



(86) Date de dépôt PCT/PCT Filing Date: 2003/02/26
(87) Date publication PCT/PCT Publication Date: 2003/09/04
(85) Entrée phase nationale/National Entry: 2004/08/27
(86) N° demande PCT/PCT Application No.: IB 2003/001425
(87) N° publication PCT/PCT Publication No.: 2003/072090
(30) Priorité/Priority: 2002/02/27 (60/359,652) US

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 38/00
(71) Demandeur/Applicant:
AB SCIENCE, FR
(72) Inventeurs/Inventors:
MOUSSY, ALAIN, FR;
KINET, JEAN-PIERRE, US
(74) Agent: SMART & BIGGAR

(54) Titre : UTILISATION D'INHIBITEURS DE TYROSINE KINASE POUR LE TRAITEMENT DE TROUBLES DU SNC
(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING CNS DISORDERS

(57) **Abrégé/Abstract:**

The present invention relates to a method for treating CNS disorders, more particularly selected from the group consisting of depression, schizophrenia, anxiety, migraine, memory loss, pain and neurodegenerative diseases, comprising administering a compound capable of depleting mast cells to a human in need of such treatment. Such compounds can be chosen from tyrosine kinase inhibitors and more particularly non-toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 September 2003 (04.09.2003)

PCT

(10) International Publication Number
WO 03/072090 A3

(51) International Patent Classification⁷: A61K 38/00

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

(21) International Application Number: PCT/IB03/01425

(22) International Filing Date: 26 February 2003 (26.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/359,652 27 February 2002 (27.02.2002) US

(71) Applicant (for all designated States except US): **AB SCI-
ENCE** [FR/FR]; 3, avenue George V, F-75008 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MOUSSY, Alain**
[FR/FR]; 22 bis, passage Dauphine, F-75006 Paris (FR).
KINET, Jean-Pierre [FR/US]; 3 Hunt Road, Lexington,
MA 02421 (US).

(74) Agents: **MARTIN, Jean-Jacques** et al.; Cabinet Regim-
beau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).

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

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:

13 November 2003

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.

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING CNS DISORDERS

(57) Abstract: The present invention relates to a method for treating CNS disorders, more particularly selected from the group consisting of depression, schizophrenia, anxiety, migraine, memory loss, pain and neurodegenerative diseases, comprising administering a compound capable of depleting mast cells to a human in need of such treatment. Such compounds can be chosen from tyrosine kinase inhibitors and more particularly non-toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.



WO 03/072090 A3

Use of tyrosine kinase inhibitors for treating CNS disorders

The present invention relates to a method for treating CNS disorders, more particularly
5 selected from the group consisting of depression, schizophrenia, anxiety, migraine,
memory loss, pain and neurodegenerative diseases, comprising administering a
compound capable of depleting mast cells to a human in need of such treatment. Such
compounds can be chosen from tyrosine kinase inhibitors and more particularly non-
toxic, selective and potent c-kit inhibitors. Preferably, said inhibitor is unable to promote
10 death of IL-3 dependent cells cultured in presence of IL-3.

Neurons propagate a signal in the form of an action potential along its axon to other
neurons or to effector cells. Many positive or negative signals are exchanged between
neurons and are integrated to produce meaningful firing patterns. The communication
15 between two neurons is based on the action of numerous neurotransmitters on specific
receptors located at the synapses. A disruption in the regulation of neurotransmission is
responsible for neurologic and psychiatric diseases. Furthermore, the activity of
neurotransmitters on their respective receptor is normally time limited so that receptors
can respond repeatedly to the next waves of stimuli. In this regard, different mechanisms
20 abolish the action of neurotransmitters : they can be pumped back into the presynaptic
nerve terminals by active processes (reuptake), they can be destroyed by enzymes, or
they simply diffuse into the surrounding area.

According to The Merck Manual of Diagnosis and Therapy, Section 14, Neurologic
25 Disorders, changes in neurotransmitter synthesis, storage, release, or degradation or
changes in the number and affinity of receptors can affect neurotransmission and cause
clinical disorders.

Among neurotransmitters, we can cite glutamate and aspartate, which are the major excitatory neurotransmitters, whereas aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. But, the first theory about depression concerned the noradrenergic system (NS) (Shildkraut J. et al. 1965, Am J. Psychiat. 122, 509-522). At 5 that time, it was observed that tricyclic compounds (ADT) and monoamine-oxidase inhibitors modified the level of noradrenaline. Later on, in 1978, Sulser F. et al., Biochem Pharmacol. 27, 257-261 showed that these antidepressants lead to a decrease in the number of post-synaptic β -adrenergic receptors. Therefore, it was thought that 10 depression was due to the deregulation of the noradrenergic pre-synaptic stimuli as well as the post-synaptic receptors (Siever LJ. et al. (1985), Am. J. Psychiat. 142, 1017-1031). In 1986, Rasmussen et al., Brain Res. 385, 395-400 demonstrated the presence of serotonin (5-hydroxytryptamin, 5-HT) receptors in NA neurones. Treatments with ADT was shown to provoke also a down-regulation of the 5-HT₂ receptors in Sugrue M.F. et 15 al, 1981, Pharmacol. Ther. 13, 219-247. As a consequence, it appears that the NA and 5-HT systems play a crucial role in the regulation of mood and behaviour.

In the nineties, research has focused on the finding of specific serotonin re-uptake inhibitors (SSRI), such as fluoxetine, paroxetine or sertraline (Pinder R.M. et al., 1993, Med. 20 Res. Rev. 13, 259-325). Serotonin (5-hydroxytryptamine, or 5-HT) levels are controlled by the uptake of tryptophan and intraneuronal monoamine oxidase activity.

In the meantime, a decrease in the level of HVA, the main catabolic of dopamine (DA), 25 was observed in depressed patients (Kapur S. et al., 1992, Biol. Psychiat. 32, 1-17).

GABA was also shown to be involved in the pathophysiology of depression since (i) unipolar patients display decreased level of GABA, (ii) some antidepressants induce the

release of GABA in vivo and (iii) agonists of GABA receptors have antidepressant effects (Lloyd K.G. et al., 1989, Prog. Neuro-Psychopharmacol. Biol. Psychiat. 13, 341-351).

5 More recently, it has been reported that other factors may be involved in CNS disorders. For example, it has been observed from 30 to 70 % of patients afflicted with melancholia have high level of plasmatic cortisol and escape to the test with dexamethasone described in Carroll B.J. et al. (1981), Arch. Gen. Psychiat. 38, 15.

In addition, corticosteroids modify (i) the expression of serotonergic receptors and (ii)
10 the activity of tryptophan hydroxylase, which is the key enzyme in the synthesis of 5-HT (Biegon A., 1990, Ann. NY Acad. Sci. 600, 427-431).

Regarding post-partum or post-menopause depression, repeated administration of oestrogene induces a down-regulation of dopaminergic D2 receptors (Munemura M. et al., 1989, Endocrinology 124, 346-355 and Roy E.J. et al., 1990, Brain. Res. Bull. 25,
15 221-227).

Other neurotransmitters include the well known acetylcholine, norepinephrine which interacts with adrenergic receptors and which is regulated by tyrosine hydroxylase and monoamine oxidase, endorphins which are polypeptides that activate many central
20 neurons and interact with opioid receptors, enkephalins, dynorphins, histamine, vasopressin, vasoactive intestinal peptide, carnosine, bradykinin, cholecystokinin, bombesin, somatostatin, corticotropin releasing factor, neurotensin, and adenosine.

As mentioned above, any imbalance in these neurotransmitters or any deregulation of
25 associated receptors may lead to the development of CNS disorders ranging from psychiatric diseases to migraine, pain, memory loss and nerve cells degeneracy.

