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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2014 (05.06.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/085494 A1

(51) International Patent Classification:
A61K 31/357 (2006.01)

(21) International Application Number:
PCT/US2013/072076

(22) International Filing Date:
26 November 2013 (26.11.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/730,761 28 November 2012 (28.11.2012) US

(71) Applicant: **APHIOS CORPORATION** [US/US]; 3e-gill Street, Woburn, MA 01801 (US).

(72) Inventor: **CASTOR, Trevor, Percival**; 469 Mystic Street, Arlington, MA 02174 (US).

(74) Agents: **JANIUK, Anthony, J.** et al.; Lathrop & Gage LLP, 28 State Street, Boston, MA 02109 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: COMBINATION THERAPEUTICS AND METHODS FOR THE TREATMENT OF NEURODEGENERATIVE AND OTHER DISEASES

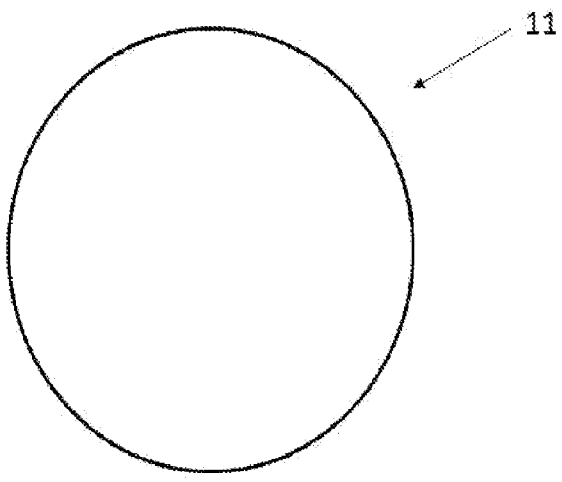


Figure 1

(57) Abstract: Embodiments of the present invention are directed to the administration of bryostatins and bryostatins and retinoids for the treatment of disease responsive to increases in alpha secretase activity. Inventions of the present application are directed to the treatment of neuro-degenerative diseases such as Hutchinson Disease, Parkinson's disease, Down's syndrome and Alzheimer's disease and virus latency diseases such as HIV and Herpes, cancers such as prostate, melanomas, lymphomas and renal cancers, esophageal and opthalmic diseases such as glaucoma.

WO 2014/085494 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

COMBINATION THERAPEUTICS AND METHODS FOR THE TREATMENT OF NEURODEGENERATIVE AND OTHER DISEASES

Related Application

This application claims the benefit of U.S. Provisional Patent Application No. 61/730,761, filed November 28, 2012, the entire contents of which are incorporated herein by reference.

Statement Regarding Federal Support

This invention was made with Federal support including National Institutes of Health Grant No. 1R44AG034760-01A1.

Field of Invention

Inventions of the present application are directed to the treatment of neuro-degenerative diseases such as Hutchinson Disease, Parkinson's disease, Down's syndrome and Alzheimer's disease and virus latency diseases such as HIV and Herpes, cancers such as prostate, melanomas, lymphomas and renal cancers, esophageal and opthalmic diseases such as glaucoma.

Background of the Invention

Neurodegenerative diseases, such as Alzheimer's disease, Hutchinson's Disease, Parkinson's disease, Kuru, Creutzfeldt - Jakob disease and other spongiform encephalopathies remain major health problems. With respect to Alzheimer's, Hutchinson's and Parkinson's diseases, these diseases tend to manifest themselves in older individuals and as the diseases progress; the afflicted individuals are less able to care for themselves. With respect to cancers such as, by way of example, without limitation, prostate cancer, Bryostatin 1 inhibits phorbol ester-induced apoptosis in prostate cancer cells by differentially modulating protein kinase C (PKC) delta translocation and preventing PKCdelta-mediated release of tumor necrosis factor-alpha. With respect to virus latency diseases such as HIV latency, Bryostatin-1, as well as many PKC agonists, activates cellular transcription factors such as NF-kB that binds the HIV-1 promoter and regulates its transcriptional activity. In HIV-1 latency the viral

promoter is less accessible to cellular transcription factors because nuclear histones surrounding the viral promoter are deacetylated (compacted chromatin). Thus HDAC inhibitors may increase the aceytation of histones (relaxed chromatin) and then transcription factors may have an easy access to the HIV promoter. With respect to opthalmic disease, such as by way of example, without limitation, glaucoma, the presence of beta amyloid is associated with elevated intra ocular pressure. Currently there are very limited means to treat these diseases.

It is therefore highly desirable to have effective therapeutics, combination therapeutics that act synergistically, effective methods of formulation and simple methods of administration (e.g. oral formulations) without the need for specially trained healthcare providers.

Summary of the Invention

Embodiments of the present invention are directed to single and combination therapies, drug delivery systems, dosage forms and methods for the treatment of neurodegenerative diseases, cancers, viral latencies and optical diseases. The neurodegenerative diseases which are the object of treatment in the present invention are exemplified by Alzheimer's disease, Hutchinson's Disease, Parkinson's disease, Kuru, Creutzfeldt - Jakob disease, Down's syndrome and spongiform encephalopathies. Other diseases include cancers such as prostate cancer and viral latencies such as HIV and herpes. The embodiments directed to an article of manufacture comprise a dosage form comprising an effective amount of a byostatin or Bryoid with an effective amount of a Retinoid. As used herein, the term "dosage form" refers to a means for administering a drug, such as by way of example, without limitation, capsules, tablets, pills, films ,ointments, creams, solutions, suspensions, aerosals, pastes, drops, suppositories, powders for reconstitution, injectables, intravenous solutions and the like.

As used herein, the term "a bryostatin" or "Bryoid" refers to any and all bryostatins and derivatives thereof. Twenty bryostatins have been identified and certain examples feature a bryostatin that is bryostatin-1. Embodiments of the present invention feature a first Bryoid composition having a molecular weight of approximately 896 – 898 Amu (Mass + Sodium) having a purity of approximately 50 % to a crystal forming purity. The first Bryoid composition can also be characterized as a Bryoid compound having a molecular weight of approximately 873-875 Amu (monoisotopic mass) having a purity of approximately 50% and

a crystal forming purity. The first Bryoid composition has a measured mass plus sodium of 897.2 Amu and a measured monoisotopic mass of 874.2 Amu. The detailed discussion which follows will refer to this Bryoid as B10.

Embodiments of the present invention feature a second Bryoid composition having a molecular weight of approximately 910 – 912 Amu (Mass + Sodium) having a purity of approximately 50 % to a crystal forming purity. The second Bryoid composition can also be characterized as a Bryoid compound having a molecular weight of approximately 888-890 Amu (monoisotopic mass) having a purity of approximately 50% and a crystal forming purity. The second Bryoid composition has a measured mass plus sodium of 911.5 Amu and a measured monoisotopic mass of 888.9 Amu. The detailed discussion which follows will refer to this Bryoid as B12.

Embodiments of the present invention feature a third Bryoid composition having a molecular weight of approximately 868 – 870 Amu (Mass + Sodium) having a purity of approximately 50 % to a crystal forming purity. The third Bryoid composition can also be characterized as a Bryoid compound having a molecular weight of approximately 846 - 848 Amu (monoisotopic mass) having a purity of approximately 50% and a crystal forming purity. The third Bryoid composition has a measured mass plus sodium of 869.5 Amu and a measured monoisotopic mass of 846.6 Amu. The detailed discussion which follows will refer to this Bryoid as B14B.

Embodiments of the present invention feature a fourth Bryoid composition having a molecular weight of approximately 895 – 897 Amu (Mass + Sodium) having a purity of approximately 50 % to a crystal forming purity. The fourth Bryoid composition can also be characterized as a Bryoid compound having a molecular weight of approximately 872 - 874 Amu (monoisotopic mass) having a purity of approximately 50% and a crystal forming purity. The fourth Bryoid composition has a measured mass plus sodium of 895.5 Amu and a measured monoisotopic mass of 872.6 Amu. The detailed discussion which follows will refer to this Bryoid as B14C.

These Bryoid compounds of the present invention have molecular weights that are different than the molecular weights of bryostatins 1-20.

As used herein, crystal forming purity means the composition has a purity which enables the composition to form crystals. Normally, such purity is greater than 90%, and more often greater than 95% purity. Examples presented in this application feature compositions having a purity greater than 99%. Crystal purity would comprise compositions in which no impurities can be detected, but is not so limited.

The first Bryoid, second Bryoid, third Bryoid, and fourth Bryoid described above are the subject of a co-pending patent application of the present inventor and applicant filed November 27, 2012, serial number US 61/730,227. The entire contents of the co-pending application are incorporated by reference.

Embodiments of the present invention feature a Bryoid present in an amount to stimulate the production of alpha secretase. For example, a Bryoid is present for administration in a dose of 0.1 – 50 micrograms per square meter of surface area per week. Another embodiment of the present invention features a Bryoid present for administration in a dose of 5- 10 micrograms per square meter of surface area per week.

Further embodiments of the article of manufacture comprise a Retinoid. The Retinoid is bioavailable in an oral form and selected from the group comprising retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene. The Examples will feature the retinoid, retinoic acid. The Examples feature the Retinoid in an amount to increase expression of alpha secretase. For example, without limitation, the retinoid is present in a dose of 1.0 – 240 mg per day.

Embodiments of the present invention feature nanospheres comprising a biopolymer which is resistant to acid. For example, without limitation, one biopolymer is a poly (D, L-lactide-coglycoside). This biopolymer has two components. Embodiments of the present invention feature a poly (D, L-lactide-co-glycoside) having a ratio of lactide and glycoside of 25-75 % lactide with the remaining comprising glycoside. A common ratio is 50:50 lactide to glycoside as determined by weight. This biopolymer is resistant to gastric acid degradation and allows oral delivery of the drug to the small intestine for absorption. Nanospheres are about 1 to 1000 nanometers in diameter.

Embodiments of the present invention feature spheres that are lyophilized for reconstitution in an aqueous solution. Another embodiment features spheres held in suspension for oral administration and/or held in an oral dosage form selected from the group of tablets, capsules, gel caps, and powders. Suspensions for oral administration are preferably flavored to improve patient acceptance.

A further embodiment of the present invention is directed to a method of treating neuro-degenerative disease. The method comprises the steps of administering an effective amount of a bryostatin held in a plurality of spheres, each sphere comprising a biopolymer and bryostatin, and each sphere having a diameter of one to 1000 nanometers.

Embodiments of the present method feature a bryostatin selected from the group consisting of Bryostatins 1-20. Certain examples feature a bryostatin that is bryostatin-1. Several bryostatins of great potency are bryostatin-3; and the first Bryoid, second Bryoid and third bryoid referenced above.

Embodiments of the present invention feature bryostatin administered in an amount to stimulate the production of alpha secretase. For example, bryostatin is administered in a dose of 0.1 – 50 micrograms per square meter of surface area per week. Another embodiment of the present invention features a bryostatin administered in a dose of 5- 10 micrograms per square meter of surface area per week.

Further embodiments of the method comprise co-administration of a retinoid. The retinoid is bioavailable in an oral form and selected from the group comprising retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene. The Examples will feature the retinoid, retinoic acid. The Examples feature the retinoid in an amount to increase expression of alpha secretase. For example, without limitation, the retinoid is administered in a dose of 1.0 – 240 mg per day.

One embodiment of the present invention features a biopolymer which is resistant to acid. For example, without limitation, one acid resistant biopolymer is a poly (D, L-lactide-coglycoside). Poly (D, L-lactide-co-glycoside) has a ratio of lactide and glycoside. A preferred ratio is 25-75 % lactide with the remaining comprising glycoside.

Preferably, the microspheres are lyophilized for reconstitution in an aqueous solution, or held in suspension for oral administration or held in an oral dosage form selected from the group of tablets, capsules, gel caps, and powders.

As a further article of manufacture, embodiments of the present invention feature an effective amount of a bryostatin dissolved in pharmaceutically acceptable oil for oral administration for the treatment of neuro-degenerative disease. As used herein, the term "pharmaceutically acceptable oil" refers to oils which are reasonably well tolerated for oral ingestion in small amounts of 5 to 10 milliliters. Embodiments of the present invention feature olive oil. Other embodiments comprise, by way of example, without limitation include, cotton seed oil, cod liver oil, castor oil, safflower oil, peanut oil, sesame oil, corn oil, vegetable oils, oils originating with animals, and other oils commonly used in the food industry. The oil is preferably administered in a gel cap.

An effective amount of Bryostatin for humans is about 0.1 to 3.0 mg per day in the pharmaceutically acceptable oil and approximately 100 micrograms to 2 mg per day as in the microsphere.

