COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS THEREOF FOR THE TREATMENT OF DISEASES

FIELD OF THE INVENTION

[0001] The present invention relates to compounds, methods for the production of the compounds of the invention, pharmaceutical compositions comprising the compounds of the invention, uses and methods for the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases by administering the compounds of the invention. In particular, the compounds of the invention may inhibit Salt-Inducible Kinases ("SIK" kinases).

BACKGROUND OF THE INVENTION

[0002] Protein kinases belong to a large family of structurally related enzymes which are responsible for the control of a wide variety of cellular signal transduction processes. In particular, they have been shown to be key regulators in cellular functions including for example proliferation, metabolism, and apoptosis. Consequently, defective control of protein phosphorylation which leads to uncontrolled signaling is involved in a number of diseases, including for example, inflammation, allergies, cancer, autoimmune diseases, CNS disorders, and angiogenesis.

[0003] In healthy individuals inflammation is self-limiting, and resolution is controlled by the release of anti-inflammatory mediators and cytokines, such as interleukin-10 (IL-10), produced by "suppressive" or "regulatory" cells, which are produced as part of a negative feedback loop.

[0004] During the process of inflammation, an initial pro-inflammatory response is followed by a pro-resolution response which turns the inflammation off after the insult has been resolved, leading to the reduction of pro-inflammatory cytokines such as TNFα and IL-12, coupled with increased levels of anti-inflammatory cytokines such as IL-10 and TGF-β, resulting in the generation of a so-called tolerogenic environment.

[0005] Salt-Inducible Kinases (SIKs) are a family of serine/threonine kinases widely expressed in the body, and involved in particular in cellular energy homeostasis. Three SIK isoforms have been identified, named SIK1 (also referred as SNF1-Like Kinase (SNF1LK) or Myocardial SNF1-Like Kinase (MSK)), SIK2 (SNF1LK2 or KIAA0781) and SIK3 (KIAA0999) (Katoh et al. 2004).

[0006] The SIKs play a number of roles in different cell types. They have been found to phosphorylate a number of substrates including CREB-responsive transcriptional co-activator (CRTC) proteins and histone de-acetylase (HDAC) proteins, thereby regulating the transcription of a number of different genes. HDAC and CRTC proteins control the phenotype of activated macrophages by reducing the proinflammatory

cytokine secretion (TNFα and IL-12) and increase the production of anti-inflammatory cytokines like IL-10 (Clark et al. 2012; Ozanne et al. 2015).

[0007] The regulation of ALK5 by SIK1 (Yu et al. 2013), the identification of the *SIK2* gene as a risk locus for primary sclerosing cholangitis (Liu et al. 2013), and the modulation by SIKs of gene expression induced by TGF-β of factors implicated in the pathology of fibrosis such as plasminogen activator inhibitor 1 (PAI-1) (Hutchinson et al. 2020) suggest a role for SIK proteins in fibrotic diseases. SIK2 and SIK3 have recently been identified to play a role in inflammation through the secretion of high levels of anti-inflammatory cytokines, in particular IL-10, and very low levels of pro-inflammatory cytokines such as TNFα upon inhibition of their kinase activity (Darling et al. 2017; Darling et al. 2021).

[0008] A role for SIK2 in T helper (Th)1 cell differentiation has been described through the regulation of IFNγ and IL-12 signaling, suggesting SIK2 may be an interesting target for inflammatory diseases (Yao et al. 2013).

[0009] SIK1 has recently been shown to be involved in skeletal muscle sensitivity in obese mice, and may be an interesting target to prevent type II diabetes (Nixon et al. 2016), and diabetic nephropathy (Hsu et al. 2020).

[0010] Recently, it has also been shown that like PTH (parathyroid hormone), small molecule SIK inhibitors cause decreased phosphorylation and increased nuclear translocation of HDAC4/5 and CRTC2. Treatment with the small molecule SIK inhibitor YKL-05-099 increased bone formation and bone mass in mice (Wein et al. 2016), confirming the relevance of SIK inhibition in the treatment of bone turnover diseases (Nishimori et al. 2019; Sato et al. 2022).

[0011] Inducible knock-out or mutation of SIK isoforms and pharmacological inhibition in mice and in human embryonic stem cell–derived kidney organoids led to induction of Cyp27b1 expression and 1,25-vitamin D synthesis. Thus, SIK isoforms, in particular SIK2 and SIK3, modulate renal 1,25-vitamin D production downstream of PTH (Yoon et al. 2023), which could be beneficial for the treatment of chronic kidney disease–mineral bone disorder (CKD-MBD).

[0012] Furthermore, it was shown that inhibition of SIK2 after oxygen-glucose deprivation enhances neuron survival (Sasaki et al. 2011) or promotes melanogenesis in melanoma cells (Kumagai et al. 2011). In this context, since therapeutic strategies are needed to modulate the cellular stress response, such as during ischaemia and post reperfusion of tissue, in the chronic phase of cardiac remodeling, in diabetes and neurodegenerative conditions, the rapid inhibition or degradation of the SIK proteins, following multiple kinds of stresses, makes them interesting targets in inflammatory, cardiac or metabolic diseases and neurodegenerative disorders. SIK inhibition might also have application in cosmetology or pigmentation-related diseases to induce melanogenesis (Mujahid et al. 2017).

[0013] Besides the pivotal function in cellular energy homeostasis, the SIK proteins have also been involved in the regulation of the cell cycle. Higher expression of SIK2 significantly correlated with poor survival in patients with high-grade serous ovarian cancers (Ahmed et al. 2010), moreover, expression of SIK3 was elevated in ovarian cancers, particularly in the serous subtype and at later stages (Charoenfuprasert et al. 2011). Therefore SIK inhibition may be useful in the treatment of cancer.

[0014] Despite great advances over the past two decades in the treatments of patients affected by auto-immune disorders based on antibodies targeting pro-inflammatory cytokines, such as anti-TNFα, a significant proportion of patients do not respond to these therapies or experience serious adverse events such as opportunistic infections. Therefore a large unmet medical need still exists for the treatment of these diseases, and new agents for the prophylaxis and/or treatment of the above mentioned diseases are required.

SEQUENCE LISTING

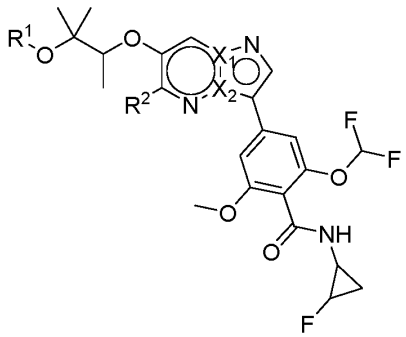
[0015] **SEQ ID 1**: AMARA peptide (Kaneca Eurogentec S.A.).

[0016] **SEQ ID 2**: SIK3 FL Gst-TEV-HsSIK3 (Uniprot# Q9Y2K2-5, amino acids 59-1321)-Thr-6His protein.

SUMMARY OF THE INVENTION

[0017] The present invention is based on the identification of novel compounds, and their use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases. In particular, the compounds of the invention may be SIK inhibitors, and more particularly SIK2 and/or SIK3 inhibitors. The present invention also provides methods for the production of these compounds, pharmaceutical compositions comprising these compounds and methods for the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases by administering the compounds of the invention.

[0018] Accordingly, in a first aspect of the invention, the compounds of the invention are provided having a Formula I:



I

wherein

one of X_1 and X_2 is N and the other is C;

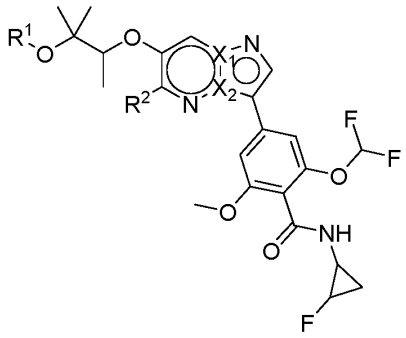
R^1 is H or -P(=O)$(OH)_2$;

R^2 is

- H,
- C_{1-4} alkyl optionally substituted with one or more independently selected halo,
- -$NR^{3a}R^{3b}$; and

Each R^{3a} and R^{3b} is independently selected from H, C_{1-4} alkyl optionally substituted with one or more independently selected halo, or phenyl.

[0019] Accordingly, in a first aspect of the invention, the compounds of the invention are provided having a Formula I:



I

wherein

one of X_1 and X_2 is N and the other is C;

R^1 is H or -P(=O)$(OH)_2$; and

R^2 is H or C_{1-4} alkyl.

[0020] In a particular aspect, the compounds of the invention are provided for use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.

[0021] Furthermore, the compounds of the invention exhibit potent inhibition against SIKs, particularly SIK2 and SIK3, more particularly SIK3, which may result in a tolerogenic therapy (*i.e.*, reduction of pro-inflammatory cytokines such as TNFα and IL-12, coupled with increased levels of anti-inflammatory cytokines such as IL-10 and TGF-β). Particularly, the compounds of the invention exhibit enhanced inhibition of SIK3, with improved selectivity over SIK1 and SIK2.

[0022] Additionally, the compounds of the invention may exhibit a low human pregnane X receptor (PXR, NR1I2) activation liability and therefore a more stable metabolic profile and reduced risk of harmful drug-drug interaction.

[0023] Certain compounds according to the invention may also exhibit one or more benefits including, inter alia, advantageous levels of biological activity which may be useful in the prophylaxis and/or treatment of one or more disease, improved safety characteristics (e.g. relating to hERG inhibition, drug-drug interaction (DDI) or CYP-interaction characteristics, etc.), improved selectivity for one or more disease-associated biological target (e.g. reduced off-target effects, etc.), improved pharmacokinetic properties (e.g. relating to dosing, solubility, absorption, etc.), improved pharmacodynamic properties (e.g. relating to permeability, efflux, etc.) or superior properties for use as pharmaceutical active ingredients alone or in pharmaceutical compositions (e.g. stability), or advantageous physico-chemical properties useful in the manufacturability of such aforementioned pharmaceutical compositions.

[0024] In a further aspect, the present invention provides pharmaceutical compositions comprising a compound of the invention, and a pharmaceutical carrier, excipient or diluent. In a particular aspect, the pharmaceutical composition may additionally comprise further therapeutically active ingredients suitable for use in combination with the compounds of the invention. In a more particular aspect, the further therapeutically active ingredient is an agent for the treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.

[0025] Moreover, the compounds of the invention, useful in the pharmaceutical compositions and treatment methods disclosed herein, are pharmaceutically acceptable as prepared and used.

[0026] In a further aspect of the invention, this invention provides a method of treating a mammal, in particular humans, afflicted with a condition selected from among those listed herein, and particularly inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases, which method comprises administering an effective amount of the pharmaceutical composition or compounds of the invention as described herein.

[0027] The present invention also provides pharmaceutical compositions comprising a compound of the invention, and a suitable pharmaceutical carrier, excipient or diluent for use in medicine. In a particular aspect, the pharmaceutical composition is for use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases,

transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.

[0028] In additional aspects, this invention provides methods for synthesizing the compounds of the invention, with representative synthetic protocols and pathways disclosed later on herein.

[0029] Other objects and advantages will become apparent to those skilled in the art from a consideration of the ensuing detailed description.

[0030] It will be appreciated that compounds of the invention may be metabolized to yield biologically active metabolites.

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] 'Alkyl' means straight or branched aliphatic hydrocarbon having the specified number of carbon atoms. Particular alkyl groups have 1 to 6 carbon atoms or 1 to 4 carbon atoms. Branched means that one or more alkyl groups such as methyl, ethyl or propyl is attached to a linear alkyl chain. Particular alkyl groups are methyl ($-CH_3$), ethyl ($-CH_2-CH_3$), *n*-propyl ($-CH_2-CH_2-CH_3$), isopropyl ($-CH(CH_3)_2$), *n*-butyl ($-CH_2-CH_2-CH_2-CH_3$), *tert*-butyl ($-C(CH_3)_3$), *sec*-butyl ($-CH(CH_3)-CH_2CH_3$), isobutyl ($-CH_2-CH(CH_3)_2$), *n*-pentyl ($-CH_2-CH_2-CH_2-CH_2-CH_3$), *n*-hexyl ($-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$), and 1,2-dimethylbutyl ($-CH(CH_3)-CH(CH_3)-CH_2-CH_3$). Particular alkyl groups have between 1 and 4 carbon atoms.

[0035] 'Substituted' refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituent(s).

[0036] As used herein, term 'substituted with one or more' refers to one to four substituents. In particular, it refers to one to three substituents. More particularly, it refers to one or two substituents. Most particularly, it refers to one substituent.

[0037] '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.

[0038] 'Pharmaceutically acceptable salt' refers to a salt of a compound of the invention that is pharmaceutically acceptable and that retains the biological activity of the given compound, and which are is not biologically or otherwise undesirable. In particular, such salts may be inorganic or organic acid addition salts and base addition salts. For example, pharmaceutically acceptable salts are described in Handbook of Pharmaceutical Salts: Properties, Selection, and Use (Stahl & Wermuth 2011). The salts can be prepared *in situ* during the final isolation and purification of the compounds described herein or separately, *e.g.*, by reacting the free base group with a suitable inorganic or organic acid. The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases are well known in the art, such as, *e.g.*, hydrochloric acid for forming acid addition salts, and such as, *e.g.*, sodium hydroxide for forming basic salts. 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.

[0039] 'Pharmaceutically acceptable vehicle' refers to a diluent, adjuvant, excipient or carrier with which a compound of the invention is administered.

[0040] 'Prodrugs' refers to compounds, including derivatives of the compounds of the invention, which have cleavable groups and become by solvolysis or under physiological conditions the compounds 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.

[0041] '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, EtOH, acetic acid and the like. The compounds 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.

[0042] 'Subject' includes humans. The terms 'human', 'patient' and 'subject' are used interchangeably herein.

[0043] 'Effective amount' means the amount of a compound of the invention that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The "effective amount" can vary depending on the compound, the disease and its severity, and the age, weight, etc., of the subject to be treated.

[0044] '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.

[0045] 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.

[0046] '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.

[0047] As used herein the term 'inflammatory disease(s)' refers to the group of conditions including, rheumatoid arthritis, osteoarthritis, juvenile idiopathic arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis, allergic airway disease (*e.g.* asthma, rhinitis), chronic obstructive pulmonary disease (COPD), inflammatory bowel diseases (*e.g.* Crohn's disease, ulcerative colitis), endotoxin-driven disease states (*e.g.* complications after bypass surgery, acute kidney injury (AKI), Alport syndrome or chronic endotoxin states contributing to *e.g.* chronic cardiac failure), and related diseases involving cartilage, such as that of the joints. Particularly the term refers to rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel diseases. More particularly the term refers to rheumatoid arthritis and inflammatory bowel diseases.

[0048] As used herein the term 'autoinflammatory diseases(s)' refers to the group of diseases including cryopyrin-associated periodic syndromes (CAPS), familial Mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), Behçet's disease, systemic-onset juvenile idiopathic arthritis (SJIA), or Still's disease.

[0049] 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, infantile asthma) particularly chronic or inveterate asthma (for example late asthma

and airway hyperresponsiveness), bronchitis, including bronchial asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, autoimmune liver diseases (e.g. autoimmune hepatitis, primary sclerosing cholangitis, and primary biliary cirrhosis), Sjögren's syndrome, multiple sclerosis, psoriasis, dry eye disease, type I diabetes mellitus and complications associated therewith, atopic eczema (atopic dermatitis), thyroiditis (Hashimoto's and autoimmune thyroiditis), pemphigus vulgaris, contact dermatitis and further eczematous dermatitis, inflammatory bowel disease (*e.g.* Crohn's disease and ulcerative colitis), atherosclerosis, type I diabetic kidney disease, anti-GBM (Goodpasture's) disease, IgA nephropathy (Berger's disease), focal segmental glomerulosclerosis, or thin basement membrane disease and amyotrophic lateral sclerosis. Particularly, the term refers to COPD, asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, Sjögren's syndrome, psoriasis, dry eye disease, and inflammatory bowel disease.

[0050] 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 leukemia, acute and chronic lymphoblastic leukemia), multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular, the term refers to psoriasis, scleroderma and fibrosis.

[0051] 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 (metastasize) to distant organs, for example to bone, liver, lung or the brain. As used herein the term cancer includes both metastatic tumor cell types (such as but not limited to, melanoma, lymphoma, leukemia, 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, and uterine leiomyosarcoma). In particular, the term 'cancer' refers to acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, anal cancer, appendix cancer, astrocytomas, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer (osteosarcoma and malignant fibrous histiocytoma), brain stem glioma, brain tumors, brain and spinal cord tumors, breast cancer, bronchial tumors, Burkitt lymphoma, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, embryonal tumors, endometrial cancer, ependymoblastoma, ependymoma, esophageal cancer, Ewing sarcoma family of tumors, eye cancer, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), gastrointestinal stromal cell tumor, germ cell tumor, glioma, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, hypopharyngeal cancer, intraocular melanoma, islet cell tumors (endocrine pancreas), Kaposi's sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, hairy cell leukemia, liver cancer, non-small cell lung cancer, small cell lung cancer, cutaneous T-cell lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, lymphoma, Waldenström macroglobulinemia, medulloblastoma, medulloepithelioma, melanoma,

mesothelioma, mouth cancer, myeloid leukemia, multiple myeloma, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma, malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, papillomatosis, parathyroid cancer, penile cancer, pharyngeal cancer, pineal parenchymal tumors of intermediate differentiation, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, Ewing sarcoma family of tumors, sarcoma, Sézary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilms tumor.

[0052] 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 leukemia (AML), and acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL).

[0053] As used herein the term 'fibrotic disease(s)' refers to diseases characterized by excessive scarring due to excessive production, deposition, and contraction of extracellular matrix, and that are associated with the abnormal accumulation of cells and/or fibronectin and/or collagen and/or increased fibroblast recruitment and include but are not limited to fibrosis of individual organs or tissues such as the heart, kidney, liver, joints, lung, pleural tissue, peritoneal tissue, skin, cornea, retina, musculoskeletal and digestive tract. In particular, the term fibrotic diseases refers to idiopathic pulmonary fibrosis (IPF); cystic fibrosis, other diffuse parenchymal lung diseases of different etiologies including iatrogenic drug-induced fibrosis, occupational and/or environmental induced fibrosis, granulomatous diseases (sarcoidosis, hypersensitivity pneumonia), collagen vascular disease, alveolar proteinosis, Langerhans cell granulomatosis, lymphangioleiomyomatosis, inherited diseases (Hermansky-Pudlak syndrome, tuberous sclerosis, neurofibromatosis, metabolic storage diseases, familial interstitial lung disease); radiation induced fibrosis; chronic obstructive pulmonary disease (COPD); scleroderma; bleomycin induced pulmonary fibrosis; chronic asthma; silicosis; asbestos induced pulmonary fibrosis; acute respiratory distress syndrome (ARDS); kidney fibrosis, autosomal dominant polycystic kidney disease, tubulointerstitium fibrosis; glomerular nephritis; diabetic nephropathy, focal segmental glomerular sclerosis; IgA nephropathy; hypertension; Alport syndrome; gut fibrosis; liver fibrosis; cirrhosis; alcohol induced liver fibrosis; toxic/drug induced liver fibrosis; hemochromatosis; nonalcoholic steatohepatitis (NASH); biliary duct injury; primary biliary cirrhosis; infection induced liver fibrosis; viral induced liver fibrosis; and autoimmune hepatitis; corneal scarring; hypertrophic scarring; Dupuytren's disease, keloids, cutaneous fibrosis; cutaneous scleroderma; systemic sclerosis, spinal cord injury/fibrosis; myelofibrosis; Duchenne muscular dystrophy (DMD) associated musculoskeletal fibrosis, vascular restenosis; atherosclerosis; arteriosclerosis; Wegener's granulomatosis; Peyronie's disease, or chronic lymphocytic.

More particularly, the term 'fibrotic diseases' refers to idiopathic pulmonary fibrosis (IPF), radiation induced fibrosis, nonalcoholic steatohepatitis (NASH), scleroderma, bleomycin induced pulmonary fibrosis, cutaneous scleroderma, kidney fibrosis and systemic sclerosis.

[0054] 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 esophagus, or graft-versus-host disease (GvHD). More particularly the term refers to graft-versus-host disease (GvHD).

[0055] 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 joint disease and Handigodu disease; degeneration resulting from fibromyalgia, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0056] As used herein the term 'congenital cartilage malformation(s)' includes conditions such as hereditary chondrolysis, chondrodysplasias and pseudo chondrodysplasias, in particular, but without limitation, microtia, anotia, metaphyseal chondrodysplasia, and related disorders.

[0057] As used herein the term 'diseases involving impairment of bone turnover' includes conditions such as osteoporosis (including postmenopausal osteoporosis, male osteoporosis, glucocorticosteroid induced osteoporosis and juvenile osteoporosis), osteoporosis caused through neoplastic bone marrow disorders, osteopenia, hormone deficiency (vitamin D deficiency, male and female hypogonadism), hormone excess (hyperprolactinemia, excess glucocorticoid, hyperthyroidism, hyperparathyroidism), Paget's disease, osteoarthritis, renal bone disease, osteogenesis imperfecta, hypophosphatasia and chronic kidney disease mineral bone disorders (CKD-MBD).

[0058] As used herein the term 'disease(s) associated with hypersecretion of IL-6' 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 TNFα, interferons, IL-12, IL-17 and/or IL-23 includes conditions such as systemic and cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, spondyloarthropathies (particularly ankylosing spondylitis) and/or Crohn's disease.

[0060] As used herein , the term 'respiratory disease(s)' refers to diseases affecting the organs that are involved in breathing, such as the nose, throat, larynx, eustachian tubes, trachea, bronchi, lungs, related muscles (e.g., diaphragm and intercostals), and nerves. In particular, examples of respiratory diseases include asthma, adult respiratory distress syndrome and allergic (extrinsic) asthma, non-allergic (intrinsic) asthma, acute severe asthma, chronic asthma, clinical asthma, nocturnal asthma, allergen-induced asthma,

aspirin-sensitive asthma, exercise-induced asthma, isocapnic hyperventilation, child onset asthma, adult-onset asthma, cough-variant asthma, occupational asthma, steroid-resistant asthma, seasonal asthma, seasonal allergic rhinitis, perennial allergic rhinitis, chronic obstructive pulmonary disease (COPD), including chronic bronchitis or emphysema, pulmonary hypertension, interstitial lung fibrosis and/or airway inflammation, cystic fibrosis, and hypoxia.

[0061] As used herein the term 'endocrine diseases' refers to disorders of the endocrine system and hormonal secretion. In particular, the term refers to hypothyroidism, congenital adrenal hyperplasia, diseases of the parathyroid gland, diabetes mellitus, diseases of the adrenal glands (including Cushing's syndrome and Addison's disease), and ovarian dysfunction (including polycystic ovary syndrome).

[0062] As used herein the term 'metabolic diseases' refers to disorders that disrupt normal metabolism, the process of converting food to energy on a cellular level. Metabolic diseases affect the ability to perform critical biochemical reactions that involve the processing or transport of proteins (amino acids), carbohydrates (sugars and starches), or lipids (fatty acids). In particular, the term refers to cystic fibrosis, phenylketonuria (PKU), diabetes, hyperlipidemia, gout, type II diabetic kidney disease and rickets. In more particular, the term refers to obesity and/or type II diabetes.

[0063] As used herein the term 'cardiovascular disease(s)' refers to diseases affecting the heart or blood vessels or both. In particular, cardiovascular disease includes arrhythmia (atrial or ventricular or both); atherosclerosis and its sequelae; angina; cardiac rhythm disturbances; myocardial ischemia; myocardial infarction; cardiac or vascular aneurysm; vasculitis (e.g., giant cell arteritis (GCA), retinal vasculitis, rheumatoid vasculitis), stroke; peripheral obstructive arteriopathy of a limb, an organ, or a tissue; reperfusion injury following ischemia of the brain, heart, kidney or other organ or tissue; endotoxic, surgical, or traumatic shock; hypertension, valvular heart disease, heart failure, abnormal blood pressure; vasoconstriction (including that associated with migraines); vascular abnormality, inflammation, or insufficiency limited to a single organ or tissue. More particularly, cardiovascular disease refers to atherosclerosis or giant cell arteritis.

[0064] As used herein the term 'dermatological disease(s)' refers to a skin disorder. In particular, dermatological disorders include proliferative or inflammatory disorders of the skin such as atopic dermatitis, bullous disorders, collagenoses, psoriasis, psoriatic lesions, dermatitis, contact dermatitis, eczema, vitiligo, pruritus, scleroderma, wound healing, scarring, hypertrophic scarring, keloids, Kawasaki disease, rosacea, Sjögren-Larsson syndrome, or urticaria. More particularly, the term dermatological disorders refers to psoriasis, psoriatic lesions, scleroderma, and vitiligo.

[0065] As used herein the term 'abnormal angiogenesis associated disease(s)' refers to diseases caused by the dysregulation of the processes mediating angiogenesis. In particular, abnormal angiogenesis associated disease refers to atherosclerosis, hypertension, tumor growth, inflammation, rheumatoid arthritis, wet-form macular degeneration, choroidal neovascularization, retinal neovascularization, and diabetic retinopathy.

[0066] 'Compound(s) of the invention', and equivalent expressions, are meant to embrace compounds of the Formula(e) as herein described, which expression includes the 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.

[0067] 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.

[0068] Other derivatives of the compounds 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 (Bundgaard 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_{1-8} alkyl, C_{2-8} alkenyl, C_{6-10} optionally substituted aryl, and (C_{6-10} aryl)-(C_{1-4} alkyl) esters of the compounds of the invention.

[0069] The present disclosure includes all isotopic forms of the compounds of the invention provided herein, whether in a form (i) wherein all atoms of a given atomic number have a mass number (or mixture of mass numbers) which predominates in nature (referred to herein as the "natural isotopic form") or (ii) wherein one or more atoms are replaced by atoms having the same atomic number, but a mass number different from the mass number of atoms which predominates in nature (referred to herein as an "unnatural variant isotopic form"). It is understood that an atom may naturally exists as a mixture of mass numbers. The term "unnatural variant isotopic form" also includes embodiments in which the proportion of an atom of given atomic number having a mass number found less commonly in nature (referred to herein as an "uncommon isotope") has been increased relative to that which is naturally occurring e.g. to the level of >20%, >50%, >75%, >90%, >95% or> 99% by number of the atoms of that atomic number (the latter embodiment referred to as an "isotopically enriched variant form"). The term "unnatural variant isotopic form" also includes embodiments in which the proportion of an uncommon isotope has been reduced relative to that which is naturally occurring. Isotopic forms may include radioactive forms (*i.e.* they incorporate radioisotopes) and non-radioactive forms. Radioactive forms will typically be isotopically enriched variant forms.

[0070] An unnatural variant isotopic form of a compound may thus contain one or more artificial or uncommon isotopes such as deuterium (^{2}H or D), carbon-11 (^{11}C), carbon-13 (^{13}C), carbon-14 (^{14}C), nitrogen-13 (^{13}N), nitrogen-15 (^{15}N), oxygen-15 (^{15}O), oxygen-17 (^{17}O), oxygen-18 (^{18}O), phosphorus-32 (^{32}P), sulfur-35 (^{35}S), chlorine-36 (^{36}Cl), chlorine-37 (^{37}Cl), fluorine-18 (^{18}F) iodine-123 (^{123}I), iodine-125 (^{125}I) in one or more atoms or may contain an increased proportion of said isotopes as compared with the proportion that predominates in nature in one or more atoms.

[0071] Unnatural variant isotopic forms comprising radioisotopes may, for example, 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.

Unnatural variant isotopic forms which incorporate deuterium i.e. ^{2}H or D may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances. Further, unnatural variant isotopic forms may be prepared which incorporate positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, and would be useful in Positron Emission Tomography (PET) studies for examining substrate receptor occupancy.

[0072] 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'.

[0073] 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, Ingold 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'.

[0074] '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.

[0075] Tautomeric forms may be relevant to the attainment of the optimal chemical reactivity and biological activity of a compound of interest.

[0076] The compounds of the invention may possess one or more asymmetric centers; such compounds can therefore be produced as individual (R)- or (S)- stereoisomers or as mixtures thereof.

[0077] 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.

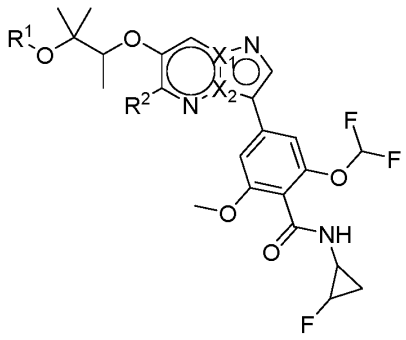
THE INVENTION

[0078] The present invention is based on the identification of novel compounds, and their use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-

12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases. In particular, the compounds of the invention may be SIK inhibitors, and more particularly SIK2 and/or SIK3 inhibitors.

[0079] The present invention also provides methods for the production of these compounds, pharmaceutical compositions comprising these compounds and methods for the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases by administering the compounds of the invention.

[0080] Accordingly, in a first aspect of the invention, the compounds of the invention are provided having a Formula I:



I

wherein

one of X_1 and X_2 is N and the other is C;

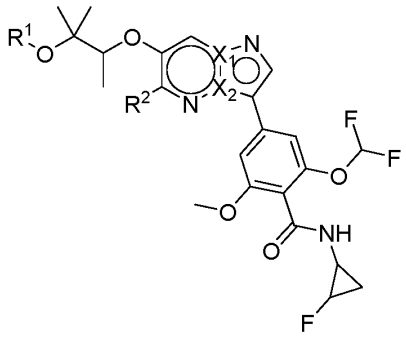
R^1 is H or $-P(=O)(OH)_2$;

R^2 is

- H,
- C_{1-4} alkyl optionally substituted with one or more independently selected halo,
- $-NR^{3a}R^{3b}$; and

Each R^{3a} and R^{3b} is indenpently selected from H, C_{1-4} alkyl optionally substituted with one or more independently selected halo, or phenyl.

[0081] Accordingly, in a second aspect of the invention, the compounds of the invention are provided having a Formula I:



I

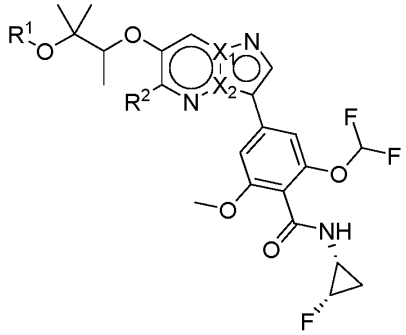
wherein

one of X_1 and X_2 is N and the other is C;

R^1 is H or -P(=O)$(OH)_2$; and

R^2 is H or C_{1-4} alkyl.

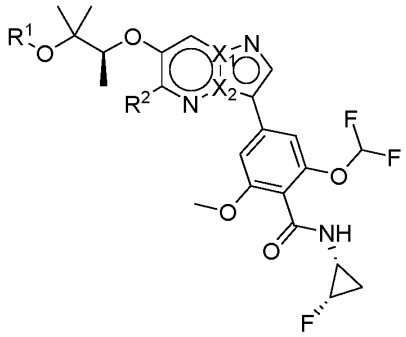
[0082] In one embodiment, the compound of the invention is according to Formula II:



II

wherein X_1, X_2, R^1, and R^2 are as described above.

[0083] In one embodiment, the compound of the invention is according to Formula III:



III

wherein X_1, X_2, R^1, and R^2 are as described above.

[0084] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein R^2 is H.

[0085] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein R^2 is C_{1-4} alkyl. In a particular embodiment, R^2 is $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$. In a more particular embodiment, R^2 is $-CH_3$ or $-CH_2CH_3$. In a further more particular embodiment R^2 is $-CH_3$.

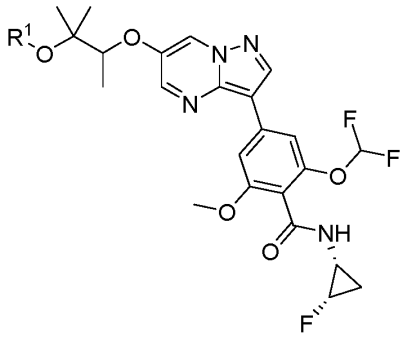
[0086] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein R^2 is C_{1-4} alkyl substituted with one or more independently selected halo. In a particular embodiment, R^2 is $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$, each of which is substituted with one or more independently selected halo. In a particular embodiment, R^2 is $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$, each of which is substituted with one or more independently selected F. In a more particular embodiment, R^2 is $-CH_3$ or $-CH_2CH_3$, each of which is substituted with one or more independently selected F. In a further more particular embodiment R^2 is $-CHF_3$.

[0087] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein R^2 is $-NR^{3a}R^{3b}$, wherein R^{3a} and R^{3b} are as previously defined. In a particular embodiment, R^2 is $-NR^{3a}R^{3b}$, and each R^{3a} and R^{3b} is independently selected from H, $-CH_3$, $-CH_2CH_3$, and $-CH_2$Phenyl. In more particular embodiment, R^2 is $-NH_2$, $-NHCH_3$, $-NH_2$, $-N(CH_3)_2$, $-NH(CH_2$Phenyl), or $-NCH_3(CH_2$Phenyl).

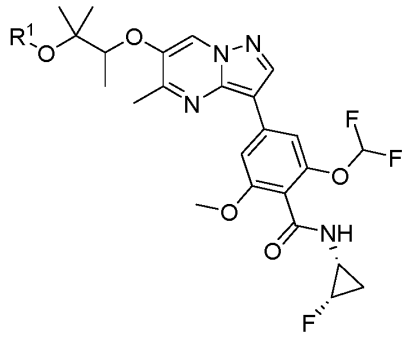
[0088] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein X_1 is N and X_2 is C.

[0089] In one embodiment, the compound of the invention is according to Formula I, II, or III, wherein X_1 is C and X_2 is N.

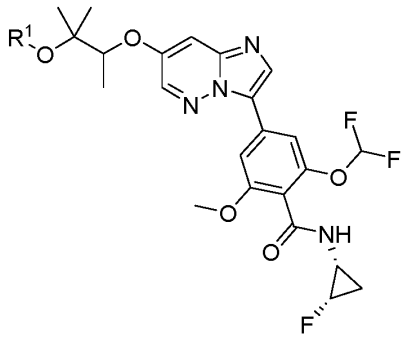
[0090] In one embodiment, the compound of the invention is according to Formula IVa, IVb, IVc, or IVd:



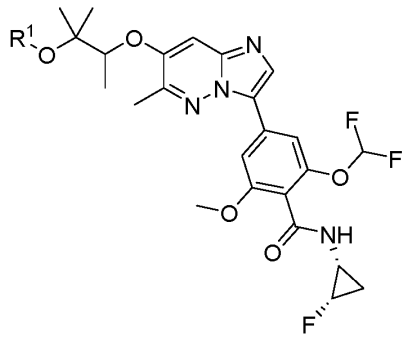
IVa



IVb



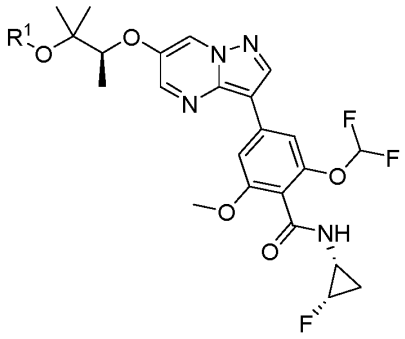
IVc



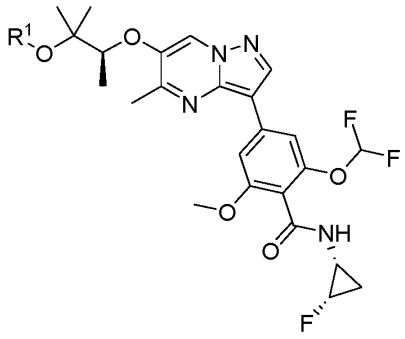
IVd

wherein R^1 is as described above.

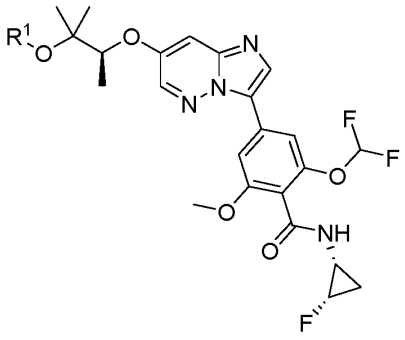
[0091] In one embodiment, the compound of the invention is according to Formula Va, Vb, Vc, or Vd:



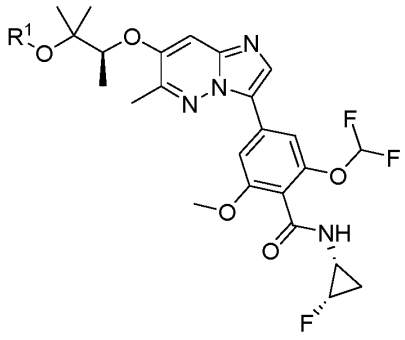
Va



Vb



Vc



Vd

wherein R^1 is as described above.

[0092] In one embodiment, the compound of the invention is according to any one of Formulae I-Vd, wherein R^1 is H.

[0093] In one embodiment, the compound of the invention is according to any one of Formulae I-Vd, wherein R^1 is $-P(=O)(OH)_2$.

[0094] In one embodiment, the compound of the invention is selected from:

2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-4-[6-[(2S)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide,

2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide,

2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-(3-hydroxy-3-methylbutan-2-yl)oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide,

2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-(3-hydroxy-3-methylbutan-2-yl)oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide,

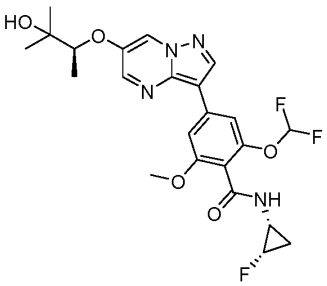
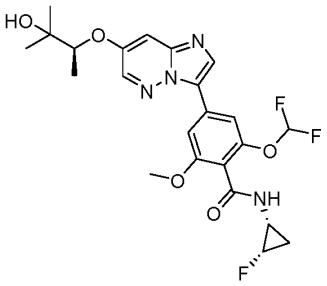
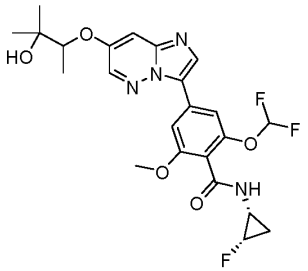
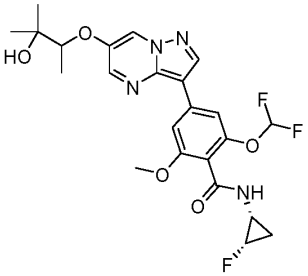
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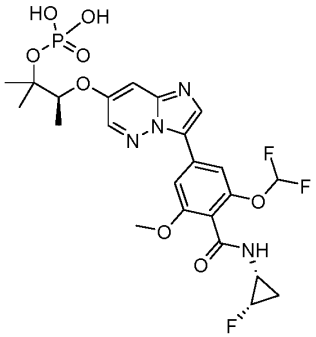
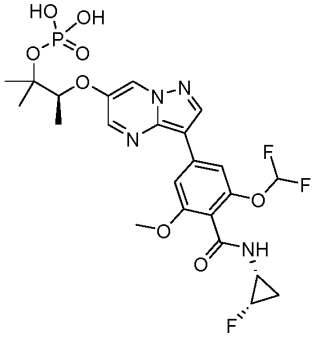
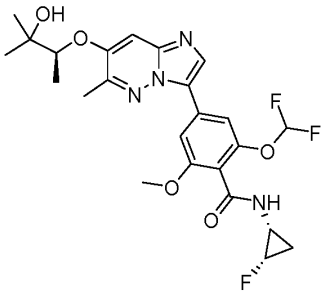
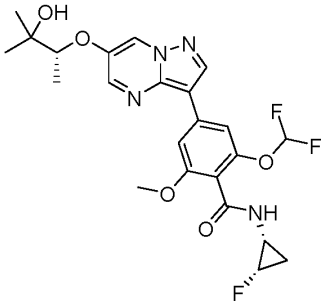
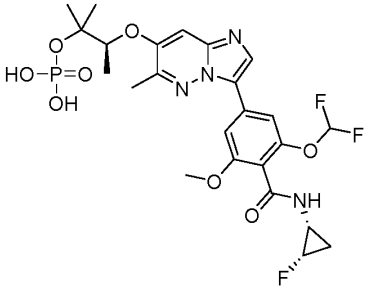
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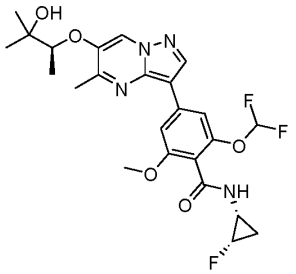
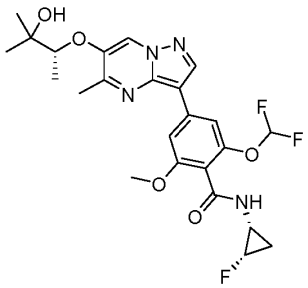
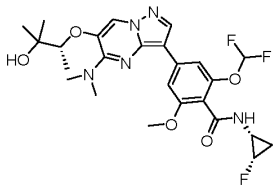
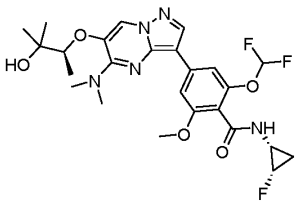
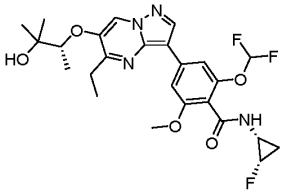
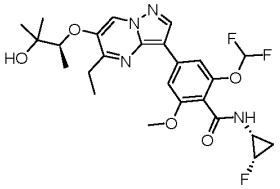
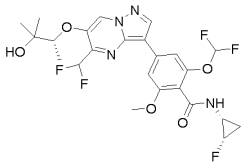
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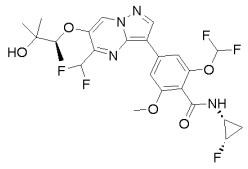
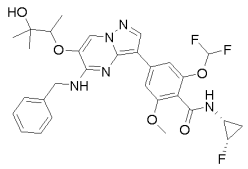
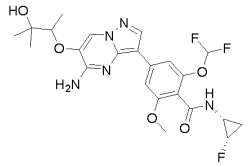
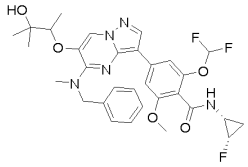
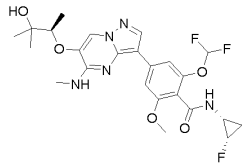
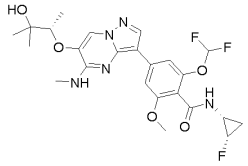
2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R*)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide,
[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]-6-methylimidazo[1,2-b]pyridazin-7-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate,
2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide, and
2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R*)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide.

[0095] In one embodiment, the compound of the invention is selected from

Cpd#	Structure	Name
1		2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-4-[6-[(2S)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide
2		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide
3		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-(3-hydroxy-3-methylbutan-2-yl)oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide
4		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-(3-hydroxy-3-methylbutan-2-yl)oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide

Cpd#	Structure	Name
5		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]imidazo[1,2-b]pyridazin-7-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate
6		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]pyrazolo[1,5-a]pyrimidin-6-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate
7		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxy-6-methylimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide
8		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R*)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide
9		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]-6-methylimidazo[1,2-b]pyridazin-7-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate

Cpd#	Structure	Name
10		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*S**)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide
11		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R**)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide
12		2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
13		2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
14		2-(difluoromethoxy)-4-(5-ethyl-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
15		2-(difluoromethoxy)-4-(5-ethyl-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
16		2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide

Cpd#	Structure	Name
17		2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
18		4-(5-(benzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
19		4-(5-amino-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
20		4-(5-(benzyl(methyl)amino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide
21		2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-4-(6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)-5-(methylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-6-methoxybenzamide
22		2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-4-(6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)-5-(methylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-6-methoxybenzamide

Wherein * in R* or S* means that the stereochemistry is arbitrarily assigned.

[0096] In one embodiment, the compound of the invention is 2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-4-[6-[(2S)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide.

[0097] In one embodiment, the compound of the invention is not 2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-4-[6-[(2S)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide.

