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Compounds and related methods for treatment of neurodegenerative diseases

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Jinwal et al.

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(54) **COMPOUNDS AND RELATED METHODS
FOR TREATMENT OF
NEURODEGENERATIVE DISEASES**

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6, 2013.

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A61K 31/222 (2006.01)
A61K 31/395 (2006.01)

(52) **U.S. Cl.**
CPC **A61K 31/395** (2013.01); **A61K 31/222**
(2013.01)

(58) **Field of Classification Search**
CPC A61K 31/222
See application file for complete search history.

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Primary Examiner — James D Anderson

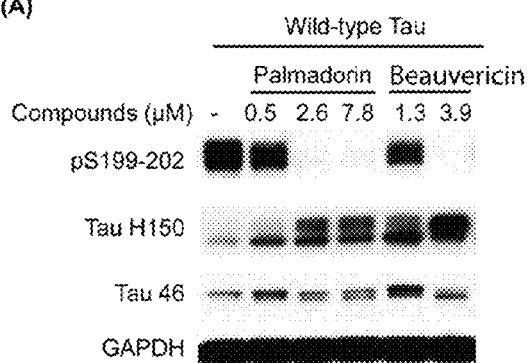
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Eisenschenk

(57) **ABSTRACT**

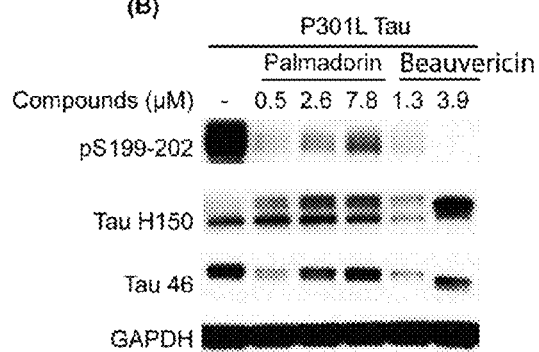
Aspects of the invention pertain to chemical compounds,
therapeutic compositions, and methods for treating neurode-
generative diseases, in particular, neurodegenerative diseases
associated with abnormal accumulation of protein tau.

6 Claims, 1 Drawing Sheet

(A)



(B)



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COMPOUNDS AND RELATED METHODS FOR TREATMENT OF NEURODEGENERATIVE DISEASES

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Ser. No. 61/819,701, filed May 6, 2013, which is hereby incorporated by reference in its entirety.

BACKGROUND

Intracellular aggregation of abnormal species of phosphorylated tau (protein tau) is a major pathologic feature of a family of neurodegenerative diseases collectively referred to as the tauopathies. Tau normally functions to stabilize microtubules in neurons; however, it pathologically aggregates more than 15 neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease. The most common tauopathy is Alzheimer's disease, in which paired helical filaments (PHFs) of mis-folded protein tau aggregates in neurofibrillary tangles, in dystrophic neuritis of senile plaques, and in cell processes in the neuropil. Abnormal accumulation of protein tau is closely linked with postsymptomatic progression in Alzheimer's disease. Abnormal accumulation of protein tau in the cytoplasm of neuronal and glial cells also represents major structural hallmarks in the pathology of Pick's disease, corticobasal degeneration, and progressive supranuclear palsy.

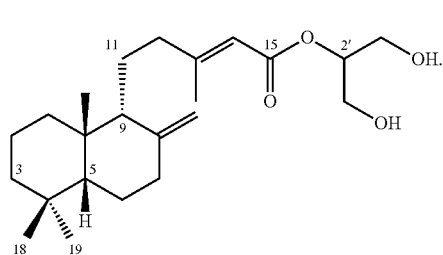
At present, researchers on the development of therapeutics for tauopathies focus primarily on agents that prevent abnormal phosphorylation or aggregation of tau proteins. However, it has been discovered that while aggregation of hyperphosphorylated protein tau is visible evidence of tauopathies, these neurofibrillary tangles appear to be less toxic than soluble intermediates of protein tau. High levels of tau intermediates, particularly aberrant tau species failed to be cleared from cells, cause cognitive dysfunction in AD and tauopathies. Therefore, agents that degrade or destabilize tau intermediates, clear aberrant tau species from cells, or otherwise reduce intracellular tau levels, are promising therapeutics for AD and tauopathies.

Existing therapeutics for the treatment tauopathies (such as AD) only demonstrate limited efficacy; as such, additional therapeutics for the treatment of tauopathies are needed.

BRIEF SUMMARY

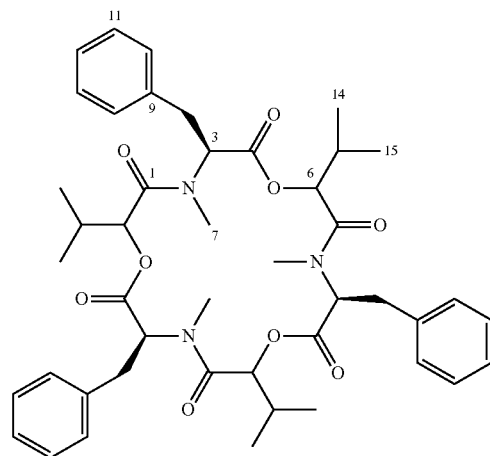
Aspects of the invention provide chemical compounds for treating neurodegenerative diseases, in particular, neurodegenerative diseases associated with abnormal accumulation of protein tau.

One aspect of the invention provides a method of reducing intracellular tau levels comprising administering, to cells comprising protein tau, an effective amount of compound A:



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In another aspect, the invention provides a method of reducing intracellular tau levels comprising administering, to cells comprising protein tau, an effective amount of compound B:



In some embodiments of aspects of the invention, the cells are in a subject, such as a human, in need of treatment for a neurodegenerative disease. The neurodegenerative disease may be a tauopathy. In some embodiments, the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease, frontotemporal dementia, frontotemporal dementia with Parkinsonism, frontotemporal lobe dementia, pallidopontonigral degeneration, progressive supranuclear palsy, multiple system tauopathy, multiple system tauopathy with presenile dementia, Wilhelmsen-Lynch disease, Pick's disease, or Pick's disease-like dementia. In one embodiment, the neurodegenerative disease is Alzheimer's disease.

