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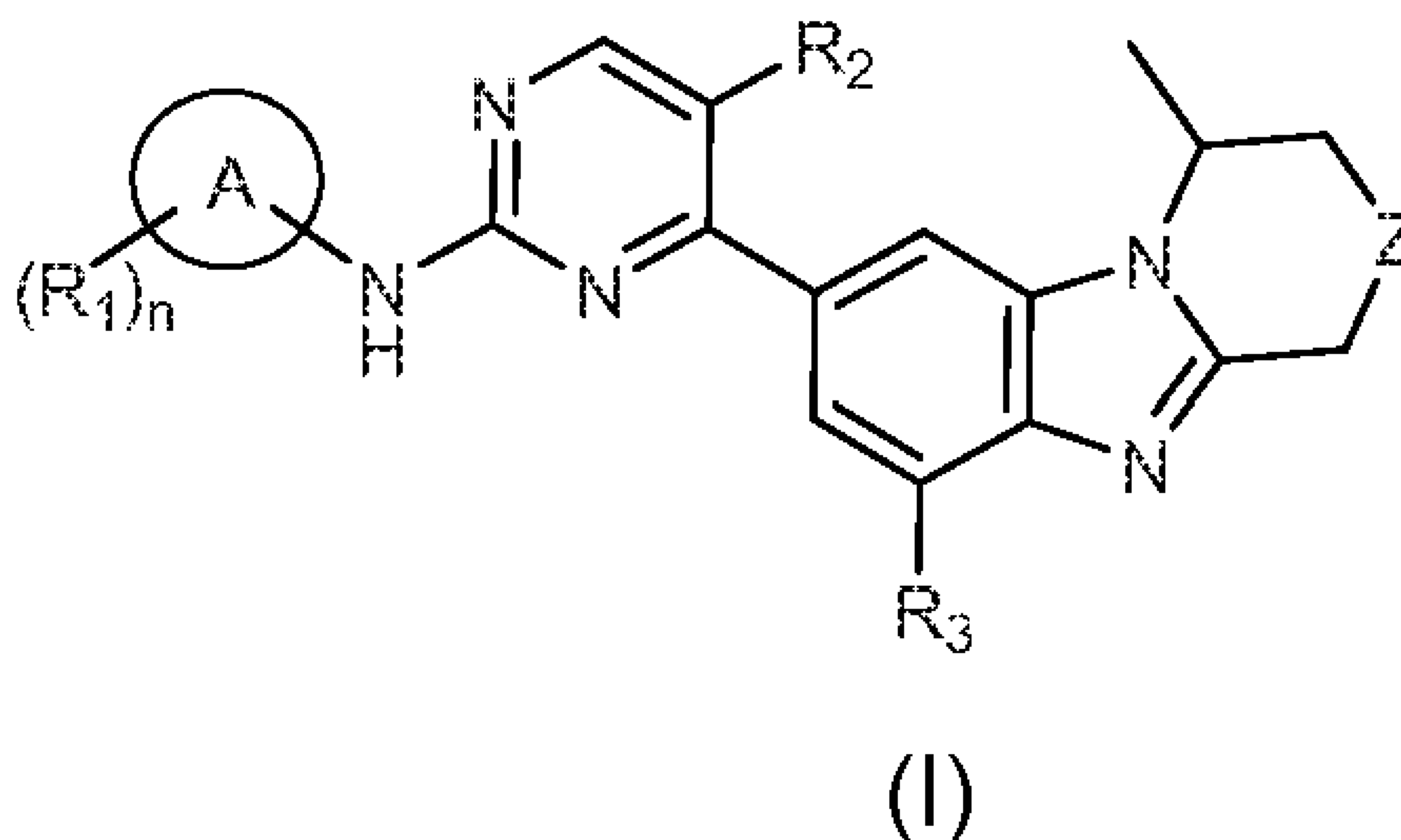
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(54) Titre : DERIVES DE BENZIMIDAZOLE, LEURS PROCEDES DE PREPARATION ET LEURS UTILISATIONS

(54) Title: BENZIMIDAZOLE DERIVATIVES, PREPARATION METHODS AND USES THEREOF



(57) Abrégé/Abstract:

The present invention relates to benzimidazole compounds useful in treating for protein kinase-associated disorders. There is also a need for compounds useful in the treatment or prevention of one or more symptoms of cancer, transplant rejections. Furthermore, there is a need for methods for modulating the activity of protein kinases, such as CDK4 and/or CDK6, using the compounds provided herein.

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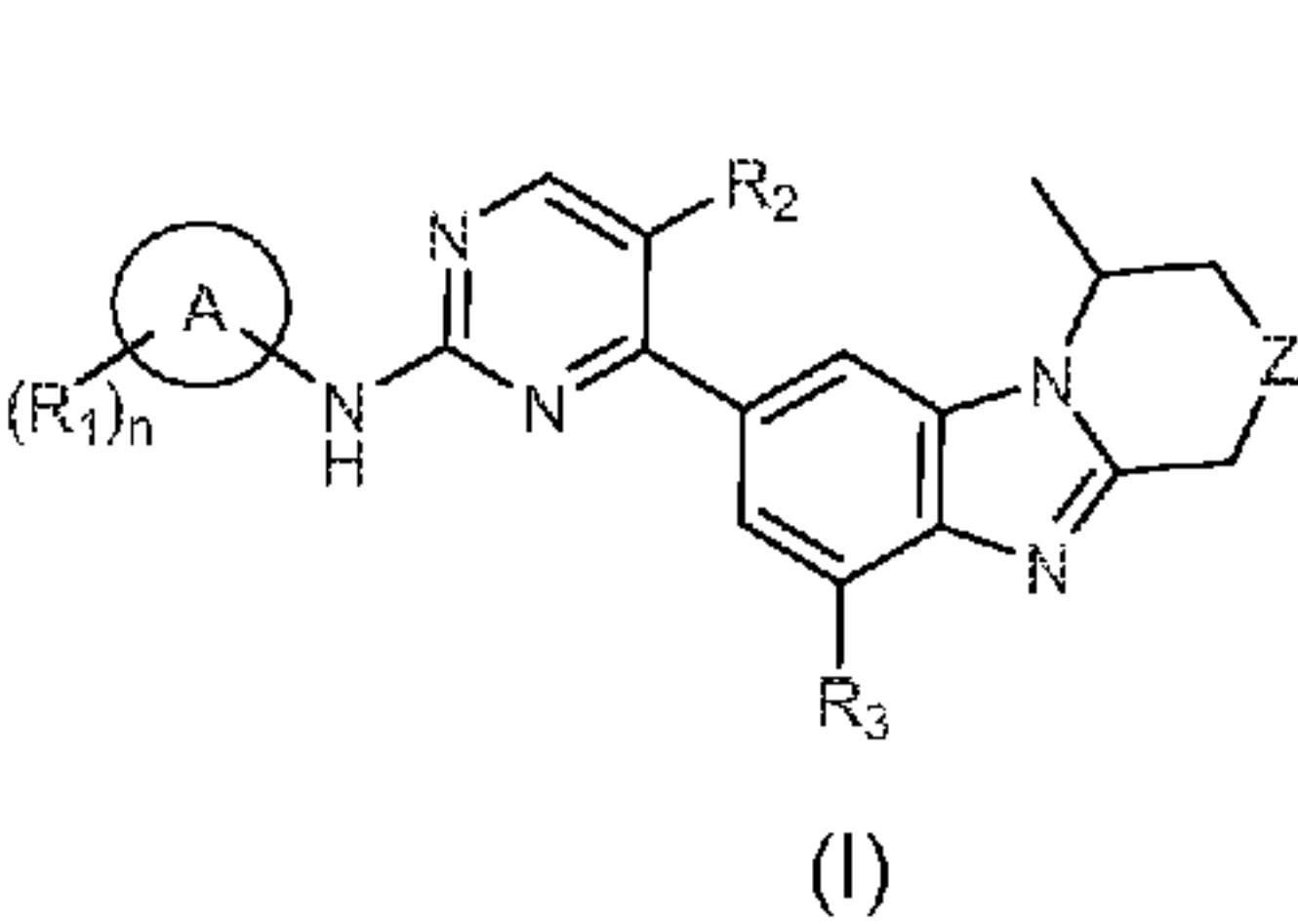
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(54) Title: BENZIMIDAZOLE DERIVATIVES, PREPARATION METHODS AND USES THEIROF



(57) Abstract: The present invention relates to benzimidazole compounds useful in treating for protein kinase-associated disorders. There is also a need for compounds useful in the treatment or prevention of one or more symptoms of cancer, transplant rejections. Furthermore, there is a need for methods for modulating the activity of protein kinases, such as CDK4 and/or CDK6, using the compounds provided herein.

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BENZIMIDAZOLE DERIVATIVES, PREPARATION METHODS AND USES THEREOF

Technical Field

The present invention relates to benzimidazole compounds which are useful for inhibiting
5 cyclin-dependent kinase. More specially, the invention provides compounds and pharmaceutical
compositions thereof which are used as CDK4/6 inhibitors, and methods of treatment for diseases
mediated by CDK4/6, such as cancer.

Background Art

Cyclin-dependent kinases (CDKs) mediate cell cycle progression, regulating transition from G1
10 to S phase and G2 to M phase. CDK activity is tightly controlled throughout the cell cycle by
posttranscriptional modifications as well as the expression of cyclins and CDK inhibitors. There
are four proliferative CDKs: CDK1, which predominantly regulates the transition from G2 to M
phase, and CDK2/4/6, which regulate the transition from G1 to S phase.

Progression through the cell cycle is a highly regulated process. In the absence of appropriate
15 growth signals, a family of pocket proteins including retinoblastoma protein (pRb) prevents cells
from entering the DNA replication phase (S phase). The replication cycle begins when mitogens
trigger signal transduction pathways, leading to increase of cellular levels of D-cyclins. D-cyclins,
in turn, activate cyclin dependent kinases 4/6 (CDK4/6), which phosphorylates and inactivates pRb.

Uncontrolled cell proliferation is one of the hallmarks of cancer, and pRb inactivation is the
20 key event that enables tumor cells to progress through the cell cycle unchecked. While some
tumors delete the pRb gene itself, the majority maintains a functional pRb and instead activates
CDK4/6 kinase activity. Ablation of CDK4/6 kinase activity led to complete tumor growth
inhibition in many cancer types such as HR+ breast cancer, mantle cell lymphoma, glioblastoma and
squamous lung cancer. Furthermore, normal fibroblast cells were shown to overcome the absence
25 of CDK4/6 due to compensation by CDK1, whose absence is not tolerated. Taken together, this
evidence suggests that a selective inhibitor of CDK4/6 may have a wider therapeutic window than
pan-CDK inhibitors.

In addition to direct antineoplastic effects, CDK4/6 inhibitors are found to treat inflammatory diseases, bone diseases, metabolic diseases, neurological and neurodegenerative diseases, cardiovascular diseases, allergies and asthma, Alzheimer's diseases, and hormone related diseases. Accordingly, there has been a substantial effort in medicinal chemistry to find CDK4/6 inhibitors
5 that are effective as therapeutic agents.

CDK1 is a key determinant of mitotic progression and it is the only CDK that can initiate the onset of mitosis. Mouse knockout experiments have shown that CDK1 is required for mammalian cell proliferation.^[1] Since CDK1 is critical to the cell proliferation, toxicity caused by inhibition of CDK1 will limit the ability to achieve therapeutic level, so it is necessary to keep the selectivity of
10 CDK1 again drug target CDK4/6.

CDK2 is structurally and functionally related to CDK1; it has a considerably broader substrate profile than CDK4 and CDK6, and phosphorylates a large number of proteins involved in cell cycle progression (for example, p27KIP1 and RB), DNA replication (for example, replication factors A and C), histone synthesis (for example, NPAT), centrosome duplication (for example,
15 nucleophosmin (NPM)), among other processes. In contrast to CDK4 and CDK6, CDK2 is not regulated by INK4 proteins but by the CDK-interacting protein/kinase inhibitory protein (CIP/KIP) class of CDK inhibitors, which bind to CDK2 cyclin complexes and render them inactive.^[1] When we design a CDK4/6 inhibitor as drug for cancer, it is better to keep the selectivity against CDK2.

In addition to the CDKs that directly promote cell cycle progression (for example, CDK4, CDK6, CDK2 and CDK1), an additional family of CDKs that regulate transcription was identified, which include CDK7, CDK8 and CDK9. CDK7 has a general role in the phosphorylation of the RNA polymerase II carboxyterminal domain that contributes to the initiation of transcription, and CDK9 also phosphorylates RNA polymerase II, thereby promoting elongation of transcription.^[1] The first generation of CDK inhibitors are pan-CDK inhibitors and failed to succeed due to
20 unmanageable toxicities. For example, flavopiridol is the most extensively investigated CDK inhibitor so far. Although flavopiridol can induce cell cycle arrest in G1 and G2 phases, in certain contexts it also induces a cytotoxic response, probably as a result of CDK7 and CDK9 inhibition that leads to suppression of transcription.^[2] So, it is necessary to avoid CDK7/9 when drugs targeting CDK4/6 are designed.

30 So far, a variety of CDK inhibitors have been evaluated preclinically and clinically. Given the evidence described above, many research groups have embarked on the discovery of a CDK4/6 selective inhibitor, with the well-documented being Palbociclib (PD-0332991), Ribociclib (LEE-011)

and Abemaciclib (LY2835219). However, there remains a need to provide more potent, selective and safer CDK4/6 inhibitors which can be used in the treatment of cell proliferative disorders such as cancer.

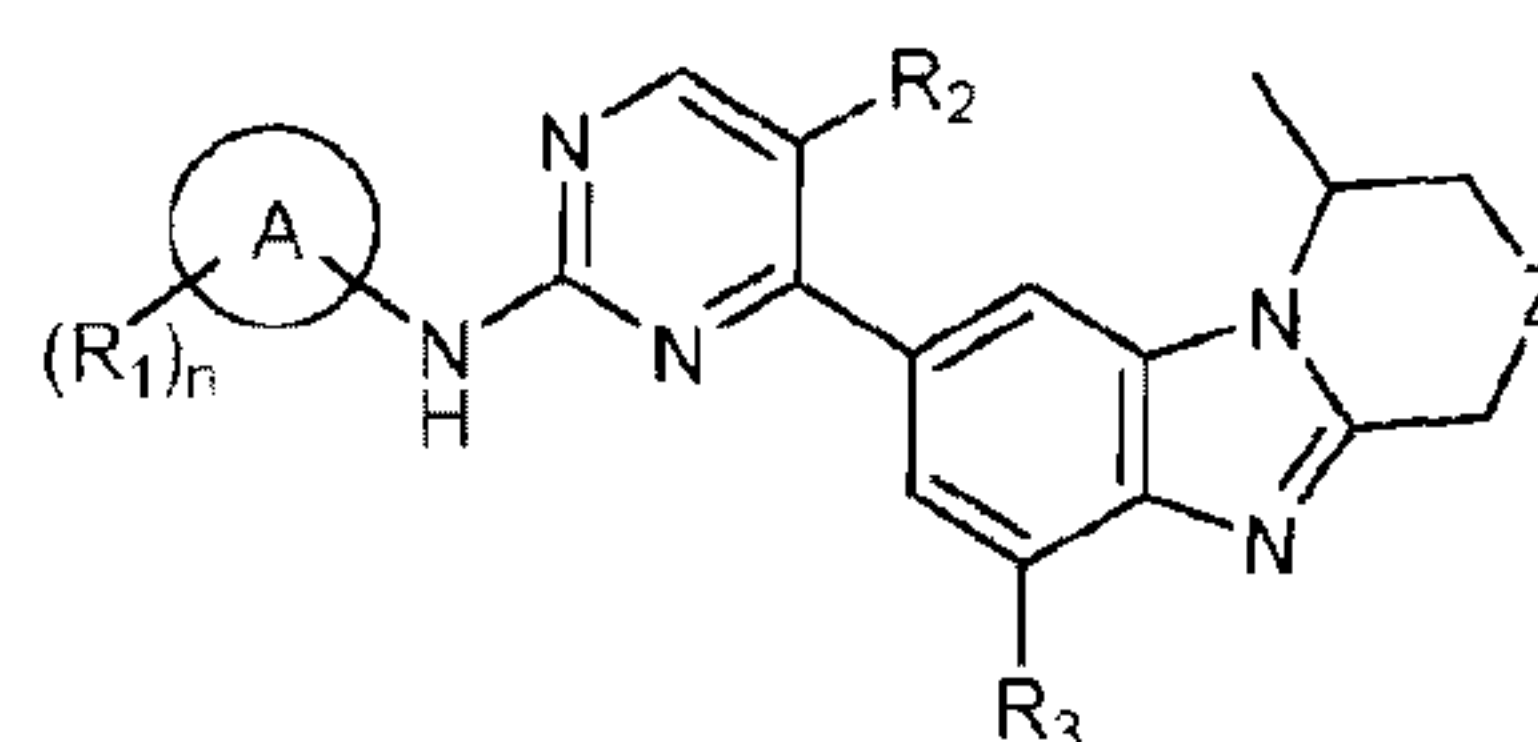
Reference:

5 [1]. Uzma Asghar, Agnieszka K. Witkiewicz, Nicholas C. Turner, Et al. Nat Rev Drug Discov. 2015, 14(2): 130-146.

[2]. Prithviraj Bose, Gary L Simmons, Steven Grant. Expert Opin Investig Drugs. 2013, 22(6): 723-738.

Summary of Invention

10 The present invention relates to benzimidazole compounds that are used as CDK4/6 inhibitors and for the treatment of diseases mediated by CDK4/6. The compounds of the invention have the general structures as Formula I. A compound of Formula I, or a stereoisomer, a tautomer, a polymorph, a solvate, a pharmaceutically acceptable salt, or a prodrug thereof,



Formula I

wherein,

ring A is aryl or heteroaryl;

Z is selected from the group consisting of CH₂, NH, O and S;

15 R₁ is independently selected from the group consisting of hydrogen, halogen, CN, NO₂, OH, NH₂, C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclyl-(CH₂)_m-, aryl-C₁₋₆alkyl-, heteroaryl-C₁₋₆alkyl-, -NR₁₂R₁₃, -NR₁₂-C₁₋₆alkylene-NR₁₂R₁₃, and heterocyclyl-C(O)-, wherein the C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclyl-(CH₂)_m-, aryl-C₁₋₆alkyl-, heteroaryl-C₁₋₆alkyl-, or heterocyclyl-C(O)- are each unsubstituted or substituted with at least one substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, heterocyclyl, 20 -NR₁₂R₁₃, or -(CH₂)_r-OH;

R₂ and R₃ are each independently selected from H, OH, CN, NO₂, NH₂, halogen, C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl; wherein the C₁₋₈alkyl, C₁₋₈alkoxy,

C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl are each unsubstituted or substituted with at least one substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, or heterocyclyl;

R₁₂ and R₁₃ are each independently selected from H, C₁₋₈alkyl, aryl, heteroaryl, heterocyclyl, or C₃₋₈cycloalkyl; wherein the C₁₋₈alkyl, aryl, heteroaryl, heterocyclyl, or C₃₋₈cycloalkyl are each
 5 unsubstituted or substituted with at least one substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, or heterocyclyl;

m is 0, 1, 2, 3 or 4;

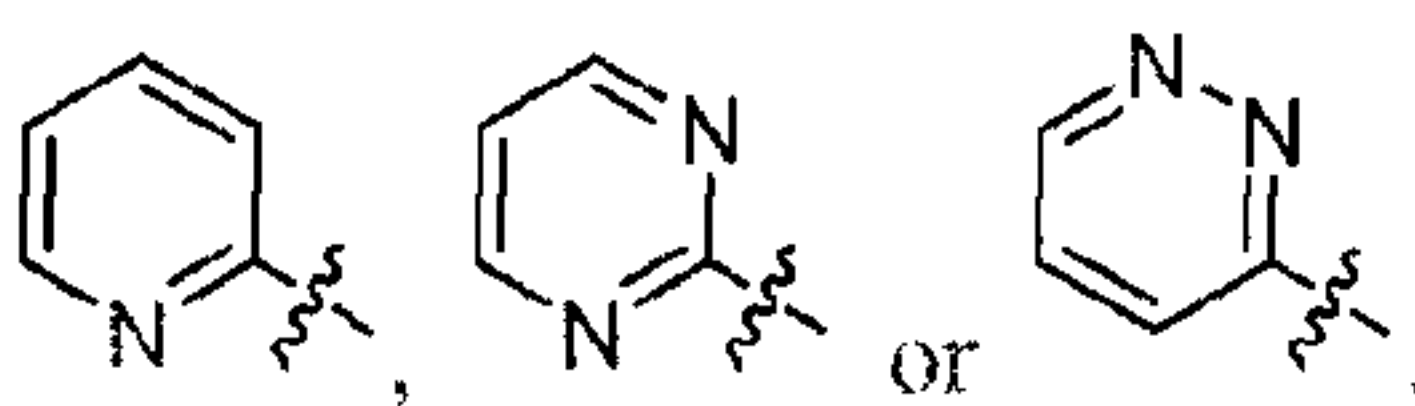
n is 0, 1, 2, 3 or 4;

t is 0, 1, 2, 3 or 4.

10 In some embodiments of Formula I, Z is CH₂.

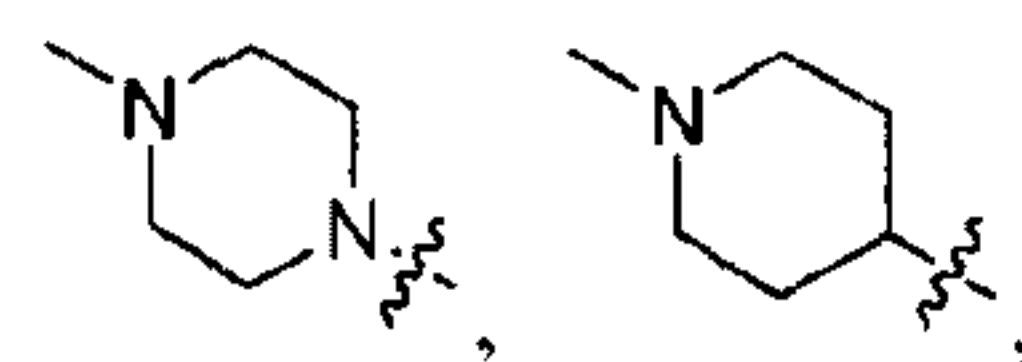
In some embodiments of Formula I, Z is O.

In some embodiments of Formula I, ring A is a 6-membered heteroaryl comprising one or two heteroatoms of N, for example, pyridyl, pyrimidinyl, pyridazinyl and the like.

In other embodiments of Formula I, ring A is .

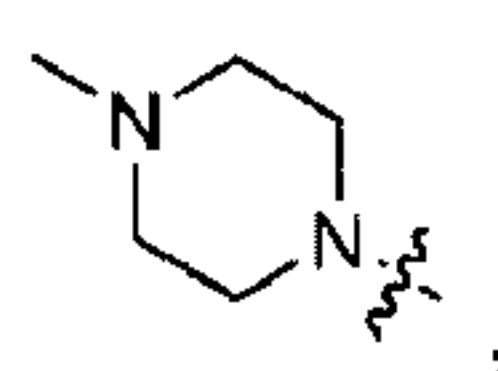
15 In some embodiments of Formula I, R₁ is heterocyclyl-(CH₂)_m-, or heterocyclyl-(CH₂)_m- substituted with C₁₋₈alkyl, NR₁₂R₁₃, 4 to 6-membered heterocyclyl, C₃₋₆cycloalkyl, or -(CH₂)_t-OH.

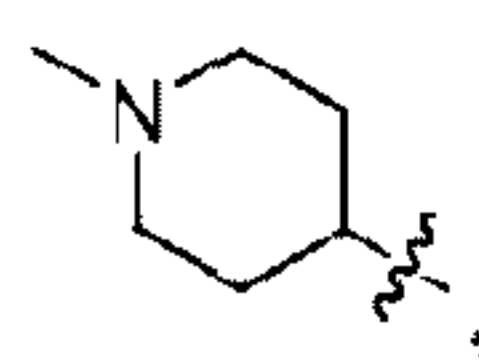
In other embodiments of Formula I, R₁ is 5 to 6-membered heterocyclyl-CH₂-, or 5 to 6-membered heterocyclyl-CH₂- substituted with C₁₋₃alkyl, -N(CH₃)₂, -N(CH₂CH₂OH)CH₃, .

, , -CH₂OH, -CH₂CH₂OH, or -OH.

20 In other embodiments of Formula I, R₁ is 6-membered heterocyclyl-CH₂-, or 6-membered heterocyclyl-CH₂- substituted with methyl or ethyl.

In some embodiments of Formula I, R₁ is heterocyclyl, or heterocyclyl substituted with C₁₋₈alkyl, NR₁₂R₁₃, 4 to 6-heterocyclyl, C₃₋₆cycloalkyl, or -(CH₂)_t-OH.

In other embodiments of Formula I, R₁ is 5 to 6-membered heterocyclyl, or 5 to 6-membered
 25 heterocyclyl substituted with C₁₋₃alkyl, -N(CH₃)₂, -N(CH₂CH₂OH)CH₃, , ,

, , -CH₂OH, -CH₂CH₂OH, or OH.

In other embodiments of Formula I, R₂ and R₃ are both F.

The present invention further provides some preferred technical solutions with regard to compound of Formula I, and the compound is:

- 1) 4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;
- 2) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 3) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;
- 4) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(6-((4-methylpiperazin-1-yl)methyl)pyridin-3-yl)pyrimidin-2-amine;
- 5) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-yl)pyrimidin-2-amine;
- 6) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyrimidin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 7) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 8) N-(5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 9) (2-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyrimidin-5-yl)(4-methylpiperazin-1-yl)methanone;
- 10) (6-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyridin-3-yl)(4-methylpiperazin-1-yl)methanone;
- 11) N5-(2-(diethylamino)ethyl)-N2-(5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)-N5-methylpyridine-2,5-diamine;
- 12) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-(trifluoromethyl)pyrimidin-2-amine;
- 13) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-methylpyrimidin-2-amine;
- 14) 5-chloro-N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;

- 15) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;
- 16) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 5 17) N-(5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 18) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine ;
- 19) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine ;
- 10 20) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-ylmethyl)pyridin-2-yl)pyrimidin-2-amine;
- 21) N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)-6-(4-methylpiperazin-1-yl)pyridazin-3-amine ;
- 15 22) 6-((4-ethylpiperazin-1-yl)methyl)-N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)pyridazin-3-amine;
- 23) 1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)pyrrolidin-3-yl)methanol;
- 24) 1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)pyrrolidin-3-yl)methanol ;
- 20 25) N-(5-(4-cyclopropylpiperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 26) N-(5-((4-cyclopropylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 25 27) 2-((1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)piperidin-4-yl)(methylamino)ethan-1-ol;
- 28) 1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)-3-methylpyrrolidin-3-ol;
- 29) 1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)-3-methylpyrrolidin-3-ol;
- 30

30) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(oxetan-3-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine;

31) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-(oxetan-3-yl)piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;

5 32) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyrazin-7-yl)pyrimidin-2-amine;

33) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4'-methyl-[1,1'-bipiperazin]-4-yl)methyl)pyridin-2-yl)pyrimidin-2-amine;

34) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(1-methylpiperidin-4-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine.

Surprisingly, the highly purified (-) enantiomer of the compound of Formula I is advantageous over the (+) enantiomer in biological activity. For example, the optically pure (-) enantiomer of compound 2 (N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine) is more potent than its (+) enantiomer.

Unless otherwise indicated, "(-)" in the present invention means that the optical rotation is a negative value; and "(+)" means that the optical rotation is a positive value. The present compound described herein can be (-) isomer of the compound and/or (+) isomer of the compound.

The present invention also provides pharmaceutical compositions comprising a therapeutically effective amount of a compound of Formula I or a therapeutically acceptable salt thereof and a pharmaceutically acceptable excipient.

In some embodiments, the said compound in a weight ratio to the said excipient within the range from about 0.001 to about 10.

