NOVEL COMPOUND USEFUL FOR THE TREATMENT OF DEGENERATIVE AND INFLAMMATORY DISEASES

RELATED APPLICATION

[0001] The present application claims the benefit under 35 U.S.C. § 119 of U.S. Provisional Application No. 61/479,956, filed April 28, 2011, the contents of which is hereby incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a compound that is an inhibitor of JAK, a family of tyrosine kinases that are involved in inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons. In particular, the compound of the invention inhibits JAK1 and/or JAK2. The present invention also provides methods for the production of the compound of the invention, pharmaceutical compositions comprising the compound of the invention, methods for the prophylaxis and/or treatment of diseases involving inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons by administering the compound of the invention.

[0003] Janus kinases (JAKs) are cytoplasmic tyrosine kinases that transduce cytokine signaling from membrane receptors to STAT transcription factors. Four JAK family members are described, JAK1, JAK2, JAK3 and TYK2. Upon binding of the cytokine to its receptor, JAK family members auto- and/or transphosphorylate each other, followed by phosphorylation of STATs that then migrate to the nucleus to modulate transcription. JAK-STAT intracellular signal transduction serves the interferons, most interleukins, as well as a variety of cytokines and endocrine factors such as EPO, TPO, GH, OSM, LIF, CNTF, GM-CSF and PRL (Vainchenker W. *et al.* (2008)).

[0004] The combination of genetic models and small molecule JAK inhibitor research revealed the therapeutic potential of several JAKs. JAK3 is validated by mouse and human genetics as an immune-suppression target (O'Shea J. *et al.* (2004)). JAK3 inhibitors were successfully taken into clinical development, initially for organ transplant rejection but later also in other immuno-inflammatory indications such as rheumathoid arthritis (RA), psoriasis and Crohn's disease (http://clinicaltrials.gov/).

[0005] TYK2 is a potential target for immuno-inflammatory diseases, being validated by human genetics and mouse knock-out studies (Levy D. and Loomis C. (2007)).

[0006] JAK1 and/or JAK2 is a target in the immuno-inflammatory disease area. JAK1 and/or JAK2 heterodimerizes with the other JAKs to transduce cytokine-driven pro-inflammatory signaling. Therefore, inhibition of JAK1 and/or JAK2 is of interest for immuno-inflammatory diseases with

pathology-associated cytokines that use JAK1 and/or JAK2 signaling, such as IL-6, IL-4, IL-5, IL-12, IL-13, IL-23, or IFNgamma, as well as for other diseases driven by JAK-mediated signal transduction.

BACKGROUND OF THE INVENTION

[0007] The degeneration of cartilage is the hallmark of various diseases, among which rheumatoid arthritis and osteoarthritis are the most prominent. Rheumatoid arthritis (RA) is a chronic joint degenerative disease, characterized by inflammation and destruction of the joint structures. When the disease is unchecked, it leads to substantial disability and pain due to loss of joint functionality and even premature death. The aim of an RA therapy, therefore, is not only to slow down the disease but to attain remission in order to stop the joint destruction. Besides the severity of the disease outcome, the high prevalence of RA (~ 0.8% of adults are affected worldwide) means a high socio-economic impact. (For reviews on RA, we refer to Smolen and Steiner (2003); Lee and Weinblatt (2001); Choy and Panayi (2001); O'Dell (2004) and Firestein (2003)).

[0008] JAK1 and JAK2 are implicated in intracellular signal transduction for many cytokines and hormones. Pathologies associated with any of these cytokines and hormones can be ameliorated by JAK1 and/or JAK2 inhibitors. Hence, several allergy, inflammation and autoimmune disorders might benefit from treatment with compounds described in this invention including rheumatoid arthritis, systemic lupus erythematosis, juvenile idiopathic arthritis, osteoarthritis, asthma, chronic obstructive pulmonary disease (COPD), tissue fibrosis, eosinophilic inflammation, eosophagitis, inflammatory bowel diseases (*e.g.* Crohn's, ulcerative colitis), transplant, graft-versus-host disease, psoriasis, myositis, psoriatic arthritis, ankylosing spondylitis, juvenile ideopathic arthrits, multiple sclerosis (Kopf et al., 2010).

[0009] Osteoarthritis (also referred to as OA, or wear-and-tear arthritis) is the most common form of arthritis and is characterized by loss of articular cartilage, often associated with hypertrophy of the bone and pain. For an extensive review on osteoarthritis, we refer to Wieland *et al.* (2005).

[0010] Osteoarthritis is difficult to treat. At present, no cure is available and treatment focuses on relieving pain and preventing the affected joint from becoming deformed. Common treatments include the use of non-steroidal anti-inflammatory drugs (NSAIDs). Although dietary supplements such as chondroitin and glucosamine sulphate have been advocated as safe and effective options for the treatment of osteoarthritis, a recent clinical trial revealed that both treatments did not reduce pain associated to osteoarthritis. (Clegg *et al.,* 2006).

[0011] Stimulation of the anabolic processes, blocking catabolic processes, or a combination of these two, may result in stabilization of the cartilage, and perhaps even reversion of the damage, and therefore prevent further progression of the disease. Therapeutic methods for the correction of the articular cartilage lesions that appear during the osteoarthritic disease have been developed, but so far none of them have been able to mediate the regeneration of articular cartilage *in situ* and *in vivo.* Taken together, no disease modifying osteoarthritic drugs are available.

[0012] Vandeghinste *et al.* (WO 2005/124342) discovered JAK1 as a target whose inhibition might have therapeutic relevance for several diseases including OA. Knockout of the JAK1 gene in mice demonstrated that JAK1 plays essential and non-redundant roles during development: JAK1-/- mice died within 24h after birth and lymphocyte development was severely impaired. Moreover, JAK1 -/- cells were not, or less, reactive to cytokines that use class II cytokine receptors, cytokine receptors that use the gamma-c subunit for signaling and the family of cytokine receptors that use the gp130 subunit for signaling (Rodig *et al.,* 1998).

[0013] Various groups have implicated JAK-STAT signaling in chondrocyte biology. Li *et al.* (2001) showed that Oncostatin M induces MMP and TIMP3 gene expression in primary chondrocytes by activation of JAK/STAT and MAPK signaling pathways. Osaki *et al.* (2003) showed that interferon-gamma mediated inhibition of collagen II in chondrocytes involves JAK-STAT signaling. IL1-beta induces cartilage catabolism by reducing the expression of matrix components, and by inducing the expression of collagenases and inducible nitric oxide synthase (NOS2), which mediates the production of nitric oxide (NO). Otero *et al.*, (2005) showed that leptin and IL1-beta synergistically induced NO production or expression of NOS2 mRNA in chondrocytes, and that that was blocked by a JAK inhibitor. Legendre *et al.* (2003) showed that IL6/IL6 Receptor induced downregulation of cartilage-specific matrix genes collagen II, aggrecan core and link protein in bovine articular chondrocytes, and that this was mediated by JAK/STAT signaling. Therefore, these observations suggest a role for JAK kinase activity in cartilage homeostasis and therapeutic opportunities for JAK kinase inhibitors.

[0014] JAK family members have been implicated in additional conditions including myeloproliferative disorders (O'Sullivan *et al*, 2007, Mol Immunol. 44(10):2497-506), where mutations in JAK2 have been identified. This indicates that inhibitors of JAK in particular JAK2 may also be of use in the treatment of myeloproliferative disorders. Additionally, the JAK family, in particular JAK1, JAK2 and JAK3, has been linked to cancers, in particular leukaemias *e.g.* acute myeloid leukaemia (O'Sullivan *et al*, 2007, Mol Immunol. 44(10):2497-506; Xiang *et al.*, 2008, "Identification of somatic *JAK1* mutations in patients with acute myeloid leukemia" Blood First Edition Paper, prepublished online December 26, 2007; DOI 10.1182/blood-2007-05-090308) and acute lymphoblastic leukaemia (Mullighan *et al,* 2009) or solid tumours *e.g.* uterine leiomyosarcoma (Constantinescu *et al.*, 2007, Trends in Biochemical Sciences 33(3): 122-131), prostate cancer (Tam *et al.*, 2007, British Journal of Cancer, 97, 378 – 383). These results indicate that inhibitors of JAK, in particular of JAK1 and/or JAK2, may also have utility in the treatment of cancers (leukaemias and solid tumours *e.g.* uterine leiomyosarcoma, prostate cancer).

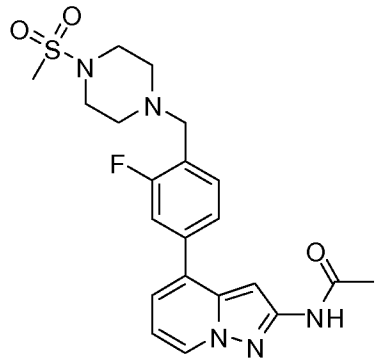
[0015] In addition, Castleman's disease, multiple myeloma, mesangial proliferative glomerulonephritis, psoriasis, and Kaposi's sarcoma are likely due to hypersecretion of the cytokine IL-6, whose biological effects are mediated by intracellular JAK-STAT signaling (Tetsuji Naka, Norihiro Nishimoto and Tadamitsu Kishimoto, Arthritis Res 2002, 4 (suppl 3):S233-S242). This result shows that inhibitors of JAK, may also find utility in the treatment of said diseases.

[0016] The current therapies are not satisfactory and therefore there remains a need to identify further compounds that may be of use in the treatment of inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, *e.g.* osteoarthritis, and rheumatoid arthritis , in particular rheumatoid arthritis. The present invention therefore provides a compound, methods for its manufacture and pharmaceutical compositions comprising the compound of the invention together with a suitable pharmaceutical carrier. The present invention also provides for the use of the compound of the invention in the preparation of a medicament for the treatment of inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, *e.g.* osteoarthritis, and rheumatoid arthritis , in particular rheumatoid arthritis.

SUMMARY OF THE INVENTION

[0017] The present invention is based on the discovery that the compound of the invention is able to act as inhibitor of JAK and that it is useful for the treatment of inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons. In a specific aspect the compound of the invention is an inhibitor of JAK1 and/or JAK2. The present invention also provides methods for the production of this compound, pharmaceutical compositions comprising this compound and methods for treating inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, by administering the compound of the invention.

[0018] Accordingly, in a first aspect of the invention, the compound of the invention provided is according to Formula (I):



I .

[0019] In a particular embodiment the compound of the invention is an inhibitor of JAK1 and/or JAK2.

[0020] In a further aspect, the present invention provides pharmaceutical compositions comprising the compound of the invention, and a pharmaceutical carrier, excipient or diluent. Moreover, the compound of the invention, useful in the pharmaceutical compositions and treatment methods disclosed herein, is pharmaceutically acceptable as prepared and used. In this aspect of the invention, the pharmaceutical composition may additionally comprise further active ingredients suitable for use in combination with the compound of the invention.

[0021] In a further aspect of the invention, this invention provides a method of treatment or prophylaxis of a mammal susceptible to or afflicted with a condition from among those listed herein, and particularly, inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and diseases associated with hypersecretion of IL6 or interferons, which method comprises administering an effective amount of the pharmaceutical composition or compound of the invention as described herein. In a particular embodiment the condition is associated with aberrant JAK activity, and more particularly JAK1 and/or JAK2 activity.

[0022] In a further aspect, the invention provides the compound of the invention or a pharmaceutical composition comprising the compound of the invention for use as a medicament. In a specific embodiment, said pharmaceutical composition additionally comprises a further active ingredient.

[0023] In a further aspect, the present invention provides the compound of the invention for use in the treatment or prophyaxis of a condition selected from those listed herein, particularly inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

[0024] In yet another method of treatment aspect, this invention provides a method of treatment or prophylaxis of a mammal susceptible to or afflicted with a selected from those listed herein, particularly inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, and comprises administering an effective amount of the pharmaceutical composition or the compound of the invention described herein for the treatment or prophylaxis of said condition. In a specific aspect the condition is causally related to abnormal JAK activity and more particularly JAK1 and/or JAK2 activity.

[0025] In a further aspect, the present invention provides the compound of the invention for use in the treatment or prophylaxis of a condition selected from those listed herein, particularlyinflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

[0026] In additional aspects, this invention provides methods for synthesizing the compound of the invention, with representative synthetic protocols and pathways disclosed later on herein.

[0027] Accordingly, it is a principal object of this invention to provide a novel compound, which can modify the activity of JAK and thus prevent or treat any conditions that may be causally related thereto. In a specific aspect the compound of the invention modulate the activity of JAK1 and/or JAK2.

[0028] It is further an object of this invention to provide a compound that can treat or alleviate conditions or symptoms of same, such as inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons,.

[0029] A still further object of this invention is to provide a pharmaceutical composition that may be used in the treatment or prophylaxis of a variety of disease states, such as inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons. In a specific embodiment the disease is associated with JAK1 and/or JAK2 activity, in particular inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

[0030] Other objects and advantages will become apparent to those skilled in the art from a consideration of the ensuing detailed description.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0031] The following terms are intended to have the meanings presented therewith below and are useful in understanding the description and intended scope of the present invention.

[0032] When describing the invention, which may include compounds, pharmaceutical compositions containing such compounds and methods of using such compounds and compositions, the following terms, if present, have the following meanings unless otherwise indicated. It should also be understood that when described herein any of the moieties defined forth below may be substituted with a variety of substituents, and that the respective definitions are intended to include such substituted moieties within their scope as set out below. Unless otherwise stated, the term "substituted" is to be defined as set out below. It should be further understood that the terms "groups" and "radicals" can be considered interchangeable when used herein.

[0033] The articles "a" and "an" may be used herein to refer to one or to more than one (*i.e.* at least one) of the grammatical objects of the article. By way of example "an analogue" means one analogue or more than one analogue.

[0034] As used herein the term 'JAK' relates to the family of Janus kinases (JAKs) which are cytoplasmic tyrosine kinases that transduce cytokine signaling from membrane receptors to STAT transcription factors. Four JAK family members are described, JAK1, JAK2, JAK3 and TYK2 and the

term JAK may refer to all the JAK family members collectively or one or more of the JAK family members as the context indicates.

[0035] 'Alkoxy' refers to the group $-OR^{26}$ where R^{26} is C_{1-8} alkyl. Particular alkoxy groups are methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, and 1,2-dimethylbutoxy. Particular alkoxy groups are lower alkoxy, i.e. with between 1 and 6 carbon atoms. Further particular alkoxy groups have between 1 and 4 carbon atoms.

[0036] 'Alkyl' means straight or branched aliphatic hydrocarbon having 1 to 20 carbon atoms. Particular alkyl has 1 to 8 carbon atoms. More particular is lower alkyl which has 1 to 6 carbon atoms. A further particular group has 1 to 4 carbon atoms. Exemplary straight chained groups include methyl, ethyl n-propyl, and n-butyl. Branched means that one or more lower alkyl groups such as methyl, ethyl, propyl or butyl is attached to a linear alkyl chain, exemplary branched chain groups include isopropyl, iso-butyl, t-butyl and isoamyl.

[0037] 'Aryl' refers to a monovalent aromatic hydrocarbon group derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. In particular aryl refers to an aromatic ring structure, monocyclic or polyyclic, with the number of ring atoms specified. Specifically, the term includes groups that include from 6 to 10 ring members. Where the aryl group is a monocyclic ring system it preferentially contains 6 carbon atoms. Particularly aryl groups include phenyl, naphthyl, indenyl, and tetrahydronaphthyl.

[0038] 'Substituted' refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituent(s).

[0039] 'Pharmaceutically acceptable' means approved or approvable by a regulatory agency of the Federal or a state government or the corresponding agency in countries other than the United States, or that is listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

[0040] 'Pharmaceutically acceptable salt' refers to a salt of the compound of the invention that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts are non-toxic may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.* an

alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of non toxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. The term 'pharmaceutically acceptable cation' refers to an acceptable cationic counter-ion of an acidic functional group. Such cations are exemplified by sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium cations, and the like.

[0041] 'Pharmaceutically acceptable vehicle' refers to a diluent, adjuvant, excipient or carrier with which the compound of the invention is administered.

[0042] 'Prodrugs' refers to compounds, including derivatives of the compound of the invention,which have cleavable groups and become by solvolysis or under physiological conditions the compound of the invention which are pharmaceutically active *in vivo*. Such examples include, but are not limited to, choline ester derivatives and the like, N-alkylmorpholine esters and the like.

[0043] 'Solvate' refers to forms of the compound that are associated with a solvent, usually by a solvolysis reaction. This physical association includes hydrogen bonding. Conventional solvents include water, ethanol, acetic acid and the like. The compound of the invention may be prepared *e.g.* in crystalline form and may be solvated or hydrated. Suitable solvates include pharmaceutically acceptable solvates, such as hydrates, and further include both stoichiometric solvates and non-stoichiometric solvates. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. 'Solvate' encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanolates and methanolates.

[0044] 'Subject' includes humans. The terms 'human', 'patient' and 'subject' are used interchangeably herein.

[0045] 'Therapeutically effective amount' means the amount of a compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" can vary depending on the compound, the disease and its severity, and the age, weight, etc., of the subject to be treated.

[0046] 'Preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (*i.e.* causing at least one of the clinical symptoms of the disease not to develop in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset.

[0047] The term 'prophylaxis' is related to 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine, in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

[0048] 'Treating' or 'treatment' of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (*i.e.* arresting the disease or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment 'treating' or 'treatment' refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In yet another embodiment, 'treating' or 'treatment' refers to modulating the disease or disorder, either physically, (*e.g.* stabilization of a discernible symptom), physiologically, (*e.g.* stabilization of a physical parameter), or both. In a further embodiment, "treating" or "treatment" relates to slowing the progression of the disease.