In additional aspects, the invention provides pharmaceutical compositions comprising any of compounds A, B, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a western blot analysis of phosphorylated tau following Palmadorin M and Beauvericin treatment in HeLa cells transfected with (A) wild type human tau and (B) mutant P301L human tau.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is an amino acid sequence of a tau protein isoform (tau 352) useful according to aspects of the invention.

SEQ ID NO:2 is an amino acid sequence of a tau protein isoform (tau 441) useful according to aspects of the invention.

SEQ ID NO:3 is an amino acid sequence of a tau protein isoform (tau 383) useful according to aspects of the invention.

SEQ ID NO:4 is an amino acid sequence of a tau protein isoform (tau 758) useful according to aspects of the invention.

SEQ ID NO:5 is an amino acid sequence of a tau protein isoform (tau 776) useful according to aspects of the invention.

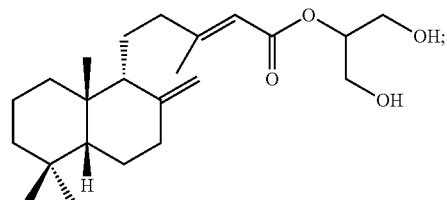
SEQ ID NO:6 is an amino acid sequence of a tau protein isoform (tau 412) useful according to aspects of the invention.

DETAILED DESCRIPTION

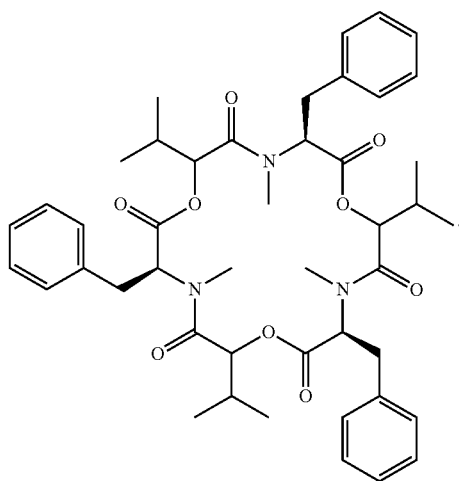
Aspects of the invention provide chemical compounds and compositions for treating neurodegenerative diseases, in particular, neurodegenerative diseases associated with abnormal accumulation of protein tau.

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In one aspect, the invention provides compounds A and B, or salts thereof:

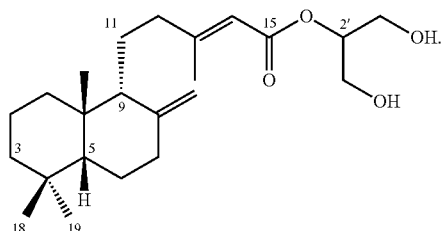


Palmadorin M
Chemical Formula: $C_{23}H_{38}O_4$
Molecular Weight: 378.5454



Chemical Formula: $C_{45}H_{57}N_3O_9$
Molecular Weight: 783.9488
Beauvericin

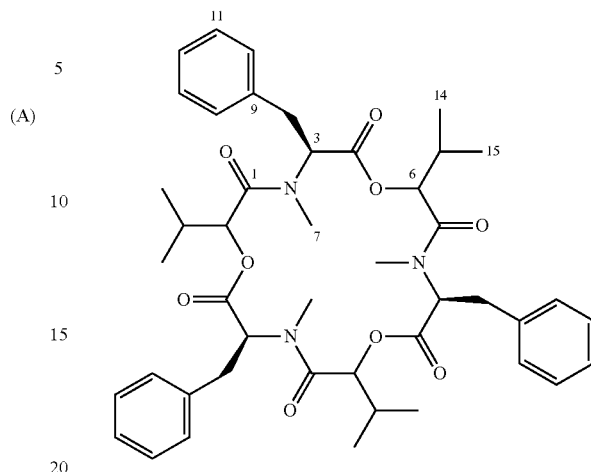
In one embodiment, the invention provides an isolated or substantially pure compound A, or a salt thereof,



In one embodiment, the invention provides an isolated or substantially pure compound B, or a salt thereof,

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(B)



(B)

In certain embodiments, the invention pertains to the uses of compound A (palmadorin M) and/or compound B (beauvericin), or salts thereof, for treating neurodegenerative diseases, in particular, neurodegenerative diseases associated with abnormal accumulation of protein tau.

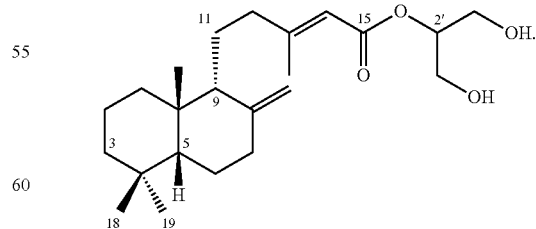
In some embodiments, the compounds of the present invention are at least 75% pure, preferably at least 90% pure, more preferably are more than 95% pure, and most preferably are more than 99% pure (substantially pure). The compounds of the present invention can also be synthesized.

The compounds and compositions of the present invention, through administration to a subject, are useful for treating or ameliorating neurodegenerative diseases or conditions, in particular, neurodegenerative diseases or conditions associated with abnormally high levels of protein tau and/or abnormal accumulation of protein tau in neurons. In some embodiments, the compounds and compositions are useful to treat or ameliorate Alzheimer's disease or Parkinson's disease.

In one aspect, the invention provides methods for treating or ameliorating a neurodegenerative disease or condition, particularly a disease or condition associated with abnormally high levels of protein tau and/or abnormal accumulation of protein tau in neurons, wherein the method comprises administering, to a subject in need of such treatment, an effective amount of a composition comprising a compound selected from compound A and compound B, or salts thereof.

Another aspect of the invention provides a method of reducing intracellular tau levels comprising administering, to cells comprising protein tau, an effective amount of compound A:

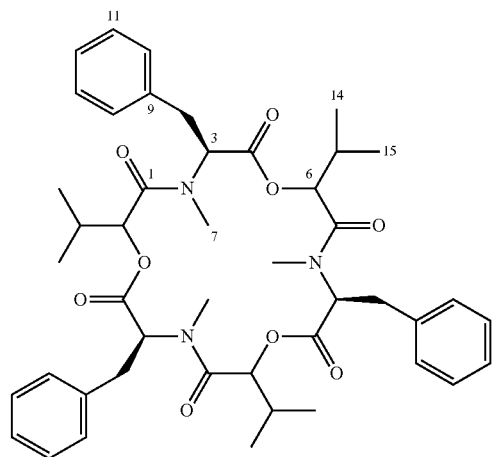
(A)



(A)

In another aspect, the invention provides a method of reducing intracellular tau levels comprising administering, to cells comprising protein tau, an effective amount of compound B:

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In yet another aspect, the invention provides a method of reducing intracellular tau levels comprising administering, to cells comprising protein tau, an effective amount of compounds selected from compound A (Palmadorin M) and compound B (Beauvericin), and combinations thereof.

