(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 September 2021 (10.09.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/178991 A1

(51) International Patent Classification:
C07D 471/04 (2006.01) *A61P 11/00* (2006.01)
A61K 31/437 (2006.01)

(21) International Application Number:
PCT/US2021/070207

(22) International Filing Date:
01 March 2021 (01.03.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/983,931 02 March 2020 (02.03.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

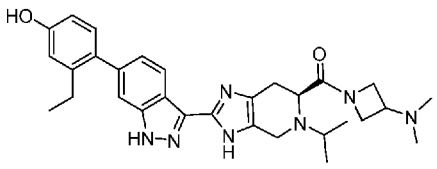
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*

(54) Title: CRYSTALLINE HYDRATE OF A JAK INHIBITOR COMPOUND

(57) Abstract: Provided herein is a crystalline hydrate of the compound of formula 1:. Also provides herein are pharmaceutical compositions comprising such crystalline hydrate, methods of using such crystalline hydrate to treat respiratory diseases, and processes useful for preparing such crystalline hydrate.



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CRYSTALLINE HYDRATE OF A JAK INHIBITOR COMPOUND

BACKGROUND OF THE INVENTION

Field of the Invention

Provided herein is a crystalline hydrate form of a JAK inhibitor compound useful for treating respiratory and other diseases. Also provided herein are pharmaceutical compositions comprising such crystalline form, methods of using the crystalline form to treat, for example, respiratory diseases, and processes useful for preparing such crystalline form.

State of the Art

Cytokines are intercellular signaling molecules which include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factor. Cytokines are critical for normal cell growth and immunoregulation but also drive immune-mediated diseases and contribute to the growth of malignant cells. Elevated levels of many cytokines have been implicated in the pathology of a large number of disease or conditions, particularly those diseases characterized by inflammation. Many of the cytokines implicated in disease act through signaling pathways dependent upon the Janus family of tyrosine kinases (JAKs), which signal through the Signal Transducer and Activator of Transcription (STAT) family of transcription factors.

The JAK family comprises four members, JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). Binding of cytokine to a JAK-dependent cytokine receptor induces receptor dimerization which results in phosphorylation of tyrosine residues on the JAK kinase, effecting JAK activation. Phosphorylated JAKs, in turn, bind and phosphorylate various STAT proteins which dimerize, internalize in the cell nucleus and directly modulate gene transcription, leading, among other effects, to the downstream effects associated with inflammatory disease. The JAKs usually associate with cytokine

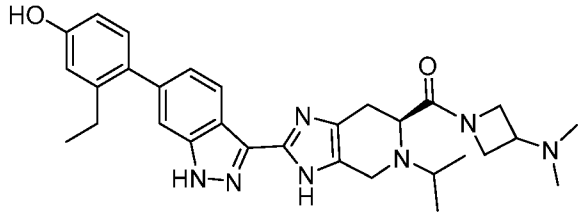
receptors in pairs as homodimers or heterodimers. Specific cytokines are associated with specific JAK pairings. Each of the four members of the JAK family is implicated in the signaling of at least one of the cytokines associated with inflammation.

Asthma is a chronic disease of the airways for which there are no preventions or cures. The disease is characterized by inflammation, fibrosis, hyperresponsiveness, and remodeling of the airways, all of which contribute to airflow limitation. An estimated 300 million people worldwide suffer from asthma and it is estimated that the number of people with asthma will grow by more than 100 million by 2025. Although most patients can achieve control of asthma symptoms with the use of inhaled corticosteroids that may be combined with a leukotriene modifier and/or a long acting beta agonist, there remains a subset of patients with severe asthma whose disease is not controlled by conventional therapies. Cytokines implicated in asthma inflammation which signal through the JAK-STAT pathway include IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-13, IL-23, IL-31, IL-27, thymic stromal lymphopoietin (TSLP), interferon-γ (IFNγ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Inflammation of the airways is characteristic of other respiratory diseases in addition to asthma. Chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pneumonitis, interstitial lung diseases (including idiopathic pulmonary fibrosis), acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, and bronchiolitis obliterans are also respiratory tract diseases in which the pathophysiology is believed to be related to JAK-signaling cytokines.

JAK-signaling cytokines also play a major role in the activation of T cells, a sub-type of immune cells that is central to many immune processes. Pathological T cell activation is critical in the etiology of multiple respiratory diseases. Autoreactive T cells play a role in bronchiolitis obliterans organizing pneumonia (also termed COS). Similar to COS, the etiology of lung transplant rejections is linked to an aberrant T cell activation of the recipient's T cells by the transplanted donor lung. Lung transplant rejections may occur early as Primary Graft Dysfunction (PGD), organizing pneumonia (OP), acute rejection (AR) or lymphocytic bronchiolitis (LB) or they may occur years after lung transplantation as Chronic Lung Allograft Dysfunction (CLAD). CLAD was previously known as bronchiolitis obliterans (BO) but now is considered a syndrome that can have different pathological manifestations including BO, restrictive CLAD (rCLAD or RAS) and neutrophilic allograft dysfunction. Chronic lung allograft dysfunction (CLAD) is a major challenge in long-term management of lung transplant recipients as it causes a

transplanted lung to progressively lose functionality (Gauthier et al., *Curr. Transplant. Rep.*, **2016**, 3(3), 185–191). CLAD is poorly responsive to treatment and therefore, there remains a need for effective compounds capable of preventing or treating this condition. Several JAK-dependent cytokines such as IFNγ and IL-5 are up-regulated in CLAD and lung transplant rejection (Berastegui et al, *Clin. Transplant.* **2017**, 31, e12898). Moreover, high lung levels of CXCR3 chemokines such as CXCL9 and CXCL10 which are downstream of JAK-dependent IFN signaling, are linked to worse outcomes in lung transplant patients (Shino et al, *PLOS One*, **2017**, 12 (7), e0180281). Systemic JAK inhibition has been shown to be effective in kidney transplant rejection (Vicenti et al., *American Journal of Transplantation*, **2012**, 12, 2446-56). Therefore, JAK inhibitors have the potential to be effective in preventing or delaying lung transplant rejection and CLAD. Similar T cell activation events as described as the basis for lung transplant rejection also are considered the main driver of lung graft-versus-host disease (GVHD) which can occur post hematopoietic stem cell transplants. Similar to CLAD, lung GVHD is a chronic progressive condition with extremely poor outcomes and no treatments are currently approved. A retrospective, multicenter survey study of 95 patients with steroid-refractory acute or chronic GVHD who received the systemic JAK inhibitor ruxolitinib as salvage therapy demonstrated complete or partial response to ruxolitinib in the majority of patients including those with lung GVHD (Zeiser et al, *Leukemia*, **2015**, 29, 10, 2062-68).

Commonly assigned U.S. Application Serial No. 16/559,077, filed on September 3, 2019 discloses some dimethyl amino azetidine amide compounds useful as JAK inhibitors. In particular, the compound (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound **1**):



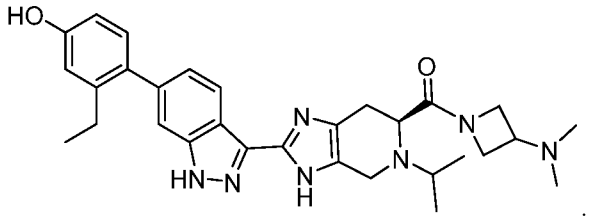
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is specifically disclosed in the application as a potent non-systemic pan-JAK inhibitor suitable for treating, preventing and/or delaying inflammatory respiratory diseases, including asthma and lung transplant rejection.

To effectively use this compound as a therapeutic agent, it would be desirable to have a crystalline solid-state form. For example, it would be highly desirable to have a physical form that is thermally stable at reasonably high temperature, thereby facilitating processing and storage of the material. Crystalline solids are generally preferred over amorphous forms, for enhancing purity and stability of the manufactured product. However, the formation of crystalline forms of organic compounds is highly unpredictable. No reliable methods exist for predicting which, if any, form of an organic compound will be crystalline. Moreover, no methods exist for predicting which, if any, crystalline form will have the physically properties desired for use as pharmaceutical agents. Accordingly, a need exists for crystalline forms of compound **1.**

SUMMARY OF THE INVENTION

Provided herein is a crystalline hydrate of the compound of formula **1**:



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Also provided herein are pharmaceutical compositions comprising the crystalline hydrate of the disclosure, and a pharmaceutically-acceptable carrier.

Provided herein is a method of preparing the crystalline hydrate of the disclosure, as well as methods of treating, preventing, delaying, and/or ameliorating diseases amenable to treatment with a JAK inhibitor, in particular respiratory diseases and lung transplant rejection.

Also provided herein are uses of the crystalline hydrate form in medical therapy and in the manufacture of a formulation or medicament for treating, preventing, delaying, and/or ameliorating diseases amenable to treatment with a JAK inhibitor, in particular respiratory diseases and lung transplant rejection.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects of the present invention are illustrated by reference to the accompanying drawings.

Figure 1 shows a powder x-ray diffraction (PXRD) pattern of the crystalline hydrate of compound **1**.

Figure 2 shows a differential scanning calorimetry (DSC) thermogram of the crystalline hydrate of compound **1.**

Figure 3 shows a thermal gravimetric analysis (TGA) plot of the crystalline hydrate of compound **1**.

Figure 4 shows a dynamic moisture sorption (DMS) isotherm of the crystalline hydrate of compound **1** observed at a temperature of about 25 °C.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

When describing this disclosure, including its various aspects and embodiments, the following terms have the following meanings, unless otherwise indicated.

The term "about" means ± 5 percent of the specified value.

The term "hydrate" means a complex or aggregate, typically in crystalline form, formed by molecules of water and the compound of the disclosure where the ratio of water molecules to compound molecules may be 1:1, less than 1:1 or more than 1:1.

The term "substantially" when referring, for example, to an X-ray diffraction pattern, a DSC trace, or a TGA trace includes a pattern, or trace that is not necessarily identical to those depicted herein, but that falls within the limits of experimental error or deviations when considered by one of ordinary skill in the art.

The term "therapeutically effective amount" means an amount sufficient to effect treatment when administered to a patient in need of treatment.

The term "treating" or "treatment" means ameliorating or suppressing the medical condition, disease or disorder being treated (e.g., a respiratory disease) in a patient (particularly a human); or alleviating the symptoms of the medical condition, disease or disorder.

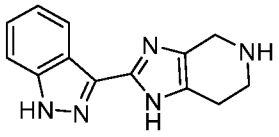
It must be noted that, as used in the specification and appended claims, the singular forms "a", "an", "one", and "the" may include plural references, unless the content clearly dictates otherwise.

Naming Convention

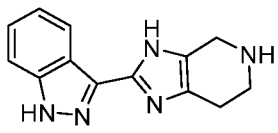
Compound **1** is designated as (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-

c]pyridin-6-yl)methanone according to IUPAC conventions as implemented in ChemDraw software (PerkinElmer, Inc., Cambridge, MA).

Furthermore, the imidazo portion of the tetrahydroimidazopyridine moiety in the structure of compound **1** exists in tautomeric forms, illustrated below for a fragment of the compound of Example **1**



A

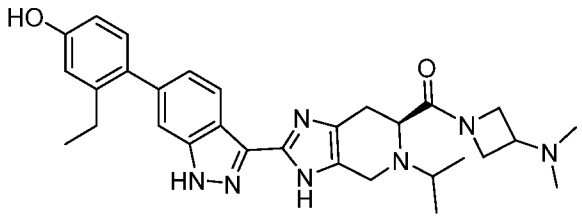


B

According to the IUPAC convention, these representations give rise to different numbering of the atoms of the imidazole portion: (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridin-6-yl)methanone (structure A) versus (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (structure B). It will be understood that although structures are shown, or named, in a particular form, the invention also includes the tautomer thereof.

Crystalline Form of compound **1**

Provided herein is a crystalline hydrate (Form I) of the compound of formula **1**:



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In one embodiment, the crystalline hydrate is characterized by a powder X-ray diffraction (PXRD) pattern comprising diffraction peaks at 2θ values of 5.68±0.20, 10.43±0.20, 10.94±0.20, and 13.08±0.20. The crystalline hydrate may be further characterized by a PXRD pattern having one additional diffraction peak at a 2θ value of 8.49±0.20. The crystalline hydrate may be further characterized by a PXRD pattern having two or more additional diffraction peaks at 2θ values selected from 11.55±0.20, 12.20±0.20, 17.06±0.20, and 26.29±0.20. The crystalline hydrate may be further characterized by a PXRD pattern having additional diffraction peaks at 2θ values of 11.55±0.20, 12.20±0.20, 17.06±0.20, and 26.29±0.20.

The crystalline hydrate may be characterized by a PXRD pattern having 2, 3, 4, 5, 6, 7, 8, 9, or 10 diffraction peaks at 2θ values selected from 5.68±0.20, 8.49±0.20, 10.43±0.20, 10.94±0.20, 11.55±0.20, 12.20±0.20, 13.08±0.20, 15.94±0.20, 16.24±0.20, 17.06±0.20, 17.60±0.20, 18.41±0.20, 18.82±0.20, 18.96±0.20, 21.90±0.20, 22.08±0.20, 22.27±0.20, 24.55±0.20, and 26.29±0.20.

As is well known in the field of powder X-ray diffraction, peak positions of PXRD spectra are relatively less sensitive to experimental details, such as details of sample preparation and instrument geometry, than are the relative peak heights. Thus, in one embodiment, the crystalline hydrate is characterized by a powder x-ray diffraction pattern in which the peak positions are substantially in accordance with those shown in Figure 1.

In another embodiment, the crystalline hydrate is characterized by its behavior when exposed to high temperature. As demonstrated in Figure 2, the differential scanning calorimetry (DSC) trace recorded at a heating rate of 10 °C per minute exhibits a dehydration endotherm with an onset at about 55.1 °C and a peak at about 139.4 °C, and a peak in endothermic heat flow, identified as a melt transition with an onset at about 198.6 °C, and a peak at about 212.4 °C. In one embodiment, the crystalline hydrate form (Form I) is characterized by a differential scanning calorimetry trace recorded at a heating rate of 10 °C per minute which shows a maximum in endothermic heat flow at a temperature of 212.4±3 °C. In another embodiment, the maximum in endothermic heat flow is at a temperature of 212.4±2 °C. In another embodiment, the maximum in endothermic heat flow is at a temperature of 212.4±1 °C.

In one embodiment, the crystalline hydrate is a monohydrate.

The thermal gravimetric analysis (TGA) trace of Figure 3 shows a weight loss of about 3.4% by 100.0 °C. The compound dehydrates at an onset temperature of about 27°C. Weight loss associated with decomposition can be seen at an onset temperature of about 248.2 °C.

The present crystalline hydrate has been demonstrated to have a reversible sorption/desorption profile. The solid is moderately hygroscopic. The crystalline hydrate exhibited a total moisture uptake of about 5.3 % when exposed to a range of relative humidity between 0 % and 90 % at room temperature as shown in Figure 4.

Form I is characterized by a unit cell as determined by single crystal X-ray crystallography of the following dimensions: a = 20.8736(5) Å; b = 9.15021(19) Å; c = 15.7412(3) Å; a = 90°; b =98.4786(18)°; and g = 90°. A single crystal of Form I, analyzed

at a temperature of 100(2) K, is characterized by a monoclinic crystal system with dimensions: a = 20.8736(5) Å; b = 9.15021(19) Å; c = 15.7412(3) Å; a = 90°; b =98.4786(18)°; g = 90°; a cell volume (V) of 2973.67(11) $Å^3$, and a space group C2.

Form I exhibited good stability under accelerated conditions of temperature and relative humidity as shown in Example 10.

Synthetic Procedures

Compound **1,** can be prepared from readily available starting materials using the procedures described in the Examples below, or using the procedures described in the commonly-assigned U.S. application listed in the Background section of this application.

Form I can be prepared by:

(a) dissolving (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone in an alcohol solvent at a temperature of 55°C ±10°C to give a solution;

(b) cooling down the solution obtained in step (a) to 10°C ±10°C to produce a suspension;

(c) isolating the solid from the suspension of step (b) under inert gas conditions;

(d) drying the solid obtained in step (c) at 60°C ±15°C;

(e) subjecting the solid obtained in step (d) to ambient humidity and temperature conditions to give the crystalline hydrate.

In some embodiments, the alcohol solvent is methanol. In some embodiments, the alcohol solvent is ethanol.

In some embodiments, seeds of Form I are added to initiate formation of the suspension in step (b).

Pharmaceutical Compositions

The crystalline hydrate solid form of the disclosure can be used in the form of a pharmaceutical composition or formulation. Such pharmaceutical compositions may advantageously be administered to a patient by inhalation. In addition, pharmaceutical compositions may be administered by any acceptable route of administration including, but not limited to, oral, topical (including transdermal), rectal, nasal, and parenteral modes of administration.

Accordingly, in one embodiment, provided herein is a pharmaceutical composition comprising a pharmaceutically-acceptable carrier or excipient and the crystalline hydrate of compound **1** (Form I). Optionally, such pharmaceutical

compositions may contain other therapeutic and/or formulating agents if desired. When discussing compositions and uses thereof, the crystalline hydrate of compound **1** (Form I) may also be referred to herein as the "active agent".

The pharmaceutical compositions of the disclosure typically contain a therapeutically effective amount of the crystalline hydrate of compound **1** (Form I). Those skilled in the art will recognize, however, that a pharmaceutical composition may contain more than a therapeutically effective amount, i.e., bulk compositions, or less than a therapeutically effective amount, i.e., individual unit doses designed for multiple administration to achieve a therapeutically effective amount.

Typically, such pharmaceutical compositions will contain from about 0.01 to about 95% by weight of the active agent; including, for example, from about 0.05 to about 30% by weight; and from about 0.1 % to about 10% by weight of the active agent.

Any conventional carrier or excipient may be used in the pharmaceutical compositions of the disclosure. The choice of a particular carrier or excipient, or combinations of carriers or excipients, will depend on the mode of administration being used to treat a particular patient or type of medical condition or disease state. In this regard, the preparation of a suitable pharmaceutical composition for a particular mode of administration is well within the scope of those skilled in the pharmaceutical arts. Additionally, the carriers or excipients used in the pharmaceutical compositions of this disclosure are commercially-available. By way of further illustration, conventional formulation techniques are described in Remington: The Science and Practice of Pharmacy, 20th Edition, Lippincott Williams & White, Baltimore, Maryland (2000); and H.C. Ansel et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Edition, Lippincott Williams & White, Baltimore, Maryland (1999).

Representative examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, the following: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, such as microcrystalline cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's

solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical compositions.

Pharmaceutical compositions are typically prepared by thoroughly and intimately mixing or blending the active agent with a pharmaceutically-acceptable carrier and one or more optional ingredients. The resulting uniformly blended mixture can then be shaped or loaded into tablets, capsules, pills and the like using conventional procedures and equipment.

In one embodiment, the pharmaceutical composition is suitable for inhaled administration. Pharmaceutical compositions for inhaled administration are typically in the form of an aerosol or a powder. Such compositions are generally administered using inhaler delivery devices, such as a dry powder inhaler (DPI), a metered-dose inhaler (MDI), a nebulizer inhaler, or a similar delivery device.

In a particular embodiment, the pharmaceutical composition is administered by inhalation using a dry powder inhaler. Such dry powder inhalers typically administer the pharmaceutical composition as a free-flowing powder that is dispersed in a patient's air-stream during inspiration. In order to achieve a free-flowing powder composition, the therapeutic agent is typically formulated with a suitable excipient such as lactose, starch, mannitol, dextrose, polylactic acid (PLA), polylactide-co-glycolide (PLGA) or combinations thereof. Typically, the therapeutic agent is micronized and combined with a suitable carrier to form a composition suitable for inhalation.

A representative pharmaceutical composition for use in a dry powder inhaler comprises lactose and the crystalline hydrate of compound **1** in micronized form. Such a dry powder composition can be made, for example, by combining dry milled lactose with the therapeutic agent and then dry blending the components. The composition is then typically loaded into a dry powder dispenser, or into inhalation cartridges or capsules for use with a dry powder delivery device.

Dry powder inhaler delivery devices suitable for administering therapeutic agents by inhalation are described in the art and examples of such devices are commercially available. For example, representative dry powder inhaler delivery devices or products include Aeolizer (Novartis); Airmax (IVAX); ClickHaler (Innovata Biomed); Diskhaler (GlaxoSmithKline); Diskus/Accuhaler (GlaxoSmithKline); Ellipta (GlaxoSmithKline); Easyhaler (Orion Pharma); Eclipse (Aventis); FlowCaps (Hovione); Handihaler (Boehringer Ingelheim); Pulvinal (Chiesi); Rotahaler (GlaxoSmithKline);

SkyeHaler/Certihaler (SkyePharma); Twisthaler (Schering-Plough); Turbuhaler (AstraZeneca); Ultrahaler (Aventis); and the like.

In another particular embodiment, the pharmaceutical composition is administered by inhalation using a metered-dose inhaler. Such metered-dose inhalers typically discharge a measured amount of a therapeutic agent using a compressed propellant gas. Accordingly, pharmaceutical compositions administered using a metered-dose inhaler typically comprise a suspension of the therapeutic agent in a liquefied propellant. Any suitable liquefied propellant may be employed including hydrofluoroalkanes (HFAs), such as 1,1,1,2-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoro-*n*-propane, (HFA 227); and chlorofluorocarbons, such as CCl_3F. In a particular embodiment, the propellant is hydrofluoroalkanes. In some embodiments, the hydrofluoroalkane formulation contains a co-solvent, such as ethanol or pentane, and/or a surfactant, such as sorbitan trioleate, oleic acid, lecithin, and glycerin.

A representative pharmaceutical composition for use in a metered-dose inhaler comprises from about 0.01% to about 5% by weight of the crystalline hydrate of compound **1** (Form I); from about 0% to about 20% by weight ethanol; and from about 0% to about 5% by weight surfactant; with the remainder being an HFA propellant. Such compositions are typically prepared by adding chilled or pressurized hydrofluoroalkane to a suitable container containing the therapeutic agent, ethanol (if present) and the surfactant (if present). To prepare a suspension, the therapeutic agent is micronized and then combined with the propellant. The composition is then loaded into an aerosol canister, which typically forms a portion of a metered-dose inhaler device.

Metered-dose inhaler devices suitable for administering therapeutic agents by inhalation are described in the art and examples of such devices are commercially available. For example, representative metered-dose inhaler devices or products include AeroBid Inhaler System (Forest Pharmaceuticals); Atrovent Inhalation Aerosol (Boehringer Ingelheim); Flovent (GlaxoSmithKline); Maxair Inhaler (3M); Proventil Inhaler (Schering); Serevent Inhalation Aerosol (GlaxoSmithKline); and the like.

In another particular embodiment, the pharmaceutical composition is administered by inhalation using a nebulizer inhaler. Such nebulizer devices typically produce a stream of high velocity air that causes the pharmaceutical composition to spray as a mist that is carried into the patient's respiratory tract. Accordingly, when formulated for use in a nebulizer inhaler, the therapeutic agent can be micronized or nanomilled and combined with a suitable carrier to form a suspension.

A representative pharmaceutical composition for use in a nebulizer inhaler comprises a suspension comprising from about 0.05 μg/mL to about 20 mg/mL of the crystalline hydrate of the disclosure and excipients compatible with nebulized formulations. In one embodiment, the solution has a pH of about 3 to about 8.

Nebulizer devices suitable for administering therapeutic agents by inhalation are described in the art and examples of such devices are commercially available. For example, representative nebulizer devices or products include the Respimat Softmist Inhaler (Boehringer Ingelheim); the AERx Pulmonary Delivery System (Aradigm Corp.); the PARI LC Plus Reusable Nebulizer (Pari GmbH); and the like.

In yet another embodiment, the pharmaceutical compositions of the disclosure may alternatively be prepared in a dosage form intended for oral administration. Suitable pharmaceutical compositions for oral administration may be in the form of capsules, tablets, pills, lozenges, cachets, dragees, powders, granules; or as a suspension in an aqueous or non-aqueous liquid; and the like; each containing a predetermined amount the crystalline hydrate of compound **1** (Form I) as an active ingredient.

When intended for oral administration in a solid dosage form, the pharmaceutical compositions of the disclosure will typically comprise the active agent and one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate. Optionally or alternatively, such solid dosage forms may also comprise: fillers or extenders, binders, humectants, solution retarding agents, absorption accelerators, wetting agents, absorbents, lubricants, coloring agents, and buffering agents. Release agents, wetting agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the pharmaceutical compositions of the disclosure.

Form I may also be formulated as a sterile aqueous suspension for ocular injection. Useful excipients that may be included in such an aqueous formulation include polysorbate 80, carboxymethylcellulose, potassium chloride, calcium chloride, magnesium chloride, sodium acetate, sodium citrate, histidine, α-α-trehalose dihydrate, sucrose, polysorbate 20, hydroxypropyl-β-cyclodextrin, and sodium phosphate. Benzyl alcohol may serve as a preservative and sodium chloride may be included to adjust tonicity. In addition, hydrochloric acid and/or sodium hydroxide may be added to the solution for pH adjustment. Aqueous formulations for ocular injection may be prepared as preservative-free.

