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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2016 (17.03.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/040858 A1

(51) International Patent Classification:
A61K 31/519 (2006.01)

(21) International Application Number:
PCT/US2015/049777

(22) International Filing Date:
11 September 2015 (11.09.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/050,043 12 September 2014 (12.09.2014) US

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

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(54) Title: COMBINATIONS AND DOSING REGIMES TO TREAT RB-POSITIVE TUMORS

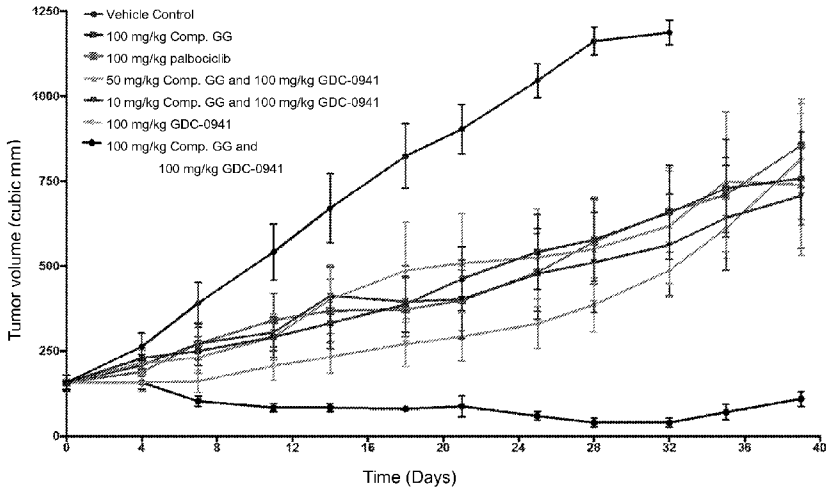


FIG. 20

(57) Abstract: This invention directed to methods for treating select RB-positive cancers and other Rb- positive abnormal cellular proliferative disorders using CDK4/6 inhibitors in specific dosing and combination or alternation regimes. In one aspect, treatments of select RB-positive cancers are disclosed using specific CDK4/6 inhibitors in combination or alternation with another chemotherapeutic, for example, an additional kinase inhibitor, PD-1 inhibitor, or BCL-2 inhibitor, or combination thereof.

WO 2016/040858 A1

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

Published:

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

ANTI-NEOPLASTIC COMBINATIONS AND DOSING REGIMENS USING CDK4/6 INHIBITOR COMPOUNDS TO TREAT RB-POSITIVE TUMORS

This application claims the benefit of U.S. Provisional Application No. 62/050,043 filed September 12, 2014. The entirety of this application is hereby incorporated by reference for all purposes.

GOVERNMENT INTEREST

The U.S. Government has rights in this invention by virtue of support under NCI Contract No. 1R44CA174074-01A1.

FIELD

This invention is in the area of methods for treating select RB-positive cancers and other Rb-positive abnormal cellular proliferative disorders using the described CDK4/6 inhibitors in specific dosing and combination or alternation regimes. In one aspect, treatments of select RB-positive cancers are disclosed using specific CDK4/6 inhibitors in combination or alternation with another chemotherapeutic, for example, an additional kinase inhibitor, PD-1 inhibitor, or BCL-2 inhibitor, or combination thereof.

BACKGROUND

The regulation of the cell cycle is governed and controlled by specific proteins, which are activated and deactivated mainly through phosphorylation/dephosphorylation processes in a precisely timed manner. The key proteins that coordinate the initiation, progression, and completion of cell-cycle program are cyclin dependent kinases (CDKs). Cyclin-dependent kinases belong to the serine–threonine protein kinase family. They are heterodimeric complexes composed of a catalytic kinase subunit and a regulatory cyclin subunit. CDK activity is controlled by association with their corresponding regulatory subunits (cyclins) and CDK inhibitor proteins (Cip & Kip proteins, INK4s), by their phosphorylation state, and by ubiquitin-mediated proteolytic degradation (see D.G. Johnson, C.L. Walker, Annu. Rev. Pharmacol. Toxicol 39 (1999) 295–312; D.O. Morgan, Annu. Rev. Cell Dev. Biol. 13 (1997) 261–291; C.J. Sherr, Science 274 (1996) 1672–1677; T. Shimamura et al., Bioorg. Med. Chem. Lett. 16 (2006) 3751–3754).

Sherr, Science 274 (1996) 1672–1677; T. Shimamura et al., Bioorg. Med. Chem. Lett. 16 (2006) 3751–3754).

There are four CDKs that are significantly involved in cellular proliferation: CDK1, which predominantly regulates the transition from G2 to M phase, and CDK2, CDK4, and CDK6, which regulate the transition from G1 to S phase (Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat. Rev. Cancer 2009;9(3):153-166). In early to mid G1 phase, when the cell is responsive to mitogenic stimuli, activation of CDK4-cyclin D and CDK6-cyclin D induces phosphorylation of the retinoblastoma protein (pRb). Phosphorylation of pRb releases the transcription factor E2F, which enters the nucleus to activate transcription of other cyclins which promote further progression of the cell cycle (see J.A. Diehl, Cancer Biol. Ther. 1 (2002) 226–231; C.J. Sherr, Cell 73 (1993) 1059–1065). CDK4 and CDK6 are closely related proteins with basically indistinguishable biochemical properties (see M. Malumbres, M. Barbacid, Trends Biochem. Sci. 30 (2005) 630–641).

A number of CDK 4/6 inhibitors have been identified, including specific pyrido[2,3-d]pyrimidines, 2-anilinopyrimidines, diaryl ureas, benzoyl-2,4-diaminothiazoles, indolo[6,7-a]pyrrolo[3,4-c]carbazoles, and oxindoles (see P.S. Sharma, R. Sharma, R. Tyagi, Curr. Cancer Drug Targets 8 (2008) 53–75). For example, WO 03/062236 identifies a series of 2-(pyridin-2-ylamino-pyrido[2,3]pyrimidin-7-ones for the treatment of Rb positive cancers that show selectivity for CDK4/6, including 6-acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylammino)-8H-pyrido-[2,3-d]-pyrimidin-7-one (PD0332991), which is currently being tested by Pfizer in late stage clinical trials as an anti-neoplastic agent against estrogen-positive, HER2-negative breast cancer. Tate, et al. describe the antitumor activity of the CDK4/6 inhibitor abemaciclib (LY2835219) ("Semi-Mechanistic Pharmacokinetic/Pharmacodynamic Modeling of the Antitumor Activity of LY2835219, a New Cyclin-Dependent Kinase 4/6 Inhibitor, in Mice Bearing Human Tumor Xenografts", Clin Cancer Res (July 15, 2014) 20; 3763-74). Rader, et al. describe the reduced proliferation in neuroblastoma-derived cell lines using the CDK4/6 inhibitor ribociclib (LEE011) ("Dual CDK4/CDK6 Inhibition Induces Cell Cycle Arrest and Senescence in Neorbalstoma", Clin Cancer Res (Nov. 15, 2013) 19(22): 6173-82). VanderWel et al. describe an iodine-containing pyrido[2,3-d]pyrimidine-7-one (CKIA) as a potent and selective CDK4 inhibitor (see VanderWel et al., J. Med. Chem. 48 (2005) 2371–2387). WO 99/15500 filed by Glaxo Group Ltd discloses protein kinase and serine/threonine kinase

inhibitors. WO 2010/020675 filed by Novartis AG describes pyrrolopyrimidine compounds as CDK inhibitors. WO 2011/101409 also filed by Novartis describes pyrrolopyrimidines with CDK 4/6 inhibitory activity. WO 2005/052147 filed by Novartis and WO 2006/074985 filed by Janssen Pharma disclose additional CDK4 inhibitors. WO 2012/061156 filed by Tavares and assigned to G1 Therapeutics describes CDK inhibitors. WO 2013/148748 filed by Francis Tavares and assigned to G1 Therapeutics describes Lactam Kinase Inhibitors. PCT Patent Application No. PCT/US2014/029073 filed by Strum et al. and assigned to G1 Therapeutics describes compounds and methods for protection of hematopoietic stem and progenitor cells against ionizing radiation using CDK4/6 inhibitors. PCT Patent Application No. PCT/US2014/028685 filed by Strum et al. and assigned to G1 Therapeutics describes compounds and methods for protection of normal cells during chemotherapy using CDK4/6 inhibitors. PCT Patent Application No. PCT/US2014/029429 filed by Strum et al. and assigned to G1 Therapeutics describes compounds and methods for treating Rb-positive cancers using CDK4/6 inhibitors. PCT Patent Application No. PCT/US2014/029274 filed by Strum et al. and assigned to G1 Therapeutics describes compounds and methods for treating certain cancers with CDK4/6 inhibitors.

It has recently been reported that the Pfizer CDK4/6 inhibitor palbociclib in combination with letrozole, while increasing progression free survival (PFS) compared with letrozole alone in post-menopausal women with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-) locally advanced or metastatic breast cancer, failed to statistically extend overall survival (OS), an important secondary endpoint. Based on the events at the time of the assessment, a median OS of 37.5 months was observed in the combination (paalbociclib + letrozole) arm versus 33.3 months for those who received letrozole alone, a difference of 4.2 months (HR = 0.813, 95% CI: 0.492, 1.345). This OS observation at the time of final PFS analysis was not statistically significant.

Accordingly, there is an ongoing need for methods, combinations, and dosing regimens to treat patients with select Rb-positive cancers and abnormal cellular proliferative disorders.

SUMMARY OF THE INVENTION

Methods, combinations, and dosing regimens are provided to treat select Rb-positive abnormal cellular proliferation disorders in a host including an Rb-positive cancer using a CDK4/6 inhibitor described herein.

In one aspect, a method is provided that includes the administering to a host in need thereof an effective amount of a CDK4/6 inhibitor described herein in combination or alternation with at least one additional chemotherapeutic agent. The treatment regimen includes the administration of a CDK4/6 inhibitor described herein in combination or alternation with at least one additional kinase inhibitor. In one embodiment, the at least one additional kinase inhibitor is selected from a phosphoinositide 3-kinase (PI3K) inhibitor, a Bruton's tyrosine kinase (BTK) inhibitor, a spleen tyrosine kinase (Syk) inhibitor, a Mitogen-activated protein kinase kinase (MEK) inhibitor, a Rapidly Accelerated Fibrosarcoma (Raf) kinase inhibitor, a B-cell lymphoma 2 (Bcl-2) protein inhibitor, a programmed death protein 1 (PD-1) inhibitor, or a combination thereof.

Without wanting to be bound by any particular theory, the use of the CDK4/6 inhibitors described herein, in combination with the kinase inhibitors described herein, provide enhanced additive anticancer effects while believed to reduce disease progression associated with kinase inhibitor resistance that commonly develops in kinase inhibitor therapy which leads to disease progression.

In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the at least one additional chemotherapeutic agent.

As contemplated herein, the CDK4/6 inhibitor to be administered is selected from the compounds of Formula I, II, III, IV, or V as described herein, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof. In one non-limiting example, a CDK4/6 inhibitor can be selected from the compounds of Table 1 below, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof. In a further embodiment, the CDK4/6 inhibitor is selected from compounds Q, T, U, or GG, and the chemotherapeutic agent is selected from a phosphoinositide 3-kinase (PI3K) inhibitor, a Bruton's tyrosine kinase (BTK) inhibitor, a spleen tyrosine kinase (Syk) inhibitor, a Raf inhibitor, a MEK inhibitor, a programmed death protein 1 (PD-1) inhibitor, or a B-cell lymphoma 2 (Bcl-2) protein inhibitor. In an additional embodiment, the CDK4/6 inhibitor is selected from compounds X or BB, and the chemotherapeutic agent is selected from a phosphoinositide 3-kinase (PI3K) inhibitor, a Bruton's

tyrosine kinase (BTK) inhibitor, a spleen tyrosine kinase (Syk) inhibitor, a Raf inhibitor, a MEK inhibitor, a programmed death protein 1 (PD-1) inhibitor, or a B-cell lymphoma 2 (Bcl-2) protein inhibitor.

In a specific embodiment, a CDK4/6 inhibitor selected from the compounds of Formula I, II, III, IV, or V as described herein, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof, is administered in combination or alternation with the PI3 kinase inhibitor pictilisib (GDC-0941; Genentech Inc., South San Francisco, CA) to a host suffering from colorectal, breast, soft tissue sarcoma, melanoma, ovarian, gastric, or prostate cancer. In a specific embodiment, the CDK4/6 inhibitor is selected from compound Q, T, U, GG, X, or BB. In a more particular embodiment, the CDK4/6 inhibitor is compound GG and the host is suffering from breast cancer.

As further contemplated herein, the CDK4/6 inhibitor is combined or alternated with another chemotherapeutic agent to treat a lymphohematopoietic malignancy, for example, but not limited to, B-cell lineage leukemia or lymphoma, T-cell lineage leukemia or lymphoma, or myeloid-lineage leukemia or lymphoma, for example, but not limited to acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL). In one embodiment, the CDK4/6 inhibitor is selected from Compound Q, T, U, or GG and the at least one additional chemotherapeutic agent is a kinase inhibitor. In one embodiment, the CDK4/6 inhibitor is selected from Compound X or BB and the at least one additional chemotherapeutic agent is a kinase inhibitor. In one embodiment, the kinase inhibitor is a BTK inhibitor or a Syk inhibitor. In one embodiment, the BTK inhibitor is ibrutinib. In one embodiment, the BTK inhibitor is ACP-196.

In one aspect contemplated herein, the CDK4/6 inhibitor is combined or alternated with another chemotherapeutic agent to treat a solid tumor. In one embodiment, the solid tumor is Erb-2/human epidermal growth factor receptor (HER)-2-positive breast cancer. In one embodiment, the solid tumor is a non-small cell lung cancer. In one embodiment, the solid tumor is a colorectal cancer. In one embodiment, the CDK4/6 inhibitor is selected from Compound Q, T, U, or GG and the at least one additional chemotherapeutic agent is a kinase inhibitor. In one embodiment, the CDK4/6 inhibitor is selected from Compound X or BB and the at least one additional chemotherapeutic agent is a kinase inhibitor. In one embodiment, the kinase inhibitor is a BTK inhibitor or a Syk inhibitor. In one embodiment, the BTK inhibitor is ibrutinib. In one embodiment, the BTK inhibitor is ACP-196.

In one aspect, a method is provided for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a Bruton's tyrosine kinase (BTK) inhibitor to a host in need thereof, wherein the Bruton's tyrosine kinase (BTK) inhibitor is selected from ibrutinib or ACP-196.

In another aspect, a method is provided for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a spleen tyrosine kinase (SYK) inhibitor.

In an additional aspect, a method is provided for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a PI3 kinase inhibitor to a host in need thereof, wherein the PI3 kinase inhibitor is pictilisib.

In one aspect, a method is provided for the treatment of an Rb-positive cancer in a host comprising: a) administering to the host having an Rb-positive cancer an effective amount of a CDK 4/6 inhibitor compound of the Formula I, II, III, IV, or V, wherein the compound is capable of arresting a significant portion of the Rb-positive abnormal cells in the G0 or G1 phase of the cell cycle; b) administering to the host at least one additional chemotherapeutic agent within about 48 hours of administration of the CDK 4/6 inhibitor.

In one aspect, a method is provided comprising 1) administering to a host having an Rb-positive abnormal cellular proliferation disorder a CDK4/6 inhibitor described herein, wherein the CDK4/6 inhibitor is capable of arresting a significant portion of the Rb-positive abnormal cells in the G0 or G1 phase of the cell cycle, 2) administering to the host at least one additional chemotherapeutic agent just prior to, or in one alternative concomitantly to, a significant portion of the Rb-positive abnormal cells, for example at least 50%, at least 60%, at least 70%, at least 80%, reentering the cell cycle, for example, within about 12 hours, within about 14 hours, within about 16 hours, within about 18 hours, within about 20 hours, within about 24 hours, within about 30 hours, within about 36 hours, within about 40 hours, or within about 48 hours of administration of the CDK 4/6 inhibitor, wherein, due to the synchronous reentry of the abnormal cells into the cell cycle, the efficacy of the chemotherapeutic agent is enhanced. Accordingly, the CDK4/6 inhibitors described herein can be combined or alternated with another chemotherapeutic described herein to treat a select Rb-positive abnormal cellular proliferation

disorder, such as a cancer, in order to synchronize the Rb-positive abnormal cells so that more abnormal cells are exposed to the chemotherapeutic agent at the cell-cycle stage that the chemotherapeutic agent is most effective, e.g., G1 phase, S phase, G2 phase, or M phase. Specifically, the invention includes administering to a patient having an Rb-positive abnormal cellular proliferation disorder, such as cancer, an effective amount of a CDK4/6 inhibitor described herein, wherein the compound has a pharmacokinetic and enzymatic half-life that provides for a transient, reversible G1 arrest of the Rb-positive abnormal cells, allowing for an initial halt of a significant portion of the cells in the G0 or G1 phase of the cell cycle, followed by the synchronous reentry of these cells into the cell cycle, wherein the administration of at least one additional chemotherapeutic agent is timed so that the abnormal cells are entering a cell-cycle stage that the chemotherapeutic agent is most effective. The chemotherapeutic agent can be any of those described in this application or utilized in the standards of care used to treat abnormal cellular proliferation. In one embodiment, the chemotherapeutic agent is a kinase inhibitor.

Non-limiting examples of CDK4/6 inhibitors useful in the present invention are described in Table 1, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof as provided below. In one embodiment, the solid tumor is a colorectal cancer. In one embodiment, the CDK4/6 inhibitor is selected from Compound Q, T, U, or GG and the at least one additional chemotherapeutic agent is a kinase inhibitor. In one embodiment, the CDK4/6 inhibitor is selected from Compound X or BB and the at least one additional chemotherapeutic agent is a kinase inhibitor.

Thus in one embodiment, the invention includes administering a CDK4/6 inhibitor described herein, for example a CDK4/6 inhibitor selected from Table 1, in an effective amount to treat a host suffering from an Rb-positive abnormal cellular proliferation disorder in a treatment regimen, wherein (either alone or in any combination thereof, each of which is considered specifically and independently described): (i) a substantial portion of the abnormal cells (e.g., at least 50%, at least 60%, at least 70%, at least 80% or greater) are arrested in G0 or G1 and re-enter the cell cycle in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the last administration of a CDK4/6 inhibitor described herein; (ii) a substantial portion of the abnormal cells reenter the cell-cycle synchronously in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the last administration of the CDK4/6 inhibitor described herein; (iii) the

dissipation of the CDK4/6 inhibitor's inhibitory effect on the abnormal cells occurs in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the administration of the CDK4/6 inhibitor; (iv) a substantial portion of the abnormal cells reenter the cell-cycle in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the dissipation of the CDK4/6 inhibitor's inhibitory effect; or (vi) a substantial portion of the abnormal cells reenter the cell-cycle within less than about 24 hours, about 30 hours, about 36 hours, or about 48 hours from the point in which the administered CDK4/6 inhibitor compound's concentration level in the subject's blood drops below a therapeutic effective concentration, and, either just prior to or concomitantly with the re-entry of the abnormal cells as described in (i)-(vi), administering at least one additional chemotherapeutic agent.

In a central embodiment of the invention, a compound described herein can be administered in a concerted regimen with another agent such as a DNA- or non-DNA-damaging, anti-neoplastic agent, for example as described below, for beneficial, additive, or synergistic effect against RB-positive abnormal cellular proliferation. By combining the administration of a CDK4/6 inhibitor described herein with the timely administration of additional chemotherapeutic agents, for example, at the time point wherein a larger number of abnormal cells are most susceptible to the at least one additional chemotherapeutic agent's deleterious effects due to synchronous re-entry into the cell cycle caused by administration of the CDK4/6 inhibitor, it is possible for the health care practitioner to decrease the amount of the additional chemotherapeutic agent to minimize the unwanted adverse effects while achieving an increased efficacy of the desired therapeutic benefit.

In certain embodiments, a CDK4/6 inhibitor described herein is administered to the host prior to treatment with another chemotherapeutic agent, during treatment with another chemotherapeutic agent, after administration of another chemotherapeutic agent, or a combination thereof. In one embodiment, a CDK4/6 inhibitor described herein is administered to the subject less than about 48 hours, 40 hours, 36 hours, 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, or 4 hours or less prior to treatment with the other chemotherapeutic agent in order to sensitize the Rb-positive cancer to the chemotherapeutic agent. In one embodiment, a CDK4/6 inhibitor described herein is administered up to 48 hours prior to treatment with the other chemotherapeutic agent.

In another aspect of the present invention, provided herein is a method for treating T-cell malignancies using CDK4/6 inhibitors described herein. In one embodiment of the present invention, provided herein is a method of treating a host suffering from a T-cell malignancy comprising a) administering to the host a CDK4/6 inhibitor of the Formula I, II, III, IV, or V, b) analyzing the cell cycle status of T-cell malignant cells and at least one subset of non-diseased hematopoetic cells, c) adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are in G0 or G1 cell cycle arrest and at least one subset of non-diseased hematopoietic cells are not in G0 or G1 arrest.

In one embodiment of the present invention, provided herein is a method of treating a host suffering from a T-cell malignancy comprising, a) administering to the host a CDK4/6 inhibitor of the Formula I, II, III, IV, or V, b) analyzing the cell cycle status of T-cell malignant cells and at least one subset of non-diseased hematopoetic cells, c) adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are not in G0 or G1 arrest and at least one subset of non-diseased hematopoietic cells are in G0 or G1 arrest, d) administering a chemotherapeutic agent.

In one aspect of the invention, provided herein is a method of treating an Rb-positive T-cell malignancy in a host by administering a CDK4/6 inhibitor described herein at a dose that provides for the long term inhibition of the proliferation of the T-cell malignancy, but provides for a differential inhibition of non-diseased Rb-positive hematopoietic cells, for example, hematopoietic stem cells (HSCs), multipotent progenitors (MPPs), hematopoietic progenitor cells (HPCs), and other hematological cells, which allows these non-diseased hematopoietic cells to reenter the cell-cycle sooner than the diseased T-cell, thus allowing for the prolonged use of these CDK4/6 inhibitors to treat the T-cell malignancy while providing for a reduction in the side-effects associated with the use of CDK4/6 inhibitors generally. In one embodiment of the invention, provided herein is a method of treating a host suffering from a T-cell malignancy comprising administering to the host a CDK4/6 inhibitor of the Formula I, II, III, IV, or V, measuring or analyzing the cell cycle status of T-cell malignant cells and non-diseased hematopoetic cells, for example but not limited to, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors

(CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, monocyte progenitors, and megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, erythroid progenitors, or a combination thereof, adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are in G0 or G1 cell cycle arrest and at least one subset of non-diseased hematopoietic cells are not in G0 or G1 arrest. In a particular embodiment, the host is a human, and the non-diseased hematological lineage cells are selected from HSC/MPPs (CD45dim/CD34+/CD38-), OPPs (CD45dim/CD34+/CD38+), monocyte progenitors (CD45+/CD14+/CD11b+), granulocyte progenitors (CD45+/CD14-/CD11b+), erythroid progenitors (CD45-/CD71+), and megakaryocyte progenitors (CD45+/CD61+).

In an alternative embodiment, a CDK 4/6 inhibitor can be administered in an amount so that a significant portion, for example at least 50%, at least 60%, at least 70%, at least 80%, of the diseased T-cells are in active cell cycling while a significant portion of at least one subset of non-diseased hematopoietic cell lineage cells, due to the effects of the CDK 4/6 inhibitor, are arrested in the G0 and/or G1 phase of the cell cycle, and subsequently administering a second chemotherapeutic agent.

It has been discovered that hematological cells respond to CDK4/6 inhibitors in a differential fashion. Accordingly, this differential response provides for the differential dosing of a CDK4/6 inhibitor based on the specific hematological disorder the host may be suffering from. Thus, in one aspect of the invention, provided herein is a method of treating a host suffering from a hematological cellular proliferation disorder comprising 1) identifying the specific hematological deficiency, 2) administering to the host a CDK4/6 inhibitor in an amount sufficient to inhibit the proliferation of the specific hematological deficiency, 3) analyzing the cell cycle status of the specific diseased hematological cells and one or more non-diseased hematological cell lineages 4) adjusting the dose of the CDK4/6 inhibitor to the minimal amount necessary so that the diseased cells remain inhibited, while allowing at least one subset of non-diseased hematological cells to proliferate. As contemplated herein, the non-diseased hematopoetic cells can be, for example, but not limited to, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors,

monocyte progenitors, and megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, erythroid progenitors, or a combination thereof, adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are in G0 or G1 cell cycle arrest and at least one subset of non-diseased hematopoietic cells are not in G0 or G1 arrest. In a particular embodiment, the host is a human, and the non-diseased hematological lineage cells are selected from HSC/MPPs (CD45dim/CD34+/CD38-), OPPs (CD45dim/CD34+/CD38+), monocyte progenitors (CD45+/CD14+/CD11b+), granulocyte progenitors (CD45+/CD14-/CD11b+), erythroid progenitors (CD45-/CD71+), and megakaryocyte progenitors (CD45+/CD61+).

In one aspect of the present invention, the differential effect the CDK4/6 inhibitors described herein have on hematological cells at the same dosage provides for a method of protecting certain cell linages during chemotherapeutic treatment using a DNA-damaging agent to treat a proliferative disorder, for example, an Rb-positive cancer, wherein the CDK4/6 inhibitor is used to protect certain hematopoetic cell lineages, and wherein the dosing of the CDK4/6 inhibitor can be differentially adjusted based on the effect the chemotherapeutic agent has on a particular hematological cell line. Thus, for example, if a certain hematological cell line is adversely effected by the chemotherapeutic, the CDK4/6 inhibitor dose can be adjusted to allow the particular lineage to replicate while still affording some chemoprotection to other hematopoetic cells. As contemplated herein, the non-diseased hematopoetic cells can be, for example, but not limited to, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, monocyte progenitors, and megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, erythroid progenitors, or a combination thereof, adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are in G0 or G1 cell cycle arrest and at least one subset of non-diseased hematopoietic cells are not in G0 or G1 arrest. In a particular embodiment, the host is a human, and the non-diseased hematological lineage cells are selected from HSC/MPPs (CD45dim/CD34+/CD38-), OPPs (CD45dim/CD34+/CD38+), monocyte progenitors

(CD45+/CD14+/CD11b+), granulocyte progenitors (CD45+/CD14-/CD11b+), erythroid progenitors (CD45-/CD71+), and megakaryocyte progenitors (CD45+/CD61+).

In some embodiments, the subject or host is a mammal, including a human. The compound can be administered to the subject by any desired route, including intravenous, sublingual, buccal, oral, intraaortal, topical, intranasal, parenteral, transdermal, systemic, intramuscular, or via inhalation.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a graph of the percentage of cells in the G0-G1 phase of the cell cycle vs. time after washout of the compound (hours) in human fibroblast (Rb-positive) cells. FIG. 1B is a graph of the percentage of cells in the S phase of the cell cycle vs. time after washout of the compound (hours) in human fibroblast (Rb-positive) cells. FIG. 1C is a graph of the percentage of cells in the G0-G1 phase of the cell cycle vs. time after washout of the compound (hours) in human renal proximal tubule epithelial (Rb-positive) cells. FIG. 1D is a graph of the percentage of cells in the S phase of the cell cycle vs. time after washout of the compound (hours) in human renal proximal tubule epithelial (Rb-positive) cells. These cellular wash out experiments demonstrated that the inhibitor compounds of the present invention have a short, transient G1-arresting effect in different cell types. The effect on the cell cycle following washing out of the compounds was determined at 24, 36, 40, and 48 hours. As described in Example 153, the results show that cells treated with

FIGS. 2-4 illustrate several exemplary embodiments of R^2 of the compounds useful in the present invention.

FIGS. 5A-5C, 6A-6D, 7A-7C, 8A-8B, and 9A-9F illustrate several exemplary embodiments of the core structure of the compounds useful in the present invention.

FIG. 10A is a graph of the percentage of cells in G2-M phase (open circles), S phase (triangles), G0-G1 phase (squares), <2N (diamonds) vs. variable concentration (nM) of compound T in tHS68 cells. The CDK4/6-dependent cell line (tHS68)(Rb-positive) was treated with the indicated concentrations of Compound T for 24 hours. Following treatment of Compound T, cells were harvested and analyzed for cell cycle distribution. As described in Example 154, tHS68 cells show a clean G1 arrest accompanied by a corresponding decrease in the number of cells in S-phase.

FIG. 10B is a graph of the number of tHS68 cells (Rb-positive) vs. the DNA content of the cells (as measured by propidium iodide). Cells were treated with DMSO for 24 hours, harvested, and analyzed for cell cycle distribution.

FIG. 10C is a graph of the number of WM2664 cells (Rb-positive) vs. the DNA content of the cells (as measured by propidium iodide). Cells were treated with DMSO for 24 hours, harvested, and analyzed for cell cycle distribution.

FIG. 10D is a graph of the number of A2058 cells (CDK4/6-independent cell line) vs. the DNA content of the cells (as measured by propidium iodide). Cells were treated with DMSO for 24 hours, harvested, and analyzed for cell cycle distribution.

FIG. 10E is a graph of the number of tHS68 cells (Rb-positive) vs. the DNA content of the cells (as measured by propidium iodide) after treatment with Compound T. Cells were treated with Compound T (300 nM) for 24 hours, harvested, and analyzed for cell cycle distribution. As described in Example 154, treatment of tHS68 cells with Compound T causes a loss of the S-phase peak (indicated by arrow).

FIG. 10F is a graph of the number of WM2664 cells (Rb-positive) vs. the DNA content of the cells (as measured by propidium iodide) after treatment with Compound T. Cells were treated with Compound T (300 nM) for 24 hours, harvested, and analyzed for cell cycle distribution. As described in Example 154, treatment of WM2664 cells with Compound T causes a loss of the S-phase peak (indicated by arrow).

FIG. 10G is a graph of the number of A2058 cells (Rb-negative) vs. the DNA content of the cells (as measured by propidium iodide) after treatment with Compound T. Cells were treated with Compound T (300 nM) for 24 hours, harvested, and analyzed for cell cycle distribution. As described in Example 154, treatment of A2058 cells with Compound T does not cause a loss of the S-phase peak (indicated by arrow).

FIG. 11 is a Western blot showing the phosphorylation levels of Rb at Ser807/811 and Ser780 after treatment with Compound T. Rb-positive (tHS68 or WM2664) and Rb-negative cell lines (A2058) were treated with Compound T (300 nM) for the indicated times (0, 4, 8, 16, and 24 hours). MAPK levels are shown as a control for protein levels. Following treatment, cells were harvested and analyzed for Rb-phosphorylation by western blot analysis. As described in Example 155, Compound T treatment resulted in reduced Rb-phosphorylation

starting 16 hours after treatment in CDK4/6-dependent cell lines (tHS68 and WM2664), but not in the CDK4/6-independent cell line (A2058).

FIG. 12 is a graph of 5'-ethynyl-2'-deoxyuridine (EdU) incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice. Compound T (50 mg/kg or 100 mg/kg) was administered by i.p. injection to assess the temporal effect of transient CDK4/6 inhibition on bone marrow arrest. As described in Example 156, a single i.p. dose of Compound T results in a dose and time dependent reduction and recovery of proliferating cells.

FIG. 13 is a graph of EdU incorporation into thymocytes vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg).

FIG. 14 is a graph illustrating Mac1+/Gr1+ cells and EdU incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg). Mac1+/Gr1+ is a marker of of myeloid cells.

FIG. 15 is a graph illustrating B220+ cells and EdU incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg). B220+ is a marker of B lymphoid cells.

FIG. 16 is a graph illustrating Ter119+ cells and EdU incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg). Ter119+ is a marker of erythroid cells.

FIG. 17 is a graph illustrating LK+ cells and EdU incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg). LK+ (LK (Lin- Sca-1- C-kit+) is a marker of myeloid progenitor cells.

FIG. 18 is a graph illustrating LSK+ cells and EdU incorporation vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice (circles; 50 mg/kg), (squares; 100mg/kg). LSK+ (Lin- Sca-1+ c-kit+) is a marker of hematopoietic stem cells.

FIG. 19 is a graph of EdU incorporation into hematopoietic stem cells (HSCs) vs. time after administration (minutes) of Compound T to healthy C57BL/6 female mice. Compound T (50 mg/kg or 100 mg/kg) was administered by i.p. injection.

FIG. 20 is a graph showing tumor volume (cubic mm) vs. time after initiation of treatment (days) in a mouse model of breast cancer. Immunodeficient mice were implanted with the human breast cancer cell line MCF7 (an Rb-positive cell line). Once the tumors reached

sufficient size, the mice were randomized into treatment cohorts. Mice were treated with vehicle control (small circles), 100 mg/kg Compound GG (days 1-28) (small squares), 100 mg/kg palbociclib (days 1-28) (large squares), 50 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (days 15-28) (triangles), 10 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (days 15-28) (upside down triangles), 100 mg/kg GDC-0941 (days 1-28) (diamonds), or 100 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (days 1-28) (large circles). As discussed in Example 157, mice treated with a combination of 100 mg/kg Compound GG and 100 mg/kg GDC-0941 for 28 days showed enhanced efficacy toward the MCF7 breast cancer cell line implants, as compared to mice treated with vehicle control, 100 mg/kg Compound GG, 100 mg/kg palbociclib, 50 mg/kg Compound GG and 100 mg/kg GDC-0941, 10 mg/kg Compound GG and 100 mg/kg GDC-0941, or 100 mg/kg GDC-0941.

FIG. 21A is a graph showing white blood cell counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

FIG. 21B is a graph showing lymphocyte counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

FIG. 21C is a graph showing neutrophil counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

FIG. 21D is a graph showing monocyte counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

FIG. 21E is a graph showing platelet counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

FIG. 21F is a graph showing red blood cell counts (cells/μl) at Day 14 in mice (human breast cancer cell line MCF7 implant model) treated with vehicle only (circles), 10 mg/kg Compound GG (squares), 50 mg/kg Compound GG (triangles), or 100 mg/kg Compound GG (upside down triangles). See Example 157.

DETAILED DESCRIPTION OF THE INVENTION

Methods, combinations, and dosing regimens are provided to treat select Rb-positive abnormal cellular proliferation disorders in a host including an Rb-positive cancer using a CDK4/6 inhibitor described herein in combination with at least one additional chemotherapeutic agent, for example, a specific kinase inhibitor. In an alternative aspect, the CDK4/6 inhibitors described herein are used to induce a G0-G1 cell-cycle arrest in Rb-positive tumors in order to synchronize the tumor cell upon dissipation of the inhibitory CDK4/6 effect, resulting in an increased exposure by a larger number of cells during a second chemotherapeutic's most efficacious period, e.g., G1, S, G2, or M phase. In a further aspect, provided herein is a method of treating an Rb-positive T-cell malignancy by administering a CDK4/6 inhibitor compound described herein at a dose that provides for an extended period of the inhibition of the growth of the T-cell malignancy, but provides for a quicker reentry into the cell-cycle of non-diseased Rb-positive hematopoietic cells, for example, hematopoietic stem and progenitor cells (HSPCs). In a further aspect, provided herein is a method for differentially protecting hematopoetic cell lineages during treatment of a cancer using a DNA-damaging agent based on the particular dose or timing of the dose of the CDK4/6 inhibitor administered to the host.

Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg (2007) *Advanced Organic Chemistry* 5^{th} *Ed.* Vols. A and B, Springer Science+Business Media LLC, New York. The practice of the present invention will employ, unless otherwise indicated, conventional methods of synthetic organic chemistry, mass spectroscopy, preparative and analytical methods of chromatography, protein

chemistry, biochemistry, recombinant DNA techniques and pharmacology. Conventional methods of organic chemistry include those included in *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 6th Edition*, M.B. Smith and J. March, John Wiley & Sons, Inc., Hoboken, NJ, 2007.

The term "alkyl," either alone or within other terms such as "haloalkyl" and "alkylamino," embraces linear or branched radicals having one to about twelve carbon atoms. "Lower alkyl" radicals have one to about six carbon atoms. Examples of such radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, *tert*-butyl, pentyl, isoamyl, hexyl and the like. The term "alkylene" embraces bridging divalent linear and branched alkyl radicals. Examples include methylene, ethylene, propylene, isopropylene and the like.

The term "alkenyl" embraces linear or branched radicals having at least one carbon-carbon double bond of two to about twelve carbon atoms. "Lower alkenyl" radicals having two to about six carbon atoms. Examples of alkenyl radicals include ethenyl, propenyl, allyl, propenyl, butenyl and 4-methylbutenyl. The terms "alkenyl" and "lower alkenyl," embrace radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations.

The term "alkynyl" denotes linear or branched radicals having at least one carbon-carbon triple bond and having two to about twelve carbon atoms. "Lower alkynyl" radicals having two to about six carbon atoms. Examples of such radicals include propargyl, butynyl, and the like.

Alkyl, alkenyl, and alkynyl radicals may be optionally substituted with one or more functional groups such as halo, hydroxy, nitro, amino, cyano, haloalkyl, aryl, heteroaryl, heterocyclo and the like.

The term "alkylamino" embraces "N-alkylamino" and "N,N-dialkylamino" where amino groups are independently substituted with one alkyl radical and with two alkyl radicals, respectively. "Lower alkylamino" radicals have one or two alkyl radicals of one to six carbon atoms attached to a nitrogen atom. Suitable alkylamino radicals may be mono or dialkylamino such as N-methylamino, N-ethylamino, N.N-dimethylamino, N,N-diethylamino and the like.

The term "halo" means halogens such as fluorine, chlorine, bromine or iodine atoms.

The term "haloalkyl" embraces radicals wherein any one or more of the alkyl carbon atoms is substituted with one or more halo as defined above. Examples include monohaloalkyl, dihaloalkyl and polyhaloalkyl radicals including perhaloalkyl. A monohaloalkyl radical, for one example, may have an iodo, bromo, chloro or fluoro atom within the radical. Dihalo and

polyhaloalkyl radicals may have two or more of the same halo atoms or a combination of different halo radicals. "Lower haloalkyl" embraces radicals having 1-6 carbon atoms. Examples of haloalkyl radicals include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl and dichloropropyl. "Perfluoroalkyl" means an alkyl radical having all hydrogen atoms replaced with fluoro atoms. Examples include trifluoromethyl and pentafluoroethyl.

The term "aryl", alone or in combination, means a carbocyclic aromatic system containing one or two rings wherein such rings may be attached together in a fused manner. The term "aryl" embraces aromatic radicals such as phenyl, naphthyl, indenyl, tetrahydronaphthyl, and indanyl. More preferred aryl is phenyl. Said "aryl" group may have 1 or more substituents such as lower alkyl, hydroxyl, halo, haloalkyl, nitro, cyano, alkoxy, lower alkylamino, and the like. An aryl group may be optionally substituted with one or more functional groups such as halo, hydroxy, nitro, amino, cyano, haloalkyl, aryl, heteroaryl, heterocyclo and the like.

The term "heterocyclyl" (or "heterocyclo") embraces saturated, and partially saturated heteroatom-containing ring radicals, where the heteroatoms may be selected from nitrogen, sulfur and oxygen. In one embodiment, heterocyclic rings comprise monocyclic 6-8 membered rings, as well as 5-16 membered bicyclic ring systems (which can include bridged fused and spiro-fused bicyclic ring systems). It does not include rings containing -O-O-.-O-S- or -S-S- portions. Said "heterocyclyl" group may have one or more substituents such as hydroxyl, Boc, halo, haloalkyl, cyano, lower alkyl, lower aralkyl, oxo, lower alkoxy, amino, lower alkylamino, and the like. In one embodiment, said "heterocyclyl" group may have 1 to 3 substituents such as hydroxyl, Boc, halo, haloalkyl, cyano, lower alkyl, lower aralkyl, oxo, lower alkoxy, amino, lower alkylamino, and the like. In an alternate embodiment, a heterocyclic ring comprises a monocyclic 3-6 membered ring.

Examples of saturated heterocyclo groups include saturated 3- to 6-membered heteromonocyclic groups containing 1 to 4 nitrogen atoms [e.g. pyrrolidinyl, imidazolidinyl, piperidinyl, pyrrolinyl, piperazinyl]; saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms [e.g. morpholinyl]; saturated 3 to 6-membered heteromonocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms

[e.g., thiazolidinyl]. Examples of partially saturated heterocyclyl radicals include dihydrothienyl, dihydropyranyl, dihydrofuryl, dihydrothiazolyl, and the like.

Particular examples of partially saturated and saturated heterocyclo groups include pyrrolidinyl, imidazolidinyl, piperidinyl, pyrrolinyl, pyrazolidinyl, piperazinyl, morpholinyl, tetrahydropyranyl, thiazolidinyl, dihydrothienyl, 2,3-dihydro-benzo[l,4]dioxanyl, indolinyl, isoindolinyl, dihydrobenzothienyl, dihydrobenzofuryl, isochromanyl, chromanyl, 1,2-dihydroquinolyl, 1,2,3,4- tetrahydro-isoquinolyl, 1 ,2,3,4-tetrahydro-quinolyl, 2,3,4,4a,9,9a-hexahydro-lH-3-aza-fluorenyl, 5,6,7- trihydro-l,2,4-triazolo[3,4-a]isoquinolyl, 3,4-dihydro-2H-benzo[l,4]oxazinyl, benzo[l,4]dioxanyl, 2,3- dihydro-lH-lλ'-benzo[d]isothiazol-6-yl, dihydropyranyl, dihydrofuryl and dihydrothiazolyl, and the like.

Heterocyclo groups also includes radicals where heterocyclic radicals are fused/condensed with aryl radicals: unsaturated condensed heterocyclic group containing 1 to 5 nitrogen atoms, for example, indolyl, isoindolyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, tetrazolopyridazinyl [e.g., tetrazolo [l,5-b]pyridazinyl]; unsaturated condensed heterocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms [e.g. benzoxazolyl, benzoxadiazolyl]; unsaturated condensed heterocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms [e.g., benzothiazolyl, benzothiadiazolyl]; and saturated, partially unsaturated and unsaturated condensed heterocyclic group containing 1 to 2 oxygen or sulfur atoms [e.g. benzofuryl, benzothienyl, 2,3-dihydro-benzo[l,4]dioxinyl and dihydrobenzofuryl].

The term "heteroaryl" denotes aryl ring systems that contain one or more heteroatoms selected from the group O, N and S, wherein the ring nitrogen and sulfur atom(s) are optionally oxidized, and nitrogen atom(s) are optionally quarternized. Examples include unsaturated 5 to 6 membered heteromonocyclyl group containing 1 to 4 nitrogen atoms, for example, pyrrolyl, imidazolyl, pyrazolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, triazolyl [e.g., 4H-l,2,4-triazolyl, IH-1 ,2,3-triazolyl, 2H-l,2,3-triazolyl]; unsaturated 5- to 6-membered heteromonocyclic group containing an oxygen atom, for example, pyranyl, 2-furyl, 3-furyl, etc.; unsaturated 5 to 6-membered heteromonocyclic group containing a sulfur atom, for example, 2-thienyl, 3-thienyl, etc.; unsaturated 5- to 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, oxazolyl, isoxazolyl, oxadiazolyl [e.g., 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5- oxadiazolyl]; unsaturated 5 to 6-membered

heteromonocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms, for example, thiazolyl, thiadiazolyl [e.g., 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl].

The term "heteroarylalkyl" denotes alkyl radicals substituted with a heteroaryl group. Examples include pyridylmethyl and thienylethyl.

The term "sulfonyl", whether used alone or linked to other terms such as alkylsulfonyl, denotes respectively divalent radicals $-SO_2-$.

The terms "carboxy" or "carboxyl", whether used alone or with other terms, such as "carboxyalkyl", denotes –C(O)–OH.

The term "carbonyl", whether used alone or with other terms, such as "aminocarbonyl", denotes -C(O)-.

The term "aminocarbonyl" denotes an amide group of the Formula $-C(O)–NH_2$.

The terms "heterocycloalkyl" embrace heterocyclic-substituted alkyl radicals. Examples include piperidylmethyl and morpholinylethyl.

The term "arylalkyl" embraces aryl-substituted alkyl radicals. Examples include benzyl, diphenylmethyl and phenylethyl. The aryl in said aralkyl may be additionally substituted with halo, alkyl, alkoxy, halkoalkyl and haloalkoxy.

The term "cycloalkyl" includes saturated carbocyclic groups of 3 to 10 carbons. Lower cycloalkyl groups include C_3-C_6 rings. Examples include cyclopentyl, cyclopropyl, and cyclohexyl. Cycloalkyl groups may be optionally substituted with one or more functional groups such as halo, hydroxy, nitro, amino, cyano, haloalkyl, aryl, heteroaryl, heterocyclo and the like.

The term "cycloalkylalkyl" embraces cycloalkyl-substituted alkyl radicals. "Lower cycloalkylalkyl" radicals are cycloalkyl radicals attached to alkyl radicals having one to six carbon atoms. Examples of include cyclohexylmethyl. The cycloalkyl in said radicals may be additionally substituted with halo, alkyl, alkoxy and hydroxy.

The term "cycloalkenyl" includes carbocyclic groups having one or more carbon-carbon double bonds including "cycloalkyldienyl" compounds. Examples include cyclopentenyl, cyclopentadienyl, cyclohexenyl and cycloheptadienyl.

The term "comprising" is meant to be open ended, including the indicated component but not excluding other elements.

The term "oxo" as used herein contemplates an oxygen atom attached with a double bond.

The term "nitro" as used herein contemplates $-NO_2$.

The term "cyano" as used herein contemplates –CN.

As used herein, the term "prodrug" means a compound which when administered to a host *in vivo* is converted into the parent drug. As used herein, the term "parent drug" means any of the presently described chemical compounds that are useful to treat any of the disorders described herein, or to control or improve the underlying cause or symptoms associated with any physiological or pathological disorder described herein in a host, typically a human. Prodrugs can be used to achieve any desired effect, including to enhance properties of the parent drug or to improve the pharmaceutic or pharmacokinetic properties of the parent. Prodrug strategies exist which provide choices in modulating the conditions for *in vivo* generation of the parent drug, all of which are deemed included herein. Nonlimiting examples of prodrug strategies include covalent attachment of removable groups, or removable portions of groups, for example, but not limited to acylation, phosphorylation, phosphonylation, phosphoramidate derivatives, amidation, reduction, oxidation, esterification, alkylation, other carboxy derivatives, sulfoxy or sulfone derivatives, carbonylation or anhydride, among others.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist, unless otherwise noted.

The current invention is directed to combinations and methods using CDK4/6 inhibitors and at least one additional chemotherapeutic agent for use in the treatment of Rb-positive proliferation disorders.

The term "selective CDK4/6 inhibitor" used in the context of the compounds described herein includes compounds that inhibit CDK4 activity, CDK6 activity, or both CDK4 and CDK6 activity at an IC_{50} molar concentration at least about 500 times less (or in alternative embodiments, at least 1000, 1500 or 2000 times less) than the IC50 molar concentration necessary to inhibit to the same degree of CDK2 activity in a standard phosphorylation assay.

As used herein the term "chemotherapy" or "chemotherapeutic agent" refers to treatment with a cytostatic or cytotoxic agent (i.e., a compound) to reduce or eliminate the growth or proliferation of undesirable cells, for example cancer cells. Thus, as used herein, "chemotherapy" or "chemotherapeutic agent" refers to a cytotoxic or cytostatic agent used to treat a proliferative disorder, for example cancer.

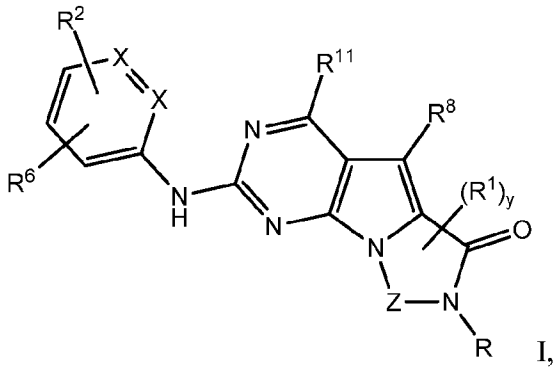
By "induces G1-arrest" is meant that the inhibitor compound induces a quiescent state in a substantial portion of a cell population at the G1 phase of the cell cycle.

By "synchronous reentry into the cell cycle" and similar phrases is meant that CDK 4/6 replication dependent cells, for example an Rb-positive abnormal proliferative cell or hematopoietic stem cell, in G1-arrest due to the effect of a CDK4/6 inhibitor compound reenters the cell-cycle within relatively the same collective timeframe or at relatively the same rate upon dissipation of the CDK 4/6 inhibitor compound's effect. Comparatively, by "asynchronous reentry into the cell cycle" is meant that cells in G1 arrest due to the effect of a CDK4/6 inhibitor compound reenter the cell cycle within relatively different collective timeframes or at relatively different rates upon dissipation of the CDK 4/6 inhibotor compound's effect.

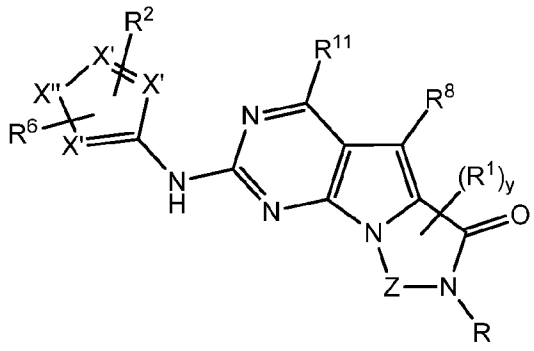
The subject or host treated is typically a human subject, although it is to be understood the methods described herein are effective with respect to other animals, such as mammals and vertebrate species. More particularly, the term subject can include animals used in assays such as those used in preclinical testing including but not limited to mice, rats, monkeys, dogs, pigs and rabbits; as well as domesticated swine (pigs and hogs), ruminants, equine, poultry, felines, bovines, murines, canines, and the like.

Active Compounds

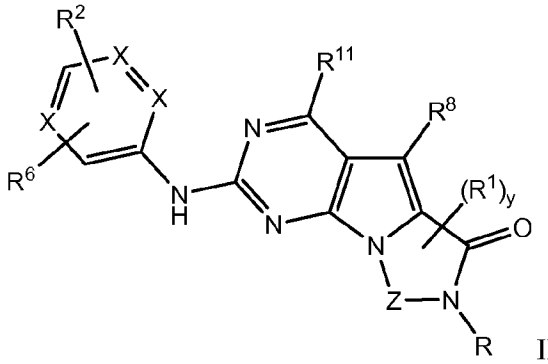
In one embodiment, the inventions described herein use the CDK4/6 inhibitors of Formula I, II, III, IV, or V:



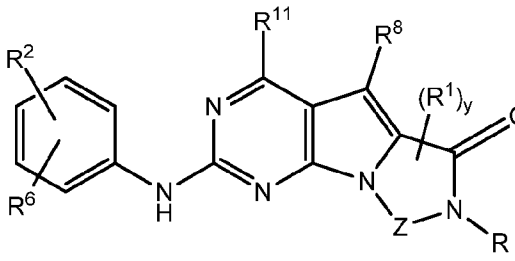
I,



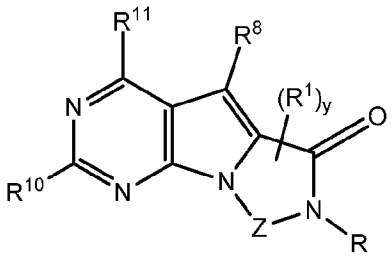
II,



III,



IV, or



V,

or a pharmaceutically acceptable salt thereof;

wherein:

Z is $-(CH_2)_x-$ wherein x is 1, 2, 3 or 4 or $-O-(CH_2)_z-$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is –$(alkylene)_m$–heterocyclo, –$(alkylene)_m$–heteroaryl, –$(alkylene)_m$–NR^3R^4, –$(alkylene)_m$–C(O)–NR^3R^4; –$(alkylene)_m$–C(O)–O-alkyl; –$(alkylene)_m$–O–R^5, –$(alkylene)_m$–$S(O)_n$–R^5, or –$(alkylene)_m$–$S(O)_n$–NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^5* at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, -(alkylene)$_m$-OR^5, -(alkylene)$_m$-O-alkylene-OR^5, -(alkylene)$_m$-S(O)$_n$–R^5, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-CN, -(alkylene)$_m$-C(O)–R^5, -(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5, -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*, -(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*, -(alkylene)$_m$-SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO_2–R^5*, -(alkylene)$_m$-N(R^3*)–SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*, -(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^{3*} and R^{4*} together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

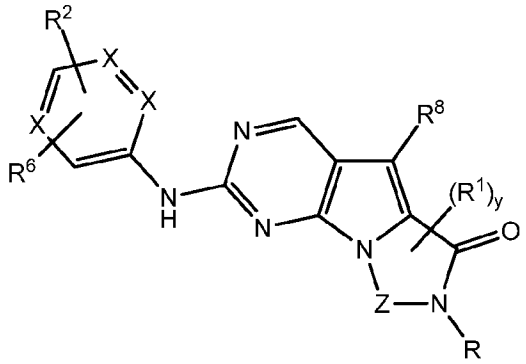
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -$(alkylene)_m$-heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$-$S(0)_n$-R^5, or -$(alkylene)_m$-$S(0)_n$-NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C_1-C_8 alkyl, cycloalkylalkyl, or –TT-RR, C_1-C_8 cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C_1-C_8 alkyl or C_3-C_8 cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C_1-C_6 alkoxy, amino, unsubstituted or substituted C_1-C_6 alkylamino, unsubstituted or substituted di-C_1-C_6 alkylamino, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13} , wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

or a pharmaceutically acceptable salt, prodrug or isotopic variant, for example, partically or fully deuterated form thereof.

In some aspects, the CDK4/6 inhibitor used is of Formula I, Formula II, Formula III, or Formula IV wherein R^6 is absent.

In some aspects, the CDK4/6 inhibitor used is of Formula III:



and the variables are as defined for compounds of Formulae I and II and pharmaceutically acceptable salts thereof.

In some aspects, R^x is not further substituted.

In some aspects, R^2 is $-(alkylene)_m-heterocyclo$, $-(alkylene)_m-heteroaryl$, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$; $-(alkylene)_m-O-R^5$, $-(alkylene)_m-S(O)_n-R^5$, or $-(alkylene)_m-S(O)_n-NR^3R^4$ any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2.

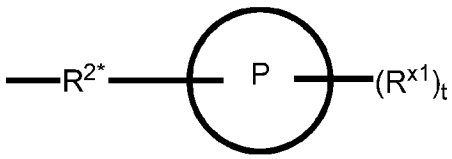
In some aspects, R^8 is hydrogen or C_1-C_3 alkyl.

In some aspects, R is hydrogen or C_1-C_3 alkyl.

In some aspects, R^2 is $-(alkylene)_m-heterocyclo$, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$, $-(alkylene)_m-C(O)-O$-alkyl or $-(alkylene)_m-OR^5$ any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring.

In some aspects, R^2 is $-(alkylene)_m-heterocyclo$, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$, $-(alkylene)_m-C(O)-O$-alkyl or $-(alkylene)_m-OR^5$ without further substitution.

In some aspects, m in R^2 is 1. In a further aspect, the alkylene in R^2 is methylene.

In some aspects, R^2 is  wherein:

R^{2*} is a bond, alkylene, -(alkylene)$_m$-O-(alkylene)$_m$-, -(alkylene)$_m$-C(O)-(alkylene)$_m$-, -(alkylene)$_m$-S(O)$_2$-(alkylene)$_m$- or -(alkylene)$_m$-NH-(alkylene)$_m$- wherein each m is independently 0 or 1;

P is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group;

each R^{x1} is independently -(alkylene)$_m$-(C(O))$_m$-(alkylene)$_m$-(N(R^N))$_m$-(alkyl)$_m$ wherein each m is independently 0 or 1 provided at least one m is 1, -(C(O))-O-alkyl, -(alkylene)$_m$-cycloalkyl wherein m is 0 or 1, -N(R^N)-cycloalkyl, -C(O)-cycloalkyl, -(alkylene)$_m$-heterocyclyl wherein m is 0 or 1, or -N(R^N)-heterocyclyl, -C(O)-heterocyclyl,-S(O)$_2$-(alkylene)$_m$ wherein m is 1 or 2, wherein:

R^N is H, C_1 to C_4 alkyl or C_1 to C_6 heteroalkyl, and

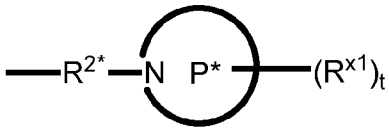
wherein two R^{x1} can, together with the atoms to which they attach on P, which may be the same atom, form a ring; and

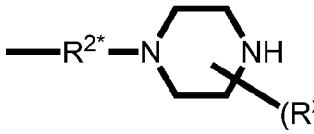
t is 0, 1 or 2.

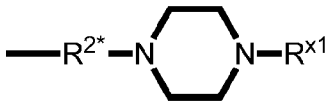
In some aspects, each R^{x1} is only optionally substituted by unsubstituted alkyl, halogen or hydroxy.

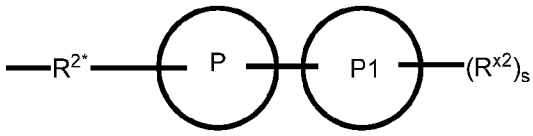
In some aspects, R^{x1} is hydrogen or unsubstituted C_1-C_4 alkyl.

In some aspects, at least one R^{x1} is -(alkylene)$_m$-heterocyclyl wherein m is 0 or 1.

In some aspects, R^2 is  wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some aspects, R^2 is .

In some aspects, R^2 is .

In some aspects, R^2 is  wherein:

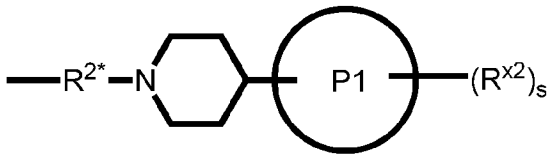
R^{2*} is a bond, alkylene, -(alkylene)$_m$-O-(alkylene)$_m$-, -(alkylene)$_m$-C(O)-(alkylene)$_m$-, -(alkylene)$_m$-S(O)$_2$-(alkylene)$_m$- and -(alkylene)$_m$-NH-(alkylene)$_m$- wherein each m is independently 0 or 1;

P is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group;

P1 is a 4- to 6-membered monocyclic saturated heterocyclyl group;

each R^{x2} is independently hydrogen or alkyl; and

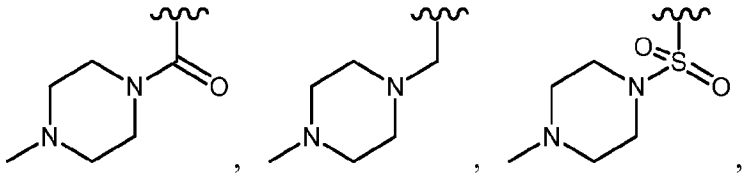
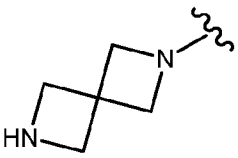
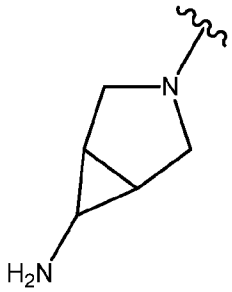
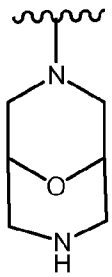
s is 0, 1 or 2.

In some aspects, R^2 is .

In some aspects, P1 includes at least one nitrogen.

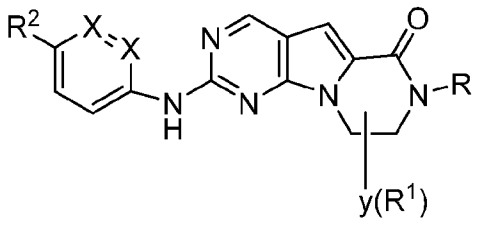
In some aspects, any alkylene in R^{2*} in any previous aspect is not further substituted.

In some aspects, R^2 is selected from the structures depicted in FIGS. 2-4.

In some aspects, R^2 is , , , ,  or .

In some aspects, the compound has general Formula I and more specifically one of the general structures in FIGS. 5-9 wherein the variables are as previously defined.

In some aspects, the CDK4/6 inhibitor used has general Formula Ia:



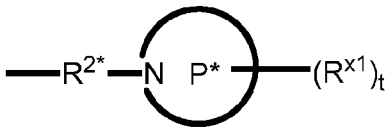
Ia

wherein R^1, R^2, R and y are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ia and R is alkyl.

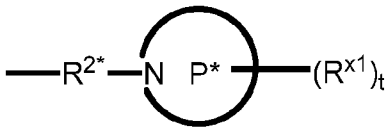
In some embodiments, the CDK4/6 inhibitor used has Formula Ia and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ia and R^2 is



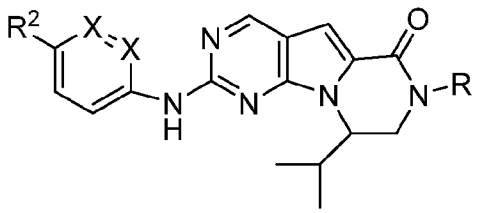
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ia and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or unsubstituted C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ib:



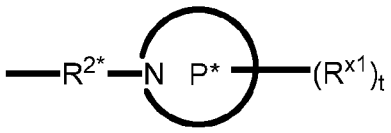
Ib

wherein R^2 and R are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ib and R is alkyl.

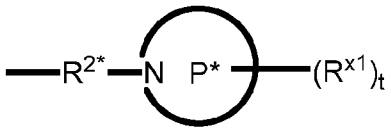
In some embodiments, the CDK4/6 inhibitor used has Formula Ib and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ib and R^2 is



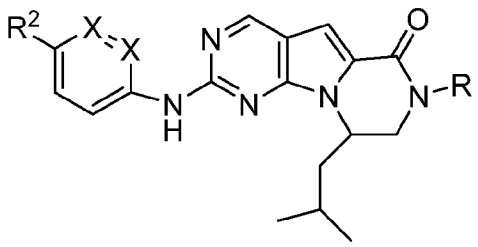
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ib and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ic:



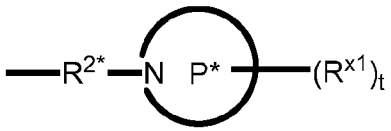
Ic

wherein R^2 and R are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ic and R is alkyl.

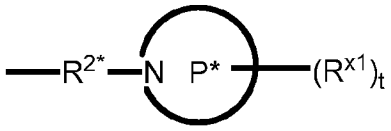
In some embodiments, the CDK4/6 inhibitor used has Formula Ic and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ic and R^2 is



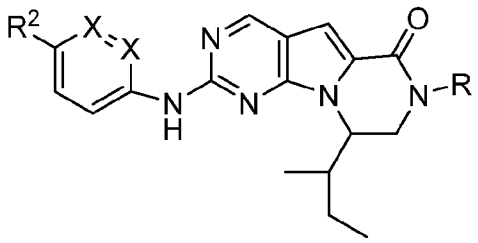
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ic and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Id:



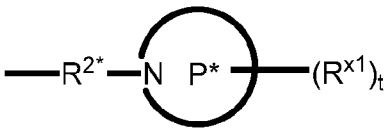
Id

wherein R^2 and R are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Id and R is alkyl.

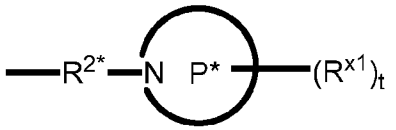
In some embodiments, the CDK4/6 inhibitor used has Formula Id and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Id and R^2 is



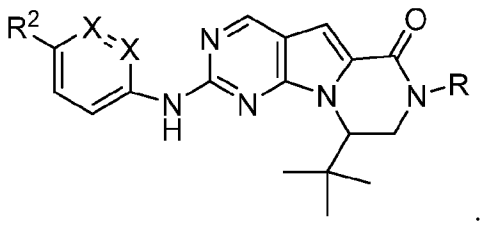
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Id and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ie:

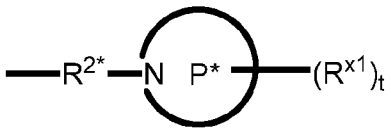


.

In some embodiments, the CDK4/6 inhibitor used has Formula Ie and R is alkyl.

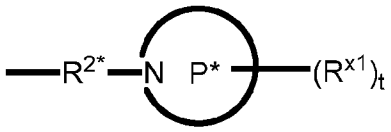
In some embodiments, the CDK4/6 inhibitor used has Formula Ie and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ie and R^2 is



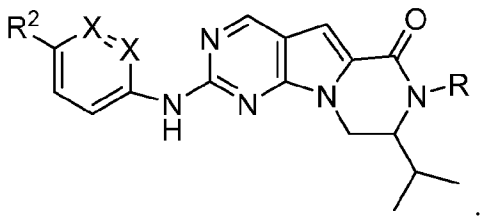
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ie and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula If:

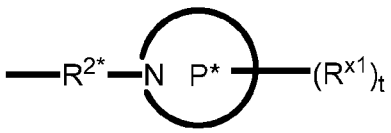


.

In some embodiments, the CDK4/6 inhibitor used has Formula If and R is alkyl.

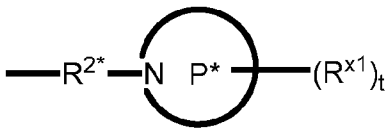
In some embodiments, the CDK4/6 inhibitor used has Formula If and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula If and R^2 is



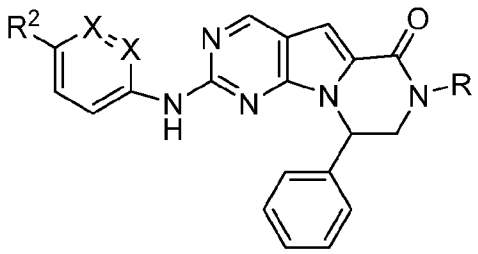
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula If and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ig:

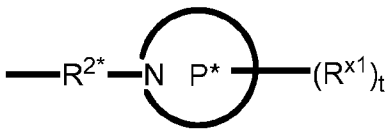


.

In some embodiments, the CDK4/6 inhibitor used has Formula Ig and R is alkyl.

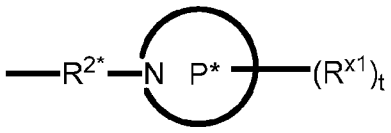
In some embodiments, the CDK4/6 inhibitor used has Formula Ig and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ig and R^2 is



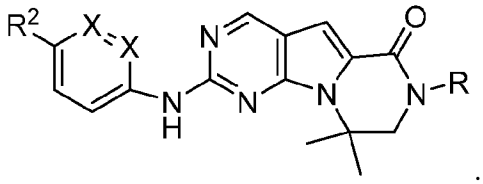
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ig and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ih:

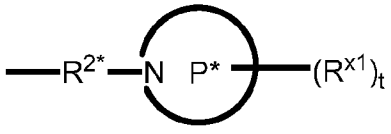


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In some embodiments, the CDK4/6 inhibitor used has Formula Ih and R is alkyl.

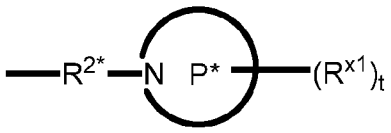
In some embodiments, the CDK4/6 inhibitor used has Formula Ih and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ih and R^2 is



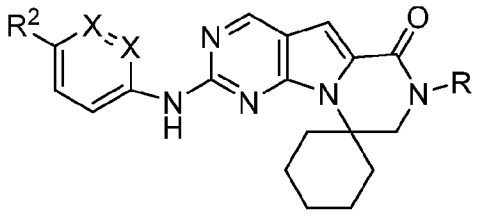
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ih and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ii:

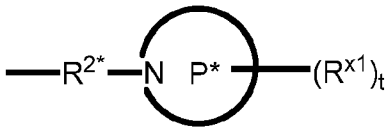


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In some embodiments, the CDK4/6 inhibitor used has Formula Ii and R is alkyl.

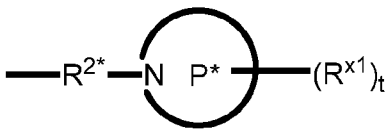
In some embodiments, the CDK4/6 inhibitor used has Formula Ii and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ii and R^2 is



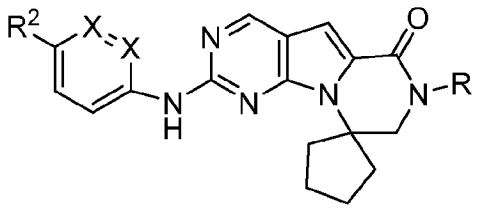
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group and R^{2*}, R^{x1} and t are as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ii and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl and R^{2*} is as previously defined.

In some embodiments, the CDK4/6 inhibitor used has Formula Ij:

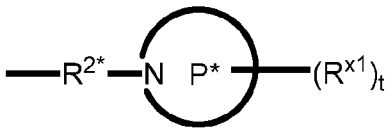


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In some embodiments, the CDK4/6 inhibitor used has Formula Ij and R is alkyl.

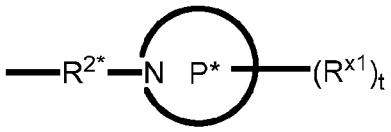
In some embodiments, the CDK4/6 inhibitor used has Formula Ij and R is H.

In some embodiments, the CDK4/6 inhibitor used has Formula Ij and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

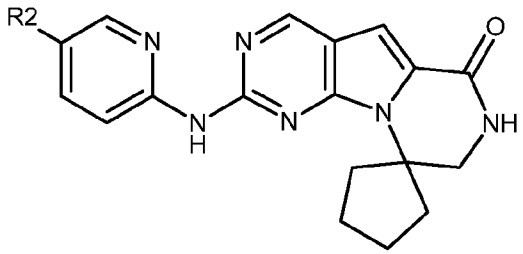
In some embodiments, the CDK4/6 inhibitor used has Formula Ij and R^2 is



wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

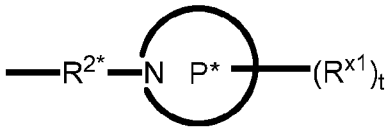
In some embodiments, the CDK4/6 inhibitor used has Formula Ij and R is H, and both X are N.

In some embodiments, the CDK4/6 inhibitor used has the structure:



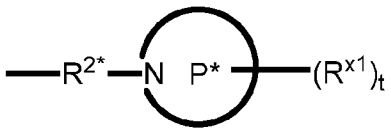
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In some embodiments, the CDK4/6 inhibitor used has Formula Ik and R^2 is



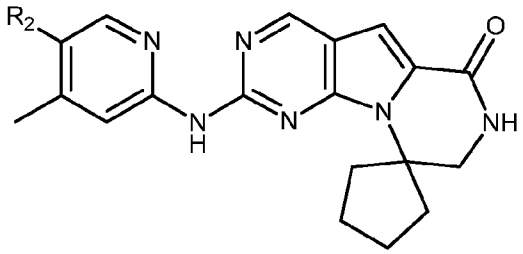
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some embodiments, the CDK4/6 inhibitor used has Formula Ik and R^2 is



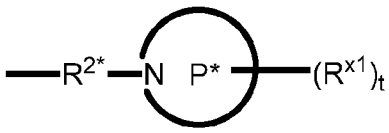
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

In some embodiments, the CDK4/6 inhibitor used has Formula II:



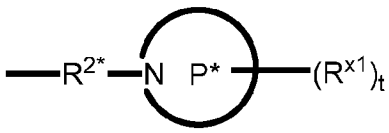
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In some embodiments, the CDK4/6 inhibitor used has Formula II and R^2 is



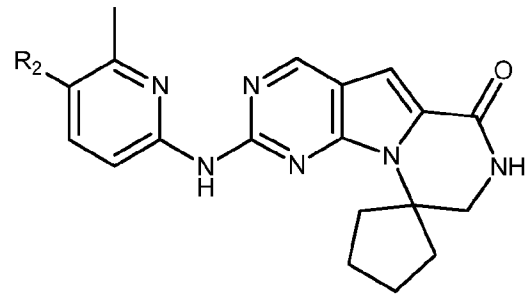
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some embodiments, the CDK4/6 inhibitor used has Formula II and R^2 is



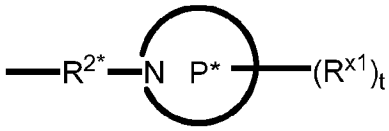
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

In some embodiments, the CDK4/6 inhibitor used has Formula Im:



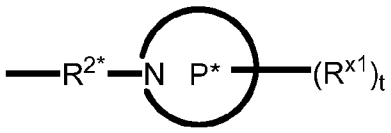
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In some embodiments, the CDK4/6 inhibitor used has Formula Im and R^2 is



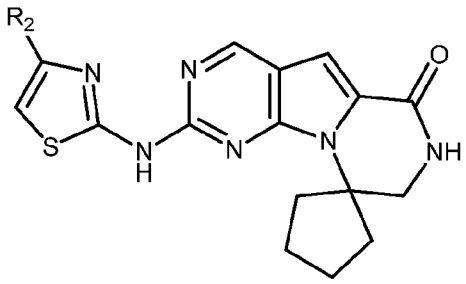
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some embodiments, the CDK4/6 inhibitor used has Formula Im and R^2 is



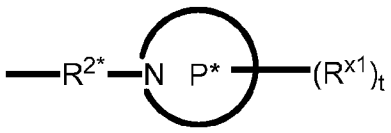
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

In some embodiments, the CDK4/6 inhibitor used has Formula IIa:



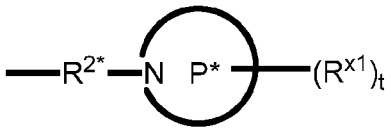
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In some embodiments, the CDK4/6 inhibitor used has Formula IIa and R^2 is



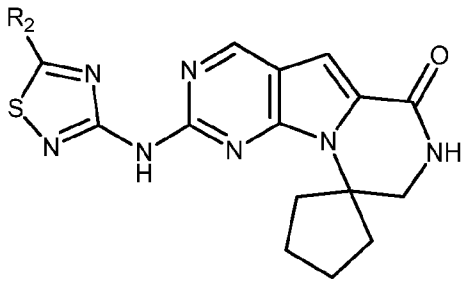
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some embodiments, the CDK4/6 inhibitor used has Formula IIa and R^2 is



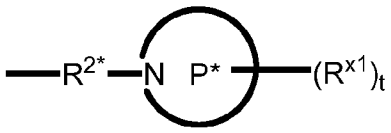
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

In some embodiments, the CDK4/6 inhibitor used has Formula IIb:



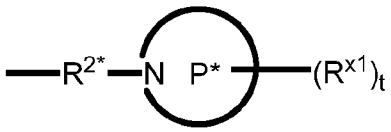
.

In some embodiments, the CDK4/6 inhibitor used has Formula Im and R^2 is



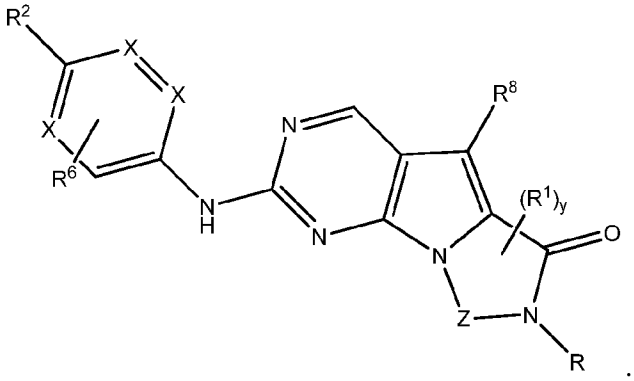
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group.

In some embodiments, the CDK4/6 inhibitor used has Formula Im and R^2 is



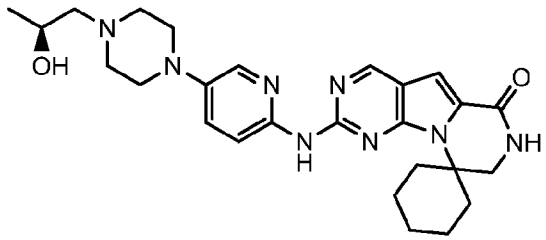
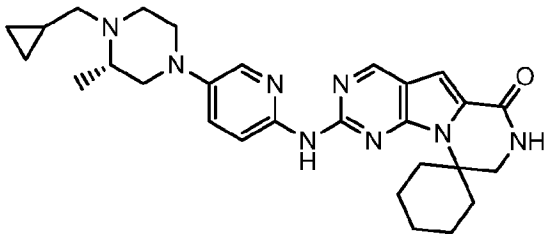
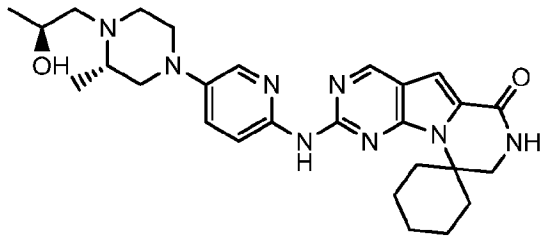
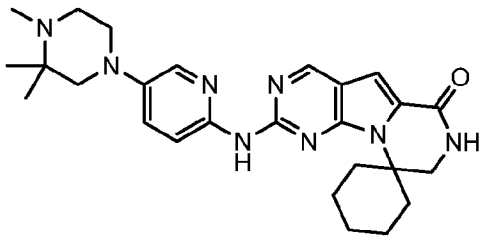
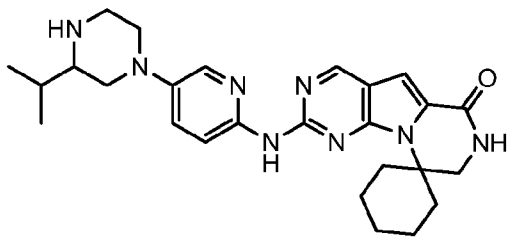
wherein P* is a 4- to 8-membered mono- or bicyclic saturated heterocyclyl group, R^{x1} is hydrogen or C_1-C_4 alkyl.

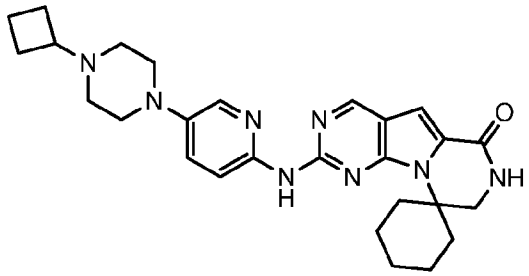
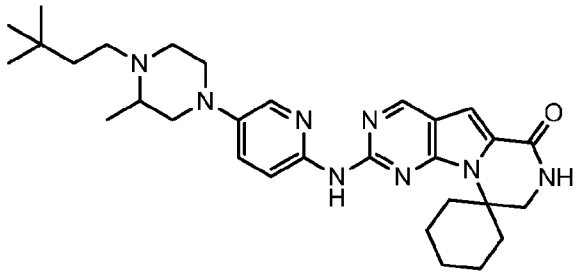
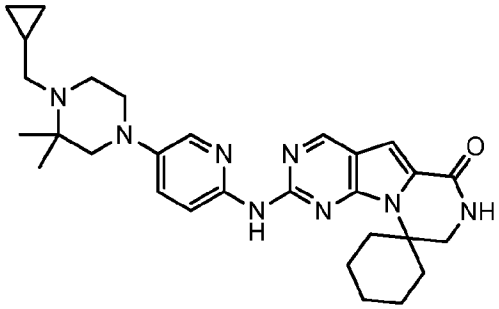
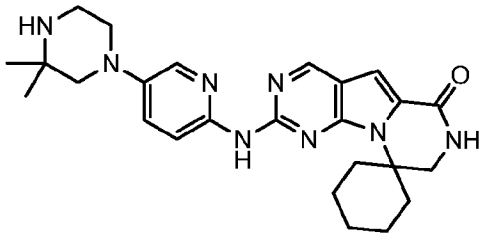
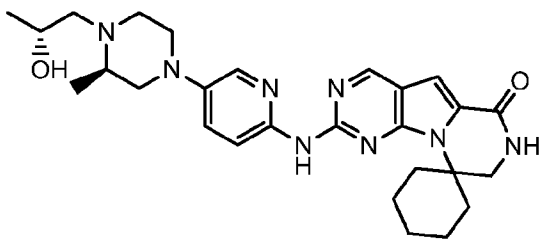
In some aspects, the CDK4/6 inhibitor used is:

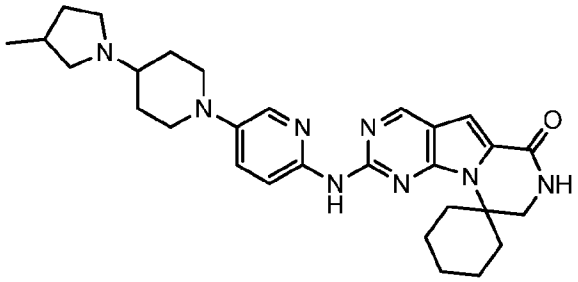
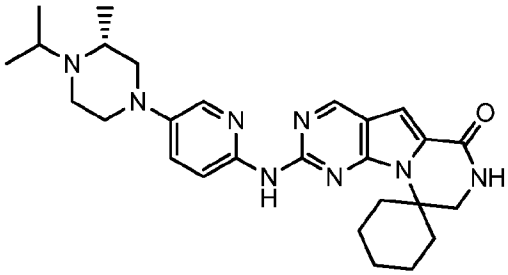
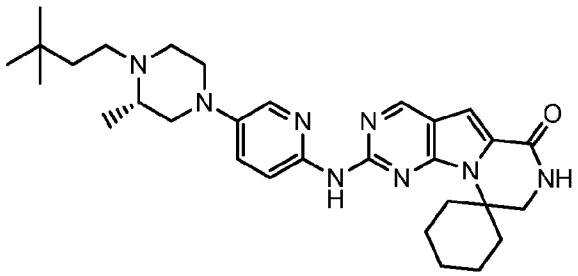
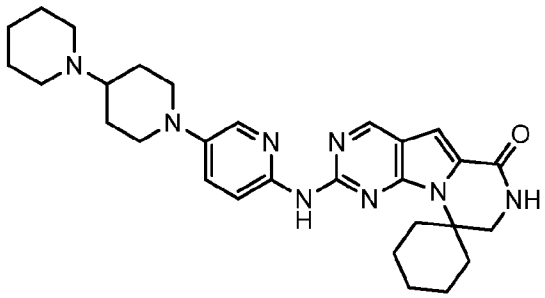
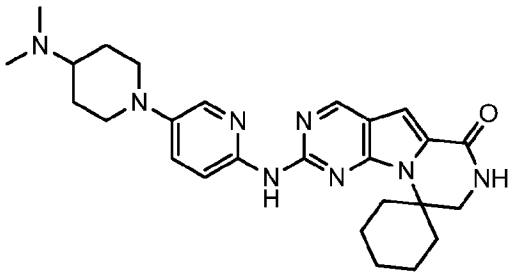


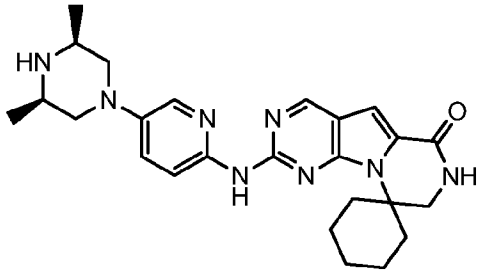
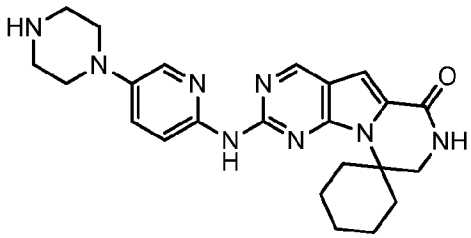
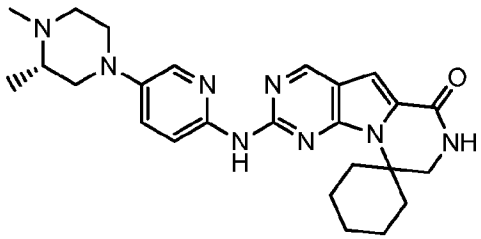
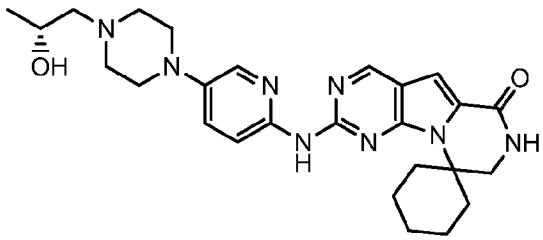
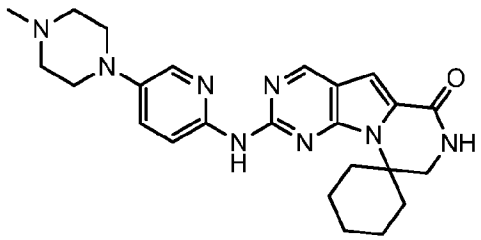
Further specific CDK4/6 inhibitor compounds that fall within the present invention and that can be used in the disclosed methods of treatment and combinations include the structures listed in Table 1 below.

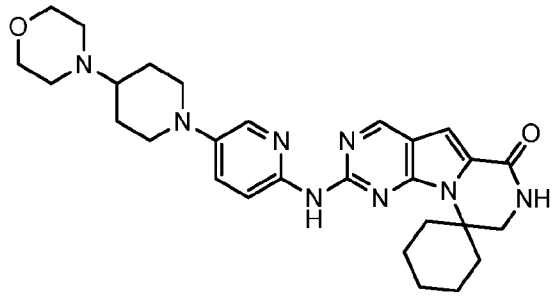
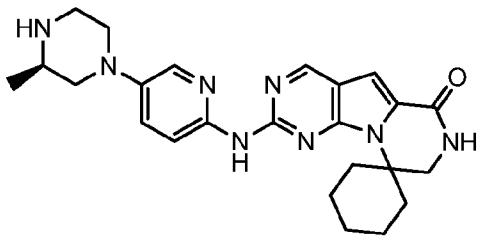
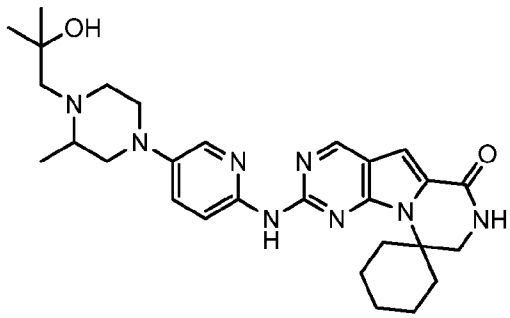
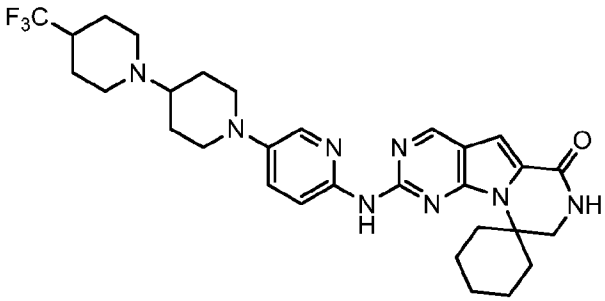
Table 1: Nonlimiting Examples of Compounds of Formula I, II, III, IV, or V

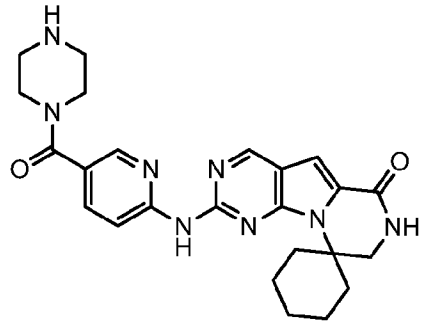
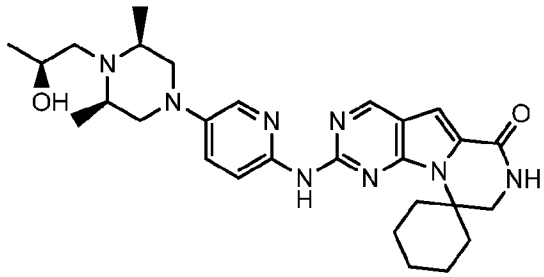
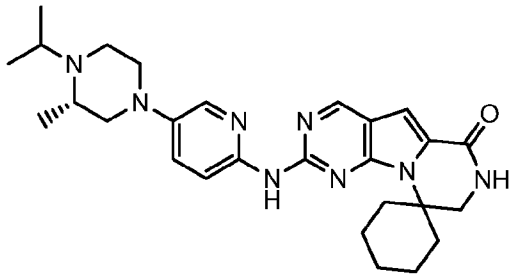
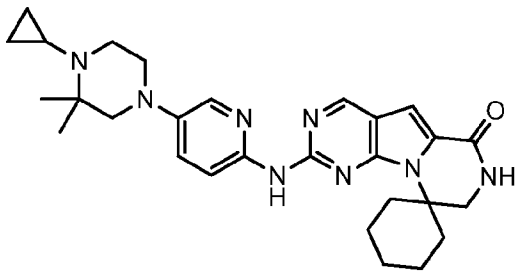
Structure Reference	Structure
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B	
C	
D	
E	

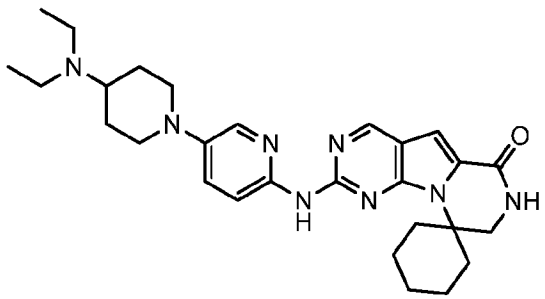
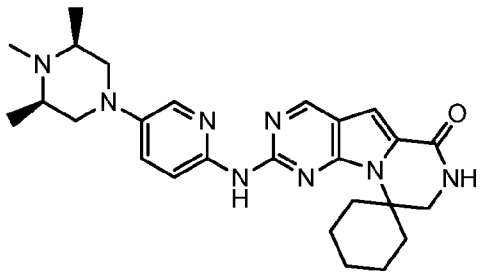
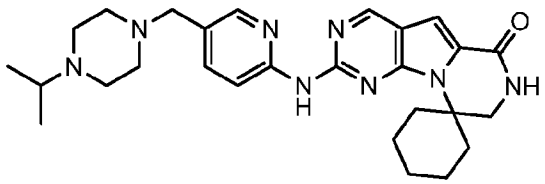
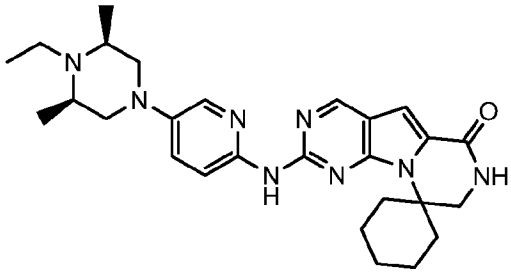
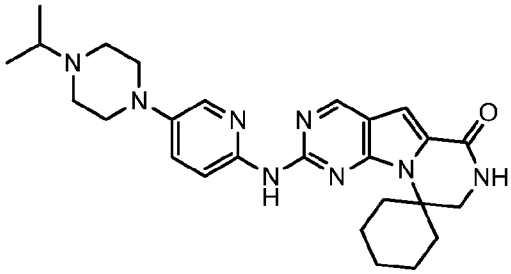
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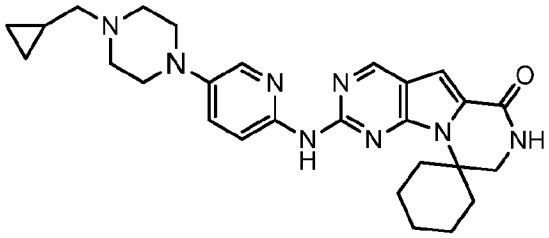
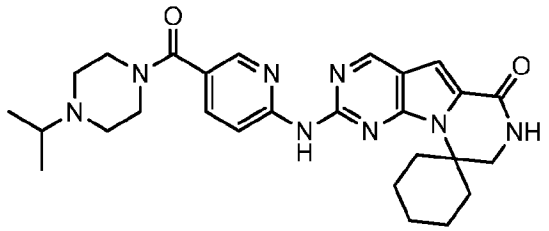
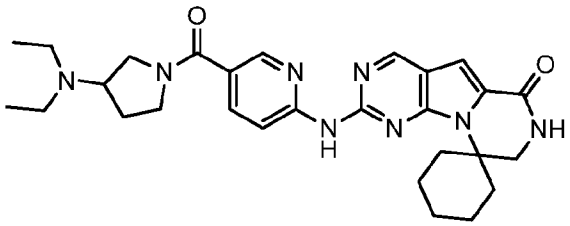
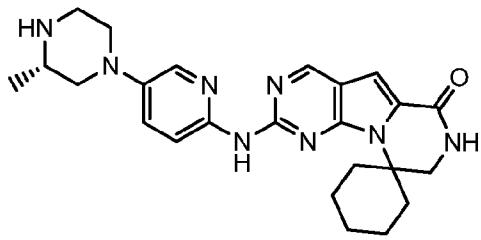
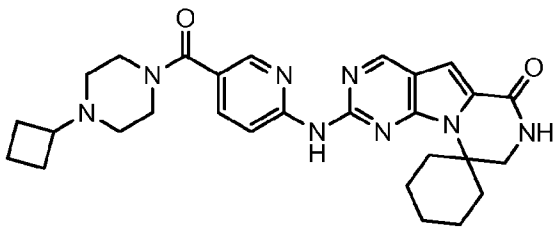
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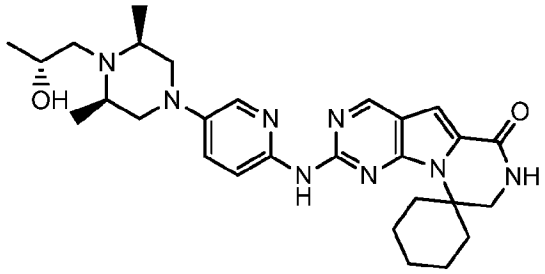
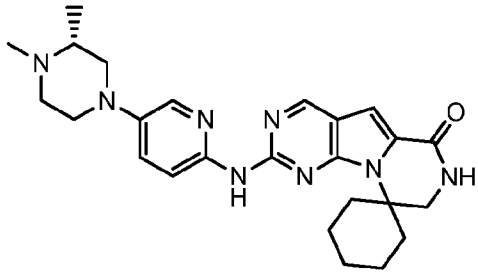
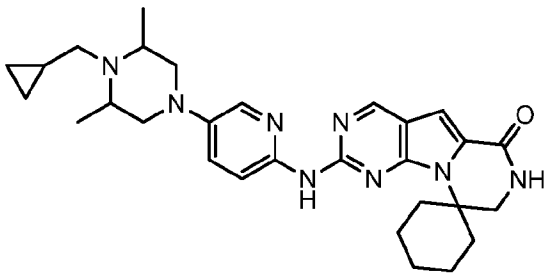
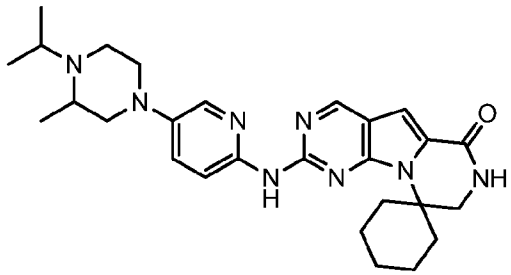
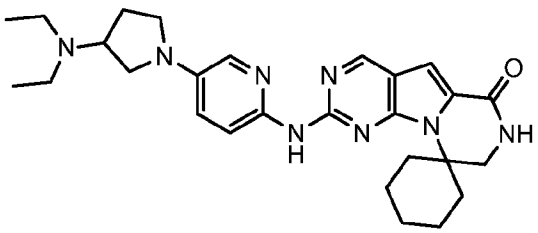
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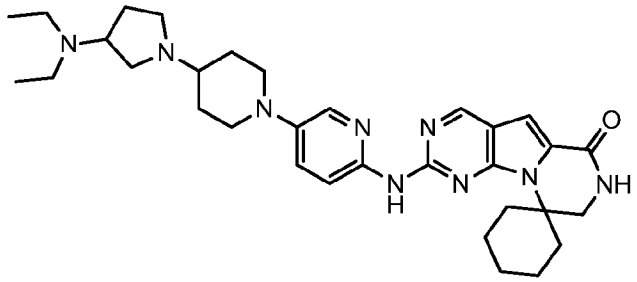
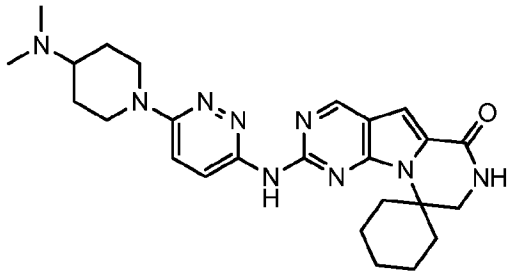
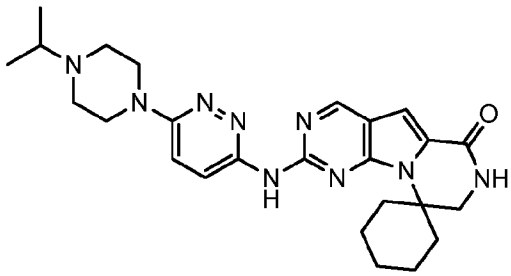
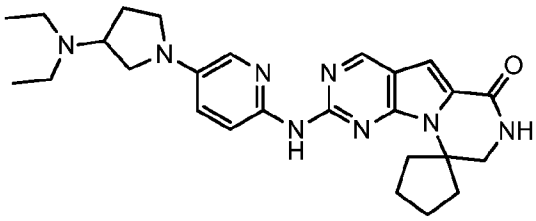
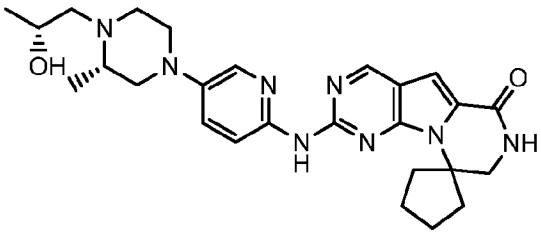
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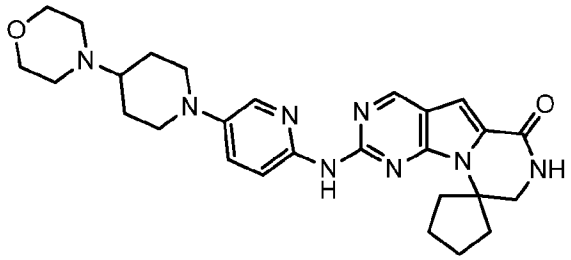
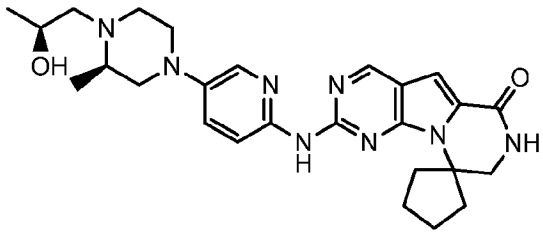
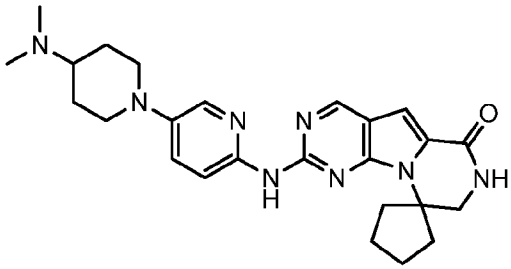
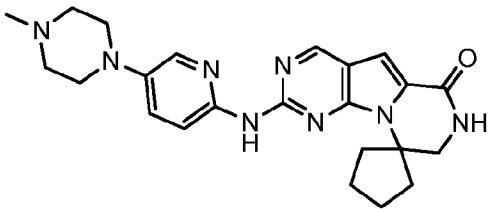
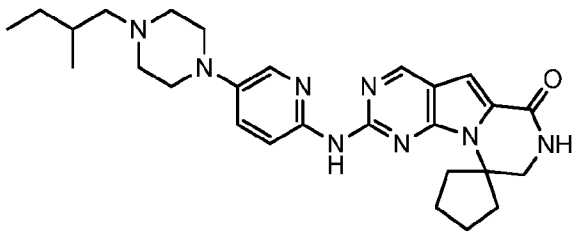
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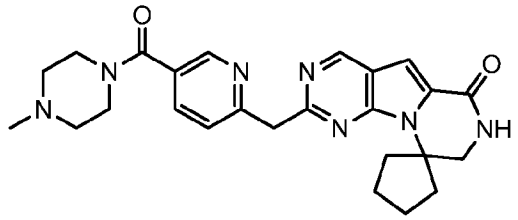
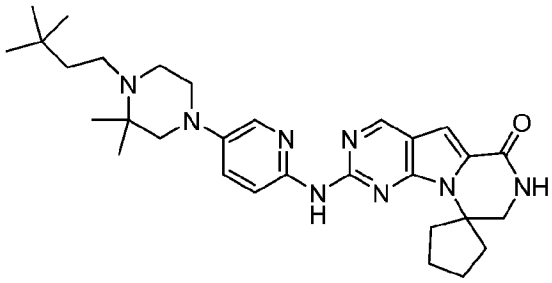
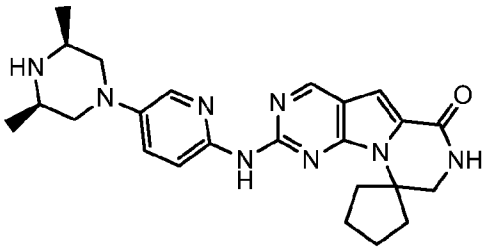
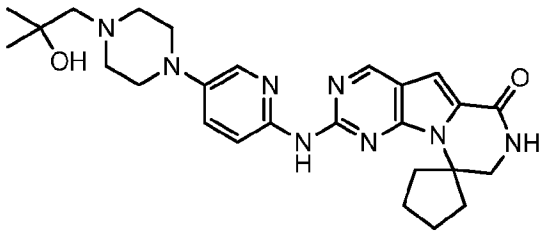
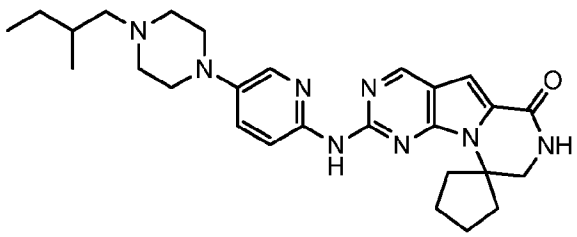
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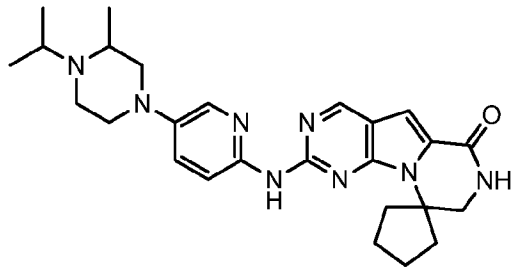
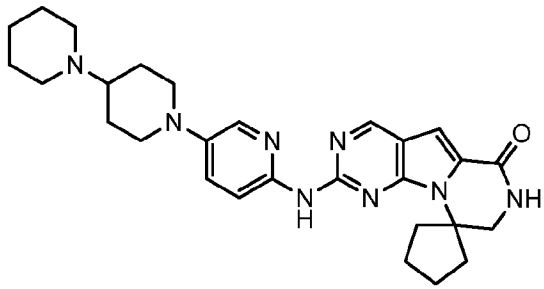
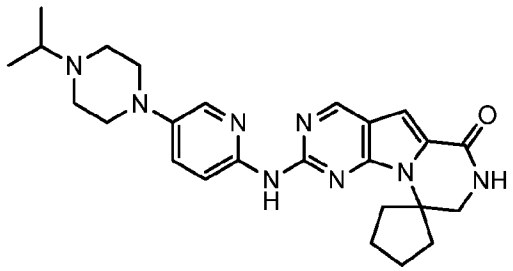
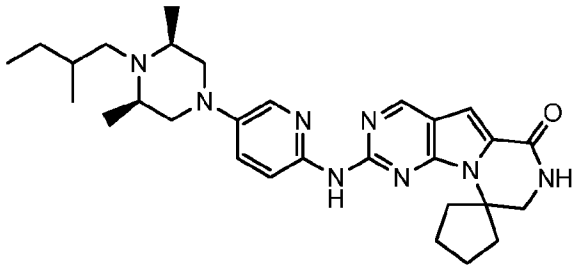
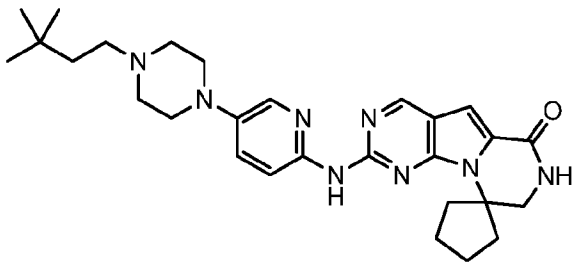
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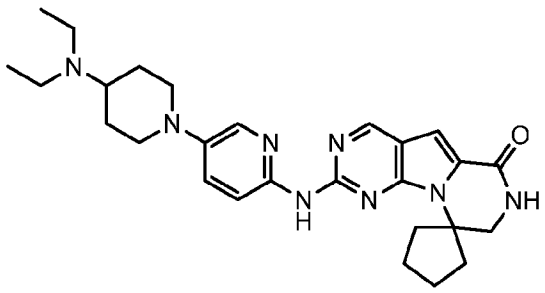
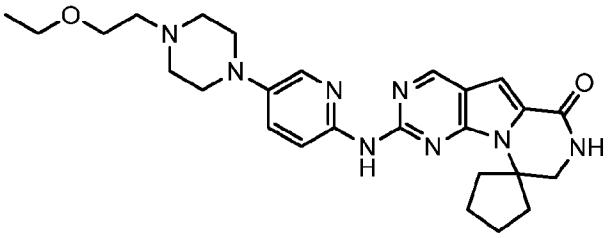
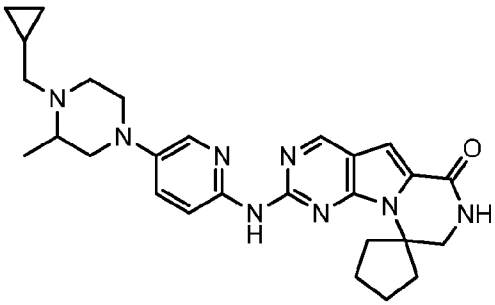
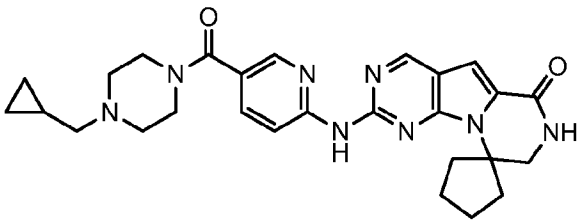
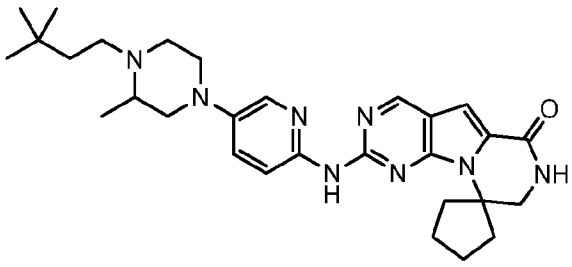
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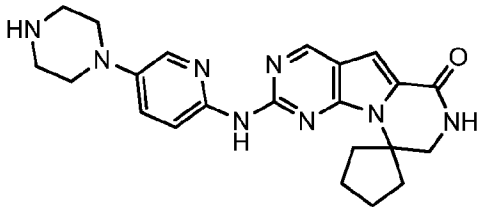
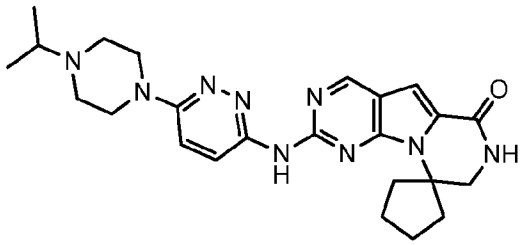
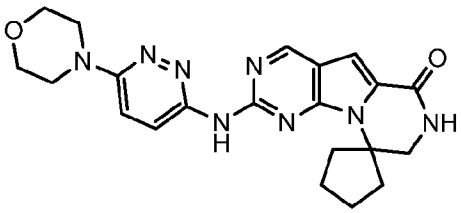
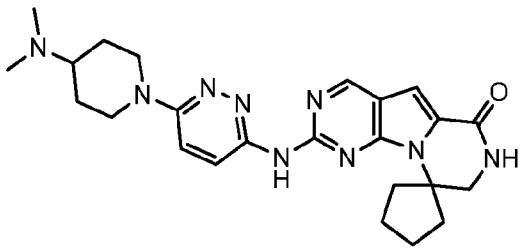
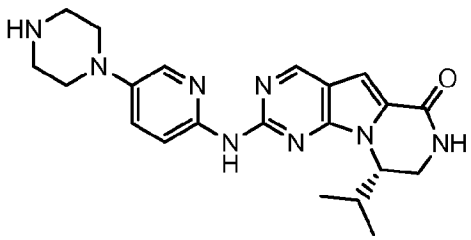
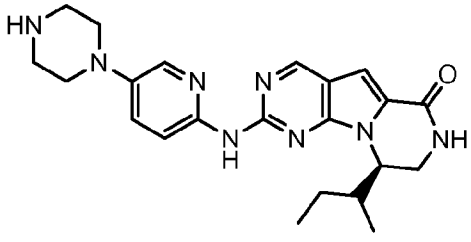
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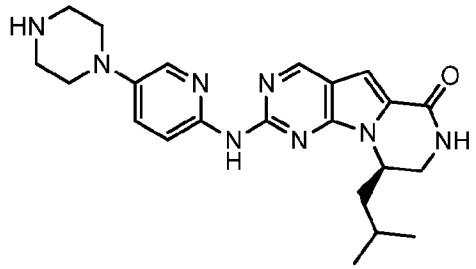
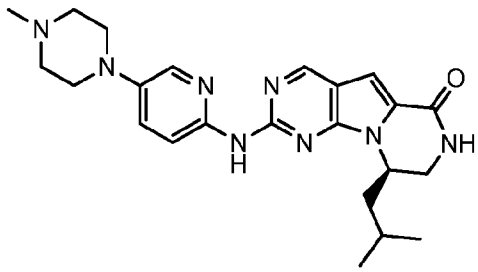
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The CDK4/6 inhibitors for use in the described methods are highly selective, potent CDK4/6 inhibitors, with minimal CDK2 inhibitory activity. In a range of embodiments, a compound for use in the methods described herein has a CDK4/CycD1 IC50 inhibitory concentration value that is >1500 times, >1800 times, >2000 times, >2200 times, >2500 times, >2700 times, >3000 times, >3200 times or greater lower than its respective IC50 concentration value for CDK2/CycE inhibition. In another range of embodiments, a compound for use in the methods described herein has an IC50 concentration value for CDK4/CycD1 inhibition that is about <1.50 nM, <1.25 nM, <1.0 nM, <0.90 nM, <0.85 nM, <0.80 nM, <0.75 nM, <0.70 nM, <0.65 nM, <0.60 nM, <0.55 nM, or less. In yet a range of embodiments, a CDK4/6 inhibitor for use in the methods described herein has an IC50 concentration value for CDK2/CycE inhibition that is about >1.0 μM, >1.25 μM, >1.50 μM, >1.75 μM, >2.0 μM, >2.25 μM, >2.50 μM, >2.75 μM, >3.0 μM, >3.25 μM, >3.5 μM or greater. In still other embodiments, a compound for use in the methods described herein has an IC50 concentration value for CDK2/CycA IC50 that is >0.80 μM, >0.85 μM, >0.90 μM, >0.95 μM, >.1.0 μM, >1.25 μM, >1.50 μM, >1.75 μM, >2.0 μM, >2.25 μM, >2.50 μM, >2.75 uM, >3.0 μM or greater.

