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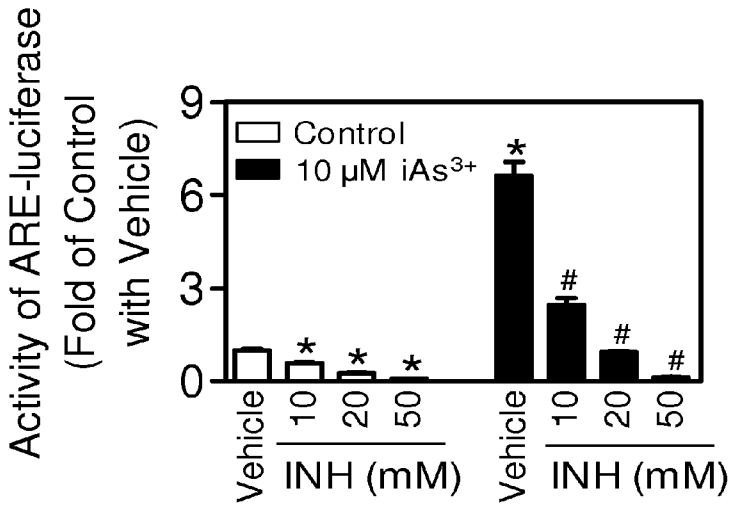
[Continued on next page]

(54) Title: COMPOSITIONS FOR MODULATING NRF2-ARE ACTIVITY AND THEIR METHODS OF USE

FIG. 2A



(57) Abstract: The invention provides the identification of compounds having a novel activity of Nrf2 inhibition. Provided is use of the Nrf2 inhibitors in pharmaceutical compositions and in treatment modalities, and methods of use thereof, to treat cells having constitutive Nrf2 activation such as in solid nonlymphoid tumors and in viral infections.

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COMPOSITIONS FOR MODULATING NRF2-ARE ACTIVITY AND THEIR METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to the discovery of compounds which can inhibit Nrf2 activity, and more particularly Nrf2-ARE activity, as well as relates to pharmaceutical compositions containing them as an active ingredient, and their use as medicaments in a method for treating cancer or viral disease having constitutive activation of Nrf2.

BACKGROUND OF THE INVENTION

One of the most critical cytoprotective mechanisms against oxidative/electrophilic stress in vertebrates is the Keap1 (Kelch-like ECH protein 1)-Nrf2 (Nuclear factor E2-related factor 2) pathway. Nrf2, characterized by the amino acid sequence comprising SEQ ID NO:1 including isoforms thereof (see, e.g., SEQ ID NOs:2 & 3), recognizes a unique DNA sequence known as the antioxidant response element (ARE). Keap1 binds to Nrf2 in the cytoplasm of a cell, resulting in Nrf2 degradation. Thus, under normal homeostatic conditions, a low amount of cellular Nrf2 is mainly controlled by Keap1-mediated ubiquitination and subsequent proteasomal degradation. However, following exposure to electrophiles or oxidative stress (including reactive oxygen species), Keap 1 is inactivated, and Nrf2 is stabilized ("Nrf2 activation"). Nrf2, as a potent transcription activator, translocates into the nucleus and activates (by binding ARE) transcription of a number of genes having functional ARE, including cytoprotective genes, such as encoding antioxidant enzymes, phase II detoxification enzymes, and multidrug resistant proteins. Thus, NRF2-mediated adaptive antioxidant response plays pivotal roles against oxidative/electrophilic stress and in chemical detoxification. As a result, transient or controlled activation of NRF2 can be an effective approach for cancer chemoprevention.

However, in conditions or circumstances in which Keap1 is not sufficiently repressing Nrf2 activity, dysregulation of Nrf2 occurs which leads to persistent or constitutive (individually or collectively, "constitutive") activation of Nrf2. Dysregulation of Nrf2 activity, including its downstream genes, can lead to promotion of a disease. For example, it has been demonstrated that one or more regulatory proteins produced by hepatitis B virus (HBV), such as HBx and LHBs, result in a constitutive activation of Nrf2, and better protection of HBV-infected cells against oxidative stress resulting from viral infection and/or inflammation in the hepatitis disease process. Nrf2 activation has also

been observed in human cytomegalovirus (HCMV)-infected cells, resulting in better protection of HCMV-infected cells against oxidative stress resulting from viral infection and/or inflammation in promoting disease in immunocompromised individuals.

Constitutive activation of Nrf2 has been observed in many human solid nonlymphoid tumors, including but not limited to, nonsmall cell lung cancer, head and neck cancer, ovarian cancer, breast cancer, pancreatic cancer, hepatocellular carcinoma, gallbladder cancer, prostate cancer, esophageal cancer, skin cancer, gliomas, and colon cancer. Constitutively stabilized Nrf2 promotes cell proliferation, and production of detoxifying and antioxidant proteins which confer survival of cancer cells from chemotherapy and radiation therapy; and has been associated with poor prognosis for individuals carrying tumors expressing Nrf2 (Nrf2-positive because of constitutive Nrf2 activation). For example, constitutive activation of Nrf2 has been associated with development of doxorubicin resistance by ovarian cancer cells, and tamoxifen resistance by breast cancer cells. There are several reported mechanisms by which dysregulation of Nrf2 activity occurs, resulting in persistent or constitutive ("constitutive") Nrf2 activation, including but not limited to, somatic mutations in the Keap1 gene or Nrf2 gene; oncometabolite regulation of Keap1; oncogene-dependent signaling resulting in upregulation of Nrf2 gene transcription; accumulation of proteins that disrupt Keap1 regulation of Nrf2 activity; and hypermethylation at the promoter region of the Keap1 gene. One or more of disease- associated phenomena comprising metabolite regulation of Keap1, upregulation of Nrf2 gene transcription, and accumulation of proteins that disrupt Keap1 regulation of Nrf2 activity, may be mechanisms that also play a role in dysregulation of Nrf2 activity.

For example, mutations in the Keap1 gene, particularly in the DC domain that is essential for binding with Nrf2, have been identified in several human nonlymphoid tumors, including tumors of the lung, liver, prostate, breast, and gallbladder. Similarly, mutations in the Nrf2 gene, particularly within the regions of the gene that encode critical sites for binding of Nrf2 to Keap1 (e.g., DLG motif) have been observed in several human nonlymphoid tumors, including tumors of the lung, esophagus, and head and neck. Disruption of Keap1 binding to Nrf2 results in constitutively stabilized Nrf2. Hypermethylation of the promoter region of Keap1 gene, resulting in inhibition of Keap1 gene expression and Nrf2 accumulation (including constitutive Nrf2 activation) has been observed in several human nonlymphoid tumors, including tumors of the lung, prostate, brain, and colon. Certain proteins that aberrantly accumulate in some cancers, such as

p21 and p62, compete for binding sites of Keap1 or Nrf2, thereby disrupting binding between Keap1 and Nrf2, leading to constitutive Nrf2 activation. Constitutive activation of Nrf2 through p62 has been observed in hepatocellular carcinoma. Oncogene-dependent signaling (e.g., mediated by oncogenes such as K-Ras, Braf and c-Myc) can result in upregulation of Nrf2 gene transcription, and constitutive Nrf2 activation. Constitutive Nrf2 activation also appears in cancers having fumarate as an oncometabolite as a result of a mutation in the fumarate hydratase gene. Certain chemotherapeutic agents, such as 5-fluorouracil, have been shown to activate Nrf2-ARE pathway in human colorectal cancer cells.

It has been reported that inhibition of the NRF2-dependent antioxidant response through overexpression of KEAP1, knockdown of NRF2 or chemical suppression, renders cancer cells more susceptible to chemotherapeutic agents. For example, it was found that silencing NRF2 in three independent human non-small cell lung cancer-derived cell lines, and mouse insulinoma MIN6 cells, sensitized them to multiple chemotherapeutic agents, including etoposide, doxorubicin, and arsenic trioxide (As_2O_3) or arsenite. While As_2O_3 has also shown efficacy in treating leukemia and other malignancies, particularly multiple myeloma and myelodysplastic syndromes, many cancer cells of nonlymphoid solid tumors are relatively resistant to As_2O_3 therapy, which may be associated with their constitutive NRF2 activation.

Taken together, Nrf2, constitutively expressed in cancer comprised of solid nonlymphoid tumors, may be targeted by inhibitors of Nrf2-ARE activity. Inhibitors of Nrf2-ARE activity in such diseases can result in inhibition of cell proliferation, and chemosensitization and radiation sensitization. Thus, inhibitors of Nrf2-ARE activity may be used to enhance the effectiveness of (by increasing sensitivity to) other treatment modalities in anticancer treatment of solid nonlymphoid tumors when used in a combination.

SUMMARY OF THE INVENTION

Methods and products are provided that inhibit the activity of the Nrf2, and can be used to sensitize cells having constitutive Nrf2 activation to anticancer or antiviral treatment in ameliorating or inhibiting the respective disease symptoms or severity. Treatment with the compounds of the present invention, comprising inhibitors of Nrf2-ARE activity ("Nrf2 inhibitors") may result in enhancing the effectiveness of other agents (e.g., therapeutic agents) or treatment modalities (e.g. radiation treatment for cancer) that

would otherwise be less effective or ineffective for treating cancer or viral disease characterized by constitutive activation of Nrf2. The Nrf2 inhibitors sensitize the affected cells to be more susceptible to other therapeutic agents (e.g., "chemosensitization") or treatment modalities (e.g., "radiation sensitization"). The methods comprise adding an Nrf2 inhibitor of the invention in combination with one or more other therapeutic agents or treatment modalities, in treating a disease having constitutive Nrf2 activation comprising one or more of cancer, and viral infections.

The compound, or composition containing the compound, preferably downregulates or inhibits Nrf2 activity, including inhibiting Nrf2 transactivation of genes downstream from Nrf2, particularly genes having an ARE (antioxidant response element) in their promoter. These newly identified NRF2 inhibitors do not reduce the level of mRNA and/or protein expression of NRF2, but suppress Nrf2 activity (e.g., affect Nrf2 function) which subsequently affects induction of ARE-driven gene expression.

Compounds of the present invention that have been identified to modulate Nrf2 activity, as Nrf2 inhibitors, include antitubercular agents (agents used in the treatment of tuberculosis), and other small molecule agents, including 4-aminobenzoic hydrazide, aminopyrazine, cyclohexanecarboxamide, 2-furoic hydrazide, phenylhydrazine, phenylacetic hydrazide, pyrazinecarboxamide, p-toluic hydrazide, 4-(aminomethyl)piperidine, isonicotinamide, and 2-amino- isonicotinamide (see, e.g., Formulas I & II). Antitubercular agents comprising Nrf2 inhibitors include, but are not limited to, isoniazid, ethionamide, ethambutol dihydrochloride, ethionamide, a rifamycin (rifampicin), and sparfloxacin.

In one aspect of the invention, the Nrf2 inhibitor comprises an amide of isonicotinic acid, or a pharmaceutically acceptable salt or solvate thereof. Amides of isonicotinic acid include, but are not limited to, isoniazid, iproniazid, nialamide, ethionamide, isonicotinamide, and 2-amino- isonicotinamide; or pharmaceutically acceptable salt or solvate thereof. In another aspect of the invention, the Nrf2 inhibitor comprises a quinolone, or a pharmaceutically acceptable salt or solvate thereof. Some antitubercular agents comprise quinolones. Quinolones include, but are not limited to, ofloxacin, levofloxacin, ciprofloxacin, and sparfloxacin.

In another aspect of the invention provided are pharmaceutical products or compositions containing a Nrf2 inhibitor selected from the group consisting of compounds represented by Formula I or Formula II, or an antitubercular drug consisting of ethambutol dihydrochloride, rifamycin (e.g., rifampicin), or an antitubercular quinolone (e.g.,

sparfloxacin), and a combination thereof; and at least anticancer treatment in the treatment of cancer, as a combined preparation for simultaneous use, separate use, or sequential use. The use is to treat solid nonlymphoid tumor, and the use may further comprise selecting an individual for treatment because the individual has solid nonlymphoid tumor characterized as having constitutive activation of Nrf2. The anticancer treatment may be selected from a cancer-treating agent, treatment modality (e.g. radiation therapy), and a combination thereof. The Nrf2 inhibitor is in an amount effective to enhance effectiveness of (render more susceptible to) anticancer treatment of solid nonlymphoid tumors expressing Nrf2, when used with at least one cancer-treating agent in the treatment of such cancer, characterized by sensitizing cancer cells to the anticancer effect of the at least one cancer-treating agent or cancer treatment modality. Enhancing the effectiveness of anticancer treatment may be observed by one or more of inhibiting the progression of the disease, a reduction in tumor mass, inhibiting tumor cell proliferation or migration, or amelioration of a pathological feature or symptom of the disease. In another aspect of the invention, provided is a method of inhibiting activity of Nrf2 in an individual having solid nonlymphoid tumor expressing Nrf2 ("Nrf2-positive" characterized by constitutive activation of Nrf2, as detected by conventional imaging or immunostaining techniques for Nrf2 protein, or by nucleic acid amplification for quantifying Nrf2 mRNA levels whether in tumor cells or virally infected cells). The method comprises selecting an individual having solid nonlymphoid tumor expressing Nrf2 characterized by constitutive activation of Nrf2, administering to the individual an Nrf2 inhibitor of the invention in an amount effective to inhibit one or more of cancer cell proliferation, sensitize such tumor cells to anticancer drug treatment, or sensitize such tumor cells to radiation treatment.

