SALTS OF THE JANUS KINASE INHIBITOR (R)-3-(4-(7H-PYRROLO[2,3-d]PYRIMIDIN-4-YL)-1H-PYRAZOL-1-YL)-3-CYCLOPENTYLPROPANENITRILE

FIELD OF THE INVENTION

The present invention provides salt forms of (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile that are useful in the modulation of Janus kinase activity and are useful in the treatment of diseases related to activity of Janus kinases including, for example, immune-related diseases, skin disorders, myeloid proliferative disorders, cancer, and other diseases.

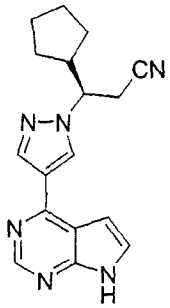
BACKGROUND OF THE INVENTION

Protein kinases (PKs) are a group of enzymes that regulate diverse, important biological processes including cell growth, survival and differentiation, organ formation and morphogenesis, neovascularization, tissue repair and regeneration, among others. Protein kinases exert their physiological functions through catalyzing the phosphorylation of proteins (or substrates) and thereby modulating the cellular activities of the substrates in various biological contexts. In addition to the functions in normal tissues/organs, many protein kinases also play more specialized roles in a host of human diseases including cancer. A subset of protein kinases (also referred to as oncogenic protein kinases), when dysregulated, can cause tumor formation and growth, and further contribute to tumor maintenance and progression (Blume-Jensen P et al, Nature 2001, 411(6835):355-365). Thus far, oncogenic protein kinases represent one of the largest and most attractive groups of protein targets for cancer intervention and drug development.

The Janus Kinase (JAK) family plays a role in the cytokine-dependent regulation of proliferation and function of cells involved in immune response. Currently, there are four known mammalian JAK family members: JAK1 (also known as Janus kinase-1), JAK2 (also known as Janus kinase-2), JAK3 (also known as Janus kinase, leukocyte; JAKL; L-JAK and Janus kinase-3) and TYK2 (also known as protein-tyrosine kinase 2). The JAK proteins range in size from 120 to 140 kDa and comprise seven conserved JAK homology (JH) domains; one of these is a functional catalytic kinase domain, and another is a pseudokinase domain

potentially serving a regulatory function and/or serving as a docking site for STATs (Scott, Godshall *et al.* 2002, *supra*).

Blocking signal transduction at the level of the JAK kinases holds promise for developing treatments for human cancers. Inhibition of the JAK kinases is also envisioned to have therapeutic benefits in patients suffering from skin immune disorders such as psoriasis, and skin sensitization. Accordingly, inhibitors of Janus kinases or related kinases are widely sought and several publications report effective classes of compounds. For example, certain JAK inhibitors, including (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile depicted below, are reported in U.S. Ser. No. 11/637,545, filed December 12, 2006.



Thus, new or improved forms of existing Janus kinase inhibitors are continually needed for developing new, improved, and more effective pharmaceutical formulations for the treatment of cancer and other diseases. The salt forms and methods described herein are directed toward these needs and other ends.

SUMMARY OF THE INVENTION

The present invention provides, *inter alia*, salts selected from:

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile maleic acid salt;

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile sulfuric acid salt; and

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphoric acid salt.

The present invention further provides methods of preparing a salt of the invention comprising combining (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile with maleic acid, sulfuric acid, or phorphoric acid.

The present invention further provides compositions comprising a salt form of the invention and at least one pharmaceutically acceptable carrier.

The present invention further provides methods of modulating an activity of JAK comprising contacting JAK with a salt of the invention.

The present invention further provides methods of treating a disease in a patient, wherein the disease is associated with JAK activity, comprising administering to the patient a therapeutically effective amount of a salt of the invention.

The present invention further provides methods of treating cancer, skin disorders, or inflammation in a patient, comprising administering to the patient a therapeutically effective amount of a salt of the invention.