The present invention additionally provides a compound of the present invention, a pharmaceutically acceptable salt thereof or a pharmaceutical composition above mentioned for the preparation of a medicament.

In some embodiments, the medicament is used for the treatment of cancer, such as colon cancer, rectal cancer, mantle cell lymphoma, multiple myeloma, breast cancer, prostate cancer, glioblastoma, squamous cell esophageal cancer, liposarcoma, T-cell lymphoma melanoma, pancreatic cancer, brain cancer or lung cancer.

In some embodiments, the medicament is used as an inhibitor of CDK, preferably CDK4 and/or CDK6.

The present invention provides a method of treating of cancer in a subject, comprising administering to the subject in need of a therapeutically effective amount of a compound of the present invention, a pharmaceutically acceptable salt thereof or above-mentioned pharmaceutical composition. In particular, the cancer is selected from the group consisting of colon cancer, rectal cancer, mantle cell lymphoma, multiple myeloma, breast cancer, prostate cancer, glioblastoma, squamous cell esophageal cancer, liposarcoma, T-cell lymphoma, melanoma, pancreatic cancer, brain cancer or lung cancer.

This invention further provides a method of treating a disease mediated by CDK, for example CDK4 and/or CDK 6 in a subject, comprising administering to the subject in need of a therapeutically effective amount of a compound of the present invention, a pharmaceutically acceptable salt thereof or above-mentioned pharmaceutical composition.

The general chemical terms used in the formula above have their usual meanings. For example, the term "halogen", as used herein, unless otherwise indicated, means fluoro, chloro, bromo or iodo. The preferred halogen groups include F, Cl and Br.

As used herein, unless otherwise indicated, alkyl includes saturated monovalent hydrocarbon radicals having straight, or branched moieties. For example, alkyl radicals include methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, 3- (2-methyl) butyl, 2-pentyl, 2-methylbutyl, neopentyl, n- hexyl, 2-hexyl, 2-methylpentyl and the like. Similarly, C₁₋₈, as in C₁₋₈alkyl is defined to identify the group as having 1, 2, 3, 4, 5, 6, 7 or 8 carbon atoms in a linear or branched arrangement.

Alkenyl and alkynyl groups include straight, or branched chain alkenes and alkynes. Likewise, "C₂₋₈ alkenyl" and "C₂₋₈ alkynyl" means an alkenyl or alkynyl radicals having 2, 3, 4, 5, 6, 7 or 8 carbon atoms in a linear or brached arrangement.

Alkoxy are oxygen ethers formed from the previously described straight, or branched chain alkyl groups, that is -O-alkyl.

As used herein, "a", "an", "the", "at least one", and "one or more" are used interchangeably. Thus, for example, a composition comprising "a" pharmaceutically acceptable excipient can be interpreted to mean that the composition includes "one or more" pharmaceutically acceptable excipients.

The term “aryl”, as used herein, unless otherwise indicated, refers to an unsubstituted or substituted mono- or polycyclic ring system containing carbon ring atoms. The preferred aryls are mono cyclic or bicyclic 6-10 membered aromatic ring systems. Phenyl and naphthyl are preferred aryls. The most preferred aryl is phenyl.

5 The term “heterocyclyl”, as used herein, unless otherwise indicated, represents an unsubstituted or substituted stable three to eight membered monocyclic saturated ring system which consists of carbon atoms and from one to three heteroatoms selected from N, O or S, and wherein the nitrogen or sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heterocyclyl group may be attached at any heteroatom or carbon atom which
10 results in the creation of a stable structure. Examples of such heterocyclyl groups include, but are not limited to azetidiny, pyrrolidinyl, piperidinyl, piperazinyl, oxopiperazinyl, oxopiperidinyl, tetrahydrofuranyl, dioxolanyl, tetrahydroimidazolyl, tetrahydrothiazolyl, tetrahydrooxazolyl, tetrahydropyranyl, morpholinyl, thiomorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone and tetrahydrooxadiazolyl.

15 The term “heteroaryl”, as used herein, unless otherwise indicated, represents an unsubstituted or substituted stable five or six membered monocyclic aromatic ring system or an unsubstituted or substituted nine or ten membered benzo-fused heteroaromatic ring system or bicyclic heteroaromatic ring system which consists of carbon atoms and from one to four heteroatoms selected from N, O or S, and wherein the nitrogen or sulfur heteroatoms may optionally be oxidized, and the nitrogen
20 heteroatom may optionally be quaternized. The heteroaryl group may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of heteroaryl groups include, but are not limited to thienyl, furanyl, imidazolyl, isoxazolyl, oxazolyl, pyrazolyl, pyrrolyl, thiazolyl, thiadiazolyl, triazolyl, pyridyl, pyridazinyl, indolyl, azaindolyl, indazolyl, benzimidazolyl, benzofuranyl, benzothienyl, benzisoxazolyl, benzoxazolyl, benzopyrazolyl, benzothiazolyl,
25 benzothiadiazolyl, benzotriazolyl adeninyl, quinolinyl or isoquinolinyl.

The term “cycloalkyl” refers to a cyclic saturated alkyl chain having from 3 to 12 carbon atoms, for example, cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

The term “substituted” refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituent(s). Typical substituents include, but
30 are not limited to, halogen (F, Cl, Br or I), C₁₋₈ alkyl, C₃₋₁₂ cycloalkyl, -OR¹, SR¹, =O, =S, -C(O)R¹, -C(S)R¹, =NR¹, -C(O)OR¹, -C(S)OR¹, -NR¹R², -C(O)NR¹R², cyano, nitro, -S(O)₂R¹, -OS(O₂)OR¹, -OS(O)₂R¹, -OP(O)(OR¹)(OR²); wherein R¹ and R² is independently selected from -H, C₁₋₆ alkyl,

C₁₋₆ haloalkyl. In some embodiments, the substituent(s) is independently selected from the group consisting of -F, -Cl, -Br, -I, -OH, trifluoromethoxy, ethoxy, propyloxy, iso-propyloxy, n-butyloxy, isobutyloxy, t-butyloxy, -SCH₃, -SC₂H₅, formaldehyde group, -C(OCH₃), cyano, nitro, CF₃, -OCF₃, amino, dimethylamino, methyl thio, sulfonyl and acetyl.

5 Examples of substituted alkyl groups include, but not limited to, 2-aminoethyl, 2-hydroxyethyl, pentachloroethyl, trifluoromethyl, methoxymethyl, pentafluoroethyl and piperazinylmethyl.

 Examples of substituted alkoxy groups include, but not limited to, aminomethoxy, trifluoromethoxy, 2-diethylaminoethoxy, 2-ethoxycarbonylethoxy, 3-hydroxypropoxy.

 The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids. When the compound of the present invention is acidic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic bases, including inorganic bases and organic bases. Salts derived from such inorganic bases include aluminum, ammonium, calcium, copper (ic and ous), ferric, ferrous, lithium, magnesium, manganese (ic and ous), potassium, sodium, zinc and the like salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, as well as cyclic amines and substituted amines such as naturally occurring and synthesized substituted amines. Other pharmaceutically acceptable organic non-toxic bases from which salts can be formed include ion exchange resins such as, for example, arginine, betaine, caffeine, choline, *N,N'*-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

25 When the compound of the present invention is basic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, formic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Preferred are citric, hydrobromic, formic, hydrochloric, maleic, phosphoric, sulfuric and tartaric acids, particularly preferred are formic and hydrochloric acid. Since the compounds of Formula I are intended for pharmaceutical use they are

preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure, especially at least 98% pure (% are on a weight for weight basis).

The compounds of the present invention may also be present in the form of pharmaceutically acceptable salts. For use in medicine, the salts of the compounds of this invention refer to non-toxic
5 “pharmaceutically acceptable salts”. The pharmaceutically acceptable salt forms include pharmaceutically acceptable acidic/anionic or basic/cationic salts. The pharmaceutically acceptable acidic/anionic salt generally takes a form in which the basic nitrogen is protonated with an inorganic or organic acid. Representative organic or inorganic acids include hydrochloric, hydrobromic, hydriodic, perchloric, sulfuric, nitric, phosphoric, acetic, propionic, glycolic, lactic, succinic, maleic,
10 fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroxyethanesulfonic, benzenesulfonic, oxalic, pamoic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, salicylic, saccharinic or trifluoroacetic. Pharmaceutically acceptable basic/cationic salts include, and are not limited to aluminum, calcium, chlorprocaine, choline, diethanolamine, ethylenediamine, lithium, magnesium, potassium, sodium and zinc.

15 The present invention includes within its scope the prodrugs of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds that are readily converted in vivo into the required compound. For example, any pharmaceutically acceptable salt, ester, ester salt or other derivative of a compound of this application that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of the present application or a
20 pharmaceutically active metabolite or residues. Particularly preferred derivatives or prodrugs are those compounds that can increase the bioavailability of a compound of the present application when administered to a patient (eg, which can make an orally administered compound more readily absorbed into the blood), or facilitate delivery of the parent compound to a biological organism or those that are delivered by a site of action (eg, the brain or lymphatic system). Thus, in the
25 methods of treatment of the present invention, the term “administering” shall encompass the treatment of the various disorders described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound in vivo after administration to the subject. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in “Design of Prodrugs”, ed. H. Bundgaard, Elsevier,
30 1985.

It is intended that the definition of any substituent or variable at a particular location in a molecule be independent of its definitions elsewhere in that molecule. It is understood that

substituents and substitution patterns on the compounds of this invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth herein.

The present invention includes compounds described herein can contain one or more asymmetric
5 centers and may thus give rise to diastereomers and optical enantiomers. The present invention includes all such possible diastereomers as well as their racemic mixtures, their substantially pure resolved enantiomers, all possible geometric enantiomers, and pharmaceutically acceptable salts thereof.

It has now been discovered that the optically pure (-) enantiomer of the present compound is a
10 more potent CDK4/6 inhibitor. The present invention includes methods for treating a disease mediated by CDK4/6 in a subject, which comprises administering to said subject an amount of (-) enantiomer or a pharmaceutically acceptable salt thereof, substantially free of its (+) enantiomer, said amount being sufficient to alleviate the disease, but insufficient to cause said adverse effects.

The term "substantially free of its (+) enantiomer" as used herein means that the composition
15 contains a greater proportion or percentage of the (-) enantiomer in relation to the (+) enantiomer, said percentage being based on the total amount of the mixture. In an embodiment, the term "substantially free of its (+) enantiomer" means that the composition contains at least 60% by weight of (-) enantiomer, and 40% by weight or less of (+) enantiomer. In a preferred embodiment, the term "substantially free of its (+) enantiomer" means that the composition contains at least 70%
20 by weight of (-) enantiomer, and 30% by weight or less of (+) enantiomer. In another embodiment, the term "substantially free of its (+) enantiomer" means that the composition contains at least 80% by weight of (-) enantiomer, and 20% by weight or less of (+) enantiomer. Furthermore, the term "substantially free of its (+) enantiomer" means that the composition contains at least 90% by weight of (-) enantiomer, and 10% by weight or less of (+) enantiomer. Even further, the term
25 "substantially free of its (+) enantiomer" means that the composition contains at least 95% by weight of (-) enantiomer, and 5% by weight or less of (+) enantiomer. Moreover, the term "substantially free of its (+) enantiomer" means that the composition contains at least 99% by weight of (-) enantiomer, and 1% by weight or less of (+) enantiomer.

The above Formula I is shown without a definitive stereochemistry at certain positions. The
30 present invention includes all stereoisomers of Formula I and pharmaceutically acceptable salts thereof. Further, mixtures of stereoisomers as well as isolated specific stereoisomers are also included. During the course of the synthetic procedures used to prepare such compounds, or in using

racemization or epimerization procedures known to those skilled in the art, the products of such procedures can be a mixture of stereoisomers.

When a tautomer of the compound of Formula I exists, the present invention includes any possible tautomers and pharmaceutically acceptable salts thereof, and mixtures thereof, except where
5 specifically stated otherwise.

When the compound of Formula I and pharmaceutically acceptable salts thereof exist in the form of solvates or polymorphic forms, the present invention includes any possible solvates and polymorphic forms. A type of a solvent that forms the solvate is not particularly limited so long as the solvent is pharmacologically acceptable. For example, water, ethanol, propanol, acetone or the
10 like can be used.

The term "composition" is herein meant to include products that include the specified amounts of the specified ingredients, as well as any product that is produced, directly or indirectly, from the specified combination of specified ingredients. Therefore, pharmaceutical compositions containing the compounds of the present invention as active ingredients and methods of preparing the
15 compounds of the invention are also parts of this invention. In addition, some of the crystalline forms of the compounds may exist as polymorphs, and such polymorphs are included in the present invention. In addition, some of the compounds may form solvates with water (ie, hydrates) or common organic solvents, and such solvates also fall within the scope of the present invention.

The pharmaceutical compositions of the present invention comprise a compound represented by
20 Formula I (or a pharmaceutically acceptable salt thereof) as an active ingredient, a pharmaceutically acceptable carrier and optionally other therapeutic ingredients or adjuvants. The compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active
25 ingredient is being administered. The pharmaceutical compositions may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

In practice, the compounds represented by Formula I, or a prodrug, or a metabolite, or pharmaceutically acceptable salts thereof, of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical
30 compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). Thus, the pharmaceutical compositions of the present invention can be presented as discrete units suitable for

oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous liquid, as an oil-in-water emulsion, or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, the compound
5 represented by Formula I, or a pharmaceutically acceptable salt thereof, may also be administered by controlled release means and/or delivery devices. The compositions may be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid
10 carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

Thus, the pharmaceutical compositions of this invention may include a pharmaceutically acceptable carrier and a compound, a stereoisomer, a tautomer, a polymorph, a solvate, a pharmaceutically acceptable salt, or a prodrug of Formula I. The compounds of Formula I, or
15 pharmaceutically acceptable salts thereof, can also be included in pharmaceutical compositions in combination with one or more other therapeutically active compounds.

The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include such as lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers include such as sugar syrup, peanut oil, olive
20 oil, and water. Examples of gaseous carriers include such as carbon dioxide and nitrogen. In preparing the compositions for oral dosage form, any convenient pharmaceutical media may be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like may be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating
25 agents, lubricants, binders, disintegrating agents, and the like may be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets may be coated by standard aqueous or nonaqueous techniques.

A tablet containing the composition of this invention may be prepared by compression or
30 molding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or

dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Each tablet preferably contains from about 0.05mg to about 5g of the active ingredient and each cachet or capsule preferably containing from about 0.05mg to about 5g of the active ingredient. For example, a formulation intended for the oral administration to humans may contain from about 0.5mg to about 5g of active agent, compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Unit dosage forms will generally contain between from about 1 mg to about 2g of the active ingredient, typically 25mg, 50mg, 100mg, 200mg, 300mg, 400mg, 500mg, 600mg, 800mg, or 1000mg.

10 Pharmaceutical compositions of the present invention suitable for parenteral administration may be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

15 Pharmaceutical compositions of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In all cases, the final injectable form must be sterile and must be effectively fluid for easy syringability. The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

25 Pharmaceutical compositions of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, or the like. Further, the compositions can be in a form suitable for use in transdermal devices. These formulations may be prepared, utilizing a compound represented by Formula I of this invention, or a pharmaceutically acceptable salt thereof, via conventional processing methods. As an example, a cream or ointment is prepared by admixing hydrophilic material and water, together with about 5wt% to about 10wt% of the compound, to produce a cream or ointment having a desired consistency.

30 Pharmaceutical compositions of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories. Suitable

carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by first admixing the composition with the softened or melted carrier(s) followed by chilling and shaping in molds.

In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including antioxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing a compound described by Formula I, or pharmaceutically acceptable salts thereof, may also be prepared in powder or liquid concentrate form.

Generally, dosage levels on the order of from about 0.01mg/kg to about 150mg/kg of body weight per day are useful in the treatment of the above-indicated conditions, or alternatively about 0.5mg to about 7g per patient per day. For example, colon cancer, rectal cancer, mantle cell lymphoma, multiple myeloma, breast cancer, prostate cancer, glioblastoma, squamous cell esophageal cancer, liposarcoma, T-cell lymphoma melanoma, pancreatic cancer, glioblastoma or lung cancer, may be effectively treated by the administration of from about 0.01 to 50mg of the compound per kilogram of body weight per day, or alternatively about 0.5mg to about 3.5g per patient per day.

It is understood, however, that lower or higher doses than those recited above may be required. Specific dose level and treatment regimens for any particular subject will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, the severity and course of the particular disease undergoing therapy, the subject disposition to the disease, and the judgment of the treating physician.

These and other aspects will become apparent from the following written description of the invention.

Examples

It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. All parts and percentages are by weight and all temperatures are degrees Celsius, unless explicitly stated otherwise. The compounds described herein can be obtained from commercial sources or

synthesized by conventional methods as shown below using commercially available starting materials and reagents. The following abbreviations have been used in the examples:

- ATP: Adenosine triphosphate;
- Boc₂O: Di-tert-butyl dicarbonate;
- 5 con-H₂SO₄: concentrated sulfuric acid;
- Crk: CT10 (Chicken Tumor Retrovirus 10);
- DCM: dichloromethane;
- DEA: Diethylamine;
- DEAD: Diethyl azodicarboxylate;
- 10 DIEA: N,N-Diisopropylethylamine;
- DMEM : Dulbecco's Modified Eagle Media;
- DMF: N,N-Dimethylformamide;
- DMA: N,N-Dimethylacetamide;
- DMAP: 4-N,N-Dimethylamipryidine;
- 15 DMSO: Dimethyl sulfoxide;
- DTT: DL-Dithiothreitol;
- EA: Ethyl acetate;
- EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide;
- EDTA: eathylene diamine tetraacetic acid;
- 20 EtOH: ethyl alcohol;
- FBS: fetal bovine serum;
- GSR: Glutathione-S-Transferase;
- HATU: O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate;
- HEPES: 4- (2-hydroxyerhyl) piperazine-1-erhaesulfonic acid;
- 25 Hex: n-hexane;
- h or hr: Hour;
- IPA: isopropanol
- KOAc: potassium acetate;
- KTB: potassium tert-butoxide;
- 30 MeOH: methanol;
- min: Minute;
- MsCl: methylsufonyl chloride;

MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium;

NaBH₄: sodium borohydride;

NaBH(OAc)₃: Sodium triacetoxyborohydride;

5 P(Cy)₃: Tricyclohexyl phosphine;

Pd₂(dba)₃: Tris(dibenzylideneacetone)dipalladium

Pd(dppf)Cl₂: [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium;

Pd(OAc)₂: Palladium acetate;

PE: Petroleum ether;

10 PMS: phenazine methosulfate;

POCl₃: phosphorus oxychloride;

P/S: Penicillin/Streptomycin Solution;

RT or rt: room temperature;

SDS: Sodium Dodecyl Sulfate;

15 SDS-PAGE: Sodium Dodecyl Sulfate PolyAcrylamide Electrophoresis Gel;

TBAB: Tetrabutyl ammonium bromide;

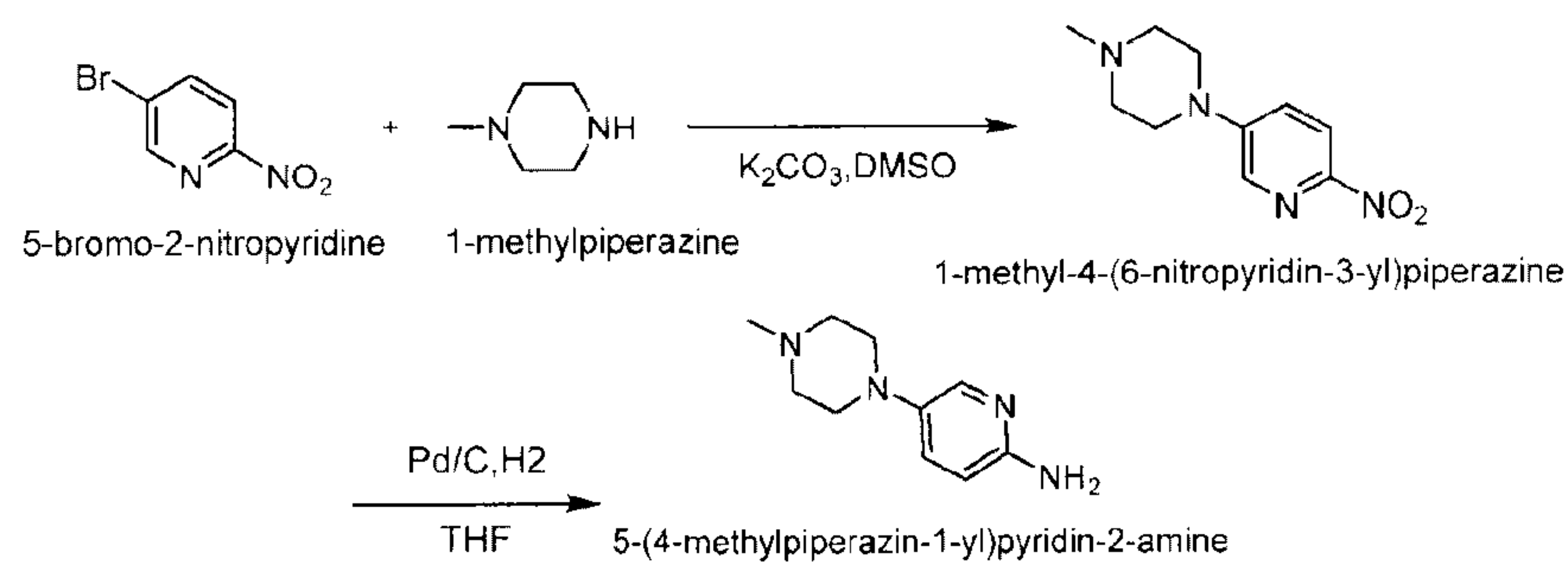
TEA: Triethylamine;

THF: tetrahydrofuran;

TLC: Thin layer chromatography;

20 Tol: Toluene.

Preparation 1: 5-(4-methylpiperazin-1-yl)pyridin-2-amine (Intermediate M1)



Add 1-Methylpiperazine (1.180g) and K₂CO₃ (2.720g) successively to a solution of 5-bromo-2-nitropyridine (2.010g) in DMSO (20mL). Let the reaction stir at 82°C for 15hrs in an oil bath. Add water (50mL), extract with DCM (20mL×8), the combined organic phase was dried over

anhydrous Na_2SO_4 , concentrated under reduced pressure, purified by column chromatography (DCM/MeOH=10/1) to give 1.940g of 1-methyl-4-(6-nitropyridin-3-yl)piperazine.

Add Pd/C (0.194g) to a solution of 1-methyl-4-(6-nitropyridin-3-yl)piperazine (1.940g) in THF (25mL) under hydrogen for 2 hrs at RT. The filtrate was collected by filtration and then concentrated to give 1.480g of 5-(4-methylpiperazin-1-yl)pyridin-2-amine.

MS(ES^+):m/z=193.1 ($\text{M}+\text{H}$) $^+$.

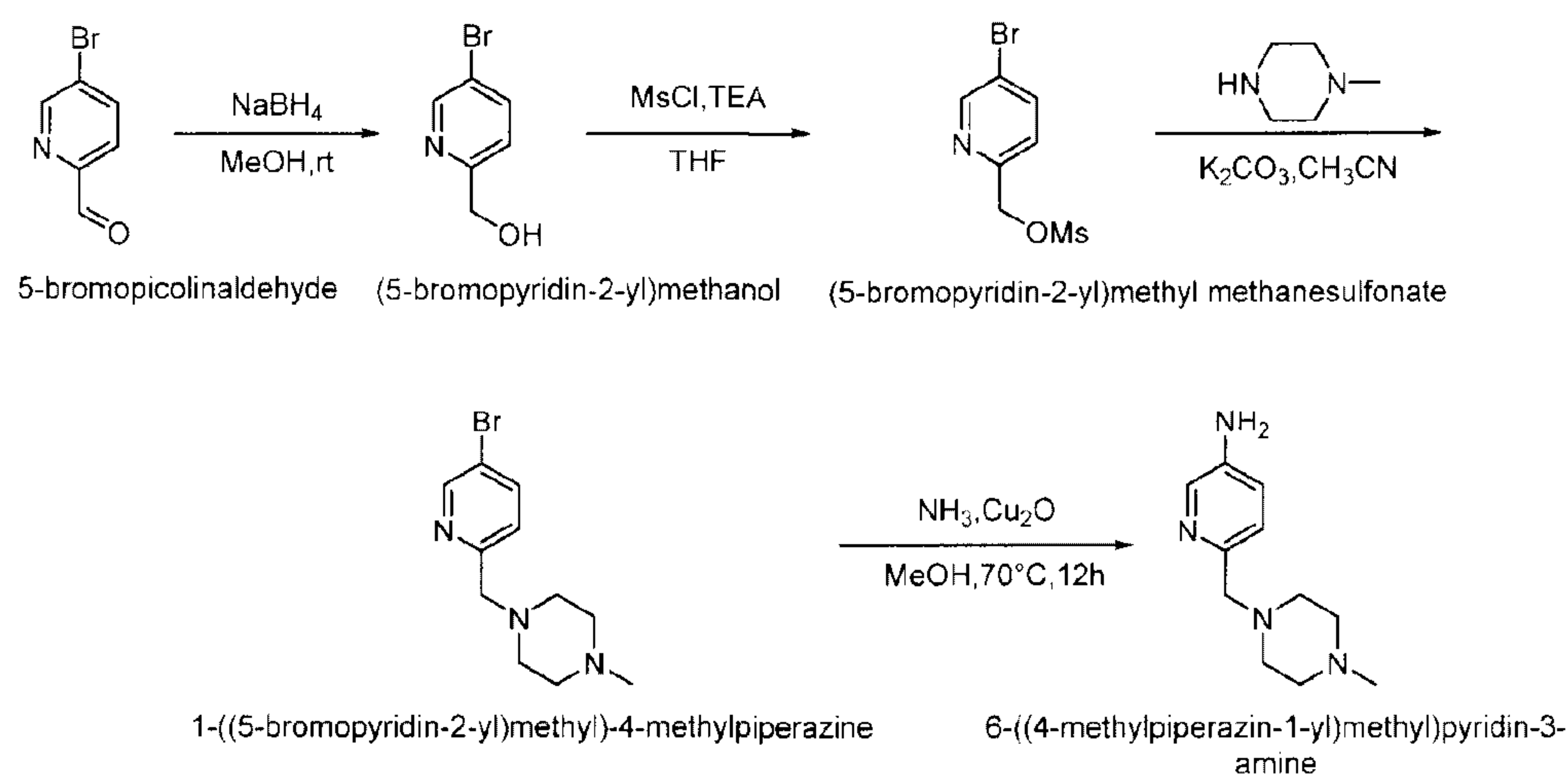
Prepare the following intermediates (shown in Table 1) essentially as described for 5-(4-methylpiperazin-1-yl)pyridin-2-amine (herein referred as Intermediate M1) using the corresponding piperazine derivative.

10

Table 1

Intermediate	Compound	Structure	Physical Data (MS) ($\text{M}+\text{H}$) $^+$
M2	5-(piperazin-1-yl)pyridin-2-amine		179.1
M3	2-(4-(6-aminopyridin-3-yl)piperazin-1-yl)ethan-1-ol		223.1

Preparation 4: 6-((4-methylpiperazin-1-yl)methyl)pyridin-3-amine (Intermediate M4)



Add NaBH_4 (1.220g) to a solution of 5-Bromopicolinaldehyde (2.010g) in MeOH (30mL) at 0°C in an ice bath, after the addition of NaBH_4 is complete, remove the ice bath, warm to room temperature

naturally. After stirring for 2hrs at RT, the reaction mixture was quenched with water (50mL) at 0°C. Extract with EA (50mL×2), the combined organic phase washed with saturated NaCl solution and dried over anhydrous Na₂SO₄, concentrated to give 1.940g of (5-bromopyridin-2-yl) methanol.

