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(54) Title: COMPOUNDS FOR INFLAMMATION AND IMMUNE-RELATED USES

(57) Abstract: The invention relates to certain compounds or pharmaceutically acceptable salts, solvates, clathrates, or prodrugs thereof, that are useful as immunosuppressive agents and for treating and preventing inflammatory conditions, allergic disorders, and immune disorders.

COMPOUNDS FOR INFLAMMATION AND IMMUNE-RELATED USES

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/552,662, filed October 28, 2011, the entire teachings of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates to biologically active chemical compounds that may be used for immunosuppression or to treat or prevent inflammatory conditions and immune disorders.

BACKGROUND OF THE INVENTION

[0003] Inflammation is a mechanism that protects mammals from invading pathogens. However, while transient inflammation is necessary to protect a mammal from infection, uncontrolled inflammation causes tissue damage and is the underlying cause of many illnesses. Inflammation is typically initiated by binding of an antigen to a T-cell antigen receptor. Antigen binding by a T-cell initiates calcium influx into the cell via calcium ion channels, such as Ca^{2+}-release-activated Ca^{2+} channels (CRAC). Calcium ion influx in turn initiates a signaling cascade that leads to activation of these cells and an inflammatory response characterized by cytokine production.

[0004] Interleukin 2 (IL-2) is a cytokine that is secreted by T-cells in response to calcium ion influx into the cell. IL-2 modulates immunological effects on many cells of the immune system. For example, it is a potent T-cell mitogen that is required for T-cell proliferation, promoting their progression from G1 to S phase of the cell cycle; it stimulates the growth of NK cells; and it acts as a growth factor to B-cells and stimulates antibody synthesis.

[0005] IL-2, although useful in the immune response, can cause a variety of problems. IL-2 damages the blood-brain barrier and the endothelium of blood vessels in the brain. These effects may be the underlying causes of neuropsychiatric side effects observed under

IL-2 therapy, *e.g.*, fatigue, disorientation, and depression. It also alters the electrophysiological behavior of neurons.

[0006] Due to its effects on both T and B-cells, IL-2 is a major central regulator of immune responses. It plays a role in inflammatory reactions, tumor surveillance, and hematopoiesis. It also affects the production of other cytokines, inducing IL-1, TNFα, and TNF-β secretion, as well as stimulating the synthesis of IFN-γ in peripheral leukocytes.

[0007] T-cells that are unable to produce IL-2 become inactive (anergic). This renders them potentially inert to any antigenic stimulation they might receive in the future. As a result, agents which inhibit IL-2 production can be used for immunosuppression or to treat or prevent inflammation and immune disorders. This approach has been clinically validated with immunosuppressive drugs such as cyclosporin, FK506, and RS61443. Despite this proof of concept, agents that inhibit IL-2 production remain far from ideal. Among other problems, efficacy limitations and unwanted side effects (including dose-dependant nephrotoxicity and hypertension) hinder their use.

[0008] Over-production of proinflammatory cytokines other than IL-2 has also been implicated in many autoimmune diseases. For example, interleukin 5 (IL-5), a cytokine that increases the production of eosinophils, is increased in asthma. Overproduction of IL-5 is associated with the accumulation of eosinophils in the asthmatic bronchial mucosa, a hall mark of allergic inflammation. Thus, patients with asthma and other inflammatory disorders involving the accumulation of eosinophils would benefit from the development of new drugs that inhibit the production of IL-5.

[0009] Interleukin 4 (IL-4) and interleukin 13 (IL-13) have been identified as mediators of the hypercontractility of smooth muscle found in inflammatory bowel disease and asthma. Thus, patients with asthma and inflammatory bowel disease would benefit from the development of new drugs that inhibit IL-4 and IL-13 production.

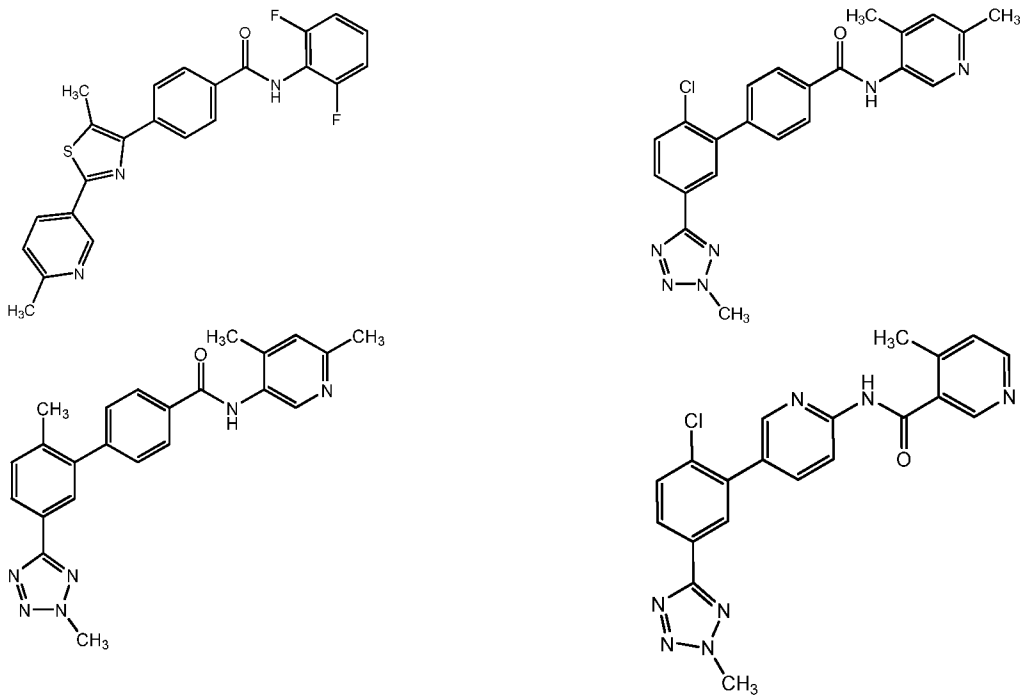
[0010] Granulocyte macrophage-colony stimulating factor (GM-CSF) is a regulator of maturation of granulocyte and macrophage lineage population and has been implicated as a key factor in inflammatory and autoimmune diseases. Anti-GM-CSF antibody blockade has been shown to ameliorate autoimmune disease. Thus, development of new drugs that

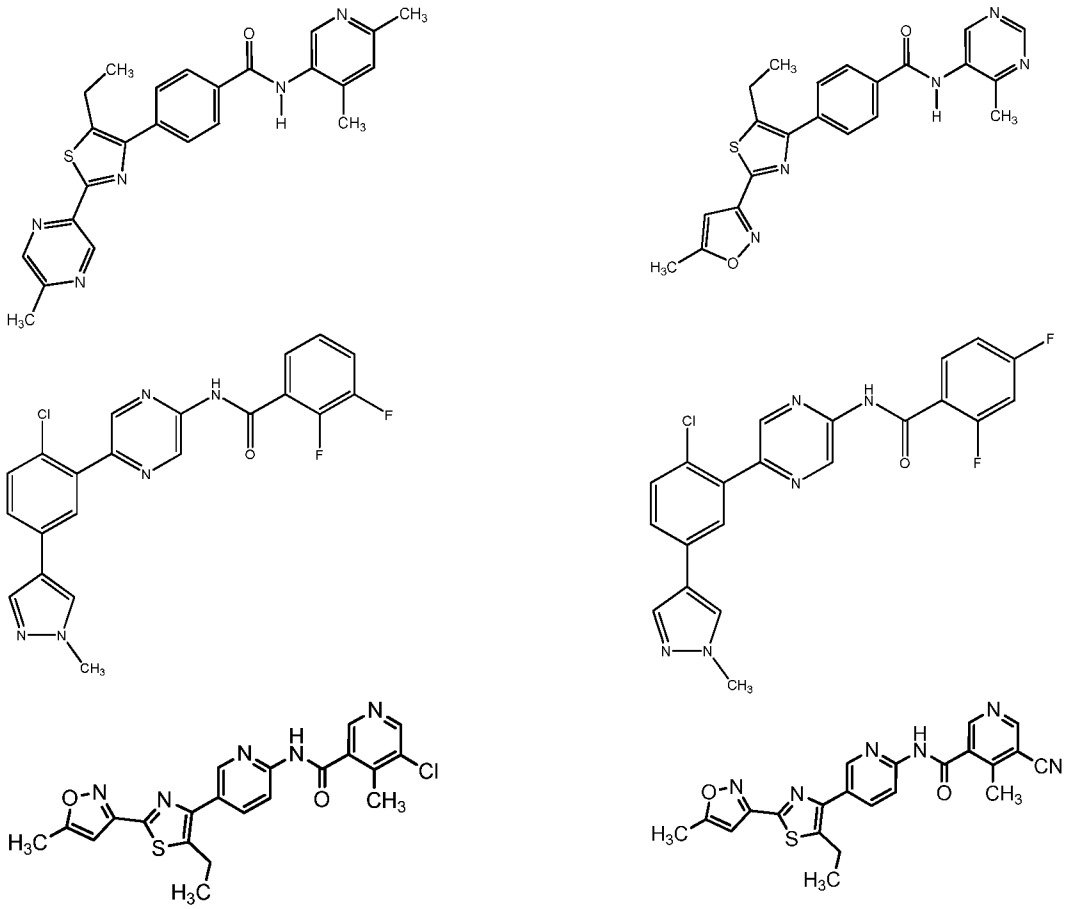
inhibit the production of GM-CSF would be beneficial to patients with an inflammatory or autoimmune disease.

SUMMARY OF THE INVENTION

[0011] The present disclosure, in an aspect, addresses the continuing need for new drugs which overcome one or more of the shortcomings of drugs currently used for immunosuppression or in the treatment or prevention of inflammatory disorders, allergic disorders, and autoimmune disorders. Desirable properties of such drugs include efficacy against diseases or disorders that are currently untreatable or poorly treatable, new mechanism of action, oral bioavailability and/or reduced side effects. Accordingly, compounds that inhibit the activity of CRAC ion channels and inhibit the production of IL-2, IL-4, IL-5, IL-13, GM-CSF, TNFα, and IFN-γ are disclosed herein. These compounds are particularly useful for immunosuppression and/or to treat or prevent inflammatory conditions and immune disorders. The particular genus of compounds described herein are particularly advantageous in that they are believed to combine inhibition of CRAC ion channels (*e.g.*, as measured by modulated I_{CRAC} current) and cytokines including IL-2, low incidence of off-target effects, and a favorable toxicity profile.

[0012] The present invention features compounds of the following formulae:





[0013] The compounds exemplified herein have especially desirable properties as a whole that have been heretofore unavailable in compounds of differing or similar class. These properties include one or more of the following: higher chemical stability which provides resistance to degradation of the compound *in vivo* that results in genotoxic fragments that are undesirable in the intended methods of administration; a longer half life *in vivo*; and improved metabolic stability, especially in reducing or eliminating CYP induction, which may result in time- or concentration-dependent loss of drug, all of which otherwise reduce drug efficacy.

[0014] In other aspects, pharmaceutical compositions including a pharmaceutically acceptable carrier and a compound of the invention are disclosed. The composition may further include one or more additional therapeutic agents, *e.g.*, immunosuppressive agents, anti-inflammatory agents, and suitable mixtures thereof. Other additional therapeutic agents include steroids, non-steroidal anti-inflammatory agents, antihistamines, analgesics, and suitable mixtures thereof.

[0015] Compounds as disclosed herein, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, are particularly useful inhibiting immune cell (*e.g.*, T-cells and/or B-cells) activation (*e.g.*, activation in response to an antigen). In particular, these compounds or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof can inhibit the production of certain cytokines that regulate immune cell activation. For example, a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof can inhibit the production of IL-2, IL-4, IL-5, IL-13, GM-CSF, TNFα, IFN-γ, or combinations thereof. Moreover, a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof can modulate the activity of one or more ion channels involved in activation of immune cells, such as CRAC ion channels.

[0016] A compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof is particularly useful for immunosuppression or for treating or preventing inflammatory conditions, allergic disorders, and immune disorders.

[0017] The invention also encompasses pharmaceutical compositions comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof; and a pharmaceutically acceptable carrier or vehicle. These compositions may further comprise additional agents. These compositions are useful for immunosuppression and treating or preventing inflammatory conditions, allergic disorders, and immune disorders.

[0018] The invention further encompasses methods for treating or preventing inflammatory conditions, allergic disorders, and immune disorders, comprising administering to a subject in need thereof an effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, or a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof. These methods may also comprise administering to the subject an additional agent separately or in a combination composition with the compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

[0019] The invention further encompasses methods for suppressing the immune system of a subject, comprising administering to a subject in need thereof an effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof, or a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof. These methods may also comprise administering to the subject an additional agent separately or in a combination composition with the compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

[0020] The invention further encompasses methods for inhibiting immune cell activation, including inhibiting proliferation of T-cells and/or B-cells, *in vivo* or *in vitro* comprising administering to the cell an effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof or a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

[0021] The invention further encompasses methods for inhibiting cytokine production in a cell (*e.g.*, IL-2, IL-4, IL-5, IL-13, GM-CSF, TNFα, and/or IFN-γ production) *in vivo* or *in vitro* comprising administering to a cell an effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof or a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

[0022] The invention further encompasses methods for modulating ion channel activity (*e.g.*, CRAC) *in vivo* or *in vitro* comprising administering an effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof or a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof.

[0023] All of the methods of this invention may be practiced with a compound of the invention alone, or in combination with other agents, such as other immunosuppressive agents, anti-inflammatory agents, agents for the treatment of allergic disorders or agents for the treatment of immune disorders.

[0024] The invention further encompasses a compound in Table 1, for use in therapy. Additionally, the invention encompasses use of a compound in Table 1, for treating a subject with an immune disorder. The invention encompasses use of a compound of Table 1, for treating an inflammatory condition. The invention encompasses use of a compound of Table 1, for suppressing the immune system. The invention further encompasses use of a compound of Table 1, for treating an allergic disorder.

DETAILED DESCRIPTION OF THE INVENTION

[0025] As used herein, the terms "subject," "patient," and "animal", are used interchangeably and include, but are not limited to, a cow, monkey, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig, or human. The preferred subject, patient, or animal is a human.

[0026] The compounds of the invention are defined herein by their chemical structures and/or chemical names. Where a compound is referred to by both a chemical structure and a chemical name, and the chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity.

[0027] The compounds of the invention can comprise isotopes of the elements which are explicitly disclosed. For example, each hydrogen substituent on compounds of the invention is independently selected from ^{1}H, ^{2}H, and ^{3}H isotopes.

[0028] Choices and combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable," as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (*e.g.*, therapeutic or prophylactic administration to a subject). Typically, such compounds are stable at a temperature of 40 °C or less, in the absence of excessive moisture, for at least one week. Such choices and combinations will be apparent to those of ordinary skill in the art and may be determined without undue experimentation.