In another aspect of the invention, provided is a method of improving or enhancing the effectiveness of concomitant anticancer treatment (i.e., one or more of a therapeutic agent for treating cancer, and a cancer treatment modality) directed towards cancer comprising solid nonlymphoid tumors characterized by constitutive Nrf2 activity, the method comprising administering to an individual in need thereof a sensitizing effective amount of an Nrf2 inhibitor of the invention in combination with the anticancer treatment. A therapeutic agent for treating cancer ("cancer-treating agent") is known to those skilled in the art to comprise a chemotherapeutic agent, immunotherapeutic agent, radiotherapeutic agent, hormonal therapeutic agent, and a biological agent (e.g., virus with the ability to kill tumor cells). A treatment modality is known to those skilled in the

art to include, but is not limited to, one or more of chemotherapy, radiation therapy, immunotherapy, biological therapy, gene therapy, hormonal therapy, anti-angiogenic therapy, demethylation therapy, targeted therapy, toxin therapy, pro-drug activating enzyme therapy, small molecule therapy, and epigenetic therapy. A sensitizing effective amount of an Nrf2 inhibitor is a dose that sensitizes tumor cells, characterized by constitutive activation of Nrf2, to a therapeutic agent or cancer treatment modality administered in conjunction with the Nrf2 inhibitor such that the anticancer effect of the therapeutic agent or cancer treatment modality is enhanced.

Another aspect of the invention is pharmaceutical compositions, or medicaments, comprising at least one Nrf2 inhibitor of the invention in a particular dosage or formulation for delivering an amount of the compound effective to inhibit Nrf2 activity in tumor cells or virally infected cells characterized by constitutive activation of Nrf2, and a pharmaceutically acceptable carrier. The composition may comprise a medically effective amount (e.g., therapeutically effective amount, or prophylactically effective amount, sensitizing effective amount, or a combination thereof) as such disease warrants and as known to a skilled medical practitioner) of a compound according to the invention, as an Nrf2 inhibitor, in modulating (one or more of inhibiting, ameliorating, treating, or preventing) such disease characterized by constitutive Nrf2 activation. The composition, in addition to comprising a compound according to the invention, may also contain at least one additional therapeutic agent (i.e., other than an Nrf2 inhibitor) in a medically effective amount to modulate such disease characterized by constitutive Nrf2 activation.

In another aspect of the invention, provided is the use of an Nrf2 inhibitor compound of the invention (or a pharmaceutical composition comprising such compound) in an amount effective to sensitize cells characterized by constitutive activation of Nrf2 comprising as solid nonlymphoid tumor cells or virally infected cells. The compound is administered to an individual (a mammal, such as a human) selected for treatment because the individual has one or more of solid nonlymphoid tumor or a viral disease characterized by constitutive activation of Nrf2 (an individual in need thereof).

In these methods, one or more compounds of the invention may be administered in a medically effective amount as the sole pharmaceutical agent, or may be administered in combination therapy wherein a sensitizing effective amount of a compound of the invention is administered with a medically effective amount of at least one additional therapeutic agent. Such combination therapy may comprise (a) a single pharmaceutical composition comprised of a compound of the invention, at least one additional therapeutic

agent, and a pharmaceutically acceptable carrier; or (b) two separate compositions, which can be administered simultaneously or sequentially, comprising a first composition comprising a compound of the invention and a pharmaceutically acceptable carrier; and a second composition comprising at least one additional therapeutic agent and a pharmaceutically acceptable carrier.

Other aspects, objects and features of the invention will be apparent from the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1A is a graph showing that isoniazid ("INH") suppresses Nrf2-ARE activity in 3T3-L1 preadipocytes in a concentration-dependent manner under basal ("Veh") conditions and under Nrf2 activated conditions (conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes).

FIG.1B is a graph showing that 10 mM of isoniazid ("INH") suppresses Nrf2-ARE-dependent gene expression of glutamate-cysteine ligase catalytic subunit ("Gclc") in 3T3-L1 preadipocytes in a concentration-dependent manner under basal ("Veh") conditions and in conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG.1C is a graph showing that 10 mM of isoniazid ("INH") suppresses Nrf2-ARE-dependent gene expression of NAD(P)H dehydrogenase [quinone] 1 ("Nqo1") in 3T3-L1 preadipocytes in a concentration-dependent manner under basal ("Veh") conditions and in conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG.1D is a graph showing that 10 mM of isoniazid ("INH") suppresses Nrf2-ARE-dependent gene expression of Heme oxygenase ("Ho1") in 3T3-L1 preadipocytes in a concentration-dependent manner under basal ("Veh") conditions and in conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG 2A is a graph showing that isoniazid ("INH") inhibits Nrf2-ARE activity in human hepatocellular liver carcinoma HepG2 cells in a concentration-dependent manner under basal ("Veh") conditions and in conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG. 2B is a graph showing that isoniazid ("INH") suppresses Nrf2-ARE-dependent gene expression of Heme oxygenase ("Ho1") in HepG2 cells in a concentration-dependent manner under basal ("Veh") conditions and in conditions of iAs^{3+}-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG. 3 is a graph showing that ethionamide (ETH) suppresses Nrf2-ARE activity in HepG2 cells in a concentration-dependent manner under basal ("Vehicle") conditions and under Nrf2 activated conditions (iAs^{3+}).

FIG.4A is a graph showing that ethionamide (ETH) suppresses Nrf2-ARE-dependent gene expression of Heme oxygenase ("HO1") in THP-1 cells in a concentration-dependent manner under basal ("Veh") conditions and in conditions of arsenic trioxide (As_2O_3)-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG.4B is a graph showing that ethionamide (ETH) suppresses Nrf2-ARE-dependent gene expression of glutamate-cysteine ligase catalytic subunit ("GCLM") in THP-1 cells in a concentration-dependent manner under basal ("Veh") conditions and in conditions of arsenic trioxide (As_2O_3)-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG.4C is a graph showing that ethionamide (ETH) suppresses Nrf2-ARE-dependent gene expression of sulfiredoxin ("SRX") in THP-1 cells in a concentration-dependent manner under basal ("Veh") conditions and in conditions of arsenic trioxide (As_2O_3)-induced mRNA expression of Nrf2-ARE-dependent genes.

FIG. 5 is an illustration of chemical structures of compounds identified as Nrf2 inhibitors according to the invention.

FIG. 6 is a graph showing that isoniazid (INH) can sensitize cancer cells (as represented by THP-1 cells) to treatment and cell death mediated by a chemotherapeutic agent (as represented by arsenic trioxide, As_2O_3).

FIG. 7 is a graph showing that ethionamide (ETH) can sensitize cancer cells to treatment and cell death by a chemotherapeutic agent (as represented by arsenic trioxide, As_2O_3). FIG. 7A is a graph showing cell viability after 24 hours of treatment with ETH in concentrations of 1 mM and 2 mM, and as compared to an assay control (without ETH). FIG. 7B is a graph showing cell viability after 48 hours of treatment with ETH in concentrations of 1mM and 2 mM, and as compared to an assay control (without ETH). FIG. 7C is a graph showing cell viability after 48 hours of treatment with ETH in concentrations ranging from 0.1 mM to 1 mM, and as compared to an assay control (without ETH).

FIG. 8 is a graph showing that an Nrf2 inhibitor of the invention can sensitize cancer cells to treatment and cell death by a chemotherapeutic agent. FIG. 8A is a graph showing ETH concentration-dependently increases As_2O_3-induced cytotoxicity in human leukemic U937 cells.

FIG. 8B is a graph showing ETH concentration-dependently increases As_2O_3-induced cytotoxicity in human lung carcinoma A549 cells. FIG. 8C is a graph showing ETH concentration-dependently increases As_2O_3-induced cytotoxicity in and hepatocarcinoma HepG2 cells.

FIG. 9 is a graph showing that the sensitization effect of an Nrf2 inhibitor on cancer cell treatment with a chemotherapeutic agent is dependent on Nrf2 expression. FIG. 9A is a graph showing the effect of ETH on As_2O_3-induced cytotoxicity in THP-1 cells containing a negative control shRNA. FIG. 9B is a graph showing the effect of ETH on As_2O_3-induced cytotoxicity in THP-1 cells containing shRNA against human Nrf2 that causes stable knockdown of Nrf2.

DETAILED DESCRIPTION OF THE INVENTION

While the terms used in the description of the invention are believed to be well understood by one of ordinary skill in the pharmaceutical arts, definitions, where provided herein, are set forth to facilitate description of the invention, and to provide illustrative examples for use of the terms.

As used herein, the terms “a”, “an”, and “the” mean “one or more”, unless the singular is expressly specified (e.g., singular is expressly specified, for example, in the phrase “a single formulation”).

As used herein, “constitutive activation”, in relation to Nrf2, means an occurrence of abnormally elevated levels of Nrf2 in a cell, or level of Nrf2 activity in a cell, greater than that in a cell in which regulation of Nrf2 activity is not disrupted or dysregulated. Constitutive activation of Nrf2 activity can result from dysregulation or disruption of regulation of Nrf2 activity. For purposes of illustration, constitutive activation may be a level and or period of activation that is an increase of at least about at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or at least 10-fold as compared to a cell in which regulation of Nrf2 activity is not disrupted or dysregulated. As an example, using methods known to those skilled in the art, one can determine if Nrf2 is constitutively active in a tumor. One method involves the use of immunohistochemistry or immunostaining with anti-Nrf2 antibody on tumor tissue. In these methods, constitutive activation of Nrf2 is characterized by prominent nuclear accumulation of Nrf2 protein in tumor cells. Another method involves quantifying the amount of Nrf2 mRNA in the cytoplasm of tumor cells, using methods and primers known to those skilled in the art, in cases where increased transcription of the Nrf2 gene underlies constitutive activation.

As used herein, the term “dysregulation”, in the context of Nrf2 activity, refers to disruption of a regulatory mechanism or pathway or protein that normally controls the level of Nrf2 activity in a cell such that Nrf2 is constitutively activated as compared to a cell in which such dysregulation is absent. As noted above, dysregulation of Nrf2 activity occurs by means including, but not limited, to somatic mutations in the Keap1 gene or Nrf2 gene; disease associated metabolite regulation of Keap1 or Nrf2; disease-dependent signaling resulting in upregulation of Nrf2 gene transcription; accumulation of disease-related proteins that disrupt Keap1 regulation of Nrf2 activity; and hypermethylation at the promoter region of the Keap1 gene.

The terms "first" and “second” are used herein for purposes of distinguishing between two compounds, or between two compositions, as will be clearer from the description.

The phrase “medically effective amount” means an amount of a composition or compound that treats the particular disease, condition or disorder; ameliorates, relieves, or decreases one or more symptoms associated with the particular disease, condition or disorder; delays or prevents the onset of symptoms of, or a pathological process associated, with the particular disease, condition or disorder; or sensitizes cancer cells to additional treatment; as described herein in more detail.

The phrase “sensitizing effective amount” means an amount or dose of a composition or compound that produces a sensitizing effect, in solid nonlymphoid tumor cells having constitutive activation of Nrf2 or virally infected cells having constitutive activation of Nrf2, to a therapeutic agent used in treatment of such cells (e.g., cancer treatment, or a treatment modality used in cancer treatment; or antiviral therapy).

The term “pharmaceutically acceptable carrier” is used herein to mean any compound or composition or carrier medium useful in any one or more of administration, delivery, storage, stability of a composition or compound described herein. These carriers are known in the art to include, but are not limited to, a diluent, water, saline, suitable vehicle (e.g., liposome, microparticle, nanoparticle, emulsion, capsule), buffer, medical parenteral vehicle, excipient, aqueous solution, suspension, solvent, emulsions, detergent, chelating agent, solubilizing agent, salt, colorant, polymer, hydrogel, surfactant, emulsifier, adjuvant, filler, preservative, stabilizer, oil, binder, disintegrant, absorbant, flavor agent, and the like as broadly known in the pharmaceutical art.

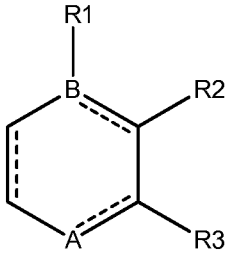
The term “solid nonlymphoid tumor” is used herein, for purposes of the specification and claims, to mean any primary tumor of ductal epithelial cell origin, including head and neck tumors or tumors originating in an organ or gland such as liver, lung, brain, thyroid,

adrenal gland, breast, colon, bladder, gall bladder, pancreas, stomach, prostate, testes, gastrointestinal tract, or reproductive tract (cervix, ovaries, endometrium etc.), or metastases thereof. For the purposes of the present invention, "solid non-lymphoid tumor" also includes melanoma.

The terms "treat", "treats", or "treating" , as used herein, embrace one or more of preventative (prophylactically) or therapeutically (palliative).

