Abstract:
The invention concerns novel synthetic triterpenoids, such as CDDO-EA and CDDO-TFEA, and their use for the treatment and prevention of diseases, such as MS.

Title:
NOVEL AMIDE DERIVATIVES OF CDDO AND METHODS OF USE THEREOF

Abstract:
The invention concerns novel synthetic triterpenoids, such as CDDO-EA and CDDO-TFEA, and their use for the treatment and prevention of diseases, such as MS.
DESCRIPTION

NOVEL AMIDE DERIVATIVES OF CDDO AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

The present application claims benefit of priority to U.S. Provisional Application Serial No. 60/916,273, filed May 4, 2007, the entire contents of which are hereby incorporated by reference.

The U.S. government owns rights in the present invention pursuant to grant number ROI CA78814 from the National Institutes of Health.

I. Field of the Invention

The present invention relates generally to the fields of biology and medicine. More particularly, it concerns compositions and methods for the treatment and prevention of diseases, including multiple sclerosis and cancer.

II. Description of Related Art

Multiple sclerosis (MS) continues to be a devastating neurological disease with fatal consequences in many patients. MS is believed to be an inflammatory autoimmune disease in which the patient's own T lymphocytes attack neurons, resulting in demyelination and subsequent neuronal failure. Multiple sclerosis may take several different forms, with new symptoms occurring either in discrete attacks or slowly accruing over time. Between attacks, symptoms may resolve completely, but permanent neurologic problems often persist, especially as the disease advances. MS currently does not have a cure, though several treatments are available that may slow the appearance of new symptoms.

MS causes gradual destruction of myelin (demyelination) and transection of neuron axons in patches throughout the brain and spinal cord, causing symptoms that vary widely depending upon which signals are interrupted. While there is no known definitive cure for multiple sclerosis, several types of treatments are used, depending on the MS type. The treatments include β-interferons, glatiramer acetate, mitoxantrone, natalizumab, and prednisone. Each of these therapies has significant side effects and limitations. For example, β-interferons reduce but don't eliminate flare-ups of multiple sclerosis. They have not been shown to reverse damage or significantly alter the long-term development of permanent disability. Also, some patients develop antibodies to β-interferons, which may make them
less effective. The side effects of β-interferons may include flu-like symptoms. Glatiramer acetate (Copaxone) is an alternative treatment to beta interferons for patients suffering from remitting MS. As had been suspected, it has recently been reported ineffective against the primary progressive types of the disease (Wolinsky et al, 2007), at least as a single agent treatment. Side effects of glatiramer can include flushing and shortness of breath after injections, which are usually taken daily. Aggressive forms of relapsing remitting MS are often treated with mitoxantrone (tradename Novantrone), a chemotherapy drug used for many cancers. The medication, while effective, is limited by cardiac toxicity. Finally, the use of the once promising treatment, natalizumab (trade name Tysabri), has been sharply limited by the FDA, due to reports that it may lead to a rare, often fatal, brain disorder called progressive multifocal leukoencephalopathy.

Given the side effects and other limitation of the above methods of treating MS, and the lack of approved treatments for primary progressive multiple sclerosis, a need exists for new and more effective compounds and methods of treating and preventing this disease.

Separately, synthetic triterpenoids (TPs) have been developed as anti-inflammatory agents and their anti-inflammatory effects have been reported. Much of the research has focused on their chemotherapeutic potential. The connection between inflammation and carcinogenesis (Balkwill et al, 2005) led to synthesis and testing of anti-inflammatory triterpenoids for the treatment of cancer. The most potent of these agents, such as 2-cyano-3,12-dioxooleana-1,9(1 l)-dien-28-oic acid (CDDO), its methylester (CDDO-Me), and CDDO-Imidazolide (CDDO-Im), are some of the strongest known inhibitors of the de novo synthesis of inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase 2 (Honda et al, 1998; Honda et al, 1999; Suh et al, 1999; Honda et al, 2000; Bore et al, 2002; Honda et al, 2002; Place et al, 2003; U.S. Patents 6,326,507, 6,552,075 and 6,974,801). The compounds are shown below.
In addition to their anti-inflammatory actions, CDDO and its derivatives are also multifunctional compounds that induce differentiation, inhibit cell proliferation, and selectively induce apoptosis of a wide variety of cancer cells, including human lung cancer cells (Suh et al., 1999; Ito et al., 2000; Konopleva et al., 2002; Kim et al., 2002). Both CDDO and CDDO-Me are currently in phase I clinical trials for treatment of leukemia and solid tumors. However, treatment of a neurodegenerative disease, such as MS, requires an agent to be able to readily penetrate the blood brain barrier (BBB). Similarly, optimal treatment of brain cancer requires an agent to penetrate the BBB, leading to a lack of effective therapies for both primary and metastatic brain cancer. Most of the triterpenoids that have been made previously do not achieve high concentrations in the brain, thus their utility as treatments for brain cancer and for disorders of the central nervous system, such as MS, remained uncertain.

**SUMMARY OF THE INVENTION**

The present invention overcomes limitation of the prior art by providing new methods for the treatment of neurodegenerative diseases, such as multiple sclerosis (MS), psychiatric disorders such as psychosis, bipolar disorder, and depression, neuropathic pain and related conditions involving CNS-mediated chronic pain, and by providing new synthetic triterpenoid derivatives, having a improved ability to penetrate the blood brain barrier.

In one aspect, the invention provides methods for treating multiple sclerosis (MS) in mammalian subjects comprising, administering to the subjects pharmaceutically effective amounts of a compound, according to formula I, shown below.
In certain embodiments, \( R_1 \) is a heteroatom-substituted or heteroatom-unsubstituted \( \text{C}_1^\text{-C}_{15}^- \) acyl.

In another aspect, the method comprises treating with a pharmaceutically acceptable salt or hydrate of the compound. In yet another aspect, the compound, salt, or hydrate may be a single enantiomer that is substantially free from other optical isomers. In still another aspect, the compound, salt, or hydrate is a racemic mixture.

In certain embodiments, the MS may be primary progressive, relapsing-remitting secondary progressive or progressive relapsing. In other embodiments, the treatment may suppress the demyelination of neurons in the mammalian subject's brain or spinal cord. In further embodiments, the treatment may suppress the following in the brains or spinal cords of mammalian subjects: inflammatory demyelination, transection of neuron axons, transection of neurites, and/or neuronal apoptosis. Examples of mammalian subjects include, for example, cows, horses, dogs, cats, pigs, mice, rats, guinea pigs or primates—for example, humans.

In other embodiments, the treatment may stimulate the remyelination of neuron axons in the brains or spinal cords of mammalian subjects. In further embodiments, the treatment may restore lost function after an MS attack, prevent new MS attacks, and/or treat disability resulting from an MS attack.

In another aspect, the invention provides methods for treating multiple sclerosis (MS) in mammalian subjects comprising, administering to the subjects pharmaceutically effective amounts of a compound, according to formula II, shown below.
In certain embodiments, the group $Y$ is $-$H, hydroxy, amino, halo, or a heteroatom-substituted or heteroatom-unsubstituted $\text{C}_1$-$\text{C}_{14}$-alkoxy, $\text{C}_2$-$\text{C}_{14}$-alkenyloxy, $\text{C}_2$-$\text{C}_{14}$-alkynylloxy, $\text{C}_1$-$\text{C}_{14}$-aryloxy, $\text{C}_2$-$\text{C}_{14}$-aralkoxy, $\text{C}_1$-$\text{C}_{14}$-alkylamino, $\text{C}_2$-$\text{C}_{14}$-alkenylamino, $\text{C}_2$-$\text{C}_{14}$-alkynylamino, $\text{C}_1$-$\text{C}_{14}$-arylamino, or $\text{C}_2$-$\text{C}_{14}$-aralkylamino. In some embodiments, the $Y$ is a heteroatom-substituted or heteroatom-unsubstituted $\text{C}_2$-$\text{C}_{4}$-alkylamino having at least one fluorine atom.

In other embodiments, $Y$ is a heteroatom-substituted or heteroatom-unsubstituted $\text{C}_1$-$\text{C}_{4}$-alkoxy.

In another embodiment, treatment with compounds of the invention may be effective in alleviating symptoms of mental illness such as psychosis, major depression, bipolar disorder, or other neuropsychiatric disorders such as autism, attention deficit disorder, and related disorders.

In yet another embodiment, treatment with compounds of the invention may be effective in alleviating symptoms of neuropathic pain and other pain syndromes including fibromyalgia, as well as related conditions such as tinnitus that also involve chronic activation of peripheral or CNS sensory pathways.
In still another embodiment, treatment with compounds of the invention may be effective in treating epilepsy and other seizure disorders.

In still another embodiment, treatment with compounds of the invention may be effective in treating primary brain cancers such as glioblastoma and other gliomas, as well as metastatic brain cancer that develops secondary to non-CNS primary cancers such as breast cancer, lung cancer, prostate cancer, lymphoma, and melanoma.

Non-limiting examples of triterpenoids that may be used in accordance with the methods of this invention are shown here.

A further aspect of the invention provides a method for treating multiple sclerosis (MS) in a mammalian subject comprising, administering to said subject a) a first amount of a first compound according to formula I, wherein $R_1$ is a heteroatom-substituted or heteroatom-unsubstituted Ci-Cis-acyl; or a pharmaceutically acceptable salt or hydrate thereof; and b) a second amount of a compound selected from the group consisting of interferon $\beta$-la,
interferon β-lb, glatiramer acetate, mitoxantrone, natalizumab, uric acid, and methylprednisolone; wherein the combined first and second amounts are effective to treat the MS.

In another aspect of this invention, new synthetic triterpenoids according to formula II, shown below, are provided.

In certain embodiments, the group Y is ethylamino or a heteroatom-substituted C₁-C₅-alkylamino having at least one fluorine atom. In other embodiments, the Y is a heteroatom-substituted or heteroatom-unsubstituted C₂-C₄-alkylamino having at least one fluorine atom.

In further embodiments, the invention provides pharmaceutically acceptable salts and hydrates of these new synthetic triterpenoids. In yet further embodiments, the invention provides single enantiomers of these new synthetic triterpenoids or their salts or hydrates that are substantially free from other optical isomers. In still further embodiments, racemic mixtures of these new synthetic triterpenoids as well as their salts and hydrates are provided.