Further embodiments of the article of manufacture comprise a retinoid dissolved in pharmaceutically acceptable oil for oral administration for the treatment of neurodegenerative disease, cancer and viral latencies. Preferably, the pharmaceutically acceptable oil has the retinoid bioavailable in an oral form and selected from the group comprising retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene. The Examples will feature the retinoid, retinoic acid. The Examples feature the retinoid in an amount to increase expression of alpha secretase. For example, without limitation, the retinoid is present in a dose of 1.0 – 240 mg per day.

Further embodiments of the article of manufacture comprise a bryostatin and retinoid dissolved in pharmaceutically acceptable oil for oral administration for the treatment of neuro-degenerative disease, cancer, viral latencies and optical diseases.

A further embodiment of the present invention is directed to a method of treating neurodegenerative disease, cancer, viral latency and/or optical diseases comprising the steps

of administering orally an effective amount of a bryostatin dissolved in pharmaceutically acceptable oil.

Thus, as a treatment for neurodegenerative diseases, embodiments of the present invention feature dosage forms and methods for the oral administration of an effective amount of a bryostatin with and without a retinoid. These and other features and advantages of the present invention will be apparent upon reading the text of the detailed description below as well as viewing the accompanying drawings.

Brief Description of the Drawings

Figure 1 depicts a microsphere embodying features of the present invention;

Figure 2 depicts an apparatus for making one or more microspheres of the present invention;

Figure 3 shows that Bryostatin-1 induced sAPP-a formation in SH-SY5Y neuroblastoma cells is increased at 3h by 2 μM and 4 μM retinoic acid. Retinoic acid, an inducer of ADAM10/alpha-secretase increased the generation of sAPP-a by SH-SY5Y cells. Left panel shows 6E10 western blots for sAPP-a, Right panel shows densitometric analysis. *-$p<0.05$ vs control, **-$p<0.01$ vs control, n=3; and

Figure 4 depicts the effect of retinoic acid (RA, 2 μM) and Bryostatin-1 on the level of sAPPα in SH-SY5Y cells. Cells were treated with RA, Bryostatin-1 (10^{-9}M) + RA, Bryostatin-1 (10^{-10}M), RA+Bryostatin-1 (10^{-10}M) for 3h. Representative western blots show sAPPα recovered from culture medium. Both Bryostatin-1 and RA significantly increased sAPPα compared to control ($p<0.05$, $p<0.01$). Combining RA and Bryostatin-1 significantly increased sAPPα recovered from culture medium.

Detailed Description of the Invention

Embodiments of the present invention will be described with respect to single and combination therapy, drug delivery system, dosage form and method for the treatment of neuro-degenerative diseases exemplified by Alzheimer's disease, with the understanding that

the discussion relates to other neuro-degenerative diseases as well, cancers such as prostate cancer and viral latencies such as HIV and herpes. This discussion will feature the preferred embodiments of the invention with the understanding that features of the invention are capable of modification and alteration without departing from the teaching.

Turning first to Figure 1, a microsphere, generally designated by the numeral 11 embodying features of the present invention is depicted. The microsphere 11, when combined with an adequate number of like microspheres comprises an effective dose of a bryostatin in a biopolymer. Each microsphere 11 has a diameter of one to 1000 nanometers. Although depicted as a microsphere, the article of manufacture may have an irregular shape, roughness, or be filamentous in form.

As used herein, the term "a bryostatin" refers to any and all bryostatins and Bryoids and derivatives thereof. Bryostatins and Bryoids are isolated in accordance with Castor, U.S Patent 5,750,709 and Castor "Supercritical fluid Isolation of Bryostatin-1, Phase II Final Report, SBIR Grant No. 5 R44 CA64017-03, April 21 2001.

Certain examples feature a bryostatin that is bryostatin-1. Bryostatin-1 is well characterized in the art and the structure of such compound need not be reproduced here. Several bryostatins of high potency are bryostatin-3 and first bryostatin, second bryostatin and third bryostatin referenced previously and the subject of the co-pending patent application incorporated by reference.

The bryostatin is administered in an amount to stimulate the production of alpha secretase. For example, bryostatin is administered in a dose of 0.1 – 50 micrograms per square meter of surface area per week. Another embodiment of the present invention features a bryostatin administered in a dose of 5- 10 micrograms per square meter of surface area per week.

In embodiments which feature the co-administration of a retinoid, the retinoid is present in the same biopolymer, or made separately and combined prior to administration, or administered at the same time or close in time to have a combined effect with the bryostatin.

The retinoid is bioavailable in an oral form and selected from the group comprising retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene. Retinoic

acid is available as an orally administered drug and is sold under the pharmaceutical name Tretinoin and tradename Retin -A®. Without being bound to a particular theory, it is believed that the retinoid acts synergistically with the bryostatin to increase expression of alpha secretase. For this purpose, the retinoid is administered in a dose of 1.0 – 240 mg per day.

Embodiments of the present invention feature a biopolymer resistant to acid. For the purpose of the present discussion, resistance to acid refers to stomach acid at a pH of approximately 1 to 3 for a period of time of about 0.5 to 4.0 hours. One biopolymer is a poly (D, L-lactide-coglycoside). This biopolymer has two components, a lactide and a glycoside component. Embodiments of the present invention feature a poly (D, L-lactide-co-glycoside) having a ratio of lactide and glycoside of 25-75 % lactide with the remaining comprising glycoside. A common ratio is 50:50 lactide to glycoside as determined by weight. This biopolymer is resistant to acid degradation and allows oral delivery of the drug to the small intestine for absorption.

Embodiments of the present invention feature microspheres that are lyophilized for reconstitution in an aqueous solution. Another embodiment features microspheres held in suspension for oral administration and/or held in an oral dosage form selected from the group of tablets, capsules, gel caps, and powders. Methods of making tablets, capsules, gel caps and powders are well known in the art. (Remington, 'The Science and Practice of Pharmacy'- 20th Edition Lippincott, Williams and Williams). Suspensions for oral administration are preferably flavored to improve patient acceptance.

Another embodiment of the present invention features pharmaceutically orally acceptable oil containing an effective amount of bryostatin. An amount of oil for administration is determined, and an effective amount of bryostatin is dissolved in such oil in a manner known in the art. Preferably, the amount of oil which is intended for oral administration is enclosed in a gel cap in a manner known in the art. For example, Vitamin D and Vitamin E supplements are often enclosed in gel cap formulations.

The present method and apparatus will be described with respect to Figure 2 which depicts in schematic form a polymer sphere apparatus, generally designated by the numeral 13. The polymer sphere apparatus is comprised of the following major elements: a polymer vessel 15,

a Bryostatin drug injection assembly 17, an admixture chamber 19, a depressurization vessel 21, and an orifice nozzle 23.

Polymer vessel 15 is in fluid communication with a supercritical critical or near critical syringe pump 25 via conduits 27a, 27b and 27c. Supercritical, critical or near critical pump 25 is in fluid communication with a source of supercritical, critical or near critical fluid.

Polymer vessel 15 is also in fluid communication with a modifier syringe pump 31 via conduit 33 which intersects with conduit 27a at junction 35. Modifier syringe pump 31 is in communication with a source of modifiers and/or entrainers (not shown).

Polymer vessel 15 is loaded with polymer. This polymer vessel receives supercritical, critical or near critical fluid from supercritical critical or near critical pump 25 via conduits 27a, 27b and 27c. Polymer vessel 15 receives modifiers and/or entrainers from modifier pump 31 via conduit 33. Polymer is dissolved in the supercritical, critical or near critical fluid and modifier to form a polymer solution. Formation of the polymer solution is facilitated by circulating the polymers and supercritical, critical or near critical fluid in a loop with a conduits 27d, 27d, 27e, 27f, and 27g, a master valve 29, a static mixer 31, and a circulation pump 33.

Polymer vessel 15 is in fluid communication with admixture chamber 19 via conduits 37 and 39. Admixture chamber 19 is also in fluid communication with bryostatin drug injection assembly 17. Bryostatin drug injection assembly 17 comprises bryostatin drug syringe pump 43, a source of a bryostatin 41 and conduit 45. Bryostatin drug syringe pump 43 is in communication with a source of bryostatin material and pressurizes and compels such material through conduit 45. Conduit 45 is in communication with admixture chamber via conduits 39 which intersects conduit 45 at junction 47. Preferably, junction 47 is a mixing "T".

Admixture vessel 19 is in the nature of an inline mixer and thoroughly mixes incoming streams from the polymer vessel 15 and bryostatin drug injection assembly 17. Admixture vessel 19 is in communication with orifice nozzle 23 via conduit 49. Orifice nozzle 23 is in the nature of a back pressure regulator and has a nozzle defining one or more orifices which discharge into depressurization vessel 21 via conduit 51. Preferably, orifice nozzle 23

controls pressure and decompression rates such that a supercritical critical or near critical carbon dioxide enters the orifice at a rate of about 0.425 mls/min and 0.075 mls/min acetone or about 0.5 mls/min carbon dioxide and ethanol combined to maintain system pressure at 2,500 psig.

The operating pressure of the system can be preset at a precise level via a computerized controller (not shown) that is part of the syringe pumps. Temperature control in the system is achieved by enclosing the apparatus 11 in 1/4" Lexan sheet while utilizing a Neslab heating/cooling system coupled with a heat exchanger (not shown) to maintain uniform temperature throughout the system.

In operation, polymeric materials are first packed into the polymer vessel 15. Supercritical critical or near critical fluid and an ethanolic solution of one or more bryostatin compounds are charged into the supercritical, critical or near critical syringe pumps 25 and 31, respectively, and brought to the desired operating pressure. In the alternative, an ethanol solution of one or more bryostatin compounds is charged into bioactive syringe pump 43.

In formulations featuring one or more bryostatin compounds and one or more retinoid compounds, supercritical critical or near critical fluid and an ethanolic solution of bryostatin and a retinoid compounds are charged into the supercritical, critical or near critical syringe pumps 25 and 31, respectively, and brought to the desired operating pressure. In the alternative, an ethanol solution of bryostatin and retinoid compounds is charged into bioactive syringe pump 43.

The system is pressurized with the supercritical critical or near critical fluid via supercritical, critical or near critical syringe pump 25 to the pressure level equal to that set in modifier syringe pump 31 and bioactive syringe pump 43, and maintained at this level with the nozzle orifice 23. The dynamic operating mode for all pumps is set so that each pump can be operated at its own desired flow rate. The supercritical critical or near critical stream flows through the polymer vessel 15, dissolves polymer and contacts the one or more bryostatin compounds, or one or more bryostatin and retinoid compounds, stream at junction 47. The mixture of supercritical critical nears critical fluid, bryostatin drug, or bryostatin and retinoid, or retinoid alone and polymer materials is then passed through admixture chamber 19 for further mixing. Finally, the mixed solution enters orifice nozzle 23 and is injected into a 10%

sucrose solution containing 0.1% polyvinyl alcohol, 40 % ethanol with trace acetic acid in the depressurization vessel 21. As a result of supercritical fluid decompression, polymer spheres containing one or more bryostatin compounds, or polymer spheres containing one or more bryostatin and retinoid compounds, or polymer compounds containing retinoid compounds are formed in the 10% sucrose solution, 0.1% polyvinyl alcohol, 40 % ethanol with trace acetic acid. The expanded supercritical fluid exits the system via a vent line on the depressurization vessel 21.

The polymer nanospheres are in the nature of microspheres 11. These microspheres 11 are frozen at -80° degrees Centigrade and lyophilized.

Examples

Retinoic acid enhances Bryostatin-1 mediated alpha secretase activity. Figure 3 shows that Bryostatin-1 induced sAPP-a formation in SH-SY5Y neuroblastoma cells is increased at 3h by 2 μM and 4 μM retinoic acid. Retinoic acid, an inducer of ADAM10/alpha-secretase increased the generation of sAPP-a by SH-SY5Y cells. Left panel shows 6E10 western blots for sAPP-a, Right panel shows densitometric analysis. *-$p<0.05$ vs control, **-$p<0.01$ vs control, n=3; and

Because Bryostatin-1 was able to enhance memory and cognition in our AD model, and was able to potently stimulate the activity of alpha-secretase in SH-SY5Y neuroblastoma cells, we investigated whether co-treatment of these cells with retinoic acid (RA, 2 or 4 uM) to increase a-secretases (ADAM10, and (and possibly ADAM17, -9, -19) expression should lead to enhanced overall APP processing. In fact we did find that SH-SY5Y cells, which were treated with both Bryostatin-1 and 2 μM or 4 μM RA showed greater sAPP-α release (measured by sAPP-α release) .

Bryostatin-1 plus retinoic acid 2 μM (red bar) or 4 μM (blue bar) retinoic acid (RA) for 24 h showed an enhanced alpha secretase processing of APP to sAPP-α. These findings show that co-treatment of neuron cultures with RA plus Bryostatin-1 exhibit enhanced APP processing to sAPP-α and suggest that *in vivo* combinations of RA with Bryostatin-1 may synergistically enhance α-secretase activation (Figure 1).