Isotopic Substitution

In certain aspects of the present invention, included is the use of CDK4/6 inhibitors and/or a chemotherapeutic agent with desired isotopic substitutions of atoms, at amounts above the natural abundance of the isotope, i.e., enriched. Isotopes are atoms having the same atomic number but different mass numbers, i.e., the same number of protons but a different number of neutrons. By way of general example and without limitation, isotopes of hydrogen, for example, deuterium (^{2}H) and tritium (^{3}H) may be used anywhere in described structures. Alternatively or in addition, isotopes of carbon, e.g., ^{13}C and ^{14}C, may be used. A preferred isotopic substitution is deuterium for hydrogen at one or more locations on the molecule to improve the performance of the drug. The deuterium can be bound in a location of bond breakage during metabolism (an α-deuterium kinetic isotope effect) or next to or near the site of bond breakage (a β-deuterium kinetic isotope effect).

Substitution with isotopes such as deuterium can afford certain therapeutic advantages resulting from greater metabolic stability, such as, for example, increased in vivo half-life or reduced dosage requirements. Substitution of deuterium for hydrogen at a site of metabolic break down can reduce the rate of or eliminate the metabolism at that bond. At any position of the compound that a hydrogen atom may be present, the hydrogen atom can be any isotope of hydrogen, including protium (^{1}H), deuterium (^{2}H) and tritium (^{3}H). Thus, reference herein to a compound encompasses all potential isotopic forms unless the context clearly dictates otherwise.

The term "isotopically-labeled" analog refers to an analog that is a "deuterated analog", a "^{13}C-labeled analog," or a "deuterated/^{13}C-labeled analog." The term "deuterated analog" means a compound described herein, whereby a H-isotope, i.e., hydrogen/protium (^{1}H), is substituted by a H-isotope, i.e., deuterium (^{2}H). Deuterium substitution can be partial or complete. Partial deuterium substitution means that at least one hydrogen is substituted by at least one deuterium. In certain embodiments, the isotope is 90, 95 or 99% or more enriched in an isotope at any location of interest. In some embodiments it is deuterium that is 90, 95 or 99% enriched at a desired location.

Further specific compounds that fall within the present invention and that can be used in the disclosed methods of treatment and compositions include the structures of Formula I, II, III, IV, or V, and those listed above in Table 1.

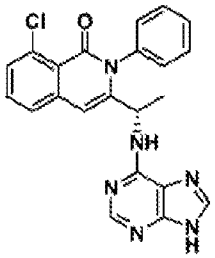
Combination Therapy

In one aspect, provided is a treatment regimen comprising the administration of a CDK4/6 inhibitor described herein in combination or in alternation with at least one additional chemotherapeutic agent. The combinations and/or alternations disclosed herein can be administered for beneficial, additive, or synergistic effect in the treatment of Rb-positive abnormal cellular proliferative disorders.

In specific embodiments, the treatment regimen includes the administration of a CDK4/6 inhibitor described herein in combination or alternation with at least one additional kinase inhibitor. In one embodiment, the at least one additional kinase inhibitor is selected from a phosphoinositide 3-kinase (PI3K) inhibitor, a Bruton's tyrosine kinase (BTK) inhibitor, or a spleen tyrosine kinase (Syk) inhibitor, or a combination thereof.

PI3K inhibitors that may be used in the present invention are well known. Examples of PI3 kinase inhibitors include but are not limited to Wortmannin, demethoxyviridin, perifosine, idelalisib, Pictilisib , Palomid 529, ZSTK474, PWT33597, CUDC-907, and AEZS-136, duvelisib, GS-9820, BKM120, GDC-0032 (Taselisib) (2-[4-[2-(2-Isopropyl-5-methyl-1,2,4-triazol-3-yl)-5,6-dihydroimidazo[1,2-d][1,4]benzoxazepin-9-yl]pyrazol-1-yl]-2-methylpropanamide), MLN-1117 ((2R)-1-Phenoxy-2-butanyl hydrogen (S)-methylphosphonate; or Methyl(oxo) {[(2R)-l-phenoxy-2-butanyl]oxy}phosphonium)), BYL-719 ((2S)-N1-[4-Methyl-5-[2-(2,2,2-trifluoro-1,1-dimethylethyl)-4-pyridinyl]-2-thiazolyl]-1,2-pyrrolidinedicarboxamide), GSK2126458 (2,4-Difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide) (omipalisib), TGX-221 ((±)-7-Methyl-2-(morpholin-4-yl)-9-(l-phenylaminoethyl)-pyrido[l,2-a]-pyrimidin-4-one), GSK2636771 (2-Methyl-1-(2-methyl-3-(trifluoromethyl)benzyl)-6-morpholino-lH-benzo[d]imidazole-4-carboxylic acid dihydrochloride), KIN-193 ((R)-2-((l-(7-methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidin-9-yl)ethyl)amino)benzoic acid), TGR-1202/RP5264, GS-9820 ((S)- l-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-mohydroxypropan- 1 -one), GS-1101 (5-fluoro-3-phenyl-2-([S)]-1-[9H-purin-6-ylamino]-propyl)-3H-quinazolin-4-one), AMG-319, GSK-2269557, SAR245409 (N-(4-(N-(3-((3,5-dimethoxyphenyl)amino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4 methylbenzamide), BAY80-6946 (2-amino-N-(7-methoxy-8-(3-morpholinopropoxy)-2,3-dihydroimidazo[l,2-c]quinaz), AS 252424 (5-[l-[5-(4-Fluoro-2-hydroxy-phenyl)-furan-2-yl]-meth-(Z)-ylidene]-thiazolidine-2,4-dione), CZ 24832 (5-(2-amino-8-fluoro-[l,2,4]triazolo[l,5-

a]pyridin-6-yl)-N-tert-butylpyridine-3-sulfonamide), Buparlisib (5-[2,6-Di(4-morpholinyl)-4-pyrimidinyl]-4-(trifluoromethyl)-2-pyridinamine), GDC-0941 (2-(lH-Indazol-4-yl)-6-[[4-(methylsulfonyl)-l-piperazinyl]methyl]-4-(4-morpholinyl)thieno[3,2-d]pyrimidine), GDC-0980 ((S)-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6 yl)methyl)piperazin-l-yl)-2-hydroxypropan-l-one (also known as RG7422)), SF1126 ((8S,14S,17S)-14-(carboxymethyl)-8-(3-guanidinopropyl)-17-(hydroxymethyl)-3,6,9,12,15-pentaoxo-1-(4-(4-oxo-8-phenyl-4H-chromen-2-yl)morpholino-4-ium)-2-oxa-7,10,13,16-tetraazaoctadecan-18-oate), PF-05212384 (N-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-N'-[4-(4,6-di-4-morpholinyl-l,3,5-triazin-2-yl)phenyl]urea) (gedatolisib), LY3023414, BEZ235 (2-Methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-lH-imidazo[4,5-c]quinolin-l-yl]phenyl}propanenitrile) (dactolisib), XL-765 (N-(3-(N-(3-(3,5-dimethoxyphenylamino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide), and GSK1059615 (5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidenedione), PX886 ([(3aR,6E,9S,9aR,10R,11aS)-6-[[bis(prop-2-enyl)amino]methylidene]-5-hydroxy-9-(methoxymethyl)-9a,11a-dimethyl-l,4,7-trioxo-2,3,3a,9,10,ll-hexahydroindeno[4,5h]isochromen- 10-yl] acetate (also known as sonolisib)), LY294002, AZD8186, PF-4989216, pilaralisib, GNE-317, PI-3065, PI-103, NU7441 (KU-57788), HS 173, VS-5584 (SB2343), CZC24832, TG100-115, A66, YM201636, CAY10505, PIK-75, PIK-93, AS-605240, BGT226 (NVP-BGT226), AZD6482, voxtalisib, alpelisib, IC-87114, TGI100713, CH5132799, PKI-402, copanlisib (BAY 80-6946), XL 147, PIK-90, PIK-293, PIK-294, 3-MA (3-methyladenine), AS-252424, AS-604850, apitolisib (GDC-0980; RG7422), and the structure described in WO2014/071109 having the formula:



Compound 292 . In a particular embodiment, the CDK4/6 inhibitors described herein are administered in combination or alternation with pictilisib, and the host is suffering from a cancer selected from colorectal, breast, soft tissue sarcoma, melenoma, ovarian, gastric, or prostate. In one embodiment, the cancer is breast cancer.

In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the PIk3 inhibitor.

BTK inhibitors for use in the present invention are well known. Examples of BTK inhibitors include ibrutinib (also known as PCI-32765)(Imbruvica™)(1-[(3R)-3-[4-amino-3-(4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one), dianilinopyrimidine-based inhibitors such as AVL-101 and AVL-291/292 (N-(3-((5-fluoro-2-((4-(2-methoxyethoxy)phenyl)amino)pyrimidin-4-yl)amino)phenyl)acrylamide) (Avila Therapeutics) (*see* US Patent Publication No 2011/0117073, incorporated herein in its entirety), Dasatinib ([N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide], LFM-A13 (alpha-cyano-beta-hydroxy-beta-methyl-N-(2,5-ibromophenyl) propenamide), GDC-0834 ([R-N-(3-(6-(4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenylamino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide], CGI-560 4-(tert-butyl)-N-(3-(8-(phenylamino)imidazo[1,2-a]pyrazin-6-yl)phenyl)benzamide, CGI-1746 (4-(tert-butyl)-N-(2-methyl-3-(4-methyl-6-((4-(morpholine-4-carbonyl)phenyl)amino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)benzamide), CNX-774 (4-(4-((4-((3-acrylamidophenyl)amino)-5-fluoropyrimidin-2-yl)amino)phenoxy)-N-methylpicolinamide), CTA056 (7-benzyl-1-(3-(piperidin-1-yl)propyl)-2-(4-(pyridin-4-yl)phenyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one), GDC-0834 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), GDC-0837 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), HM-71224, ACP-196, ONO-4059 (Ono Pharmaceuticals), PRT062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), QL-47 (1-(1-acryloylindolin-6-yl)-9-(1-methyl-1H-pyrazol-4-yl)benzo[h][1,6]naphthyridin-2(1H)-one), and RN486 (6-cyclopropyl-8-fluoro-2-(2-hydroxymethyl-3-{1-methyl-5-[5-(4-methyl-piperazin-1-yl)-pyridin-2-ylamino]-6-oxo-1,6-dihydro-pyridin-3-yl}-phenyl)-2H-isoquinolin-1-one), and other molecules capable of inhibiting BTK activity, for example those BTK inhibitors disclosed in Akinleye et ah, Journal of Hematology & Oncology, 2013, 6:59, the entirety of which is incorporated herein by reference. In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the BTK inhibitor.

Syk inhibitors for use in the present invention are well known, and include, for example, Cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino)pyrimidine-5-carboxamide), entospletinib (6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine), fostamatinib ([6-({5-Fluoro-2-[(3,4,5-trimethoxyphenyl)amino]-4-pyrimidinyl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b][1,4]oxazin-4-yl]methyl dihydrogen phosphate), fostamatinib disodium salt (sodium (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-3-oxo-2H-pyrido[3,2-b][1,4]oxazin-4(3H)-yl)methyl phosphate), BAY 61-3606 (2-(7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HCl), RO9021 (6-[(1R,2S)-2-Amino-cyclohexylamino]-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide), imatinib (Gleevac; 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), staurosporine, GSK143 (2-(((3R,4R)-3-aminotetrahydro-2H-pyran-4-yl)amino)-4-(p-tolylamino)pyrimidine-5-carboxamide), PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine), PRT-060318 (2-(((1R,2S)-2-aminocyclohexyl)amino)-4-(m-tolylamino)pyrimidine-5-carboxamide), PRT-062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), R112 (3,3'-((5-fluoropyrimidine-2,4-diyl)bis(azanediyl))diphenol), R348 (3-Ethyl-4-methylpyridine), R406 (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one), piceatannol (3-Hydroxyresveratol), YM193306(see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643), 7-azaindole, piceatannol, ER-27319 (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), Compound D (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), PRT060318 (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), luteolin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), apigenin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), quercetin (see Singh et al.

Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), fisetin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), myricetin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), morin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein). In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the Syk inhibitor.

MEK inhibitors for use in the present invention are well known, and include, for example, trametinib/GSK1120212 (N-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-l(2H-yl}phenyl)acetamide), selumetinib (6-(4-bromo-2-chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide), pimasertib/AS703026/MSC 1935369 ((S)-N-(2,3-dihydroxypropyl)-3-((2-fluoro-4- iodophenyl)amino)isonicotinamide), XL-518/GDC-0973 (l-({3,4-difluoro-2-[(2-fluoro-4- iodophenyl)amino]phenyl}carbonyl)-3-[(2S)-piperidin-2-yl]azetidin-3-ol), refametinib/BAY869766/RDEAl 19 (N-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-6-methoxyphenyl)-1-(2,3-dihydroxypropyl)cyclopropane-1-sulfonamide), PD-0325901 (N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide), TAK733 ((R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione), MEK162/ARRY438162 (5-[(4-Bromo-2-fluorophenyl)amino]-4-fluoro-N-(2- hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide), R05126766 (3-[[3-Fluoro-2- (methylsulfamoylamino)-4-pyridyl]methyl]-4-methyl-7-pyrimidin-2-yloxychromen-2-one), WX-554, R04987655/CH4987655 (3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-N-(2-hydroxyethoxy)-5-((3-oxo-l,2-oxazinan-2yl)methyl)benzamide), or AZD8330 (2-((2-fluoro-4-iodophenyl)amino)-N-(2 hydroxyethoxy)-1,5-dimethyl-6-oxo-l,6-dihydropyridine-3-carboxamide), U0126-EtOH, PD184352 (CI-1040), GDC-0623, BI-847325, cobimetinib, PD98059, BIX 02189, BIX 02188, binimetinib, SL-327, TAK-733, PD318088, and additional MEK inhibitors as described below. In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the MEK inhibitor.

Raf inhibitors for use in the present invention are well known, and include, for example, Vemurafinib (N-[3-[[5-(4-Chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]carbonyl]-2,4-difluorophenyl]-1-propanesulfonamide), sorafenib tosylate (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide;4-methylbenzenesulfonate), AZ628 (3-(2-cyanopropan-2-yl)-N-(4-methyl-3-(3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylamino)phenyl)benzamide), NVP-BHG712 (4-methyl-3-(1-methyl-6-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)-N-(3-(trifluoromethyl)phenyl)benzamide), RAF-265 (1-methyl-5-[2-[5-(trifluoromethyl)-1H-imidazol-2-yl]pyridin-4-yl]oxy-N-[4-(trifluoromethyl)phenyl]benzimidazol-2-amine), 2-Bromoaldisine (2-Bromo-6,7-dihydro-1H,5H-pyrrolo[2,3-c]azepine-4,8-dione), Raf Kinase Inhibitor IV (2-chloro-5-(2-phenyl-5-(pyridin-4-yl)-1H-imidazol-4-yl)phenol), Sorafenib N-Oxide (4-[4-[[[[4-Chloro-3(trifluoroMethyl)phenyl]aMino]carbonyl]aMino]phenoxy]-N-Methyl-2pyridinecarboxaMide 1-Oxide), PLX-4720, dabrafenib (GSK2118436), GDC-0879, RAF265, AZ 628, SB590885, ZM336372, GW5074, TAK-632, CEP-32496, LY3009120, and GX818 (Encorafenib). In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the Raf inhibitor.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the CDK4/6 inhibitor is a programmed death protein 1 (PD-1) inhibitor or programmed death protein ligand 1 or 2 inhibitor. PD-1 inhibitors are known in the art, and include, for example, nivolumab (BMS), pembrolizumab (Merck), pidilizumab (CureTech/Teva), AMP-244 (Amplimmune/GSK), BMS-936559 (BMS), and MEDI4736 (Roche/Genentech), and MPDL3280A (Genentech). In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the PD-1 inhibitor.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the CDK4/6 inhibitor is a B-cell lymphoma 2 (Bcl-2) protein inhibitor. BCL-2 inhibitors are known in the art, and include, for example, ABT-199 (4-[4-[[2-(4-Chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl]piperazin-l-yl]-N-[[3-nitro-4-[[(tetrahydro-2H-pyran-4-yl)methyl]amino]phenyl]sulfonyl]-2-[(lH- pyrrolo[2,3-b]pyridin-5-yl)oxy]benzamide), ABT-737 (4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4- [[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl] amino]-3- nitrophenyl]sulfonylbenzamide) (navitoclax), ABT-263

((R)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[l, l'-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide), GX15-070 (obatoclax mesylate, (2Z)-2-[(5Z)-5-[(3,5- dimethyl-lH-pyrrol-2-yl)methylidene]-4-methoxypyrrol-2-ylidene]indole; methanesulfonic acid))), 2-methoxy-antimycin A3, YC137 (4-(4,9-dioxo-4,9-dihydronaphtho[2,3-d]thiazol-2-ylamino)-phenyl ester), pogosin, ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate, Nilotinib-d3, TW-37 (N-[4-[[2-(1,1-Dimethylethyl)phenyl]sulfonyl]phenyl]-2,3,4-trihydroxy-5-[[2-(1-methylethyl)phenyl]methyl]benzamide), Apogossypolone (ApoG2), HA14-1, AT101, sabutoclax, gambogic acid, or G3139 (Oblimersen). In one embodiment, the CDK4/6 inhibitor is combined in a single dosage form with the at least one BCL-2 inhibitor.

Additional Combinations/Alternations

In one embodiment, a CDK4/6 inhibitor combination or alternation as described above, that is a CDK4/6 inhibitor as described herein combined or alternated with a kinase inhibitor, PD-1 inhibitor, or BCL-2 inhibitor, can be further combined with an additional therapeutic to treat the Rb-positive cancer. The second therapy can be an immunotherapy. As discussed in more detail below, the CDK4/6 inhibitor or combination agent can be conjugated to an antibody, radioactive agent, or other targeting agent that directs the CDK4/6 inhibitor to the diseased or abnormally proliferating cell. In another embodiment, the CDK4/6 inhibitor combination is used in combination with another pharmaceutical or a biologic agent (for example an antibody) to increase the efficacy of treatment with a combined or a synergistic approach. In an embodiment, CDK4/6 inhibitor combination can be used with T-cell vaccination, which typically involves immunization with inactivated autoreactive T cells to eliminate an Rb-positive cancer cell population as described herein. In another embodiment, the CDK4/6 inhibitor combination is used in combination with a bispecific T-cell Engager (BiTE), which is an antibody designed to simultaneously bind to specific antigens on endogenous T cells and Rb-positive cancer cells as described herein, linking the two types of cells.

In one embodiment, the additional therapy is a monoclonal antibody (MAb). Some MAbs stimulate an immune response that destroys cancer cells. Similar to the antibodies produced naturally by B cells, these MAbs "coat" the cancer cell surface, triggering its

destruction by the immune system. For example, bevacizumab targets vascular endothelial growth factor(VEGF), a protein secreted by tumor cells and other cells in the tumor's microenvironment that promotes the development of tumor blood vessels. When bound to bevacizumab, VEGF cannot interact with its cellular receptor, preventing the signaling that leads to the growth of new blood vessels. Similarly, cetuximab and panitumumab target the epidermal growth factor receptor (EGFR), and trastuzumab targets the human epidermal growth factor receptor 2 (HER-2). MAbs that bind to cell surface growth factor receptors prevent the targeted receptors from sending their normal growth-promoting signals. They may also trigger apoptosis and activate the immune system to destroy tumor cells.

Another group of cancer therapeutic MAbs are the immunoconjugates. These MAbs, which are sometimes called immunotoxins or antibody-drug conjugates, consist of an antibody attached to a cell-killing substance, such as a plant or bacterial toxin, a chemotherapy drug, or a radioactive molecule. The antibody latches onto its specific antigen on the surface of a cancer cell, and the cell-killing substance is taken up by the cell. FDA-approved conjugated MAbs that work this way include ado-trastuzumab emtansine, which targets the HER-2 molecule to deliver the drug DM1, which inhibits cell proliferation, to HER-2 expressing metastatic breast cancer cells.

Immunotherapies with T cells engineered to recognize cancer cells via bispecific antibodies (bsAbs) or chimeric antigen receptors (CARs) are approaches with potential to ablate both dividing and non/slow-dividing subpopulations of cancer cells.

Bispecific antibodies, by simultaneously recognizing target antigen and an activating receptor on the surface of an immune effector cell, offer an opportunity to redirect immune effector cells to kill cancer cells. The other approach is the generation of chimeric antigen receptors by fusing extracellular antibodies to intracellular signaling domains. Chimeric antigen receptor-engineered T cells are able to specifically kill tumor cells in a MHC-independent way.

In some embodiments, the CDK4/6 inhibitor combination or alternation described herein can be administered to the subject in further combination or alternation with other chemotherapeutic agents. If convenient, the CDK4/6 inhibitor combination or alternation described herein can be administered at the same time as another chemotherapeutic agent, in order to simplify the treatment regimen. In some embodiments, the CDK4/6 inhibitor combination and the other chemotherapeutic can be provided in a single formulation. In one

embodiment, the use of the CDK4/6 inhibitor combination described herein is combined in a therapeutic regime with other agents. Such agents may include, but are not limited to, at least one of tamoxifen, midazolam, letrozole, bortezomib, anastrozole, goserelin, an mTOR inhibitor, a PI3 kinase inhibitor as described above, a dual mTOR-PI3K inhibitor, a MEK inhibitor, a RAS inhibitor, ALK inhibitor, an HSP inhibitor (for example, HSP70 and HSP 90 inhibitor, or a combination thereof), a BCL-2 inhibitor as described above, apopototic inducing compounds, an AKT inhibitor, including but not limited to, MK-2206, GSK690693, Perifosine, (KRX-0401), GDC-0068, Triciribine, AZD5363, Honokiol, PF-04691502, and Miltefosine, a PD-1 inhibitor as described above including but not limited to, Nivolumab, CT-011, MK-3475, BMS936558, and AMP-514 or a FLT-3 inhibitor, including but not limited to, P406, Dovitinib, Quizartinib (AC220), Amuvatinib (MP-470), Tandutinib (MLN518), ENMD-2076, and KW-2449, or a combination thereof. Examples of mTOR inhibitors include but are not limited to rapamycin and its analogs, everolimus (Afinitor), temsirolimus, ridaforolimus, sirolimus, and deforolimus. Examples of MEK inhibitors include but are not limited to tametinib/GSKl120212 (N-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-l(2H-yl}phenyl)acetamide), selumetinob (6-(4-bromo-2-chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide), pimasertib/AS703026/MSC1935369 ((S)-N-(2,3-dihydroxypropyl)-3-((2-fluoro-4-iodophenyl)amino)isonicotinamide), XL-518/GDC-0973 (l-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-[(2S)-piperidin-2-yl]azetidin-3-ol) (cobimetinib), refametinib/BAY869766/RDEAl19 (N-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-6-methoxyphenyl)-1-(2,3-dihydroxypropyl)cyclopropane-1-sulfonamide), PD-0325901 (N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide), TAK733 ((R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3d]pyrimidine-4,7(3H,8H)-dione), MEK162/ARRY438162 (5-[(4-Bromo-2-fluorophenyl)amino]-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzimidazole-6 carboxamide), R05126766 (3-[[3-Fluoro-2-(methylsulfamoylamino)-4-pyridyl]methyl]-4-methyl-7-pyrimidin-2-yloxychromen-2-one), WX-554, R04987655/CH4987655 (3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-N-(2-hydroxyethoxy)-5-((3-oxo-l,2-oxazinan-2 yl)methyl)benzamide), or AZD8330 (2-((2-fluoro-4-iodophenyl)amino)-N-(2-hydroxyethoxy)-1,5-dimethyl-6-oxo-l,6-dihydropyridine-3-carboxamide). Examples of RAS inhibitors include

but are not limited to Reolysin and siG12D LODER. Examples of ALK inhibitors include but are not limited to Crizotinib, Ceritinib (Zykadia), AP26113, and LDK378. HSP inhibitors include but are not limited to Geldanamycin or 17-N-Allylamino-17-demethoxygeldanamycin (17AAG), and Radicicol. In a particular embodiment, a compound described herein is administered in combination with letrozole and/or tamoxifen. Other chemotherapeutic agents that can be used in combination with the compounds described herein include, but are not limited to, chemotherapeutic agents that do not require cell cycle activity for their anti-neoplastic effect.

In one embodiment, a CDK4/6 inhibitor combination described herein can be combined with a chemotherapeutic selected from, but are not limited to, Imatinib mesylate (Gleevac®), Dasatinib (Sprycel®), Nilotinib (Tasigna®), Bosutinib (Bosulif®), Trastuzumab (Herceptin®), trastuzumab-DM1, Pertuzumab (PerjetaTM), Lapatinib (Tykerb®), Gefitinib (Iressa®), Erlotinib (Tarceva®), Cetuximab (Erbitux®), Panitumumab (Vectibix®), Vandetanib (Caprelsa®), Vemurafenib (Zelboraf®), Vorinostat (Zolinza®), Romidepsin (Istodax®), Bexarotene (Tagretin®), Alitretinoin (Panretin®), Tretinoin (Vesanoid®), Carfilizomib (KyprolisTM), Pralatrexate (Folotyn®), Bevacizumab (Avastin®), Ziv-aflibercept (Zaltrap®), Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Regorafenib (Stivarga®), and Cabozantinib (CometriqTM).

In certain aspects, the additional therapeutic agent is an anti-inflammatory agent, a chemotherapeutic agent, a radiotherapeutic, an additional therapeutic agent, or an immunosuppressive agent.

Suitable chemotherapeutic agents include, but are not limited to, a radioactive molecule, a toxin, also referred to as cytotoxin or cytotoxic agent, which includes any agent that is detrimental to the viability of cells, and liposomes or other vesicles containing chemotherapeutic compounds. General anticancer pharmaceutical agents include: Vincristine (Oncovin®) or liposomal vincristine (Marqibo®), Daunorubicin (daunomycin or Cerubidine®) or doxorubicin (Adriamycin®), Cytarabine (cytosine arabinoside, ara-C, or Cytosar®), L-asparaginase (Elspar®) or PEG-L-asparaginase (pegaspargase or Oncaspar®), Etoposide (VP-16), Teniposide (Vumon®), 6-mercaptopurine (6-MP or Purinethol®), Methotrexate, Cyclophosphamide (Cytoxan®), Prednisone, Dexamethasone (Decadron), imatinib (Gleevec®), dasatinib (Sprycel®), nilotinib (Tasigna®), bosutinib (Bosulif®), and ponatinib (Iclusig™). Examples of additional suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone,

5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, an alkylating agent, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC)), an anti-mitotic agent, cis-dichlorodiamine platinum (II) (DDP) cisplatin), diamino dichloro platinum, anthracycline, an antibiotic, an antimetabolite, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucouorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclothosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytoxan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorucbicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxy anthracin dione, Docetaxel, dolasetron mesylate, doxorubicin HCL, dronabinol, *E. coli* L-asparaginase, emetine, epoetin-α, *Erwinia* L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon α-2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptipurine, mesna, methotrexate, methyltestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 with carmustine implant, porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, teniposide, tenoposide, testolactone, tetracaine, thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate, trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

Additional therapeutic agents that can be administered in combination with a CDK4/6 inhibitor combination disclosed herein can include bevacizumab, sutinib, sorafenib, 2-methoxyestradiol or 2ME2, finasunate, vatalanib, vandetanib, aflibercept, volociximab, etaracizumab (MEDI-522), cilengitide, erlotinib, cetuximab, panitumumab, gefitinib, trastuzumab, dovitinib, figitumumab, atacicept, rituximab, alemtuzumab, aldesleukine, atlizumab, tocilizumab, temsirolimus, everolimus, lucatumumab, dacetuzumab, HLL1, huN901-

DM1, atiprimod, natalizumab, bortezomib, carfilzomib, marizomib, tanespimycin, saquinavir mesylate, ritonavir, nelfinavir mesylate, indinavir sulfate, belinostat, panobinostat, mapatumumab, lexatumumab, dulanermin, ABT-737, oblimersen, plitidepsin, talmapimod, P276-00, enzastaurin, tipifarnib, perifosine, imatinib, dasatinib, lenalidomide, thalidomide, simvastatin, celecoxib, bazedoxifene, AZD4547, rilotumumab, oxaliplatin (Eloxatin), PD0332991, ribociclib (LEE011), amebaciclib (LY2835219), HDM201, fulvestrant (Faslodex), exemestane (Aromasin), PIM447, ruxolitinib (INC424), BGJ398, necitumumab, pemetrexed (Alimta), and ramucirumab (IMC-1121B).

In one aspect of the present invention, a CDK4/6 inhibitor combination described herein can be combined with at least one immunosuppressive agent. The immunosuppressive agent is preferably selected from the group consisting of a calcineurin inhibitor, e.g. a cyclosporin or an ascomycin, e.g. Cyclosporin A (NEORAL®), FK506 (tacrolimus), pimecrolimus, a mTOR inhibitor, e.g. rapamycin or a derivative thereof, e.g. Sirolimus (RAPAMUNE®), Everolimus (Certican®), temsirolimus, zotarolimus, biolimus-7, biolimus-9, a rapalog, e.g.ridaforolimus, azathioprine, campath 1H, a S1P receptor modulator, e.g. fingolimod or an analogue thereof, an anti IL-8 antibody, mycophenolic acid or a salt thereof, e.g. sodium salt, or a prodrug thereof, e.g. Mycophenolate Mofetil (CELLCEPT®), OKT3 (ORTHOCLONE OKT3®), Prednisone, ATGAM®, THYMOGLOBULIN®, Brequinar Sodium, OKT4, T10B9.A-3A, 33B3.1, 15-deoxyspergualin, tresperimus, Leflunomide ARAVA®, CTLAI-Ig, anti-CD25, anti-IL2R, Basiliximab (SIMULECT®), Daclizumab (ZENAPAX®), mizorbine, methotrexate, dexamethasone, ISAtx-247, SDZ ASM 981 (pimecrolimus, Elidel®), CTLA4lg (Abatacept), belatacept, LFA3lg,, etanercept (sold as Enbrel® by Immunex), adalimumab (Humira®), infliximab (Remicade®), an anti-LFA-1 antibody, natalizumab (Antegren®), Enlimomab, gavilimomab, antithymocyte immunoglobulin, siplizumab, Alefacept efalizumab, pentasa, mesalazine, asacol, codeine phosphate, benorylate, fenbufen, naprosyn, diclofenac, etodolac and indomethacin, aspirin and ibuprofen.

In certain embodiments, a CDK4/6 inhibitor combination described herein is administered to the subject prior to treatment with another chemotherapeutic agent, during treatment with another chemotherapeutic agent, after administration of another chemotherapeutic agent, or a combination thereof.

In some embodiments, the selective CDK4/6 inhibitor combination can be administered to the subject such that the other chemotherapeutic agent can be administered either at higher doses (increased chemotherapeutic dose intensity) or more frequently (increased chemotherapeutic dose density). Dose-dense chemotherapy is a chemotherapy treatment plan in which drugs are given with less time between treatments than in a standard chemotherapy treatment plan. Chemotherapy dose intensity represents unit dose of chemotherapy administered per unit time. Dose intensity can be increased or decreased through altering dose administered, time interval of administration, or both.

In one embodiment of the invention, the CDK4/6 inhibitor combination described herein can be administered in a concerted regimen with another agent such as a non-DNA-damaging, targeted anti-neoplastic agent or a hematopoietic growth factor agent. It has recently been reported that the untimely administration of hematopoietic growth factors can have serious side effects. For example, the use of the EPO family of growth factors has been associated with arterial hypertension, cerebral convulsions, hypertensive encephalopathy, thromboembolism, iron deficiency, influenza like syndromes and venous thrombosis. The G-CSF family of growth factors has been associated with spleen enlargement and rupture, respiratory distress syndrome, allergic reactions and sickle cell complications. By combining the administration of the CDK4/6 inhibitor combination as described herein with the timely administration of hematopoietic growth factors, for example, at the time point wherein the affected cells are no longer under growth arrest, it is possible for the health care practitioner to decrease the amount of the growth factor to minimize the unwanted adverse effects while achieving the desired therapeutic benefit. As such, in one embodiment, the use of the CDK4/6 inhibitor combination or methods described herein is combined with the use of hematopoietic growth factors including, but not limited to, granulocyte colony stimulating factor (G-CSF, for example, sold as Neupogen (filgrastin), Neulasta (peg-filgrastin), or lenograstin), granulocyte-macrophage colony stimulating factor (GM-CSF, for example sold as molgramostim and sargramostim (Leukine)), M-CSF (macrophage colony stimulating factor), thrombopoietin (megakaryocyte growth development factor (MGDF), for example sold as Romiplostim and Eltrombopag) interleukin (IL)-12, interleukin-3, interleukin-11 (adipogenesis inhibiting factor or oprelvekin), SCF (stem cell factor, steel factor, kit-ligand, or KL) and erythropoietin (EPO), and their derivatives (sold as for example epoetin-α as Darbopoetin, Epocept, Nanokine, Epofit, Epogin, Eprex and Procrit;

epoetin-β sold as for example NeoRecormon, Recormon and Micera), epoetin-delta (sold as for example Dynepo), epoetin- omega (sold as for example Epomax), epoetin zeta (sold as for example Silapo and Reacrit) as well as for example Epocept, EPOTrust, Erypro Safe, Repoeitin, Vintor, Epofit, Erykine, Wepox, Espogen, Relipoeitin, Shanpoietin, Zyrop and EPIAO). In one embodiment, the CDK4/6 inhibitor combination is administered prior to administration of the hematopoietic growth factor. In one embodiment, the hematopoietic growth factor administration is timed so that the CDK4/6 inhibitor combination's effect on HSPCs has dissipated. In one embodiment, the growth factor is administered at least 20 hours after the administration of a CDK4/6 inhibitor combination described herein.

If desired, multiple doses of a CDK4/6 inhibitor combination described herein can be administered to the subject. Alternatively, the subject can be given a single dose of a CDK4/6 inhibitor combination described herein.

In one embodiment, the activity of an active compound for a purpose described herein can be augmented through conjugation to an agent that targets the diseased or abnormally proliferating cell or otherwise enhances activity, delivery, pharmacokinetics or other beneficial property.

For example, the CDK4/6 inhibitor or other therapeutic agent the CDK4/6 inhibitor is combined with during treatment, or combined in a treatment regimen, can be administered as an antibody-drug conjugate (ADC). In certain embodiments, one or more of the combination agents described herein can be administered in conjugation or combination with an antibody or antibody fragment. Fragments of an antibody can be produced through chemical or genetic mechanisms. The antibody fragment can be an antigen binding fragment. For example, the antigen binding fragment can be selected from an Fab, Fab', (Fab')2, or Fv. The antibody fragment can be a Fab. Monovalent F(ab) fragments have one antigen binding site. The antibody can be a divalent (Fab')2 fragment, which has two antigen binding regions that are linked by disulfide bonds. In one embodiment, the antigen fragment is a (Fab'). Reduction of F(ab')2 fragments produces two monovalent Fab' fragments, which have a free sulfhydryl group that is useful for conjugation to other molecules.

A selected compound described herein can be administered in conjugation or combination with a Fv fragment. Fv fragments are the smallest fragment made from enzymatic cleavage of IgG and IgM class antibodies. Fv fragments have the antigen-binding site made of

the VH and VC regions, but they lack the CH1 and CL regions. The VH and VL chains are held together in Fv fragments by non-covalent interactions.

In one embodiment, a selected compound as described herein can be administered in combination with an antibody fragment selected from the group consisting of an ScFv, domain antibody, diabody, triabody, tetrabody, Bis-scFv, minibody, Fab2, or Fab3 antibody fragment. In one embodiment, the antibody fragment is a ScFv. Genetic engineering methods allow the production of single chain variable fragments (ScFv), which are Fv type fragments that include the VH and VL domains linked with a flexible peptide. When the linker is at least 12 residues long, the ScFv fragments are primarily monomeric. Manipulation of the orientation of the V-domains and the linker length creates different forms of Fv molecules. Linkers that are 3-11 residues long yield scFv molecules that are unable to fold into a functional Fv domain. These molecules can associate with a second scFv molecule, to create a bivalent diabody. In one embodiment, the antibody fragment administered in combination with a selected compound described herein is a bivalent diabody. If the linker length is less than three residues, scFv molecules associate into triabodies or tetrabodies. In one embodiment, the antibody fragment is a triabody. In one embodiment, the antibody fragment is a tetrabody. Multivalent scFvs possess greater functional binding affinity to their target antigens than their monovalent counterparts by having binding to two more target antigens, which reduces the off-rate of the antibody fragment. In one embodiment, the antibody fragment is a minibody. Minibodies are scFv-CH3 fusion proteins that assemble into bivalent dimers. In one embodiment, the antibody fragment is a Bis-scFv fragment. Bis-scFv fragments are bispecific. Miniaturized ScFv fragments can be generated that have two different variable domains, allowing these Bis-scFv molecules to concurrently bind to two different epitopes.

In one embodiment, a selected compound described herein is administered in conjugation or combination with a bispecific dimer (Fab2) or trispecific dimer (Fab3). Genetic methods are also used to create bispecific Fab dimers (Fab2) and trispecific Fab trimers (Fab3). These antibody fragments are able to bind 2 (Fab2) or 3 (Fab3) different antigens at once.

In one embodiment, a selected compound described herein is administered in conjugation or combination with an rIgG antibody fragment. rIgG antibody fragments refers to reduced IgG (75,000 daltons) or half-IgG. It is the product of selectively reducing just the hinge-region disulfide bonds. Although several disulfide bonds occur in IgG, those in the hinge-region are

most accessible and easiest to reduce, especially with mild reducing agents like 2-mercaptoethylamine (2-MEA). Half-IgG are frequently prepared for the purpose of targeting the exposing hinge-region sulfhydryl groups that can be targeted for conjugation, either antibody immobilization or enzyme labeling.

In other embodiments, a selected active compound described herein can be linked to a radioisotope to increase efficacy, using methods well known in the art. Any radioisotope that is useful against Rb-positive cancer cells can be incorporated into the conjugate, for example, but not limited to, ^{131}I, ^{123}I, ^{192}Ir, ^{32}P, ^{90}Sr, ^{198}Au, ^{226}Ra, ^{90}Y, ^{241}Am, ^{252}Cf, ^{60}Co, or ^{137}Cs.

Of note, the linker chemistry can be important to efficacy and tolerability of the drug conjugates. The thio-ether linked T-DM1 increases the serum stability relative to a disulfide linker version and appears to undergo endosomal degradation, resulting in intra-cellular release of the cytotoxic agent, thereby improving efficacy and tolerability, *See*, Barginear, M.F. and Budman, D.R., Trastuzumab-DM1: A review of the novel immune-conjugate for HER2-overexpressing breast cancer, The Open Breast Cancer Journal, 1:25-30, 2009.

Examples of early and recent antibody-drug conjugates, discussing drugs, linker chemistries and classes of targets for product development that may be used in the present invention can be found in the reviews by Casi, G. and Neri, D., Antibody-drug conjugates: basic concepts, examples and future perspectives, J. Control Release 161(2):422-428, 2012, Chari, R.V., Targeted cancer therapy: conferring specificity to cytotoxic drugs, Acc. Chem. Rev., 41(1):98-107, 2008, Sapra, P. and Shor, B., Monoclonal antibody-based therapies in cancer: advances and challenges, Pharmacol. Ther., 138(3):452-69, 2013, Schliemann, C. and Neri, D., Antibody-based targeting of the tumor vasculature, Biochim. Biophys. Acta., 1776(2):175-92, 2007, Sun, Y., Yu, F., and Sun, B.W., Antibody-drug conjugates as targeted cancer therapeutics, Yao Xue Xue Bao, 44(9):943-52, 2009, Teicher, B.A., and Chari, R.V., Antibody conjugate therapeutics: challenges and potential, Clin. Cancer Res., 17(20):6389-97, 2011, Firer, M.A., and Gellerman, G.J., Targeted drug delivery for cancer therapy: the other side of antibodies, J. Hematol. Oncol., 5:70, 2012, Vlachakis, D. and Kossida, S., Antibody Drug Conjugate bioinformatics: drug delivery through the letterbox, Comput. Math. Methods Med., 2013; 2013:282398, Epub 2013 Jun 19, Lambert, J.M., Drug-conjugated antibodies for the treatment of cancer, Br. J. Clin. Pharmacol., 76(2):248-62, 2013, Concalves, A., Tredan, O., Villanueva, C. and Dumontet, C., Antibody-drug conjugates in oncology: from the concept to trastuzumab

emtansine (T-DM1), Bull. Cancer, 99(12):1183-1191, 2012, Newland, A.M., Brentuximab vedotin: a CD-30-directed antibody-cytotoxic drug conjugate, Pharmacotherapy, 33(1):93-104, 2013, Lopus, M., Antibody-DM1 conjugates as cancer therapeutics, Cancer Lett., 307(2):113-118, 2011, Chu, Y.W. and Poison, A., Antibody-drug conjugates for the treatment of B-cell non-Hodgkin's lymphoma and leukemia, Future Oncol., 9(3):355-368, 2013, Bertholjotti, I., Antibody-drug conjugate - - a new age for personalized cancer treatment, Chimia, 65(9): 746-748, 2011, Vincent, K.J., and Zurini, M., Current strategies in antibody engineering: Fc engineering and pH – dependent antigen binding, bispecific antibodies and antibody drug conjugates, Biotechnol. J., 7(12):1444-1450, 2012, Haeuw, J.F., Caussanel, V., and Beck, A., Immunoconjugates, drug-armed antibodies to fight against cancer, Med. Sci., 25(12):1046-1052, 2009 and Govindan, S.V., and Goldenberg, D.M., Designing immunoconjugates for cancer therapy, Expert Opin. Biol. Ther., 12(7):873-890, 2012.

Pharmaceutical Compositions and Dosage Forms

An active compound described herein, or its salt, isotopic analog, or prodrug can be administered in combination in an effective amount to the host using any suitable approach which achieves the desired therapeutic result. The amount and timing of active compound administered will, of course, be dependent on the host being treated, the instructions of the supervising medical specialist, on the time course of the exposure, on the manner of administration, on the pharmacokinetic properties of the particular active compound, and on the judgment of the prescribing physician. Thus, because of host to host variability, the dosages given below are a guideline and the physician can titrate doses of the compound to achieve the treatment that the physician considers appropriate for the host. In considering the degree of treatment desired, the physician can balance a variety of factors such as age and weight of the host, presence of preexisting disease, as well as presence of other diseases. Pharmaceutical formulations can be prepared for any desired route of administration including, but not limited to, oral, intravenous, or aerosol administration, as discussed in greater detail below.

The therapeutically effective dosage of any active compound described herein will be determined by the health care practitioner depending on the condition, size and age of the patient as well as the route of delivery. In one non-limited embodiment, a dosage from about 0.1 to about 200 mg/kg has therapeutic efficacy, with all weights being calculated based upon the

weight of the active compound, including the cases where a salt is employed. In some embodiments, the dosage can be the amount of compound needed to provide a serum concentration of the active compound of up to between about 1 and 5, 10, 20, 30, or 40 μM. In some embodiments, a dosage from about 10 mg/kg to about 50 mg/kg is employed for oral administration. A dosage from about 0.5 mg/kg to 5 mg/kg can be employed for intramuscular injection. In some embodiments, dosages can be from about 1 μmol/kg to about 50 μmol/kg, or, optionally, between about 22 μmol/kg and about 33 μmol/kg of the compound for intravenous or oral administration. An oral dosage form can include any appropriate amount of active material, including for example from 5 mg to, 50, 100, 200, 250, 300, 400 or 500 mg per tablet or other solid dosage form.

In accordance with the presently disclosed methods, pharmaceutically active compounds as described herein can be administered orally as a solid or as a liquid, or can be administered intramuscularly, intravenously, or by inhalation as a solution, suspension, or emulsion. In some embodiments, the compounds or salts also can be administered by inhalation, intravenously, or intramuscularly as a liposomal suspension. When administered through inhalation the active compound or salt can be in the form of a plurality of solid particles or droplets having any desired particle size, and for example, from about 0.01, 0.1 or 0.5 to about 5, 10, 20 or more microns, and optionally from about 1 to about 2 microns. Compounds as disclosed in the present invention have demonstrated good pharmacokinetic and pharmacodynamics properties, for instance when administered by the oral or intravenous routes.

The pharmaceutical formulations can comprise an active compound described herein or a pharmaceutically acceptable salt thereof, in any pharmaceutically acceptable carrier. If a solution is desired, water may be the carrier of choice for water-soluble compounds or salts. With respect to the water-soluble compounds or salts, an organic vehicle, such as glycerol, propylene glycol, polyethylene glycol, or mixtures thereof, can be suitable. In the latter instance, the organic vehicle can contain a substantial amount of water. The solution in either instance can then be sterilized in a suitable manner known to those in the art, and for illustration by filtration through a 0.22-micron filter. Subsequent to sterilization, the solution can be dispensed into appropriate receptacles, such as depyrogenated glass vials. The dispensing is optionally done by an aseptic method. Sterilized closures can then be placed on the vials and, if desired, the vial contents can be lyophilized.

In addition to the active compounds or their salts, the pharmaceutical formulations can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids, such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium gluconate. Further, the formulations can contain antimicrobial preservatives. Useful antimicrobial preservatives include methylparaben, propylparaben, and benzyl alcohol. An antimicrobial preservative is typically employed when the formulations is placed in a vial designed for multi-dose use. The pharmaceutical formulations described herein can be lyophilized using techniques well known in the art.

For oral administration a pharmaceutical composition can take the form of solutions, suspensions, tablets, pills, capsules, powders, and the like. Tablets containing various excipients such as sodium citrate, calcium carbonate and calcium phosphate may be employed along with various disintegrants such as starch (e.g., potato or tapioca starch) and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate, and talc are often very useful for tableting purposes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules. Materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the compounds of the presently disclosed host matter can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

In yet another embodiment, there are provided injectable, stable, sterile formulations comprising combinations of active compound as described herein, or a salt thereof, in a unit dosage form in a sealed container. The combination is provided in the form of a lyophilizate, which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form liquid formulation suitable for injection thereof into a host. When the combination is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. Particularly useful emulsifying agents include phosphatidyl cholines and lecithin.

Additional embodiments provided herein include liposomal formulations of the active compounds disclosed herein. The technology for forming liposomal suspensions is well known in the art. When the compound is an aqueous-soluble salt, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the active compound, the active compound can be substantially entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the active compound of interest is water-insoluble, again employing conventional liposome formation technology, the salt can be substantially entrained within the hydrophobic lipid bilayer that forms the structure of the liposome. In either instance, the liposomes that are produced can be reduced in size, as through the use of standard sonication and homogenization techniques. The liposomal formulations comprising the active compounds disclosed herein can be lyophilized to produce a lyophilizate, which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

Pharmaceutical formulations also are provided which are suitable for administration as an aerosol by inhalation. These formulations comprise a solution or suspension of a desired compound or combination described herein or a salt thereof, or a plurality of solid particles of the compound or salt or combination. The desired formulations can be placed in a small chamber and nebulized. Nebulization can be accomplished by compressed air or by ultrasonic energy to form a plurality of liquid droplets or solid particles comprising the compounds or salts or combinations. The liquid droplets or solid particles may for example have a particle size in the range of about 0.5 to about 10 microns, and optionally from about 0.5 to about 5 microns. In one embodiment, the solid particles provide for controlled release through the use of a degradable polymer. The solid particles can be obtained by processing the solid compound or a salt thereof, in any appropriate manner known in the art, such as by micronization. Optionally, the size of the solid particles or droplets can be from about 1 to about 2 microns. In this respect, commercial nebulizers are available to achieve this purpose. The compounds can be administered via an aerosol suspension of respirable particles in a manner set forth in U.S. Pat. No. 5,628,984, the disclosure of which is incorporated herein by reference in its entirety.

Pharmaceutical formulations also are provided which provide a controlled release of a one or both compounds of the combinations described herein, including through the use of a degradable polymer, as known in the art.

When the pharmaceutical formulations suitable for administration as an aerosol is in the form of a liquid, the formulations can comprise a water-soluble active compound in a carrier that comprises water. A surfactant can be present, which lowers the surface tension of the formulations sufficiently to result in the formation of droplets within the desired size range when hosted to nebulization.

The term "pharmaceutically acceptable salts" as used herein refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with hosts (e.g., human hosts) without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the presently disclosed host matter.

Thus, the term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the presently disclosed compounds. These salts can be prepared during the final isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Basic compounds are capable of forming a wide variety of different salts with various inorganic and organic acids. Acid addition salts of the basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form can be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms may differ from their respective salt forms in certain physical properties such as solubility in polar solvents. Pharmaceutically acceptable base addition salts may be formed with metals or amines, such as alkali and alkaline earth metal hydroxides, or of organic amines. Examples of metals used as cations, include, but are not limited to, sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines include, but are not limited to, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine. The base addition salts of acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form can be regenerated by contacting the salt form with an acid and isolating the free acid

in a conventional manner. The free acid forms may differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents.

Salts can be prepared from inorganic acids sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorus, and the like. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate, laurylsulphonate and isethionate salts, and the like. Salts can also be prepared from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. and the like. Representative salts include acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Pharmaceutically acceptable salts can include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Also contemplated are the salts of amino acids such as arginate, gluconate, galacturonate, and the like. See, for example, Berge et al., J. Pharm. Sci., 1977, 66, 1-19, which is incorporated herein by reference.

Rb-positive Cancers and Proliferative Disorders

In particular, the CDK4/6 inhibitor combinations and methods described herein can be used to treat a subject suffering from an Rb-positive cancer or other Rb-positive abnormal cellular proliferative disorder. In some embodiments, the cancer or cellular proliferation disorder is a CDK4/6-replication dependent cancer or cellular proliferation disorder, which refers to a cancer or cellular proliferation disorder that requires the activity of CDK4/6 for replication or proliferation, or which may be growth inhibited through the activity of a selective CDK4/6

inhibitor. Cancers and disorders of such type can be characterized by (e.g., that has cells that exhibit) the presence of a functional Retinoblastoma protein. Such cancers and disorders are classified as being Rb-positive. Rb-positive abnormal cellular proliferation disorders, and variations of this term as used herein, refer to disorders or diseases caused by uncontrolled or abnormal cellular division which are characterized by the presence of a functional Retinoblastoma protein, which can include cancers. In one aspect of the present invention, the use of CDK4/6 inhibitors in combination with additional therapeutic agents and methods described herein can be used to treat a non-cancerous Rb-positive abnormal cellular proliferation disorder. Examples of such disorders may include non-malignant lymphoproliferation, non-malignant breast neoplasms, psoriasis, arthritis, dermatitis, pre-cancerous colon lesions or pulps, angiogenesis disorders, immune mediated and non-immune mediated inflammatory diseases, arthritis, age-related macular degeneration, diabetes, and other non-cancerous or benign cellular proliferation disorders.

Targeted cancers suitable for administration of a compound described herein may include Rb-positive: estrogen-receptor positive cancer, HER2-negative advanced breast cancer, late-line metastatic breast cancer, liposarcoma, non-small cell lung cancer, liver cancer, ovarian cancer, glioblastoma, refractory solid tumors, retinoblastoma positive breast cancer as well as retinoblastoma positive endometrial, vaginal and ovarian cancers and lung and bronchial cancers, adenocarcinoma of the colon, adenocarcinoma of the rectum, central nervous system germ cell tumors, teratomas, estrogen receptor-negative breast cancer, estrogen receptor-positive breast cancer, familial testicular germ cell tumors, HER2-negative breast cancer, HER2-positive breast cancer, male breast cancer, ovarian immature teratomas, ovarian mature teratoma, ovarian monodermal and highly specialized teratomas, progesterone receptor-negative breast cancer, progesterone receptor-positive breast cancer, recurrent breast cancer, recurrent colon cancer, recurrent extragonadal germ cell tumors, recurrent extragonadal non-seminomatous germ cell tumor, recurrent extragonadal seminomas, recurrent malignant testicular germ cell tumors, recurrent melanomas, recurrent ovarian germ cell tumors, recurrent rectal cancer, stage III extragonadal non-seminomatous germ cell tumors, stage III extragonadal seminomas, stage III malignant testicular germ cell tumors, stage III ovarian germ cell tumors, stage IV breast cancers, stage IV colon cancers, stage IV extragonadal non-seminomatous germ cell tumors, stage IV extragonadal seminoma, stage IV melanomas, stage IV ovarian germ cell tumors, stage

IV rectal cancers, testicular immature teratomas, testicular mature teratomas. In particular embodiments, the targeted cancers included estrogen-receptor positive, HER2-negative advanced breast cancer, late-line metastatic breast cancer, liposarcoma, non-small cell lung cancer, liver cancer, ovarian cancer, glioblastoma, refractory solid tumors, retinoblastoma positive breast cancer as well as retinoblastoma positive endometrial, vaginal and ovarian cancers and lung and bronchial cancers, metastatic colorectal cancer, metastatic melanoma with CDK4 mutation or amplification, or cisplatin-refractory, unresectable germ cell tumors.

In one embodiment, the Rb-positive cancer is selected from an Rb-positive carcinoma, sarcoma, including, but not limited to, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, or a combination of one or more of the foregoing cancers.

In one embodiment, the Rb-positive cancer is selected from the group consisting of Rb-positive: fibrosarcoma, myxosarcoma, chondrosarcoma, osteosarcoma, chordoma, malignant fibrous histiocytoma, hemangiosarcoma, angiosarcoma, lymphangiosarcoma. Mesothelioma, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma; epidermoid carcinoma, malignant skin adnexal tumors, adenocarcinoma, hepatoma, hepatocellular carcinoma, renal cell carcinoma, hypernephroma, cholangiocarcinoma, transitional cell carcinoma, choriocarcinoma, seminoma, embryonal cell carcinoma, glioma anaplastic; glioblastoma multiforme,, neuroblastoma, medulloblastoma, malignant meningioma, malignant schwannoma, neurofibrosarcoma, parathyroid carcinoma, medullary carcinoma of thyroid, bronchial carcinoid, pheochromocytoma, Islet cell carcinoma, malignant carcinoid, malignant paraganglioma, melanoma, Merkel cell neoplasm, cystosarcoma phylloide, salivary cancers, thymic carcinomas, bladder cancer, and Wilms tumor.

In more particular embodiments, the Rb-positive cancer or disorder includes a blood disorder or a hematologic malignancy, including, but not limited to, myeloid disorder, lymphoid disorder, leukemia, lymphoma, myelodysplastic syndrome (MDS), myeloproliferative disease (MPD), mast cell disorder, and myeloma (e.g., multiple myeloma), among others. Abnormal proliferation of T-cells, B-cells, and/or NK-cells can result in a wide range of diseases such as cancer, proliferative disorders and inflammatory/immune diseases. A host, for example a human, afflicted with any of these disorders can be treated with an effective amount of a combination as described herein to achieve a decrease in symptoms (a palliative agent) or a decrease in the underlying disease (a disease modifying agent).

Examples include T-cell or NK-cell lymphoma, for example, but not limited to: peripheral T-cell lymphoma; anaplastic large cell lymphoma, for example anaplastic lymphoma kinase (ALK) positive, ALK negative anaplastic large cell lymphoma, or primary cutaneous anaplastic large cell lymphoma; angioimmunoblastic lymphoma; cutaneous T-cell lymphoma, for example mycosis fungoides, Sézary syndrome, primary cutaneous anaplastic large cell lymphoma, primary cutaneous CD30+ T-cell lymphoproliferative disorder; primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma; primary cutaneous gamma-delta T-cell lymphoma; primary cutaneous small/medium CD4+ T-cell lymphoma, and lymphomatoid papulosis; Adult T-cell Leukemia/Lymphoma (ATLL); Blastic NK-cell Lymphoma; Enteropathy-type T-cell lymphoma; Hematosplenic gamma-delta T-cell Lymphoma; Lymphoblastic Lymphoma; Nasal NK/T-cell Lymphomas; Treatment-related T-cell lymphomas; for example lymphomas that appear after solid organ or bone marrow transplantation; T-cell prolymphocytic leukemia; T-cell large granular lymphocytic leukemia; Chronic lymphoproliferative disorder of NK-cells; Aggressive NK cell leukemia; Systemic EBV+ T-cell lymphoproliferative disease of childhood (associated with chronic active EBV infection); Hydroa vacciniforme-like lymphoma; Adult T-cell leukemia/ lymphoma; Enteropathy-associated T-cell lymphoma; Hepatosplenic T-cell lymphoma; or Subcutaneous panniculitis-like T-cell lymphoma.

In one embodiment, a combination disclosed herein can be used in an effective amount to treat a host, for example a human, with a lymphoma or lymphocytic or myelocytic proliferation disorder or abnormality. For example, the compounds as described herein can be administered to a host suffering from a Hodgkin Lymphoma or a Non-Hodgkin Lymphoma. For example, the

host can be suffering from a Non-Hodgkin Lymphoma such as, but not limited to: an AIDS-Related Lymphoma; Anaplastic Large-Cell Lymphoma; Angioimmunoblastic Lymphoma; Blastic NK-Cell Lymphoma; Burkitt's Lymphoma; Burkitt-like Lymphoma (Small Non-Cleaved Cell Lymphoma); Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma; Cutaneous T-Cell Lymphoma; Diffuse Large B-Cell Lymphoma; Enteropathy-Type T-Cell Lymphoma; Follicular Lymphoma; Hepatosplenic Gamma-Delta T-Cell Lymphoma; Lymphoblastic Lymphoma; Mantle Cell Lymphoma; Marginal Zone Lymphoma; Nasal T-Cell Lymphoma; Pediatric Lymphoma; Peripheral T-Cell Lymphomas; Primary Central Nervous System Lymphoma; T-Cell Leukemias; Transformed Lymphomas; Treatment-Related T-Cell Lymphomas; or Waldenstrom's Macroglobulinemia.

Alternatively, a combination disclosed herein, or its salt, prodrug, or isotopic variant can be used in an effective amount to treat a host, for example a human, with a Hodgkin Lymphoma, such as, but not limited to: Nodular Sclerosis Classical Hodgkin's Lymphoma (CHL); Mixed Cellularity CHL; Lymphocyte-depletion CHL; Lymphocyte-rich CHL; Lymphocyte Predominant Hodgkin Lymphoma; or Nodular Lymphocyte Predominant HL.

Alternatively, a combination disclosed herein, or its salt, prodrug, or isotopic variant can be used in an effective amount to treat a host, for example a human with a specific B-cell lymphoma or proliferative disorder such as, but not limited to: multiple myeloma; Diffuse large B cell lymphoma; Follicular lymphoma; Mucosa-Associated Lymphatic Tissue lymphoma (MALT); Small cell lymphocytic lymphoma;Mediastinal large B cell lymphoma; Nodal marginal zone B cell lymphoma (NMZL); Splenic marginal zone lymphoma (SMZL); Intravascular large B-cell lymphoma; Primary effusion lymphoma; or Lymphomatoid granulomatosis;; B-cell prolymphocytic leukemia; Hairy cell leukemia; Splenic lymphoma/leukemia, unclassifiable; Splenic diffuse red pulp small B-cell lymphoma; Hairy cell leukemia-variant; Lymphoplasmacytic lymphoma; Heavy chain diseases, for example, Alpha heavy chain disease, Gamma heavy chain disease, Mu heavy chain disease; Plasma cell myeloma; Solitary plasmacytoma of bone; Extraosseous plasmacytoma; Primary cutaneous follicle center lymphoma; T cell/histiocyte rich large B-cell lymphoma; DLBCL associated with chronic inflammation; Epstein-Barr virus (EBV)+ DLBCL of the elderly; Primary mediastinal (thymic) large B-cell lymphoma; Primary cutaneous DLBCL, leg type; ALK+ large B-cell lymphoma; Plasmablastic lymphoma; Large B-cell lymphoma arising in HHV8-associated multicentric;

Castleman disease; B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma; or B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

In one embodiment, a combination disclosed herein, or its salt, prodrug, or isotopic variant can be used in an effective amount to treat a host, for example a human with leukemia. For example, the host may be suffering from an acute or chronic leukemia of a lymphocytic or myelogenous origin, such as, but not limited to: Acute lymphoblastic leukemia (ALL); Acute myelogenous leukemia (AML); Chronic lymphocytic leukemia (CLL); Chronic myelogenous leukemia (CML); juvenile myelomonocytic leukemia (JMML); hairy cell leukemia (HCL); acute promyelocytic leukemia (a subtype of AML); large granular lymphocytic leukemia; or Adult T-cell chronic leukemia. In one embodiment, the patient suffers from an acute myelogenous leukemia, for example an undifferentiated AML (M0); myeloblastic leukemia (M1; with/without minimal cell maturation); myeloblastic leukemia (M2; with cell maturation); promyelocytic leukemia (M3 or M3 variant [M3V]); myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]); monocytic leukemia (M5); erythroleukemia (M6); or megakaryoblastic leukemia (M7).

The presence or normal functioning of the retinoblastoma (Rb) tumor suppressor protein (Rb-positive) can be determined through any of the standard assays known to one of ordinary skill in the art, including but not limited to Western Blot, ELISA (enzyme linked immunoadsorbent assay), IHC (immunohistochemistry), and FACS (fluorescent activated cell sorting). The selection of the assay will depend upon the tissue, cell line or surrogate tissue sample that is utilized e.g., for example Western Blot and ELISA may be used with any or all types of tissues, cell lines or surrogate tissues, whereas the IHC method would be more appropriate wherein the tissue utilized in the methods of the present invention was a tumor biopsy. FACs analysis would be most applicable to samples that were single cell suspensions such as cell lines and isolated peripheral blood mononuclear cells. See for example, US 20070212736 "Functional Immunohistochemical Cell Cycle Analysis as a Prognostic Indicator for Cancer". Alternatively, molecular genetic testing may be used for determination of retinoblastoma gene status. Molecular genetic testing for retinoblastoma includes the following as described in Lohmann and Gallie "Retinoblastoma. Gene Reviews" (2010): "A

comprehensive, sensitive and economical approach for the detection of mutations in the RB1 gene in retinoblastoma" Journal of Genetics, 88(4), 517-527 (2009).

In some embodiments, the cancer to be treated is selected from estrogen-receptor positive, HER2-negative advanced breast cancer, late-line metastatic breast cancer, liposarcoma, non-small cell lung cancer, liver cancer, ovarian cancer, glioblastoma, refractory solid tumors, retinoblastoma positive breast cancer as well as retinoblastoma positive endometrial, vaginal and ovarian cancers and lung and bronchial cancers.

Dosing Regimen

In one aspect of the invention, dosing regimens are provided wherein a host suffering from an Rb-positive cellular proliferative disorder is administered a CDK4/6 inhibitor described herein to arrest the cells in G0 or G1 phase, thus synchronizing the cells, wherein upon dissipation of the cell cycle arrest caused by the CDK4/6 inhibitor, the host is administered a second chemotherapeutic agent that takes advantage of the cells reentry into the cell cycle so that the administered second agent is more effective against a larger number of cells, as more cells will be in the effective cell-cycle state that the second chemotherapeutic is most efficacious in, for example, within the G1, S, G2, or M phase. Thus in one embodiment, the invention includes administering in combination or alternation a CDK4/6 inhibitor described herein, for example a CDK4/6 inhibitor selected from Table 1, in an effective amount to a host suffering from an Rb-positive abnormal cellular proliferation disorder in a treatment regimen, wherein (either alone or in any combination thereof, each of which is considered specifically and independently described): (i) a substantial portion of the abnormal cells (e.g., at least 80% or greater) are arrested in G0 or G1 and re-enter the cell cycle in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the last administration of a CDK4/6 inhibitor described herein; (ii) a substantial portion of the abnormal cells reenter the cell-cycle synchronously in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the last administration of the CDK4/6 inhibitor described herein; (iii) the dissipation of the CDK4/6 inhibitor's inhibitory effect on the abnormal cells occurs in less than about 24 hours, 30 hours, 36 hours, or 48 hours from the administration of the CDK4/6 inhibitor; (iv) a substantial portion of the abnormal cells reenter the cell-cycle in less than abou t24 hours, 30 hours, 36 hours, or 48 hours from the dissipation of the CDK4/6 inhibitor's inhibitory effect; or (vi) a substantial portion of the abnormal cells reenter the cell-

cycle within less than about 24 hours, about 30 hours, about 36 hours, or about 48 hours from the point in which the administered CDK4/6 inhibitor compound's concentration level in the subject's blood drops below a therapeutic effective concentration, and, either just prior to, concomitantly with, or shortly after the re-entry of the abnormal cells as described in (i)-(vi), administering at least one additional chemotherapeutic agent.

For example, a CDK4/6 inhibitor compound can be administered so that CDK4/6-replication dependent abnormal cells are G1 arrested as they proceed through the cell cycle, wherein, due to the dissipation of the G1-arresting effect of the CDK4/6 inhibitor, a significant number of cells reenter the cell-cycle in a synchronous manner and are capable of replicating shortly after exposure, for example, within about 24-48 hours or less, and continue to replicate as they are exposed to the second chemotherapeutic agent. In one embodiment, the CDK4/6 inhibitor compound is administered to allow for the cycling of the CDK4/6-replication dependent abnormal cells between G1-arrest and reentry into the cell-cycle to accommodate a repeated-dosing treatment regimen, for example a long term repeated-dosing treatment regime.

By synchronizing the abnormal cells, more cells can be exposed to an agent that acts at a particular cell-cycle phase, allowing for dose intensification (e.g., more therapy can be given in a fixed period of time) and fewer dosing requirements, which will translate to better efficacy. Therefore, the presently disclosed methods can result in regimens that are less toxic and more effective. When appropriate, the small molecules can be formulated for oral, topical, intranasal, inhalation, intravenous or any other desired form of administration.

A compound useful in the methods described herein is a selective CDK4/6 inhibitor compound that selectively inhibit at least one of CDK4 and CDK6 or through the inhibition of cellular replication of an Rb-positive cancer. In one embodiment, the compounds described herein have an IC_{50} for CDK4 as measured in a CDK4/CycD1 IC_{50} phosphorylation assay that is at least 500 times or greater lower than the compound's IC_{50}s for CDK2 as measured in a CDK2/CycE IC_{50} phosphorylation assay.

The use of a compound as described herein can induce selective G1 arrest in CDK4/6-dependent abnormal cells such as Rb-positive abnormal cells (e.g., as measured in a cell-based in vitro assay). In one embodiment, the CDK4/6 inhibitor is capable of increasing the percentage of CDK4/6-dependent abnormal cells in the G1 phase, while decreasing the percentage of CDK4/6-dependent abnormal cells in the G2/M phase and S phase. In one embodiment, the

compound induces substantially pure (i.e., "clean") G1 cell cycle arrest in the CDK4/6-dependent abnormal cells, e.g., wherein treatment with the CDK4/6 inhibitor compound induces cell cycle arrest such that the majority of abnormal cells are arrested in G1 as defined by standard methods (e.g. propidium iodide (PI) staining or others) with the population of cells in the G2/M and S phases combined being less than about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 3% or less of the total cell population. Methods of assessing the cell phase of a population of cells are known in the art (see, for example, in U.S. Patent Application Publication No. 2002/0224522) and include cytometric analysis, microscopic analysis, gradient centrifugation, elutriation, fluorescence techniques including immunofluorescence, and combinations thereof. Cytometric techniques include exposing the cell to a labeling agent or stain, such as DNA-binding dyes, e.g., PI, and analyzing cellular DNA content by flow cytometry. Immunofluorescence techniques include detection of specific cell cycle indicators such as, for example, thymidine analogs (e.g., 5-bromo-2-deoxyuridine (BrdU) or an iododeoxyuridine), with fluorescent antibodies.

In certain embodiments, the compound administered to induce G1 arrest is selected from the group consisting of the compound or a composition comprising Formula I, Formula II, Formula III, Formula IV, or Formula V, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof. In certain embodiments, the compound administered is selected from a compound contained in Table 1, or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

T-Cell Neoplasm Dosing Regimen

In another aspect of the present invention, provided herein is a differential dosing method for treating certain malignancies. For example, in one aspect of the invention, provided herein is a method of treating an Rb-positive T-cell malignancy by administering a CDK4/6 inhibitor described herein at a dose that provides for the extended inhibition of the proliferation of the T-cell malignancy, but provides for the differential proliferation of non-diseased Rb-positive hematopoietic cells, for example, hematopoietic stem and progenitor cells (HSPCs).

It has been discovered that T-cell lineages are particularly sensitive to the CDK4/6 inhibitors described herein. Accordingly, provided herein is a method for treating patients with particular T-cell malignancies with a concentration of a CDK4/6 inhibitor which inhibits the

proliferation of the T-cell malignancy, but differentially inhibits, for example, inhibits for a shorter period or at a lesser rate, other hematopoietic lineage cells. Because of this, the use of a CDK4/6 inhibitor to treat T-cell malignancies allows for an extended period of treatment while reducing the side effects, such as myelosuppression, associated with long term use of CDK4/6 inhibitors.

In one embodiment, the dose administered to the host having a T-cell malignancy in a dose effective enough to inhibit the abnormal T-cell proliferation while differentially inhibiting other hematopoietic cell lineages so that the other cell lineages are allowed to re-enter the cell cycle at a faster rate than the T-cell malignancy, allowing for the continued replication of hematopoetic cells. In one embodiment of the invention, provided herein is a method of treating a host suffering from a T-cell malignancy comprising 1) administering to the host a CDK4/6 inhibitor in an amount sufficient to inhibit the proliferation of the specific T-cell malignancy, 2) measuring or analyzing the cell cycle status of the specific cell T-cell malignancy and at least one subset of non-diseased hematopoietic cell lineages, for example, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), and megakaryocyte-erythroid progenitors (MEPs), macrophage progenitors, granulocyte progenitors (GP), monocyte progenitors (MP), megakaryocyte progenitors (MP), erythroid progenitors (EP), B cell progenitors, T cell and NK cell progenitors (TNKP), T cell progenitors (TCP), NK cell progenitors (TNKP), mast cell progenitors, macrophage-dendritic cell progenitors, dendritic cell progenitors, osteoclast precursor cells, basophil progenitors, eosinophil pregenitors, neutrophil progenitors, oligodendrocyte pre-progenitors (OPPs), or a combination thereof, 3) adjusting the dose of the CDK4/6 inhibitor so that at least one subset of non-diseased hematopoietic cell lineages, for example, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), and megakaryocyte-erythroid progenitors (MEPs), macrophage progenitors, granulocyte progenitors (GP), monocyte progenitors (MP), megakaryocyte progenitors (MP), erythroid progenitors (EP), B cell progenitors, T cell and NK cell progenitors (TNKP), T cell progenitors (TCP), NK cell progenitors (TNKP), mast cell progenitors,

macrophage-dendritic cell progenitors, dendritic cell progenitors, osteoclast precursor cells, basophil progenitors, eosinophil pregenitors, neutrophil progenitors, oligodendrocyte pre-progenitors (OPPs), or a combination thereof,reenter the cell cycle while a significant portion of the T-cell malignant cells remain in cell cycle arrest. In a particular embodiment, the host is a human, and the non-diseased hematological lineage cells are selected from HSC/MPPs (CD45dim/CD34+/CD38-), OPPs (CD45dim/CD34+/CD38+), monocyte progenitors (CD45+/CD14+/CD11b+), granulocyte progenitors (CD45+/CD14-/CD11b+), erythroid progenitors (CD45-/CD71+), and megakaryocyte progenitors (CD45+/CD61+). The measurement of cell cycle status of cell lineages is described further below and routine in the art. Cell lineages are readily assayable by analyzing specific cell markers on their cell surface: see Stelzer et al., CD45 gating for routine flow cytometric analysis of human bone marrow specimens, Annals of New York Academy of Sciences, Mar 20, 1993, Vol. 677;pg. 265-280; Spangrude et al., Purification and Characterization of Mouse Hematopoietic Stem Cells, Science (1988) Vol. 241:58-62.

Thus, as contemplated herein, provided is a method of treating a T-cell disorder comprising administering a CDK4/6 inhibitor at a dose wherein abnormal T-cells, or a significant portion of abnormal T-cells, for example 50%, 60%, 70%, 80% or greater, are arrested in the G0 and/or G1 phase of the cell cycle while at least a significant number, for example 50%, 60%, 70%, 80% or greater, of one non-diseased hematopoietic cell lineage is not arrested at the G0 and/or G1 phase of the cell cycle.

Hematological Malignances Differential Dosing

It has been discovered that hematological malignancies respond to CDK4/6 inhibitors in a differential fashion. Accordingly, this differential response provides for the differential dosing of a CDK4/6 inhibitor based on the specific hematological disorder the host may be suffering from. Thus, in one aspect of the invention, provided herein is a method of treating a host suffering from a hematological cellular proliferation disorder comprising 1) identifying the specific hematological deficiency, 2) administering to the host a CDK4/6 inhibitor in an amount sufficient to inhibit the proliferation of a significant portion of, for example, at least 50%, at least 60%, at least 70%, at least 80%, the specific hematological deficiency, 3) measuring, or analyzing the results of a measurement of, the cell cycle status of the specific hematological

deficient cells as well as one or more subsets of non-diseased hematological cell lineages, 4) adjusting the dose of the CDK4/6 inhibitor to the minimal amount necessary so that a significant portion of the diseased cells remain in G0 and/or G1 cell cycle arrest, while a significant portion, for example, at least 50%, at least 60%, at least 70%, at least 80%, or at least one subset of non-diseased hematological cells are mpt om G0 and/or G1 cell cycle arrest. Determining the cell cycle status of specific hematological cell lineages is well known in the art and described further in the Examples below.

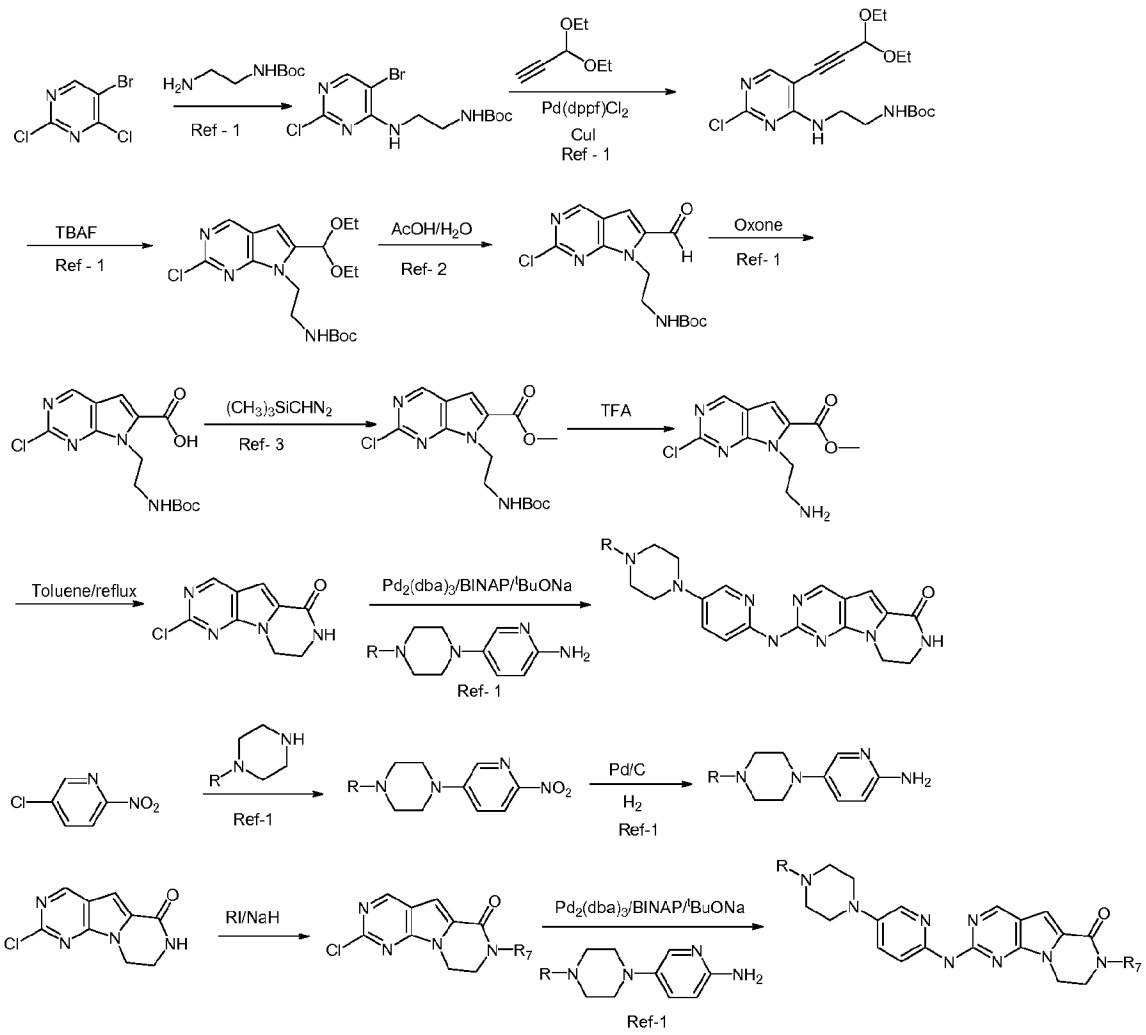
Optimizing dosage and time regimens using CDK4/6 Inhibitor for Chemoprotection

As indicated in Figures 12-19, cells of the bone marrow are differentially affected by the CDK4/6 inhibitors described herein. This differential response should be taken into account when treating patients through the selection of optimal dosage and timing. For example, if a patient has depleted macrophages due to the administration of a DNA-damaging chemotherapeutic agent for the treatment of an Rb-negative cancer, for example, it may be appropriate to use a CDK4/6 inhibitor as a chemoprotectant for some hematopoietic cell lineages while sparing the macrophage progenitor cells the inhibition caused by the use of the CDK4/6 inhibitor. For example, as Figure 14 indicates, macrophage lineages are less sensitive to CDK4/6 inhibition than other hematological cell lines. Accordingly, a method for differential dosing that takes into account the sparing of this cell lineage when needed, while still protecting other cell lines from the damage associated with the chemotherapeutic is provided.

Preparation of Active Compounds

Syntheses

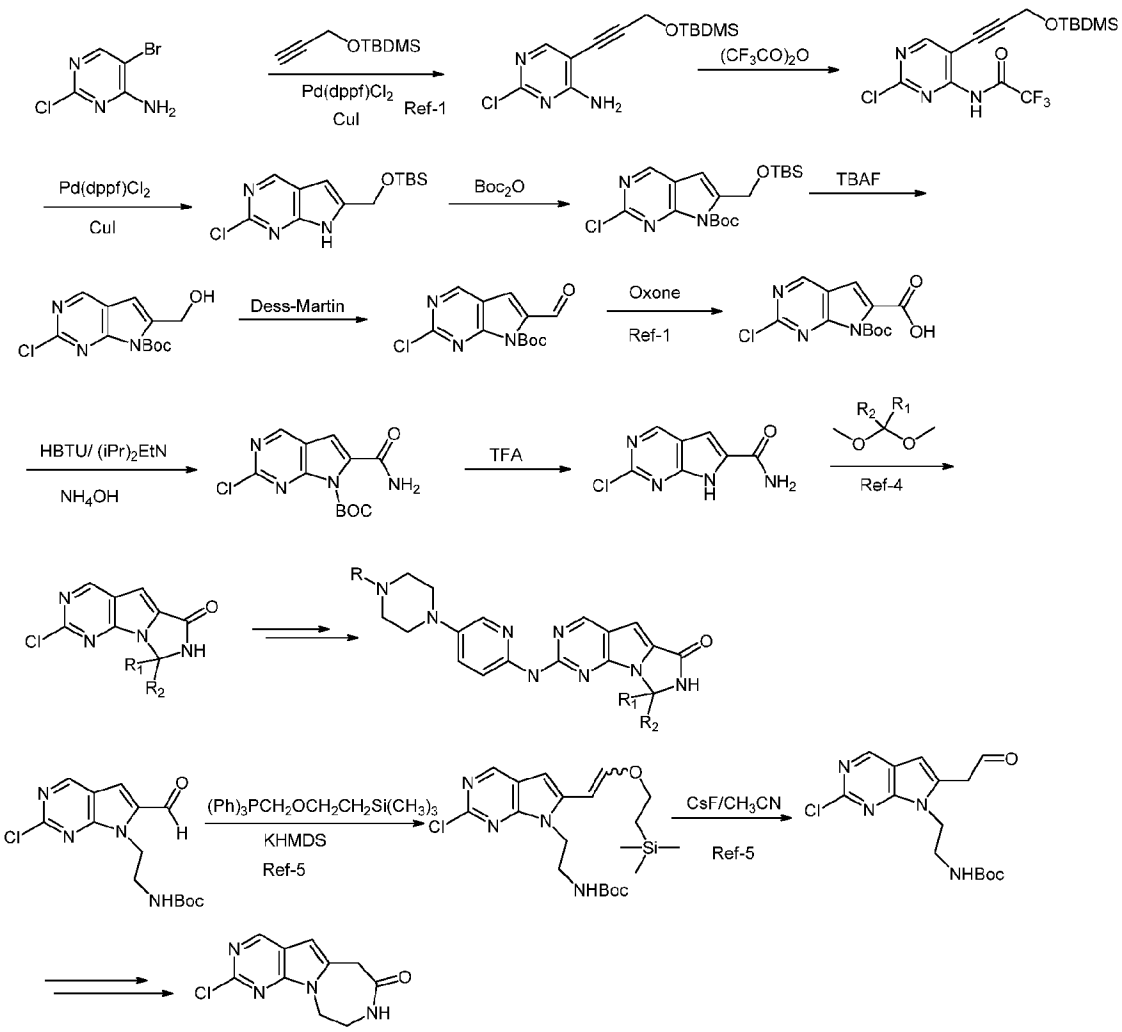
The disclosed compounds can be made by the following general schemes:



Scheme 1

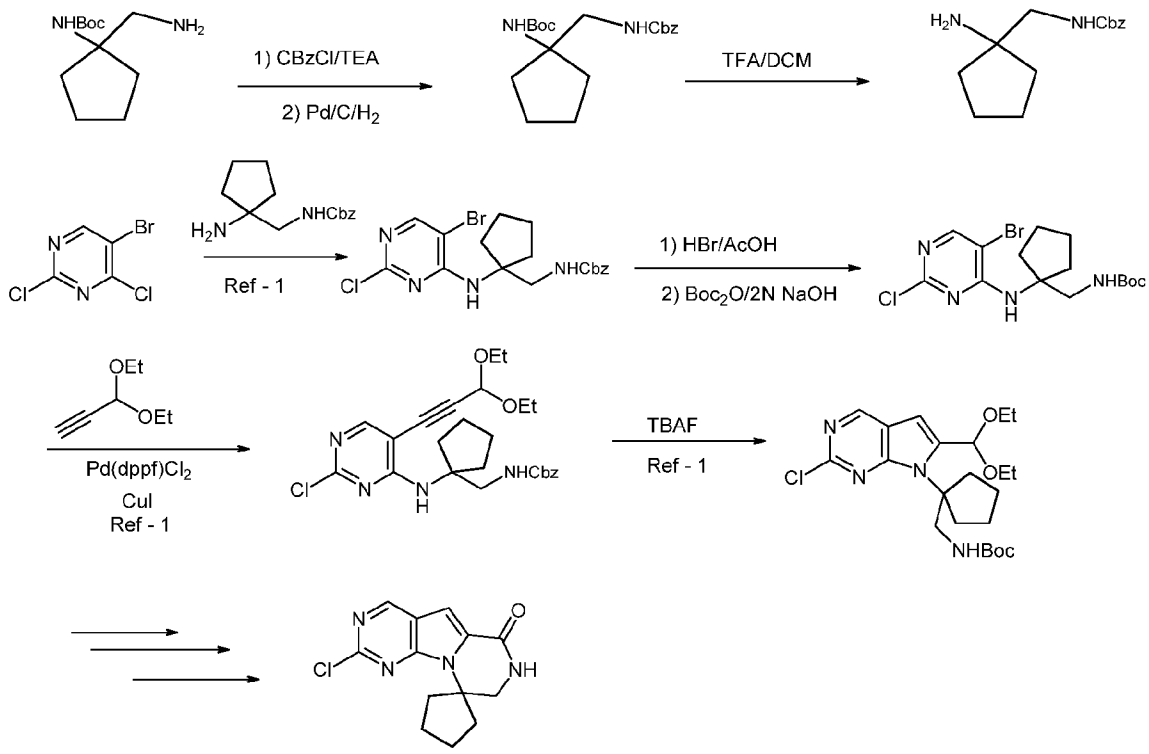
In Scheme 1, Ref-1 is WO 2010/020675 A1; Ref-2 is White, J. D.; *et al. J. Org. Chem.* 1995, 60, 3600; and Ref-3 Presser, A. and Hufner, A. *Monatshefte für Chemie* 2004, 135, 1015.

5



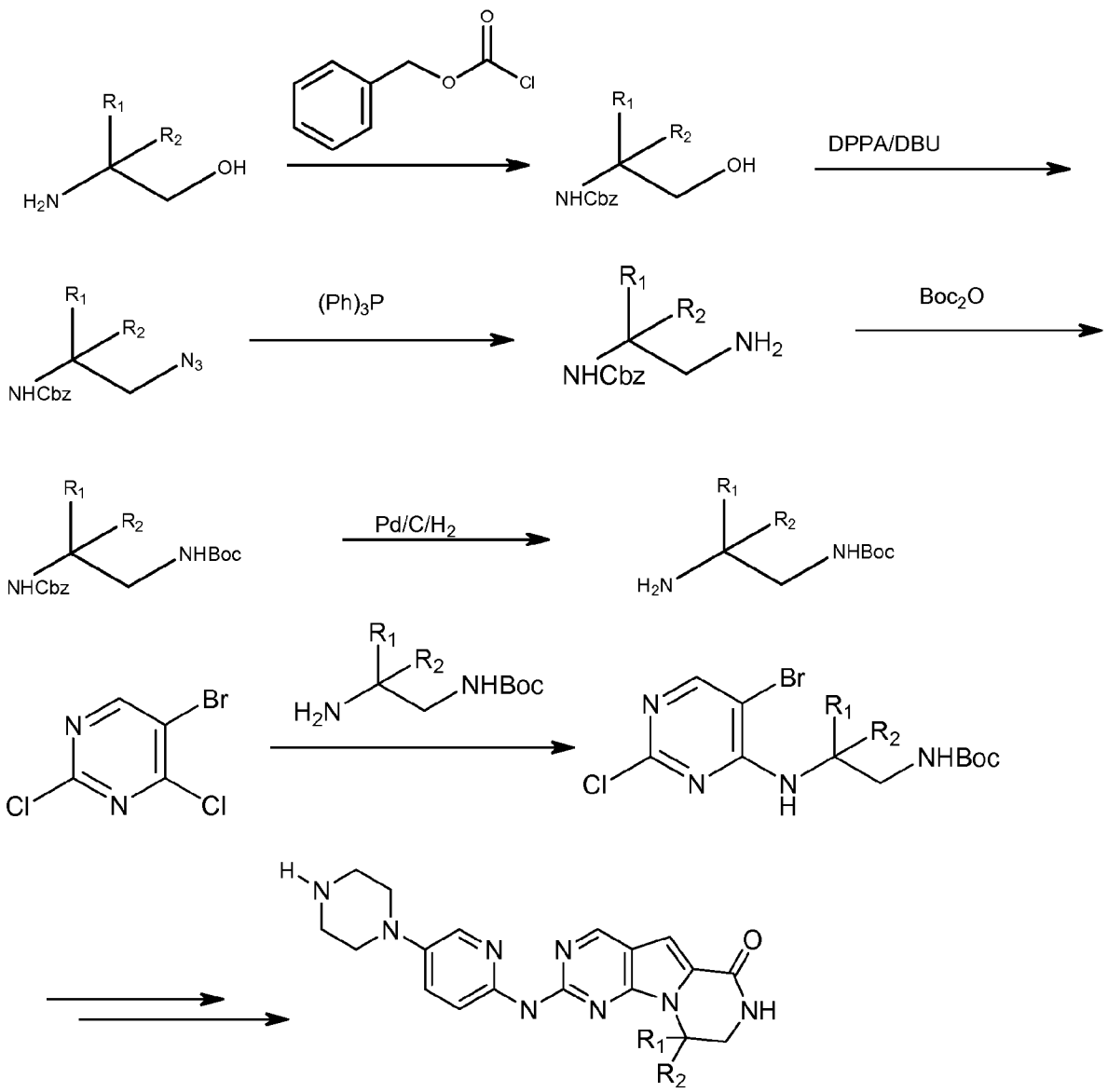
Scheme 2

In Scheme 2, Ref-1 is WO 2010/020675 A1; Ref-4 is WO 2005/040166 A1; and Ref-5 is Schoenauer, K and Zbiral, E. *Tetrahedron Letters* 1983, 24, 573.

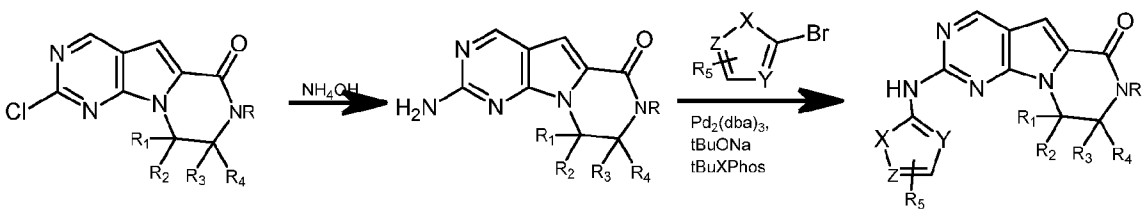


Scheme 3

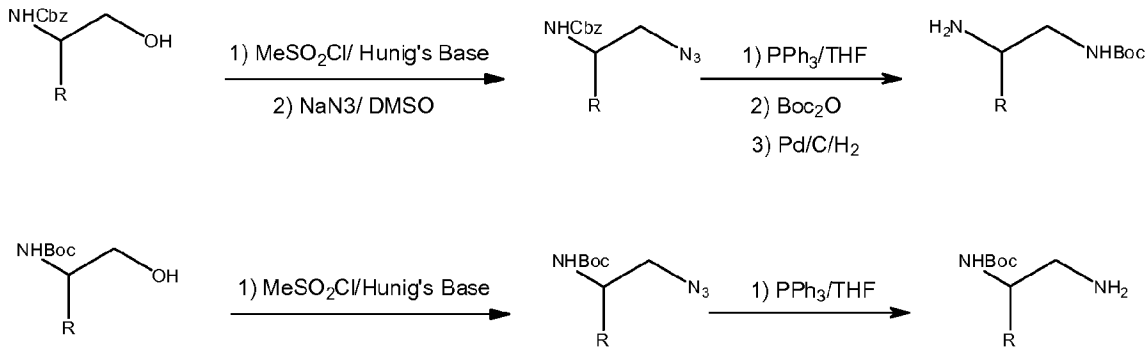
In Scheme 3, Ref – 1 is WO 2010/020675 A1.



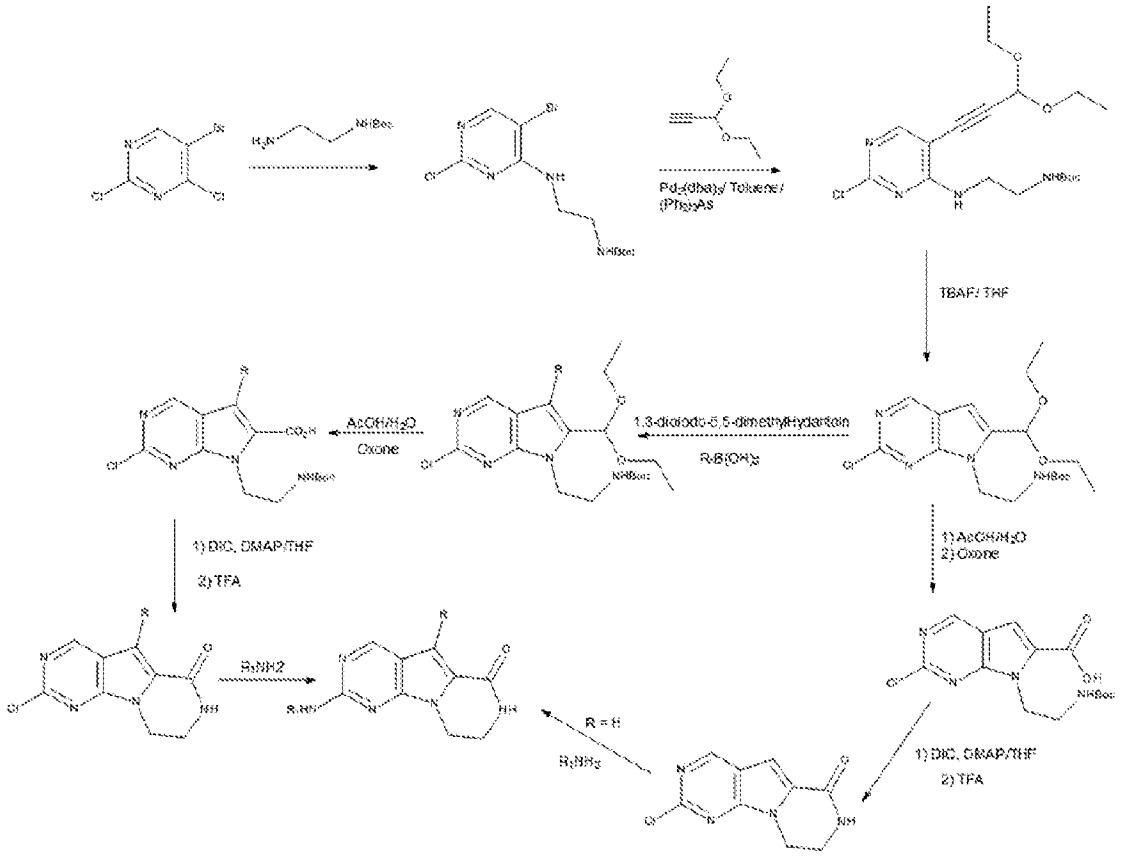
Scheme 4



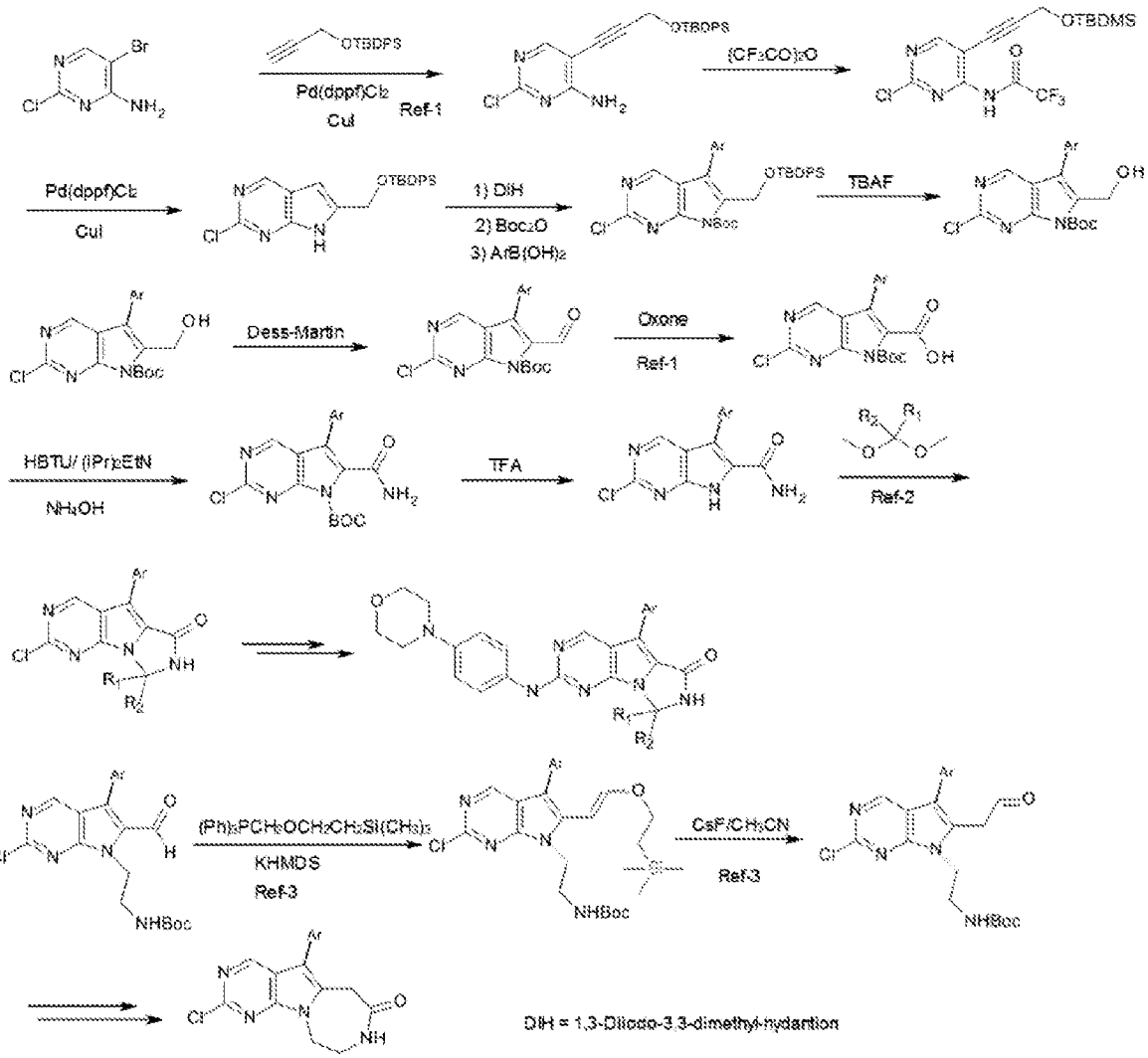
Scheme 5



Scheme 6



Scheme 7



Scheme 8

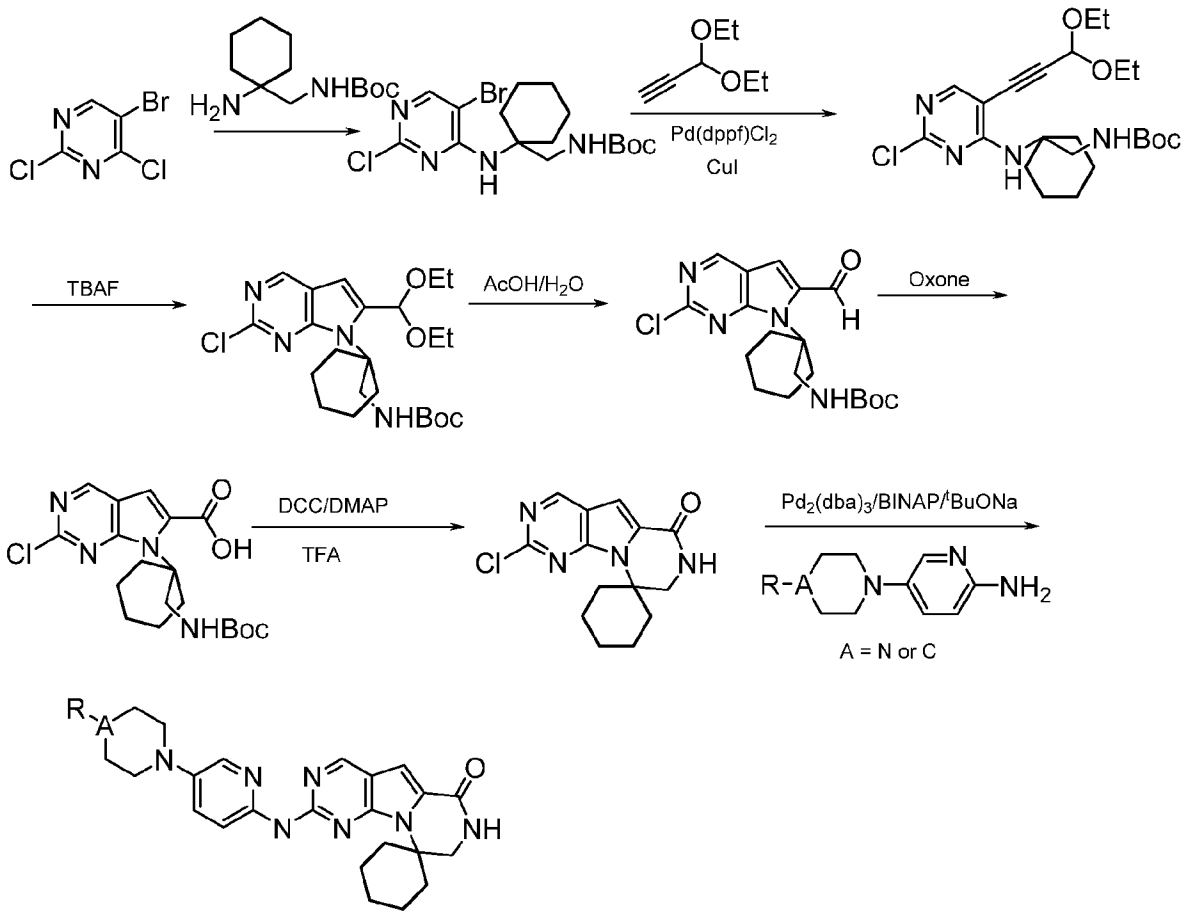
In Scheme 8, Ref-1 is WO 2010/020675 A1; Ref-2 is WO 2005/040166 A1; and Ref-3 is Schoenauer, K and Zbiral, E. Tetrahedron Letters 1983, 24, 573.

Alternatively, the lactam can be generated by reacting the carboxylic acid with a protected amine in the presence of a strong acid and a dehydrating agent, which can be together in one moiety as a strong acid anhydride. Examples of strong acid anhydrides include, but are not limited to, trifluoroacetic acid anhydride, tribromoacetic acid anhydride, trichloroacetic acid anhydride, or mixed anhydrides. The dehydrating agent can be a carbodiimide based compound such as but not limited to DCC (N,N-dicyclohexylcarbodiimide), EDC (1-ethyl-3-(3-

dimethylaminopropyl)carbodiimide or DIC (N,N-diisopropylcarbodiimide). An additional step may be necessary to take off the N-protecting group and the methodologies are known to those skilled in the art.

Alternatively, the halogen moiety bonded to the pyrimidine ring can be substituted with any leaving group that can be displaced by a primary amine, for example to create an intermediate for a final product such as Br, I, F, SMe, SO_2Me, SOalkyl, SO_2alkyl. See, for Exmaple PCT /US2013/037878 to Tavares.

Other amine intermediates and final amine compounds can be synthesized by those skilled in the art. It will be appreciated that the chemistry can employ reagents that comprise reactive functionalities that can be protected and de-protected and will be known to those skilled in the art at the time of the invention. See for example, Greene, T.W. and Wuts, P.G.M., Greene's Protective Groups in Organic Synthesis, 4th edition, John Wiley and Sons.



Scheme 9

CDK4/6 Inhibitors of the present invention can be synthesized according to the generalized Scheme 9. Specific synthesis and characterization of the Substituted 2-aminopyrmidines can be found in, for instance, WO2012/061156.

