(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 September 2006 (14.09.2006)

PCT

(10) International Publication Number
WO 2006/096405 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2006/007257

(22) International Filing Date: 1 March 2006 (01.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/658,505 4 March 2005 (04.03.2005) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/096405 A2

(54) Title: MODULATION OF NEURODEGENERATIVE DISEASES

(57) Abstract: Methods and compositions are disclosed for selectively interfering with protein synthesis in a central nervous system, meningial, or muscle cell by administrating a pharmacological agent. In particular, methods and compositions that interfere with SOD-I protein synthesis are disclosed.

Modulation of Neurodegenerative Diseases

Related Applications

This application claims benefit of priority to U.S. Provisional Application No. 60/658,505, filed March 4, 2005, the entire disclosure of which is incorporated herein by reference.

Background of the Invention

Amyotrophic lateral sclerosis (ALS) is the most commonly diagnosed progressive motor neuron disease. The disease is characterized by degeneration of motor neurons in the cortex, brainstem and spinal cord (Principles of Internal Medicine, 1991 McGraw-Hill, Inc., New York; Tandan *et al.* (1985) *Ann. Neurol*, 18:271-280, 419-431). The cause of the disease is unknown and ALS may only be diagnosed when the patient begins to experience asymmetric limb weakness and fatigue, localized fasciculation in the upper limbs and/or spasticity in the legs which typifies onset. There is a genetic component to at least some incidences of ALS.

In almost all instances, sporadic ALS and autosomal dominant familial ALS (FALS) are clinically similar (Mulder *et al.* (1986) *Neurology*, 36:511-517). It has been shown that in some but not all FALS pedigrees the disease is linked to a genetic defect on chromosome 21q (Siddique *et al.*, (1991) *New Engl. J. Med.*, 324:1381-1384).

In particular, mutations in the SOD-1 gene which is localized on chromosome 21q, appear to be associated with the familial form of ALS. The deleterious effects of various mutations on SOD-1 are most likely mediated through a gain of toxic function rather than a loss of SOD-1 activity (Al-Chalabi and Leigh, (2000) *Curr. Opin. Neurol.*, 13, 397-405; Alisky *et al.* (2000) *Hum. Gene Ther.*, 11, 2315-2329). While the toxicity is unclear, there exists evidence to suggest that elimination of the protein itself will ameliorate the toxicity.

A need exists to develop therapies that can alter the course of neurodegenerative diseases or prolong the survival time of patients with such diseases. In particular, a need exists to reduce the SOD-1 protein produced in the brain and spinal cord of ALS patients. Preventing the formation of wild type or mutant SOD-1 protein may stop disease progression and allow for amelioration of ALS symptoms.

Summary of the Invention

Methods and compositions are disclosed for interfering with protein synthesis in the brain, spinal cord, meningia and muscle cells by administrating a pharmacological agent. In particular, methods and compositions that decrease SOD-1 gene expression are disclosed.

The methods and compositions of the invention can be used to reduce or inhibit the expression of a protein associated with a neurodegenerative disease, e.g., SOD-1 by administering an agent e.g., pyrimethamine, to inhibit SOD-1 mRNA transcription or the stability of the transcript. The decreases in SOD-1 mRNA then lead to decreased protein levels of SOD-1, which reduce its accumulation in the cell and ameliorate the disease. The expression and accumulation of mutant SOD-1 is a widely accepted pathophysiological mechanism underlying familial ALS, and might also play a role in the sporadic form of the disease.

Accordingly, in one aspect, the invention pertains to a method for reducing the production of an SOD protein in a cell comprising administering a nuclear receptor modulating pharmacological agent to the cell, such that the agent interacts with a nuclear receptor and inhibits transcription of a gene encoding the SOD protein. The cell can be a neural cell, or any cell in the spinal cord, the meningial tissue, or a muscle cell, for example in a subject with ALS (e.g., familial ALS). The SOD protein can be the SOD-1 protein. Examples of cells include, but are not limited to, neurons, interneurons, glial cells, microglia cells, muscle cells, cells involved in the immune response, and the like.

In one embodiment, the pharmacological agent is pyrimethamine and functional analogs thereof. In another embodiment, the pharmacological agent is pyrimethamine with at least one modification in the benzene ring. In yet another embodiment, the pharmacological agent is pyrimethamine with at least one modification in the pyrimidine ring.

The inhibition of transcription of the gene comprises monitoring by measuring the expression levels of the SOD protein, e.g., the SOD-1 protein. Alternatively, the inhibition of transcription of the gene comprises monitoring the levels of a nucleic acid molecule that encodes the SOD protein, for example by monitoring the ribonucleic acid or deoxynucleic acid levels.

In another aspect, the invention pertains to a method for preventing, ameliorating or treating the symptoms or progression of ALS in a subject by administering a therapeutically effective amount of a pharmacological agent to the subject, wherein the agent interacts with the nucleus of the cell and inhibits transcription of a gene encoding a SOD-1 protein. The ameliorating of symptoms can be monitored by measuring the survival prolongation of the subject, for example by monitoring a neurological score of the subject. alternatively, the amelioration can be determined by monitoring the expression levels of the SOD-1 protein or the levels of a nucleic acid molecule that encodes SOD-1 protein.

Brief Description of Drawings

Fig. 1 is a graph showing the reduction of SOD-1 protein expression by pyrimethamine.

Fig. 2 is a bar graph showing the reduced expression of SOD-1 mRNA with pyrimethamine and norethindrone.

Fig. 3 is a schematic showing a few representative pyrimethamine functional analogs of the invention.

Fig. 4 is a bar graph showing reduced expression of mRNA for alpha synuclein in HeLa cells following treatment with pyrimethamine and norethindrone.

Fig. 5 is a bar graph showing the reduction of SOD-1 protein expression in male and female SOD-93A mice with chronic pyrimethamine treatment (TX).

Fig. 6 is a bar graph showing the decrease in expression of alpha synuclein in mouse lymphocytes with chronic pyrimethamine treatment.

Fig. 7 is a bar graph showing the decreased expression of spinal SOD-1 in SOD-93A mice following oral administration of pyrimethamine.

Fig. 8 is a bar graph showing a decrease in lymphocyte SOD-1 levels in a familial SOD-1 patient following 30 days of oral administration of pyrimethamine.

Detailed Description

The practice of the present invention employs, unless otherwise indicated, conventional methods of microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. (*See, e.g.*, Sambrook, *et al.* Molecular Cloning: A Laboratory Manual (Current Edition); DNA Cloning: A Practical Approach, Vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (*N. Gait, ed.*, Current Edition); Nucleic Acid Hybridization (*B. Hames & S. Higgins, eds.*, Current Edition); Transcription and Translation (*B. Hames & S. Higgins, eds.*, Current Edition); CRC Handbook of Parvoviruses, vol. I & II (*P. Tijessen*, ed.); Fundamental Virology, 2nd Edition, Vol. I & II (*B. N. Fields and D. M. Knipe, eds.*)).

So that the invention is more clearly understood, the following terms are defined:

The term "neurodegenerative disorder" or "neurodegenerative disease" are used interchangeably herein and refer to an impairment or absence of a normal neurological function, or presence of an abnormal neurological function in a subject, or group of subjects. For example, neurological disorders can be the result of disease, injury, and/or aging. As used herein, neurodegenerative disorder also includes neurodegeneration which causes morphological and/or functional abnormality of a neural cell or a population of neural cells. Non-limiting examples of morphological and functional abnormalities include physical deterioration and/or death of neural cells, abnormal growth patterns of neural cells, abnormalities in the physical connection between neural cells, under- or over production of a substance or substances, *e.g.*, a neurotransmitter, by neural cells, failure of neural cells to produce a substance or substances which it normally produces, production of substances, *e.g.*, neurotransmitters, and/or transmission of electrical impulses in abnormal patterns or at abnormal times. Neurodegeneration can occur in any area of the brain of a subject and is seen with many disorders including, for example, Amyotrophic Lateral Sclerosis (ALS), multiple sclerosis, Huntington's disease, Parkinson's disease, Alzheimer's disease, prion

associated disease (CJD), spinal muscular atrophy, spinal cerebellar ataxia, and spinal cord injury.

The terms "pharmacological agent" and "nuclear receptor modulating pharmacological agent" as used herein, are intended to be used interchangeably, and these terms refer to the compound, or compounds, that interfere selectively with protein synthesis in a neural spinal cord, menengial, or muscle cell. In particular, interfere with protein synthesis of an SOD protein, e.g., SOD-1 protein. Preferably, the pharmacological agent is pyrimethamine and analogs thereof.

The terms "modulate" or "modulating" or "modulated" are used interchangeable herein also refer to a change SOD-1 activity, or the expression, i.e., an increase or decrease in SOD-1 activity, or expression, such that the modulation produces a therapeutic effect in a subject, or group of subjects. A therapeutic effect is one that results in an amelioration in the symptoms, or progression of ALS. The change in activity can be measured by quantitative or qualitative measurements of the SOD-1 protein level for example by Western blot analysis. The quantitative assay can be used to measure downregulation or upregulation of SOD-1 protein levels in the presence of a pharmacological agent, such as pyrimethamine and analogs thereof. A suitable pharmacological agent can be one that down-regulates SOD-1 expression by about 5 percent to about 50 percent compared with a control. The change in expression can also be measured by quantitative or qualitative measurements of the nucleic acid level associated with SOD-1, for example by measuring the expression level of RNA or DNA.

The effect of SOD-1 modulation on a subject, or group of subjects, can also be investigated by examining the survival of the subject, or group of subjects. For example, by measuring the change in the survival, or the prolongation of survival in one or more animal models for a neurodegenerative disease, e.g., ALS. The change in the survival can be due to the administration of pharmacological agent such as pyrimethamine or functional analog that is administered to an ALS murine model. The effect of the pharmacological agent can be determined based on the increase in days of survival of a test group of ALS mice compared with a control group of ALS mice that have been given a control agent, or no agent. In one embodiment, the pharmacological agent or functional analog thereof increases the percentage effect on survival of the subject, or a population of subjects (e.g., a male population, or a female population) by at least 2% to

about 100%. Preferably the percentage effect on survival of the subject, or a population of subjects, is by at least 5% to about 50%, by at least 10% to about 25%. Even more preferably, the percentage effect on survival of the subject, or a population of subjects, is by at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26% 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48% and 50%. The effect of SOD-1 modulation may also determined by examining the neurological score of a subject, or group of subjects for example, by assessing the improvement in muscular movement, or by examining the alleviation or amelioration of the disease symptoms. In a preferred embodiment, the neurological score of a subject, or group of subjects is significantly different from that of the untreated control subjects, with a level of significance between $p<0.05$ and $p<0.0001$, as determined using standard statistical analysis procedures.

The terms may also be used to refer to a change in the nuclear receptor upon interaction with a pharmacological agent, i.e., a change in nuclear receptor activity, structure, or the expression of a nuclear receptor, or a subunit of the nuclear receptor, i.e., an increase or decrease in nuclear receptor activity, or expression, such that the modulation produces a therapeutic effect in a subject, or group of subjects.

The term "inhibit" or "inhibiting" as used herein refers to a measurable reduction of expression of a target gene or a target protein, e.g., SOD-1. The term also refers to a measurable reduction in the activity of a target protein. Preferably a reduction in expression is at least about 10%. More preferably the reduction of expression is about 20%, 30%, 40%, 50%, 60%, 80%, 90% and even more preferably, about 100%.

The phrase "a disorder associated with SOD activity" or "a disease associated with SOD activity" as used herein refers to any disease state associated with the expression of SOD protein (e.g., SOD-1, SOD-2, SOD-3, and the like). In particular, this phrase refers to the gain of toxic function associated with SOD protein production. The SOD protein can be a wild type SOD protein or a mutant SOD protein and can be derived from a wild type SOD gene or an SOD gene with at least one mutation.

