RADIOLABELLED DERIVATIVES OF A 2-AMINO-6-FLUORO-N-[5-FLUORO-PYRIDIN-3-YL]-PYRAZOLO[1,5-A]PYRIMIDIN-3-CARBOXAMIDE COMPOUND USEFUL AS ATR KINASE INHIBITOR, THE PREPARATION OF SAID COMPOUND AND DIFFERENT SOLID FORMS THEREOF

BACKGROUND OF THE INVENTION

[0001] ATR ("ATM and Rad3 related") kinase is a protein kinase involved in cellular responses to DNA damage. ATR kinase acts with ATM ("ataxia telangiectasia mutated") kinase and many other proteins to regulate a cell's response to DNA damage, commonly referred to as the DNA Damage Response ("DDR"). The DDR stimulates DNA repair, promotes survival and stalls cell cycle progression by activating cell cycle checkpoints, which provide time for repair. Without the DDR, cells are much more sensitive to DNA damage and readily die from DNA lesions induced by endogenous cellular processes such as DNA replication or exogenous DNA damaging agents commonly used in cancer therapy.

[0002] Healthy cells can rely on a host of different proteins for DNA repair including the DDR kinase ATR. In some cases these proteins can compensate for one another by activating functionally redundant DNA repair processes. On the contrary, many cancer cells harbour defects in some of their DNA repair processes, such as ATM signaling, and therefore display a greater reliance on their remaining intact DNA repair proteins which include ATR.

[0003] In addition, many cancer cells express activated oncogenes or lack key tumour suppressors, and this can make these cancer cells prone to dysregulated phases of DNA replication which in turn cause DNA damage. ATR has been implicated as a critical component of the DDR in response to disrupted DNA replication. As a result, these cancer cells are more dependent on ATR activity for survival than healthy cells. Accordingly, ATR inhibitors may be useful for cancer treatment, either used alone or in combination with DNA damaging agents, because they shut down a DNA repair mechanism that is more important for cellular survival in many cancer cells than in healthy normal cells.

[0004] In fact, disruption of ATR function (e.g. by gene deletion) has been shown to promote cancer cell death both in the absence and presence of DNA damaging agents. This suggests that ATR inhibitors may be effective both as single agents and as potent sensitizers to radiotherapy or genotoxic chemotherapy.

[0005] For all of these reasons, there is a need for the development of potent and selective ATR inhibitors for the treatment of cancer, either as single agents or as combination therapies with radiotherapy or genotoxic chemotherapy. Furthermore, it would be desirable to have a

synthetic route to ATR inhibitors that is amenable to large-scale synthesis and improves upon currently known methods.

[0006] ATR peptide can be expressed and isolated using a variety of methods known in the literature (see e.g., Ünsal-Kaçmaz et al, *PNAS* 99: 10, pp6673-6678, May 14, 2002; see also Kumagai et al. Cell 124, pp943-955, March 10, 2006; Unsal-Kacmaz et al. Molecular and Cellular Biology, Feb 2004, p1292-1300; and Hall-Jackson et al. Oncogene 1999, 18, 6707-6713).

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1a: XRPD Compound **I-I** anhydrous free base

FIGURE 2a: TGA Compound **I-1** anhydrous free base

FIGURE 3a: DSC Compound **I-1** anhydrous free base

FIGURE 1b: XRPD Compound **I-1** hydrate

FIGURE 2b: TGA Compound **I-1** hydrate

FIGURE 3b: DSC Compound **I-1** hydrate

FIGURE 1c: XRPD Compound **I-1** tartaric acid

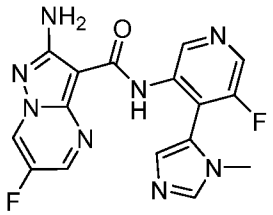
FIGURE 2c: TGA Compound **I-1** tartaric acid

FIGURE 3c: DSC Compound **I-1** tartaric acid

SUMMARY OF THE INVENTION

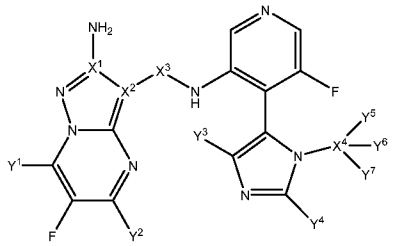
[0007] The present invention relates to solid forms of ATR inhibitors as well as deuterated ATR inhibitors. The present invention also relates to processes and intermediates for preparing an aminopyrazolopyrimidine compound useful as a potent inhibitor of ATR kinase. Amino-pyrazolopyrimidine derivatives are useful as ATR inhibitors and are also useful for preparing ATR inhibitors.

[0008] One aspect of the invention provides a process for preparing compound **I-1**:



I-1

[0009] Another aspect of the present invention comprises a compound of formula **I-A**:



I-A

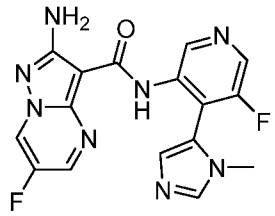
or a pharmaceutically acceptable salt or derivative thereof, wherein:

each Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is independently hydrogen or deuterium; provided at least one of Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is deuterium;

each X^1, X^2, and X^4 is independently selected from ^{12}C or ^{13}C; and

X^3 is independently selected from $-^{12}C(O)-$ or $-^{13}C(O)-$.

[0010] Yet another aspect of the invention provides solid forms of a compound of formula **I-1:**



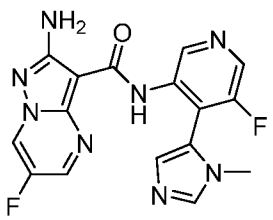
I-1

[0011] Other aspects of the invention are set forth herein.

DETAILED DESCRIPTION OF THE INVENTION

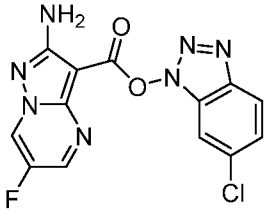
Processes

[0012] Another aspect of the present invention comprises a process for preparing a compound of formula **I-1**:



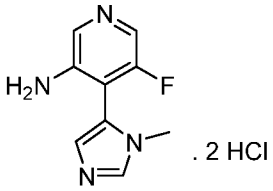
I-1

comprising the step of reacting the compound of formula **6b**:



6b

with a compound of formula **11**:

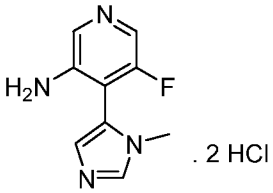


11

under suitable conditions to form an amide bond.

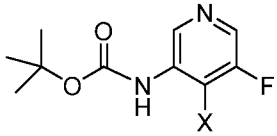
[0013] Suitable conditions for forming the amide bond comprises reacting the compound of formula **6b** with the substituted 3-amino pyridine **11** in the presence of a solvent and an organic base. In one embodiment, the solvent can be selected from NMP, DMF or anisole (preferred). In another embodiment, the organic base is an aliphatic amine independently selected from triethylamine or DIPEA (preferred).

[0014] Still other embodiments of the present invention comprises a process for preparing the compound of formula **11**:



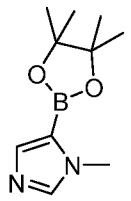
11

by reacting the compound of formula **9**:



9

with a compound of formula **10**:



10

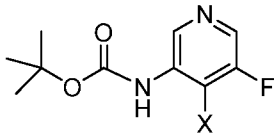
under suitable metal catalysed cross-coupling conditions to form an adduct containing a protected amine group; and

subjecting the resulting adduct to suitable deprotection conditions.

[0015] Suitable metal catalysed cross-coupling conditions include a metal catalyst, a suitable solvent, and a suitable base. In some embodiments, the metal catalyst is a palladium catalyst. Examples of suitable palladium catalysts include, but are not limited to, $PdCl_2(PPh_3)_2$, $Pd(Ph_3)_4$, and $PdCl_2$(dppf) (wherein each Ph is phenyl, and dppf is 1,1-bis(diphenylphosphino)ferrocene). Suitable bases include, but are not limited to, potassium phosphate, K_2CO_3, tBuOK and Na_2CO_3. Suitable solvents include, but are not limited to, DME, tetrahydrofuran, toluene, and ethanol.

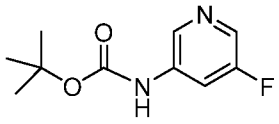
[0016] Suitable deprotection conditions for removing the protecting group comprises reacting the protected species in the presence of a strong acid, such as HCl (preferred), HBr, sulfuric acid or trifluoroacetic acid.

[0017] Another embodiment provides a process for preparing a compound of formula **9**:



9

by reacting the compound of formula **8:**



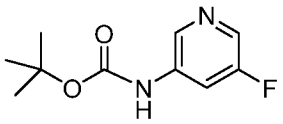
8

under suitable halogenation conditions.

[0018] Suitable halogenation conditions comprises reacting compound **8** in an aprotic solvent, in the presence of a strong base, and an electrophilic source of halogen. In one embodiment, the solvent can be selected from DCM, diethylether or THF (preferred). In

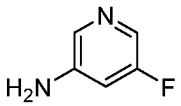
another embodiment, the strong base is selected from *tert*-BuLi, sec-BuLi or n-BuLi (preferred). In yet another embodiment, the electrophilic species used to introduce the halogen atom can, for example, be selected from I_2 (preferred), CF_3I, diiodoethane, Br_2, CBr_4.

[0019] Still other embodiments of the present invention provides a process for preparing a compound of formula **8**:



8

by reacting a compound of formula **7**:



7

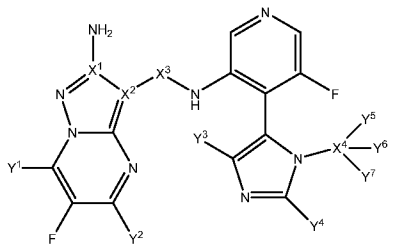
under suitable conditions to generate a protected amine group.

[0020] Suitable conditions for introducing the protecting group comprises reacting the amino species **7** in an aprotic solvent, in the presence of Boc_2O. Such reaction can be conducted in the presence of a base. In one embodiment, the solvent can be selected from diethylether or THF (preferred). In another embodiment, the strong base can be selected from DMAP, n-BuLi, LHMDS or NaHMDS (preferred).

Deuterated Compounds

[0021] Isotopes can be introduced on compound **I-1** by selecting building blocks that contain the isotopic atoms (either commercial or that can be prepared according to the literature) and engaging them into a sequence similar to the novel and inventive process reported for the unlabelled material (described above).

[0022] Another aspect of the present invention provides a compound of Formula **I-A**:



I-A

or a pharmaceutically acceptable salt or derivative thereof, wherein:

each Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is independently hydrogen or deuterium; provided at least one of Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is deuterium;

each X^1, X^2, and X^4 is independently selected from ^{12}C or ^{13}C; and

X^3 is independently selected from -$^{12}C(O)$- or -$^{13}C(O)$-.

[0023] The following labelled building blocks, which can be used in the synthetic route for preparing the compound of Formula **I-A**, are all commercially available:

- 1, 2-Di^{13}C-2-cyanoacetic acid;
- 1-^{13}C-2-cyano(^{13}C)acetic acid ethyl ester;
- 2-^{13}C-2-cyano(^{13}C)acetic acid ethyl ester;
- 1-(trideuteromethyl)-1H-imidazole;
- 2,4,5-trideutero-1-(methyl)-1H-imidazole; and
- 2,4,5-trideutero-1-(trideuteromethyl)-1H-imidazole.