The present invention further provides the salts of the invention for use in methods of treating the human or animal body by therapy.

The present invention further provides the salts of the invention for use in treating cancer, skin disorders, or inflammation.

The present invention further provides use of the salts of the invention for the preparation of a medicament for use in the treatment of any of the diseases or disorders recited herein.

DETAILED DESCRIPTION

The present invention provides, *inter alia*, salts of the JAK inhibitor (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile selected from the maleic acid salt, sulfuric acid salt, and phosphoric acid salt. These salts modulate the activity of one or more JAKs and are useful, for example, in the treatment of diseases associated with JAK expression or activity.

The salts of the invention have numerous advantageous properties over the free base form and other salt forms. In particular, these salts were highly crystalline which would facilitate the preparation of pharmaceutical formulations and improve general handling, manipulation, and storage of the active ingredient. The salts of the invention also have superior aqueous solubility, rate of dissolution, chemical stability (with a longer shelf life), compatibility with excipients, and reproducibility compared with the free base form.

In some embodiments, the salts of the invention are substantially isolated. By "substantially isolated" is meant that the salt is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the salt of the invention. Substantial separation can

include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the salt.

Salts of the invention also include all isotopes of atoms occurring in the salts. Isotopes include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium.

Salts of the invention can be prepared using known techniques. Conventionally, a salt form is prepared by combining in solution the free base compound and an acid containing the anion of the salt form desired, and then isolating the solid salt product from the reaction solution (e.g., by crystallization, precipiatation, evaporation, etc.). Other salt-forming techniques can be employed.

Methods of Use

Salts of the invention can modulate activity of one or more Janus kinases (JAKs). The term "modulate" is meant to refer to an ability to increase or decrease the activity of one or more members of the JAK family of kinases. Accordingly, compounds of the invention can be used in methods of modulating a JAK by contacting the JAK with any one or more of the compounds or compositions described herein. In some embodiments, salts of the present invention can act as inhibitors of one or more JAKs. In some embodiments, compounds of the present invention can act to stimulate the activity of one or more JAKs. In further embodiments, the compounds of the invention can be used to modulate activity of a JAK in an individual in need of modulation of the receptor by administering a modulating amount of a salt of the invention.

JAKs to which the present salts bind and/or modulate include any member of the JAK family. In some embodiments, the JAK is JAK1, JAK2, JAK3 or TYK2. In some embodiments, the JAK is JAK1 or JAK2. In some embodiments, the JAK is JAK2. In some embodiments, the JAK is JAK3.

The salts of the invention can be selective. By "selective" is meant that the compound binds to or inhibits a JAK with greater affinity or potency, respectively, compared to at least one other JAK. In some embodiments, the compounds of the invention are selective inhibitors of JAK1 or JAK2 over JAK3 and/or TYK2. In some embodiments, the salts of the invention are selective inhibitors of JAK2 (*e.g.*, over JAK1, JAK3 and TYK2). Without wishing to be bound by theory, because inhibitors of JAK3 can lead to immunosuppressive effects, a compound which is selective for JAK2 over JAK3 and which is useful in the

treatment of cancer (such as multiple myeloma, for example) can offer the additional advantage of having fewer immunosuppressive side effects. Selectivity can be at least about 5-fold, 10-fold, at least about 20-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 500-fold or at least about 1000-fold. Selectivity can be measured by methods routine in the art. In some embodiments, selectivity can be tested at the Km of each enzyme. In some embodiments, selectivity of salts of the invention for JAK2 over JAK3 can be determined by the cellular ATP concentration.

Another aspect of the present invention pertains to methods of treating a JAK-associated disease or disorder in an individual (*e.g.*, patient) by administering to the individual in need of such treatment a therapeutically effective amount or dose of a salt of the present invention or a pharmaceutical composition thereof. A JAK-associated disease can include any disease, disorder or condition that is directly or indirectly linked to expression or activity of the JAK, including overexpression and/or abnormal activity levels. A JAK-associated disease can also include any disease, disorder or condition that can be prevented, ameliorated, or cured by modulating JAK activity.