[0049] As used herein the term 'allergy' refers to the group of conditions characterized by a hypersensitivity disorder of the immune system including, allergic airway disease (*e.g.* asthma, rhinitis), sinusitis, eczema and hives, as well as food allergies or allergies to insect venom.

[0050] As used herein the term 'inflammatory condition(s)' refers to the group of conditions including, rheumatoid arthritis, osteoarthritis, juvenile idiopathic arthritis, psoriasis, psoriatic arthritis, allergic airway disease (*e.g.* asthma, rhinitis), inflammatory bowel diseases (*e.g.* Crohn's disease, ulcerative colitis), endotoxin-driven disease states (*e.g.* complications after bypass surgery or chronic endotoxin states contributing to *e.g.* chronic cardiac failure), and related diseases involving cartilage, such as that of the joints. Partcicularly the term refers to rheumatoid arthritis, osteoarthritis, allergic airway disease (*e.g.* asthma) and inflammatory bowel diseases.

[0051] As used herein the term 'autoimmune disease(s)' refers to the group of diseases including obstructive airways disease, including conditions such as COPD, asthma (e.g intrinsic asthma, extrinsic asthma, dust asthma, infantily asthma) particularly chronic or inveterate asthma (for example late asthma and airway hyperreponsiveness), bronchitis, including bronchial asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythrematosis, lupus nephritis, dermatomyositis, Sjogren's syndrome, multiple sclerosis, psoriasis, dry eye disease, type I diabetes mellitus and complications associated therewith, atopic eczema (atopic dermatitis), contact dermatitis and further eczematous dermatitis, inflammatory bowel disease (*e.g.* Crohn's disease and ulcerative colitis), psoriatic arthritis, ankylosing spondylitis, juvenile ideopathic arthritis, atherosclerosis and amyotrophic lateral sclerosis. Particularly the term refers to COPD, asthma, systemic lupus erythematosis, type I diabetes mellitus and inflammatory bowel disease.

[0052] As used herein the term 'proliferative disease(s)' refers to conditions such as cancer (*e.g.* uterine leiomyosarcoma or prostate cancer), myeloproliferative disorders (*e.g.* polycythemia vera, essential thrombocytosis and myelofibrosis), leukemia (*e.g.* acute myeloid leukaemia, acute and chronic lymphoblastic leukemia), multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular the term refers to cancer, leukemia, multiple myeloma and psoriasis.

[0053] As used herein, the term 'cancer' refers to a malignant or benign growth of cells in skin or in body organs, for example but without limitation, breast, prostate, lung, kidney, pancreas, stomach or bowel. A cancer tends to infiltrate into adjacent tissue and spread (metastasise) to distant organs, for example to bone, liver, lung or the brain. As used herein the term cancer includes both metastatic rumour cell types, such as but not limited to, melanoma, lymphoma, leukaemia, fibrosarcoma, rhabdomyosarcoma, and mastocytoma and types of tissue carcinoma, such as but not limited to, colorectal

cancer, prostate cancer, small cell lung cancer and non-small cell lung cancer, breast cancer, pancreatic cancer, bladder cancer, renal cancer, gastric cancer, glioblastoma, primary liver cancer, ovarian cancer, prostate cancer and uterine leiomyosarcoma.

[0054] As used herein the term 'leukemia' refers to neoplastic diseases of the blood and blood forming organs. Such diseases can cause bone marrow and immune system dysfunction, which renders the host highly susceptible to infection and bleeding. In particular the term leukemia refers to acute myeloid leukaemia (AML) and acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukaemia (CLL).

[0055] As used herein the term 'transplant rejection' refers to the acute or chronic rejection of cells, tissue or solid organ allo- or xenografts of *e.g.* pancreatic islets, stem cells, bone marrow, skin, muscle, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus, or graft-versus-host diseases.

[0056] As used herein the term 'diseases involving impairment of cartilage turnover' includes conditions such as osteoarthritis, psoriatic arthritis, juvenile rheumatoid arthritis, gouty arthritis, septic or infectious arthritis, reactive arthritis, reflex sympathetic dystrophy, algodystrophy, Tietze syndrome or costal chondritis, fibromyalgia, osteochondritis, neurogenic or neuropathic arthritis, arthropathy, endemic forms of arthritis like osteoarthritis deformans endemica, Mseleni disease and Handigodu disease; degeneration resulting from fibromyalgia, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0057] As used herein the term 'congenital cartilage malformation(s)' includes conditions such as hereditary chondrolysis, chondrodysplasias and pseudochondrodysplasias, in particular, but without limitation, microtia, anotia, metaphyseal chondrodysplasia, and related disorders.

[0058] As used herein the term 'disease(s) associated with hypersecretion of IL6' includes conditions such as Castleman's disease, multiple myeloma, psoriasis, Kaposi's sarcoma and/or mesangial proliferative glomerulonephritis.

[0059] As used herein the term 'disease(s) associated with hypersecretion of interferons includes conditions such as systemic and cutaneous lupus erythematosis, lupus nephritis, dermatomyositis, Sjogren's syndrome, psoriasis, rheumatoid arthritis.

[0060] 'Compound of the invention', and equivalent expressions, are meant to embrace the compound of the Formula as herein described, which expression includes the biologically active metabolites, pharmaceutically acceptable salts, and the solvates, *e.g.* hydrates, and the solvates of the pharmaceutically acceptable salts where the context so permits. Similarly, reference to intermediates, whether or not they themselves are claimed, is meant to embrace their salts, and solvates, where the context so permits.

[0061] When ranges are referred to herein, for example but without limitation, C_{1-8} alkyl, the citation of a range should be considered a representation of each member of said range.

[0062] Other derivatives of the compound of this invention have activity in both their acid and acid derivative forms, but in the acid sensitive form often offers advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (see, Bundgard, H., Design of Prodrugs, pp. 7-9, 21-24,

Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well know to practitioners of the art, such as, for example, esters prepared by reaction of the parent acid with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a substituted or unsubstituted amine, or acid anhydrides, or mixed anhydrides. Simple aliphatic or aromatic esters, amides and anhydrides derived from acidic groups pendant on the compounds of this invention are particularly useful prodrugs. In some cases it is desirable to prepare double ester type prodrugs such as (acyloxy)alkyl esters or ((alkoxycarbonyl)oxy)alkylesters. Particular such prodrugs are the C_1 to C_8 alkyl, C_{2-8} alkenyl, aryl, C_{7-12} substituted aryl, and C_{7-12} arylalkyl esters of the compound of the invention.

[0063] As used herein, the term 'isotopic variant' refers to a compound that contains unnatural proportions of isotopes at one or more of the atoms that constitute such compound. For example, an 'isotopic variant' of a compound can contain one or more non-radioactive isotopes, such as for example, deuterium (^{2}H or D), carbon-13 (^{13}C), nitrogen-15 (^{15}N), or the like. It will be understood that, in a compound where such isotopic substitution is made, the following atoms, where present, may vary, so that for example, any hydrogen may be ^{2}H/D, any carbon may be ^{13}C, or any nitrogen may be ^{15}N, and that the presence and placement of such atoms may be determined within the skill of the art. Likewise, the invention may include the preparation of isotopic variants with radioisotopes, in the instance for example, where the resulting compounds may be used for drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, *i.e.* ^{3}H, and carbon-14, *i.e.* ^{14}C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Further, compounds may be prepared that are substituted with positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, and would be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

[0064] All isotopic variants of the compounds provided herein, radioactive or not, are intended to be encompassed within the scope of the invention.

[0065] It is also to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed 'isomers'. Isomers that differ in the arrangement of their atoms in space are termed 'stereoisomers'.

[0066] Stereoisomers that are not mirror images of one another are termed 'diastereomers' and those that are non-superimposable mirror images of each other are termed 'enantiomers'. When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (*i.e.* as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a 'racemic mixture'.

[0067] 'Tautomers' refer to compounds that are interchangeable forms of a particular compound structure, and that vary in the displacement of hydrogen atoms and electrons. Thus, two structures may be in equilibrium through the movement of π electrons and an atom (usually H). For example, enols and ketones are tautomers because they are rapidly interconverted by treatment with either acid or base.

Another example of tautomerism is the aci- and nitro- forms of phenylnitromethane, that are likewise formed by treatment with acid or base.

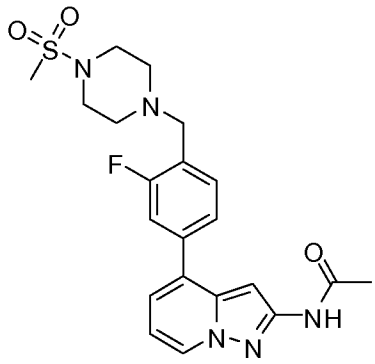
[0068] Tautomeric forms may be relevant to the attainment of the optimal chemical reactivity and biological activity of a compound of interest.

[0069] Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art.

THE COMPOUND

[0070] The present invention is based on the identification that the compound of the invention is an inhibitor of JAK and that it may be useful for the treatment of inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons. The present invention also provides methods for the production of the compound of the invention, pharmaceutical compositions comprising the compound of the invention and methods for treating inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, by administering the compound of the invention. In a specific embodiment the compound of the invention is an inhibitor of JAK1 and/or JAK2.

[0071] Accordingly, in a first aspect of the invention, the compound of the invention is according to Formula (I):



I

[0072] In one embodiment the compound of the invention is not an isotopic variant.

[0073] In one aspect the compound of the invention is present as the free base.

[0074] In one aspect the compound of the invention is a pharmaceutically acceptable salt.

[0075] In one aspect the compound of the invention is a solvate of the compound.

[0076] In one aspect the compound of the invention is a solvate of a pharmaceutically acceptable salt of a compound.

[0077] In certain aspects, the present invention provides prodrugs and derivatives of the compound of the invention. Prodrugs are derivatives of the compound of the invention, which have metabolically cleavable groups and become by solvolysis or under physiological conditions the compound of the invention, which are pharmaceutically active, *in vivo*. Such examples include, but are not limited to, choline ester derivatives and the like, N-alkylmorpholine esters and the like.

[0078] Other derivatives of the compound of this invention have activity in both their acid and acid derivative forms, but the acid sensitive form often offers advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (see, Bundgard, H., Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well know to practitioners of the art, such as, for example, esters prepared by reaction of the parent acid with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a substituted or unsubstituted amine, or acid anhydrides, or mixed anhydrides. Simple aliphatic or aromatic esters, amides and anhydrides derived from acidic groups pendant on the compounds of this invention are preferred prodrugs. In some cases it is desirable to prepare double ester type prodrugs such as (acyloxy)alkyl esters or ((alkoxycarbonyl)oxy)alkylesters. Particularly useful are the C_1 to C_8 alkyl, C_{2-8} alkenyl, aryl, C_{7-12} substituted aryl, and C_{7-12} arylalkyl esters of the compound of the invention.

[0079] The compound of the invention is a novel inhibitor of JAK. In particular, the compound is a potent inhibitor of JAK1 and/or JAK2, however it may inhibit TYK2 and JAK3 with a lower potency.

PHARMACEUTICAL COMPOSITIONS

[0080] When employed as a pharmaceutical, the compound of the invention is typically administered in the form of a pharmaceutical composition. Such compositions can be prepared in a manner well known in the pharmaceutical art and comprise at least one active compound. Generally, the compound of this invention is administered in a pharmaceutically effective amount. The amount of the compound actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[0081] The pharmaceutical compositions of the invention can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intra-articular, intravenous, intramuscular, and intranasal. Depending on the intended route of delivery, a compound of this invention is preferably formulated as either injectable or oral compositions or as salves, as lotions or as patches all for transdermal administration.

[0082] The compositions for oral administration can take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term 'unit dosage forms' refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined

quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient, vehicle or carrier. Typical unit dosage forms include prefilled, premeasured ampules or syringes of the liquid compositions or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the compound of the invention is usually a minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

[0083] Liquid forms suitable for oral administration may include a suitable aqueous or nonaqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. Solid forms may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0084] Injectable compositions are typically based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. As before, the active compound in such compositions is typically a minor component, often being from about 0.05 to 10% by weight with the remainder being the injectable carrier and the like.

[0085] Transdermal compositions are typically formulated as a topical ointment or cream containing the active ingredient(s), generally in an amount ranging from about 0.01 to about 20% by weight, preferably from about 0.1 to about 20% by weight, preferably from about 0.1 to about 10% by weight, and more preferably from about 0.5 to about 15% by weight. When formulated as a ointment, the active ingredients will typically be combined with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with, for example an oil-in-water cream base. Such transdermal formulations are well-known in the art and generally include additional ingredients to enhance the dermal penetration of stability of the active ingredients or the formulation. All such known transdermal formulations and ingredients are included within the scope of this invention.

[0086] The compound of the invention can also be administered by a transdermal device. Accordingly, transdermal administration can be accomplished using a patch either of the reservoir or porous membrane type, or of a solid matrix variety.

[0087] The above-described components for orally administrable, injectable or topically administrable compositions are merely representative. Other materials as well as processing techniques and the like are set forth in Part 8 of Remington's Pharmaceutical Sciences, 17th edition, 1985, Mack Publishing Company, Easton, Pennsylvania, which is incorporated herein by reference.

[0088] The compound of the invention can also be administered in sustained release forms or from sustained release drug delivery systems. A description of representative sustained release materials can be found in Remington's Pharmaceutical Sciences.

[0089] The following formulation examples illustrate representative pharmaceutical compositions that may be prepared in accordance with this invention. The present invention, however, is not limited to the following pharmaceutical compositions.

Formulation 1 - Tablets

[0090] The compound of the invention may be admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ratio. A minor amount of magnesium stearate may be added as a lubricant. The mixture may be formed into 240-270 mg tablets (80-90 mg of active amide compound per tablet) in a tablet press.

Formulation 2 - Capsules

[0091] The compound of the invention may be admixed as a dry powder with a starch diluent in an approximate 1:1 weight ratio. The mixture may be filled into 250 mg capsules (125 mg of active amide compound per capsule).

Formulation 3 - Liquid

[0092] The compound of the invention (125 mg), may be admixed with sucrose (1.75 g) and xanthan gum (4 mg) and the resultant mixture may be blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of microcrystalline cellulose and sodium carboxymethyl cellulose (11:89, 50 mg) in water. Sodium benzoate (10 mg), flavor, and color may be diluted with water and added with stirring. Sufficient water may then be added with stirring. Further sufficient water may be then added to produce a total volume of 5 mL.

Formulation 4 - Tablets

[0093] The compound of the invention may be admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ratio. A minor amount of magnesium stearate may be added as a lubricant. The mixture may be formed into 450-900 mg tablets (150-300 mg of active amide compound) in a tablet press.

Formulation 5 - Injection

[0094] The compound of the invention may be dissolved or suspended in a buffered sterile saline injectable aqueous medium to a concentration of approximately 5 mg/mL.

Formulation 6 - Topical

[0095] Stearyl alcohol (250 g) and a white petrolatum (250 g) may be melted at about 75°C and then a mixture of the compound of the invention (50 g) methylparaben (0.25 g), propylparaben (0.15 g), sodium lauryl sulfate (10 g), and propylene glycol (120 g) dissolved in water (about 370 g) may be added and the resulting mixture may be stirred until it congeals.

METHODS OF TREATMENT

[0096] The compound of the invention may be used as a therapeutic agent for the treatment or prophylaxis of conditions in mammals that are causally related or attributable to aberrant activity of JAK. In particular, conditions related to aberrant activity of JAK1 and/or JAK2.

[0097] Accordingly, the compounds and pharmaceutical compositions of the invention find use as therapeutics for the treatment or prophylaxis of inflammatory conditions, autoimmune diseases, proliferative diseases, allergy, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and diseases associated with hypersecretion of IL6 or interferons, in mammals including humans.

[0098] In one aspect, the present invention provides the compound of the invention, or a pharmaceutical composition comprising the compound of the invention for use as a medicament.

[0099] In a method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with an allergic reaction. In a specific embodiment, the invention provides methods of treatment or prophylaxis ofallergic airway disease, sinusitis, eczema and/or hives, food allergies or allergies to insect venom.

[00100] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of an allergic reaction. In a specific embodiment, the invention provides methods of treatment or prophylaxis of allergic airway disease, sinusitis, eczema and/or hives, food allergies or allergies to insect venom.

[00101] In additional method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with an inflammatory condition. The methods comprise administering an effective amount of one or more of the pharmaceutical compositions or compound of the invention herein described for the treatment or prophylaxis of said condition. In a specific embodiment, the inflammatory condition is selected from rheumatoid arthritis, osteoarthritis, allergic airway disease (*e.g.* asthma) and inflammatory bowel diseases.

[00102] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of an inflammatory condition. In a specific embodiment, the inflammatory condition is selected from rheumatoid arthritis, osteoarthritis, allergic airway disease (*e.g.* asthma) and inflammatory bowel diseases.

[00103] In additional method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with an autoimmune disease. The methods comprise administering an effective amount of one or more of the pharmaceutical compositions or compound of the invention herein described for the treatment or prophylaxis of said condition. In a specific embodiment, the autoimmune disease is selected from COPD, asthma, systemic lupus erythematosis, type I diabetes mellitus, psoriatic arthritis, ankylosing spondylitis, juvenile ideopathic arthritis, and inflammatory bowel disease.

