

Tyrosine Kinase Inhibitors and Methods Thereof

Technical Field

- 5 The present invention relates, in general terms, to tyrosine kinase inhibitors and their methods of use thereof.

Background

- 10 Acute myeloid leukaemia (AML) is the cancer of white blood cells and affects a group of cells called myeloid cells. It is characterized by rapid expansion of immature blast cells which affect development of other blood cells in the bone marrow. If left untreated, AML leads to life threatening complications and is fatal. Global incidence of AML has been on the rise since 1990 and can afflict any age group. AML was the most diagnosed
15 leukaemia in the USA in 2017. Although the mechanism of AML development is not fully understood, it is generally believed that mutations in certain genes provide selective advantage for clonal expansion of immature blast cells. Alterations in genes such as Flt3, IDH1/2, DNMT3A and NPM1 have been frequently associated with the development of AML. Of these, Flt3 mutations were identified in 30% of the AML patients. Flt3 (FMS-
20 like tyrosine kinase) is a receptor tyrosine kinase and plays an important role in proliferation, survival and differentiation hematopoietic cells.

- Although several Flt3 inhibitors have been approved for clinical use such as Quizartinib, Midostaurin, Gilteritinib and Crenolanib, mutations that lead to resistance to these
25 inhibitors have been identified in patients. For instance, mutations at the D835, Y842 or the gatekeeper mutation F691L have been identified in patients who relapse following Quizartinib (type II inhibitor) treatment due to development of resistance. A combination of Flt3-ITD+D835Y mutations also showed resistance to Quizartinib. Crenolanib exhibit inhibitory activity against Flt3-ITD and FLT3-TKD mutations but
30 shows digestive toxicity.

- FLT3 overactivity has also been implicated lymphocytic leukemia, myeloproliferative disease and in autoimmune diseases, such as rheumatoid arthritis. Emergence of resistance due to mutations (or combinations of mutations) has been an issue for the
35 currently available inhibitors (for instance D835, Y842, and F691 and their

combinations).

Hence, there is a clinical need for inhibitors that act against such resistant mutations and cause minimal toxicity.

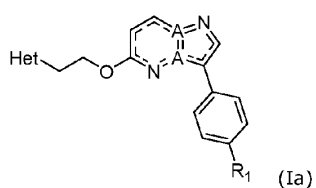
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It would be desirable to overcome or ameliorate at least one of the above-described problems, or at least to provide a useful alternative.

Summary

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The present disclosure provides a method of treating acute myeloid leukemia in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof to the subject:



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wherein

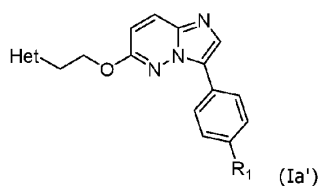
A is independently C or N, and at least one A is N;

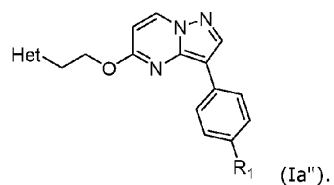
Het is optionally substituted 6-membered heterocycyl; and

R₁ is halo substituted alkyl, oxo optionally substituted alkyoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy.

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In some embodiments, compound of Formula (Ia) is represented by Formula (Ia') or (Ia''):





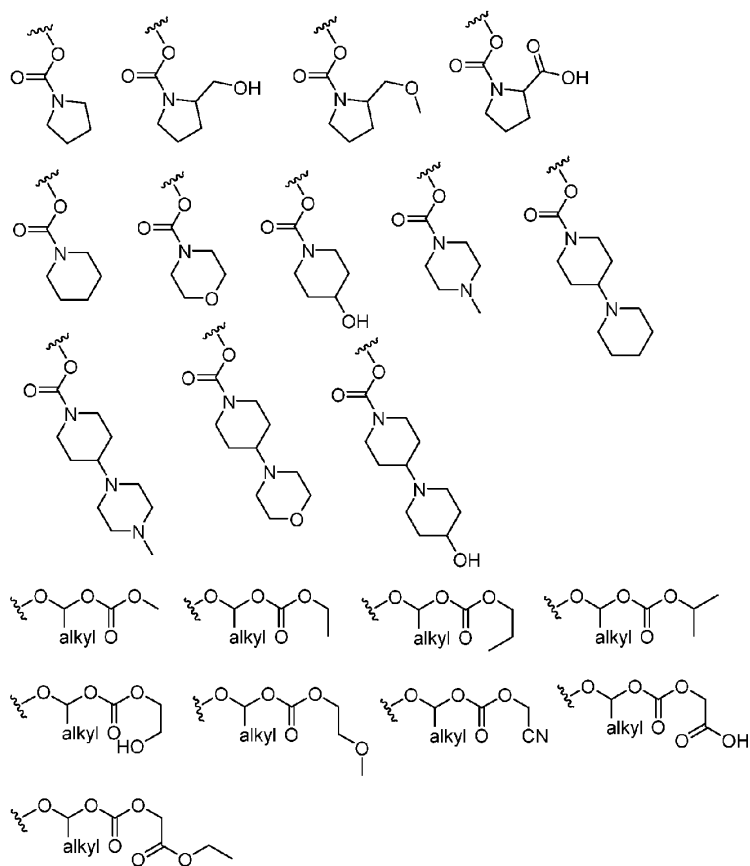
In some embodiments, Het is optionally substituted 6-membered N-heterocycl.

- 5 In some embodiments, Het is selected from optionally substituted piperidinyl, optionally substituted piperazinyl, and optionally substituted morpholinyl.

In some embodiments, the optional substituent on Het is selected from halo, cyano, oxo, and C₁-C₆ alkyl.

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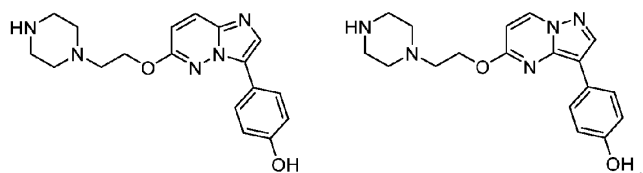
In some embodiments, R₁ is selected from -OH,



wherein $\sim$ denotes a bond to the Markush structure at R₁; and
alkyl is C₁-C₆ alkyl.

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In some embodiments, the compound of Formula (Ia) is selected from

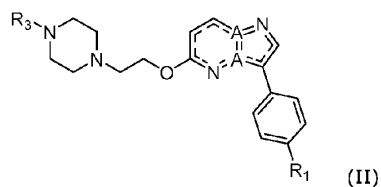


The present disclosure also provides a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof for use in treating acute myeloid leukemia in a subject in need thereof.

5

The present disclosure also provides a use of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating acute myeloid leukemia.

10 The present disclosure also provides a compound of Formula (IIa) or a salt, solvate or prodrug thereof:



wherein

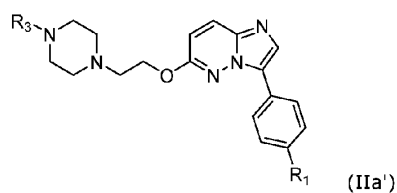
A is independently C or N;

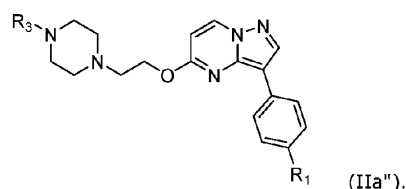
15 at least one A is N;

R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy;

R₃ is H, optionally substituted alkyl or optionally substituted acyl.

20 In some embodiments, compound of Formula (IIa) is represented by Formula (IIa') or (IIa''):





In some embodiments, R₃ is H or C₁-C₆ alkyl.

- 5 The present disclosure also provides a pharmaceutical composition comprising a compound of Formula (Ia), (IIa) or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Brief description of the drawings

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Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

- Figure 1.** Expression of human active internal tandem duplications of Flt3 (Flt3-ITD) leads to loss of viability in engineered yeast. Human Flt3-ITD and its kinase dead version were under the control of yeast GAL1 promoter was integrated at the TRP1 locus. (A) Growth retardation in 5 different clones expressing human Flt3-ITD protein. (B) Expression of Kinase-dead version of human Flt3 fails to cause loss of viability in yeast.
- Figure 2.** Effect of the hit compounds F1, F2 and F3 on the viability of acute lymphoblastic leukaemia cell line (SEM) and MV-4-11 leukemia cell lines. Imatinib and Quizartinib were used as positive controls. Acute Lymphoblastic Leukemia (ALL) cell line SEM expresses high levels of Flt3 while Acute Myeloid leukemia cell line MV-4-11 expresses Flt3-ITD, a constitutive active mutant version of Flt3. Compounds of the present invention are represented by initial hit compound F3.
- Figure 3.** Overview of the structure of Flt3 kinase in its (A) active and (B) inactive form. (C) Predicted binding mode of one of the hit molecule (Salmon) with the structure of Flt3 kinase in its in-active form. The N-lobe (magenta), C-lobe (grey), helix αC (yellow), the activation loop (cyan), hinge (green), and DFG motif (blue) highlighted.
- Figure 4.** 2nd generation derivatives of Flt3 inhibitors were tested for their ability to inhibit proliferation of acute myeloid cell line MV4-11. This cell line harbours Flt3^{ITD} version of the Flt3 gene. The extent of inhibition is expressed as % cytotoxicity. Of the
- 15
20
25
30

19 derivatives, Compounds 9, 13 and 27 showed promising inhibitory activity against Flt3^{ITD} (IC50 140 nm and 100 nm, respectively).

Figure 5. Selectivity profile of the Flt3 inhibitors Compound 13 and 27 against 340 kinase in the human proteome. The profile analysis was conducted at 1 μ M.

5 **Figure 6.** Representative Kd profile of Compound 13 and 27 against various WT and mutant versions of Flt3.

Figure 7. Effect of Flt3 inhibitors Compound 13 and 27 in subcutaneous xenograft model. Compound 13 was able to reduce tumor volume by 40%.

Figure 8. Effect of Flt3 inhibitors Compound 13 and 27 on body weight in mouse model.

10 No significant toxic effects of these inhibitors were observed.

Detailed description

"Alkyl" refers to monovalent alkyl groups which may be straight chained or branched
15 and preferably have from 1 to 10 carbon atoms or more preferably 1 to 6 carbon atoms. Examples of such alkyl groups include methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

"Alkoxy" refers to the group alkyl-O- where the alkyl group is as described above.
20 Examples include, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

25 "Oxo/hydroxy" refers to groups =O, HO-.

"Aryl" refers to an unsaturated aromatic carbocyclic group having a single ring (eg. phenyl) or multiple condensed rings (eg. naphthyl or anthryl), preferably having
30 from 6 to 14 carbon atoms. Examples of aryl groups include phenyl, naphthyl and the like.

"Acyl" refers to groups H-C(O)-, alkyl-C(O)-, cycloalkyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclyl-C(O)-, where alkyl, cycloalkyl, aryl, heteroaryl and heterocyclyl
35 are as described herein.

"Acyloxy" refers to the groups -OC(O)-alkyl, -OC(O)-aryl, -C(O)O-heteroaryl, and -C(O)O-heterocyclyl where alkyl, aryl, heteroaryl and heterocyclyl are as described herein.