The term "subject" as used herein refers to any living organism in which an immune response is elicited. The term subject includes, but is not limited to, humans, nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs

and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered.

As used herein, "alkyl" groups include saturated hydrocarbons having one or more carbon atoms, including straight-chain alkyl groups (*e.g.*, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, etc.), cyclic alkyl groups (or "cycloalkyl" or "alicyclic" or "carbocyclic" groups) (*e.g.*, cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, etc.), branched-chain alkyl groups (isopropyl, *tert*-butyl, *sec*-butyl, isobutyl, etc.). Unless otherwise specified the term alkyl includes both "unsubstituted alkyls" and "substituted alkyls," the latter of which refers to alkyl groups having substituents replacing one or more hydrogens on one or more carbons of the hydrocarbon backbone.

The term "alkoxy group" as used herein means an alkyl group having an oxygen atom attached thereto. Representative alkoxy groups include groups having 1-10 carbon atoms, preferably 1-6 carbon atoms, *e.g.*, methoxy, ethoxy, propoxy, *tert*-butoxy, and the like. Examples of alkoxy groups include methoxy, ethoxy, propoxy, *iso*-propoxy, butoxy, pentoxy.

The term "aromatic group" or "aryl group" includes unsaturated and aromatic cyclic hydrocarbons as well as unsaturated and aromatic heterocycles containing one or more rings. Aryl groups may also be fused or bridged with alicyclic or heterocyclic rings that are not aromatic so as to form a polycycle (*e.g.*, tetralin). Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin).

An "arylalkyl" group is an alkyl group substituted with an aryl group (*e.g.*, phenylmethyl (*i.e.*, benzyl)). An "alkylaryl" moiety is an aryl group substituted with an alkyl group (*e.g.*, *p*-methylphenyl (*i.e.*, *p*-tolyl)). An "alkoxyphenyl" group (or "alkyloxyphenyl" group) is a phenyl group substituted with an alkoxy group (*e.g.*, *p*-methoxyphenyl). An "arylalkoxy" group is an alkoxy group substituted with a phenyl group (*e.g.*, benzyloxy), An "aryloxyalkyl" group is an alkyl group substituted with an oxyaryl group (*e.g.*, phenylmethyl ether (*i.e.*, phenoxymethyl)), An "aryloxyphenyl" group is an phenyl group substituted with a phenoxy group (*e.g.*, biphenyl ether (*i.e.*, phenoxyphenyl)), A "phenoxy" group is an oxygen atom attached to a phenyl group.

The term "heteroaryl group" includes unsaturated and aromatic cyclic groups in which one or more of the carbon atoms in the ring is an element other than carbon, for example, nitrogen, sulfur, or oxygen.

The term "heterocyclic group" includes closed ring structures analogous to carbocyclic groups in which one or more of the carbon atoms in the ring is an element other than carbon, for example, nitrogen, sulfur, or oxygen. Heterocyclic groups may be saturated or unsaturated. Additionally, heterocyclic groups (such as pyrrolyl, pyridyl, isoquinolyl, quinolyl, purinyl, and furyl) may have aromatic character, in which case they may be referred to as "heteroaryl" or "heteroaromatic" groups.

An "heteroarylalkyl" group is an alkyl group substituted with a heteroaryl group (*e.g.*, 4-methylpyridine).

An "heteroarylalkoxy" group is an alkoxy group substituted with a heteroaryl group (*e.g.*, 4-methoxypyridine).

I. Neurodegenerative Diseases

In one aspect, the invention pertains to altering the expression of an SOD protein in a cell by administering a pharmacological agent, e.g., a nuclear receptor modulating pharmacological agent. The cell can be a neural cell associated in a neurodegenerative disease that involves an SOD protein, such as amyotrophic lateral sclerosis (ALS). The nuclear receptor can be a ligand activated transcription factor that binds a ligand, e.g., pyrimethamine, and its functional analogues with high affinity and acts on genomic DNA to inhibit or activate the expression of a broad spectrum of genes. Nuclear receptors (e.g., estrogen receptors, progesterone receptors or orphan nuclear receptors) are found in the spinal cord and nearly all cells in both males and females, and thus constitutes a useful therapeutic target for neurodegenerative diseases, e.g., ALS. A change in function of the nuclear receptor may be at the heart of many neurodegenerative conditions, including, for example, ALS, Alzheimer's disease, Parkinson's disease, Huntington's disease, and Multiple Sclerosis, each of which is described below.

Amyotrophic Lateral Sclerosis (ALS), also called Lou Gehrig's disease, is a fatal neurodegenerative disease affecting motor neurons of the cortex, brain stem and spinal cord. (Hirano, (1996) *Neurology*, 47(4 Suppl. 2): S63-6). Onset of ALS occurs in the

fourth or fifth decade of life (median age of onset is 57) and is fatal within two to five years after diagnosis (Williams, *et al.* (1991) *Mayo Clin. Proc.*, 66: 54-82). ALS affects approximately 30,000 Americans with nearly 8,000 deaths reported in the US each year. ALS patients progressively lose all motor function – unable to walk, speak, or breathe on their own.

The cardinal feature of ALS is the loss of spinal motor neurons, which causes the muscles under their control to weaken and waste away leading to paralysis. ALS has both familial (5-10%) and sporadic forms and the familial forms have now been linked to several distinct genetic loci (Deng, *et al.* (1995) *Hum. Mol. Genet.*, 4: 1113-16; Siddique, *et al.* (1995) *Clin. Neurosci.*, 3: 338-47; Siddique, *et al.*, (1997) *J. Neural Transm. Suppl.*, 49: 219-33; Ben Hamida, *et al.* (1990) *Brain*, 113: 347-63; Yang, *et al.* (2001) *Nat. Genet.* 29: 160-65; Hadano, *et al.* (2001) *Nat. Genet.* 29: 166-73). About 15-20% of familial cases are due to mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) (Siddique, *et al.* (1991) *N. Engl. J. Med.*, 324: 1381-84; Rosen, *et al.* (1993) *Nature*, 362: 59-62).

Although the etiology of the disease is unknown, one theory is that neuronal cell death in ALS is the result of over-excitement of neuronal cells due to excess extracellular glutamate. Glutamate is a neurotransmitter that is released by glutaminergic neurons, and is taken up into glial cells where it is converted into glutamine by the enzyme glutamine synthetase, glutamine then re-enters the neurons and is hydrolyzed by glutaminase to form glutamate, thus replenishing the neurotransmitter pool. In a normal spinal cord and brain stem, the level of extracellular glutamate is kept at low micromolar levels in the extracellular fluid because glial cells, which function in part to support neurons, use the excitatory amino acid transporter type 2 (EAAT2) protein to absorb glutamate immediately. A deficiency in the normal EAAT2 protein in patients with ALS, was identified as being important in the pathology of the disease (*See e.g.*, Meyer *et al.* (1998) *J. Neurol. Neurosurg. Psychiatry*, 65: 594-596; Aoki *et al.* (1998) *Ann. Neurol.* 43: 645-653; Bristol *et al.* (1996) *Ann Neurol.* 39: 676-679). One explanation for the reduced levels of EAAT2 is that EAAT2 is spliced aberrantly (Lin *et al.* (1998) *Neuron*, 20: 589-602). The aberrant splicing produces a splice variant with a deletion of 45 to 107 amino acids located in the C-terminal region of the EAAT2 protein (Meyer *et al.* (1998) *Neureosci Lett.* 241: 68-70). Due to the lack of, or defectiveness of

EAAT2, extracellular glutamate accumulates, causing neurons to fire continuously. The accumulation of glutamate has a toxic effect on neuronal cells because continual firing of the neurons leads to early cell death.

Although a great deal is known about the pathology of ALS little is known about the pathogenesis of the sporadic form and about the causative properties of mutant SOD protein in familial ALS (Bruijn, *et al.* (1996) *Neuropathol. Appl. Neurobiol.*, 22: 373-87; Bruijn, *et al.* (1998) *Science* 281: 1851-54). Many models have been speculated, including glutamate toxicity, hypoxia, oxidative stress, protein aggregates, neurofilament and mitochondrial dysfunction Cleveland, *et al.* (1995) *Nature* 378: 342-43; Cleveland, *et al. Neurology*, 47(4 Suppl. 2): S54-61, discussion S61-2(1996); Cleveland, (1999) *Neuron*, 24: 515-20; Cleveland, *et al.* (2001) *Nat. Rev. Neurosci.*, 2: 806-19; Couillard-Despres, *et al.* (1998) *Proc. Natl. Acad. Sci. USA*, 95: 9626-30; Mitsumoto, (1997) *Ann. Pharmacother.*, 31: 779-81; Skene, *et al.* (2001) *Nat. Genet.* 28: 107-8; Williamson, *et al.* (2000) *Science*, 288: 399).

Presently, there is no cure for ALS, nor is there a therapy that has been proven effective to prevent or reverse the course of the disease. Several drugs have recently been approved by the Food and Drug Administration (FDA). To date, attempts to treat ALS have involved treating neuronal degeneration with long-chain fatty alcohols which have cytoprotective effects (*See* U.S. Pat. No. 5,135,956); or with a salt of pyruvic acid (*See* U.S. Pat. No. 5,395,822); and using a glutamine synthetase to block the glutamate cascade (*See* U.S. patent 5,906,976). For example, Riluzole™, a glutamate release inhibitor, has been approved in the U.S. for the treatment of ALS, and appears to extend the life of at least some patients with ALS. However, some reports have indicated that even though Riluzole™ therapy can prolong survival time, it does not appear to provide an improvement of muscular strength in the patients. Therefore, the effect of Riluzole™ is limited in that the therapy does not modify the quality of life for the patient (Borras-Blasco *et al.* (1998) *Rev. Neurol.*, 27: 1021-1027).

II. SOD and SOD Mutations

The invention pertains to decreasing the SOD-1 protein (e.g., mutant SOD-1 protein) in cells by reducing or eliminating the expression of the protein with pharmacological modulating agents and their functional analogs. The SOD-1 gene is

localized to chromosome 21q22.1. SOD-1 sequences are disclosed in PCT publication WO 94/19493 are oligonucleotide sequences encoding SOD-1 and generally claimed is the use of an antisense DNA homolog of a gene encoding SOD-1 in either mutant and wild-type forms in the preparation of a medicament for treating a patient with a disease. The nucleic acid sequence of human SOD-1 gene can be found at Genbank accession no. NM_000454. The nucleotide sequence of human SOD-1 is also presented in SEQ ID NO: 1. The corresponding SOD-1 protein sequence is presented in SEQ ID NO: 2.

III. Nuclear Receptors and Ligands

In one aspect, the invention pertains to using pharmacological agents that alter gene expression or protein production of SOD, e.g., SOD-1. The pharmacological agent can be a nuclear receptor pharmacological agent. The nuclear receptor can be a ligand activated transcription factor that binds a ligand with high affinity and acts on genomic DNA to inhibit or activate the expression of a broad spectrum of genes. Nuclear receptors, such as the estrogen receptor and progesterone receptor haves been implicated in neurodegenerative disorders.

Approximately 70 members of the nuclear receptor superfamily members have been identified (Moras & Gronemeyer 1998). Only some of them are ligand-binding receptors, while others belong to the subfamily of so-called orphan receptors for which specific ligands have not yet been identified or may not even exist (O'Malley & Conneely 1992). Many of these nuclear receptors can modulate gene expression directly by interacting with specific elements in the regulatory regions of target genes or indirectly by activating various growth factor signaling pathways.