[00104] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of an autoimmune disease. In a specific embodiment, the autoimmune disease is selected from COPD, asthma, systemic lupus erythematosis, type I diabetes mellitus, psoriatic arthritis, ankylosing spondylitis, juvenile ideopathic arthritis, and inflammatory bowel disease. In a more specific embodiment, the autoimmune disease is systemic lupus erythematosis.

[00105] In further method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with a proliferative disease, in particular cancer (*e.g.* solid tumors such as uterine leiomyosarcoma or prostate cancer), leukemia (*e.g.* AML, ALL or CLL), multiple myeloma and/or psoriasis.

[00106] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of a proliferative disease, in particular cancer (*e.g.* solid tumors such as uterine leiomyosarcoma or prostate cancer), leukemia (*e.g.* AML, ALL or CLL), multiple myeloma and/or psoriasis.

[00107] In further method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with transplant rejection. In a specific embodiment, the invention provides methods of treatment or prophylaxis of organ transplant rejection.

[00108] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of transplant rejection. In a specific embodiment, the invention provides methods of treatment or prophylaxis of organ transplant rejection.

[00109] In a method of treatment aspect, this invention provides a method of treatment or prophylaxis in a mammal susceptible to or afflicted with diseases involving impairment of cartilage turnover, which method comprises administering a therapeutically effective amount of the compound of the invention, or one or more of the pharmaceutical compositions herein described.

[00110] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of diseases involving impairment of cartilage turnover.

[00111] The present invention also provides a method of treatment or prophylaxis of congenital cartilage malformations, which method comprises administering an effective amount of one or more of the pharmaceutical compositions or compound of the invention herein described.

[00112] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of congenital cartilage malformations.

[00113] In further method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with diseases associated with hypersecretion of IL6, in particular Castleman's disease or mesangial proliferative glomerulonephritis.

[00114] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of diseases associated with hypersecretion of IL6, in particular Castleman's disease or mesangial proliferative glomerulonephritis.

[00115] In further method of treatment aspects, this invention provides methods of treatment or prophylaxis of a mammal susceptible to or afflicted with diseases associated with hypersecretion of

interferons, in particular systemic and cutaneous lupus erythematosis, lupus nephritis, dermatomyositis, Sjogren's syndrome, psoriasis, rheumatoid arthritis.

[00116] In another aspect the present invention provides the compound of the invention for use in the treatment or prophylaxis of diseases associated with hypersecretion of interferons, in particular systemic and cutaneous lupus erythematosis, lupus nephritis, dermatomyositis, Sjogren's syndrome, psoriasis, rheumatoid arthritis.

[00117] As a further aspect of the invention there is provided the compound of the invention for use as a pharmaceutical especially in the treatment or prophylaxis of the aforementioned conditions and diseases. Also provided herein is the use of the present compounds in the manufacture of a medicament for the treatment or prophylaxis of one of the aforementioned conditions and diseases.

[00118] A particular regimen of the present method comprises the administration to a subject suffering from a disease involving inflammation, in particular rheumatoid arthritis, osteoarthritis, allergic airway disease (*e.g.* asthma) and inflammatory bowel diseases, of an effective amount of the compound of the invention for a period of time sufficient to reduce the level of inflammation in the subject, and preferably terminate the processes responsible for said inflammation. A special embodiment of the method comprises administering of an effective amount of the compound of the invention to a subject patient suffering from or susceptible to the development of rheumatoid arthritis, for a period of time sufficient to reduce or prevent, respectively, inflammation in the joints of said patient, and preferably terminate, the processes responsible for said inflammation.

[00119] A further particular regimen of the present method comprises the administration to a subject suffering from a disease condition characterized by cartilage or joint degradation (*e.g.* rheumatoid arthritis and/or osteoarthritis) of an effective amount of the compound of the invention for a period of time sufficient to reduce and preferably terminate the self-perpetuating processes responsible for said degradation. A particular embodiment of the method comprises administering of an effective amount of the compound of the invention to a subject patient suffering from or susceptible to the development of osteoarthritis, for a period of time sufficient to reduce or prevent, respectively, cartilage degradation in the joints of said patient, and preferably terminate, the self-perpetuating processes responsible for said degradation. In a particular embodiment said compound may exhibit cartilage anabolic and/or anti-catabolic properties.

[00120] Injection dose levels range from about 0.1 mg/kg/h to at least 10 mg/kg/h, all for from about 1 to about 120 h and especially 24 to 96 h. A preloading bolus of from about 0.1 mg/kg to about 10 mg/kg or more may also be administered to achieve adequate steady state levels. The maximum total dose is not expected to exceed about 2 g/day for a 40 to 80 kg human patient.

[00121] For the prophylaxis and/or treatment of long-term conditions, such as degenerative conditions, the regimen for treatment usually stretches over many months or years so oral dosing is preferred for patient convenience and tolerance. With oral dosing, one to five and especially two to four and typically three oral doses per day are representative regimens. Using these dosing patterns, each dose provides

from about 0.01 to about 20 mg/kg of the compound of the invention, with particular doses each providing from about 0.1 to about 10 mg/kg and especially about 1 to about 5 mg/kg.

[00122] Transdermal doses are generally selected to provide similar or lower blood levels than are achieved using injection doses.

[00123] When used to prevent the onset of a condition, the compound of the invention will be administered to a patient at risk for developing the condition, typically on the advice and under the supervision of a physician, at the dosage levels described above. Patients at risk for developing a particular condition generally include those that have a family history of the condition, or those who have been identified by genetic testing or screening to be particularly susceptible to developing the condition.

[00124] The compound of the invention can be administered as the sole active agent or it can be administered in combination with other therapeutic agents, including other compounds that demonstrate the same or a similar therapeutic activity and that are determined to safe and efficacious for such combined administration. In a specific embodiment, co-administration of two (or more) agents allows for significantly lower doses of each to be used, thereby reducing the side effects seen.

[00125] In one embodiment, the compound of the invention or a pharmaceutical composition comprising the compound of the invention is administered as a medicament. In a specific embodiment, said pharmaceutical composition additionally comprises a further active ingredient.

[00126] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of a disease involving inflammation; particular agents include, but are not limited to, immunoregulatory agents *e.g.* azathioprine, corticosteroids (*e.g.* prednisolone or dexamethasone), cyclophosphamide, cyclosporin A, tacrolimus, Mycophenolate Mofetil, muromonab-CD3 (OKT3, *e.g.* Orthocolone®), ATG, aspirin, acetaminophen, ibuprofen, naproxen, and piroxicam.

[00127] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of arthritis (*e.g.* rheumatoid arthritis); particular agents include but are not limited to analgesics, non-steroidal anti-inflammatory drugs (NSAIDS), steroids, synthetic DMARDS (for example but without limitation methotrexate, leflunomide, sulfasalazine, auranofin, sodium aurothiomalate, penicillamine, chloroquine, hydroxychloroquine, azathioprine, Tofacitinib, Fostamatinib, and cyclosporin), and biological DMARDS (for example but without limitation Infliximab, Etanercept, Adalimumab, Rituximab, and Abatacept).

[00128] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of proliferative disorders; particular agents include but are not limited to: methotrexate, leukovorin, adriamycin, prenisone, bleomycin, cyclophosphamide, 5-fluorouracil, paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, doxorubicin, tamoxifen, toremifene, megestrol acetate, anastrozole, goserelin, anti-HER2 monoclonal antibody (*e.g.* Herceptin™), capecitabine, raloxifene hydrochloride, EGFR inhibitors (*e.g.* Iressa®, Tarceva™, Erbitux™), VEGF inhibitors (*e.g.* Avastin™), proteasome inhibitors (*e.g.* Velcade™), Glivec® and hsp90 inhibitors (*e.g.* 17-AAG). Additionally, the compound of the invention may be administered in combination with other

therapies including, but not limited to, radiotherapy or surgery. In a specific embodiment the proliferative disorder is selected from cancer, myeloproliferative disease or leukaemia.

[00129] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of autoimmune diseases, particular agents include but are not limited to: glucocorticoids, cytostatic agents (*e.g.* purine analogs), alkylating agents, (e.g nitrogen mustards (cyclophosphamide), nitrosoureas, platinum compounds, and others), antimetabolites (*e.g.* methotrexate, azathioprine and mercaptopurine), cytotoxic antibiotics (*e.g.* dactinomycin anthracyclines, mitomycin C, bleomycin, and mithramycin), antibodies (*e.g.* anti-CD20, anti-CD25 or anti-CD3 (OTK3) monoclonal antibodies, Atgam® and Thymoglobuline®), cyclosporin, tacrolimus, rapamycin (sirolimus), interferons (*e.g.* IFN-β), TNF binding proteins (*e.g.* infliximab (Remicade™), etanercept (Enbrel™), or adalimumab (Humira™)), mycophenolate, Fingolimod and Myriocin.

[00130] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of transplant rejection, particular agents include but are not limited to: calcineurin inhibitors (*e.g.* cyclosporin or tacrolimus (FK506)), mTOR inhibitors (*e.g.* sirolimus, everolimus), anti-proliferatives (*e.g.* azathioprine, mycophenolic acid), corticosteroids (*e.g.* prednisolone, hydrocortisone), Antibodies (*e.g.* monoclonal anti-IL-2Rα receptor antibodies, basiliximab, daclizumab), polyclonal anti-T-cell antibodies (*e.g.* anti-thymocyte globulin (ATG), anti-lymphocyte globulin (ALG)).

[00131] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of asthma and/or rhinitis and/or COPD, particular agents include but are not limited to: $beta_2$-adrenoceptor agonists (*e.g.* salbutamol, levalbuterol, terbutaline and bitolterol), epinephrine (inhaled or tablets), anticholinergics (*e.g.* ipratropium bromide), glucocorticoids (oral or inhaled) Long-acting β_2-agonists (*e.g.* salmeterol, formoterol, bambuterol, and sustained-release oral albuterol), combinations of inhaled steroids and long-acting bronchodilators (*e.g.* fluticasone/salmeterol, budesonide/formoterol), leukotriene antagonists and synthesis inhibitors (*e.g.* montelukast, zafirlukast and zileuton), inhibitors of mediator release (*e.g.* cromoglycate and ketotifen), biological regulators of IgE response (*e.g.* omalizumab), antihistamines (*e.g.* ceterizine, cinnarizine, fexofenadine) and vasoconstrictors (*e.g.* oxymethazoline, xylomethazoline, nafazoline and tramazoline).

[00132] Additionally, the compound of the invention may be administered in combination with emergency therapies for asthma and/or COPD, such therapies include oxygen or heliox administration, nebulized salbutamol or terbutaline (optionally combined with an anticholinergic (*e.g.* ipratropium), systemic steroids (oral or intravenous, *e.g.* prednisone, prednisolone, methylprednisolone, dexamethasone, or hydrocortisone), intravenous salbutamol, non-specific beta-agonists, injected or inhaled (*e.g.* epinephrine, isoetharine, isoproterenol, metaproterenol), anticholinergics (IV or nebulized, *e.g.* glycopyrrolate, atropine, ipratropium), methylxanthines (theophylline, aminophylline, bamiphylline), inhalation anesthetics that have a bronchodilatory effect (*e.g.* isoflurane, halothane, enflurane), ketamine and intravenous magnesium sulfate.

[00133] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of inflammatory bowel disease (IBD), particular agents include but are not limited to: glucocorticoids (*e.g.* prednisone, budesonide) synthetic disease modifying, immunomodulatory agents (*e.g.* methotrexate, leflunomide, sulfasalazine, mesalazine, azathioprine, 6-mercaptopurine and cyclosporin) and biological disease modifying, immunomodulatory agents (infliximab, adalimumab, rituximab, and abatacept).

[00134] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of SLE, particular agents include but are not limited to: Disease-modifying antirheumatic drugs (DMARDs) such as antimalarials (*e.g.* plaquenil, hydroxychloroquine), immunosuppressants (*e.g.* methotrexate and azathioprine), cyclophosphamide and mycophenolic acid; immunosuppressive drugs and analgesics, such as nonsteroidal anti-inflammatory drugs, opiates (*e.g.* dextropropoxyphene and co-codamol), opioids (*e.g.* hydrocodone, oxycodone, MS Contin, or methadone) and the fentanyl duragesic transdermal patch.

[00135] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of psoriasis, particular agents include but are not limited to: topical treatments such as bath solutions, moisturizers, medicated creams and ointments containing coal tar, dithranol (anthralin), corticosteroids like desoximetasone (Topicort™), fluocinonide, vitamin D_3 analogues (for example, calcipotriol), Argan oiland retinoids (etretinate, acitretin, tazarotene), systemic treatments such as methotrexate, cyclosporine, retinoids, tioguanine, hydroxyurea, sulfasalazine, mycophenolate mofetil, azathioprine, tacrolimus, fumaric acid esters or biologics such as Amevive™, Enbrel™, Humira™, Remicade™, Raptiva™ and ustekinumab (a IL-12 and IL-23 blocker). Additionally, the compound of the invention may be administered in combination with other therapies including, but not limited to phototherapy, or photochemotherapy (*e.g.* psoralen and ultraviolet A phototherapy (PUVA)).

[00136] In one embodiment, the compound of the invention is co-administered with another therapeutic agent for the treatment and/or prophylaxis of allergic reaction, particular agents include but are not limited to: antihistamines (*e.g.* cetirizine, diphenhydramine, fexofenadine, levocetirizine), glucocorticoids (*e.g.* prednisone, betamethasone, beclomethasone, dexamethasone), epinephrine, theophylline or anti-leukotrienes (*e.g.* montelukast or zafirlukast), anti-cholinergics and decongestants.

[00137] By co-administration is included any means of delivering two or more therapeutic agents to the patient as part of the same treatment regime, as will be apparent to the skilled person. Whilst the two or more agents may be administered simultaneously in a single formulation, *i.e.* as a single pharmaceutical composition, this is not essential. The agents may be administered in different formulations and at different times.

GENERAL SYNTHETIC PROCEDURES

General

[00138] The compound of the invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (*i.e.* reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00139] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and P. G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

[00140] The following methods are presented with details as to the preparation of the compound of the invention as defined hereinabove and the comparative examples. The compound of the invention may be prepared from known or commercially available starting materials and reagents by one skilled in the art of organic synthesis.

[00141] All reagents were of commercial grade and were used as received without further purification, unless otherwise stated. Commercially available anhydrous solvents were used for reactions conducted under inert atmosphere. Reagent grade solvents were used in all other cases, unless otherwise specified. Column chromatography was performed on silica gel 60 (35-70 μm). Thin layer chromatography was carried out using pre-coated silica gel F-254 plates (thickness 0.25 mm). ^{1}H NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer (400 MHz). Chemical shifts (δ) for ^{1}H NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or the appropriate residual solvent peak, *i.e.* $CHCl_3$ (δ 7.27), as internal reference. Multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Coupling constants (J) are given in Hz. Electrospray MS spectra were obtained on a Micromass platform LC/MS spectrometer. Columns Used for LCMS analysis: Hichrom, Kromasil Eternity, 2.5μm C18, 150 x 4.6mm, Waters Xbridge 5μm C18 (2), 250 x 4.6mm (ref 86003117), Waters Xterra MS 5μm C18, 100 x 4.6mm (Plus guard cartridge) (ref 186000486), Gemini-NX 3 μm C18 100 x 3.0 mm (ref 00D-4453-Y0), Phenomenex Luna 5μm C18 (2), 100 x 4.6mm. (Plus guard cartridge) (ref 00D-4252-E0), Kinetix fused core 2.7μm C18 100 x 4.6 mm (ref 00D-4462-E0), Supelco, Ascentis® Express C18 (ref 53829-U), or Hichrom Halo C18, 2.7μm C18, 150 x 4.6mm (ref 92814-702). LC-MS were recorded on a Waters Micromass ZQ coupled to a HPLC Waters 2795, equipped with a UV detector Waters 2996. LC were also run on a HPLC Agilent 1100 coupled to a UV detector Agilent G1315A. Preparative HPLC: Waters XBridge Prep C18 5μm ODB 19mm ID x 100mm L (Part No.186002978). All the methods are using MeCN/H_2O gradients. H_2O contains either 0.1% TFA or 0.1% NH_3.