As of today, available treatments include selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, paroxetine, and fluvoxamine. Other compounds include nefazodone which blocks the 5-HT₂ receptor and inhibits reuptake of 5-HT and norepinephrine, trazodone which is a 5-HT₂ receptor blocker and a 1-noradrenergic
5 blocker, mirtazapine which blocks 2-adrenergic autoreceptors as well as 5-HT₂, 5-HT₃ and H₁ receptors, tricyclic compounds such as imipramine and desipramine, tetracyclic compounds which increase the level of free norepinephrine and of 5-HT, and monoamine oxidase inhibitors (MAOI) which inhibit the oxidative deamination of norepinephrine, dopamine, and 5-HT. We can also cite lithium-antidepressants for
10 treating bipolar disorder.

However, these compounds are only effective in about 65% of depressed patients, which implies a large population afflicted with the so-called "refractory depression". In some cases, the life of patients is in jeopardy at the extent that hospitalization and
15 electroconvulsive therapy is required. This shows the seriousness of these diseases. Furthermore, the above mentioned compounds display numerous side effect such as tachycardia, sedation and weight gain.

Schizophrenia is also a serious mental disorder affecting about 1% of western countries
20 population. Antipsychotic (neuroleptic) drugs available include chlorpromazine and haloperidol which show affinity for the dopamine 2 receptor. But, adverse side effects such as sedation, dystonia, tremors and akathisia have been commonly observed and a significant percentage of patients do not respond to the treatments.

25 Therefore, the problem is to find alternative solutions to provide a relief and a cure for the numerous patients afflicted with these diseases.

In connection with the present invention, we propose that mast cells are involved in or contribute to CNS disorders. Mast cells (MC) are tissue elements derived from a particular subset of hematopoietic stem cells that express CD34, c-kit and CD13
5 antigens (Kirshenbaum et al, Blood. 94: 2333-2342, 1999 and Ishizaka et al, Curr Opin Immunol. 5: 937-43, 1993). Immature MC progenitors circulate in the bloodstream and differentiate in tissues. These differentiation and proliferation processes are under the influence of cytokines, one of utmost importance being Stem Cell Factor (SCF), also termed Kit ligand (KL), Steel factor (SL) or Mast Cell Growth Factor (MCGF). SCF
10 receptor is encoded by the protooncogene c-kit, that belongs to type III receptor tyrosine kinase subfamily (Boissan and Arock, J Leukoc Biol. 67: 135-48, 2000). This receptor is also expressed on others hematopoietic or non hematopoietic cells. Ligation of c-kit receptor by SCF induces its dimerization followed by its transphosphorylation, leading to the recruitment and activation of various intracytoplasmic substrates. These activated
15 substrates induce multiple intracellular signaling pathways responsible for cell proliferation and activation (Boissan and Arock, 2000). Mast cells are characterized by their heterogeneity, not only regarding tissue location and structure but also at the functional and histochemical levels (Aldenborg and Enerback., Histochem. J. 26: 587-96, 1994 ; Bradding et al. J Immunol. 155: 297-307, 1995 ; Irani et al, J Immunol. 147:
20 247-53, 1991 ; Miller et al, Curr Opin Immunol. 1: 637-42, 1989 and Welle et al, J Leukoc Biol. 61: 233-45, 1997).

Here, it is postulated that the activation of mast cells by different stimuli such as stress, trauma, infection as well as neurotransmitters, participate in the exacerbation of the
25 chemical imbalance causing CNS disorders.

More specifically, mast cell degranulation is stimulated by common neurotransmitters such as neurotensin, somatostatin, substance P and acetylcholine, by growth or survival factors, notably NGF, TGF β 1 (see figure 1). Mast cells involved in the response to such stimulus can be brain mast cells but also other mast cells releasing the content of their granules in the blood stream that ultimately reach sensory, motor or brain neurons. Brain mast cells staining is CTMC staining-like but they show the secretory pattern of MMC, implying that they constitute a particular subset of mast cells presenting specificities.

Following mast cells activation, released granules liberate various factors capable of modulating and altering neurotransmission and neurons survival. Among such factors, serotonin is important since an increase of the level of free serotonin has been observed in depressed patients. Alternatively, the sudden burst of serotonin may be followed by a period of serotonin shortage, leading to pain and migraine. As a consequence, we believe that mast cells exacerbate in autocrine or paracrine manner the deregulation of neurotransmission. For example, anxiety or stress-induced release of neurotransmitters such as serotonin activates mast cells, which in turn release the content of their granules, further contributing to the chemical imbalance in the brain leading to CNS disorders. Other mediators released by mast cells can be categorized into vasoactive, nociceptive, proinflammatory and other neurotransmitters. Taken together, these factors are able to induce great disturbance in the activity of neurons, whether they are sensory, motor, or CNS neurons.

We also observed that patients afflicted with mastocytosis are more inclined to develop CNS disorders than the normal population. This can be explained by the presence of activating mutations in the c-kit receptor, which induce degranulation of mast cells and a burst of factors contributing to chemical imbalance and neurotransmission alteration.

In some cases, activated mast cells can also participate in the destruction of neuronal tissues by releasing a cocktail of different proteases and mediators categorized into three groups: preformed granule-associated mediators (histamine, proteoglycans, and neutral proteases), lipid-derived mediators (prostaglandins, thromboxanes and leucotrienes), and various cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF- α , GM-CSF, MIP-1a, MIP-1b, MIP-2 and IFN- γ). Then, liberation by activated mast cells of mediators (TNF- α , histamine, leucotrienes, prostaglandines etc...) as well as proteases is proposed here i) to induce inflammation and vasodilatation and ii) to participate in the neuronal tissue destruction process.

10

As a consequence, the present invention proposes to deplete mast cells using compounds that are substantially specific to mast cells. In this regard, tyrosine kinase inhibitors and more particularly c-kit specific kinase inhibitors are proposed to inhibit mast cell proliferation, survival and activation.

15

A new route for treating CNS disorders is provided, which consists of destroying mast cells involved in and contributing to the pathogenesis of these disorders. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited to reach this goal.

20

Description

The present invention relates to a method for treating CNS disorders comprising administering a compound capable of depleting mast cells to a human in need of such treatment.

25

Said method for treating CNS disorders can comprise administering a tyrosine kinase inhibitor to a human in need of such treatment.

Tyrosine kinase inhibitors are selected for example from bis monocyclic, bicyclic or
5 heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO
94/14808) and 1-cyclopropyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds
(US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), selenindoles and
selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and
benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (US
10 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones
(US 5,792,783, EP 934 931, US 5,834,504, US 5,883,116, US 5,883,113, US 5,
886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and
heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline
derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940) and aryl and
15 heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

20 In another embodiment, the invention is directed to a method for treating CNS disorders comprising administering a c-kit inhibitor to a human in need of such treatment.