The structural features of the nuclear receptor superfamily are similar. Each have four major functional regions: the N-terminal transactivation domain (TAD), a central DNA-binding domain (DBD), a C-terminal ligand-binding domain (LBD), and a hinge region connecting the DBD and LBD (Mangelsdorf et al. 1995). Two autonomous transactivation functions, a constitutively active activation function (AF-1) originating in the N-terminal and a ligand-dependent activation function (AF-2) arising in the LBD, are responsible for the transcriptional activity of nuclear receptors (Gronemeyer & Laudet 1995).

The DBD of nuclear receptors exhibits a high degree of amino acid sequence identity to other members of the subfamily. Consequently, the four receptors recognize very similar, if not identical, hormone response elements (HREs) in nuclear DNA.

Conformation changes resulting from the binding of a ligand (e.g., pyrimethamine, progesterone or estrogen) to the LBD located at the C-terminal end of the molecule are responsible for activating the ligand response. Despite the low sequence identity of as low as 20% between the LBDs of different nuclear receptor families, all nuclear receptors share a similar fold in this region. They are comprised of up to 12 helices and a small -sheet arranged in a so-called α-helical sandwich. The transactivation functions of AF-1 and AF-2 are located in the TAD and the LBD, respectively, of nuclear receptors, and the activity of them is dependent on the recruitment of coactivator molecules to form active preinitiation sites for gene transcription (Onate et al. 1998, Bevan et al. 1999). Receptors with a deletion of their LBD are constitutively active, suggesting that the AF-1 is ligand-independent. Strong AF-2 was demonstrated in LBDs of retinoic acid receptor (RAR) (Durand et al. 1994), retinoic-X receptor (RXR) (vom Baur et al. 1998), vitamin D receptor (Jimenéz et al. 1999), GR (Sheldon et al. 1999), PR (Onate et al. 1998), Peroxisome proliferatoractivated receptor (PPARγ) (Nolte et al. 1998), estrogen receptor (ER) (Tora et al. 1989), and thyroid hormone receptor (THR) (Barettino et al. 1994), but not in AR (Berrevoets et al. 1998, Bevan et al. 1999).

The transcriptional activity of nuclear receptors is affected by coregulators that influence a number of functional properties of nuclear receptor, including ligand selectivity and DNA binding capacity. Nuclear receptor coregulators participate in DNA modification of target genes, either directly through modification of histones or indirectly by the recruitment of chromatin-modifying complexes, as well as functioning in the recruitment of the basal transcriptional machinery (Heinlein & Chang 2002). Some of the better characterized coregulators are members of the p160 family, ARA70, ARA55, ARA54, ARA267-α, Smad-3, and AIB1 (Yeh et al. 1999). ARA55 and ARA70 both allow the activation of androgen receptor by 17β -estradiol (E2), with ARA70 being the most effective coactivator for conferring androgenic activity to E2 (Miyamoto et al. 1998, Yeh et al. 1998, Fujimoto et al. 1999). Furthermore, both ARA55 and Smad-3 have been suggested to function as bridges for cross-talk between transforming growth

factor-β signalling pathway and androgen/androgen receptor action (Fujimoto et al. 1999, Kang et al. 2001).

(i) *Ligand dependent activation*

Ligands, e.g., estrogen/progesterone, and pyrimethamine can enter the target cells and bind to the nuclear receptors. Ligand-binding initiates a series of events leading to the regulation of target genes by the receptor. The occupied receptor undergoes an allosteric change in its LBD, and is dissociated from heat shock proteins, such as hsp90, hsp70, and hsp56 (Roy et al. 2001), complexed, e.g., dimerized, and translocated, if it is not already present into the nucleus. Upon binding to response element, e.g., a hormone response element (HRE) in nuclear DNA, the receptor dimer recruits coactivators such as p160 family to form an active pre-initiation complex and interacts with basal transcription machinery to inhibit or trigger the transcription of the target genes.

(ii) *Ligand-independent activation*

Nuclear receptors may also be activated by signaling pathways that originated at the cell surface. Nuclear receptors, along with other transcription factors, are regulated by reversible phosphorylation (Orti et al. 1992). Kinase-mediated signal transduction pathways could affect the activity of nuclear receptors (Burnstein & Cidlowski 1993). Certain consensus phosphorylation sites can be a substrate for the DNA-dependent protein kinase, protein kinase A, protein kinase C, mitogen-activited kinase, and casein kinase II (Blok et al. 1996).

Several naturally occurring ligands for nuclear receptors are known. For example, the natural ligand for the estrogen receptor is the estrogen ligand, but synthetic compounds, such as estradiol, have been made which also serves as a ligand. The natural ligand for the progesterone receptor is progesterone ligand, but synthetic compounds, such as norethindrone, have been made which also serves as a ligand. In addition to natural ligands, other agents such as pyrimethamine, may also act as a ligand for nuclear receptors.

The ligand binds to the nuclear receptor to create a receptor/ligand complex. This complex binds to specific gene promoters present in nuclear DNA. Once bound to the DNA the complex modulates the production of mRNA and protein encoded by that gene. Thus, the nuclear receptor modulating agents can be FDA approved therapeutic agents that are currently being used for diseases not associated with SOD-1 function, e.g., antimalarial drugs, and modified variants thereof. The nuclear receptor modulating agents can also be newly synthesized compounds that alter SOD-1 expression.

IV. Pyrimethamine and Its Functional Analogs

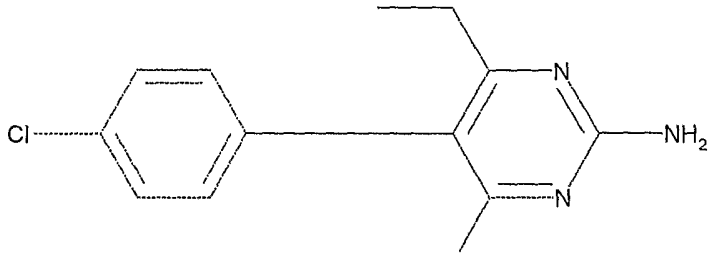
In one aspect, the invention pertains to using pyrimethamine and its functional analogs as pharmacological agents that interfere with protein synthesis. Pyrimethamine is an antimalarial drug, that readily penetrates cells in the body and brain. Pyrimethamine has been used for the treatment of malaria, toxoplasmosis, and several other microbial infections (for review see Schweitzer, *et al.* (1990) *FASEB J* 4:2441-2452). The antimicrobial effect of pyrimethamine is a result of its inhibition of dihydrofolate reductase (DHFR), and enzymes involved in the folate synthesis pathway. The malaria parasite synthesizes folates de novo whereas the human host must obtain preformed folates and cannot synthesize folate. The inability of the parasite to utilize exogenous folates makes folate biosynthesis a good drug target. DHFR is an ubiquitious enzyme that participates in the recycling of folates by reducing dihydrofolate to tetrahydofolate. The tetrahydrofolate is then oxidized back to dihydrofolate as it participates in biosynthetic reactions (e.g.., thymidylate synthase). Inhibiting DHFR will prevent the formation of thymidylate and lead to an arrest in DNA synthesis and subsequent parasite death. Pyrimethamine is the most common DHFR inhibitor used as antimalarials. Other DHFR inhibitors include, but are not limited to, sulfadoxine, trimethoprim, sulfadiazine, trimethoprim, and sulfamethoxazole.

In one aspect, the invention pertains to lowering SOD-1 expression by administration of pharmacological modulating agents, such a pyrimethamine. Pyrimethamine is a potent inhibitor of SOD-1 expression in the HeLa cell and in the mouse Neuro2A cell lines as shown in the Examples. The mechanism of action for reduction of SOD-1 is not known at this time, but levels of both the protein and mRNA coding for SOD-1 are dose-dependently reduced. Pyrimethamine, however, does not act

via dihydrofolate reductase inhibition, because its effects could not be prevented or reversed using folinic acid (the enzymatic product of DHFR). Furthermore, methotrexate, a potent DHFR inhibitor with an unrelated chemical structure, did not reduce SOD-1 protein in the HeLa cell. Finally, pyrimethamine is very weak inhibitor of human DHFR with an $EC_{50} > 70$ μM. (Schweitzer *et al.*, (1990) *Supra*). Pyrimethamine has been documented to act on centromeric DNA and most likely inhibits transcription of the SOD-1 gene by an action on a transcription factor or less likely by a direct action on the genomic DNA itself.

While not required to provide a mechanism, it is believed that for inhibition of SOD-1 expression, pyrimethamine and its functional analogs putatively acts to reduce the expression of human SOD1 via an as yet unidentified nuclear receptor. Briefly, pyrimethamine binds with high affinity to the receptor protein, activating it and causing it to dimerize with another activated receptor. The dimerized receptors are transported into the cell's nucleus, or are already in the nucleus and bound to genomic DNA 5' to the start codon of hSOD1. The transported receptors bind to a stretch of palindromic DNA in the promoter region of the SOD-1 gene where the receptor-ligand-DNA complexes exert steric hindrance to prevent the initiation of transcription. Thus, less RNA coding for SOD1 is made, and consequently less protein is made. Reductions in the amount of mutant SOD1 protein produced would then prevent its neurotoxic effects and ameliorate ALS disease progression.

In one embodiment, the pharmacological modulating agent is pyrimethamine. The pyrimethamine pharmacophore as shown in formula I.

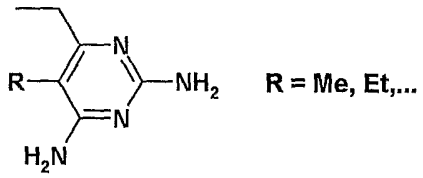


formula I

In another embodiment, the phamacological modulating agent is a functional analog of pyrimethamine. The structure-activity relationship (SAR) within pyrimethamine series of compounds can be established with a group of the representative 30 compounds shown in Figure 3. A systematic SAR study may demonstrate that these compounds do act by a specific cell target. Some of these compounds are commercially available, while others have been synthesized using standard organic chemistry methods. The design of the compounds is based on the lead molecule pyrimethamine may be broken into two main structural features and modified separately. The two main structural features are (1) the benzene ring, and (2) the pyrimidine ring. A number of analogues or derivatives have been designed, as described below:

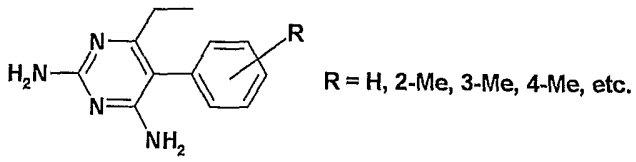
(1) Modification optimization of the benzene ring

The benzene ring of formula I can be modified in a number of different ways. In one embodiment, by replacing R in formula II with a alkyl group selected from the group consisting of Me, Et, and the like.