5 "Aminoacyloxy" refers to the groups -OC(O)N(R'')₂, -OC(O)NR''-alkyl, -OC(O)NR''-aryl, -OC(O)NR''-heteroaryl, and -OC(O)NR''-heterocyclyl where R'' is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl and where each of alkyl, cycloalkyl, aryl, heteroaryl and heterocyclyl is as described herein.

10 "Heteroaryl" refers to a monovalent aromatic heterocyclic group which fulfils the Hückel criteria for aromaticity (ie. contains $4n + 2$ π electrons) and preferably has from 2 to 10 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen, selenium, and sulfur within the ring (and includes oxides of sulfur, selenium and nitrogen). Such heteroaryl groups can have a single ring (eg. pyridyl, pyrrolyl or N-
15 oxides thereof or furyl) or multiple condensed rings (eg. indoliziny, benzoimidazolyl, coumarinyl, quinolinyl, isoquinolinyl or benzothienyl).

Examples of heteroaryl groups include, but are not limited to, oxazole, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole,
20 indole, indazole, purine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, isothiazole, phenoxazine, phenothiazine, thiazole, thiadiazoles, oxadiazole, oxatriazole, tetrazole, thiophene, benzo[b]thiophene, triazole, imidazopyridine and the like.

25 "Cycloalkyl" refers to cyclic alkyl groups having a single cyclic ring or multiple condensed rings, preferably incorporating 3 to 11 carbon atoms. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and the like, or multiple ring
30 structures such as adamantanyl, indanyl, 1,2,3,4-tetrahydronaphthalenyl and the like.

"Heterocyclyl" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, preferably from 1 to 8 carbon atoms and from 1 to
35 4 hetero atoms selected from nitrogen, sulfur, oxygen, selenium or phosphorous

within the ring. The most preferred heteroatom is nitrogen. It will be understood that where, for instance, R₂ or R¹ is an optionally substituted heterocyclyl which has one or more ring heteroatoms, the heterocyclyl group can be connected to the core molecule of the compounds of the present invention, through a C-C or C-heteroatom bond, in particular a C-N bond.

Examples of heterocyclyl and heteroaryl groups include, but are not limited to, oxazole, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, isothiazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiadiazoles, oxadiazole, oxatriazole, tetrazole, thiazolidine, thiophene, benzo[b]thiophene, morpholino, piperidinyl, pyrrolidine, tetrahydrofuranyl, triazole, and the like.

In this specification "optionally substituted" is taken to mean that a group may or may not be further substituted or fused (so as to form a condensed polycyclic group) with one or more groups selected from hydroxyl, acyl, alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl, alkynyloxy, amino, aminoacyl, thio, arylalkyl, arylalkoxy, aryl, aryloxy, carboxyl, acylamino, cyano, halogen, nitro, phosphono, sulfo, phosphorylamino, phosphinyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heterocyclyl, heterocyclylalkyl, heterocyclioxy, oxyacyl, oxime, oxime ether, hydrazone, oxyacylamino, oxysulfonylamino, aminoacyloxy, trihalomethyl, trialkylsilyl, pentafluoroethyl, trifluoromethoxy, difluoromethoxy, trifluoromethanethio, trifluoroethenyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclyl amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, aryl, heteroaryl and heterocyclyl, and the like, and may also include a bond to a solid support material, (for example, substituted onto a polymer resin). For instance, an "optionally substituted amino" group may include amino acid and peptide residues.

Cluster of differentiation antigen 135 (CD135) also known as fms-like tyrosine kinase 3

(Flt-3 with fms standing for "feline McDonough sarcoma"), receptor-type tyrosine-protein kinase Flt3, or fetal liver kinase-2 (Flk2) is a protein that in humans is encoded by the FLT3 gene. Flt3 is a cytokine receptor which belongs to the receptor tyrosine kinase class III. CD135 is the receptor for the cytokine Flt3 ligand (Flt3L). It is expressed
5 on the surface of many hematopoietic progenitor cells. Signalling of Flt3 is important for cell survival, cell proliferation and differentiation of hematopoietic progenitor cells.

FLT3 is dimerized by the binding of FL to its extracellular domain. Tyrosine residues in the activation-loop (A-loop) are subsequently trans-phosphorylated. Activated FLT3
10 induces multiple intracellular signaling pathways, leading to hematopoietic cell survival, proliferation and differentiation. As FLT3 activates pathways in hematopoietic cells, FLT3 is frequently mutated in acute myeloid leukemia, other hematologic malignancies, and colorectal cancer. In Acute Myeloid Leukemia (AML), FLT3 mutation is the most frequent gene mutation in the protein-coding regions. FLT3-ITD and tyrosine kinase domain of
15 Flt3 (FLT3-TKD) occur in approximately 20 and 10% of AML, respectively.

Dysregulation of the Flt3 signalling pathway may result in anti-apoptosis, abnormal cell growth, and/or differentiation block. The FLT3 gene is one of the most frequently mutated genes in acute myeloid leukemia (AML). High levels of wild-type FLT3 have
20 been reported for blast cells of some AML patients without FLT3 mutations. These high levels may be associated with worse prognosis.

Without wanting to be bound by theory, the inventors believed that mutations in Flt3 are commonly found in more aggressive cases of AML. This is based on their work
25 genotyping AML patients, in which Flt3-ITD mutation was found in at least one of the samples. Using a yeast-based drug discovery platform to identify inhibitors against proteins implicated in human disease, the inventors constructed a genetically engineered yeast strain for this patient specific Flt3-ITD mutation so as to be able to conduct a screening campaign to identify inhibitors against this mutation. This platform
30 includes a screening module (constructing humanised yeast, testing the efficacy of the humanised yeast strains, run a high throughput screen against chemical libraries and identify dose response of potential hits), a lead optimization module (in-silico modelling and simulation for cellular efficacy and target selectivity), testing the optimised molecules in animal model studies (pharmacokinetics, pharmacodynamics, toxicity, etc)
35 and selection of a preclinical drug candidate. Accordingly, the inventors have identified

initial hit compound F3 and other small molecule inhibitors of Flt3 that inhibit not only the wild type Flt3 but also a number of activating mutant-versions of Flt3 (including Flt3-ITD mutant) and resistance mutations found in AML patients. These inhibitors exhibit higher target specificity for Flt3 with IC50 in nM range. MTD (Maximum Tolerable
5 Dose) studies in mice show that these compounds are non-toxic even at high concentrations. The *in vitro* PK parameters for these inhibitors fall well within the acceptable range assigned to potentially good drug candidates.

In particular, the Flt3 inhibitors Compound 13 and 27 are active against wider range of
10 Flt3 mutations (especially notable is the combination of ITD mutation and the gatekeeper mutation F691L) identified in AML patients.

Compared to the clinically approved Flt3 inhibitor, the compounds of the present disclosure (and in particular Compound 13 and 27) act against the WT Flt3 and its
15 mutant versions reported in AML patients, such as FLT3 WT, FLT3 (D835H), FLT3 (D835V), FLT3 (D835Y), FLT3 (ITD), FLT3 (ITD, D835V), FLT3 (ITD, F691L), FLT3 (N841I), FLT3 (R834Q), and FLT3 (autoinhibited).

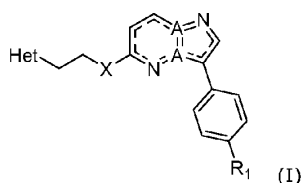
These inhibitors have narrow target specificity compared to the previously reported Flt3
20 inhibitors.

Flt3 has been implicated in haematological malignancies (such as acute myeloid leukaemia), colorectal cancer, colon adenocarcinoma and lung adenocarcinoma. Hence these inhibitors can be developed into drugs to treat these malignancies. These
25 inhibitors would be useful in treating AML patients that have relapsed due to emergence of resistance to the current anti-Flt3 therapies.

These inhibitors may be developed into anti-cancer drugs against Flt3 mutations in AML patient that have relapsed due to emergence of resistance to the current therapies.
30

Accordingly, the present disclosure provides a method of treating a disease or condition associated with Flt3. The Flt3 may be mutated. The diseases or conditions may be acute myeloid leukemia, myelodysplastic syndromes, acute lymphoblastic leukemia, chronic myeloid leukemia, and chronic myelomonocytic leukemia.
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The present disclosure provides a method of treating acute myeloid leukemia in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof to the subject:



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wherein

A is independently C or N, and at least one A is N;

Het is optionally substituted 6-membered heterocyclyl;

X is selected from O or NR₂;

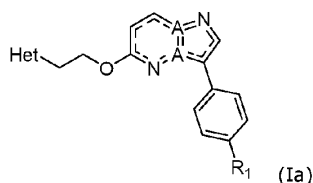
10 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy; and

R₂ is H or optionally substituted alkyl.

15 Acute myeloid leukemia (AML) is a type of blood cancer that affects the cells that normally develop into different types of blood cells, such as red blood cells, white blood cells, and platelets. AML can cause symptoms such as fatigue, shortness of breath, bleeding, infections, and bone pain. In general, AML is diagnosed by blood tests and bone marrow tests, and it is treated with chemotherapy, targeted therapy, and stem cell transplant. AML may be classified into different subtypes based on the genetic and

20 molecular features of the abnormal cells. The subtype of AML may affect the prognosis and treatment options for each patient. AML associated with Flt3 mutation is one subtype.

25 The present disclosure provides a method of treating acute myeloid leukemia in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof to the subject:



wherein

A is independently C or N, and at least one A is N;

Het is optionally substituted 6-membered heterocyclyl; and

- 5 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy.

It was found that compound of Formula (I) are particular effective as Flt3 inhibitors, and also as inhibitors of Flt3 mutants. Without wanting to be bound by theory, the
 10 inventors have found that the 2 carbon linker between the heterocyclyl and pyrazolopyrimidinyl moiety is advantageous in providing a compound which acts against resistant mutations and also cause minimal toxicity. This is because the 2 carbon linker is the optimal length for the heterocyclyl and in particular piperazine to interact with the Flt3 residues.

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Additionally, a para substituted oxo moiety on the phenyl ring relative to the pyrazolopyrimidinyl moiety is advantageous as the oxo moiety at the para position interacts with the conserved positively charged Lys from the active site of Flt3. It was found that replacement of the OH by triazole, cyanide or CL resulted in loss of activity.

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Oxo moiety at the para position is involved in hydrogen bond interaction with the positively charged lysine side chain from Flt3. Such an interaction is not possible with substitution at meta or ortho position.

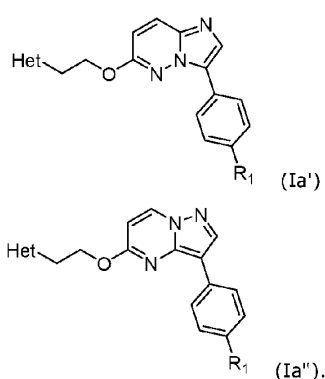
Further, it was found that additional meta and/or ortho substitution may reduce the efficacy of the compounds as Flt3 inhibitors. The phenyl ring is sandwiched between two phenylalanine residues from Flt3, with the ortho and meta position points towards these two phenylalanine residues and any substitution at these positions would result in steric clash with these residues.