[0029] Unless indicated otherwise, the compounds of the invention containing reactive functional groups (such as, without limitation, carboxy, hydroxy, and amino moieties) also include protected derivatives thereof. "Protected derivatives" are those compounds in which a reactive site or sites are blocked with one ore more protecting groups. Suitable protecting groups for carboxy moieties include benzyl, tert-butyl, and the like. Suitable protecting groups for amino and amido groups include acetyl, tert-butoxycarbonyl, benzyloxycarbonyl, and the like. Suitable protecting groups for hydroxy include benzyl and the like. Other suitable protecting groups are well known to those of ordinary skill in the art and include those found in T. W. Greene, *Protecting Groups in Organic Synthesis*, John Wiley & Sons, Inc. 1981, the entire teachings of which are incorporated herein by reference.

[0030] As used herein, the term "compound(s) of this invention" and similar terms refers to a compound of formula (I) or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof and also include protected derivatives thereof.

[0031] As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide a compound of this invention. Prodrugs may only become active upon such reaction under biological conditions, but they may have activity in their unreacted forms. Examples of prodrugs contemplated in this invention include, but are not limited to, analogs or derivatives of compounds of the invention that comprise biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Other examples of prodrugs include derivatives of compounds of the invention that include -NO, -NO_2, -ONO, or -ONO_2 moieties. Prodrugs can typically be prepared using well-known methods, such as those described in *Burger's Medicinal Chemistry and Drug Discovery* (1995) 172-178, 949-982 (Manfred E. Wolff ed., 5th ed), the entire teachings of which are incorporated herein by reference.

[0032] As used herein, the term "pharmaceutically acceptable salt," is a salt formed from an acid and a basic group of one of the compounds of the invention. Illustrative salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate,

tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. The term "pharmaceutically acceptable salt" also refers to a salt prepared from a compound of the invention having an acidic functional group, such as a carboxylic acid functional group, and a pharmaceutically acceptable inorganic or organic base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or trialkylamines; dicyclohexylamine; tributyl amine; pyridine; N-methyl,N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-hydroxy-lower alkyl amines), such as mono-, bis-, or tris-(2-hydroxyethyl)- amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N, N,-di-lower alkyl-N-(hydroxy lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)- amine, or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like. The term "pharmaceutically acceptable salt" also refers to a salt prepared from a compound of the invention having a basic functional group, such as an amino functional group, and a pharmaceutically acceptable inorganic or organic acid. Suitable acids include, but are not limited to, hydrogen sulfate, citric acid, acetic acid, oxalic acid, hydrochloric acid, hydrogen bromide, hydrogen iodide, nitric acid, phosphoric acid, isonicotinic acid, lactic acid, salicylic acid, tartaric acid, ascorbic acid, succinic acid, maleic acid, besylic acid, fumaric acid, gluconic acid, glucaronic acid, saccharic acid, formic acid, benzoic acid, glutamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, and *p*-toluenesulfonic acid.

[0033] When a disclosed compound is named or depicted by structure, it is to be understood that solvates (*e.g.*, hydrates) of the compound or its pharmaceutically acceptable salts are also included. "Solvates" refer to crystalline forms wherein solvent molecules are incorporated into the crystal lattice during crystallization. Solvate may include water or nonaqueous solvents such as ethanol, isopropanol, DMSO, acetic acid, ethanolamine, and EtOAc. Solvates, wherein water is the solvent molecule incorporated into the crystal lattice,

are typically referred to as "hydrates." Hydrates include a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

[0034] When a disclosed compound is named or depicted by structure, it is to be understood that the compound, including solvates thereof, may exist in crystalline forms, non-crystalline forms or a mixture thereof. The compounds or solvates may also exhibit polymorphism (*i.e.*, the capacity to occur in different crystalline forms). These different crystalline forms are typically known as "polymorphs." It is to be understood that when named or depicted by structure, the disclosed compounds and solvates (*e.g.*, hydrates) also include all polymorphs thereof. As used herein, the term "polymorph" means solid crystalline forms of a compound of the present invention or complex thereof. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (*e.g.*, to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (*e.g.*, differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (*e.g.*, tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (*e.g.*, tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing. For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it. In addition, one polymorph may spontaneously convert to another polymorph under certain conditions.

[0035] When a disclosed compound is named or depicted by structure, it is to be understood that clathrates ("inclusion compounds") of the compound or its pharmaceutically acceptable salts, solvates or polymorphs are also included. As used herein, he term "clathrate" means a compound of the present invention or a salt thereof in the form of a crystal lattice that contains spaces (*e.g.*, channels) that have a guest molecule (*e.g.*, a solvent or water) trapped within.

[0036] As used herein, the term "asthma" means a pulmonary disease, disorder or condition characterized by reversible airway obstruction, airway inflammation, and increased airway responsiveness to a variety of stimuli.

[0037] "Immunosuppression" refers to impairment of any component of the immune system resulting in decreased immune function. This impairment may be measured by any conventional means including whole blood assays of lymphocyte function, detection of lymphocyte proliferation and assessment of the expression of T-cell surface antigens. The antisheep red blood cell (SRBC) primary (IgM) antibody response assay (usually referred to as the plaque assay) is one specific method. This and other methods are described in Luster, M.I., Portier, C., Pait, D.G., White, K.L., Jr., Gennings, C., Munson, A.E., and Rosenthal, G.J. (1992). "Risk Assessment in Immunotoxicology I: Sensitivity and Predictability of Immune Tests." Fundam. Appl. Toxicol., 18, 200-210. Measuring the immune response to a T-cell dependent immunogen is another particularly useful assay (Dean, J.H., House, R.V., and Luster, M.I. (2001). "Immunotoxicology: Effects of, and Responses to, Drugs and Chemicals" in *Principles and Methods of Toxicology*: Fourth Edition (A.W. Hayes, Ed.), pp. 1415-1450, Taylor & Francis, Philadelphia, Pennsylvania).

[0038] The compounds of this invention can be used to treat subjects with immune disorders. As used herein, the term "immune disorder" and like terms means a disease, disorder or condition caused by the immune system of an animal, including autoimmune disorders. Immune disorders include those diseases, disorders or conditions that have an immune component and those that are substantially or entirely immune system-mediated. Autoimmune disorders are those wherein the animal's own immune system mistakenly attacks itself, thereby targeting the cells, tissues, and/or organs of the animal's own body. For example, the autoimmune reaction is directed against the nervous system in multiple sclerosis and the gut in Crohn's disease. In other autoimmune disorders such as systemic lupus erythematosus (lupus), affected tissues and organs may vary among individuals with the same disease. One person with lupus may have affected skin and joints whereas another may have affected skin, kidney, and lungs. Ultimately, damage to certain tissues by the immune system may be permanent, as with destruction of insulin-producing cells of the pancreas in Type 1 diabetes mellitus. Specific autoimmune disorders that may be ameliorated using the compounds and methods of this invention include without limitation,

autoimmune disorders of the nervous system (*e.g.*, multiple sclerosis, myasthenia gravis, autoimmune neuropathies such as Guillain-Barré, and autoimmune uveitis), autoimmune disorders of the blood (*e.g.*, autoimmune hemolytic anemia, pernicious anemia, and autoimmune thrombocytopenia), autoimmune disorders of the blood vessels (*e.g.*, temporal arteritis, anti-phospholipid syndrome, vasculitides such as Wegener's granulomatosis, and Behcet's disease), autoimmune disorders of the skin (*e.g.*, psoriasis, dermatitis herpetiformis, pemphigus vulgaris, and vitiligo), autoimmune disorders of the gastrointestinal system (*e.g.*, Crohn's disease, ulcerative colitis, primary biliary cirrhosis, and autoimmune hepatitis), autoimmune disorders of the endocrine glands (*e.g.*, Type 1 or immune-mediated diabetes mellitus, Grave's disease. Hashimoto's thyroiditis, autoimmune oophoritis and orchitis, and autoimmune disorder of the adrenal gland); and autoimmune disorders of multiple organs (including connective tissue and musculoskeletal system diseases) (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, polymyositis, dermatomyositis, spondyloarthropathies such as ankylosing spondylitis, and Sjogren's syndrome). In addition, other immune system mediated diseases, such as graft-versus-host disease and allergic disorders, are also included in the definition of immune disorders herein. Because a number of immune disorders are caused by inflammation, there is some overlap between disorders that are considered immune disorders and inflammatory disorders. For the purpose of this invention, in the case of such an overlapping disorder, it may be considered either an immune disorder or an inflammatory disorder. "Treatment of an immune disorder" herein refers to administering a compound or a composition of the invention to a subject, who has an immune disorder, a symptom of such a disease or a predisposition towards such a disease, with the purpose to cure, relieve, alter, affect, or prevent the autoimmune disorder, the symptom of it, or the predisposition towards it.

[0039] As used herein, the term "allergic disorder" means a disease, condition or disorder associated with an allergic response against normally innocuous substances. These substances may be found in the environment (such as indoor air pollutants and aeroallergens) or they may be non-environmental (such as those causing dermatological or food allergies). Allergens can enter the body through a number of routes, including by inhalation, ingestion, contact with the skin or injection (including by insect sting). Many allergic disorders are linked to atopy, a predisposition to generate the allergic antibody IgE.

Because IgE is able to sensitize masT-cells anywhere in the body, atopic individuals often express disease in more than one organ. For the purpose of this invention, allergic disorders include any hypersensitivity that occurs upon re-exposure to the sensitizing allergen, which in turn causes the release of inflammatory mediators. Allergic disorders include without limitation, allergic rhinitis (*e.g.*, hay fever), sinusitis, rhinosinusitis, chronic or recurrent otitis media, drug reactions, insect sting reactions, latex reactions, conjunctivitis, urticaria, anaphylaxis and anaphylactoid reactions, atopic dermatitis, asthma, and food allergies.

[0040] The compounds of this invention can be used to prevent or to treat subjects with inflammatory disorders. As used herein, an "inflammatory disorder" means a disease, disorder or condition characterized by inflammation of body tissue or having an inflammatory component. These include local inflammatory responses and systemic inflammation. Examples of such inflammatory disorders include: transplant rejection, including skin graft rejection; chronic inflammatory disorders of the joints, including arthritis, rheumatoid arthritis, osteoarthritis and bone diseases associated with increased bone resorption; inflammatory bowel diseases such as ileitis, ulcerative colitis, Barrett's syndrome, and Crohn's disease; inflammatory lung disorders such as asthma, adult respiratory distress syndrome, and chronic obstructive airway disease; inflammatory disorders of the eye including corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic inflammatory disorders of the gums, including gingivitis and periodontitis; tuberculosis; leprosy; inflammatory diseases of the kidney including uremic complications, glomerulonephritis and nephrosis; inflammatory disorders of the skin including sclerodermatitis, psoriasis and eczema; inflammatory diseases of the central nervous system, including chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and viral or autoimmune encephalitis; autoimmune disorders, immune-complex vasculitis, systemic lupus and erythematodes; systemic lupus erythematosus (SLE); and inflammatory diseases of the heart such as cardiomyopathy, ischemic heart disease hypercholesterolemia, atherosclerosis); as well as various other diseases with significant inflammatory components, including preeclampsia; chronic liver failure, brain and spinal cord trauma, cancer). There may also be a systemic

inflammation of the body, exemplified by gram-positive or gram negative shock, hemorrhagic or anaphylactic shock, or shock induced by cancer chemotherapy in response to pro-inflammatory cytokines, *e.g.*, shock associated with pro-inflammatory cytokines. Such shock can be induced, *e.g.*, by a chemotherapeutic agent used in cancer chemotherapy. "Treatment of an inflammatory disorder" herein refers to administering a compound or a composition of the invention to a subject, who has an inflammatory disorder, a symptom of such a disorder or a predisposition towards such a disorder, with the purpose to cure, relieve, alter, affect, or prevent the inflammatory disorder, the symptom of it, or the predisposition towards it.

[0041] An "effective amount" is the quantity of compound in which a beneficial outcome is achieved when the compound is administered to a subject or alternatively, the quantity of compound that possess a desired activity *in vivo* or *in vitro*. In the case of inflammatory disorders and autoimmune disorders, a beneficial clinical outcome includes reduction in the extent or severity of the symptoms associated with the disease or disorder and/or an increase in the longevity and/or quality of life of the subject compared with the absence of the treatment. The precise amount of compound administered to a subject will depend on the type and severity of the disease or condition and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of inflammatory disorder or autoimmune disorder or the degree of immunosuppression sought. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. Effective amounts of the disclosed compounds typically range between about 1 mg/m^2 per day and about 10 grams/m^2 per day, and preferably between 10 mg/m^2 per day and about 1 gram/m^2.

[0042] The compounds of the invention may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (*i.e.*, geometric isomers), enantiomers, or diastereomers. According to this invention, the chemical structures depicted herein, including the compounds of this invention, encompass all of the corresponding compounds' enantiomers and stereoisomers, that is, both the stereomerically pure form (*e.g.*, geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric, diastereomeric, and geometric isomeric mixtures. In some cases, one enantiomer, diastereomer, or geometric isomer will possess

superior activity or an improved toxicity or kinetic profile compared to others. In those cases, such enantiomers, diastereomers, and geometric isomers of a compound of this invention are preferred.

[0043] The term "inhibit production of IL-2" and like terms means inhibiting IL-2 synthesis (*e.g.*, by inhibiting transcription (mRNA expression), or translation (protein expression)) and/or inhibiting IL-2 secretion in a cell that has the ability to produce and/or secrete IL-2 (*e.g.*, T lymphocyte). Likewise, the term "inhibiting production of IL-4, IL-5, IL-13, GM-CSF, TNFα, or IFN-γ means inhibiting the synthesis (*e.g.*, by inhibiting transcription, or translation) and/or inhibiting the secretion in a cell that has the ability to produce and/or secrete these cytokines.

[0044] As used herein, a racemic mixture means about 50% of one enantiomer and about 50% of is corresponding enantiomer relative to all chiral centers in the molecule. The invention encompasses all enantiomerically-pure, enantiomerically-enriched, diastereomerically pure, diastereomerically enriched, and racemic mixtures of the compounds of the invention.

[0045] Enantiomeric and diastereomeric mixtures can typically be resolved into their component enantiomers or stereoisomers by well known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and diastereomers can also be obtained from diastereomerically- or enantiomerically-pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[0046] When administered to a patient, *e.g.*, to a non-human animal for veterinary use or for improvement of livestock, or to a human for clinical use, the compounds of the invention are typically administered in isolated form or as the isolated form in a pharmaceutical composition. As used herein, "isolated" means that the compounds of the invention are separated from other components of either (a) a natural source, such as a plant or cell, preferably bacterial culture, or (b) a synthetic organic chemical reaction mixture. Preferably, via conventional techniques, the compounds of the invention are purified. As used herein,

"purified" means that when isolated, the isolate contains at least 95%, preferably at least 98%, of a single compound of the invention by weight of the isolate.