[0024] Other labelled building blocks, which can be used in the synthetic route for preparing the compound of Formula **I-A**, are known to those skilled in the art. These may include, but are not limited to, the following labelled building blocks:

- 2-cyano(^{13}C)acetic acid; Triplett et al., J Labelled Comp Radiopharm, 1978, 14(1), 35;
- 1-^{13}C-2-cyanoacetic acid; Matsumoto et al., Heterocycles, 1985, 23(8), 2041;
- 2-^{13}C-2-cyanoacetic acid; Baldwin et al., J Am Chem Soc, 1989, 111(9), 3319;
- 1-deutero-3-(diethylamino)-2-fluoroacrylaldehyde; Funabiki et al., Chem Lett, 1997, (8), 739;
- 2-deutero-1-(methyl)-1H-imidazole; Torregrosa et al., Tetrahedron, 2005, 61(47), 11148-11155;
- 4,5-dideutero-1-(methyl)-1H-imidazole; Pavlik et al., J. Org. Chem., 1991, 56(22), 6313-6320;
- 4,5-dideutero-1-(trideuteromethyl)-1H-imidazole; Mamer et al., Rapid Communications in Mass Spectrometry, 2005, 19(12), 1771-1774;

- 2-tritio-1-(methyl)-1H-imidazole; Buncel et al., Can. J. Chem., 1986, 64(6), 1240-1245;
- 2,4,5-tritritio-1-(methyl)-1H-imidazole; Grimmett, Scien of Synthesis, 2002, 325-528; and
- 1-(^{13}C-methyl)-1H-imidazole; Van Thuijl et al., Organic Mass Spectrometry, 1973, 7(10), 1165-1172.

[0025] In one or more embodiments of the present invention, Y^1, Y^2, Y^3, and Y^4 are independently selected from deuterium or hydrogen; and Y^5, Y^6, and Y^7 are deuterium.

[0026] In some embodiments, Y^1 and Y^2 are independently selected from deuterium or hydrogen; and Y^3, Y^4, Y^5, Y^6, and Y^7 are deuterium.

[0027] In another embodiment, Y^1, Y^2, Y^5, Y^6, and Y^7 are independently selected from deuterium or hydrogen; and Y^3 and Y^4 are deuterium.

[0028] In other embodiments, Y^1, Y^3, and Y^4 are independently selected from deuterium or hydrogen; and Y^2, Y^5, Y^6, and Y^7 are deuterium.

[0029] In still other embodiments, Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^4 is ^{13}C.

[0030] In yet another embodiment, Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^1 and X^4 are ^{13}C.

[0031] In some embodiments, Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^3 is -^{13}C(O)-.

[0032] In another embodiment, Y^1, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; Y^2 is deuterium; and X^4 is ^{13}C.

[0033] In other embodiments, Y^1, Y^2, Y^3, and Y^4 are hydrogen; Y^5, Y^6, and Y^7 are deuterium; and X^1 is ^{13}C.

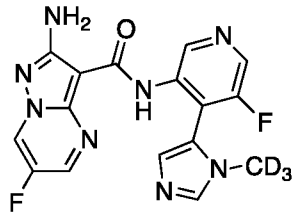
[0034] In still other embodiments, Y^1, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; Y^2 is deuterium; and X^1 is ^{13}C.

[0035] In yet another embodiment, Y^1, Y^2, Y^3, Y^5, Y^6, and Y^7 are hydrogen; Y^4 is deuterium; and X^1 is ^{13}C.

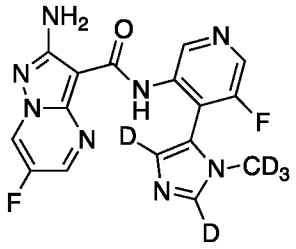
[0036] In another embodiment, Y1 is hydrogen; Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are deuterium; X^2 is ^{13}C; and X^3 is -^{13}C(O)-.

[0037] In another example, the compounds of formula **I-A** of this invention are represented in Table 1. It will be appreciated by those skilled in the art that the compounds of the present invention may be represented in varying tautomeric forms.

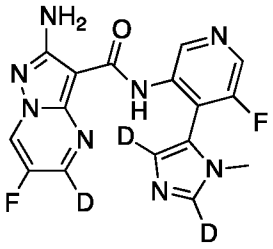
Table 1



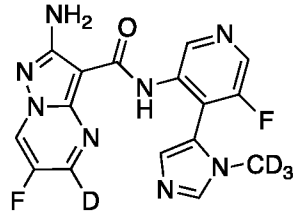
I-2



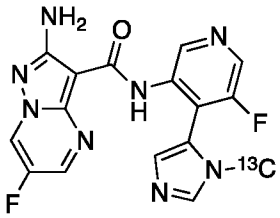
I-3



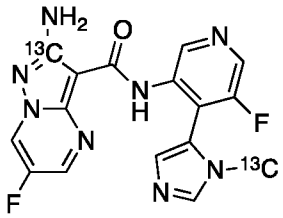
I-4



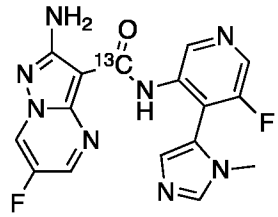
I-5



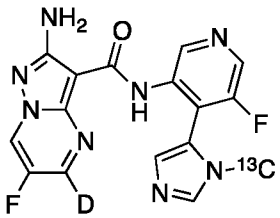
I-6



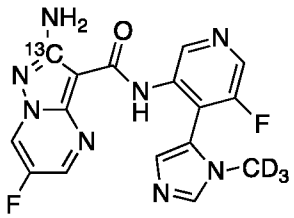
I-7



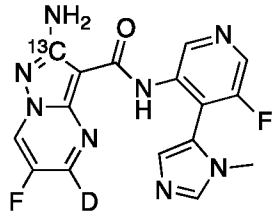
I-8



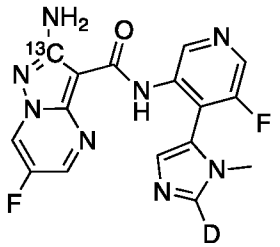
I-9



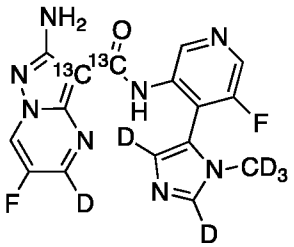
I-10



I-11



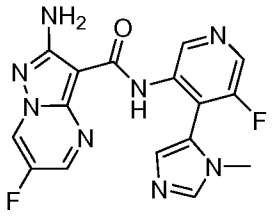
I-12



I-13 .

Solid Forms

[0038] Another aspect of the present invention provides a solid form of a compound of formula **I-1**:



I-1

wherein, the form is selected from the group consisting of Compound **I-1** anhydrous free base, Compound **I-1** hydrate, or Compound **I-1** tartaric acid **.**

*Compound **I-1** anhydrous free base*

[0039] In some aspects of the present inventions, the solid form is Compound **I-1** anhydrous free base. In another aspect of the present invention, the solid form is crystalline Compound **I-1** anhydrous free base. In some embodiments, the solid form is characterized by one or more peaks expressed in 2-theta ± 0.2 at about 9.9, 12.8, 15.4, 17.0, 23.1, 27.8, 29.0, and 30.1 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation. In other embodiments, the solid form is characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1a.

Compound I-1 hydrate

[0040] In some aspects of the present invention, the solid form is Compound **I-1** hydrate. In another aspect of the present invention, the solid form is crystalline Compound **I-1** hydrate. In other embodiments, the crystalline Compound **I-1** hydrate has a Compound **I-1** to water ratio of 1:3. In still other embodiments, Compound **I-1** hydrate is characterized by a weight loss of from about 12.6% in a temperature range from about 40°C and about 100°C. In some embodiments, the solid form is characterized by one or more peaks expressed in 2-theta ± 0.2 at about 27.5, 20.6, and 9.7 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation. In yet other embodiments, the solid form is characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1b.

Compound I-1 tartaric acid

[0041] In some aspects of the present invention, the solid form is Compound **I-1** tartaric acid. In another aspect of the present invention, the solid form is crystalline Compound **I-1** tartaric acid. In other embodiments, the crystalline Compound **I-1** tartaric acid has a Compound **I-1** to tartaric acid ratio of 1:1. In some embodiments, the solid form is

characterized by one or more peaks expressed in 2-theta ± 0.2 at about 7.1, 18.3, and 13.2 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation. In yet other embodiments, the solid form is characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1c.

[0042] For purposes of this application, it will be understood that the terms embodiment, example, and aspect are used interchangeably.

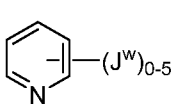
[0043] Compounds of this invention include those described generally herein, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0044] As described herein, a specified number range of atoms includes any integer therein. For example, a group having from 1-4 atoms could have 1, 2, 3, or 4 atoms.

[0045] As described herein, compounds of the invention may optionally be substituted with one or more substituents, such as are illustrated generally herein, or as exemplified by particular classes, subclasses, and species of the invention. It will be appreciated that the phrase "optionally substituted" is used interchangeably with the phrase "substituted or unsubstituted." In general, the term "substituted", whether preceded by the term "optionally" or not, refers to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. Unless otherwise indicated, an optionally substituted group may have a substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds.

[0046] Unless otherwise indicated, a substituent connected by a bond drawn from the center of a ring means that the substituent can be bonded to any position in the ring. In example **i** below, for instance, J^w can be bonded to any position on the pyridyl ring. For bicyclic rings,

a bond drawn through both rings indicates that the substituent can be bonded from any position of the bicyclic ring. In example **ii** below, for instance, J^w can be bonded to the 5-membered ring (on the nitrogen atom, for instance), and to the 6-membered ring.



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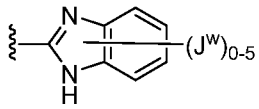


ii

[0047] The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, recovery, purification, and use for one or more of the purposes disclosed herein. In some embodiments, a stable compound or chemically feasible compound is one that is not substantially altered when kept at a temperature of 40°C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

[0048] The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched), branched, or cyclic, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation that has a single point of attachment to the rest of the molecule.

[0049] Unless otherwise specified, aliphatic groups contain 1-20 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms, and in yet other embodiments aliphatic groups contain 1-4 aliphatic carbon atoms. Aliphatic groups may be linear or branched, substituted or unsubstituted alkyl, alkenyl, or alkynyl groups. Specific examples include, but are not limited to, methyl, ethyl, isopropyl, n-propyl, sec-butyl, vinyl, n-butenyl, ethynyl, and *tert*-butyl. Aliphatic groups may also be cyclic, or have a combination of linear or branched and cyclic groups. Examples of such types of aliphatic groups include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, -CH_2-cyclopropyl, $CH_2CH_2CH(CH_3)$-cyclohexyl.

[0050] The term "cycloaliphatic" (or "carbocycle" or "carbocyclyl") refers to a monocyclic C_3-C_8 hydrocarbon or bicyclic C_8-C_{12} hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule wherein any individual ring in said bicyclic ring system has 3-7 members. Examples of cycloaliphatic groups include, but are not limited to,

cycloalkyl and cycloalkenyl groups. Specific examples include, but are not limited to, cyclohexyl, cyclopropyl, and cyclobutyl.

[0051] The term "heterocycle", "heterocyclyl", or "heterocyclic" as used herein means non-aromatic, monocyclic, bicyclic, or tricyclic ring systems in which one or more ring members are an independently selected heteroatom. In some embodiments, the "heterocycle", "heterocyclyl", or "heterocyclic" group has three to fourteen ring members in which one or more ring members is a heteroatom independently selected from oxygen, sulfur, nitrogen, or phosphorus, and each ring in the system contains 3 to 7 ring members.

[0052] Examples of heterocycles include, but are not limited to, 3-1H-benzimidazol-2-one, 3-(1-alkyl)-benzimidazol-2-one, 2-tetrahydrofuranyl, 3-tetrahydrofuranyl, 2-tetrahydrothiophenyl, 3-tetrahydrothiophenyl, 2-morpholino, 3-morpholino, 4-morpholino, 2-thiomorpholino, 3-thiomorpholino, 4-thiomorpholino, 1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl, 1-tetrahydropiperazinyl, 2-tetrahydropiperazinyl, 3-tetrahydropiperazinyl, 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 1-pyrazolinyl, 3-pyrazolinyl, 4-pyrazolinyl, 5-pyrazolinyl, 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-piperidinyl, 2-thiazolidinyl, 3-thiazolidinyl, 4-thiazolidinyl, 1-imidazolidinyl, 2-imidazolidinyl, 4-imidazolidinyl, 5-imidazolidinyl, indolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, benzothiolane, benzodithiane, and 1,3-dihydro-imidazol-2-one.