[0098] In one embodiment, the compound of the invention is 2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxy-6-methylimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide.

[0099] In one embodiment, the compound of the invention is not 2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxy-6-methylimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide.

[0100] In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$.

[0101] In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$ in an amorphous form.

[0102] In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$ in a crystalline form.

[0103] In one embodiment, In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$, wherein the compound is an hydrate. In a particular embodiment, the hydrate is an hemihydrate or a monohydrate. In a more particular embodiment, the hydrate is an hemihydrate or a monohydrate in a crystalline form.

[0104] In one embodiment, In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$, wherein the compound is a monohydrate in a crystalline form characterized by an X-ray powder diffraction pattern comprising peaks at 9.1, 10.0, 13.2, 13.6, and 14.9 ± 0.2° 2θ using Cu Kα radiation. In a particular embodiment, the X-ray powder diffraction pattern further comprises peaks at 15.7, 15.9, 16.6, 17.2, 19.0, 19.3, 20.4, 20.8, 24.2, 24.8, and 26.5 ± 0.2° 2θ.

[0105] In one embodiment, In one embodiment, the compound of the invention is according to Formula Va wherein R^1 is $-P(=O)(OH)_2$, wherein the compound is a hemihydrate in a crystalline form characterized by an X-ray powder diffraction pattern comprising peaks at 9.1, 9.6, 15.1 15.4, and 19.7 ± 0.2° 2θ using Cu Kα radiation. In a particular embodiment, the X-ray powder diffraction pattern further comprises peaks at 10.6, 12.4, 14.1, 18.1, 18.5, 18.8, 20.0, and 20.3 ± 0.2° 2θ.

[0106] In one embodiment, a compound of the invention is provided in a natural isotopic form.

[0107] In one embodiment, a compound of the invention is provided in an unnatural variant isotopic form. In a specific embodiment, the unnatural variant isotopic form is a form in which deuterium (*i.e.* ^2H or D) is incorporated where hydrogen is specified in the chemical structure in one or more atoms of a compound of the invention. In one embodiment, the atoms of the compounds of the invention are in an isotopic form which is not radioactive. In one embodiment, one or more atoms of the compounds of the invention are in an isotopic form which is radioactive. Suitably radioactive isotopes are stable isotopes. Suitably, the unnatural variant isotopic form is a pharmaceutically acceptable form.

[0108] In one embodiment, a compound of the invention is provided whereby a single atom of the compound exists in an unnatural variant isotopic form. In another embodiment, a compound of the invention is provided whereby two or more atoms exist in an unnatural variant isotopic form.

[0109] Unnatural isotopic variant forms can generally be prepared by conventional techniques known to those skilled in the art or by processes described herein *e.g.* processes analogous to those described in the accompanying Examples for preparing natural isotopic forms. Thus, unnatural isotopic variant forms could be prepared by using appropriate isotopically variant (or labelled) reagents in place of the normal reagents employed in the illustrative example as examples.

[0110] In one aspect a compound of the invention according to any one of the embodiments herein described is present as the free base.

[0111] In one aspect a compound of the invention according to any one of the embodiments herein described is a pharmaceutically acceptable salt.

[0112] In one aspect a compound of the invention according to any one of the embodiments herein described is a solvate of the compound.

[0113] In one aspect a compound of the invention according to any one of the embodiments herein described is a solvate of a pharmaceutically acceptable salt of a compound.

[0114] While specified groups for each embodiment have generally been listed above separately, a compound of the invention includes one in which several or each embodiment in the above Formula, as well as other formulae presented herein, is selected from one or more of particular members or groups designated respectively, for each variable. Therefore, this invention is intended to include all combinations of such embodiments within its scope.

[0115] While specified groups for each embodiment have generally been listed above separately, a compound of the invention may be one for which one or more variables (for example, R groups) is selected from one or more embodiments according to any of the Formula(e) listed above. Therefore, the present invention is intended to include all combinations of variables from any of the disclosed embodiments within its scope.

[0116] Alternatively, the exclusion of one or more of the specified variables from a group or an embodiment, or combinations thereof is also contemplated by the present invention.

[0117] In certain aspects, the present invention provides prodrugs and derivatives of the compounds according to the formulae above. Prodrugs are derivatives of the compounds of the invention, which have metabolically cleavable groups and become by solvolysis or under physiological conditions the compounds 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.

[0118] Other derivatives of the compounds 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 (Bundgaard 1985). Prodrugs include acid derivatives well known 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-C_8 alkenyl, aryl, C_7-C_{12} substituted aryl, and C_7-C_{12} arylalkyl esters of the compounds of the invention.

PHARMACEUTICAL COMPOSITIONS

[0119] When employed as a pharmaceutical, a 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 of the invention according to Formula I.

Generally, a compound of the invention is administered in a pharmaceutically effective amount. The amount of compound of the invention 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 of the invention administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[0120] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent.

[0121] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases treatment agent.

[0122] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an inflammatory diseases treatment agent. In particular, the term inflammatory diseases refers to rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel diseases. More particularly, the term refers to rheumatoid arthritis and inflammatory bowel diseases.

[0123] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an autoinflammatory diseases treatment agent. In particular, the term autoinflammatory diseases refers to cryopyrin-associated periodic syndromes (CAPS), familial mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), Behçet's disease, systemic-onset juvenile idiopathic arthritis (SJIA) or Still's disease. More particularly, the term refers to CAPS, FMF, TRAPS and Still's disease.

[0124] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an autoimmune diseases treatment agent. Particularly, the term autoimmune diseases refers to COPD, asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, Sjögren's syndrome, psoriasis, dry eye disease, and inflammatory bowel disease. More particularly, the term refers to COPD, asthma, systemic lupus erythematosus, and inflammatory bowel disease.

[0125] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a proliferative diseases treatment agent. In particular, the term proliferative diseases refers to cancer, myeloproliferative disorders, leukemia, multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular, the term refers to psoriasis, scleroderma, and fibrosis.

[0126] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a fibrotic diseases treatment agent. In particular, the term fibrotic diseases refers to idiopathic pulmonary fibrosis (IPF), radiation induced fibrosis, nonalcoholic steatohepatitis (NASH), scleroderma, bleomycin induced pulmonary fibrosis, cutaneous scleroderma, and systemic sclerosis.

[0127] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a transplant rejection treatment agent. In particular, the term transplant rejection refers to 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 esophagus, or graft-versus-host disease. More particularly, the term refers to graft-versus-host disease.

[0128] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a disease involving impairment of cartilage turnover treatment agent. In particular, the term diseases involving impairment of cartilage turnover refers to 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. More particularly, the term refers to osteoarthritis, psoriatic arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0129] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a congenital cartilage malformation treatment agent. In particular, the term congenital cartilage malformation refers to hereditary chondrolysis, chondrodysplasias and pseudochondrodysplasias, microtia, anotia, metaphyseal chondrodysplasia. More particularly, the term refers to microtia, anotia, metaphyseal chondrodysplasia.

[0130] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a diseases involving impairment of bone turnover treatment agent. In particular, the term diseases involving impairment of bone turnover refers to osteoporosis, osteopenia, hormone deficiency, hormone excess, Paget's disease, osteoarthritis, renal bone disease, osteogenesis imperfecta, and hypophosphatasia. More particularly, the term refers to osteoporosis.

[0131] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a diseases associated with hypersecretion of IL-6 treatment agent. In particular, the term diseases associated with hypersecretion of IL-6 refers to Castleman’s disease, multiple myeloma, psoriasis, Kaposi’s sarcoma and/or mesangial proliferative glomerulonephritis.

[0132] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23 treatment agent. In particular, the term diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23 refers to systemic and cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, spondyloarthropathies (particularly ankylosing spondylitis) and/or Crohn's disease. More particularly, the term refers to Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, ankylosing spondylitis and/or Crohn's disease.

[0133] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a respiratory diseases treatment agent. In particular, the term respiratory diseases refers to asthma, adult respiratory distress syndrome, isocapnic hyperventilation, seasonal asthma, seasonal allergic rhinitis, perennial allergic rhinitis, chronic obstructive pulmonary disease, emphysema, pulmonary hypertension, interstitial lung fibrosis, cystic fibrosis, or hypoxia. More particularly, the term refers to pulmonary hypertension or interstitial lung fibrosis.

[0134] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an endocrine diseases treatment agent. In particular, the term endocrine diseases refers to hypothyroidism, congenital adrenal hyperplasia, diseases of the parathyroid gland, diabetes mellitus, diseases of the adrenal glands, Cushing's syndrome and Addison's disease, ovarian dysfunction polycystic ovary syndrome.

[0135] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a metabolic diseases treatment agent. In particular, the term metabolic diseases refers to cystic fibrosis, phenylketonuria (PKU), diabetes, hyperlipidemia, gout, type II diabetic kidney disease and rickets. More particularly, the term refers to obesity and/or type II diabetes.

[0136] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a cardiovascular diseases treatment agent. In particular, the term cardiovascular diseases refers to arrhythmia (atrial or ventricular or both); atherosclerosis and its sequelae; angina; cardiac rhythm disturbances; myocardial ischemia; myocardial infarction; cardiac or vascular aneurysm; vasculitis, stroke; peripheral obstructive arteriopathy of a limb, an organ, or a tissue; reperfusion injury following ischemia of the brain, heart, kidney or other organ or tissue; endotoxic, surgical, or traumatic shock; hypertension, valvular heart disease, heart failure, abnormal blood pressure; vasoconstriction (including that associated with migraines); vascular abnormality, inflammation, or insufficiency limited to a single organ or tissue. More particularly, the term refers to atherosclerosis or giant cell arteritis.

[0137] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is a dermatological

diseases treatment agent. In particular, the term dermatological diseases refers to atopic dermatitis, bullous disorders, collagenoses, psoriasis, psoriatic lesions, dermatitis, contact dermatitis, eczema, vitiligo, pruritus, scleroderma, wound healing, scarring, hypertrophic scarring, keloids, Kawasaki disease, rosacea, Sjögren-Larsson syndrome, or urticaria. More particularly, the term refers to a psoriasis, psoriatic lesions, scleroderma, and vitiligo.

[0138] In one embodiment, the present invention provides pharmaceutical compositions comprising a compound of the invention and another therapeutic agent, which other therapeutic agent is an abnormal angiogenesis associated diseases treatment agent. In particular, the term abnormal angiogenesis associated diseases refers to atherosclerosis, hypertension, tumor growth, inflammation, rheumatoid arthritis, wet-form macular degeneration, choroidal neovascularization, retinal neovascularization, and diabetic retinopathy. More particularly, the term refers to atherosclerosis, hypertension, or diabetic retinopathy.

[0139] The pharmaceutical compositions of this 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 the invention is preferably formulated as either injectable or oral compositions or as salves, as lotions or as patches all for transdermal administration.

[0140] 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 according to Formula I 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.

[0141] Liquid forms suitable for oral administration may include a suitable aqueous or non-aqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and the like. Solid forms may include, for example, any of the following ingredients, or compound of the inventions 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 or orange flavoring.

[0142] 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 of the invention according to Formula I 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.

[0143] 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 an 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 or stability of the active ingredients or the formulation. All such known transdermal formulations and ingredients are included within the scope of this invention.

[0144] A 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.

[0145] 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.

[0146] A 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 (Remington & Gennaro 1985).

[0147] 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

[0148] A compound of the invention according to Formula I 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 compound of the invention according to Formula I per tablet) in a tablet press.

Formulation 2 - Capsules

[0149] A compound of the invention according to Formula I 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 compound of the invention according to Formula I per capsule).

Formulation 3 - Liquid

[0150] A compound of the invention according to Formula I (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

[0151] A compound of the invention according to Formula I 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 compound of the invention according to Formula I) in a tablet press.

Formulation 5 - Injection

[0152] A compound of the invention according to Formula I may be dissolved or suspended in a buffered sterile saline injectable aqueous medium to a concentration of approximately 5 mg/mL.

Formulation 6 - Topical

[0153] Stearyl alcohol (250 g) and a white petrolatum (250 g) may be melted at about 75 ºC and then a mixture of a compound of the invention according to Formula I (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

[0154] In one embodiment, the present invention provides compounds of the invention, or pharmaceutical compositions comprising a compound of the invention, for use in medicine.

[0155] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.

[0156] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.

[0157] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said conditions.

[0158] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of inflammatory diseases. In particular, the term inflammatory diseases refers to rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel diseases. More particularly, the term refers to rheumatoid arthritis and inflammatory bowel diseases.

[0159] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of inflammatory diseases. In particular, the term inflammatory diseases refers to rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel diseases. More particularly, the term refers to rheumatoid arthritis and inflammatory bowel diseases.

[0160] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with inflammatory diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term inflammatory diseases refers to rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis and inflammatory bowel diseases. More particularly, the term refers to rheumatoid arthritis and inflammatory bowel diseases.

[0161] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of autoinflammatory diseases. In particular, the term autoinflammatory diseases refers to cryopyrin-associated periodic syndromes (CAPS), familial mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), Behçet's disease, systemic-onset juvenile idiopathic arthritis (SJIA) or Still's disease. More particularly, the term refers to CAPS, FMF, TRAPS and Still's disease.

[0162] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of autoinflammatory diseases. In particular, the term autoinflammatory diseases refers to cryopyrin-associated periodic syndromes (CAPS), familial mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), Behçet's disease, systemic-

onset juvenile idiopathic arthritis (SJIA) or Still's disease. More particularly, the term refers to CAPS, FMF, TRAPS and Still's disease.

[0163] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with autoinflammatory diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term autoinflammatory diseases refers to cryopyrin-associated periodic syndromes (CAPS), familial mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), Behçet's disease, systemic-onset juvenile idiopathic arthritis (SJIA) or Still's disease. More particularly, the term refers to CAPS, FMF, TRAPS and Still's disease.

[0164] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of autoimmune diseases. Particularly, the term autoimmune diseases refers to COPD, asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, Sjögren's syndrome, psoriasis, dry eye disease, and inflammatory bowel disease. More particularly, the term refers to COPD, asthma, systemic lupus erythematosus, and inflammatory bowel disease.

[0165] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of autoimmune diseases. Particularly, the term autoimmune diseases refers to COPD, asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, Sjögren's syndrome, psoriasis, dry eye disease, and inflammatory bowel disease. More particularly, the term refers to COPD, asthma, systemic lupus erythematosus, and inflammatory bowel disease.

[0166] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with autoimmune diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. Particularly, the term autoimmune diseases refers to COPD, asthma, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, lupus nephritis, Sjögren's syndrome, psoriasis, dry eye disease, and inflammatory bowel disease. More particularly, the term refers to COPD, asthma, systemic lupus erythematosus, and inflammatory bowel disease.

[0167] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of proliferative diseases. In particular, the term proliferative diseases refers to cancer, myeloproliferative disorders, leukemia, multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular, the term refers to psoriasis, scleroderma, and fibrosis.

[0168] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament

for the prophylaxis and/or treatment of proliferative diseases. In particular, the term proliferative diseases refers to cancer, myeloproliferative disorders, leukemia, multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular, the term refers to psoriasis, scleroderma, and fibrosis.

[0169] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with proliferative diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term proliferative diseases refers to cancer, myeloproliferative disorders, leukemia, multiple myeloma, psoriasis, restenosis, scleroderma or fibrosis. In particular, the term refers to psoriasis, scleroderma, and fibrosis.

[0170] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of fibrotic diseases. In particular, the term fibrotic diseases refers to idiopathic pulmonary fibrosis (IPF), radiation induced fibrosis, nonalcoholic steatohepatitis (NASH), scleroderma, bleomycin induced pulmonary fibrosis, cutaneous scleroderma, and systemic sclerosis.

[0171] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of fibrotic diseases. In particular, the term fibrotic diseases refers to idiopathic pulmonary fibrosis (IPF), radiation induced fibrosis, nonalcoholic steatohepatitis (NASH), scleroderma, bleomycin induced pulmonary fibrosis, cutaneous scleroderma, and systemic sclerosis.

[0172] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with fibrotic diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term fibrotic diseases refers to idiopathic pulmonary fibrosis (IPF), radiation induced fibrosis, nonalcoholic steatohepatitis (NASH), scleroderma, bleomycin induced pulmonary fibrosis, cutaneous scleroderma, and systemic sclerosis.

[0173] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of transplant rejection. In particular, the term transplant rejection refers to 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 esophagus, or graft-versus-host disease. More particularly, the term refers to graft-versus-host disease.

[0174] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of transplant rejection. In particular, the term transplant rejection refers to 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 esophagus, or graft-versus-host disease. More particularly, the term refers to graft-versus-host disease.

[0175] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with transplant rejection, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term transplant rejection refers to 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 esophagus, or graft-versus-host disease. More particularly, the term refers to graft-versus-host disease.

[0176] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of diseases involving impairment of cartilage turnover. In particular, the term diseases involving impairment of cartilage turnover refers to 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. More particularly, the term refers to osteoarthritis, psoriatic arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0177] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of diseases involving impairment of cartilage turnover. In particular, the term diseases involving impairment of cartilage turnover refers to 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. More particularly, the term refers to osteoarthritis, psoriatic arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0178] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with diseases involving impairment of cartilage turnover, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term diseases involving impairment of cartilage turnover refers to 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. More particularly, the term refers to osteoarthritis, psoriatic arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, scleroderma and ankylosing spondylitis.

[0179] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of congenital cartilage malformation. In particular, the term congenital cartilage malformation refers to hereditary chondrolysis, chondrodysplasias and pseudochondrodysplasias, microtia, anotia, metaphyseal chondrodysplasia. More particularly, the term refers to microtia, anotia, metaphyseal chondrodysplasia.

[0180] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of congenital cartilage malformation. In particular, the term congenital cartilage malformation refers to hereditary chondrolysis, chondrodysplasias and pseudochondrodysplasias, microtia, anotia, metaphyseal chondrodysplasia. More particularly, the term refers to microtia, anotia, metaphyseal chondrodysplasia.

[0181] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with congenital cartilage malformation, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term congenital cartilage malformation refers to hereditary chondrolysis, chondrodysplasias and pseudochondrodysplasias, microtia, anotia, metaphyseal chondrodysplasia. More particularly, the term refers to microtia, anotia, metaphyseal chondrodysplasia.

[0182] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of diseases involving impairment of bone turnover. In particular, the term diseases involving impairment of bone turnover refers to osteoporosis, osteopenia, hormone deficiency, hormone excess, Paget's disease, osteoarthritis, renal bone disease, osteogenesis imperfecta, and hypophosphatasia. More particularly, the term refers to osteoporosis.

[0183] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of diseases involving impairment of bone turnover. In particular, the term diseases involving impairment of bone turnover refers to osteoporosis, osteopenia, hormone deficiency, hormone excess, Paget's disease, osteoarthritis, renal bone disease, osteogenesis imperfecta, and hypophosphatasia. More particularly, the term refers to osteoporosis.

[0184] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with diseases involving impairment of bone turnover, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term diseases involving impairment of bone turnover refers to osteoporosis, osteopenia,

hormone deficiency, hormone excess, Paget's disease, osteoarthritis, renal bone disease, osteogenesis imperfecta, and hypophosphatasia. More particularly, the term refers to osteoporosis.

[0185] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of diseases associated with hypersecretion of IL−6. In particular, the term diseases associated with hypersecretion of IL-6 refers to Castleman's disease, multiple myeloma, psoriasis, Kaposi's sarcoma and/or mesangial proliferative glomerulonephritis.

[0186] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of diseases associated with hypersecretion of IL-6. In particular, the term diseases associated with hypersecretion of IL-6 refers to Castleman's disease, multiple myeloma, psoriasis, Kaposi's sarcoma and/or mesangial proliferative glomerulonephritis.

[0187] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with diseases associated with hypersecretion of IL-6, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term diseases associated with hypersecretion of IL-6 refers to Castleman's disease, multiple myeloma, psoriasis, Kaposi's sarcoma and/or mesangial proliferative glomerulonephritis.

[0188] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23. In particular, the term diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23 refers to systemic and cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease. More particularly, the term refers to Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease.

[0189] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23. In particular, the term diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23 refers to systemic and cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease. More particularly, the term refers to Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease.

[0190] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, which methods comprise the administration of an effective amount of a compound of

the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23 refers to systemic and cutaneous lupus erythematosus, lupus nephritis, dermatomyositis, Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease. More particularly, the term refers to Sjögren's syndrome, psoriasis, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Down syndrome, ulcerative colitis, and/or Crohn's disease.

[0191] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of respiratory diseases. In particular, the term respiratory diseases refers to asthma, adult respiratory distress syndrome, isocapnic hyperventilation, seasonal asthma, seasonal allergic rhinitis, perennial allergic rhinitis, chronic obstructive pulmonary disease, emphysema, pulmonary hypertension, interstitial lung fibrosis, cystic fibrosis, or hypoxia. More particularly, the term refers to pulmonary hypertension or interstitial lung fibrosis.

[0192] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of respiratory diseases. In particular, the term respiratory diseases refers to asthma, adult respiratory distress syndrome, isocapnic hyperventilation, seasonal asthma, seasonal allergic rhinitis, perennial allergic rhinitis, chronic obstructive pulmonary disease, emphysema, pulmonary hypertension, interstitial lung fibrosis, cystic fibrosis, or hypoxia. More particularly, the term refers to pulmonary hypertension or interstitial lung fibrosis.

[0193] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with respiratory diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term respiratory diseases refers to asthma, adult respiratory distress syndrome, isocapnic hyperventilation, seasonal asthma, seasonal allergic rhinitis, perennial allergic rhinitis, chronic obstructive pulmonary disease, emphysema, pulmonary hypertension, interstitial lung fibrosis, cystic fibrosis, or hypoxia. More particularly, the term refers to pulmonary hypertension or interstitial lung fibrosis.

[0194] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of endocrine diseases. In particular, the term endocrine diseases refers to hypothyroidism, congenital adrenal hyperplasia, diseases of the parathyroid gland, diabetes mellitus, diseases of the adrenal glands, Cushing's syndrome and Addison's disease, ovarian dysfunction polycystic ovary syndrome.

[0195] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of endocrine diseases. In particular, the term endocrine diseases refers to hypothyroidism, congenital adrenal hyperplasia, diseases of the parathyroid gland, diabetes mellitus,

diseases of the adrenal glands, Cushing's syndrome and Addison's disease, ovarian dysfunction polycystic ovary syndrome.

[0196] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with endocrine diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term endocrine diseases refers to hypothyroidism, congenital adrenal hyperplasia, diseases of the parathyroid gland, diabetes mellitus, diseases of the adrenal glands, Cushing's syndrome and Addison's disease, ovarian dysfunction polycystic ovary syndrome.

[0197] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of metabolic diseases. In particular, the term metabolic diseases refers to cystic fibrosis, phenylketonuria (PKU), diabetes, hyperlipidemia, gout, type II diabetic kidney disease and rickets. More particularly, the term refers to obesity and/or type II diabetes.

[0198] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of metabolic diseases. In particular, the term metabolic diseases refers to cystic fibrosis, phenylketonuria (PKU), diabetes, hyperlipidemia, gout, type II diabetic kidney disease and rickets. More particularly, the term refers to obesity and/or type II diabetes.

[0199] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with metabolic diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term metabolic diseases refers to cystic fibrosis, phenylketonuria (PKU), diabetes, hyperlipidemia, gout, type II diabetic kidney disease and rickets. More particularly, the term refers to obesity and/or type II diabetes.

[0200] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of cardiovascular diseases. In particular, the term cardiovascular diseases refers to arrhythmia (atrial or ventricular or both); atherosclerosis and its sequelae; angina; cardiac rhythm disturbances; myocardial ischemia; myocardial infarction; cardiac or vascular aneurysm; vasculitis, stroke; peripheral obstructive arteriopathy of a limb, an organ, or a tissue; reperfusion injury following ischemia of the brain, heart, kidney or other organ or tissue; endotoxic, surgical, or traumatic shock; hypertension, valvular heart disease, heart failure, abnormal blood pressure; vasoconstriction (including that associated with migraines); vascular abnormality, inflammation, or insufficiency limited to a single organ or tissue. More particularly, the term refers to atherosclerosis or giant cell arteritis.

[0201] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of cardiovascular diseases. In particular, the term cardiovascular

diseases refers to arrhythmia (atrial or ventricular or both); atherosclerosis and its sequelae; angina; cardiac rhythm disturbances; myocardial ischemia; myocardial infarction; cardiac or vascular aneurysm; vasculitis, stroke; peripheral obstructive arteriopathy of a limb, an organ, or a tissue; reperfusion injury following ischemia of the brain, heart, kidney or other organ or tissue; endotoxic, surgical, or traumatic shock; hypertension, valvular heart disease, heart failure, abnormal blood pressure; vasoconstriction (including that associated with migraines); vascular abnormality, inflammation, or insufficiency limited to a single organ or tissue. More particularly, the term refers to atherosclerosis or giant cell arteritis.

[0202] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with cardiovascular diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term cardiovascular diseases refers to arrhythmia (atrial or ventricular or both); atherosclerosis and its sequelae; angina; cardiac rhythm disturbances; myocardial ischemia; myocardial infarction; cardiac or vascular aneurysm; vasculitis, stroke; peripheral obstructive arteriopathy of a limb, an organ, or a tissue; reperfusion injury following ischemia of the brain, heart, kidney or other organ or tissue; endotoxic, surgical, or traumatic shock; hypertension, valvular heart disease, heart failure, abnormal blood pressure; vasoconstriction (including that associated with migraines); vascular abnormality, inflammation, or insufficiency limited to a single organ or tissue. More particularly, the term refers to atherosclerosis or giant cell arteritis.

[0203] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of dermatological diseases. In particular, the term dermatological diseases refers to atopic dermatitis, bullous disorders, collagenoses, psoriasis, psoriatic lesions, dermatitis, contact dermatitis, eczema, vitiligo, pruritus, scleroderma, wound healing, scarring, hypertrophic scarring, keloids, Kawasaki disease, rosacea, Sjögren-Larsson syndrome, or urticaria. More particularly, the term refers to psoriasis, psoriatic lesions, scleroderma, and vitiligo.

[0204] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of dermatological diseases. In particular, the term dermatological diseases refers to atopic dermatitis, bullous disorders, collagenoses, psoriasis, psoriatic lesions, dermatitis, contact dermatitis, eczema, vitiligo, pruritus, scleroderma, wound healing, scarring, hypertrophic scarring, keloids, Kawasaki disease, rosacea, Sjögren-Larsson syndrome, or urticaria. More particularly, the term refers to psoriasis, psoriatic lesions, scleroderma, and vitiligo.

[0205] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with dermatological diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term dermatological diseases refers to atopic dermatitis, bullous disorders, collagenoses, psoriasis, psoriatic lesions, dermatitis, contact dermatitis, eczema, vitiligo, pruritus, scleroderma, wound healing, scarring, hypertrophic scarring,

keloids, Kawasaki disease, rosacea, Sjögren-Larsson syndrome, or urticaria. More particularly, the term refers to psoriasis, psoriatic lesions, scleroderma, and vitiligo.

[0206] In one embodiment, the present invention provides compounds of the invention or pharmaceutical compositions comprising a compound of the invention, for use in the prophylaxis and/or treatment of abnormal angiogenesis associated diseases. In particular, the term abnormal angiogenesis associated diseases refers to atherosclerosis, hypertension, tumor growth, inflammation, rheumatoid arthritis, wet-form macular degeneration, choroidal neovascularization, retinal neovascularization, and diabetic retinopathy. More particularly, the term refers to atherosclerosis, hypertension, or diabetic retinopathy.

[0207] In another embodiment, the present invention provides the use of compounds of the invention or pharmaceutical compositions comprising a compound of the invention in the manufacture of a medicament for the prophylaxis and/or treatment of abnormal angiogenesis associated diseases. In particular, the term abnormal angiogenesis associated diseases refers to atherosclerosis, hypertension, tumor growth, inflammation, rheumatoid arthritis, wet-form macular degeneration, choroidal neovascularization, retinal neovascularization, and diabetic retinopathy. More particularly, the term refers to atherosclerosis, hypertension, or diabetic retinopathy.

[0208] In additional methods of treatment aspects, this invention provides methods of prophylaxis and/or treatment of a mammal afflicted with abnormal angiogenesis associated diseases, which methods comprise the administration of an effective amount of a compound of the invention or one or more of the pharmaceutical compositions herein described for the treatment or prophylaxis of said condition. In particular, the term abnormal angiogenesis associated diseases refers to atherosclerosis, hypertension, tumor growth, inflammation, rheumatoid arthritis, wet-form macular degeneration, choroidal neovascularization, retinal neovascularization, and diabetic retinopathy. More particularly, the term refers to atherosclerosis, hypertension, or diabetic retinopathy.

[0209] 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 titled to exceed about 1 g/day for a 40 to 80 kg human patient.

[0210] 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 four (1-4) regular doses daily, especially one to three (1-3) regular doses daily, typically one to two (1-2) regular doses daily, and most typically one (1) regular dose daily are representative regimens. Alternatively for long lasting effect drugs, with oral dosing, once every other week, once weekly, and once a day are representative regimens. In particular, dosage regimen can be every 1-14 days, more particularly 1-10 days, even more particularly 1-7 days, and most particularly 1-3 days.

[0211] Using these dosing patterns, each dose provides from about 1 to about 1000 mg of a compound of the invention, with particular doses each providing from about 10 to about 500 mg and especially about 30 to about 250 mg.

[0212] Transdermal doses are generally selected to provide similar or lower blood levels than are achieved using injection doses.

[0213] When used to prevent the onset of a condition, a 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.

[0214] A 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 compound of the inventions that demonstrate the same or a similar therapeutic activity and that are determined to be 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.

[0215] In one embodiment, a compound of the invention or a pharmaceutical composition comprising a compound of the invention is administered as a medicament. In a specific embodiment, said pharmaceutical composition additionally comprises a further active ingredient.

[0216] In one embodiment, a 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.

[0217] In one embodiment, a 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, JAK inhibitors (for example tofacitinib, baricitinib, Upadacitinib, ruxolitinib, or filgotinib), fostamatinib, and cyclosporin), and biological DMARDS (for example but without limitation infliximab, etanercept, adalimumab, rituximab, and abatacept).

[0218] In one embodiment, a 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, leucovorin, adriamycin, prednisone, 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 according to Formula I 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 leukemia.

[0219] In one embodiment, a 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 compound of the inventions, 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, etanercept, or adalimumab), mycophenolate, fingolimod and myriocin.

[0220] In one embodiment, a 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)).

[0221] In one embodiment, a 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: beta2-adrenoceptor agonists (e.g. salbutamol, levalbuterol, terbutaline and bitolterol), epinephrine (inhaled or tablets), anticholinergics (e.g. ipratropium bromide), glucocorticoids (oral or inhaled), long-acting β2-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. cetirizine, cinnarizine, fexofenadine) and vasoconstrictors (e.g. oxymethazoline, xylomethazoline, nafazoline and tramazoline).

[0222] Additionally, a 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.

[0223] In one embodiment, a 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).

[0224] In one embodiment, a 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: human monoclonal antibodies (belimumab (Benlysta®)), Disease-modifying antirheumatic drugs (DMARDs) such as antimalarials (e.g. 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.

[0225] In one embodiment, a 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 D3 analogues (for example, calcipotriol), argan oil and 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 (an IL-12 and IL-23 blocker). Additionally, a 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)).

[0226] In one embodiment, a 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.

[0227] 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.

CHEMICAL SYNTHETIC PROCEDURES

General

[0228] 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.

[0229] 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 (Wuts & Greene 2006).

[0230] The following methods are presented with details as to the preparation of a compound of the invention as defined hereinabove and the comparative examples. A 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.

[0231] All reagents are of commercial grade and are used as received without further purification, unless otherwise stated. Commercially available anhydrous solvents are used for reactions conducted under inert atmosphere. Reagent grade solvents are used in all other cases, unless otherwise specified. Column chromatography is performed on silica gel 60 (35-70 μm) or with Biotage® SNAP KP-NH, Biotage® SNAP Ultra, or Interchim® PuriFlash® Si HC flash chromatography cartridges. Thin layer chromatography is carried out using pre-coated silica gel F-254 plates (thickness 0.25 mm). Biotage® ISOLUTE® phase separators (*e.g.*, Cat# 120-1907-E) are used for aqueous phase separation. ^{1}H NMR spectra are recorded on a Bruker DPX 400 NMR spectrometer (400 MHz), a Bruker Avance 300 NMR spectrometer (300 MHz), or a Bruker Avance III HD 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, *e.g.* $CHCl_3$ (δ 7.27), as internal reference. Multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), multiplet (m) and broad (br). Electrospray MS spectra are obtained on a Waters Acquity H-Class or I-Class UPLC system coupled to a UV PDA detector and to a Waters SQD, SQD2 or QDa mass spectrometer. Columns used: Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm ID × 30/50 mm L; Waters Acquity UPLC CSH C18 1.7 μm, 2.1 mm ID × 100 mm L; Waters Acquity UPLC CSH PhenylHexyl 1.7 μm, 2.1 mm ID × 100 mm L; Waters Acquity UPLC HSS PFP 1.8 μm, 2.1 mm ID × 100 mm L. The methods are using ACN/water or MeOH/water gradients with either 0.1% formic acid in both mobile phases or 0.05% NH_3 in both mobile phases. Preparative HPLC is performed on a Waters AutoPurification system with UV and MS detection using Waters XBridge BEH C18 OBD 30 mm ID × 100 mm L columns and ACN/water gradients with either 0.1% formic acid in both mobile phases or 0.1% NH_4OH in both mobile phases. Microwave heating is performed with a Biotage® Initiator.

Table I. List of abbreviations used in the experimental section:

Abbreviation	Definition
ACN	acetonitrile
AcOH	acetic acid
ANOVA	analysis of variance
aq.	aqueous
ATP	adenosine 5′-triphosphate

Abbreviation	Definition
b.i.d.	bis in die (twice a day)
B_2pin_2	bis(pinacolato)diboron (CAS# 73183-34-3)
BUN	blood urea nitrogen
Cpd	compound

Abbreviation	Definition
d	doublet
DCM	dichloromethane
dd	doublet of doublets
DMF	dimethylformamide
DMSO	dimethylsulfoxide
dppf	1,1′-bis(diphenylphosphino)ferrocene (CAS# 12150-46-8)
DSS	dextran sodium sulfate
dt	doublet of triplets
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eq.	equivalent
% e.e.	Enantiomeric excess percentage
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
h	hour
HPLC	high-performance liquid chromatography
i.n.	intranasal
Int	intermediate
i.p.	intraperitoneal
$[Ir(OCH_3)(COD)]_2$	(1,5-cyclooctadiene)(methoxy)iridium(I)dimer (CAS# 12148-71-9)
i.v.	intravenous
LCMS	liquid chromatography-mass spectrometry

Abbreviation	Definition
LPS	lipopolysaccharide
m	multiplet
2-MeTHF	2-methyltetrahydrofuran
MeOH	methanol
min	minute
mmol	millimole
MS	mass spectrometry
MTBE	methyl *tert*-butyl ether
Mtd	method
MW	molecular weight
MW (calcd)	molecular weight calculated
MW (obsd)	molecular weight observed
N-ter	N-terminal
NA	not available
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
$Pd_2(dba)_3$	tris(dibenzylideneacetone)dipalladium(0) (CAS# 51364-51-3)
Pd/C	palladium on carbon
$Pd(dppf)Cl_2$	[1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (CAS# 72287-26-4)
$Pd(dppf)Cl_2 \cdot DCM$	[1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (CAS# 95464-05-4)
PIN	percentage inhibition

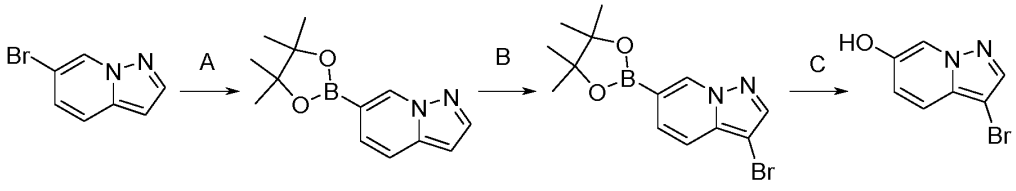
Abbreviation	Definition
p.o.	*per os*
ppm	parts-per-million
q	quartet
q.d.	*quaque die* (once a day)
RuPhos	2-dicyclohexylphosphino-2′,6′-diisopropoxy-1,1′-biphenyl (CAS# 787618-22-8)
RT	room temperature
s	singlet
sat.	saturated
sc	subcutaneous
SEM	standard error of the mean
sept.	septet
SFC	supercritical fluid chromatography

Abbreviation	Definition
SM	starting material
t	triplet
t-BuOH	*tert*-butanol
t-BuOK	potassium *tert*-butoxide
td	triplet of doublets
THF	tetrahydrofuran
XPhos	2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl (CAS# 564483-18-7)
R* S*	* next to R or/and S stereochemistry indicators means that the stereochemistry has been arbitrarily assigned

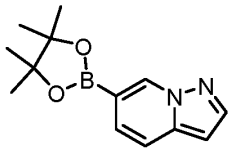
SYNTHETIC PREPARATION OF THE COMPOUNDS OF THE INVENTION

Example 1. Preparation of the compounds of the invention and comparative compounds

1.1. Int 1



1.1.1. Step A: 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine



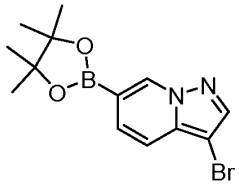
[0232] To a suspension of 6-bromopyrazolo[1,5-a]pyridine (CAS# 1264193-11-4; 148 g, 751.15 mmol, 1 eq.) and bis(pinacol)diborane (209.82 g, 826.26 mmol, 1.1 eq.) in 2-MeTHF (1.5 L) were added $Pd(OAc)_2$ (4.22 g, 18.78 mmol, 0.025 eq.), KOAc (221.15 g, 2.25 mol, 3 eq.) and XPhos (17.90 g, 37.56 mmol, 0.05 eq.) at 25 °C. The suspension was degassed and purged with N_2 three times and stirred at 90 °C under N_2

for 12 h. The reaction mixture was cooled down to 25 °C, diluted with DCM (1.5 L), filtered through a thin layer of celite. The mother liquor was extracted with DCM (2 L) and H_2O (2 L), dried with $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The crude product was purified by silica gel chromatography (eluting with EtOAc/petroleum ether 12/88) to give 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine.

[0233] LCMS: MW (calcd): 401.2; m/z MW (obsd): 162.9 (M+H of corresponding boronic acid)

[0234] ^{1}H NMR (400 MHz, CDCl3): δ 8.79 (d, 1H), 7.90 (d, 1H), 7.41 (dd, 1H), 7.28 (dd, 1H), 6.40 (d, 1H), 1.28 (s, 12H)

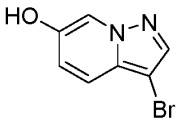
1.1.2. Step B: 3-bromopyrazolo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine



[0235] To a solution of 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine (173 g, 708.74 mmol, 1 eq.) in ACN (1.6 L) was added NBS (126.14 g, 708.74 mmol, 1 eq.), and the mixture was stirred at 25 °C for 12 h. The reaction mixture was diluted with EtOAc (2 L) at 25 °C, washed with H_2O (1 L) and brine (1 L), and the organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to give 3-bromopyrazolo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine.

[0236] ^{1}H NMR: (400 MHz, $CDCl_3$): δ 8.74 (s, 1 H), 7.89 (s, 1H), 7.39 (s, 2 H), 1.28 (s, 12 H)

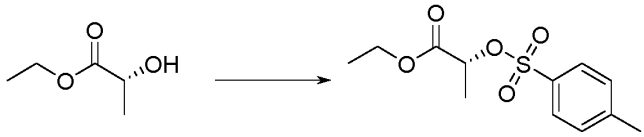
1.1.3. Step C: Int 1



[0237] To a solution of 3-bromopyrazolo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyridine (142 g, 439.64 mmol, 1 eq.) in THF (1.25 L) was added dropwise NaOH (2 M in water, 659.46 mL, 3 eq.) and H_2O_2 (168.63 g, 1.49 mol, 142.90 mL, 30% w/w in H_2O, 3.38 eq.) at 0 °C. The reaction was stirred at 0-5 °C for 2 h. The mixture was quenched with 10% Na_2SO_3 (2 L) and stirred at 25 °C for 2 h, then checked by potassium iodide starch paper. The mixture was acidified with 2N HCl to pH 5, filtered and concentrated under reduced pressure to give product. The crude product was purified by column chromatography on silica gel (eluting with DCM/THF 9/1 to 7/3) and further purified by SFC (column: Daicel CHIRALPAK IG (250 mm L × 50 mm ID, 10 μm); CO_2 back pressure: 100 bar; co-solvent: 0.1% NH_4OH in EtOH; flowrate: 220 mL/min; mode: isocratic 15%; temperature: 40 °C) to give **Int 1**.

[0238] ^{1}H NMR: (400 MHz, DMSO-d6): δ 9.85 (s, 1H), 8.15 (dd, 1H), 7.95 (s, 1H), 7.46 (d, 1H), 7.10 (dd, 1H)

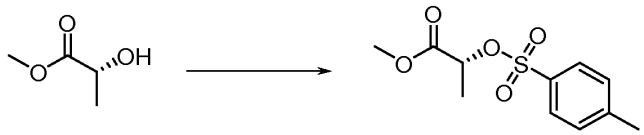
1.2. Int 2



[0239] In a 15 L single jacketed process reactor equipped with mechanical stirring (200 rpm) and baffle were charged DCM (4 L, 6.7V) and 4-methylbenzenesulfonyl chloride (873 g, 4571 mmol, 0.9 eq.). Then 4-dimethylaminopyridine (73 g, 598 mmol, 0.2 eq.) was added. DCM (1.5 L, 2.5V) was used to rinse the containers and funnels and was added to the reaction mixture. The reaction mixture was cooled down to 0 °C and ethyl (R)-(+) lactate (CAS# 7699-00-5; 600 g, 5079 mmol, 1.00 eq.) was added in one portion. DCM (0.5 L, 0.8V) was used to rinse the containers and funnels used, and added to the reaction mixture. Triethylamine (1.42 L, 10200 mmol, 2.01 eq.) was added dropwise, while maintaining reaction temperature below 5 °C. The reaction mixture was stirred (250 rpm) at 0 °C for a further 1.5 h. HCl 2N (3.7 L, 6V) was slowly added under cooling, maintaining reaction temperature below 5 °C, until pH reached 2-3. The layers were separated and the organic layer washed with water (2 L, 3V) and 10% NaCl in water (2 L, 3V), then concentrated under vacuum to give 1.228 kg of crude product. The crude product was purified by flash chromatography using heptane/EtOAc 100/0 to 70/30 as eluting system to afford **Int 2**.

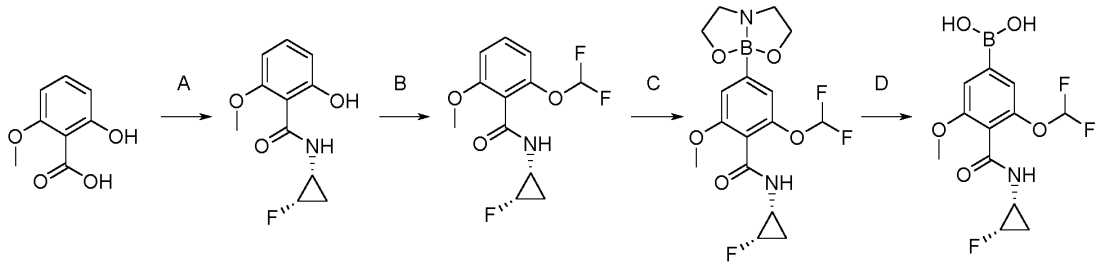
[0240] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 – 7.77 (m, 2H), 7.53 – 7.45 (m, 2H), 4.99 (q, 1H), 4.05 (qd, 2H), 2.43 (s, 3H), 1.38 (d, 3H), 1.12 (t, 3H)

1.3. Int 3

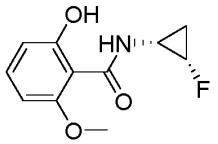


[0241] In a 5 L reactor equipped with baffles and under N_2 atmosphere, triethylamine (250.32 mL, 182.24 g, 1800.87 mmol, 2 eq.) was added to a solution of methyl (R)-(+)-lactate (CAS# 17392-83-5; 93.74 g, 86 mL, 900.44 mmol, 1 eq.), tosyl chloride (163.076 g, 855.42 mmol, 0.95 eq.) and DMAP (20 g, 163.705 mmol, 0.18 eq.) in DCM (1000 mL) cooled at -5 °C (jacket temperature). The addition lasted 1 h with reaction temperature kept under 7 °C. The reaction mixture was then stirred at 0-5 °C for 2.2 h. HCl 2 M (500 mL, 6V) was added until pH <4 while keeping the reaction mixture temperature below 10 °C. The organic phase was washed with water (500 mL, 5V) and a 10% aqueous NaCl solution (300 mL, 3V). The organic phase was concentrated and purified by chromatography on silica gel (eluting with heptane/EtOAc 100/0 to 70/30) to afford **Int 3**.