30 These compounds were found to be very potent and highly selective. In addition they inhibit the activity of several oncogenic mutations and resistant mutations in Flt3.

The combination of the above features makes compound of Formula (I) particularly effective as a Flt3 and Flt3 mutant inhibitor. In this regard, compound of Formula (I) may be used to treat a subclass of patients having Flt3 related diseases or conditions.

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In some embodiments, compound of Formula (Ia) is represented by Formula (Ia') or (Ia''):



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In some embodiments, the heterocyclyl is connected to the Markush structure via the heteroatom. In some embodiments, the heteroatom in the heterocyclyl is N. In some embodiments, Het is optionally substituted 6-membered N-heterocyclyl. In some embodiments, Het is selected from optionally substituted piperidinyl, optionally substituted piperazinyl, and optionally substituted morpholinyl.

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In some embodiments, the optional substituent on Het is selected from halo, cyano, oxo, acyl, and C₁-C₆ alkyl.

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In some embodiments, R₁ is selected from halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy. In some embodiments, R₁ is selected from oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy. In some embodiments, when R₁ is oxo, the oxo may be protected.

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By way of non-limiting example, hydroxyl protecting groups include methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM),
 5 guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyl, diphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl,
 25 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylhexylsilyl, t-butyl, dimethylsilyl (TBDMS), t-butyl, diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butylmethoxyphenylsilyl (TBMPs), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate,
 35 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate

(mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, 5 alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 10 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinate, (E)-2-methyl-2-butenolate, o-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, 15 phenylcarbamate, sulfate, methanesulfonate (mesylate), benzyisulfonate, and tosylate (Ts).

Exemplary protecting groups are detailed herein, however, it will be appreciated that the present disclosure is not intended to be limited to these protecting groups; rather, 20 a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in Protecting Groups in Organic Synthesis, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference.

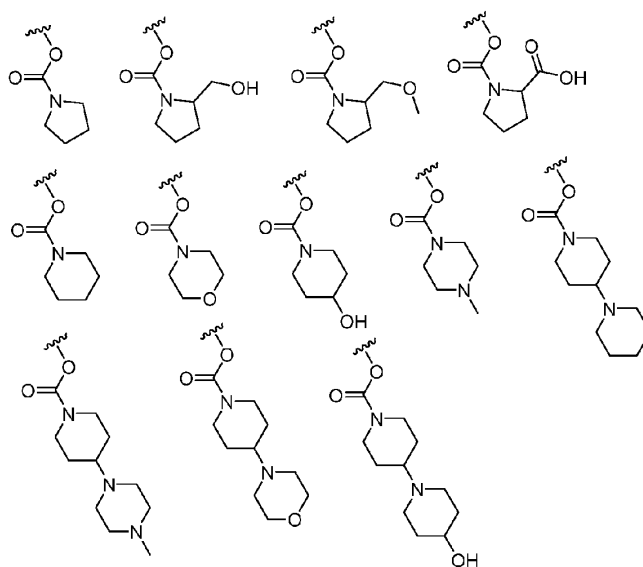
25 In some embodiments, R₁ is -CF₃, oxo or optionally substituted C₁-C₆ alkoxy. In some embodiments, R₁ is oxo or optionally substituted alkoxy. In some embodiments, R₁ is -OH. In some embodiments, R₁ is methoxy, ethoxy or propoxy.

30 In some embodiments, R₁ is selected from optionally substituted acyloxy, or optionally substituted aminoacyloxy. These moieties may be used to protect oxo.

In some embodiments, the optional substituent is selected from optionally substituted alkyl, optionally substituted alkoxy, optionally substituted oxyalkyl, optionally 35 substituted amino, optionally substituted aminoalkyl, optionally substituted cyano,

optionally substituted cyanoalkyl, and optionally substituted oxyacylalkyl. In some embodiments, the optional substituent is selected from methyl, ethyl, n-propyl, i-propyl, t-butyl, oxyethyl, methyloxyethyl, cyanomethyl, ethyloxyacetylmethyl, oxyacetylmethyl optionally substituted with methyl, ethyl, n-propyl or i-propyl, -CH₂CH(OH)COOH, or -CH₂CH(OH)CH₂OH. In some embodiments, the optional substituent is selected from aminomethyl, methylaminomethyl, dimethylaminomethyl, amino(methyl)methyl, oxy(amino)ethyl, or (oxy)(amino)propyl.

In some embodiments, R₁ is optionally substituted aminoacyloxy. The optional substituent may be linked to form an optionally substituted N-heterocyclyl. For example, the N-heterocyclyl may be azetidiny, pyrrolidinyl, pyrazolidinyl, piperidinyl, piperazinyl, morpholinyl. The optional substituent may be oxo, optionally substituted alkyl, optionally substituted oxyalkyl, optionally substituted oxyacyl, or optionally substituted N-heterocyclyl. In some embodiments, R₁ is

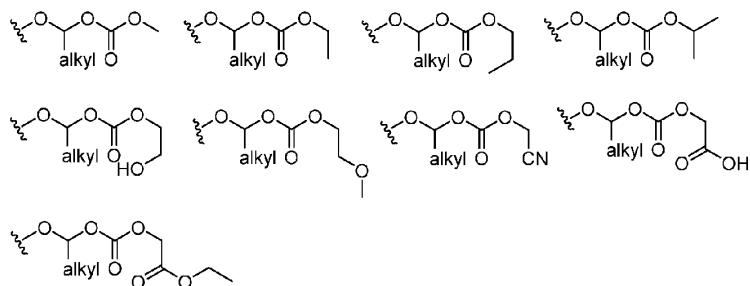


15

wherein  denotes a bond to the Markush structure at R₁.

In some embodiments, R₁ is optionally substituted alkoxy. The alkoxy may be methoxy, ethoxy, or propoxy. The optional substituent may be optionally substituted oxyacyloxy.

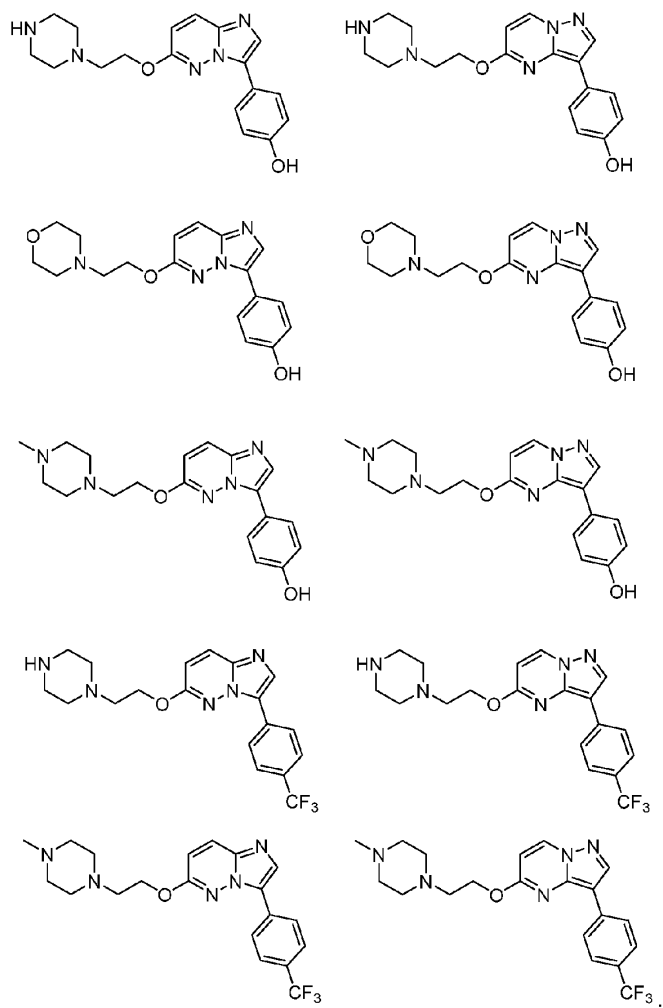
The optional substituent may be oxyacyloxy substituted with optionally substituted alkyl, optionally substituted oxyalkyl, optionally substituted cyanoalkyl, or optionally substituted oxyacylalkyl. In some embodiments, the optional substituent is selected from methyl, ethyl, n-propyl, i-propyl, oxyethyl, methoxyethyl, cyanomethyl, oxyacetylmethyl, or ethoxyacetylmethyl. In some embodiments, R₁ is selected from



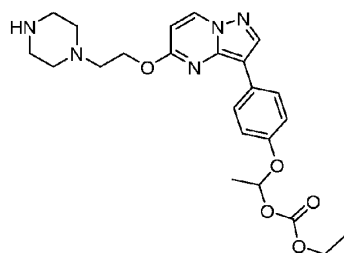
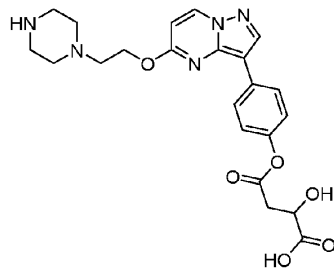
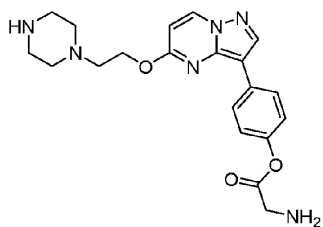
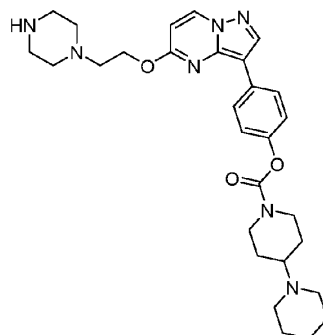
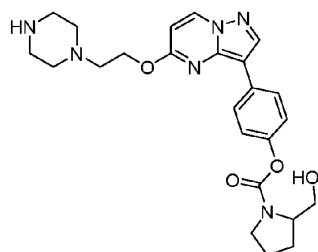
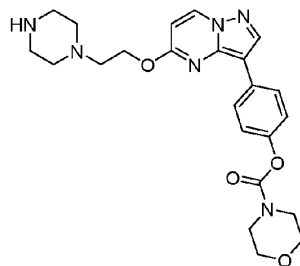
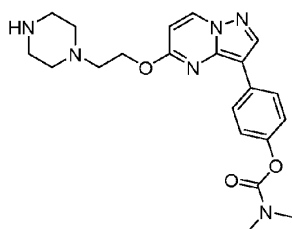
wherein denotes a bond to the Markush structure at R₁; and alkyl is C₁-C₆ alkyl.

