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April 09, 2003

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APPLICATION NUMBER: 60/362,080
FILING DATE: March 07, 2002
RELATED PCT APPLICATION NUMBER: PCT/US03/07101

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle (if any)) Family Name or Surname Residence (City and Either State or Foreign Country)

Rene Daniel L. Etcheberrigaray Columbia, Maryland

Additional inventors are being named on page 2 attached hereto.

TITLE OF THE INVENTION (280 characters max)

ACTIVATION OF PKC ISOENZYMES FOR COGNITIVE ENHANCEMENT

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 8 ☒ Small Entity Status Claimed As:

☐ Independent Inventor ☒ Small Business Concern ☐ Nonprofit Organization ☐ Non-Inventor Supporting Claim By Another

☒ Drawing(s) Number of Sheets 4 ☐ Other (specify) 

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: 

☒ No.

Respectfully submitted, 

Date March 6, 2002

By Robert M. Schultman

Telephone (202) 955-1928

Registration No. 31,196
Complete if Known

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- Plant Filing Fee $  
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**3. ADDITIONAL FEES**

- Surcharge - late filing fee or oath $  
- Surcharge - late provisional filing fee or cover sheet $  
- ______, Month Extension of Time $  
- Notice of Appeal $  
- Filing Brief in Support of Appeal $  
- Request for Oral Hearing $  
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- Plant Issue Fee $  
- Petition to Commissioner $  
- Petition to Revive (Unavoidable) $  
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**2. EXTRA CLAIMS FEES**

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**SUBMITTED BY**

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ACTIVATION OF PKC ISOENZYMES FOR COGNITIVE ENHANCEMENT

BACKGROUND OF THE INVENTION

(i) Field of the Invention

The present invention relates to the activation of PKC isoenzymes and cognitive enhancement.

(ii) Background of the Invention

Various disorders and diseases exist which affect cognition. Cognition can be generally described as including at least three different components: attention, learning, and memory. Each of these components and their respective levels effect the overall level of a subject’s cognitive ability. For instance, while Alzheimer’s Disease patients suffer from a loss of overall cognition and thus deterioration of each of these characteristics, it is the loss of memory that is most often associated with the disease. In other diseases patients suffer from cognitive loss but the loss is more predominately associated with different characteristics of cognition, for instance Attention Deficit Hyperactivity Disorder (ADHD), focuses on the individual’s ability to maintain an attentive state. Other conditions include general dementias associated with other neurological diseases, aging, treatment of conditions which can cause deleterious effects on mental capacity, such as cancer treatments, and mental retardation.

Ways to improve the cognitive abilities of diseased individuals have been the subject of various studies. Recently the cognitive state related to Alzheimer’s Disease and different ways to improve patient’s memory have been the subject of various approaches and strategies. Unfortunately, these approaches and strategies only improve symptomatic and transient cognition in diseased individuals but have not addressed the progression of the disease. In the case of Alzheimer’s Disease, efforts to improve cognition, typically through the cholinergic pathways or though other brain transmitter pathways, have been investigated. This approach relies on the inhibition of acetyl cholinesterase enzymes through drug therapy. Acetyl cholinesterase is a major brain enzyme and manipulating its levels can result in various changes to other neurological functions and cause side effects.

While these and other methods may improve cognition, at least transiently, they do not address the disease state of the patient. For instance, Alzheimer’s Disease is typically associated with the formation of
plaques through the accumulation of amyloid precursor protein. Attempts to elicit an immunological response through treatment against amyloid and plaque formation has been done in animal models, but has not been successfully extended to humans.

Furthermore, cholinesterase inhibitors only produce some symptomatic improvement for a short time. Additionally, the use of cholinergic inhibitors only produces an improvement in a fraction of the Alzheimer’s Disease patients with mild to moderate symptoms and is thus only a useful treatment for a small portion of the overall patient population. Even more critical is that present efforts at improving cognition do not result in treatment of the disease condition, but are merely ameliorative of the symptoms. Current treatments do not modify the disease progression. These treatments have also included the use of a “vaccine” to treat the symptoms of Alzheimer’s Disease patients. While theoretically plausible and effective in mice tests, however, these tests have been shown to cause severe adverse reactions in humans.

As a result, use of the cholinergic pathway for the treatment of cognitive impairment, particularly in Alzheimer’s Disease, has proven to be inadequate. Additionally, the current treatments for cognitive improvement are limited to specific neurodegenerative diseases and have not proven effective in the treatment of other cognitive conditions.

There still exists a need for the development of methods for the treatment for improved overall cognition, either through a specific characteristic of cognitive ability or general cognition. There also still exists a need for the development of methods for the improvement of cognitive enhancement whether or not it is related to specific disease state or cognitive disorder.