[0053] Cyclic groups, (e.g. cycloaliphatic and heterocycles), can be linearly fused, bridged, or spirocyclic.

[0054] The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[0055] The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation. As would be known by one of skill in the art, unsaturated groups can be partially unsaturated or fully unsaturated. Examples of partially unsaturated groups include, but are not limited to, butene, cyclohexene, and tetrahydropyridine. Fully unsaturated groups can be aromatic, anti-aromatic, or non-aromatic. Examples of fully unsaturated groups include, but are not limited to, phenyl, cyclooctatetraene, pyridyl, thienyl, and 1-methylpyridin-2(1H)-one.

[0056] The term "alkoxy", or "thioalkyl", as used herein, refers to an alkyl group, as previously defined, attached through an oxygen ("alkoxy") or sulfur ("thioalkyl") atom.

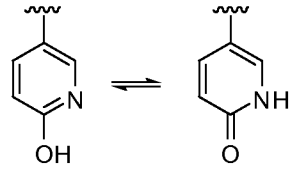
[0057] The terms "haloalkyl", "haloalkenyl", "haloaliphatic", and "haloalkoxy" mean alkyl, alkenyl or alkoxy, as the case may be, substituted with one or more halogen atoms. This term includes perfluorinated alkyl groups, such as $-CF_3$ and $-CF_2CF_3$.

[0058] The terms "halogen", "halo", and "hal" mean F, Cl, Br, or I.

[0059] The term "aryl" used alone or as part of a larger moiety as in "arylalkyl", "arylalkoxy", or "aryloxyalkyl", refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 7 ring members. The term "aryl" may be used interchangeably with the term "aryl ring".

[0060] The term "heteroaryl", used alone or as part of a larger moiety as in "heteroarylalkyl" or "heteroarylalkoxy", refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic, at least one ring in the system contains one or more heteroatoms, and wherein each ring in the system contains 3 to 7 ring members. The term "heteroaryl" may be used interchangeably with the term "heteroaryl ring" or the term "heteroaromatic". Examples of heteroaryl rings include, but are not limited to, 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, benzimidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, N-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, pyridazinyl (e.g., 3-pyridazinyl), 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, tetrazolyl (e.g., 5-tetrazolyl), triazolyl (e.g., 2-triazolyl and 5-triazolyl), 2-thienyl, 3-thienyl, benzofuryl, benzothiophenyl, indolyl (e.g., 2-indolyl), pyrazolyl (e.g., 2-pyrazolyl), isothiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,3-triazolyl, 1,2,3-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, purinyl, pyrazinyl, 1,3,5-triazinyl, quinolinyl (e.g., 2-quinolinyl, 3-quinolinyl, 4-quinolinyl), and isoquinolinyl (e.g., 1-isoquinolinyl, 3-isoquinolinyl, or 4-isoquinolinyl).

[0061] It shall be understood that the term "heteroaryl" includes certain types of heteroaryl rings that exist in equilibrium between two different forms. More specifically, for example, species such hydropyridine and pyridinone (and likewise hydroxypyrimidine and pyrimidinone) are meant to be encompassed within the definition of "heteroaryl."



[0062] The term "protecting group" and "protective group" as used herein, are interchangeable and refer to an agent used to temporarily block one or more desired functional groups in a compound with multiple reactive sites. In certain embodiments, a protecting group has one or more, or preferably all, of the following characteristics: a) is added selectively to a functional group in good yield to give a protected substrate that is b) stable to reactions occurring at one or more of the other reactive sites; and c) is selectively removable in good yield by reagents that do not attack the regenerated, deprotected functional group. As would be understood by one skilled in the art, in some cases, the reagents do not attack other reactive groups in the compound. In other cases, the reagents may also react with other reactive groups in the compound. Examples of protecting groups are detailed in Greene, T.W., Wuts, P. G in "Protective Groups in Organic Synthesis", Third Edition, John Wiley & Sons, New York: 1999 (and other editions of the book), the entire contents of which are hereby incorporated by reference. The term "nitrogen protecting group", as used herein, refers to an agent used to temporarily block one or more desired nitrogen reactive sites in a multifunctional compound. Preferred nitrogen protecting groups also possess the characteristics exemplified for a protecting group above, and certain exemplary nitrogen protecting groups are also detailed in Chapter 7 in Greene, T.W., Wuts, P. G in "Protective Groups in Organic Synthesis", Third Edition, John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

[0063] In some embodiments, a methylene unit of an alkyl or aliphatic chain is optionally replaced with another atom or group. Examples of such atoms or groups include, but are not limited to, nitrogen, oxygen, sulfur, -C(O)-, -C(=N-CN)-, -C(=NR)-, -C(=NOR)-, -SO-, and $-SO_2-$. These atoms or groups can be combined to form larger groups. Examples of such larger groups include, but are not limited to, -OC(O)-, -C(O)CO-, $-CO_2-$, -C(O)NR-, -C(=N-CN), -NRCO-, -NRC(O)O-, $-SO_2NR-$, $-NRSO_2-$, -NRC(O)NR-, -OC(O)NR-, and $-NRSO_2NR-$, wherein R is, for example, H or C_{1-6}aliphatic. It should be understood that these groups can be bonded to the methylene units of the aliphatic chain via single, double, or triple bonds. An example of an optional replacement (nitrogen atom in this case) that is bonded to the aliphatic chain via a double bond would be $-CH_2CH=N-CH_3$. In some cases,

especially on the terminal end, an optional replacement can be bonded to the aliphatic group via a triple bond. One example of this would be $CH_2CH_2CH_2C{\equiv}N$. It should be understood that in this situation, the terminal nitrogen is not bonded to another atom.

[0064] It should also be understood that, the term "methylene unit" can also refer to branched or substituted methylene units. For example, in an isopropyl moiety [$-CH(CH_3)_2$], a nitrogen atom (e.g. NR) replacing the first recited "methylene unit" would result in dimethylamine [$-N(CH_3)_2$]. In instances such as these, one of skill in the art would understand that the nitrogen atom will not have any additional atoms bonded to it, and the "R" from "NR" would be absent in this case.

[0065] Unless otherwise indicated, the optional replacements form a chemically stable compound. Optional replacements can occur both within the chain and/or at either end of the chain; i.e., both at the point of attachment and/or also at the terminal end. Two optional replacements can also be adjacent to each other within a chain so long as it results in a chemically stable compound. For example, a C_3 aliphatic can be optionally replaced by 2 nitrogen atoms to form $-C-N{\equiv}N$. The optional replacements can also completely replace all of the carbon atoms in a chain. For example, a C_3 aliphatic can be optionally replaced by -NR-, -C(O)-, and -NR- to form -NRC(O)NR- (a urea).

[0066] Unless otherwise indicated, if the replacement occurs at the terminal end, the replacement atom is bound to a hydrogen atom on the terminal end. For example, if a methylene unit of $-CH_2CH_2CH_3$ were optionally replaced with -O-, the resulting compound could be $-OCH_2CH_3$, $-CH_2OCH_3$, or $-CH_2CH_2OH$. It should be understood that if the terminal atom does not contain any free valence electrons, then a hydrogen atom is not required at the terminal end (e.g., $-CH_2CH_2CH{=}O$ or $-CH_2CH_2C{\equiv}N$).

[0067] The term "cross-coupling reaction", as used herein, refers to a reaction in which a carbon-carbon bond is formed with the aid of a metal catalyst. Usually, one of the carbon atoms is bonded to a functional group (a "cross-coupling group") while the other carbon atom is bonded to a halogen. Examples of cross coupling reactions include, but are not limited to, Suzuki couplings, Stille couplings, and Negishi couplings.

[0068] The term "cross-coupling group", as used herein, refers to a functional group capable of reacting with another functional group (e.g., halo) in a cross coupling reaction to form a carbon-carbon ("C-C") bond. In some embodiments, the C-C bond is formed between two aromatic groups.

[0069] The term "cross coupling condition", as used herein, refers to the chemical conditions (e.g., temperature, length of time of reaction, volume of solvent required) required in order to enable the cross coupling reaction to occur.

[0070] Examples of cross-coupling groups and their respective cross-coupling conditions include, but are not limited to, boronic acids and boronic esters with Suzuki coupling conditions, $SnBu_3$ (Bu: butyl) with Stille coupling conditions, and ZnX (X: halogen) with Negishi coupling conditions.

[0071] All three of these coupling conditions typically involve the use of a catalyst, a suitable solvent, and optionally a base. Suzuki coupling conditions involve the use of a palladium catalyst and a suitable solvent. Examples of suitable palladium catalysts include, but are not limited to, $PdCl_2(PPh_3)_2$, $Pd(Ph_3)_4$, and $PdCl_2$(dppf) (wherein each Ph is phenyl, and dppf is 1,1-bis(diphenylphosphino)ferrocene). Suitable bases include, but are not limited to, K_2CO_3 and Na_2CO_3. Suitable solvents include, but are not limited to, tetrahydrofuran, toluene, and ethanol.

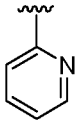
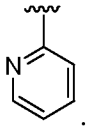
[0072] Stille coupling conditions involve the use of a catalyst (usually palladium, but sometimes nickel), a suitable solvent, and other optional reagents. Examples of suitable catalysts include, but are not limited to, $PdCl_2(PPh_3)_2$, $Pd(Ph_3)_4$, and $PdCl_2$(dppf). Suitable solvents include, but are not limited to, tetrahydrofuran, toluene, and dimethylformamide.

[0073] Negishi coupling conditions involve the use of a catalyst (palladium or nickel) and a suitable solvent. Examples of suitable catalysts include, but are not limited to $Pd_2(dba)_3$, $Ni(PPh_3)_2Cl_2$, $PdCl_2(PPh_3)_2$, and $Pd(Ph_3)_4$ (where “dba” is tris(dibenzylideneacetone)dipalladium). Suitable solvents include, but are not limited to, tetrahydrofuran, toluene, and dimethylformamide.

[0074] Suzuki, Stille, and Negishi conditions are known to one skilled in the art and are described in more detail in a variety of references, including "March's Advanced Organic Chemistry".

[0075] As would be understood by one skilled in the art, cross-coupling groups are formed from coupling group precursors. A coupling group precursor is a reagent or group of reagents used to form a cross-coupling group. Examples include, but are not limited to, bis(pinacolato)diborane for the formation of boronate esters, trimethylborates for the formation of boronic acids, Bu_3SnCl for the formation of stannanes, and $ZnCl_2$ for the formation zincates in Negishi coupling reactions. Examples of suitable coupling group

formation conditions include, but are not limited to, making boronic esters via palladium-mediated catalysis; making boronic acids by hydrolyzing boronic esters; making stannanes via a two step process: 1) halogen metal exchange followed by 2) transmetallation with Bu_3SnCl and making zincates via a two step process: 1) halogen metal exchange followed by 2) addition of $ZnCl_2$.

[0076] Unless otherwise indicated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, geometric, conformational, and rotational) forms of the structure. For example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers are included in this invention. As would be understood to one skilled in the art, a substituent can freely rotate around any rotatable bonds. For example, a substituent drawn as  also represents .

[0077] Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, geometric, conformational, and rotational mixtures of the present compounds are within the scope of the invention.

[0078] Unless otherwise indicated, all tautomeric forms of the compounds of the invention are within the scope of the invention.

[0079] In the compounds of this invention any atom not specifically designated as a particular isotope is meant to represent any stable isotope of that atom. Unless otherwise stated, when a position is designated specifically as "H" or "hydrogen", the position is understood to have hydrogen at its natural abundance isotopic composition. Also unless otherwise stated, when a position is designated specifically as "D" or "deuterium", the position is understood to have deuterium at an abundance that is at least 3340 times greater than the natural abundance of deuterium, which is 0.015% (i.e., at least 50.1% incorporation of deuterium).

