is an inhibitor for a fatty acid transporter (e.g., a transporter that transports fatty acids into the cell, or from the cytoplasm into the mitochondria for metabolism). In yet another embodiment, the inhibitor may react or otherwise inhibit key steps within the fatty acid metabolism pathway. In still another embodiment, the inhibitor may be an inhibitor of fatty acids as a source of energy in the mitochondria. For example, the inhibitor may inhibit the breakdown of intermediates such as butyryl CoA, glutaryl CoA, or isovaleryl CoA.

2,4-dienoyl-CoA reductase is an enzyme within the fatty acid metabolism pathway that catalyzes reduction reactions involved in the metabolism of

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polyunsaturated fatty acids. Certain fatty acids are substrates for 2,4-dienoyl-CoA reductases located within the mitochondria. In some cases, fatty acids may be transported into the mitochondria through uncoupling proteins. The uncoupling protein may, in certain instances, increase the mitochondrial metabolism to increase the availability of fatty acids within the mitochondria and/or increase the throughput of beta-oxidation within the mitochondria.

The enzyme 2,4-dienoyl-CoA isomerase is an enzyme within the fatty acid metabolism pathway that catalyzes isomerization of certain fatty acids. One step in the metabolism of certain polyunsaturated fatty acids may be protective against reactive oxygen intermediates ("ROI"). Thus, by generating substrates and antagonists for the activity of 2,4-dienoyl-CoA isomerase, the metabolic production of reactive oxygen intermediates may be enhanced and/or reduced. This, in turn, affects the levels of fatty acids in the cell.

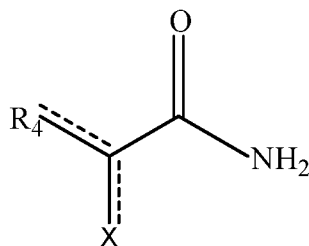
Thus, it is to be understood that, as used herein, compounds useful for inhibiting fatty acid metabolism (i.e., "fatty acid metabolism inhibitors") are also useful for altering cellular production of reactive oxygen; compounds described in reference to fatty acid metabolism inhibition should also be understood herein to be able to alter reactive oxygen production within a cell. For example, by altering the ability of a cell to metabolize a fatty acid, the ability of the cell to produce reactive oxygen may also be affected, since one pathway for a cell to produce reactive oxygen intermediates is through the metabolism of fatty acids. Thus, in some cases, the production of reactive oxygen can be affected by exposing a cell to, or removing a cell from, a fatty acid metabolism inhibitor.

The inhibitor of fatty acid metabolism may be an inhibitory nucleic acid. The inhibitory nucleic acid may be, for instance, specific for an enzyme selected from the group consisting of 2,4-dienoyl-CoA reductase, 2,4-dienoyl-CoA isomerase, and butyryl dehydrogenase.

In other embodiments the inhibitor of fatty acid metabolism is oxamate. The oxamate may be, for instance an alkyl oxamate such as, ethyl oxamate or sodium oxamate.

In yet another embodiment, the inhibitor of fatty acid metabolism is a compound having the following structure:

- 18 -

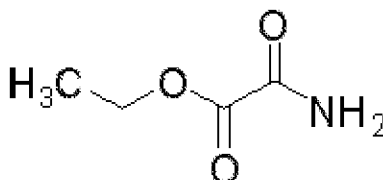


or a pharmaceutically acceptable salt or prodrug thereof, wherein R_4 is defined herein; wherein the dashed line is a double bond at one of the indicated positions and a
 5 single bond in the other; wherein R_4 is $O-C-CH_3$, $-ONa$, $-OH$, $-O-(CH_2)_3-CH_3$, $-CH_2-C(O)-C(O)-O-R_8$ or $-CH=C(OH)-C(O)-O-R_8$, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocyclyl, heterocycloalkyl, substituted alkyl, substituted cycloalkyl or substituted aryl, substituted aralkyl, substituted heteroaryl, substituted heteroaralkyl, substituted heterocyclyl, substituted heterocycloalkyl; wherein X is: $=O$, $=N-O R_2$; and
 10 wherein R_2 is independently selected from hydrogen, H_2 , alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

In some preferred embodiments the fatty acid inhibitor is an oxamate including, for example, each of the following:

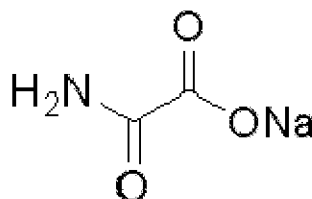
Ethyl oxamate

15



Sodium oxamate

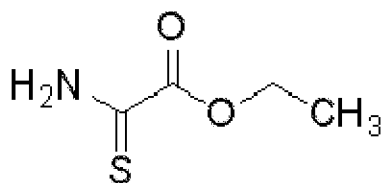
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Ethyl thiooxamate

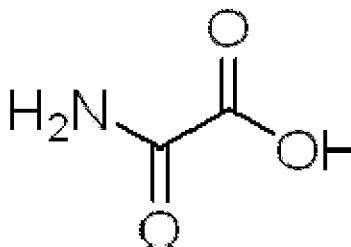
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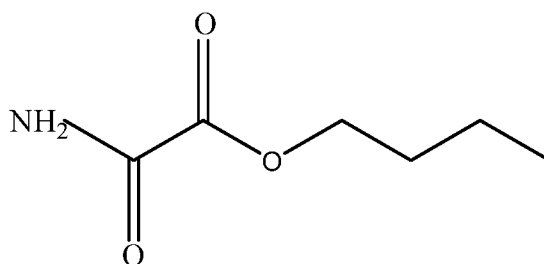


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Oxamic acid



Butyl oxamate

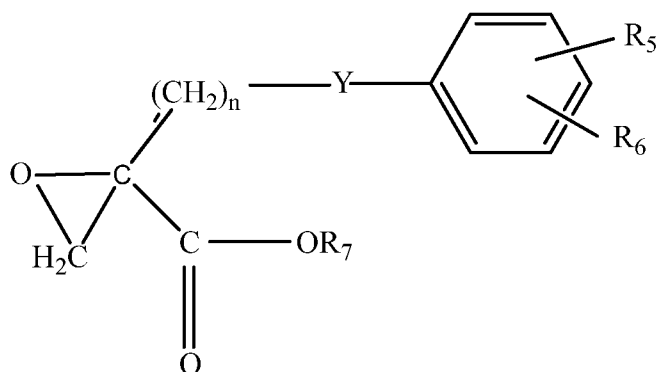


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Pyruvate derivatives have been described in the art and are useful for inhibiting fatty acid production. For instance, US Patents, such as 5,395,822; 6,916,850; 6,086,789; 5,968,727; 5,047,427 and 5,256,697 (the specific pyruvate derivatives, salts etc are incorporated by reference), describe pyruvate derivatives, conjugates and salts.

The method involves the use of a fatty acid metabolism inhibitor that is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof in some embodiments. The subject may not have an indication otherwise indicated for treatment with the compound. In some embodiments the oxirane carboxylic acid compound has the formula:

- 20 -



or a pharmaceutically acceptable salt or prodrug thereof, wherein R_5 , R_6 and R_7 are herein; wherein R_5 represents a hydrogen atom, a halogen atom, a 1–4C alkyl group, a 1–4C alkoxy group, a nitro group or a trifluoromethyl group, R_6 has one of the meanings of R_5 , R_7 represents a hydrogen atom or a 1–4C alkyl group, Y represents the grouping $—O—(CH_2)_m—$, m is 0 or a whole number from 1 to 4, and n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8. R_5 in some embodiments is a halogen atom, R_6 is a hydrogen atom, m is 0, and n is 6. In other embodiments R_7 is an ethyl group. The oxirane carboxylic acid compound is etomoxir in some embodiments.

It is most particularly preferred to use etomoxir, i.e., 2-(6-(4-chlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester. Examples of other oxirane carboxylic acid compounds useful in the invention are 2-(4-(3-chlorophenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(4-(3-trifluoromethylphenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(5-(4-chlorophenoxy)-pentyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(3,4-dichlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(4-fluorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, and 2-(6-phenoxyhexyl)-oxirane-2-carboxylic acid ethyl ester, the corresponding oxirane carboxylic acids, and their pharmacologically acceptable salts.

The foregoing class of oxirane carboxylic acid compounds, including etomoxir, has been described by Horst Wolf and Klaus Eistetter in United States Patent 4,946,866 for the prevention and treatment of illnesses associated with increased cholesterol and/or triglyceride concentration, and by Horst Wolf in United States Patent 5,739,159 for treating heart insufficiency. The preparation of oxirane carboxylic acid compounds, and their use for blood glucose lowering effects as an ant diabetic agent, is described in Jew *et al* United States Patent 6,013,666. Etomoxir has been described as an inhibitor of

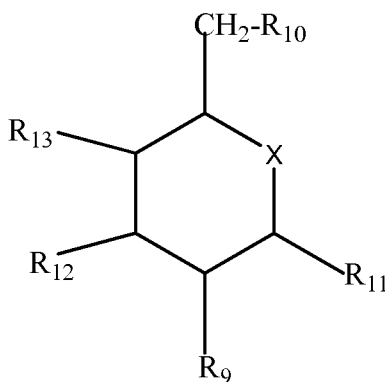
- 21 -

mitochondrial carnitine palmitoyl transferase-I by Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. De Schepper "Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats," J. Biol. Chem. 254:4585-4595, 1979.

5 The foregoing United States Patents 4,946,866, 5,739,159, and 6,013,666, United States Patent Application 20030036199, and the foregoing publication by Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. De Schepper, are incorporated herein by reference. In addition, U.S. Patent Application Serial No. 10/272,432, filed October 15, 2002, entitled "Methods for Regulating Co-Stimulatory Molecule Expression with Reactive
10 Oxygen," by M. K. Newell, et al. is incorporated herein by reference in its entirety.

A glycolytic inhibitor may also be used in the methods of the invention. Preferred glycolytic inhibitors are 2-deoxyglucose compounds, defined herein as homologs, analogs, and/or derivatives of 2-deoxy-D-glucose. Glycolytic inhibitors particularly useful herein can have the formula:

15



or a pharmaceutically acceptable salt or prodrug thereof, wherein R₉, R₁₀, R₁₁, R₁₂, and R₁₃ are herein; wherein X represents an O or S atom; R₉ represents a hydrogen atom or a halogen atom; R₁₀ represents a hydroxyl group, a halogen atom, a thiol group, or CO-R₆; R₁₁, R₁₂, and R₁₃ each represent a hydroxyl group, a halogen atom, or CO-
20 R₁₄, R₁₄ represents an alkyl group of from 1 to 20 carbon atoms, and at least two of R₁₁, R₁₂, and R₁₃ are hydroxyl groups. In one embodiment the 2-deoxyglucose compound is 2-deoxy-D-glucose.

Other, non-limiting examples of fatty acid metabolism inhibitors include fatty
25 acid transporter inhibitors, beta-oxidation process inhibitors, reductase inhibitors, and/or

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isomerase inhibitors within the fatty acid metabolism pathway. Specific examples of other fatty acid metabolism inhibitors include, but are not limited to, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocamitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, and beta-hydroxy butyrate. As another example, the inhibitor may be a non-hydrolyzable analog of camitine.

In one embodiment, the fatty acid metabolism inhibitor is a carboxylic acid. In some cases, the carboxylic acid may have the structure:

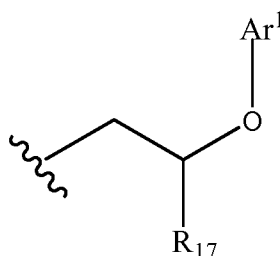


where R₁₄ comprises an organic moiety, as further described below. In some cases, R₁₄ may include at least two nitrogen atoms, or R₁₄ may include an aromatic moiety (as further described below), such as a benzene ring, a furan, etc.