Alternative formulations may also include controlled release formulations, transdermal patches, and parenteral formulations. Conventional excipients and methods

of preparation of such alternative formulations are described, for example, in the reference by Remington, *supra.*

The following non-limiting examples illustrate representative pharmaceutical compositions of the present disclosure.

Dry Powder Composition

A micronized crystalline hydrate of compound **1** (Form I) (1 g) is blended with milled lactose (25 g). This blended mixture is then loaded into individual blisters of a peelable blister pack in an amount sufficient to provide between about 0.1 mg to about 4 mg of the compound of formula 1 per dose. The contents of the blisters are administered using a dry powder inhaler.

Dry Powder Composition

A micronized crystalline hydrate of compound **1** (Form I) (1 g) is blended with milled lactose (20 g) to form a bulk composition having a weight ratio of compound to milled lactose of 1:20. The blended composition is packed into a dry powder inhalation device capable of delivering between about 0.1 mg to about 4 mg of the compound of formula 1 per dose.

Metered-Dose Inhaler Composition

A micronized crystalline hydrate of compound **1**, Form I (10 g) is dispersed in a solution prepared by dissolving lecithin (0.2 g) in demineralized water (200 mL). The resulting suspension is spray dried and then micronized to form a micronized composition comprising particles having a mean diameter less than about 1.5μm. The micronized composition is then loaded into metered-dose inhaler cartridges containing pressurized 1,1,1,2-tetrafluoroethane in an amount sufficient to provide about 0.1 mg to about 4 mg of compound **1** per dose when administered by the metered dose inhaler.

Nebulizer Composition

The crystalline hydrate of the disclosure, Form I (25 mg) is suspended in a solution containing 1.5-2.5 equivalents of hydrochloric acid, followed by addition of sodium hydroxide to adjust the pH to 3.5 to 5.5 and 3% by weight of glycerol. The suspension is administered using a nebulizer device that provides about 0.1 mg to about 4 mg of compound **1** per dose.

Aqueous formulation for ocular injection

Each mL of a sterile aqueous suspension includes from 5 mg to 50 mg of the crystalline hydrate of compound **1** (Form I), sodium chloride for tonicity, 0.99 % (w/v) benzyl alcohol as a preservative, 0.75 % carboxymethylcellulose sodium, and 0.04 %

polysorbate. Sodium hydroxide or hydrochloric acid may be included to adjust pH to 5 to 7.5.

Aqueous formulation for ocular injection

A sterile preservative-free aqueous suspension includes from 5 mg/mL to 50 mg/mL of the crystalline hydrate of compound **1** (Form I) in 10 mM sodium phosphate, 40 mM sodium chloride, 0.03 % polysorbate 20, and 5 % sucrose.

Utility

Compound **1** has been designed for the treatment of inflammatory and fibrotic disease of the respiratory tract. In particular, the compound has been designed to enable delivery of a potent anti-cytokine agent directly to the site of action of respiratory disease in the lung while limiting systemic exposure.

Compound **1** has been shown to be a potent inhibitor of the JAK family of enzymes: JAK1, JAK2, JAK3, and TYK2. In addition, compound **1** has demonstrated potent inhibition of pro-inflammatory and pro-fibrotic cytokines. It has been recognized that the broad anti-inflammatory effect of JAK inhibitors could suppress normal immune cell function, potentially leading to increased risk of infection. The present compound has been optimized to limit absorption from the lung into the plasma, thus minimizing the risk of immunosuppression.

As described in the experimental section below, the absorption and distribution of compound **1** has been profiled in preclinical assays. Compound **1** was tested in mice and showed at 5 hours post-dosing high concentration in lung tissue and low absorption into plasma. Compound **1** has been shown to inhibit an effect of the pro-inflammatory cytokine IL-13 in mouse lung tissue. Specifically, the compound has demonstrated inhibition of IL-13-induced phosphorylation of STAT6 in lung tissue which provides evidence of local lung JAK target engagement *in vivo*. This effect has been observed when the pro-inflammatory cytokine IL-13 is administered 4 hours after administration of the test compound, providing further evidence of significant retention in the lung.

Compound **1** has been demonstrated to exhibit both potent inhibitory activity at the cellular level and significant retention in lung tissue. Extensive investigation by the present inventors has determined that while it is possible to identify compounds that are potent at the cellular level or compounds that show significant retention in the lung, it is far more difficult to discover compounds that exhibit both desirable characteristics at the same time.

The anti-inflammatory activity of JAK inhibitors has been robustly demonstrated in preclinical models of asthma (Malaviya et al., *Int. Immunopharmacol.*, **2010,** *10*, 829-836; Matsunaga et al., *Biochem. and Biophys. Res. Commun.*, **2011,** *404*, 261-267; Kudlacz et al., *Eur. J. Pharmacol*, **2008**, *582*, 154-161). Cytokines implicated in asthma inflammation which signal through the JAK-STAT pathway include IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-13, IL-23, IL-31, IL-27, thymic stromal lymphopoietin (TSLP), interferon-γ (IFNγ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Accordingly, the compound **1**, and its crystalline hydrate, Form I, are expected to be useful for the treatment of inflammatory respiratory disorders, in particular, asthma. Asthma has been classified as Th2 low and Th2 high subtypes (Simpson et al, *Respirology*, **2006**, 11, 54-61). IL-4, IL-13, IL-5, and TSLP are implicated in Th2 high asthma, while IL-23/IL-12, IL-6, IL-27, and IFNgamma are implicated in Th2 low asthma. Based on its pan JAK inhibitory profile, compound **1** potently inhibits mediators of both Th2 high and Th2 low asthma. It is therefore expected that Form I of compound **1** will be useful in the treatment of both Th2 high and Th2 low asthma.

Inflammation and fibrosis of the lung is characteristic of other respiratory diseases in addition to asthma such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pneumonitis, interstitial lung diseases (including idiopathic pulmonary fibrosis), acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, bronchiolitis obliterans, and sarcoidosis. Compound **1**, and its crystalline hydrate, Form I, therefore, are also expected to be useful for the treatment of chronic obstructive pulmonary disease, cystic fibrosis, pneumonitis, interstitial lung diseases (including idiopathic pulmonary fibrosis), acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, bronchiolitis obliterans, and sarcoidosis.

When compared to its corresponding fluoro analog (compound **C-1**), compound **1** has been shown to have similar JAK activity. However, it has the advantage of giving rise to significantly less sulfation metabolism, as demonstrated in the assay section. This is significant as sulfation metabolism occurs in the lungs, which could lead to a rapid decrease in exposure of the active parent compound.

Compound **1** has demonstrated inhibition of cytokines associated with inflammation. Therefore, Form I is likely to be useful for the treatment of certain specific respiratory diseases, as detailed below.

Eosinophilic airway inflammation is a characteristic feature of diseases collectively termed eosinophilic lung diseases (Cottin et al., *Clin. Chest. Med.*, **2016**,

37(3), 535-56). Eosinophilic diseases have been associated with IL-4, IL-13 and IL-5 signaling. Eosinophilic lung diseases include infections (especially helminthic infections), drug-induced pneumonitis (induced for example by therapeutic drugs such as antibiotics, phenytoin, or l-tryptophan), fungal-induced pneumonitis (e.g. allergic bronchopulmonary aspergillosis), hypersensitivity pneumonitis and eosinophilic granulomatosis with polyangiitis (formerly known as Churg-Strauss syndrome). Eosinophilic lung diseases of unknown etiology include idiopathic acute eosinophilic pneumonia, idiopathic chronic eosinophilic pneumonia, hypereosinophilic syndrome, and Löffler syndrome.

A polymorphism in the IL-6 gene has been associated with elevated IL-6 levels and an increased risk of developing pulmonary arterial hypertension (PAH) (Fang et al., *J. Am. Soc. Hypertens.*, **2017**, 11(3), 171-177). Corroborating the role of IL-6 in PAH, inhibition of the IL-6 receptor chain gp130 ameliorated the disease in a rat model of PAH (Huang et al., *Can. J. Cardiol.*, **2016**, 32(11), 1356.e1-1356.e10).

Cytokines such as IFNγ, IL-12 and IL-6 have been implicated in a range of non-allergic lung diseases such as sarcoidosis, and lymphangioleiomyomatosis (El-Hashemite et al., *Am. J. Respir. Cell. Mol. Biol.*, **2005**, 33, 227-230, and El-Hashemite et al., *Cancer Res.*, **2004**, 64, 3436-3443). The compound of the disclosure has also been shown to inhibit IL-6 and IFNγ signaling.

Bronchiectasis and infiltrative pulmonary diseases are diseases associated with chronic neutrophilic inflammation.

Pathological T cell activation is critical in the etiology of multiple respiratory diseases. Autoreactive T cells play a role in bronchiolitis obliterans organizing pneumonia (also termed COS). Similar to COS the etiology of lung transplant rejections is linked to an aberrant T cell activation of the recipients T cells by the transplanted donor lung. Lung transplant rejections may occur early as Primary Graft Dysfunction (PGD), organizing pneumonia (OP), acute rejection (AR) or lymphocytic bronchiolitis (LB) or they may occur years after lung transplantation as Chronic Lung Allograft Dysfunction (CLAD). CLAD was previously known as bronchiolitis obliterans (BO) but now is considered a syndrome that can have different pathological manifestations including BO, restrictive CLAD (rCLAD or RAS) and neutrophilic allograft dysfunction. Chronic lung allograft dysfunction (CLAD) is a major challenge in long-term management of lung transplant recipients as it causes a transplanted lung to progressively lose functionality (Gauthier et al., Curr Transplant Rep., **2016**, 3(3), 185–191). CLAD is poorly responsive to treatment

and therefore, there remains a need for effective compounds capable of preventing or treating this condition. Several JAK-dependent cytokines such as IFNγ and IL-5 are up-regulated in CLAD and lung transplant rejection (Berastegui et al, *Clin. Transplant.* **2017**, 31, e12898). Moreover, high lung levels of CXCR3 chemokines such as CXCL9 and CXCL10 which are downstream of JAK-dependent IFN signaling, are linked to worse outcomes in lung transplant patients (Shino et al, *PLOS One*, **2017**, 12 (7), e0180281). Systemic JAK inhibition has been shown to be effective in kidney transplant rejection (Vicenti et al., *American Journal of Transplantation*, **2012**, 12, 2446-56). Therefore, JAK inhibitors have the potential to be effective in preventing or delaying lung transplant rejection and CLAD. Similar T cell activation events as described as the basis for lung transplant rejection also are considered the main driver of lung graft-versus-host disease (GVHD) which can occur post hematopoietic stem cell transplants. Similar to CLAD, lung GVHD is a chronic progressive condition with extremely poor outcomes and no treatments are currently approved. A retrospective, multicenter survey study of 95 patients with steroid-refractory acute or chronic GVHD who received the systemic JAK inhibitor ruxolitinib as salvage therapy demonstrated complete or partial response to ruxolitinib in the majority of patients including those with lung GVHD (Zeiser et al, *Leukemia*, **2015**, 29, 10, 2062-68). As systemic JAK inhibition is associated with serious adverse events and a small therapeutic index, the need remains for an inhaled lung-directed, non-systemic JAK inhibitor to prevent or delay lung transplant rejection or lung GVHD. Compound **1** has the characteristics required to meet this need. More recently, immune-checkpoint inhibitor induced pneumonitis, another T cell mediated lung disease emerged with the increased use of immune-checkpoint inhibitors. In cancer patients treated with these T cell stimulating agents, fatal pneumonitis can develop.

The mixed lymphocyte reaction assay is an in-vitro assay that mimics transplant rejection. Compound **1** was shown to effectively inhibit IFNγ secretion.

In one embodiment, therefore, the disclosure provides a method of treating a respiratory disease in a mammal (e.g., a human), the method comprising administering to the mammal (or human) a therapeutically-effective amount of the crystalline hydrate of compound **1** (Form I), or of a pharmaceutical composition comprising the crystalline hydrate of compound **1** (Form I).

In one embodiment, the respiratory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonitis, idiopathic pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, bronchitis,

emphysema, sarcoidosis, an eosinophilic disease, a lung infection, a helminthic infection, pulmonary arterial hypertension, lymphangioleiomyomatosis, bronchiectasis, an infiltrative pulmonary disease, drug-induced pneumonitis, fungal induced pneumonitis, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, eosinophilic granulomatosis with polyangiitis, idiopathic acute eosinophilic pneumonia, idiopathic chronic eosinophilic pneumonia, hypereosinophilic syndrome, Löffler syndrome, bronchiolitis obliterans organizing pneumonia, lung graft-versus-host disease, and immune-checkpoint-inhibitor induced pneumonitis. In some embodiments, the respiratory disease is asthma. In some embodiments, the asthma is moderate to severe asthma. In some embodiments, the asthma is mild to moderate asthma. In some embodiments, the pharmaceutical composition is administered by inhalation. In some embodiments, the asthma is Th2 high asthma. In some embodiments, the asthma is Th2 low asthma.

In one embodiment, the disclosure provides a method of preventing or delaying lung transplant rejection in a mammal (e.g., a human), the method comprising administering to the mammal (or human) a therapeutically-effective amount of the crystalline hydrate of compound **1** (Form I), or of a pharmaceutical composition comprising the crystalline hydrate of compound **1** (Form I). In some embodiments, the lung transplant rejection is selected from the group consisting of primary graft dysfunction, organizing pneumonia, acute rejection, lymphocytic bronchiolitis, and chronic lung allograft dysfunction. In some embodiments, the lung transplant rejection is acute lung transplant rejection. In some embodiments, the lung transplant rejection is chronic lung allograft dysfunction. In some embodiments, the lung transplant rejection is selected from the group consisting of bronchiolitis obliterans, restrictive chronic lung allograft dysfunction, and neutrophilic allograft dysfunction. In some embodiments, the pharmaceutical composition is administered by inhalation.

Also provided herein are uses of Form I in medical therapy and in the manufacture of a formulation or medicament for treating, preventing, delaying, or ameliorating diseases amenable to treatment with a JAK inhibitor, in particular respiratory diseases and lung transplant rejection.

The disclosure further provides a method of treating asthma in a mammal, the method comprising administering to the mammal a therapeutically-effective amount of the crystalline hydrate of compound **1** (Form I), or of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I).

When used to treat asthma, the crystalline hydrate of compound **1** (Form I), will typically be administered in a single daily dose or in multiple doses per day, although other forms of administration may be used. The amount of active agent administered per dose or the total amount administered per day will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

The disclosure further provides a method of treating a respiratory disease (including but not limited to the disease described herein) in a mammal, the method comprising administering to the mammal a therapeutically-effective amount of the crystalline hydrate of compound **1** (Form I), or of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I).

When used to treat a respiratory disease (including but not limited to the disease described herein), the crystalline hydrate of compound **1** (Form I) will typically be administered in a single daily dose or in multiple doses per day, although other forms of administration may be used. The amount of active agent administered per dose or the total amount administered per day will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

Human coronavirus is a common respiratory pathogen and typically induces mild upper respiratory disease. The two highly pathogenic viruses, Severe Acute Respiratory Syndrome associated- Coronavirus (SARS-CoV-1) and Middle East Respiratory Syndrome-associated Coronavirus (MERS-CoV), caused severe respiratory syndromes resulting in more than 10% and 35% mortality, respectively (Assiri et al., N Engl J Med., **2013**, 369, 407–1). The recent emergence of Coronavirus Disease 2019 (COVID-19 and subsequent pandemic has created a global health care emergency. Similar to SARS-CoV-1 and MERS-CoV, a subset of patients (about 16%) can develop a severe respiratory illness manifested by acute lung injury (ALI) leading to ICU admission (about 5%), respiratory failure (about 6.1%) and death (Wang et al., JAMA, **2020**, 323, 11, 1061-1069; Guan et al., *N Engl J Med.*, **2020**, 382, 1708-1720; Huang et al., *The Lancet*, **2020**.

395 (10223), 497–506; Chen et al., *The Lancet*, **2020**, 395(10223), 507–13). A subgroup of patients with COVID-19 appears to have a hyperinflammatory "cytokine storm" resulting in acute lung injury and acute respiratory distress syndrome (ARDS). This cytokine storm may also spill over into the systemic circulation and produce sepsis and ultimately, multi-organ dysfunction syndrome. The dysregulated cytokine signaling that appears in COVID-19 is characterized by increased expression of interferons (IFNs), interleukins (ILs), and chemokines, resulting in ALI and associated mortality. This hyperinflammatory response can potentially be modulated and treated by a lung-selective pan-Janus Kinase (JAK) inhibitor. Monoclonal antibodies directed against IL-6 (tocilizumab and sarilumab) appear to be effective in treating patients with ALI from COVID-19 (Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective Treatment of Severe COVID-19 Patients with Tocilizumab, **2020**, *PNAS*, https://doi.org/10.1073/pnas.2005615117). Though in-vivo models of COVID-19 have yet to be published, infection with mouse adapted strains of the 2003 SARS-CoV-1 and 2012 MERS-CoV, as well as a transgenic mouse expressing the human SARS-CoV-1 receptor hACE2 infected with human SARS-CoV-1, demonstrate elevations of JAK-dependent cytokines, such as IFNγ, IL-6, and IL-12, and downstream chemokines, such as chemokine (C-C motif) ligand 10 (CCL10), CCL2, and CCL7 (McCray et al., *J Virol.*, **2007**, 81(2), 813–21; Gretebeck et al., *Curr Opin Virol.* **2015**, 13, 123–9.; Day et al., *Virology*. **2009**, 395(2), 210–22. JAK inhibitors have also been shown to be beneficial in mouse models of lipopolysaccharide-or ganciclovir-induced ALI (Severgnini et al., *Am J Respir Crit Care Med.*, **2005**, 171(8), 858-67; Jin et al., *Am J Physiol-Lung Cell Mol Physiol.*, **2018**, 314(5), L882–92). Finally, based on the results of clinical trials, baricitinib, a JAK inhibitor, has received an emergency use authorization (EUA) in combination with remdesivir, for the treatment of COVID-19 in patients requiring supplemental oxygen, invasive mechanical ventilation, or extracorporeal membrane oxygenation (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-drug-combination-treatment-covid-19#:~:text=Today%2C%20the%20U.S.%20Food%20and,or%20older%20requiring%20supplemental%20oxygen%2C). In a clinical trial of hospitalized patients with COVID-19, baricitinib, in combination with remdesivir, was shown to reduce time to recovery within 29 days after initiating treatment compared to patients who received a placebo with remdesivir.

Therefore, compound **1**, which is a lung-selective inhaled pan-JAK inhibitor, could be uniquely suited to dampen the cytokine storm associated with COVID-19. By delivering to the lung and avoiding systemic immunosuppression, additional infections that lead to worsened mortality may also be avoided. This is particularly true in those patients requiring ventilatory support. As major causes of death in subjects with COVID-19 appear to be comorbidities and superinfection, an inhaled medication may be a way to avoid systemic immunosuppression that would pre-dispose patients to these risks.

Therefore, the present disclosure provides a method of treating a mammal (or patient) infected with a coronavirus such as SARS-CoV-1, SARS-CoV-2, and MERS-CoV, or the symptoms thereof, the method comprising administering to the mammal (or patient) the crystalline hydrate of compound **1** (Form I) of the present disclosure, or of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I) of the present disclosure. The present disclosure also provides a method of treating ALI and/or ARDS in a mammal (or a patient) caused by a coronavirus infection (such as SARS-CoV-1, SARS-CoV-2, and MERS-CoV), the method comprising administering to the mammal (or patient) the crystalline hydrate of compound **1** (Form I) of the present disclosure, or of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I) of the present disclosure.

The mechanism of action of JAK inhibitors has been linked to the treatment of nasal inflammatory diseases (Therapeutic Effects of Intranasal Tofacitinib on Chronic Rhinosinusitis with Nasal Polyps in Mice, Joo et al., *The Laryngoscope*, **2020**, https://doi.org/10.1002/lary.29129). Further, Dupilumab, which acts by blocking the IL-4 and IL-13 signaling pathways, has been approved for the treatment of chronic rhinosinusitis with nasal polyps.

Therefore, also provided herein is a method of treating nasal inflammatory diseases in a mammal (e.g. a human), the method comprising administering to the mammal (or human) the crystalline hydrate of compound **1** (Form I) of the present disclosure, or of a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I) of the present disclosure. In some embodiments, the nasal inflammatory disease is selected from the group consisting of chronic rhinosinusitis with or without nasal polyps, nasal polyposis, sinusitis with nasal polyps, and rhinitis (non-allergic, allergic, perenial, and vasomotor rhinitis).

As a JAK inhibitor, the crystalline hydrate of compound **1** (Form I) of the disclosure may also be useful for a variety of other diseases. The crystalline hydrate of compound **1** (Form I) of the disclosure may be useful for a variety of gastrointestinal inflammatory indications that include, but are not limited to, inflammatory bowel disease, ulcerative colitis (proctosigmoiditis, pancolitis, ulcerative proctitis and left-sided colitis), Crohn's disease, collagenous colitis, lymphocytic colitis, Behcet's disease, celiac disease, immune checkpoint inhibitor induced colitis, ileitis, eosinophilic esophagitis, graft versus host disease-related colitis, and infectious colitis. Ulcerative colitis (Reimund et al., *J. Clin. Immunology,* **1996**, *16*, 144-150), Crohn's disease (Woywodt et al., *Eur. J. Gastroenterology Hepatology*, **1999**, *11*, 267-276), collagenous colitis (Kumawat et al., *Mol. Immunology*, **2013**, *55*, 355-364), lymphocytic colitis (Kumawat et al., **2013**), eosinophilic esophagitis (Weinbrand-Goichberg et al., *Immunol. Res.*, **2013**, *56*, 249-260), graft versus host disease-related colitis (Coghill et al., *Blood*, **2001**, *117*, 3268-3276), infectious colitis (Stallmach et al., *Int. J. Colorectal Dis.*, **2004**, *19*, 308–315), Behcet's disease (Zhou et al., *Autoimmun. Rev.*, **2012**, *11*, 699-704), celiac disease (de Nitto et al., *World J. Gastroenterol.*, **2009**, *15*, 4609-4614), immune checkpoint inhibitor induced colitis (e.g., CTLA-4 inhibitor-induced colitis; (Yano et al., *J. Translation. Med.*, **2014**, *12*, 191), PD-1- or PD-L1-inhibitor-induced colitis), and ileitis (Yamamoto et al., *Dig. Liver Dis.*, **2008**, *40*, 253-259) are characterized by elevation of certain pro-inflammatory cytokine levels. As many pro-inflammatory cytokines signal via JAK activation, compounds described in this application may be able to alleviate the inflammation and provide symptom relief. In particular, the crystalline hydrate of compound **1** (Form I) of the disclosure may be useful for the induction and maintenance of remission of ulcerative colitis, and for the treatment of Crohn's disease, immune checkpoint inhibitor induced colitis, and the gastrointestinal adverse effects in graft versus host disease. In one embodiment, therefore, the disclosure provides a method of treating a gastrointestinal inflammatory disease in a mammal (e.g., a human), the method comprising administering to the mammal the crystalline hydrate of compound **1** (Form I) of the disclosure, or a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and the crystalline hydrate of compound **1** (Form I).

Atopic dermatitis and other inflammatory skin diseases have been associated with elevation of proinflammatory cytokines that rely on the JAK-STAT pathway. Therefore, the crystalline hydrate of compound **1** (Form I) of the disclosure, may be beneficial in a number of dermal inflammatory or pruritic conditions that include, but are not limited to

atopic dermatitis, alopecia areata, vitiligo, psoriasis, dermatomyositis, cutaneous T cell lymphoma (Netchiporouk et al., *Cell Cycle* **2014**; *13*, 3331-3335) and subtypes (Sezary syndrome, mycosis fungoides, pagetoid reticulosis, granulomatous slack skin, lymphomatoid papulosis, pityriasis lichenoides chronica, pityriasis lichenoides et varioliformis acuta, CD30+ cutaneous T-cell lymphoma, secondary cutaneous CD30+ large cell lymphoma, non-mycosis fungoides CD30− cutaneous large T-cell lymphoma, pleomorphic T-cell lymphoma, Lennert lymphoma, subcutaneous T-cell lymphoma, angiocentric lymphoma, blastic NK-cell lymphoma), prurigo nodularis, lichen planus, primary localized cutaneous amyloidosis, bullous pemphigoid, skin manifestations of graft versus host disease, pemphigoid, discoid lupus, granuloma annulare, lichen simplex chronicus, vulvar/scrotal/perianal pruritus, lichen sclerosus, post herpetic neuralgia itch, lichen planopilaris, and foliculitis decalvans. In particular, atopic dermatitis (Bao et al., *JAK-STAT*, **2013**, *2*, e24137), alopecia areata (Xing et al., *Nat. Med.* **2014**, *20*, 1043-1049), vitiligo (Craiglow et al, *JAMA Dermatol.* **2015**, *151*, 1110-1112), prurigo nodularis (Sonkoly et al., *J. Allergy Clin. Immunol.* **2006**, *117*, 411-417), lichen planus (Welz-Kubiak et al., *J. Immunol. Res.* **2015**, ID:854747), primary localized cutaneous amyloidosis (Tanaka et al., *Br. J. Dermatol.* **2009**, *161*, 1217-1224), bullous pemphigoid (Feliciani et al., *Int. J. Immunopathol. Pharmacol.* **1999**, *12*, 55-61), and dermal manifestations of graft versus host disease (Okiyama et al., *J. Invest. Dermatol.* **2014**, *134*, 992-1000) are characterized by elevation of certain cytokines that signal via JAK activation. Accordingly, the crystalline hydrate of compound **1** (Form I) of the disclosure, may be able to alleviate associated dermal inflammation or pruritus driven by these cytokines. In particular, the crystalline hydrate of compound **1** (Form I) of the disclosure, may be expected to be useful for the treatment of atopic dermatitis and other inflammatory skin diseases. In one embodiment, therefore, the disclosure provides a method of treating an inflammatory skin disease in a mammal (e.g., a human), the method comprising applying a pharmaceutical composition comprising the crystalline hydrate of compound **1** (Form I) of the disclosure, and a pharmaceutical carrier to the skin of the mammal. In one embodiment, the inflammatory skin disease is atopic dermatitis.