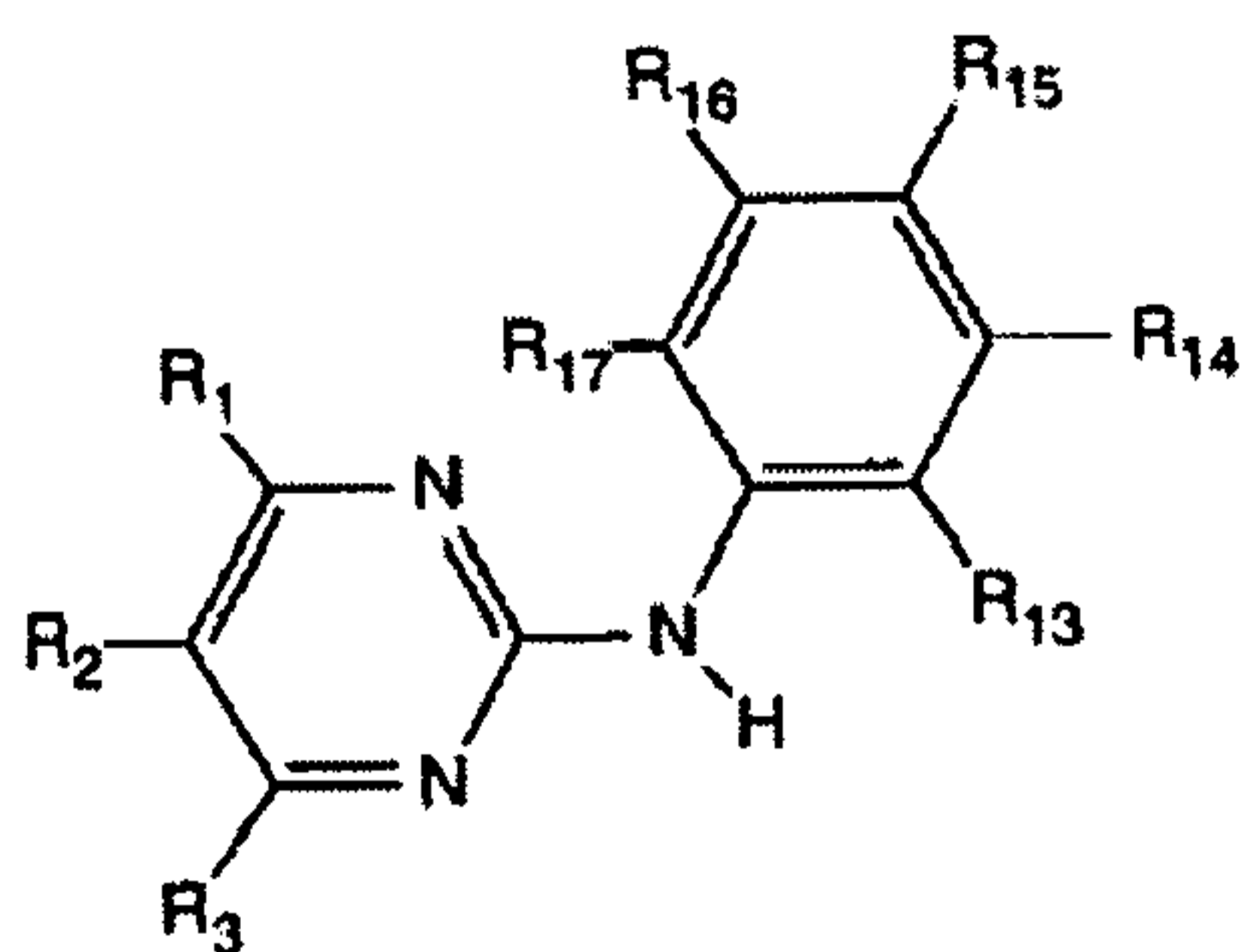
Preferably, said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor. Such inhibitors can be selected from the group consisting of indolinones, pyrimidine
25 derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl

compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as
 5 N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520
 10 722, US 3,772,295 and US 4,343,940), 4-amino-substituted quinazolines (US 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (US 3,800,039), aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (US 4,464,375), and 4-thienyl-2-(1H)-quinazolones (US 3,551,427).

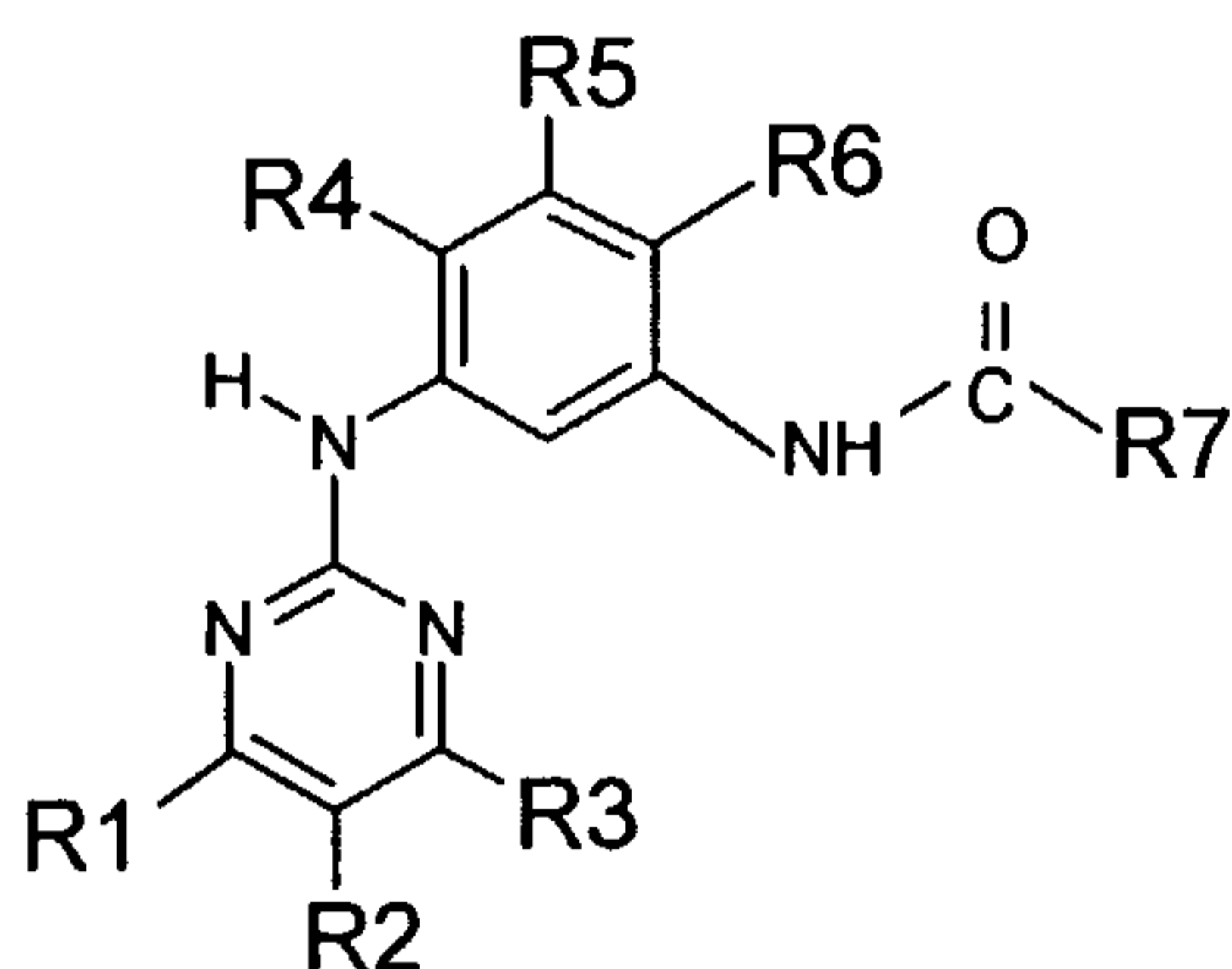
15

So, preferably, the invention relates to a method for treating CNS disorders comprising administering a non toxic, potent and selective c-kit inhibitor is a pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I :



20 wherein the R1, R2, R3, R13 to R17 groups have the meanings depicted in EP 564 409 B1, incorporated herein in the description.

Preferably, the N-phenyl-2-pyrimidine-amine derivative is selected from the compounds corresponding to formula II :



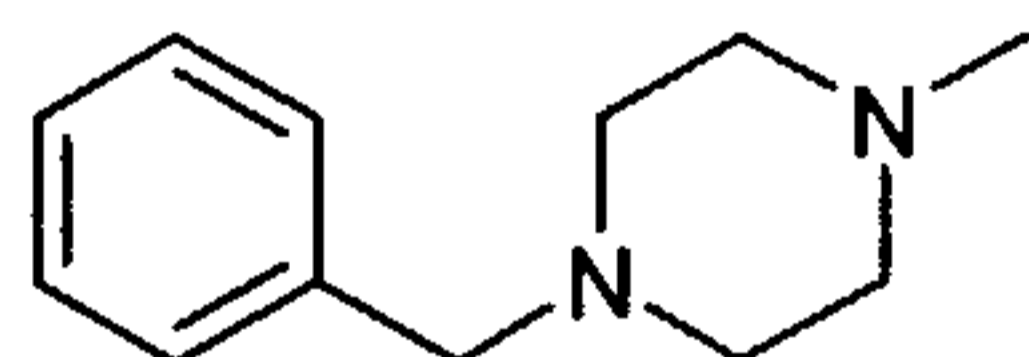
5

Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

10 and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function.