**SUMMARY OF THE INVENTION**

The present invention relates to compounds, compositions, and methods for the treatment of conditions associated with enhancement/improvement of cognitive ability. In a preferred embodiment, the present invention further relates to compounds, compositions and methods for the treatment of conditions associated with amyloid processing, such as Alzheimer’s Disease, which provides for improved/enhanced cognitive ability in the subject treated.
In another aspect the present invention relates to bryostatin-1, a PKC activator, to alter conditions associated with amyloid processing in order to enhance the \( \alpha \)-secretase pathway to generate soluble \( \alpha \)-amyloid precursor protein (\( \alpha \)APP) so as to prevent \( \beta \)-amyloid aggregation and improve/enhance cognitive ability. Such activation, for example, can be employed in the treatment of Alzheimer’s Disease.

In another aspect, the present invention relates to a method for treating plaque formation, such as that associated with Alzheimer’s Disease, and improving/enhancing the cognitive state of the subject comprising administering to the subject an effective amount of a PKC activator. In a preferred embodiment, the PKC activator is bryostatin-1.

Another aspect of the present invention relates to a composition for treating plaque formation and improving/enhancing cognitive ability comprising: (i) bryostatin-1 in an amount effective to generate soluble \( \alpha \)APP and prevent \( \beta \)-amyloid aggregation; and (ii) a pharmaceutically effective carrier. In a preferred embodiment the composition is used to improve/enhance cognitive ability associated with Alzheimer’s Disease.

In one embodiment of the invention the activation of PKC isoenzymes results in improved cognitive abilities. In one embodiment the improved cognitive ability is memory. In another embodiment the improved cognitive ability is learning. In another embodiment the improved cognitive ability is attention.

In another aspect the invention comprises a composition of a PKC isoenzyme activator administered in an amount effective to improve cognitive abilities. In a preferred embodiment the PKC isoenzyme activator is selected from bryostatin-1, a benzolactam, or a 2-pyrrolidinone. In a preferred embodiment the amount of PKC activator administered is in an amount effective to increase the production of sAPP. In a more preferred embodiment the amount of bryostatin administered does not cause myalgia.

In a preferred embodiment the PKC isoenzymes are activated in subjects which are suffering or have suffered from neurological diseases, strokes or hypoxia. In a more preferred embodiment the PKC isoenzyme is activated in Alzheimer’s Disease subjects or models.

In another embodiment of the invention the PKC activation results in the modulation of amyloid precursor protein metabolism. Further the modulation by the PKC activation results in an increase in the
alpha secretase pathway. The alpha secretase pathway results in non-toxic, non-amyloidogenic fragments related to cognitive impairment. As a result the cognitive condition of the subject improves. In another embodiment of the invention the PKC activation reduces the amyloidogenic and toxic fragments Abeta 40 and Ab42.

Another embodiment of the invention is a method of improving cognitive ability through the activation of PKC isoenzymes. In another embodiment of the invention the PKC activation occurs in "normal" subjects. In another embodiment of the invention the PKC activation occurs in subjects suffering from a disease, deteriorating cognitive faculties, or malfunctioning cognition. In a preferred embodiment the method is a method for treating Alzheimer's Disease.

In another embodiment of the invention the modulation of PKC is through the use of a non-tumor promoting agent resulting in improved cognitive abilities. In a preferred embodiment the PKC activator is Bryostatin-1.

In another embodiment of the invention, the modulation of PKC through bryostatin is used in vitro for the testing of conditions associated with Alzheimer's Disease.

In a preferred embodiment of the invention the PKC isoenzyme activator is administered through oral and/or injectable forms including intravenously and intraventricularly.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the effect of different PKC inhibitors on sAPP resonance with Bryostatin-1 showing greater efficacy at lower concentrations than controls and Benzolactam.

Fig. 2 illustrates the effect of different concentrations of Bryostatin-1 on the PKC isoform.

Fig. 3 illustrates the effect of different concentrations of Bryostatin-1 on sAPP secretion.

Fig. 4 illustrates the amount of time required for treated rats versus controls to learn a water maze.

Fig. 5(a) illustrates the amount of time control rats spent swimming in the different quadrants.
Fig. 5(b) illustrates the amount of time treated rats spent swimming in the different quadrants.

Fig. 5(c) illustrates the difference between the amount of time the treated rats spent in target quadrant compared to control rats.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

Protein kinases serve a regulatory function which is crucial for all aspects of cellular development, differentiation and transformation. PKC was identified as one of the largest gene families of non-receptor serine-threonine protein kinases. The PKC gene family consists presently of 11 genes which are divided into four subgroups: 1) classical PKC, \( \beta_1 \), \( \beta_2 \) (\( \beta_1 \) and \( \beta_2 \) are alternatively spliced forms of the same gene) and \( \gamma \), 2) novel PKC\( \delta \), \( \varepsilon \), \( \eta \) and \( \theta \), 3) atypical PKC\( \zeta \), \( \lambda \), \( \eta \) and \( \iota \) and 4) PKC\( \mu \). Due to the differences in structural features, and the diversity of PKC isoforms, these proteins can play specialized roles in signal transduction cascades. The PKC isoenzyme provides a critical, specific and rate limiting molecular target through which a unique correlation of biochemical, biophysical, and behavioral efficacy can be demonstrated and applied to subjects to improve cognitive ability.