In another embodiment, the fatty acid metabolism inhibitor has the structure:

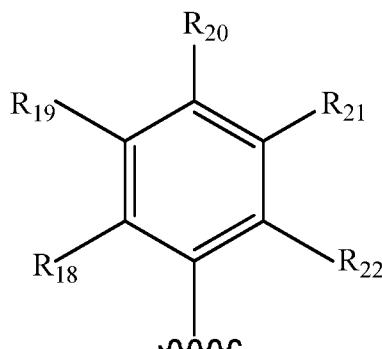


where each of R₁₅ and R₁₆ independently comprises organic moiety. In some instances, either or both of R₁₅ and R₁₆ may independently be an alkyl, such as a straight-chain alkyl, for instance, methyl, ethyl, propyl, etc. In certain cases, R₁₆ may have at least 5 carbon atoms, at least 10 carbon atoms, or at least 15 or more carbon atoms. For example, in one embodiment, R₁₆ may be a tetradecyl moiety. In other cases, R₁₆ may include an aromatic moiety, for example, a benzene ring. In still other cases, R₁₆ may have the structure:



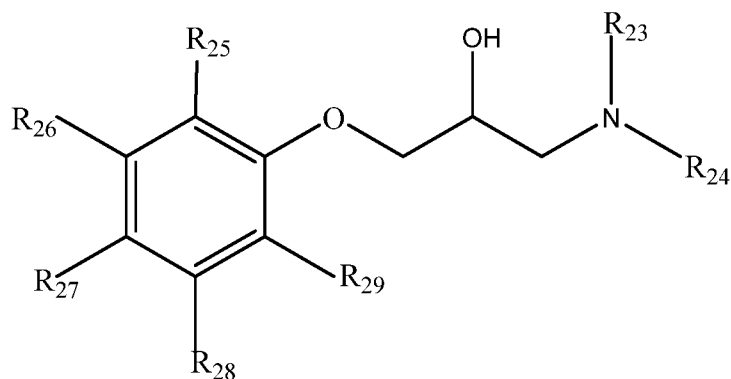
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where R^3 comprises an organic moiety and Ar^1 comprises an aromatic moiety. R_{17} may be an alkyl, such as a straight-chain alkyl. In some instances, Ar^1 may be a benzene ring or a derivative thereof, i.e., having the structure:

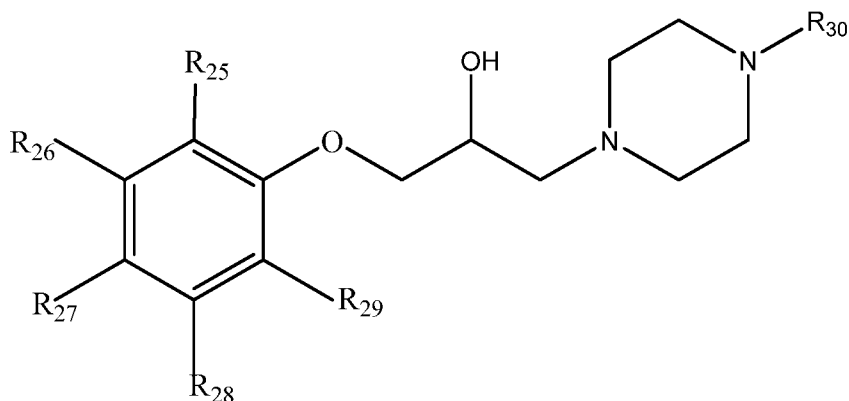


5 wherein each of R_{18} , R_{19} , R_{20} , R_{21} , and R_{22} is hydrogen, a halogen, an alkyl, an alkoxy, etc.

In yet another embodiment, the fatty acid metabolism inhibitor has the structure:

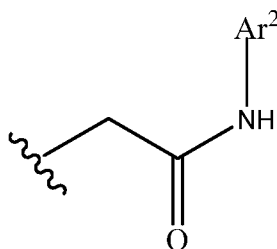


10 where each of R_{23} , R_{24} , R_{25} , R_{26} , R_{27} , R_{28} and R_{29} independently comprises hydrogen, a halogen, or an organic moiety, such as an alkyl, an alkoxy, etc. In some cases, R_{23} and R_{24} together may define an organic moiety, such as a cyclic group. For example, the fatty acid metabolism inhibitor may have the structure:



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wherein R_{30} comprises an organic moiety, such as an alkyl, an alkoxy, an aromatic moiety, an amide, etc. An example, of R_{30} is:



wherein Ar^2 comprises an aromatic moiety, such as a benzene ring or a benzene derivative, as previously described.

In another set of embodiments, the cells may be exposed to an agent that inhibits the synthesis or production of one or more enzymes within the fatty acid metabolism pathway. Exposure of the cells to the agent thus inhibits fatty acid metabolism within the cell. For example, in one embodiment, an inhibitory oligonucleotide such as a RNAi or antisense oligonucleotide may be used that selectively binds to regions encoding enzymes present within the fatty acid metabolism pathway, such as 2,4-dienoyl-CoA reductase or 2,4-dienoyl-CoA isomerase.

Thus, agents that inhibit enzymes of the fatty acid metabolism pathway include enzymes of the fatty acid metabolism pathway expression inhibitors. A enzymes of the fatty acid metabolism pathway expression inhibitor as used herein is molecule that knocks down expression of an enzyme of the fatty acid metabolism pathway. Thus, the invention also features the use of small nucleic acid molecules, referred to as short interfering nucleic acid (siNA) that include, for example: microRNA (miRNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), and short hairpin RNA (shRNA) molecules to knockdown expression of proteins such as enzymes of the fatty acid metabolism pathway. An siNA of the invention can be unmodified or chemically-modified. An siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through, for example, increased resistance to nuclease degradation in vivo and/or through improved cellular uptake. Furthermore, siNA having multiple chemical modifications

- 25 -

may retain its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic applications.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases can increase
5 their potency (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al, 1990 Nature 344, 565; Pieken et al., 1991, Science 253, 314; Usman and Cedergren, 1992, Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162; and Sproat, U.S. Pat. No. 5,334,711; all of these describe various chemical
10 modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules herein). Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate
15 modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, TIBS. 17,
20 34; Usman et al., 1994, Nucleic Acids Symp. Ser. 31, 163; Burgin et al., 1996, Biochemistry, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. Nature, 1990, 344, 565 568; Pieken et al. Science, 1991, 253, 314317; Usman and Cedergren, Trends in Biochem. Sci., 1992, 17, 334 339; Usman
25 et al. International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, J. Biol. Chem., 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al.).

In one embodiment, one of the strands of the double-stranded siNA molecule
30 comprises a nucleotide sequence that is complementary to a nucleotide sequence of a target RNA or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence identical to the nucleotide sequence or a

- 26 -

portion thereof of the targeted RNA. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is substantially complementary to a nucleotide sequence of a target RNA or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the target RNA. In another embodiment, each strand of the siNA molecule comprises about 19 to about 23 nucleotides, and each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

In some embodiments an siNA is an shRNA, shRNA-mir, or microRNA molecule encoded by and expressed from a genomically integrated transgene or a plasmid-based expression vector. Thus, in some embodiments a molecule capable of inhibiting mRNA expression, or microRNA activity, is a transgene or plasmid-based expression vector that encodes a small-interfering nucleic acid. Such transgenes and expression vectors can employ either polymerase II or polymerase III promoters to drive expression of these shRNAs and result in functional siRNAs in cells. The former polymerase permits the use of classic protein expression strategies, including inducible and tissue-specific expression systems. In some embodiments, transgenes and expression vectors are controlled by tissue specific promoters. In other embodiments transgenes and expression vectors are controlled by inducible promoters, such as tetracycline inducible expression systems.

In another embodiment, a small interfering nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. The recombinant mammalian expression vector may be capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the myosin heavy chain promoter, albumin promoter, lymphoid-specific promoters, neuron specific promoters, pancreas specific promoters, and mammary gland specific promoters. Developmentally-regulated promoters are also encompassed, for example the murine hox promoters and the α -fetoprotein promoter.

Other inhibitor molecules that can be used include sense and antisense nucleic acids (single or double stranded), ribozymes, peptides, DNazymes, peptide nucleic acids

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(PNAs), triple helix forming oligonucleotides, antibodies, and aptamers and modified form(s) thereof directed to sequences in gene(s), RNA transcripts, or proteins. Antisense and ribozyme suppression strategies have led to the reversal of a tumor phenotype by reducing expression of a gene product or by cleaving a mutant transcript at the site of the mutation (Carter and Lemoine *Br. J. Cancer*. 67(5):869-76, 1993; Lange et al.,
5 *Leukemia*. 6(11):1786-94, 1993; Valera et al., *J. Biol. Chem.* 269(46):28543-6, 1994; Dosaka-Akita et al., *Am. J. Clin. Pathol.* 102(5):660-4, 1994; Feng et al., *Cancer Res.* 55(10):2024-8, 1995; Quattrone et al., *Cancer Res.* 55(1):90-5, 1995; Lewin et al., *Nat Med.* 4(8):967-71, 1998). For example, neoplastic reversion was obtained using a
10 ribozyme targeted to an H-Ras mutation in bladder carcinoma cells (Feng et al., *Cancer Res.* 55(10):2024-8, 1995). Ribozymes have also been proposed as a means of both inhibiting gene expression of a mutant gene and of correcting the mutant by targeted trans-splicing (Sullenger and Cech *Nature* 371(6498):619-22, 1994; Jones et al., *Nat. Med.* 2(6):643-8, 1996). Ribozyme activity may be augmented by the use of, for
15 example, non-specific nucleic acid binding proteins or facilitator oligonucleotides (Herschlag et al., *Embo J.* 13(12):2913-24, 1994; Jankowsky and Schwenzer *Nucleic Acids Res.* 24(3):423-9, 1996). Multitarget ribozymes (connected or shotgun) have been suggested as a means of improving efficiency of ribozymes for gene suppression (Ohkawa et al., *Nucleic Acids Symp Ser.* (29):121-2, 1993).

20 Anti-sense oligonucleotides may be designed to hybridize to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of an enzymes of the fatty acid metabolism pathway encoded by a given DNA sequence (e.g. either native polypeptide or a mutant form thereof), so that its expression is reduce or prevented altogether. Anti-sense techniques may be used to target a coding sequence;
25 a control sequence of a gene, e.g. in the 5' flanking sequence, whereby the anti-sense oligonucleotides can interfere with control sequences. Anti-sense oligonucleotides may be DNA or RNA and may be of around 14-23 nucleotides, particularly around 15-18 nucleotides, in length. The construction of antisense sequences and their use is described in Peyman and Ullman, *Chemical Reviews*, 90:543-584, (1990), and Crooke, *Ann. Rev. Pharmacol. Toxicol.*, 32:329-376, (1992).
30

It may be preferable that there is complete sequence identity in the sequence used for down-regulation of expression of a target sequence, and the target sequence, though

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total complementarity or similarity of sequence is not essential. One or more nucleotides may differ in the sequence used from the target gene. Thus, a sequence employed in a down-regulation of gene expression in accordance with the present invention may be a wild-type sequence (e.g. gene) selected from those available, or a mutant, derivative,
5 variant or allele, by way of insertion, addition, deletion or substitution of one or more nucleotides, of such a sequence.

The sequence need not include an open reading frame or specify an RNA that would be translatable. It may be preferred for there to be sufficient homology for the respective sense RNA molecules to hybridize. There may be down regulation of gene
10 expression even where there is about 5%, 10%, 15% or 20% or more mismatch between the sequence used and the target gene.