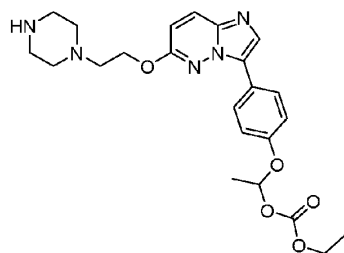
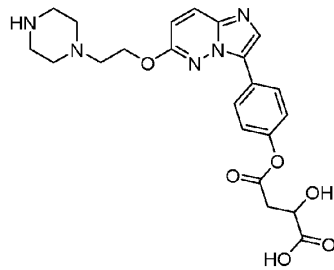
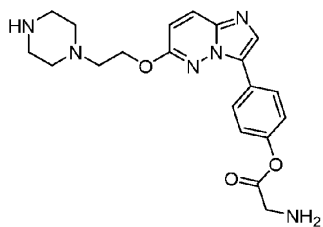
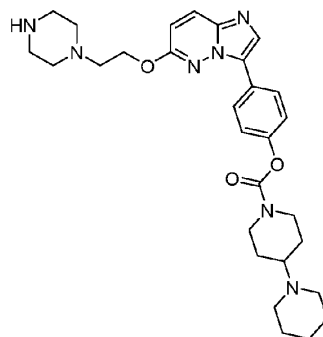
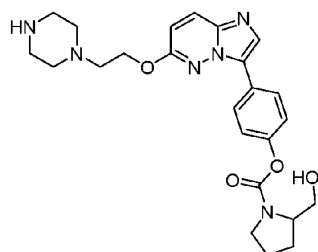
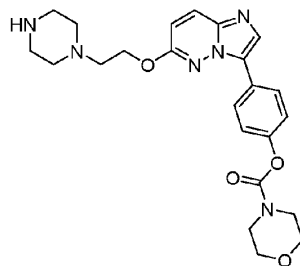
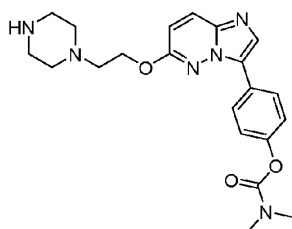
- 10 It was found that modifying R₁ to the above mentioned moieties may help with the solubility and permeability of the compounds in a subject, thereby improving the pharmacokinetic profile of the compounds. The modifications add negligibly to the cost of synthesising the compounds, and the moieties are readily cleaved in systemic circulation or in the desired tissue or cellular compartment to provide the -OH analog.
- 15 In some cases, the cleavage is rapid and complete. In other cases, prolonged, slow cleavage allows for a controlled release mechanism. Moreover, the modification may be tailored to accumulate and /or cleave specifically in cancerous tissue leading to superior efficacy. There is also no additional side effect or toxicity burden.
- 20 In some embodiments, R₂ is H, or optionally substituted C₁-C₆ alkyl. In some embodiments, R₂ is H. In some embodiments, R₂ is methyl, ethyl or propyl.

In some embodiments, the compound of Formula (Ia) is selected from

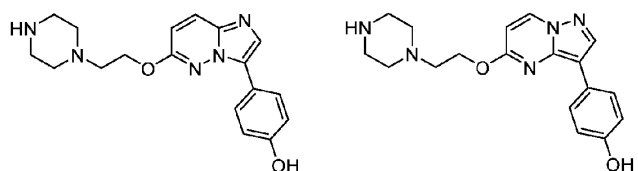


In some embodiments, the compound of Formula (Ia) is selected from

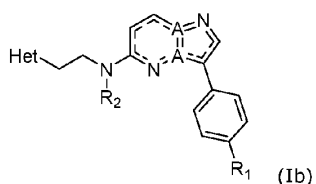




In some embodiments, the compound of Formula (Ia) is selected from



The present disclosure provides a method of treating acute myeloid leukemia in a
 5 subject in need thereof, comprising administering a therapeutically effective amount of
 a compound of Formula (Ib) or a pharmaceutically acceptable salt, solvate or prodrug
 thereof to the subject:

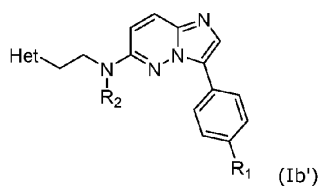


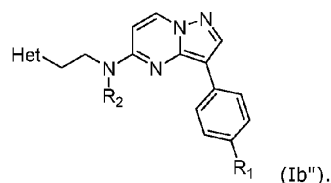
wherein

- 10 A is independently C or N, and at least one A is N;
 Het is optionally substituted 6-membered heterocyclyl;
 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted
 acyloxy, or optionally substituted aminoacyloxy; and
 R₂ is H or optionally substituted alkyl.

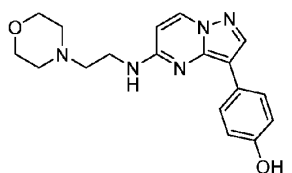
15

In some embodiments, compound of Formula (Ib) is represented by Formula (Ib') or
 (Ib''):





In some embodiments, the compound of Formula (Ia) is selected from

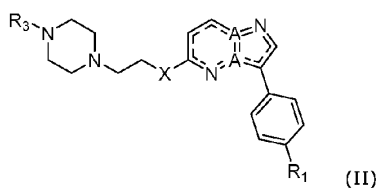


5

The present disclosure also provides a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof for use in treating acute myeloid leukemia in a subject in need thereof.

- 10 The present disclosure also provides a use of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating acute myeloid leukemia.

15 The present disclosure also provides a compound of Formula (II) or a salt, solvate or prodrug thereof:



wherein

A is independently C or N;

at least one A is N;

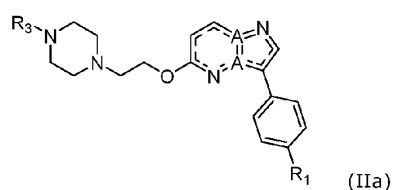
- 20 X is O or NR₂;

R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy;

R₂ is H or optionally substituted alkyl; and

R₃ is H, optionally substituted alkyl or optionally substituted acyl.

The present disclosure also provides a compound of Formula (IIa) or a salt, solvate or
5 prodrug thereof:



wherein

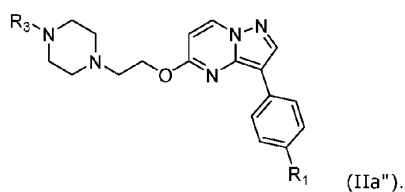
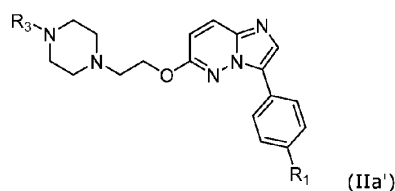
A is independently C or N;

at least one A is N;

10 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy;

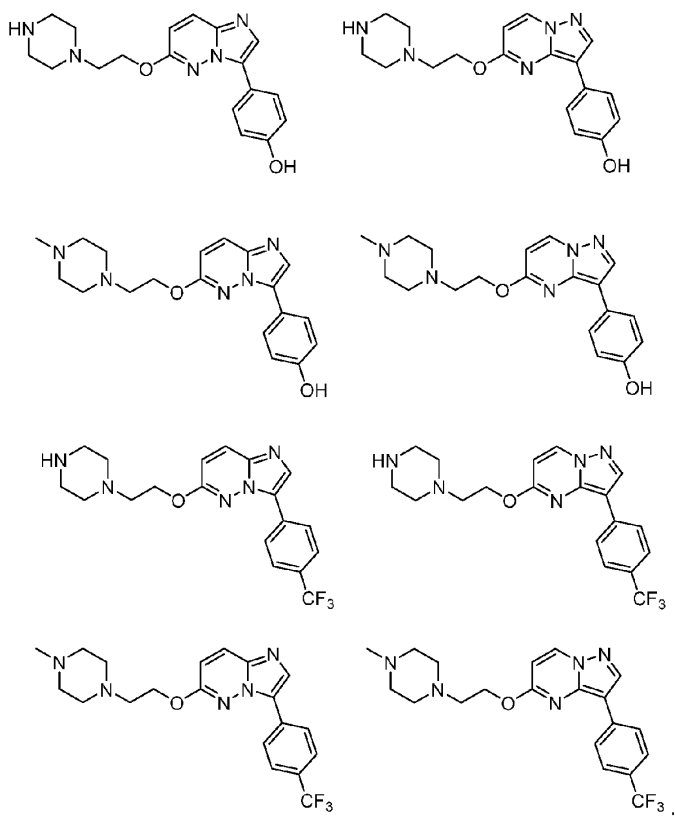
R₃ is H, optionally substituted alkyl or optionally substituted acyl.

In some embodiments, compound of Formula (IIa) is represented by Formula (IIa') or
15 (IIa''):

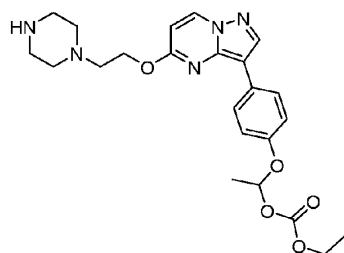
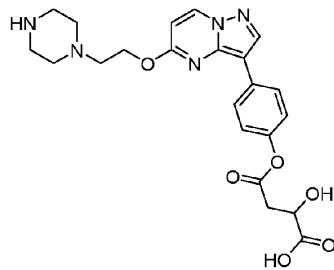
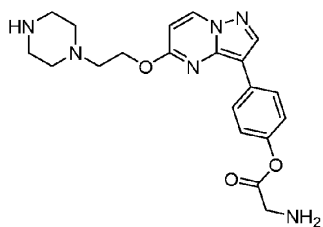
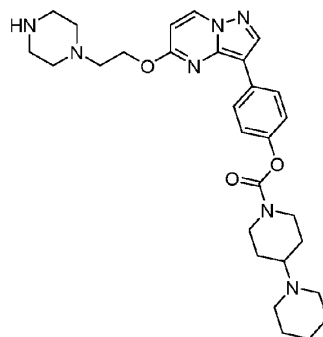
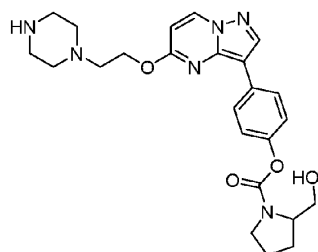
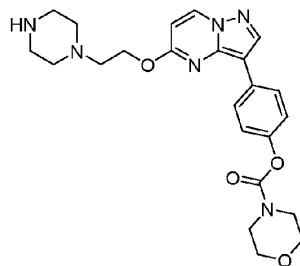
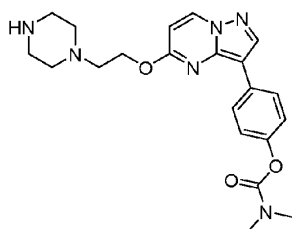


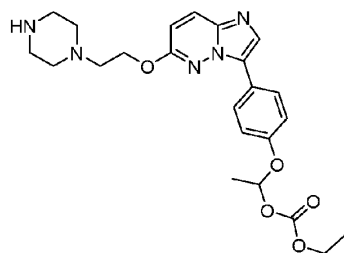
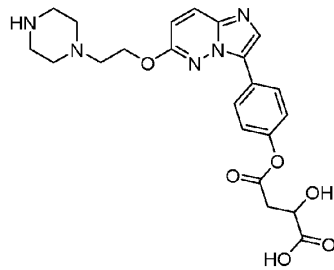
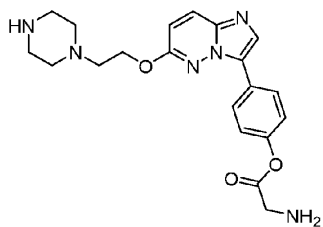
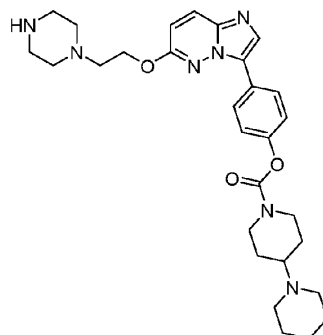
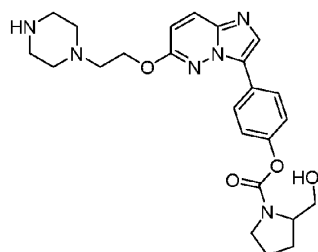
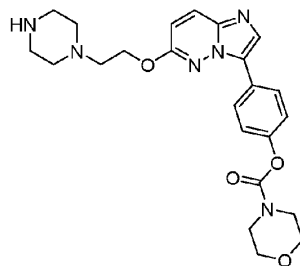
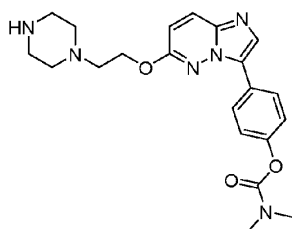
20 In some embodiments, R₃ is H, optionally substituted C₁-C₆ alkyl, -C(O)H or optionally substituted C₁-C₆ acyl. In some embodiments, R₃ is H. In some embodiments, R₃ is methyl, ethyl or propyl.