A solution of (5-bromopyridin-2-yl) methanol (1.940g) in THF (20mL) is cooled to 0°C in an ice bath, then add methylsulfonyl chloride (1.780g) drop wise to the solution. After the addition of methylsulfonyl chloride is complete, remove the ice bath, warm to room temperature naturally. After stirring for 2hrs at RT, the reaction mixture was quenched with water (50mL). Extract with EA (50mL×2), the combined organic phase washed with saturated NaCl solution (50mL) and dried over anhydrous Na₂SO₄, concentrated to give 2.750g of crude product (5-bromopyridin-2-yl) methyl methanesulfonate.

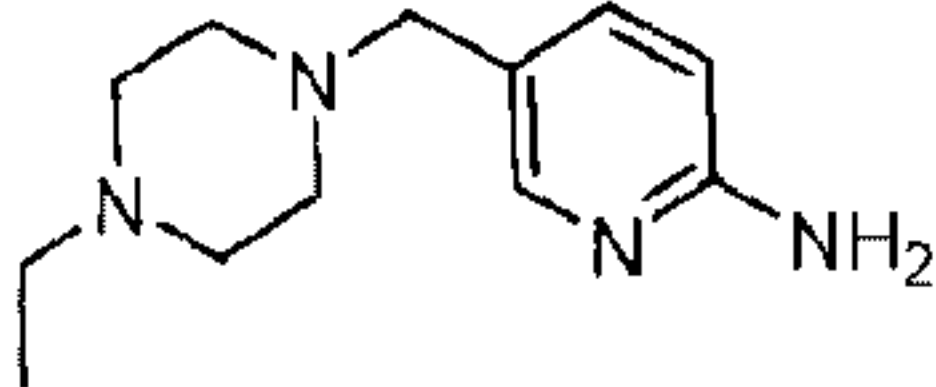
Add K₂CO₃ (2.870g) and 1-Methylpiperazine (1.560g) successively to a solution of (5-bromopyridin-2-yl) methyl methanesulfonate (2.750g) in acetonitrile (30mL), heat to 50°C in an oil bath and react for 2hrs. Then cool to room temperature, add water, extract with EA (50mL×3), the combined organic phase washed with saturated NaCl solution (50mL) and dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 2.010g of 1-((5-bromopyridin-2-yl)methyl)-4-methylpiperazine.

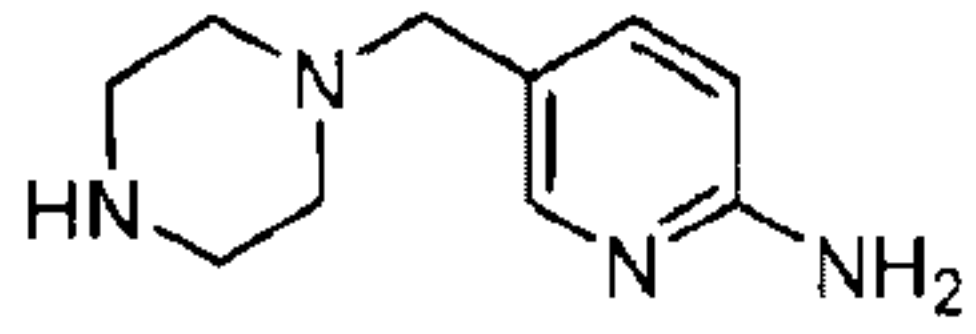
Add MeOH (20mL) to a 100mL sealed tube under ammonia at -78°C, then add 1-((5-bromopyridin-2-yl)methyl)-4-methylpiperazine (1.000g) and cuprous oxide (0.532g) successively until the volume of solution rising to 30mL. Remove the outside bath, warm to room temperature naturally, then heat to 70°C and react for 12hrs. The filtrate was collected by filtration, concentrated and purified by column chromatography (DCM/MeOH=15/1) to give 0.730g of 6-((4-methylpiperazin-1-yl)methyl)pyridin-3-amine.

MS(ES⁺):m/z=207.2(M+H)⁺.

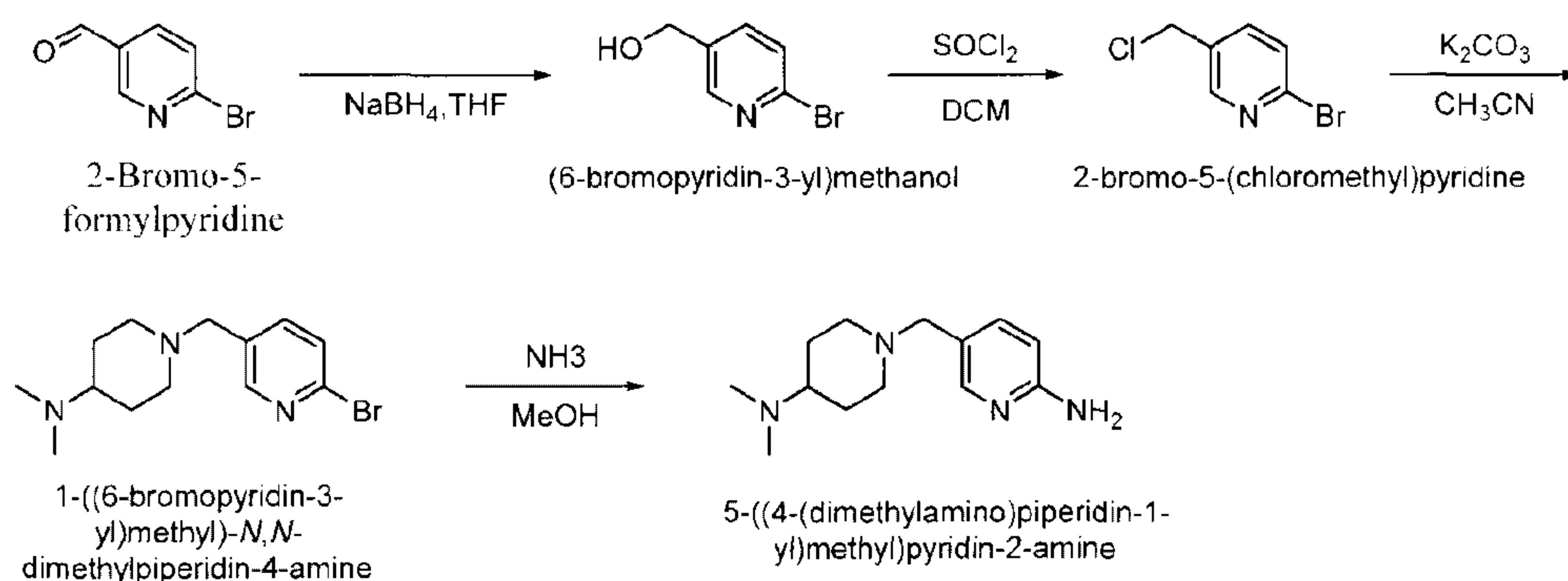
Prepare the following intermediates (shown in Table 2) essentially as described for 6-((4-methylpiperazin-1-yl)methyl)pyridin-3-amine (herein referred as Intermediate M4) using the corresponding piperazine derivative.

Table 2

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M5	5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-amine		221.2

M6	5-((piperazin-1-ylmethyl)pyridin-2-amine		193.1
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Preparation 7: 5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-amine (Intermediate M7)



Add NaBH_4 (1.640g) to a solution of 2-Bromo-5-formylpyridine (2.010g) in THF (20mL) at 0°C in an ice bath, after the addition of NaBH_4 is complete, remove the ice bath, warm to room temperature naturally. After stirring for 2hrs at RT, the reaction mixture was quenched with water (50mL), extract with EA (50mL $\times$ 2), the combined organic phase dried over anhydrous Na_2SO_4 , concentrated and purified by column chromatography (PE/EA=5/1) to give 1.900g of (6-bromopyridin-3-yl)methanol.

A solution of (6-bromopyridin-3-yl)methanol (1.000g) in DCM (10mL) is cooled to 0°C in an ice bath and added to thionyl chloride (1.260g) dropwise, after the addition is complete, remove the ice bath, the solution is warmed to room temperature naturally with stirring for 2hrs, then directly concentrated to give 1.050g of 2-bromo-5-(chloromethyl)pyridine.

Add *N,N*-Dimethylpiperidin-4-amine (0.586g) and K_2CO_3 (1.160g) to a solution of 2-bromo-5-(chloromethyl)pyridine (0.853g) in acetonitrile (10mL). Add water (30mL), extract with EA (50mL $\times$ 3), the combined organic phase dried over anhydrous Na_2SO_4 , concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 0.730g of 1-((6-bromopyridin-3-yl)methyl)-*N,N*-dimethylpiperidin-4-amine.

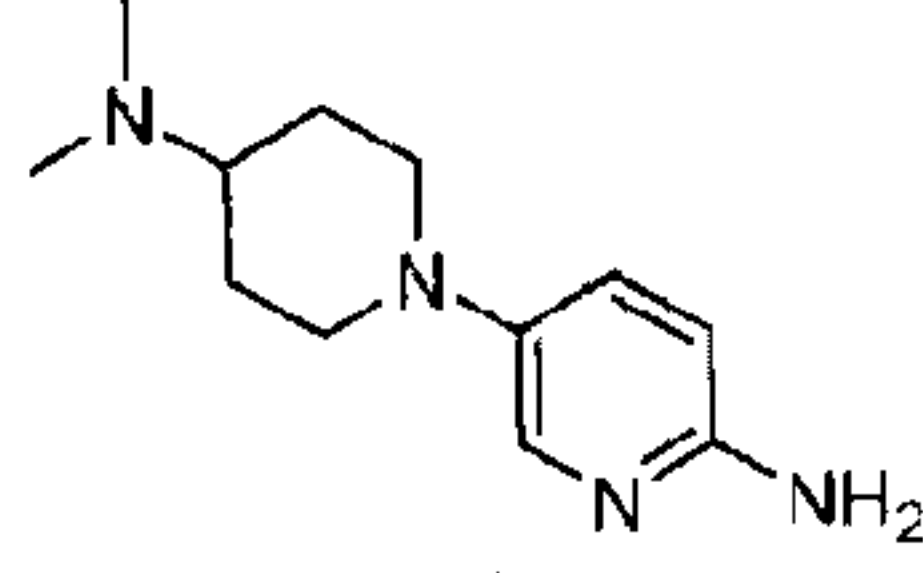
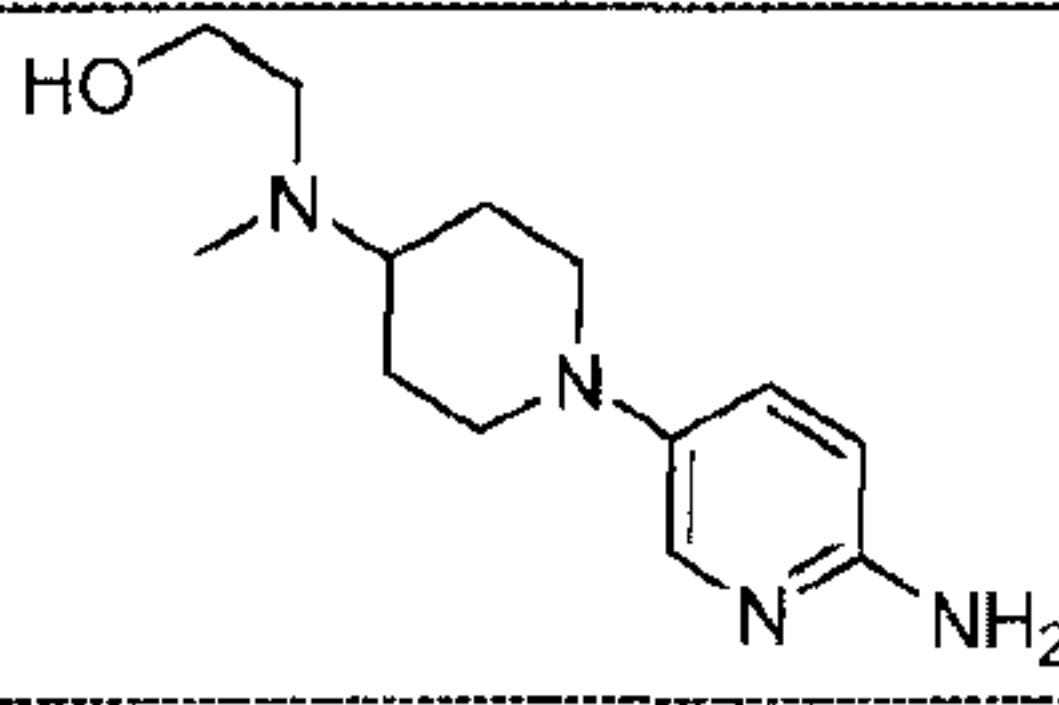
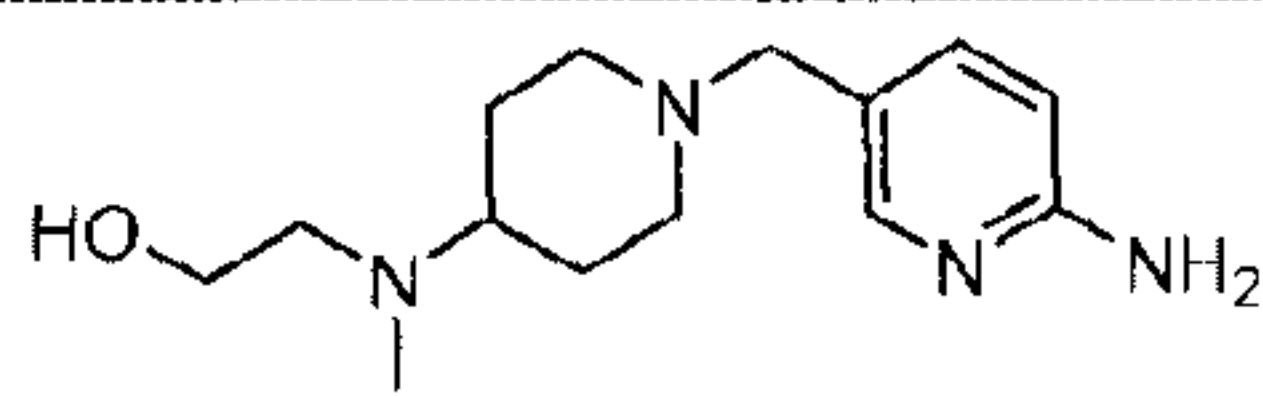
Add MeOH (20mL) to a 100mL sealed tube under ammonia at -78°C, then add 1-((6-bromopyridin-3-yl)methyl)-*N,N*-dimethylpiperidin-4-amine (0.35mg) and cuprous oxide (0.168g) successively until the volume of solution rising to 30mL. Remove the outside bath, warm to room temperature naturally, then heat to 70°C and react for 12hrs. The filtrate was collected by

filtration, concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 0.260g of 5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-amine.

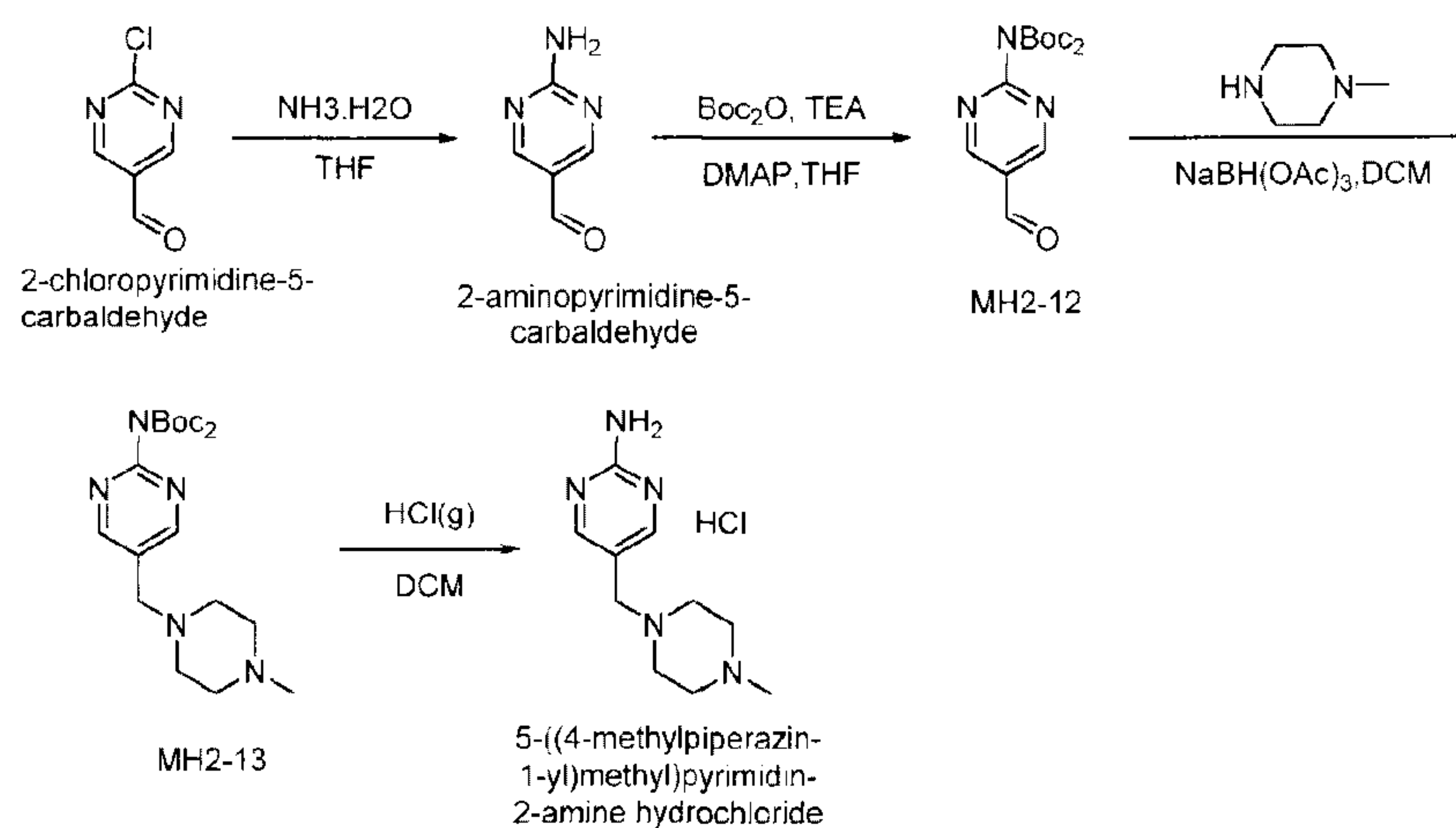
MS(ES⁺):m/z=235.2(M+H)⁺.

Prepare the following intermediates (shown in Table 3) essentially as described for 5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-amine (herein referred as Intermediate M7) using the corresponding piperidine derivative.

Table 3

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M8	5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-amine		221.3
M9	2-((1-(6-aminopyridin-3-yl)piperidin-4-yl)(methylamino)ethan-1-ol		251.2
M10	2-((1-((6-aminopyridin-3-yl)methyl)piperidin-4-yl)(methylamino)ethan-1-ol		265.2

Preparation 11: 5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-amine hydrochloride (Intermediate M11)



Add aqueous ammonia (25%) (1.200g) to a solution of 2-Chloropyrimidine-5-carbaldehyde (0.500g) in THF (50mL), stirring for 12hrs. Add water (80mL), extract with DCM (80mL×8), the combined organic phase dried over anhydrous Na₂SO₄, concentrated to give 0.540g of crude product 2-aminopyrimidine-5-carbaldehyde.

5 Add Boc₂O (2.817g), triethylamine (1.310g) and DMAP (0.054g) successively to a solution of 2-aminopyrimidine-5-carbaldehyde (0.540g) in THF (30mL) with stirring for 2hrs. Add water, extract with EA (50mL×2), the combined organic phase dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (PE/EA=5/1) to give 0.514g of compound MH2-12.

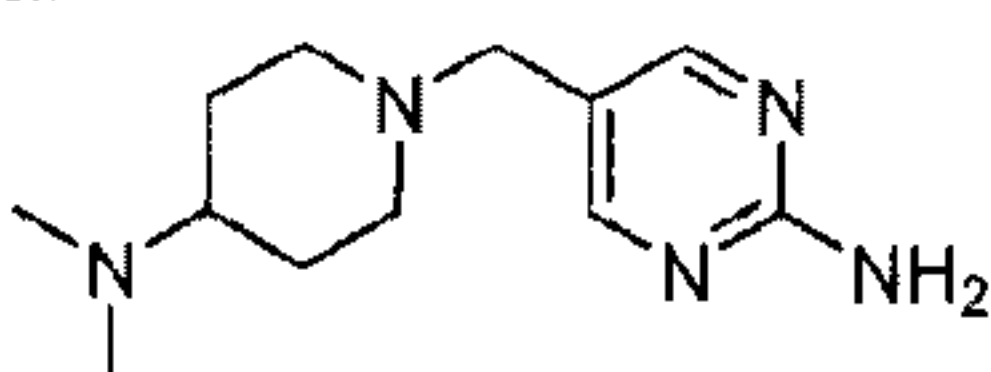
Add 1-Methylpiperazine) (0.109g) and anhydrous magnesium sulfate (0.216g) successively to a
10 solution of compound MH2-12 (0.290g) in DCM (10mL) with stirring for 2hrs, then react for 3hrs at RT after adding Sodium triacetoxyborohydride, the reaction mixture was quenched with water (20mL), extract with DCM (20mL×3), the combined organic phase dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 0.350g of compound MH2-13.

15 A solution of compound MH2-13(0.350g) in DCM is reacted for 2hrs under hydrochloric acid gas at RT, the reaction mixture is concentrated to give 0.210g of 5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-amine hydrochloride.

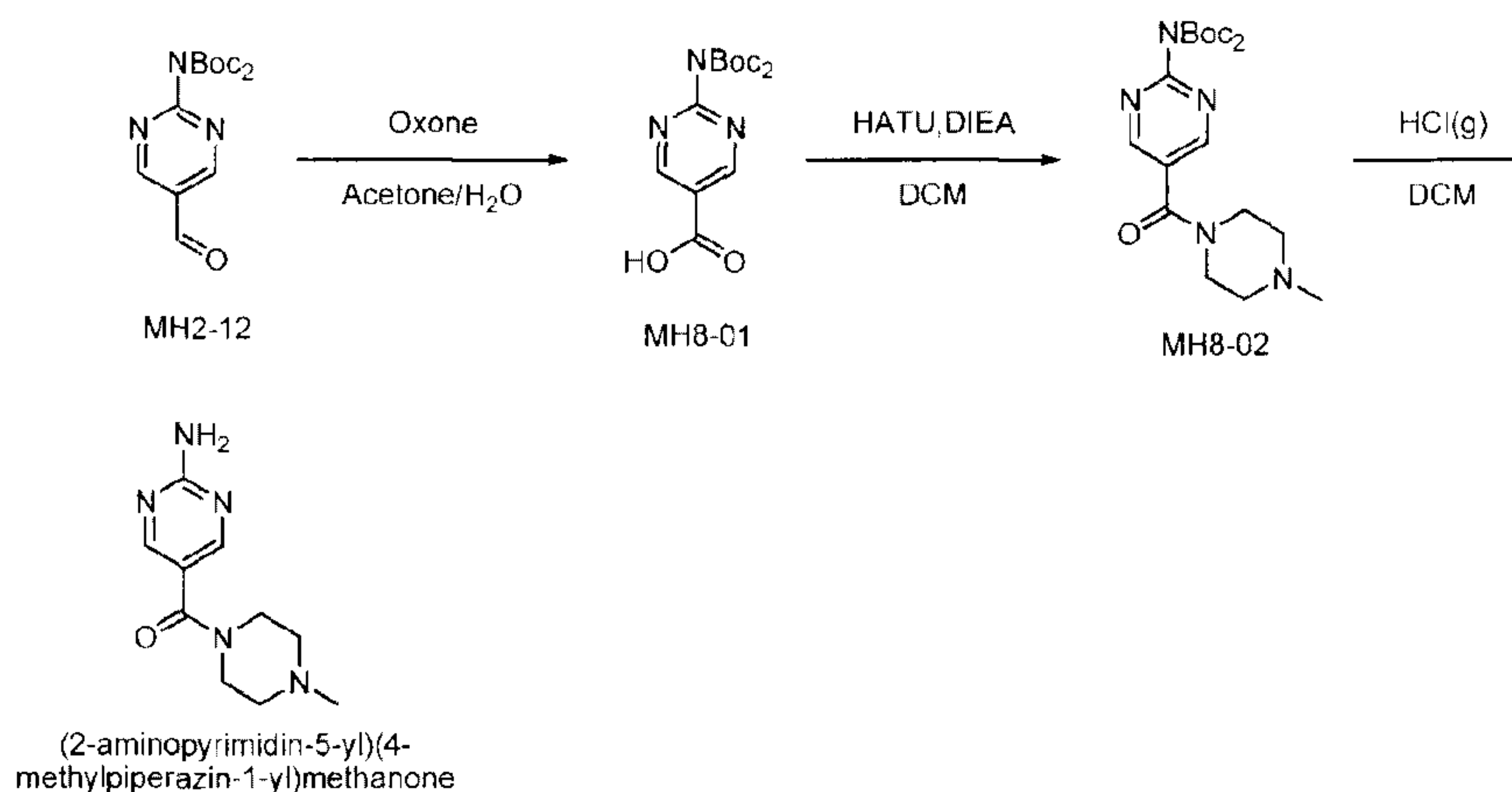
MS(ES⁺):m/z=244.1 (M+H)⁺.

20 Prepare the following intermediate (shown in Table 4) essentially as described for 5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-amine (herein referred as Intermediate M11) hydrochloride using the corresponding piperidine derivative instead of piperazine derivative.

Table 4

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M12	5-((4-(dimethylamino)piperidin-1-yl)methyl)pyrimidin-2-amine		236.2

Preparation 13: (2-aminopyrimidin-5-yl)(4-methylpiperazin-1-yl)methanone (Intermediate M13)



Add Oxone (1.810g) to a mixture of compound MH2-12 (0.315g) in acetone (10mL) and water (3mL) with stirring for 2hrs at RT. Add water (20mL), extract with DCM (25mL×3), the combined organic phase dried over anhydrous Na₂SO₄, concentrated to give 0.290g of compound MH8-01.

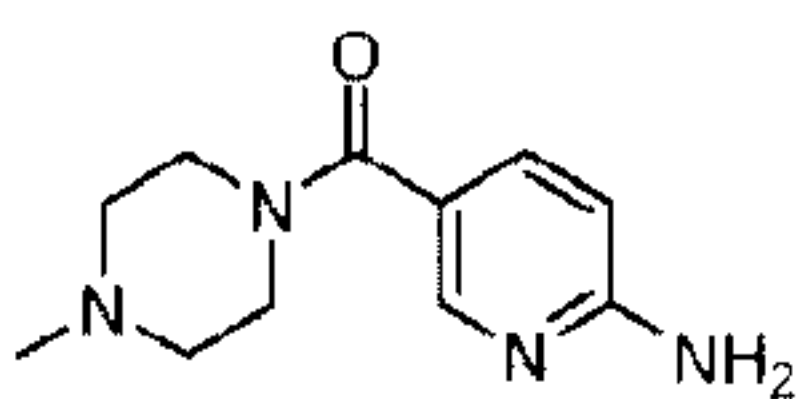
Add HATU (0.488g) and DIEA (0.221g) successively to a solution of compound MH8-01 (0.290g) in DCM (10mL) with stirring for 1h at RT, the solution is reacted for 2hrs after adding 1-Methylpiperazine (0.105g) at RT. Add water (20mL), extract with DCM (20mL×3), the combined organic phase dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (DCM/MeOH=50/1) to give 0.250g of compound MH8-02.

A solution of compound MH8-02 (0.150g) in DCM (10mL) is reacted for 2hrs under hydrochloric acid gas at RT, add water (10mL), then adjust pH to 8~9 with Na₂CO₃, the resulting aqueous solution was extracted with mixed solvent (DCM/MeOH=10/1) (20mL×5). The combined organic phase was dried over anhydrous Na₂SO₄, concentrated to give 0.060g of (2-aminopyrimidin-5-yl)(4-methylpiperazin-1-yl)methanone.