Examples of JAK-associated diseases include diseases involving the immune system including, for example, organ transplant rejection (*e.g.*, allograft rejection and graft versus host disease).

Further examples of JAK-associated diseases include autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, juvenile arthritis, type I diabetes, lupus, psoriasis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, myasthenia gravis, immunoglobulin nephropathies, autoimmune thyroid disorders, and the like. In some embodiments, the autoimmune disease is an autoimmune bullous skin disorder such as pemphigus vulgaris (PV) or bullous pemphigoid (BP).

Further examples of JAK-associated diseases include allergic conditions such as asthma, food allergies, atopic dermatitis and rhinitis. Further examples of JAK-associated diseases include viral diseases such as Epstein Barr Virus (EBV), Hepatitis B, Hepatitis C, HIV, HTLV 1, Varicella-Zoster Virus (VZV) and Human Papilloma Virus (HPV).

Further examples of JAK-associated diseases or conditions include skin disorders such as psoriasis (for example, psoriasis vulgaris), atopic dermatitis, skin rash, skin irritation, skin sensitization (*e.g.*, contact dermatitis or allergic contact dermatitis). For example, certain substances including some pharmaceuticals when topically applied can cause skin sensitization. In some embodiments, co-administration or sequential administration of at least one JAK inhibitor of the invention together with the agent causing unwanted

sensitization can be helpful in treating such unwanted sensitization or dermatitis. In some embodiments, the skin disorder is treated by topical administration of at least one JAK inhibitor of the invention.

In further embodiments, the JAK-associated disease is cancer including those characterized by solid tumors (*e.g.*, prostate cancer, renal cancer, hepatic cancer, pancreatic cancer, gastric cancer, breast cancer, lung cancer, cancers of the head and neck, thyroid cancer, glioblastoma, Kaposi's sarcoma, Castleman's disease, melanoma etc.), hematological cancers (*e.g.*, lymphoma, leukemia such as acute lymphoblastic leukemia, acute myelogenous leukemia (AML), or multiple myeloma), and skin cancer such as cutaneous T-cell lymphoma (CTCL) and cutaneous B-cell lymphoma. Example cutaneous T-cell lymphomas include Sezary syndrome and mycosis fungoides.

JAK-associated diseases can further include those characterized by expression of a mutant JAK2 such as those having at least one mutation in the pseudo-kinase domain (*e.g.*, JAK2V617F).

JAK-associated diseases can further include myeloproliferative disorders (MPDs) such as polycythemia vera (PV), essential thrombocythemia (ET), myeloid metaplasia with myelofibrosis (MMM), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), hypereosinophilic syndrome (HES), systemic mast cell disease (SMCD), and the like.

Further JAK-associated diseases include inflammation and inflammatory diseases. Example inflammatory diseases include inflammatory diseases of the eye (*e.g.*, iritis, uveitis, scleritis, conjunctivitis, or related disease), inflammatory diseases of the respiratory tract (*e.g.*, the upper respiratory tract including the nose and sinuses such as rhinitis or sinusitis or the lower respiratory tract including bronchitis, chronic obstructive pulmonary disease, and the like), inflammatory myopathy such as myocarditis, and other inflammatory diseases. Other inflammatory diseases treatable by the compounds of the invention include systemic inflammatory response syndrome (SIRS) and septic shock.

The JAK inhibitors described herein can further be used to treat ischemia reperfusion injuries or a disease or condition related to an inflammatory ischemic event such as stroke or cardiac arrest. The JAK inhibitors described herein can further be used to treat anorexia, cachexia, or fatigue such as that resulting from or associated with cancer. The JAK inhibitors described herein can further be used to treat restenosis, sclerodermitis, or fibrosis. The JAK inhibitors described herein can further be used to treat conditions associated with hypoxia or astrogliosis such as, for example, diabetic retinopathy, cancer, or neurodegeneration. See,

e.g., Dudley, A.C. *et al. Biochem. J.* 2005, 390(Pt 2):427-36 and Sriram, K. *et al. J. Biol. Chem.* 2004, 279(19):19936-47. Epub 2004 Mar 2.