Compounds T, Q, GG, and U were prepared as above and were characterized by mass spectrometry and NMR as shown below:

Compound T

1H NMR (600 MHz, DMSO- d6) ppm 1.47 (br. s., 6 H) 1.72 (br. s., 2 H) 1.92 (br. s., 2 H) 2.77 (br. s., 3 H) 3.18 (br. s., 2 H) 3.46 (br. s., 2 H) 3.63 (br. s., 2 H) 3.66 (d, J=6.15 Hz, 2 H) 3.80 (br. s., 2 H) 7.25 (s, 1 H) 7.63 (br. s., 2 H) 7.94 (br. s., 1 H) 8.10 (br. s., 1 H) 8.39 (br. s., 1 H) 9.08 (br. s., 1 H) 11.59 (br. s., 1 H). LCMS ESI (M + H) 447.

Compound Q

1H NMR (600 MHz, DMSO-d6) ppm 0.82 (d, J=7.32 Hz, 2 H) 1.08 - 1.37 (m, 3 H) 1.38 - 1.64 (m, 2 H) 1.71 (br. s., 1 H) 1.91 (br. s., 1 H) 2.80 (br. s., 1 H) 3.12 (s, 1 H) 3.41 (br. s., 4 H) 3.65 (br. s., 4 H) 4.09 (br. s., 1 H) 7.26 (s, 1 H) 7.52 - 7.74 (m, 2 H) 7.94 (br. s., 1 H) 8.13 (br. s., 1 H) 8.40 (br. s., 1 H) 9.09 (br. s., 1 H) 9.62 (br. s., 1 H) 11.71 (br. s., 1 H). LCMS ESI (M + H) 433.

Compound GG

1H NMR (600 MHz, DMSO-d6) ppm 0.85 (br. s., 1 H) 1.17 - 1.39 (m, 7 H) 1.42 - 1.58 (m, 2 H) 1.67 - 1.84 (m,3 H) 1.88 - 2.02 (m, 1 H) 2.76 - 2.93 (m, 1 H) 3.07 - 3.22 (m, 1 H) 3.29 - 3.39 (m, 1 H) 3.41 - 3.61 (m, 4 H) 3.62 - 3.76 (m, 4 H) 3.78 - 3.88 (m, 1 H) 4.12 (br. s., 1H) 7.28 (s, 1 H) 7.60 - 7.76 (m, 2 H) 7.98 (s, 1 H) 8.13 (br. s., 1 H) 8.41 (s, 1 H) 9.10 (br. s., 1 H) 11.21 (br. s., 1 H) 11.54 (s, 1 H). LCMS ESI (M + H) 475.

Compound U

1H NMR (600 MHz, DMSO-d6) ppm 0.84 (t, J=7.61 Hz, 2 H) 1.13 - 1.39 (m, 4 H) 1.46 (d, J=14.05 Hz, 2 H) 1.64 - 1.99 (m, 6 H) 2.21 (br. s., 1 H) 2.66 - 2.89 (m, 2 H) 3.06 (br. s., 1 H) 3.24 - 3.36 (m, 1 H) 3.37 - 3.50 (m, 2 H) 3.56 - 3.72 (m, 2 H) 3.77 - 4.00 (m, 4 H) 4.02 - 4.19 (m, 2 H) 7.25 (s, 1 H) 7.50 - 7.75 (m, 2 H) 7.89 (d, J=2.93 Hz, 1 H) 8.14 (d, J=7.32 Hz, 1 H) 8.38 (br. s., 1 H) 9.06 (s, 1 H) 11.53 (br. s., 1 H). LCMS ESI (M + H) 517.

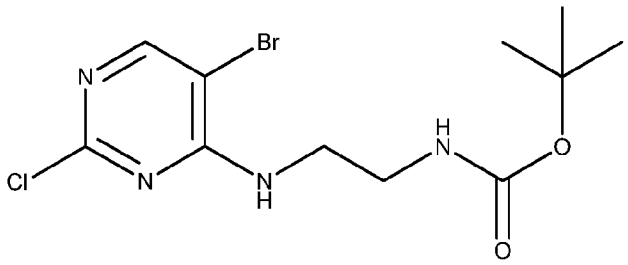
EXAMPLES

Intermediates B, E, K, L, 1A, 1F and 1CA were synthesized according to US 8,598,186 entitled CDK Inhibitors to Tavares, F.X. and Strum, J.C..

The patents WO 2013/148748 entitled Lactam Kinase Inhibitors to Tavares, F.X., WO 2013/163239 entitled Synthesis of Lactams to Tavares, F.X., and US 8,598,186 entitled CDK Inhibitors to Tavares, F.X. and Strum, J.C. are incorporated by reference herein in their entirety.

Example 1

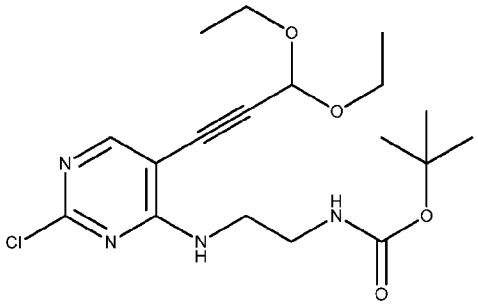
Synthesis of *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4yl)amino]ethyl]carbamate, Compound 1



To a solution of 5-bromo-2,4-dichloropyrimidine (3.2 g, 0.0135 mol) in ethanol (80 mL) was added Hunig's base (3.0 mL) followed by the addition of a solution of N-(*tert*-butoxycarbonyl)-1,2-diaminoethane (2.5 g, 0.0156 mole) in ethanol (20 mL). The contents were stirred overnight for 20 hrs. The solvent was evaporated under vacuum. Ethyl acetate (200 mL) and water (100 mL) were added and the layers separated. The organic layer was dried with magnesium sulfate and then concentrated under vacuum. Column chromatography on silica gel using hexane/ethyl acetate (0- 60%) afforded *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. ^{1}HNMR (d6-DMSO) δ ppm 8.21 (s, 1H), 7.62 (brs, 1H), 7.27 (brs, 1H), 3.39 (m, 2H), 3.12 (m, 2H), 1.34 (s, 9H). LCMS (ESI) 351 (M + H).

Example 2

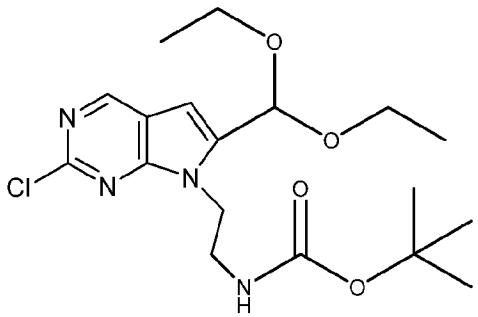
Synthesis of *tert*-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate, Compound 2



To *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate (1.265 g, 3.6 mmol) in THF (10 mL) was added the acetal (0.778 mL, 5.43 mmol), Pd(dppf)CH_2Cl_2 (148 mg), and triethylamine (0.757 mL, 5.43 mmol). The contents were degassed and then purged with nitrogen. To this was then added CuI (29 mg). The reaction mixture was heated at reflux for 48 hrs. After cooling, the contents were filtered over CELITE™ and concentrated. Column chromatography of the resulting residue using hexane/ethyl acetate (0- 30%) afforded *tert*-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate. ^{1}HNMR (d6-DMSO) δ ppm 8.18 (s, 1H), 7.63 (brs, 1H), 7.40 (brs, 1H), 5.55 (s, 1H), 3.70 (m, 2H), 3.60 (m, 2H), 3.42 (m, 2H), 3.15 (m, 2H), 1.19 – 1.16 (m, 15H). LCMS (ESI) 399 (M + H).

Example 3

Synthesis of *tert*-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate, Compound 3

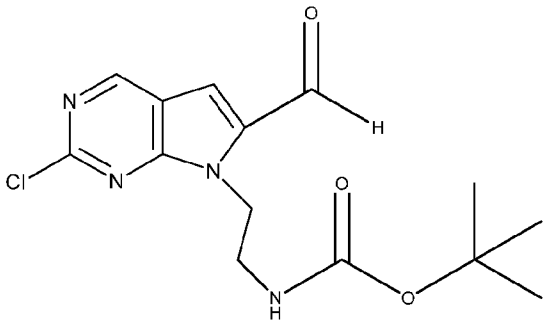


To a solution of the coupled product (2.1 g, 0.00526 mole) in THF (30 mL) was added TBAF solid (7.0 g). The contents were heated to and maintained at 65 degrees for 2 hrs. Concentration followed by column chromatography using ethyl acetate/hexane (0-50%) afforded

tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate as a pale brown liquid (1.1 g). ^{1}HNMR (d6-DMSO) δ ppm 8.88 (s, 1H), 6.95 (brs, 1H), 6.69 (s, 1H), 5.79 (s, 1H), 4.29 (m, 2H), 3.59 (m, 4H), 3.34 (m, 1H), 3.18 (m, 1H), 1.19 (m, 9H), 1.17 (m, 6H). LCMS (ESI) 399 (M + H).

Example 4

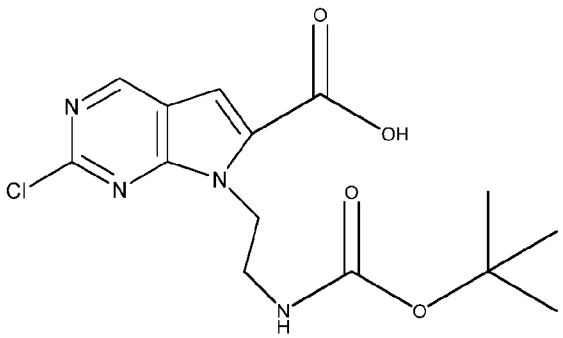
Synthesis of *tert*-butyl N-[2-(2-chloro-6-formyl-pyrrolo[2,3-d]pyrimidin-7-yl)ethyl]carbamate, Compound 4



To the acetal (900 mg) from the preceeding step was added AcOH (8.0 mL) and water (1.0 mL). The reaction was stirred at room temperature for 16 hrs. Conc. and column chromatography over silica gel using ethyl acetate/hexanes (0- 60%) afforded *tert*-butyl N-[2-(2-chloro-6-formyl-pyrrolo[2,3-d]pyrimidin-7-yl)ethyl]carbamate as a foam (0.510 g). ^{1}HNMR (d6-DMSO) δ ppm 9.98 (s, 1H), 9.18 (s, 1H), 7.66 (s, 1H), 6.80 (brs, 1H), 4.52 (m, 2H), 4.36 (m, 2H), 1.14 (s, 9H). LCMS (ESI) 325 (M + H).

Example 5

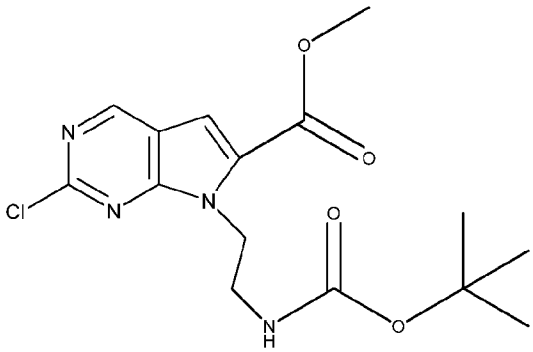
Synthesis of 7-[2-(*tert*-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 5



To the aldehyde (0.940 g) from the preceeding step in DMF (4 mL) was added oxone (1.95 g, 1.1 eq). The contents were stirred at room temp for 7 hrs. Silica gel column chromatography using hexane/ethyl acetate (0- 100%) afforded 7-[2-(*tert*-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (0.545 g). ^{1}HNMR (d6-DMSO) δ ppm 9.11 (s, 1H), 7.39 (s, 1H), 4.38 (m, 2H), 4.15 (m, 2H), 1.48 (m, 9H). LCMS (ESI) 341(M + H).

Example 6

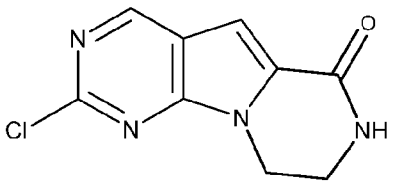
Synthesis of methyl 7-[2-(*tert*-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylate, Compound 6



To a solution of 2-chloro-7-propyl-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (0.545 g, 0.00156 mole) from the preceeding step in toluene (3.5 mL) and MeOH (1 mL) was added TMS-diazomethane (1.2 mL). After stirring overnight at room temperature, the excess of TMS-diazomethane was quenched with acetic acid (3 mL) and the reaction was concentrated under vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (0- 70%) to afford methyl 7-[2-(*tert*-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylate as an off white solid (0.52 g). ^{1}HNMR (d6-DMSO) δ ppm 9.10 (s, 1H), 7.45 (s, 1H), 6.81 (brs, 1H) 4.60 (m, 2H), 3.91 (s, 3H), 3.29 (m, 2H), 1.18 (m, 9H) LCMS (ESI) 355 (M + H).

Example 7

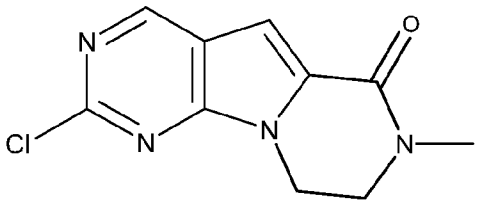
Synthesis of Chloro tricyclic amide, Compound 7



To methyl 7-[2-(*tert*-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylate (0.50 g, 0.0014 mole) from the preceeding step in dichloromethane (2.0 mL) was added TFA (0.830 mL). The contents were stirred at room temperature for 1 hr. Concentration under vacuum afforded the crude amino ester which was suspended in toluene (5 mL) and Hunig's base (0.5 mL). The contents were heated at reflux for 2 hrs. Concentration followed by silica gel column chromatography using hexane/ethyl acetate (0- 50%) afforded the desired chloro tricyclic amide (0.260 g). ^{1}HNMR (d6-DMSO) δ ppm 9.08 (s, 1H), 8.48 (brs, 1H), 7.21 (s, 1H) 4.33 (m, 2H), 3.64 (m, 2H). LCMS (ESI) 223 (M + H).

Example 8

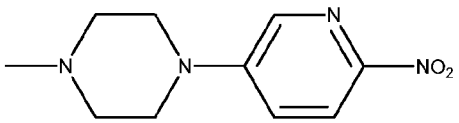
Synthesis of chloro-N-methyltricyclic amide, Compound 8



To a solution of the chloro tricycliclactam, Compound 7, (185 mg, 0.00083 mole) in DMF (2.0 mL) was added sodium hydride (55% dispersion in oil, 52 mg). After stirring for 15 mins, methyl iodide (62 μL, 1.2 eq). The contents were stirred at room temperature for 30 mins. After the addition of methanol (5 mL), sat $NaHCO_3$ was added followed by the addition of ethyl acetate. Separation of the organic layer followed by drying with magnesium sulfate and concentration under vacuum afforded the N-methylated amide in quantitative yield. ^{1}HNMR (d6-DMSO) δ ppm 9.05 (s, 1H), 7.17 (s, 1H) 4.38 (m, 2H), 3.80 (m, 2H), 3.05 (s, 3H). LCMS (ESI) 237 (M + H).

Example 9

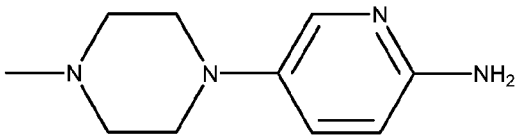
Synthesis of 1-methyl-4-(6-nitro-3-pyridyl)piperazine, Compound 9



To 5-bromo-2-nitropyridine (4.93 g, 24.3 mmole) in DMF (20 mL) was added N-methylpiperazine (2.96 g, 1.1 eq) followed by the addition of DIPEA (4.65 mL, 26.7 mmole). The contents were heated at 90 degrees for 24 hrs. After addition of ethyl acetate (200 mL), water (100 mL) was added and the layers separated. Drying followed by concentration afforded the crude product which was purified by silica gel column chromatography using (0-10%) DCM/Methanol. ^{1}HNMR (d6-DMSO) δ ppm 8.26 (s, 1H), 8.15 (1H, d, J = 9.3 Hz), 7.49 (1H, d, J = 9.4 Hz), 3.50 (m, 4H), 2.49 (m, 4H), 2.22 (s, 3H).

Example 10

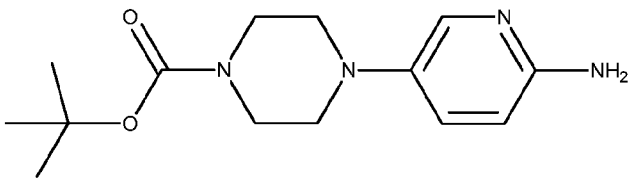
Synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine, Compound 10



To 1-methyl-4-(6-nitro-3-pyridyl)piperazine (3.4 g) in ethyl acetate (100 mL) and ethanol (100 mL) was added 10% Pd/C (400 mg) and then the reaction was stirred under hydrogen (10 psi) overnight. After filtration through CELITE^{TM}, the solvents were evaporated and the crude product was purified by silica gel column chromatography using DCM/ 7N ammonia in MeOH (0- 5%) to afford 5-(4-methylpiperazin-1-yl)pyridin-2-amine (2.2 g). ^{1}HNMR (d6-DMSO) δ ppm 7.56 (1H, d, J = 3 Hz), 7.13 (1H, m), 6.36 (1H, d, J = 8.8 Hz), 5.33 (brs, 2H), 2.88 (m, 4H), 2.47 (m, 4H), 2.16 (s, 3H).

Example 11

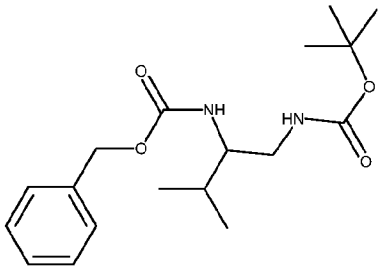
Synthesis of tert-butyl 4-(6-amino-3-pyridyl)piperazine-1-carboxylate, Compound 11



This compound was prepared as described in WO 2010/020675 A1.

Example 12

Synthesis of *tert*-butyl N-[2-(benzyloxycarbonylamino)-3-methyl-butyl] carbamate, Compound 12



To benzyl N-[1-(hydroxymethyl)-2-methyl-propyl]carbamate (11.0 g, 0.0464 mole) in dioxane (100 mL) cooled to 0 °C was added diphenylphosphoryl azide (10.99 mL, 1.1 eq) followed by the addition of DBU (8.32 mL, 1.2 eq). The contents were allowed to warm to room temperature and stirred for 16 hrs. After the addition of ethyl acetate (300 mL) and water (100 mL), the organic layer was separated and washed with satd. $NaHCO_3$ (100 mL). The organic layer was then dried (magnesium sulfate) and concentrated under vacuum. To this intermediate in DMSO (100 mL) was added sodium azide (7.54 g) and the contents then heated to 90 degrees for 2 hrs. After addition of ethyl acetate and water the layers were separated. The organic layer was dried with magnesium sulfate followed by concentration under vacuum to afford an oil that was purified by silica gel column chromatography using hexane/ethyl acetate (0- 70%) to afford benzyl N-[1-(azidomethyl)-2-methyl-propyl] carbamate 6.9 g as a colorless oil.

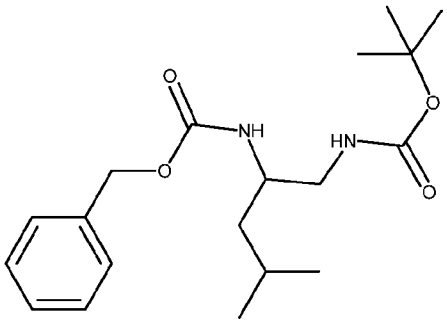
To benzyl N-[1-(azidomethyl)-2-methyl-propyl] carbamate (6.9 g, 0.0263 mole) in THF (100 mL) was added triphenyl phosphine (7.59 g, 1.1 eq). The contents were stirred for 20 hrs. After addition of water (10 mL), and stirring for an additional 6 hrs, ethyl acetate was added and the layers separated. After drying with magnesium sulfate and concentration under vacuum, the crude product was purified by silica gel column chromatography using DCM/MeOH (0 – 10%) to afford benzyl N-[1-(aminomethyl)-2-methyl-propyl] carbamate as a yellow oil.

To benzyl N-[1-(aminomethyl)-2-methyl-propyl] carbamate (4.65 g, 0.019 mole) in THF (70 mL) was added 2N NaOH (20 mL) followed by the addition of di-*tert*-butyl dicarbonate (5.15 g, 1.2 eq). After stirring for 16 hrs, ethyl acetate was added and the layers separated. After drying with magnesium sulfate and concentration under vacuum, the crude product was purified

using hexane/ethyl acetate (0- 40%) over a silica gel column to afford intermediate A, *tert*-butyl N-[2-(benzyloxycarbonylamino)-3-methyl-butyl] carbamate, (6.1 g). ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.89 (d, *J*=6.73 Hz, 3 H) 0.92 (d, *J*=6.73 Hz, 3 H) 1.38 (s, 9 H) 1.70 - 1.81 (m, 1 H) 3.18 (d, *J*=5.56 Hz, 2 H) 3.47 - 3.60 (m, 1 H) 4.76 (s, 1 H) 4.89 (d, *J*=7.90 Hz, 1 H) 5.07 (s, 2 H) 7.25 - 7.36 (m, 5 H). LCMS (ESI) 337 (M + H).

Example 13

Synthesis of *tert*-butyl N-[2-(benzyloxycarbonylamino)-4-methyl-pentyl] carbamate, Compound 13



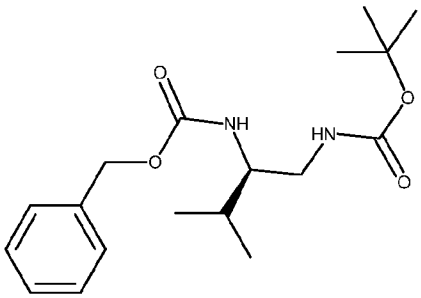
To a solution of benzyl N-[1-(hydroxymethyl)-3-methyl-butyl]carbamate (6.3 g, 0.025 mole) in DCM (100 mL) was added diisopropylethyl amine (5.25 mL, 1.2 eq) followed by the addition of methane sulfonylchloride (2.13 mL, 1.1 eq) at 0 degrees. After stirring for 3 hrs, water (100 mL) was added and the organic layer separated. After drying with magnesium sulfate and concentration under vacuum, the crude [2-(benzyloxycarbonylamino)-4-methyl-pentyl] methanesulfonate which was taken directly to the next step.

To the crude [2-(benzyloxycarbonylamino)-4-methyl-pentyl] methanesulfonate from the above reaction in DMF (50 mL), was added sodium azide 2.43 g. The reaction mixture was then heated to 85 degrees for 3 hrs. After cooling, ethyl acetate (300 mL) and water was added. The organic layer was separated, dried with magnesium sulfate and then concentrated under vacuum to afford the crude benzyl N-[1-(azidomethyl)-3-methyl-butyl] carbamate. To this crude intermediate was added THF (100 mL) followed by triphenylphosphine 7.21 g and stirred under nitrogen for 16 hrs. After addition of water (10 mL), and stirring for an additional 6 hrs, ethyl acetate was added and the layers separated. After drying with magnesium sulfate and concentration under vacuum, the crude product was columned using DCM/MeOH (0 – 10%) to afford benzyl N-[1-(aminomethyl)-3-methyl-butyl] carbamate (4.5 g).

To benzyl N-[1-(aminomethyl)-3-methyl-butyl] carbamate (4.5 g, 0.018 mole) in THF (60 mL) was added 2N NaOH (18 mL) followed by the addition of di-*tert*-butyl dicarbonate (4.19 g, 1.07 eq). After stirring for 16 hrs, ethyl acetate was added and the layers separated. After drying with magnesium sulfate and concentration under vacuum, the crude product was taken to the next step. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.89 (d, *J*=6.73 Hz, 6 H) 1.25 - 1.34 (m, 1 H) 1.39 (s, 9 H) 1.57 - 1.71 (m, 2 H) 3.04 - 3.26 (m, 2 H) 3.68 - 3.80 (m, 1 H) 4.72 - 4.89 (m, 2 H) 5.06 (s, 2 H) 7.25 - 7.38 (m, 5 H). LCMS (ESI) 351 (M + H).

Example 14

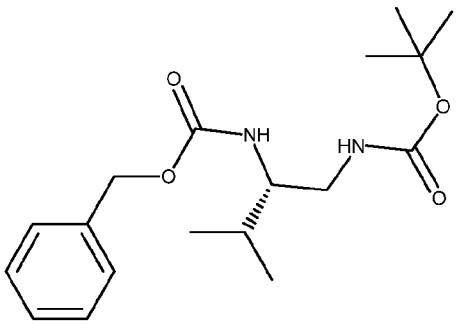
Synthesis of *tert*-butyl N-[(2R)-2-(benzyloxycarbonylamino)-3-methyl-butyl] carbamate, Compound 14



Compound 14 was synthesized from benzyl N-[(1R)-1-(hydroxymethyl)-2-methyl-propyl] carbamate using similar synthetic steps as that described for Compound 13. The analytical data (NMR and mass spec) was consistent with that for Compound 12.

Example 15

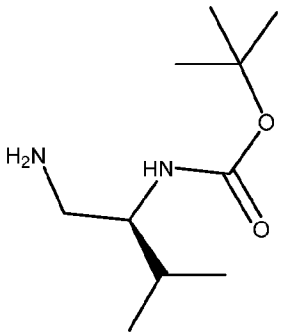
Synthesis of *tert*-butyl N-[(2S)-2-(benzyloxycarbonylamino)-3-methyl-butyl]carbamate, Compund 15



Compound 15 was synthesized from benzyl N-[(1S)-1-(hydroxymethyl)-2-methyl-propyl] carbamate using similar synthetic steps as that described for Compound 13. The analytical data (NMR and mass spec) was consistent with that for Compound 12.

Example 16

Synthesis of *tert*-butyl N-[(1S)-1-(aminomethyl)-2-methyl-propyl]carbamate, Compound 16

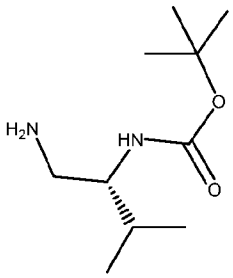


To a solution of *tert*-butyl N-[(1S)-1-(hydroxymethyl)-2-methyl-propyl]carbamate carbamate (6.3 g, 0.025 mole) in THF (100 mL) was added diisopropylethyl amine (5.25 mL, 1.2 eq) followed by the addition of methane sulfonylchloride (2.13 mL, 1.1 eq) at 0 degrees. After stirring for 3 hrs, water (100 mL) was added and the organic layer separated. After drying with magnesium sulfate and concentration under vacuum, the crude [(2S)-2-(*tert*-butoxycarbonylamino)-3-methyl-butyl] methanesulfonate was taken directly to the next step.

To the crude [(2S)-2-(*tert*-butoxycarbonylamino)-3-methyl-butyl] methanesulfonate from the above reaction in DMSO (50 mL), was added sodium azide (2.43 g). The reaction mixture was then heated to 85 degrees for 3 hrs. After cooling, ethyl acetate (300 mL) and water were added. The organic layer was separated, dried with magnesium sulfate and then concentrated under vacuum to afford the crude benzyl N-[1-(azidomethyl)-3-methyl-butyl] carbamate. To this crude intermediate was added THF (100 mL) followed by triphenylphosphine (7.21 g) and the reation was stirred under nitrogen for 16 hrs. After addition of water (10 mL), and stirring for an additional 6 hrs, ethyl acetate was added and the layers separated. After drying with magnesium sulfate and concentration under vacuum, the crude product was purified by silica gel column chromatography using DCM/MeOH (0 – 10%) to afford benzyl N-[1-(aminomethyl)-3-methyl-butyl] carbamate (4.5 g). LCMS (ESI) 203 (M + H).

Example 17

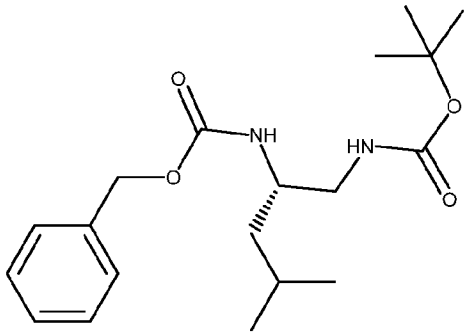
Synthesis of *tert*-butyl N-[(1R)-1-(aminomethyl)-2-methyl-propyl]carbamate, Compound 17



Compound 17 was synthesized from *tert*-butyl N-[(1R)-1-(hydroxymethyl)-2-methyl-propyl] carbamate using a similar synthetic sequence as described for Compound 16. The analytical data (NMR and mass spec) was consistent with Compound 16.

Example 18

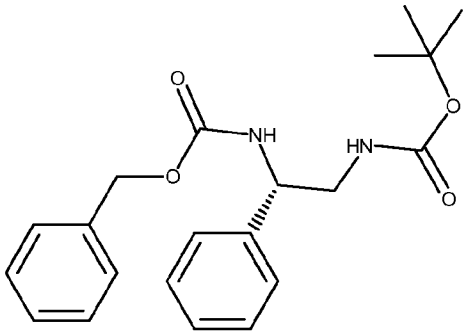
Synthesis of *tert*-butyl N-[(2S)-2-(benzyloxycarbonylamino)-4-methyl-pentyl] carbamate, Compound 18



Compound 18 was synthesized from benzyl N-[(1S)-1-(hydroxymethyl)-3-methyl-butyl]carbamate using a similar synthetic sequence as described for Compound 13. The analytical data (NMR and mass spec) was consistent with Compound 13.

Example 19

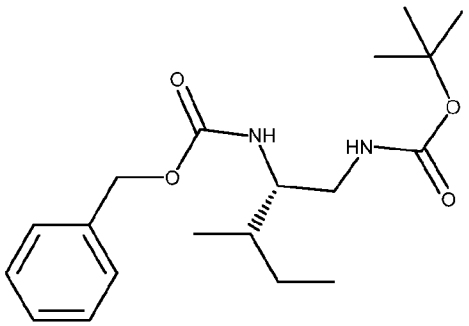
Synthesis of *tert*-butyl N-[(2S)-2-(benzyloxycarbonylamino)-2-phenyl-ethyl] carbamate, Compound 19



Compound 19 was synthesized from benzyl N-[(1S)-2-hydroxy-1-phenyl-ethyl] carbamate using a similar synthetic sequence as described for Compound 13. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.20 - 1.33 (m, 9 H) 3.11 (t, *J*=6.29 Hz, 2 H) 4.59 - 4.68 (m, 1 H) 4.88 - 5.01 (m, 2 H) 6.81 (t, *J*=5.42 Hz, 1 H) 7.14 - 7.35 (m, 10 H) 7.69 (d, *J*=8.49 Hz, 1 H). LCMS (ESI) 371 (M + H).

Example 20

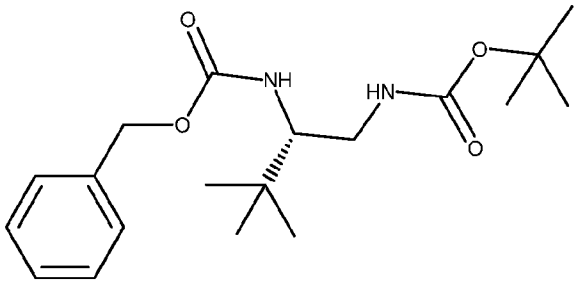
Synthesis of *tert*-butyl N-[(2S)-2-(benzyloxycarbonylamino)-3-methyl-pentyl] carbamate, Compound 20



Compound 20 was synthesized from benzyl N-[(1S)-1-(hydroxymethyl)-2-methyl-butyl] carbamate using a similar synthetic sequence as described for Compound 13. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.85 - 0.92 (m, 6 H) 1.05 - 1.15 (m, 1 H) 1.35 - 1.41 (m, 9 H) 1.45 - 1.56 (m, 2 H) 3.14 - 3.24 (m, 2 H) 3.54 - 3.64 (m, 1 H) 4.78 (s, 1 H) 4.96 (d, *J*=7.91 Hz, 1 H) 5.06 (s, 2 H) 7.27 - 7.37 (m, 5 H). LCMS (ESI) 351 (M + H).

Example 21

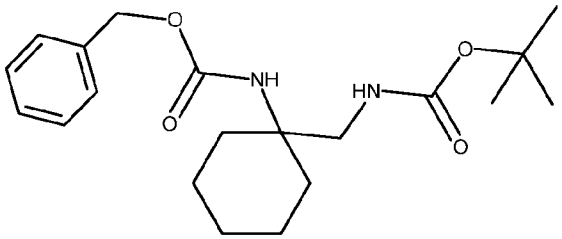
Synthesis of *tert*-butyl N-[(2S)-2-(benzyloxycarbonylamino)-3,3-dimethyl-butyl] carbamate, Compound 21



Compound 21 was synthesized from benzyl N-[(1S)-1-(hydroxymethyl)-2,2-dimethyl-propyl]carbamate using a similar synthetic sequence as described for Compound 13. LCMS (ESI) 351.

Example 22

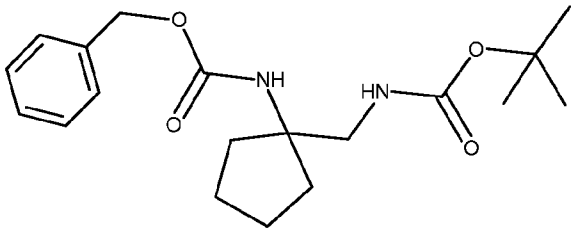
Synthesis of *tert*-butyl N-[[1-(benzyloxycarbonylamino)cyclohexyl]methyl] carbamate, Compound 22



To a solution of benzyl N-[1-(aminomethyl)cyclohexyl]carbamate (10.0 g, 0.0381 mole) in THF (150 mL) was added di-*tert*-butyl dicarbonate (9.15 g, 1.1 eq) and the contents were stirred at room temperature for 16 hrs. Ethyl acetate and water were then added. The organic layer was separated, dried over magnesium sulfate and then concentrated under vacuum to afford *tert*-butyl N-[[1-(benzyloxycarbonylamino)cyclohexyl]methyl] carbamate (13.1 g). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.92 - 1.54 (m, 17 H) 1.76 - 2.06 (m, 2 H) 3.09 (d, J=6.15 Hz, 2 H) 4.92 (s, 2 H) 6.63 (d, J=17.27 Hz, 1 H) 7.16 - 7.49 (m, 6 H). LCMS (ESI) 363 (M + H).

Example 23

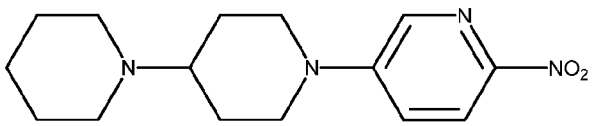
Synthesis of *tert*-butyl N-[[1-(benzyloxycarbonylamino)cyclopentyl]methyl] carbamate, Compound 23



tert-butyl N-[[1-(benzyloxycarbonylamino)cyclopentyl]methyl]carbamate was synthesized in an analogous manner to *tert*-butyl N-[[1-(benzyloxycarbonylamino) cyclohexyl]methyl] carbamate. LCMS (ESI) 349 (M + H).

Example 24

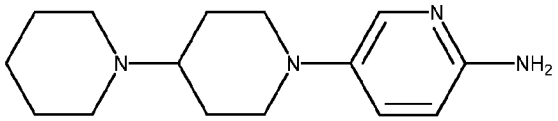
Synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine, Compound 24



To 5-bromo-2-nitropyridine (1.2 g, 5.9 mmol) in DMSO (4 mL) was added 1-(4-piperidyl)piperidine (1.0 g, 5.9 mmole) and triethylamine (0.99 mL, 7.1 mmole). The contents were heated to 120 °C in a CEM Discovery microwave system for 3 hours. The crude reaction was then purified by silica gel column chromatography with DCM/methanol (0-20%) to afford 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine as an oil (457 mg). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.26 - 1.36 (m, 2 H) 1.43 (m, 6 H) 1.76 (m, 2 H) 2.37 (m, 5 H) 2.94 (t, *J*=12.74 Hz, 2 H) 4.06 (d, *J*=13.47 Hz, 2 H) 7.41 (dd, *J*=9.37, 2.64 Hz, 1 H) 8.08 (d, *J*=9.37 Hz, 1 H) 8.20 (d, *J*=2.64 Hz, 1 H).

Example 25

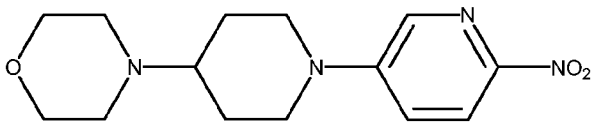
Synthesis of 5-[4-(1-piperidyl)-1-piperidyl]pyridin-2-amine, Compound 25



5-[4-(1-piperidyl)-1-piperidyl]pyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.13 - 1.37 (m, 6 H) 1.40 - 1.63 (m, 6 H) 1.71 (m, 2 H), 2.24 (m, 1H) 2.43 (m, 2 H) 3.33 (d, *J*=12.30 Hz, 2 H) 5.31 (s, 2 H) 6.33 (d, *J*=8.78 Hz, 1 H) 7.10 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.55 (d, *J*=2.64 Hz, 1 H). LCMS (ESI) 261 (M + H).

Example 26

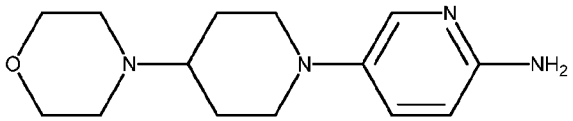
Synthesis of 4-[1-(6-nitro-3-pyridyl)-4-piperidyl] morpholine, Compound 26



4-[1-(6-nitro-3-pyridyl)-4-piperidyl]morpholine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.41 (m, 2 H) 1.82 (m, 2 H) 2.42 (m, 5 H) 2.98 (t, *J*=12.44 Hz, 2 H) 3.52 (s, 4 H) 4.04 (d, *J*=12.88 Hz, 2 H) 7.42 (d, *J*=9.37 Hz, 1 H) 8.08 (d, *J*=9.08 Hz, 1 H) 8.21 (s, 1 H).

Example 27

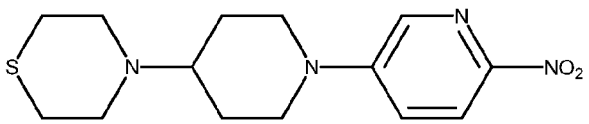
Synthesis of 5-(4-morpholino-1-piperidyl) pyridin-2-amine, Compound 27



5-(4-morpholino-1-piperidyl)pyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.34 - 1.52 (m, 2 H) 1.78 (m, 2 H) 2.14 (m, 1 H) 2.43 (m, 4 H) 3.32 (d, *J*=12.30 Hz, 4 H) 3.47 - 3.59 (m, 4 H) 5.32 (s, 2 H) 6.34 (d, *J*=8.78 Hz, 1 H) 7.11 (dd, *J*=8.93, 2.78 Hz, 1 H) 7.47 - 7.62 (m, 1 H). LCMS (ESI) 263 (M + H).

Example 28

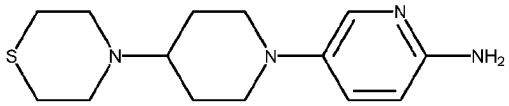
Synthesis of 4-[1-(6-nitro-3-pyridyl)-4-piperidyl] thiomorpholine, Compound 28



4-[1-(6-nitro-3-pyridyl)-4-piperidyl] thiomorpholine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.40 - 1.52 (m, 2 H) 1.71 (m, 2 H) 2.49 - 2.55 (m, 4 H) 2.56 - 2.63 (m, 1 H) 2.68 - 2.75 (m, 4 H) 2.88 - 2.98 (m, 2 H) 4.09 (d, *J*=13.18 Hz, 2 H) 7.42 (dd, *J*=9.22, 3.07 Hz, 1 H) 8.08 (d, *J*=9.37 Hz, 1 H) 8.20 (d, *J*=3.22 Hz, 1 H).

Example 29

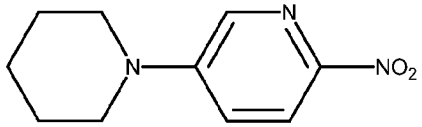
Synthesis of 5-(4-thiomorpholino-1-piperidyl) pyridin-2-amine, Compound 29



5-(4-thiomorpholino-1-piperidyl) pyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.47 - 1.59 (m, 2 H) 1.65 (m, 2 H) 2.22 - 2.38 (m, 1 H) 2.50 - 2.59 (m, 6 H) 2.68 - 2.82 (m, 4 H) 3.33 (d, *J*=12.00 Hz, 2 H) 5.31 (s, 2 H) 6.33 (d, *J*=9.08 Hz, 1 H) 7.10 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.55 (d, *J*=2.64 Hz, 1 H). LCMS (ESI) 279 (M + H).

Example 30

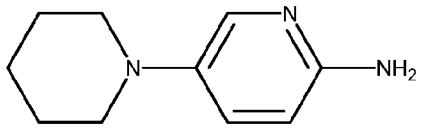
Synthesis of 2-nitro-5-(1-piperidyl)pyridine, Compound 30



2-nitro-5-(1-piperidyl) pyridine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.56 (m, 6 H) 3.49 (d, *J*=4.39 Hz, 4 H) 7.30 - 7.47 (m, 1 H) 8.02 - 8.12 (m, 1 H) 8.15 - 8.26 (m, 1 H).

Example 31

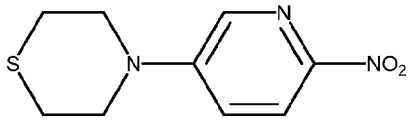
Synthesis of 5-(1-piperidyl)pyridin-2-amine, Compound 31



5-(1-piperidyl) pyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.39 - 1.46 (m, 2 H) 1.51 - 1.62 (m, 4 H) 2.75 - 2.92 (m, 4 H) 5.30 (s, 2 H) 6.34 (d, *J*=8.78 Hz, 1 H) 7.09 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.54 (d, *J*=2.93 Hz, 1 H). LCMS (ESI) 178 (M + H).

Example 32

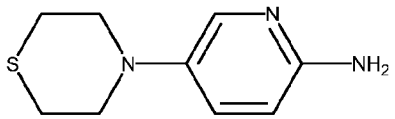
Synthesis of 4-(6-nitro-3-pyridyl) thiomorpholine, Compound 32



4-(6-nitro-3-pyridyl) thiomorpholine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 2.56 - 2.69 (m, 4 H) 3.79 - 3.92 (m, 4 H) 7.43 (dd, *J*=9.22, 3.07 Hz, 1 H) 8.10 (d, *J*=9.37 Hz, 1 H) 8.20 (d, *J*=2.93 Hz, 1 H).

Example 33

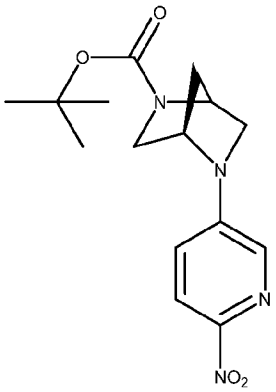
Synthesis of 5-thiomorpholinopyridin-2-amine, Compound 33



5-thiomorpholinopyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl) pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 2.59 - 2.73 (m, 4 H) 3.04 - 3.20 (m, 4 H) 5.41 (s, 2 H) 6.35 (d, *J*=8.78 Hz, 1 H) 7.10 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.57 (d, *J*=2.64 Hz, 1 H). LCMS (ESI) 196 (M + H).

Example 34

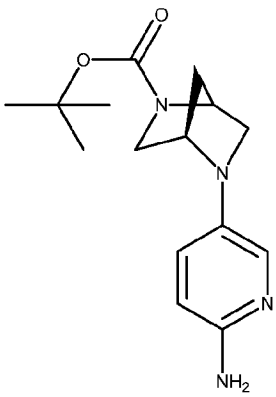
Synthesis of *tert*-butyl (4R)-5-(6-nitro-3-pyridyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, Compound 34



tert-butyl (4R)-5-(6-nitro-3-pyridyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.33 (d, J=32.21 Hz, 11 H) 1.91 (m, 2 H) 3.15 (d, J=10.25 Hz, 1 H) 3.58 (m, 1 H) 4.46 (m, 1 H) 4.83 (s, 1 H) 7.16 (s, 1 H) 7.94 (s, 1 H) 8.05 - 8.16 (m, 1 H).

Example 35

Synthesis of *tert*-butyl (4R)-5-(6-amino-3-pyridyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, Compound 35

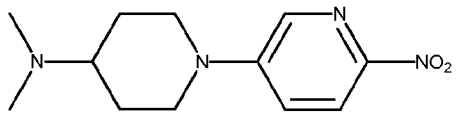


tert-butyl (4R)-5-(6-amino-3-pyridyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.31 (d, J=31.91 Hz, 11 H) 1.83 (m, 2 H) 2.71 -

2.82 (m, 1 H) 3.44 (m,1 H) 4.30 (d, 2H) 5.08 (s, 2 H) 6.35 (d, *J*=8.78 Hz, 1 H) 6.77 - 6.91 (m, 1 H) 7.33 (s, 1 H). LCMS (ESI) 291 (M + H).

Example 36

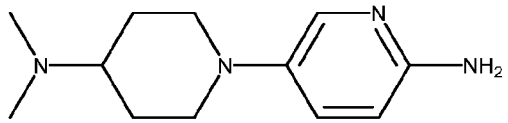
Synthesis of N,N-dimethyl-1-(6-nitro-3-pyridyl) piperidin-4-amine, Compound 36



N,N-dimethyl-1-(6-nitro-3-pyridyl)piperidin-4-amine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.30 - 1.45 (m, 2 H) 1.79 (m, 2 H) 2.14 (s, 6 H) 2.33 (m, 1 H) 2.92 - 3.04 (m, 2 H) 4.03 (d, *J*=13.76 Hz, 2 H) 7.42 (dd, *J*=9.22, 3.07 Hz, 1 H) 8.04 - 8.11 (m, 1 H) 8.21 (d, *J*=2.93 Hz, 1 H).

Example 37

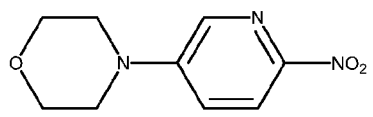
Synthesis of 5-[4-(dimethylamino)-1-piperidyl] pyridin-2-amine, Compound 37



5-[4-(dimethylamino)-1-piperidyl]pyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.35 - 1.50 (m, 2 H) 1.69 - 1.81 (m, 2 H) 2.00 - 2.10 (m, 1 H) 2.11 - 2.22 (s, 6 H) 3.17 - 3.36 (m, 4 H) 5.19 - 5.38 (s, 2 H) 6.34 (d, *J*=8.78 Hz, 1 H) 7.10 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.55 (d, *J*=2.63 Hz, 1 H). LCMS (ESI) 221 (M + H).

Example 38

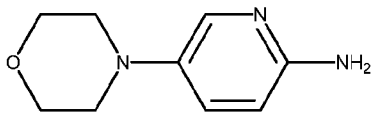
Synthesis of 4-(6-nitro-3-pyridyl) morpholine, Compound 38



4-(6-nitro-3-pyridyl) morpholine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl] pyridine.

Example 39

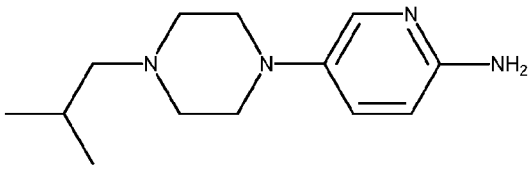
Synthesis of 5-morpholinopyridin-2-amine, Compound 39



5-morpholinopyridin-2-amine was prepared in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl) pyridin-2-amine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 2.91 - 3.00 (m, 4 H) 3.76 - 3.84 (m, 4 H) 4.19 (br. s., 2 H) 6.45 (d, *J*=8.78 Hz, 1 H) 7.12 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.72 (d, *J*=2.93 Hz, 1 H).

Example 40

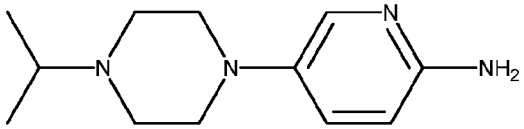
Synthesis of 5-(4-isobutylpiperazin-1-yl) pyridin-2-amine, Compound 40



1-isobutyl-4-(6-nitro-3-pyridyl)piperazine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine which was then converted 5-(4-isobutylpiperazin-1-yl)pyridin-2-amine in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.88 (d, *J*=6.73 Hz, 6 H) 1.71 - 1.84 (m, 1 H) 2.10 (d, *J*=7.32 Hz, 2 H) 2.46 - 2.58 (m, 4 H) 2.97 - 3.07 (m, 4 H) 4.12 (s, 2 H) 6.45 (d, *J*=8.78 Hz, 1 H) 7.14 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.75 (d, *J*=2.93 Hz, 1 H). LCMS (ESI) 235 (M + H).

Example 41

Synthesis of 5-(4-isopropylpiperazin-1-yl) pyridin-2-amine, Compound 41

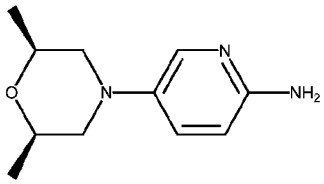


1-isopropyl-4-(6-nitro-3-pyridyl)piperazine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine which was then converted to 5-(4-isopropylpiperazin-1-yl)pyridin-2-amine in a manner similar to that used in the synthesis

of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 1.06 (d, *J*=6.44 Hz, 6 H) 2.59 - 2.75 (m, 5 H) 2.97 - 3.10 (m, 4 H) 4.13 (s, 2 H) 6.45 (d, *J*=8.78 Hz, 1 H) 7.15 (dd, *J*=9.08, 2.93 Hz, 1 H) 7.76 (d, *J*=2.93 Hz, 1 H). LCMS (ESI) 221 (M + H).

Example 42

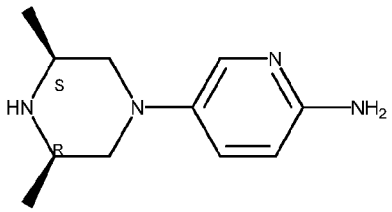
Synthesis of 5-[(2R,6S)-2,6-dimethylmorpholin-4-yl]pyridin-2-amine, Compound 42



(2S,6R)-2,6-dimethyl-4-(6-nitro-3-pyridyl)morpholine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine which was then converted to 5-[(2R,6S)-2,6-dimethylmorpholin-4-yl]pyridin-2-amine in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 1.20 (d, *J*=6.44 Hz, 6 H) 2.27 - 2.39 (m, 2 H) 3.11 - 3.21 (m, 2 H) 3.70 - 3.84 (m, 2 H) 4.15 (s, 2 H) 6.45 (d, *J*=8.78 Hz, 1 H) 7.12 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.72 (d, *J*=2.63 Hz, 1 H). LCMS (ESI) 208 (M + H).

Example 43

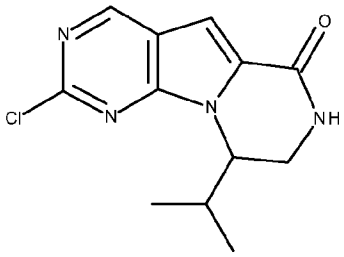
Synthesis of 5-[(3R,5S)-3,5-dimethylpiperazin-1-yl]pyridin-2-amine, Compound 43



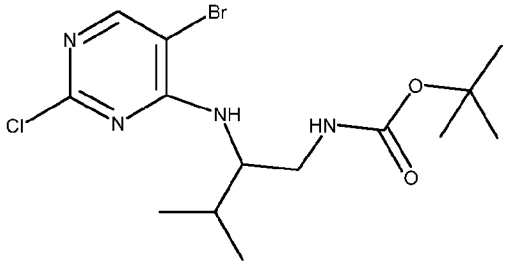
(3S,5R)-3,5-dimethyl-1-(6-nitro-3-pyridyl)piperazine was synthesized in a manner similar to that used in the synthesis of 2-nitro-5-[4-(1-piperidyl)-1-piperidyl]pyridine which was then converted to 5-[(3R,5S)-3,5-dimethylpiperazin-1-yl]pyridin-2-amine in a manner similar to that used in the synthesis of 5-(4-methylpiperazin-1-yl)pyridin-2-amine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 1.09 (d, *J*=6.44 Hz, 6 H) 2.20 (t, *J*=10.83 Hz, 2 H) 2.95 - 3.08 (m, 2 H) 3.23 (dd, *J*=11.71, 2.05 Hz, 2 H) 4.13 (s, 2 H) 6.45 (d, *J*=8.78 Hz, 1 H) 7.14 (dd, *J*=8.78, 2.93 Hz, 1 H) 7.73 (d, *J*=2.63 Hz, 1 H). LCMS (ESI) 207 (M + H).

Example 44

Synthesis of Compound 44



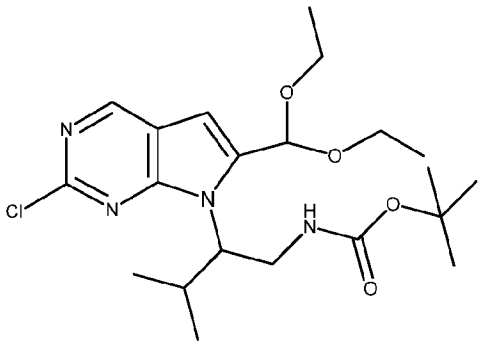
tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl] carbamate



A solution of intermediate A in ethanol (100 mL) was hydrogenated under 30 psi of hydrogen using 10% Pd/C (0.7 g) in a pressure bomb for 7 hrs. After filtration of the reaction mixture through CELITE™, the organic layer was concentrated under vacuum to afford *tert*-butyl N-(2-amino-3-methyl-butyl) carbamate (3.8 g).

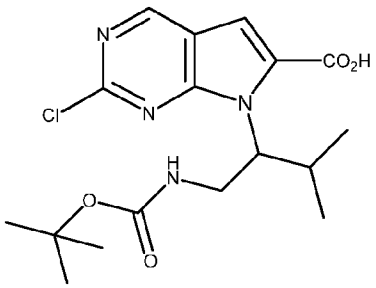
To a solution of 5-bromo-2,4-dichloro-pyrimidine (7.11 g, 0.0312 mole) in ethanol (100 mL) was added diisopropylethyl amine (5.45 mL, 1.0 eq) and *tert*-butyl N-(2-amino-3-methyl-butyl) carbamate (6.31 g, 0.0312 mole). The reaction mixture was stirred at room temperature for 20 hrs. After concentration under vacuum, ethyl acetate and water were added. The organic layer was separated, dried with magnesium sulfate and then concentrated under vacuum. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (0-30%) to afford *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl] carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.77 - 0.85 (d, J=6.5 Hz, 3 H) 0.87 (d, J=6.73 Hz, 3 H) 1.31 - 1.39 (m, 9 H) 1.82 - 1.93 (m, 1 H) 2.94 (d, J=5.56 Hz, 1 H) 3.08 - 3.22 (m, 2 H) 3.98 (d, J=8.20 Hz, 1 H) 6.96 (d, J=8.78 Hz, 1 H) 8.21 (s, 1 H). LCMS (ESI) 393 (M + H).

tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]-3-methyl-butyl]carbamate



tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]-3-methyl-butyl]carbamate was synthesized by hosting *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate to Sonogoshira conditions as described for *tert*-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate followed by subsequent treatment with TBAF as described in the synthesis of *tert*-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.11 (d, *J*=6.44 Hz, 3 H) 1.18 (t, *J*=7.03 Hz, 6 H) 1.21 - 1.26 (m, 12 H) 2.88 (br. s., 1 H) 3.43 - 3.78 (m, 6 H) 3.97 - 4.08 (m, 1 H) 5.61 (s, 1 H) 6.65 (s, 1 H) 6.71 - 6.78 (m, 1 H) 8.87 (s, 1 H). LCMS (ESI) 441 (M + H).

7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



To a solution *tert*-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate in THF was added TBAF and the contents were heated at reflux for 3 hrs. Ethyl acetate and water were then added and the organic layer separated, dried with magnesium sulfate and then concentrated under vacuum. To this crude reaction was added acetic acid/water (9:1) and the contents were stirred for 12 hrs at room temperature. After

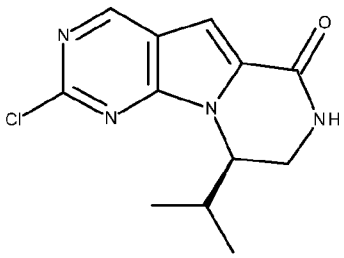
concentration under vacuum, sat $NaHCO_3$ and ethyl acetate were added. The organic layer was separated, dried and then concentrated under vacuum. The crude reaction product thus obtained was dissolved in DMF, oxone was then added and the contents stirred for 3 hrs. After addition of ethyl acetate, the reaction mixture was filtered through CELITE™ and concentrated under vacuum. Column chromatography of the crude product over silica gel using hexane/ethyl acetate (0- 100%) afforded 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.85 (d, *J*=7.03 Hz, 3 H) 0.97 (d, *J*=6.73 Hz, 3 H) 1.52 (s, 9 H) 1.99 - 2.23 (m, 1 H) 3.98 (dd, *J*=14.05, 3.51 Hz, 1 H) 4.47 - 4.71 (m, 2 H) 7.47 (s, 1 H) 9.17 (s, 1 H). LCMS (ESI) 383 (M + H).

Compound 44

To 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (0.050 g, 0.00013 mole) in DCM (1.5 mL) was added DIC (32.7 mg) and DMAP (10 mg). The contents were stirred for 2 hrs. Trifluoroacetic acid (0.4 mL) was then added and stirring continued for an additional 30 minutes. After addition of satd $NaHCO_3$ to neutralize the excess acid, ethyl acetate was added and the organic layer separated, dried using magnesium sulfate and then concentrated under vacuum. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (0- 100%) to afford the product . ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.72 (d, *J*=6.73 Hz, 3 H) 0.97 (d, *J*=6.73 Hz, 3 H) 2.09 - 2.22 (m, 1 H) 3.57 (dd, *J*=13.18, 4.98 Hz, 1 H) 3.72 (dd, *J*=13.61, 4.25 Hz, 1 H) 4.53 (dd, *J*=8.05, 3.95 Hz, 1 H) 7.20 (s, 1 H) 8.34 (d, *J*=4.98 Hz, 1 H) 9.08 (s, 1 H). LCMS (ESI) 265 (M + H).

Example 45

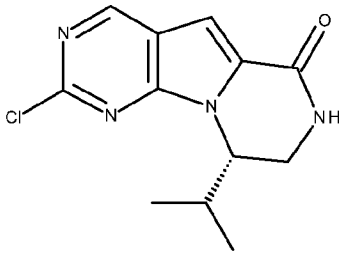
Synthesis of Compound 45



Compound 14 was hydrogenated with 10% Pd/C to afford the intermediate *tert*-butyl N-[(2R)-2-amino-3-methyl-butyl] carbamate, which was then treated with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for Compound 44 to afford Compound 45 The analytical data is consistent with that reported for the racemate (Intermediate 1A).

Example 46

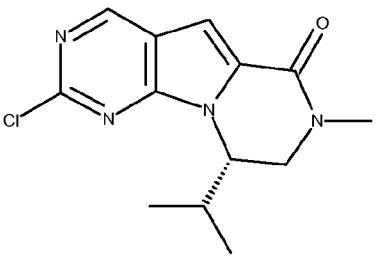
Synthesis of Compound 46



Compound 15 was hydrogenated with 10% Pd/C to afford the intermediate *tert*-butyl N-[(2S)-2-amino-3-methyl-butyl]carbamate, which was then treated with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for Compound 44 to afford Compound 46. The analytical data (NMR and LCMS) was consistent with that reported for the racemate Compound 44.

Example 47

Synthesis of Compound 47

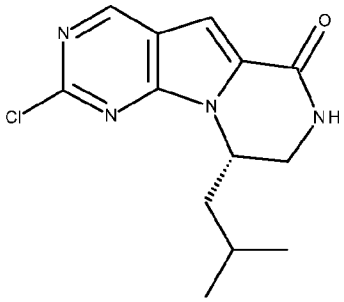


To a solution of Compound 44 (80 mg, 0.00030 mole) in DMF (3 mL) was added a 60% dispersion of sodium hydride in oil (40 mg). After stirring for 15 minutes, methyl iodide (37 μL, 2eq) was added. The contents were stirred at room temperature for 30 minutes. Saturated

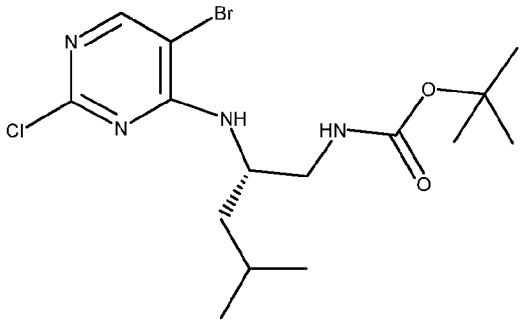
$NaHCO_3$ was then added followed by ethyl acetate. The organic layer was dried with magnesium sulfate and then concentrated under vacuum to afford the product . ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.74 (d, *J*=6.73 Hz, 3 H) 0.91 (d, *J*=6.73 Hz, 3 H) 2.04 - 2.20 (m, 1 H) 3.04 (s, 3 H) 3.69 (dd, *J*=13.76, 1.17 Hz, 1 H) 3.96 (dd, *J*=13.76, 4.68 Hz, 1 H) 4.58 (dd, *J*=7.32, 3.51 Hz, 1 H) 7.16 (s, 1 H) 9.05 (s, 1 H). LCMS (ESI) 279 (M + H).

Example 48

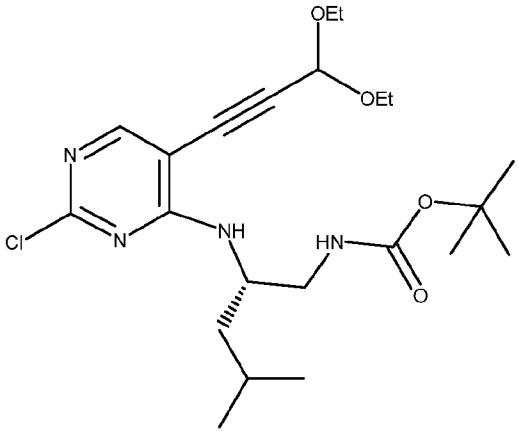
Synthesis of Compound 48



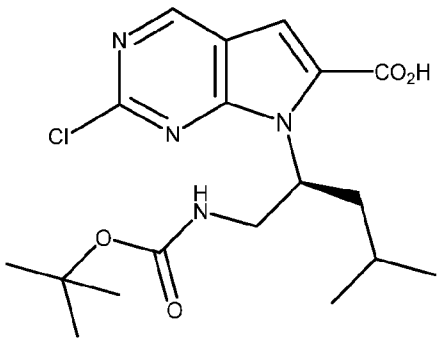
tert-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-4-methyl-pentyl]carbamate



Compound 18 was hydrogenated with 10% Pd/C in ethanol under a blanket of hydrogen at 50 psi in a pressure bomb to afford *tert*-butyl N-[(2S)-2-amino-4-methyl-pentyl]carbamate which was then reacted with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate to afford *tert*-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-4-methyl-pentyl]carbamate. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.91 (d, *J*=6.44 Hz, 3 H) 0.94 (d, *J*=6.44 Hz, 3 H) 1.32 - 1.51 (m, 11 H) 1.55 - 1.67 (m, 1 H) 3.28 (t, *J*=5.86 Hz, 2 H) 4.21 - 4.42 (m, 1 H) 4.84 (s, 1 H) 5.84 (d, *J*=7.32 Hz, 1 H) 8.07 (s, 1 H). LCMS (ESI) 407 (M + H).



To a solution of *tert*-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-4-methyl-pentyl]carbamate (5.0 g, 12.3 mmole) in tolune (36 mL) and triethylamine (7.2 mL) was added under nitrogen, 3,3-diethoxyprop-1-yne (2.8 mL, 19.7 mmole), $Pd_2(dba)_3$ (1.1g, 1.23 mmole), and triphenylarsine (3.8 g, 12.3 mmole). The contents were heated to 70 degrees for 24 hrs. After cooling to room temperature, the reaction mixture was filtered through CELITE™ and then concentrated under vacuum. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (0- 30%) to afford (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. LCMS (ESI) 455 (M + H).

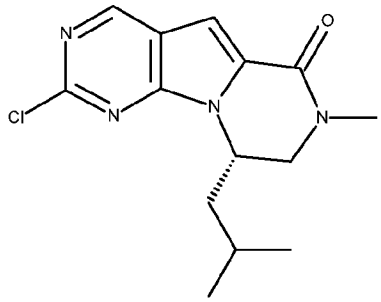


7-[(1S)-1-[(*tert*-butoxycarbonylamino)methyl]-3-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.88 (d, *J*=6.44 Hz, 3 H) 0.97 (d, *J*=6.44 Hz, 3 H) 1.47 (s, 9 H) 1.49 - 1.54 (m, 1 H) 1.56 (t, *J*=7.17 Hz, 2 H) 3.98 (dd, *J*=13.91, 3.07 Hz, 1 H) 3.76 (dd, *J*=13.31, 4.13 Hz, 1 H) 4.38 (d, *J*=14.05 Hz, 1 H) 4.90 (t, *J*=7.17 Hz, 1 H) 7.41 (s, 1 H) 9.11 (s, 1 H). LCMS (M + H) 397.

Compound 48 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.82 (d, J=6.73 Hz, 3 H) 0.97 (d, J=6.44 Hz, 3 H) 1.34 - 1.46 (m, 1 H) 1.48 - 1.65 (m, 2 H) 3.40 (dd, J=13.32, 5.42 Hz, 1 H) 3.76 (dd, J=13.47, 4.10 Hz, 1 H) 4.76 - 4.92 (m, 1 H) 7.17 (s, 1 H) 8.34 (d, J=5.27 Hz, 1 H) 9.04 (s, 1 H). LCMS (ESI) 279 (M + H).

Example 49

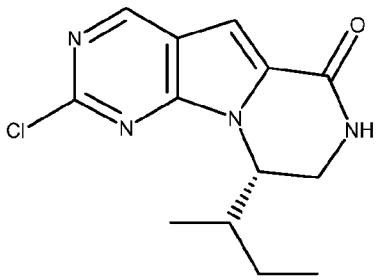
Synthesis of Compound 49



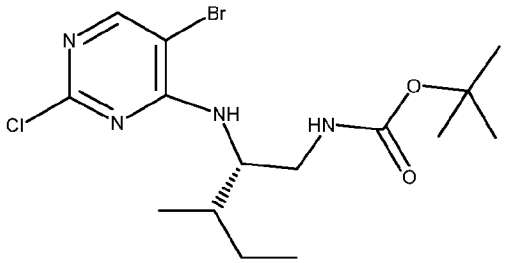
Compound 49 was synthesized in a manner similar to that described for Compound 47. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.82 (d, J=6.44 Hz, 3 H) 0.97 (d, J=6.44 Hz, 3 H) 1.37 - 1.68 (m, 3 H) 3.04 (s, 3 H) 3.56 (d, J=13.47 Hz, 1 H) 4.00 (dd, J=13.32, 4.25 Hz, 1 H) 4.82 - 4.94 (m, 1 H) 7.16 (s, 1 H) 9.03 (s, 1 H). LCMS (ESI) 293 (M + H).

Example 50

Synthesis of Compound 50

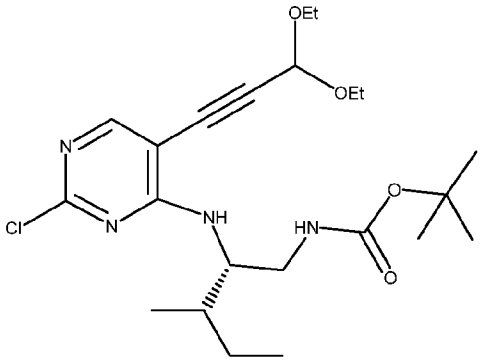


tert-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-pentyl]carbamate



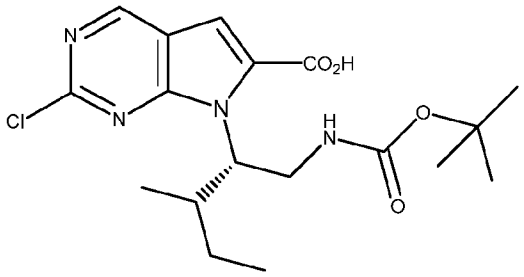
Compound 20 was hydrogenated using 10% Pd/C under hydrogen at 50 psi in a pressure vessel to afford *tert*-butyl N-[(2S)-2-amino-3-methyl-pentyl]carbamate which was reacted with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate to afford *tert*-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-pentyl]carbamate. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.88 - 0.95 (m, 6 H) 1.11 - 1.20 (m, 1 H) 1.34 (s, 9 H) 1.44 - 1.54 (m, 1 H) 1.64 - 1.72 (m, 1 H) 3.17 - 3.27 (m, 1 H) 3.33 - 3.43 (m, 1 H) 4.11 - 4.21 (m, 1 H) 4.81 (s, 1 H) 5.92 (d, *J*=8.20 Hz, 1 H) 8.05 (s, 1 H). LCMS (ESI) 407.

tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-3-methyl-pentyl]carbamate



tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-3-methyl-pentyl]carbamate was synthesized using similar experimental conditions to that used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.76 - 0.89 (m, 6 H) 1.03 (q, *J*=7.22 Hz, 3 H) 1.10 - 1.17 (m, 3 H) 1.25 - 1.42 (m, 11 H) 1.59 - 1.73 (m, 1 H) 3.35 - 3.47 (m, 4 H) 3.51 - 3.73 (m, 2 H) 3.99 - 4.11 (m, 1 H) 5.52 - 5.56 (m, 1 H) 6.76 - 7.03 (m, 2 H) 8.12 - 8.23 (m, 1 H). LCMS (ESI) 455 (M + H).

7-[(1S)-1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid

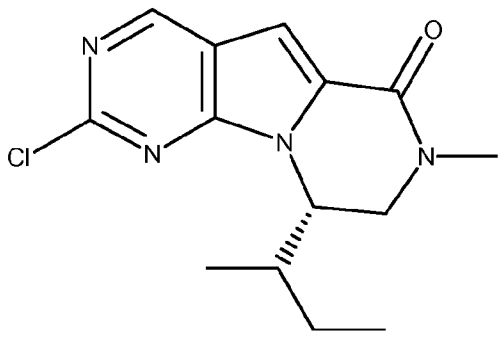


7-[(1S)-1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.80 (t, *J*=7.47 Hz, 3 H) 0.86 (d, *J*=7.03 Hz, 3 H) 1.06 - 1.30 (m, 2 H) 1.48 (s, 9 H) 1.79 - 1.96 (m, 1 H) 3.95 (dd, *J*=14.05, 3.22 Hz, 1 H) 4.52 (d, *J*=14.35 Hz, 1 H) 4.61 - 4.73 (m, 1 H) 7.43 (s, 1 H) 9.13 (s, 1 H). LCMS (ESI) 397 (M + H).

Compound 50 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.74 (t, *J*=7.32 Hz, 3 H) 0.89 (d, *J*=6.73 Hz, 3 H) 1.00 - 1.12 (m, 2 H) 1.82 - 1.94 (m, 1 H) 3.55 (dd, *J*=13.91, 4.83 Hz, 1 H) 3.70 (dd, *J*=13.61, 4.25 Hz, 1 H) 4.57 (dd, *J*=7.91, 4.10 Hz, 1 H) 7.17 (s, 1 H) 8.31 (d, *J*=5.27 Hz, 1 H) 9.05 (s, 1 H). LCMS (ESI) 279 (M + H).

Example 51

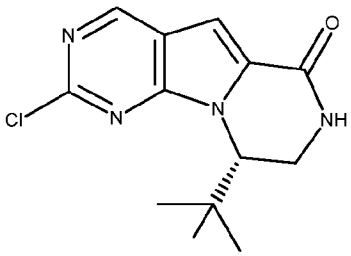
Synthesis of Compound 51



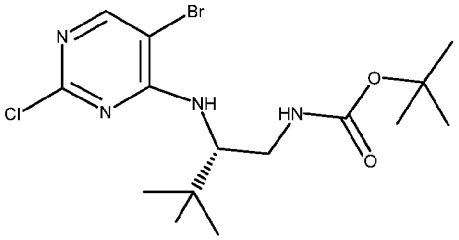
Compound 51 was synthesized in a manner similar to Compound 47. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.77 (t, *J*=7.47 Hz, 3 H) 0.84 (d, *J*=6.73 Hz, 3 H) 1.07 - 1.16 (m, 2 H) 1.82 - 1.95 (m, 1 H) 3.03 (s, 3 H) 3.68 (d, *J*=13.76 Hz, 1 H) 3.96 (dd, *J*=13.76, 4.39 Hz, 1 H) 4.59 - 4.70 (m, 1 H) 7.16 (s, 1 H) 9.04 (s, 1 H). LCMS (ESI) 293 (M + H).

Example 52

Synthesis of Compound 52

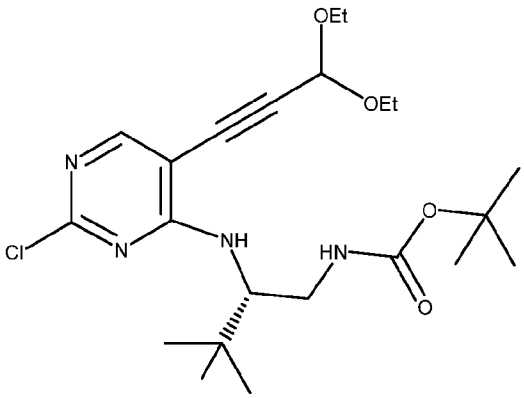


tert-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3,3-dimethyl-butyl] carbamate



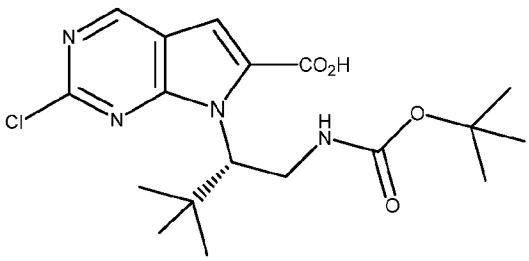
Compound 21 was hydrogenated using 10% Pd/C under hydrogen at 50 psi in a pressure vessel to afford *tert*-butyl N-[(2S)-2-amino-3,3-dimethyl-butyl]carbamate which was then reacted with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate to afford *tert*-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3,3-dimethyl-butyl]carbamate. LCMS (ESI) 407 (M + H).

tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-3,3-dimethyl-butyl] carbamate



tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-3,3-dimethyl-butyl]carbamate was synthesized using similar experimental conditions to that used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. LCMS (ESI) 455 (M + H).

7-[(1S)-1-[(*tert*-butoxycarbonylamino)methyl]-2,2-dimethyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid

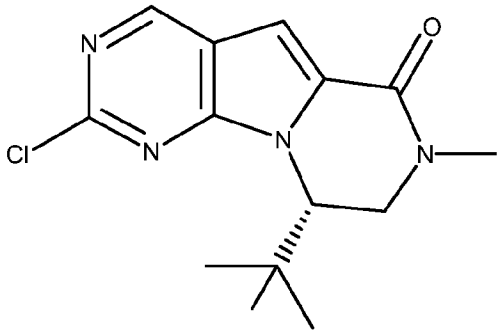


7-[(1S)-1-[(*tert*-butoxycarbonylamino)methyl]-2,2-dimethyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 397 (M + H).

Intermediate 1F was synthesized using an analogous synthetic sequence as that described for intermediate 1A. LCMS (ESI) 279 (M + H).

Example 53

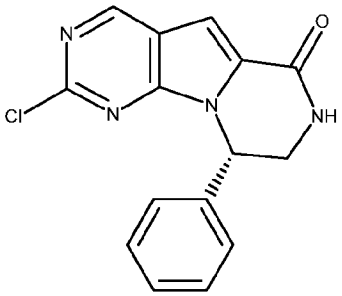
Synthesis of Compound 53



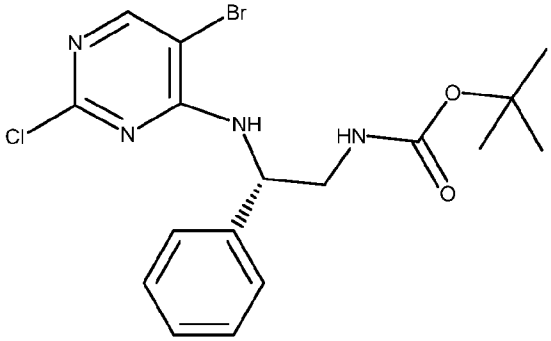
Compound 53 was synthesized in a manner similar to that described for Intermediate 1CA. LCMS (ESI) 293 (M + H).

Example 54

Synthesis of Compound 54



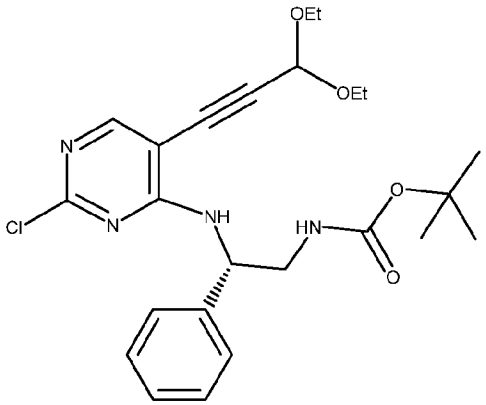
tert-butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-phenyl-ethyl] carbamate



Compound 21 was hydrogenated using 10% Pd/C under hydrogen at 50 psi in a pressure vessel to afford *tert*-butyl N-[(2S)-2-amino-2-phenyl-ethyl]carbamate which was then reacted with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate to afford *tert*-

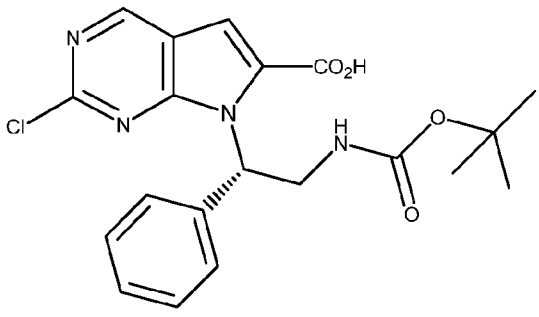
butyl N-[(2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-phenyl-ethyl]carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.32 (s, 9 H) 3.29 - 3.50 (m, 2 H) 5.12 - 5.24 (m, 1 H) 7.10 (t, *J*=5.27 Hz, 1 H) 7.21 (t, *J*=6.88 Hz, 1 H) 7.26 - 7.34 (m, 4 H) 7.89 (d, *J*=7.32 Hz, 1 H) 8.24 (s, 1 H). LCMS (ESI) 427 (M + H).

tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-phenyl-ethyl]carbamate



tert-butyl N-[(2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-phenyl-ethyl]carbamate was synthesized using similar experimental conditions to those used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.14 (t, *J*=7.03 Hz, 6 H) 1.32 (s, 9 H) 3.39 (s, 2 H) 3.52 - 3.61 (m, 2 H) 3.64 - 3.73 (m, 2 H) 5.17 - 5.26 (m, 1 H) 5.57 (s, 1 H) 7.07 - 7.14 (m, 1 H) 7.20 - 7.25 (m, 1 H) 7.26 - 7.33 (m, 4 H) 7.90 (d, *J*=7.61 Hz, 1 H) 8.19 (s, 1 H). LCMS (ESI) 475 (M + H).

7-[(1S)-2-(*tert*-butoxycarbonylamino)-1-phenyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



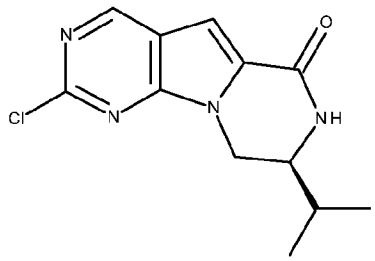
7-[(1S)-2-(*tert*-butoxycarbonylamino)-1-phenyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 417 (M + H).

Compound 54

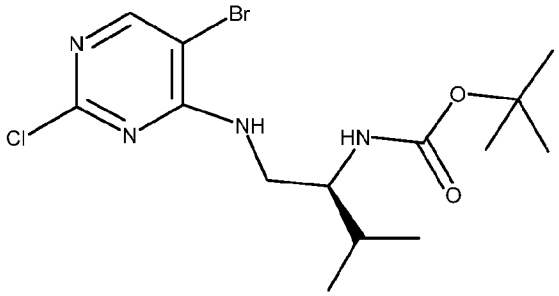
Compound 54 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 3.58 - 3.69 (m, 1 H) 4.13 (dd, *J*=13.47, 4.39 Hz, 1 H) 6.07 (d, *J*=3.81 Hz, 1 H) 6.85 (d, *J*=7.32 Hz, 2 H) 7.19 - 7.31 (m, 3 H) 7.34 (s, 1 H) 8.27 (d, *J*=5.27 Hz, 1 H) 9.13 (s, 1 H). LCMS (ESI) 299 (M + H).

Example 55

Synthesis of Compound 55



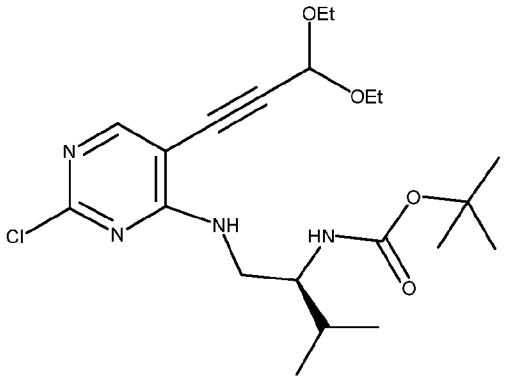
tert-butyl N-[(1S)-1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]-2-methyl-propyl] carbamate



tert-butyl N-[(1S)-1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]-2-methyl-propyl]carbamate was synthesized using 5-bromo-2,4-dichloro-pyrimidine and Intermediate E using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ

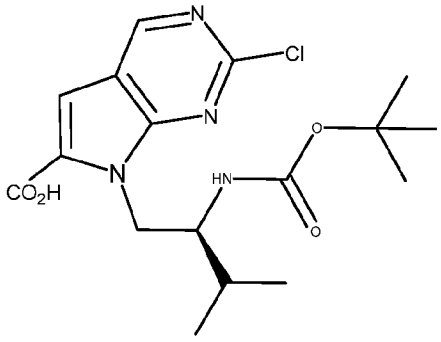
ppm 0.95 - 1.02 (m, 6 H) 1.35 - 1.45 (m, 9 H) 1.75 - 1.90 (m, 1 H) 3.35 - 3.48 (m, 1 H) 3.52 - 3.61 (m, 1 H) 3.64 - 3.76 (m, 1 H) 4.56 (d, *J*=8.49 Hz, 1 H) 6.47 (s, 1 H) 8.07 (s, 1 H). LCMS (ESI) 393 (M + H).

tert-butyl N-[(1S)-1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]-2-methyl-propyl] carbamate



tert-butyl N-[(1S)-1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]-2-methyl-propyl]carbamate was synthesized using similar experimental conditions to those used in the synthesis (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.90 - 1.00 (m, 6 H) 1.18 - 1.25 (m, 6 H) 1.34 - 1.36 (m, 9 H) 1.69 - 1.90 (m, 1 H) 3.34 - 3.82 (m, 6 H) 4.53 - 4.77 (m, 1 H) 5.45 - 5.55 (m, 1 H) 6.37 (dd, *J*=15.37, 6.59 Hz, 1 H) 6.56 (s, 1 H) 8.05 (s, 1 H). LCMS (ESI) 441 (M + H).

7-[(2S)-2-(*tert*-butoxycarbonylamino)-3-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



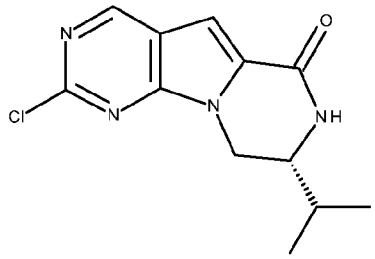
7-[(2S)-2-(*tert*-butoxycarbonylamino)-3-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 0.90 (d, *J*=6.73 Hz, 3 H) 0.96 (d, *J*=7.03 Hz, 3 H) 1.55 - 1.66 (m, 10 H) 4.14 (dd, *J*=13.61, 3.95 Hz, 1 H) 4.52 - 4.63 (m, 1 H) 4.84 (dd, *J*=13.61, 1.32 Hz, 1 H) 7.37 (s, 1 H) 8.95 (s, 1 H). LCMS (ESI) 383 (M + H).

Compound 55

Compound 55 was synthesized using an analogous synthetic sequence as that described for Compound 44. LCMS (ESI) 265 (M + H).

Example 56

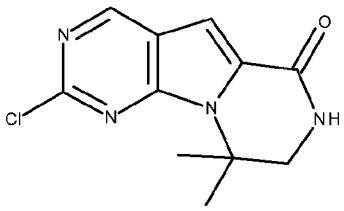
Synthesis of Compound 56



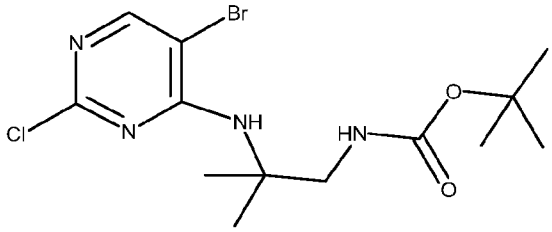
Compound 56 was synthesized using 5-bromo-2,4-dichloro-pyrimidine and Compound 17 as starting materials, and following a similar sequence of synthetic steps as for Compound 55. The analytical data was consistent with that described for its antipode (Compound 55). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.88 (d, *J*=6.44 Hz, 6 H) 1.73 - 1.86 (m, 1 H) 3.67 - 3.76 (m, 2 H) 4.11 - 4.21 (m, 1 H) 7.13 - 7.19 (m, 1 H) 8.56 (s, 1 H) 9.05 (s, 1 H). LCMS (ESI) 265 (M + H).

Example 57

Synthesis of Compound 57

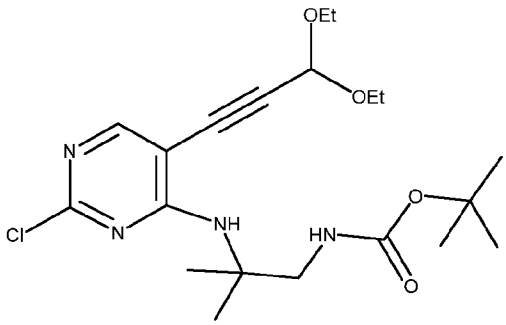


tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-methyl-propyl]carbamate



tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-methyl-propyl]carbamate was synthesized using 5-bromo-2,4-dichloro-pyrimidine and *tert*-butyl N-(2-amino-2-methyl-propyl)carbamate using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate. LCMS (ESI) 379 (M + H).

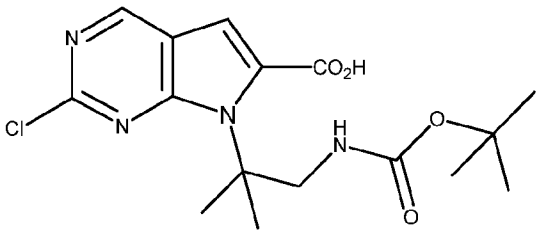
tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate



tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate was synthesized using similar experimental conditions to those used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.11 - 1.22 (m, 6 H) 1.31 - 1.45 (m, 15 H)

3.10 - 3.24 (m, 2 H) 3.51 - 3.76 (m, 4 H) 5.60 (s, 1 H) 6.94 (s, 1 H) 7.33 (t, *J*=6.44 Hz, 1 H) 8.18 (s, 1 H). LCMS (ESI) 427 (M + H).

7-[2-(*tert*-butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



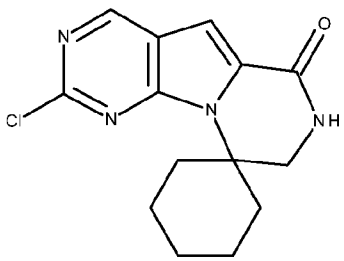
7-[2-(*tert*-butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.43 (s, 9H) 1.73 (s, 6 H) 4.06 (s, 2 H) 7.46 (s, 1 H) 9.23 (s, 1H). LCMS (ESI) 369 (M + H).

Compound 57

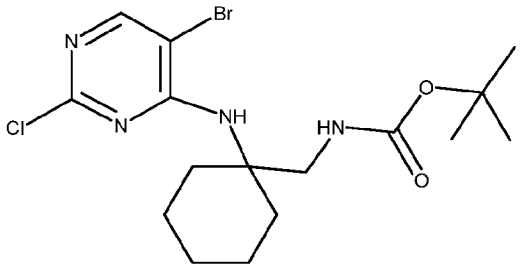
Compound 57 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.73 (s, 6 H) 3.50 (d, *J*=2.93 Hz, 2 H) 7.25 (s, 1 H) 8.46 - 8.55 (m, 1 H) 9.07 (s, 1 H). LCMS (ESI) 251 (M + H).

Example 58

Synthesis of Compound 58

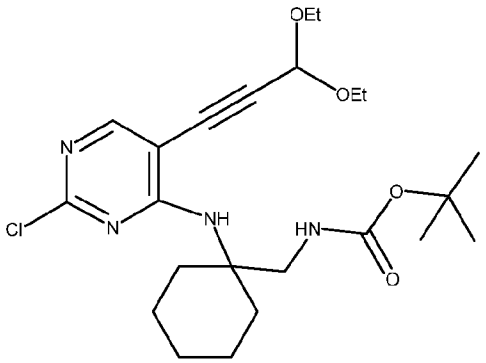


tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclohexyl]methyl]carbamate



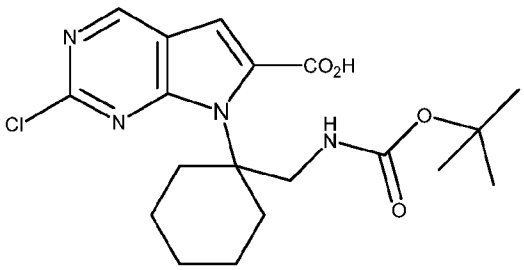
tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclohexyl]methyl] carbamate was synthesized using 5-bromo-2,4-dichloro-pyrimidine and Intermediate K using the analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl] carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.18 - 1.54 (m, 17 H) 2.23 (d, *J*=14.35 Hz, 2 H) 3.36 (d, *J*=6.44 Hz, 2 H) 5.82 (s, 1 H) 6.93 (s, 1 H) 8.22 (s, 1 H). LCMS (ESI) 419 (M + H).

tert-butyl N-[[1-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclohexyl] methyl] carbamate



tert-butyl N-[[1-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclohexyl]methyl] carbamate was synthesized using similar experimental conditions to those used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.08 - 1.16 (m, 6 H) 1.17 - 1.54 (m, 17 H) 2.13 (br. s., 2 H) 3.36 (d, *J*=6.73 Hz, 2 H) 3.50 - 3.69 (m, 4 H) 5.72 (s, 1 H) 6.94 (s, 1 H) 5.72 (br. s., 1H) 8.17 (s, 1 H). LCMS (ESI) 467 (M + H).

7-[1-[(*tert*-butoxycarbonylamino)methyl]cyclohexyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



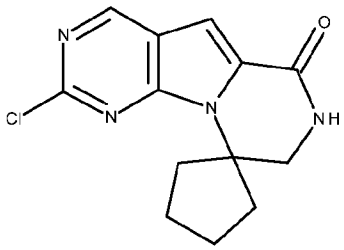
7-[1-[(*tert*-butoxycarbonylamino)methyl]cyclohexyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.37 - 1.54 (m, 13 H) 1.75 (br. s., 4 H) 2.74 (br. s., 2 H) 3.78 - 3.84 (m, 2 H) 7.44 - 7.51 (m, 1 H) 8.23 (s, 1 H) 9.11 (s, 1 H). LCMS (ESI) 409 (M + H).

Compound 58

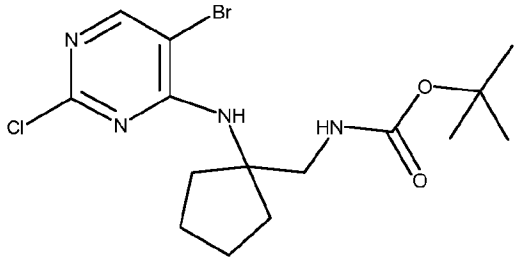
Compound 58 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.28 (br. s., 2 H) 1.42 (br. s., 2 H) 1.70 (br. s., 4 H) 1.85 - 1.95 (m, 2 H) 2.69 (m, 2 H) 7.16 - 7.25 (m, 1 H) 8.41 (br. s., 1 H) 9.04 (s, 1 H). LCMS 291 (M + H).

Example 59

Synthesis of Compound 59

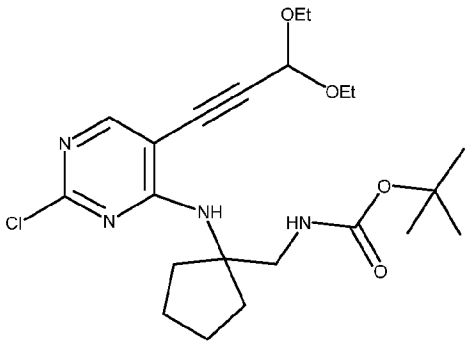


tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl]methyl] carbamate



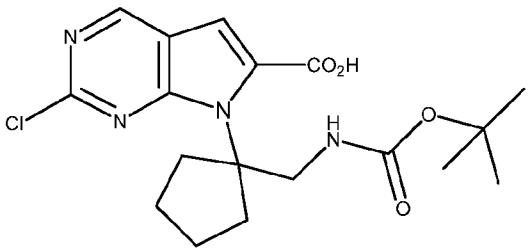
tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl] methyl] carbamate was synthesized using 5-bromo-2,4-dichloro-pyrimidine and Intermediate L using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.34 (s, 9 H) 1.50 - 1.58 (m, 2 H) 1.63 - 1.78 (m, 4 H) 1.96 - 2.06 (m, 2 H) 3.25 (d, *J*=6.15 Hz, 2 H) 6.71 (s, 1 H) 7.18 (t, *J*=6.29 Hz, 1 H) 8.20 (s, 1 H). LCMS (ESI) 405 (M + H).

tert-butyl N-[[1-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclopentyl] methyl] carbamate



tert-butyl N-[[1-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclopentyl]methyl]carbamate was synthesized using similar experimental conditions to that used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. LCMS (ESI) 453 (M + H).

7-[1-[(*tert*-butoxycarbonylamino)methyl]cyclopentyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



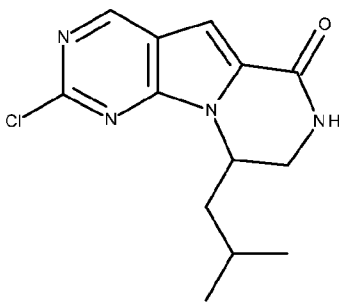
7-[1-[(*tert*-butoxycarbonylamino)methyl]cyclopentyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.47 (s, 9 H) 1.74 (br. s., 2 H) 1.88 (br. s., 2 H) 2.04 (br. s., 2 H) 2.41 - 2.45 (m, 2 H) 4.06 (s, 2 H) 7.45 (s, 1 H) 9.11 (s, 1 H). LCMS (ESI) 395 (M + H).

Compound 59

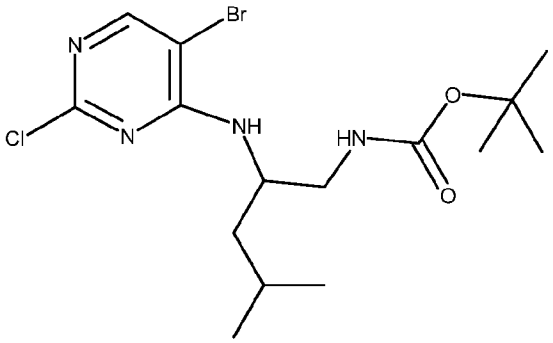
Compound 59 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.72 (br. s., 2 H) 1.86 - 1.93 (m, 2 H) 1.99 (d, *J*=3.81 Hz, 2 H) 2.40 (br. s., 2 H) 3.48 (d, *J*=2.34 Hz, 2 H) 7.22 (s, 1 H) 8.53 (br. s., 1 H) 9.05 (s, 1 H). LCMS (ESI) 277 (M + H).

Example 60

Synthesis of Compound 60

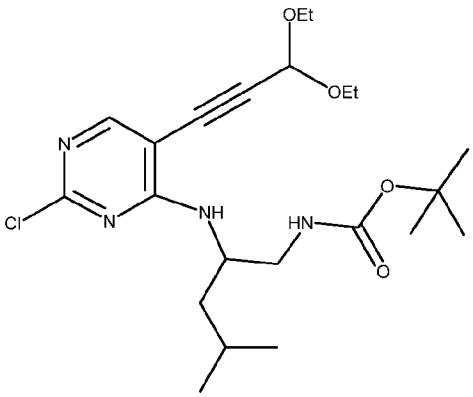


tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-4-methyl-pentyl] carbamate



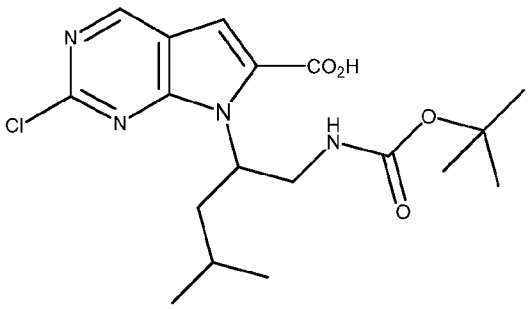
tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-4-methyl-pentyl]carbamate was synthesized using 5-bromo-2,4-dichloro-pyrimidine and Intermediate B using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate. The analytical data is consistent with that described for the L-enantiomer.

tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-4-methyl-pentyl] carbamate



tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-4-methyl-pentyl]carbamate was synthesized using similar experimental conditions to that used in the synthesis of *tert*-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate. ^{1}HNMR (600 MHz, CHLOROFORM-*d*) δ ppm 1.21 – 1.31 (m, 12 H) 1.38 – 1.46 (m, 11 H) 1.70 (m, 1H) 3.24 (m, 2 H) 3.65 - 3.82 (m, 4 H) 4.86 (br s., 1H), 5.65 (s, 1 H) 5.85 (br s., 1H) 6.94 (s, 1 H) 8.21 (s, 1 H). LCMS (ESI) 455 (M + H).

7-[1-[(*tert*-butoxycarbonylamino)methyl]-3-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



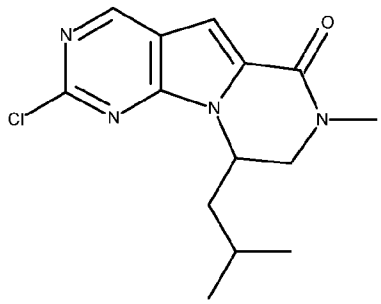
7-[1-[(*tert*-butoxycarbonylamino)methyl]-3-methyl-butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. The analytical data was consistent with that described for the L-isomer.

Compound 60

Compound 60 was synthesized using an analogous synthetic sequence as that described for Compound 44. The analytical data was consistent with that described for the L-isomer.

Example 61

Synthesis of Compound 61

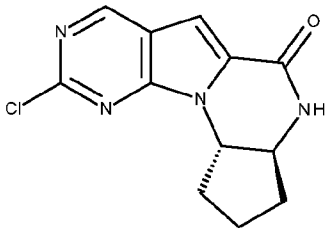


To a solution of Compound 60 (100 mg, 0.00024 mole) in DMF (3.0 mL) was added sodium hydride (60 % dispersion in oil), (27.6 mg, 3 eq). After stirring for 15 mins, methyl iodide (30, 2 eq) was added. The contents were stirred at room temperature for 30 mins. After the addition of sat $NaHCO_3$, ethyl acetate was added. Separation of the organic layer followed

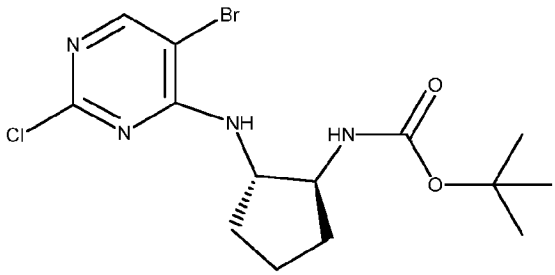
by drying with magnesium sulfate and concentration under vacuum afforded the product. Analytical data was similar to the Compound 49.

Example 62

Synthesis of Compound 62

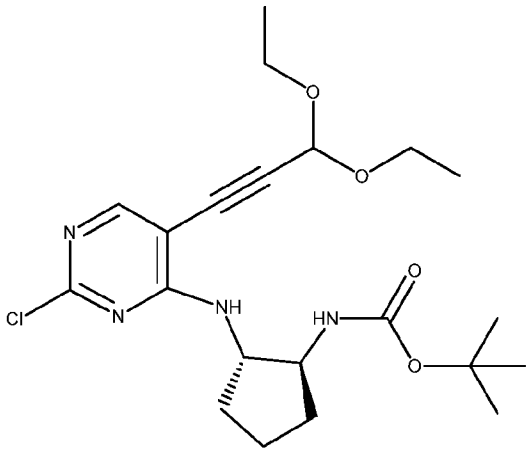


tert-butyl N-[(1S,2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl]carbamate



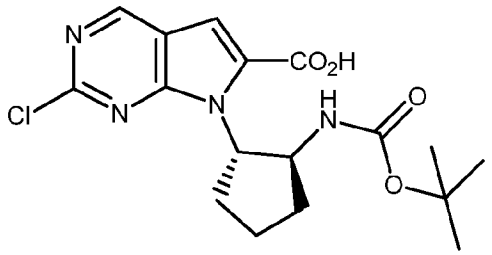
tert-butyl N-[(1S,2S)-2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl]carbamate was synthesized by treating *tert*-butyl N-[(1S,2S)-2-aminocyclopentyl]carbamate with 5-bromo-2,4-dichloro-pyrimidine using analogous reaction conditions as described for *tert*-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-3-methyl-butyl]carbamate. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.27 (s, 9 H) 1.42 - 1.54 (m, 2 H) 1.56 - 1.65 (m, 2 H) 1.80 - 1.88 (m, 1 H) 1.96 - 2.01 (m, 1 H) 3.88 - 3.96 (m, 1 H) 4.03 - 4.09 (m, 1 H) 6.91 (d, *J*=8.20 Hz, 1 H) 7.41 (d, *J*=7.32 Hz, 1 H) 8.18 (s, 1 H). LCMS (ESI) 391 (M + H).

tert-butyl N-[(1S,2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclopentyl]carbamate



tert-butyl N-[(1S,2S)-2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclopentyl]carbamate was synthesized using similar experimental conditions to that used in the synthesis of (2S)-N2-[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]-4-methyl-pentane-1,2-diamine. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.13 (t, 6 H) 1.28 (s, 9 H) 1.42 - 1.52 (m, 2 H) 1.58 - 1.65 (m, 2 H) 1.81 - 1.90 (m, 1 H) 1.99 - 2.08 (m, 1 H) 3.49 - 3.60 (m, 2 H) 3.63 - 3.71 (m, 2 H) 3.84 - 3.93 (m, 1 H) 3.96 - 4.04 (m, 1 H) 5.53 (s, 1 H) 6.96 (d, *J*=7.90 Hz, 1 H) 7.34 (d, *J*=7.03 Hz, 1 H) 8.14 (s, 1 H). LCMS (ESI) 439 (M + H).

7-[(1S,2S)-2-(*tert*-butoxycarbonylamino)cyclopentyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid



7-[(1S,2S)-2-(*tert*-butoxycarbonylamino)cyclopentyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using the analogous synthetic sequence as that described for 7-[1-[(*tert*-butoxycarbonylamino)methyl]-2-methyl-propyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.41 - 1.52 (m, 9 H) 1.55 - 1.68 (m, 1

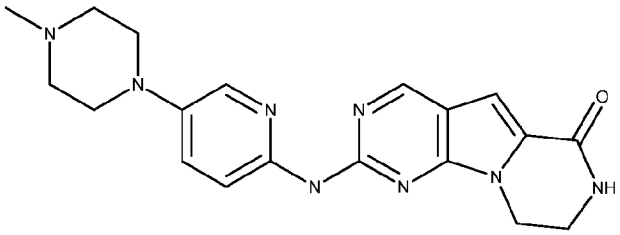
H) 1.88 - 2.00 (m, 2 H) 2.05 - 2.15 (m, 1 H) 2.26 - 2.35 (m, 1 H) 2.71 - 2.89 (m, 1 H) 4.01 - 4.16 (m, 1 H) 4.28 - 4.45 (m, 1 H) 7.41 (s, 1 H) 9.11 (s, 1 H). LCMS (ESI) 381 (M + H).

Compound 62

Compound 62 was synthesized using an analogous synthetic sequence as that described for Compound 44. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.48 - 1.60 (m, 1 H) 1.88 - 1.98 (m, 3 H) 1.99 - 2.08 (m, 1 H) 2.66 - 2.75 (m, 1 H) 3.63 - 3.74 (m, 1 H) 3.99 - 4.12 (m, 1 H) 7.21 (s, 1 H) 8.89 (s, 1 H) 9.04 (s, 1 H). LCMS (ESI) 263 (M + H).

Example 63

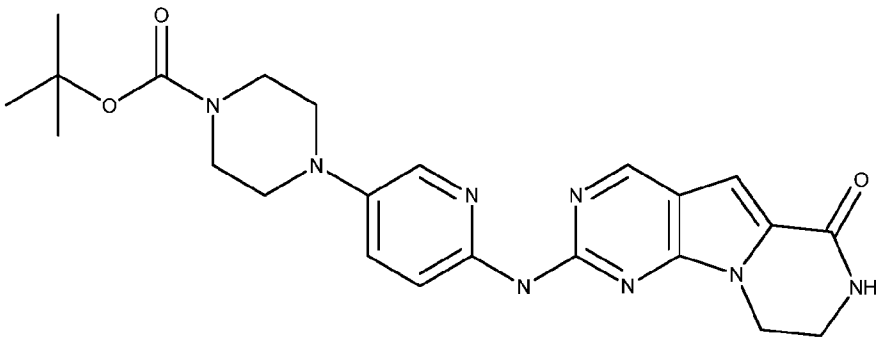
Synthesis of Compound 63



To chloro tricycliclactam (0.050 g, 0.225 mmole) in dioxane (2.0 mL) under nitrogen was added 5-(4-methylpiperazin-1-yl)pyridin-2-amine (0.052 g, 1.2 eq, 0.270 mmole) followed by the addition of $Pd_2(dba)_3$ (18.5 mg), BINAP (25 mg) and sodium-*tert*-butoxide (31 mg , 0.324 mmole). The contents of the flask are degassed for 10 minutes and then heated to 100 degrees for 12 hours. The crude reaction was loaded on a silica gel column and eluted with DCM/MeOH (0- 15%) to afford the desired product (26 mg). To this compound dissolved in DCM/MeOH (10%) was added 3N HCl in iso-propanol (2 eq) and the reaction was stirred overnight. Concentration under vacuum afforded the hydrochloride salt. ^{1}HNMR (d6-DMSO) δ ppm 11.13 (brs, 1H), 9.07 (s, 1H), 8.42 (s, 1H), 8.03 (br m 1H), 7.99 (s, 1H), 7.67 (brm, 1H), 7.18 (s, 1H), 4.33 (m, 2H), 3.79 (m, 2H), 3.64 (m, 2H), 3.50 (m, 2H), 3.16 (m, 4H), 2.79 (s, 3H). LCMS (ESI) 379 (M + H).