The term "subject," as used herein, describes an organism, including mammals, to which treatment with the compositions and compounds according to the subject invention can be administered. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and other animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters. Typically, the subject is a human.

The term "tau protein" or any grammatical variation thereof (e.g., protein tau and tau etc.), as used herein, refers generally to any protein of the microtubule-associated tau protein family. Members of the tau family share the common features of a characteristic N-terminal segment, sequences of approximately 50 amino acids inserted in the N-terminal segment, a characteristic tandem repeat region consisting of 3 or 4 tandem repeats of 31-32 amino acids, and a C-terminal tail. Tau proteins of the present invention may be in a form of soluble tau intermediates, functional, aberrant, abnormally-truncated, mis-folded or mis-processed tau, and phosphorylated tau.

In some embodiments, tau protein of the invention is of mammalian origin, and more preferably, of human origin. Specifically, tau proteins of the subject invention include microtubule-associated protein translated from the human chromosomal sequence of GenBank Accession No. AH005895 and naturally-occurring mammalian variants or isoforms thereof. Six human brain tau isoforms are currently known, including tau352 (GenBank Accession No. NP_058525) (SEQ ID NO:1), tau441 (GenBank Accession No. NP_005901) (SEQ ID NO:2), tau383 (GenBank Accession No. NP_058518) (SEQ ID NO:3), tau758 (GenBank Accession No. NP_058519) (SEQ ID NO:4), tau776 (GenBank Accession No. NP_001116538) (SEQ ID NO:5), and tau412 (GenBank Accession No. NP_001116539) (SEQ ID NO:6).

The term "treatment" or any grammatical variation thereof (e.g., treat, treating and treatment etc.), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity,

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(B)

and/or scope of a condition, chance of re-occurrence or returning of a disease after a remission. For instance, the term "treatment" includes (i) ameliorating a symptom associated with a neurodegenerative disease in a patient diagnosed with the neurodegenerative disease; and/or (ii) relieving (such as attenuating the progress of) or remedying a neurodegenerative disease in a patient diagnosed with the neurodegenerative disease.

In some embodiments, the treatment methods of the present invention reduce tau levels and/or improve tau clearance. Normal, functional tau is less affected by clearance pathways in the cell than aberrant tau. In one embodiment, the treatment methods of the present invention modulate tau clearance by selectively targeting abnormal tau.

In an embodiment, the therapeutic composition is administered to a human subject who has symptoms of, or is diagnosed with, a neurodegenerative disease. In some embodiments, the therapeutic composition is administered to a human subject who has symptoms of, or is diagnosed with, a neurodegenerative disease associated with abnormal accumulation of protein tau. For instance, the therapeutic composition is administered to a human subject who has elevated levels of soluble protein tau and/or hyperphosphorylated protein tau in the nervous system, such as in the brain or cytoplasm of neuronal and glial cells. In addition, the therapeutic composition is administered to a human subject who exhibits pathologic features such as neurofibrillary tangles or senile plaques in neuronal cells and/or cell processes. In a specific embodiment, the therapeutic composition is administered to a human subject who has symptoms of, or is diagnosed with, Alzheimer's disease.

The identification of subjects who are in need of treatment for a neurodegenerative disease is well within the knowledge and ability of one skilled in the art. By way of example, a clinician skilled in the art can readily identify, by the use of clinical tests, neurologic and physical examination, and medical/family history, those patients who are suffering from a neurodegenerative disease as well as those who are predisposed to developing a neurodegenerative disease and thus readily determine if an individual is in need of treatment. For instance, neurofibrillary tangles or senile plaques present in neuronal cells and/or cell processes can be determined using electron microscopy (EM) or other clinical techniques known in the art. In addition, spinal fluid or cerebral fluid samples or tissues samples from hippocampal tissue or frontal cortex tissue samples may be obtained from a subject and levels of protein tau present in the samples can be determined using routine techniques such as enzyme-linked immunosorbent assay (ELISA), western blot, and immunological assays.

The compounds and compositions of the present invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject. In a one embodiment, the compounds and compositions of the subject invention are administered orally.

The term "effective amount" or "therapeutically effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect. For instance, the effective amount of the compounds and compo-

sitions of the present invention is an amount capable of reducing levels of protein tau in a subject. In certain embodiments, the effective amount enables a 5%, 25%, 50%, 75%, 90%, 95%, 99% and 100% reduction of levels of protein tau (e.g. soluble protein tau intermediates and/or aberrant protein tau) in a subject.

The amount of the therapeutic or pharmaceutical composition which is effective in treatment of a neurodegenerative disease will depend on the nature of the disease, condition or disorder and can be determined by standard clinical techniques. Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to about 20 mg/kg; intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally 0.01 to about 200 mg/kg and preferably about 1 to 100 mg/kg; intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

Once improvement of the subject's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced as a function of the symptoms to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Subjects may however require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation/composition will also depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

The compounds and compositions of the present invention can be used to treat or ameliorate neurodegenerative diseases including, but not limited to, Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, multiple sclerosis, Pick's disease, frontotemporal dementia, cortico-basal degeneration, progressive supranuclear palsy, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, and Kuru.

The compounds and compositions of the present invention can also be used to treat or ameliorate neurodegenerative diseases including, but not limited to, Down's syndrome, Argyrophilic grain disease, parkinsonism dementia complex of Guam, non-Guamanian motor neurone disease with NFT, Niemann-Pick disease type C, subacute sclerosing panencephalitis, postencephalitic parkinsonism, dementia pugilistica, myotonic dystrophy, prion protein amyloid antipathy, and Hallervorden-Spatz disease.