Many ocular diseases have been shown to be associated with elevations of proinflammatory cytokines that rely on the JAK-STAT pathway. The crystalline hydrate of compound **1** (Form I), therefore, may be useful for the treatment of a number of ocular diseases that include, but are not limited to, uveitis, diabetic retinopathy, diabetic macular edema, dry eye disease, age-related macular degeneration, and atopic

keratoconjunctivitis. In particular, uveitis (Horai and Caspi, *J. Interferon Cytokine Res.*, **2011**, *31*, 733-744), diabetic retinopathy (Abcouwer, *J. Clin. Cell. Immunol.*, **2013**, *Suppl 1*, 1-12), diabetic macular edema (Sohn et al., *American Journal of Ophthalmology*, **2011,** *152*, 686-694), dry eye disease (Stevenson et al, *Arch. Ophthalmol.*, **2012**, *130*, 90-100), and age-related macular degeneration (Knickelbein et al, *Int. Ophthalmol. Clin.*, **2015**, *55(3)*, 63-78) are characterized by elevation of certain pro-inflammatory cytokines that signal via the JAK-STAT pathway. Retinal vein occlusion (RVO) is a highly prevalent visually disabling disease. Obstruction of retinal blood flow can lead to damage of the retinal vasculature, hemorrhage, and tissue ischemia. Although the causes for RVO are multifactorial, both vascular as well as inflammatory mediators have been shown to be important (Deobhakta et al, *International Journal of Inflammation*, **2013**, article ID 438412). Cytokines which signal through the JAK-STAT pathway, such as IL-6 and IL-13, as well as other cytokines, such as MCP-1, whose production is driven in part by JAK-STAT pathway signaling, have been detected at elevated levels in ocular tissues of patients with RVO (Shchuko et al, *Indian Journal of Ophthalmology*, **2015**, 63(12), 905-911). Accordingly, the crystalline hydrate of compound **1** (Form I) of the disclosure may be able to alleviate the associated ocular inflammation and reverse disease progression or provide symptom relief in this disease. While many patients with RVO are treated by photocoagulation, this is an inherently destructive therapy. Anti-VEGF agents are also used, but they are only effective in a fraction of patients. Steroid medications that reduce the level of inflammation in the eye (Triamcinolone acetonide and dexamethasone implants) have also been shown to provide beneficial results for patients with certain forms of RVO, but they have also been shown to cause cataracts and increased intraocular pressure/glaucoma.

Accordingly, the crystalline hydrate of compound **1** (Form I) of the disclosure may be able to alleviate the associated ocular inflammation and reverse disease progression or provide symptom relief. In one embodiment, therefore, the disclosure provides a method of treating an ocular disease in a mammal, the method comprising administering a pharmaceutical composition comprising the crystalline hydrate of compound **1** (Form I) of the disclosure and a pharmaceutical carrier to the eye of the mammal. In one embodiment, the ocular disease is uveitis, diabetic retinopathy, diabetic macular edema, dry eye disease, age-related macular degeneration, retinal vein occlusion or atopic keratoconjunctivitis. In one embodiment, the method comprises administering the crystalline hydrate of compound **1** (Form I) of the disclosure by intravitreal injection.

The crystalline hydrate of compound **1** (Form I) may also be used in combination with one or more compound useful to ocular diseases.

The crystalline hydrate of compound **1** (Form I) may also be useful to treat other diseases such as other inflammatory diseases, autoimmune diseases or cancers. The crystalline hydrate of compound **1** (Form I) of the disclosure may be useful to treat one or more of arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, transplant rejection, xerophthalmia, psoriatic arthritis, diabetes, insulin dependent diabetes, motor neurone disease, myelodysplastic syndrome, pain, sarcopenia, cachexia, septic shock, systemic lupus erythematosus, leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, ankylosing spondylitis, myelofibrosis, B-cell lymphoma, hepatocellular carcinoma, Hodgkins disease, breast cancer, Multiple myeloma, melanoma, non-Hodgkin lymphoma, non-small-cell lung cancer, ovarian clear cell carcinoma, ovary tumor, pancreas tumor, polycythemia vera, Sjogren's syndrome, soft tissue sarcoma, sarcoma, splenomegaly, T-cell lymphoma, and thalassemia major.

Combination therapy

The crystalline hydrate of compound **1** (Form I) of the disclosure may be used in combination with one or more agents which act by the same mechanism or by different mechanisms to treat a disease. The different agents may be administered sequentially or simultaneously, in separate compositions or in the same composition. Useful classes of agents for combination therapy include, but are not limited to, a beta 2 adrenoceptor agonist, a muscarinic receptor antagonist, a glucocorticoid agonist, a G-protein coupled receptor-44 antagonist, a leukotriene D4 antagonist, a muscarinic M3 receptor antagonist, a histamine H1 receptor antagonist, an immunoglobulin E antagonist, a PDE 4 inhibitor, an IL-4 antagonist, a muscarinic M1 receptor antagonist, a histamine receptor antagonist, an IL-13 antagonist, an IL-5 antagonist, a 5-Lipoxygenase inhibitor, a beta adrenoceptor agonist, a CCR3 chemokine antagonist, a CFTR stimulator, an immunoglobulin modulator, an interleukin 33 ligand inhibitor, a PDE 3 inhibitor, a phosphoinositide-3 kinase delta inhibitor, a thromboxane A2 antagonist, an elastase inhibitor, a Kit tyrosine kinase inhibitor, a leukotriene E4 antagonist, a leukotriene antagonist, a PGD2 antagonist, a TNF alpha ligand inhibitor, a TNF binding agent, a complement cascade inhibitor, an eotaxin ligand inhibitor, a glutathione reductase inhibitor, an histamine H4 receptor antagonist, an IL-6 antagonist, an IL2 gene stimulator, an immunoglobulin gamma Fc receptor IIB modulator, an interferon gamma ligand, an interleukin 13 ligand inhibitor, an

interleukin 17 ligand inhibitor, a L-Selectin antagonist, a leukocyte elastase inhibitor, a leukotriene C4 antagonist, a Leukotriene C4 synthase inhibitor, a membrane copper amine oxidase inhibitor, a metalloprotease-12 inhibitor, a metalloprotease-9 inhibitor, a mite allergen modulator, a muscarinic receptor modulator, a nicotinic acetylcholine receptor agonist, a nuclear factor kappa B inhibitor, a p-Selectin antagonist, a PDE 5 inhibitor, a PDGF receptor antagonist, a phosphoinositide-3 kinase gamma inhibitor, a TLR-7 agonist, a TNF antagonist, an Abl tyrosine kinase inhibitor, an acetylcholine receptor antagonist, an acidic mammalian chitinase inhibitor, an ACTH receptor agonist, an actin polymerization modulator, an adenosine A1 receptor antagonist, an adenylate cyclase stimulator, an adrenoceptor antagonist, an adrenocorticotrophic hormone ligand, an alcohol dehydrogenase 5 inhibitor, an alpha 1 antitrypsin stimulator, an alpha 1 proteinase inhibitor, an androgen receptor modulator, an angiotensin converting enzyme 2 stimulator, an ANP agonist, a Bcr protein inhibitor, a beta 1 adrenoceptor antagonist, a beta 2 adrenoceptor antagonist, a beta 2 adrenoceptor modulator, a beta amyloid modulator, a BMP10 gene inhibitor, a BMP15 gene inhibitor, a calcium channel inhibitor, a cathepsin G inhibitor, a CCL26 gene inhibitor, a CCR3 chemokine modulator, a CCR4 chemokine antagonist, a cell adhesion molecule inhibitor, a chaperonin stimulator, a chitinase inhibitor, a collagen I antagonist, a complement C3 inhibitor, a CSF-1 antagonist, a CXCR2 chemokine antagonist, a cytokine receptor common beta chain modulator, a cytotoxic T-lymphocyte protein-4 stimulator, a deoxyribonuclease I stimulator, a deoxyribonuclease stimulator, a dipeptidyl peptidase I inhibitor, a DNA gyrase inhibitor, a DP prostanoid receptor modulator, an E-Selectin antagonist, an EGFR family tyrosine kinase receptor inhibitor, an elastin modulator, an Endothelin ET-A antagonist, an Endothelin ET-B antagonist, an epoxide hydrolase inhibitor, a FGF3 receptor antagonist, a Fyn tyrosine kinase inhibitor, a GATA 3 transcription factor inhibitor, a Glucosylceramidase modulator, a Glutamate receptor modulator, a GM-CSF ligand inhibitor, a Guanylate cyclase stimulator, a H+ K+ ATPase inhibitor, an hemoglobin modulator, an Heparin agonist, an Histone deacetylase inhibitor, an Histone deacetylase-2 stimulator, an HMG CoA reductase inhibitor, an I-kappa B kinase beta inhibitor, an ICAM1 gene inhibitor, an IL-17 antagonist, an IL-17 receptor modulator, an IL-23 antagonist, an IL-4 receptor modulator, an Immunoglobulin G modulator, an Immunoglobulin G1 agonist, an Immunoglobulin G1 modulator, an Immunoglobulin epsilon Fc receptor IA antagonist, an Immunoglobulin gamma Fc receptor IIB antagonist, an Immunoglobulin kappa modulator, an Insulin sensitizer, an Interferon beta ligand, an

Interleukin 1 like receptor antagonist, an Interleukin 18 ligand inhibitor, an Interleukin receptor 17A antagonist, an Interleukin-1 beta ligand inhibitor, an Interleukin-5 ligand inhibitor, an Interleukin-6 ligand inhibitor, a KCNA voltage-gated potassium channel-3 inhibitor, a Kit ligand inhibitor, a Laminin-5 agonist, a Leukotriene CysLT1 receptor antagonist, a Leukotriene CysLT2 receptor antagonist, a LOXL2 gene inhibitor, a Lyn tyrosine kinase inhibitor, a MARCKS protein inhibitor, a MDR associated protein 4 inhibitor, a Metalloprotease-2 modulator, a Metalloprotease-9 modulator, a Mineralocorticoid receptor antagonist, a Muscarinic M2 receptor antagonist, a Muscarinic M4 receptor antagonist, a Muscarinic M5 receptor antagonist, a Natriuretic peptide receptor A agonist, a Natural killer cell receptor modulator, a Nicotinic ACh receptor alpha 7 subunit stimulator, a NK cell receptor modulator, a Nuclear factor kappa B modulator, an opioid growth factor receptor agonist, a P-Glycoprotein inhibitor, a P2X3 purinoceptor antagonist, a p38 MAP kinase inhibitor, a Peptidase 1 modulator, a phospholipase A2 inhibitor, a phospholipase C inhibitor, a plasminogen activator inhibitor 1 inhibitor, a platelet activating factor receptor antagonist, a PPAR gamma agonist, a prostacyclin agonist, a protein tyrosine kinase inhibitor, a SH2 domain inositol phosphatase 1 stimulator, a signal transduction inhibitor, a sodium channel inhibitor, a STAT-3 modulator, a Stem cell antigen-1 inhibitor, a superoxide dismutase modulator, a T cell surface glycoprotein CD28 inhibitor, a T-cell surface glycoprotein CD8 inhibitor, a TGF beta agonist, a TGF beta antagonist, a thromboxane synthetase inhibitor, a thymic stromal lymphoprotein ligand inhibitor, a thymosin agonist, a thymosin beta 4 ligand, a TLR-8 agonist, a TLR-9 agonist, a TLR9 gene stimulator, a Topoisomerase IV inhibitor, a Troponin I fast skeletal muscle stimulator, a Troponin T fast skeletal muscle stimulator, a Type I IL-1 receptor antagonist, a Type II TNF receptor modulator, an ion channel modulator, a uteroglobin stimulator, and a VIP agonist.

Specific agents that may be used in combination with the present crystalline hydrate of compound **1** (Form I) include, but are not limited to rosiptor acetate, umeclidinium bromide, secukinumab, metenkefalin acetate, tridecactide acetate, fluticasone propionate, alpha-cyclodextrin-stabilized sulforaphane, tezepelumab, mometasone furoate, BI-1467335, dupilumab, aclidinium, formoterol, AZD-1419, HI-1640V, rivipansel, CMP-001, mannitol, ANB-020, omalizumab, tregalizumab, Mitizax, benralizumab, golimumab, roflumilast, imatinib, REGN-3500, masitinib, apremilast, RPL-554, Actimmune, adalimumab, rupatadine, parogrelil, MK-1029, beclometasone dipropionate, formoterol fumarate, mogamulizumab, seratrodast, UCB-4144, nemiralisib,

CK-2127107, fevipiprant, danirixin, bosentan, abatacept, EC-18, duvelisib, dociparstat, ciprofloxacin, salbutamol HFA, erdosteine, PrEP-001, nedocromil, CDX-0158, salbutamol, enobosarm, R-TPR-022, lenzilumab, fluticasone furoate, vilanterol trifenatate, fluticasone propionate, salmeterol, PT-007, PRS-060, remestemcel-L, citrulline, RPC-4046, nitric oxide, DS-102, gerilimzumab, Actair, fluticasone furoate, umeclidinium, vilanterol, AG-NPP709, Gamunex, infliximab, Ampion, acumapimod, canakinumab, INS-1007, CYP-001, sirukumab, fluticasone propionate, mepolizumab, pitavastatin, solithromycin, etanercept, ivacaftor, anakinra, MPC-300-IV, glycopyrronium bromide, aclidinium bromide, FP-025, risankizumab, glycopyrronium, formoterol fumarate, Adipocell, YPL-001, tiotropium bromide, glycopyrronium bromide, indacaterol maleate, andecaliximab, olodaterol, esomeprazole, dust mite vaccine, mugwort pollen allergen vaccine, vamorolone, gefapixant, revefenacin, gefitinib, ReJoin, tipelukast, bedoradrine, SCM-CGH, SHP-652, RNS-60, brodalumab, BIO-11006, umeclidinium bromide, vilanterol trifenatate, ipratropium bromide, tralokinumab, PUR-1800, VX-561, VX-371, olopatadine, tulobuterol, formoterol fumarate, triamcinolone acetonide, reslizumab, salmeterol xinafoate, fluticasone propionate, beclometasone dipropionate, formoterol fumarate, tiotropium bromide, ligelizumab, RUTI, bertilimumab, omalizumab, glycopyrronium bromide, SENS-111, beclomethasone dipropionate, CHF-5992, LT-4001, indacaterol, glycopyrronium bromide, mometasone furoate, fexofenadine, glycopyrronium bromide, azithromycin, AZD-7594, formoterol, CHF-6001, batefenterol, OATD-01, olodaterol, CJM-112, rosiglitazone, salmeterol, setipiprant, inhaled interferon beta, AZD-8871, plecanatide, fluticasone, salmeterol, eicosapentaenoic acid monoglycerides, lebrikizumab, RG-6149, QBKPN, Mometasone, indacaterol, AZD-9898, sodium pyruvate, zileuton, CG-201, imidafenacin, CNTO-6785, CLBS-03, mometasone, RGN-137, procaterol, formoterol, CCI-15106, POL-6014, indacaterol, beclomethasone, MV-130, GC-1112, Allergovac depot , MEDI-3506, QBW-251, ZPL-389, udenafil, GSK-3772847, levocetirizine, AXP-1275, ADC-3680, timapiprant, abediterol, AZD-7594, ipratropium bromide, salbutamol sulfate, tadekinig alfa, ACT-774312, dornase alfa, iloprost, batefenterol, fluticasone furoate, alicaforsen, ciclesonide, emeramide, arformoterol, SB-010, Ozagrel, BTT-1023, Dectrekumab, levalbuterol, pranlukast, hyaluronic acid, GSK-2292767, Formoterol, NOV-14, Lucinactant, salbutamol, prednisolone, ebastine, dexamethasone cipecilate, GSK-2586881, BI-443651, GSK-2256294, VR-179, VR-096, hdm-ASIT+, budesonide, GSK-2245035, VTX-1463, Emedastine, dexpramipexole, levalbuterol, N-6022, dexamethasone sodium phosphate,

PIN-201104, OPK-0018, TEV-48107, suplatast, BI-1060469, Gemilukast, interferon gamma, dalazatide, bilastine, fluticasone propionate, salmeterol xinafoate, RP-3128, bencycloquidium bromide, reslizumab, PBF-680, CRTH2 antagonist, Pranlukast, salmeterol xinafoate, fluticasone propionate, tiotropium bromide monohydrate, masilukast, RG-7990, Doxofylline, abediterol, glycopyrronium bromide, TEV-46017, ASM-024, fluticasone propionate, glycopyrronium bromide, salmeterol xinafoate, salbutamol, TA-270, Flunisolide, sodium chromoglycate, Epsi-gam, ZPL-521, salbutamol, aviptadil, TRN-157, Zafirlukast, Stempeucel, pemirolast sodium, nadolol, fluticasone propionate + salmeterol xinafoate, RV-1729, salbutamol sulfate, carbon dioxide + perfluorooctyl bromide, APL-1, dectrekumab + VAK-694, lysine acetylsalicylate, zileuton, TR-4, human allogenic adipose-derived mesenchymal progenitor cell therapy, MEDI-9314, PL-3994, HMP-301, TD-5471, NKTT-120, pemirolast, beclomethasone dipropionate, trantinterol, monosodium alpha luminol, IMD-1041, AM-211, TBS-5, ARRY-502, seratrodast, recombinant midismase, ASM-8, deflazacort, bambuterol, RBx-10017609, ipratropium + fenoterol, fluticasone + formoterol, epinastine, WIN-901X, VALERGEN-DS,OligoG-COPD-5/20, tulobuterol, oxis Turbuhaler, DSP-3025, ASM-024, mizolastine, budesonide + salmeterol, LH-011, AXP-E, histamine human immunoglobulin, YHD-001, theophylline, ambroxol + erdosteine, ramatroban, montelukast, pranlukast, AG-1321001, tulobuterol, ipratropium + salbutamol, tranilast, methylprednisolone suleptanate, colforsin daropate, repirinast, and doxofylline.

Also provided, herein, is a pharmaceutical composition comprising the crystalline hydrate of compound **1** (Form I) of the disclosure and one or more other therapeutic agents. The therapeutic agent may be selected from the class of agents specified above and from the list of specific agents described above. In some embodiments, the pharmaceutical composition is suitable for delivery to the lungs. In some embodiments, the pharmaceutical composition is suitable for inhaled or nebulized administration. In some embodiments, the pharmaceutical composition is a dry powder, or a liquid suspension.

Further, in a method embodiment, the disclosure provides a method of treating a disease or disorder in a mammal comprising administering to the mammal the crystalline hydrate of compound **1** (Form I) of the disclosure and one or more other therapeutic agents.

When used in combination therapy, the agents may be formulated in a single pharmaceutical composition, or the agents may be provided in separate compositions that are administered simultaneously or at separate times, by the same or by different routes of administration. Such compositions can be packaged separately or may be packaged together as a kit. The two or more therapeutic agents in the kit may be administered by the same route of administration or by different routes of administration.

EXAMPLES

The following synthetic and biological examples are offered to illustrate the invention, and are not to be construed in any way as limiting the scope of the invention. In the examples below, the following abbreviations have the following meanings unless otherwise indicated. Abbreviations not defined below have their generally accepted meanings.

ACN = acetonitrile
calcd = calculated
DCM = dichloromethane
DIPEA= *N,N*-diisopropylethylamine
DMF = *N,N*-dimethylformamide
EtOAc = ethyl acetate
h = hour(s)
HATU= *N,N,N',N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate
IPA = isopropyl alcohol
IPAc = isopropylacetate
KOAc = potassium acetate
MeOH= methanol
min = minute(s)
$PdCl_2$ (dppf) = dichloro(1,1'-bis(diphenylphosphino)-ferrocene)dipalladium(II)
$Pd(PPh_3)_4$ = tetrakis(triphenylphosphine)palladium(0)
RT = room temperature
TEA = triethylamine
TFA = trifluoroacetic acid
THF = tetrahydrofuran

bis(pinacolato)diboron = 4,4,5,5,4',4',5',5'-octamethyl-[2,2']bi[[1,3,2]dioxaborolanyl]

Reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka, Sigma, etc.), and used without further purification. Progress of reaction mixtures was monitored by thin layer chromatography (TLC), analytical high performance liquid chromatography (anal. HPLC), and mass spectrometry. Reaction mixtures were worked up as described specifically in each reaction; commonly they were purified by extraction and other purification methods such as temperature-, and solvent-dependent crystallization, and precipitation. In addition, reaction mixtures were routinely purified by column chromatography or by preparative HPLC, typically using C18 or BDS column packings and conventional eluents. Typical preparative HPLC conditions are described below.

Characterization of reaction products was routinely carried out by mass and ^{1}H-NMR spectrometry. For NMR analysis, samples were dissolved in deuterated solvent (such as CD_3OD, $CDCl_3$, or d_6-DMSO), and ^{1}H-NMR spectra were acquired with a Varian Gemini 2000 instrument (400 MHz) under standard observation conditions. Mass spectrometric identification of compounds was performed by an electrospray ionization method (ESMS) with an Applied Biosystems (Foster City, CA) model API 150 EX instrument or a Waters (Milford, MA) 3100 instrument, coupled to autopurification systems.

Preparative HPLC Conditions

Column:	C18, 5 µm. 21.2 x 150 mm or C18, 5 µm 21 x 250 or C14, 5 µm 21x150 mm
Column temperature:	Room Temperature
Flow rate:	20.0 mL/min
Mobile Phases:	A = Water + 0.05 % TFA B = ACN + 0.05 % TFA,
Injection volume:	(100-1500 µL)
Detector wavelength:	214 nm

Crude compounds were dissolved in 1:1 water:acetic acid at about 50 mg/mL . A 4 minute analytical scale test run was carried out using a 2.1 x 50 mm C18 column followed by a 15 or 20 minute preparative scale run using 100 µL injection with the gradient based on the % B retention of the analytical scale test run. Exact gradients were sample dependent. Samples with close running impurities were checked with a

21 x 250 mm C18 column and/or a 21 x 150 mm C14 column for best separation. Fractions containing desired product were identified by mass spectrometric analysis.

Analytic HPLC Conditions

Method A

Column: Agilent Zorbax Bonus-RP C18, 150 x 4.60 nm, 3.5 micron
Column temperature: 40 °C
Flow rate: 1.5 mL/min
Injection volume: 5 µL
Sample preparation: Dissolve in 1:1 ACN:1 M HCl
Mobile Phases: A = Water: TFA (99.95:0.05)
B = ACN:TFA (99.95:0.05)
Detector wavelength: 254 nm and 214 nm
Gradient: 26 min total (time (min)/ % B): 0/5, 18/90, 22/90, 22.5/90, 26/5

Method B

Column: Agilent Poroshell 120 Bonus-RP, 4.6 x 150 mm, 2.7 µm
Column temperature: 30 °C
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Mobile Phases: A = ACN:Water:TFA (2:98:0.1)
B = ACN:Water:TFA (90:10:0.1)
Sample preparation: Dissolve in Mobile phase B
Detector wavelength: 254 nm and 214 nm
Gradient: 60 min total (time (min)/ % B): 0/0, 50/100, 55/100, 55.1/0, 60/0

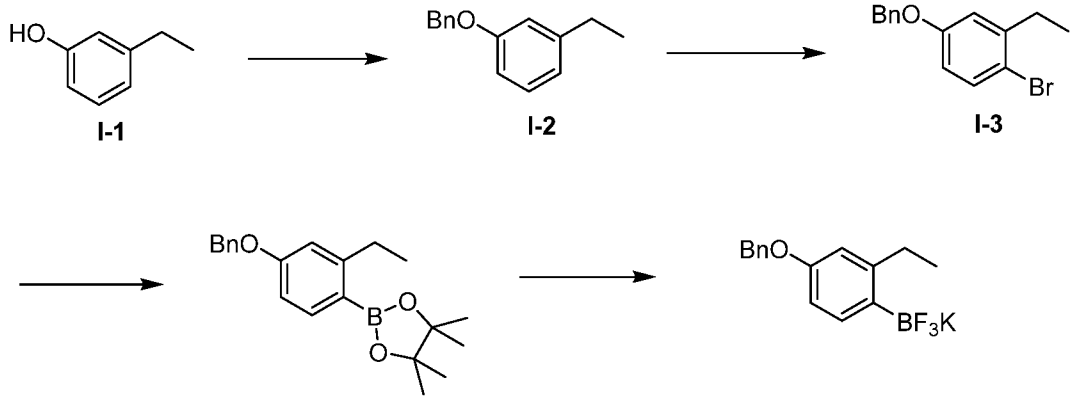
Method C

Column: Agilent Poroshell 120 Bonus-RP, 4.6 x 150 mm, 2.7 µm
Column temperature: 30 °C
Flow rate: 1.5 mL/min
Injection volume: 10 µL
Mobile Phases: A = ACN:Water:TFA (2:98:0.1)
B = ACN:Water:TFA (90:10:0.1)
Sample preparation: Dissolve in Mobile phase B (0.15 mL) then dilute with Mobile phase A (0.85 mL)

Detector wavelength: 245 nm

Gradient: 46 min total (time (min)/ % B): 0/0, 25/50, 35/100,40/100, 40.1/0, 46/0

Preparation 1: (4-(benzyloxy)-2-ethylphenyl)trifluoro-λ^4-borane, potassium salt I-5



I-1 I-2 I-3 I-4 I-5

(a) 1-(benzyloxy)-3-ethylbenzene **(I-2)**

To a stirred solution of 3-ethylphenol **(I-1)** (25.0 g, 204.0 mmol) in ACN (250 mL, 10 vol) was added potassium carbonate (42.0 g, 306 mmol) at room temperature. The resulting reaction mass was stirred at room temperature for 15 minutes, followed by the addition of benzyl bromide (24.0 mL, 204 mmol) in drop wise manner. The resulting reaction mixture was stirred for 6 hours at room temperature. After completion of the reaction (TLC monitoring), the resulting reaction mass was poured into water (1.0 L) followed by the extraction of compound with EtOAc (2 x 2L). The combined organics were washed with cold water, brine solution and dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was then purified by column chromatography over silica gel (100-200M) by using eluents 2% EtOAc in hexane to get the desired product **(I-2)** as light yellow oily compound (35.0 g, 81%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.46-7.44 (m, 2H), 7.39 (t, J = 7.6 Hz, 2H), 7.34-7.31 (m, 1H), 7.21 (t, J = 7.6 Hz), 6.86-6.80 (m, 3H), 5.07 (s, 2H), 2.64 (q, J = 7.6 Hz, 2H), 1.24 (t, J = 7.6 Hz, 3H).

