CYCLIN-DEPENDENT KINASE 2 BIOMARKERS AND USES THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/806,265, filed February 15, 2019, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been filed electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 23, 2020, is named 20443-0588WO1_SL.txt and is 15,865 bytes in size.

TECHNICAL FIELD

This invention relates generally to biomarkers and cancer.

BACKGROUND

Cyclin-dependent kinases ("CDKs") are a family of serine/threonine kinases. Heterodimerized with regulatory subunits known as cyclins, CDKs become fully activated and are the driving force behind cell cycle and cell division. Uncontrolled proliferation is a hallmark of cancer cells, and misregulation of CDK function occurs with high frequency in many tumors. CDK2 and CDK4 are of particular interest because their activities are frequently dysregulated in a wide variety of human cancers. CDKs are therefore recognized as an attractive target for the design and development of compounds that can specifically bind and inhibit CDK activity in cancer cells, and thus can serve as therapeutic agents. Potent and highly selective CDK4/6 inhibitors, palbociclib, abemaciclib, and ribociclib have been developed and licensed by the U.S. Food and Drug Administration ("FDA") for treatment of ER+ advanced breast tumor. Despite significant efforts, there are no FDA-licensed agents targeting CDK2 to date. The lack of a biomarker that reliably reports CDK2 enzymatic and/or oncogenic activity has hampered the development of effective target-associated assays for lead discovery and optimization. There is a clear need to identify biomarkers of CDK2-mediated oncogenesis to provide rapid and effective means for development and evaluation of CDK2-targeted anti-cancer therapies.

SUMMARY

The present invention is based, at least in part, on the discovery that the functional status of cyclin dependent kinase inhibitor 2A ("CDKN2A"; also referred to as "p16") is a biomarker for predicting sensitivity to CDK2-targeting therapies in G1/S-specific cyclin-E1- ("CCNE1-") amplified cells suitable for use in patient stratification. In addition, the present invention is based, at least in part, on the discovery that, in CCNE1-amplified cell lines, the level of human retinoblastoma associated protein ("Rb") phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is a pharmacodynamic marker for CDK2 activity and is suitable for use in measuring CDK2 enzymatic activity in cellular assay or preclinical and clinical applications, such as, e.g., monitoring the progress of or responsiveness to treatment with a CDK2 inhibitor.

The disclosure features a method of treating a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2, comprising administering to the human subject a CDK2 inhibitor, wherein the human subject has been previously determined to: (i) (a) have a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) have a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) express a p16 protein, and (ii) (a) have an amplification of the CCNE1 gene and/or (b) have an expression level of CCNE1 in a biological sample obtained from the human subject that is higher than a control expression level of CCNE1. In some embodiments, the subject has a disease or disorder associated with CDK2. In some embodiments, the subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In some embodiments, the human subject has been previously determined to: (i) (a) have a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 and/or (b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and (ii) have an amplification of the CCNE1 gene in a biological sample obtained from the human subject. In some embodiments, the CDKN2A gene encodes a protein comprising the amino acid sequence of SEQ ID NO:1. In some embodiments, a second therapeutic agent is administered to the human subject in combination with the CDK2 inhibitor. In some embodiments, the second therapeutic agent is a BCL2 inhibitor or a CDK4/6 inhibitor.

The disclosure also features a method of treating a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2, comprising: (i) identifying, in a biological sample obtained from the human subject: (a) a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions, and/or (c) the presence of a p16 protein; (ii) identifying, in a biological sample obtained from the human subject: (a) an amplification of the CCNE1 gene and/or (b) an expression level of CCNE1 that is higher than a control expression level of CCNE1; and (iii) administering a CDK2 inhibitor to the human subject. In some embodiments, the subject has a disease or disorder associated with CDK2. In some embodiments, the subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In some embodiments, the method comprises: (i) identifying, in a biological sample obtained from the human subject: (a) a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) the presence of a p16 protein; (ii) identifying, in a biological sample obtained from the human subject: (a) an amplification of the CCNE1 gene; and (iii) administering a CDK2 inhibitor to the human subject. In some embodiments, the CDKN2A gene encodes a protein comprising the amino acid sequence of SEQ ID NO:1. In some embodiments, a second therapeutic agent is administered to the human subject in combination with the CDK2 inhibitor. In some embodiments, the second therapeutic agent is a BCL2 inhibitor or a CDK4/6 inhibitor.

The disclosure also features a method of predicting the response of a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor, comprising: (i) determining, from a biological sample obtained from the human subject: (a) the nucleotide sequence of a CDKN2A gene, (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) the presence of a p16 protein; and (ii) determining, from a biological sample obtained from the human subject: (a) the copy number of the CCNE1 gene and/or (b) the expression level of CCNE1, wherein (1) (a) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c)

the presence of a p16 protein, and (2) (a) an amplification of the CCNE1 gene and/or (b) an expression level of CCNE1 that is higher than a control expression level of CCNE1, is predictive that the human subject will respond to the CDK2 inhibitor. In some embodiments, the subject has a disease or disorder associated with CDK2. In some embodiments, the subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In some embodiments, the method comprises: (i) determining, from a biological sample obtained from the human subject: (a) the nucleotide sequence of a CDKN2A gene and/or (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions; and (ii) determining, from a biological sample obtained from the human subject: (a) the copy number of the CCNE1 gene, wherein (1) (a) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 and/or (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and (2) (a) an amplification of the CCNE1 gene, is predictive that the human subject will respond to the CDK2 inhibitor.

In some embodiments of the foregoing methods, the amplification of the CCNE1 gene comprises a gene copy number of at least 3. In some embodiments of the foregoing methods, the amplification of the CCNE1 gene comprises a gene copy number of at least 5. In some embodiments of the foregoing methods, the amplification of the CCNE1 gene comprises a gene copy number of at least 21.

In some embodiments of the foregoing methods, the control expression level of CCNE1 is a pre-established cut-off value. In some embodiments of the foregoing methods, the control expression level of CCNE1 is the expression level of CCNE1 in a sample or samples obtained from one or more subjects that have not responded to treatment with the CDK2 inhibitor.

