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(54) Titre : UTILISATION D'INHIBITEURS DE TYROSINE KINASE POUR TRAITER DES TROUBLES LIES A L'UTILISATION DE SUBSTANCES

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

(57) Abrégé/Abstract:
The present invention relates to a method for treating substance use disorders, more particularly drug addiction, drug habituation, drug dependence, withdrawal syndrome and overdose, 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.

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

(57) Abstract: The present invention relates to a method for treating substance use disorders, more particularly drug addiction, drug habituation, drug dependence, withdrawal syndrome and overdose, 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.

Use of tyrosine kinase inhibitors for treating substance use disorders

The present invention relates to a method for treating substance use disorders, more particularly drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose, 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.

Drug dependence is the result of a phenomenon called tolerance, which is the need to increase the dose of the drug to maintain its full effect, and of physical dependence, which is the habituation of the body to a drug. When the intake of a drug is discontinued, individual may experience unpleasant withdrawal syndrome. This syndrome is difficult to qualify or quantify but it can be illustrated by a strong feeling of unmet satisfaction. This episode has been described by former drug addicted individuals as “a strong scream and complaint emanating from the body”. This shows the seriousness and the difficulties encountered by these individuals. In addition, it must be emphasized that drug addiction is accompanied with or may follow psychiatric disorders such as anxiety, depression, and schizophrenia.

We can classify two types of drugs leading to dependence :

- Drugs such as cocaine, marijuana, amphetamine, and hallucinogens are responsible for psychologic dependence.

- Other drugs such as heroin, alcohol and nicotine are more prone to physical dependence but one must not rule out psychologic dependence as well.

Of course, any drug that acts on the CNS may involved a risk of dependence. For example, it is well known that one of the side effect of benzodiazepine derivatives is dependence. In animal models, it has been observed that administration of drugs such as opioids, cocaine, amphetamine, nicotine, and benzodiazepines is associated with enhanced dopaminergic transmission. The problem is that the increased level of DA may be followed by a down regulation of DA receptors. This might explain in part the observed withdrawal symptoms that are sometimes associated with depression, mood disorders, insomnia…and other unwanted dependence disorders.

Drug addiction may be responsible for or arise from job pressure or a familial problems resulting in anxiety or depression. At the extreme of the spectrum, is can result in hospitalization for overdose, withdrawal episodes and associated substance use disorders.

Finally, statistics show that anxiolytics such as benzodiazepines are more and more consumed in western countries, for example in France. Therefore, it is urgent to find solutions to prevent and manage drug dependence and withdrawal symptoms. The socioeconomic consequences of reliable solutions will have a huge impact in modern societies since addiction is often accompanied not only with susceptibility to HIV infection and hepatitis but it is would also have a positive socioeconomic impact.

Consequently, research programs aimed at developing compounds capable of alleviating drug dependence and withdrawal symptoms must be encouraged and considered as a top priority.

Substance abuse and drug addiction introduce changes in neurotransmitter synthesis, storage, release, or in the number and affinity of receptors. This can affect neurotransmission and cause drug dependence and withdrawal symptoms.

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,
- dopamin (DA) which was observed in depressed patients (Kapur S. et al., 1992, Biol. Psychiat. 32, 1-17),
- GABA which was also shown to be involved in the physiopathology of depression (Lloyd K.G. et al., 1989, Prog. Neuro-Psychopharmacol. Biol. Psychiat. 13, 341-351),
- serotonin (5-HT) (Biegon A., 1990, Ann. NY Acad. Sci. 600, 427-431),
- 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 neurons and interact with opioid receptors,
- and others neurotransmitters such as 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 associated receptors due to drug intake may lead to the development of drug dependence and withdrawal symptoms.

But, as of today, there is no treatment providing relief and help to individuals to withdraw from their addiction are available.

Against all odds, we identified that mast cells are involved in or contribute to drug dependence and withdrawal symptoms.