(b) 4-(benzyloxy)-1-bromo-2-ethylbenzene **(I-3)**

To an ice cold stirred solution of 1-(benzyloxy)-3-ethylbenzene **(I-2)** (35.0 g, 164 mmol) in ACN (525 mL, 15 vol) was added N-bromosuccinimide (32.0 g 181 mmol) in portions over a period of 15 minutes. The resulting reaction mixture was stirred for next 1 hour at room temperature. After completion of reaction (TLC monitoring), the resulting

reaction mass was poured into ice cold water (1.50 L) followed by the extraction of compound with EtOAc (2 x 1L). The combined organics were washed with water and dried over sodium sulfate, filtered and evaporated under reduced pressure to obtain the crude product. n-Hexane (250 mL) was added to the crude material, resulting in a slurry, followed by filtration through a sintered funnel. Mother liquor was evaporated under reduced pressure to obtain the desired product **I-3** as light yellow oily compound (42.0 g, 87%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.52-7.29 (m, 7H), 6.88 (s, 1H), 6.68 (d, J = 6.0 Hz, 1H), 5.04 (s, 2H), 2.69 (q, J = 7.6 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H).

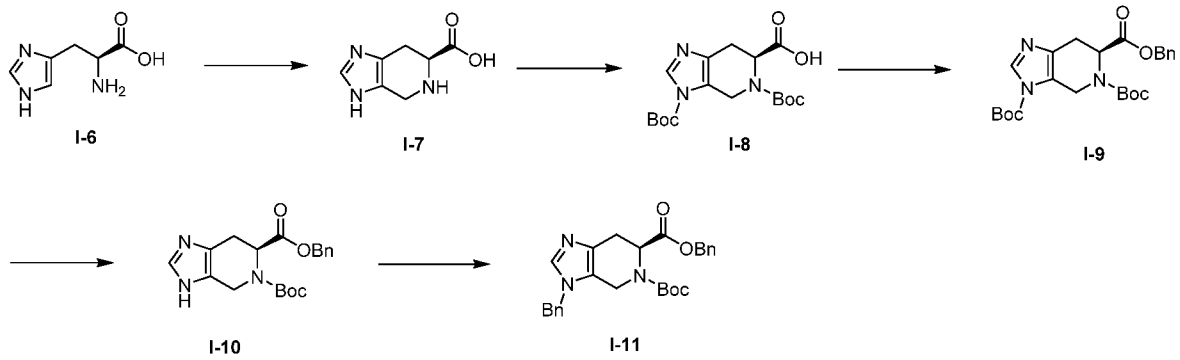
(c) 2-(4-(benzyloxy)-2-ethylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(I-4)**

A stirred solution of 4-(benzyloxy)-1-bromo-2-ethylbenzene **(I-3)** (42.0 g, 144 mmol), bis(pinacolato) diboron (44.0 g, 173 mmol), and potassium acetate (28 g, 288 mmol) in dioxane (440 mL) was degassed by purging N2 (g) for 15 min followed by addition of $PdCl_2$(dppf) DCM complex (11.0 g, 15 mmol). The resulting reaction mixture was heated up to 80°C for next 16h. After completion of the reaction (TLC monitoring), the reaction mass was filtered through celite bed and mother liquor was evaporated under reduced pressure to obtain the crude product. Crude residue was purified by column chromatography over silica gel (100-200M) by using eluents 1% EtOAc in hexane to get the desired product **(I-4)** as light yellow oily compound (32.0 g, 66%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.74 (d, J = 8.4 Hz, 1H), 7.45-7.36 (m, 5H), 6.84-6.78 (m, 2H), 5.08 (s, 2H), 2.91 (q, J = 7.6 Hz), 1.33 (s, 12H), 1.19 (t, J = 7.6 Hz, 3H).

(d) (4-(benzyloxy)-2-ethylphenyl)trifluoro-λ^4-borane, potassium salt **(I-5)**

To a stirred solution of compound 2-(4-(benzyloxy)-2-ethylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **(I-4)** (20 g, 59.0 mmol), in acetone:methanol (200 mL, 1:1 ratio, 10 vol), was added a 3M solution of potassium hydrogen fluoride (23.0 g, 295 mmol, dissolved in 98.0 mL of water). The resulting reaction mixture was stirred at room temperature for 16 hours. After completion of the reaction (TLC monitoring), the resulting reaction mass was evaporated under reduced pressure. The solid thus obtained was taken up in water (100 mL) and stirred at room temperature for 30 min. The resulting reaction mass was filtered through a sintered funnel, washed with n-hexane and dried under reduced pressure to provide the desired product **(I-5)** as a white solid (14.0 g, 74%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.43 (d, J = 7.2 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.1 Hz, 1H), 7.22 (d, J = 8.0 Hz), 6.58 (s, 1H), 6.53 (d, J = 7.9 Hz, 1H), 5.00 (s, 2H), 2.65 (q, J = 7.5 Hz, 2H), 1.07 (t, J = 7.4 Hz, 3H).

Preparation 2: 6-benzyl 5-(tert-butyl) (*S*)-3-benzyl-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate (I-11)



(a) (*S*)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, hydrochloride salt **(I-7)**

To an ice cold stirred suspension of L-histidine **(I-6)** (5.0 kg, 32.14mol) in water (40 L, 8 vol.) was added concentrated hydrochloric acid (3.93 L, 33.75 mol), followed by the addition of formaldehyde (5.50 L, 67.5 mol, 37% aq. solution) in drop wise manner. The resulting solution was stirred for 30 minutes at same temperature and then heated at 80°C for 8 hours. Reaction progress was monitored by LCMS. Water was removed under reduced pressure to obtain the crude product, and the resulting crude was stirred for 2 hours in Toluene (20 L). Organics were removed under reduced pressure to remove excess water and the compound was azeotropically dried. The resulting material was then taken in diethyl ether (20 L) and stirred for 2 hours. The solid material was then filtered and air dried to obtain the desired product **(I-7)** as an off-white solid (6.50 Kg, 85%). ^{1}H NMR (400 MHz, D2O) δ 8.69 (s, 1H), 4.56 (d, J = 15.4 Hz, 1H), 4.42 (d, J = 15.5 Hz, 1H), 4.20 (dd, J = 5.5, 5.2 Hz, 1H), 3.42 (dd, J = 5.0, 17.0 Hz, 1H), 3.11 (dd, J = 10.2, 16.8 Hz, 1H).

(b) (*S*)-3,5-bis(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid **(I-8)**

To an ice cold stirred solution of (*S*)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid di-hydrochloride **(I-7)** (6.10 Kg, 25.40mol) in 1,4-dioxane (48 L, 8 vol) and water (48 L, 8 vol) was added triethylamine (12.36 L, 89 mol) drop wise followed by the addition of di-tert-butyl dicarbonate (18.07 L, 78.74 mol, dissolved in 5 L of 1,4-dioxane) over a period of 30 min. The resulting reaction mixture was stirred at room temperature for next 16 hours. After completion of reaction (TLC & LCMS

monitoring), the yellowish reaction mixture was diluted with water (10 L) and washed successively with diethyl ether (2 x 10 L) and EtOAc (2 x 7.50 L). The organic phase was discarded. The aqueous layer was cooled and brought to pH ~3 with 6N HCl solution; the aqueous phase was extracted with EtOAc (3 x 10 L). The combined organics were washed with brine solution, dried over sodium sulfate, and concentrated under reduced pressure. The oily residue was crystallized from 30% EtOAc:Hexanes to afford the desired product **(I-8)** as off-white solid (5.1 Kg, 55%). (m/z): [M+H]+ calcd for $C_{17}H_{25}N_3O_6$ 368.18 found 368.21.

(c) 6-benzyl 3,5-di-*tert*-butyl (*S*)-6,7-dihydro-3H-imidazo[4,5-c]pyridine-3,5,6(4H)-tricarboxylate **(I-9)**

To an ice cold solution of (*S*)-3,5-bis(*tert*-butoxycarbonyl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid **(I-8)** (5.1 Kg, 13.88 mol) in DCM (51 L, 10 vol) was added sequentially saturated aqueous sodium bicarbonate (41.0 L, 8 vol), tetra-butyl ammonium iodide (5.13 Kg, 13.88 mol) and benzyl bromide (2.47 L, 20.82 mol). The resulting reaction mixture was stirred at room temperature for next 16 hours. After completion of the reaction (TLC & LCMS monitoring), the biphasic solution was separated. The aqueous layer was extracted with DCM (3 x 10 L). The combined organics were washed with brine solution, dried over sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product, which was purified by column chromatography through silica gel (100-200M) by using eluents 40% EtOAc in hexane to get the desired product **(I-9)** as viscous oil (4.50 Kg, 72%). (m/z): [M+H]+ calcd for $C_{24}H_{31}N_3O_6$ 458.22 found 458.60.

(d) 6-benzyl 5-(*tert*-butyl) (*S*)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-10)**

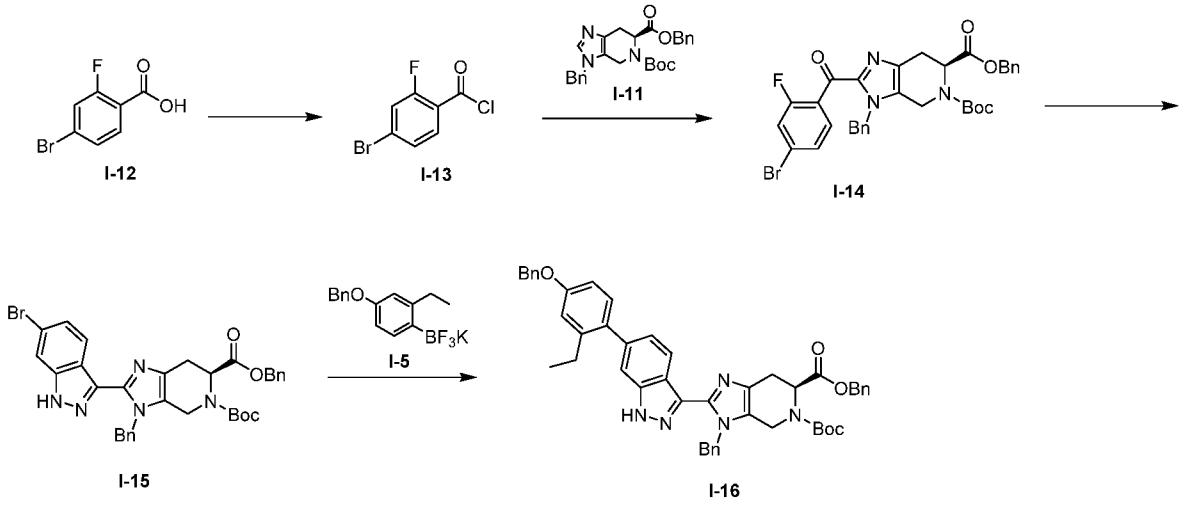
To an ice cold solution of 6-benzyl 3,5-di-*tert*-butyl (*S*)-6,7-dihydro-3H-imidazo[4,5-c]pyridine-3,5,6(4H)-tricarboxylate **(I-9)** (4.50 Kg, 9.84 mol) in IPA (45 L, 10 vol) was added ammonium hydroxide (36 L, 8 vol) drop wise. The resulting reaction mixture was further stirred at room temperature for the next 16 hours. After completion of the reaction (TLC & LCMS monitoring), the resulting mixture was diluted with water (25 L) followed by extraction with EtOAc (3 x 20 L). The combined organics were washed with brine solution, dried over sodium sulfate, filtered and concentrated under reduced pressure to yield the crude product which was purified by column chromatography through silica gel (100-200M) by using eluents 2% MeOH in DCM to obtain the desired

product **(I-10)** as a thick viscous oil (2.70 Kg, 77%). (m/z): [M+H]+ calcd for $C_{19}H_{23}N_3O_4$ 358.17 found 358.33.

(e) 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-11)**

To an ice cold solution of 6-benzyl 5-(*tert*-butyl) (*S*)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-10)** (2.70 kg, 7.55 mol) in DCM (32.4 L, 12 vol) was added aqueous 1N sodium hydroxide (24.3 L, 9 vol) followed by the sequential addition of tetra-butyl ammonium iodide (2.80 Kg, 7.55 mol) and benzyl bromide (0.99 L, 8.31 mol). The resulting reaction mixture was stirred at room temperature for next 2 hours. After completion of the reaction (TLC & LCMS monitoring), the biphasic solution was separated. The aqueous layer was extracted with DCM (3 x 10 L). The combined organics were washed with brine solution, dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield the crude product which was purified by column chromatography over silica gel (100-200M) by using eluents 40% EtOAc in hexane to obtain the desired product **(I-11)** as a viscous oil (1.70 Kg, 63%). (m/z): [M+H]+ calcd for $C_{26}H_{29}N_3O_4$ 448.22 found 448.20.

Preparation 3: 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate (I-16)



(a) 4-bromo-2-fluorobenzoyl chloride **(I-13)**

To an ice cold stirred solution of 4-bromo-2-fluorobenzoic acid **(I-12)** (1.25 Kg, 5.71 mol) in DCM (12.5 L, 15 vol), was added oxalyl chloride (0.98 L, 11.42 mol) in a drop wise manner. The resulting reaction mixture was stirred for 10 min at the same

temperature. DMF (150 mL) was then added in a drop wise manner to the reaction mixture. The resulting reaction mass was allowed to warm to room temperature and stirred for 2 hours. After completion of the reaction (by TLC monitoring), excess oxalyl chloride was removed under reduced pressure under a nitrogen atmosphere to obtain the crude product **(I-13)** (1.08 Kg, 80%), which was used in the next step without further purification.

(b) 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(4-bromo-2-fluorobenzoyl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-14)**

To a stirred solution of 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-11)** (1.70 Kg, 3.80 mol) in ACN (13.6 L, 8 vol) was added triethylamine (2.11 L, 15.2 mol) followed by the addition of 4-bromo-2-fluorobenzoyl chloride **(I-13)** (1.08 Kg, 4.56 mol in 3.4 L ACN, 2 vol) at room temperature. After completion of addition, the resulting reaction mixture color turned brown from light yellow. The resulting reaction mixture was stirred at same temperature for 30 min, and reaction progress was monitored by TLC. The resulting reaction mixture was quenched with ice cold water (10 L), followed by extraction with EtOAc (3 x 5 L) and combined organics were washed with brine solution. The organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure to yield the crude product which was purified by column chromatography over silica gel (100-200M) by using eluents 20% EtOAc in hexane to obtain the desired product **(I-14)** (1.74 Kg, 71%). %). (m/z): [M+H]+ calcd for $C_{33}H_{31}BrFN_3O_5$ 648.14 found 648.20.

(c) 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(6-bromo-1H-indazol-3-yl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-15)**

To a stirred solution of 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(4-bromo-2-fluorobenzoyl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-14)** (1.74 Kg, 2.68 mol) in THF (26.0 L, 15 vol) was added hydrazine hydrate (0.705 L, 13.4 mol) at room temperature. The resulting reaction mixture was heated at 60°C for 3 hours. After completion of the reaction (TLC monitoring), the resulting reaction mass was poured into ice cold water (10 L) followed by extraction of compound with EtOAc (3 x 10L). The combined organics were washed with brine and dried over sodium sulfate, filtered, and evaporated under reduced pressure to yield the crude product, which was purified by column chromatography over silica gel (100-200M) by using eluents 20% EtOAc in hexane to obtain the desired product **(I-15)** as an off-white solid (1.12 Kg, 65%). (m/z): [M+H]+ calcd for $C_{33}H_{32}BrN_5O_4$ 642.16 found 642.21.

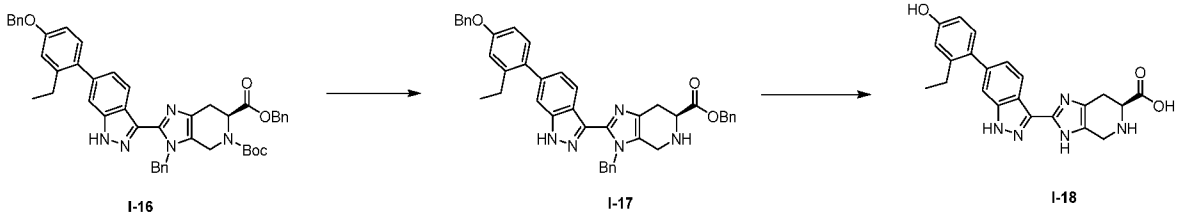
(d) 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-16)**

Bis(pinacolato)diboron (250 g, 984 mmol) was charged to a 5 L 3-neck single walled flask previously etched using fluoride chemistry, along with propan-2-ol (1882 mL, 2.46E+04 mmol) and the mixture was stirred until fully dissolved. Dissolution was endothermic (-4 °C). In a 4 L Erlenmeyer flask, previously etched using fluoride chemistry, potassium fluoride hydrofluoride (538 g, 6891 mmol) was dissolved in water (2.306 L, 1.28E+05 mmol) to form a 3M solution. The dissolution was endothermic (-12 °C). The solution was then filtered to remove a small amount of insoluble material from the potassium fluoride hydrofluoride. Once both solutions were fully dissolved, the contents of the Erlenmeyer flask were charged into the single walled flask portion-wise over 15 minutes. A moderate exotherm was observed (+10 °C). The solution became a thick and translucent semi-opaque gray slurry during the addition and stirring was increased to keep the contents well mixed. The mixture was stirred for 1.5 h, and then filtered through a coarse glass fritted funnel (4 L, previously etched). The filtration required 30-45 minutes to complete. The clear biphasic filtrate was discarded. The white solids were dried for 10 minutes on the filter (cracking of the cake was observed). The solids were transferred back into a cleaned 5 L 3-neck single walled flask and re-slurried with water (1.33 L, 7.38E+04 mmol). The slurry was stirred for 2 h after which time it formed a clear homogenous hydrogel. The solution was stirred for another 1 h whereupon the solids/gel were filtered out using a 4 L coarse glass funnel (previously etched). The solids were allowed to dry on the filter for 30 minutes. The solids were transferred back to a cleaned 5 L 3-neck single walled flask and re-slurried in acetone (1.084 L, 1.48E+04 mmol). The white/gray slurry was stirred for 1 h and was then filtered on a 4 L coarse glass funnel (previously etched). The filtration required 20 minutes to complete, and was then dried on the funnel for another 1 h. During this time, the solids were occasionally agitated to ensure homogenous drying. A light white powder remained after drying on the filter. The solids were dried for 20 h at 55 °C under vacuum with a slow nitrogen bleed to afford a fluffy white solid (200.3 g were collected).

To a stirred solution of 6-benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(6-bromo-1H-indazol-3-yl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-15)** (10.0 g, 16.0 mmol) in 2-methyl tetrahydrofuran (100 mL, 10 vol) was added (4-(benzyloxy)-2-ethylphenyl)trifluoro-λ^4-borane, potassium salt **(I-5)** (8.0 g, 20 mmol) and

the fluffy white solid obtained above (0.20 g). The resulting reaction mixture was degassed with nitrogen gas for 30 minutes. To this solution, a prepared aqueous solution of cesium carbonate (20.0 g, 62.0 mmol in 60 mL water, 6 vol) was added. The resulting reaction mixture was further degassed for 15 minutes followed by addition of bis(di-*tert*-butyl(4-dimethylaminophenyl)phosphine)dichloropalladium(II) (0.66 g, 0.93 mmol), and the reaction mixture was evacuated under vacuum and flushed by nitrogen. The resulting reaction mixture was heated at 110°C for 20 hours. After completion of the reaction (TLC & LCMS monitoring), the resulting reaction mixture was cooled to room temperature and filtered through a celite bed, then further washed with EtOAc (3 x 0.5 L). The combined organics were washed with 1N sodium hydroxide solution (3 x 0.5 L). The combined organics were then washed with brine and dried over sodium sulfate, filtered, and evaporated under reduced pressure to yield the crude product, which was purified by column chromatography over silica gel (100-200M) by using eluents 20% EtOAc in hexane to obtain the desired product **(I-16)** (as mixture of N-benzyl regioisomers) as light yellow solid (8.0 g, 66%). (m/z): [M+H]+ calcd for $C_{48}H_{47}N_5O_5$ 774.36 found 774.59.

Preparation 4: (*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, hydrochloride (I-18)



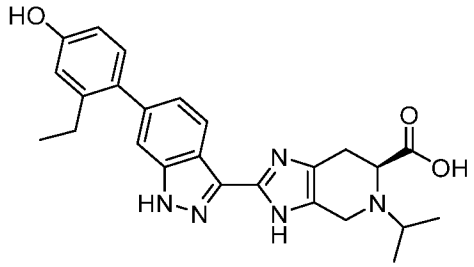
(a) Benzyl (*S*)-3-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylate, hydrochloride **(I-17)**

6-Benzyl 5-(*tert*-butyl) (*S*)-3-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-3,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridine-5,6-dicarboxylate **(I-16)** (1.0 g, 1.292 mmol) was dissolved in dioxane (8 mL) and water (1.5 mL), then hydrogen chloride solution, 4 M in dioxane (7 mL, 28.0 mmol) was added and the reaction mixture was stirred at room temperature for 3 hours (reaction progress monitored by LCMS). The reaction mixture was then frozen and lyophilized, and the crude product **(I-17)** was used directly in the next reaction (quantitative yield was assumed). (m/z): [M+H]+ calcd for $C_{43}H_{39}N_5O_3$ 674.31 found 674.3.

(b) (*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, hydrochloride **(I-18)**

Benzyl (*S*)-3-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylate, hydrochloride **(I-17)** (0.918 g, 1.292 mmol) was dissolved in 2-propanol (15 mL), hydrogen chloride solution, 5 M in water (0.258 mL, 1.292 mmol), and water (0.25 mL) at 50 °C, then palladium, 10% wt. on carbon, 50% water (0.138 g, 0.065 mmol) was added. The reaction flask was then purged with nitrogen, a hydrogen balloon was attached, and the reaction mixture was stirred at 50 °C for 4 days with the hydrogen balloon being replenished as needed (reaction progress monitored by LCMS). All solids were then removed by filtration and the resulting solution was concentrated. The residue was dissolved in 1:1 ACN/Water, frozen, and lyophilized. The resulting powder **(I-18)** was used without further purification (quantitative yield was assumed). (m/z): [M+H]+ calcd for $C_{22}H_{21}N_5O_3$ 404.17 found 404.2.

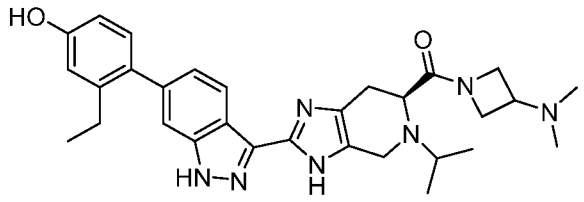
Preparation 5: (*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid (I-19)



I-19

(*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, HCl **(I-18)** (0.25 g, 0.568 mmol) was suspended in DMF (2.5 mL) and acetone (2.5 mL), then acetic acid (0.098 mL, 1.705 mmol) and sodium cyanoborohydride (0.179 g, 2.84 mmol) were added and the reaction mixture was stirred at room temperature for 24 hours (reaction progress was monitored by LCMS). The reaction mixture was concentrated, then the crude product was purified by reverse phase chromatography (5-70% ACN/Water gradient, 50 g C18aq column) to provide the TFA salt of the title compound (149 mg, 47% yield). (m/z): [M+H]+ calcd for $C_{25}H_{27}N_5O_3$ 446.21 found 446.3.