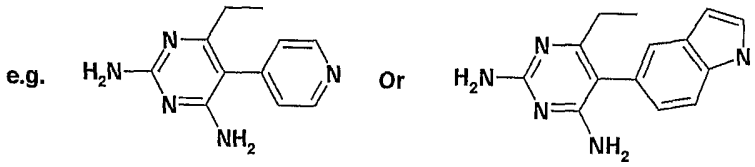
formula II

In another embodiment, by optimizing substituents at the benzene ring with respect to the position and the nature of the substituents. This may be performed by methyl scanning of the benzene ring at various positions as shown in formula III.



formula III

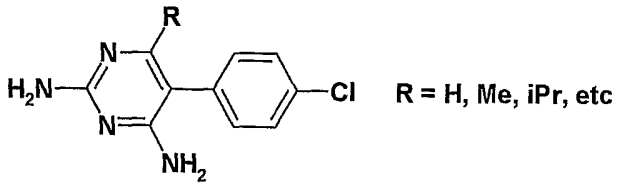
In another embodiment, the compound can be produced by replacing the ring with another ring including a heterocyclic ring as shown in formula IV



formula IV

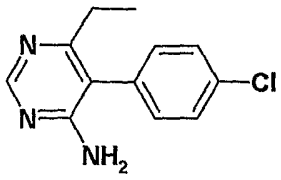
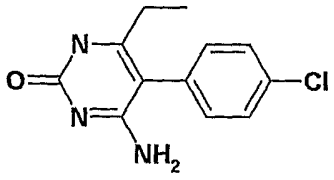
(2) Modification of the pyrimidine ring:

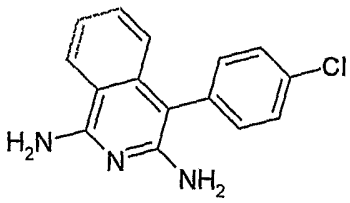
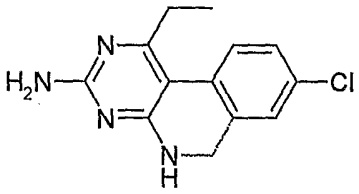
The pyrimidine ring can be modified alone or in combination with the modifications to the benzene ring. In one embodiment, the pyrimidine ring is modified by removing or replacing the alkyl (R) group of formula V with other functional groups selected from the group consisting of H, Me, Isopropyl (iPr) or the like.



formula V

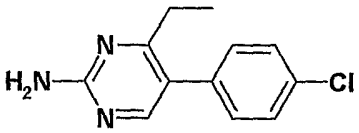
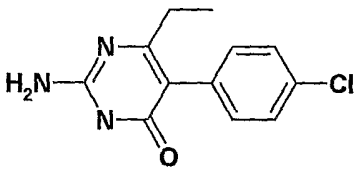
In another embodiment, the para-amino group (with respect to the benzene ring) can be removed or replaced with oxygen as shown in formula VI

e.g  and 

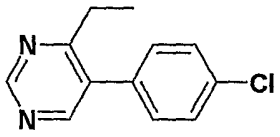
formula VI

In another embodiment, the ortho-amino group (with respect to the benzene ring) can be removed or replaced with oxygen as shown in formula VII

e.g.  and 

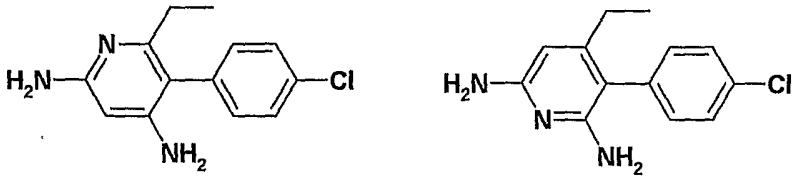
formula VII

In another embodiment, both of the primary amine groups can be eliminated from the molecule as shown in formula VIII



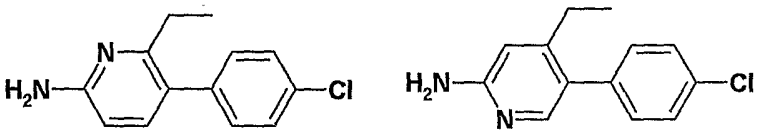
formula VIII

In another embodiment, one of the endocyclic nitrogens can be removed from the molecule as shown in formula IX



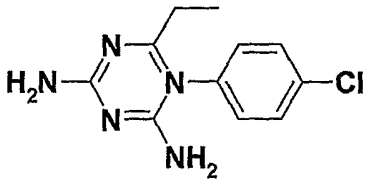
formula IX

In another embodiment, the primary amines and the endocyclic nitrogen can be removed simultaneously as shown in formula X.



formula X

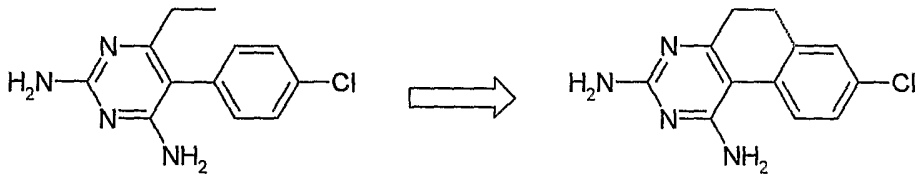
In another embodiment, the pyrimidine ring is replaced with a pyrazine ring system as shown in formula XI.



formula XI

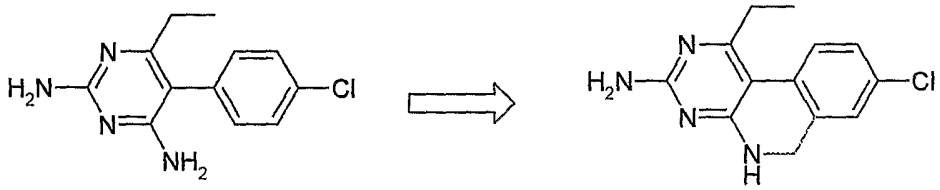
The compounds all contain the pharmachophoric features that are present in the lead structure shown in Formula I. These modifications are made to retain or improve the potency, oral activity and the drug property of the molecule. In other embodiments entirely new compounds are synthesized using standard organic synthetic chemistry. These new compounds retain the original pharmachophoric features. Analogs of the

new compounds can also be synthesized. In one embodiment, a structure that removes rotational freedom between the two rings can be synthesized, as shown in formula XII.



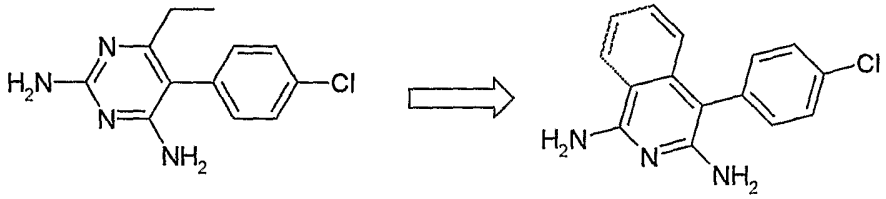
formula XII

In another embodiment, a structure that ties up the two rings at the ortho-amino group can be synthesized, as shown in formula XIII.



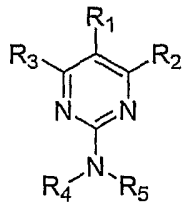
formula XIII

In another embodiment, a structure that replaces the pyrimidine with iso-quinolines can be synthesized, as shown in formula XIIII.



formula XIV

In some embodiments, the invention pertains, at least in part, to compounds of Formula XV:



XV

wherein R_1 is selected from the group consisting of hydrogen, C1-C10 straight chain alkyls, branched C3-C7 alkyls, substituted or unsubstituted cycloalkyl groups, aryl groups optionally substituted with one or two substituents selected from the group consisting of C1-C3 alkyls, halogen, nitro, cyano and alkoxy, substituted or unsubstituted aryloxyphenyl groups, substituted or unsubstituted alkyloxyphenyl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted alkylaryls, substituted or unsubstituted arylalkoxy groups, substituted or unsubstituted aryloxyalkyl groups, substituted or unsubstituted heteroaryl groups, substituted or unsubstituted heteroarylalkyls, substituted or unsubstituted heteroarylalkoxy groups, halogen, cyano, and NR'R"; wherein R' and R" are each independently selected from the group consisting of hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, and substituted or unsubstituted arylalkyl groups.

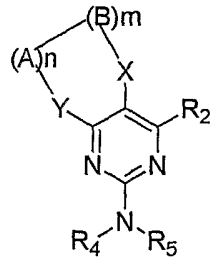
R_2, is selected from the group consisting of hydrogen, C1-C10 alkyls, hydroxyl, substituted or unsubstituted aryl groups, amino, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted heteroaryl groups, C3-C6 cycloalkyl groups, and NR'R"; wherein R' and R" are each independently selected from the group consisting of hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups.

R_3, is selected from the group consisting of hydrogen, C1-C10 alkyls, hydroxyl groups, hydroxyl-C1-C6-alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted phenoxy groups, and NR'R"; wherein R' and R" are each independently selected from the group

consisting of hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups.

R_4 and R_5 are each independently selected from the group consisting of hydrogen, hydroxyl, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, straight or branched halo-C1-C6-alkyl groups, straight or branched halo-C1-C6-alkoxy groups, alkoxy-C1-C6-alkyl groups, hydroxyl-C1-C6-alkyl groups, or carboxy-C1-C6-alkyl groups, or alternatively R4 and R5 can form a ring together with the nitrogen atom to which they are bonded, said ring can be selected, for example, from groups consisting of pyridyl, piperadino, morpholino, pyrrolidino, benzylamino and piperazino that can be optionally substituted with one or two substituents selected from the group consisting of C1-C6 alkyls, carboxyl (which may be protected), amino, cyano, hydroxyl, halogen, substituted or unsubstituted benzoyl, and substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

In some embodiment, the invention pertains, at least in part, to compounds of Formula XVI:



XVI

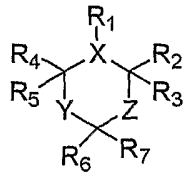
wherein:

X and Y form part of a saturated or unsaturated ring; wherein each of X and Y are each independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; and wherein carbon and nitrogen atoms can be substituted or unsubstituted; A is a carbon atom either substituted or unsubstituted; B is selected from a group consisting of carbon, nitrogen, oxygen, and sulfur; and wherein n is an integer between 1 and 3 and m is an integer between 0 and 2; and wherein n + m is equal to or less than 7.

R_2, is selected from the group consisting of hydrogen, C1-C10 alkyl, hydroxyl, substituted or unsubstituted aryl groups, substituted or unsubstituted heteroaryl groups, C3-C6 cycloalkyl groups, NR'R"; wherein R' and R" are each independently hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted arylalkoxy groups, substituted or unsubstituted aryloxyalkyl groups, substituted or unsubstituted phenoxy groups.

R_4 and R_5 are each independently selected from the group consisting of hydrogen, hydroxyl, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, straight or branched halo-C1-C6-alkyl groups, straight or branched halo-C1-C6-alkoxy groups, alkoxy-C1-C6-alkyl group, hydroxyl-C1-C6-alkyl groups, or carboxy-C1-C6-alkyl groups, or alternatively R4 and R5 form a ring together with the nitrogen atom to which they are bonded, said ring selected from groups consisting of pyridyl, piperadino, morpholino, pyrrolidino and piperazino that can be optionally substituted with one or two substituents selected from the group consisting of C1-C6 alkyl, carboxyl (which may be protected), amino, cyano, hydroxyl, halogen, substituted or unsubstituted benzoyl, substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

In another embodiments, the invention pertains, at least in part, to compounds of Formula XVII:



XVII

wherein:

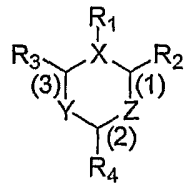
X, Y, and Z form part of a saturated or unsaturated ring; wherein each of X, Y, and Z are each independently selected from the group consisting of carbon, nitrogen,

oxygen, and sulfur; and wherein R_1, is selected from the group consisting of hydrogen, substituted or unsubstituted C1-C10 alkyls, branched C1-C6 alkyls, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted phenoxy groups, substituted or unsubstituted alkoxyphenyl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, substituted or unsubstituted heteroaryl groups, substituted or unsubstituted heteroarylalkyls, substituted or unsubstituted heteroarylalkoxy groups, halogen, cyano, substituted or unsubstituted aryloxyalkyl groups.