[0047] Only those choices and combinations of substituents that result in a stable structure are contemplated. Such choices and combinations will be apparent to those of ordinary skill in the art and may be determined without undue experimentation.

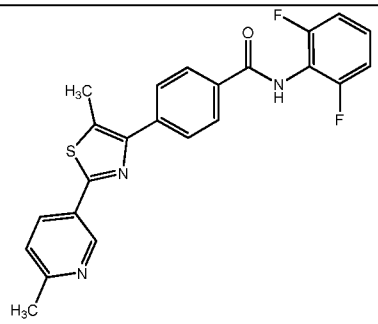
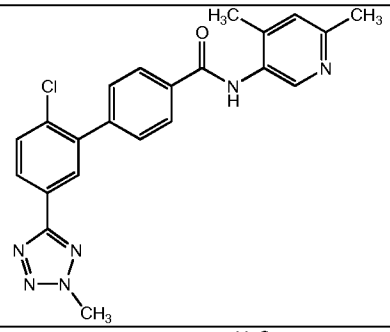
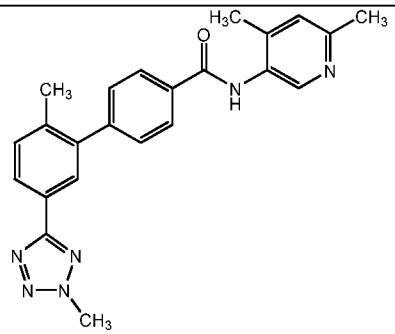
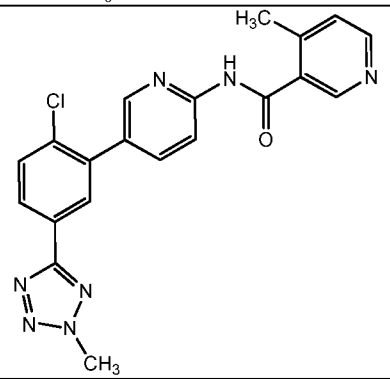
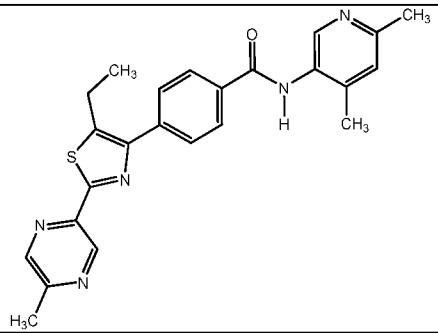
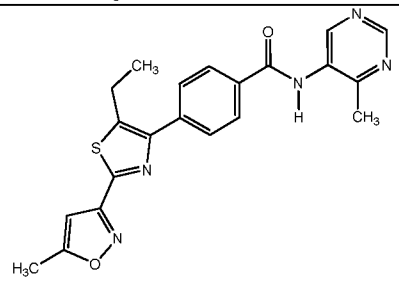
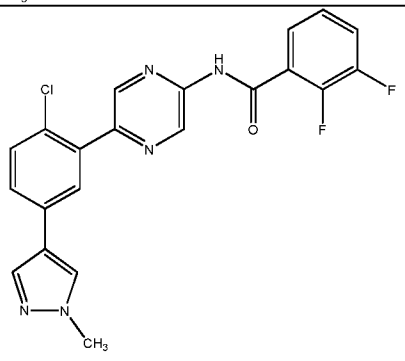
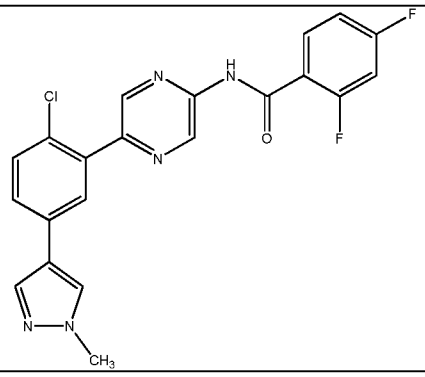
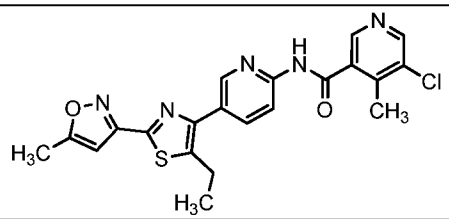
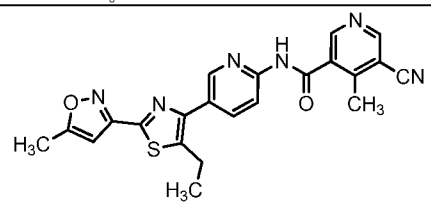
[0048] The invention can be understood more fully by reference to the following detailed description and illustrative examples, which are intended to exemplify non-limiting embodiments of the invention.

SPECIFIC EMBODIMENTS

[0049] The invention relates to compounds as described herein, compounds in Table 1, and pharmaceutical compositions that are particularly useful for immunosuppression or to treat or prevent inflammatory conditions, immune disorders, and allergic disorders.

EXEMPLARY COMPOUNDS

[0050] Exemplary compounds of the invention, that have been made in accordance with the descriptions in the examples below, are depicted in Table 1 below.

Compound number	Structure	Compound number	Structure
1		2	
3		4	
5		6	
7		8	
9		10	

MECHANISM OF ACTION

[0051] Activation of T-lymphocytes in response to an antigen is dependent on calcium ion oscillations. Calcium ion oscillations in T-lymphocytes are triggered through stimulation of the T-cell antigen receptor, and involve calcium ion influx through the stored-operated Ca^{2+}-release-activated Ca^{2+} (CRAC) channel. Although a detailed electrophysiological profile of the channel exists, the molecular structure of the CRAC ion channel had not been identified till the recent identification of the pore-forming unit, named Orai1/CRACM1 (Vig, *Science* (2006), *312*:1220-3, Feske, *Nature* (2006), *441*:179-85). Thus, inhibition of CRAC ion channels can be measured by measuring inhibition of the I_{CRAC} current. Calcium ion oscillations in T-cells have been implicated in the activation of several transcription factors (*e.g.*, NFAT, Oct/Oap and NFκB) which are critical for T-cell activation (Lewis, *Biochemical Society Transactions* (2003), *31*:925-929, the entire teachings of which are incorporated herein by reference). Without wishing to be bound by any theory, it is believed that because the compounds of the invention inhibit the activity of CRAC ion channels, they inhibit immune cell activation.

METHODS OF TREATMENT AND PREVENTION

[0052] A effective amount of a compound of the invention or a pharmaceutically acceptable salt, solvate, clathrate, and prodrug thereof, or a pharmaceutical composition comprising a compound of the invention, or a pharmaceutically acceptable salt, solvate, clathrate, and prodrug thereof, is administered to a patient in need of immunosuppression or in need of treatment or prevention of an inflammatory condition, an immune disorder, or an allergic disorder. Such patients may be treatment naïve or may experience partial or no response to conventional therapies.

[0053] Responsiveness of a particular inflammatory condition, immune disorder, or allergic disorder in a subject can be measured directly (*e.g.*, measuring blood levels of inflammatory cytokines (such as IL-2, IL-4, IL-5, IL-13, GM-CSF, TNFα, IFN-γ and the like) after administration of a compound of this invention), or can be inferred based on an understanding of disease etiology and progression. The compounds of the invention, or pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof can be assayed

in vitro or *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, known animal models of inflammatory conditions, immune disorders, or allergic disorders can be used to demonstrate the safety and efficacy of compounds of this invention.

PHARMACEUTICAL COMPOSITIONS AND DOSAGE FORMS

[0054] Pharmaceutical compositions and dosage forms of the invention comprise one or more active ingredients in relative amounts and formulated in such a way that a given pharmaceutical composition or dosage form can be used for immunosuppression or to treat or prevent inflammatory conditions, immune disorders, and allergic disorders. Preferred pharmaceutical compositions and dosage forms comprise a compound of the invention, or a pharmaceutically acceptable prodrug, salt, solvate, or clathrate thereof, optionally in combination with one or more additional active agents.

[0055] Single unit dosage forms of the invention are suitable for oral, mucosal (*e.g.*, nasal, sublingual, vaginal, buccal, or rectal), parenteral (*e.g.*, subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (*e.g.*, nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (*e.g.*, aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (*e.g.*, crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

[0056] The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form suitable for mucosal administration may contain a smaller amount of active ingredient(s) than an oral dosage form used to treat the same indication. This aspect of the invention will be readily apparent

to those skilled in the art. *See, e.g., Remington's Pharmaceutical Sciences* (1990) 18th ed., Mack Publishing, Easton PA.

[0057] Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms.

[0058] The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients can be accelerated by some excipients such as lactose, or when exposed to water. Active ingredients that comprise primary or secondary amines (*e.g.*, N-desmethylvenlafaxine and N,N-didesmethylvenlafaxine) are particularly susceptible to such accelerated decomposition. Consequently, this invention encompasses pharmaceutical compositions and dosage forms that contain little, if any, lactose. As used herein, the term "lactose-free" means that the amount of lactose present, if any, is insufficient to substantially increase the degradation rate of an active ingredient. Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmacopeia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise active ingredients, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise active ingredients, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

[0059] This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (*e.g.*, 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. *See, e.g.*, Jens T. Carstensen (1995) *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, NY, 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the

effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

[0060] Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient including a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

[0061] An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (*e.g.*, vials), blister packs, and strip packs.

[0062] The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizer" include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

[0063] Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention include a compound of the invention, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof in an amount of from about 1 mg to about 1000 mg, preferably in an amount of from about 50 mg to about 500 mg, and most preferably in an amount of from about 75 mg to about 350 mg. The typical total daily dosage of a compound of the invention, or a pharmaceutically acceptable salt, solvate, clathrate, or prodrug thereof can range from about 1 mg to about 5000 mg per day, preferably in an amount from about 50 mg to about 1500 mg per day, more preferably from about 75 mg to about 1000 mg per

day. It is within the skill of the art to determine the appropriate dose and dosage form for a given patient.

ORAL DOSAGE FORMS

[0064] Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (*e.g.*, chewable tablets), caplets, capsules, and liquids (*e.g.*, flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences* (1990) 18th ed., Mack Publishing, Easton PA.

[0065] Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (*e.g.*, powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

[0066] Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

[0067] For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded

tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

[0068] Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (*e.g.*, ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (*e.g.*, Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

[0069] Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, PA), and mixtures thereof. One specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103J and Starch 1500 LM.

[0070] Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (*e.g.*, granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

[0071] Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily

discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

[0072] Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

[0073] Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (*e.g.*, peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, MD), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, TX), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, MA), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

CONTROLLED RELEASE DOSAGE FORMS

[0074] Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in

varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

[0075] All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (*e.g.*, adverse) effects.

[0076] Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

PARENTERAL DOSAGE FORMS

[0077] Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral

dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

[0078] Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

[0079] Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

TRANSDERMAL, TOPICAL, AND MUCOSAL DOSAGE FORMS

[0080] Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, *e.g.*, *Remington's Pharmaceutical Sciences* (1980 & 1990) 16th and 18th eds., Mack Publishing, Easton PA and *Introduction to Pharmaceutical Dosage Forms* (1985) 4th ed., Lea & Febiger, Philadelphia. Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

[0081] Suitable excipients (*e.g.*, carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate,

isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, *e.g.*, *Remington's Pharmaceutical Sciences* (1980 & 1990) 16th and 18th eds., Mack Publishing, Easton PA.

[0082] Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

[0083] The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

COMBINATION THERAPY

[0084] The methods for immunosuppression or for treating or preventing inflammatory conditions and immune disorders in a patient in need thereof can further comprise administering to the patient being administered a compound of this invention, an effective

amount of one or more other active agents. Such active agents may include those used conventionally for immunosuppression or for inflammatory conditions or immune disorders. These other active agents may also be those that provide other benefits when administered in combination with the compounds of this invention. For example, other therapeutic agents may include, without limitation, steroids, non-steroidal anti-inflammatory agents, antihistamines, analgesics, immunosuppressive agents and suitable mixtures thereof. In such combination therapy treatment, both the compounds of this invention and the other drug agent(s) are administered to a subject (*e.g.*, humans, male or female) by conventional methods. The agents may be administered in a single dosage form or in separate dosage forms. Effective amounts of the other therapeutic agents and dosage forms are well known to those skilled in the art. It is well within the skilled artisan's purview to determine the other therapeutic agent's optimal effective-amount range.

[0085] In one embodiment of the invention where another therapeutic agent is administered to a subject, the effective amount of the compound of this invention is less than its effective amount when the other therapeutic agent is not administered. In another embodiment, the effective amount of the conventional agent is less than its effective amount when the compound of this invention is not administered. In this way, undesired side effects associated with high doses of either agent may be minimized. Other potential advantages (including without limitation improved dosing regimens and/or reduced drug cost) will be apparent to those of skill in the art.

[0086] In one embodiment relating to autoimmune and inflammatory conditions, the other therapeutic agent may be a steroid or a non-steroidal anti-inflammatory agent. Particularly useful non-steroidal anti-inflammatory agents, include, but are not limited to, aspirin, ibuprofen, diclofenac, naproxen, benoxaprofen, flurbiprofen, fenoprofen, flubufen, ketoprofen, indoprofen, piroprofen, carprofen, oxaprozin, pramoprofen, muroprofen, trioxaprofen, suprofen, aminoprofen, tiaprofenic acid, fluprofen, bucloxic acid, indomethacin, sulindac, tolmetin, zomepirac, tiopinac, zidometacin, acemetacin, fentiazac, clidanac, oxpinac, mefenamic acid, meclofenamic acid, flufenamic acid, niflumic acid, tolfenamic acid, diflurisal, flufenisal, piroxicam, sudoxicam, isoxicam; salicylic acid derivatives, including aspirin, sodium salicylate, choline magnesium trisalicylate, salsalate, diflunisal, salicylsalicylic acid, sulfasalazine, and olsalazin; para-aminophenol derivatives

including acetaminophen and phenacetin; indole and indene acetic acids, including indomethacin, sulindac, and etodolac; heteroaryl acetic acids, including tolmetin, diclofenac, and ketorolac; anthranilic acids (fenamates), including mefenamic acid, and meclofenamic acid; enolic acids, including oxicams (piroxicam, tenoxicam), and pyrazolidinediones (phenylbutazone, oxyphenthartazone); and alkanones, including nabumetone and pharmaceutically acceptable salts thereof and mixtures thereof. For a more detailed description of the NSAIDs, see Paul A. Insel, "Analgesic-Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout" in *Goodman & Gilman's The Pharmacological Basis of Therapeutics* 617-57 (Perry B. Molinhoff and Raymond W. Ruddon eds., 9th ed 1996) and Glen R. Hanson, "Analgesic, Antipyretic and Anti-Inflammatory Drugs" in *Remington: The Science and Practice of Pharmacy Vol II* 1196-1221 (A.R. Gennaro ed. 19th ed. 1995) which are hereby incorporated by reference in their entireties.

