Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2562035 C 2015/01/06

(11)(21) 2 562 035

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2005/05/18
(87) Date publication PCT/PCT Publication Date: 2005/12/08
(45) Date de délivrance/Issue Date: 2015/01/06
(85) Entrée phase nationale/National Entry: 2006/10/02
(86) N° demande PCT/PCT Application No.: US 2005/017158
(87) N° publication PCT/PCT Publication No.: 2005/115548
(30) Priorité/Priority: 2004/05/18 (US60/571,892)

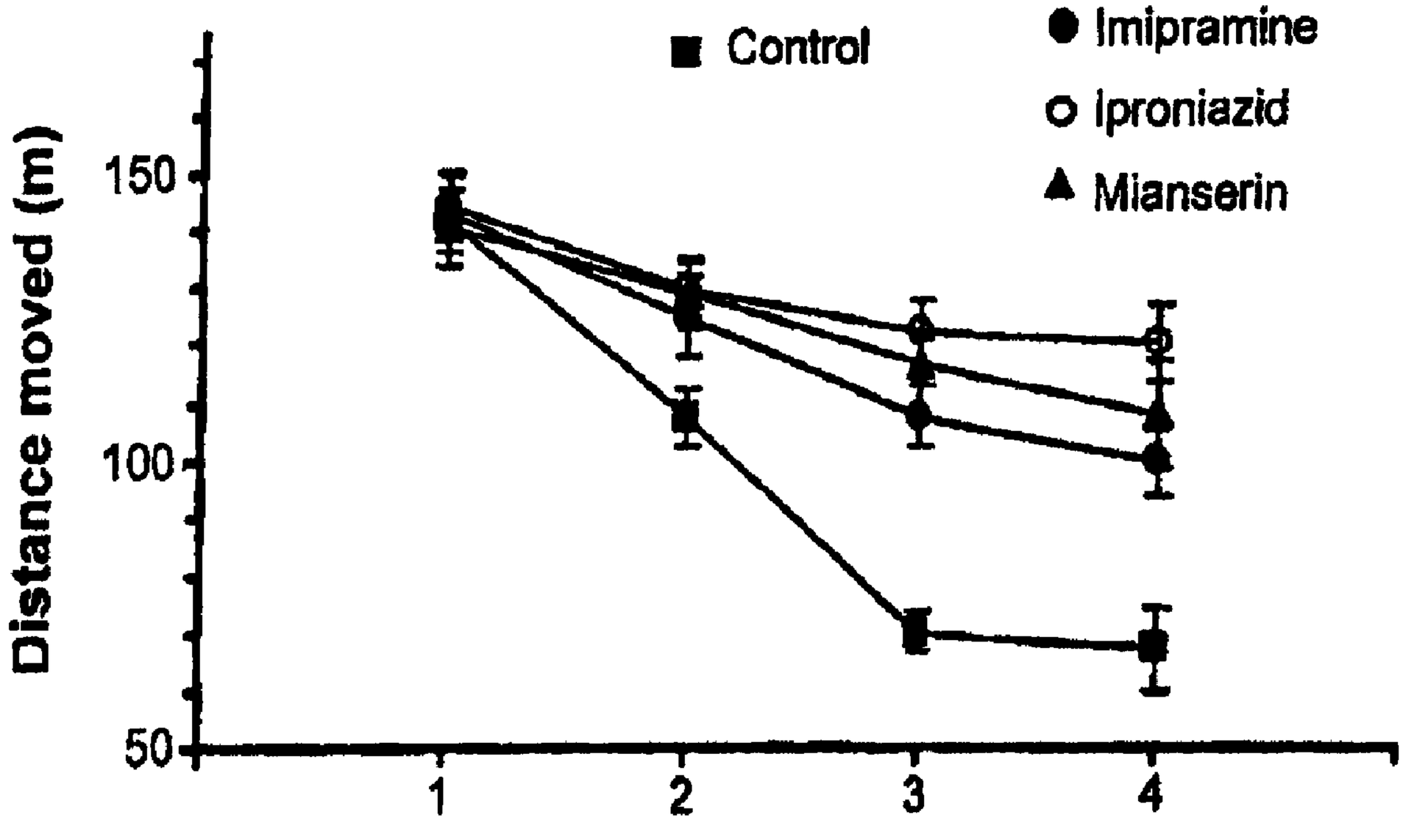
(51) Cl.Int./Int.Cl. *A61K 38/18* (2006.01), *A61K 31/137* (2006.01), *A61K 31/198* (2006.01), *A61K 31/365* (2006.01), *A61K 31/415* (2006.01), *A61K 31/417* (2006.01), *A61P 25/24* (2006.01), *C12Q 1/00* (2006.01)

(72) Inventeurs/Inventors:
SUN, MIAO-KUN, US;
ALKON, DANIEL L., US

(73) Propriétaire/Owner:
BLANCHETTE ROCKEFELLER NEUROSCIENCES INSTITUTE, US

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : TRAITEMENT DE TROUBLES DEPRESSIFS
(54) Title: TREATMENT OF DEPRESSIVE DISORDERS



(57) Abrégé/Abstract:
The invention provides for the use of carbonic anhydrase activators; protein kinase C activators and FGF-18 to treat depressive disorders. The invention also relates to improved animal models and methods for screening and identifying compounds the treatment of depressive disorders.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2005 (08.12.2005)

PCT

(10) International Publication Number
WO 2005/115548 A2

(51) International Patent Classification[7]: A61P 25/24, A61K 31/365, 31/192, 31/196, 31/045, 31/05, 31/4164, 31/4172

(21) International Application Number: PCT/US2005/017158

(22) International Filing Date: 18 May 2005 (18.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/571,892 18 May 2004 (18.05.2004) US

(71) Applicant *(for all designated States except US)*: **BLANCHETTE ROCKEFELLER NEUROSCIENCES INSTITUTE** [US/US]; P.O. Box 9301, Medical Center Drive, Morgantown, WV 26596-9301 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SUN, Miao-Kun** [US/US]; 16012 Howard Landing Drive, Gaithersburg, MD 20878 (US). **ALKON, Daniel, L.** [US/US]; 6701 Bonaventura Court, Bethesda, MD 20817 (US).

(74) Agent: **LONGTON, Enrique, D.**; Milbank, Tweed, Hadley & McCloy LLP, International Square Building, 1825 Eye Street, N.W., Suite 1100, Washington, D.C. 20006 (US).

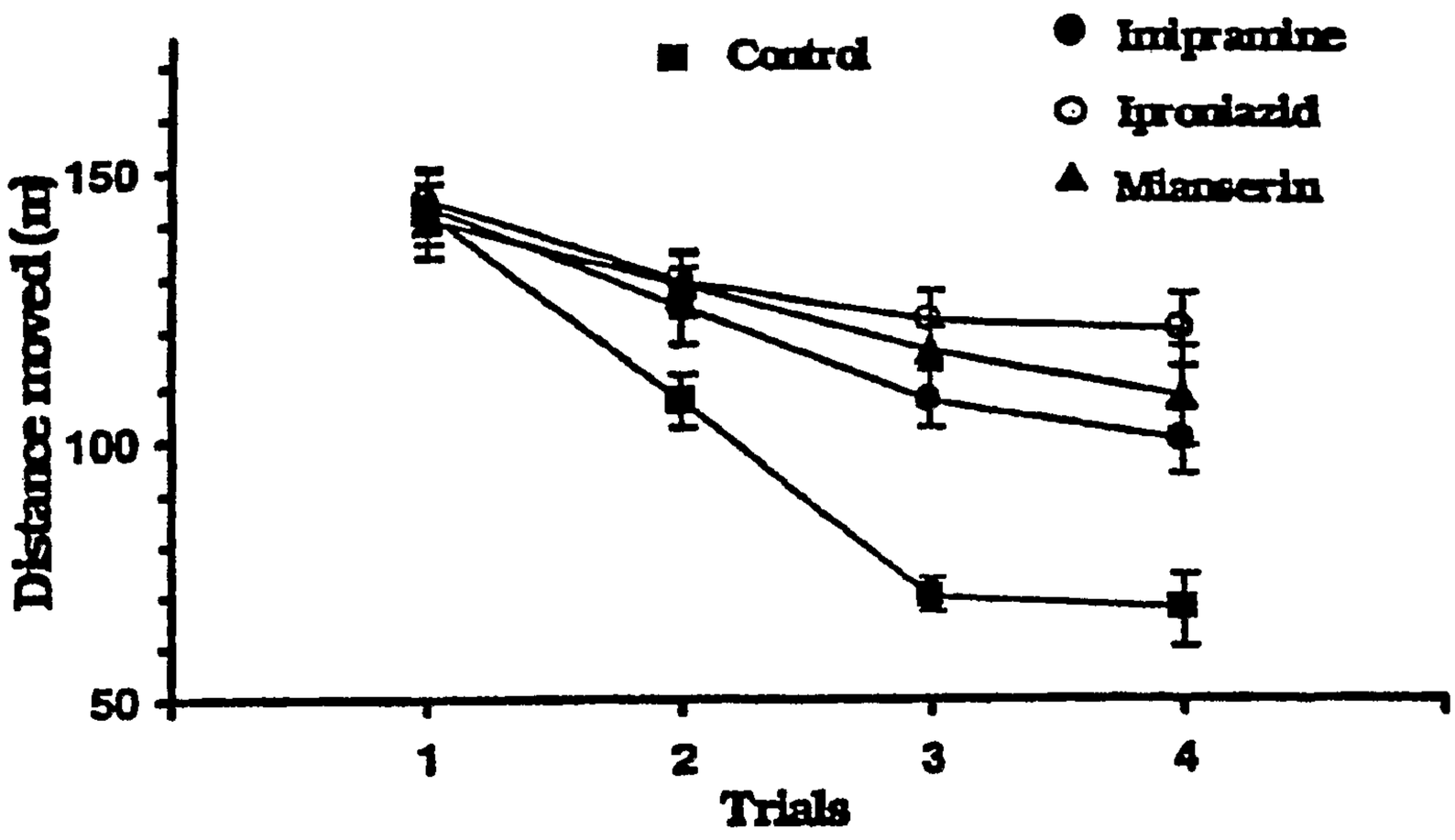
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK,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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: TREATMENT OF DEPRESSIVE DISORDERS



(57) Abstract: The invention provides for the use of carbonic anhydrase activators; protein kinase C activators and FGF-18 to treat depressive disorders. The invention also relates to improved animal models and methods for screening and identifying compounds the treatment of depressive disorders.

WO 2005/115548 A2

WO 2005/115548 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TREATMENT OF DEPRESSIVE DISORDERS

FIELD OF THE INVENTION

[0001] The present invention relates to the treatment of depressive disorders with compounds that improve learning and memory. Specifically, the invention relates to the fields of carbonic anhydrase activators, protein kinase C activators and fibroblast growth factors as pharmaceutical agents in the treatment of depressive disorders. The present invention also relates to the field of animal models for depressive disorders.

