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(54) **Title:** MODULATION OF CELL FATES AND ACTIVITIES BY PHTHALAZINEDIONES

(57) **Abstract:** Phthalazinediones that function as intracellular redox modulators are useful in treating cells in various disease states where intracellular redox status is impaired. By buffering aberrant redox states, phthalazinediones enable cellular processes essential for survival and augment medical treatments. The phthalazinediones of the invention can modulate functions related to cell growth, differentiation, activity, or death, to correct aberrations and restore homeostasis, and can serve as adjunctive therapy in treating various disease conditions.

MODULATION OF CELL FATES AND ACTIVITIES BY PHTHALAZINEDIONES

BACKGROUND AND INVENTION

Current medical treatments generally focus on the disease and strive to eliminate the inciting agent or the symptoms, often injuring healthy tissue in the process. The present invention focuses instead on the patient, to enable self-repair mechanisms by supporting the patient's body in controlling or stabilizing its cellular functions without toxic side effects. The methods and compositions of the invention comprise phthalazinedione compounds that buffer intracellular reduction and oxidation (redox) reactions and thereby modulate cellular functions of growth, differentiation, activity, and death in various disease states.

In healthy cells, a balance of redox reactions maintains a physiologically appropriate environment for various cellular functions related to growth, differentiation, activity, and death. The proper coordination of such functions ensures homeostasis and the health of cells. Research has shown that alterations in cellular redox status affect activities such as cellular signaling, suggesting that altering the cellular redox status could also affect cellular activation, which results from certain cellular signals (U.S. Pat. No. 5,994,402). Altering the intracellular redox state by depleting cells of glutathione (GSH), an endogenous "redox agent," has also been shown to protect cells from certain injury and to promote their survival (U.S. Pat. No. 5,994,402), again suggesting a link between alterations in the cellular redox state and cellular functions.

Stresses that perturb a cell's redox status may be internal or external. For example, a genetic mutation may produce defective protein products that function abnormally or not at all. These defective proteins could disrupt certain cellular processes, including redox reactions. Cellular redox reactions may also be disrupted by microbes, toxins, allergens, or other agents external to the cell. The external stress could trigger defensive responses that leave the cell's redox system depleted and unstable.

An imbalanced redox state, even if not the cause of a particular disease condition, may facilitate that condition by providing an "unhealthy" environment in which necessary cellular functions become impaired. Cellular redox status may become impaired in numerous disease conditions. Under the stress of a disease state, the rate of redox reactions increases or decreases as needed by the cell. Significant or prolonged deviations in the intracellular redox status disable cellular processes, including defense mechanisms. When such cellular functions are impaired, the survival of the cell becomes uncertain. Maintenance of the proper redox status is thus critical to the fate of the cell.

To counter and correct disturbances in the redox status, cells require agents that can modulate redox imbalances, to facilitate reduction or oxidation reactions as appropriate. Agents currently available for correcting redox imbalances are inadequate in that they are labile, quickly oxidized, or unable to translocate to the proper region of the cell. Examples of such exogenous redox agents include cysteine, reduced lipoates or thiols, glucocorticoids, and other antioxidants. Redox agents that remain stable, active, and functional in the cellular environment are necessary.

Although their role in modulating intracellular redox status was not recognized, phthaloylhydrazide, phthalazinedione, and phthalazine derivatives have been described as effective against inflammation, cancer, arrythmia, hyperlipidemia, and hypoxia (U.S. Pat. Nos. 6,686,347; 6,489,326; 5,874,444; 5,543,410; 5,512,573; 4,861,778; 4,250,180; Hall et al., *Biomed. Biochim. Acta.* 47: 423-433 (1988); Hall et al., *J. Pharm. Pharmacol.* 41: 394-397 (1989); Hall et al., *Anticancer Drugs.* 3: 55-62 (1992); Butner et al., *Int. J. Tissue React.* 18: 47-55 (1996)). However, toxicity and the lack of pharmacological activity of certain phthaloylhydrazides, including 2,3-dihydrophthalazine-1,4-dione and 5-amino-2,3-dihydrophthalazine-1,4-dione, were also noted (U.S. Pat. Nos. 6,489,326; 5,543,410; 5,512,573).

Phthalazinediones have also been described as starting materials in the manufacture of pesticides (U.S. Pat. App. Pub. No. 2005/0033050) and as starting or intermediate compounds in the preparation of the structurally related phthalazinone compounds having pharmacological activity (U.S. Pat. Nos. 4,769,369; 4,665,181; 4,250,180; 3,957,776; U.S. Pat. App. Pub. No. 2003/0073692). Luminol, also known as *o*-aminophthaloylhydrazide, 3-aminophthalhydrazide, 5-aminophthaloylhydrazide, or 5-amino-2,3-dihydro-1,4-phthalazinedione, was considered toxic and used in photothermographic imaging, chemiluminescent assays and labeling of cellular structures, detection of copper, iron, peroxides, or cyanides, and forensic science to detect traces of blood (U.S. Pat. Nos. 5,279,940; 4,729,950; 4,226,993; Merck Index, 13th ed. (2001), monograph no. 5622).

Nonetheless, luminol has been described for use in treating inflammation, cancer, ulcerative colitis, Crohn's disease, diffuse sclerosis, diarrhea, proctitis, hemorrhoids, anal fissures, dyspepsia, intestinal infection, Alzheimer's disease, osteoarthritis, muscular dystrophy, macular degeneration, and proctosigmoiditis (U.S. Pat. Nos. 5,874,444; 5,543,410; EP 617024; RU 2211036), as well as for use in treating psoriasis, infarct, and transplant rejection (U.S. Pat. Nos. 6,489,326; 5,512,573). Luminol has also been described as the chemiluminescent moiety in a composite prodrug compound useful as antiviral and anticancer agents (U.S. Pat. App. Pub. No. 2005/0080260).

Other phthaloylhydrazide derivatives identified as having pharmacological activity include 2,3-dihydrophthalazine-1,4-dione, 2-amino-1,2,3,4-tetrahydrophthalazine-1,4-dione sodium salt dihydrate, 4-aminophthaloylhydrazide, 4,5-aminophthaloylhydrazide, 4,5-methylaminophthaloylhydrazide, and their derivatives (U.S. Pat. Nos. 6,489,326; 5,512,573; U.S. Pat. App. Pub. No. 2003/0195183; RU 2113222; Butner et al., *Int'l J. Tissue Reactions* 18: 47-55, 1996; de Wazieres et al., *Immunopharmacology* 39: 51-59 1998).

Phthalazinedione compounds, including luminol, have also been described as an inhibitor of poly (ADP-ribose) polymerase, an enzyme that responds to DNA damage, and for treating conditions involving the functions of poly (ADP-ribose) polymerase (U.S. Pat. Nos. 6,358,975; 6,348,475; 5,874,444; 5,719,151; 5,633,282; 5,589,483). A method of manufacturing the sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione and its pharmaceutical use for immunomodulation, inflammation, and antioxidant treatment have been described (U.S. Pat. No. 6,489,326; RU 2222327).

Phthalazinediones of the invention may be used to modulate redox imbalances and to support a patient's body in a variety of disease states and in treating metabolic distress, inflammation, infectious conditions, neurological disorders, immune disorders, proliferative diseases, and senescence. The phthalazinediones and their pharmaceutical compositions may also be used in conjunction with standard medical treatments and devices to enhance treatment methods such as chemotherapy, radiation, nutrition, pharmaceutical therapy, and surgery, including angioplastic procedures using catheters or stents.

The present invention describes the use of phthalazinedione compounds in treating disorders involving imbalanced or deficient intracellular redox states. Phthalazinediones can act as efficient substrates for reaction with reactive oxygen species (ROS) and radicals that are inevitably generated under metabolic distress. By buffering redox imbalances or deficiencies, phthalazinediones can reversibly and selectively modulate cellular functions, e.g., upregulating mitochondrial aerobic metabolism when a cell under stress needs energy for defense or repair, or downregulating metabolism when the stressed cell is overactive. Phthalazinediones can modulate cellular processes such as proliferation, secretion, differentiation, transformation, migration, and apoptosis, without toxic side effects on healthy cells.

Under any stress, the intracellular redox balance inevitably falters as aerobic metabolism is necessarily overworked. Any stress to the cell, especially if prolonged, will deplete the cell of endogenous redox agents, including thiols, glutathione, thioredoxins, iron-sulfur proteins, cysteine, and thiol proteins, as well as redox-sensitive proteins such as catalase. Redox imbalance is often etiologic in inducing multiple cell damages. Chronic stress leads to cellular and organelle thiol deficiencies, as blood cysteine is limited. In turn, since many cellular pathways are controlled by or depend on intracellular redox activities, thiol deficiencies lead rapidly to impaired energy production, with increased oxidant production and progressive mitochondrial and cell death.

Thus, the fate of growing cells is highly dependent on oxygen redox status. Permeant redox agents that can control oxygen metabolism and maintain redox homeostasis have therapeutic implications. Phthalazines, tetracyclines, or thiols can potentially dictate and control cell fate in activated or stressed cells in redox imbalance (Tikka and Kolstinaho, *J. Immunol.* 166: 7527-7533, 2001). In addition to controlling proliferation and activation pathways, these redox modulators also scavenge destructive oxygen radicals and thereby prevent apoptotic and necrotic pathways. Potential therapeutic usefulness of these redox modulators in astroglia induced neurodegenerative diseases, renal allografts, and inflammation-induced cell damages has been recognized (Tikka and Kolstinaho, *J. Immunol.* 166: 7527-7533, 2001; Husman et al., *FASEB J.* 10: 1135-1141, 2002; Ryan et al., *Curr. Opinion in Rheumatology* 8: 238-247, 1996). Permeant extracellular reductants and oxidants – reduced thiols, tetracyclines, phthalazines, O_2^-, gamma radiation, doxorubicin, glucocorticoids, *cis*-platinum, doxorubicin, etc. – may prove useful.

With prolonged thiol deficiencies, replacement therapy with available thiols is difficult and usually inadequate. Cysteine and other reduced thiols are labile and rapidly oxidized to toxic

metabolites in the presence of oxygen. Most antioxidants, which dissipate oxygen-based oxidants, are unable to penetrate to the electron-transporting inner mitochondrial membrane to modulate the iron-sulfur protein mediated electron flow in mitochondrial Complex III or to stabilize disulfide cross-linkages that control permeability of the mitochondrial megapores and channels. Antioxidants also cannot supply the cysteine required in the manufacture of most proteins or the energy required to combat chronic stresses or repair cellular damages.