In some embodiments of the foregoing methods, the expression level of CCNE1 is the expression level of CCNE1 mRNA. In some embodiments of the foregoing methods, the expression level of CCNE1 is the expression level of CCNE1 protein. In some embodiments in which the expression level of CCNE1 is the expression level of CCNE1 mRNA, the expression level of CCNE1 is measured by RNA sequencing, quantitative polymerase chain reaction (PCR), in situ hybridization, nucleic acid array or RNA sequencing. In some embodiments in which the expression level of CCNE1 is the expression level of CCNE1 protein, the expression level of

CCNE1 is measured by western blot, enzyme-linked immunosorbent assay, or immunohistochemistry staining.

The disclosure also features a method for assessing the CDKN2A gene and the CCNE1 gene, comprising determining, from a biological sample or biological samples obtained from a human subject having a disease or disorder associated with CDK2, (i) (a) the nucleotide sequence of a CDKN2A gene or (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and (ii) the copy number of the CCNE1 gene.

The disclosure also features a method of evaluating the response of a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor, comprising: (a) administering a CDK2 inhibitor to the human subject, wherein the human subject has been previously determined to have an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1; (b) measuring, in a biological sample of obtained from the subject subsequent to the administering of step (a), the level of retinoblastoma (Rb) protein phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, wherein a reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, is indicative that the human subject responds to the CDK2 inhibitor. In some embodiments, the subject has a disease or disorder associated with CDK2. In some embodiments, the subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In some embodiments, the biological sample comprises a blood sample or a tumor biopsy sample.

The disclosure also features a method for measuring the amount of a protein in a sample, comprising: (a) providing a biological sample obtained from a human subject having a disease or disorder associated with CDK2; and (b) measuring the level of Rb protein phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 in the biological sample. In some embodiments, the biological sample comprises a blood sample or a tumor biopsy sample.

In some embodiments of the foregoing methods, the CDK2 inhibitor is a compound described *infra*, or a pharmaceutically acceptable salt thereof.

In some embodiments of the foregoing methods, the disease or disorder associated with CDK2 is a cancer.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIGS. 1A-B: Characterization of ovarian and endometrial cell lines. **FIG. 1A**: Cell lines used for study included four cell lines with CCNE1 amplification and three cell lines with no CCNE1 amplification. CCNE1 amplification copy numbers are indicated. **FIG. 1B**: The expression of CCNE1 was determined by Western blot in indicated cell lines. This blot show cell lines with CCNE1 gain of function by copy number (CN>2) expressed higher levels of CCNE1 protein compared with cell lines with copy neutral or loss of function of the gene (CN≤2). GAPDH was detected as a loading control. Non-Amp, non-amplification; Amp, amplification.

FIGS. 2A-B: siRNA mediated CDK2 knockdown inhibits proliferation in CCNE1 amplified cell lines. **FIG. 2A**: CCNE1 amplified Fu-ov1 (upper) and KLE (lower) cells were harvested and subjected to cell cycle analysis 72 hours after transfection with either scrambled siRNAs ("Ctl") or CDK2 siRNAs. The cell cycle phase distribution was evaluated by FACS. Shown are representative images of three separate experiments. **FIG. 2B**: CDK2 knockdown was confirmed by Western blot analysis after transfection with CDK2 siRNA. GAPDH was used as a loading control.

FIGS. 3A-B: CDK2 knockdown does not inhibit proliferation in CCNE1 Non-Amp lines. **FIG. 3A**: CCNE1 non-amplified COV504 and Igrov1 cells were harvested and subjected to cell cycle analysis 72 hours after transfection with Ctl siRNAs and CDK2 siRNAs. The cell cycle phase distribution was evaluated by FACS. Shown are representative images of three separate experiments. **FIG. 3B**: CDK2 knockdown was confirmed by Western blot analysis after transfection with CDK2 siRNA. GAPDH was used as a loading control.

FIG. 4: CDK2 knockdown by siRNA inhibits proliferation in CCNE1 amplified, but not in CCNE1 non-amplified, human cancer cell lines. Percentage of cells at the S phase 3 days after transfection of CDK2 siRNAs, relative to Ctl siRNA .

The cell cycle phase distribution was evaluated by FACS. Means represent three independent experiments in four CCNE1 Amp cell lines and three Non-Amp lines.

FIG. 5: Palbociclib treatment induces dose-dependent inhibition of proliferation in CCNE1 non-amplified, but not in amplified cell lines. Cell cycle analysis of CCNE1 non-amplified cell line COV504 (upper) and CCNE1 amplified OVCAR3 cells (lower) after Palbociclib treatment for 16 hours. The cell cycle phase distribution was evaluated by FACS.

FIG. 6: Palbociclib treatment selectively inhibits proliferation in CCNE1 non-amplified cancer cell lines. Percentage of cells at the S phase after 16 hours of Palbociclib with the indicated doses, relative to DMSO.

FIGS. 7A-B: CDK2 knockdown by siRNAs blocks RB phosphorylation at S780 in CCNE1 amplified, but not in non-amplified ovarian cells. **FIG. 7A**: Four CCNE1 Amp cell lines, COV318, Fu-OV1, OVCAR3 and KLE cells, were transfected with CDK2 siRNAs for 72 hours. **FIG. 7B**: Three CCNE1 Non-Amp cell lines, COV504, OV56 and Igrov1, were transfected with CDK2 siRNAs for 72 hours. The total proteins were extracted from CDK2 siRNA or Ctl siRNA transfected cells and subjected to western blotting. GAPDH was used as a loading control.

FIGS. 8A-B: Palbociclib blocks RB phosphorylation at S780 in CCNE1 non-amplified, but not in amplified ovarian cells. **FIG. 8A**: CCNE1 Amp OVCAR3 and COV318 cells were treated at various concentrations of Palbociclib as indicated for 1 hour or 15h. **FIG. 8B**: CCNE1 Non-Amp COV504 and OV56 were treated at various concentrations of Palbociclib as indicated for 1 hour or 15h. The total proteins were extracted from these Palbociclib or DMSO (controls) treated cells and subjected to western blotting. p-RB, phosphorylated retinoblastoma protein. GAPDH was used as a loading control.