1.4. Int 4

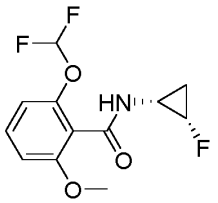


1.4.1. Step A: N-[(1R,2S)-2-fluorocyclopropyl]-2-hydroxy-6-methoxy-benzamide



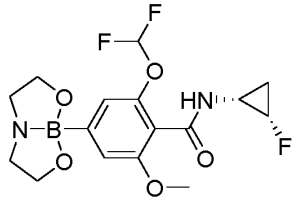
[0242] To a solution of 2-hydroxy-6-methoxy-benzoic acid (CAS# 3147-64-6; 50 g, 297 mmol, 1 eq.) in DCM (300 mL) was added 1,1'-carbonyldiimidazole (CAS# 530-62-1; 49 g, 297 mmol, 1 eq.) portion wise. Then (1R,2S)-2-fluorocyclopropanamine 4-methylbenzenesulfonate (CAS# 143062-84-4; 80 g, 312 mmol, 1.05 eq.) and triethylamine (50 mL, 359 mmol, 1.21 eq.) were added to the mixture. The resulting solution was stirred at RT for 2.5 h. Water was added and the pH adjusted to 2 using a 12N HCl solution (60 mL). The organic layer was separated and the aqueous layer extracted twice with DCM. The combined organic layers were washed three times with a sat. $NaHCO_3$ solution, and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (eluting with DCM/EtOAc 100/0 to 90/10) to afford *N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-2-hydroxy-6-methoxy-benzamide.

1.4.2. Step B: 2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-6-methoxy-benzamide



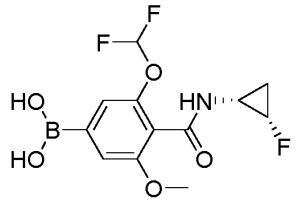
[0243] In a 3-necked round-bottom flask equipped with a temperature probe and a funnel, containing a solution of *N*-[(1*R*,2S)-2-fluorocyclopropyl]-2-hydroxy-6-methoxy-benzamide (29 g, 130 mmol, 1 eq.) in a mixture ACN/water (146 mL/146 mL) at 5 °C was added potassium hydroxide (73 g, 1298 mmol, 10 eq.) over 1 h. Bromodifluoromethyl diethylphosphonate (CAS# 65094-22-6; 46 mL, 260 mmol, 2 eq.) was added neat dropwise over 40 min while keeping the reaction temperature below 12 °C. The reaction mixture was allowed to warm to RT and was stirred at RT for 40 min. The reaction mixture was then extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was resuspended in MTBE (80 mL). The suspension was filtered, the solid washed with MTBE (20 mL) and dried to afford 2-(difluoromethoxy)-N-[(1R, 2S)-2-fluorocyclopropyl]-6-methoxy-benzamide.

1.4.3. Step C: 2-(difluoromethoxy)-4-(2,8-dioxa-5-azonia-1-boranuidabicyclo[3.3.0]octan-1-yl)-N-[(1R,2S)-2-fluorocyclopropyl]-6-methoxy-benzamide



[0244] To a solution of 2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-6-methoxy-benzamide (20.8 g, 76 mmol, 1.0 eq.) in THF (85 mL, 1040 mmol, 13.8 eq.) were added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (CAS# 73183-34-3; 19.2 g, 76 mmol, 1.0 eq.), 4,4'-di-*tert*-butyl-2,2'-bipyridine (CAS# 72914-19-3; 0.83 g, 3.0 mmol, 0.04 eq.) and $[Ir(OCH_3)(COD)]_2$ (CAS# 12148-71-9; 0.51 g, 0.76 mmol, 0.01 eq.). The reaction mixture was heated to reflux for 1 h, then cooled to RT. Diethanolamine (CAS# 111-42-2; 7.3 mL, 76 mmol, 1.0 eq.) was added and the reaction mixture was stirred at RT for 30 min. The suspension was filtered. The cake was washed with THF (80 mL) and dried to give 2-(difluoromethoxy)-4-(2,8-dioxa-5-azonia-1-boranuidabicyclo[3.3.0]octan-1-yl)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-6-methoxy-benzamide.

1.4.4. Step D: Int 4



[0245] A suspension of 2-(difluoromethoxy)-4-(2,8-dioxa-5-azonia-1-boranuidabicyclo[3.3.0]octan-1-yl)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-6-methoxy-benzamide (29.2 g, 76 mmol, 1.0 eq.) in hydrochloric acid (1 mol/L) in deionized water (CAS# 7647-01-0; 115 mL, 115 mmol, 1.5 eq) was stirred at RT for 40 min. The suspension was filtered. The cake was washed with water (200 mL, pH 5) and the powder was dried overnight in a 45 °C oven to afford **Int 4**.

1.5. Int 5

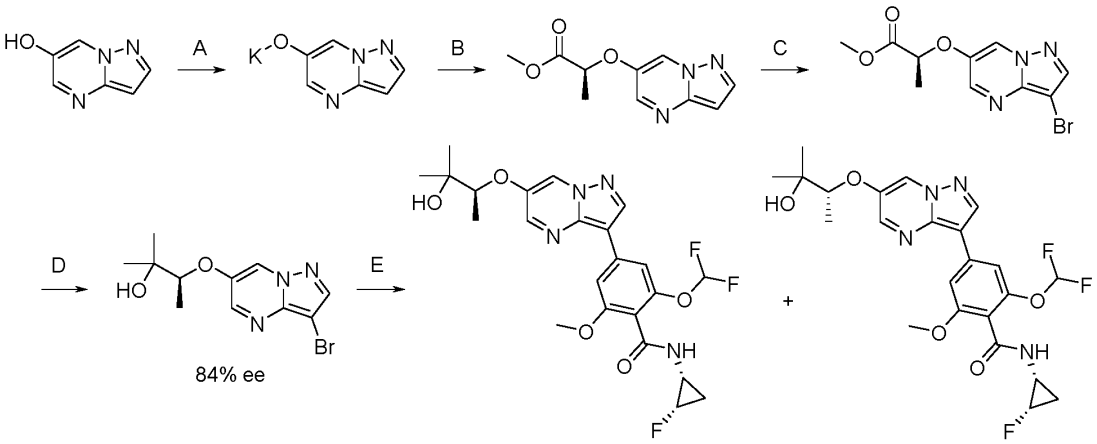


[0246] In a 15 L single jacketed process reactor equipped with mechanical stirring (200 rpm) and baffle, were charged THF (8.5 L, 17V) and **Int 1** (500 g, 2347 mmol, 1.0 eq.). The reaction mixture was stirred for 10 min at RT, until full dissolution of substrate, then cooled down to 11 °C. *t*-BuOK (85 g, 742 mmol, 0.32 eq.) was added. When the reaction temperature reached 15 °C the jacket temperature was increased from 5 °C to 10 °C and the stirring was increased to 250 rpm. When the reaction temperature reached 12 °C, additional *t*BuOK (170 g, 1484 mmol, 0.64 eq.) was added. Following the addition of the base, a thick

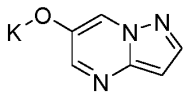
suspension formed rapidly. The jacket temperature was set at 22 °C and the stirring increased to 300 rpm. The reaction mixture was stirred for one further hour after reaching a temperature of 20 °C. The reaction mixture was filtered on a sintered funnel, the solid was washed with THF (3V), triturated with MTBE (3V), then filtered and collected. The resulting solid was dried under vacuum (3 days at 35 °C, then one day at RT) to afford **Int 5**.

[0247] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (s, 1H), 7.21 (d, 1H), 6.90 (d, 1H), 6.59 (dd, 1H)

1.6. Cpd 1 & Cpd 8



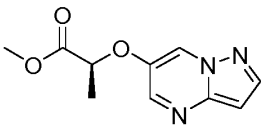
1.6.1. Step A: potassium pyrazolo[1,5-a]pyrimidin-6-olate



[0248] In a round bottom flask, a suspension of pyrazolo[1,5-a]pyrimidin-6-ol (CAS# 1580489-59-3; 25.0 g, 185 mmol, 1.00 eq.) in THF (500 mL) was heated at 60 °C for 1 h. The suspension was cooled down to 0 °C and potassium *tert*-butoxide (19.7 g, 176 mmol, 0.95 eq.) was added in two portions (first 9.0 g and 10 min later, 10.7 g). The reaction mixture was stirred at RT for 1.5 h. The suspension was filtered and the solid was washed with THF. The solid was triturated in MTBE directly on the fritted funnel. The solid was collected and dried under vacuum at 35 °C for 3 h.

[0249] ^{1}H NMR (400 MHz, DMSO-d6) δ 7.79 (d, 1H), 7.43 (d, 1H), 7.30 (dd, 1H), 6.09 (dd, 1H)

1.6.2. Step B: methyl (2S)-2-pyrazolo[1,5-a]pyrimidin-6-yloxypropanoate

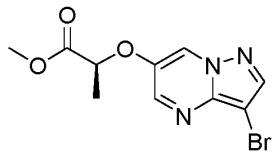


[0250] In a round bottom flask, to a solution of potassium pyrazolo[1,5-a]pyrimidin-6-olate (4.0 g, 23 mmol, 1.0 eq.) in DMF (32 mL) at 0 °C was added a solution of **Int 3** (6.6 g, 25 mmol, 1.1 eq.) in DMF (8.0 mL). The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched by slow addition of aq. K_2CO_3 10%. Water and EtOAc were then added, and the layers were separated. The aqueous

layer was extracted with EtOAc (four times), the organic layers were combined, washed with aq. LiCl 10% (twice), NaCl 10%, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified by flash chromatography with a Biotage® Sfär HC 100 g cartridge, eluting with heptane/EtOAc 100/0 to 0/100 to afford the titled product.

[0251] LCMS: MW (calcd): 221.0; m/z MW (obsd): 221.8 (M+H)

1.6.3. Step C: methyl (2S)-2-(3-bromopyrazolo[1,5-a]pyrimidin-6-yl)oxypropanoate

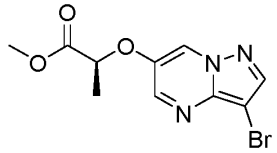


[0252] In a round bottom flask, to a solution of methyl (2S)-2-pyrazolo[1,5-a]pyrimidin-6-yloxypropanoate (2.4 g, 11 mmol, 1.0 eq.) in ACN (48 mL) at 0 °C was added NBS (2.1 g, 1.0 mL, 12 mmol, 1.1eq.). The reaction mixture was stirred at RT for 45 min. The mixture was diluted in DCM and the organic layer was washed with aq. $NaHCO_3$ 5%, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography with a Biotage® Sfär HC 100 g cartridge, eluting with heptane/EtOAc 100/0 to 75/25 to afford the titled product.

[0253] LCMS: MW (calcd): 299.0; m/z MW (obsd): 299.7 / 301.7 (M+H)

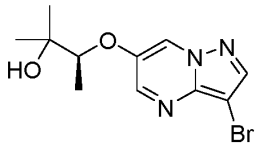
[0254] ^{1}H NMR (400 MHz, CD3OD) δ 8.62 (d, 1H), 8.51 (d, 1H), 8.06 (s, 1H), 5.02 (q, 1H), 3.78 (s, 3H), 1.66 (d, 3H)

1.6.4. Alternative Step C: methyl (2S)-2-(3-bromopyrazolo[1,5-a]pyrimidin-6-yl)oxypropanoate



[0255] In a 15 L single jacketed reactor were charged acetonitrile (3500 mL), 3-bromopyrazolo[1,5-a]pyrimidin-6-ol (350 g, 1635 mmol, 1.0 eq.), methyl (2R)-2-[(4-methylbenzenesulfonyl)oxy]propanoate (CAS 1314893-97-4, 412 g, 1595 mmol, 1.0 eq., 94.8 % e.e.) and potassium carbonate (315 g, 2279 mmol, 1.4 eq,). The resulting mixture was heated to 55°C in 30 minutes, stirred at 55 °C for 17h, then cooled down to 20°C. The suspension was filtered, and the cake was washed with EtOAc (4000 mL). The filtrate was washed with NaCl 10% solution (1200 mL). The organic layer was recovered and concentrated until 1-1.1kg, then MTBE (1000 mL) was added and stirred at 0-5°C for 30 min. The suspension was filtered and washed with MTBE (1000 mL), the resulting cake was dried under vacuum at 40°C to afford the title product.

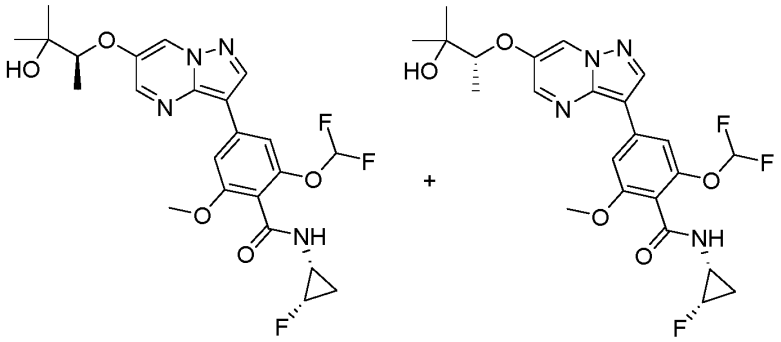
1.6.5. Step D: (3S)-3-(3-bromopyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol



[0256] In a round bottom flask, to a suspension of methyl (2S)-2-(3-bromopyrazolo[1,5-a]pyrimidin-6-yl)oxypropanoate (3100 mg, 10 mmol, 1.0 eq.) at 0 °C in 2-MeTHF (31 mL) was added dropwise a methylmagnesium bromide solution (3.0 mol/L in 2-MeTHF; 10 mL, 31 mmol, 3.0 eq.). The reaction mixture was stirred at 0 °C for 15 min. The mixture was then quenched with hydrochloric acid (1 mol/L) in water (36 mL, 36 mmol, 3.5eq.) and extracted with 2-MeTHF (twice). The yellow organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography with a Biotage® Sfär HC 100 g cartridge, eluting with heptane/EtOAc 100/0 to 0/100 to afford the titled product, with a measured enantiomeric excess of 84%.

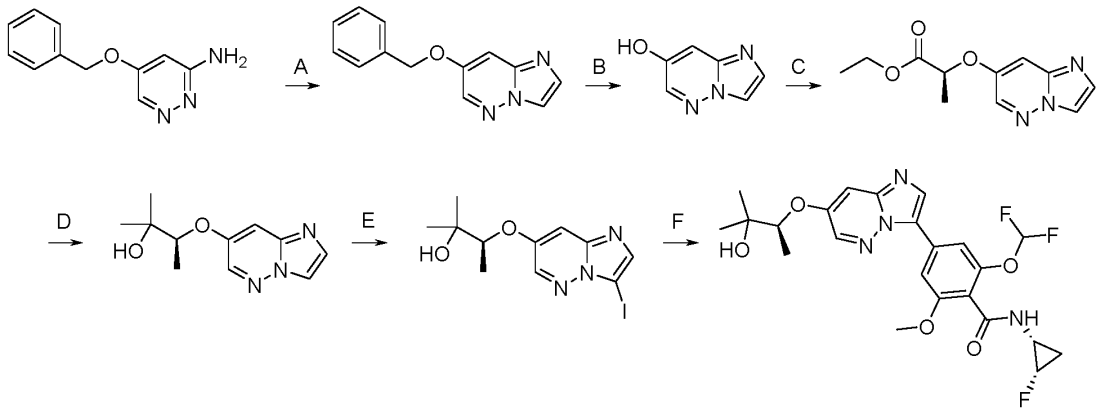
[0257] LCMS: MW (calcd): 299.0; m/z MW (obsd): 299.8 / 301.8 (M+H)

1.6.6. Step E: Cpd 1 & Cpd 8

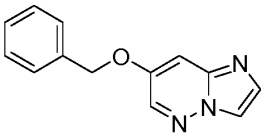


[0258] In a round bottom flask, to a solution of (3S)-3-(3-bromopyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol (1000 mg, 3.33 mmol, 1,00 eq.) in a mixture of 1,4-dioxane (8 mL) and water (2 mL) were added **Int 4** (1382 mg, 4.33 mmol, 1.30 eq.), sodium carbonate (1059 mg, 10.0 mmol, 3.00 eq.), RuPhos (CAS# 787618-22-8; 31.7 mg, 0.067 mmol, 0.02 eq.) and $Pd(OAc)_2$ (7.5 mg, 0.033 mmol, 0.010 eq.). The reaction mixture was stirred at 90 °C for 1 h. RuPhos (31.7 mg, 0.067 mmol, 0.02eq.) and $Pd(OAc)_2$ (7.48 mg, 0.033 mmol, 0.01eq.) were added and the reaction mixture was stirred at 90 °C for 1 h. The reaction mixture was filtered on Clarcel® diatomaceous earth and rinsed with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography with a Biotage® Sfär HC 100 g cartridge, eluting with heptane/EtOAc 100/0 to 0/100. **Cpd 1** and **Cpd 8** were separated by chiral SFC (**Cpd 8** retention time: 3.70 min, **Cpd 1** retention time: 5.65 min) using the following conditions: Chiralpak IA, 150 × 4.6 mm, 5 µm column; CO_2 back pressure: 1500 psi; co-solvent: EtOH; flowrate: 3 mL/min; mode: isocratic 30%; temperature: 40 °C.

1.7. Cpd 2



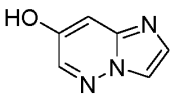
1.7.1. Step A: 7-benzyloxyimidazo[1,2-b]pyridazine



[0259] In a round bottom flask were introduced 3-amino-5-(benzyloxy)pyridazine (CAS# 2155875-87-7; 1 g, 4.72 mmol, 1.00 eq.), sodium bicarbonate (0.793 g, 9.44 mmol, 2.00 eq.) and 2-propanol (20 mL). Then a chloroacetaldehyde solution (55 mass%) in water (6.74 g, 5.45 mL, 47.21 mmol, 10.00 eq.) was added. The resulting mixture was heated to 90 °C. The reaction mixture was quenched by addition of water and then extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a Biotage® Sfär HC 25 g column, diluting with DCM, and eluting with heptane/EtOAc 98/2 to 50/50. The corresponding fractions were pooled together and concentrated *in vacuo* to deliver the titled product.

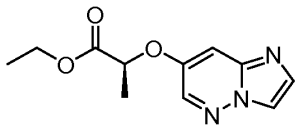
[0260] LCMS: MW (calcd): 225,0; m/z MW (obsd): 226.3 (M+H)

1.7.2. Step B: imidazo[1,2-b]pyridazin-7-ol



[0261] In a round bottom flask, under N_2 atmosphere, were introduced 7-benzyloxyimidazo [1,2-b]pyridazine (0.89 g, 3.95 mmol, 1.0 eq.) and MeOH (15 mL). Palladium hydroxide 20 wt% on carbon (0.555 g, 0.79 mmol, 0.20 eq.) was then added. The reaction mixture was degassed, backfilled with H_2 and stirred at RT for 1.5 h. The mixture was filtered on Clarcel® diatomaceous earth. The residue was washed with MeOH, and the filtrate was concentrated *in vacuo* to afford the titled product.

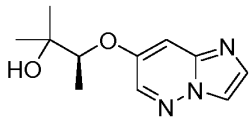
1.7.3. Step C: ethyl (2S)-2-imidazo[1,2-b]pyridazin-7-yloxypropanoate



[0262] To a solution of imidazo[1,2-b]pyridazin-7-ol (0.46 g, 3.41 mmol, 1.00 eq.) in DMF (6.8 mL) under N_2 were added **Int 2** (0.881 g, 3.23 mmol, 0.95 eq.) and potassium carbonate (0.941 g, 6.81 mmol, 2.00 eq.). The mixture was stirred at 70 °C for 2.5 h. The mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a Biotage® Sfär HC 25 g column, diluting with DCM, and eluting with heptane/EtOAc 98/2 to 0/100. The corresponding fractions were pooled together and concentrated *in vacuo* to deliver the titled product.

[0263] LCMS: MW (calcd): 235.1; m/z MW (obsd): 236.1 (M+H)

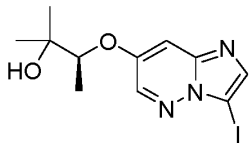
1.7.4. Step D: (3S)-3-imidazo[1,2-b]pyridazin-7-yloxy-2-methyl-butan-2-ol



[0264] To an iced cold solution of ethyl (2S)-2-imidazo[1,2-b]pyridazin-7-yloxypropanoate (560 mg, 2.38 mmol, 1.00 eq.) in THF (4.8 mL) was added a methylmagnesium bromide solution (3.0 mol/L) in diethyl ether (1.7 mL, 5.24 mmol, 2.20eq.). The reaction mixture was stirred at 0 °C for 45 min, then allowed to warm to RT. The reaction mixture was quenched after 1 h by addition of water and then extracted with EtOAc and a few drops of MeOH. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a Biotage® Sfär KP-NH 11 g column, diluting with DCM, eluting with DCM/MeOH 100/0 to 90/10. The corresponding fractions were pooled together and concentrated *in vacuo* to deliver the titled product.

[0265] LCMS: MW (calcd): 221.2; m/z MW (obsd): 222.2 (M+H)

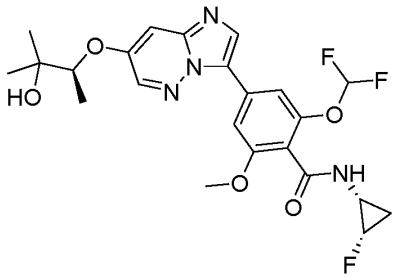
1.7.5. Step E: (3S)-3-(3-iodoimidazo[1,2-b]pyridazin-7-yl)oxy-2-methyl-butan-2-ol



[0266] To a solution of (3S)-3-imidazo[1,2-b]pyridazin-7-yloxy-2-methyl-butan-2-ol (0.385 g, 1.74 mmol, 1.00 eq.) in DMF (7 mL) was added NIS (0.419 g, 1.83 mmol, 1.05 eq.) The mixture was stirred at RT for 40 min, then quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the titled product.

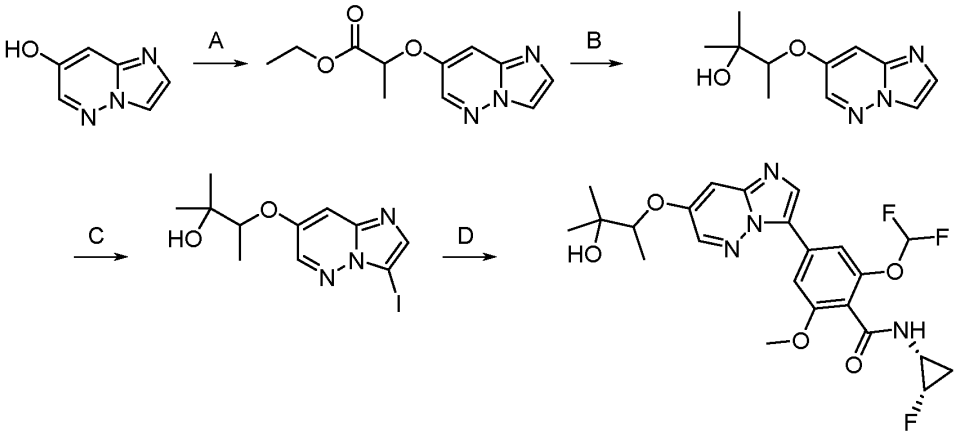
[0267] LCMS: MW (calcd): 347.0; m/z MW (obsd): 348.0 (M+H)

1.7.6. Step F: Cpd 2

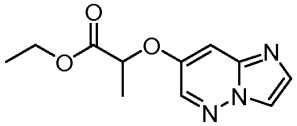


[0268] Ta solution of (3S)-3-(3-iodoimidazo[1,2-b]pyridazin-7-yl)oxy-2-methyl-butan-2-ol (0.604 g, 1.74 mmol, 1.00 eq.) in 1,4-dioxane (5.8 mL) and water (0.6 mL) were added **Int 4** (0.610 g, 1.914 mmol, 1.10 eq.), cesium carbonate (1.70 g, 5.22 mmol, 3.00eq.) and Pd(dppf)Cl_2·DCM (CAS#95464-05-4; 0.15 g, 0.174 mmol, 0.10 eq.). The reaction was heated at 100 °C for 1 h. The reaction mixture was concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a Biotage® Sfär KP-NH 28 g column, diluting with DCM, eluting with DCM/MeOH 100/0 to 95/5. The corresponding fractions were pooled together and concentrated *in vacuo* to deliver the titled product.

1.8. Cpd 3



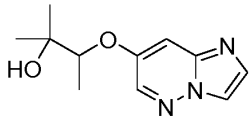
1.8.1. Step A: ethyl 2-imidazo[1,2-b]pyridazin-7-yloxypropanoate



[0269] In a microwave vial were placed imidazo[1,2-b]pyridazin-7-ol (CAS# 2410998-35-3; 0.12 g, 0.888 mmol, 1.00 eq.) and ethyl 2-hydroxypropionate (CAS# 97-64-3; 0.243 g, 1.95 mmol, 2.20 eq.) in dry toluene (2.14 mL) under an N_2 atmosphere. Cyanomethylenetributylphosphorane (CAS#157141-27-0; 0.486 g, 0.549 mL, 1.95 mmol, 2.20 eq.) was added using a syringe. The reaction mixture was heated at 80 °C for 6 h. The mixture was concentrated under reduced pressure. The crude was purified by column chromatography on a 10 g Biotage® Sfär HC column, eluting with DCM/MeOH 100/0 to 90/10, to afford the titled product.

[0270] LCMS: MW (calcd): 235.1; m/z MW (obsd): 236.1 (M+H)

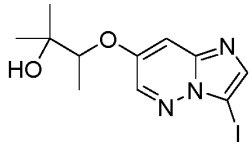
1.8.2. Step B: 3-imidazo[1,2-b]pyridazin-7-yloxy-2-methyl-butan-2-ol



[0271] To a 0 °C solution of ethyl 2-imidazo[1,2-b]pyridazin-7-yloxypropanoate (200 mg, 0.850 mmol, 1.00 eq.), in THF (1.7 mL) was added a methylmagnesium bromide solution (3.0 mol/L) in Et2O (0.62 mL, 1.8704 mmol, 2.20 eq.). The reaction mixture was stirred at 0 °C and allowed to warm to RT overnight. The reaction mixture was quenched by addition of water and then extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude residue was purified by column chromatography on a 5 g Biotage® Sfär HC column, eluting with DCM/MeOH 100/0 to 90/10 to afford the titled product.

[0272] LCMS: MW (calcd): 221.1; m/z MW (obsd): 222.0 (M+H)

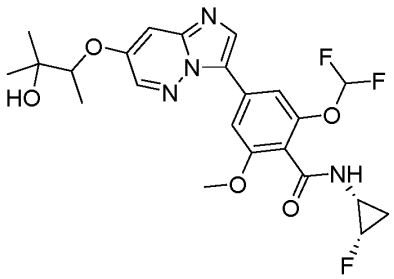
1.8.3. Step C: 3-(3-iodoimidazo[1,2-b] pyridazin-7-yl)oxy-2-methyl- butan-2-ol



[0273] To a solution of 3-imidazo[1,2-b]pyridazin-7-yloxy-2-methyl-butan-2-ol (0.047 g, 0.212 mmol, 1.00 eq.) in DMF (0.425 mL) was added NIS (0.0536 g, 0.234 mmol, 1.10 eq.) The mixture was stirred 40 min at RT. The mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the titled product.

LCMS: MW (calcd): 347.0; m/z MW (obsd): 348.4 (M+H)

1.8.4. Step D: Cpd 3

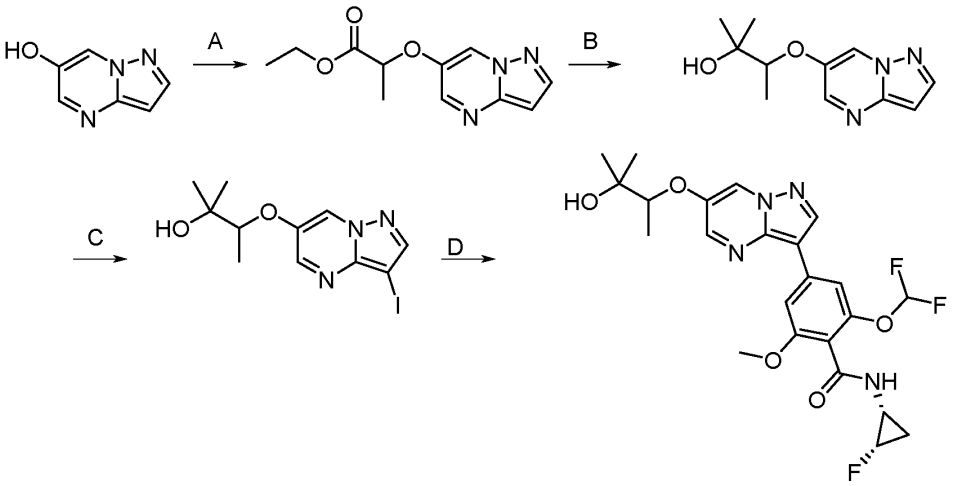


[0274] To a solution of 3-(3-iodoimidazo[1,2-b]pyridazin-7-yl)oxy-2-methyl-butan-2-ol (0.073 g, 0.210 mmol, 1.00 eq.) in 1,4-dioxane (0.70 mL) and water (0.07 mL) were added **Int 4** (0.06708 g, 0.210 mmol, 1.00 eq.), cesium carbonate (0.13703 g, 0.420 mmol, 2.00 eq.), and $Pd(dppf)Cl_2 \cdot DCM$ (0.0181 g, 0.0210 mmol, 0.10 eq.). The reaction was heated to 95 °C for 8 h. The mixture was concentrated under reduced pressure and purified by preparative LCMS to afford the titled product.

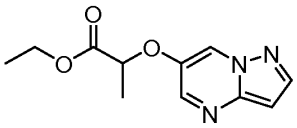
Preparative LCMS conditions: Waters XBridge Prep C18 OBD 30 × 100 mm 5 µm column with XBridge Prep C18 30 × 10mm 5 µm pre-column; time: 8 min; solvents: A=water + 0.1% formic acid, B=ACN + 0.1% formic acid; flow : 50 mL/min; gradient:

Time (min)	0	1.20	5.70	5.80	7.80	7.90	8.00
%B	5	25	40	100	100	5	5
%A	95	75	60	0	0	95	95

1.9. Cpd 4



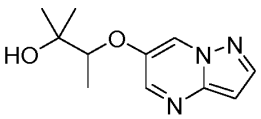
1.9.1. Step A: ethyl 2-pyrazolo[1,5-a] pyrimidin-6-yloxypropanoate



[0275] In a microwave vial were placed pyrazolo[1,5-a]pyrimidin-6-ol (CAS# 1580489-59-3; 0.1 g, 0.703 mmol, 1.00 eq.) and ethyl 2-hydroxypropionate (CAS# 97-64-3; 0.192 g, 1.547 mmol, 2.20 eq.) in dry toluene (1.70 mL) under an N_2 atmosphere. Cyanomethylenetributylphosphorane (CAS# 157141-27-0; 0.385 g, 0.434 mL, 1.547 mmol, 2.20 eq.) was added using a syringe. The reaction mixture was heated at 80 °C for 6 h. The mixture was concentrated under reduced pressure. The crude was purified by column chromatography on a 10 g Biotage® Sfär HC column, eluting with DCM/MeOH 100/0 to 90/10, to afford the titled product.

[0276] LCMS: MW (calcd): 235.1; m/z MW (obsd): 236.1 (M+H)

1.9.2. Step B: 2-methyl-3-pyrazolo[1,5-a] pyrimidin-6-yloxy-butan-2-ol

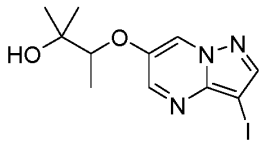


[0277] To a 0 °C solution of ethyl 2-pyrazolo[1,5-a]pyrimidin-6-yloxypropanoate (44 mg, 0.187 mmol, 1.00 eq.), in THF (0.374 mL) was added a methylmagnesium bromide solution (3.0 mol/L) in Et2O (0.16

mL, 0.467 mmol, 2.50 eq.). The reaction mixture was stirred at 0°C and allowed to warm to RT overnight. The reaction mixture was quenched by addition of water and then extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude residue was purified by flash chromatography on a 5 g Biotage® Sfär HC column, eluting with DCM/MeOH 100/0 to 90/10 to afford the titled product.

[0278] LCMS: MW (calcd): 221.1; m/z MW (obsd): 222.0 (M+H)

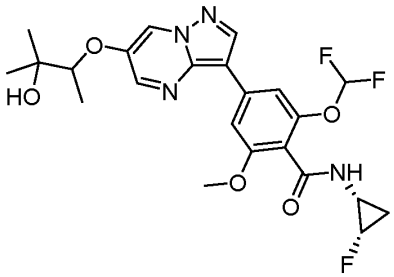
1.9.3. Step C: 3-(3-iodopyrazolo[1,5-a] pyrimidin-6-yl)oxy-2-methyl- butan-2-ol



[0279] To a solution of 2-methyl-3-pyrazolo[1,5-a]pyrimidin-6-yloxy-butan-2-ol (0.03 g, 0.135 mmol, 1.00 eq.) in DMF (0.271 mL) was added NIS (0.0342 g, , 0.149 mmol, 1.10 eq.) The mixture was stirred 30 min at RT. The mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the titled product.

[0280] LCMS: MW (calcd): 347.0; m/z MW (obsd): 348.0 (M+H)

1.9.4. Step D: Cpd 4

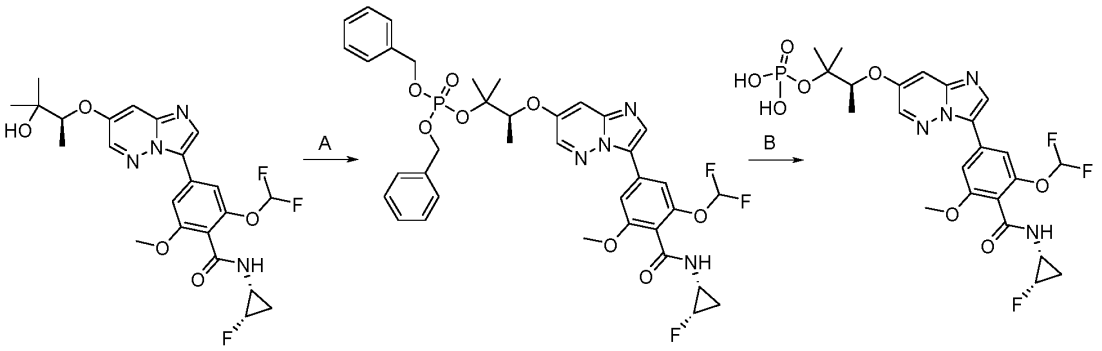


[0281] To a solution of 3-(3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol (0.047 g, 0.135 mmol, 1.00 eq.) in 1,4-dioxane (0.45 mL) and water (0.045 mL) were added **Int 4** (0.043 g, 0.135mmol, 1.00 eq.), cesium carbonate (0.088 g, , 0.27 mmol, 2.00 eq.), and $Pd(dppf)Cl_2 \cdot DCM$ (0.0116 g, 0.0135 mmol, 0.10 eq.). The reaction was heated to 95 °C for 8 h. The reaction mixture was concentrated under reduced pressure and purified by preparative LCMS to afford the titled product.

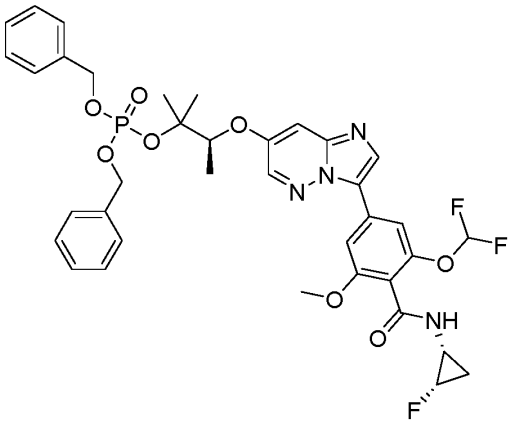
Preparative LCMS conditions: Waters XBridge Prep C18 OBD 30 × 100 mm 5 µm column with XBridge Prep C18 30 × 10mm 5 µm pre-column; time: 8 min; solvents: A=water + 0.1% formic acid, B=ACN + 0.1% formic acid; flow : 50 mL/min; gradient:

Time (min)	0	1.20	5.70	5.80	7.80	7.90	8.00
%B	5	35	50	100	100	5	5
%A	95	65	50	0	0	95	95

1.10. Cpd 5



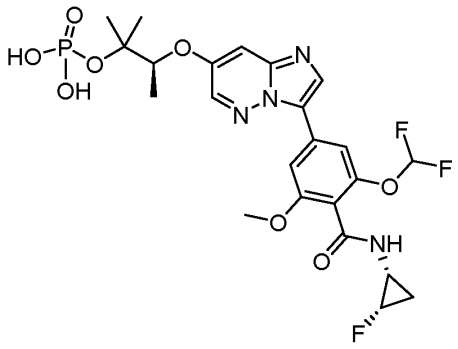
1.10.1. Step A: dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]imidazo[1,2-b]pyridazin-7-yl]oxy-1,1-dimethyl-propyl] phosphate



[0282] To a solution of Cpd 2 (800 mg, 1.62 mmol, 1.00 eq.) in DMF (4 mL) under N_2 was added slowly 1H-tetrazole (0.45 mol/L) in ACN (7.2 mL) and then dropwise dibenzyl diisopropylphosphoramidite (931.3 mg, 0.91 mL, 2.43 mmol, 1.50 eq.). The resulting mixture was stirred at RT overnight. The reaction mixture was cooled to 0 °C, and then 3-chloroperoxybenzoic acid (595.6 mg, 2.59 mmol, 1.60 eq.) was added. The resulting mixture was stirred for 15 min at 0 °C, and then warmed to RT for 30 min. The reaction mixture was quenched by addition of water and then extracted with EtOAc. The combined organic layers were washed with a saturated aq. $NaHCO_3$ solution, a 10% aq. $Na_2S_2O_3$ solution, water, then brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude residue was purified by flash chromatography on silica gel using a Biotage® Sfär 25 g column, diluting with DCM, and eluting with DCM/MeOH 100/0 to 90/10. The corresponding fractions were pooled together and concentrated in vacuo to afford the titled product.

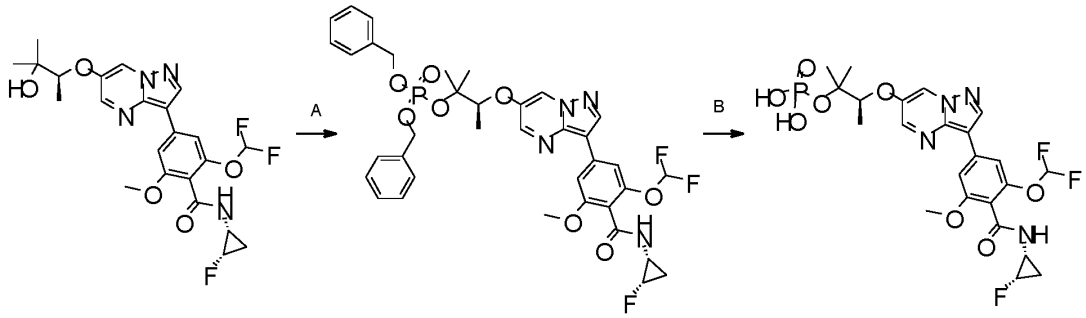
[0283] LCMS: MW (calcd): 754.2; m/z MW (obsd): 755.4 (M+H)

1.10.2. Step B: Cpd 5

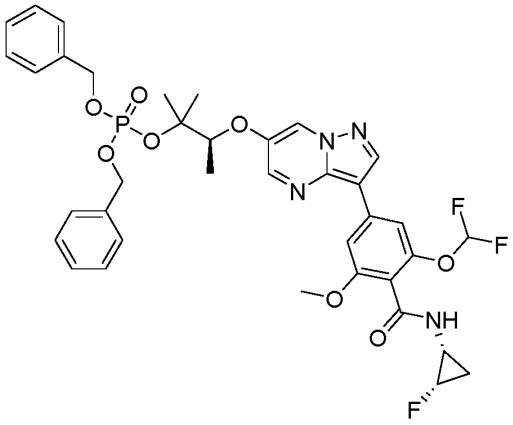


[0284] In a round bottom flask, to a solution of dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]imidazo[1,2-b]pyridazin-7-yl]oxy-1,1-dimethyl-propyl] phosphate (0.653 g, 0.865 mmol, 1.00 eq.) in a mixture of EtOH (10.5 mL) and water (2.6 mL), was added Pd/C 10% (0.074 g, 0.035 mmol, 0.04 eq.). The reaction mixture was put under vacuum and backfilled with H_2 three times. The reaction mixture was stirred at RT under H_2 atmosphere for 2.5 h. The reaction was purged with N_2 and additional Pd/C 10% (30 mg, 0.014 mmol, 0.016 eq.) was added. The reaction was placed again under vacuum and backfilled with H_2 3 times and was stirred over two days at RT. The reaction vessel was purged with nitrogen, heated to 55 °C, and filtered over a PTFE membrane (0.2 µm). The filtration cake was washed with hot (60 °C) EtOH. The filtrate was concentrated under reduce pressure. Purification by flash chromatography on a Biotage® Sfär C18 Duo 30 g column, eluting with H_2O+0.1%HCOOH/ACN+0.1%HCOOH 100/0 to 0/100 afforded the titled product.

1.11. Cpd 6



1.11.1. Step A: dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]pyrazolo[1,5-a]pyrimidin-6-yl]oxy-1,1-dimethyl-propyl] phosphate

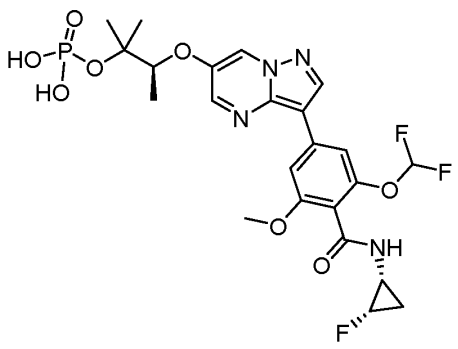


[0285] In a round bottom flask, to a suspension of Cpd 1 (1270 mg, 2.57 mmol, 1.00eq.) and dibenzyl diisopropylphosphoramidite (1480 mg, 1.44 mL, 3.85 mmol, 1.50 eq.) in DCM (12.7 mL). The suspension was cooled in an ice bath and solid 4,5-dicyanoimidazole (551 mg, 4.62 mmol, 1.80 eq.) was added portionwise. The reaction mixture was left to warm up to RT. After 1 h, hydrogen peroxide (30 mass%) in water (582 mg, 0.525 mL, 5.14 mmol, 2.00 eq.) was added dropwise at 0 °C and the reaction mixture was stirred at 0 °C for 2.5 h. The reaction mixture was quenched with 10% aq. Na2SO3 and extracted with DCM, washed with aq. $NaHCO_3$ 10% and then with NaCl 10%. The organic layer was filtered on a phase separator and concentrated under reduced pressure. The crude was purified by flash chromatography with a Biotage® Sfär C18 HD 120 g cartridge, eluting with H_2O+0.1%HCOOH/ACN+0.1%HCOOH 100/0 to 0/100 to afford the titled product.