[0080] "D" and "d" both refer to deuterium.

[0081] Additionally, unless otherwise indicated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched

atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools or probes in biological assays.

[0082] As used herein "crystalline" refers to a solid that has a specific arrangement and/or conformation of the molecules in the crystal lattice.

[0083] As used herein the term "amorphous" refers to solid forms that consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice.

[0084] As used herein, the term "solvate" refers to a crystalline solid adduct containing either stoichiometric or nonstoichiometric amounts of a solvent incorporated within the crystal structure. If the incorporated solvent is water, such adduct is refered to as a "hydrate".

Abbreviations

[0085] The following abbreviations are used:

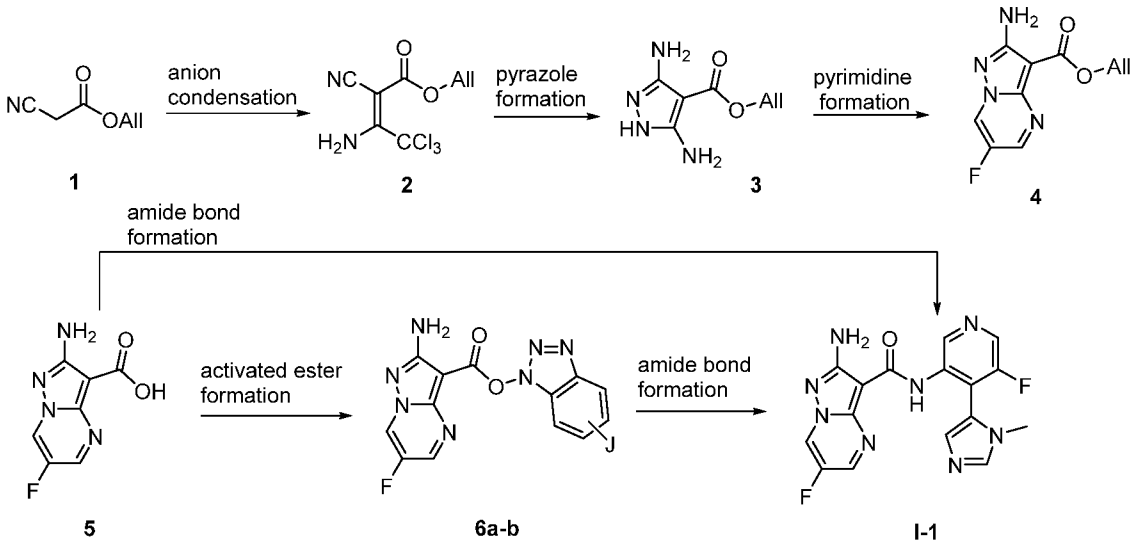
DMSO	dimethyl sulfoxide
DCM	dichloromethane
ATP	adenosine triphosphate
TFA	trifluoroacetic acid
^{1}HNMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
LCMS	liquid chromatography-mass spectrometry
Rt	retention time
XRPD	X-Ray Powder Diffraction
DSC	Differential scanning calorimetry
TGA	Thermogravimetric analysis
RT	room temperature
NMP	N-methyl-2-pyrrolidone
Bp	boiling point
DMF	dimethylformamide
PTSA	*p*-Toluenesulfonic acid
DIPEA	N,N-diisopropylethylamine
HOBT	hydroxybenzotriazole

HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
T3P	Propylphosphonic anhydride
COMU	1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino)]uroniumhexafluorophosphate
TCTU	[(6-chlorobenzotriazol-1-yl)oxy-(dimethylamino)methylene]-dimethyl-ammonium tetrafluoroborate
HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
DME	Dimethoxyethane
THF	tetrahydrofuran
TMEDA	tetramethylethylenediamine
NaHMDS	sodium hexamethyldisilazane
LHMDS	Lithium bis(trimethylsilyl)amide

Processes

[0086] Processes and compounds described herein are useful for producing ATR inhibitors that contain an aminopyrazolopyrimidine core. The general synthetic procedures shown in schemes herein are useful for generating a wide array of chemical species which can be used in the manufacture of pharmaceutical compounds.

SCHEME A



[0087] Compounds of this invention can be synthesised according to methods similar to the

one depicted in **Scheme A**.

Step 1

[0088] The anion of commercially available allyl cyanoacetate **1** can react with, e.g., trichloroacetonitrile to provide intermediate **2**. In the anion condensation step, the anion of commercially available allyl cyanoacetate **1** can be generated with a base such as potassium acetate in an appropriate solvent such as an alcohol (e.g., isopropylalcohol). The anion then reacts with trichloroacetonitrile at room temperature.

Step 2

[0089] Intermediate **2** then reacts with hydrazine to form the diaminopyrazole **3**. In the pyrazole formation step, intermediate **2** is reacted with hydrazine (or its hydrate) in an aprotic solvent, such as DMF, to provide the diaminopyrazole **3**. The reaction occurs under basic conditions (e.g., in the presence of potassium acetate or AcONa) with heating (e.g.,≥ 110°C) to ensure complete cyclisation.

Step 3

[0090] Intermediate **3** can further be condensed with a dielectrophilic coupling partner to form the pyrimidine **4**. In the pyrimidine formation step, intermediate **3** is reacted with a 1,3-dielectrophilic species (e.g., a 1,3-dialdehyde or a 3-(dialkylamino)-prop-2-enal) in various types of solvents (e.g., DMF or DMSO/water) to furnish the bicyclic cores **4**. When one or two of the electrophilic centers is protected/masked (e.g., aldehyde masked as a ketal), introduction of a sulfonic acid (e.g., PTSA) is required to liberate the reactive functional group.

Step 4

[0091] Deprotection, e.g, via hydrolysis, of the allyl ester leads to the carboxylic acids **5**. In the deprotection step, compound **4** is subjected to hydrolytic conditions that are known to those skilled in the art. For example, treatment of **4** with phenylsilane or 4-methylbenzenesulfinate in the presence of a catalytic amount of palladium (e.g., $Pd(PPh_3)_4$) leads to the formation of the corresponding carboxylic acid **5**. Alternatively, compounds **4** could be treated with aqueous alkali (e.g., NaOH, LiOH, or KOH) to produce acids **5**.

Step 5

[0092] In the activated ester formation step, the carboxylic acids **5** are reacted with amide coupling agents known to those skilled in the art. Suitable amide coupling partners include,

but are not limited to TBTU, TCTU, HATU, T3P, and COMU. When the coupling agent is chosen appropriately, the reactions can proceed rapidly (~1hr.) at room temperature in the presence of an organic base such as an aliphatic amine (e.g., triethylamine, DIPEA) to provide the activated esters **6a-b**. For example, when the amide coupling agents TBTU [J=H] or TCTU[J=Cl] are used, compounds **6a-b** are obtained readily by filtration of the reaction mixture.

[0093] Formation of the activated esters **6a-b** prior to the amide bond formation to prepare **I-A** is generally preferred, although a direct conversion of **5** into the compounds of formula **I-A** of this invention is also possible. Alternative activated esters can also be utilised (isolated or formed *in situ*) and will be known to those skilled in the art (e.g., using TBTU, TCTU, HATU, T3P, COMU coupling agents).

Step 6

[0094] In the amide bond formation step, activated esters **6a-b** can react with substituted 3-aminopyridine **11** to provide compound **I-1** of this invention. The reaction conditions for the amide coupling are in a solvent (e.g. anisole, NMP, pyridine, DMF, etc …) with heating (e.g., $\geq 90°C$).

[0095] Alternatively, the two steps described above can be combined: carboxylic acid **5** can be used as starting points for the amide bond formation, the activated esters being generated *in situ*, using the same amide couplings agents as those described above. Compounds of this invention are isolated in a similar manner to the one described above.

PREPARATIONS AND EXAMPLES

[0096] All commercially available solvents and reagents were used as received. Microwave reactions were carried out using a CEM Discovery microwave. Flash Chromatography, e.g., was carried out on an ISCO© Combiflash^{R} Companion^{TM} system eluting with a 0 to 100% EtOAc/petroleum ether gradient. Other methods known in the art were also utilized to perform Flash Chromotography. Samples were applied pre-absorbed on silica. Where stated, supercritical fluid chromatography (SFC) was performed on a Berger Minigram SFC machine. All ^{1}H NMR spectra were recorded using a Bruker Avance III 500 instrument at 500 MHz. MS samples were analyzed on a Waters SQD mass spectrometer with electrospray ionization operating in positive and negative ion mode. Samples were introduced into the mass spectrometer using chromatography. All final products had a purity ≥95%, unless specified otherwise in the experimental details. HPLC purity was measured on a Waters

Acquity UPLC system with a Waters SQD MS instrument equipped with a Waters UPLC BEH C8 1.7 µm, 2.1 x 50 mm column and a Vanguard BEH C8 1.7 µm, 2.1 x 5 mm guard column.

[0097] As used herein, the term "Rt(min)" refers to the HPLC retention time, in minutes, associated with the compound. Unless otherwise indicated, the HPLC methods utilized to obtain the reported retention times are as described below:

HPLC Method B

Instrument: Waters Acquity UPLC-MS;

Column: Waters UPLC BEH C8 1.7 µm, 2.1 x 50 mm with Vanguard BEH C8 1.7 µm, 2.1 x 5 mm guard column;

Column temperature: 45°C;

Mobile Phase A: 10mM ammonium formate in water:acetonitrile 95:5, pH 9;

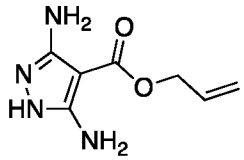
Mobile Phase B: acetonitrile;

Detection: 210-400 nm;

Gradient: 0-0.40 min: 2% B, 0.40-4.85 min: 2% B to 98% B, 4.85-4.90 min: 98% B to 2% B, 4.90-5.00 min: hold at 2% B;

Flow rate: 0.6 mL/minute.

Preparation 1: Allyl 3,5-diamino-1*H*-pyrazole-4-carboxylate



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Step 1: allyl 3-amino-4,4,4-trichloro-2-cyanobut-2-enoate **2**

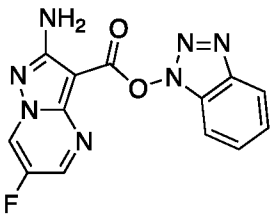
[0098] To a solution of KOAc (589.4 g, 6.006 mol) in isopropanol (3 L) was added allyl cyanoacetate (429.4 g, 403.2 mL, 3.432 mol) and the reaction mixture was cooled to 5°C. Trichloroacetonitrile (495.5 g, 3.432 mol) was added in 50 mL portions, maintaining temperature below 15°C. The reaction mixture was then allowed to warm to 20°C and stirred

for 3 hr. Water (~4 L) was added to dissolve the inorganic materials and precipitate out the desired product. The mixture was stirred for 20 minutes and the solid was isolated by filtration under vacuum. This solid was filtered, washed with water (2 x 0.5 L) and dried in a vacuum oven overnight at 40°C to afford allyl 3-amino-4,4,4-trichloro-2-cyanobut-2-enoate **2** as an off-white powder (787 g, 85%).

Step 2: Allyl 3,5-diamino-1H-pyrazole-4-carboxylate **3**

[0099] To a suspension of allyl 3-amino-4,4,4-trichloro-2-cyano-but-2-enoate **2** (619 g, 2.297 mol) and KOAc (676.3 g, 6.891 mol) in DMF (2.476 L) at 0°C was slowly added hydrazine hydrate (172.5 g, 167.6 mL, 3.446 mol) over 15 min. The reaction mixture was then stirred at ambient temperature for 2 hr., at which stage ^{1}H NMR shows complete consumption of the starting material. Reaction mixture was then heated overnight at 110°C before being allowed to cool to ambient and stirred for another 48hr. The mixture was filtered through a sintered glass funnel to remove the precipitated solid and the filtrate was evaporated under reduced pressure to give a thick liquid. DCM (approx 2 L) was added, and the mixture filtered again to remove additional solids that have precipitated. The filtrate was purified through a 1 kg silica gel plug (gradient of DCM/MeOH as an eluent), and the solvent was removed to afford an orange solid which was suspended in acetonitrile and heated at about 70°C until all the solid went into solution, at which point the solution was allowed to cool to ambient temperature, then to 2°C. The precipitate that formed was isolated by filtration under vacuum, washed with chilled MeCN (~50 mL) and dried to constant mass in a vacuum oven to furnish the title compound as an off-white powder (171.2 g, 41%).