FIGS. 9A-B: CDK2 degradation by dTAG decreases RB phosphorylation at S780. **FIG. 9A**: Chemical structure of dTAG. **FIG. 9B**: CDK2-FKBP12(F36V) degradation by CDK2-dTAG treatment for 14 hours inhibited RB phosphorylation at S780 in CDK2 knockout OVCAR3 (right, Cas9+, CDK2-FKBP12(F36V)–HA+, CDK2-gRNA) cells, but not in OVCAR3 cells with endogenous CDK2 (left, Cas9+, CDK2-FKBP12(F36V)–HA+, Ctl-gRNA).

FIGS. 10A-B: p-RB S780 HTRF cellular Assay for identification of CDK2 inhibitors. **FIG. 10A**: IC_{50} in CDK2 biochemical kinase activity assay. **FIG. 10B**: Concentration response analysis of reference compounds tested in the p-RB S780

HTRF cellular assay. HTRF, homogeneous time-resolved fluorescence. IC_{50} from HTRF cellular Assay correlates with IC_{50} in CDK2 enzymatic assay.

FIG. 11: Bioinformatics analysis of CCLE dataset reveals the sensitivity to CDK2 inhibition in CCNE1 amplified cells relies on functional p16. **FIG. 11** shows the status of p16 in CDK2 sensitive verse insensitive cell lines. CCLE: Broad Institute Cancer Cell Line Encyclopedia (*see* Barretina, below).

FIGS. 12A-B: CCNE1 amplified cells with dysfunctional p16 do not respond to CDK2 inhibition. **FIG. 12A**: Western blot analysis of p16 in three gastric cell lines with CCNE1 Amp. **FIG. 12B**: Percentage of cells at the S phase 3 days after transfection of CDK2 siRNAs, relative to Ctl siRNA . The cell cycle phase distribution was evaluated by FACS.

FIG. 13: p16 knockdown by siRNA abolishes CDK2 inhibition induced cell cycle suppression in CCNE1 amplified cells. The percentage of S phase cells following p16 knockdown and CDK2 inhibitor treatment, normalized to cell with Ctl siRNA and DMSO treatment. CCNE1 amplified COV318 cells were transfected with either Ctl siRNAs or p16 siRNA. 72 hours after transfection, cells were treated with 100nM CDK2 inhibitor Compound A. Cells were harvested and subjected to cell cycle analysis 16 hours after treatment.

DETAILED DESCRIPTION

The disclosure provides predictive markers (e.g., biomarkers and pharmacodynamic markers, e.g., gene copy number, gene sequence, expression levels, or phosphorylation levels) to identify those human subjects having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 for whom administering a CDK2 inhibitor is likely to be effective. The disclosure also provides pharmacodynamic markers (e.g., phosphorylation levels) to identify those human subjects having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 whom are responding to a CDK2 inhibitor. This disclosure also provides methods for treating a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 (e.g., cancer), comprising administering to the human subject a CDK2 inhibitor.

Diseases and Disorders Associated with CDK2

Diseases or disorders associated with CDK2 are those in which the underlying pathology is, wholly or partially, mediated by CDK2. Such diseases include cancer and other diseases with proliferation disorder. In certain embodiments, diseases or disorders associated with CDK2 are those that are treatable with a CDK2 inhibitor.

In some embodiments, the disease or disorder associated with CDK2 is a cancerous tumor comprising an aberration that activates the CDK2 kinase activity. This includes, but is not limited to, cancers that are characterized by amplification or overexpression of CCNE1 such as ovarian cancer, uterine carcinosarcoma and breast cancer and p27 inactivation such as breast cancer and melanomas.

In some embodiments, the disease or disorder associated with CDK2 is a N-myc amplified neuroblastoma (see Molenaar, et al., Proc Natl Acad Sci USA 106(31): 12968-12973), a K-Ras mutant lung cancer (see Hu, S., et al., *Mol Cancer Ther,* 2015. 14(11): p. 2576-85), or a cancer with a FBW7 mutation and CCNE1 overexpression (see Takada, et al., *Cancer Res*, 2017. 77(18): p. 4881-4893).

In some embodiments, the disease or disorder associated with CDK2 is lung squamous cell carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, bladder urothelial carcinoma, mesothelioma, or sarcoma.

In some embodiments, the disease or disorder associated with CDK2 is lung adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, or stomach adenocarcinoma.

In some embodiments, the disease or disorder associated with CDK2 is an adenocarcinoma, carcinoma, or cystadenocarcinoma.

In some embodiments, the disease or disorder associated with CDK2 is uterine cancer, ovarian cancer, stomach cancer, esophageal cancer, lung cancer, bladder cancer, pancreatic cancer, or breast cancer.

In some embodiments, the disease or disorder associated with CDK2 is a cancer.

In some embodiments, the cancer is characterized by amplification or overexpression of CCNE1. In some embodiments, the cancer is ovarian cancer or breast cancer, characterized by amplification or overexpression of CCNE1.

In some embodiments, the breast cancer is chemotherapy or radiotherapy resistant breast cancer, endocrine resistant breast cancer, trastuzumab resistant breast cancer, or breast cancer demonstrating primary or acquired resistance to CDK4/6 inhibition. In some embodiments, the breast cancer is advanced or metastatic breast cancer.

Examples of cancers that are treatable with a CDK2 inhibitor using the methods of the present disclosure include, but are not limited to, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, endometrial cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, 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, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or urethra, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, Merkel cell carcinoma, and combinations of said cancers. The methods of the present disclosure are also useful for the treatment of metastatic cancers, especially metastatic cancers that express PD-Ll.