The JAK inhibitors described herein can further be used to treat gout and increased prostate size due to, e.g., benign prostatic hypertrophy or benign prostatic hyperplasia.

As used herein, the term "contacting" refers to the bringing together of indicated moieties in an *in vitro* system or an *in vivo* system. For example, "contacting" a JAK with a salt of the invention includes the administration of a salt of the present invention to an individual or patient, such as a human, having a JAK, as well as, for example, introducing a salt of the invention into a sample containing a cellular or purified preparation containing the JAK.

As used herein, the term "individual" or "patient," used interchangeably, refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

As used herein, the phrase "therapeutically effective amount" refers to the amount of active salt or pharmaceutical agent that elicits the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician.

As used herein, the term "treating" or "treatment" refers to one or more of (1) preventing the disease; for example, preventing a disease, condition or disorder in an individual who may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease; (2) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder; and (3) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as decreasing the severity of disease.

Combination Therapies

One or more additional pharmaceutical agents such as, for example, chemotherapeutics, anti-inflammatory agents, steroids, immunosuppressants, as well as Bcr-Abl, Flt-3, RAF and FAK kinase inhibitors such as, for example, those described in WO 2006/056399, or other agents can be used in combination with the salts of the present invention for treatment of JAK-associated diseases, disorders or conditions. The one or more

additional pharmaceutical agents can be administered to a patient simultaneously or sequentially.

Example chemotherapeutics include proteosome inhibitors (*e.g.*, bortezomib), thalidomide, revlimid, and DNA-damaging agents such as melphalan, doxorubicin, cyclophosphamide, vincristine, etoposide, carmustine, and the like.

Example steroids include coriticosteroids such as dexamethasone or prednisone.

Example Bcr-Abl inhibitors include the compounds, and pharmaceutically acceptable salts thereof, of the genera and species disclosed in U.S. Pat. No. 5,521,184, WO 04/005281, EP2005/009967, EP2005/010408, and U.S. Ser. No. 60/578,491.

Example suitable Flt-3 inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 03/037347, WO 03/099771, and WO 04/046120.

Example suitable RAF inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 00/09495 and WO 05/028444.

Example suitable FAK inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 04/080980, WO 04/056786, WO 03/024967, WO 01/064655, WO 00/053595, and WO 01/014402.

In some embodiments, the salt forms of the invention can be used in combination with other kinase inhibitors such as imatinib, particularly for the treatment of patients resistant to imatinib or other kinases.

In some embodiments, one or more salt forms of the invention can be used in combination with a chemotherapeutic in the treatment of cancer, such as multiple myeloma, and may improve the treatment response as compared to the response to the chemotherapeutic agent alone, without exacerbation of its toxic effects. Examples of additional pharmaceutical agents used in the treatment of multiple myeloma, for example, can include, without limitation, melphalan, melphalan plus prednisone [MP], doxorubicin, dexamethasone, and Velcade (bortezomib). Further additional agents used in the treatment of multiple myeloma include Bcr-Abl, Flt-3, RAF and FAK kinase inhibitors. Additive or synergistic effects are desirable outcomes of combining a JAK inhibitor of the present invention with an additional agent. Furthermore, resistance of multiple myeloma cells to agents such as dexamethasone may be reversible upon treatment with a JAK inhibitor of the present invention. The agents can be combined with the present compounds in a single or continuous dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms.

In some embodiments, a corticosteroid such as dexamethasone is administered to a patient in combination with at least one JAK inhibitor where the dexamethasone is administered intermittently as opposed to continuously.