The compounds and compositions of the present invention are particularly useful to treat or ameliorate a neurodegenerative disease involving tau pathologies (i.e., tauopathies) including, but not limited to, Alzheimer's disease, Parkinson's disease, frontotemporal dementia, frontotemporal dementia with Parkinsonism, frontotemporal lobe dementia, pallidopontonigral degeneration, progressive supranuclear palsy, multiple system tauopathy, multiple system tauopathy with presenile dementia, Wilhelmsen-Lynch disease, Pick's disease, and Pick's disease-like dementia.

Specifically, the compounds and compositions of the present invention are particularly useful to treat or ameliorate a disease or condition arising, at least in part, from abnormally high levels of protein tau in the nervous system, such as in cytoplasm of neuronal and glial cells and in neuronal and

glial cell processes. Thus, the subject invention is particularly useful for treatment of neurodegenerative diseases and disorders, in which reduction of levels of protein tau in the nervous system would be beneficial.

In addition, the compounds and compositions of the subject invention are useful for alleviating or attenuating symptoms arising from or associated with neurodegenerative diseases, including cognitive dysfunction, impaired memory, impaired mental capacities, emotional disturbances, speech dysfunction, incontinence, tremor, postural instability, rigidity or stiff movement, muscle paralysis, and pain.

In additional aspects, the invention provides pharmaceutical compositions comprising a therapeutically effective amount of any of compounds A and/or B and a pharmaceutically acceptable carrier or adjuvant.

The terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as used herein, include compositions, carriers, diluents and reagents, are used interchangeably, and represent that the materials are capable of administration to or upon a subject such as mammal.

The term "carrier" refers to an adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Particularly preferred pharmaceutical carriers for treatment of or amelioration of a neurodegenerative disease are carriers that can penetrate the blood/brain barrier.

Suitable carriers also include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, sorbitol, inositol, xylitol, D-xylose, mannitol, powdered cellulose, microcrystalline cellulose, talc, colloidal silicon dioxide, calcium carbonate, calcium phosphate, calcium aluminium silicate, aluminium hydroxide, sodium starch phosphate, lecithin, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The amount of active ingredient (i.e., one or more of compounds A or B) that may be combined with the carrier materials to produce a single dosage form will vary, depending on the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80% or about 30% to about 70% active ingredient (w/w).

The compounds and compositions can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, Remington's Pharmaceutical Science by E. W. Martin describes formulations which can be used in connection with the present invention. In general, the compositions of the present invention will be formulated such that an effective amount of the bioactive compound(s) is/are combined with a suitable carrier in order to facilitate effective administration of the composition.

The therapeutic or pharmaceutical compositions of the invention can also be formulated as neutral or salt forms.

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Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables, either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspensions, in liquid prior to use also can be prepared. The preparation also can be emulsified, such as oil-in-water emulsion.

The compounds and compositions of the present invention in prescription amounts can be readily made into any form of drug, suitable for administering to humans or other animals. Suitable forms include, for example, tinctures, decoctions, and dry extracts. These can be taken orally, applied through venous injection, mucous membranes or inhalation. The active ingredient(s) can also be formulated into capsules, powder, pellets, pastille, suppositories, oral solutions, pasteurized gastroenteric suspension injections, small or large amounts of injection, frozen powder injections, pasteurized powder injections and the like.

EXAMPLES

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

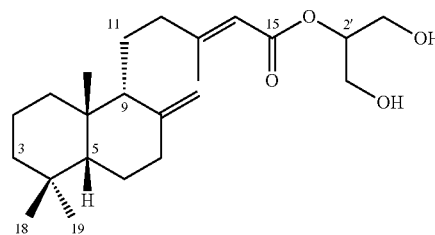
Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

Palmadorin M Extraction and Characterization

600 g of freeze-dried Antarctic nudibranchs, *Austrodoris kerguelensis*, collected by hand via SCUBA off the Palmer Station in the Western Antarctic Peninsula (3-40 m depth range), were first extracted by DCM/MeOH (1:1) then EtOAc/H₂O (1:1). Both resulting extracts were combined and fractionated by normal phase MPLC using gradient conditions from 100% hexanes to 100% ethyl acetate to 100% methanol. The separation provided 10 MPLC fractions. The last 8 fractions were combined and purified by reversed-phase semi-preparative HPLC (40-100% MeOH—H₂O over 70 minutes) to provide 16 fractions. Fraction 13 (47 min) yielded 10.1 mg of palmadorin M (Maschek J. A., 2011 et Maschek J. A. et al., 2012).

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Palmadorin M
Chemical Formula: C₂₃H₃₈O₄

Colorless oil; $[\alpha]_D^{20}$ -18 (c 0.1, CHCl₃); UV (MeOH) λ_{max} (ϵ) 230 (4.38) nm; IR (thin film) 3429 (br), 2935, 1696, 1645, 1214 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 5.72 (1H, s, H-14), 4.93 (1H, s, H-2'), 4.71 (1H, t, H-17b), 4.52 (1H, t, H-17a), 3.83 (2H, m, H₂-1'), 3.83 (2H, m, H₂-3'), 2.18 (1H, m, H-7b), 2.15 (3H, s, H₃-16), 2.04 (1H, m, H-7a), 2.04 (1H, m, H-12b), 1.87 (1H, m, H-12a), 1.70 (1H, m, H-11b), 1.63 (1H, m, H-2b), 1.62 (1H, m, H-6b), 1.53 (1H, m, H-1b), 1.51 (1H, m, H-9), 1.47 (1H, m, H-11a), 1.44 (1H, m, H-2a), 1.42 (1H, m, H-3b), 1.29 (1H, m, H-6a), 1.28 (1H, m, H-5), 1.18 (1H, m, H-3a), 1.06 (1H, m, H-1a), 0.92 (3H, s, H₃-20), 0.88 (3H, s, H₃-19), 0.81 (3H, s, H₃-18); ¹³C NMR (125 MHz, CDCl₃) 166.8 (C-15), 163.2 (C-13), 148.7 (C-8), 114.5 (C-14), 109.9 (C-17), 74.2 (C-2'), 62.3 (C-1'), 62.3 (C-3'), 57.8 (C-9), 45.8 (C-5), 42.6 (C-3), 39.8 (C-12), 38.0 (C-10), 36.7 (C-1), 33.4 (C-19), 33.2 (C-4), 31.5 (C-7), 24.7 (C-11), 23.5 (C-6), 22.3 (C-20), 22.1 (C-18), 19.4 (C-16), 19.1 (C-2); HRESIMS m/z 401.2648 [M+Na]⁺ (C₂₃H₃₈NaO₄ requires 401.2668).