In some embodiments, the compound of Formula (IIa) is selected from

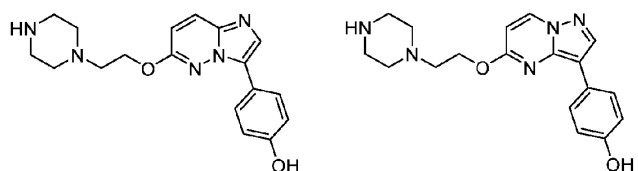


In some embodiments, the compound of Formula (IIa) is selected from

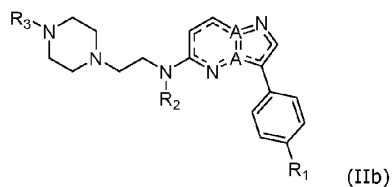




In some embodiments, the compound of Formula (IIa) is selected from



The present disclosure also provides a compound of Formula (IIb) or a salt, solvate or
5 prodrug thereof:



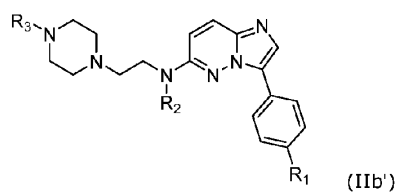
wherein

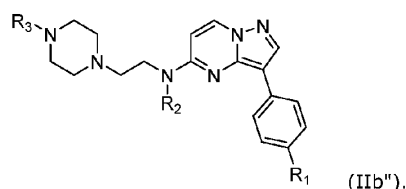
A is independently C or N;

at least one A is N;

- 10 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy;
R₂ is H or optionally substituted alkyl; and
R₃ is H, optionally substituted alkyl or optionally substituted acyl.

- 15 In some embodiments, compound of Formula (IIb) is represented by Formula (IIb') or (IIb''):





The present disclosure also provides a pharmaceutical composition comprising a compound of Formula (I) or (II), or a pharmaceutically acceptable salt, solvate or
 5 prodrug thereof. The pharmaceutical composition may further comprise a pharmaceutically acceptable excipient.

The compound of the invention can be administered to a subject as a pharmaceutically acceptable salt thereof. Suitable pharmaceutically acceptable salts include, but are not
 10 limited to salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic,
 15 ascorbic and valeric acids.

Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and
 20 alkylammonium. In particular, the present invention includes within its scope cationic salts eg sodium or potassium salts, or alkyl esters (eg methyl, ethyl) of the phosphate group.

Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl
 25 halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

It will be appreciated that any compound that is a prodrug of the compound of formula (I) or (II) is also within the scope and spirit of the invention. Thus the compound of the
 30 invention can be administered to a subject in the form of a pharmaceutically acceptable pro-drug. The term "pro-drug" is used in its broadest sense and encompasses those

derivatives that are converted *in vivo* to the compound of the invention. Such derivatives would readily occur to those skilled in the art. Other texts which generally describe prodrugs (and the preparation thereof) include: *Design of Prodrugs*, 1985, H. Bundgaard (Elsevier); *The Practice of Medicinal Chemistry*, 1996, Camille G. Wermuth *et al.*, Chapter 31 (Academic Press); and *A Textbook of Drug Design and Development*, 1991, Bundgaard *et al.*, Chapter 5, (Harwood Academic Publishers). For example, the hydroxyl moiety on the phenyl may be protected as methoxymethyl ether, tetrahydropyranyl ether, t-butyl ether, benzyl ether, acetate, pivalic acid ester, or benzoic acid ester. The amino moiety on the heterocyclyl may be protected as acetamide, t-butyl carbamate, trifluoroacetamide, 9-fluorenylmethyl carbamate, or benzylamine.

The compound of the invention may be in crystalline form either as the free compound or as a solvate (e.g. hydrate) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

The compound of the invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof is administered to the patient in a therapeutically effective amount. As used herein, a therapeutically effective amount is intended to include at least partially attaining the desired effect, or delaying the onset of, or inhibiting the progression of, or halting or reversing altogether the onset or progression of macular degeneration.

As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired therapeutic activity. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage may be in the range of 1 mg to 1000 mg per kg of body weight per dosage. In another embodiment, the dosage may be in the range of 1 mg to 800 mg per kg of body weight per dosage. In yet another embodiment, the dosage may be in the range of 1 mg to 500 mg per kg of body weight per dosage, such as up to 250 mg per body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician and may depend on the severity of the condition as well as the general age, health and weight of the patient to be treated.

5 The compound of the invention may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition. The formulation of such compositions is well known to those skilled in the art. The composition may contain any suitable carriers, diluents or excipients. These include all
10 conventional solvents, dispersion media, fillers, solid carriers, coatings, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

15 The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the patient. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or
20 more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Injectables for such use can be prepared in conventional forms, either as a liquid
25 solution or suspension or in a solid form suitable for preparation as a solution or suspension in a liquid prior to injection, or as an emulsion. Carriers can include, for example, water, saline (e.g., normal saline (NS), phosphate-buffered saline (PBS), balanced saline solution (BSS)), sodium lactate Ringer's solution, dextrose, glycerol, ethanol, and the like; and if desired, minor amounts of auxiliary substances, such as
30 wetting or emulsifying agents, buffers, and the like can be added. Proper fluidity can be maintained, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants. By way of example, the compound, composition or combination can be dissolved in a pharmaceutically effective carrier and be injected into the vitreous of the eye with a fine
35 gauge hollow bore needle (e.g., 30 gauge, 1/2 or 3/8 inch needle) using a temporal

approach (e.g., about 3 to about 4 mm posterior to the limbus for human eye to avoid damaging the lens).

5 The compound or composition of the invention may also be suitable for intravenous administration. For example, a compound of formula (I), (II) or a pharmaceutically acceptable salt, solvate or prodrug thereof may be administered intravenously at a dose of up to 100 mg/m².

10 The compound or composition of the invention may also be suitable for oral administration and may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. In another embodiment, the
15 compound of formula (I), (II) or a pharmaceutically acceptable salt, solvate or prodrug is orally administerable.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a
20 suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent, preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound
25 moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

30 The compound or composition of the invention may be suitable for topical administration in the mouth including lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerin, or sucrose and acacia gum; and
35 mouthwashes comprising the active ingredient in a suitable liquid carrier.

The compound or composition of the invention may be suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like.

5 Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal patches may also be used to administer the compounds of the invention.

10 The compound or composition of the invention may be suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the compound, composition or combination isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending
15 agents and thickening agents. The compound, composition or combination may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from
20 sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage composition or combinations are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

25 It should be understood that in addition to the active ingredients particularly mentioned above, the composition or combination of this invention may include other agents conventional in the art having regard to the type of composition or combination in question, for example, those suitable for oral administration may include such further
30 agents as binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include cornstarch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of
35 wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include

polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Examples

General protocol for compound identification and activity testing

- 10 1. Hit molecules against Flt3-ITD were identified using a yeast-based screening campaign
2. The hit molecules were tested for their efficacy in human AML cells expressing Flt-ITD mutant
3. Subsequently, the 'top performers' were subjected to a reiterative structural simulation exercise to predict chemical modifications to the hit-molecule-structure that would improve their target binding efficacy and selectivity
- 15 4. The predicted chemical modifications were introduced by chemical synthesis and the resulting derivatives were retested in the human AML cell line for their efficacy
5. The top performing candidates were further tested for their target-selectivity by determining their binding-profile against 340 kinases in human proteome. The most highly selective derivatives were chosen for further development.
- 20 6. Dissociation constant (Kd) of the best performing candidates was determined for various Flt3 mutants.
7. in vitro ADME properties and in vivo PK properties were determined for the top candidates
- 25 8. The best performing candidates were selected for mouse xenograft studies

Figure 1 shows the results from a humanized yeast-based drug discovery platform used to identify inhibitors against the Flt3-ITD mutation. Yeast strains were engineered such that expression of human FLT-3-ITD mutant cause loss of viability. Genetically, all five clones were the same i.e. they all had FLT3-ITD allele integrated at the TRP1 locus. These clones exhibited small variation in the extent of Flt3-ITD expression. This phenotypical variability is very often seen in biological systems that are genetically identical. The clone that exhibited maximum Flt3-ITD expression was selected for further development.

A chemical compound library (80K compounds) was screened against the Flt3-ITD strain to identify hit molecules that allow Flt3-ITD expressing cells to grow by inhibiting Ftl-3-ITD mutant protein. Table 1 shows examples of the initial hit compounds.

5

Table 1. Initial hit compounds

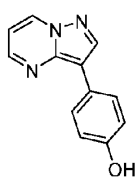
Compound	% increase in growth relative to control	SD (+/-)
F1	51.3	0.032
F2	64.4	0.085
F3	48.9	0.086

The initial hit compounds are all structurally different. The hit molecules exhibited EC50 in nM range when tested for their efficacy against patient-derived SEM (WT Flt3 expressing) and MV4-11 cell lines (Flt3-ITD expressing) (Figure 2). F3 was selected as it is the smallest molecule, possessed the most structures for functionality based on present understanding, and provides the most "space" for modifications. Hence a larger library of compounds may be generated and there may be better conformity with of predicted results compared to actual results.

15

The selected hit molecules were put through reiterative cycle of structural modelling and dynamic simulations to predict chemical modifications that would enhance selectivity and efficacy of the selected hits (Figure 3).