Examples of new CDDO derivatives provided by the present invention include CDDO-TFEA and CDDO-EA, shown below.

Any embodiment discussed herein with respect to one aspect of the invention applies to other aspects of the invention as well, unless specifically noted.
Other objects, features and advantages of the present invention will become apparent from the following detailed description and any accompanying drawings. It should be understood, however, that the detailed description and any specific examples or drawings provided, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 - CDDO-Me (TP-155) is Detectable in the Brains of Mice Fed Very Low Levels of Compound for One Week. Three male mice were in each group. The concentration in picograms (pg) of TP-155 per milligrams (mg) of mouse brain is shown as a function of the amount of Tp-155 in the diet, normalized to the weight of the individual mouse.

FIG. 2 - Significant Concentrations of CDDO Methyl Amide (TP-224) in Brains of Mice After Feeding 800 mg/kg Diet. The nanomolar concentration of TP-244 in the brains of mice is shown as a function of the number of days the mice were fed a 800 mg/kg diet of TP-224.

FIG. 3 - Feeding CDDO-Ethyl Amide (TP-319) for Two Days Results in Significantly Higher Brain Levels Than CDDO Methyl Amide (TP-224). Four CD-I mice per group were fed triterpenoids (800 mg/kg diet) for 48 hrs, and triterpenoid levels in brain were analyzed by LC/MS.

FIG. 4 - Brain Levels of CDDO-Ethyl Amide (TP-319) Are Dose Responsive and Higher Than For CDDO Methyl Amide (TP-224). Male CD-I mice were fed triterpenoids (200-800 mg/kg diet) for 3.5 days, and triterpenoid levels in the brains of the mice were analyzed by LC/MS. The number of mice in each experiment is indicated by "n".

FIG. 5 - CDDO-TFEA (TP-500) Is Detected at Higher Levels in Mouse Brain than CDDO-EA (TP-319). Three CD-I mice per group were fed TPs (200-400 mg/kg diet) for 3.5 days, and TP levels in the brains of the mice were analyzed by LC/MS.
FIG. 6 - Brain Levels of CDDO-TFEA (TP-500) Remain Significantly Higher Than CDDO-EA (TP-319). Four CD-I mice per group were fed TPs (400 mg/kg diet) for 10 weeks (CDDO-EA) or 6 weeks (CDDO-TFEA), and TP levels in the brains of the mice were analyzed by LC/MS.

FIG. 7 - Brain Levels of Triterpenoids in Gavaged CD-I Mice. Male CD-I mice, which each group containing "n" mice, were gavaged with TPs (2 μmol/mouse) daily for 3 consecutive days. Six hours after the final dose, TP levels in brain were analyzed by LC/MS.

FIG. 8 - CDDO-EA (TP-319) in CD-I Mouse Tissues. Four male CD-1 mice per group were gavaged once daily for 3 consecutive days with 1 μmol TP-319 (CDDO-EA). Six hours after the final gavage, the mice were sacrificed and TP levels were analyzed by LC/MS.

FIG. 9 - CDDO-TFEA (TP-500) in CD-I Mouse Tissues. Four male CD-1 mice per group were gavaged once daily for 3 consecutive days with 1 μmol TP-500 (CDDO-EA). Six hours after the final gavage, the mice were sacrificed and TP levels were analyzed by LC/MS.

FIGS. 10 and 11 - CDDO-TFEA (RTA 404) and CDDO-Me (RTA-402) Induce Full Recovery of Mice in Rapidly Progressive EAE Model. All animals (n=2/group) of varying clinical scores (CS) were immunized with myelin oligodendrocyte glycoprotein (MOG). The dose of MOG Peptide was 200 μg (divided into two injections, 100 μl each). The animals were then treated intraperitoneally (IP) with 100 nmol (~2.8 mg/kg) of RTA-402 or RTA-404 in 7.5% PBST (Phosphate Buffered Saline Tween-20) on a Q2Dx4 (4 doses, one every other day) schedule. A CS score of 0 indicates no symptoms, and score of 6 indicates quadriplegia.

FIGS. 12, 13, 14, 15, 16 and 17 - Untreated Animals do not Survive and Treated Animals Fully Recover. "CDDO-CF₃" refers to CDDO-TFEA. All animals (n=2/group) of varying clinical scores (CS) were immunized with myelin oligodendrocyte glycoprotein (MOG). The dose of MOG Peptide was 200 μg (divided into two injections, 100 μl each). The animals were then treated intraperitoneally (IP) with 100 nmol (~2.8 mg/kg) of RTA-402 or RTA-404 in 7.5% PBST (Phosphate Buffered Saline Tween-20) on a Q2Dx4 (4 doses, one every other day) schedule. A CS score of 0 indicates no symptoms, and score of 6 indicates quadriplegia.

FIG. 18 - Histologic Evidence of Resolution of Inflammatory Lesions After CDDO-TFEA (TP-500) Treatment. The three panels show H&E stains of tissue harvested from the brain stems of mice. The left panel shows the H&E stain from the control group, a
mouse that was neither immunized with MOG nor treated with TP. The middle panel shows extensive inflammation (here in the brainstem, but present in spinal cord and brain cortex as well) of a mouse that had been immunized with 200 µg of MOG (divided into two injections, 100 µl each) and had expired approximately 15 to 18 days later. The H&E stain reveals significant perivascular infiltrates (indicated by arrows) and infiltrates along the surface of the brain (subdural). These are gone in a treated animal (vessels encircled are free of surrounding infiltrates as is the surface of the brainstem), as shown in the right panel. The tissue of the brain stem of the treated animal was harvested after the mouse had recovered to a CS of 0 after having been first immunized with 200 µg of MOG (divided into two injections, 100 µl each), second allowed to degenerate to a CS of 6, third treated intraperitoneally (IP) with 100 nmol (-2.8 mg/kg) of CDDO-TFEA in 7.5% PBST (Phosphate Buffered Saline Tween-20) on a Q2Dx4 (4 doses, one every other day) schedule, and fourth allowed to recover to a CS of 0.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. The Present Invention

The present invention concerns new methods and compounds for the treatment and prevention of diseases, including multiple sclerosis (MS), involving the use of both novel triterpenoids that have been shown to be effective at penetrating into a mammalian subject's central nervous system, as well as known triterpenoids, whose effectiveness for the treatment of MS was unknown.

II. Definitions

As used herein, the term "amino" means -NH₂; the term "nitro" means -NO₂; the term "halo" designates -F, -Cl, -Br or -I; the term "mercapto" means -SH; the term "cyano" means -CN; the term "silyl" means -SiH₃, and the term "hydroxy" means -OH.

The term "heteroatom-substituted," when used to modify a class of organic radicals (e.g., alkyl, aryl, acyl, etc.), means that one, or more than one, hydrogen atom of that radical has been replaced by a heteroatom, or a heteroatom containing group. Examples of heteroatoms and heteroatom containing groups include: hydroxy, cyano, alkoxy, =O, =S, -NO₂, -N(CH₃)₂, amino, or -SH. Specific heteroatom-substituted organic radicals are defined more fully below.

The term "heteroatom-unsubstituted," when used to modify a class of organic radicals (e.g., alkyl, aryl, acyl, etc.) means that none of the hydrogen atoms of that radical have been
replaced with a heteroatom or a heteroatom containing group. Substitution of a hydrogen atom with a carbon atom, or a group consisting of only carbon and hydrogen atoms, is not sufficient to make a group heteroatom-substituted. For example, the group \(-\text{CeH}_4\text{C}≡\text{CH}\) is an example of a heteroatom-unsubstituted aryl group, while \(-\text{C}_6\text{H}_4\text{F}\) is an example of a heteroatom-substituted aryl group. Specific heteroatom-unsubstituted organic radicals are defined more fully below.

The term "heteroatom-unsubstituted \(\text{C}_n\)-alkyl" refers to a radical, having a linear or branched, cyclic or acyclic structure, further having no carbon-carbon double or triple bonds, further having a total of \(n\) carbon atoms, all of which are nonaromatic, 3 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted \(\text{Ci-Cio-alkyl}\) has 1 to 10 carbon atoms. The term "alkyl" includes straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl heteroatom-substituted cycloalkyl groups, and cycloalkyl heteroatom-substituted alkyl groups. The groups, \(-\text{CH}_2\text{F}\), \(-\text{CH}_2\text{Cl}\), \(-\text{CH}_2\text{Br}\), \(-\text{CH}_2\text{OH}\), \(-\text{CH}_2\text{OCH}_3\), \(-\text{CH}_2\text{OCH}_2\text{CH}_3\), \(-\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{NH}_2\), \(-\text{CH}_2\text{NHCH}_3\), \(-\text{CH}_2\text{N(CH}_3)_2\), \(-\text{CH}_2\text{NHCH}_2\text{CH}_3\), \(-\text{CH}_2\text{N(CH}_2\text{CH}_3)_2\), \(-\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{CH}_2\text{F}\), \(-\text{CH}_2\text{CH}_2\text{Cl}\), \(-\text{CH}_2\text{CH}_2\text{Br}\), \(-\text{CH}_2\text{CH}_2\text{I}\), \(-\text{CH}_2\text{CH}_2\text{OH}\), \(-\text{CH}_2\text{CH}_2\text{OCOCH}_3\), \(-\text{CH}_2\text{CH}_2\text{NH}_2\), \(-\text{CH}_2\text{CH}_2\text{N(CH}_3)_2\), \(-\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_3\), \(-\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_3)_2\), \(-\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_3\), \(-\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2\text{CH}_3)_2\), \(-\text{CH}_2\text{CH}_2\text{NHCOCH}_3\text{O}(\text{CH}_3)_3\), and \(-\text{CH}_2\text{Si(\text{CH}_3)_3}\).

