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(71) Applicant: **SYNAPTOGENIX, INC.** [US/US]; 205 East 42nd Street, 16th Floor, New York, New York 10017 (US).

(72) Inventor: **ALKON, Daniel L.**; 4604 Dorset Avenue, Chevy Chase, Maryland 20815 (US).

(74) Agent: **GROLZ, Edward W.**; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Suite 300, Garden City, New York 11530 (US).

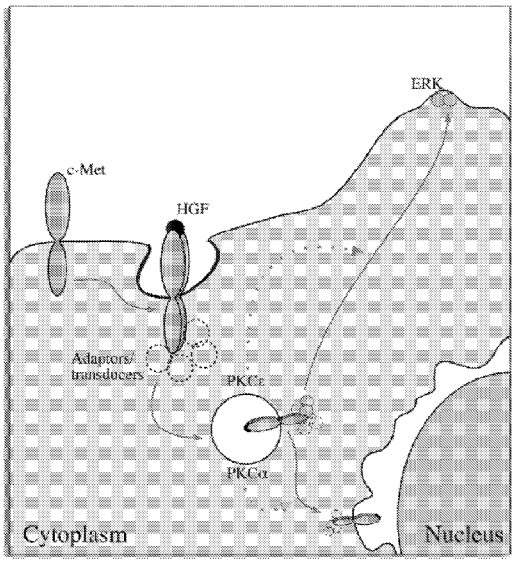
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(54) **Title:** METHOD OF INDUCING SYNAPTOGENESIS BY ADMINISTERING ANGIOTENSIN AND ANALOGUES THEREOF

(57) **Abstract:** A method for inducing synaptogenesis in a subject having a neurodegenerative disease, the method comprising administering an angiotensin or analogue thereof in a therapeutically effective amount to induce synaptogenesis in said subject. The neurodegenerative disease may be, for example, Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, dementia, or mild cognitive impairment. The methods may also be generally directed to improving or enhancing cognitive ability, preventing or treating cognitive impairment, preventing or treating a neurodegenerative disease or condition, and/or preventing or treating a disease or condition associated with neuronal or synaptic loss according to the disclosed regimens.



S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005

FIG. 1

WO 2023/278364 A1

METHOD OF INDUCING SYNAPTOGENESIS BY ADMINISTERING ANGIOTENSIN AND ANALOGUES THEREOF

BACKGROUND

[0001] The debilitating effects of neurodegenerative (neurological) diseases are well known. A neurodegenerative disease is generally associated with β-amyloidogenic processing of amyloid precursor protein (APP) in the central nervous system (CNS) or peripheral nervous system (PNS). This may result in neuronal or glial cell defects including but not limited to neuronal loss, neuronal degeneration, neuronal demyelination, gliosis (i.e., astrogliosis), or neuronal or extraneuronal accumulation of aberrant proteins or toxins (e.g., amyloid beta peptide, i.e., Aβ). Some examples of neurodegenerative diseases include Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, dementia, Huntington's Disease, amyotrophic lateral sclerosis (ALS), mild cognitive impairment, and early or late stages of these diseases.

[0002] Alzheimer's disease (AD), in particular, is a neurodegenerative disorder generally characterized by the progressive decline of mental functioning. More specifically, AD is characterized clinically by the progressive loss of memory, cognition, reasoning, judgment, and emotional stability that gradually leads to profound mental deterioration and, ultimately, death. Although there are many hypotheses for the possible mechanisms of AD, one central theory is that the excessive formation and accumulation of toxic beta-amyloid (Aβ) peptides either directly or indirectly affects a variety of cellular events and leads to neuronal damage and cell death. Selkoe, *Neuron*. 1991;6(4):487-98 1991; Selkoe, *J. Clin Invest*. 2002;110(10): 1375-81. Dementia associated with AD is referred to as senile dementia of the Alzheimer's type (SDAT) in usage with Alzheimer's disease.

[0003] AD is a progressive disorder with a mean duration of around 8-15 years between onset of clinical symptoms and death. AD is believed to represent the seventh most common medical

cause of death and affects about 5 million people in the United States. There are three general stages of Alzheimer's disease: mild (early) stage, moderate (middle) stage and severe (late) stage. Each stage is associated with a worsening of neurological abilities. In the early (mild) stage, the subject may function independently, but experiences mild changes in cognitive functioning, such as memory lapses of recent events. The moderate stage, which is typically the longest stage and can last for many years, can be characterized by increased cognitive decline, significantly impacting memory and thinking, and interfering with routine functioning. The severe (late) stage of AD is characterized by further decline of mental functioning, such as losing the ability to communicate, to respond to surroundings, and to control movement and physical abilities.

[0004] Protein kinase C (PKC) is one of the largest gene families of protein kinase. Several PKC isozymes are expressed in the brain, including PKCα, PKCβ1, PKCβII, PKCδ, PKCε, and PKCγ. PKC is primarily a cytosolic protein, but with stimulation it translocates to the membrane. PKC activators have been associated with prevention and treatment of various diseases and conditions. For example, PKC has been shown to be involved in numerous biochemical processes relevant to AD, and PKC activators have demonstrated neuroprotective activity in animal models of AD. PKC activation has a crucial role in learning and memory enhancement, and PKC activators have been shown to increase memory and learning. Sun and Alkon, *Eur J Pharmacol.* 2005;512:43-51; Alkon et al., *Proc Natl Acad Sci USA.* 2005;102:16432-16437. PKC activation also has been shown to induce synaptogenesis in rat hippocampus, suggesting the potential of PKC-mediated antiapoptosis and synaptogenesis during conditions of neurodegeneration. Sun and Alkon, *Proc Natl Acad Sci USA.* 2008; 105(36): 13620-13625. In fact, synaptic loss appears to be a pathological finding in the brain

that is closely correlated with the degree of dementia in AD patients. PKC activation has further been shown to protect against traumatic brain injury-induced learning and memory deficits, (*see* Zohar et al., *Neurobiology of Disease*, 2011, 41: 329-337), has demonstrated neuroprotective activity in animal models of stroke, (*see* Sun et al., *Eur. J. Pharmacol.*, 2005, 512: 43-51), and has shown neuroprotective activity in animal models of depression, (*see* Sun et al., *Eur. J. Pharmacol.*, 2005, 512: 43-51).

[0005] Neurotrophins, particularly brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), are key growth factors that initiate repair and regrowth of damaged neurons and synapses. Activation of some PKC isoforms, particularly PKCε and PKCα, protect against neurological injury, most likely by upregulating the production of neurotrophins such as BDNF. Weinreb et al., *FASEB Journal.* 2004;18:1471- 1473). The activation of PKCε also increases brain postsynaptic density anchoring protein (PSD-95) which is an important marker for synaptogenesis.

[0006] In addition, changes in dendritic spine density form the basis of learning- and memory-induced changes in synaptic structure that increase synaptic strength. Abnormalities in the number and morphology of dendritic spines have been observed in many cognitive disorders, such as attention deficit hyperactivity disorder, schizophrenia, autism, mental retardation, and fragile X syndrome. For example, the brains of schizophrenic patients and people suffering from cognitive-mood disorders show a reduced number of dendritic spines in the brain areas associated with these diseases. In mental retardation and autism, the shapes of the dendritic spines are longer and appear more immature.

[0007] In view of the limited existing options for treating neurodegenerative disease, new methodologies for treating, preventing, or slowing the onset of neurodegenerative disease are needed. There is a particular need for methodologies that not only treat the neurophysiological deterioration and mental decline resulting from these diseases, but that mitigate or halt the progression by interfering with the mechanism associated with the initiation and/or progression of these diseases and which can also repair damaged synapses.

SUMMARY

[0008] The present disclosure is directed to a method for treating or preventing a neurodegenerative disease in a subject by administering an angiotensin in a therapeutically effective amount to the subject to result in treatment (e.g., mitigation) or prevention of symptoms of the neurodegenerative disease. More specifically, the present disclosure is directed to a method of inducing synaptogenesis in a subject by administering an angiotensin in a therapeutically effective amount to the subject. The neurodegenerative disease may be any of the diseases enumerated earlier above, such as, for example, Alzheimer's Disease, Parkinson's Disease, ALS, multiple sclerosis, dementia, and mild cognitive impairment. The angiotensin may be any of the known an angiotensins, e.g., any of angiotensin I, II, III, or IV.

[0009] The present method operates by activating HGF in a subject and potentiating HGF activity at its receptor, c-Met (i.e., by administration of the angiotensin, which binds to HGF and induces c-Met phosphorylation). It is also known that PKC-α and PKC-ε isozymes control HGF signaling efficacy and vice-versa. Thus, HGF can be considered as immediately downstream from PKC-α and PKC-ε, and these two isozymes are also activated by HGF, which thus functions as a PKC activator (e.g., S. Kermogant, P. J. Parker, *Cell Cycle*, 4(3),

352-355; 2005; Z. Xie et al., *eNeuro*, 3(4), 2016; S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734, November 2004; and G. D. Sharma et al., *Exp Eye Res.*, 85(2), 289-297, August 2007, all of the contents of which are herein incorporated by reference).

[0010] The interrelationship between PKC, Met, and HGF is conveniently captured in the schematic shown in FIG. 1, as adopted from S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005. Significantly, FIG. 1 shows that the HGF-Met pathway is a PKC epsilon activation process. As particularly noted in the aforesaid publication, it was previously reported that c-Met signaling to the MAPkinase(s), ERK 1/2 (extracellular signal regulated kinases 1 and 2), occurs in the internalised endosomal compartments and requires continued signal output to sustain elevated levels of activated ERK1/2—inactivation of the upstream kinase MEK rapidly leads to the loss of active phosphorylated ERK1/2 even 1.8 hours after the initial stimulation by HGF. From this internalised compartment, the activated ERK1/2 is translocated to the plasma membrane and becomes associated with focal complexes, as evidenced by colocalization with paxillin, vinculin and actin at sites of membrane ruffling (S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734; S. Ishibe et al., *Mol Cell* 2003, 12:1275-85).