[0286] LCMS: MW (calcd): 754.2; m/z MW (obsd): 755.1 (M+H)

[0287] ^{1}H NMR (400 MHz, CD_3OD) δ 8.67 (d, 1H), 8.53 (s, 1H), 8.49 (d, 1H), 7.72 (t, 1H), 7.60 (s, 1H), 7.26 (dd, 10H), 6.83 (t, 1H), 4.96 (d, 2H), 4.94 (d, 2H), 4.81 – 4.78 (m, 0.5H), 4.63 (ddd, 0.5H), 4.58 (q, 1H), 3.94 (s, 3H), 2.93 – 2.87 (m, 1H), 1.63 (s, 3H), 1.59 (s, 3H), 1.35 (d, 3H), 1.23 – 1.14 (m, 1H), 1.05 (ddd, 0.5H), 0.99 (ddd, 0.5H)

1.11.2. Step B: Cpd 6



[0288] In a round bottom flask, to a solution of dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]pyrazolo[1,5-a]pyrimidin-6-yl]oxy-1,1-dimethyl-propyl] phosphate (1.79 g, 2.37 mmol, 1.00 eq.) in a mixture of EtOH (28.6 mL) and water (7.2 mL) was added Pd/C 10% (0.202 g, 0.095 mmol, 0.04eq.). The reaction mixture was put under vacuum and backfilled with H_2 three times. The reaction mixture was stirred at RT under H_2 atmosphere for 4.5 h. The reaction vessel was purged with N_2, the reaction mixture was heated to 55 °C and filtered over a PTFE membrane (0.2 µm). The filtration cake was washed with hot (60 °C) EtOH. The filtrate was concentrated under reduced pressure. The solid was triturated with ACN, filtered and dried at 45 °C under reduced pressure overnight to afford **Cpd 6**.

1.12. Alternative Step B – Cpd 6

[0289] A tri-neck flask was charged with dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]pyrazolo[1,5-a]pyrimidin-6-yl]oxy-1,1-dimethylpropyl]

[0290] phosphate (117 g, 155 mmol, 1.0 eq.), ethanol (1450 mL) and water (360 mL). The reaction mixture was put under N_2 and Pd/C 10%, 50% wet (11.11 g, 5.22 mmol, 0.03 eq.) was added.

[0291] The reaction mixture was put under vacuum and backfilled with H_2 twice, then H_2 was bubbled through the reaction mixture for 1 min. The reaction mixture was stirred at room temperature under 1 atmosphere of H_2 for 23 h.

[0292] The reaction vessel was purged with nitrogen. NaOH 1 M (169 mL, 169 mmol, 1.1 eq.) and water (360 mL) were added to the reaction mixture, which was heated to 60°C. The solution was filtered through a few cm high pad of dicalite. The resulting cake was rinsed with a mixture EtOH/H_2O 3/2 (250 mL).

[0293] The filtrate was combined with another filtrate of production (same scale) and heated to 60°C. Then HCl 1 M (339 mL, 339 mmol, 2.2 eq.) was added and precipitation started. The suspension was cooled down to room temperature in 30 minutes and filtered. The resulting solid was dried to afford **Cpd 6**.

[0294] A single jacket reactor was charged with **Cpd 6** (630 g, 1097 mmol) and ethyl acetate (6300 mL). The yellow suspension was stirred at 50°C for 1h. Then the suspension was left cooling down to room temperature for 16h. The solid was filtered and dried under vacuum (40°C, 24h) to give **Cpd 6** as a crystalline monohydrate (pattern A).

[0295] Water content (by coulometric Karl-Fischer(Rivera-Quintero et al. 2024)): 3.9 wt%, equating to 1.3 mol eq.

[0296] Diffractograms were obtained on a Panalytical Aeris reflection diffractometer with a Cu (λ = 1.54 Å) X-Ray generator operating at 15 mA and 40 kV. The samples were held on low background silicon substrates and analyzed from 5-40° 2θ with a 0.022° 2θ step size and a 48.84 s step time, samples were spinning during measurements to reduce preferred orientation effects.

[0297] XRPD (°2θ): Major peaks: 9.1, 10.0, 13.2, 13.6, and 14.9. Other peaks: 15.7, 15.9, 16.6, 17.2, 19.0, 19.3, 20.4, 20.8, 24.2, 24.8, and 26.5.

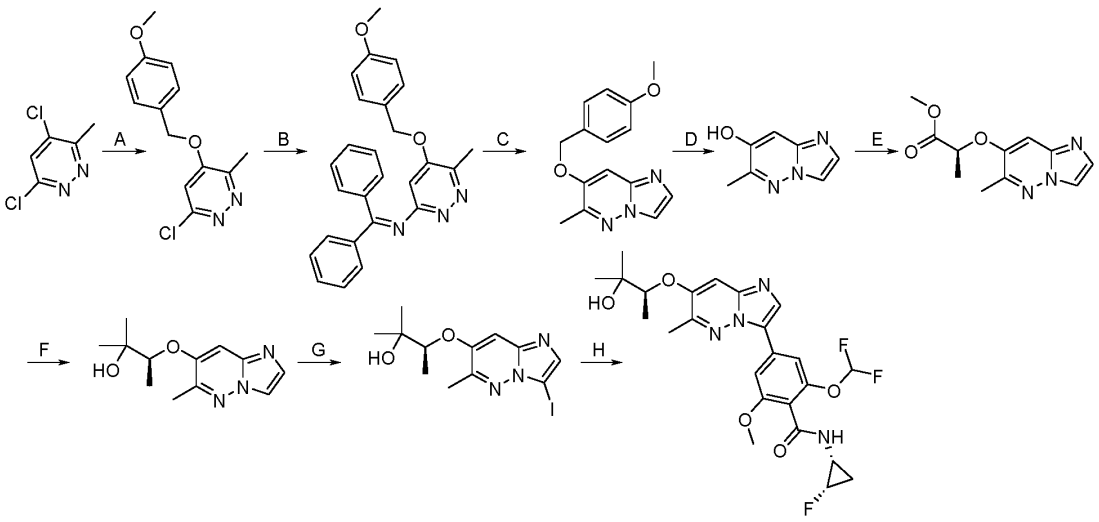
1.12.1. Preparation of Pattern C

[0298] **Cpd 6**, Pattern A (1 g) was weighed into a round bottom flask. Anhydrous ethanol was added (30 mL) and the mixture was heated to 50 °C, 750 rpm for 72 hours. The material was filtered and isolated under vacuum and washed with anhydrous EtOH (5 mL). The isolated solid was dried for 1 hour under vacuum. **Cpd 6** was obtained as a crystalline hemihydrate (pattern C).

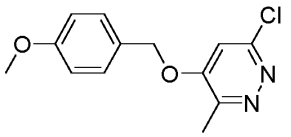
[0299] Water content (by coulometric Karl-Fischer (Rivera-Quintero et al. 2024)): 2.0 wt%, equating to 0.65 mol eq.

[0300] XRPD (°2θ): Major peaks: 9.1, 9.6, 15.1 15.4, and 19.7. Other peaks: 10.6, 12.4, 14.1, 18.1, 18.5, 18.8, 20.0, and 20.3.

1.13. Cpd 7



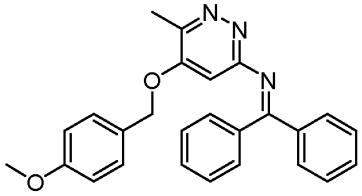
1.13.1. Step A: 6-chloro-4-[(4- methoxyphenyl)methoxy]-3- methyl-pyridazine



[0301] Under argon atmosphere, at 0 °C, to a mixture of 4-methoxybenzyl alcohol (CAS# 105-13-5; 1.1 mL, 8.7 mmol, 1.5 eq.) in DMF (15 mL) was added sodium hydride (60 mass%) in mineral oil (310 mg, 7.8 mmol, 1.3 eq.). The resulting mixture was stirred at 0 °C for 15 min and 4,6-dichloro-3-methylpyridazine (CAS# 68240-43-7; 1.0 g, 6.0 mmol, 1.0 eq.) dissolved in DMF (4 mL) was added dropwise. The resulting mixture was allowed to warm to RT and was stirred for 2 h. At 0 °C, sat. aq. NH_4Cl and water were added. Precipitation was observed. The resulting mixture was filtered off and the solid was washed with water and dissolved with DCM. The resulting filtrate was filtered on a phase separator and concentrated under reduced pressure. The resulting solid was purified by chromatography on a Biotage® Sfär HC 50 g column, eluting with n-heptane/EtOAc 100/0 to 60/40 to afford the titled product.

LCMS: MW (calcd): 264.1; m/z MW (obsd): 575.3 (M+H)

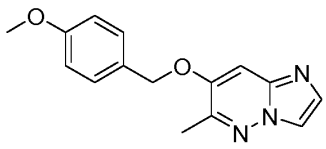
1.13.2. Step B: N-[5-[(4-methoxyphenyl) methoxy]-6-methyl-pyridazin-3-yl]-1,1-diphenyl-methanimine



[0302] Under argon atmosphere, to a mixture of 6-chloro-4-[(4-methoxyphenyl)methoxy]-3-methyl-pyridazine (500 mg, 1.9 mmol, 1.0 eq.) and benzophenone imine (CAS# 1013-88-3) (0.38 mL, 2.3 mmol, 1.2 eq.) in 1,4-dioxane (9.4 mL) were added cesium carbonate (1.25 g, 3.84 mmol, 2.0 eq.) and XantPhos Pd G3 (CAS# 1445085-97-1; 190 mg, 0.19 mmol, 0.10 eq.). The resulting mixture was heated at 90 °C overnight, then filtered on a pad of Celite® and concentrated under reduced pressure to afford the titled product.

LCMS: MW (calcd): 409.2; m/z MW (obsd): 410.3 (M+H)

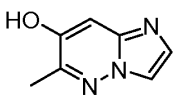
1.13.3. Step C: 7-[(4-methoxyphenyl) methoxy]-6-methyl-imidazo[1,2-b]pyridazine



[0303] To a solution of N-[5-[(4-methoxyphenyl)methoxy]-6-methyl-pyridazin-3-yl]-1,1-diphenyl-methanimine (1.9 mmol, 1.9 mmol, 1.0 eq.) dissolved in THF (10 mL) was added dropwise hydrochloric acid (2 mol/L) in water (10 mL, 20 mmol, 11 eq.). The resulting solution was stirred at RT for 1 h. The resulting solution was quenched by a sat. aq. $NaHCO_3$ solution and extracted twice with EtOAc. The resulting organic layer was filtered on a phase separator and concentrated under reduced pressure. To the resulting oil dissolved in EtOH (10 mL) was added sodium bicarbonate (0.5 g, 6 mmol, 3 eq.) and a chloroacetaldehyde solution (45 mass%) in water (0.54 mL, 3.8 mmol, 2.0 eq.). The resulting mixture was refluxed for 2 h. The resulting mixture was then concentrated under reduced pressure and the residue was diluted between water and EtOAc. The organic layer was filtered on a phase separator and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on a Biotage® Sfär HC 25 g column, eluting with *n*-heptane/EtOAc 100/0 to 20/80, to afford the titled product.

LCMS: MW (calcd): 269.2; m/z MW (obsd): 270.1 (M+H)

1.13.4. Step D: 6-methylimidazo[1,2-b]pyridazin-7-ol

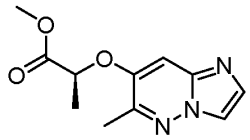


[0304] Under argon atmosphere, to a solution of 7-[(4-methoxyphenyl)methoxy]-6-methyl-imidazo[1,2-b]pyridazine (150 mg, 0.56 mmol, 1.0 eq.) in MeOH (3.0 mL) were added palladium hydroxide on carbon (CAS# 12135-22-7; 15 mg, 0.11 mmol, 0.19 eq.). The reaction mixture was put under vacuum and

backfilled with H_2, and then stirred at RT for 2 h. The resulting mixture was filtered off a pad of Celite®, washed with EtOAc, and concentrated under reduced pressure to afford the titled product.

LCMS: MW (calcd): 149.1; m/z MW (obsd): 149.9 (M+H)

1.13.5. Step E: methyl (2S)-2-(6- methylimidazo[1,2-b]pyridazin-7-yl)oxypropanoate

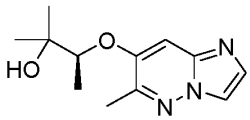


[0305] Preparation of potassium salt of 6-methylimidazo[1,2-b]pyridazin-7-ol: to a solution of 6-methylimidazo[1,2-b]pyridazin-7-ol (530 mg, 3.6 mmol, 1.0 eq.) in THF (18 mL) was added *t*-BuOK (280 mg, 2.4 mmol, 0.68 eq.) in one portion. The resulting mixture was stirred at RT for 1 h. The resulting mixture was filtered and the resulting solid was washed with THF and then Et_2O to afford potassium 6-methylimidazo[1,2-b]pyridazin-7-olate.

[0306] Under argon atmosphere, at 0 °C, to a solution of potassium 6-methylimidazo[1,2-b]pyridazin-7-olate (990 mg, 5.3 mmol, 1.0 eq.) in DMF (26 mL) was added dropwise **Int 3** (1.43 g, 5.54 mmol, 1.0 eq.) dissolved in DMF (2 mL). The resulting solution was stirred at 0 °C for 1 h. The resulting mixture was quenched at 0 °C by aq. NH_4Cl and extracted with EtOAc several times. The combined organic layers were filtered on a phase separator and concentrated under reduced pressure. The residue was purified by flash chromatography on a Biotage® Sfär HC 25 g column, eluting with DCM/MeOH 100/0 to 96/04 to afford the titled product.

LCMS: MW (calcd): 249.1; m/z MW (obsd): 250.2 (M+H)

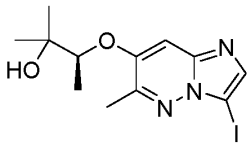
1.13.6. Step F: (3S)-2-methyl-3-(6- methylimidazo[1,2-b] pyridazin-7-yl)oxy-butan-2-ol



[0307] Under argon atmosphere, at 0 °C, to a solution of methyl (2S)-2-(6- methylimidazo[1,2-b]pyridazin-7-yl)oxypropanoate (580 mg, 2.47 mmol, 1.0 eq.) in THF (12 mL) was added a methylmagnesium bromide solution (3.0 mol/L) in Et_2O (2.5 mL, 7.5 mmol, 3.0 eq.). The resulting mixture was stirred at 0 °C for 1 h. The resulting solution was quenched by sat. aq. NH_4Cl, then basified by $NaHCO_3$ and finally extracted three times with EtOAc. The combined organic layers were filtered on a phase separator and concentrated under reduced pressure. The resulting oil was purified by flash chromatography on a Biotage® SNAP HC 25 g column, eluting with CH_2Cl_2/MeOH 100/0 to 95/05 to afford the titled product.

LCMS: MW (calcd): 235.1; m/z MW (obsd): 236.2 (M+H)

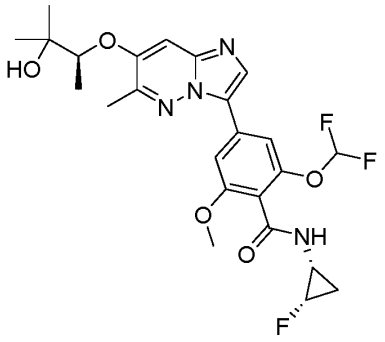
1.13.7. Step G: (3S)-3-(3-iodo-6-methyl-imidazo[1,2-b]pyridazin-7-yl) oxy-2-methyl-butan-2-ol



[0308] To a solution of (3S)-2-methyl-3-(6-methylimidazo[1,2-b]pyridazin-7-yl)oxy-butan-2-ol (800 mg, 3.4 mmol, 1.0 eq.) in DMF (15 mL) was added NIS (1.0 g, 4.4 mmol, 1.3 eq.) in one portion. The resulting solution was stirred at RT for 1 h. At 0 °C, the resulting mixture was quenched by $Na_2S_2O_3$, basified with $NaHCO_3$, and extracted three times with EtOAc. The combined organic layers were filtered on a phase separator and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on a Biotage® SNAP HC 25 g column, eluting with CH_2Cl_2/MeOH 100/0 to 96/04 to afford the titled product.

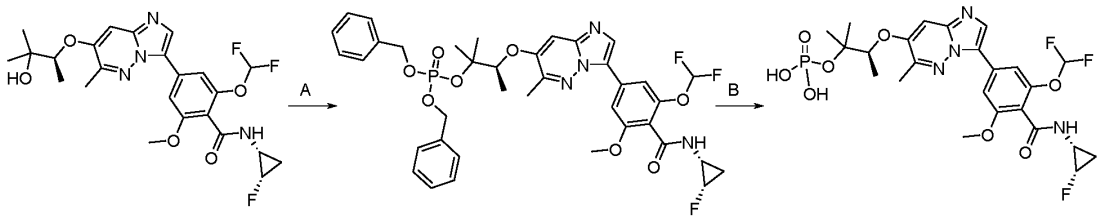
LCMS: MW (calcd): 361.0; m/z MW (obsd): 362.0 (M+H)

1.13.8. Step H: Cpd 7

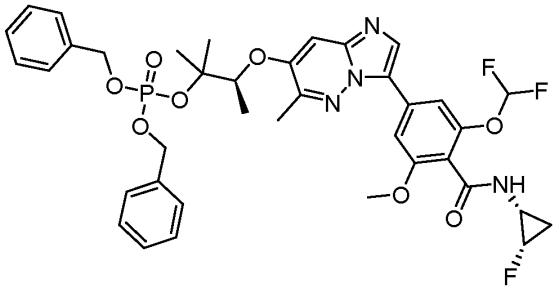


[0309] Under argon atmosphere, to a solution of (3S)-3-(3-iodo-6-methyl-imidazo[1,2-b]pyridazin-7-yl)oxy-2-methyl-butan-2-ol (1.03 g, 2.85 mmol, 1.00 eq.) and **Int 4** (1.09 g, 3.42 mmol, 1.20 eq.) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2 (CAS# 72287-26-4; 220 mg, 0.28 mmol, 0.10 eq.) and potassium carbonate (1.18 g, 8.54 mmol, 3.00 eq.) dissolved in water (4.5 mL). The resulting mixture was heated at 80 °C for 2 h. The mixture was then diluted with EtOAc and water. The organic layer was filtered on a phase separator and concentrated under reduced pressure. The residue was purified by flash chromatography on a Biotage® Sfär HC 50 g column, eluting with n-heptane/(EtOAc/EtOH, 3/1) 100/0 to 0/100 to afford the titled product.

1.14. Cpd 9

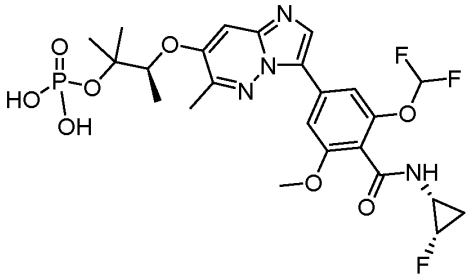


1.14.1. Step A: dibenzyl [(2S)-2-[3-[3- (difluoromethoxy)-4-[[(1R,2S) -2-fluorocyclopropyl]carbamoyl]-5-methoxy- phenyl]-6-methyl-imidazo [1,2-b]pyridazin-7-yl]oxy-1,1- dimethyl-propyl] phosphate



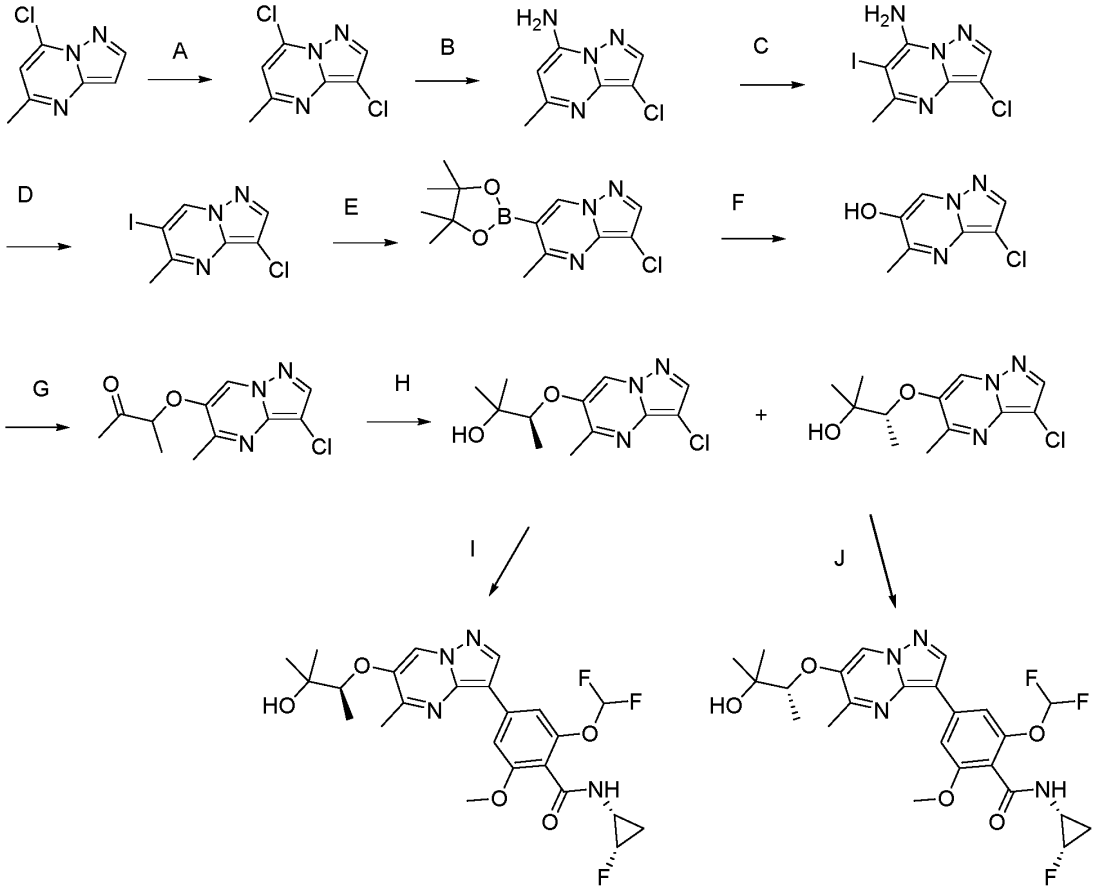
[0310] At 0 °C, to a suspension of **Cpd 7** (670 mg, 1.3 mmol, 1.0 eq.) and dibenzyl diisopropylphosphoramidite (0.74 mL, 2.0 mmol, 1.5 eq.) in DCM (7.0 mL) was added 4,5-dicyanoimidazole (280 mg, 2.3 mmol, 1.8 eq.) in one portion. The reaction mixture was stirred at RT for 1 h. hydrogen peroxide (30 mass%) in water (298.8 mg, 0.269 mL, 2.64 mmol, 2.0 eq.) was added dropwise at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with 10% aq. Na_2SO_3 and extracted with DCM, washed with aq. $NaHCO_3$ 10% and then with aq. NaCl 10%. The organic layer was filtered on hydrophobic filter and concentrated under reduced pressure. The resulting oil was purified by flash chromatography with a Biotage® Sfär C18 HD 120 g cartridge, eluting with H_2O+0.1%HCOOH/ACN+0.1%HCOOH 100/0 to 0/100 to afford the titled product.
LCMS: MW (calcd): 768.2; m/z MW (obsd): 769.3 (M+H)

1.14.2. Step B: Cpd 9

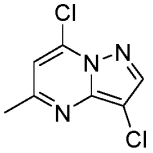


[0311] Under argon atmosphere, to a solution of dibenzyl [(2S)-2-[3-[3-(difluoromethoxy)-4-[[(1R,2S)-2-fluorocyclopropyl]carbamoyl]-5-methoxy-phenyl]-6-methyl-imidazo[1,2-b]pyridazine -7-yl]oxy-1,1-dimethyl-propyl] phosphate (950 mg, 1.2 mmol, 1.0 eq.) in EtOH (20 mL) and water (3 mL), was added Pd/C 10% (100 mg, 0.047 mmol, 0.038 eq.) in one portion. The reaction mixture was put under vacuum and backfilled with H_2 three times. The resulting black mixture was stirred at RT overnight, then heated at 60 °C, and filtered over a PTFE membrane (0.2 µm). The filtration cake was washed with hot EtOH. The resulting filtrate was concentrated and the solid residue was triturated with Et_2O and dried under high vacuum overnight to afford the titled product.

1.15. Cpd 10 & Cpd 11



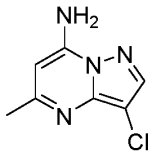
1.15.1. Step A: 3,7-dichloro-5-methyl-pyrazolo[1,5-a]pyrimidine



[0312] A 100 mL round bottom flask was charged under N_2 atmosphere with 7-chloro-5-methylpyrazolo[1,5-a]pyrimidine (CAS# 16082-27-2; 2 g, 11.93 mmol, 1 eq.) and anhydrous DMF (18.4 mL). To the solution was added NCS (1.75 g, 13.13 mmol, 1.1 eq.). The mixture was stirred at 60 °C for 2 h. To the mixture was added 10V (180 mL) of water and the mixture was stirred at RT for 10 min. A precipitate formed. The suspension was filtered under vacuum to afford a solid which was taken up in acetone. The suspension was filtered. Solids were washed and rinsed with acetone. The filtrate was concentrated *in vacuo* to afford the titled product.

[0313] LCMS: MW (calcd): 201.0; m/z MW (obsd): 202.1-204.0 (M+H)

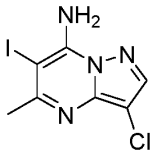
1.15.2. Step B: 3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-7-amine



[0314] A pressure tube at RT was charged with 3,7-dichloro-5-methyl-pyrazolo[1,5-a]pyrimidine (1.49 g, 7.37 mmol, 1 eq.) and ammonium hydroxide (15 mL). The mixture was stirred at RT and warmed up to 120 °C for 2 h. The mixture was cooled to RT, 10V of water (150 mL) were added to the mixture, which was stirred at RT for 15 min. A precipitate was formed. The suspension was filtered under vacuum to afford the titled product.

[0315] LCMS: MW (calcd): 182.0; m/z MW (obsd): 183.1-185.0 (M+H)

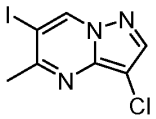
1.15.3. Step C: 3-chloro-6-iodo-5-methyl-pyrazolo[1,5-a]pyrimidin-7-amine



[0316] A 50 mL round bottom flask was charged at RT under N_2 atmosphere with 3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-7-amine (0.76 g, 4.13 mmol, 1 eq.) and anhydrous DMF (7 mL). To the solution was added NIS (1.12 g, 4.96 mmol, 1.2 eq.). The mixture was cooled to RT and 10V of water (70 mL) were added. The reaction mixture was stirred at RT for 15 min, a precipitate was formed. The suspension was filtered under vacuum to afford the titled product.

[0317] LCMS: MW (calcd): 307.9; m/z MW (obsd): 308.9-310.9 (M+H)

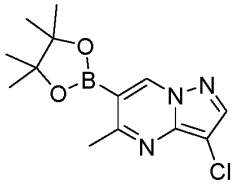
1.15.4. Step D: 3-chloro-6-iodo-5-methyl-pyrazolo[1,5-a]pyrimidine



[0318] A 50 mL round bottom flask was charged under N_2 atmosphere with 3-chloro-6-iodo-5-methyl-pyrazolo[1,5-a]pyrimidin-7-amine (1.16 g, 3.76 mmol, 1 eq.) and anhydrous THF (10 mL). To the solution was added salicylic acid (0.104 g, 0.75 mmol, 0.2 eq.) and dropwise t-butyl nitrite (3.88 g, 4.509 mL, 37.6 mmol, 10 eq.). The mixture was warmed up to 70 °C and stirred for 2 hours. The mixture was cooled to RT and water (70 mL) was added. The mixture was extracted with EtOAc. The organic layer was washed with 50 mL of $Na_2S_2O_3$ and 50 mL of brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to afford a solid, which was taken up in ACN to afford the titled product.

[0319] LCMS: MW (calcd): 292.9; m/z MW (obsd): 294.0-295.9 (M+H)

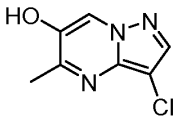
1.15.5. Step E: 3-chloro-5-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyrimidine



[0320] A screw cap vial was charged at RT under N_2 atmosphere with 3-chloro-6-iodo-5-methyl-pyrazolo[1,5-a]pyrimidine (686 mg, 2.34 mmol, 1 eq.), bis(pinacolato)diboron (CAS# 73183-34-3; 712 mg, 2.80 mmol, 1.2 eq.) and anhydrous dioxane (10 mL), bubbling N_2 into the mixture for 10 min. To this mixture was added Pd(dppf)Cl_2·DCM (CAS# 95464-05-4; 191 mg, 0.23 mmol, 0.1 eq.) and potassium pivalate (CAS# 19455-23-3; 983 mg, 7.01 mmol, 3 eq.). The mixture was stirred at 100 °C for 50 min, then cooled to RT and filtered through a prepacked pad of Celite®. The solid was rinsed with EtOAc. The filtrate was concentrated in vacuo to afford the titled product, which was directly used in the next step.

[0321] LCMS: MW (calcd): 293.1; m/z MW (obsd): 294.0-296.1 (M+H)

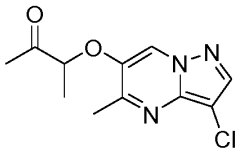
1.15.6. Step F: 3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-ol



[0322] A 100 mL round bottom flask under N_2 atmosphere was charged at 0 °C with 3-chloro-5-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyrimidine (1.93 g, 2.35 mmol, 1 eq.) and acetone (10 mL). To the mixture was added a solution of potassium peroxymonosulfate (0.46 g, 3.05 mmol, 1.3 eq.) in water (20 mL). After 5 min, a 10 mass% aq. solution of $Na_2S_2O_3$ (20 mL) was added to the mixture. The mixture was extracted with EtOAc (20 mL). The organic layer was washed with a 10 mass% solution of $Na_2S_2O_3$, brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash chromatography, eluting with heptane/EtOAc 100/0 to 0/100 to afford the titled product.

[0323] LCMS: MW (calcd): 183.0; m/z MW (obsd): 184.0-185.9 (M+H)

1.15.7. Step G: 3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxybutan-2-one

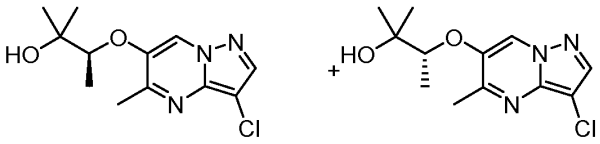


[0324] A 50 mL bottom flask was charged at 0 °C under N_2 atmosphere with 3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-ol (235 mg, 1.28 mmol, 1 eq.) and anhydrous DMF (3 mL). To the mixture was added K_2CO_3 (265.34 mg, 1.92 mmol, 1.5 eq.) and the mixture was stirred at RT for 10 min. To the mixture was added dropwise 3-chloro-2-butanone (205 mg, 0.19 mL, 1.92 mmol, 1.5 eq.). The mixture was stirred at RT for 1.2 h. To the mixture was added 15 mL of an aq. K_2CO_3 solution 10 mass% and 15 mL of water.

The mixture was extracted with EtOAc. The organic layer was washed with brine and concentrated in vacuo. The crude product was purified by flash chromatography, eluting with heptane/EtOAc 100/0 to 50/50 to afford the titled product.

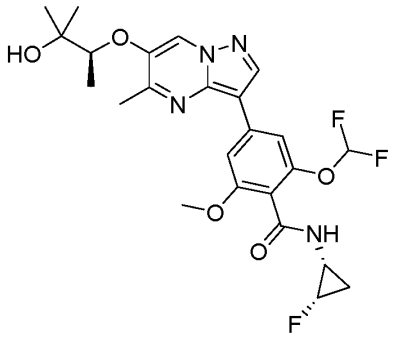
[0325] LCMS: MW (calcd): 253.1; m/z MW (obsd): 254.1-256.1 (M+H)

1.15.8. Step H: (3S)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol & (3R)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol



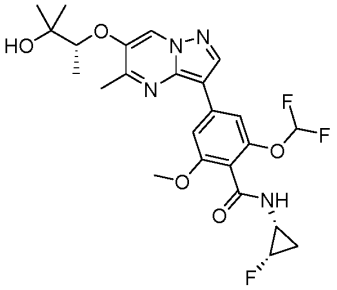
[0326] To a solution of 3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxybutan-2-one (235 mg, 0.86 mmol, 1 eq.) and anhydrous MeTHF (2.79 mL) in a 100 mL round bottom flask under N_2 atmosphere at -20 °C was added dropwise a solution of MeMgBr (3 M in Et_2O) (0.34 mL, 1.034 mmol, 1.2 eq.). The mixture was stirred at -20 °C. The reaction mixture was then hydrolyzed at 0 °C with a sat. aq. solution of ammonium chloride and stirred at RT for 5 min. The mixture was extracted with EtOAc. The organic layer was washed twice with a sat. aq. solution of ammonium chloride, brine, dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The crude product was purified by flash chromatography, eluting with DCM/(EtOAc/EtOH 3/1) 100/0 to 80/20 to afford a mixture of enantiomers. This mixture of enantiomers was separated by SFC on a Waters ACQUITY UPC2® system. Column: Chiralpak IA, 150 × 21 mm, 5 µm with guard of 50 × 21 mm, 5 µm, CO_2 back pressure: 100 bar, co-solvent: EtOH, % of co-solvent: 20%, flowrate: 60 mL/min, isocratic mode, temperature: 40 °C. First eluting compound: (3R*)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol, second eluting compound: (3S*)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol.

1.15.9. Step I: Cpd 10



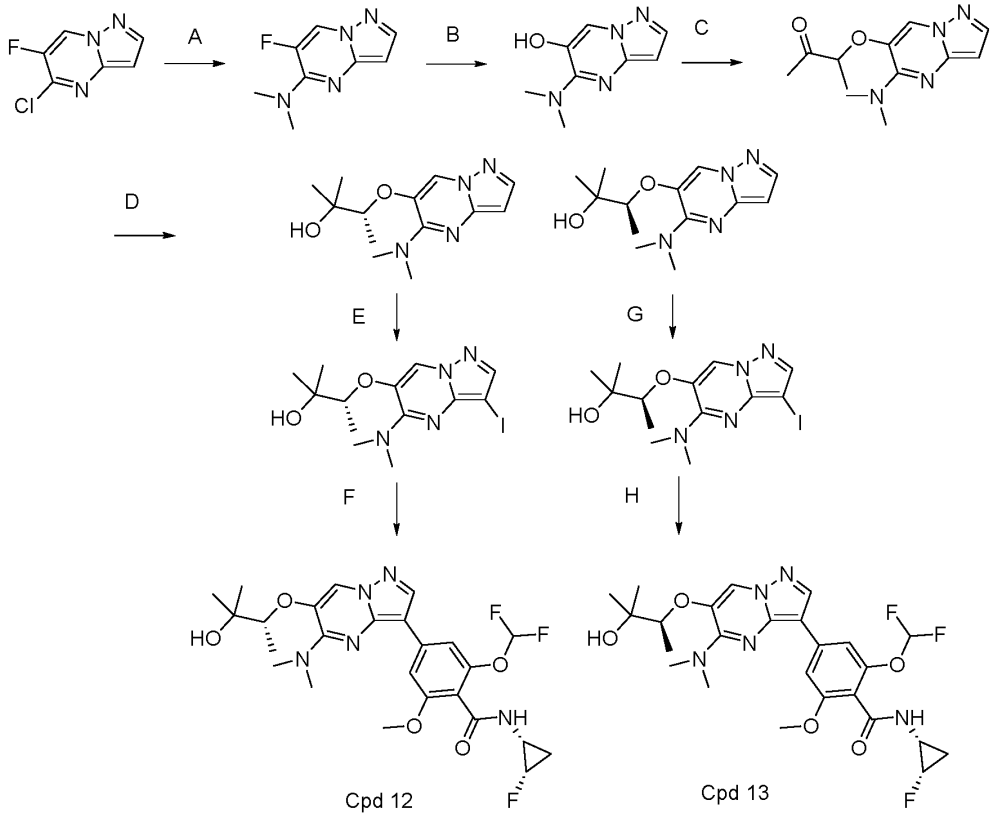
[0327] In a sealed tube, a mixture of (3S*)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol (60 mg, 0.22 mmol, 1 eq.), **Int 4** (85.16 mg, 0.27 mmol, 1.2 eq.), K_2CO_3 (92.2 mg, 0.67 mmol, 3 eq.), XPhos Pd G3 (CAS# 1445085-55-1; 18.8 mg, 0.022 mmol, 0.1 eq.), dioxane (1.28 mL) and water (0.32 mL) was degassed with N_2 and stirred at 100 °C for 2 h. EtOAc and water were added. The organic layer was washed with brine, dried with $MgSO_4$, filtered, evaporated and purified by flash chromatography eluting with heptane/EtOAc 100/0 to 0/100 to afford the titled product.

1.15.10. Step J: Cpd 11



[0328] A screw vial cap was charged at RT with **Int 4** (87.43 mg, 0.27 mmol, 1.2 eq.), (3R*)-3-(3-chloro-5-methyl-pyrazolo[1,5-a]pyrimidin-6-yl)oxy-2-methyl-butan-2-ol (61.6 mg, 0.23 mmol, 1 eq.), K_2CO_3 (94.69 mg, 0.69 mmol, 3 eq.), anhydrous dioxane (4 mL) and water (1 mL). To the mixture was added at RT XPHOS Pd G3 (CAS# 1445085-55-1; 19.3 mg, 0.023 mmol, 0.1 eq.). The mixture was warmed up to 110 °C and stirred at 110 °C for 3.5 h. The mixture was filtered through a pad of Celite®, the solid was rinsed with EtOAc, and the filtrate was concentrated *in vacuo*. Water was added to the crude. The mixture was extracted with DCM through a phase separator. The organic layer was concentrated *in vacuo*. The crude product was purified by flash chromatography eluting with heptane/(EtOAc/EtOH 3/1) 100/0 to 50/50. The product was further purified by flash chromatography, eluting with DCM/ACN 100/0 to 50/50 to afford the expect product.

1.16. Cpd 12 & 13



1.16.1. Step A:

[0329] In a sealed tube, under air, a mixture of 5-chloro-6-fluoropyrazolo[1,5-a]pyrimidine (1 eq., 1.5 g, 8.74 mmol), Me_2NH in THF (3.6 eq., 15.74 mL, 31.48 mmol) and THF (30 mL) was stirred at 70 °C for 1h. DCM was added and the mixture concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 50:50 to afford 6-fluoro-N,N-dimethylpyrazolo[1,5-a]pyrimidin-5-amine (1,42 g, 90 % yield).

[0330] LCMS: MW (calcd): 180.2; m/z MW (obsd): 181.1 (M+H)

1.16.2. Step B:

[0331] In a round sealed tube, under air, a mixture of 6-fluoro-N,N-dimethylpyrazolo[1,5-a]pyrimidin-5-amine (1 eq., 1,400 mg, 7.77 mmol) and NaOH 10M in H_2O (19.31 eq., 15 mL, 150 mmol) in MeOH (30 mL) was stirred at 100 °C for 3 h. The mixture was filtered, concentrated *in vacuo*, then was dissolved in MeOH, celite was added and the mixture was evaporated and purified by flash chromatography on silica gel: elution with a gradient DCM/MeOH 100:0 to 90:10 then DCM/MeOH 50:50 to afford 6-fluoropyrazolo[1,5-a]pyrimidin-5-ol (530 mg, 38 % yield).

[0332] LCMS: MW (calcd): 178.2; m/z MW (obsd): 179.1 (M+H)

1.16.3. Step C:

[0333] In a sealed tube, under air, a mixture of 6-fluoropyrazolo[1,5-a]pyrimidin-5-ol (1 eq., 530 mg, 2.97 mmol), 3-chloro-2-butanone (1.5 eq., 475.4 mg, 0.45 mL, 4.46 mmol), K_2CO_3 (1.5 eq., 616.6 mg, 4.46 mmol), and DMF (6 mL) was stirred at RT for 1 h. EtOAc (20 mL) was added and the organic layer was washed with water (3 x 15 mL) and brine (2 x 15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (416 mg, 56% yield).

[0334] LCMS: MW (calcd): 248.3; m/z MW (obsd): 249.2 (M+H)

1.16.4. Step D:

[0335] In a round bottom flask, under N_2, at -20 °C, 3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (1 eq., 410 mg, 1.65 mmol) in MeTHF (26.78 mL) was added. The reaction mixture was degassed through vacuum / N_2 cycles 3 times, kept under N_2 atmosphere and MeMgBr 3M in Et_2O (2 eq., 1.10 mL, 3.30 mmol) was added at -20°C. The reaction mixture was stirred at -20°C for 1 h. EtOAc (30 mL) and NH_4Cl aq. (30 mL) were added. The organic layer was washed with brine (20 mL x 2), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford the titled mixture of enantiomers which were then separated by Chiral SFC on a Waters ACQUITY UPC2® system. Column: Lux Cellulose-4, 150 x 21.2mm, 5µm, CO_2 back pressure: 100bar, co-solvent: EtOH, % of co-solvent: 15%, flowrate: 60 mL/min, isocratic mode, temperature: 40 °C *(Lux Cellulose-4, 150 x 21.2mm, 5µm to give the first eluting compound (R*)-3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-

methylbutan-2-ol (109 mg, ee 100%) arbitrary assigned configuration R and second eluting compound (S*)-3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (115 mg, ee 99.9%) arbitrary assigned configuration S.

[0336] LCMS: MW (calcd): 264.3; m/z MW (obsd): 265.2 (M+H) for both enantiomers

1.16.5. Step E:

[0337] In a sealed tube, under air, (R*)-3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 109 mg, 0.41 mmol) was added in DMF (1.5 mL). Then, NIS (1.1 eq., 102.0 mg, 0.45 mmol) was added and the reaction mixture was stirred at rt for 1h . EtOAc (10 mL) was added and the organic layer was washed with water (3 x 5 mL) and brine (2 x 5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford (R*)-3-((5-(dimethylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (131.5 mg, 82% yield).

[0338] LCMS: MW (calcd): 390.2; m/z MW (obsd): 391.1 (M+H)

1.16.6. Step F:

[0339] In a sealed tube, under N_2, a mixture of (R*)-3-((5-(dimethylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 130 mg, 0.33 mmol), Int_4 (1.2 eq., 127.54 mg, 0.4 mmol), Cs_2CO_3 (1.2 eq., 130.2 mg, 0.4 mmol) and $PdCl_2$dppf.DCM (0.1 eq., 27.21 mg, 0.033 mmol) was dissolved in dioxane (10.5 mL) and water (2.5 mL). This mixture was degassed with N_2 for 10min. Then, the reaction mixture was degassed through vacuum / N_2 cycles 3 times, kept under N_2 atmosphere. The reaction mixture was stirred at 90°C for 1h. The reaction mixture was concentrated *in vacuo* and the crude residue was purified by preparative LCMS to afford 2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide, **Cpd 12** (67 mg, 37% yield)

[0340] LCMS: MW (calcd): 537.5; m/z MW (obsd): 538.3 (M+H)

[0341] ee%= 100

1.16.7. Step G:

[0342] In a sealed tube, under air, (S*)-3-((5-(dimethylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 115 mg, 0.44 mmol) was added in DMF (1.5 mL). Then, NIS (1.1 eq., 107.67 mg, 0.48 mmol) was added and the reaction mixture was stirred at rt for 1h. EtOAc (10 mL) was added and the organic layer was washed with water (3 x 5 mL) and brine (2 x 5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford (S*)-3-((5-(dimethylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (139.4 mg, 82% yield).