The term "heteroatom-unsubstituted \(\text{C}_n\)-alkenyl" refers to a radical, having a linear or branched, cyclic or acyclic structure, further having at least one nonaromatic carbon-carbon double bond, but no carbon-carbon triple bonds, a total of \(n\) carbon atoms, three or more
hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted \( \text{C}_6\text{H}_4\text{C}=\text{CH} \) has 2 to 10 carbon atoms. Heteroatom-unsubstituted alkenyl groups include:

\(-\text{CH}=\text{CH}_2, \text{-CH}=\text{CHCH}_3, \text{-CH}=\text{CHCH}_2\text{CH}_3, \text{-CH}=\text{CHCH(CH)}_3\text{CH}_3, \text{-CH}=\text{CHCH(CH)}_2\text{CH}_2\text{CH}_3, \text{-CH}=\text{CHCHCH}_2\text{CH}_2\text{CH}_3,\)

\(-\text{CH}_2\text{CH}=\text{CH}, \text{-CH}_2\text{CH}=\text{CHCH}_3, \text{-CH}_2\text{CH}=\text{CHCH}\text{(CH)}_2\text{CH}_3, \text{-CH}_2\text{CH}=\text{CHCHCH}_2\text{CH}_2\text{CH}_3,\)

The term "heteroatom-substituted \( \text{C}_n\text{-alkenyl} \)" refers to a radical, having a single nonaromatic carbon atom as the point of attachment and at least one nonaromatic carbon-carbon double bond, but no carbon-carbon triple bonds, further having a linear or branched, cyclic or acyclic structure, further having a total of \( n \) carbon atoms, 0, 1, or more than one hydrogen atom, and at least one heteroatom, wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted \( \text{C}_2\text{-C}_1\text{O-alkenyl} \) has 2 to 10 carbon atoms. The groups, \(-\text{CH}=\text{CHF, -CH}=\text{CHCl, and -CH}=\text{CHBr} \) are examples of heteroatom-substituted alkenyl groups.

The term "heteroatom-unsubstituted \( \text{C}_n\text{-alkynyl} \)" refers to a radical, having a linear or branched, cyclic or acyclic structure, further having at least one carbon-carbon triple bond, a total of \( n \) carbon atoms, at least one hydrogen atom, and no heteroatoms. For example, a heteroatom-unsubstituted \( \text{C}_2\text{-C}_1\text{O-alkynyl} \) has 2 to 10 carbon atoms. The groups, \(-\text{C}=\text{CH, -C}=\text{CCH}_3\) and \(-\text{C}=\text{CC}_6\text{H}_5 \) are examples of heteroatom-unsubstituted alkynyl groups.

The term "heteroatom-substituted \( \text{C}_n\text{-alkynyl} \)" refers to a radical, having a single nonaromatic carbon atom as the point of attachment and at least one carbon-carbon triple bond, further having a linear or branched, cyclic or acyclic structure, and having a total of \( n \) carbon atoms, 0, 1, or more than one hydrogen atom, and at least one heteroatom, wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted \( \text{C}_2\text{-C}_1\text{O-alkynyl} \) has 2 to 10 carbon atoms. The group, \(-\text{C}=\text{C}	ext{Si(CH)}_3\) is an example of a heteroatom-substituted alkynyl group.

The term "heteroatom-unsubstituted \( \text{C}_n\text{-aryl} \)" refers to a radical, having a single carbon atom as a point of attachment, wherein the carbon atom is part of an aromatic ring structure containing only carbon atoms, further having a total of \( n \) carbon atoms, 5 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted \( \text{C}_6\text{-C}_1\text{O-aryl} \) has 6 to 10 carbon atoms. Examples of heteroatom-unsubstituted aryl groups include phenyl, methylphenyl, (dimethyl)phenyl, \( \text{C}_6\text{H}_4\text{CH}_2\text{CH}_3, \text{C}_6\text{H}_4\text{CH}_2\text{CH}_2\text{CH}_3, \text{C}_6\text{H}_4\text{CH(CH)}_3\text{CH}_3, \text{C}_6\text{H}_4\text{CH(CH)}_2\text{CH}_2\text{CH}_3, \text{C}_6\text{H}_4\text{CH}=\text{CH}, \text{C}_6\text{H}_4\text{CH}=\text{CHCH}_3, \text{C}_6\text{H}_4\text{C}=\text{CH}, \text{C}_6\text{H}_4\text{C}=\text{CCH}_3, \text{naphthyl, quinolyl, indolyl, and the radical derived from biphenyl. The term
"heteroatom-unsubstituted aryl" includes carbocyclic aryl groups, biaryl groups, and radicals derived from polycyclic fused hydrocarbons (PAHs).

The term "heteroatom-substituted Cₙ-aryl" refers to a radical, refers to a radical, having either a single aromatic carbon atom or a single aromatic heteroatom as the point of attachment, further having a total of n carbon atoms, at least one hydrogen atom, and at least one heteroatom, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-unsubstituted C₁₋₁₀-heteroaryl has 1 to 10 carbon atoms. The term "heteroatom-substituted aryl" includes heteroaryl and heterocyclic aryl groups. It also includes those groups derived from the compounds: pyrrole, furan, thiophene, imidazole, oxazole, isoxazole, thiazole, isothiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like. Further examples of heteroatom-substituted aryl groups include the groups: -C₆H₄F, -C₆H₄Cl, -C₆H₄Br, -C₆H₄I, -C₆H₄OH, -C₆H₄OCH₃, -C₆H₄OH₂CH₃, -C₆H₄OCOCH₃, -C₆H₄OC₆H₅, -C₆H₄NH₂, -C₆H₄NHCH₃, -C₆H₄NCH₂CH₃, -C₆H₄Cl₂, -C₆H₄CH₂Br, -C₆H₄CH₂OH, -C₆H₄CH₂OCOCH₃, -C₆H₄CH₂NH₂, -C₆H₄N(CH₃)₂, -C₆H₄CH₂CH₂Cl, -C₆H₄CH₂CH₂OH, -C₆H₄CH₂CH₂OCOCH₃, -C₆H₄CH₂CH₂NH₂, -C₆H₄CH₂CH₂NH₂, -C₆H₄CH₂CH=CH₂, -C₆H₄CF₃, -C₆H₄CN, -C₆H₄C≡CSi(CH₃)₃, -C₆H₄COH, -C₆H₄COCH₃, -C₆H₄COCH₂CH₃, -C₆H₄COCH₂CF₃, -C₆H₄COC₆H₅, -C₆H₄CO₂H, -C₆H₄CO₂CH₃, -C₆H₄CONH₂, -C₆H₄CONHCH₃, -C₆H₄CON(CH₃)₂, furanyl, thienyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, and imidazoyl.

The term "heteroatom-unsubstituted Cₙ-aralkyl" refers to a radical, having a single saturated carbon atom as the point of attachment, further having a total of n carbon atoms, wherein at least 6 of the carbon atoms form an aromatic ring structure containing only carbon atoms, 7 or more hydrogen atoms, and no heteroatoms. For example, a heteroatom-unsubstituted C₇-Cio-aralkyl has 7 to 10 carbon atoms. An "aralkyl" includes an alkyl heteroatom-substituted with an aryl group. Examples of heteroatom-unsubstituted aralkyls include phenylmethyl (benzyl) and phenylethyl.

The term "heteroatom-substituted Cₙ-aralkyl" refers to a radical, having a single saturated carbon atom as the point of attachment, further having a total of n carbon atoms, o, 1, or more than one hydrogen atom, and at least one heteroatom, wherein at least one of the carbon atoms is incorporated an aromatic ring structures, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C₂-C₇-o-heteroaralkyl has 2 to 10 carbon atoms.

The term "heteroatom-unsubstituted Cₙ-acyl" refers to a radical, having a single carbon atom of a carbonyl group as the point of attachment, further having a linear or
branched, cyclic or acyclic structure, further having a total of n carbon atoms, 1 or more hydrogen atoms, a total of one oxygen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted Ci-Cio-acyl has 1 to 10 carbon atoms. The groups, -COH, -COCH₃, -COCH₂CH₃, -COCH₂CH₂CH₃, -COCH(CH₃)₂, -COCH(CH₂)₂, -COCH₂H₅, -COCH₆H₄CH₂CH₃, -COCH₆H₄CH₂CH₂CH₃, -COCH₆H₄CH(CH₃)₂, -COCH₆H₄CH(CH₂)₂, and -COCH₆H₃(CH₃)₂, are examples of heteroatom-unsubstituted acyl groups.

The term "heteroatom-substituted Cₙ-acyl" refers to a radical, having a single carbon atom as the point of attachment, the carbon atom being part of a carbonyl group, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom in addition to the oxygen of the carbonyl group, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted Ci-Cio-acyl has 1 to 10 carbon atoms. The term heteroatom-substituted acyl includes carbamoyl, thiocarboxylic, and thiocarboxylic acid groups. The groups, -COCH₂CF₃, -CO₂H, -CO₂CH₃, -CO₂CH₂CH₃, -CO₂CH₂CH₂CH₃, -CO₂CH(CH₃)₂, -CO₂CH(CH₂)₂, -CONH₂, -CONHCH₃, -CONHCH₂CH₃, -CONHCH₂CH₂CH₃, -CONHCH₃(CH₂)₂, -CONHCH(CH₃)₂, -CON(CH₂CH₃)₃, -CON(CH₂CH₂CH₃)₂ and -CONHCH₂CF₃, are examples heteroatom-substituted acyl groups.

The term "heteroatom-unsubstituted Cₙ-alkoxy" refers to a group, having the structure -OR, in which R is a heteroatom-unsubstituted Cₙ-alkyl, as that term is defined above. Heteroatom-unsubstituted alkoxy groups include: -OCH₃, -OCH₂CH₃, -OCH₂CH₂CH₃, -OCH(CH₃)₂, and -OCH(CH₂)₂.

The term "heteroatom-substituted Cₙ-alkenyl" refers to a group, having the structure -OR, in which R is a heteroatom-substituted Cₙ-alkenyl, as that term is defined above. For example, -OCH₂CF₃ is a heteroatom-substituted alkenyl group.

The term "heteroatom-unsubstituted Cₙ-alkenyl" refers to a group, having the structure -OR, in which R is a heteroatom-unsubstituted Cₙ-alkenyl, as that term is defined above.