BACKGROUND OF THE INVENTION

A. Depression and Traditional Treatment

[0002] Depression is one of the most prevalent and pervasive forms of mental illness that affects individuals across age and gender lines. (Gainotti *et al.* (2001) *J. Neural Neurosurg. Psychiatr.* 71: 258-261; Wong *et al.* (2001) *Nature Rev. Neurosci.* 2: 343-351; Nestler *et al.* (2002) *Neuron* 34: 13-25). The lifetime risk of major depression is about 12% in men and about 25% in women, generally, (Kessler *et al.* (1994) *Arch. Gen. Psychiatry* 51: 8). In addition, about 5 to 10% of all patients in the primary care environment, present with major depression, whereas about 3 to 5% of patients are diagnosed with dysthymia. (Barrett *et al.* (1988) *Arch. Gen. Psychiatry* 45: 1100). In an in-patient setting, however, between 10 and 14% of all patients are diagnosed with major depression. (Blackburn *et al.* (1997) *Br. J. Psychiatry* 171: 328). Major depression is a particularly disabling and pernicious, in part, because it is recurring. The rate of relapse for patients with major depression is about 40% over a two-year period after a first episode. The occurrence of relapse increases to about 75% within a five year period after the diagnosis of a second episode of major depression. (Solomon *et al.* (2000) *Am. J. Psychiatry* 157: 229).

[0003] Depressive disorders are most commonly treated with three main classes of compounds: 1) monamine oxidase inhibitors; 2) heterocyclic antidepressants; and 3) selective serotonin reuptake inhibitors (SSRIs). The known and currently prescribed antidepressants are by numerous side effects. Monoamine oxidase inhibitors were the first class of antidepressants used clinically. Monoamine oxidase inhibitors, including isocarboxazid, phenelzine, and tranylcypromine, inhibit the metabolism of phenylethylamine and catabolism of dopamine, serotonin and norepinephrine. As a consequence of numerous

dietary restrictions associated with the use of monoamine oxidase inhibitors, extensive side effects, including hypertension, headache, myoclonic jerk, sleep disruption, and gastrointestinal complications, monoamine oxidase inhibitors are currently not used as a first-line antidepressant. The tricyclic antidepressants, including, imipramine, desipramine, nortrypline, amitrypline, doxepin and protrypline, produce a variety of anticholinergic side effects, drowsiness, orthostatic hypotension, cardiac arrhythmias and weight gain. Although generally milder than the monoamine oxidase inhibitors and the tricyclic antidepressants, SSRIs also produce numerous side effects. For example, SSRIs, including fluoxetine, paroxetine, fluvoxamine, sertraline, and citalopram, are associated with gastrointestinal distress, jitteriness, agitation and sleep disruption.

[0004] In addition to the numerous side effects associated with traditional antidepressant medications, these therapeutics are also characterized by marginal efficacy. Several studies on the efficacy of antidepressant therapy for major depression have concluded that the treatment of acute disease or maintenance therapy is associated with a 50-60% response rate. (Schulberg *et al.* (1998) *Arch. Gen. Psychiatry* 55: 1121). The average absolute response rate between antidepressants and placebo is about 20-25%. (Williams *et al.* (2000) *Ann. Intern. Med.* 132: 743). Consequently, there is a current need for new antidepressant therapies.

[0005] In view of the sometimes severe adverse side effects and marginal efficacy of numerous antidepressant therapies, there is a great need for improved pharmaceuticals that effectively treat depressive disorders without producing the side effects associated with treatments of depression. The present invention identifies those compounds that enhance or improve learning and memory as a new class of therapeutics for the treatment of depressive disorders.

B. Carbonic Anhydrase

[0006] Carbonic anhydrase, a zinc-containing enzyme that catalyzes the interconversion of carbon dioxide and bicarbonate anion, is present throughout the body, including the brain. (Sun *et al.* (2002) *Trends in Pharm. Sci.* 23(2): 83-89)

Carbonic anhydrase II, the most active of the seven human isozymes, is a 23.9 kDa enzyme found primarily in erythrocytes, glial cells and brain

neurons. (*Id.*). In addition to its involvement in pH regulation, bicarbonate reabsorption and carbon dioxide expiration, carbonic anhydrase plays a crucial role in signal processing, long-term synaptic transformation and attentional gating of memory storage. (*Id.*).

[0007] Carbonic anhydrase dysfunction has been associated with mental retardation, Alzheimer's disease, and impaired cognition. Conversely, the activation of carbonic anhydrase has been demonstrated to improve learning and memory. (*Id.*; U.S. Patent Application Serial Nos. PCT/US02/13784; PCT/US03/07102; 60/287,721; 60/362,081; 10/172,005; and 10/476,459) Prior to the present disclosure, however, the carbonic-anhydrase-mediated improvement of learning and memory has not been recognized as a mechanism for the treatment of depressive disorders. Although a recent biochemical study using isolated carbonic anhydrase identified three SSRIs, fluoxetine, sertraline and citalopram, as activators of carbonic anhydrase, these experiments did not demonstrate that carbonic anhydrase activation is the mechanism whereby the symptoms of depressive disorders are ameliorated. (Casini *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13: 2765-2768).

[0008] Carbonic anhydrase activity is regulated by signaling pathways that include ryanodine receptor-mediated signaling pathways. The intracellular release of calcium through ryanodine receptors, for example, is involved in the GABA-mediated synaptic switch. (Sun *et al.* (2002) *Trends in Pharmacol. Sci.* 23(2): 83-89). Activation of ryanodine receptors in CA1 pyramidal cells, combined with depolarization induced calcium loading, transforms GABA-mediated responses, an effect that is blocked by ryanodine-receptor antagonists or carbonic anhydrase inhibitors. (Sun *et al.* (2000) *Proc. Nat'l Acad. Sci USA* 97: 12300-12305). The effect of calcium on carbonic anhydrase, however, appears to be indirect. For example, early studies show that calcium potentiates the activation of either purified carbonic anhydrase or gastric mucosa carbonic anhydrase by histamine and other agents. (Puscas *et al.* (1996) *J. Pharmacol. Exp. Ther.* 277: 1464-1466). In addition, the dose-dependent inhibition of carbonic anhydrase by verapamil also implicates calcium in the activation of carbonic anhydrase. Furthermore, in human myelomonocytic cell lines, the synthesis of carbonic anhydrase II is activated by protein kinase C. (Sun *et al.* (2002) *Trends in Pharmacol. Sci.* 23(2): 83-89). Consequently, the PKC-mediated increase in carbonic

anhydrase synthesis can increase carbonic anhydrase activity that has a resultant antidepressant effect.

C. Protein Kinase C

[0009] PKC has been identified as one of the largest gene families of non-receptor serine-threonine protein kinases. Since the discovery of PKC in the early eighties by Nishizuka and coworkers (Kikkawa *et al.* (1982) *J. Biol. Chem.* 257: 13341), and its identification as a major receptor for phorbol esters (Ashendel *et al.* (1983) *Cancer Res.*, 43: 4333), a multitude of physiological signaling mechanisms have been ascribed to this enzyme. The intense interest in PKC stems from its unique ability to be activated *in vitro* by calcium and diacylglycerol (and its phorbol ester mimetics), an effector whose formation is coupled to phospholipid turnover by the action of growth and differentiation factors.

[0010] The activation of PKC has been shown to improve learning and memory. (U.S. Patent Application Serial Nos. PCT/US02/13784; PCT/US03/07102; 60/287,721; 60/362,081; 10/172,005; and 10/476,459)

Prior to the present disclosure, however, the PKC-mediated improvement of learning and memory has not been recognized as a mechanism for the treatment of depressive disorders. Also, the PKC activators disclosed herein, specifically those compounds that improve learning and memory, were not recognized as possessing antidepressant activity.

D. Fibroblast Growth Factor-18 (FGF-18)

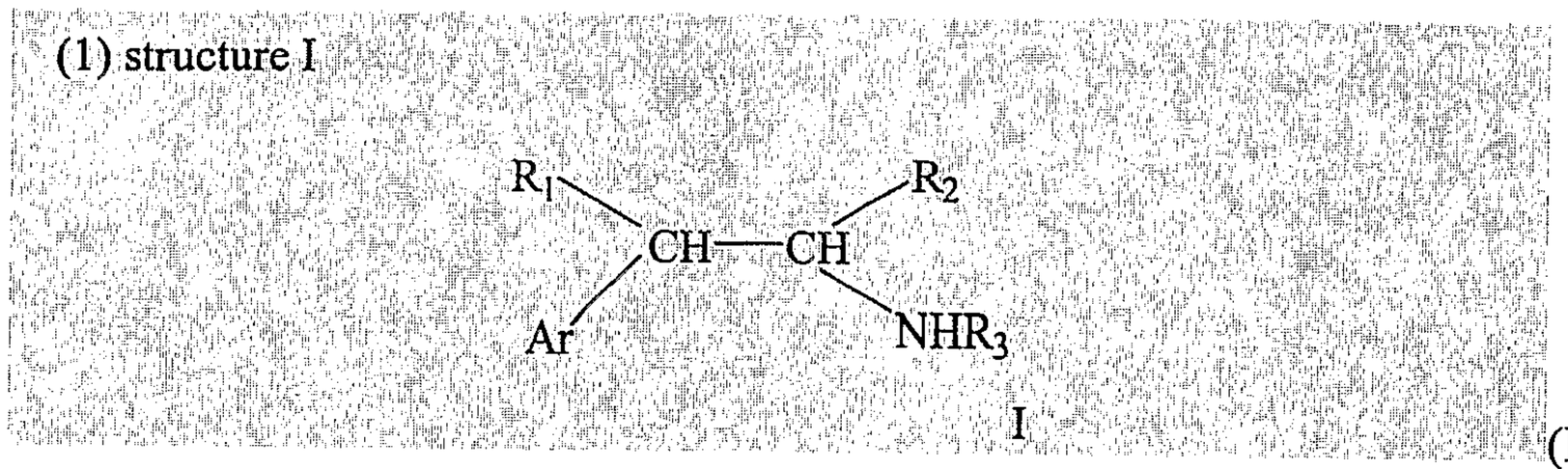
[0011] Fibroblast Growth Factor-18 (FGF-18) has been shown to improve learning and memory. (U.S. Provisional Application Serial No. 60/429,321 and PCT/IB03/05408) Binding of FGF-18 to its cognate receptor activates a PKC-mediated signaling pathway that implicates both PKC and carbonic anhydrase. Prior to the present disclosure, however, the FGF-18-mediated improvement of learning and memory has not been recognized as a mechanism for the treatment of depressive disorders. Also, FGF-18 has not been recognized as possessing antidepressant activity.

SUMMARY OF THE INVENTION

[0012] The present invention provides a new class of therapeutics, compounds and compositions that enhance or improve learning and memory, for the treatment of depressive disorders. The present invention provides methods of treating depressive disorders comprising the administration of compositions comprising compounds that enhance or improve learning and memory.