Mast cells (MC) are tissue elements derived from a particular subset of hematopoietic stem cells that express CD34, c-kit and CD13 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 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 recruitement and activation of various intracytoplasmic substrates. These activated 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: 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 drugs, especially salicylic derivatives, morphine derivatives, opioids, heroin, amphetamines, alcohol, nicotine, analgesics, anesthetics, and anxiolytics results in the degranulation of mast cells, which participate in the exacerbation of the chemical imbalance responsible for drug habituation and withdrawal syndrome.

Indeed, once activated, mast cells release the content of their granules at proximity of neurons which further stimulate neurons and participate to the feeling of satisfaction. 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.

In addition, following mast cells activation, released granules liberate various factors capable of modulating and altering neurotransmission. Among such factors, we can cite morphine which is bound or stored in mast cells granules. This was shown in morphine perfused dogs by Akcasu A. et al, Int J Clin Pharmacol Ther Toxicol 1985 Jan;23(1):33-7.

Thomas PS et al, Am J Physiol 1992 Jul ; 263 : 67-72 also observed that tobacco smoke induces the release of mediators from canine mast cells and modulates prostaglandin production leading to asthma.

Here, we postulate that mast cells exacerbate in paracrine manner the deregulation of neurotransmission. For example, modulation of neurotransmitters such as serotonin by drug intake activates mast cells, which in turn release the content of their granules,

further contributing to the chemical imbalance in the brain leading to dependence 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 even go further in postulating that mast cells may constitute a reservoir of drugs and that activation of mast cells leads to the release of such drugs such as morphine and other substances, such as histamine for example, that contribute to the prolonged synaptic plasticity engaged initially by the drugs.

We also observed that patients afflicted with mastocytosis are more incline to develop substance use 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.

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. Indeed, once mast cells are removed, no exacerbation or prolonged neural excitation will take place so that drug dependence is alleviated. In addition, removing mast cells is also of interest for preventing death due to overdose. Indeed, adventitial mast cells have been suggested to potentiate atherosclerosis and vasospasm, thrombosis and premature sudden death in long-term cocaine abusers (Kolodgie FD et al, J Am Coll Cardiol 1991 Jun;17(7):1553-60).

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

Description

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

Said method for treating substance use 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 heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO 94/14808) and 1-cycloproppyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and benzylphosphonic acid compounds (WO 91/15495), pyrimidine 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 520 722, US 3,772,295 and US 4,343,940) and aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

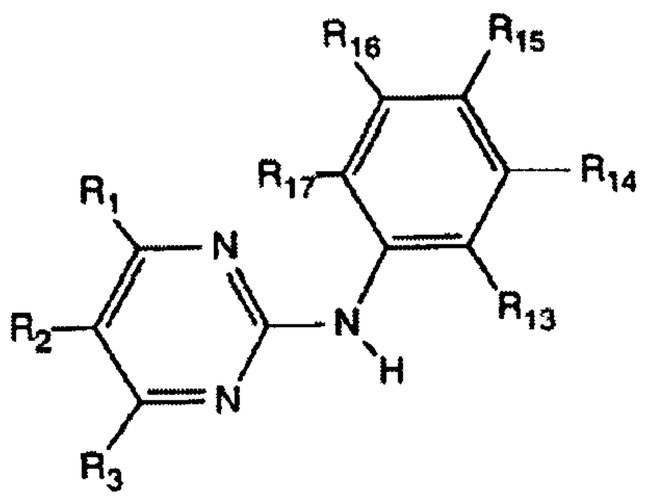
Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

In another embodiment, the invention is directed to a method for treating substance use 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 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.

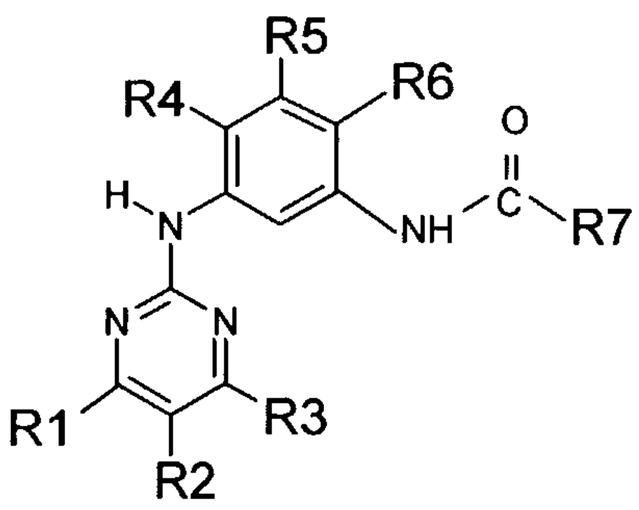
Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, 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 520 722, 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).