R_2,R_3, R_4, and R_5, are each independently selected from the group consisting of hydrogen, C1-C10 alkyls, C3-C7 branched alkyls, hydroxyl, cyano, amino, alkylaminoalkyl, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted heteroaryl groups, C3-C6 cycloalkyl groups, and NR'R"; wherein R' and R" are each independently selected from the group consisting of hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups. Alternatively both R_2 and R_3, and/or both R_4 and R_5 can be oxygen atoms.

R_6 and R7 are each independently selected from the group consisting of hydroxyl, alkoxy, phenoxy, aryloxyalkyl, arylalkoxy, amino, NR'R"; R' and R" are each independently selected from the group consisting of hydrogen, hydroxyl, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, straight or branched halo-C1-C6-alkyl groups, straight or branched halo-C1-C6-alkoxy groups, alkoxy-C1-C6-alkyl groups, hydroxyl-C1-C6- alkyl groups, or carboxy-C1-C6-alkyl groups, or alternatively R' and R" can form a ring together with the nitrogen atom to which they are bonded, said ring can be selected, for example, from the group consisting of pyridyl, piperadino, morpholino, pyrrolidino and piperazino and can be optionally substituted with one or two substituents selected from the group consisting of C1-C6 alkyl, carboxyl (which may be protected), amino, cyano, hydroxyl, halogen, substituted or unsubstituted benzoyl, substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

In other embodiments, the invention pertains, at least in part, to compounds of Formula XVIII:



XVIII

wherein:

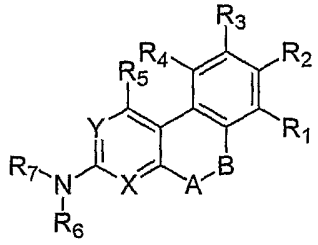
X, Y, and Z form part of an unsaturated ring having at least one, two or three alternating double bonds; wherein each of X, Y, and Z are independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; such that a double bond between $C_{(1)}$-Z would require $C_{(2)}$ and $C_{(3)}$ to be optionally substituted with two substituents; wherein double bonds between $C_{(1)}$-Z and $C_{(2)}$-Y would require $C_{(3)}$ to be optionally substituted with two substituents, and wherein double bonds between $C_{(1)}$-Z, $C_{(2)}$-Y and $C_{(3)}$-X would require R_2, R_3 and R_4 to each independently be selected from the group consisting of hydrogen, substituted or unsubstituted C1-C10 straight chain alkyls, branched C1-C6 alkyls, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted benzylic groups, substituted or unsubstituted aryloxyphenyl groups, substituted or unsubstituted alkyloxyphenyl groups, substituted or unsubstituted alkoxyphenyl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups substituted or unsubstituted heteroaryl groups, substituted or unsubstituted heteroarylalkyls, substituted or unsubstituted heteroarylalkoxy groups halogen, cyano, substituted or unsubstituted benzylic groups.

R_1 is selected from the group consisting of hydrogen, substituted or unsubstituted C1-C10 straight chain alkyls, branched C1-C6 alkyls, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted aryloxyphenyl grous, substituted or unsubstituted alkyloxyphenyl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, substituted or

unsubstituted heteroaryl groups, substituted or unsubstituted heteroarylalkyls, substituted or unsubstituted heteroarylalkoxy groups, halogen, and cyano.

Alternatively, R_2,R_3, and R_4, can each independently be selected from the group consisting of hydrogen, C1-C10 alkyl, C3-C7 branched alkyls, hydroxyl, cyano, amino, alkylaminoalkyls, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted heteroaryl groups, C3-C6 cycloalkyl groups, and NR'R"; wherein R' and R" are each independently selected from the group consisting of hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups.

In still another embodiments, the invention pertains, at least in part, to compounds of Formula XIX:



XIX

wherein:

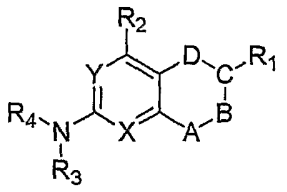
X, Y, A and B are each independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; and wherein R_1, R_2, R_3, and R_4 are each independently selected from the group consisting of C1-C10 alkyls, C3-C7 branched alkyls, hydroxyls, halogen, cyano, alkylaminoalkyls, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl groups, substituted or unsubstituted heteroaryl groups, C3-C6 cycloalkyl groups, and NR'R"; wherein R' and R" are each independently hydrogen, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

R_5 is selected from the group consisting of hydrogen, halogen, C1-C10 alkyl, C3-C7 branched alkyls, straight or branched halo-C1-C6-alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, and NR'R"; wherein R' and R" are each independently hydrogen, straight or branched C1-C6 alkyl groups,

substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

R_6 and R_7 are each independently selected from the group consisting of hydrogen, hydroxyl, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, straight or branched halo-C1-C6-alkyl groups, straight or branched halo-C1-C6-alkoxy groups, alkoxy-C1-C6-alkyl groups, hydroxyl-C1-C6-alkyl groups, or carboxy-C1-C6-alkyl groups, or alternatively R_6 and R_7 form a ring together with the nitrogen atom to which they are bonded, said ring selected from, for example, the group consisting of pyridyl, piperadino, morpholino, pyrrolidino and piperazino that can be optionally substituted with one or two substituents selected from the group consisting of C1-C6 alkyl carboxyl (which may be protected), amino, cyano, hydroxyl, halogen, substituted or unsubstituted benzoyl, substituted or unsubstituted arylalkyl (*e.g.*, benzylic) groups.

In still another embodiments, the invention pertains, at least in part, to compounds of Formula XX:



XX

wherein:

X, Y, A, B, C and D are each independently selected from the group consisting of carbon, nitrogen, oxygen, and sulfur; and wherein A, B, C and D form part of a saturated or unsaturated ring.

R_1, R_2 are selected from the group consisting of hydrogen, substituted or unsubstituted alkyl having C1-C10 atoms, substituted or unsubstituted branched alkyl having C3-C7 atoms, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted arylalkoxy, substituted or unsubstituted heteroaryl, substituted or unsubstituted heteroarylalkyl, substituted or unsubstituted

heteroarylalkoxy, substituted or unsubstituted aryloxyphenyl, substituted or unsubstituted alkoxyphenyl.

R_3 and R_4 are each independently selected from the group consisting of hydrogen, hydroxyl, straight or branched C1-C6 alkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted arylalkyls, substituted or unsubstituted arylalkoxy groups, straight or branched halo-C1-C6-alkyl groups, straight or branched halo-C1-C6-alkoxy groups, alkoxy-C1-C6-alkyl groups, hydroxyl-C1-C6-alkyl groups, or carboxy-C1-C6-alkyl groups, or alternatively R_3 and R_4 form a ring together with the nitrogen atom to which they are bonded, said ring selected from, for example, the group consisting of pyridyl, piperadino, morpholino, pyrrolidino and piperazino that can be optionally substituted with one or two substituents selected from the group consisting of C1-C6 alkyl, carboxyl (which may be protected), amino, cyano, hydroxyl, halogen, substituted or unsubstituted benzoyl, substituted or unsubstituted benzylic group.

In other embodiments, the structures shown above and in Figure 3 and their analogs can be synthesized.

Some of these structures may be commercially available from comprehensive chemicals databases such as Available Chemical Directory. However, many of the structures are novel an unavailable on the databases. The synthesis of those compounds that are not commercially available can be carried out by well established synthetic methodologies. There are many synthesis papers and patents that describe the synthesis of all of the desired analogues. (See e.g., U.S. 3,940,393; Sardarian, *et al.*, (2003) *Org. Biomol. Chem.* 21: 960-964; Ross, *et al.*, (1976) *J. Med. Chem,* 19: 723-725).

V. Modulation of Neurodegenerative Disorders Using Pharmacological Agents

The role of the nuclear receptor in the neurodegenerative diseases such as ALS, and modulation of the pathway associated with the nuclear receptor maybe a target of a clinical investigation in ALS or other neurodegenerative disease. The data shown in the Examples section indicate that the pyrimethamine and its analogs play a role in decreasing the expression of SOD-1.

The SOD1 G93A (high copy) mouse model for ALS is a suitable mouse that carries 23 copies of the human G93A SOD mutation and is driven by the endogenous promoter. Survival in the mouse is copy dependent. The high copy G93A has a median survival of around 128 days. High molecular weight complexes of mutant SOD protein are seen in the spinal cord beginning around day 30. At day 60 reactive astrocytosis (GFAP reactive) are observed; activated microglia are observed from day 90 onwards. Studies by Gurney *et al.* showed that at day 90 reactive astrocytosis loses statistical significance while microglial activation is significantly elevated and continues to be elevated through the end stage of the disease (*See* Gurney, *et al.* (1996) *Ann. Neurol.*, 39: 147-5739).

Many drugs that have shown efficacy in this model have moved forward into human clinical trials. Experience with riluzole, the only approved drug in the treatment of ALS, indicates that the mouse ALS model is a good predictor of clinical efficacy. Other drugs such as Creatine, Celebrex, Co-enzyme Q10, and Minocycline are under clinical evaluation based on studies in this model.

VI. Delivery of the Nuclear Receptor Modulating Pharmacological Agents

The pharmacological agent of the present invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a nuclear receptor modulating pharmacological agent, e.g., pyrimethamine and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the pharmacological agent.

The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the pharmacological agent is administered by an intraperitoneal injection.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, (see, for example, Langer, *Science* 249, 1527 (1990) and Hanes, *Advanced Drug Delivery Reviews* 28, 97-119 (1997). The agents of this invention can also be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient. The depot injection or implant preparation can, for example, comprise one or more of the pyrimethamine compounds or functional analogs, or comprise a combination of different agents (e.g., pyrimethamine and norethindrone).

The pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, the pharmacological agent) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any

additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The nuclear receptor modulating pharmacological agent can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. (*See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978; U.S. Patent Nos. 6,333,051 to Kabanov *et al.*, and 6,387,406 to Kabanov *et al.*).

In certain embodiments, a nuclear receptor modulating pharmacological agent may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

In certain embodiments, a nuclear receptor modulating pharmacological agent can be administered in a liquid form. The pharmacological agent should be soluble in a variety of solvents, such as for example, methanol, ethanol, and isopropanol. A variety of methods are known in the art to improve the solubility of the pharmacological agent

in water and other aqueous solutions. For example, U.S. Patent No. 6,008,192 to Al-Razzak *et al.* teaches a hydrophilic binary system comprising a hydrophilic phase and a surfactant, or mixture of surfactants, for improving the administration of compounds.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a nuclear receptor modulating pharmacological agent can be coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for improving the pharmacokinetics of the pharmacological agent. A variety of methods are known in the art to improve the pharmacokinetics of the pharmacological agent of the present invention. (See e.g., U.S. Patent No. 6,037,157 to Norbeck *et al.*).

Other methods of improving the pharmacokinetics of the pharmacological agent have been disclosed, for example, in U.S. Patent Nos. 6,342,250 to Masters, 6,333,051 to Kabanov *et al.*, 6,395,300 to Straub *et al.*, 6,387,406 to Kabanov *et al.*, and 6,299,900 to Reed *et al.* Masters discloses a drug delivery device and method for the controlled release of pharmacologically active agents. The drug delivery device disclosed by Masters is a film comprising one or more biodegradable polymeric materials, one or more biocompatible solvents, and one or more pharmacologically active agents dispersed uniformed throughout the film. In U.S. Patent No. 6,333,051, Kabanov *et al.* disclose a copolymer networking having at least one cross-linked polyamine polymer fragment, at least one nonionic water-soluble polymer fragment, and at least one suitable biological agent, including a pharmacological agent. According to the teachings of this patent, this network, referred to as a nanogel network, improves the therapeutic effect of the pharmacological agent by decreasing side effects and increasing therapeutic action. In another patent, U.S. Patent No. 6,387,406, Kabanov *et al.* also disclose another composition for improving the oral delivery of numerous pharmacological agents.