Example 2

Beauvericin Extraction and Characterization

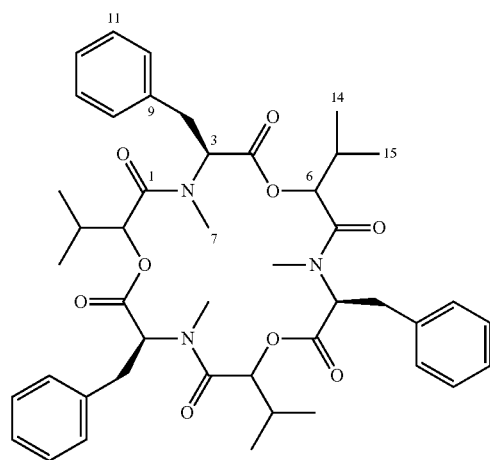
Fungi Collection, Culture Preparation, Isolation and Scale Up:

Segments of approximately 1-2 mm² of mangrove samples were plated on malt extract freshwater agar. Fungal growth was examined every day for two weeks and then twice a week for at least one month for isolation of pure fungal strain. Pure fungal cultures were grown for scale up to 2 L total volume in liquid medium which contained 1% w/v glucose, 0.1% w/v yeast extract and 0.2% w/v peptone (3 weeks) for extraction, separation and pure metabolite identification (Calcul et al. 2013)

Beauvericin:

2.3 g from a freeze-dried scale up of fungus strain collection #EG09-15B-2, isolated from the Florida Everglades bark of *Coccoloba uvifera*, was extracted by MeOH. The methanolic extract was separated by reverse phase MPLC using gradient from H₂O/10% MeOH to 100% MeOH to give 6 fractions. Fraction 5 was selected to be further chromatographed by normal phase MPLC using a gradient from 100% hexane to 100% EtOAc and provided 6 fractions. Fraction 4 was purified on reverse phase semi-preparative HPLC using isocratic H₂O/55% ACN yielding 206.2 mg of Beauvericin (trimer). (Beau J. 2012)

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Beauvericin
Chemical Formula: C₄₅H₅₇N₃O₉

Colorless film; ¹H NMR (500 MHz, CDCl₃) 7.26-7.14 (3H, m, H-12), 7.26-7.14 (6H, m, H-11), 7.26-7.14 (6H, m, H-10), 5.62 (3H, s, H-6), 4.81 (3H, d, H-3), 3.39 (3H, dd, H-8a), 3.03 (9H, s, H-7), 2.93 (3H, dd, H-8b), 1.93 (3H, dq, H-13), 0.78 (9H, d, H-14), 0.35 (9H, d, H-15); ¹³C NMR (125 MHz, CDCl₃) 170.0 (C-1), 169.9 (C-4), 136.4 (C-9), 128.7 (C-11), 128.5 (C-10), 126.8 (C-12), 75.7 (C-6), 56.8 (C-3), 34.7 (C-7), 31.9 (C-8), 29.7 (C-13), 18.4 (C-14), 17.1 (C-15); LRESIMS m/z 784.4 [M+H]⁺—Note: the ¹H and ¹³C NMR data matched to the data from the literature (Gupta, S. et al. 1991);

Example 3

Palmadorin M and Beauvericin Preferentially Reduce Phosphorylated Tau

Compounds effects on tau activity/level were determine by a previously described method (Jinwal et al *J. Neurosci.* 2009 Sep. 30; 29(39):12079-88). In brief, human tau transfected

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HeLa Cells were treated with Compounds for 24 hours. Cells were harvested by using MPER reagent containing protease inhibitor and phosphatase inhibitor. Samples were analyzed by western blot. pS199-202 antibody was used to detect phospho tau level. Total tau levels were detected by tau H-150 &/or tau 46 antibodies.

HeLa cells were transfected with wild-type human tau (A) and mutant P301L human tau (B) (FIG. 1). After 24 hours, cells were treated with either palmadorin M or beauvericin for 24 hours. Western blot analysis of samples showed potent reduction in phosphorylated (pS199-202) wild type and mutant P301L tau at various doses of palmadorin M and beauvericin. Tau H-150 antibody tested for N-terminal tau showed increase in tau level. Tau 46 antibody tested for C-terminal tau showed moderate change in tau level.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

REFERENCES

- 30 Maschek J. A. "Chemical Investigation of the Antarctic Marine Invertebrates *Austrodoris kerguelensis* & *Dendrilla membranosa* and the Antarctic Red Alga *Gigartina skottsbergii*" Ph. D. Thesis, Department of Chemistry, College of Art and Sciences, University of South Florida, Tampa, Fla., USA, 7 Jun. 2011.
- 35 Maschek J. A. 2012, *Tetrahedron*, 9095-9104.
- Beau J. "Drug Discovery from Floridian Mangrove Endophytes" Ph D. Thesis, Department of Chemistry, College of Art and Sciences, University of South Florida, Tampa, Fla., USA, 12 Jul. 2012.
- 40 Gupta S. et al. *Mycopathologia*, 1991, 185-189
- Calcul L. et al. *Mar. Drugs*, 2013, 5036-5050.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 6

<210> SEQ ID NO 1

<211> LENGTH: 352

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala
35          40          45

Gly Ile Gly Asp Thr Pro Ser Leu Glu Asp Glu Ala Ala Gly His Val
50          55          60