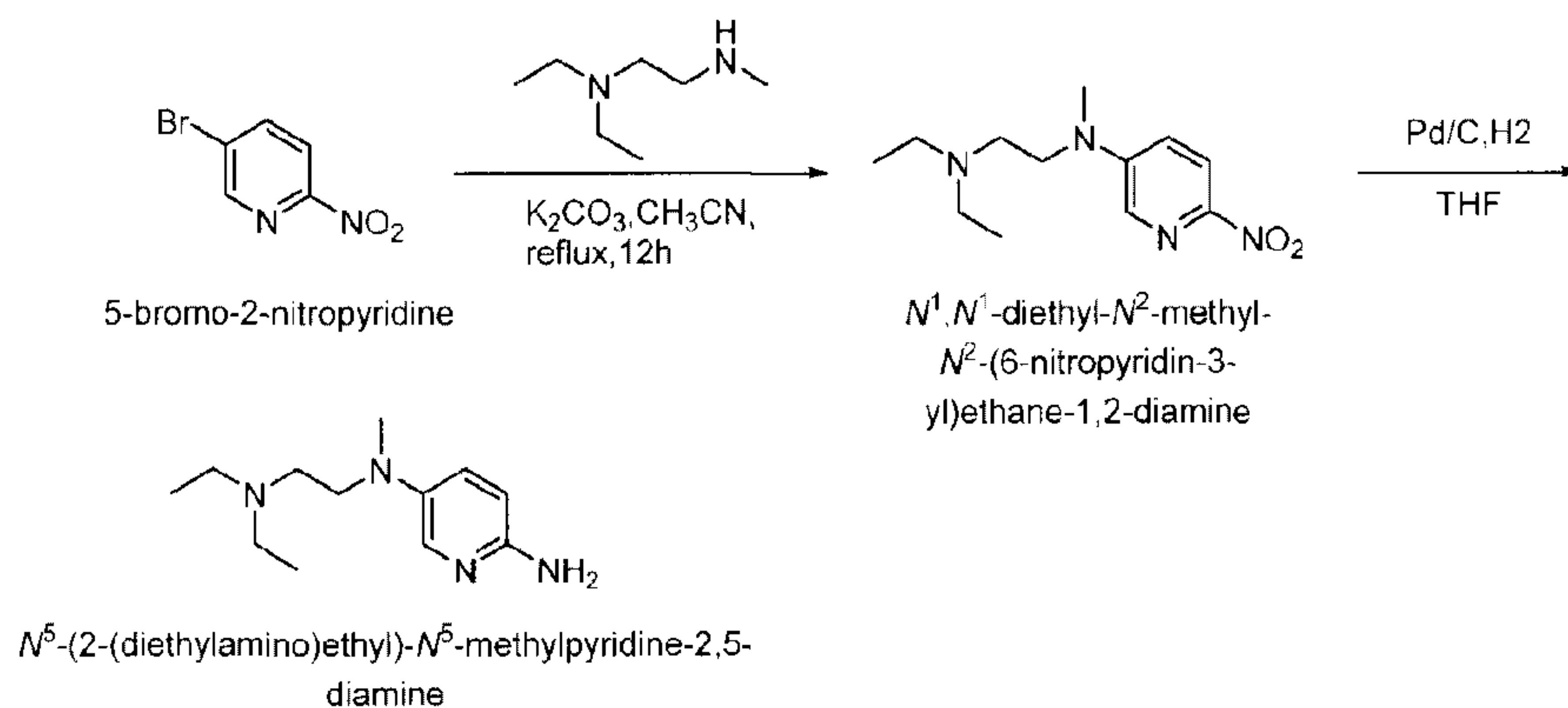
MS(ES⁺):m/z=223.1(M+H)⁺.

Prepare the following intermediate (shown in Table 5) essentially as described for (2-aminopyrimidin-5-yl)-(4-methylpiperazin-1-yl)methanone (herein referred as Intermediate M13) using the corresponding pyridine derivative instead of pyrimidine derivative.

Table 5

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M14	(6-aminopyridin-3-yl)(4-methylpiperazin-1-yl)methanone		221.1

Preparation 15: *N*⁵-(2-(diethylamino)ethyl)-*N*⁵-methylpyridine-2,5-diamine (Intermediate M15)

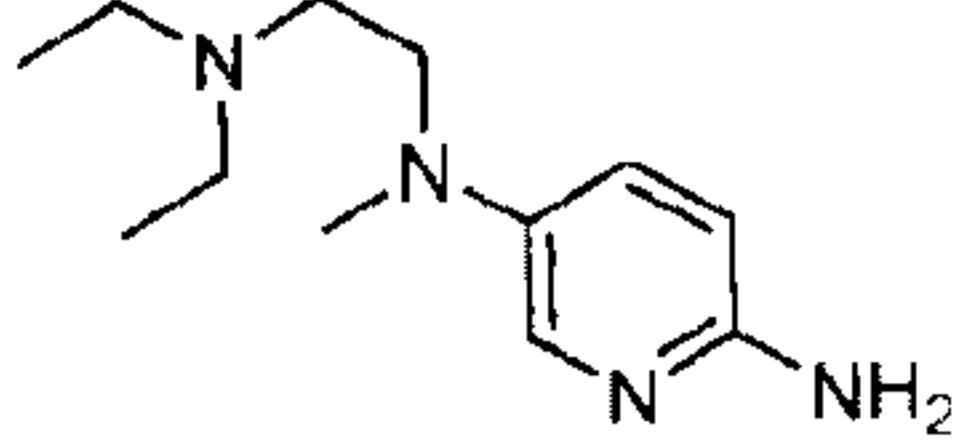


- 5 Add *N,N*-Diethyl-*N'*-methylethylenediamine (0.305g) and K₂CO₃ (0.679g) successively to a solution of 2-Nitro-5-bromopyridine(0.500g) in acetonitrile (10mL). Let the reaction stir at 82°C for 15hrs in an oil bath. Add water (50mL), extract with DCM (80mL×3), the combined organic phase was dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 0.400g of 1-methyl-4-(6-nitropyridin-3-yl)- piperazine.
- 10 Add Pd/C (0.040g) to a solution of 1-methyl-4-(6-nitropyridin-3-yl)piperazine in THF (15mL) with stirring for 2hrs at RT under hydrogen gas. The filtrate was collected by filtration and then concentrated to give 0.350g of *N*⁵-(2-(diethylamino)ethyl)-*N*⁵-methylpyridine-2,5- diamine.

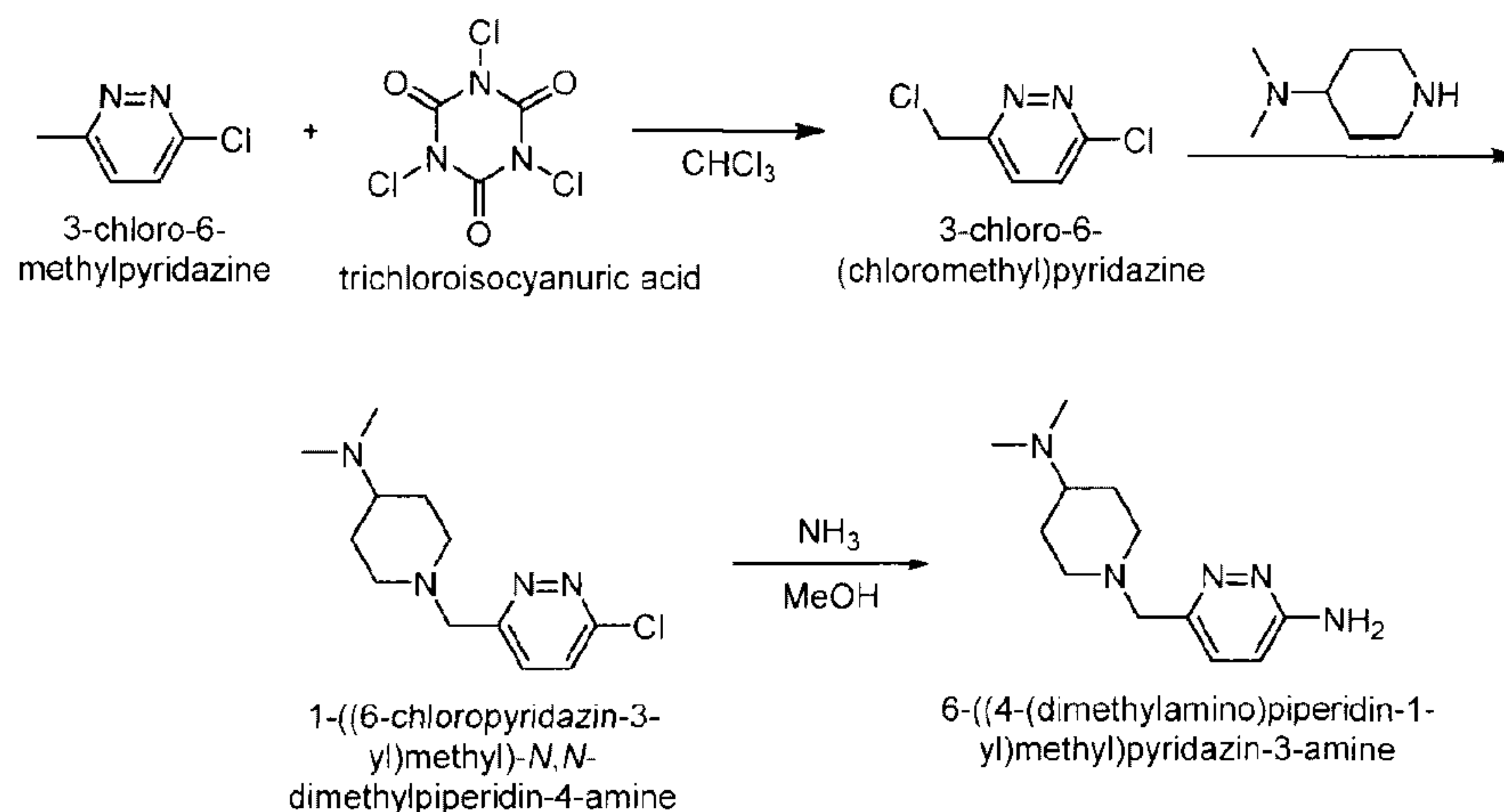
MS(ES⁺):m/z=223.2(M+H)⁺.

- 15 Prepare the following intermediate (shown in Table 6) essentially as described for *N*⁵-(2-(diethylamino)ethyl)- *N*⁵-methylpyridine-2,5-diamine(herein referred as Intermediate M15) using *N*¹,*N*¹-diethyl-*N*²,*N*²-dimethylethane-1,2-diamine instead of *N,N*-Diethyl-*N'*-methylethylenediamine.

Table 6

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M16	<i>N</i> ⁵ -(2-((diethylamino)ethyl)- <i>N</i> ⁵ -methylpyridine-2,5-diamine		223.2

Preparation 17: 6-((4-(dimethylamino)piperidin-1-yl)methyl)pyridazin-3-amine (Intermediate M17)



5 Add trichloroisocyanuric acid (0.189g) to a solution of 3-Chloro-6-methylpyridazine (0.208g) in CHCl_3 (10mL), heat to 60°C for 12hrs in an oil bath. Cool to room temperature, the filtrate was collected by filtration, concentrated and purified by column chromatography (PE/EA=10/1) to give 0.201g of 3-chloro-6-(chloromethyl)pyridazine.

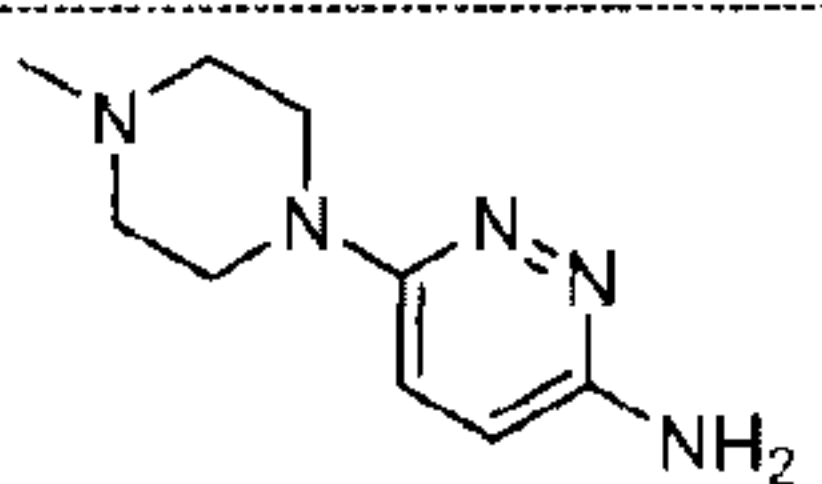
10 Add K_2CO_3 (0.578g), KI (0.070g) and *N,N*-Dimethylpiperidin-4-amine (0.322g) successively to a solution of 3-chloro-6-(chloromethyl)pyridazine (0.340g) in DMF (15mL), heat to 50°C for 1h in an oil bath. Cool to room temperature, add DCM (50mL), wash combined organic layers with saturated NaCl solution and dried over anhydrous Na_2SO_4 , concentrated and purified by column chromatography (DCM/MeOH=10/1) to give 0.370g of 1-((6-chloropyridazin-3-yl)methyl)-*N,N*-dimethylpiperidin-4-amine.

15 Add MeOH (20mL) to a 100mL sealed tube under ammonia at -78°C, then add 1-((6-chloropyridazin-3-yl)methyl)-*N,N*-dimethylpiperidin-4-amine (0.370g) and cuprous oxide (0.532g) successively until the volume of solution rising to 30mL. Remove the outside bath, warm to room temperature naturally, then heat to 70°C and react for 12hrs. The filtrate was collected by

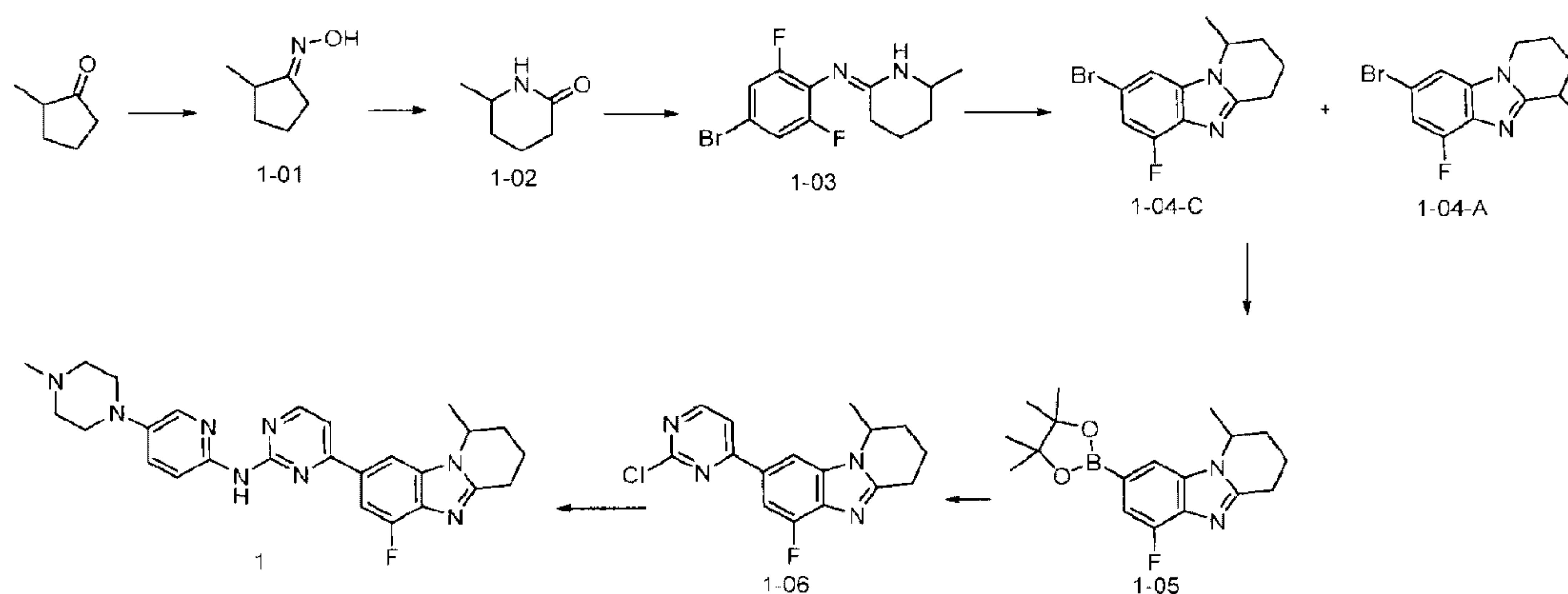
filtration, concentrated and purified by column chromatography (DCM/MeOH=15/1) to give 0.230g of 6-((4-(dimethylamino)piperidin-1-yl)methyl)pyridazin-3-amine. MS(ES⁺);m/z=236.2(M+H)⁺.

Prepare the following intermediate (shown in Table 7) essentially as described for 6-((4-(dimethylamino)piperidin-1-yl)methyl)pyridazin-3-amine (herein referred as Intermediate
5 M17) using piperazine derivative instead of piperidin derivative.

Table 7

Intermediate	Compound	Structure	Physical Data (MS) (M+H) ⁺
M18	6-(4-methylpiperazin-1-yl)pyridazin-3-amine		194.1

Example 1: Synthesis of compound 1



1. Compound 1-01

10 A mixture of 2-Methylcyclopentanone (5.200g), Hydroxylamine hydrochloride (9.200g) and Triethylamine (16.080g) in anhydrous ethanol (70mL) was stirred at 85°C overnight in an oil bath. Then, the reaction solution was concentrated; the residue was washed with EA. The filtrate was collected by filtration and then concentrated to give 5.820g of crude compound 1-01.

2. Compound 1-02

15 The crude compound 1-01 (5.820g) was dissolved in sulfuric acid solution (con-H₂SO₄: H₂O=20mL:5mL), the resulting mixture was stirred at 90°C in an oil bath for 90min, water (10mL) was added, then adjusted pH to 8~9 with Na₂CO₃, the resulting aqueous solution was extracted with DCM (20mL×5), the combined organic phase was dried over anhydrous Na₂SO₄, concentrated under reduced pressure to give 4.110g of crude compound 1-02.

3. Compound 1-03

A mixture of crude Compound 1-02 (4.110g) and 4-bromo-2,6-difluoroaniline (3.780g) in methylbenzene (40mL) was added POCl₃ (4.180g) and was heated in an oil bath, TEA (2.770g) was added when the temperature was raised to 110°C, the resulting mixture was reacting at 110°C for 20min. A part of methylbenzene was removed, then adjusted pH to 8~9 with Na₂CO₃, extracted with EA, the combined organic phase was washed with saturated NaCl solution and dried over anhydrous Na₂SO₄, concentrated under reduced pressure to give 6.550g crude Compound 1-03.

4. Compound 1-04

A mixture of crude Compound 1-03 (6.100g) and potassium tert-butoxide (4.520g) in DMF (60mL) was stirred at 100°C for 20min in an oil bath and then extracted with 300mL EA, the combined organic phase was washed with saturated NaCl solution (120mL×3) and dried over anhydrous Na₂SO₄, concentrated and then purified by column chromatography(PE/EA=1/5) to give 0.705g of compound 1-04-A, and 1.500g of crude compound 1-04-C.

5. Compound 1-05

A mixture of crude compound 1-04-C (0.957g), Bis(pinacolato)diboron (1.290g), tricyclohexyl phosphine (0.047g), Palladium acetate (0.038g) in DMSO (20mL) was stirred under nitrogen for 1h at 90°C in an oil bath. The resulting mixture was extracted with EA (60mL), the combined organic phase was washed with saturated NaCl solution (30mL×3) and dried over anhydrous Na₂SO₄, concentrated under reduced pressure to give 2.290g crude Compound 1-05.

6. Compound 1-06

A mixture of crude compound 1-05 (2.290g), 2,4-Dichloropyrimidine (0.755g), K₂CO₃ (1.400g) and Pd(dppf)Cl₂·DCM (0.138g) in 1,4- dioxane (30mL) and water (3mL) was stirred under nitrogen for 2h at 60°C in an oil bath. The resulting mixture was extracted with EA (30mL×2), the combined organic phase was washed with saturated NaCl solution (30mL×1) and dried over anhydrous Na₂SO₄, concentrated and then purified by column chromatography (PE/EA=1/1) to give 0.927g of Compound 1-06.

7. Compound 1

A mixture of compound 1-06 (0.400g), intermediate M1 (0.291g), Cs₂CO₃ (0.822g), Xanphos (0.017g) and Pd₂(dba)₃ (0.027g) in 1,4- dioxane (12mL) was stirred under nitrogen gas for 1h at 110°C in an oil bath, continued to react under microwave for 0.5h at 110°C. The resulting mixture was added water (10mL), then extracted with DCM (20mL×3), the combined organic phase was washed with saturated NaCl solution (30mL×1) and dried over anhydrous Na₂SO₄, concentrated and

then purified by column chromatography (DCM/MeOH=20/1), the solid was washed with methyl tert butyl ether (10mL) and n-hexane(10mL) to give 290mg of Compound 1.

MS(ES⁺):m/z=473.2(M+H)⁺.

H-NMR(CDCl₃): δ8.498-8.511(d,1H,CH), 8.373-8.396(d,1H,CH), 8.167(s,1H,CH),
 5 8.040-8.047(d,1H,CH), 7.963(s,1H,CH), 7.631-7.660 (d,1H,CH), 7.346-7.660(dd,1H,CH),
 7.177-7.190(d,1H,CH), 4.687-4.719(m,1H,CH), 3.190-3.10(m,4H,CH₂), 2.651-2.675(m,4H,CH₂),
 2.999-3.024(m,1H,CH₂), 2.403(s,3H,CH₃), 2.337-2.356(m,1H,CH₂), 2.267-2.357(m,1H,CH₂),
 2.219-2.248(m,1H,CH₂), 2.010-2.056(m,2H,CH₂), 1.602-1.618(d,3H,CH₃).

Example 1-1 Chiral separation of Compound 1-06

10 Techniques useful for the separation of isomers, e.g., enantiomers are within skill of the art and are described in Eliel, E.L.; Wilen, S.H.; Mander, L.N. *stereochemistry of Organic Compounds*. Wiley Interscience, NY,1994. For example compound 1, 2 or 15 can be resolved to a high enantiomeric excess (e.g., 60%, 70%, 80%, 90%, 95%, 99% or greater) via high performance liquid chromatography using a chiral column. In some embodiments, the crude compound 1-06 of the
 15 Example 1 is purified directly on a chiral column to provide enantiomerically enriched compound.

Chiral HPLC conditions:

Column	CHIRALPAK IE
Column size	2cm *25cm
Injection	0.7mL
Mobile phase	Hex: EtOH =65:35(v/v)
Flow rate	19mL/min
Wave length	UV 220nm
Temperature	35 °C
Sample solution	80mg/mL in Mobile phase
Prep- HPLC equipment	Prep- Gilson-HPLC
Sample name	Compound 1-06

Example 1-2 Synthesis of Compound 1a and Compound 1b

The crude Compound 1-06 is purified by chiral column under the above conditions to give Compound 1-06-A and Compound 1-06-B.

20 Prepare Compound 1a and Compound 1b essentially as described for step 7 of Example 1 using Compound 1-06-A and Compound 1-06-B respectively.

Furthermore, Optical rotations were measured 3 times for each compound shown as below on a Rudolf polarimeter.

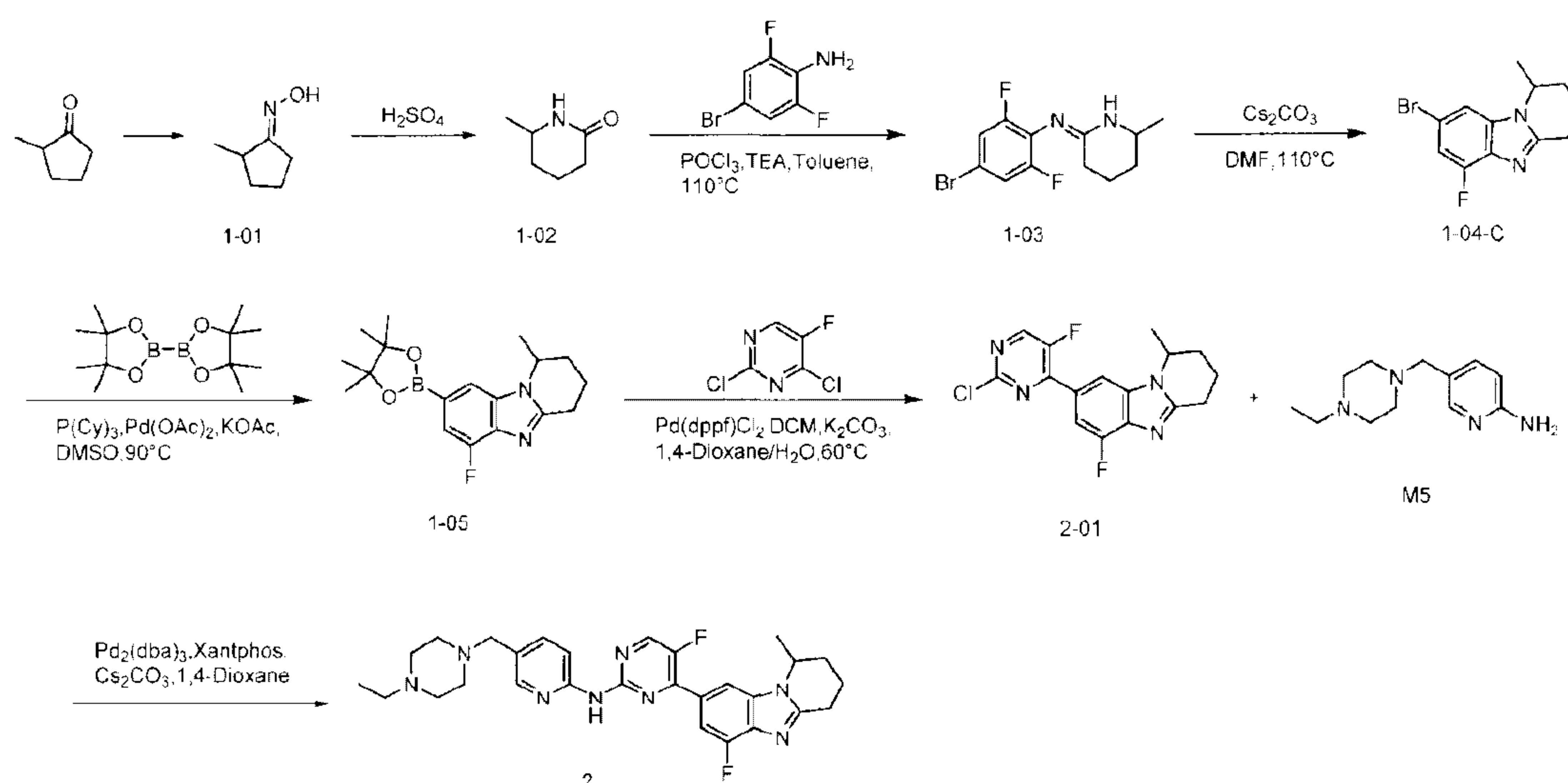
Conditions :

Polarimeter tube length	100mm
Temperature	20 °C
Sample solution	3.0mg/mL in EtOH
Sample name	Compound 1a and Compound 1b

Results:

	1 st (°)	2 nd (°)	3 rd (°)	Average (°)
Compound 1a	30.357	30.711	29.906	30.325
Compound 1b	-35.430	-35.453	-35.298	-35.394

5 Example 2: Synthesis of compound 2



1. Compound 1-01

A mixture of 2-Methylcyclopentanone (5.200g), Hydroxylamine hydrochloride (9.200g) and Triethylamine (16.080g) in anhydrous ethanol (70mL) was stirred at 85°C overnight in an oil bath. Then, the reaction solution was concentrated; the residue was washed with EA. The filtrate was collected by filtration and then concentrated to give 5.820g of crude compound 1-01.