Preparation 2a: 1*H*-benzo[*d*][1,2,3]triazol-1-yl 2-amino-6-fluoropyrazolo[1,5-*a*]pyrimidine-3-carboxylate



6a

Step 1: allyl 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate **4**

[00100] To a suspension of allyl 3,5-diamino-1H-pyrazole-4-carboxylate **3** (42.72 g, 234.5 mmol) in DMSO (270.8 mL) / Water (270.8 mL), was added p-TsOH hydrate (46.72 g, 245.6 mmol) and 3-(diisopropylamino)-2-fluoro-prop-2-enal (described in *Tetrahedron Letters*, 33(3), 357-60; 1992) (38.69 g, 223.3 mmol). The reaction mixture was heated to 100°C for 3hr. during which time a solid slowly precipitated out of solution. The orange suspension was allowed to cool down to RT overnight. The solid was filtered, washed with water and dried under vacuum to give allyl 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate **4** as a sand solid (45.05 g, 85% yield).

Step 2: 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid **5**

[00101] To a suspension of allyl 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate **4** (45 g, 190.5 mmol) in DCM (1.35 L) was added phenylsilane (41.23 g, 46.96 mL, 381.0 mmol), followed by $Pd(PPh_3)_4$ (8.805 g, 7.620 mmol). The reaction was stirred at room temperature for 2hr. 30min. The reaction mixture was filtered and the solid was washed with DCM to give a light yellow solid (43.2g). This solid was triturated further in DCM (225 mL) at RT for 45 min, then filtered and dried overnight under vacuum to provide 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid **5** as a light yellow solid (37.77g, 100% yield).

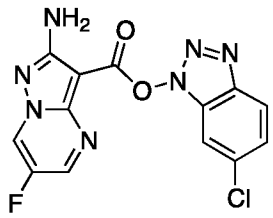
[00102] In an alternative method, 4-methylbenzenesulfinate (anhydrous, 1.2 eqv, 22.6g, 127mmol) was suspended in dry DMSO (20 vol, 500ml). The stirred mixture was warmed to 30°C under a nitrogen atmosphere. Upon complete dissolution $Pd(PPh_3)_4$ (2 mol%, 2.4g, 2.1 mmol) was added. The mixture was stirred for 10 min at 25-30°C after which time a turbid yellow solution was present. Allyl 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate **4** (25g, 105.8mmol) was added portionwise, maintaining the temperature at 25-30°C. Once addition was complete the cloudy solution was stirred until the reaction was complete by HPLC (2-3 hrs). A heavy precipitate formed after 15 minutes post addition of the substrate. The mixture became thicker as the reaction proceeded. The reaction mixture was diluted with water (125 ml) and 2M HCl (66 ml) was added slowly, maintaining the temperature at 25-30°C. The slurry was stirred for 30 minutes, then filtered. The filtration was slow (2hrs). The resulting solid was washed with water, then dried on the sinter. The solid was slurried in DCM (8 vol) for 1hr. The solid was filtered (rapid filtration) and washed with DCM. The solid was re-slurried in chloroform (8 vol) for 1 hr. The acid was filtered and

dried on the sinter. It was further dried in a vacuum oven at 50°C for 24 hrs. The product **5** was obtained as an off-white solid (18.6g, 85%); ^{1}H NMR (500 MHz, DMSO-d6) δ 12.14 (1H, brs), 9.31 (1H, dd), 8.69 (1H, m), 6.47 (2H, brS); 19F NMR (500 MHz, DMSO-d6) δ -153.65; MS (ES+) 197.1.

Step 3: 1H-benzo[d][1,2,3]triazol-1-yl 2-amino-6-fluoropyrazolo[1,5-a]pyrimidine-3-carboxylate **6a**

[00103] To a suspension of 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid **5** (20 g, 102.0 mmol) in chloroform (300 mL) was added Et_3N (11.35 g, 15.63 mL, 112.2 mmol). The suspension was stirred for ~ 5mins and then (benzotriazol-1-yloxy-dimethylamino-methylene)-dimethyl-ammonium Boron Tetrafluoride was added (32.75 g, 102.0 mmol). The suspension was heated to 60°C for 1hr. before the thick suspension was allowed to cool down to RT. The resulting suspension was filtered, washed with chloroform (200 mL) and dried under vacuum overnight to afford the title compound **6a** as a light yellow powder (32.5g, 88%).

Preparation 2b: ***(6-chlorobenzotriazol-1-yl)-2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate*** **6b**



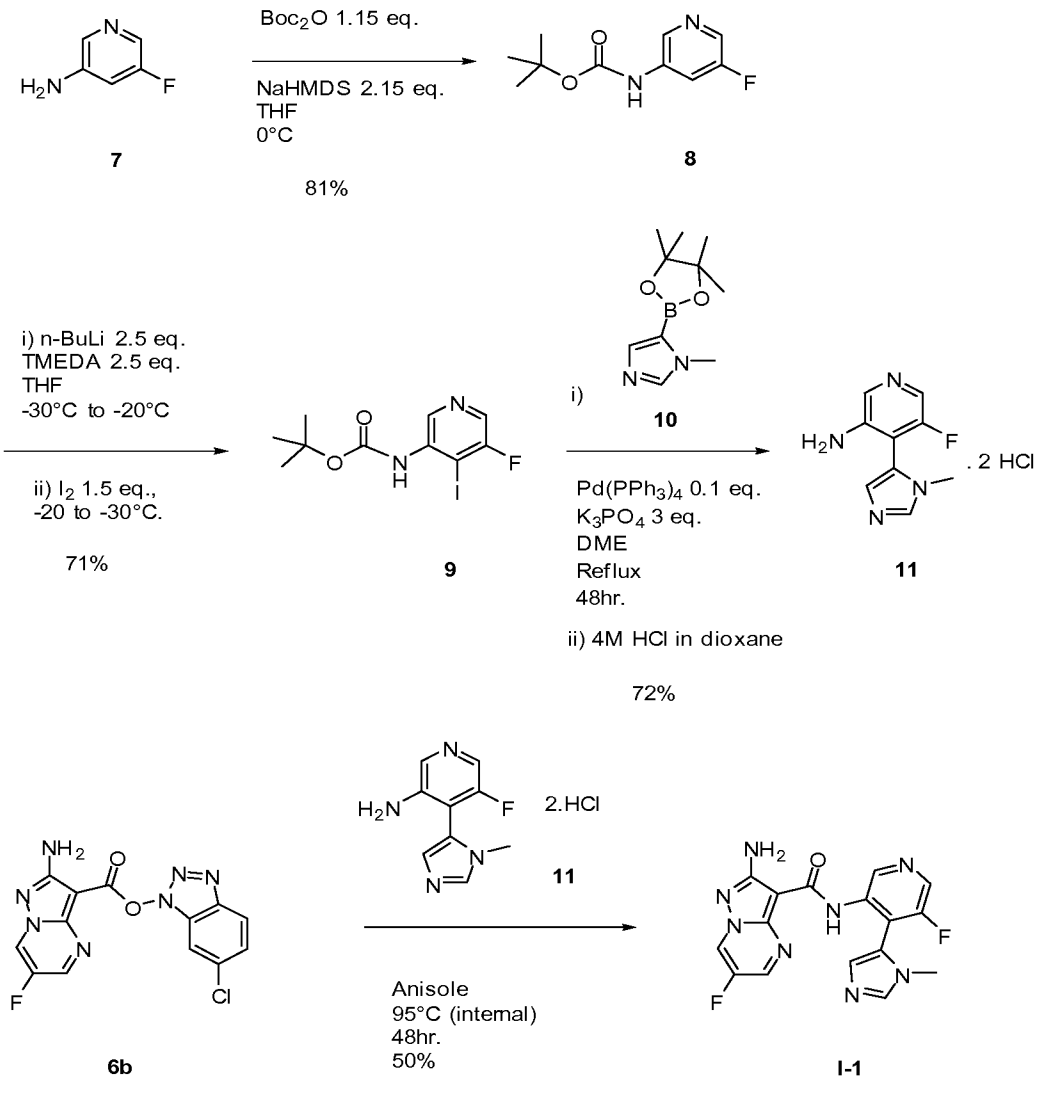
6b

[00104] In a 2.5 L three-necked flask equipped with stirrer bar, condenser, nitrogen line and Hanna temperature probe was charged 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid **5** (60 g, 305.9 mmol), chloroform (900.0 mL) and triethylamine (32.44 g, 44.68 mL, 320.6 mmol). [(6-chlorobenzotriazol-1-yl)oxy-(dimethylamino)methylene]-dimethyl-ammonium (Boron Tetrafluoride Ion (1)) (87.00 g, 244.7 mmol) was added portionwise over 5 mins (internal dropped from 22.7 to 21.5°C on complete addition). Mixture heated at 60°C (internal temp) for 2hr., still a cream suspension. Mixture cooled to room temperature then solid collected by filtration, washed well with chloroform (until filtrate runs essentially colourless) and dried by suction to leave product **6b** as a cream solid (82.2g, 77% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (dd, 1H), 8.91 (d, 1H), 8.22 (dd,

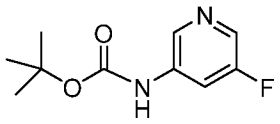
1H), 8.09 (dd, 1H), 7.57 (dd, 1H) and 6.87 (s, 2H). MS (ES+) 348.1.

[00105] In an alternative method, 2-Amino-6-fluoropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid **5** (30g, 153 mmol) was slurried in acetonitrile (540ml). Triethylamine (22.5ml, 153mmol) was added, followed by [(6-chlorobenzotriazol-1yl)oxy-(dimethylamino)methylene]-dimethylammonium tetrafluoroborate (TCTU, 54.4g, 153mmol). The mixture was stirred at room temperature for 2 hrs. The product was isolated by filtration-the filter cake was washed with acetonitrile (2x60ml). The product was obtained as a brown solid (49.3g, 93%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (dd, 1H), 8.91 (d, 1H), 8.22 (dd, 1H), 8.09 (dd, 1H), 7.57 (dd, 1H) and 6.87 (s, 2H); 19F NMR (500 MHz, DMSO-d6) δ -150.1; MS (ES+) 348.1.

Example 1: Synthesis of 2-amino-6-fluoro-N-(5-fluoro-4-(1-methyl-1H-imidazol-5-yl)pyridin-3-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (Compound I-1)



Step 1: tert-butyl (5-fluoropyridin-3-yl)carbamate



8

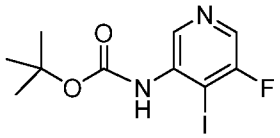
[00106] In a 50 L jacketed vessel was added THF (2.5 L), 5-fluoropyridin-3-amine 1 (500 g, 4.460 mol) then additional THF (5 L). To this stirred mixture was added a solution of *tert*-butoxycarbonyl *tert*-butyl carbonate (1.119 kg, 5.129 mol) in THF (2.5 L), pumped in *via* a vacuum line. The line was then rinsed with THF (1 L) in to the reaction vessel. The reaction temperature was cooled to 0°C before NaHMDS (4.794 L of 2 M in THF, 9.589 mol) was added in 12 x 400 mL portions (approx. 5°C exotherm after each addition, dosing continued once internal cooled to 0°C). Addition was completed after 1 hr. The internal temperature was raised to 5°C and stirred at this temperature for 1 hr. The reaction was carefully quenched by slow addition of a saturated ammonium chloride aqueous solution (1 L) (exothermic). The internal was raised to 10°C and additional saturated ammonium chloride aqueous solution (3 L) was added. The internal was raised to 25°C and the reaction mixture was extracted with EtOAc (1 x 5 L then 1 x 2.5 L). The combined organic layers were washed with water (1 x 5.5 L then 1 x 3 L) then with brine (3 L).