Thr Gln Ala Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp
65          70          75          80
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Asp	Lys	Lys	Ala	Lys	Gly	Ala	Asp	Gly	Lys	Thr	Lys	Ile	Ala	Thr	Pro
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Arg	Gly	Ala	Ala	Pro	Pro	Gly	Gln	Lys	Gly	Gln	Ala	Asn	Ala	Thr	Arg
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Ile	Pro	Ala	Lys	Thr	Pro	Pro	Ala	Pro	Lys	Thr	Pro	Pro	Ser	Ser	Gly
			115				120					125			
Glu	Pro	Pro	Lys	Ser	Gly	Asp	Arg	Ser	Gly	Tyr	Ser	Ser	Pro	Gly	Ser
	130					135					140				
Pro	Gly	Thr	Pro	Gly	Ser	Arg	Ser	Arg	Thr	Pro	Ser	Leu	Pro	Thr	Pro
145					150					155					160
Pro	Thr	Arg	Glu	Pro	Lys	Lys	Val	Ala	Val	Val	Arg	Thr	Pro	Pro	Lys
				165					170					175	
Ser	Pro	Ser	Ser	Ala	Lys	Ser	Arg	Leu	Gln	Thr	Ala	Pro	Val	Pro	Met
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Pro	Asp	Leu	Lys	Asn	Val	Lys	Ser	Lys	Ile	Gly	Ser	Thr	Glu	Asn	Leu
		195					200					205			
Lys	His	Gln	Pro	Gly	Gly	Gly	Lys	Val	Gln	Ile	Val	Tyr	Lys	Pro	Val
	210					215					220				
Asp	Leu	Ser	Lys	Val	Thr	Ser	Lys	Cys	Gly	Ser	Leu	Gly	Asn	Ile	His
225					230					235					240
His	Lys	Pro	Gly	Gly	Gly	Gln	Val	Glu	Val	Lys	Ser	Glu	Lys	Leu	Asp
				245					250					255	
Phe	Lys	Asp	Arg	Val	Gln	Ser	Lys	Ile	Gly	Ser	Leu	Asp	Asn	Ile	Thr
			260					265					270		
His	Val	Pro	Gly	Gly	Gly	Asn	Lys	Lys	Ile	Glu	Thr	His	Lys	Leu	Thr
		275					280					285			
Phe	Arg	Glu	Asn	Ala	Lys	Ala	Lys	Thr	Asp	His	Gly	Ala	Glu	Ile	Val
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Tyr	Lys	Ser	Pro	Val	Val	Ser	Gly	Asp	Thr	Ser	Pro	Arg	His	Leu	Ser
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Asn	Val	Ser	Ser	Thr	Gly	Ser	Ile	Asp	Met	Val	Asp	Ser	Pro	Gln	Leu
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<400> SEQUENCE: 2

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			20					25					30		
Gln	Asp	Gln	Glu	Gly	Asp	Thr	Asp	Ala	Gly	Leu	Lys	Glu	Ser	Pro	Leu
		35					40					45			
Gln	Thr	Pro	Thr	Glu	Asp	Gly	Ser	Glu	Glu	Pro	Gly	Ser	Glu	Thr	Ser
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Asp	Ala	Lys	Ser	Thr	Pro	Thr	Ala	Glu	Asp	Val	Thr	Ala	Pro	Leu	Val
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Asp	Glu	Gly	Ala	Pro	Gly	Lys	Gln	Ala	Ala	Ala	Gln	Pro	His	Thr	Glu
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Ile	Pro	Glu	Gly	Thr	Thr	Ala	Glu	Glu	Ala	Gly	Ile	Gly	Asp	Thr	Pro
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Ser Leu Glu Asp Glu Ala Ala Gly His Val Thr Gln Ala Arg Met Val
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 Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly
 130 135 140
 Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro
 145 150 155 160
 Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro
 165 170 175
 Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly
 180 185 190
 Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
 195 200 205
 Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys
 210 215 220
 Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys
 225 230 235 240
 Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val
 245 250 255
 Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly
 260 265 270
 Gly Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln
 275 280 285
 Ser Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly
 290 295 300
 Ser Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser
 305 310 315 320
 Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln
 325 330 335
 Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser
 340 345 350
 Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn
 355 360 365
 Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala
 370 375 380
 Lys Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser
 385 390 395 400
 Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser
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<210> SEQ ID NO 3

<211> LENGTH: 383

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

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Gly 50	Ile	Gly	Asp	Thr	Pro	Ser	Leu	Glu	Asp	Glu	Ala	Ala	Gly	His
Thr 65	Gln	Ala	Arg	Met	Val	Ser	Lys	Ser	Lys	Asp	Gly	Thr	Gly	Ser
Asp	Lys	Lys	Ala	Lys	Gly	Ala	Asp	Gly	Lys	Thr	Lys	Ile	Ala	Thr
Arg	Gly	Ala	Ala	Pro	Pro	Gly	Gln	Lys	Gly	Gln	Ala	Asn	Ala	Thr
Ile	Pro	Ala	Lys	Thr	Pro	Pro	Ala	Pro	Lys	Thr	Pro	Pro	Ser	Ser
Glu	Pro	Pro	Lys	Ser	Gly	Asp	Arg	Ser	Gly	Tyr	Ser	Ser	Pro	Gly
Pro	Gly	Thr	Pro	Gly	Ser	Arg	Ser	Arg	Thr	Pro	Ser	Leu	Pro	Thr
Pro	Thr	Arg	Glu	Pro	Lys	Lys	Val	Ala	Val	Val	Arg	Thr	Pro	Pro
Ser	Pro	Ser	Ser	Ala	Lys	Ser	Arg	Leu	Gln	Thr	Ala	Pro	Val	Pro
Pro	Asp	Leu	Lys	Asn	Val	Lys	Ser	Lys	Ile	Gly	Ser	Thr	Glu	Asn
Lys	His	Gln	Pro	Gly	Gly	Gly	Lys	Val	Gln	Ile	Ile	Asn	Lys	Lys
Asp	Leu	Ser	Asn	Val	Gln	Ser	Lys	Cys	Gly	Ser	Lys	Asp	Asn	Ile
His	Val	Pro	Gly	Gly	Gly	Ser	Val	Gln	Ile	Val	Tyr	Lys	Pro	Val
Leu	Ser	Lys	Val	Thr	Ser	Lys	Cys	Gly	Ser	Leu	Gly	Asn	Ile	His
Lys	Pro	Gly	Gly	Gly	Gln	Val	Glu	Val	Lys	Ser	Glu	Lys	Leu	Asp
Lys	Asp	Arg	Val	Gln	Ser	Lys	Ile	Gly	Ser	Leu	Asp	Asn	Ile	Thr
Val	Pro	Gly	Gly	Gly	Asn	Lys	Lys	Ile	Glu	Thr	His	Lys	Leu	Thr
Arg	Glu	Asn	Ala	Lys	Ala	Lys	Thr	Asp	His	Gly	Ala	Glu	Ile	Val
Lys	Ser	Pro	Val	Val	Ser	Gly	Asp	Thr	Ser	Pro	Arg	His	Leu	Ser
Val	Ser	Ser	Thr	Gly	Ser	Ile	Asp	Met	Val	Asp	Ser	Pro	Gln	Leu
Thr	Leu	Ala	Asp	Glu	Val	Ser	Ala	Ser	Leu	Ala	Lys	Gln	Gly	Leu