This data here suggest that RA plus Bryostatin-1 represent an innovative combination which (1) increases alpha-secretase levels and (2) activates the more abundant alpha-secretase to (3) achieve higher overall levels of APP processing to sAPP-α

Retinoic Acid Plus Bryostatin-1

Figure 2 shows that in SH-SY5Y neuroblastoma cells, sAPP formation is increased by 2 μM retinoic acid ($p<0.01$) similar to 10^{-9}M Bryostatin-1. An additive effect was observed when retinoic acid (RA) was added at 2 μM concentration with Bryostatin-1 at 10^{-9}M and 10^{-10}M.

Interestingly, Figure 2 shows that both RA and Bryostatin-1 can each significantly increase expression of ADAM10 (the major form of α-secretase) and that in combination there is at least an additive effect on expression. This would be the first demonstration that Bryostatin-1 increases the expression of -secretase, which is a novel and unanticipated effect of Bryostatin-1. Therefore, Bryostatin-1 may increase -secretase processing through 2 independent mechanisms: (i) activation of PKCs which stimulate secretase; and (ii) increased expression of -secretase.

It is also possible that the increased abundance of ADAM10 might reflect increased ADAM10 stability and not necessarily greater protein synthesis. In any case, the effect on ADAM10 is novel. ADAM10 mRNA synthesis may be increased by Bryostatin-1 ultimately leading to greater α-secretase.

Oil Based Bryostatin Solutions

Oil based bryostatin solutions are made with the desired amount of bryostatin dissolved in olive oil with vitamin E as a preservative and lecithin and medium chain triglyceride emulsifiers to increase bioavailability. The oil with the dissolved bryostatin is encapsulated in gel capsules with a nitrogen purge and head. In the alternative, the oil with dissolved bryostatin is administered as a liquid dosage form. In the alternative, the oil with dissolved bryostatin may also be emulsified and administered as a liquid formulation. Emulsification

may mask some of the less desirable taste and texture associated with oil based oral formulations.

Oil based Bryostatin and Retinoic acid Solutions

Oil based bryostatin and retinoic acid solutions are made with the desired amount of bryostatin and retinoic acid dissolved in olive oil with vitamin E as a preservative and lecithin and medium chain triglyceride emulsifiers to increase bioavailability. The oil with the dissolved bryostatin and retinoic acid is encapsulated in gel capsules with a nitrogen purge and head. In the alternative, the oil with dissolved bryostatin and retinoic acid is administered as a liquid dosage form. In the further alternative, the oil with dissolved bryostatin and retinoic acid may also be emulsified and administered as a liquid formulation. Emulsification may mask some of the less desirable taste and texture associated with oil based oral formulations.

Bryostatin Microspheres

Microspheres comprising polymers and bryostatin 1 were prepared in accordance with the methods described above. The results are summarized in Table 1 below.

Table 1: Summary of Polymer Nanoencapsulation of Bryostatin-1 Experiments

Expt. No.	SFS	P (bars)	T (°C)	Particle Size (nm)	Bryo-1 (mg/100 mls)	Encapsulation (%)
ALZ-01-01	CO_2:Acetone::95:5	171	45	259	0.0511	11.4
ALZ-02-01	Freon-22	205	22	973	0.3089	16.8
ALZ-03-01	CO_2:Ethanol::85:15	171	45	246*	0.0027	71.3
ALZ-04-01	CO_2:Acetone::95:5	171	45	215*	0.0160	50.8
ALZ-05-01	CO_2:Acetone::95:5	171	45	254*	0.1323	84.0

ALZ-06-01	CO_2:Acetone::85:15	171	45	251*	0.2374	82.3

* After lyophilization and reconstitution

The nanospheres appear stable at 4-25°C (Centigrade) for at least one week duration. Further, the nanospheres appear stable in solutions at about pH 1.13 at 37°C (Centigrade), similar to a stomach environment.

Results further suggest that nanospheres with bryostatins and bryostatin 1, in particular, induce alpha-secretase processing of amyloid precursor protein (APP) to s-APP alpha, and activate protein kinase C (PKC) isoforms alpha, delta and epsilon (measured by membrane translocation) in the SH-SY5Y neuroblastoma cell line. These events are well-described cell and pharmacological events associated with prevention of beta-secretase mediated formation of beta-amyloid, the presumptive cause of dementia in human Alzheimer's disease and in the sweAPP/PS1 mouse model of Alzheimer's disease.

Bryostatin and Retinoid Microspheres

Microspheres comprising polymers and one or more bryostatin and retinoid compounds are prepared in accordance with the methods described above.

Water Maze Studies

Mouse strain B6C3-Tg carrying mutant Swedish Amyloid precursor protein (sweAPP) and PS1 (presenilin-1) genes associated with early onset Alzheimer's disease were subjected to water maze tests at 5-6 months of age. These tests suggest that mice that received bryostatin-1 at a dose of 5 micrograms/mouse on alternative days orally in an oil formulation showed significant protection against Alzheimer's disease mediated memory loss produced by the APP/PS1 mutations as compared with memory acquisition skills seen in control animals.

Therefore, we have described the present invention with respect to preferred embodiments with the understanding that these embodiments are capable of modification and alteration without departing from the teaching herein. Therefore, the present invention should not be

limited to the precise details, but should encompass the subject matter of the claims that follow and their equivalents.

Claims:

1. A dosage form comprising an effective amount of Bryoid and an effective amount of a Retinoid for the treatment of one or more diseases for which an enhanced alpha secretase processing is beneficial.

2. The article of manufacture of claim 1 wherein said Bryoid is selected from the group consisting of Bryostatin 1-20 and including the first Bryoid, second Bryoid and third Bryoid.

3. The article of manufacture of claim 3 wherein said Retinoid is retinoic acid.

4. The article of manufacture of claim 4 wherein said Retinoid is present in an amount to increase expression of alpha secretase.

5. The article of manufacture of claim 1 wherein said Bryoid is present in an amount to stimulate the production of alpha secretase.

6. The article of manufacture of claim 1 wherein said Bryoid is present for administration in a dose of 0.1 – 50 micrograms per square meter of surface area per week.

7. The article of manufacture of claim 7 wherein said Bryoid is present for administration in a dose of 5- 10 micrograms per square meter of surface area per week.

8. The article of manufacture of claim 3 wherein said Retinoid is present in a dose of 1.0 – 240 mg per day.

9. The article of manufacture of claim 9 wherein said Retinoid is selected from the group of oral retinoids consisting of retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene.

10. As an article of manufacture, an effective amount of a Bryoid and Retinoid in a biopolymer said biopolymer comprising a plurality of spheres in which said spheres have a diameter of one to 1000 nanometers, for the treatment of a disease responsive to increases in alpha secretase activity.

11. The article of manufacture of claim 10 wherein said biopolymer is resistant to acid.

12. The article of manufacture of claim 11 wherein said biopolymer is a poly (D, L-lactide-coglycoside).

13. The article of manufacture of claim 12 wherein said poly (D, L-lactide-co-glycoside) has a ratio of lactide and glycoside of 25-75 % lactide.

14. The article of manufacture of claim 10 wherein said microspheres are lyophilized for reconstitution in an aqueous solution.

15. The article of manufacture of claim 10 wherein said microspheres are held in suspension for oral administration.

16. The article of manufacture of claim 10 wherein said microspheres are held in an oral dosage form selected from the group of tablets, capsules, gel caps, and powders.

17. A method of treating neurodegenerative disease comprising the steps of administering an effective amount of a Bryoid and Retinoid held in a plurality of microspheres, said microspheres comprising a biopolymer and having a diameter of one to 1000 nanometers.

18. The method of claim 17 wherein said Bryoid is selected from the group consisting of bryostatin 1-20 and including a first Bryoid, second Bryoid and third Bryoid.

19. The method of claim 17 wherein said retinoid is retinoic acid.

20. The method of claim 19 wherein said retinoid is present in an amount to increase expression of alpha secretase.

21. The method of claim 17 wherein said Bryoid is present in an amount to stimulate the production of alpha secretase.

22. The method of claim 17 wherein said Bryoid is administered in a dose of 0.1 – 50 micrograms per square meter of surface area per week.

23. The method of claim 22 wherein said Bryoid is administered in a dose of 5- 10 micrograms per square meter of surface area per week.

24. The method of claim 20 wherein said retinoid is administered in a dose of 1.0 – 240 mg per day.

26. The article of method of claim 20 wherein said retinoid is selected from the group of oral retinoids consisting of retinoic acid, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and bexarotene.

27. The method of claim 17 wherein said biopolymer is resistant to acid.

28. The method of claim 27 wherein said biopolymer is a poly (D, L-lactide-coglycoside).

29. The method of claim 28 wherein said poly (D, L-lactide-co-glycoside) has a ratio of lactide and glycoside of 25-75 % lactide.

30. The method of claim 17 wherein said microspheres are lyophilized for reconstitution in an aqueous solution.

31. The method of claim 17 wherein said microspheres are held in suspension for oral administration.

32. The method of claim 17 wherein said microspheres are held in an oral dosage form selected from the group of tablets, capsules, gelatin capsules, and powders.

33. As an article of manufacture, an effective amount of a Bryoid and Retinoid dissolved in an orally acceptable oil medium for oral administration for the treatment of neurodegenerative disease, cancers, viral latency and opthalmic disease.

34. The article of manufacture of claim 33 wherein said neurodegenerative disease in selected from the group consisting of Alzheimer's Disease, Hutchinson's Disease, Parkinson's Disease, Kuru, Creutzfeldt-Jakob Disease, Down's syndrome and spongiform encephalopathies.

35. The article of manufacture of claim 33 wherein said cancers is prostate cancer, melanomas, lymphomas and renal cancers, esophageal and other cancers.

36. The article of manufacture of claim 33 wherein said viral latency is associated with HIV and herpes.

37. The article of manufacture of claim 33 wherein said effective amount of Bryoid is approximately 3-10 ug per kilogram body weight per day.

38. The article of manufacture of claim 33 wherein said effective amount of Retinoid is administered in a dose of 1.0 – 240 mg per day.

39. A method of treating neurodegenerative disease comprising the steps of administering orally an effective amount of a Bryoid and Retinoid dissolved in an orally acceptable oil medium.

40. A method of treating neurodegenerative disease comprising the steps of administering orally an effective amount of a Bryoid and Retinoid by intravenous administration.

41. The method of claim 39 wherein said effective amount of Bryoid is approximately 3-10 ug per kilogram body weight per day.

42. The method of claim 39 wherein said effective amount of Retinoid is administered in a dose of 1.0 – 240 mg per day.

43. A method of treating cancers comprising the steps of administering orally an effective amount of a Bryoid and Retinoid dissolved in an orally acceptable oil medium.

44. A method of treating cancers comprising the steps of administering orally an effective amount of a Bryoid and Retinoid by intravenous administration.

45. The method of claim 43 wherein said effective amount of Bryoid is approximately 3-10 ug per kilogram body weight per day.

46. The method of claim 43 wherein said effective amount of Retinoid is administered in a dose of 1.0 – 240 mg per day.

47. A method of treating virus latency comprising the steps of administering orally an effective amount of a Bryoid and Retinoid dissolved in an orally acceptable oil medium.

48. A method of treating virus latency comprising the steps of administering orally an effective amount of a Bryoid and Retinoid by intravenous administration.

49. The method of claim 48 wherein said effective amount of Bryoid is approximately 3-10 ug per kilogram body weight per day.