The term "heteroatom-substituted Cₙ-alkenyl" refers to a group, having the structure -OR, in which R is a heteroatom-substituted Cₙ-alkenyl, as that term is defined above.
The term "heteroatom-unsubstituted $C_n$-alkynyloxy" refers to a group, having the structure -OR, in which R is a heteroatom-unsubstituted $C_n$-alkynyl, as that term is defined above.

The term "heteroatom-substituted $C_n$-alkynyloxy" refers to a group, having the structure -OR, in which R is a heteroatom-substituted $C_n$-alkynyl, as that term is defined above.

The term "heteroatom-unsubstituted $C_n$-aryloxy" refers to a group, having the structure -OAr, in which Ar is a heteroatom-unsubstituted $C_n$-aryl, as that term is defined above. An example of a heteroatom-unsubstituted aryloxy group is -OC$_6$H$_5$.

The term "heteroatom-substituted $C_n$-aryloxy" refers to a group, having the structure -OAr, in which Ar is a heteroatom-substituted $C_n$-aryl, as that term is defined above.

The term "heteroatom-unsubstituted $C_n$-aralkyloxy" refers to a group, having the structure -OAr, in which Ar is a heteroatom-unsubstituted $C_n$-aralkyl, as that term is defined above.

The term "heteroatom-substituted $C_n$-aralkyloxy" refers to a group, having the structure -OAr, in which Ar is a heteroatom-substituted $C_n$-aralkyl, as that term is defined above.

The term "heteroatom-unsubstituted $C_n$-acyloxy" refers to a group, having the structure -OAc, in which Ac is a heteroatom-unsubstituted $C_n$-acyl, as that term is defined above. A heteroatom-unsubstituted acyloxy group includes alkylcarbonyloxy and arylcarbonyloxy groups. For example, -OCOCH$_3$ is an example of a heteroatom-unsubstituted acyloxy group.

The term "heteroatom-substituted $C_n$-acyloxy" refers to a group, having the structure -OAc, in which Ac is a heteroatom-substituted $C_n$-acyl, as that term is defined above. A heteroatom-substituted acyloxy group includes alkoxy carbonyloxy, aryloxy carbonyloxy, carboxylate, alkyl carbonyl, alkoxy carbonyl, aminocarbonyl, and alkylthiocarbonyl groups.

The term "heteroatom-unsubstituted $C_n$-alkylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two saturated carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, containing a total of n carbon atoms, all of which are nonaromatic, 4 or more hydrogen atoms, a total of 1 nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted Ci-Cio-alkylamino has 1 to 10 carbon atoms. The term "heteroatom-unsubstituted $C_n$-alkylamino" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted $C_n$-alkyl, as that term is defined above. A heteroatom-unsubstituted
alkylamino group would include \(-\text{NHCH}_3\), \(-\text{NHCH}_2\text{CH}_3\), \(-\text{NHCH}_2\text{CH}_2\text{CH}_3\), \(-\text{NHCH}(\text{CH}_3)_2\), 
\(-\text{NHCH}(\text{CH}_2)_2\), \(-\text{NHCH}_2\text{CH}_2\text{CH}_3\), \(-\text{NHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3\), 
\(-\text{NHCH}_2\text{CH}(\text{CH}_3)_2\), \(-\text{NH}(\text{CH}_3)_3\), \(-\text{N}(\text{CH}_3)_2\), 
\(-\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_3\), \(-\text{N}(\text{CH}_2\text{CH}_3)_2\), \text{iV-pyrrolidinyl}, and \text{N-piperidinyl}.

The term "heteroatom-substituted \(\text{C}_n\)-alkylamino" refers to a radical, having a single 
nitrogen atom as the point of attachment, further having one or two saturated carbon atoms 
attached to the nitrogen atom, no carbon-carbon double or triple bonds, further having a linear 
or branched, cyclic or acyclic structure, further having a total of \(n\) carbon atoms, all of which 
are nonaromatic, 0, 1, or more than one hydrogen atom, and at least one additional 
heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein each 
additional heteroatom is independently selected from the group consisting of \(\text{N}, \text{O}, \text{F}, \text{Cl}, \text{Br}, \text{I}, \text{Si}, \text{P}, \text{and S}\). For example, a heteroatom-substituted \(\text{Ci-Cio-alkylamino}\) has 1 to 10 carbon 
atoms. The term "heteroatom-substituted \(\text{C}_n\)-alkylamino" includes groups, having the structure 
\(-\text{NHR}\), in which \(R\) is a heteroatom-substituted \(\text{C}_n\)-alkyl, as that term is defined 
above.

The term "heteroatom-unsubstituted \(\text{C}_n\)-alkenylamino" refers to a radical, having a single 
nitrogen atom as the point of attachment, further having one or two carbon atoms 
attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, 
containing at least one nonaromatic carbon-carbon double bond, a total of \(n\) carbon atoms, 4 
or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For 
example, a heteroatom-unsubstituted \(\text{C}_2\)-\text{Cio-alkenylamino} has 2 to 10 carbon atoms. The term 
"heteroatom-unsubstituted \(\text{C}_n\)-alkenylamino" includes groups, having the structure 
\(-\text{NHR}\), in which \(R\) is a heteroatom-unsubstituted \(\text{C}_n\)-alkenyl, as that term is defined above. 
Examples of heteroatom-unsubstituted \(\text{C}_n\)-alkenylamino groups also include dialkenylamino 
and alkyl(alkenyl)amino groups.

The term "heteroatom-substituted \(\text{C}_n\)-alkenylamino" refers to a radical, having a single 
nitrogen atom as the point of attachment and at least one nonaromatic carbon-carbon double 
bond, but no carbon-carbon triple bonds, further having one or two carbon atoms attached to 
the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further 
having a total of \(n\) carbon atoms, 0, 1, or more than one hydrogen atom, and at least one 
additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, 
wherein each additional heteroatom is independently selected from the group consisting of \(\text{N}, \text{O}, \text{F}, \text{Cl}, \text{Br}, \text{I}, \text{Si}, \text{P}, \text{and S}\). For example, a heteroatom-substituted \(\text{C}_2\)-\text{Ci-o-alkenylamino} has 2 
to 10 carbon atoms. The term "heteroatom-substituted \(\text{C}_n\)-alkenylamino" includes groups,
having the structure -NHR, in which R is a heteroatom-substituted C_n-alkenyl, as that term is defined above.

The term "heteroatom-unsubstituted C_n-alkynylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two carbon atoms attached to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, containing at least one carbon-carbon triple bond, a total of n carbon atoms, at least one hydrogen atom, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C_2-C_10-alkynylamino has 2 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-alkynylamino" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted C_n-alkynyl, as that term is defined above. An alkynylamino group includes dialkynylamino and alkyl(alkynyl)amino groups.

The term "heteroatom-substituted C_n-alkynylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two carbon atoms attached to the nitrogen atom, further having at least one nonaromatic carbon-carbon triple bond, further having a linear or branched, cyclic or acyclic structure, and further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, and at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C_2-C_10-alkynylamino has 2 to 10 carbon atoms. The term "heteroatom-substituted C_n-alkynylamino" includes groups, having the structure -NHR, in which R is a heteroatom-substituted C_n-alkynyl, as that term is defined above.

The term "heteroatom-unsubstituted C_n-arylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having at least one aromatic ring structure attached to the nitrogen atom, wherein the aromatic ring structure contains only carbon atoms, further having a total of n carbon atoms, 6 or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C6-C10-arylamino has 6 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-arylamino" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted C_n-aryl, as that term is defined above. A heteroatom-unsubstituted arylamino group includes diarylamino and alkyl(aryl)amino groups.

The term "heteroatom-substituted C_n-arylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having a total of n carbon atoms, at least one hydrogen atom, at least one additional heteroatoms, that is, in addition to the nitrogen atom at the point of attachment, wherein at least one of the carbon atoms is incorporated into one or
more aromatic ring structures, further wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted Ce-Cio-arylamino has 6 to 10 carbon atoms. The term "heteroatom-substituted C_6-arylamino" includes groups, having the structure -NHR, in which R is a heteroatom-substituted C_6-aryl, as that term is defined above. A heteroatom-substituted arylamino group includes heteroarylamino groups.

The term "heteroatom-unsubstituted C_n-aralkylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having one or two saturated carbon atoms attached to the nitrogen atom, further having a total of n carbon atoms, wherein at least 6 of the carbon atoms form an aromatic ring structure containing only carbon atoms, 8 or more hydrogen atoms, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted C_6-Cio-aralkylamino has 7 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-aralkylamino" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted C_n-aralkyl, as that term is defined above. An aralkylamino group includes diaralkylamino groups.

The term "heteroatom-substituted C_n-aralkylamino" refers to a radical, having a single nitrogen atom as the point of attachment, further having at least one or two saturated carbon atoms attached to the nitrogen atom, further having a total of n carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom, that is, in addition to the nitrogen atom at the point of attachment, wherein at least one of the carbon atom incorporated into an aromatic ring, further wherein each heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C_6-Cio-aralkylamino has 7 to 10 carbon atoms. The term "heteroatom-substituted C_n-aralkylamino" includes groups, having the structure -NHR, in which R is a heteroatom-substituted C_n-aralkyl, as that term is defined above. The term "heteroatom-substituted aralkylamino" includes the term "heteroaralkylamino."

The term "heteroatom-unsubstituted C_n-amido" refers to a radical, having a single nitrogen atom as the point of attachment, further having a carbonyl group attached via its carbon atom to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further having a total of n carbon atoms, 1 or more hydrogen atoms, a total of one oxygen atom, a total of one nitrogen atom, and no additional heteroatoms. For example, a heteroatom-unsubstituted Ci-Cio-amido has 1 to 10 carbon atoms. The term "heteroatom-unsubstituted C_n-amido" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted C_n-acyl, as that term is defined above. The term amido includes
JV-alkyl-amido, JV-aryl-amido, JV-aralkyl-amido, acylamino, alkylcarbonylamino, arylcarbonylamino, and ureido groups. The group, -NHCOCH3, is an example of a heteroatom-unsubstituted amido group.