[0087] Of particular relevance to allergic disorders, the other therapeutic agent may be an antihistamine. Useful antihistamines include, but are not limited to, loratadine, cetirizine, fexofenadine, desloratadine, diphenhydramine, chlorpheniramine, chlorcyclizine, pyrilamine, promethazine, terfenadine, doxepin, carbinoxamine, clemastine, tripelennamine, brompheniramine, hydroxyzine, cyclizine, meclizine, cyproheptadine, phenindamine, acrivastine, azelastine, levocabastine, and mixtures thereof. For a more detailed description of antihistamines, see Goodman & Gilman's *The Pharmacological Basis of Therapeutics* (2001) 651-57, 10th ed).

[0088] Immunosuppressive agents include glucocorticoids, corticosteroids (such as Prednisone or Solumedrol), T-cell blockers (such as cyclosporin A and FK506), purine analogs (such as azathioprine (Imuran)), pyrimidine analogs (such as cytosine arabinoside), alkylating agents (such as nitrogen mustard, phenylalanine mustard, busulfan, and cyclophosphamide), folic acid antagonists (such as aminopterin and methotrexate), antibiotics (such as rapamycin, actinomycin D, mitomycin C, puramycin, and chloramphenicol), human IgG, antilymphocyte globulin (ALG), and antibodies (such as anti-CD3 (OKT3), anti-CD4 (OKT4), anti-CD5, anti-CD7, anti-IL-2 receptor, anti-alpha/beta TCR, anti-ICAM-1, anti-CD20 (Rituxan), anti-IL-12 and antibodies to immunotoxins).

[0089] The foregoing and other useful combination therapies will be understood and appreciated by those of skill in the art. Potential advantages of such combination therapies include a different efficacy profile, the ability to use less of each of the individual active ingredients to minimize toxic side effects, synergistic improvements in efficacy, improved ease of administration or use and/or reduced overall expense of compound preparation or formulation.

OTHER EMBODIMENTS

[0090] The compounds of this invention may be used as research tools (for example, as a positive control for evaluating other potential CRAC inhibitors, or IL-2, IL-4, IL-5, IL-13, GM-CSF, TNFα, and/or IFN-γ inhibitors). These and other uses and embodiments of the compounds and compositions of this invention will be apparent to those of ordinary skill in the art.

[0091] The invention is further defined by reference to the following examples describing in detail the preparation of compounds of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the purpose and interest of this invention. The following examples are set forth to assist in understanding the invention and should not be construed as specifically limiting the invention described and claimed herein. Such variations of the invention, including the substitution of all equivalents now known or later developed, which would be within the purview of those skilled in the art, and changes in formulation or minor changes in experimental design, are to be considered to fall within the scope of the invention incorporated herein.

EXAMPLES

Experimental Rationale

[0092] Without wishing to be bound by theory, it is believed that the compounds of this invention inhibit CRAC ion channels, thereby inhibiting production of IL-2 and other key cytokines involved with inflammatory and immune responses. The examples that follow demonstrate these properties.

Materials and General Methods

[0093] Reagents and solvents used below can be obtained from commercial sources such as Aldrich Chemical Co. (Milwaukee, Wisconsin, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 300MHz NMR spectrometer. Significant peaks are tabulated in the order: δ (ppm): chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet), coupling constant(s) in Hertz (Hz) and number of protons.

[0094] Manual patch clamp experiments are conducted in the tight-seal whole-cell configuration at room temperature (21-25 °C). Patch pipettes are fashioned from borosilicate glass capillary tubes and have resistances between 2-4 MΩ after filling with standard intracellular solution. High resolution current recordings are acquired with a computer-based patch clamp amplifier system (EPC-10, HEKA, Lambrecht, Germany). All voltages are corrected for a liquid junction potential of 10 mV between external and internal solutions with glutamate as the intracellular anion. Currents are filtered at 2.9 kHz and digitized at 10 μs intervals. Capacitive currents and series resistance are determined and corrected before each voltage ramp using the automatic capacitance compensation of the EPC-10.

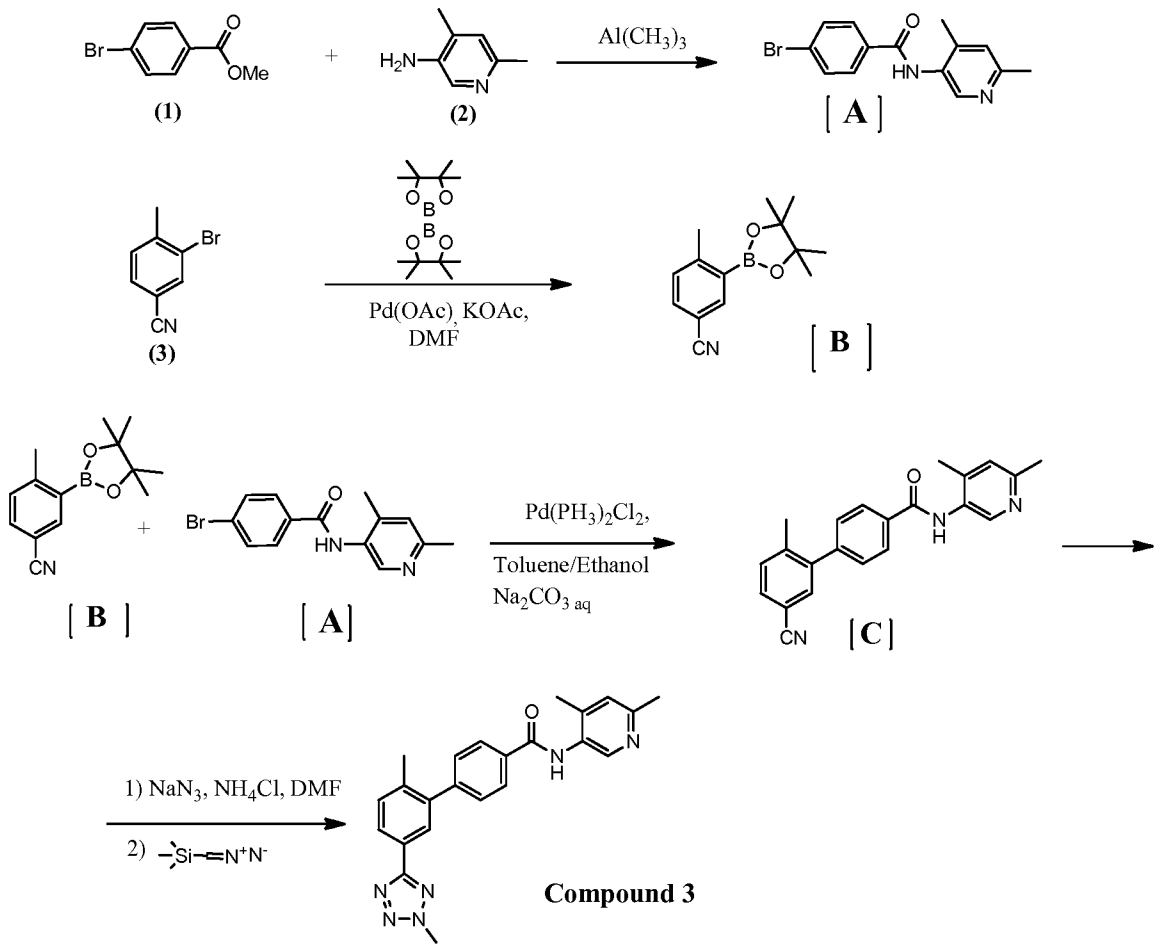
[0095] Automated patch clamp experiments are conducted with the QPatch 16 (Sophion Bioscience, Ballerup, Denmark) at room temperature (21-25 °C). Immediately following the establishment of giga-seal whole-cell configuration, the cells membrane potential is clamped at 0 mV. Voltage ramps of 50 ms duration spanning the voltage range of -100 to +100 mV are then stimulated at a rate of 0.33 Hz. Currents are filtered at 2.9 kHz and digitized at 200 μs intervals. Capacitive currents and series resistance are determined and corrected before each voltage ramp using the automatic capacitance compensation.

EXAMPLE 1

Synthesis of Exemplary Compounds of the Invention:

Representative Synthetic Procedures:

Synthesis of Compound 3, N-(4,6-dimethylpyridin-3-yl)-2'-methyl-5'-(2-methyl-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-carboxamide:



[0096] A solution of methyl-4-bromobenzoate **(1)** (430 mg, 2mmol) and 4,6-dimethylpyridin-3-amine **(2)** (250 mg, 2.1 mmol) in toluene (8 mL) was prepared and the flask was closed. It was purged with nitrogen, then 2M solution of $Al(CH_3)_3$ in toluene (1.5 mL) was added drop-wise into the reaction mixture. After the addition was finished, the reaction was microwaved for 15 min at 110°C. The reaction mixture was cooled down to room temperature, diluted with EtOAc (20mL), washed with 2N solution of NaOH (2 x 10mL) then brine (1x10 mL). Organic phase was collected and dried over Na_2SO_4. Column chromatography afforded 4-bromo-N-(4,6-dimethylpyridin-3-yl)benzamide **(A)** (yield over 80%).

[0097] The solution of 3-bromo-4-methylbenzonitrile **(3)** (2.0 g, 10 mmol), in DMF (12 mL) with Bis(pinacolato)diboron (3.4 g, 13 mmol), and $Pd(OAc)_2$ (0.5 g, 2 mmol), and KOAc (3 g, 30 mmol) was microwaved for 3 h at 80°C. The mixture was then diluted with H_2O (25 mL) and extracted with EtOAc (2 x 30 mL).

[0098] The organic phase was collected and dried over Na_2SO_4. Column chromatography (in Hexane/Ethyl acetate 9:1) afforded 4-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (**B**) as an oil, which solidified during the drying *in vacuo* (yield: 96%)

[0099] To a solution of **(A)** (200 mg, 0.65 mmol) and **(B)** (250 mg, 1 mmol) and $(Pd(PPh_3)_2Cl_2$ (130 mg, 0.2 mmol) in toluene (6 mL) was added Na_2CO_3 (2 N, 2 mL) and ethanol (2 mL). The reaction mixture was stirred at 80°C for 12 h. The solution was cooled down to room temperature and was then diluted with EtOAc (30 mL); washed with water (20 mL). The organic layer was dried over Na_2SO_4 and the product 5'-cyano-N-(4,6-dimethylpyridin-3-yl)-2'-methyl-[1,1'-biphenyl]-4-carboxamide **(C)** was isolated by column chromatography as a white solid. (Yield: ~60 %)

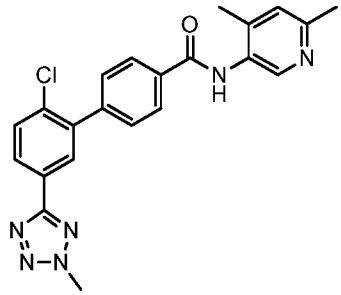
[00100] A solution/suspension of **(C)** (140 mg, 0.4 mmol), NaN_3 (160 mg, 2.4 mmol) and NH_4Cl (130 mg, 2.4 mmol) in DMF (5 mL) was stirred for 6 h at 90-96°C (until the substrate **C** was consumed). The reaction mixture was cooled down to room temperature, the solids were filtered off and washed with DMF (2 x 1mL). Into the DMF-solution an excess of (trimethylsilyl)diazomethane was added portion-wise (2M solution in hexane, 4-5 mL, until gas evolution stopped).

[00101] Reaction mixture was then diluted with EtOAc (20 mL), washed with water (10 mL), dried over Na_2SO_4, concentrated and chromatographed to give N-(4,6-dimethylpyridin-3-yl)-2'-methyl-5'-(2-methyl-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-carboxamide (main isomer).

[00102] ^{1}H-NMR (DMSO) δ 10.14 (s, 1H), 8.36 (s, 1H), 8.09-7.92 (m, 4H,), 7.72-7.53 (m, 3H), 7.17 (s, 1H,), 4.41 (s, 3H), 2.42 (s, 3H), 2.31 (s, 3H,), 2.21 (s, 3H), ppm; ESMS calcd for $C_{23}H_{22}N_6O$: 398.2; found: 399.3 ($M + H^+$).

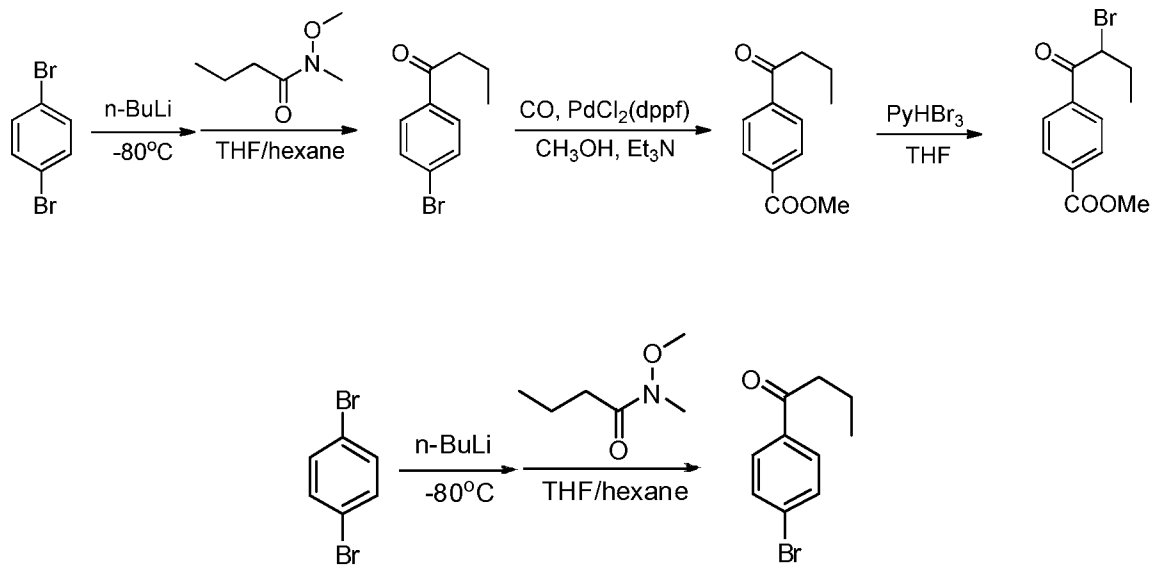
[00103] The following analogs were synthesized in a similar manner according to the above described procedures:

[00104] *Compound 2,* 2'-chloro-N-(4,6-dimethylpyridin-3-yl)-5'-(2-methyl-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-carboxamide:



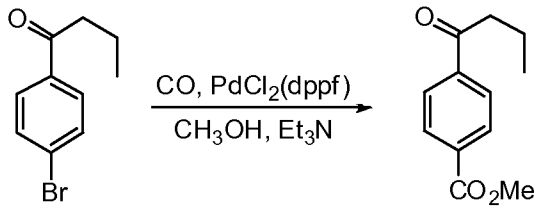
[00106] ^{1}H-NMR (DMSO) δ 10.14 (s, 1H), 8.35 (s, 1H), 8.13-8.07 (m, 4H), 7.82 (d, 1H, J=6.3), 7.69 (d, 2H, J=6), 7.2 (s, 1H), 4.45 (s, 3H), 2.45 (s, 3H), 2.23 (s, 3H), ppm; ESMS calcd for $C_{22}H_{19}ClN_6O$: 418.1; found: 419.2 ($M + H^+$).