50. The method of claim 48 wherein said effective amount of Retinoid is administered in a dose of 1.0 – 240 mg per day.

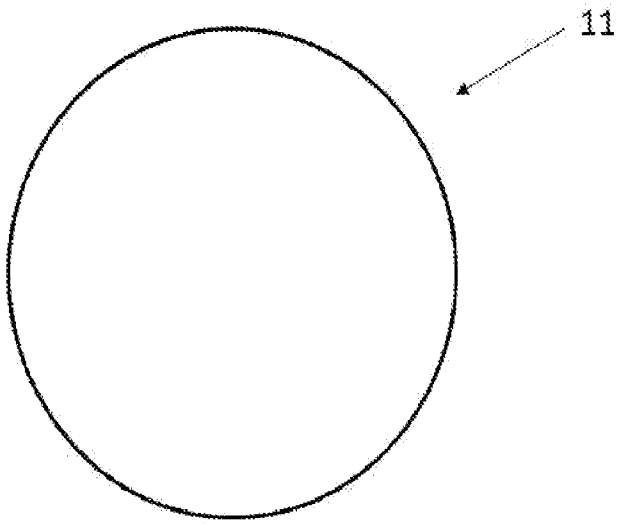


Figure 1

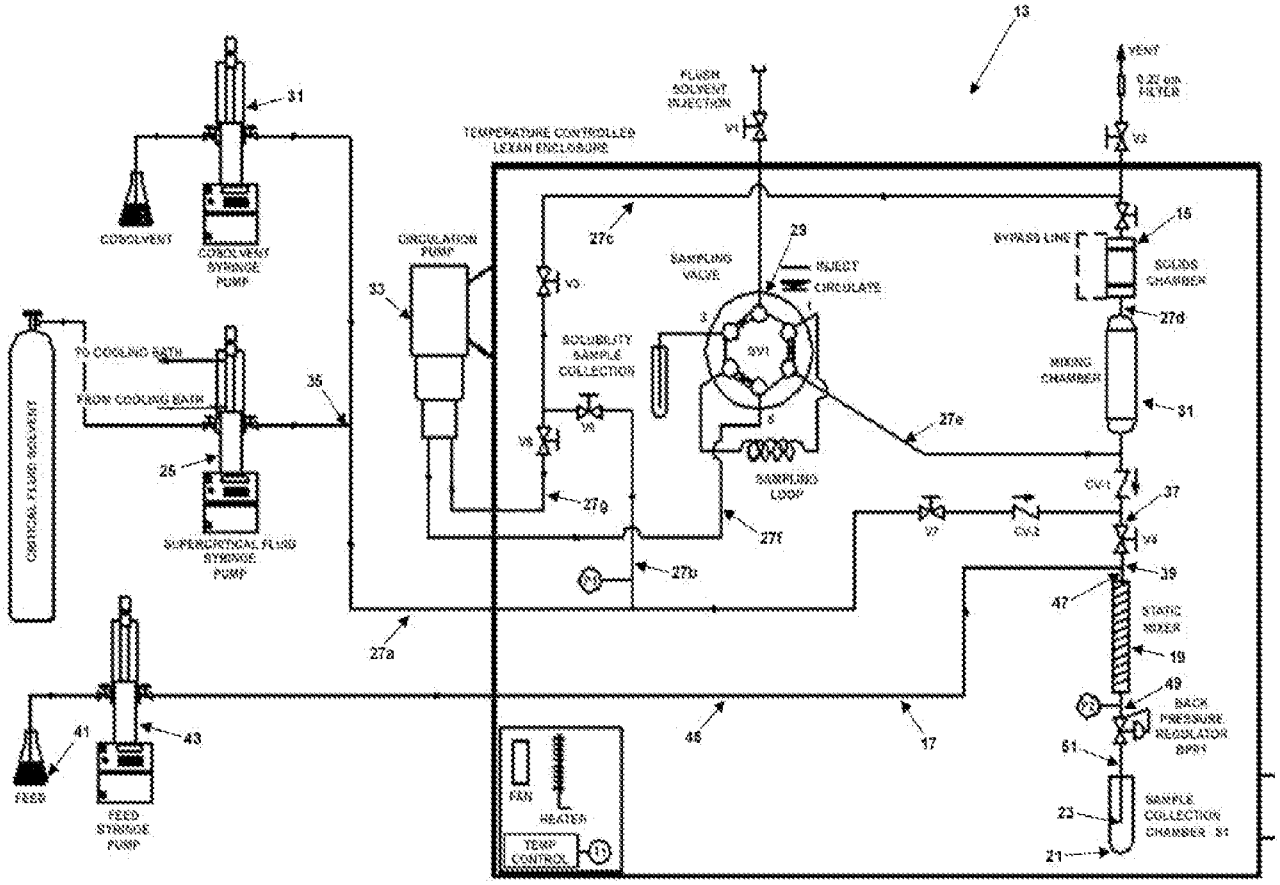

13
31
CO-SOLVENT
CO-SOLVENT SYRINGE PUMP
TEMPERATURE CONTROLLED LEXAN ENCLOSURE
FLUSH SOLVENT INJECTION
V1
VENT
0.2? um FILTER
V2
27c
CIRCULATION PUMP
33
V3
SAMPLING VALVE
29
INJECT
CIRCULATE
BYPASS LINE
15
SOLIDS CHAMBER
27d
SOLUBILITY SAMPLE COLLECTION
V5
V6
SV1
MIXING CHAMBER
31
27e
SAMPLING LOOP
27g
27f
CV-1
37
V7
CV-2
V8
39
47
STATIC MIXER
19
27b
49
BACK PRESSURE REGULATOR BPR1
51
SAMPLE COLLECTION CHAMBER S1
23
21
TO COOLING BATH
FROM COOLING BATH
35
25
SUPERCRITICAL FLUID SYRINGE PUMP
CRITICAL FLUID SOLVENT
27a
45
17
41
43
FEED
FEED SYRINGE PUMP
FAN
HEATER
TEMP CONTROL
T1

Figure 2

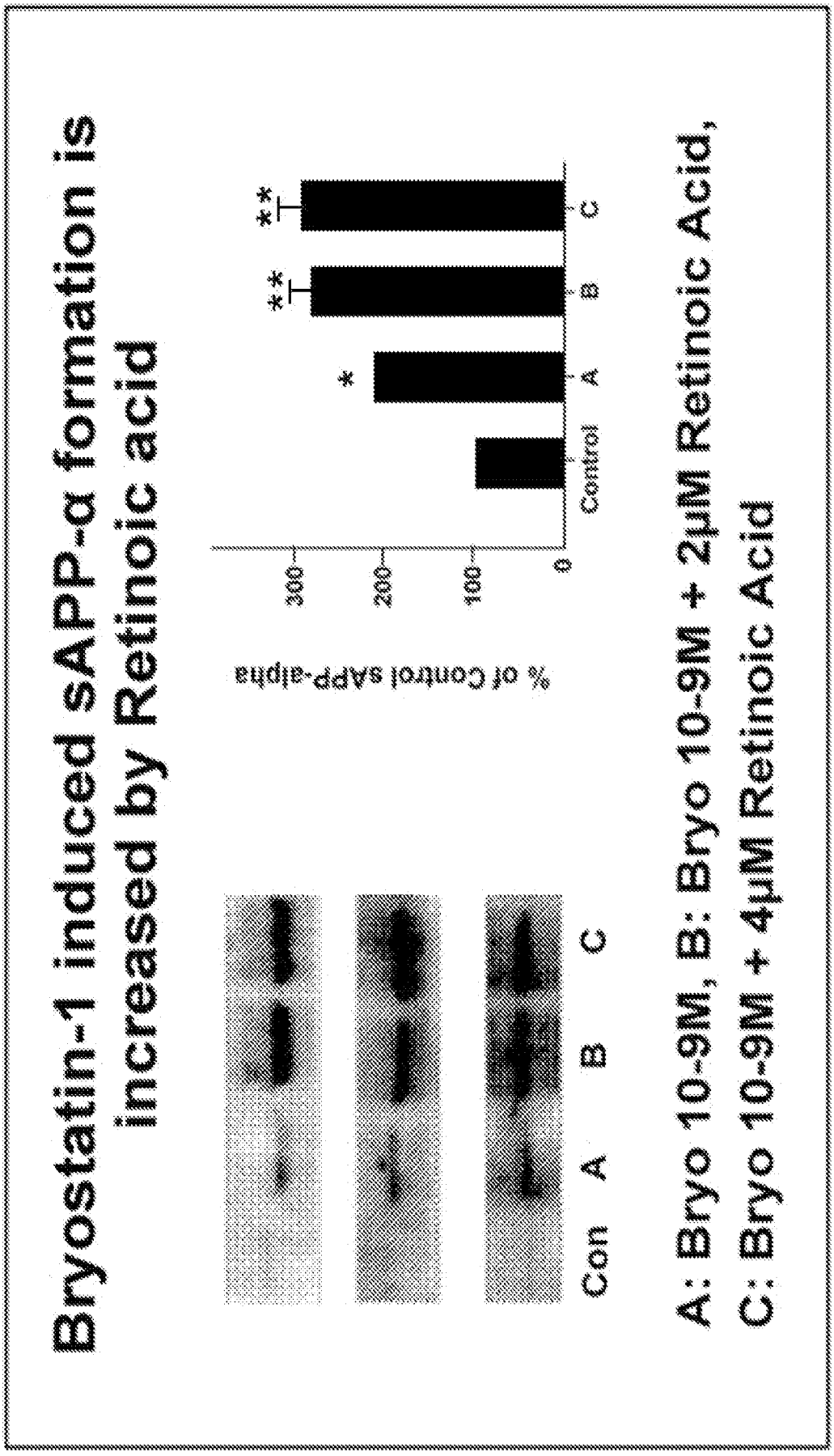

Bryostatin-1 induced sAPP-α formation is increased by Retinoic acid
% of Control sAPP-alpha
300
200
100
0
Control
A
B
C
*
**
**
Con
A
B
C
A: Bryo 10-9M, B: Bryo 10-9M + 2μM Retinoic Acid,
C: Bryo 10-9M + 4μM Retinoic Acid

Figure 3

Effect of Retinoic acid (RA, 2μM) plus Bryostatin-1 on sAPPα Release in SH-SY5Y neuroblastoma cells



RA: Retinoic Acid 2μM
B9: Bryostatin-1 10^{-9}M
B10: Bryostatin-1 10^{-10}M

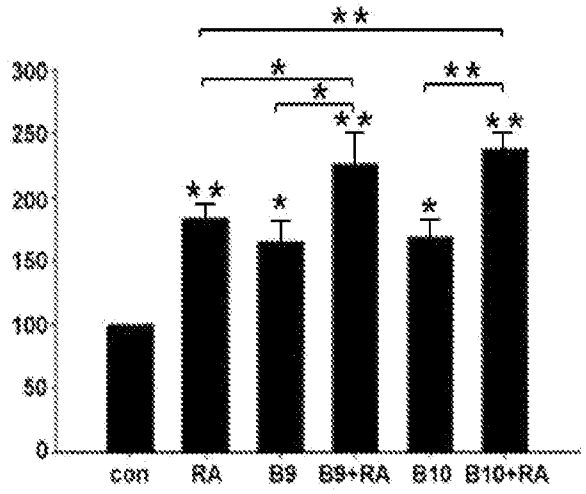


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/072076

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/357 (2014.01)
USPC - 514/450
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)
IPC(8) - A61K 31/357; A61P 25/16, 25/28, 27/02, 31/12, 31/18, 35/00, 37/00; C07D 323/00, 493/22 (2014.01)
USPC - 514/3.7, 3.8, 4.2, 17.8, 19.3, 20.8, 450; 549/267, 348

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 38/00; C07D 321/00, 493/18; C07H 19/01 (2014.02)

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

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 2009/0270492 A1 (WENDER) 29 October 2009 (29.10.2009) entire document	1, 3, 6-17, 19, 22, 23, 27-50 --- 2, 4, 5, 18, 20, 21, 24, 26
Y	US 2011/0129450 A1 (LAZAROV et al) 02 June 2011 (02.06.2011) entire document	4, 5, 20, 21, 24, 26
Y	DALE et al. 'Comparison of Effects of Bryostatins 1 and 2 and 12-0-Tetradecanoyl phorbol-13-acetate on Protein Kinase C Activity in A549 Human Lung Carcinoma Cells.' Cancer Res 49: 3242-3245. 15 June 1989. entire document	2, 18
A	US 4,560,774 A (PETTIT et al) 24 December 1985 (24.12.1985) entire document	1-50
A	US 4,611,066 A (PETTIT et al) 09 September 1986 (09.09.1986) entire document	1-50
A	MEHLA et al. "Bryostatin Modulates Latent HIV-1 Infection via PKC and AMPK Signaling but Inhibits Acute Infection in a Receptor Independent Manner" . PLoS ONE 5(6): 1-15 (e11160). 16 June 2010. entire document	1-50

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"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
18 March 2014	15 APR 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105228614 A
(43)申请公布日 2016.01.06

(21)申请号 201380070968.5
(22)申请日 2013.11.26
(30)优先权数据
61/730761 2012.11.28 US
(85)PCT国际申请进入国家阶段日
2015.07.20
(86)PCT国际申请的申请数据
PCT/US2013/072076 2013.11.26
(87)PCT国际申请的公布数据
WO2014/085494 EN 2014.06.05
(71)申请人 阿菲欧斯公司
地址 美国马萨诸塞州
(72)发明人 T.P. 卡斯托尔
(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 刘辛 李炳爱
(51)Int.Cl.
A61K 31/357(2006.01)