[0013] The present invention provides a method comprising the steps of a) identifying a subject with a depressive disorder; and b) administering an effective amount of a composition comprising a carbonic anhydrase activator and a pharmaceutically acceptable carrier to said subject, wherein the carbonic anhydrase activator being selected from the group consisting of:

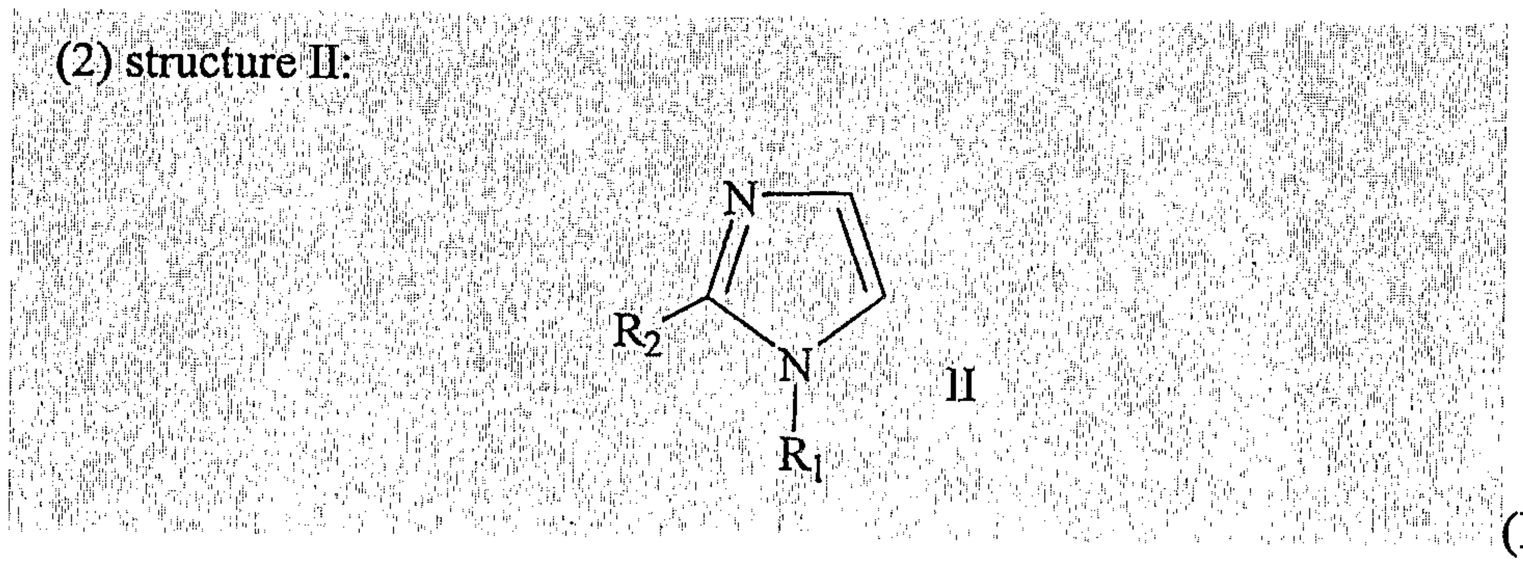
(1) structure I



I (I)

wherein R_1 is H or OH; R_2 and R_3 are independent H, COOH or lower alkyl, for example linear, branched or cyclic C_1-C_6 alkyl or C_1-C_4 alkyl; and Ar is phenyl, imidizolyl or phenyl or imidizolyl substituted with one or more halo, hydroxy, amino or lower alkyl groups for example linear, branched or cyclic C_1-C_6 group or C_1-C_4 alkyl group;

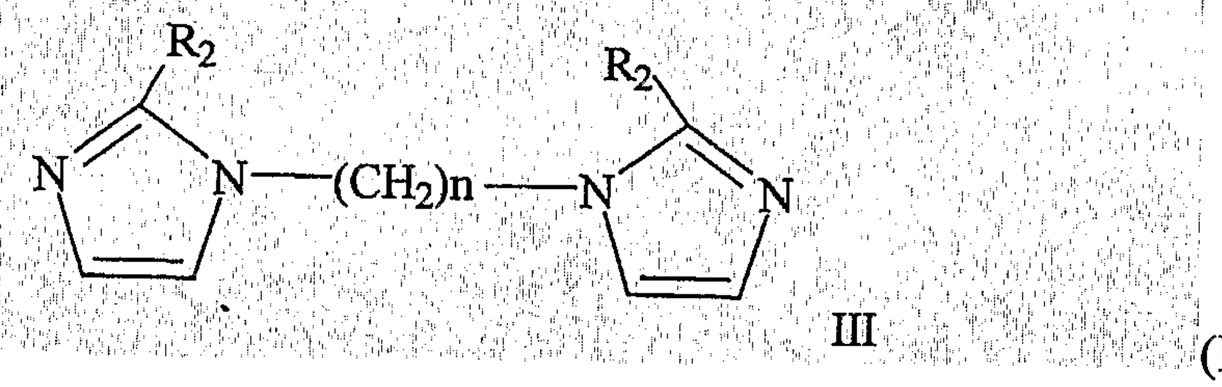
(2) structure II:



II (II)

wherein R1 and R_2 are independently H or lower alkyl, for example linear, branched or cyclic C_1-C_6 alkyl or C_1-C_4 alkyl;

(3) structure III:



(III)

[0014] wherein *n* is 1 or 2 and R_2 is H or lower alkyl, for example linear, branched or cyclic C_1-C_6 alkyl or C_1-C_4 alkyl; and pharmaceutically acceptable salts of I, II, or III. In one embodiment of the present invention, the activator has structure I, wherein R_1 is H or OH; R_2 is H, CH_3 or COOH; R_3 is H or CH_3; and Ar is phenyl, or a substituted phenyl. In a preferred embodiment, the substituted phenyl is 4-hydroxyphenyl, 4-fluorophenyl, 4-aminophenyl, 3-amino-4-hydroxyphenyl, or 3,4-dihydroxyphenyl.

[0015] The present invention also contemplates methods using derivatives and analogs of the carbonic anhydrase activators disclosed herein, wherein the derivatives and analogs increase the potency of the carbonic anhydrase activating effect, increase the specificity to carbonic anhydrase as compared to other targets, reduce toxicity, improve stability in an oral dosage form, and/or enhance the ability of the compound to cross the blood brain barrier (pro-drugs). Derivatives are compounds formed by adding or removing side chains from the listed compounds. Analogs are structural variants of the compounds having enhanced similar physical and/or chemical properties with respect to the binding site of carbonic anhydrase. Derivates and analogs according to the invention are those which are able to deliver the activator compounds of the invention to the brain of a subject.

[0016] In one embodiment of the present invention, the carbonic anhydrase activators provide neuronal carbonic anhydrase activity of at least about 110, 115, 125, 135, 150, 170, 180, 190, 200, 210, 220, 220, 230, 240 and 250% that of alanine.

[0017] In one embodiment, the activator of the present invention is an aromatic amine or an aromatic amino acid of structure I, II or III. In a preferred embodiment, the activator activates intraneuronal carbonic anhydrase. In another embodiment, the activator activates carbonic anhydrase II between 1.5- and 2-times more than alanine, *in vitro*.

[0018] In another embodiment of the present invention, the activator has structure I wherein R_1 is H or OH; R_2 is H, CH_3 or COOH; R_3 is H or CH_3; and Ar is imidazole or a substituted imidazole. In a preferred embodiment, the substituted imidazole is imadazol-4-yl-, or 5-methylimidazole-4-yl-.

[0019] In a preferred embodiment, the activator is selected from the group consisting of: imidazole, phenylalanine, a substituted ethylamine, phenethylamine, histamine, histidine, a linked di-imidazole, a triazole, and pharmaceutically acceptable salts thereof. More preferably the activator is histidine; histamine; phenylalanine; 4-hydroxy phenylalanine; 4-fluoro phenylalanine; 3, 4-dihydroxy phenylalanine; 3-amino-4-hydroxyphenylalanine; 4-amino phenylalanine; tyrosine; dopamine; noradrenaline; adrenaline or 5-methyl histamine.

[0020] The present invention also provides a method comprising the steps of a) identifying a subject with a depressive disorder; and b) administering an effective amount of a composition comprising a carbonic anhydrase activator and a pharmaceutically acceptable carrier to said subject, wherein the activator has structure II and further wherein R_1 is H, methyl, ethyl or propyl; and R_2 is H or methyl. In one embodiment, the activator activates carbonic anhydrase II between 1.5- and 2-times more than alanine, *in vitro*.

[0021] The present invention also provides a method comprising the steps of a) identifying a subject with a depressive disorder; and b) administering an effective amount of a composition comprising a carbonic anhydrase activator and a pharmaceutically acceptable carrier to said subject, wherein the activator is structure III and further wherein *n* is 1 or 2; and R^2 is H or methyl. In one embodiment, the activator activates carbonic anhydrase II between 1.5- and 2-times more than alanine, *in vitro*.

[0022] The present invention also provides methods of treating depression in a subject in need thereof, comprising administering an effective amount of a composition comprising a carbonic anhydrase activator and a pharmaceutically acceptable carrier, wherein the activator is selected from the group consisting of: an aromatic amine or an aromatic amino acid, wherein the aromatic amine or aromatic amino acid contains a single aromatic group. In one embodiment, the aromatic amine or aromatic amino acid activates carbonic anhydrase II between 1.5- and 2-times more than alanine. In a preferred embodiment, the activator is an aromatic amino acid selected from the group consisting of: phenylalanine, a

substituted phenylalanine, histidine, a substituted histidine, a substituted phenylalanineimidazole, a substituted imidazole, a linked di-imidazole, and a linked substituted di-imidazole. Preferably, the activator is an aromatic amine selected from the group consisting of dopamine, noradrenaline, adrenaline, histamine, and 5-methyl histamine.

[0023] The present invention provides a method comprising the steps of a) identifying a subject with a depressive disorder and b) administering an effective amount of a composition comprising a protein kinase C activator and a pharmaceutically acceptable carrier to said subject, wherein the PKC activator is selected from a group consisting of: FGF-18, a macrocyclic lactone, a benzolactam, a pyrrolidinone, or a combination thereof. In a preferred embodiment, the macrocyclic lactone is a bryostatin or neristatin. In a more preferred embodiment, the bryostatin is selected from a group consisting of bryostatin-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18. Most preferably, the bryostatin is bryostatin-1 and the neristatin is neristatin-1.

[0024] The present invention also provides methods of treating depression in a subject in need thereof, comprising administering an effective amount of a composition comprising a protein kinase C activator and a pharmaceutically acceptable carrier, wherein the activator is selected from the group consisting of: FGF-18, a macrocyclic lactone, a benzolactam, a pyrrolidinone, or a combination thereof. In a preferred embodiment, the macrocyclic lactone is a bryostatin or neristatin. In a more preferred embodiment, the bryostatin is selected from a group consisting of bryostatin-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18. Most preferably, the bryostatin is bryostatin-1 and the neristatin is neristatin-1.

[0025] The present invention also provides methods of treating depression in a subject in need thereof, comprising administering an effective amount of a composition comprising one or more of the following: tacrine (marketed under the tradename COGNEX®), velnacrine, donepezil (marketed under the tradename ARICEPT®), galantamine (marketed under the tradename REMINYL®), memantine (marketed under the tradename NAMENDA®), or pharmaceutically acceptable salts thereof.

[0026] The present invention also provides methods for screening an agent for antidepressant activity, comprising the steps of:

a) administering an agent in a pharmaceutically acceptable carrier to a test subject and administering the pharmaceutically acceptable carrier to the control subject;

b) individually placing said test and control subject into a pool of water and measuring the distance and/or duration of swimming during a testing period; and

c) comparing the distance or duration of swimming of the test subject to a control subject, wherein increased distance or duration of swimming of the test subject compared to the control subject is indicative of antidepressant activity.

[0027] In a preferred embodiment, the water pool is round. Preferably, the pool has a diameter of between 100 and 200 cm. More preferably, the pool has a diameter of 150 cm. Preferably, the pool provides no escape.

