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(12) **United States Patent**
Moussy et al.(10) **Patent No.:** **US 7,678,805 B2**(45) **Date of Patent:** ***Mar. 16, 2010**(54) **USE OF TYROSINE KINASE INHIBITORS
FOR TREATING INFLAMMATORY BOWEL
DISEASES (IBD)**(75) Inventors: **Alain Moussy**, Paris (FR); **Jean-Pierre
Kinet**, Lexington, MA (US)(73) Assignee: **AB Science**, Paris (FR)(*) Notice: Subject to any disclaimer, the term of this
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See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to a method for treating inflam-
 matory bowel diseases (IBD), such as crohn's disease, com-
 prising administering a tyrosine kinase inhibitor to a human
 in need of such treatment, more particularly a non-toxic,
 selective and potent c-kit inhibitor. Preferably, said inhibitor
 is unable to promote death of IL-3 dependent cells cultured in
 presence of IL-3.

4 Claims, No Drawings

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- Non-Final Office Action mailed Apr. 10, 2009 in U.S. Appl. No. 10/482,039.
- Notice of Allowance mailed Aug. 26, 2009 in U.S. Appl. No. 10/482,039.
- Non-Final Office Action dated Mar. 3, 2009, in U.S. Appl. No. 10/482,177.
- Final Office Action mailed Mar. 4, 2009, in U.S. Appl. No. 10/482,758.
- 1.111 Amendment filed Dec. 22, 2008, in U.S. Appl. No. 10/490,334.
- Non-Final Office Action mailed Apr. 28, 2009 in U.S. Appl. No. 10/490,334.
- Non-Final Office Action mailed Mar. 19, 2009, in U.S. Appl. No. 10/490,348.
- Restriction Requirement mailed Jan. 29, 2009, in U.S. Appl. No. 10/505,899.
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USE OF TYROSINE KINASE INHIBITORS FOR TREATING INFLAMMATORY BOWEL DISEASES (IBD)

This application claims benefit to U.S. Provisional Appli- 5 cation 60/301,405, filed Jun. 29, 2001.

The present invention relates to a method for treating inflammatory bowel diseases (IBD), such as Crohn's disease, comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non- 10 toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Inflammatory bowel disease is the term generally applied to four diseases of the bowel, namely Crohn's disease, ulcer- 15 ative colitis, indeterminate colitis, and infectious colitis.

It is estimated that there may be up to 1,000,000 Americans with IBD. Males and females appear to be affected equally. While Crohn's disease afflicts people of all ages, it is primarily a disease of the young. Most cases are diagnosed before 20 age 30, but the disease can occur in the sixth, seventh, and later decades.

Ulcerative colitis is a chronic inflammatory disease of unknown etiology afflicting the large intestine. The course of the disease may be continuous or relapsing, mild or severe. 25 The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkuhn. A separation of the overlying mucosa from its blood supply leading to ulceration is observed. Signs and symptoms of the disease include cramping, lower abdominal pain, rectal bleeding, and frequent, loose discharges consisting mainly of blood, pus, and mucus with scanty fecal particles. A total colectomy may be required for acute severe or chronic ulcer- ative colitis.

Crohn's disease is also a chronic inflammatory disease of unknown etiology but, unlike ulcerative colitis, it can affect any part of the bowel. The most prominent feature of the disease is the granular, reddish-purple edematous thickening of the bowel wall. With the development of inflammation, these granulomas often lose their circumscribed borders and 30 integrate with the surrounding tissue. Diarrhea and obstruction of the bowel are the predominant clinical features. Most patients with Crohn's disease require surgery at some point, but subsequent relapse is common. Consequently, continuous medical treatment is necessary as of today.

Mucositis involves ulcerative breakdown of mucosal epithelial tissue, and is literally defined as inflammation of the mucous membrane. The physiopathology of mucositis involves a cascade of interactions among cells, cytokines and microflora. Early inflammatory phase is characterized by 35 release of inflammatory cytokines in response to local tissue damage caused for example by cytotoxic agent(s);

The symptoms and signs of gastrointestinal mucositis include pain, bleeding, diarrhea, neovascularization, and progression to ulceration. Early signs of diarrhea include increased stool frequency, loose or watery stool, food aver- 40 sion, increased bowel sounds, abdominal pain, and some loss of skin turgor indicative of dehydration. When the diarrhea is severe it may be associated with mucosal ulceration, bleeding, intestinal perforation and proctitis. Stool exam may reveal occult blood and fecal leukocytes.