In some further embodiments, combinations of one or more JAK inhibitors of the invention with other therapeutic agents can be administered to a patient prior to, during, and/or after a bone marrow transplant or stem cell transplant.

Pharmaceutical Formulations and Dosage Forms

When employed as pharmaceuticals, the salts of the invention can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes, depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including transdermal, epidermal, ophthalmic and to mucous membranes including intranasal, vaginal and rectal delivery), pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal or intranasal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal, intramuscular or injection or infusion; or intracranial, *e.g.*, intrathecal or intraventricular, administration. Parenteral administration can be in the form of a single bolus dose, or may be, for example, by a continuous perfusion pump. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of the invention above in combination with one or more pharmaceutically acceptable carriers (excipients). In making the compositions of the invention, the active ingredient is typically mixed with an excipient, diluted by an excipient or enclosed within such a carrier in the form of, for example, a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and

hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, the active compound can be milled to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it can be milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size can be adjusted by milling to provide a substantially uniform distribution in the formulation, *e.g.* about 40 mesh.

The compounds of the invention may be milled using known milling procedures such as wet milling to obtain a particle size appropriate for tablet formation and for other formulation types. Finely divided (nanoparticulate) preparations of the compounds of the invention can be prepared by processes known in the art, for example see International Patent Application No. WO 2002/000196.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions can be formulated in a unit dosage form, each dosage containing from about 5 to about 1000 mg (1 g), more usually about 100 to about 500 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The active compound can be effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the compound actually administered will usually be determined by a physician, according to the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, the active ingredient is typically dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, about 0.1 to about 1000 mg of the active ingredient of the present invention.

The tablets or pills of the present invention can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the compounds and compositions of the present invention can be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions can be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device can be attached to a face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can be administered orally or nasally from devices which deliver the formulation in an appropriate manner.

The amount of salt or composition administered to a patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy,

the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions can be administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. Effective doses will depend on the disease condition being treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the disease, the age, weight and general condition of the patient, and the like.

The compositions administered to a patient can be in the form of pharmaceutical compositions described above. These compositions can be sterilized by conventional sterilization techniques, or may be sterile filtered. Aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the compound preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of pharmaceutical salts.

The therapeutic dosage of the salts of the present invention can vary according to, for example, the particular use for which the treatment is made, the manner of administration of the compound, the health and condition of the patient, and the judgment of the prescribing physician. The proportion or concentration of a salt of the invention in a pharmaceutical composition can vary depending upon a number of factors including dosage, chemical characteristics (*e.g.*, hydrophobicity), and the route of administration. For example, the salts of the invention can be provided in an aqueous physiological buffer solution containing about 0.1 to about 10% w/v of the compound for parenteral administration. Some typical dose ranges are from about 1 μg/kg to about 1 g/kg of body weight per day. In some embodiments, the dose range is from about 0.01 mg/kg to about 100 mg/kg of body weight per day. The dosage is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, formulation of the excipient, and its route of administration. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The compositions of the invention can further include one or more additional pharmaceutical agents such as a chemotherapeutic, steroid, anti-inflammatory compound, or immunosuppressant, examples of which are listed hereinabove.

Labeled Compounds and Assay Methods

Another aspect of the present invention relates to labeled salts of the invention (radio-labeled, fluorescent-labeled, etc.) that would be useful not only in imaging techniques but also in assays, both *in vitro* and *in vivo*, for localizing and quantitating JAK in tissue samples, including human, and for identifying JAK ligands by inhibition binding of a labeled compound. Accordingly, the present invention includes JAK assays that contain such labeled compounds.