[0028] In another embodiment, steps (a), (b), and (c) are repeated. Preferably, the steps are repeated three times.

[0029] In the methods for screening an agent for antidepressant activity of the present invention, the distance and/or duration of swimming is preferably measured by video means.

BRIEF DESCRIPTION OF THE FIGURES

[0030] **Figure 1** depicts the effect of three antidepressants, imipramine, iproniazid and mianserin, on the mobility of rats in the open-space-swimming test.

[0031] **Figure 2** depicts the effect of alaproclate on the mobility and duration of active swimming of rats in the open-space-swimming test.

[0032] **Figure 3** compares the effect of imipramine and the carbonic anhydrase activator, phenylalanine, on the mobility of rats in the open-space-swimming test.

[0033] **Figure 4** compares the effect of imipramine and the PKC activator, bryostatin-1, on the mobility of rats in the open-space-swimming test.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

[0034] As used herein, "administration" of a composition includes any route of administration, including oral subcutaneous, intraperitoneal, and intramuscular.

[0035] As used herein, the term "aromatic" means a cyclically conjugated molecular entity with a stability significantly greater than that of a hypothetical localized structure. As used herein, aromatic compounds include polycyclic and heterocyclic compounds.

[0036] As used herein, "carbonic anhydrase activator" means a substance that increases the rate of the reaction catalyzed by carbonic anhydrase by binding to the carbonic anhydrase at or near a zinc-bound water.

[0037] As used herein, "depressive disorder" means major depression, dysthymia, and atypical depression or depression not otherwise specified.

[0038] As used herein, "an effective amount" is an amount sufficient to reduce one or more symptoms associated with a depressive disorder.

[0039] As used herein, "protein kinase C activator" or "PKC activator" means a substance that increases the rate of the reaction catalyzed by protein kinase C by binding to the protein kinase C.

[0040] As used herein, the term "a single aromatic group" means only one monocyclic or one polycyclic aromatic group.

[0041] As used herein, the term "subject" means a mammal.

[0042] As used herein, the term "substituted imidazole" means an imidazole moiety with one or more substituent groups attached to the imidazole ring.

[0043] As used herein, the term "substituted phenyl" means a phenyl moiety with one or more substituent groups attached to the phenyl ring.

[0044] As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition with which the active ingredient may be combined and which,

following the combination, can be used to administer the active ingredient to a subject. As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

[0045] As used herein, "pharmaceutically acceptable carrier" also includes, but is 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" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.

[0046] The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0047] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the

pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, and other mammals.

[0048] The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient. In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers. Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

[0049] The effective dose for administration of the compounds is one that enhances carbonic anhydrase activity in cells of neuronal signaling pathways. Typically dosages of the compound of the invention which may be administered to an animal, preferably a human, range in amount from 1 mg to about 100 grams check the invention disclosure per kilogram of body weight of the animal. While the precise dosage administered will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration. Preferably, the dosage of the compound will vary from about 1 mg to about 10 g per kilogram of body weight of the animal. More preferably, the dosage will vary from about 10 mg to about 1 g per kilogram of body weight of the animal. Most preferably, the dosage is between 50 and 100 mg per kilogram of body weight of the animal and is administered orally three times daily.

[0050] Extrapolating from rat dosing, which predictive of human dosing, effective does of a phenylalanine (50 mM) or imidazole (0.5 M) agents for treating humans may include the equivalent of 0.1, 0.3, 1, 3 or 10 ml/kg body weight taken thrice daily.

[0051] The invention encompasses derivatives and analogs of these compounds which increase the potency of the carbonic anhydrase activating effect, increase the specificity to carbonic anhydrase as compared to other targets, reduce toxicity, improve

stability in an oral dosage form, and/or enhance the ability of the compound to cross the blood brain barrier (pro-drugs). Derivatives are compounds formed by adding or removing side chains from the listed compounds. Analogs are structural variants of the compounds having enhanced similar physical and/or chemical properties with respect to the binding site of carbonic anhydrase. Derivates and analogs according to the invention are those which are able to deliver the activator compounds of the invention to the brain of a subject.

[0052] The compound may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the memory, attention or learning deficit being treated, the type and age of the animal, etc.

B. Depressive Disorders

[0053] Depressive disorders encompass the diagnoses of major depression, dysthymia, and atypical depression or depression not otherwise specified ("minor depression"). The different subgroups of depressive disorders are categorized and defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). (American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Ed., Primary Care Version (DSM-IV-PC). American Psychiatric Association Press, Washington, DC 1995). According to the DSM-IV, a diagnosis of "major depression" requires that a patient present with at least five of the following nine symptoms during the diagnostic period: 1) depressed mood most of the day (most acute in the morning); 2) markedly diminished interest or pleasure in nearly all activities (anhedonia); 3) significant weight loss or gain; 4) insomnia or hypersomnia; 5) psychomotor agitation or retardation; 6) fatigue or energy loss; 7) feelings of guilt and worthlessness; 8) impaired concentration and indecisiveness; and 9) recurring thoughts of death or suicide. To support a diagnosis of major depression, a depressed mood or loss of interest (anhedonia) must be one of the five observed symptoms. In contrast, a diagnosis of "atypical depression" or "depression not otherwise specified" (also referred to as "minor depression"), the most common form of depression, requires between 2 and 4 depressive symptoms that are present daily or for most of the day

for at least a two week period. Dysthymia is a chronic, low intensity mood disorder characterized by anhedonia, low self esteem and low energy that persists for more than two years, consecutively. Seasonal affective disorder is considered to be a form of major depression characterized by seasonal variation.

C. Animal Models

[0054] Despite progress toward the development of new therapeutic agents and availability of several animal models, there is still a pressing need for improved animal models for screening antidepressant activity of candidate compounds. (Cryan *et al.* 2002 *Trends Pharmacol* 23: 238-45). Currently, the most widely used animal models of depression include the forced-swimming tests, tail-suspension test, and olfactory bulbectomy (Cryan *et al.* (2002) *Trends Pharmacol.* 23: 238-45). The forced-swimming test measures immobility of animals in an inescapable cylinder of water, 24 h after a 15 minute pretest in the same cylinder. It is the most widely-used model for preclinical prediction of antidepressant activity, but requires judgments and scoring by the investigators. The test does not reliably predict the antidepressant activity of selective serotonin-reuptake inhibitors (Porsolt (1990) Behavioral Despair: Present Status and Future Perspectives, In: Antidepressants: Thirty Years On, CNS Publishers 85-94; Porsolt *et al.* (1991) Pharmacological Models of Depression, In: Animal Models in Psychopharmacology, Advances in Pharmacological Sciences. Basel: Birkhauser 137-59; Takamori *et al.* (2001) *Pharmacology* 73: 147-53; Cryan *et al.* (2002) *Trends Pharmacol.* 23: 238-45) and requires a modified scoring to improve detection (Detke *et al.* (1995) *Psychopharmacology* 121: 66-72; Detke *et al.* (1996) *Behav. Brain Res.* 73: 43-46; Cryan *et al.* (2002) *Trends Pharmacol.* 23: 238-245). The tail-suspension test induces a state of a lack of despair characterized by a lack of effort or struggle to escape, that is acutely reversed by antidepressants. Some animals, however climb their tails. Olfactory bulbectomy, bilateral removal of the olfactory bulbs, on the other hand, results in a behavioral change correlated with changes in the depression. A hyperactive response in a novel, brightly lit open field apparatus, is reversed chronically, but not acutely, by antidepressant treatment (Kelly *et al.* (1997) *Pharmacol. Ther.* 74: 299-316). However, the test is based on the behavioral similarity to depression. Its mechanism of action is poorly understood. For more detailed discussion of a variety of animal models, the

reader is referred to two recent review articles (Cryan *et al.* 2000 *Trends Pharmacol.* 23: 238-245; Nestler *et al.* (2002) *Neuron* 34: 13-25).

[0055] To improve the understanding of the causal mechanisms of depression, we need animal models that mirror the situation in patients (Cryan *et al.* 2000 *Trends Pharmacol.* 23: 238-245). One difference between human depression and the standard forced swimming model (also called the behavioral-despair model) is that human depression is manifested in a majority of cases as a lack of motivation (hopelessness) and does not have a direct counterpart to the main feature of the forced-swing model - a lack of "physical space" that operates as the inducer of depression.

[0056] The present invention provides an improved animal model of depression with increased predictive power. To elucidate whether the open-space-swimming model has "predictive validity", rats were treated with all three major antidepressants. These experiments demonstrated a time-dependent restoration of behavioral parameters as compared with untreated animals. Imipramine, a tricyclic antidepressant (inhibitor of serotonin and norepinephrine reuptake transporters), iproniazid, a monoamine oxidase inhibitor, and mianserin, an atypical antidepressant were used in the study of, for their effectiveness in clinics and representative of each class. It is a common practice to evaluate the suitability of animal models of depression by examining effectiveness of all three prototypical antidepressants. Unlike the forced swimming test, the restricted space would not be a determining factor in the open space swimming test and the mobility is directly measured without an involvement of human judgment and scoring. Our results show that the test has a high predictivity of antidepressant drugs, including an SSRI, and improved sensitivity to antidepressant treatment. The distance moved measures active swimming status of the animals during the trial period. In addition, it is sensitive to SSRIs without any requirement of a scoring modification. It may have value as a choice of depressive models in revealing pathophysiological mechanism(s) of depression and searching for new classes of antidepressants.

[0057] Animal models are indispensable in searching for new antidepressants and for clarifying pathophysiology that underlies depression. On the other hand, the availability of clinically active antidepressants has also made it possible to develop and validate a wide

range of behavioral tests to study depression-like phenotypes in animal models. In our open space model, rats quickly and reproducibly became immobile, exhibiting increased "floating" inactivity over the course of the trials. The validity of the open-space-swimming test for depression was confirmed by results obtained by testing animals treated with known antidepressants. All three major classes of antidepressants were represented in this study: imipramine, a tricyclic antidepressant (inhibition of serotonin or norepinephrine reuptake transporters); iproniazid, a monoamine oxidase inhibitor; and mianserin, an atypical antidepressant, and alaproclate, an SSRI. The applied dose in our study was similar/identical to those used in rats in antidepressant studies without inducing changes in non-specific locomotor activity (Bai *et al.* (2001) *Pharmacol. Biochem. Behav.* 70: 187-192; Kroczka *et al.* (2001) *Brain Res. Bull.* 55: 297-300; Takamori *et al.* (2001) Pharmacology 63: 147-153; Kitamura *et al.* (2002) *Pharmacol. Biochem. Behav.*71: 63-69).

[0058] Results from the open-space-swimming test can be compared with previously reported data (Porsolt *et al.* (1977) *Nature* 266: 730-730-732; Porsolt *et al.* (1978) *Eur J. Pharmacol.* 47: 379-391), in which three similar i.p. doses (15 mg/kg) of these traditional antidepressants were applied to the same age of rats in the forced-swimming test. The percentages of decreases in the distance moved on the third day (using the decrease of control groups as 100%) in our study for the imipramine, iproniazid, and mianserin groups were 49.8 ± 7.6% (n = 8), 44.0± 7.7% (n = 8), and 33.3 ± 6.3% (n = 8), respectively, compared with immobility% as control of 61.5 ± 6.5% (n = 5; 15 mg/kg x 3 per day; $P < 0.01$; Porsolt *et al.* (1977) *Nature* 266: 730-730-732; 87.6 ± 7.3% (n = 5, 15 mg/kg x 3 per day; $P < 0.01$; Porsolt *et al.* (1978) *Eur J. Pharmacol.* 47: 379-391), and 66.5 ± 7.0% (n= 5, 15 mg/kg x 3 per day; $P < 0.01$; Porsolt *et al.* (1977) *Nature* 266: 730-730-732) in the forced swimming test, respectively. Thus, the rats tested using the open-space-swimming test were more sensitive to the antidepressant treatments with statistically significant differences of the iproniazid ($F_{1, 12} = 11.65$; $P < 0.01$) as compared with the previously reported data.