The term "heteroatom-substituted Cn-amido" refers to a radical, having a single nitrogen atom as the point of attachment, further having a carbonyl group attached via its carbon atom to the nitrogen atom, further having a linear or branched, cyclic or acyclic structure, further having a total of n aromatic or nonaromatic carbon atoms, 0, 1, or more than one hydrogen atom, at least one additional heteroatom in addition to the oxygen of the carbonyl group, wherein each additional heteroatom is independently selected from the group consisting of N, O, F, Cl, Br, I, Si, P, and S. For example, a heteroatom-substituted C1-C3-amido has 1 to 10 carbon atoms. The term "heteroatom-substituted Cn-amido" includes groups, having the structure -NHR, in which R is a heteroatom-unsubstituted Cn-acyl, as that term is defined above. The group, -NHCO2CH3, is an example of a heteroatom-substituted amido group.

The term "pharmaceutically acceptable salts," as used herein, refers to salts of compounds of this invention that are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of a compound of this invention with an inorganic or organic acid, or an organic base, depending on the substituents present on the compounds of the invention.

Examples of inorganic acids which may be used to prepare pharmaceutically acceptable salts include: hydrochloric acid, phosphoric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, phosphorous acid and the like. Examples of organic acids which may be used to prepare pharmaceutically acceptable salts include: aliphatic mono- and dicarboxylic acids, such as oxalic acid, carbonic acid, citric acid, succinic acid, phenyl-heteroatom-substituted alkanoic acids, aliphatic and aromatic sulfuric acids and the like. Pharmaceutically acceptable salts prepared from inorganic or organic acids thus include hydrochloride, hydrobromide, nitrate, sulfate, pyrosulfate, bisulfate, sulfite, bisulfate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, hydroiodide, hydrofluoride, acetate, propionate, formate, oxalate, citrate, lactate, p-toluenesulfonate, methanesulfonate, maleate, and the like. Other suitable salts are known to one of ordinary skill in the art.

Suitable pharmaceutically acceptable salts may also be formed by reacting the agents of the invention with an organic base such as methylamine, ethylamine, ethanolamine, lysine, ornithine and the like. Other suitable salts are known to one of ordinary skill in the art.
Pharmaceutically acceptable salts include the salts formed between carboxylate or sulfonate groups found on some of the compounds of this invention and inorganic cations, such as sodium, potassium, ammonium, or calcium, or such organic cations as isopropylammonium, trimethylammonium, tetramethylammonium, and imidazolium.

It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable and as long as the anion or cation does not contribute undesired qualities or effects. Further, additional pharmaceutically acceptable salts are known to those skilled in the art, and may be used within the scope of the invention. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Pharmaceutical Salts: Properties, Selection and Use—A Handbook* (2002), which is incorporated herein by reference.

As used herein, the term "patient" is intended to include living organisms in which certain conditions as described herein can occur. Examples include humans, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. In a preferred embodiment, the patient is a primate. In an even more preferred embodiment, the primate is a human. Other examples of subjects include experimental animals such as mice, rats, dogs, cats, goats, sheep, pigs, and cows. The experimental animal can be an animal model for a disorder, *e.g.*, a transgenic mouse with an Alzheimer's-type neuropathology. A patient can be a human suffering from a neurodegenerative disease, such as Alzheimer's disease, or Parkinson's disease.

As used herein, the term "IC50" refers to an inhibitory dose which is 50% of the maximum response obtained.

Other abbreviations used herein are as follows: DMSO, dimethyl sulfoxide; 1NOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; NGF, nerve growth factor; IBMX, isobutylmethylxanthine; FBS, fetal bovine serum; GPDH, glycerol 3-phosphate dehydrogenase; PvXR, retinoid X receptor; TGF-β, transforming growth factor-β; IFN-γ, interferon-γ; LPS, bacterial endotoxic lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TCA, trichloroacetic acid; HO-I, inducible heme oxygenase.
As used herein, the term "water soluble" means that the compound dissolves in water at least to the extent of 0.010 mole/liter or is classified as soluble according to literature precedence.

As used herein, "predominantly one enantiomer" means that the compound contains at least 85% of one enantiomer, or more preferably at least 90% of one enantiomer, or even more preferably at least 95% of one enantiomer, or most preferably at least 99% of one enantiomer. Similarly, the phrase "substantially free from other optical isomers" means that the composition contains at most 5% of another enantiomer or diastereomer, more preferably 2% of another enantiomer or diastereomer, and most preferably 1% of another enantiomer or diastereomer.

The term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to "and/or." When used in conjunction with the word "comprising" or other open language in the claims, the words "a" and "an" denote "one or more," unless specifically noted. The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

III. Synthetic Triterpenoids

Triterpenoids, biosynthesized in plants by the cyclization of squalene, are used for medicinal purposes in many Asian countries; and some, like ursolic and oleanolic acids, are known to be anti-inflammatory and anti-carcinogenic (Huang et al., 1994; Nishino et al., 1988). However, the biological activity of these naturally-occurring molecules is relatively weak, and therefore the synthesis of new analogs to enhance their potency was undertaken (Honda et al., 1997; Honda et al., 1998). Subsequent research has identified a number of synthetic compounds that have improved activity as compared to the naturally-occurring triterpenoids.

The ongoing efforts for the improvement of anti-inflammatory and antiproliferative activity of oleanolic and ursolic acid analogs led to the discovery of 2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid (CDDO) and related compounds (e.g., CDDO-Me, TP-
In the case of inducing cytoprotective genes through Keapl-Nrf2-antioxidant response element (ARE) signaling, a recent structure activity evaluation of 15 triterpenoids confirmed the importance of Michael acceptor groups on both the A and C rings, the requirement for a nitrile group at C-2 of the A ring, and that substituents at C-17 dramatically affected pharmacodynamic action in vivo (Yates et al., 2007).

In general, CDDO is the prototype for a large number of compounds in a family of agents that have been shown useful in a variety of contexts. For example, CDDO-Me and CDDO-Im are reported to possess the ability to modulate transforming growth factor-β (TGF-βySmad signaling in several types of cells (Suh et al., 2003; Minns et al., 2004; Mix et al., 2004). Both are known to be potent inducers of heme-oxygenase-1 and Nrf2/ARE signaling (Liby et al., 2005). For example, one important activity of the triterpenoids is their ability to activate the Keap/Nrf2/ARE pathway because activation of this phase 2 enzyme cytoprotective response is highly correlated to their anti-inflammatory activity (Liby et al., 2005, Dinkova-Kostova et al, 2005; Thimmulappa et al, 2006; Yu and Kensler, 2005; Na and Surh, 2006). In this regard, a series of synthetic triterpenoid (TP) analogs of oleanolic acid have also been shown to be potent inducers of the phase 2 response, that is elevation of NAD(P)H-quinone oxidoreductase and heme oxygenase 1 (HO-I), which is a major protector of cells against oxidative and electrophile stress. See Dinkova-Kostova et al, 2005. Like previously identified phase 2 inducers, the TP analogs were shown to use the antioxidant response element-Nrf2-Keapl signaling pathway.

Other pathways that CDDO-type compounds have been shown to affect include the blocking of NF-κB. It has been suggested that NF-κB activity may lead to enhancement of...
the cell cycle by its ability to activate cyclin D1 (Guttridge et al., 1999; Hinz et al., 1999; Joyce et al., 1999). Inhibition of IKK-driven NF-κB activation offers a strategy for treatment of different malignancies and can convert inflammation-induced tumor growth to inflammation-induced tumor regression. Luo et al., 2005, is incorporated herein by reference. For example, as reported by Shishodia et al. (2006), CDDO-Me modulates nuclear factor κB (NF-κB) activity and NF-κB-regulated gene expression. Using human leukemia cell lines and patient samples, it was shown that CDDO-Me potently inhibits both constitutive and inducible NF-κB activated by tumor necrosis factor (TNF), interleukin (IL)-1β, phorbol ester, okadaic acid, hydrogen peroxide, lipopolysaccharide, and cigarette smoke. NF-κB suppression occurred through inhibition of IkBα kinase activation, IkBα phosphorylation, IkBα degradation, p65 phosphorylation, p65 nuclear translocation, and NF-κB-mediated reporter gene transcription. This inhibition was shown to correlate with suppression of NF-κB-dependent genes involved in antiapoptosis (IAP2, cFLIP, TRAF1, survivin, and bcl-2), proliferation (cyclin d1 and c-myc), and angiogenesis (VEGF, cox-2, and mmp-9). CDDO-Me was also shown to potentiate the cytotoxic effects of TNF and chemotherapeutic agents. Overall, the results suggested that CDDO-Me inhibits NF-κB through inhibition of IkBα kinase, leading to the suppression of expression of NF-κB-regulated gene products and enhancement of apoptosis induced by TNF and chemotherapeutic agents. In general, it is known that CDDO and its congeners form Michael adducts with thiol groups on cysteine residues of target proteins. Some of these such as Keapl (Dinkova-Kostova et al., 2005), an inhibitor of the Nrf2 transcription factor that regulates the phase 2 cytoprotective response, and IκB kinase (Ahmad et al., 2006; Yore et al., 2006) have already been identified. Subsequent reports confirmed that CDDO-Me and CDDO-Im are direct inhibitors of IKKb activity, via binding to Cys179 (Ahmad et al., 2006; Yore et al., 2006). Given that triterpenoids form reversible Michael adducts with thiol groups, there are undoubtedly other targets, some of which may be implicated in the MS treatment effects presented in this application.

Because optimal treatment of brain cancer requires a therapeutic agent to cross the BBB in effective concentrations, and because the majority of chemotherapy agents are unable to penetrate the BBB and cannot reach effective concentrations in the brain, few effective agents for the treatment of brain cancer are available. Primary brain cancers such as glioblastoma multiforme are among the deadliest cancers due to their rapid progression and the lack of effective treatments. Brain metastases arising from common primary cancers such
as breast and lung cancer are also a major source of morbidity and mortality, not least because agents that are effective in treating these tumors outside the CNS cannot cross the BBB. Brain metastases, therefore, are sheltered from exposure to agents that otherwise would effectively inhibit their growth.