20 Based on these studies, initial hit compound F3 (also labelled as Compound 1) was selected for further studies.

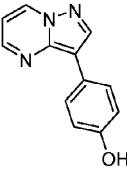
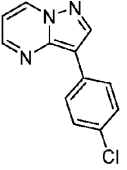
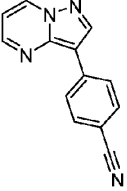
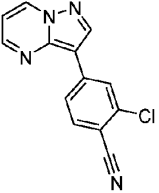


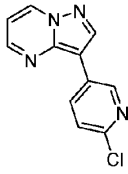
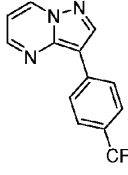
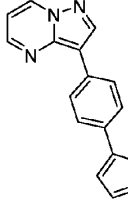
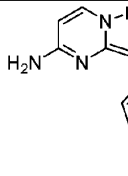
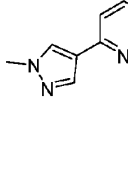
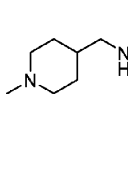
(Compound 1)

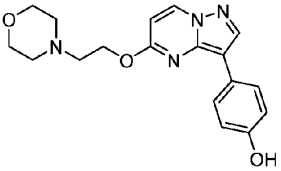
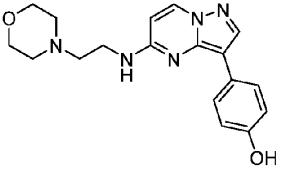
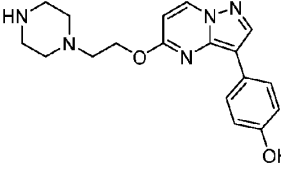
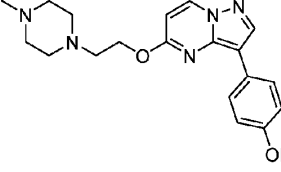
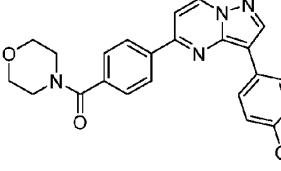
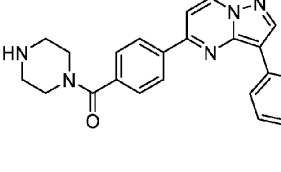
Table 2 shows in vitro enzyme inhibition profile and cell viability of the derivatives.

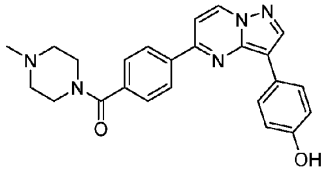
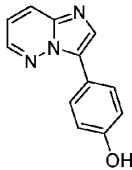
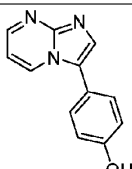
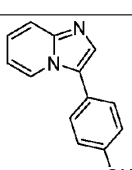
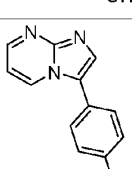
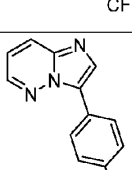
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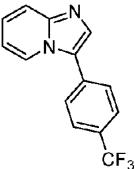
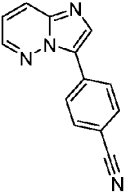
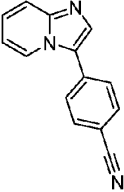
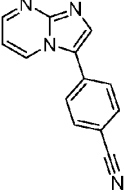
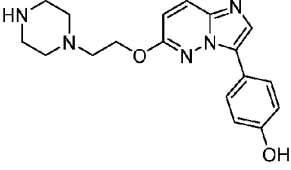
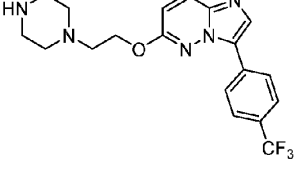
Table 2. in vitro enzyme inhibition profile and cell viability (+++: < 250nM; ++: 251nM – 1000nM ; +: > 1000nM)

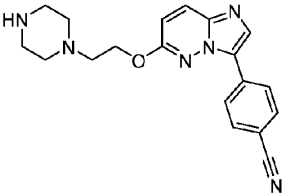
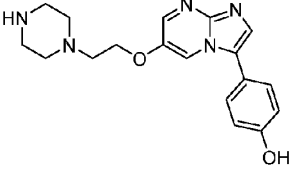
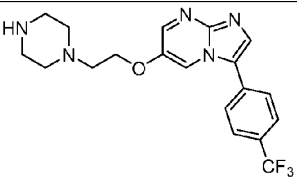
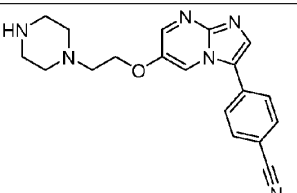
Compound	Structure	In-vitro enzyme inhibition IC ₅₀ (μM)			Cell viability IC ₅₀ (μM)
		Flt3 ^{WT}	Flt3 ^{ITD}	Flt3 ^{D635Y}	MV411
1		+	+	+	++
2		+	+	+	+
3		+	+	+	+
4		+	+	+	+

5		+	+	+	+
6		+++	+++	+++	+++
7		+	+	+	+
8		++	++	++	+
9		+++	+++	+++	++
10		> 5	> 5	> 5	>10

11		++	+	++	+
12		+++	+	+++	+++
13		+++	+++	+++	+++
14		++	++	+	++
15		++	++	+++	++
16		++	++	++	++

17		++	++	+	++
18		++	++	++	-
19		+	+	+	-
20		+	+	+	-
21		+	+	+	-
22		+	+	+	-

23		+	+	+	-
24		+	+	+	-
25		+	+	+	-
26		+	+	+	-
27		++	+++	+++	-
28		+	+	+	-

29		+	+	++	-
30		+	+	+	-
31		+	+	+	-
32		+	+	+	-

Three derivatives (Compounds 9, 13 and 27) were selected for further testing (Figure 4).

- 5 Table 3 shows *in vitro* ADME profile parameters for Compounds 13 and 27.

Table 3 (+++: < 250nM; ++: 251nM – 1000nM ; +: > 1000nM)

	Compound 13	Compound 27	
Flt3 (ITD) IC ₅₀ <200 nM	+++	+++	Y
Cell activity ≤ 500 nM	+++	+++	Y
Kinase selectivity against 320 WT kinases (1μM)	0.03	0.03	Y

Stab (H/MLM), $t_{1/2}$ > 30 min	>60/90	>60/>60	Y
Cyp (six isoforms)	< 50%	<50%	Y
MW < 500	339.4	339.4	Y
Solubility (ph 7.4) ($\mu\text{g/mL}$)	312.4	1193.1	Y
Lipophilicity (logD < 4)	0.5	0.4	Y
Protein binding	72%	76%	Y
Permeability determined (PAMPA)	0.15 (cm/sec)	0.03 (cm/sec)	U

Y: met, no issues; U: met, potential issues; N: not met

Selectivity profile was determined for Compounds 13 and 27 against 340 kinases in the human proteome (Figure 5).

5

Table 4 shows dissociation constant (Kd) for Compounds 13 and 27.

Table 4 (+++: < 500nM; ++: 501nM – 1000nM ; +: > 1000nM)

Target	Compound 13	Compound 27
Gene Symbol	Kd (nM)	Kd (nM)
FLT3	+++	+++
FLT3(D835H)	+++	+++
FLT3(D835V)	+++	+++
FLT3(D835Y)	+++	+++
FLT3(ITD)	+++	+++
FLT3(ITD,D835V)	+++	+++
FLT3(ITD,F691L)	+++	+++
FLT3(N841I)	+++	+++
FLT3(R834Q)	+++	+++
FLT3-autoinhibited	+++	+++

Matrix of Kds for Compound 13 and 27

10 FLT3: WT

FLT3(D835H/V/Y): activating mutations

FLT3(ITD): internal tandem duplication

FLT3(F691L): Gatekeeper mutation

FLT3(R834Q): activating mutation

15

Representative Kd profile of Compounds 13 and 27 against various WT and mutant

versions of FLT3 (Figure 6).

Table 5-7 shows the maximum tolerable dose (MTD-single) study for Flt3^{ITD} inhibitor Compound 13. In general, administration of a single dose of Compound 13 at 200, 400 and 800 mg/kg orally to C57BL/6 mice was well tolerated. There were no Compound 13-related effects on body weight, body weight gain, feed consumption and gross pathology up to 800 mg/kg.

Table 5. Mortality and clinical signs – Compound 13

No. of Animals/ Mortality/ Clinical Signs	Day 1 (Hours)					Treatment Day		
	0.5	1	2	4	6	2	3	4
Group: G1						Dose: 0 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G2						Dose: 200 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G3						Dose: 400 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G4						Dose: 800 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3

Table 6. Body weight (g) – Compound 13

Mean/SD/N	Treatment Day			
	1	2	3	4
Group: G1				
	Dose: 0 mg/kg			
Mean	22.43	21.50	21.13	22.43
SD	1.78	1.67	1.52	0.90
N	3	3	3	3
Group: G2				
	Dose: 200 mg/kg			
Mean	22.07	21.07	21.63	22.43
SD	1.18	1.10	0.99	1.44
N	3	3	3	3
Group: G3				
	Dose: 400 mg/kg			
Mean	22.40	20.57	21.07	20.77
SD	2.04	1.21	1.50	2.10
N	3	3	3	3
Group: G4				
	Dose: 800 mg/kg			
Mean	23.00	21.73	22.10	22.67
SD	1.73	1.53	1.47	1.46
N	3	3	3	3

Table 7. Gross pathology findings – Compound 13

Group	G1	G2	G3	G4
Dose (mg/kg)	0	200	400	800
Number of Animals Examined	3	3	3	3
Mode of Death				
Terminal Sacrificed	3	3	3	3
External Abnormalities				
No Abnormality Detected	3	3	3	3
Internal Abnormalities				
No Abnormality Detected	3	3	3	3

Table 8-10 shows the maximum tolerable dose (MTD-single) study for Flt3^{ITD} inhibitor Compound 27. In general, administration of a single dose of Compound 27 at 200, 400 and 800 mg/kg orally to C57BL/6 mice was well tolerated. There were no Compound 27-related effects on body weight, body weight gain, feed consumption and gross pathology up to 800 mg/kg.

Table 8. Mortality and clinical signs – Compound 27

No. of Animals/ Mortality/ Clinical Signs	Day 1 (Hours)					Treatment Day		
	0.5	1	2	4	6	2	3	4
Group: G1						Dose: 0 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G2						Dose: 200 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G3						Dose: 400 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3
Group: G4						Dose: 800 mg/kg		
No. of animals	3	3	3	3	3	3	3	3
Mortality	0	0	0	0	0	0	0	0
Normal	3	3	3	3	3	3	3	3

10

Table 9. Body weight (g) – Compound 27

Mean/SD/N	Treatment Day			
	1	2	3	4
Group: G1				
	Dose: 0 mg/kg			
Mean	22.33	21.70	21.50	21.33
SD	1.89	2.82	2.96	0.25
N	3	3	3	3
Group: G2				
	Dose: 200 mg/kg			
Mean	22.67	21.83	22.33	21.60
SD	0.60	0.87	0.83	0.36
N	3	3	3	3
Group: G3				
	Dose: 400 mg/kg			
Mean	22.50	21.53	21.93	22.33
SD	0.44	0.21	0.12	0.59
N	3	3	3	3
Group: G4				
	Dose: 800 mg/kg			
Mean	23.20	21.90	22.13	22.73
SD	0.56	0.30	0.45	0.35
N	3	3	3	3

Table 10. Gross pathology findings – Compound 27

Group	G1	G2	G3	G4
Dose (mg/kg)	0	200	400	800
Number of Animals Examined	3	3	3	3
Mode of Death				
Terminal Sacrificed	3	3	3	3
External Abnormalities				
No Abnormality Detected	3	3	3	3
Internal Abnormalities				
No Abnormality Detected	3	3	3	3

Comparatively, the compounds of the present invention exhibits better selectivity profile and activity against the oncogenic and resistant mutations of Flt3, such as Quizartinib, Midostaurin, Gilteritinib and Crenolanib.