[0343] LCMS: MW (calcd): 390.2; m/z MW (obsd): 391.1 (M+H)

1.16.8. Step H

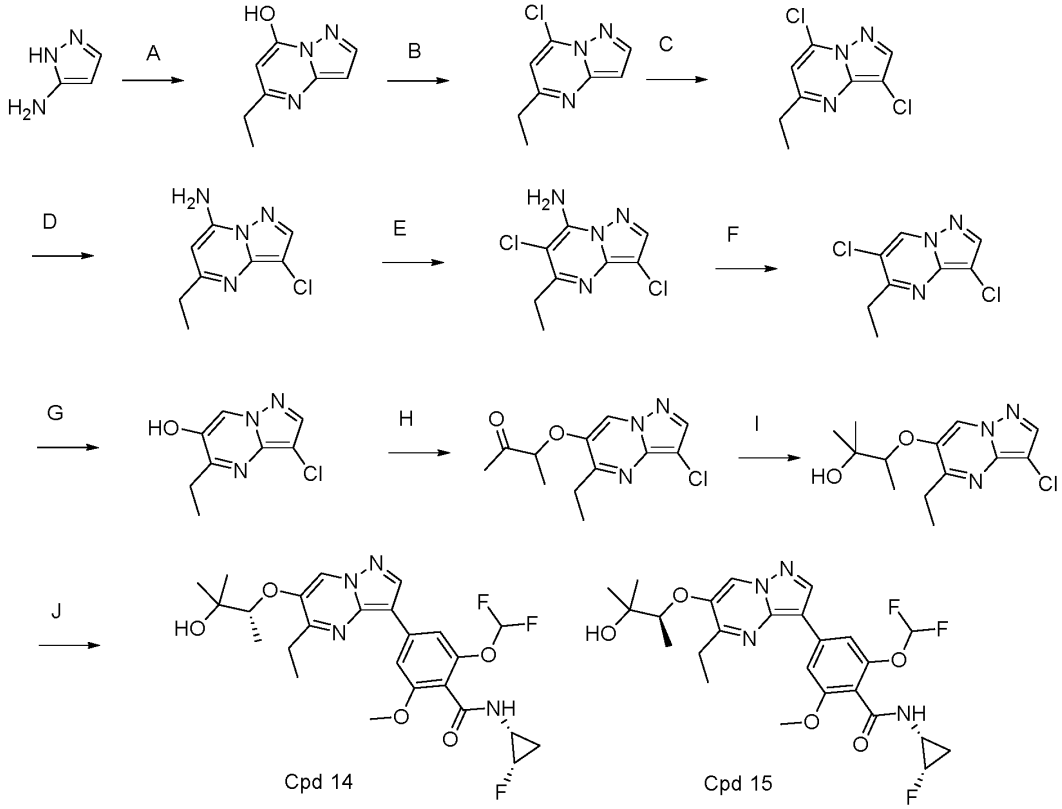
[0344] In a sealed tube, under N_2, a mixture of (S*)-3-((5-(dimethylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 138 mg, 0.35 mmol), Int_4 (1.2 eq., 135.4 mg, 0.42 mmol), Cs_2CO_3 (1.2 eq., 138.3 mg, 0.42 mmol and $PdCl_2$dppf.DCM (0.1 eq., 28.9 mg, 0.035 mmol) was

dissolved in dioxane (10.5 mL) and water (2.5 mL). This mixture was degassed with N_2 for 10min. Then, the reaction mixture was degassed through vacuum / N_2 cycles 3 times, kept under N_2 atmosphere. The reaction mixture was stirred at 90°C for 1h. The reaction mixture was concentrated *in vacuo* and the crude residue was purified by preparative LCMS to afford 2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide, **Cpd 13** (67 mg, 35% yield)

[0345] LCMS: MW (calcd): 537.5; m/z MW (obsd): 538.5(M+H)

[0346] ee%= 100%

Cpd 14 &15



1.16.9. Step A:

[0347] A 250 mL round bottom flask under air was charged with 3-aminopyrazole (1 eq., 10 g, 120.35 mmol) and AcOH (60 mL) and stirred for 5min at RT. To the resulting solution, ethyl propionylacetate (1 eq., 17.35 g, 17.18 mL, 120.35 mmol) was added at RT. The reaction mixture was heated at reflux for 3h. The reaction was stopped and cooled down to RT. The resulting suspension was filtered. The white solid obtained was washed with MeOH (twice, 2x 1 mL), dried *in vacuo* to afford 5-ethylpyrazolo[1,5-a]pyrimidin-7-ol (13.51 g, 68% yield).

[0348] LCMS: MW (calcd): 163.2; m/z MW (obsd): 164.2 (M+H)

1.16.10. Step B:

[0349] A 25mL round bottom flask was charged with 5-ethylpyrazolo[1,5-a]pyrimidin-7-ol (1 eq., 500 mg, 3.06 mmol) and placed under nitrogen atmosphere. Then anhydrous dioxane (2.8 mL) was added at RT followed by Diethylaniline (2 eq., 914.5 mg, 0.98 mL, 6.13 mmol) and $POCl_3$ (5 eq., 2,35 g, 1.43 mL, 15.32 mmol). The mixture was heated at 105°C for 2 h. Reaction was stopped and cooled down to RT. The reaction mixture was concentrated *in vacuo* ($POCl_3$ evaporated was carefully quenched). The residue evaporated was taken up in DCM (12mL) and basified with $NaHCO_3$ sat (pH = 7-8 reached V=20mL). The 2 phases were separated and the aqueous phase was further extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was then purified by flash chromatography on silica gel: elution with Heptane/AcOEt 100 to 80/20 in to afford 7-chloro-5-ethylpyrazolo[1,5-a]pyrimidine (493.5 mg, 89% yield).

[0350] LCMS: MW (calcd): 181.6; m/z MW (obsd): 183.9 (M+H)

1.16.11. Step C:

[0351] Into a 100 mL round bottom flask was placed under nitrogen 7-chloro-5-ethylpyrazolo[1,5-a]pyrimidine (1 eq., 2 g, 11.012 mmol) in anhydrous DMF (16.91 mL) and NCS (1.1 eq., 1.62 g, 12.11 mmol) was added to the solution. The reaction mixture was heated at 60°C for 2 h. The reaction was stopped and cooled at RT. To the reaction mixture 10V of water (160mL) was added and mixture was stirred to RT for 10 min. The resulting mixture was extracted with EtOAc (40mL), the two phases were separated and the aqueous phase was further extracted with EtOAc (2 times with 40 mL). The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was then purified by flash chromatography on silicagel: elution with Heptane/AcOEt 100 to 80/20 to afford 3,7-dichloro-5-ethylpyrazolo[1,5-a]pyrimidine (2.09 g, 88% yield).

[0352] LCMS: MW (calcd): 216.07; m/z MW (obsd): 216.0 – 218.0 (M+H)

1.16.12. Step D

[0353] A 10mL round bottom flask under air was charged at RT with 3,7-dichloro-5-ethylpyrazolo[1,5-a]pyrimidine (1 eq., 2 g, 9.26 mmol) and NH_4OH (19.83 mL). The reaction mixture was heated at 100°C for 2 h. 10 mL of NH_4OH were added and the reaction mixture was heated at 100°C for another 2 h. The heating was stopped and the reaction mixture was stirred at RT for 1.5 days. The reaction was stopped. The resulting suspension was filtered. The yellow solid was washed with water (twice with 4 mL), dried *in vacuo* to afford 3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-7-amine (1.8 g, 99% yield).

[0354] LCMS: MW (calcd): 196.6; m/z MW (obsd): 197.1 (M+H)

1.16.13. Step E:

[0355] Into a 250 mL round bottom flask was placed under Nitrogen 3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-7-amine (1 eq., 1.8 g, 9.15 mmol) in anhydrous DMF (18 mL) and NCS (1.3 eq., 1.59 g, 11.9 mmol) was added to the solution. The reaction mixture was heated at 50°C for 2.5 h. The reaction was stopped and cooled at RT. To the reaction mixture 10V of water (180mL) was added and mixture was

stirred to RT for 10 min. The resulting suspension was filtered. The white solid was washed with water (0.2 mL), dried *in vacuo* to afford 3,6-dichloro-5-ethylpyrazolo[1,5-a]pyrimidin-7-amine (1.87 g, 88% yield).

[0356] LCMS: MW (calcd): 231.1; m/z MW (obsd): 231.0 – 233.0 (M+H)

1.16.14. Step F:

[0357] Into a 10 mL round bottom flask was placed under nitrogen 3,6-dichloro-5-ethylpyrazolo[1,5-a]pyrimidin-7-amine (1 eq., 1.85 g, 8.01 mmol) in anhydrous THF (20.5 mL) and t-butyl nitrite (10 eq., 8.26 g, 9.6 mL, 80.06 mmol) was added dropwise to the solution, followed by salicylic acid (0.2 eq., 0.22 g, 1.61 mmol) . The reaction mixture was heated at 70°C for 1h. Reaction was stopped and cooled at RT. To the reaction mixture water (205 mL) was added and the resulting mixture was extracted with EtOAc (150 mL), the two phases were separated and the aqueous phase was further extracted with EtOAc (2 times with 50 mL). The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was then purified by flash chromatography on silica gel: elution with Heptane/AcOEt 100 to 80/20 to afford 3,6-dichloro-5-ethylpyrazolo[1,5-a]pyrimidine (957.5 mg, 55% yield).

[0358] LCMS: MW (calcd): 216.1; m/z MW (obsd): 216.0 – 218.0 (M+H)

1.16.15. Step G

[0359] A 100 mL round bottom flask under nitrogen 3,6-dichloro-5-ethylpyrazolo[1,5-a]pyrimidine (1 eq., 950 mg, 4.4 mmol) in methanol (21.0 mL) and NaOH 2N (15 eq., 33.0 mL, 66.0 mmol) was added to the solution. The reaction mixture was heated at 65°C for 1.15 h. The reaction mixture was heated at 65°C for another 2 h, was stopped and cooled at RT then was quenched at RT with HCl 2N (15 eq., 33.0 mL, 66.0 mmol) and stirred at RT for 5 min. The resulting suspension was filtered. The white solid was washed with water (twice with 10mL), dried *in vacuo* to afford 3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-ol (675.8 mg, 78% yield).

[0360] LCMS: MW (calcd): 197.6; m/z MW (obsd): 198.0 – 200.0 (M+H)

1.16.16. Step H:

[0361] A 10 mL round bottom flask was charged with 3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-ol (1 eq., 670 mg, 3.4 mmol) and placed under nitrogen atmosphere. Then anhydrous DMF (13.4 mL) was added at 0° (ice/water bath) followed by K_2CO_3 (1.5 eq., 702.8 mg, 5.09 mmol). The reaction mixture was stirred at RT for 10 min Then 3-chloro-2-butanone (1.5 eq., 541.9 mg, 0.52 mL, 5.09 mmol) was added dropwise. The reaction mixture was stirred at RT for 3 h and was stopped. To the mixture was added 25 mL of a K_2CO_3 solution10% mass and 25 mL of water. The mixture was extracted with EtOAc . Organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash chromatography on silica gel: elution with Heptane/AcOEt 100 to50/50 to afford 3-((3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (819.9 mg, 90% yield).

[0362] LCMS: MW (calcd): 267.7; m/z MW (obsd): 267.8 – 270.1 (M+H); 266.1 – 268.1 (M-H)

1.16.17. Step I:

[0363] To a solution of 3-((3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (1 eq., 700 mg, 2.61 mmol) and anhydrous MeTHF (10.4 mL) in a 100 mL round bottom flask under nitrogen to -20°C was added dropwise a solution of MeMgBr (3M Et_2O) (1.2 eq., 1.05 mL, 3.14 mmol). The mixture was stirred at -20°C for 10 min. The reaction was stopped. The reaction mixture was hydrolyzed at 0°C with a saturated solution of ammonium chloride stirred at RT for 5 min. The mixture was extracted with EtOAc. Organic layer was washed with twice with a saturated solution of ammonium chloride, brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was then purified by flash chromatography on silicagel: elution with Heptane/AcOEt 100 to 40/60 to afford 3-((3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (461.9 mg, 62% yield).

[0364] LCMS: MW (calcd): 283.8; m/z MW (obsd): 284.2 – 286-1 (M+H)

1.16.18. Step J:

[0365] A vial was charged with Int_4 (1.2 eq., 0.27 g, 0.85 mmol), 3-((3-chloro-5-ethylpyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 0.2 g, 0.71 mmol), XPhosPdG3 (0.05 eq., 0.03 g, 0.035 mmol), Cs_2CO_3 (1.2 eq., 0.28 g, 0.85 mmol), and degassed with argon. Anhydrous degassed (bubbling Nitrogen for 15min) water (0.75 mL) and dioxane (3 mL) were added at RT and reaction mixture was degassed through vacuum / Argon cycles 4 times, kept under argon atmosphere. Then the reaction mixture was heated at reflux under argon atmosphere for 2 h and the reaction was stopped. The reaction mixture was filtered through a pad of celite and the solid was rinsed with EtOAc, the filtrate was washed twice with water, and with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was then purified by SFC chiral separation on a Waters ACQUITY UPC2® system. Column: Lux Cellulose-1, 150 x 21.2mm, 5µm, CO_2 back pressure: 100 bar, co-solvent: EtOH, % of co-solvent: 15%, flowrate: 60 mL/min, isocratic mode, temperature: 40 °C. First eluting diastereoisomer (arbitrarily assigned as R as indicated), **Cpd 14** was isolated in 30% yield (103.8mg); Second eluting diastereoisomer (arbitrarily assigned as S), **Cpd 15** was isolated in 16% yield (53.7 mg).

1.16.19. Cpd 14

[0366] LCMS: MW (calcd): 522.5; m/z MW (obsd): 523.3 (M+H)

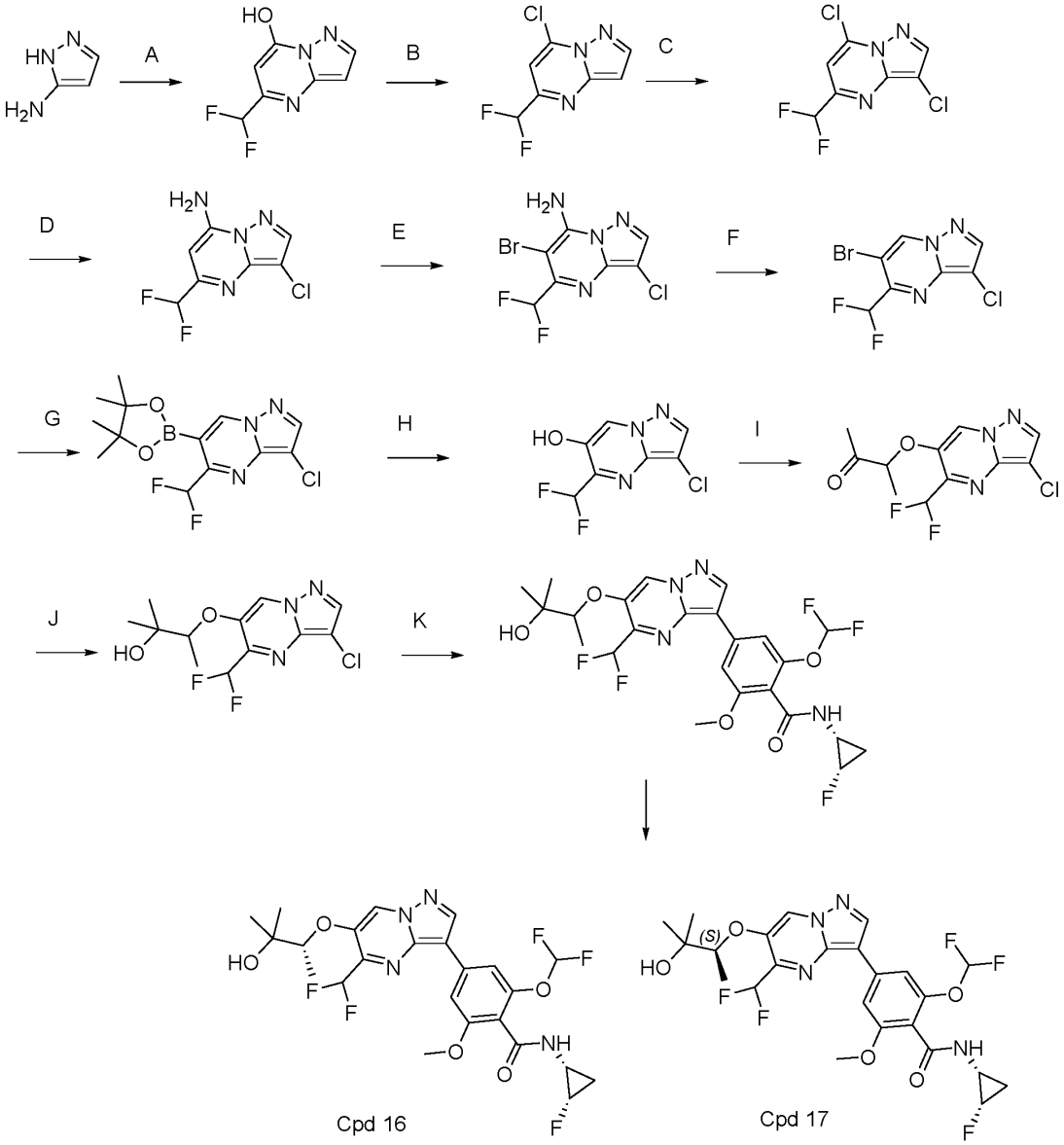
[0367] ee%=100%

1.16.20. Cpd 15

[0368] LCMS: MW (calcd): 522.5; m/z MW (obsd): 523.4 (M+H)

[0369] ee%=93.6%

1.17. Cpd 16 &Cpd 17



1.17.1. Step A

[0370] A 2,500 mL round bottom flask under air was charged with 3-aminopyrazole (1 eq., 10 g, 120.4 mmol) and AcOH (60 mL) and stirred for 5 min at rt. To the resulting solution ethyl 4,4-difluoro-3-oxobutanoate (1 eq., 20.0 g, 120.4 mmol) was added at RT. The reaction mixture was heated at reflux The formation of a white precipitate was quickly seen. 60 mL of NH_4OH were added and the reaction mixture was heated at 100°C for 2 hours then stirred at RT overnight. The resulting suspension was filtered. The yellow solid was washed with water (twice with 25 mL), dried *in vacuo* to afford 5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-ol (11.12 g, 99% yield).

[0371] LCMS: MW (calcd): 185.1; m/z MW (obsd): 186.2 (M+H)

1.17.2. Step B

[0372] A 50 mL round bottom flask was charged with 5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-ol (1 eq., 2 g, 10.80 mmol) and placed under nitrogen atmosphere. Then anhydrous dioxane (10 mL) was added at RT followed by diethylaniline (2 eq., 3.22 g, 3.46 mL, 21.6 mmol) and $POCl_3$ (5 eq., 8.28 g, 5.0 mL, 54.0 mmol). The reaction mixture was heated at 105°C for 2 h, then stopped and cooled down to RT. The reaction mixture was concentrated *in vacuo* ($POCl_3$ evaporated was carefully quenched). The residue evaporated was taken up in DCM (60 mL) and basified with $NaHCO_3$ sat (pH= 7-8 reached). The 2 phases were separated and the aqueous phase was further extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude was then purified by flash chromatography on silicagel: elution with heptane/AcOEt 100 to 80/20 to afford 7-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (1.67 g, 76% yield).

[0373] LCMS: MW (calcd): 203.6; m/z MW (obsd): 203.9 – 205.9 (M+H)

1.17.3. Step C

[0374] A 500 mL bottom flask was charged at RT under nitrogen with 7-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (1 eq., 12.73 g, 62.53 mmol) and anhydrous DMF (400 mL). To the mixture was added NCS (1.3 eq., 10.85 g, 81.3 mmol). The mixture was warmed up to 60°C and the mixture was stirred at 60°C for 2 h. To the mixture was added water, and the mixture was stirred at RT for 20 min. A precipitate was formed and filtered to afford 3,7-dichloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (13.54 g, 91% yield).

[0375] LCMS: MW (calcd): 238.0; m/z MW (obsd): 238.1 – 239.1 (M+H)

1.17.4. Step D

[0376] A 1,000 mL round bottom flask was charged at RT under nitrogen with 3,7-dichloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (1 eq., 15.3 g, 64.2 mmol) and ammonium hydroxide (500 mL). The mixture was stirred at RT and warmed up to 100°C for 1.75 h. The mixture was cooled to RT and to the mixture was added water (300 mL), and stirred at RT for 15 min, a yellow precipitate was formed. The suspension was filtered *in vacuo* to afford 3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-amine (14.56 g, 100% yield).

[0377] LCMS: MW (calcd): 218.6; m/z MW (obsd): 219.0 – 221.0 (M+H)

1.17.5. Step E

[0378] A 250 mL round bottom flask, was charged at RT under nitrogen with 5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-amine (1 eq., 14.56 g, 66.6 mmol) and anhydrous DMF (150 mL). To the mixture was added NBS (1.5 eq., 17.8 g, 99.90 mmol). The mixture was stirred at 50°C for 1 h and was cooled to RT and then to this mixture was added water (300 mL). The mixture was stirred at RT for 15 min. a grey precipitate was formed and filtered to afford 6-bromo-3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-amine (18.03 g, 91% yield).

[0379] LCMS: MW (calcd): 297.5; m/z MW (obsd): 297.0-299.0 (M+H)

1.17.6. Step F

[0380] A 100 mL round bottom flask was charged at RT under nitrogen 6-bromo-3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-7-amine (1 eq., 1.1 g, 3.7 mmol) and anhydrous THF (11 mL). To the mixture was added dropwise t-butyl nitrite (10 eq., 3.81 g, 4.43 mL, 36.98 mmol) and DMSO (0.1 eq., 0.029 g, 0.026 mL, 0.37 mmol). The mixture was warmed up to 70°C and stirred at 70°C for 2 h. The mixture was cooled to RT and to the mixture was added water. The mixture was extracted with EtOAc. The organic layer was washed with twice with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography, elution with a gradient heptane 100%, heptane / EtOAc 100 to 90/10 and DCM 100% to afford 6-bromo-3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (0.62 g, 59 % yield).

[0381] LCMS: MW (calcd): 282.5; m/z MW (obsd): 282.0-284.0 (M+H)

1.17.7. Step G

[0382] A 10-20 mL screw cap vial was charged at RT under nitrogen with bis(pinacolato)diboron (1.2 eq., 1011.91 mg, 3.98 mmol), 6-bromo-3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidine (1 eq., 938 mg, 3.32 mmol) and anhydrous dioxane (10 mL), bubbling nitrogen into the mixture for 10 min, and to the mixture was added $Pd(dppf)Cl_2$.DCM (0.1 eq., 271.2 mg, 0.33 mmol) and potassium pivalate (3 eq., 1,4 g, 9.96 mmol) . The mixture was stirred at 100°C for 15 min. The reaction was stopped. The mixture was cooled to RT and filtered through on a prepacked pad of celite, the solid was rinsed with EtOAc. The filtrate was concentrated *in vacuo* to afford 3-chloro-5-(difluoromethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyrimidine (3.13 g) directly used in the step H.

1.17.8. Step H

[0383] A 100 mL round bottom flask under nitrogen was charged at 0°C (ice/ water bath) with 3-chloro-5-(difluoromethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazolo[1,5-a]pyrimidine (1 eq., 3.13 g, 3.31 mmol) and acetone (15 mL). To the mixture was added a solution of Oxone (1.3 eq., 0.66 g, 4.31 mmol) in water (30 mL) at 0°C and directly monitored by LCMS. The reaction was stopped and to the mixture at 0°C was added a 10% mass solution of $Na_2S_2O_3$ (20 mL). The mixture was extracted with EtOAc. Organic layer was washed with a 10% mass solution of $Na_2S_2O_3$, brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel: elution with a gradient DCM 100%, DCM / (EtOAc/EtOH 3/1)) 100 to 90/10 to afford 3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-ol (495 mg, 68% yield).

[0384] LCMS: MW (calcd): 219.6; m/z MW (obsd): 220.0-222.0 (M+H).

1.17.9. Step I

[0385] A 50 mL round bottom flask was charged under nitrogen at 0° with 3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-ol (1 eq., 495 mg, 2.25 mmol) and anhydrous DMF (5 mL). To the mixture was added K_2CO_3 (1.5 eq., 467.33 mg, 3.38 mmol), the mixture was stirred at RT for 10 min. To the mixture was added dropwise 3-chloro-2-butanone (1.5 eq., 360.29 mg, 0.34 mL, 3.38 mmol).

The mixture was stirred at RT for 3.5 h. To the mixture was added water. The mixture was extracted with EtOAc . Organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, concentrated *in vacuo* and purified by flash chromatography on silica gel: elution with a gradient Heptane 100%, Heptane / EtOAc 100 to 50/50 to afford 3-((3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (376 mg, 58% yield).

[0386] LCMS: MW (calcd): 289.7; m/z MW (obsd): 290.1-292.1 (M+H).

1.17.10. Step J

[0387] To a solution of 3-((3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (1 eq., 376 mg, 1.3 mmol) and anhydrous MeTHF (5.18 mL) in a 100 mL round bottom flask under nitrogen to -20°C was added dropwise a solution of MeMgBr (3M Et_2O) (1.2 eq., 0.52 mL, 1.56 mmol). The mixture was stirred at -20°C and monitored by LCMS just after the addition of MeMgBr. The reaction was stopped and hydrolyzed at 0°C with a saturated solution of ammonium chloride stirred at RT for 5 min. The mixture was extracted with EtOAc. Organic layer was washed with twice with a saturated solution of ammonium chloride, brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* and purified by flash chromatography on silica gel: elution with a gradient: DCM 100%, DCM / (EtOAc / EtOH 3 / 1) 100 to 80/20 to afford 3-((3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (176 mg, 44% yield).

[0388] LCMS: MW (calcd): 305.7; m/z MW (obsd): 306.1 – 308.1 (M+H).

1.17.11. Step K

[0389] A screw vial cap was charged under nitrogen at RT Int_4 (1.2 eq., 95.2 mg, 0.3 mmol), 3-((3-chloro-5-(difluoromethyl)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1.16 eq., 88 mg, 0.29 mmol), K_2CO_3 (3 eq., 103.1 mg, 0.75 mmol), anhydrous dioxane (4 mL) and water (1 mL). To the mixture was added at RT XPHOS Pd G3 (0.1 eq., 21.0 mg, 0.025 mmol, CAS# 1445085-55-1). The mixture was warmed up to 110°C and stirred at 110°C for 1 h. The mixture was filtered through on a pad of celite and the solid was rinsed with EtOAc, the filtrate was concentrated *in vacuo* to afford a black oil. The mixture was extracted with EtOAc. Organic layer was washed with twice with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* and directly purified by flash chromatography on silica gel: elution with a gradient: Heptane 100%, Heptane / EtOAc 100 to 50/50 and DCM 100%, DCM / ((EtOAc/EtOH 3/1)) 100 to 90/10 to afford 2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide (86.8 mg, 55% yield)

[0390] LCMS: MW (calcd): 544.5; m/z MW (obsd): 545.2 (M+H).

1.17.12. Step L Chiral separation

[0391] Two diastereoisomers were separated by chiral SFC on a Waters ACQUITY UPC2® system. Column: Lux Cellulose-1, 150 x 21.2mm, 5µm, CO_2 back pressure: 100bar, co-solvent: EtOH, % of co-solvent: 15%, flowrate: 60 mL/min, isocratic mode, temperature: 40 °C to afford the first eluting

diastereomer arbitrarily assigned as 2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide **Cpd 16** (34.1 mg) and second eluting diastereomer arbitrarily assigned as 2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo [1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide, **Cpd 17** (23.4 mg).

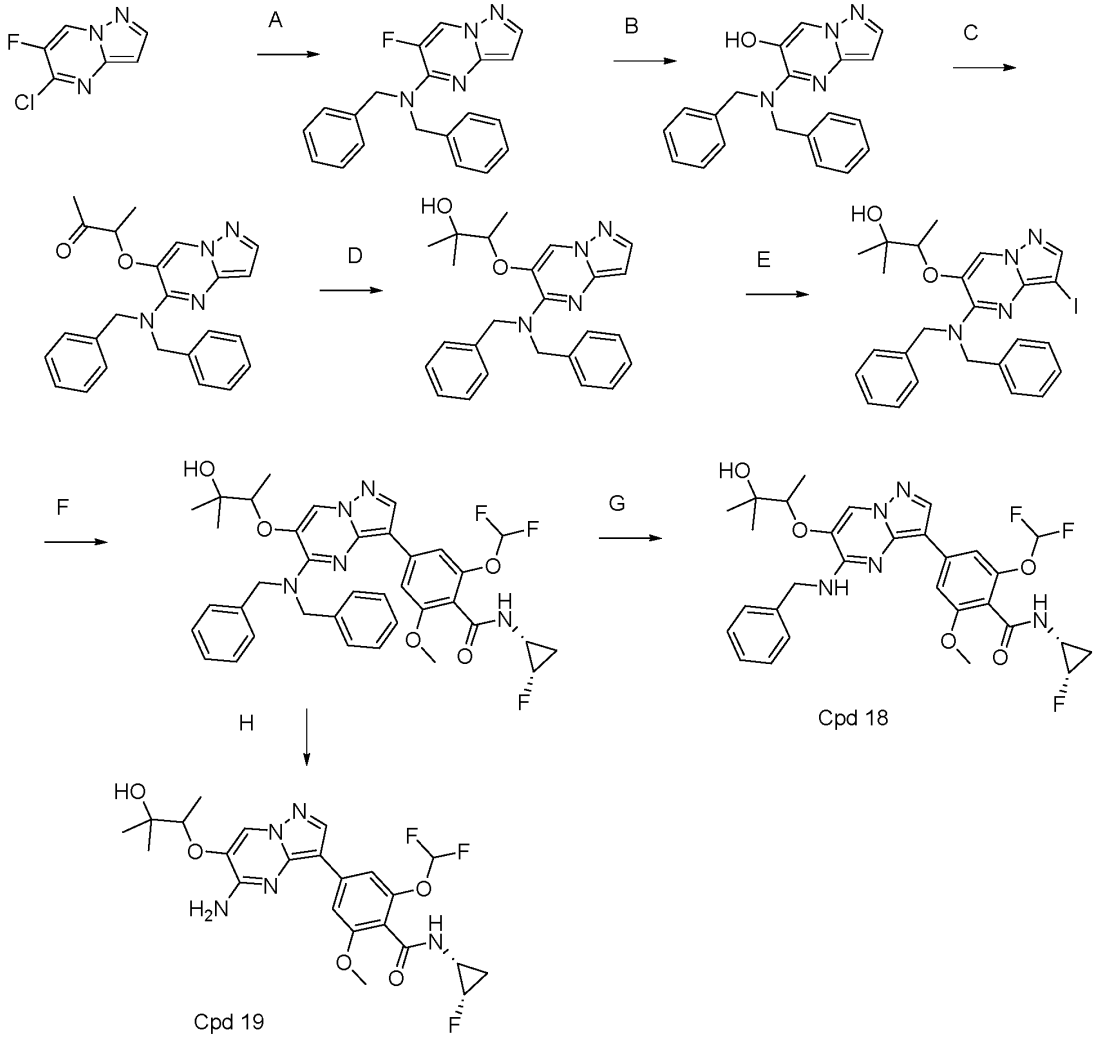
[0392] **Cpd 16**:

[0393] % e.e.: 100

[0394] **Cpd 17**:

[0395] % e.e.: 97.0

1.18. Cpd 18 & Cpd 19



1.18.1. Step A

[0396] In a sealed tube under air, a mixture of 5-chloro-6-fluoropyrazolo[1,5-a]pyrimidine (1 eq., 500 mg, 2.91 mmol), dibenzylamine (2 eq., 1,150 mg, 1.13 mL, 5.83 mmol) and DIPEA (2 eq., 753.4 mg, 1.0 mL,

5.83 mmol) in isopropanol (5 mL) was stirred at 100 °C for 24 h. The mixture was evaporated. Water and DCM were added. The organic layer was washed with brine, dried with $MgSO_4$, filtered, evaporated and then purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 50:50 to afford N,N-dibenzyl-6-fluoropyrazolo[1,5-a]pyrimidin-5-amine (970 mg, 88% yield).

[0397] LCMS: MW (calcd): 332.4; m/z MW (obsd): 333.1 (M+H).

1.18.2. Step B

[0398] In a sealed tube, under air, a mixture of N,N-dibenzyl-6-fluoropyrazolo[1,5-a]pyrimidin-5-amine (1 eq., 920 mg, 2.44 mmol) and NaOH 10M in H_2O (19.47 eq., 4.74 mL, 47.43 mmol) in MeOH (9.49 mL) was stirred at 100 °C in a sealed vial for 6 h. The mixture was evaporated and purified by flash chromatography on silica gel: elution with a gradient DCM/MeOH 100:0 to 90:10 in 20 to afford a yellow solid which was triturated with MeOH and filtered to afford 5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-ol (215 mg, 27% yield).

[0399] LCMS: MW (calcd): 330.4; m/z MW (obsd): 331.1 (M+H).

1.18.3. Step C

[0400] In a round bottom flask, under air, a mixture of 5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-ol (1 eq., 600 mg, 1.82 mmol), 3-chloro-2-butanone (1.5 eq., 290.2 mg, 0.28 mL, 2.72 mmol), K_2CO_3 (1.5 eq., 376.5 mg, 2.72 mmol), and DMF (3.63 mL) was stirred at RT for 2 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (571 mg, 79% yield).

[0401] LCMS: MW (calcd): 400.5; m/z MW (obsd): 401.3 (M+H).

1.18.4. Step D

[0402] In a round bottom flask, under N_2, at -20 °C, MeMgBr 3M in Et_2O (2 eq., 0.95 mL, 2.85 mmol) was added to 3-((5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (1 eq., 571 mg, 1.43 mmol) in MeTHF (17.2 mL) . The mixture was stirred at -20 °C for 2 h. EtOAc and NH_4Cl aq. were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (418 mg, 70% yield).

[0403] LCMS: MW (calcd): 416.5; m/z MW (obsd): 417.3 (M+H).

1.18.5. Step E

[0404] In a sealed tube, a mixture of 3-((5-(dibenzylamino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 418 mg, 1.01 mmol) and NIS (1.1 eq., 248.4 mg, 1.11 mmol) in DMF (4 mL) was stirred at RT for 1 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(dibenzylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (528 mg, 97% yield).

[0405] LCMS: MW (calcd): 542.4; m/z MW (obsd): 543.2 (M+H).

1.18.6. Step F

[0406] In a sealed tube, a mixture of 3-((5-(dibenzylamino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 528 mg, 0.97 mmol), Int_4 (1.2 eq., 372.7 mg, 1.17 mmol) , Cs_2CO_3 (1.2 eq., 380.6 mg, 1.17 mmol), $PdCl_2$dppf.DCM (0.1 eq., 79.5 mg, 0.097 mmol), dioxane (7.5 mL) and water (1.5 mL) was degassed with N_2 and stirred at 90 °C for 2 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 4-(5-(dibenzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide (342 mg, 51% yield).

[0407] LCMS: MW (calcd): 689.7; m/z MW (obsd): 690.7 (M+H).

1.18.7. Step G

[0408] In a round bottom flask at 0 °C, trifluoromethanesulfonic acid (20 eq., 217.6 mg, 0.13 mL, 1.45 mmol) was added to a solution of 4-(5-(dibenzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide (1 eq., 50 mg, 0.072 mmol) in DCM (0.9 mL). The mixture was stirred at 0 °C for 10 min. then at rt for 18 h. DCM and $NaHCO_3$ sat aq. were added until pH 7 and the mixture was stirred et RT. The organic layer was separated with a phase separator (Isolute) and evaporated. The crude residue was purified by preparative LCMS to afford 4-(5-(benzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide **Cpd 18** (10 mg, 23% yield).

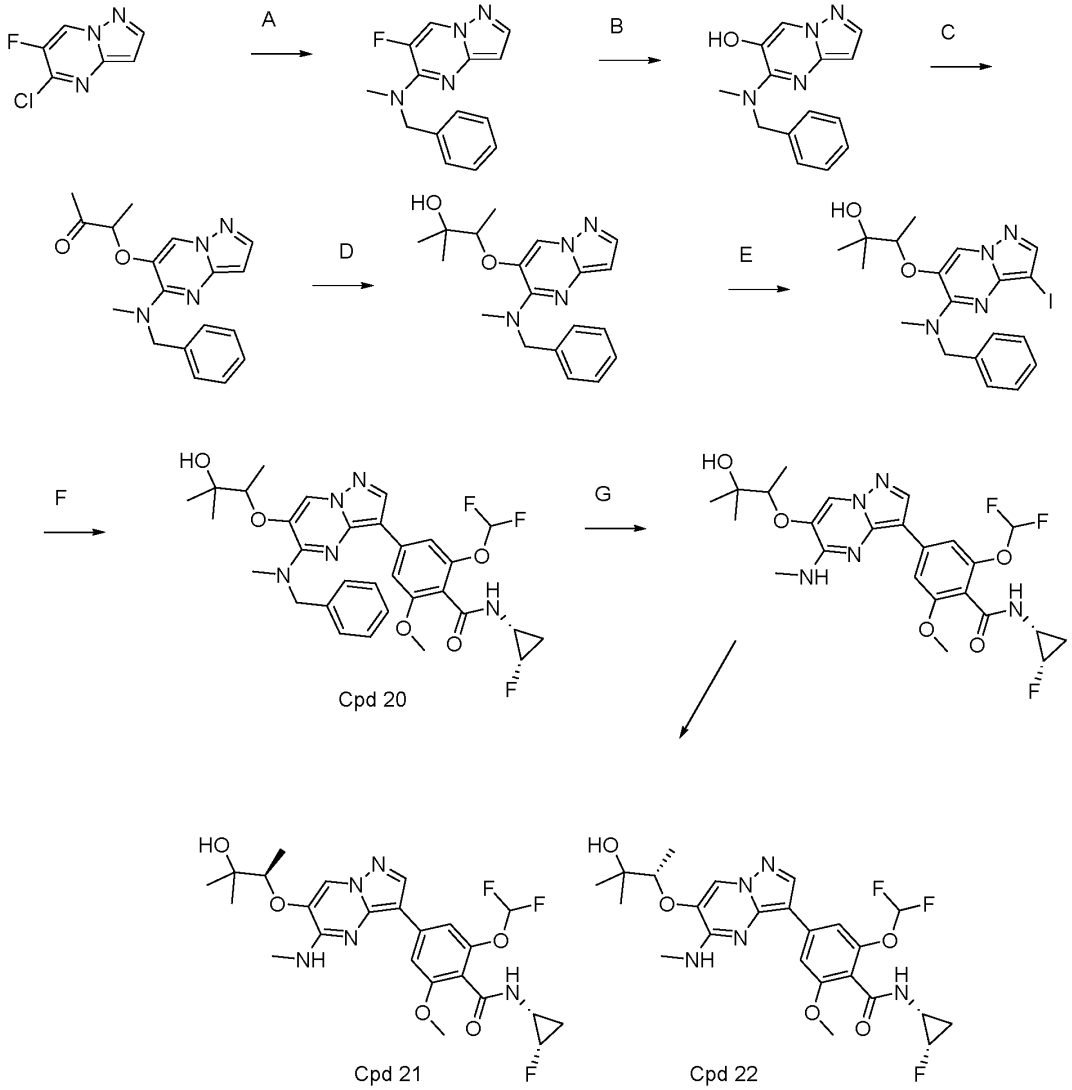
[0409] LCMS: MW (calcd): 599.6; m/z MW (obsd): 600.4 (M+H).

Step H

[0410] A H-Cube Nano was charged with a cartridge of $Pd(OH)_2$. A solution of 4-(5-(dibenzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide (1 eq., 50 mg, 0.072 mmol) in isopropanol (2 mL) was run at 1 mL/min, 1 bar of hydrogen and 70 °C. The resulting mixture was used for 2nd run at 1 mL/min, 1 bar of hydrogen and 70 °C. The resulting mixture was used for 3rd run at 1 mL/min, 80 bar of hydrogen and 70 °C. The resulting mixture was used for 4th run at 1 mL/min, 80 bar of hydrogen and 100 °C. The mixture was evaporated and purified by preparative LCMS (PrepB_50mL_35-50_8_min) to afford (5-amino-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide **Cpd 19** (1 mg, 3% yield).

[0411] LCMS: MW (calcd): 509.5; m/z MW (obsd): 510.2 (M+H).

1.19. Cpd 20, Cpd 21 & Cpd 22



1.19.1. Step A

[0412] In a sealed tube, under air, a mixture of 5-chloro-6-fluoropyrazolo[1,5-a]pyrimidine (1 eq., 500 mg, 2.91 mmol), N-methylbenzylamine (1.2 eq., 423.82 mg, 0.45 mL, 3.5 mmol) and THF (10 mL) was stirred at 70 °C for 18 h. N-methylbenzylamine (0.5 eq., 176.59 mg, 0.19 mL, 1.46 mmol), was added and the mixture was stirred at 70 °C for 18 h. The mixture was evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 50:50 to afford N-benzyl-6-fluoro-N-methylpyrazolo[1,5-a]pyrimidin-5-amine (658 mg, 88%).

[0413] LCMS: MW (calcd): 256.3; m/z MW (obsd): 257.2 (M+H).

1.19.2. Step B

[0414] In a round sealed tube, under air, a mixture of N-benzyl-6-fluoro-N-methylpyrazolo[1,5-a]pyrimidin-5-amine (1 eq., 658 mg, 2.57 mmol) and NaOH 10M in H_2O (19.47 eq., 5 mL, 50 mmol) in

MeOH (10 mL) was stirred at 100 °C in a sealed vial for 3 h. The mixture was evaporated and purified by flash chromatography elution with a gradient DCM/MeOH 100:0 to 90:10 to afford 5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-ol (325 mg, 50%).

[0415] LCMS: MW (calcd): 254.3; m/z MW (obsd): 255.4 (M+H).

1.19.3. Step C

[0416] In a round bottom flask, a mixture of 5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-ol (1 eq., 325 mg, 1.28 mmol), 3-chloro-2-butanone (1.5 eq., 204.26 mg, 0.19 mL, 1.92 mmol), K_2CO_3 (1.5 eq., 264.95 mg, 1.92 mmol), and DMF (2.56 mL) was stirred at RT for 1 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (300 mg, 72% yield).

[0417] LCMS: MW (calcd): 324.4; m/z MW (obsd): 325.2 (M+H).

1.19.4. Step D

[0418] In a round bottom flask, under N_2, at -20 °C, MeMgBr 3M in Et_2O (2 eq., 0.62 mL, 1.85 mmol) was added to 3-((5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)butan-2-one (1 eq., 300 mg, 0.92 mmol) in MeTHF (15 mL) . The mixture was stirred at -20 °C for 1 h. MeMgBr 3M in Et_2O (2 eq., 0.62 mL, 1.85 mmol) was added at -20 °c and the mixture was stirred and slowly heated to RT for 18 h. EtOAc and NH_4Cl aq. were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (147 mg, 47% yield).

[0419] LCMS: MW (calcd): 340.4; m/z MW (obsd): 341.2 (M+H).

1.19.5. Step E

[0420] In a round bottom flask, a mixture of 3-((5-(benzyl(methyl)amino)pyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 135 mg, 0.4 mmol) and NIS (1.1 eq., 98.14 mg, 0.44 mmol) in DMF (1.35 mL) was stirred at rt for 18 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 to afford 3-((5-(benzyl(methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (152 mg, 82%).

[0421] LCMS: MW (calcd): 466.3; m/z MW (obsd): 467.1 (M+H).

1.19.6. Step F

[0422] In a sealed tube, a mixture of 3-((5-(benzyl(methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-6-yl)oxy)-2-methylbutan-2-ol (1 eq., 14 mg, 0.03 mmol), Int_4 (1.2 eq., 11.5 mg, 0.036 mmol) , Cs_2CO_3 (1.2 eq., 11.74 mg, 0.036 mmol), $PdCl_2$dppf.DCM (0.1 eq., 2.45 mg, 0.003 mmol), dioxane (0.5 mL) and water (0.1 mL) was degassed with N_2 and stirred at 90 °C for 1.5 h. EtOAc and water were added. The organic layer was washed with brine, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on

silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 then 100% EtOAc to afford 4-(5-(benzyl(methyl)amino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide **Cpd 20** (7 mg, 38% yield)

[0423] LCMS: MW (calcd): 613.6; m/z MW (obsd): 614.4 (M+H).