The aberrant or excessive expression of either iNOS or cyclooxygenase-2 (COX-2) has been implicated in the pathogenesis of many disease processes. For example, it is clear that NO is a potent mutagen (Tamir and Tannebaum, 1996), and that nitric oxide can also activate COX-2 (Salvemini et al., 1994). Furthermore, there is a marked increase in iNOS in rat colon tumors induced by the carcinogen, azoxymethane (Takahashi et al., 1997). A series of synthetic triterpenoid analogs of oleanolic acid have been shown to be powerful inhibitors of cellular inflammatory processes, such as the induction by IFN-γ of inducible nitric oxide synthase (iNOS) and of cyclooxygenase 2 in mouse macrophages. See Honda et al. (2000a); Honda et al. (2000b), and Honda et al. (2002), which are all incorporated herein by reference.

Induction of HO-I, in particular, is known to be therapeutic in animal models of many different diseases, including myocardial infarction, renal failure, transplant failure and rejection, stroke, cardiovascular disease, and autoimmune disease.

The inventors contemplate the use of the compounds of this invention for treating a subject having a condition caused by elevated levels of oxidative stress in one or more tissues. The oxidative stress may be accompanied by either acute or chronic inflammation. The oxidative stress may be caused by acute exposure to an external agent such as ionizing radiation or a cytotoxic chemotherapy agent {e.g., doxorubicin}, by trauma or other acute tissue injury, by ischemia/reperfusion injury, by poor circulation or anemia, by localized or systemic hypoxia or hyperoxia, or by other abnormal physiological states such as hyperglycemia or hypoglycemia.

In animal models of many such conditions, stimulating expression of inducible heme oxygenase (HO-I) has been shown to have a significant therapeutic effect {e.g., Sacerdoti et al., 2005; Abraham & Kappas, 2005; Bach, 2006; Araujo et al., 2003; Liu et al., 2006; Ishikawa et al., 2001; Kruger et al., 2006; Satoh et al., 2006; Zhou et al., 2005; Morse and Choi, 2005; Morse and Choi, 2002.). This enzyme breaks free heme down into iron, carbon monoxide (CO), and biliverdin (which is subsequently converted to the potent antioxidant molecule, bilirubin). It was shown that at nanomolar concentrations, CDDO and CDDO-Im rapidly increase the expression of the cytoprotective heme oxygenase-1 (HO-I) enzyme in vitro and in vivo. See Liby et al. (2005). Transfection studies using a series of reporter constructs showed that activation of the human HO-I promoter by the triterpenoids requires
an antioxidant response element (ARE), a cyclic AMP response element, and an E Box sequence. Inactivation of one of these response elements alone was shown to partially reduce HO-I induction, but mutations in all three sequences entirely eliminated promoter activity in response to the triterpenoids.

Therefore, the compounds of this invention, especially given their structural similarities with CDDO and other CDDO derivatives, may be useful in preventing or treating tissue damage or organ failure, acute and chronic, resulting from oxidative stress exacerbated by inflammation. Examples of diseases that fall in this category include: heart failure, liver failure, transplant failure and rejection, renal failure, pancreatitis, fibrotic lung diseases (cystic fibrosis and COPD, among others), diabetes (including complications), atherosclerosis, ischemia-reperfusion injury, glaucoma, stroke, autoimmune disease, autism, macular degeneration, and muscular dystrophy. For example, in the case of autism, studies suggest that increased oxidative stress in the central nervous system may contribute to the development of the disease (Chauhan and Chauhan, 2006).

Evidence also links oxidative stress and inflammation to the development and pathology of many other disorders of the central nervous system, including psychiatric disorders such as psychosis, major depression, and bipolar disorder; seizure disorders such as epilepsy; pain and sensory syndromes such as migraine, neuropathic pain or tinnitus; and behavioral syndromes such as the attention deficit disorders. See, e.g., Dickerson et al, 2007; Hanson et al, 2005; Kendall-Tackett, 2007; Lenz et al, 2007; Dudhgaonkar et al, 2006; Lee et al, 2007; Morris et al., 2002; Ruster et al, 2005; McIver et al, 2005; Sarchielli et al, 2006; Kawakami et al, 2006; Ross et al, 2003, which are all incorporated by reference herein. For example, elevated levels of inflammatory cytokines, including TNF, interferon-γ, and IL-6, are associated with major mental illness (Dickerson et al, 2007). Microglial activation has also been linked to major mental illness. Therefore, downregulating inflammatory cytokines and inhibiting excessive activation of microglia could be beneficial in patients with schizophrenia, major depression, bipolar disorder, autism-spectrum disorders, and other neuropsychiatric disorders. Therefore, both the new and the known TP compounds, may be useful in preventing or treating the above described disorders.

Accordingly, in pathologies involving oxidative stress alone or oxidative stress exacerbated by inflammation, treatment may comprise administering to a subject a therapeutically effective amount of a compound of this invention, such as those described above or throughout this specification. Treatment may be administered preventively, in advance of a predictable state of oxidative stress {e.g., organ transplantation or the
administration of radiation therapy to a cancer patient), or it may be administered therapeutically in settings involving established oxidative stress and inflammation.

The invention contemplates that the compounds of this invention can also be generally applied to the treatment of inflammatory conditions, such as sepsis, dermatitis, autoimmune disease and osteoarthritis. Other conditions that may be treatable with the compounds of this invention include inflammatory pain and neuropathic pain. The effects here may rely on induction of Nrf2 and inhibition of NF-κB.

Newer amide derivatives of CDDO have now also been found to be promising agents, for example for their ability to pass through the blood brain barrier, as discussed in greater detail below. In addition to the methyl amide of CDDO (CDDO-MA), as reported in (Honda et al., 2002), the invention provides additional CDDO amide derivatives, such as the ethyl amide (CDDO-EA), as well fluorinated amide derivative of CDDO, such as the 2,2,2-trifluoroethyl amide derivative of CDDO (CDDO-TFEA).

CDDO compounds corresponding to formulas I and II can be prepared according to the methods taught by Honda et al. (1998), Honda et al. (2000b), Honda et al. (2002) and Yates et al. (2007), which are all incorporated herein by reference.

Specific examples of triterpenoids that may be used in accordance with the methods of this invention are shown here.

Examples of new CDDO derivatives provided by the present invention include the following compounds:
The synthesis of CDDO-MA is discussed in Honda et al. (2002), which is incorporated herein by reference. The syntheses of CDDO-EA and CDDO-TFEA are presented in Yates et al. (2007), which is incorporated herein by reference, and shown in the Scheme 1 below.

Given their structural similarity with other synthetic triterpenoids, such as CDDO-Me, these new CDDO derivatives, such as CDDO-TFEA and CDDO-EA are expected to have utility not only in the treatment and prevention of MS, as discussed below, but also for the treatment and prevention of other diseases such as cancer, inflammation, Alzheimer's disease, Parkinson's disease, multiple sclerosis, autism, amyotrophic lateral sclerosis, rheumatoid arthritis, and inflammatory bowel disease, all other diseases whose pathogenesis is believed to
involve excessive production of either nitric oxide or prostaglandins, and pathologies involving oxidative stress alone or oxidative stress exacerbated by inflammation.

For example, the invention contemplates that the compounds of the present invention, will function, through one or more of the mechanisms described in this application to induce apoptosis in tumor cells, induce differentiation, inhibit cancer cell proliferation, inhibit inflammatory response, and/or function in a chemopreventative capacity. For example, the invention contemplates that the new CDDO derivatives may have one or more of the following properties: (1) the ability to induce apoptosis and differentiate both malignant and non-malignant cells, (2) activity at sub-micromolar or nanomolar levels as an inhibitor of proliferation of many malignant or premalignant inhibitors of proliferation of many malignant or premalignant cells, (3) the ability to suppress the de novo synthesis of the inflammatory enzyme inducible nitric oxide synthase (iNOS), (4) the ability to inhibit NF-κB activation, and (5) the ability to induce heme oxygenase-1 (HO-I).

IV. Use of Triterpenoids for the Treatment of Multiple Sclerosis

Multiple sclerosis (MS) is known to be an inflammatory condition of the central nervous system (Williams et al., 1994; Merrill and Benvenist, 1996; Genain and Nauser, 1997). In general, inflammatory, oxidative, or immune mechanisms may be involved in the pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and MS (Bagasra et al., 1995; McGeer and McGeer, 1995; Simonian and Coyle, 1996; Kaltschmidt et al., 1997). Both reactive astrocytes and activated microglia have been implicated in causation of neurodegenerative disease (NDD) and neuroinflammatory disease (NID); there has been a particular emphasis on microglia as cells that synthesize both NO and prostaglandins as products of the respective enzymes, iNOS and COX-2. De novo formation of these enzymes may be driven by inflammatory cytokines such as interferon-γ or interleukin-1. In turn, excessive production of NO may lead to inflammatory cascades and/or oxidative damage in cells and tissues of many organs, including neurons and oligodendrocytes of the nervous system, with consequent manifestations in AD and MS, and possible PD and ALS (Coyle and Puttfarcken, 1993; Beal, 1996; Merrill and Benvenist, 1996; Simonian and Coyle, 1996; Vodovotz et al., 1996). Epidemiologic data indicate that chronic use of NSAID's which block synthesis of prostaglandins from arachidonate, markedly lower the risk for development of AD (McGeer et al., 1996; Stewart et al., 1997). Thus, agents that block formation of NO and prostaglandins, may be used in approaches to prevention and treatment of NDD.
Based on the results presented in this application, the compounds and methods of this invention are expected to have substantial utility for treating multiple sclerosis (MS) in mammalian subjects. In general, the method will comprise administering to the subjects pharmaceutically effective amounts of a compound, having the structure:

wherein R₁ is a heteroatom-substituted or heteroatom-unsubstituted Ci-Cis-acyl. For example, both CDDO-TFEA and CDDO-Me have been shown induce full recovery of mice in a rapidly progressive experimental autoimmune encephalomyelitis (EAE) model. The results presented in Example 2 and FIGS. 1 - 9 demonstrate that triterpenoids vary in their ability to cross the blood-brain barrier and reach significant concentrations in the brain. Further, the data shown in FIGS. 1 - 9 demonstrate that CDDO-TFEA reaches significantly higher concentrations in the brain than other triterpenoids such as the related amides CDDO-EA and CDDO-MA. In addition, FIG. 1 shows that CDDO-Me is able to reach appreciable levels in the brain after one week of feeding (100 mg/kg diet). The levels measured are comparable to those reached by TP-224 (CDDO-MA) after only 2 days of feeding at a higher dose. Furthermore, the brain levels achieved after oral administration of CDDO-TFEA (TP-500) are comparable to those achieved in other tissue compartments such as lung (FIG. 9). In contrast, brain levels of CDDO-Me achieved in primates (see Example 2) after oral dosing were approximately 10-fold less than the levels achieved in lung, indicating that CDDO-Me is less efficient in crossing the blood-brain barrier than CDDO-TFEA. The more rapid and durable effects achieved with CDDO-TFEA in the EAE model are likely due to this enhanced penetration of the CNS. See FIGS. 10 - 17. In general, animal models of human diseases which closely resemble their human counterparts are be studied with a view to better understanding and treating the human form. Experimental Autoimmune Encephalomyelitis (EAE), also called Experimental Allergic Encephalomyelitis, is an animal model of Multiple Sclerosis. While EAE is not multiple sclerosis, nor is it a single disease in a single species,
but its different forms resemble the various forms and stages of MS very closely in a large number of ways.