In some embodiments, cancers treatable with a CDK2 inhibitor using the methods of the present disclosure include melanoma (e.g., metastatic malignant melanoma, BRAF and HSP90 inhibition-resistant melanoma), renal cancer (e.g., clear cell carcinoma), prostate cancer (e.g., hormone refractory prostate adenocarcinoma), breast cancer, colon cancer, lung cancer (e.g., non-small cell lung cancer and small cell lung cancer), squamous cell head and neck cancer, urothelial cancer (e.g., bladder) and cancers with high microsatellite instability (MSI^{high}). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the compounds of the disclosure.

In some embodiments, cancers that are treatable with a CDK2 inhibitor using the methods of the present disclosure include, but are not limited to, solid tumors (*e.g.*,

prostate cancer, colon cancer, esophageal cancer, endometrial cancer, ovarian cancer, uterine cancer, renal cancer, hepatic cancer, pancreatic cancer, gastric cancer, breast cancer, lung cancer, cancers of the head and neck, thyroid cancer, glioblastoma, sarcoma, bladder cancer, etc.), hematological cancers (*e.g.*, lymphoma, leukemia such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), DLBCL, mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma or multiple myeloma) and combinations of said cancers.

In some embodiments, cancers that are treatable with a CDK2 inhibitor using the methods of the present disclosure include, but are not limited to, cholangiocarcinoma, bile duct cancer, triple negative breast cancer, rhabdomyosarcoma, small cell lung cancer, leiomyosarcoma, hepatocellular carcinoma, Ewing's sarcoma, brain cancer, brain tumor, astrocytoma, neuroblastoma, neurofibroma, basal cell carcinoma, chondrosarcoma, epithelioid sarcoma, eye cancer, Fallopian tube cancer, gastrointestinal cancer, gastrointestinal stromal tumors, hairy cell leukemia, intestinal cancer, islet cell cancer, oral cancer, mouth cancer, throat cancer, laryngeal cancer, lip cancer, mesothelioma, neck cancer, nasal cavity cancer, ocular cancer, ocular melanoma, pelvic cancer, rectal cancer, renal cell carcinoma, salivary gland cancer, sinus cancer, spinal cancer, tongue cancer, tubular carcinoma, urethral cancer, and ureteral cancer.

In some embodiments, diseases and indications that are treatable with a CDK2 inhibitor using the methods of the present disclosure include, but are not limited to hematological cancers, sarcomas, lung cancers, gastrointestinal cancers, genitourinary tract cancers, liver cancers, bone cancers, nervous system cancers, gynecological cancers, and skin cancers.

Exemplary hematological cancers include lymphomas and leukemias such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), acute promyelocytic leukemia (APL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma, myeloproliferative diseases (e.g., primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET)), myelodysplasia syndrome (MDS), T-cell acute lymphoblastic lymphoma (T-ALL) and multiple myeloma (MM).

Exemplary sarcomas include chondrosarcoma, Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma, angiosarcoma, fibrosarcoma, liposarcoma, myxoma, rhabdomyoma, rhabdosarcoma, fibroma, lipoma, harmatoma, and teratoma.

Exemplary lung cancers include non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), bronchogenic carcinoma, squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma, alveolar (bronchiolar) carcinoma, bronchial adenoma, chondromatous hamartoma, and mesothelioma.

Exemplary gastrointestinal cancers include cancers of the esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Kaposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), and colorectal cancer.

Exemplary genitourinary tract cancers include cancers of the kidney (adenocarcinoma, Wilm's tumor [nephroblastoma]), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), and testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma).

Exemplary liver cancers include hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, and hemangioma.

Exemplary bone cancers include, for example, osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma, and giant cell tumors

Exemplary nervous system cancers include cancers of the skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma, glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), and spinal cord

(neurofibroma, meningioma, glioma, sarcoma), as well as neuroblastoma and Lhermitte-Duclos disease.

Exemplary gynecological cancers include cancers of the uterus (endometrial carcinoma), cervix (cervical carcinoma, pre -tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), and fallopian tubes (carcinoma).

Exemplary skin cancers include melanoma, basal cell carcinoma, Merkel cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, and keloids. In some embodiments, diseases and indications that are treatable using the compounds of the present disclosure include, but are not limited to, triple-negative breast cancer (TNBC), myelodysplastic syndromes, testicular cancer, bile duct cancer, esophageal cancer, and urothelial carcinoma.

In some embodiments, the disease or disorder associated with CDK2 is an infection, e.g., a viral infection, a bacterial infection, a fungus infection or a parasite infection.

Biomarkers and Methods of Predicting Responsiveness to a CDK2 Inhibitor

Provided herein are biomarkers that are useful in predicting responsiveness (improvement in disease status as evidenced by, e.g., disease remission/resolution) of a subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor. Thus, provided herein are methods of predicting the response of a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor. In certain embodiments, the predictive methods described herein predict that the subject will respond to treatment with the CDK2 inhibitor with at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98% or 100% accuracy. For example, in some embodiments, if the predictive methods described herein are applied to 10 subjects having, suspected of having, or at risk of developing a disease or disorder associated with CDK2, and 8 of those 10 subjects are predicted to respond

to treatment with a CDK2 inhibitor based on a predictive method described herein, and 7 of those 8 subjects do indeed respond to treatment with a CDK2 inhibitor, then the predictive method has an accuracy of 87.5% (7 divided by 8). A subject is considered to respond to the CDK2 inhibitor if the subject shows any improvement in disease status as evidenced by, e.g., reduction or alleviation in symptoms, disease remission/resolution, etc.