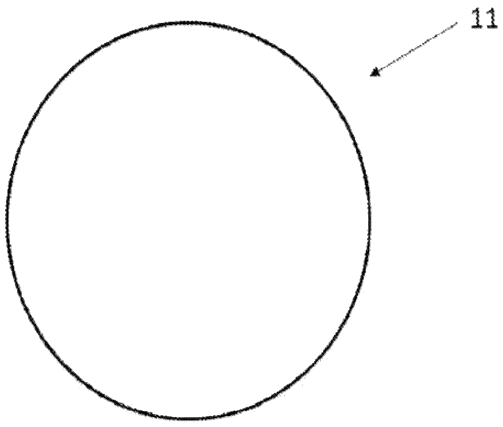
权利要求书3页 说明书8页 附图4页

(54)发明名称

用于治疗神经退化疾病和其它疾病的组合治疗剂和方法

(57)摘要

本发明的实施方案是针对施用苔藓抑素以及苔藓抑素和视黄素用于治疗响应 α 分泌酶活性增加的疾病。本申请的发明是针对治疗神经退化疾病,诸如哈钦森病、帕金森氏病、唐氏综合症以及阿尔茨海默氏病;以及病毒潜伏疾病,诸如HIV和疱疹;癌症,诸如前列腺癌、黑素瘤、淋巴瘤以及肾癌;食管和眼部疾病,诸如青光眼。



CN 105228614 A

1.一种包含有效量的类苔藓抑素和有效量的视黄素的剂型,其用于治疗增强的 α 分泌酶加工有益的一种或多种疾病。

2.如权利要求1所述的制品,其中所述类苔藓抑素选自由苔藓抑素1-20组成的群组并且包括第一类苔藓抑素、第二类苔藓抑素以及第三类苔藓抑素。

3.如权利要求 3 所述的制品,其中所述视黄素是视黄酸。

4.如权利要求 4 所述的制品,其中所述视黄素以增加 α 分泌酶表达的量存在。

5.如权利要求 1 所述的制品,其中所述类苔藓抑素以刺激 α 分泌酶产生的量存在。

6.如权利要求1所述的制品,其中所述类苔藓抑素以每周每平方米表面积0.1至50微克的剂量存在以供施用。

7.如权利要求7所述的制品,其中所述类苔藓抑素以每周每平方米表面积5至10微克的剂量存在以供施用。

8.如权利要求 3 所述的制品,其中所述视黄素以每天 1.0 至 240 毫克的剂量存在。

9.如权利要求 9 所述的制品,其中所述视黄素选自由视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13- 顺视黄酸以及蓓萨罗丁组成的口服视黄素的群组。

10.一种有效量的类苔藓抑素和视黄素于生物聚合物中的制品,所述生物聚合物包含多个球,其中所述球具有 1 至 1000 纳米的直径,所述制品用于治疗响应 α 分泌酶活性增加的疾病。

11.如权利要求 10 所述的制品,其中所述生物聚合物对酸具抗性。

12.如权利要求 11 所述的制品,其中所述生物聚合物是聚 (D,L- 丙交酯 - 共乙交酯)。

13.如权利要求 12 所述的制品,其中所述聚 (D,L- 丙交酯 - 共 - 乙交酯) 的丙交酯与乙交酯的比率为 25-75%丙交酯。

14.如权利要求 10 所述的制品,其中所述微球被冻干用于在水溶液中复原。

15.如权利要求 10 所述的制品,其中所述微球保持于供口服施用的悬浮液中。

16.如权利要求 10 所述的制品,其中所述微球保持于选自片剂、胶囊、凝胶帽以及粉末的群组的口服剂型中。

17.一种治疗神经退化疾病的方法,其包括施用有效量的保持于多个微球中的类苔藓抑素和视黄素的步骤,所述微球包含生物聚合物并且具有 1 至 1000 纳米的直径。

18.如权利要求17所述的方法,其中所述类苔藓抑素选自由苔藓抑素1-20组成的群组并且包括第一类苔藓抑素、第二类苔藓抑素以及第三类苔藓抑素。

19.如权利要求 17 所述的方法,其中所述视黄素是视黄酸。

20.如权利要求 19 所述的方法,其中所述视黄素以增加 α 分泌酶表达的量存在。

21.如权利要求 17 所述的方法,其中所述类苔藓抑素以刺激 α 分泌酶产生的量存在。

22.如权利要求17所述的方法,其中所述类苔藓抑素以每周每平方米表面积0.1至50微克的剂量施用。

23.如权利要求22所述的方法,其中所述类苔藓抑素以每周每平方米表面积5至10微克的剂量施用。

24.如权利要求 20 所述的方法,其中所述视黄素以每天 1.0 至 240 毫克的剂量施用。

26.如权利要求 20 所述的方法,其中所述视黄素选自由视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13- 顺视黄酸以及蓓萨罗丁组成的口服视黄素的群组。

27. 如权利要求 17 所述的方法，其中所述生物聚合物对酸具抗性。

28. 如权利要求 27 所述的方法，其中所述生物聚合物是聚 (D,L- 丙交酯 - 共乙交酯)。

29. 如权利要求 28 所述的方法，其中所述聚 (D,L- 丙交酯 - 共 - 乙交酯) 的丙交酯与乙交酯的比率为 25-75％丙交酯。

30. 如权利要求 17 所述的方法，其中所述微球被冻干用于在水溶液中复原。

31. 如权利要求 17 所述的方法，其中所述微球保持于供口服施用的悬浮液中。

32. 如权利要求 17 所述的方法，其中所述微球保持于选自片剂、胶囊、明胶胶囊以及粉末的群组的口服剂型中。

33. 一种有效量的类苔藓抑素和视黄素溶解于口服可接受的油介质中的制品，其用于口服施用以供治疗神经退化疾病、癌症、病毒潜伏以及眼部疾病。

34. 如权利要求 33 所述的制品，其中所述神经退化疾病选自由阿尔茨海默氏病、哈钦森氏病、帕金森氏病、库鲁病、库贾氏病、唐氏综合症以及海绵状脑病组成的群组。

35. 如权利要求 33 所述的制品，其中所述癌症是前列腺癌、黑素瘤、淋巴瘤和肾癌、食管癌以及其它癌症。

36. 如权利要求 33 所述的制品，其中所述病毒潜伏与 HIV 和疱疹相关。

37. 如权利要求 33 所述的制品，其中所述有效量的类苔藓抑素为每天每公斤体重约 3 至 10 微克。

38. 如权利要求 33 所述的制品，其中所述有效量的视黄素以每天 1.0 至 240 毫克的剂量施用。

39. 一种治疗神经退化疾病的方法，其包括口服施用有效量的溶解于口服可接受的油介质中的类苔藓抑素和视黄素的步骤。

40. 一种治疗神经退化疾病的方法，其包括通过静脉内施用来口服施用有效量的类苔藓抑素和视黄素的步骤。

41. 如权利要求 39 所述的方法，其中所述有效量的类苔藓抑素为每天每公斤体重约 3 至 10 微克。

42. 如权利要求 39 所述的方法，其中所述有效量的视黄素以每天 1.0 至 240 毫克的剂量施用。

43. 一种治疗癌症的方法，其包括口服施用有效量的溶解于口服可接受的油介质中的类苔藓抑素和视黄素的步骤。

44. 一种治疗癌症的方法，其包括通过静脉内施用来口服施用有效量的类苔藓抑素和视黄素的步骤。

45. 如权利要求 43 所述的方法，其中所述有效量的类苔藓抑素为每天每公斤体重约 3 至 10 微克。

46. 如权利要求 43 所述的方法，其中所述有效量的视黄素以每天 1.0 至 240 毫克的剂量施用。

47. 一种治疗病毒潜伏的方法，其包括口服施用有效量的溶解于口服可接受的油介质中的类苔藓抑素和视黄素的步骤。

48. 一种治疗病毒潜伏的方法，其包括通过静脉内施用来口服施用有效量的类苔藓抑素和视黄素的步骤。

49. 如权利要求 48 所述的方法，其中所述有效量的类苔藓抑素为每天每公斤体重约 3 至 10 微克。

50. 如权利要求 48 所述的方法，其中所述有效量的视黄素以每天 1.0 至 240 毫克的剂量施用。

用于治疗神经退化疾病和其它疾病的组合治疗剂和方法

[0001] 相关申请

[0002] 本申请要求2012年11月28日提交的美国临时专利申请号61/730,761的权益，其全部内容以引用的方式并入本文中。

[0003] 关于联邦支持的声明

[0004] 本发明是在包括国家卫生研究院(National Institutes of Health)批准号1R44AG034760-01A1的联邦支持下进行。

技术领域

[0005] 本申请的发明是针对治疗神经退化疾病，诸如哈钦森病(Hutchinson Disease)、帕金森氏病(Parkinson′ s disease)、唐氏综合症(Down′ s syndrome)以及阿尔茨海默氏病(Alzheimer′ s disease)；以及病毒潜伏疾病，诸如HIV和疱疹；癌症，诸如前列腺癌、黑素瘤、淋巴瘤以及肾癌；食管和眼部疾病，诸如青光眼。

背景技术

[0006] 神经退化疾病，诸如阿尔茨海默氏病、哈钦森氏病(Hutchinson′ s Disease)、帕金森氏病、库鲁病(Kuru)、库贾氏病(Creutzfeldt-Jakob disease)以及其它海绵状脑病仍然是主要的健康问题。关于阿尔茨海默氏病、哈钦森氏病以及帕金森氏病，这些疾病倾向于在老年个体中并且随着疾病发展而自己显现出来；罹病的个体很少能够自理。关于癌症，诸如（但不限于）前列腺癌，苔藓抑素1(Bryostatin 1)通过差异化调节蛋白激酶C(PKC)δ易位并且防止PKCδ介导的肿瘤坏死因子-α释放来抑制佛波酯(phorbol ester)诱导的前列腺癌细胞凋亡。关于病毒潜伏疾病，诸如HIV潜伏，苔藓抑素-1以及许多PKC激动剂活化细胞转录因子，诸如结合HIV-1启动子并且调控其转录活性的NF-kB。在HIV-1潜伏中，病毒启动子较难接近细胞转录因子，因为围绕病毒启动子的核组蛋白被脱乙酰化（致密的染色质）。因此，HDAC抑制剂可以增加组蛋白的乙酰化（松弛的染色质），并且然后转录因子可以容易接近HIV启动子。关于眼部疾病，诸如（但不限于）青光眼，β淀粉样蛋白的存在与升高的眼内压相关。当前存在非常有限的手段来治疗这些疾病。

[0007] 因此，极其需要具有有效的治疗剂、协同起作用的组合治疗剂、有效的配制方法以及简单的施用方法（例如，口服制剂），而无需受过专门训练的保健提供者。

发明内容

[0008] 本发明的实施方案是针对用于治疗神经退化疾病、癌症、病毒潜伏以及眼病的单一和组合疗法、药物递送系统、剂型以及方法。作为本发明的治疗对象的神经退化疾病由阿尔茨海默氏病、哈钦森氏病、帕金森氏病、库鲁病、库贾氏病、唐氏综合症以及海绵状脑病例示。其它疾病包括癌症，诸如前列腺癌；以及病毒潜伏，诸如HIV和疱疹。针对制品的实施方案包括一种剂型，其包含有效量的苔藓抑素或类苔藓抑素(Bryoid)与有效量的视黄素(Retinoid)。如本文所用的术语“剂型”指的是用于施用药物的工具，诸如（但不限于）胶

囊、片剂、丸剂、薄膜、软膏、乳膏、溶液、悬浮液、气雾剂、糊剂、滴剂、栓剂、用于复原的粉末、可注射剂、静脉内溶液，等等。

[0009] 如本文所用的术语“苔藓抑素”或“类苔藓抑素”指的是任何和所有苔藓抑素以及其衍生物。已经鉴别出二十种苔藓抑素，并且某些实施例关于作为苔藓抑素 -1 的苔藓抑素。本发明的实施方案关于分子量为约 896 至 898Amu（质量 + 钠）并且纯度为约 50％至晶体成形纯度的第一类苔藓抑素组合物。第一类苔藓抑素组合物还可以特征在于分子量为约 873 至 875Amu（单同位素质量）并且纯度为约 50％和晶体成形纯度的类苔藓抑素化合物。第一类苔藓抑素组合物的测量质量 + 钠为 897.2Amu 并且测量单同位素质量为 874.2Amu。以下详细论述将这种类苔藓抑素称作 B10。

[0010] 本发明的实施方案关于分子量为约 910 至 912Amu（质量 + 钠）并且纯度为约 50％至晶体成形纯度的第二类苔藓抑素组合物。第二类苔藓抑素组合物还可以特征在于分子量为约 888 至 890Amu（单同位素质量）并且纯度为约 50％和晶体成形纯度的类苔藓抑素化合物。第二类苔藓抑素组合物的测量质量 + 钠为 911.5Amu 并且测量单同位素质量为 888.9Amu。以下详细论述将这种类苔藓抑素称作 B12。

[0011] 本发明的实施方案关于分子量为约 868 至 870Amu（质量 + 钠）并且纯度为约 50％至晶体成形纯度的第三类苔藓抑素组合物。第三类苔藓抑素组合物还可以特征在于分子量为约 846 至 848Amu（单同位素质量）并且纯度为约 50％和晶体成形纯度的类苔藓抑素化合物。第三类苔藓抑素组合物的测量质量 + 钠为 869.5Amu 并且测量单同位素质量为 846.6Amu。以下详细论述将这种类苔藓抑素称作 B14B。