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



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 :

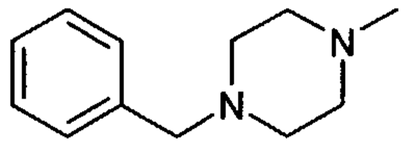


Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

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

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 :



Among these compounds, the preferred are defined as follows:

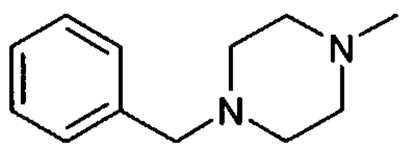
R1 is a heterocyclic group, especially a pyridyl group,

R2 and R3 are H,

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

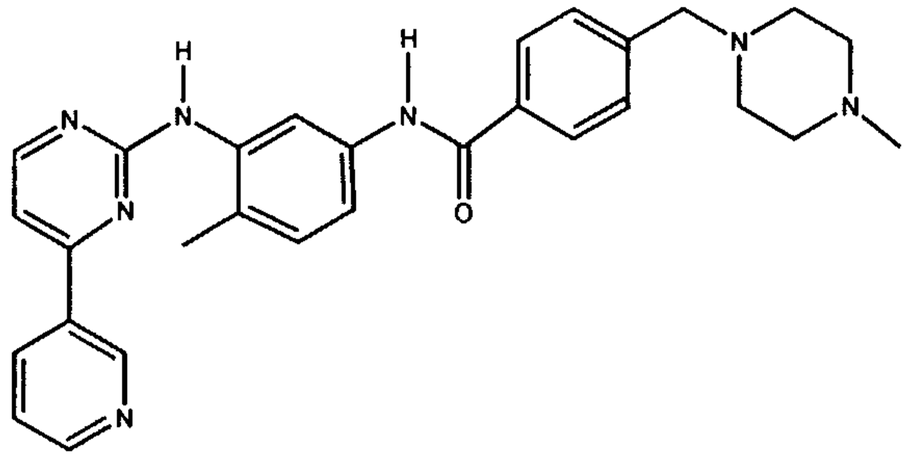
R5 and R6 are H,

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 substance use disorders comprising the administration of an effective amount of the compound known in the art as CGP57148B :

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



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,
- monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phenyl-6,7-dimethoxy quinaxoline.

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

The substance use disorders as referred herein include but are not limited to drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.

Therefore, in a preferred embodiment, the method of the invention is applicable to the treatment or prevention of drug addiction.

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

In another preferred embodiment, the method of the invention is applicable to the treatment or prevention of drug habituation.

In another preferred embodiment, the method of the invention is applicable to the treatment or prevention of drug dependence.

In another preferred embodiment, the method of the invention is applicable to the treatment or prevention of withdrawal syndrome and drug craving.

In another preferred embodiment, the method of the invention is applicable to the treatment or prevention of overdose.

Among drugs that are particularly addictive, we can cite alcohol, nicotine, opioids, cocaine, heroin, anxiolytics and hypnotics such as benzodiazepine, methaqualone and barbiturates, cannabinoids (tetrahydrocannabinol, cannabigerol, cannabinol cannabichromene, cannabidiol, cannabinoid acids), amphetamine such ecstasy, hallucinogen such as LSD, phencyclidine (PCP), mescaline, volatile solvent and volatile nitrites.

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 substance use 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 μM in step a). Relevant concentrations are for example 10, 15, 20, 25, 30, 35 or 40 μM.

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 :

- 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 hematopoetic cells by 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 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 % CO_2 atmosphere at a concentration of 10^5 cells per ml in the medium MCCM (α-MEM supplemented with L-glutamine, penicillin, streptomycin, 5 10^{-5} M β-mercaptoethanol, 20 % veal fœtal 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 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 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 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,
- 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 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.