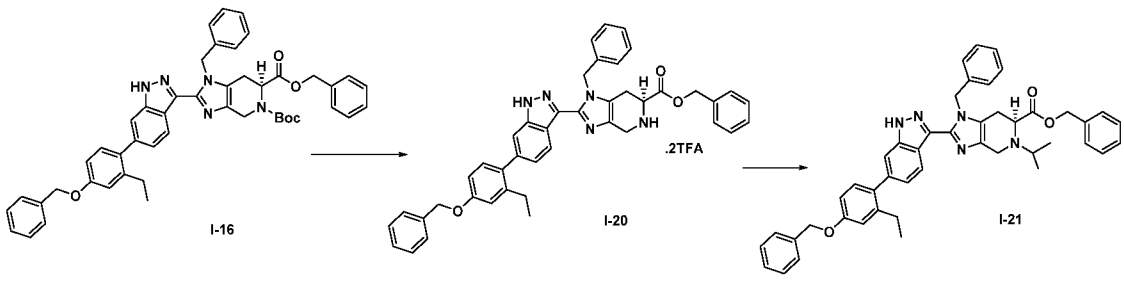
Example 1: (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone



1

(*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, TFA **(I-19)** (50 mg, 0.089 mmol), 3-(Dimethylamino)azetidine dihydrochloride (23.20 mg, 0.134 mmol), and DIPEA (0.078 mL, 0.447 mmol) were dissolved in DMF (1.5 mL), then HATU (51.0 mg, 0.134 mmol) was added and the reaction mixture was stirred at room temperature for 3 hours (reaction progress was monitored by LCMS). Hydrazine (0.014 mL, 0.447 mmol) was added to cleave undesired byproducts, and the solution was stirred at room temperature for 10 minutes. The reaction mixture was then concentrated and the crude product was purified by preparative HPLC (5-70% ACN/Water gradient, C18 column) to provide the TFA salt of the title compound (25 mg, 37% yield). (m/z): [M+H]+ calcd for $C_{30}H_{37}N_7O_2$ 528.30 found 528.3. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.09 (s, 1H), 9.40 (s, 1H), 8.27 (d, J = 8.31, 1H), 7.30 (s, 1H), 7.04 (m, 2H), 6.71 (d, J = 2.54, 1H), 6.64 (dd, J = 2.53, 8.26, 1H), 4.26 (m, 1H), 4.06 (m, 2H), 3.82 (m, 2H), 3.64 (m, 2H), 3.03 (m, 2H), 2.74 (m, 2H), 2.47 (q, J = 7.56, 2H), 2.07 (d, J = 3.69, 6H), 1.07 (m, 6H), 1.00 (t, J = 7.50, 3H).

Preparation 6: benzyl (*S*)-1-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridine-6-carboxylate (I-21)



Step 1

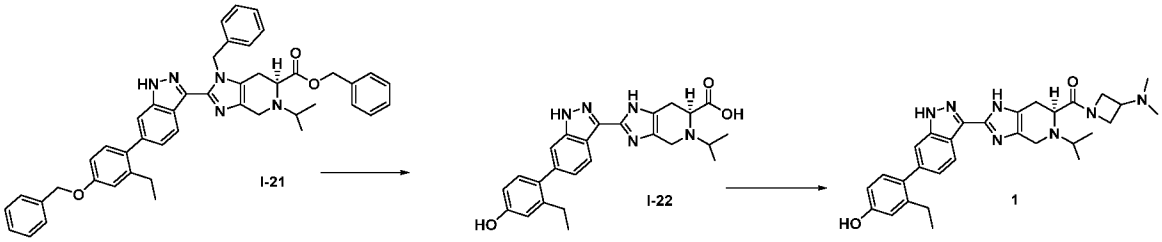
In a 3 neck dried round bottom flask was added I-16 (550 g, 0.71 moles). The compound was dissolved in DCM (4125 mL) at room temperature (24-25°C) to give a clear pale yellow solution. The solution was cooled to 0°C (ice salt mixture) over next 30 minutes (the outer temperature was -5°C). TFA (1375 mL) was then added slowly over 45 min via a dropping funnel. The reaction was stirred for 10 min at the same temperature (0°C). The reaction mass was warmed up to 30°C and stirred at the same temperature for 1.5 h. Reaction progress was monitored by TLC as well as by LCMS. After completion of the reaction, the reaction mixture was evaporated under reduced pressure at 40°C to get the crude material as a light brown thick viscous mass. Heptane stripping was conducted twice by adding 2.5 L of heptane each time and evaporating to dryness. Then compound was dried under high vacuum. The desired product **I-20** was obtained as a thick viscous liquid (641 g, 100% yield, LCMS= 94.35% pure).

Step 2

In a 3 neck dried round bottom flask, molecular sieves 4A° (641 g, w/w) were charged followed by addition of 8 volumes of Acetone (5.13 L) in a single portion under inert atmosphere at room temperature (25°C). A white suspension was observed. The material obtained in step 1 (641 g, 0.71 mol, dissolved in 7.690 L (12 volumes) of acetone) was slowly added into the flask over 10 minutes to avoid formation of a gummy mass inside the flask. A light yellow color was observed for the reaction mass. CH_3COOH (15.4 mL, 0.266 mol) was added dropwise at 25°C and the resulting reaction mass was stirred at the same temperature for 30 min. A dark yellow suspension was observed. Sodium triacetoxyborohydride (297 g, 1.4 mol) was added portion-wise at 25°C over a period of 30 min. The temperature rose to 31°C from 25°C and the reaction mass color also changed from dark yellow to light yellow. The resulting reaction mixture was stirred at 30°C for next 30 min. Reaction progress was monitored by LCMS and TLC. After completion of the reaction, the reaction mixture was cooled to 10°C and stirred for an additional 20 min. The reaction mass was then filtered through a celite bed to remove molecular sieves and sodium triacetoxyborohydride. The celite bed used for filtration was washed with 2-Me-THF 10Volumes (6.41 L) and 2-Me-THF was taken separately. The acetone mother liquor obtained above was evaporated under reduced pressure up to dryness to get a thick viscous liquid. The viscous liquid obtained was dissolved in 2-Me-THF (6.41 L) that was used for celite bed washing. To the organic layer (2-Me-THF), 10% Aq. $NaHCO_3$ (~5.5 L) was added to bring the pH to about 7.02. At this point, slight gas evolution was observed which ceased in 5 minutes. The biphasic

layer and the organic layer were separated. The organic layer was further washed with water (2.0 L twice). The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure at 45°C to give benzyl (*S*)-1-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridine-6-carboxylate **(I-21)** as a light yellowish foamy solid (500 g) 95.73% pure by HPLC. 11 batches on the same scale were performed and the materials obtained were combined. A slurry of the compound in Heptane (5 Volumes) was prepared and stirred at room temperature for 2 hrs. The compound was filtered and washed with heptane (2 Volumes). The wet cake was dried in a vacuum tray dryer for 24 hrs without applying any external temperature to get 5.40 kg of compound **I-21**.

Preparation 7: (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone (1)



Step 1:

Into a 3-neck round bottom flask was charged benzyl (S)-1-benzyl-2-(6-(4-(benzyloxy)-2-ethylphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylate (1,350 g, 1886 mmol) and tetrahydrofuran (2025 mL) with stirring at 25°C to afford a homogeneous clear yellow/orange solution. To the solution was added propan-2-ol (11,475 mL) and 6M hydrogen chloride(aq) (1257 mL) with stirring to give a homogeneous solution at 25°C. Nitrogen bubble was used to de-gas the freely stirring yellow slurry for 30 min. 10 wt % Pd/C, 50 wt % H_2O (202.5 g, 95 mmol) were added to the reactor. The nitrogen gas was shut off whereupon the reaction solution was purged with H_2 bubbling and warmed to 65°C internal temperature. The HPLC showed that the reaction was complete after 210 min. The reaction mixture was filtered through a 10 gram celite pad to remove the bulk amount of catalyst. A clear, yellow solution was collected. To the filtrate was charged 10%(wXw) SiliaMetS Thiol (101.25 g, 953 mmol) (white solid) and the mixture was stirred at 50°C over 1 hour to scavenge the remainder of the Pd. After 1 hour, the SiliaMetS Thiol was filtered off through a 0.2micron filter to afford

a light yellow colored homogeneous solution. The filtrate was then concentrated to 3X (vol) and held at 50°C. To the 50°C concentrated solution was added 3 equivalents of 12M HCl (471 ml, 5657 mmol)). After stirring at 50°C over 5 min, 2.025grams of seed was added and held at 50°C over 1 hour. The slurry thickened significantly. 15X (vol) of acetonitrile (20250 mL) was added slowly over 120 min. The batch was then held at 50°C over 2 hours then cooled to 20°C over 3 hours and held overnight with stirring. The slurry was then filtered and the cake was rinsed five times with ACN and dried under high vacuum at 50°C overnight.

Once off loaded, the cake afforded a pale orange solid as (*S*)-2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, 2HCl (850 g, 1640 mmol, 87 % yield).

A similar scheme was conducted on 1.115 kg of compound **I-21** to give an additional 700g of compound **I-22**. The two lots obtained were combined.

Step 2:

To a 20L jacketed reactor was charged N,N-dimethylazetidin-3-amine, 2HCl (432 g, 2497 mmol) and N,N-dimethylacetamide (3750 ml, 4.00E+04 mmol) at 20°C. DIPEA (2699 ml, 1.55E+04 mmol) was added at 20°C over about 5 mins. The mixture was stirred for 15 mins at 20°C to give a hazy solution which was cooled down to 10-15°C with agitation at 200rpm. (S)-2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-6-carboxylic acid, 2.2HCl (1250 g, 2378 mmol) was added to the batch while maintaining the temperature below 20°C. Exothermic reaction was observed during the addition (an exotherm of about 7°C was observed). A slurry was obtained which was agitated for about 15 mins at 15-25°C. After 15 min, a thin slurry was still observed. With an internal temperature at 17°C, HATU (994 g, 2615 mmol) was added over about 5 minutes while mixing at 350 rpm at 20 ± 5°C. The temperature rose to 22°C during the addition. A thin slurry was obtained after HATU was added. The batch was kept at 20-25°C and agitated with stirring at 215rpm until the reaction reached completion, i.e. 2h. To the completed reaction was charged a premixed cooled (5°C) solution of: 1M aq. HCl (4993 ml, 4993 mmol) diluted with 1 volume of water (1.25L), while maintaining the batch temperature below 30°C.

5 volumes of isopropyl acetate (6,250 mL) were charged to the batch and the mixture was agitated for about 15 mins. The agitation was stopped. The aqueous acid layer was separated and transferred to the reactor. The organic layer was discarded. The pH of the aqueous layer was determined to be 4-5.0. 5 volumes of isopropyl acetate (6,250 mL)

were added to the batch and agitation was conducted for about 15 mins at 20°C. The mixture was allowed to settle at 20°C. The aqueous acid layer was separated and transferred to the reactor. The organic layer was discarded. 5 volumes isopropyl acetate (6,250 mL) were added to the batch which was agitated for about 15 mins at 20°C then allowed to settle at 20°C. The aqueous acid layer was separated and transferred to the reactor. The organic layer was discarded. The aqueous acid layer was stored at 20°C overnight. 30 volumes of water (37.5kg) was charged to a reactor. Sodium bicarbonate (1238 g, 1.47E+04 mmol) was added and the mixture was agitated until full dissolution was achieved. Agitation was stopped, and the solution held at 20°C overnight. With stirring 10 volumes of 2-methyltetrahydrofuran (12,500 mL) was added to the aqueous solution. An exothermic event was observed. The internal temperature was adjusted to 20 ± 5°C. The aqueous acidic layer was added to a reactor over about 10 mins. A mild exotherm of 1°C was observed. The mixture was agitated at 20°C for about 15 min then allowed to settle at 20°C. The bottom aqueous layer was removed and added to the reactor. The organic layer was removed and extracted by charging 5 volumes of 2-methyltetrahydrofuran (6,250 mL) and mixing for about 15 min at 20°C. The combined organic layers were concentrated to 3 volumes (3.75L) with a rotovap with a bath temperature at 50°C. 10 volumes of 2-methyltetrahydrofuran (12,500 mL) were added and the mixture was concentrated to 3 volumes (3.75L) with a rotovap with a bath temperature at 50°C. Acetonitrile (2.00E+04 mL) was added to a 25L jacketed reactor and its internal temperature was adjusted to 0-5°C with mixing at 215rpm. The concentrated batch (about 3.75L) was added into the cooled acetonitrile over about 1hr at 0-5°C. The mixture was allowed to age overnight at 0°C while mixing at 215rpm. It was filtered and the cake was washed with 4 volumes of pre-cooled acetonitrile (5,000 mL) at 0°C. The cake was dried on a filter under N_2 gas pressure over 2 hours. The wet cake was further dried under high vacuum (with slight N_2 purge) at 50°C overnight to afford (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone (1150 g, 2179 mmol, 92 % yield) as an amorphous solid. A second batch of material was prepared following the same method to give 1036g of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone as an amorphous solid (confirmed by PXRD) and the two batches were combined.

Example 2: Crystalline hydrate of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (Form I)

(S)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone (95 g, 180 mmol) was taken up into 1M HCl (aq) (950 mL) and isopropyl acetate (950 ml) at 30°C. The batch was held at 30°C over 30 min then the layers were separated. This was repeated three times, always keeping the bottom aqueous layer and discarding the isopropyl acetate layer. After the fourth isopropyl acetate extraction of the aqueous layer, 2-methyltetrahydrofuran (950 ml, 9419 mmol) was added, followed by enough 10% sodium bicarbonate to bring the pH=7.5. The mixture was stirred over 30 min and the layers were separated. The aqueous layer was extracted with 2-methyltetrahydrofuran (250mL). The 2-methyltetrahydrofuran layer were combined. A solvent swap from 2-methyltetrahydrofuran to acetonitrile was conducted to precipitate the product out of solution. After filtration and drying, the cake afforded 89 grams of crude (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone. This solid (89 grams) was taken up into 445mL of methanol. After stirring to complete dissolution, the batch was filtered through a 0.2µm filter. The filtrate was then heated to an internal temperature of 55°C and held with stirring at this temperature over 60 min. Some precipitate started to form to give a slightly hazy solution. The temperature was then cooled linearly at 0.2°C/min down to a final internal temperature of 15°C then held at 15°C over 8 hours. At this time, the batch thickened significantly, and the slurry was filtered under nitrogen blanket using high vacuum. The wet cake was off-loaded and dried over 18 hours under high vacuum (28mm Hg) in a vacuum oven with the temperature set to 65°C. The dry cake was then off-loaded and allowed to equilibrate to ambient humidity and temperature to afford a final white to off-white crystalline cake of (S)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone hydrate Form I (75 g, 140 mmol, 78 % yield).

Example 3: Crystalline hydrate of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound 1), Form I

Into a 20L jacketed reactor, was added the (S)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound **1**, 2186 g, 4143 mmol) obtained in Preparation 7, followed by methanol (10930 mL). The slurry obtained was stirred and heated to 55°C to afford a clear homogeneous solution. The solution was held at 55°C over 30 min and seeded (with 2.186 g of the material obtained in the previous Example). The solution was held an additional hour at 55°C. Crystallization and thickening occurred. The mixture was then cooled to 10°C over 450 minutes. The batch was held at 10°C internally over 12 hours then filtered. The wet cake was washed with pre-cooled methanol (2186 mL) at 5°C. The cake was dried on a filter dryer under vacuum with a nitrogen purge and heated jacket set to 55°C. The cake was held under these drying conditions over 19 hours. The cake was then off-loaded and spread out in a drying tray and allowed to equilibrate to ambient RH conditions over 30 hours to afford a crystalline hydrate of (S)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone (1411.6 g, 2587 mmol, 62.4 % yield), Form I, as an off-white to pale yellow solid.

Example 4: Crystalline hydrate of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound 1), Form I

Methanol (1.250 mL) was added to 250 g of crude (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound **1**), obtained in a similar way as shown in Example 2, and the slurry obtained was heated to 55°C for about 30 minutes to afford an homogeneous solution. The solution was filtered through a 0.2μm filter. The filtrate was stirred at 55°C for about 3 hours to initiate crystallization. The temperature of the resulting thin slurry was adjusted to 5°C for about 250 minutes. The thickened slurry was stirred at 5°C overnight. The solids were then isolated using a pressure filter. The filter cake was washed with methanol (250 mL) pre-cooled at 5°C and then dried under high vacuum at 55°C overnight to afford 180 g of crystalline material. To the 180 g of crystalline material obtained, was added methanol (900 mL). The stirred slurry's temperature was adjusted to 55°C for about 30 minutes. The slurry was stirred at 55°C for about 3 hours. The temperature of the slurry was then adjusted to 5-15°C for about 200 minutes. The slurry was stirred at 5-15°C for about 2 more hours. The solids were isolated using a pressure filter under inert conditions. The

filter cake was washed with methanol pre-cooled at 5-15°C (300 mL) and then dried under high vacuum in a vacuum oven for about 12 hours at 55-65°C. The dry cake was held under ambient temperature, pressure and relative humidity for about 12 hours to afford 165.7 g of crystalline monohydrate (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (Form I) as an off-white to light yellow solid with 97.7% purity based on HPLC data.

Example 5: Crystalline hydrate of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound 1), Form I

(S)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridin-6-yl)methanone was obtained by following a similar preparation as shown in Preparation 7 step 2. The solid obtained (0.100 g, 0.183 mmol) was dissolved in ethanol (0.5 ml, 0.183 mmol)) at 50°C over 5 hours at which time a slurry formed. The slurry was filtered at that temperature under inert conditions and dried in an oven under *vacuum* at 60°C over 12hours followed by equilibration under ambient room temperature and pressure over 12 hours to afford Form I (0.06g).

Examples 6-10: Properties of the Crystalline Form

Samples of the crystalline hydrate of (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone (compound 1), Form I, were analyzed by powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), dynamic moisture sorption (DMS), and single crystal X-Ray diffraction.

Example 6 Powder X-Ray Diffraction

The powder X-ray diffraction patterns of Figure 1 was obtained with a Bruker D8-Advance X-ray diffractometer equipped with a Lynxeye 1D detector using Cu-*Kα* radiation (λ = 1.54051 Å) with output voltage of 45 kV and current of 40 mA. The instrument was operated in Bragg-Brentano geometry with incident, divergence, and scattering slits set to maximize the intensity at the sample. For measurement, a small amount of powder (5-25 mg) was gently pressed onto a sample holder to form a smooth surface and subjected to X-ray exposure. The samples were scanned in 2θ-2θ mode from 2° to 35° in 2θ with a step size of 0.02° and a scan speed of 2 seconds per step for a total of 1 hour scan time. The data acquisition was controlled by Bruker DiffracSuite

measurement software and analyzed by Jade software (version 7.7). Observed PXRD 2θ peak positions and *d*-spacings are shown in Table 1 for the crystalline hydrate of compound **1**, Form I.

Table 1: PXRD Data for the Crystalline Hydrate Form I

2θ	d(Å)	Area	A%
5.68	15.56	40.1	100.0
8.49	10.40	10.6	26.4
10.43	8.47	18.2	45.5
10.94	8.08	13.2	32.9
11.55	7.65	8.1	20.3
12.20	7.25	6.9	17.3
13.08	6.76	13.7	34.3
15.94	5.56	15.1	37.6
16.24	5.45	3.3	8.2
17.06	5.19	6.2	15.6
17.60	5.04	745.5	4.8
18.41	4.82	2.3	5.6
18.82	4.71	2.3	5.8
18.96	4.68	4.3	10.6
21.90	4.06	2.5	6.1
22.08	4.02	4.4	11.1
22.27	3.99	5	12.6
24.55	3.62	4.3	10.9
26.29	3.39	3.4	8.4

Example 7: Thermal Analysis

Differential scanning calorimetry (DSC) was performed using a TA Instruments Model Discovery DSC. Data was collected with TRIOS software and was analyzed using TA Instruments Universal Analysis software. A sample of the crystalline form was accurately weighed into an aluminum pan covered with a TZero hermetic pinhole lid. The sample was initially cooled to -20°C after which the sample was heated using a linear heating ramp of 10 °C/min from -20 °C to 250 °C. A representative DSC thermogram of Form I is shown in Figure 2.

Thermogravimetric analysis (TGA) measurements were performed using a TA Instruments Model Discovery TGA module equipped with high resolution capability. Data were collected using TA Instruments TRIOS software and analyzed using TA Instruments Universal Analysis software. A weighed sample was placed onto a platinum pan and scanned with a heating rate of 10 °C per minute from ambient temperature to 260 °C. The balance and furnace chambers were purged with nitrogen flow during use. A representative TGA trace of Form I is shown in Figure 3. A weight loss of about 3.4% between 27°C and 100°C was observed and corresponds to dehydration of a monohydrate form.

Example 8: Dynamic Moisture Sorption Assessment

Dynamic moisture sorption (DMS) measurement was performed using a VTI atmospheric microbalance, SGA-100 system (VTI Corp., Hialeah, FL 33016). A weighed sample was used and the humidity was lowest possible value (close to 0% RH) at the start of the analysis. The DMS analysis consisted of an initial drying step (~0%RH) for 16 hours, followed by two cycles of sorption and desorption with a scan rate of 5 % RH/step over the humidity range of 5 % RH to 90 % RH. The DMS run was performed isothermally at 25 °C. A representative DMS trace for Form I is shown in Figure 4.

Example 9: Single Crystal X-Ray Diffraction

A crystal of the crystalline hydrate of compound **1** (Form I) having dimensions of 0.28 x 0.11 x 0.02 mm was mounted on a glass fiber.

Data were collected on a Rigaku Atlas CCD diffractometer equipped with an Oxford Cryosystems Cobra cooling device. The data were collected using Cu Kα radiation and the crystal structure was solved and refined using the Bruker AXS SHELXTL software. Hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to the heteroatoms were located in a difference Fourier map and were allowed to refine freely with an isotropic displacement parameter. Unit cell parameters, along with crystal system and space group details, are provided in Table 2. The data confirmed that Form I is a monohydrate.

Table 2: Data from Single Crystal X-Ray Diffraction Analysis

Temperature of Data Collection	100(2) K
Wavelength used for Data Collection	1.54178 Å
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	a = 20.8736(5) Å
	b = 9.15021(19) Å
	c = 15.7412(3) Å
	a = 90°
	b = 98.4786(18)°
	g = 90°
Unit cell volume	2973.67(11) Å^3
Final R indices [$F^2 > 2\sigma(F^2)$]	R_1 = 0.0368, wR_2 = 0.0975

Example 10: Stability Study

Samples of Form I stored at accelerated conditions of 25 ºC and 60 % relative humidity (RH), as well as at accelerated conditions of 40 ºC and 75 % relative humidity (RH) were analyzed by HPLC for compound **1** content and impurity profile as shown in Tables 3 and 4.

RRT = Relative Retention Time (with respect to compound **1**)

LOQ = Limit of Quantitation (0.05% a/a), only peaks at or above LOQ are reported

Table 3: Compound 1 and Impurities Percentages at 40°C/75% RH

	T=0	T=1 month 40°C/75% RH	T=3 month 40°C/75% RH
RRT	% a/a	% a/a	% a/a
0.77	0.17	0.18	0.18
0.84	0.05	0.06	0.05
0.95			0.05
0.97	0.06	0.07	0.07
Compound **1**	97.5%	97.5%	97.5%
1.10		0.09	
1.11	0.07	0.12	0.10
1.13			0.05

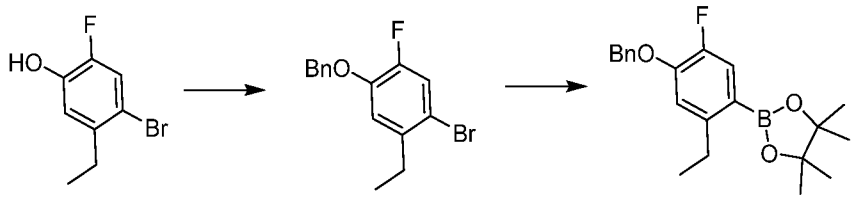
	T=0	T=1 month 40°C/75% RH	T=3 month 40°C/75% RH
1.18			0.06
1.25	0.11	0.11	0.14
1.28	0.36	0.35	0.36
1.30	0.21	0.23	0.22
1.35	0.06	0.07	0.11
1.77		0.05	
1.84	0.32	0.32	0.31
1.85	0.13	0.14	0.20
2.11	0.06	0.05	0.05
2.27	0.07	0.09	0.07

Table 4: Compound 1 and Impurities Percentages at 25°C/60% RH

	T=0	T=3 month 25°C/60% RH
RRT	% a/a	% a/a
0.77	0.17	0.18
0.84	0.05	0.05
0.95		0.05
0.97	0.06	0.06
Compound **1**	97.5%	97.6%
1.11	0.07	0.08
1.25	0.11	0.14
1.28	0.36	0.36
1.30	0.21	0.22
1.35	0.06	0.10
1.84	0.32	0.31
1.85	0.13	0.20
2.11	0.06	0.06
2.27	0.07	0.07

Form I exhibited good stability under these conditions.