Preferably, R7 is the following group :



15 Among these compounds, the preferred are defined as follows :

R1 is a heterocyclic group, especially a pyridyl group,

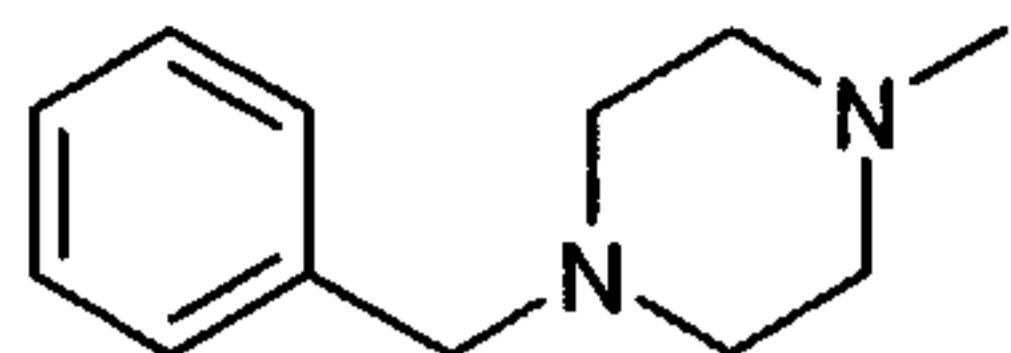
R2 and R3 are H,

R4 is a C1-C3 alkyl, especially a methyl group,

R5 and R6 are H,

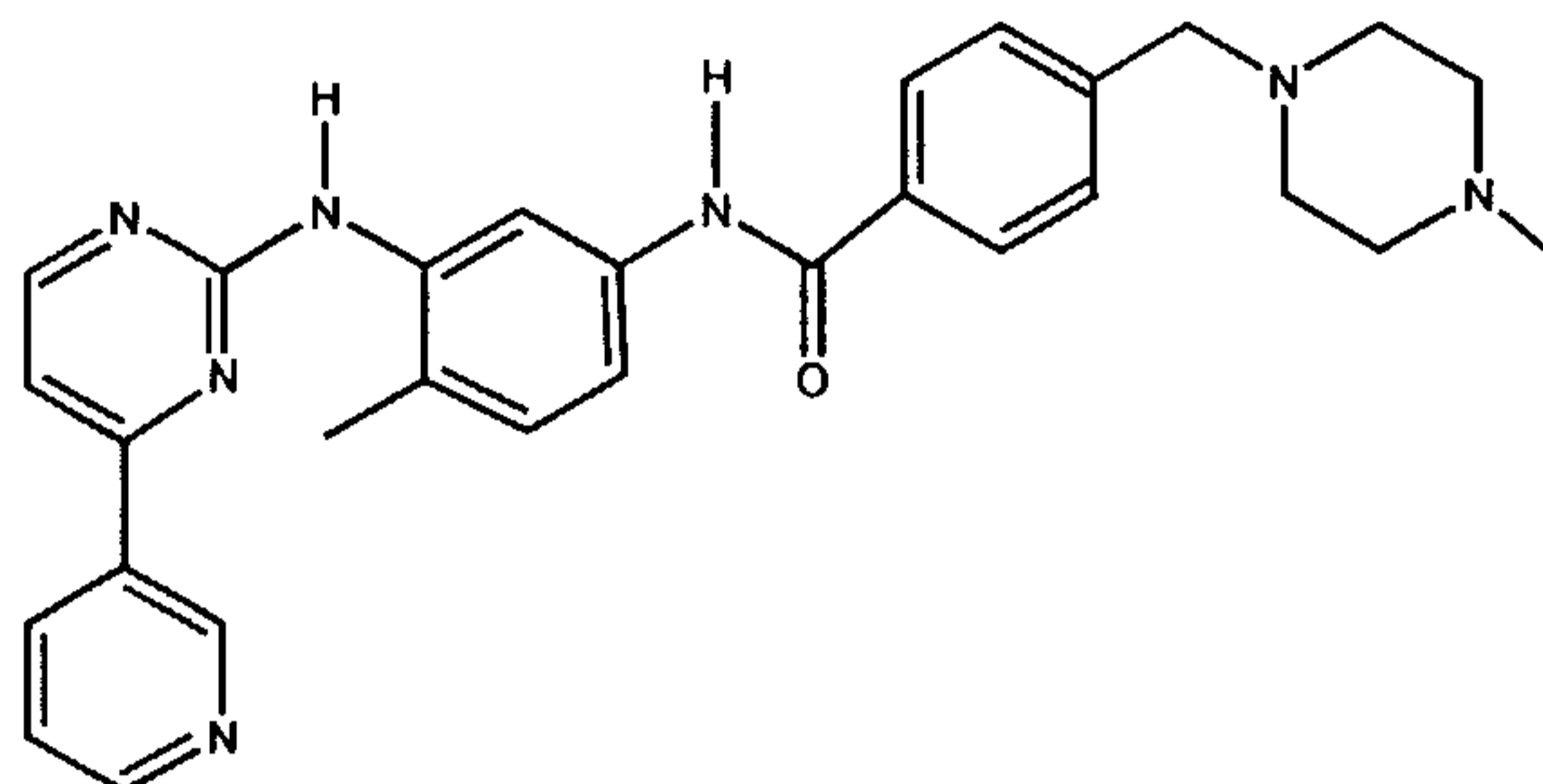
20 and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one

basic site, such as an amino function, for example the group :



Therefore, in a preferred embodiment, the invention relates to a method for treating CNS disorders comprising the administration of an effective amount of the compound known
 5 in the art as CGP57148B :

4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2-ylamino]phényl]-benzamide corresponding to the following formula :



10 The preparation of this compound is described in example 21 of EP 564 409 and the β -form, which is particularly useful is described in WO 99/03854.

Alternatively, the c-kit inhibitor can be selected from :

- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- 15 - monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phényl-6,7-dimethoxy quinaxoline.

In a preferred aspect, the invention contemplated the method mentioned above, wherein
 20 said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The CNS disorders as referred herein include but are not limited to psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

More particularly, the method according to the invention is useful for the treatment of the following disorders :

- 5 - Depression including dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, refractory depression, seasonal depression, anorexia, bulimia, premenstrual syndrome, post-menopause syndrome.
- Other syndromes such as mental slowing and loss of concentration, pessimistic
10 worry, agitation, self-deprecation, decreased libido,
- Pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, psychogenic pain syndromes,
- Anxiety disorders including anxiety associated with hyperventilation and cardiac
15 arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, generalized anxiety disorder,
- Psychiatric emergencies such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, seizures
- 20 - Severe psychiatric emergencies including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis,
- Schizophrenia including paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia,
- Neurodegenerative diseases including Alzheimer's disease , Parkinson's disease,
25 Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

Therefore, in a preferred embodiment, the method of the invention is applicable to the treatment of depression.

In another preferred embodiment, the method of the invention is applicable to the treatment of pain.

In another preferred embodiment, the method of the invention is applicable to the treatment of anxiety disorders.

In another preferred embodiment, the method of the invention is applicable to the treatment of psychiatric disorders.

In another preferred embodiment, the method of the invention is applicable to the treatment of schizophrenia.

In another preferred embodiment, the method of the invention is applicable to the treatment of neurodegenerative diseases.

The method as depicted above is also useful for treating memory loss.

In still another preferred embodiment, the method of the invention is applicable to the treatment of migraine.