[00142] List of abbreviations used in the experimental section:

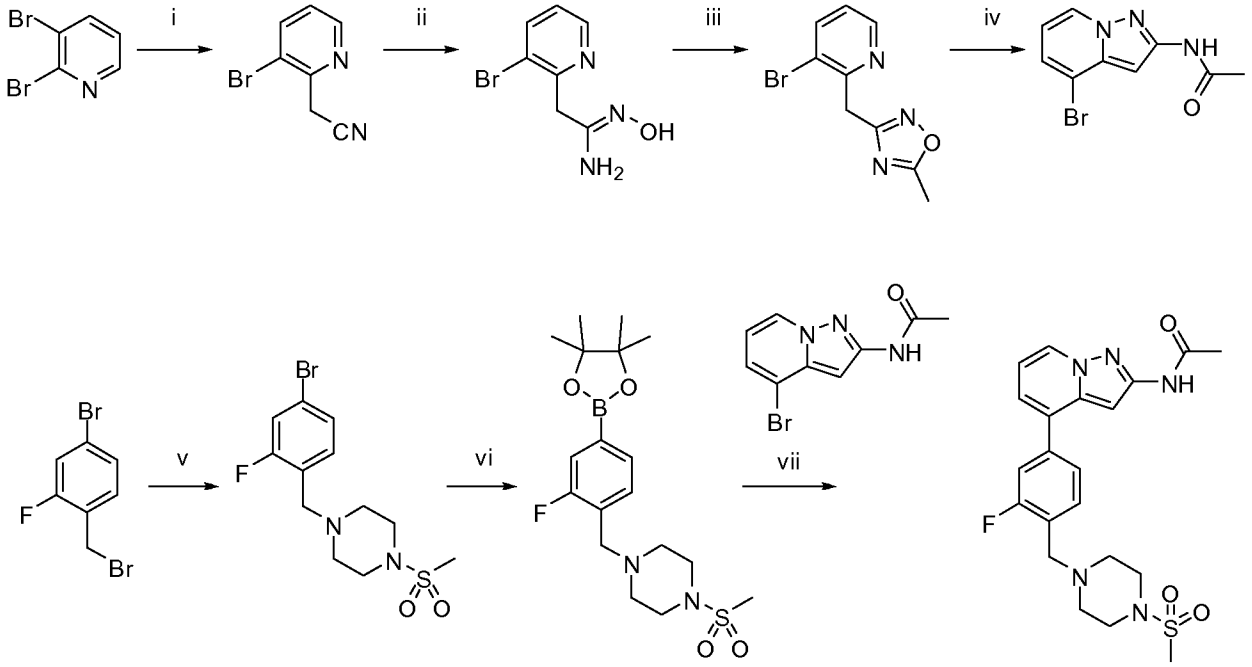
DCM	Dichloromethane
DiPEA	N,N-diisopropylethylamine
MeCN	Acetonitrile
BOC	tert-Butyloxy-carbonyl
DMF	N,N-dimethylformamide
Cat.	Catalytic amount
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
NMR	Nuclear Magnetic Resonnance
DMSO	Dimethylsulfoxide
LC-MS	Liquid Chromatography- Mass Spectrometry
Ppm	part-per-million
Pd/C	Palladium on Charcoal 10%
PMB	Para-methoxy-benzyl
PyBOP	benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoroborate
EtOAc	ethyl acetate
APCI	atmospheric pressure chemical ionization
Rt	retention time
s	singlet
br s	broad singlet
m	multiplet
min	minute
mL	milliliter
µL	microliter
g	gram
mg	milligram
$PdCl_2dppf$	[1,1'-Bis(diphenylphosphino)ferrocene] dichloropalladium(II)
TEA	Triethylamine
MMP	Matrix Metallo Proteinase

NHAC	Normal Human Articular Chondrocytes
shRNA	short hairpin RNA
RNA	Ribonucleic acid
Ad-Si RNA	Adenoviral encoded siRNA
PBST	Phosphate buffered saline with Tween 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, 0.05% Tween 20, pH 7.4
APMA	4-aminophenylmercuric acetate
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
hCAR	human cellular adenovirus receptor
3- MOI	multiplicity of infection of 3
dNTP	deoxyribonucleoside triphosphate
QPCR	quantitative polymerase chain reaction
cDNA	copy deoxyribonucleic acid
GAPDH	Glyceraldehyde phosphate dehydrogenase
h	hour
mmol	millimoles
HATU	O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HPLC	High pressure liquid chromatography
PS-NMe_3BH_3CN	Polymer supported-NMe_3BH_3CN
NMP	N-Methylpyrrolidone

Synthetic Preparation of the Compound of the Invention

[00143] The compound of the invention can be produced according to the following scheme.

Compound 1: N-4-(3-fluoro-4-((4-(methylsulfonyl)piperazin-1-yl)methyl)phenyl)pyrazolo[1,5-a]pyridin-2-yl)acetamide



Step i): 3-bromo-2-cyanomethylpyridine (Intermediate 1)

[00144] Dry THF (100 mL) was cooled to –78 °C and n-butyllithium (2.5 M in hexanes; 23 mL, 58 mmol) was added. Acetonitrile (3.3 mL, 64 mmol) was added dropwise maintaining the temperature below –60 °C. A white precipitate formed and the reaction mixture was stirred at –78 °C for 45 min. A solution of 2,3-dibromopyridine (2.0 g, 8.4 mmol) in dry THF (10 mL) was added dropwise and the reaction mixture stirred at –78 °C for 1.5 h then allowed to warm to room temperature. The reaction was quenched by the dropwise addition of water. The aqueous was extracted with DCM (x3) and the combined organics washed with brine, dried ($MgSO_4$) and concentrated in vacuo. (3-Bromo-pyridin-2-yl)-acetonitrile (Intermediate 1) was purified by flash column chromatography.

Step ii) 2-(3-Bromo-pyridin-2-yl)-N-hydroxy-acetamidine (Intermediate 2)

[00145] A vigorously stirred mixture of hydroxylamine hydrochloride (924 mg, 13.3 mmol), water (4 mL), $NaHCO_3$ (1.12 g, 13.3 mmol), 3-bromo-2-cyanomethylpyridine (Intermediate 1) (1.31 g, 6.65 mmol) and ethanol (14 mL) was heated to reflux for 5 h. The reaction mixture was allowed to cool to room temperature, the ethanol was removed *in vacuo* and the white solid collected by filtration, washing with water (3 x 10 mL). The wet solid was dried *in vacuo* at 40 °C for 1 hour to yield 2-(3-Bromo-pyridin-2-yl)-N-hydroxy-acetamidine as a solid (Intermediate 2).

Step iii): 3-Bromo-2-(5-methyl-[1,2,4]oxadiazol-3-ylmethyl)-pyridine (Intermediate 3)

[00146] A mixture of 2-(3-bromo-pyridin-2-yl)-N-hydroxy-acetamidine obtained in Step ii) (1.24 g, 5.39 mmol) and acetic anhydride (552 mg, 5.41 mmol) were stirred in dry THF (10 mL) at 20 °C for 1.5 h. The reaction mixture was concentrated *in vacuo* and the residue was stirred vigorously in a two-phase system of 2 M Na_2CO_3 (10 mL) and DCM (10 mL) at 20 °C for 10 min. The resultant white precipitate

was collected by filtration, washing with water (2 x 5 mL) and DCM (2 x 5 mL), then dried *in vacuo* at 40 °C for 1 h, to yield *O*-acetyl amidoxime as a powder.

[00147] The *O*-acetyl amidoxime obtained (610 mg, 2.24 mmol) and K_2CO_3 (1.56 g, 11.3 mmol) were stirred vigorously in $CHCl_3$ (6 mL) at 60 °C for 41 h. Water (5 mL) and further $CHCl_3$ (5 mL) were added to the mixture with vigorous stirring. The layers were separated and the organic extract concentrated *in vacuo*. Crude 3-bromo-2-(5-methyl-[1,2,4]oxadiazol-3-ylmethyl)-pyridine was obtained as a yellow liquid and used in subsequent reactions without further purification (Intermediate 3).

Step iv): 3-(4-Bromo-pyrazolo[1,5-a]pyridin-2-yl)-acetamide (Intermediate 4)

[00148] The 3-bromo-2-(5-methyl-[1,2,4]oxadiazol-3-ylmethyl)-pyridine obtained in step iii) (663 mg, 2.61 mmol) was stirred at 150 °C in toluene (5.2 mL) in a sealed tube for 41 h. The reaction mixture was allowed to cool to room temperature, the toluene was removed *in vacuo* and the residue purified by column chromatography (gradient: 0-10% MeOH in DCM). The solid obtained was triturated with diethyl ether to yield 3-(4-bromo-pyrazolo[1,5-a]pyridin-2-yl)-acetamide (Intermediate 4) as a solid.

Step v): 1-(4-Bromo-2-fluoro-benzyl)-4-methanesulfonyl-piperazine (Intermediate 5)

[00149] A mixture of 4-bromo-2-fluorobenzyl bromide (30.4 g, 114 mmol, 1 eq), 1-methylsulfonylpiperazine (20.0 g, 114 mmol, 1 eq) and K_2CO_3 (31.6 g, 228 mmol, 2 eq) in acetonitrile (500 mL) was stirred at 40 °C for 18 h. The reaction mixture was allowed to cool to room temperature, the mixture was filtered through Celite, washing with EtOAc. The filtrate was concentrated *in vacuo* to yield the target amine.

[00150] A mixture of 4-bromo-2-fluorobenzyl bromide (30.4 g, 114 mmol, 1 eq), 1-methylsulfonylpiperazine (20.0 g, 114 mmol, 1 eq) and K_2CO_3 (31.6 g, 228 mmol, 2 eq) in acetonitrile (500 mL) was stirred at 40 °C for 18 h. The reaction mixture was allowed to cool to room temperature, the mixture was filtered through Celite, and washed with EtOAc. The filtrate was concentrated *in vacuo* to yield 1-(4-Bromo-2-fluoro-benzyl)-4-methanesulfonyl-piperazine (Intermediate 5).

Step vi): 1-[2-Fluoro-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl]-4-methanesulfonyl-piperazine (Intermediate 6)

[00151] A mixture of the aryl bromide (20.0 g, 57 mmol, 1 eq), bis(pinacolato)diboron (16.0 g, 63 mmol, 1.1 eq), $PdCl_2$(dppf) (2.3 g, 2.9 mmol, 0.05 eq) and potassium acetate (6.2 g, 63 mmol, 1.1 eq) in degassed dioxane (200 mL) was stirred under nitrogen in a sealed tube at 90 °C for 18 h. The reaction mixture was allowed to cool to room temperature, the mixture was filtered through a plug of silica, and washed with DCM. The filtrate was concentrated *in vacuo*. The residue was mixed with isohexane and diethyl ether was added until the product crystallized. The product was collected by filtration as a solid.

[00152] Alternatively, A mixture of the aryl bromide (309 g, 879.7 mmol,), bis(pinacolato)diboron (245.7 g, 967.7 mmol), $PdCl_2$(dppf) (35.9 g, 43.9 mmol) and potassium acetate (94.9 g, 967.7 mmol) in degassed dioxane (3 L) were stirred under nitrogen at 100°C for 6 h. The reaction mixture was allowed to

cool to room temperature, the mixture was filtered through a plug of silica, and washed with DCM. The filtrate was concentrated *in vacuo*. DCM and hexane (2 L) were added to the viscous residue during which the product precipitated. The suspension was filtered affording the desired compound.

Step vii): N-4-(3-fluoro-4-((4-(methylsulfonyl)piperazin-1-yl)methyl)phenyl)pyrazolo[1,5-a]pyridin-2-yl)acetamide (Compound 1).

3 methods were used.

Step vii): first method

[00153] 1-[2-Fluoro-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl]-4-methanesulfonyl-piperazine (26.4 g, 66 mmol, 1.1 eq) was added to a solution of aryl bromide (16.0 g, 63 mmol, 1 eq) in 1,4-dioxane/water (540 mL, 4:1; v/v). Na_2CO_3 (13.4 g, 126 mmol, 2.0 eq) and $Pd(dppf)Cl_2$ (2.6 g, 3 mmol, 0.05 eq) (dppf = 1,1'-Bis(diphenylphosphino)ferrocene) were added to the degassed solution. The resultant mixture was heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc, dried with $MgSO_4$, and concentrated in vacuo. Purification by preparative HLPC yielded the target compounds

Step vii): second method

[00154] 1-[2-Fluoro-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl]-4-methanesulfonyl-piperazine obtained in step(ii) above (7.5 g, 18 mmol, 1 eq) was added to a solution of aryl bromide (4.5 g, 18 mmol, 1 eq) in 1,4-dioxane/water (150 mL, 4:1; v/v). Na_2CO_3 (3.8 g, 36 mmol, 2.0 eq) and $Pd(dppf)Cl_2$ (0.7 g, 0.9 mmol, 5%) (dppf = 1,1'-Bis(diphenylphosphino)ferrocene) were added to the degassed solution. The resultant mixture was heated at 100 °C for 2 h. The two reaction mixtures were combined and the solvent removed *in vacuo*. The residue was mixed with DCM and water and the insoluble material removed by filtration. (The insoluble material was reserved and later re-combined with the organic residue before purification.) The aqueous layer was separated and extracted with DCM (× 3). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo*. This residue was combined with the previously collected insoluble material and adsorbed onto silica. Purification by column chromatography (gradient: 0 - 5% MeOH in EtOAc) or preparative HLPC yielded the target as a brown solid. The brown solid was mixed with ethanol and the suspension heated to reflux. The mixture was allowed to cool to room temperature and then further cooled with an ice-bath. The target material was collected by filtration and dried *in vacuo*.

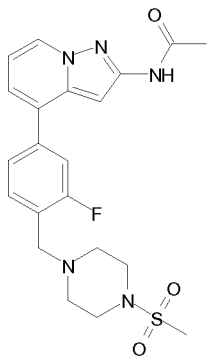
Step vii): third method

[00155] A mixture of 1-*N*-(4-bromo-pyrazolo[1,5-a]pyridine-2-yl)-acetamide (149 g, 0.586 mol) and Na_2CO_3 (124 g, 1.172 mol) in a solvent mixture of 1,4–dioxane (2 L)/water (0.5 L) at room temperature

was degassed with N_2. To this mixture, boronic ester (270 g, 1.15 eq) and $PdCl_2$(dppf) (24 g, 5 mol%) were added. The reactor's jacket was heated to 100°C, degassing with N_2 was stopped and the mixture was stirred under heating for 45 min. UPLC revealed complete consumption of the boronic ester. An additional amount of boronic ester (10 g, 0.05 eq) was added and stirring was continued at 100°C for an additional 30 min. UPLC revealed complete conversion and single peak of the expected product. The solvent volume was removed under reduced pressure to 0.5 L of and the resulting suspension was left at 4°C overnight. Water (0.5 L) was added to the suspension and stirred for 30 min and filtered. The resulting cake was washed with water (0.5 L) and dried under suction for 1h. The cake was transferred to a Petri dish (425 g) and left to dry in air overnight to obtain the crude desired compound. The powder was put on a pad of silicagel and washed with DCM/MeOH (9:1 mixture). The first fraction (2.5 L) was concentrated to dryness giving a resinous solid that was discarded. The second fraction (10 L of DCM/MeOH 9:1 mixture, 10 L of 5:1 mixture and 3L of 3:1 mixture) was concentrated to dryness giving the desired product. The solid was suspended in EtOH (1.1 L), heated to reflux, stirred for 30 min, cooled to 5°C, stirred for 30 min and filtered. The cake was washed with cold EtOH (300 mL) and transferred to a Petri dish giving the expected product.

[00156] The compound of the invention that has been prepared according to the synthetic method described here above is listed in Table I below, and the NMR spectral data of the compound of the invention is given in Table II.

[00157] **Table I**

Cpd #	Structures	Name	MW	MS Mes'd
1		N-4-(3-fluoro-4-((4-(methylsulfonyl)piperazin-1-yl)methyl)phenyl)pyrazolo[1,5-a]pyridin-2-yl)acetamide	445	446

[00158] **Table II: NMR Data of Representative Compound of the invention**

Cpd #	(δ) NMR data
1	^{1}H NMR δ (ppm)(DMSO-d_6): 10.86 (1 H, s, NH), 8.59 (1 H, d, ArH), 7.65-7.48 (3 H, m, ArH), 7.36 (1 H, d, ArH), 6.99 (1 H, s, ArH), 7.00-6.92 (1 H, m, ArH), 3.69 (2 H, s, CH2), 3.21-3.14 (4 H, m, CH), 2.91 (3 H, s, CH3), 2.64-2.51 (4 H, m, CH), 2.11 (3 H, s, CH3).

Biological Examples

Example 1: *In-vitro* assays

1.1 JAK1 inhibition assay

1.1.1 JAK1 assay polyGT substrate

[00159] Recombinant human JAK1 catalytic domain (amino acids 866-1154; catalog number PV4774) is purchased from Invitrogen. 25 ng of JAK1 is incubated with 6.25 µg polyGT substrate (Sigma catalog number P0275) in kinase reaction buffer (15 mM Hepes pH7.5, 0,01% Tween20, 10mM $MgCl_2$, 2 µM non-radioactive ATP, 0.25 µCi ^{33}P-gamma-ATP (Perkin Elmer, catalog number NEG602K001MC) final concentrations) with or without 5µL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 25 µL, in a polypropylene 96-well plate (Greiner, V-bottom). After 75 min at 30 °C, reactions are stopped by adding of 25 µL/well of 150 mM phosphoric acid. All of the terminated kinase reaction is transferred to prewashed (75 mM phosphoric acid) 96 well filter plates (Perkin Elmer catalog number 6005177) using a cell harvester (Perkin Elmer). Plates are washed 6 times with 300 µL per well of a 75 mM phosphoric acid solution and the bottom of the plates is sealed. 40 µL/well of Microscint-20 is added, the top of the plates was sealed and a readout was performed using the Topcount (Perkin Elmer). Kinase activity is calculated by subtracting counts per min (cpm) obtained in the presence of a positive control inhibitor (10 µM staurosporine) from cpm obtained in the presence of vehicle. The ability of a test compound to inhibit this activity is determined as:

[00160] Percentage inhibition = ((cpm determined for sample with test compound present – cpm determined for sample with positive control inhibitor) divided by (cpm determined in the presence of vehicle – cpm determined for sample with positive control inhibitor)) * 100.

[00161] Dose dilution series are prepared for the compounds enabling the testing of dose-response effects in the JAK1 assay and the calculation of the IC_{50} for the compound. The compound is tested at a concentration of 20µM followed by a 1/3 serial dilution, 8 points (20µM - 6.67µM - 2.22µM - 740nM - 247nM - 82nM - 27nM - 9nM) in a final concentration of 1% DMSO. When compound potency increases, more dilutions are prepared and/or the top concentration is lowered (*e.g.* 5 µM, 1 µM).