Triple helix approaches have also been investigated for sequence-specific gene suppression. Triple helix forming oligonucleotides have been found in some cases to bind in a sequence-specific manner (Postel et al., Proc. Natl. Acad. Sci. U.S.A.
15 88(18):8227-31, 1991; Duval-Valentin et al., Proc. Natl. Acad. Sci. U.S.A. 89(2):504-8, 1992; Hardenbol and Van Dyke Proc. Natl. Acad. Sci. U.S.A. 93(7):2811-6, 1996; Porumb et al., Cancer Res. 56(3):515-22, 1996). Similarly, peptide nucleic acids have been shown to inhibit gene expression (Hanvey et al., Antisense Res. Dev. 1(4):307-17, 1991; Knudsen and Nielson Nucleic Acids Res. 24(3):494-500, 1996; Taylor et al., Arch.
20 Surg. 132(11):1177-83, 1997). Minor-groove binding polyamides can bind in a sequence-specific manner to DNA targets and hence may represent useful small molecules for future suppression at the DNA level (Trauger et al., Chem. Biol. 3(5):369-77, 1996). In addition, suppression has been obtained by interference at the protein level using dominant negative mutant peptides and antibodies (Herskowitz Nature
25 329(6136):219-22, 1987; Rimsky et al., Nature 341(6241):453-6, 1989; Wright et al., Proc. Natl. Acad. Sci. U.S.A. 86(9):3199-203, 1989). In some cases suppression strategies have led to a reduction in RNA levels without a concomitant reduction in proteins, whereas in others, reductions in RNA have been mirrored by reductions in protein.

30 The diverse array of suppression strategies that can be employed includes the use of DNA and/or RNA aptamers that can be selected to target, for example, a protein of interest such as enzymes of the fatty acid metabolism pathway.

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2,4-dienoyl-CoA reductase has been described in for instance Koivuranta et al Biochemical Journal 1994, 304, p. 787. It is also disclosed in NCBI gene ID 1666 (DECRI) as well as NCBI genbank Accession number U78302 (SEQ ID NO. 1). The sequence of 2,4-dienoyl-CoA isomerase is disclosed in NCBI gene ID 1891 (ECH1).

5

SEQ ID NO.	Sequence information	Sequence
1	Nucleic acid sequence for 2,4-dienoyl-CoA reductase	taagctttaa aaacatgtaa aaaggacatt aaattgacat cttttttgtg ttaggtcacc aaggagcagt gggacacatc agaagaactc atcaggaaga caaaagggtc ctaagaccac ttggccttc atcttggtta cagaaaaggg aatagaaatg aaacaaatta tctctcatct ttgactatt tcaagtctaa taaattctta attaacaac attcattgaa tatgtattat gtgccaggcc agtgatagcc attgtatatt caaagataaa taaaatgaaa tatagtcttc aaaacattaa aaaaaaaagg agggcatggg gagagtaggt aaaggctcct cttacctattt
2	Amino acid sequence for 2,4-dienoyl-CoA reductase	MKLPARVFFTLGSRLLPCGLAPRRFFSYGTKILYQNTEALQS KFFSPLQKAMLPNSFQGVAFITGGGTGLGKGM TLLSSL GAQCVIASRKMDVLKATAEQISSQTGNKVHAIQCDVRDPD MVQNTVSELIKVAGHPNIVINNAAGNFISPTERLSPNAWKTI TDIVLNGTAFVTLEIGKQLIKAQKGA AFLSITTIYAETGSGF VVPSASAKAGVEAMSKSLAAEWGKYGMRFNVIQPGPIKTK GAFSRLDPTGTFEKEMIGRIPCGR LGTVEELANLAAFLCSD YASWINGAVIKFDGGEEVLISGEFNDLRKVTK EQWDTIEEL IRKTKG

The molecules useful herein are isolated molecules. As used herein, the term "isolated" means that the referenced material is removed from its native environment, *e.g.*, a cell. Thus, an isolated biological material can be free of some or all cellular components, *i.e.*, components of the cells in which the native material is occurs naturally (*e.g.*, cytoplasmic or membrane component). The isolated molecules may be substantially pure and essentially free of other substances with which they may be found in nature or *in vivo* systems to an extent practical and appropriate for their intended use.

In particular, the molecules are sufficiently pure and are sufficiently free from other

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biological constituents of their hosts cells so as to be useful in, for example, producing pharmaceutical preparations or sequencing. Because an isolated peptide of the invention may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the peptide may comprise only a small percentage by weight of the preparation. The peptide is nonetheless substantially pure in that it has been substantially separated from the substances with which it may be associated in living systems. In some embodiments, the peptide is a synthetic peptide.

The term "purified" in reference to a protein or a nucleic acid, refers to the separation of the desired substance from contaminants to a degree sufficient to allow the practitioner to use the purified substance for the desired purpose. Preferably this means at least one order of magnitude of purification is achieved, more preferably two or three orders of magnitude, most preferably four or five orders of magnitude of purification of the starting material or of the natural material. In specific embodiments, a purified thymus derived peptide is at least 60%, at least 80%, or at least 90% of total protein or nucleic acid, as the case may be, by weight. In a specific embodiment, a purified thymus derived peptide is purified to homogeneity as assayed by, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis, or agarose gel electrophoresis.

The active agents of the invention are administered to the subject in an effective amount for treating the subject. An "effective amount", for instance, is an amount necessary or sufficient to realize a desired biologic effect. For instance an effective amount is that amount sufficient to prevent or inhibit autophagy. An effective amount for treating precancerous tissue may be an amount sufficient to prevent, delay or inhibit the development of a tumor in the subject compared to the levels in the absence of treatment. According to some aspects of the invention, an effective amount is that amount of a compound of the invention alone or in combination with another medicament, which when combined or co-administered or administered alone, results in a biological affect associated with treating the precancerous tissue. Prevention or inhibition as used in this context refers to any reduction or delay in tumor formation as a result of the treatment when compared to an untreated subject.

The effective amount of a compound of the invention in the treatment of a subject may vary depending upon the specific compound used, the mode of delivery of the compound, and whether it is used alone or in combination. The effective amount for any

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particular application can also vary depending on such factors as the type and/or amount of catastrophic trigger to which the subject is exposed, the particular compound being administered for treatment, the size of the subject, or the severity of the disorder. One of ordinary skill in the art can empirically determine the effective amount of a particular molecule of the invention without necessitating undue experimentation. Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity in and of itself and yet is entirely effective to treat the particular subject.

Toxicity and efficacy of the prophylactic and/or therapeutic protocols of the present invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Prophylactic and/or therapeutic agents that exhibit large therapeutic indices are preferred. While prophylactic and/or therapeutic agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays, animal studies and human studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

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In particular a number of the autophagy and fatty acid metabolism inhibitors described herein have been safely administered to humans. Safe doses for chronic or acute therapies of these compounds are known to the skilled artisan. For example chloroquine and hydroxychloroquine have been chronically administered to humans for the treatment of malaria infection as well as some forms of autoimmune disease. Dichloroacetate (DCA) has been administered to subjects for the treatment of metabolic disorders. Chronic therapy with these compounds at doses effective for inhibiting autophagy have proven to be safe in long term administration protocols. Chloroquine typically is administered in a dosage of 300mg-600mg to adults for the treatment of malarial infection. DCA can be used, for example, in dosages of 1- 25 mg/kg of body weight per day, 1- 15 mg/kg of body weight per day, or 5- 10 mg/kg of body weight per day.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein.

Subject doses of the compounds described herein typically range from about 0.1 µg to 10,000 mg, more typically from about 1 µg/day to 8000 mg, and most typically from about 10 µg to 100 µg. Stated in terms of subject body weight, typical dosages range from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above. The absolute amount will depend upon a variety of factors including

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the concurrent treatment, the number of doses and the individual patient parameters including age, physical condition, size and weight. These are factors well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is preferred generally that a maximum dose be used, that is, the
5 highest safe dose according to sound medical judgment.

Multiple doses of the molecules of the invention on the same or different days are also contemplated. The autophagy inhibitor may be administered by any schedule and route of administration. In some embodiments the autophagy inhibitor is administered once a week, twice a week, three times a week, four times a week, five times a week, six
10 times a week or every day. In other embodiments it is administered once every two weeks, three weeks, 4 weeks, 5 weeks or 6 weeks. In some embodiments the autophagy inhibitor therapy is initiated within a year of exposure to the catastrophic trigger.

Pharmaceutical compositions of the present invention comprise an effective amount of one or more agents, dissolved or dispersed in a pharmaceutically acceptable
15 carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards
20 as required by FDA Office of Biological Standards. The compounds are generally suitable for administration to humans. This term requires that a compound or composition be nontoxic and sufficiently pure so that no further manipulation of the compound or composition is needed prior to administration to humans.

As used herein, "pharmaceutically acceptable carrier" includes any and all
25 solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for
30 example, Remington's Pharmaceutical Sciences (1990), incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

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The agent may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration. The present invention involves administration of the therapeutic compounds orally.

5 In any case, the composition may comprise various antioxidants to retard oxidation of one or more components. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

10 The agent may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free
15 carboxyl groups also can be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine.

 In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (*e.g.*,
20 glycerol, propylene glycol, liquid polyethylene glycol, *etc.*), lipids (*e.g.*, triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or
25 combinations thereof such methods. In many cases, it will be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof.

 The compounds of the invention may be administered directly to a tissue. Direct tissue administration may be achieved by direct injection. The compounds may be administered once, or alternatively they may be administered in a plurality of
30 administrations. If administered multiple times, the compounds may be administered via different routes. For example, the first (or the first few) administrations may be made directly into the affected tissue while later administrations may be systemic.

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The formulations of the invention are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

5 According to the methods of the invention, the compound may be administered in a pharmaceutical composition. In general, a pharmaceutical composition comprises the compound of the invention and a pharmaceutically-acceptable carrier. Pharmaceutically-acceptable carriers are well-known to those of ordinary skill in the art. As used herein, a pharmaceutically-acceptable carrier means a non-toxic material that does not interfere
10 with the effectiveness of the biological activity of the active ingredients.

Pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers and other materials which are well-known in the art. Exemplary pharmaceutically acceptable carriers for peptides in particular are described in U.S. Patent No. 5,211,657. Such preparations may routinely contain salt, buffering agents,
15 preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from
20 the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

The compounds of the invention may be formulated into preparations in solid,
25 semi-solid, liquid or gaseous forms such as tablets, capsules, powders, granules, ointments, solutions, depositories, inhalants and injections, and usual ways for oral, parenteral or surgical administration. The invention also embraces pharmaceutical compositions which are formulated for local administration, such as by implants.

Compositions suitable for oral administration may be presented as discrete units,
30 such as capsules, tablets, lozenges, each containing a predetermined amount of the active agent. Other compositions include suspensions in aqueous liquids or non-aqueous liquids, such as a syrup, an elixir or an emulsion.

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For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral
5 ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including
10 lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or
polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for
15 neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer
20 solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in
25 admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres
30 have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

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For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation
5 from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder
10 mix of the compound and a suitable powder base such as lactose or starch. Techniques for preparing aerosol delivery systems are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the active agent (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712;
15 incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing aerosols without resort to undue experimentation.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or
20 continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

25 Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles
30 include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the

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like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Lower doses will result from other forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses
5 (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the agents of the invention to the subject. Biodegradable matrices are preferred.
10 Such polymers may be natural or synthetic polymers. Synthetic polymers are preferred. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its
15 weight in water and further, optionally is cross-linked with multivalent ions or other polymers.

In general, the agents of the invention may be delivered using the bioerodible implant by way of diffusion, or more preferably, by degradation of the polymeric matrix. Exemplary synthetic polymers which can be used to form the biodegradable delivery
20 system include: polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, poly-vinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic
25 esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate),
30 poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol),

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poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, poly vinyl chloride, polystyrene and polyvinylpyrrolidone.

Examples of non-biodegradable polymers include ethylene vinyl acetate, poly(meth)acrylic acid, polyamides, copolymers and mixtures thereof.

5 Examples of biodegradable polymers include synthetic polymers such as polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof (substitutions, additions of chemical
10 groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water *in vivo*, by surface or bulk erosion.

15 Bioadhesive polymers of particular interest include bioerodible hydrogels described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, 1993, 26, 581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, gluten, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl
20 methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the
25 compound, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are
30 described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release

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systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the platelet reducing agent is contained in a form within a matrix such as those described in U.S.