In a further embodiment, c-kit inhibitors as mentioned above are inhibitors of activated c-kit. In frame with the invention, the expression "activated c-kit" means a constitutively activated-mutant c-kit including at least one mutation selected from point mutations, deletions, insertions, but also modifications and alterations of the natural c-kit sequence (SEQ ID N°1). Such mutations, deletions, insertions, modifications and alterations can occur in the transphosphorylase domain, in the juxtamembrane domain as well as in any domain directly or indirectly responsible for c-kit activity. The expression "activated c-kit" also means herein SCF-activated c-kit. Preferred and optimal SCF concentrations for activating c-kit are comprised between 5.10^{-7} M and 5.10^{-6} M, preferably around

2.10⁻⁶ M. In a preferred embodiment, the activated-mutant c-kit in step a) has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants. In another preferred embodiment, the activated-mutant c-kit in step a) has a deletion in the juxtamembrane domain of c-kit. Such a deletion is for example between codon 573 and 579 called c-kit d(573-579). The point mutation V559G proximal to the juxtamembrane domain c-kit is also of interest.

In this regard, the invention contemplates a method for treating CNS disorders as defined above comprising administering to a human in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

- a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,
- b) selecting compounds that inhibit activated c-kit,
- c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

This screening method can further comprise the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit (for example in the transphosphorylase domain), which are also capable of inhibiting SCF-activated c-kit wild.

Alternatively, in step a) activated c-kit is SCF-activated c-kit wild.

A best mode for practicing this method consists of testing putative inhibitors at a concentration above 10 μ M in step a). Relevant concentrations are for example 10, 15, 20, 25, 30, 35 or 40 μ M.

In step c), IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

Examples of IL-3 dependent cells include but are not limited to :

- 5 - cell lines naturally expressing and depending on c-kit for growth and survival. Among such cells, human mast cell lines can be established using the following procedures : normal human mast cells can be infected by retroviral vectors containing sequences coding for a mutant c-kit comprising the c-kit signal peptide and a TAG sequence allowing to differentiate mutant c-kits from c-kit wild expressed in hematopoietic cells by
10 means of antibodies.

This technique is advantageous because it does not induce cellular mortality and the genetic transfer is stable and gives satisfactory yields (around 20 %). Pure normal human mast cells can be routinely obtained by culturing precursor cells originating from blood obtained from human umbilical vein. In this regard, heparinated blood from umbilical
15 vein is centrifuged on a Ficoll gradient so as to isolate mononucleated cells from other blood components. CD34+ precursor cells are then purified from the isolated cells mentioned above using the immunomagnetic selection system MACS (Miltenyi biotech). CD34+ cells are then cultured at 37°C in 5 % CO₂ atmosphere at a concentration of 10⁵ cells per ml in the medium MCCM (α -MEM supplemented with L-glutamine, penicillin,
20 streptomycin, 5 10⁻⁵ M β -mercaptoethanol, 20 % veal foetal serum, 1 % bovine albumin serum and 100 ng/ml recombinant human SCF. The medium is changed every 5 to 7 days. The percentage of mast cells present in the culture is assessed each week, using May-Grünwal Giemsa or Toluidine blue coloration. Anti-tryptase antibodies can also be used to detect mast cells in culture. After 10 weeks of culture, a pure cellular population
25 of mast cells (> 98 %) is obtained.

It is possible using standard procedures to prepare vectors expressing c-kit for transfecting the cell lines established as mentioned above. The cDNA of human c-kit has been described in Yarden et al., (1987) EMBO J.6 (11), 3341-3351. The coding part of c-kit (3000 bp) can be amplified by PCR and cloned, using the following
5 oligonucleotides :

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No2) sens
- 5'CTGCTTCGCGGCCGCGTTAACTCTTCTCAACCA3' (SEQ ID No3)
antisens

The PCR products, digested with Not1 and Xho1, has been inserted using T4 ligase in
10 the pFlag-CMV vector (SIGMA), which vector is digested with Not1 and Xho1 and dephosphorylated using CIP (Biolabs). The pFlag-CMV-c-kit is used to transform bacterial clone XL1-blue. The transformation of clones is verified using the following primers :

- 5'AGCTCGTTTAGTGAACCGTC3' (SEQ ID No4) sens,
- 15 - 5'GTCAGACAAAATGATGCAAC3' (SEQ ID No5) antisens.

Directed mutagenesis is performed using relevant cassettes is performed with routine and common procedure known in the art..

The vector Migr-1 (ABC) can be used as a basis for constructing retroviral vectors used for transfecting mature mast cells. This vector is advantageous because it contains the
20 sequence coding for GFP at the 3' and of an IRES. These features allow to select cells infected by the retrovirus using direct analysis with a fluorocytometer. As mentioned above, the N-terminal sequence of c-kit c-DNA can be modified so as to introduce a Flag sequence that will be useful to discriminating heterogeneous from endogenous c-kit.

25 Other IL-3 dependent cell lines that can be used include but are not limited to:

- BaF3 mouse cells expressing wild-type or mutated form of c-kit (in the juxtamembrane and in the catalytic sites) are described in Kitayama et al, (1996), Blood 88, 995-1004 and Tsujimura et al, (1999), Blood 93, 1319-1329.

- IC-2 mouse cells expressing either c-kit^{WT} or c-kit^{D814Y} are presented in Piao et al, (1996), Proc. Natl. Acad. Sci. USA 93, 14665-14669.

IL-3 independent cell lines are :

- HMC-1, a factor-independent cell line derived from a patient with mast cell leukemia, expresses a juxtamembrane mutant c-kit polypeptide that has constitutive kinase activity

(Furitsu T et al, J Clin Invest. 1993;92:1736-1744 ; Butterfield et al, Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345-355 and Nagata et al, Proc Natl Acad Sci U S A. 1995;92:10560-10564).

- P815 cell line (mastocytoma naturally expressing c-kit mutation at the 814 position) has been described in Tsujimura et al, (1994), Blood 83, 2619-2626.

The extent to which component (ii) inhibits activated c-kit can be measured *in vitro* or *in vivo*. In case it is measured *in vivo*, cell lines expressing an activated-mutant c-kit, which has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants, are preferred.

Example of cell lines expressing an activated-mutant c-kit are as mentioned above.

In another preferred embodiment, the method further comprises the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μ M. This can be measured *in vitro* or *in vivo*.

Therefore, compounds are identified and selected according to the method described above are potent, selective and non-toxic c-kit wild inhibitors.

Alternatively, the screening method as defined above can be practiced *in vitro*. In this regard, the inhibition of mutant-activated c-kit and/or c-kit wild can be measured using standard biochemical techniques such as immunoprecipitation and western blot. Preferably, the amount of c-kit phosphorylation is measured.

In a still further embodiment, the invention contemplates a method for treating CNS disorders as depicted above wherein the screening comprises :

- a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an $IC_{50} < 10 \mu M$, by measuring the extent of cell death,
- b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,
- c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an $IC_{50} < 10 \mu M$, preferably an $IC_{50} < 1 \mu M$, by measuring the extent of cell death.

Here, the extent of cell death can be measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide. These are common techniques routinely practiced in the art.

The method according to the invention includes preventing, delaying the onset and/or treating CNS disorders in humans. Regarding the prion diseases, the invention encompasses the treatment of mammals such as bovine and ovine species.

5 In the method defined above, any compound capable of depleting mast cells can be used. Such compounds can belong to, as explicated above, tyrosine kinase inhibitors, such as c-kit inhibitors, but are not limited to any particular family so long as said compound shows capabilities to deplete mast cells. Depletion of mast cells can be evaluated using for example one of the mast cell lines depicted above using routine procedure.

10 Best compounds are compounds exhibiting the greatest selectivity.

Control cell lines include other hematopoietic cells that are not mast cells or related cells or cell lines. These control cell lines include SCF independent expanded human CD34+ normal cells. These control cells also include but are not limited to the human T lymphocyte Jurkat cell line (ATCC N° TIB-152 and mutant cell lines derived thereof),
15 the human B lymphocyte Daudi or Raji cell line (ATCC N° CCL-213 and CCL-86 respectively), the human monocytic U 937 cell line (ATCC N° CRL-1593.2) and the human HL-60 cell line (ATCC N° CCL-240) and mutant cell lines derived thereof CRL-2258 and CRL-2392).