Necrotizing enterocolitis is an inflammatory disease of unknown etiology that afflicts between 1-5% of all infants admitted to neonatal intensive care units, most of whom are premature infants. Signs and symptoms include abdominal 45 distention, gastrointestinal hemorrhage, and feeding intolerance. The disease most often involves the ileum and colon,

and is characterized by loss of epithelium and submucosal edema, ulcerations, and, in severe cases, transmural necrosis.

Today, the following groups of drugs are available for therapy of IBD:

Aminosalicylates: aspirin-like drugs, which include sul- fasalazine and mesalamine, given both orally and rec- tally.

Corticosteroids: prednisone and methylprednisolone, available orally and rectally.

Immune modifiers: azathioprine, 6MP, methotrexate.

Antibiotics: metronidazole, ampicillin, ciprofloxacin, and others.

However, many of these anti-inflammatory compounds are ineffective in the treatment of IBD and can exacerbate experi- mental colitis in animals and activate quiescent inflammatory bowel disease in humans (Wallace et al., *Gastroenterology*, 102:18-27 (1992); Kaufmann et al., *Annals of Internal Medi- cine*, 107:513-516(1987)).

Because there are many mediators involved in IBD and since inflammation occurs in different areas having different mechanisms of action, it is difficult to predict what the correct therapy is for any specific inflammatory response. The use of histamine H.sub.3-receptor agonists for the treatment of IBD has been proposed in U.S. Pat. No. 6,028,095, but this treat- ment, while alleviating symptoms, does not respond to the causes of inflammatory bowel diseases.

Therefore, there is a need for alternative treatments of IBD that would provide a specific solution adapted to IBD.

More recently, IL-11 has been proposed for treating inflammatory bowel diseases (U.S. Pat. No. 6,126,933). IL-11 was discovered as a new cytokine stimulating the func- tion of cells of the immune and hematopoietic systems (U.S. Pat. No. 5,854,028), such as macrophages (Burstin et al., *Journal of Cellular Physiology* (1992) 153:312; Bazan, *Neu- ron* (1991) 7:197; and Yang et al., *BioFactors* (1992) 4:15-21).

The invention goes to the opposite direction since it has been found that the causes of inflammation in IBD in directly or indirectly due to the presence of numerous mast cells in the bowel, leading to the activation of the immune system medi- ated inflammatory response.

Mast cells (MC) are tissue elements derived from a par- ticular 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 cyto- kines, 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 recruitment and activa- tion 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 heteroge- neity, 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).

Several inflammatory disorders of the intestine are characterised by enhanced expression of tumour necrosis factor alpha (TNF-alpha). Monocytes and macrophages have been suggested as a major cellular source of TNF-alpha in human gut, whereas mast cells, although known to be capable of producing TNF-alpha, have been poorly examined in this respect. One of the first cytokines found to be produced by mast cells was TNF-a which was detected primarily in rodent mast cells and mast cell lines (Gordon et al. (1991), J Exp Med 174: 103-107). In animal models, mast cells derived TNF-a was found to be responsible for the regulation of bacterial infection, and for the influx of neutrophils observed during immune complex peritonitis and IgE dependent cutaneous or gastric inflammation (Furuta et al. (1997) Gastroenterology 113: 1560-1569; Zhang et al. (1992), Science 258: 1957-1959; Wershil et al. (1991) J Clin Invest 87: 446-453 and Bischoff et al. (1999). Gut 44: 643-652. It was shown that mast cells are an important source of TNF-a in the human intestinal mucosa. Moreover, they also demonstrated that % of TNF-a positive cells and the % of tryptase positive TNF producing cells were higher in inflamed tissue (in Crohn's disease) compared with macroscopically normal tissue. Former electron microscopic studies performed in patients with Crohn's disease revealed that number of mast cells was markedly increased and were found predominantly in edematous submucosa and between smooth muscle in the muscular coats of the involved gut (Dvorak et al. (1980). Hum Pathol 11(6): 606-619).