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 μM. 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 substance use 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 IC50 < 10 μM, 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 IC50 < 10 μM, preferably an IC50 < 1 μM, 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.

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.

Best compounds are compounds exhibiting the greatest selectivity.

Control cell lines include other hematopoeitic 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), 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).

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 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 substance use disorders such as drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.

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.

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 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 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.

Pharmaceutical compositions suitable for use in the invention include compositions 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 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 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.

CLAIMS

1. A method for treating substance use 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 Substance use disorders comprising administering a tyrosine kinase inhibitor to a human in need of such treatment.

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 Substance use disorders comprising administering a c-kit inhibitor to a human in need of such treatment.

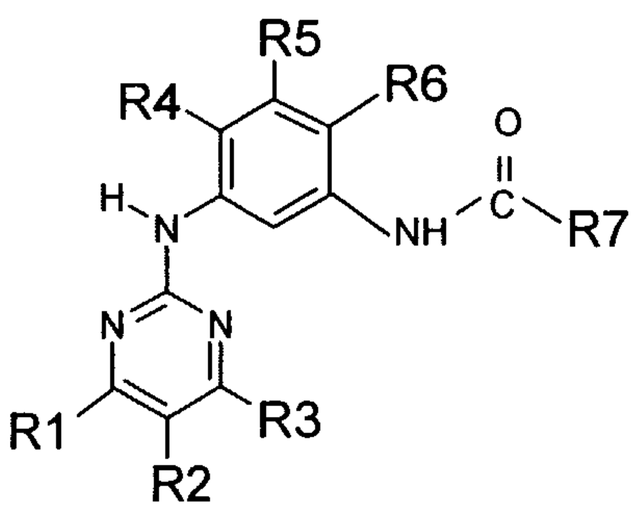
5. A method according to claim 4, wherein said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor.

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, 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,
- monocyclic, bicyclic aryl and heteroaryl compounds,
- and quinazoline derivatives.

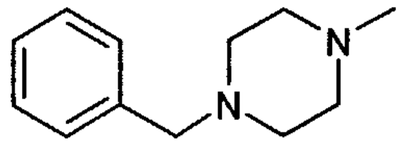
8. A method according to claim 5, wherein said inhibitor is selected from the group consisting of N-phenyl-2-pyrimidine-amine derivatives having the formula II :



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;

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 :



9. A method according to claim 8, wherein said inhibitor is the 4-(4-méhylpipérazine-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.

11. A method according to one of claims 4 to 9, 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.

13. A method according to one of claims 4 to 12, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

14. A method for treating substance use disorders 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.

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 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 at a concentration above 10 μM 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.

19. A method according to claim 17, 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 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 μM.

22. A method according to claim 14 or 21, wherein the testing is performed *in vitro* or *in vivo*.

23. A method according to one of claims 14 to 21, wherein the inhibition of mutant-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 21, wherein the amount of c-kit phosphorylation is measured.

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

26. A method for treating substance use 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 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 IC50 < 10 μM, 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 IC50 < 10 μM, preferably an IC50 < 1 μM, by measuring the 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.

28. A method according to one of claims 1 to 27 for preventing and/or treating substance use disorders in human, more particularly drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.

29. A method according to claim 28, wherein said drug is selected from the group consisting of alcohol, nicotine, opioids, cocaine, heroin, anxiolytics and hypnotics such

as benzodiazepine, methaqualone and barbiturates, cannabinoids (tetrahydrocannabinol, cannabigerol, cannabinol cannabichromene, cannabidiol, cannabinoid acids), amphetamine such ecstasy, hallucinogen such as LSD, phencyclidine (PCP), mescaline, volatile solvent and volatile nitrites.

30. Use of a c-kit inhibitor to manufacture a medicament for preventing and/or treating substance use disorders in human, more particularly drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.

31. 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 preventing and/or treating substance use disorders in human, more particularly drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.

32. A composition suitable for intravenous, intramuscular, intra-arterial, intramedullary, 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 preventing and/or treating substance use disorders in human, more particularly drug addiction, drug abuse, drug habituation, drug dependence, withdrawal syndrome and overdose.