[00107] The organic phase was concentrated *in vacuo* to a total volume of approx. 6 L, dried ($MgSO_4$), filtered through filter paper and concentrated *in vacuo* (on a rotary evaporator, 40°C bath temp) until product crystallised out (approx. 2 L of solvent remaining). Heptane (2.5 L) was added and the mixture rotated on a rotary evaporator at 40°C. The solution was concentrated *in vacuo* (on a rotary evaporator, 40°C bath temp) to remove more EtOAc until the product crystallised out of solution. The mixture was then left to cool and stand at ambient temperature overnight. The solid was collected by filtration through Whatman № 1 filter paper, washed with heptane until filtrate ran essentially colourless. The solid was dried for approx. 5 hr. to leave crop 1 of product as an off white solid, 382.51 g.

[00108] The mother liquor was concentrated slowly *in vacuo* (on a rotary evaporator, 40°C bath temp) until a solid crystallised out. The mixture was left to stand at ambient overnight and the solid collected by filtration, washed with heptane and dried by suction to leave crop 2 of product **8** as an off white solid, 203.5 g. The process was repeated on the mother liquor to give crop 3 as an off white solid, 178.7 g. Total yield of product, 764.71 g, 81%. ^{1}H NMR (500 MHz, DMSO-d6) δ 9.86 (s, 1H), 8.44 (s, 1H), 8.17 (d, J = 2.6 Hz, 1H), 7.83 (d, J = 11.6

Hz, 1H), 3.30 (s, 1H). MS (ES+) 213.0.

Step 2: tert-butyl (5-fluoro-4-iodopyridin-3-yl)carbamate



9

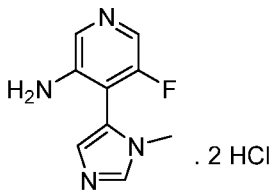
[00109] In a 50 L jacket vessel was added THF (2.5 L), *tert*-butyl N-(5-fluoro-3-pyridyl)carbamate **8** (400 g, 1.885 mol) in THF (2.5 L), additional THF (3 L) and N,N,N',N'-tetramethylethane-1,2-diamine (547.6 g, 711.2 mL, 4.712 mol). The reaction mixture was cooled to -28°C (internal temperature), then n-BuLi (1.885 L of 2.5 M in hexanes, 4.712 mol) was added *via* canula at such a rate as to keep internal temperature below -20°C (i.e., over 2 hr.). On complete addition, the reaction mixture was stirred at between -30 and -20°C (internal temperature) for a further 50 mins. Solid molecular iodine (765.5 g, 3.016 mol) was slowly added in 12 roughly equal portions over 1 hr. (approx. 2/3°C delayed exotherm after each portion added) keeping the internal temperature below -20°C. On complete addition of iodine, the reaction mixture was stirred at -30°C (internal temperature) for a further 45 mins.

[00110] The reaction was then quenched by the slow addition of a saturated ammonium chloride aqueous solution (2 L) (exothermic). Water (2 L) was then added and the reaction mixture warmed to 20°C (internal temperature) and left to stand overnight. To the reaction mixture was added EtOAc (5 L) and stirring continued for 10 mins. The aqueous phase was removed then a saturated sodium thiosulfate aqueous solution (2 L) was added to the organic phase, stirred vigorously for 10 mins. Additional EtOAc (2.5 L) and water (2 L) was added and stirring continued for 10 mins. The aqueous phase was removed and the organic phase washed further with a saturated sodium thiosulfate aqueous solution (2 L) and water (1 x 2 L then 1 x 2.5 L) and then brine (2 L). The organic phase was concentrated *in vacuo* (rotary evaporator) to such a volume that the product started to crystallise out to give a thick suspension. The mixture was left to stand at room temperature overnight.

[00111] The solid was collected by filtration, washed with minimal EtOAc (a few hundred mL) then washed well with heptane, dried by suction for 3hr. to leave crop 1 of product **9** as a white solid, 311.99 g. The mother liquor was concentrated *in vacuo* (rotary evaporator) to dryness leaving a dark green solid. (approx 200 g) which was dissolved in EtOAc (750 mL) by heating under reflux. Activated carbon (20 g) was then added and the mixture stirred

under reflux for 10 mins. The mixture was filtered through filter paper then concentrated slowly on rotary evaporator until a thick suspension formed. The resulting solid was collected by filtration, washed with minimal EtOAc then heptane, dried by suction then in a vacuum oven at 40°C for 2 hr., leaving crop 2 as a white solid, 103.9 g. The mother liquor was concentrated again until a thick suspension formed. The solid was collected by filtration, washed with heptane and dried by suction *in vacuo* (rotary evaporator) then in a vacuum oven at 40°C for a few hours to leave product crop 3 as a white solid, 39.4 g. Total yield = 455.29 g, 71%. ^{1}H NMR (500 MHz, DMSO-d6) δ 8.98 (s, 1H), 8.27 (dd, J = 1.2, 0.6 Hz, 2H), 1.47 (s, 9H). MS (ES+) 338.9.

Step 3: 5-fluoro-4-(1-methyl-1H-imidazol-5-yl)pyridin-3-amine dihydrochloride



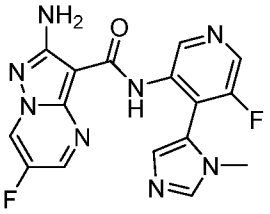
11

[00112] To a degassed (3 x vacuum/nitrogen cycles) mixture of *tert*-butyl N-(5-fluoro-4-iodo-3-pyridyl)carbamate **9** (190 g, 561.9 mmol), 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazole **10** (175.4 g, 842.8 mmol) and potassium phosphate (226.0 g, 1.686 mol) in DME (2.28 L) was added $Pd(PPh_3)_4$ (64.93 g, 56.19 mmol). The reaction vessel was again flushed with nitrogen *via* vacuum/nitrogen cycles (3x). The mixture was heated under reflux and under a nitrogen atmosphere for 48 hr. The mixture was cooled to room temperature then passed through a pad of celite, rinsing through with EtOAc until filtrate almost colourless (approx. 1.5 L). The filtrate was concentrated *in vacuo* to leave a sticky brown solid, 339.7 g.

[00113] The crude product was dissolved in dioxane (950 mL) and methanol (431.1 mL) and the solution cooled on ice bath (internal of 10°C), HCl (4 M in 1,4-dioxane) (842.8 mL of 4 M, 3.371 mol) was then added in 8 roughly equal portions over 20 mins. (approximately 3 to 4°C exotherm observed on each addition). On complete addition, the mixture was warmed to 40°C and stirred at this temperature for 3 hr., then left to cool to room temperature overnight with stirring. The solid was collected by filtration, washed with 1,4-dioxane and dried under vacuum for 1 hr. to leave product **11** as a sand/brown solid (107.9 g, 72% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 9.09 (s, 1H), 8.24 (s, 1H), 8.15 (br s, 1H), 7.91 – 7.90 (1H, br

s), 7.88 (m, 1H), 3.85 (s, 3H). MS (ES+) 193.1.

Step 4: 2-amino-6-fluoro-N-(5-fluoro-4-(1-methyl-1H-imidazol-5-yl)pyridin-3-yl)pyrazolo[1,5-a]pyrimidine-3-carboxamide **(Compound I-1)**



[00114] A mixture of 5-fluoro-4-(3-methylimidazol-4-yl)pyridin-3-amine dihydrochloride **11** (8.006 g, 30.2 mmol) and (6-chlorobenzotriazol-1-yl) 2-amino-6-fluoro-pyrazolo[1,5-a]pyrimidine-3-carboxylate **6b** (10 g, 28.76 mmol) was suspended in anisole (100 mL). To this suspension was added DIPEA (8.177 g, 11.02 mL, 63.27 mmol) and the mixture was heated at 95°C (internal temperature) for 44 hr. then allowed to cool to room temperature overnight. The solid was collected by filtration, washed with minimal anisole (approx 20 mL), dried under vacuum for 1 hr., then the solid dried in a vacuum oven at 45°C (internal temperature) for 2 hr. to leave product as a light yellow solid, 7.8 g. This solid was suspended in water (78 mL) and MeCN (117 mL) and TFA (2.4 g, 1.62 mL, 1 eq.) was added. The reaction mixture was stirred at room temperature for 10 mins. then filtered through filter paper, washed through with small amount of water. The filtrate was basified to pH = 8 by addition of 2 M sodium carbonate whilst stirring. The solid was collected by filtration, washed with water then dried under vacuum for 1hr. The solid was then dried in vacuum oven at 45°C (internal temperature) overnight leaving product **I-1** as a pale yellow solid, 5.29 g. ^{1}H NMR (500 MHz, DMSO-*d6*) δ 9.68 (s, 2H), 9.42 (dd, J = 4.8, 2.5 Hz, 1H), 8.46 (s, 1H), 8.31 (d, J = 2.5 Hz, 1H), 8.07 (s, 1H), 7.25 (d, J = 1.0 Hz, 1H), 6.71 (s, 2H), 3.46 (s, 3H). MS (ES+) 371.0.

Compound Analytical Data

Cmpd No.	LCMS ES +	LCMS (Rt min)	HNMR
I-1	371.0	1.80	^{1}H NMR (500 MHz, DMSO-*d6*) δ 9.68 (s, 2H), 09.42 (dd, J = 4.8, 2.5 Hz, 1H), 8.46 (s, 1H), 8.31 (d, J = 2.5 Hz, 1H), 8.07 (s, 1H), 7.25 (d, J = 1.0 Hz, 1H), 6.71(s, 2H), 3.46 (s, 3H).

Solid Forms of Compound I-1

[00115] Compound **I-1** has been prepared in various solid forms, including anhydrous forms. The solid forms of the present invention are useful in the manufacture of medicaments for the treatment of cancer. One embodiment provides use of a solid form described herein for treating cancer. In some embodiments, the cancer is triple negative breast cancer, pancreatic cancer, small cell lung cancer, colorectal cancer, ovarian cancer, or non-small cell lung cancer. Another embodiment provides a pharmaceutical composition comprising a solid form described herein and a pharmaceutically acceptable carrier.

[00116] Applicants describe herein a novel solid form of Compound **I-1**. The name and stoichiometry of the solid form is provided in Table 2 below:

Table 2

Example	Forms	Stoichiometry
Example 2	Compound **I-1** anhydrous free base	N/A
Example 3	Compound **I-1** hydrate	1:3
Example 4	Compound **I-1** tartaric acid	1:1

Example 2: Compound I-1 (anhydrous free base)

[00117] Compound **I-1** anhydrous free base can be prepared according to the methods described in **Example 1**, *Step 4*.

XRPD of Compound **I-1** (anhydrous free base)

[00118] The XRPD pattern of compound **I-1** anhydrous free base was recorded at room temperature in reflection mode using a *PANalytical* diffractometer equipped with an Empyrean tube source and a PIXcel 1D detector (*PANalytical,* The Netherlands). The X-ray generator was operating at a voltage of 45 kV and a current of 40 mA. The powder sample was placed in a silicon holder. The data were recorded over the range of 3°-39° 2 theta with a step size of 0.013° and a dwell time of 0.5s per step. Figure 1a shows the X-ray powder diffractogram of the sample which is characteristic of crystalline drug substance.