Under certain conditions, loss of redox control may cause: (1) cross-linking of thiols in the adenine nucleotide translocase and other proteins, which then opens the mitochondrial transmembrane pores and channels and leads to a decline in mitochondrial voltage and energy production (Constantini et al., *J. Biol. Chem.* 271: 6746-6751, 1996; Larochette et al., *Exp. Cell Res.* 249: 413-421, 1999; Zamzami et al., *Oncogene* 16: 1055, 1998); (2) increases in intracellular calcium levels; (3) activation of redox defenses and heat shock proteins; (4) activation of redox-sensitive cell cycling factor AP-1 and E2F/Rb pathway; (5) activation of apoptotic pathways via AsK-1, with liberation of caspases, cytochrome *c*, and apoptosis inducing factor (AIF) from the failing mitochondrion; (6) a decline in ADP-dependent electron flow, as well as alteration of mobility of redox sensitive iron-sulfur proteins at mitochondrial Complex III (Zhang et al., *J. Biol. Chem.* 275: 7656-7662, 2000); (7) oxidation of macromolecules, including redox-sensitive proteins such as glutamate transporters (Trotti et al., *J. Biol. Chem.* 271: 5976-5979, 1996), mitochondrial DNA, and membrane lipids; (8) a failure in modulation of redox-sensitive phosphatases PTB-1, SHP-1, and SHP-2 (Doza et al., *Oncogene* 17: 19-26, 1998); and (9) dysregulation of the thiol-sensitive MAP kinase-Ras pathway, which controls cellular proliferation.

Acute diseases represent acute imbalances in redox controls, specifically an imbalance in nitric oxide (NO) and superoxide (O_2^-), the two major signal molecules in the cell. This NO/O_2^-

redox imbalance may result in defects in blood flow, blood pressures, rhythmic heart rates, and hemostasis, as well as sensory, motor, cognitive, emotive, and neurotransmission defects as occur in certain conditions such as multiple chemical sensitivity syndrome (MCS) due to acute poisoning with inhaled toxins.

Both NO and O_2^- are continuously produced at low levels in active cells or respiring tissues. Under various cellular redox stresses, NO and O_2^- act as potent counter redox agents as they are rapidly produced to quickly modulate responses to the stresses. For example, during muscle contraction with its obligatory consumption of energy and production of O_2^- by respiring mitochondria, the NO/O_2^- redox balance is under rapid flux. By cyclically nitrosylating mitochondrial thiols and metals, NO can suppress respiration and its production of O_2^- (Beltrain et al., *PNAS*, 97: 14602-14607, 2000; Clementi et al., *PNAS*, 95: 7631-7636, 1998). NO can also directly scavenge O_2^-, and O_2^- can directly scavenge NO, both as OONO. Superoxide can also oxidize and regulate activity of local thiol proteins.

Thus, by multiple interactions, NO and O_2^- can maintain and buffer intracellular thiol redox potentials at desired levels. If levels of the highly toxic NO become too high, NO can be temporarily stored as nitrosylated proteins and peptides, then subsequently reduced to less toxic ammonium ions plus oxidized glutathione by a glutathione-dependent formaldehyde dehydrogenase (Liu et al., *Cell*, 116: 617-628, 2004). Nitric oxide is also readily oxidized to nitrates. If levels of the highly toxic O_2^- or its products (H_2O_2) become too high, O_2^- can be rapidly dissipated by reversibly oxidizing various thiols or dismutated to less toxic oxidants. Cells thus have multiple means to both produce and destroy nitric oxide and superoxide and thereby maintain desirable nitroso-oxo-thiol redox balance (Harc, J.M., *NEJM*, 351: 2112-2113, 2004).

The major source of chemical energy and heat in aerobic cells is mitochondria. The permeable pores and channels in mitochondria are exquisitely sensitive to thiol redox status. The specific mitochondrial channel is composed of two thiol redox sensitive proteins located in the inner membrane – adenine nucleotide translocase (ANT) and voltage dependent anion channel (VDAC) – and other coproteins such as cyclophilin D, hexokinase, benzodiazepine receptors, and the Bcl-2/Bax family of peptides. These proteins together control the permeability and transport of mitochondrial transmembrane channels and pores, which control ADP entry, proton exit, electron flow, intracellular calcium concentration, and O_2^- production.

Proteins such as Bax, benzodiazepine receptors, and hexokinases bind to the outer membrane of mitochondria and regulate transport and pore formation in these membranes. Major physiologic modulators of this mitochondrial transmembrane pore include: (1) transmembrane voltage, which is generated by electron and proton gradients; (2) inducible membrane proteins, Bcl-2 and Bax; and (3) thiol redox status, the redox state of Cys-56 on the channel protein ANT being a major regulator of the permeability of mitochondrial transmembrane pores (He and Lemasters, *FEBS Letters* 512: 1-7, 2002).

Thiol oxidants or cross-linking agents such as diamide or diethyl maleate distort and open mitochondrial pores and channels, and uncouple electron flow, allowing oxygen to trap electrons and produce O_2^-, H_2O_2, and other radicals. Energy production declines, and mitochondria release cytochrome *c*, caspases, and AIF. Destructive cytochrome *c*, redox-sensitive proteases, and caspases are activated in the cytoplasm and the nucleus, causing cell death, both apoptotic and necrotic. Agents that can stabilize ANT sulfhydryls and maintain pore permeability status, e.g., reduced thiols, dithiothreatol, glutathione, N-acetyl cysteine, Bcl-2, Bongkrekic acid, cyclosporine A, or chaperone cyclophilins, can completely prevent the electron leak and cell

death (Armstrong and Jones, *FASEB J.* 16: [online], June 7, 2002; Castantini et al., *Oncogene* 19: 307-314, 2000; Hong et al., *FASEB J.* 16: 1633-1636, 2002).

Under conditions of oxidant stress where thiol redox status is not optimal, as in radiation, chemotherapy, occlusive vascular diseases, leptin-deficient or resistin-induced obesity, caloric excesses, and type II diabetes, therapeutic support by external thiol redox buffers will be acutely necessary, at least until the host can repair and buffer the stressed and imbalanced thiol redox status and fully activate its hypoxia-inducible transcription factors (Wenger, *FASEB J.* 16: 1151-1162, 2002; De Giorgi et al., *FASEB J.* 10: 607-609, 2002). With oxidative stress, labile vicinal cysteinyl residues on ANT undergo cyclic oxidation, ionization, and eventually cross-linking.

The oxidations and cross-linking of protein thiols greatly perturb channel functions, especially by thiol cross-linking cyclic amines, diazenes (diamide), or phenylarsines. Uptake of ADP fails, protons are released with collapse of the inner membrane potential, ordered electron flow at mitochondrial Complex III falters, and O_2 now accepts the fluxing electrons with production of O_2^- and other radicals. The oxidant-producing mitochondria release cytochrome *c* and AIF, and downstream oxidation of NF_KB, AP1 (major transcription factor for proliferation), AsK-1 (apoptosis stimulating kinase), glutathione, Bax, HDAC (histone deacetylase in nucleus), PTEN (phosphatase in cytoplasm), and ATM occurs. Apoptosis, senescence, quiescence, or necrosis results, depending largely on the extent and duration of the redox stress.

Apoptosis (nuclear or programmed cell death) and necrosis (plasma membrane linked cell death) represent conditions of aberrant or uncontrolled proteolysis by thiol redox dependent caspases, endonucleases, and histone deacetylases. Thiol redox modulators such as thioredoxin can either upregulate or downregulate regulatory proteases that process and digest the caspases, endonucleases, and deacetylases, e.g., hydrolyzing procaspase 3 to the active caspase fragments that initiate the apoptotic cascade in the nucleus. Diamide is a phthalazinedione with activity

similar to the oxidized 4-amino-phthalazinedione and can activate and cross-link such proteases. Since such cross-linking agents can also oxidize essential membrane proteins, pores can form in the mitochondrial membrane, with increased ROS and cell destruction (Ueda et al., *J. Immunol.* 161: 6689-6695, 1998).

A photoactive diamine fluorescent cation, tetramethyl-rhodamine, which accumulates in mitochondria and releases free radicals when photoactivated, is a potent agonist of the mitochondrial transmembrane pore. When tetramethyl-rhodamine is activated, all downstream effects of oxidation and cross-linking of ANT's labile cysteinyl residues occur, including translocation and polymerization of Bax in mitochondrial membranes. These effects are fully inhibited by Bongkrekic acid, a specific inhibitor of mitochondrial transmembrane pores (De Giorgi et al., *FASEB J.* 10: 607-609, 2002), as well as by reduced thiols, reduced phthalazines, cyclophilins, and pterines. Such observations suggest that the fate of cells under stress is largely dictated by mitochondrial thiol redox status, and that host and cell fates are readily buffered or controlled by permeant lipophilic redox-sensitive amines, such as phthalazinediones, tetrahydrobiopterin, and permeant thiols.

In general, a therapeutically effective amount of a phthalazinedione of the invention that is sufficient to ameliorate redox imbalance or stress will depend on the acuteness of the disease condition, the particular redox status or deficiency of the patient, the developmental condition of the stressed cell, and also the state of oxidation of the phthalazinedione, but will be in the range of about 0.01-100.0 mg per kg of body weight or about 1.0-10,000.0 mg per day, e.g., administered in amounts of 1.0, 10.0, 50.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, 700.0, 800.0, 900.0, 1000.0, 2000.0, 3000.0, 4000.0, 5000.0, 6000.0, 7000.0, 8000.0, 9000.0, or 10,000.0 mg.

The phthalazinedione compounds of the present invention are preferably incorporated into pharmaceutical forms suitable for administration by oral, nasal, mucosal, vaginal, rectal, transdermal, or parenteral routes, including subcutaneous, intramuscular, intravenous, and intraperitoneal, e.g., tablet, capsule, granule, powder, solution, suspension, microsphere, liposome, colloid, lyophilized composition, gel, lotion, ointment, cream, spray, and suppository, and preferably include pharmaceutically acceptable excipients, carriers, adjuvants, diluents, or stabilizers as is well known to the skilled in the art.

The phthalazinedione may be a derivative compound containing a substituent that enhances the activity, stability, or other property of the compound. Such a derivative compound may be an amino derivative or a phthalazinedione comprising a haloamino, alkylamino, acylamino, alkanolamino, alkenylamino, alkoxyamino, haloalkylamino, allylamino, or sulfhydrylamino (thiolamino or mercaptoamino) group or other substituents that confer a preferred function. Furthermore, the phthalazinedione may be a bromoamino, chloroamino, fluoroamino, iodoamino, methylamino, ethylamino, propylamino, isopropylamino, methanoylamino (formylamino), ethanoylamino (acetylamino), propanoylamino, hydroxylamino, carboxylamino, methanolamino, ethanolamino, propanolamino, methenylamino, ethenylamino, propenylamino, methoxyamino, ethoxyamino, propoxyamino, or dimethylamino derivative.

Examples of such phthalazinedione derivatives include, but are not limited to, 5-amino-2,3-dihydrophthalazine-1,4-dione (luminol), 6-amino-2,3-dihydrophthalazine-1,4-dione (isoluminol), 5-amino-2,3-dihydrophthalazine-1,4-dion-8-yl (luminyl), N-bromo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-chloro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-fluoro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-iodo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethyl-5-amino-2,3-dihydrophthalazine-1,4-

dione, N-propyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-isopropyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-hydroxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-carboxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N,N-dimethyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-acetylcysteine-5-amino-2,3-dihydrophthalazine-1,4-dione, N-acetylglutathione-5-amino-2,3-dihydrophthalazine-1,4-dione, and a phthalazinedione manufactured by the method disclosed herein. Enantiomers, isomers, tautomers, esters, amides, salts, solvates, hydrates, analogues, metabolites, free bases, or prodrugs of the phthalazinedione or its derivative are also contemplated by the invention.