1.19.7. Step G

[0424] In a round bottom flask, under air, at 0 °C Trifluoromethanesulfonic acid (20 eq., 464.7 mg, 0.27 mL, 3.1 mmol) was added to a solution of 4-(5-(benzyl(methyl)amino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide **Cpd 20** (1 eq., 95 mg, 0.15 mmol) in DCM (1.9 mL). The mixture was stirred at 0 °C for 2h then at RT. DCM and aq. sat. $NaHCO_3$ were added (until pH 7). The organic layer was separated, dried ($MgSO_4$), filtered, evaporated and purified by flash chromatography on silica gel: elution with a gradient Heptane/EtOAc 100:0 to 0:100 then 100% EtOAc to afford the titled racemic mixture (50 mg, 62% yield) as a white solid witch was then purified by chiral SFC on a Waters ACQUITY UPC2® system. Column: Chiralpak IA, 150 x 21mm, 5µm with Guard of 50 x 21mm, 5µm, CO_2 back pressure: 100bar, co-solvent: Isopropanol, % of co-solvent: 30%, flowrate: 60 mL/min, isocratic mode, temperature: 40 °C to afford **Cpd 21**, the first eluting arbitrarily assigned with the (R) configuration (14.8 mg, 18% yield) and **Cpd 22**, the second eluting arbitrarily assigned with the (S) configuration (14.2 mg, 18%)

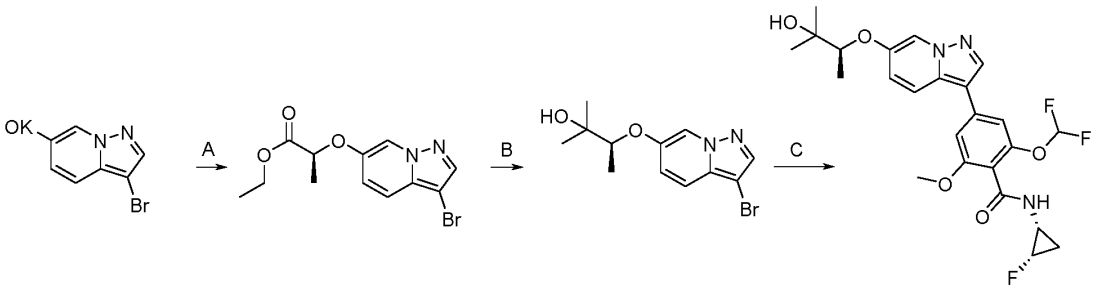
[0425] **Cpd 21**: % e.e.: 100

[0426] LCMS: MW (calcd): 523.5; m/z MW (obsd): 524.5 (M+H).

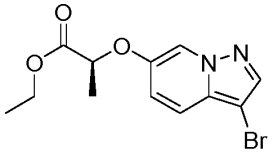
[0427] **Cpd 22**: % e.e.: 96.0

[0428] LCMS: MW (calcd): 523.5; m/z MW (obsd): 524.5 (M+H).

1.20. Cpd B



1.20.1. Step A: ethyl (2S)-2-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxypropanoate

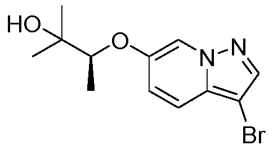


[0429] In a 15 L single jacketed process reactor equipped with mechanical stirring (200 rpm) and baffle, were charged DMF (3.5 L, 8V) and **Int 5** (430 g, 1,712 mmol, 1 eq.). The resulting solution was stirred 10 min at RT then cooled down to 0 °C. A solution of **Int 2** (490 g, 1763 mmol, 1.03 eq.) in DMF (860 mL,

2V) was slowly added over 1.3 h while maintaining the process temperature between 0 and 2°C. Over the course of the addition, the stirring was increased to 275 rpm. Following the addition, the reaction mixture was stirred for a further 3 h at 0 °C. The reaction mixture was quenched by slow addition (approx. 45 min) of aq. K_2CO_3 10% (1.075 L, 2.5V) while maintaining the process temperature below 5 °C. Water (2.2 L, 5V) and EtOAc (4.4L, 10V) were added in 3 portions each, alternating between the two liquids. The jacket temperature was set to 22 °C and the biphasic mixture was stirred for 5 min at 275 rpm. The layers were separated and the aqueous layer extracted with EtOAc (2×1.1 L, 2×2.5V), the organic layers were combined, washed with aq. K_2CO_3 10% (860 mL, 2V), aq. LiCl 10% (1.7 L, 4V), water (1.3 L, 3V) and aq. NaCl 10% (860 mL, 2V); and concentrated under vacuum to afford ethyl (2S)-2-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxypropanoate.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (dd, 1H), 8.05 (s, 1H), 7.53 (dd, 1H), 7.22 (dd, 1H), 5.12 (q, 1H), 4.16 (qd, 2H), 1.54 (d, 3H), 1.18 (t, 3H).

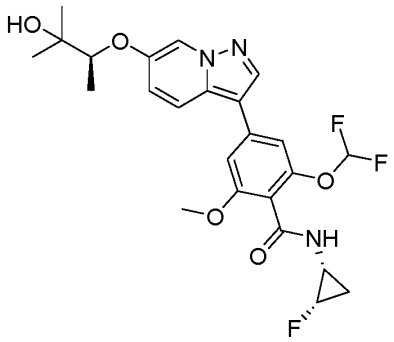
1.20.2. Step B: (3S)-3-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxy-2-methyl-butan-2-ol



[0430] In a 15 L single jacketed process reactor equipped with mechanical stirring (200 rpm) and baffle, were charged ethyl (2S)-2-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxypropanoate (432 g, 1170 mmol, 1 eq.) and 2-MeTHF (4.3 L, 10V). The resulting solution was cooled to 0 °C. A solution of MeMgBr (3.4 M in 2-MeTHF, 1 L, 3400 mmol, 2.9 eq.) was slowly added over 1.25 h while maintaining the process temperature between 0 and 2 °C. Following the addition, the reaction mixture was stirred for a further 30 min at this temperature. The jacket temperature was set at -15 °C and the reaction mixture was quenched by slow addition over 55 min of aq. HCl 1 M (5.5 L, 5500 mmol, 1V) while maintaining the process temperature below 10 °C, with the jacket temperature set to warm to 30 °C over 30 min after addition of 3.5 L of HCl. The stirring was set to 250 rpm and the biphasic mixture was stirred 30 min, with jacket temperature set at 30 °C for 20 min, then cooled down to 23 °C. The layers were separated, and the aqueous layer was extracted with 2-MeTHF (4V). The organic layers were combined, washed with aq. $NaHCO_3$ 10% (2V), brine (3V), filtered over cellulose and concentrated under vacuum to give (3S)-3-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxy-2-methyl-butan-2-ol.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (dd, 1H), 8.02 (s, 1H), 7.53 – 7.42 (m, 1H), 7.18 (dd, 1H), 4.54 (s, 1H), 4.19 (q, 1H), 1.22 (d, 3H), 1.18 (s, 3H), 1.16 (s, 3H)

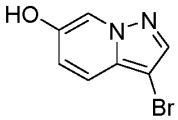
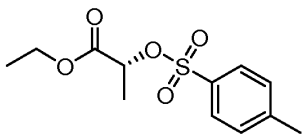
1.20.3. Step C: Cpd B



[0431] In a 15 L single jacketed process reactor equipped with condenser, nitrogen inlet, gas bubbler, mechanical stirring (150 rpm) and baffle, was charged at 20 °C a solution of (3S)-3-(3-bromopyrazolo[1,5-a]pyridin-6-yl)oxy-2-methyl-butan-2-ol (562 g, 1550 mmol, 1.0 eq.) in 1,4-dioxane (4.5 L, 8V), followed by **Int 4** (494 g, 1550 mmol, 1.0 eq.). Water (1.25 L, 2V) and sodium carbonate (498 g, 4651 mmol, 3.0 eq.) were then added. The reaction mixture was then degassed by nitrogen sparge for 15 min, the remaining of the procedure was performed under nitrogen flow. XPhos (59.7 g, 124 mmol, 8 mol%) and $Pd_2(dba)_3$ (28.4 g, 31 mmol, 2 mol%) were then added. The stirring was increased to 200 rpm and the jacket temperature set at 75 °C. The process temperature gradually increased over 2 h to reach a maximum at 77.4 °C, after which, it decreased to 72 °C. The reaction mixture was stirred at this temperature for a further 1.25 h, and then cooled down to 30-35 °C. After cooling, the reaction mixture was filtered over cellulose and washed with 2-MeTHF (2 × 5V). The organic layer was transferred into a 15 L reactor and washed with water (3V). The layers were separated, and the aqueous layer was extracted with 2-MeTHF (2 × 1V). The organic layers were combined, washed with aq. NaCl 10% (3V), and concentrated partially under vacuum to obtain a suspension. This suspension was transferred into a 15 L reactor and reslurried in MTBE (8V). The solid was filtered, washed with MTBE and dried in a vacuum oven (48 h at 45 °C) to afford **Cpd B**.

Table II. Intermediates used towards the compounds of the invention.

SM = Starting Material, Mtd = Method, MS Mes'd = Measured mass, NA = not measured

Int#	Structure	SM	Mtd	MW	MS Mes'd
1		CAS# 1264193-11-4	Ex. 1.1	212.0	212.9 214.9
2		CAS# 7699-00-5	Ex. 1.2	272.1	272.8

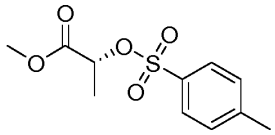
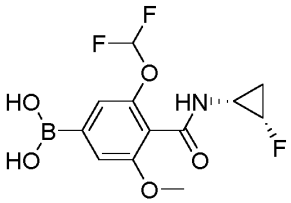
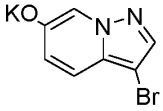
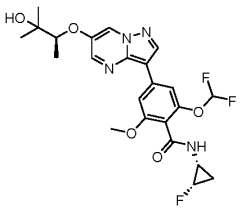
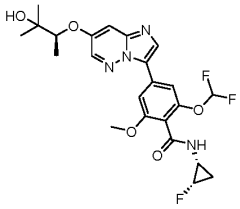
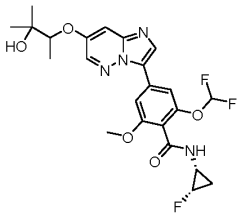
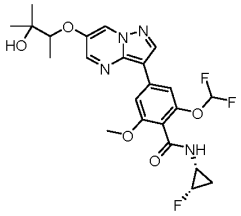
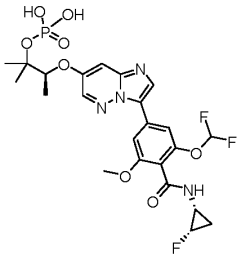
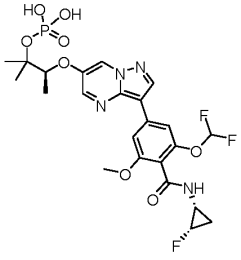
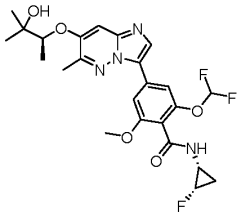
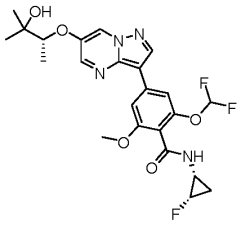
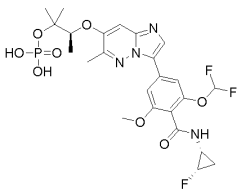
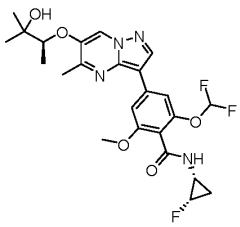
Int#	Structure	SM	Mtd	MW	MS Mes'd
3		CAS# 17392-83-5	Ex. 1.3	258.1	258.8
4		CAS# 3147-64-6	Ex. 1.4	319.1	320.0
5		**Int 1**	Ex. 1.5	249.9	212.9 214.9

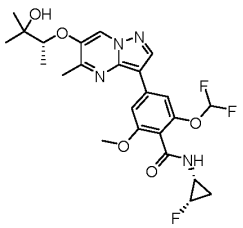
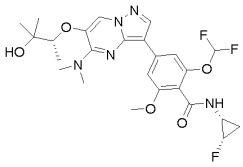
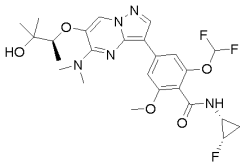
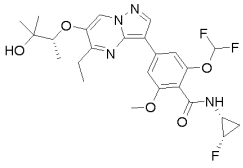
Table III. Illustrative compounds of the invention.

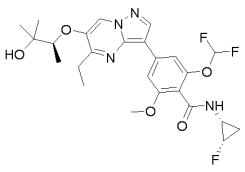
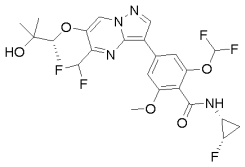
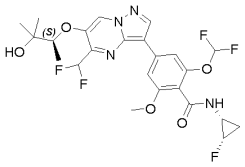
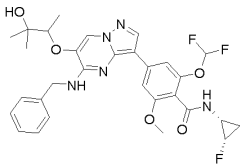
SM = Starting Material, Mtd = Method, MS Mes'd = Mesured mass

Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
1		2-(difluoromethoxy)-N-[(1R,2S)-2-fluorocyclopropyl]-4-[6-[(2S)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide	CAS# 1580489-59-3	Ex. 1.6	494.2	495.0
2		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide	CAS# 2155875-87-7	Ex. 1.7	494.2	495.2

Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
3		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-(3-hydroxy-3-methylbutan-2-yl)oxyimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide	CAS# 2410998-35-3	Ex. 1.8	494.2	495.3
4		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-(3-hydroxy-3-methylbutan-2-yl)oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide	CAS# 1580489-59-3	Ex. 1.9	494.2	495.3
5		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]imidazo[1,2-b]pyridazin-7-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate	Cpd 2	Ex. 1.10	574.1	575.3
6		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]pyrazolo[1,5-a]pyrimidin-6-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate	Cpd 1	Ex. 1.11	574.1	575.0

Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
7		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[7-[(2*S*)-3-hydroxy-3-methylbutan-2-yl]oxy-6-methylimidazo[1,2-b]pyridazin-3-yl]-6-methoxybenzamide	CAS# 68240-43-7	Ex. 1.12	508.2	509.2
8		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R*)-3-hydroxy-3-methylbutan-2-yl]oxypyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide	CAS# 1580489-59-3	Ex. 1.6	494.2	495.0
9		[(3*S*)-3-[3-[3-(difluoromethoxy)-4-[[(1*R*,2*S*)-2-fluorocyclopropyl]carbamoyl]-5-methoxyphenyl]-6-methylimidazo[1,2-b]pyridazin-7-yl]oxy-2-methylbutan-2-yl] dihydrogen phosphate	Cpd 7	Ex. 1.13	588.2	589.3
10		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*S**)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide	CAS# 16082-27-2	Ex. 1.14	508.2	509.2

Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
11		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(2*R**)-3-hydroxy-3-methylbutan-2-yl]oxy-5-methylpyrazolo[1,5-a]pyrimidin-3-yl]-6-methoxybenzamide	CAS# 16082-27-2	Ex. 1.14	508.2	509.2
12		2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.16	537.5	538.5
13		2-(difluoromethoxy)-4-(5-(dimethylamino)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.16	537.5	538.5
14		2-(difluoromethoxy)-4-(5-ethyl-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.16	522.5	523.3

Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
15		2-(difluoromethoxy)-4-(5-ethyl-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.17	522.5	523.4
16		2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.17	544.5	545.2
17		2-(difluoromethoxy)-4-(5-(difluoromethyl)-6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.18	544.5	545.2
18		4-(5-(benzylamino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.18	599.6	600.4

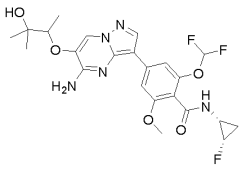
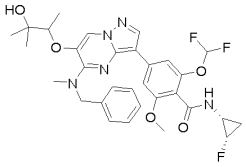
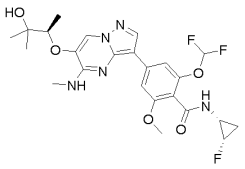
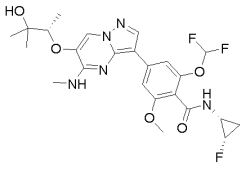
Cpd#	Structure	Name	SM	Mtd	MW	MS Mes'd
19		4-(5-amino-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.19	509.5	510.2
20		4-(5-(benzyl(methyl)amino)-6-((3-hydroxy-3-methylbutan-2-yl)oxy)pyrazolo[1,5-a]pyrimidin-3-yl)-2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-6-methoxybenzamide	Described above	Ex 1.19	613.6	614.4
21		2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-4-(6-(((R*)-3-hydroxy-3-methylbutan-2-yl)oxy)-5-(methylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-6-methoxybenzamide	Described above	Ex 1.20	523.5	524.5
22		2-(difluoromethoxy)-N-((1R,2S)-2-fluorocyclopropyl)-4-(6-(((S*)-3-hydroxy-3-methylbutan-2-yl)oxy)-5-(methylamino)pyrazolo[1,5-a]pyrimidin-3-yl)-6-methoxybenzamide	Described above	Ex 1.20	523.5	524.5

Table IV. Comparative compounds.

SM = Starting Material, Mtd = Method, MS Mes'd = Measured mass

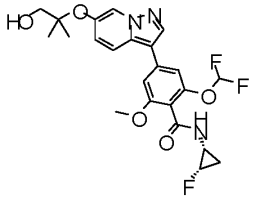
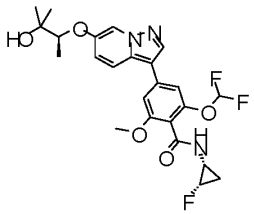
Cpd#	Structure	Name	SM / Mtd	MW	MS Mes'd
A		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-(2-hydroxy-1,1-dimethyl-ethoxy)pyrazolo[1,5-a]pyridin-3-yl]-6-methoxy-benzamide	Cpd A was synthesized according to the procedure on page 74 of WO 2020/239660 A1	479.2	480.3
B		2-(difluoromethoxy)-*N*-[(1*R*,2*S*)-2-fluorocyclopropyl]-4-[6-[(1*S*)-2-hydroxy-1,2-dimethyl-propoxy]pyrazolo[1,5-a]pyridin-3-yl]-6-methoxy-benzamide	Int 5 Ex. 1.20	493.2	494.2

Table V. NMR data of illustrative compounds of the invention and comparative compounds.

Cpd#	NMR
1	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, 1H), 8.38 (d, 1H), 8.32 (s, 1H), 7.62 (d, 1H), 7.46 (t, 1H), 6.67 (t, 1H), 6.35 (d, 1H), 4.77 (dtd, 1H), 4.16 (q, 1H), 3.96 (s, 3H), 3.18 – 3.00 (m, 1H), 1.43 – 1.33 (m, 9H), 1.33 – 1.21 (m, 1H), 1.08 (dddd, 1H)
2	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, 2H), 8.21 (s, 1H), 7.70 – 7.60 (m, 3H), 7.09 (t, 1H), 4.81 (td, 1H), 4.65 (td, 1H), 4.46 (q, 1H), 3.88 (s, 3H), 2.90 – 2.79 (m, 1H), 1.27 (d, 3H), 1.19 (d, 6H), 1.17 – 1.03 (m, 1H), 0.94 (dddd, 1H)
3	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 – 8.47 (m, 2H), 8.21 (s, 1H), 7.70 – 7.60 (m, 3H), 7.09 (t, 1H), 4.85 – 4.62 (m, 1H), 4.63 (s, 1H), 4.46 (q, 1H), 3.88 (s, 3H), 2.84 (dd, 1H), 1.38 – 1.22 (m, 3H), 1.19 (d, 6H), 1.10 (dq, 1H), 1.01 – 0.84 (m, 1H)
4	^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, 1H), 8.77 (s, 1H), 8.64 (d, 1H), 8.44 (d, 1H), 7.70 – 7.63 (m, 2H), 7.06 (t, 1H), 4.85 – 4.63 (m, 1H), 4.62 (s, 1H), 4.31 (q, 1H), 3.88 (s, 3H), 2.83 (dd, 1H), 1.30 – 1.14 (m, 9H), 1.09 (dd, 1H), 1.04 – 0.68 (m, 1H)
5	^{1}H NMR (400 MHz, CD_3OD) δ 8.62 (d, 1H), 8.08 (s, 1H), 7.81 – 7.72 (m, 1H), 7.54 (d, 2H), 6.81 (t, 1H), 4.98 (d, 1H), 4.85 – 4.60 (m, 1H), 3.94 (s, 3H), 2.93 (dd, 1H), 1.64 (s, 6H), 1.44 (d, 3H), 1.22 (sept., 1H), 1.15 – 1.05 (m, 1H)

Cpd#	NMR
6	^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, 1H), 8.60 (d, 1H), 8.53 (s, 1H), 7.74 (d, 1H), 7.60 (q, 1H), 6.92 (d, 1H), 4.81 – 4.77 (m, 0.5H), 4.63 (ddd, 0.5H), 4.57 (q, 1H), 3.94 (s, 3H), 2.92 – 2.86 (m, 1H), 1.63 (s, 3H), 1.59 (s, 3H), 1.41 (d, 3H), 1.18 (dtd, 1H), 0.99 – 1.05 (dddd, 1H)
7	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, 1H), 8.15 (s, 1H), 7.70 (d, 2H), 7.57 (s, 1H), 7.11 (t, 1H), 4.88 – 4.58 (m, 2H), 4.46 (q, 1H), 3.88 (s, 3H), 2.84 (td, 1H), 1.27 (d, 3H), 1.20 (d, 6H), 1.14 – 1.03 (m, 1H), 0.99 – 0.87 (m, 1H)
8	^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, 1H), 8.76 (s, 1H), 8.63 (d, 1H), 8.44 (d, 1H), 7.72 – 7.58 (m, 2H), 7.05 (t, 1H), 4.83 – 4.52 (m, 2H), 4.30 (q, 1H), 3.87 (s, 3H), 2.82 (q, 1H), 1.26 (d, 3H), 1.19 (d, 6H), 1.09 (tt, 1H), 0.93 (dtd, 1H)
9	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, 1H), 8.17 (s, 1H), 7.70 (d, 2H), 7.59 (s, 1H), 7.11 (t, 1H), 4.85 – 4.61 (m, 2H), 3.87 (s, 3H), 2.83 (dt, 1H), 1.51 (d, 6H), 1.31 (d, 3H), 1.16 – 1.02 (m, 1H), 1.01 – 0.85 (m, 1H)
10	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, 2H), 7.68 (d, 1H), 7.58 – 7.50 (m, 1H), 6.66 (t, 1H), 6.24 (d, 1H), 4.74 (dtd, 1H), 4.17 (q, 1H), 3.95 (s, 3H), 3.07 (dq, 1H), 2.62 (s, 3H), 1.36 (dd, 9H), 1.31 – 1.18 (m, 1H), 1.05 (dddd, 1H)
11	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, 2H), 7.70 – 7.63 (m, 1H), 7.52 (d, 1H), 6.66 (t, 1H), 6.23 (d, 1H), 4.84 – 4.65 (m, 1H), 4.18 (q, 1H), 3.96 (s, 3H), 3.07 (dq, 1H), 2.63 (s, 3H), 1.37 (dd, 9H), 1.35 – 0.97 (m, 2H)
12	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.44 (s, 1H), 8.36 (d, J = 4.5 Hz, 1H), 7.66 (d, J = 1.3 Hz, 1H), 7.63 – 7.61 (m, 1H), 7.06 (t, J = 74.6 Hz, 1H), 4.81 – 4.59 (m, 1H), 4.62 (s, 1H), 4.31 (q, J = 6.2 Hz, 1H), 3.85 (s, 3H), 3.25 (s, 6H), 2.86 – 2.78 (m, 1H), 1.24 (d, J = 6.2 Hz, 3H), 1.21 (s, 3H), 1.20 (s, 3H), 1.13 – 1.01 (m, 1H), 0.99 – 0.86 (m, 1H)
13	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.44 (s, 1H), 8.36 (d, J = 4.5 Hz, 1H), 7.66 (d, J = 1.3 Hz, 1H), 7.64 – 7.61 (m, 1H), 7.06 (t, J = 74.6 Hz, 1H), 4.81 – 4.59 (m, 1H), 4.62 (s, 1H), 4.31 (q, J = 6.2 Hz, 1H), 3.85 (s, 3H), 3.25 (s, 6H), 2.86 – 2.78 (m, 1H), 1.24 (d, J = 6.2 Hz, 3H), 1.21 (s, 3H), 1.20 (s, 3H), 1.13 – 1.02 (m, 1H), 0.99 – 0.86 (m, 1H)
14	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.67 (s, 1H), 8.41 (d, J = 4.5 Hz, 1H), 7.80 (d, J = 1.3 Hz, 1H), 7.73 (q, J = 1.1 Hz, 1H), 6.97 (d, J = 74.4 Hz, 1H), 4.87 – 4.57 (m, 2H), 4.36 (q, J = 6.1 Hz, 1H), 3.88 (s, 3H), 3.04 – 2.77 (m, 3H), 1.34 (t, J = 7.3 Hz, 3H), 1.23 (dd, J = 20.5, 7.4 Hz, 9H), 1.14 – 1.03 (m, 1H), 0.99 – 0.87 (m, 1H)

Cpd#	NMR
15	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.67 (s, 1H), 8.41 (d, J = 4.5 Hz, 1H), 7.80 (d, J = 1.2 Hz, 1H), 4.61 (s, 2H), 4.36 (q, J = 6.1 Hz, 1H), 3.88 (s, 3H), 3.02 – 2.77 (m, 3H), 1.34 (t, J = 7.3 Hz, 3H), 1.23 (dd, J = 20.4, 7.5 Hz, 9H), 1.12 – 0.86 (m, 2H), 7.73 (q, J = 1.1 Hz, 1H), 7.07 (t, J = 74.5 Hz, 1H)
16	^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.88 (s, 1H), 8.45 (d, J = 4.5 Hz, 1H), 7.79 (d, J = 1.3 Hz, 1H), 7.70 – 7.64 (m, 1H), 7.39 – 6.88 (m, 2H), 4.83 – 4.62 (m, 2H), 4.45 (q, J = 6.2 Hz, 1H), 3.88 (s, 3H), 2.89 – 2.78 (m, 1H), 1.29 (d, J = 6.2 Hz, 3H), 1.20 (d, J = 5.1 Hz, 6H), 1.14 – 1.04 (m, 1H), 0.99 – 0.89 (m, 1H)
17	^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.88 (s, 1H), 8.45 (d, J = 4.5 Hz, 1H), 7.79 (d, J = 1.3 Hz, 1H), 7.67 (d, J = 1.2 Hz, 1H), 7.37 – 6.89 (m, 2H), 4.83 – 4.63 (m, 2H), 4.45 (q, J = 6.1 Hz, 1H), 3.88 (s, 3H), 2.84 (q, J = 4.7 Hz, 0H), 1.29 (d, J = 6.2 Hz, 3H), 1.20 (d, J = 5.1 Hz, 6H), 1.13 – 1.04 (m, 1H), 1.00 – 0.88 (m, 1H)
18	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.36 (s, 1H), 8.32 (d, J = 4.5 Hz, 1H), 8.09 (t, J = 6.3 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H), 7.52 (d, J = 1.2 Hz, 1H), 7.38 – 7.28 (m, 4H), 7.25 – 7.19 (m, 1H), 6.93 (t, J = 74.8 Hz, 1H), 4.80 (d, J = 6.3 Hz, 2H), 4.68 (s, 1H), 4.79 – 4.56 (m, 1H), 4.32 (q, J = 6.1 Hz, 1H), 3.62 (s, 3H), 2.82 – 2.75 (m, 1H), 1.27 (d, J = 6.1 Hz, 3H), 1.23 (s, 3H), 1.14 (s, 3H), 1.11 – 1.00 (m, 1H), 0.96 – 0.84 (m, 1H)
19	^{1}H NMR (400 MHz, CD_3OD) δ 8.24 – 8.13 (m, 2H), 7.80 – 7.69 (m, 1H), 7.52 – 7.44 (m, 1H), 7.04 – 6.56 (m, 1H), 4.78 – 4.57 (m, 1H), 4.27 (q, J = 6.2 Hz, 1H), 3.94 (s, 3H), 2.93 – 2.84 (m, 1H), 1.37 (d, J = 6.2 Hz, 3H), 1.33 (s, 3H), 1.30 (s, 3H), 1.25 – 1.14 (m, 1H), 1.09 – 0.95 (m, 1H)
20	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 – 8.61 (m, 1H), 8.28 – 8.09 (m, 1H), 7.57 – 7.27 (m, 7H), 6.75 – 6.35 (m, 1H), 6.24 – 6.13 (m, 1H), 5.17 – 4.89 (m, 2H), 4.86 – 4.59 (m, 1H), 4.30 – 4.19 (m, 1H), 3.97 – 3.65 (m, 3H), 3.51 – 3.21 (m, 3H), 3.04 (dt, J = 9.8, 4.9 Hz, 1H), 1.41 – 0.93 (m, 11H)
21	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 – 8.31 (m, 3H), 7.71 (d, J = 1.2 Hz, 1H), 7.68 (s, 1H), 7.53 (d, J = 4.9 Hz, 1H), 7.06 (t, J = 74.6 Hz, 1H), 4.82 – 4.58 (m, 2H), 4.33 – 4.26 (m, 1H), 3.85 (s, 3H), 3.02 (d, J = 4.7 Hz, 3H), 2.87 – 2.77 (m, 1H), 1.28 – 1.18 (m, 6H), 1.13 (s, 3H), 1.11 – 1.06 (m, 1H), 0.99 – 0.87 (m, 1H)
22	^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 – 8.29 (m, 3H), 7.71 (d, J = 1.2 Hz, 1H), 7.68 (d, J = 1.2 Hz, 1H), 7.53 (d, J = 4.8 Hz, 1H), 7.06 (t, J = 74.6 Hz, 1H), 4.82 – 4.57 (m, 2H), 4.32 – 4.25 (m, 1H), 3.85 (s, 3H), 3.02 (d, J = 4.7 Hz, 3H), 2.85 – 2.79 (m, 1H), 1.25 – 1.20 (m, 6H), 1.13 (s, 3H), 1.11 – 1.06 (m, 1H), 0.99 – 0.87 (m, 1H)

Cpd#	NMR
B	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, 1H), 8.09 (s, 1H), 7.70 (dd, 1H), 7.17 (dd, 1H), 7.05 (q, 1H), 6.98 (d, 1H), 6.67 (t, 1H), 6.24 (d, 1H), 4.77 (dtd, 1H), 4.16 (q, 1H), 3.95 (s, 3H), 3.22 – 2.92 (m, 1H), 1.38-1.33 (m, 9H), 1.32-1.23 (m, 1H), 1.07 (dddd, 1H)

BIOLOGICAL EXAMPLES

Example 2. ***In vitro*** **assays**

2.1. Biochemical assays

2.1.1. ADP-Glo™ SIK Kinase Assay

2.1.1.1. Overview

[0432] The ADP-Glo™ kinase assay is a luminescent technology assay which measures the ADP formed from a kinase reaction. In this specific study, the kinase reactions consisted of the phosphorylation of the AMARA peptide substrate (SignalChem, Cat# A11-58, or Eurogentec, SEQ ID 1) by SIK1 (Carna Biosciences, Cat# 02-131), SIK2 (Invitrogen, Cat# PV4792, or Cat# PR8353A), or SIK3 FL (SEQ ID 2). In a second step the kinase reactions are terminated, and all the remaining ATP is depleted. In a final step the ADP is converted into ATP and this newly synthesized ATP is measured by using a luciferase/luciferin reaction. The generated light is measured using an Envision plate reader, wherein the luminescent signal obtained positively correlates with the kinase activity.

2.1.1.2. Protocol without pre-incubation

[0433] The test compounds were prepared as a serial dilution of 10 point dose responses with 1/5 dilution steps in 100% DMSO starting from 2 mM highest concentration, diluted 1/20 in water and 1 µL was transferred to the assay plates (PerkinElmer Inc., Cat# 6007290).

[0434] 1% DMSO and 10 µM staurosporine final concentrations were used as negative and positive controls.

[0435] The enzyme-substrate mixture was prepared by adding the enzyme and the substrate to the assay buffer.

[0436] 2 µL enzyme-substrate mixture was added to the assay plates.

[0437] The reaction was started by adding 2 µL of ATP (diluted in the assay buffer) on the assay plates. Plates were centrifuged for a few seconds at 1000 rpm and gently shaken for 2 min followed by an incubation at RT for 120 min.

[0438] The reactions were stopped, and the unconsumed ATP was depleted by adding 5 µL ADP-Glo Reagent (Promega, Cat# V912B) to the reaction. The plates were centrifuged for a few seconds at 1000 rpm and incubated at RT for 40 min (ATP depletion).

[0439] The ADP was converted to ATP and luciferase and luciferin was introduced to detect ATP by adding 10 µL Kinase Detection Reagent (Promega, Cat# V913B + V914B) to the reaction. The plates were centrifuged for a few seconds at 1000 rpm and incubated at RT for 30 min (ADP detection).

[0440] Luminescence was measured on an Envision plate reader (PerkinElmer Inc.).

2.1.1.3. Preparation of SIK3 FL Gst-TEV-HsSIK3 protein

2.1.1.3.1 Protein expression

[0441] Construct design and bacmid preparation: The coding sequence from amino acid 59 to 1321 of the human SIK3 protein (UniProt Q9Y2K2-5) was cloned into the pFastBac1 (Cat# 10360014, ThermoFisher) with N-ter (Gst) and C-ter (6His) affinity tags + N-ter (Tobacco Etch virus, TEV) and Cter (Thrombin, Thr) protease sites giving pFastBac-Gst-TEV-HsSIK3(59-1321)-Thr-6His construct. The expression cassette of the pFastBac-Gst-TEV-HsSIK3(59-1321)-Thr-6His was recombined with the parent bacmid in DH10Bac *E. coli* Competent Cells (Invitrogen) and the parent bacmid in EmbacY_DH10Bac *E. coli* Competent Cells (Geneva Biotech) to form expression bacmids.

[0442] Bacmid transfection and viral stock preparation: Sf9 insect cells (Life Technologies) were transfected either by DH10Bac or EmbacY_DH10Bac DNA using CellFectin II reagent (Life Technologies) to get P0 viral stocks. P0 viral stocks were used to infect Sf9 cells to make P1 viral stocks, and this latest was used to get P2 viral stocks.

[0443] Recombinant protein production: Sf9 insect cells were infected with P2 viral stocks and after 4 to 6 days, the cells were harvested and the pellets stored at -20 °C.

2.1.1.3.2 Protein purification

[0444] Lysis and sample clarification: The pellet cells were resuspended (5 vol/g of cell paste) on ice with Equilibration buffer (50 mM Tris pH 8, 250 mM NaCl; 2 mM DTT, 1 mM EDTA, 10% glycerol, 0.05% Brij-35) + EDTA-free Protease Inhibitor cocktail (Roche). After homogenization by sonication, Benzonase Nuclease (Merck Millipore) was added to remove DNA viscosity. The sample was clarified by ultracentrifugation and filtration on 22 µm (Polyethersulfone) low binding filters (Corning).

[0445] GST Trap affinity: The sample was applied on a GST-Trap 4B column (GE), pre-equilibrated with 50 mM Tris pH 8, 250 mM NaCl; 2 mM DTT, 1 mM EDTA, 10% glycerol, 0.05% Brij-35 with a recirculation of the sample though the column for 24 h to improve the binding efficiency. The column was washed with 10 CV of Equilibration buffer. Elution was done by steps and the POI was successfully eluted after 2 CV of 10 mM of reduced glutathione. The sample was stored at -80 °C after dialysis against (50 mM Tris pH 8, 250 mM NaCl; 1 mM DTT, 1 mM EDTA, 10% glycerol, 0.05% Brij-35), aliquoted and flash frozen in liquid nitrogen.

[0446] His Trap affinity: The sample was applied to pre-equilibrated HisTrap HP (GE). The column was washed with 10 CV of equilibration buffer and eluted with a 300 mM imidazole step over 6 CV. Elution fractions were pooled and the sample was submitted to size exclusion chromatography after centrifugation to remove potential insoluble aggregates.

[0447] Preparative Size Exclusion chromatography: The injection was done on a pre-equilibrated (50 mM Tris pH 8 + 250 mM NaCl + 1 mM DTT + 1 mM EDTA +10% glycerol + 0.05% Brij-35) Hiload 16/600 Superdex 75 preparative grade (GE). A unique peak at ~48 mL retention volume was detected. Elution fractions were pooled and the sample was aliquoted and stored -80 °C after being flash frozen in liquid nitrogen.

Table VI. Conditions for human SIK kinase ADP-Glo™ assays

Kinase, [Kinase]	Substrate, [Substrate]	ATP	Assay buffer	Incubation time
SIK1 (Carna Biosciences, Cat# 02-131), 0.25 or 0.125 ng/µL	AMARA peptide (SignalChem, Cat# A11-58), or (Eurogentec, SEQ ID 1), 45 µM	5 µM ATP (Promega, Cat# V915B)	25 mM Tris pH 7.5 0.01% Triton X-100 0.5 mM EGTA 2.5 mM DTT 5 mM $MgCl_2$	120 min
SIK2 (Invitrogen, Cat# PV4792), 0.0625 ng/µL, or (Invitrogen, Cat# PR8353A), 0.03125 ng/µL	AMARA peptide (SignalChem, Cat# A11-58), or (Eurogentec, SEQ ID 1), 45 µM	5 µM ATP (Promega, Cat# V915B)	25 mM Tris pH 7.5 0.01% Triton X-100 0.5 mM EGTA 5 mM $MgCl_2$ 2.5 mM DTT	120 min
SIK3 FL (SEQ ID 2), 0.1 or 0.05 ng/µL	AMARA peptide (SignalChem, Cat# A11-58), 45 µM	5 µM ATP (Promega, Cat# V915B)	25 mM Tris pH 7.5 0.01% Triton X-100 0.5 mM EGTA 5 mM $MgCl_2$ 2.5 mM DTT	120 min

2.1.1.4. Data analysis and Results

[0448] Raw data were generated following the read-out performed on the TopCount, plotted to generate dose response curves to calculate percentage inhibition (PIN) and average IC_{50} for each SIK homologue which are reported in the table below.

Table VII. ADP-Glo™ SIK kinase assay IC_{50} of illustrative compounds of the invention and comparative compounds.

Cpd#	SIK1 IC_{50} (nM)	SIK2 IC_{50} (nM)	SIK3 FL IC_{50} (nM)	SIK1/SIK3 IC_{50} ratio	SIK2/SIK3 IC_{50} ratio
1	806.7	102.6	0.82	984	125
2	1342.32	164.06	0.72	1864	228
3	1920.88	206.54	1.1	1746	188
4	504.66	90.37	1.36	371	66
7	2060.63	156.68	0.82	2513	191
8	2666.86	356.45	5.09	524	70
10	772.29	119.02	1.16	666	103
11	>974.99	280.33	4.19	>233	67
12	>19999	291.07	4.01	>4981	73
13	>19999	857.03	11.39	>1,756	75
14	>19999	946.24	6.02	>3321	157
15	>3999.45	173.98	1.63	2453	107
16	2485.05	1834.42	23.24	107	79
17	>3999.45	515.82	3.85	>1038	134
18	2137.96	622.3	14.84	144	42
19	162.6	23.82	0.54	301	44
20	>3999.45	2015.27	54.32	74	37
21	919.39	49.74	0.91	1015	55
22	1438.8	126.57	1.44	998	88
A	1078.35	253.79	3.62	298	70
B	410.58	98.58	0.85	483	116

2.1.1.5. Conclusions

[0449] As shown in Table VII, the illustrative compounds of the invention exhibit enhanced inhibition of SIK3, and/or improved selectivity for SIK3 over SIK1 and SIK2, relative to comparative prior art compound **Cpd A**.

2.2. Cellular assays

2.2.1. MdM assay: LPS-triggered TNFα/IL-10 (ELISA)

2.2.1.1. Overview

[0450] SIK inhibition inhibits TNFα and increases IL-10 release in LPS triggered monocyte-derived macrophages (MdM) and dendritic cells (MdDCs) (Clark et al. 2012; Sundberg et al. 2014; Ozanne et al.

2015). This assay evaluates illustrative compounds of the invention for their inhibition of LPS-induced TNFα and LPS triggered IL-10 secretion in monocyte-derived macrophages.

2.2.1.2. Protocols

[0451] PBMCs are isolated from human blood samples (buffycoats). The buffy coat is aseptically transferred into a 50 mL Falcon tube, and diluted 1/2 in PBS. Falcon tubes are filled with 20 mL Lymphoprep™, on top of which 25 mL of the buffy coat is carefully added, tubes are centrifuged for 35 min at 400 × g in temperature controlled centrifuge, without brake, at 25 °C. PBMCs are aspirated from the white interface layer between sample and Lymphoprep™. PBMCs are washed five times in PBS. Cells are resuspended in RPMI 1640 complete medium supplemented with 10% FBS, 1% P/S, and cell density is determined using a hematologic analyzer (Sysmex XS-500i).

[0452] PBMCs are centrifuged at 300 × g for 10 min and resuspended at a density of $1.0*10^7$ cells/80 µL Miltenyi buffer (PBS, pH 7.4, 1% FBS, 2 mM EDTA).

2.2.1.2.1 Positive labelling of CD14+ monocytes.

[0453] Starting from this point of the protocol all steps are performed on ice. 20 µL of CD14+ micro-beads are added per $1.0*10^7$ cells, the tube is mixed and incubated for 15 min in the fridge at 4 °C. Cell suspension volume is adjusted to total volume of 100 mL using Miltenyi buffer, mixed gently and subsequently centrifuged for 10 min at 300 × g. Supernatant is discarded and cell pellet is resuspended in 12 mL of Miltenyi buffer.

2.2.1.2.2 Magnetic cell sorting

[0454] Four LS columns are placed in the MACS Separator (magnet) from Miltenyi Biotec, and are prewet by rinsing with 3 mL of MACS buffer per column. Three mL of cell suspension is added onto the column (max $1*10^8$ of labelled cells/column), and columns are subsequently washed 3 times with 3 mL of Miltenyi buffer.

[0455] The columns are removed from the magnets, and 5 mL of Miltenyi buffer are added to the column to flush out the CD14+ fraction by pushing the plunger into the column. The flushed fractions are collected in a fresh 50 mL Falcon and volume is adjusted to 30 mL using Miltenyi buffer, cells are centrifuged for 10 min at 300 × g. The obtained cell pellet is resuspended in 10 mL RPMI w/o FBS, and cell density is determined using a hematologic analyzer (Sysmex XS-500i). 100 000 cells are seeded per well of a 96-well plate for differentiation to MdM in RPMI 1640 medium supplemented with 10% FBS, 1% P/S and 100 ng/mL rhM-CSF. On day 5 the medium is refreshed with 100 µL RPMI 1640 medium supplemented with 10% FBS, 1% P/S and 100 ng/mL rhM-CSF.

[0456] On day 10, MdMs are triggered and compound is added.

[0457] A compound dilution plate is made in 100% DMSO by 3-fold dilution of 10 mM stock solution. An intermediate dilution plate (10x final concentration) is made by diluting the compound dilution plate 50-fold in RPMI medium.

[0458] Medium is carefully removed from cell plates using multichannel pipette, and replaced by 80 µL fresh medium. 10 µL of the 10x final concentration compound is added to the cells and incubated for 1 hour

at 37 °C before addition of trigger. No trigger conditions/trigger conditions are spiked with equal final DMSO concentrations of 0.2% DMSO. 10 µL of 10 x LPS (final conc. 200 ng/mL) solution are added to all wells except for the 'no trigger wells' where 10 µL medium is added. Supernatant is collected after 2 h (IL-10 determination) and after 20 h (TNFα determination) of LPS triggering.

2.2.1.2.3 TNFα ELISA

[0459] A Lumitrac 600 Greiner 384-well plate is coated with 40 µL of capture antibody (BD Pharmingen, Cat# 551220) reaching a final concentration of 1 µg/mL in 1x PBS and stored overnight at 4 °C.

[0460] The plate is then washed once with PBST (PBS + 0.05% Tween20) and once with PBS followed by the addition of 100 µL of blocking buffer (1% Bovine Serum Albumin (BSA) - 5% Sucrose) and plates are sealed and incubated for at least 4 h at RT. After washing the plate once with PBST and once with PBS, 40 µL of standard or sample are added (TNFα standard curve is prepared using a 1/2 serial dilution starting from 16000 pg/mL; dilutions are made in dilution buffer (PBS + 1% BSA)). Plates are washed twice with PBST, and once with PBS, after which 35 µL of the detection antibody is added (final concentration 0.25 µg/mL diluted in dilution buffer) and plates are incubated for at least 2 h at RT. Plates are washed twice with PBST, and once with PBS, where after 35 µL of Strep-HRP conjugate (0.5 µg/mL final concentration diluted in dilution buffer) is added. Plates are incubated in the dark, at RT for at least 45 min but no longer than 1 hour. Plates are washed twice with PBST, and once with PBS. Thereafter, 50 µL of luminol substrate is added to each well (prepared according to manufacturer's instructions), and incubated for 5 min at RT protected from light. Chemiluminescence is measured on the Envision 2104.