[0012] 本发明的实施方案关于分子量为约 895 至 897Amu（质量 + 钠）并且纯度为约 50％至晶体成形纯度的第四类苔藓抑素组合物。第四类苔藓抑素组合物还可以特征在于分子量为约 872 至 874Amu（单同位素质量）并且纯度为约 50％和晶体成形纯度的类苔藓抑素化合物。第四类苔藓抑素组合物的测量质量 + 钠为 895.5Amu 并且测量单同位素质量为 872.6Amu。以下详细论述将这种类苔藓抑素称作 B14C。

[0013] 本发明的这些类苔藓抑素化合物具有不同于苔藓抑素 1-20 的分子量的分子量。

[0014] 如本文所用的晶体成形纯度意味着组合物具有使组合物能够形成晶体的纯度。所述纯度通常高于 90％，并且更常高于 95％纯度。本申请中所呈现的实施例关于纯度高于 99％的组合物。晶体纯度将包含没有杂质能被检测到的组合物，但并不限于此。

[0015] 上文所描述的第一类苔藓抑素、第二类苔藓抑素、第三类苔藓抑素以及第四类苔藓抑素是本发明者和申请者于 2012 年 11 月 27 日提交的共同未决专利申请序列号 US 61/730,227 的主题。这个共同未决申请的全部内容以引用的方式并入。

[0016] 本发明的实施方案关于以刺激 α 分泌酶产生的量存在的类苔藓抑素。举例来说，类苔藓抑素以每周每平方米表面积 0.1 至 50 微克的剂量存在以供施用。本发明的另一个实施方案关于以每周每平方米表面积 5 至 10 微克的剂量存在以供施用的类苔藓抑素。

[0017] 制品的其它实施方案包括视黄素。视黄素以口服形式是生物可利用的并且选自包含视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13- 顺视黄酸以及蓓萨罗丁 (bexarotene) 的群组。实施例将关于视黄素、视黄酸。实施例关于增加 α 分泌酶表达的量的视黄素。举例来说（但不限于此），视黄素以每天 1.0 至 240 毫克的剂量存在。

[0018] 本发明的实施方案关于包含对酸具抗性的生物聚合物的纳米球。举例来说（但不

限于此），一种生物聚合物是聚（D，L- 丙交酯 - 共乙交酯）。这种生物聚合物具有两种组分。本发明的实施方案关于丙交酯与乙交酯的比率为 25-75％丙交酯并且其余部分包含乙交酯的聚（D，L- 丙交酯 - 共 - 乙交酯）。如以重量测定，常用比率为 50 ： 50 的丙交酯：乙交酯。这种生物聚合物对胃酸降解具抗性并且允许口服递送药物至小肠以供吸收。纳米球的直径为约 1 至 1000 纳米。

[0019] 本发明的实施方案关于被冻干用于在水溶液中复原的球。另一个实施方案关于保持于供口服施用的悬浮液中和 / 或保持于选自片剂、胶囊、凝胶帽以及粉末的群组的口服剂型中的球。供口服施用的悬浮液优选地加以调味以改善患者接受性。

[0020] 本发明的另一个实施方案是针对一种治疗神经退化疾病的方法。这种方法包括施用有效量的保持于多个球中的苔藓抑素的步骤，每个球包含生物聚合物和苔藓抑素，并且每个球具有 1 至 1000 纳米的直径。

[0021] 本发明方法的实施方案关于选自由苔藓抑素 1-20 组成的群组的苔藓抑素。某些实施例关于作为苔藓抑素 -1 的苔藓抑素。具有很大效力的几种苔藓抑素是苔藓抑素 -3 ；以及上文所提及的第一类苔藓抑素、第二类苔藓抑素以及第三类苔藓抑素。

[0022] 本发明的实施方案关于以刺激 α 分泌酶产生的量施用的苔藓抑素。举例来说，苔藓抑素以每周每平方米表面积 0.1 至 50 微克的剂量施用。本发明的另一个实施方案关于以每周每平方米表面积 5 至 10 微克的剂量施用的苔藓抑素。

[0023] 这种方法的其它实施方案包括共同施用视黄素。视黄素以口服形式是生物可利用的并且选自包含视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13- 顺视黄酸以及蓓萨罗丁的群组。实施例将关于视黄素、视黄酸。实施例关于增加 α 分泌酶表达的量的视黄素。举例来说（但不限于此），视黄素以每天 1.0 至 240 毫克的剂量施用。

[0024] 本发明的一个实施方案关于对酸具抗性的生物聚合物。举例来说（但不限于此），一种抗酸的生物聚合物是聚（D，L- 丙交酯 - 共乙交酯）。聚（D，L- 丙交酯 - 共 - 乙交酯）具有丙交酯与乙交酯的比率。优选的比率为 25-75％丙交酯并且其余部分包含乙交酯。

[0025] 优选地，微球被冻干用于在水溶液中复原，或保持于供口服施用的悬浮液中或保持于选自片剂、胶囊、凝胶帽以及粉末的群组的口服剂型中。

[0026] 作为另一种制品，本发明的实施方案关于有效量的溶解于医药学上可接受的油中的苔藓抑素，其用于口服施用以供治疗神经退化疾病。如本文所用的术语“医药学上可接受的油”指的是对于以 5 至 10 毫升少量口服摄取合理地良好耐受的油。本发明的实施方案关于橄榄油。其它实施方案包括例如，但不限于包括棉籽油、鱼肝油、蓖麻油、红花油、花生油、芝麻油、玉米油、植物油、起源于动物的油，以及通常用于食品工业中的其它油。油优选地于凝胶帽中施用。

[0027] 用于人类的苔藓抑素的有效量为于医药学上可接受的油中每天约 0.1 至 3.0 毫克以及于微球中每天约 100 微克至 2 毫克。

[0028] 制品的其它实施方案包括溶解于医药学上可接受的油中的视黄素，其用于口服施用以供治疗神经退化疾病、癌症以及病毒潜伏。优选地，医药学上可接受的油具有以口服形式生物可利用的视黄素并且选自包含视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13- 顺视黄酸以及蓓萨罗丁的群组。实施例将关于视黄素、视黄酸。实施例关于增加 α 分泌酶表达的量的视黄素。举例来说（但不限于此），视黄素以每天 1.0 至 240 毫克的剂量存

在。

[0029] 制品的其它实施方案包括溶解于医药学上可接受的油中的苔藓抑素和视黄素，其用于口服施用以供治疗神经退化疾病、癌症、病毒潜伏以及眼病。

[0030] 本发明的另一个实施方案是针对一种治疗神经退化疾病、癌症、病毒潜伏和/或眼病的方法，其包括口服施用有效量的溶解于医药学上可接受的油中的苔藓抑素的步骤。

[0031] 因此，作为神经退化疾病的治疗，本发明的实施方案关于用于口服施用有效量的伴有和不伴有视黄素的苔藓抑素的剂型和方法。本发明的这些和其它特征和优点在阅读以下具体实施方式的正文以及查看随附图式后将显而易见。

附图说明

[0032] 图 1 描绘了体现本发明的特征的微球；

[0033] 图 2 描绘了用于制造本发明的一个或多个微球的装置；

[0034] 图 3 示出了在 SH-SY5Y 成神经细胞瘤细胞中苔藓抑素 -1 诱导的 sAPP-a 形成在 3 小时由 2μM 和 4μM 视黄酸增加。视黄酸，一种 ADAM10/α- 分泌酶诱导剂，增加由 SH-SY5Y 细胞产生 sAPP-a。左图示出了 sAPP-a 的 6E10 西方墨点，右图示出了光密度分析。*-p ＜ 0.05 对比对照，**-p ＜ 0.01 对比对照，n ＝ 3；以及

[0035] 图 4 描绘了视黄酸 (RA，2μM) 和苔藓抑素 -1 对 SH-SY5Y 细胞中 sAPPα 的水平的影响。用 RA、苔藓抑素 -1(10^{-9}M)+RA、苔藓抑素 -1(10^{-10}M)、RA+ 苔藓抑素 -1(10^{-10}M) 处理细胞 3 小时。代表性的西方墨点示出了从培养基回收的 sAPPα。苔藓抑素 -1 与 RA 相较于对照都显著增加 sAPPα (p ＜ 0.05，p ＜ 0.01)。组合 RA 与苔藓抑素 -1 显著增加从培养基回收的 sAPPα。

具体实施方式

[0036] 本发明的实施方案将关于用于治疗由阿尔茨海默氏病例示的神经退化疾病的单一和组合疗法、药物递送系统、剂型以及方法来描述，应了解，论述也涉及其它神经退化疾病；癌症，诸如前列腺癌；以及病毒潜伏，诸如 HIV 和疱疹。这个论述将关于本发明的优选实施方案，应了解，本发明的特征能够在不脱离教义的情况下进行修改和变更。

[0037] 首先转向图 1，描绘了体现本发明的特征的微球，一般以数字 11 指示。微球 11 当与足够数量的同样的微球组合时，包括有效剂量的于生物聚合物中的苔藓抑素。每个微球 11 具有 1 至 1000 纳米的直径。尽管以微球描绘，但制品可以具有不规则形状、粗糙度，或形式呈丝状。

[0038] 如本文所用的术语“苔藓抑素”指的是任何和所有苔藓抑素和类苔藓抑素以及其衍生物。根据 Castor 的美国专利 5,750,709 和 Castor 的“苔藓抑素 -1 的超临界流体分离 (Supercritical fluid Isolation of Bryostatin-1)”，第 1I 阶段最终报告 (Phase II Final Report)，SBIR 批准号 5R44CA64017-03，2001 年 4 月 21 日来分离苔藓抑素和类苔藓抑素。

[0039] 某些实施例关于作为苔藓抑素 -1 的苔藓抑素。苔藓抑素 -1 在本领域中良好表征，并且所述化合物的结构无须在这里重现。具有很高效力的几种苔藓抑素是苔藓抑素 -3，以及先前所提及的第一苔藓抑素、第二苔藓抑素和第三苔藓抑素，以及以引用的方式并入的

共同未决专利申请的主题。

[0040] 苔藓抑素以刺激 α 分泌酶产生的量施用。举例来说，苔藓抑素以每周每平方米表面积 0.1 至 50 微克的剂量施用。本发明的另一个实施方案关于以每周每平方米表面积 5 至 10 微克的剂量施用的苔藓抑素。

[0041] 在关于共同施用视黄素的实施方案中，视黄素存在于同一生物聚合物中，或在施用之前独立地制得并组合，或同时或在时间上接近地施用以与苔藓抑素具有组合效应。

[0042] 视黄素以口服形式是生物可利用的并且选自包含视黄酸、视黄醇、视黄醇乙酸酯、视黄醇棕榈酸酯、13-顺视黄酸以及蓓萨罗丁的群组。视黄酸可用作口服施用的药物并且以药品名维甲酸 (Tretinoin) 和商标名 Retin-A® 出售。不受特定理论约束，据信视黄素与苔藓抑素协同起作用以增加 α 分泌酶的表达。出于这个目的，视黄素以每天 1.0 至 240mg 的剂量施用。

[0043] 本发明的实施方案关于对酸具抗性的生物聚合物。出于本发明论述的目的，对酸具抗性指的是在 pH 为约 1 至 3 的胃酸中维持约 0.5 至 4.0 小时的时段。一种生物聚合物是聚 (D,L- 丙交酯 - 共乙交酯)。这种生物聚合物具有两种组分，丙交酯和乙交酯组分。本发明的实施方案关于丙交酯与乙交酯的比率为 25-75％丙交酯并且其余部分包含乙交酯的聚 (D,L- 丙交酯 - 共 - 乙交酯)。如以重量测定，常用比率为 50 ： 50 的丙交酯：乙交酯。这种生物聚合物对酸降解具抗性并且允许口服递送药物至小肠以供吸收。

[0044] 本发明的实施方案关于被冻干用于在水溶液中复原的微球。另一个实施方案关于保持于供口服施用的悬浮液中和 / 或保持于选自片剂、胶囊、凝胶帽以及粉末的群组的口服剂型中的微球。制造片剂、胶囊、凝胶帽以及粉末的方法在本领域中广为熟知。(Remington，‘制药科学与实践 (The Science and Practice of Pharmacy)’ - 第 20 版 Lippincott，Williams and Williams)。供口服施用的悬浮液优选地加以调味以改善患者接受性。

[0045] 本发明的另一个实施方案关于含有有效量的苔藓抑素的医药学上口服可接受的油。确定供施用的油量，并且以本领域中已知的方式将有效量的苔藓抑素溶解于所述油中。优选地，以本领域中已知的方式将意图用于口服施用的油量封入凝胶帽中。举例来说，常常将维生素 D 和维生素 E 补充物封入凝胶帽制剂中。