[00107] General synthetic procedure for compounds 1, 5 and 6::

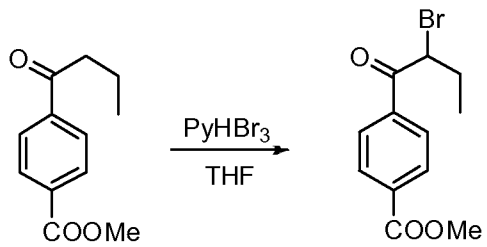


[00108] Into a 2000-mL 4-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed a solution of 1,4-dibromobenzene (50 g, 211.86 mmol, 1.00 equiv) in tetrahydrofuran/hexane=1:10 (1000 mL). The resulting solution was cooled to -80°C in a liquid nitrogen bath. This was followed by the addition of n-butyllithium (88.8 mL, 1.05 equiv) dropwise with stirring at -80°C. The resulting solution

was stirred for 30 min at -80°C in a liquid nitrogen bath. To this was added a solution of N-methoxy-N-methylbutyramide (41.7 g, 318.32 mmol, 1.50 equiv) in tetrahydrofuran/hexane=1:10 (100 mL). The resulting solution was allowed to react, with stirring, for an additional 15 min while the temperature was maintained at -80°C in a liquid nitrogen bath. The reaction was then quenched by the addition of 400 mL of water. The resulting solution was extracted with 3x400 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 3x400 mL of water and 3x400 mL of brine. The mixture was dried over anhydrous magnesium sulfate. The solids were filtered out. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column eluting with ethyl acetate/petroleum ether (0:100~1:100). This resulted in 35 g (73%) of 1-(4-bromophenyl)butan-1-one as a white solid.

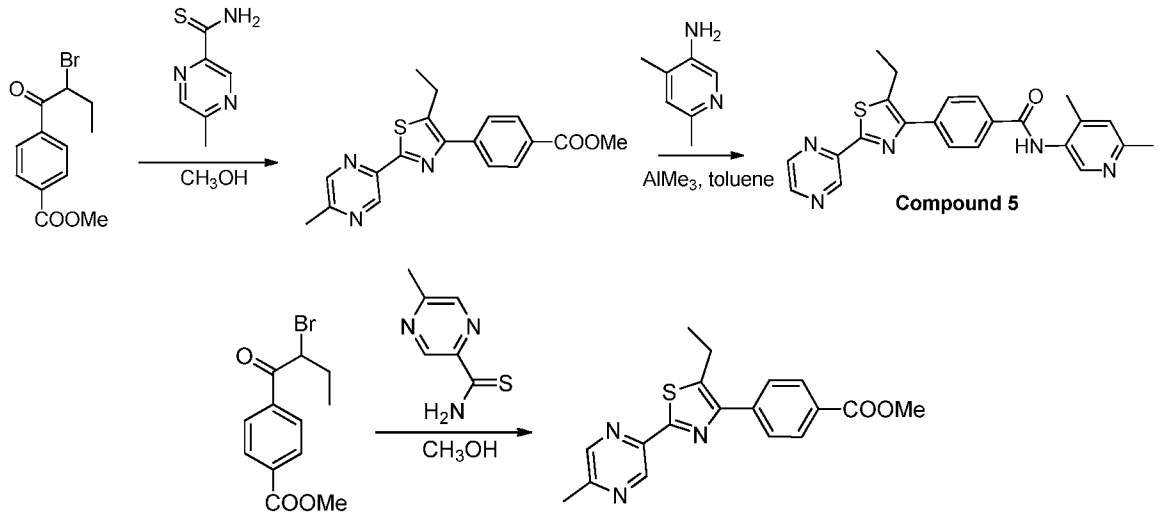


[00109] Into a 2000-mL pressure tank reactor (CO, 15 atm), was placed a solution of 1-(4-bromophenyl) butan-1-one (58 g, 255.51 mmol, 1.00 equiv) in methanol (1200 mL), triethylamine (51 g, 504.95 mmol, 2.00 equiv), 1,1'-Bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (9.42 mg, 0.01 mmol, 0.05 equiv). The resulting solution was stirred for 12 h at 90°C. The solids were filtered out. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column eluting with ethyl acetate/petroleum ether (1:20~1:10). This resulted in 45 g (85%) of methyl 4-butyrylbenzoate as a white solid.

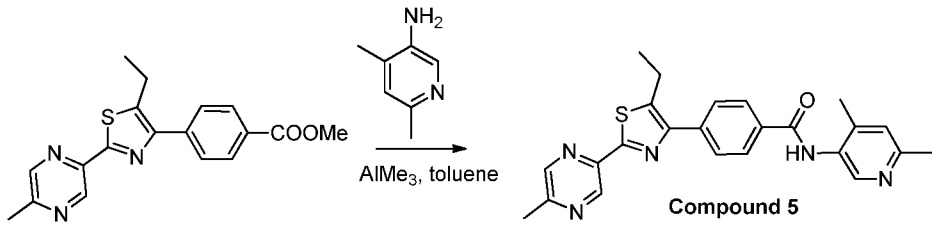


[00110] Into a 250-mL round-bottom flask, was placed a solution of methyl 4-butyrylbenzoate (8 g, 36.89 mmol, 1.00 equiv, 95%) in tetrahydrofuran (150 mL), pyridinium tribromide (19.2 g, 60.00 mmol, 1.50 equiv). The resulting solution was stirred for 2 h at 75°C

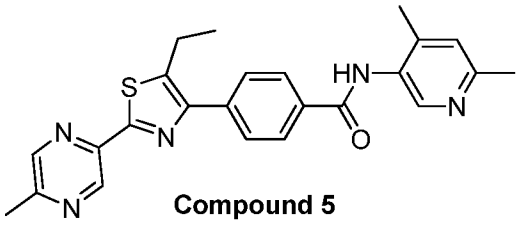
in an oil bath. The reaction was then quenched by the addition of 200 mL of water. The pH value of the solution was adjusted to 8~9 with saturated sodium bicarbonate. The resulting solution was extracted with 3x200 mL of ethyl acetate and the organic layers combined. The resulting mixture was washed with 3x200 mL of water and 3x200 mL of brine. The mixture was dried over anhydrous magnesium sulfate. This resulted in 10 g (95%) of methyl 4-(2-bromobutanoyl)benzoate as a red brown liquid.



[00111] The solution of methyl 4-(2-bromobutanoyl)benzoate (850 mg, 2.98 mmol) and 5-methylpyrazine-2-carbothioamide (500 mg, 3.28 mmol) in MeOH (15 mL) was heated in microwave at 120 °C for 60 min. The solution was concentrated and column chromatography (Hexanes/EtOAc=3/1)) gave methyl 4-(5-ethyl-2-(5-methylpyrazin-2-yl)thiazol-4-yl)benzoate (0.76 g, 2.24 mmol) in 75% yield.

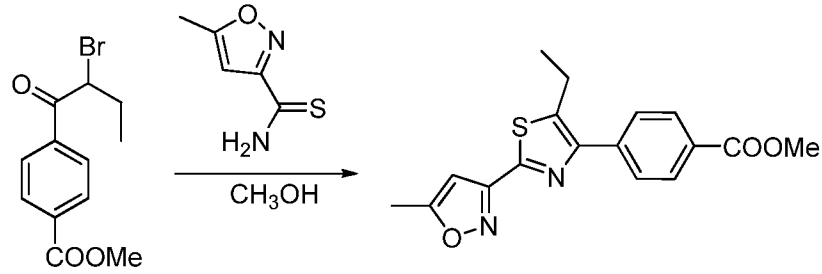


[00112] To the solution of 4-(5-ethyl-2-(5-methylpyrazin-2-yl)thiazol-4-yl)benzoate (75 mg, 0.22 mmol) in toluene (4 mL) was added 4,6-dimethylpyridin-3-amine (53 mg, 0.42 mmol) and $AlMe_3$ (2M solution in toluene, 0.21 mL, 0.42 mmol). The reaction was heated in microwave at 110 °C for 15 min before it was diluted with EtOAc (15 mL) and washed with 1N NaOH (25 mL × 2). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Column chromatography gave compound 5 in 81% yield.

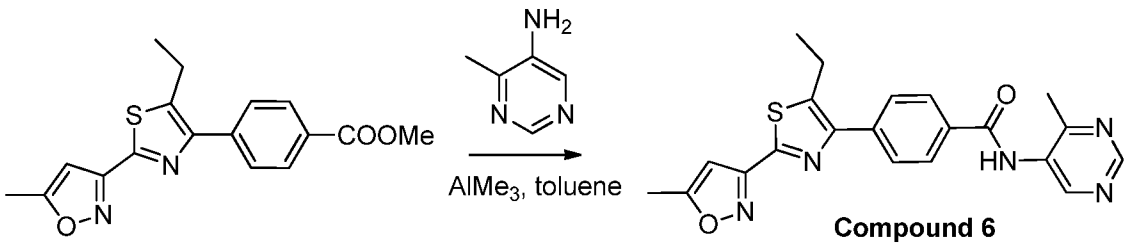


Compound 5

[00113] ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, *J* = 1.5 Hz, 1H), 8.75 (s, 1H), 8.43 (d, *J* = 1.5 Hz, 1H), 8.04 – 7.99 (m, 2H), 7.89 – 7.83 (m, 2H), 7.73 (s, 1H), 7.08 (s, 1H), 3.08 (q, *J* = 7.5 Hz, 2H), 2.64 (s, 3H), 2.54 (s, 3H), 2.32 (s, 3H), 1.43 (t, *J* = 7.4 Hz, 3H). ESMS cacld ($C_{24}H_{23}N_5OS$): 429.2; found: 430.3 (M+H).

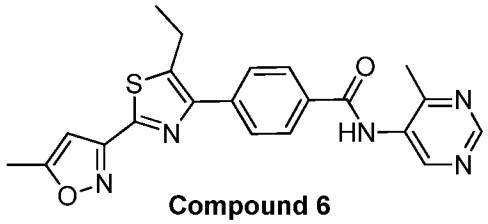


[00114] A solution of methyl 4-(2-bromobutanoyl)benzoate (850 mg, 2.98 mmol) and 5-methylisoxazole-3-carbothioamide (550 mg, 3.87 mmol) in MeOH (15 mL) was heated in microwave at 120 °C for 60 min. The solution was concentrated and column chromatography (Hexanes/EtOAc=3/1)) gave methyl 4-(5-ethyl-2-(5-methylisoxazol-3-yl)thiazol-4-yl)benzoate in 61% yield.



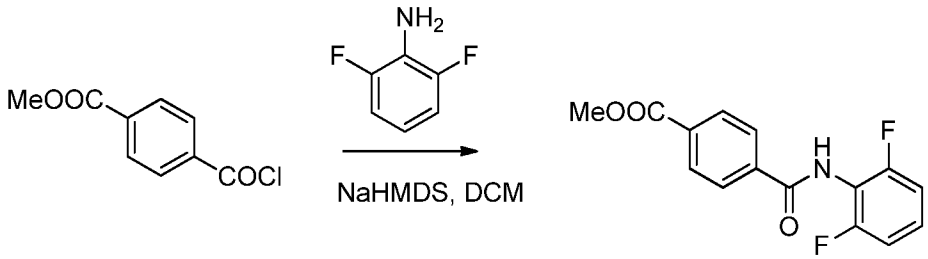
Compound 6

[00115] To the solution of methyl 4-(5-ethyl-2-(5-methylisoxazol-3-yl)thiazol-4-yl)benzoate (73 mg, 0.22 mmol) in toluene (4 mL) was added 4-methylpyrimidin-5-amine (46 mg, 0.42 mmol) and $AlMe_3$ (2M solution in toluene, 0.21 mL, 0.42 mmol). The reaction was heated in microwave at 110°C for 15 min before it was diluted with EtOAc (15 mL) and washed with 1N NaOH (25 mL × 2). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Column chromatography gave **Compound 6** in 73% yield.

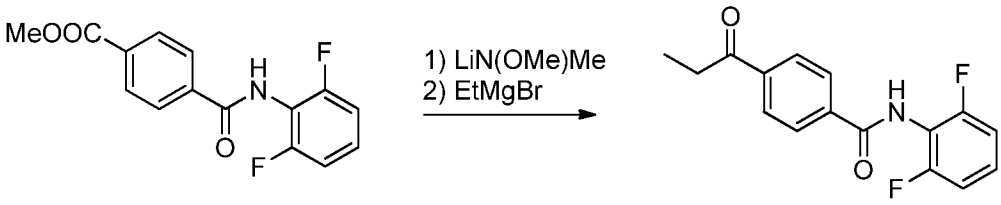


Compound 6

[00116] ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.96 (s, 1H), 8.03 – 7.96 (m, 2H), 7.87 – 7.81 (m, 2H), 7.70 (s, 1H), 6.60 (d, *J* = 1.1 Hz, 1H), 3.07 (q, *J* = 7.5 Hz, 2H), 2.61 (s, 3H), 2.52 (d, *J* = 1.0 Hz, 3H), 1.42 (t, *J* = 7.5 Hz, 3H). ESMS cacld ($C_{21}H_{19}N_5O_2S$): 405.1; found: 406.3 (M+H).

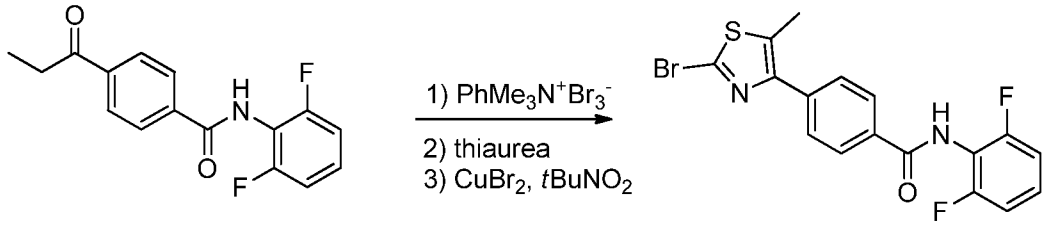


[00117] To a solution of methyl 4-(chlorocarbonyl)benzoate (1 g, 5 mmol) in DCM (50 mL) was added 2,6-difluoroaniline (1.3 g, 10 mmol) followed by NaHMDS (1 M, 10 mL, 10 mmol) at room temperature. The reaction was stirred at room temperature for 3 hr before it was quenched with NH_4Cl (sat. 50 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Column chromatography gave methyl 4-((2,6-difluorophenyl)carbamoyl)benzoate in 63% yield.