To survive any stress, cells must replace depleted thiols and maintain optimum mitochondrial redox potentials and activities. In one embodiment of the invention, therapy includes combined treatment with phthalazinediones and compounds to replace the lost thiols, oxidatively protect the phthalazinedione, eliminate the source of stress, or otherwise support the subject. Such multiple agent or combination therapeutic approaches may employ a phthalazinedione with one or more other pharmacologically active ingredients that elicit desirable complementary or supplementary effects, administered simultaneously, separately, or sequentially.

For example, combined therapy with a compound that is an amino acid, antibiotic, antiviral agent, antiinflammatory agent, antioxidant, immunomodulator, reductant, oxidative protector, steroid, or vitamin may be beneficial or necessary. Compounds such as a cysteine (e.g., acetyl cysteine, N-acetylcysteineamide), glutathione, lipoic acid (e.g., alpha lipoic acid, dehydrolipoic acid), hydralazine, thioredoxin, biopterin (e.g., tetrahydropterin, sepiapterin), glucocorticoid, dexamethasone, rasagiline, ferulic acid, minocyline, menadione, tetracycline, isosorbate dinitrate, dextromethorphan, dithiothreitol, carnosine, clomethiazole, or mixtures thereof may be used.

The preferred active ingredients may also be formulated into a pharmaceutical composition with one or more pharmaceutically acceptable excipients. For example, a pharmaceutical composition may contain a phthalazinedione, a glutathione, and one or more pharmaceutically acceptable excipients. The pharmaceutical composition may be in the form of a tablet, capsule, granule, powder, solution, suspension, microsphere, liposome, colloid, lyophilized composition, gel, lotion, ointment, cream, spray, or suppository and administered intravenously, intramuscularly, intraperitoneally, subcutaneously, orally, nasally, mucosally, transdermally, parenterally, vaginally, or rectally. A therapeutically effective amount of the phthalazinedione or a pharmaceutical composition comprising a therapeutically effective amount of the phthalazinedione is administered to a subject in metabolic distress, to maintain the desired redox status and mitochondrial energy production, as well as the redox-sensitive signal transduction pathways.

Furthermore, because of their antioxidant, anti-inflammatory, antiproliferative, immunomodulatory, redox-buffering, and non-toxic properties, phthalazinediones can be beneficial as adjunctive support therapy for the stressed cell regardless of the compromising stress or its downstream symptoms. Redox therapy may provide sufficient support for the

diseased cell to treat itself, but in some situations, the cell will also need the mechanical, pharmacological, or genetic support of standard medical treatments such as radiation, chemotherapy, laser therapy, surgery, medication, and nutrition used in treating the particular disease conditions. As adjunct therapy, the phthalazinediones of the invention may be administered simultaneously, separately, or sequentially for a combined treatment regimen.

The amount of phthalazinedione needed or effective at any one point is cell- and stress-dependent. Optimum dosage and treatment require proper diagnosis of the thiol redox status of the patient's aerobic metabolism in the stressed mitochondria. Administration sufficiently early on in cell or stress development, such that cellular structures or functions have not deteriorated beyond repair, e.g., mitochondria swollen and leaky, cells entering apoptosis, would be particularly beneficial. The thiol redox status must also be frequently monitored, since phthalazinediones can be oxidatively very labile and rapidly expended.

Redox support therapy may be utilized in various disease states, as in:

(1) conditions of metabolic distress, such as redox imbalance or deficiency, metabolic syndrome (Syndrome X), intoxication, diabetes, insulin resistance, hyperglycemia, hypoglycemia, hyperinsulinemia, hypoinsulinemia, hypoadiponectinemia, hyper fatty acidemia, inflammation, tissue injury, and burns;

(2) inflammatory conditions where overactive cells, e.g., lymphocytes, macrophages, astrocytes, or microglia, strain redox defenses and energy production, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis (MS), Guillain-Barré syndrome (GBS, acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculneuritis, acute idiopathic polyneuritis, or Landry's ascending paralysis), Lyme disease, Crohn's disease, ulcer, colitis, hemorrhoids, diarrhea, proctitis, arthritis, osteoarthritis, rheumatoid arthritis, stroke, myocardial infarction, auricular or atrial fibrillation, preexcitation

syndrome (Wolff-Parkinson-White syndrome), arteriosclerosis, atherosclerosis, inflammation of blood vessels that characterize vascular disease in heart and brain, thromboangiitis obliterans (Winiwarter-Buerger disease), other inflammatory conditions of the vascular system, inflammatory conditions of the skin such as dermatitis, eczema, and psoriasis, postoperative complications, peritonitis, bronchitis, and pleurisy;

(3) infectious conditions such as HIV infection, acquired immunodeficiency disease (AIDS), hepatitis, herpes, Lyme disease, toxic shock syndrome, dysentery, erysipelas, hantavirus pulmonary syndrome, respiratory syndromes such as pneumonia and tuberculosis, and other viral, bacterial, or pathogen related conditions or diseases;

(4) neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Cockayne syndrome, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), MS, Bloom's disease, dementia, dystonia, Charcot-Marie-Tooth syndrome (CMT), Dejerine-Sottas syndrome, Roussy-Levy syndrome, Rosenberg Chutorian syndrome, Korsakoff syndrome, Friedreich ataxia, Machado-Joseph disorder, progressive supranuclear palsy (PSP or Steele-Richardson-Olszewski syndrome), GBS, neurally mediated hypotension, pain syndromes such as fibromyalgia, reflex sympathetic dystrophy syndrome (RSDS or complex regional pain syndrome, CRPS), myofascial pain syndrome (MPS), patellofemoral pain syndrome, and other neurodegenerative conditions;

(5) immune disorders, including multiple chemical sensitivity syndrome (MCS), leukemia, GBS, immune deficiency diseases such as AIDS, transplant or graft rejection as in graft-vs-host disease, allergies or allergic reactions, sinusitis or sinus conditions, eczema, psoriasis, asthma, autoimmune diseases or disorders such as systemic lupus erythematosus, scleroderma, and rheumatoid arthritis, Wegener's granulomatosis, diabetes mellitus, Crohn's disease, and Wiskott-Aldrich syndrome;

(6) proliferative diseases, such as cancer, including leukemia, lymphoma, and myeloma, tumors, melanoma, carcinoma, sarcoma, prostatic hypertrophy or adenoma, atherosclerosis, angiogenesis, restenosis, proliferative diseases of the vascular system or endometrium, and other syndromes of uncontrolled cell proliferation or clonal expansion; and

(7) senescence, such as that linked to or caused by genetic abnormalities as in Down syndrome, trichothiodystrophy, ataxia telangiectasia (AT), Bloom's disease, xeroderma pigmentosum (XPD deficiency), and p53 overactivity, and other premature aging or wasting diseases such as muscular dystrophy, age-related macular degeneration (AMD), metabolic syndrome, and aging.

In vitro, at low doses between 20-50 μM, amino derivative phthalazinediones facilitate electron flow at mitochondrial Complex III, thereby increasing ATP production, DNA synthesis, and cell cycling, for cell growth. At an intermediate dose of 100 μM, amino derivative phthalazinediones slow down electron flow, with concomitant effects on ATP production, DNA synthesis, and cell cycling, so that differentiation can proceed. At the high dose of 200 μM, amino derivative phthalazinediones completely stop ATP production, DNA synthesis, and cell cycling in the stressed cell, such that the cell becomes quiescent but does not die.

In tissue culture, small doses of less than 1 μg/ml of an amino phthalazinedione are effective for conditions with chronic losses of cells, especially of stem or developing cells, as in neuroimmunodegenerative syndromes. In conditions where proliferation and apoptotic rates are out of control, including cancer, autoimmunity, infection, and traumas, doses greater than 50 μg/ml of amino phthalazinediones may be required. Successful treatment with the phthalazinedione compounds of the invention therefore depends both on redox diagnosis with repeated assessment of cellular thiol redox status and on maintenance of proper dosage of the phthalazinedione over time. Treatment with phthalazinediones is directed at cells or organs in

which stress has dysregulated thiol redox homeostasis, with resulting energy deprivation and oxidant stress.

In one embodiment of the invention, phthalazinediones may be used to either facilitate or inhibit electron flow in mitochondria, and thus control ATP production and cell fates. Phthalazinediones can serve as redox buffers for the redox- and thiol-sensitive energy producing pathways in the mitochondrion, signaling pathways at the cell plasma membrane, and glutamate uptake and cytokine secretion by astrocytes in the central nervous system (Trotti et al., *J. Biol. Chem.* 271: 5976-5979, 1996). In particular, amino derivative phthalazinediones catalyze disulfide cross-linkages in the adenine nucleotide translocase (ANT) of the mitochondrial anion channels and in the megapores, which prevents energy production, increases production of the potent signal transducers hydrogen peroxide and superoxide (Zamzami et al., *Oncogene* 16: 1055-1063, 1998; Constantini et al., *J. Biol. Chem.* 271: 6746-6751, 1996), and liberates the apoptosis-inducing factors cytochrome *c* and AIF.

With redox support to buffer the redox stress and restore the redox status, the mitochondrion can resume energy production. The cell can then repair stress-induced damages, restock essential substrates, and remove all offenders, in essence treating its own disease. To be successful, any exogenous redox agent must therefore enable the cell to correct the redox aberration, remove the cellular stress, and repair mechanical damages, without toxic side effects. Accordingly, in an embodiment of the invention, phthalazinediones primarily support metabolically distressed cells in a subject, by buffering the intracellular redox status without toxic side effects, to enable the subject's cellular repair or defense functions, rather than treat a particular condition in terms of trying to eliminate the disease or its cause. The following examples further illustrate the invention.

Example 1

Acute Redox Imbalance

In acute metabolic distress, redox-sensitive transcription factors are rapidly activated or under-activated if the oxygen deprivation is not too severe. These transcription factors are triggered by the alternate redox-sensitive mammalian target of rapamycin (mTOR) signal transduction pathway, which is upregulated by low levels of oxygen, ATP, and amino acids. Activated mTOR markedly upregulates DNA synthesis and cellular proliferation, especially in endothelial and vascular smooth muscle cells. Consequently, mTOR is involved in many redox-sensitive proliferative diseases of vascular tissues, including diabetic retinopathy, hypoxia, psoriasis, rheumatoid arthritis, certain tumors, and arteriosclerosis (Humar, *FASEB J.* 16: 771-780, 2002).