2.2.1.2.1 IL-10 ELISA

[0461] An Immulon 2HB 96-well plate (Thermo Electron Co. , Cat# 3455) is coated with 40 µL of capture antibody (final concentration of 2 µg/mL diluted in Tris buffer (50 mM Tris; 150 mM NaCl; pH 9 (adjusted with HCl)) and stored overnight at 4 °C. The next day the plate is washed three times with PBST, and subsequently 200 µL blocking buffer (1% BSA + 5% sucrose in PBS-T) is added. After an incubation of 30 min at 37 °C, the plate is washed three times with PBST, and 100 µL of standard or sample are added (IL-10 standard curve samples are prepared using a 1/2 serial dilution starting from 1000 pg/mL; dilutions are made in dilution buffer: PBS + 1% BSA). After 1 hour incubation at 37 °C, plates are washed three times with PBST, after which 100 µL of the detection antibody (BD Pharmingen, Cat# 554499) is added (final concentration 0.25 µg/mL diluted in Tris buffer) and plates are incubated for at least 2 h at RT. Plates are washed three times with PBST, where after 100 µL of Strep-HRP conjugate (0,5 µg/mL final concentration diluted in dilution buffer) is added. Plates are incubated in the dark, at 37 °C for 30 min. Plates are washed three times with PBST. A substrate solution is made, for a total volume of 20 mL, 18 mL H_2O; 2 mL citrate acetate buffer; 200 µL TMB mix (tetramethil benzidine (TMB) 101 mg, DMSO 10 mL stored at 4 °C); 2.5 µL 30% H_2O_2 are mixed. 100 µL of substrate solution is added to each well and incubated until brilliant blue color develops. The reaction is stopped by adding 50 µL of 1 M H_2SO_4, after which absorbance is measured at 450 nm on the SpectraMax i3, Molecular Devices.

2.2.1.3. Data analysis and results

2.2.1.3.1 TNFα inhibition calculation

[0462] To measure the inhibition of LPS induced TNFα, percentage inhibition (PIN) values are calculated for all concentrations tested, compared to controls. Unstimulated samples (no trigger/vehicle (0.2% DMSO)) are used as negative control (100% inhibition). As a positive control (0% inhibition), the stimulated samples (trigger/vehicle)) are used.

$$PIN = \frac{(\mathrm{RLUp} - \mathrm{RLUtest\ compound})}{\mathrm{RLUp} - \mathrm{RLUn}} \times 100$$

[0463] Wherein RLU = Relative Chemiluminescent Light Units (background subtracted) and p and n subscripts refer to the average of positive and negative controls, respectively.

[0464] PIN values are plotted in concentration-response and EC_{50} values are derived using GraphPad Prism Software, applying 4-parameter nonlinear regression (sigmoidal) curve fitting. Because no clear bottom plateau is obtained, bottom of the curve is constrained to be equal to 0.

2.2.1.3.2 IL-10 induction calculation

[0465] IL-10 is induced upon SIK inhibition. To quantify these inductions fold changes (FC) compared to 'LPS only' are calculated for each concentration tested and the maximal FC is calculated (IL-10FCmax):

$$\mathrm{IL-10\ FCmax} = \frac{\max \mathrm{ABStest\ compound}}{\mathrm{ABStrigger}}$$

wherein ABS = Absorbance measured at 450 nm.

[0466] The median maximal FC for test compounds across two or more assays is reported (IL-10FCmax median).

2.2.1.3.3 Results & Outcome

[0467] The data obtained when subjecting illustrative compounds of the invention are described in the table below.

Table VIII. MdM TNFα inhibition and IL-10 induction of illustrative compounds of the invention and comparative compounds.

*	> 5000 nM	+	≤ 1.5
**	> 1000 - 5000 nM	++	> 1.5 - 4.5
***	> 100 - 1000 nM	+++	> 4.5
****	0.1 - 100 nM	NA	not measured

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
1	***	++
2	***	++

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
7	****	+
10	***	NA

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
12	NA	NA
13	*	+
14	**	+
15	NA	+
16	*	+

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
17	NA	++
21	***	++
22	***	++
A	***	++
B	***	++

2.2.2. Monocytes assay: LPS-triggered TNFα/IL-10 (ELISA)

2.2.2.1. Overview

[0468] SIK inhibition inhibits TNFα and increases IL-10 release in LPS triggered monocyte-derived macrophages (MdM) and dendritic cells (MdDCs) (Clark et al. 2012; Sundberg et al. 2014; Ozanne et al. 2015). This assay evaluates illustrative compounds of the invention for their inhibition of LPS-induced TNFα and LPS triggered IL-10 secretion in monocytes.

2.2.2.2. Protocols

[0469] PBMCs are isolated from human blood samples (buffycoats). The buffy coat is aseptically transferred into a 50 mL Falcon tube, and diluted 1/2 in PBS. Falcon tubes are filled with 20 mL Lymphoprep™, on top of which 25 mL of the buffy coat is carefully added, tubes are centrifuged for 35 min at 400 × g in temperature controlled centrifuge, without brake, at 25 °C. PBMCs are aspirated from the white interface layer between sample and Lymphoprep™. PBMCs are washed five times in PBS. Cells are resuspended in RPMI 1640 complete medium supplemented with 10% FBS, 1% P/S, and cell density is determined using a hematologic analyzer (Sysmex XS-500i).

[0470] PBMCs are centrifuged at 300 × g for 10 min and resuspended at a density of $1.0*10^7$ cells/80 µL Miltenyi buffer (PBS, pH 7.4, 1% FBS, 2 mM EDTA).

2.2.2.2.1 Positive labelling of CD14+ monocytes.

[0471] Starting from this point of the protocol all steps are performed on ice. 20 µL of CD14+ micro-beads are added per $1.0*10^7$ cells, the tube is mixed and incubated for 15 min in the fridge at 4 °C. Cell suspension volume is adjusted to total volume of 100 mL using Miltenyi buffer, mixed gently and subsequently centrifuged for 10 min at 300 × g. Supernatant is discarded and cell pellet is resuspended in 12 mL of Miltenyi buffer.

2.2.2.2.2 Magnetic cell sorting

[0472] Four LS columns are placed in the MACS Separator (magnet) from Miltenyi Biotec, and are prewet by rinsing with 3 mL of MACS buffer per column. Three mL of cell suspension is added onto the column

(max $1*10^8$ of labelled cells/column), and columns are subsequently washed 3 times with 3 mL of Miltenyi buffer.

[0473] The columns are removed from the magnets, and 5 mL of Miltenyi buffer are added to the column to flush out the CD14+ fraction by pushing the plunger into the column. The flushed fractions are collected in a fresh 50 mL Falcon and volume is adjusted to 30 mL using Miltenyi buffer, cells are centrifuged for 10 min at 300 × g. The obtained cell pellet is resuspended in 10 mL RPMI w/o FBS, and cell density is determined using a hematologic analyzer (Sysmex XS-500i). 100 000 cells are seeded in 80 µL per well of a 96-well plate in RPMI 1640 medium supplemented with 10% FBS, 1% P/S.

[0474] A compound dilution plate is made in 100% DMSO by 3-fold dilution of 10 mM stock solution. An intermediate dilution plate (10x final concentration) is made by diluting the compound dilution plate 50-fold in RPMI medium.

[0475] 10 µL of the 10x final concentration compound is added to the cells and incubated for 1 h at 37 °C before addition of trigger. No trigger conditions/trigger conditions are spiked with equal final DMSO concentrations of 0.2% DMSO. 10 µL of 10 x LPS (final conc. 200 ng/mL) solution are added to all wells except for the 'no trigger wells' where 10 µL medium is added. Supernatant is collected after 4 h of LPS triggering.

2.2.2.2.3 TNFα ELISA

[0476] A Lumitrac 600 Greiner 384-well plate is coated with 40 µL of capture antibody (BD Pharmingen, Cat# 551220) reaching a final concentration of 1 µg/mL in 1x PBS and stored overnight at 4 °C.

[0477] The plate is then washed once with PBST (PBS + 0.05% Tween20) and once with PBS followed by the addition of 100 µL of blocking buffer (1% Bovine Serum Albumin (BSA) - 5% Sucrose) and plates are sealed and incubated for at least 4 h at RT. After washing the plate once with PBST and once with PBS, 100 µL of blocking buffer (1% BSA - 5 % Sucrose) is added and plates are sealed and incubated for at least 4 h at RT. Plates are washed twice with PBST, and once with PBS, after which 35 µL of the detection antibody is added (final concentration 0.25 µg/mL diluted in dilution buffer) and plates are incubated for at least 2 h at RT. Plates are washed twice with PBST, and once with PBS, whereafter 35 µL of Strep-HRP conjugate (0.5 µg/mL final concentration diluted in dilution buffer) is added. Plates are incubated in the dark, at RT for at least 45 min but no longer than 1 h. Plates are washed twice with PBST, and once with PBS. Thereafter, 50 µL of luminol substrate is added to each well (prepared according to manufacturer's instructions), and incubated for 5 min at RT protected from light. Chemiluminescence is measured on the Envision 2104.

2.2.2.2.4 IL-10 ELISA

[0478] A Lumitrac 600 Greiner 384-well plate is coated with 40 µL of capture antibody (final concentration of 1 µg/mL in 1x PBS) and stored overnight at 4 °C. The next day the plate is washed three times with PBST (PBS + 0.05% Tween20) and once with PBS followed by the addition of 100 µL of blocking buffer (1% BSA - 5 % Sucrose) and plates are sealed and incubated for at least 4 h at RT. After washing the plate once with PBST and once with PBS, 40 µL of standard or sample are added (IL-10

standard curve is prepared using a 1/2 serial dilution starting from 2000 pg/mL; dilutions are made in dilution buffer (PBS + 1% BSA)). Plates are washed twice with PBST, and once with PBS, after which 35 µL of the detection antibody is added (final concentration 0.143 µg/mL diluted in dilution buffer) and plates are incubated for at least 2 h at RT. Plates are washed twice with PBST, and once with PBS, whereafter 35 µL of Strep-HRP conjugate (0.5 µg/mL final concentration diluted in dilution buffer) is added. Plates are incubated in the dark, at RT for at least 45 min but no longer than 1 h. Plates are washed twice with PBST, and once with PBS. Thereafter, 50 µL of luminol substrate is added to each well (prepared according to manufacturer's instructions), and incubated for 5 min at RT protected from light. Chemiluminescence is measured on the Envision 2104.

2.2.2.3. Data analysis and results

2.2.2.3.1 TNFα inhibition calculation

[0479] To measure the inhibition of LPS induced TNFα, percentage inhibition (PIN) values are calculated for all concentrations tested, compared to controls. Unstimulated samples (no trigger/vehicle (0.2% DMSO)) are used as negative control (100% inhibition). As a positive control (0% inhibition), the stimulated samples (trigger/vehicle)) are used.

$$PIN = \frac{(\mathrm{RLUp} - \mathrm{RLUtest\ compound})}{\mathrm{RLUp} - \mathrm{RLUn}} \times 100$$

[0480] Wherein RLU = Relative Chemiluminescent Light Units (background subtracted) and p and n subscripts refer to the average of positive and negative controls, respectively.

[0481] PIN values are plotted in concentration-response and EC_{50} values are derived using GraphPad Prism Software, applying 4-parameter nonlinear regression (sigmoidal) curve fitting. When no clear bottom plateau is obtained, bottom of the curve is constrained to be equal to 0.

2.2.2.3.2 IL-10 induction calculation

[0482] LPS-induced IL-10 is increased upon SIK inhibition. To quantify these increases, fold changes (FC) compared to 'LPS only' are calculated for each concentration tested and the maximal FC is calculated (IL-10 FCmax):

$$\text{IL-10 FCmax} = max\frac{(\mathrm{RLUtest\ compound})}{\mathrm{RLU\ trigger}}$$

[0483] The median maximal FC for test compounds across two or more runs is reported (IL-10FCmax median).

2.2.2.3.3 Results & Outcome

[0484] The data obtained when subjecting illustrative compounds of the invention are described in the table below.

Table IX. Monocytes TNFα inhibition and IL-10 induction of illustrative compounds of the invention and comparative compounds.

*	> 5000 nM	+	≤ 1.5
**	> 1000 - 5000 nM	++	> 1.5 - 4.5
***	> 100 - 1000 nM	+++	> 4.5
****	0.1 - 100 nM	NA	not measured

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
1	****	++
2	***	++
7	***	++
8	***	+++
10	***	++
11	***	NA
12	***	++
13	NA	++

Cpd#	TNFα EC_{50} (nM)	IL-10 FCmax median
14	NA	++
15	***	+++
16	**	++
17	**	++
21	***	++
22	***	++
A	***	+
B	****	++

Example 3. ***Pharmacokinetic, ADME and Toxicity Assays***

3.1. Human pregnane X receptor (PXR) transactivation assay

3.1.1. Overview

[0485] PXR, a member of the nuclear receptor superfamily, acts as a transcriptional regulator for the cytochrome P450 gene CYP3A4 by forming a heterodimer with the 9-cis retinoic acid receptor RXR and binding to the CYP3A4 promoter's response element. PXR transactivation assay data are therefore useful for categorizing the DDI potential of compounds with respect to CYP3A induction (Chu et al. 2009).This assay utilizes the Human PXR Reporter Assay Kit (Indigo Biosciences, Cat# IB07002) with engineered human cells constitutively expressing a functional hybrid form of human PXR wherein the N-terminal sequence encoding the PXR DNA binding domain (DBD) has been substituted with that of the yeast GAL4-DBD. Upon ligand activation, PXR binds to the GAL4 DNA binding sequence linked to a luciferase reporter gene. The change in PXR activity is quantified by luminescence detection on a plate reader.

3.1.2. Experimental Procedure

[0486] Test compounds were incubated with the Human PXR reporter cells (from the Indigo PXR kit) for 24 h. At the end of the incubation period, cell viability and transcriptional activation were measured.

[0487] The PXR reporter cells were seeded in 384 well plates and recovered at 37 °C in 5% CO_2 for 4-6 h prior to dosing. The cells were dosed with increasing concentrations of test compound (final DMSO concentration 0.4%; final test compound concentrations of 0.15, 0.48, 1.5, 4.9, 15.6, and 50 µM; two replicates per concentration), positive control or vehicle control and incubated at 37 °C for 22-24 h. Rifampicin was used as a positive control (final control compound concentrations of 0.032, 0.1, 0.32, 1.0, 3.2, and 10 µM).

[0488] At the end of the incubation period, the culture media was discarded and cell viability was assessed fluorimetrically using the Live Cell Multiplex Assay Kit (Indigo Biosciences, Cat# LCM01), and luciferase activity was measured using the luciferase detection reagent provided in the Human PXR Reporter Assay Kit.

3.1.3. Data analysis and results

[0489] Transcriptional activation was monitored by luminescence. Data were expressed as fold activation relative to the vehicle control, following normalization of luciferase activity against cell viability. The use of five or more doses of test compound and positive control allows for the derivation of EC_{50} (*i.e.*, concentration causing half the maximal effect) and E_{max} (*i.e.*, maximum fold activation vs. vehicle) values from nonlinear regression analysis of the log dose response curves. The maximum fold activation was also calculated as a percentage activation of 10 µM rifampicin (%Act) to allow for interday comparison and ranking of test compounds, using the equation below:

$$\%\text{Act} = \frac{(\text{Fold activation of test compound}) - 1}{(\text{Fold activation of rifampicin (10 µM)}) - 1} \text{ x } 100$$

The following %Act categories are generally accepted for this assay: >40% for high CYP3A4 inducing potential; between 15 and 40% for moderate potential; and <15% for low potential (Chu et al. 2009; Sinz et al. 2006).

Table X. Human pregnane X receptor activation of illustrative compounds of the invention and comparative compounds.

Cpd#	EC_{50} (µM)	Emax	%Act	Prediction of clinical induction
1	14.4	1.8	5.33	Low
	>40	1.6	12	Low
2	8.8	2.0	5.0	Low
	>40	1.2	9.0	Low
7	15.5	2.2	4.0	Low
10	23.0	1.43	5.0	Low
B	18.7	5.5	41	High

3.1.4. Conclusions

[0490] As shown in Table X, the illustrative compounds of the invention exhibit surprisingly low human pregnane X receptor transactivation potential, relative to comparative compound **Cpd B**.

Example 4. ***In vivo*** **assays**

4.1. Inflammatory bowel disease: DSS model (mice)

[0491] The mouse chronic DSS-induced inflammatory bowel disease model (IBD) is a well validated disease model for inflammatory bowel disease (Sina et al. 2009; Wirtz et al. 2007).

[0492] To induce a chronic colitis, female BALB/c mice are fed with drinking water containing 4% dextran sodium sulfate (DSS) for 4 days, followed by 3 days of regular drinking water. This cycle is repeated until sacrifice on day 12 or 18. Animals are divided into several groups:

a. intact water; vehicle alone, n=10),
b. diseased (DSS; vehicle alone, n=10),
c. sulfasalazine used as reference (DSS; 20 mg/kg/day, *p.o.*, n=10) and
d. the tested compound (DSS; e.g., 1, 3, 10, 30 mg/kg/day, *p.o.*, n=10).

[0493] Clinical parameters are measured every other day. The disease activity index (DAI) is a composite measure combining the individual scores for weight loss, stool consistency and blood presence in stools. Mice are sacrificed according to the protocol introduced by Sina *et al.* (Sina et al. 2009). At sacrifice time, the complete colon is removed and rinsed with sterile PBS. Segments of the distal colon are dissected for histological analysis, gene expression and protein level measurement.

4.2. CIA model

4.2.1. Materials

[0494] Completed Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) are purchased from Difco. Bovine collagen type II (CII), lipopolysaccharide (LPS), and Enbrel® (etanercept) are obtained from Chondrex (L'Isle d'Abeau, France); Sigma (P4252, L'Isle d'Abeau, France), Wyeth (25 mg injectable syringe, France), respectively. All other reagents used are of reagent grade and all solvents are of analytical grade.

4.2.2. Animals

[0495] DBA1/J mice (male, 7-8 weeks old) are obtained from Charles River Laboratories (Écully, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 °C, and food and water are provided *ad libitum*.

4.2.3. Collagen induced arthritis (CIA)

[0496] One day before the experiment, CII solution (2 mg/mL) is prepared with 0.05 M AcOH and stored at 4 °C. Just before the immunization, a homogenizer in a pre-cooled glass bottle in an ice water bath mixes equal volumes of adjuvant (IFA) and CII. 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

mouse 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 (I.-Ming Jou et al. 2005; Sims et al. 2004).

4.2.4. Study design

[0497] The therapeutic effects of the compounds are tested in the mouse CIA model. Mice are randomly divided into equal groups and each group contains 10 mice. All mice are immunized on day 1 and boosted on day 21. The negative control group is treated with vehicle (MC 0.5%) and the positive control group with Enbrel® (10 mg/kg, 3x week., sc). At day 32, randomization between groups is performed with respect with clinical score and animals are therapeutically treated regarding their group until day 47. Body weight and clinical score are recorded at least twice a week.

4.2.5. Clinical assessment of arthritis

[0498] Arthritis is scored according to the method of Khachigian 2006, Lin et al 2007 and Nishida et al. 2004 (Khachigian 2006; H.-S. Lin et al. 2007; Nishida, Komiyama, S.-I. Miyazawa, 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).

4.2.5.1. Change in body weight (%) after onset of arthritis

[0499] Clinically, body weight loss is associated with arthritis (J. M. Argilés & López-Soriano 1998; Rall & Roubenoff 2004; Shelton et al. 2005; 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 mouse model. The change in body weight (%) after onset of arthritis is calculated as follows:

$$\text{Mice: } \frac{\text{Body Weigh t}_{(\text{week6})} - \text{Body Weigh t}_{(\text{week5})}}{\text{Body Weigh t}_{(\text{week5})}} \times 100\%$$

4.2.5.2. Radiology

[0500] 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 (Bush et al. 2002; I.-Ming Jou et al. 2005; D. Salvemini et al. 2001; Sims et al. 2004).

4.2.5.3. Steady State PK

[0501] At day 7, 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 h. 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 ionization electrospray mode.

4.3. Murine model of psoriatic-like epidermal hyperplasia induced by topical applications of imiquimod, a TLR7/8 agonist.

4.3.1. Materials

[0502] Aldara® 5% imiquimod cream is obtained from MEDA.

[0503] Anti-mouse IL 12/IL 23 p40purified antibody (C17.8) (Cat# 16 7123 85) is obtained from eBioscience (Frankfurt, Germany).

4.3.2. Animals

[0504] Balb/cJ mice (female, 18-20 g body weight) are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 ± 2 °C, food and water are provided *ad libitum*.

4.3.3. Study design

[0505] The design of the study is adapted from Van der Fits L. et al. (van der Fits et al. 2009).

[0506] On the first day, the mice are shaved around the two ears under light anaesthesia.

[0507] 30 mg of commercially available imiquimod cream (Aldara® 5% cream) are applied on both internal and external surfaces of each ear for 4 consecutive days, corresponding to a daily dose of 1.5 mg of the active compound. Control animals received the same quantity of vaseline.

[0508] From day 1 to day 5, mice are dosed with test compound, p.o., b.i.d. in methyl cellulose 0.5%, before application of imiquimod (on day 5, the mice are dosed only once, 2 h before euthanasia).

[0509] In a positive reference group, the animals receive two intraperitoneal injections of anti mouse IL-12/IL-23 p40 antibody, 10 mg/kg, on day 1 and 3 days before day 1.

4.3.4. Assessment of disease

[0510] The thickness of both ears is measured daily with a thickness gage (Mitutoyo, Absolute Digimatic, 547 321). Body weight is assessed at initiation of the experiment and at sacrifice. At day 5, 2 h after the last dosing, the mice are sacrificed. The pinnae of the ear are cut, excluding cartilage. The pinnae are weighed and then immersed in a vial containing 1 mL of RNA*later*® solution to assess gene expression.

[0511] The results are expressed as mean ± SEM and statistical analysis is performed using one way ANOVA followed by Dunnett's post hoc test versus imiquimod vehicle group.

4.3.5. Gene expression analysis

[0512] Ears are removed from the RNA*later*® solution and put in Trizol® after disruption with 1.4 mm ceramic beads in a Precellys® device. Total RNA is then purified using NucleoSpin® RNA kit. cDNA is prepared and quantitative PCR is performed with gene-specific primers from Qiagen using SYBR Green technology in a ViiA7 real-time PCR system (Applied Biosystems). Expression levels of each gene (are calculated relative to the cyclophilin A housekeeping gene expression level. Data are expressed as mean ± SEM of the relative quantity. The statistical test used is ANOVA analysis of variance with Dunnett's post-hoc test versus imiquimod vehicle group.

4.4. Murine model of psoriatic-like epidermal hyperplasia induced by intradermal injections of IL-23

4.4.1. General overview

[0513] Overexpression of IL-23 in mice (highly expressed in psoriatic plaques, synovium and enthesis) leads to axial and peripheral enthesitis, entheseal new bone formation and hallmarks of psoriatic arthritis (Sherlock et al., 2012).

4.4.2. Materials

[0514] Mouse recombinant IL-23 (cat#1887-ML/CF), is provided by R&D Systems.

4.4.3. Animals

[0515] Balb/c mice (female, 18-20 g body weight) are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 °C, food and water are provided *ad libitum*.

4.4.4. Study design

The design of the study is adapted from Rizzo HL. *et al*. (Rizzo et al. 2011). On the first day (D1), the mice are shaved around the two ears. For 4 consecutive days (D1 to D4), the mice receive a daily intradermal dose of mouse recombinant IL-23 (1 µg/20 µL in PBS/0.1% BSA) in the right pinna ear and 20 µL of PBS/0.1% BSA in the left pinna ear under anesthesia.

[0516] From D1 to D5, mice are dosed with test-compound or with vehicle, 1 h prior IL-23 injection.

4.4.5. Assessment of disease

[0517] The thickness of the right ear is measured daily using a thickness gauge (Mitutoyo, Absolute Digimatic, Cat# 547-321). Body weight is assessed at initiation and at sacrifice. On fifth day, 2 h after the last dosing, the mice are sacrificed. The pinnae of the right ear is cut, excluding cartilage. The pinnae is placed in a vial containing 1 mL of RNA*later*® solution.

[0518] At D4, blood samples are also collected from the retro-orbital sinus for PK profiling just before dosing (T0) and 1 h, 3 h, 6 h post-dosing.

[0519] There are 10 mice per group. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus IL-23 vehicle groups.

4.4.6. Gene expression analysis

[0520] Half ears are removed from RNA*later*® solution and put in Trizol® after disruption with 1.4 mm ceramic beads in a Precellys® device. Total RNA is then purified using NucleoSpin® RNA kit. cDNA is prepared and quantitative PCR is performed with gene-specific primers from Qiagen using SYBR Green technology in a ViiA7 real-time PCR system (Applied Biosystems). Expression levels of each gene are calculated relative to the cyclophilin A housekeeping gene expression level. Data are expressed as mean ± SEM of the relative quantity. The statistical test used is ANOVA analysis of variance with Dunnett's post-hoc test versus the IL-23 vehicle group.

4.5. Murine model of systemic lupus erythematosus induced by epicutaneous applications of imiquimod

4.5.1. Materials

[0521] Aldara® 5% imiquimod cream is obtained from MEDA.

[0522] Mouse anti-double-stranded DNA antibodies ELISA kits are obtained from Alpha Diagnostic International (Cat# 5120). Mouse urinary albumin ELISA kits are obtained from Abcam (Cat# ab108792). Urine creatinine assay kits are obtained from Abnova (Cat# KA4344).

4.5.2. Animals

[0523] BALB/cJ mice (female, 18-20 g body weight) are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 ± 2 °C, food and water are provided *ad libitum*.

4.5.3. Study design

[0524] The design of the study is adapted from Yokogawa M. et al. (Yokogawa et al. 2014).

[0525] On the first day (D1), the mice are shaved around the right ears.

[0526] The mice receive an epicutaneous application of 1.25 mg of imiquimod 3 times per week on the right pinna ear for 12 consecutive weeks (D1 to D86). The control group receives the same quantity of vaseline.

[0527] From D1 to D86, mice are dosed with test compound (p.o., q.d. in methylcellulose 0.5%) or with vehicle (10 mL/kg).

4.5.4. Assessment of disease

[0528] The thickness of the ears is measured once a week with an automatic gage (Mitutoyo, Absolute Digimatic, 547-321).

[0529] Body weight is assessed at initiation and once a week until sacrifice. At necropsy, the spleen weight is also measured. The mice are sacrificed 2 h after the last dosing.

[0530] At different time points (e.g., on days D28, D56 and D84), the mice are individually placed in a metabolic cage to perform urinalysis and assess proteinuria (albumin to creatinine ratio).

[0531] Serums are collected at different time points (e.g., on D28, D56 and D86) to assess anti-double stranded-DNA IgG levels.

[0532] At D13, blood samples are also collected from the retro-orbital sinus for PK profiling just before dosing (T0) and 1 h, 3 h, and 6 h post-dosing.

[0533] There are 8-19 mice per group. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus imiquimod vehicle groups.

4.5.5. Quantification of compound levels in plasma

[0534] Plasma concentrations of each test compound are determined by an LC-MS/MS method in which the mass spectrometer is operated in positive or negative electrospray mode.

4.5.5.1. Histopathology

[0535] In each glomerulus, 4 different readouts including mesangioproliferation, endocapillary proliferation, mesangial matrix expansion and segmental sclerosis are graded on a scale of 0 to 2 and then summed. For each kidney, about 50 glomeruli are scored and then averaged giving one glomerular lesion score (Yokogawa et al. 2014). Data are expressed as mean ± SEM and statistical analysis is performed using the Kruskal-Wallis test followed by Dunn's post-hoc test versus imiquimod vehicle group.

4.5.5.2. Cellular quantifications

[0536] For each cell type, immunohistochemical analysis is performed using image analysis (CaloPix software, TRIBVN Healthcare) on the whole tissue section at a magnification of ×20. Data are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus imiquimod vehicle group.

4.5.5.3. Gene expression analysis

[0537] At sacrifice, the second part of the left kidneys is placed in tubes containing 1.4 mm ceramic beads and disrupted in 1% DTT RLT lysis buffer (Qiagen, Cat# 79216) with a Bertin Instruments Precellys® homogenizer. Total RNA is then purified with a QIAcube using an RNeasy® 96 QIAcube® HT Kit (Qiagen, Cat# 74171). cDNA is prepared and quantitative PCR performed with gene-specific primers from Qiagen using SYBR Green technology in a ViiA 7 real-time PCR system (Applied Biosystems). Expression levels of each gene of interest (GOI = CD3, CD68, CD20, OAS1, Mx1, IFIT1, CXCL11 and Usp18) are calculated relative to the cyclophilin, GAPDH and β-actin housekeeping gene expression levels.

[0538] At sacrifice, one-third of the spleen is placed into tubes containing 1.4 mm ceramic beads and disrupted in Trizol® with a Bertin Instruments Precellys® homogenizer. Total RNA is extracted using a phenol/chloroform process and then purified with a QIAcube using an RNeasy® 96 QIAcube® HT Kit (Qiagen, Cat# 74171). cDNA is prepared and quantitative PCR performed with gene-specific primers from Qiagen using SYBR Green technology in a ViiA 7 real-time PCR system (Applied Biosystems). Expression levels of each gene of interest are calculated relative to the cyclophilin, GAPDH and β-actin housekeeping gene expression levels.

4.6. Systemic lupus erythematosus model in NZB/WF1/J mice

[0539] The purpose of this study is to evaluate the activity of test compounds of the invention in the treatment of systemic lupus erythematosus (SLE). The NZB/W F1 model is the first murine model

described for lupus nephritis, which consists of the F1 hybrid between New Zealand Black and New Zealand White (NZB/W) mice. They can develop lymphadenopathy, splenomegaly, and elevated serum antinuclear autoantibodies (ANA). Particularly, they develop renal lesions that are remarkably similar to the pathology described in human lupus (Tejon et al. 2020; Zampeli et al. 2017).

4.6.1. Materials

[0540] The test compounds are stored as dry matters in the dark and formulated weekly as suspensions using magnetic stirring in the vehicle solution (aqueous methyl cellulose 5%). The resulting suspensions are kept under magnetic stirring protected from light.

[0541] Dexamethasone (DEX; VetOne, Cat# 501012) is prepared in 1% carboxymethylcellulose for PO dosing at 10 mL/kg.

4.6.2. Animals

[0542] NZBW/F1/J mice (female, 20-week old) and NZW mice (female, 8-week old) are obtained from the Jackson laboratory (USA). The mice are 28 weeks old at the time of first treatment.

4.6.3. Study design

[0543] At 27 weeks old (study day 0), the mice with developing disease are randomized by animal body weight into each group.

[0544] Treatment is initiated after randomization when the animals are 28 weeks old and continued until the animals are sacrificed at 39 weeks of age.

[0545] The animals are observed daily for significant clinical signs, morbidity and mortality.

[0546] The activity of test compounds of the invention is evaluated based on weight, proteinuria levels, tissue weights at necropsy (kidney, spleen, and lymph nodes); anti-dsDNA Ab, Igs, cytokine/chemokine and gene expression levels; and histopathology and immunohistochemistry.

[0547] The study is carried out on the following groups (15 mice/group):

Group (n=15)	**Treatment**	**Dose Level (mg/kg)**	**Dose Route**	**Regimen**	**Dose Vol (mL/kg)[3]**	**Dose Conc (mg/mL)**
1	Non-Diseased Vehicle Control	N/A	PO	BID*	5	N/A
2	Diseased Vehicle Control	N/A	PO	BID*	5	N/A
3	DEX Positive Control	1	PO	QD*	10	0.1

*BID dosing to occur at approximately 10–12 h intervals - QD dosing at approximately 24 h intervals

The test compound doses to be administered are calculated daily in mg/kg based on the latest body weight of the animal.

4.6.4. End points

[0548] Proteinuria score is recorded for all animals once a week starting on week 28 until week 39, from fresh urine samples using colorimetric Albustix® test strips (Siemens, Cat# 2872A).

[0549] The resulting score is obtained matching the color to the code scale within 1 to 2 min from sampling, giving the following endpoints:

0 = none
1 = 1 to 30 mg/dL
2 = 31 to 99 mg/dL
3 = 100 to 299 mg/dL
4 = 300 to 1999 mg/dL
5 = > 2000 mg/dL

[0550] Body weight is recorded once a week for all animals from week 28 to week 39.

[0551] Blood is collected under anesthesia on week 27, 33 and 38 for all animals for blood dsDNA Ab and Igs.

[0552] Blood is collected for PK analysis in the test compound treated animal group on week 29 at the following time points: pre-dose, and 0.25 h, 1 h, 3 h, and 6 h post dosing.

[0553] At sacrifice, spleen, kidneys and lymph nodes are weighed and kept for anti-dsDNA Ab, Igs, cytokine/chemokine and gene expression levels; and histopathology and immunohistochemistry analysis.

4.6.5. Statistical analysis

[0554] Based on individual animal raw data, the means for each group are determined and percent change from disease controls is calculated. Treatment groups are compared to disease controls using a one-way analysis of variance (1-way ANOVA) with a Dunnett's post-hoc analysis for measured (parametric) data or a Kruskal-Wallis test with a Dunn's post-hoc analysis for scored (non-parametric) data.

[0555] Data is reported as 1) all animals including those that died interim and 2) only animals that survived to study termination (surviving animals). Statistical analysis is performed using Prism 6.0d software (GraphPad).

[0556] Significance for all tests is set at $p < 0.05$, and p values are rounded to the third decimal place. Percent inhibition is calculated using the following formula:

$$percentage\ change = \frac{\text{mean [treated]} - \text{mean [disease control]}}{0 - \text{mean [disease control]}} * 100$$

4.7. Murine model of psoriatic arthritis induced by overexpression of IL-23

4.7.1. Materials

[0557] Mouse IL-23 enhanced episomal expression vector (EEV) is obtained from System Biosciences (Cat# EEV651A-1). Mouse IL-23 Quantikine ELISA Kits are obtained from R&D Systems (Cat# M2300). ProSense® 680 and OsteoSense® 750EX are obtained from PerkinElmer (Cat# NEV10003 and NEV10053EX). RNA*later*® is obtained from Ambion (Cat# AM7021). Imalgene® 1000 (Merial) and Rompun® 2% (Bayer) are obtained from Centravet (Cat# IMA004-6827812 and ROM001-6835444).

4.7.2. Animals

[0558] B10.RIII mice (male, 8-week old) are obtained from Charles River (Écully, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 ± 2 °C, food and water are provided *ad libitum*.

4.7.3. Study design

[0559] The design of the study is adapted from Sherlock JP. et al. (Sherlock et al. 2012).

[0560] On the first day (D1), the mice undergo a hydrodynamic injection of Ringer or IL-23 EEV in Ringer into the tail vein.

[0561] As of D5, twice a week, the mice are scored for clinical symptoms until the end of the experiment.

[0562] On D5, blood is collected by puncture in the submandibular vein to assess the serum IL-23 concentration.

[0563] On D9, mice from all groups receive ProSense® 680 probe (0.8 nmol/10 g, i.p.). On D10, the mice are anesthetized. Granulocyte infiltration is then measured using in vivo molecular imaging (Bruker In-Vivo Xtreme imaging system).

[0564] On D11, randomization is performed according to ProSense® 680 molecular imaging and scoring.

[0565] As of D12, mice are dosed with test compound or with vehicle.

[0566] On D19, blood is sampled at time T0, T1h, T3h and T6h after last dosing. Plasma is separated and kept at 20 °C until bioanalysis.

[0567] On D36, mice from all groups are sacrificed 2 h after last administration of compound.

[0568] Total blood is collected in a serum blood tube and mixed by gentle inversion 8-10 times. After clotting, blood samples are centrifuged 10 min at 1800 × g. After centrifugation, serum is stored at -80 °C.

4.7.4. Assessment of disease

[0569] Body weight is assessed at initiation of the study, then twice a week and at sacrifice.

[0570] Twice weekly, clinical signs of inflammation are scored: 0 for normal paw; 1 if swelling of one digit; 2 if swelling of two or more digits; 3 if swelling of the entire paw. The scores of all limbs are summed up to produce a global score.

[0571] On D32, mice from all groups receive ProSense® 680 probe (0.8 nmol/10 g, i.p.) and OsteoSense® 750EX probe (0.8 nmol/10 g, i.p.). On D33, the mice are anesthetized and granulocyte infiltration and bone remodelling are measured using in vivo molecular imaging (Bruker In-Vivo Xtreme imaging system).

[0572] There are 10 mice per group. Statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus diseased vehicle group for scoring and imaging analysis, versus sham vehicle group for body weight.

4.7.5. Results

[0573] When subjected to this protocol, **Cpd 6** dosed at 10 and 30 mg/kg b.i.d. showed a statistically significant decrease of the clinical score compared to diseased vehicle group.

4.8. Murine Collagen-antibody induced arthritis model (CAIA)

4.8.1. Materials

[0574] ArthritoMab™ antibody cocktail for inducing arthritis and lipopolysaccharide (LPS) from *Escherichia Coli* serotype O55:B5 are purchased from MD Biosciences (Oakdale, MN, USA, Cat# CIA-MAB-50); PBS 1X (GIBCO, Cat# 140190-086) is obtained from ThermoFisher Scientific, and Enbrel® (etanercept) is purchased from Chondrex (L'Isle d'Abeau, France, Cat# 3771910).

4.8.2. Animals

[0575] Five to seven week old BALBc female mice are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 °C, food and water are provided *ad libitum*.

4.8.3. Study design

[0576] The therapeutic effects of the compounds of the invention are tested in the mouse CAIA model (MD Biosciences, Inc. 2008; Nandakumar et al. 2003). At day 1 (D1), mice are randomly divided into equal groups containing 10 mice. All mice including vehicle, except the non-treated group, are immunized with ArthritoMab™ cocktail (100 mg/kg, i.v., 200 µL/mouse) and treatment with compound or vehicle starts. Bodyweight and clinical score of each mouse is assessed every day except the weekend until the end of the study. At D4, all mice, except the non-treated group, receive a challenge of LPS (2.5 mg/kg, i.p.). At D11, all mice are sacrificed and blood is sampled on serum tube. After centrifugation, serum is collected and frozen at -80 °C pending analysis (*e.g.*, cytokine levels, gene expression, compound levels). For histology readouts, right and left hind paws are individually collected in vials (25 mL minimum) filled with 4% buffered formaldehyde for a minimum of 24 h to a maximum of 4 days at RT.

4.8.4. Clinical assessment of arthritis

[0577] Arthritis is scored according to the method of Khachigian 2006; Lin et al 2007 and Nishida et al. 2004 (Khachigian 2006; H.-S. Lin et al. 2007; Nishida, Komiyama, S.-I. Miyazawa, et al. 2004). The swelling of each of the four paws is ranked with the arthritic score as follows:

Score	Definition
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

[0578] The final clinical score is the cumulative score of the four paws (maximum cumulative clinical arthritis score 16 per animal) (Nishida et al. 2004). A curve of cumulative clinical score is drawn for each group, and the area under the curve is calculated. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus vehicle groups.

4.9. Murine therapeutic model of atopic dermatitis induced by topical application of MC903

4.9.1. General overview

[0579] Topical application of the low-calcemic analog of vitamin D3 MC903 (calcipotriol) to mice induced development of a chronic eczematous dermatitis with histopathology changes resembling human atopic dermatitis (Li et al. 2006).

4.9.2. Materials

[0580] Methylcellulose 0.5% (Cat# AX021233) is obtained from VWR. MC903 (calcipotriol, Cat# 2700/50) is obtained from Tocris Bioscience (Bristol, UK). ProSense® 680 (Cat# NEV10003) is obtained from PerkinElmer (Massachusetts, USA). RNA*later*® (Cat# AM7021) is obtained from Ambion (California, USA).

4.9.3. Animals

[0581] BALB/cN mice (female, 18-20 g body weight) or CD1/Swiss mice (female, 24-26 g body weight) are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 ± 2 °C, food and water are provided *ad libitum*.

4.9.4. Study design

[0582] The design of the study is adapted from Li M. et al. (Li et al. 2006). On the first day (D1), the mice are anesthetized and shaved around the two ears. As of D1, either 20 µL EtOH or 2 nmol of MC903 (in 20 µL EtOH) are topically applied on each ear of the mice up to D9, D11 or D15 (except during the weekend).

[0583] From D5, the mice are dosed with test compound (*p.o.*, *b.i.d.* in methylcellulose 0.5%) or dexamethasone (5 mg/kg, *p.o.*, *q.d.* in methylcellulose 0.5%), or with vehicle, until D12.

4.9.5. Quantification of compound levels in plasma

[0584] Plasma concentrations of each test compound are determined by an LC-MS/MS method in which the mass spectrometer is operated in positive or negative electrospray mode.

4.9.6. Determination of pharmacokinetic parameters

[0585] Pharmacokinetic parameters are calculated using Phoenix® WinNonlin® (Pharsight®, USA).

4.9.7. Assessment of disease

[0586] The thickness of each ear is measured immediately before first application of MC903 (baseline), three times a week, and at sacrifice using a thickness gauge (Mitutoyo, Absolute Digimatic, Cat# 547-321).

[0587] Body weight is assessed at immediately before first application of EtOH (baseline), three times a week and at sacrifice.

[0588] On D10, isoflurane-anaesthetized mice from all groups receive ProSense® 680 probe (0.8 nmol/10 g, IV retroorbital sinus). On the next day (D11), the mice are anesthetized. Granulocyte infiltration is then measured using *in vivo* molecular imaging (Bruker In-Vivo Xtreme imaging system, excitation wavelength: 630 nm, emission wavelength: 700 nm, acquisition time: 5 seconds).

[0589] On D12, 2 h after the last dosing, the mice are sacrificed, total blood is collected in EDTA-coated tubes and plasma is frozen for further measurements (including circulating compound).

[0590] The pinnae of the ears are collected. One ear is cut longitudinally into 2 halves. One half is fixed in formaldehyde buffer 3.7% for histology; the other one is immersed in RNA*later*® to assess gene expression.

[0591] There are 10 mice per group. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus MC903 vehicle groups (MC903 treated mice dosed with vehicle alone) for ear thickness and/or versus EtOH vehicle group (EtOH treated mice dosed with vehicle alone) for body weight.

[0592] There are 8 mice per group. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus MC903 vehicle groups (MC903 treated mice dosed with vehicle alone) for ear thickness and weight, and/or versus EtOH vehicle group (EtOH treated mice dosed with vehicle alone) for body weight.

4.9.8. Histology

[0593] After sacrifice, half ears are collected and fixed in 3.7% formaldehyde before embedding in paraffin. 4 µm thick sections are immunostained by immunohistochemistry with anti-CD3 antibody. The immunostained cell areas from a whole section per mouse are measured by image analysis (CaloPix software, TRIBVN Healthcare, France). Data are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus MC903 vehicle group.

4.9.9. Gene expression analysis

[0594] Ears are removed from RNA*later*® solution and placed in Trizol® after disruption with 1.4 mm ceramic beads in a Bertin Instruments Precellys® homogenizer. Total RNA is then extracted using a phenol/chloroform protocol and purified with a QIAcube using a RNeasy® 96 QIAcube® HT Kit (Qiagen, Cat# 74171). cDNA is prepared and quantitative PCR performed with gene-specific primers from Qiagen using SYBR Green technology in a ViiA 7 real-time PCR system (Applied Biosystems). Expression levels of each gene of interest (GOI = IL4, IL5, IL13, TSLP, IL33, ST2, IL25, IL31, IFNγ, IL6, IL10, LCN2, S100A8, and S100A9) are calculated relative to the housekeeping gene expression levels (HPRT, GAPDH and β-actin).

[0595] All qPCR data are expressed as mean ± SEM of the normalized relative quantity (NRQ) calculated according to the following steps:

1- Calculate the geometric mean of NRQ for each group of animals

$$\mathrm{NRQ_{sample}} = \frac{2^{-\mathrm{Cq\ GOI}}}{\mathrm{Geometric\ mean}(2^{-\mathrm{Cq\ hPRT}}, 2^{-\mathrm{Cq\ GADPH}}, 2^{-\mathrm{Cq\ \beta-actin}})}$$

2- Calculate NRQ-scaled compared to the MC903 vehicle group

$$\mathrm{NRQscaled} = \frac{\mathrm{NRQ_{sample}}}{\mathrm{Geometric\ mean}\ (\mathrm{NRQ_{samples\ MC903\ vehicle\ group}})}$$

[0596] The statistical test used is ANOVA analysis of variance with Dunnett's post-hoc test versus the EtOH vehicle group and/or MC903 vehicle group.