[0011] As further noted in S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005, a key regulator of this sorting of signals at the endosome and the consequent ERK1/2 translocation to the plasma membrane is PKCε; **in the absence of PKCε, HGF no longer causes ERK1/2 to accumulate at focal complexes. PKCε is required for c-Met dependent cell migration as is ERK1/2 activation. For example, sustained ERK1/2 activation is required for the disassembly of adherens junctions, one of the early steps in cell dissociation (S. Potempa et al., *Mol. Biol. Cell*, 9:2185-2200, 1998). These studies indicate that it is the combination**

of ERK1/2 activation and its PKCε dependent translocation to focal complexes. [foregoing statements reprinted from S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005].

[0012] FIG. 2, as adopted from S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734, November 2004, further illustrates the elements in the HGF-induced c-Met pathway under the control of PKC. As stated in the foregoing publication: "It is shown that c-Met signalling to the ERK cascade occurs within endosomal compartments and that it is in this compartment that PKCε specifically exerts its control on the pathway with the consequent accumulation of ERK in focal complexes. These events are clearly separated from the subsequent microtubule-dependent sorting of c-Met to its perinuclear destination, which is shown to be under the control of PKCα. Thus while it is shown that traffic to endosomes is essential for HGF/c-Met to trigger an ERK response, the subsequent traffic and signalling of c-Met controlled by these two PKC isotypes are unconnected events. The dynamic properties conferred by the PKCε control are shown to be essential for a normal HGF-dependent migratory response. Thus PKCs are shown to control both receptor traffic and signal traffic to relay HGF/c-Met responses." [reprinted from S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734, November 2004]

[0013] The ELAV (HUD) pathway is also known, by which PKC activation activates HGF and other growth factors (e.g., J. Hongpaisan and D. L. Alkon, *PNAS*, 104(49), December 4, 2007). Hongpaisan and Alkon, 2007 (Ibid) also demonstrate that PKC activation by bryostatin enhances associative learning and increases the number of fully mature mushroom spine synapses. As the HGF-Met pathway is ultimately a PKC-ε activation process, the presently described method provides an alternative methodology for treating neurodegenerative disease

by directly interfering with the mechanism associated with initiation and/or progression of neurodegeneration, particularly Alzheimer's Disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1, as reprinted from S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005, illustrates the activation of the HGF receptor at the plasma membrane, its association with various efffectors (adapters/transducers) and traffic to endosomes. Within the endosomal compartment, PKC isoforms exert selective influences on c-Met traffic to the perinuclear compartment and on the movement of ERK1/2 (and associated proteins) to the leading edge of the cell where focal complexes accumulate.

[0015] FIG. 2, as reprinted from S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734, November 2004, illustrates the elements in the HGF-induced c-Met pathway under the control of PKC.

DETAILED DESCRIPTION

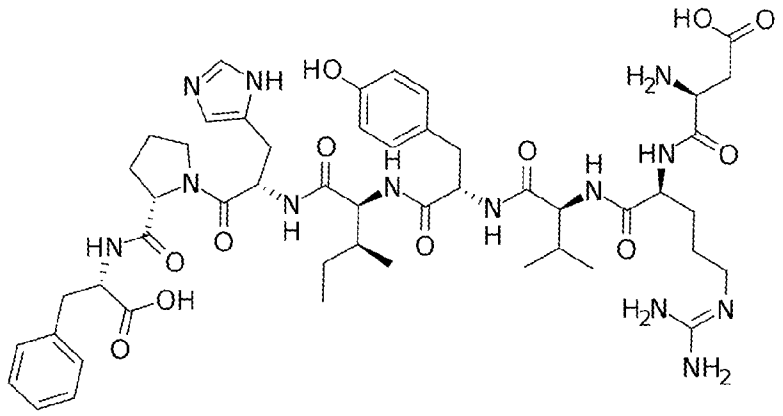
[0016] As used herein, the singular forms "a," "an," and "the" include plural reference.

[0017] As used herein, the term "HGF activator" refers to a substance that increases the rate of the reaction catalyzed by HGF. HGF is well known in the art, as described in, for example, T. Nakamura et al., *Proc., Jpn. Acad. Ser. B Phys. Biol. Sci.*, 86(6), 588-610, 2010.

[0018] For purposes of the present invention, at least one HGF activator being administered to the subject is an angiotensin. The angiotensin may be any of the known angiotensins, e.g., angiotensin I, II, III, or IV, or an analogue thereof, or a combination thereof. Typically, the

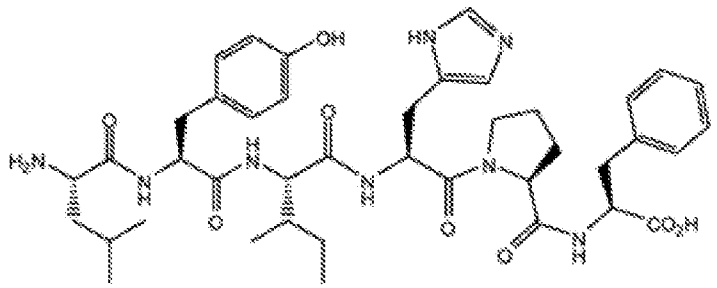
angiotensin is an oligopeptide containing six to ten peptide units (i.e., a hexapeptide, heptapeptide, octapeptide, nonapeptide, or decapeptide). The term "peptide unit" is herein meant to be synonymous with the term "amino acid". The term "angiotensin analogue" herein refers to an oligopeptide molecule containing at least two, three, four, five, six, seven, eight, nine, or ten of the peptides found in one of the angiotensins I-IV, along with either or both of: i) one, two, or three additional natural or synthetic peptide units (amino acids), and/or ii) one or more existing peptide units found in angiotensin I-IV is derivatized, such as by esterification or amidation of a terminal carboxy group or acylation of a terminal amino group or chemical modification of a peptide side chain (e.g., acylation of a tyrosine hydroxy group), wherein one or more additional peptide units may or may not be present.

[0019] In particular embodiments, the angiotensin is angiotensin II, having the following structure:



[0020] The angiotensin may also be an analogue of angiotensin II.

[0021] In other particular embodiments, the angiotensin is angiotensin IV, having the following structure:



[0022] The angiotensin may also be an analogue of angiotensin IV.

[0023] As used herein, the term "synaptogenesis" refers to a process involving the formation of synapses. As used herein, the term "synaptic networks" refer to a multiplicity of neurons and synaptic connections between the individual neurons. Synaptic networks may include extensive branching with multiple interactions. Synaptic networks can be recognized, for example, by confocal visualization, electron microscopic visualization, and electrophysiologic recordings.

[0024] The phrases "cognitive ability" and "cognitive function" are used interchangeably in this application and refer to cerebral activities that encompass, for example, reasoning, memory, attention, and language. These phrases also encompass mental processes, such as awareness, perception, reasoning, and judgment. In one example, these phrases refer to brain-based skills necessary to carry out any task from the simplest to the most complex, such as learning, remembering, problem-solving, and paying attention.

[0025] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce adverse reactions when administered to a subject. The pharmaceutically acceptable substance is typically approved by a regulatory agency or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term

"pharmaceutically acceptable carrier" generally refers to a chemical substance in which the active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject. The carrier can also be, for example, a diluent, adjuvant, excipient, or vehicle for the compound being administered.

[0026] The term "therapeutically effective amount" refers to an amount of a therapeutic agent that results in a measurable or observable therapeutic response, particularly an induction of synaptogenesis. A therapeutic response may include, for example, any response that a person of sound medical adjustment (e.g., a clinician or physician) will recognize as an effective response to the therapy, including improvement of symptoms and surrogate clinical markers. Thus, a therapeutic response can include a mitigation, amelioration, or inhibition of one or more symptoms of a disease or condition. A measurable therapeutic response also includes a finding that a symptom or disease is prevented or has a delayed onset, or is otherwise attenuated by the therapeutic agent.

[0027] The term "subject," as used herein, refers to a human or other mammal having or at risk of neuronal or synaptic loss. Some examples of mammals other than humans include dogs, cats, monkeys, and apes.

[0028] As used herein, the term "Alzheimer's disease" includes any of the stages of Alzheimer's disease, such as mild or early stage, moderate or middle stage, and severe or late-stage.

[0029] The terms "approximately" and "about" mean to be nearly the same as a referenced number or value including an acceptable degree of error for the quantity measured given the nature or precision of the measurements. As used herein, the terms "approximately" and

"about" should be generally understood to encompass ± 20% or ± 10% of a specified amount, frequency or value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated. For example, the term "about 20 μg" may be interpreted as precisely that amount or as being within a margin of 16-24 μg or 18-22 μg.

[0030] The terms "administer," "administration," or "administering," as used herein, refer to (1) providing, giving, dosing and/or prescribing by either a health practitioner or his/her authorized agent or under his/her direction a composition according to the disclosure, and (2) putting into, taking or consuming by the patient or person himself or herself, a composition according to the disclosure. As used herein, "administration" of a composition includes any route of administration, including oral, intravenous, subcutaneous, intraperitoneal, and intramuscular.

[0031] The phrase "weekly dosing regimen" is used when the subject is administered a dose of a therapeutic agent (drug) every week for a predetermined number of consecutive weeks. For example, the subject may receive a single dose of a therapeutic agent each week for three consecutive weeks.

[0032] The phrases "spaced dosing regimen" and "intermittent dosing regimen" are herein used interchangeably and refer to an on/off dosing regimen of a defined periodicity. In some embodiments, a spaced dosing regimen or intermittent dosing regimen may be used for administering the angiotensin to a subject. The spaced or intermittent dosing regimen may entail, for example, administering the angiotensin to the subject once a week for two or three consecutive weeks, followed by cessation of administration or dosing for two or three

consecutive weeks. In further embodiments, the administration may continue in alternating intervals of administering the angiotensin once a week for two or three consecutive weeks, followed by cessation of administration or dosing for two or three consecutive weeks, and continuing those alternating intervals over a period of about 4 months, about 8 months, about 1 year, about 2 years, about 5 years, or otherwise for the duration of therapy with the angiotensin.