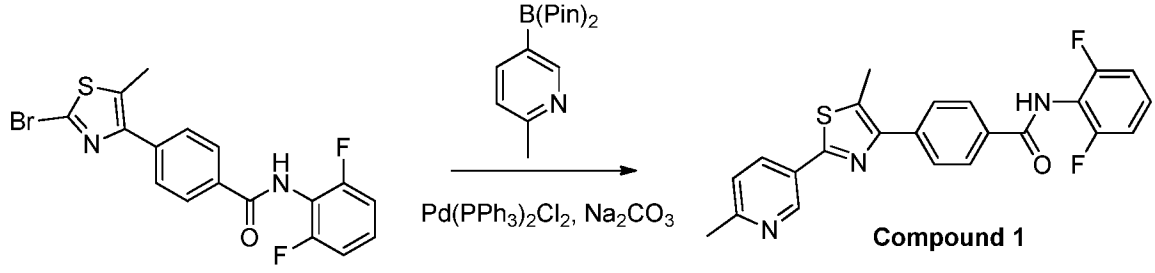


[00118] To a solution of LiN(OMe)Me.HCl (0.78 g, 8 mmol) in THF (50 mL) was added n-BuLi (1.6 M, 10 mL, 16 mmol) at -78°C. The cold bath was removed and the reaction was stirred for 30 min before it was cooled back to -78°C. To the reaction solution was added methyl 4-((2,6-difluorophenyl)carbamoyl)benzoate (0.6 g, 2 mmol) as solid. The reaction was stirred at -78°C for 60 min before it was quenched with H_2O (50 mL). The mixture was extracted with EtOAc (50 mL). The organic phase was dried over magnesium sulfate,

filtered, and concentrated to give the crude product. The resulting crude product was dissolved in THF (50 mL) and to the solution was added EtMgBr (3 M, 2.7 mL, 8.1 mmol) at -78°C. The reaction was slowly warmed up room temperature before it was quenched with H_2O (50 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Recrystallization (hexanes/EtOAc) gave *N*-(2,6-difluorophenyl)-4-propionylbenzamide as yellow solid in 75% yield.

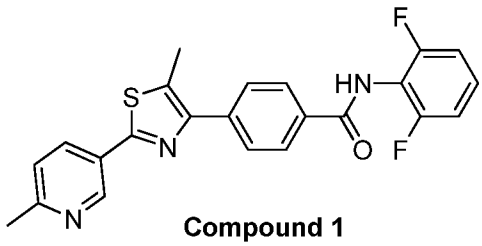


[00119] A solution of *N*-(2,6-difluorophenyl)-4-propionylbenzamide (0.4 g, 1.38 mmol) and $PhMe_3N^+Br_3^-$ (1 g, 2.66 mmol) in THF (80 mL) was heated at 60°C for 1 hr. The solution was diluted with H_2O (100 mL) and EtOAc (100 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated to give the crude product which was dissolved in EtOH (80 mL). To the resulting solution was added thiourea (0.1 g, 1.36 mmol) and heated at 60°C for 1 hr. The solution was diluted with 1 N NaOH (100 mL) and EtOAc (100 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated to give the crude product. To the solution of $CuBr_2$ (0.34 g, 1.52 mmol) in CH_3CN (20 mL) was added tBuNO$_2$ (0.23 mL, 1.94 mmol) at 0°C. After 5 min, the solution of above crude product in CH_3CN (20 mL) was added. The reaction mixture was stirred at room temperature for 60 min before it was quenched with sat. $NaHCO_3$ (50 mL) and EtOAc (50 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Column chromatography gave 4-(2-bromo-5-methylthiazol-4-yl)-N-(2,6-difluorophenyl)benzamide in 33% overall yield.



Compound 1

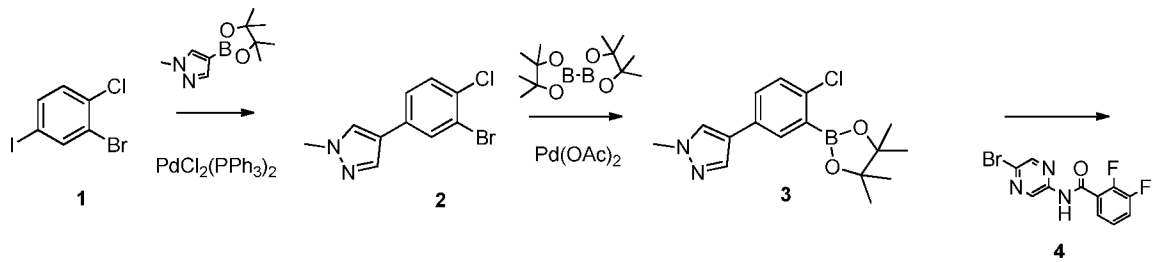
[00120] To a solution of 4-(2-bromo-5-methylthiazol-4-yl)-*N*-(2,6-difluorophenyl)benzamide (40 mg, 0.1 mmol), dichlorobis (triphenylphosphine)palladium (II) ($Pd(PPh_3)_2Cl_2$, 15 mg, 0.15 mmol), and 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (33 mg, 0.15 mmol) in THF (5 mL) was added Na_2CO_3 (2 N, 1.0 mL). The stirred mixture was heated up to 70°C for 16 hr. The solution was cooled to room temperature and diluted with H_2O (10 mL) and EtOAc (10 mL). The organic phase was dried over Na_2SO_4, concentrated, and chromatographied to give **Compound 1** in 65% yield.

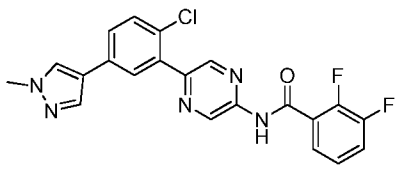


Compound 1

[00121] ^1H NMR (400 MHz, $CDCl_3$) δ 9.05 – 9.00 (m, 1H), 8.16 (dd, *J* = 8.1, 2.4 Hz, 1H), 8.04 (d, *J* = 8.3 Hz, 2H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.55 (s, 1H), 7.27 – 7.21 (m, 2H), 7.01 (t, *J* = 8.1 Hz, 2H), 2.67 (s, 3H), 2.62 (s, 3H). ESMS cacld ($C_{23}H_{17}F_2N_3OS$): 421.1; found: 422.2 (M+H).

[00122] Procedure for the syntheses of Compounds 7 and 8:





Compound 7

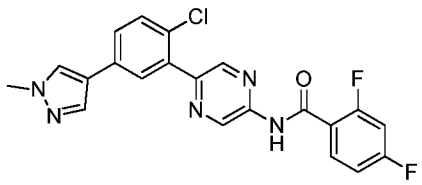
[00123] Compound 7 (N-(5-(2-chloro-5-(1-methyl-1H-pyrazol-4-yl)phenyl)pyrazin-2-yl)-2,3-difluorobenzamide):

[00124] A mixture of 2-bromo-1-chloro-4-iodo benzene (10 mmol), 1-methyl-4-tetramethyldioxaborolanyl-pyrazole (10 mmol), bis(triphenylphosphine)-palladium(II)dichloride (0.4 mmol), and K_2CO_3 (10 mmol) in dioxane/water (10:1, 20 ml) was heated in microwave at 90°C for 2 hr., the reaction mixture was poured over water, the crude product was extracted with DCM, and purified with silica gel column to give pure 4-(3-bromo-4-chlorophenyl)-1-methyl-1H-pyrazole (2.1g).

[00125] A suspension solution of 4-(3-bromo-4-chlorophenyl)-1-methyl-1H-pyrazole (5 mmol), bis(pinacolato)diboron(6.25 mmol), KOAc (2 mmol), and $Pd(OAc)_2$ (1 mmol) in DMF (15ml) was heated at 86 °C for 3h, and the reaction was quenched with water, the mixture was extracted with DCM. The organic layer was filtered with a small silica gel funnel and eluted with EtOAc to get the crude 4-(4-chloro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1-methyl-1H-pyrazole (yield ~60-80%), which was used directly for the next step

[00126] A mixture of 4-(4-chloro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1-methyl-1H-pyrazole (2 mmol), N-(5-bromopyrazin-2-yl)-2,3-difluorobenzamide (2 mmol), bis(triphenylphosphine)-palladium(II)dichloride (0.1mmol), and K_2CO_3 (2 mmol) in dioxane/water (10:1, 20 ml) was heated in microwave at 100 °C for 2 hr., the reaction mixture was poured over water, the crude product was extracted with DCM, and purified with silica gel column to give pure Compound 7 (420mg) ^{1}H NMR($CDCl_3$) δ 9.8 (d, 1H, J=1.5), 9.0 (d, 1H, J=12.5), 8.8 (d, 1H, 1.5), 7.95 (m, 1H), 7.79 (s, 1H), 7.75 (br. 1H), 7.67 (s, 1H), 7.5-727 (m, 4H), 3.96 (3, 3H). ESMS calcd for $C_{21}H_{14}ClF_2N_5O$: 425.1; found: 426.1 ($M+H^+$)

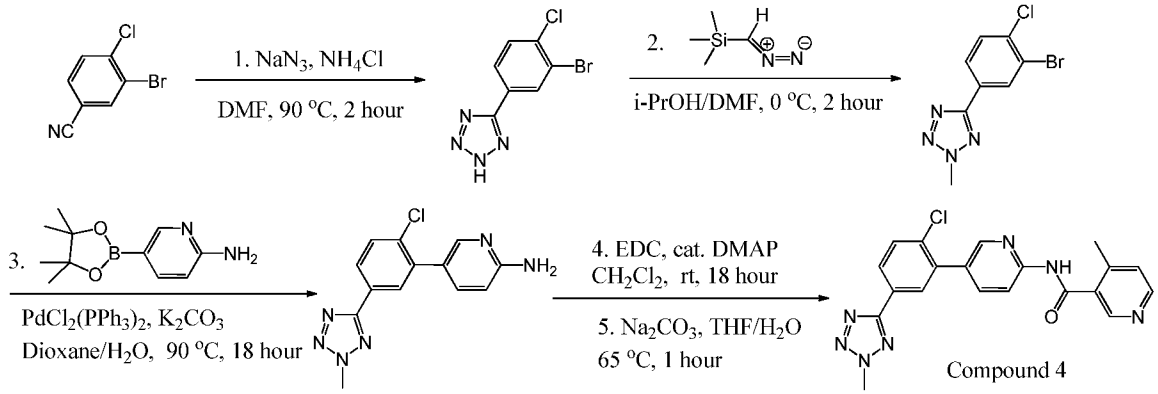
[00127] N-(5-bromopyrazin-2-yl)-2,3-difluorobenzamide was formed by 2-amino-5-bromopyrazine (10 mmol) and 2,3-difluorobenzoyl chloride (30 mmol) and DMAP (30 mmol) in DCM at room temperature for 5 hr (85%).



[00128] Compound 8 (N-(5-(2-chloro-5-(1-methyl-1H-pyrazol-4-yl)phenyl)pyrazin-2-yl)-2,4-difluorobenzamide) was prepared using a similar procedure to Compound 7.

[00129] ^{1}H NMR($CDCl_3$) ^{1}H NMR($CDCl_3$) δ 9.8 (d, 1H, J=1.5), 9.1 (d, 1H, J=15), 8.8 (d, 1H, 1.5), 8.3 (m, 1H), 7.8-6.97 (m, 7H), 3.96 (3, 3H) ESMS calcd for $C_{21}H_{14}ClF_2N_5O$: 425.1; found: 426.1 ($M+H^+$)

[00130] Procedure for Compound 4 synthesis:



[00131] One-pot reaction for step 1 & 2

[00132] 3-Bromo-4-chlorobenzonitrile (1.08 g, 5.00 mmol, 1.0 equiv), sodium azide (1.95 g, 30.0 mmol, 6.0 equiv), ammonium chloride (1.60 g, 30.0 mmol, 6.0 equiv) and 15 mL DMF were mixed in a 50 mL round bottom flask. The mixture was placed in 90°C oil bath, and stirred for 2 hours. The reaction was cooled to room temperature, and diluted with 50 mL EtOAc to crash out inorganic solids. Solids were removed by filtration, and solution was concentrated on rotary evaporator to remove EtOAc. The clear DMF solution was diluted with 20 mL i-PrOH, and treated with (trimethylsilyl)diazomethane (5 mL 2M in hexane, 10.00 mmol, 2.0 equiv.) at 0°C for 2 hours. The crude product obtained from routine EtOAc-aqueous workup was purified by flash chromatography, and yielded 5-(3-bromo-4-chlorophenyl)-2H-tetrazole as white solid (less polar spot, major, 0.74 g, 2.70 mmol, 54%). The more polar spot was region-isomer (minor).

[00133] Step 3

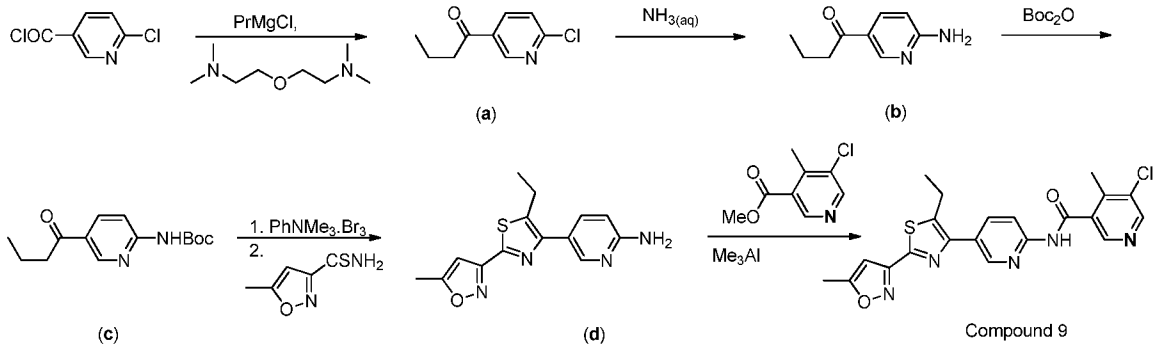
[00134] 5-(3-Bromo-4-chlorophenyl)-2H-tetrazole (0.41 g, 1.50 mmol, 1.00 equiv.), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (0.363 g, 1.65 mmol, 1.10 equiv.), bis(triphenylphosphine)palladium(II) dichloride (0.042 g, 0.06 mmol, 0.04 eq), potassium carbonate (0.41 g, 3.00 mmol, 2.0 eq), dioxane (7 mL), and water (3 mL) were

mixed in a 20 mL sealed tube. The mixture was subjected to three cycles of vacuum/nitrogen gas treatment, and then placed and stirred in 90 °C oil bath for 18 hours. Routing EtOAc-aqueous workup and flash chromatography purification gave 5-(2-chloro-5-(2-methyl-2H-tetrazol-5-yl)phenyl)pyridin-2-amine as off-white solid (0.43 g, 1.50 mmol, 100%).