EAE is an acute or chronic-relapsing, acquired, inflammatory and demyelinating autoimmune disease. The animals are injected with the whole or parts of various proteins that make up myelin, the insulating sheath that surrounds nerve cells (neurons). These proteins induce an autoimmune response in the animals, causing the animal's immune system to mount an attack on its own myelin as a result of exposure to the injection. The animals develop a disease process that closely resembles MS in humans.

Several proteins or parts of proteins (antigens) are used to induce EAE including: Myelin Basic Protein (MBP), Proteolipid Protein (PLP), and Myelin Oligodendrocyte Glycoprotein (MOG). The EAE model continues to provide a crucial tool for improving our understanding and treatment of MS, and results obtained in rapidly progressive EAE models have been shown to be very relevant to understanding the effectiveness of treatments for many subtypes of MS. See Gold et al., (2006); Juedes et al., (2000); Owens (2006); Virley (2005), which are all incorporated herein by reference.

V. Pharmaceutical Formulations and Routes of Administration

The compounds of the present invention may be administered by a variety of methods, e.g., orally or by injection (e.g. subcutaneous, intravenous, intraperitoneal, etc.). Depending on the route of administration, the active compounds may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. They may also be administered by continuous perfusion/infusion of a disease or wound site.

To administer the therapeutic compound 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. For example, the therapeutic compound may be administered to a patient in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al., 1984).

The therapeutic compound may also be administered parenterally, intraperitoneally, intraspinally, or intracerebrally. Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.
Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (such as, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic compound 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 therapeutic compound into a sterile carrier which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients 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 therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of
course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

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 subjects to be treated; each unit containing a predetermined quantity of therapeutic 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 therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of a selected condition in a patient.

Active compounds are administered at a therapeutically effective dosage sufficient to treat a condition associated with a condition in a patient. A "therapeutically effective amount" preferably reduces the amount of symptoms of the condition in the infected patient by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. For example, the efficacy of a compound can be evaluated in an animal model system that may be predictive of efficacy in treating the disease in humans, such as the model systems shown in the examples and drawings.

VI. Combination Therapy

In addition to being used as a monotherapy, the compounds of the present invention will also find use in combination therapies. Effective combination therapy may be achieved with a single composition or pharmacological formulation that includes both agents, or with two distinct compositions or formulations, at the same time, wherein one composition includes the synthetic triterpenoid (TP) according to the methods of this invention, and the other includes the second agent(s). Alternatively, the TP therapy may precede or follow the other agent treatment by intervals ranging from minutes to months.

Various combinations may be employed, TP therapy is "A" and the secondary agent, such as β-interferons treatment, is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/B/A/B A/B/B/A B/B/A/B A/A/B/A B/A/B/A B/A/B/B A/B/B/B B/A/B/A
Administration of the TP compounds of the present invention to a patient will follow general protocols for the administration of pharmaceuticals, taking into account the toxicity, if any, of the drug. It is expected that the treatment cycles would be repeated as necessary.

Beta interferons may be suitable secondary agents. These are medications derived from human cytokines which help regulate the immune system. They include interferon β-lb (Betaseron) and interferon β-la (Avonex, Rebif). Betaseron has been approved by the FDA for relapsing forms of secondary progressive MS. Furthermore, the FDA has approved the use of several β-interferons as treatments for people who have experienced a single attack that suggests multiple sclerosis, and who may be at risk of future attacks and developing definite MS. For example, risk of MS may be suggested when an MRI scan of the brain shows lesions that predict a high risk of conversion to definite MS.

Glatiramer acetate (Copaxone) is a further example of a secondary agent that may be used in combination with a TP treatment. Glatiramer is presently used to treat relapsing remitting MS. It is made of four amino acids that are found in myelin. This drug is reported to stimulate T cells in the body's immune system to change from harmful, pro-inflammatory agents to beneficial, anti-inflammatory agents that work to reduce inflammation at lesion sites.

Another potential secondary agent is mitoxantrone (tradename Novantrone), a chemotherapy drug used for many cancers. This drug is also FDA-approved for treatment of aggressive forms of relapsing remitting MS, as well as certain forms of progressive MS. It is given intravenously, typically every three months. This medication is effective, but is limited by cardiac toxicity. Novantrone has been approved by the USA’s FDA for secondary progressive, progressive-relapsing, and worsening relapsing-remitting MS.

Another potential secondary agent is natalizumab (trade name Tysabri). In general, natalizumab works by blocking the attachment of immune cells to brain blood vessels, which is a necessary step for immune cells to cross into the brain, thus reducing the immune cells' inflammatory action on brain neurons. Natalizumab has been shown to significantly reduce the frequency of attacks in people with relapsing MS. While there are reports that in combination with other immunotherapies the use can lead to progressive multifocal leukoencephalopathy (PML), the combination treatment of Natalizumab and a TP, may not have that side effect because TPs are expected not to function as a conventional immunosuppressive agents, but rather according to one of the mechanisms discussed above. Immunosuppressive agents can be broadly characterized by their ability to either deplete
resting and/or activated immune cells or to inhibit the activation and effector function of immune cells. In most instances, these effects lead to a general state of immunosuppression, and in some cases lead to increased susceptibility to infection and cancer. Continuous exposure to synthetic triterpenoids does not lead to a reduction in number of immune cells and has not been associated with increased infection. Furthermore, based on the extensive data regarding the potential of these agents as chemopreventatives for cancer, the expectation is that continuous exposure would minimize the risk for malignancy.

In the case of relapsing remitting MS, patients may be given intravenous corticosteroids, such as methylprednisolone, as a secondary agent, to end the attack sooner and leave fewer lasting deficits.

Other common drugs for multiple sclerosis (MS) that may be used in combination with the triterpenoid derivatives include immunosuppressive drugs such as azathioprine (Imuran), cladribine (Leustatin), and Cyclophosphamide (Cytoxan).

It is contemplated that other anti-inflammatory agents may be used in conjunction with the TP treatments of the current invention. Other COX inhibitors may be used, including arylcarboxylic acids (salicylic acid, acetylsalicylic acid, diflunisal, choline magnesium trisalicylate, salicylate, benorylate, flufenamic acid, mefenamic acid, meclofenamic acid and triflumic acid), arylalkanoic acids (diclofenac, fenclofenac, alclofenac, fentiazac, ibuprofen, flurbiprofen, ketoprofen, naproxen, fenoprofen, fenbufen, suprofen, indoprofen, tiaprofenic acid, benoxaprofen, pirprofen, tolmetin, zomepirac, clopinac, indomethacin and sulindac) and enolic acids (phenylbutazone, oxyphenbutazone, azapropazone, feprazone, piroxicam, and isoxicam. U.S. Pat. No. 6,025,395.

Histamine H2 receptor blocking agents may also be used in conjunction with the TP derivatives of the current invention, including cimetidine, ranitidine, famotidine and nizatidine.

Treatment with acetylcholinesterase inhibitors such as tacrine, donepezil, metrifonate and rivastigmine for the treatment of Alzheimer's and other disease in conjunction with the TP derivatives of the present invention is contemplated. Other acetylcholinesterase inhibitors may be developed which may be used once approved include rivastigmine and metrifonate. Acetylcholinesterase inhibitors increase the amount of neurotransmitter acetylcholine at the nerve terminal by decreasing its breakdown by the enzyme cholinesterase.

MAO-B inhibitors such as selegilene (Eldepryl or Deprenyl) may be used in conjunction with the TP derivatives of the current invention. Selegilene is used for Parkinson's disease and irreversibly inhibits monoamine oxidase type B (MAO-B).
Monoamine oxidase is an enzyme that inactivates the monoamine neurotransmitters norepinephrine, serotonin and dopamine.

Dietary and nutritional supplements with reported benefits for treatment or prevention of Parkinson's, Alzheimer's, multiple sclerosis, amyotrophic lateral sclerosis, rheumatoid arthritis, inflammatory bowel disease, and all other diseases whose pathogenesis is believed to involve excessive production of either nitric oxide (NO) or prostaglandins, such as acetyl-L-carnitine, octacosanol, evening primrose oil, vitamin B6, tyrosine, phenylalanine, vitamin C, L-dopa, or a combination of several antioxidants may be used in conjunction with the TP derivatives of the current invention.

VII. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 - Materials and Methods

Chemicals. Triterpenoids were synthesized as previously described in Honda et al. (2002), Honda et al. (1998), and Honda et al. (2000b). The various amide derivatives were synthesized by the condensation of CDDO acid chloride with the respective amine hydrochlorides (or free amines) using previously published methods Honda et al. (2002). The synthesis of CDDO-MA is discussed in Honda et al. (2002), which is incorporated herein by reference. The syntheses of CDDO-EA and CDDO-TFEA are presented in Yates et al. (2007), which is incorporated herein by reference, and shown in the Scheme 1 above.