[0033] The angiotensin may be administered according to any suitable dosing schedule or regimen. In some embodiments, the angiotensin may be administered in an amount ranging from about 0.01 $\mu g/m^2$ to about 100 $\mu g/m^2$. In different embodiments, the amount administered is precisely, about, up to, or less than 0.01 $\mu g/m^2$, 0.05 $\mu g/m^2$, 0.1 $\mu g/m^2$, 0.5 $\mu g/m^2$, 1 $\mu g/m^2$, 5 $\mu g/m^2$, 10 $\mu g/m^2$, 15 $\mu g/m^2$, 20 $\mu g/m^2$, 25 $\mu g/m^2$, 30 $\mu g/m^2$, 35 $\mu g/m^2$, 40 $\mu g/m^2$, 45 $\mu g/m^2$, 50 $\mu g/m^2$, 55 $\mu g/m^2$, 60 $\mu g/m^2$, 65 $\mu g/m^2$, 70 $\mu g/m^2$, 75 $\mu g/m^2$, 80 $\mu g/m^2$, 85 $\mu g/m^2$, 90 $\mu g/m^2$, 95 $\mu g/m^2$, or 100 $\mu g/m^2$, or an amount within a range bounded by any two of the foregoing amounts, e.g., 0.01-100 $\mu g/m^2$, 0.1-100 $\mu g/m^2$, 1-100 $\mu g/m^2$, 5-100 $\mu g/m^2$, 10-100 $\mu g/m^2$, 0.01-50 $\mu g/m^2$, 0.1-50 $\mu g/m^2$, 1-50 $\mu g/m^2$, 5-50 $\mu g/m^2$, 10-50 $\mu g/m^2$, 0.01-20 $\mu g/m^2$, 0.1-20 $\mu g/m^2$, 1-20 $\mu g/m^2$, 5-20 $\mu g/m^2$, or 10-20 $\mu g/m^2$. In particular embodiments, the amount may range from about 10 - 50 $\mu g/m^2$, or more particularly, about 15 $\mu g/m^2$, about 20 $\mu g/m^2$, about 25 $\mu g/m^2$, about 30 $\mu g/m^2$, about 35 $\mu g/m^2$, or about 40 $\mu g/m^2$, or about 45 $\mu g/m^2$, or about 50 $\mu g/m^2$, or an amount within a range bounded by any two of the foregoing values. Notably, any of the amounts above or below expressed as "$\mu g/m^2$" may alternatively be interpreted in terms of micrograms (µg) or micrograms per 50 kg body weight (µg/50 kg). For example, 25 $\mu g/m^2$ may be interpreted as 25 µg or 25 µg/50 kg.

[0034] In some embodiments, the angiotensin is administered as a dose in the range of about 0.01 to 100 μg/m^2/week. For example, the dose may be administered each week in a range of about 0.01 to about 25 μg/m^2/week; about 1 to about 20 μg/m^2/week, about 5 to about 20 μg/m^2/week, or about 10 to about 20 μg/m^2/week. In particular embodiments, the dose may be about or less than, for example, 5 μg/m^2/week, 10 μg/m^2/week, 15 μg/m^2/week, 20 μg/m^2/week, 25 μg/m^2/week, or 30 μg/m^2/week. Any of the foregoing dosages may be administered over a suitable time period, e.g., three weeks, four weeks, (approximately 1 month), two months, three months (approximately 12 or 13 weeks), four months, five months, six months, or a year. Notably, any of the amounts above or below expressed as "μg/m^2" may alternatively be interpreted in terms of micrograms (μg) or micrograms per 50 kg body weight (μg/50 kg).

[0035] In some embodiments, the angiotensin is administered in an amount of precisely or about 20 μg, 30 μg, or 40 μg (20 μg/m^2, 30 μg/m^2, or 40 μg/m^2) every week or every two weeks for a total period of time of, e.g., four weeks, (approximately 1 month), five weeks, six weeks, eight weeks, ten weeks, twelve weeks, four months, five months, six months, or a year. The administration may alternatively start with an initial single higher amount (e.g., 10%, 15%, 20%, or 25% higher amount than successive administrations). For example, in some embodiments, the angiotensin may be administered in an amount of precisely or about 15 μg, 24 μg, or 48 μg for the first week, or first two or three consecutive weeks, followed by administrations of 12 μg, 20 μg or 40 μg, respectively, every week or alternately every two or three weeks for at least four weeks (approximately 1 month), six weeks, eight weeks, ten weeks, twelve weeks, fifteen weeks, eighteen weeks, or for at least three months, four months, five months, six months, or a year. The term "alternately," as used herein, indicates a period of

time in which the angiotensin is not being administered. For example, "alternately every two or three weeks" indicates, respectively, regular one-week periods of no administration or regular two-week periods of no administration, also referred to herein as "1 on/1 off" and "1 on/2 off" dosing regimens, respectively. Other alternating dosing regimens are possible, including, for example, "2 on/1 off", "2 on/2 off", "1 on/3 off", "2 on/3 off", "3 on/3 off", "3 on/1 off", and "3 on/2 off". Notably, any of the amounts above or below expressed as μg may alternatively be interpreted in terms of $\mu g/m^2$ or micrograms per 50 kg body weight (μg/50 kg).

[0036] In a further aspect, the role of such intermittent dosing of an angiotensin on restoring or upregulating BDNF, increasing the postsynaptic density of the anchoring protein PSD-95, and lowering or preventing the downregulation of PCK-ε, is disclosed. BDNF is a peptide that is implicated to induce mitogenesis and improve cognitive function. Although evidence for BDNF polymorphisms in AD is still inconclusive, synaptic loss is the single most important correlate of AD. Lower BDNF levels are associated in AD cases with apathy, a noncognitive symptom common to many forms of dementia (Alvarez et al., Apathy and APOE4 are associated with reduced BDNF levels in Alzheimer's disease, *J. Alzheimers Dis.,* 42:1347–1355, 2014). While BDNF expression is regulated by at least nine promoters (Aid et al., Mouse and rat BDNF gene structure and expression revisited, *J. Neurosci. Res.;* 85:525–535, 2007; Pruunsild et al., Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters, *Genomics*, 90:397– 406, 2007), promoter IV (PIV) is most responsive to neuronal activity (Tao et al., Ca2 influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism, *Neuron,* 20:709 –726, 1998). PKCε, which is decreased in AD (Hongpaisan et al., PKC epsilon activation prevents synaptic loss, Abeta elevation, and cognitive deficits in Alzheimer's disease transgenic mice, *J. Neurosci.,* 31:630–

643, 2011; Khan et al., PKC-epsilon deficits in Alzheimer's disease brains and skin fibroblasts, *J. Alzheimers Dis.,* 43:491–509, 2015), also regulates BDNF expression (Lim and Alkon, 2012; Corbett et al., 2013; Hongpaisan et al., PKC activation during training restores mushroom spine synapses and memory in the aged rat, *Neurobiol. Dis.,* 55:44–62, 2013; Neumann et al., Increased BDNF protein expression after ischemic or PKC epsilon preconditioning promotes electrophysiologic changes that lead to neuroprotection, *J. Cereb. Blood Flow Metab.,* 35:121–130, 2015).

[0037] Other embodiments of the present disclosure are directed to a method for improving or enhancing cognitive ability of a subject, preventing or treating cognitive impairment of a subject in need thereof, treating or preventing a neurodegenerative disorder in a subject in need thereof, and/or preventing or treating a disease or condition associated with neuronal or synaptic loss in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an angiotensin. In some embodiments, the therapeutically effective amount of angiotensin is administered according to any suitable dosing schedule or regimen described. In some embodiments, administration of the angiotensin results in enhanced associative learning and increases the number of fully mature mushroom spine synapses. In other embodiments, administration of the angiotensin results in at least partial or full restoration of mature mushroom spines or mushroom spine synapses in a subject having a neurodegenerative disorder in which mushroom spine synapses have been deformed, such as in Fragile X Syndrome.

[0038] The subject may be in need of treatment for a neurodegenerative disorder, such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), dementia (e.g., frontotemporal dementia or vascular dementia), mild cognitive impairment, chronic traumatic

encephalopathy (CTE), traumatic brain injury, Fragile X, Niemann-Pick C, depression, bipolar disorder, ALS, schizophrenia, Post-Traumatic Stress Disorder, stroke, mental retardation, or brain injury.

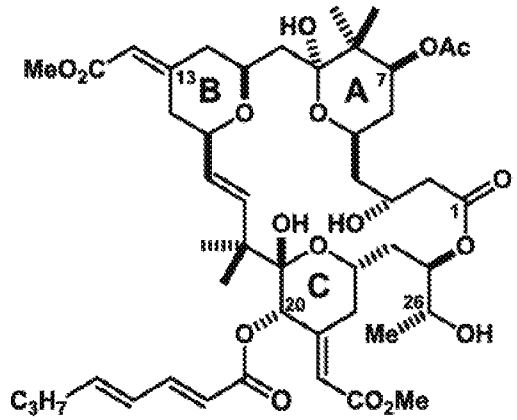
[0039] In particular embodiments, the method described herein is used to treat or prevent Alzheimer's Disease (AD) or a neurodegenerative disorder associated with or related to AD. In one embodiment, the subject has moderate-to-severe or severe (i.e., late-stage or advanced) AD. In another embodiment, the subject has early stage AD. In another embodiment, the subject is not diagnosed with AD, but is deemed at-risk for AD by exhibiting certain cognitive changes or deficits that indicate a reasonable likelihood of developing AD. The method described herein may also be used to treat or prevent a range of other neurodegenerative diseases, such as any of those mentioned earlier above. The method may be used preventatively for any of these neurodegenerative diseases for a subject that has been determined to be at risk for any of these neurodegenerative diseases.

[0040] In some embodiments, the angiotensin is combined with a PKC activator or HGF activator. The PKC activator or HGF activator may be selected from, for example, macrocyclic lactones, bryologs, diacylglycerols, isoprenoids, octylindolactam, gnidimacrin, ingenol, iripallidal, napthalenesulfonamides, diacylglycerol inhibitors, growth factors, polyunsaturated fatty acids, monounsaturated fatty acids, cyclopropanated polyunsaturated fatty acids, cyclopropanated monounsaturated fatty acids, fatty acid alcohols and derivatives, and fatty acid esters. In particular embodiments, the PKC activator or HGF activator is a macrocyclic lactone selected from bryostatins and neristatin, such as neristatin-1. In a further embodiment, the PKC activator or HGF activator is a bryostatin, such as bryostatin-1, bryostatin-2, bryostatin-3, bryostatin-4, bryostatin-5, bryostatin-6, bryostatin-7, bryostatin-8, bryostatin-9, bryostatin-10,

bryostatin-11, bryostatin-12, bryostatin-13, bryostatin-14, bryostatin-15, bryostatin-16, bryostatin-17, or bryostatin-18. In a further embodiment, the PKC activator or HGF activator is bryostatin-1. The PKC activator or HGF activator can be administered in any of the amounts or dosing regimens described above for the angiotensin. In one embodiment, the therapeutically effective amount of the PKC activator or HGF activator, such as bryostatin-1, is about 25 $\mu g/m^2$.