In connection with the invention, evidence of focal and complete degranulation of mast cells was frequently observed. Besides, mast cells produce a large variety of mediators categorized here 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-a, GM-CSF, MIP-1a, MIP-1b and IFN-g). Then, liberation by activated mast cells of mediators (TNF-a, histamine, leucotrienes, prostaglandins etc. . . .) is proposed here to induce acute or chronic inflammation as it can be observed in Crohn's disease.

So, a new route for treating inflammatory bowel diseases, such as crohn's disease, is provided, which consists of destroying mast cells playing a role in IBD pathogenesis. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited for treating these diseases.

DESCRIPTION

The present invention relates to a method for treating inflammatory bowel diseases (IBD) comprising 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 derivative (WO 94/14808) and 1-cyclopropyl-4-pyridyl-quinolones (U.S. Pat. No. 5,330,992), Styryl compounds (U.S. Pat. No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Pat. No. 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (U.S. Pat. No. 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (U.S. Pat. No. 5,792,783, EP 934 931, U.S. Pat. No. 5,834,504, U.S. Pat. No. 5,883,116, U.S. Pat. No. 5,883,113, U.S. Pat. No. 5,886,020, WO 96/40116 and WO 00/38519), as well

as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, U.S. Pat. No. 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, U.S. Pat. No. 3,772,295 and U.S. Pat. No. 4,343,940) and aryl and heteroaryl quinazoline (U.S. Pat. No. 5,721,237, U.S. Pat. No. 5,714,493, U.S. Pat. No. 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.

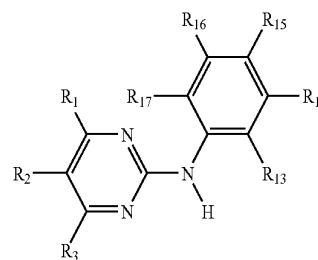
In another embodiment, the invention is directed to a method for treating inflammatory bowel diseases comprising administering a c-kit inhibitor to a human in need of such treatment.

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 (U.S. Pat. No. 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (U.S. Pat. No. 5,792,783, EP 934 931, U.S. Pat. No. 5,834,504), U.S. Pat. No. 5,883,116, U.S. Pat. No. 5,883,113, U.S. Pat. No. 5,886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, U.S. Pat. No. 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 722, U.S. Pat. No. 3,772,295 and U.S. Pat. No. 4,343,940), 4-amino-substituted quinazolines (U.S. Pat. No. 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (U.S. Pat. No. 3,800,039), aryl and heteroaryl quinazoline (U.S. Pat. No. 5,721,237, U.S. Pat. No. 5,714,493, U.S. Pat. No. 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (U.S. Pat. No. 4,464,375), and 4-thienyl-2-(1H)-quinazolones (U.S. Pat. No. 3,551,427).

So, preferably, the invention relates to a method for treating inflammatory bowel diseases comprising administering to a human in need of such treatment a non toxic, potent and selective c-kit inhibitor.

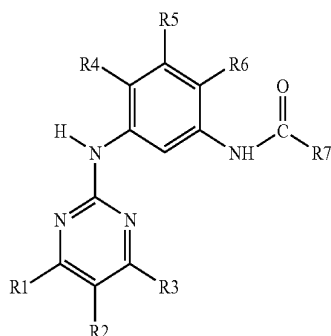
Such inhibitor can be selected from pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I:



wherein the R1, R2, R3, R13 to R17 groups have the meaning 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