5 Patent Nos. 4,452,775, 4,675,189, and 5,736,152 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Therapeutic formulations of the active compounds may be prepared for storage
10 by mixing an active compound having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers
15 such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10
20 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions
25 such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

A peptide, for instance, may be administered directly to a cell or a subject, such as a human subject alone or with a suitable carrier. Alternatively, a peptide may be delivered to a cell *in vitro* or *in vivo* by delivering a nucleic acid that expresses the
30 peptide to a cell. Various techniques may be employed for introducing nucleic acid molecules of the invention into cells, depending on whether the nucleic acid molecules are introduced *in vitro* or *in vivo* in a host. Such techniques include transfection of

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nucleic acid molecule-calcium phosphate precipitates, transfection of nucleic acid molecules associated with DEAE, transfection or infection with the foregoing viruses including the nucleic acid molecule of interest, liposome-mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid molecule to particular
5 cells. In such instances, a vehicle used for delivering a nucleic acid molecule of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid molecule delivery vehicle.

10 Especially preferred are monoclonal antibodies. Where liposomes are employed to deliver the nucleic acid molecules of the invention, proteins that bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins
15 which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used successfully to deliver nucleic acid molecules into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acid molecules.

Definitions of specific functional groups and chemical terms are described in
20 more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University
25 Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

30 Certain compounds as described herein can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, e.g., enantiomers and/or diastereomers. The compounds provided herein can be in the form of an individual

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enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. In certain embodiments, the compounds as described herein are enantiopure compounds. In certain other embodiments, mixtures of stereoisomers are provided.

Furthermore, certain compounds, as described herein may have one or more double bonds that can exist as either the *cis* or *trans*, or the E or Z isomer, unless otherwise indicated. The invention additionally encompasses the compounds as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers, *e.g.*, racemic mixtures of E/Z isomers or mixtures enriched in one E/Z isomer.

The terms "enantiomerically enriched," "enantiomerically pure" and "non-racemic," as used interchangeably herein, refer to compositions in which the percent by weight of one enantiomer is greater than the amount of that one enantiomer in a control mixture of the racemic composition (*e.g.*, greater than 1:1 by weight). For example, an enantiomerically enriched preparation of the (S)-enantiomer, means a preparation of the compound having greater than 50% by weight of the (S)-enantiomer relative to the (R)-enantiomer, more preferably at least 75% by weight, and even more preferably at least 80% by weight. In some embodiments, the enrichment can be much greater than 80% by weight, providing a "substantially enantiomerically enriched," "substantially enantiomerically pure" or a "substantially non-racemic" preparation, which refers to preparations of compositions which have at least 85% by weight of one enantiomer relative to other enantiomer, more preferably at least 90% by weight, and even more preferably at least 95% by weight. In preferred embodiments, the enantiomerically enriched composition has a higher potency with respect to therapeutic utility per unit mass than does the racemic mixture of that composition. Enantiomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred enantiomers can be prepared by asymmetric syntheses. See, for example, Jacques, et al., *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen, S.H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E.L. *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S.H.

Tables of Resolving Agents and Optical Resolutions p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example “C₁₋₆ alkyl” is intended to encompass, C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅, and C₅₋₆ alkyl.

As used herein, alone or as part of another group, “alkyl” refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 6 carbon atoms (“C₁₋₆ alkyl”). In some embodiments, an alkyl group has 1 to 5 carbon atoms (“C₁₋₅ alkyl”). In some embodiments, an alkyl group has 1 to 4 carbon atoms (“C₁₋₄ alkyl”). In some embodiments, an alkyl group has 1 to 3 carbon atoms (“C₁₋₃ alkyl”). In some embodiments, an alkyl group has 1 to 2 carbon atoms (“C₁₋₂ alkyl”). In some embodiments, an alkyl group has 1 carbon atom (“C₁ alkyl”). In some embodiments, an alkyl group has 2 to 6 carbon atoms (“C₂₋₆ alkyl”). Examples of C₁₋₆ alkyl groups include methyl (C₁), ethyl (C₂), n-propyl (C₃), isopropyl (C₃), n-butyl (C₄), tert-butyl (C₄), sec-butyl (C₄), iso-butyl (C₄), n-pentyl (C₅), 3-pentanyl (C₅), amyl (C₅), neopentyl (C₅), 3-methyl-2-butanyl (C₅), tertiary amyl (C₅), and n-hexyl (C₆). Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an “unsubstituted alkyl”) or substituted (a “substituted alkyl”) are substituted with one or more substituents. In certain embodiments, the alkyl group is an unsubstituted C₁₋₆ alkyl (*e.g.*, -CH₃). In certain embodiments, the alkyl group is a substituted C₁₋₆ alkyl.

As used herein, “alkyloxy” refers to an alkyl group, as defined herein, substituted with an oxygen atom, wherein the point of attachment is the oxygen atom. In certain embodiments, the alkyl group has 1 to 6 carbon atoms (“C₁₋₆ alkyloxy”). In some embodiments, the alkyl group has 1 to 4 carbon atoms (“C₁₋₄ alkyloxy”). Examples of C₁₋₄ alkyloxy groups include methoxy (C₁), ethoxy (C₂), propoxy (C₃), isopropoxy (C₃), butoxy (C₄), tert-butoxy (C₅) and the like. Examples of C₁₋₆ alkyloxy groups include the aforementioned C₁₋₄ alkyloxy groups as well as pentyloxy (C₅), isopentyloxy (C₅), neopentyloxy (C₅), hexyloxy (C₆) and the like. Unless otherwise specified, each instance of the alkyl moiety of the alkyloxy group is independently unsubstituted (an “unsubstituted alkyloxy”) or substituted (a “substituted alkyloxy”) with one or more

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substituents. In certain embodiments, the alkyloxy group is an unsubstituted C₁₋₆ alkyloxy. In certain embodiments, the alkyloxy group is a substituted C₁₋₆ alkyloxy.

As used herein, “alkylcarboxy” refers to a group of the formula $-C(=O)OR^a$ or $-OC(=O)R^a$, wherein R^a is an alkyl group as defined herein. In certain embodiments, the alkyl of the alkylcarboxy group has 1 to 6 carbon atoms (“C₁₋₆ alkylcarboxy”). In some embodiments, the alkyl of the alkylcarboxy group has 1 to 5 carbon atoms (“C₁₋₅ alkylcarboxy”). In some embodiments, the alkyl of the alkylcarboxy group has 1 to 4 carbon atoms (“C₁₋₄ alkylcarboxy”). In some embodiments, the alkyl of the alkylcarboxy group has 1 to 3 carbon atoms (“C₁₋₃ alkylcarboxy”). In some embodiments, the alkyl of the alkylcarboxy group has 1 to 2 carbon atoms (“C₁₋₂ alkylcarboxy”). Unless otherwise specified, each instance of the alkyl of the alkylcarboxy group is independently unsubstituted (an “unsubstituted alkylcarboxy”) or substituted (a “substituted alkylcarboxy”) with one or more substituents. In certain embodiments, the alkylcarboxy group is an unsubstituted C₁₋₆ alkylcarboxy. In certain embodiments, the alkylcarboxy group is a substituted C₁₋₆ alkylcarboxy.

As used herein, alone or as part of another group, “alkenyl” refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 6 carbon atoms and one or more carbon-carbon double bonds (“C₂₋₆ alkenyl”). In some embodiments, an alkenyl group has 2 to 5 carbon atoms (“C₂₋₅ alkenyl”). In some embodiments, an alkenyl group has 2 to 4 carbon atoms (“C₂₋₄ alkenyl”). In some embodiments, an alkenyl group has 2 to 3 carbon atoms (“C₂₋₃ alkenyl”). In some embodiments, an alkenyl group has 2 carbon atoms (“C₂ alkenyl”). The one or more carbon-carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of C₂₋₄ alkenyl groups include ethenyl (C₂), 1-propenyl (C₃), 2-propenyl (C₃), 1-butenyl (C₄), 2-butenyl (C₄), butadienyl (C₄) and the like. Examples of C₂₋₆ alkenyl groups include the aforementioned C₂₋₄ alkenyl groups as well as pentenyl (C₅), pentadienyl (C₅), hexenyl (C₆) and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an “unsubstituted alkenyl”) or substituted (a “substituted alkenyl”) with one or more substituents. In certain embodiments, the alkenyl group is an unsubstituted C₂₋₆ alkenyl. In certain embodiments, the alkenyl group is a substituted C₂₋₆ alkenyl.

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As used herein, alone or as part of another group, “alkynyl” refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 6 carbon atoms and one or more carbon-carbon triple bonds (“C₂₋₆ alkynyl”). In some embodiments, an alkynyl group has 2 to 5 carbon atoms (“C₂₋₅ alkynyl”). In some embodiments, an
5 alkynyl group has 2 to 4 carbon atoms (“C₂₋₄ alkynyl”). In some embodiments, an alkynyl group has 2 to 3 carbon atoms (“C₂₋₃ alkynyl”). In some embodiments, an alkynyl group has 2 carbon atom (“C₂ alkynyl”). The one or more carbon-carbon triple bonds can be internal (such as in 2-butynyl) or terminal (such as in 1-butynyl). Examples of C₂₋₄ alkynyl groups include, without limitation, ethynyl (C₂), 1-propynyl
10 (C₃), 2-propynyl (C₃), 1-butynyl (C₄), 2-butynyl (C₄) and the like. Examples of C₂₋₆ alkenyl groups include the aforementioned C₂₋₄ alkynyl groups as well as pentynyl (C₅), hexynyl (C₆) and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an “unsubstituted alkynyl”) or substituted (a “substituted alkynyl”) with one or more substituents. In certain embodiments, the alkynyl group is an
15 unsubstituted C₂₋₆ alkynyl. In certain embodiments, the alkynyl group is a substituted C₂₋₆ alkynyl.

As used herein, a “saturated or unsaturated acyclic hydrocarbon” refers to radical of a saturated or unsaturated, straight-chain or branched, hydrocarbon group having from 1 to 20 carbon atoms and optionally one or more carbon-carbon double or triple bonds.
20 In certain embodiments, the hydrocarbon group is saturated. In some embodiments, the hydrocarbon group is unsaturated, and contains one or more carbon-carbon double or triple bonds. In some embodiments, the hydrocarbon group contains 1-10 carbon atoms. In certain embodiments, the hydrocarbon group contains 1-5 carbon atoms. In some embodiments, the hydrocarbon group contains 1-4 carbon atoms. In some embodiments,
25 the hydrocarbon group contains 1-3 carbon atoms. In some embodiments, the hydrocarbon group contains 1-2 carbon atoms.

As used herein, “hydroxyl” or “hydroxy” refers to the group -OH.

As used herein, “oxo” refers to the group =O.

Alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl and heteroaryl, referred to
30 without the suffix “-ene,” describe a monoradical of alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, respectively, and as defined herein, wherein the

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monoradical is attached to another group by only one single bond. Groups referred to with the suffix “-ene”, such as alkylene, alkenylene, alkynylene, carbocyclylene, heterocyclylene, arylene and heteroarylene groups, describe a diradical of alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, or heteroaryl, respectively, and as defined
5 herein, wherein the diradical is attached to one or two groups by two single bonds.

As used herein, the term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio.

10 Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1–19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group
15 formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate,
20 borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2–hydroxy–ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate,
25 palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium,
30 magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed

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using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

As used herein, the term “prodrug” means a biologically active derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the pharmacologically active compound. In this instance, the “prodrug” is a compound administered to a subject, and the pharmacologically active compound is the “active metabolite thereof.” In certain cases, a prodrug has improved physical and/or delivery properties over the parent compound. Prodrugs are typically designed to enhance pharmaceutically and/or pharmacokinetically based properties associated with the parent compound. The advantage of a prodrug can lie in its physical properties, such as enhanced water solubility for parenteral administration at physiological pH compared to the parent compound, or it enhances absorption from the digestive tract, or it may enhance drug stability for long-term storage.

The present invention is further illustrated by the following Examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Example 1

Materials and Methods:

Cell Culture Rat lung microvascular endothelial cells (RLMEC) were grown in complete MCDB-131 medium (VEC Technologies, NY).