Example 64

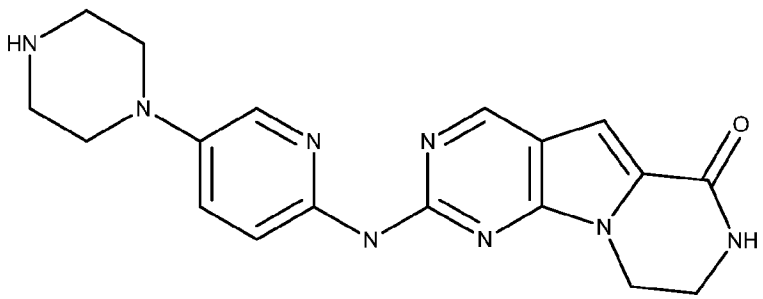
Synthesis of Compound 64



To chloro tricycliclactam (0.075 g, 0.338 mmole) in dioxane (3.5 mL) under nitrogen was added *tert*-butyl 4-(6-amino-3-pyridyl)piperazine-1-carboxylate (0.098 g, 1.05 eq) followed by the addition of $Pd_2(dba)_3$ (27 mg), BINAP (36 mg) and sodium- *tert*-butoxide (45 mg). The contents were heated at reflux for 11 hrs. The crude reaction was loaded onto a silica gel column and eluted with DCM/MeOH (0- 10 %) to afford the desired product (32 mg). ^{1}HNMR (d6-DMSO) δ ppm 9.48 (s, 1H), 8.84 (s, 1H), 8.29 (s, 1H), 8.18 (s, 1H), 7.99 (s, 1H), 7.42 (m, 1H), 6.98 (s, 1H), 4.23 (m, 2H), 3.59 (m, 2H), 3.45 (m, 4H), 3.50 (m, 2H), 3.05 (m, 4H). LCMS (ESI) 465 (M + H).

Example 65

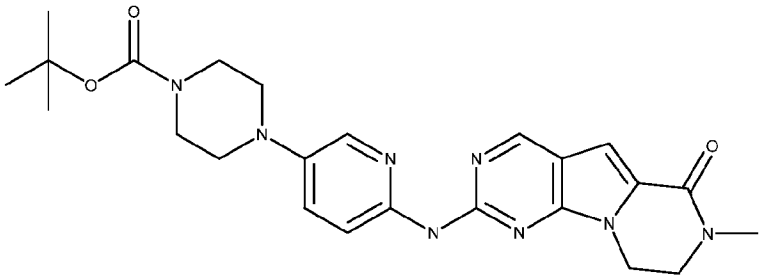
Synthesis of Compound 65



To a solution of Compound 64 (23 mg) in 10% DCM/MeOH was added 10 mL of a 3M solution of HCl in iso-propanol. The contents were stirred for 16 hrs. Concentration of the reaction mixture afforded the hydrochloride salt. ^{1}HNMR (d6-DMSO) δ ppm 9.01 (s, 1H), 7.94 (m, 1H), 7.86 (m, 1H), 7.23 (s, 1H), 4.30 (m, 2H), 3.64 (m, 2H), 3.36 (m, 4H), 3.25 (m, 4H). LCMS (ESI) 465 (M + H).

Example 66

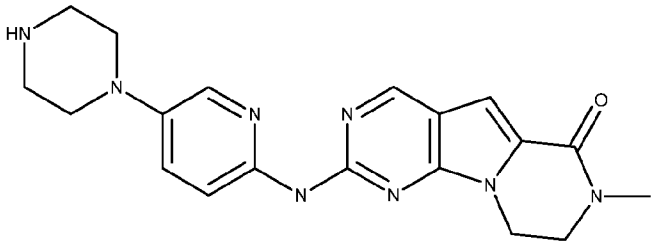
Synthesis of Compound 66



To chloro-N-methyltricyclic amide (0.080 g, 0.338 mmole) in dioxane (3.5 mL) under nitrogen was added *tert*-butyl 4-(6-amino-3-pyridyl)piperazine-1-carboxylate 0.102 g (1.1 eq) followed by the addition of $Pd_2(dba)_3$ (27 mg), BINAP (36 mg) and sodium- *tert*-butoxide (45 mg). The contents were heated at reflux for 11 hrs. The crude product was purified using silica gel column chromatography with an eluent of dichloromethane/methanol (0- 5%) to afford the desired product (44 mg). ^{1}HNMR (d6-DMSO) δ ppm 9.49 (s, 1H), 8.85 (s, 1H), 8.32 (m, 1H), 8.02 (s, 1H), 7.44 (m, 1H), 7.00 (s, 1H), 4.33 (m, 2H), 3.80 (m, 2H), 3.48 (m, 4H), 3.07 (m, 4H), 3.05 (s, 3H), 1.42 (s, 9H). LCMS (ESI) 479 (M + H).

Example 67

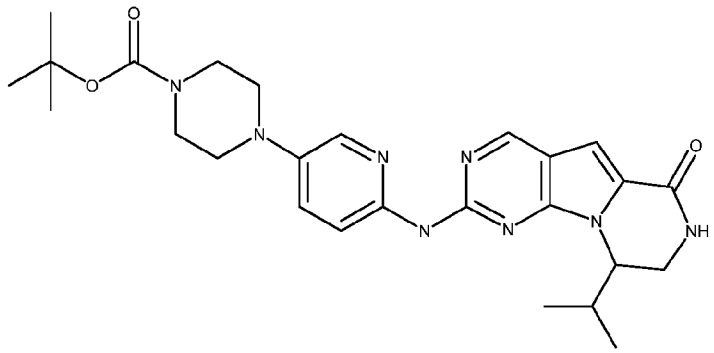
Synthesis of Compound 67



To Compound 66 (32 mg) was added 3N HCL (10 mL) in isopropanol and the contents were stirred at room temperature overnight for 16 hrs. Concentration afforded the hydrochloride salt. ^{1}HNMR (d6-DMSO) δ ppm 9.13 (m, 2H), 8.11 (m, 1H), 8.10 (s, 1H), 7.62 (m, 1H), 7.21 (s, 1H), 4.43 (m, 2H), 3.85 (m, 2H), 3.41 (m, 4H), 3.28 (m, 4H), 3.08 (s, 3H). LCMS (ESI) 379 (M + H).

Example 68

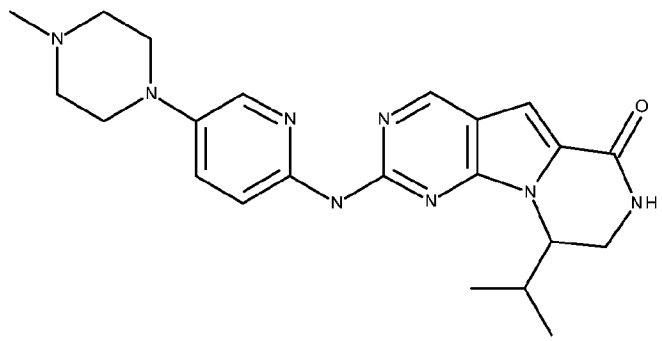
Synthesis of Compound 68



Compound 68 was synthesized using similar experimental conditions to that described for compound 64. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.79 (d, J=7.03 Hz, 3 H) 1.01 (d, J=6.73 Hz, 3 H) 1.35 - 1.48 (m, 9 H) 2.16 (dd, J=14.64, 6.73 Hz, 1 H) 3.00 - 3.14 (m, 4 H) 3.40 - 3.51 (m, 4 H) 3.51 - 3.60 (m, 1 H) 3.63 - 3.74 (m, 1 H) 4.44 (dd, J=7.90, 3.81 Hz, 1 H) 6.99 (s, 1 H) 7.46 (dd, J=8.93, 2.78 Hz, 1 H) 7.94 - 8.09 (m, 2 H) 8.31 (dd, J=9.08, 1.46 Hz, 1 H) 8.85 (s, 1 H) 9.46 (s, 1 H). LCMS (ESI) 507 (M + H) .

Example 69

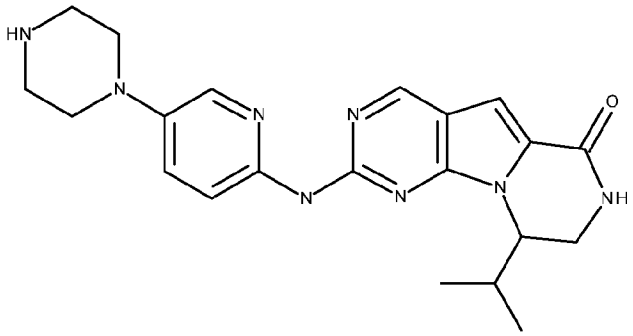
Synthesis of Compound 69



Compound 69 was synthesized using similar experimental conditions to those described for compound 63 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.77 - 0.86 (m, 3 H) 0.96 (d, J=7.03 Hz, 3 H) 2.10 - 2.24 (m, 1 H) 3.07 (s, 3 H) 3.37 - 3.79 (m, 8 H) 4.00 (dd, J=13.61, 4.54 Hz, 2 H) 4.63 - 4.73 (m, 1 H) 7.20 (s, 1 H) 7.58 - 7.71 (m, 1 H) 7.99 (d, J=2.34 Hz, 1 H) 8.12 (d, J=9.37 Hz, 1 H) 9.11 (s, 1 H) 9.41 (br. s., 2 H) 11.76 (br. s., 1 H). LCMS (ESI) 421 (M + H).

Example 70

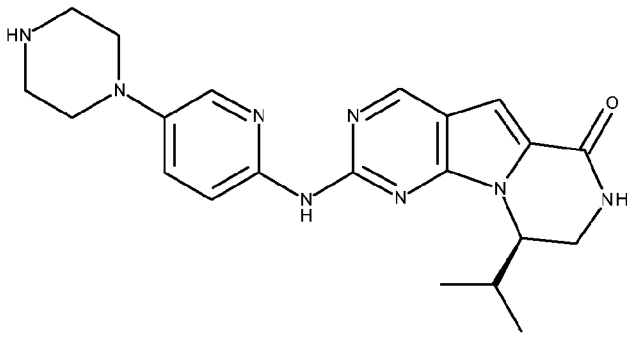
Synthesis of Compound 70



Compound 70 was synthesized using similar experimental conditions to those described for compounds 64 and 65 and was recovered as an HCl salt. The characterization data (NMR and LCMS) was consistent with that reported for compound 71 .

Example 71

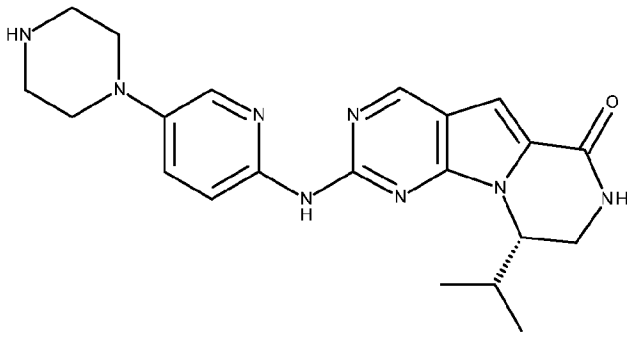
Synthesis of Compound 71



Compound 71 was synthesized using similar experimental conditions to those described for compounds 64 and 65 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.79 (d, J=6.73 Hz, 3 H) 1.01 (d, J=6.73 Hz, 3 H) 2.18 (dd, J=14.49, 7.17 Hz, 1 H) 3.18 - 3.84 (m, 10 H) 4.53 - 4.71 (m, 1 H) 7.24 (s, 1 H) 7.65 (d, J=9.37 Hz, 1 H) 8.01 (d, J=2.64 Hz, 1 H) 8.14 (d, J=1.46 Hz, 1 H) 8.35 (d, J=5.27 Hz, 1 H) 9.14 (s, 1 H) 9.46 (s, 2 H) 11.80 (s, 1 H) LCMS (ESI) 407 (M+H).

Example 72

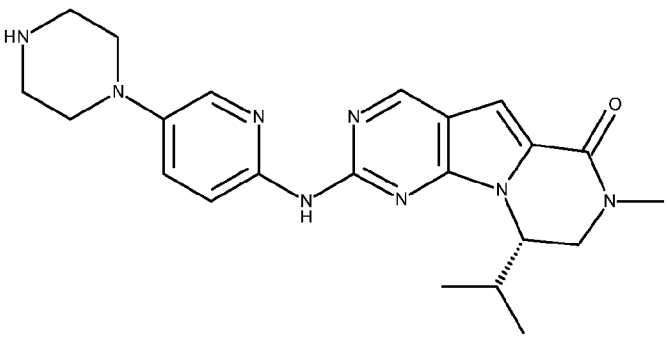
Synthesis of Compound 72 (Compound UUU)



Compound 72 was synthesized using similar experimental conditions to that described for compounds 64 and 65 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.77 (d, *J*=7.03 Hz, 3 H) 0.99 (d, *J*=6.73 Hz, 3 H) 2.10 - 2.24 (m, 1 H) 3.18 - 3.81 (m, 10 H) 4.54 - 4.69 (m, 1 H) 7.22 (s, 1 H) 7.63 (d, *J*=9.08 Hz, 1 H) 7.99 (d, *J*=2.63 Hz, 1 H) 8.11 (s, 1 H) 8.33 (d, *J*=5.27 Hz, 1 H) 9.12 (s, 1 H) 9.43 (s, 2 H) 11.77 (s, 1 H). LCMS (ESI) 407 (M+H).

Example 73

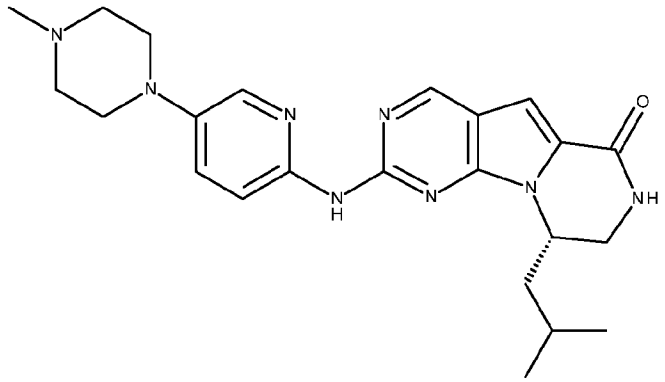
Synthesis of Compound 73



Compound 73 was synthesized using similar experimental conditions to those described for compounds 64 and 65 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.84 (d, *J*=6.73 Hz, 3 H) 0.98 (d, *J*=6.73 Hz, 3 H) 2.12 - 2.26 (m, 1 H) 3.09 (s, 3 H) 3.22 - 3.81 (m, 8 H) 4.01 (dd, *J*=13.61, 4.25 Hz, 2 H) 4.59 - 4.72 (m, 1 H) 7.19 (s, 1 H) 7.74 (s, 1 H) 7.96 - 8.10 (m, 2 H) 9.08 (s, 1 H) 9.22 (s, 2 H). LCMS (ESI) 421 (M+H).

Example 74

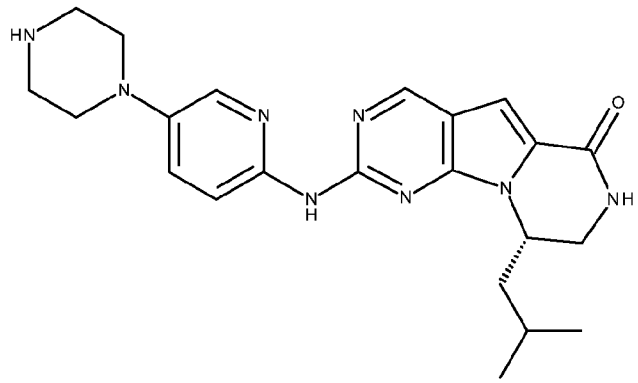
Synthesis of Compound 74



Compound 74 was synthesized using similar experimental conditions to those described for compound 63 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.85 (d, J=4.98 Hz, 3 H) 0.95 (d, J=4.98 Hz, 3 H) 1.42 - 1.70 (m, 3 H) 2.77 (d, J=2.93 Hz, 3 H) 3.07 - 4.14 (m, 10 H) 4.95 (s, 1 H) 7.20 (s, 1 H) 7.66 (d, J=9.66 Hz, 1 H) 7.94 (s, 1 H) 8.08 - 8.16 (m, 1 H) 8.33 (d, J=4.68 Hz, 1 H) 9.09 (s, 1 H) 11.38 (s, 1 H) 11.71 (s, 1 H). LCMS (ESI) 435 (M+H).

Example 75

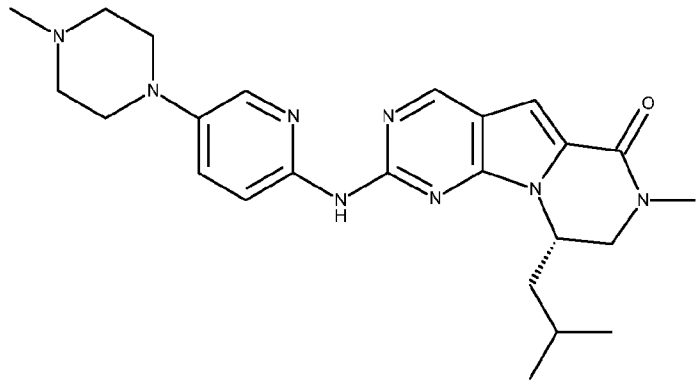
Synthesis of Compound 75



Compound 75 was synthesized using similar experimental conditions to those described for compounds 64 and 65 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.87 (d, J=6.15 Hz, 3 H) 0.94 (d, J=6.15 Hz, 3 H) 1.57 (d, J=84.61 Hz, 3 H) 3.05 (s, 3 H) 3.13 - 3.55 (m, 8 H) 3.69 (d, J=78.17 Hz, 2 H) 4.90 (s, 1 H) 7.15 (s, 1 H) 7.63 - 7.85 (m, 1 H) 7.93 (s, 1 H) 8.26 (s, 1 H) 9.03 (s, 1 H) 9.20 (s, 2 H). LCMS (ESI) 421 (M+H).

Example 76

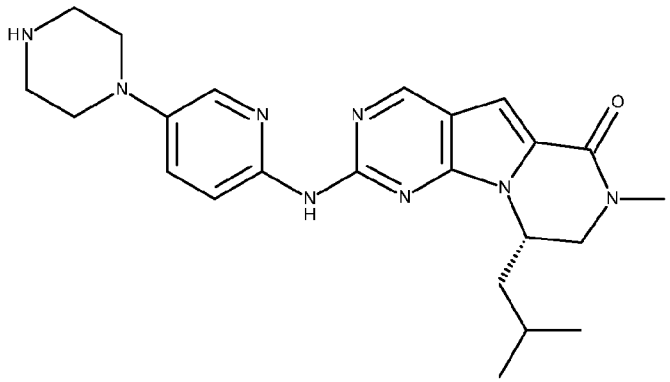
Synthesis of Compound 76



Compound 76 was synthesized using similar experimental conditions to those described for compound 63 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.85 (d, J=6.44 Hz, 3 H) 0.95 (d, J=6.44 Hz, 3 H) 1.43 - 1.70 (m, 3 H) 2.78 (d, J=2.93 Hz, 3 H) 3.05 (s, 3 H) 3.24 - 3.84 (m, 8 H) 4.01 (d, J=9.66 Hz, 2 H) 4.89 - 5.01 (m, 1 H) 7.15 (s, 1 H) 7.77 (s, 1 H) 7.91 - 8.05 (m, 2 H) 9.03 (s, 1 H) 10.96 - 11.55 (m, 2 H). LCMS (ESI) 449 (M+H).

Example 77

Synthesis of Compound 77

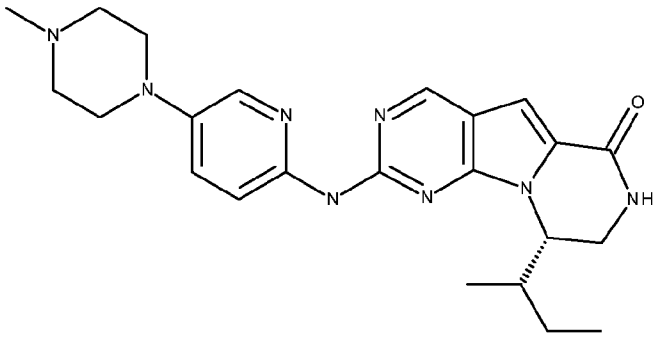


Compound 77 was synthesized using similar experimental conditions to those described for compounds 64 and 65 and was recovered as an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.83 - 0.88 (d, J=6.15 Hz, 3 H) 0.95 (d, J=6.15 Hz, 3 H) 1.40 - 1.71 (m, 3 H) 3.28 - 3.83 (m,

8 H) 4.00 (d, *J*=3.22 Hz, 2 H) 4.91 - 5.08 (m, 1 H) 7.17 (s, 1 H) 7.68 (d, *J*=9.66 Hz, 1 H) 7.93 (s, 1 H) 8.07 (s, 1 H) 9.06 (s, 1 H) 9.40 (s, 2 H) 11.59 (s, 1 H). LCMS (ESI) 435 (M+H).

Example 78

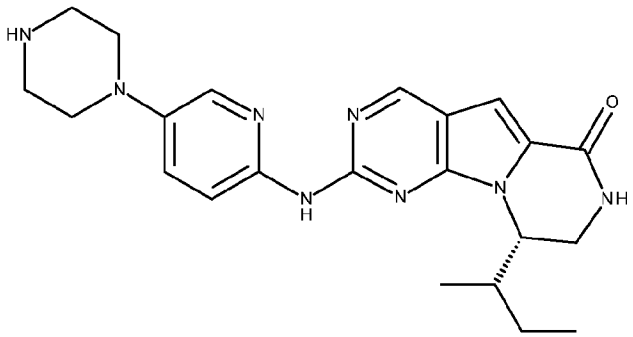
Synthesis of Compound 78



To Compound 50 0.060 g (0.205 mmole) was added 5-(4-methylpiperazin-1-yl)pyridin-2-amine (35.42 mg, 0.9 eq) followed by the addition of 1,4-dioxane (3 mL). After degassing with nitrogen, Pd_2dba_3 (12 mg), BINAP (16 mg) and sodium *tert*-butoxide (24 mg) were added. The contents were then heated at 90 degrees in a CEM Discovery microwave for 3 hrs. The reaction was then loaded onto a silica gel column and purified by eluting with DCM/MeOH (0-15%). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.75 (t, *J*=7.47 Hz, 3 H) 0.91 (d, *J*=6.73 Hz, 3 H) 1.04 - 1.20 (m, 2 H) 1.80 - 1.98 (m, 1 H) 2.77 (d, *J*=3.81 Hz, 3 H) 2.94 - 3.90 (m, 10 H) 4.54 - 4.68 (m, 1 H) 7.06 - 7.23 (m, 2 H) 7.56 - 7.75 (m, 1 H) 7.90 - 8.12 (m, 2 H) 8.29 (s, 1 H) 9.07 (s, 1 H) 10.98 - 11.74 (m, 2 H). LCMS (ESI) 435 (M + H).

Example 79

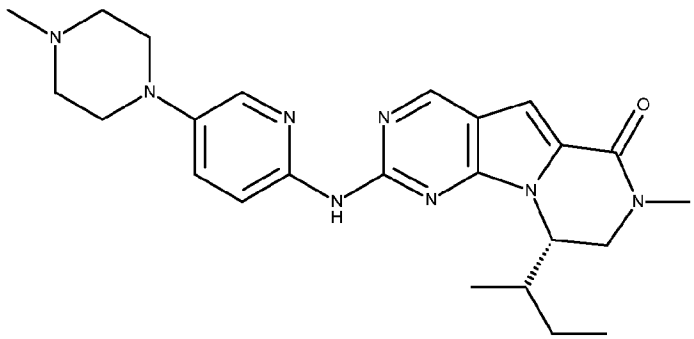
Synthesis of Compound 79



Compound 79 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.75 (t, J=7.32 Hz, 3 H) 0.90 (d, J=6.73 Hz, 3 H) 1.07 - 1.15 (m, 2 H) 1.85 - 1.94 (m, 1 H) 3.17 - 3.75 (m, 10 H) 4.58 - 4.67 (m, 1 H) 7.17 (s, 1 H) 7.71 (s, 1 H) 7.96 (s, 1 H) 7.98 - 8.05 (m, 1 H) 8.28 (d, J=4.10 Hz, 1 H) 9.06 (s, 1 H) 9.39 (s, 2 H). LCMS (ESI) 421 (M+H).

Example 80

Synthesis of Compound 80

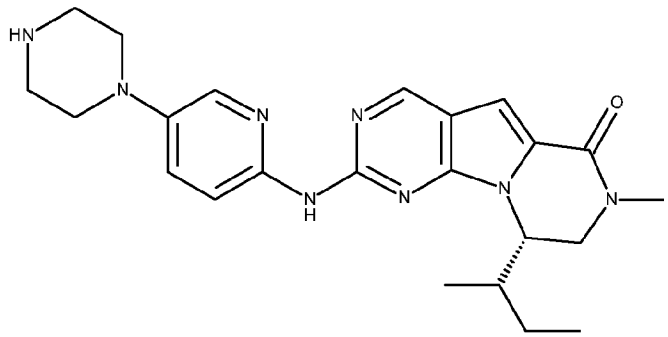


Compound 80 was synthesized in a similar manner to that described for compound 78. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.78 (t, J=7.32 Hz, 3 H) 0.86 (d, J=6.73 Hz, 3 H) 1.13 - 1.21 (m, 2 H) 1.84 - 1.96 (m, 1 H) 2.77 (d, J=4.39 Hz, 3 H) 3.04 (s, 3 H) 3.11 - 3.84 (m, 8 H) 3.98 (dd, J=13.61, 4.25 Hz, 2 H) 4.66 - 4.74 (m, 1 H) 7.17 (s, 1 H) 7.64 (s, 1 H) 7.96 (d, J=2.34

Hz, 1 H) 8.03 - 8.13 (m, 1 H) 9.08 (s, 1 H) 11.26 (s, 1 H) 11.66 (s, 1 H). LCMS (ESI) 449 (M+H).

Example 81

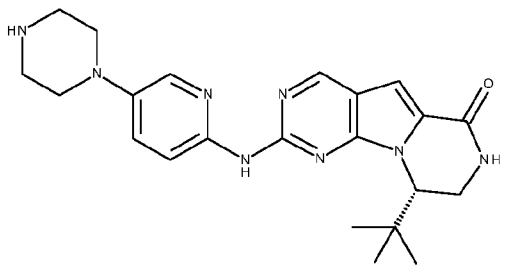
Synthesis of Compound 81



The compound was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.78 (t, J=7.32 Hz, 3 H) 0.85 (d, J=6.73 Hz, 3 H) 1.10 - 1.27 (m, 2 H) 1.82 - 1.99 (m, 1 H) 3.04 (s, 3 H) 3.28 - 3.77 (m, 8 H) 3.97 (dd, J=13.91, 4.54 Hz, 2 H) 4.62 - 4.75 (m, 1 H) 7.07 - 7.24 (m, 1 H) 7.62 - 7.75 (m, 1 H) 7.94 (d, J=2.34 Hz, 1 H) 7.97 - 8.08 (m, 1 H) 9.05 (s, 1 H) 9.29 (s, 2 H). LCMS (ESI) 435 (M+H).

Example 82

Synthesis of Compound 82

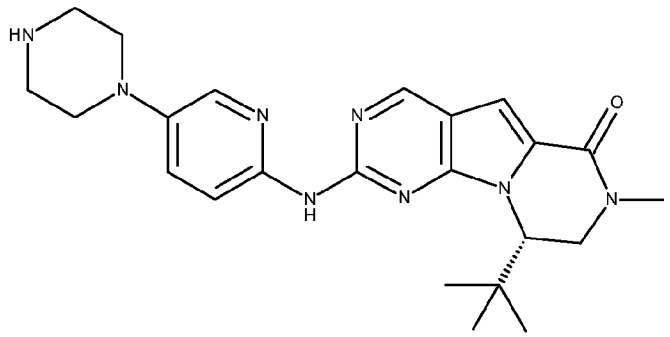


The compound was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.96 (s, 9 H) 3.15 - 3.87 (m, 10 H) 4.42 - 4.53 (m, 1 H)

6.99 (s, 1 H) 7.24 (s, 1 H) 8.06 (s, 1 H) 8.11 - 8.21 (m, 1 H) 8.79 - 8.98 (m, 2 H) 9.25 (s, 2 H) 9.88 (s, 1 H). LCMS (ESI) 421 (M+H).

Example 83

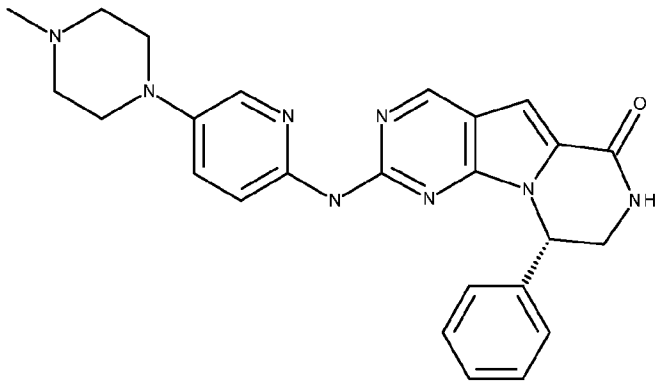
Synthesis of Compound 83



Compound 83 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.95 (s, 9 H) 2.79 (d, J=4.10 Hz, 3 H) 3.06 - 3.86 (m, 10 H) 4.56 - 4.67 (m, 1 H) 7.17 (s, 1 H) 7.70 (s, 1 H) 7.96 (d, J=2.63 Hz, 1 H) 7.99 - 8.08 (m, 1 H) 8.26 (s, 1 H) 9.06 (s, 1 H) 10.80 (s, 1 H). LCMS (ESI) 435 (M+H).

Example 84

Synthesis of Compound 84

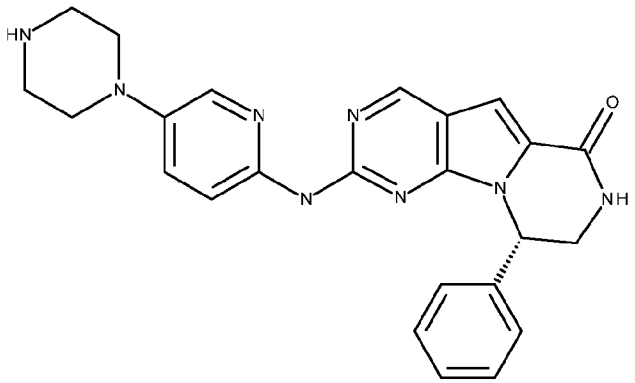


Compound 84 was synthesized in a similar manner to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 2.75 - 2.81 (m, 3 H)

3.12 - 3.16 (m, 2 H) 3.46 - 3.54 (m, 4 H) 3.60 - 3.69 (m, 2 H) 3.72 - 3.79 (m, 1 H) 4.07 - 4.18 (m, 2 H) 6.06 - 6.09 (m, 1 H) 6.90 (d, *J*=7.61 Hz, 2 H) 7.20 - 7.31 (m, 3 H) 7.33 (s, 1 H) 7.49 - 7.55 (m, 1 H) 7.62 - 7.70 (m, 1 H) 7.92 (d, *J*=2.93 Hz, 1 H) 8.22 (s, 1 H) 9.14 (s, 1 H). LCMS (ESI) 455 (M + H).

Example 85

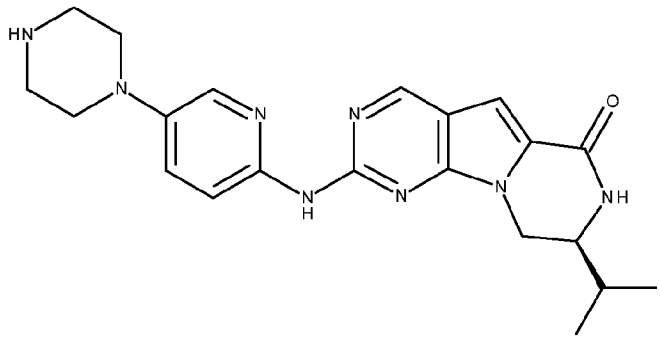
Synthesis of Compound 85



Compound 85 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 3.21 (s, 4 H) 3.35 - 3.67 (m, 5 H) 4.07 - 4.20 (m, 2 H) 6.13 (s, 1 H) 6.90 (d, *J*=7.32 Hz, 2 H) 7.22 - 7.31 (m, 3 H) 7.36 (s, 1 H) 7.48 (d, *J*=9.37 Hz, 1 H) 7.93 (d, *J*=2.34 Hz, 1 H) 8.04 - 8.11 (m, 1 H) 8.25 (d, *J*=4.98 Hz, 1 H) 9.17 (s, 1 H) 11.77 (br, s., 1H). LCMS (ESI) 441 (M + H).

Example 86

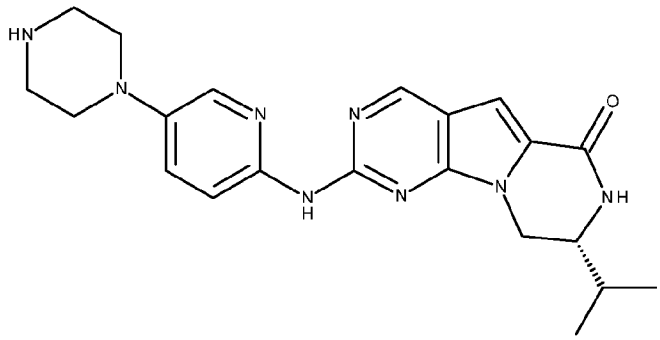
Synthesis of Compound 86



Compound 86 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.90 (d, J=6.15 Hz, 6 H) 1.72 - 1.89 (m, 1 H) 3.15 - 3.92 (m, 9 H) 4.10 - 4.46 (m, 2 H) 7.18 (s, 1 H) 7.59 (d, J=8.78 Hz, 1 H) 8.00 (s, 1 H) 8.13 (d, J=9.37 Hz, 1 H) 8.55 (s, 1 H) 9.09 (s, 1 H) 9.67 (s, 2 H) 11.91 (s, 1 H). LCMS (ESI) 407 (ESI).

Example 87

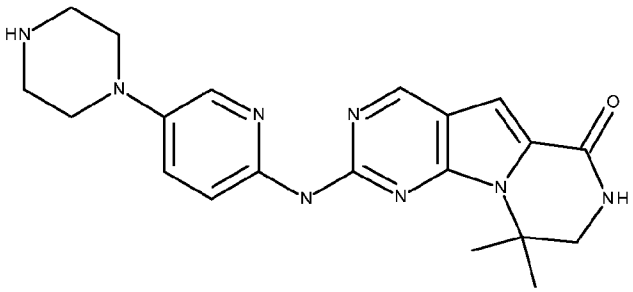
Synthesis of Compound 87



Compound 87 was synthesized in a manner similar to compound 86 and was converted to an HCl salt. The characterization data (NMR and LCMS) was similar to that obtained for the antipode compound 86 .

Example 88

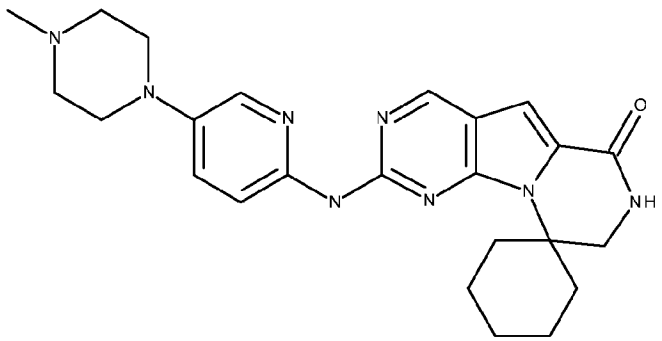
Synthesis of Compound 88



Compound 88 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 ~~3~~-and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.78 (s, 6 H) 3.40 - 3.53 (m, 6 H) 3.64 - 3.73 (m, 4 H) 7.27 (s, 1 H) 7.66 (d, J=9.37 Hz, 1 H) 7.98 (d, J=2.34 Hz, 1 H) 8.12 (br. s., 1 H) 8.47 (br. s., 1 H) 9.11 (s, 1 H) 9.45 (br. s., 2 H) 11.62 (br. s., 1 H). LCMS (ESI) 393 (M + H).

Example 89

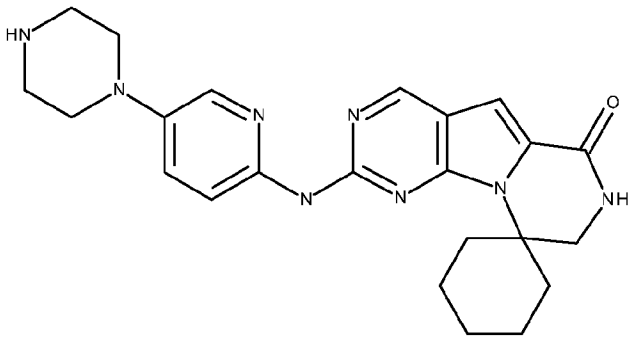
Synthesis of Compound 89 (also referred to as Compound T)



Compound 89 was synthesized in a similar manner to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.47 (br. s., 6 H) 1.72 (br. s., 2 H) 1.92 (br. s., 2 H) 2.77 (br. s., 3 H) 3.18 (br. s., 2 H) 3.46 (br. s., 2 H) 3.63 (br. s., 2 H) 3.66 (d, J=6.15 Hz, 2 H) 3.80 (br. s., 2 H) 7.25 (s, 1 H) 7.63 (br. s., 2 H) 7.94 (br. s., 1 H) 8.10 (br. s., 1 H) 8.39 (br. s., 1 H) 9.08 (br. s., 1 H) 11.59 (br. s., 1 H). LCMS (ESI) 447 (M + H).

Example 90

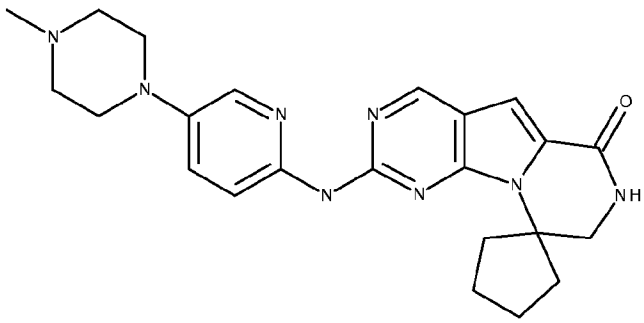
Synthesis of Compound 90 (also referred to as Compound Q)



Compound 90 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.27 - 1.64 (m, 6 H) 1.71 (br. s., 2 H) 1.91 (br. s., 2 H) 2.80 (br. s., 1 H) 3.17 - 3.24 (m, 2 H) 3.41 (br. s., 4 H) 3.65 (br. s., 4 H) 7.26 (br. s., 1 H) 7.63 (br. s., 1 H) 7.94 (br. s., 1 H) 8.13 (br. s., 1 H) 8.40 (br. s., 1 H) 9.09 (br. s., 1 H) 9.62 (br. s., 1 H) 11.71 (br. s., 1 H). LCMS (ESI) 433 (M + H).

Example 91

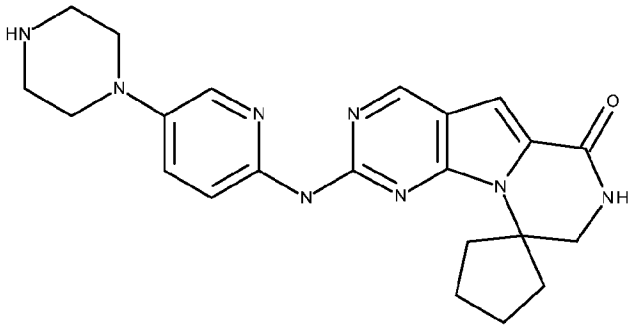
Synthesis of Compound 91 (also referred to as Compound ZZ)



Compound 91 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.64 - 1.75 (m, 2 H) 1.83 - 1.92 (m, 2 H) 1.96 - 2.06 (m, 2 H) 2.49 - 2.58 (m, 2 H) 2.79 (d, J=3.81 Hz, 3 H) 3.06 - 3.18 (m, 4 H) 3.59 - 3.69 (m, 2 H) 3.73 - 3.83 (m, 2 H) 4.04 - 4.12 (m, 2 H) 7.17 (br. s., 1 H) 7.60 - 7.70 (m, 2 H) 7.70 - 7.92 (m, 2 H) 7.96 (br. s., 1 H) 8.41 (br. s., 1 H) 8.98 (br. s., 1 H) 10.77 (br. s., 1 H). LCMS (ESI) 433 (M + H).

Example 92

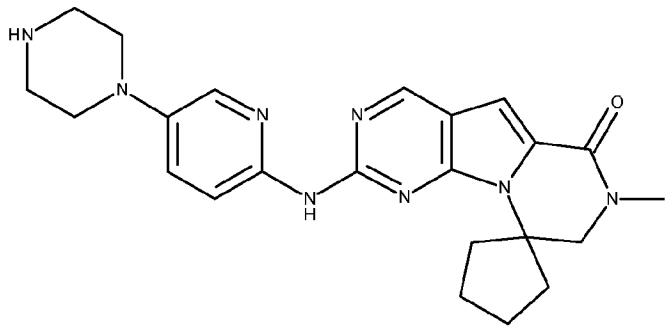
Synthesis of Compound 92



Compound 92 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.64 - 1.75 (m, 2 H) 1.84 - 1.92 (m, 2 H) 1.96 - 2.05 (m, 2 H) 2.48 - 2.56 (m, 2 H) 3.22 (br. s., 4 H) 3.42 - 3.48 (m, 4 H) 3.60 - 3.69 (m, 2 H) 4.05 - 4.13 (m, 1 H) 7.18 (s, 1 H) 7.65 (d, *J*=13.47 Hz, 1 H) 7.70 - 7.77 (m, 1 H) 7.94 (d, *J*=1.76 Hz, 1 H) 8.42 (br. s., 1 H) 9.00 (s, 1 H) 9.15 (br. s., 2 H). LCMS (ESI) 419 (M + H).

Example 93

Synthesis of Compound 93

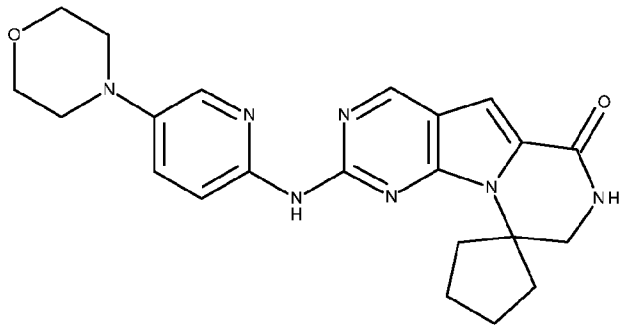


Compound 93 was synthesized in a similar manner to that described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.76 (br. s., 2 H) 1.89 (br. s., 2 H) 2.03 (br. s., 2 H) 2.47 - 2.58 (m, 2 H) 3.04 (s, 3 H) 3.22 (br. s., 4 H) 3.39 (br. s., 4 H) 3.66 (s, 2 H) 7.21 (s, 1 H) 7.67 (d,

J=9.37 Hz, 1 H) 7.93 (br. s., 1 H) 7.98 - 8.09 (m, 1 H) 9.04 (s, 1 H) 9.34 (br. s., 2 H) 11.31 (br. s., 1 H). LCMS (ESI) 433 (M + H).

Example 94

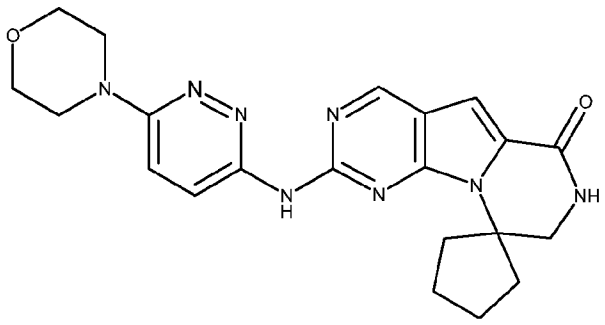
Synthesis of Compound 94



Compound 94 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.66 - 1.77 (m, 2 H) 1.84 - 1.94 (m, 2 H) 1.96 - 2.08 (m, 2 H) 2.48 - 2.57 (m, 2 H) 3.36 - 3.52 (m, 4 H) 3.60 - 3.80 (m, 6 H) 7.21 (s, 1 H) 7.53 - 7.74 (m, 2 H) 7.86 (s, 1 H) 8.02 (s, 1 H) 8.45 (s, 1 H) 9.03 (s, 1 H) 11.19 (br. s., 1 H). LCMS (ESI) 420 (M+H).

Example 95

Synthesis of Compound 95

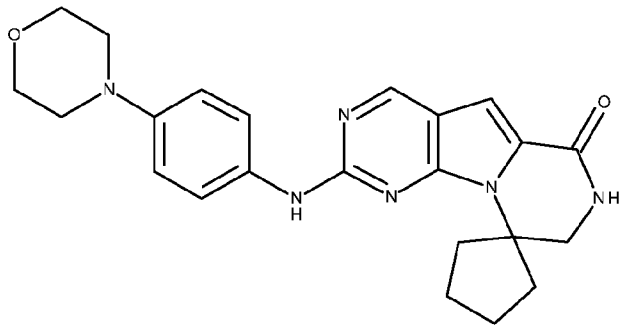


Compound 95 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.65 - 1.79 (m, 2 H) 1.85 - 1.95 (m, 2 H) 1.97 - 2.08 (m, 2 H) 2.47 - 2.54 (m, 2 H) 3.40 - 3.58 (m, 5 H) 3.65 (dd,

J=21.67, 5.56 Hz, 1 H) 3.69 - 3.78 (m, 4 H) 7.24 (s, 1 H) 7.97 - 8.17 (m, 2 H) 8.48 (s, 1 H) 9.08 (s, 1 H) 11.81 (s, 1 H). LCMS (ESI) 421 (M+H).

Example 96

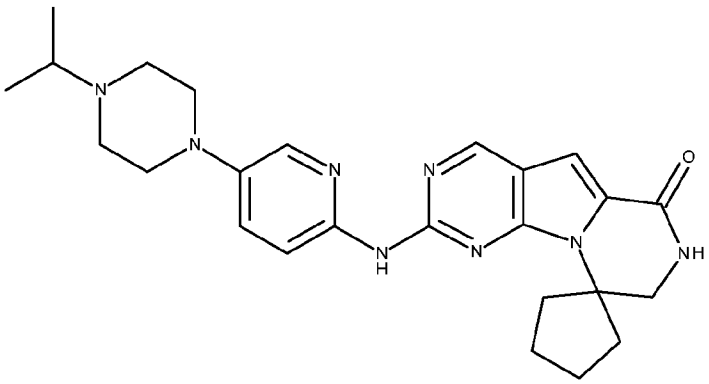
Synthesis of Compound 96



Compound 96 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.55 - 1.74 (m, 2 H) 1.80 - 1.98 (m, 4 H) 2.48 - 2.60 (m, 2 H) 3.40 - 3.50 (m, 4 H) 3.57 - 3.72 (m, 2 H) 3.90 - 4.20 (m, 4 H) 7.08 (s, 1 H) 7.37 - 7.57 (m, 2 H) 7.70 (m, 2 H) 8.32 (s, 1 H) 8.88 (s, 1 H) 9.98 (s, 1 H). LCMS (ESI) 419 (M+H).

Example 97

Synthesis of Compound 97 (also referred to as Compound III)

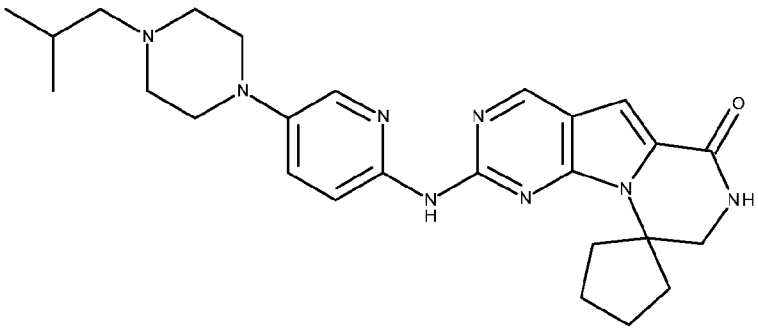


Compound 97 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.30 (d, *J*=5.27 Hz, 6 H) 1.65 - 1.78 (m, 2 H) 1.83 - 1.95 (m, 2 H) 1.97 - 2.10 (m, 2 H) 2.45 - 2.55 (m, 2H) 3.25 -

3.36 (m, 1 H) 3.39 - 3.48 (m, 4 H) 3.60 – 3.70 (m, 4 H) 3.75 - 4.15 (m, 2 H) 7.24 (s, 1 H) 7.54 - 7.75 (m, 2 H) 7.95 (s, 1 H) 8.10 (s, 1 H) 8.49 (s, 1 H) 9.07 (s, 1 H) 11.25 (s, 1 H) 11.48 (s, 1 H). LCMS (ESI) 461 (M+H).

Example 98

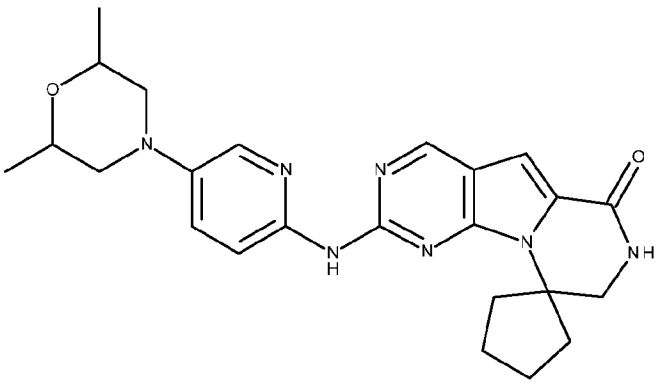
Synthesis of Compound 98



Compound 98 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.99 (d, J=6.15 Hz, 6 H) 1.65 - 1.78 (m, 2 H) 1.90 (m, 2 H) 1.97 - 2.08 (m, 2 H) 2.08 - 2.17 (m, 1 H) 2.45 - 2.55 (m, 2H) 2.88 - 3.02 (m, 2 H) 3.33 - 3.48 (m, 4 H) 3.50 - 3.90 (m, 6 H) 7.24 (s, 1 H) 7.67 (s, 2 H) 7.94 (s, 1 H) 8.12 (s, 1 H) 8.49 (s, 1 H) 9.07 (s, 1 H) 10.77 (s, 1 H) 11.51 (s, 1 H). LCMS (ESI) 475 (M+H).

Example 99

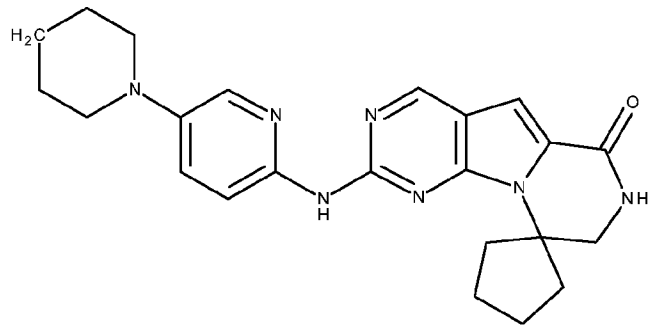
Synthesis of Compound 99



Compound 99 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.13 (d, J=5.86 Hz, 6 H) 1.66 - 1.77 (m, 2 H) 1.84 - 1.94 (m, 2 H) 1.97 - 2.09 (m, 2 H) 2.40 - 2.53 (m, 2 H) 3.37 - 3.49 (m, 2 H) 3.50 - 3.59 (m, 2 H) 3.59 - 3.73 (m, 4 H) 7.23 (s, 1 H) 7.64 (m, 3 H) 7.85 (s, 1 H) 8.11 (s, 1 H) 8.47 (s, 1 H) 9.05 (s, 1 H). 11.35 (br s., 1H). LCMS (ESI) 448 (M+H).

Example 100

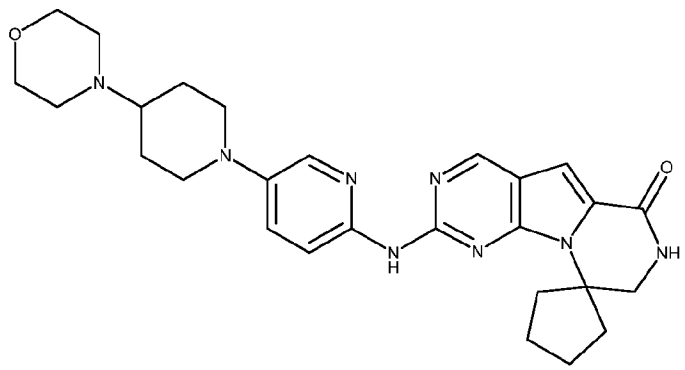
Synthesis of Compound 100



Compound 100 was synthesized using similar conditions to that described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.50 - 1.57 (m, 2 H) 1.62 - 1.68 (m, 3 H) 1.68 - 1.75 (m, 2 H) 1.84 - 1.92 (m, 2 H) 1.97 - 2.08 (m, 2 H) 2.48 - 2.53 (m, 2 H) 3.14 - 3.23 (m, 4 H) 3.43 - 3.47 (m, 2 H) 3.58 - 3.70 (m, 2 H) 7.22 (s, 1 H) 7.58 - 7.70 (m, 2 H) 7.85 - 8.00 (m, 1 H) 8.16 (d, 1 H) 8.46 (s, 1 H) 9.04 (s, 1 H) 11.37 (br s., 1H). LCMS (ESI) 418 (M + H).

Example 101

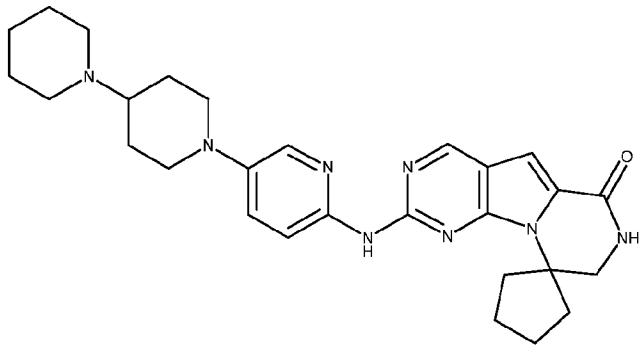
Synthesis of Compound 101 (also referred to as Compound WW)



Compound 101 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.72 (s, 2 H) 1.90 (s, 4 H) 2.03 (s, 2 H) 2.21 (s, 2 H) 2.48 - 2.54 (m, 2 H) 2.73 (s, 2 H) 3.03 (s, 2 H) 3.25 - 3.35 (m, 1 H) 3.38 - 3.48 (m, 4 H) 3.65 - 3.99 (m, 5 H) 7.23 (s, 1 H) 7.63 (d, *J*=9.66 Hz, 1 H) 7.90 (s, 1 H) 8.13 (s, 1 H) 8.47 (s, 1 H) 9.06 (s, 1 H) 10.50 (br s., 1H). LCMS (ESI) 503 (M + H).

Example 102

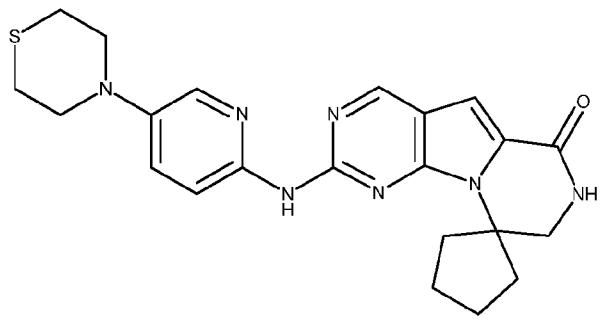
Synthesis of Compound 102 (also referred to as Compound HHH)



Compound 102 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.63 - 1.85 (m, 6 H) 1.87 - 1.92 (m, 2 H) 1.99 - 2.06 (m, 2 H) 2.15 - 2.23 (m, 2 H) 2.47 - 2.53 (m, 1 H) 2.69 - 2.79 (m, 2 H) 2.81 - 2.91 (m, 2 H) 2.98 - 3.08 (m, 2 H) 3.32 - 3.48 (m, 4 H) 3.57 - 3.72 (m, 4 H) 3.77 - 3.85 (m, 2 H) 7.22 (s, 1 H) 7.60 - 7.68 (m, 2 H) 7.90 (s, 1 H) 8.07 (s, 1 H) 8.46 (s, 1 H) 9.04 (s, 1 H). 11.41 (br s., 1H). LCMS (ESI) 501 (M + H).

Example 103

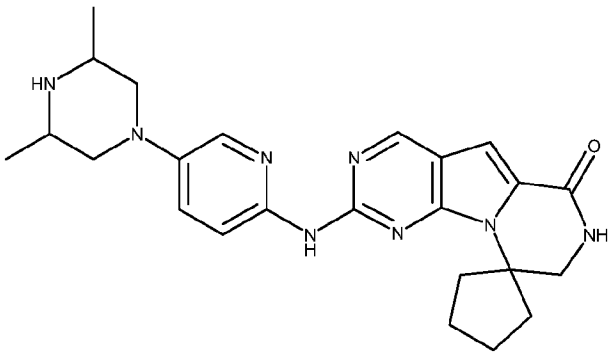
Synthesis of Compound 103



Compound 103 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.64 - 1.76 (m, 2 H) 1.87 - 1.93 (m, 2 H) 2.00 - 2.07 (m, 2 H) 2.48 - 2.53 (m, 2 H) 2.67 - 2.72 (m, 4 H) 3.44 - 3.47 (m, 2 H) 3.50 - 3.55 (m, 4 H) 7.24 (s, 1 H) 7.61 (d, *J*=9.37 Hz, 2 H) 7.86 (d, *J*=2.63 Hz, 1 H) 8.09 (d, *J*=12.88 Hz, 1 H) 8.48 (s, 1 H) 9.06 (s, 1 H) 11.41 (br s., 1H). LCMS (ESI) 436 (M + H).

Example 104

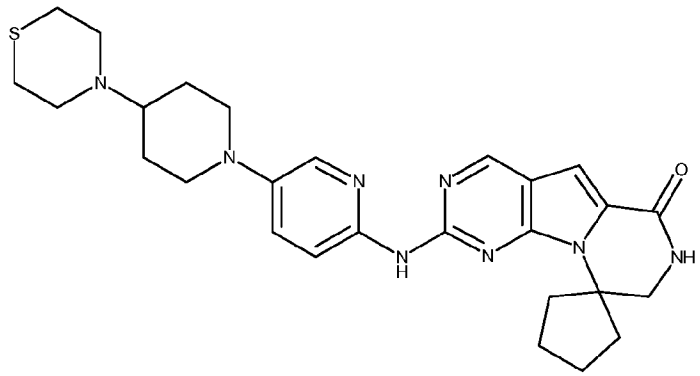
Synthesis of Compound 104



Compound 104 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.29 (d, *J*=6.73 Hz, 6 H) 1.66 - 1.79 (m, 2 H) 1.84 - 1.95 (m, 2 H) 1.98 – 2.09 (m, 2 H) 2.46 - 2.55 (m, 2 H) 3.29 – 3.39 (m, 2H) 3.58 – 3.70 (m, 4H) 3.77 – 3.86 (m, 4H) 7.24 (s, 1 H) 7.66 (d, *J*=9.37 Hz, 1 H) 7.96 (d, *J*=2.93 Hz, 1 H) 8.08 (s, 1 H) 8.48 (s, 1 H) 9.06 (s, 1 H) 9.28 (s, 1 H) 9.67 (s, 1 H) 11.36 (s, 1H). LCMS (ESI) 447 (M + H).

Example 105

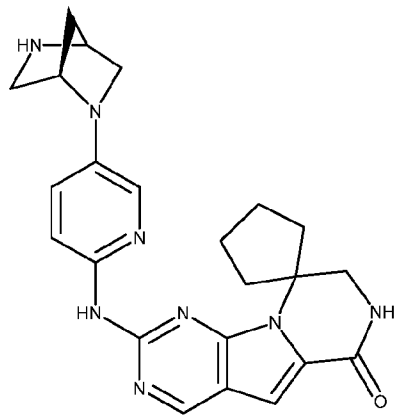
Synthesis of Compound 105



Compound 105 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.73 (s, 2 H) 1.76 - 1.85 (m, 2 H) 1.85 - 1.94 (m, 2 H) 1.98 - 2.07 (m, 2 H) 2.19 - 2.26 (m, 2 H) 2.48 - 2.52 (m, 1 H) 2.70 - 2.81 (m, 4 H) 3.13 - 3.20 (m, 1 H) 3.30 - 3.48 (m, 3 H) 3.58 - 3.71 (m, 4 H) 3.78 - 3.84 (m, 4 H) 7.24 (s, 1 H) 7.62 (d, *J*=9.37 Hz, 2 H) 7.89 (d, *J*=1.17 Hz, 1 H) 8.09 - 8.18 (m, 1 H) 8.48 (s, 1 H) 9.06 (s, 1 H) 11.46 (br s., 1H). LCMS (ESI) 519 (M + H).

Example 106

Synthesis of Compound 106

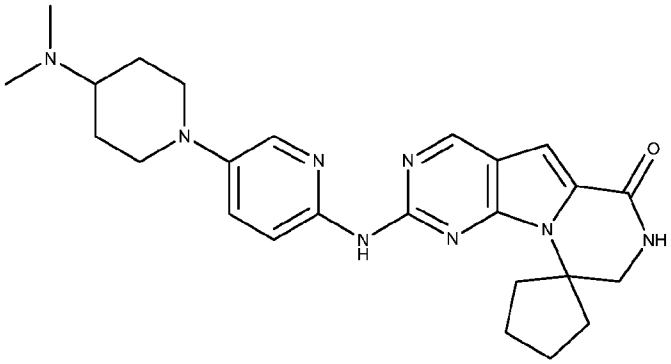


Compound 106 was synthesized using similar conditions to those described for compound 78 followed by the deblocking step described for compound 65 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.65 - 1.75 (m, 2 H) 1.85 - 1.93 (m, 2 H) 1.93 - 1.99 (m, 1 H) 2.00 - 2.06 (m, 2 H) 2.08 - 2.14 (m, 1 H) 2.47 - 2.55 (m, 2 H) 3.07 - 3.25 (m,

2 H) 3.25 - 3.69 (m, 5 H) 4.46 (s, 1 H) 4.67 (s, 1 H) 7.22 (s, 1 H) 7.58 - 7.69 (m, 2 H) 8.46 (s, 1 H) 9.02 (s, 1 H) 9.34 (s, 1 H) 9.65 (s, 1 H). LCMS (ESI) 431 (M + H).

Example 107

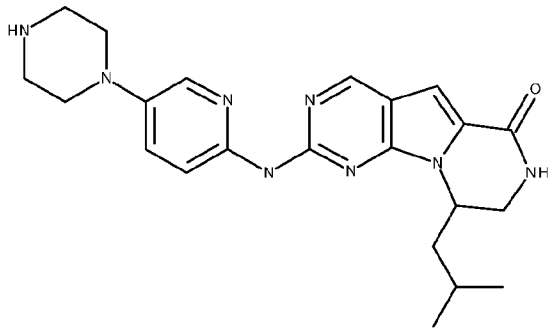
Synthesis of Compound 107 (also referred to as Compound YY)



Compound 107 was synthesized using similar conditions to those described for compound 78 and was converted to an HCl salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.65 - 1.82 (m, 3 H) 1.89 (br. s., 2 H) 1.98 - 2.08 (m, 2 H) 2.13 (br. s., 2 H) 2.47 - 2.55 (m, 2 H) 2.68 (d, *J*=4.98 Hz, 6 H) 2.71 - 2.80 (m, 2 H) 3.29 - 3.71 (m, 10 H) 7.16 - 7.26 (m, 1 H) 7.67 (d, *J*=9.66 Hz, 2 H) 7.91 (d, *J*=2.05 Hz, 1 H) 8.14 (br. s., 1 H) 8.48 (br. s., 1 H) 9.05 (s, 1 H) 11.14 (br. s., 1 H) 11.43 (br. s., 1 H). LCMS (ESI) 461 (M + H).

Example 108

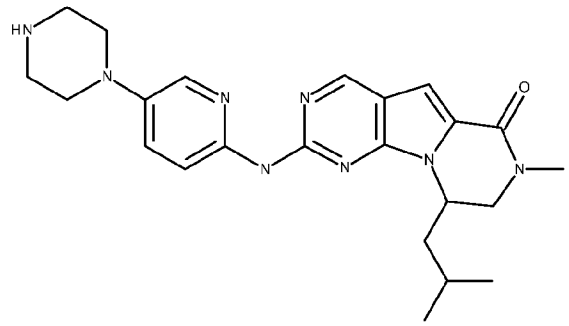
Synthesis of Compound 108



Compound 108 was synthesized in a manner similar to that described for compounds 64 and 65 and was recovered as an HCl salt. The analytical data was consistent with that described for the antipode compound 75 .

Example 109

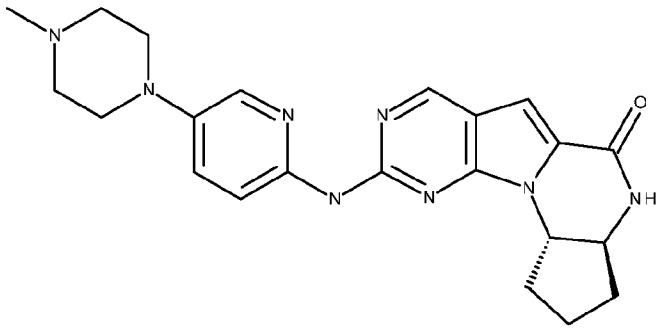
Synthesis of Compound 109



Compound 109 was synthesized in a manner similar to that described for compounds 64 and 65 and was recovered as an HCl salt. The analytical data was consistent with that described for the antipode compound 75.

Example 110

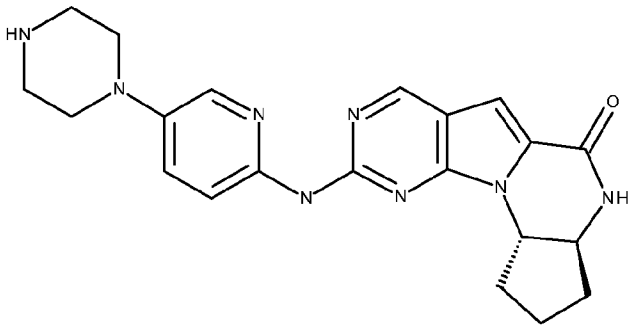
Synthesis of Compound 110



Compound 110 was synthesized in a similar manner to that described for compound 78 and then converted to its hydrochloride salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.50 - 1.65 (m, 1 H) 1.92 - 2.02 (m, 3 H) 2.06 - 2.15 (m, 1 H) 2.78 (d, *J*=3.81 Hz, 4 H) 3.10 - 3.20 (m, 4 H) 3.47 - 3.51 (m, 2 H) 3.64 - 3.71 (m, 1 H) 3.76 - 3.83 (m, 2 H) 3.98 - 4.14 (m, 1 H) 7.20 (s, 2 H) 7.77 (s, 1 H) 7.97 (s, 2 H) 8.81 (s, 1 H) 9.03 (s, 1 H) 10.97 (br s., 1H). LCMS (ESI) 419 (M + H).

Example 111

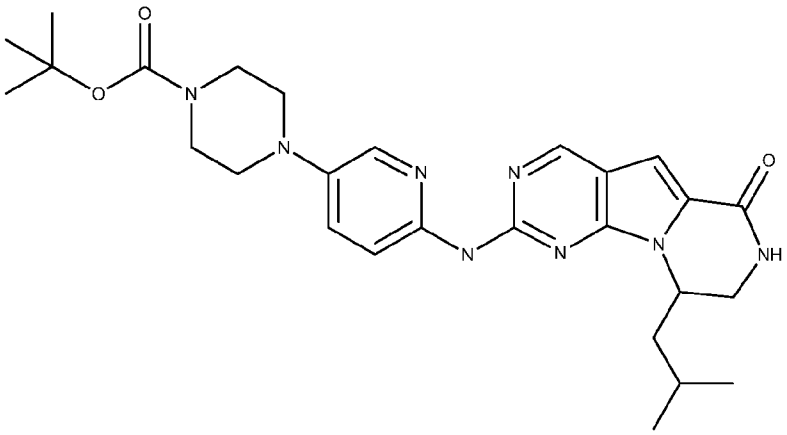
Synthesis of Compound 111



Compound 111 was synthesized in a similar manner to that described for compound 78 and then converted to its hydrochloride salt. ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.54 - 1.59 (m, 1 H) 1.92 - 2.01 (m, 3 H) 2.06 - 2.15 (m, 1 H) 2.76 - 2.84 (m, 1 H) 3.17 - 3.24 (m, 6 H) 3.64 - 3.71 (m, 2 H) 4.02 - 4.11 (m, 2 H) 7.22 (s, 2 H) 7.64 (s, 1 H) 7.97 (s, 2 H) 8.75 (s, 1 H) 8.97 (s, 1 H) 9.21 (s, 1 H). LCMS (ESI) 405 (M + H).

Example 112

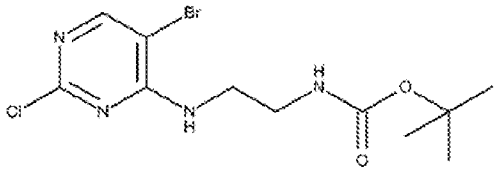
Synthesis of Compound 112



Compound 112 was synthesized using similar experimental conditions to that described for compound 64.

Example 113

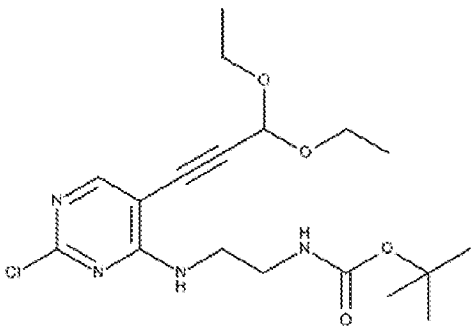
Synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate, Compound 113



To a solution of 5-bromo-2,4-dichloropyrimidine (12.80 g, 0.054 mole) in ethanol (250 mL) was added Hunig's base (12.0 mL) followed by the addition of a solution of N-(tert-butoxycarbonyl)-1,2-diaminoethane (10 g, 0.0624 mole) in ethanol (80 mL). The contents were stirred overnight for 20 hrs. The solvent was evaporated under vacuum. Ethyl acetate (800 mL) and water (300 mL) were added and the layers separated. The organic layer was dried with magnesium sulfate and then concentrated under vacuum. Column chromatography on silica gel using hexane/ethyl acetate (0- 60%) afforded tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. LCMS (ESI) 351 (M + H).

Example 114

Synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4 yl]amino]ethyl]carbamate, Compound 114

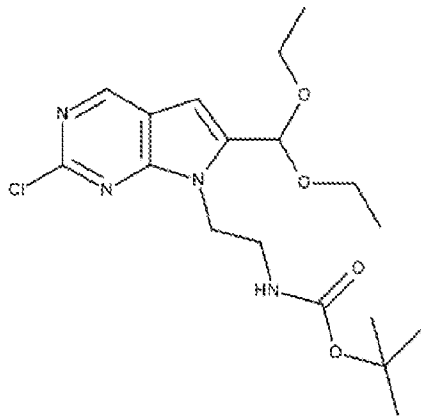


To tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate (5 g, 14.23 mmole) in toluene (42 mL) and triethylamine (8.33 mL) under nitrogen was added triphenyl

arsine (4.39 g), 3,3-diethoxyprop-1-yne (3.24 mL) and Pddba (1.27 g). The contents were heated at 70 degrees for 24 hrs. After filtration through CELITE®, the crude reeacton was columned using hexane/ethyl acetate (0- 20%) to afford the desired product 3.9 g). Column chromatography of the resulting residue using hexane/ethyl acetate (0- 30%) afforded tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate. LCMS (ESI) 399 (M + H).

Example 115

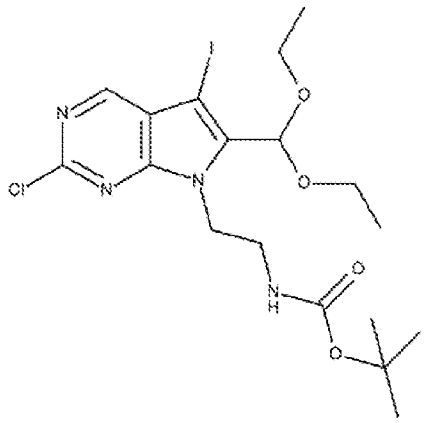
Synthesis of tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate, Compound 115



To a solution of Compound 114 (3.9 g, 0.00976 mole) in THF (60 mL) was added TBAF (68.3 mL, 7eq). The contents were heated to 45 degrees for 2 hrs. Concentration followed by column chromatography using ethyl acetate/hexane (0-50%) afforded tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate as a pale brown liquid (1.1 g). ^{1}HNMR (d6-DMSO) δ ppm 8.88 (s, 1H), 6.95 (brs, 1H), 6.69 (s, 1H), 5.79 (s, 1H), 4.29 (m, 2H), 3.59 (m, 4H), 3.34 (m, 1H), 3.18 (m, 1H), 1.19 (m, 9H), 1.17 (m, 6H). LCMS (ESI) 399 (M+H).

Example 116

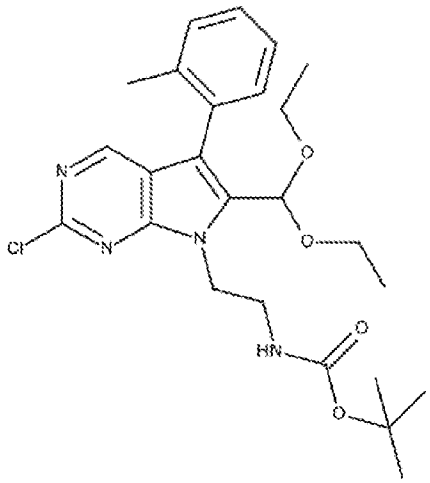
Synthesis of tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate, Compound 116



To tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate (0.1 g, 0.00025 mol) in acetonitrile (2 mL) was added 1,3-diiodo-5,5-dimethylhydantoin (95 mg, 1 eq), and solid $NaHCO_3$ (63 mg, 3 eq). The reacation was stirred at room tempreature for 16 hrs. The reaction was filtered and concentrated in vacuo. The product was purified by silica gel column chromatography using hexane/ethylacetate (0 – 50%) to afford tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate as a pale yellow solid (0.03 g). LCMS (ESI) 525 (M + H).

Example 117

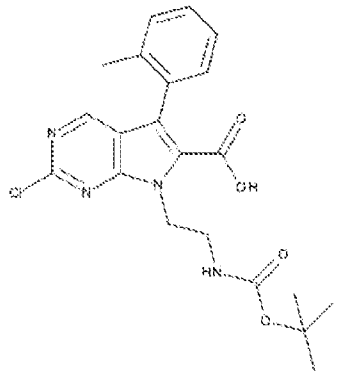
Synthesis of tert-Butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-(o-tolyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate, Compound 117



To tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-iodo-pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate (0.1g , 0.19 mmole) in dioxane (3 mL) was added 2-methylphenylboronic acid (28 mg), tetrakis(triphenylphosphine)palladium (25 mg) and potassium phosphate (250 mg) in water (0.3 mL). The reaction was heated in a CEM Discovery microwave at 90 °C for 3 hrs. The crude reaction was loaded onto silica gel and columned using hexane/ethyl acetate (0 – 30%) to afford tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-(o-tolyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate (0.06 g). LCMS (ESI) 489 (M + H).

Example 118

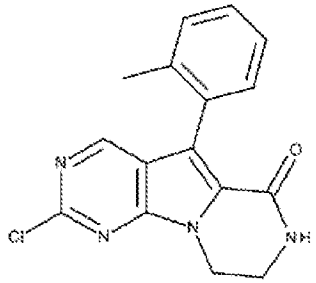
Synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 118



To tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)-5-(o-tolyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate (0.85 g, 1.74 mmole) in AcOH (10 mL) was added water (1.5 mL). The reation was stirred at room tempreature for 16 hrs. The crude reaction was then concentrated under vacuum. After the addition of ethyl acetate (50 mL), the organic layer was washed with satd. $NaHCO_3$. The organic layer was dried with magnesium sulfate and then concentrated under vacuum to afford the crude intermediate, tert-butyl N-[2-[2-chloro-6-formyl-5-(o-tolyl)pyrrolo[2,3-d]pyrimidin-7-yl]ethyl]carbamate. To this crude intermediate in DMF (5 mL) was added oxone (1.3 g). After stirring for 2.5 hrs, water (20 mL) and ethyl acetate (100 mL) were added. The organic layer was separated, dried and then concentrated under vacuum to afford the crude product which was columned over silica gel using hexane/ethyl acetate (0 – 50%) to afford 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (0.112 g). LCMS (ESI) 431 (M + H).

Example 119

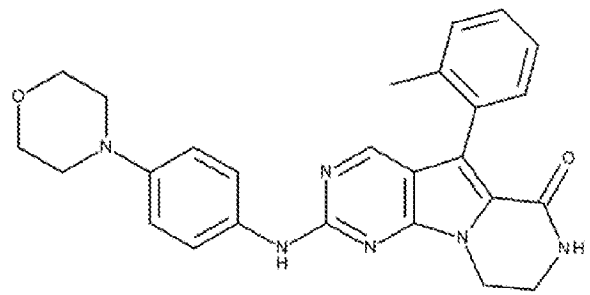
Synthesis of Compoound 119



To 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (0.1 g, 0.261 mmol) in DCM (4.1 mL) was added DMAP (20 mg) followed by the addition of N,N'-diisopropylcarbodiimide (0.081 mL, 2eq). After stirring for 3 hrs, TFA (0.723 mL) was added. Stirring was then continued for another 30 minutes. The reaction mixture was neutralized with satd. $NaHCO_3$. DCM (20 mL) was then added and the organic layer separated, dried with magnesium sulfate and then concentrated under vacuum to afford the crude product which was columned using hexane/ethylacetate (0–100%) to afford chloro tricyclic amide Compound 119 (0.65 g). LCMS (ESI) 313 (M + H).

Example 120

Synthesis of Compound 120

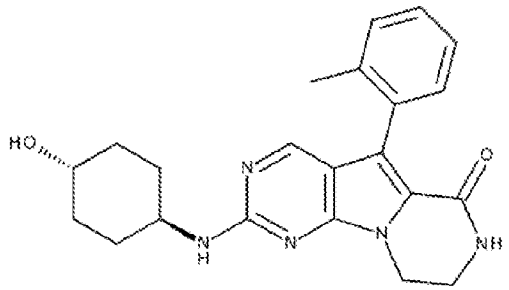


To the chloro tricyclic amide (0.040 g, 0.128 mmole) (Compound 119) in dioxane (2.5 mL) under nitrogen was added $Pd_2(dba)_3$ (12 mg), sodium tert-butoxide (16 mg), BINAP (16 mg) and 4-morpholinoaniline (22.7 mg, 1eq). The reaction mixture was heated at 90 °C in a CEM Discovery microwave for 3.0 hrs. The crude reaction was loaded onto a silica gel column and the contents eluted with DCM/MeOH (0-6%) to afford the product (10 mg). LCMS (ESI)

455 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 2.14 (s, 3 H) 3.23 - 3.50 (m, 2 H) 3.57 - 3.73 (m, 2 H), 3.81 – 3.92 (m, 8H), 7.11 - 7.31 (m, 4 H) 7.31 - 7.48 (m, 1 H) 7.58 - 7.73 (m, 1 H) 7.77 - 7.95 (m, 2 H) 8.05 - 8.21 (m, 1 H) 8.44 (s, 1 H) 9.85 - 10.01 (m, 1 H).

Example 121

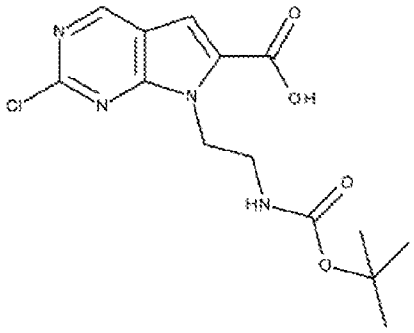
Synthesis of Compound 121



To the chloro tricyclic amide (0.024 g) (Compound 119) in N-methyl-2-pyrrolidone (NMP) (1.5 mL) was added trans-4-aminocyclohexanol (0.0768 mmol, 26.54 mg, 3 eq) and Hunig's base (0.4 mL). The reaction was heated in a CEM Discovery microwave vessel at 150 °C for 1.2 hrs. The crude reaction was loaded onto a silica gel column and the contents eluted with DCM/MeOH (0 - 10%) to afford the product (21 mg). LCMS (ESI) 392 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.23 (d, J=8.78 Hz, 4 H) 1.84 (br. s., 4 H) 2.11 (s, 3 H) 3.34 - 3.43 (m, 1 H) 3.55 (br. s., 2 H) 3.72 (br. s., 1 H) 4.13 (br. s., 2 H) 4.50 (br. s., 1 H) 7.03 (br. s., 1 H) 7.12 - 7.28 (m, 4 H) 7.96 (br. s., 1 H) 8.18 (br. s., 1 H).

Example 122

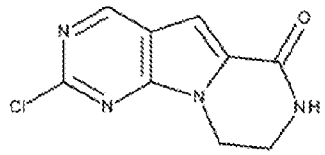
Synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 122



7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using a similar experimental procedure as that described for the synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 341 (M + H).

Example 123

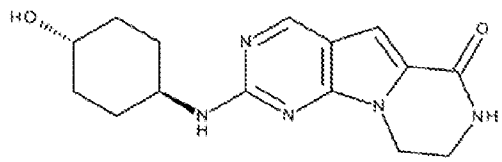
Synthesis of Compound 123



Chloro tricyclic amide, Compound 123, was synthesized using a similar experimental procedure as that described for the synthesis of chloro tricyclic amide (Compound 119). LCMS (ESI) 223 (M + H).

Example 124

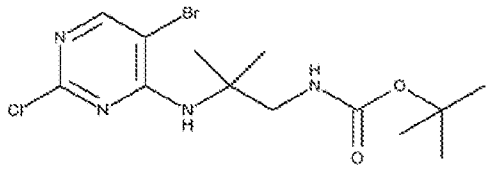
Synthesis of Compound 124



To the chloro tricyclic amide, Compound 123 (0.035 g, 0.00157 mole) in NMP (1.5 mL) was added Hunig's base (0.3 mL) followed by the addition of the trans-4-aminocyclohexanol (54.2 mg). The reaction mixture was heated at 150 °C for 1.5 hrs. The crude reaction was loaded onto a silica gel column and the column was eluted with DCM/MeOH (0 - 10%) to afford the product (5 mg). LCMS (ESI) 302 (M + H).

Example 125

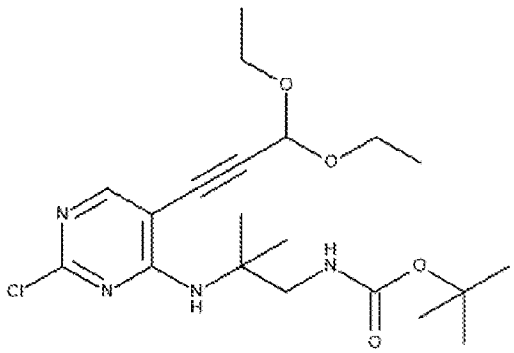
Synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-methyl-propyl]carbamate, Compound 125



tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-methyl-propyl]carbamate was synthesized by treating 5-bromo-2,4-dichloropyrimidine with tert-butyl N-(2-amino-2-methyl-propyl)carbamate using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. LCMS (ESI) (M+H) 379.

Example 126

Synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate, Compound 126

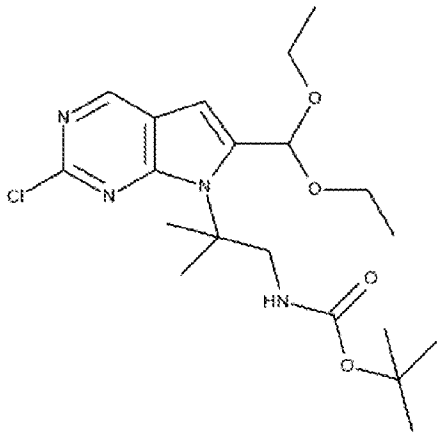


tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate was synthesized by treating tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-

yl)amino]-2-methyl-propyl]carbamate with 3,3-diethoxyprop-1-yne in the presence of a catalyst such as Pddba using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4 yl]amino]ethyl]carbamate.
LCMS (ESI) (M+H) 427.

Example 127

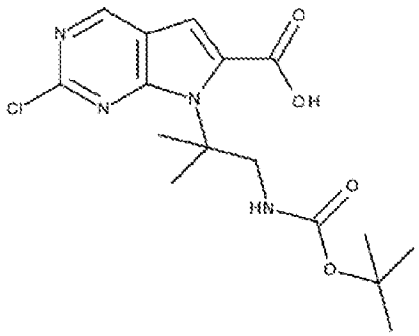
Synthesis of tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]-2-methyl-propyl]carbamate, Compound 127



tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]-2-methyl-propyl]carbamate was synthesized by treating tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate with TBAF using similar experimental conditions as described for the synthesis tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3- d]pyrimidin-7-yl]ethyl]carbamate. LCMS (ESI) (M+H) 427.

Example 128

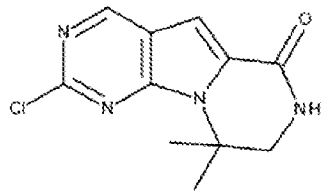
Synthesis of 7-[2-(tert-butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 128



7-[2-(tert-butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using a similar experimental procedure as that described for the synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 369 (M + H).

Example 129

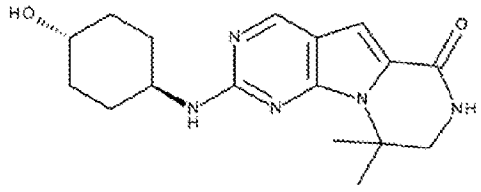
Synthesis of Compound 129



Chloro tricyclic amide, Compound 129, was synthesized using a similar procedure as that described for the synthesis of chloro tricyclic amide, Compound 119. LCMS (ESI) 251 (M + H).

Example 130

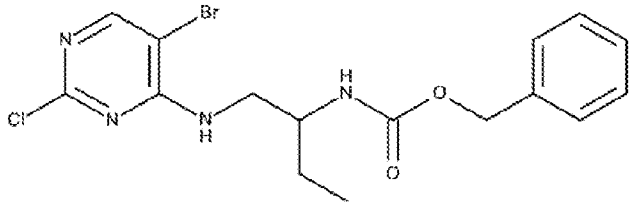
Synthesis of Compound 130



Compound 130 was synthesized by treating chlorotricyclic amine Compound 129 with trans-4-aminocyclohexanol using similar experimental conditions as for compound 124. LCMS (ESI) 330 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.07 - 1.34 (m, 4 H) 1.47 - 2.05 (m, 10 H) 3.09 (m, 1H) 3.51 (d, J = 2.91 Hz, 2 H) 3.57 (m, 1H) 4.50 (br. s., 1 H) 6.89 (s, 1 H) 6.94 - 7.05 (m, 1 H) 8.04 (br. s., 1 H) 8.60 (s, 1 H) 9.00 (br. s., 1 H).

Example 131

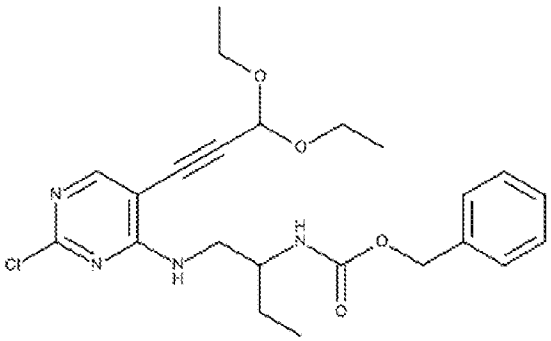
Synthesis of benzyl N-[1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]propyl]carbamate, Compound 131



Benzyl N-[1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]propyl]carbamate was synthesized by treating 5-bromo-2,4-dichloropyrimidine with benzyl N-[1-(aminomethyl)propyl]carbamate using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. LCMS (ESI) (M+H) 413.

Example 132

Synthesis of benzyl N-[1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]propyl]carbamate, Compound 132

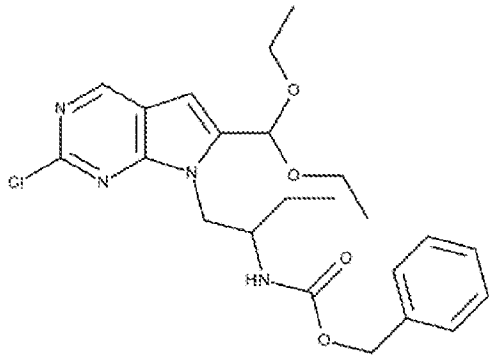


Benzyl N-[1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]propyl]carbamate was prepared by treating benzyl N-[1-[[(5-bromo-2-chloro-

pyrimidin-4-yl)amino]methyl]propyl]-carbamate with 3,3-diethoxyprop-1-yne in the presence of a catalyst such as Pddba using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]ethyl]carbamate LCMS (ESI) (M+H) 461.

Example 133

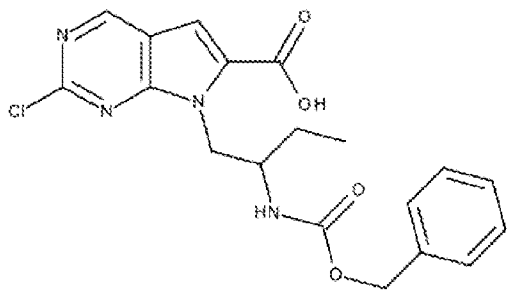
Synthesis of benzyl N-[1-[[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl] methyl]propyl]carbamate, Compound 133



Benzyl N-[1-[[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]methyl]propyl]carbamate was synthesized by treating benzyl N-[1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]propyl]carbamate with TBAF using similar experimental conditions as described for the synthesis tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3 d]pyrimidin-7-yl]ethyl]carbamate. LCMS (ESI) (M+H) 461.

Example 134

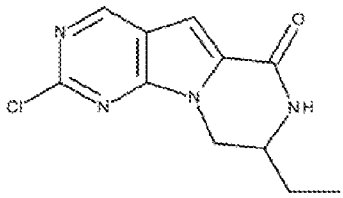
Synthesis of 7-[2-(benzyloxycarbonylamino)butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 134



7-[2-(benzyloxycarbonylamino)butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using a similar experimental procedure as that described for the synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 403 (M + H).

Example 135

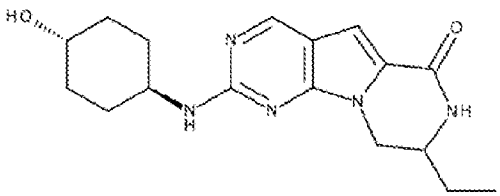
Synthesis of Compound 135



To a solution of 7-[2-(benzyloxycarbonylamino)butyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid in dichloromethane was added HBr, the reaction was stirred at 45 degrees for 3 hrs. After concentration, 2N NaOH was added to basify (pH = 8.0) the reaction followed by the addition of THF (20 mL). Boc_2O was then added (1.2 eq) and the reacation was stirred for 16 hrs. To the crude reaction mixture was then added ethyl acetate (100 mL) and water (50 mL) and the organic phase was separated, dried (magnesium sulfate) and then concentrated under vacuum. To the crude product was added dichloromethane (30 mL) followed by DIC and DMAP. After stirring for 2 hrs, TFA was added and the contents stirred for an hour. The solvents were evaporated under vacuum and the residue basified with satd. $NaHCO_3$. Ethyl acetate was then added and the organic layer separated, dried (magnesium sulfate) and then concentrared under vacuum. Colum chromatography with hexane/ethyl acetate (0 -100%) afforded the desired chlorotricyclic core, Compound 135. LCMS (ESI) 251 (M + H).

Example 136

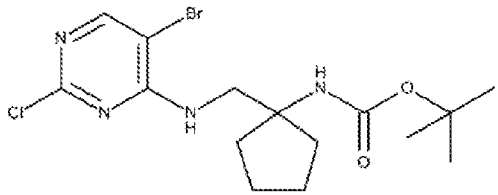
Synthesis of Compound 136



Compound 136 was synthesized by treating chlorotricyclic amine, Compound 135, with trans-4-aminocyclohexanol using similar experimental conditions as for compound 124. LCMS (ESI) 330 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 0.80 - 0.95 (m, 3 H) 1.35 – 1.92 (m, 10 H) 3.66 (br. m., 3 H) 4.17 (br. s., 2 H) 4.47 (br. s., 1 H) 6.85 (s, 1 H) 6.96 (br. s., 1 H) 8.15 (br. s., 1 H) 8.62 (br. s., 1 H).

Example 137

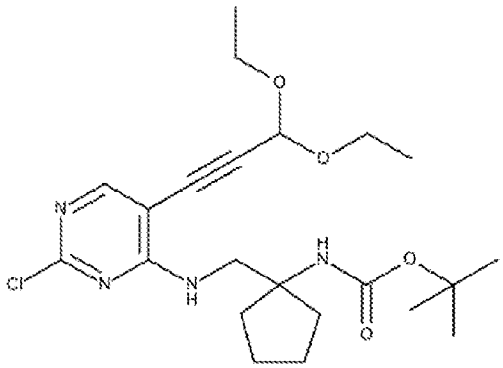
Synthesis of tert-butyl N-[1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]cyclopentyl]carbamate, Compound 137



tert-butyl N-[1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]cyclopentyl]carbamate was synthesized by treating 5-bromo-2,4-dichloropyrimidine with tert-butyl N-[1-(aminomethyl)cyclopentyl]carbamate using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. LCMS (ESI) 405 (M+H).

Example 138

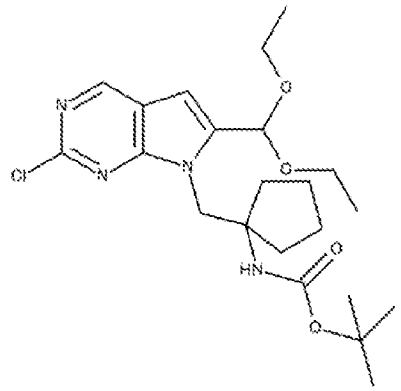
Synthesis of tert-butyl N-[1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]cyclopentyl]carbamate, Compound 138



tert-butyl N-[1-[[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]methyl]cyclopentyl]carbamate was synthesized by treating tert-butyl N-[1-[[(5-bromo-2-chloro-pyrimidin-4-yl)amino]methyl]cyclopentyl]carbamate with with 3,3-diethoxyprop-1-yne in the presence of a catalyst such as Pddba using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4 yl]amino]ethyl]carbamate LCMS (ESI) 453 (M+H).

Example 139

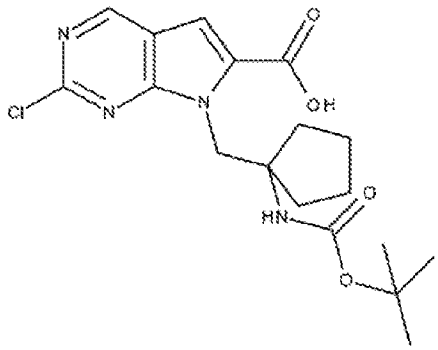
Synthesis of tert-butyl N-[1-[[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]methyl]cyclopentyl]carbamate, Compound 139



tert-butyl N-[1-[[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]methyl]cyclopentyl]carbamate was synthesized by treating tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate with TBAF using similar experimental conditions as described for the synthesis tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3 d]pyrimidin-7-yl]ethyl]carbamate. LCMS (ESI) 453 (M+H).

Example 140

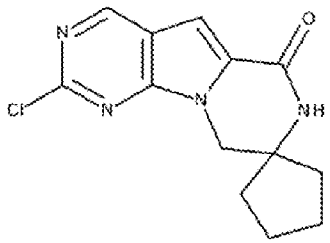
Synthesis of 7-[[1-(tert-butoxycarbonylamino)cyclopentyl]methyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid, Compound 140



7-[[1-(tert-butoxycarbonylamino)cyclopentyl]methyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using a similar experimental procedure as that described for the synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 395 (M + H).

Example 141

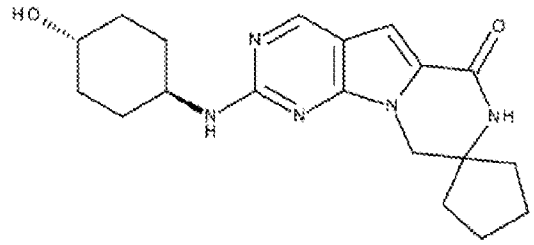
Synthesis of Compound 141



Chlorotricyclic core Compound 141 was synthesized using a similar experimental procedure as that described for the synthesis of chloro tricyclic amide Compound 119. LCMS (ESI) 277 (M + H).

Example 142

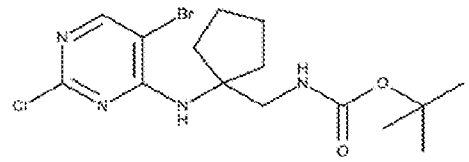
Synthesis of Compound 142



Compound 142 was synthesized by treating chlorotricyclic amine, Compound 141, with trans-4-aminocyclohexanol using similar experimental conditions as for Compound 124 . LCMS (ESI) 356 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.08 - 1.32 (m, 8 H) 1.60 - 2.09 (m, 8 H) 3.03 - 3.17 (m, 1 H) 3.35 (s, 2 H) 3.54 - 3.62 (m, 1 H) 4.51 (d, *J*=4.39 Hz, 1 H) 6.88 (s, 1 H) 6.96 (br. s., 1 H) 8.07 (br. s., 1 H) 8.58 (s, 1 H).

Example 143

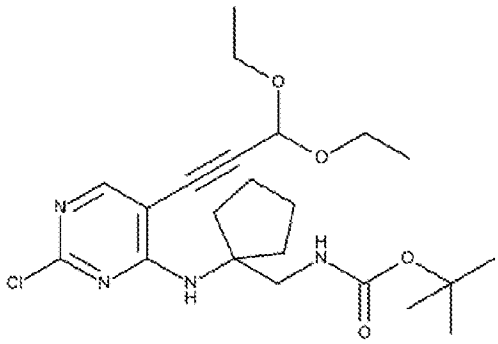
Synthesis of tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl]methyl]carbamate, Compound 143



tert-butyl N-[[1-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]cyclopentyl]methyl]carbamate was synthesized by treating 5-bromo-2,4-dichloropyrimidine with tert-butyl N-[(1-aminocyclopentyl)methyl]carbamate using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]ethyl]carbamate. LCMS (ESI) 405 (M+H).

Example 144

Synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate, Compound 144

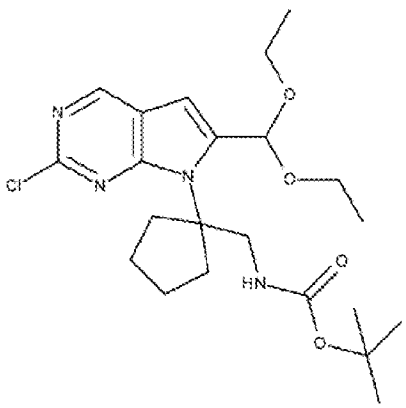


tert-butyl N-[[1-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]cyclopentyl]methyl]carbamate was synthesized by treating tert-butyl N-[2-[(5-bromo-2-chloro-pyrimidin-4-yl)amino]-2-methyl-propyl]carbamate with 3,3-diethoxyprop-1-yne in the presence of a catalyst such as Pddba using similar experimental conditions as described for the synthesis of tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4 yl]amino]ethyl]carbamate.

LCMS (ESI) 453 (M+H).

Example 145

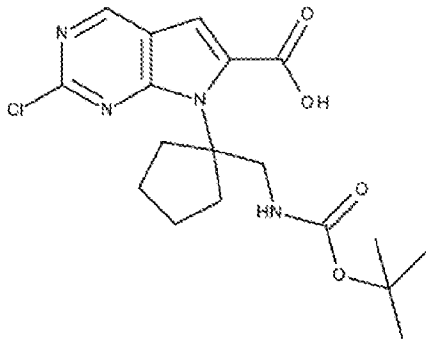
Synthesis of tert-butyl N-[[1-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]cyclopentyl]methyl]carbamate, Compound 145



tert-Butyl N-[[1-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3-d]pyrimidin-7-yl]cyclopentyl]methyl]carbamate was synthesized by treating tert-butyl N-[2-[[2-chloro-5-(3,3-diethoxyprop-1-ynyl)pyrimidin-4-yl]amino]-2-methyl-propyl]carbamate with TBAF using similar experimental conditions as described for the synthesis tert-butyl N-[2-[2-chloro-6-(diethoxymethyl)pyrrolo[2,3 d]pyrimidin-7-yl]ethyl]carbamate. LCMS (ESI) 4534 (M+H).

Example 146

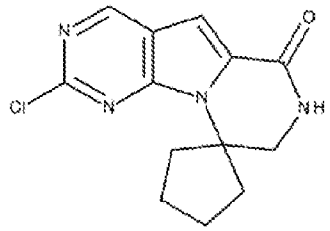
Synthesis of 7-[2-(tert-butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6carboxylic acid, Compound 146



7-[2-(tert-Butoxycarbonylamino)-1,1-dimethyl-ethyl]-2-chloro-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid was synthesized using a similar experimental procedure as that described for the synthesis of 7-[2-(tert-butoxycarbonylamino)ethyl]-2-chloro-5-(o-tolyl)pyrrolo[2,3-d]pyrimidine-6-carboxylic acid. LCMS (ESI) 395 (M + H).

Example 147

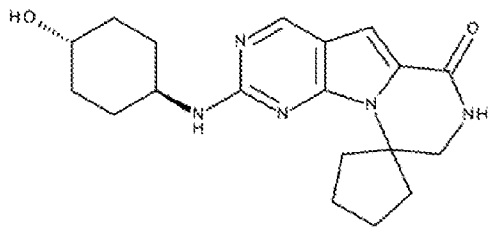
Synthesis of Compound 147



Chloro tricyclic amide, Compound 147 was synthesized using a similar experimental procedure as that described for the chloro tricyclic amide, Compound 119. LCMS (ESI) 277 (M + H).

Example 148

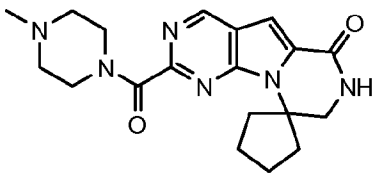
Synthesis of Compound 148



Compound 148 was synthesized by treating chlorotricyclic amine, Compound 147, with trans-4-aminocyclohexanol using similar experimental conditions as for Compound 124. LCMS (ESI) 356 (M + H). ^{1}HNMR (600 MHz, DMSO-d_6) δ ppm 1.06 - 1.35 (m, 8 H) 1.45 - 1.95 (m, 8 H) 3.10 (m, 1 H) 3.58 (br. s., 2 H) 3.95 (br. s., 1 H) 4.49 (br. s., 1 H) 6.84 (s, 1 H) 6.85 - 6.93 (m, 1 H) 8.29 (s, 1 H) 8.61 (br. s., 1 H).

Example 149:

Synthesis of Compound 149



Step 1: Compound 59 is Boc protected according to the method of A. Sarkar et al. (JOC, 2011, 76, 7132-7140).

Step 2: Boc-protected Compound 59 is treated with 5 mol% $NiCl_2(Ph_3)_2$, 0.1 eq triphenylphosphine, 3 eq Mn, 0.1 eq tetraethylammonium iodide, in DMI under CO_2 (1 atm) at 25 °C for 20 hours to convert the aryl halide derivative into the carboxylic acid.

Step 3: The carboxylic acid from Step 2 is converted to the corresponding acid chloride using standard conditions.

Step 4: The acid chloride from Step 3 is reacted with N-methyl piperazine to generate the corresponding amide.

Step 5: The amide from Step 4 is deprotected using trifluoroacetic acid in methylene chloride to generate the target compound. Compound 149 is purified by silica gel column chromatography eluting with a dichloromethane – methanol gradient to provide Compound 149.

Each of Compounds 119 through 147 and corresponding compounds with various R^8, R^1 and Z definitions may be reacted with sodium hydride and an alkyl halide or other halide to insert the desired R substitution prior to reaction with an amine, such as described above for the synthesis of Compound 120, to produce the desired product of Formulae I, II, III, IV, or V.

Example 150

CDK4/6 Inhibition *In Vitro* Assay

Selected compounds disclosed herein were tested in CDK4/cyclinD1, CDK2/CycA and CDK2/cyclinE kinase assays by Nanosyn (Santa Clara, CA) to determine their inhibitory effect on these CDKs. The assays were performed using microfluidic kinase detection technology (Caliper Assay Platform). The compounds were tested in 12-point dose-response format in singlicate at Km for ATP. Phosphoacceptor substrate peptide concentration used was 1 μM for all assays and Staurosporine was used as the reference compound for all assays. Specifics of each assay are as described below:

CDK2/CyclinA: Enzyme concentration: 0.2 nM; ATP concentration: 50 μM; Incubation time: 3 hr.

CDK2/CyclinE: Enzyme concentration: 0.28 nM; ATP concentration: 100 μM; Incubation time: 1 hr.

CDK4/CyclinD1: Enzyme concentration: 1 nM; ATP concentration: 200 μM; Incubation time: 10 hr.

The inhibitory IC_{50} values for the compounds in Table 1 for CDK4/CycD1, CDK2/CycE, CDK2/CycA, as well as fold selectivity are presented in Table 2.