[0041] In some embodiments, the PKC activator or HGF activator is a macrocyclic lactone. Macrocyclic lactones (also known as macrolides) generally comprise 14-, 15-, or 16-membered lactone rings. Macrolides belong to the polyketide class of natural products. Macrocyclic lactones and derivatives thereof are described, for example, in U.S. Patent Nos. 6,187,568; 6,043,270; 5,393,897; 5,072,004; 5,196,447; 4,833,257; and 4,611,066; and 4,560,774; each incorporated by reference herein in its entirety. Those patents describe various compounds and various uses for macrocyclic lactones including their use as an anti-inflammatory or anti-tumor agents. *See also* Szallasi et al. *J. Biol. Chem.* (1994), vol. 269, pp. 2118-2124; Zhang et al., *Cancer Res.* (1996), vol. 56, pp. 802-808; Hennings et al. *Carcinogenesis* (1987), vol. 8, pp. 1343-1346; Varterasian et al. *Clin. Cancer Res.* (2000), vol. 6, pp. 825-828; Mutter et al. *Bioorganic & Med. Chem.* (2000), vol. 8, pp. 1841-1860; each incorporated by reference herein in its entirety. In particular embodiments, the macrocyclic lactone is a bryostatin. Bryostatins include, for example, Bryostatin-1, Bryostatin-2, Bryostatin-3, Bryostatin-4, Bryostatin-5, Bryostatin-6, Bryostatin-7, Bryostatin-8, Bryostatin-9, Bryostatin-10, Bryostatin-11, Bryostatin-12, Bryostatin-13, Bryostatin-14, Bryostatin-15, Bryostatin-16, Bryostatin-17, and Bryostatin-18.

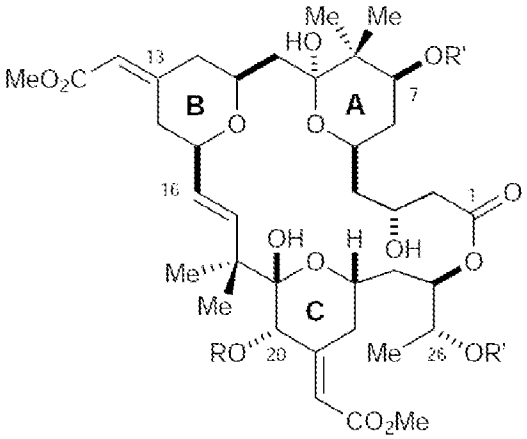
[0042] In one embodiment, the bryostatin is Bryostatin-1 (shown below).



Bryostatin 1

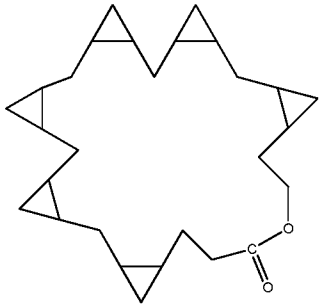
Ki = 1.35 nM

[0043] In another embodiment, the bryostatin is Bryostatin-2 (shown below; R = COC_7H_{11}, R' = H).



[0044] In another embodiment, the macrocyclic lactone is a neristatin, such as neristatin-1. In another embodiment, the macrocyclic lactone is selected from macrocyclic derivatives of

cyclopropanated PUFAs such as, 24-octaheptacyclononacosan-25-one (cyclic DHA-CP6) (shown below).



[0045] In another embodiment, the macrocyclic lactone is a bryolog, wherein bryologs are analogues of bryostatin. Bryologs can be chemically synthesized or produced by certain bacteria. Different bryologs exist that modify, for example, the rings A, B, and C (see Bryostatin-1, figure shown above) as well as the various substituents. As a general overview, bryologs are considered less specific and less potent than bryostatin but are easier to prepare.

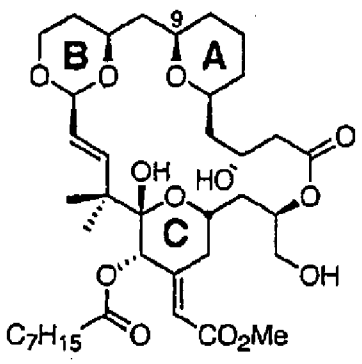
[0046] Table 1 summarizes structural characteristics of several bryologs and their affinity for PKC (ranging from 0.25 nM to 10 μM). While Bryostatin-1 has two pyran rings and one 6-membered cyclic acetal, in most bryologs one of the pyrans of Bryostatin-1 is replaced with a second 6-membered acetal ring. This modification may reduce the stability of bryologs, relative to Bryostatin-1, for example, in either strong acid or base, but has little significance at physiological pH. Bryologs also tend to have a lower molecular weight (ranging from about 600 g/mol to 755 g/mol), as compared to Bryostatin-1 (988), a property which may facilitate transport across the blood-brain barrier.

Table 1: Bryologs

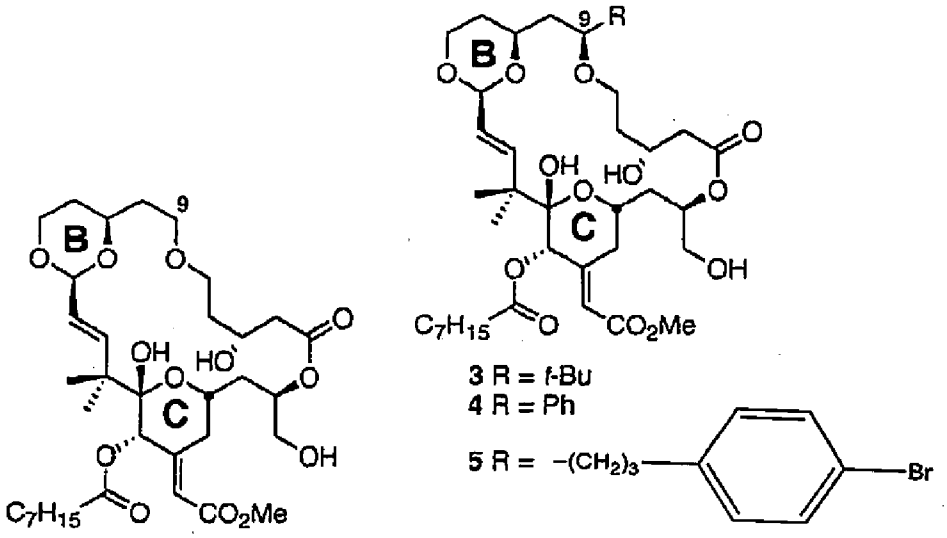
Name	PKC Affin (nM)	MW	Description
Bryostatin-1	1.35	988	2 pyran + 1 cyclic acetal + macrocycle
Analog 1	0.25	737	1 pyran + 2 cyclic acetal + macrocycle
Analog 2	6.50	723	1 pyran + 2 cyclic acetal + macrocycle
Analog 7a	-	642	1 pyran + 2 cyclic acetals + macrocycle
Analog 7b	297	711	1 pyran + 2 cyclic acetals + macrocycle
Analog 7c	3.4	726	1 pyran + 2 cyclic acetals + macrocycle
Analog 7d	10000	745	1 pyran + 2 cyclic acetals + macrocycle, acetylated
Analog 8	8.3	754	2 cyclic acetals + macrocycle
Analog 9	10000	599	2 cyclic acetals

[0047] Analog 1 exhibits the highest affinity for PKC. Wender et al., *Curr. Drug Discov. Technol.* (2004), vol. 1, pp. 1-11; Wender et al. *Proc. Natl. Acad. Sci.* (1998), vol. 95, pp. 6624-6629; Wender et al., *J. Am. Chem. Soc.* (2002), vol. 124, pp. 13648-13649, each incorporated

by reference herein in their entireties. Only Analog 1 exhibits a higher affinity for PKC than Bryostatin-1. Analog 2, which lacks the A ring of Bryostatin-1, is the simplest analog that maintains high affinity for PKC. In addition to the active bryologs, Analog 7d, which is acetylated at position 26, has virtually no affinity for PKC.

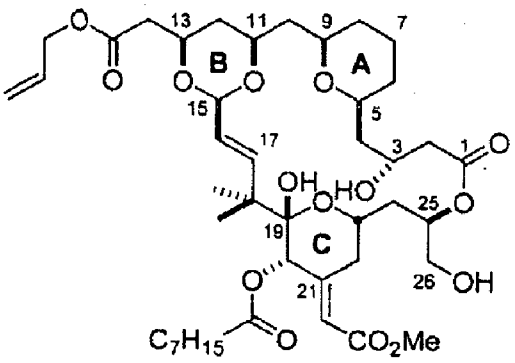


Analogue 1: K_i = 0.25 nM

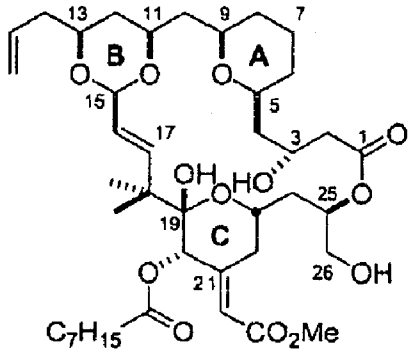


Analogue 2: K_i = 8.0 nM

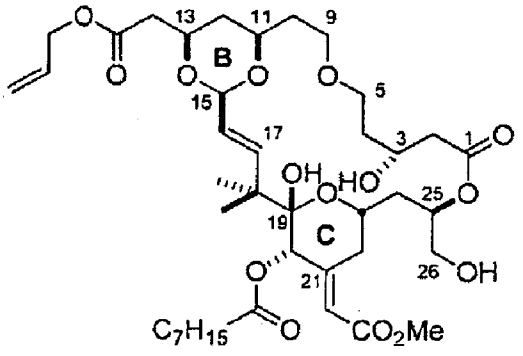
[0048] B-ring bryologs may also be used in the present disclosure. These synthetic bryologs have affinities in the low nanomolar range. Wender et al., *Org Lett.* (2006), vol. 8, pp. 5299-5302, incorporated by reference herein in its entirety. B-ring bryologs have the advantage of being completely synthetic, and do not require purification from a natural source.