Treatments *Figures 1-4:* RLMECs were trypsonized, washed in PBS and transferred into tubes at 1×10^6 cells/mL in warm medium. Cells were first transfected with BCL-XL protein using TranIT-Pro (Mirus) transfection kit according to the manufacturers directions. Transfected and untransfected cells were treated with etomoxir (Sigma-Aldrich), 0.4mM, glucose free media (Invitrogen) or both for 10 minutes in a CO₂ incubator at 37° C.

Figures 5: For the 10 minute time point, RLMECs were trypsonized, washed in PBS and transferred into tubes at 1×10^6 cells/mL in warm medium. Cells were treated

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with 0.4mM etomoxir (Sigma-Aldrich) or 50 μ M 6-diazo-5-oxo-l-norleucine (DON, Sigma-Aldrich) or both for 10 minutes in a CO₂ incubator at 37⁰ C. For the 24 hour time points cells were grown to confluence and then the original media was removed and the cells washed. Next, 1mL of media containing 0.4mM etomoxir (Sigma-Aldrich), 50 μ M
5 6-diazo-5-oxo-l-norleucine (DON, Sigma-Aldrich) or both was added and incubated for 1 hour. The volume was brought up to 10mL and cells incubated for a total of 24 hours.

Flow Cytometry RLMECs were trypsonized to remove them from the dish and washed twice in PBS. Cells were stained for mitochondrial membrane potential using MitoTracker Red (CM-H₂XROS, Invitrogen). Cells were resuspended in warm PBS and
10 stained at 5 μ g/mL for 20 minutes at 37⁰ C in the CO₂ incubator. Cells were washed in PBS and resuspended for analysis. To stain for intracellular H₂O₂ cells 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCF-DA, Invitrogen) was used. Cells were resuspended in warm PBS and stained at 1 μ g/mL for 20 minutes at 37⁰ C in the CO₂ incubator. To stain lysosomal acidity cells were stained with Lysosensor Green
15 (Invitrogen). Cells were resuspended in warm PBS and stained at 1 μ g/mL for 20 minutes at 37⁰ C in the CO₂ incubator. Cells were washed in PBS and resuspended for analysis. Cells are analyzed on a Beckman Coulter FC500 flow cytometer or a Becton Dickinson FACS CantoII flow cytometer. Cells were analyzed using the 488nm laser on both cytometers measuring fluorescence in the FL1, FITC (Lysosensor and DCF-DA) or
20 FL2, PE (MitoTracker). Data was analyzed using FlowJo software.

Results: Bcl-Xl overcomes Fatty Acid Inhibitor and Glucose Free Medium promoted cell death

A series of experiments was conducted to examine the effects of compounds on mitochondrial membrane potential and cell death. The studies were performed using
25 flow cytometry analysis. Endothelial cells were treated with fatty acid metabolism inhibitors (etomoxir), glucose free medium, Bcl-Xl protein, combinations thereof or control treatments. Mitochondrial membrane potential and cell death were assessed in each group.

Figure 1 demonstrates that a fatty acid inhibitor (etomoxir) increases
30 mitochondrial membrane potential and promotes cell death in endothelial cells. While the control cells only had a percent cell death of about 35%, the etomoxir treatment

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resulted in about 61% cell death. Etomoxir increases mitochondrial membrane potential and promotes cell death in endothelial cells.

The data in Figure 2 demonstrate that Bcl-Xl protein lowers mitochondrial membrane potential and protects endothelial cells from apoptosis. The Bcl-Xl protein treatment resulted in a population cell death percentage of about 31%, as compared to a population cell death percentage of about 35% in the control cells. Bcl-Xl protein lowers mitochondrial membrane potential and protects the cell from apoptosis, likely because BCLX creates a pore through the cell membrane.

Figure 3 demonstrate that removal of all glucose from endothelial cell medium causes an increase in mitochondrial membrane potential, and thus promotes cell death.

The combination of Bcl-Xl plus etomoxir or Bcl-Xl plus glucose free medium treatment on endothelial cells was also assessed. The data in Figure 4 demonstrate that Bcl-Xl mixed with etomoxir or Bcl-Xl plus glucose free medium results in lower mitochondrial membrane potential and protects the cell from apoptosis, when compared with levels ordinarily induced by etomoxir or glucose free medium treatment. Bcl-Xl appears to be dominant and overcome problems associated with etomoxir and removing all glucose.

The experiment in Figure 5 involved the harvesting of RLMECs as indicated at either 10 minutes or 24 hours post the indicated treatment. The live populations of cells were identified using forward versus side scatter profiles for size and granularity. Mitotracker, LysoSensor, or DCF-da were used as stains for mitochondrial membrane potential, acidity of lysosomal vesicles, and levels of reactive intermediates, respectively. The Y axis indicates relative amounts of fluorescence from each stain and the bars reflect at least three replicates in each treatment group as indicated where etomoxir or "DON" as described in methods above were added. The results suggest that endothelial cells depend on both fatty acid oxidation (as inhibited by etomoxir) and glutaminolysis (as indicated by inhibition with DON). Therefore, these compounds can cause inhibition of fatty acid and glutaminolysis leading to cell death.

Example 2

Materials and Methods:

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Cell Culture: HTB-77 and Daudi cells were obtained from ATCC. These cells were grown and treated in 10% FBS RPMI (Invitrogen). The cells were treated with hydroxy-chloroquine at 0.1mM (Sigma-Aldrich) and Etomoxir sodium salt hydrate at 0.5mM (Sigma-Aldrich).

5 Treatments: The following treatment groups were set up: 1) No Treatment, 2) Etomoxir, 3) Chloroquine, 4) Etomoxir + Chloroquine, 5) Etomoxir followed by Chloroquine, and 6) Chloroquine followed by Etomoxir.

At the start (T=0) each group of cells received treatment, with group 4 receiving both groups 2 and 5 receiving Etomoxir and Chloroquine groups 3 and 6 receiving
10 Chloroquine. At 24 hours (T=24) Chloroquine was added to group 5 and Etomoxir was added to group 6. At 48 hours (T=48) the cells were stained with B7-H1 (BD Biosciences) and HLA-DR (BD Biosciences) for HTB-77 or CLIP for Daudi cells. A Becton Dickinson FACS CantoII Cytometer was used for data acquisition and FlowJo (Tree Star, Inc.) was utilized for data analysis.

15 Results:

The results of the treatment are shown in Figures 6 (HTB-77 cells) and Figure 7 (Daudi cells). In Figure 6A and 7A the results of Etomoxir and Chloroquine alone or in various combinations on the expression levels of B7H1 and HLA-DR is shown. The effect of treatment on cell death is shown in Figures 6B and 7B. In the HTB-77 cells the
20 most effective treatment for inducing cell death involves the pretreatment with Etomoxir followed by Chloroquine. Interestingly, the most effective treatment in the Daudi cell line for inducing cell death involves pretreatment with Chloroquine followed by Etomoxir.

Having thus described several aspects of at least one embodiment of this
25 invention, it is to be appreciated various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

30 What is claimed is:

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CLAIMS

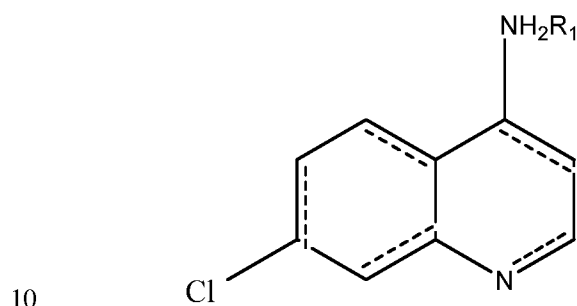
1. A method for treating a subject, comprising
administering to a subject that has been exposed to a catastrophic trigger an
autophagy inhibitor, with the proviso that the subject be not otherwise in need of
5 treatment with an autophagy inhibitor and wherein the subject has not been diagnosed
with cancer.
2. A method for treating a subject, comprising
administering to a subject that has been exposed to a catastrophic trigger selected
10 from the group consisting of radiation, severe burns, severe oxidative stress, severe
hypoxia, and severe sunburn an acute dosage regimen of an autophagy inhibitor.
- 2.1. A method for treating a subject, comprising
administering to a subject that has been exposed to radiation an autophagy
15 inhibitor.
3. The method of claim 1, wherein the catastrophic trigger is radiation,
electromagnetic waves, severe burns, severe oxidative stress, severe hypoxia, and severe
sunburn.
20
4. The method of any one of claims 1-3, further comprising identifying the
subject as one who has been exposed to radiation, electromagnetic waves, severe burns,
severe oxidative stress, severe hypoxia, or severe sunburn.
- 25 5. The method of any one of claims 1-4, wherein the method involves preventing
tumor formation by blocking the process of autophagy.
6. The method of any one of claims 1-5, wherein the autophagy inhibitor is
administered once a week.
30
7. The method of any one of claims 1-5, wherein the autophagy inhibitor is
administered once every two weeks.

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8. The method of any one of claims 1-7, wherein the autophagy inhibitor therapy is initiated within a year of exposure to the catastrophic trigger.

5 9. The method of any one of claims 1, 2.1, and 3-8, wherein the autophagy inhibitor is administered chronically.

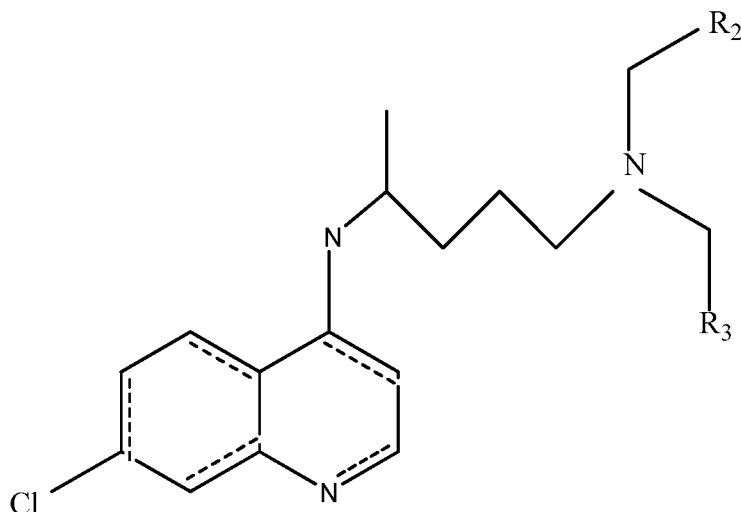
10. The method of any one of claims 1-9, wherein the autophagy inhibitor is a 4-aminoquinoline having the following structure:



or a pharmaceutically acceptable salt or prodrug thereof, wherein each instance of the dotted line independently represents a single bond or a double bond which can be in the *cis* or *trans* configuration; and R₁ is 1 or 2 hydrogens, alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

15

11. The method of any one of claims 1-10, wherein the autophagy inhibitor has the following structure:

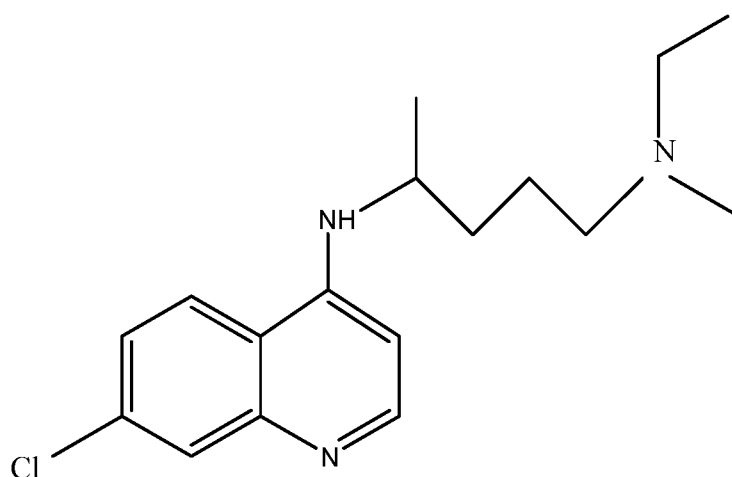


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or a pharmaceutically acceptable salt or prodrug thereof, wherein each instance of the dotted line independently represents a single bond or a double bond which can be in the *cis* or *trans* configuration; R₂ and R₃ is independently a hydroxalkyl, an alkyl, alkyloxy, alkylcarboxy, alkylene or alkenylene having from one to six carbon atoms.