[0059] The open-space-swimming tests satisfies three of the four minimum requirements (McKinney *et al.* (1969) *Arch Gen Psychiatr* Vol. 21, 240-8; Cryan *et al.* (2002) *Trends Pharmacol* 23: 238-45) for an animal model of depression. These requirements for a suitable animal model include (1) reasonably analogous to the human disorder in its manifestations or symptomatology; (2) the existence of a behavioral change

that can be monitored objectively; (3) reversibility of the behavioral change by the same treatments that are effective in human; and (4) reproducible between investigators. Reliability across laboratories remains to be tested. The rat forced swimming test, which has been used for many years, is generally viewed as a test with high predictivity of antidepressant efficacy in human depression (Porsolt *et al.* (1977) *Nature* 266: 730-730-732). Its value in searching for new types of effective drugs is not clear.

[0060] The open-space-swimming model has several advantages. First, one obvious advantage of this test over the leading forced swimming test is that, unlike those time-sampling judgment and scoring in the forced swimming test (Kroczka *et al.* (2001) *Brain Res Bull* 55: 297-300; Rénéric *et al.* (2002) *Eur. Neuropsychopharmacol.* 12: 159-71), no human judging and scoring are involved in this test so that the monitoring is more objective. Second, objective monitoring offers the possibility of improving the reproducibility across different laboratories. Third, the forced-swimming test does not reflect the extent or degree of a behavior, such as vigorous, moderate, or mild swimming mobility. In contrast. These characteristics are directly measured in the open-space-swimming test. In addition, no climbing behavior was observed in the open space model, probably due to the large space available. This removes another artificial judgment as whether climbing should account for more than or equal to active swimming in the forced-swimming test. Fourth, this test does not limit the animals' movement due to space restriction and mimics the human disorder more closely. It is the lack of motivation (opportunities or hope) rather than the restricted "physical space" that largely (though not exclusively) defines the human disease.

D. Carbonic Anhydrase

[0061] Carbonic anhydrase, a zinc-containing enzyme that catalyzes the interconversion of carbon dioxide and bicarbonate anion, is present throughout the body, including the brain. (Sun *et al.* (2002) *Trends in Pharm. Sci.* 23(2): 83-89)

Carbonic anhydrase II, the most active of the seven human isozymes, is a 23.9 kDa enzyme found primarily in erythrocytes, glial cells and brain neurons. (*Id.*). In addition to its involvement in pH regulation, bicarbonate reabsorption and carbon dioxide expiration, carbonic anhydrase plays a crucial role in signal processing, long-term synaptic transformation and attentional gating of memory storage. (*Id.*). Carbonic anhydrase dysfunction has been associated with mental retardation, Alzheimer's disease, and

impaired cognition, and the activation of carbonic anhydrase has been demonstrated to improve memory and learning. (*Id.*; U.S. Patent Application Serial Nos. PCT/US02/13784; PCT/US02/14378; PCT/US03/07102; 60/287,271; 60/289,137; 60/362,081; 10/172,005; 10/476,459; and 10/477,121)

[0062] Carbonic anhydrase catalyzes a reversible reaction between CO_2 hydration and HCO_3^- dehydration. Recent studies indicate that activation of this enzyme provides a rapid and efficient mechanism to raise HCO_3^- concentrations in memory-related neural structures. Increased HCO_3^- flux through synaptic $GABA_A$ receptor channels alters postsynaptic neuronal responses to GABA and thus neuronal responses to diverse signal inputs. In this way, carbonic anhydrase functions as an effective attentional gate that controls signal transfer through the neural network. Alterations in carbonic anhydrase activity in hippocampal CA1 neurons provide a mechanism for switching between operational states at GABA releasing synapses, thereby gating signal transfer through the hippocampal network.

[0063] Carbonic anhydrase activity is at least partially activated by intracellular release of Ca^{2+} through the ryanodine receptors (Rye). For example, the RyR is involved in the GABA-mediated synaptic switch. The effect of Ca^{2+} on carbonic anhydrase appears to be indirect. In human myelomonocytic cell lines, synthesis of carbonic anhydrase II is activated by protein kinase C, an effect that is blocked by 0.1 μm staurosporine. Hormones also regulate the activity of carbonic anhydrase via cAMP. Thus, the increase in carbonic anhydrase activity induced by adrenaline and dibutyryl-cAMP in erythrocytes is enhanced by theophylline, and phosphorylation by a cAMP-dependent protein kinases activates carbonic anhydrase.

[0064] There are at least seven isozymes of carbonic anhydrase in humans. (Lindskog (1997)*Pharmacol. Ther.* 74(1): P1-20). The structure of the CAII binding site for acetazolamide and some other inhibitors is known. This knowledge allows rational design of derivatives and analogs of the compounds contemplated herein.

[0065] Based on structural, biochemical and medicinal chemistry studies, the pharmacological profile of carbonic anhydrase has been refined and specific activators have been developed. Activators of carbonic anhydrase provided an important tool for the treatment of genetic carbonic anhydrase deficiencies and memory disorders. Many amines

and amino acids (*e.g.*, dopamine, seratonin, noradrenaline, adrenaline, histamine, histidine, imidazoles, phenylalanine or derivatives thereof (*See*, WO 00/56760)

are carbonic anhydrase activators. Specifically, carbonic anhydrase activators increase the rate of interconversion of carbon dioxide and bicarbonate ion (Reaction I) by acting directly as proton acceptors.

EZn^{2+}--OH_2 + Activator ←→ [EZn^{2+}--OH_2----ActivatorH^+] ←→ [EZn^{2+}--OH^----- ActivatorH^+] ←→ EZn^{2+}--OH^- + ActivatorH^+] (Reaction I)

[0066] Carbonic anhydrase activators encompassed by the present invention and the activation by each of human carbonic anhydrase II activity (relative to the activation of the enzyme by alanine) is depicted in Table I.

TABLE 1. Carbonic Anhydrase Activators

Effector	Ar	R1	R2	R3	% Activity/Control CAII Activity
1	H	H	COOH	H	100
2	Phenyl	H	COOH	H	186.7
3	Phenyl	H	H	H	109.5
4	4-Hydroxyphenyl	H	COOH	H	189.1
5	4-Fluorophenyl	H	COOH	H	167.7
6	4-Aminophenyl	H	COOH	H	159.4
7	3-Amino-4-hydroxyphenyl	H	COOH	H	176.3
8	3,4-Dihydroxyphenyl	H	COOH	H	134.3
9	3,4-Dihydroxyphenyl	H	H	H	137.5
10	3,4-Dihydroxyphenyl	OH	H	H	115.5
11	3,4-Dihydroxyphenyl	OH	H	CH_3	135.0
12	3,4-Dihydroxyphenyl	OH	CH_3	H	129.0
13	Phenyl	OH	CH_3	CH_3	134.5
14	Imidazole	(Ar only, no rest C – C chain)			230.0
15	Imadazol-4-yl	H	H	H	150.0
16	Imadazol-4-yl	H	COOH	H	170.0
17	5-Methylimidazole-4-yl	H	H	H	130.5

E. Protein Kinase C (PKC)

[0067] The PKC gene family consists presently of 11 genes which are divided into four subgroups: 1) classical PKCα, β_1, β_2 (β_1 and β_2 are alternatively spliced forms of the same gene) and γ, 2) novel PKCδ, ε, η, and θ, 3) atypical PKCζ, λ, η and *i* and 4) PKC μ. PKC μ resembles the novel PKC isoforms but differs by having a putative transmembrane domain (reviewed by Blohe *et al.* (1994) *Cancer Metast. Rev.* 13: 411; Ilug *et al.* (1993) Biochem J. 291: 329; Kikkawa *et al.*(1989) *Ann. Rev. Biochem.* 58: 31). The α, β_1, β_2 and γ isoforms are C^{2+}, phospholipid and diacylglycerol-dependent and represent the classical isoforms of PKC, whereas the other isoforms are activated by phospholipid and diacylglycerol but are not dependent on Ca^{2+}. All isoforms encompass 5 variable (V1-V5) regions, and the α, β and γ isoforms contain four (C1-C4) structural domains which are

highly conserved. All isoforms except PKC α, β and γ lack the C2 domain, the λ η and isoforms also lack nine of two cysteine-rich zinc finger domains in C1 to which diacylglycerol binds. The C1 domain also contains the pseudosubstrate sequence which is highly conserved among all isoforms, and which serves an autoregulartory function by blocking the substrate-binding site to produce an inactive conformation of the enzyme (House *et al.* (1987) *Science* 238, 1726).

[0068] Because of these structural features, diverse PKC isoforms are thought to have highly specialized roles in signal transduction in response to physiological stimuli (Nishizuka (1989) *Cancer* 10: 1892), as well as in neoplastic transformation and differentiation (Glazer (1994) *Protein Kinase C*, J.F. Kuo, ed., Oxford U. Press at pages 171-198). For a discussion of known PKC modulators see PCT/US97/08141, U.S. Patent Nos. 5,652,232; 6,080,784; 5,891,906; 5,962,498; 5,955,501; 5,891,870 and 5,962,504.

[0069] The anti-depressant effect of PKC activators is mediated directly by activation of PKC and/or the increased synthesis of carbonic anhydrase observed following PKC activation.

[0070] There is increasing evidence that the individual PKC isozymes play different, sometimes opposing, roles in biological processes, providing two directions for pharmacological exploitation. One is the design of specific (preferably, isozyme specific) inhibitors of PKC. This approach is complicated by the act that the catalytic domain is not the domain primarily responsible for the isotype specificity of PKC. The other approach is to develop isozyme-selective, regulatory site-directed PKC activators. These may provide a way to override the effect of other signal transduction pathways with opposite biological effects. Alternatively, by inducing down-regulation of PKC after acute activation, PKC activators. These may provide a way to override the effect of other signal transduction pathways with opposite biological effects. Alternatively, by inducing down-regulation of PKC after acute activation, PKC activators may cause long term antagonism. Bryostatin is currently in clinical trials as an anti-cancer agent. The bryostatins are know to bind to the regulatory domain of PKC and to activate the enzyme. Bryostatins an example of isozyme-selective activators of PKC. Compounds in addition to bryostatins have been found to

modulate PKC. (see for example WO 97/43268)

For a discussion of known PKC modulators see PCT/US97/08141, U.S. Patent Nos. 5,652,232; 6,043,270; 6,080,784; 5,891,906; 5,962,498; 5,955,501; 5,891,870 and 5,962,504.

[0071] Several classes of PKC activators have been identified. Phorbol esters, however, are not suitable compounds for eventual drug development because of their tumor promotion activity, (Ibarreta *et al.* (1999) *Neuro Report* 10(5&6): 1035-40). Of particular interest are macrocyclic lactones (i.e. bryostatin class and neristatin class) that act to stimulate PKC. Of the bryostatin class compounds., 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 isozymes, including PKCα, PKCδ and PKCε. Bryostatin-1 has undergone toxicity and safety studies in animals and humans and is actively 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.