The term "Nrf2 inhibitor" is a compound selected from the group consisting of compounds represented by Formulas I-II, and an antitubercular agent (e.g., quinolone such as sparfloxacin, a rifamycin (i.e., rifampin, rifapentine and rifabutin), or ethambutol dihydrochloride); or a pharmaceutically acceptable salt or solvate thereof; wherein the compound has the ability to downregulate or inhibit Nrf2 activity, including inhibiting Nrf2 transactivation of genes downstream from Nrf2, particularly genes having an ARE in their promoter (hence, suppress Nrf2-ARE activity). The ability to inhibit or suppress Nrf2-ARE activity can be demonstrated in a number of ways known in the art. For example, ARE-reporter assays, such as the ARE-luciferase assay described herein, are assays validated to model cellular ARE- driven gene expression by Nrf2, and can be used to demonstrate Nrf2-ARE-inhibitory activity using methods known in the art. A preferred Nrf2 inhibitor may be used to the exclusion of an Nrf2 inhibitor other than the preferred Nrf2 inhibitor. The Nrf2 inhibitor may be selected from a naturally occurring compound or a non-naturally occurring compound. For example, in one aspect, when an Nrf2 inhibitor is administered by itself (e.g., is the sole therapeutic agent in a pharmaceutical composition, and is not combined with at least one cancer-treating agent and/or a second Nrf2 inhibitor, to produce a pharmaceutical composition), such Nrf2 inhibitor may be selected from a non-naturally occurring compound. In another example, in one aspect, when an Nrf2 inhibitor is administered in combination with one or more of a second Nrf2 inhibitor or a cancer-treating agent (e.g., administered as a pharmaceutical composition comprising a combination of the Nrf2 inhibitor and one or more of a second Nrf2 inhibitor and a cancer-treating agent), such Nrf2 inhibitor may be selected from a non-naturally occurring compound or a naturally occurring compound. As shown herein, in one example, an Nrf2 inhibitor comprises a heterocyclic compound having a hydrazide moiety or carboxamide moiety (typically, as a side chain), and is selected from a compound represented by Formulas I-II.

In one aspect of the invention, an Nrf2 inhibitor is selected from compounds represented by Formula I, and a pharmaceutically acceptable salt thereof.



Formula I

wherein:

A is N or C;

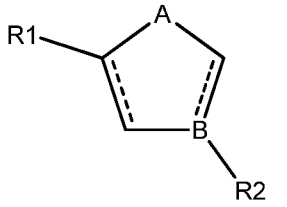
B is N or C;

R1 or R2 or R3 are each independently selected from H, (C_1-C_6)alkyl, $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, $CHCHCONHNH_2$, or $COCH_3$;

wherein at least one of R1, R2, and R3 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; and

the dashed lines represent optional double bonds.

In one aspect of the invention, an Nrf2 inhibitor is selected from compounds represented by Formula II, and a pharmaceutically acceptable salt thereof.



Formula II

wherein:

A is O or N;

B is N or C;

R1 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$;

R2 is absent if B is NH;

if B is C, R2 is absent or selected from CH_3, CH_2CH_3, NH_2, or $NHNH_2$; and

the dashed lines represent optional double bonds.

The term “non-naturally occurring” used in reference to a compound means that the compound is not known to exist in nature or that does not exist in nature. The term “naturally occurring” when used in connection with compounds refers to a compound which is found in nature. It is apparent to those skilled in the art that a naturally occurring

compound can be modified or engineered by a human or by an engineered organism to be structurally or chemical different to form a non-naturally occurring compound.

The terms “purified” or “isolated” for a compound or composition refers to the physical state of the compound or composition following isolation from a synthetic process or purification step described herein or well known to those in the art, and in sufficient purity to be characterizable by standard analytical methods described herein or well known in the art.

The terms “salt” or pharmaceutically acceptable salt”, as used herein, refers to inorganic or organic salts of a compound. These salts can be prepared, for example, by reacting a compound comprising an Nrf2 inhibitor with an amount of acid or base, such as an equivalent amount, and in a medium such as one in which the salt formed then precipitates, or in an aqueous medium followed by lyophilization. Representative salts include bisulfate, sulfate, benzene sulfonate, camphorsulfonate, laurylsulphonate, methanesulfonate, toluenesulfonate, naphthalenesulformate, acetate, trifluoracetate, benzoate, borate, butyrate, citrate, formate, fumarate, hydorbromide, hydrochloride, hydroiodide, lactate, laurate, maleate, malonate, mesylate, nitrate, oxalate, phosphate, hexafluorophosphate, propionate, salicylate, stearate, succinate, tartrate, thiocyanate, and the like. The salts may include base salts based on the alkali and alkaline earth metals, such as calcium, sodium, lithium, magnesium, and potassium; or with organic bases such as with organic amines (e.g., dicyclohexylamine, t-butyl amine, methylamine, dimethylamine, triethylamine, ethylamine, procaine, morpholine, N-methylpiperidine, dibenzylamine, and the like); or as an ammonium salt. The compounds disclosed herein may exist in a solvated form or unsolvated form. Solvates of a compound disclosed in the invention may be formed in the synthetic process in which the compound becomes physically associated with one or more solvent molecules (e.g., such as by ionic and/or covalent bonding) or, optionally, may be converted to a solvate such as by dissolving the compound in desired amounts of a solvent of choice (e.g., organic solvent, water, or mixtures thereof) in forming a solution, heating the solution to a temperature higher that ambient temperature, and cooling the solution at a rate sufficient to form crystals of the solvate, which may then be further isolated using methods known the art. Examples of suitable solvents include methanolates, ethanolates, hydrates (where the solvent molecule is water), and the like.

The compounds of Formulas I-II may contain asymmetric or chiral centers, and thus exist in different stereoisomeric forms. All stereoisomers (e.g., geometric isomers, optical

isomers, and the like), enantiomeric forms, diastereomeric forms, tautomeric forms, positional isomers, of the compounds disclosed in the invention are embraced within the scope of the invention. A first conformational form of a compound can be separated from a second and different conformational form of the compound using methods well known in the chemical arts such as by chromatography, crystallization, and methods of synthesis which selectively result in a particular desired conformational form.

A medically effective amount or sensitizing effective amount of a compound of the invention, or a composition comprising a compound of the invention, will depend on such factors as the mode of administration, the formulation for administration, disease to be modulated, the size and health of the individual to receive such a composition, and other factors which can be taken into consideration by a medical practitioner whom is skilled in the art of determining appropriate dosages for treatment. An amount of compound of the invention in a composition to be administered may vary from 0.01 milligrams to about 500 milligrams, and more typically from about 1 milligram per day to about 200 milligram per day. In another example, the amount of a compound according to the invention to be administered is an amount which results in a blood concentration of from about 0.01mM to 50 mM in an individual receiving the compound. One skilled in the art can apply known principles and models of drug delivery and pharmacokinetics to ascertain a likely range of dosages to be tested in preclinical and clinical studies for determining a medically effective amount of a compound of the invention. A pharmaceutically acceptable carrier, used in a composition of the invention, may facilitate one or more of storage, stability, administration, and delivery, of the composition. The carrier may be particulate, so that the composition may be in, for example, powder or solid form. The carrier may be in a semi-solid, gel, or liquid formula, so that the composition may be ingested, injected, applied, or otherwise administered. The carrier may be gaseous, so that the composition may be inhaled.

For oral administration of a composition containing a compound of the invention, suitable formulations may be presented in the form of tablets, caplets, capsules, and the like, in which typically the compound of the invention may be present in a predetermined amount as a powder, granules, solution, or suspension as the sole active agent, or in combination with an additional one or more pharmaceutical agents. As known in the art, such oral formulations typically involve one or more of a binder (e.g., syrup, sorbitol, gum, corn starch, gelatin, acacia), a filler (e.g., lactose, sugar, starch, calcium phosphate), an excipient (e.g., dicalcium phosphate), a disintegrating agent (e.g., vegetable starch,

alginic acid), a lubricant (e.g., magnesium stearate), a flavoring agent (sweetening agent, natural or artificial flavors). Such oral formulations may be coated or uncoated to modify their disintegration and/or absorption. Coating may be performed using conventional coating agents and methods known in the art.

The mode of administration of a compound or composition of the invention to an individual (such as a human) in need of such composition or compound may be any mode known in the art to be suitable for delivering a pharmaceutical composition, and particularly suitable for treating solid nonlymphoid tumor or a viral infection in which there is constitutive activation of Nrf2, and may include but is not limited to, intravenously, intraperitoneally, orally, subcutaneously, intramuscularly, intranasally, transdermally, by perfusion, and by peristaltic techniques. The compositions of the invention may also be combined with other therapies, such as one or more additional pharmaceutical agents, to treat such disease having constitutive activation of Nrf2. Such combination therapy may be administered in concurrently, sequentially, or in regimen alternating between one or more doses of the composition of the invention and at least one additional therapy (one or more of therapeutic agent or treatment modality). Such combination therapies may include administering an Nrf2 inhibitor of the invention with one or more additional therapeutic agents, for treating cancer or viral infection in which there is constitutive activation of Nrf2. The structure such additional therapeutic agents, and for an Nrf2 inhibitor of the invention, and their generic or trademark names, are readily available to those skilled in the art, such as from the standard compendium of drugs (e.g., The Merck Index) or from the applicable pharmaceutical company's web site, as well as dosages applicable for treatment (see also The Physician's Desk Reference). Alternatively, the doses and dosage regimen of an additional therapeutic agent, used in conjunction with an Nrf2 inhibitor of the invention in combination therapy, can be determined by a physician, taking into account the medical literature, the health, age and sex of the patient, the disease or condition or disorder to be treated, the mode of administration and dosing schedule of the therapeutic agent, and other relevant considerations. Generally, dosages of such agents can range from about 0.1 mg to 1000 mg per day, with more specific dosages dependent on the aforementioned factors.

Accordingly, provided herein is a pharmaceutical composition or medicament comprising a sensitizing effective amount of an Nrf2 inhibitor of the invention, in combination with a medically effective amount of one or more therapeutic agents for treating solid nonlymphoid tumors or viral disease, treatment modalities directed towards

solid nonlymphoid tumors or viral disease, or a combination thereof; and optionally further comprising a pharmaceutically acceptable carrier. Also provided herein is a pharmaceutical composition or medicament comprising a sensitizing effective amount of an Nrf2 inhibitor of the invention, and a pharmaceutically acceptable carrier.

EXAMPLE 1

In this Example, illustrated is the discovery that Nrf2-ARE signaling protects various cancer cells against diverse chemotherapeutic agents, and that treating solid nonlymphoid tumor cells using a sensitizing amount of an Nrf2 inhibitor of the invention inhibits Nrf2 activity and sensitizes the treated tumor cells to anticancer treatment, including by one or more chemotherapeutic agents, including etoposide, doxorubicin, and As_2O_3.

The present invention identified a novel class of compounds with strong inhibitory effects on NRF2 activity including downstream genes (ARE activity). These compounds include a panel of antitubercular agents, such as isoniazid, ethionamide, ethambutol dihydrochloride, rifampicin, ethionamide, and sparfloxacin; and other compounds represented by Formula I or Formula II, particularly heterocyclic compounds having a hydrazide side chain or carboxamide side chain, including 4-aminobenzoic hydrazide, aminopyrazine, cyclohexanecarboxamide, 2-furoic hydrazide, phenylhydrazine, phenylacetic hydrazide, pyrazinecarboxamide, p-toluic hydrazide, 4-(aminomethyl)piperidine; isonicotinamide, and 2-amino- isonicotinamide (see, e.g., Table 1). These compounds decrease ARE-luciferase activity, in a concentration-dependent manner in treated cells, under basal and arsenite-treated conditions. These newly identified NRF2 inhibitors suppress ARE activity and induction of ARE-driven gene expression, and sensitize treated cells to therapeutic agent-induced cancer cell death by inhibiting Nrf2 activity.

To identify novel chemical modulators of Nrf2 activity, performed was a series of chemical screening using an assay in which an ARE-luciferase reporter is stably expressed in cells in which there is confirmed constitutive activation of Nrf2 activity. These cells include mouse preadipocyte 3T3-L1 cell line; mouse insulinoma MIN6 cell line; human keratinocyte HaCaT cell line; and human hepatocellular cancer cell line, HepG2 cells. A commercially available ARE-luciferase reporter, in ready-to-transduce lentiviral particles, was used for assessing when the Nrf2 pathway is activated or inhibited by a drug or chemical, via detection of any modulation of luciferase reporter activity which

can then be measured quantitatively. Lentiviral transduction of 3T3-L1, HaCaT and HepG2 cells was performed based on manufacturer's protocol. Briefly, 24 hours before transduction, the cells to be transduced were plated in 6-well plates at 40–50% confluency in complete cell culture medium. The following day, hexadimethrine bromide, a transduction enhancer, was added to each well at a concentration of 8 µg/ml, and viral particles were added to each well at a concentration of 2 x 10^5 transducing units/ml. After overnight incubation, medium containing viral particles was removed and replaced with fresh medium containing 2 µg/ml puromycin. Cells were grown to ~90% confluence and sub-cultured in medium containing puromycin. The 3T3-L1 cells, HaCaT cells, MIN6, and HepG2 cells, with stable expression of ARE-luciferase reporter, were used to assess or identify ARE activators and inhibitors, and more particularly, Nrf2 inhibitors.

Briefly, chemicals were individually added to the cells, and incubated for 24 hours under basal conditions (no added Nrf2 activator), or with tBHQ-treated or sodium arsenite-treated cells (6 hour treatment, 5-10 µM iAs^{3+} or 50 µM tBHQ), a known activator of Nrf2 activity, and assessed for luciferase activity, as compared to assay controls. The luciferase activity was measured by a commercially available luciferase reporter assay system according to the manufacturer's protocol. The luciferase activity was normalized to protein content or cell viability. To confirm an inhibitory effect on Nrf2-ARE activity as observed by a decrease in luciferase activity (as compared to the assay control), the chemical was also tested for its ability to inhibit, in a concentration dependent manner, cell expression of multiple ARE-dependent genes, including one or more of *HO1* (*Heme oxygenase*), *GCLC* (*Glutamate—cysteine ligase catalytic subunit, also known as GCLM*), *Nqo1 (NAD(P)H dehydrogenase [quinone])* and *SRX* (*sulfiredoxin)* by using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) and commercially available primers. An inhibitory effect on expression of multiple ARE-dependent genes was used as an indication of inhibition of Nrf2 activity.