5

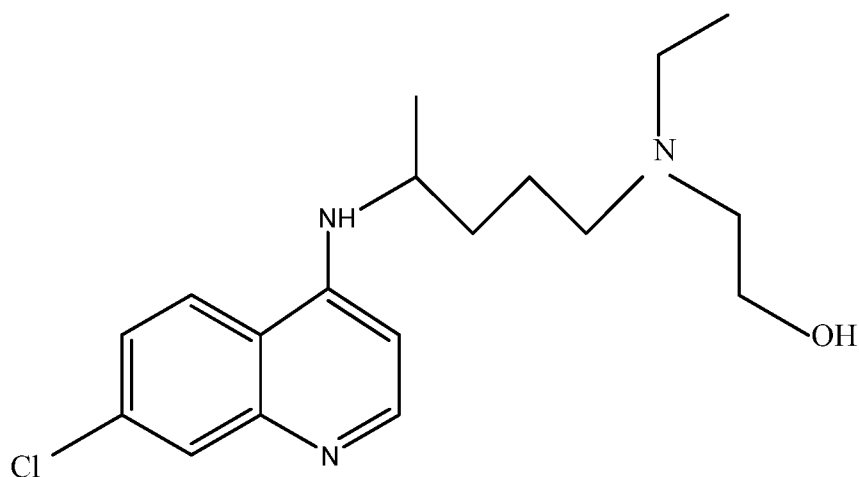
12. The method of any one of claims 1-10, wherein the autophagy inhibitor has the following structure:



or a pharmaceutically acceptable salt or prodrug thereof.

10

13. The method of any one of claims 1-10, wherein the autophagy inhibitor has the following structure:



or a pharmaceutically acceptable salt or prodrug thereof.

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14. The method of any one of claims 1-10, wherein the autophagy inhibitor is selected from the group consisting of chloroquine, 2-hydroxychloroquine, amodiaquine, mondesethylchloroquine, quinoline phosphate, and chloroquine phosphate or mixtures thereof.

5 15. The method of any one of claims 1-10, wherein the autophagy inhibitor is selected from the group consisting of chloroquine compounds, aminoquinoline derivatives, 3-methyladenine, bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate
10 cAMP levels, adenosine, N6-mercaptopurine riboside, wortmannin, and vinblastine.

16. The method of claim 15, wherein the chloroquine compound is selected from the group consisting of chloroquine phosphate; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline (hydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino]-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 7-chloro-2-(o-chlorostyryl)-4-[4-diethylamino-1-methylbutyl]aminoquinoline phosphate; 3,4-dihydro-1
25 (2H)-quinolinecarboxyaldehyde; 1,r-pentamethylenediquinoleinium diiodide; and 8-quinolinol sulfate, enantiomers thereof, as well as suitable pharmaceutical salts thereof.

17. The method of claim 15, wherein the aminoquinoline derivative is selected from the group consisting of (S)-N₂-(7-Chloro-quinolin-4-yl) Ni, N, -dimethyl-propane-1,2-diamine; (R)- N₂-(7-chloroquinolin-4-yl)- Ni, Ni-dimethyl-propane-1, 2-diamine; 5 Ni-(7-chloro-quinolin-4-yl)-2, N₂, N₂-trimethyl-propane-1,2-diamine; N₃-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1,3-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-piperidin-3-yl)-amine; (RS)-(7-chloro-quinolin-4-yl)-(1-methyl-pyrrolidin-3-yl)-amine; (RS)-N₂-(7-Chloroquinolin-4-yl)- Ni, Nidimethyl-propane-1,- 2-diamine; (RS)-N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1, 2-diamine; (S)- N₂-(7-chloro-quinolin-4-yl)- N₁, N₁-diethyl-propane- 1,2-diamine; (R)- N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-diethyl-propane-1,2- diamine; (RS)-7-chloro-quinolin-4-yl)-(1-methyl-2- 10 pyrrolidin-1-yl-ethyl)-amine; N₂-(7-chloro-quinolin-4-yl)- Ni, Ni-dimethyl-ethane-1,- 2-diamine; N₂-(7-chloro-quinolin-4-yl) Ni, Ni-diethylethane-1,2-diamine; N₃-(7-chloro-quinolin-4-yl) Ni, N₁-dimethyl-propane-1,3-diamine; (R) Ni-(7-chloroquinolin-4-yl) N₂, 15 N₂-dimethyl-propane-1, 2-diamine; (S) Ni-(7-chloro-quinoline-4-yl) N₂, N₂-dimethyl-propane-1-2-diamine; (RS)-(7-chloro-quinolin-4-yl)-(1 -methyl-pyrrolidin-2-yl-methyl)-amine; Ni .1 -(7-Chloro-quinolin-4-yl) N₂-(3-chloro-benzyl)-2-methyl-propane-1 , 2-diamine; N)-(7-chloro-quinolin-4-yl) N₂-(benzyl)-2-methyl-propane-1,2-diamine; Nr(7-chloro-quinolin-4-yl)-N₂-(2-hydroxy-3-methoxy-benzyl)-2-methyl-propane 1,2-diamine; 20 Ni-(7-chloro-quinolin-4-yl)- N₂-(2-hydroxy-5-methoxy-benzyl)-2-methyl-propane- 1,2-diamine; and Ni-(7-chloro-quinolin-4-yl)- N₂-(4-hydroxy-3-methoxy-benzyl)-2-methyl-propane-1,2-diamine; (1 S,2S)-Ni-(7-chloro-quinolin-4-yl)- N₂-(benzyl)-cyclohexane-1,- 2-diamine; (1S,2S)- Ni-(7-chloro-quinolin-4-yl)- N₂-(4-chlorobenzyl)-cyclohexane- 1,2-diamine; (1S,2S)- Ni-(7-chloro-quinolin-4-yl)- N₂-(4-dimethylamino-benzyl)- 25 cyclohexane1,2-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(3-chloro-benzyl)-cyclohexane-1,4-diamine; cis- Nj-(7-chloro-quinolin-4-yl)- N₄-(2-hydroxy-4-methoxy-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(3,5-dimethoxy-benzyl)-cyclo hexane-1,4-diamine; cis- Nj-(7-chloro-quinolin-4-yl)- N₄-(4- 30 methylsulphanyl-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-(4-diethylamino-benzyl)-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-yl)- N₄-

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(biphenyl-4-yl)methyl-cyclohexane-1,4-diamine; trans- Ni-(7-chloro-quinolin-4-yl)- N₄-
[2-(3,5-dimethoxy-phenyl)~ ethyl]-cyclohexane-1,4-diamine; cis- Ni-(7-chloro-quinolin-4-
yl)-N₄-(4-methoxy-benzyl)-cyclohexane-1,4-diamine; trans- Ni-(7-chloro-quinolin-4-
yl)- N₄-(4-dimethylamino-benzyl)-cyclohexane-1,4-diamine; and trans- N₁-(7-chloro-
5 quinolin-4-yl)-N₄-(2,6-difluoro-benzyl)-cyclohexane- 1,4-diamine.

18. The method of claim 2, wherein the autophagy inhibitor is balifomycin A.

19. A method for treating a subject, comprising
10 administering to a subject that has been exposed to a catastrophic trigger a
dichloroacetate compound, with the proviso that the subject be not otherwise in need of
treatment with a dichloroacetate compound and wherein the subject has not been
diagnosed with cancer.

15 20. The method of claim 19, wherein the dichloroacetate compound is sodium
dichloroacetate.

21. A method for treating a subject, comprising
administering to a subject that has been exposed to a catastrophic trigger a fatty
20 acid metabolism inhibitor, with the proviso that the subject be not otherwise in need of
treatment with a fatty acid metabolism inhibitor and wherein the subject has not been
diagnosed with cancer.

22. The method of claim 21, wherein the fatty acid metabolism inhibitor is
25 an inhibitor of fatty acid oxidation, a fatty acid transporter inhibitor, a reductase
inhibitor, or an isomerase inhibitor within the fatty acid metabolism pathway.

23. The method of claim 22 wherein the reductase is 2,4-dienoyl-CoA
reductase.

30

24. The method of claim 22 wherein the isomerase is 2,4-dienoyl-CoA
isomerase.

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25. The method of claim 21, wherein the inhibitor of fatty acid metabolism is an inhibitor of fatty acid oxidation and is selected from the group consisting of an oxirane carboxylic acid compound, such as etomoxir (2-(6-(4-chlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester), 2-(4-(3-chlorophenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(4-(3-trifluoromethylphenoxy)-butyl)-oxirane-2-carboxylic acid ethyl ester, 2-(5(4-chlorophenoxy)-pentyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(3,4-dichlorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-(4-fluorophenoxy)-hexyl)-oxirane-2-carboxylic acid ethyl ester, 2-(6-phenoxyhexyl)-oxirane-2-carboxylic acid ethyl ester, cerulenin, 5-(tetradecyloxy)-2-furoic acid, oxfenicine, methyl palmoxirate, metoprolol, amiodarone, perhexiline, aminocamitine, hydrazonopropionic acid, 4-bromocrotonic acid, trimetazidine, ranolazine, hypoglycin, dichloroacetate, methylene cyclopropyl acetic acid, beta-hydroxy butyrate, and a non-hydrolyzable analog of camitine or pharmacologically acceptable salts thereof.

15

26. The method of claim 21, wherein the inhibitor of fatty acid metabolism is an inhibitory nucleic acid.

27. The method of claim 26, wherein the inhibitory nucleic acid is specific for an enzyme selected from the group consisting of 2,4-dienoyl-CoA reductase, 2,4-dienoyl-CoA isomerase, and butyryl dehydrogenase.

20

28. The method of claim 21, wherein the inhibitor of fatty acid metabolism is oxamate.

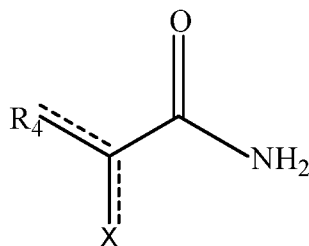
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29. The method of claim 28, wherein the oxamate is selected from the group consisting of ethyl oxamate and sodium oxamate.

30. The method of claim 21, wherein the inhibitor of fatty acid metabolism is a compound having the following structure:

30

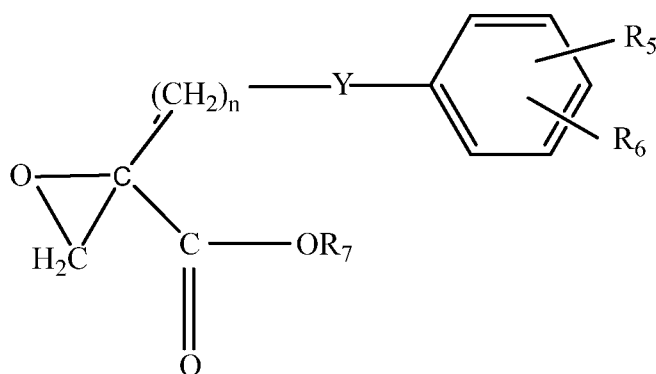
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or a pharmaceutically acceptable salt or prodrug thereof; wherein the dashed line is a double bond at one of the indicated positions and a single bond in the other; wherein R₄ is O-C-CH₃, -ONa, -OH, -O-(CH₂)₃-CH₃, -CH₂-C(O)-C(O)-O- R₈ or -CH=C(OH)-C(O)-O- R₈, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocyclyl, heterocycloalkyl, substituted alkyl, substituted cycloalkyl or substituted aryl, substituted aralkyl, substituted heteroaryl, substituted heteroaralkyl, substituted heterocyclyl, substituted heterocycloalkyl; wherein X is: =O, =N--O R₂; and wherein R₈ is independently selected from hydrogen, H₂, alkyl, cycloalkyl, aryl, substituted alkyl, substituted cycloalkyl or substituted aryl.