1.1.2 JAK1 Ulight-JAK1 peptide assay

[00162] Recombinant human JAK1 (catalytic domain, amino acids 866-1154; catalog number PV4774) was purchased from Invitrogen. 1 ng of JAK1 was incubated with 20 nM Ulight-JAK1(tyr1023) peptide (Perkin Elmer catalog number TRF0121) in kinase reaction buffer (25mM MOPS pH6.8, 0.01% Brij-35, 5mM $MgCl_2$, 2mM DTT, 7µM ATP) with or without 4µL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 20 µL, in a white 384 Opti plate (Perkin Elmer, catalog number 6007290). After 60 min at room temperature, reactions were stopped by adding 20 µL/well of detection mixture (1 x detection buffer (Perkin Elmer, catalog number CR97-100C), 0.5nM Europium-anti-phosphotyrosine (PT66) (Perkin Elmer, catalog number AD0068), 10 mM EDTA). Readout is performed using the Envision with excitation at 320nm and measuring emission at 615 nm (Perkin Elmer). Kinase activity was calculated by subtracting relative fluorescence units (RFU) obtained

in the presence of a positive control inhibitor (10 μM staurosporine) from RFU obtained in the presence of vehicle. The ability of a test compound to inhibit this activity was determined as:

[00163] Percentage inhibition = ((RFU determined for sample with test compound present – RFU determined for sample with positive control inhibitor) divided by (RFU determined in the presence of vehicle – RFU determined for sample with positive control inhibitor)) * 100.

[00164] A dose dilution series was prepared for the compound enabling the testing of dose-response effects in the JAK1 assay and the calculation of the IC_{50} for the compound. The compound is routinely tested at a concentration of 20μM followed by a 1/5 serial dilution, 10 points in a final concentration of 1% DMSO. When compound potency increases, more dilutions are prepared and/or the top concentration is lowered (*e.g.* 5 μM, 1 μM). The data are expressed as the average IC_{50} from the assays ± standard error of the mean.

[00165] The activity of the compound of the invention against JAK1 has been determined in accordance with the assay described above, thus returning IC_{50} values of 8.18, 11.5, 7.71, 35.2, and 7.85 nM.

1.2 JAK1 Ki determination assay

[00166] For the determination of Ki, different amounts of compound are mixed with the enzyme and the enzymatic reaction is followed as a function of ATP concentration. The Ki is determined by means of double reciprocal plotting of Km vs compound concentration (Lineweaver-Burk plot). 1 ng of JAK1 (Invitrogen, PV4774) is used in the assay. The substrate is 20nM Ulight-JAK-1 (Tyr1023) Peptide (Perkin Elmer, TRF0121). The reaction is performed in 25mM MOPS pH 6.8, 0.01%, Brij-35, 2 mM DTT, 5 mM $MgCl_2$ with varying concentrations of ATP and compound. Phosphorylated substrate is measured using an Europium-labeled anti-phosphotyrosine antibody PT66 (Perkin Elmer, AD0068) as described in 1.1.2. Readout is performed on the envision (Perkin Elmer) with excitation at 320 nm and emission followed at 615 nm and 665 nm.

1.2 JAK2 inhibition assay

1.2.1 JAK2 assay polyGT substrate

[00167] Recombinant human JAK2 catalytic domain (amino acids 808-1132; catalog number PV4210) is purchased from Invitrogen. 0.05mU of JAK2 is incubated with 2.5 μg polyGT substrate (Sigma catalog number P0275) in kinase reaction buffer (10mM MOPS pH 7.5, 0.5mM EDTA, 0.01% Brij-35 , 1mM DTT , 15mM MgAc, 1 μM non-radioactive ATP, 0.25 μCi ^{33}P-gamma-ATP (Perkin Elmer, catalog number NEG602K001MC) final concentrations) with or without 5μL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 25 μL, in a polypropylene 96-well plate (Greiner, V-bottom). After 90 min at 30 °C, reactions are stopped by adding of 25 μL/well of 150 mM phosphoric acid. All of the terminated kinase reaction is transferred to prewashed (75 mM phosphoric acid) 96 well filter plates (Perkin Elmer catalog number 6005177) using a cell harvester (Perkin Elmer).

Plates are washed 6 times with 300 µL per well of a 75 mM phosphoric acid solution and the bottom of the plates is sealed. 40 µL/well of Microscint-20 is added, the top of the plates is sealed and readout is performed using the Topcount (Perkin Elmer). Kinase activity is calculated by subtracting counts per min (cpm) obtained in the presence of a positive control inhibitor (10 µM staurosporine) from cpm obtained in the presence of vehicle. The ability of a test compound to inhibit this activity is determined as:

[00168] Percentage inhibition = ((cpm determined for sample with test compound present – cpm determined for sample with positive control inhibitor) divided by (cpm determined in the presence of vehicle – cpm determined for sample with positive control inhibitor)) * 100.

[00169] Dose dilution series are prepared for the compounds enabling the testing of dose-response effects in the JAK2 assay and the calculation of the IC_{50} for each compound. The compound is tested at concentration of 20µM followed by a 1/3 serial dilution, 8 points (20µM - 6.67µM - 2.22µM - 740nM - 247nM - 82nM - 27nM - 9nM) in a final concentration of 1% DMSO. When potency of compound series increases, more dilutions are prepared and/or the top concentration is lowered (*e.g.* 5 µM, 1 µM).

1.2.2 JAK2 Ulight-JAK1 peptide assay

[00170] Recombinant human JAK2 (catalytic domain, amino acids 866-1154; catalog number PV4210) was purchased from Invitrogen. 0.0125mU of JAK2 was incubated with 25 nM Ulight-JAK1(tyr1023) peptide (Perkin Elmer catalog number TRF0121) in kinase reaction buffer (25mM HEPES pH7.0, 0.01% Triton X-100, 7.5mM $MgCl_2$, 2mM DTT, 7.5µM ATP) with or without 4µL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 20 µL, in a white 384 Opti plate (Perkin Elmer, catalog number 6007290). After 60 min at room temperature, reactions were stopped by adding 20 µL/well of detection mixture (1xdetection buffer (Perkin Elmer, catalog number CR97-100C), 0.5nM Europium-anti-phosphotyrosine (PT66) (Perkin Elmer, catalog number AD0068), 10 mM EDTA). Readout is performed using the Envision with excitation at 320nm and measuring emission at 615 nm (Perkin Elmer). Kinase activity was calculated by subtracting relative fluorescence units (RFU) obtained in the presence of a positive control inhibitor (10 µM staurosporine) from RFU obtained in the presence of vehicle. The ability of a test compound to inhibit this activity was determined as:

[00171] Percentage inhibition = ((RFU determined for sample with test compound present – RFU determined for sample with positive control inhibitor) divided by (RFU determined in the presence of vehicle – RFU determined for sample with positive control inhibitor)) * 100.

[00172] Dose dilution series are prepared for compound enabling the testing of dose-response effects in the JAK2 assay and the calculation of the IC_{50} for the compound. The compound is tested at concentration of 20µM followed by a 1/5 serial dilution, 10 points in a final concentration of 1% DMSO. When compound potency increases, more dilutions are prepared and/or the top concentration is lowered (*e.g.* 5 µM, 1 µM). The data are expressed as the average IC_{50} from the assays ± standard error of the mean.

[00173] The activity of the compound of the invention against JAK2 has been determined in accordance with the assay described above, thus returning IC_{50} values of 17.3, 11.3, 9.57, 26, and 18.2 nM.

1.4 JAK2 Kd/Ki determination assay

1.4.1. JAK2 Ki determination assay

[00174] For the determination of Ki, different amounts of compound are mixed with the enzyme and the enzymatic reaction is followed as a function of ATP concentration. The Ki is determined by means of double reciprocal plotting of Km vs compound concentration (Lineweaver-Burk plot). 0.0125mU of JAK1 (Invitrogen, PV4210) is used in the assay. The substrate is 25nM Ulight-JAK-1 (Tyr1023) Peptide (Perkin Elmer, TRF0121). The reaction is performed in 25mM HEPES pH7.0, 0.01% Triton X-100, 7.5mM $MgCl_2$, 2mM DTT with varying concentrations of ATP and compound. Phosphorylated substrate is measured using a Europium-labeled anti-phosphotyrosine antibody PT66 (Perkin Elmer, AD0068) as described in 1.2.2. Readout was performed on the envision (Perkin Elmer) with excitation at 320 nm and emission followed at 615 nm and 665 nm.

1.4.2. JAK2 Kd determination assay

[00175] JAK2 (Invitrogen, PV4210) is used at a final concentration of 2.5 nM. The binding experiment is performed in 50mM Hepes pH 7.5, 0.01% Brij-35, 10mM $MgCl_2$, 1mM EGTA using 25nM kinase tracer 236 (Invitrogen, PV5592) and 2 nM Europium-anti-GST (Invitrogen, PV5594) with varying compound concentrations. Detection of tracer is performed according to the manufacturer's procedure.

1.5 JAK3 inhibition assay

[00176] Recombinant human JAK3 catalytic domain (amino acids 795-1124; catalog number 08-046) was purchased from Carna Biosciences. 0.5 ng JAK3 protein was incubated with 2.5 µg polyGT substrate (Sigma catalog number P0275) in kinase reaction buffer (25 mM Tris pH 7.5, 0.5 mM EGTA, 10mM $MgCl_2$, 2.5mM DTT, 0.5 mM Na_3VO_4, 5 mM b-glycerolphosphate, 0.01% Triton X-100, 1 µM non-radioactive ATP, 0.25 µCi 33P-gamma-ATP (Perkin Elmer, catalog number NEG602K001MC) final concentrations) with or without 5µL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 25 µL, in a polypropylene 96-well plate (Greiner, V-bottom). After 45 min at 30 °C, reactions were stopped by adding 25 µL/well of 150 mM phosphoric acid. All of the terminated kinase reaction was transferred to prewashed (75 mM phosphoric acid) 96 well filter plates (Perkin Elmer catalog number 6005177) using a cell harvester (Perkin Elmer). Plates were washed 6 times with 300 µL per well of a 75 mM phosphoric acid solution and the bottom of the plates was sealed. 40 µL/well of Microscint-20 was added, the top of the plates was sealed and readout was performed using the Topcount (Perkin Elmer). Kinase activity was calculated by subtracting counts per min (cpm) obtained in the presence of a positive control inhibitor (10 µM staurosporine) from cpm obtained in the presence of vehicle. The ability of a test compound to inhibit this activity was determined as:

[00177] Percentage inhibition = ((cpm determined for sample with test compound present – cpm determined for sample with positive control inhibitor) divided by (cpm determined in the presence of vehicle – cpm determined for sample with positive control inhibitor)) * 100.

[00178] Dose dilution series were prepared for the compounds enabling the testing of dose-response effects in the JAK3 assay and the calculation of the IC_{50} for the compound. The compound was tested at concentration of 20μM followed by a 1/5 serial dilution, 10 points in a final concentration of 1% DMSO. When compound increased, more dilutions were prepared and/or the top concentration was lowered (*e.g.* 5 μM, 1 μM).

[00179] The activity of the compound of the invention against JAK3 has been determined in accordance with the assay described above, thus returning IC_{50} values of 271, 299, 213, and 428 nM.

1.6 JAK3 Ki determination assay

[00180] For the determination of Ki, different amounts of compound are mixed with the enzyme and the enzymatic reaction is followed as a function of ATP concentration. The Ki is determined by means of double reciprocal plotting of Km *vs* compound concentration (Lineweaver-Burk plot). JAK3 (Carna Biosciences, 08-046) was used at a final concentration of 20 ng/ml. The substrate was Poly(Glu,Tyr)sodium salt (4:1) , MW 20 000 - 50 000 (Sigma, P0275) The reaction was performed in 25mM Tris pH 7.5 , 0.01% Triton X-100 , 0.5mM EGTA, 2.5mM DTT, 0.5mM Na_3VO_4, 5mM b-glycerolphosphate, 10mM $MgCl_2$ with varying concentrations of ATP and compound and stopped by addition of 150 mM phosphoric acid. Measurement of incorporated phosphate into the substrate polyGT was done by loading the samples on a filter plate (using a harvester, Perkin Elmer) and subsequent washing. Incorporated ^{33}P in polyGT is measured in a Topcount scintillation counter after addition of scintillation liquid to the filter plates (Perkin Elmer).

[00181] For example, when tested in this assay, the compound of the invention returned a Ki = 227nM

1.7 TYK2 inhibition assay

[00182] Recombinant human TYK2 catalytic domain (amino acids 871-1187; catalog number 08-147) was purchased from Carna biosciences. 4 ng of TYK2 was incubated with 12.5 μg polyGT substrate (Sigma catalog number P0275) in kinase reaction buffer (25 mM Hepes pH 7.2, 50 mM NaCl, 0.5mM EDTA, 1mM DTT, 5mM $MnCl_2$, 10mM $MgCl_2$, 0.1% Brij-35, 0.1 μM non-radioactive ATP, 0.125 μCi ^{33}P-gamma-ATP (Perkin Elmer, catalog number NEG602K001MC) final concentrations) with or without 5μL containing test compound or vehicle (DMSO, 1% final concentration), in a total volume of 25 μL, in a polypropylene 96-well plate (Greiner, V-bottom). After 90 min at 30 °C, reactions were stopped by adding 25 μL/well of 150 mM phosphoric acid. All of the terminated kinase reaction was transferred to prewashed (75 mM phosphoric acid) 96 well filter plates (Perkin Elmer catalog number 6005177) using a cell harvester (Perkin Elmer). Plates were washed 6 times with 300 μL per well of a 75 mM phosphoric acid solution and the bottom of the plates was sealed. 40 μL/well of Microscint-20 was added, the top of the plates was sealed and readout was performed using the Topcount (Perkin Elmer). Kinase activity was

calculated by subtracting counts per min (cpm) obtained in the presence of a positive control inhibitor (10 μM staurosporine) from cpm obtained in the presence of vehicle. The ability of a test compound to inhibit this activity was determined as:

[00183] Percentage inhibition = ((cpm determined for sample with test compound present – cpm determined for sample with positive control inhibitor) divided by (cpm determined in the presence of vehicle – cpm determined for sample with positive control inhibitor)) * 100.

[00184] Dose dilution series were prepared for the compounds enabling the testing of dose-response effects in the TYK2 assay and the calculation of the IC50 for each compound. Each compound was routinely tested at concentration of 20μM followed by a 1/3 serial dilution, 8 points (20μM - 6.67μM - 2.22μM - 740nM - 247nM - 82nM - 27nM - 9nM) in a final concentration of 1% DMSO. When potency of compound series increased, more dilutions were prepared and/or the top concentration was lowered (*e.g.* 5 μM, 1 μM).

[00185] The activity of the compound of the invention against TYK2 has been determined in accordance with the assay described above, thus returning IC_{50} values of 167, 135, 119, and 157 nM

1.8 TYK2 Kd/Ki determination assay

1.8.1 TYK2 Ki determination assay

[00186] For the determination of Ki, different amounts of compound are mixed with the enzyme and the enzymatic reaction is followed as a function of ATP concentration. The Ki is determined by means of double reciprocal plotting of Km *vs* compound concentration (Lineweaver-Burk plot). TYK2 (Carna Biosciences, 08-147) was used at a final concentration of 160 ng/ml. The substrate was Poly(Glu,Tyr)sodium salt (4:1) , MW 20 000 - 50 000 (Sigma, P0275) The reaction was performed in 25 mM Hepes pH 7.2, 50 mM NaCl, 0.5mM EDTA, 1mM DTT, 5mM MnCl2, 10mM $MgCl_2$, 0.1% Brij-35 with varying concentrations of ATP and compound and stopped by addition of 150 mM phosphoric acid. Measurement of incorporated phosphate into the substrate polyGT was done by loading the samples on a filter plate (using a harvester, Perkin Elmer) and subsequent washing. Incorporated ^{33}P in polyGT is measured in a Topcount scintillation counter after addition of scintillation liquid to the filter plates (Perkin Elmer).

[00187] For example, when tested in this assay, the compound of the invention returned a Ki = 110nM

1.8.2 TYK2 Kd determination assay

[00188] TYK2 (Carna Biosciences, 08-147) is used at a final concentration of 50 nM. The binding experiment is performed in 50mM Hepes pH 7.5, 0.01% Brij-35, 10mM $MgCl_2$, 1mM EGTA using 15nM kinase tracer 236 (Invitrogen, PV5592) and 10 nM Europium-anti-GST (Invitrogen, PV5594) with varying compound concentrations. Detection of tracer is performed according to the manufacturers' procedure.

Example 2. Cellular assays:

2.1 JAK-STAT signalling assay:

[00189] HeLa cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin. HeLa cells were used at 70 % confluence for transfection. 20,000 cells in 87 µL cell culture medium were transiently transfected with 40 ng pSTAT1(2)-luciferase reporter (Panomics), 8 ng of LacZ reporter as internal control reporter and 52 ng of pBSK using 0.32 µL Jet-PEI (Polyplus) as transfection reagent per well in 96-well plate format. After overnight incubation at 37°C, 5% CO_2, transfection medium was removed. 81 µL of DMEM + 1.5% heat inactivated fetal calf serum was added. 9 µL compound at 10 x concentration was added for 60 min and then 10 µL of human OSM (Peprotech) at 33 ng/mL final concentration.

[00190] The compound was tested in duplicate starting from 20 µM followed by a 1/3 serial dilution, 8 doses in total (20 µM – 6.6 µM – 2.2 µM – 740 nM – 250 nM – 82 nM – 27 nM – 9 nM) in a final concentration of 0.2% DMSO.