4.10. Mouse LPS-induced endotoxic shock

4.10.1. General overview

[0597] Injection of lipopolysaccharide (LPS) induces a rapid release of soluble tumor necrosis factor (TNFα) into the periphery. This model was used to assess prospective blockers of TNFα release *in vivo*.

4.10.2. Materials

[0598] Lipopolysaccharide (LPS) from *Escherichia Coli* serotype O111:B4 was obtained from Sigma Aldrich (Cat# L2630).

4.10.3. Animals

[0599] BALB/cAnNCrl mice (female, 18-20 g body weight) were obtained from Charles River (Calco, Italy). Mice were kept on a 12 h light/dark cycle. Temperature was maintained at 22 ± 2 °C, food and water were provided *ad libitum*.

4.10.4. Study design

[0600] Mice were dosed once by oral (p.o.) or subcutaneous (sc) route with the tested compound in the appropriate vehicle.

[0601] At the Tmax of compound, 100 µg of LPS (in H_2O) was injected intraperitoneally to mice. A control group was included which comprises administering the vehicle without an LPS challenge.

[0602] Mice were sacrificed 90 min after LPS challenge and blood was collected in heparinised tubes. Plasma was obtained by centrifugation for 15 min, 2000 × g at +4 °C and frozen at -80 °C before cytokine analysis.

4.10.5. Assessment of disease

[0603] TNFα and IL-10 were quantified in plasma by AlphaLISA detection kits obtained from PerkinElmer, Cat# AL505C and AL502C, respectively.

[0604] Statistics were performed with Prism 5.03 software (GraphPad).

[0605] Active compounds were defined as showing a statistically significant decrease in TNFα with or without a statistically significant induction of IL-10, compared to the vehicle group.

4.10.6. Results

Score	Definition
+	statistically significant inhibition of TNFα
	statistically significant induction of IL-10
-	no significant effect on the level of TNFα
	no significant effect on the level of IL-10

Cpd	dose	TNFα	IL-10
6	0.1 mg/kg, *p.o.*	-	-
	0.3 mg/kg, *p.o.*	-	-
	1 mg/kg, *p.o.*	-	+
	3 mg/kg, *p.o.*	-	+
	10 mg/kg, *p.o.*	-	+
	30 mg/kg, *p.o.*	+	+
9	0.3 mg/kg, *p.o.*	-	-
	1 mg/kg, *p.o.*	-	-
	3 mg/kg, *p.o.*	-	+
	10 mg/kg, *p.o.*	-	+
	30 mg/kg, *p.o.*	+	+
	100 mg/kg, *p.o.*	+	+

4.11. MultiDrug Resistance-1a-ablated (MDRa1) model (mice)

4.11.1. General overview

[0606] Mice deficient in Abcb1a ***(MDRa1)*** develop spontaneous colitis that can be accelerated by infection with *Helicobacter bilis*. This model is used to evaluate the ability of a compound to treat or prevent colitis (Maxwell et al. 2015).

4.11.2. Materials

[0607] Sterile PBS (Gibco, Cat# 20012027) is obtained from ThermoFisher Scientific (Massachusetts, USA); Brucella agar (Cat# 211086) is obtained from Becton Dickinson (New Jersy, USA); Brucella broth base (Cat# B3051-500g) is obtained from Sigma Aldrich (Missouri, USA). Defibrinated sheep blood (Cat# SR0051) and Campygen (Cat# CN0025) are obtained from ThermoFisher Scientific (Massachusetts, USA). *H. bilis* ATCC 51360 is obtained from LGC Standards (Molsheim, France) and Combur testE (Cat# 11896857) is obtained from Roche Diagnostics (Basel, Switzerland).

4.11.3. Animals

[0608] Seven to nine week old MDR1a (FVB.129P2- Abcb1atm1Bor N7) female mice are obtained from Taconic (Rensselaer, NY, USA) and seven to nine week old FVB female mice are obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice are kept on a 12 h light/dark cycle. Temperature is maintained at 22 °C, food and water are provided *ad libitum*.

4.11.4. H. bilis inoculum preparation

[0609] Frozen vial of *H. bilis* is thawed, put in Brucella broth and incubated in Brucella agar slant containing 5% of defibrinated sheep blood under microaerophily at 37 °C for 4 to 5 days. At D1, just before administration, a part of *H. bilis* culture is diluted in PBS in order to obtain 10^7 cfu/mouse and a second part is put in fresh Brucella broth and incubated as previously for 7 days. At D8, just before administration, *H. bilis* culture is diluted in PBS in order to obtain 10^7 cfu/mouse.

4.11.5. Study design

[0610] After a 10 days acclimatization period, the disease activity index (DAI) of each MDR1a mouse is determined in order to constitute homogene groups regarding the DAI score between groups. All mice (10 mice per group), except for the sham group (n=10), are then administered by oral route with an inoculum of *H. bilis* (10^7 cfu/mouse) and treatment starts accordingly to the protocol for six weeks. Seven days after the start of treatment, a second administration of *H. bilis* is performed. During the whole treatment period, the DAI is determined twice a week. Six weeks after the start of treatment, mice are sacrificed, blood is sampled and the complete colon is collected and rinsed with sterile PBS. Collected colons are measured and weighed in order to determine colon weight/length ratio, and histological analysis, gene expression, protein level measurement and/or FACs immunophenotyping analysis are performed on the samples. The results are expressed as mean ± SEM and statistical analysis is performed using one-way ANOVA followed by Dunnett's post-hoc test versus vehicle groups.

4.11.6. Disease Activity Index (DAI) determination

[0611] The DAI score of each mouse (sum of scores for weight loss, stool consistency and rectal bleeding) is monitored during the entire treatment period and a DAI score progression curve is obtained.

DAI	Weight evolution	Stool consistency	Rectal bleeding*
0 point	No weight loss (vs D1)	Well molded stools	no erythrocyte no haemoglobin
1 point	1 to 5%	Semi-soft stools	~10 erythrocytes/µL
2 points	5 to 10%	Soft stools that do not stick to the anus	~25 erythrocytes/µL
3 points	10 to 20%	Semi-liquid stools not sticking to the anus	~50 erythrocytes/µL
4 points	> 20%	Liquid stools remaining stuck to the anus	~250 erythrocytes/µL

*A little piece of stool is deposited on a vial containing 1 mL of D-PBS and homogenized, deposited on a test strip (Combur TestE), a color appears according to the blood intensity in the stool, a score is given according to this intensity, from 0 to 4 points.

4.12. Radiation induced fibrosis mouse model

4.12.1. Study overview

[0612] Pneumonitis and lung fibrosis are the major radiation-induced complications following thoracic radiotherapy, which is one of the major treatment of lung and breast cancers, lymphomas and hematopoietic transplant conditioning. The objective of this model is to evaluate the effect of a compound of the invention in lung fibrosis induced by radiation in mice (Favaudon et al. 2014), in particular on lung functionality (flexiVent®) and fibrosis marker (Collagen I).

4.12.2. Animals

[0613] 7 weeks old (18-22 g) female C57BL/6J mice from Charles River (Écully, France), batch number S1672) are maintained on 12 h light/dark cycle at 22 °C with *ad libitum* access to tap water and food.

4.12.3. Materials

[0614] The test compounds are dissolved/suspended in appropriate vehicle prior to using and kept light-free, under agitation at RT.

[0615] An aliquot of the formulation (~200 µL) is frozen at T0 (day of preparation) and all the formulations are checked (daily) for any change in aspect.

[0616] The dose volume administered is 10 mL/kg and the volume is adapted following mean (body weight (BW) of the group as follows: 200 µL if mean BW < 22.5 g, 250 µL if mean BW ≥ 22.5 g; 300 µL if mean BW > 27.5 g.

4.12.4. In vivo experimental procedure

[0617] On day 1 of week 1, the animals are exposed at the thorax to a 17 Gray irradiation dose, under isoflurane anesthesia.

[0618] At the beginning of week 13 post radiation (D1), animals are randomized into 6 study groups (15 subjects per group): 1) sham (vehicle: methylcellulose (MC) 0.5%), 2) diseased (vehicle: methylcellulose (MC) 0.5%), 3) positive control (nintedanib dosed 60 mg/kg in 0.1% Natrosol™), and 4) 3 groups test compound (3 mg/kg, 10 mg/kg, or 30 mg/kg in Solutol / MC, 2:98 ratio), and dosed p.o. b.i.d. until D42 (week 19).

[0619] Body weight is recorded once a week,

[0620] At week 19 post radiation, Lungs are collected and fixed in 4% formaldehyde for 24 h before embedding in paraffin. 4 µm thick sections are immunostained with anti-collagen I antibody (LSBio, 2401 Fourth Avenue Suite 900, Seattle WA 98121, USA, Cat# LS-343921). The sections are deparaffinized and processed by heat antigen retrieval before incubation for 1 h with the primary antibody. The anti-collagen I antibody is detected and amplified by ImmPress kit (Vector Laboratories, 3, Accent Park, Bakewell Road, Orton Southgate, Peterborough, PE2 6XS, United Kingdom, Cat# MP-7401). The immunostained sections are then scanned (Nanozoomer, Hamamatsu) before quantification by image analysis (CaloPix software, TRIBVN Healthcare). Data are expressed as percentage collagen I area per area of lung tissue.

[0621] Values of all mice from the same group are averaged. Data are expressed as mean ± sem and are compared with a one-way ANOVA on Log-transformed data and Dunnett's *post-hoc* test. Significance levels are defined as * ($p<0.05$), ** ($p<0.01$), or *** ($p<0.001$) *versus* irradiated control group.

4.13. Therapeutic bleomycin induced pulmonary fibrosis 21-day mice model

[0622] The aim of the study is to test the efficacy of a test compound at three different doses in a 21-day model of bleomycin induced pulmonary fibrosis in mice.

4.13.1. Animals

[0623] This study is carried out on C57BL/6N male mice, supplied by Charles River (Calco, Italy), which are acclimatized for at least 5 days in an environment maintained at 22 °C, at 55% relative humidity, with 15-20 air changes per hour under light cycles of 12 h. Mice pelleted food and water are provided *ad libitum*.

[0624] At least one day prior to start of experiment, all animals are allocated randomly into groups as indicated in the table below.

Table XI. Study groups

Groups	Purpose	n	Dose	Treatment schedule Days (Frequency)	Route	Vehicle
1 PBS+Vehicle	control	15 or 6	-	D7-D21 (b.i.d.)	NA	NA
2 BLM+Vehicle	control	15	-	D7-D21 (b.i.d.)	p.o.	PEG400 or PEG200/MC 0.5% 20/80 (v/v)
3 BLM+ Nintedanib	control	15	60 mg/kg	D7-D21 (q.d. or b.i.d.)	p.o.	0.1% Natrosol™
4 BLM+ test compound	active	15	3 mg/kg	D7-D21 (b.i.d.)	p.o.	PEG400 or PEG200/MC 0.5% 20/80 (v/v)
5 BLM+ test compound	active	15	10 mg/kg	D7-D21 (q.d.)	p.o.	Solutol / MC 0.5% (2/98, v/v)
6 BLM+ test compound	active	15	3 mg/kg	D7-D21 (b.i.d.)	p.o.	Solutol / MC 0.5% (2/98, v/v)
7 BLM+ test compound	active	15	10 mg/kg	D7-D21 (b.i.d.)	p.o.	Solutol / MC 0.5% (2/98, v/v)
8 BLM+ test compound satellite for PK	active	10	10 mg/kg	D7-D14 (b.i.d.)	p.o.	Solutol / MC 0.5% (2/98, v/v)

4.13.2. Materials

[0625] The solvent for the test solutions is prepared by adding 0.5 g of hydroxyethyl cellulose (Natrosol™) into 500 mL aqua distillate (0.1%) under continuous stirring without heating for 5 h on a magnetic stirrer.

[0626] To prepare a solution for intranasal (i.n.) challenge, 0.8 mg/mL stock solutions of bleomycin (Cat# BML-AP302-0010, Enzo Life Sciences, Inc., USA) are thawed and diluted in 330 µL of saline. Prior to i.n. administration, mice are anesthetized i.p.

[0627] Fresh nintedanib formulation is prepared daily in 0.1% Natrosol™ formulations to a final concentration of 5 mg/mL. Before dosing, animals are weighed and the nintedanib amount administered is adjusted accordingly to individual weights corresponding to 10 mL/kg body weight, once daily p.o..

[0628] Finally, test compound solutions are prepared by dissolving the suitable amount of said test compound in PEG 400 or PEG 200 (20% of the final volume) then MC 0.5% (80% of the final volume) to reach final concentrations of 1 mg/mL, 0.3 mg/mL and 0.1 mg/mL, thus yielding compound for a doses of 10 mg/kg, 3 mg/kg and 1 mg/kg. Prior to dosing, animals are weighed and the amount administered adjusted accordingly to individual weights.

[0629] The application volume of the test doses corresponds to 10 mL/kg body weight, and is the test compounds are administered p.o. twice daily, with 7.5 h interval between two administrations.

4.13.3. Study

[0630] Animals are examined clinically twice daily. List of clinical signs and parameters are indicated in human endpoints table. Animals are weighed daily starting from D0.

[0631] On day 21, 2 h post dosing with vehicle, nintedanib or test compound, mice are sacrificed.

[0632] The lungs are excised and weighed individually. For all groups: the whole superior right lung lobe is placed into a Precellys® tube containing silica beads and immediately snap frozen in liquid nitrogen and subjected to gene expression analysis.

[0633] All remaining lungs are placed into marked bottles containing 10% buffered formalin for further histopathological evaluation.

4.13.4. Sample analysis, data processing and statistical evaluation

[0634] Body weight data and lung weight data are processed using MS Excel. Statistical analysis and graphical presentation are performed using GraphPad Prism software. One-way ANOVA or Mann-Whitney test are employed for lung weights. Two-way ANOVA are employed for body weight changes.

[0635] Differences between groups are considered statistically significant when $p<0.05$.

[0636] For histopathological evaluation, whole lungs (except sampled superior right lung) are embedded in paraffin and stained with Mallory's trichrome.

[0637] Pulmonary histological changes are assessed using Matsuse modification of Ashcroft score (Ashcroft et al. 1988; Matsuse et al. 1999). Statistical analysis and graphical presentation is performed using GraphPad Prism software. A Mann-Whitney test is employed.

[0638] Differences between groups are considered statistically significant when $p<0.05$.

Ashcroft Score	**Definition**
1	Normal lungs (no fibrosis)
2	Minimal fibrotic thickening of alveolar or bronchial walls (network of fine collagen fibrils)
3	Moderate fibrotic thickening of walls without obvious damage to lung architecture
4	Fibrosis with damage of pulmonary structure (coarse fibrous bands or small fibrous masses, intra-alveolar collagen fibrils)
5	Large fibrous area with severe distortion of lung structure

4.13.5. PK analysis – Group 7

4.13.5.1. Protocol

[0639] Animals in group 7 (n=10) are included for PK study only and are not subjected to clinical sign scoring.

[0640] These animals are induced with the disease at the start of treatment at day 0 (D0) and are sequentially sacrificed on D7 at 1 h, 3 h, 6 h, 8 h, 24 h after the first administration of test compound.

[0641] A blood sample (50 µL) is collected from the tail vein into Li-heparin anticoagulant tubes for each time point and kept on ice until separation. Within maximum 30 min after collection, blood samples are centrifuged at 2000 × g for 10 min at 4 °C and the resulting plasma samples are aliquoted into polypropylene tubes (1 × 25 µL). The samples are stored frozen at –20 °C until analysis.

[0642] The lung tissue is collected at sacrifice after blood sampling for each animal, then weighed and placed into polypropylene tubes prior to freezing. The samples are stored frozen at –80 °C until analysis.

4.13.5.2. Plasma concentration and pharmacokinetic analysis

[0643] Plasma and lung concentrations are measured via LC-MS/MS. Samples are prepared for LC-MS/MS analysis via protein precipitation. The plasma concentrations measured below the lower limit of quantification (LLOQ) are reported as below the limit of quantification (BLQ). The test compound concentrations in plasma are expressed in ng/mL. Mean plasma concentrations are calculated. For mean calculation, the concentrations below the LLOQ are set to zero. Therefore, mean values may be BLQ. Standard deviation (SD), standard error of the mean (SE) and coefficient of variation (CV, %) are tabulated when at least three plasma concentration values are above the LLOQ.

[0644] Non-compartmental analysis on individual plasma concentrations is performed using Phoenix™ WinNonlin® 6.3 (Pharsight Corporation) to determine at least, the following pharmacokinetic parameters:

- Maximum plasma concentration, Cmax (µg/mL) with the corresponding time, tmax (h),
- Area under the plasma concentration versus time curve up to the last quantifiable concentration AUC_{0-t} or up to 24 h AUC_{0-24h} (µg.h/mL) (if compound is quantifiable up to 24 h postdose), and/or up to infinity $AUC_{0-\infty}$, (µg.h/mL) is calculated according to the linear up/log down trapezoidal rule. Partial AUC may be calculated if deemed necessary. Concentrations below the limit of quantification (BLQ) are set to zero. No AUC is calculated if there are less than three quantifiable time points. AUC0-∞ is considered if %AUCextra < 20%,
- Apparent terminal elimination half-life, t1/2 (h) is only reported if three or more time points, excluding tmax is used for linear regression, and if the adjusted $R^2 > 0.80$.
- Normalized AUC and Cmax dose.
- Mean pharmacokinetic parameters are calculated. Standard deviation (SD) and coefficient of variation (CV, %) are tabulated if at least three values are available.

4.14. T cell transfer model (mice)

4.14.1. General overview

[0645] T cell transfer model is a model using immunocompromised mice (SCID) which are injected with CD4+CD25- cells, T cell which are depressed of regulatory cell (Treg). Mice are then developing colitis in response to this injection. Compound efficacy is then evaluated by measuring activity on decreasing colitis features.

4.14.2. Materials

[0646] DynaMag (Cat# 12321D and 123203D) was obtained from Life Technologies Invitrogen (California, USA); DynabeadsFlowComp Mouse CD4+CD25-treg cells (Cat# 11463D) were obtained from Life Technologies Invitrogen (California, USA), or EasySep Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II (cat# 18783A) was obtained from StemCell Technologies (7100 Cambridge Research Park Beach Drive, Cambridge CB25 9TL, UK), Fetal Bovine Serum (GIBCO), Cat# 10270-106; RPMI (Gibco) Cat# 32404-014 and D-PBS 1X without $CaCl_2$ without $MgCl_2$ (GIBCO), Cat# 14190-086 were obtained from ThermoFisher Scientific (Massachusetts, USA). Red Blood Cell (RBC) lysis buffer 10 X, Cat# BLE420301 obtained from Ozyme (Montigny-le-Bretonneux, France). Cell strainer (70 µm grid), Cat# 352350, obtained from Corning (New York, USA). Bovine Serum Albumin (BSA), Cat# A9647-50g and EDTA, Cat# EDS-100g obtained from Sigma Aldrich (Missouri, USA) and Combur^3 Test E, Cat# 11896857, obtained from Roche Diagnostics (Basel, Switzerland).

4.14.3. Animals

[0647] Five to seven week old BALBc/N female mice and five to seven week old SCID female mice were obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice were kept on a 12 h light/dark cycle. Temperature was maintained at 22 °C, food and water were provided *ad libitum*.

4.14.4. Study design

[0648] The therapeutic effects of the compounds were tested in the mouse T cell transfer model model (Lindebo Holm et al. 2012). After a 5 to 7-day acclimatization period, BALBc/N mice were sacrificed, spleens were removed, homogenized, rinsed with D-PBS and centrifuged. Cell pellets were resuspended in RBC lysis isolation buffer (D-PBS, EDTA, BSA, 1/1/1) and centrifuged, then resuspended in isolation buffer and processed following DynabeadsFlowComp Mouse CD4+CD25-Treg cells Dynabeads kit protocol or following EasySep Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II protocol. The obtained cells were resuspended in RPMI and 0.2 mL were injected to SCID mice by intra-peritoneal injection after checking the purity of T cells (Tcell/Treg > 95%). Sham group mice received RPMI alone.

[0649] Treatment started on day 15 or day 16, with groups homogenized by level of disease activity index (DAI). Disease activity index was determined twice a week. Three to six weeks after the start of treatment, mice were sacrificed, blood was sampled and the complete colon was removed and rinsed with sterile PBS, it was measured and weighed in order to determine colon weight/length ratio. Segments of colon were dissected for histological analysis, gene expression, protein level measurement and/or totally sampled for immunophenotyping by FACs.

[0650] There were 12 mice per group. The results were expressed as mean ± SEM.

4.14.5. Disease Activity Index (DAI) determination

[0651] The DAI score of each mouse (sum of scores for weight loss, stool consistency and rectal bleeding) was monitored during the entire treatment period and a DAI score progression curve was obtained.

DAI	Weight evolution	Stool consistency	Rectal bleeding*
0 Point	No weight loss vs D1	Well molded stools	no erythrocyte no haemoglobin
1 point	1 to 5%	Semi-soft stools	~10 erythrocytes/µL
2 points	5 to 10%	Soft stools that do not stick to the anus	~25 erythrocytes/µL
3 points	10 to 20%	Semi-liquid stools not sticking to the anus	~50 erythrocytes/µL
4 Points	> 20%	Liquid stools remaining stuck to the anus	~250 erythrocytes/µL

*A little piece of stool was deposited in a vial containing 1 mL of D-PBS and homogeneized, deposited on a test strip (Combur TestE), a color appears according to the blood intensity in the stool, a score is given according to this intensity, from 0 to 4 points.

4.14.6. Statistical analysis

[0652] Statistical analysis was performed using a one-way ANOVA with Dunnett's post-hoc test versus vehicle group.

4.14.7. Results

[0653] When subjected to this protocol, using DynabeadsFlowComp Mouse CD4+CD25-treg cells Dynabeads kit, **Cpd 6** dosed at 10 or 30 mg/kg p.o. b.i.d. over 39 days, or dosed at 15 or 30 mg/kg p.o. b.i.d. or 30 or 60 mg/kg p.o. q.d. over 34 days showed a statistically significant decrease of the AUC of the DAI score compared to the vehicle group.

[0654] When subjected to this protocol, using EasySep Mouse CD4+CD25+ Regulatory T Cell Isolation Kit II, **Cpd 6** dosed at 10 or 15 mg/kg p.o. b.i.d. or 20 or 30 mg/kg q.d. over 41 days showed a statistically significant decrease of the AUC of the DAI score compared to the vehicle group.

4.15. Surgical destabilization of the medial meniscus (DMM) mouse model of osteoarthritis

4.15.1. General overview

[0655] The experiment assesse disease-modifying osteoarthritis drug (DMOAD) effect by prophylactic treatment of compounds that inhibits the structural disease progression of OA and ideally also improves symptoms and/or function.

4.15.2. Animals

[0656] DMM surgery is performed in the right knees of 10-week old male C57BL/6 mice. For the prophylactic study, systemic (p.o.) treatment starts at the time of surgery. Mice are sacrificed 8 weeks after surgery, and another group are sacrificed 12 weeks after surgery.

4.15.3. Study design

[0657] Knees are harvested for detailed histopathological assessment(Glasson et al. 2007). Thus, the DMM model uniquely captures the chronic progressive nature of OA and associated sensitization and pain-related behaviors. Knees are collected for histology, following standard methods (Miller et al. 2016).

4.16. Ovariectomized (OVX) mouse model

4.16.1. General overview

[0658] The OVX model is used widely for investigating problems related to postmenopausal bone loss, a primary risk factor for osteoporosis

4.16.2. Animals

[0659] A cohort of C57Bl6 female mice of 12 weeks of age are subjected to sham surgery, or to OVX. Animals are kept for a period of 8 weeks, during which time hypogonadal bone loss is established. Then, at 20 weeks of age (8 weeks after sham or OVX surgery), the OVX mice are treated once daily over the course of 4 weeks (Dempster et al. 2013).

4.16.3. Study design

[0660] The following skeletal-directed endpoints are used at the completion of the 4 weeks treatment period: µ-CT of the femur and L5 vertebrae to assess bone mass and micro-architecture.

4.17. Murine sclerodermatous chronic graft-versus host disease (cGvHD)

4.17.1. General overview

[0661] In this cGvHD model, fibrosis is induced in BALB/c (H_2^d) mice by allogeneic transplantation of bone marrow cells and splenocytes from B10.D2 (H_2^d) donor mice (minor HLA mismatch). The recipient mice develop inflammation-driven dermal and pulmonary fibrosis resembling patients with rapidly progressive diffuse cutaneous systemic sclerosis (Zerr et al. 2012).

[0662] The treatment is provided only after the onset of first clinical symptoms of sclerodermatous cGvHD.

4.17.2. Study groups

[0663] The following groups with each eight mice are used in this study

- *Syngeneically transplanted, placebo-treated control group:*
  Syngeneic bone marrow and splenocyte transplantation (BALB/c (H_2^d) → BALB/c (H_2^d)). Application of methyl cellulose 0.5% from day 21 to day 56 post transplantation.
- *Vehicle-treated fibrosis group:*
  Allogeneic bone marrow and splenocyte transplantation (B10.D2 (H_2^d) → BALB/c (H_2^d)). Application of methyl cellulose 0.5% from day 21 to day 56 post transplantation
- *Control group to assess pretreatment levels of fibrosis induced by allogeneic transplantation:*
  Allogeneic bone marrow and splenocyte transplantation (B10.D2 (H_2^d) → BALB/c (H_2^d)). Sacrifice at day 21, before treatment is initiated in the other groups.

- *Treatment group:*

  Allogeneic bone marrow and splenocyte transplantation (B10.D2 (H_2^d) → BALB/c (H_2^d)). Application of a test compound of the invention at 10 mg/kg po b.i.d. in 0.5% methyl cellulose from day 21 to day 56 post transplantation.

- *Positive control group:*

  Allogeneic bone marrow and splenocyte transplantation (B10.D2 (H_2^d) → BALB/c (H_2^d)). Application of 50 mg/kg qd nintedanib from day 21 to day 56 post transplantation.

4.17.3. Steady state PK

[0664] On D20, for the groups receiving test compounds, blood is collected from the tail vein from 2 animals per timepoint, at the following timepoints: pre-dose, 1, 3 and 6 h with anticoagulant Li-heparin.

[0665] The blood samples are kept on ice and centrifuged at approx. 3500 × g, for 10 min at +4 °C, within 1 h after blood sampling; plasma is transferred in labelled polypropylene tubes and stored at -20 °C.

4.17.4. Sampling and analysis

[0666] Animals are sacrificed 2 h post last dose, and samples of skin (3 mm punch biopsies), lung, spleen and blood are collected for histology and gene expression analysis.

4.17.5. Main readouts

[0667] The anti-fibrotic effects on skin are analyzed by determination of dermal thickness, quantification of lesional collagen and staining for myofibroblasts.

[0668] In case of positive effects on skin fibrosis, effects on pulmonary fibrosis are analyzed by Ashcroft scoring, hydroxyproline content, and quantification of the collagen covered area using SirCol staining.

4.17.6. Analysis

[0669] Based on individual animal raw data, the means for each group are determined and percent change from disease controls is calculated. Treatment groups are compared to disease controls using a one-way analysis of variance (1-way ANOVA) with a Dunnett's post-hoc analysis for measured (parametric) data or a Kruskal-Wallis test with a Dunn's post-hoc analysis for scored (non-parametric) data.

4.18. Chronic Kidney Disease (CKD) rat model

4.18.1. General overview

[0670] The CKD model evaluates the therapeutic potential of the compounds of the invention on adenine-induced kidney injury and fibrosis in Sprague-Dawley Rats.

4.18.2. Animals

[0671] This study is carried out on Sprague-Dawley rats (male) weighing approximately 276-300g (Plato BioPharma, Denver, Colorado, USA). The animals are allowed *ad libitum* access to food (Teklad 8940 rodent chow) and water. Prior to starting the study, rats are placed into weight-matched study enrollment sets over four consecutive enrollment days with the heaviest animals enrolling first.

Table XII. Study groups

Group	Endpoint Group	# animals	Dose	Route & Administration	Diet	Challenge Route & Timing	Duration
A	1	n = 10	None	None	Control Diet	*Ad lib* in chow d0 – d15	15 Days
	2	n = 10	Vehicle	p.o. QD d 15 – d 56	Control Diet	*Ad lib* in chow d0 – d56	56 Days
B	3	n = 10	None	None	0.25% Adenine Diet	*Ad lib* in chow d0 – d15	15 Days
	4	n = 15	Vehicle	p.o. QD d 15 – d 56	0.25% Adenine Diet	*Ad lib* in chow d0 – d56	56 Days
	5	n = 15	test compound	p.o. QD d 15 – d 56	0.25% Adenine Diet	*Ad lib* in chow d0 – d56	56 Days
	6	n = 15	SB-525334, CAS# 356559-20-1), 3 mg/kg	p.o. QD d 15 – d 56	0.25% Adenine Diet	*Ad lib* in chow d0 – d56	56 Days

4.18.3. Study

[0672] On day 0, rats have blood collected for evaluation of plasma renal injury biomarkers (blood urea nitrogen (BUN), creatinine). Immediately following the day 0 blood collection, rats incept challenge administration. Animals in Group A (endpoint groups 1 and 2) receive *ad libitum* access to control chow on day 0 through endpoint. Animals in Group B (endpoint groups 3 – 5) receive *ad libitum* access to 0.25% adenine ad-mix chow on day 0 through endpoint.

[0673] On day 7, rats have blood collected for evaluation of plasma renal injury biomarkers (BUN, creatinine).

[0674] On day 12, all rats are placed into metabolic cage housing for a 24-hour collection period. Upon exiting from metabolic cages on day 13, rats have blood collected for evaluation of BUN and creatinine.

[0675] On day 14, all animals have renal function assessed by evaluating Fluorescein-Isothiocyanate-Sinistrin (FITC-Sinistrin) clearance and half-life using a MediBeacon device (MB1279mini, MediBeacon, Mannheim, Germany).

[0676] Within each enrollment set, the following data from Group A and B rats are randomized for homogeneization of average values on the following quantified baseline readouts: a) Day 12 body weight, b) Change in body weight from Day 0 to Day 12, c) Day 13 plasma BUN, d) Day 13 plasma creatinine, e) Day 13 creatinine clearance, f) Day 13 urinary albumin:creatinine and g) Day 14 FITC-Sinistrin half-life. The randomization is used to place rats from Groups A and B to Endpoint Groups 1 or 2 and to Endpoint Groups 3 – 6, respectively, ensuring there are no statistical differences across the seven parameters listed above. Rats are enrolled to groups in a balanced design such that an approximately equal number of animals are enrolled per group per set.

[0677] On day 15, rats placed to Groups 1 and 3 are sacrificed and tissue specimens are collected. All remaining rats receive vehicle/compound administration. Briefly, animals in Groups 2 and 4 receive oral administration (5 mL/kg) of vehicle once daily from day 15 to day 56. Rats in Group 5 receive the test compound (p.o.) once daily from day 15 to day 56. And rats in Group 6 receive the reference compound SB-525334 (CAS# 356559-20-1) (3 mg/kg, p.o.) once daily from day 15 to day 56.

[0678] On day 21 (7 days of dosing), three rats of Endpoint Group 5 have blood collected via conscious tail venous puncture prior to dosing (trough PK; BUN, creatinine). All remaining rats in Endpoint Group 5 are timed dosed and subsequently four rats per group per timepoint have blood collected at 0.25 h, 1.5 h, or 6 h post-dosing (PK; BUN, creatinine). Rats in Groups 2, 4 and 6 have blood collected for evaluation of plasma renal injury biomarkers (BUN, creatinine).

[0679] On days 28, 35, and 42, rats have blood collected for evaluation of plasma renal injury biomarkers (BUN, creatinine).

[0680] On day 49 (35 days of dosing), three rats per each of Group 5 have blood collected prior to dosing (trough PK; BUN, creatinine). All remaining rats in Group 5 are timed dosed and subsequently four rats per group per timepoint have blood collected at 0.25 h, 1.5 h, or 6 h post-dosing (PK; BUN, creatinine). Rats in Groups 2, 4 and 6 have blood collected for evaluation of plasma renal injury biomarkers (BUN, creatinine).

[0681] On day 53, all remaining rats are placed into metabolic cage housing for a 24-hour collection period. Upon exiting from metabolic cage on day 54, animals have blood collected for evaluation of plasma renal injury biomarkers (BUN, creatinine).

[0682] On day 55, all remaining rats have renal function assessed by evaluating FITC-Sinistrin clearance using the MediBeacon device.

[0683] On the day of sacrifice (day 15 or day 56, as appropriate), rats are sacrificed, blood and tissues are collected. For animals in Groups 2 and 4 – 6, blood collection is timed so as to occur 5 h post final dose. Both left and right kidneys are immediately placed in ice-cold 0.9% NaCl. The left and right kidneys are de-encapsulated and weighed.

[0684] Urine samples are immediately flash-frozen in liquid N_2 and stored at -80 °C until analyzed.

4.18.4. Sampling and analysis

4.18.4.1. Urine Specimens:

[0685] On days 13 and 54, 24-hour urine volumes are determined and five 500 µL urine samples are collected, aliquoted, immediately flash-frozen in liquid N_2 and stored at -80 °C until assayed.

[0686] Urine is aliquoted as follows:

- Clinical Chemistry: 500 µL subjected to clinical chemistry analysis (creatinine, protein) and to rat-specific albumin ELISA
- Kidney Injury Molecule-1 (KIM-1): 500 µL subjected to rat specific KIM-1 ELISA
- Neutrophil gelatinase-associated lipocalin (NGAL): 500 µL subjected to rat specific NGAL ELISA

4.18.4.2. Plasma Samples for PK

[0687] For Groups 5 and 6 only, on day 21 (7 days of dosing) and day 49 (35 days of dosing), whole blood (400 µL) is collected on Li-Heparin. Each rat will have blood collected at one of the following timepoints per study day: pre-dosing (n = 3 rats per group), 0.25 h post-dosing (n = 4 rats per group), 1.5 h post-dosing (n = 4 rats per group), or 6 h post-dosing (n = 4 rats per group).

[0688] Samples are processed appropriately for plasma. Plasma samples (2 x 100 µL) are transferred to 96-well plates (Micronic Cat # MP52540, 1.1mL) on dry ice. All samples are stored at -80 °C until further needed.

4.18.4.3. Kidney samples:

[0689] Immediately following the endpoint blood collection (5 h post-final dose), left and right kidneys are collected. Histological analysis is performed for

a. tissue hydroxy proline (OH-P) content in each left kidney
b. tissue TGF-β content in each left kidney
c. histological analysis of renal cortical fibrosis in each left kidney by Picrosirius Red quantitation

4.18.4.3.1 Endpoint Histological Specimens

[0690] Two 3-4 mm mid-transverse sections of each left kidney and one 3-4 mm mid-transverse section of each right kidney from all animals will be collected.

[0691] The sections are immersion-fixed in 10% neutral buffered formalin for 48 h and then transferred to 70% EtOH for processing. One section of each left kidney section is paraffin-embedded and one serial section is obtained at each of three anatomically-distinct (separated by ≥300µm) depths. Sections are mounted on slides in consistent orientation of depths 1 – 3. Sections are 5 µm thick unless otherwise specified. Sections from each depth are stained with Picrosirius Red and subjected to quantitative histological analyses or renal collagen volume fraction using standard color spectral segmentation analyses. The other section of each left kidney and the section collected from the right kidney section are paraffin-embedded and kept.

4.18.4.3.2 Endpoint Kidney Tissue Specimens:

[0692] All remaining renal cortical tissue (after mid-transverse sections for histology) from each left and right kidney are collected and aliquoted as described below.

4.18.4.3.2.1. Left Kidney

[0693] 50-75 mg left kidney biopsy for analysis of mRNA expression is taken. Specimen weight is recorded and placed in chilled RNAlater for 24 h at 4 °C, excess RNAlater is then decanted, and specimens are stored at -80 °C until needed for analysis .

[0694] All remaining left renal cortical tissue (1 tube/kidney/rat) are snap frozen in liquid N_2 and stored at -80 °C. Tissue is cryopowdered and mixed thoroughly. Sufficient tissue powder for subsequent analysis of tissue hydroxy proline (OH-P) and TGF-β content analyses is taken and all remaining tissue powder is stored at -80 °C until needed for analysis .

4.18.4.3.2.2. Right Kidney

[0695] 50-75 mg right kidney biopsy for analysis of mRNA expression is taken. Specimen weight is recorded and in chilled RNAlater for 24 h at 4 °C, excess RNAlater then decanted, and specimens stored at -80 °C until further needed.

[0696] All remaining left renal cortical tissue (1 tube/kidney/rat) is snap frozen in liquid N_2 and stored at -80 °C. Tissue is then cryopowdered and mixed thoroughly. All tissue powder is stored at -80 °C until needed for analysis.

FINAL REMARKS

[0697] 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. Through routine experimentation, an artisan will recognize apparent modifications and variations that 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. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

[0698] 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 are specifically and individually indicated to be incorporated by reference herein as though fully set forth.

[0699] 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.

[0700] 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 OpenEye Scientific 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.

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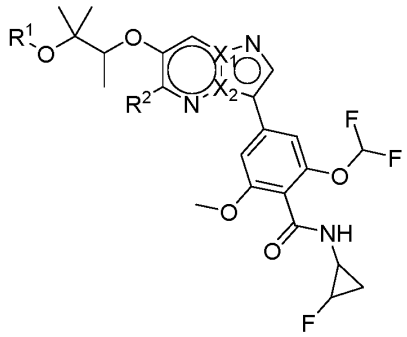
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CLAIMS

1. A compound according to Formula I:



I

wherein

one of X_1 and X_2 is N and the other is C;

R^1 is H or -P(=O)$(OH)_2$; and

R^2 is

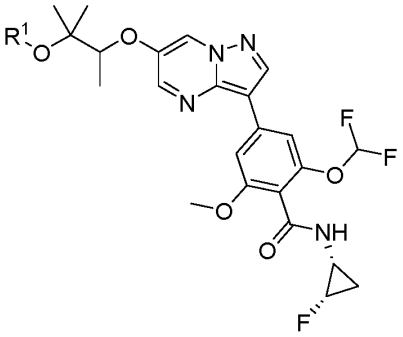
- H,
- C_{1-4} alkyl optionally substituted with one or more independently selected halo,
- -$NR^{3a}R^{3b}$; and

Each R^{3a} and R^{3b} is indenpently selected from H, C_{1-4} alkyl optionally substituted with one or more independently selected halo, or phenyl;

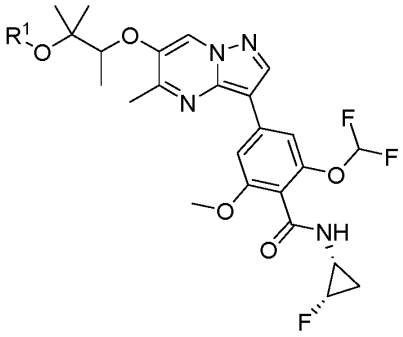
or a pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof.

2. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to claim 1, wherein R^2 is C_{1-4} alkyl.
3. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-2, wherein R^2 is -CH_3 or -CH_2CH_3.
4. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to claim 1, wherein R^2 is H.
5. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-5, wherein X_1 is N and X_2 is C.
6. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-5, wherein X_1 is C and X_2 is N.

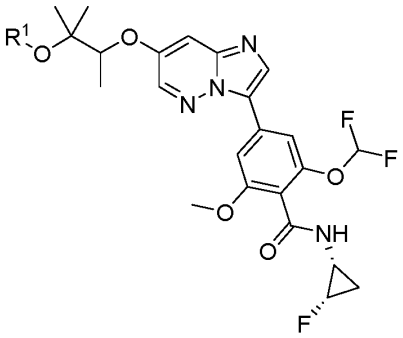
7. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is according to Formula IVa, IVb, IVc, or IVd:



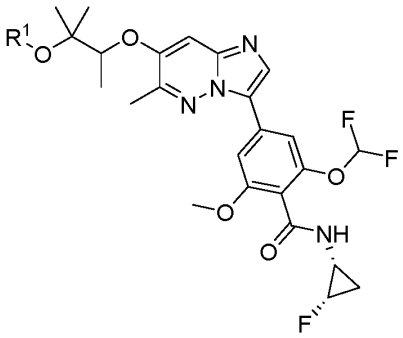
IVa



IVb

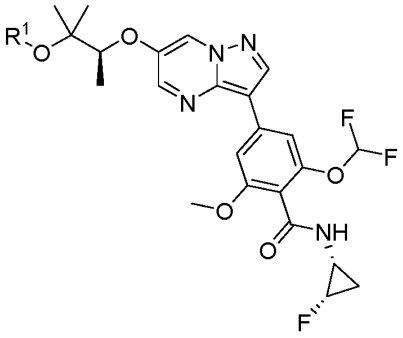


IVc

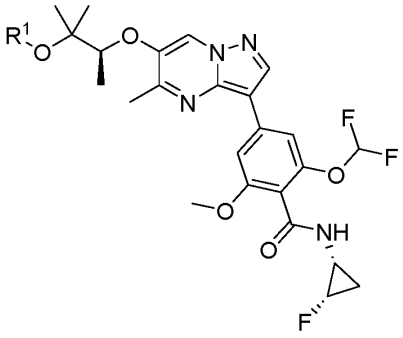


IVd.

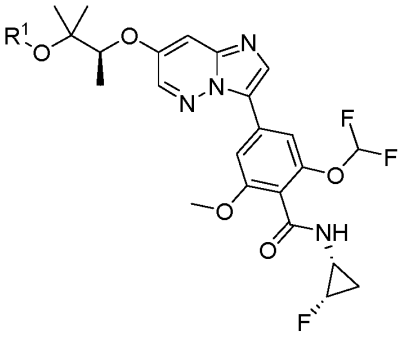
8. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is according to Formula Va, Vb, Vc, or Vd:



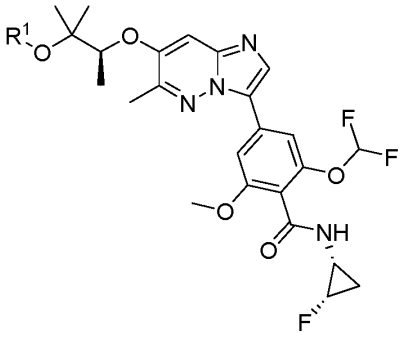
Va



Vb



Vc



Vd.

9. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-8, wherein R^1 is H.
10. The compound, pharmaceutically acceptable salt, solvate, or solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-8, wherein R^1 is $-P(=O)(OH)_2$.
11. The compound according to Formula Va of claim 8, wherein R^1 is $-P(=O)(OH)_2$, wherein the compound is a monohydrate in a crystalline form characterized by an X-ray powder diffraction pattern comprising peaks at 9.1, 10.0, 13.2, 13.6, and 14.9 ± 0.2° 2θ using Cu Kα radiation.
12. The compound according to Formula Va of claim 8, wherein R^1 is $-P(=O)(OH)_2$, wherein the compound is a hemihydrate in a crystalline form characterized by an X-ray powder diffraction pattern comprising peaks at 9.1, 9.6, 15.1 15.4, and 19.7 ± 0.2° 2θ using Cu Kα radiation.
13. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a pharmaceutically effective amount of a compound, a pharmaceutically acceptable salt, a solvate, or a solvate of a pharmaceutically acceptable salt thereof according to any one of claims 1-12.
14. A compound, a pharmaceutically acceptable salt, a solvate, or a solvate of a pharmaceutically acceptable salt thereof, according to any one of claims 1-12, or a pharmaceutical composition according to claim 13 for use in medicine.
15. A compound, a pharmaceutically acceptable salt, a solvate, or a solvate of a pharmaceutically acceptable salt thereof, according to any one of claims 1-12, or a pharmaceutical composition according to claim 13 for use in the prophylaxis and/or treatment of inflammatory diseases, autoinflammatory diseases, autoimmune diseases, proliferative diseases, fibrotic diseases, transplant rejection, diseases involving impairment of cartilage turnover, congenital cartilage malformation, diseases involving impairment of bone turnover, diseases associated with hypersecretion of IL-6, diseases associated with hypersecretion of TNFα, interferons, IL-12, IL-17 and/or IL-23, respiratory diseases, endocrine diseases, metabolic diseases, cardiovascular diseases, dermatological diseases, and/or abnormal angiogenesis associated diseases.