31. The method of claim 21 in which the fatty acid metabolism inhibitor is an oxirane carboxylic acid compound capable of inhibiting fatty acid metabolism, or a pharmacologically acceptable salt thereof, wherein the subject is not otherwise indicated for treatment with the compound.

32. The method of claim 31 wherein the oxirane carboxylic acid compound has the formula:



20

or a pharmaceutically acceptable salt or prodrug thereof, wherein R₅, R₆ and R₇ are herein; wherein R₅ represents a hydrogen atom, a halogen atom, a 1-4C alkyl group,

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a 1-4C alkoxy group, a nitro group or a trifluoromethyl group, R_6 has one of the meanings of R_5 , R_7 represents a hydrogen atom or a 1-4C alkyl group, Y represents the grouping $—O—(CH_2)_m—$, m is 0 or a whole number from 1 to 4, and n is a whole number from 2 to 8 wherein the sum of m and n is a whole number from 2 to 8.

5 33. The method of claim 32 wherein R_1 is a halogen atom, R_2 is a hydrogen atom, m is 0, and n is 6.

 34. The method of claim 33 wherein R_3 is an ethyl group.

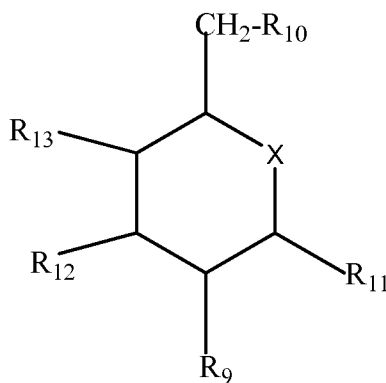
 35. The method of claim 32 wherein the oxirane carboxylic acid compound is etomoxir.

10

 36. The method of claim 21 further comprising administering a glycolytic inhibitor to the subject.

 37. The method of claim 36 in which the glycolytic inhibitor is a 2-
15 deoxyglucose compound.

 38. The method of claim 37 in which the 2-deoxyglucose compound has the formula:



20 or a pharmaceutically acceptable salt or prodrug thereof, wherein R_9 , R_{10} , R_{11} , R_{12} , and R_{13} are herein; wherein X represents an O or S atom; R_9 represents a hydrogen atom or a halogen atom; R_{10} represents a hydroxyl group, a halogen atom, a thiol group, or CO-R_6 ; R_{11} , R_{12} , and R_{13} each represent a hydroxyl group, a halogen atom, or CO-R_{14} , R_{14} represents an alkyl group of from 1 to 20 carbon atoms, and at least two of R_{11} ,
25 R_{12} , and R_{13} are hydroxyl groups.

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39. The method of claim 38 in which the 2-deoxyglucose compound is 2-deoxy-D-glucose.

Figure 1

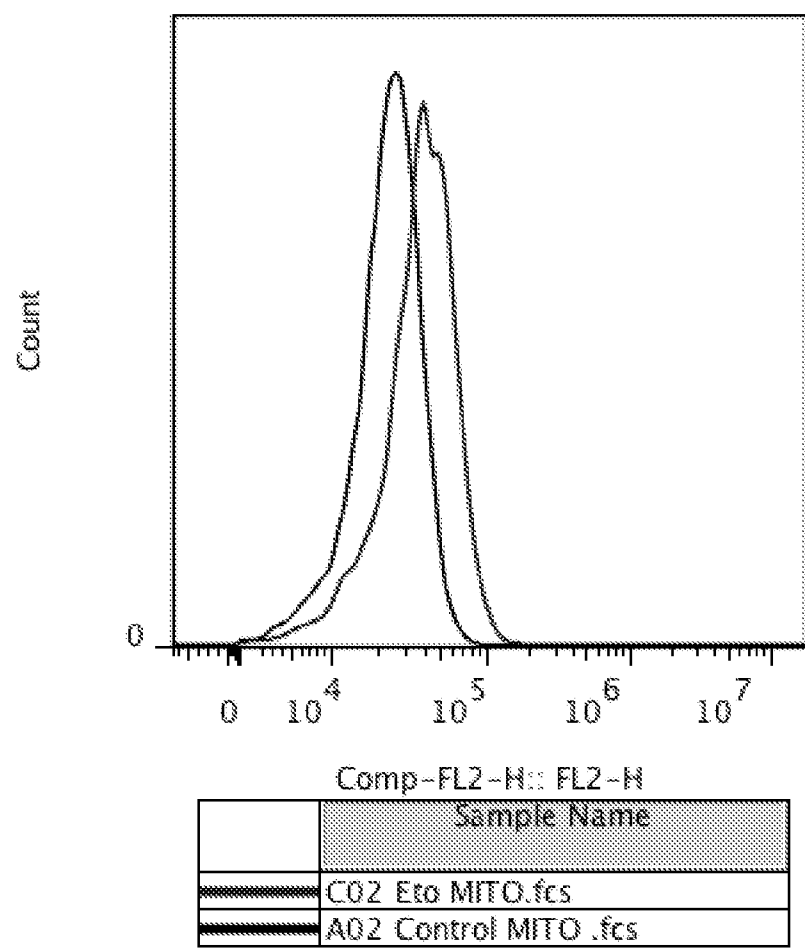


Figure 2

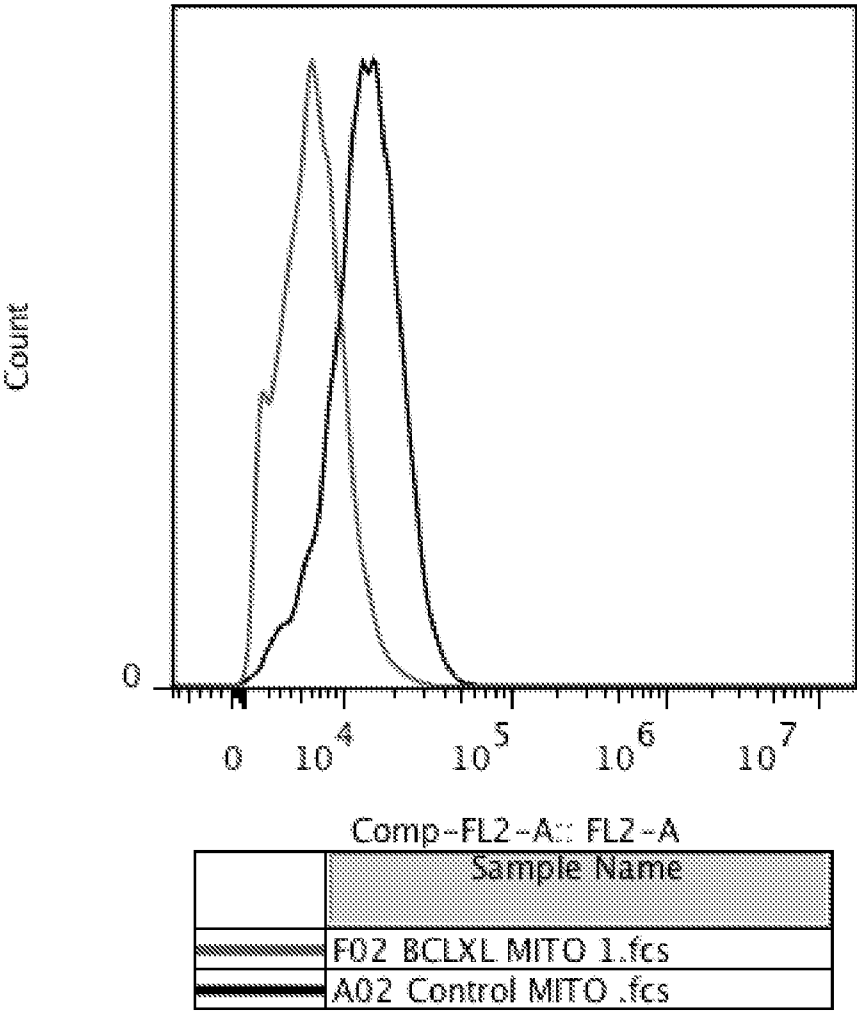


Figure 3

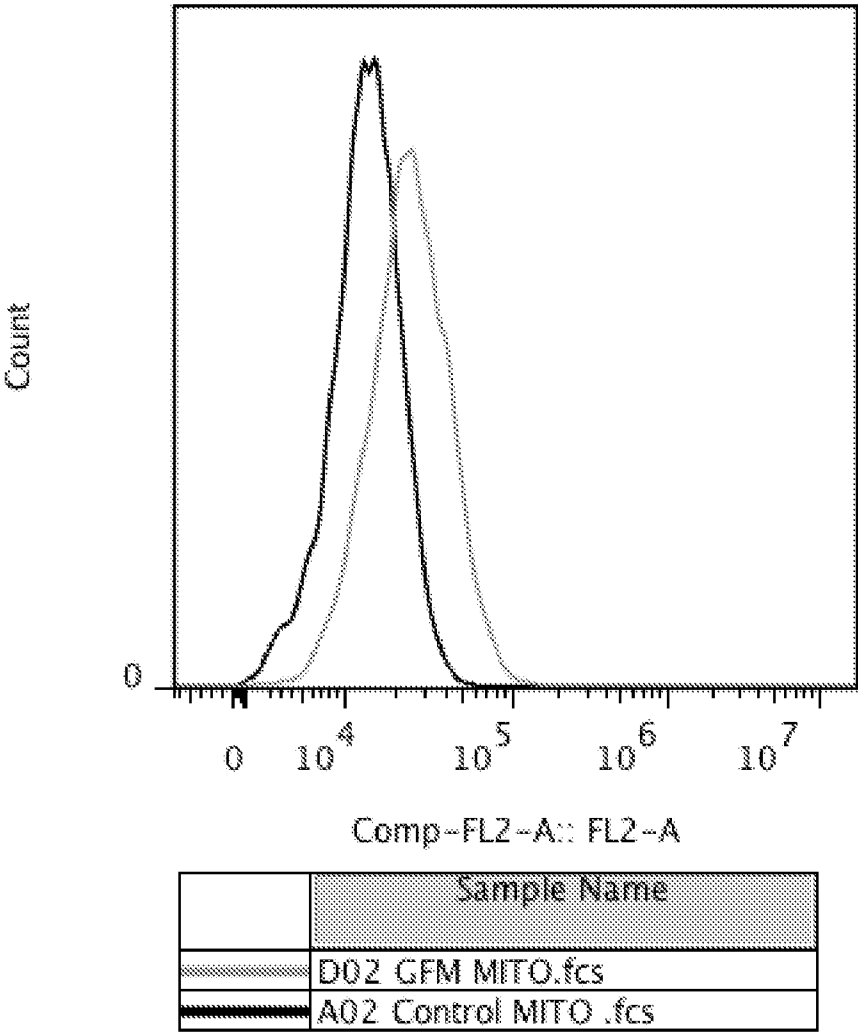
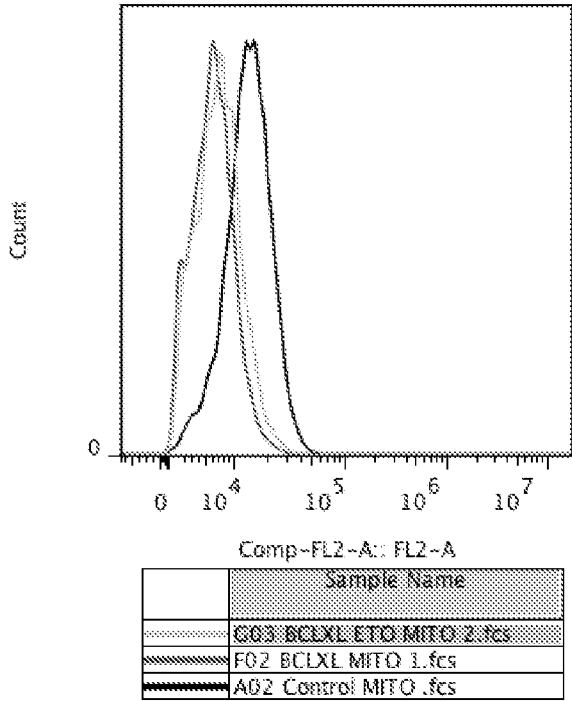