Of particular interest is the PKC activator bryostatin-1. Bryostatin-1 has been shown to activate PKC and proven to be devoid of tumor promotion activity. Bryostatin-1, as a PKC activator is also particularly useful since the dose response curve of bryostatin-1 is biphasic. Additionally, bryostatin-1 demonstrates differential regulation of PKC isoforms, including PKC\( \alpha \), PKC\( \delta \), and PKC\( \zeta \). Bryostatin-1 has undergone toxicity and safety studies in animals and humans and is actively being investigated as an anti-cancer agent. Bryostatin-1’s use in the studies has determined that the main adverse reaction in humans is myalgia, limiting the maximum dose to 40 mg/m\(^2\). The present invention has utilized concentrations of 0.1 nM of bryostatin-1 to cause a dramatic increase of sAPP secretion. Bryostatin-1 has been compared to a vehicle alone and to another PKC activator, benzo lactam (BL), used at a concentration 10,000 times higher. Bryostatin used at 0.01 nM also still proved effective to increase sAPP secretion. (See Figure 1). PKC translocation shows that a measure of activation is maximal at 30 min, followed by a partial decline, which remains higher than basal translocation levels up to six hours. (see Figures 2 & 3). The use of the PKC inhibitor staurosporin completely
prevents the effect of bryostatin on sAPP secretion. The data further demonstrates that PKC activation mediates the effect of the bryostatin on sAPP secretion. (see Figures 1-3)

The effect of PKC activators on cognition was demonstrated by the Morris Water Maze paradigm. In the present example, rats were injected intraventricularly with bryostatin and trained for 4 days (following standard protocols). Retention was assessed on the 5th day. Learning was measured as the reduction of escape latency from trial to trial, which was significantly lower in the treated animals. Acquisition of memory was measured as time spent in the relevant quadrant (5th day). Memory or retention was significantly enhanced in treated animals, compared to sham injection animals (see Figures 4 through 5(a)-5(c)). The rats treated with bryostatin-1 showed improved cognition over control rats within 2 days of treatment (see Figure 4).

Bryostatin-1 is capable of being used at concentrations to improve cognition which are 300 to 300,000 times lower than the concentration used to treat tumors. The above example further shows that cognitive ability can be improved in non-diseased subjects as compared to other non-diseased subjects through the administration of bryostatin-1.

Because of the previously conducted safety, toxicology and phase II clinical studies for cancer, one can conclude that the use of PKC activators, particularly bryostatin-1, would be viewed as safe and that phase II studies for AD treatment/cognitive enhancement could be expedited. Furthermore, bryostatin-1’s lipophilic nature provides increased blood brain barrier transport. The present invention would allow for intravenous, oral, intraventricular, and other known methods of administration.

Test of sAPP secretion experiments, PKC activation experiments, and animal behavior experiments have shown that increases in sAPP secretion follow increased PKC activation and result in improved cognition in animal behavior studies.
ABSTRACT

The present invention relates to the use of PKC activators, particularly bryostatin-1, to improve cognitive ability. The invention further relates the improved/enhanced cognitive ability in diseased individuals, particularly Alzheimer's Disease patients, and treatment thereof through increased sAPP production.
Claims:

1. A method for enhancing cognitive ability comprising administering an effective amount of a PKC activator in a pharmacologically suitable carrier.

2. The method of claim 1 wherein the PKC activator selectively activates PKCa, PKCδ, and PKCε.

3. The method of claim 1, wherein the PKC activator is selected from benzolactam, 2-pyrollidinone, bryostatin-1.

4. The method of claim 1, wherein the cognitive ability enhancer is selected from learning, memory, and attention.

5. The method of claim 1 wherein the PKC activator is administered to a subject.

6. The method of claim 5, wherein the subject is selected from a non-primate and a primate.

7. The method of claim 1, wherein the amount of PKC activator administered is in an amount effective to treat cognitive impairment of a neurological disease or disorder.

8. The method of claim 7, wherein the neurological disease is selected from Alzheimer’s disease and attention deficit hyperactivity disorder.

9. The method of claim 1, wherein the administration of the PKC activator further causes an increase in sAPP.

10. A method of treating neurtumors comprising administering an effective amount of bryostatin-1.
Fig 1

sAPP-α Secretion
(Human Fibroblast AG06848)
Fig 2

PKC-α

- Bryo 0.1 nM
- DMSO
- Bryo 0.01 nM

P/S (normalized)

Time (min)

0 20 40 60 80 100 120 140 160 180
Fig. 3

sAPP\(\alpha\) secretion

- DMSO
- Bryo-0.1 nM
- Bryo-0.01 nM

Relative Units (normalized to controls)

Time (mins)
Bryostatin-1 (i.c.v.; 1 μl/site of 2 μM solution; ~0.5 hr prior to the 1st and 5th training trials); 10 rats/group.