[0046] 本发明方法和装置将关于图 2 来描述，图 2 以示意图形式描绘了聚合物球装置，一般以数字 13 指示。聚合物球装置由以下主要元件组成 :聚合物容器 15、苔藓抑素药物注射组合件 17、掺合室 19、减压容器 21 以及孔口式喷嘴 23。

[0047] 聚合物容器 15 经由管道 27a、27b 以及 27c 与超临界、临界或近临界注射泵 25 流体连通。超临界、临界或近临界泵 25 与超临界、临界或近临界流体的来源流体连通。

[0048] 聚合物容器 15 还经由管道 33 与改性剂注射泵 31 流体连通，管道 33 在接点 35 处与管道 27a 相交。改性剂注射泵 31 与改性剂和 / 或夹带剂的来源 (未示出) 连通。

[0049] 聚合物容器 15 加载有聚合物。这个聚合物容器经由管道 27a、27b 以及 27c 从超临界、临界或近临界泵 25 接收超临界、临界或近临界流体。聚合物容器 15 经由管道 33 从改性剂泵 31 接收改性剂和 / 或夹带剂。将聚合物溶解于超临界、临界或近临界流体和改性剂中以形成聚合物溶液。通过使聚合物和超临界、临界或近临界流体在具有管道 27d、27d、27e、27f 以及 27g、总阀 29、静态混合器 31 以及循环泵 33 的回路中循环来促进聚合物溶液

的形成。

[0050] 聚合物容器 15 经由管道 37 和 39 与掺合室 19 流体连通。掺合室 19 还与苔藓抑素药物注射组合件 17 流体连通。苔藓抑素药物注射组合件 17 包括苔藓抑素药物注射泵 43、苔藓抑素来源 41 以及管道 45。苔藓抑素药物注射泵 43 与苔藓抑素物质的来源连通并且加压并迫使所述物质通过管道 45。管道 45 经由管道 39 与掺合室连通，管道 39 在接点 47 处与管道 45 相交。优选地，接点 47 是混合“T”。

[0051] 掺合容器 19 具有在线混合器的性质并且充分混合来自聚合物容器 15 和苔藓抑素药物注射组合件 17 的引入流。掺合容器 19 经由管道 49 与孔口式喷嘴 23 连通。孔口式喷嘴 23 具有背压调节器的性质并且具有界定经由管道 51 排料至减压容器 21 中的一个或多个孔口的喷嘴。优选地，孔口式喷嘴 23 控制压力和减压速率以使得超临界、临界或近临界二氧化碳以约 0.425ml/min 与 0.075ml/min 丙酮或约 0.5ml/min 二氧化碳与乙醇组合的速率进入孔口以维持 2,500psig 的系统压力。

[0052] 可以经由作为注射泵的一部分的计算机控制器（未示出）将系统的操作压力预置为精确水平。通过将装置 11 封入 1/4″ Lexan 板材中，同时利用与热交换器（未示出）耦接的 Neslab 加热 / 冷却系统以维持整个系统中的均匀温度来达成系统中的温度控制。

[0053] 在操作中，将聚合物质首先包装于聚合物容器 15 中。将超临界、临界或近临界流体以及一种或多种苔藓抑素化合物的乙醇溶液分别馈入超临界、临界或近临界注射泵 25 和 31 中，并且使其达到所需操作压力。在替代方案中，将一种或多种苔藓抑素化合物的乙醇溶液馈入生物活性注射泵 43 中。

[0054] 在关于一种或多种苔藓抑素化合物和一种或多种视黄素化合物的制剂中，将超临界、临界或近临界流体以及苔藓抑素和视黄素化合物的乙醇溶液分别馈入超临界、临界或近临界注射泵 25 和 31 中，并且使其达到所需操作压力。在替代方案中，将苔藓抑素和视黄素化合物的乙醇溶液馈入生物活性注射泵 43 中。

[0055] 经由超临界、临界或近临界注射泵 25 用超临界、临界或近临界流体将系统加压至等于在改性剂注射泵 31 和生物活性注射泵 43 中设置的压力水平的压力水平，并且用喷嘴孔口 23 维持在这个水平下。设置所有泵的动态操作模式以使得每个泵可以在其自身所需的流动速率下操作。超临界、临界或近临界流流经聚合物容器 15，溶解聚合物，并且在接点 47 处接触一种或多种苔藓抑素化合物、或一种或多种苔藓抑素和视黄素化合物的流。超临界、临界、近临界流体、苔藓抑素药物、或苔藓抑素和视黄素、或单独的视黄素与聚合物物质的混合物然后通过掺合室 19 以供进一步混合。最后，混合溶液进入孔口式喷嘴 23 并且被注射至减压容器 21 中的含有 0.1％聚乙烯醇、40％乙醇与微量乙酸的 10％蔗糖溶液中。作为超临界流体减压的结果，在 10％蔗糖溶液、0.1％聚乙烯醇、40％乙醇与微量乙酸中形成含有一种或多种苔藓抑素化合物的聚合物球、或含有一种或多种苔藓抑素和视黄素化合物的聚合物球、或含有视黄素化合物的聚合物化合物。膨胀的超临界流体经由减压容器 21 上的排气管线退出系统。

[0056] 聚合物纳米球具有微球 11 的性质。这些微球 11 在 -80℃（摄氏度）下冷冻并且冻干。

[0057] 实施例

[0058] 视黄酸增强苔藓抑素 -1 介导的 α 分泌酶活性。图 3 示出了在 SH-SY5Y 成神经细

胞瘤细胞中苔藓抑素 -1 诱导的 sAPP-a 形成在 3 小时由 2 μ M 和 4 μ M 视黄酸增加。视黄酸，一种 ADAM10/ α - 分泌酶诱导剂，增加由 SH-SY5Y 细胞产生 sAPP-a。左图示出了 sAPP-a 的 6E10 西方墨点，右图示出了光密度分析。*-p ＜ 0.05 对比对照，**-p ＜ 0.01 对比对照，n ＝ 3 ；以及

[0059] 因为苔藓抑素 -1 能够增强我们的 AD 模型的记忆和认知，并且能够有效力地刺激 SH-SY5Y 成神经细胞瘤细胞中 α - 分泌酶的活性，所以我们研究了用视黄酸 (RA，2 或 4 μ M) 共同处理这些细胞以增加 a- 分泌酶 (ADAM10，以及可能的 ADAM17、ADAM9、ADAM19) 表达是否将使得总体 APP 加工增强。实际上，我们确实发现用苔藓抑素 -1 与 2 μ M 或 4 μ M RA 处理的 SH-SY5Y 细胞显示较高 sAPP- α 释放 (如由 sAPP- α 释放所测量)。

[0060] 苔藓抑素 -1+ 视黄酸 2 μ M(红条) 或 4 μ M(蓝条) 视黄酸 (RA) 为时 24 小时显示 APP 至 sAPP- α 的 α 分泌酶加工增强。这些发现显示用 RA+ 苔藓抑素 -1 共同处理神经元培养物展现 APP 加工成 sAPP- α 的增强，并且表明体内 RA 与苔藓抑素 -1 的组合可以协同增强 α - 分泌酶活化 (图 1)。

[0061] 这个资料在这里表明 RA+ 苔藓抑素 -1 代表创新的组合，其 (1) 提高 α - 分泌酶水平，以及 (2) 活化更丰富的 α - 分泌酶至 (3) 达成 APP 加工成 sAPP- α 的较高总体水平。

[0062] 视黄酸 + 苔藓抑素 -1

[0063] 图 2 示出了在 SH-SY5Y 成神经细胞瘤细胞中，sAPP □形成由 2 μ M 视黄酸增加 (p ＜ 0.01)，类似于 10^{-9}M 苔藓抑素 -1。当视黄酸 (RA) 以 2 μ M 浓度与 10^{-9}M 和 10^{-10}M 的苔藓抑素 -1 一起添加时，观测到相加效应。

[0064] 令人感兴趣的是，图 2 示出了 RA 与苔藓抑素 -1 都可以各自显著增加 ADAM10(α - 分泌酶的主要形式) 的表达，并且组合起来对表达至少存在相加效应。这将首次证实苔藓抑素 -1 增加□ - 分泌酶的表达，其为苔藓抑素 -1 的新颖并且未预期的作用。因此，苔藓抑素 -1 可以经由 2 个独立的机制增加□ - 分泌酶加工 :(i) 刺激□□分泌酶的 PKC 活化 ；以及 (ii) □ - 分泌酶的表达增加。

[0065] 还有可能的是，增加的 ADAM10 丰度可能反映增加的 ADAM10 稳定性并且未必反映更多的蛋白质合成。在任何情况下，对 ADAM10 的作用是新颖的。ADAM10 mRNA 合成可以由苔藓抑素 -1 增加，最终得到更多 α - 分泌酶。

[0066] 油基苔藓抑素溶液

[0067] 用所需量的溶解于橄榄油中的苔藓抑素制得油基苔藓抑素溶液，其中维生素 E 作为防腐剂并且卵磷脂和中链甘油三酯乳化剂用以增加生物利用度。用氮气吹洗和压头将溶解有苔藓抑素的油囊封于凝胶胶囊中。在替代方案中，将溶解有苔藓抑素的油作为液体剂型施用。在替代方案中，还可以乳化溶解有苔藓抑素的油并且作为液体制剂施用。乳化可以掩蔽一些与油基口服制剂相关的不合需要的味道和质地。

[0068] 油基苔藓抑素和视黄酸溶液

[0069] 用所需量的溶解于橄榄油中的苔藓抑素和视黄酸制得油基苔藓抑素和视黄酸溶液，其中维生素 E 作为防腐剂并且卵磷脂和中链甘油三酯乳化剂用以增加生物利用度。用氮气吹洗和压头将溶解有苔藓抑素和视黄酸的油囊封于凝胶胶囊中。在替代方案中，将溶解有苔藓抑素和视黄酸的油作为液体剂型施用。在另一个替代方案中，还可以乳化溶解有苔藓抑素和视黄酸的油并且作为液体制剂施用。乳化可以掩蔽一些与油基口服制剂相关的

不合需要的味道和质地。

[0070] 苔藓抑素微球

[0071] 根据上文所描述的方法制备包含聚合物和苔藓抑素 1 的微球。结果概述于下表 1 中。

[0072] 表 1 :苔藓抑素 -1 实验的聚合物纳米囊封的概述

[0073]

实验编号	SFS	P	T	粒度	Bryo-1	囊封

[0074]

		(巴)	(℃)	(nm)	(mg/100ml)	(%)
ALZ-01-01	CO_2：丙酮 : :95 : 5	171	45	259	0.0511	11.4
ALZ-02-01	Freon-22	205	22	973	0.3089	16.8
ALZ-03-01	CO_2：乙醇 : :85 : 15	171	45	246*	0.0027	71.3
ALZ-04-01	CO_2：丙酮 : :95 : 5	171	45	215*	0.0160	50.8
ALZ-05-01	CO_2：丙酮 : :95 : 5	171	45	254*	0.1323	84.0
ALZ-06-01	CO_2：丙酮 : :85 : 15	171	45	251*	0.2374	82.3

[0075] * 在冻干和复原之后

[0076] 纳米球似乎在 4-25℃（摄氏度）下稳定至少一周的持续时间。此外，纳米球似乎在 37℃（摄氏度）下在约 pH 1.13 的溶液中稳定，类似于胃环境。

[0077] 结果进一步表明，具有苔藓抑素和苔藓抑素 1 的纳米球特别地诱导淀粉样前体蛋白 (APP) 至 s-APPα 的 α - 分泌酶加工，并且活化 SH-SY5Y 成神经细胞瘤细胞系中的蛋白激酶 C(PKC) 同种型 α 、δ 以及 ε（由膜易位测量）。这些事件是与防止 β - 分泌酶介导的 β - 淀粉样蛋白形成相关的良好描述的细胞和药理事件，β - 淀粉样蛋白形成是人类阿尔茨海默氏病和阿尔茨海默氏病的 sweAPP/PS1 小鼠模型的痴呆的推定原因。

[0078] 苔藓抑素和视黄素微球

[0079] 根据上文所描述的方法制备包含聚合物以及一种或多种苔藓抑素和视黄素化合物的微球。

[0080] 水迷宫研究

[0081] 对带有与早发型阿尔茨海默氏病相关的突变瑞典淀粉样前体蛋白 (sweAPP) 和 PS1(早老素 -1(presenilin-1)) 基因的小鼠品系 B6C3-Tg 在 5-6 月龄进行水迷宫测试。这些测试表明，与在对照动物中观察的记忆获得技能相比，隔天口服接收于油制剂中的剂量为 5 微克 / 小鼠的苔藓抑素 -1 的小鼠显示出对抗阿尔茨海默氏病介导的由 APP/PS1 突变产生的记忆丧失的显著保护。