20 Such compounds can be selected with a method for identifying compounds capable of depleting mast cells, said compound being non-toxic for cell types other than mast cells, comprising the step consisting of :

- a) culturing mast cells in vitro in a culture medium suitable for mast cells,
- b) adding to said culture medium at least one compound to be tested and incubating said
25 cells for a prolonged period of time,
- c) selecting compounds that promote mast cells death,
- d) identifying a subset of compounds selected in step c) that are unable to promote death of cells selected from the above mentioned control cell lines.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament for treating CNS disorders such as psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

5

The invention is also directed to the use of the compounds defined above to manufacture a medicament for treating a disorders selected from the subgroups consisting of :

- 10 - Depression including dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, seasonal depression, anorexia, bulimia, premenstrual syndrome, post-menopause syndrome.
- Other syndromes such as mental slowing and loss of concentration, pessimistic worry, agitation, self-deprecation, decreased libido,
- Pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, psychogenic pain syndromes,
- 15 - Anxiety disorders including anxiety associated with hyperventilation and cardiac arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, generalized anxiety disorder,
- Psychiatric emergencies such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes
20 including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, seizures
- Severe psychiatric emergencies including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis,
- Schizophrenia including paranoid schizophrenia, disorganized schizophrenia,
25 catatonic schizophrenia, and undifferentiated schizophrenia,

- Neurodegenerative diseases including Alzheimer's disease , Parkinson's disease, Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

5 The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

10 In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack
15 Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated
20 as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

More particularly, the invention relates to a pharmaceutical composition intended for oral administration.

25

Regarding the treatment of pain, topical administration may be most suitable in some cases. Here, the compositions according to the invention may be presented in the form of a gel, paste, ointment, cream, lotion, liquid suspension aqueous, aqueous-alcoholic or,

oily solutions, or dispersions of the lotion or serum type, or anhydrous or lipophilic gels, or emulsions of liquid or semi-solid consistency of the milk type, obtained by dispersing a fatty phase in an aqueous phase or vice versa, or of suspensions or emulsions of soft, semi-solid consistency of the cream or gel type, or alternatively of microemulsions, of
5 microcapsules, of microparticles or of vesicular dispersions to the ionic and/or nonionic type. These compositions are prepared according to standard methods.

The composition according to the invention comprises any ingredient commonly used in dermatology and cosmetic. It may comprise at least one ingredient selected from
10 hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preservatives, emollients, viscosity enhancing polymers, humectants, surfactants, preservatives, antioxidants, solvents, and fillers, antioxidants, solvents, perfumes, fillers, screening agents, bactericides, odor absorbers and coloring matter.

15 As oils which can be used in the invention, mineral oils (liquid paraffin), vegetable oils (liquid fraction of shea butter, sunflower oil), animal oils, synthetic oils, silicone oils (cyclomethicone) and fluorinated oils may be mentioned. Fatty alcohols, fatty acids (stearic acid) and waxes (paraffin, carnauba, beeswax) may also be used as fatty substances.

20

As emulsifiers which can be used in the invention, glycerol stearate, polysorbate 60 and the PEG-6/PEG-32/glycol stearate mixture are contemplated.

As hydrophilic gelling agents, carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, polysaccharides such as
25 hydroxypropylcellulose, clays and natural gums may be mentioned, and as lipophilic gelling agents, modified clays such as bentones, metal salts of fatty acids such as aluminum stearates and hydrophobic silica, or alternatively ethylcellulose and polyethylene may be mentioned.

As hydrophilic active agents, proteins or protein hydrolysates, amino acids, polyols, urea, allantoin, sugars and sugar derivatives, vitamins, starch and plant extracts, in particular those of Aloe vera may be used.

5

As lipophilic active agents, retinol (vitamin A) and its derivatives, tocopherol (vitamin E) and its derivatives, essential fatty acids, ceramides and essential oils may be used. These agents add extra moisturizing or skin softening features when utilized.

10 In addition, a surfactant can be included in the composition so as to provide deeper penetration of the compound capable of depleting mast cells, such as a tyrosine kinase inhibitor, preferably a c-kit inhibitor.

Among the contemplated ingredients, the invention embraces penetration enhancing
15 agents selected for example from the group consisting of mineral oil, water, ethanol, triacetin, glycerin and propylene glycol; cohesion agents selected for example from the group consisting of polyisobutylene, polyvinyl acetate and polyvinyl alcohol, and thickening agents.

20 Chemical methods of enhancing topical absorption of drugs are well known in the art. For example, compounds with penetration enhancing properties include sodium lauryl sulfate (Dugard, P. H. and Sheuplein, R. J., "Effects of Ionic Surfactants on the Permeability of Human Epidermis: An Electrometric Study," J. Invest. Dermatol., V.60, pp. 263-69, 1973), lauryl amine oxide (Johnson et. al., US 4,411,893), azone
25 (Rajadhyaksha, US 4,405,616 and 3,989,816) and decylmethyl sulfoxide (Sekura, D. L. and Scala, J., "The Percutaneous Absorption of Alkylmethyl Sulfides," Pharmacology of the Skin, Advances In Biology of Skin, (Appleton-Century Craft) V. 12, pp. 257-69, 1972). It has been observed that increasing the polarity of the head group in amphoteric

molecules increases their penetration-enhancing properties but at the expense of increasing their skin irritating properties (Cooper, E. R. and Berner, B., "Interaction of Surfactants with Epidermal Tissues: Physiochemical Aspects," Surfactant Science Series, V. 16, Reiger, M. M. ed. (Marcel Dekker, Inc.) pp. 195-210, 1987).

5

A second class of chemical enhancers are generally referred to as co-solvents. These materials are absorbed topically relatively easily, and, by a variety of mechanisms, achieve permeation enhancement for some drugs. Ethanol (Gale et. al., U.S. Pat. No. 4,615,699 and Campbell et. al., U.S. Pat. Nos. 4,460,372 and 4,379,454), dimethyl
10 sulfoxide (US 3,740,420 and 3,743,727, and US 4,575,515), and glycerine derivatives (US 4,322,433) are a few examples of compounds which have shown an ability to enhance the absorption of various compounds.

Pharmaceutical compositions suitable for use in the invention include compositions
15 wherein compounds for depleting mast cells, such as tyrosine kinase inhibitors and c-kit inhibitors, are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be
20 determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. As
25 mentioned above, a tyrosine kinase inhibitor and more particularly a c-kit inhibitor according to the invention is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The invention also contemplates a product comprising at least one compound capable of depleting mast cells, such as a tyrosine kinase inhibitors, more particularly a non-toxic, selective and potent c-kit inhibitor and at least one antidepressant, antipsychotic, or anxiolytic for simultaneous, separate or sequential use for the treatment of CNS
5 disorders as defined above.