Whether mTOR or its upstream activators are redox sensitive is not clear. Nonetheless, oxygen at low dose increases proliferation, whereas oxygen at very low dose (<1%) stops proliferation and activates cell death pathways. A phthalazinedione of the invention, e.g., an amino derivative, increases proliferation at low doses and stops proliferation and activates cell death pathways at high doses. Vascular cell fates are clearly dependent on external redox agents that modulate internal redox status, and the responses and fates of these cells are readily controlled in a dose-dependent manner by external redox agents such as oxygen, amino phthalazinedione, diamide, or permeant thiols that modulate the mTOR-signaling pathway. These redox agents should therefore be useful as redox buffers in controlling the redox-sensitive mTOR pathway, ameliorating vascular proliferative inflammatory diseases, and controlling angiogenesis in tumor growth and inflammatory syndromes, particularly in the brain.

In auricular or atrial fibrillation (AF), arrhythmia results in inefficient pumping of blood, especially to brain, kidney, and coronary vessels. This results in activation of multiple defenses, including increased vascular resistance and inflammatory responses. The loss of blood

perfusion, with resultant anoxia, may also activate the intravascular blood clotting system with further worsening of perfusion, anoxia, and embolization of the blood clots. The lack of blood and food with the resultant nitroso-thiol (NO/O_2^-) imbalance in the cardio-neuro-vascular system leads to nitrosative and oxidative stresses with acute pathologic results.

Multiple hydrophobic amines, e.g., propafenone, sotalol, amiodarone, anticatechols (beta blockers), acetaminophen with diphenylhydrazine, as well as calcium channel blockers and NO donors, have been found to be partially effective in temporarily suppressing the fibrillations in AF by recoupling the failing excitation-contraction coupling (ECC). Therapy to suppress the fibrillatory activity in AF include surgical removal of the overexcitatory input around the pulmonary veins leading to the auricle for medically uncontrollable AF, removal of psychic or emotional stresses that activate catechol secretory pathways, and vasoactive amines and nitric oxide that lower the vascular resistance and thereby lower the cardiac workload. Although many approaches to suppressing the fibrillations in AF are available, none are permanently successful even when used collectively. In addition, none of these treatments are designed to rebalance the failing and deficient NO/O_2^-/sulfhydryl redox system.

However, NO donors plus an amine like hydralazine reduce production of O_2^- by NADH oxidases and reverse the heart failure as well as lower the reactive vascular resistance and increase blood flow to other organs. This suggests that maintenance of a physiologic NO/O_2^- redox potential is crucial for efficient operation of ECC in the cardiovascular system. Effective therapy for maintaining the proper physiologic NO/O_2^- redox balance in the overworked failing cardiovascular system may lie in sustained treatment with a phthalazinedione of the invention.

Example 2

Chronic Redox Imbalance

Metabolic syndrome (Syndrome X) is a condition marked by excessive abdominal fat, diabetes, high blood pressure, and cholesterol problems. The syndrome is caused by the body's inability to use insulin efficiently, due primarily to overeating and inactivity. Food intake with excess deposition of fat in adipose cells causes production and secretion of large amounts of the adipose tissue defense peptide hormones – resistin, leptin, tumor necrosis factor, adiponectin. These collagen- and complement-like peptides facilitate uptake of glucose and combustion of long-chain fatty acids via peroxisome proliferator receptors (PPAR) and mitochondria, with production of heat in the muscle mitochondria, facilitated by activating uncoupling proteins in mitochondria. This removal of the excess fatty acids relieves the fatty acid-induced stress in adipocytes and also lowers levels of toxic, free fatty acids in blood.

Prolonged intake of fatty and sugary foods, with consequent excessive storage of fat in adipose cells and overaccumulation of glucose in blood, causes the overstuffed cells to produce and secrete more inflammatory cytokines, tumor necrosis factor, and resistin (a redox-sensitive adipokine), at the expense of secretion of adiponectin. In aging individuals with overstuffed fat cells, blood levels of tumor necrosis factor and resistin are high; adiponectin and plasminogen-activator inhibitors are low; glucose, free fatty acids, triglyceride, and insulin are high; and the PPARγ/RXR (retinoid X receptor) complexes in fat and muscle cells are under-activated. Vascular accidents in heart and brain, with atherosclerotic plaques, are also greatly increased in these insulin-resistant individuals.

A rapid change in the redox potential from sudden or repeated ingestion of sugars produces superoxide in mitochondria, with the resultant oxidation of mitochondrial membrane lipids and liberation of mitochondrial stress signals including superoxide and hydrogen peroxide, and with subsequent activation of downstream effectors such as protein kinases and NF_KB. The tricarboxylic acid cycle consequently stops, and mitochondria slowly swell and release their

apoptosis signals. The oxidative stress from increased intracellular superoxide irreversibly damages mitochondria and subsequently activates many cellular defenses, including upregulation of superoxide dismutase (SOD), catalase, and mitochondrial NADH oxidase (Vincent et al., *FASEB J.* 19: 638-640, 2005). The multiple cell damages seen in metabolic syndrome and diabetes may be due to repeated and periodic elevations in the redox-labile glucose.

Metabolic syndrome is currently and partially treated with various benzolated thiazolidinediones. These cyclic nitrogenous diketones, which are structurally similar to the phthalazinediones of the present invention, bind to the promoters of PPARγ in the nucleus and activate multiple gene families that activate peroxisomal fatty acid oxidation with increased production of adiponectin and catalase, increased glucose uptake, and increased production of enzymes required for fatty acid synthesis and oxidation and for terminal differentiation in adipocytic precursor cells. At high concentrations, these diketone ligands of PPARγ also block proliferation and activities of activated macrophages, endothelial cells, microglia in brain, and probably proliferating smooth muscle cells in atheromatous plaques.

Thus, benzolated thiazolidinediones are useful in preventing metabolic syndrome and its downstream sequelae, including insulin resistance, vascular degeneration with hypertension, and macrophage proliferation and hyperactivity, with plaque formation and type II diabetes. Like benzolated thiazolidinediones, amino derivative phthalazinediones also stop proliferation and suppress destructive overactivity by inflammatory and adipose cells, with production of many inflammogens. Whether amino phthalazinediones are actually a ligand for PPARγ, can suppress tumor necrosis factor and resistin secretion in adipocytes and macrophages, and increase secretion of adiponectin by adipocytes are under investigation. Whether benzolated thiazolidinediones, like amino phthalazinediones, can bind to benzodiazepine receptors in

mitochondria and alter activity of ion channels and megapores in mitochondria are also not presently known.

Since benzolated thiazolidinediones are very poor redox agents, it is not likely that they directly modulate thiol redox status in mitochondrial voltage-dependent channels or in the permeability pores. In contrast, since the phthalazinediones of the invention possess the dual defensive functions of redox agents, as a redox buffer in mitochondria and as a PPAR activator in the nucleus, phthalazinediones promise a better and more complete therapy for all symptoms of metabolic syndrome. Combination therapy with benzolated thiazolidinediones and phthalazinediones, plus thiols and other redox adjuvants, may be the treatment of choice for prevention of downstream sequelae of metabolic syndrome, such as hyperglycemia, hyper fatty acidemia, increased tumor necrosis factor and resistin levels, hypo-adiponectin-emia, hyper- or hypo-insulin-emia, impaired thiol redox status (hypo-glutathione and cysteine-emia), PPARγ inactivity, and mitochondrial energy uncoupling with elevated H_2O_2, $OHOO^{\bullet}$, and cytoplasmic cytochrome *c*.

Furthermore, recent study has shown that alpha lipoic acid, a thiol source, plus catalase, a potent peroxidase, can acutely prevent the glucose-induced oxidative stress in neurons in vitro (Vincent et al., *FASEB J.* 19: 638-640, 2005). The chronic hyperglycemic oxidative stress in metabolic syndrome or insulin-resistant type II diabetes may be alleviated by combined treatment with a phthalazinedione and alpha lipoic acid or other thiols. The phthalazinedione may be administered at 20 mg/ml in isotonic NaCl intranasally four times daily and injected intramuscularly once daily for three days with 25 mg capsules of alpha lipoic acid at each meal for three days.

Example 3

Acute Inflammation

In inflammatory conditions such as acute infections, wounds, and immune responses, phthalazinediones of the invention can quickly ameliorate the painful redox-induced edematous swelling, facilitate healing, and protect cells from toxic effects of conventional medications or treatments, thus reducing side effects. Edematous inflammatory lesions in intestines, such as duodenal ulcers, ulcerative colitis, and acute vascular injury, are all suppressed to some degree by thiol redox modulators, including dihydrolipoates, reduced biopterins, amino phthalazinediones, and more slowly by glucocorticoids. Healing rates increase, with replacement of the injured epithelial cells by thiol redox-stimulated new cell growth. Thus, phthalazinediones may be utilized as thiol redox modulators to suppress injurious over-reactive inflammatory responses and also facilitate healing and replacement of injured cells.

Example 4

Chronic Inflammation

Where foreign fats such as oxidized fatty acids or cholesterol accumulate, a chronic inflammatory reaction ensues. Signaling and transport processes in lipid-laden membranes falter, and lipid-laden activated macrophages accumulate. Oxidant stress follows, due to deficiency in glucose transport in the lipid-laden membranes and the increased production of oxidants and proteases by the influx of activated macrophages. Chronic localized abscesses form. In vascular tissue, atherosclerosis with occlusive diseases, stroke, myocardial infarction, cystic mastitis, wet macular degeneration, and engorged activated adipocytes result. In all these syndromes, thiol redox homeostasis becomes gravely perturbed and cellular redox damage occurs. Metabolic syndrome with insulin resistance is an early sequela.

Therapies known to modulate the above lipid- and redox-induced syndromes include: (1) thiol redox modulators, especially amino phthalazinediones, to buffer the aberrant thiol redox status; (2) anti-proteases, especially minocycline, to block the excess proteolytic activity and

suppress O_2^- production by the induced NO synthase by macrophages; (3) peroxisome proliferators, to accelerate oxidation of accumulating lipids; (4) caloric restriction, to block input and accumulation of the aberrant lipids and O_2^-; (5) glucocorticoids, to deplete thiols by excretion, inhibit growth, and accelerate death of the overactivated macrophages and microglia; and (6) sepiapterin, to prevent superoxide (O_2^-) production by iNOS in the brain and to prevent activation of the apoptosis stimulating kinase AsK-1, especially in the brain.

Many external therapies are therefore available to modulate and prevent the chronic abscess formations induced by accumulation of aberrant oxidized fats in cell membranes. To fully maintain optimum redox status, various combinations and doses of all six redox approaches may be used. With optimum redox support, the host will repair most damages and induce the means – for example, peroxisome proliferator receptors (PPARs) and adiponectin – to remove the offending fats. In severe defects, specific anti-proteases and antioxidants as those listed above are essential for optimal therapy.