[0082] 因此，我们已经关于优选的实施方案描述本发明，应了解，这些实施方案能够在不脱离本文中的教义的情况下进行修改和变更。因此，本发明不应限于精确的细节，而应涵盖以上权利要求书和其等效物的主题。

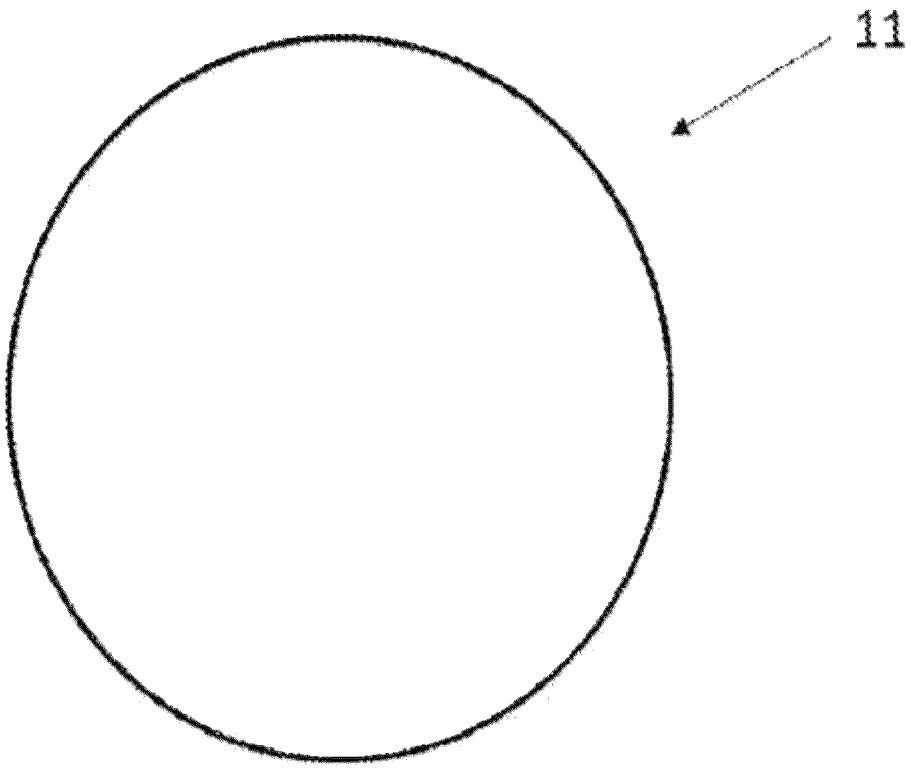


图 1

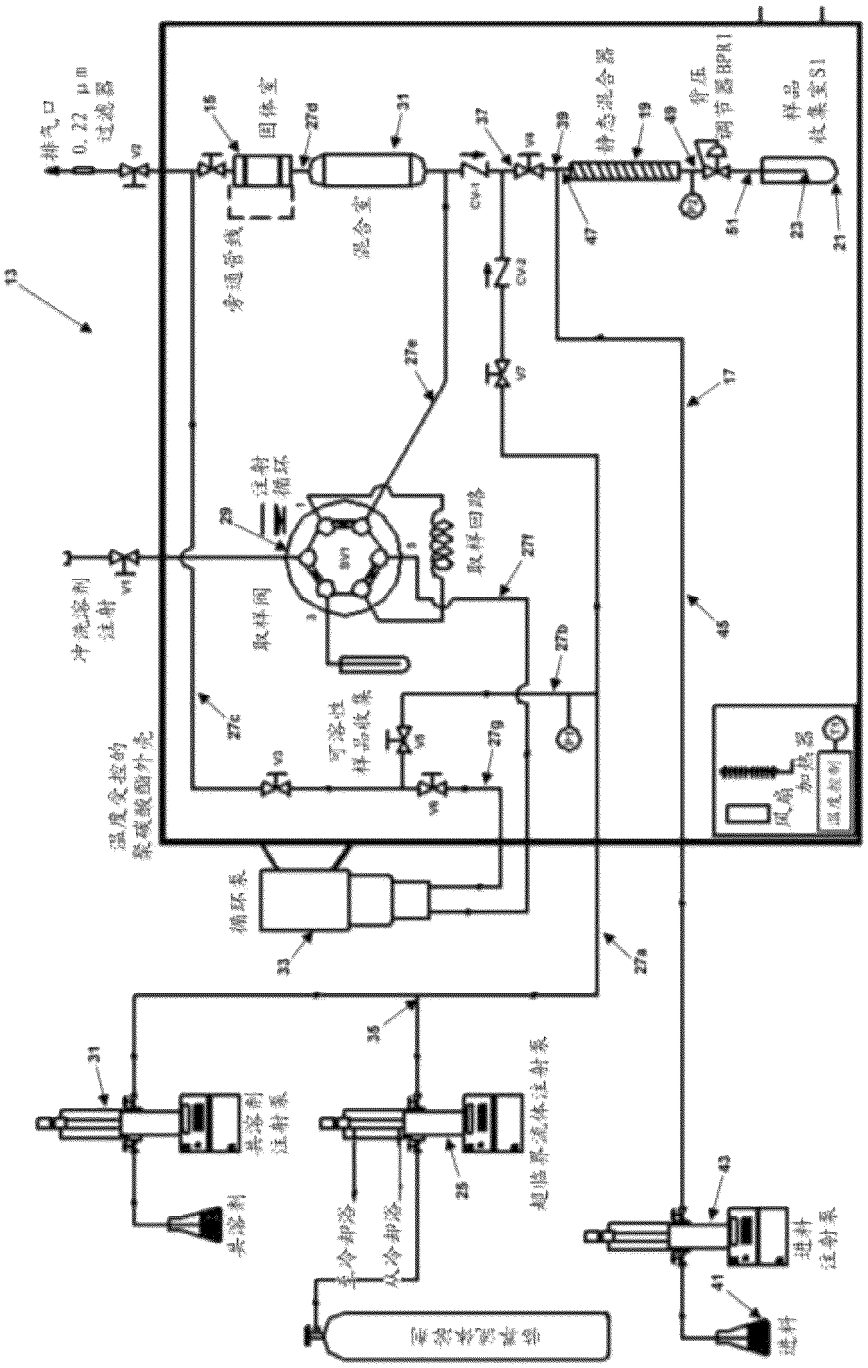

排气口
0.22 μm
过滤器
V0
15
固体室
27a
31
混合室
37
CV1
V4
39
静态混合器
19
49
背压
调节器BPR1
样品
收集室S1
47
51
23
21
13
旁通管线
CV2
V7
27e
17
29
注射
循环
取样回路
V1
取样阀
27f
冲洗溶剂
注射
45
27d
27c
可溶性
样品收集
V5
27b
V3
V6
温度受控的
聚碳酸酯外壳
风扇
加热器
循环泵
33
27a
35
31
共溶剂
注射泵
共溶剂
28
超临界流体注射泵
主冷却液
从冷却液
43
进料
注射泵
41
进料
收集溶液容器

图 2

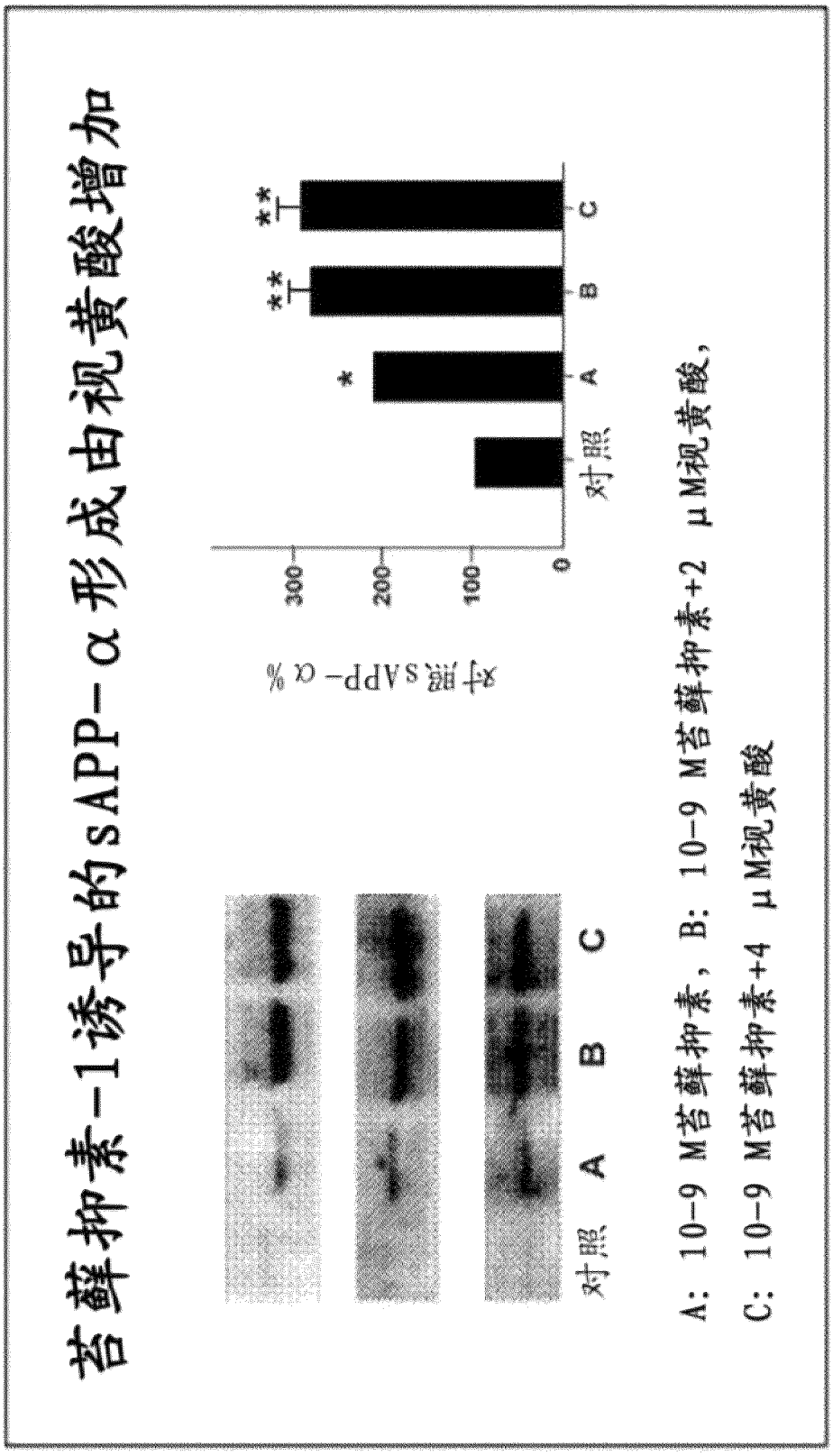

苔藓抑素-1诱导的sAPP-α形成由视黄酸增加
对照 A B C
对照 sAPP-α %
300
200
100
0
对照
A
B
C
*
**
**
A: 10-9 M苔藓抑素, B: 10-9 M苔藓抑素+2 μM视黄酸,
C: 10-9 M苔藓抑素+4 μM视黄酸

图 3

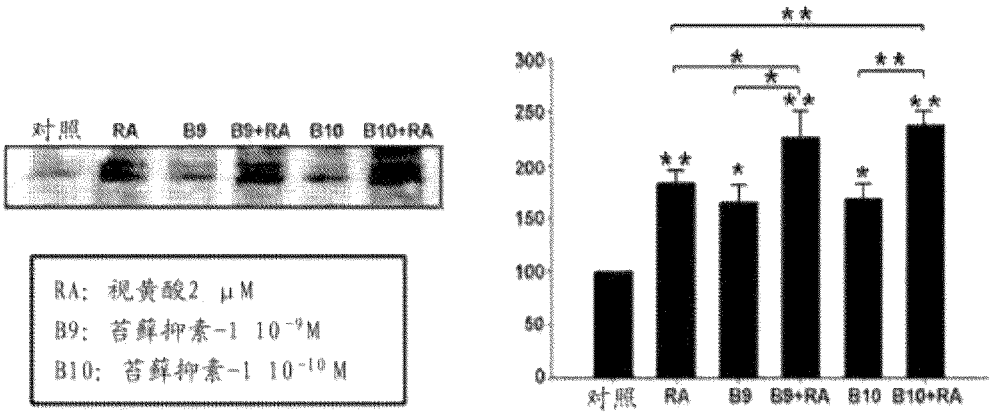

视黄酸（RA，2 μM）+苔藓抑素-1对
SH-SY5Y成神经细胞瘤细胞中sAPPα释放的影响
对照 RA B9 B9+RA B10 B10+RA
RA：视黄酸2 μM
B9：苔藓抑素-1 10^{-9}M
B10：苔藓抑素-1 10^{-10}M
300
250
200
150
100
50
0
对照 RA B9 B9+RA B10 B10+RA

图 4