The present invention further includes isotopically-labeled salts of the invention. An "isotopically" or "radio-labeled" compound is a salt of the invention where one or more atoms are replaced or substituted by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature (*i.e.*, naturally occurring). Suitable radionuclides that may be incorporated in compounds of the present invention include but are not limited to ^{2}H (also written as D for deuterium), ^{3}H (also written as T for tritium), ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{18}F, ^{35}S, ^{36}Cl, ^{82}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{123}I, ^{124}I, ^{125}I and ^{131}I. The radionuclide that is incorporated in the instant radio-labeled compounds will depend on the specific application of that radio-labeled compound. For example, for *in vitro* metalloprotease labeling and competition assays, compounds that incorporate ^{3}H, ^{14}C, ^{82}Br, ^{125}I , ^{131}I, ^{35}S or will generally be most useful. For radio-imaging applications ^{11}C, ^{18}F, ^{125}I, ^{123}I, ^{124}I, ^{131}I, ^{75}Br, ^{76}Br or ^{77}Br will generally be most useful.

It is understood that a "radio-labeled " or "labeled compound" is a salt that has incorporated at least one radionuclide. In some embodiments the radionuclide is selected from the group consisting of ^{3}H, ^{14}C, ^{125}I , ^{35}S and ^{82}Br.

The present invention can further include synthetic methods for incorporating radio-isotopes into compounds of the invention. Synthetic methods for incorporating radio-isotopes into organic compounds are well known in the art, and a person of ordinary skill in the art will readily recognize the methods applicable for the compounds of invention.

A labeled salt of the invention can be used in a screening assay to identify/evaluate compounds. For example, a newly synthesized or identified compound (*i.e.*, test compound) which is labeled can be evaluated for its ability to bind a JAK by monitoring its concentration variation when contacting with the JAK, through tracking of the labeling. For example, a test compound (labeled) can be evaluated for its ability to reduce binding of another compound which is known to bind to a JAK (*i.e.*, standard compound). Accordingly, the ability of a test compound to compete with the standard compound for binding to the JAK directly correlates to its binding affinity. Conversely, in some other screening assays, the standard compound is

labeled and test compounds are unlabeled. Accordingly, the concentration of the labeled standard compound is monitored in order to evaluate the competition between the standard compound and the test compound, and the relative binding affinity of the test compound is thus ascertained.

Kits

The present invention also includes pharmaceutical kits useful, for example, in the treatment or prevention of JAK-associated diseases or disorders, such as cancer, inflammation, or skin disorders, which include one or more containers containing a pharmaceutical composition comprising a therapeutically effective amount of a salt of the invention. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

EXAMPLES

Example 1: Preparation of (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile maleic acid salt

To a test tube was added (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile (153.7 mg, 0.5 mmol) and maleic acid (61.7 mg) followed by isopropyl alcohol (IPA) (4 mL). The resulting mixture was heated to clear, cooled to room temperature, and then stirred for another 2.5 hours. The precipitate was collected by filtration and the cake was washed with 0.8 mL of cold IPA. The cake was dried under vacuum to constant weight to provide the final salt product (173 mg).

The maleic acid salt was shown to be a 1:1 salt by H^1 NMR and crystallinity was confirmed by X-ray powder diffraction (XRPD). Differential scanning calorimetry (DSC) gave a sharp melting peak at about 175.96 °C (onset at 175.67 °C). The product showed only slight weight loss up to 150 °C by thermogravimetric analysis (TGA).

Example 2: Preparation of (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphoric acid salt

To a test tube was added (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile (153.5 mg) and phosphoric acid (56.6 mg) followed by isopropyl alcohol (IPA) (5.75 mL). The resulting mixture was heated to clear, cooled to room temperature, and then stirred for another 2 hours. The precipitate was collected by filtration and the cake was washed with 0.6 mL of cold IPA. The cake was dried under vacuum to constant weight to provide the final salt product (171.7 mg).

The phosphroic acid salt was shown to be a 1:1 salt by ^{1}H NMR and crystallinity was confirmed by X-ray powder diffraction (XRPD). Differential scanning calorimetry (DSC) gave a sharp melting peak at about 198.66 °C. The product showed little weight loss up to 200 °C by TGA.