CCNE1 and p16

CCNE1 and p16 have been identified in the Examples as genes, in combination, useful in predicting responsiveness (e.g., improvement in disease as evidenced by disease remission/resolution) of a subject having a disease or disorder associated with CDK2 to a CDK2 inhibitor.

p16 (also known as cyclin-dependent kinase inhibitor 2A, cyclin-dependent kinase 4 inhibitor A, multiple tumor suppressor 1, and p16-INK4a) acts as a negative regulator of the proliferation of normal cells by interacting with CDK4 and CDK6. p16 is encoded by the *cyclin dependent kinase inhibitor 2A* ("*CDKN2A*") gene (GenBank Accession No. NM_000077). The cytogenic location of the *CDKN2A* gene is 9p21.3, which is the short (p) arm of chromosome 9 at position 21.3 The molecular location of the *CDKN2A* gene is base pairs 21,967,752 to 21,995,043 on chromosome 9 (Homo sapiens Annotation Release 109, GRCh38.p12). Genetic and epigenetic abnormalities in the gene encoding p16 are believed to lead to escape from senescence and cancer formation (Okamoto et al., 1994, PNAS 91(23):11045-9). Nonlimiting examples of genetic abnormalities in the gene encoding p16 are described in Table 1, below. The amino acid sequence of human p16 is provided below (GenBank Accession No. NP_000068 / UniProtKB Accession No. P42771):

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  1 MEPAAGSSME PSADWLATAA ARGRVEEVRA LLEAGALPNA PNSYGRRPIQ VMMMGSARVA
 61 ELLLLHGAEP NCADPATLTR PVHDAAREGF LDTLVVLHRA GARLDVRDAW GRLPVDLAEE
121 LGHRDVARYL RAAAGGTRGS NHARIDAAEG PSDIPD
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(SEQ ID NO:1).

CCNE1 is a cell cycle factor essential for the control of the cell cycle at the G1/S transition (Ohtsubo et al., 1995, Mol. Cell. Biol. 15:2612-2624). CCNE1 acts as a regulatory subunit of CDK2, interacting with CDK2 to form a serine/threonine kinase holoenzyme complex. The CCNE1 subunit of this holoenzyme complex provides the substrate specificity of the complex (Honda et al., 2005, EMBO 24:452-463). CCNE1 is encoded by the *cyclin E1* ("*CCNE1*") gene (GenBank Accession No.

NM_001238). The amino acid sequence of human CCNE1 is provided below (GenBank Accession No. NP_001229 / UniProtKB Accession No. P24864):

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  1 mprerrerda kerdtmkedg gaefsarsrk rkanvtvflq dpdeemakid rtardqcgsq
 61 pwdnnavcad pcsliptpdk edddrvypns tckpriiaps rgsplpvlsw anreevwkim
121 lnkektylrd qhfleqhpll qpkmrailld wlmevcevyk lhretfylaq dffdrymatq
181 envvktllql igisslfiaa kleeiyppkl hqfayvtdga csgdeiltme lmimkalkwr
241 lspltivswl nvymqvayln dlhevllpqy pqqifiqiae lldlcvldvd clefpygila
301 asalyhfsss elmqkvsgyq wcdiencvkw mvpfamvire tgssklkhfr gvadedahni
361 qthrdsldll dkarakkaml seqnrasplp sglltppqsg kkqssgpema
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(SEQ ID NO:2).

The Examples demonstrate CDK2-knockdown inhibits proliferation of CCNE1-amplified cell lines, but not of CCNE1-non-amplified cell lines. Conversely, the Examples show that CDK4/6 inhibition inhibits proliferation of CCNE1-non-amplified cell lines, but not of CCNE1-amplified cell lines. The Examples further demonstrate that presence of a normal (e.g., non-mutated or non-deleted) p16 gene is required for the observed inhibition of cell proliferation in CCNE1-amplified cells treated with a CDK2-inhibitor. Accordingly, CCNE1 and p16 are, together, a combination biomarker: cells that respond to treatment with a CDK2 inhibitor display an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, and have a nucleotide sequence (e.g., a gene or an mRNA) that encodes the p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1) and/or have p16 protein present, while control cells that do not respond to treatment with a CDK2 inhibitor do not have an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, and tend to have a mutated or deleted gene that encodes the p16 protein and/or lack expression of p16 protein. Thus, provided herein are methods relating to the use of: (i) the amplification of the CCNE1 gene and/or expression level of CCNE1; and (ii) presence of a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or expression of a p16 protein, in a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 as a biomarker for predicting the response of the subject to a CDK2 inhibitor. In specific embodiments, the human subject has a disease or disorder

associated with CDK2. In specific embodiments, the human subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2.

In a specific embodiment, provided herein is a method of predicting the response of a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor, comprising: (i) determining, from a biological sample obtained from the human subject: (a) the nucleotide sequence of a CDKN2A gene, (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) the presence of a p16 protein; and (ii) determining, from a biological sample obtained from the human subject: (a) the copy number of the CCNE1 gene and/or (b) the expression level of CCNE1, wherein (1) (a) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) the presence of a p16 protein, and (2) (a) an amplification of the CCNE1 gene and/or (b) an expression level of CCNE1 that is higher than a control expression level of CCNE1, is predictive that the human subject will respond to the CDK2 inhibitor. In specific embodiments, the human subject has a disease or disorder associated with CDK2. In specific embodiments, the human subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In specific embodiments, the (i) determining of (a) the nucleotide sequence of a CDKN2A gene, (b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) the presence of a p16 protein is performed before (e.g., at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks, or from 6 hours to 16 hours, from 6 hours to 20 hours, or from 6 hours to 24 hours, from 2 days to 3 days, from 2 days to 4 days, from 2 days to 5 days, from 2 days to 6 days, from 2 days to 7 days, from 1 week to 2 weeks, from 1 week to 3 weeks, or from 1 week to 4 weeks before) administering to the human subject the CDK2 inhibitor. In specific embodiments, the (ii) determining of (a) the copy number of the CCNE1 gene and/or (b) the expression level of CCNE1 in the biological sample obtained from the human subject is performed before (e.g., at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks, or from 6 hours to 16 hours, from 6 hours to 20 hours, or from 6 hours to 24 hours, from 2 days

to 3 days, from 2 days to 4 days, from 2 days to 5 days, from 2 days to 6 days, from 2 days to 7 days, from 1 week to 2 weeks, from 1 week to 3 weeks, or from 1 week to 4 weeks before) administering to the human subject the CDK2 inhibitor.

An amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, combined with the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or the presence of a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1), is indicative/predictive that a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 will respond to a CDK2 inhibitor.

In some embodiments, the CCNE1 gene is amplified to a gene copy number from 3 to 25. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 3. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 5. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 7. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 10. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 12. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 14. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 21.

In specific embodiments, the expression level of CCNE1 is the level of CCNE1 mRNA. In specific embodiments, the expression level of CCNE1 is the level of CCNE1 protein.

In specific embodiments, the CDKN2A gene encodes a protein comprising the amino acid sequence of SEQ ID NO:1.

In specific embodiments, the one or more inactivating nucleic acid substitutions and/or deletions in the CDKN2A gene is as described in Table 1. In specific embodiments, the one or more inactivating nucleic acid substitutions and/or deletions in the CDKN2A gene is as described in Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574, 1999; Liggett and Sidransky, Biology of Neoplasia, Journal of Oncology, 16(3):1197-1206, 1998, and Cairns et al., Nature

Genetics, 11:210-212, 1995, each of which is incorporated by reference herein in its entirety.

Table 1. CDKN2A gene substitutions, deletions, and modifications

Description	**Reference(s)**
C to T transition converting codon 232 of the CDKN2A gene from an arginine codon to a stop codon	RefSNP Accession No. rs121913388; Kamb et al., Science 264: 436-440, 1994
19-basepair germline deletion at nucleotide 225 causing a reading-frame shift predicted to severely truncate p16 protein	RefSNP Accession No. rs587776716; Gruis et al., Nature Genet. 10: 351-353, 1995
6-basepair deletion at nucleotides 363-368 of the CDKN2A gene	ClinVar Accession No. RCV000010017.2; Liu et al., Oncogene 11: 405-412, 1995
Mutation at chromosome 9:21971058 predicted to substitute glycine corresponding to amino acid position 101 of SEQ ID NO:1 with a tryptophan	RefSNP Accession No. rs104894094; Ciotti et al., Am. J. Hum. Genet. 67: 311-319, 2000
Germline mutation constituting an in-frame 3-basepair duplication at nucleotide 332 in exon 2 of the CDKN2A gene	ClinVar Accession No. RCV000010020.3; Borg et al., Cancer Res. 56: 2497-2500, 1996
Mutation predicted to substitute methionine corresponding to amino acid position 53 of SEQ ID NO:1 with an isoleucine	RefSNP Accession No. rs104894095; Harland et al., Hum. Molec. Genet. 6: 2061-2067, 1997
Mutation predicted to substitute arginine corresponding to amino acid position 24 of SEQ ID NO:1 with a proline	RefSNP Accession No. rs104894097; Monzon et al., New Eng. J. Med. 338: 879-887, 1998
24-basepair repeat inserted at chromosome 9 between 21974795 and 21974796 (forward strand)	RefSNP Accession No. rs587780668; Pollock et al., Hum. Mutat. 11: 424-431, 1998)
G-to-T transversion at nucleotide -34 of the CDKN2A gene	ClinVar Accession No. RCV000010024.5; Liu et al., Nature Genet. 21: 128-132, 1999
Deletion of the p14(ARF)-specific exon 1-beta of CDKN2A	ClinVar Accession No. RCV000010026.2; Randerson-Moor et al., Hum. Molec. Genet. 10: 55-62, 2001
Mutation predicted to substitute valine corresponding to amino acid position 126 of SEQ ID NO:1 with an isoleucine	RefSNP Accession No. rs104894098; Goldstein et al., Brit. J. Cancer 85: 527-530, 2001
Transition (IVS2-105 A-G) in intron 2 of the CDKN2A gene creating a false GT splice donor site 105 bases 5-prime of exon 3 resulting in aberrant splicing of the mRNA	ClinVar Accession No. RCV000010028.3; Harland et al., Hum. Molec. Genet. 10: 2679-2686, 2001
Mutation predicted to result in substitution of glycine corresponding to amino acid position 122 of SEQ ID NO:1 with an arginine	RefSNP Accession No. rs113798404; Hewitt et al., Hum. Molec. Genet. 11: 1273-1279, 2002

Description	Reference(s)
Mutation predicted to result in substitution of valine corresponding to amino acid position 59 of SEQ ID NO:1 with an arginine	RefSNP Accession No. rs113798404; Yakobson et al., Melanoma Res. 11: 569-570, 2001
Tandem germline339G-C transversion and a 340C-T transition in the CDKN2A gene resulting in substitution of proline corresponding to amino acid position 114 of SEQ ID NO:1 with a serine	RefSNP Accession Nos. rs113798404 and rs104894104; Kannengiesser et al., Genes Chromosomes Cancer 46: 751-760, 2007
Mutation predicted to result in substitution of serine corresponding to amino acid position 56 of SEQ ID NO:1 with an isoleucine	RefSNP Accession No. rs104894109; Kannengiesser et al., Genes Chromosomes Cancer 46: 751-760, 2007
Mutation predicted to result in substitution of glycine corresponding to amino acid position 89 of SEQ ID NO:1 with an aspartic acid	RefSNP Accession No. rs137854599; Goldstein et al., J. Med. Genet. 45: 284-289, 2008
Heterozygous A-to-G transition in exon 1B of the CDKN2A gene, affecting splicing of the p14(ARF) isoform	ClinVar Accession no. RCV000022943.3; Binni et al., Clin. Genet. 77: 581-586, 2010
Heterozygous 5-bp duplication (19_23dup) in the CDKN2A gene, resulting in a frameshift and premature termination	ClinVar Accession No. RCV000030680.6; Harinck, F., Kluijt et al., J. Med. Genet. 49: 362-365, 2012
Mutation predicted to result in substitution of aspartic acid corresponding to amino acid position 84 of SEQ ID NO:1 with a valine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of aspartic acid corresponding to amino acid position 84 of SEQ ID NO:1 with a glycine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of arginine corresponding to amino acid position 87 of SEQ ID NO:1 with a proline	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of proline corresponding to amino acid position 48 of SEQ ID NO:1 with a leucine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of aspartic acid corresponding to amino acid position 74 of SEQ ID NO:1 with a asparagine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of arginine corresponding to amino acid position 87 of SEQ ID NO:1 with a leucine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of asparagine corresponding to amino acid position 71 of SEQ ID NO:1 with a serine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574
Mutation predicted to result in substitution of arginine corresponding to amino acid position 80 of SEQ ID NO:1 with a leucine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574