SEQUENCE LISTING

<110> AB Science

<120> Use of tyrosine kinase inhibitors for treating CNS disorders

<130> D20044 NT

<150> US 60/359,652

<151> 2002-02-27

<160> 5

<170> PatentIn Ver. 2.1

<210> 1

<211> 976

<212> PRT

<213> Homo sapiens

<220>

<223> Human c-kit

<400> 1

```

Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu
  1           5           10           15

Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly
           20           25           30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val
           35           40           45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
  50           55           60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
  65           70           75           80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr
           85           90           95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg
           100           105           110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
           115           120           125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
           130           135           140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu
           145           150           155           160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
           165           170           175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly
           180           185           190

Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe
           195           200           205

Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
           210           215           220

```

Glu 225	Gly	Glu	Glu	Phe	Thr 230	Val	Thr	Cys	Thr	Ile 235	Lys	Asp	Val	Ser	Ser 240
Ser	Val	Tyr	Ser	Thr 245	Trp	Lys	Arg	Glu	Asn 250	Ser	Gln	Thr	Lys	Leu 255	Gln
Glu	Lys	Tyr	Asn 260	Ser	Trp	His	His	Gly 265	Asp	Phe	Asn	Tyr	Glu 270	Arg	Gln
Ala	Thr	Leu 275	Thr	Ile	Ser	Ser	Ala 280	Arg	Val	Asn	Asp	Ser 285	Gly	Val	Phe
Met	Cys 290	Tyr	Ala	Asn	Asn	Thr 295	Phe	Gly	Ser	Ala	Asn 300	Val	Thr	Thr	Thr
Leu 305	Glu	Val	Val	Asp	Lys 310	Gly	Phe	Ile	Asn	Ile 315	Phe	Pro	Met	Ile	Asn 320
Thr	Thr	Val	Phe	Val 325	Asn	Asp	Gly	Glu	Asn 330	Val	Asp	Leu	Ile	Val 335	Glu
Tyr	Glu	Ala	Phe 340	Pro	Lys	Pro	Glu	His 345	Gln	Gln	Trp	Ile	Tyr 350	Met	Asn
Arg	Thr	Phe 355	Thr	Asp	Lys	Trp	Glu	Asp 360	Tyr	Pro	Lys	Ser 365	Glu	Asn	Glu
Ser	Asn 370	Ile	Arg	Tyr	Val	Ser 375	Glu	Leu	His	Leu	Thr 380	Arg	Leu	Lys	Gly
Thr 385	Glu	Gly	Gly	Thr	Tyr 390	Thr	Phe	Leu	Val	Ser 395	Asn	Ser	Asp	Val	Asn 400
Ala	Ala	Ile	Ala	Phe 405	Asn	Val	Tyr	Val	Asn 410	Thr	Lys	Pro	Glu	Ile 415	Leu
Thr	Tyr	Asp	Arg 420	Leu	Val	Asn	Gly	Met 425	Leu	Gln	Cys	Val	Ala	Ala	Gly
Phe	Pro	Glu 435	Pro	Thr	Ile	Asp	Trp 440	Tyr	Phe	Cys	Pro	Gly 445	Thr	Glu	Gln
Arg	Cys 450	Ser	Ala	Ser	Val	Leu 455	Pro	Val	Asp	Val	Gln 460	Thr	Leu	Asn	Ser
Ser 465	Gly	Pro	Pro	Phe	Gly 470	Lys	Leu	Val	Val	Gln 475	Ser	Ser	Ile	Asp	Ser 480
Ser	Ala	Phe	Lys	His 485	Asn	Gly	Thr	Val	Glu 490	Cys	Lys	Ala	Tyr	Asn 495	Asp
Val	Gly	Lys	Thr 500	Ser	Ala	Tyr	Phe	Asn 505	Phe	Ala	Phe	Lys	Gly 510	Asn	Asn
Lys	Glu	Gln	Ile	His	Pro	His	Thr 520	Leu	Phe	Thr	Pro	Leu 525	Leu	Ile	Gly
Phe 530	Val	Ile	Val	Ala	Gly	Met 535	Met	Cys	Ile	Ile	Val 540	Met	Ile	Leu	Thr
Tyr 545	Lys	Tyr	Leu	Gln	Lys 550	Pro	Met	Tyr	Glu	Val 555	Gln	Trp	Lys	Val	Val 560
Glu	Glu	Ile	Asn	Gly 565	Asn	Asn	Tyr	Val	Tyr 570	Ile	Asp	Pro	Thr	Gln 575	Leu

Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly
 580 585 590
 Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
 595 600 605
 Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
 610 615 620
 Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu
 625 630 635 640
 Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu
 645 650 655
 Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr
 660 665 670
 Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
 675 680 685
 Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
 690 695 700
 Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu
 705 710 715 720
 Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
 725 730 735
 Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val
 740 745 750
 Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
 755 760 765
 Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala
 770 775 780
 Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
 785 790 795 800
 Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp
 805 810 815
 Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
 820 825 830
 Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
 835 840 845
 Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
 850 855 860
 Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
 865 870 875 880
 Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
 885 890 895
 Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
 900 905 910
 Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
 915 920 925

Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
 930 935 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
 945 950 955 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
 965 970 975

<210> 2
 <211> 30
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Primer

<400> 2
 aagaagagat ggtacctcga ggggtgaccc 30

<210> 3
 <211> 33
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Primer

<400> 3
 ctgcttcgcg gccgcgtaa ctcttctcaa cca 33

<210> 4
 <211> 20
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Primer

<400> 4
 agctcgttta gtgaaccgtc 20

<210> 5
 <211> 20
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Primer

<400> 5
 gtcagacaaa atgatgcaac 20

CLAIMS

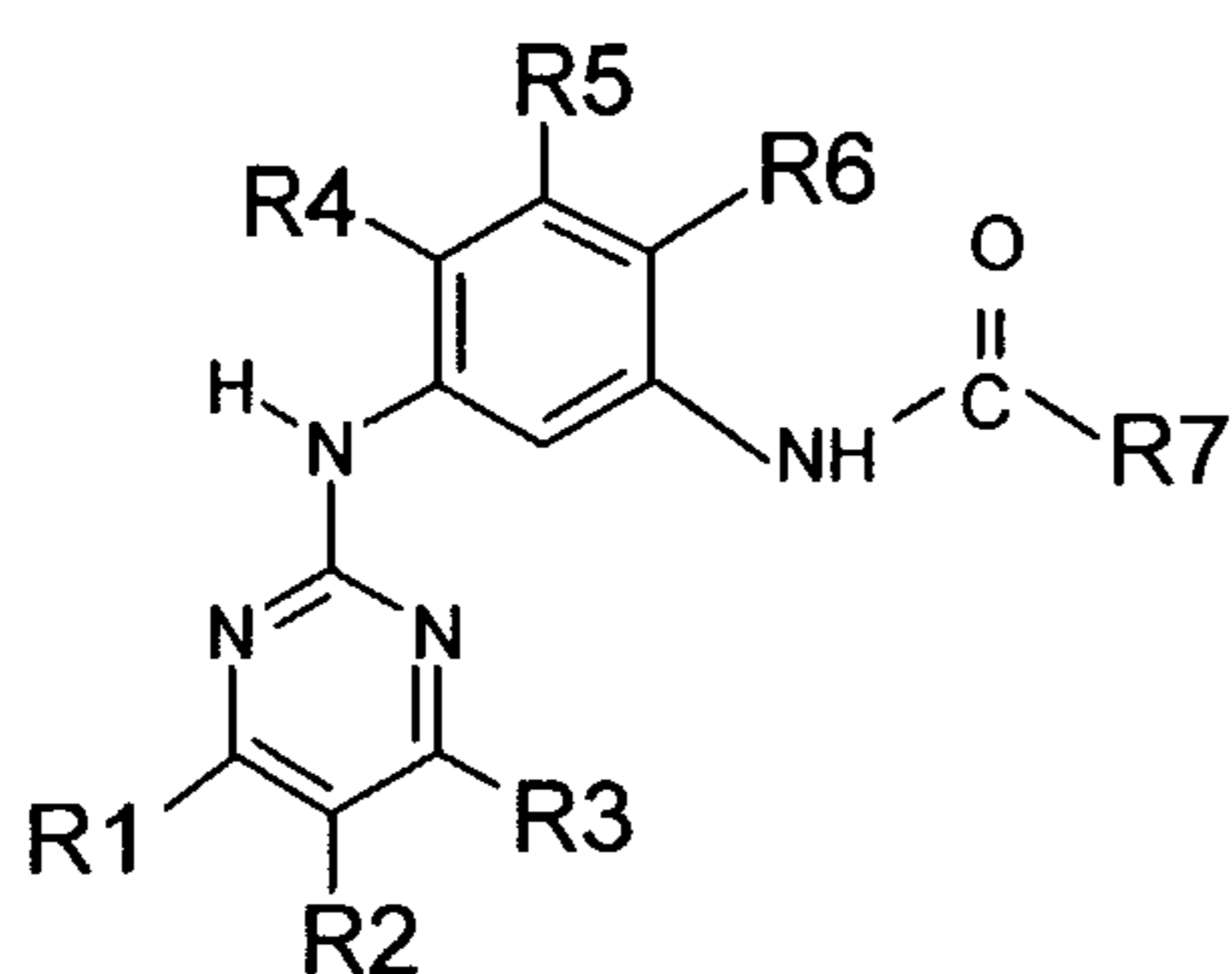
- 5 1. A method for treating CNS disorders comprising administering a compound capable of depleting mast cells to a human in need of such treatment.
2. A method according to claim 1 for treating CNS disorders comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.
- 10 3. A method according to claim 2, wherein said tyrosine kinase inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.
4. A method according to claim 2 for treating CNS disorders comprising administering a
- 15 c-kit inhibitor to a human in need of such treatment.
5. A method according to claim 4, wherein said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor.
- 20 6. A method according to claim 5, wherein said inhibitor is selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds,
- 25 seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