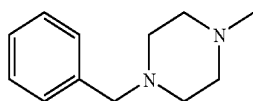


Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

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

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,

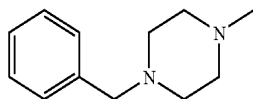
R2 and R3 are H,

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

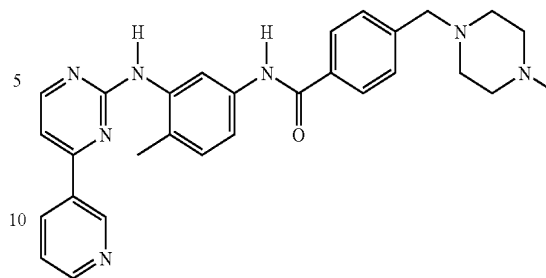
basic site, such as an amino function, for example the group:



Therefore, in a preferred embodiment, the invention relates to a method for treating IBD, more particularly crohn's disease, comprising the administration of an effective amount of the compound known in the art as CGP57148B:

4-(4-méthylpiperazine-1-ylméthyl)-N-(4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 yl amino)phényl-benzamide corresponding to the following formula:

6



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.

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 No1). 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 inflammatory bowel diseases 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:

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 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% CO₂ atmosphere at a concentration of 10⁵ cells per ml in the medium MCCM (α -MEM supplemented with L-glutamine, penicillin, streptomycin, 5, 10⁻⁵ M β -mercaptoethanol, 20% veal fetal 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' CTGCTTCGCGGCCGCTTAACCTCTTCAACCA3' (SEQ ID No3) antisens

The PCR products, digested with NotI and XhoI, has been inserted using T4 ligase in the pFlag-CMV vector (SIGMA), which vector is digested with NotI and XhoI 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' AGCTCGTTTAGTGAAACCGTC3' (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.

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 inflammatory bowel diseases as depicted above wherein the screening comprises:

a) performing a cellular assay with cells selected from 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 all $IC_{50} < 10 \mu M$, by measuring the extent of cell death,

b) performing a cellular 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 cellular assay with cells selected from 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 IBD.

More particularly, the invention contemplates the method defined above for treating Crohn's disease, mucositis, ulcerative colitis, and necrotizing enterocolitis.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament for treating IBD, such as Crohn's disease, mucositis, ulcerative colitis, and necrotizing enterocolitis.

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. Controlled as well as prolonged release formulation are contemplated.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

The pharmaceutical composition may be provided as a salt and can be formed with many acids including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids, etc. Salts tend to be more soluble in aqueous or other protic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Pharmaceutical compositions suitable for use in the invention include compositions wherein 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., ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (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, LD_{50}/ED_{50} . 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.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 5

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<211> LENGTH: 976

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: Human c-kit

<400> SEQUENCE: 1

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Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
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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
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Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
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Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
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 145          150          155          160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
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Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
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Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
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Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
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 Thr Tyr Asp Arg Leu Val Asn Gly Met Leu Gln Cys Val Ala Ala Gly
 420 425 430
 Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
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 Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
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 Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe Ala Phe Lys Gly Asn Asn
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 Lys Glu Gln Ile His Pro His Thr Leu Phe Thr Pro Leu Leu Ile Gly
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 Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr
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 Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
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 Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
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 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
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 Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
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 Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
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 Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
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 Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp
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 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
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 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
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<210> SEQ ID NO 5
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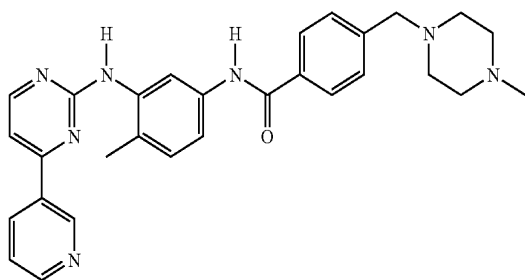
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The invention claimed is:

1. A method for treating inflammatory bowel diseases (IBD) comprising administering an effective amount of the following compound



4-(4-methylpiperazine-1-ylmethyl)-N-[4-methyl-3-[(4-pyridine-3-ylpyrimidine-2-yl)amino]phenyl]-benzamide, or a pharmaceutically acceptable salt thereof, to a human in need of such treatment.

2. The method according to claim 1 wherein the inflammatory bowel disease is Crohn's disease.

3. The method according to claim 1, wherein the inflammatory bowel disease is ulcerative colitis.

4. The method according to claim 1, wherein the inflammatory bowel disease is necrotizing enterocolitis.

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