2. Compound 1-02

The crude compound 1-01 (5.820g) was dissolved in sulfuric acid solution (con-H₂SO₄: H₂O=20mL:5mL), the resulting mixture was stirred at 90°C in an oil bath for 90min, water (10mL)

was added, then adjusted pH to 8~9 with Na_2CO_3 , the resulting aqueous solution was extracted with DCM ($20\text{mL}\times 5$), the combined organic phase was dried over anhydrous Na_2SO_4 , concentrated under reduced pressure to give 4.110g of crude compound 1-02.

3. Compound 1-03

5 A mixture of crude Compound 1-02 (4.110g) and 4-bromo-2,6-difluoroaniline (3.780g) in methylbenzene (40mL) was added POCl_3 (4.180g) and was heated in an oil bath, TEA (2.770g) was added when the temperature was raised to 110°C , the resulting mixture was reacting at 110°C for 20min. A part of methylbenzene was removed, then adjusted pH to 8~9 with Na_2CO_3 , extracted with EA, the combined organic phase was washed with saturated NaCl solution and dried over
10 anhydrous Na_2SO_4 , concentrated under reduced pressure to give 6.550g crude Compound 1-03.

4. Compound 1-04

A mixture of crude Compound 1-03 (6.100g) and potassium tert-butoxide (4.520g) in DMF (60mL) was stirred at 100°C for 20min in an oil bath and then extracted with 300mL EA, the combined organic phase was washed with saturated NaCl solution ($120\text{mL}\times 3$) and dried over
15 anhydrous Na_2SO_4 , concentrated and then purified by column chromatography (PE/EA=1/5) to give 0.705g of compound 1-04-A, and 1.500g of crude compound 1-04-C.

5. Compound 1-05

A mixture of crude compound 1-04-C (0.200g), Bis(pinacolato)diboron (0.270g), tricyclohexyl phosphine (0.039g), Palladium acetate (0.031g) in DMSO (5mL) was stirred under nitrogen for 1h at
20 90°C in an oil bath. The resulting mixture was extracted with EA (50mL), the combined organic phase was washed with saturated NaCl solution ($20\text{mL}\times 3$) and dried over anhydrous Na_2SO_4 , concentrated under reduced pressure to give 0.398g crude Compound 1-05.

6. Compound 2-01

A mixture of crude compound 1-05 (0.398g), 2,4-Dichloropyrimidine (0.304g), K_2CO_3 (0.502g)
25 and $\text{Pd(dppf)Cl}_2\cdot\text{DCM}$ (0.050g) in 1,4- dioxane (10mL) and water (1mL) was stirred under nitrogen for 80min at 60°C in an oil bath. The resulting mixture was added water (10mL) and then extracted with EA ($20\text{mL}\times 2$), the combined organic phase was washed with saturated NaCl solution ($20\text{mL}\times 1$) and dried over anhydrous Na_2SO_4 , concentrated and then purified by column chromatography (PE/EA=1/1) to give 0.165g of Compound 2-01.

7. Compound 2

30 A mixture of compound 2-01 (0.030g), Intermediate M5 (0.025g), Cs_2CO_3 (0.067g), Xanphos (0.005g) and $\text{Pd}_2(\text{dba})_3$ (0.005g) in 1,4- dioxane (2mL) was stirred under nitrogen gas for 3.5h at

110°C in an oil bath. The resulting mixture was added 20mL of mixture solvent (DCM/MeOH =10/1), then filtered, concentrated and purified by column chromatography (DCM/MeOH=8/1), the solid was washed with 1,4-dioxane (5mL) to give 0.024g of Compound 2.

MS(ES⁺):m/z=519.3(M+H)⁺.

5 H-NMR(CDCl₃):δ8.587(s,1H,NH), 8.448-8.457(d,1H,CH), 8.383-8.405(d,1H,CH),
8.280-8.286(d,1H,CH), 8.009-8.013(d,1H,CH), 7.804-7.837(dd,1H,CH), 7.6867.714(dd,1H,CH),
4.681-4.728(m,1H,CH), 3.532(s,2H,CH₂), 3.189-3.256(m,1H,CH₂), 3.008-3.091(m,1H,CH₂),
2.654(s,8H,CH₂), 2.571-2.608(m,2H,CH₂), 2.226-2.299(m,1H,CH₂), 2.111-2.15(m,1H,CH₂),
2.012-2.053(m,2H,CH₂), 1.596-1.612(d,3H,CH₃), 1.174-1.210(t,3H,CH₃).

10 **Example 2-1 Chiral separation of compound 2-01**

In this embodiment, the crude compound 2-01 of the Example 2 is purified directly on a chiral column to provide compound 2-01-A and compound 2-01-B under the following conditions.

Chiral HPLC conditions:

Column	CHIRALPAK AD-H
Column size	2cm *25cm,5μm
Injection	1.0mL
Mobile phase	Hex: IPA =90:10(v/v)
Flow rate	20mL/min
Wave length	UV 220nm
Temperature	25 °C
Sample solution	25.9mg/mL in EtOH:CAN=3:1
Prep- HPLC equipment	Prep- YMC-HPLC
Sample name	Compound 2-01

Example 2-2 Synthesis of compound 2a and compound 2b

15 The crude compound 2-01 is purified by chiral column under the above conditions to give Compound 2-01-A and Compound 2-01-B.

Prepare Compound 2a and Compound 2b essentially as described for step 7 of Example 2 using Compound 2-01-A and Compound 2-01-B respectively.

Furthermore, Optical rotations were measured 3 times for each compound shown as below on a
20 Rudolf polarimeter.

Conditions :

Polarimeter tube length	100mm
Temperature	20 °C
Sample solution	4.0mg/mL in DCM
Sample name	Compound 2a and Compound 2b

Results:

	1 st (°)	2 nd (°)	3 rd (°)	Average (°)
Compound 2a	38.074	38.111	38.078	38.088
Compound 2b	-32.070	-32.134	-31.903	-32.036

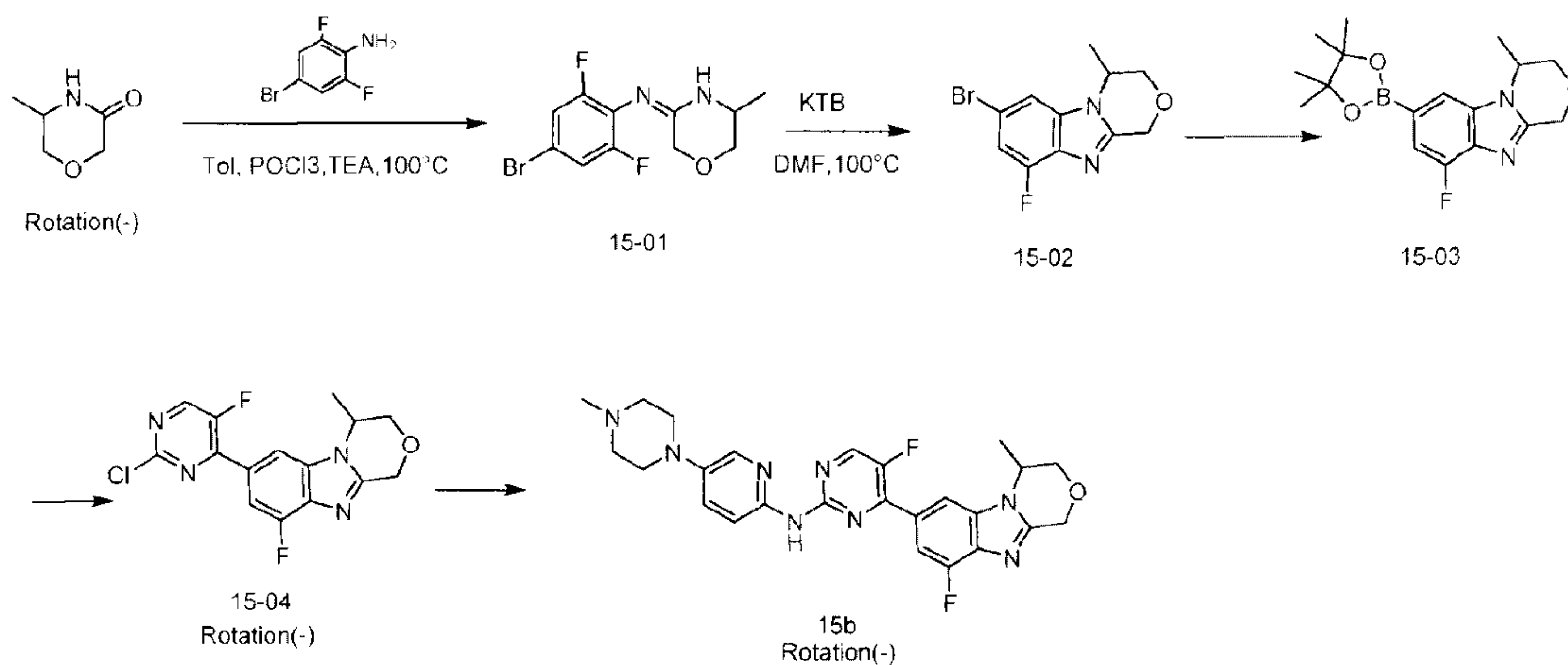
Prepare the following examples (shown in Table 8) essentially as described for Example 2 using the corresponding intermediates. Wherein the two enantiomers of each compound are separated on a chiral column, then test their optical rotation respectively essentially as described for Example 2-2.

Table 8

EX No.	Chemical Name	Structure	Physical Data (MS) (M+H) ⁺	Enantiomers (optical rotation)
1	4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		473.2	1a (+) 1b (-)
2	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		519.3	2a (+) 2b (-)
3	5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		491.2	3a (+) 3b (-)

4	5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(6-((4-methylpiperazin-1-yl)methyl)pyrimidin-3-yl)pyrimidin-2-amine		505.3	4a (+) 4b (-)
5	5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-yl)pyrimidin-2-amine		506.3	5a (+) 5b (-)
6	N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyrimidin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		534.3	6a (+) 6b (-)
7	N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyrimidin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		533.3	7a (+) 7b (-)
8	N-(5-(4-(dimethylamino)piperidin-1-yl)pyrimidin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		519.3	8a (+) 8b (-)
9	(2-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyrimidin-5-yl)(4-methylpiperazin-1-yl)methanone		520.2	9a (+) 9b (-)
10	(6-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyridin-3-yl)(4-methylpiperazin-1-yl)methanone		519.2	10a (+) 10b (-)

11	N5-(2-(diethylamino)ethyl)-N2-(5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)-N5-methylpyridine-2,5-diamine		521.3	11a (+) 11b (-)
12	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-(trifluoromethyl)pyrimidin-2-amine		571.3	12a (+) 12b (-)
13	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-methylpyrimidin-2-amine		517.3	13a (+) 13b (-)
14	5-chloro-N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		535.2	14a (+) 14b (-)

Example 15: Synthesis of compound 15b**1. Compound 15-01**

A mixture of (-) enantiomer of 5-methylmorpholin-3-one (0.200g) and 4-bromo-2,6-difluoroaniline (0.210g) in methylbenzene (20mL) was added POCl₃ (0.510g) and was

heated in an oil bath, TEA (0.210g) was added when the temperature was raised to 110°C, the resulting mixture was reacting at 110°C for 40min. A part of methylbenzene was removed, adjusted pH to 8~9 with Na₂CO₃ after adding water (10mL). Then extracted with EA (20mL×2), the combined organic phase was washed with saturated NaCl (20mL×1) solution and dried over anhydrous Na₂SO₄, concentrated to give 0.280g of Compound 15-01.

2. Compound 15-02

A mixture of Compound 15-01 (0.275g) in DMF (5mL) was added potassium tert-butoxide (0.482g), then stirred for 30min at 70°C in an oil bath. The resulting mixture was extracted with EA (20mL×3), the combined organic phase was washed with saturated NaCl solution (30mL×4) and dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (PE/EA=5/1) to give 0.260g of Compound 15-02.

3. Compound 15-03

A mixture of Compound 15-02 (0.260g), Bis(pinacolato)diboron (0.320g), tricyclohexyl phosphine (0.050g), Palladium acetate (0.040g) in DMSO (6mL) was stirred under nitrogen for 50min at 90°C in an oil bath. The resulting mixture was extracted with EA (20mL×2), the combined organic phase was washed with saturated NaCl solution (10mL×3) and dried over anhydrous Na₂SO₄, concentrated to give 0.258g of Compound 15-03.

4. Compound 15-04

A mixture of Compound 15-03 (0.120g), 2, 4-Dichloro-5-fluoropyrimidine (0.060g), K₂CO₃ (0.099g) and Pd(dppf)Cl₂·DCM (0.012g) in 1,4- dioxane (10mL) and water (1mL) was stirred under nitrogen for 1h at 60°C in an oil bath. The resulting mixture was extracted with EA (20mL×2), the combined organic phase was washed with saturated NaCl solution (20mL×1) and dried over anhydrous Na₂SO₄, concentrated and then purified by preparative HPLC (DCM/MeOH=30/1) to give 0.052g of (-) enantiomer of Compound 15-04.

5. Compound 15b

(-) enantiomer of Compound 15-04(20mg), intermediate M1(0.017g), Cs₂CO₃ (0.056g), Xanphos (0.005g) and Pd₂(dba)₃ (0.005g) in 1,4- dioxane (2mL) was reacted under microwave at 110°C for 1.5hrs through nitrogen stream. The resulting mixture was added 20mL of mixture solvents (DCM/MeOH=10/1), the filtrate was collected by filtration and then concentrated, purified by preparative HPLC (DCM/MeOH=20/1), the resulting solid was washed with n-hexane (10mL) to give 0.014g of Compound 15b.

MS(ES⁺):m/z=493.2(M+H)⁺.

H-NMR(CDCI₃): δ 8.409-8.418(d,1H,CH), 8.211-8.288(m,2H,CH), 8.070-8.077(d,1H,CH),
 8.019-8.023(d,1H,CH), 7.833-7.868(m,1H,CH), 5.118-5.158(d,1H,CH₂), 4.912-5.002(d,1H,CH₂),
 4.522-4.584(m,1H,CH), 4.111-4.168(m,1H,CH₂), 4.039-4.075(m,1H,CH₂),
 3.249-3.275(m,4H,CH₂), 2.724-2.748(m,4H,CH₂), 2.462(s,3H,CH₃), 1.662-1.678(d,3H,
 5 CH₃).

Example 15-1 Synthesis of Compound 15a and Compound 15

Compound 15a and Compound 15b are enantiomers. Prepare Compound 15a essentially as described for Example 15 using (+) enantiomer of 5-methylmorpholin-3-one as starting material.

In another embodiment, crude 5-methylmorpholin-3-one is used as starting material; the crude
 10 compound 15 including Compound 15a and Compound 15b will be obtained in the end.

Furthermore, Optical rotations were measured 3 times for each compound shown as below on a Rudolf polarimeter.

Conditions :

Polarimeter tube length	100mm
Temperature	20 °C
Sample solution	4.6mg/mL in DCM/MeOH(1:1)
Sample name	Compound 15a and Compound 15b

Results:

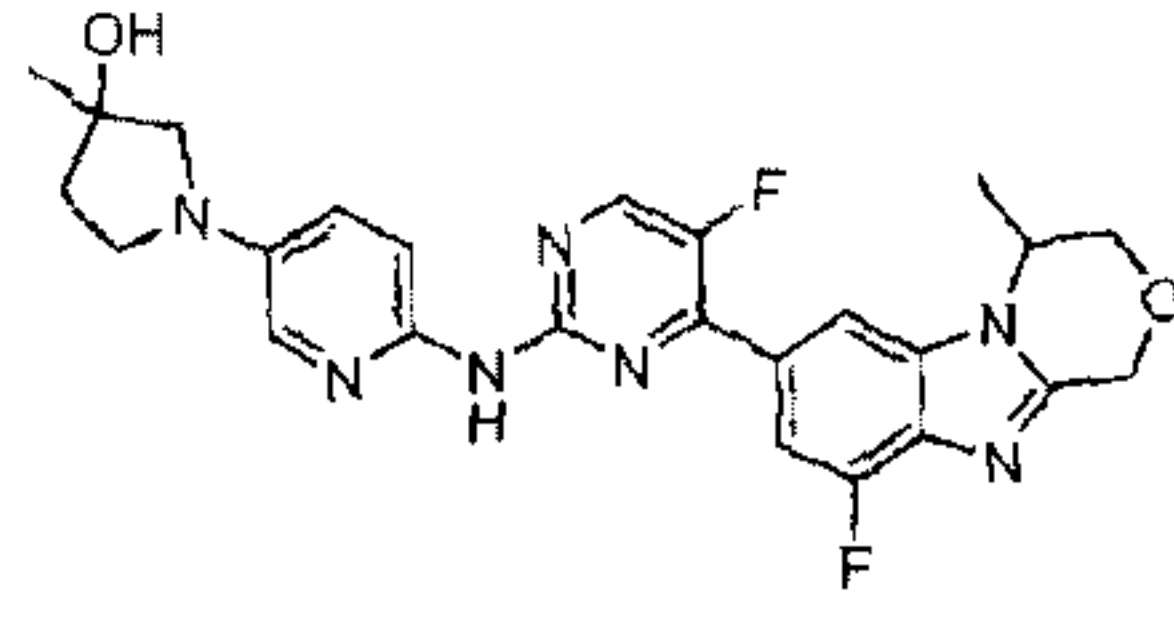
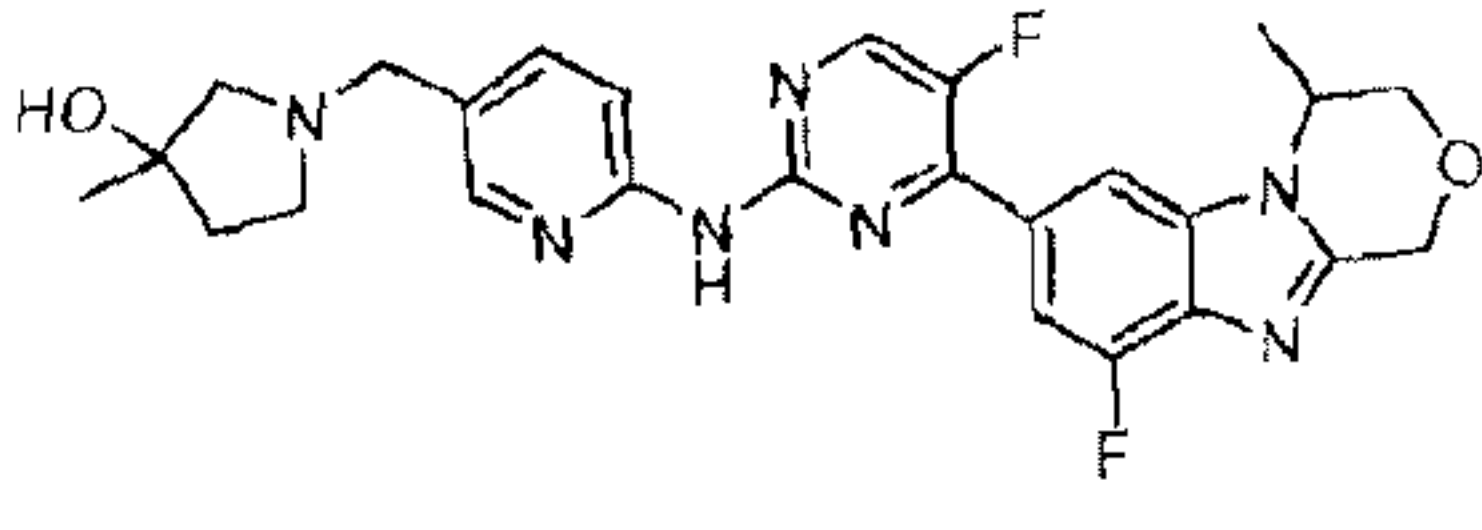
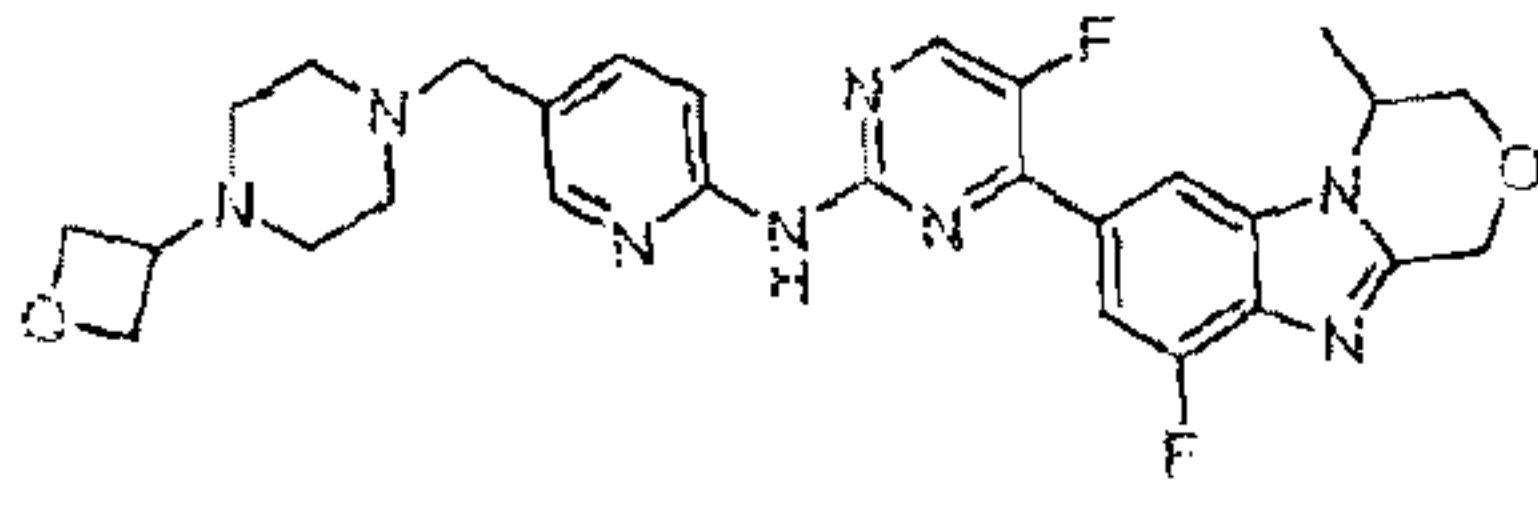
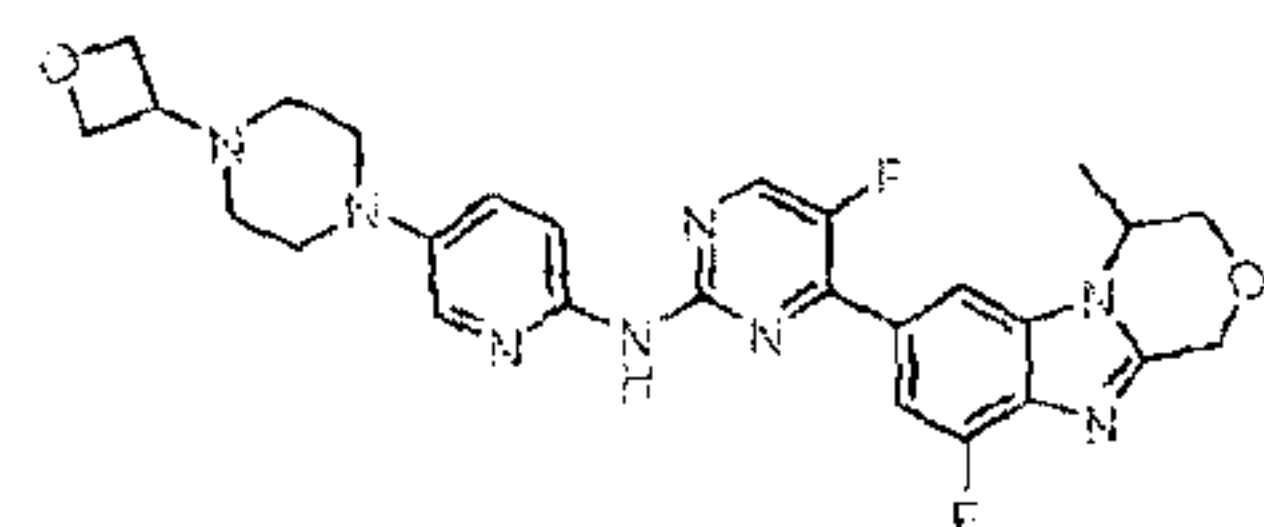
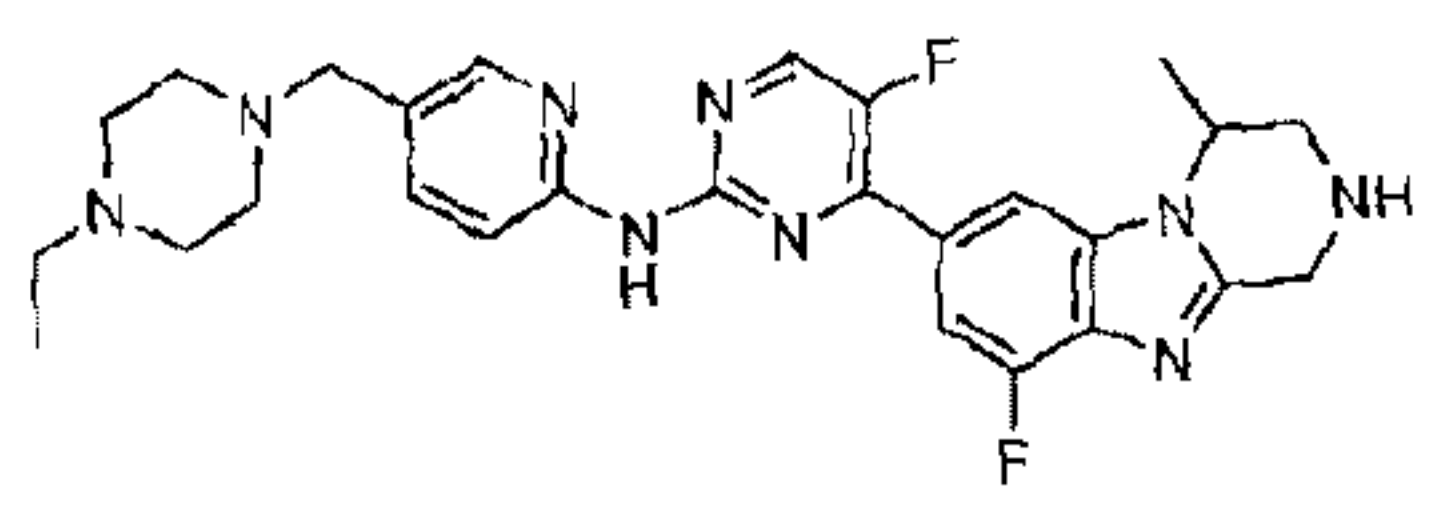
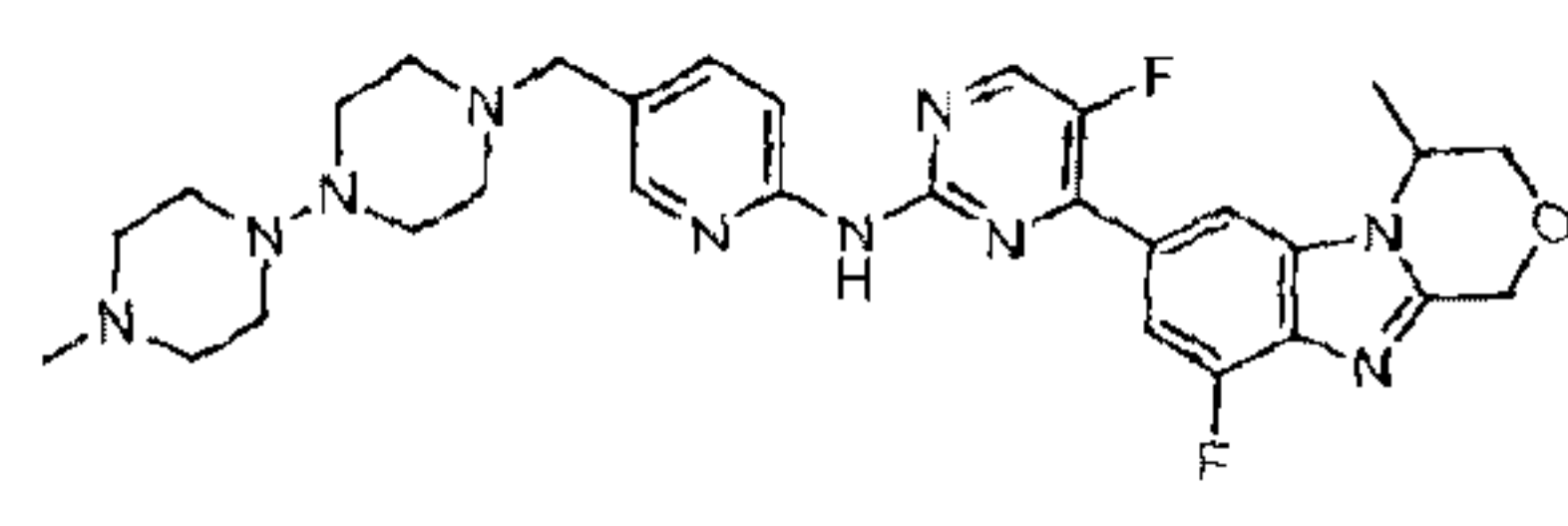
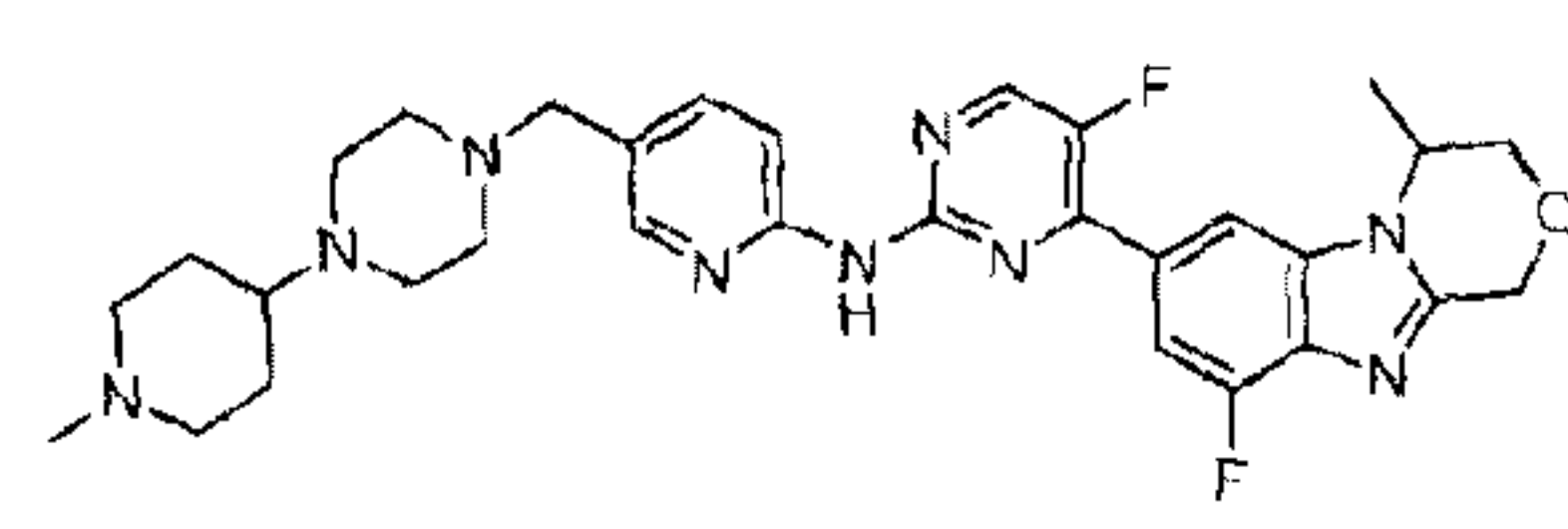
	1 st (°)	2 nd (°)	3 rd (°)	Average (°)
Compound 15a	32.859	32.818	32.809	32.829
Compound 15b	-28.979	-28.840	-28.967	-28.929