[00135] Step 4 and 5, one-pot reaction

[00136] 5-(2-chloro-5-(2-methyl-2H-tetrazol-5-yl)phenyl)pyridin-2-amine (0.0.086 g, 0.30 mmol, 1.00 equiv.), 4-methylnicotinic acid (0.082 g, 0.60 mmol, 2.00 equiv.), EDC (0.116 g, 0.60 mmol, 2.00 equiv.), and DMAP (0.018 g, 0.15 mmol, 0.50 equiv.) were placed in 25 mL flask. CH_2Cl_2 (10 mL) was added, and the mixture was stirred at room temperature for 18 hours. Solvent was removed, and the residue was treated with 10 mL THF and 5 mL 5% Na_2CO_3 solution at 65 °C for 1 hour to hydrolyze double-acylated amide to desired product. Routine workup and flash chromatography purification gave N-(5-(2-chloro-5-(2-methyl-2H-tetrazol-5-yl)phenyl)pyridin-2-yl)-4-methylnicotinamide as off-white solid (0.043 g, 0.11 mmol, 35%). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm):11.25 (s, 1H); 8.67 (s, 1H); 8.52-8.55 (m, 2H); 8.35 (d, *J* = 6.3 Hz, 1H); 8.05-8.12 (m, 3H); 7.83 (d, *J* = 6.3 Hz, 1H); 7.36 (d, *J* = 3.9 Hz, 1H); 4.45 (s, 3H); 2.45 (s, 3H). ESMS calcd. for $C_{20}H_{17}ClN_7O$ $(M + H)^+$: 406; Found: 406.

[00137] Representative procedure of Compound 9, 5-cyano-N-(5-(5-ethyl-2-(5-methylisoxazol-3-yl)thiazol-4-yl)pyridin-2-yl)-4-methylnicotinamide:



[00138] A mixture of propyl-magnesium chloride (42 mmol, 21 mL x 2.0 M in THF) and 2,2'-oxybis(N,N-dimethylethanamine) (42 mmol) in THF (40 mL) was cooled to 0°C and 6-chloronicotinoyl chloride (28 mmol) was added in one portion and the mixture was kept at

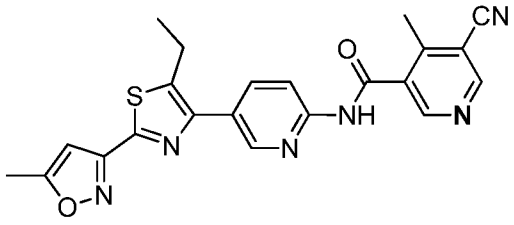
0°C for 40 min. The mixture was poured over water (200 mL) and extracted with DCM (200 mL). The organic layer was dried and concentrated to give 1-(6-chloropyridin-3-yl)butan-1-one (**a**, 5.1 g) as white crude product.

[00139] 3.5 g of the above ketone **a** was placed in a microwave reactor and 20 mL of NH_3 (aq, conc.) was added and the reactor was sealed and heated at 140°C for 2 h. The reaction mixture was poured over water (50 mL), filtered and rinsed with EtOH/water (1:1) to give 1-(6-aminopyridin-3-yl)butan-1-one (**b**, 3.5 g) as white crude product.

[00140] The above amine **b** (2.0 g) was dissolved in THF (50 mL) and Boc_2 (1.5 eq) and DMAP (0.1 eq) was added and the mixture was stirred at rt for 2 h. The mixture was concentrated, distributed in DCM/water (50 mL each) and DCM layer passed through a plug of silica gel to give a crude product (2.4g) which was triturated in EA/Hexanes (10 mL/ 40 mL) to give tert-butyl (5-butyrylpyridin-2-yl)carbamate (**c**, 1.5 g) as pure white powder.

[00141] 5.0 g of **c** was treated with tribromide (20.0 mmoL) in boiling THF (80 mL) at 80°C for 4h. The mixture was cooled down and pass through a plug of silica gel and eluted with EA/hexanes (1:1) to give crude bromide intermediate as orange solids (6.0 g) which was heated with 5-methylisoxazole-3-carbothioamide (16 mmol) in EtOH (100 mL)/ AcOH (1 mL) for 6 h. The reaction mixture was concentrated, neutralized with $NaHCO_3$ and extracted with DCM. Organic layer passed through silica gel plug and eluted from MeOH/DCM (1:9) followed by recrystallization of the resulted crude product gave 5-(5-ethyl-2-(5-methylisoxazol-3-yl)thiazol-4-yl)pyridin-2-amine (**d**, 2.3 g) as white solids.

[00142] To a mixture of 80 mg of the above amine **d** and methyl 5-chloro-4-methylnicotinate (50 mg) in toluene (5 mL) was added a solution of Me_3Al (0.8 mmol, 0.4 mL x 2.0 M) and the resulting solution was heated at 110°C for 2 h. The mixture was diluted with DCM (5 mL)/ 1N NaOH (5 mL) and organic layer was purified by column to give Compound 9 as white solids (10 mg). ^{1}H-NMR ($CDCl_3$) δ 8.91 (s, 1H), 8.6 (m, 2H), 8.4 (m, 2H), 8.1 (dd, 1H, J_1=8, J_2=2), 6.61 (s, 1H), 3.0 (q, 2H, J=8), 2.57 (s, 3H), 2.52 (s, 3H), 1.4 (t, 3H, J=8) ppm; ESMS calcd for $C_{21}H_{18}ClN_5O_2S$: 439.0; found: 440.4 ($M + H^+$).

[00143] Compound 10, 5-cyano-N-(5-(5-ethyl-2-(5-methylisoxazol-3-yl)thiazol-4-yl)pyridin-2-yl)-4-methylnicotinamide  , was prepared using a similar procedure to Compound 9.

[00145] ^{1}H-NMR ($CDCl_3$) δ 8.94 (s, 1H), 8.91 (s, 1H), 8.6 (d, 1H, J=2), 8.4 (m, 2H), 8.1 (dd, 1H, J_1=8, J_2=2), 6.61 (s, 1H), 3.0 (q, 2H, J=8), 2.78 (s, 3H), 2.52 (s, 3H), 1.4 (t, 3H, J=8) ppm; ESMS calcd for $C_{22}H_{18}N_6O_2S$: 430.1; found: 431.4 (M + H^+).

EXAMPLE 2: INHIBITION OF IL-2 PRODUCTION

[00146] JurkaT-cells were placed in a 96 well plate (0.5 million cells per well in 1% FBS medium), and then a test compound of this invention was added at different concentrations. After 10 minutes, the cells were activated with PHA (final concentration 2.5 μg/mL) and incubated for 20 hours at 37 °C under 5% CO_2. The final volume was 200 μL. Following incubation, the cells were centrifuged, and the supernatants collected and stored at -70 °C prior to assaying for IL-2 production. A commercial ELISA kit (IL-2 Eli-pair, Diaclone Research, Besancon, France) was used to detect production of IL-2, from which dose response curves were obtained. The IC_{50} value was calculated as the concentration at which 50% of maximum IL-2 production after stimulation was inhibited versus a non-stimulation control.

[00147] Inhibition of other cytokines, such as IL-4, IL-5, IL-13, GM-CSF, TNFα, and IFN-γ, can be tested in a similar manner using a commercially available ELISA kit for each cytokine.

Compound No.	IL-2 inhibition Jurkat/PHA/1%FBS IC50 (nM)	I_{CRAC} current inhibition RBL-2H3 Cells Qpatch at 100n M
1	27	80
2	33	61
3	46	36

4	18	36
5	32	40
6	38	93*
7	10	98
8	14	82
9	10	73
10	13	NA

EXAMPLE 3: MANUAL PATCH CLAMP STUDIES OF INHIBITION OF I_{CRAC} CURRENT IN RBL CELLS, JURKA T-CELLS, AND PRIMARY T-CELLS

[00148] In general, a whole cell patch clamp method is used to examine the effects of a compound of the invention on a channel(s) that mediates I_{CRAC}. In such experiments, a baseline I_{CRAC} measurement is established within the first 70 voltage ramps, or 140 seconds, for a patched cell. Then the cells are perfused with the compound to be tested and the effect of the compound on I_{CRAC} is measured for at least an additional 440 to 500 seconds. A compound that modulates I_{CRAC} (*e.g.*, inhibits) is a compound that is useful in the invention for modulating CRAC ion channel activity.

1. <u>RBL and JurkaT-cells</u>

Cells

[00149] Rat basophilic leukemia cells (RBL-2H3) are grown in DMEM media supplemented with 10% fetal bovine serum in an atmosphere of 95% air/5% CO_2. Cells are seeded on glass coverslips 1-3 days before use.

[00150] Jurkat T-cells are grown in RPMI media supplemented with 10% fetal bovine serum in an atmosphere of 95% air/5% CO_2. Cells are harvested by centrifugation and transferred to a recording chamber just prior to each experiment.

Recording Conditions

[00151] Membrane currents of individual cells are recorded using the manual patch clamp technique in the whole-cell configuration.

Intracellular pipette solution

[00152] The intracellular pipette solution contains Cs-Glutamate 100 mM; CsCl 20 mM; NaCl 8 mM; $MgCl_2$ 3 mM; D-*myo*-Inositol 1,4,5-trisphosphate (InsP3) 0.02 mM; CsBAPTA 10 mM; HEPES 10 mM; pH=7.2 adjusted with CsOH. The solution is kept on ice and shielded from light before the experiments are preformed.

Extracellular solution

[00153] The extracellular solution contains NaCl 140 mM; KCl 5.4 mM; CsCl 10 mM; $CaCl_2$ 10 mM; $MgCl_2$ 1 mM; HEPES 10 mM; Glucose 5.5 mM; at pH=7.4 adjusted with NaOH.

Compound treatment

[00154] Each compound is diluted from a 10 mM stock in series using DMSO. The final DMSO concentration is always kept at 0.1 %.

Experimental procedure

[00155] I_{CRAC} currents are measured using 50 msec voltage ramps between -100 mV to +100 mV. The voltage ramps are stimulated every 2 seconds for the first 70 sweeps, then every 5 seconds for the remainder of the experiment. The membrane potential is held at 0 mV between the test ramps. In a typical experiment, the peak inward currents will develop within 50-100 seconds. Once the I_{CRAC} current is stabilized, the cells are perfused with a test compound in the extracellular solution for at least an additional 500 seconds.

Data analysis

[00156] Off-line analysis with the Heka PatchMaster software is used to separate the I_{CRAC} membrane current from the cells basal background currents. In a typical recording, InsP3 stimulated I_{CRAC} currents begin to develop in 6 to 12 seconds after whole cell is established. Therefore, the first 1-4 voltage ramps represent the basal membrane currents in the absence of I_{CRAC} and the average value is subtracted from all subsequent traces. The current value at -80 mV for each ramp trace is then measured and plotted against time. The resulting current versus time data is exported into a Microsoft Excel spreadsheet. The % I_{CRAC} inhibition in each cell is calculated by comparing the amount of current just prior to the compound perfusion to the amount of current after the cells has been perfused with the

compound for 440-500 seconds. The IC_{50} value and Hill coefficient for each compound is estimated by fitting all the individual data points to a single-site Hill equation.

2. Primary T-cells

Preparation of Primary T-cells

[00157] Primary T-cells are obtained from human whole blood samples by adding 100μL of RosetteSep® human T-cell enrichment cocktail to 2 mL of whole blood. The mixture is incubated for 20 minutes at room temperature, then diluted with an equal volume of PBS containing 2% FBS. The mixture is layered on top of RosetteSep® DM-L density medium and then centrifuged for 20 minutes at 1200 g at room temperature. The enriched T-cells are recovered from the plasma/density medium interface, then washed with PBS containing 2% FBS twice, and used in patch clamp experiments following the procedure described for RBL cells.

EXAMPLE 4: AUTOMATED PATCH CLAMP STUDIES OF INHIBITION OF I_{CRAC}

RBL-2H3 Cells.

Cells

[00158] RBL-2H3 are grown in DMEM media supplemented with 10% fetal bovine serum, penicillin 100 U/ml and streptomycin 100 g/ml in an atmosphere of 95% air/5% CO_2. Cells are grown to confluence in 175 cm^2 tissue culture flask. On the experimental day, cells harvested with 0.25% trypsin/0.02% EDTA and resuspended in extracelllular solution at density of $5x10^6$ cells/ml.

Intracellular Solution

[00159] The intracellular solution contains Cs-Glutamate 90 mM; NaCl 8 mM; $MgCl_2$ 3 mM; CsCl 20 mM; CsBAPTA 20 mM; HEPES 10 mM; InsP3 0.02mM; pH=7.2 adjusted with CsOH.

Extracellular Solution

[00160] The extracellular solution contains NMDGCl 120 mM; KCl 5.4 mM; CsCl 10 mM; $CaCl_2$ 10 mM; $MgCl_2$ 1 mM; HEPES 10 mM; Glucose 5.5 mM; at pH=7.4 adjusted with HCl.

Experimental Procedure

[00161] I_{CRAC} currents are measured using 50 msec voltage ramps between -100 mV to +100 mV. The voltage ramps are stimulated every 3 seconds for at least 570 seconds. The maximum I_{CRAC} current is allowed to develop for at least 135 seconds. Compounds diluted in extracellular solutions are then applied twice, 30 seconds apart. After incubating the cells with compound for 435 seconds, a reference solution is applied at the end of the experiment. The reference solution is a Ca^{2+} free extracellular solution.

Data Analysis

[00162] Off-line analysis with the Qpatch software is used to plot the current value at -80 mV for each ramp trace against time. The resulting current versus time data is then exported into a Microsoft Excel spreadsheet. The I_{CRAC} membrane currents are separated from the cells basal background currents by either subtracting out the average membrane current values during the first 1-3 traces, or the average membrane current values obtained with the reference solution at the end of the experiment. The % I_{CRAC} inhibition in each cell is calculated by comparing the amount of current just prior to the first compound addition to the amount of current after the cells has been perfused with the compound for at least 400 seconds.

EXAMPLE 5: INHIBITION OF MULTIPLE CYTOKINES IN PRIMARY HUMAN PBMCs

[00163] Human peripheral blood mononuclear cells (PBMCs) were prepared from heparinized human blood by separation over a Ficoll density gradient.