Table 2: Selective Inhibition of CDK4

Structure	CDK4/ CycD1 IC_{50} [nM]	CDK2/ CycE IC_{50} [nM]	Fold Selectivity (CDK2/CycE/CDK4)	CDK2/CycA IC_{50} [nM]	Fold Selectivity (CDK2/CycA/CDK4)
A	4.2	6350	1516	3160	754
B	0.4	3040	6862	1890	4266
C	1.4	1920	1333	616	428
D	0.9	3480	3779	1500	1629
E	1	695	688	204	202
F	1.5	628	419	190	127
G	1.5	2580	1767	646	442
H	1.5	1520	1013	377	251
I	2	2120	1065	1130	568
J	0.7	5110	7707	4340	6546
K	1	1070	1019	738	703
L	5.7	4530	789	1490	260
M	2.3	2280	1004	1410	621
N	1	1500	1500	ND	ND
O	2.5	41410	1636	3150	1245
P	3.3	3560	1085	1010	308
Q	0.6	1080	1722	3030	4833
R	0.5	1920	3918	1360	2776
S	1.7	1250	718	342	197
T	0.8	1660	2022	1670	2034
U	0.7	1460	2229	857	1308
V	2.9	3500	1224	2130	745
W	2.7	3970	1481	539	201

Structure	CDK4/ CycD1 IC_{50} [nM]	CDK2/ CycE IC_{50} [nM]	Fold Selectivity (CDK2/CycE/CDK4)	CDK2/CycA IC_{50} [nM]	Fold Selectivity (CDK2/CycA/CDK4)
X	0.9	11600	12975	1840	2058
Y	2.5	124	50	61	25
Z	3.2	3710	1174	647	205
AA	0.5	6100	13319	4630	10109
BB	0.8	1680	2017	502	603
CC	1.6	1250	791	755	478
DD	1.9	9620	5200	8360	4519
EE	3.8	1660	432	1110	289
FF	1.2	4620	3949	1400	1197
GG	1	3580	3377	1510	1425
HH	1.7	1280	766	265	159
II	2	367	184	239	120
JJ	1.4	288	204	ND	ND
KK	2.3	1760	762	915	396
LL	2	202	103	108	55
MM	1.8	3390	1863	597	328
NN	3.7	4700	1274	1560	423
OO	9	3980	442	570	63
PP	3.1	3600	1146	3090	984
QQ	4.1	3060	746	2570	627
RR	1.2	1580	1374	693	603
SS	0.8	1460	1865	1390	1775
TT	0.8	1260	1550	596	733
UU	7.3	3960	542	ND	ND
VV	3.3	2630	809	789	243
WW	0.7	1350	204	ND	ND
XX	1.3	7300	5615	6290	4838
YY	4.6	6900	1490	ND	ND
ZZ	10.5	9960	949	ND	ND
AAA	2.3	6010	2591	2130	918

Structure	CDK4/ CycD1 IC_{50} [nM]	CDK2/ CycE IC_{50} [nM]	Fold Selectivity (CDK2/CycE/CDK4)	CDK2/CycA IC_{50} [nM]	Fold Selectivity (CDK2/CycA/CDK4)
BBB	2.8	187	68	85	31
CCC	2	2170	1074	457	226
DDD	9.5	9350	986	ND	ND
EEE	0.2	2950	1266	943	405
FFF	4.7	4540	966	1370	291
GGG	13.7	7610	555	ND	ND
HHH	6.8	2840	419	ND	ND
III	6	3770	626	ND	ND
JJJ	3.2	5200	1620	2830	882
KKK	1.3	291	231	87.3	69
LLL	3.2	1620	509	4530	1425
MMM	3.2	1890	600	990	314
NNN	1.4	2930	2154	1010	743
OOO	2.4	393	164	203	85
PPP	0.8	16500	21263	2640	3402
QQQ	10.5	11100	1057	ND	ND
RRR	2.6	4500	1758	ND	ND
SSS	2	2280	1112	1880	917
TTT	3.4	3030	899	ND	ND
UUU	18	16460	914	ND	ND
VVV	7.4	4380	589	ND	ND
WWW	18.5	2500	135	ND	ND
XXX	11.4	6620	581	ND	ND

To further characterize its kinase activity, Compound T was screened against 456 (395 non-mutant) kinases using DiscoveRx's KINOMEscan™ profiling service. The compound was screened using a single concentration of 1000 nM (> 1000 times the IC50 on CDK4). Results from this screen confirmed the high potency against CDK4 and high selectivity versus CDK2. Additionally, the kinome profiling showed that Compound T was relatively selective for CDK4 and CDK6 compared to the other kinases tested. Specifically, when using an inhibitory

threshold of 65%, 90%, or 99%, Compound T inhibited 92 (23.3%), 31 (7.8%) or 6 (1.5%) of 395 non-mutant kinases respectively.

In addition to CDK4 kinase activity, several compounds were also tested against CDK6 kinase activity. The results of the CDK6/CycD3 kinase assays, along with the CDK4/cyclinD1, CDK2/CycA and CDK2/cyclinE kinase assays, are shown for PD0332991 (Reference) and the compounds T, Q, GG, and U in Table 3. The IC_{50} of 10 nM for CDK4/cyclinD1 and 10uM for CDK12/ CyclinE agrees well with previously published reports for PD0332991 (Fry et al. Molecular Cancer Therapeutics (2004) 3(11)1427-1437; Toogood et al. Journal of Medicinal Chemistry (2005) 48, 2388-2406). Compounds T, Q, GG, and U are more potent (lower IC_{50}) with respect to the reference compound (PD0332991) and demonstrate a higher fold selectivity with respect to the reference compound (CDK2/CycE IC_{50} divided by CDK4/CycD1 IC_{50}).

Table 3: Inhibition of CDK kinases by Compounds T, Q, GG, and U

Formula	CDK4/CycD1 IC_{50}(nM)	CDK2/CycE IC_{50}(uM)	Fold Selectivity CDK2/CDK4	CDK2/CycA IC_{50}(uM)	CDK6/CycD3 IC50(nM)
PD0332991 Reference	10	10	1000	Not determined	Not determined
Compound T	0.821	1.66	2022	1.67	5.64
Compound Q	0.627	1.08	1722	3.03	4.38
Compound GG	1.060	3.58	3377	1.51	4.70
Compound U	0.655	1.46	2229	.857	5.99

Example 151

G1 Arrest (Cellular G1 and S-phase) Assay

To demonstrate the ability to synchronize the cell cycle of Rb-positive cells following administration of a CDK4/6 inhibitor, HS68 cells (human skin fibroblast cell line (Rb-positive)) were stained with propidium iodide staining solution and run on Dako Cyan Flow Cytometer. The fraction of cells in G0-G1 DNA cell cycle versus the fraction in S-phase DNA cell cycle was determined using FlowJo 7.2 .2 analysis.

The compounds listed in Table 1 were tested for their ability to arrest HS68 cells at the G1 phase of the cell cycle. From the results of the cellular G1 arrest assay, the range of the

inhibitory EC_{50} values necessary for G1 arrest of HS68 cells was from 22 nM to 1500 nM (see column titled "Cellular G1 Arrest EC_{50}" in Table 4).

Example 152

Inhibition of Cellular Proliferation

Cellular proliferation assays were further conducted using the following cancer cell lines: MCF7 (breast adenocarcinoma – Rb-positive), ZR-75-1 (breast ductal carcinoma - Rb-positive), H69 (human small cell lung cancer - Rb-negative) cells, or A2058 (human metastatic melanoma cells – Rb-negative). These cells were seeded in Costar (Tewksbury, Massachusetts) 3093 96 well tissue culture treated white walled/ clear bottom plates. Cells were treated with the compounds of Table 1 as nine point dose response dilution series from 10uM to 1nM. Cells were exposed to compounds and then cell viability was determined after either four (H69) or six (MCF7, ZR75-1, A2058) days as indicated using the CellTiter-Glo® luminescent cell viability assay (CTG; Promega, Madison, Wisconsin, United States of America) following the manufacturer's recommendations. Plates were read on BioTek (Winooski, Vermont) Syngergy2 multi-mode plate reader. The Relative Light Units (RLU) were plotted as a result of variable molar concentration and data was analyzed using Graphpad (LaJolla, Californaia) Prism 5 statistical software to determine the EC_{50} for each compound.

The results of the cellular inhibition assays for the two Rb-positive breast cancer cell lines (MCF7 and ZR75-1) are shown in Table 4. The range of the inhibitory EC_{50} values necessary for inhibition of MCF7 breast cancer cell proliferation was 28 nM to 257 nM. The range of the inhibitory EC_{50} values necessary for inhibition of ZR75-1 breast cancer cell proliferation was 24 nM to 581 nM.

In addition to breast cancer cell lines, a number of the compounds disclosed herein were also evaluated against a small cell lung cancer cell line (H69) and a human metastatic melanoma cell line (A2058), two Rb-deficient (Rb-negative) cell lines. The results of these cellular inhibition assays are shown in Table 4. The range of the inhibitory EC_{50} values necessary for inhibition of H69 small cell lung cancer cells was 2040 nM to >3000 nM. The range of the inhibitory EC_{50} values necessary for inhibition of A2058 malignant melanoma cell proliferation was 1313 nM to >3000 nM. In contrast to the significant inhibition seen on the two Rb-positive

breast cancer cell lines, it was found that the compounds tested were not significantly effective at inhibiting proliferation of the small cell lung cancer or melanoma cells.

Table 4: Inhibition of Cancer Cell Proliferation

Structure	Cellular G1 Arrest EC_{50} (nM)	MCF7 Cellular EC_{50} [nM]	ZR75-1 Cellular EC_{50} [nM]	H69 Cellular EC_{50} [nM]	A2058 Cellular EC_{50} [nM]
A	110	75	44	>3000	ND
B	90	201	245	ND	ND
C	95	88	73	ND	ND
D	50	57	46	2911	1670
E	75	53	62	2580	1371
F	175	ND	ND	ND	ND
G	175	ND	ND	ND	ND
H	85	85	120	2040	1313
I	80	61	40	2950	1062
J	110	70	82	>3000	>3000
K	28	43	ND	>3000	1787
L	65	506	ND	2161	>3000
M	100	ND	ND	ND	ND
N	25	28	24	>3000	1444
O	40	56	29	>3000	2668
P	30	60	43	>3000	>3000
Q	100	49	35	>3000	2610
R	70	36	50	>3000	2632
S	150	76	ND	>3000	>3000
T	100	49	36	>3000	>3000
U	25	70	59	>3000	>3000
V	70	50	29	>3000	1353
W	160	294	ND	>3000	>3000
X	65	ND	ND	>3000	>3000
Y	350	ND	ND	ND	ND

Structure	Cellular G1 Arrest EC_{50} (nM)	MCF7 Cellular EC_{50} [nM]	ZR75-1 Cellular EC_{50} [nM]	H69 Cellular EC_{50} [nM]	A2058 Cellular EC_{50} [nM]
Z	110	141	54	ND	ND
AA	70	47	47	>3000	ND
BB	75	ND	ND	2943	1635
CC	90	50	38	>3000	>3000
DD	100	ND	ND	ND	ND
EE	125	216	203	ND	ND
FF	80	140	ND	ND	ND
GG	80	52	62	2920	2691
HH	110	ND	ND	ND	ND
II	40	94	33	>3000	>3000
JJ	90	122	ND	>3000	>3000
KK	22	333	ND	2421	1379
LL	125	96	ND	>3000	>3000
MM	100	73	77	>3000	>3000
NN	110	ND	ND	ND	ND
OO	95	120	229	>3000	>3000
PP	100	164	66	ND	ND
QQ	120	ND	ND	>3000	>3000
RR	90	72	ND	2888	1617
SS	80	94	53	2948	1658
TT	75	ND	ND	ND	ND
UU	300	ND	ND	ND	ND
VV	200	ND	ND	ND	ND
WW	400	ND	ND	ND	ND
XX	225	ND	ND	ND	ND
YY	175	257	581	ND	ND
ZZ	500	ND	ND	ND	ND
AAA	275	320	ND	>3000	>3000
BBB	230	123	ND	>3000	>3000
CCC	250	ND	ND	ND	ND

Structure	Cellular G1 Arrest EC_{50} (nM)	MCF7 Cellular EC_{50} [nM]	ZR75-1 Cellular EC_{50} [nM]	H69 Cellular EC_{50} [nM]	A2058 Cellular EC_{50} [nM]
DDD	350	ND	ND	ND	ND
EEE	250	453	ND	>3000	>3000
FFF	650	ND	ND	ND	ND
GGG	350	ND	ND	ND	ND
HHH	250	ND	ND	ND	ND
III	250	ND	ND	ND	ND
JJJ	240	ND	ND	ND	ND
KKK	190	ND	ND	ND	ND
LLL	250	ND	ND	ND	ND
MMM	200	134	141	>3000	>3000
NNN	210	ND	ND	ND	ND
OOO	200	138	ND	>3000	>3000
PPP	275	ND	ND	ND	ND
QQQ	500	ND	ND	ND	ND
RRR	400	ND	ND	ND	ND
SSS	1500	ND	ND	ND	ND
TTT	350	ND	ND	ND	ND
UUU	300	ND	ND	ND	ND
VVV	300	ND	ND	ND	ND
WWW	300	ND	ND	ND	ND
XXX	300	ND	ND	ND	ND

Example 153

Synchronization of Rb-positive Cells

HS68 cells were seeded out at 40,000 cells/ well in 60mm dish on day 1 in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin/ streptomycin and 1x Glutamax (Invitrogen) as described (Brookes et al. EMBO J, 21(12)2936-2945 (2002) and Ruas et al. Mol Cell Biol, 27(12)4273-4282 (2007)). 24hrs post seeding, cells are treated with compound T, compound Q, compound GG, compound U, PD0332991, or DMSO vehicle alone at 300nM final concentration of test compounds. On day 3, one set of treated cell samples were harvested in

triplicate (0 Hour sample). Remaining cells were washed two times in PBS-CMF and returned to culture media lacking test compound. Sets of samples were harvested in triplicate at 24, 40, and 48 hours.

Alternatively, the same experiment was done using normal Renal Proximal Tubule Epithelial Cells (Rb-positive) obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in an incubator at 37°C in a humidified atmosphere of 5% CO2 in Renal Epithelial Cell Basal Media (ATCC) supplemented with Renal Epithelial Cell Growth Kit (ATCC) in 37°C humidified incubator.

Upon harvesting cells, samples were stained with propidium iodide staining solution and samples run on Dako Cyan Flow Cytometer. The fraction of cells in G0-G1 DNA cell cycle versus the fraction in S-phase DNA cell cycle was determined using FlowJo 7.2 .2 analysis.

Figure 1 shows cellular wash-out experiments which demonstrate the inhibitor compounds of the present invention have a short, transient G1-arresting effect in different cell types. Compounds T, Q, GG, and U were compared to PD0332991 in either human fibroblast cells (Rb-positive) (FIG. 1A & 1B) or human renal proximal tubule epithelial cells (Rb-positive) (FIG. 1C & 1D) and the effect on cell cycle following washing out of the compounds was determined at 24, 36, 40, and 48 hours.

As shown in Figure 1, using CDK4/6 inhibitors as described herein provides the ability to synchronize Rb-positive cells.

Example 154

Cell cycle arrest by compound T in CDK4/6-dependent cells

To test the ability of CDK4/6 inhibitors to induce a clean G1-arrest in Rb-positive cells, a cell based screening method was used consisting of two CDK4/6-dependent cell lines (tHS68 and WM2664; Rb-positive) and one CDK4/6-independent (A2058; Rb-negative) cell line. Twenty-four hours after plating, each cell line was treated with Compound T in a dose dependent manner for 24 hours. At the conclusion of the experiment, cells were harvested, fixed, and stained with propidium iodide (a DNA intercalator), which fluoresces strongly red (emission maximum 637 nm) when excited by 488nm light. Samples were run on Dako Cyan flow cytometer and > 10,000 events were collected for each sample. Data were analyzed using FlowJo 2.2 software developed by TreeStar, Inc.

In Figure 10A, results show that Compound T induces a robust G1 cell cycle arrest, as nearly all cells are found in the G0-G1 phase upon treatment with increasing amounts of Compound T. In Figure 10A, the results show that in CDK4/6-dependent cell lines, Compound T induced a robust G1 cell cycle arrest with an EC_{50} of 80 nM in tHS68 cells with a corresponding reduction in S-phase ranging from 28% at baseline to 6% at the highest concentration shown. Upon treatment with Compound T (300 nM), there was a similar reduction in the S-phase population and an increase in G1-arrested cells in both CDK4/6-dependent cell lines (tHS68 (Compare Figures 10B and 10E) and WM2664 (Compare Figures 10C and 10F)), but not in the CDK4/6-independent (A2058; Compare Figures 10D and 10G) cell line. The CDK4/6-independent cell line shows no effect in the presence of inhibitor.

Example 155

Compound T Inhibits Phosphorylation of RB

The CDK4/6-cyclin D complex is essential for progression from G1 to the S-phase of the DNA cell cycle. This complex phosphorylates the retinoblastoma tumor suppressor protein (Rb). To demonstrate the impact of CDK4/6 inhibition on Rb phosphorylation (pRb), Compound T was exposed to three cell lines, two CDK4/6 dependent (tHS68, WM2664; Rb-positive) and one CDK4/6 independent (A2058; Rb-negative). Twenty four hours after seeding, cells were treated with Compound T at 300nM final concentration for 4, 8, 16, and 24 hours. Samples were lysed and protein was assayed by western blot analysis. Rb phosphorylation was measured at two sites targeted by the CDK4/6-cyclin D complex, Ser780 and Ser807/811 using species specific antibodies. Results demonstrate that Compound T blocks Rb phosphorylation in Rb-dependent cell lines by 16 hours post exposure, while having no effect on Rb-independent cells (Figure 11).

Example 156

Differential Effects of a CDK4/6 Inhibitor on Hematopoietic Cells

Healthy C57BL/6 female mice received Compound T (50 mg or 100 mg) or vehicle control by i.p. injection. At the designated time: 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h or 48 h, mice received an injection of 5'-ethynyl-2'-deoxyuridine (EdU). Two hours post EdU injection, mice were sacrificed. As illustrated in Figures 12-19, cell lines were isolated and incorporation of EdU was measured. Hematopoietic cell lineages were identified using known

cell lineage markers. (See, for example, Challen, et al. "Mouse Hematopoietic Stem Cell Identification and Analysis", Cytometry A. (2009) 75(1): 14–24).

As evidence by Figures 12-19, hematopoietic cells are differentially inhibited by the same dose of CDK4/6 inhibitor based on cell-type. The different cell types examined include bone marrow cells, thymocytes, myeloid cells (Mac1+/Gr1+), B lymphoid cells (B220+), erythroid cells (Ter119+), myeloid progenitor cells (LK+: Lin- Sca-1- c-kit+), and hematopoietic stem cells (LSK+: Lin- Sca-1+ c-kit+). This differential inhibitory effect can be utilized to adjust the dose of the CDK4/6 inhibitor used during a treatment strategy to provide for inhibition of certain lineages, for example diseased hematological lineages, while not inhibiting others.

Example 157

Enhanced efficacy of a PI3 kinase inhibitor in combination with a CDK 4/6 inhibitor in a mouse model of breast cancer

The potential advantageous, additive, or synergistic effect of a PI3 kinase inhibitor (pictilisib; GDC-0941) in combination with a CDK 4/6 inhibitor (Compound GG) was examined in a mouse model of breast cancer. Immunodeficient mice were implanted with the human breast cancer cell line MCF7 (an Rb-positive cell line). Once the tumors reached sufficient size, the mice were randomized into treatment cohorts as outlined in Table 5.

Table 5. Cohort Table

Group	Subjects (N)	Agents	Dose (mg/kg/dose)	Route/Schedule
1	10	Vehicle	--	PO/QDX28
2	10	Compound GG	100	PO/QDXD1-D28
3	10	Compound GG	50	PO/QDXD1-D28
		GDC-0941	100	PO/QDXD15-D28
4	10	Compound GG	10	PO/QDXD1-D28
		GDC-0941	100	PO/QDXD15-D28
5	10	GDC-0941	100	IP/QDXD1-D14 PO/QDXD15-D28
6	10	Compound GG	100	PO/QDX28
		GDC-0941	100	IP/QDXD1-D14 PO/QDXD15-D28
7	10	PD0332991	100	PO/QDX28

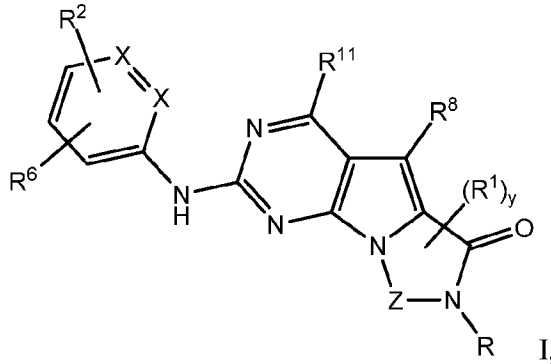
Mice were treated with vehicle control (days 1-28) (group 1), 100 mg/kg Compound GG (days 1-28) (group 2), 50 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (days 15-28) (group 3), 10 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (days 15-28) (group 4), 100 mg/kg GDC-0941 (IP dose days 1-14; PO dose days 15-28) (group 5), or 100 mg/kg Compound GG (days 1-28) and 100 mg/kg GDC-0941 (IP dose days 1-14; PO dose days 15-28) (group 6), and 100 mg/kg palbociclib (PD0332991) (days 1-28) (group 7). Compound GG was administered immediately prior to administration of GDC-0941. Tumors were measured twice a week for 60 days post treatment or until tumor burden endpoint was reached.

As seen in Figure 20, mice treated with a combination of 100 mg/kg Compound GG and 100 mg/kg GDC-0941 (pictilisib) for 28 days showed enhanced efficacy toward the MCF7 breast cancer cell line implants, as compared to mice treated with vehicle control, 100 mg/kg Compound GG, 100 mg/kg palbociclib, 50 mg/kg Compound GG and 100 mg/kg GDC-0941, 10 mg/kg Compound GG and 100 mg/kg GDC-0941, or 100 mg/kg GDC-0941. As shown in Figure 21, peripheral blood from mice in compound GG cohorts was obtained on day 14 and CBCs were analyzed. Specific cell counts analyzed were white blood cells (WBC: Fig. 21A), lymphocytes (Fig. 21B), neutrophils (Fig. 21C), monocytes (Fig. 21D), platelets (Fig. 21E), and red blood cells (Fig. 21F). As shown in Figure 21, white blood cells, lymphocytes, neutrophils, platelets, and red blood cells showed reduced cell counts on Day 14 in mice treated with 100 mg/kg Compound GG.

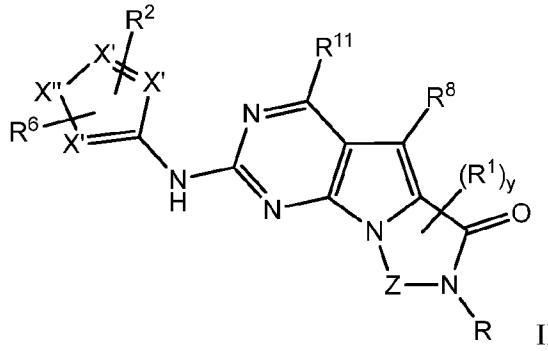
This specification has been described with reference to embodiments of the invention. The invention has been described with reference to assorted embodiments, which are illustrated by the accompanying Examples. The invention can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Given the teaching herein, one of ordinary skill in the art will be able to modify the invention for a desired purpose and such variations are considered within the scope of the invention.

We claim:

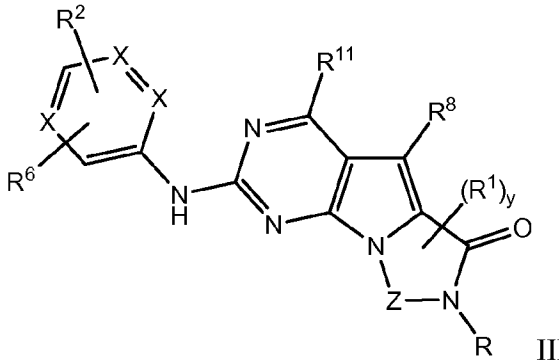
1. A method for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a Bruton's tyrosine kinase (BTK) inhibitor to a host in need thereof, wherein the Bruton's tyrosine kinase (BTK) inhibitor is selected from ibrutinib or ACP-196, wherein Formula I, II, III, IV, or V has the structure:



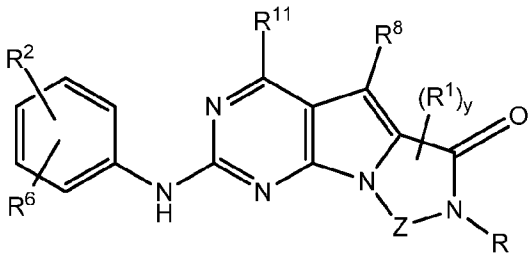
I,



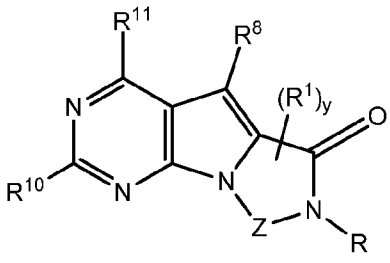
II,



III,



IV, or



V,

wherein:

Z is $-(CH_2)_x-$ wherein x is 1, 2, 3 or 4 or $-O-(CH_2)_z-$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is $-(alkylene)_m-$heterocyclo, $-(alkylene)_m-$heteroaryl, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$; $-(alkylene)_m-C(O)-O$-alkyl; $-(alkylene)_m-O-R^5$, $-(alkylene)_m-S(O)_n-R^5$, or $-(alkylene)_m-S(O)_n-NR^3R^4$ any of which may be optionally

independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^{5*} at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, -(alkylene)$_m$-OR^5, -(alkylene)$_m$-O-alkylene-OR^5, -(alkylene)$_m$-S(O)$_n$–R^5, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-CN, -(alkylene)$_m$-C(O)–R^5, -(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5, -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups

may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*, -(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*, -(alkylene)$_m$-SO$_2$–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO$_2$–R^5*, -(alkylene)$_m$-N(R^3*)–SO$_2$–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*, -(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO$_2$–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

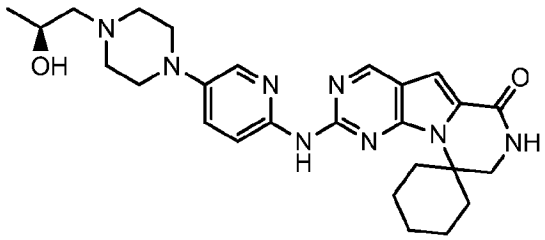
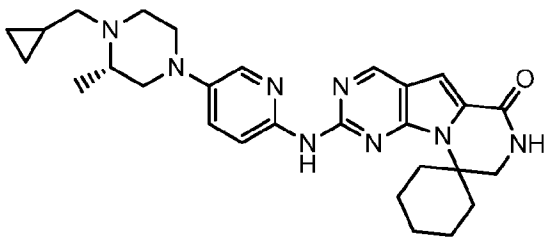
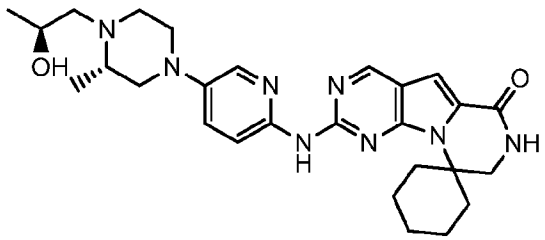
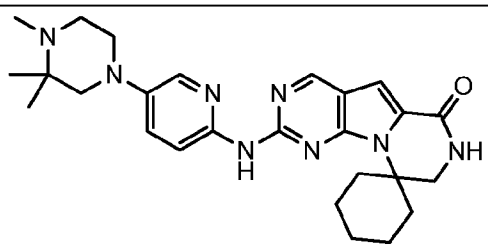
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -(alkylene)$_m$-heteroaryl, -(alkylene)$_m$-NR^{3}R^4, -(alkylene)$_m$-C(0)-NR^{3}R^4; -(alkylene)$_m$-0-R^5, -(alkylene)$_m$-S(0)$_n$-R^5, or -(alkylene)$_m$-S(0)$_n$-NR^{3}R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

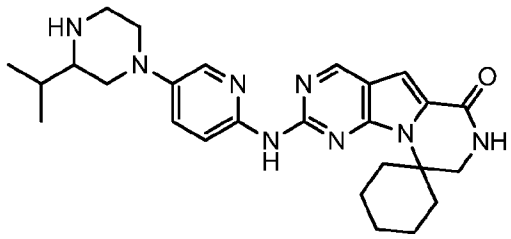
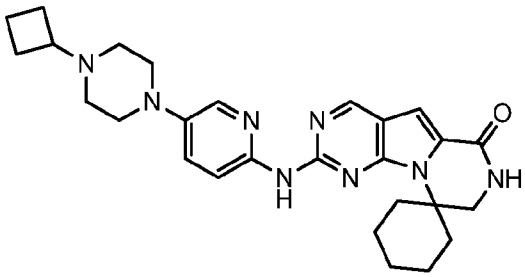
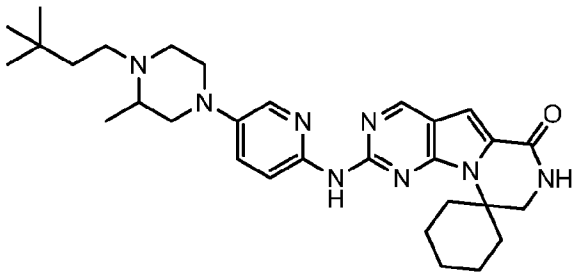
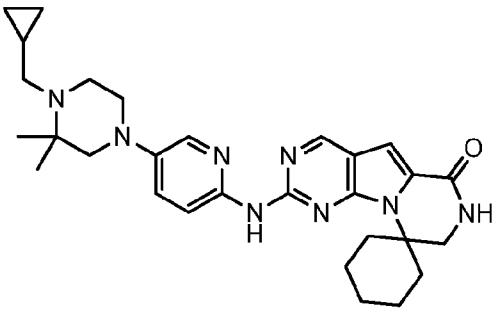
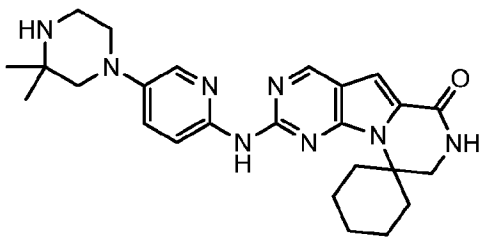
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C$_1$-C$_8$ alkyl, cycloalkylalkyl, or –TT-RR, C$_1$-C$_8$ cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C$_1$-C$_8$ alkyl or C$_3$-C$_8$ cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C$_1$-C$_6$ alkoxy, amino, unsubstituted or substituted C$_1$-C$_6$ alkylamino, unsubstituted or substituted di-C$_1$-C$_6$ alkylamino, unsubstituted or substituted C$_6$-C$_{10}$

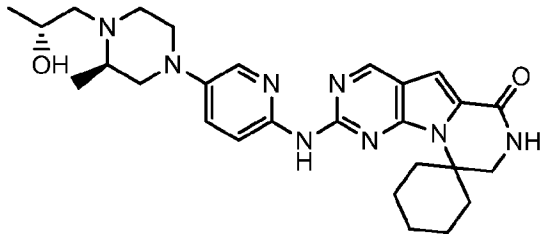
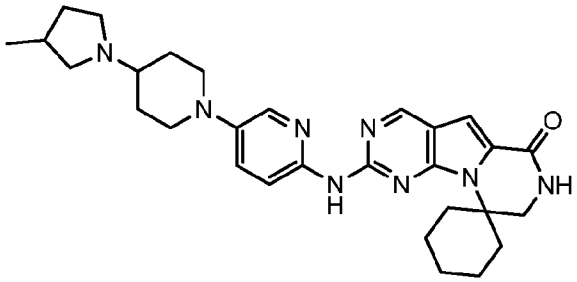
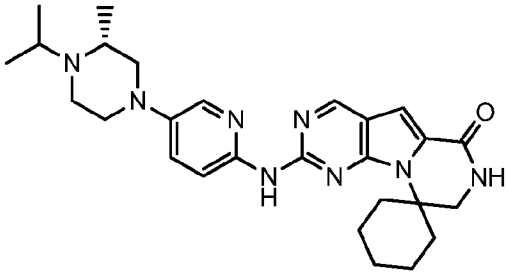
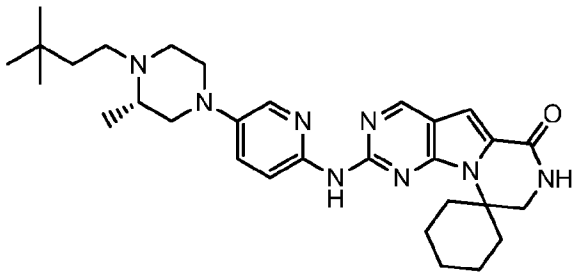
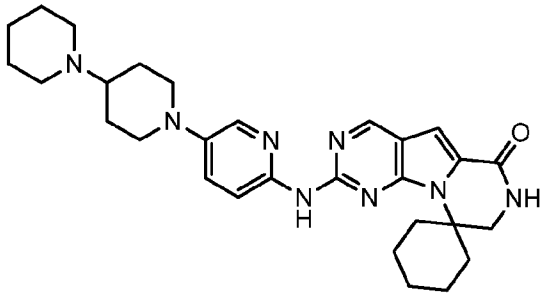
aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13} , wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

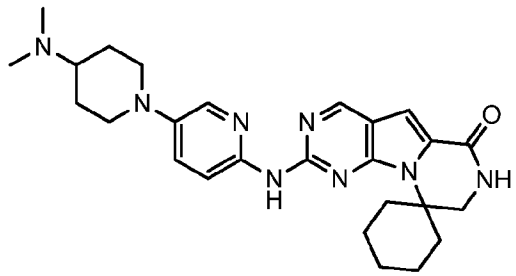
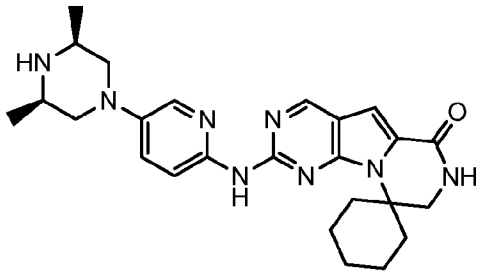
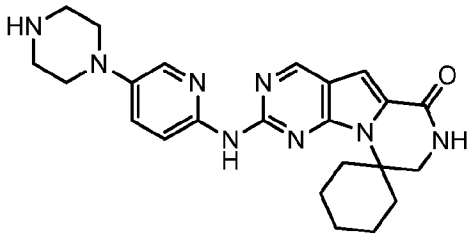
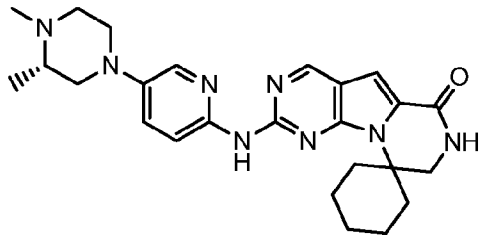
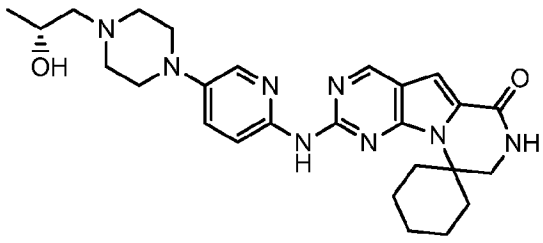
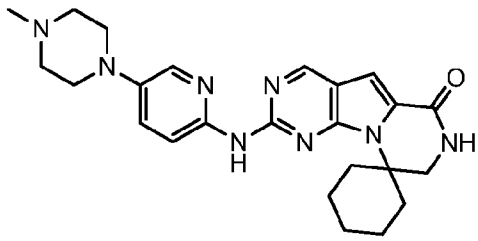
or a pharmaceutically acceptable salt thereof.

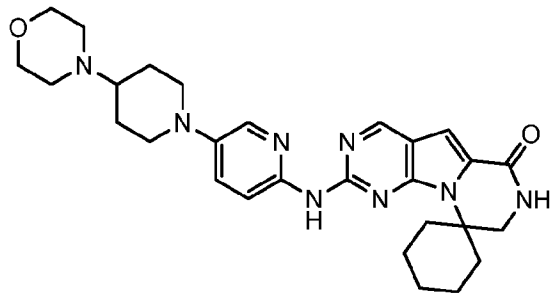
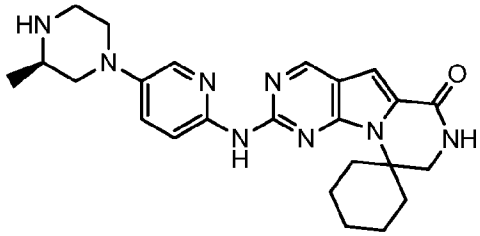
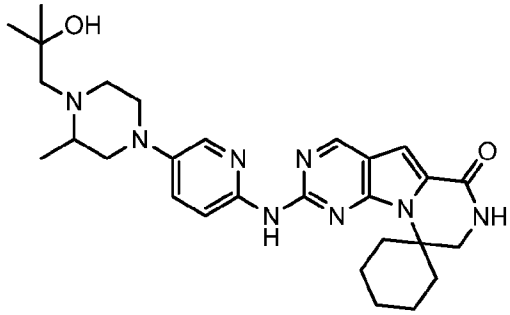
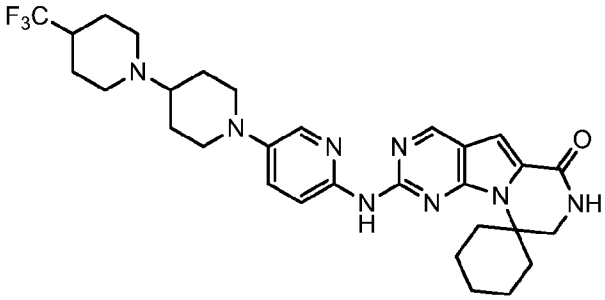
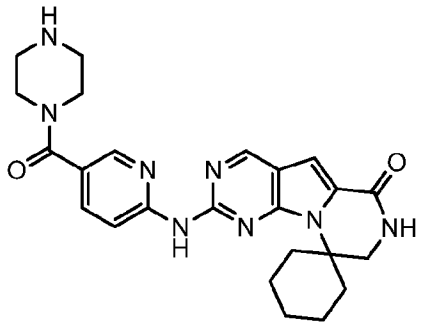
2. The method of claim 1, wherein the CDK4/6 inhbitor compound is selected from the group consisting of:

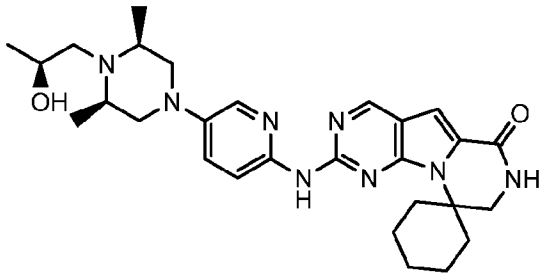
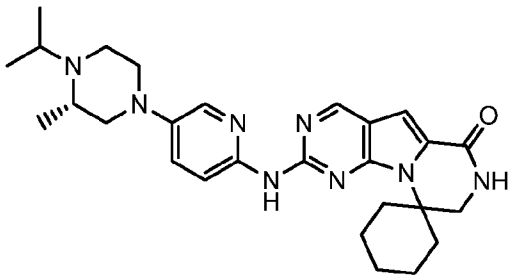
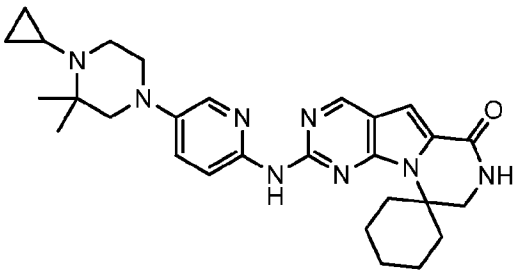
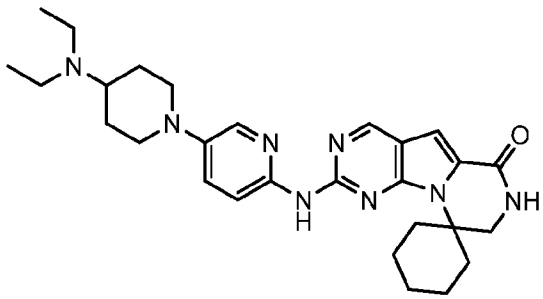
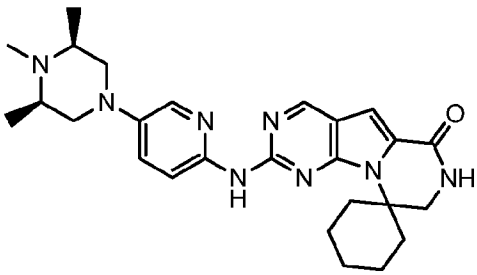
Structure Reference	Structure
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D	

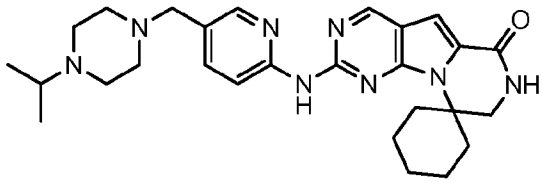
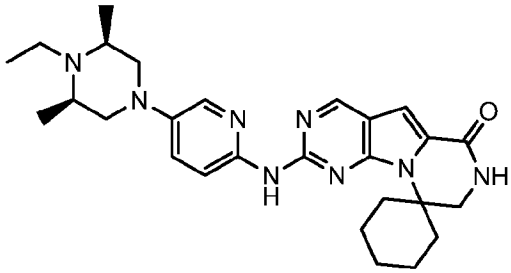
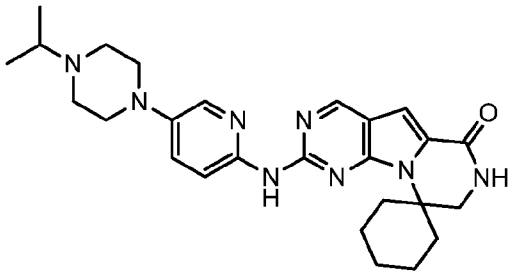
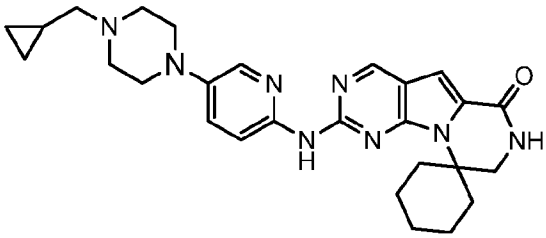
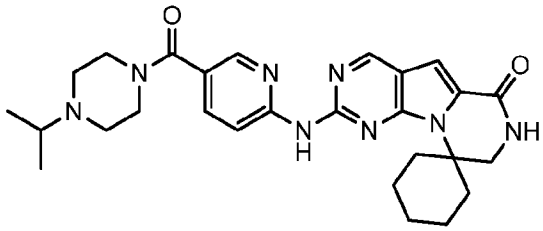
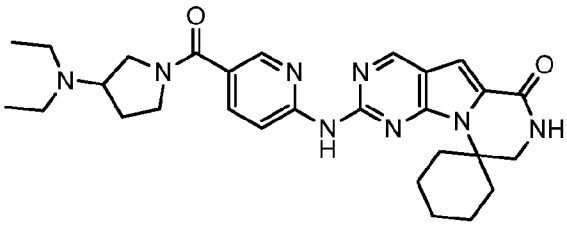
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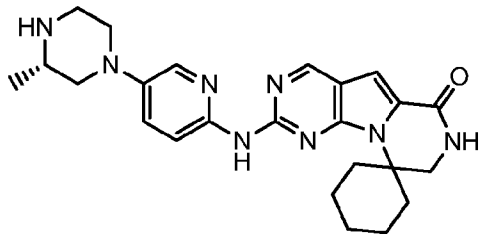
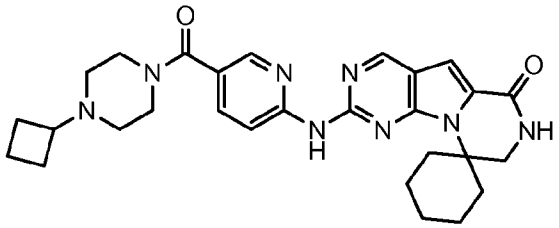
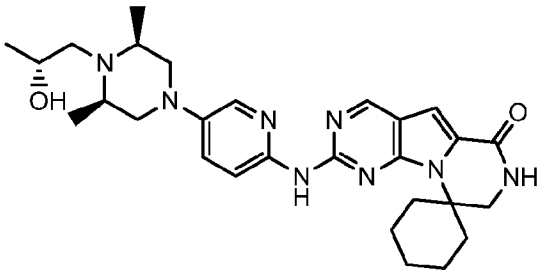
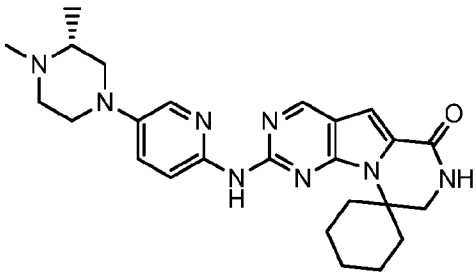
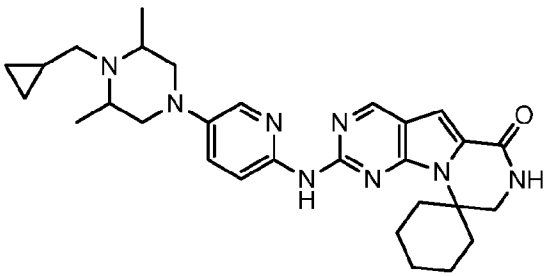
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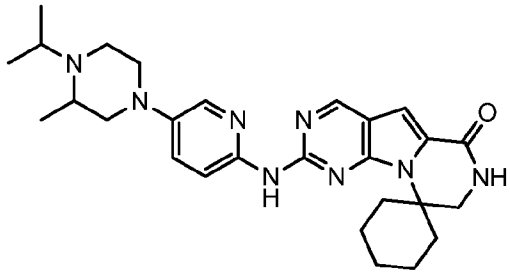
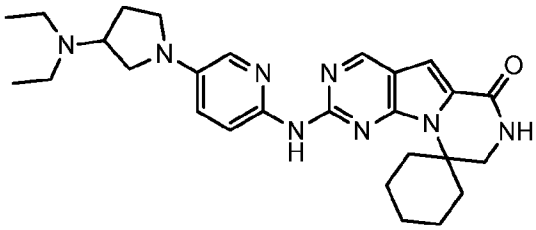
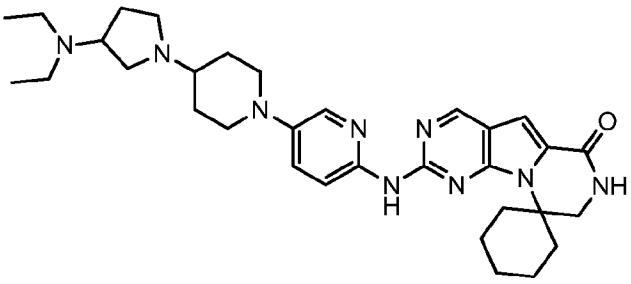
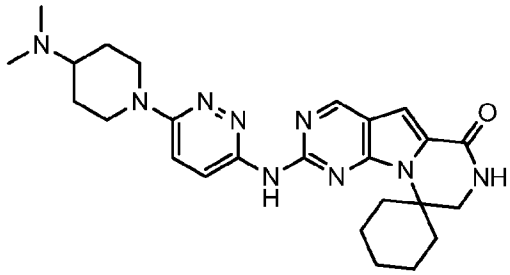
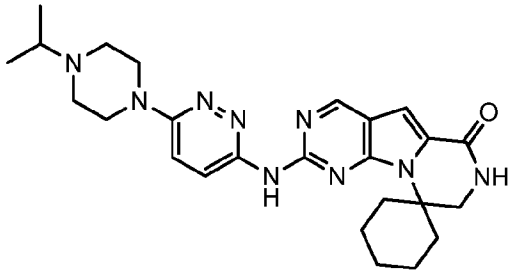
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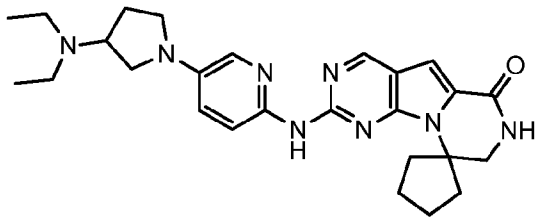
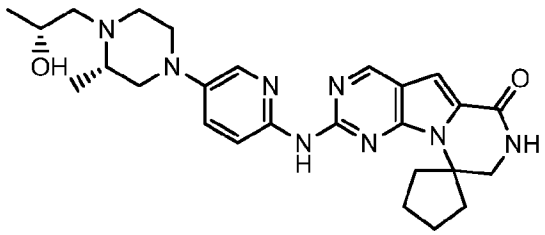
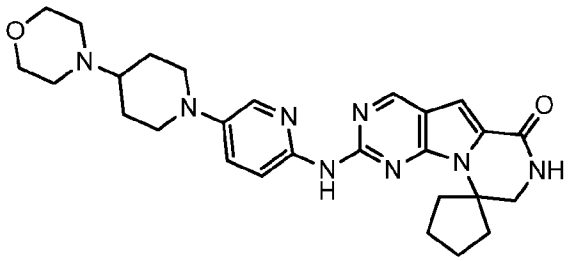
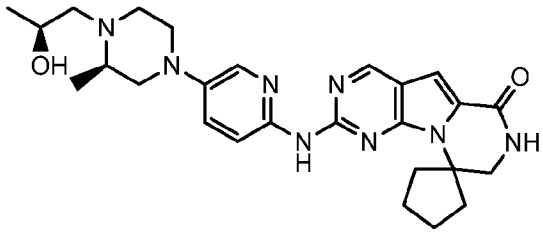
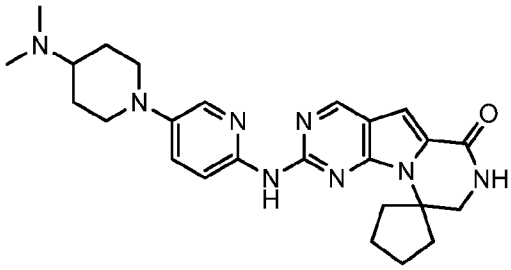
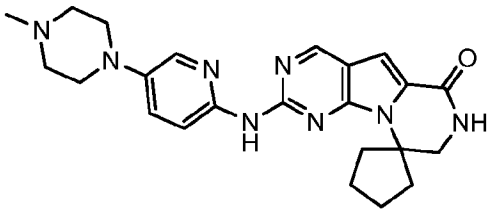
Structure Reference	Structure
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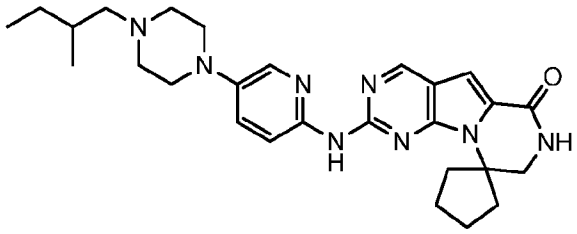
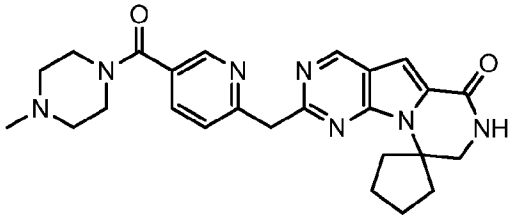
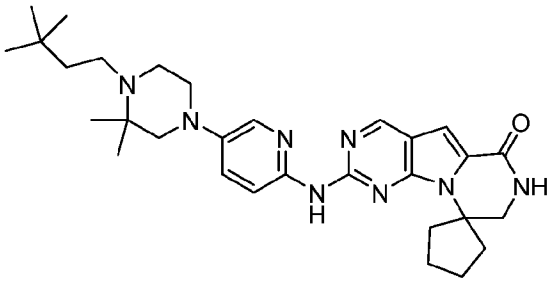
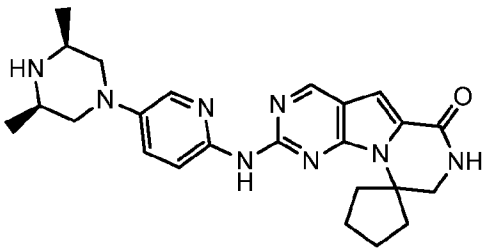
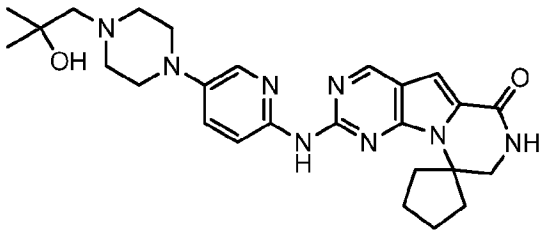
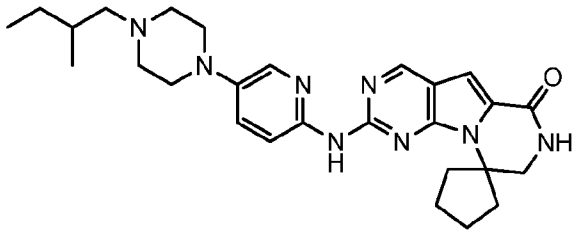
Structure Reference	Structure
Z	
AA	
BB	
CC	
DD	

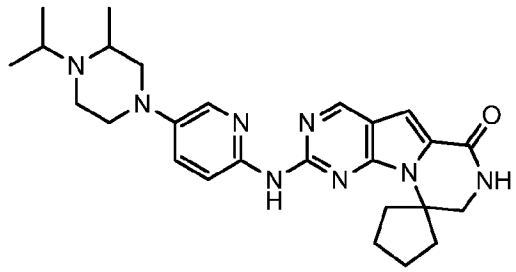
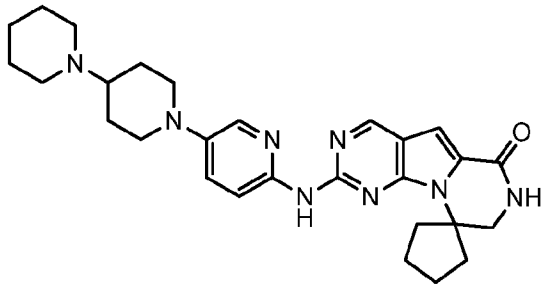
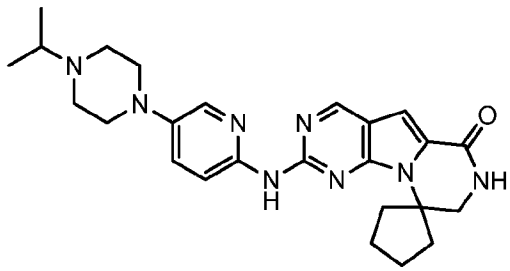
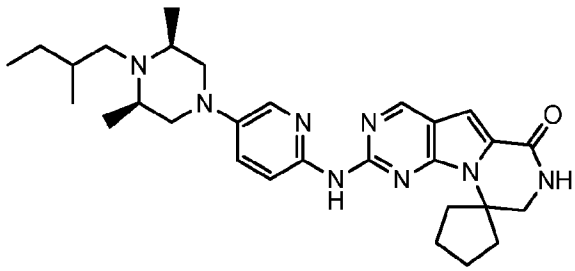
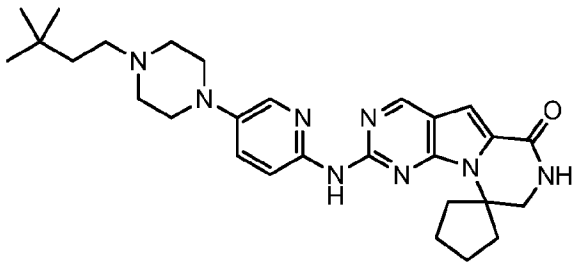
Structure Reference	Structure
EE	
FF	
GG	
HH	
II	
JJ	

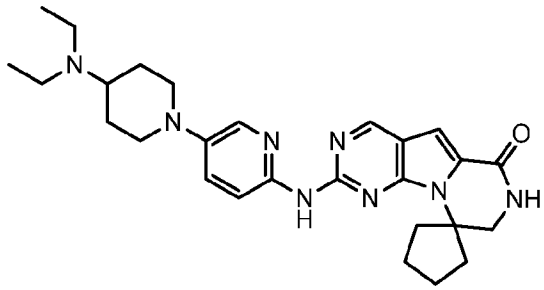
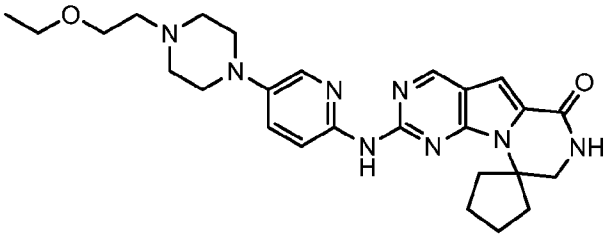
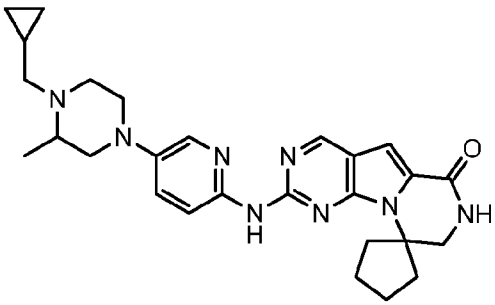
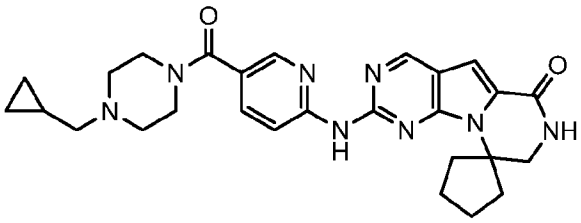
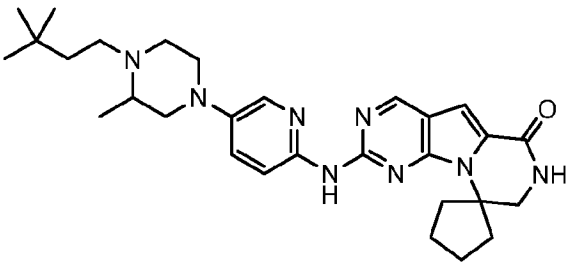
Structure Reference	Structure
KK	
LL	
MM	
NN	
OO	

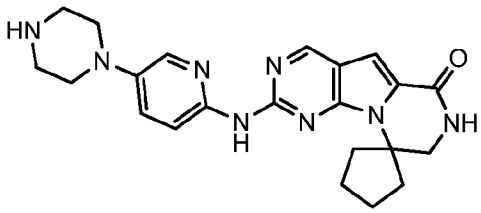
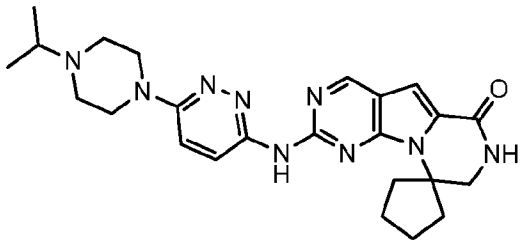
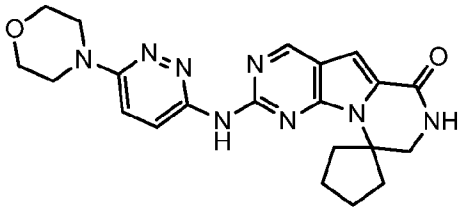
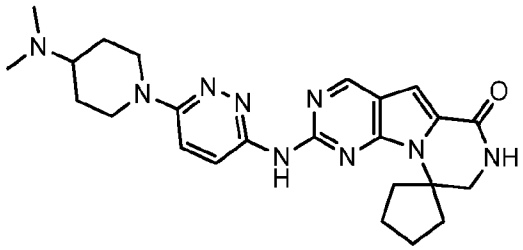
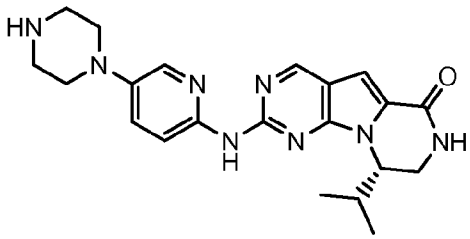
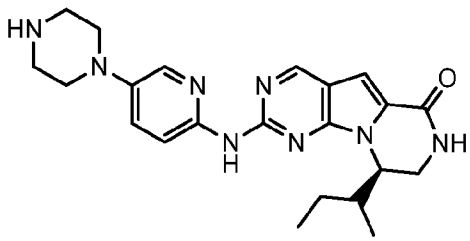
Structure Reference	Structure
PP	
QQ	
RR	
SS	
TT	

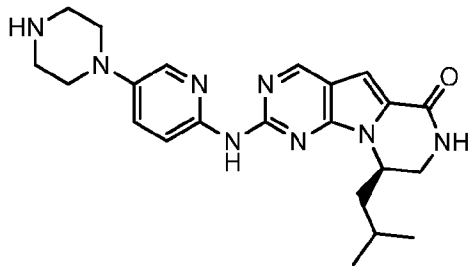
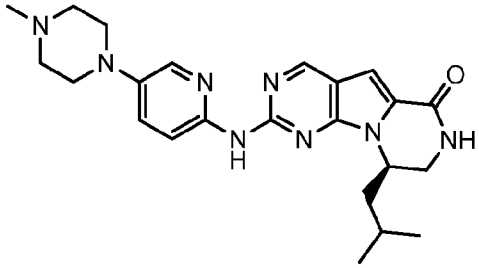
Structure Reference	Structure
UU	
VV	
WW	
XX	
YY	
ZZ	

Structure Reference	Structure
AAA	
BBB	
CCC	
DDD	
EEE	
FFF	

Structure Reference	Structure
GGG	
HHH	
III	
JJJ	
KKK	

Structure Reference	Structure
LLL	
MMM	
NNN	
OOO	
PPP	

Structure Reference	Structure
QQQ	
RRR	
SSS	
TTT	
UUU	
VVV	

Structure Reference	Structure
WWW	
XXX	

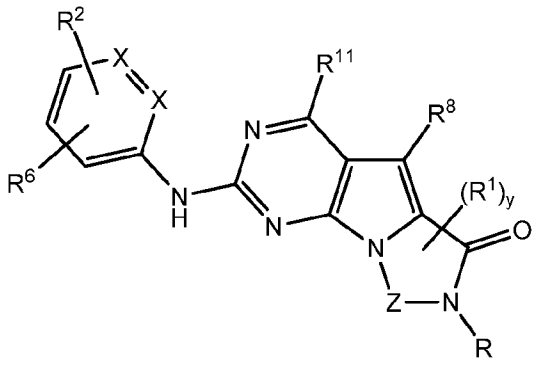
3. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.
4. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.
5. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.
6. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.
7. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.
8. The method of claim 2, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.
9. The method of claim 2, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.
10. The method of claim 2, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.

11. The method of claim 2, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.
12. The method of any of claims 1-11, wherein the Rb-positive cancer is a solid tumor.
13. The method of any of claims 1-12, wherein the solid tumor is selected from non-small cell lung cancer, breast cancer, colorectal cancer, liver cancer, ovarian cancer, or glioblastoma.
14. The method of claim 13, wherein the solid tumor is Erb-2/human epidermal growth factor receptor (HER)-2-positive breast cancer.
15. The method of any of claims 1-11, wherein the Rb-positive cancer is a hematological cancer.
16. The method of claim 15, wherein the hematological cancer is acute myeloid leukemia (AML).
17. The method of claim 15, wherein the hematological cancer is a T-cell cancer.
18. The method of any of claims 1-17, wherein the CDK 4/6 inhibitor compound is conjugated to a targeting agent.
19. The method of claim 18, wherein the targeting agent is an antibody or antibody fragment.
20. The method of any of claims 1-18, wherein the compound is conjugated to a radioisotope.
21. The method of claim 1-20, wherein the host is a human.
22. A method for the treatment of an Rb-positive cancer in a host comprising:

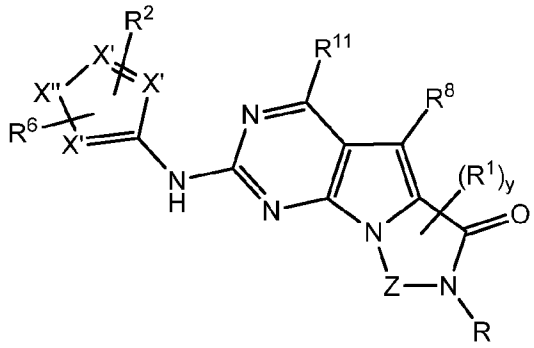
a) administering to the host having an Rb-positive cancer an effective amount of a CDK 4/6 inhibitor compound of the Formula I, II, III, IV, or V, wherein the compound is capable of arresting a significant portion of the Rb-positive abnormal cells in the G0 or G1 phase of the cell cycle;

b) administering to the host at least one additional chemotherapeutic agent within about 48 hours of administration of the CDK 4/6 inhibitor;

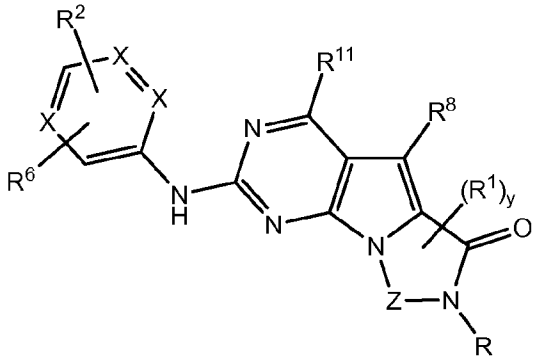
wherein the compound of the Formula I, II, III, IV, or V has the following structure:



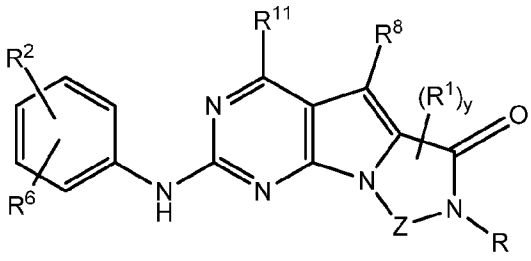
I,



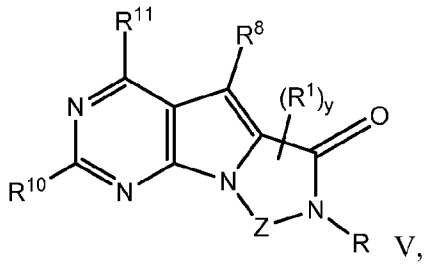
II,



III,



IV, or



V,

wherein:

Z is $–(CH_2)_x–$ wherein x is 1, 2, 3 or 4 or $–O–(CH_2)_z–$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is $–(alkylene)_m–$heterocyclo, $–(alkylene)_m–$heteroaryl, $–(alkylene)_m–NR^3R^4$, $–(alkylene)_m–C(O)–NR^3R^4$; $–(alkylene)_m–C(O)–O$-alkyl; $–(alkylene)_m–O–R^5$, $–(alkylene)_m–S(O)_n–R^5$, or $–(alkylene)_m–S(O)_n–NR^3R^4$ any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^5* at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, -(alkylene)$_m$-OR^5, -(alkylene)$_m$-O-alkylene-OR^5, -(alkylene)$_m$-S(O)$_n$–R^5, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-CN, -(alkylene)$_m$-C(O)–R^5, -(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5, -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*,

-(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*,
-(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*,
-(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*,
-(alkylene)$_m$-SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,
-(alkylene)$_m$-N(R^3*)–SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*,
-(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

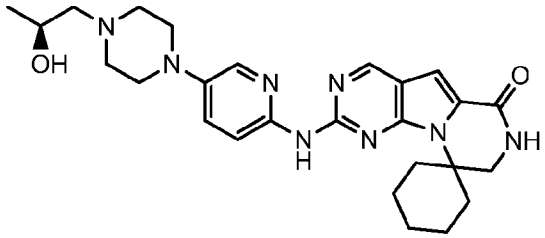
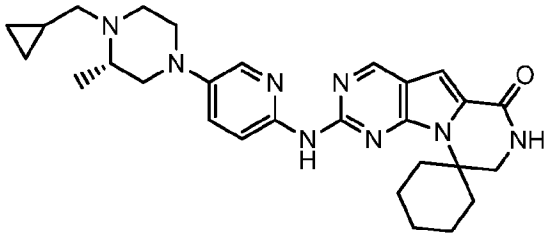
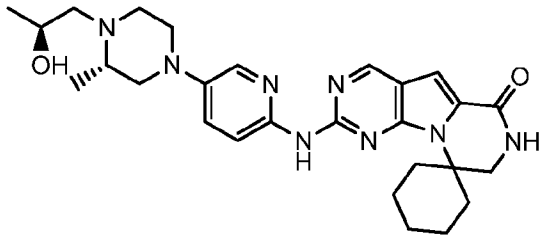
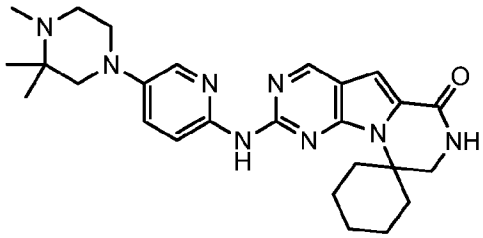
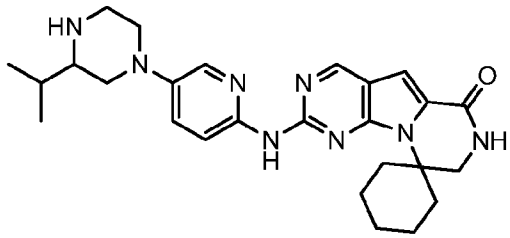
(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

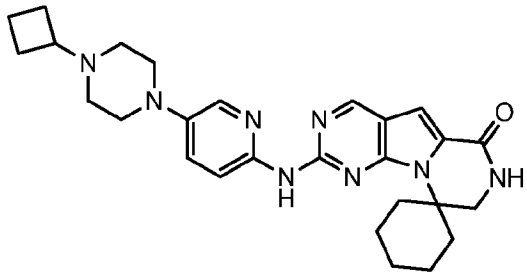
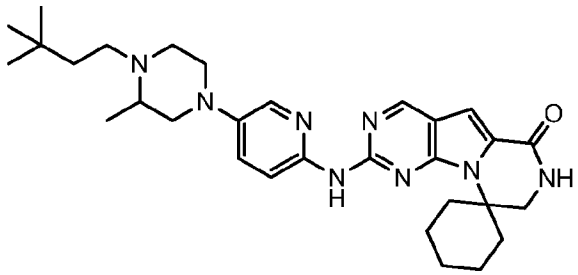
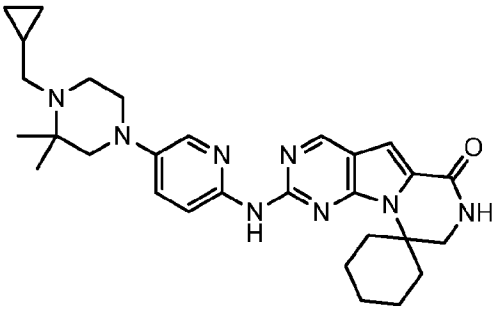
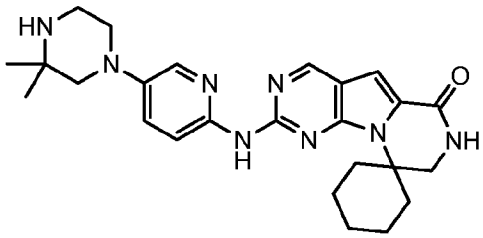
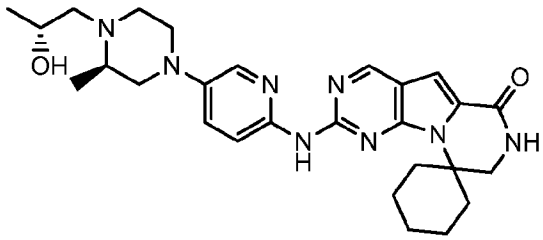
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -(alkylene)$_m$-heteroaryl, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(0)-NR^3R^4; -(alkylene)$_m$-0-R^5, -(alkylene)$_m$-S(0)$_n$-R^5, or -(alkylene)$_m$-S(0)$_n$-NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

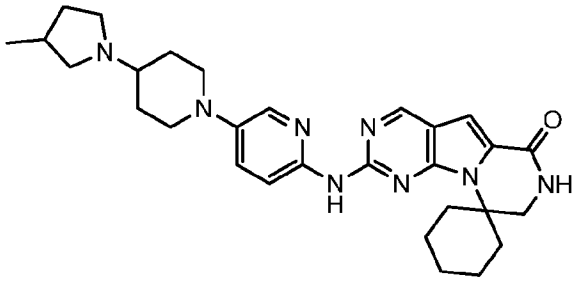
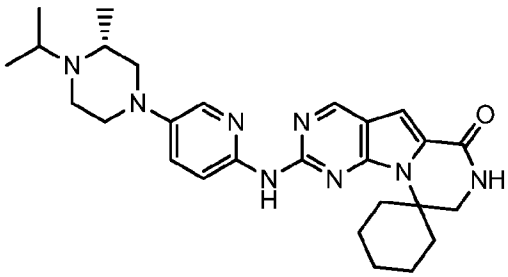
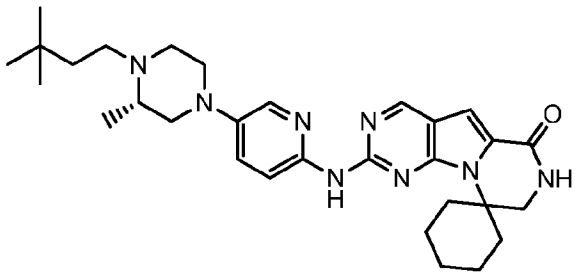
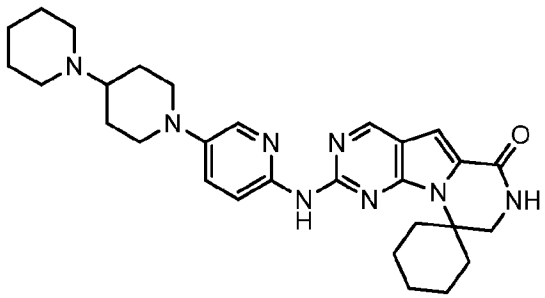
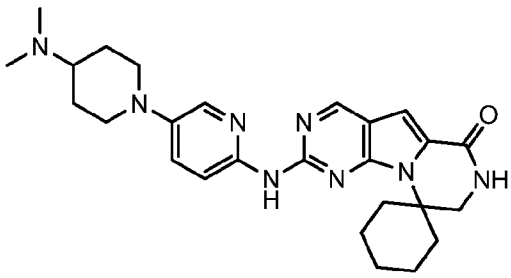
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C_1-C_8 alkyl, cycloalkylalkyl, or –TT-RR, C_1-C_8 cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C_1-C_8 alkyl or C_3-C_8 cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C_1-C_6 alkoxy, amino, unsubstituted or substituted C_1-C_6 alkylamino, unsubstituted or substituted di-C_1-C_6 alkylamino, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13} , wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

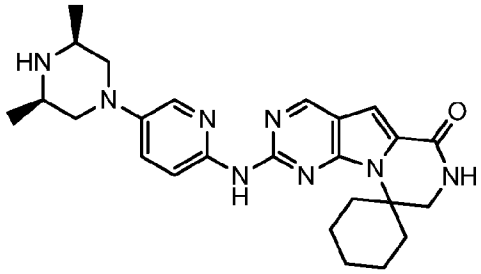
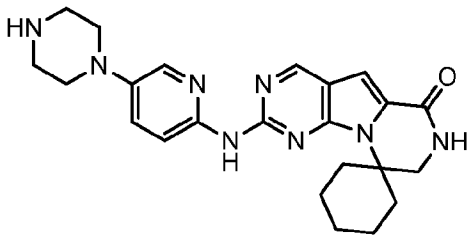
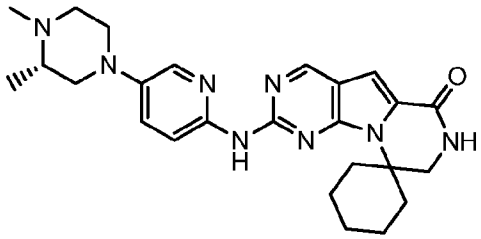
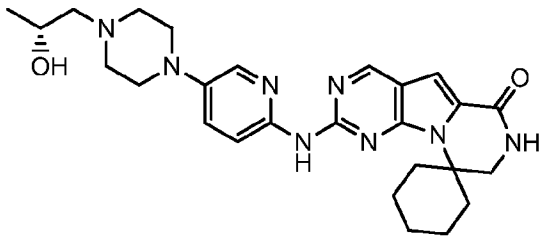
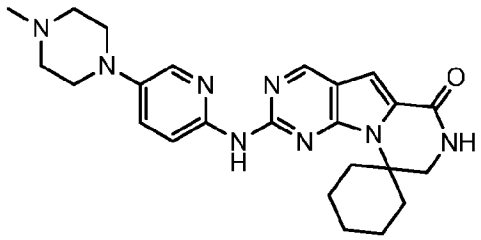
or a pharmaceutically acceptable salt thereof.

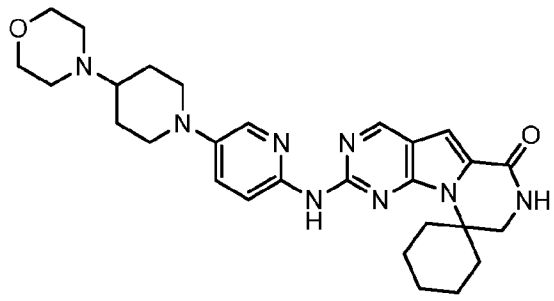
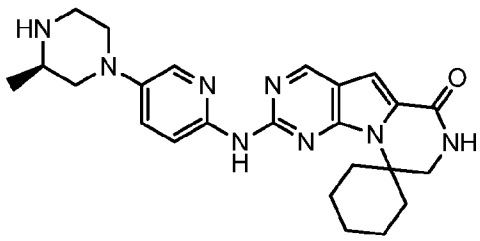
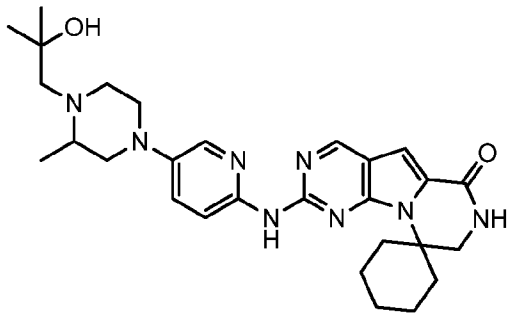
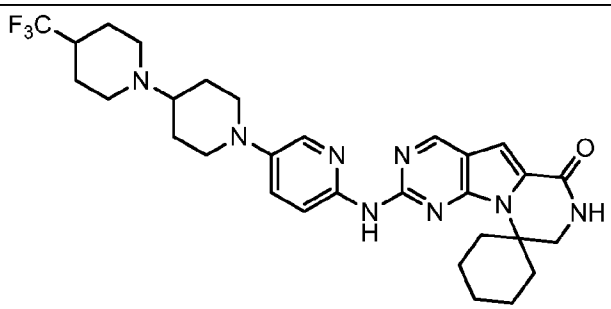
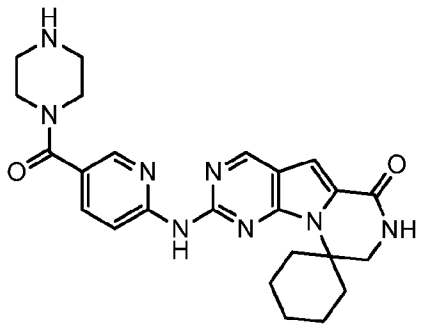
23. The method of claim 22, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of:

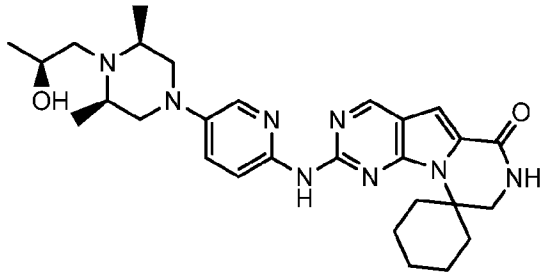
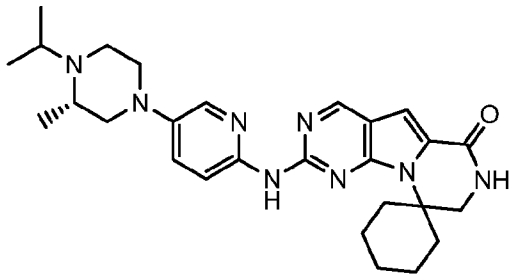
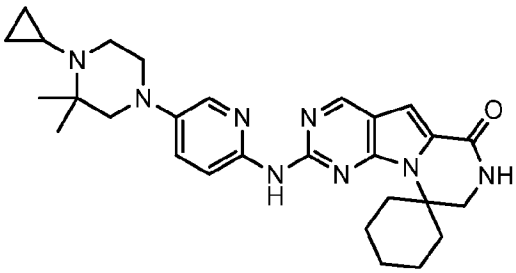
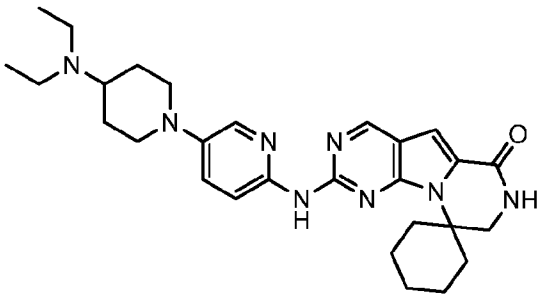
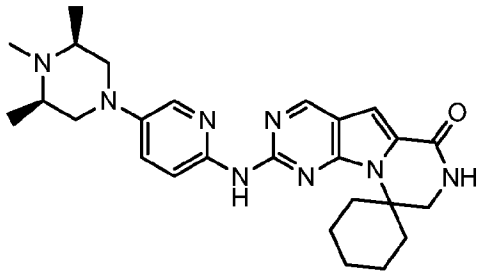
Structure Reference	Structure
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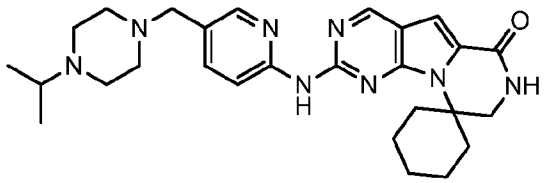
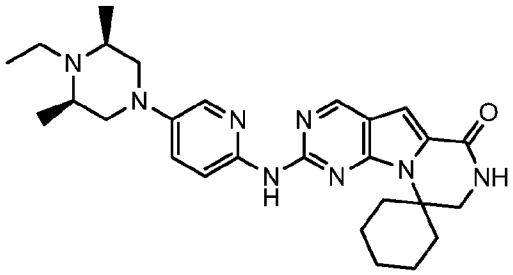
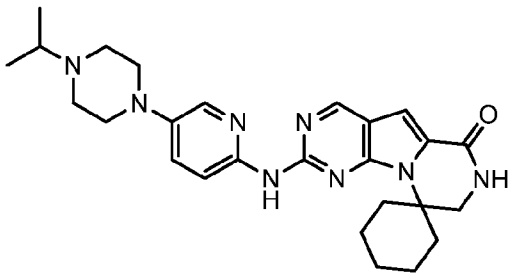
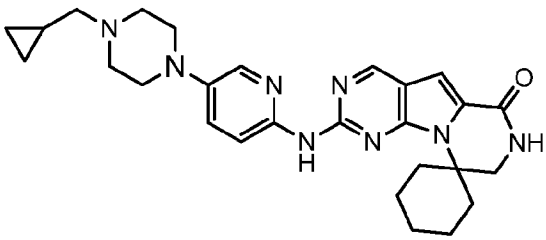
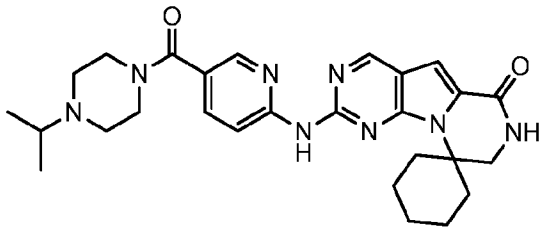
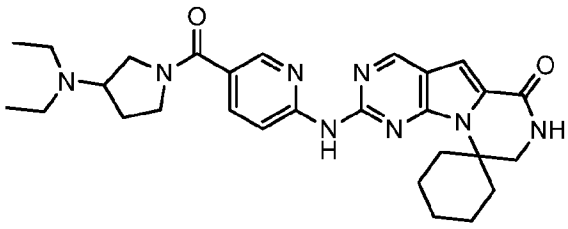
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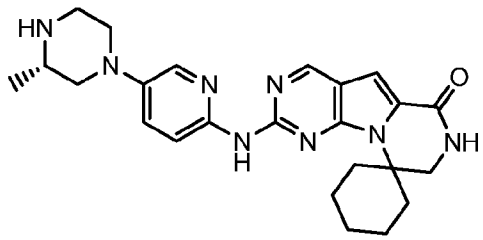
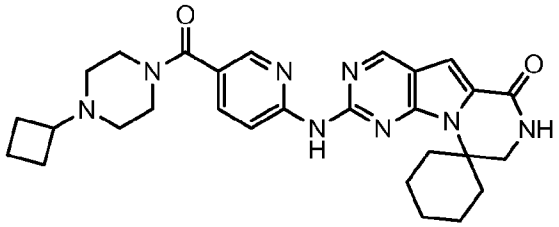
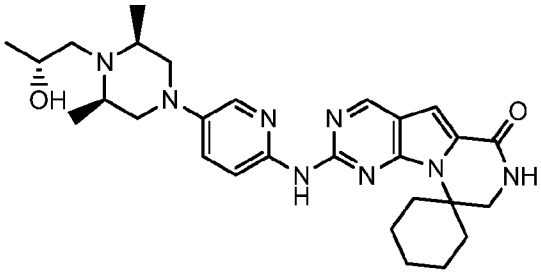
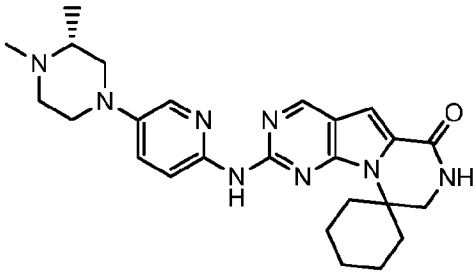
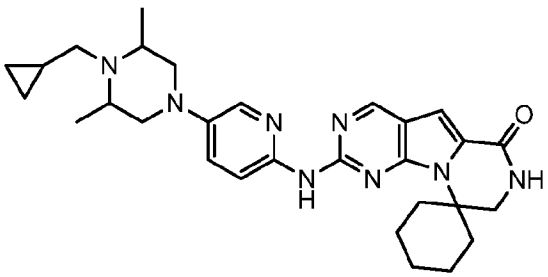
Structure Reference	Structure
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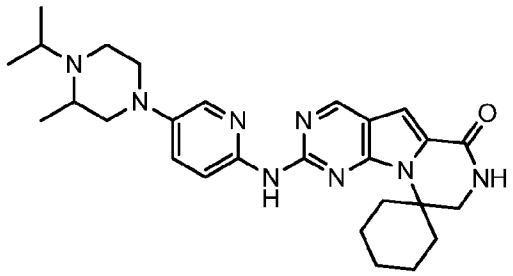
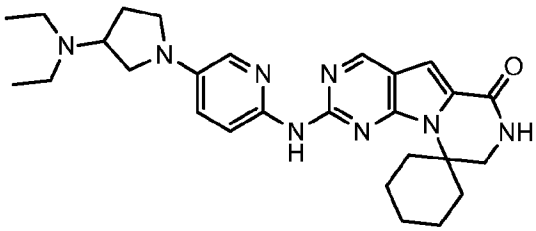
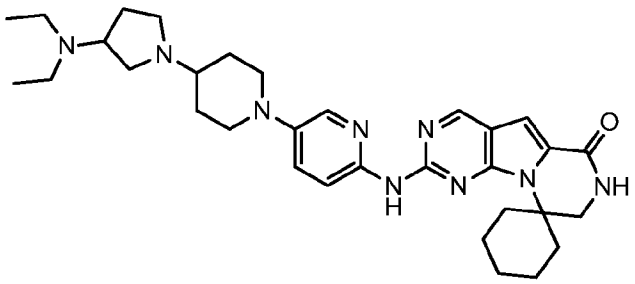
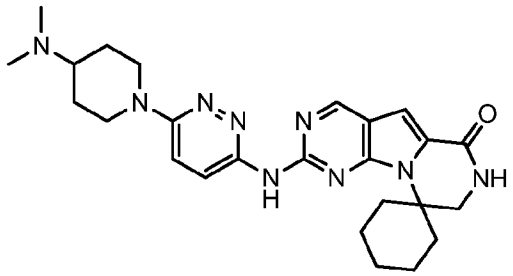
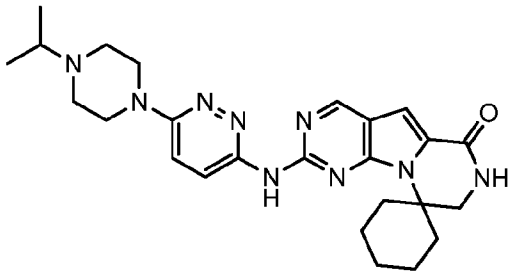
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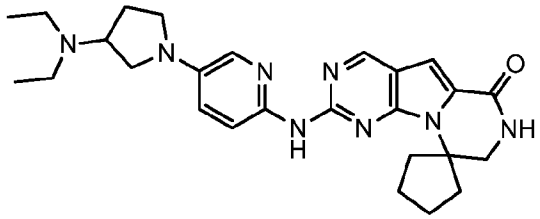
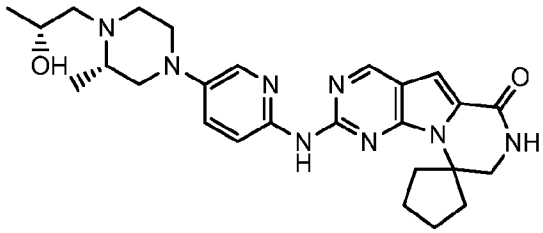
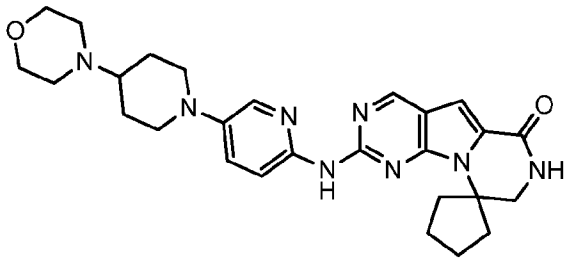
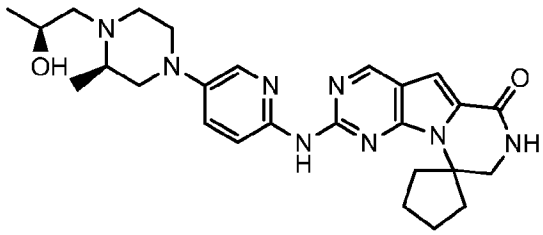
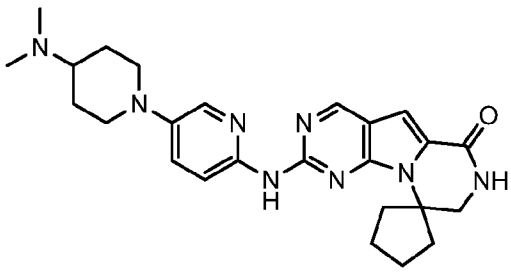
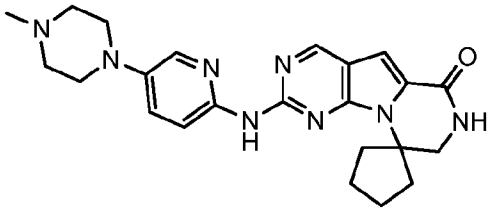
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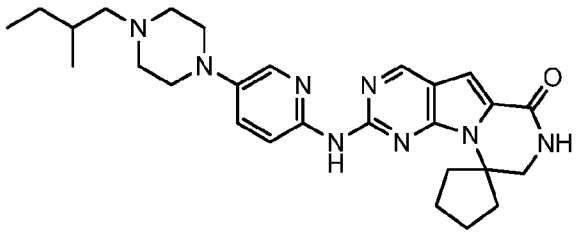
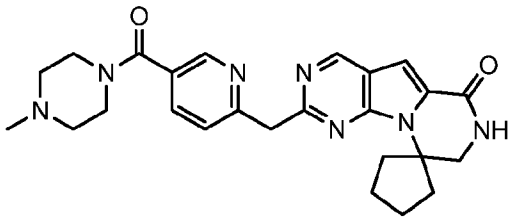
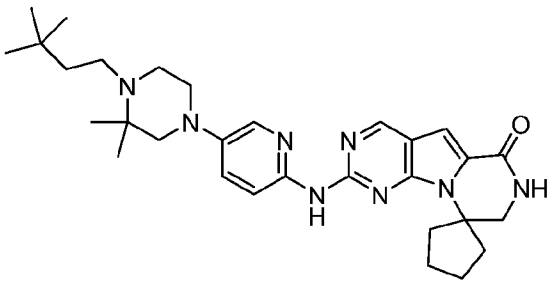
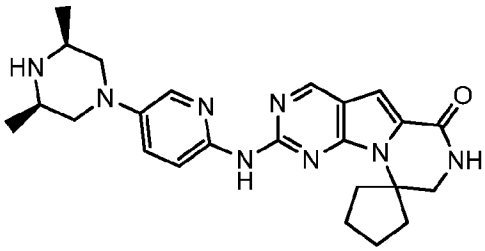
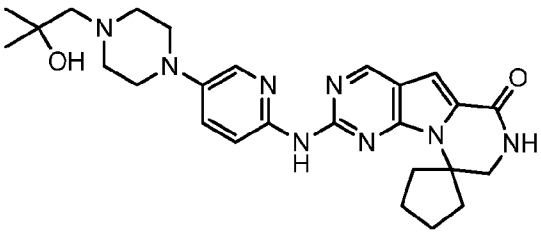
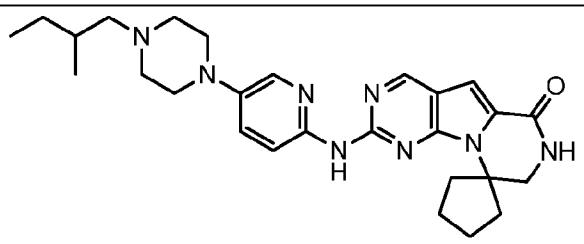
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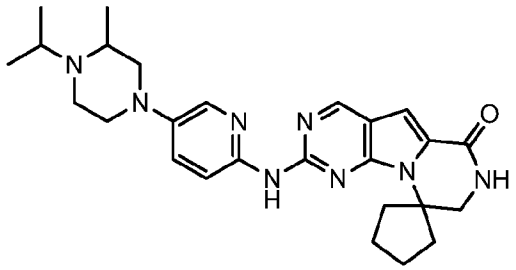
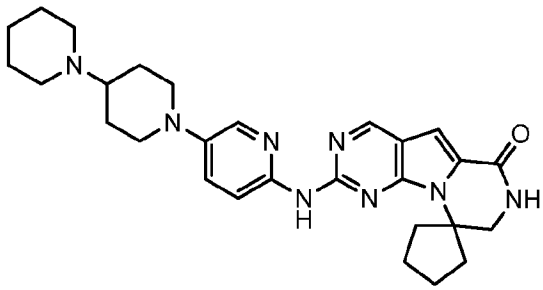
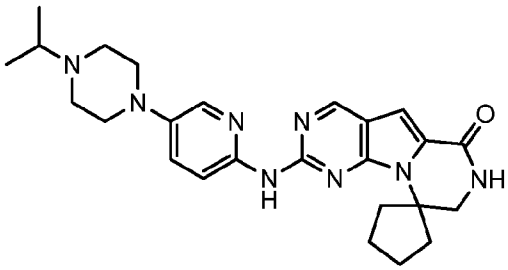
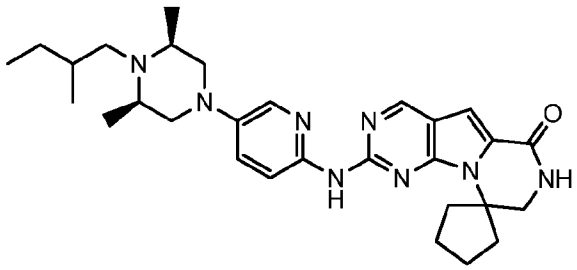
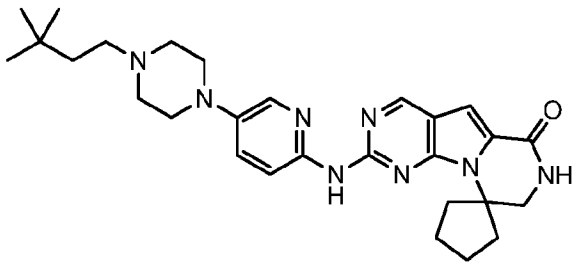
Structure Reference	Structure
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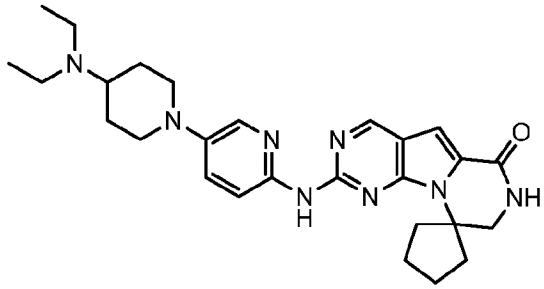
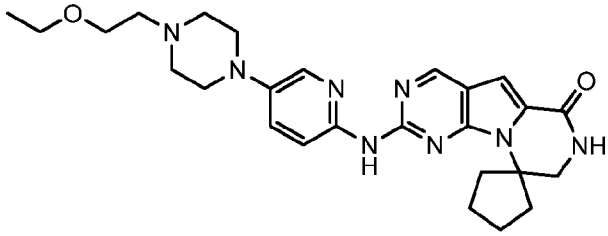
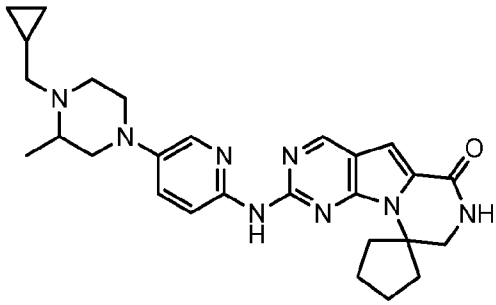
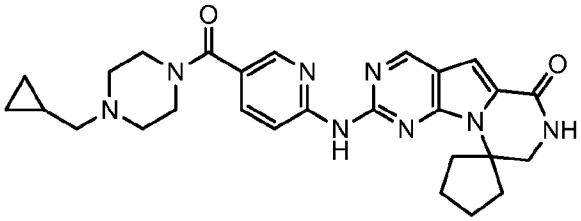
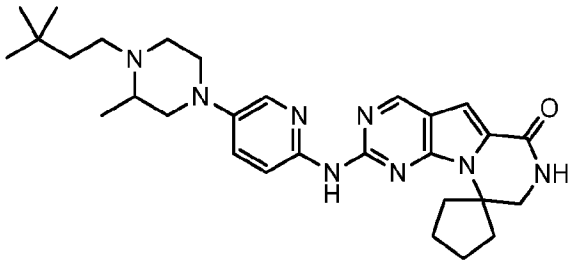
Structure Reference	Structure
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NN	
OO	

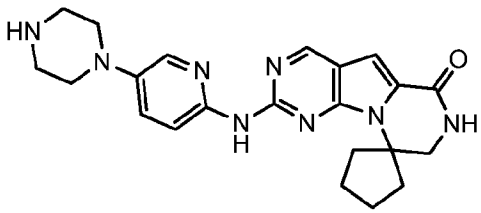
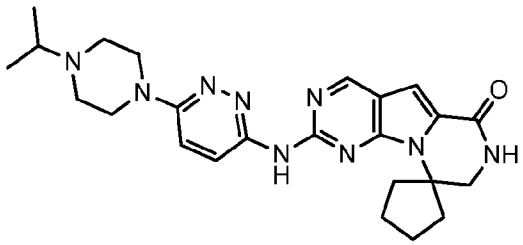
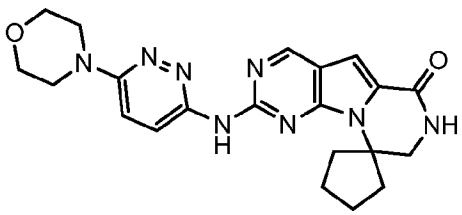
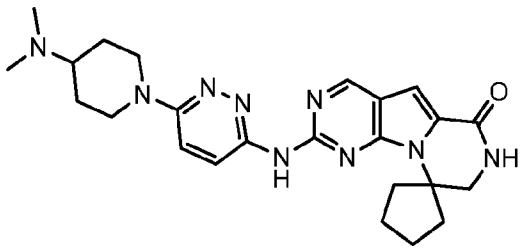
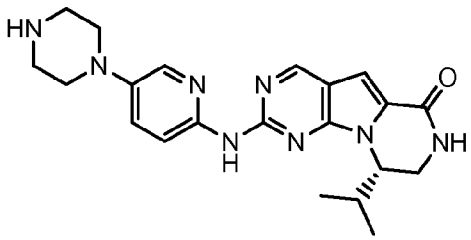
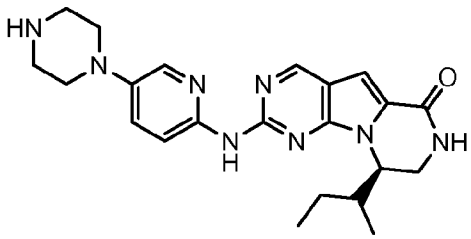
Structure Reference	Structure
PP	
QQ	
RR	
SS	
TT	

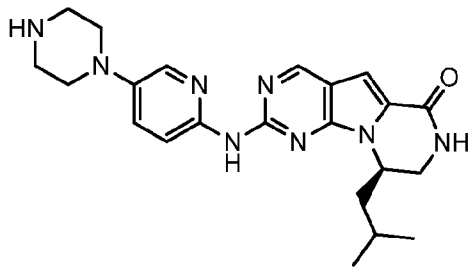
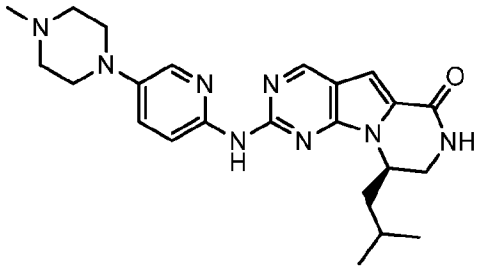
Structure Reference	Structure
UU	
VV	
WW	
XX	
YY	
ZZ	

Structure Reference	Structure
AAA	
BBB	
CCC	
DDD	
EEE	
FFF	

Structure Reference	Structure
GGG	
HHH	
III	
JJJ	
KKK	

Structure Reference	Structure
LLL	
MMM	
NNN	
OOO	
PPP	

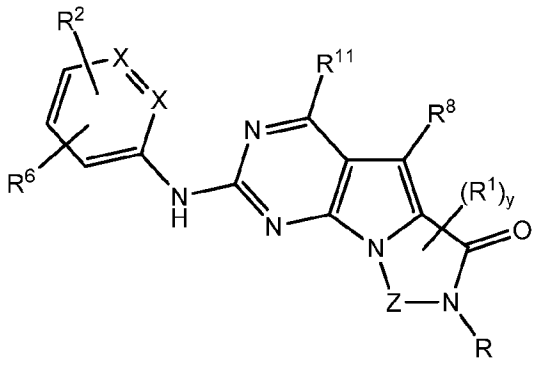
Structure Reference	Structure
QQQ	
RRR	
SSS	
TTT	
UUU	
VVV	

Structure Reference	Structure
WWW	
XXX	

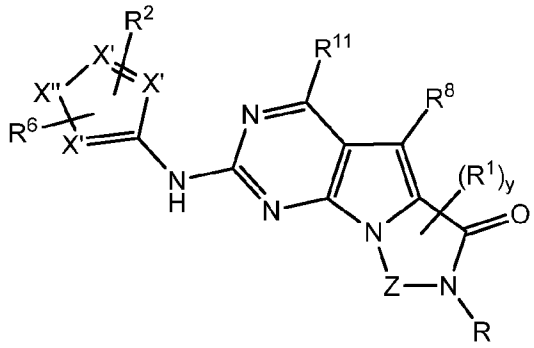
24. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.
25. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.
26. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.
27. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.
28. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.
29. The method of claim 23, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.
30. The method of claim 23, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.
31. The method of claim 23, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.