15 Prepare the following examples (shown in Table 9) essentially as described for Example 15 using the corresponding intermediates, and using (+) and/or (-) enantiomer of 5-methylmorpholin-3-one as starting material. Their optical rotations were essentially tested as described for Example 15-1.

Table 9

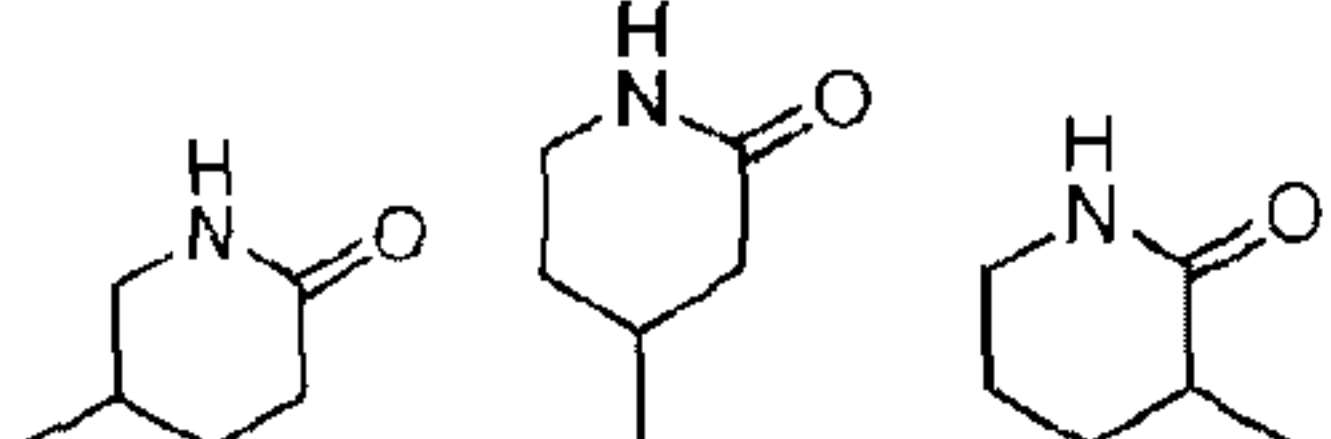
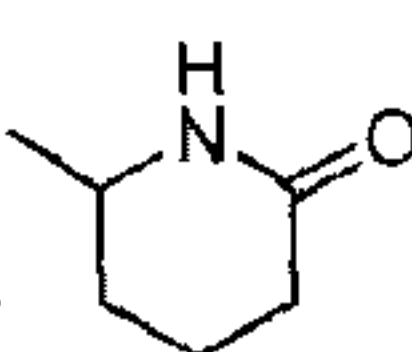
EX No.	Chemical Name	Structure	Physical Data (MS) (M+H) ⁺	Enantiomers (optical rotation)
15	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		493.2	15a (+) 15b (-)
16	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		521.3	16a (+) 16b (-)
17	N-(5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		521.3	17a (+) 17b (-)
18	N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		535.3	18a (+) 18b (-)
19	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		479.2	19a (+) 19b (-)
20	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-ylmethyl)pyridin-2-yl)pyrimidin-2-amine		493.2	20a (+) 20b (-)

21	N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)-6-(4-methylpiperazin-1-yl)pyridazin-3-amine		494.2	21a (+) 21b (-)
22	6-((4-ethylpiperazin-1-yl)methyl)-N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)pyridazin-3-amine		522.2	22a (+) 22b (-)
23	(1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)pyrrolidin-3-yl)methanol		494.2	23a (+) 23b (-)
24	(1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)pyrrolidin-3-yl)methanol		508.2	24a (+) 24b (-)
25	N-(5-(4-cyclopropylpiperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		519.2	25a (+) 25b (-)
26	N-(5-((4-cyclopropylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		533.3	26a (+) 26b (-)
27	2-((1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)piperidin-4-yl)(methyl)amino)ethan-1-ol		565.3	27a (+) 27b (-)

28	1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)-3-methylpyrrolidin-3-ol		494.2	28a (+) 28b (-)
29	1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)-3-methylpyrrolidin-3-ol		508.2	29a (+) 29b (-)
30	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(oxetan-3-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine		549.2	30a (+) 30b (-)
31	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-(oxetan-3-yl)piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		535.2	31a (+) 31b (-)
32	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyrazin-7-yl)pyrimidin-2-amine		520.3	32 (+) 32b (-)
33	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-methyl-[1,1'-bipiperazin]-4-yl)methyl)pyridin-2-yl)pyrimidin-2-amine		591.3	33a (+) 33b (-)
34	5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(1-methylpiperidin-4-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine		590.3	34a (+) 34b (-)

EXAMPLES FOR COMPARISON

Prepare the following comparison examples essentially as described for Example 1, 2 or 15 using the corresponding intermediates or starting materials. For example, Prepare the following comparison example 8, 9 and 10 (shown in Table 10) essentially as described for Example 2 using

5  instead of . And prepare the following comparison example

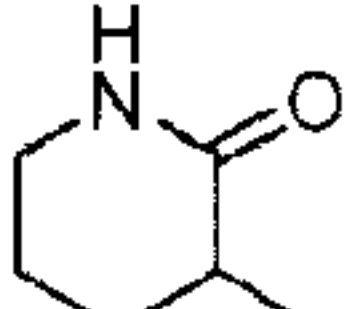
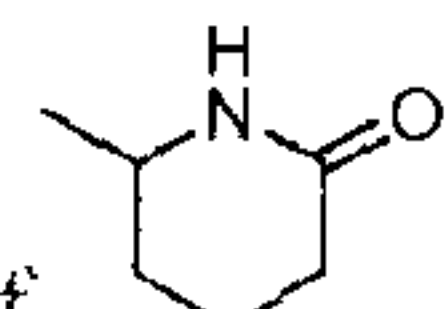
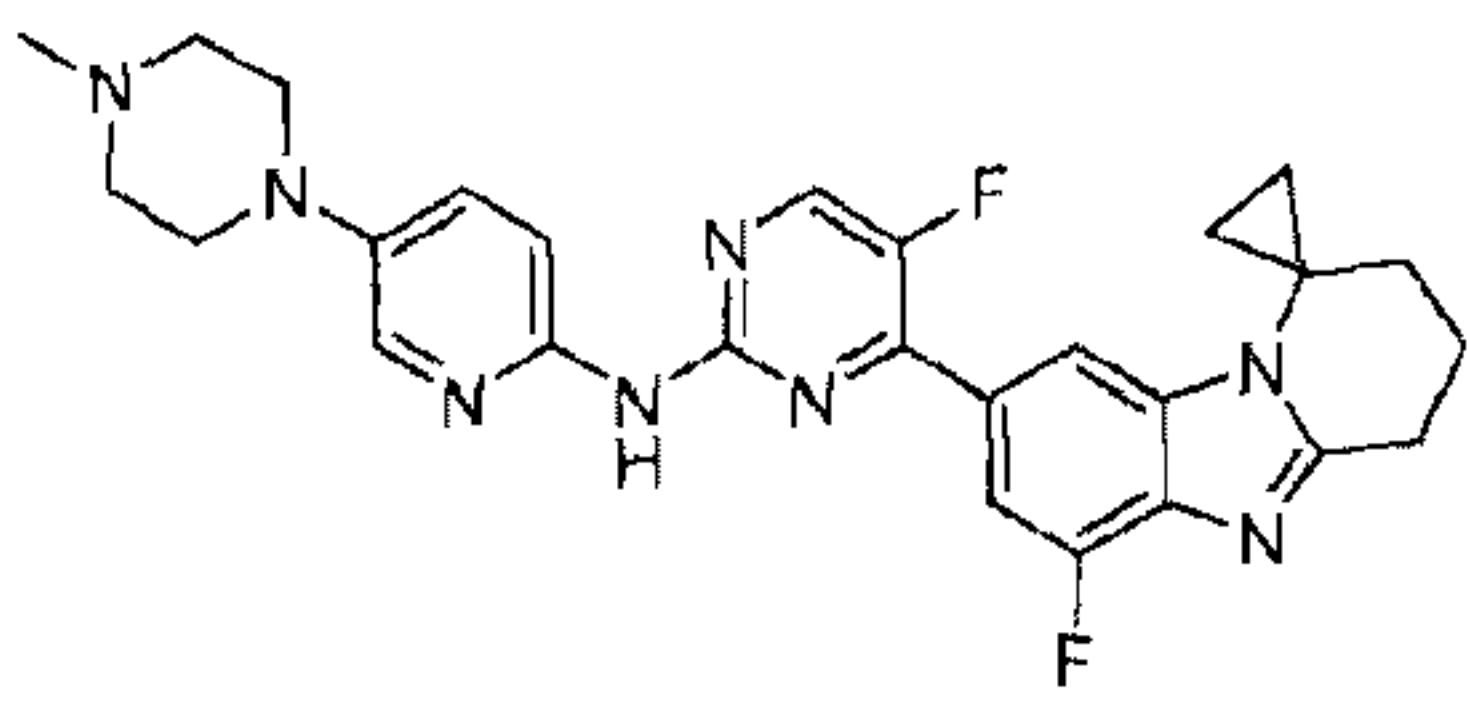
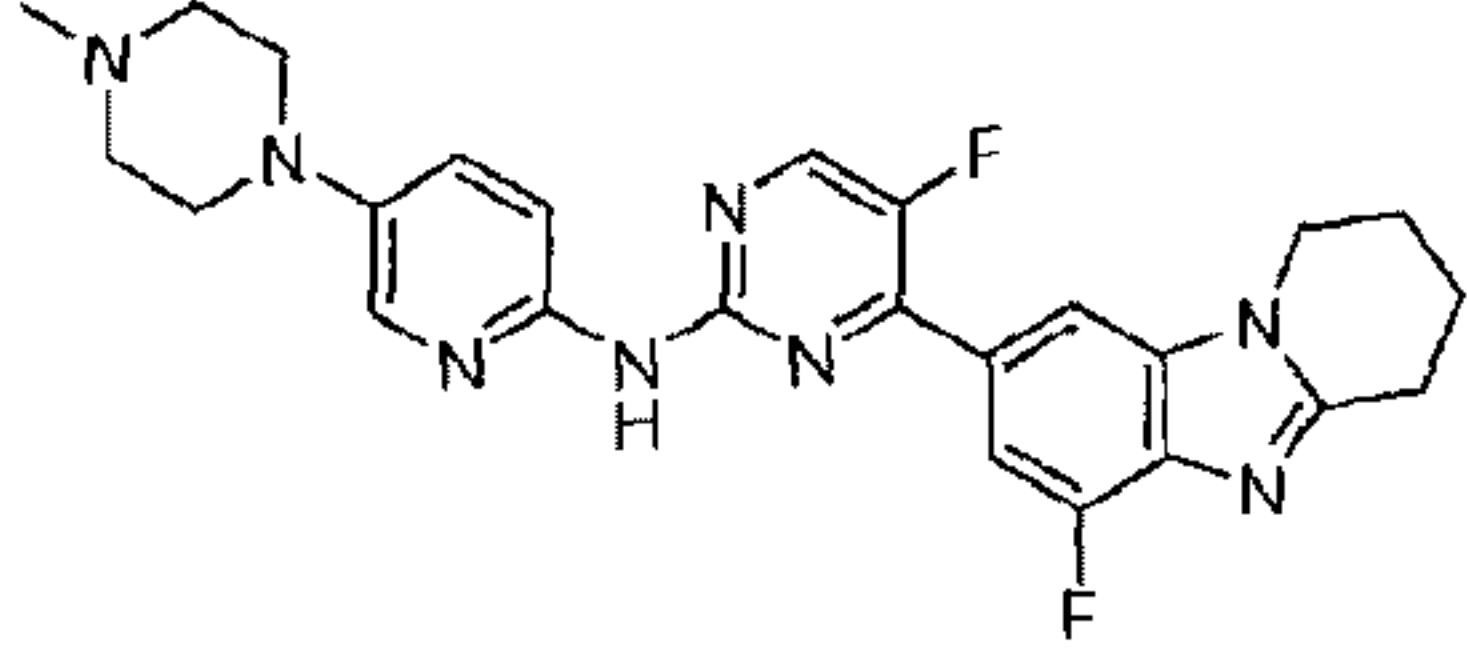
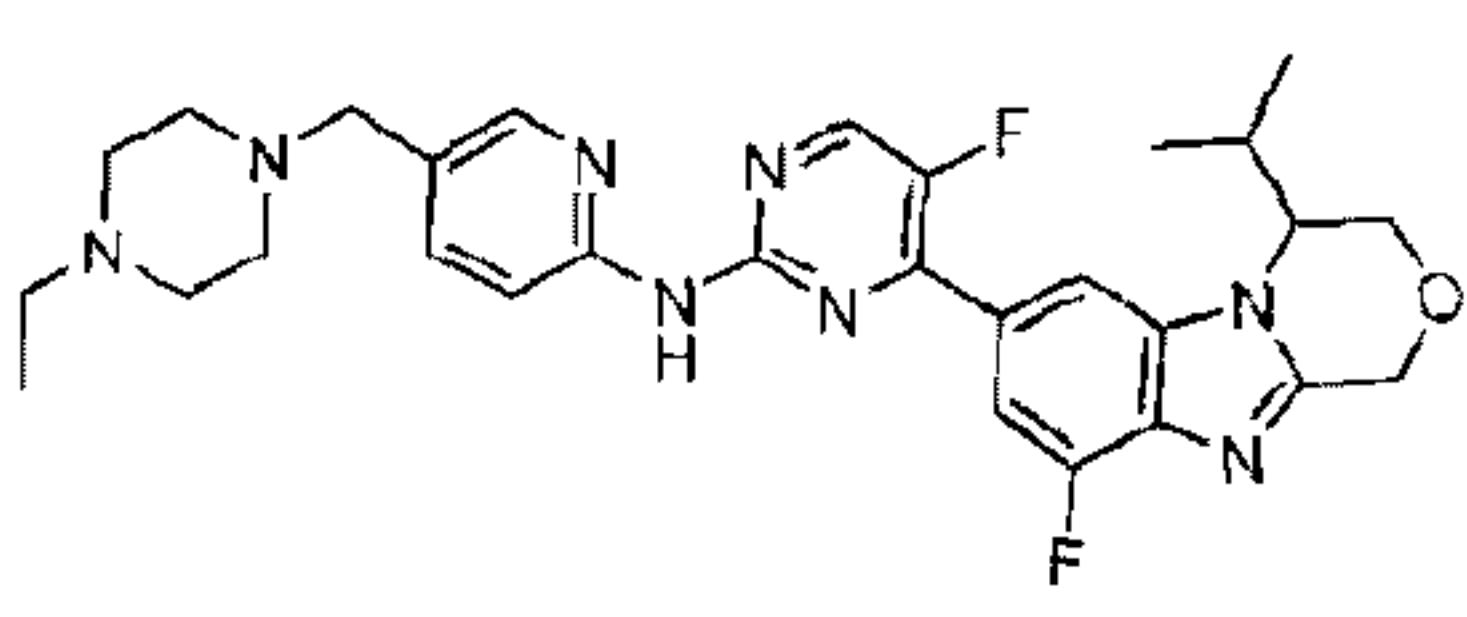
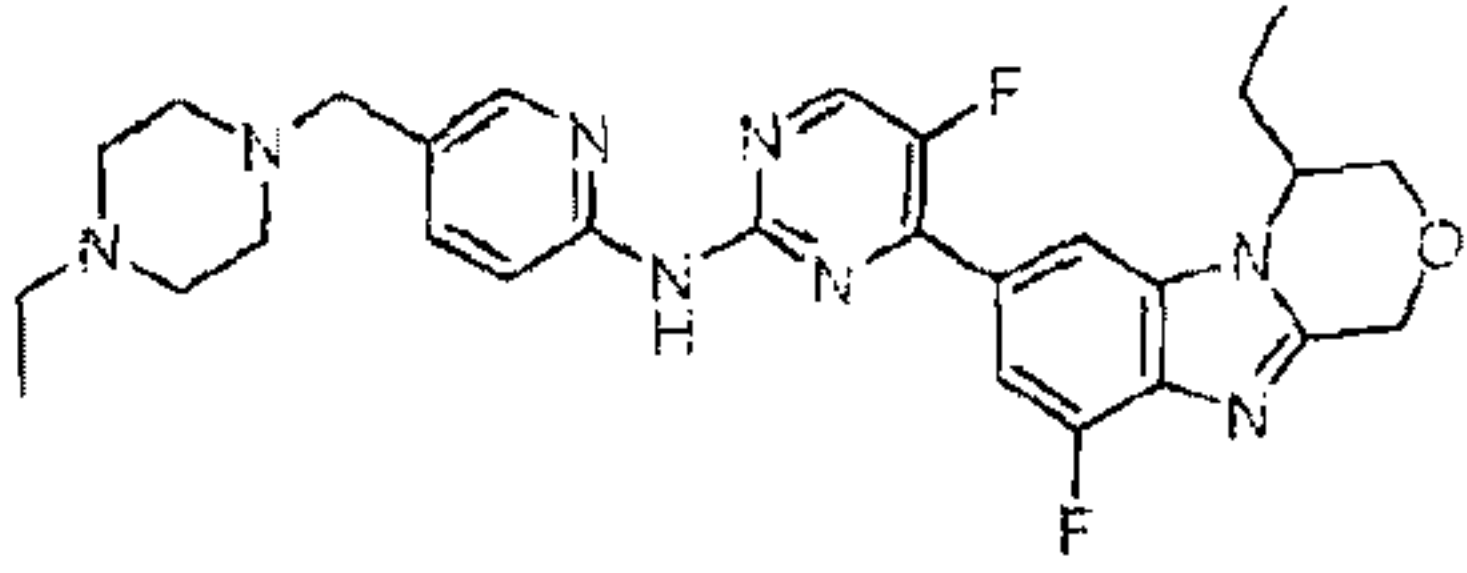
7 essentially as described for Example 1 using  instead of .

Table 10

Com. EX. No.	Chemical Name	Structure	Physical Data (MS) (M+H) ⁺
1	5-fluoro-4-(6-fluoro-3,4-dihydro-2H-spiro[benzo[4,5]imidazo[1,2-a]pyridine-1,1'-cyclopropan]-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		503.2
2	5-fluoro-4-(6-fluoro-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		476.2
3b	(R)-N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-isopropyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine		548.3
4b	(R)-4-(4-ethyl-9-fluoro-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoropyrimidin-2-amine		534.3

5	5-fluoro-4-(6-fluoro-1,1-dimethyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-(oxetan-3-yl)piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		546.3
6	5-fluoro-4-(6-fluoro-1,1-dimethyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(piperazin-1-ylmethyl)pyridin-2-yl)pyrimidin-2-amine		504.3
7	4-(6-fluoro-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine		473.3
8	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-2-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		519.3
9	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-3-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		519.3
10	N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine		519.3

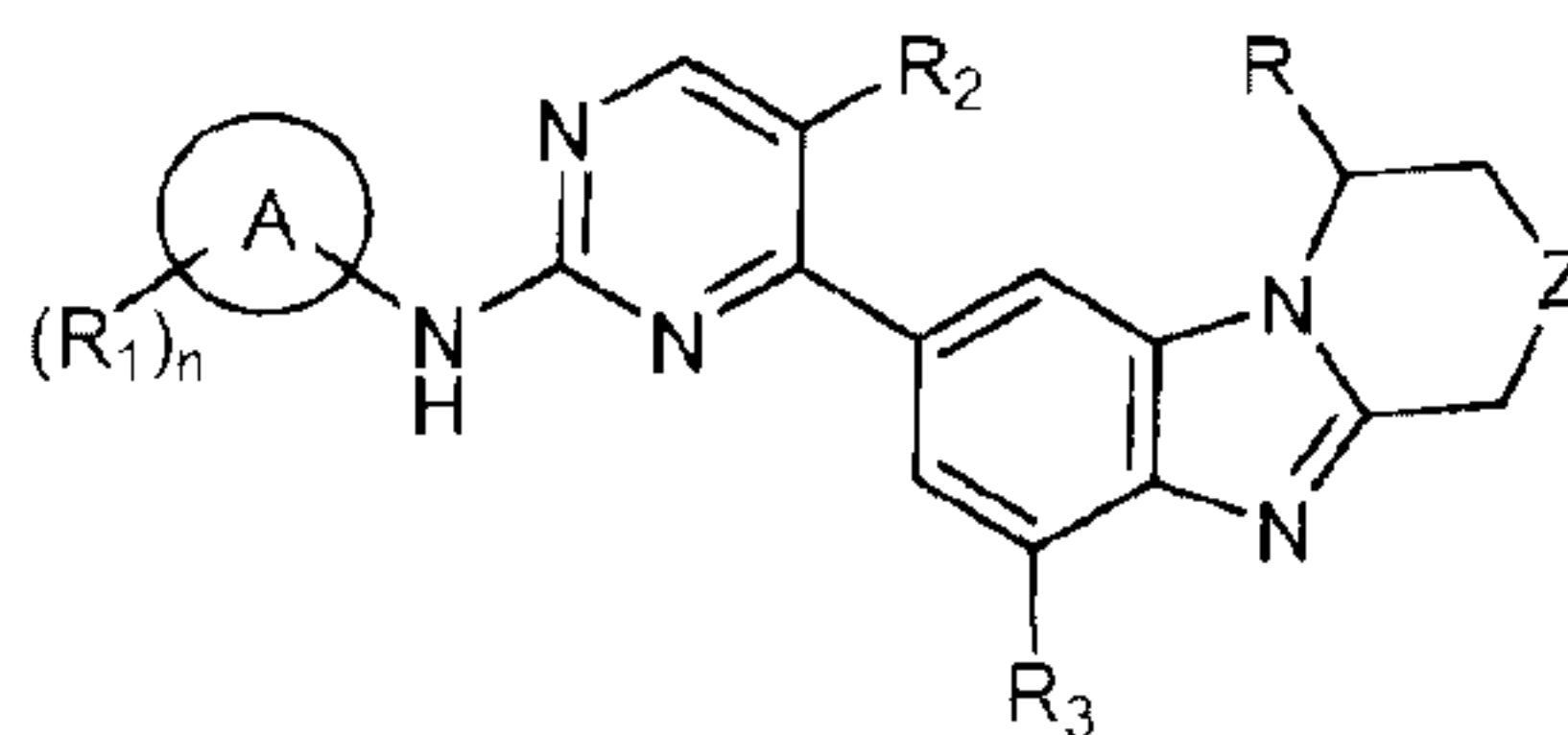
In the above table, The Com. EX. No.3b and 4b are synthesis essentially as described for Example 15, and then test their optical rotation respectively essentially as described for Example 15-1. The Com. EX. No.3b and 4b both show negative optical rotation.

PHARMACOLOGICAL TESTING

- 5 The results of the following assays demonstrate evidence that the compounds exemplified herein are useful as specific CDK4/6 inhibitors and as anticancer agents. As used herein, "IC₅₀"

refers to the concentration of an agent which produces 50% of the maximal inhibitory response possible for the agent.

For illustration conveniently, the following general structure is showed below. Surprisingly, we found that “R” has a critical influence on biological activity, selectivity and safety.



5

Test 1 Comparison of different substituent by Cell Proliferation Assay

Effects of test compounds on *in vitro* proliferation were measured by MTS cell viability assay.

Cell culture

Human colorectal cancer cells (colo-205) is expanded in culture (colo-205 is grown in DMEM media with 12%FBS, 1%P/S, and 1% L-glutamine).

10

MTS cell viability assay:

1. Seed cells at density of 4×10^3 cells per well of 96-wells plates, grow for 24 h;
2. Add varying concentrations of test compounds to the cells;
3. Incubate for 7 days of exposure;
- 15 4. Prepare reagents following the instructions in the Cell Proliferation Assay kit (Promega);
5. Change to serum-free medium with a final volume of 100 μ l/well. Prepare a set of wells with medium only for background subtraction;
6. Add 20 μ l MTS solution containing PMS to each well (final concentration of MTS will be 0.33 mg/mL);
- 20 7. Incubate 1 to 4 h at 37°C in a humidified, 5% CO₂ atmosphere;
8. Record absorbance at 490nm using VICTORTMX5 plate reader (PerkinElmer).