[0072] Macrocyclic lactones, and particularly bryostatin-1 is described in U.S. Patent 4,560,774. Macrocyclic lactones and their derivatives are described elsewhere in U.S. Patent 6,187,568, U.S. Patent 6,043,270, U.S. Patent 5,393,897, U.S. Patent 5,072,004, U.S. Patent 5,196,447, U.S. Patent 4,833,257, and U.S. Patent 4,611,066. The above patents describe various compounds and various uses for macrocyclic lactones including their use as an anti-inflammatory or anti-tumor agent. (Szallasi *et al.* (1994) *Journal of Biological Chemistry* 269(3): 2118-24; Zhang *et al.* (1996) *Caner Research* 56: 802-808; Hennings *et al.* (1987) *Carcinogenesis* 8(9): 1343-1346; Varterasian et al. (2000) *Clinical Cancer Research* 6: 825-828; Mutter *et al.* (2000) *Bioorganic & Medicinal Chemistry* 8: 1841-1860)

[0073] As will also be appreciated by one of ordinary skill in the art, macrocyclic lactone compounds and their derivatives, particularly the bryostatin class, are amenable to combinatorial synthetic techniques and thus libraries of the compounds can be generated to

optimize pharmacological parameters, including, but not limited to efficacy and safety of the compositions. Additionally, these libraries can be assayed to determine those members that preferably modulate α-secretase and/or PKC.

[0074] Combinatorial libraries high throughput screening of natural products and fermentation broths has resulted in the discovery of several new drugs. At present, generation and screening of chemical diversity is being utilized extensively as a major technique for the discovery of lead compounds, and this is certainly a major fundamental advance in the area of drug discovery. Additionally, even after a "lead" compound has been identified, combinatorial techniques provide for a valuable tool for the optimization of desired biological activity. As will be appreciated, the subject reaction readily lend themselves to the creation of combinatorial libraries of compounds for the screening of pharmaceutical, or other biological or medically-related activity or material-related qualities. A combinatorial library for the purposes of the present invention is a mixture of chemically related compounds, which may be screened together for a desired property; said libraries may be in solution or covalently linked to a solid support. The preparation of many related compounds in a single reaction greatly reduces and simplifies the number of screening processes that need to be carried out. Screening for the appropriate biological property may be done by conventional methods. Thus, the present invention also provides methods for determining the ability of one or more inventive compounds to bind to effectively modulate α-secretase and/or PKC.

[0075] A variety of techniques are available in the art for generating combinatorial libraries described below, but it will be understood that the present invention is not intended to be limited by the foregoing examples and descriptions. (*See, for example,* Blondelle *et al.* (1995) *Trends Anal. Chem.* 14: 83; U.S. Patents 5,359,115; 5,362,899; U.S. 5,288,514: PCT publication WO 94/08051; Chen *et al.* (1994) *JACCS* 1 6:266 1: Kerr *et al.* (1993) *JACCS I* 1 5:252; PCT publications W092/10092, W093/09668; W091/07087; and W093/20242). Accordingly, a variety of libraries on the order of about 16 to 1,000,000 or more diversomers can be synthesized and screened for a particular activity or property.

F. Fibroblast Growth Factor 18 (FGF-18)

[0076] Fibroblast Growth Factors ("FGF") are among the proteins that play vital roles in controlling embryonic development, cell growth, morphogenesis, and tissue repair in animals. (Hu et al. (1999) Oncogene, 18 (16): 2635-42). FGF-18 is one member of this family of proteins; it is a peptide consisting of 207 amino acids, encoded by a single memory related gene associated with spatial learning. It is expressed primarily in the lungs and kidneys and at lower levels in the heart, testes, spleen, skeletal muscle, and brain. (Hu et al (1998) Molecular Cellular Biology, 18(10): 6063-6074). Sequence comparison studies indicated that FGF-18 is highly conserved between humans and mice and is most homologous to FGF-8 among the FGF family members. In continuing studies investigating the full role of FGF-18 in cellular and tissue development, FGF-18 has thus far been identified as a signaling molecule for proliferation in the adult lung and developing tissue, and it has been linked to cancerous cells.

[0077] The present invention contemplates the use of FGF-18, modified forms of FGF-18 and fragments of FGF-18 that each maintain me ability to improve learning an memory as described in U.S. Provisional Application Serial No. 60/429,321 and PCT/IB03/05408.

[0078] Reference to any compound herein includes the racemate as well as the single enantiomers.

EXAMPLES

[0079] The following Examples serve to further illustrate the present invention and are not to be construed as limiting its scope in any way.

EXAMPLE 1: Open-Space-Swimming Test for Depression

[0080] Adult Wistar rats (180-200 g) were housed in a temperature-controlled (20-24 ^{Q}C) room for at least a week prior to experimentation, allowed free access to food and water, and kept on a 12-h light/dark cycle. They were randomly assigned to different groups (eight each) and were moved to the test room in their home cages at least 1 h before trials. Rats were individually placed into a round pool with a diameter of 152 cm and height of 60 cm

and was filled with 40 cm H_2O (22±1 °C). The room and pool are part of the started set-up used for spatial water maze task. (Sun *et al.* (2002) *J. Pharmacol. Exp. Ther.* 300: 408-416). The tests for different drug treatment and control groups were in a counterbalanced order. No escape was available in these trials. Rats were free to swim (or not to swim) for 15 min and then removed and returned to their home cage after drying. Observers were obscured from sight of the rats during the trials, but were able to observe the animals' behaviors on a video screen monitor during trials. The same procedure (15 min session per day) was repeated 24 h later and continued for 3 additional days. Drugs were dissolved in a saline solution for test groups and saline alone was used in the control groups. Imipramine (10 mg/kg x 3 day, i.p.), iproniazid (10 mg/kg x 3 per day, i.p.), mianserin (10 mg/kg x 3 per day, i.p.), alaproclate (10 mg/kg x 3 per day), or saline were administered between the swimming trial sessions at 23, 2, and 1 h before the second, third, and fourth trial sessions, respectively. The rationale for the three doses before test trials is the more consistent predictive effects than those of a single dose (Porsolt *et al.* (1977) *Nature* 266: 730-730-732; Porsolt *et al.* (1978) *Eur J. Pharmacol.* 47: 379-391; Poncelet *et al,* (1986) Psychopharmacology 90: 139-141). The swimming/drifting path was recorded with a video-tracking system that was also used for the spatial water maze task in other studies (Sun et al., 2002; Sun and Alkon, 2002a). The system tracks the animal's position by recording sequential *x/y*-coordinates of trigger events (video signal above track level) and calculating the distance, in the pool (sample period: 0.055 s) and adds the distance at pre-set intervals (15 min) during each trial. The tracking system, however, neither distinguishes nor determines the duration of any period of mobility or immobility. To evaluate whether the distance moved, as recorded by the tracking system, reflects the duration of active swimming, the investigator(s) also recorded the duration of active swimming on-line via the video screen monitor for a direct comparison of the two parameters.

EXAMPLE 2. Open space swimming test induces immobility in rats.

[0081] The rats injected with saline (eight per group) showed a gradual and significant reduction (Fig. 1) in mobility (distance moved) ($F_{3,31} = 49.717$; $P < 0.001$). The distance moved includes all the distance moved during the entire 15 min, as caused by active swimming/searching as well as slow drifts, which were caused by apparently non-searching movement of the legs, in the overall movement. Active swimming is defined as when a rat is

making active swimming motions as those to move around in the pool. Unlike the behavior patterns reported in the forced swimming test (e.g. Rénéric *et al.*, (2002) *Eur. Neuropsychopharmacol.* 12: 159-71), no climbing on the wall was observed, probably due to the large space available to the rats. As the trials progressed, the control rats showed progressively less and briefer intermittent periods of active swimming. A maximal reduction in their mobility was reached at the 3rd trial in these control rats (Fig. 1). Typically, a control rat did not make any movements other than those just sufficient to keep its head above the water surface (immobility: not shown), a characteristic behaviour that is taken as an indicator of depression in the forced swimming test.

EXAMPLE 3. Induced immobility sensitivity to traditional antidepressant treatments.

[0082] Treatment with antidepressants significantly decreased the reduction in the distance moved over trials (Fig. 1), compared with that of the control group. Statistical analysis revealed significant effects of groups ($F_{3, 16}$ =25,071; P <0.001) indicating that the mobility in rats that were injected with the antidepressants was significantly higher than that of the rats that were injected with vehicle only. These rats showed more and long lasting periods of active swimming/searching, measured as the distance swum, although the periods of active swimming were not quantified individually in the study. Moreover, a post hoc analysis revealed a significant difference from the 2nd trial to the 4th trial (P <0.05), confirming higher mobility of the rat groups that received the antidepressant treatment. For the three drug groups, statistical analysis revealed no significant inter-group difference ($F_{2, 12}$ = 4.199; P <0.05), although iproniazid appeared more effective at the dose given than mianserin and imipramine in reducing the immobility. (**Figure 1**). A comparison between the present results and previously reported data obtained by using the forced swimming test reveals that the present test shows an improved antidepressant effect in general.

EXAMPLE 4. Induced immobility sensitivity to SSRI-antidepressant treatment.

[0083] Alaproclate, an SSRI, was effective in reducing the immobility in the open space swimming test (**Figure 2A**), as compared with that of a control group. Statistical analysis yielded a significant difference between the groups ($F_{1, 8}$ = 32.60; P < 0.001) indicating that the mobility of the rats that were injected with alaproclate was significantly higher than that of the rats that were injected with vehicle only.

[0084] Whether the observed difference in the distance moved would reflect different duration of active swimming was evaluated by recording the periods during which the rats were clearly performing active swimming, *i.e.*, making those obvious movements to swim around in the pool. A comparison of the results of the different groups was illustrated in **Figure 2B.** The duration of active swimming of the alaproclate group was significantly longer (group difference: $F_{1,\ 8} = 31.51$; $P < 0.001$) than that of the control group. The %change in distance moved (using corresponding change of the control group as 100%) did not significantly differ ($P > 0.05$, unpaired *t*-test) from the %change in the duration of active swimming (using corresponding change of the control group as 100%). Thus, the results show that the parameter, "distance moved", indeed reflects duration of mobility during the test.

EXAMPLE 5: Induced immobility sensitivity to phenylalanine.

[0085] Depressive behavior in rats was induced by placing the animals in an open-space swimming apparatus, as described herein, for 15 minutes per trial per day. The animals were subjected to three trials over the course of three days. The animals were divided into three groups: control rats (8); phenylalanine rats (10); and imipramine rats (10). The control rats received a single i.v. dose of saline in the tail vein, 3.5 hours before the second trial. Rats in the phenylalanine group received a single i.v. dose of phenylalanine in the tail vein, 3.5 hours before the second trial. Rats in the imipramine group received 3 i.p. doses (10 mg/kg) at 23, 2 and 1 hour before the first, second and third trials, respectively.

[0086] Phenylalanine, a carbonic anhydrase activator, was effective in reducing the immobility in the open-space-swimming test, as compared with a control group and a group treated with the antidepressant imipramine. (**Figure 3**). Statistical analysis demonstrates a significant difference between the phenylalanine, imipramine and control groups, thereby indicating that the mobility of the rats injected with phenylalanine is significantly higher than that of the rats receiving either imipramine or saline.