32. The method of claim 23, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.
33. The method of any of claims 22-32, wherein the Rb-positive cancer is a solid tumor.
34. The method of any of claims 22-33, wherein the solid tumor is selected from non-small cell lung cancer, breast cancer, colorectal cancer, liver cancer, ovarian cancer, or glioblastoma.
35. The method of claim 33, wherein the solid tumor is Erb-2/human epidermal growth factor receptor (HER)-2-positive breast cancer.
36. The method of any of claim 22-32, wherein the Rb-positive cancer is a hematological cancer.
37. The method of claim 36, wherein the hematological cancer is acute myeloid leukemia (AML).
38. The method of claim 36, wherein the hematological cancer is a T-cell cancer.
39. The method of any of claims 22-38, wherein the CDK 4/6 inhibitor compound is conjugated to a targeting agent.
40. The method of claim 39, wherein the targeting agent is an antibody or antibody fragment.
41. The method of any of claims 22-38, wherein the CDK 4/6 inhibitor compound is conjugated to a radioisotope.
42. The method of any of claims 22-41, wherein the host is human.
43. A method of treating a host suffering from a T-cell malignancy comprising
    a) administering to the host a CDK4/6 inhibitor of the Formula I, II, III, IV, or V,
    b) analyzing the cell cycle status of T-cell malignant cells and at least one subset of non-diseased hematopoetic cells,
    c) adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are in G0 or G1 cell cycle arrest and at least one subset of non-diseased hematopoietic cells are not in G0 or G1 arrest,

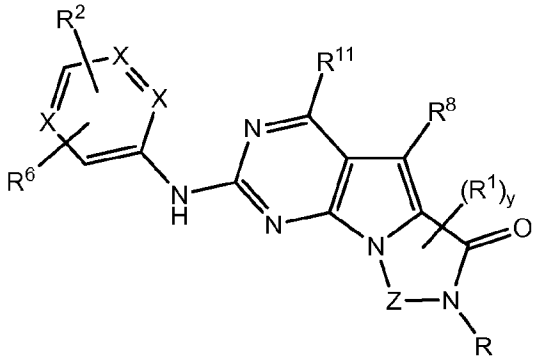
    wherein Formula I, II, III, IV, or V has the following structure:



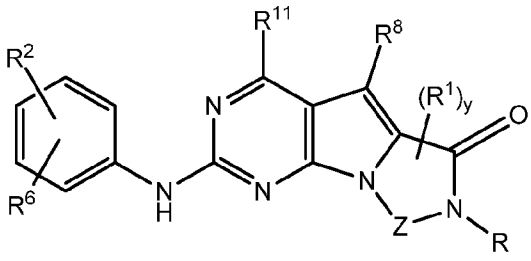
I,



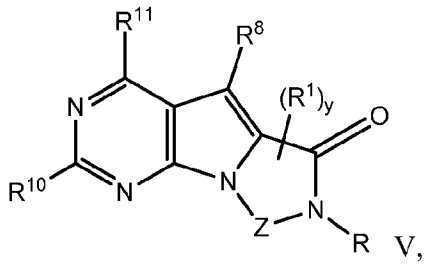
II,



III,



IV, or



V,

wherein:

Z is $–(CH_2)_x–$ wherein x is 1, 2, 3 or 4 or $–O–(CH_2)_z–$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is $–(alkylene)_m–$heterocyclo, $–(alkylene)_m–$heteroaryl, $–(alkylene)_m–NR^3R^4$, $–(alkylene)_m–C(O)–NR^3R^4$; $–(alkylene)_m–C(O)–O$-alkyl; $–(alkylene)_m–O–R^5$, $–(alkylene)_m–S(O)_n–R^5$, or $–(alkylene)_m–S(O)_n–NR^3R^4$ any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^5* at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, -(alkylene)$_m$-OR^5, -(alkylene)$_m$-O-alkylene-OR^5, -(alkylene)$_m$-S(O)$_n$–R^5, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-CN, -(alkylene)$_m$-C(O)–R^5, -(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5, -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*,

-(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*,
-(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*,
-(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*,
-(alkylene)$_m$-SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,
-(alkylene)$_m$-N(R^3*)–SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*,
-(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

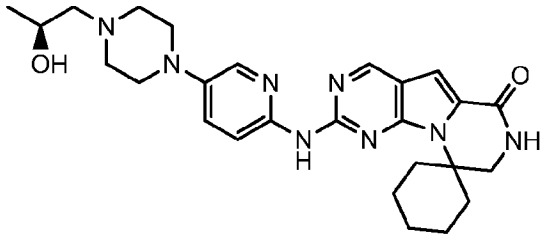
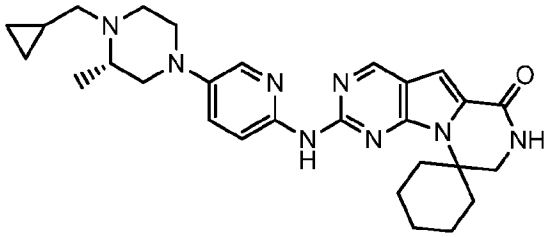
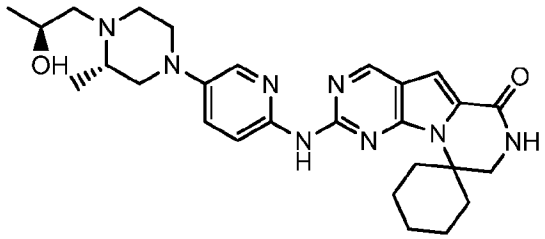
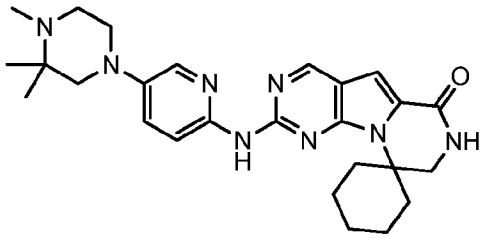
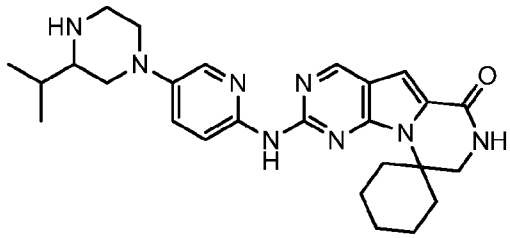
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -(alkylene)$_m$-heteroaryl, -(alkylene)$_m$-NR^3R^4,

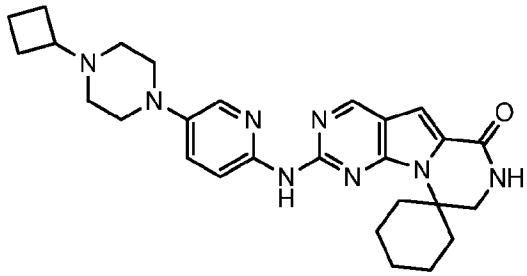
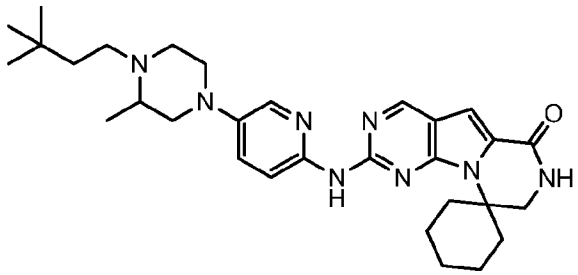
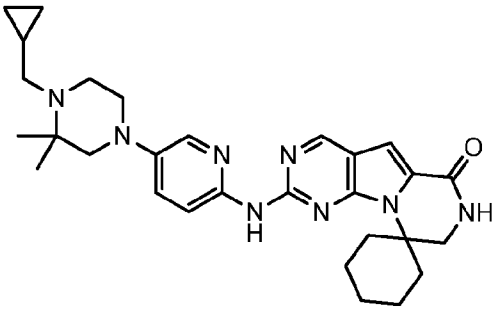
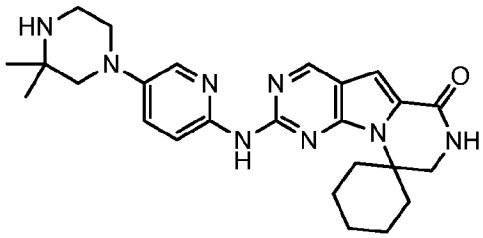
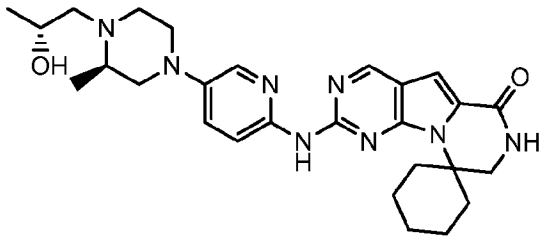
-(alkylene)$_m$-C(0)-NR^3R^4; -(alkylene)$_m$-0-R^5, -(alkylene)$_m$-S(0)$_n$-R^5, or -(alkylene)$_m$-S(0)$_n$-NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

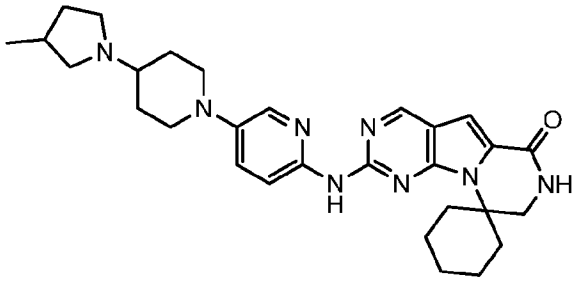
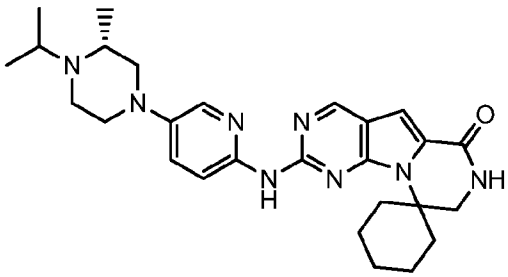
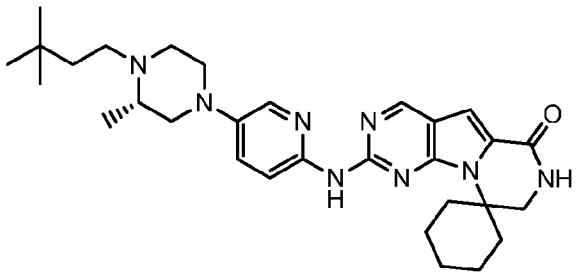
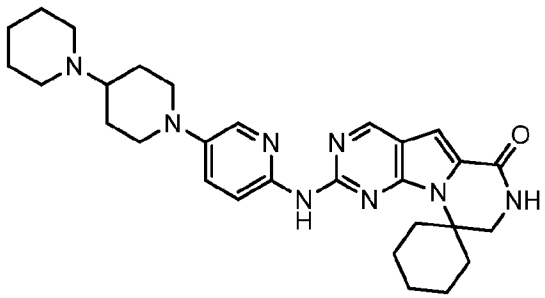
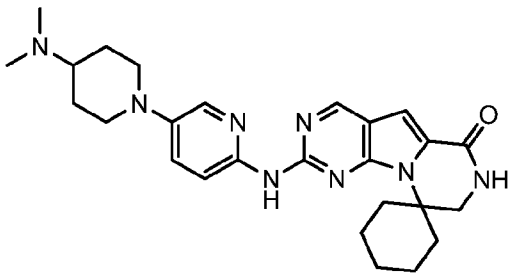
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C_1-C_8 alkyl, cycloalkylalkyl, or –TT-RR, C_1-C_8 cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C_1-C_8 alkyl or C_3-C_8 cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C_1-C_6 alkoxy, amino, unsubstituted or substituted C_1-C_6 alkylamino, unsubstituted or substituted di-C_1-C_6 alkylamino, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13}, wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

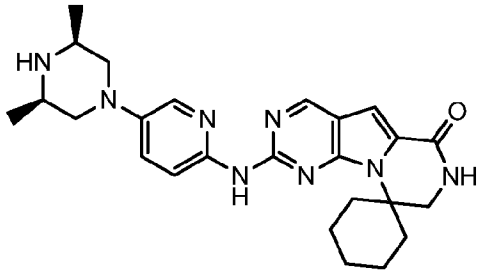
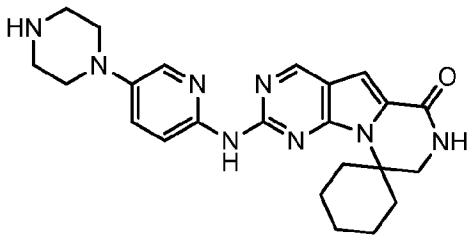
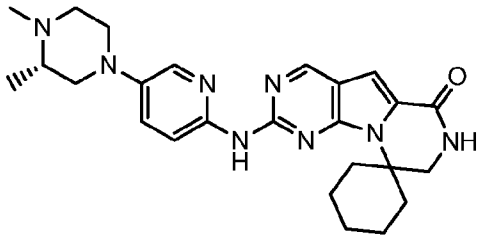
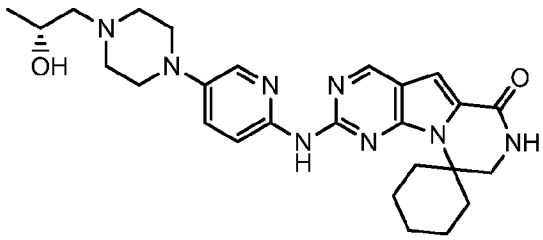
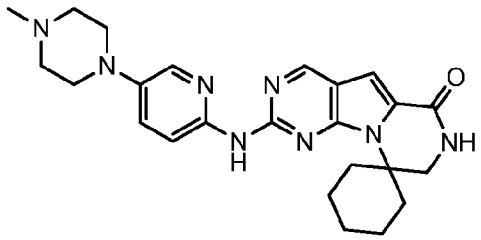
or a pharmaceutically acceptable salt thereof.

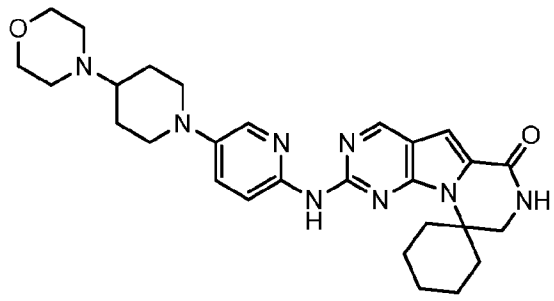
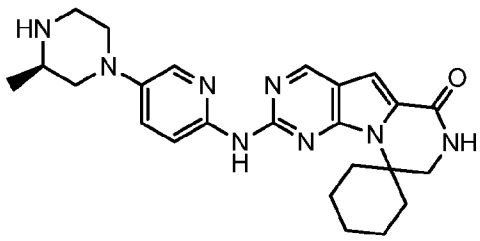
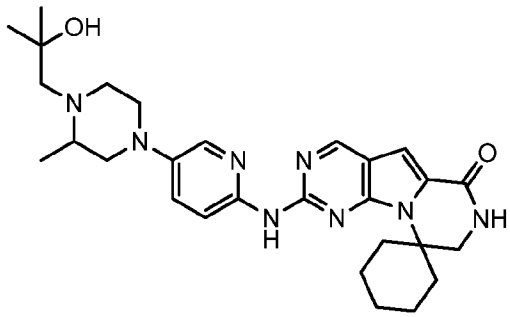
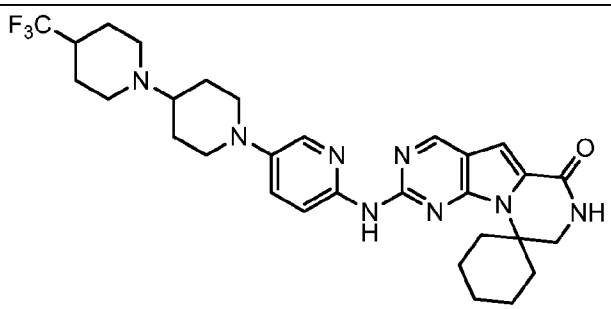
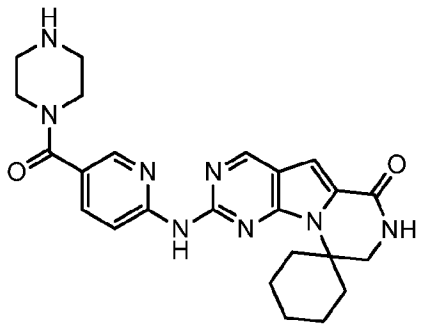
44. The method of claim 43, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of:

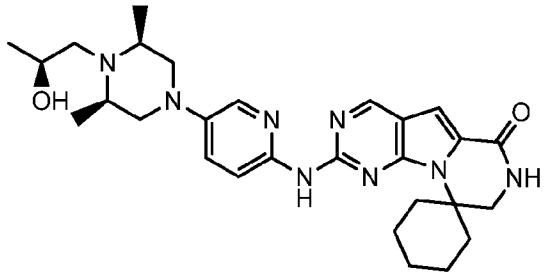
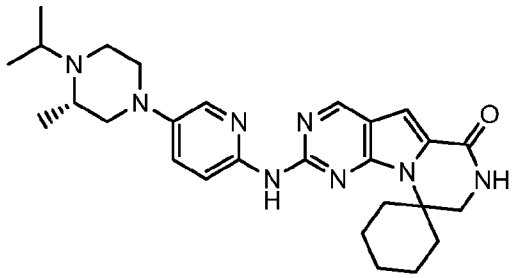
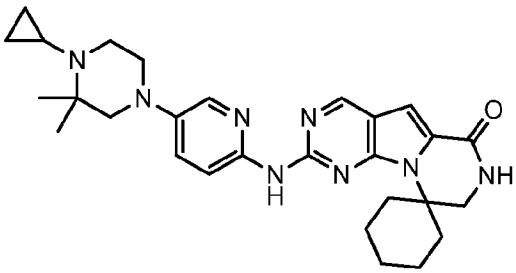
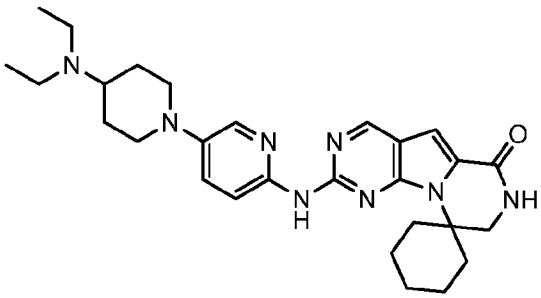
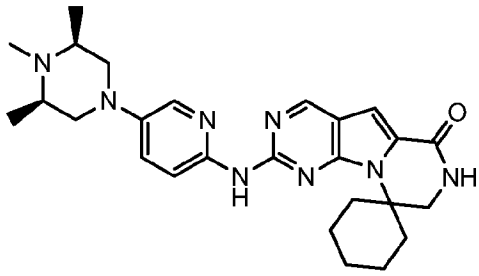
Structure Reference	Structure
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D	
E	

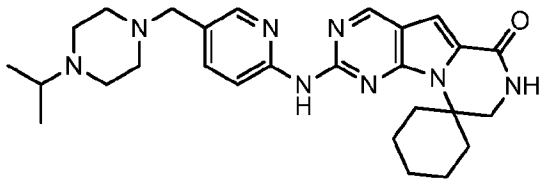
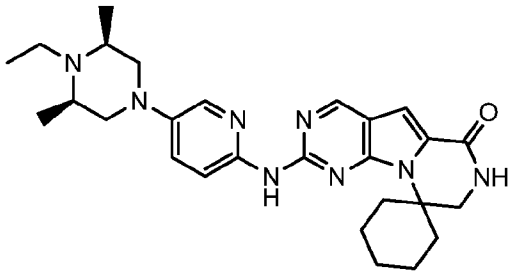
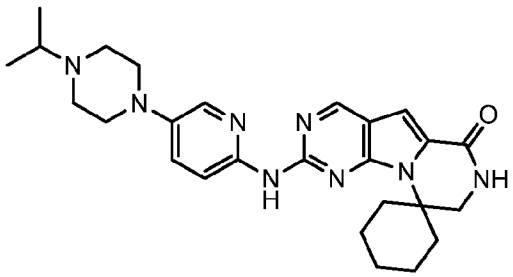
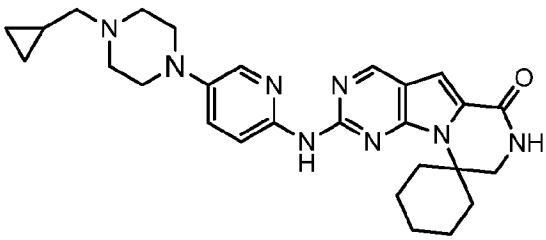
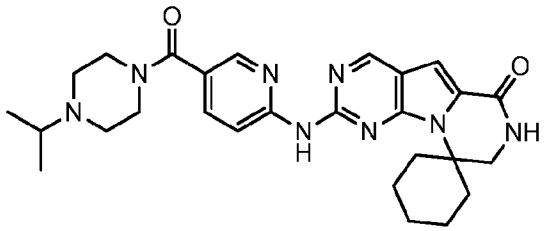
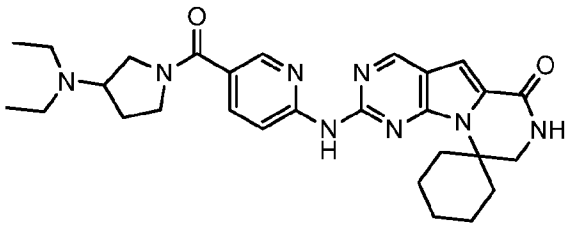
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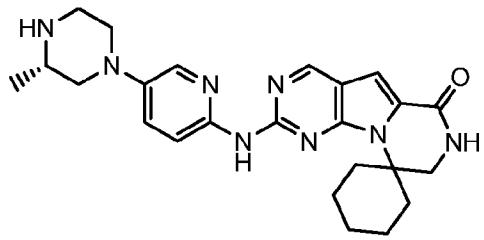
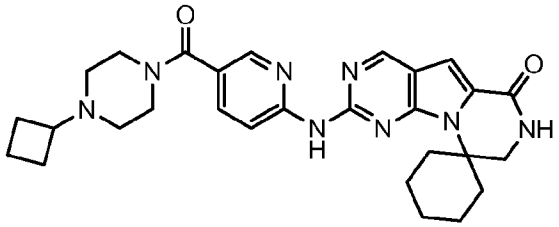
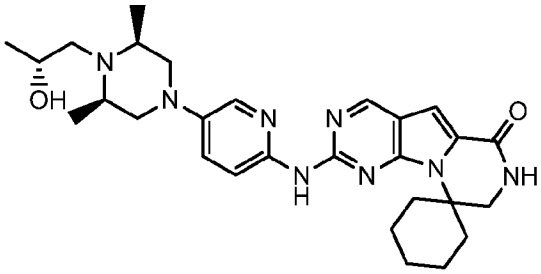
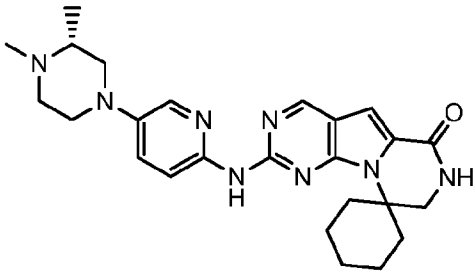
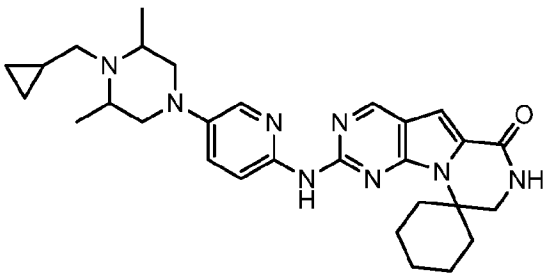
Structure Reference	Structure
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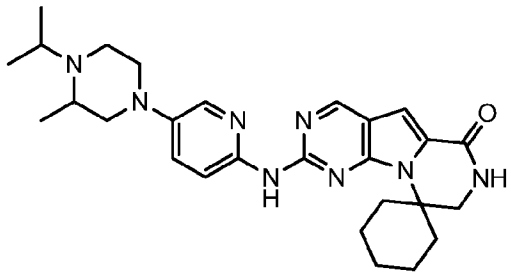
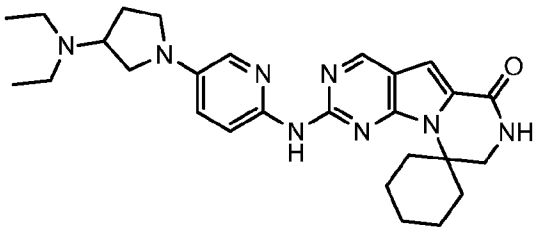
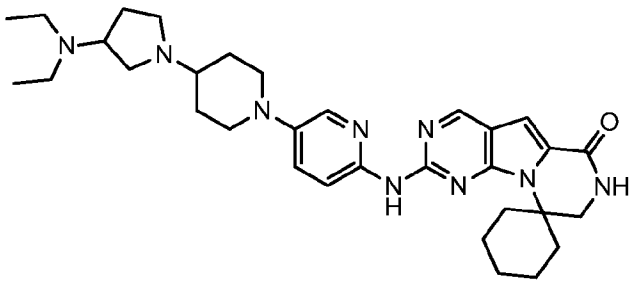
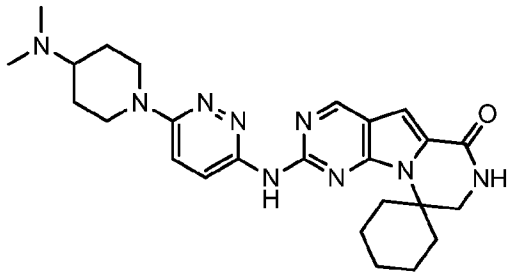
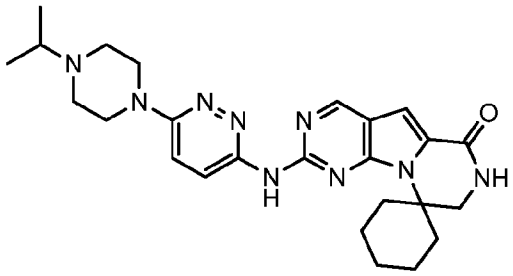
Structure Reference	Structure
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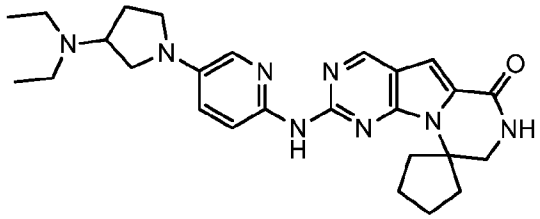
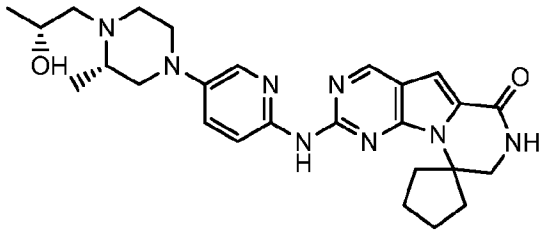
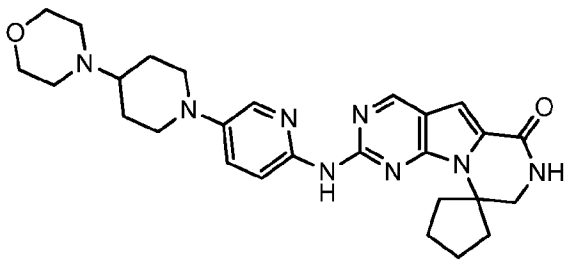
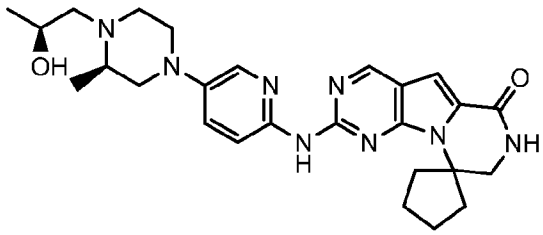
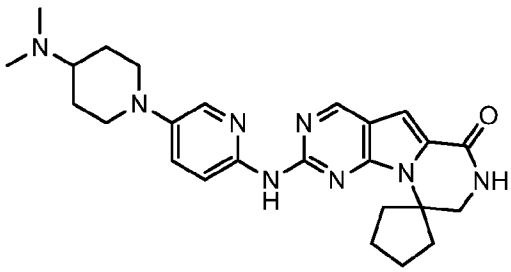
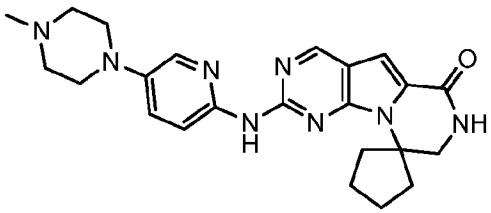
Structure Reference	Structure
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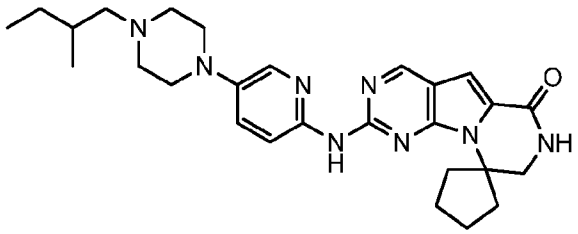
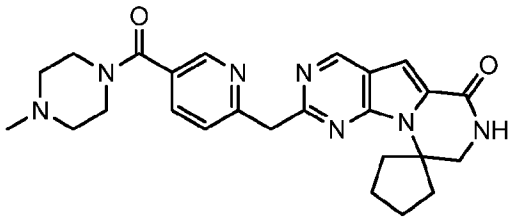
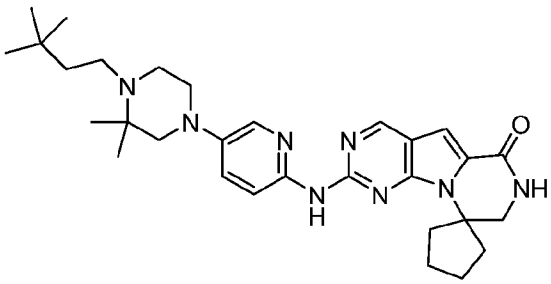
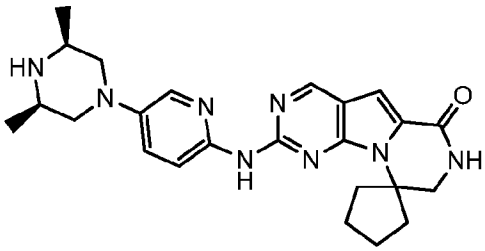
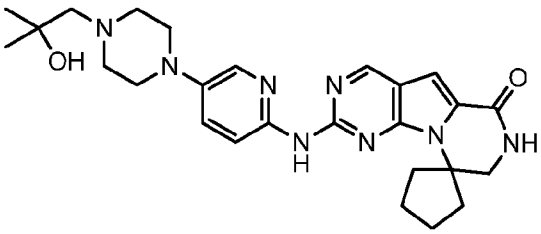
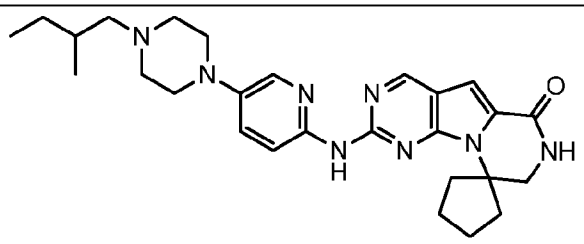
Structure Reference	Structure
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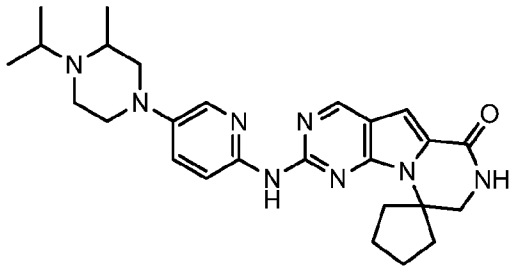
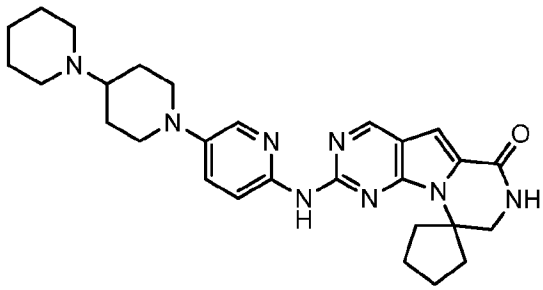
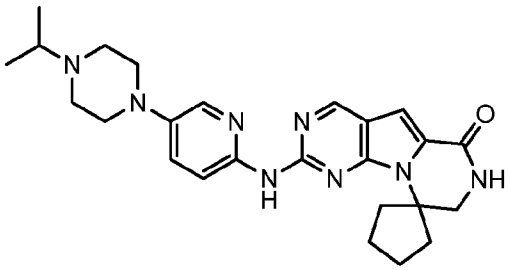
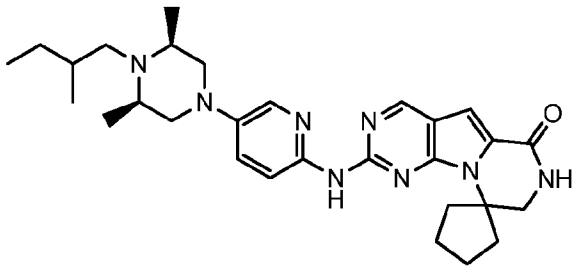
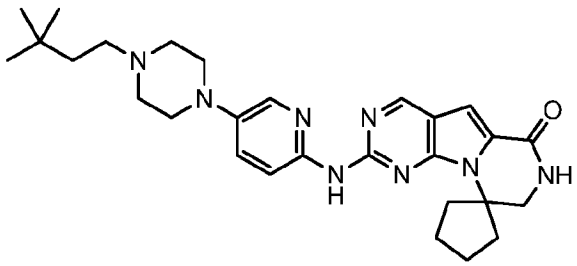
Structure Reference	Structure
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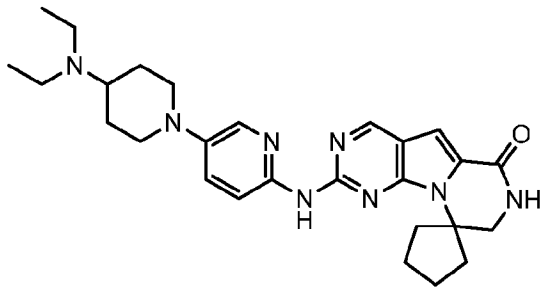
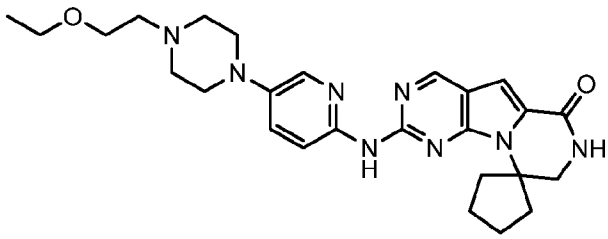
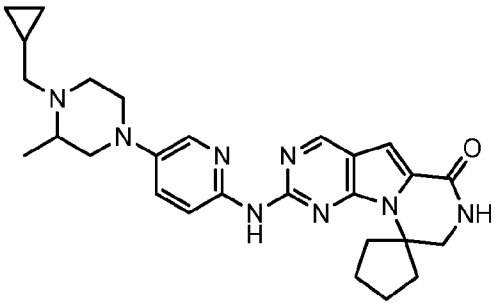
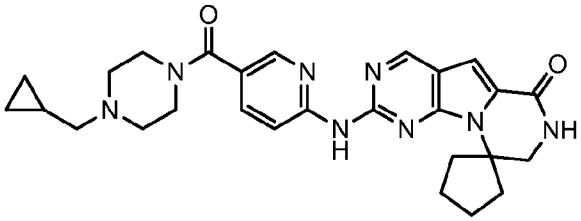
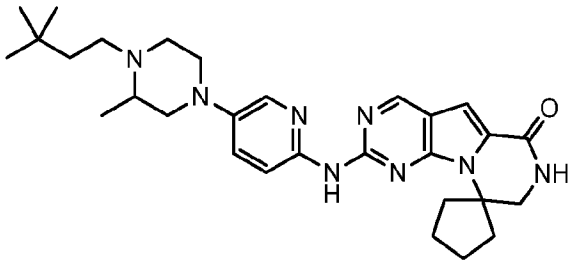
Structure Reference	Structure
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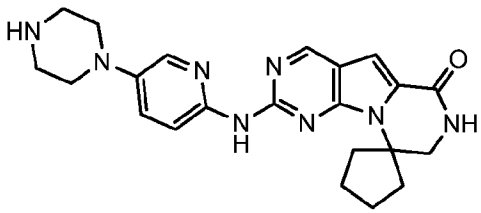
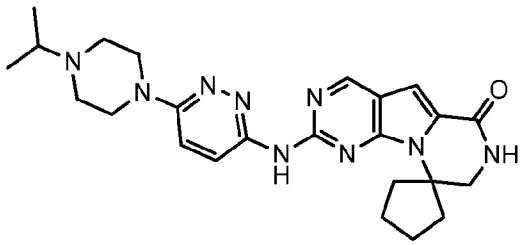
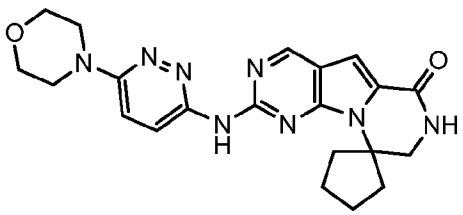
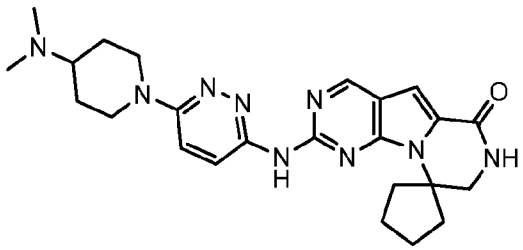
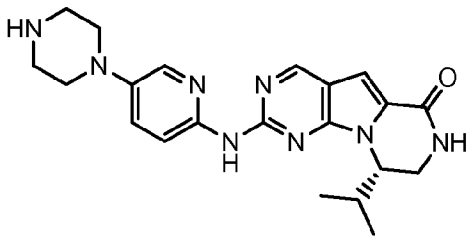
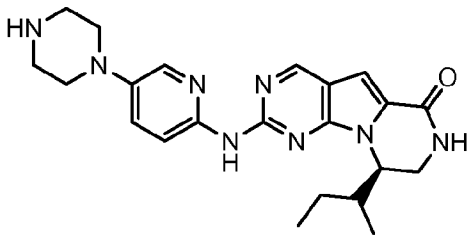
Structure Reference	Structure
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RR	
SS	
TT	

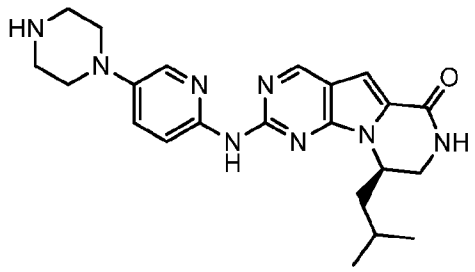
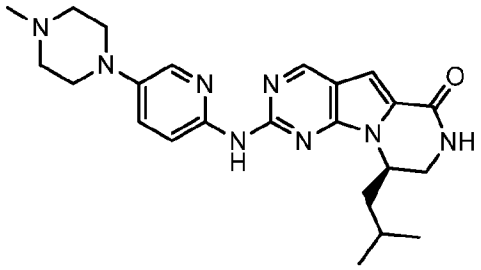
Structure Reference	Structure
UU	
VV	
WW	
XX	
YY	
ZZ	

Structure Reference	Structure
AAA	
BBB	
CCC	
DDD	
EEE	
FFF	

Structure Reference	Structure
GGG	
HHH	
III	
JJJ	
KKK	

Structure Reference	Structure
LLL	
MMM	
NNN	
OOO	
PPP	

Structure Reference	Structure
QQQ	
RRR	
SSS	
TTT	
UUU	
VVV	

Structure Reference	Structure
WWW	
XXX	

45. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.
46. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.
47. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.
48. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.
49. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.
50. The method of claim 43, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.
51. The method of claim 43, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.
52. The method of claim 43, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.

53. The method of claim 43, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.

54. The method of any of claims 43-53, wherein the non-diseased hematological cell lineage is selected from long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, monocyte progenitors, megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, or erythroid progenitors.

55. The method of claim 54, wherein the non-diseased hematological cell lineage are long term hematopoietic stem cells (LT-HSCs).

56. The method of claim 54, wherein the non-diseased hematological cell lineage are short term hematopoietic stem cells (ST-HSCs).

57. The method of claim 54, wherein the non-diseased hematological cell lineage are hematopoietic progenitor cells (HPCs).

58. The method of claim 54, wherein the non-diseased hematological cell lineage are multipotent progenitors (MPPs).

59. The method of claim 54, wherein the non-diseased hematological cell lineage are common myeloid progenitors (CMPs).

60. The method of claim 54, wherein the non-diseased hematological cell lineage are common lymphoid progenitors (CLPs).

61. The method of claim 54, wherein the non-diseased hematological cell lineage are granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, or monocyte progenitors.

62. The method of claim 54, wherein the non-diseased hematological cell lineage are megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, or erythroid progenitors.

63. The method of claim 54, wherein the non-diseased hematological cell lineage are oligodendrocyte pre-progenitors (OPPs).

64. The method of any of claims 43-63, wherein the host is human.

65. A method of treating a host suffering from a T-cell malignancy comprising

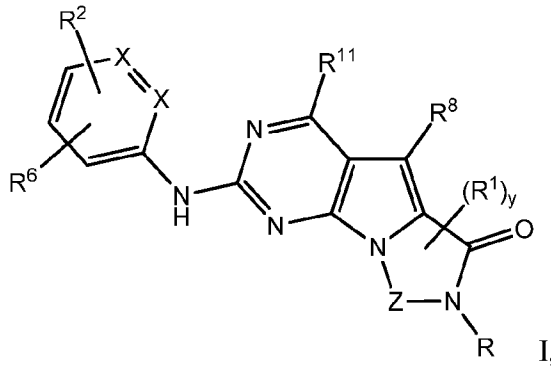
a) administering to the host a CDK4/6 inhibitor of the Formula I, II, III, IV, or V,

b) analyzing the cell cycle status of T-cell malignant cells and at least one subset of non-diseased hematopoetic cells,

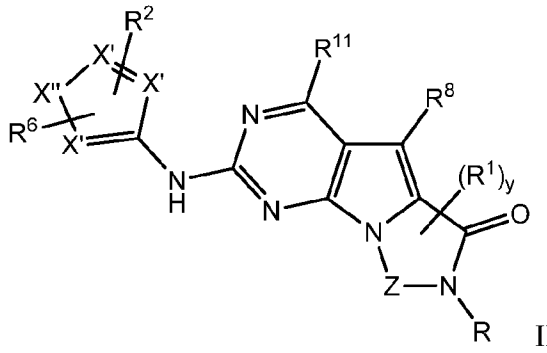
c) adjusting the dose of the CDK4/6 inhibitor so that a significant portion of the T-cell malignant cells are not in G0 or G1 arrest and at least one subset of non-diseased hematopoietic cells are in G0 or G1 arrest,

d) administering a chemotherapeutic agent,

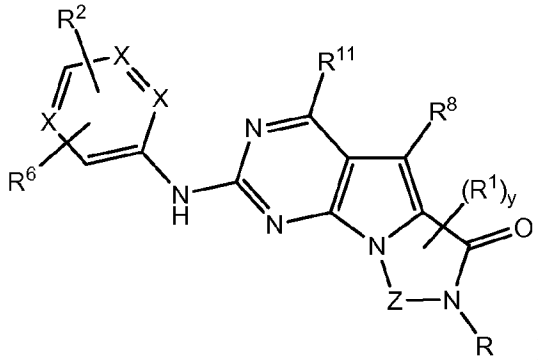
wherein Formula I, II, III, IV, or V has the following structure:



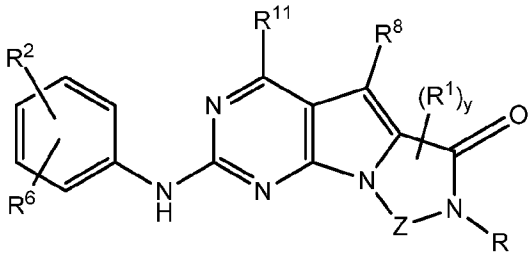
I,



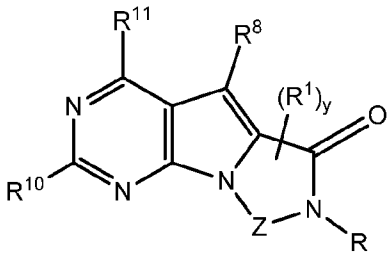
II,



III,



IV, or



V,

wherein:

Z is $–(CH_2)_x–$ wherein x is 1, 2, 3 or 4 or $–O–(CH_2)_z–$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-$C(0)$-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups

as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is $-(alkylene)_m-$heterocyclo, $-(alkylene)_m-$heteroaryl, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$; $-(alkylene)_m-C(O)-O$-alkyl; $-(alkylene)_m-O-R^5$, $-(alkylene)_m-S(O)_n-R^5$, or $-(alkylene)_m-S(O)_n-NR^3R^4$ any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^{5*} at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, $-(alkylene)_m-OR^5$, $-(alkylene)_m-O-alkylene-OR^5$, $-(alkylene)_m-S(O)_n-R^5$, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-CN$, $-(alkylene)_m-C(O)-R^5$,

-(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5) -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*, -(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*, -(alkylene)$_m$-SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO_2–R^5*, -(alkylene)$_m$-N(R^3*)–SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*, -(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

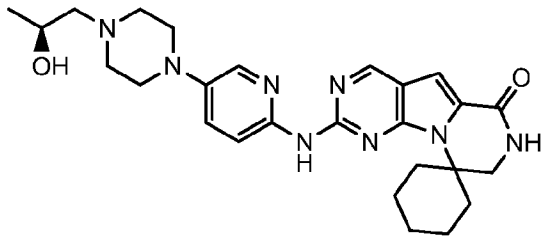
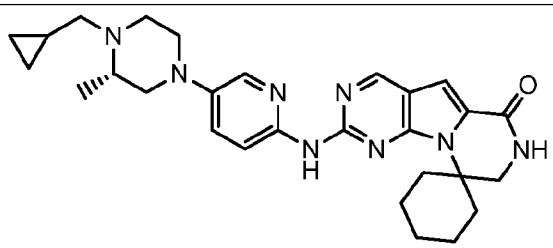
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -(alkylene)$_m$-heteroaryl, -(alkylene)$_m$-NR^3R^4,

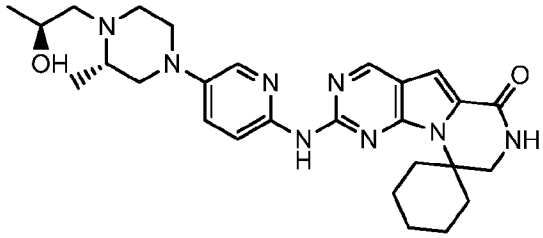
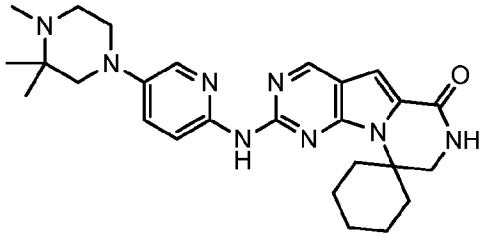
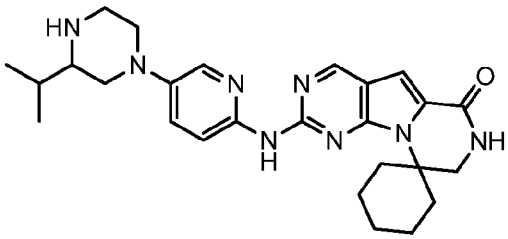
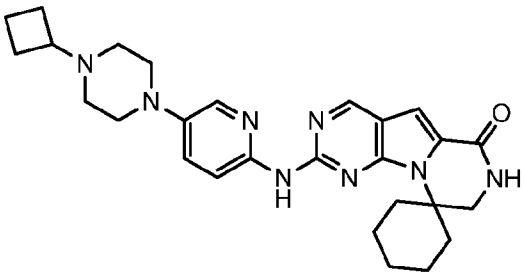
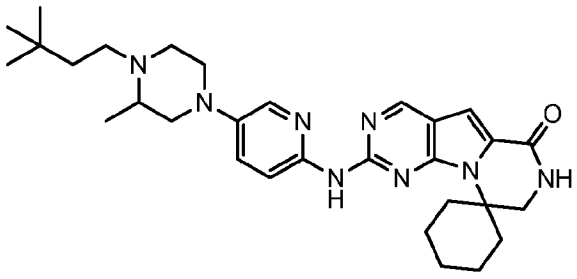
-(alkylene)$_m$-C(0)-NR^{3}R^4; -(alkylene)$_m$-0-R^5, -(alkylene)$_m$-S(0)$_n$-R^5, or -(alkylene)$_m$-S(0)$_n$-NR^{3}R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

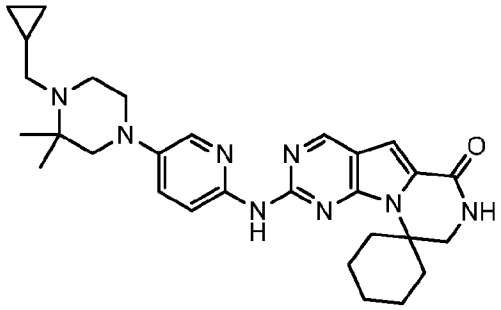
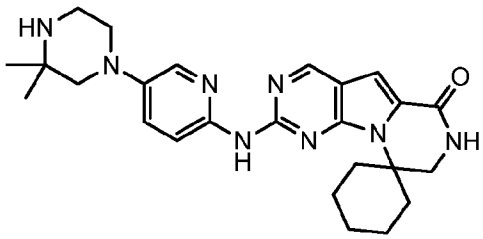
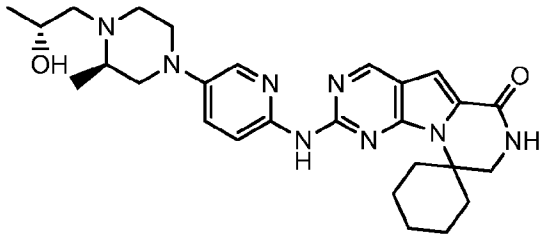
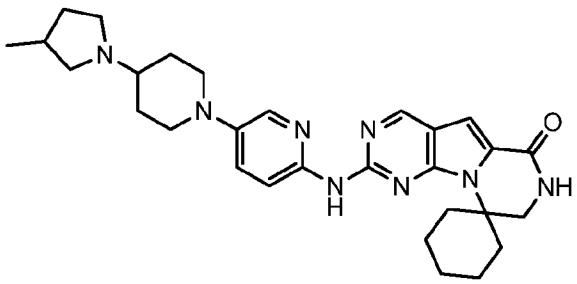
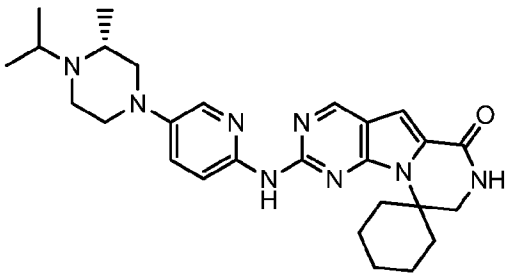
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C_1-C_8 alkyl, cycloalkylalkyl, or –TT-RR, C_1-C_8 cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C_1-C_8 alkyl or C_3-C_8 cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C_1-C_6 alkoxy, amino, unsubstituted or substituted C_1-C_6 alkylamino, unsubstituted or substituted di-C_1-C_6 alkylamino, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13}, wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

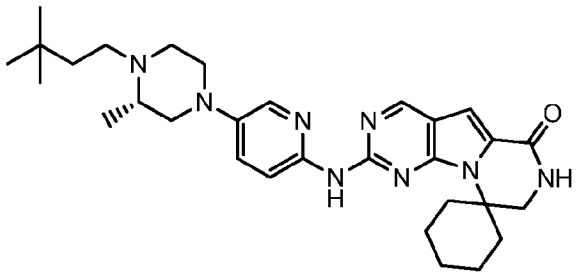
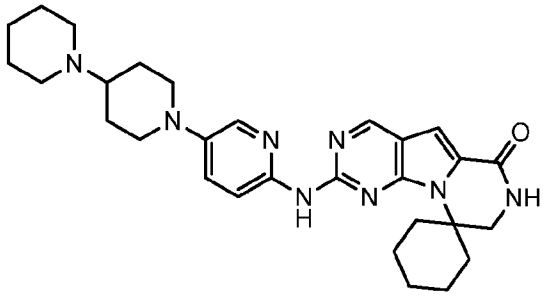
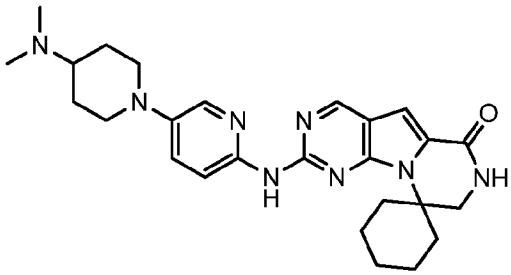
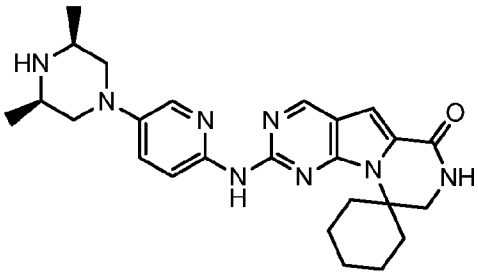
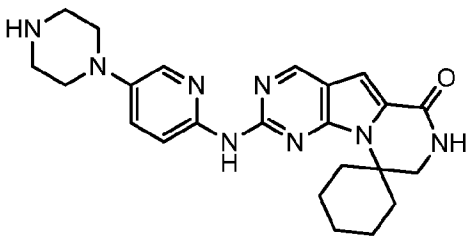
or a pharmaceutically acceptable salt thereof.

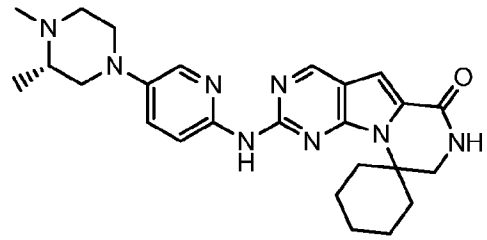
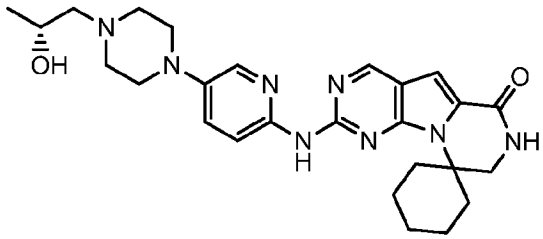
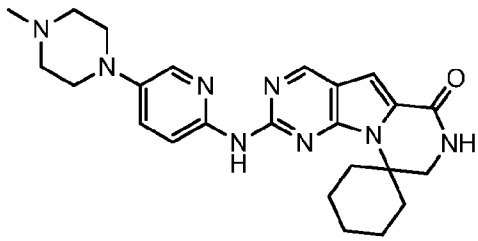
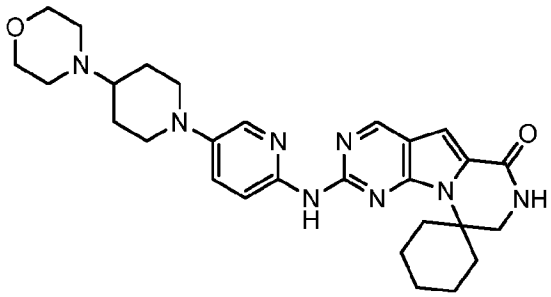
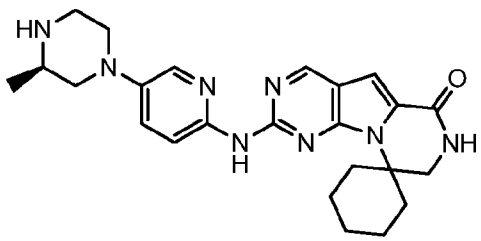
66. The method of claim 65, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of:

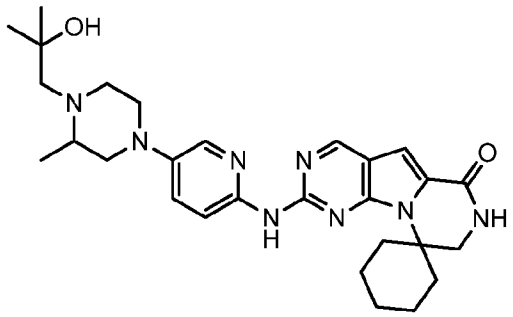
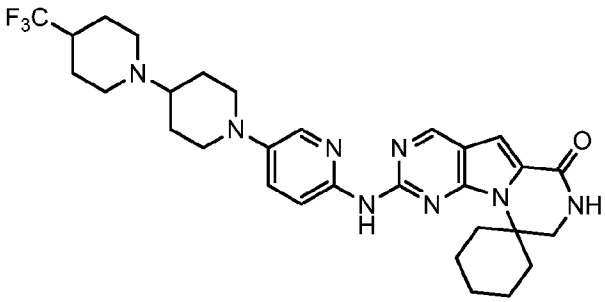
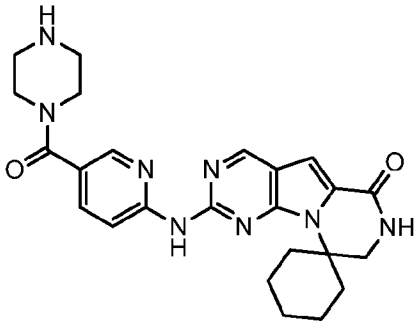
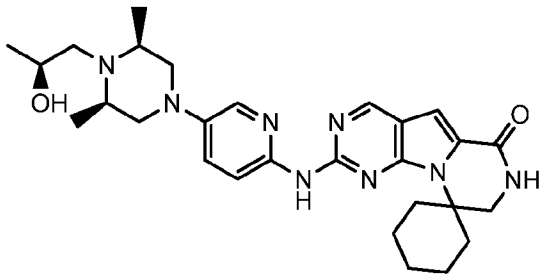
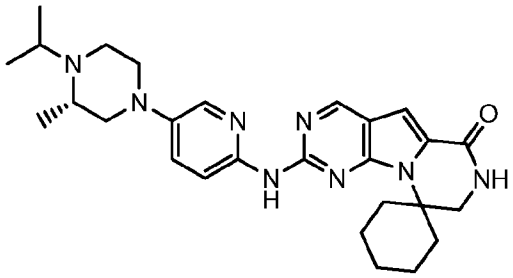
Structure Reference	Structure
A	
B	

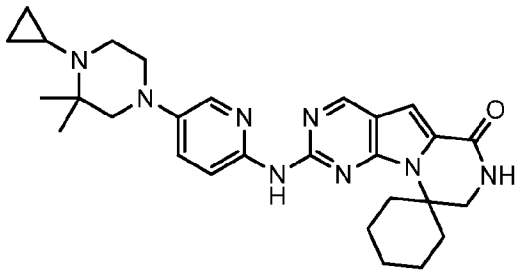
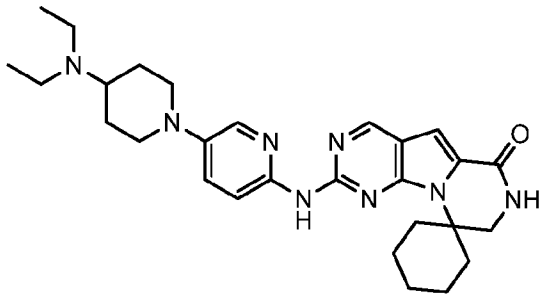
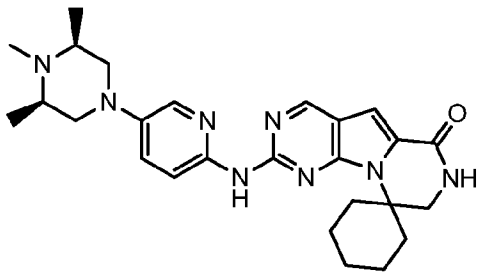
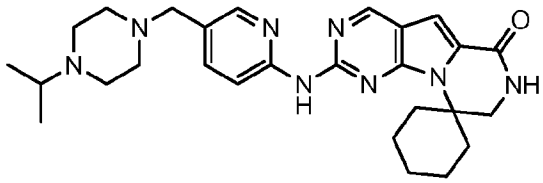
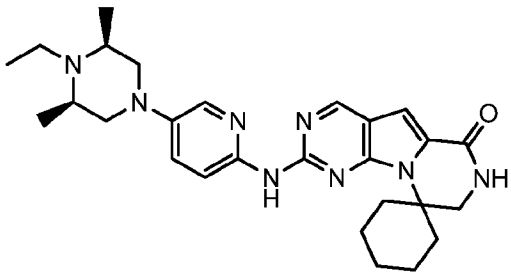
Structure Reference	Structure
C	
D	
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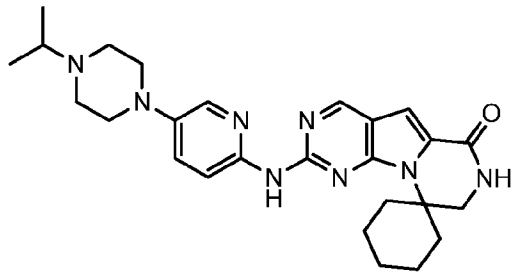
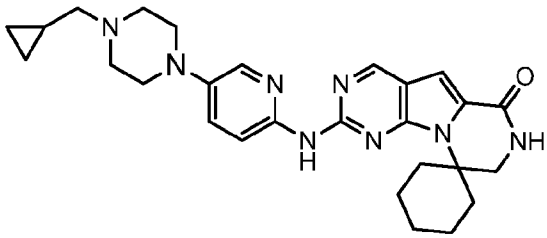
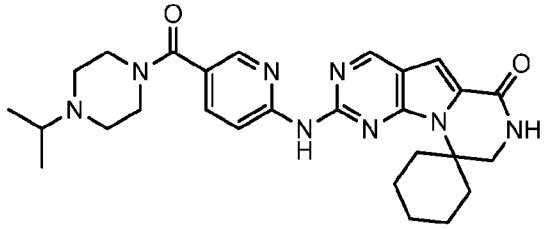
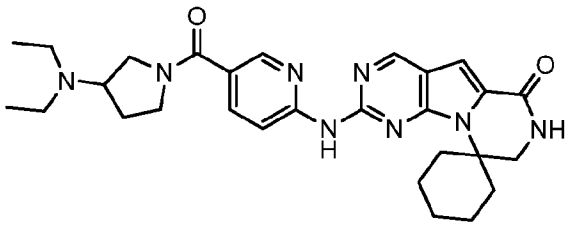
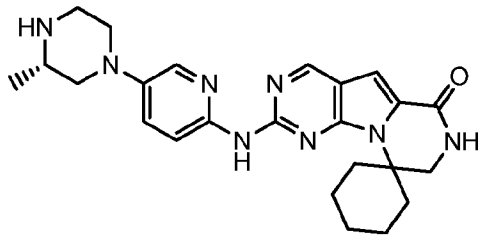
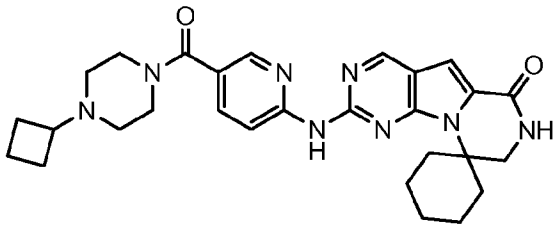
Structure Reference	Structure
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K	
L	

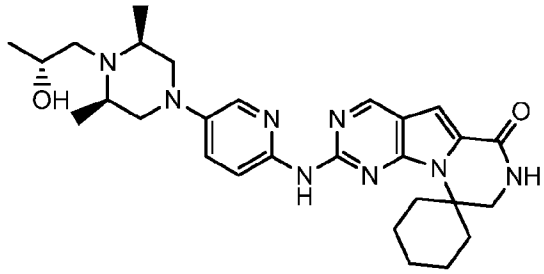
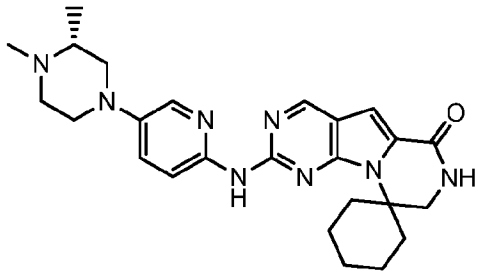
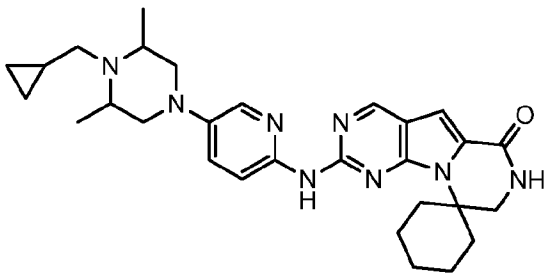
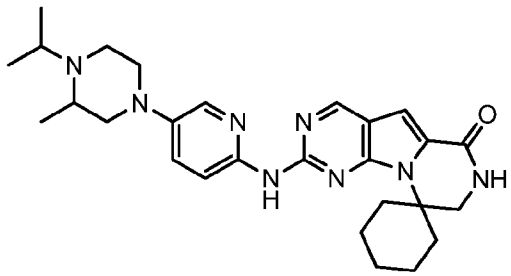
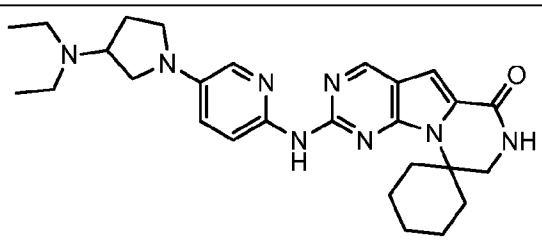
Structure Reference	Structure
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P	
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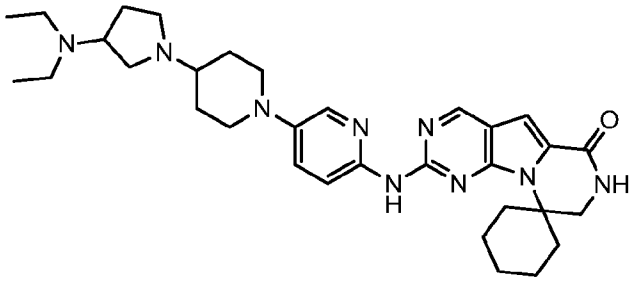
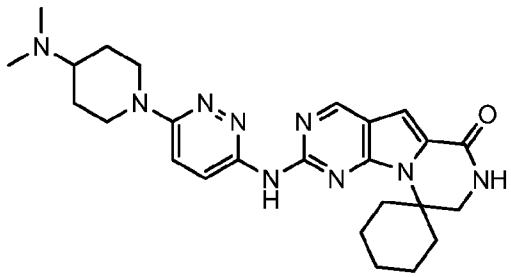
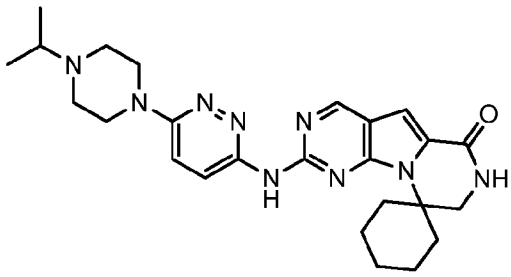
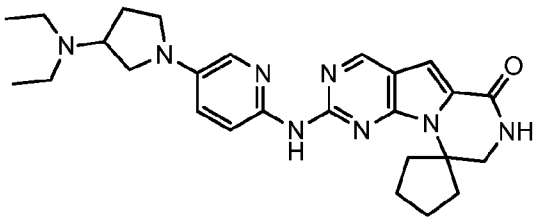
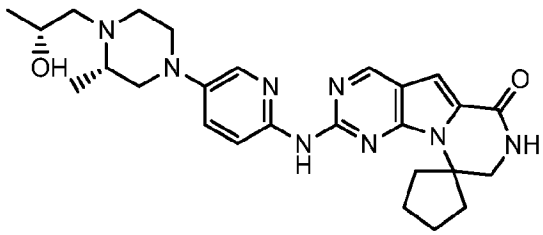
Structure Reference	Structure
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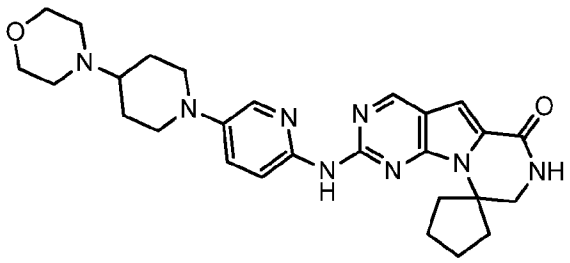
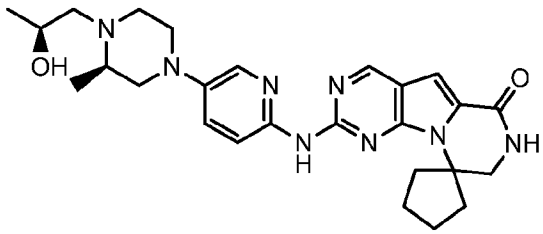
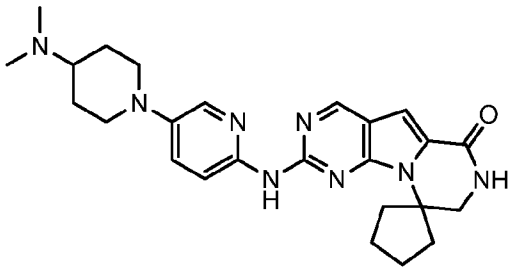
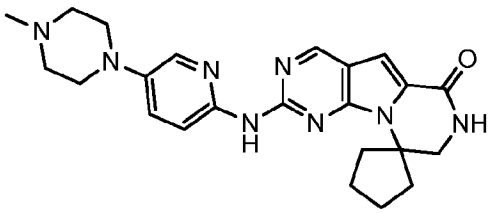
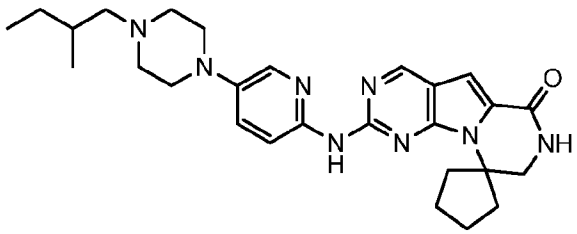
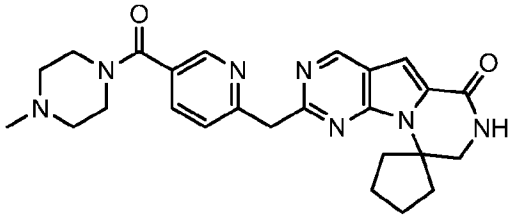
Structure Reference	Structure
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Y	
Z	
AA	

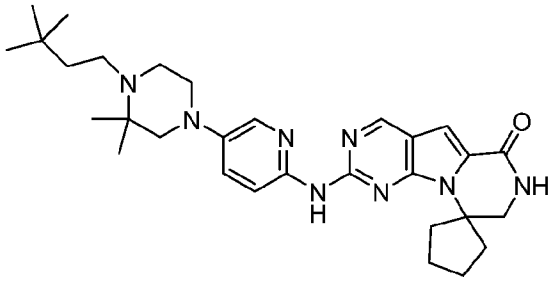
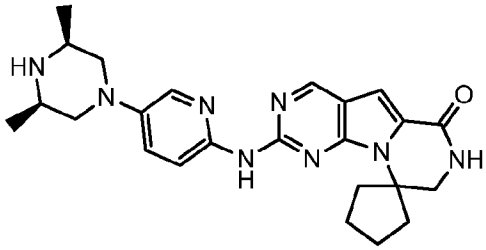
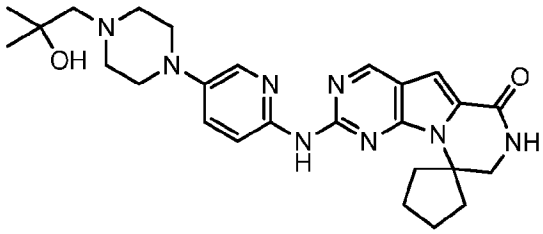
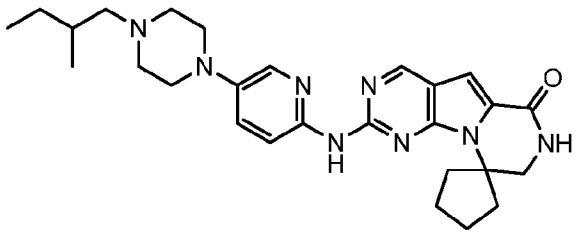
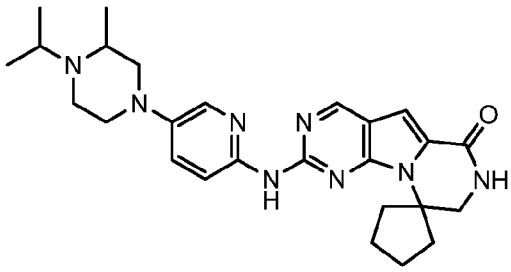
Structure Reference	Structure
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EE	
FF	

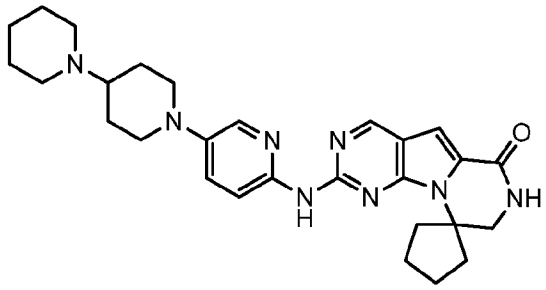
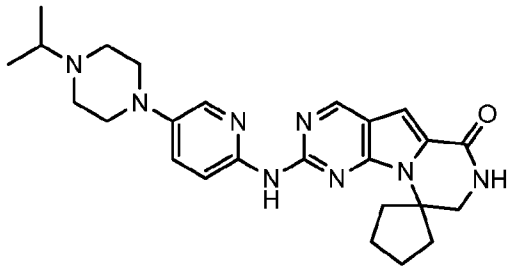
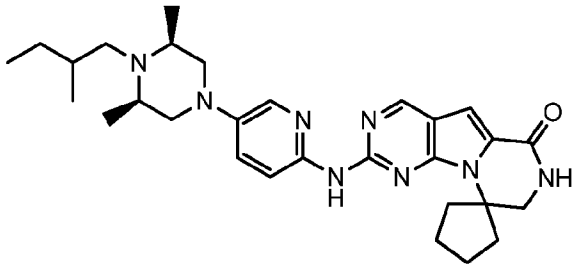
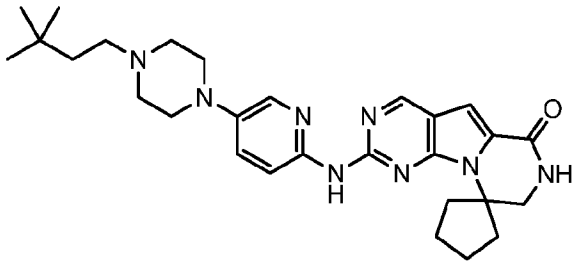
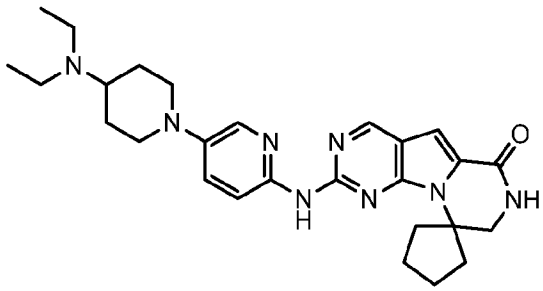
Structure Reference	Structure
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JJ	
KK	
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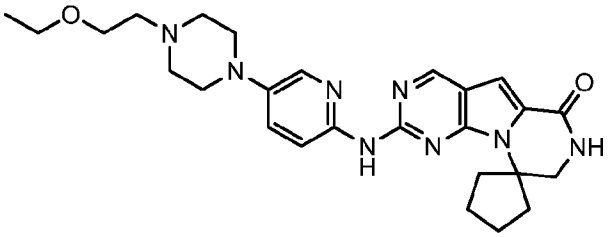
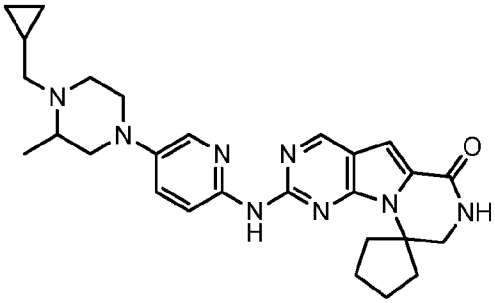
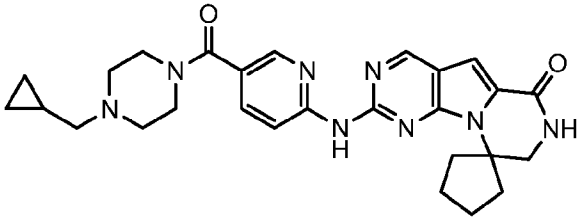
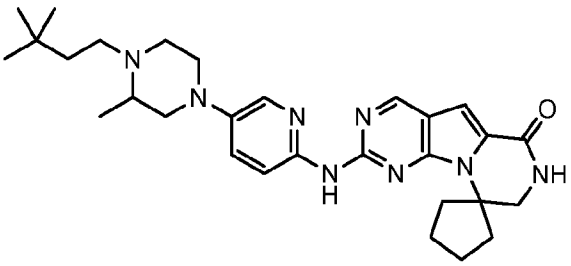
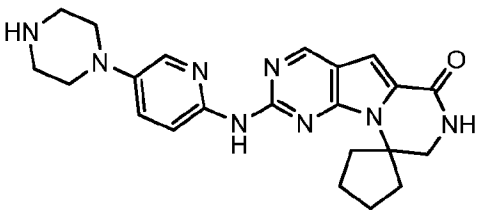
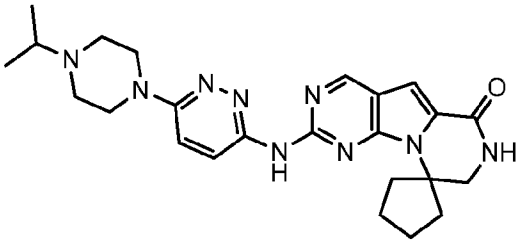
Structure Reference	Structure
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NN	
OO	
PP	
QQ	

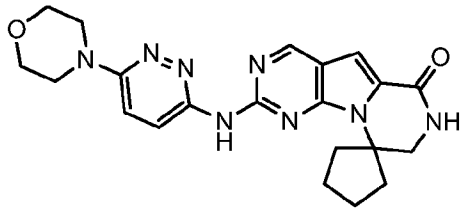
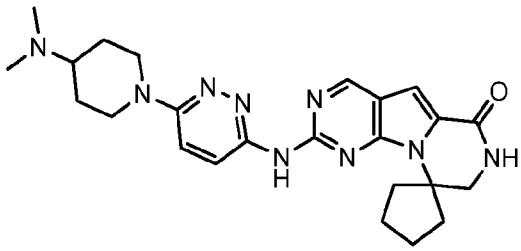
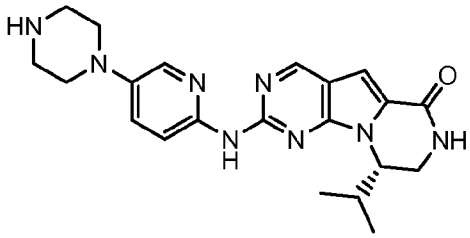
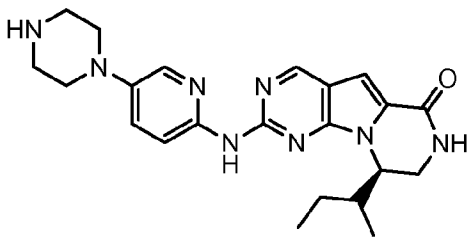
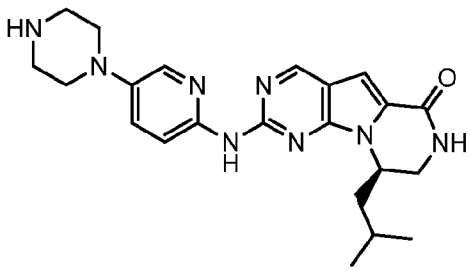
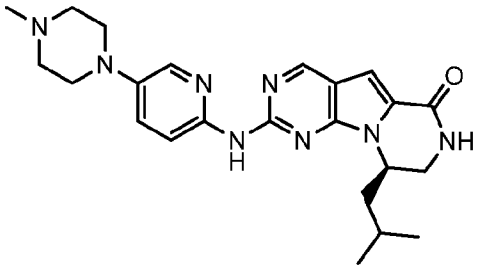
Structure Reference	Structure
RR	
SS	
TT	
UU	
VV	

Structure Reference	Structure
WW	
XX	
YY	
ZZ	
AAA	
BBB	

Structure Reference	Structure
CCC	
DDD	
EEE	
FFF	
GGG	

Structure Reference	Structure
HHH	
III	
JJJ	
KKK	
LLL	

Structure Reference	Structure
MMM	
NNN	
OOO	
PPP	
QQQ	
RRR	

Structure Reference	Structure
SSS	
TTT	
UUU	
VVV	
WWW	
XXX	

67. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.
68. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.
69. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.
70. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.
71. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.
72. The method of claim 65, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.
73. The method of claim 65, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.
74. The method of claim 65, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.
75. The method of claim 65, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.
76. The method of any of claims 65-75, wherein the non-diseased hematological cell lineage is selected from long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, monocyte progenitors, and megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, or erythroid progenitors.
77. The method of claim 76, wherein the non-diseased hematological cell lineage are long term hematopoietic stem cells (LT-HSCs).

78. The method of claim 76, wherein the non-diseased hematological cell lineage are short term hematopoietic stem cells (ST-HSCs).

79. The method of claim 76, wherein the non-diseased hematological cell lineage are hematopoietic progenitor cells (HPCs).

80. The method of claim 76, wherein the non-diseased hematological cell lineage are multipotent progenitors (MPPs).

81. The method of claim 76, wherein the non-diseased hematological cell lineage are common myeloid progenitors (CMPs).

82. The method of claim 76, wherein the non-diseased hematological cell lineage are common lymphoid progenitors (CLPs).

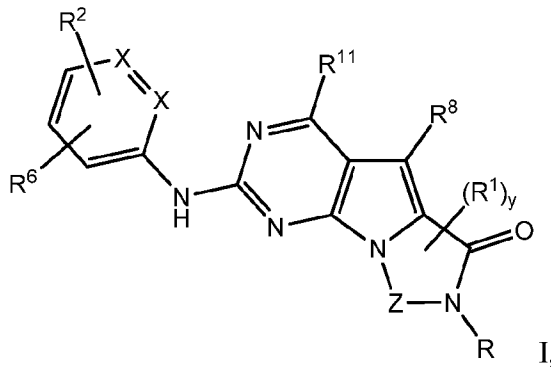
83. The method of claim 76, wherein the non-diseased hematological cell lineage are granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, or monocyte progenitors.

84. The method of claim 76, wherein the non-diseased hematological cell lineage are megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, or erythroid progenitors.

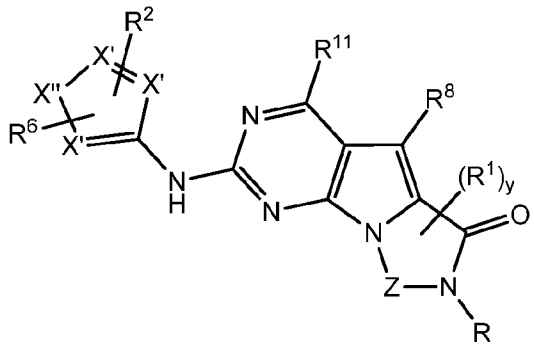
85. The method of claim 76, wherein the non-diseased hematological cell lineage are oligodendrocyte pre-progenitors (OPPs).

86. The method of any of claims 65-85, wherein the host is human.

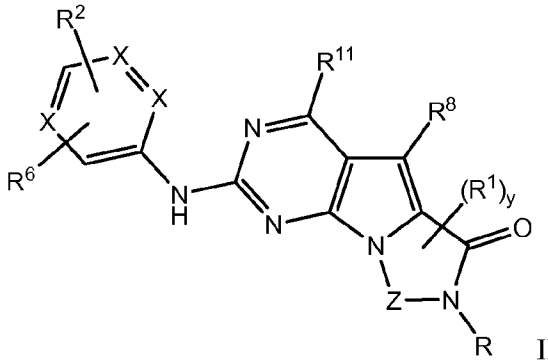
87. A method for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a spleen tyrosine kinase (SYK) inhibitor to a host in need thereof, wherein Formula I, II, III, IV, or V has the structure:



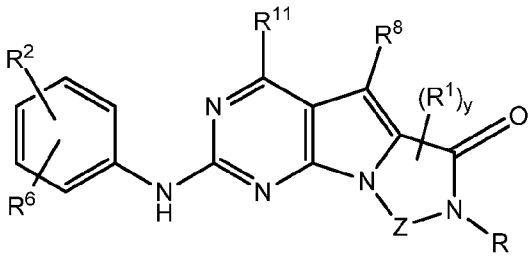
I,



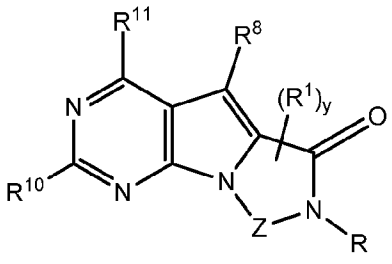
II,



III,



IV, or



V,

wherein:

Z is $–(CH_2)_x–$ wherein x is 1, 2, 3 or 4 or $–O–(CH_2)_z–$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is –$(alkylene)_m$–heterocyclo, –$(alkylene)_m$–heteroaryl, –$(alkylene)_m$–NR^3R^4, –$(alkylene)_m$–C(O)–NR^3R^4; –$(alkylene)_m$–C(O)–O-alkyl; –$(alkylene)_m$–O–R^5, –$(alkylene)_m$–$S(O)_n$–R^5, or –$(alkylene)_m$–$S(O)_n$–NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^{5*} at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, -(alkylene)$_m$-OR^5, -(alkylene)$_m$-O-alkylene-OR^5, -(alkylene)$_m$-S(O)$_n$–R^5, -(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-CN, -(alkylene)$_m$-C(O)–R^5, -(alkylene)$_m$-C(S)–R^5, -(alkylene)$_m$-C(O)–OR^5, -(alkylene)$_m$-O–C(O)–R^5, -(alkylene)$_m$-C(S)–OR^5, -(alkylene)$_m$-C(O)-(alkylene)$_m$-NR^3R^4, -(alkylene)$_m$-C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(S)–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–R^5, -(alkylene)$_m$-N(R^3)–C(S)–R^5, -(alkylene)$_m$-O–C(O)–NR^3R^4, -(alkylene)$_m$–O–C(S)–NR^3R^4, -(alkylene)$_m$–SO_2–NR^3R^4, -(alkylene)$_m$–N(R^3)–SO_2–R^5, -(alkylene)$_m$-N(R^3)–SO_2–NR^3R^4, -(alkylene)$_m$-N(R^3)–C(O)–OR^5, -(alkylene)$_m$-N(R^3)–C(S)–OR^5, or -(alkylene)$_m$-N(R^3)–SO_2–R^5; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*, -(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*, -(alkylene)$_m$-SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO_2–R^5*, -(alkylene)$_m$-N(R^3*)–SO_2–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*, -(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO_2–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

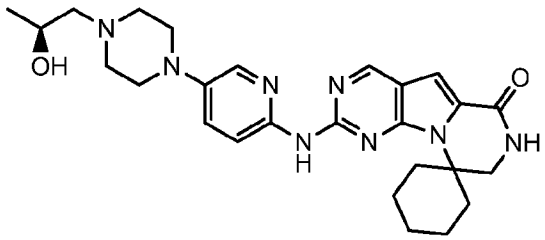
(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

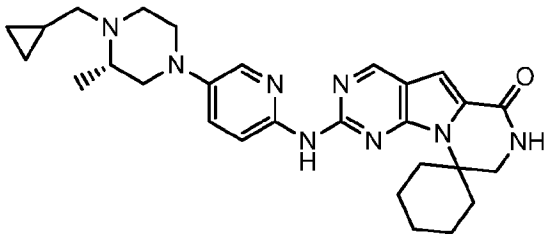
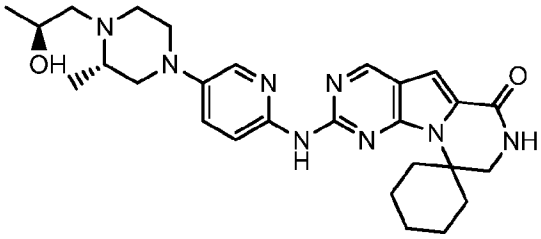
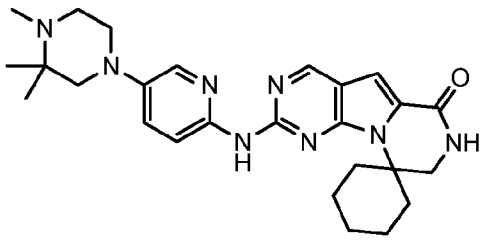
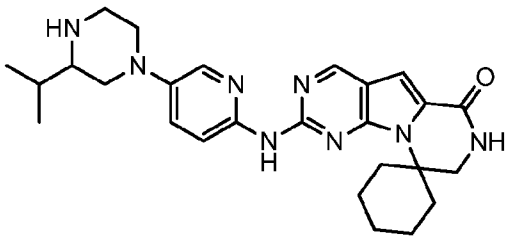
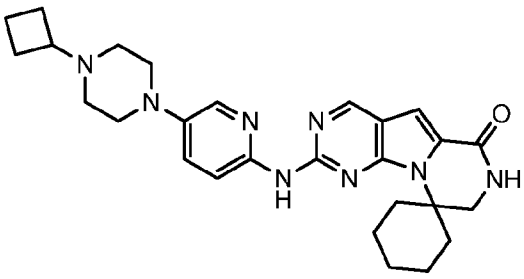
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -$(alkylene)_m$-heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$-$S(0)_n$-R^5, or -$(alkylene)_m$-$S(0)_n$-NR^3R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

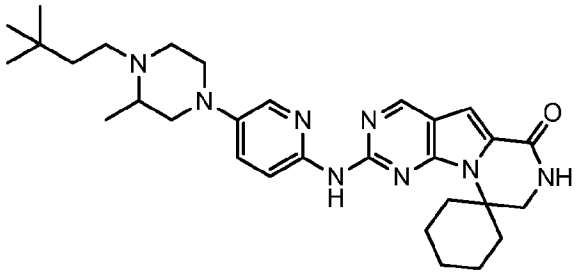
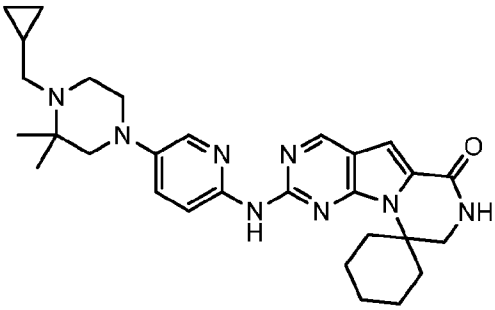
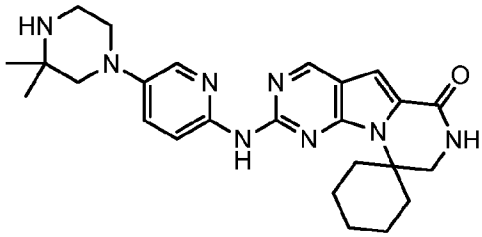
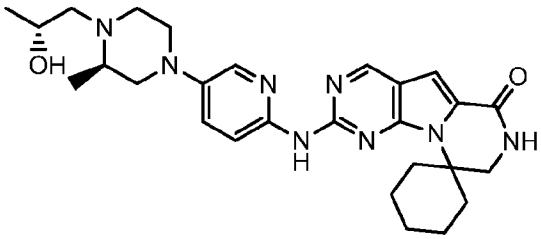
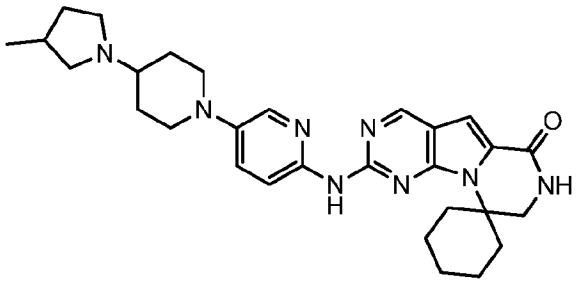
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C_1-C_8 alkyl, cycloalkylalkyl, or –TT-RR, C_1-C_8 cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C_1-C_8 alkyl or C_3-C_8 cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C_1-C_6 alkoxy, amino, unsubstituted or substituted C_1-C_6 alkylamino, unsubstituted or substituted di-C_1-C_6 alkylamino, unsubstituted or substituted C_6-C_{10} aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13} , wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

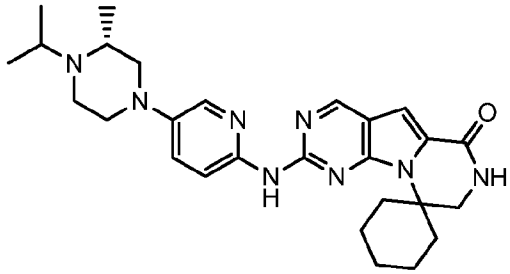
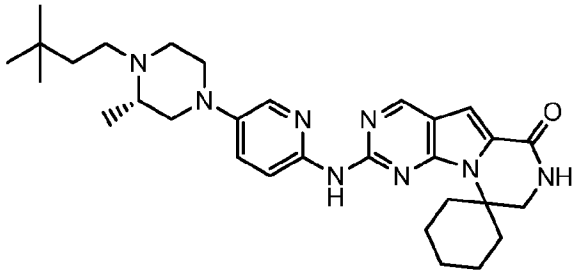
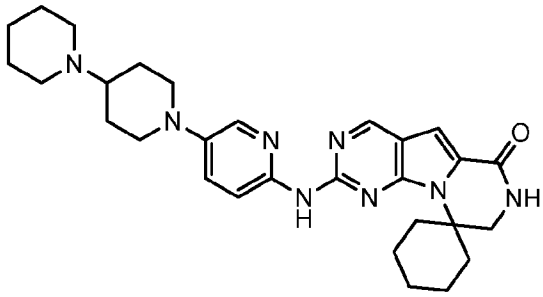
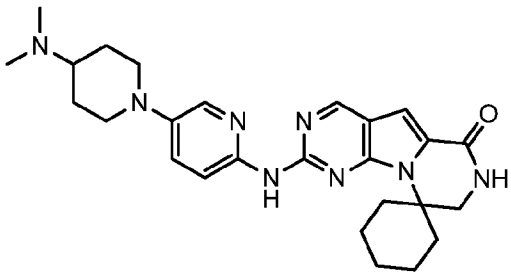
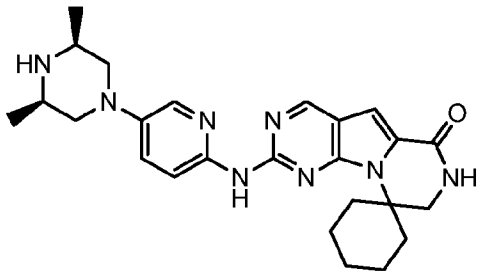
or a pharmaceutically acceptable salt thereof.

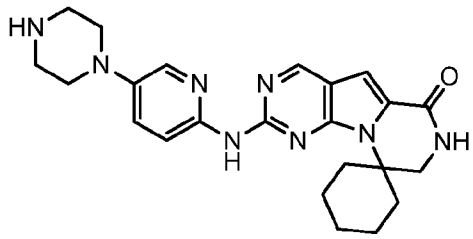
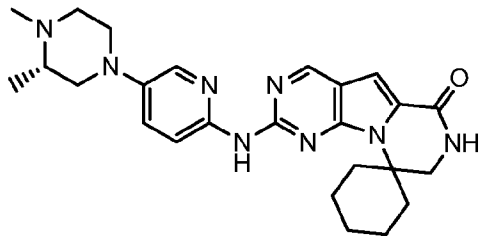
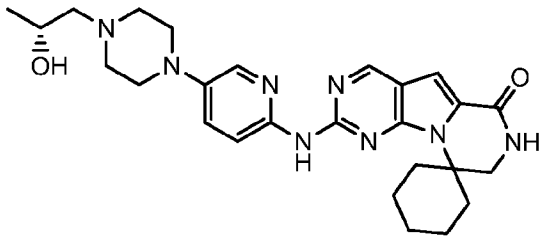
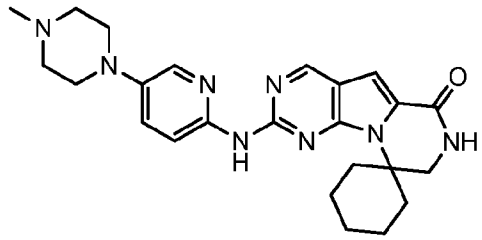
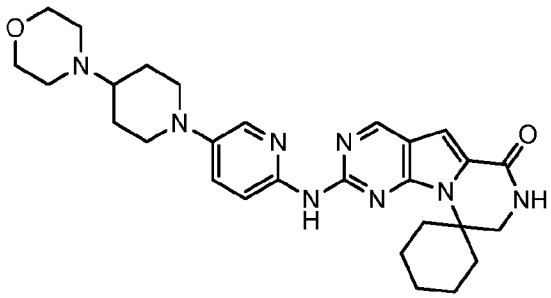
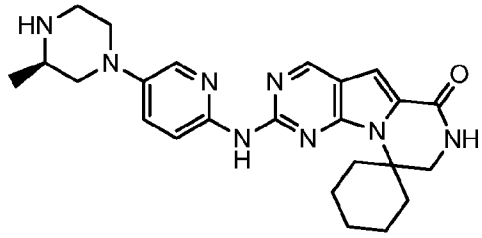
88. The method of claim 87, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of:

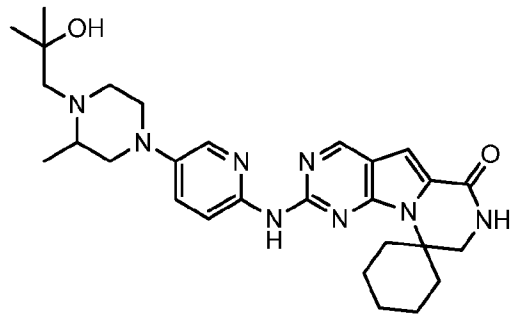
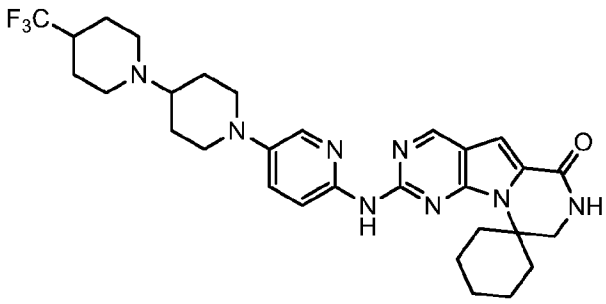
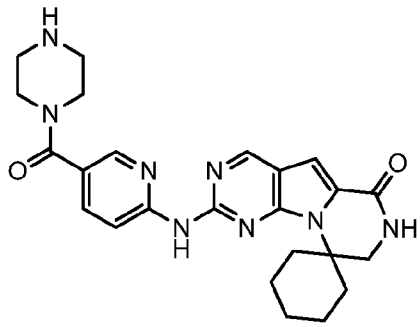
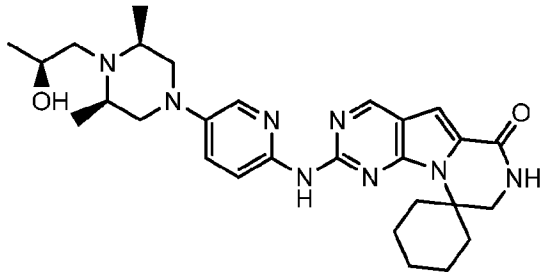
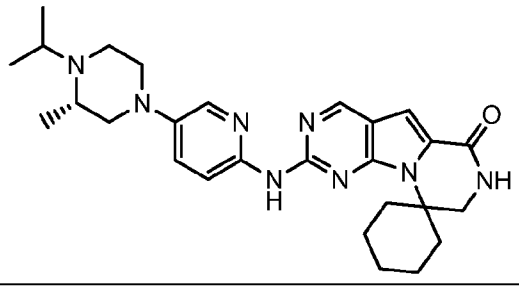
Structure Reference	Structure
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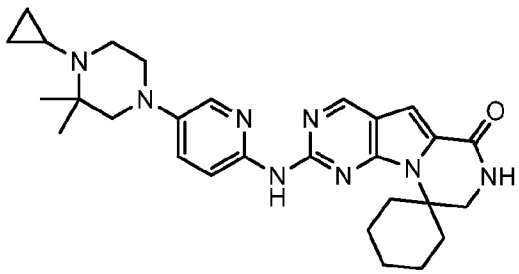
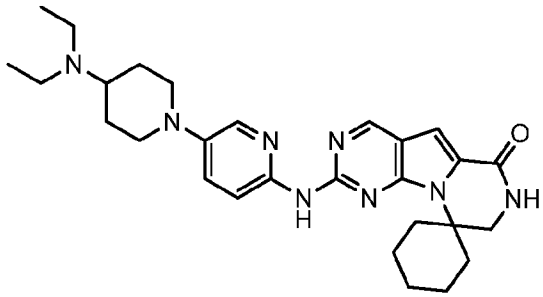
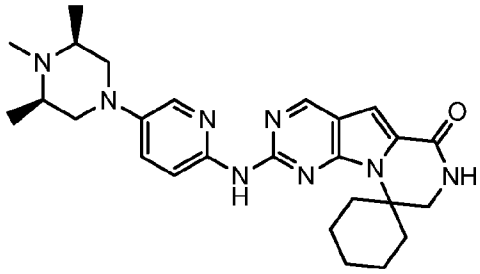
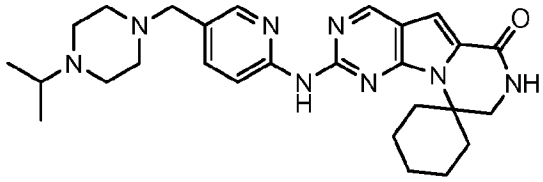
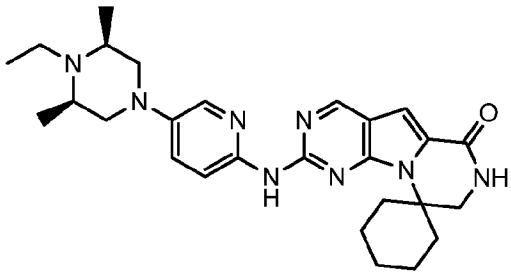
Structure Reference	Structure
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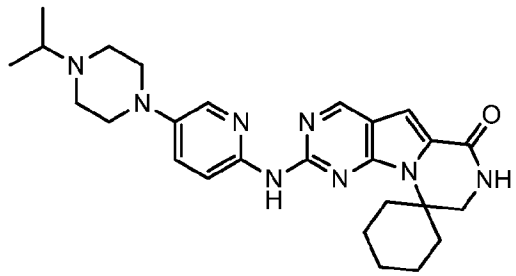
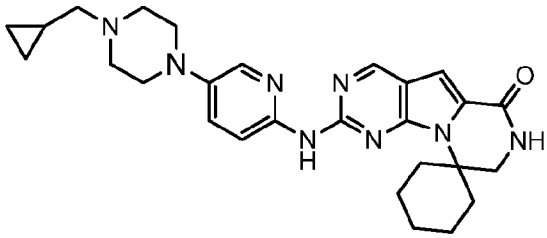
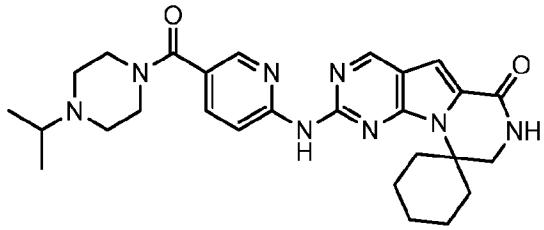
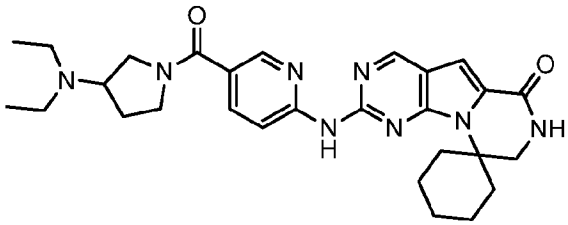
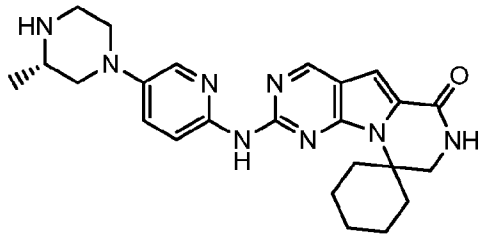
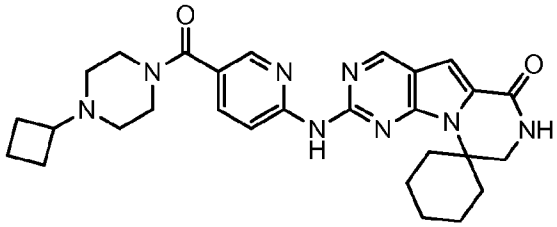
Structure Reference	Structure
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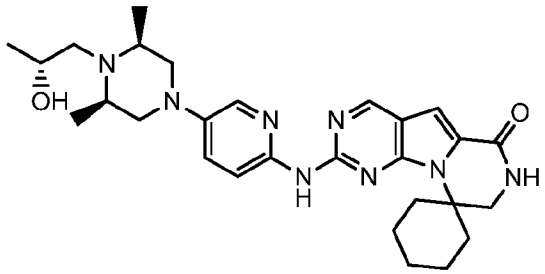
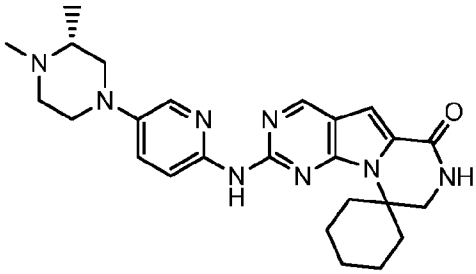
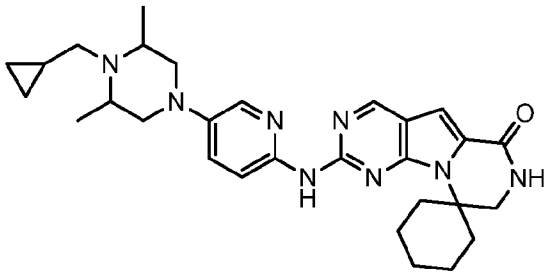
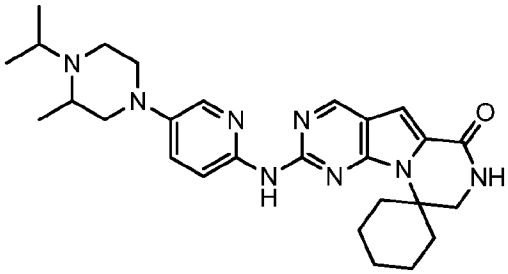
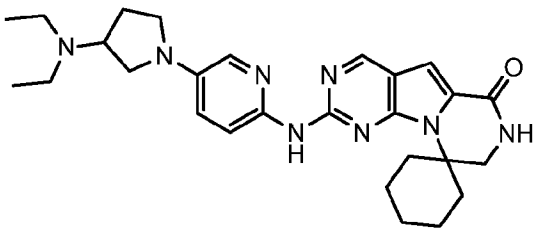
Structure Reference	Structure
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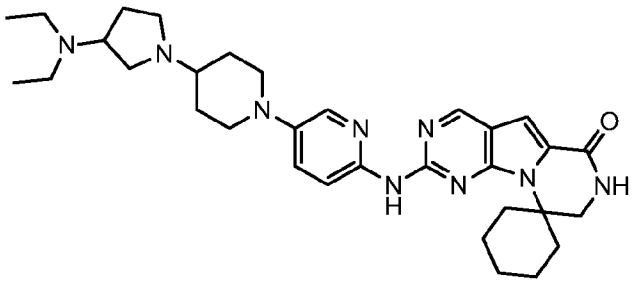
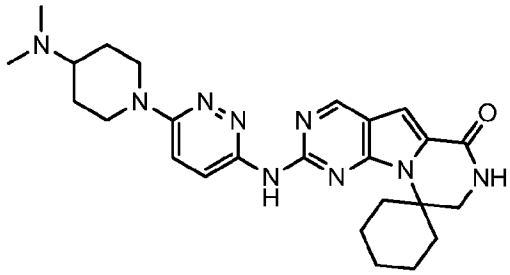
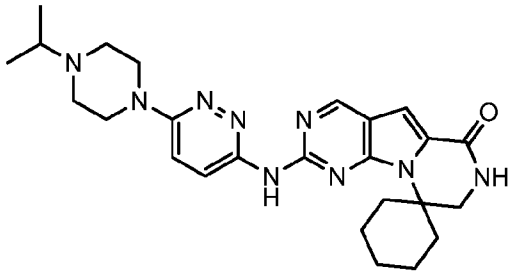
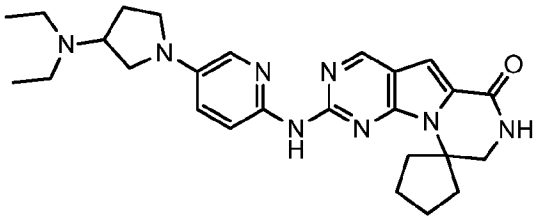
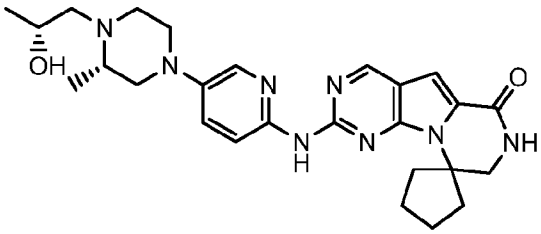
Structure Reference	Structure
Q	
R	
S	
T	
U	
V	

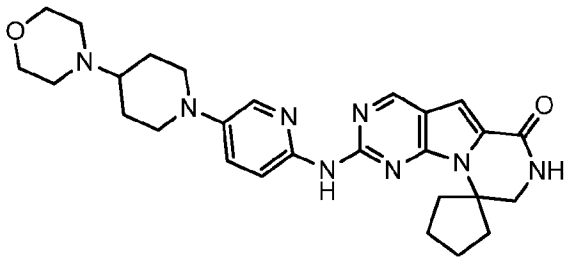
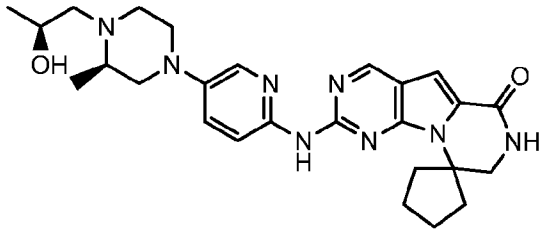
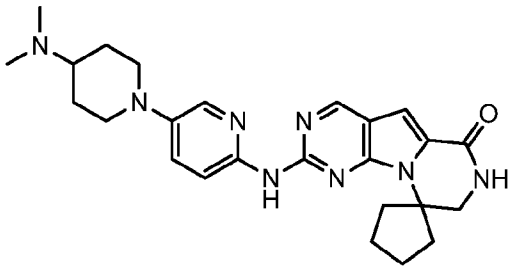
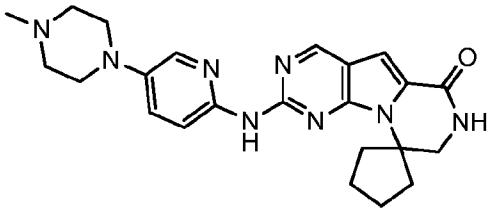
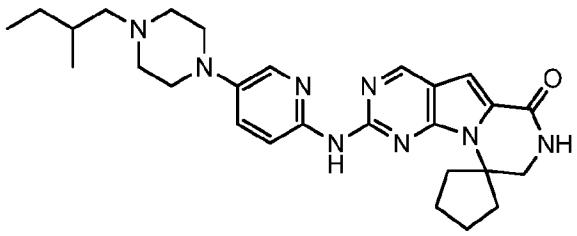
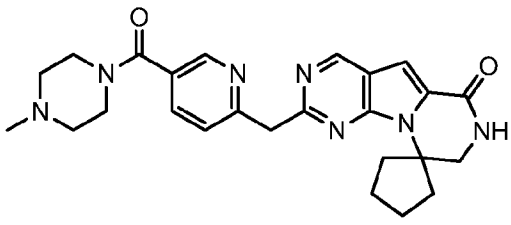
Structure Reference	Structure
W	
X	
Y	
Z	
AA	

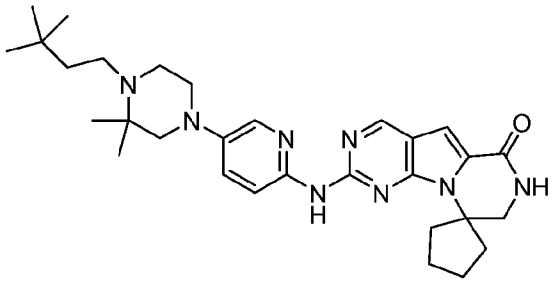
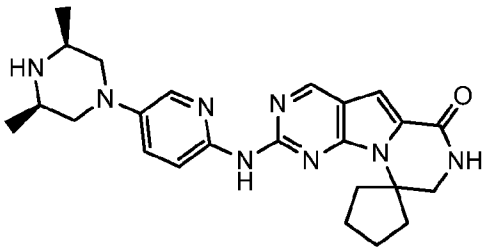
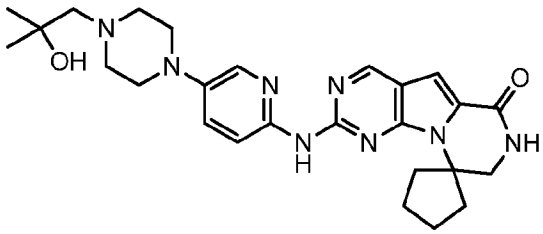
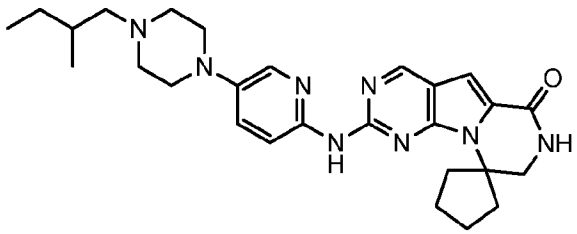
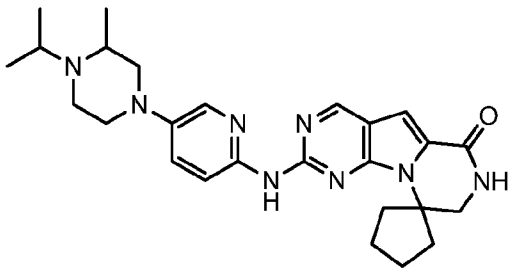
Structure Reference	Structure
BB	
CC	
DD	
EE	
FF	

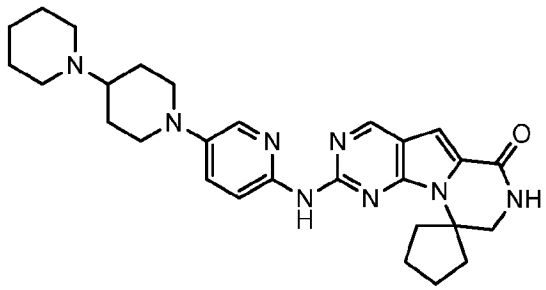
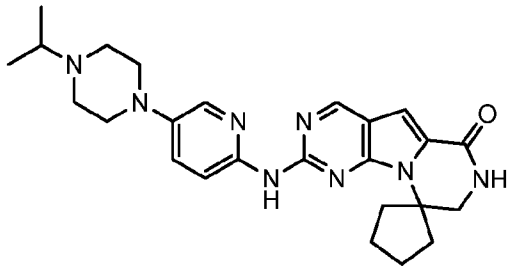
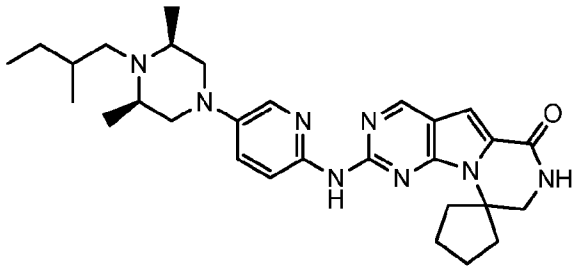
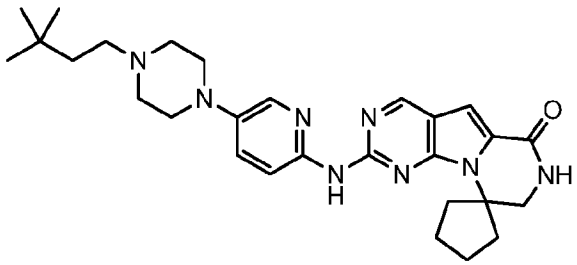
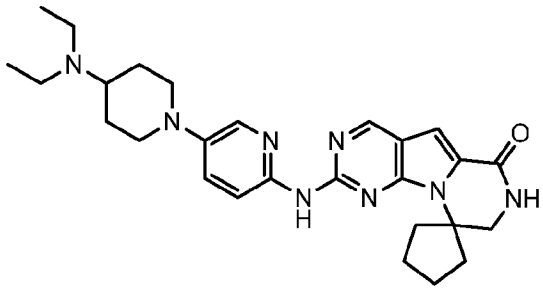
Structure Reference	Structure
GG	
HH	
II	
JJ	
KK	
LL	

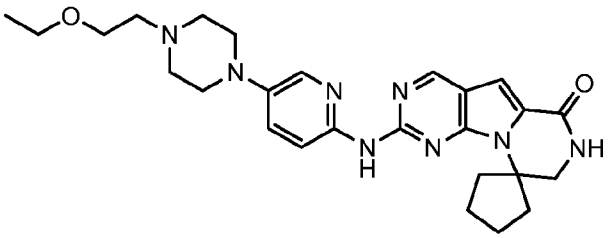
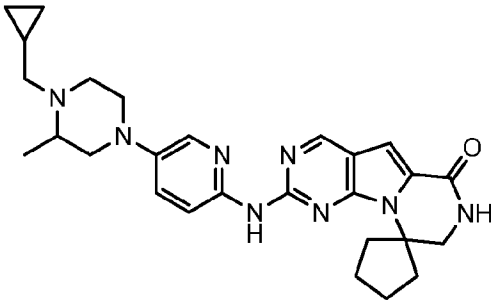
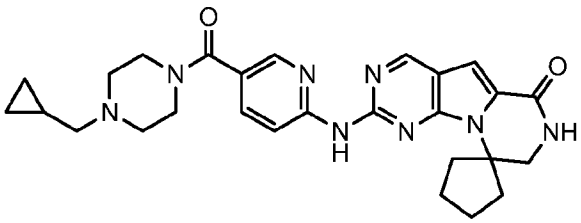
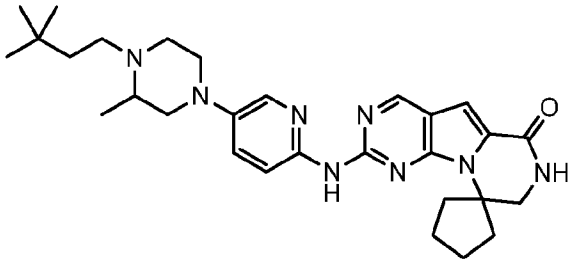
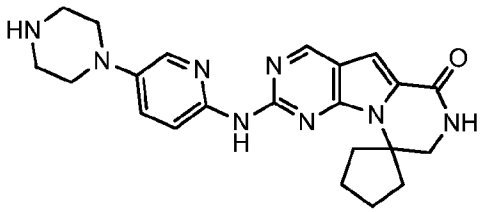
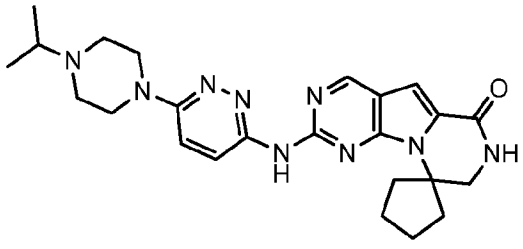
Structure Reference	Structure
MM	
NN	
OO	
PP	
QQ	

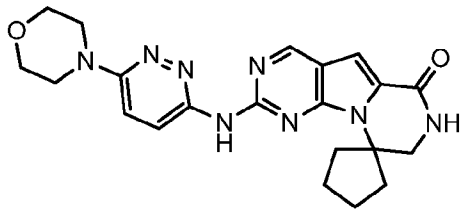
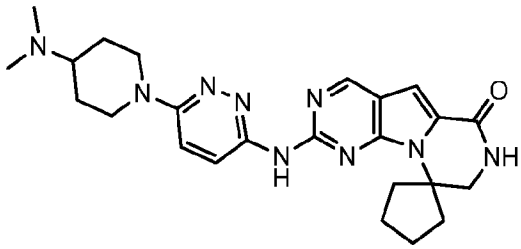
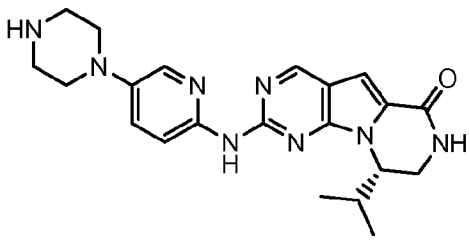
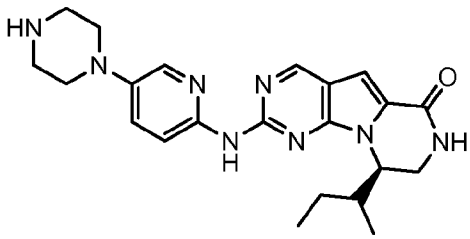
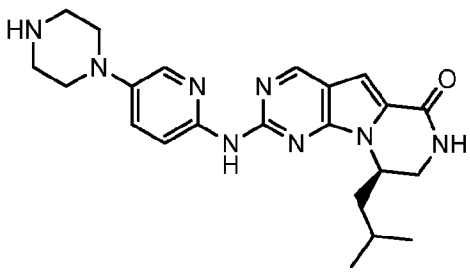
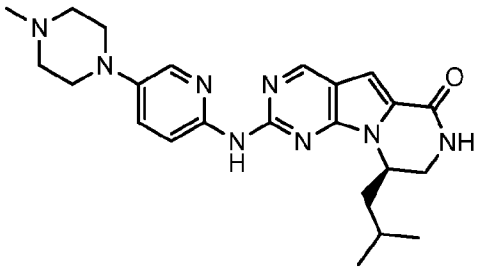
Structure Reference	Structure
RR	
SS	
TT	
UU	
VV	

Structure Reference	Structure
WW	
XX	
YY	
ZZ	
AAA	
BBB	

Structure Reference	Structure
CCC	
DDD	
EEE	
FFF	
GGG	

Structure Reference	Structure
HHH	
III	
JJJ	
KKK	
LLL	

Structure Reference	Structure
MMM	
NNN	
OOO	
PPP	
QQQ	
RRR	

Structure Reference	Structure
SSS	
TTT	
UUU	
VVV	
WWW	
XXX	

89. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.
90. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.
91. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.
92. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.
93. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.
94. The method of claim 88, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.
95. The method of claim 88, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.
96. The method of claim 88, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.
97. The method of claim 88, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.
98. The method of any of claims 87-97, wherein the Rb-positive cancer is a solid tumor.
99. The method of any of claims 87-98, wherein the solid tumor is selected from non-small cell lung cancer, breast cancer, colorectal cancer, liver cancer, ovarian cancer, or glioblastoma.
100. The method of claim 99, wherein the solid tumor is Erb-2/human epidermal growth factor receptor (HER)-2-positive breast cancer.
101. The method of any of claim 87-100, wherein the Rb-positive cancer is a hematological cancer.
102. The method of claim 101, wherein the hematological cancer is acute myeloid leukemia (AML).