All experimental points were set up in three wells and all experiments were repeated at least three times. IC₅₀ value is calculated from dose-response curve by using software (Graphpad prism 6), and the results are shown in Table 11.

Table 11

Sample	IC ₅₀ (colo-205)/ μ M
LY2835219	0.136
Example 1	0.078
Example 2	0.053
Com. EX. 1	6.644
Com. EX.2	8.897

The above exemplified compounds display anti-tumor activity in this model as shown in Table 11, thus demonstrating that exemplified compounds of the present invention have more potent *in vivo* activity against Rb⁺ tumors. Compared with the known compound LY2835219 (Abemaciclib), the compound of the present invention, for example the compound of Example 1 or 2 has more potent inhibition against Rb⁺ tumors. Compared with the comparison example 1 (hereinafter referred to as Com.EX.1; R is spiro) and the comparison example 2 (hereinafter referred to as Com.EX.2; R is H), the compound of the present invention, for example the compound of Example 1 or 2 (R is methyl) has much more potent inhibition against Rb⁺ tumors.

The above exemplified compounds also display that R is methyl but not H or spiro has much more potent biological activity in this model. From the above results, we can see that type of substituents has significant influence on inhibiting against Rb⁺ tumors.

Test 2 Comparison of different substituent by safety test

Select compounds prepared as described above were assayed the safety according to the change on body weight and whether mortality happened, and the procedures were described herein. Testing compound is prepared in an appropriate vehicle and is administered to BALB/c mice (22-23g) by oral gavage. Body weight and mortality are taken as general measurement of toxicity. Weight loss (%body weight changed) is calculated twice a week by comparing treated groups to vehicle control group during the course of treatment. The compound 2 and 16 demonstrates almost little weight loss, in these models when dosed at 200 mg/kg (qd). But at the same dosage, the comparison examples, for example, Com. EX. 3b and Com. EX. 4b are able to cause much more weight loss, even cause high mortality with 4 and 5 mice death in each group (6 mice) after treatment for 2 weeks.

The results were given in table 12. “*” stands for “weight loss less than 5%”; “**” stands for “weight loss more than 5% and less than 10%”; “***” stands for “weight loss more than 10% and less

than 30%"; "*****" stands for "weight loss more than 30%". "+" stands for "mortality occurred"; "-" stands for "No mortality occurred".

Table 12

Sample	Body weight	observation
LY2835219	*	-
Example 2b	*	-
Example 16b	*	-
Com. EX. 3b	***	+
Com. EX. 4b	***	+

Surprisingly, we found that Com. EX.3b (R is isopropyl) or Com. EX.4b (R is ethyl) has more side effects and toxicity. However, the exemplified compounds of the present invention, for example compound 2b or 16b (R is methyl), are much safer, thus demonstrating that type of substituents has significant influence on safety.

Test 3 the effect of number of substituent by CDK kinase assays

To demonstrate that the compounds exhibit affinity for CDK kinases (CDK2/CycA2, CDK4/CycD3, CDK6/cycD3), CDK kinase assays were performed.

Reaction buffers were prepared as follows: kinase base buffer for CDK2,6 (50 mM HEPES, pH 7.5; 0.0015% Brij-35; 10 mM MgCl₂; 2 mM DTT); Kinase base buffer for CDK4 (20 mM HEPES, pH 7.5; 0.01% Triton X-100; 10 mM MgCl₂; 2 mM DTT); Stop buffer (100 mM HEPES, pH 7.5; 0.015% Brij-35; 0.2% Coating Reagent #3; 50 mM EDTA).

Enzyme reaction protocol:

1) Dilute the compound to 50X of the final desired highest concentration in reaction by 100% DMSO. Transfer 100μL of this compound dilution to a well in a 96-well plate. Then, serially dilute the compound by transferring 30μL to 60μL of 100% DMSO in the next well and so forth for a total of 10 concentrations. Add 100μL of 100% DMSO to two empty wells for no compound control and no enzyme control in the same 96-well plate. Mark the plate as source plate.

2) Prepare intermediate plate by transferring 10μL of compound from source plate to a new 96-well plate containing 90μL of kinase buffer as the intermediate plate.

3) Transfer 5μL of compound from the 96-well intermediate plate to a 384-well plate in duplicates.

4) Add 10μL of 2.5x enzyme solution to each well of the 384-well assay plate.

5) Incubate at room temperature for 10min.

6) Add 10 μ L of 2.5x substrate solution prepared by adding FAM-labeled peptide and ATP in the kinase base buffer. Reaction concentrations for enzymes and substrates as following table (Table 13):

Table 13

Enzyme	Enzyme (nM)	ATP (μ M)	Peptide	Peptide concentration(μ M)
CDK2	10	30	P18	3
CDK4	10	280	P8	3
CDK6	15	800	P8	3

5 7) Incubate at 28°C for specified period of time.

8) Add 25 μ L of stop buffer to stop reaction.

9) Collect data on Caliper. Then convert conversion values to inhibition values.

Percent inhibition = (max-conversion)/(max-min)*100.

“max” stands for DMSO control and “min” stands for low control herein.

10 10) Curve fitting using percent inhibition in XLFit excel add-in version 4.3.1 to obtain IC₅₀ values. Equation used is: $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (\text{IC}_{50}/X)^{\text{HillSlope}})$. Wherein, Y is inhibition percentage (%); X is concentration of the test compound.

The results are expressed as IC₅₀ value which is shown in Table 14.

Table 14

Sample	IC ₅₀ (CDK2)/ μ M	IC ₅₀ (CDK4)/ μ M	IC ₅₀ (CDK6)/ μ M
LY2835219	0.039	0.002	0.022
Example 1	>0.3	0.002	0.023
Example 2	>0.3	0.002	0.031
Example 16b	>0.3	0.003	0.040
Com. EX. 5	0.040	0.003	0.017
Com. EX. 6	0.040	0.007	0.014

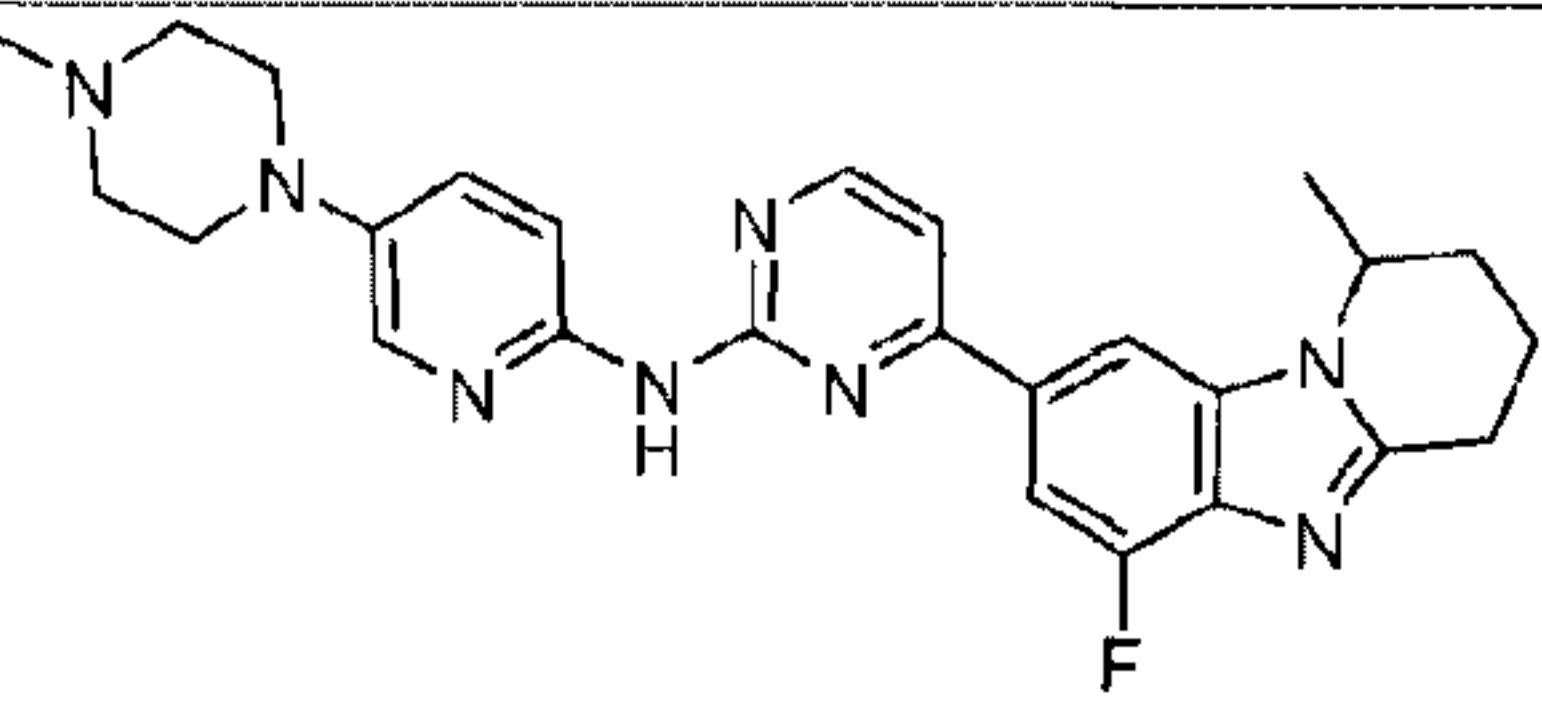
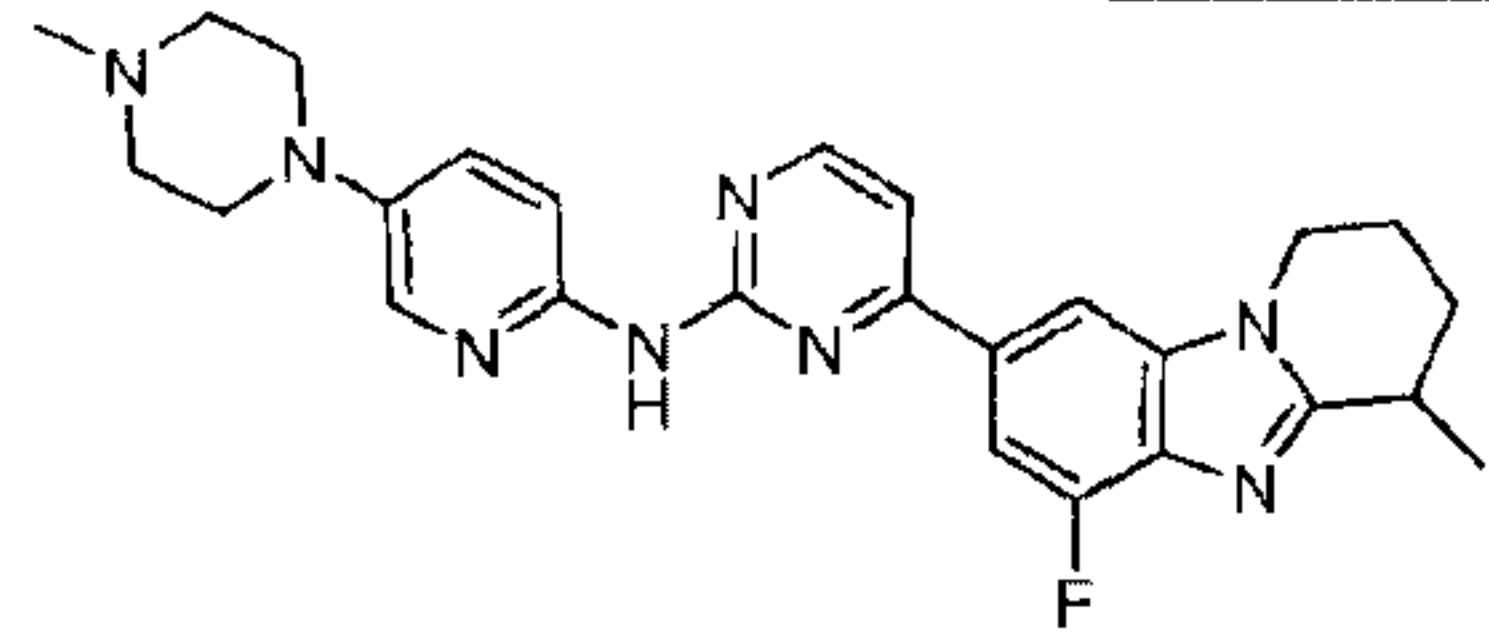
15 As shown in the above table, we can see the number of methyl has very important influence on selectivity. Surprisingly, the exemplified compounds of the present invention display an IC₅₀ of >0.3 μ M in the above CDK2 kinase inhibition assay and an IC₅₀ of $\leq$ 0.04 μ M in the above CDK4/6 kinase inhibition assay as shown in Table 14. It demonstrates that the exemplified compounds of the present invention are more selective inhibitors of CDK4/6 kinase activity. Thus it shows that
20 the exemplified compounds (R represents only one methyl) are more specific inhibitors of CDK4/6,

compared with the known compound LY2835219 and comparison example, for example Com. EX. 5 or Com. EX. 6 (R represents two methyl).

Test 4 the effect of substituent site

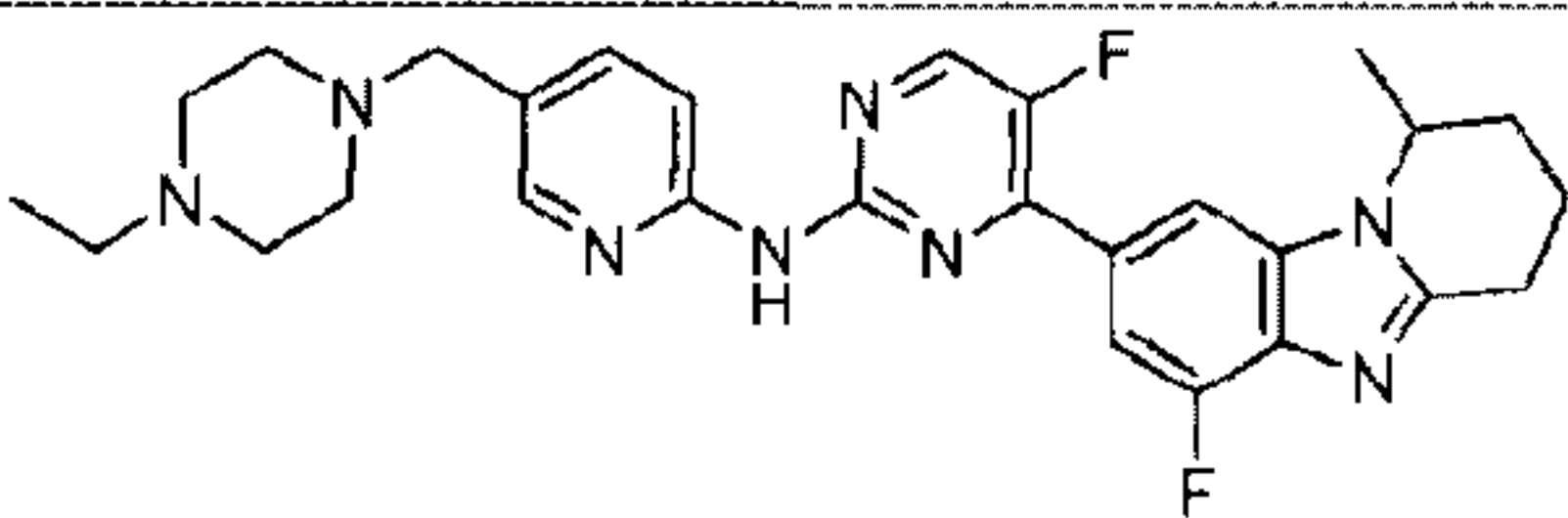
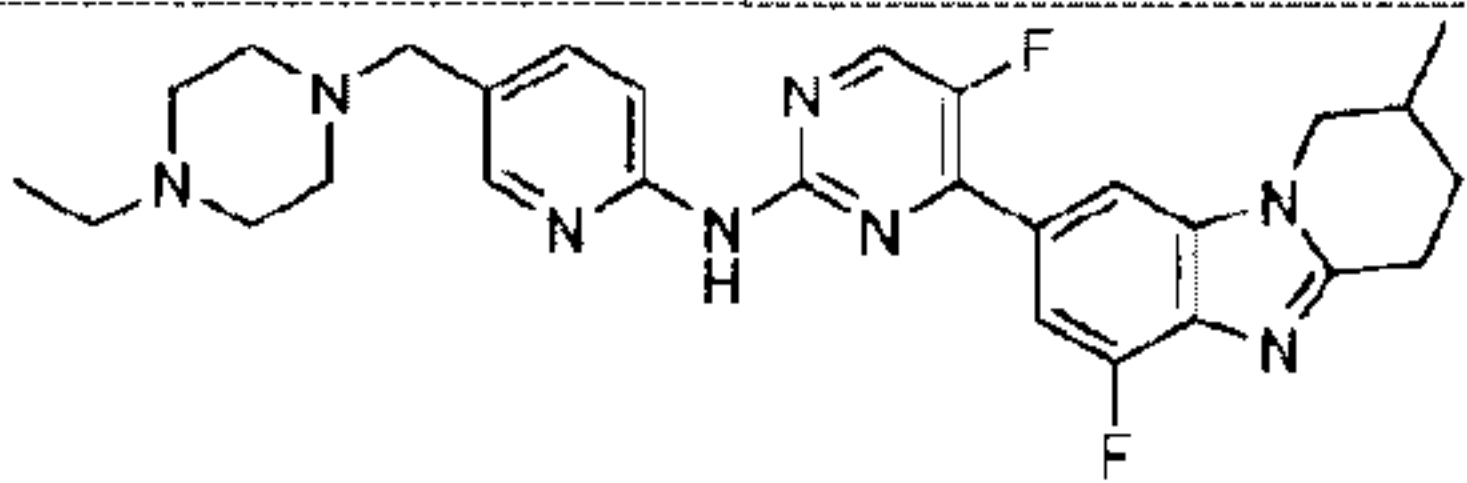
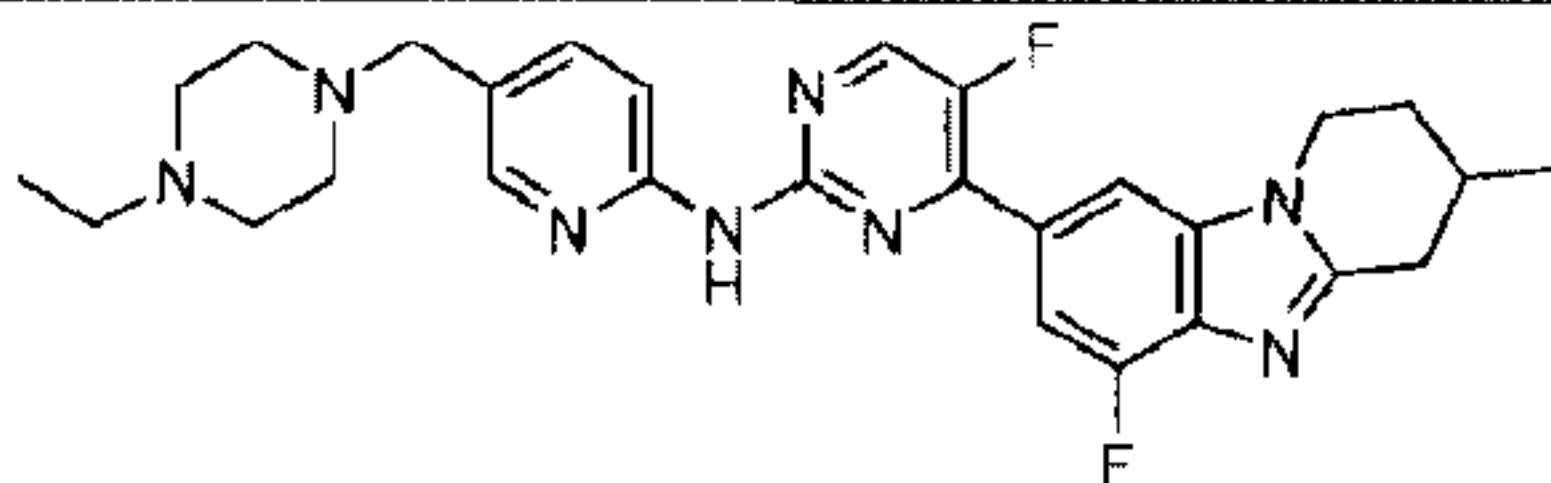
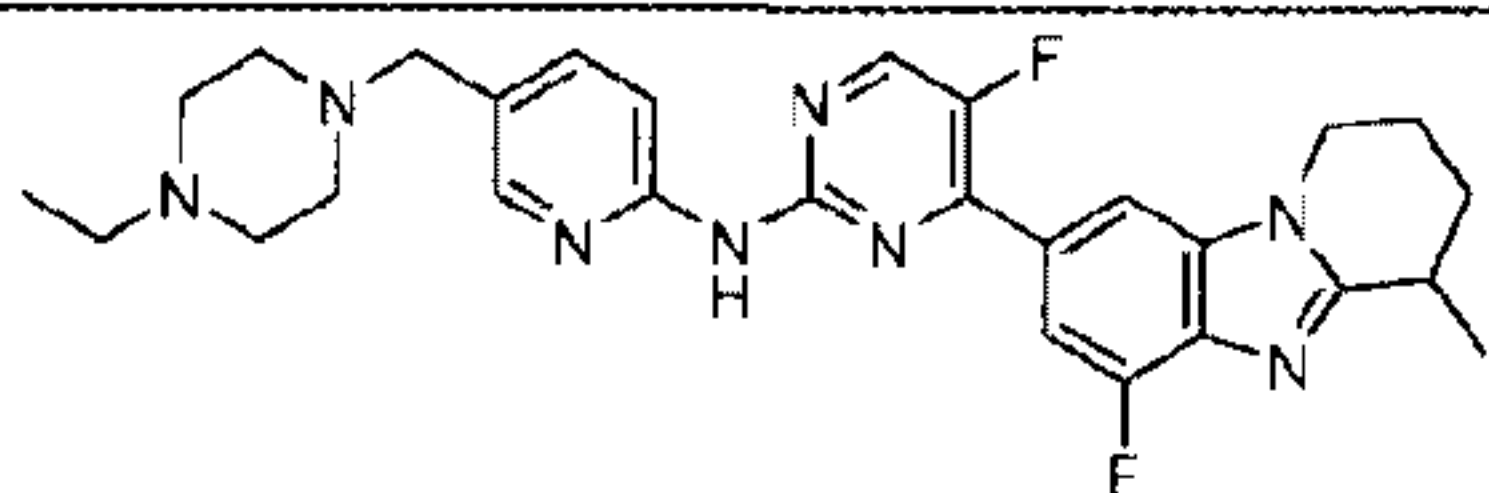
Select compound prepared as described above were assayed according to the biological procedures described herein. The results were showed as in below table.

Table 15

Sample	Structure	IC ₅₀ (CDK4)/μM	IC ₅₀ (CDK6)/μM
Example 1		0.002	0.023
Com. EX. No.7		0.057	0.110

Further, a Head-to-Head comparison of Example 2 and its comparison example 8, 9 or 10 was shown in the following table. The results were given in table 16. “++++” stands for “IC₅₀ value less than 0.2μM”; “+++” stands for “IC₅₀ value more than 0.2μMand less than 1.0μM”; “++” stands for “IC₅₀ value more than 1.0μMand less than 2.0μM”; “+” stands for “IC₅₀ value more than 2.0μM”.

Table 16

Sample	Structure	IC ₅₀ (colo-205)/μM
Example 2		++++
Com. EX. No.8		++
Com. EX. No.9		++
Com. EX. No.10		++

As shown in the above table 15 and 16, we can see substituent site also plays an important role on biological activity. The effect on biological activity resulting from the site change of substituents is significantly unexpected.

As shown in the results presented above, we can know that "R" of the 6-membered heterocyclic ring has a critical influence on biological activity, selectivity and safety. Surprisingly, when there is one and only one methyl in the said 6-membered heterocyclic ring, the effects will be obtained at least as follows:

- ① improved biological activity;
- ② good selective; and
- ③ low side effects.

Test 5 the effect of opticity

Select compounds prepared as described above were assayed according to the biological procedures described as Test 3 (CDK kinase assays). The results were showed as in below table.

Table 17

Example	IC ₅₀ (CDK4)/nM	IC ₅₀ (CDK6)/nM	Example	IC ₅₀ (CDK4)/nM	IC ₅₀ (CDK6)/nM
LY2835219	2	22			
Compound 1a	3.0	121	Compound 1b	1.7	23
Compound 2a	4.1	108	Compound 2b	1.9	22
Compound 15a	6.7	230	Compound 15b	1.8	23
Compound 16a	17.6	>300	Compound 16b	3.4	45

As shown in the above table, we can see compound 1b, 2b, 15b, 16b is more potent than compound 1a, 2a, 15a, 16a in inhibiting CDK4/6 respectively, thus demonstrating that (-) enantiomers of the present compounds are advantageous over the (+) enantiomers.

In some instances, the compound disclosed herein is administered where one enantiomer [e.g., the (-) enantiomer or (+) enantiomer] is present in high enantiomeric excess. In one instance, the enantiomer of compound 1b having a negative optical rotation, e.g., -35.394° (c=3.0mg/mL, EtOH) has greater activity against CDK4/6 enzyme than the enantiomer (compound 1a) that has a positive optical rotation of +30.325° (c=3.0mg/mL, EtOH). In another instance, the enantiomer of compound 2b having a negative optical rotation, e.g., -32.036° (c=4.0mg/mL, DCM) has greater activity against CDK4/6 enzyme than the enantiomer that has a positive optical rotation of +38.088° (c=4.0mg/mL,

DCM). In other instances, the enantiomer of compound 15b having a negative optical rotation, e.g., -28.929° (c=4.6mg/mL, DCM/MeOH=1:1) has greater activity against CDK4/6 enzyme than the enantiomer that has a positive optical rotation of +32.829° (c=4.6mg/mL, DCM/MeOH=1:1).

Test 6 Inhibitory activity and selectivity test on other subtypes of CDK kinase at molecular level

5 The representative compound 2b of the present invention was used as a test compound, and compared with the positive control drug LY2835219 (Abemaciclib) to compare CDK kinase inhibitory activity and selective specificity between them.

The mechanism of this method is shown in formula (II). The kinase catalyzes the phosphorylation of the protein substrate to label the ³³P on the ³³P-labeled ATP (γ-³³P-ATP) to the protein substrate in the reaction system; the reaction system was spotted on P81 ion-exchange membrane, and the membrane was washed extensively with 0.75% phosphate buffer; the radioactively-phosphorylated substrate was left on the membrane, and the kinase activity was reflected by recording the intensity of the substrate protein radiolabel.



15 Formula (II)

Data was processed with Prism4 Software (GraphPad), and the curve fitting formula was:

$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogIC}_{50} - X) * \text{HillSlope}))}$; wherein, Y is percent inhibition (%); X is logarithm of concentration of the inhibitor.

Results: Through the screening of various CDK kinases, it was found that the representative compound 2, 2a, and 2b have an IC₅₀ of greater than 0.4μM for inhibiting CDK1/2/7/9, which is tens to thousands of fold higher than that of CDK4/6. (See table 18)

Table 18: CDK kinase inhibitory activity

Kinase	IC ₅₀ (nM)			
	LY2835219	Compound 2	Compound 2a	Compound 2b
CDK1/cyclin B	308	2350	3573	1683
CDK2/cyclin E	90	474	596	441
CDK7/cyclin H	2071	1050	2370	664
CDK9/cyclin TI	111	572	779	649

Conclusion: At the molecular level, the representative compound 2 and compound 2b of the present invention showed strong inhibitory effect on CDK4/6 and weak inhibitory effect on CDK1/2/7/9, indicating that compound 2 and compound 2b is a CDK4/6 kinase inhibitor with excellent selectivity. Compound 2a showed strong inhibitory effect on CDK4, slight inhibitory

effect on CDK6, and very weak inhibitory effect on CDK1/2/7/9, indicating that compound 2a is a CDK4 kinase inhibitor with extreme selectivity and a CDK6 kinase inhibitor with good selectivity. Additionally, the selectivity of the representative compounds of the present invention between CDK1/2/9 and CDK4/6 was significantly higher than that of LY2835219 (Abemaciclib).