Multiple sclerosis (MS) is due to inflammatory reactions to foreign or misfolded proteins in multiple areas of the brain and retina. This disease results in demyelination of neurons and their oxidative death. Astrocytes surrounding the lesions of demyelinated neurons are highly activated and overexpress the human endogenous retrovirus gene HERV-W, which produces the envelope peptide syncytin. When HERV-W is activated, astrocytes release syncytin and the oxidants NO and O_2^-, which can kill the myelin-producing cells, oligodendrocytes, but not the local neurons, in the corpus callosum. Neurons in the MS brain lack their protective insulating covering, and, in the presence of the glial produced oxidants, die an oxidative (mitochondria-induced) death. Vacuolar neurodegenerative lesions are surrounded by activated and proliferating astrocytes producing cytotoxic oxidants and syncytin.

In the mouse model of astrocytes overproducing syncytin, treatment with antioxidants such as ferulic acid or with inhibitors of NO production was shown to fully prevent the syncytin-induced oligodendrocytic and neuronal death (Antony et al., *Nature Neuroscience*, 7: 1088-1094, 2004; Miller and Greg, *Science, News Focus*, 308: 778-781, May 6, 2005). N-propargyl amines, e.g., rasagiline, were shown to be neuroprotective and to prevent various oxidant-associated degenerative processes in the central nervous system (CNS) (Mandel et al., *Brain Research Reviews*, 48: 379-387, 2005). Antioxidants and nitric-oxide releasing derivatives of nonsteroidal inflammatory drugs also prevent the glia-induced neurodegeneration induced in vivo by lipopolysaccharides, excitotoxins, ethylated pyridines, synucleins, or amyloid peptides that accumulate in Alzheimer's disease.

Recent findings implicate hydrophobic peptide-induced glial malfunctions as a primary cause of oligodendrocytic and neuronal degeneration in CNS diseases such as MS, Parkinson's disease, and Alzheimer's disease (Mandel et al., *Brain Research Reviews*, 48: 379-387, 2005; Chen and Feany, *Nature Neurosci.*, 8: 657-663, 2005; Glasson et al., *Science*, 290: 985-989, 2000). Since antioxidants or drugs that induce production of antioxidant defenses, e.g., N-propargyl-aminoindans, can largely prevent the degenerations in many of these CNS diseases, it is likely that glia-mediated uncontrolled oxidant stress is responsible for the progressive loss of neurons or oligodendrocytes.

Treatment for these diseases may be effective with agents that can: (1) localize in the brain; (2) sop up free radicals; (3) prohibit production of ROS by mitochondria; (4) activate cellular defenses against oxidant stresses; (5) inhibit solvation (phosphorylation or nitration) or hyperproduction of the cytotoxic hydrophobic protein fragments such as synucleins, amyloids, polyglutamates, and prions; or (6) maintain appropriate redox equilibrium, especially in mitochondria, and thereby inhibit activation of apoptotic pathways and sustain energy pathways.

A phthalazinedione such as N-acetyl-5-amino-2,3-dihydrophthalazine-1,4-dione in combination with reduced thiols or rasagiline, can gain access to the CNS and maintain physiologic redox potentials to prevent the above neurodegenerative diseases of the CNS. The phthalazinedione may be administered intranasally three times per day as a solution containing 100 mg of phthalazinedione and 100 mg glutathione in 5 ml of isotonic saline at pH 7.5. In addition, rectal suppositories containing 100 mg of phthalazinedione may be given every other day.

Example 5

Virus-Induced Redox Imbalance

Infection with the human retrovirus HIV and the murine retrovirus MOMU-LV-ts1 results in chronic syndromes of neuroimmunodegeneration from NO/O_2^- redox imbalance. Oncogenic retroviral infections from HIV or MOMU-LV-ts1 cause degenerative changes with severe losses of brain cells, immune cells, and germ cells. Other cells like astrocytes and microglia in brain become activated, secrete nitric oxide and superoxide, and grow and accumulate excessively. This imbalance in cell growth and death rates eventually leads to fatal immune and neuronal deficiency syndromes with subsequent transformation in some cells.

In humans, viruses infect and proliferate primarily in the mature developed T cells. In mice, viruses infect and proliferate primarily in undeveloped T cells and glia. The viruses prevent cellular development and cause premature cell death or slow transformation. Chronic bacterial infections and dementia occur. In both human and murine diseases, the loss of T cells and neurons is mediated in part by oxidative stress. In brain, the neurotoxic stress is induced by infected microglia; in thymus, the immunotoxic fatal stress occurs in infected T cells. In both T cells and neurons, microglial NADPH oxidase activated by the viruses, superoxide, and

peroxides induces the oxidative damage (Qin et al., *FASEB J.* 19: 550-557, 2005; Li et al., *FASEB J.* 19: 489-496, 2005).

In mice infected at two days of age with the tsl virus, hind limb paralysis occurs with severe wasting, especially of immune organs. In humans infected with HIV, severe immune deficiency with sensory and motor neuropathy also results. In these wasting syndromes with disordered life and death pathways in various cells, some therapeutic attempts with thiol redox modulators other than phthalazinediones have been partially successful (Lynn and Wong, *Neuroimmunomodulation* 4: 277-284, 1997; Yan et al., *FASEB J.* 15: 1132-1138, 2001). In these studies, phthalazinediones combined with thiol redox modulators appear to be sufficient to maintain survival and an adequate intracellular thiol redox potential in brain and thymus.

Since retroviruses activate caspase-dependent apoptosis, and since thiol redox modulators, oxidized and reduced, regulate caspase production from procaspases (Nobel et al., *Chem. Res. Toxicol.* 10: 636-643, 1997), thiol redox modulators in mitochondria, especially an amino derivative phthalazinedione together with dexamethasone, will prevent both the loss and the hyperplasia of cells dysregulated by the viruses. In ts1 viral infection, daily treatment with an amino phthalazinedione prevents both thymic atrophy and brain lesions from neuronal degeneration with astroglial reaction. Viral titers in brainstem, but not in thymus, are markedly decreased.

Example 6

Redox Imbalance Induced by Oxidant Stress

Cellular signaling pathways for survival and growth involve the phosphorylation of proteins such as epidermal growth factor receptor (EGFR), mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases (ERK), phosphoinositol-3 kinase, protein kinase B, and inhibitor $_{K}$B kinase (IKK). Signal pathways for cell death involve the phosphorylation of

proteins such as c-Jun N-terminal kinase (JNK), p38, and p53. Such phosphorylation effects stress-inducible transcription factors, e.g., p53 and NF_KB.

Oxidants such as H_2O_2 activate intracellular phosphorylation cascades (Wang et al., *J. Biol. Chem.* 275: 14624-14631, 2000). Low doses of H_2O_2 rapidly activate survival pathways and downregulate apoptotic factors Bad and caspase 9. Higher doses of or prolonged exposure to H_2O_2 activate cell death pathways. Thus, oxidants can activate pathways for either cell survival or cell death. However, H_2O_2 is not a buffer and cannot maintain optimal redox potentials sufficient to maintain cell signaling and growth. H_2O_2 also does not scavenge the excess ROS produced by activated cell growth pathways.

In the presence of oxidants, the redox-sensitive nuclear poly (ADP-ribose) polymerase is rapidly activated. Oxidants also open the redox-sensitive permeability transition pores and anion channels in mitochondrial membranes. This releases AIF, which is then taken up by the poly (ADP-ribose) polymerase-activated nucleus to initiate chromatin condensation. Chromatin condenses, mitochondria swell, and mitochondrial processes become uncoupled. Mitochondria then produce more oxidants and less ATP. In addition, the oxidants rapidly induce reshuffling of plasma membrane ionic phospholipids with surface exposure of phosphatidyl serine, which rapidly alters permeability and transport activities in the plasma, mitochondrial, endoplasmic reticulum, and nuclear membranes. The plasma and endoplasmic reticulum membranes leak calcium, which activates innumerable signal transduction pathways, including ATM, mTOR, and p38 MAPK (Yu et al., *Science* 297: 259-263, 2002; De Giorgi et al., *FASEB J.* 10: 607-609, 2002).

Thus, redox potentials of cellular membranes can be rapidly altered by brief exposure to permeant oxidants, and cell death rapidly ensues through both apoptotic and necrotic changes. Membrane-permeant reductants, such as the phthalazinediones of the invention combined with

reduced biopterins and thiols, should be able to buffer and maintain the proper redox status in membranes of oxidant-stressed organelles, e.g., in acute neurodegenerative syndromes such as hypoxia, glucose deficient states, or chronic inflammatory states such as Parkinson's disease, Alzheimer's disease, ALS, MS, AT, or aging.

Moreover, the ability of phthalazinediones, especially amino derivatives, to provide both oxidizing and reducing potential to mitochondria, peroxisomes, and cytoplasmic signaling pathways makes these compounds an ideal in vivo redox buffer capable of dictating cell fates. The phthalazinediones of the invention may thus be used in modulating aberrant phosphorylation signaling syndromes involved in cell growth and death, e.g., in disease states where signal-induced cell death rates exceed cell growth rates (various neurodegenerative syndromes such as Alzheimer's disease, AT, Parkinson's disease, multiple system atrophy, or AIDS, etc.) or autonomous growth signaling rates exceed cell death rates (cancers, AT, trichothiodystrophy, or hyperinflammatory syndromes, etc.).

Example 7

Neurodegeneration

In disease states where aberrant peptides slowly accumulate in the brain, early neuronal death with glial hypertrophy occurs. In Huntington's disease, polyglutamine sequences or tracts accumulate in the huntingtin protein. These tracts bind to and inhibit transcription complexes containing Sp-l and TAFII130 coactivators. Transcription rates decrease, and dysregulated neurons slowly die, first in the caudate nucleus and later in the hippocampus. Alzheimer's and Parkinson's diseases are both due to overactivation of microglia by aggregates of synuclein or amyloid with resultant oxidant stress. In these neurodegenerative syndromes, presenilin or synucleins may be responsible for accumulation of the Lewy bodies and B-amyloid peptides in plasma, mitochondrial, or endoplasmic reticulum membranes of the cell and also responsible for

the neuronal losses in these syndromes. These toxic peptides, like the polyglutamine proteins in Huntington's disease, also lead to astroglia-induced imbalances in thiol redox metabolism, with cell swelling, membrane leakiness, and mitochondrial necrosis.

Non-proliferating, non-replaceable neurons usually die from metabolic redox imbalances, rather than from programmed death. Therefore, neuronal death in Huntington's disease is likely to be redox-mediated and induced by activation of redox-sensitive cytokines, metalloproteases, and ROS by activated migroglia and astrocytes (Chen et al., *Nature Med.* 6: 797-801, 2000). In that case, reduced thiol redox-modulators, which at low doses promote cell growth and longevity in redox-suppressed cells, should prove to be useful therapy (Dunah et al., *Science* 296: 2238-2243, 2002). Maintenance of the thiol redox status with reduced thiol redox modulators, especially an amino derivative phthalazinedione and cysteine, should prevent or delay the neuronal death in these degenerative diseases (Wolfe and Selkoe, *Science* 296: 2156-2157, 2002; Welhofen et al., *Science* 296: 2215-2218, 2002).