Example 3: Preparation of (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile sulfuric acid salt

To a test tube was added (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropanenitrile (153.0 mg) and sulfuric acid (56.1 mg) followed by acetonitrile (7.0 mL). The resulting mixture was heated to clear, cooled to room temperature, and then stirred for another 2 hours. The precipitate was collected by filtration and the cake was washed with 0.8 mL of cold acetonitrile. The cake was dried under vacuum to constant weight to provide the final salt product (180 mg).

The sulfuric acid salt was shown to be a 1:1 salt by ^{1}H NMR and crystallinity was confirmed by X-ray powder diffraction (XRPD). Differential scanning calorimetry (DSC) gave a sharp melting peak at about 186.78 °C. The product showed little weight loss up to 175 °C by TGA.

Example A

***In vitro* JAK Kinase Assay**

Inhibitory activity of test compounds on JAK targets can be tested according to the following *in vitro* assay described in Park *et al.*, *Analytical Biochemistry* **1999**, *269*, 94-104. The catalytic domains of human JAK1 (a.a. 837-1142), Jak2 (a.a. 828-1132) and Jak3 (a.a. 781-1124) with an N-terminal His tag are expressed using baculovirus in insect cells and purified. The catalytic activity of JAK1, JAK2 or JAK3 is assayed by measuring the

phosphorylation of a biotinylated peptide. The phosphorylated peptide was detected by homogenous time resolved fluorescence (HTRF). IC_{50}s of compounds are measured for each kinase in the reactions that contain the enzyme, ATP and 500 nM peptide in 50 mM Tris (pH 7.8) buffer with 100 mM NaCl, 5 mM DTT, and 0.1 mg/mL (0.01%) BSA. The ATP concentration in the reactions is 90 μM for Jak1, 30 μM for Jak2 and 3 μM for Jak3. Reactions are carried out at room temperature for 1 hr and then stopped with 20 μL 45 mM EDTA, 300 nM SA-APC, 6 nM Eu-Py20 in assay buffer (Perkin Elmer, Boston, MA). Binding to the Europium labeled antibody takes place for 40 minutes and HTRF signal is measured on a Fusion plate reader (Perkin Elmer, Boston, MA). Both the phosphoric acid salt of the invention, and the corresponding free base compound, were found to have IC_{50} values of less than 50 nM for each of JAK1, JAK2, and JAK3.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference cited in the present application is incorporated herein by reference in its entirety.

MCSLLP

Claims:

1. A salt selected from:

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile maleic acid salt;

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile sulfuric acid salt; and

(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile phosphoric acid salt.

2. The salt of claim 1 that is (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile maleic acid salt.

3. The salt of claim 1 that is (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile sulfuric acid salt.

4. The salt of claim 1 that is (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile phosphoric acid salt.

5. A method of preparing a salt of any one of claims 1 to 4 comprising combining (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile with maleic acid, sulfuric acid, or phosphoric acid.

6. A composition comprising at least one salt of any one of claims 1 to 4 and at least one pharmaceutically acceptable carrier.

7. The composition of claim 6 which is suitable for oral or topical administration.

8. The composition of claim 6 which is suitable for topical administration.

9. A method of modulating an activity of JAK comprising contacting JAK *ex vivo* with a salt of any one of claims 1 to 4.

10. The method of claim 9 wherein said modulating is inhibiting.

11. The salt of any one of claims 1 to 4 for use in treating a disease in a patient wherein said disease is assocated with JAK activity.

12. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating a disease in a patient, wherein said disease is associated with JAK activity, and wherein said disease is: allograft rejection, graft versus host disease, an autoimmune disease, atopic dermatitis, psoriasis, skin sensitization, skin irritation, skin rash, contact dermatitis, allergic contact sensitization, cancer, a myeloproliferative disorder, or an inflammatory disease.