First, cytotoxicity of isoniazid (a widely used antitubercular drug) in 3T3-L1 cells and HepG2 cells was determined by exposing the cells to various concentrations of isoniazid, ranging from 1 mM to 200 mM, for 24 hours, and subsequently determining cell viability by a commercially available MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. From this determination, non-cytotoxic concentrations of isoniazid ranging from 1 mM to 50 mM were tested, as was an equal volume of cell culture without isoniazid ("Vehicle") for comparison, in the ARE-luciferase reporter assay. Isoniazid exhibited a concentration-dependent inhibitory effect on ARE-

luciferase activity in 3T3-L1 cells (Fig. 1A) and HepG2 cells (Fig. 2A) under basal (no stressor) and in the presence of an Nrf2 activator (iAs^{3+}-treated). The Nrf2 inhibitory effect was also observed in HaCaT cells stably expressing the same ARE-luciferase reporter assay. The inhibitory activity was confirmed by decreased expression of multiple ARE-dependent genes, including *GCLC* (FIG. 1B), *NQO1* (FIG. 1C) and *HO1* (FIG. 1 D, and FIG. 2B) under basal conditions and stressed conditions (6 hour treatment with Nrf2 activator *tert*-butylhydroquinone (tBHQ) or iAs^{3+}).

Using these methods and the ARE-luciferase reporter assay described herein, another antitubercular agent ethionamide (ETH), in non-cytotoxic concentrations, also displayed a concentration-dependent inhibitory effect on ARE-luciferase activity under basal conditions and iAs^{3+}-treated conditions in HepG2 cells (FIG. 3). Confirmation of the Nrf2 inhibitory effect mediated by was shown by the ability of ETH treatment to significantly decrease the expression of multiple ARE-dependent genes, including *HO1* (FIG. 4A), *GCLM* (FIG. 4B), and sulfiredoxin (*SRX,* FIG. 4C) in THP-1 cells under basal conditions and upon treatment with a known Nrf2 activator (arsenic trioxide or (As_2O_3)).

By using the same methods, surprisingly it was discovered that antitubercular agents, including ethambutol dihydrochloride, ethionamide, rifampicin, and sparfloxacin, are Nrf2 inhibitors as demonstrated by the concentration-dependent inhibitory effect on ARE-luciferase activity under basal and iAs^{3+}-treated or tBHQ-treated conditions (Table 1). In addition, a number of other compounds represented by either Formula I or Formula II, particularly heterocyclic compounds having a hydrazide side chain or carboxamide side chain, including 4-aminobenzoic hydrazide, aminopyrazine, 2-furoic hydrazide, cyclohexanecarboxamide, phenylhydrazine, phenylacetic hydrazide, pyrazinecarboxamide, and p-toluic hydrazide, were discovered to exhibit Nrf2 inhibitory activity as demonstrated by the concentration-dependent inhibitory effect on ARE-luciferase activity under basal conditions and iAs^{3+}-treated or tBHQ-treated conditions (Table 1). As apparent from FIG. 5 showing the chemical structure of newly discovered Nrf2 inhibitors, many of these compounds are represented by Formula I or Formula II, particularly heterocyclic compounds having a hydrazide side chain or carboxamide side chain, suggesting a structure-function relationship between such chemical representation and the ability to inhibit Nrf2-ARE activity.

Table 1

Compound	CAS number	Concentration tested & showing inhibition on basal ARE activity	Concentration tested & showing inhibition on inducible ARE activity
4-Aminobenzoic hydrazide	5351-17-7	1 mM	0.1-1 mM
Aminopyrazine	5049-61-6	1.0-10 mM	1.0-10 mM
Cyclohexanecarboxamide	1122-56-1	10 mM	0.1-10 mM
Ethambutol dihydrochloride	1070-11-7	10 mM	0.1-10 mM
Ethionamide	536-33-4	0.1-2 mM	0.1-2 mM
2-Furoic hydrazide	3326-71-4	10 mM	0.1-10 mM
Phenylhydrazine	100-63-0	1-10 mM	0.1-10 mM
Isoniazid	54-85-3	1-50 mM	1-50 mM
Isonicotinamide	1453-82-3	1-50 mM	1-50 mM
2- Amino-isonicotinamide	13538-42-6	1-50 mM	1-50 mM
Phenylacetic hydrazide	937-39-3	10 mM	1-10 mM
Pyrazinecarboxamide (including pyrazinamide)	98-96-4	1-10 mM	1-10 mM
Rifampicin	13292-46-1	0.5 mM	0.1-0.5 mM
Sparfloxacin	110871-86-8	0.1-1 mM	0.1-1 mM
p-Toluic hydrazide	3619-22-5	10 mM	0.1-1 mM
4-(Aminomethyl)piperidine	7144-05-0	---	0.1-1 mM

EXAMPLE 2

In this Example, illustrated is use of an Nrf2 inhibitor of the invention in combination with an additional therapeutic agent, in a medically effective amount, and more particularly in a sensitizing effective amount, to treat tumor cells having constitutive Nrf2 activation. In one demonstration of combination therapy, an Nrf2 inhibitor of the invention was used to sensitize tumor cells to arsenic trioxide (As_2O_3). As_2O_3 is an anti-cancer drug approved by the U.S. Food and Drug Administration to specifically treat acute promyleocytic leukemia; however, many tumor cells from solid nonlymphoid tumors, are relatively resistant to As_2O_3 therapy, which may be associated with their enhanced Nrf2 activity.

In this illustration, in each well of a 96 well plate was cultured 1×10^4 of cells. Different wells of the plate were treated with either As_2O_3 in concentration range depending on the sensitivity/resistance of the cancer cells to the anti-cancer drug (from 0.25 μM to 2.5 μM for THP-1 cancer cells; 1-16 μM for human leukemic U937 cells; 5-80 μM for human lung carcinoma A549 cells; and 0.25-4 μM for human hepatocellular cancer HepG2 cells); or with As_2O_3 in combination with an Nrf2 inhibitor of the invention (exemplified by INH, and ETH) in a concentration range of from 0.1 mM to 10 mM; or an

equal amount of cell culture medium (as an assay negative control). After 48 hours from initiation of As_2O_3 treatment, cell viability was then assessed by MTT assay.

As evident from FIGs. 6, 7 A-C and 8 A-C, an Nrf2 inhibitor of the invention, at concentrations that are non-cytotoxic but capable of significantly suppressing Nrf2-ARE activity, markedly enhanced the efficacy of (by sensitizing tumor cells to the activity of) a chemotherapeutic agent in inducing cytotoxicity of a wide variety of cancer cells, and particularly in cancer cells which have constitutive Nrf2 activation. As shown in FIG. 6, the Nrf2 inhibitor, isoniazid (INH), significantly sensitized THP-1 cells to treatment by arsenic trioxide (▲, and ▼) as compared to the control (○). As shown by FIGs. 7A, B, and C, the Nrf2 inhibitor, ethionamide (ETH), significantly sensitized THP-1 cells (▲,▼, and ■) to treatment by arsenic trioxide at various time periods following treatment (24 hours, FIG. 7A; 48 hours, FIGs. 7B and 7C) and at various concentrations of ETH and arsenic trioxide, as compared to the controls (○). ETH treatment also significantly sensitized human acute monocytic leukemia U937 cells (Fig. 8A, ●, and ▼) to treatment by arsenic trioxide, as compared to the controls (○).

Recent studies have demonstrated that human non-small cell lung carcinoma A549 cells have constitutive activation of NRF2, and hence, are resistant to a variety of chemotherapeutic drugs, including As_2O_3, etoposide and doxorubicin. Likewise, it has been demonstrated that there is constitutive activation of Nrf2 in HepG2 cells, a human hepatocellular carcinoma cell line; and that HepG2 cells are resistant to a variety of chemotherapeutic drugs. Both A549 cells and HepG2 cells are representative of cells of solid nonlymphoid tumors. As shown here, ETH treatment significantly sensitized A549 cells (Fig. 8B, ●, and ▼) and HepG2 cells (Fig. 8C, ●) to treatment by arsenic trioxide, as compared to the controls (○).

EXAMPLE 3

In this Example, shown is that the Nrf2 inhibitors of the invention do not reduce the mRNA and/or protein expression of NRF2, but rather suppress Nrf2 activity and induction of ARE-driven gene expression. Evaluated was the ability to silence Nrf2 using shRNA against human Nrf2. With shRNA against human Nrf2, and as compared to a scrambled nontarget negative control shRNA (demonstrated not to knockdown Nrf2), demonstrated was at least 60% stable knockdown of Nrf2 mRNA expression in human leukemic THP-1 cells, and statistically significant knockdown of ARE-dependent genes needing Nrf2 transcriptional activation (e.g., Nqo1, and Ho1). Having established stable

knockdown of Nrf2 mRNA expression, use of Nrf2 knockdown was applied to show that Nrf2 inhibitors suppress Nrf2-ARE activity. In this Example, the combination therapy comprising treatment of cancer cells with an Nrf2 inhibitor of the invention and an additional therapeutic agent was performed on various human cancers, as described in Example 2 herein, except that prior to treatment, the cancer cells were transduced with either a scrambled nontarget negative control shRNA, or shRNA against human Nrf2. As shown in FIG. 9A, in the presence of scrambled nontarget negative control shRNA, the Nrf2 inhibitor of the invention (as exemplified by ETH) significantly sensitized human cancer cells (Fig. 9A, ●) to treatment by arsenic trioxide, as compared to the controls (○). However, as shown in FIG. 9B, the sensitization of human cancer cells to treatment by arsenic trioxide by the Nrf2 inhibitor (Fig. 9B, ●) was lost in the presence of stable knockdown of Nrf2 mRNA expression as compared to the presence of Nrf2 mRNA expression ((Fig. 9A, ●). Thus, the role of the Nrf2 inhibitor in sensitization of cells, having constitutive Nrf2 activation, to treatment by a therapeutic agent is highly dependent on Nrf2.

EXAMPLE 4

In this Example, illustrated is the use of an Nrf2 inhibitor of the invention, or a pharmaceutical composition comprising such compound, in a sensitizing effective amount to enhance effectiveness of anticancer treatment of solid nonlymphoid tumors characterized by constitutive Nrf2 activation. Provided is a method of improving or enhancing the effectiveness of concomitant anticancer treatment of cancer comprising solid nonlymphoid tumors characterized by constitutive Nrf2 activity, the method comprising administering to an individual in need thereof a sensitizing effective amount of an Nrf2 inhibitor of the invention in combination with the anticancer treatment. A sensitizing effective amount of an Nrf2 inhibitor is a dose that sensitizes tumor cells, characterized by constitutive activation of Nrf2, to a therapeutic agent or cancer treatment modality administered in conjunction with the Nrf2 inhibitor such that the anticancer effect of the therapeutic agent or cancer treatment modality is enhanced. In that regard, tumor cells treated with the Nrf2 inhibitor become more susceptible to the anticancer treatment as compared to the absence of treatment with the Nrf2 inhibitor. Provided is use of an Nrf2 inhibitor compound of the invention (or a pharmaceutical composition comprising such compound) in an amount effective to sensitize cells (solid nonlymphoid tumor cells or virally infected cells) characterized by constitutive activation of Nrf2. The compound is

administered to an individual (a mammal, such as a human) selected for treatment because the individual has one or more of solid nonlymphoid tumor or a viral disease characterized by constitutive activation of Nrf2 (as an individual in need thereof). The method may comprise selecting an individual having such disease to be treated or to whom the composition is to be administered (e.g., to an individual in need thereof), followed by administration of the compound or composition of the invention. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, or a pharmaceutically acceptable salt of the Nrf2 inhibitor. Also provided is an Nrf2 inhibitor of the invention, used in the manufacture of a medicament for treating a disease having constitutive activation of Nrf2 that is selected from solid nonlymphoid tumors, and viral infections.