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Gln	Thr	Pro	Thr	Glu	Asp	Gly	Ser	Glu	Glu	Pro	Gly	Ser	Glu	Thr	Ser
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Asp	Ala	Lys	Ser	Thr	Pro	Thr	Ala	Glu	Asp	Val	Thr	Ala	Pro	Leu	Val
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Asp	Glu	Gly	Ala	Pro	Gly	Lys	Gln	Ala	Ala	Ala	Gln	Pro	His	Thr	Glu
				85				90						95	
Ile	Pro	Glu	Gly	Thr	Thr	Ala	Glu	Glu	Ala	Gly	Ile	Gly	Asp	Thr	Pro
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Ser	Leu	Glu	Asp	Glu	Ala	Ala	Gly	His	Val	Thr	Gln	Glu	Pro	Glu	Ser
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Gly	Lys	Val	Val	Gln	Glu	Gly	Phe	Leu	Arg	Glu	Pro	Gly	Pro	Pro	Gly
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Leu	Ser	His	Gln	Leu	Met	Ser	Gly	Met	Pro	Gly	Ala	Pro	Leu	Leu	Pro
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Glu	Gly	Pro	Arg	Glu	Ala	Thr	Arg	Gln	Pro	Ser	Gly	Thr	Gly	Pro	Glu
				165					170					175	
Asp	Thr	Glu	Gly	Gly	Arg	His	Ala	Pro	Glu	Leu	Leu	Lys	His	Gln	Leu
			180					185					190		
Leu	Gly	Asp	Leu	His	Gln	Glu	Gly	Pro	Pro	Leu	Lys	Gly	Ala	Gly	Gly
	195						200					205			
Lys	Glu	Arg	Pro	Gly	Ser	Lys	Glu	Glu	Val	Asp	Glu	Asp	Arg	Asp	Val
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Asp	Glu	Ser	Ser	Pro	Gln	Asp	Ser	Pro	Pro	Ser	Lys	Ala	Ser	Pro	Ala
225					230					235					240
Gln	Asp	Gly	Arg	Pro	Pro	Gln	Thr	Ala	Ala	Arg	Glu	Ala	Thr	Ser	Ile
				245					250					255	
Pro	Gly	Phe	Pro	Ala	Glu	Gly	Ala	Ile	Pro	Leu	Pro	Val	Asp	Phe	Leu
		260						265					270		
Ser	Lys	Val	Ser	Thr	Glu	Ile	Pro	Ala	Ser	Glu	Pro	Asp	Gly	Pro	Ser
	275						280					285			
Val	Gly	Arg	Ala	Lys	Gly	Gln	Asp	Ala	Pro	Leu	Glu	Phe	Thr	Phe	His
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Val	Glu	Ile	Thr	Pro	Asn	Val	Gln	Lys	Glu	Gln	Ala	His	Ser	Glu	Glu
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His	Leu	Gly	Arg	Ala	Ala	Phe	Pro	Gly	Ala	Pro	Gly	Glu	Gly	Pro	Glu
				325					330					335	
Ala	Arg	Gly	Pro	Ser	Leu	Gly	Glu	Asp	Thr	Lys	Glu	Ala	Asp	Leu	Pro
			340					345					350		
Glu	Pro	Ser	Glu	Lys	Gln	Pro	Ala	Ala	Ala	Pro	Arg	Gly	Lys	Pro	Val
	355						360					365			
Ser	Arg	Val	Pro	Gln	Leu	Lys	Ala	Arg	Met	Val	Ser	Lys	Ser	Lys	Asp
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Gly	Thr	Gly	Ser	Asp	Asp	Lys	Lys	Ala	Lys	Thr	Ser	Thr	Arg	Ser	Ser
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Ala	Lys	Thr	Leu	Lys	Asn	Arg	Pro	Cys	Leu	Ser	Pro	Lys	His	Pro	Thr
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Pro	Gly	Ser	Ser	Asp	Pro	Leu	Ile	Gln	Pro	Ser	Ser	Pro	Ala	Val	Cys
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 Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg
 515 520 525
 Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys Lys Val Ala
 530 535 540
 Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys Ser Arg Leu
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 Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys
 565 570 575
 Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly Lys Val
 580 585 590
 Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln Ser Lys Cys
 595 600 605
 Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly Ser Val Gln
 610 615 620
 Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys Cys Gly
 625 630 635 640
 Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln Val Glu Val
 645 650 655
 Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile Gly
 660 665 670
 Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys Ile
 675 680 685
 Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp
 690 695 700
 His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr
 705 710 715 720
 Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp Met
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 Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val Ser Ala Ser
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 Leu Ala Lys Gln Gly Leu
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<210> SEQ ID NO 5
 <211> LENGTH: 776
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

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 35 40 45
 Gln Thr Pro Thr Glu Asp Gly Ser Glu Glu Pro Gly Ser Glu Thr Ser
 50 55 60