Other methods for improving the delivery and administration of the pharmacological agent include means for improving the ability of the pharmacological agent to cross membranes, and in particular, to cross the blood-brain barrier. In one embodiment, the pharmacological agent can be modified to improve its ability to cross the blood-brain barrier, and in an alternative embodiment, the pharmacological agent can be co-administered with an additional agent, such as for example, an anti-fungal compound, that improves the ability of the pharmacological agent to cross the blood-

brain barrier. Alternatively, precise delivery of the pharmacological agent into specific sites of the brain, can be conducted using stereotactic microinjection techniques. For example, the subject being treated can be placed within a stereotactic frame base (MRI-compatible) and then imaged using high resolution MRI to determine the three-dimensional positioning of the particular region to be treated. The MRI images can then be transferred to a computer having the appropriate stereotactic software, and a number of images are used to determine a target site and trajectory for pharmacological agent microinjection. The software translates the trajectory into three-dimensional coordinates that are precisely registered for the stereotactic frame. In the case of intracranial delivery, the skull will be exposed, burr holes will be drilled above the entry site, and the stereotactic apparatus used to position the needle and ensure implantation at a predetermined depth. The pharmacological agent can be delivered to regions, such as the cells of the spinal cord, brainstem, or brain that are associated with the disease or disorder. For example, target regions can include the medulla, pons, and midbrain, cerebellum, diencephalon (*e.g.*, thalamus, hypothalamus), telencephalon (*e.g.*, corpus stratium, cerebral cortex, or within the cortex, the occipital, temporal, parietal or frontal lobes), or combinations, thereof.

Pharmacological agents can be used alone or in combination to treat neurodegenerative disorders. For example, the pharmacological agent can be used in conjunction with other existing nuclear receptor modulators, for example, to produce an additive or synergistic effect. Likewise, the pharmacological agent can be used alone or in combination with an additional agent, *e.g.*, an agent which imparts a beneficial attribute to the therapeutic composition, *e.g.*, an agent which effects the viscosity of the composition. The combination can also include more than one additional agent, *e.g.*, two or three additional agents if the combination is such that the formed composition can perform its intended function. In some embodiments, the invention includes administrating a pyrimethamine compound of the present invention, or functional analog thereof, together with for example, at least one progesterone related compound, such as norethindrone, or at least one estrogen related compound, such as estradiol. For descriptions of these compounds and admininstration, see co-pending applications entitled "Modulation of Neurodegenerative Diseases through the Progesterone

Receptor" and "Modulation of Neurodegenerative Diseases through the Estrogen Receptor" filed March 1, 2006.

The compounds of the present invention can be conjugated with pharmaceutically acceptable acid salts to facilitate their long storage and dosing as aqueous solutions. For example, the salt can be derived from a pharmaceutically acceptable acid (e.g., HCl) with or without the use of a pharmaceutically acceptable carrier (e.g., water). Such salts can be derived from either inorganic or organic acids, including for example hydrochloric, hydrobromic, acetic, citric, fumaric, maleic, benzenesulfonic, and ascorbic acids. The pharmaceutical compositions obtained by the combination of the carrier and the salt will generally be used in a dosage necessary to elicit the desired biological effect. This includes its use in a therapeutically effective amount or in a lesser amount when used in combination with other biologically active agents.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of a pharmacological agent of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the pharmacological agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the pharmacological agent to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmacological agent are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used

herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a pharmacological agent (e.g., pyrimethamine) is between 5 mg/day to about 200 mg/day administered to a subject, or group of subjects, preferably about 10 mg/day to about 150 mg/day, more preferably about 5 mg/day to about 20 mg/day, and most preferably about 3 mg/day to 10 mg/day. Preferably, administration of a therapeutically effective amount of pharmacological agent (e.g., pyrimethamine), results in a concentration of pharmacological agent in the bloodstream in the range of 1 nanomolar (nM) to 100 millimolar (mM) concentration. For example, a concentration range of about 100nM to about 10mM, about, 1nM to about 1mM, about 1nM to about 100 micromolar (μM), about 1μM to about 500μM, about 1μM to about 200μM, or about 10μM to about 50μM. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

One skilled in the art will appreciate further features and advantages of the invention based on the above-described embodiments. Accordingly, the invention is not to be limited by what has been particularly shown and described, except as indicated by the appended claims. All publications and references cited herein are expressly incorporated herein by reference in their entirety.

Examples

Example 1: Materials and Methods:

(i) Cell Culture

The human cervical carcinoma derived HeLa cell line (ATCC) was found to express SOD-1 protein and mRNA and was used as the model system to identify compounds that inhibit SOD-1 expression. Briefly, cells were maintained in Dulbecco's Minimal Essential Medium, with high glucose, supplemented with glutamine, 4 mM, certified fetal bovine serum, 10%, and penicillin, streptomycin, and nystatin (all from Invitrogen). Incubation conditions were 37 degrees and 99% relative humidity, with CO_2 at 5%. Cultures were passaged when they reached 90% confluence. For pharmacological experiments, cells were plated into sterile tissue culture treated 96 well plates at a density of 3,500 cells/well in 150 µl medium.

(ii) Drugs:

All compounds were dissolved in 100% DMSO, at a stock concentration of 10 mM. Drugs were obtained from Microsource Discovery or from Sigma Aldrich.

(iii) Experimental Protocol:

After plating and 6 hours for attachment, drugs were added to the medium in a concentration of 10 µM. Following 72 hours of incubation with the drugs, the cells were photographed at 100X using an inverted microscope and digital camera, so that cytotoxicity could be evaluated. After photodocumentation, the medium was removed and the cells were washed once with phosphate buffered saline, and then 50 µl molecular biology grade water containing a protease inhibitor cocktail was added. After 10 min incubation, the plates were placed in –80 degrees to induce complete lysis. Plates were then thawed and 25 µl was transferred from each well into a maxsorp ELISA plate coated with anti-human SOD-1 antibody, which contained 75 µl phosphate buffered saline. A second antibody pair (a polyclonal anti-SOD-1/HRP conjugated goat anti-rabbit) was then added to the well, and incubation was conducted for 1 hour at room temperature. At the conclusion of the incubation, the plate was washed three times (wash buffer from KPL Inc.) and Sure Blue Reserve HRP Substrate was added. Following a 5-10 min incubation, the reaction (which had turned blue to varying degrees) was stopped by the addition of a stop reagent (KPL). The plate was then

shaken gently for 5 seconds and the absorbance at 450 nm read on a Tecan Plate reader. Absorbance from each sample were compared to standard curve of purified recombinant human SOD-1 assayed on the same ELISA plate, and SOD-1 immunoreactivity (ng/ml) was estimated by comparison with the standard curve.

(iv) Bradford Protein Assay:

To determine if decrements found in the SOD-1 assay were simply the result of cytotoxic effects of the drug treatment, total protein was determined for each well. While the ELISA incubation was ongoing, 10 µl of the remaining lysate was removed from each well and placed into another empty plate, and BioRad Bradford reagent (100 µl) was added to the protein. After a 15 min incubation at room temperature the plate was shaken gently for 5 seconds and the absorbance was read at 595 nm in a Tecan Sunrise plate reader. Protein concentrations in each well were thus determined by comparison with protein standards that were run on the same plate.

(v) Quantitative RT-PCR:

HeLa cells at 3500 cells/well in a 96 well plate were treated with a compound of the present invention for 72 h as above and then cells were lysed and total RNA extracted using the Gentra RNA extraction protocol and reagents. The purified RNA was then used as the template in a reverse transcription reaction using Superscript III MMLV Transcriptase primed with oligoDT. A PCR reaction was performed on the resultant cDNA to amplify the cDNA corresponding to human SOD-1, human TATA-box binding protein, and human Beta-2 microglobulin. The PCR reactions were run in separate tubes for 20, 25, and 30 cycles and the amplicons were then run on a 2% agarose gel containing ethidium bromide. The fluorescence emitted by the ethidium bromide stained bands following stimulation by a UV light source was captured using a digital camera. The digitized images were analyzed using ImageJ (NIH) and the bands for SOD-1 were compared with the bands for TATA-box binding protein and Beta2 Microglobulin (these housekeeping genes were unaffected by the drugs) while in the linear range of cycles, 25 cycles under these conditions, for increases or decreases relative to controls.

(vi) GeneChip Experiments:

Total cellular mRNA was prepared from HeLa cells with or without treatment using a Qiagen RNA mini kit followed by oligotex mRNA mini kit. Double-stranded cDNAs were synthesized from 2 μg total mRNA using the Superscript Choice System for cDNA synthesis (Invitrogen) with the T7-(dT)24 primer following the manufacturer's recommendations. cDNAs were cleaned up by phase lock gel (PLG) phenol/chloroform extraction and concentrated by ethanol precipitation. Biotin-labeled cRNA was synthesized from cDNA by in vitro transcription using the Bioarray HighYield RNA transcript Labeling Kit (Affymetrix) following vendor's recommendation. In vitro transcription products were cleaned up using RNeasy spin columns (Qiagen) and fragmented by metal-induced hydrolysis in fragmentation buffer (40 mM Tris-acetate, pH 8.1, 100 mM KOAc, 30 mM MgOAc). Fragmented cRNA was then subjected to Affymetrix GeneChip sets in hybridization buffer (100 mM MES, 1M NaCl, 20 mM EDTA, 0.01% Tween-20). GeneChip images were analyzed with Affymetrix Microarray Suite V5.0 and Affymetrix Data Mining Tool V3.0. Signal intensities of all probe sets were scaled to a target value of 150. Results of Detection Call, Change Call and Signal Log Ratio were obtained by applying the default parameters to statistical algorithms for both absolute and comparison analyses.

(vii) Western blotting.

Animals were overdosed with sodium pentobarbital (250 mg/kg, i.p.). Spinal cords were dissected and homogenized in 20 mM Tris-HCl, pH 7.5, 2 mM DTT, 0.1 mg of leupeptin, 1 mM EDTA, and 1 mM EGTA. The homogenate was then centrifuged at 14,000 × g to pellet debris. Protein concentration was measured using the BCA protein assay (Pierce, Rockford, IL). Protein (25 μg) from each sample was run on a 4-20% Tris-glycine gel (Invitrogen, San Diego, CA). After transfer, membranes were washed in PBS, followed by overnight incubation in blocking buffer (0.2% I-block (Applied Biosystems, Foster City, CA), PBS, and 0.1% Tween 20). The membrane was then probed with a polyclonal rabbit anti-bovine SOD1 (Sigma) antibody at 1:4000 dilution. After several washes, membranes were incubated with an alkaline phosphatase-conjugated secondary antibody (1:5000 in blocking buffer), and the immunoreactive signals were visualized using an enhanced chemiluminescent reagent, CDP Star

(Western Star kit; Tropix). After exposure, films were scanned and then imported into NIH Image for quantitation of band density.

Example 2: Testing the Effects of an Antimalarial Agent

This example describes how to examine the *in vitro* effects of the antimalarial drug, pyrimethamine, on SOD-1 activity. The human cervical carcinoma derived HeLa cell line (ATCC) were cultured in Dulbecco's Minimal Essential Medium, with high glucose, supplemented with glutamine, 4 mM, certified fetal bovine serum, 10%, and penicillin, streptomycin, and nystatin (all from Invitrogen). Incubation conditions were 37°C and 99% relative humidity, with CO_2 at 5%. Cultures were passaged when they reached 90% confluence. For pharmacological experiments, cells were plated into sterile tissue culture treated 96 well plates at a density of 3,500 cells/well in 150 µl medium.