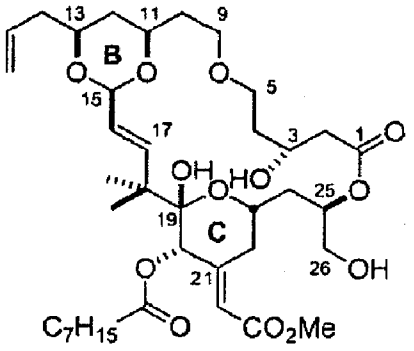
3: PKC K_i = 1.2 ± 0.6 nM



4: PKC K_i = 0.67 ± 0.5 nM



5: PKC K_i = 3.0 ± 0.5 nM



6: PKC K_i = 2.6 ± 0.5 nM

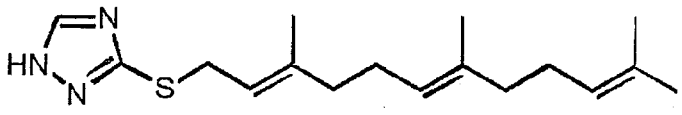
[0049] A third class of suitable bryostatin analogs are the A-ring bryologs. These bryologs have slightly lower affinity for PKC than Bryostatin-1 (6.5 nM, 2.3 nM, and 1.9 nM for bryologs 3, 4, and 5, respectively) and a lower molecular weight. A-ring substituents are important for non-tumorigenesis.

[0050] Bryostatin analogs are described, for example, in U.S. Patent Nos. 6,624,189 and 7,256,286. Methods using macrocyclic lactones to improve cognitive ability are also described in U.S. Patent No. 6,825,229 B2.

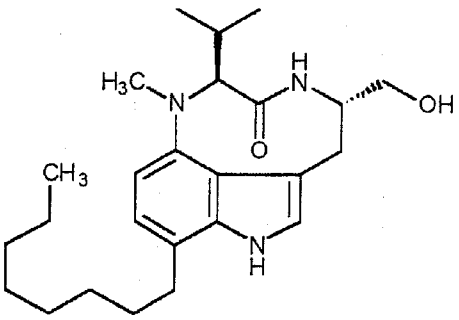
[0051] The PKC activator or HGF activator may also include derivatives of diacylglycerols (DAGs). *See, e.g.,* Niedel et al., *Proc. Natl. Acad. Sci.* (1983), vol. 80, pp. 36-40; Mori et al., *J. Biochem.* (1982), vol. 91, pp. 427-431; Kaibuchi et al., *J. BioI. Chem.* (1983), vol. 258, pp. 6701-6704. The fatty acid substitution on the diacylglycerol derivatives may determine the strength of activation. Diacylglycerols having an unsaturated fatty acid may be most active. The stereoisomeric configuration is important; fatty acids with a 1,2-sn configuration may be active while 2,3-sn-diacylglycerols and 1,3-diacylglycerols may not bind to HGF or PKC. Cis-unsaturated fatty acids may be synergistic with diacylglycerols. In some embodiments, the PKC activator or HGF activator excludes DAG or DAG derivatives.

[0052] The PKC activator or HGF activator may also include isoprenoids. Farnesyl thiotriazole, for example, is a synthetic isoprenoid that activates PKC with a K_d of 2.5 μM. Farnesyl thiotriazole, for example, is equipotent with dioleoylglycerol, but does not possess hydrolyzable esters of fatty acids. Gilbert et al., *Biochemistry* (1995), vol. 34, pp. 3916-3920; incorporated by reference herein in its entirety. Farnesyl thiotriazole and related compounds

represent a stable, persistent PKC activator. Because of its low molecular weight (305.5 g/mol) and absence of charged groups, farnesyl thiotriazole may readily cross the blood-brain barrier.



[0053] Yet other types of the PKC activator or HGF activator include octylindolactam V, gnidimacrin, and ingenol. Octylindolactam V is a non-phorbol protein kinase C activator related to teleocidin. The advantages of octylindolactam V (specifically the (-)-enantiomer) may include greater metabolic stability, high potency (EC_{50} = 29 nM) and low molecular weight that facilitates transport across the blood brain barrier. Fujiki et al. *Adv. Cancer Res.* (1987), vol. 49 pp. 223-264; Collins et al. *Biochem. Biophys. Res. Commun.* (1982), vol. 104, pp. 1159-4166, each incorporated by reference herein in its entirety.

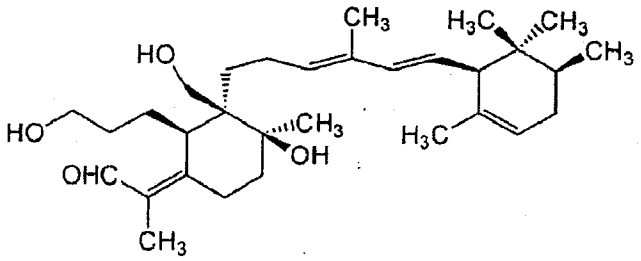


(7-octylindolactam V)

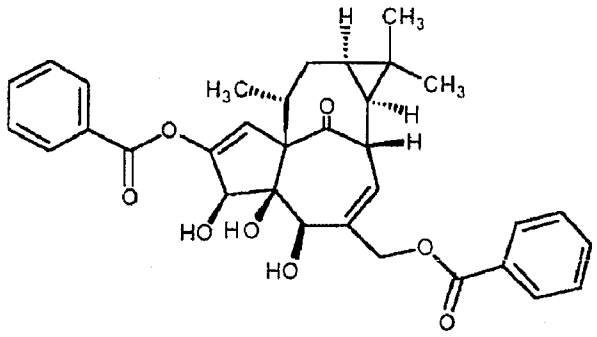
[0054] Gnidimacrin is a daphnane-type diterpene that displays potent antitumor activity at concentrations of 0.1 nM - 1 nM against murine leukemias and solid tumors. It may act as a HGF or PKC activator at a concentration of 0.3 nM in K562 cells, and regulate cell cycle progression at the G1/S phase through the suppression of Cdc25A and subsequent inhibition of cyclin-dependent kinase 2 (Cdk2) (100% inhibition achieved at 5 ng/ml). Gnidimacrin is a

heterocyclic natural product similar to Bryostatin-1, but somewhat smaller (MW = 774.9 g/mol).

[0055] Iripallidal is a bicyclic triterpenoid isolated from Iris pallida. Iripallidal displays anti-proliferative activity in a NCI 60 cell line screen with GI_{50} (concentration required to inhibit growth by 50%) values from micromolar to nanomolar range. It binds to PKCα with high affinity (K_i = 75.6 nM). It may induce phosphorylation of Erk1/2 in a RasGRP3-dependent manner. Its molecular weight is 486.7 g/mol. Iripallidal is about half the size of Bryostatin-1 and lacks charged groups.



[0056] Ingenol is a diterpenoid related to phorbol but less toxic. It is derived from the milkweed plant Euphorbia peplus. Ingenol 3,20-dibenzoate, for example, competes with [3H] phorbol dibutyrate for binding to PKC (K_i = 240 nM). Winkler et al., *J. Org. Chem.* (1995), vol. 60, pp. 1381-1390, incorporated by reference herein. Ingenol-3-angelate exhibits antitumor activity against squamous cell carcinoma and melanoma when used topically. Ogbourne et al. *Anticancer Drugs* (2007), vol. 18, pp. 357-362, incorporated by reference herein.



[0057] The PKC activator or HGF activator may also include the class of napthalenesulfonamides, including N-(n-heptyl)-5-chloro-1-naphthalenesulfonamide (SC-10) and N-(6-phenylhexyl)-5-chloro-1-naphthalenesulfonamide. SC-10 may activate PKC in a calcium-dependent manner, using a mechanism similar to that of phosphatidylserine. Ito et al., *Biochemistry* (1986), vol. 25, pp. 4179-4184, incorporated by reference herein. Naphthalenesulfonamides act by a different mechanism than bryostatin and may show a synergistic effect with bryostatin or member of another class of HGF activators. Structurally, naphthalenesulfonamides are similar to the calmodulin (CaM) antagonist W-7, but are reported to have no effect on CaM kinase.

[0058] The PKC activator or HGF activator may also include the class of diacylglycerol kinase inhibitors, which indirectly activate PKC. Examples of diacylglycerol kinase inhibitors include, but are not limited to, 6-(2-(4-[(4-fluorophenyl)phenylmethylene]-1-piperidinyl)ethyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (R59022) and [3-[2-[4-(bis-(4-fluorophenyl)methylene]piperidin-1-yl)ethyl]-2,3-dihydro-2-thioxo-4(1H)-quinazolinone (R59949).

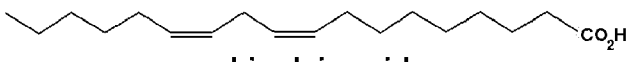
[0059] The PKC activator or HGF activator may also be a growth factor, such as fibroblast growth factor 18 (FGF-18) and insulin growth factor, which function through the PKC

pathway. FGF-18 expression is up-regulated in learning, and receptors for insulin growth factor have been implicated in learning. Activation of the PKC signaling pathway by these or other growth factors offers an additional potential means of activating PKC.

[0060] The PKC activator or HGF activator may also include hormones and growth factor activators, including 4-methyl catechol derivatives, such as 4-methylcatechol acetic acid (MCBA), which stimulate the synthesis and/or activation of growth factors, such as NGF and BDNF, which also activate PKC as well as convergent pathways responsible for synaptogenesis and/or neuritic branching.

[0061] The PKC activator or HGF activator (being combined with an angiotensin) may also include polyunsaturated fatty acids ("PUFAs"). PUFAs are essential components of the nervous system and have numerous health benefits. In general, PUFAs increase membrane fluidity, rapidly oxidize to highly bioactive products, produce a variety of inflammatory and hormonal effects, and are rapidly degraded and metabolized. The inflammatory effects and rapid metabolism is likely the result of their active carbon-carbon double bonds.