103. The method of claim 101, wherein the hematological cancer is a T-cell cancer.

104. The method of any of claims 87-103, wherein the CDK 4/6 inhibitor compound is conjugated to a targeting agent.

105. The method of claim 104, wherein the targeting agent is an antibody or antibody fragment.

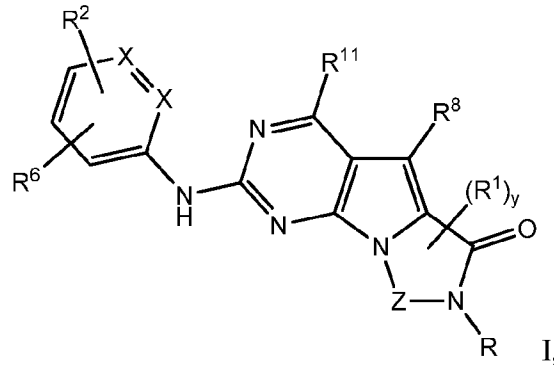
106. The method of any of claims 87-103, wherein the CDK 4/6 inhibitor compound is conjugated to a radioisotope.

107. The method of any of claims 87-106, wherein the SYK inhibitor is selected from Cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino)pyrimidine-5-carboxamide), entospletinib (6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine), fostamatinib ([6-({5-Fluoro-2-[(3,4,5-trimethoxyphenyl)amino]-4-pyrimidinyl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b][1,4]oxazin-4-yl]methyl dihydrogen phosphate), fostamatinib disodium salt (sodium (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-3-oxo-2H-pyrido[3,2-b][1,4]oxazin-4(3H)-yl)methyl phosphate), BAY 61-3606 (2-(7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HCl), RO9021 (6-[(1R,2S)-2-Amino-cyclohexylamino]-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide), imatinib (Gleevac; 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), staurosporine, GSK143 (2-(((3R,4R)-3-aminotetrahydro-2H-pyran-4-yl)amino)-4-(p-tolylamino)pyrimidine-5-carboxamide), PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine), PRT-060318 (2-(((1R,2S)-2-aminocyclohexyl)amino)-4-(m-tolylamino)pyrimidine-5-carboxamide), PRT-062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), R112 (3,3'-((5-fluoropyrimidine-2,4-diyl)bis(azanediyl))diphenol), R348 (3-Ethyl-4-methylpyridine), R406 (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one), piceatannol (3-Hydroxyresveratol), YM193306(see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643), 7-azaindole, piceatannol, ER-

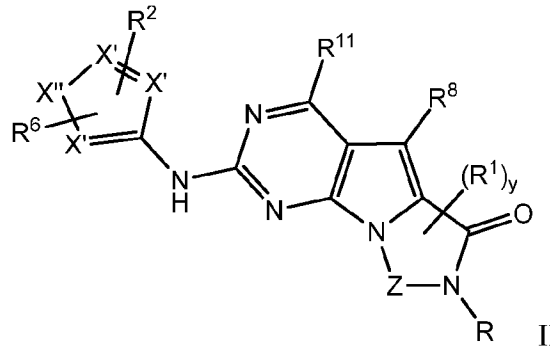
27319, Compound D, PRT060318, luteolin, apigenin, quercetin, fisetin, myricetin, or morin.

108. The method of any of claims 87-107, wherein the host is a human.

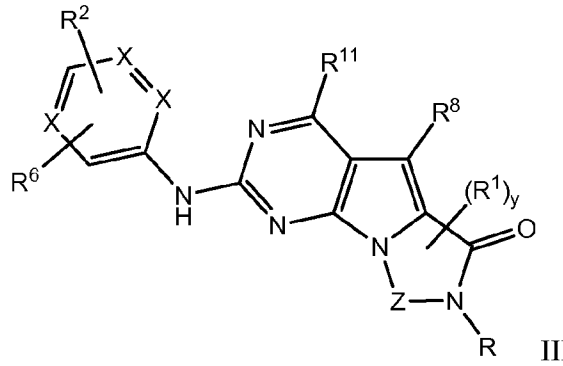
109. A method for the treatment of an Rb-positive cancer comprising administering an effective amount of a CDK 4/6 inhibitor compound of Formula I, II, III, IV, or V in combination with a PI3 kinase inhibitor to a host in need thereof, wherein the PI3 kinase inhibitor is pictilisib, wherein Formula I, II, III, IV, or V has the structure:



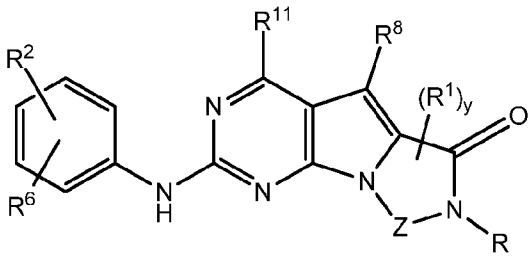
I,

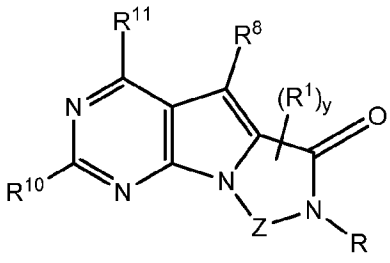


II,



III,


IV, or


V,

wherein:

Z is $-(CH_2)_x-$ wherein x is 1, 2, 3 or 4 or $-O-(CH_2)_z-$ wherein z is 2, 3 or 4;

each X is independently CH or N;

each X′ is independently, CH or N;

X″ is independently CH_2, S or NH, arranged such that the moiety is a stable 5-membered ring;

R, R^8, and R^{11} are independently H, C_1-C_3 alkyl or haloalkyl, cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O or S; -(alkylene)m- C_3-C_8 cycloalkyl, -$(alkylene)_m$-aryl, -$(alkylene)_m$-heterocyclo, -$(alkylene)_m$- heteroaryl, -$(alkylene)_m$-NR^3R^4, -$(alkylene)_m$-C(0)-NR^3R^4; -$(alkylene)_m$-0-R^5, -$(alkylene)_m$- $S(0)_n$-R^5, or -$(alkylene)_m$-S(0)n-NR^3R^4 any of which may be optionally independently substituted with one or more R groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring;

each R^1 is independently aryl, alkyl, cycloalkyl or haloalkyl, wherein each of said alkyl, cycloalkyl and haloalkyl groups optionally includes O or N heteroatoms in place of a carbon in the chain and two R^1's on adjacent ring atoms or on the same ring atom together with the ring atom(s) to which they are attached optionally form a 3-8-membered cycle;

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

R^2 is $-(alkylene)_m-$heterocyclo, $-(alkylene)_m-$heteroaryl, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(O)-NR^3R^4$; $-(alkylene)_m-C(O)-O$-alkyl; $-(alkylene)_m-O-R^5$, $-(alkylene)_m-S(O)_n-R^5$, or $-(alkylene)_m-S(O)_n-NR^3R^4$ any of which may be optionally

independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring and wherein m is 0 or 1 and n is 0, 1 or 2;

R^3 and R^4 at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring; or R^3 and R^4 together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atom may optionally combine to form a ring;

R^5 and R^{5*} at each occurrence is:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance;

R^x at each occurrence is independently, halo, cyano, nitro, oxo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, heterocycloalkyl, $-(alkylene)_m-OR^5$, $-(alkylene)_m-O-alkylene-OR^5$, $-(alkylene)_m-S(O)_n-R^5$, $-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-CN$, $-(alkylene)_m-C(O)-R^5$, $-(alkylene)_m-C(S)-R^5$, $-(alkylene)_m-C(O)-OR^5$, $-(alkylene)_m-O-C(O)-R^5$, $-(alkylene)_m-C(S)-OR^5$, $-(alkylene)_m-C(O)-(alkylene)_m-NR^3R^4$, $-(alkylene)_m-C(S)-NR^3R^4$, $-(alkylene)_m-N(R^3)-C(O)-NR^3R^4$, $-(alkylene)_m-N(R^3)-C(S)-NR^3R^4$, $-(alkylene)_m-N(R^3)-C(O)-R^5$, $-(alkylene)_m-N(R^3)-C(S)-R^5$, $-(alkylene)_m-O-C(O)-NR^3R^4$, $-(alkylene)_m-O-C(S)-NR^3R^4$, $-(alkylene)_m-SO_2-NR^3R^4$, $-(alkylene)_m-N(R^3)-SO_2-R^5$, $-(alkylene)_m-N(R^3)-SO_2-NR^3R^4$, $-(alkylene)_m-N(R^3)-C(O)-OR^5$, $-(alkylene)_m-N(R^3)-C(S)-OR^5$, or $-(alkylene)_m-N(R^3)-SO_2-R^5$; wherein:

said alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocyclo, aryl, heteroaryl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, and heterocycloalkyl groups

may be further independently substituted with one or more -(alkylene)$_m$-CN, -(alkylene)$_m$-OR^5*, -(alkylene)$_m$-S(O)$_n$–R^5*, -(alkylene)$_m$-NR^3*R^4*, -(alkylene)$_m$-C(O)–R^5*, -(alkylene)$_m$-C(=S)R^5*, -(alkylene)$_m$-C(=O)O R^5*, -(alkylene)$_m$-OC(=O)R^5*, -(alkylene)$_m$-C(S)–OR^5*, -(alkylene)$_m$-C(O)–NR^3*R^4*, -(alkylene)$_m$-C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(S)–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–R^5*, -(alkylene)$_m$-N(R^3*)–C(S)–R^5*, -(alkylene)$_m$-O–C(O)–NR^3*R^4*, -(alkylene)$_m$-O–C(S)–NR^3*R^4*, -(alkylene)$_m$-SO$_2$–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–SO$_2$–R^5*, -(alkylene)$_m$-N(R^3*)–SO$_2$–NR^3*R^4*, -(alkylene)$_m$-N(R^3*)–C(O)–OR^5*, -(alkylene)$_m$-N(R^3*)–C(S)–OR^5*, or -(alkylene)$_m$-N(R^3*)–SO$_2$–R^5*,

n is 0, 1 or 2, and

m is 0 or 1;

R^3* and R^4* at each occurrence are independently:

(i) hydrogen or

(ii) alkyl, alkenyl, alkynyl cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkylalkyl, heterocycloalkyl, arylalkyl, or heteroarylalkyl any of which may be optionally independently substituted with one or more R^x groups as allowed by valance; or R^3* and R^4* together with the nitrogen atom to which they are attached may combine to form a heterocyclo ring optionally independently substituted with one or more R^x groups as allowed by valance; and

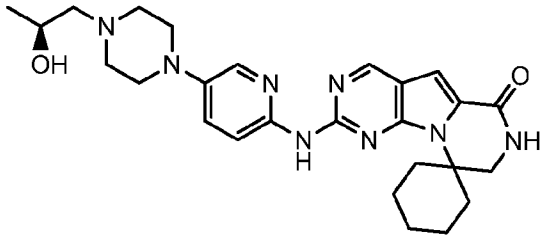
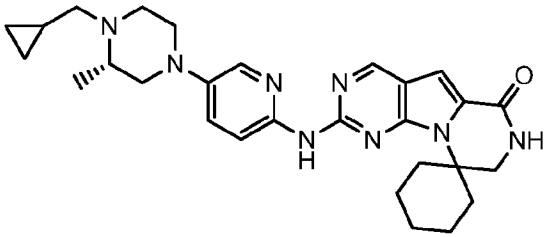
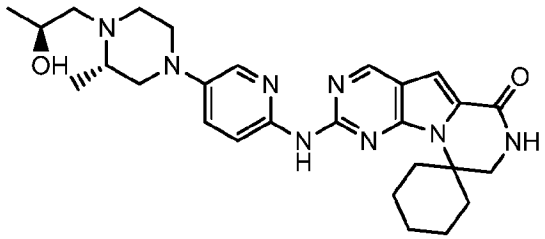
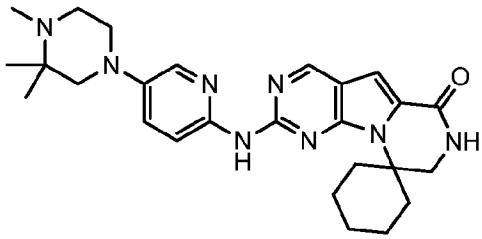
R^6 is H or lower alkyl, -(alkylene)m-heterocyclo, -(alkylene)$_m$-heteroaryl, -(alkylene)$_m$-NR^{3}R^4, -(alkylene)$_m$-C(0)-NR^{3}R^4; -(alkylene)$_m$-0-R^5, -(alkylene)$_m$-S(0)$_n$-R^5, or -(alkylene)$_m$-S(0)$_n$-NR^{3}R^4 any of which may be optionally independently substituted with one or more R^x groups as allowed by valance, and wherein two R^x groups bound to the same or adjacent atoms may optionally combine to form a ring; and

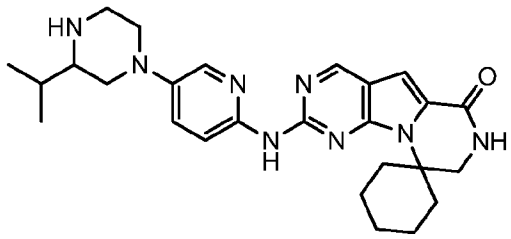
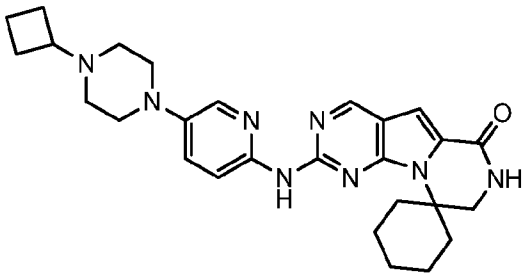
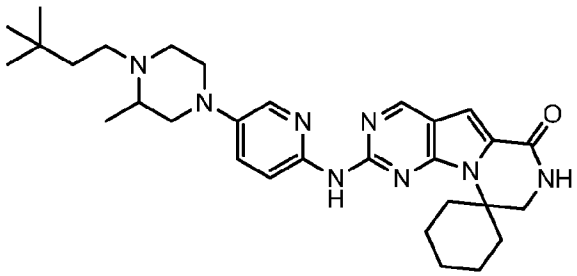
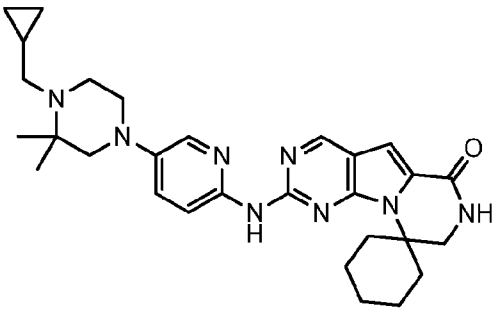
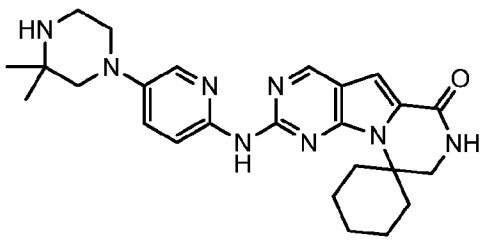
R^{10} is (i) NHR^A, wherein R^A is unsubstituted or substituted C$_1$-C$_8$ alkyl, cycloalkylalkyl, or –TT-RR, C$_1$-C$_8$ cycloalkyl or cycloalkyl containing one or more heteroatoms selected from N, O, and S; TT is an unsubstituted or substituted C$_1$-C$_8$ alkyl or C$_3$-C$_8$ cycloalkyl linker; and RR is a hydroxyl, unsubstituted or substituted C$_1$-C$_6$ alkoxy, amino, unsubstituted or substituted C$_1$-C$_6$ alkylamino, unsubstituted or substituted di-C$_1$-C$_6$ alkylamino, unsubstituted or substituted C$_6$-C$_{10}$

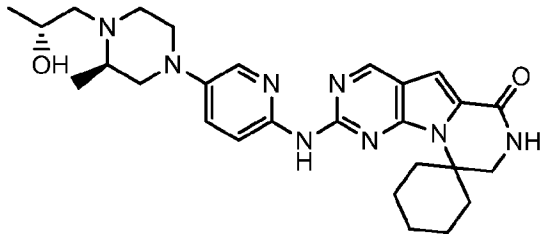
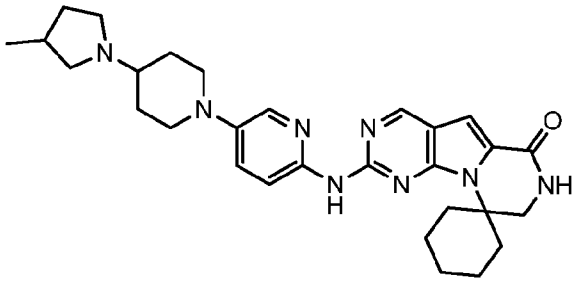
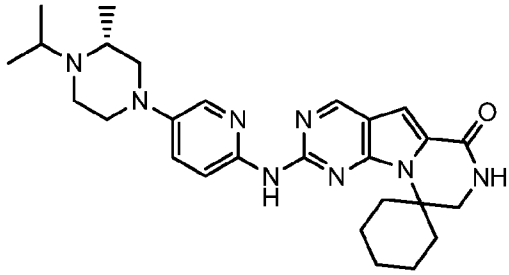
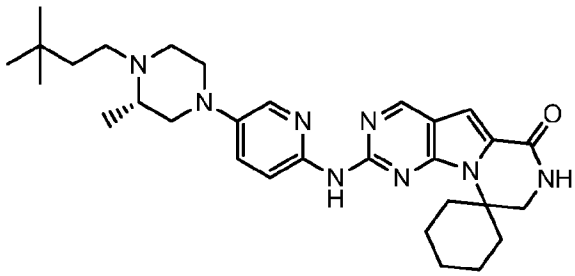
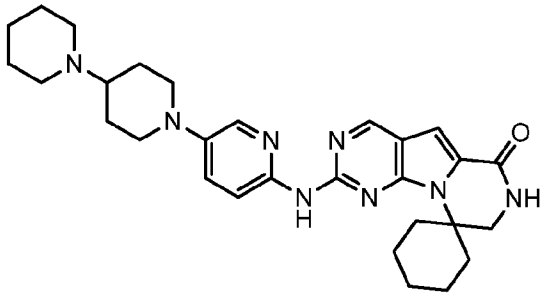
aryl, unsubstituted or substituted heteroaryl comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S, unsubstituted or substituted C_3-C_{10} carbocycle, or unsubstituted or substituted heterocycle comprising one or two 5- or 6-member rings and 1-4 heteroatoms selected from N, O and S; or (ii) -C(O)-R^{12} or –C(O)O-R^{13} , wherein R^{12} is NHR^A or R^A and R^{13} is R^A;

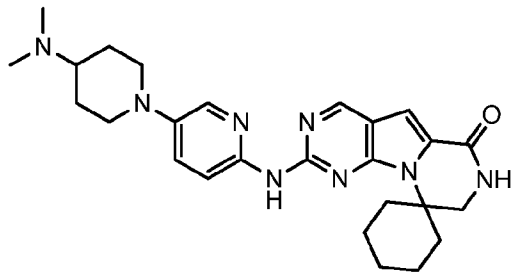
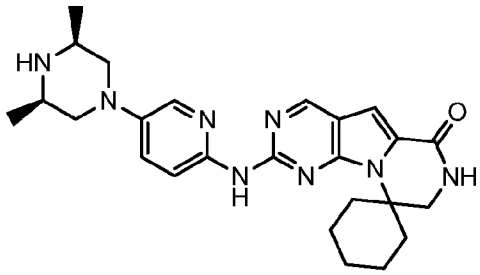
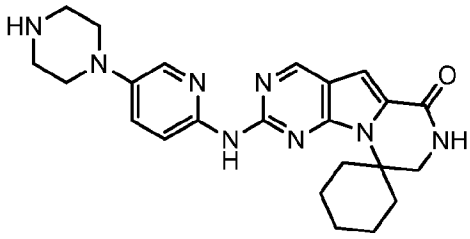
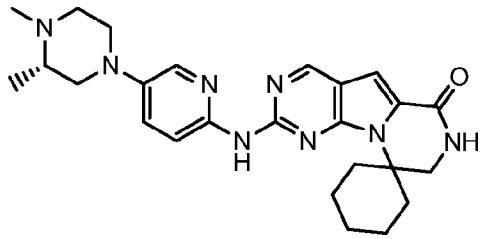
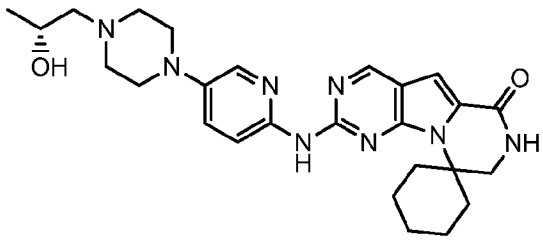
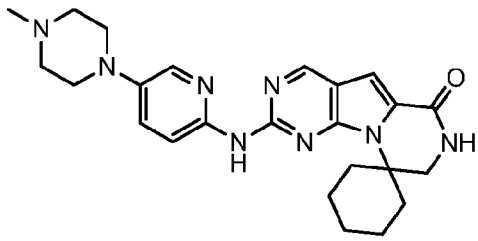
or a pharmaceutically acceptable salt thereof.

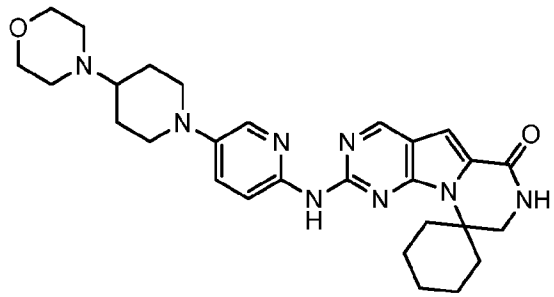
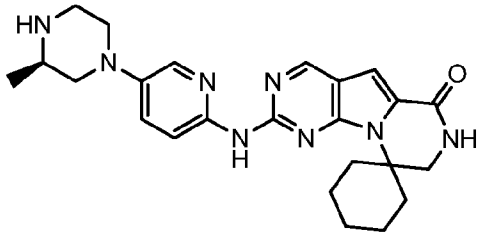
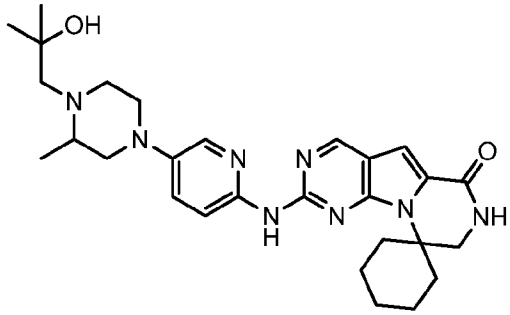
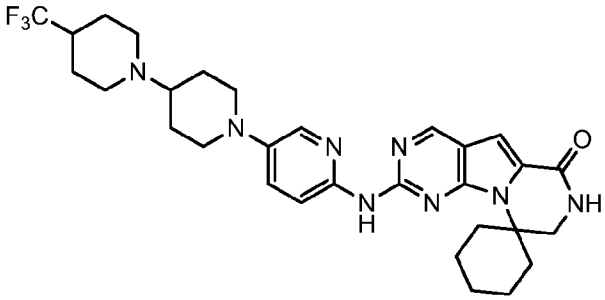
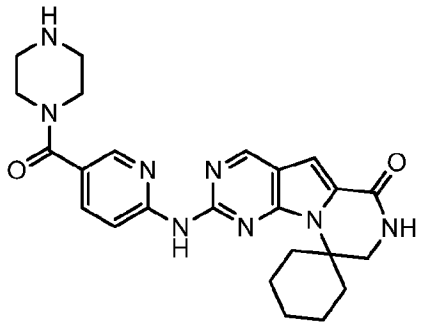
110. The method of claim 109, wherein the CDK4/6 inhbitor compound is selected from the group consisting of:

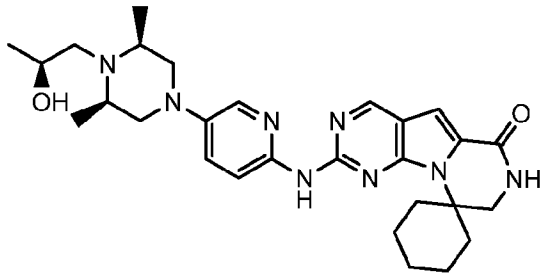
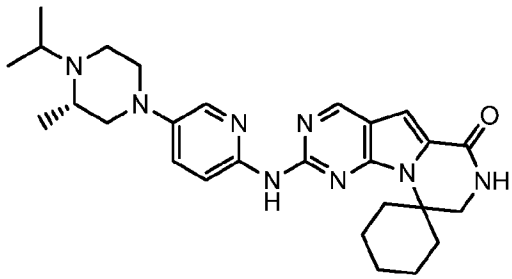
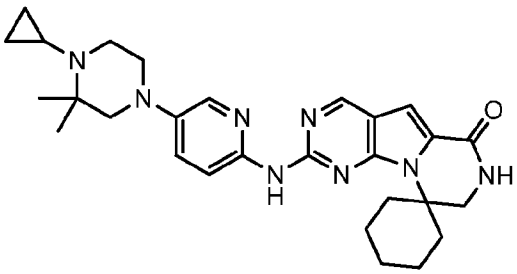
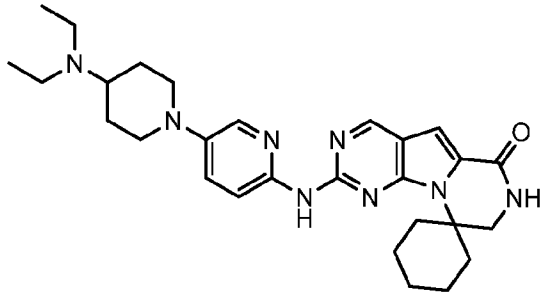
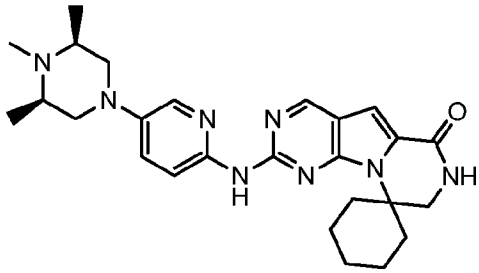
Structure Reference	Structure
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B	
C	
D	

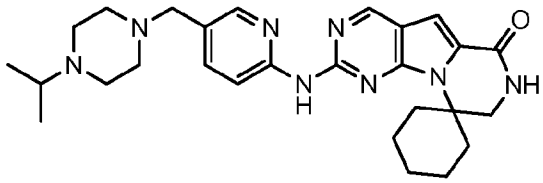
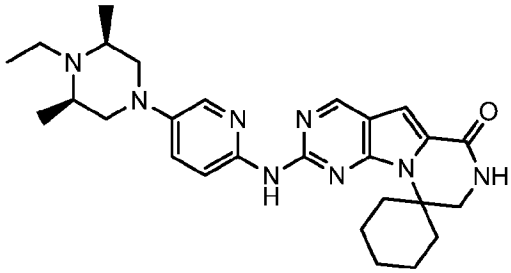
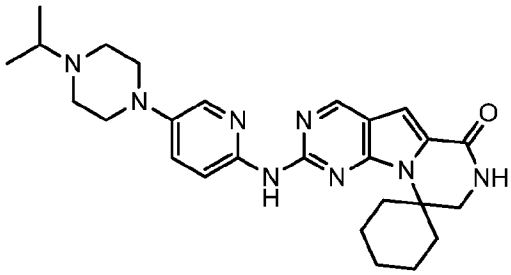
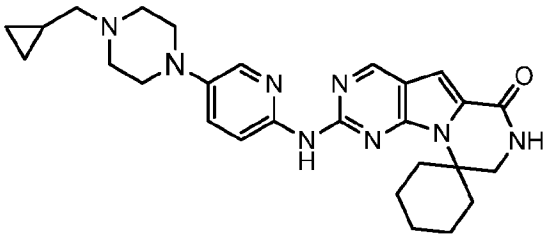
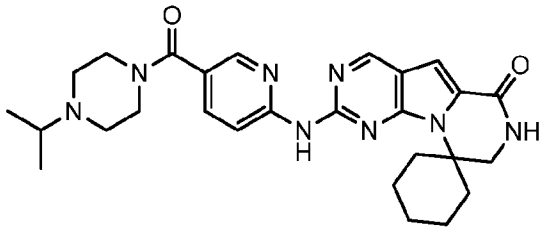
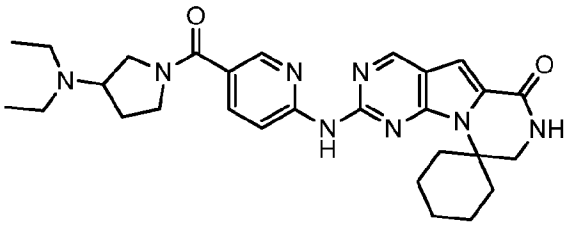
Structure Reference	Structure
E	
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G	
H	
I	

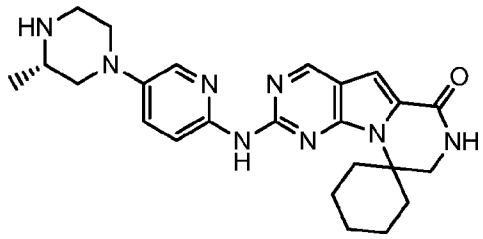
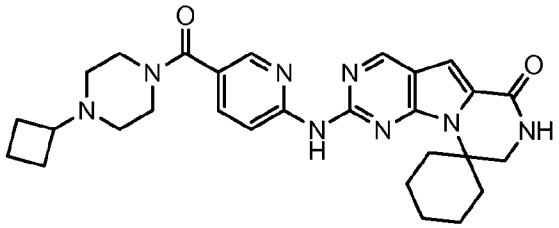
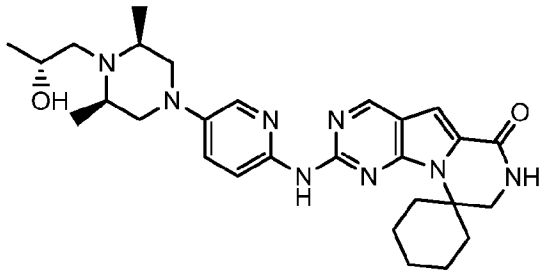
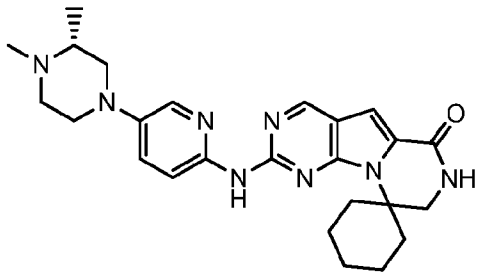
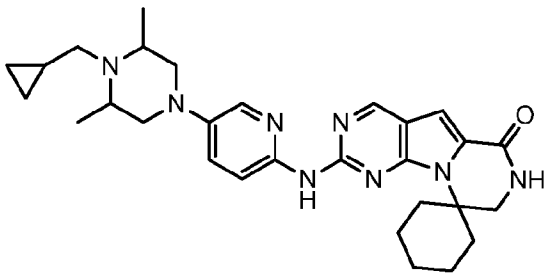
Structure Reference	Structure
J	
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M	
N	

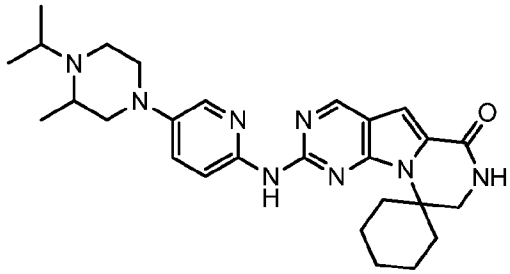
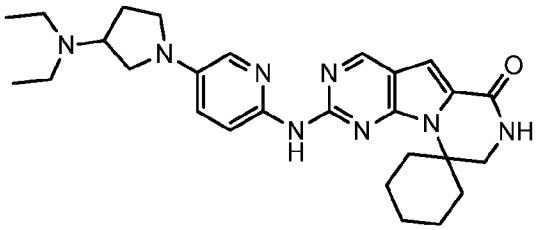
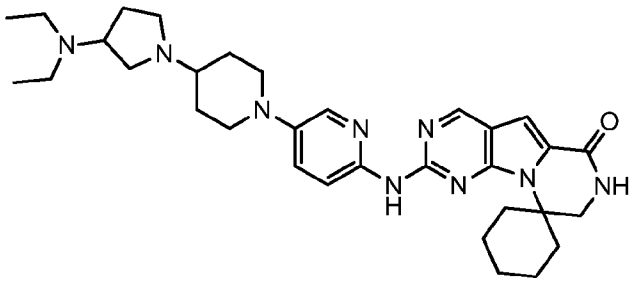
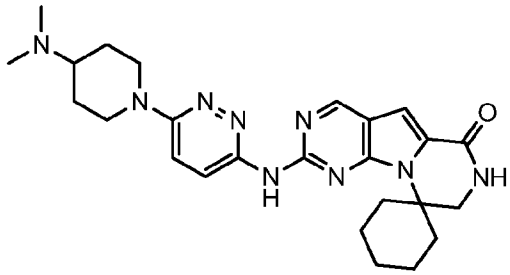
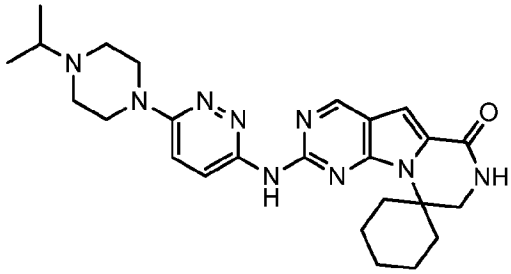
Structure Reference	Structure
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R	
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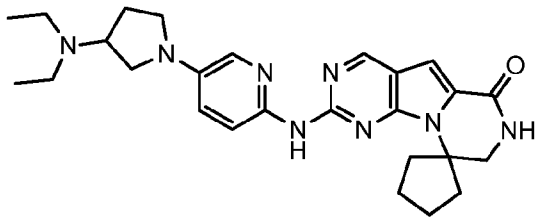
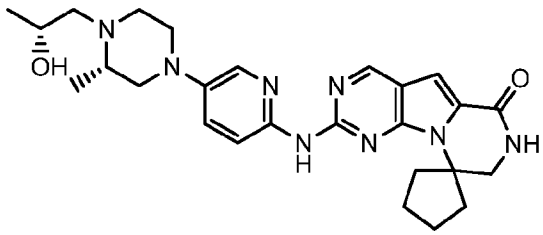
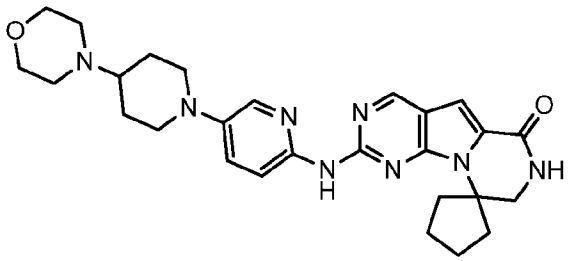
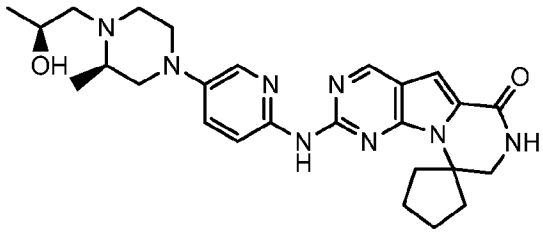
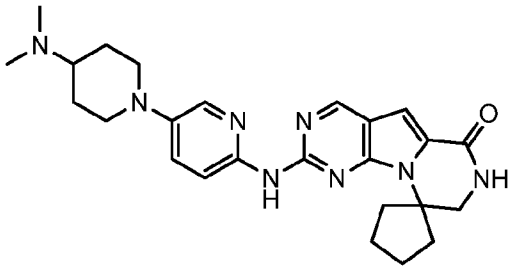
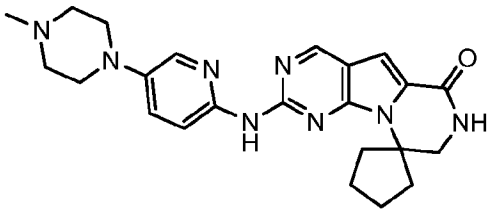
Structure Reference	Structure
U	
V	
W	
X	
Y	

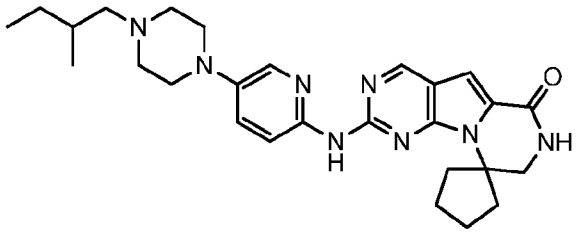
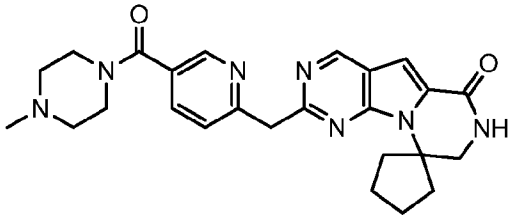
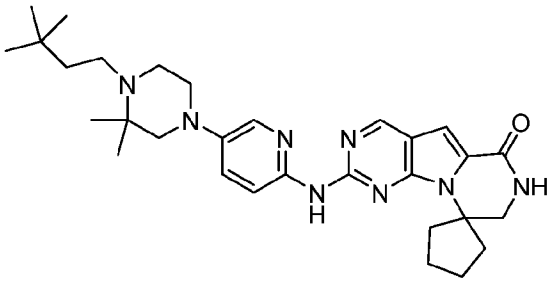
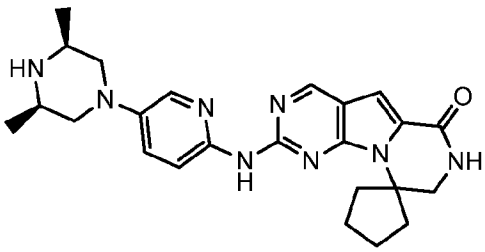
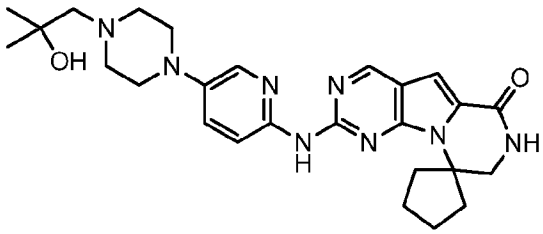
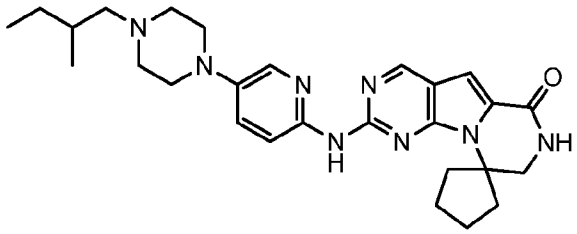
Structure Reference	Structure
Z	
AA	
BB	
CC	
DD	

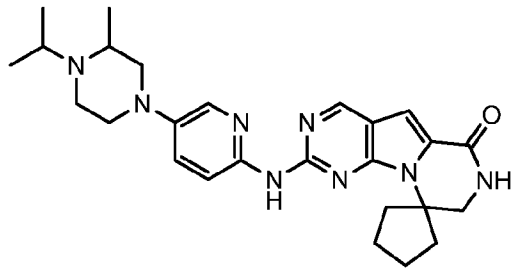
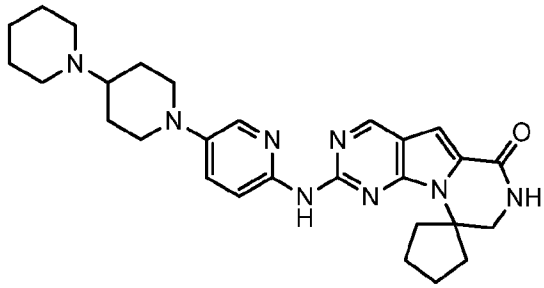
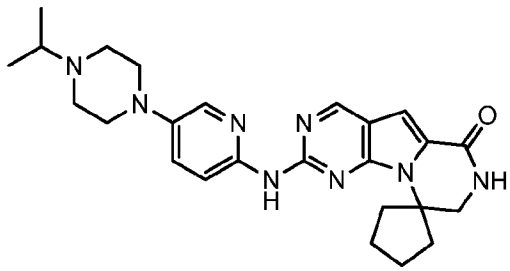
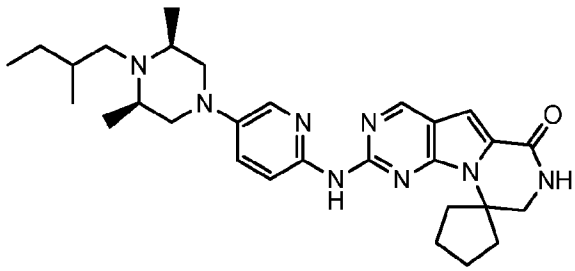
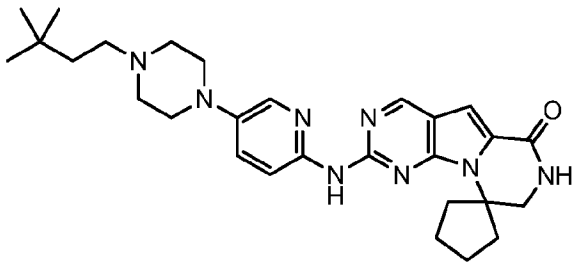
Structure Reference	Structure
EE	
FF	
GG	
HH	
II	
JJ	

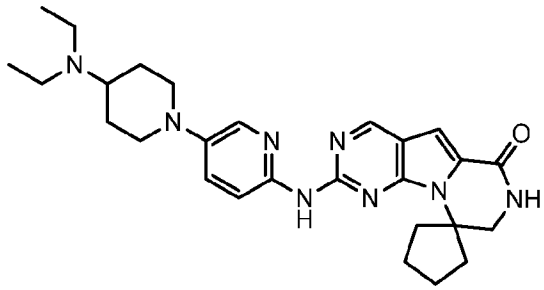
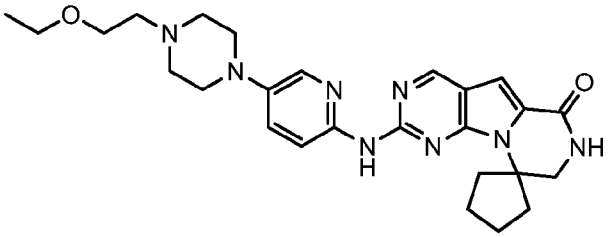
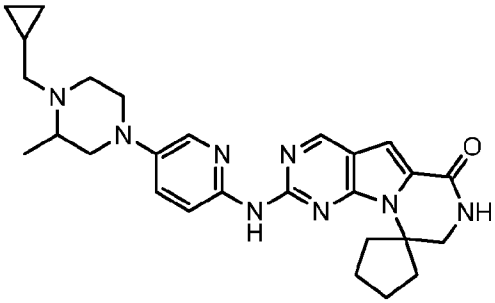
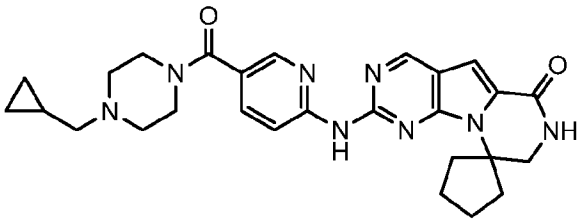
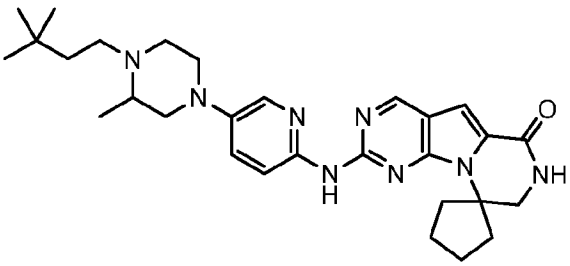
Structure Reference	Structure
KK	
LL	
MM	
NN	
OO	

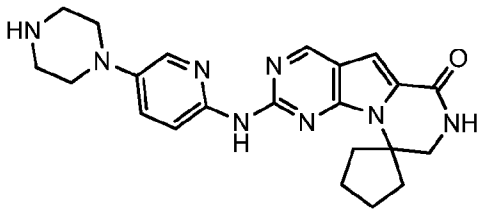
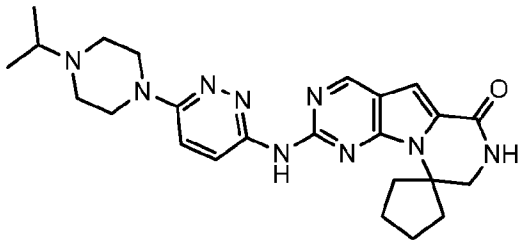
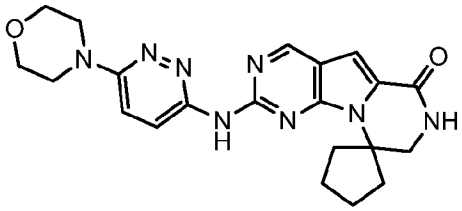
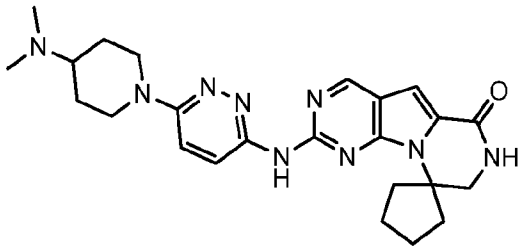
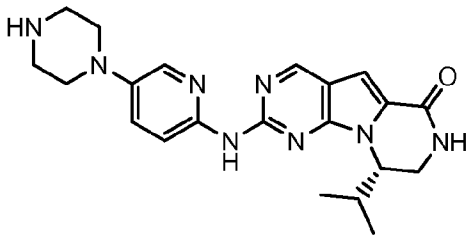
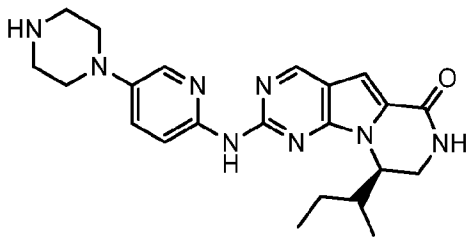
Structure Reference	Structure
PP	
QQ	
RR	
SS	
TT	

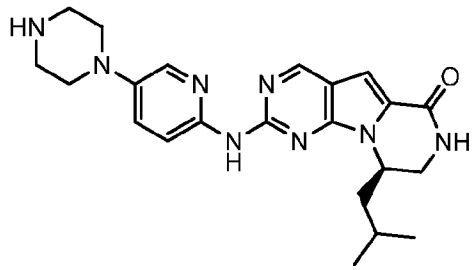
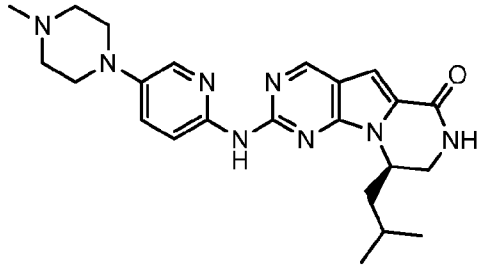
Structure Reference	Structure
UU	
VV	
WW	
XX	
YY	
ZZ	

Structure Reference	Structure
AAA	
BBB	
CCC	
DDD	
EEE	
FFF	

Structure Reference	Structure
GGG	
HHH	
III	
JJJ	
KKK	

Structure Reference	Structure
LLL	
MMM	
NNN	
OOO	
PPP	

Structure Reference	Structure
QQQ	
RRR	
SSS	
TTT	
UUU	
VVV	

Structure Reference	Structure
WWW	
XXX	

111. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound Q or its pharmaceutically acceptable salt.

112. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound T or its pharmaceutically acceptable salt.

113. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound U or its pharmaceutically acceptable salt.

114. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound GG or its pharmaceutically acceptable salt.

115. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound X or its pharmaceutically acceptable salt.

116. The method of claim 110, wherein the CDK 4/6 inhibitor compound is Compound BB or its pharmaceutically acceptable salt.

117. The method of claim 110, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound A through Compound Z, or its pharmaceutically acceptable salt.

118. The method of claim 110, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AA through ZZ, or its pharmaceutically acceptable salt.

119. The method of claim 110, wherein the CDK 4/6 inhibitor compound is selected from the group consisting of Compound AAA through XXX, or its pharmaceutically acceptable salt.
120. The method of any of claims 109-119, wherein the Rb-positive cancer is a solid tumor.
121. The method of any of claims 109-119, wherein the solid tumor is selected from colorectal, breast, soft tissue sarcoma, melanoma, ovarian, gastric, or prostate cancer.
122. The method of claim 121, wherein the solid tumor is colorectal cancer.
123. The method of claim 121, wherein the solid tumor is a breast cancer.
124. The method of claim 123, wherein the solid tumor is Erb-2/human epidermal growth factor receptor (HER)-2-positive breast cancer.
125. The method of claim 121, wherein the solid tumor is a soft tissue sarcoma.
126. The method of claim 121, wherein the solid tumor is melanoma.
127. The method of claim 121, wherein the solid tumor is ovarian cancer.
128. The method of claim 121, wherein the solid tumor is gastric cancer.
129. The method of claim 121, wherein the solid tumor is prostate cancer.
130. The method of any of claims 109-119, wherein the Rb-positive cancer is a hematological cancer.
131. The method of claim 130, wherein the hematological cancer is a T-cell cancer.
132. The method of any of claims 109-130, wherein the CDK 4/6 inhibitor compound is conjugated to a targeting agent.
133. The method of claim 132, wherein the targeting agent is an antibody or antibody fragment.
134. The method of any of claims 109-130, wherein the compound is conjugated to a radioisotope.
135. The method of claim 109-134, wherein the host is a human.

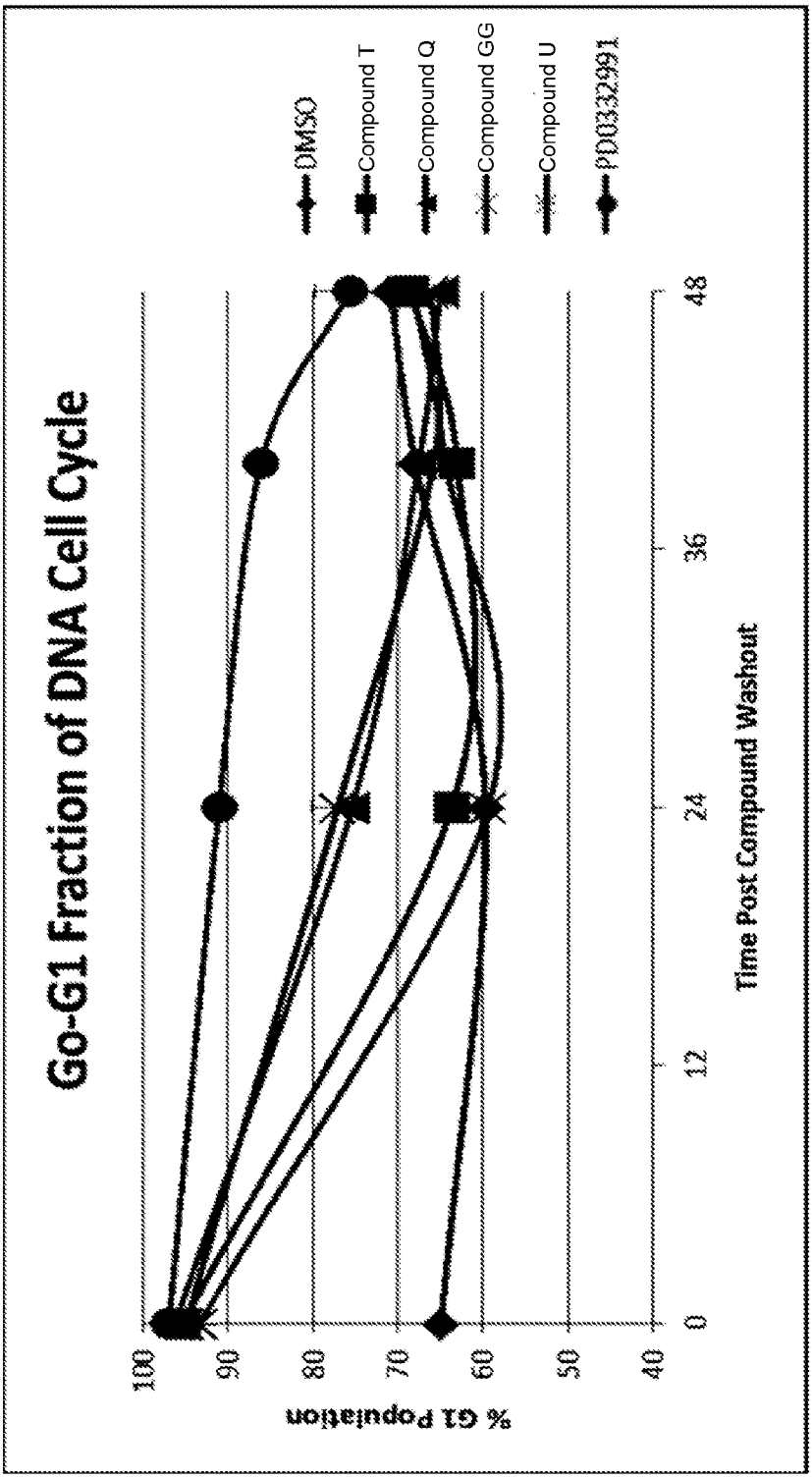

Go-G1 Fraction of DNA Cell Cycle
DMSO
Compound T
Compound Q
Compound GG
Compound U
PD0332991
% G1 Population
100
90
80
70
60
50
40
0
12
24
36
48
Time Post Compound Washout

FIG. 1A

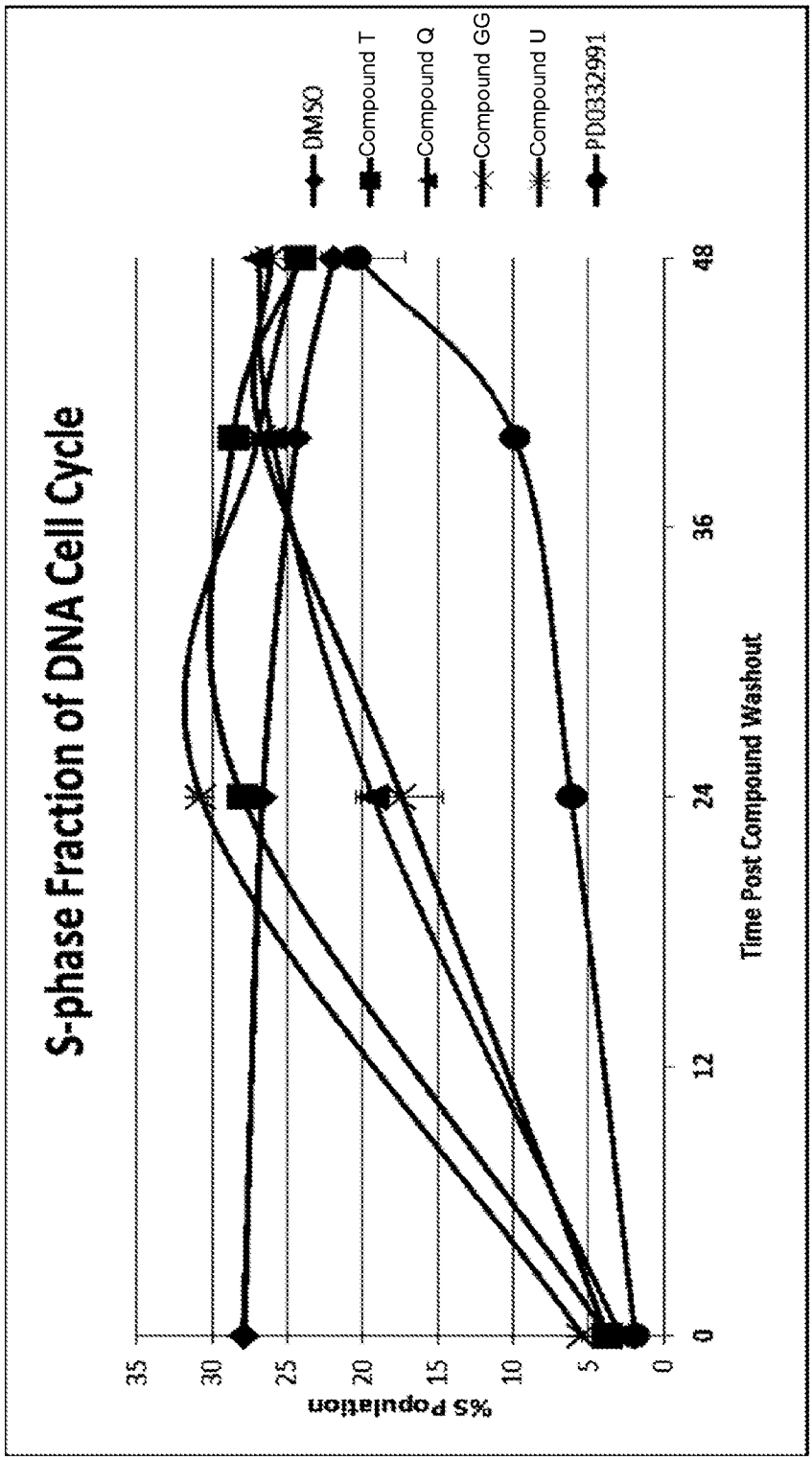

S-phase Fraction of DNA Cell Cycle
DMSO
Compound T
Compound Q
Compound GG
Compound U
PD0332991
%S Population
35
30
25
20
15
10
5
0
0
12
24
36
48
Time Post Compound Washout

FIG. 1B

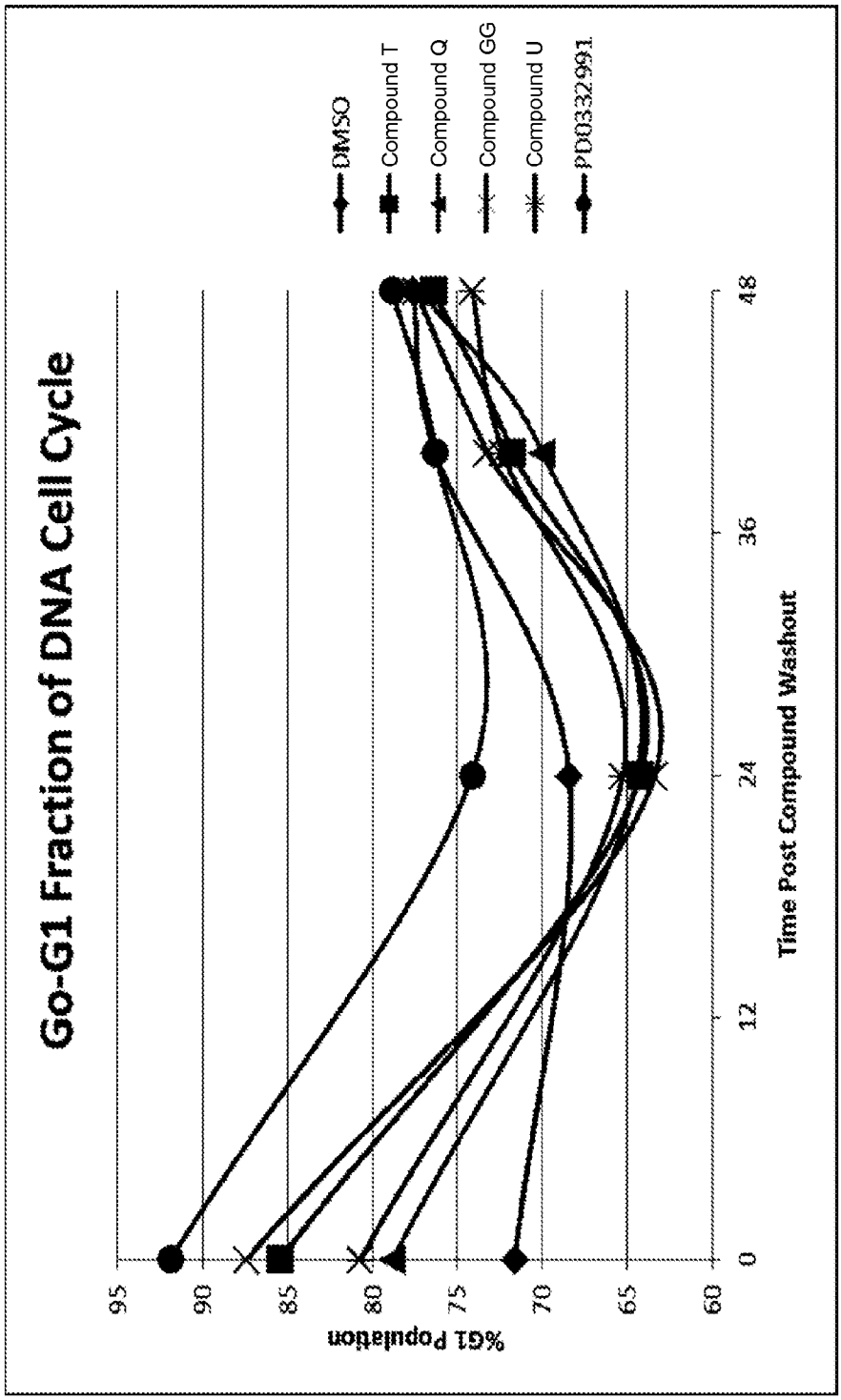

Go-G1 Fraction of DNA Cell Cycle
DMSO
Compound T
Compound Q
Compound GG
Compound U
PD0332991
%G1 Population
95
90
85
80
75
70
65
60
0
12
24
36
48
Time Post Compound Washout

FIG. 1C

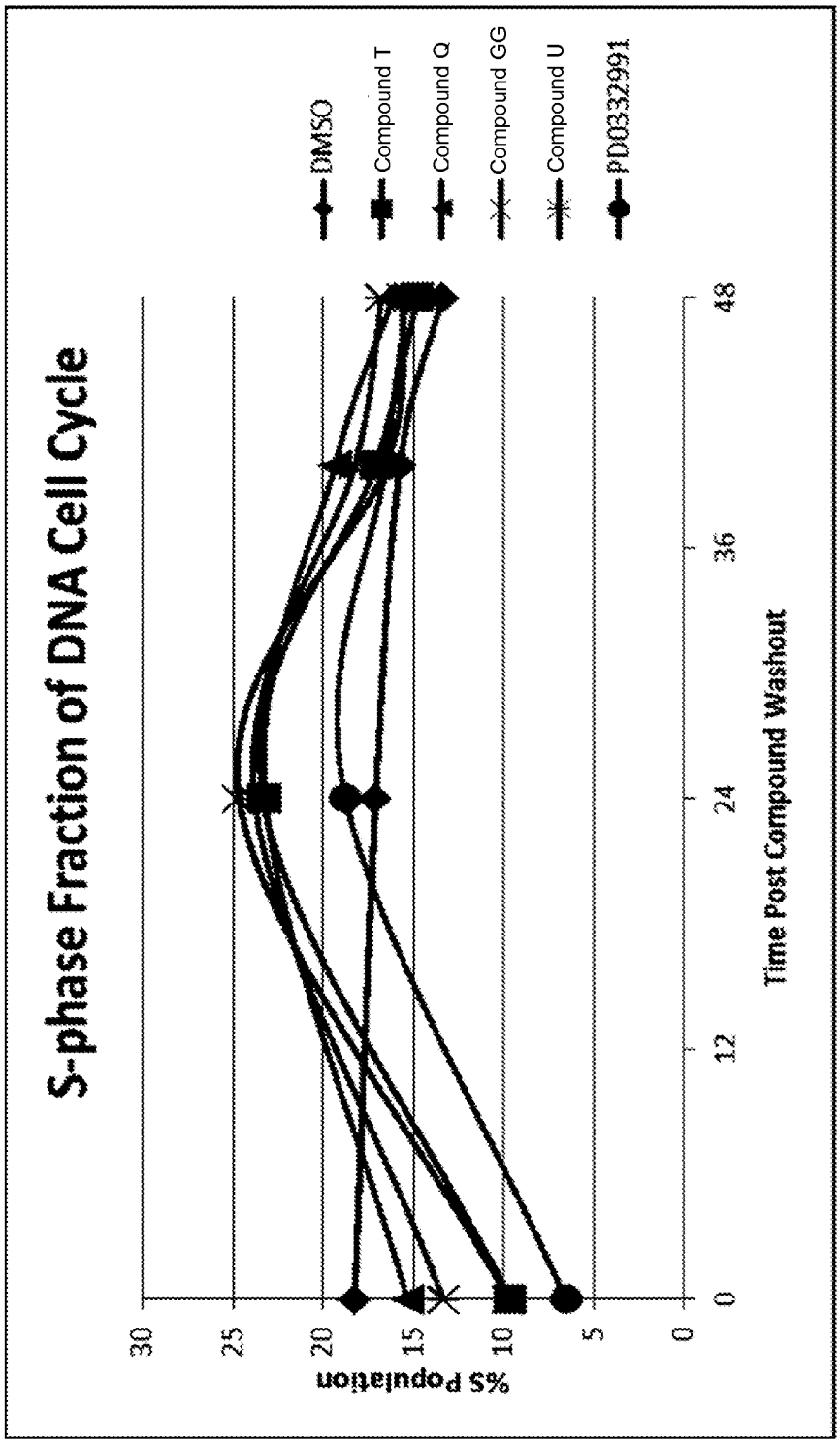

S-phase Fraction of DNA Cell Cycle
DMSO
Compound T
Compound Q
Compound GG
Compound U
PD0332991
%S Population
30
25
20
15
10
5
0
0
12
24
36
48
Time Post Compound Washout