Example 8

Neuronal Overactivity and Excitotoxicity

In Parkinson's disease, neurons in the subthalamic nucleus (STN) are overactive. Downstream effects of STN neuronal overactivity in the substantia nigra reticulata, globus pallidus, and motor thalamus are likely responsible for multiple movement disorders – bradykinesia, rigidity, and tremors – accompanying the disease. Although the cause of overactivity in only a few STN neurons is not known (Levy et al., *Brain* 124: 2105-2118, 2001; Luo et al., *Science* 298: 425-429, 2002; Limousin et al., *New England J. of Med.* 339: 1105-1111, 1998; Alvarez et al., *Movement Disorders* 16: 72-78, 2001), the parkinsonism symptoms can be promptly relieved with suppression of the oscillatory neuronal activity by injecting agents such as lidocaine and muscimol intra-STN or by chronic electrical stimulation.

When used in treating the symptoms of Parkinson's disease, agents such as the amino derivative phthalazinediones of the invention can modulate thiol redox status, downregulate mitochondrial energy production, and gain access to the overactivated STN neurons. The phthalazinedione can thus potentially suppress the neuronal overactivity and the increased energy expenditures required to maintain the excessive and imbalanced electrical oscillation, thereby also modulating the downstream network processes responsible for the parkinsonism symptoms. In ts1 virus infected mice, for example, daily intraperitoneal injections of 200 μg of 4-sodium-amino-phthalazinedione significantly delay the progress of the associated movement disorder with paralysis. Whether this is due to suppression of oscillatory activity in neurons, suppression of virus-induced astrocytic inflammatory responses, or both, is under investigation. Amino derivate phthalazinediones should, however, suppress both the neuronal and astrocytic overactivity.

In NMDA-induced neuronal excitotoxicity, microglial cells secrete the inflammatory products – glutamate, quinolinic acid, inflammatory cytokines, tumor necrosis factor, IL-1B, superoxide, and nitric oxide – that are likely responsible for neuronal necrosis (Tikka and Kolstinaho, *J. Immunol.* 166: 7527-7533, 2001). These excitotoxins rapidly perturb redox homeostasis in neurons, which slowly die, and in astroglia, which become activated and proliferate. Minocycline, a cyclic polyhydroxy ketonic amide that suppresses mitochondrial activity, has been shown to prevent both the NMDA-induced proliferation of astrocytes and toxic secretions by activated astrocytes, as well as the subsequent neuronal death (Tikka and Kolstinaho, *J. Immunol.* 166: 7527-7533, 2001). This suggests that cell death in neurons, secretory proliferative activation of astroglia, and proliferative responses in spinal cord astrocytes are all mitochondrial redox-mediated. Therefore, correction of thiol redox status by phthalazinediones of the invention should modulate the fate of these brain cells.

Example 9

Immune Deficiency

In immune deficiency syndromes such as AIDS, antioxidant treatment with thiols has been attempted with minimal success. Morphine derivatives such as dextromethorphan bind with high affinity to peptide receptors on astroglia and inhibit activation and oxidant production by lipopolysaccharide (LPS)-stimulated microglia to prevent neuronal degeneration (Li et al., *FASEB J.* 19: 489-494, 2005). Effective combination therapy for immune deficiency may include antioxidants with antimicroglial agents and antiviral therapies, to provide proper redox support to enable cells to mobilize their own defenses sufficiently to eliminate the virus. For example, therapy may include administering an isotonic solution containing 25 mg/ml of a phthalazinedione of the invention and 12 mg/ml of sodium glutathione intranasally four times a day, an isotonic solution containing 20 mg/ml of a phthalazinedione by intramuscular injection every other day, a suppository containing 100 mg of phthalazinedione on alternate days, and 15 mg of dextromethorphan twice a day.

Example 10

Hyperproliferation

Controlling entry and exit of small molecules – Ca^{2+}, H^+, O_2^- and substrate anions – through the redox- and voltage-sensitive mitochondrial channels and pores affects cell fates. These channels and pores modulate concentrations of intracellular cations Ca^{2+} and H^+, intracellular anions ADP, ATP, malate, and glutamate, and intracellular thiols, glutathione, cysteine, thioredoxin, and biopterin. These channels can indirectly modulate redox-sensitive sites in signal transduction, proliferation, development, transcription, apoptosis pathways, and necrosis pathways, thereby dictating cell fates.

Many agents that can directly modulate these pores are in use for antiproliferative therapies, notably as treatments for hyperproliferative syndromes and cancer (Miccoli et al., *J. Nat. Cancer Inst.* 90: 1401-1406, 1998; Ravagnan et al., *Oncogene* 18: 2537-2546, 1999; Larochette et al., *Exp. Cell Res.* 249: 413-471, 1999). Three broad classes of modulators are in use – lipophilic peptides, lipophilic amines, and thiol redox-reactive cyclic amines.

Lipophilic peptides are useful as antiproliferative and anti-inflammatory therapies. These peptides, primarily Bax, Bcl-2, and cyclosporine A, either block or bypass mitochondrial transmembrane channels by creating pores of oxidized polymerized peptides of variable permeability in the mitochondrial outer membranes (De Giorgi et al., *FASEB J.* 10: 607-609, 2002). The redox-insensitive lipophilic benzo amines are useful in cancer therapy. Diazepam and lonidamine, for example, bind to mitochondrial benzodiazepine receptors in the mitochondrial matrix, block mitochondrial electron flow and ATP synthesis, and induce apoptotic and necrotic death in rapidly growing cells (Miccoli et al., *J. Nat. Cancer Inst.* 90: 1401-1406, 1998). As for the thiol redox-sensitive cyclic amines, their usefulness in mitochondrial transmembrane pore modulation has not been fully explored.

Diamide (diazenedicarboxylic acid), the thiol cross-linking non-cyclic amine, completely opens mitochondrial transmembrane pores, which causes the mitochondrial transmembrane potential to collapse, with dissipation of H^+ (pH) gradients, production of O_2^-, and release of the apoptosis inducing factors cytochrome *c* and AIF. Consequently, cells slowly die, depending on their supplies of reduced thiols, primarily glutathione (Zamzami et al., *Oncogene* 16: 1055-1062, 1998). However, although a potent eradicator of cancer cells and other proliferating cells of the host, this cross-linking non-cyclic amine is too toxic for clinical uses.

Amino phthalazinediones of the present invention, as cyclic lipophilic amines like biopterins and rhodamines, accumulate electrostatically in mitochondrial transmembrane pores

and accept and release both electrons and protons, and thereby reversibly serve as both electron and pH buffers in the polarized channels and pores, with toxic side effects. Consequently, both the ionic and oxidative status of the labile sulfhydryl in ANT can be maintained. The cyclic amines thus affect voltage in the channels, and fluxing electrons are either trapped by O_2 as O_2^- or proceed downstream with production of H_2O and ATP. At low doses of these compounds, electron flow increases, electrons proceed downstream to H_2O, ATP production increases, DNA synthesis and cell proliferation increase, and cell death is aborted. At high doses, electron flow to H_2O decreases, substrate anion translocations falter, membrane potential declines, ATP production ceases, as does electron flow, and cells go into a quiescent G_0/G1 phase or apoptosis.

With the lipophilic tetramethyl-rhodamine, many electrons are shunted directly to O_2, with the result that O_2 accumulates, mitochondrial transmembrane pores open with loss of membrane potential, and apoptotic and necrotic pathways become activated (De Giorgi et al., *FASEB J.* 10: 607-609, 2002). Phthalazinediones, such as amino derivatives, combined with reduced biopterins, thiols, or lipoic acid, can modulate electron flow to O_2^- or H_2O (Lynn et al., unpublished). Specifically, at low doses, amino phthalazinediones upregulate the host's immune responses to eradicate cancerous cells. At high doses, amino phthalazinediones stop proliferation of hyperproliferating cancerous cells. Thus, by upregulating or downregulating particular cells, amino phthalazinediones are useful in cancer treatment (Tzyb et al., *Int. J. Immunorehabilitation* 12: 398-403, 1999).

Modulation of mitochondria by these bifunctional cyclic phthalazines is most effective in controlling cell fate in proliferating cells that are deficient in biological thiol redox buffers (Armstrong and Jones, *FASEB J.* 16: [online], June 7, 2002; Larochette et al., *Exp. Cell Res.* 249: 413-471, 1999) or in proliferating cells deficient in cell cycle checkpoint genes (Yan et al., *Genes and Dev.*, in press). Thus, redox- and pH-sensitive amines that buffer by dually

modulating mitochondrial transmembrane pores and anion channels are clinically useful both in preventing and treating hyperproliferative states such as cancers.

Example 11

Premature Aging

Age-related macular degeneration (AMD) results in irreversible loss of central vision from a premature degeneration of specific neurons with extracellular accumulation of lipofuscin and protein aggregates known as drusen bodies. Its causes are not clear, but risk factors include old age, smoking, hypertension, obesity, diet, excess light, ischemic conditions, and genetic defects (Klein et al., *Science* 308: 385-389, 2005; Haines et al., *Science* 308: 419-421, 2005). AMD pathology is reminiscent of other neurodegenerative diseases in which aggregates of various aberrant peptides and their binding partners accumulate excessively, as in Parkinson's, Alzheimer's, and Huntington's diseases.

In all these syndromes, inflammatory cells in the lesions are scarce, suggesting that the neurotoxicity may be due to toxic oxidants or nutrient deficiencies in blood. In AMD, as well as in other age-related syndromes, peripheral signs of early inflammation with oxidant stress are usually present. Redox therapy to restore some vision and prevent progression of the neurodegeneration includes correcting the blood and macular redox imbalance, to induce the stressed microglia to remove the retinal drusen bodies and to prevent the redox-induced death of the metabolically highly active macular neurons. Therapy may include 100 mg of a phthalazinedione of the invention combined with 50 mg glutathione in 8 ml isotonic sodium chloride by intranasal inhalation four times per day for two weeks and 20 mg tablets of dinitroisosorbide, a source of nitric oxide, twice a day.

Example 12

Gene-Induced Premature Aging

In regulatory gene-dependent syndromes of premature aging, including ataxia telangiectasia (AT), xeroderma pigmentosum (XPD deficiency), Down syndrome, trichothiodystrophy, Bloom's disease, and p53 over-activity (De Boer et al., *Science* 296: 1276-1281, 2002; Tyner et al., *Nature* 415: 45-50, 2002), life and death cycles of specific cell types are aberrant. Treatments in vitro and in vivo with thiol redox modulators have been partially successful.

In mice with ataxia telangiectasia gene (ATM) deficiency, the atm-/- cells show early signs of oxidative aging and degeneration in the cerebellum, gonads, thymus, and venules or capillaries. Ataxia, immune deficiency with oncogeneity, sterility, and angiogenic failures are the clinical results. Research shows that atm-/- lymphoid cells, which in culture or in vivo rapidly die an oxidative death, especially when oxidatively or mitogenically stressed, can be fully protected by thiol or phthalazine antioxidants, i.e., by supporting the redox deficient host and thus modulating the gene-induced NO/O_2^- redox imbalance. This research suggests that a primary function of ATM is to regulate intracellular redox potentials. ATM most likely regulates redox potentials by increasing availability of thiols to the cell.