[0062] In one embodiment, the PUFA is selected from linoleic acid (shown below).



Linoleic acid

[0063] The PKC activator or HGF activator may also be a PUFA or MUFA derivative. In particular embodiments, the PUFA or MUFA derivative is a cyclopropanated derivative. Certain cyclopropanated PUFAs, such as DCPLA (i.e., linoleic acid with cyclopropane at both double bonds), may be able to selectively activate HGF or PKC-ε. *See Journal of Biological*

Chemistry, 2009, 284(50): 34514-34521; *see also* U.S. Patent Application Publication No. 2010/0022645 A1. Like their parent molecules, PUFA derivatives are thought to activate PKC by binding to the PS site.

[0064] Cyclopropanated fatty acids exhibit low toxicity and are readily imported into the brain where they exhibit a long half-life ($t_{1/2}$). Conversion of the double bonds into cyclopropane rings prevents oxidation and metabolism to inflammatory byproducts and creates a more rigid U-shaped 3D structure that may result in greater HGF or PKC activation. Moreover, this U-shape may result in greater isoform specificity. For example, cyclopropanated fatty acids may exhibit potent and selective activation of HGF or PKC-ε.

[0065] The Simmons-Smith cyclopropanation reaction is an efficient way of converting double bonds to cyclopropane groups. This reaction, acting through a carbenoid intermediate, preserves the *cis*-stereochemistry of the parent molecule. Thus, the HGF-activating properties are increased while metabolism into other molecules, such as bioreactive eicosanoids, thromboxanes, or prostaglandins, is prevented.

[0066] A particular class of HGF-activating fatty acids is Omega-3 PUFA derivatives. In at least one embodiment, the Omega-3 PUFA derivatives are selected from cyclopropanated docosahexaenoic acid, cyclopropanated eicosapentaenoic acid, cyclopropanated rumelenic acid, cyclopropanated parinaric acid, and cyclopropanated linolenic acid (CP3 form shown below).



[0067] Another class of HGF-activating fatty acids is Omega-6 PUFA derivatives. In at least one embodiment, the Omega-6 PUFA derivatives are selected from cyclopropanated linoleic acid ("DCPLA," CP2 form shown below),



cyclopropanated arachidonic acid, cyclopropanated eicosadienoic acid, cyclopropanated dihomo-gamma-linolenic acid, cyclopropanated docosadienoic acid, cyclopropanated adrenic acid, cyclopropanated calendic acid, cyclopropanated docosapentaenoic acid, cyclopropanated jacaric acid, cyclopropanated pinolenic acid, cyclopropanated podocarpic acid, cyclopropanated tetracosatetraenoic acid, and cyclopropanated tetracosapentaenoic acid.

[0068] Vernolic acid is a naturally occurring compound. However, it is an epoxyl derivative of linoleic acid and therefore, as used herein, is considered an Omega-6 PUFA derivative. In addition to vernolic acid, cyclopropanated vernolic acid (shown below) is an Omega-6 PUFA derivative.



[0069] Another class of HGF-activating fatty acids is Omega-9 PUFA derivatives. In at least one embodiment, the Omega-9 PUFA derivatives are selected from cyclopropanated eicosenoic acid, cyclopropanated mead acid, cyclopropanated erucic acid, and cyclopropanated nervonic acid.

[0070] Yet another class of HGF-activating fatty acids is monounsaturated fatty acid ("MUFA") derivatives. In at least one embodiment, the MUFA derivatives are selected from cyclopropanated oleic acid (shown below),



[0071] and cyclopropanated elaidic acid (shown below).



[0072] HGF-activating MUFA derivatives include epoxylated compounds such as trans-9,10-epoxystearic acid (shown below).



[0073] Another class of HGF-activating fatty acids is Omega-5 and Omega-7 PUFA derivatives. In at least one embodiment, the Omega-5 and Omega-7 PUFA derivatives are selected from cyclopropanated rumenic acid, cyclopropanated alpha-elostearic acid, cyclopropanated catalpic acid, and cyclopropanated punicic acid.

[0074] Another class of HGF activators is fatty acid alcohols and derivatives thereof, such as cyclopropanated PUFA and MUFA fatty alcohols. It is thought that these alcohols activate

PKC by binding to the PS site. These alcohols can be derived from different classes of fatty acids.

[0075] In at least one embodiment, the HGF-activating fatty alcohols are derived from Omega-3 PUFAs, Omega-6 PUFAs, Omega-9 PUFAs, and MUFAs, especially the fatty acids noted above. In at least one embodiment, the fatty alcohol is selected from cyclopropanated linolenyl alcohol (CP3 form shown below),



[0076] cyclopropanated linoleyl alcohol (CP2 form shown below),



[0077] cyclopropanated elaidic alcohol (shown below),



[0078] cyclopropanated DCPLA alcohol, and cyclopropanated oleyl alcohol.

[0079] Another class of HGF activators includes fatty acid esters and derivatives thereof, such as cyclopropanated PUFA and MUFA fatty esters. In at least one embodiment, the cyclopropanated fatty esters are derived from Omega-3 PUFAs, Omega-6 PUFAs, Omega-9 PUFAs, MUFAs, Omega-5 PUFAs, and Omega-7 PUFAs. These compounds are thought to activate PKC through binding on the PS site. One advantage of such esters is that they are generally considered to be more stable that their free acid counterparts.

[0080] In one embodiment, the HGF-activating fatty acid esters derived from Omega-3 PUFAs are selected from cyclopropanated eicosapentaenoic acid methyl ester (CP5 form shown below)



[0081] and cyclopropanated linolenic acid methyl ester (CP3 form shown below).



[0082] In another embodiment, the Omega-3 PUFA esters are selected from esters of DHA-CP6 and aliphatic and aromatic alcohols. In at least one embodiment, the ester is cyclopropanated docosahexaenoic acid methyl ester (CP6 form shown below).



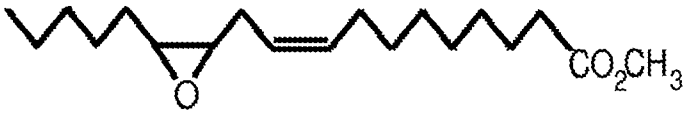
[0083] In one embodiment, HGF-activating fatty esters derived from Omega-6 PUFAs are selected from cyclopropanated arachidonic acid methyl ester (CP4 form shown below),



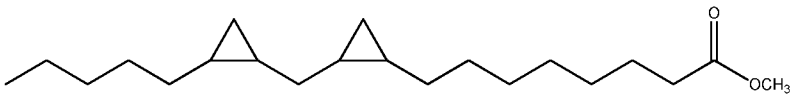
[0084] cyclopropanated vernolic acid methyl ester (CP1 form shown below),



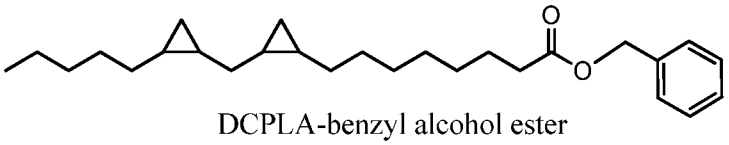
[0085] and vernolic acid methyl ester (shown below).



[0086] In particular embodiments, the PKC activator or HGF activator is an ester derivative of DCPLA (CP6-linoleic acid). In one embodiment, the ester of DCPLA is an alkyl ester. The alkyl group of the DCPLA alkyl esters may be linear, branched, and/or cyclic. The alkyl groups may be saturated or unsaturated. When the alkyl group is an unsaturated cyclic alkyl group, the cyclic alkyl group may be aromatic. The alkyl group may be selected from, for example, methyl, ethyl, propyl (e.g., isopropyl), and butyl (e.g., tert-butyl) esters. DCPLA in the methyl ester form ("DCPLA-ME") is shown below.



[0087] In another embodiment, the esters of DCPLA are derived from a benzyl alcohol (unsubstituted benzyl alcohol ester shown below). In yet another embodiment, the esters of DCPLA are derived from aromatic alcohols such as phenols used as antioxidants and natural phenols with pro-learning ability. Some specific examples include estradiol, butylated hydroxytoluene, resveratrol, polyhydroxylated aromatic compounds, and curcumin.



DCPLA-benzyl alcohol ester

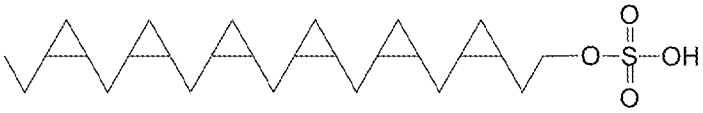
[0088] Another class of PKC activator or HGF activator includes fatty esters derived from cyclopropanated MUFAs. In at least one embodiment, the cyclopropanated MUFA ester is selected from cyclopropanated elaidic acid methyl ester (shown below),



[0089] and cyclopropanated oleic acid methyl ester (shown below).



[0090] Another class of PKC activator or HGF activator includes sulfates and phosphates derived from PUFAs, MUFAs, and their derivatives. In at least one embodiment, the sulfate is selected from DCPLA sulfate and DHA sulfate (CP6 form shown below).



[0091] In one embodiment, the phosphate is selected from DCPLA phosphate and DHA phosphate (CP6 form shown below).



[0092] The angiotensin (and, if present, PKC or HGF activator) according to the present disclosure may be administered to a patient/subject in need thereof by conventional methods, such as oral, parenteral, transmucosal, intranasal, inhalation, or transdermal administration. Parenteral administration includes intravenous, intra-arteriolar, intramuscular, intradermal, subcutaneous, intraperitoneal, intraventricular, intrathecal, ICV, intracisternal injections or infusions and intracranial administration. A suitable route of administration may be chosen to permit crossing the blood-brain barrier. *See e.g., J. Lipid Res.* (2001) vol. 42, pp. 678-685, incorporated by reference herein.

[0093] The angiotensin and/or PKC and/or HGF activator can be compounded into a pharmaceutical composition suitable for administration to a subject using general principles of pharmaceutical compounding. In one aspect, the pharmaceutically acceptable composition comprises an angiotensin and a pharmaceutically acceptable carrier.