[00119] Representative XRPD peaks from Compound **I-1** anhydrous free base:

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
1	8.704	35.67
2*	9.8727	100
3*	12.7565	34.37
4*	15.4224	31.96
5*	16.9295	29.04
6	17.4518	6.14

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
7	18.6901	21.74
8	20.5734	9.04
9	21.2755	9.98
10	21.7139	5.54
11*	23.0565	29.6
12	24.3907	14.96
13	25.9089	3.38
14*	27.8453	28.56
15*	28.9558	17.14
16*	30.1162	9.76
17	31.7775	6.85
18	32.2508	2.88
19	33.04	3.17
20	33.7887	4.71
21	36.5878	2.64
22	37.6243	0.33

Thermo Analysis of Compound **I-1** (anhydrous free base)

[00120] A thermogravimetric analysis of compound **I-1** anhydrous free base was performed to determine the percent weight loss as a function of temperature using the Discovery TGA (TA Instruments Trios). A sample (2.84mg) was added to a pre-tared aluminum pan and heated from ambient temperature to 400°C at 10°C/min. The TGA results seen in Figure 2a show very little observed weight loss prior to melting or thermal degradation. From ambient temperature to 261°C, the weight loss is 0.60%. The onset temperature of melting/degradation is 299°C.

Differential Scanning Calorimetry of Compound **I-1** (anhydrous free base)

[00121] Differential scanning calorimetry of compound **I-1** anhydrous free base was measured using the TA Instrument DSC Q2000. A sample (1.71 mg) was weighed in a pinholed hermetic aluminum pan and heated from ambient temperature to 400°C at 10°C/min. The DSC results seen in Figure 3a show a single melting endotherm at 302°C (onset).

Example 3: Compound I-1 (hydrate)

[00122] Compound **I-1** anhydrous free base, prepared according to the methods described in **Example 1**, *Step* 4, was slurried in water or organic solvent water mixtures to produce Compound **I-1** hydrate.

XRPD of Compound **I-1** (hydrate)

[00123] The XRPD pattern of Compound **I-1** hydrate was recorded at room temperature in reflection mode using a *PANalytical* diffractometer equipped with an Empyrean tube source and a PIXcel 1D detector (*PANalytical,* The Netherlands). The X-ray generator was operating at a voltage of 45 kV and a current of 40 mA. The powder sample was placed in a silicon holder. The data were over the range of 3°-39° 2 theta with a step size of 0.013° and a dwell time of 0.5s per step. Figure 1b shows the X-ray powder diffractogram of the sample which is characteristic of crystalline drug substance.

[00124] Representative XRPD peaks from Compound **I-1** hydrate:

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
1	7.5	100.0
*2	27.5	52.7
3	6.4	43.1
*4	20.6	23.2
5	27.9	22.4
6	18.3	16.2
7	17.1	15.2
*8	9.7	13.5
9	30.3	13.4
10	11.8	12.8
11	28.5	12.8
12	15.6	12.7
13	16.7	11.6
14	18.7	10.8
15	22.8	10.3

Thermo Analysis of Compound **I-1** (hydrate)

[00125] A thermal gravimetric analysis (TGA) of Compound **I-1** hydrate was performed to determine the percent weight loss as a function of temperature using the Discovery TGA (TA Instruments Trios). A sample (4.74 mg) was added to a pre-tared aluminum pan and heated from ambient temperature to 400°C at 10°C/min. The TGA results seen in Figure 2b show a large weight loss of 12.6% below 100°C. This weight loss corresponds to approximately 3 molar equivalents of water. The subsequent weight loss above 250°C is a result of melting and degradation.

Differential Scanning Calorimetry of Compound **I-1** (hydrate)

[00126] Differential scanning calorimetry (DSC) of Compound **I-1** hydrate was measured using the TA Instrument DSC Q2000. A sample (2.78 mg) was weighed in a pinholed aluminum hermetic pan and heated from ambient temperature to 370°C at 10°C/min. The DSC results seen in Figure 3b show a broad desolvation endotherm below 100°C followed by a exotherm recrystallization to Compound **I-1** anhydrous free base between 100-150°C.The endotherm peak between 300-305°C indicates the melting of Compound **I-1** anhydrous free base.

Example 4: Compound I-1 (tartaric acid)

[00127] Compound **I-1** anhydrous free base, prepared according to the methods described in **Example 1**, *Step* 4, was slurried with tartaric acid and ethanol to produce Compound **I-1** tartaric acid.

XRPD of Compound **I-1** (tartaric acid)

[00128] The XRPD pattern of Compound **I-1** tartaric acid form was recorded at room temperature in reflection mode using a *PANalytical* diffractometer equipped with an Empyrean tube source and a PIXcel 1D detector (*PANalytical,* The Netherlands). The X-ray generator was operating at a voltage of 45 kV and a current of 40 mA. The powder sample was placed in a silicon holder. The data were over the range of 4.5°-39° 2 theta with a step size of 0.013° and a dwell time of 299.6s per step. Figure 1c shows the X-ray powder diffractogram of the sample which is characteristic of crystalline drug substance.

[00129] Representative XRPD peaks from Compound **I-1** tartaric acid:

XRPD Peaks	Angle (2-Theta ± 0.2)	Intensity %
*1	7.1	100.0
*2	18.3	36.7
3	19.2	36.2
*4	13.2	29.2
5	28.0	25.8
6	24.8	24.2
7	20.3	20.3
8	22.2	16.9
9	28.9	16.4
10	23.7	15.7
11	28.4	14.3
12	10.6	14.1
13	10.3	12.0

Thermo Analysis of Compound **I-1** (tartaric acid)

[00130] A thermal gravimetric analysis (TGA) of Compound **I-1** tartaric acid form was performed to determine the percent weight loss as a function of temperature using the Discovery TGA (TA Instruments Trios). A sample (3.35 mg) was added to a pre-tared aluminum pan and heated from ambient temperature to 330°C at 10°C/min. The TGA results seen in Figure 2c show three step weight losses of 12.4%, 12.6%, and 8.5% between 150-330°C.

Differential Scanning Calorimetry of Compound **I-1** (tartaric acid)

[00131] Differential scanning calorimetry (DSC) of Compound **I-1** tartaric acid was measured using the TA Instrument DSC Q2000. A sample (1.08 mg) was weighed in a pinholed aluminum hermetic pan and heated from ambient temperature to 350°C at 10°C/min. The DSC results seen in Figure 3c show the first 2 exotherm peaks between 200-275°C corresponding to the first 2 step weight losses in TGA, and the last endoterm peak above 275°C corresponding to the last step weight loss in TGA.

Example 5: Cellular ATR Inhibition Assay:

[00132] Compounds can be screened for their ability to inhibit intracellular ATR using an immunofluorescence microscopy assay to detect phosphorylation of the ATR substrate histone H2AX in hydroxyurea treated cells. HT29 cells are plated at 14,000 cells per well in 96-well black imaging plates (BD 353219) in McCoy's 5A media (Sigma M8403) supplemented with 10% foetal bovine serum (JRH Biosciences 12003), Penicillin/Streptomycin solution diluted 1:100 (Sigma P7539), and 2mM L-glumtamine (Sigma G7513), and allowed to adhere overnight at 37°C in 5% CO_2. Compounds are then added to the cell media from a final concentration of 25μM in 3-fold serial dilutions and the cells are incubated at 37°C in 5% CO_2. After 15min, hydroxyurea (Sigma H8627) is added to a final concentration of 2mM.

[00133] After 45min of treatment with hydroxyurea, the cells are washed in PBS, fixed for 10min in 4% formaldehyde diluted in PBS (Polysciences Inc 18814), washed in 0.2% Tween-20 in PBS (wash buffer), and permeabilised for 10min in 0.5% Triton X-100 in PBS, all at room temperature. The cells are then washed once in wash buffer and blocked for 30min at room temperature in 10% goat serum (Sigma G9023) diluted in wash buffer (block buffer). To detect H2AX phosphorylation levels, the cells are then incubated for 1h at room temperature in primary antibody (mouse monoclonal anti-phosphorylated histone H2AX

Ser139 antibody; Upstate 05-636) diluted 1:250 in block buffer. The cells are then washed five times in wash buffer before incubation for 1h at room temperature in the dark in a mixture of secondary antibody (goat anti-mouse Alexa Fluor 488 conjugated antibody; Invitrogen A11029) and Hoechst stain (Invitrogen H3570); diluted 1:500 and 1:5000, respectively, in wash buffer. The cells are then washed five times in wash buffer and finally 100ul PBS is added to each well before imaging.

[00134] Cells are imaged for Alexa Fluor 488 and Hoechst intensity using the BD Pathway 855 Bioimager and Attovision software (BD Biosciences, Version 1.6/855) to quantify phosphorylated H2AX Ser139 and DNA staining, respectively. The percentage of phosphorylated H2AX-positive nuclei in a montage of 9 images at 20x magnification is then calculated for each well using BD Image Data Explorer software (BD Biosciences Version 2.2.15). Phosphorylated H2AX-positive nuclei are defined as Hoechst-positive regions of interest containing Alexa Fluor 488 intensity at 1.75-fold the average Alexa Fluor 488 intensity in cells not treated with hydroxyurea. The percentage of H2AX positive nuclei is finally plotted against concentration for each compound and IC50s for intracellular ATR inhibition are determined using Prism software (GraphPad Prism version 3.0cx for Macintosh, GraphPad Software, San Diego California, USA).

[00135] The compounds described herein can also be tested according to other methods known in the art (see Sarkaria et al, "Inhibition of ATM and ATR Kinase Activities by the Radiosensitizing Agent, Caffeine: *Cancer Research* 59: 4375-5382 (1999); Hickson et al, "Identification and Characterization of a Novel and Specific Inhibitor of the Ataxia-Telangiectasia Mutated Kinase ATM" *Cancer Research* 64: 9152-9159 (2004); Kim et al, "Substrate Specificities and Identification of Putative Substrates of ATM Kinase Family Members" *The Journal of Biological Chemistry*, 274(53): 37538-37543 (1999); and Chiang et al, "Determination of the catalytic activities of mTOR and other members of the phosphoinositide-3-kinase-related kinase family" *Methods Mol. Biol.* 281:125-41 (2004)).

Example 6: ATR Inhibition Assay:

[00136] Compounds can be screened for their ability to inhibit ATR kinase using a radioactive-phosphate incorporation assay. Assays are carried out in a mixture of 50mM Tris/HCl (pH 7.5), 10mM $MgCl_2$ and 1mM DTT. Final substrate concentrations are 10μM [γ-33P]ATP (3mCi 33P ATP/mmol ATP, Perkin Elmer) and 800 μM target peptide (ASELPASQPQPFSAKKK).

[00137] Assays are carried out at 25°C in the presence of 5 nM full-length ATR. An assay stock buffer solution is prepared containing all of the reagents listed above, with the exception of ATP and the test compound of interest. 13.5 µL of the stock solution is placed in a 96 well plate followed by addition of 2 µL of DMSO stock containing serial dilutions of the test compound (typically starting from a final concentration of 15 µM with 3-fold serial dilutions) in duplicate (final DMSO concentration 7%). The plate is pre-incubated for 10 minutes at 25°C and the reaction initiated by addition of 15 µL [γ-33P]ATP (final concentration 10 µM).

[00138] The reaction is stopped after 24 hours by the addition of 30µL 0.1M phosphoric acid containing 2mM ATP. A multiscreen phosphocellulose filter 96-well plate (Millipore, Cat no. MAPHN0B50) is pretreated with 100µL 0.2M phosphoric acid prior to the addition of 45µL of the stopped assay mixture. The plate is washed with 5 x 200µL 0.2M phosphoric acid. After drying, 100 µL Optiphase 'SuperMix' liquid scintillation cocktail (Perkin Elmer) is added to the well prior to scintillation counting (1450 Microbeta Liquid Scintillation Counter, Wallac).

[00139] After removing mean background values for all of the data points, Ki(app) data are calculated from non-linear regression analysis of the initial rate data using the Prism software package (GraphPad Prism version 3.0cx for Macintosh, GraphPad Software, San Diego California, USA).

[00140] In general, the compounds of the present invention are effective for inhibiting ATR. Compound **I-1** inhibits ATR at Ki values below 1 µM.