[00191] After overnight incubation at 37°C, 5% CO_2 cells were lysed by adding 100 µL lysis buffer/well (PBS, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10% Trehalose, 0.05% Tergitol NP9, 0.3% BSA).

[00192] 40 µL of cell lysate was used to read β-galactosidase activity by adding 180 µL β-Gal solution (30µl ONPG 4mg/mL + 150 µL β-Galactosidase buffer (0.06 M Na_2HPO_4, 0.04 M NaH_2PO_4, 1 mM $MgCl_2$)) for 20 min. The reaction was stopped by addition of 50 µL Na_2CO_3 1 M. Absorbance was read at 405 nm.

[00193] Luciferase activity was measured using 40 µL cell lysate plus 40 µL of Steadylite® as described by the manufacturer (Perkin Elmer), on the Envision (Perkin Elmer).

[00194] Omitting OSM was used as a positive control (100% inhibition). As negative control 0.5% DMSO (0% inhibition) was used. The positive and negative controls were used to calculate z' and 'percent inhibition' (PIN) values.

[00195] Percentage inhibition = ((fluorescence determined in the presence of vehicle - fluorescence determined for sample with test compound present) divided by (fluorescence determined in the presence of vehicle – fluorescence determined for sample without trigger)) * 100.

[00196] PIN values were plotted for compounds tested in dose-response and EC_{50} values were derived.

[00197] The activity of the compound of the invention against JAK-STAT has been determined in accordance with the assay described above, thus returning an IC_{50} value of 970 nM.

Example 2.2 OSM/IL-1β signaling Assay

[00198] OSM and IL-1β are shown to synergistically upregulate MMP13 levels in the human chondrosarcoma cell line SW1353. The cells are seeded in 96 well plates at 15,000 cells/well in a volume of 120 µL DMEM (Invitrogen) containing 10% (v/v) FBS and 1% penicillin/streptomycin (InVitrogen) incubated at 37°C 5% CO_2. Cells are preincubated with 15 µL of compound in M199 medium with 2% DMSO 1 hr before triggering with 15 µL OSM and IL-1β to reach 25 ng/mL OSM and 1 ng/mL IL-1β, and MMP13 levels are measured in conditioned medium 48 h after triggering. MMP13 activity is

measured using an antibody capture activity assay. For this purpose, 384 well plates (NUNC, 460518, MaxiSorb black) are coated with 35 µL of a 1.5 µg/mL anti-human MMP13 antibody (R&D Systems, MAB511) solution for 24 hrs at 4°C. After washing the wells 2 times with PBS + 0.05% Tween, the remaining binding sites are blocked with 100 µL 5% non-fat dry milk (Santa Cruz, sc-2325, Blotto) in PBS for 24 hr at 4°C. Next, the wells are washed twice with PBS + 0.05% Tween and 35 µL of 1/10 dilution of culture supernatant containing MMP13 in 100-fold diluted blocking buffer is added and incubated for 4 hr at room temperature. Next the wells are washed twice with PBS + 0.05% Tween followed by MMP13 activation by addition of 35 µL of a 1.5 mM 4-Aminophenylmercuric acetate (APMA) (Sigma, A9563) solution and incubation at 37 °C for 1 hr. The wells are washed again with PBS + 0.05% Tween and 35 µL MMP13 substrate (Biomol, P-126, OmniMMP fluorogenic substrate) is added. After incubation for 24 hrs at 37°C fluorescence of the converted substrate is measured in a Perkin Elmer Wallac EnVision 2102 Multilabel Reader (wavelength excitation: 320 nm, wavelength emission: 405 nm).

[00199] Percentage inhibition = ((fluorescence determined in the presence of vehicle - fluorescence determined for sample with test compound present) divided by (fluorescence determined in the presence of vehicle – fluorescence determined for sample without trigger)) * 100.

Example 2.3 PBL Proliferation assay

[00200] Human peripheral blood lymphocytes (PBL) are stimulated with IL-2 and proliferation is measured using a BrdU incorporation assay. The PBL are first stimulated for 72 hrs with PHA to induce IL-2 receptor, then they are fasted for 24 hrs to stop cell proliferation followed by IL-2 stimulation for another 72 hrs (including 24hr BrdU labeling). Cells are preincubated with test compounds 1 hr before IL-2 addition. Cells are cultured in RPMI 1640 containing 10% (v/v) FBS.

Example 2.4 Whole blood assay (WBA)

2.4.1 IFNα stimulation protocol

[00201] To predict the potency of the test compounds to inhibit JAK1 or JAK2-dependent signaling pathways *in vivo*, a physiologically relevant *in vitro* model was developed using human whole blood. In the WBA assay, blood, drawn from human volunteers who gave informed consent, is treated *ex vivo* with compound (1h) and subsequently stimulated either for 30 min with interferon α (IFNα, JAK1 dependent pathway) or for 2 h with granulocyte macrophage-colony stimulating factor (GM-CSF, JAK2 dependent pathway).

2.4.1.1 Phospho – STAT1 Assay

[00202] For IFNα stimulation, increase in phosphorylation of Signal Transducers and Activators of Transcription 1 (pSTAT1) by INFα in white blood cell extracts is measured using a pSTAT1 ELISA assay. Phosphorylation of Signal Transducer and Activator of Transcription 1 (STAT1) after interferon alpha (IFNα) triggering is a JAK1-mediated event. The Phospho-STAT1 Assay, which is used to

measure Phospho-STAT1 levels in cellular extracts, is developed to assess the ability of a compound to inhibit JAK1-dependent signaling pathways.

[00203] Whole human blood, drawn from human volunteers who gave informed consent, is *ex vivo* treated with compound (1h) and subsequently stimulated for 30 min with IFNα. The increase in phosphorylation of STAT1 by INFα in white blood cell extracts was measured using a phospho-STAT1 ELISA.

[00204] The ACK lysis buffer consists of 0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA. The pH of the buffer is 7.3.

[00205] A 10x cell lysis buffer concentrate (part of the PathScan Phospho-STAT1 (Tyr701) sandwich ELISA kit from Cell Signaling) is diluted 10-fold in H_2O. Proteinase inhibitors are added to the buffer before use.

[00206] 20 µg IFNα is dissolved in 40 µL H_2O to obtain a 500 µg/mL stock solution. The stock solution is stored at -20°C.

[00207] A 3-fold dilution series of the compound is prepared in DMSO (highest concentration: 10 mM). Subsequently, the compound is further diluted in medium (dilution factor dependent on desired final compound concentration).

2.4.1.1.1 Incubation of blood with compound and stimulation with IFNα

[00208] Human blood is collected in heparinized tubes. The blood is divided in aliquots of 392 µL. Afterward, 4 µL of compound dilution is added to each aliquot and the blood samples are incubated for 1 h at 37°C. The IFNα stock solution is diluted 1000-fold in RPMI medium to obtain a 500 ng/mL working solution. 4 µL of the 500 ng/mL work solution is added to the blood samples (final concentration IFNα: 5ng/ml). The samples are incubated at 37°C for 30 min.

2.4.1.1.2 Preparation of cell extracts

[00209] At the end of the stimulation period, 7.6 mL ACK buffer is added to the blood samples to lyse the red blood cells. The samples are mixed by inverting the tubes five times and the reaction is incubated on ice for 5 min. The lysis of the RBC should be evident during this incubation. The cells are pelleted by centrifugation at 300 g, 4°C for 7 min and the supernatant is removed. 10 mL 1x PBS is added to each tube and the cell pellet is resuspended. The samples are centrifuged again for 7 min at 300 g, 4°C. The supernatant is removed and the pellet resuspended in 500 µL of 1x PBS. Then, the cell suspension is transferred to a clean 1.5 mL microcentrifuge tube. The cells are pelleted by centrifugation at 700 g for 5 min at 4°C. The supernatant is removed and the pellet is dissolved in 150 µL cell lysis buffer. The samples are incubated on ice for 15 min. After that, the samples are stored at -80°C until further processing.

2.4.1.1.3 Measurement of STAT1 phosphorylation by ELISA

[00210] The Pathscan Phospho-STAT1 (Tyr701) Sandwich ELISA kit from Cell Signaling (Cat.no: #7234) is used to determine Phospho-STAT1 levels.

[00211] The cellular extracts are thawed on ice. The tubes are centrifuged for 5 min at 16,000 g, 4°C and the cleared lysates are harvested. Meanwhile, the microwell strips from the kit are equilibrated to room temperature and wash buffer is prepared by diluting 20 x wash buffer in H_20. Samples are diluted 2-fold in sample diluent and 100 µL is added to the microwell strips. The strips are incubated overnight at 4°C.

[00212] The following day, the wells are washed 3 times with wash buffer. 100 µL of the detection antibody is added to the wells. The strips are incubated at 37°C for 1 h. Then, the wells are washed 3 times with wash buffer again. 100 µL HRP-linked secondary antibody is added to each well and the samples are incubated at 37°C. After 30 min, the wells are washed 3 times again and 100 µL TMB substrate is added to all wells. When samples turned blue, 100 µL STOP solution is added to stop the reaction. Absorbance is measured at 450 nm.

2.4.1.2 Data analysis

[00213] Inhibition of phosphoSTAT1 induction by IFNα in cell extracts is plotted against the compound concentration and IC_{50} values are derived using Graphpad software. Data are retained if R^2 is larger than 0.8 and the hill slope is smaller than 3.

2.4.1.3 IL-8 ELISA

[00214] For GM-CSF stimulation, increase in interleukin-8 (IL-8) levels in plasma is measured using an IL-8 ELISA assay. Granulocyte macrophage–colony stimulating factor (GM-CSF) - induced interleukin 8 (IL-8) expression is a JAK2-mediated event. The IL-8 ELISA, which can be used to measure IL-8 levels in plasma samples, has been developed to assess the ability of a compound to inhibit JAK2-dependent signaling pathways.

[00215] Whole human blood, drawn from human volunteers who gave informed consent, is *ex vivo* treated with compound (1h) and subsequently stimulated for 2 h with GM-CSF. The increase in IL-8 levels in plasma is measured using an IL-8 ELISA assay.

[00216] 10 µg GM-CSF is dissolved in 100 µL H_2O to obtain a 100 µg/mL stock solution. The stock solution is stored at -20°C.

[00217] A 3-fold dilution series of the test compound is prepared in DMSO (highest concentration: 10 mM). Subsequently, the compound is further diluted in medium (dilution factor dependent on desired final compound concentration).

2.4.1.3.1 Incubation of blood with compound and stimulation with GM-CSF

[00218] Human blood is collected in heparinized tubes. The blood is divided in aliquots of 245 µL. Afterwards, 2.5 µL test compound dilution is added to each aliquot and the blood samples are incubated

for 1 h at 37°C. The GM-CSF stock solution is diluted 100-fold in RPMI medium to obtain a 1 µg/mL work solution. 2.5 µL of the 1 µg/mL work solution is added to the blood samples (final concentration GM-CSF: 10 ng/mL). The samples are incubated at 37°C for 2 h.

2.4.1.3.2 Preparation of plasma samples

[00219] The samples are centrifuged for 15 min at 1,000 g, 4°C. 100 µL of the plasma is harvested and stored at -80°C until further use.

2.4.1.3.3 Measurement of IL-8 levels by ELISA

[00220] The Human IL-8 Chemiluminescent Immunoassay kit from R&D Systems (Cat.no: Q8000B) is used to determine IL-8 levels.

[00221] Wash buffer is prepared by diluting 10 x wash buffer in H_2O. Working glo reagent is prepared by adding 1 part Glo Reagent 1 to 2 parts Glo Reagent B 15 min to 4 h before use.

[00222] 100 µL assay diluent RD1-86 is added to each well. After that, 50 µL of sample (plasma) is added. The ELISA plate is incubated for 2 h at room temperature, 500 rpm. All wells are washed 4 times with wash buffer and 200 µL IL-8 conjugate is added to each well. After incubation for 3 h at room temperature, the wells are washed 4 times with wash buffer and 100 µL working glo reagent is added to each well. The ELISA plate is incubated for 5 min at room temperature (protected from light). Luminescence is measured (0.5 s/well read time).

2.4.2 IL-6 stimulation protocol

[00223] In addition, a flow cytometry analysis is performed to establish JAK1 over JAK2 compound selectivity *ex vivo* using human whole blood. Therefore, blood is taken from human volunteers who gave informed consent. Blood is then equilibrated for 30 min at 37°C under gentle rocking, then aliquoted in Eppendorf tubes. Compound is added at different concentrations and incubated at 37°C for 30 min under gentle rocking and subsequently stimulated for 20 min at 37°C under gentle rocking with interleukin 6 (IL-6) for JAK1-dependent pathway stimulation or GM-CSF for JAK2-dependent pathway stimulation. Phospho-STAT1 and phospho-STAT5 are then evaluated using FACS analysis.

2.4.2.1 *Phospho–STAT1 Assays*

[00224] For IL-6-stimulated increase of Signal Transducers and Activators of Transcription 1 (pSTAT1) phosphorylation in white blood cell, human whole blood, drawn from human volunteers who gave informed consent, is *ex vivo* treated with the compound for 30 min and subsequently stimulated for 20 min with IL-6. The increase in phosphorylation of STAT1 by IL-6 in lymphocytes is measured using anti phospho-STAT1 antibody by FACS.

[00225] The 5X Lyse/Fix buffer (BD PhosFlow, Cat. no 558049) is diluted 5-fold with distilled water and pre-warmed at 37°C. The remaining diluted Lyse/Fix buffer is discarded.

[00226] 10 µg rhIL-6 (R&D Systems, Cat no 206-IL) is dissolved in 1ml of PBS 0.1% BSA to obtain a 10µg/ml stock solution. The stock solution is aliquoted and stored at -80°C.

[00227] A 3-fold dilution series of the compound is prepared in DMSO (10 mM stock solution). Control-treated samples received DMSO instead of compound. All samples are incubated with a 1% final DMSO concentration.

2.4.2.1.1 *Incubation of blood with compound and stimulation with IL-6*

[00228] Human blood is collected in heparinized tubes. The blood is divided in aliquots of 148.5µl. Then, 1.5 µl of the test compound dilution is added to each blood aliquot and the blood samples are incubated for 30 min at 37°C under gentle rocking. IL-6 stock solution (1.5µl) is added to the blood samples (final concentration 10ng/ml) and samples are incubated at 37°C for 20 min under gentle rocking.

2.4.2.1.2 *White blood cell preparation and CD4 labeling*

[00229] At the end of the stimulation period, 3ml of 1X pre-warmed Lyse/Fix buffer is immediately added to the blood samples, vortexed briefly and incubated for 15 min at 37°C in a water bath in order to lyse red blood cells and fix leukocytes, then frozen at -80°C until further use.

[00230] For the following steps, tubes are thawed at 37°C for approximately 20 min and centrifuged for 5 min at 400xg at 4°C. The cell pellet is washed with 3ml of cold 1X PBS, and after centrifugation the cell pellet is resuspended in 100µl of PBS containing 3% BSA. FITC-conjugated anti-CD4 antibody or control FITC-conjugated isotype antibody are added and incubated for 20 min at room temperature, in the dark.

2.4.2.1.3 *Cell permeabilization and labeling with anti Phospho-STAT1 antibody*

[00231] After washing cells with 1X PBS, the cell pellet is resuspended in 100µl of ice-cold 1X PBS and 900µl ice-cold 100% methanol is added. Cells are then incubated at 4°C for 30 min for permeabilization.

[00232] Permeabilized cells are then washed with 1X PBS containing 3% BSA and finally resuspended in 80µl of 1X PBX containing 3% BSA.

[00233] 20µL of PE mouse anti-STAT1 (pY701) or PE mouse IgG2aκ isotype control antibody (BD Biosciences, Cat. no 612564 and 559319, respectively) are added and mixed, then incubated for 30 min at 4°C, in the dark.

[00234] Cells are then washed once with 1X PBS and analyzed on a FACSCanto II flow cytometer (BD Biosciences).

2.4.2.1.4 *Fluorescence analysis on FACSCanto II*

[00235] 50,000 total events are counted and Phospho-STAT1 positive cells are measured after gating on CD4+ cells, in the lymphocyte gate. Data are analyzed using the FACSDiva software and the

percentage inhibition of IL-6 stimulation calculated on the percentage of positive cells for phospho-STAT1 on CD4+ cells.

2.4.2.2 *Phospho–STAT5 Assay*

[00236] For GM-CSF-stimulated increase of Signal Transducers and Activators of Transcription 5 (pSTAT5) phosphorylation in white blood cell, human whole blood, drawn from human volunteers who gave informed consent, is *ex vivo* treated with compound for 30 min and subsequently stimulated for 20 min with GM-CSF. The increase in phosphorylation of STAT5 by GM-CSF in monocytes is measured using an anti phospho-STAT5 antibody by FACS.

[00237] The 5X Lyse/Fix buffer (BD PhosFlow, Cat. no 558049) is diluted 5-fold with distilled water and pre-warmed at 37°C. Remaining diluted Lyse/Fix buffer is discarded.

[00238] 10 µg rhGM-CSF (AbCys S.A., Cat no P300-03) is dissolved in 100µl of PBS 0.1% BSA to obtain a 100µg/ml stock solution. The stock solution is stored aliquoted at -80°C.

[00239] A 3-fold dilution series of the compound is prepared in DMSO (10 mM stock solution). Control-treated samples receive DMSO without the test compound. All samples are incubated with a 1% final DMSO concentration.