Following 72 hours of incubation with the pyrimethamine, the cells were photographed and processed as described in Example 1 (iii). The total protein of the lysates was determined by Bradford assay as described in Example 1 (iv). The results of this study are shown in Figure 1. These results show that pyrimethamine added to culture medium of HeLa cells 72 hours before harvest significantly reduced the levels of SOD-1 protein, while total protein levels were unaffected. This reduction was dose related and maximal by 10 µM, with an IC_{50} of less than 3 µM. Figure 2 shows that both norethindrone and pyrimethamine (5 µM) caused a dose-related decrease in hSOD-1 mRNA in HeLa cells following 72 h treatment.

Alpha-synuclein has been implicated in neurodegenerative disorders characterized by Lewy body inclusions such as Parkinson's disease (PD) and dementia with Lewy bodies. Lewy body-like inclusions have also been observed in spinal neurons of patients with amyotrophic lateral sclerosis (ALS) and reports suggest possible alpha-synuclein abnormalities in ALS patients alpha-Synuclein is a ubiquitous protein that shares significant physical and functional homology to the protein chaperone, 14-3--3, and is particularly abundant in the brain (Ostrerova N. et al., J. *Neurosci.*, 19:5782 (1990)). An increased rate of alpha-synuclein aggregation might contribute to the mechanisms of neurodegeneration in Lewy body diseases. Studies on transgenic animals also suggest that aggregation of alpha-synuclein is harmful to

neurons. It was reported that dopaminergic dysfunction occurred in transgenic mice expressing wild type human alpha-synuclein (Masliah, E., et at., *Science*, 287:1265-1269 (2000)) and that Drosophila over-expressing alpha-synuclein exhibited dopaminergic dysfunction and dopaminergic neuronal death associated with development of alpha-synuclein aggregates (Feany, M B, et al., *Nature* 404:394-8 (2000)). Evidence suggests that neurons with dopamine develop alpha-synuclein aggregates and degenerate as these aggregates development.

The results of the Genechip analysis shown in Figure 4 illustrates that pyrimethamine (ALG-2001) (3μM) and norethindrone (ALG-3001) (3μM) substantially decreased mRNA for alpha synuclein in HeLa cells following 4 days of treatment. Thus, the compounds of the present invention can slow neurodegeneration in Lewy body diseases. This illustrates a role for the compounds of the present invention in slowing the progression or ameliorating the effects of ALS and PD.

Example 3: Testing the Effects of Pharmacological Agents In vivo

(a) SOD-93A murine model

The effects of the pharmacological agents e.g., pyrimethamine, and analogs thereof described in Example 2 were tested *in vivo* in the SOD-93A murine model for ALS and a reduction in the SOD-1 levels was measured. The inhibition of RNA expression was monitored by isolated blood samples from the art recognized mouse model of ALS pre- and post introduction of the compound using standard RT-PCR techniques. The expression of the SOD-1 protein was determined using Western blot techniques with an anti-SOD-1 antibody from Sigma.

As shown in Figures 5 and 6, chronic treatment with pyrimethamine (10 mg/kg ip X 14 days) significantly ($P < 0.05$, $n = 7$) decreased SOD-1 protein and alpha synuclein in mouse lymphocytes. The results were show to be statistically significant using a student t-test analysis. The control was vehicle (saline).

Chronic pyrimethamine (50 mg/kg/d) significantly decreased spinal SOD-1 in G93A mice following 14 d treatment as shown in Figure 7. Pyrimethamine was administered orally for 14 days. Spinal cords were harvested and analyzed by Western blot analysis as described above.

(b) Human familial ALS patient

A 38 year old familial ALS patient volunteer showed a significant decrease in SOD-1 levels following oral treatment with pyrimethamine (100 mg/d for 30 days). Figure 8 shows the decreased lymphocyte SOD1 levels in the familial SOD1 patient following administration of the drug (post drug) compared to prior to treatment (predrug). Approximately 5-8 cc blood was collected from the patient. SOD-1 levels were analyzed by ELISA and Western blot analysis.

The *in vivo* effects can also be determined by monitoring the breathing of a subject by measuring the forced vital capacity (FVC) using a Renaissance Puritan Bennett Spirometer. The maximum inspiratory force (MIF) can also be measured using a hand held manometer.

Example 4: Neurological Scoring

The effects of the nuclear receptor modulating pharmacological agents can also be determined by a neurological score recorded on a 4-point scale:

0 = Normal reflex on the hind limbs (animal will splay its hind limbs when lifted by its tail)

1 = Abnormal reflex (Lack of splaying of hind limbs when animal is lifted by the tail).

2 = Abnormal reflex and visible evidence of paralysis

3 = Lack of reflex and total paralysis of hind limbs.

4 = Inability to right themselves when placed on the sides in 30 seconds or found dead. The animals are sacrificed at this stage if alive.

Statistical analysis on the neurological score, body weight and survival can be performed by utilizing ANOVA, Kaplan Meier, t-test, Cox's proportional hazards

regression model, log-logistic and parametric methods and mixed linear model methods. All statistical analysis was performed using standard procedures known in the art.

CLAIMS:

1. A method for reducing the production of an SOD protein in a cell comprising, administering a pharmacological agent to the cell, such that the agent interacts with a nuclear receptor and inhibits transcription of a gene encoding the SOD protein, wherein the agent is pyrimethamine or functional analogs thereof.

2. The method of claim 1, wherein the cell is selected from the group consisting of a cell in a brain, a cell in a spinal cord, a cell in a meningial tissue and, a cell in muscle.

3. The method of claim 1, wherein the cell is a neural cell in a subject with ALS.

4. The method of claim 1, wherein the SOD protein is the SOD-1 protein.

5. The method of claim 1, wherein the pharmacological agent is pyrimethamine.

6. The method of claim 1, wherein the pharmacological agent is pyrimethamine with at least one modification in the benzene ring.

7. The method of claim 1, wherein the pharmacological agent is pyrimethamine with at least one modification in the pyrimidine ring.

8. The method of claim 1, wherein the inhibition of transcription of the gene comprises monitoring expression levels of the SOD protein.

9. The method of claim 1, wherein the inhibition of transcription of the gene comprises monitoring the levels of a nucleic acid molecule that encodes the SOD protein.

10. The method of claim 9, wherein the nucleic acid molecule is selected from the group consisting of ribonucleic acid or deoxynucleic acid.

11. A method for preventing the development of symptoms, or ameliorating the symptoms or progression of amyotrophic lateral sclerosis (ALS) in a subject comprising, administering a prophylactically or therapeutically effective amount of a pharmacological agent to the subject, wherein the agent inhibits transcription of a gene encoding a SOD-1 protein.

12. The method of claim 11, wherein the pharmacological agent is pyrimethamine or functional analogs thereof.

13. The method of claim 11, wherein the pharmacological agent is pyrimethamine with at least one modification in the benzene ring.

14. The method of claim 11, wherein the pharmacological agent is pyrimethamine with at least one modification in the pyrimidine ring.

15. The method of claim 11, further comprising monitoring the amelioration of ALS by monitoring survival prolongation of the subject.

16. The method of claim 15, wherein the step of monitoring the amelioration of ALS comprises monitoring a neurological score of the subject.

17. The method of claim 15, wherein the step of monitoring the amelioration of ALS comprises monitoring expression levels of the SOD-1 protein.

18. The method of claim 15, wherein the step of monitoring the amelioration of ALS comprises monitoring the levels of a nucleic acid molecule that encodes SOD-1.

19. The method of claim 18, wherein the nucleic acid molecule is selected from the group consisting of ribonucleic acid or deoxynucleic acid.

FIGURE 1

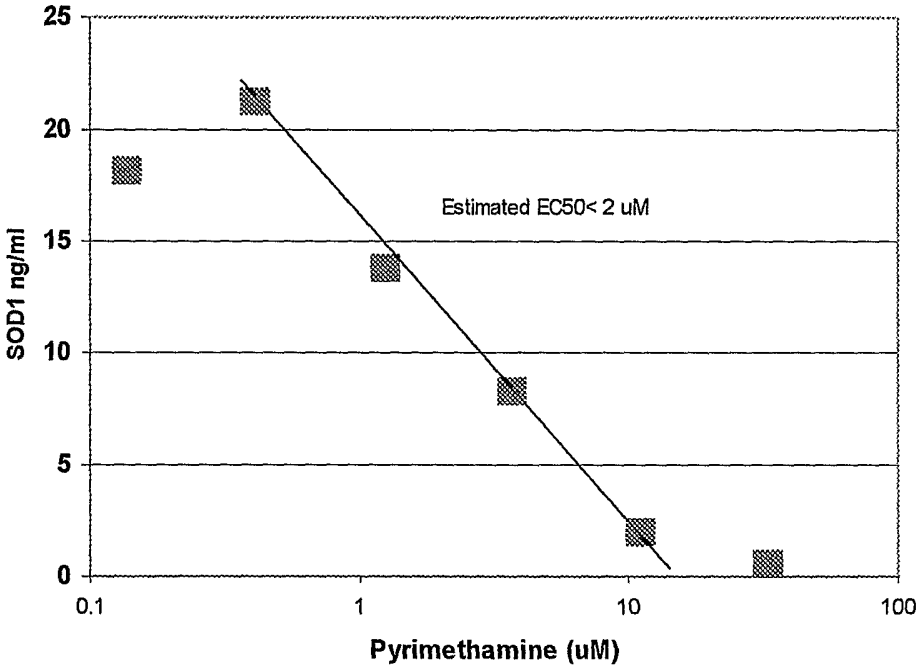

25
20
15
10
5
0
SOD1 ng/ml
Estimated EC50< 2 uM
0.1
1
10
100
Pyrimethamine (uM)

FIGURE 2

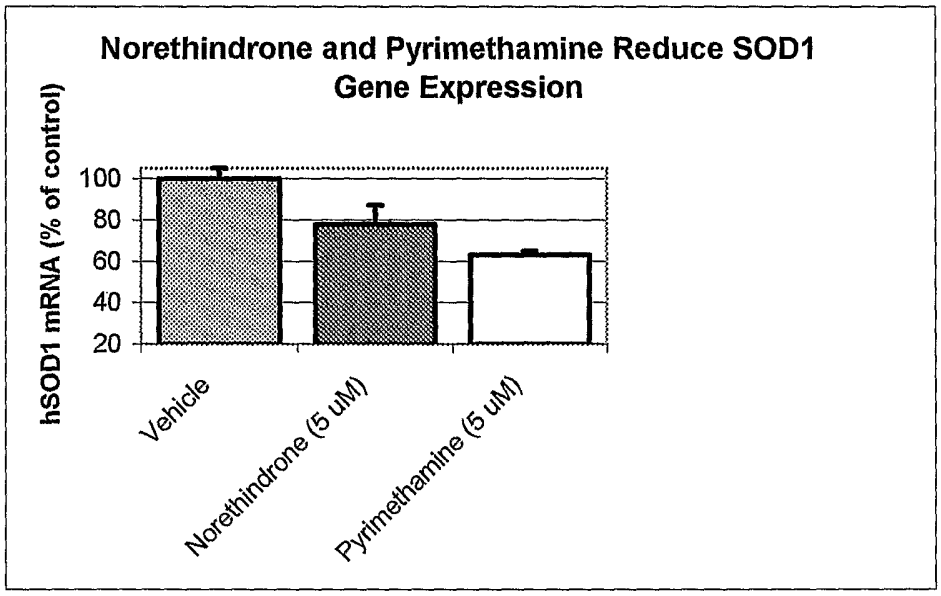

Norethindrone and Pyrimethamine Reduce SOD1 Gene Expression
hSOD1 mRNA (% of control)
100
80
60
40
20
Vehicle
Norethindrone (5 uM)
Pyrimethamine (5 uM)