Example 2 - Blood Brain Barrier Penetration Results

The ability of synthetic triterpenoids (TPs) to penetrate the brain of mammals varies according to their structure. As shown in FIG. 1, CDDO-Me (TP-155) is detectable in the brains of mice fed very low levels of the compound over a week. FIG. 2 shows that significant concentrations of CDDO Methyl Amide (TP-224) penetrate the brains of mice...
after feeding them an 800 mg/kg diet. As shown in FIG. 3, feeding CDDO-EA (TP-319) for two days results in significantly higher brain levels than when the mice are fed CDDO-MA (TP-224). However, FIG. 5 shows that CDDO-TFEA (TP-500) is detected at higher levels in mouse brain than is CDDO-EA (TP-319). The effects are dose responsive. For example, FIG. 4 shows that the brain levels of CDDO-EA (TP-319) are dose responsive and higher than for CDDO-MA (TP-224). Also the brain levels of triterpenoids in gavaged CD-I mice varied. This is shown in FIG. 7.

The ability of synthetic triterpenoids to remain in the brain also varies according to their structure. As shown in FIG. 6, the brain levels of CDDO-TFEA (TP-500) remain significantly higher than CDDO-EA (TP-319). Furthermore, as shown in FIGS. 8 and 9, the relative concentration in the brain of gavaged mice was higher for CDDO-TFEA than for CDDO-EA. FIGS. 8 and 9 also show the distribution of CDDO-EA (TP-319) and CDDO-TFEA (TP-500), respectively, in the following CD-I mouse tissues: brain, lung, liver, plasma, and whole blood.

Experiments using CDDO-Me (RTA-402) were also conducted on 1 male and 1 female cynomolgus monkey (origin: Vietnam) between the ages of 2 and 3 years and weighing approximately 1.7 kg. Each received the test article (CDDO-Me in sesame oil) at 75 mg/kg/day via oral gavage administered at a volume of 5 mL/kg on Days 1, 2, and 3. Individual doses were based on the most recently obtained body weights. Blood samples were collected from the femoral artery/vein for determination of the plasma concentrations of the test article at 0.5, 1.5, 3, and 12 hours after dosing on Days 1 and 2 and at 0.5, 1.5, and 3 hours after dosing (± 0.5 hour) on Day 3.

At the termination of the study (approximately three hours after dosing on Day 3), all animals were euthanized and tissues collected. Samples (approximately 1 g or greater) of the adipose tissue, brain, colon, cheek pouch (buccal mucosa), heart, ileum, kidney, liver, lung, mammary glands, ovaries, pancreas, prostate, and bone marrow from the femur (as much as possible) were collected and frozen at approximately -20 °C for analysis for the presence of the test article. All other tissues and organs were discarded.

Table 1 shows the average distribution of CDDO-Me (RTA-402) in tissues of cynomolgus monkeys after 3 days of oral dosing at 1800 mg/m² (vehicle is sesame seed oil).
Table 1: Average Distribution of CDDO-Me in Monkey Tissue

<table>
<thead>
<tr>
<th>Organ</th>
<th>CDDO-Me (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>4,389</td>
</tr>
<tr>
<td>Lung</td>
<td>4,992</td>
</tr>
<tr>
<td>Colon</td>
<td>4,467</td>
</tr>
<tr>
<td>Prostate</td>
<td>4,446</td>
</tr>
<tr>
<td>Ovary</td>
<td>2,115</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,603</td>
</tr>
<tr>
<td>Liver</td>
<td>926</td>
</tr>
<tr>
<td>Brain*</td>
<td>215-447</td>
</tr>
</tbody>
</table>

nM is ng/nL x 1000/505.8, where 505.8 is the molecular weight of RTA-402 and assumes the density of the tissue is that of water. *showing the range.

**Example 3 - Treatment of Multiple Sclerosis Results**

The mice used for these studies were female and either wild type or heterozygotes for the Tgf-bl gene. The latter have a more accelerated course of disease (yet are equally protected by triterpenoid treatment). The mouse strain used for these studies includes either a mixed SvEV 129 x C56BL/6 or a pure SvEV 129 strain.

As shown in FIGS. 10 - 18, CDDO-TFEA (RTA 404) and CDDO-Me (RTA-402) induce full recovery of mice in a rapidly progressive experimental autoimmune encephalomyelitis (EAE) model. All animals (n=2/group) of varying clinical score (CS) were immunized with myelin oligodendrocyte glycoprotein (MOG) and treated intraperitoneally (IP) with 100 nmol in a volume of 50 - 100 µL (-2.8 mg/kg) of RTA-402 or RTA-404 every other day for a total of four times (Q2Dx4 schedule). Further experiments showed that lower doses (10 nmol) of these TPs were also effective. A CS of 0 indicates no symptoms, and score of 6 indicates quadriplegia. The drugs may not be killing immune effector cells, which may explain the relapse. Relapsed animals do respond to additional treatment (data not shown). The drugs may not be killing immune effector cells, which may explain the relapse. It was shows that untreated animals develop severe paralysis and die within days of developing quadriplegia. Treated animals respond within a few days and fully recover to absence of any paralysis.

**************

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the
compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent 6,025,395
U.S. Patent 6,326,507
U.S. Patent 6,552,075
U.S. Patent 6,974,801


Hanson *et al.*, *BMC Medical Genetics*, 6(7), 2005.


CLAIMS

1. A compound having the structure:

   where Y is ethylamino or a heteroatom-substituted C1-C5-alkylamino having at least one fluorine atom; or a pharmaceutically acceptable salt or hydrate thereof.

2. The compound of claim 1, wherein the compound is further defined as:

3. The compound of claim 2, wherein the compound is substantially free from other optical isomers.

4. The compound of claim 1, wherein Y is a heteroatom-substituted C2-C4-alkylamino having at least one fluorine atom.

5. The compound of claim 4, wherein Y is a heteroatom-substituted C2-C4-alkylamino having at least one trifluoromethyl group.
6. The compound of claim 5, wherein the compound is further defined as:

7. The compound of claim 6, wherein the compound is substantially free from other optical isomers.

8. A pharmaceutical or veterinary composition comprising:
   (a) a compound having the structure:

   wherein Y is ethylamino or a heteroatom-substituted Ci-Cs-alkylamino having at least one fluorine atom; or
   (b) a pharmaceutically acceptable salt or hydrate thereof; and
   (b) an excipient.
9. A method of treating or preventing a disease or disorder in a mammalian subject, comprising administering to said subject a pharmaceutically effective amount of a compound having the structure:

![Chemical structure]

wherein Y is ethylamino or a heteroatom-substituted Ci-Cs-alkylamino having at least one fluorine atom; or a pharmaceutically acceptable salt or hydrate thereof.

10. Use of a compound having the structure:

![Chemical structure]

wherein Y is ethylamino or a heteroatom-substituted Ci-Cs-alkylamino having at least one fluorine atom; or a pharmaceutically acceptable salt or hydrate thereof, for the manufacture of a medicament for the prevention or treatment of a disorder or a disease in the body of a human or an animal.
FIG. 1
FIG. 2

Significant Concentrations of CDDO Methyl Amide (TP-224) in Brains of Mice After Feeding 800 mg/kg Diet

Concentration of TP-224 in Brain, nM

Expt 1, 2 days (3 mice)  Expt 2, 4 days (3 mice)  Expt 3, 2 days (6 mice)
FIG. 3

Feeding TP319 for Two Days Results in Significantly Higher Brain Levels Than TP224
Brain Levels of TP-319 Are Dose Responsive and Higher Than For TP-224

FIG. 4
CDDO-TFEA is detected at higher levels in mouse brain than CDDO-EA.

FIG. 5
Brain Levels of CDDO-TFEA Remain Significantly Higher Than CDDO-EA With Long-Term Feeding

FIG. 6
FIG. 7
FIG. 8

TP-319 Levels in CD-1 Mouse Tissues

![Bar chart showing TP-319 levels in different mouse tissues: Brain, Lung, Liver, Plasma, Whole Blood. The Liver has the highest level, followed by Plasma, Brain, Whole Blood, and Lung.]
FIG. 9

TP-500 Levels in CD-1 Mouse Tissues

[Graph showing levels of TP-500 in different mouse tissues: Lung, Liver, Pancreas, Whole Blood, and Brain. The graph indicates significantly higher levels in the Liver compared to other tissues.]
FIG. 10
FIG. 11

RTA 402-Treated Animals

Mean clinical scores

Time (in days)

CDDO-Me (100uM in 7.5%PBST)
I.p. every 48 hrs. x 4

Recovery phase

Relapse
FIG. 12
FIG. 13
FIG. 14
FIG. 15
FIG. 16

- Untreated (CS-2)
- CDDO-CF3 (CS-2)
- CDDO-Me (CS-2)
- Control

Mean clinical scores vs. time (in days):

CDDO (100 nM in 7.5% PBST)
I.p. every 48 hrs x 4

Recovery phase
Relapse
FIG. 17

- Untreated (CS-1)
- CDDO-CF3 (CS-1)
- CDDO-Me(CS-1)
- Control

Time (in days)

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Recovery phase

Relapse

CS-1

Mean clinical scores

CDDO (100µM in 7.5% FBS)
l.p. every 48 hrs. x 4
FIG. 18
### A. CLASSIFICATION OF SUBJECT MATTER
INV. C07J63/00 A61K31/575 A61K31/58 A61P25/28 A61P35/00

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

### B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07J A61P A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BEILSTEIN Data, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>YATES, M.S. ETAL.: &quot;Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes&quot; MOL CANCER THER, vol. 6, no. 1, 2007, pages 154-162, XP002456640 cited in the application page 155, paragraph CHEMICALS page 159; example amides; table 2</td>
<td>1-10</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search: 15 November 2007
Date of mailing of the international search report: 26/11/2007

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016

Authorized officer: Mezzato, Stefano
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
Continuation of Box II.1

Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.1

Claims Nos.: 9

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/071933

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 9 because they relate to subject matter not required to be searched by this Authority, namely:
   
   see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [D] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 2004002463 A1</td>
<td>01-01-2004</td>
<td>NONE</td>
<td></td>
</tr>
</tbody>
</table>

Form PCT/ISA/210 (patent family annex) (April 2008)