7. A method according to claim 5, wherein said inhibitor is selected from the group consisting of :

- pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives.
- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- 5 - monocyclic, bicyclic aryl and heteroaryl compounds,
- and quinazoline derivatives.

8. A method according to claim 4, wherein said inhibitor is selected from the group consisting of N-phenyl-2-pyrimidine-amine derivatives having the formula II :

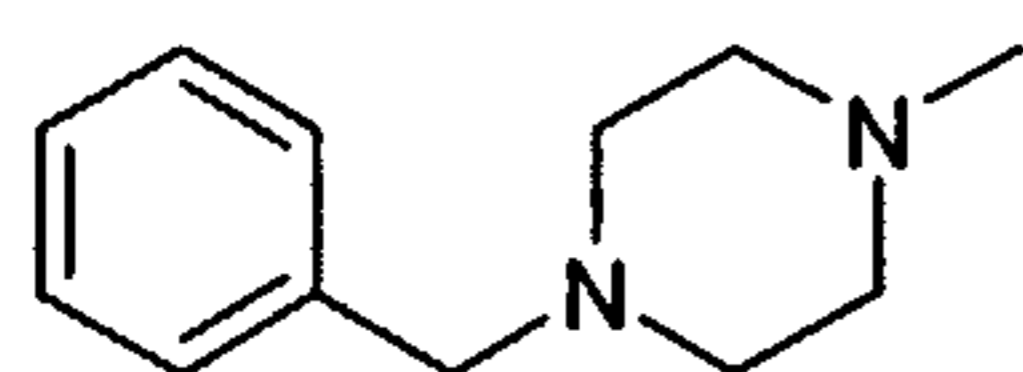
10



Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

15 R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, preferably the following group :



20

9. A method according to claim 8, wherein said inhibitor is the 4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino]phényl]-benzamide.

10. A method according to one of claims 4 to 9, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

5 11. A method according to one of claims 4 to 10, wherein said c-kit inhibitor is an inhibitor of activated c-kit.

12. A method according to claim 11, wherein said inhibitor is capable of inhibiting constitutively activated-mutant c-kit.

10

13. A method according to one of claims 4 to 10, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

14. A method for treating CNS disorders comprising administering to a human in need
15 of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,

b) selecting compounds that inhibit activated c-kit,

20 c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

15. A method according to claim 14, wherein the screening method further comprises the step consisting of testing and selecting a subset of compounds identified in step b) that
25 are inhibitors of mutant activated c-kit, which are also capable of inhibiting SCF-activated c-kit wild.

16. A method according to claim 14, wherein activated c-kit is SCF-activated c-kit wild in step a).

17. A method according to one of claims 14 to 16, wherein putative inhibitors are tested
5 at a concentration above 10 μ M in step a).

18. A method according to one of claims 14 to 16, wherein IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

10

19. A method according to claim 14, wherein IL-3 dependent cells are selected from the group consisting of mast cells, transfected mast cells, BaF3 and IC-2.

20. A method according to one of claims 14 to 19, wherein the extent to which
15 component (ii) inhibits activated c-kit is measured *in vitro* or *in vivo*.

21. A method according to one of claims 14 to 20, further comprising the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μ M.

20

22. A method according to claim 17 or 21, wherein the testing is performed *in vitro* or *in vivo*.

23. A method according to one of claims 14 to 22, wherein the inhibition of mutant-
25 activated c-kit and/or c-kit wild is measured using standard biochemical techniques such as immunoprecipitation and western blot.

24. A method according to one of claims 14 to 22, wherein the amount of c-kit phosphorylation is measured.

25. A method according to one of claims 14 to 24, wherein identified and selected
5 compounds are potent, selective and non-toxic c-kit wild inhibitors.

26. A method for treating CNS disorders comprising administering to a human in need of such treatment a c-kit inhibitor obtainable by a screening method comprising :

a) performing a proliferation assay with cells expressing a mutant c-kit (for example in
10 the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an $IC_{50} < 10 \mu M$, by measuring the extent of cell death,

b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured
15 in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,

c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an $IC_{50} < 10 \mu M$, preferably an $IC_{50} < 1 \mu M$, by measuring the
20 extent of cell death.

27. A method according to claim 26, wherein the extent of cell death is measured by 3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide.

25

28. A method according to one of claims 1 to 27 for preventing, delaying the onset and/or treating CNS disorders in human.

29. A method according to one of claims 1 to 28 for treating psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

30. A method according to one of claims 1 to 28 for treating depression including
5 dysthymic disorder, cyclothymic disorder, bipolar depression, severe or "melancholic" depression, atypical depression, refractory depression, seasonal depression, anorexia, bulimia, premenstrual syndrome and post-menopause syndrome.

31. A method according to one of claims 1 to 28 for treating mental slowing and loss of
10 concentration, pessimistic worry, agitation, self-deprecation and decreased libido.

32. A method according to one of claims 1 to 28 for treating pain including, acute pain, postoperative pain, chronic pain, nociceptive pain, cancer pain, neuropathic pain, and psychogenic pain syndromes.

15

33. A method according to one of claims 1 to 28 for treating anxiety disorders including anxiety associated with hyperventilation and cardiac arrhythmias, phobic disorders, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, and generalized anxiety disorder.

20

34. A method according to one of claims 1 to 28 for treating psychiatric disorders such as panic attacks, including psychosis, delusional disorders, conversion disorders, phobias, mania, delirium, dissociative episodes including dissociative amnesia, dissociative fugue and dissociative identity disorder, depersonalization, catatonia, and
25 seizures.

35. A method according to one of claims 1 to 28 for treating severe psychiatric disorders including suicidal behaviour, self-neglect, violent or aggressive behaviour, trauma, borderline personality, and acute psychosis.

5 36. A method according to one of claims 1 to 28 for treating schizophrenia including paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and undifferentiated schizophrenia.

10 37. A method according to one of claims 1 to 28 for treating neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, the prion diseases, Motor Neurone Disease (MND), and Amyotrophic Lateral Sclerosis (ALS).

38. A method according to one of claims 1 to 28 for treating memory loss.

15 39. A method according to one of claims 1 to 28 for treating migraine.

40. Use of a c-kit inhibitor to manufacture a medicament for treating CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

20

41. A composition suitable for topical administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treatment of pain.

25 42. A composition suitable for oral administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit

inhibitor for the treatment of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

43. A composition suitable for intravenous, intramuscular, intra-arterial, intramedullary,
5 intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, enteral, sublingual, or rectal administration comprising a compound capable of depleting mast cells, preferably a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treating of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

10

44. A product comprising at least one compound capable of depleting mast cells, such as a tyrosine kinase inhibitors, more particularly a non-toxic, selective and potent c-kit inhibitor and at least one antidepressant, antipsychotic, or anxiolytic for simultaneous,
15 separate or sequential use for the treatment of CNS disorders, more particularly for the treatment of psychiatric disorders, migraine, pain, memory loss and nerve cells degeneracy.

20

25