5

Midostaurin, Gilteritinib and Crenolanib binds to 83, 107 and 78 kinases in the human kinome at 1uM concentrations, whereas the compounds of the present invention binds to only 7 kinases at 1uM and 5uM concentration. The high specificity of the compounds of the present invention will have the reduced off-target effect as a result it will be less

10

toxic. Flt3 acquire resistance against Quizartinib through point mutations (D835Y, F691L), whereas the compounds of the present invention inhibits the activity of these activation and resistant mutations.

15

The *in vivo* effects of Flt3 inhibitors Compound 13 and 27 in mouse model are shown in Figure 7 (anti-tumor activity) and Figure 8 (chronic toxicity).

20

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

25

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

30

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel

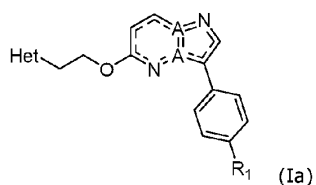
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characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or
5 information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Claims

1. A method of treating acute myeloid leukemia in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof to the subject:



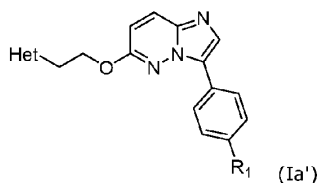
wherein

A is independently C or N, and at least one A is N;

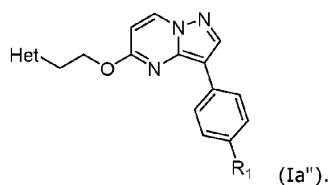
Het is optionally substituted 6-membered heterocycyl; and

- 10 R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy.

2. The method according to claim 1, wherein compound of Formula (Ia) is represented by Formula (Ia') or (Ia''):



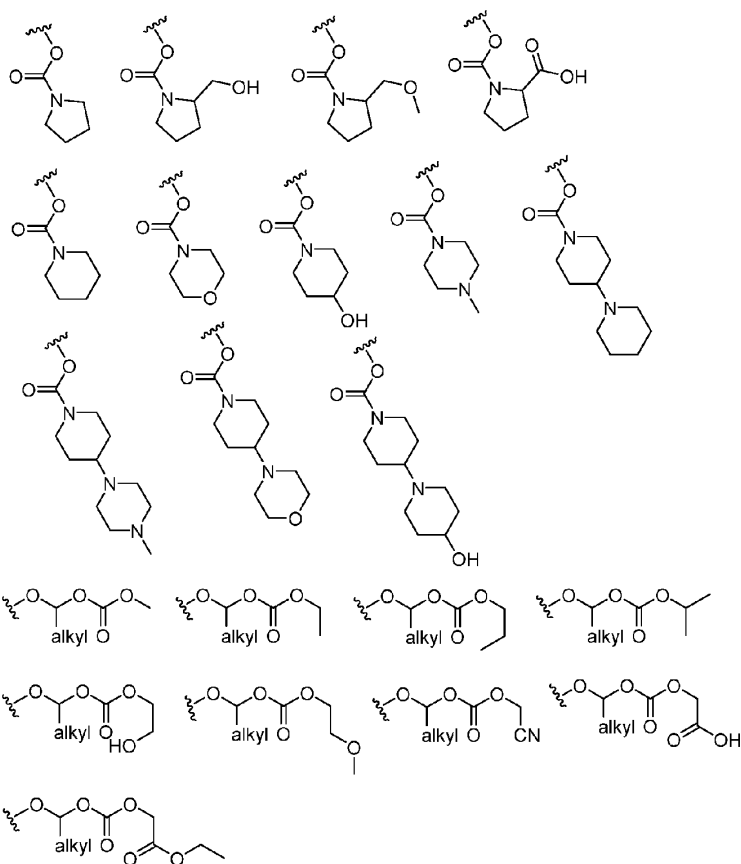
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3. The method according to claim 1 or 2, wherein Het is optionally substituted 6-membered N-heterocycyl.

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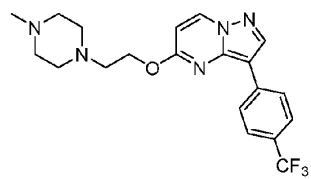
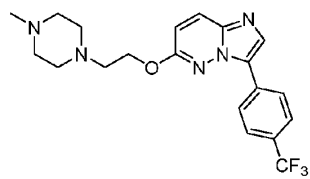
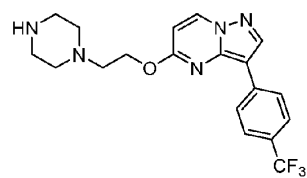
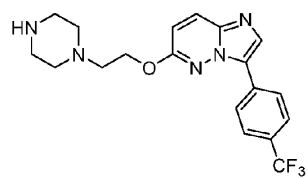
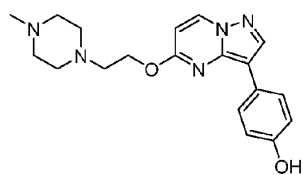
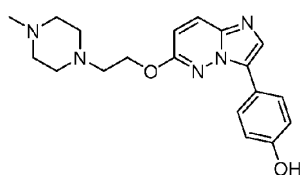
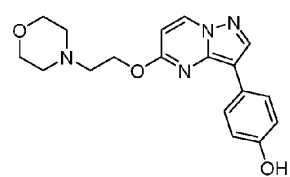
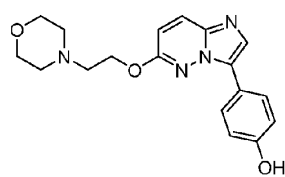
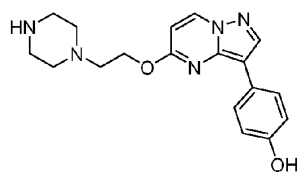
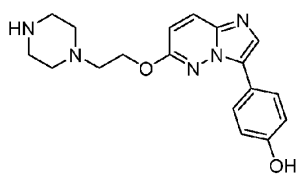
4. The method according to any one of claims 1 to 3, wherein Het is selected from optionally substituted piperidinyl, optionally substituted piperazinyl, and optionally substituted morpholinyl.
5. The method according to any one of claims 1 to 4, wherein the optional substituent on Het is selected from halo, cyano, oxo, and C₁-C₆ alkyl.
6. The method according to any one of claims 1 to 5, wherein R₁ is selected from -

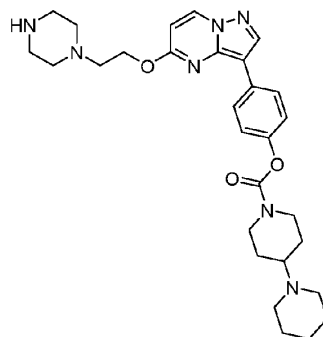
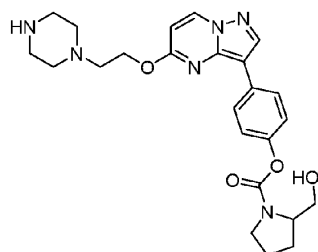
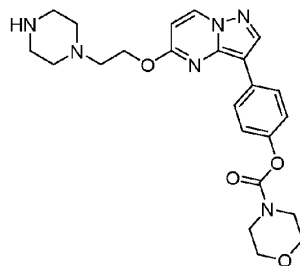
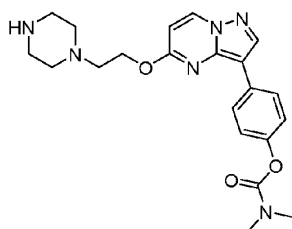


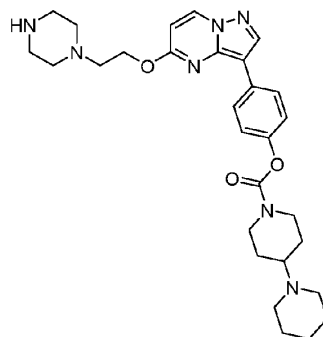
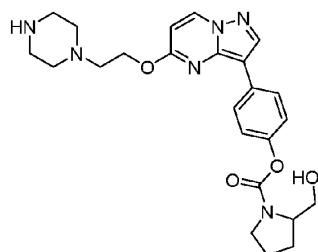
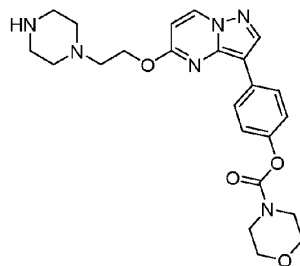
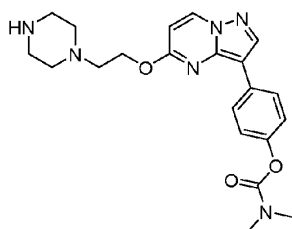
wherein denotes a bond to the Markush structure at R₁; and

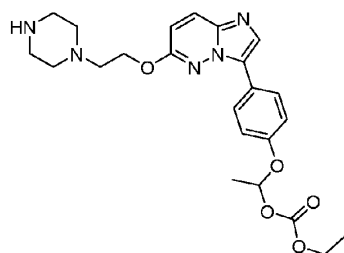
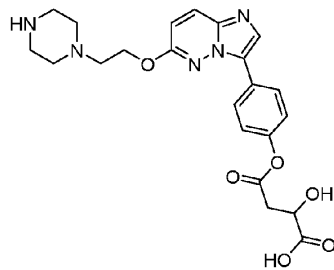
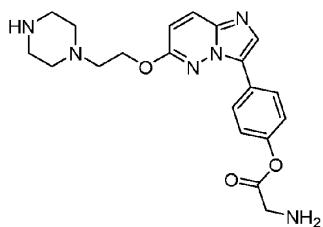
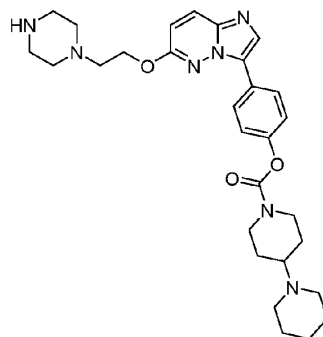
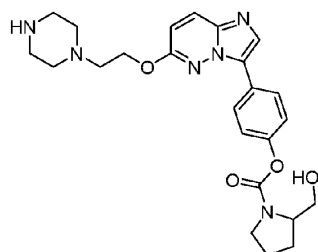
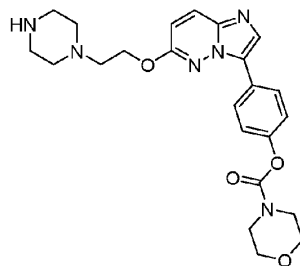
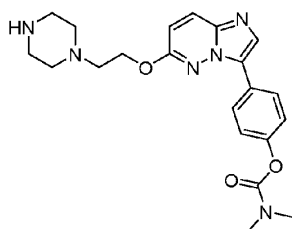
alkyl is C₁-C₆ alkyl.