13. The use of claim 12 wherein said disease is allograft rejection or graft versus host disease.

14. The use of claim 12 wherein said disease is an autoimmune disease.

15. The use of claim 14 wherein said autoimmune disease is, multiple sclerosis, rheumatoid arthritis, juvenile arthritis, type I diabetes, lupus, inflammatory bowel disease, Crohn's disease, myasthenia gravis, immunoglobulin nephropathies, myocarditis, or autoimmune thyroid disorder.

16. The use of claim 12 wherein said autoimmune disease is bullous skin disorder.

17. The use of claim 16 wherein said bullous skin disorder is pemphigus vulgaris (PV) or bullous pemphigoid (BP).

18. The use of claim 12 wherein said disease is atopic dermatitis, psoriasis, skin sensitization, skin irritation, skin rash, contact dermatitis or allergic contact sensitization.

19. The use of claim 12 wherein said disease is cancer.

20. The use of claim 19 wherein said cancer is a solid tumor

21. The use of claim 19 wherein said cancer is prostate cancer, renal cancer, hepatic cancer, breast cancer, lung cancer, thyroid cancer, Kaposi's sarcoma, Castleman's disease or pancreatic cancer.

22. The use of claim 21 wherein said cancer is prostate cancer.

23. The use of claim 19 wherein said cancer is hematological.

24. The use of claim 23 wherein said cancer is lymphoma, leukemia, or multiple myeloma.

25. The use of claim 19 wherein said cancer is a skin cancer.

26. The use of claim 25 wherein said skin cancer is cutaneous T-cell lymphoma or cutaneous B-cell lymphoma.

27. The use of claim 19 wherein said cancer is multiple myeloma.

28. The use of claim 12 wherein said disease is a myeloproliferative disorder.

29. The use of claim 28 wherein said myeloproliferative disorder (MPD) is polycythemia vera (PV), essential thrombocythemia (ET), myeloid metaplasia with myelofibrosis (MMM), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), hypereosinophilic syndrome (HES), or systemic mast cell disease (SMCD).

30. The use of claim 12 wherein said disease is an inflammatory disease.

31. The use of claim 30 wherein said inflammatory disease is an inflammatory disease of the eye.

32. The use of claim 31 wherein said inflammatory disease of the eye is iritis, uveitis, scleritis, or conjunctivitis.

33. The use of claim 30 wherein said inflammatory disease is an inflammatory disease of the respiratory tract.

34. The use of claim 30 wherein said inflammatory disease concerns the upper respiratory tract.

35. The use of claim 30 wherein said inflammatory disease concerns the lower respiratory tract.

36. The use of claim 30 wherein said inflammatory disease is an inflammatory myopathy.

37. The use of claim 30 wherein said inflammatory disease is myocarditis.

38. Use of a salt of any one of claims 1 to 4 in the preparation on a medicament for treating cancer in a patient.

39. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for topical administration for treating inflammation in a patient.

40. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating rheumatoid arthritis in a patient.

41. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating prostate cancer.

42. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating psoriasis.

43. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating multiple myeloma in a patient.

44. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating myeloid metaplasia with myelofibrosis (MMM) in a patient.

45. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating polycythemia vera (PV) in a patient.

46. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating essential thrombocythemia (ET) in a patient.

47. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating mycosis fungoides in a patient.

48. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating a hematological cancer in a patient.

49. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating chronic myelogenous leukemia (CML) in a patient.

50. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating acute lymphoblastic leukemia (ALL) in a patient.

51. Use of a salt of any one of claims 1 to 4 in the preparation of a medicament for treating chronic myelomonocytic leukemia (CMML) in a patient.

52. Use of (R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-lH-pyrazol-l-yl)-3-cyclopentylpropanenitrile phosphoric acid salt in the preparation of a medicament for treating a myeloproliferative disorder in a patient.

53. Use according to claim 52, wherein said myeloproliferative disorder is myeloid metaplasia with myelofibrosis, polycythemia vera, or essential thrombocythemia.