In one aspect of the invention, one or more compounds which inhibit Nrf2 activity, when used to treat cancer, may be used in combination with one or more additional therapeutic agents, with the potential for synergistically enhancing one or more of apoptosis of cancer cells, growth inhibition of cancer cells, or reducing the systemic toxicity or increasing the efficacy of the one or more additional therapeutic agents in the combination (e.g., by sensitizing the cancer cells to the one or more additional therapeutic agents, potentially shortening the period of treatment or reducing the amount of therapeutic agent needed for a therapeutic effect than if administered without an Nrf2 inhibitor). Examples of therapeutic agents which can be used in combination with an Nrf2 inhibitor of the invention for treatment of cancer include ("cancer-treating agent"), but are not limited to, alkylating agents (e.g., nitrogen mustards, such as mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and melphalan; nitrosoureas, such as streptozocin, carmustine, and lomustine; alkyl sulfonates, such as busulfan; triazines, such as dacarbazine and temozolomide; ethylenimines, such as thiotepa and altretamine; platinum-based drugs, such as cisplatin, carboplatin, and oxalaplatin; antimetabolites (e.g., 5-fluorouracil, 6-mercaptopurine, capecitabine, cladribine, clofarabine, cytarabine, floxuridine, fludarabine, gemcitabine, hydroxyurea, methotrexate, pemetrexed, pentostatin, and thioguanine); anti-tumor antibiotics (e.g., anthracyclines, such as daunorubicin, doxorubicin, epirubicin, and idarubicin; and actinomycin-D, bleomycin, mitomycin-C, and mitoxantrone); topoisomerase inhibitors (e.g., topoisomerase I inhibitors such as topotecan and irinotecan; topoisomerase II inhibitors, such as etoposide, teniposide, and mitoxantrone); mitotic inhibitors (e.g., taxanes, such as paclitaxel and docetaxel; epothilones such as ixabepilone; Vinca alkaloids, such as

vinblastine, vincristine, and vinorelbine; and estramustine); corticosteroids (e.g., prednisone, methylprednisolone, and dexamethasone); proteosome inhibitors (e.g., bortezomib); immunotherapeutics (e.g., imatinib, gefitinib, sunitinib, rituximab, alemtuzumab, trastuzumab, bevacizumab, and bortezomib); differentiating agents (e.g., retinoids, tretinoin, and bexarotene); and hormonal agents (e.g., anti-estrogens, such as fulvestrant, tamoxifen, and toremifene; aromatase inhibitors, such as anastrozole, exemestane, and letrozole; progestins, such as megestrol acetate; estrogens; anti-androgens, such as bicalutamide, flutamide, and nilutamde; gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH) agonists or analogs, such as leuprolide and goserelin). Also provided is a pharmaceutical composition comprising a medically or sensitizing effective amount of an Nrf2 inhibitor of the present invention, a medically effective amount of a cancer-treating agent, and a pharmaceutically acceptable carrier. Optionally, the Nrf2 inhibitor of the invention, or the combination therapy comprising the Nrf2 inhibitor and at least one additional therapeutic agent, may be used in combination with a treatment modality for treating cancer.

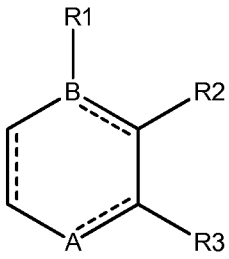
In another example, one or more compounds of the invention as an Nrf2 inhibitor, is used to sensitize a viral infection to treatment by antiviral agents. Sensitizing the cells to antiviral treatment is characterized by enhancing the effectiveness of antiviral treatment; e.g., rendering cells treated with the Nrf2 inhibitor more susceptible to the anticancer treatment as compared to the absence of treatment with the Nrf2 inhibitor. Sensitization of the treated cells may be observed by one or more of inhibition of the progression of the disease, a reduction in the number of virally-infected cells, or amelioration of a pathological feature or symptom of the viral disease. Also provided is a pharmaceutical composition comprising a sensitizing effective amount of an Nrf2 inhibitor of the present invention, and a medically effective amount of a therapeutic agent comprising one or more antiviral agents in antiviral treatment directed towards a viral infection characterized by virally infected cells having constitutive activation of Nrf2; and may further comprise a pharmaceutically acceptable carrier. In one example, the Nrf2 inhibitor may be used in combination with one or more therapeutic agents for treating virally infected cells in an individual in need thereof. For example, when the viral infection comprises HBV infection, the Nrf2 inhibitor may be used in combination with one or more therapeutic agents for treating HBV infection that include, but are not limited to, interferon alpha-2b, peginterferon alfa-2a, lamivudine, adefovir, entecavir, telbivudine, tenofovir, and

Hepatitis B immune globulin. Also provided is a pharmaceutical composition comprising a sensitizing effective amount of an Nrf2 inhibitor of the present invention, a medically effective amount therapeutic agent for treating HBV infection, and a pharmaceutically acceptable carrier.

In another example, one or more compounds of the invention as an Nrf2 inhibitor, when used to treat CMV infection, may be used in combination with one or more therapeutic agents for treating CMV infection that include, valacyclovir, valganciclovir, ganciclovir, and cytomegalovirus immune globulin. Also provided is a pharmaceutical composition comprising a sensitizing effective amount of an Nrf2 inhibitor of the present invention, a medically effective amount of a therapeutic agent for treating CMV infection, and a pharmaceutically acceptable carrier. Optionally, the Nrf2 inhibitor of the invention, or the combination therapy comprising the Nrf2 inhibitor and at least one additional therapeutic agent, may be used in combination with a treatment modality for treating a viral disease such as HBV infection, CMV infection, Hepatitis C virus (HCV) infection, or other viral disease in which a virus induced constitutive activation of Nrf2. Another example in which a Nrf2 is constitutively active in a virus infection is Hepatitis C virus (HCV) infection. It has been shown that HCV proteins core, E1, E2, NS4B, and NS5A each independently activate the Nrf2/ARE pathway. In another example, one or more compounds of the invention as an Nrf2 inhibitor, when used to treat HCV infection, may be used in combination with one or more therapeutic agents for treating HCV infection that include but are not limited to, a protease inhibitor directed towards HCV (e.g., boceprevir, telaprevir, asunaprevir, and simeprevir), an NS5A replication complex inhibitor (e.g., daclatasvir), polymerase inhibitor (e.g., sofosbuvir), peginterferon-alfa, ribavirin, and a combination thereof. Also provided is a pharmaceutical composition comprising a sensitizing effective amount of an Nrf2 inhibitor of the present invention, a medically effective amount of a therapeutic agent for treating HCV infection, and a pharmaceutically acceptable carrier. Optionally, the Nrf2 inhibitor of the invention, or the combination therapy comprising the Nrf2 inhibitor and at least one additional therapeutic agent, may be used in combination with antiviral therapy directed towards treating the viral infection.

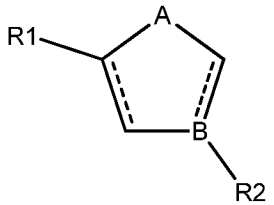
What is claimed is:

1. A pharmaceutical composition comprising an Nrf2 inhibitor selected from the group consisting of a compound represented by Formula I or Formula II, ethambutol dihydrochloride, rifamycin, an antitubercular quinolone, and a combination thereof; wherein the pharmaceutical composition is administered in combination with an anticancer treatment in an amount effective to sensitize solid nonlymphoid tumor to the anticancer treatment; wherein sensitizing to anticancer treatment is characterized by enhancing an anticancer effect of the anticancer treatment; wherein the solid nonlymphoid tumor is characterized by tumor cells having constitutive activation of Nrf2; wherein Nrf2 is comprised of an amino acid sequence comprising SEQ ID NO:1 including isoforms thereof; wherein the Nrf2 inhibitor is a non-naturally occurring compound when the pharmaceutical composition consists of the Nrf2 inhibitor as a sole therapeutic agent, and is either a naturally occurring compound or a non-naturally occurring compound when the pharmaceutical composition comprises the Nrf2 inhibitor in combination with one or more of a therapeutic agent or second Nrf2 inhibitor; with Formula I as



wherein A is N or C; B is N or C; R1 or R2 or R3 are each independently selected from H, (C_1-C_6)alkyl, $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, $CHCHCONHNH_2$, or $COCH_3$; wherein at least one of R1, R2, and R3 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof;

and with Formula II as



wherein A is O or N; B is N or C; R1 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; R2 is absent if B is NH; if B is

C, R2 is absent or selected from CH_3, CH_2CH_3, NH_2, or $NHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof.

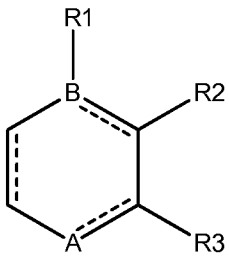
2. The pharmaceutical composition of claim 1, which is combined with anticancer treatment comprising one or more of chemotherapy, immunotherapy, and radiation therapy.

3. The pharmaceutical composition of claim 1, which contains a cancer-treating agent in a medically effective amount in combination with one or more Nrf2 inhibitors.

4. The pharmaceutical composition according to claim 1, which is administered concurrently, sequentially, or in a regimen of alternating dose, with the anticancer treatment.

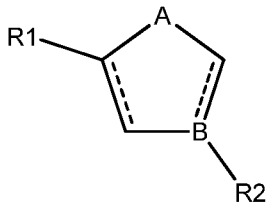
5. The pharmaceutical composition of claim 1, further comprising a pharmaceutically acceptable carrier

6. Use of an Nrf2 inhibitor in a sensitizing amount, in combination with anticancer treatment, to enhance effectiveness of anticancer treatment against solid nonlymphoid tumors characterized by constitutive activation of Nrf2; wherein Nrf2 is comprised of an amino acid sequence comprising SEQ ID NO:1 including isoforms thereof; wherein the Nrf2 inhibitor is (i) selected from the group consisting of compounds represented by Formula I or Formula II, ethambutol dihydrochloride, rifamycin, an antitubercular quinolone, and a combination thereof, (ii) a non-naturally occurring compound for administration by itself, and (iii) either a naturally occurring compound or a non-naturally occurring compound when combined together with one or more of a cancer-treating agent and a second Nrf2 inhibitor to form a pharmaceutical composition; with Formula I as



wherein A is N or C; B is N or C; R1 or R2 or R3 are each independently selected from H, (C_1-C_6)alkyl, $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, $CHCHCONHNH_2$, or $COCH_3$; wherein at least one of R1, R2, and R3 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof;

and with Formula II as



wherein A is O or N; B is N or C; R1 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; R2 is absent if B is NH; if B is C, R2 is absent or selected from CH_3, CH_2CH_3, NH_2, or $NHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof.

7. A method for enhancing the effectiveness of anticancer treatment directed towards solid nonlymphoid tumors characterized by constitutive activation of Nrf2, the method comprising administering to an individual in need thereof a sensitizing effective amount of an Nrf2 inhibitor in combination with the anticancer treatment; and wherein the Nrf2 inhibitor is (i) a non-naturally occurring compound when administered as a sole therapeutic agent, and (ii) either a naturally occurring compound or a non-naturally occurring compound when administered as a pharmaceutical composition comprising the Nrf2 inhibitor combined with one or more of a therapeutic agent or second Nrf2 inhibitor.

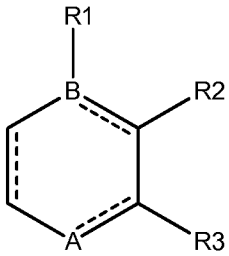
8. The method of claim 7, wherein the anticancer treatment comprises one or more of chemotherapy, immunotherapy, and radiation therapy.

9. The method of claim 7, wherein Nrf2 inhibitor is administered as a pharmaceutical composition which additionally includes a cancer-treating agent in a medically effective amount.

10. The method of claim 7, wherein the Nrf2 inhibitor is administered concurrently, sequentially, or in a regimen of alternating dose, with the anticancer treatment.

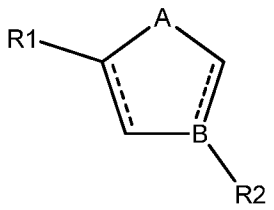
11. The method of claim 9, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

12. A pharmaceutical composition comprising an Nrf2 inhibitor selected from the group consisting of a compound represented by Formula I or Formula II, ethambutol dihydrochloride, rifamycin, an antitubercular quinolone, and a combination thereof; wherein the pharmaceutical composition is administered in combination with an antiviral treatment in an amount effective to sensitize virally infected cells to the antiviral treatment; wherein sensitizing to antiviral treatment is characterized by enhancing an antiviral effect of the antiviral treatment; wherein the virally infected cells may be characterized as having constitutive activation of Nrf2; wherein Nrf2 is comprised of an amino acid sequence comprising SEQ ID NO:1 including isoforms thereof; wherein the Nrf2 inhibitor is a non-naturally occurring compound when the pharmaceutical composition consists of the Nrf2 inhibitor as a sole therapeutic agent, and is either a naturally occurring compound or a non-naturally occurring compound when the pharmaceutical composition comprises the Nrf2 inhibitor in combination with one or more of a therapeutic agent and a second Nrf2 inhibitor; with Formula I as



wherein A is N or C; B is N or C; R1 or R2 or R3 are each independently selected from H, (C_1-C_6)alkyl, $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, $CHCHCONHNH_2$, or $COCH_3$; wherein at least one of R1, R2, and R3 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof;

and with Formula II as



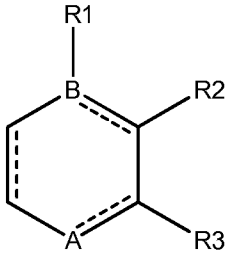
wherein A is O or N; B is N or C; R1 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; R2 is absent if B is NH; if B is C, R2 is absent or selected from CH_3, CH_2CH_3, NH_2, or $NHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof.

13. The pharmaceutical composition of claim 12, which contains an antiviral agent in a medically effective amount in combination with one or more Nrf2 inhibitors.

14. The pharmaceutical composition according to claim 12, which is administered concurrently, sequentially, or in a regimen of alternating dose, with the antiviral treatment.

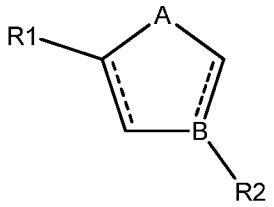
15. The pharmaceutical composition of claim 12, further comprising a pharmaceutically acceptable carrier.