Preparation 8: 2-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane



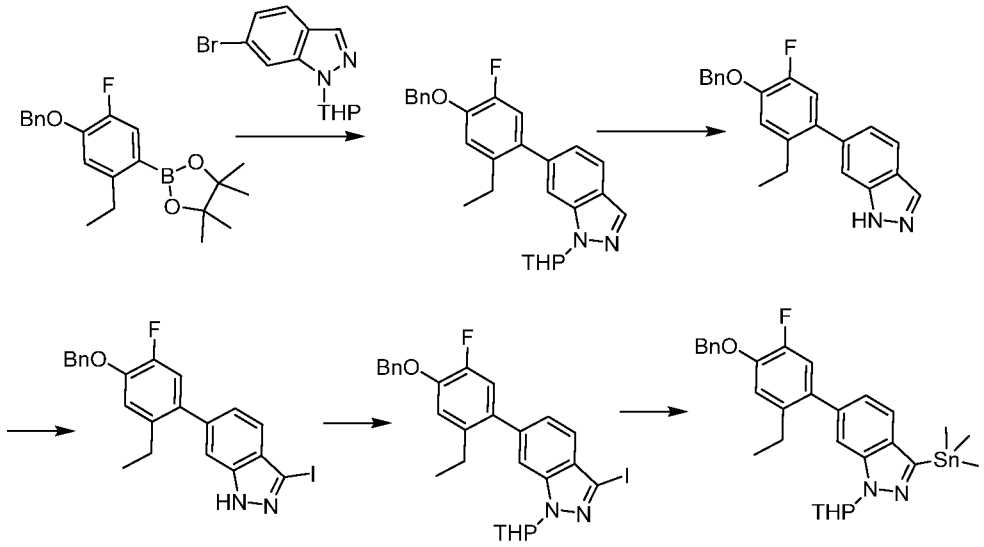
(a) 1-(Benzyloxy)-4-bromo-5-ethyl-2-fluorobenzene

To a solution of 4-bromo-5-ethyl-2-fluorophenol (20 g, 910.32 mmol) in ACN (250 mL) was added K_2CO_3 (31.55 g, 228.3 mmol) followed by benzyl bromide (13.10 mL, 109.58 mmol) drop wise. The resulting reaction mixture was stirred at 80 °C for 2 h. The aqueous layer was extracted with EtOAc (three times), combined and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to afford the title intermediate as a pale yellow oily liquid (25 g, 89 % yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.30 (m, 5H), 7.27 (d, *J* = 10.5 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 5.12 (s, 2H), 2.66 (q, *J* = 7.5 Hz, 2H), 1.16 (t, *J* = 7.5 Hz, 3H).

(b) 2-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane

To a solution of the product of the previous step (12.5 g, 40.45 mmol) in dioxane (100 mL) was added bis(pinacolato)diboron (15.40 g, 60.67 mmol) and KOAc (11.9 g, 121.35 mmol). The reaction mixture was purged with nitrogen for 15 min followed by addition of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (1.65 g, 2.023 mmol). The resulting reaction mixture was stirred and heated at 110 °C for 3 h, filtered through Celite and the residue washed with EtOAc. The filtrate was diluted with excess EtOAc (200 mL) and washed with water (100 mL) followed by brine (100 mL), dried over sodium sulfate and concentrated in vacuo to get crude product which was purified by column chromatography over (100-200) silica gel, eluted with 3-5% EtOAc: Hexane to afford the desired product as an off-white solid (9.50 g, 66 % yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.54 – 7.27 (m, 6H), 6.81 (d, *J* = 7.9 Hz, 1H), 5.16 (s, 2H), 2.84 (q, *J* = 7.5 Hz, 2H), 1.32 (s, 12H), 1.14 (t, *J* = 7.5 Hz, 3H).

Preparation 9: 6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-3-(trimethylstannyl)-1*H*-indazole



(a) 6-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole

To a solution of 6-bromo-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole (50 g, 178.57 mmol) and 2-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (76.3 g, 214.29 mmol) in DMF:H_2O (480:120 mL) was added K_3PO_4 (94.64 g, 446.86 mmol). The reaction mixture was degassed with nitrogen for 15 min, then $Pd(PPh_3)_2Cl_2$ catalyst (6.26 g, 8.93 mmol) was added and the mixture was again degassed with nitrogen for 5 min stirred, and heated at 100-110 °C for 5 h. The reaction mixture was filtered through Celite and the residue was washed with EtOAc. The filtrate was diluted with EtOAc, washed with cold water and brine, dried over sodium sulfate and concentrated in vacuo to provide crude product which was purified by flash column chromatography to afford the title intermediate as a white solid (65 g, 86 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{27}FN_2O_2$ 431.21 found 431.46. ^1H NMR (400 MHz, Chloroform-*d*) δ 8.06 – 7.98 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.51 – 7.32 (m, 5H), 7.08 (dd, *J* = 809.6, 8.3 Hz, 1H), 7.03 (d, *J* = 11.9 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 5.76 – 5.64 (m, 1H), 5.20 (s, 2H), 4.04 (d, *J* = 10.1 Hz, 1H), 3.72 (t, *J* = 9.7 Hz, 1H), 2.52 (q, *J* = 7.5 Hz, 2H), 2.22 – 2.02 (m, 3H), 1.80 – 1.71 (m, 3H), 1.06 (t, *J* = 7.5 Hz, 3H).

(b) 6-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-1*H*-indazole

To a solution of the product of the previous step (65 g, 151.16 mmol) in methanol (700 mL) was added conc. HCl (120 mL) and the resulting solution was heated at 60-65 °C for 3 h, cooled to RT, and concentrated in vacuo. The residue was dissolved in EtOAc

and washed with saturated $NaHCO_3$ aqueous solution and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the title intermediate as a white solid (52 g, 99 % (crude)). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.13 (s, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.59 – 7.30 (m, 6H), 7.10 (d, *J* = 8.3 Hz, 1H), 7.01 (d, *J* = 11.8 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 5.21 (s, 2H), 2.53 (q, *J* = 7.5 Hz, 2H), 1.05 (t, *J* = 7.5 Hz, 3H).

(c) 6-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-3-iodo-1*H*-indazole

To a solution of 6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1*H*-indazole (56 g, 161.18 mmol) in DMF (400 mL) was added KOH (36.2 g, 647.39 mmol) and the mixture was stirred for 5 min. A solution of iodine (82.2 g, 323.69 mmol) in DMF (100 mL) was added slowly at 0 °C and stirred at RT for 30 min, diluted with water (3 x 150 mL) and extracted with EtOAc (3 x 200 mL). The organic layer was washed with saturated sodium metabisulfite aqueous solution (3 x 200 mL) and water (400 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get crude product which was purified by flash column chromatography to afford the title intermediate as a brownish semi-solid (64 g, 84 %yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.49 (s, 1H), 7.57 – 7.32 (m, 7H), 7.16 (d, *J* = 8.3 Hz, 1H), 7.04 – 6.91 (m, 2H), 5.20 (s, 2H), 2.51 (q, *J* = 7.4 Hz, 2H), 1.04 (t, *J* = 7.5 Hz, 3H).

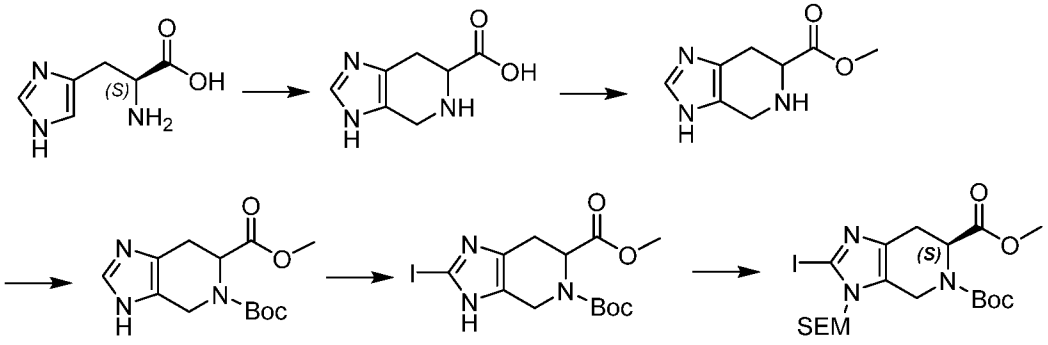
(d) 6-(4-(Benzyloxy)-2-ethyl-5-fluorophenyl)-3-iodo-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole

To an ice-cold solution of the product of the previous step (60 g, 127.12 mmol) in DCM (700 mL) was added *p*-toluensulfonic acid (4.84 g, 25.423 mmol) followed by 3,4-dihydro-2*H*-pyran (17.43 mL, 190.68 mmol) drop wise. The reaction mixture was stirred at RT overnight, diluted with DCM and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to provide crude product which was purified by flash chromatography (silica gel) to afford the title intermediate as an off white solid (64 g, 91 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{26}FIN_2O_2$ 557.10 found 557.30. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 – 7.31 (m, 7H), 7.14 (d, *J* = 8.3 Hz, 1H), 7.01 (d, *J* = 11.8 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 5.68 (d, *J* = 9.3 Hz, 1H), 5.20 (s, 2H), 4.08 – 3.99 (m, 1H), 3.77 – 3.64 (m, 1H), 2.50 (q, *J* = 7.2 Hz, 2H), 2.23 – 1.97 (m, 3H), 1.81 – 1.68 (m, 3H), 1.06 (t, *J* = 7.4 Hz, 3H).

(e) 6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-3-(trimethylstannyl)-1*H*-indazole

To a solution of 6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-3-iodo-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazole (20 g, 35.97 mmol) in toluene (150 mL) was added hexamethylditin (9.2 mL, 43.17 mmol). The reaction mixture was degassed with nitrogen for 20 min followed by addition of tetrakis (2.0 g, 1.80 mmol) and then stirred at 100 °C for 2 h, cooled to RT, filtered through Celite and residue washed with EtOAc. The filtrate was concentrated and purified by column chromatography (over neutral alumina), eluted with 2-5%. EtOAc:Hexane to afford the title compound (17.50 g, 82 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{30}H_{35}FN_2O_2Sn$ 595.17, 593.17 found 595.49, 593.55. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.68 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.29 (m, 6H), 7.13 – 7.00 (m, 2H), 6.96 (d, *J* = 8.4 Hz, 1H), 5.81 – 5.68 (m, 1H), 5.21 (s, 2H), 4.13 – 4.00 (m, 1H), 3.81 – 3.66 (m, 1H), 2.54 (q, *J* = 7.3 Hz, 2H), 2.23 – 2.00 (m, 2H), 1.87 – 1.59 (m, 4H), 1.08 (t, *J* = 7.5 Hz, 3H), 0.47 (s, 9H).

Preparation 10: 5-(*tert*-butyl) 6-methyl (*S*)-2-iodo-3-((2-trimethylsilyl)ethoxy) methyl)- 3,4,6,7-tetrahydro -5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate



(a) (*S*)-4,5,6,7-Tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid

To a stirred suspension of L-histidine (50 g, 322.24 mmol) in water (420 mL) was added conc. HCl (29 mL) drop wise at 0 °C followed by formaldehyde (55 mL, 676.72 mmol) in one portion at 0 °C. The resulting reaction mixture was stirred for 30 min and then heated at 75 °C for 6 h and concentrated. The resulting crude was stirred for 2 h with diethyl ether, filtered and washed with IPA:THF (100:300 mL) to provide the HCl salt of the title intermediate as an off white solid (75 g 99 % yield (crude)). (*m/z*): $[M+H]^+$ calcd for $C_7H_9N_3O_2$ 168.07 found 168.17.

(b) Methyl (*S*)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylate

To a stirred solution of the product of the previous step (75.0 g, 312.5 mmol) in methanol (1500 mL) was added $SOCl_2$ (45.6 mL, 625 mmol) dropwise at 0 °C and stirred at RT for 16 h, then heated up to reflux (70 °C) for 1 h. The solvent was removed by distillation and the crude product was triturated with methanol followed by diethyl ether

to provide the crude HCl salt of the title intermediate as an off white solid (80 g crude). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (s, 1H), 4.71 (dd, J = 9.4, 5.2 Hz, 1H), 4.36 (d, J = 15.5 Hz, 1H), 4.30 (d, J = 15.6 Hz, 1H), 3.82 (s, 3H), 3.44 – 3.21 (m, 2H).

(c) 5-(*tert*-Butyl) 6-methyl (*S*)-3,4,6,7-tetrahydro-5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate

To a stirred solution of the product of the previous step (80.0 g, 314.96 mmol) in methanol (1000 mL) was added DIPEA (282 mL, 1574 mmol) followed by di-*tert*-butyl dicarbonate (172 mL, 787.48 mmol) at 0 °C. The reaction mixture was stirred at RT for 16 h and then liquid NH_3 (150 mL, 25 % in water) was added and the reaction mixture was stirred again for 16 h at RT, methanol was removed by distillation and the residue was extracted in DCM (3 x 200 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, concentrated and purified by flash chromatography (100-200 mesh silica gel), eluted with 5% MeOH:DCM to afford the title intermediate (41 g, 46 %. yield). (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{19}N_3O_4$ 282.14 found 282.21. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 7.50 (s, 1H), 5.18 (dd, J = 49.3, 5.1 Hz, 1H), 4.51 (t, J = 14.2 Hz, 1H), 4.09 (dd, J = 43.9, 16.1 Hz, 1H), 3.59 (s, 3H), 3.08 (d, J = 15.5 Hz, 1H), 2.94 (d, J = 15.1 Hz, 1H), 1.45 (s, 9H).

(d) 5-(*tert*-Butyl) 6-methyl (*S*)-2-iodo-3,4,6,7-tetrahydro-5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate

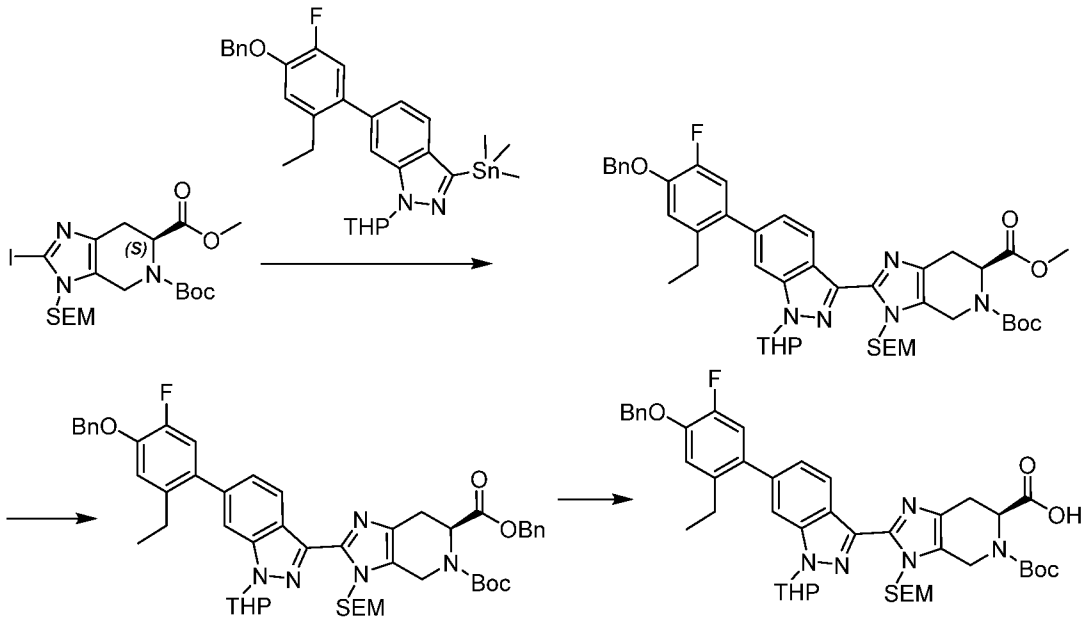
To a solution of the product of the previous step (41.0 g, 145.9 mmol) in THF (500 mL) was added *N*-iodosuccinimide (66.0 g, 291.8 mmol) at 0 °C and the resulting solution was stirred at RT for 4 h, diluted with water and extracted with ethyl acetate. The organic portion was washed with 10% sodium thiosulphate solution (3 x 200 mL). The combined organic layer was dried over anhydrous sodium sulfate, and concentrated to provide the title compound 60 g (crude), which was used in the next step without further purification. (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{18}IN_3O_4$ 408.03 found 408.31. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 5.34 – 4.97 (m, 1H), 4.67 – 4.35 (m, 1H), 4.12 – 3.95 (m, 1H), 3.60 (s, 3H), 3.14 – 2.82 (m, 2H), 1.44 (s, 9H).

(e) 5-(*tert*-Butyl) 6-methyl (*S*)-2-iodo-3-((2-trimethylsilyl)ethoxy) methyl)-3,4,6,7-tetrahydro -5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate

To a stirred solution of 5-(*tert*-butyl) 6-methyl (*S*)-2-iodo-3,4,6,7-tetrahydro-5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate (40 g, 0.098 mol) in DMF (150 mL) was added DIPEA (35.1 mL, 0.19 mol) at 0 °C. The reaction mixture was stirred for 10 min then 2-(trimethylsilyl)-ethoxymethyl chloride (19.1 mL, 0.10 mol) was added drop wise at 0 °C.

The resulting reaction mixture was stirred for 3 h at RT. After 4 h chilled water was added and the reaction mixture was extracted with EtOAc (2 x 200 mL). The organic layer was dried over anhydrous sodium sulphate, concentrated, and purified by flash column chromatography, eluted with 20-35% EtOAc:Hexane, to afford the title product as a pale yellow viscous liquid (27 g). (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{32}IN_3O_5Si$ 538.12 found 538.42. ^1H NMR (400 MHz, DMSO-d_6) δ 5.33 – 5.04 (m, 3H), 4.79 – 4.56 (m, 1H), 4.54 – 4.14 (m, 1H), 3.60 (s, 3H), 3.47 (t, J = 7.8 Hz, 2H), 3.31 – 3.16 (m, 1H), 2.97 (t, J = 18.9 Hz, 1H), 1.44 (s, 9H), 0.92 – 0.74 (m, 2H), -0.03 (s, 9H).

Preparation 11: (6*S*)-5-(*tert*-butoxycarbonyl)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazol-3-yl)-3-((2-(trimethylsilyl)ethoxy)methyl)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid



(a) 5-(*tert*-Butyl) 6-methyl (6*S*)-2-(6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazol-3-yl)-3-((2-(trimethylsilyl) ethoxy) methyl)-3,4,6,7-tetrahydro-5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate

To a stirred solution of 5-(*tert*-butyl) 6-methyl (*S*)-2-iodo-3-((2-trimethylsilyl)ethoxy) methyl)- 3,4,6,7-tetrahydro -5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate (17.0 g, 31.65 mmol) in toluene (500 mL) was added 6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-3-(trimethylstannyl)-1*H*-indazole (20 g, 34.82 mmol). The reaction mixture was purged with argon for 15 min, $Pd(PPh_3)_4$ (3.6 g, 3.16 mmol) and copper iodide (1.20 g, 6.33 mmol) were added and the reaction mixture was stirred at 120 °C for 16 h. The reaction mixture was filtered through Celite,

the filtrate was concentrated under reduced pressure and purified by silica gel column chromatography (Redisep 80 g column, eluted with DCM for 10 min and then 15-20% EtOAc in Hexane to afford the title intermediate as a yellow solid (15.10 g, 58 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{46}H_{58}FN_5O_7Si$ 840.41 found 840.54. ^1H NMR (400 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.54 – 7.33 (m, 6H), 7.20 (s, 1H), 7.05 (d, *J* = 11.4 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.09 – 5.69 (m, 3H), 5.59 – 5.36 (m, 1H), 5.20 (s, 2H), 4.97 – 4.80 (m, 1H), 4.12 – 3.90 (m, 1H), 3.68 (s, 3H), 3.57 – 3.47 (m, 2H), 3.40 (d, 1H), 3.21 – 3.05 (m, 1H), 2.74 – 2.34 (m, 4H), 2.25 – 2.07 (m, 2H), 1.94 – 1.65 (m, 4H), 1.54 (s, 9H), 1.12 – 0.99 (m, 3H), 0.91 – 0.75 (m, 2H), -0.12 (s, 9H).

(b) 6-Benzyl 5-(*tert*-butyl) (6*S*)-2-(6-(4-(benzyloxy)-2-ethyl-5-fluorophenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazol-3-yl)-3-((2-(trimethylsilyl) ethoxy) methyl)-3,4,6,7-tetrahydro-5*H*-imidazo[4,5-c]pyridine-5,6-dicarboxylate

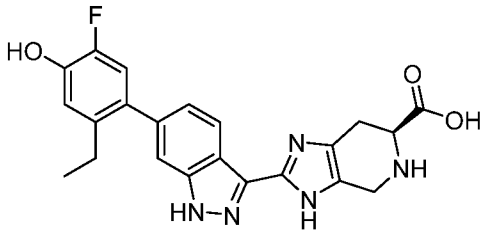
To a round bottom flask was added the product of the previous step (15.0 g, 17.85 mmol) in toluene (400 mL), benzyl alcohol (46.3 mL) and $Ti(OEt)_4$ (7.15 mL, 35.70 mmol) and the reaction mixture was refluxed vigorously (140 °C) for 48 h, diluted with water and extracted with DCM. The suspension was filtered, filtrate was dried over Na_2SO_4, concentrated under reduced pressure and purified by silica gel column chromatography (Redisep 80 g column, 0-5% EtOAc in hexanes) for 20 min to remove excess benzyl alcohol, then eluted with 10-15% EtOAc in Hexane) to provide the title intermediate. ^1H NMR consistent with structure. (*m/z*): $[M+H]^+$ calcd for $C_{52}H_{62}FN_5O_7Si$ 916.44 found 916.86.

(c) (6*S*)-5-(*tert*-butoxycarbonyl)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazol-3-yl)-3-((2-(trimethylsilyl)ethoxy)methyl)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid

To a stirred solution of the product of the previous step (21.0 g, 22.92 mmol) in 1:1 IPA:THF (400 mL)) was added $Pd(OH)_2$ (5.0 g). The reaction mixture was stirred at RT for 16 h under a hydrogen balloon, filtered through Celite, concentrated under reduced pressure, and purified by silica gel column chromatography (Redisep 80 g column, eluted with 25-40% EtOAc in Hexane) to provide the title compound (6.1 g, 8.29 mmol) as an off-white solid). (*m/z*): $[M+H]^+$ calcd for $C_{38}H_{50}FN_5O_7Si$ 736.35 found 736.5. ^1H NMR consistent with structure. (*m/z*): $[M+H]^+$ calcd for $C_{38}H_{50}FN_5O_7Si$ 736.35 found 736.5. ^1H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 9.86 (s, 1H), 8.34 (t, *J* = 7.6 Hz, 1H), 7.66 (s, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 11.8 Hz, 1H), 6.93 (d, *J* = 9.1 Hz, 1H), 6.11 – 5.77 (m, 3H), 5.33 – 5.06 (m, 1H), 4.87 – 4.56 (m, 1H), 4.52 –

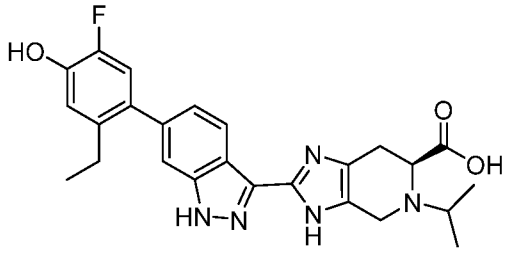
4.14 (m, 1H), 3.97 – 3.69 (m, 2H), 3.53 – 3.40 (m, 2H), 3.23 – 3.11 (m, 1H), 3.11 – 2.93 (m, 1H), 2.47 – 2.44 (m, 2H), 2.13 – 1.96 (m, 2H), 1.68 (d, J = 70.9 Hz, 4H), 1.48 (s, 9H), 1.02 (t, J = 7.5 Hz, 3H), 0.86 – 0.68 (m, 2H), -0.17 (s, 9H).

Preparation 12: (*S*)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1*H*-indazol-3-yl)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid



To a stirred solution of (6*S*)-5-(*tert*-butoxycarbonyl)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1-(tetrahydro-2*H*-pyran-2-yl)-1*H*-indazol-3-yl)-3-((2-(trimethylsilyl)ethoxy)-methyl)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid (5.7 g, 7.75 mmol) in 5:1 dioxane:water (60 mL) was added conc. HCl (20 mL) drop wise at 0°C. The reaction mixture was warmed and stirred at 90 °C for 16 h and distilled under vacuum to provide the crude residue, which was sequentially triturated with chilled diethyl ether and acetonitrile to provide the HCl salt of the title compound (3.6 g. 95 % yield) as a light brown solid. (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{20}FN_5O_3$ 422.16 found 422.24. ^{1}H NMR (400 MHz, D_2O/DMSO-d_6) δ 8.22 (d, J = 8.4 Hz, 1H), 7.49 (s, 1H), 7.19 (d, J = 8.1 Hz, 1 H), 6.99 (d, J = 11.9 Hz, 1 H), 6.91 (d, J = 9.0 Hz, 1H), 4.56 – 4.51 (m, 1H), 4.36 (d, J = 15.5 Hz, 1H), 4.30 (d, J = 15.5 Hz, 1H), 3.35 – 3.25 (m, 1H), 3.15 – 3.05 (m, 1H), 2.4 – 2.55 (m, 2H), 0.97 (t, J = 7.5 Hz, 3H).