2.4.2.2.1 *Incubation of blood with compound and stimulation with GM-CSF*

[00240] Human blood is collected in heparinized tubes. The blood is divided in aliquots of 148.5µl. Then, 1.5 µl of compound dilution is added to each aliquot and the blood samples are incubated for 30 min at 37°C under gentle rocking. GM-CSF stock solution (1.5µl) is added to the blood samples (final concentration 20pg/ml) and samples are incubated at 37°C for 20 min under gentle rocking.

2.4.2.2.2 *White blood cell preparation and CD14 labeling*

[00241] At the end of the stimulation period, 3ml of 1X pre-warmed Lyse/Fix buffer is immediately added to the blood samples, vortexed briefly and incubated for 15 min at 37°C in a water bath in order to lyse red blood cells and fix leukocytes, then frozen at -80°C until further use.

[00242] For the following steps, tubes are thawed at 37°C for approximately 20 min and centrifuged for 5 min at 400xg at 4°C. The cell pellet is washed with 3ml of cold 1X PBS, and after centrifugation the cell pellet is resuspended in 100µl of PBS containing 3% BSA. FITC mouse anti-CD14 antibody (BD Biosciences, Cat. no 345784) or control FITC mouse IgG2bκ isotype antibody (BD Biosciences, Cat. no 555057) are added and incubated for 20 min at room temperature, in the dark.

2.4.2.2.3 *Cell permeabilization and labeling with anti phospho-STAT5 antibody*

[00243] After washing cells with 1X PBS, the cell pellet is resuspended in 100µl of ice-cold 1X PBS and 900µl of ice-cold 100% methanol is added. Cells are then incubated at 4°C for 30 min for permeabilization.

[00244] Permeabilized cells are then washed with 1X PBS containing 3% BSA and finally resuspended in 80µl of 1X PBX containing 3% BSA.

[00245] 20µL of PE mouse anti-STAT5 (pY694) or PE mouse IgG1κ isotype control antibody (BD Biosciences, Cat. no 612567and 554680, respectively) are added, mixed then incubated for 30 min at 4°C, in the dark.

[00246] Cells are then washed once with 1X PBS and analyzed on a FACSCanto II flow cytometer (BD Biosciences).

2.4.2.2.4 *Fluorescence analysis on FACSCanto II*

[00247] 50,000 total events are counted and Phospho-STAT5 positive cells are measured after gating on CD14+ cells. Data are analyzed using the FACSDiva software and correspond to the percentage of inhibition of GM-CSF stimulation calculated on the percentage of positive cells for phosphor-STAT5 on CD14+ cells.

Example 3. *In vivo* models

Example 3.1 CIA model

3.1.1 Materials

[00248] Complete Freund's adjuvant (CFA) and Incomplete Freund's adjuvant (IFA) are purchased from Difco. Bovine collagen type II (CII), lipopolysaccharide (LPS), and Enbrel® is obtained from Chondrex (Isle d'Abeau, France); Sigma (P4252, L'Isle d'Abeau, France), Whyett (25mg injectable syringe, France) Acros Organics (Palo Alto, CA), respectively. All other reagents used are of reagent grade and all solvents are of analytical grade.

3.1.2 Animals

[00249] Dark Agouti rats (male, 7-8 weeks old) are obtained from Harlan Laboratories (Maison-Alfort, France). Rats are kept on a 12 hr light/dark cycle (0700 - 1900). Temperature is maintained at 22°C, and food and water are provided *ad libitum*.

3.1.3 Collagen induced arthritis (CIA)

[00250] One day before the experiment, CII solution (2 mg/mL) is prepared with 0.05 M acetic acid and stored at 4°C. Just before the immunization, equal volumes of adjuvant (IFA) and CII are mixed by a homogenizer in a pre-cooled glass bottle in an ice water bath. Extra adjuvant and prolonged homogenization may be required if an emulsion is not formed. 0.2 mL of the emulsion is injected intradermally at the base of the tail of each rat on day 1, a second booster intradermal injection (CII solution at 2 mg/mL in CFA 0.1 mL saline) is performed on day 9. This immunization method is modified from published methods (Sims *et al*, 2004; Jou *et al.*, 2005).

3.1.4 Study design

[00251] The therapeutic effects of the compounds are tested in the rat CIA model. Rats are randomly divided into equal groups and each group contained 10 rats. All rats are immunized on day 1 and boosted on day 9. Therapeutic dosing lasts from day 16 to day 30. The negative control group is treated with vehicle (MC 0.5%) and the positive control group with Enbrel® (10 mg/kg, 3x week., s.c.). A compound of interest is typically tested at 3 doses, *e.g.* 3, 10, 30 mg/kg, p.o.

3.1.5 Clinical assessment of arthritis

[00252] Arthritis is scored according to the method of Khachigian 2006, Lin et al 2007 and Nishida *et al.* 2004). The swelling of each of the four paws is ranked with the arthritic score as follows: 0-no symptoms; 1-mild, but definite redness and swelling of one type of joint such as the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2-moderate redness and swelling of two or more types of joints; 3-severe redness and swelling of the entire paw including digits; 4-maximally inflamed limb with involvement of multiple joints (maximum cumulative clinical arthritis score 16 per animal) (Nishida *et al.*, 2004).

[00253] To permit the meta-analysis of multiple studies the clinical score values are normalised as follows:

[00254] *AUC of clinical score (AUC score)*: The area under the curve (AUC) from day 1 to day 14 is calculated for each individual rat. The AUC of each animal is divided by the average AUC obtained for the vehicle in the study from which the data on that animal is obtained and multiplied by 100 (*i.e.* the AUC was expressed as a percentage of the average vehicle AUC per study).

[00255] *Clinical score increase from day 1 to day 14 (End point score)*: The clinical score difference for each animal is divided by the average clinical score difference obtained for the vehicle in the study from which the data on that animal is obtained and multiplied by 100 (*i.e.* the difference is expressed as a percentage of the average clinical score difference for the vehicle per study).

3.1.6 Change in body weight (%) after onset of arthritis

[00256] Clinically, body weight loss is associated with arthritis (Shelton *et al.*, 2005; Argiles *et al.*, 1998; Rall, 2004; Walsmith *et al.*, 2004). Hence, changes in body weight after onset of arthritis can be used as a non-specific endpoint to evaluate the effect of therapeutics in the rat model. The change in body weight (%) after onset of arthritis is calculated as follows:

[00257] Mice: $$\frac{\text{Body Weight}_{(\text{week6})} - \text{Body Weight}_{(\text{week5})}}{\text{Body Weight}_{(\text{week5})}} \times 100\%$$

[00258] Rats: $$\frac{\text{Body Weight}_{(\text{week4})} - \text{Body Weight}_{(\text{week3})}}{\text{Body Weight}_{(\text{week3})}} \times 100\%$$

3.1.7 Radiology

[00259] X-ray photos are taken of the hind paws of each individual animal. A random blind identity number is assigned to each of the photos, and the severity of bone erosion is ranked by two independent scorers with the radiological Larsen's score system as follows: 0- normal with intact bony outlines and normal joint space; 1- slight abnormality with any one or two of the exterior metatarsal bones showing slight bone erosion; 2-definite early abnormality with any three to five of the exterior metatarsal bones showing bone erosion; 3-medium destructive abnormality with all the exterior metatarsal bones as well as any one or two of the interior metatarsal bones showing definite bone erosions; 4-severe destructive abnormality with all the metatarsal bones showing definite bone erosion and at least one of the inner metatarsal joints completely eroded leaving some bony joint outlines partly preserved; 5-mutilating abnormality without bony outlines. This scoring system is a modification from Salvemini *et al.*, 2001; Bush *et al.*, 2002; Sims *et al.*, 2004; Jou *et al.*, 2005.

3.1.8 Histology

[00260] After radiological analysis, the hind paws of mice are fixed in 10% phosphate-buffered formalin (pH 7.4), decalcified with rapid bone decalcifiant for fine histology (Laboratories Eurobio) and embedded in paraffin. To ensure extensive evaluation of the arthritic joints, at least four serial sections (5 µm thick) are cut and each series of sections are 100 µm in between. The sections are stained with hematoxylin and eosin (H&E). Histologic examinations for synovial inflammation and bone and cartilage damage are performed using a double blind protocol. In each paw, four parameters are assessed using a four-point scale. The parameters are cell infiltration, pannus severity, cartilage erosion and bone erosion. Scoring is performed accordingly, as follows: 1-normal, 2-mild, 3-moderate, 4-marked. The four scores are summed together and represented as an additional score, namely the 'RA total score'.

3.1.9 Micro-computed tomography (µCT) analysis of calcaneus (heel bone):

[00261] Bone degradation observed in RA occurs especially at the cortical bone and can be revealed by µCT analysis (Sims NA *et al.*, Arthritis Rheum. 50 (2004) 2338-2346: Targeting osteoclasts with zoledronic acid prevents bone destruction in collagen-induced arthritis; Oste L *et al.*, ECTC Montreal 2007: A high throughput method of measuring bone architectural disturbance in a murine CIA model by micro-CT morphometry). After scanning and 3D volume reconstruction of the calcaneus bone, bone degradation is measured as the number of discrete objects present per slide, isolated *in silico* perpendicular to the longitudinal axis of the bone. The more the bone is degraded, the more discrete objects are measured. 1000 slices, evenly distributed along the calcaneus (spaced by about 10.8 µm), are analyzed.

3.1.10 Steady State PK

[00262] At day 7 or 11, blood samples are collected at the retro-orbital sinus with lithium heparin as anti-coagulant at the following time points: predose, 1, 3 and 6 hrs. Whole blood samples are centrifuged and the resulting plasma samples are stored at -20°C pending analysis. Plasma concentrations of each test

compound are determined by an LC-MS/MS method in which the mass spectrometer is operated in positive electrospray mode. Pharmacokinetic parameters are calculated using Winnonlin® (Pharsight®, United States) and it is assumed that the predose plasma levels are equal to the 24 hrs plasma levels.

3.1.11 Results

[00263] The compound of the invention exhibited statistically significant improvements in the normalized clinical score values (calculated as AUC or as the difference from day 1 to day14) at a dose of 0.1 mg/kg.

Example 3.2 Septic shock model

[00264] Injection of lipopolysaccharide (LPS) induces a rapid release of soluble tumour necrosis factor (TNF-alpha) into the periphery. This model is used to analyse prospective blockers of TNF release *in vivo*.

[00265] Six BALB/cJ female mice (20 g) per group are treated at the intended dosing once, po. Thirty min later, LPS (15 µg/kg; *E. Coli* serotype 0111:B4) is injected ip. Ninety min later, mice are euthanized and blood is collected. Circulating TNF alpha levels are determined using commercially available ELISA kits. Dexamethasone (5 µg/kg) is used as a reference anti-inflammatory compound.

Example 3.3 MAB model

[00266] The MAB model allows a rapid assessment of the modulation of an RA-like inflammatory response by therapeutics (Kachigian LM. Nature Protocols (2006) 2512-2516: Collagen antibody-induced arthritis). DBA/J mice are injected i.v. with a cocktail of mAbs directed against collagen II. One day later, compound treatment is initiated (vehicle: 10% (v/v) HPβCD). Three days later, mice receive an i.p. LPS injection (50 µg/mouse), resulting in a fast onset of inflammation. Compound treatment is continued until 10 days after the mAb injection. Inflammation is read by measuring paw swelling and recording the clinical score of each paw. The cumulative clinical arthritis score of four limbs is presented to show the severity of inflammation. A scoring system is applied to each limb using a scale of 0–4, with 4 being the most severe inflammation.

0 Symptom free

1 Mild, but definite redness and swelling of one type of joint such as the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits

2 Moderate redness and swelling of two or more types of joints

3 Severe redness and swelling of the entire paw including digits

4 Maximally inflamed limb with involvement of multiple joints

Example 3.4 Oncology models

[00267] *In vivo* models to validate efficacy of small molecules towards JAK2-driven myleoproliferative diseases are described by Wernig *et al.* Cancer Cell 13, 311, 2008 and Geron *et al.* Cancer Cell 13, 321, 2008.

Example 3.5 Mouse IBD model

[00268] *In vitro* and *in vivo* models to validate efficacy of small molecules towards IBD are described by Wirtz *et al.* 2007.

Example 3.6 Mouse Asthma model

[00269] *In vitro* and *in vivo* models to validate efficacy of small molecules towards asthma are described by Nials *et al.*, 2008; Ip *et al.* 2006; Pernis *et al.*, 2002; Kudlacz *et al.*, 2008.

Example 4: Pharmacokinetic, DMPK and Toxicity Assays

Example 4.1 Thermodynamic solubility

[00270] A solution of 1 mg/mL of the test compound is prepared in a 0.2M phosphate buffer pH 7.4 or a 0.1M citrate buffer pH 3.0 at room temperature in a glass vial.

[00271] The samples are rotated in a Rotator drive STR 4 (Stuart Scientific, Bibby) at speed 3.0 at room temperature for 24 hrs.

[00272] After 24 hrs, 800µL of the sample is transferred to an eppendorf tube and centrifuged 5 min at 14000rpm. 200 µL of the supernatant of the sample is then transferred to a MultiscreenR Solubility Plate (Millipore, MSSLBPC50) and the supernatant is filtered (10-12" Hg) with the aid of a vacuum manifold into a clean Greiner polypropylene V-bottom 96 well plate (Cat no.651201). 5 µL of the filtrate is diluted into 95 µL (F20) of the same buffer used to incubate in the plate containing the standard curve (Greiner, Cat no.651201).

[00273] The standard curve for the compound is prepared freshly in DMSO starting from a 10mM DMSO stock solution diluted factor 2 in DMSO (5000µM) and then further diluted in DMSO up to 19.5µM. 3µl of the dilution series as from 5000µM is then transferred to a 97µL acetonitrile-buffer mixture (50/50). The final concentration range is 2.5 to 150 µM.

[00274] The plate is sealed with sealing mats (MA96RD-04S, Kinesis, Cambs, PE19 8YX, UK) and samples are measured at room temperature on LCMS (ZQ 1525 from Waters) under optimized conditions using Quanoptimize to determine the appropriate mass of the molecule.

[00275] The samples are analyzed on LCMS with a flow rate of 1mL/min. Solvent A is 15mM ammonia and solvent B is acetonitrile. The sample is run under positive ion spray on an XBridge C18 3.5µM (2.1 x 30mm) column, from Waters. The solvent gradient has a total run time of 2 min and ranges from 5% B to 95% B.

[00276] Peak areas are analyzed with the aid of Masslynx software package and peak areas of the samples are plotted against the standard curve to obtain the solubility of the compound.

[00277] Solubility values are reported in µM or µg/mL.

Example 4.2 Aqueous Solubility

4.2.1 Aqueous Solubility2% DMSO procedure

[00278] Starting from a 10 mM stock in DMSO, a serial dilution of the compound is prepared in DMSO. The dilution series is transferred to a 96 NUNC Maxisorb plate F-bottom (Cat no. 442404) and 0.2M phosphate buffer pH7.4 or 0.1M citrate buffer pH 3.0 at room temperature is added.

[00279] The final concentration ranges from 200µM to 2.5µM in 5 equal dilution steps. The final DMSO concentration does not exceed 2%. 200µM Pyrene is added to the corner points of each 96 well plate and serves as a reference point for calibration of Z-axis on the microscope.

[00280] The assay plates are sealed and incubated for 1 hr at 37°C while shaking at 230rpm. The plates are then scanned under a white light microscope, yielding individual pictures of the precipitate per concentration. The precipitate is analyzed and converted into a number which is plotted onto a graph. The first concentration at which the compound appears completely dissolved is the concentration that is reported below, however the true concentration will lie somewhere between this concentration and one dilution step higher.

[00281] Solubility values mesured according to this protocol are reported in µg/mL.

4.2.2 Aqueous Solubility3% DMSO procedure

[00282] Starting from a 10mM stock in DMSO, a serial dilution of the compound is prepared in DMSO. The dilution series is transferred to a 96 NUNC Maxisorb plate F-bottom (Cat no. 442404) and 0.1M phosphate buffer pH7.4 or 0.1M citrate buffer pH3.0 at room temperature is added.

[00283] The final concentration will range from 300µM to 18.75µM in 5 equal dilution steps. The final DMSO concentration does not exceed 3%. 200µM Pyrene is added to the corner points of each 96 well plate and serves as a reference point for calibration of Z-axis on the microscope.

[00284] The assay plates are sealed and incubated for 1 h at 37°C while shaking at 230rpm. The plates are then scanned under a white light microscope, yielding individual pictures of the precipitate per concentration. The precipitate is analyzed and converted into a number with a software tool which can be plotted onto a graph. The first concentration at which the compound appears completely dissolved is the concentration reported; however the true concentration lies somewhere between this concentration and one dilution step higher.

[00285] Solubility values mesured according to this protocol are reported in µg/mL.

Example 4.3 Plasma Protein Binding (Equilibrium Dialysis)

[00286] A 10mM stock solution of the compound in DMSO is diluted with a factor 5 in DMSO. This solution is further diluted in freshly thawed human, rat, mouse or dog plasma (BioReclamation INC) with a final concentration of 10µM and final DMSO concentration of 0.5% (5.5µL in 1094.5µL plasma in a PP-Masterblock 96well (Greiner, Cat no. 780285))

[00287] A Pierce Red Device plate with inserts (ThermoScientific, Cat no. 89809) is prepared and filled with 750µL PBS in the buffer chamber and 500µL of the spiked plasma in the plasma chamber. The plate is incubated for 4 hrs at 37°C while shaking at 230rpm. After incubation, 120µL of both chambers is transferred to 360µL acetonitrile in a 96-well round bottom, PP deep-well plates (Nunc, Cat no. 278743) and sealed with an aluminum foil lid. The samples are mixed and placed on ice for 30 min. This plate is then centrifuged 30 min at 1200rcf at 4°C and the supernatant is transferred to a 96 v-bottom PP plate (Greiner, 651201) for analysis on LCMS.