EXAMPLE 6: Induced immobility sensitivity to bryostatin-1.

[0087] Depressive behavior in rats was induced by placing the animals in an open-space swimming apparatus, as described herein, for 15 minutes per trial per day. The animals were subjected to three trials over the course of three days. The animals were divided into

three groups: control rats (8); bryostatin-1 rats (10); and imipramine rats (10). The control rats received a single i.v. dose of saline in the tail vein, 3.5 hours before the second trial. Rats in the bryostatin-1 group received a single i.v. dose of bryostatin-1 (80 µg/kg) in the tail vein, 3.5 hours before the second trial. Rats in the imipramine group received 3 i.p. doses (10 mg/kg) at 23, 2 and 1 hour before the first, second and third trials, respectively.

[0088] Bryostatin-1, a PKC activator, was effective in reducing immobility in the open-space-swimming test, as compared with a control group and a group treated with the antidepressant, imipramine. **(Figure 4).** Statistical analysis demonstrates a significant difference between the Bryostatin-1, imipramine and control groups, thereby indicating that the mobility of the rats injected with bryostatin-1 is significantly higher than that of the rats receiving saline ($F_{2,27}$ =6.168: P=0.007). No significant difference was observed between the bryostatin-1 and imipramine groups ($F_{2,27}$ =10.128: P=0.724).

EXAMPLE 7: Induced immobility sensitivity to imidazole.

[0089] Imidazole, a carbonic anhydrase activator, is effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

EXAMPLE 8: Induced immobility sensitivity to linked diimidizole.

[0090] Linked diimidazole (structure III, wherein R_2 is H and n=1 or 2), a carbonic anhydrase activator, is effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

EXAMPLE 9: Induced immobility sensitivity to tyrosine.

[0091] Tyrosine, a carbonic anhydrase activator, is effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

EXAMPLE 10: Induced immobility sensitivity to 4-fluoro-phenylalanine.

[0092] 4-Fluoro-phenylalanine a carbonic anhydrase activator, is effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

EXAMPLE 11: Induced immobility sensitivity to carbonic anhydrase activators that provide carbonic anhydrase activity of between 150 and 250% that observed with alanine.

[0093] Compounds that activate carbonic anhydrase II between 150 and 250% relative to the activation observed for alanine, are effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

EXAMPLE 12: Induced immobility sensitivity to neristatins.

[0094] Neristatins, PKC activators, are effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

Example 13: Induced immobility sensitivity to FGF-18.

[0095] FGF-18, a PKC activator, is effective in reducing immobility in the open-space-swimming test when compared with a control group and a group treated with the antidepressant imipramine.

We claim:

1. Use of a composition comprising a protein kinase C activator as a sole active ingredient for treating a depressive disorder of a subject and a pharmaceutically acceptable carrier, wherein the protein kinase C activator is a macrocyclic lactone.

2. The use of claim 1, wherein the macrocyclic lactone is a bryostatin or neristatin.

3. The use of claim 2, wherein the bryostatin is selected from a group consisting of bryostatin-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

4. The use of claim 3, wherein the bryostatin is bryostatin-1.

5. The use of claim 2, wherein the neristatin is neristatin-1.

6. Use of a composition comprising a protein kinase C activator as a sole active ingredient for treating depression in a subject and a pharmaceutically acceptable carrier wherein the protein kinase C activator is macrocyclic lactone.

7. The use of claim 6, wherein the macrocyclic lactone is a bryostatin or neristatin.

8. The use of claim 7, wherein the bryostatin is selected from a group consisting of bryostatin-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18.

9. The use of claim 8, wherein the bryostatin is bryostatin-1.

10. The use of claim 7, wherein the neristatin is neristatin-1.

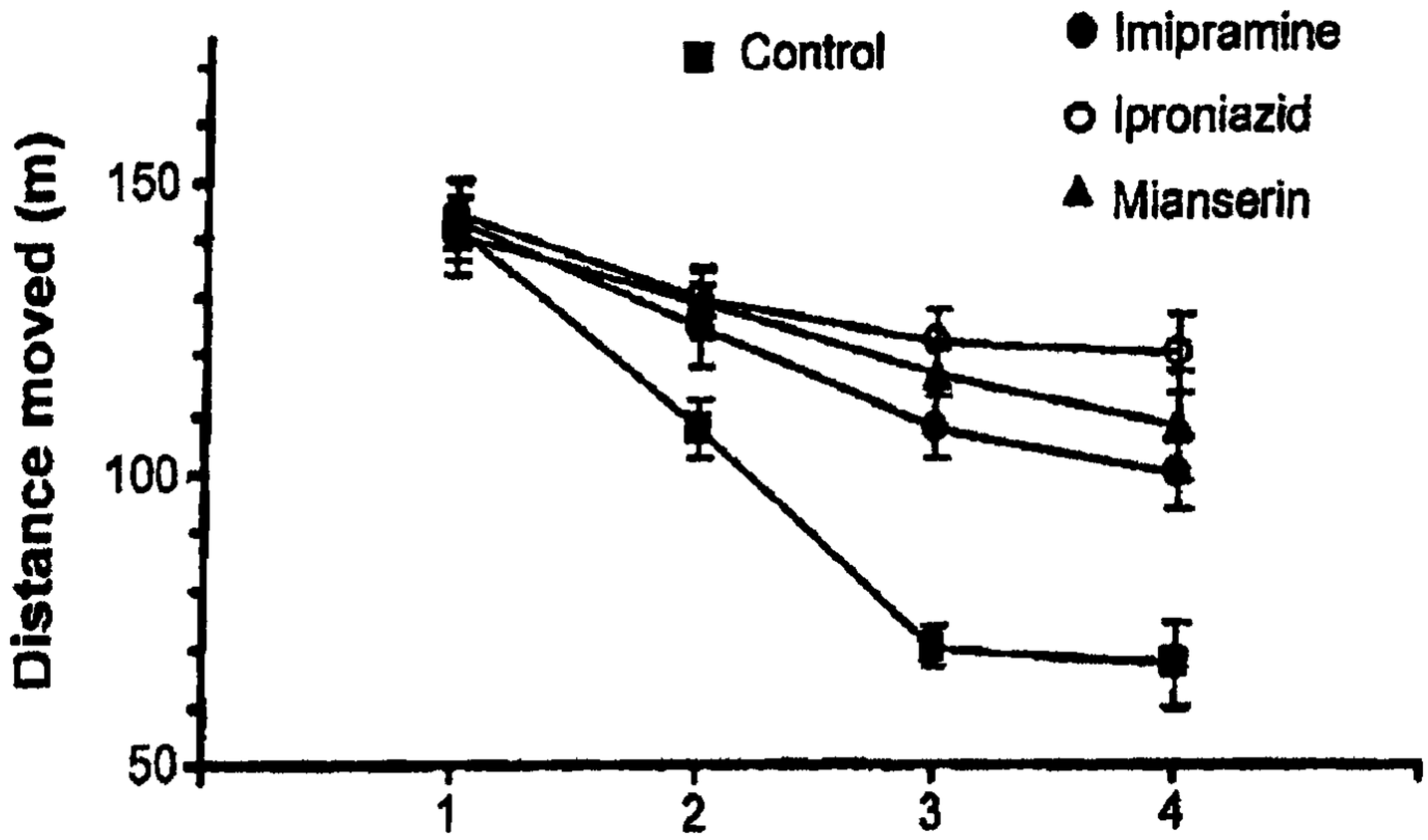

Control
Imipramine
Iproniazid
Mianserin
Distance moved (m)
150
100
50
1
2
3
4
Trials

FIG 1

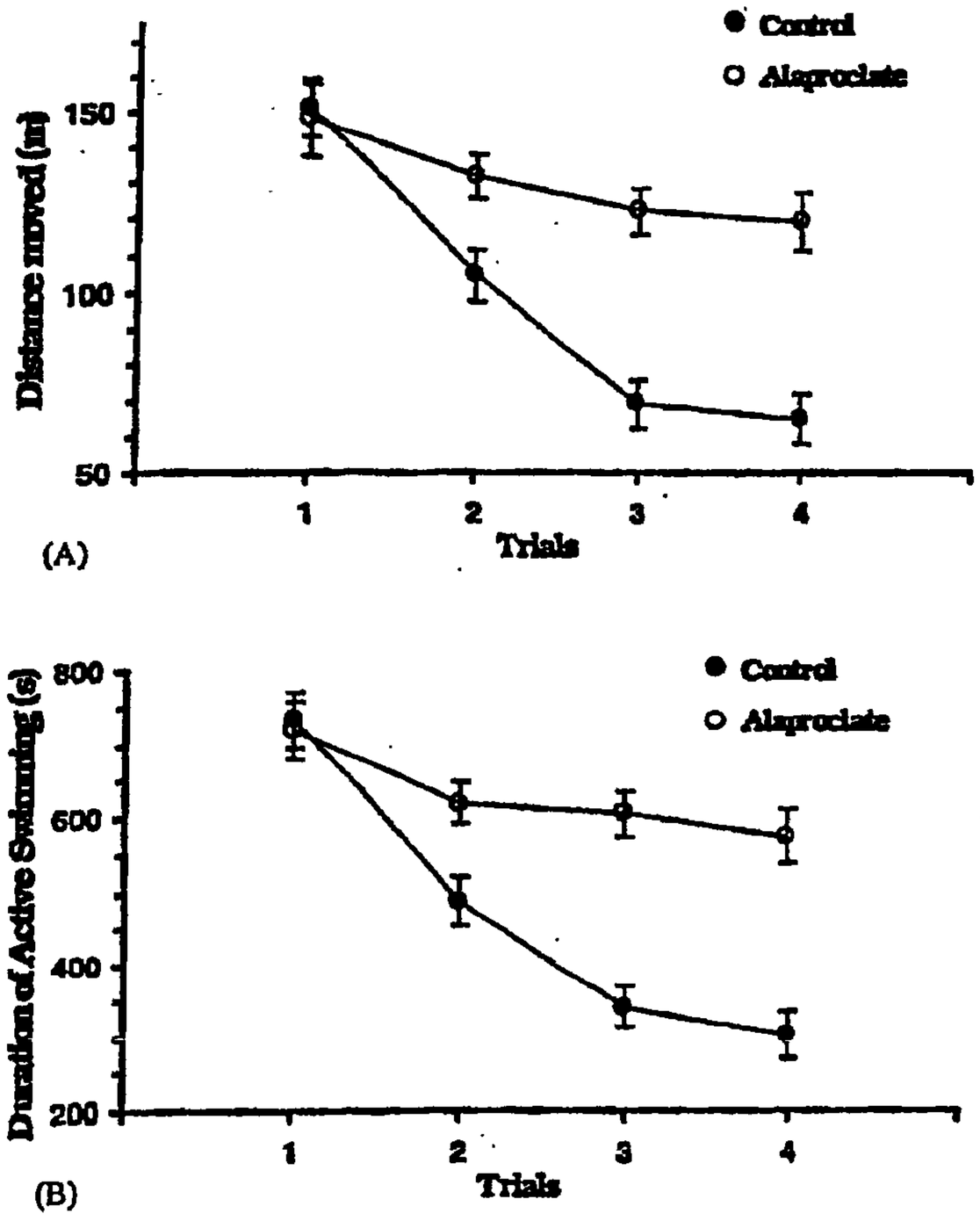

Control
Alaproclate
Distance moved (m)
150
100
50
1
2
3
4
Trials
(A)
Control
Alaproclate
Duration of Active Swimming (s)
800
600
400
200
1
2
3
4
Trials
(B)