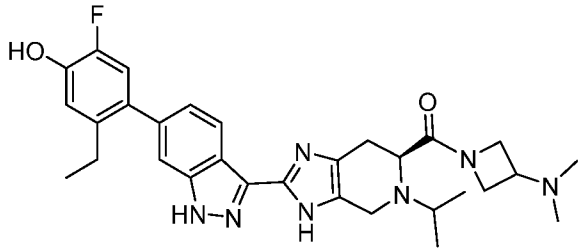
Preparation 13: (*S*)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid



To a solution of (*S*)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1H-indazol-3-yl)-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid, HCl (400 mg, 0.874 mmol), acetone (0.192 mL, 2.62 mmol), and acetic acid (0.150 mL, 2.62 mmol) in DMF (7 mL), was added sodium cyanoborohydride (274 mg, 4.37 mmol) and the reaction mixture was stirred at RT overnight. Sodium borohydride (33 mg, 0.874 mmol) was

added, the solution was concentrated, and purified by preparative HPLC to provide the TFA salt of the title compound (115 mg, 23 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{26}FN_5O_3$ 464.20 found 464.5.

Example 11: (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone C-1



C-1

To a solution of (*S*)-2-(6-(2-ethyl-5-fluoro-4-hydroxyphenyl)-1H-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridine-6-carboxylic acid, TFA (179 mg, 0.310 mmol), *N,N*-dimethylazetidin-3-amine, 2 HCl (107 mg, 0.465 mmol), and DIPEA (0.162 mL 0.930 mmol) in DMF (4 mL), was added HATU (177 mg, 0.465 mmol) and the reaction mixture was stirred at RT overnight. Hydrazine (5 eq) was added, the reaction mixture was concentrated and purified by preparative HPLC to provide the TFA salt of the title compound (63 mg, 26 % yield). (*m/z*): $[M+H]^+$ calcd for $C_{30}H_{36}FN_7O_2$ 546.29 found 546.7. ^1H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.29 (dd, 1H), 7.34 (s, 1H), 7.07 (d, 1H), 7.01 (d, 1H), 6.89 (d, 1H), 4.35 – 4.18 (m, 1H), 4.11 – 3.94 (m, 1H), 3.94 – 3.73 (m, 3H), 3.70 – 3.57 (m, 2H), 3.06 – 2.94 (m, 2H), 2.87 – 2.66 (m, 2H), 2.48 – 2.40 (m, 2H), 2.13 – 2.00 (m, 6H), 1.07 (t, 3H), 1.03 – 0.93 (m, 6H).

Biological Assays

Compound **1** has been characterized in the following biological assays.

Assay 1: Biochemical JAK Kinase Assays

A panel of four LanthaScreen JAK biochemical assays (JAK1, 2, 3 and Tyk2) were carried in a common kinase reaction buffer (50 mM HEPES, pH 7.5, 0.01% Brij-35, 10 mM $MgCl_2$, and 1 mM EGTA). Recombinant GST-tagged JAK enzymes and a GFP-tagged STAT1 peptide substrate were obtained from Life Technologies.

Serially diluted compounds were pre-incubated with each of the four JAK enzymes and the substrate in white 384-well microplates (Corning) at ambient temperature for 1h. ATP was subsequently added to initiate the kinase reactions in 10 μL total volume, with 1% DMSO. The final enzyme concentrations for JAK1, 2, 3 and Tyk2

are 4.2 nM, 0.1 nM, 1 nM, and 0.25 nM respectively; the corresponding Km ATP concentrations used are 25 μM, 3 μM, 1.6 μM, and 10 μM; while the substrate concentration is 200 nM for all four assays. Kinase reactions were allowed to proceed for 1 hour at ambient temperature before a 10 μL preparation of EDTA (10mM final concentration) and Tb-anti-pSTAT1 (pTyr701) antibody (Life Technologies, 2 nM final concentration) in TR-FRET dilution buffer (Life Technologies) was added. The plates were allowed to incubate at ambient temperature for 1h before being read on the EnVision reader (Perkin Elmer). Emission ratio signals (520nm/495nm) were recorded and utilized to calculate the percent inhibition values based on DMSO and background controls.

For dose-response analysis, percent inhibition data were plotted vs. compound concentrations, and IC_{50} values were determined from a 4-parameter robust fit model with the Prism software (GraphPad Software). Results were expressed as pIC_{50} (negative logarithm of IC_{50}) and subsequently converted to pK_i (negative logarithm of dissociation constant, Ki) using the Cheng-Prusoff equation.

Test compounds having a lower K_i value or higher pK_i value in the four JAK assays show greater inhibition of JAK activity.

Assay 2: Inhibition of IL-2 Stimulated pSTAT5 in Tall-1 T cells

The potency of test compounds for inhibition of interleukin-2 (IL-2) stimulated STAT5 phosphorylation was measured in the Tall-1 human T cell line (DSMZ) using AlphaLisa. Because IL-2 signals through JAK1/3, this assay provides a measure of JAK1/3 cellular potency.

Phosphorylated STAT5 was measured via the AlphaLISA SureFire Ultra pSTAT5 (Tyr694/699) kit (PerkinElmer). Human T cells from the Tall-1 cell line were cultured in a 37°C, 5% CO_2 humidified incubator in RPMI (Life Technologies) supplemented with 15% Heat Inactivated Fetal Bovine Serum (FBS, Life Technologies), 2mM Glutamax (Life Technologies), 25mM HEPES (Life Technologies) and 1X Pen/Strep (Life Technologies). Compounds were serially diluted in DMSO and dispensed acoustically to empty wells. Assay media (phenol red-free DMEM (Life Technologies) supplemented with 10% FBS (ATCC)) was dispensed (4 μL/well) and plates shaken at 900rpm for 10 mins. Cells were seeded at 45,000 cells/well in assay media (4 μL/well), and incubated at 37°C, 5% CO_2 for 1 hour, followed by the addition of IL-2 (R&D Systems; final concentration 300 ng/mL) in pre-warmed assay media (4 μL) for 30 minutes. After cytokine stimulation, cells were lysed with 6ul of 3x AlphaLisa Lysis Buffer

(PerkinElmer) containing 1x PhosStop and Complete tablets (Roche). The lysate was shaken at 900rpm for 10 minutes at room temperature (RT). Phosphorylated STAT5 was measured via the pSTAT5 AlphaLisa kit (PerkinElmer). Freshly prepared acceptor bead mixture was dispensed onto lysate (5μL) under green filtered <100 lux light. Plates were shaken at 900rpm for 2mins, briefly spun down, and incubated for 2hrs at RT in the dark. Donor beads were dispensed (5μL) under green filtered <100 lux light. Plates were shaken at 900rpm for 2 minutes, briefly spun down, and incubated overnight at RT in the dark. Luminescence was measured with excitation at 689 nm and emission at 570 nm using an EnVision plate reader (PerkinElmer) under green filtered <100 lux light.

To determine the inhibitory potency of test compounds in response to IL-2, the average emission intensity of beads bound to pSTAT5 was measured in a human T cell line. IC_{50} values were determined from analysis of the inhibition curves of signal intensity versus compound concentration. Data are expressed as pIC_{50} (negative decadic logarithm IC_{50}) values (mean ± standard deviation).

In Vitro Assay Results

Table 5

Compound	JAK1 pK_i	JAK2 pK_i	JAK3 pK_i	Tyk2 pK_i	Tall-1 pIC50
1	10.2	10.5	10.2	9.1	8.6
C-1	10.4	10.8	10.1	9.5	8.8

Assay 3: Murine (Mouse) model of IL-13 induced pSTAT6 induction in lung tissue

IL-13 is an important cytokine underlying the pathophysiology of asthma (Kudlacz et al. *Eur. J. Pharmacol*, **2008**, *582*,154-161). IL-13 binds to cell surface receptors activating members of the Janus family of kinases (JAK) which then phosphorylate STAT6 and subsequently activates further transcription pathways. In the described model, a dose of IL-13 was delivered locally into the lungs of mice to induce the phosphorylation of STAT6 (pSTAT6) which is then measured as the endpoint.

Adult Balb/c mice from Harlan were used in the assay. On the day of study, animals were lightly anesthetized with isoflurane and administered either vehicle or test compound (1 mg/mL, 50 μL total volume over several breaths) via oral aspiration. Animals were placed in lateral recumbency post dose and monitored for full recovery from anesthesia before being returned to their home cage. Four hours later, animals were

once again briefly anesthetized and challenged with either vehicle or IL-13 (0.03 µg total dose delivered, 50 µL total volume) via oral aspiration before being monitored for recovery from anesthesia and returned to their home cage. One hour after vehicle or IL-13 administration, whole blood and lungs were collected for both pSTAT6 detection in lung homogenates using a Perkin Elmer AlphaLISA® *SureFire® Ultra™ HV* p-STAT6 (Tyr641) assay kit and for total drug concentration analysis in both lung and plasma. Blood samples were centrifuged (Eppendorf centrifuge, 5804R) for 4 minutes at approximately 12,000 rpm at 4°C to collect plasma. Lungs were rinsed in DPBS (Dulbecco's Phosphate-Buffered Saline), padded dry, flash frozen, weighed, and homogenized at a dilution of 1:3 in 0.1% formic acid in HPLC water. Plasma and lung levels of test compound were determined by LC-MS analysis against analytical standards constructed into a standard curve in the test matrix. A lung to plasma ratio was determined as the ratio of the lung concentration in ng/g to the plasma concentration in ng/mL at 5 hours.

Activity in the model is evidenced by a decrease in the level of pSTAT6 present in the lungs of treated animals at 5 hours compared to the vehicle treated, IL-13 challenged control animals. The difference between the control animals which were vehicle- treated, IL-13 challenged and the control animals which were vehicle-treated, vehicle challenged dictated the 0% and 100% inhibitory effect, respectively, in any given experiment. Compound **1** exhibited inhibition of STAT6 phosphorylation at 5 hours after IL-13 challenge as documented below.

Table 6: pSTAT6 Inhibition and Plasma/Lung Exposure Observed

Compound	Lung Concentration (ng/g) at 5 hr	Plasma Concentration (ng/mL) at 5 hr	Lung to Plasma ratio at 5 hr	pSTAT6 inhibition at 5 hours
1	10155±1979	24.0±16.2	423	75

Observation of significant compound concentration in the mouse lung confirmed that the observed inhibition of IL-13 induced pSTAT6 induction was a result of the activity of the test compound. The lung to plasma ratio at 5 hours showed that compound **1** exhibited significantly more exposure in the lung than exposure in plasma in mice.

Assay 4: Inhibition of TSLP-Evoked TARC Release in Human Peripheral Blood Mononuclear Cells

Thymic stromal lymphopoietin (TSLP) and thymus and activation-regulated chemokine (TARC) are overexpressed in asthmatic airways, and correlate with disease severity. In the lungs, TSLP may be released by bronchial epithelial cells in response to allergens and viral infections. TSLP signals through an IL-7Rα/TSLPR heterodimer found in a broad range of tissues and cell types, including epithelial cells, endothelial cells, neutrophils, macrophages, and mast cells. The binding of TSLP to its receptor induces a conformational change that activates JAK1 and JAK2 to phosphorylate various transcription factors, including STAT3 and STAT5. In immune cells, this triggers a cascade of intracellular events that result in cell proliferation, anti-apoptosis, dendritic cell migration, and production of Th2 cytokines and chemokines. In peripheral blood mononuclear cells (PBMC), TSLP has a proinflammatory effect by activating myeloid dendritic cells to attract and stimulate T cells, a process mediated by the chemoattractant TARC.

In this assay, it was shown that TSLP stimulation induces TARC release from PBMCs, and that this response is attenuated in a dose-dependent manner upon treatment with compound. The potencies of the test compounds were measured for inhibition of TARC release.

PBMC aliquots (previously isolated from whole blood and frozen in aliquots at -80°C) from 3 to 5 donors were thawed at 37°C and added dropwise to 40 mL pre-warmed, sterile-filtered, complete RPMI media in 50 mL Falcon tubes. Cells were pelleted and resuspended in complete media at 2.24×10^6 cells/mL. Cells were seeded at 85 µL (190,000 cells) per well in a tissue culture treated 96-well flat bottom microplate. Cells were allowed to rest for 1 hour at 37°C with 5% CO_2.

Compounds were received as 10 mM stock solutions in DMSO. 3.7-fold serial dilutions were performed to generate 9 concentrations of test compound in DMSO at 300X the final assay test concentration. 150-fold intermediate dilutions were performed in complete media to generate compound at 2X the final assay test concentration with 0.2% DMSO. After the 1 hour rest period, 95 µL of 2X compound was added to each well of PBMC, for a final assay concentration range of 33.33 µM to 0.95 µM. 95 µL of 0.2% DMSO in complete media was added to the untreated control wells. Cells were pre-treated with compound for 1 hour at 37°C with 5% CO_2 prior to stimulation.

Recombinant human TSLP protein was reconstituted at 10 μg/mL in sterile DPBS with 0.1% BSA and stored in aliquots at -20°C. Immediately prior to use, an aliquot was thawed and prepared at 20X the final assay concentration in complete media. 10 μL of 20X TSLP was added to each well of PBMC, for a final assay concentration of 10 ng/mL. 10 μL of complete media was added to the unstimulated control wells. Cells were stimulated in the presence of compound for 48 hours at 37°C with 5% CO_2.

Following stimulation, the cell culture supernatants were harvested and TARC levels were detected by enzyme-linked immunosorbent assay (ELISA), using Human CCL17/TARC Quantikine ELISA Kit (R&D Systems #DDN00) according to the manufacturer's instructions.

For dose response analysis, the log [test compound (M)] was plotted versus the percent response values for each donor, and IC_{50} values were determined using a nonlinear regression analysis with GraphPad Prism Software using the 4-parameter sigmoidal dose-response algorithm with variable slope. Data are expressed as mean pIC_{50} (negative decadic logarithm IC_{50}) values calculated from pIC_{50} values of individual donors and rounded to one decimal place. The potency values for inhibition by original compounds and their des-fluoro modified analogues are summarized in Table 7.

Table 7: Potency (pIC_{50}) Values of Test Compounds for Inhibition of TSLP-evoked TARC Release in Human Peripheral Blood Mononuclear Cells

Compound	pIC50 ± st. dev.
1	7.2 ± 0.1
C-1	7.0 ± 0.1

Assay 5: Lung S9 Metabolism

The in vitro metabolic stability of compounds **1** and **C-1** were evaluated in human lung S9 fraction (1 μM compound; 1 mg/mL S9 protein). The time 0, 15, 30, and 60 minute samples were analyzed for parent compound by high resolution LC-MS/MS. Lung S9 fractions from human (lot 1410245) were purchased from XenoTech LLC (Lenexa, KS). NADPH (Sigma Aldrich, N1630) and 3-phosphoadenosine 5-phosphosulfate (PAPS) (Sigma Aldrich, A1651) were purchased from Sigma Aldrich (St. Louis, MO). Acetonitrile and water were obtained from VWR (Radnor, PA) and were of HPLC grade or better. Raloxifene and formic acid was purchased from Sigma Aldrich (St. Louis, MO).

Lung S9 incubations were performed in a water bath at 37°C in a 96-well polypropylene plate. Lung S9 solutions consisted of 100mM potassium phosphate buffered to pH 7.4 (BD Biosciences, Woburn, MA) supplemented with 1 mM NADPH (Sigma-Aldrich, St. Louis, MO), 3 mM magnesium chloride (Sigma Aldrich, M1028) and in the presence of 100 µM PAPS (Sigma-Aldrich, St. Louis, MO) cofactor, with final incubation protein concentrations of 1mg/mL. 10mM DMSO stocks of Raloxifene (n=1) and compound (n=1) were diluted in buffer and spiked into the incubations to yield 1µM substrate concentrations (0.001% DMSO v/v). Incubation volumes consisted of 400µL and time points were taken at 0, 15, 30, and 60 minutes by the removal of a 70 µL aliquot and dilution into 140µL acetonitrile (0% formic acid). All samples were centrifuged at 2250 g for 10 minutes at 5°C. Supernatant (50µL) was taken from the centrifuged samples and diluted into 100µL HPLC water containing internal standard. The samples were run on a Dionex Ultimate 3000 Auto sampler and analyzed using a Thermo Q-Exactive High Resolution Mass Spectrometer (Thermo, Waltham, MA) in Full Scan mode in conjunction with an Atlantis T3 column 3µM – 2.1 x 50 mm (Waters Inc., 186003717). Mobile Phase A consisted of Water+0.2% formic acid and Mobile Phase B consisted of acetonitrile +0.2% formic acid. Peak integration was accomplished using Gubbs GMSU software (Gubbs Inc., Alpharetta, GA). For each sample, peak area ratios were calculated by dividing the analyte peak area by the internal standard peak area. For each incubation, the peak area ratios of the analytes in each t0 was set to 100%, and the peak areas ratios from the 60-minute samples were converted to percentages remaining relative to the corresponding t0. Determination of sulfate metabolite formation was made qualitatively by observation of early-eluting peak in the parent ion channel which, based on historical internal data, corresponded to the O-sulfate metabolite of each parent compound. The results of the assay are summarized in Table 8 (n=2 replicate).

Table 8: Metabolic Stability in Human Lung S9 Fraction

Compound	Clearance (µL/min/mg)	Compound Remaining at 60 min (%)	Sulfate Appearance
1	3.5	81	Yes
C-1	49.0	6	Yes

When compared to its corresponding fluoro analog (compound **C-1**), compound **1** gave rise to significantly less sulfation metabolism.

Assay 6: Pharmacokinetics in Plasma and Lung in Mouse

Plasma and lung concentrations of test compound and ratio thereof were determined in the following manner. BALB/c mice from Charles River Laboratories were used in the assay. Compound **1** was individually formulated in 20 % propylene glycol in pH 4 citrate buffer at a concentration of 0.2 mg/mL and 50 µL of the dosing solution was introduced into the trachea of a mouse by oral aspiration. At various time points (typically 0.167, 2, 6, 24 hr) post dosing, blood samples were removed via cardiac puncture and intact lungs were excised from the mice. Blood samples were centrifuged (Eppendorf centrifuge, 5804R) for 4 minutes at approximately 12,000 rpm at 4°C to collect plasma. Lungs were padded dry, weighed, and homogenized at a dilution of 1:3 in sterile water. Plasma and lung concentrations of test compound were determined by LC-MS analysis against analytical standards constructed into a standard curve in the test matrix. A lung-to-plasma ratio was determined as the ratio of the lung AUC in µg hr/g to the plasma AUC in µg hr/mL, where AUC is conventionally defined as the area under the curve of test compound concentration vs. time.

Table 9: Plasma and Lung Tissue Exposure Following a Single Oral Aspiration Administration of Compound 1

Compound	Plasma $AUC_{(0-24)}$ (µg hr/mL)	Lung Tissue $AUC_{(0-24)}$ (µg hr/g)	Lung Tissue:Plasma AUC ratio
1	0.943	54.5	57.8

Assay 7: Inhibition of IFNγ and IL-27 induced chemokines CXCL9 and CXCL10 in human 3D airway cultures

EpiAirway tissue cultures were obtained from Mattek (AIR-100). Cultures were derived from asthmatic donors. In a cell culture insert, human derived tracheal/bronchial epithelial cells were grown and differentiated on a porous membrane support, allowing an air-liquid interface with warmed culture medium below the cells and a gaseous test atmosphere above. Tissues were cultured in maintenance media (Mattek, AIR-100-MM) in a 37°C, 5% CO2 humidified incubator. Four donors were tested. On Day 0, tissue cultures were treated with test compound in liquid interface at 10µM, 1µM and/or 0.1µM. Compound **1** was diluted in dimethyl sulfoxide (DMSO, Sigma) to a final concentration of 0.1%. DMSO at 0.1% was used as a vehicle control. Compound **1** was incubated with cultures for 1 hour at 37°C, 5% CO_2, followed by the addition of pre-warmed media

containing IFNγ (R&D Systems) or IL-27 (R&D Systems) at a final concentration of 100ng/ml. Tissue cultures were maintained for 8 days. Media was replaced every 2 days with fresh media containing Compound **1** and IFNγ or IL-27. On Day 8, tissue cultures and supernatants were collected for analysis. Supernatant samples were assayed for CXCL10 (IP-10) and CXCL9 (MIG) using luminex analysis (EMD Millipore). Data is expressed as % Inhibition +/- standard deviation (±STDV). Percent inhibition was determined by compound inhibitory potency against IFNγ or IL-27 induced CXCL10 or CXCL9 secretion compared to vehicle treated cells. Data is the average from 4 donors. Compound **1** was able to inhibit IFNγ induced CXCL10 secretion by 100% ±1.0 (at 10μM), 76% ±13 (at 1μM) and 18% ±22 (at 0.1μM) when compared to vehicle control. Compound **1** was able to inhibit IFNγ induced CXCL9 secretion by 100% ±0.1 (at 10μM), 93% ±6.9 (at 1μM) and 16% ±41 (at 0.1μM) when compared to vehicle. Compound **1** was able to inhibit IL-27 induced CXCL10 secretion by 100% ±0.0 (at 10μM), 98% ±1.0 (at 1μM) and 25% ±26 (at 0.1μM) when compared to vehicle control. Compound **1** was able to inhibit IL-27 induced CXCL9 secretion by 100% ±0.0 (at 10μM), 97% ±2.0 (at 1μM) and 52% ±18 (at 0.1μM) when compared to vehicle control.

Assay 8: Cellular JAK Potency Assay: Inhibition of IFNγ-Induced pSTAT1

The potency of compound **1** for inhibition of interferon gamma (IFNγ) stimulated STAT1 phosphorylation was measured in CD14-positive (CD14+) monocytes derived from human whole blood (Stanford Blood Center) using flow cytometry. Because IFNγ signals through JAK, this assay provides a measure of JAK cellular potency.

Monocytes were identified using a fluorescein isothiocyanate (FITC) conjugated anti-CD14 antibody (Clone RM052, Beckman Coulter), and an Alexa Fluor 647 conjugated anti-pSTAT1 antibody (pY701, Clone 4a, BD Biosciences) was used to detect STAT1 phosphorylation.

Human peripheral blood mononuclear cells (PBMC) were isolated from human whole blood of healthy donors using a ficoll gradient. Cells were cultured in a 37 °C, 5 % CO_2 humidified incubator in RPMI (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 2 mM Glutamax (Life Technologies), 25 mM HEPES (Life Technologies) and 1X Pen/Strep (Life Technologies). Cells were seeded at 250,000 cells/well in media (200 μL), cultured for 2 h and re-suspended in assay media (50 μL) (RPMI supplemented with 0.1 % bovine serum albumin (Sigma), 2 mM Glutamax, 25 mM HEPES and 1X Penstrep) containing various concentrations of test compound. The compound was serially diluted in DMSO and then diluted another 1000-

fold in media to bring the final DMSO concentration to 0.1 %. Test compound dilutions were incubated with cells at 37 °C, 5 % CO_2 for 1 h, followed by the addition of pre-warmed IFNγ (R&D Systems) in media (50 µL) at a final concentration of 0.6 ng/mL for 30 min. After cytokine stimulation, cells were fixed with pre-warmed fix solution (100 µL) (BD Biosciences) for 10 min at 37 °C, 5 % CO_2, washed twice with FACS buffer (1 mL) (1% BSA in PBS), re-suspended in 1:10 anti-CD14 FITC:FACS buffer (100 µL), and incubated at 4 °C for 15 min. Cells were washed once, and then re-suspended in ice cold Perm Buffer III (BD Biosciences) (100 µL) for 30 min at 4 °C. Cells were washed twice with FACS buffer, and then re-suspended in 1:10 anti-pSTAT1 Alexa Fluor 647:FACS buffer (100 µL) for 30 min at RT in the dark, washed twice in FACS buffer, and analyzed using a MACSQuant flow cytometer (Miltenyi).

To determine the inhibitory potency of the test compound, the median fluorescent intensity (MFI) of pSTAT1 was measured in CD14+ monocytes. IC_{50} values were determined from analysis of the inhibition curves of MFI vs compound concentration. Data are expressed as pIC_{50} (negative decadic logarithm IC_{50}) values. Compound **1** exhibited a pIC_{50} value of 7.5 in this assay.

Assay 9: Cellular JAK Potency Assay: Inhibition of GM-CSF-Induced pSTAT5

The potency of compound **1** for inhibition of granulocyte-macropage colony-stimulating factor (GM-CSF) stimulated STAT5 phosphorylation was measured in CD14-positive (CD14+) monocytes derived from human whole blood (Stanford Blood Center) using flow cytometry. Because GM-CSF signals through JAK, this assay provides a measure of JAK cellular potency.

Monocytes were identified using a fluorescein isothiocyanate (FITC) conjugated anti-CD14 antibody (Clone RM052, Beckman Coulter), and an Alexa Fluor 647 conjugated anti-pSTAT5 antibody (pY694, BD Biosciences) was used to detect STAT5 phosphorylation.