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Asp	Glu	Gly	Ala	Pro	Gly	Lys	Gln	Ala	Ala	Ala	Gln	Pro	His	Thr	Glu	85	90	95	
Ile	Pro	Glu	Gly	Thr	Thr	Ala	Glu	Glu	Ala	Gly	Ile	Gly	Asp	Thr	Pro	100	105	110	
Ser	Leu	Glu	Asp	Glu	Ala	Ala	Gly	His	Val	Thr	Gln	Glu	Pro	Glu	Ser	115	120	125	
Gly	Lys	Val	Val	Gln	Glu	Gly	Phe	Leu	Arg	Glu	Pro	Gly	Pro	Pro	Gly	130	135	140	
Leu	Ser	His	Gln	Leu	Met	Ser	Gly	Met	Pro	Gly	Ala	Pro	Leu	Leu	Pro	145	150	155	160
Glu	Gly	Pro	Arg	Glu	Ala	Thr	Arg	Gln	Pro	Ser	Gly	Thr	Gly	Pro	Glu	165	170	175	
Asp	Thr	Glu	Gly	Gly	Arg	His	Ala	Pro	Glu	Leu	Leu	Lys	His	Gln	Leu	180	185	190	
Leu	Gly	Asp	Leu	His	Gln	Glu	Gly	Pro	Pro	Leu	Lys	Gly	Ala	Gly	Gly	195	200	205	
Lys	Glu	Arg	Pro	Gly	Ser	Lys	Glu	Glu	Val	Asp	Glu	Asp	Arg	Asp	Val	210	215	220	
Asp	Glu	Ser	Ser	Pro	Gln	Asp	Ser	Pro	Pro	Ser	Lys	Ala	Ser	Pro	Ala	225	230	235	240
Gln	Asp	Gly	Arg	Pro	Pro	Gln	Thr	Ala	Ala	Arg	Glu	Ala	Thr	Ser	Ile	245	250	255	
Pro	Gly	Phe	Pro	Ala	Glu	Gly	Ala	Ile	Pro	Leu	Pro	Val	Asp	Phe	Leu	260	265	270	
Ser	Lys	Val	Ser	Thr	Glu	Ile	Pro	Ala	Ser	Glu	Pro	Asp	Gly	Pro	Ser	275	280	285	
Val	Gly	Arg	Ala	Lys	Gly	Gln	Asp	Ala	Pro	Leu	Glu	Phe	Thr	Phe	His	290	295	300	
Val	Glu	Ile	Thr	Pro	Asn	Val	Gln	Lys	Glu	Gln	Ala	His	Ser	Glu	Glu	305	310	315	320
His	Leu	Gly	Arg	Ala	Ala	Phe	Pro	Gly	Ala	Pro	Gly	Glu	Gly	Pro	Glu	325	330	335	
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Glu	Pro	Ser	Glu	Lys	Gln	Pro	Ala	Ala	Ala	Pro	Arg	Gly	Lys	Pro	Val	355	360	365	
Ser	Arg	Val	Pro	Gln	Leu	Lys	Ala	Arg	Met	Val	Ser	Lys	Ser	Lys	Asp	370	375	380	
Gly	Thr	Gly	Ser	Asp	Asp	Lys	Lys	Ala	Lys	Thr	Ser	Thr	Arg	Ser	Ser	385	390	395	400
Ala	Lys	Thr	Leu	Lys	Asn	Arg	Pro	Cys	Leu	Ser	Pro	Lys	His	Pro	Thr	405	410	415	
Pro	Gly	Ser	Ser	Asp	Pro	Leu	Ile	Gln	Pro	Ser	Ser	Pro	Ala	Val	Cys	420	425	430	
Pro	Glu	Pro	Pro	Ser	Ser	Pro	Lys	Tyr	Val	Ser	Ser	Val	Thr	Ser	Arg	435	440	445	
Thr	Gly	Ser	Ser	Gly	Ala	Lys	Glu	Met	Lys	Leu	Lys	Gly	Ala	Asp	Gly	450	455	460	
Lys	Thr	Lys	Ile	Ala	Thr	Pro	Arg	Gly	Ala	Ala	Pro	Pro	Gly	Gln	Lys	465	470	475	480

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Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro Pro Ala Pro
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 Lys Thr Pro Pro Ser Ser Ala Thr Lys Gln Val Gln Arg Arg Pro Pro
 500 505 510
 Pro Ala Gly Pro Arg Ser Glu Arg Gly Glu Pro Pro Lys Ser Gly Asp
 515 520 525
 Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg
 530 535 540
 Ser Arg Thr Pro Ser Leu Pro Thr Pro Thr Arg Glu Pro Lys Lys
 545 550 555 560
 Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys Ser
 565 570 575
 Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys
 580 585 590
 Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly
 595 600 605
 Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln Ser
 610 615 620
 Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly Ser
 625 630 635 640
 Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys
 645 650 655
 Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln Val
 660 665 670
 Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys
 675 680 685
 Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn Lys
 690 695 700
 Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys
 705 710 715 720
 Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser Gly
 725 730 735
 Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile
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<210> SEQ ID NO 6
 <211> LENGTH: 412
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

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 35 40 45
 Gln Thr Pro Thr Glu Asp Gly Ser Glu Glu Pro Gly Ser Glu Thr Ser
 50 55 60
 Asp Ala Lys Ser Thr Pro Thr Ala Glu Ala Glu Glu Ala Gly Ile Gly
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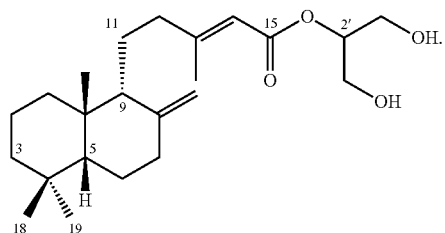
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Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys	100	105	110
Ala Lys Gly Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala	115	120	125
Ala Pro Pro Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala	130	135	140
Lys Thr Pro Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro	145	150	155
Lys Ser Gly Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr	165	170	175
Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg	180	185	190
Glu Pro Lys Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser	195	200	205
Ser Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu	210	215	220
Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln	225	230	235
Pro Gly Gly Gly Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser	245	250	255
Asn Val Gln Ser Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro	260	265	270
Gly Gly Gly Ser Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys	275	280	285
Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly	290	295	300
Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg	305	310	315
Val Gln Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly	325	330	335
Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn	340	345	350
Ala Lys Ala Lys Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro	355	360	365
Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser	370	375	380
Thr Gly Ser Ile Asp Met Val Asp Ser Pro Gln Leu Ala Thr Leu Ala	385	390	395
Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly Leu	405	410	

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We claim:

1. A method of reducing intracellular tau phosphorylation levels, wherein the method comprises administering, to cells comprising phosphorylated protein tau, an effective amount of compound A:



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2. The method according to claim 1, wherein the cells are in a subject in need of treatment for a neurodegenerative disease.

3. The method of claim 2, wherein the neurodegenerative disease is a tauopathy.

4. The method of claim 3, wherein the subject is a human.

5. The method of claim 4, wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease, frontotemporal dementia, frontotemporal dementia with Parkinsonism, frontotemporal lobe dementia, pallidop-
 10 ontonigral degeneration, progressive supranuclear palsy, multiple system tauopathy, multiple system tauopathy with presenile dementia, Wilhelmsen-Lynch disease, Pick's dis-
 15 ease, or Pick's disease-like dementia.

6. The method of claim 4, wherein the neurodegenerative disease is Alzheimer's disease.

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