Example 7: Cisplatin Sensitization Assay

[00141] Compounds can be screened for their ability to sensitize HCT116 colorectal cancer cells to Cisplatin using a 96h cell viability (MTS) assay. HCT116 cells, which possess a defect in ATM signaling to Cisplatin (see, Kim et al.; *Oncogene* 21:3864 (2002); see also, Takemura et al.; *JBC* 281:30814 (2006)) are plated at 470 cells per well in 96-well polystyrene plates (Costar 3596) in 150µl of McCoy's 5A media (Sigma M8403) supplemented with 10% foetal bovine serum (JRH Biosciences 12003), Penicillin/Streptomycin solution diluted 1:100 (Sigma P7539), and 2mM L-glumtamine (Sigma G7513), and allowed to adhere overnight at 37°C in 5% CO_2. Compounds and Cisplatin are then both added simultaneously to the cell media in 2-fold serial dilutions from

a top final concentration of 10μM as a full matrix of concentrations in a final cell volume of 200μl, and the cells are then incubated at 37°C in 5% CO_2. After 96h, 40μl of MTS reagent (Promega G358a) is added to each well and the cells are incubated for 1h at 37°C in 5% CO_2. Finally, absorbance is measured at 490nm using a SpectraMax Plus 384 reader (Molecular Devices) and the concentration of compound required to reduce the IC50 of Cisplatin alone by at least 3-fold (to 1 decimal place) can be reported.

[00142] In general, the compounds of the present invention are effective for sensitizing cancer cells to Cisplatin. Compound **I-1** have Cisplatin sensitization values of < 0.2 μM.

Example 8: Single Agent HCT116 Activity

[00143] Compounds can be screened for single agent activity against HCT116 colorectal cancer cells using a 96h cell viability (MTS) assay. HCT116 are plated at 470 cells per well in 96-well polystyrene plates (Costar 3596) in 150μl of McCoy's 5A media (Sigma M8403) supplemented with 10% foetal bovine serum (JRH Biosciences 12003), Penicillin/ Streptomycin solution diluted 1:100 (Sigma P7539), and 2mM L-glumtamine (Sigma G7513), and allowed to adhere overnight at 37°C in 5% CO_2. Compounds are then added to the cell media in 2-fold serial dilutions from a top final concentration of 10μM as a full matrix of concentrations in a final cell volume of 200μl, and the cells are then incubated at 37°C in 5% CO_2. After 96h, 40μl of MTS reagent (Promega G358a) is added to each well and the cells are incubated for 1h at 37°C in 5% CO_2. Finally, absorbance is measured at 490nm using a SpectraMax Plus 384 reader (Molecular Devices) and IC50 values can be calculated.

Example 9: ATR-complex Inhibition Assay

[00144] Compounds were screened for their ability to inhibit ATR kinase, in the presence of partner proteins ATRIP, CLK2 and TopBP1, using a radioactive-phosphate incorporation assay. Assays were carried out in a mixture of 50 mM Tris/HCl (pH 7.5), 10 mM $MgCl_2$ and 1 mM DTT. Final substrate concentrations were 10 μM [g-33P]ATP (3.5 μCi 33P ATP/nmol ATP, Perkin Elmer, Massachusetts, USA) and 800 μM target peptide (ASELPASQPQPFSAKKK, Isca Biochemicals, Cambridgeshire, UK).

[00145] Assays were carried out at 25°C in the presence of 4 nM full-length ATR, 40 nM full-length ATRIP, 40 nM full-length CLK2 and 600 nM TopBP1(A891-S1105). An enzyme stock buffer solution was prepared containing all of the reagents listed above, with the

exception of target peptide, ATP and the test compound of interest. This enzyme stock was pre-incubated for 30 minutes at 25°C. 8.5 µL of the enzyme stock solution was placed in a 96-well plate followed by addition of 5µl of target peptide and 2 µL of DMSO stock containing serial dilutions of the test compound (typically starting from a final concentration of 1.5 µM with 2.5-fold serial dilutions) in duplicate (final DMSO concentration 7%). The plate was pre-incubated for 10 minutes at 25°C and the reaction initiated by addition of 15 µL [g-33P]ATP (final concentration 10 µM).

[00146] The reaction was stopped after 20 hours by the addition of 30 µL 0.3 M phosphoric acid containing 2 mM ATP. A phosphocellulose filter 96-well plate (Multiscreen HTS MAPHNOB50, Merck-Millipore, Massachusetts, USA) was pretreated with 100 µL 0.1 M phosphoric acid prior to the addition of 45 µL of the stopped assay mixture. The plate was washed with 5 x 200 µL 0.1 M phosphoric acid. After drying, 50 µL Optiphase 'SuperMix' liquid scintillation cocktail (Perkin Elmer, Massachusetts, USA) was added to the well prior to scintillation counting (Wallac 1450 Microbeta Liquid Scintillation Counter, Perkin Elmer, Massachusetts, USA).

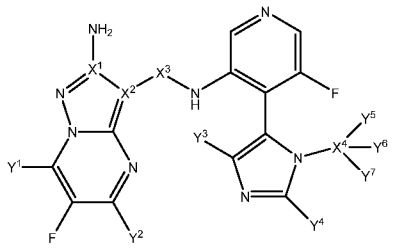
[00147] After removing mean background values for all of the data points, Ki(app) data were calculated from non-linear regression analysis of the initial rate data using the Prism software package (GraphPad Prism version 6.0c for Macintosh, GraphPad Software Inc., San Diego, USA).

[00148] While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds, methods, and processes of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example herein.

CLAIMS

We claim:

1. A compound of Formula **I-A**:



I-A

or a pharmaceutically acceptable salt or derivative thereof, wherein:

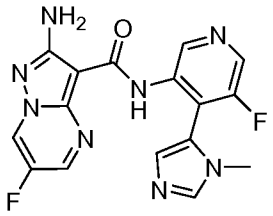
each Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is independently hydrogen or deuterium; provided at least one of Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 is deuterium;

each X^1, X^2, and X^4 is independently selected from ^{12}C or ^{13}C; and

X^3 is independently selected from -^{12}C(O)- or -^{13}C(O)-.

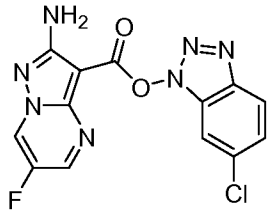
2. The compound of claim 1, wherein Y^1, Y^2, Y^3, and Y^4 are independently selected from deuterium or hydrogen; and Y^5, Y^6, and Y^7 are deuterium.
3. The compound of claim 1, wherein Y^1 and Y^2 are independently selected from deuterium or hydrogen; and Y^3, Y^4, Y^5, Y^6, and Y^7 are deuterium.
4. The compound of claim 1, wherein Y^1, Y^2, Y^5, Y^6, and Y^7 are independently selected from deuterium or hydrogen; and Y^3 and Y^4 are deuterium.
5. The compound of claim 1, wherein Y^1, Y^3, and Y^4 are independently selected from deuterium or hydrogen; and Y^2, Y^5, Y^6, and Y^7 are deuterium.
6. The compound of claim 1, wherein Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^4 is ^{13}C.
7. The compound of claim 1, wherein Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^1 and X^4 are ^{13}C.
8. The compound of claim 1, wherein Y^1, Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; and X^3 is -^{13}C(O)-.
9. The compound of claim 1, wherein Y^1, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; Y^2 is deuterium; and X^4 is ^{13}C.

10. The compound of claim 1, wherein Y^1, Y^2, Y^3, and Y^4 are hydrogen; Y^5, Y^6, and Y^7 are deuterium; and X^1 is ^{13}C.
11. The compound of claim 1, wherein Y^1, Y^3, Y^4, Y^5, Y^6, and Y^7 are hydrogen; Y^2 is deuterium; and X^1 is ^{13}C.
12. The compound of claim 1, wherein Y^1, Y^2, Y^3, Y^5, Y^6, and Y^7 are hydrogen; Y^4 is deuterium; and X^1 is ^{13}C.
13. The compound of claim 1, wherein Y^1 is hydrogen; Y^2, Y^3, Y^4, Y^5, Y^6, and Y^7 are deuterium; X^2 is ^{13}C; and X^3 is -^{13}C(O)-.
14. A process for preparing a compound of formula **I-1**:



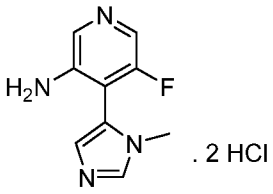
I-1

comprising the step of reacting the compound of formula **6b**:



6b

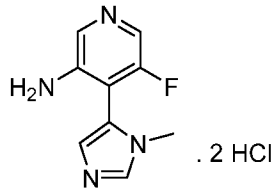
with a compound of formula **11**:



11

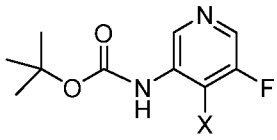
under suitable conditions to form an amide bond.

15. The process of claim 14, further comprising the step of preparing a compound of formula **11**:



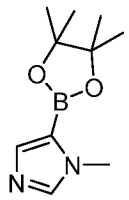
11

by reacting the compound of formula **9**:



9

with a compound of formula **10**:

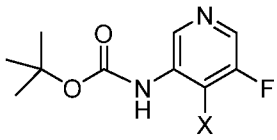


10

under suitable metal catalyzed cross-coupling conditions to form an adduct containing a protected amine group; and

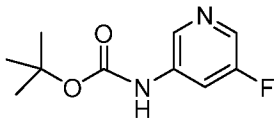
subjecting the resulting adduct to suitable deprotection conditions.

16. The process of claim 15, further comprising the step of preparing a compound of formula **9**:



9

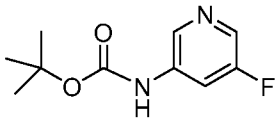
by reacting the compound of formula **8:**



8

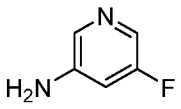
under suitable halogenation conditions.

17. The process of claim 16, further comprising the step of preparing a compound of formula **8**:



8

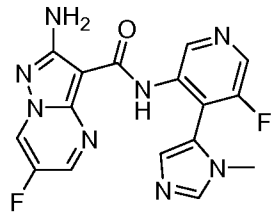
by reacting a compound of formula **7**:



7

under suitable conditions to generate a protected amine group.

18. A solid form of a compound of formula **I-1**:



I-1

wherein the form is selected from the group consisting of Compound **I-1** anhydrous free base, Compound **I-1** hydrate, or Compound **I-1** tartaric acid.

19. The solid form of claim 18, wherein the form is crystalline Compound **I-1** anhydrous free base.

20. The solid form of claim 19, characterized by one or more peaks expressed in 2-theta ± 0.2 at about 9.9, 12.8, 15.4, 17.0, 23.1, 27.8, 29.0, and 30.1 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation.

21. The solid form of claim 19, characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1a.

22. The solid form of claim 18, wherein the form is crystalline Compound **I-1** hydrate.

23. The solid form of claim 22, wherein the crystalline Compound **I-1** hydrate has a Compound **I-1** to water ratio of 1:3.

24. The solid form of claim 22, characterized by a weight loss of from about 12.6% in a temperature range from about 40°C and about 100°C.

25. The solid form of claim 22, characterized by one or more peaks expressed in 2-theta ± 0.2 at about 27.5, 20.6, and 9.7 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation.
26. The solid form of claim 22, characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1b.
27. The solid form of claim 18, wherein the form is crystalline Compound **I-1** tartaric acid.
28. The solid form of claim 27, wherein the crystalline Compound **I-1** tartaric acid has a Compound **I-1** to tartaric acid of 1:1.
29. The solid form of claim 27, characterized by one or more peaks expressed in 2-theta ± 0.2 at about 7.1, 18.3, and 13.2 degrees in an X-Ray powder diffraction pattern obtained using Cu K alpha radiation.
30. The solid form of claim 27, characterized as having an X-ray powder diffraction pattern substantially the same as that shown in Figure 1c.