Human peripheral blood mononuclear cells (PBMC) were isolated from human whole blood of healthy donors using a ficoll gradient. Cells were cultured in a 37 °C, 5 % CO_2 humidified incubator in RPMI (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 2 mM Glutamax (Life Technologies), 25 mM HEPES (Life Technologies) and 1X Pen/Strep (Life Technologies). Cells were seeded at 250,000 cells/well in media (200 µL), cultured for 2 h and re-suspended in assay media (50 µL) (RPMI supplemented with 0.1 % bovine serum albumin (Sigma), 2 mM

Glutamax, 25 mM HEPES and 1X Penstrep) containing various concentrations of test compound. The compound was serially diluted in DMSO and then diluted another 1000-fold in media to bring the final DMSO concentration to 0.1 %. Test compound dilutions were incubated with cells at 37 °C, 5 % CO_2 for 1 h, followed by the addition of pre-warmed GM-CSF (R&D Systems) in media (50 µL) at a final concentration of 0.3 ng/mL for 15 min. After cytokine stimulation, cells were fixed with pre-warmed fix solution (100 µL) (BD Biosciences) for 10 min at 37 °C, 5 % CO_2, washed twice with FACS buffer (1 mL) (1% BSA in PBS), re-suspended in 1:10 anti-CD14 FITC:FACS buffer (100 µL), and incubated at 4 °C for 15 min. Cells were washed once, and then re-suspended in ice cold Perm Buffer III (BD Biosciences) (100 µL) for 30 min at 4 °C. Cells were washed twice with FACS buffer, and then re-suspended in 1:10 anti-pSTAT1 Alexa Fluor 647:FACS buffer (100 µL) for 30 min at RT in the dark, washed twice in FACS buffer, and analyzed using a MACSQuant flow cytometer (Miltenyi).

To determine the inhibitory potency of the test compound, the median fluorescent intensity (MFI) of pSTAT5 was measured in CD14+ monocytes. IC_{50} values were determined from analysis of the inhibition curves of MFI vs compound concentration. Data are expressed as pIC_{50} (negative decadic logarithm IC_{50}) values. Compound **1** exhibited a pIC_{50} value of 6.9 in this assay.

Assay 10: Cellular JAK Potency Assay: Inhibition of IL-12-Induced pSTAT4

The potency of compound **1** for inhibition of interleukin-12 (IL-12) stimulated STAT4 phosphorylation was measured in CD3-positive (CD3+) T cells derived from human whole blood (Stanford Blood Center) using flow cytometry. Because IL-12 signals through JAK, this assay provides a measure of JAK cellular potency.

CD3+ T cells were identified using a phycoerythrin (PE) conjugated anti-CD3 antibody (clone UCHT1, BD Biosciences), and an Alexa Fluor 647 conjugated anti-pSTAT4 antibody (clone 38/p-Stat4, BD Biosciences) was used to detect STAT4 phosphorylation.

Human peripheral blood mononuclear cells (PBMC) were isolated from human whole blood of healthy donors using a ficoll gradient. Cells were cultured in RPMI (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 2 mM Glutamax (Life Technologies), 25 mM HEPES (Life Technologies), 1X Pen/Strep (Life Technologies), plate bound purified anti-CD3 antibody (5µg/ml, clone UCHT1, BD Biosciences) and soluble anti-CD28 antibody (1µg/ml, clone CD28.2, BD Biosciences) for 3 days in a 37 °C, 5 % CO_2 humidified incubator. Cells were harvested, washed with

media and then re-suspended in media containing interleukin-2 (IL-2, 10ng/ml, R&D Systems). Cells were cultured for 3 days in a 37°C, 5 % CO_2 humidified incubator. Cells were harvested, washed with RPMI and seeded at 250,000 cells/well in media (200 µL), cultured for 2 h and re-suspended in assay media (50 µL) (RPMI supplemented with 0.1 % bovine serum albumin (Sigma), 2 mM Glutamax, 25 mM HEPES and 1X Penstrep) containing various concentrations of test compound. The compound was serially diluted in DMSO and then diluted another 1000-fold in media to bring the final DMSO concentration to 0.1 %. Test compound dilutions were incubated with cells at 37 °C, 5 % CO_2 for 1 h, followed by the addition of pre-warmed IL-12 (R&D Systems) in media (50 µL) at a final concentration of 10 ng/mL for 30 min. After cytokine stimulation, cells were fixed with pre-warmed fix solution (100 µL) (BD Biosciences) for 10 min at 37 °C, 5 % CO_2, washed twice with FACS buffer (1 mL) (1% BSA in PBS), and re-suspended in ice cold Perm Buffer III (1000 µL) (BD Biosciences) for 30 min at 4°C. Cells were washed twice with FACS buffer, and then re-suspended in FACS buffer (100 µL) containing anti-CD3 PE (1:50 dilution) and anti-pSTAT4 Alexa Fluor 647 (1:10 dilution) for 45 min at room temperature in the dark. After incubation, cells were washed twice in FACS buffer before being analyzed using a MACSQuant flow cytometer (Miltenyi). To determine the inhibitory potency of the test compound, the median fluorescent intensity (MFI) of pSTAT4 was measured in CD3+ T cells. IC_{50} values were determined from analysis of the inhibition curves of MFI vs compound concentration. Data are expressed as pIC_{50} (negative decadic logarithm IC_{50}) values. Compound **1** exhibited a pIC_{50} value of 6.0 in this assay.

Assay 11: Inhibition of IFNγ secretion in a Mixed Lymphocyte Reaction Assay

The mixed lymphocyte reaction assay is an in-vitro assay that mimics transplant rejection. T cells from one donor are cultured with allogeneic dendritic cells from another donor. This reaction induces a cellular immune response such as IFNγ secretion.

CD14+ monocytes were isolated from human whole blood (Stanford blood center) of donor A using a ficoll gradient and magnetic separation (CD14 microbeads, Miltenyi). Monocytes were differentiated into dendritic cells by culturing cells in RPMI (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 2 mM Glutamax (Life Technologies), 1X Pen/Strep (Life Technologies), interleukin-4 (IL-4, 50ng/ml, R&D Systems) and granulocyte-macrophage colony-stimulating factor (GM-CSF, 50ng/ml, R&D Systems) for 6 days in a 37 °C, 5 % CO_2 humidified incubator.

Dendritic cells were harvested, washed with media and then activated by culturing cells in media containing lipopolysaccharide from *Escherichia coli* (LPS, 100ng/ml, Sigma) for 24 hours in a 37°C, 5 % CO_2 humidified incubator. Cells were harvested, washed with media, re-suspended to 400,000 cells/ml in media and plated at 10,000 cells/well/25µl. CD4+ T cells were freshly isolated from human whole blood (Stanford blood center) of donor B using a ficoll gradient and magnetic separation (CD4+ T cell isolation kit, Miltenyi). T cells were re-suspended to 4,000,000 cells/ml in RPMI (Life Technologies) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 2 mM Glutamax (Life Technologies), 25 mM HEPES (Life Technologies) and 1X Pen/Strep (Life Technologies). CD4+ T cells were mixed with the dendritic cells at 100,000 cells/well/25µl. Cells were treated with test compound (50µl at 20µM, 2µM and/or 0.2µM) to a final concentration of 10µM, 1µM and/or 0.1µM. Compound 1 was diluted in dimethyl sulfoxide (DMSO, Sigma) to a final concentration of 0.1%. DMSO at 0.1% was used as a vehicle control. Cells were maintained for 5 days in a 37°C, 5 % CO_2 humidified incubator. On day 5, supernatants were collected and measured for interferon gamma (INFγ) using enzyme linked immunosorbent assay (ELISA). Percent inhibition was determined by compound inhibitory potency against IFNγ secretion compared to vehicle treated cells. Data is the average from 4 donors. Compound **1** was able to inhibit IFNγ secretion by 99% ±0.4 (at 10µM), 76% ±10 (at µM) and 43% ±12 (at 0.1µM) when compared to vehicle control.

Assay 12: Inhibition of spontaneous periostin and IL-6 secretion in human 3D airway cultures derived from asthmatic donors

EpiAirway tissue cultures were obtained from Mattek (AIR-100). Cells were derived from asthmatic donors that spontaneously secrete periostin, a matricellular protein associated with Th2 mediated asthma (eosinophilic), and interleukin-6 (IL-6), an inflammatory cytokine that plays a role in both Th2 and non-Th2 related asthma. In a cell culture insert, human derived tracheal/bronchial epithelial cells were grown and differentiated on a porous membrane support, allowing an air-liquid interface with warmed culture medium below the cells and a gaseous test atmosphere above. Tissues were cultured in maintenance media (Mattek, AIR-100-MM) in a 37°C, 5% CO2 humidified incubator. Four donors were tested. On Day 0, tissue cultures were treated with test compound in liquid interface at 10µM, 1µM and/or 0.1µM. Compound 1 was diluted in dimethyl sulfoxide (DMSO, Sigma) to a final concentration of 0.1%. DMSO at 0.1% was used as a vehicle control. Tissue cultures were maintained for 8 days. Media

was replaced every 2 days with fresh media containing compound 1. On Day 8, supernatants were collected for analysis. Supernatant samples were assayed for periostin and interleukin-6 (IL-6) using luminex analysis (EMD Millipore). Data is expressed as % Inhibition +/- standard deviation (±STDV). Percent inhibition was determined by compound inhibitory potency against spontaneous secretion of periostin and IL-6 compared to vehicle treated cells. Data is the average from 3 or 4 donors. Compound **1** was able to inhibit spontaneous periostin secretion by 62% ±25 (at 10μM) and 40% ±28 (at 1μM) when compared to vehicle control. Compound **1** was able to inhibit spontaneous IL-6 secretion by 91% ±9.0 (at 10μM), 70% ±33 (at 1μM) and 10% ±40 (at 0.1μM) when compared to vehicle.

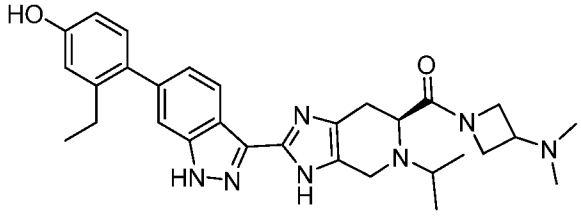
Crystal Structure

A co-crystal structure was obtained of compound **C-1** bound to human JAK1 at a resolution of 2.28 Å. The ligand was observed to bind in the ATP binding site. Seven specific hydrogen bonding interactions were identified based upon a distance of 3.5 Å or less between donor and acceptor atoms. Of particular note, a hydrogen bonding interaction was identified between the carbonyl of the exocyclic amide of the compound of **C-1** and the sidechain of Arg879 of JAK1. In earlier modeling studies, this interaction had been proposed as a way of providing selectivity for JAK1 over other tyrosine kinases, as otherwise closely related kinases (e.g. TRKA, VEGFR, ABL1) do not possess an arginine residue at the equivalent location. The observed results of the hydrogen bonding interaction in the crystal structure and improved kinome selectivity compared to series not possessing the exocyclic amide validate this design hypothesis.

While the present disclosure has been described with reference to specific aspects or embodiments thereof, it will be understood by those of ordinary skilled in the art that various changes can be made or equivalents can be substituted without departing from the true spirit and scope of the disclosure. Additionally, to the extent permitted by applicable patent statutes and regulations, all publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety to the same extent as if each document had been individually incorporated by reference herein.

WHAT IS CLAIMED IS:

1. A crystalline hydrate of the compound of formula **1**:



1

wherein the crystalline hydrate is characterized by a powder X-ray diffraction pattern comprising diffraction peaks at 2θ values of 5.68±0.20, 10.43±0.20, 10.94±0.20, and 13.08±0.20.

2. The crystalline hydrate of claim 1, wherein the powder X-ray diffraction pattern is further characterized by having one additional diffraction peak at a 2θ value of 8.49±0.20.

3. The crystalline hydrate of claim 2, wherein the powder X-ray diffraction pattern is further characterized by having two or more additional diffraction peaks at 2θ values selected from 11.55±0.20, 12.20±0.20, 17.06±0.20, and 26.29±0.20.

4. The crystalline hydrate of claim 2, wherein the powder X-ray diffraction pattern is further characterized by having additional diffraction peaks at 2θ values of 11.55±0.20, 12.20±0.20, 17.06±0.20, and 26.29±0.20.

5. The crystalline hydrate of any one of claims 1 to 4, wherein the crystalline hydrate is characterized by a powder X-ray diffraction pattern in which the peak positions are substantially in accordance with the peak positions of the pattern shown in Figure 1.

6. The crystalline hydrate of any one of claims 1 to 5, wherein the crystalline hydrate is characterized by a differential scanning calorimetry trace recorded at a heating rate of 10 °C per minute which shows a maximum in endothermic heat flow at a temperature of 212.4±3 °C.

7. The crystalline hydrate of any one of claims 1 to 6, wherein the crystalline hydrate is characterized by a differential scanning calorimetry trace substantially in accordance with that shown in Figure 2.

8. The crystalline hydrate of any one of claims 1 to 7, wherein the crystalline hydrate is a monohydrate.

9. A pharmaceutical composition comprising a crystalline hydrate of any one of claims 1 to 8, and a pharmaceutically-acceptable carrier.

10. A method of preparing the crystalline hydrate of any one of claims 1 to 8, the method comprising:

(a) dissolving (*S*)-(3-(dimethylamino)azetidin-1-yl)(2-(6-(2-ethyl-4-hydroxyphenyl)-1*H*-indazol-3-yl)-5-isopropyl-4,5,6,7-tetrahydro-3*H*-imidazo[4,5-c]pyridin-6-yl)methanone in an alcohol solvent at a temperature of 55°C ±10°C to give a solution;

(b) cooling down the solution obtained in step (a) to 10°C ±10°C to produce a suspension;

(c) isolating the solid from the suspension of step (b) under inert gas conditions;

(d) drying the solid obtained in step (c) at 60°C ±15°C;

(e) subjecting the solid obtained in step (d) to ambient humidity and temperature conditions to give the crystalline hydrate.

11. The method of claim 10, wherein the alcohol solvent is methanol or ethanol.

12. The crystalline hydrate of any one of claims 1 to 8, for treating a respiratory disease in a mammal.

13. The crystalline hydrate of claim 12, wherein the respiratory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonitis, idiopathic pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, sarcoidosis, an eosinophilic

disease, a helminthic infection, pulmonary arterial hypertension, lymphangioleiomyomatosis, bronchiectasis, an infiltrative pulmonary disease, drug-induced pneumonitis, fungal induced pneumonitis, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, eosinophilic granulomatosis with polyangiitis, idiopathic acute eosinophilic pneumonia, idiopathic chronic eosinophilic pneumonia, hypereosinophilic syndrome, Löffler syndrome, bronchiolitis obliterans organizing pneumonia, lung graft-versus-host disease, and immune-checkpoint-inhibitor induced pneumonitis.

14. The crystalline hydrate of claim 12, wherein the respiratory disease is asthma.

15. The crystalline hydrate of claim 14, wherein the asthma is moderate to severe asthma.

16. The crystalline hydrate of claim 12, wherein the crystalline hydrate is administered in a pharmaceutical composition by inhalation.

17. The crystalline hydrate of any one of claims 1 to 8, for preventing or delaying lung transplant rejection in a mammal.

18. The crystalline hydrate of claim 17, wherein the lung transplant rejection is selected from the group consisting of primary graft dysfunction, organizing pneumonia, acute rejection, lymphocytic bronchiolitis, and chronic lung allograft dysfunction.

19. The crystalline hydrate of claim 17, wherein the lung transplant rejection is acute lung transplant rejection.

20. The crystalline hydrate of claim 17, wherein the lung transplant rejection is chronic lung allograft dysfunction.

21. The crystalline hydrate of claim 17, wherein the lung transplant rejection is selected from the group consisting of bronchiolitis obliterans, restrictive chronic lung allograft dysfunction, and neutrophilic allograft dysfunction.

22. The crystalline hydrate of claim 17, wherein the crystalline hydrate is administered in a pharmaceutical composition by inhalation.

23. Use of the crystalline hydrate of any one of claims 1 to 8 in the manufacture of a medicament for the treatment of a respiratory disease in a mammal.

24. The use of claim 23, wherein the respiratory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonitis, idiopathic pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, sarcoidosis, an eosinophilic disease, a helminthic infection, pulmonary arterial hypertension, lymphangioleiomyomatosis, bronchiectasis, an infiltrative pulmonary disease, drug-induced pneumonitis, fungal induced pneumonitis, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, eosinophilic granulomatosis with polyangiitis, idiopathic acute eosinophilic pneumonia, idiopathic chronic eosinophilic pneumonia, hypereosinophilic syndrome, Löffler syndrome, bronchiolitis obliterans organizing pneumonia, lung graft-versus-host disease, and immune-checkpoint-inhibitor induced pneumonitis.

25. The use of claim 23, wherein the respiratory disease is asthma.

26. The use of claim 25, wherein the asthma is moderate to severe asthma.

27. The use of claim 23, wherein the medicament is administered by inhalation.

28. Use of the crystalline hydrate of any one of claims 1 to 8 in the manufacture of a medicament for preventing or delaying lung transplant rejection in a mammal.

29. The use of claim 28, wherein the lung transplant rejection is selected from the group consisting of primary graft dysfunction, organizing pneumonia, acute rejection, lymphocytic bronchiolitis, and chronic lung allograft dysfunction.

30. The use of claim 28, wherein the lung transplant rejection is acute lung transplant rejection.

31. The use of claim 28, wherein the lung transplant rejection is chronic lung allograft dysfunction.

32. The use of claim 28, wherein the lung transplant rejection is selected from the group consisting of bronchiolitis obliterans, restrictive chronic lung allograft dysfunction, and neutrophilic allograft dysfunction.

33. The use of claim 28, wherein the medicament is administered by inhalation.

34. A method of treating a respiratory disease in a mammal, the method comprising administering to the mammal a pharmaceutical composition comprising the crystalline hydrate of any one of claims 1 to 8 and a pharmaceutically-acceptable carrier.

35. The method of claim 34, wherein the respiratory disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, cystic fibrosis, pneumonitis, idiopathic pulmonary fibrosis, acute lung injury, acute respiratory distress syndrome, bronchitis, emphysema, sarcoidosis, an eosinophilic disease, a helminthic infection, pulmonary arterial hypertension, lymphangioleiomyomatosis, bronchiectasis, an infiltrative pulmonary disease, drug-induced pneumonitis, fungal induced pneumonitis, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, eosinophilic granulomatosis with polyangiitis, idiopathic acute eosinophilic pneumonia, idiopathic chronic eosinophilic pneumonia, hypereosinophilic syndrome, Löffler syndrome, bronchiolitis obliterans organizing pneumonia, lung graft-versus-host disease, and immune-checkpoint-inhibitor induced pneumonitis.

36. The method of claim 34, wherein the respiratory disease is asthma.

37. The method of claim 36, wherein the asthma is moderate to severe asthma.

38. The method of claim 34, wherein the pharmaceutical composition is administered by inhalation.

39. A method of preventing or delaying lung transplant rejection in a mammal, the method comprising administering to the mammal a pharmaceutical composition comprising the crystalline hydrate of any one of claims 1 to 8, and a pharmaceutically-acceptable carrier.

40. The method of claim 39, wherein the lung transplant rejection is selected from the group consisting of primary graft dysfunction, organizing pneumonia, acute rejection, lymphocytic bronchiolitis, and chronic lung allograft dysfunction.

41. The method of claim 39, wherein the lung transplant rejection is acute lung transplant rejection.

42. The method of claim 39, wherein the lung transplant rejection is chronic lung allograft dysfunction.

43. The method of claim 39, wherein the lung transplant rejection is selected from the group consisting of bronchiolitis obliterans, restrictive chronic lung allograft dysfunction, and neutrophilic allograft dysfunction.

44. The method of claim 39, wherein the pharmaceutical composition is administered by inhalation.

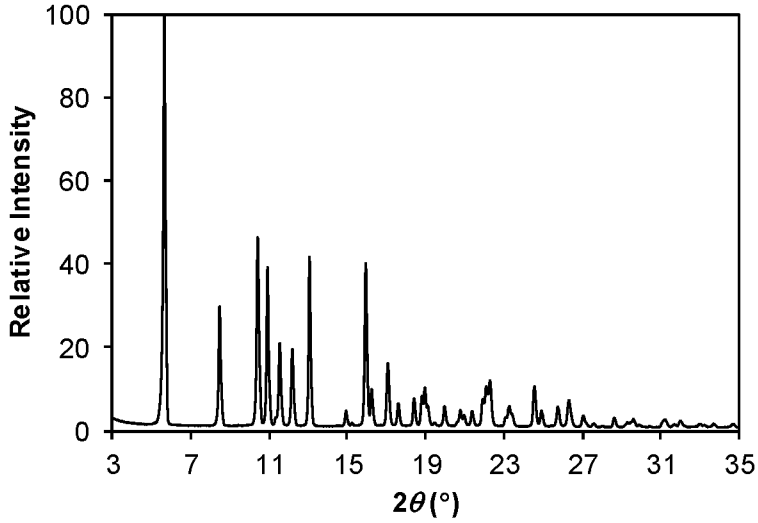

100
80
60
40
20
0
Relative Intensity
3
7
11
15
19
23
27
31
35
2θ(°)

FIG. 1

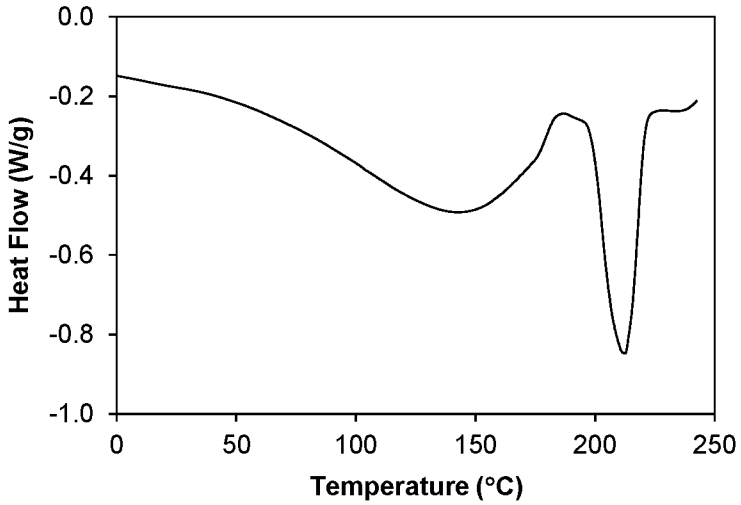

0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Heat Flow (W/g)
0
50
100
150
200
250
Temperature (°C)

FIG. 2

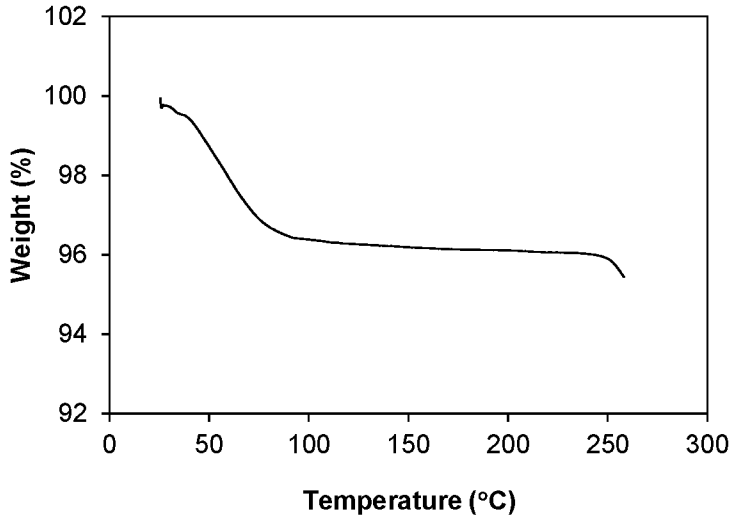

102
100
98
96
94
92
Weight (%)
0
50
100
150
200
250
300
Temperature (°C)

FIG. 3

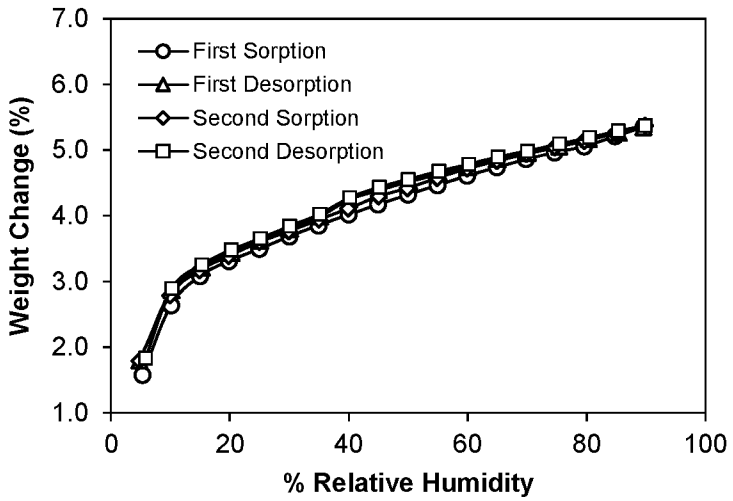

7.0
6.0
5.0
4.0
3.0
2.0
1.0
Weight Change (%)
First Sorption
First Desorption
Second Sorption
Second Desorption
0
20
40
60
80
100
% Relative Humidity

FIG. 4