[0094] The formulations of the compositions described herein may be prepared by any suitable method known in the art. In general, such preparatory methods include bringing at least one of the active ingredients into association with a carrier. If necessary or desirable, the resultant product can be shaped or packaged into a desired single- or multi-dose unit.

[0095] As discussed herein, carriers include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin;

aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other additional ingredients that may be included in the compositions of the disclosure are generally known in the art and may be described, for example, in *Remington's Pharmaceutical Sciences*, Genaro, ed., Mack Publishing Co., Easton, Pa., 1985, and *Remington's Pharmaceutical Sciences*, 20th Ed., Mack Publishing Co. 2000, both incorporated by reference herein.

[0096] In at least one embodiment, the carrier is an aqueous or hydrophilic carrier. In a further embodiment, the carrier can be water, saline, or dimethylsulfoxide. In another embodiment, the carrier is a hydrophobic carrier. Hydrophobic carriers include inclusion complexes, dispersions (such as micelles, microemulsions, and emulsions), and liposomes. Exemplary hydrophobic carriers include inclusion complexes, micelles, and liposomes. *See, e.g.*, Remington's: The Science and Practice of Pharmacy 20th ed., ed. Gennaro, Lippincott: Philadelphia, PA 2003, incorporated by reference herein. In addition, other compounds may be included either in the hydrophobic carrier or the solution, e.g., to stabilize the formulation.

[0097] In some embodiments, the compositions described herein may be formulated into oral dosage forms. For oral administration, the composition may be in the form of a tablet or capsule prepared by conventional means with, for example, carriers such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch

glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets may be coated by methods generally known in the art.

[0098] In another embodiment, the compositions herein are formulated into a liquid preparation. Such preparations may be in the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means using pharmaceutically acceptable carriers, such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl p-hydroxybenzoates, or sorbic acid). The preparations may also comprise buffer salts, flavoring, coloring, and sweetening agents as appropriate. In some embodiments, the liquid preparation is specifically designed for oral administration.

[0099] In another embodiment of the present disclosure, the compositions herein may be formulated for parenteral administration such as bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules, or in multi-dose containers, with an added preservative. The composition may be in the form of a suspension, solution, dispersion, or emulsion in oily or aqueous vehicles, and may contain a formulary agent, such as a suspending, stabilizing, and/or dispersing agent.

[00100] In another embodiment, the compositions herein may be formulated as depot preparations. Such formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. For example, the

compositions may be formulated with a suitable polymeric or hydrophobic material (for example, as an emulsion in an acceptable oil) or ion exchange resin, or as a sparingly soluble derivative, for example, as a sparingly soluble salt.

[00101] In another embodiment, at least one angiotensin (and, if present, a PKC and/or HGF activator) is delivered in a vesicle, such as a micelle, liposome, or an artificial low-density lipoprotein (LDL) particle. *See, e.g.*, U.S. Patent No. 7,682,627, the contents of which are herein incorporated by reference.

[00102] In some embodiments, at least one angiotensin (and, if present, a PKC and/or HGF activator) may be present in the pharmaceutical composition in an amount ranging from about 0.01% to about 100%, from about 0.1% to about 95%, from about 0.1% to about 90%, from about 0.1% to about 60%, from about 0.1% to about 30% by weight, from about 1% to about 90%, from about 1% to about 10% by weight, from about 5% to about 85%, from about 10% to about 80%, or from about 25% to about 75% of the final formulation.

[00103] The present disclosure further relates to kits that may be utilized for administering to a subject an angiotensin (and, if present, a PKC and/or HGF activator) according to the present disclosure. The kits may comprise devices for storage and/or administration. For example, the kits may comprise syringe(s), needle(s), needle-less injection device(s), sterile pad(s), swab(s), vial(s), ampoule(s), cartridge(s), bottle(s), and the like. The storage and/or administration devices may be graduated to allow, for example, measuring volumes. In at least one embodiment, the kit comprises at least one angiotensin and/or PKC and/or HGF activator in a container separate from other components in the system.

[00104] The kits may also comprise one or more anesthetics, such as local anesthetics. In at least one embodiment, the anesthetics are in a ready-to-use formulation, for example an injectable formulation (optionally in one or more pre-loaded syringes), or a formulation that may be applied topically. Topical formulations of anesthetics may be in the form of an anesthetic applied to a pad, swab, towelette, disposable napkin, cloth, patch, bandage, gauze, cotton ball, Q-tip™, ointment, cream, gel, paste, liquid, or any other topically applied formulation. Anesthetics for use with the present disclosure may include, but are not limited to lidocaine, marcaine, cocaine, and xylocaine.

[0100] The kits may also contain instructions relating to the use of at least one angiotensin and/or PKC and/or HGF activator. In another embodiment, the kit may contain instructions relating to procedures for mixing, diluting, or preparing formulations of at least one angiotensin and/or PKC and/or HGF activator. The instructions may also contain directions for properly diluting a formulation of at least one angiotensin and/or PKC and/or HGF activator in order to obtain a desired pH or range of pHs and/or a desired specific activity and/or protein concentration after mixing but prior to administration. The instructions may also contain dosing information. The instructions may also contain material directed to methods for selecting subjects for treatment with at least one angiotensin and/or PKC and/or HGF activator.

[0101] The angiotensin and/or PKC and/or HGF activator can be formulated in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles appropriate for each route of administration. Pharmaceutical compositions may further comprise other therapeutically active compounds approved for the treatment of neurodegenerative diseases or to reduce the risk of developing a neurodegenerative disorder.

[0102] All of the references, patents and printed publications mentioned in the instant disclosure are hereby incorporated by reference in their entirety into this application.

[0103] The following examples are provided by way of illustration to further describe certain preferred embodiments of the invention, and are not intended to be limiting of the present disclosure.

EXAMPLES

[0104] Mouse studies may be performed using an angiotensin, as described above, in accordance with the protocol described below. The following metrics may be used to evaluate dosing regimens: induction of brain postsynaptic anchoring protein PSD-95, upregulation of BDNF levels in brain, upregulation of HGF levels in brain, minimal downregulation of PKC-ε levels, and elevation of brain and plasma concentrations of the angiotensin. Groups of 2-3 mice may be formed and housed in an approved research animal facility. Water may be given *ad libitum.* A first study involves three groups of mice with animals in each group dosed weekly for 1, 2, 3, or 6 consecutive weeks. Each group has its own control group containing the same number of mice. For example, mice in the first, second and third groups may receive an intravenous (i.v.) injection of 10 $\mu g/m^2$, 15 $\mu g/m^2$, and 25 $\mu g/m^2$ dose of an angiotensin. For each dose, mice in that group may receive a single injection of the angiotensin weekly for a predetermined number of consecutive weeks. Following dosing, mice are sacrificed, and the blood and brain of each animal is collected for further analysis.

[0105] A dose of 10 $\mu g/m^2$, (i.v. administration) of angiotensin for 3 or 6 consecutive weeks may not result in elevated levels of brain BDNF. While some increase in the levels of brain BDNF may be observed at a dose of 15 $\mu g/m^2$ for three consecutive weeks, the maximum

increase in brain BDNF levels may be observed at a dose of 25 μg/m^2. At a dose of 25 μg/m^2, the levels of brain BDNF may increase with each successive week of dosing, that is, brain BDNF levels may be greatest after three consecutive weeks of dosing.

[0106] A similar observation can be made concerning the levels of the synaptogenesis marker PSD-95. Brain and blood samples of study subjects may show higher amounts of PSD-95 after three weeks at a dose of 25 μg/m^2 angiotensin, administered as i.v. as a once per week injection. In addition, 25 μg/m^2 administered in three consecutive weekly doses may not produce more PKC-ε downregulation in brain compared to three consecutive weeks of lower doses. Continued weekly dosing at 10 μg/m^2 for another three consecutive weeks (total of 6 consecutive weeks) may result in downregulation.

[0107] Although 25 μg/m^2 administered in three consecutive weekly doses may not produce more PKC-ε downregulation in brain than three consecutive weeks of lower doses, with continued dosing at this higher level, additional downregulation may occur for the "1 on/1 off" and "2 on/1 off" regimens. Since PKC-ε is a biological target of angiotensin, lower levels of this protein may result in decline in cognitive benefits in AD patients. In some embodiments, mice are dosed weekly with an angiotensin at 25 μg/m^2 for three consecutive weeks, followed by cessation of drug administration for three consecutive weeks, and then a second round of dosing at 25 μg/m^2 for an additional three consecutive weeks (that is, a "3 on/3 off/3 on" dosing regimen). In other embodiments, mice are dosed at 25 μg/m^2 at a "1 on/1 off" regimen for a total of nine weeks (e.g., one dose of angiotensin on weeks 1, 3, 5, 7, and 9, with no dosing in weeks 2, 4, 6, and 8). In other embodiments, mice are dosed at 25 μg/m^2 for another regimen starting with "2 on/1 off" immediately followed by alternating "1 on/1 off" until reaching the ninth total week (i.e., one dose of angiotensin on weeks 1, 2, 4, 6, 8, with no dosing in weeks

3, 5, 7, and 9). Increasing the duration of the rest intervals (i.e., "off" intervals) to three weeks may significantly reduce PKC downregulation. That is, the "3 on/3 off" dosing regimen may increase brain PKC-ε levels in mice over the other regimens, thus resulting in optimal cognitive benefits.

[0110] Brain BDNF in mice may reach its highest level after three consecutive weekly doses of angiotensin at 25 $\mu g/m^2$ and remain elevated after three additional consecutive weeks of no dosing, followed by three more consecutive weekly doses at 25 $\mu g/m^2$. Since BDNF is a peptide that induces synaptogenesis (i.e., the formation of new synapses), a "3 on/3 off" regimen may maximize synaptogenesis and minimize PKC downregulation.

[0111] Further evaluation may be performed on angiotensin crossing the blood-brain-barrier (BBB) and the steady state levels of angiotensin in the brain and plasma of mice. In some embodiments, angiotensin administered intravenously crosses the BBB. In that case, the concentration of angiotensin in mice brain may be less than its concentration in plasma. However, the concentration in brain may be no less than two-fold lower than the plasma concentrations for comparable doses under steady-state conditions.