7. The method according to any one of claims 1 to 6, wherein the compound of Formula (Ia) is selected from

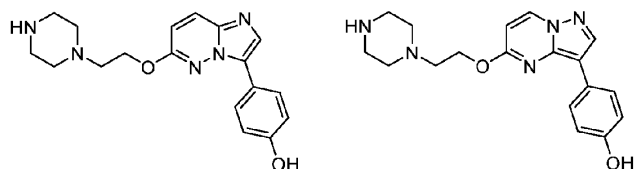








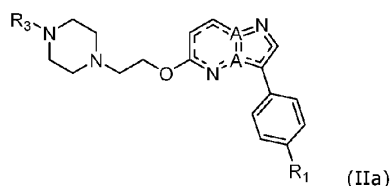
8. The method according to any one of claims 1 to 7, wherein the compound of Formula (Ia) is selected from



9. A compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof for use in treating acute myeloid leukemia in a subject in need thereof.

10. Use of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating acute myeloid leukemia,

11. A compound of Formula (IIa) or a salt, solvate or prodrug thereof:



wherein

15 A is independently C or N;

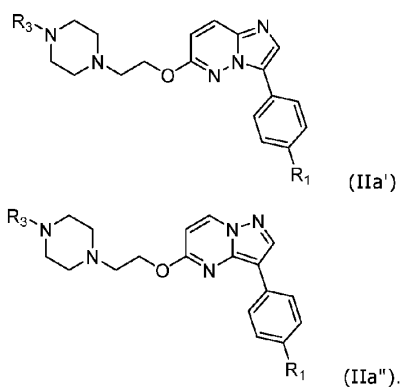
at least one A is N;

R₁ is halo substituted alkyl, oxo, optionally substituted alkoxy, optionally substituted acyloxy, or optionally substituted aminoacyloxy;

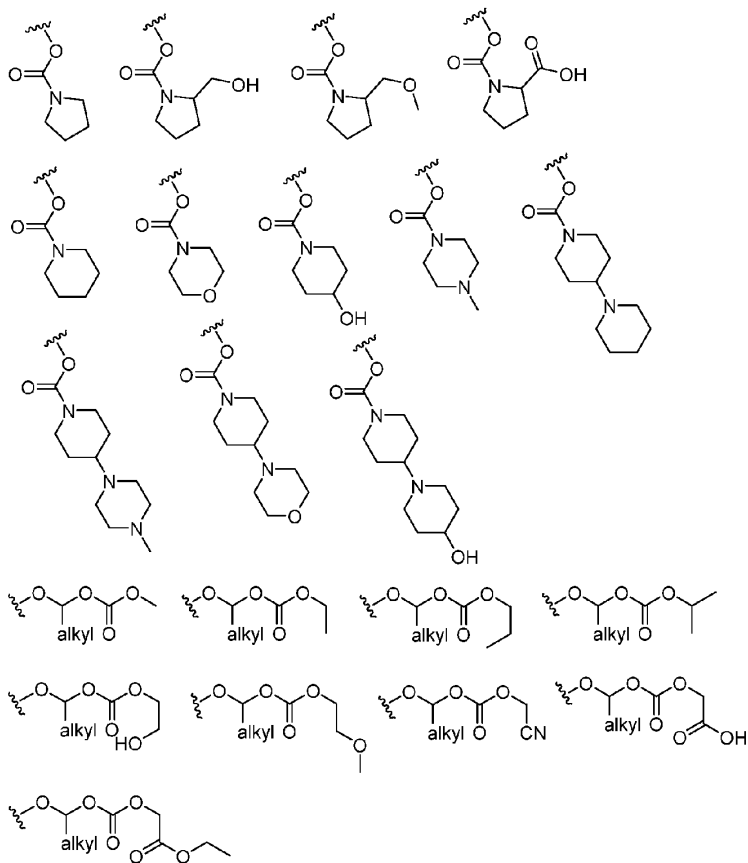
R₃ is H, optionally substituted alkyl or optionally substituted acyl.

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12. The compound according to claim 11, wherein compound of Formula (II) is represented by Formula (IIa') or (IIa''):



13. The compound according to claim 11 or 12, wherein R_1 is selected from -OH,



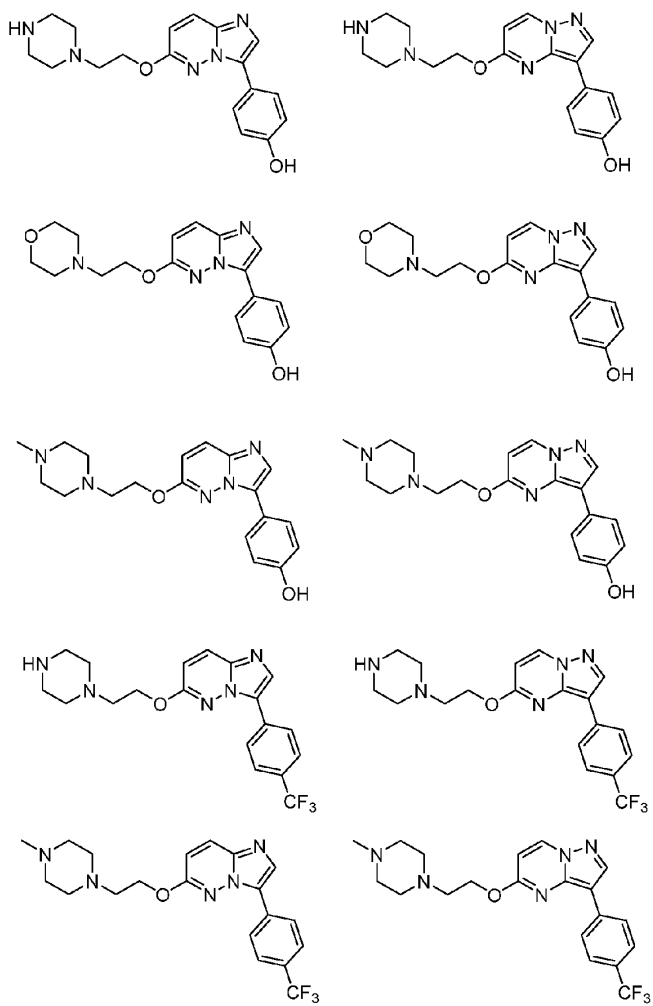
wherein  denotes a bond to the Markush structure at R₁; and
alkyl is C₁-C₆ alkyl.

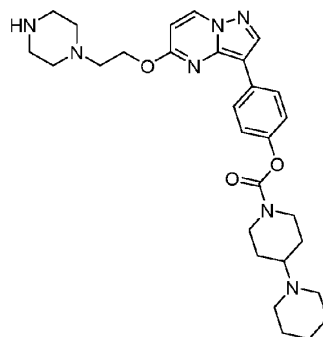
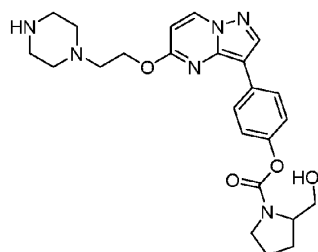
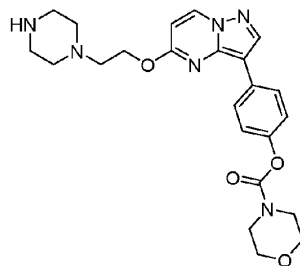
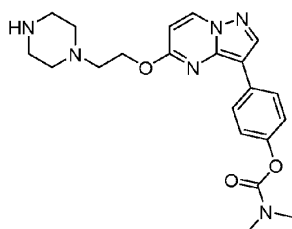
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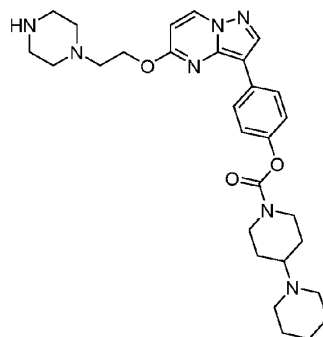
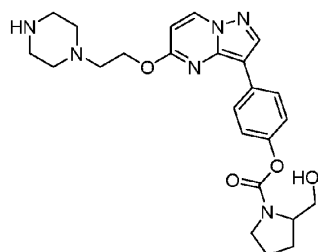
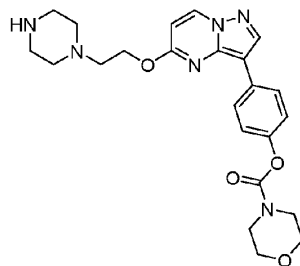
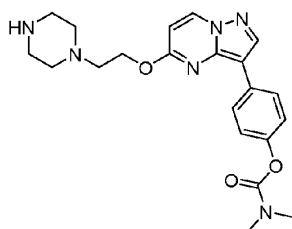
14. The compound according to any one of claims 11 to 13, wherein R₃ is H or C₁-C₆ alkyl.

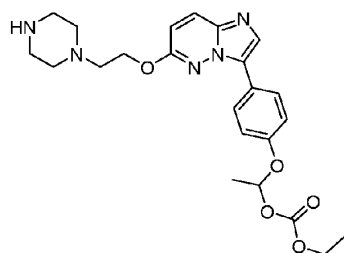
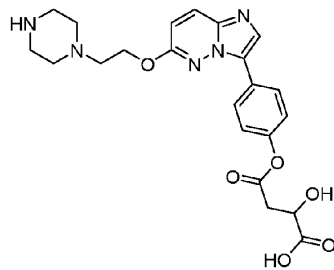
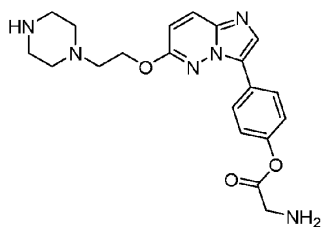
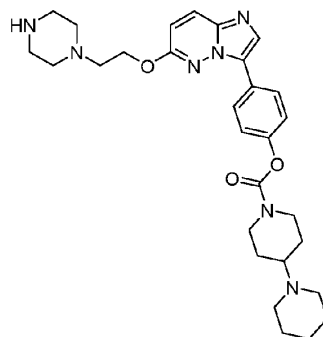
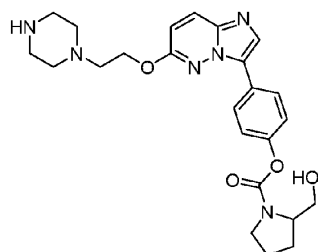
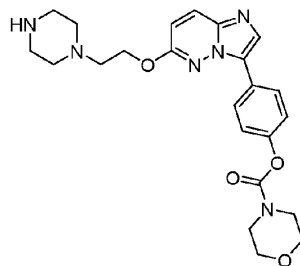
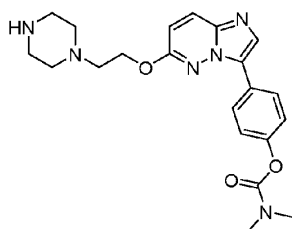
15. The compound according to any one of claims 11 to 14, wherein the compound
of Formula (IIa) is selected from

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16. A pharmaceutical composition comprising a compound of Formula (I) according to any one of claims 1 to 15, or a pharmaceutically acceptable salt, solvate or prodrug thereof.

5