In the AT model, early pretreatment with dexamethasone, the glutathione secretagogue, completely prevents the excessive proliferation and development of the fatal thymic cancer. Other thiol redox modulators, such as N-acetyl cysteine and dehydrolipoic acid, also delay the premature degeneration of cells and the thymomas. Thiol redox modulators also correct the delayed differentiation and excessive production of DNA in ATM-deficient lymphoid cells (Yan et al., *FASEB J.* 15: 1132-1138, 2001; Lynn et al., unpublished). However, in the ATM-deficient mice, treatment is fully successful only if the thiol redox modulators are applied early in development or disease progression, before two weeks of age and before tumor development.

Dexamethasone alone completely prevents tumor formation if given to 10-day old ATM-deficient mice for three weeks, but does not suppress tumor growth or increase longevity if given at physiologic doses at three months of age. Whether amino phthalazinediones with other thiol redox modulators, which suppress growth of non-transformed ATM-deficient cells in vitro, can fully suppress tumors in vivo, without toxicity, has not been rigorously evaluated. Cross-linking redox modulators such as diamide, menadione, and oxidized phthalazines are known to stop cell growth, activate caspases, and initiate apoptosis in some tumor cells (Pias and Aw, *FASEB J.* 16: 781-790, 2002).

In XPD deficiency, DNA transcription and repair functions are impaired, resulting in multiple symptoms of early aging (De Boer et al., *Science* 296: 1276-1281, 2002). The XPD gene of the xeroderma pigmentosum family codes for a helicase. In XPD-deficient mice or humans, wasting, loss of subcutaneous fat and muscle cells, gray brittle greasy hair with hyperplasia of sebaceous and mammary glands, severe osteoporosis, atrophic germ and stem cells, and immunoneurodegenerative and hyperplastic changes all occur prematurely.

The failure to maintain normal numbers of cells or normal amounts of cellular thiols, at least in the brittle hair, suggests that a global thiol redox deficiency is responsible for the progressive wasting and chronic cell losses. Since amino phthalazinediones with reduced thiol redox modulators, at low dosage, stimulate cell growth and maintain thiol redox status in cells, treatment with appropriate amounts of reduced thiol redox modulators plus the phthalazinediones of the present invention will likely prevent the premature aging in regulatory gene deficiencies such as XPD deficiency.

In p53 over-activity, cell growth and tumor formation are constantly suppressed by the growth suppressor gene p53. The p53 protein product is a potent transcription factor that suppresses cell growth and DNA synthesis and is also an activator of genes that induce oxidative

stress and apoptosis. As a consequence, signs of premature aging and replication senescence develop, with chronic cell losses in skin, hair, bone, adipose tissue, and the immune system (Tyner et al., *Nature* 415: 45-50, 2002). The phthalazinediones of the invention may be used to maintain cellular replication pathways by modulating the p53-induced thiol redox imbalance, override the p53-induced suppression, maintain a balance between apoptotic or proliferation pathways, and thereby prevent the degenerative sequelae.

Example 13

Acute Cardiovascular Redox Imbalance

Maintenance of the appropriate NO/O_2^- redox balance is acutely critical for efficient excitation-contraction coupling (ECC) in the cardiovascular system. Agents that can generate NO (nitric oxide) and also dissipate O_2^- (superoxide) and other radicals can maintain physiologic NO/O_2^- levels. Such agents ameliorate the pathologic consequences of redox stresses in vascular systems, including hypertension, heart failure, and aberrant ECC. In hypertensive subjects with dilated ventricles and severe heart failure, treatment with the NO donor known as isosorbate dinitrate, plus hydralazine, an inhibitor of the NADH oxidase that produces superoxide, ameliorates both the hypertension and the heart failure (Munzel et al., *J. Clin. Invest.* 98: 1465, 1998; Taylor et al., *NEJM* 151: 2049-2057, 2004).

Under cardiovascular mechanical stress, contracting cells may become relatively deficient in NO, and with the obligatory increase in respiration, will produce excess O_2^-. Neither NO addition nor suppression of O_2^- production is apparently able to restore the proper redox balance or improve the failed ECC (Hare, J.M., *J. Mol. Cell Cardiology* 35: 219-229, 2003) or ameliorate the severe heart failure in these patients (Taylor et al., *NEJM* 151: 2049-2057, 2004). Treatment with phthalazinediones of the invention may modulate physiologic NO/O_2^- levels and alleviate the symptoms.

Example 14

Toxin-Induced Redox Imbalance

Multiple chemical sensitivity (MCS) is a decompensating syndrome in which individuals develop hypersensitivities to multiple environmental toxins, e.g., exhaust fumes from vehicles, factories, garbage dumps, or explosives, perfumes, sulfur oxides, nitrous oxide, and cyclic hydrocarbons produced by molds or plants. Inhalation of such toxins causes symptoms like fatigue, weakness, loss of equilibrium, sensory impairments in smell, taste, hearing, vision, and sensation, cognitive impairments in memory, concentration, and motivation, and motor symptoms that vary from muscle and bone wasting to athetoid, epileptiform, or fibrillary movements. External signs of premature aging, e.g., graying or loss of hair, wrinkling of skin, bradykinesia, or signs of Syndrome X (type II diabetes, hypertension, hyperlipidemia, atrial fibrillation) are usually present at least to some extent.

The multiple incapacitating symptoms of MCS are rapidly induced by inhaling trace amounts of the noxious substance. Removal of the inciting inhalant prevents the acute symptomology but does not eliminate the hypersensitivity. The most characteristic symptom is an inability to "get up and go," or lead a productive life. The symptoms may become permanent or persist for months unless the chronic exposure is eliminated. The inhaled toxins are thought to induce redox imbalances in the naso-olfactory system and brain, and preliminary findings suggest that the functional impairments in MCS are due to acute imbalances in NO/O_2^- and thiol redox potentials in the naso-olfactory system.

Studies also suggest that the neuromuscular and cognitive dysfunctions in MCS may represent redox imbalances in pathways controlling neurotransmission. The constant neurotransmission in neurons, with its repetitive separation of charges, obligatorily produces large amounts of reactive oxygen and nitrogen species. Under stress, these radicals may

accumulate with pathologic results. The cell's major redox buffers for controlling these signaling radicals, e.g., reduced thiols such as glutathione, cysteine, and thioredoxins, are rapidly consumed under redox stresses such as anoxia, nutrient or ion deprivation, and aberrant peptide accumulation.

The symptomatic redox imbalances may be corrected by appropriate redox support to the impaired naso-olfactory system. Treatment with thiol redox support, i.e., inhaled or intramuscular glutathione, has been partially successful. Intranasal inhalation of a solution of 25 mg/ml of a phthalazinedione of the invention and 12 mg/ml of sodium glutathione in isotonic NaCl four times per day for three days should remove the incapacitating weakness and the sensory and cognitive symptoms within 48 hours. Inhalation of the reduced phthalazinedione, along with a reduced thiol, can quickly but temporarily rebalance the thiol redox imbalance in the patient's brain and quickly ameliorate some of the symptoms. Motor symptoms may be reversed by prolonged treatment with higher doses of this combined redox therapy.

The foregoing material describes various aspects of the invention and how it may be practiced. The description is not intended to be exhaustive of the many different embodiments of the invention. Although the foregoing invention has been described in some detail by way of illustration and example, to aid understanding, it will be readily apparent to those of ordinary skill in the art, in light of the teachings of this invention, that certain changes and modifications may be made to the invention without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A method for modulating metabolic distress, comprising administering to a subject a therapeutically effective amount of a phthalazinedione or its pharmaceutically acceptable salt, ester, solvate, hydrate, analogue, metabolite, enantiomer, isomer, tautomer, amide, derivative, prodrug, or free base in treating a condition selected from the group consisting of multiple chemical sensitivity syndrome, Charcot-Marie-Tooth disease, Dejerine-Sottas syndrome, Roussy-Levy syndrome, Rosenberg Chutorian syndrome, Korsakoff syndrome, Friedreich ataxia, Machado-Joseph disorder, progressive supranuclear palsy, Guillain-Barré syndrome, Hodgkin's disease, Wegener's granulomatosis, toxic shock syndrome, systemic lupus erythematosus, scleroderma, Lyme disease, auricular or atrial fibrillation, thromboangiitis obliterans, peritonitis, hantavirus pulmonary syndrome, Wiskott-Aldrich syndrome, preexcitation syndrome, insulin resistance, hyperglycemia, hyper fatty acidemia, hyperinsulinemia, hypoadiponectinemia, metabolic syndrome, xeroderma pigmentosum, ataxia telangiectasia, trichothiodystrophy, Down's syndrome, burns, psoriasis, rheumatoid arthritis, leprosy, a condition associated with a regulatory gene defect, tumors associated with the p53 transcription factor, brain, or thymus, and a neurological disorder, wherein the neurological disorder is selected from the group consisting of Parkinson's disease, Huntington's disease, Bloom's disease, amyotrophic lateral sclerosis, Cockayne syndrome, and dementia.

2. The method as in claim 1, wherein the phthalazinedione is an amino derivative.

3. The method as in claim 2, wherein the phthalazinedione is a haloamino, alkylamino, acylamino, alkanolamino, alkenylamino, alkoxyamino, haloalkylamino, allylamino, or sulfhydrylamino derivative.

4. The method as in claim 1, wherein the phthalazinedione is administered with an adjuvant, diluent, carrier, excipient, or stabilizer.

5. The method as in claim 4, wherein the phthalazinedione comprises a pharmaceutically acceptable form selected from the group consisting of tablet, capsule, granule, powder, solution, suspension, microsphere, liposome, colloid, lyophilized composition, gel, lotion, ointment, cream, spray, and suppository.

6. The method as in claim 5, wherein the phthalazinedione is administered by a means selected from the group consisting of intravenous, intramuscular, intraperitoneal, subcutaneous, oral, nasal, mucosal, transdermal, parenteral, vaginal, and rectal.

7. The method as in claim 6, wherein the method is used in combination with a standard treatment selected from the group consisting of radiation, chemotherapy, laser therapy, surgery, medication, and nutrition.

8. A method for modulating metabolic distress, comprising administering to a subject a therapeutically effective amount of a phthalazinedione or its pharmaceutically acceptable salt, ester, solvate, hydrate, analogue, metabolite, enantiomer, isomer, tautomer, amide, derivative, prodrug, or free base with a compound selected from the group consisting of a glutathione, glucocorticoid, dexamethasone, cysteine, lipoic acid, biopterin, hydralazine, rasagiline, thioredoxin, ferulic acid, minocycline, menadione, tetracycline, isosorbate dinitrate, and dextromethorphan.