FIGURE 3

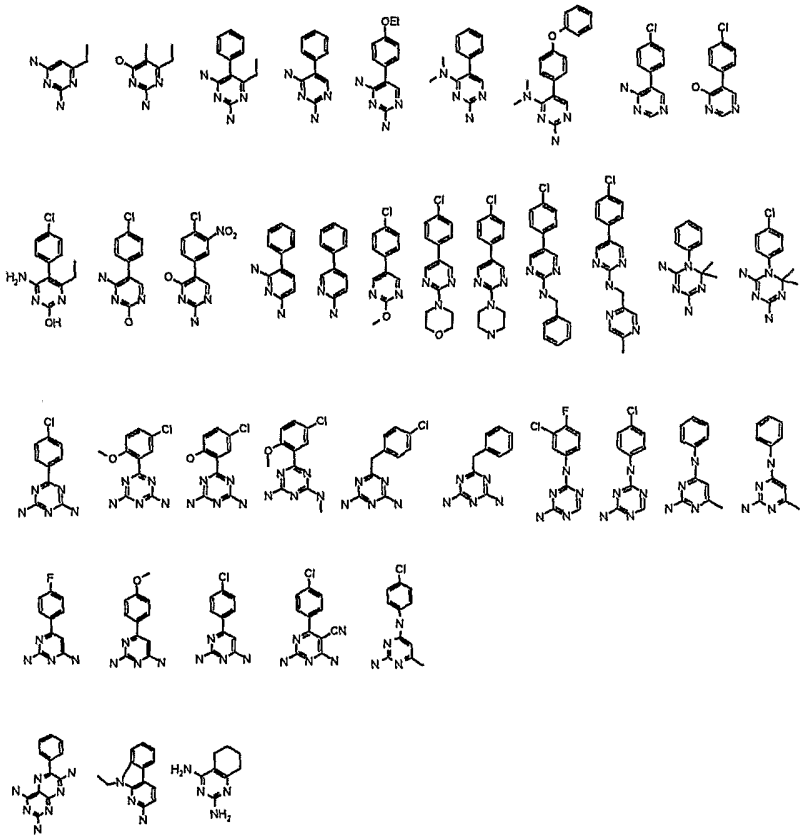


FIGURE 4

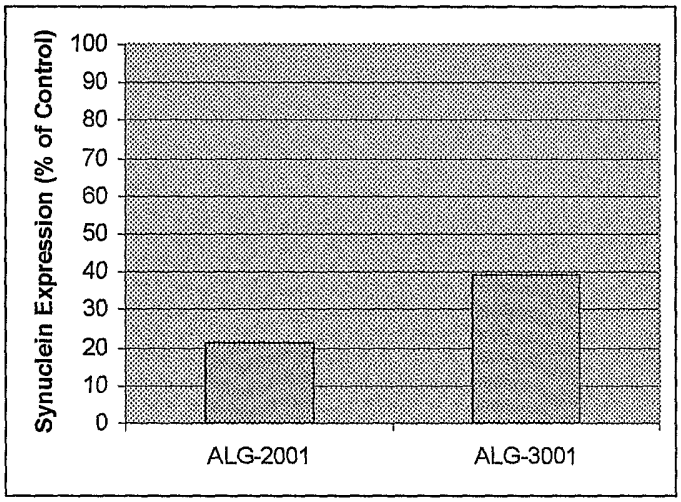

Synuclein Expression (% of Control)
100
90
80
70
60
50
40
30
20
10
0
ALG-2001
ALG-3001

FIGURE 5

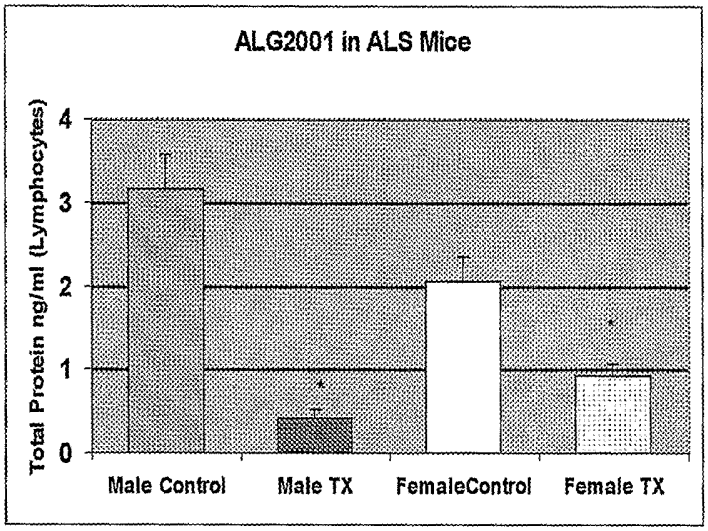

ALG2001 in ALS Mice
Total Protein ng/ml (Lymphocytes)
4
3
2
1
0
Male Control
Male TX
FemaleControl
Female TX

FIGURE 6

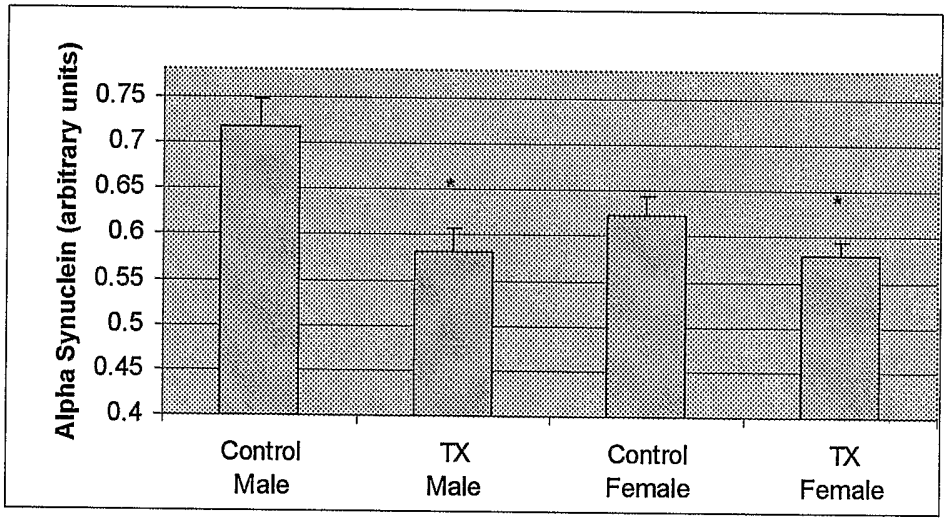

Alpha Synuclein (arbitrary units)
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
Control Male
TX Male
Control Female
TX Female

FIGURE 7

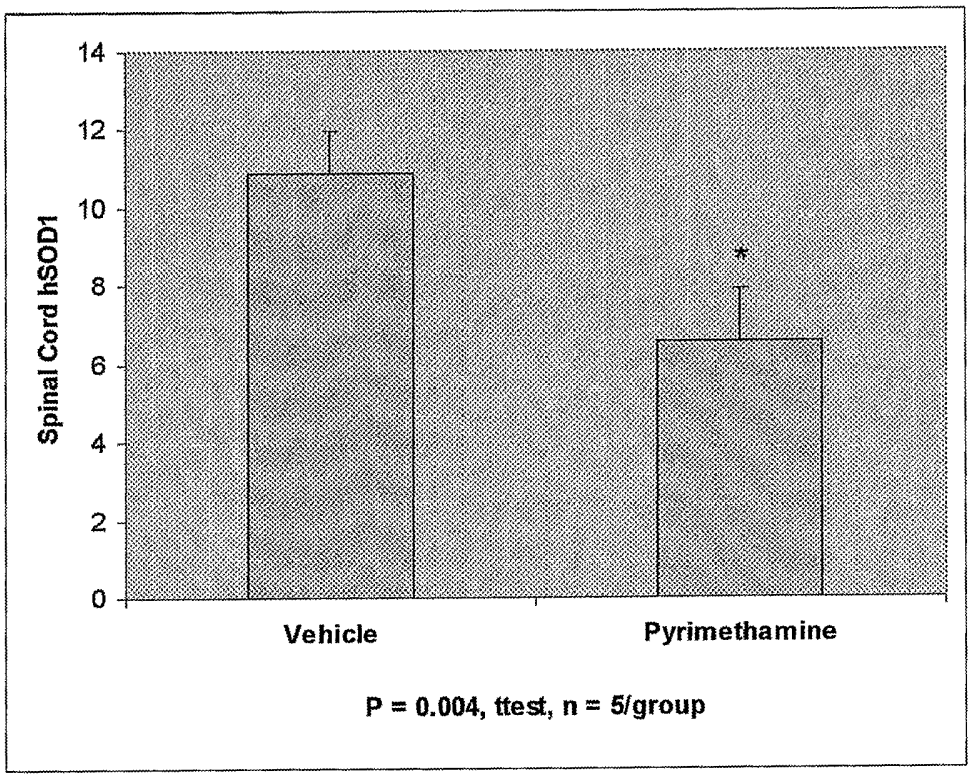
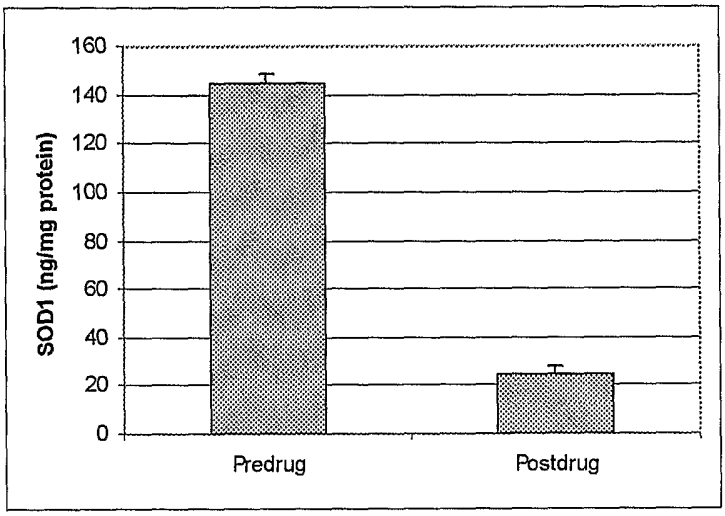

Spinal Cord hSOD1
14
12
10
8
6
4
2
0
*
Vehicle
Pyrimethamine
P = 0.004, ttest, n = 5/group

FIGURE 8


160
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100
80
60
40
20
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SOD1 (ng/mg protein)
Predrug
Postdrug

SEQUENCE LISTING

<110> Scott, Sean
Benjamin, Daniel

<120> Modulation of Neurodegenerative Diseases

<130> 106792-0009

<140> Not Yet Assigned
<141> 2006-03-01

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Asn Pro Leu Ser Arg Lys His Gly Gly Pro Lys Asp Glu Glu Arg His
65                  70                  75                  80

Val Gly Asp Leu Gly Asn Val Thr Ala Asp Lys Asp Gly Val Ala Asp
                85                  90                  95

Val Ser Ile Glu Asp Ser Val Ile Ser Leu Ser Gly Asp His Cys Ile
            100                 105                 110

Ile Gly Arg Thr Leu Val Val His Glu Lys Ala Asp Asp Leu Gly Lys
        115                 120                 125

Gly Gly Asn Glu Glu Ser Thr Lys Thr Gly Asn Ala Gly Ser Arg Leu
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Ala Cys Gly Val Ile Gly Ile Ala Gln
145                 150
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