16. Use of an Nrf2 inhibitor in a sensitizing amount, in combination with antiviral treatment, to enhance effectiveness of antiviral treatment directed towards a virus infection characterized by cells having constitutive activation of Nrf2 caused by an infecting virus; wherein Nrf2 is comprised of an amino acid sequence comprising SEQ ID NO:1; and wherein the Nrf2 inhibitor is (i) selected from the group consisting of compounds represented by Formula I or Formula II, ethambutol dihydrochloride, rifamycin, an antitubercular quinolone, and a combination thereof, (ii) a non-naturally occurring compound for administration by itself, and (iii) either a naturally occurring compound or a non-naturally occurring compound when combined together with one or more of an antiviral agent or second Nrf2 inhibitor to form a pharmaceutical composition; with Formula I as



wherein A is N or C; B is N or C; R1 or R2 or R3 are each independently selected from H, (C_1-C_6)alkyl, $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, $CHCHCONHNH_2$, or $COCH_3$; wherein at least one of R1, R2, and R3 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof;

and with Formula II as



wherein A is O or N; B is N or C; R1 is selected from $CONH_2$, $CONHNH_2$, $CSNH_2$, SO_2NH_2, NH_2, $NHNH_2$, $CHCHCONH_2$, or $CHCHCONHNH_2$; R2 is absent if B is NH; if B is C, R2 is absent or selected from CH_3, CH_2CH_3, NH_2, or $NHNH_2$; with dashed lines representing optional double bonds; and a pharmaceutically acceptable salt thereof.

17. A method for enhancing the effectiveness of antiviral treatment directed towards virally infected cells characterized as having constitutive activation of Nrf2, the method comprising administering to an individual in need thereof a sensitizing effective amount of an Nrf2 inhibitor in combination with the antiviral treatment; and wherein the Nrf2 inhibitor is (i) a non-naturally occurring compound when administered as a sole therapeutic agent, and (ii) either a naturally occurring compound or a non-naturally occurring compound when administered as a pharmaceutical composition comprising the Nrf2 inhibitor combined with one or more of an antiviral agent and a second Nrf2 inhibitor.

18. The method of claim 17, wherein Nrf2 inhibitor is administered as a pharmaceutical composition which additionally includes an antiviral agent in a medically effective amount.

19. The method of claim 17, wherein the Nrf2 inhibitor is administered concurrently, sequentially, or in a regimen of alternating dose, with the antiviral treatment.

20. The method of claim 18, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

FIG. 1A

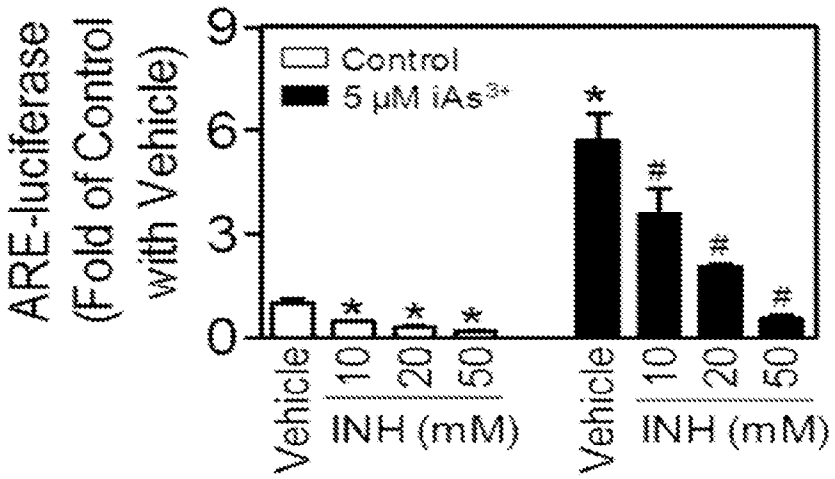

ARE-luciferase
(Fold of Control
with Vehicle)
0
3
6
9
Control
5 μM iAs3+
*
#
Vehicle
10
20
50
INH (mM)

FIG. 1B

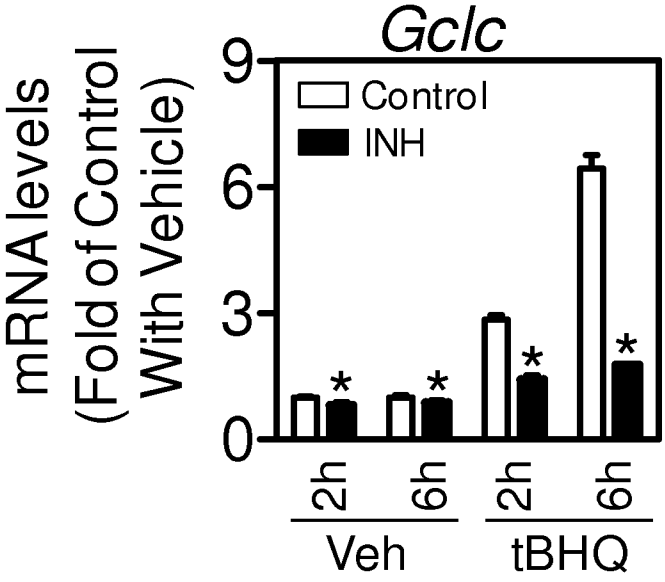

Gclc
mRNA levels
(Fold of Control
With Vehicle)
0
3
6
9
Control
INH
*
2h
6h
Veh
tBHQ

FIG. 1C

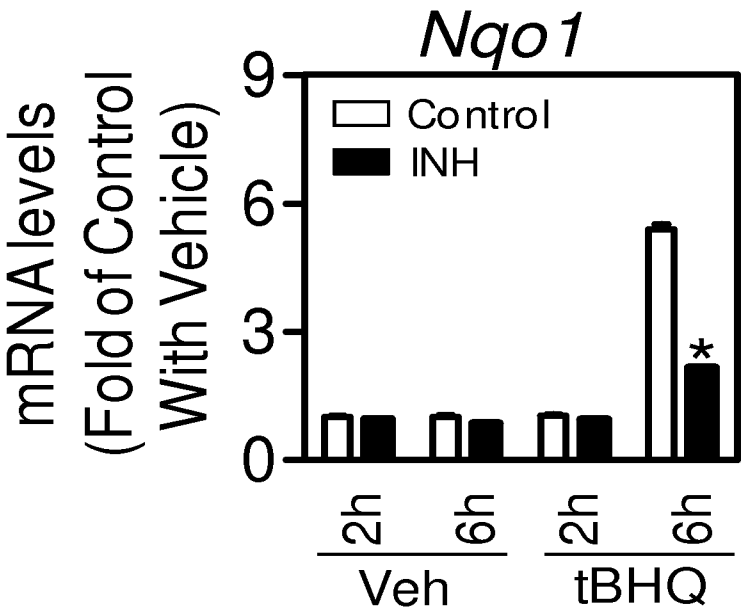

Nqo1
mRNA levels
(Fold of Control
With Vehicle)
9
6
3
0
Control
INH
*
2h
6h
2h
6h
Veh
tBHQ

FIG. 1D

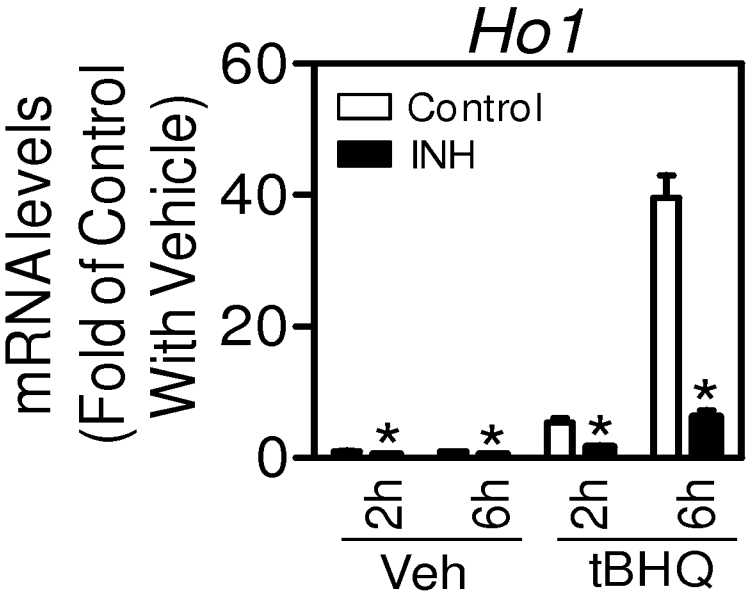

Ho1
mRNA levels
(Fold of Control
With Vehicle)
60
40
20
0
Control
INH
*
*
*
*
2h
6h
2h
6h
Veh
tBHQ

FIG. 2A

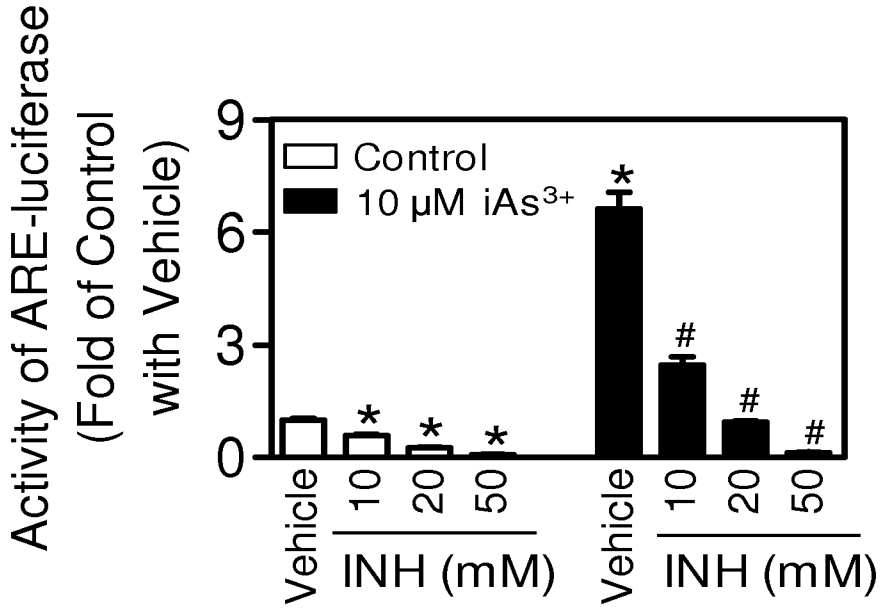

Activity of ARE-luciferase
(Fold of Control
with Vehicle)
9
6
3
0
Control
10 μM iAs3+
*
#
Vehicle
10
20
50
INH (mM)

FIG. 2B

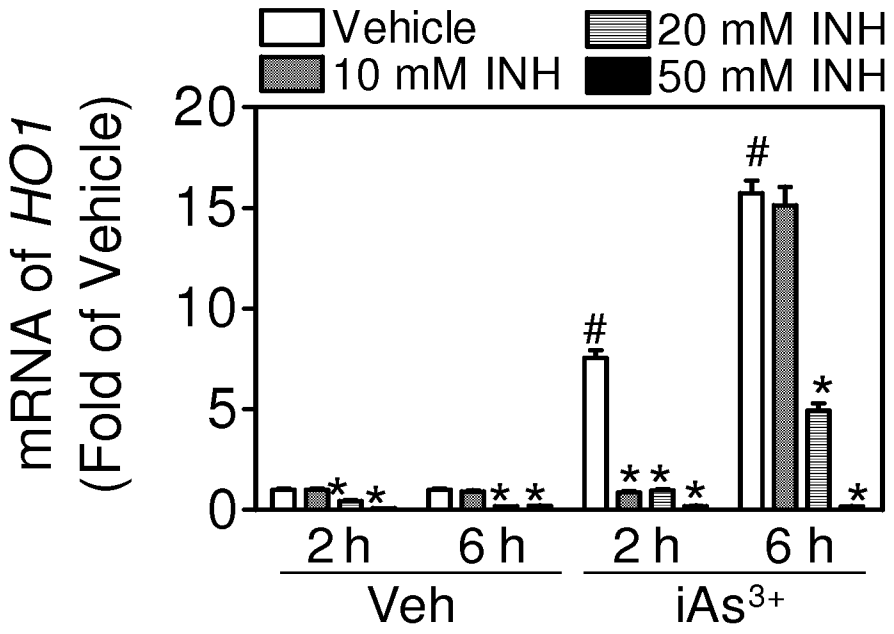

Vehicle
10 mM INH
20 mM INH
50 mM INH
mRNA of HO1
(Fold of Vehicle)
20
15
10
5
0
2 h
6 h
Veh
iAs3+