9. The method as in claim 8, wherein the method is used in treating inflammatory conditions.

10. The method as in claim 8, wherein the method is used in treating infectious conditions.

11. The method as in claim 8, wherein the method is used in treating neurological disorders.

12. The method as in claim 8, wherein the method is used in treating immune disorders.

13. The method as in claim 8, wherein the method is used in treating proliferative diseases.

14. The method as in claim 8, wherein the method is used in treating cellular senescence.

15. The method as in claim 8, wherein the phthalazinedione is an amino derivative.

16. The method as in claim 15, wherein the phthalazinedione is a haloamino, alkylamino, acylamino, alkanolamino, alkenylamino, alkoxyamino, haloalkylamino, allylamino, or sulfhydrylamino derivative.

17. The method as in claim 16, wherein the phthalazinedione is a bromoamino, chloroamino, fluoroamino, iodoamino, methylamino, ethylamino, propylamino, isopropylamino, methanoylamino, ethanoylamino, propanoylamino, hydroxylamino, carboxylamino, methanolamino, ethanolamino, propanolamino, methenylamino, ethenylamino, propenylamino, methoxyamino, ethoxyamino, propoxyamino, or dimethylamino derivative.

18. The method as in claim 15, wherein the phthalazinedione is 5-amino-2,3-dihydrophthalazine-1,4-dione, 6-amino-2,3-dihydrophthalazine-1,4-dione, or 5-amino-2,3-dihydrophthalazine-1,4-dion-8-yl.

19. The method as in claim 17, wherein the phthalazinedione is N-bromo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-chloro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-fluoro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-iodo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethyl-5-amino-2,3-

dihydrophthalazine-1,4-dione, N-propyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-isopropyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-hydroxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-carboxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, or N,N-dimethyl-5-amino-2,3-dihydrophthalazine-1,4-dione.

20. The method as in claim 8, wherein the phthalazinedione is administered with an adjuvant, diluent, carrier, excipient, or stabilizer.

21. The method as in claim 20, wherein the phthalazinedione comprises a pharmaceutically acceptable form selected from the group consisting of tablet, capsule, granule, powder, solution, suspension, microsphere, liposome, colloid, lyophilized composition, gel, lotion, ointment, cream, spray, and suppository.

22. The method as in claim 21, wherein the phthalazinedione is administered by a means selected from the group consisting of intravenous, intramuscular, intraperitoneal, subcutaneous, oral, nasal, mucosal, transdermal, parenteral, vaginal, and rectal.

23. The method as in claim 22, wherein the method is used in combination with a standard treatment selected from the group consisting of radiation, chemotherapy, laser therapy, surgery, medication, and nutrition.

24. The method as in claim 8, wherein the phthalazinedione is administered in an amount of about 0.01 mg/kg to about 100.0 mg/kg of body weight.

25. The method as in claim 24, wherein the phthalazinedione is administered in an amount of about 0.05 mg/kg to about 50.0 mg/kg of body weight.

26. The method as in claim 25, wherein the phthalazinedione is administered in an amount of about 0.1 mg/kg to about 10.0 mg/kg of body weight.

27. The method as in claim 8, wherein the phthalazinedione is administered in an amount of about 1.0 mg per day to about 10,000.0 mg per day.

28. The method as in claim 27, wherein the phthalazinedione is administered in an amount of about 50.0 mg per day to about 5000.0 mg per day.

29. The method as in claim 28, wherein the phthalazinedione is administered in an amount of about 100.0 mg per day to about 1000.0 mg per day.

30. The method as in claim 27, wherein the phthalazinedione is administered in an amount of about 1.0 mg, 10.0 mg, 50.0 mg, 100.0 mg, 200.0 mg, 300.0 mg, 400.0 mg, 500.0 mg, 600.0 mg, 700.0 mg, 800.0 mg, 900.0 mg, 1000.0 mg, 2000.0 mg, 3000.0 mg, 4000.0 mg, 5000.0 mg, 6000.0 mg, 7000.0 mg, 8000.0 mg, 9000.0 mg, or 10,000.0 mg per day.

31. A pharmaceutical composition comprising a phthalazinedione or its pharmaceutically acceptable salt, ester, solvate, hydrate, analogue, metabolite, enantiomer, isomer, tautomer, amide, derivative, prodrug, or free base; a compound that is an amino acid, antibiotic, antiviral agent, antiinflammatory agent, antioxidant, immunomodulator, reductant, oxidative protector, steroid, or vitamin; and one or more pharmaceutically acceptable excipients.

32. The pharmaceutical composition of claim 31, wherein the phthalazinedione is an amino derivative.

33. The pharmaceutical composition of claim 32, wherein the phthalazinedione is a haloamino, alkylamino, acylamino, alkanolamino, alkenylamino, alkoxyamino, haloalkylamino, allylamino, or sulfhydrylamino derivative.

34. The pharmaceutical composition of claim 33, wherein the phthalazinedione is a bromoamino, chloroamino, fluoroamino, iodoamino, methylamino, ethylamino, propylamino, isopropylamino, methanoylamino, ethanoylamino, propanoylamino, hydroxylamino, carboxylamino, methanolamino, ethanolamino, propanolamino, methenylamino, ethenylamino, propenylamino, methoxyamino, ethoxyamino, propoxyamino, or dimethylamino derivative.

35. The pharmaceutical composition of claim 32, wherein the phthalazinedione is 5-amino-2,3-dihydrophthalazine-1,4-dione, 6-amino-2,3-dihydrophthalazine-1,4-dione, or 5-amino-2,3-dihydrophthalazine-1,4-dion-8-yl.

36. The pharmaceutical composition of claim 34, wherein the phthalazinedione is N-bromo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-chloro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-fluoro-5-amino-2,3-dihydrophthalazine-1,4-dione, N-iodo-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-isopropyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanoyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-hydroxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-carboxyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propanol-5-amino-2,3-dihydrophthalazine-1,4-dione, N-methenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propenyl-5-amino-2,3-dihydrophthalazine-1,4-dione, N-

methoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-ethoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, N-propoxy-5-amino-2,3-dihydrophthalazine-1,4-dione, or N,N-dimethyl-5-amino-2,3-dihydrophthalazine-1,4-dione.

37. The pharmaceutical composition of claim 31, wherein the compound is a glutathione, glucocorticoid, dexamethasone, cysteine, lipoic acid, biopterin, hydralazine, rasagiline, thioredoxin, ferulic acid, minocycline, menadione, tetracycline, isosorbate dinitrate, or dextromethorphan.

38. The pharmaceutical composition of claim 31, further comprising a pharmaceutically acceptable form selected from the group consisting of tablet, capsule, granule, powder, solution, suspension, microsphere, liposome, colloid, lyophilized composition, gel, lotion, ointment, cream, spray, and suppository.

39. The pharmaceutical composition of claim 38, wherein the pharmaceutical composition is administered by a means selected from the group consisting of intravenous, intramuscular, intraperitoneal, subcutaneous, oral, nasal, mucosal, transdermal, parenteral, vaginal, and rectal.

40. The pharmaceutical composition of claim 39, wherein the pharmaceutical composition is used in combination with a standard treatment selected from the group consisting of radiation, chemotherapy, laser therapy, surgery, medication, and nutrition.

41. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating metabolic distress.

42. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating inflammatory conditions.

43. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating infectious conditions.

44. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating neurological disorders.

45. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating immune disorders.

46. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating proliferative diseases.

47. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is used in treating cellular senescence.

48. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 0.01 mg/kg to about 100.0 mg/kg of body weight.

49. The pharmaceutical composition of claim 48, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 0.05 mg/kg to about 50.0 mg/kg of body weight.

50. The pharmaceutical composition of claim 49, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 0.1 mg/kg to about 10.0 mg/kg of body weight.

51. The pharmaceutical composition of claim 31, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 1.0 mg per day to about 10,000.0 mg per day.

52. The pharmaceutical composition of claim 51, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 50.0 mg per day to about 5000.0 mg per day.

53. The pharmaceutical composition of claim 52, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 100.0 mg per day to about 1000.0 mg per day.

54. The pharmaceutical composition of claim 51, wherein the pharmaceutical composition is administered in a dose comprising the phthalazinedione in an amount of about 1.0 mg, 10.0 mg, 50.0 mg, 100.0 mg, 200.0 mg, 300.0 mg, 400.0 mg, 500.0 mg, 600.0 mg, 700.0 mg, 800.0 mg, 900.0 mg, 1000.0 mg, 2000.0 mg, 3000.0 mg, 4000.0 mg, 5000.0 mg, 6000.0 mg, 7000.0 mg, 8000.0 mg, 9000.0 mg, or 10,000.0 mg per day.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/29230

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/00**(2006.01),**31/56**(2006.01),**31/495**(2006.01),**31/385**(2006.01),**31/195**(2006.01),**31/19**(2006.01)
A61K 38/00(2006.01),31/56(2006.01),31/495(2006.01),31/385(2006.01),31/195(2006.01),31/19(2006.01)

USPC: 514/18,171,248,440,562,570

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/18, 171, 248, 440, 562, 570

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,420,364 B1 (EMMANUEL et al.) 16 July 2002 (16.07.02), see col. 144, lines 32-39; col. 147, lines 25, 27 and 50-60; and col. 148, lines 15 and 18-21.	1-7
Y	US 5,543,410 A (MININ et al.) 06 August 1996 (06.08.96), see col. 1, lines 10-58; col. 4, lines 17-63; col. 6, lines 35-44; and col. 11, Example 7.	1-10, 12 and 15-54
Y	US 5,874,444 A (WEST) 23 February 1999 (23.02.99), see col. 2, lines 51-63; col. 3, line 11; col. 5, lines 20-29, 32, 33, 36-38 and 40-42; col. 6, lines 12-20 and 60; and col. 7, lines 13-22 and 50-52.	8, 9 and 13-54
Y	US 6,335,361 B1 (HAMILTON) 01 January 2002 (01.01.02), see col. 6, lines 58-60 and col. 8, line 47.	1-17, 30-34 and 37-54
Y	US 6,337,065 B1 (JACOBSON et al.) 08 January 2002 (08.01.02), see the abstract; col. 10, lines 35, 36, 39, 42 and 63; and col. 22, lines 33-34.	1-54

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

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Date of the actual completion of the international search

16 July 2006 (16.07.2006)

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INTERNATIONAL SEARCH REPORT

International application No.
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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,686,347 B2 (BOLD et al.) 03 February 2004 (03.02.04), see col. 7, lines 30-34 and 58-60; col. 8, lines 41-47; col. 18, lines 20-23, 39, 40 and 46-50; col. 40, lines 41-46, 51-54, 56 and 58; and cols. 55-84.	1-17, 20-34 and 37-54
Y	Goodman and Gilman, The Pharmacological Basis of Therapeutics, 6th edition, published 1980 by MacMillan Publishing Co., pp. 1181-1191.	1-17, 30-34 and 37-54
Y	The Merck Index, 11th edition, published 1989 by Merck & Co., Inc., (NJ), pp. 463, 464 and 1224.	1-54
Y	The Merck Manual of Diagnosis and Therapy, published 1982 by Merck, Sharp & Dohme, (NJ), pp. 262-265 and 1190-1193.	1-54