5 Test 7 Tumor regression effect on JeKo-1 xenograft animal model

JeKo-1 cells were cultured in RPMI 1640 medium containing 20% fetal bovine serum. Exponentially growing JeKo-1 cells were collected and resuspended in PBS to a suitable concentration for NOD/SCID mice subcutaneous tumor inoculation. Seventy female mice were inoculated subcutaneously on the right with 5×10^6 JeKo-1 cells, resuspended in PBS and matrigel (1:1). When the average tumor volume reached 134mm^3 , the mice were randomly grouped according to the size of the tumor and were administrated. Forty-eight mice were divided into the experimental group, and the remaining twenty-two mice were not used for experiment. Tumor volume is calculated as: long diameter $\times$ short diameter²/2. The test was divided into solvent control group, test drug representative compound 2b (10mg/kg), test drug representative compound 2b (25mg/kg), test drug representative compound 2b (50mg/kg), test drug representative compound 2b (100mg/kg), a total of 6 groups with each of 8 mice, and the mice were administered orally by gavage once a day and then continuous administration for 19 days. Efficacy is evaluated according to the relative tumor growth inhibition rate of TGI.

The calculation formula is as follows: $\text{TGI}(\%) = (\text{C}-\text{T})/\text{C} \times 100\%$ (C and T are the average tumor weight of the solvent control group and the average tumor weight of the treatment group, respectively). The higher the TGI(%) value illustrates the better the potency; and vice versa.

Results: Compound 2b demonstrates excellent anti-tumor activity.

Table 19 Anti-tumor efficacy evaluation of representative compound 2b on JeKo-1 xenograft model

Group	Dose(mg/kg)	Relative tumor growth inhibition rate(TGI(%))	<i>p</i> Value ^d
Solvent control	--	--	--
Compound 2b	10	42.7	0.087
Compound 2b	25	73.8	0.003
Compound 2b	50	98.3	0.001
Compound 2b	100	104.5	0.001

Note:

a: *p* value is the comparative analysis of tumor volume for the treatment group and the solvent control group.

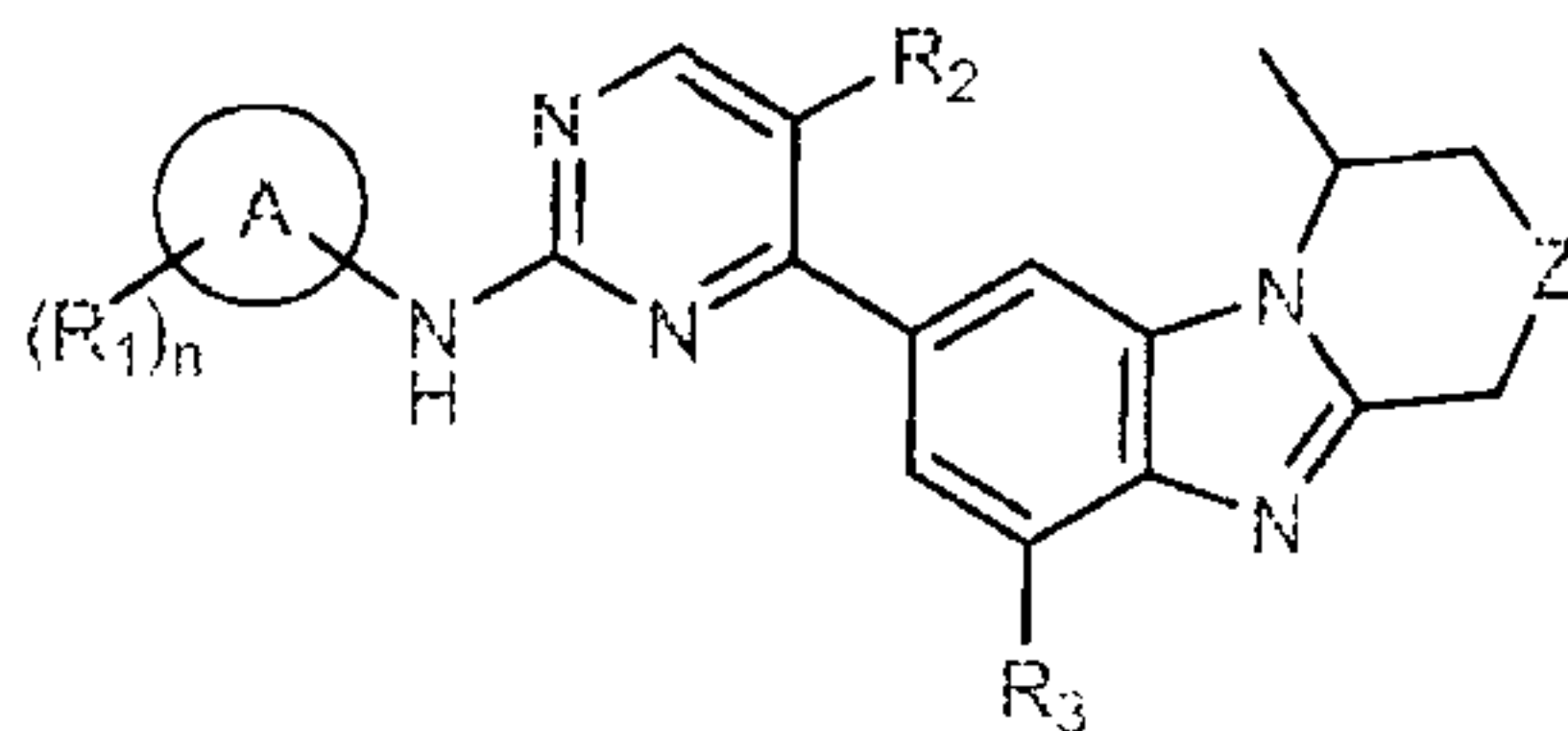
Accordingly, in some instances, it is beneficial to administer to a subject a compound 1, 2 or 15
5 having a high enantiomeric excess of the enantiomer having a negative optical rotation to treat a disease. Unexpectedly, the optically pure (-) enantiomer of the present compound, including but not limited to compound 1b, 2b or 15b is more potent drug for treating a disease mediated by CDK4/6 in a subject.

A number of embodiments of the invention have been described. Nevertheless, it will be
10 understood that various modifications may be made without departing from the spirit and scope of the invention. Other embodiments are in the claims.

THE CLAIMS

What is claimed is:

1. A compound of Formula I, or a stereoisomer, a tautomer, a polymorph, a solvate, a pharmaceutically acceptable salt, or a prodrug thereof,



Formula I

wherein,

ring A is aryl or heteroaryl;

Z is selected from the group consisting of CH₂, NH, O and S;

- 10 R₁ is independently selected from the group consisting of hydrogen, halogen, CN, NO₂, OH, NH₂, C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclyl-(CH₂)_m-, aryl-C₁₋₆alkyl-, heteroaryl-C₁₋₆alkyl-, -NR₁₂R₁₃, -NR₁₂-C₁₋₆alkylene-NR₁₂R₁₃, and heterocyclyl-C(O)-, wherein the C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclyl-(CH₂)_m-, aryl-C₁₋₆alkyl-, heteroaryl-C₁₋₆alkyl-, or heterocyclyl-C(O)- are each unsubstituted or substituted with
15 at least one substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, heterocyclyl, -NR₁₂R₁₃, or -(CH₂)_t-OH;

- R₂ and R₃ are each independently selected from H, OH, CN, NO₂, NH₂, halogen, C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl; wherein the C₁₋₈alkyl, C₁₋₈alkoxy, C₃₋₈cycloalkyl, aryl, heteroaryl, heterocyclyl are each unsubstituted or substituted with at least one
20 substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, or heterocyclyl;

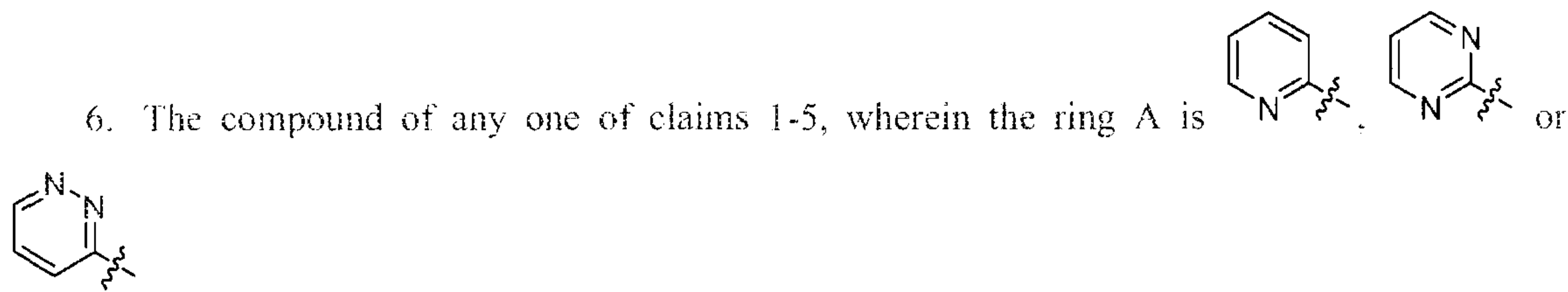
R₁₂ and R₁₃ are each independently selected from H, C₁₋₈alkyl, aryl, heteroaryl, heterocyclyl, C₃₋₈cycloalkyl; wherein the C₁₋₈alkyl, aryl, heteroaryl, heterocyclyl, or C₃₋₈cycloalkyl are each unsubstituted or substituted with at least one substituent selected from halogen, hydroxyl, C₁₋₈alkyl, C₃₋₈cycloalkyl, or heterocyclyl;

- 25 m is 0, 1, 2, 3 or 4;

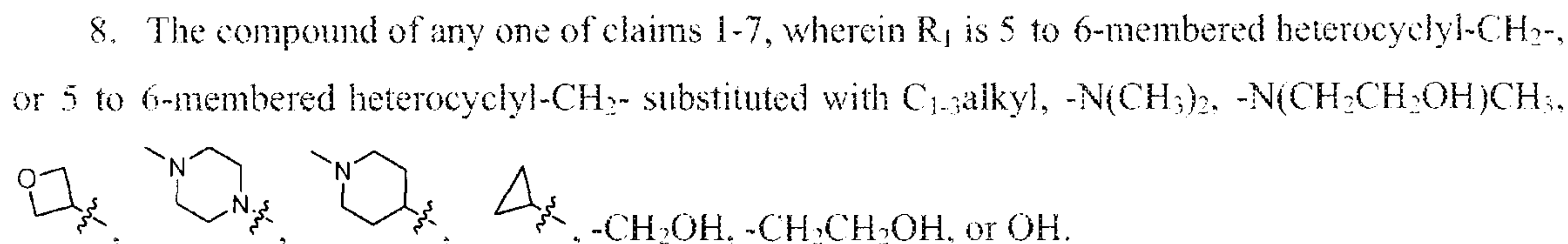
n is 0, 1, 2, 3 or 4;

t is 0, 1, 2, 3 or 4.

2. The compound of claim 1, wherein Z is CH₂.
3. The compound of claim 1, wherein Z is O.
4. The compound of any one of claims 1-3, wherein the ring A is a 6-membered heteroaryl comprising one or two heteroatoms of N.
5. The compound of any one of claims 1-4, wherein the ring A is pyridyl, pyrimidinyl, or pyridazinyl.

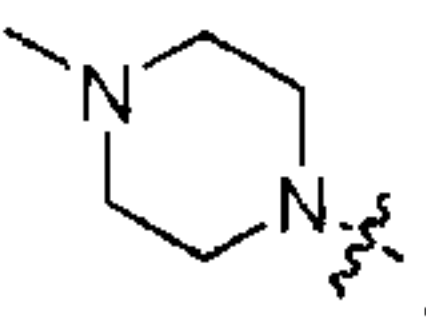
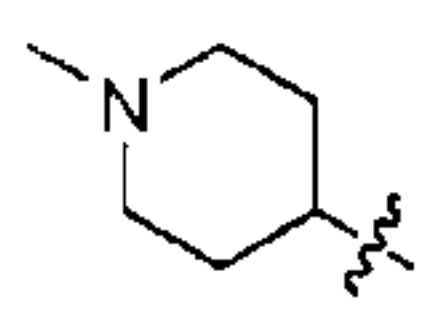
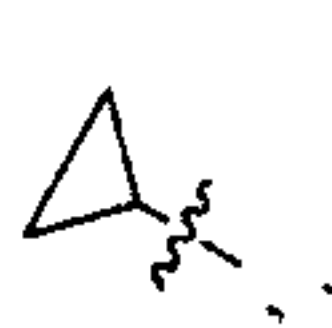


7. The compound of any one of claims 1-6, wherein R₁ is heterocyclyl-(CH₂)_m-, or heterocyclyl-(CH₂)_m- substituted with C₁₋₈alkyl, NR₁₂R₁₃, 4 to 6-membered heterocyclyl, C₃₋₆cycloalkyl, or (CH₂)_t-OH.



9. The compound of any one of claims 1-8, wherein R₁ is 6-membered heterocyclyl-CH₂-, or 6-membered heterocyclyl-CH₂- substituted with methyl or ethyl.

10. The compound of any one of claims 1-6, wherein R₁ is heterocyclyl, or heterocyclyl substituted with C₁₋₈alkyl, NR₁₂R₁₃, 4 to 6-heterocyclyl, C₃₋₆cycloalkyl, or (CH₂)_t-OH.

11. The compound of any one of claims 1-6 or 10, wherein R₁ is 5 to 6-membered heterocyclyl, or 5 to 6-membered heterocyclyl substituted with C₁₋₃alkyl, -N(CH₃)₂, -N(CH₂CH₂OH)CH₃, , , , , -CH₂OH, -CH₂CH₂OH, or OH.

12. The compound of any one of claims 1-6, 10, or 11, wherein R₁ is 6-membered heterocyclyl, or 6-membered heterocyclyl substituted with methyl or ethyl.

13. The compound of any one of claims 1-6, wherein R₁ is 6-membered heterocyclyl-C(O)- or 6-membered heterocyclyl-C(O)- substituted with C₁₋₃alkyl.

14. The compound of any one of claims 1-6 or 13, wherein R₁ is 6-membered heterocyclyl-C(O)- substituted with methyl.

15. The compound of any one of claims 1-14, wherein the heterocyclyl comprises one or two heteroatoms of N or O as ring atoms.

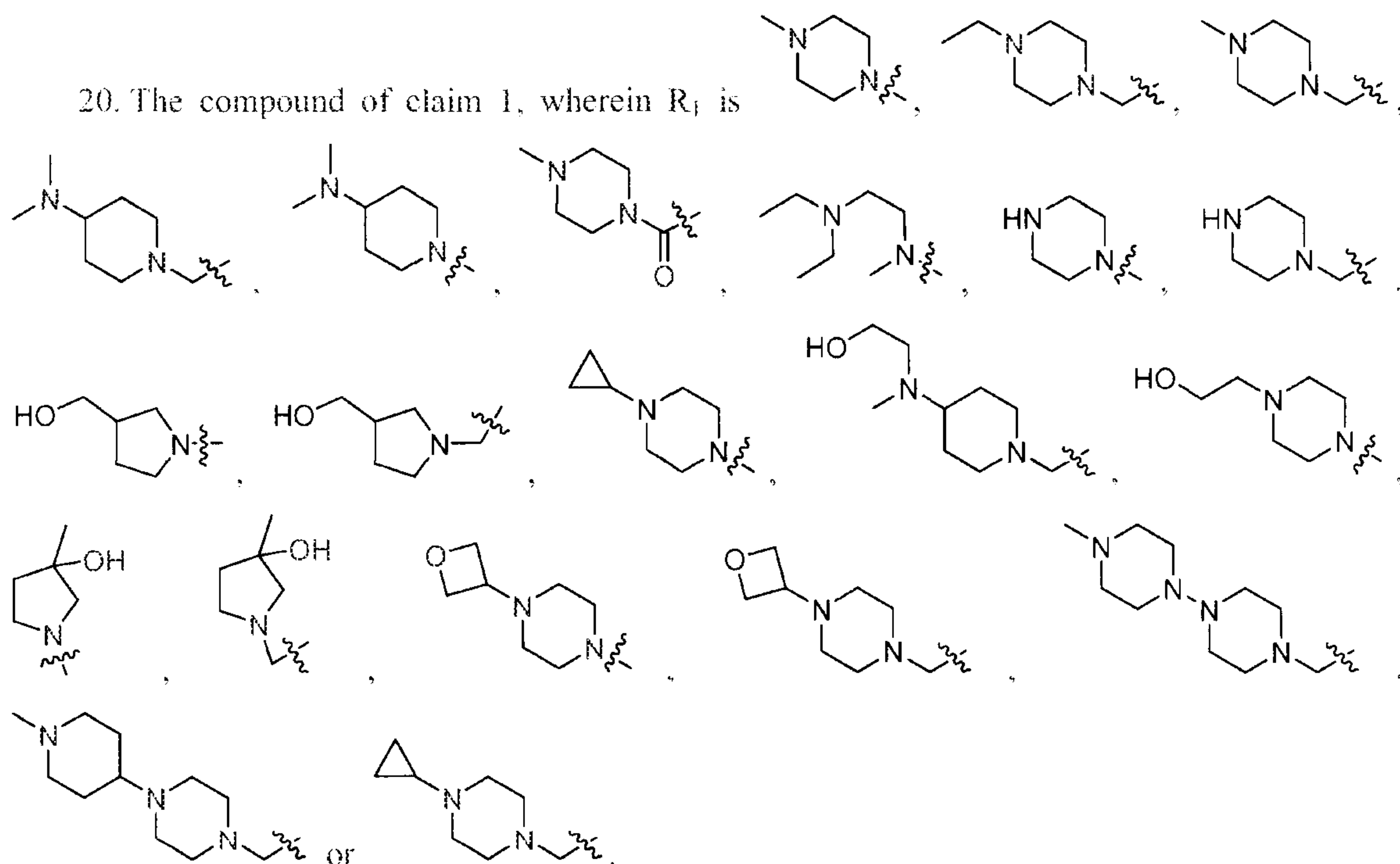
16. The compound of any one of claims 1-14, wherein the heterocyclyl comprises one or two heteroatoms of N as ring atoms.

5 17. The compound of any one of claims 1-6, wherein R_1 is $-NR_{12}-C_{1-3}\text{alkylene}-NR_{12}R_{13}$.

18. The compound of any one of claims 1-17, wherein R_{12} and R_{13} are each independently H, $(CH_2)_7-OH$ or $C_{1-3}\text{alkyl}$.

19. The compound of any one of claims 1-18, wherein R_{12} and R_{13} are each independently OH, CH_2CH_2OH , methyl or ethyl.

10 20. The compound of claim 1, wherein R_1 is



15 21. The compound of any one of claims 1-20, wherein m is 1.

22. The compound of any one of claims 1-21, wherein n is 1.

23. The compound of any one of claims 1-22, wherein t is 0, 1, or 2.

24. The compound of any one of claims 1-23, wherein R_2 and R_3 are each independently H, OH, halogen, $C_{1-6}\text{alkyl}$, $C_{1-6}\text{alkyl}$ substituted with halogen, $C_{1-6}\text{alkoxy}$, $C_{1-6}\text{alkoxy}$ substituted with halogen.

20 25. The compound of any one of claims 1-24, wherein R_2 and R_3 are each independently H, OH, F, Cl, CH_3 , CH_2CH_3 , CF_3 , OCH_3 , or OCF_3 .

26. The compound of any one of claims 1-25, wherein R_2 and R_3 are both F.

27. The compound of claim 1, wherein the compound is:

- 1) 4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;
- 2) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 3) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;
- 4) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(6-((4-methylpiperazin-1-yl)methyl)pyridin-3-yl)pyrimidin-2-amine;
- 5) 5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-N-(5-((4-methylpiperazin-1-yl)methyl)pyrimidin-2-yl)pyrimidin-2-amine;
- 6) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyrimidin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 7) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 8) N-(5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 9) (2-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyrimidin-5-yl)(4-methylpiperazin-1-yl)methanone;
- 10) (6-((5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)amino)pyridin-3-yl)(4-methylpiperazin-1-yl)methanone;
- 11) N5-(2-(diethylamino)ethyl)-N2-(5-fluoro-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-yl)-N5-methylpyridine-2,5-diamine;
- 12) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-(trifluoromethyl)pyrimidin-2-amine;
- 13) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4,4a,5-hexahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)-5-methylpyrimidin-2-amine;
- 14) 5-chloro-N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(6-fluoro-1-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyridin-8-yl)pyrimidin-2-amine;
- 15) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;

- 16) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 17) N-(5-(4-(dimethylamino)piperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 5 18) N-(5-((4-(dimethylamino)piperidin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine ;
- 19) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine ;
- 20) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine;
- 10 21) N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)-6-(4-methylpiperazin-1-yl)pyridazin-3-amine ;
- 22) 6-((4-ethylpiperazin-1-yl)methyl)-N-(5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)pyridazin-3-amine;
- 15 23) (1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)pyrrolidin-3-yl)methanol;
- 24) (1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)pyrrolidin-3-yl)methanol ;
- 25) N-(5-(4-cyclopropylpiperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 20 26) N-(5-((4-cyclopropylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-amine;
- 27) 2-((1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)piperidin-4-yl)(methyl)amino)ethan-1-ol;
- 25 28) 1-(6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)-3-methylpyrrolidin-3-ol;
- 29) 1-((6-((5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)pyrimidin-2-yl)amino)pyridin-3-yl)methyl)-3-methylpyrrolidin-3-ol;
- 30) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(oxetan-3-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine;
- 30

31) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-(4-(oxetan-3-yl)piperazin-1-yl)pyridin-2-yl)pyrimidin-2-amine;

32) N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(9-fluoro-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]imidazo[1,2-a]pyrazin-7-yl)pyrimidin-2-amine;

5 33) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4'-methyl-[1,1'-bipiperazin]-4-yl)methyl)pyridin-2-yl)pyrimidin-2-amine;

34) 5-fluoro-4-(9-fluoro-4-methyl-3,4-dihydro-1H-benzo[4,5]imidazo[2,1-c][1,4]oxazin-7-yl)-N-(5-((4-(1-methylpiperidin-4-yl)piperazin-1-yl)methyl)pyridin-2-yl)pyrimidin-2-amine.

28. The compound of any of claims 1-27, wherein the compound is the (-) enantiomer of the
10 compound.

29. The compound of any of claims 1-27, wherein the compound is the (+) enantiomer of the compound.

30. A pharmaceutical composition comprising a therapeutically effective amount of the compound according to any one of claims 1-29, and a pharmaceutically acceptable excipient.

15 31. The pharmaceutical composition according to claim 30, wherein, the said compound in a weight ratio to the said excipient within the range from about 0.001 to about 10.

32. Use of the pharmaceutical composition of claim 30 or 31, or the compound of any one of claims 1-29 for the preparation of a medicament.

33. The use of claim 32, wherein the medicament is used for the treatment or prevention of
20 cancer.

34. The use of claim 33, wherein the cancer is colon cancer, rectal cancer, mantle cell lymphoma, multiple myeloma, breast cancer, prostate cancer, glioblastoma, squamous cell esophageal cancer, liposarcoma, T-cell lymphoma, melanoma, pancreatic cancer, brain cancer or lung cancer.

35. The use of claim 32, wherein the medicament is used as an inhibitor of CDK.

25 36. The use of claim 35, wherein the medicament is used as an inhibitor of CDK4 and/or CDK6.

37. A method of treating a disease mediated by CDK in a subject, comprising administering the compound of any one of claims 1-29 or the pharmaceutical composition of claim 30 or 31.

38. The method of claim 37, wherein the CDK comprises CDK4 and/or CDK6.

39. The method of claim 37 or 38, wherein the disease mediated by CDK is cancer.

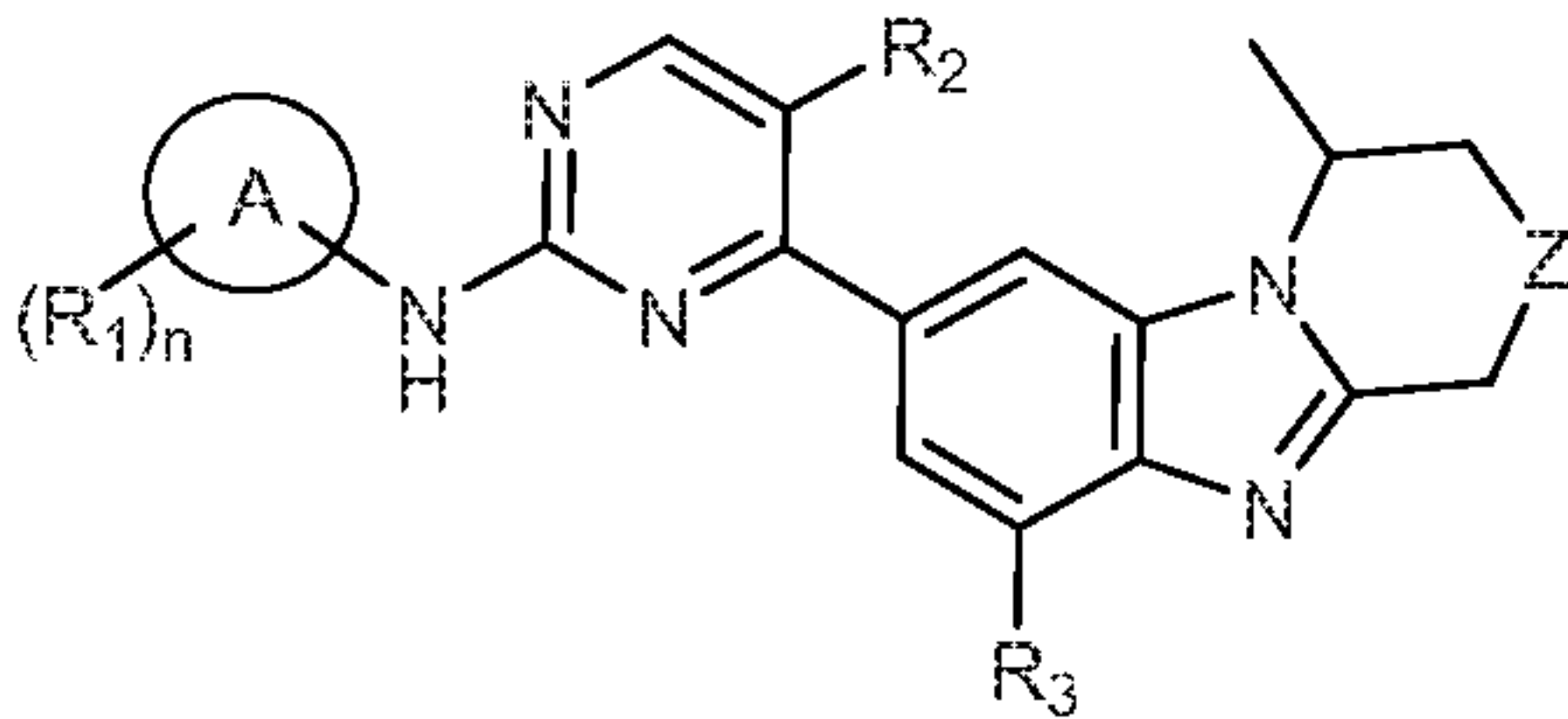
30 40. The method of claim 39 wherein the cancer is colon cancer, rectal cancer, mantle cell lymphoma, multiple myeloma, breast cancer, prostate cancer, glioblastoma, squamous cell

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esophageal cancer, liposarcoma, T-cell lymphoma melanoma, pancreatic cancer, brain cancer or lung cancer.

41. The method of any one of claims 37-40, wherein the subject is a human.



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