FIG. 3

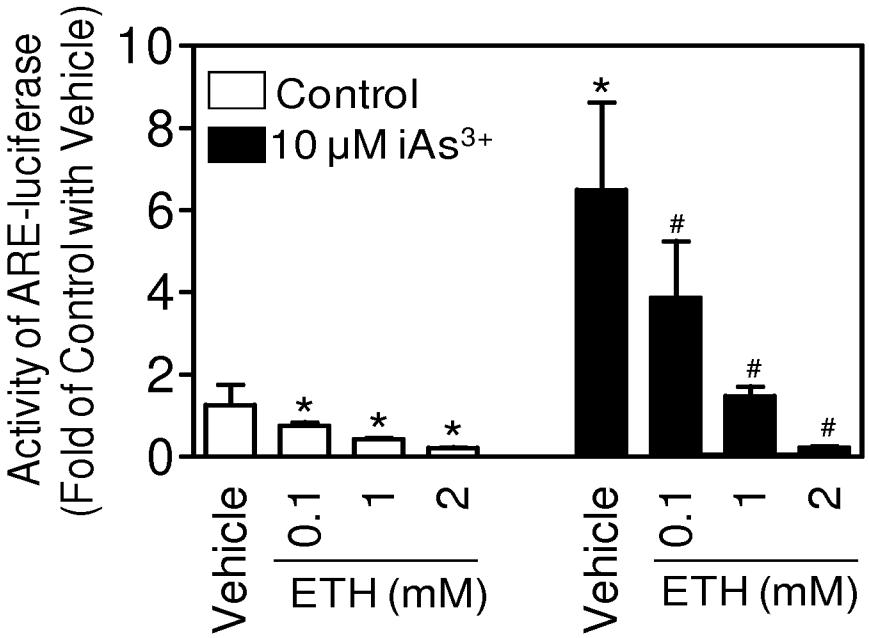

Activity of ARE-luciferase
(Fold of Control with Vehicle)
10
8
6
4
2
0
Control
10 μM iAs3+
*
#
Vehicle
0.1
1
2
ETH (mM)

FIG. 4A

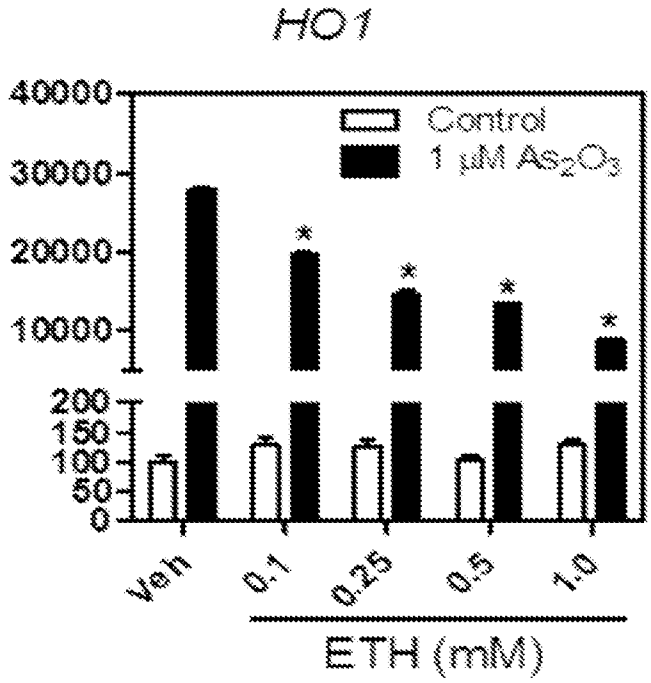

HO1
40000
30000
20000
10000
200
150
100
50
0
Control
1 μM As2O3
Veh
0.1
0.25
0.5
1.0
ETH (mM)

FIG. 4B

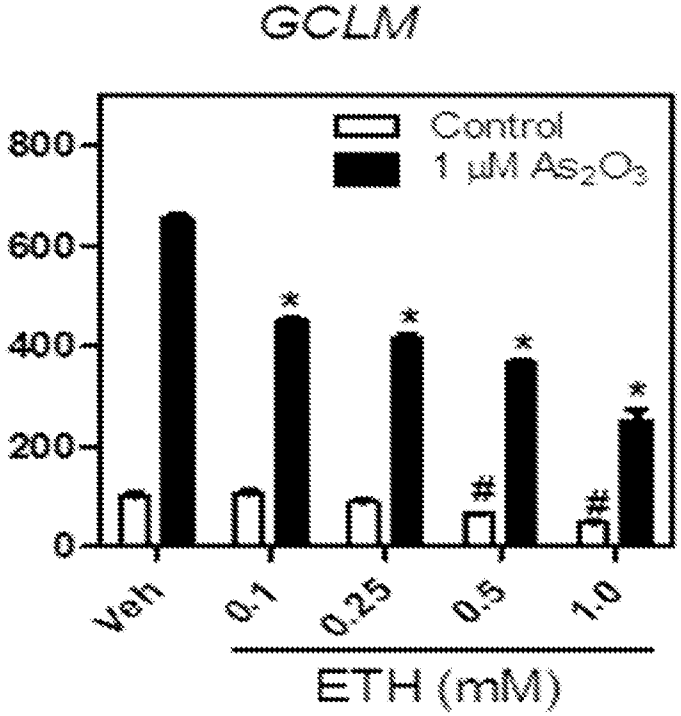
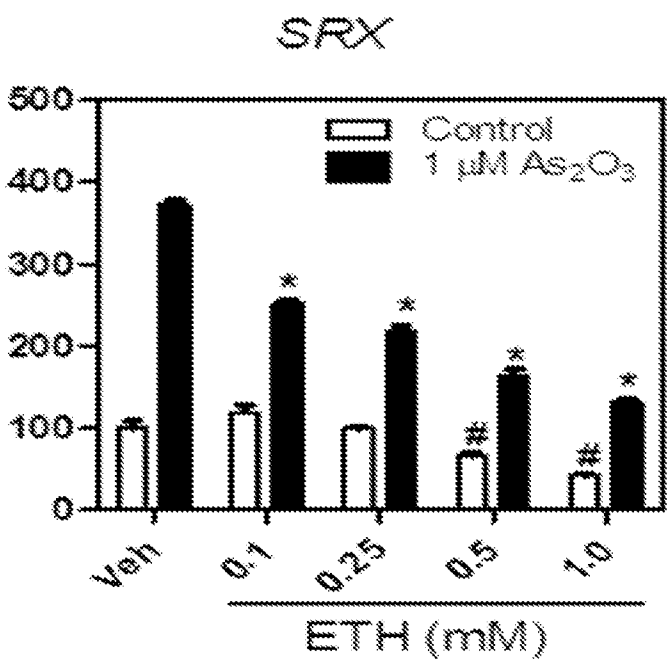

GCLM
Control
1 μM As2O3
800
600
400
200
0
*
*
*
*
#
#
Veh
0.1
0.25
0.5
1.0
ETH (mM)

FIG. 4C


SRX
Control
1 μM As2O3
500
400
300
200
100
0
*
*
*
*
#
#
Veh
0.1
0.25
0.5
1.0
ETH (mM)

6/11
FIG. 5

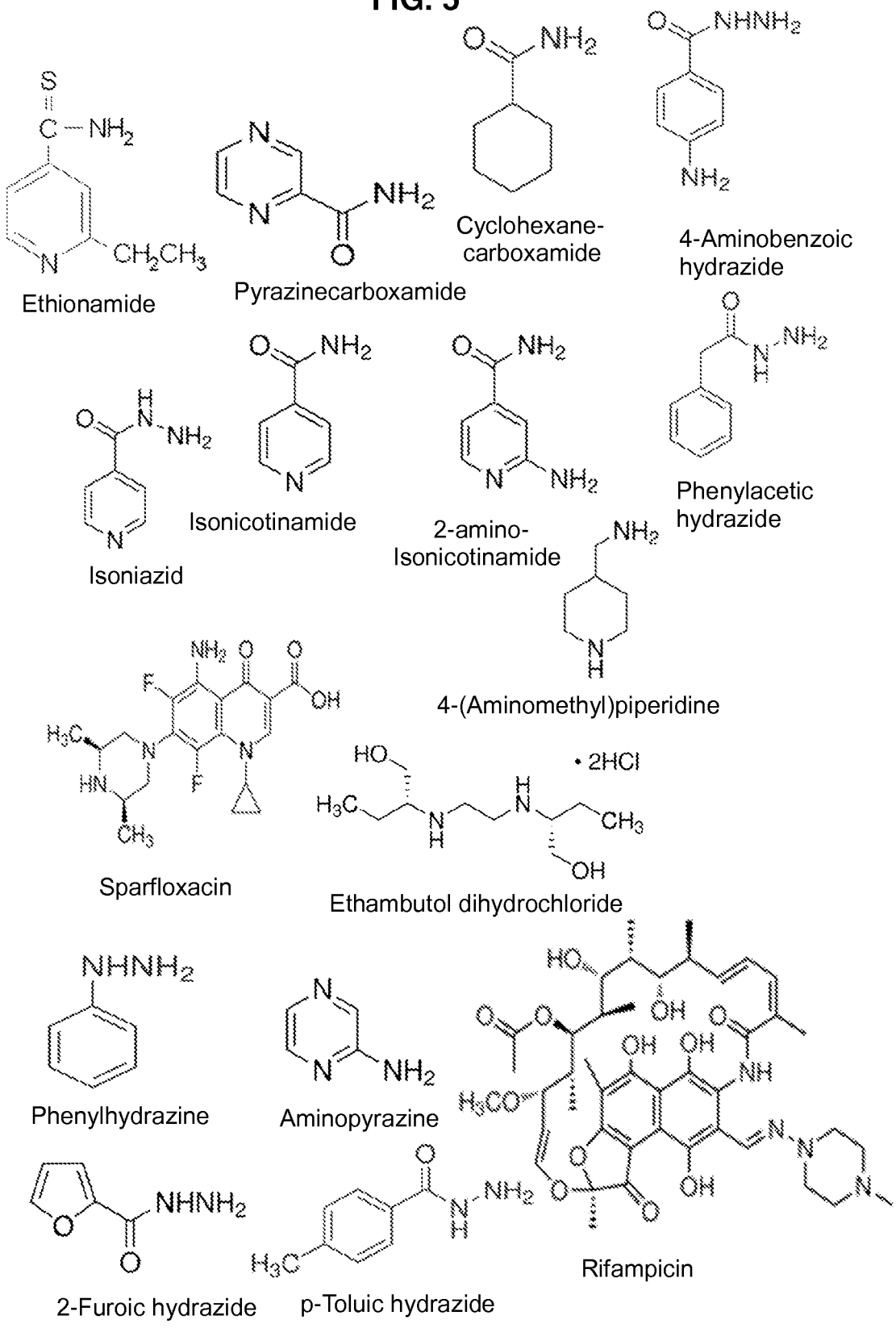

Ethionamide
Pyrazinecarboxamide
Cyclohexane-carboxamide
4-Aminobenzoic hydrazide
Isoniazid
Isonicotinamide
2-amino-Isonicotinamide
Phenylacetic hydrazide
4-(Aminomethyl)piperidine
Sparfloxacin
Ethambutol dihydrochloride
Phenylhydrazine
Aminopyrazine
Rifampicin
2-Furoic hydrazide
p-Toluic hydrazide

FIG. 6

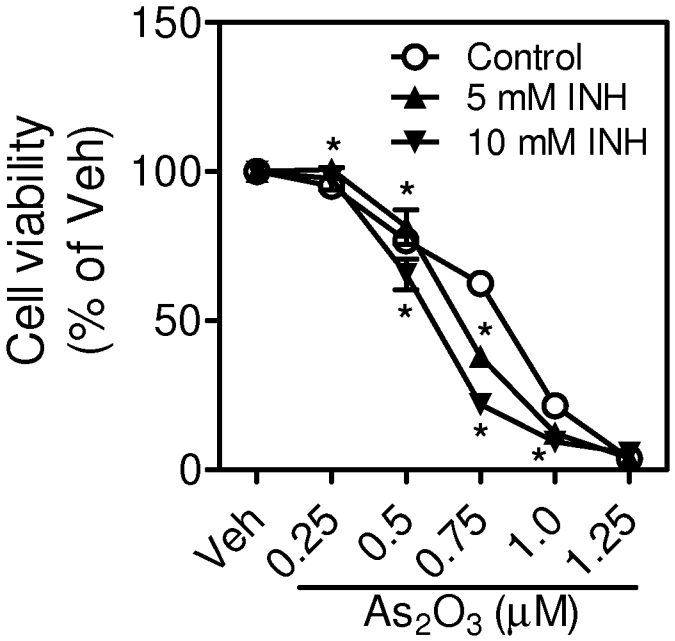

150
100
50
0
Cell viability
(% of Veh)
Control
5 mM INH
10 mM INH
*
Veh
0.25
0.5
0.75
1.0
1.25
As2O3 (μM)

FIG. 7A

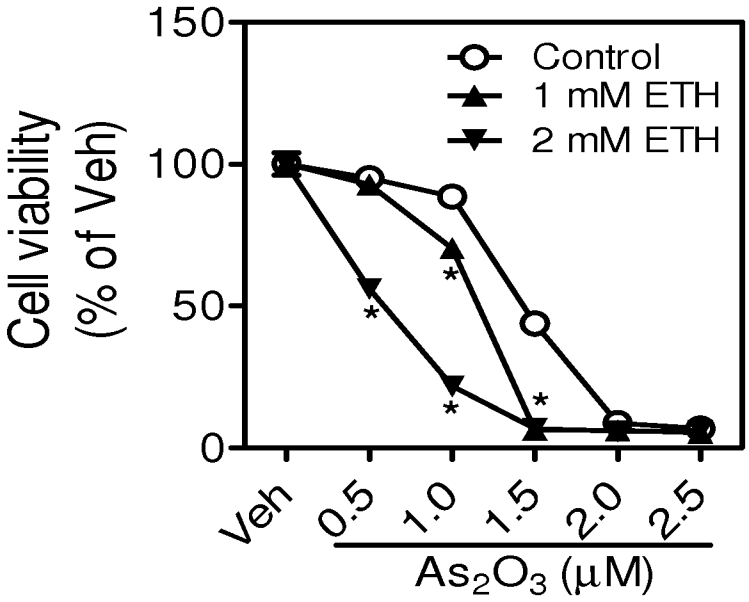

150
100
50
0
Cell viability
(% of Veh)
Control
1 mM ETH
2 mM ETH
*
Veh
0.5
1.0
1.5
2.0
2.5
As2O3 (μM)