[0112] A weekly dosing regimen of a single injection of angiotensin at a dose of 25 $\mu g/m^2$ for three consecutive weeks may be less effective at increasing angiotensin concentration in mice brain than a "1 on/1 off" or a "2 on/1 off" administration of the 25 $\mu g/m^2$ dose. In contrast, plasma concentrations of angiotensin may be greater when the drug is administered as a single injection for three consecutive weeks. Blood plasma concentrations of angiotensin may be less in mice receiving a 25 $\mu g/m^2$ dose as a "1 on/1 off" or a "2 on/1 off" administration. Without

being bound to a specific theory, it may be hypothesized that the intermittent dosing regimen facilitates the transport of angiotensin across the BBB.

[0113] While there have been shown and described what are at present considered the preferred embodiments of the invention, those skilled in the art may make various changes and modifications which remain within the scope of the invention defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for inducing synaptogenesis in a subject having a neurodegenerative disease, the method comprising administering an angiotensin or analogue thereof in a therapeutically effective amount to induce synaptogenesis in said subject.

2. The method of claim 1, wherein the angiotensin activates PKC-ε in said subject.

3. The method of claim 1, wherein the angiotensin activates HGF.

4. The method of claim 1, wherein the angiotensin activates the Met receptor involved in activation of PKC-ε.

5. The method according to any one of claims 1-4, wherein said neurodegenerative disease is selected from Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, dementia, and mild cognitive impairment.

6. The method according to any one of claims 1-5, wherein said neurodegenerative disease is Alzheimer's Disease.

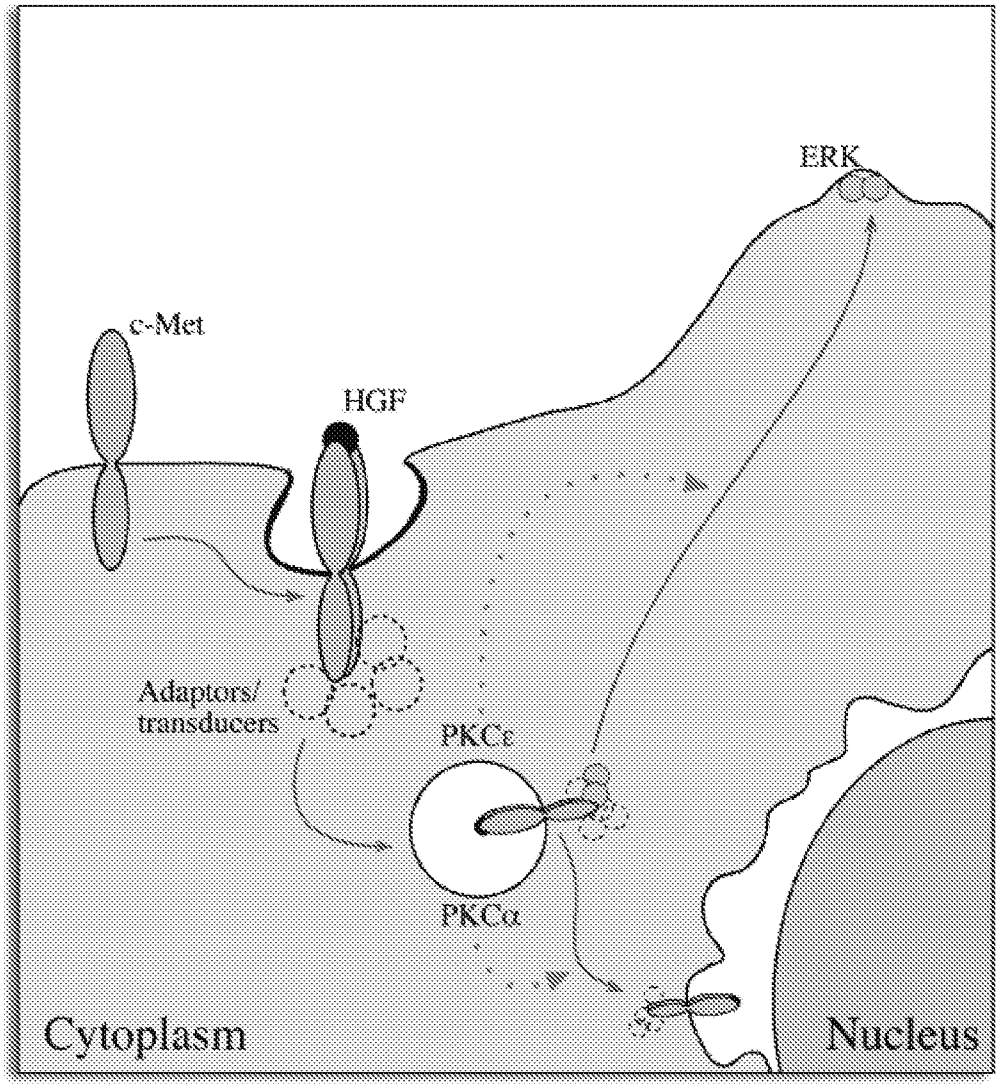
7. The method of claim 6, wherein the Alzheimer's Disease is early stage.

8. The method of claim 6, wherein the Alzheimer's Disease is late stage.

9. The method according to any one of claims 1-8, wherein the angiotensin is angiotensin II.

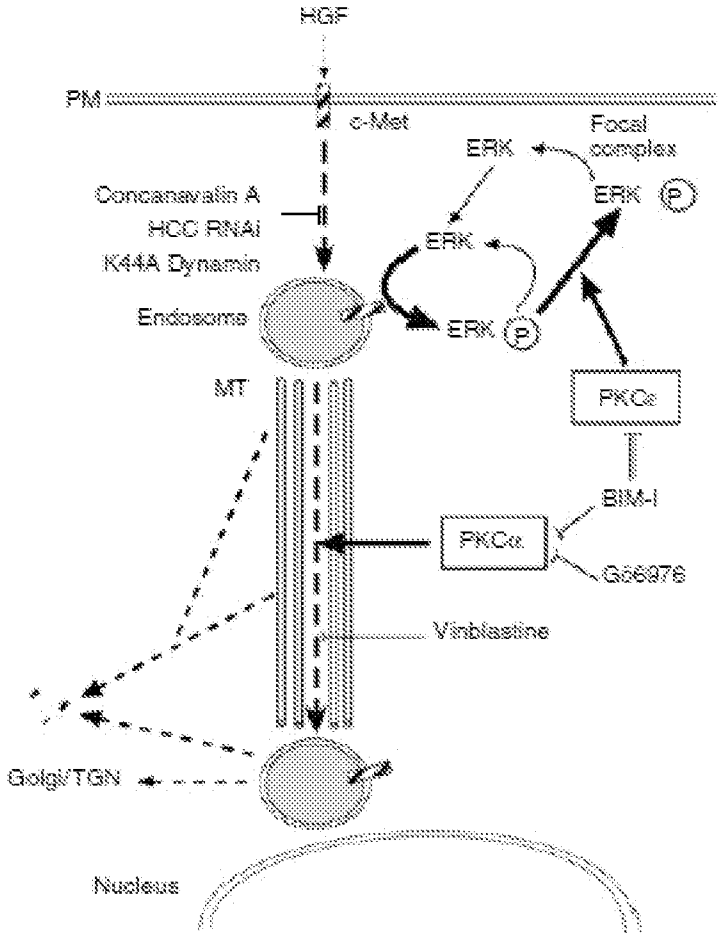
10. The method according to any one of claims 1-9, wherein the angiotensin is administered intravenously.

11. The method according to any one of claims 1-10, wherein the angiotensin is co-administered with a PKC activator or HGF activator.



S. Kermorgant et al., *Cell Cycle* 4:3, 352-355, March 2005

FIG. 1



S. Kermorgant et al., *EMBO Journal*, 23(19), 3721-3734, November 2004

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/035197

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - A61K 38/08; A61K 45/00 (2022.01)
ADD. - A61P 25/28; A61P 43/00 (2022.01)

CPC - INV. - A61K 38/085; A61P 43/00; A61P 25/28 (2022.08)
ADD. - A61K 2121/00 (2022.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0165392 A1 (WASHINGTON STATE UNIVERSITY) 27 June 2013 (27.06.2013) entire document	1, 3-5 --- 2
Y	BUTT et al., Regulation of biomechanical properties of cells in circulation by angiotensin II, The Ohio State University, 2006, [retrieved on 23.08.2022], Retrieved from internet: <URL: https://etd.ohiolink.edu/apexprod/rws_etd/send_file/send?accession=osu1155735506&disposition=inline>. Pgs. 1, 50-60, and 105-110.	2
A	BENOIST, EVALUATION OF ANGIOTENSIN IV ANALOGUE EFFECTS MEDIATED BY THE HEPATOCYTE GROWTH FACTOR AND c-MET SYSTEM, Washington State University, May 2011, Pgs. iii-ix, 1-31, [retrieved on 23.08.2022], Retrieved from internet: <URL: https://s3.amazonaws.com/na-st01.ext.exlibrisgroup.com/01ALLIANCE_WSU/storage/alma/7A/56/58/28/90/73/2F/7B/EB/CB/C8/8E/AD/E7/55/77/CarolineBenoistFinalDissertationJune0311.pdf?response-content-type=application%2Fpdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Date=20220823T230800Z&X-Amz-SignedHeaders=host&X-Amz-Expires=119&X-Amz-Credential=AKIAJN6NPMNGJALPPWAQ%2F20220823%2Fus-east-1%2Fs3%2Faws4_request&X-Amz-Signature=62305388366b310061ce15424e25949c14492cb14da29f5e8d092a9a7ea766c9>. entire document	1-5
A	WO 2007/134136 A2 (BRAINCELLS INC) 22 November 2007 (22.11.2007) entire document	1-5
A	MCCOY et al., Evaluation of Metabolically Stabilized Angiotensin IV Analogs as Procognitive/Antidementia Agents, The Journal of Pharmacology and Experimental Therapeutics, Vol. 344, Iss. 1, January 2013, Pgs. 141-154	1-5

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2022	SEP 15 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Taina Matos Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/035197

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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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. ☒ Claims Nos.: 6-11
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 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.