[00288] The plate is sealed with sealing mats (MA96RD-04S) of Kinesis, Cambs, PE19 8YX, UK and samples are measured at room temperature on LCMS (ZQ 1525 from Waters) under optimized conditions using Quanoptimize to determine the appropriate mass of the molecule.

[00289] The samples are analyzed on LCMS with a flow rate of 1ml/min. Solvent A is 15mM ammonia and solvent B is acetonitrile. The sample is run under positive ion spray on an XBridge C18 3.5µM (2.1 x 30mm) column, from Waters. The solvent gradient has a total run time of 2 min and ranges from 5% B to 95% B.

[00290] Peak area from the compound in the buffer chamber and the plasma chamber are considered to be 100% compound. The percentage bound to plasma is derived from these results and is reported as percentage bound to plasma.

[00291] The solubility of the compound in the final test concentration in PBS is inspected by microscope to indicate whether precipitation is observed or not.

Example 4.4 Microsomal stability

4.4.1 Microsomal stability 1h incubation procedure

[00292] A 10mM stock solution of compound in DMSO is diluted 1000 fold in a 182 mM phosphate buffer pH7.4 in a 96 deep well plate (Greiner, Cat no.780285) and pre-incubated at 37°C.

[00293] 40µL of deionised water is added to a well of a polypropylene Matrix 2D barcode labelled storage tube (Thermo Scientific) and pre-incubated at 37°C.

[00294] A Glucose-6-phophate-dehydrogenase (G6PDH) working stock solution is prepared in 182mM phosphate buffer pH7.4 and placed on ice before use. A co-factor containing $MgCl_2$, glucose-6-phosphate and NADP+ is prepared in deionised water and placed on ice before use.

[00295] A final working solution containing liver microsomes (Xenotech) of a species of interest (human, mouse, rat, dog), previously described G6PDH and co-factors is prepared and this mix is incubated for no longer than 20 min at room temperature.

[00296] 30µL of the pre-heated compound dilution is added to 40µL of pre-heated water in the Matrix tubes and 30µL of the microsomal mix is added. Final reaction concentrations are 3µM compound, 1mg microsomes, 0.4U/mL GDPDH, 3.3mM $MgCl_2$, 3.3mM glucose-6-phosphate and 1.3mM NADP+.

[00297] To measure percentage remaining of compound at time zero MeOH or ACN is added (1:1) to the well before adding the microsomal mix. The plates are sealed with Matrix Sepra sealsTM (Matrix, Cat. No.4464) and shaken for a few seconds ensure complete mixing of all components.

[00298] The samples which are not stopped are incubated at 37°C, 300rpm and after 1 hr of incubation the reaction is stopped with MeOH or ACN (1:1).

[00299] After stopping the reaction the samples are mixed and placed on ice for 30 min to precipitate the proteins. The plates are then centrifuged 30 min at 1200rcf at 4°C and the supernatant is transferred to a 96 v-bottom PP plate (Greiner, 651201) for analysis on LCMS.

[00300] These plates are sealed with sealing mats (MA96RD-04S) of Kinesis, Cambs, PE19 8YX, UK and samples are measured at room temperature on LCMS (ZQ 1525 from Waters) under optimized conditions using Quanoptimize to determine the appropriate mass of the parent molecule.

[00301] The samples are analyzed on LCMS with a flow rate of 1mL/min. Solvent A is 15mM ammonia and solvent B is methanol or acetonitrile, depending on the stop solution used. The samples are run under positive ion spray on an XBridge C18 3.5µM (2.1 x 30mm) column, from Waters. The solvent gradient has a total run time of 2 min and ranges from 5% B to 95% B.

[00302] Peak area from the parent compound at time 0 is considered to be 100% remaining. The percentage remaining after 1 hr incubation is calculated from time 0 and is calculated as the percentage remaining. The solubility of the compound in the final test concentration in buffer is inspected by microscope and results are reported.

[00303] The data on microsomal stability are expressed as a percentage of the total amount of compound remaining after 60 min.

4.4.2 Microsomal stability 30mn incubation procedure

[00304] A 10mM stock solution of compound in DMSO is diluted to 6µM in a 105mM phosphate buffer, pH 7.4 in a 96 deep well plate (Greiner, Cat no.780285) and pre-warmed at 37°C.

[00305] A Glucose-6-phosphate-dehydrogenase (G6PDH, Roche, 10127671001) working stock solution of 700U/ml is diluted with a factor 1:700 in a 105mM phosphate buffer, pH7.4. A co-factor mix containing 0.528M $MgCl_2.6H_2O$ (Sigma, M2670), 0.528M glucose-6-phosphate (Sigma, G-7879) and 0.208M NADP+ (Sigma,N-0505) is diluted with a factor 1:8in a 105mM phosphate buffer, pH7.4.

[00306] A working solution is made containing 1 mg/ml liver microsomes (Provider, Xenotech) of the species of interest (human, mouse, rat, dog …), 0.8U/ml G6PDH and co-factor mix (6.6mM $MgCl_2$, 6.6mM glucose-6-phosphate, 2.6mM NADP+). This mix is pre-incubated for 15 min, but never more than 20 min, at room temperature.

[00307] After pre-incubation, compound dilution and the mix containing the microsomes, are added together in equal amount and incubated for 30 min at 300 rpm. For the time point of 0 min, two volumes of methanol are added to the compound dilution before the microsome mix is added. The final concentration during incubation are: 3µM test compound or control compound, 0.5 mg/ml microsomes, 0.4U/ml G6PDH, 3.3mM $MgCl_2$, 3.3mM glucose-6-phosphate and 1.3mM NaDP+.

[00308] After 30 min of incubation, the reaction is stopped with 2 volumes of methanol.

[00309] Of both time points, samples are mixed, centrifuged and the supernatant is harvested for analysis on LC-MS/MS. The instrument responses (*i.e.* peak heights) are referenced to the zero time-point

samples (as 100%) in order to determine the percentage of compound remaining. Standard compounds Propanolol and Verapamil are included in the assay design.

[00310] The data on microsomal stability are expressed as a percentage of the total amount of compound remaining after 30 min.

Example 4.5 Caco2 Permeability

[00311] Bi-directional Caco-2 assays are performed as described below. Caco-2 cells are obtained from European Collection of Cell Cultures (ECACC, cat 86010202) and used after a 21 day cell culture in 24-well Transwell plates (Fisher TKT-545-020B).

[00312] $2x10^5$ cells/well are seeded in plating medium consisting of DMEM + GlutaMAXI + 1% NEAA + 10% FBS (FetalClone II) + 1% Pen/Strep. The medium is changed every 2 – 3 days.

[00313] Test and reference compounds (propranolol and rhodamine123 or vinblastine, all purchased from Sigma) are prepared in Hanks' Balanced Salt Solution containing 25 mM HEPES (pH7.4) and added to either the apical (125µL) or basolateral (600µL) chambers of the Transwell plate assembly at a concentration of 10 µM with a final DMSO concentration of 0.25%.

[00314] 50µM Lucifer Yellow (Sigma) is added to the donor buffer in all wells to assess integrity of the cell layers by monitoring Lucifer Yellow permeation. As Lucifer Yellow (LY) cannot freely permeate lipophilic barriers, a high degree of LY transport indicates poor integrity of the cell layer.

[00315] After a 1 hr incubation at 37°C while shaking at an orbital shaker at 150rpm, 70µL aliquots are taken from both apical (A) and basal (B) chambers and added to 100µLl 50:50 acetonitrile:water solution containing analytical internal standard (0.5µM carbamazepine) in a 96 well plate.

[00316] Lucifer yellow is measured with a Spectramax Gemini XS (Ex 426nm and Em 538nm) in a clean 96 well plate containing 150µL of liquid from basolateral and apical side.

[00317] Concentrations of compound in the samples are measured by high performance liquid-chromatography/mass spectroscopy (LC-MS/MS).

[00318] Apparent permeability (P_{app}) values are calculated from the relationship:

$$P_{app} = [compound]_{acceptor\ final} \times V_{acceptor} / ([compound]_{donor\ initial} \times V_{donor}) / T_{inc} \times V_{donor} / \text{surface area} \times 60 \times 10^{-6}\ cm/s$$

V = chamber volume

T_{inc} = incubation time.

Surface area = $0.33cm^2$

[00319] The Efflux ratios, as an indication of active efflux from the apical cell surface, are calculated using the ratio of P_{app} B>A/ P_{app} A>B.

[00320] The following assay acceptance criteria are used:

Propranolol: P_{app} (A>B) value $\geq 20(\times 10^{-6}$ cm/s)

Rhodamine 123 or Vinblastine: P_{app} (A>B) value < 5 ($\times 10^{-6}$ cm/s) with Efflux ratio ≥ 5.

Lucifer yellow permeability: ≤ 100 nm/s

Example 4.6 Pharmacokinetic study in rodents

4.6.1 Animals

[00321] Sprague-Dawley rats (male, 5-6 weeks old) are obtained from Janvier (France). Rats are acclimatized for at least 7 days before treatment and were kept on a 12 hr light/dark cycle (0700 - 1900). Temperature is maintained at approximately 22°C, and food and water are provided *ad libitum*. Two days before administration of the test compounds, rats undergo surgery to place a catheter in the jugular vein under isoflurane anesthesia. After the surgery, rats are housed individually. Rats are deprived of food for at least 16 h before oral dosing and 6 h after. Water is provided *ad libitum.*

4.6.2 Pharmacokinetic study

[00322] Compounds are formulated in PEG200/physiological saline (60/40) for the intravenous route and in 0.5% methylcellulose and 10% hydroxylpropyl-β-cyclodextrine pH3 for the oral route. Test compounds are orally dosed as a single esophageal gavage at 5 mg/kg under a dosing volume of 5 ml/kg and intravenously dosed as a bolus via the caudal vein at 1 mg/kg under a dosing volume of 5 mL/kg. Each group consisted of 3 rats. Blood samples are collected via the jugular vein with lithium heparin as anti-coagulant at the following time points: 0.05, 0.25, 0.5, 1, 3, 5 and 8 hrs (intravenous route), and 0.25, 0.5, 1, 3, 5, 8 and 24 hrs (oral route). Whole blood samples are centrifuged at 5000 rpm for 10 min and the resulting plasma samples are stored at -20°C pending analysis.

4.6.3 Quantification of compound levels in plasma

[00323] Plasma concentrations of each test compound are determined by an LC-MS/MS method in which the mass spectrometer is operated in positive electrospray mode.

4.6.4 Determination of pharmacokinetic parameters

[00324] Pharmacokinetic parameters may be calculated using Winnonlin® (Pharsight®, United States).

Example 4.7 7-Day rat toxicity study

[00325] A 7-day oral toxicity study with test compounds is performed in Sprague-Dawley male rats to assess their toxic potential and toxicokinetics, at daily doses of 100, 300 and 500 mg/kg/day, by gavage, at the constant dosage-volume of 5 mL/kg/day.

[00326] The test compounds are formulated in 30% (v/v) HPβCD in purified water. Each group includes 5 principal male rats as well as 3 satellite animals for toxicokinetics. A fourth group is given 30% (v/v) HPβCD in water only, at the same frequency, dosage volume and by the same route of administration, and acted as the vehicle control group.

[00327] The goal of the study is to determine the lowest dose that resulted in no adverse events being identified (no observable adverse effect level - NOAEL).

Example 4.8 Hepatocyte stability

[00328] Models to evaluate metabolic clearance in hepatocyte are described by McGinnity *et al.* Drug Metabolism and Disposition **2008**, 32, *11*, 1247.

Example 4.9 Liability for QT prolongation

[00329] Potential for QT prolongation is assessed in the hERG patch clamp assay.

Conventional whole-cell patch-clamp

[00330] Whole-cell patch-clamp recordings are performed using an EPC10 amplifier controlled by Pulse v8.77 software (HEKA). Series resistance is typically less than 10 MΩ and compensated by greater than 60%, recordings are not leak subtracted. Electrodes are manufactured from GC150TF pipette glass (Harvard).

[00331] The external bathing solution contains: 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 5 mM Glucose, 10 mM HEPES, pH 7.4.

[00332] The internal patch pipette solution contains: 100mM Kgluconate, 20 mM KCl, 1mM $CaCl_2$, 1 mM $MgCl_2$, 5mM Na_2ATP, 2mM Glutathione, 11 mM EGTA, 10 mM HEPES, pH 7.2.

[00333] Drugs are perfused using a Biologic MEV-9/EVH-9 rapid perfusion system.

[00334] All recordings are performed on HEK293 cells stably expressing hERG channels. Cells are cultured on 12 mm round coverslips (German glass, Bellco) anchored in the recording chamber using two platinum rods (Goodfellow). hERG currents are evoked using an activating pulse to +40 mV for 1000 ms followed by a tail current pulse to –50 mV for 2000 ms, holding potential is -80 mV. Pulses are applied every 20s and all experiments are performed at room temperature.

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[00336] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

[00337] It will be appreciated by those skilled in the art that the foregoing descriptions are exemplary and explanatory in nature, and intended to illustrate the invention and its preferred embodiments. From the foregoing description, various modifications and changes in the compositions and methods of this invention will occur to those skilled in the art, and may be made without departing from the spirit of the invention. All such modifications coming within the scope of the appended claims are intended to be included therein.

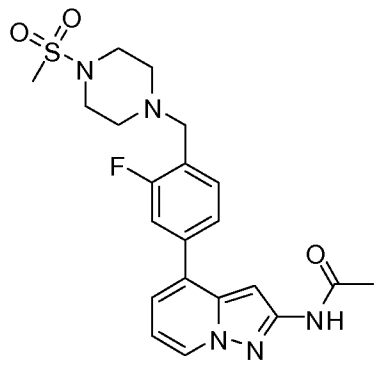
[00338] It should be understood that factors such as the differential cell penetration capacity of the various compounds can contribute to discrepancies between the activity of the compounds in the *in vitro* biochemical and cellular assays.

[00339] At least some of the chemical names of compound of the invention as given and set forth in this application, may have been generated on an automated basis by use of a commercially available chemical naming software program, and have not been independently verified. Representative programs performing this function include the Lexichem naming tool sold by Open Eye Software, Inc. and the Autonom Software tool sold by MDL, Inc. In the instance where the indicated chemical name and the depicted structure differ, the depicted structure will control.

[00340] Chemical structures shown herein were prepared using either ChemDraw® or ISIS® /DRAW. Any open valency appearing on a carbon, oxygen or nitrogen atom in the structures herein indicates the presence of a hydrogen atom. Where a chiral center exists in a structure but no specific stereochemistry is shown for the chiral center, both enantiomers associated with the chiral structure are encompassed by the structure.

WHAT IS CLAIMED IS:

1. The compound according to Formula I:



I

or a pharmaceutically acceptable salt, or a solvate, or a solvate of the pharmaceutically acceptable salt, or a biologically active metabolite thereof.

2. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a pharmaceutically effective amount of a compound or pharmaceutically acceptable salt thereof, according to claim 1.

3. The pharmaceutical composition according to claim 2 comprising a further therapeutic agent.

4. The compound or pharmaceutically acceptable salt thereof, according to claim 1, or the pharmaceutical composition according to claim 2 or 3, for use in medicine.

5. The use of the compound or pharmaceutically acceptable salt thereof, according to claim 1, or the pharmaceutical composition according to claim 2 or 3, in the manufacture of a medicament.

6. A compound or pharmaceutically acceptable salt according to claim 1, or the pharmaceutical composition according to claim 2 or 3, for use in the treatment or prophylaxis of allergy, inflammatory conditions, autoimmune diseases, proliferative diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

7. The use of the compound or pharmaceutically acceptable salt thereof, according to claim 1, or the pharmaceutical composition according to claim 2 or 3, in the manufacture of a medicament for the treatment or prophylaxis of allergy, inflammatory conditions, autoimmune diseases, proliferative diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

8. A method for the treatment or prophylaxis of allergy, inflammatory conditions, autoimmune diseases, proliferative diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons, comprising administering an amount of a compound or pharmaceutically acceptable salt thereof according to any one of claims 1, or the pharmaceutical composition according to claim 2 or 3, sufficient to effect said treatment or prophylaxis.

9. The method according to claim 8, wherein the compound or pharmaceutically acceptable salt thereof according to claim 1, or the pharmaceutical composition according to claim 2, is administered in combination with a further therapeutic agent.

10. The pharmaceutical composition according to claim 3, or the method according to claim 9, wherein the further therapeutic agent is an agent for the treatment or prophylaxis of allergy, inflammatory conditions, autoimmune diseases, proliferative diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformations, and/or diseases associated with hypersecretion of IL6 or interferons.

11. The compound for use according to claim 6, or the use of the compound according to claim 7, the method according to 8, or the pharmaceutical composition according to claim 10, wherein the autoimmune disease is selected from COPD, asthma, systemic lupus erythematosis, type I diabetes mellitus, psoriatic arthritis, ankylosing spondylitis, juvenile ideopathic arthritis, and inflammatory bowel disease.

12. The compound for use according to claim 6, or the use of the compound according to claim 7, the method according to 8, or the pharmaceutical composition according to claim 10, wherein the inflammatory disease is selected from rheumatoid arthritis, osteoarthritis, asthma and inflammatory bowel diseases.