Figure 4

4A



4B

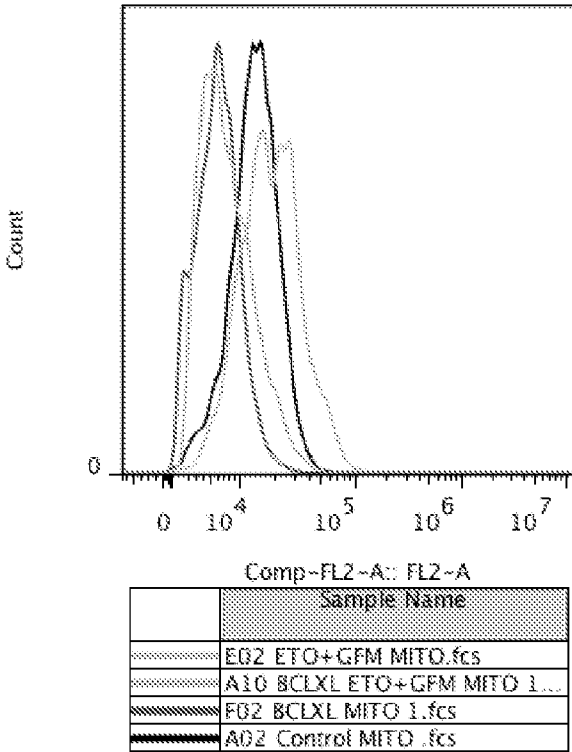
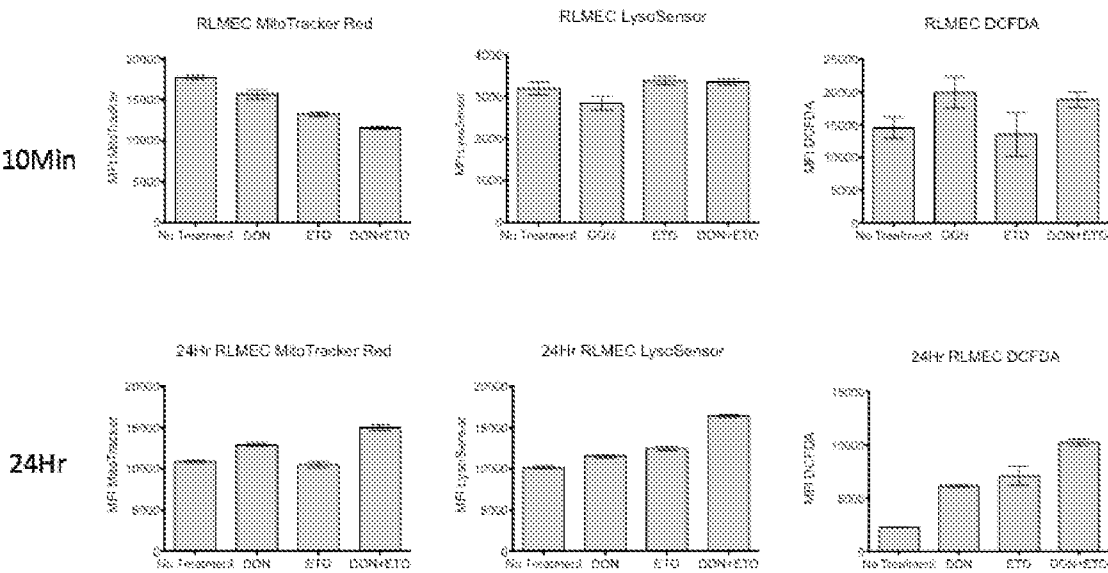


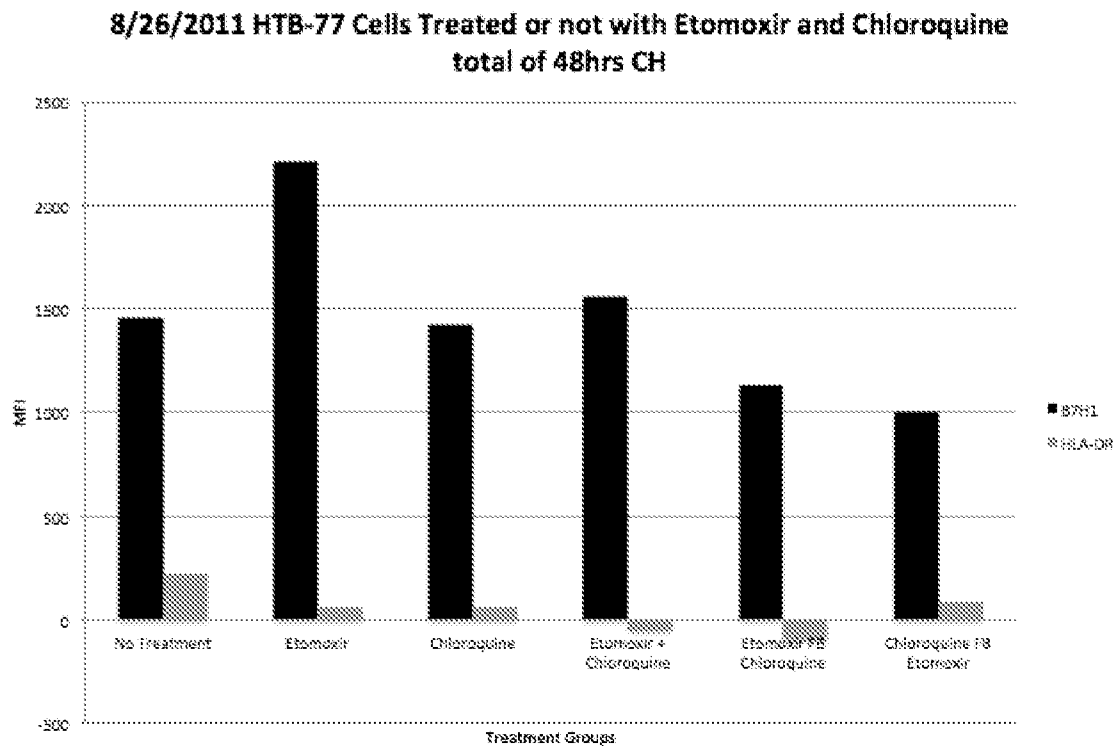
Figure 5



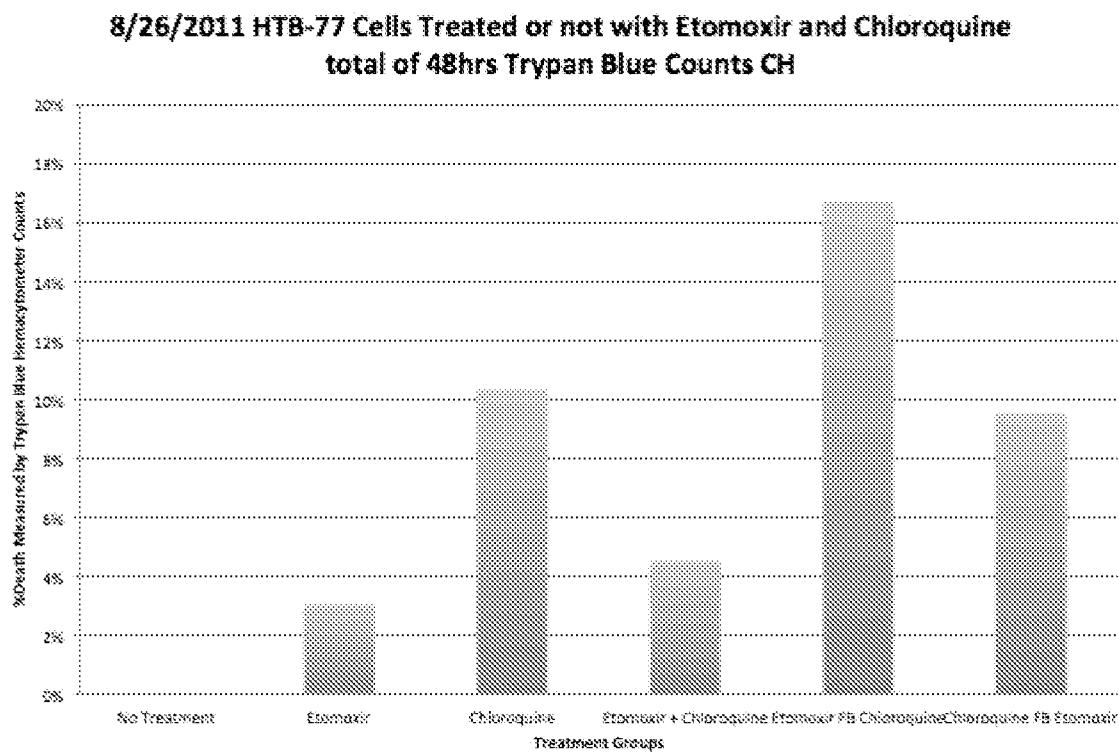
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Figure 6

6A



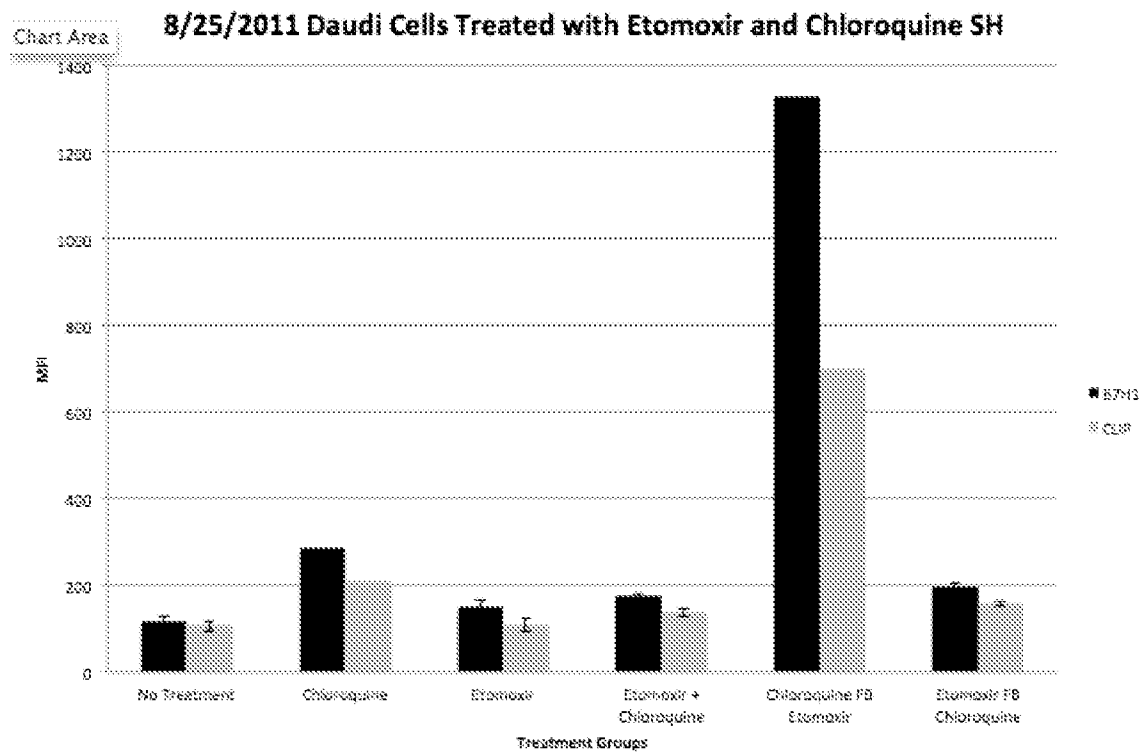
6B



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Figure 7

7A



7B