FIG. 7B

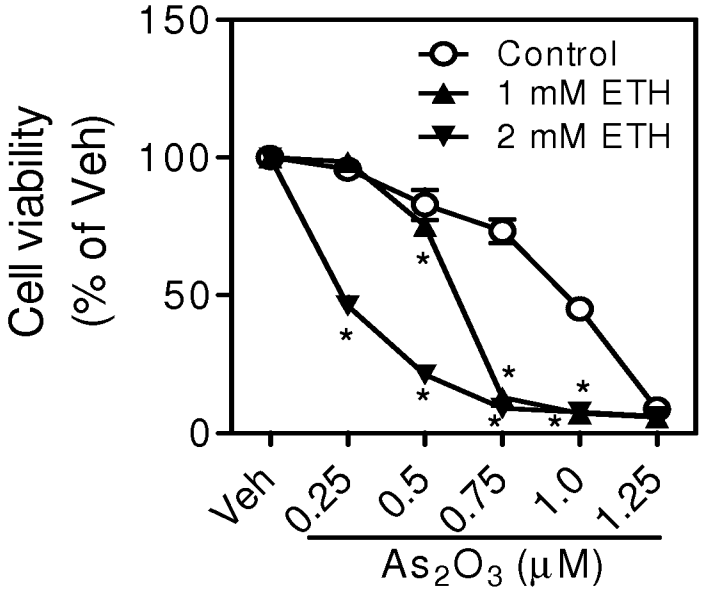

150
100
50
0
Cell viability
(% of Veh)
Control
1 mM ETH
2 mM ETH
Veh
0.25
0.5
0.75
1.0
1.25
As2O3 (μM)

FIG. 7C

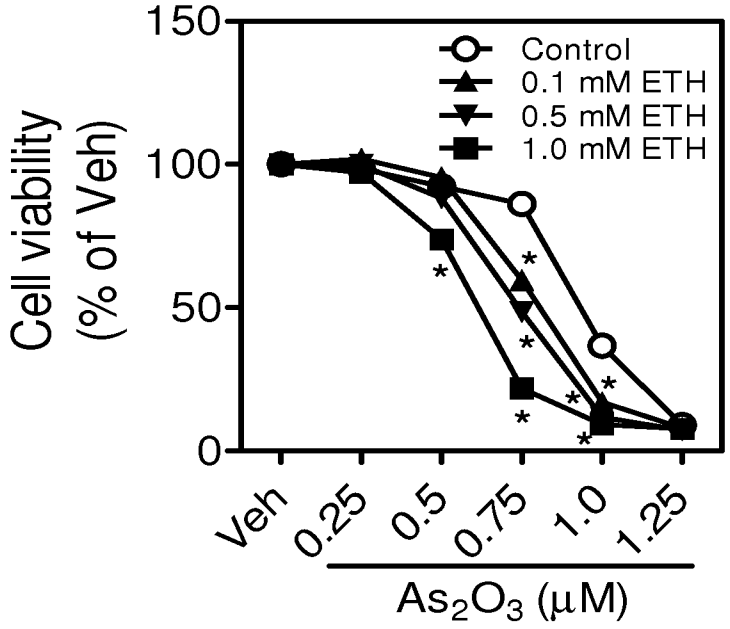

150
100
50
0
Cell viability
(% of Veh)
Control
0.1 mM ETH
0.5 mM ETH
1.0 mM ETH
Veh
0.25
0.5
0.75
1.0
1.25
As2O3 (μM)

FIG. 8A

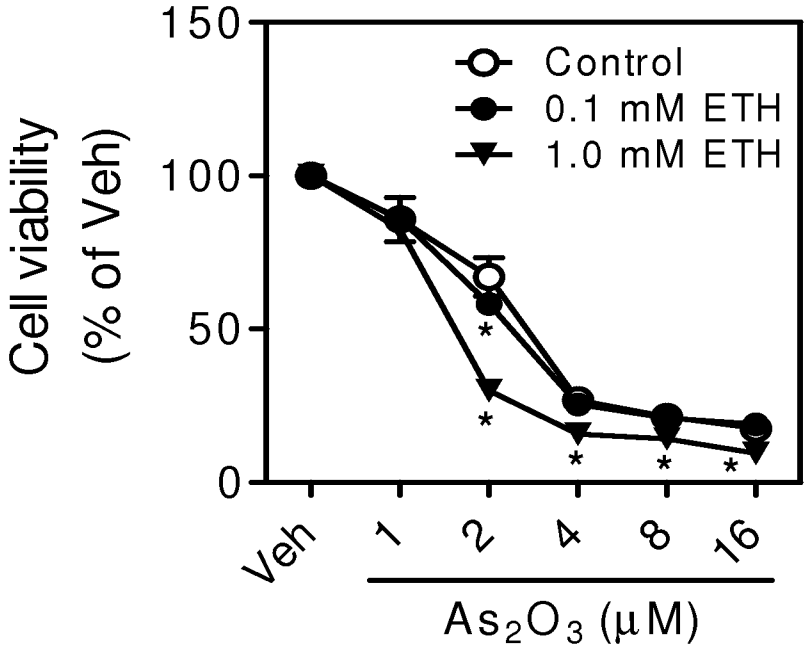

150
100
50
0
Cell viability
(% of Veh)
Control
0.1 mM ETH
1.0 mM ETH
*
*
*
*
*
Veh
1
2
4
8
16
As2O3 (μM)

FIG. 8B

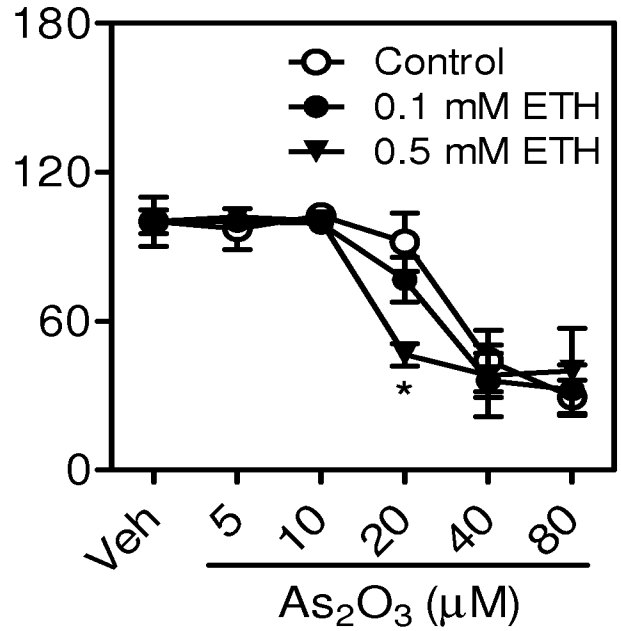

180
120
60
0
Control
0.1 mM ETH
0.5 mM ETH
*
Veh
5
10
20
40
80
As2O3 (μM)

FIG. 8C

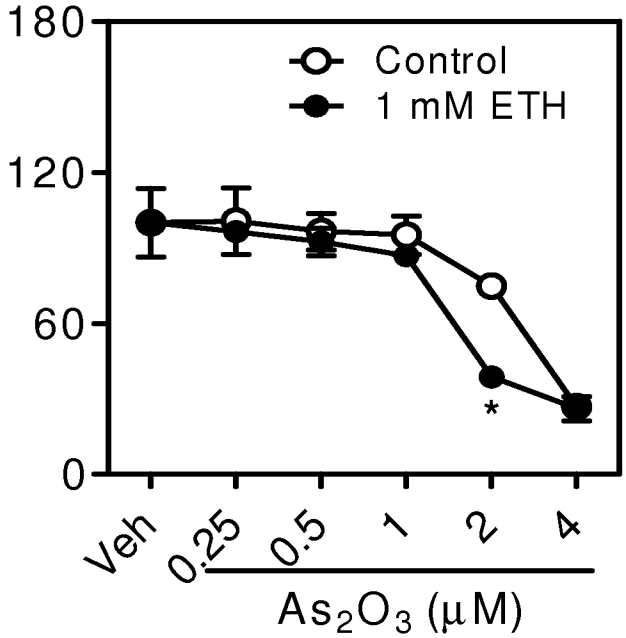

180
120
60
0
Control
1 mM ETH
*
Veh
0.25
0.5
1
2
4
As2O3 (μM)

FIG. 9A

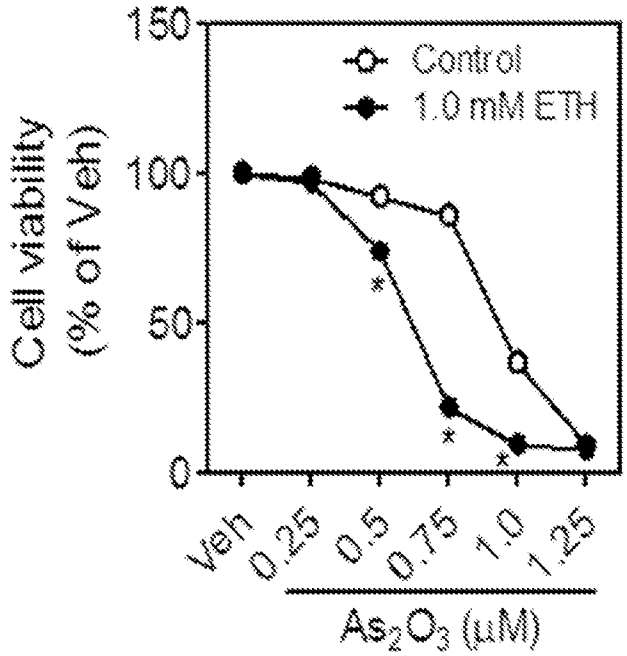
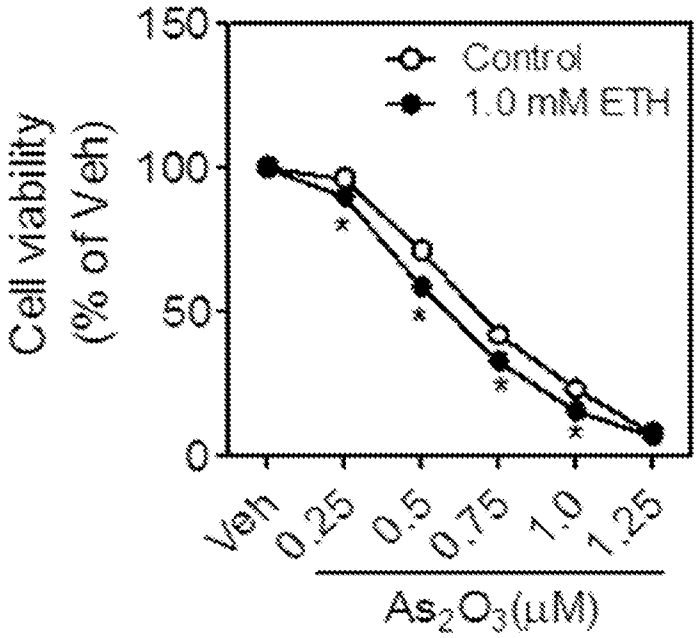

150
100
50
0
Cell viability
(% of Veh)
Control
1.0 mM ETH
*
*
*
Veh
0.25
0.5
0.75
1.0
1.25
As2O3 (μM)

FIG. 9B


150
100
50
0
Cell viability
(% of Veh)
Control
1.0 mM ETH
*
*
*
*
Veh
0.25
0.5
0.75
1.0
1.25
As2O3(μM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/046933

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/4725 (2014.01)
CPC - A61K 45/06 (2014.09)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K31/00, 31/4725, 31/506, 31/519, 31/52, 31/5377, 45/06; A61P 35/00, 35/02 (2014.01)
CPC - A61K 31/00, 45/06; C07D 471/04, 473/00, 401/12; G01N 33/574 (2014.09)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/00, 45/06; C07D 471/04, 473/00, 401/12; G01N 33/574 (keyword delimited)
US Classes: 424/133.1, 94.6; 514/171, 234.2, 256, 262.1, 263.22, 265.1, 275, 300

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

PatBase, Google Patents, Google, PubMed

Search Terms: Nrf2, antiviral, inhibit, cancer, quinolone, floxacin, nitrogen heterocycle

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2010/0255117 A1 (BISWAL et al) 07 October 2010 (07.10.2010) entire document	1-11 ----- 12-20
Y	US 2007/0098728 A1 (PETERSEN et al) 03 May 2007 (03.05.2007) entire document	12-20
A	WO 2013/067036 A1 (HU et al) 10 May 2013 (10.05.2013) entire document	1-20
A	WO 2011/046382 A2 (SONG et al) 21 April 2011 (21.04.2011) entire document	1-20
A	KAWAI et al. "Acetylation-Deacetylation of the Transcription Factor Nrf2 (Nuclear Factor Erythroid 2-related Factor 2) Regulates Its Transcriptional Activity and Nucleocytoplasmic Localization," The Journal of Biological Chemistry, 04 March 2011 (04.03.2011), Vol. 286, No. 9, Pgs. 7629-7640. entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2014	05 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/046933

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs: 1 and 2 were searched.