FIG. 1D

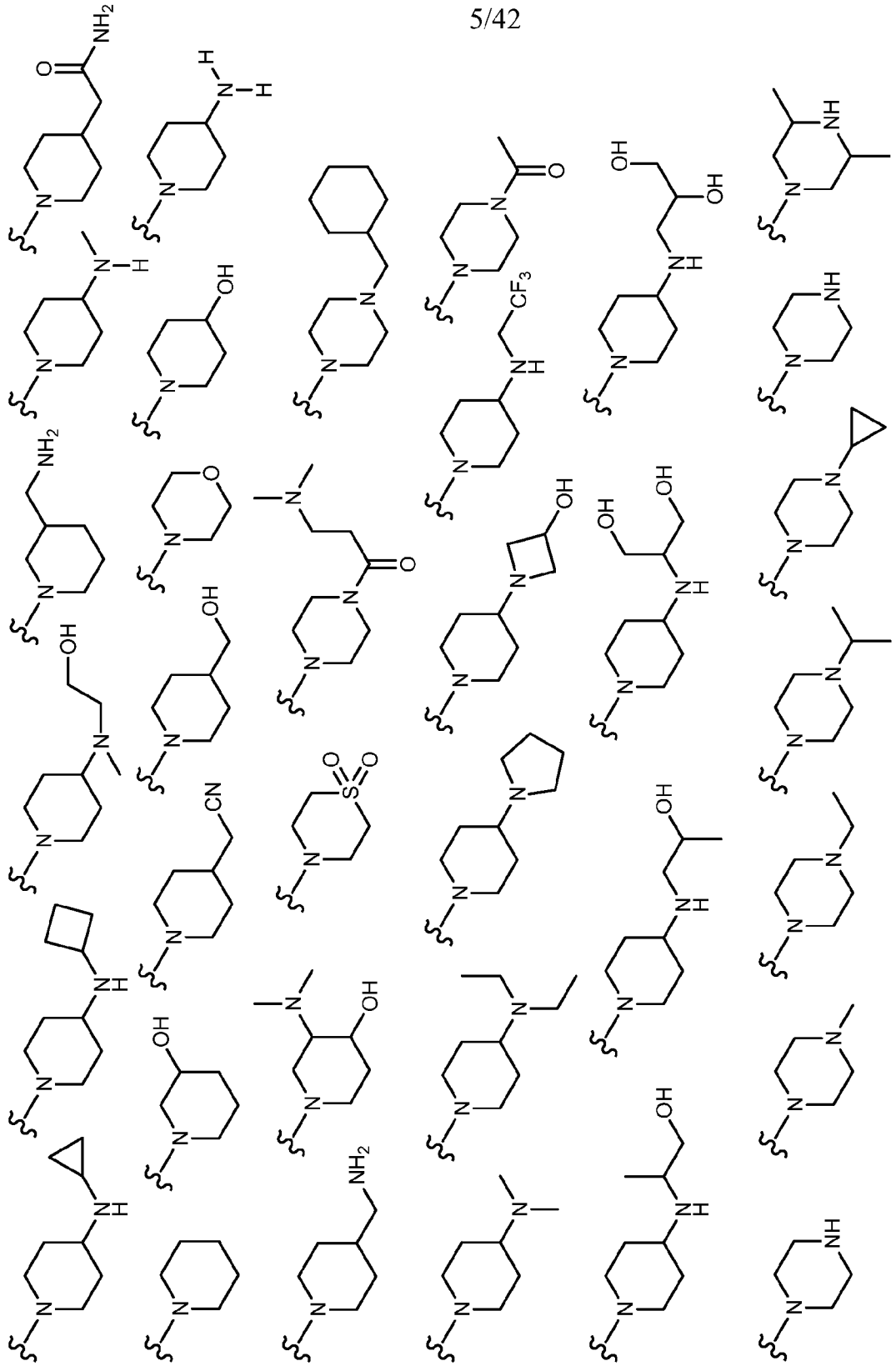


FIG. 2

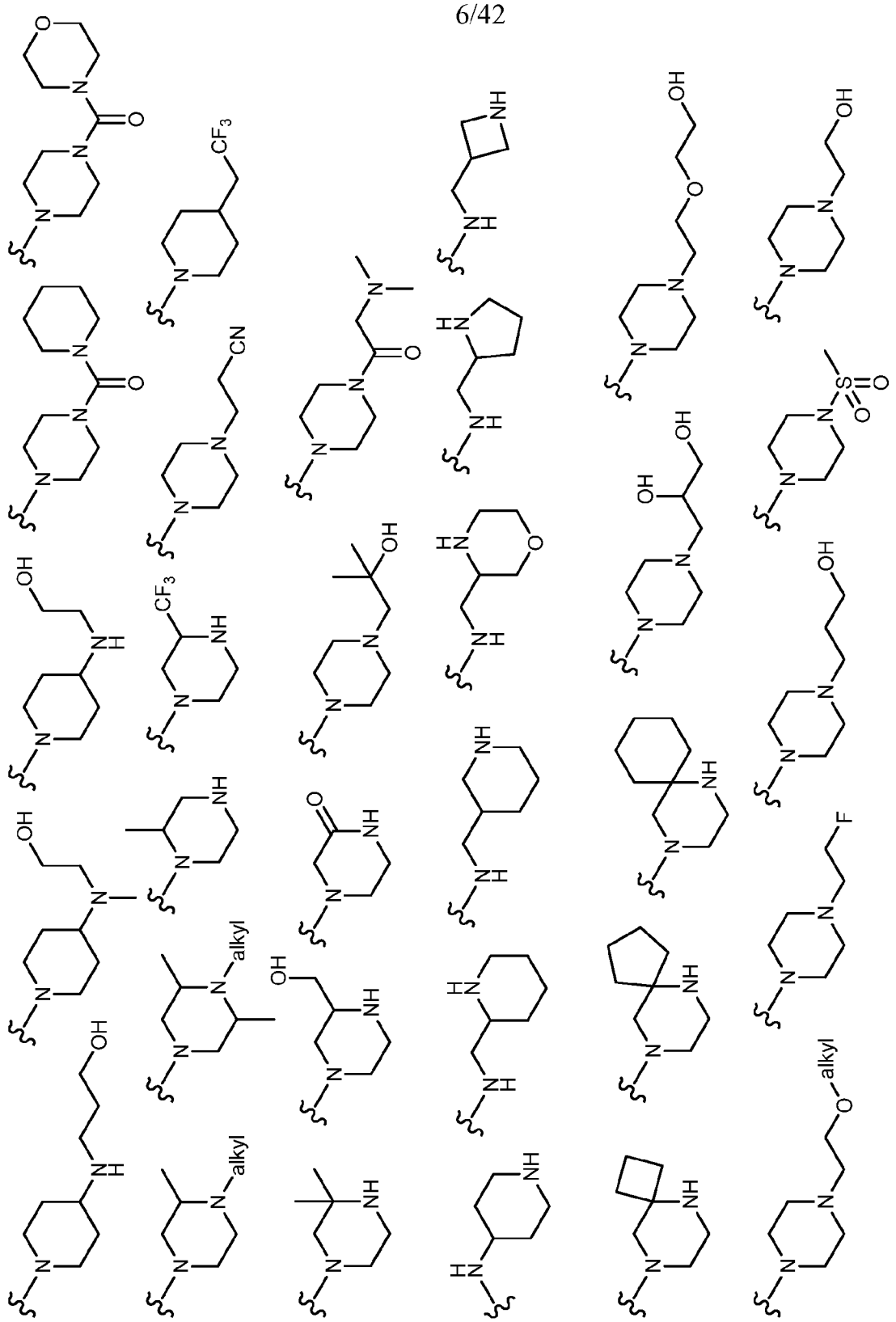


FIG. 3

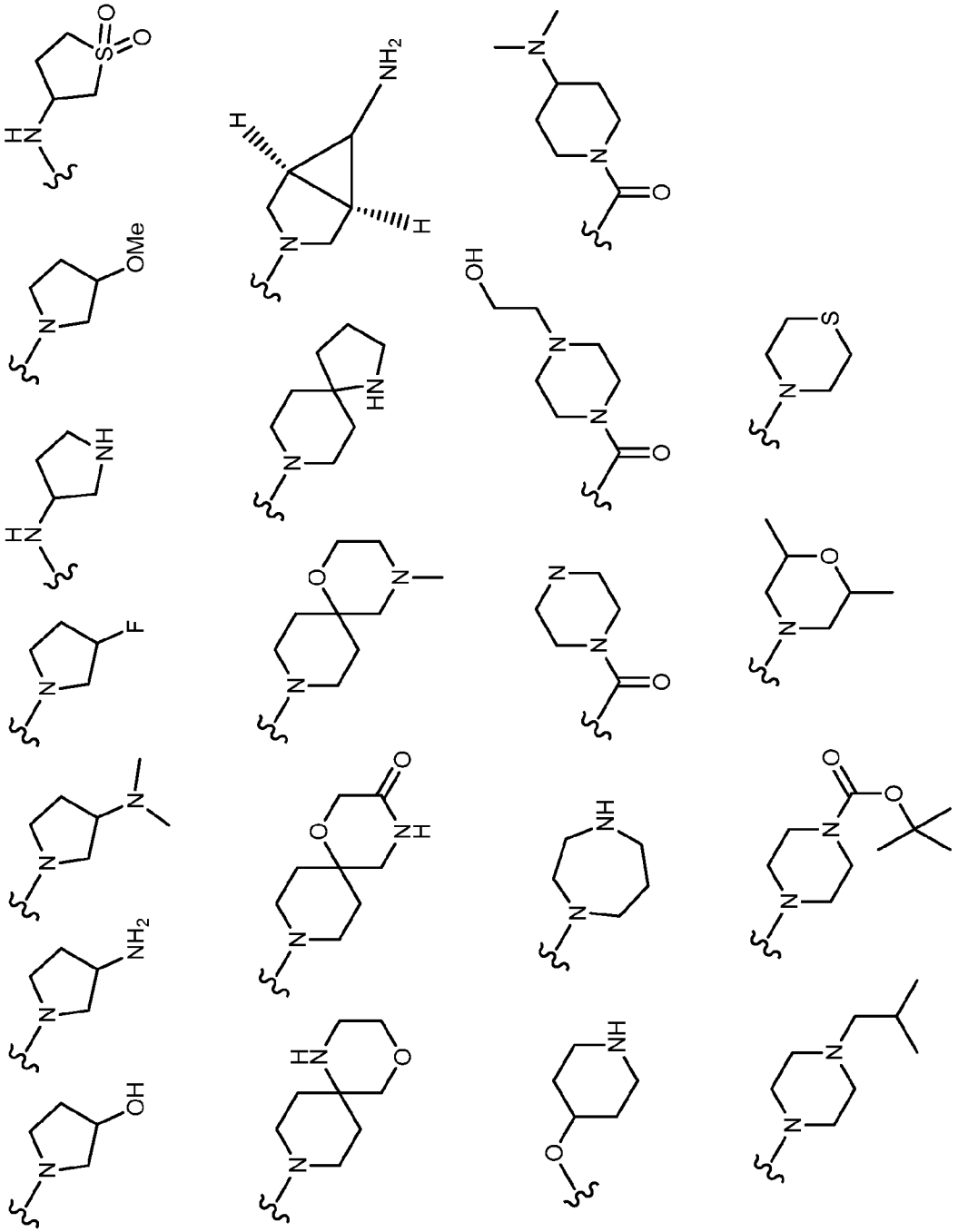


FIG. 4

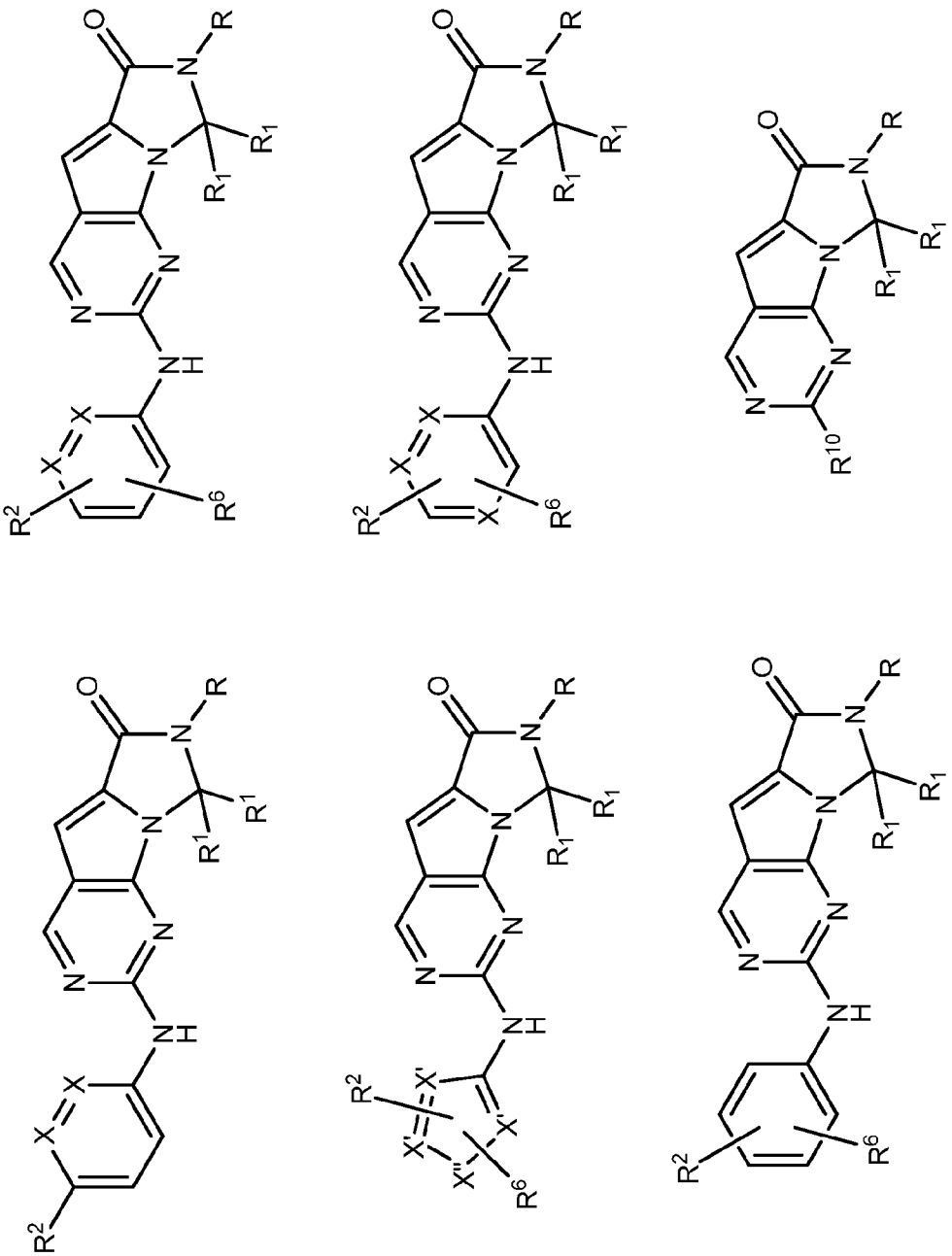


FIG. 5A

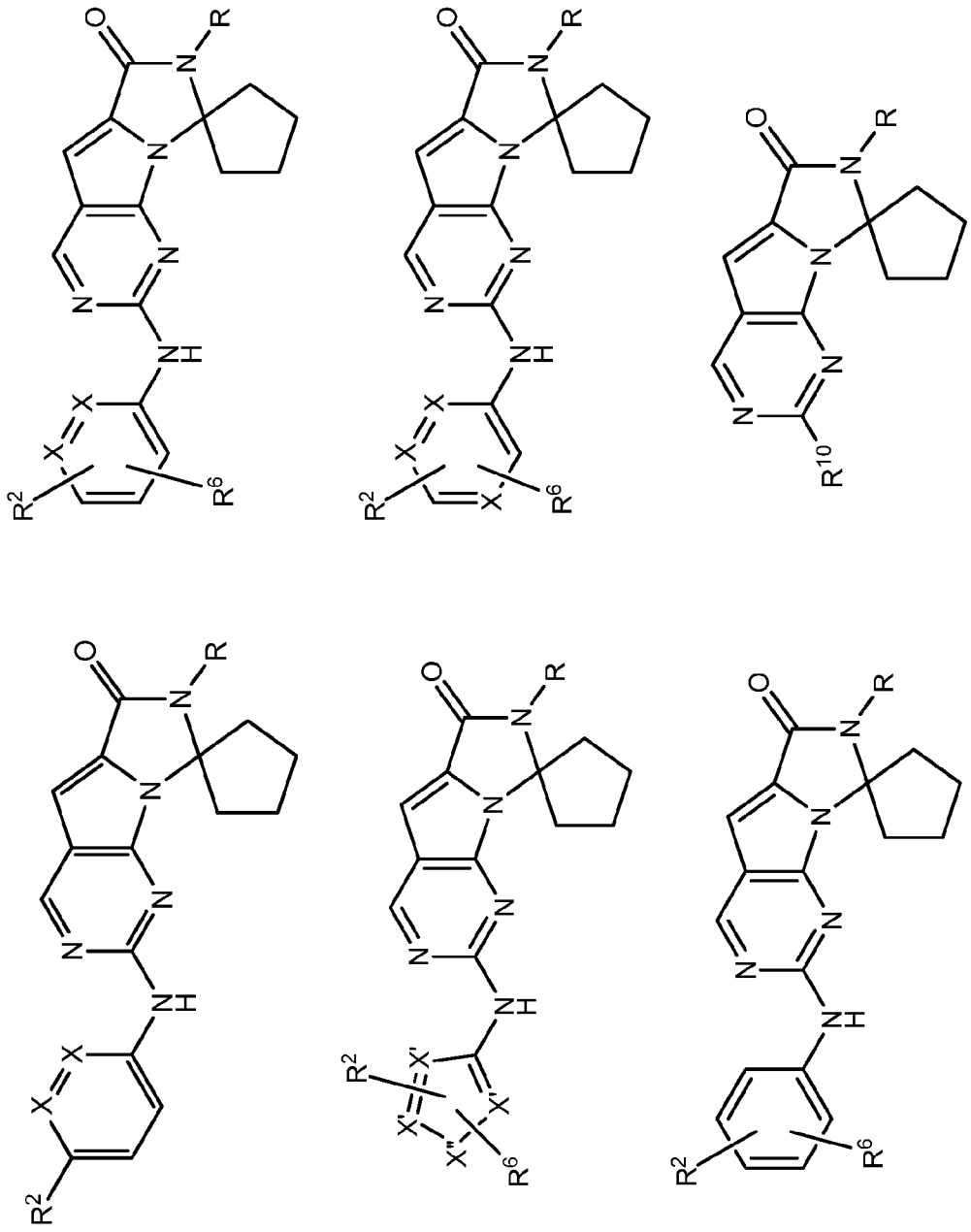


FIG. 5B

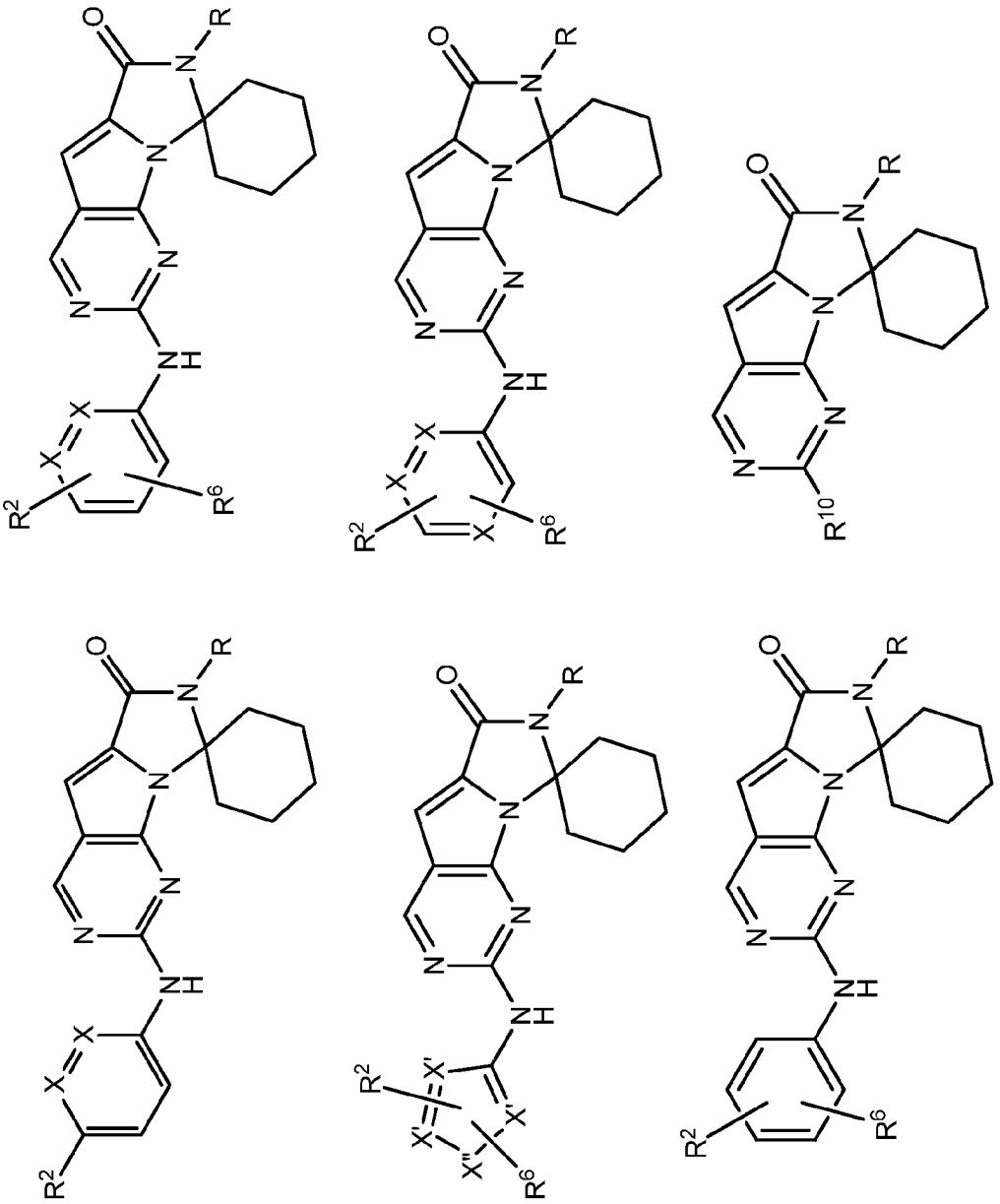


FIG. 5C

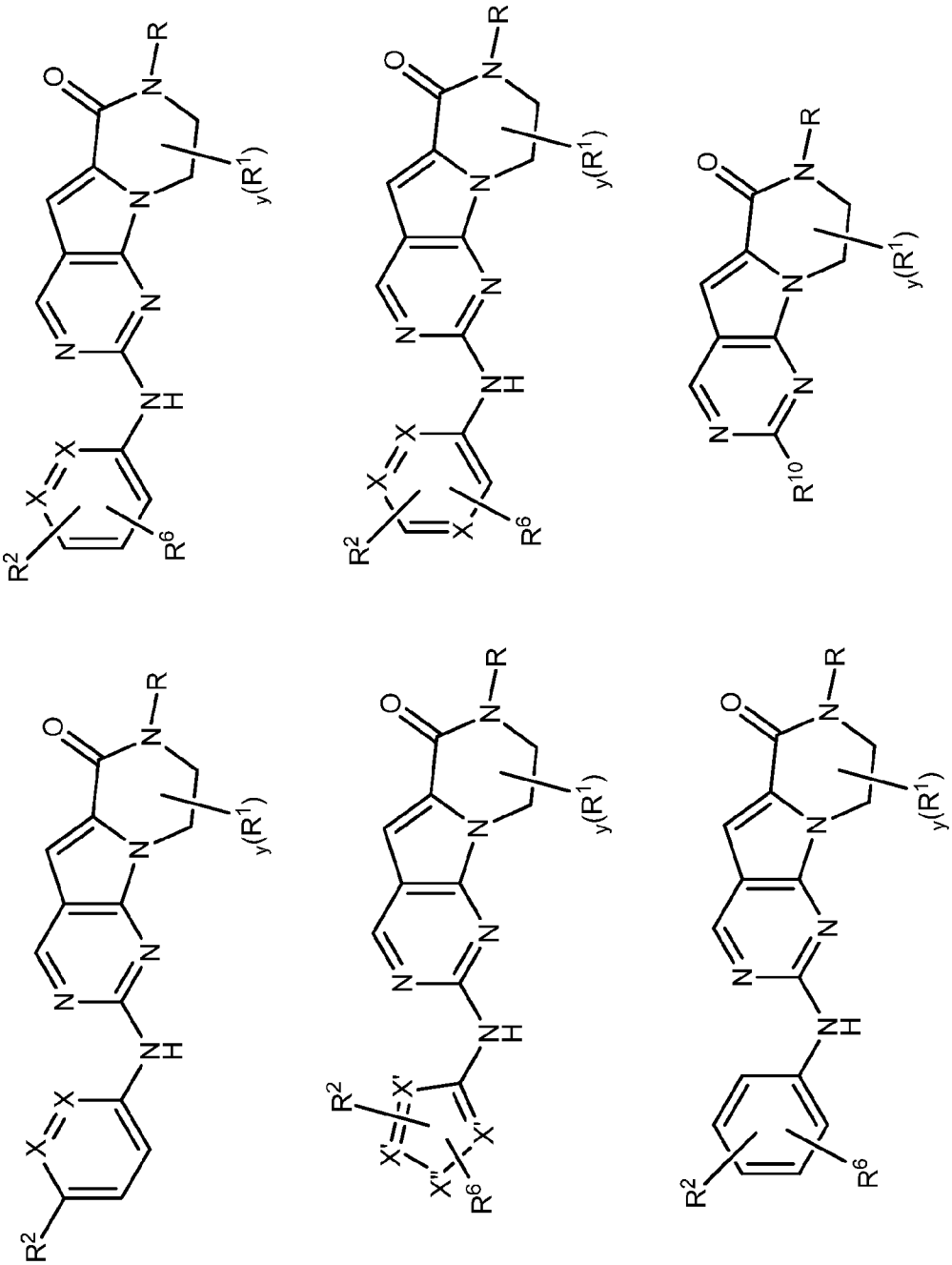


FIG. 6A

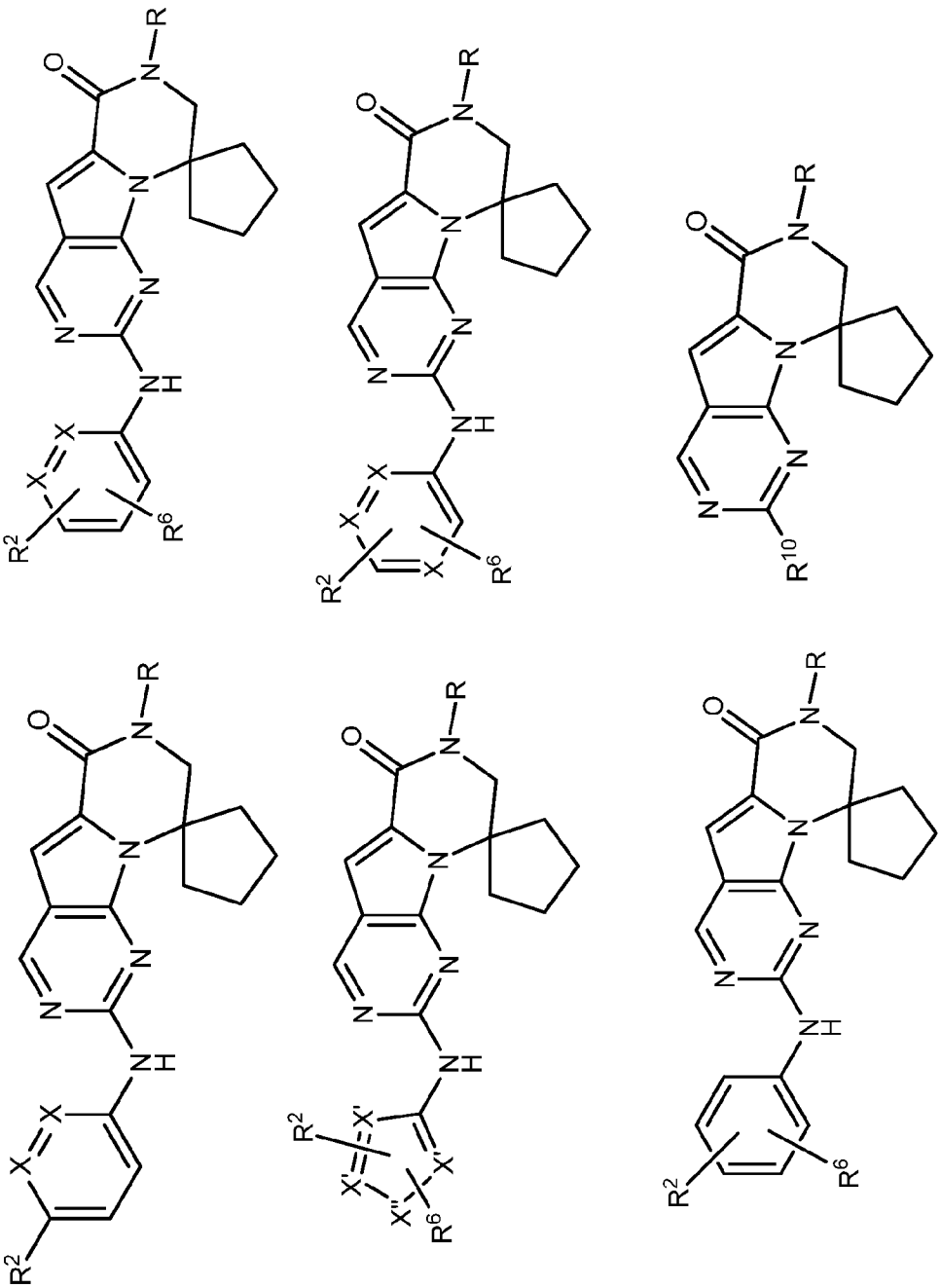


FIG. 6B

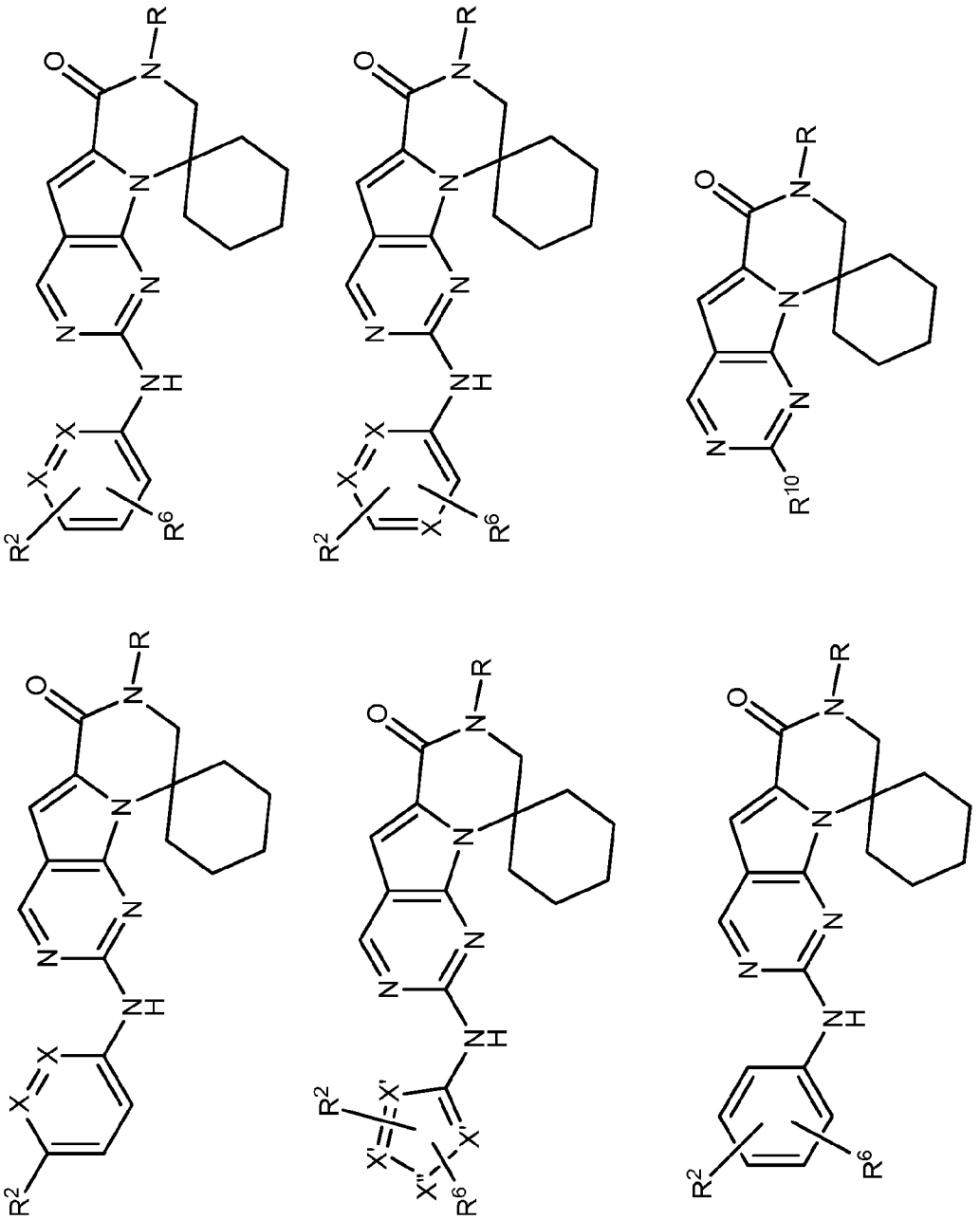


FIG. 6C

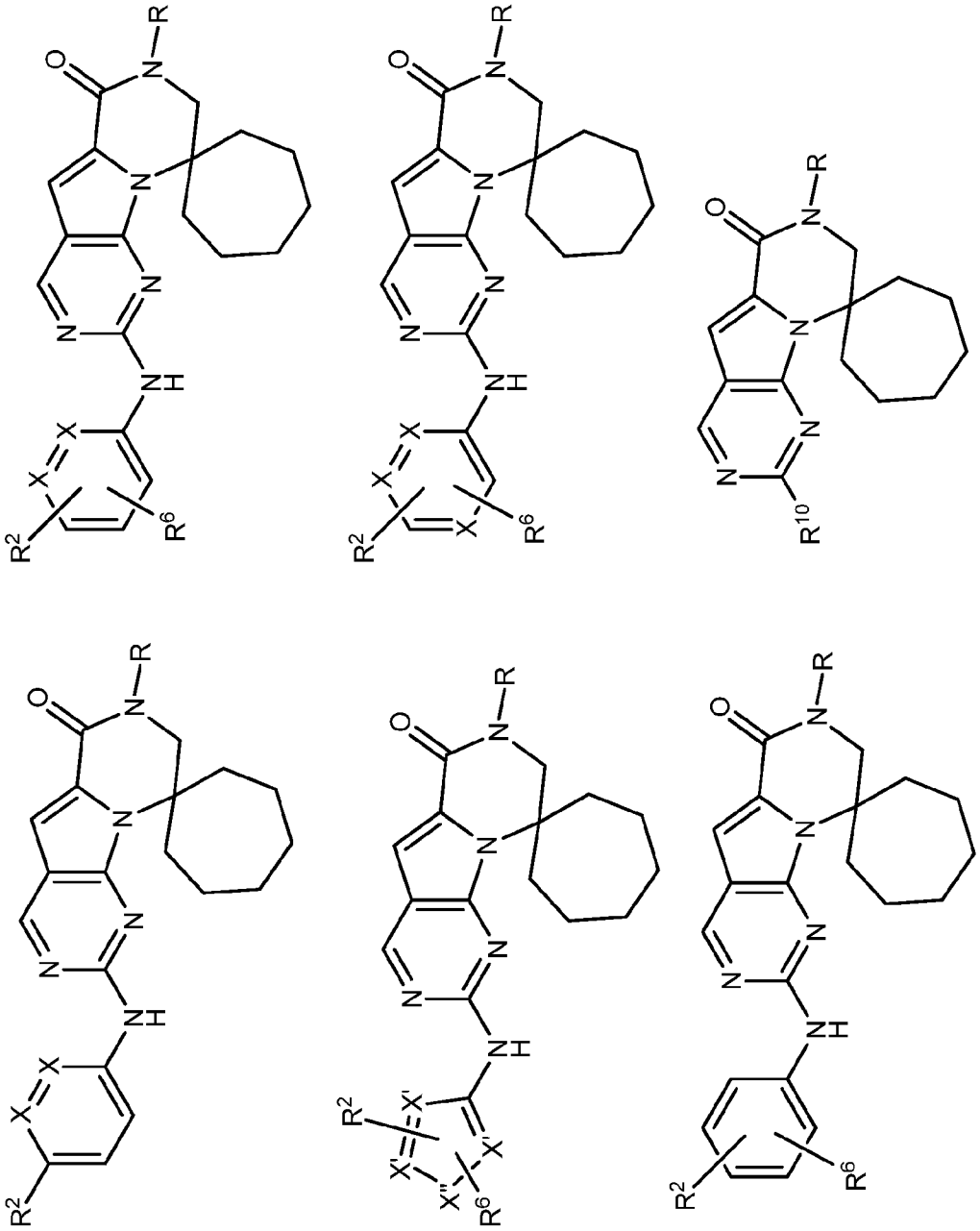


FIG. 6D

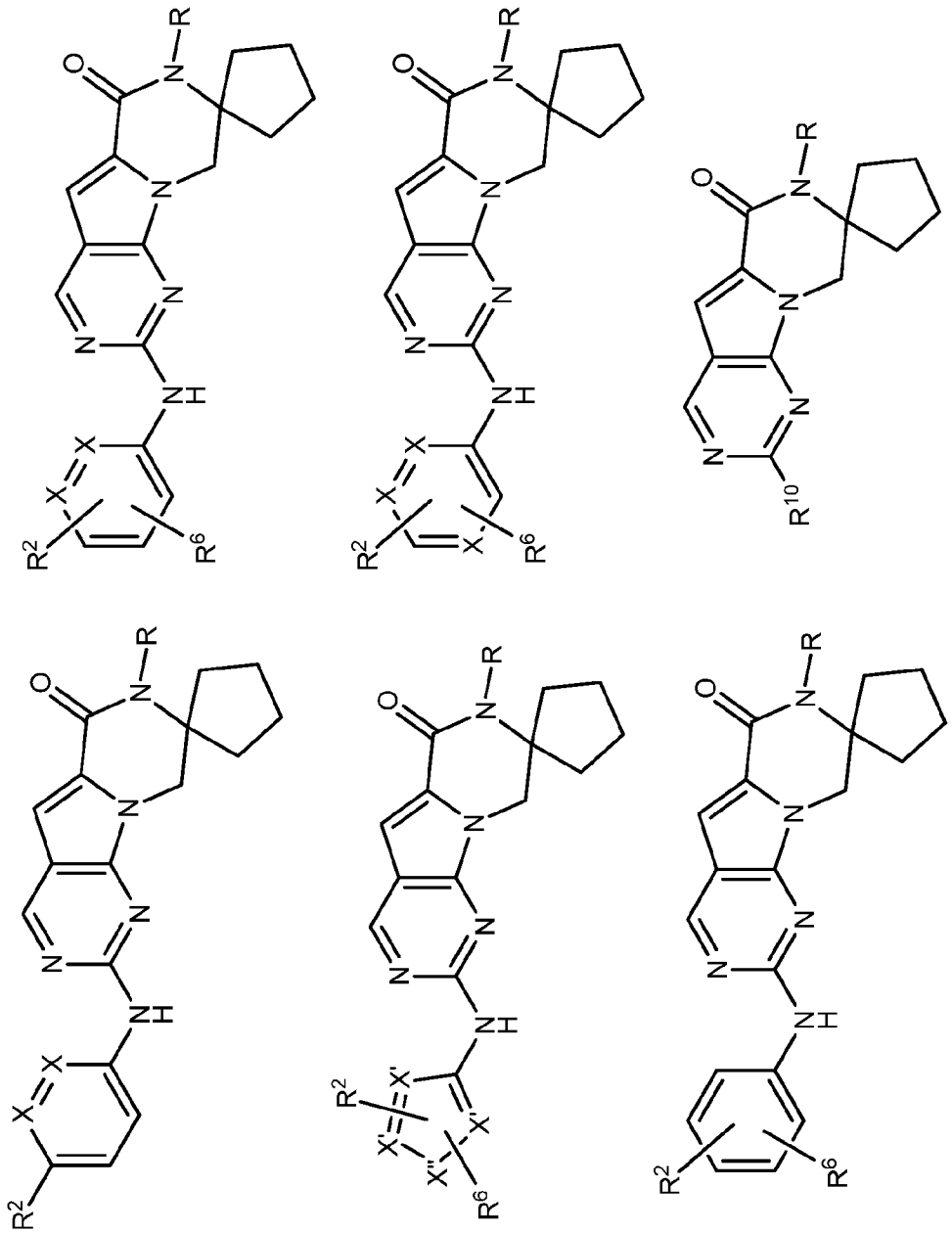


FIG. 7A

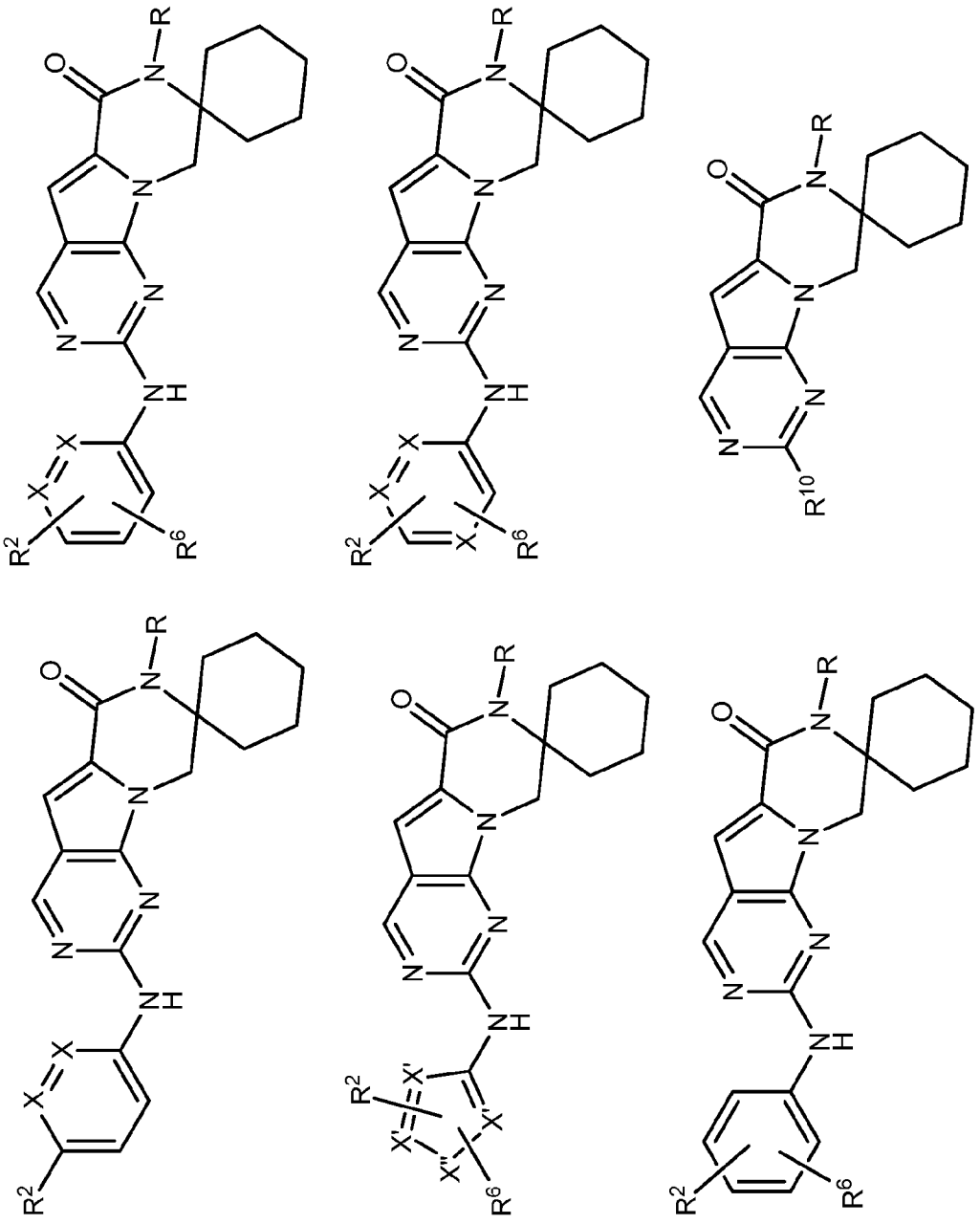


FIG. 7B

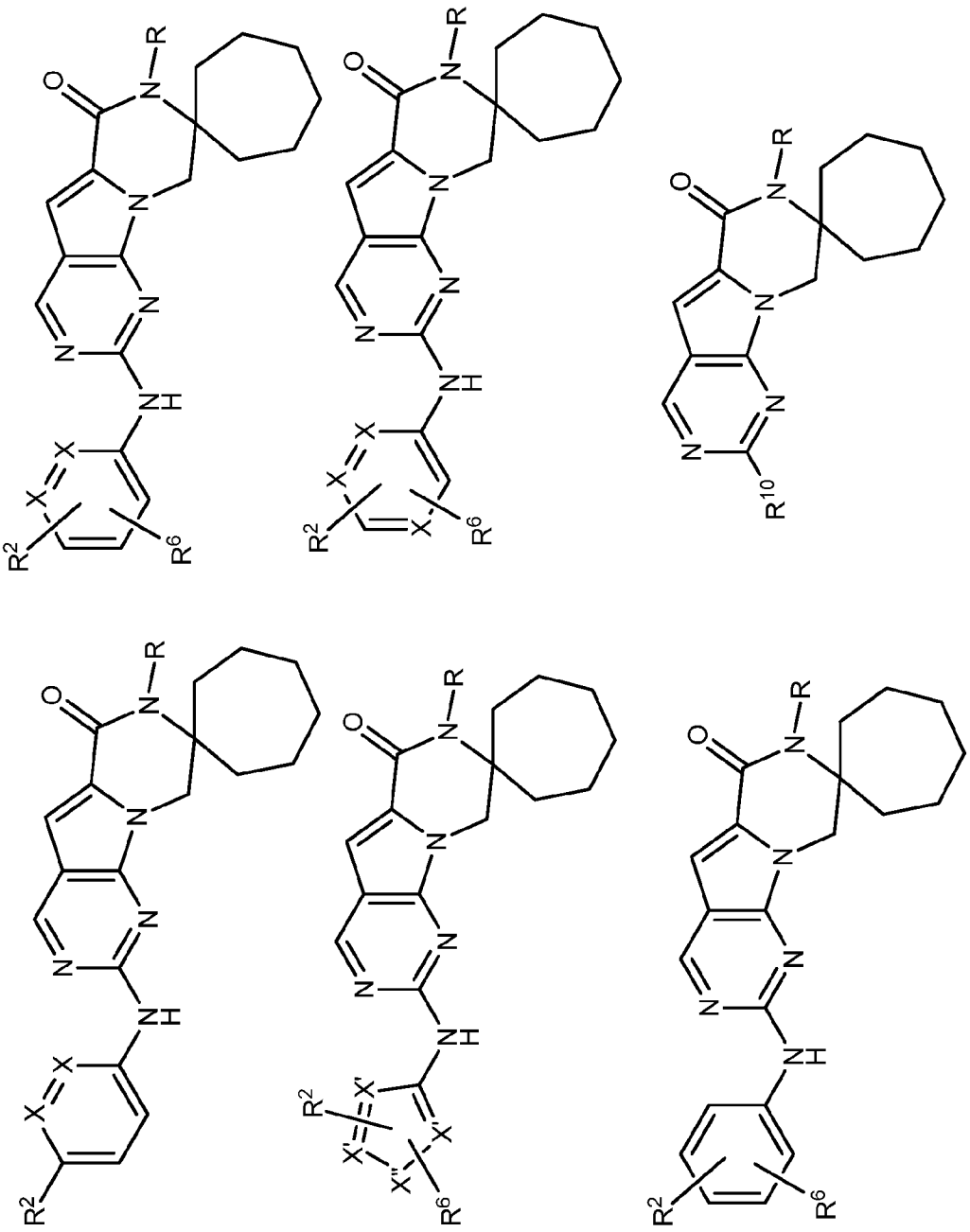


FIG. 7C

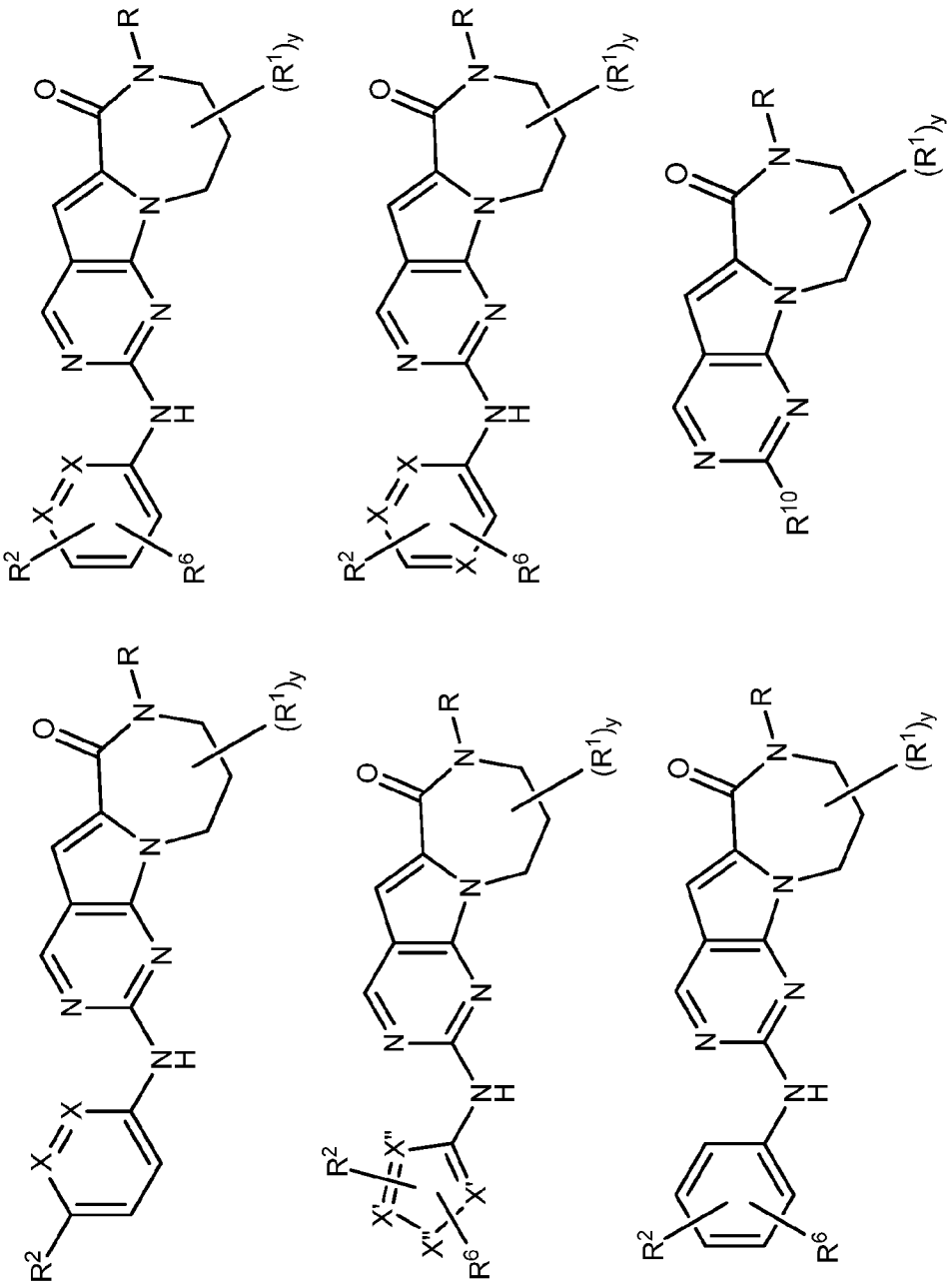


FIG. 8A

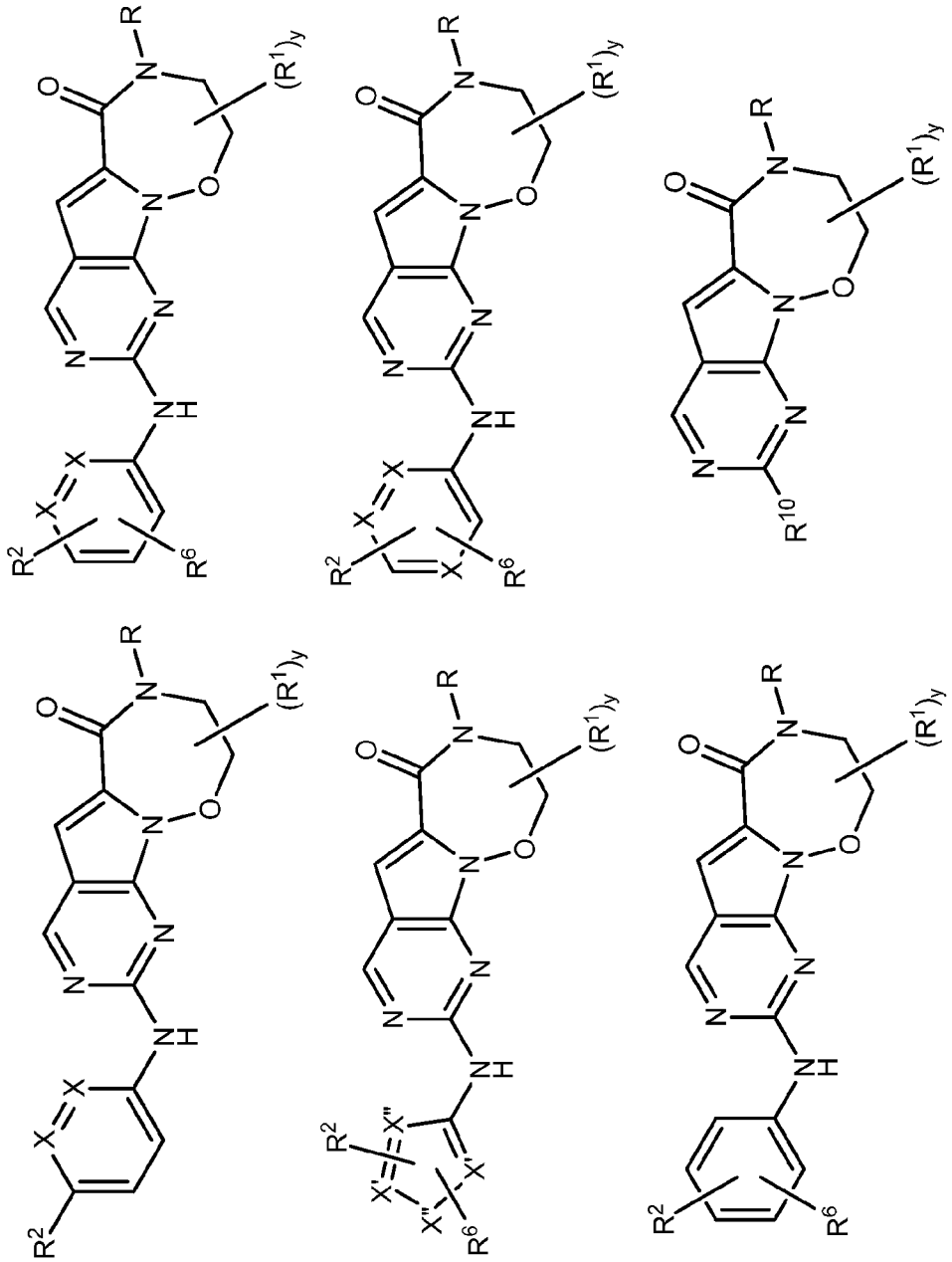


FIG. 8B

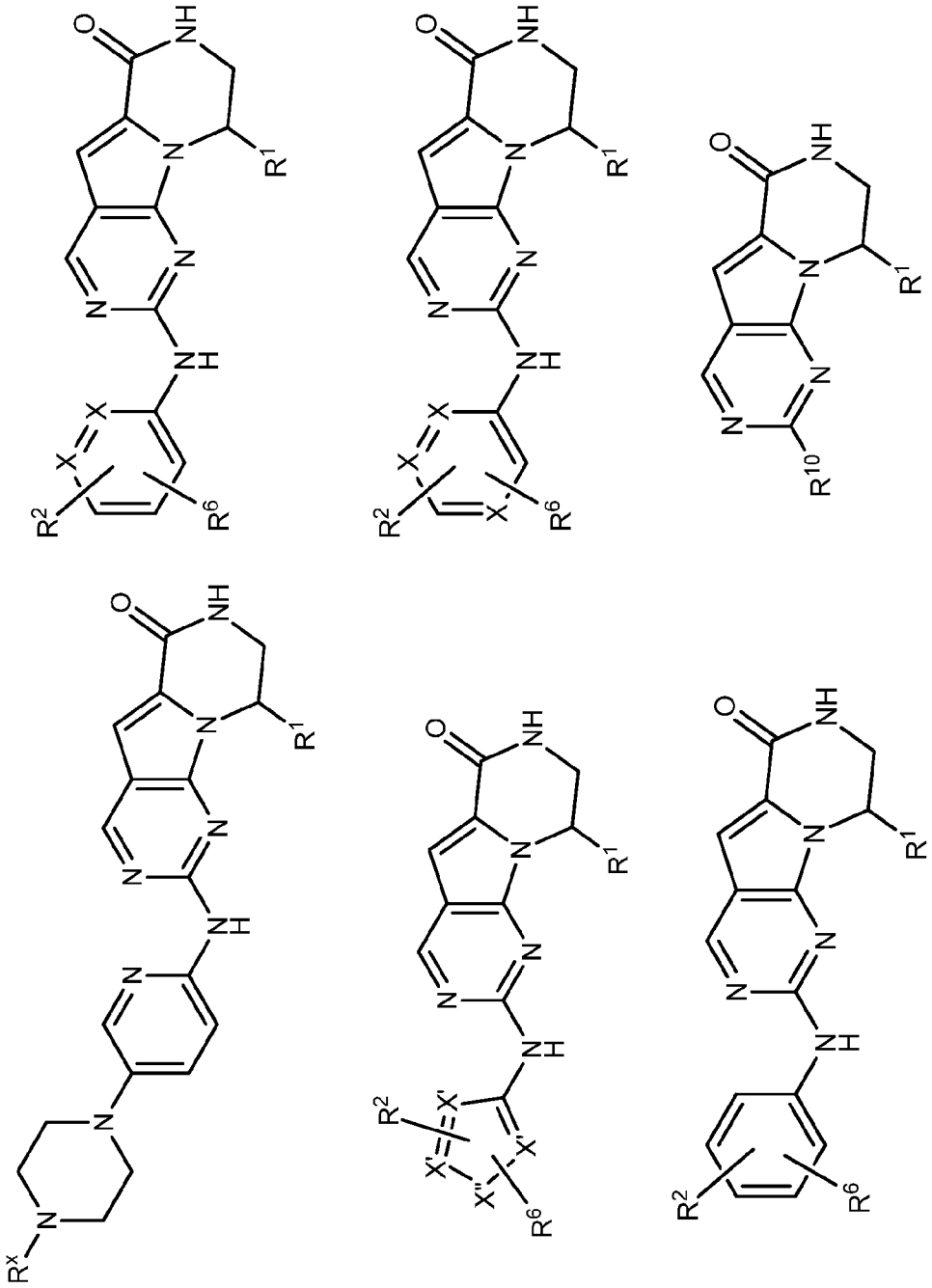


FIG. 9A

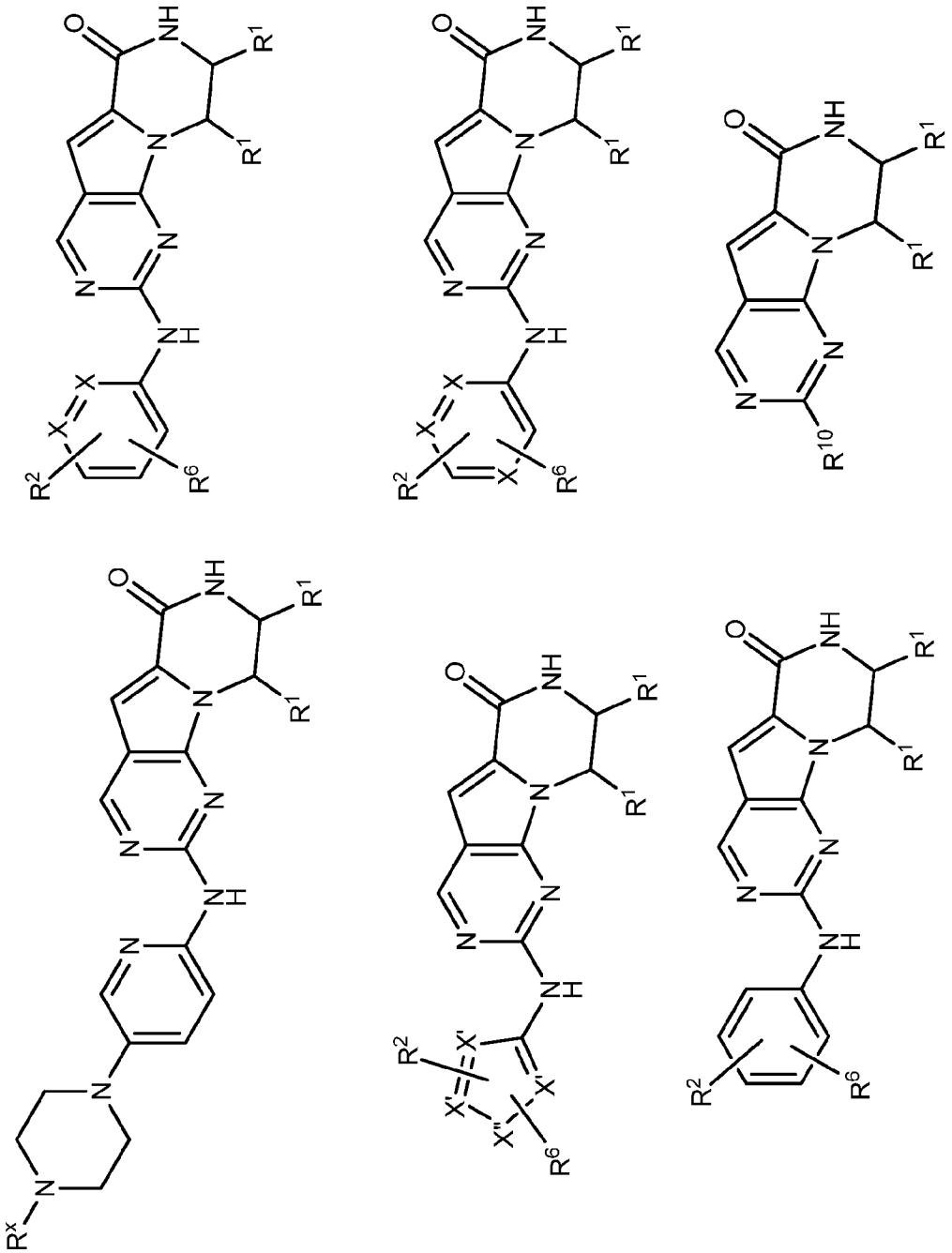


FIG. 9B

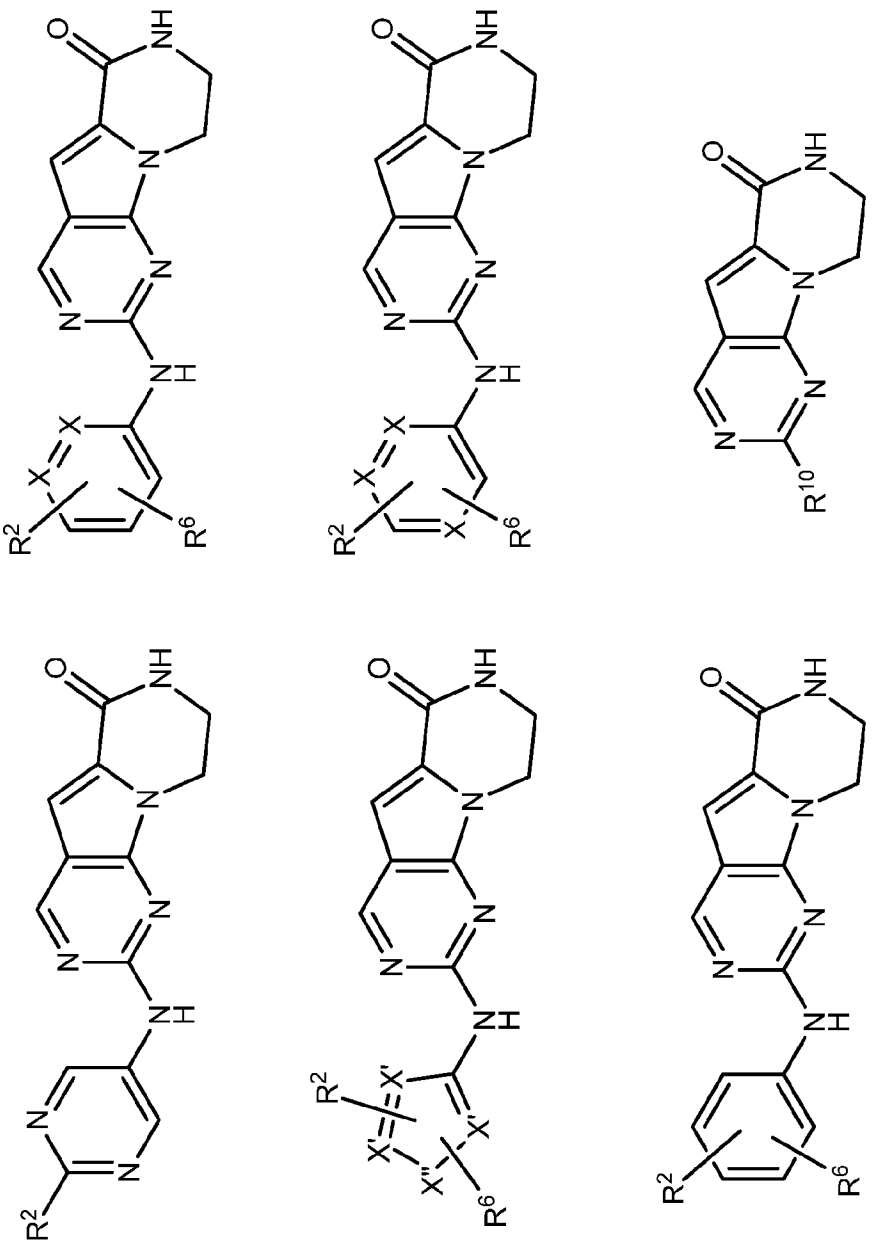


FIG. 9C

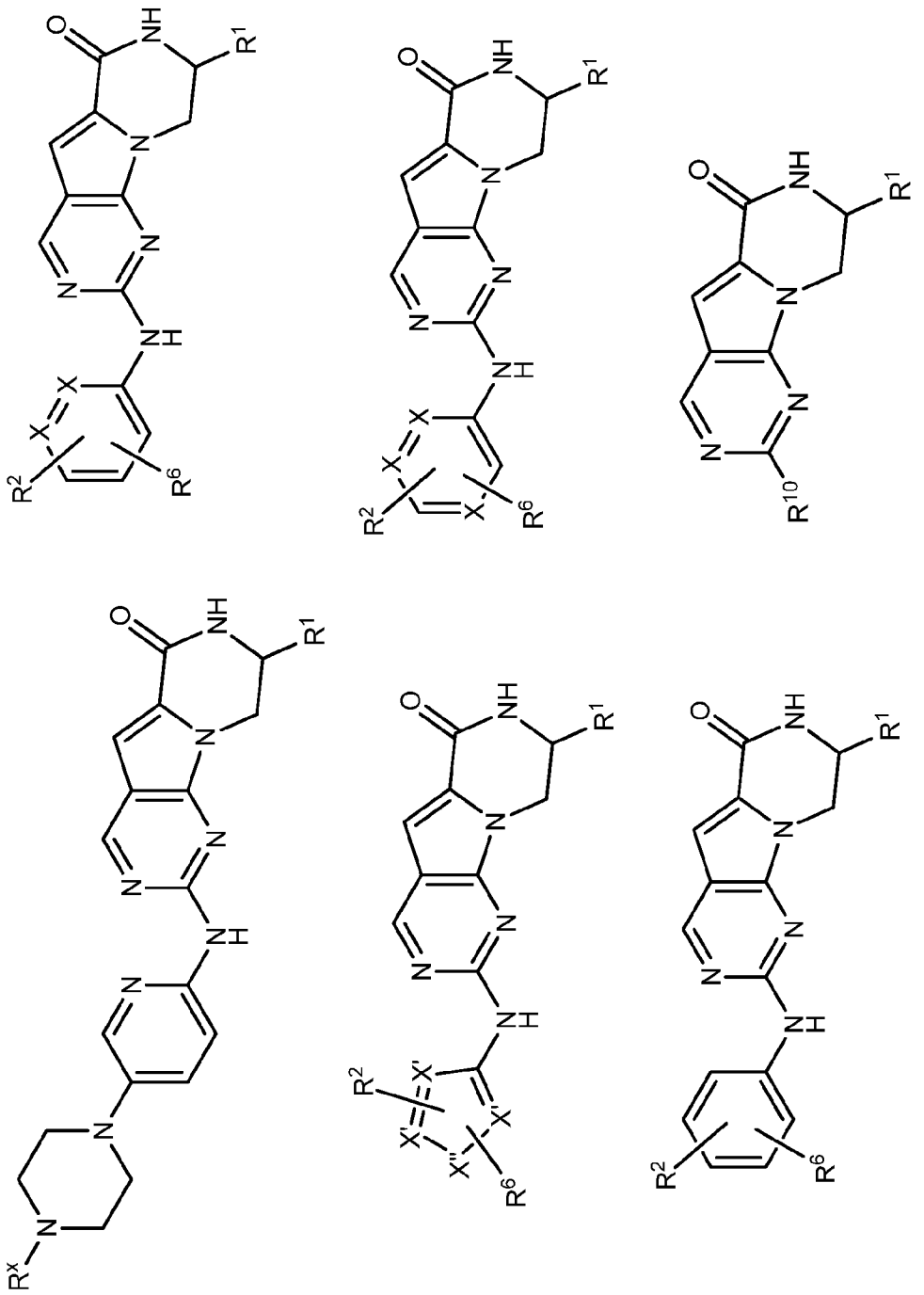


FIG. 9D

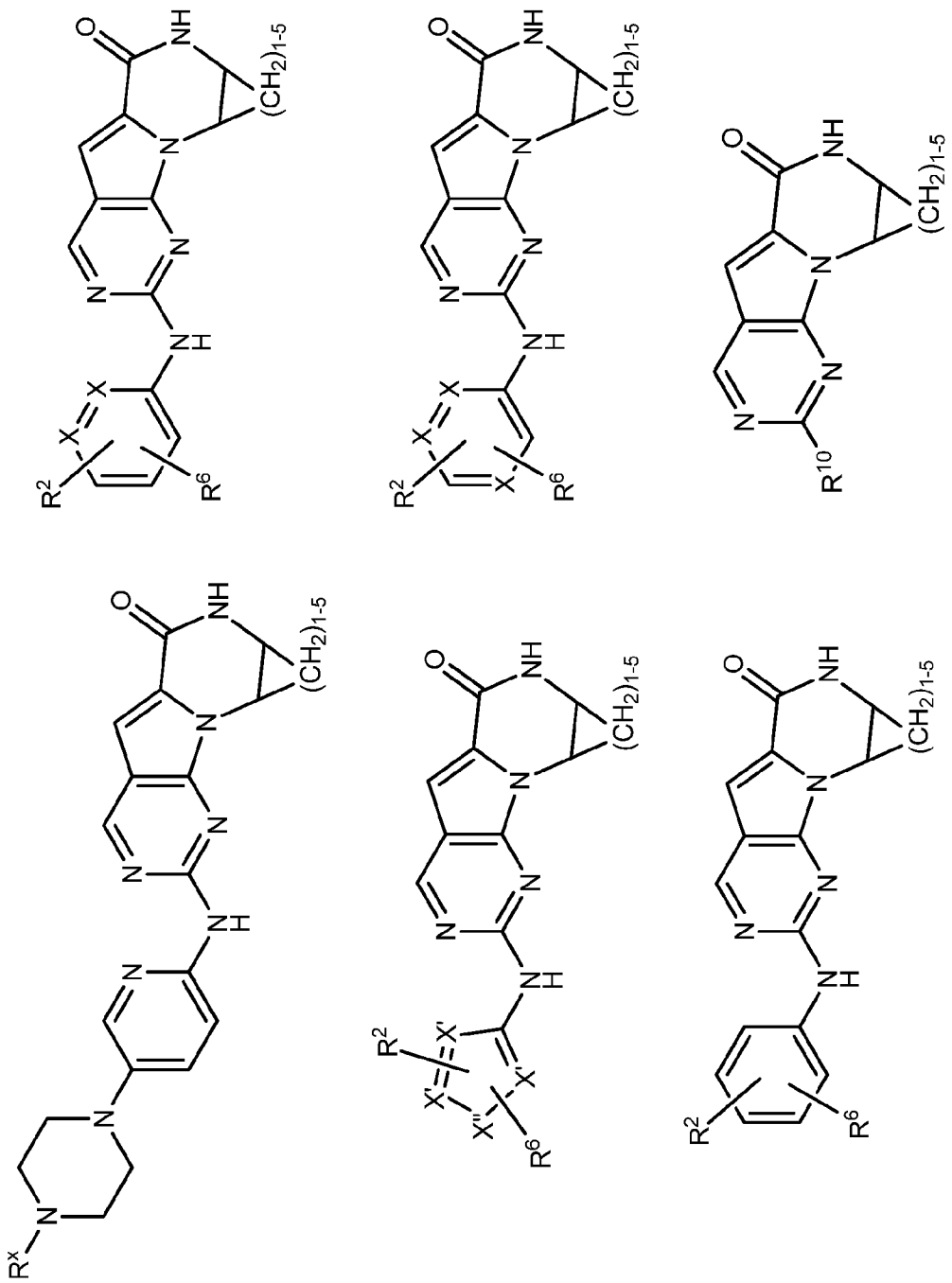


FIG. 9E

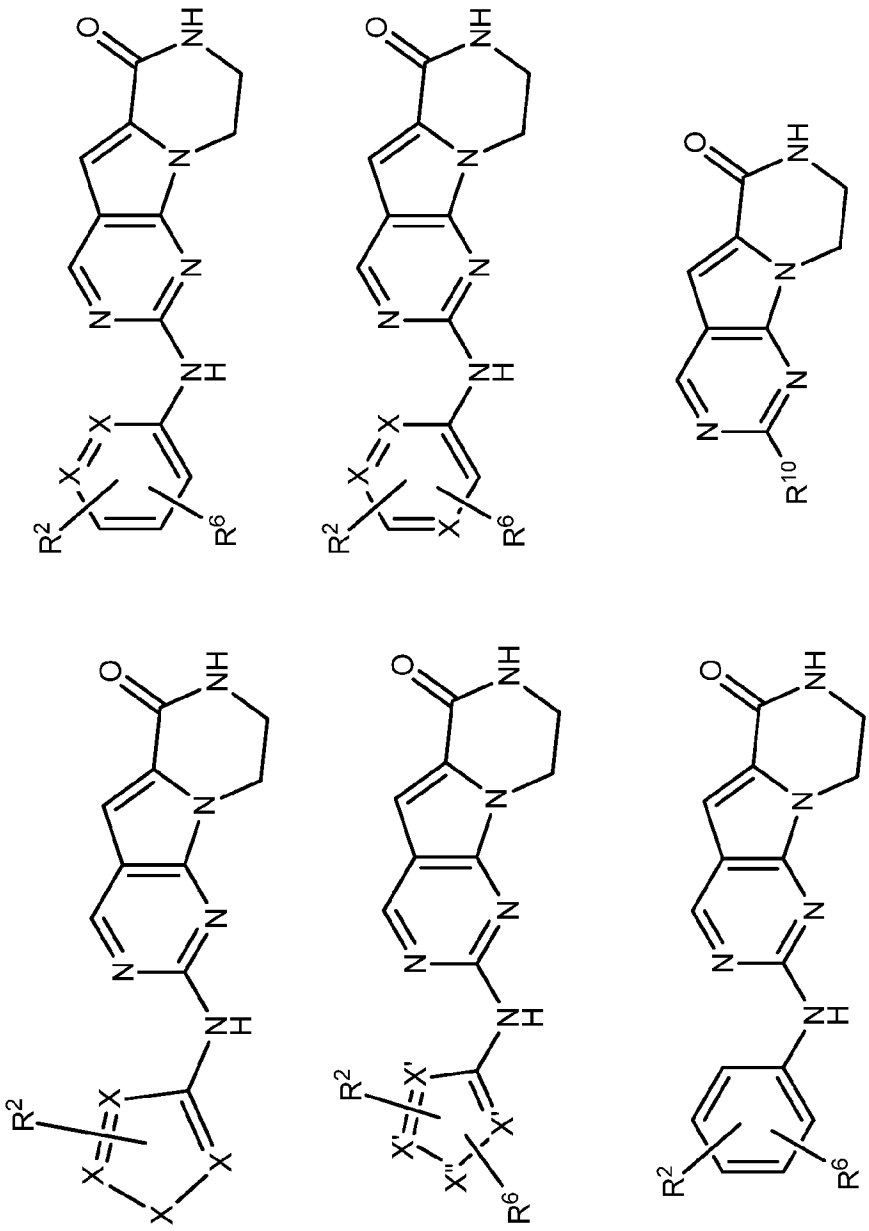


FIG. 9F

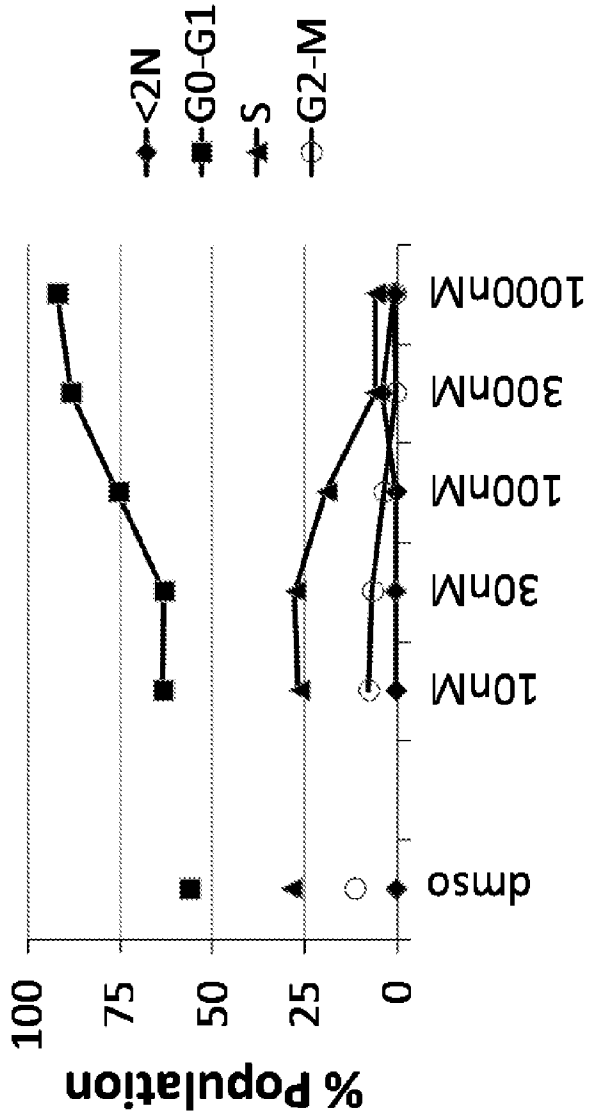

% Population
100
75
50
25
0
dmso
10nM
30nM
100nM
300nM
1000nM
<2N
G0-G1
S
G2-M

FIG. 10A

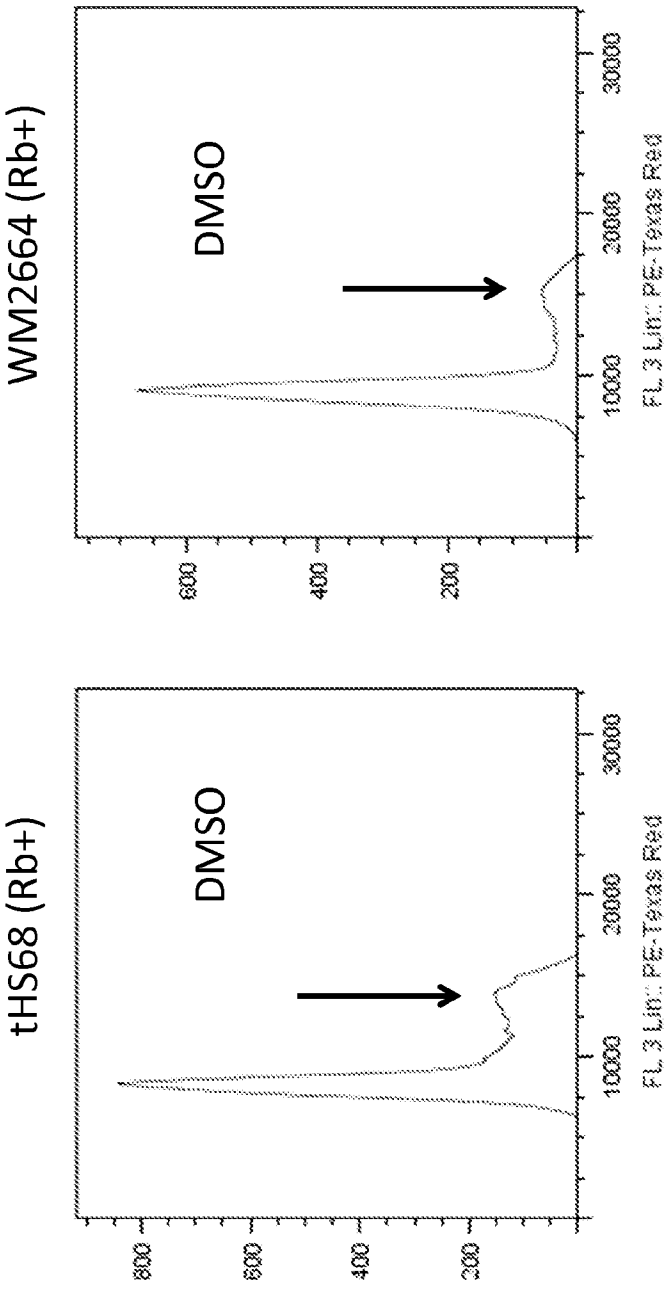


FIG. 10B

FIG. 10C

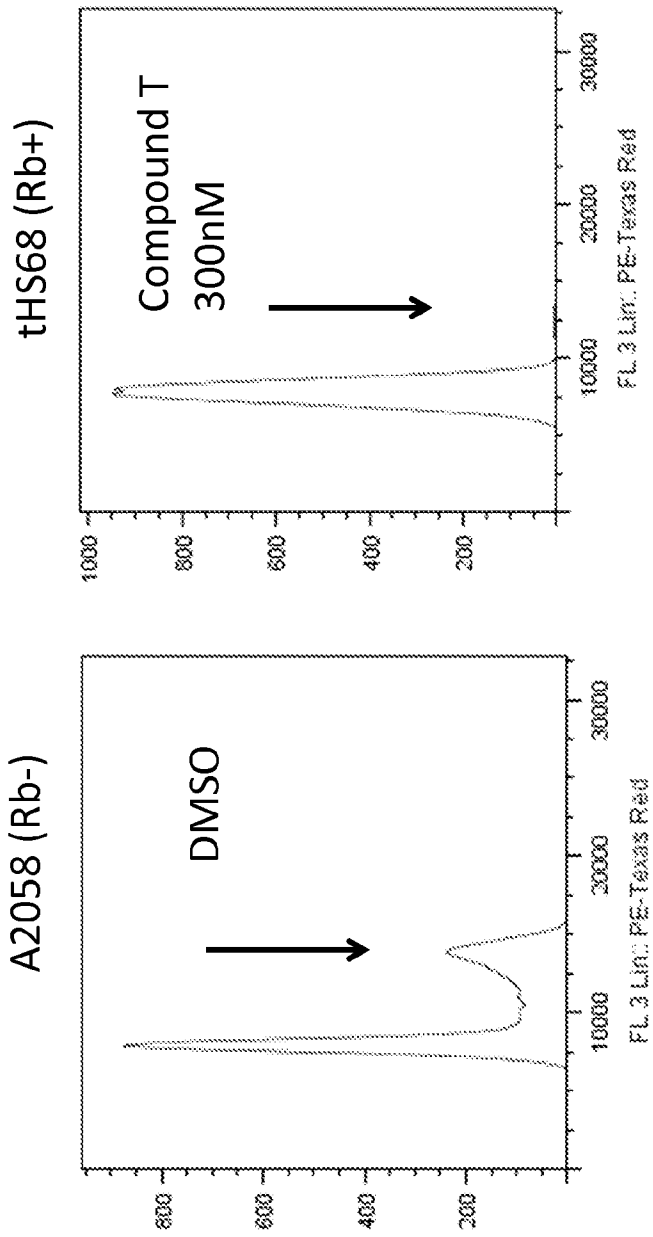


FIG. 10E

FIG. 10D

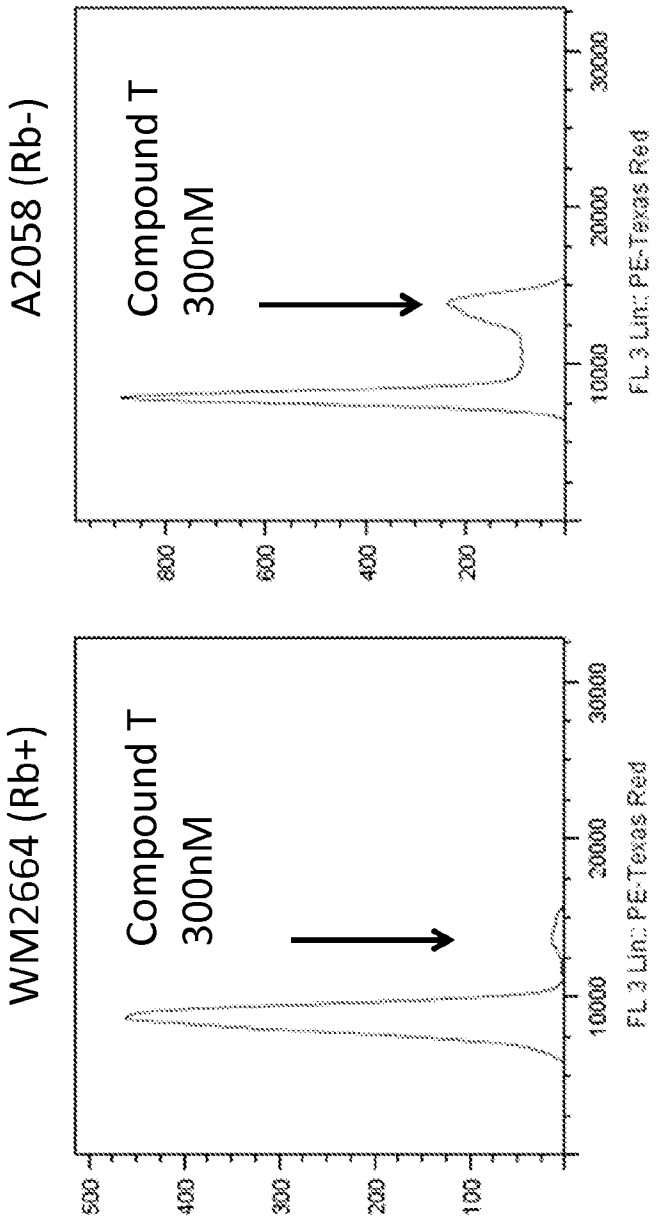


FIG. 10G

FIG. 10F

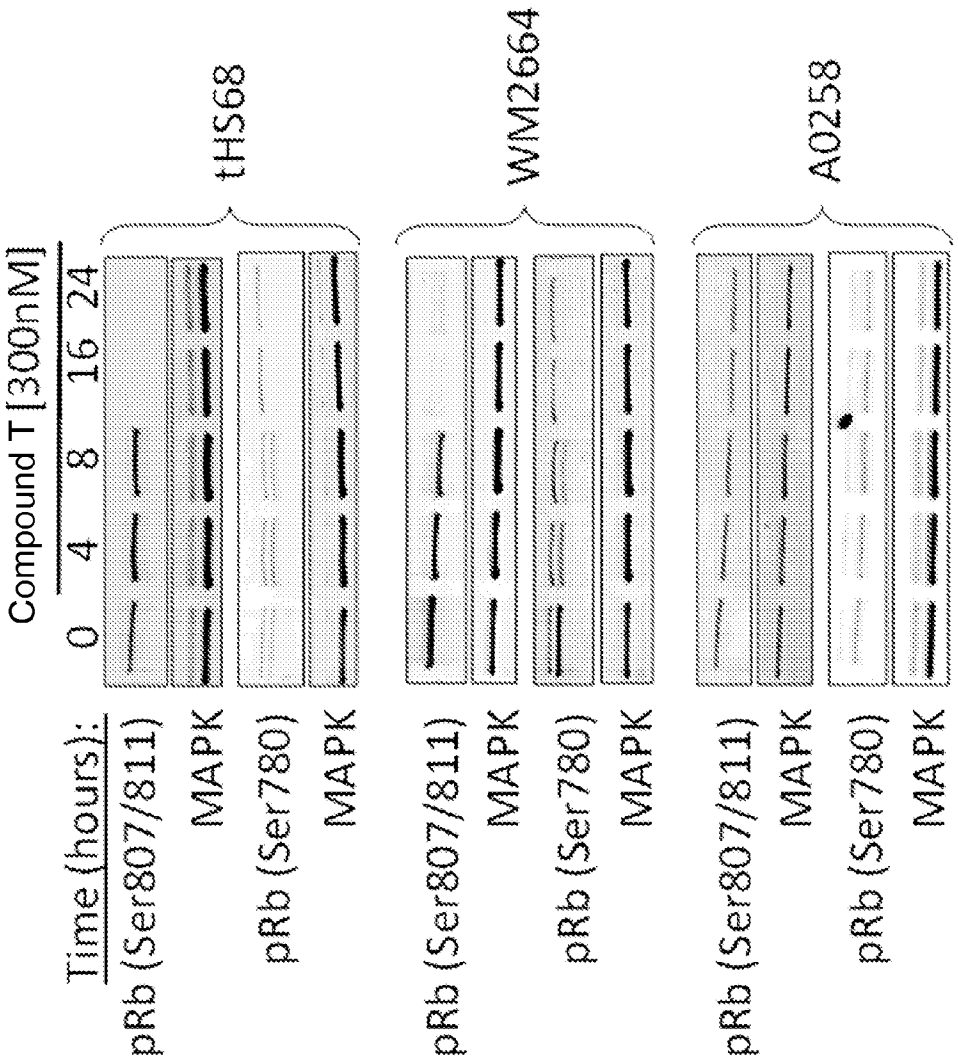

tHS68
WM2664
A0258
Compound T [300nM]
0 4 8 16 24
Time (hours):
pRb (Ser807/811)
MAPK
pRb (Ser780)
MAPK

FIG. 11

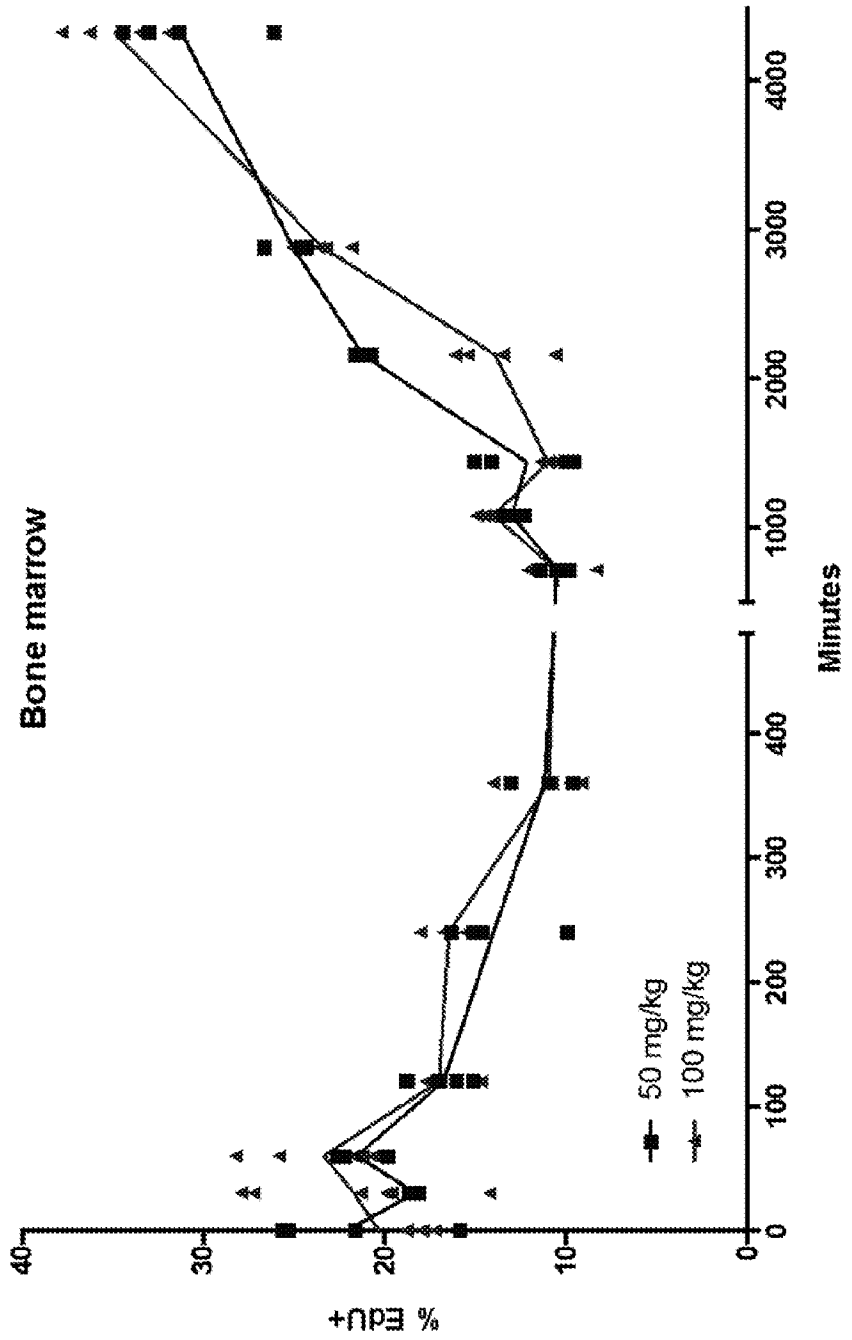

Bone marrow
% EdU+
Minutes
50 mg/kg
100 mg/kg
0
10
20
30
40
0
100
200
300
400
1000
2000
3000
4000

FIG. 12

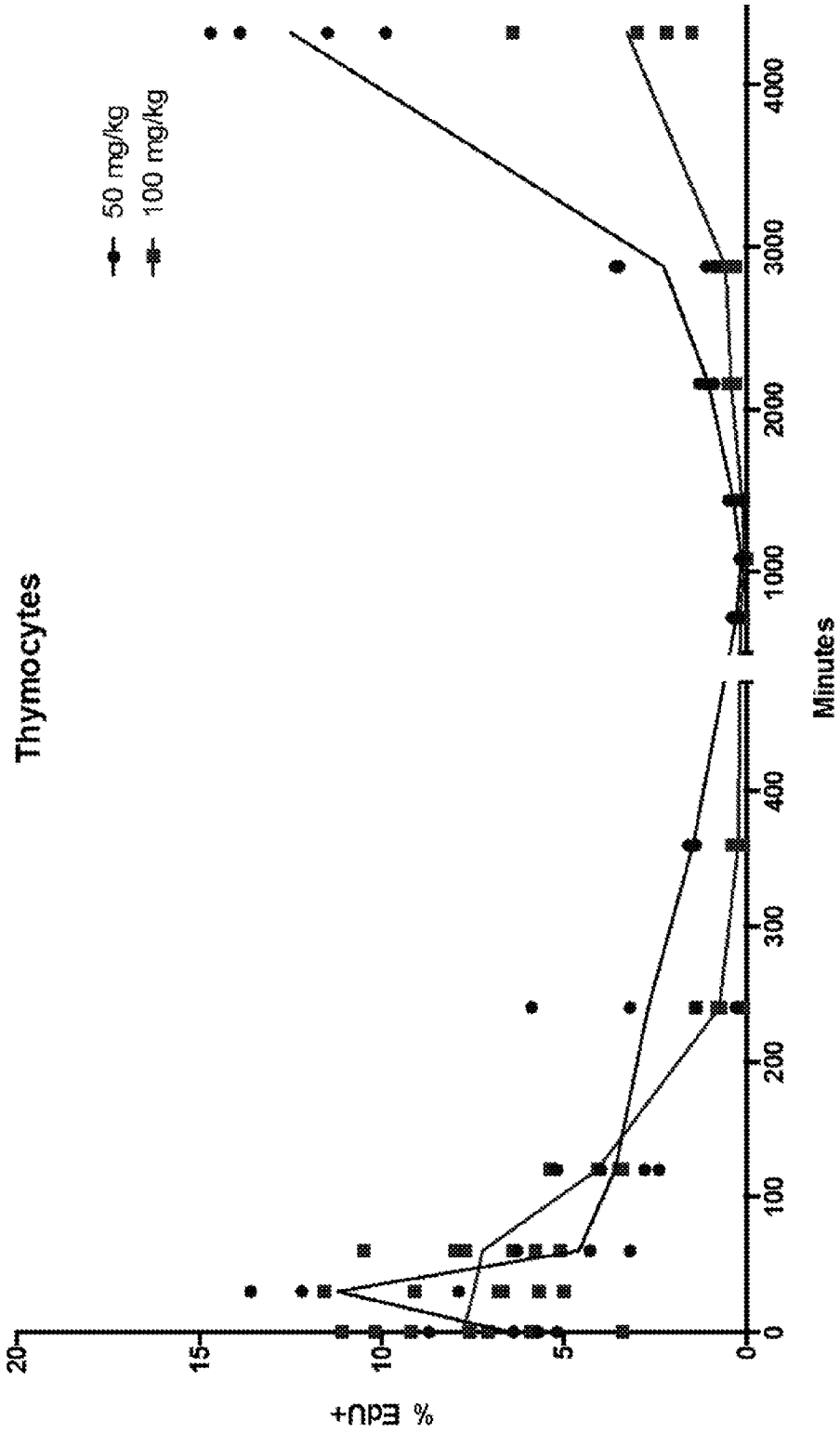

Thymocytes
50 mg/kg
100 mg/kg
% EdU+
Minutes
0
5
10
15
20
0
100
200
300
400
1000
2000
3000
4000

FIG. 13

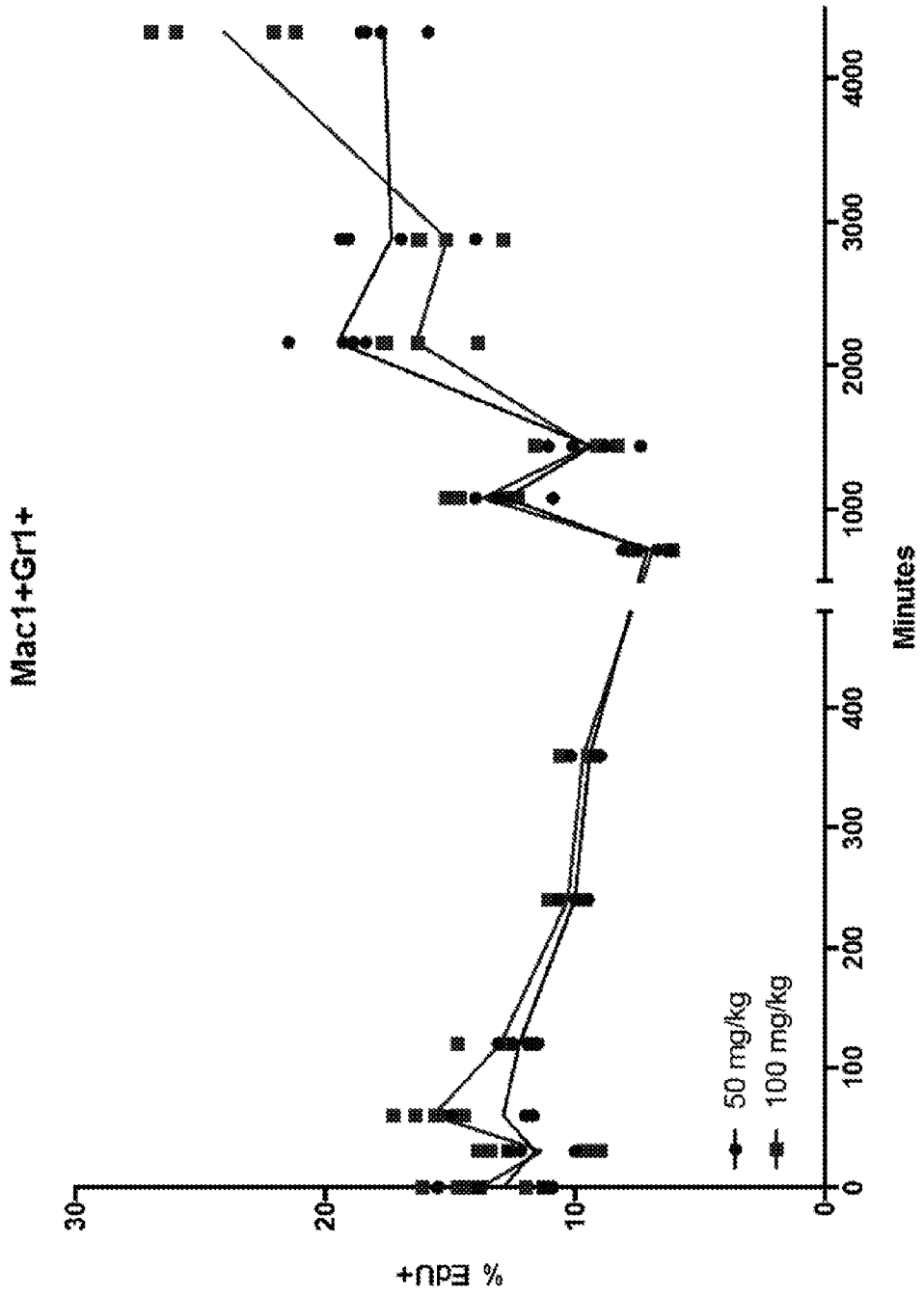

Mac1+Gr1+
% EdU+
30
20
10
0
0
100
200
300
400
1000
2000
3000
4000
Minutes
50 mg/kg
100 mg/kg

FIG. 14

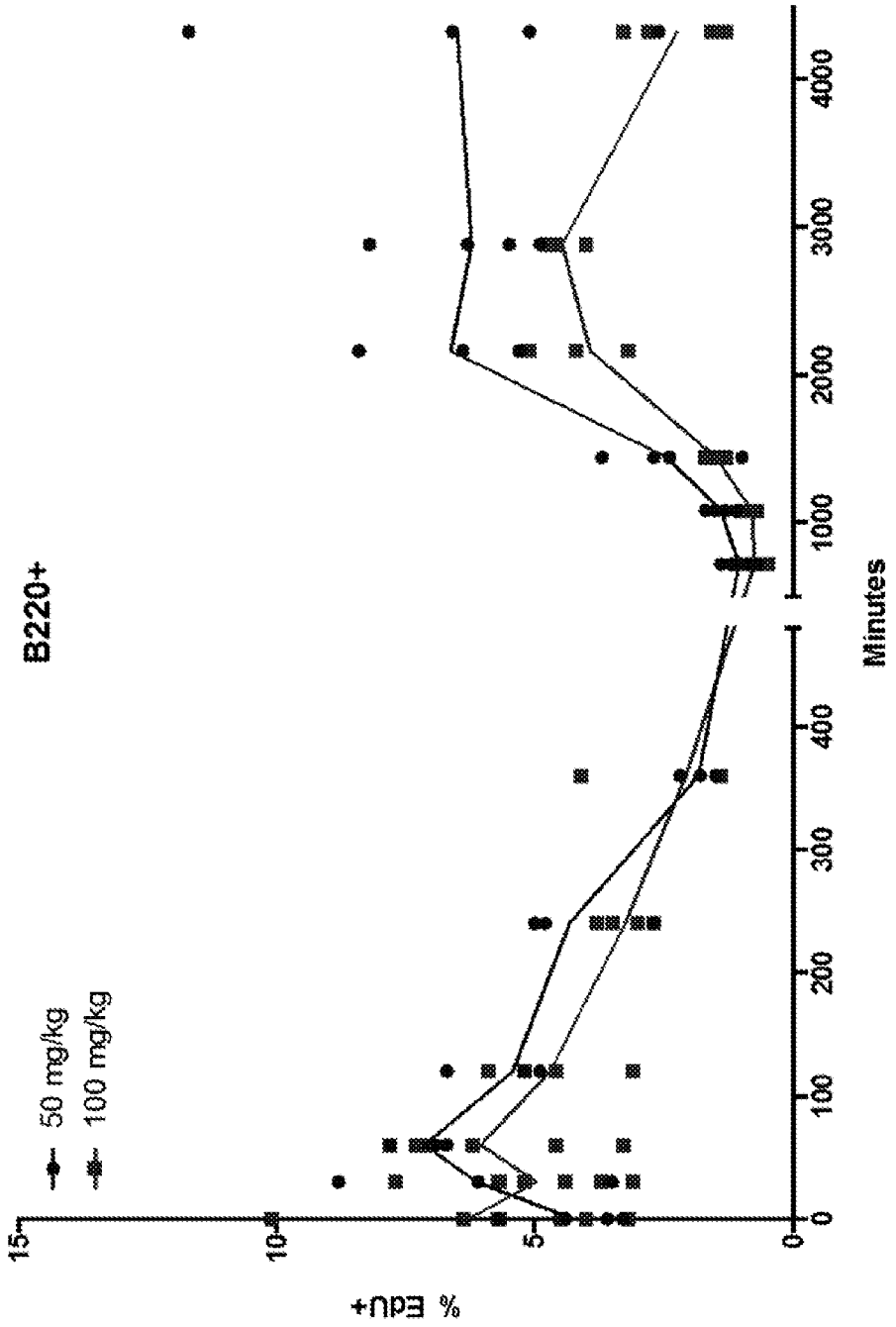

B220+
% EdU+
Minutes
50 mg/kg
100 mg/kg
0
5
10
15
100
200
300
400
1000
2000
3000
4000

FIG. 15

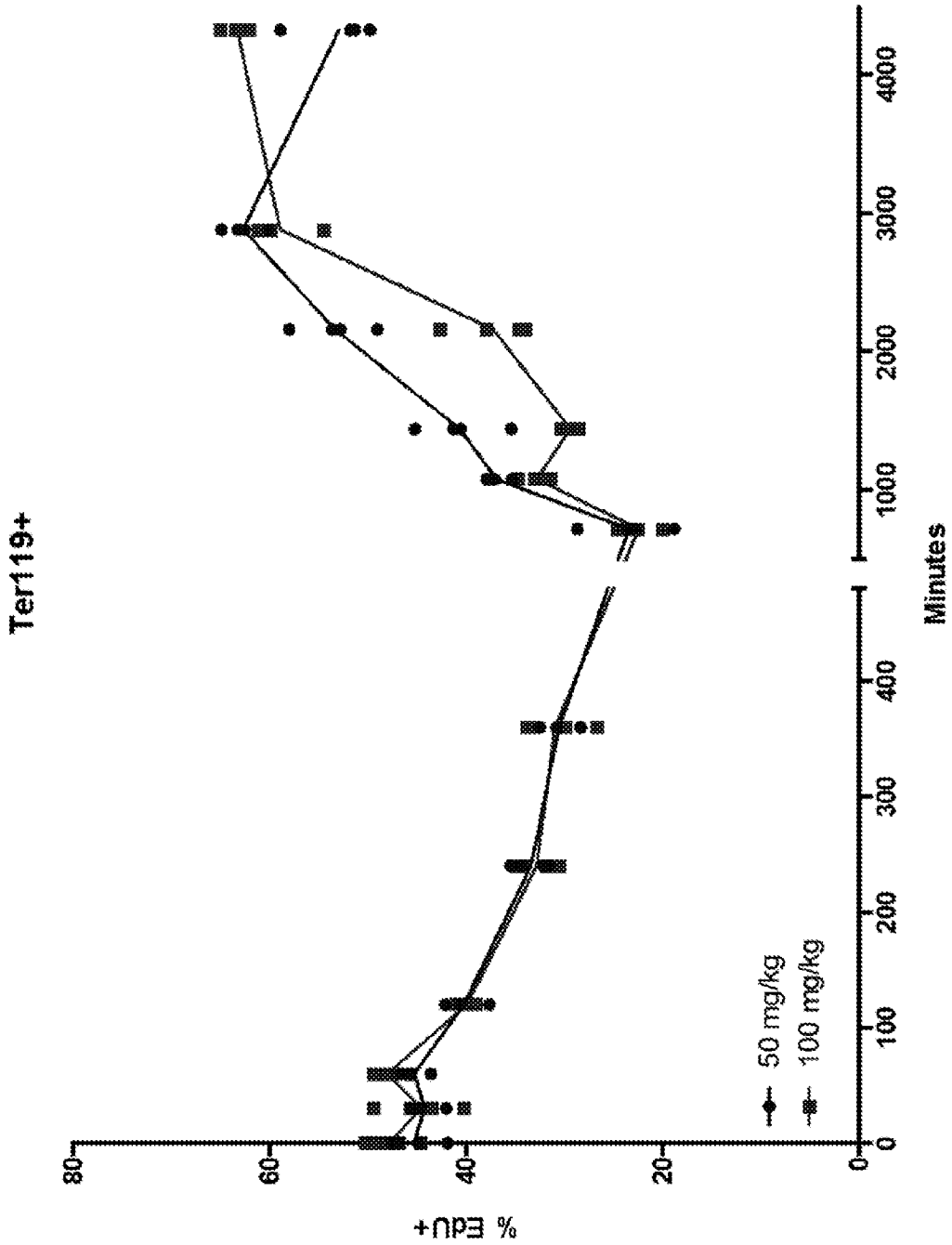

Ter119+
% EdU+
80
60
40
20
0
100
200
300
400
1000
2000
3000
4000
Minutes
50 mg/kg
100 mg/kg

FIG. 16

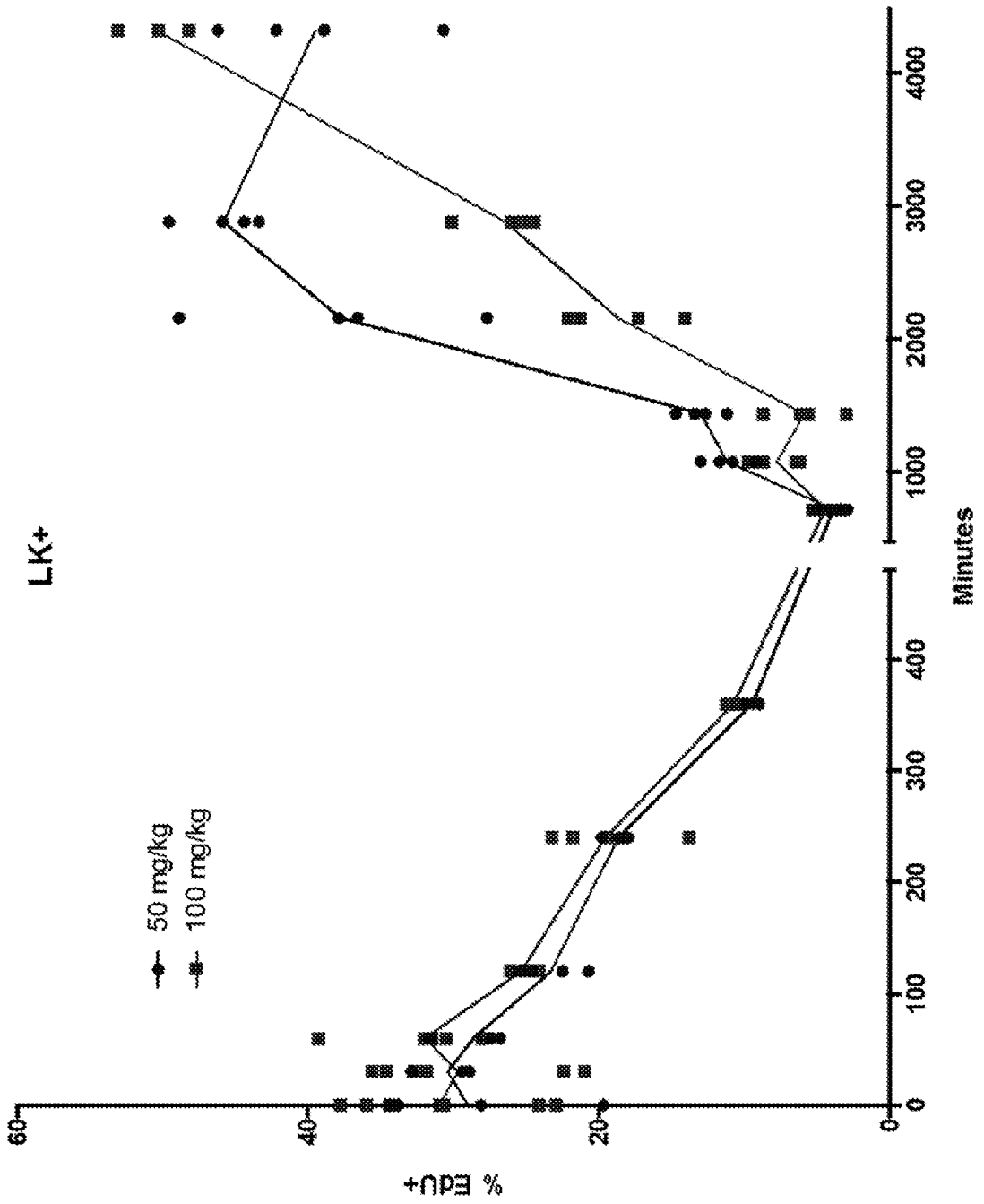

LK+
50 mg/kg
100 mg/kg
% EdU+
Minutes
0
100
200
300
400
1000
2000
3000
4000
20
40
60

FIG. 17

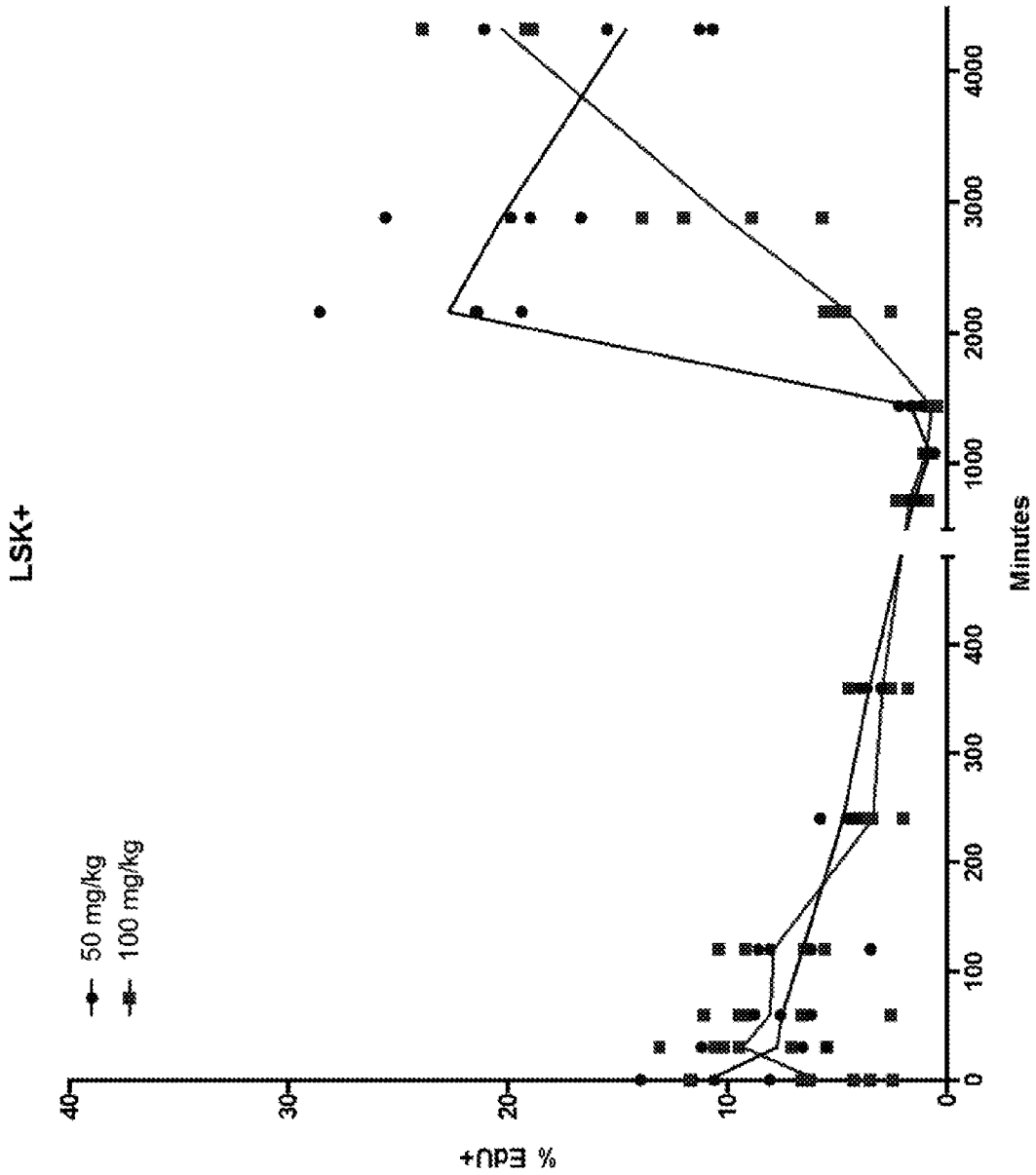

LSK+
% EdU+
Minutes
50 mg/kg
100 mg/kg
0
10
20
30
40
0
100
200
300
400
1000
2000
3000
4000

FIG. 18

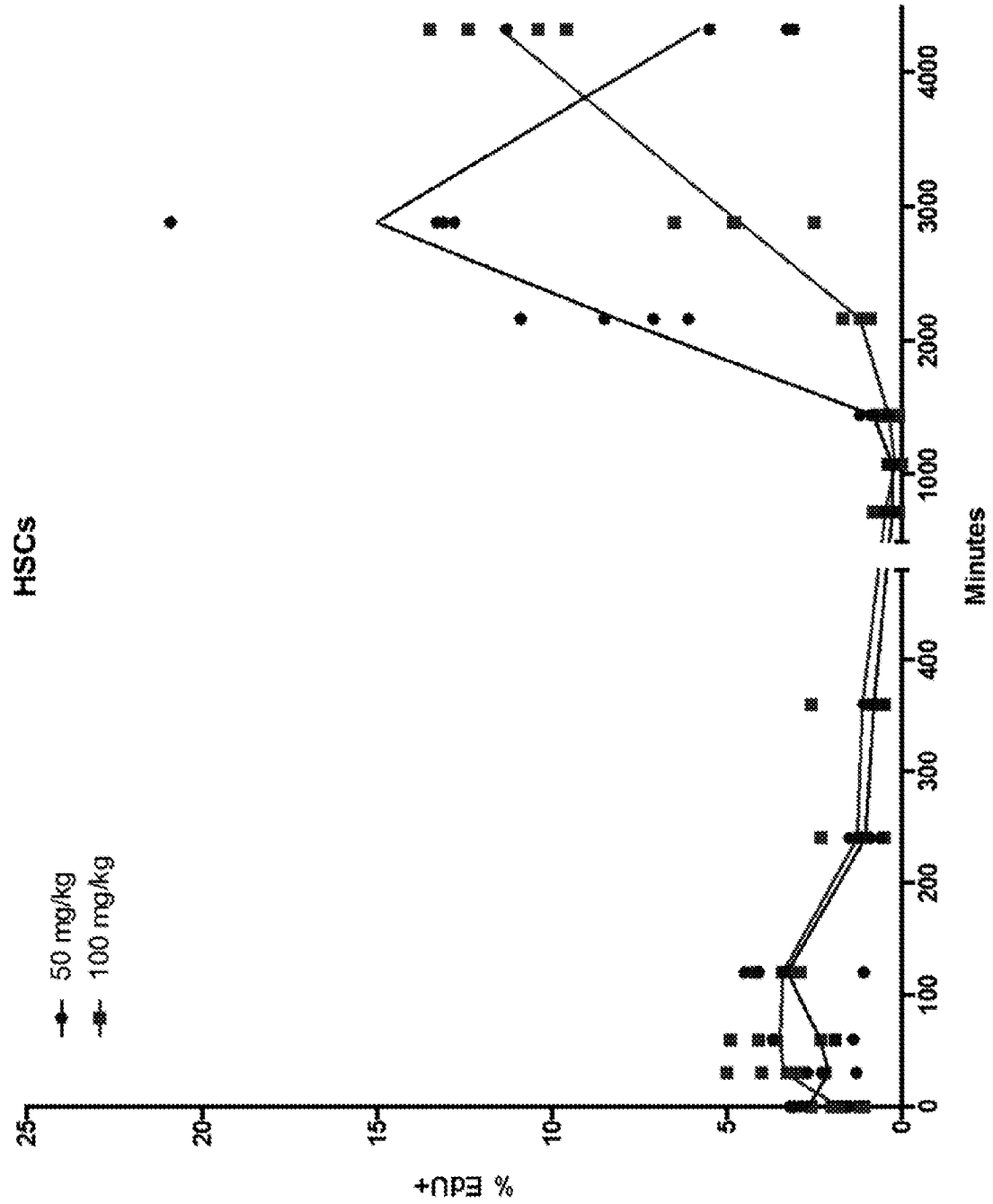

HSCs
50 mg/kg
100 mg/kg
% EdU+
0
5
10
15
20
25
0
100
200
300
400
1000
2000
3000
4000
Minutes

FIG. 19

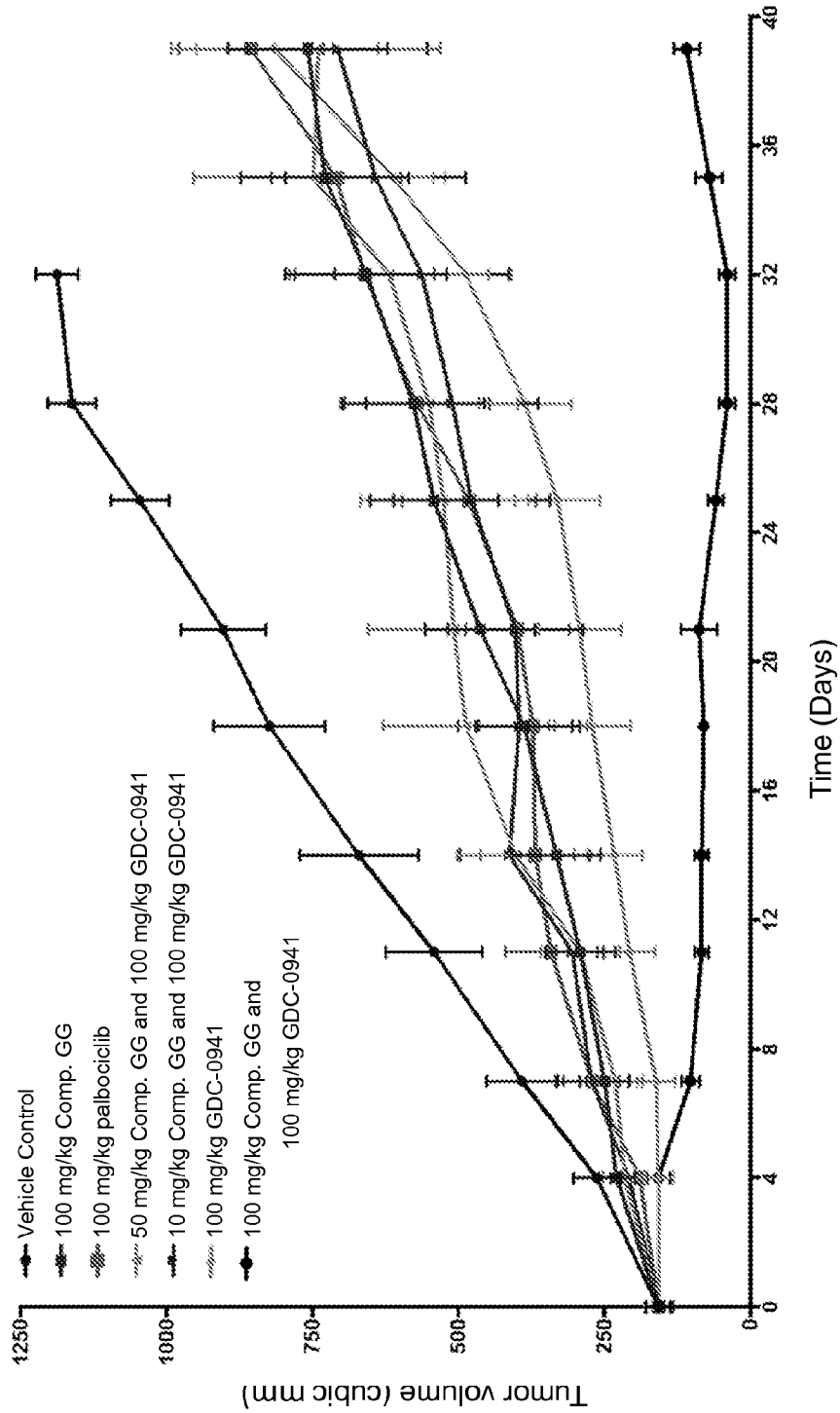

Tumor volume (cubic mm)
1250
1000
750
500
250
0
0
4
8
12
16
20
24
28
32
36
40
Time (Days)
Vehicle Control
100 mg/kg Comp. GG
100 mg/kg palbociclib
50 mg/kg Comp. GG and 100 mg/kg GDC-0941
10 mg/kg Comp. GG and 100 mg/kg GDC-0941
100 mg/kg GDC-0941
100 mg/kg Comp. GG and 100 mg/kg GDC-0941

FIG. 20

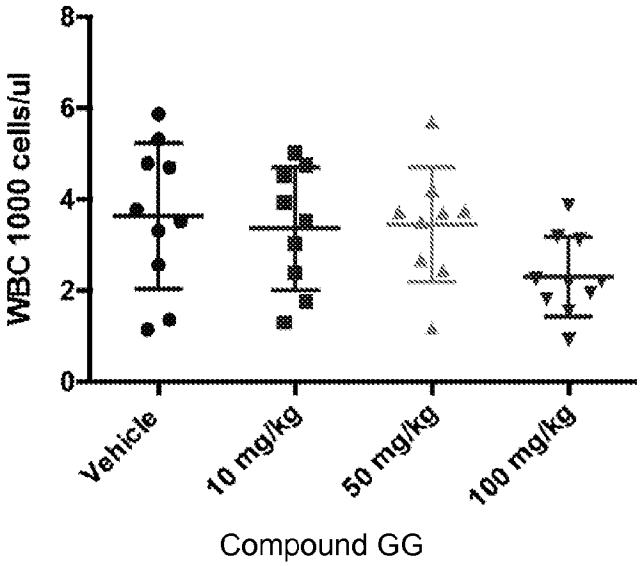


FIG. 21A

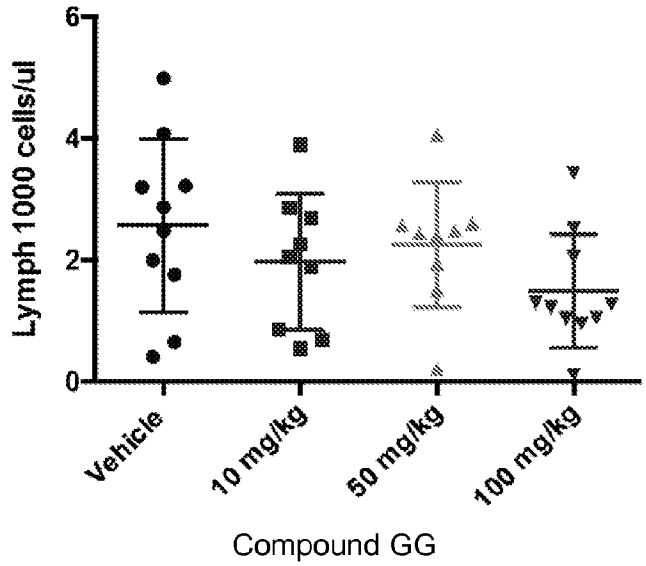


FIG. 21B

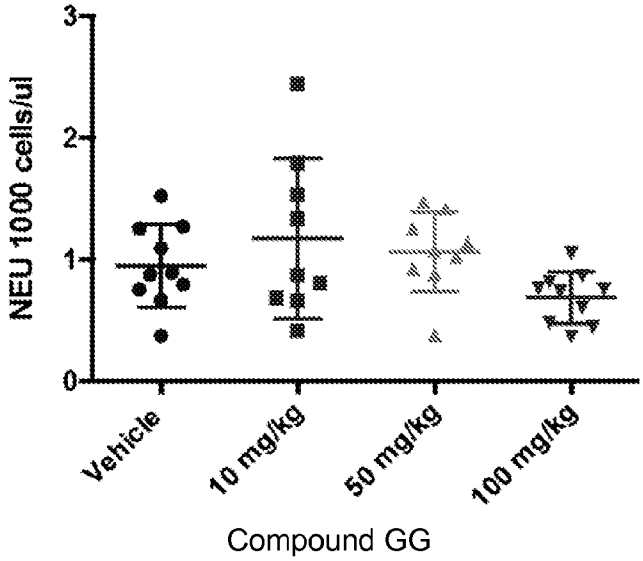


FIG. 21C

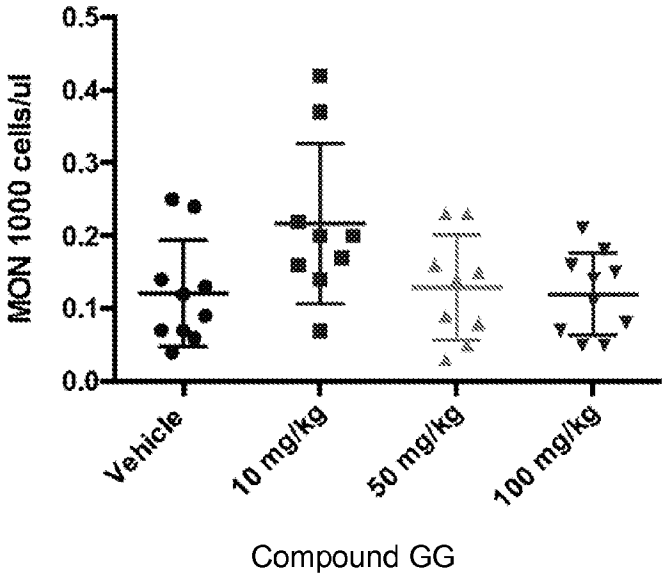


FIG. 21D

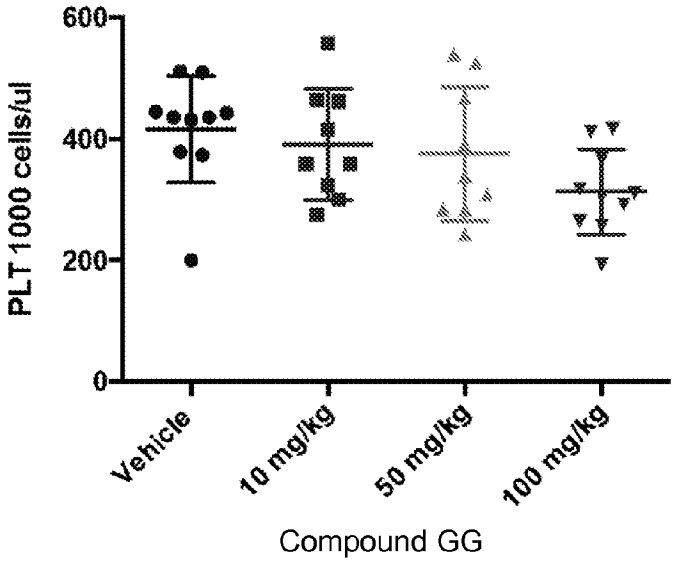


FIG. 21E

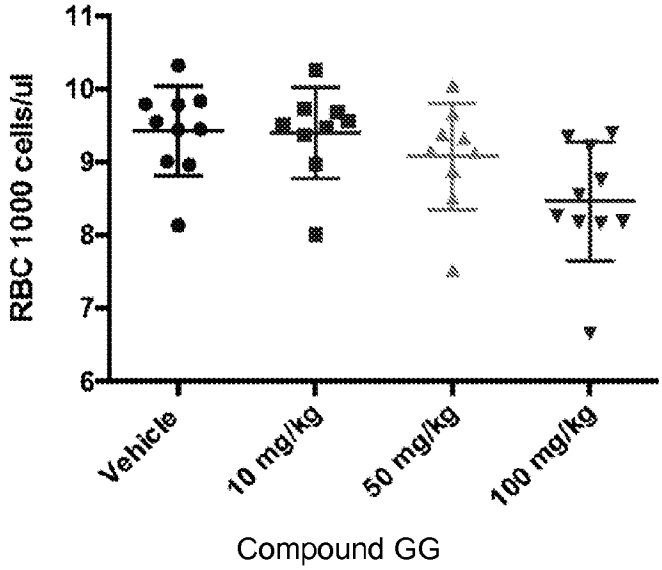


FIG. 21F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/049777

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/519 (2015.01)
CPC - A61K 31/519 (2015.10)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/519 (2015.01)
CPC - A61K 31/519 (2015.10)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/250; IPC(8) - A61K 31/519; CPC - A61K 31/519 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, PubChem, Google Scholar.
Search terms used: CDK, Bruton's , BTK, splean tyrosine, SYK, chemotherapeutic, Rb-positive, T-cell, cancer, malignancy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0009353 A1 (CHEN-KIANG et al) 13 January 2011 (13.01.2011) entire document	22-25, 30-33, 35-38
Y	US 8,598,186 B2 (TAVARES et al) 03 December 2013 (03.12.2013) entire document	22-25, 30-33, 35-38
A	US 2011/0224221 A1 (SHARPLESS et al) 15 September 2011 (15.09.2011) entire document	1-12, 15-17, 22-33, 35-38, 43-63, 65-85, 87-98, 109-131
A	US 2014/0107114 A1 (KIM et al) 17 April 2014 (17.04.2014) entire document	1-12, 15-17, 22-33, 35-38, 43-63, 65-85, 87-98, 109-131
P, A	WO 2014/168975 A1 (PHARMACYCLICS INC) 16 October 2014 (16.10.2014) entire document	1-12, 15-17, 22-33, 35-38, 43-63, 65-85, 87-98, 109-131
P, X	US 2014/0271466 A1 (G1 THERAPEUTICS INC) 18 September 2014 (18.09.2014) entire document	1-12, 15-17, 22-33, 35-38, 43-63, 65-85, 87-98, 109-131

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2015	10DEC 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/049777

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 13, 14, 18-21, 34, 39-42, 64, 86, 99-108, 132-135
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.