FIG 2A & 2B

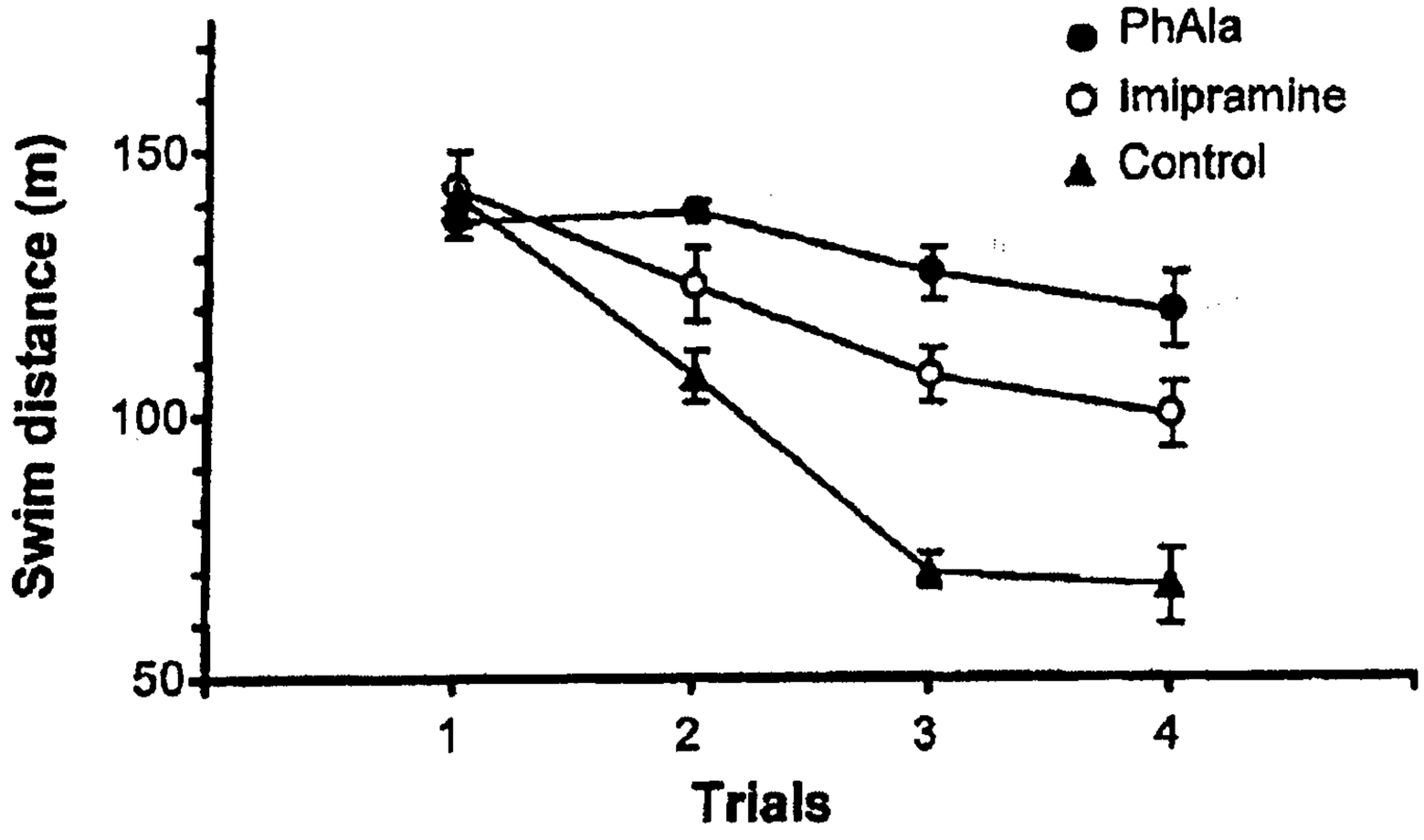

PhAla
Imipramine
Control
Swim distance (m)
150
100
50
1
2
3
4
Trials

FIG 3

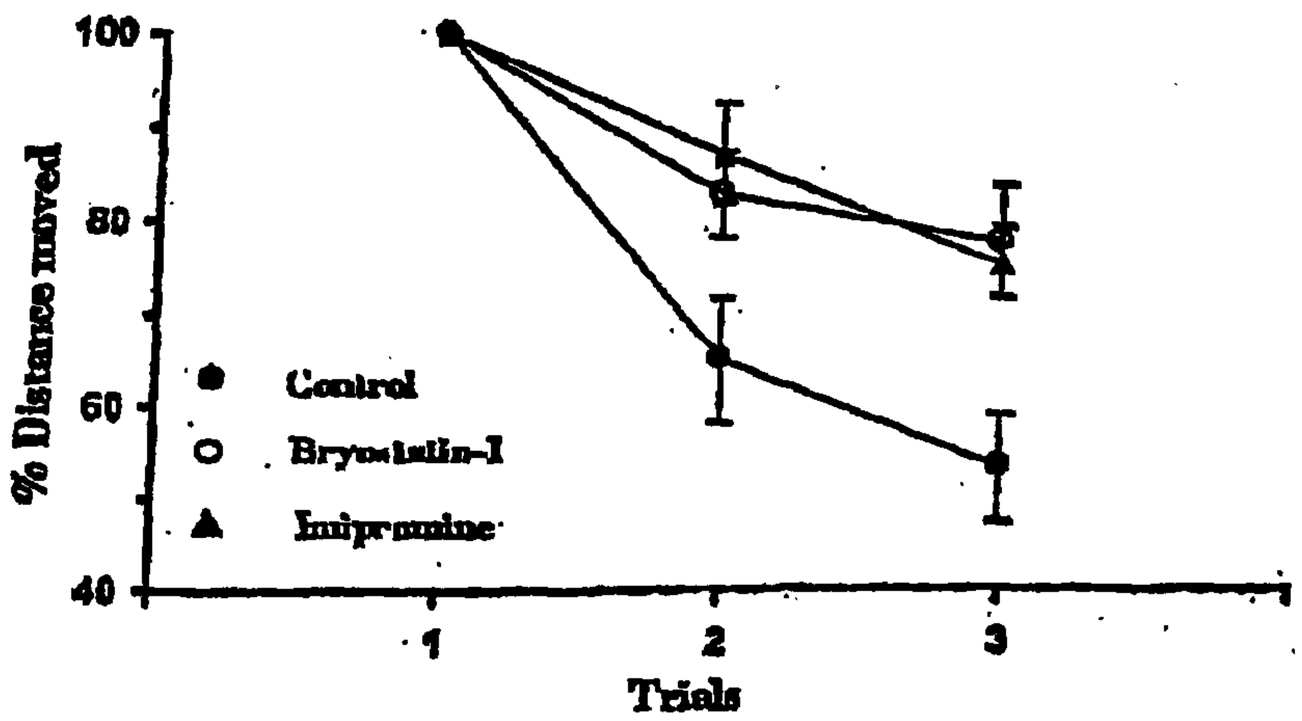
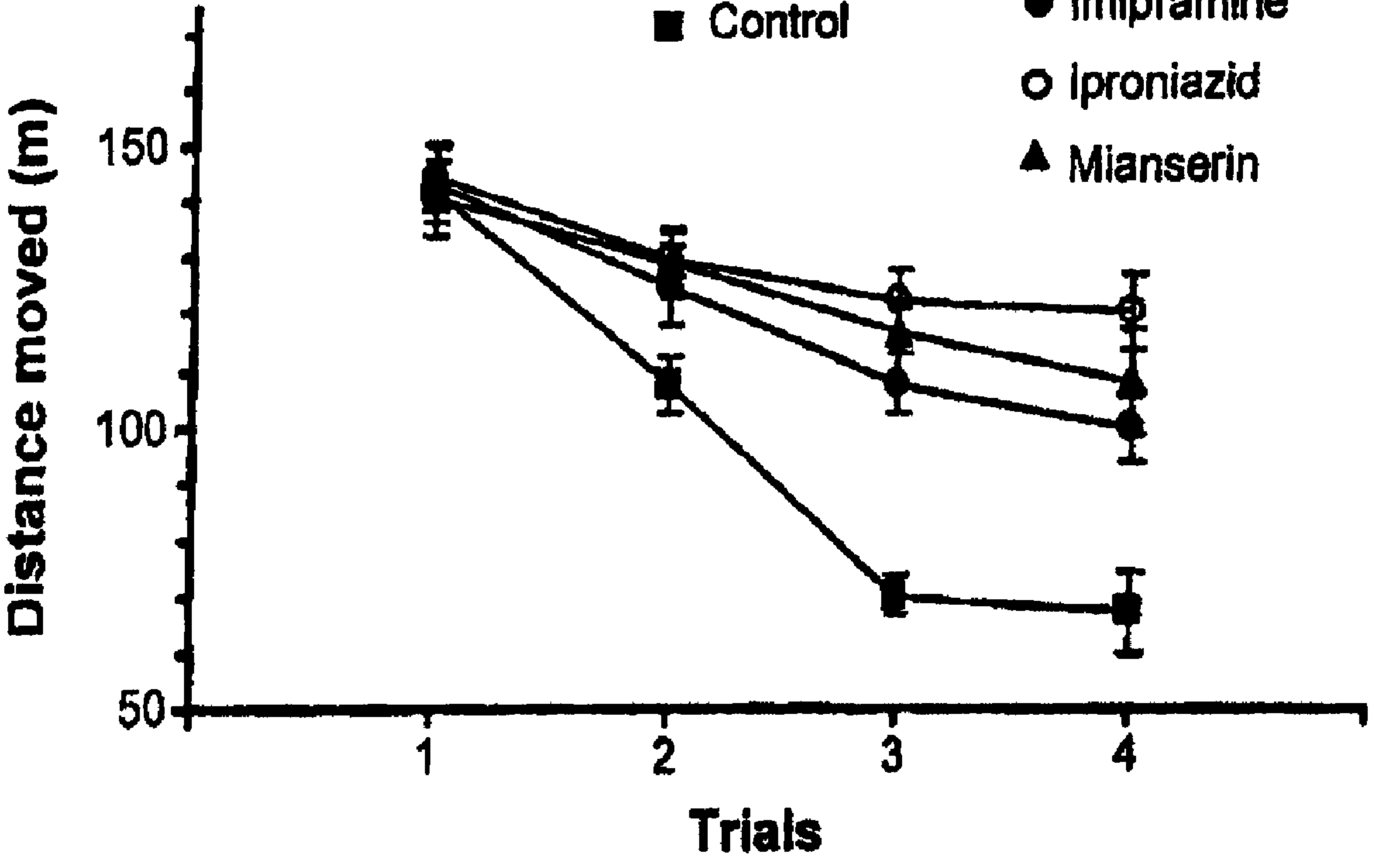

100
80
60
40
% Distance moved
Control
Bryostatin-1
Imipramine
1
2
3
Trials

FIG 4


Control
Imipramine
Iproniazid
Mianserin
Distance moved (m)
150
100
50
1
2
3
4
Trials