[00164] PBMCs are stimulated with phytohemagglutinin (PHA) in the presence of varying concentrations of compounds of the invention or cyclosporine A (CsA), a known inhibitor of cytokine production. Cytokine production is measured using commercially available human ELISA assay kits (from Cell Science, Inc.) following the manufacturers instructions.

[00165] Alternatively, PBMCs with 10% FCS at 1-2 x 10^6/ml are stimulated with pre-coated with anti-CD3 (clone UCHT1) and anti-CD28 (clone ANC28.1/5D10) at 5 μg/ml each, with or without compound or DMSO (maximun concentration: 0.1%). Cell cultures are

incubated at 37 °C, 5% CO_2. Samples of the culture supernatant are collected after 48-72 hrs. incubation for measurement of multiple cytokines. Cytokines present in the supernatants are quantified using BioRad BioPlex assays according to the manufacturer's instructions.

[00166] The compounds of the invention are expected to be potent inhibitors of IL-2, IL-4, IL-5, IL-13, GM-CSF, IFN-alpha, and TNF- alpha in primary human PBM cells. In addition, compounds of the invention are not expected to inhibit the anti-inflammatory cytokine, IL-10.

EXAMPLE 6: INHIBITION OF DEGRANULATION IN RBL CELLS

Procedure:

[00167] The day before the assay is performed, RBL cells, that have been grown to confluence in a 96 well plate, are incubated at 37 °C for at least 2 hours. The medium is replaced in each well with 100 μL of fresh medium containing 2 μLg/mL of anti-DNP IgE.

[00168] On the following day, the cells are washed once with PRS (2.6 mM glucose and 0.1% BSA) and 160 μL of PRS is added to each well. A test compound is added to a well in a 20 μL solution at 10x of the desired concentration and incubated for 20 to 40 minutes at 37 °C. 20 μL of 10x mouse anti-IgE (10 μL/mL) is added. Maximum degranulation occurs between 15 to 40 minutes after addition of anti-IgE.

[00169] Compounds of the invention are expected to inhibit degranulation.

EXAMPLE 7: INHIBITION OF CHEMOTAXIS IN T-CELLS

T-cell isolation:

[00170] Twenty ml aliquots of heparinized whole blood (2 pig, 1 human) are subjected to density gradient centrifugation on Ficoll Hypaque. The buffy coat layers representing peripheral blood mononuclear cells (PBMCs) containing lymphocytes and monocytes are washed once, resuspended in 12 ml of incomplete RPMI 1640 and then placed in gelatin-coated T75 culture flasks for 1 hr at 37 °C. The non-adherenT-cells, representing peripheral blood lymphocytes (PBLs) depleted of monocytes, are resuspended in complete RPMI media and placed in loosely packed activated nylon wool columns that have been equilibrated with

warm media. After 1 hr at 37 °C, the non-adherent T-cell populations are eluted by washing of the columns with additional media. The T-cell preparations are centrifuged, resuspended in 5 ml of incomplete RPMI, and counted using a hemocytometer.

Cell migration assay:

[00171] Aliquots of each T-cell preparation are labeled with Calcien AM (TefLabs) and suspended at a concentration of 2.4 x10^6/ml in HEPES-buffered Hank's Balanced Salt Solution containing 1.83 mM $CaCl_2$ and 0.8 mM $MgCl_2$, pH 7.4 (HHBSS). An equal volume of HHBSS containing 0, 20 nM, 200 nM or 2000 nM of compound 1 or 20 nM EDTA is then added and the cells incubated for 30 min at 37 °C. Fifty μl aliquots of the cell suspensions (60,000 cells) are placed on the membrane (pore size 5 μm) of a Neuroprobe ChemoTx 96 well chemotaxis unit that have been affixed over wells containing 10 ng/ml MIP-1α in HHBSS. The T-cells are allowed to migrate for 2 hr at 37 °C, after which the apical surface of the membrane is wiped clean of cells. The chemotaxis units are then placed in a CytoFluor 4000 (PerSeptive BioSystems) and the fluorescence of each well measured (excitation and emission wavelengths of 450 and 530 nm, respectively). The number of migrating cells in each well is determined from a standard curve generated from measuring the fluorescence of serial two-fold dilutions of the labeled cells placed in the lower wells of the chemotaxis unit prior to affixing the membrane.

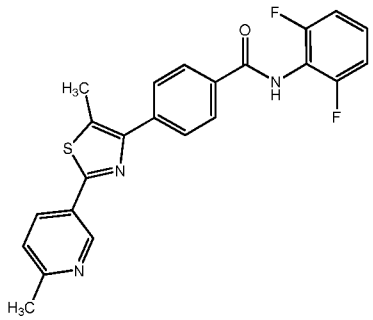
[00172] Compounds of the invention are expected to inhibit chemotactic response of T-cells.

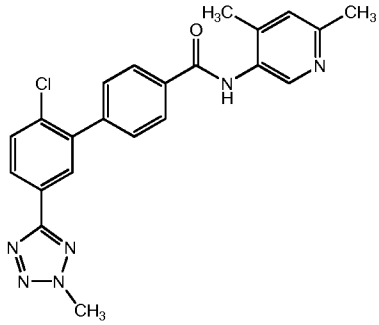
[00173] All publications, patent applications, patents, and other documents cited herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting in any way.

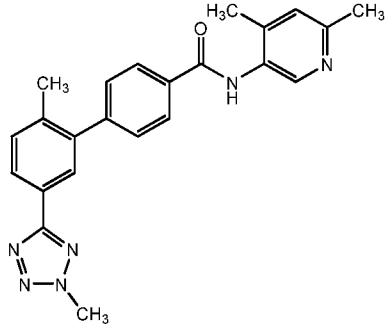
CLAIMS

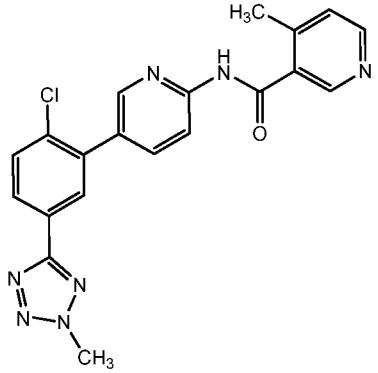
What is claimed is:

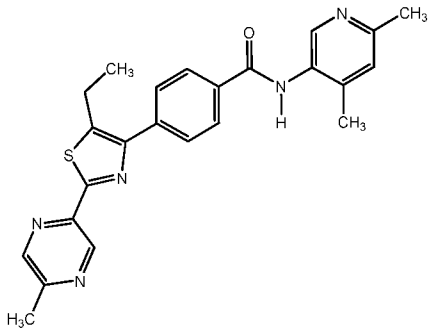
1. A compound selected from the group consisting of:

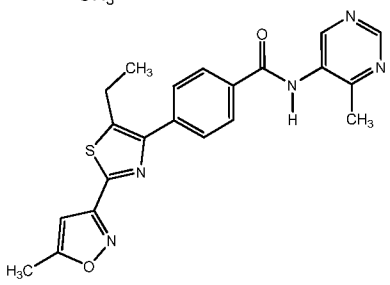


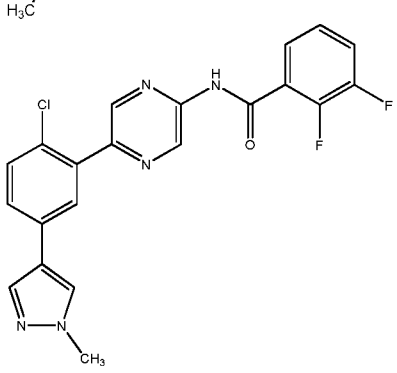


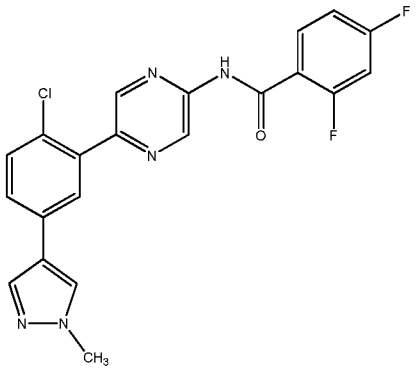


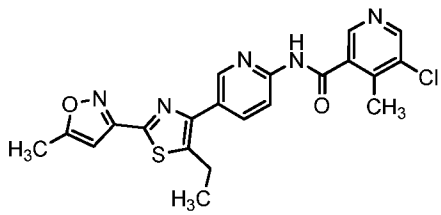


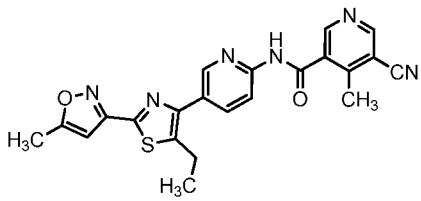












;

or pharmaceutically acceptable salts thereof.

2. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a compound of claim 1.

3. The pharmaceutical composition of claim 2, comprising a pharmaceutically acceptable carrier and a compound of claim 1.

4. The pharmaceutical composition of claims 2 or 3, further comprising one or more additional therapeutic agents selected from the group consisting of immunosuppressive agents, anti-inflammatory agents, steroids, non-steroidal anti-inflammatory agents, antihistamines, analgesics, and suitable mixtures thereof.

5. A method of inhibiting immune cell activation comprising administering to an immune cell a compound of claim 1.

6. The method of claim 5, comprising administering to an immune cell a compound of claim 1.

7. A method of inhibiting cytokine production in a cell, comprising administering to the cell a compound of claim 1.

8. The method of claim 7, comprising administering to the cell a compound of claim 26.

9. The method of claims 7 or 8, wherein the cytokine is selected from the group consisting of IL-2, IL-4, IL-5, IL-13, GM-CSF, IFN-γ, TNFα, and combinations thereof.

10. A method of modulating an ion channel in a cell, wherein the ion channel is involved in immune cell activation, comprising administering to the cell a compound of claim 1.

11. The method of claim 10, comprising administering to the cell a compound of claim 26.

12. The method of claims 10 or 11, wherein the ion channel is a Ca^{2+}-release-activated Ca^{2+} channel (CRAC).

13. A method of inhibiting T-cell and/or B-cell proliferation in response to an antigen, comprising administering to a T-cell and/or B-cell cell a compound of claim 1.

14. The method of claim 13, comprising administering to a T-cell and/or B-cell cell a compound of claim 1.

15. A method for treating or preventing an immune disorder in a subject in need thereof, comprising administering to the subject an effective amount of a compound of claim 1.

16. The method of claim 15, comprising administering to the subject an effective amount of a compound of claim 1.

17. The method of claims 15 or 16, wherein the disorder is selected from the group consisting of multiple sclerosis, myasthenia gravis, Guillain-Barré, autoimmune uveitis, autoimmune hemolytic anemia, pernicious anemia, autoimmune thrombocytopenia, temporal arteritis, anti-phospholipid syndrome, vasculitides such as Wegener's granulomatosis, Behcet's disease, psoriasis, dermatitis herpetiformis, pemphigus vulgaris, vitiligo, Crohn's disease, ulcerative colitis, primary biliary cirrhosis, autoimmune hepatitis, Type 1 or immune-mediated diabetes mellitus, Grave's disease. Hashimoto's thyroiditis, autoimmune oophoritis and orchitis, autoimmune disorder of the adrenal gland, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, polymyositis,

dermatomyositis, ankylosing spondylitis, and Sjogren's syndrome.

18. A method for treating or preventing an inflammatory condition in a subject in need thereof, comprising administering to the subject an effective amount of a compound of claim 1.

19. The method of claim 18, comprising administering to the subject an effective amount of a compound of claim 1.

20. The method of claims 18 or 19, wherein the disorder is selected from transplant rejection, skin graft rejection, arthritis, rheumatoid arthritis, osteoarthritis and bone diseases associated with increased bone resorption; inflammatory bowel disease, ileitis, ulcerative colitis, Barrett's syndrome, Crohn's disease; asthma, adult respiratory distress syndrome, chronic obstructive airway disease; corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis, endophthalmitis; gingivitis, periodontitis; tuberculosis; leprosy; uremic complications, glomerulonephritis, nephrosis; sclerodermatitis, psoriasis, eczema; chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related neurodegeneration, Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis viral or autoimmune encephalitis; autoimmune disorders, immune-complex vasculitis, systemic lupus and erythematodes; systemic lupus erythematosus (SLE); cardiomyopathy, ischemic heart disease hypercholesterolemia, atherosclerosis, preeclampsia; chronic liver failure, brain and spinal cord trauma, and cancer.

21. A method for suppressing the immune system of a subject in need thereof, comprising administering to the subject an effective amount of a compound of any one of claim 1.

22. The method of claim 21, comprising administering to the subject an effective amount of a compound of claim 1.

23. A method for treating or preventing an allergic disorder in a subject in need thereof, comprising administering to the subject an effective amount of a compound of claim 1.

24. The method of claim 23, comprising administering to the subject an effective amount of a compound of claim 1.

25. The method of claims 23 or 24, wherein the disorder is allergic rhinitis, sinusitis, rhinosinusitis, chronic otitis media, recurrent otitis media, drug reactions, insect sting reactions, latex reactions, conjunctivitis, urticaria, anaphylaxis reactions, anaphylactoid reactions, atopic dermatitis, asthma, or food allergies.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/62108

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/506 (2012.01)
USPC - 514/214.02
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 514/214.02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/214.02, 514/275, 514/320, 514/322, 514/352; 544/297; 546/312

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
*** Databases: WEST (PGPB, USPT, USOC, EPAB, JPAB); Google, Google Scholar
*** Search Terms Used: Chen, Zhang, Jiang, Kowalczyk, Xia, Zhang, CRAC ion channel, T-Cell, inflammation, interleukin 2, IL-2, GM-CSF, allergic disorder,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0130522 A1 (JIANG et al.) (27 May 2010 (27.05.2010), especially para [0012]-[0015], [0017]-[0018], [0020]-[0021], [0025], [0028], [0032]-[0036], [0040], [0053], [0055],	1-2, 5, 7, 10, 13, 15, 18, 21 and 23
A	US 2011/0112058 A1 (Muthuppalaniappan et al.) 12 May 2011 (12.05.2011), entire document	1-2, 5, 7, 10, 13, 15, 18, 21 and 23
A	US 2011/0015184 A1 (BOHNERT et al.) 20 January 2011 (20.01.2011), entire document	1-2, 5, 7, 10, 13, 15, 18, 21 and 23
A	US 2010/0249195 A1 (JIANG et al.) 30 September 2010 (30.09.2010), entire document	1-2, 5, 7, 10, 13, 15, 18, 21 and 23

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 November 2012 (29.11.2012)	28 DEC 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/62108

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 3-4, 6, 8-9, 11-12, 14, 16-17, 19-20, 22, and 24-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.