Description	Reference(s)
Mutation predicted to result in substitution of histidine corresponding to amino acid position 83 of SEQ ID NO:1 with a tyrosine	Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574

Rb S780

Phosphorylation of Rb at the serine corresponding to amino acid position 780 of SEQ ID NO:3 (referred to herein as "Ser780" or "S780") has been identified in the Examples as a pharmacodynamic marker useful in assessing responsiveness (e.g., inhibition by CDK2) of a human subject having a disease or disorder having CCNE1 amplification to a CDK2 inhibitor.

Rb is a regulator of the cell cycle and acts as a tumor suppressor. Rb is activated upon phosphorylation by cyclin D-CDK4/6 at Ser780 and Ser795 and by cyclin E/CDK2 at Ser807 and Ser811. Rb is encoded by the *RB transcriptional corepressor 1* ("*RB1*") gene (GenBank Accession No. NM_000321). The amino acid sequence of human Rb is provided below (GenBank Accession No. NP_000312 / UniProtKB Accession No. P06400) (S780 is in bold and underlined):

1 MPPKTPRKTA ATAAAAAAEP PAPPPPPPPE EDPEQDSGPE DLPLVRLEFE ETEEPDFTAL
61 CQKLKIPDHV RERAWLTWEK VSSVDGVLGG YIQKKKELWG ICIFIAAVDL DEMSFTFTEL
121 QKNIEISVHK FFNLLKEIDT STKVDNAMSR LLKKYDVLFA LFSKLERTCE LIYLTQPSSS
181 ISTEINSALV LKVSWITFLL AKGEVLQMED DLVISFQLML CVLDYFIKLS PPMLLKEPYK
241 TAVIPINGSP RTPRRGQNRS ARIAKQLEND TRIIEVLCKE HECNIDEVKN VYFKNFIPFM
301 NSLGLVTSNG LPEVENLSKR YEEIYLKNKD LDARLFLDHD KTLQTDSIDS FETQRTPRKS
361 NLDEEVNVIP PHTPVRTVMN TIQQLMMILN SASDQPSENL ISYFNNCTVN PKESILKRVK
421 DIGYIFKEKF AKAVGQGCVE IGSQRYKLGV RLYYRVMESM LKSEEERLSI QNFSKLLNDN
481 IFHMSLLACA LEVVMATYSR STSQNLDSGT DLSFPWILNV LNLKAFDFYK VIESFIKAEG
541 NLTREMIKHL ERCEHRIMES LAWLSDSPLF DLIKQSKDRE GPTDHLESAC PLNLPLQNNH
601 TAADMYLSPV RSPKKKGSTT RVNSTANAET QATSAFQTQK PLKSTSLSLF YKKVYRLAYL
661 RLNTLCERLL SEHPELEHII WTLFQHTLQN EYELMRDRHL DQIMMCSMYG ICKVKNIDLK
721 FKIIVTAYKD LPHAVQETFK RVLIKEEEYD SIIVFYNSVF MQRLKTNILQ YASTRPPTL**S**
781 PIPHIPRSPY KFPSSPLRIP GGNIYISPLK SPYKISEGLP TPTKMTPRSR ILVSIGESFG
841 TSEKFQKINQ MVCNSDRVLK RSAEGSNPPK PLKKLRFDIE GSDEADGSKH LPGESKFQQK
901 LAEMTSTRTR MQKQKMNDSM DTSNKEEK (SEQ ID NO:3).

As stated above, the Examples demonstrate CDK2-knockdown inhibits proliferation in CCNE1-amplified cell lines, but not in CCNE1-non-amplified cell lines. The Examples further demonstrate CDK2-knockdown or inhibition blocks Rb phosphorylation at the S780 in CCNE1-amplified cell lines, but not in CCNE1-non-amplified cell lines. Accordingly, Rb phosphorylation at the serine corresponding to

amino acid position 780 of SEQ ID NO:3 is a pharmacodynamic marker for assessing response to CDK2 inhibition in CCNE1 amplified cancer cells or patients with diseases or disorders having CCNE1 amplification. Thus, provided herein are methods relating to the use of the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 in a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 as a marker for indicating the response of the human subject to a CDK2 inhibitor, wherein the human subject has an increased expression level of CCNE1.

In a specific embodiment, provided herein is a method of evaluating the response of a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 to a CDK2 inhibitor, comprising:

(a) administering a CDK2 inhibitor to the human subject, wherein the human subject has been previously determined to have an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1; and

(b) measuring, in a biological sample obtained from the human subject subsequent to the administering of step (a), the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3,

wherein a reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, is indicative that the human subject responds to the CDK2 inhibitor. In specific embodiments, the human subject has a disease or disorder associated with CDK2. In specific embodiments, the human subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In a specific embodiment, the human subject has further been previously determined to have a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions preventing the CDKN2A gene from encoding a protein comprising the amino acid sequence of SEQ ID NO:1 and/or a p16 protein lacking one or more inactivating amino acid substitutions and/or deletions (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1). In a specific embodiment, the measuring of step (b) occurs at least 6 hours, at least 16 hours, at least 20 hours, or at least 24 hours after the administering of step (a). In some embodiments, the measuring of step (b) occurs at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6

days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks after the administering of step (a). In a specific embodiment, the measurement of step (b) occurs from 6 hours to 16 hours, from 6 hours to 20 hours, or from 6 hours to 24 hours after the administering of step (a). In some embodiments, the measuring of step (b) occurs from 2 days to 3 days, from 2 days to 4 days, from 2 days to 5 days, from 2 days to 6 days, from 2 days to 7 days, from 1 week to 2 weeks, from 1 week to 3 weeks, or from 1 week to 4 weeks after the administering of step (a).

A reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, combined with an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, is indicative that a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 responds to a CDK2 inhibitor. For example, in a subject having an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, a biological sample, obtained from the subject after treatment with a CDK2 inhibitor, having low (e.g., reduced as compared to a control) or undetectable levels of Rb phosphorylation at serine corresponding to amino acid position 780 of SEQ ID NO:3 is indicative that the subject responds to the CDK2 inhibitor.

A biological sample, obtained from a subject after administration of a CDK2 inhibitor to the subject, having a reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, combined with: (i) an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, and (ii) presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or presence of a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1), is indicative that a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2 responds to a CDK2 inhibitor. For example, in a human subject having (i) an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1,

and (ii) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or the presence of a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1), a biological sample, obtained from the human subject after administration of a CDK2 inhibitor to the subject, having low (e.g., reduced as compared to a control) or undetectable levels of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is indicative that the human subject responds to the CDK2 inhibitor

In some embodiments, the CCNE1 gene is amplified to a gene copy number from 3 to 25. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 3. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 5. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 7. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 10. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 12. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 14. In specific embodiments, the CCNE1 gene is amplified to a gene copy number of at least 21.

In specific embodiments, the expression level of CCNE1 is the level of CCNE1 mRNA. In specific embodiments, the expression level of CCNE1 is the level of CCNE1 protein.

In specific embodiments, the CDKN2A gene encodes a protein comprising the amino acid sequence of SEQ ID NO:1.

In specific embodiments, the one or more inactivating nucleic acid substitutions and/or deletions in the CDKN2A gene is as described in Table 1. In specific embodiments, the one or more inactivating nucleic acid substitutions and/or deletions in the CDKN2A gene is as described in Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574, 1999; Liggett and Sidransky, Biology of Neoplasia, Journal of Oncology, 16(3):1197-1206, 1998, and Cairns et al., Nature Genetics, 11:210-212, 1995, each of which is incorporated by reference herein in its entirety.

Controls

As described above, the methods of the present invention can involve, measuring one or more markers (e.g., a biomarker or a pharmacodynamics marker, e.g., the amplification of the CCNE1 gene, the expression level of CCNE1, the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, the presence of a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1), and Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3) in a biological sample from a human subject having, suspected of having or at risk of developing a disease or disorder associated with CDK2. In specific embodiments, the human subject has a disease or disorder associated with CDK2. In specific embodiments, the human subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In certain aspects, the level (e.g., amplification (e.g., for the CCNE1 gene), expression level (e.g., for CCNE1 or p16 protein), or phosphorylation level (e.g., for Rb)) of one or more biomarkers, compared to a control level of the one or more biomarkers, predicts/indicates the response of a human subject to treatment comprising a CDK2 inhibitor. In certain embodiments, when (i) the CCNE1 gene is amplified and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, and (ii) a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present, a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is present, and/or a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1) is present, the human subject is identified as likely to respond to a CDK2 inhibitor. In other embodiments, when (i) the CCNE1 gene is amplified and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1, and (ii) in a biological sample from the human subject after the human subject has been administered a CDK2 inhibitor, the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is less than the control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, the human subject is identified as responding to a CDK2 inhibitor. In yet another embodiment, when (i) the CCNE1 gene is amplified and/or an expression level of CCNE1 that is higher than a control

expression level of CCNE1, (ii) a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present, a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is present, and/or a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1) is present, and (iii) in a biological sample from the human subject after the human subject has been administered a CDK2 inhibitor, the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is less than the control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, the human subject is identified as responding to a CDK2 inhibitor. In this context, the term "control" includes a sample (from the same tissue type) obtained from a human subject who is known to not respond to a CDK2 inhibitor. The term "control" also includes a sample (from the same tissue type) obtained in the past from a human subject who is known to not respond to a CDK2 inhibitor and used as a reference for future comparisons to test samples taken from human subjects for which therapeutic responsiveness is to be predicted. The "control" level (e.g., gene copy number, expression level, or phosphorylation level) for a particular biomarker (e.g., CCNE1, p16, or Rb phosphorylation) in a particular cell type or tissue may be pre-established by an analysis of biomarker level (e.g., expression level or phosphorylation level) in one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40 or more) human subjects that have not responded to treatment with a CDK2 inhibitor. This pre-established reference value (which may be an average or median level (e.g., gene copy number, expression level, or phosphorylation level) taken from multiple human subjects that have not responded to the therapy) may then be used for the "control" level of the biomarker (e.g., CCNE1, p16, or Rb phosphorylation) in the comparison with the test sample. In such a comparison, the human subject is predicted to respond to a CDK2 inhibitor if the CCNE1 gene is amplified and/or the expression level of CCNE is higher than the pre-established reference, and a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present, a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is present, and/or a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1) is present. In another such a comparison, the human subject is predicted to respond to a CDK2 inhibitor if (i) CCNE1 gene is amplified and/or the expression level of CCNE is higher than the pre-established reference, and (ii) after administering to the human

subject a CDK2 inhibitor, the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is lower than the pre-established reference. In yet another such a comparison, the human subject is indicated to respond to a CDK2 inhibitor if (i) CCNE1 gene is amplified and/or the expression level of CCNE is higher than the pre-established reference, (ii) a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present, a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is present, and/or a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1) is present, and (iii) after administering to the human subject a CDK2 inhibitor, the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 is lower than the pre-established reference.

The "control" level for a particular biomarker in a particular cell type or tissue may alternatively be pre-established by an analysis of biomarker level in one or more human subjects that have responded to treatment with a CDK2 inhibitor. This pre-established reference value (which may be an average or median level (e.g., expression level or phosphorylation level) taken from multiple human subjects that have responded to the therapy) may then be used as the "control" level (e.g., expression level or phosphorylation level) in the comparison with the test sample. In such a comparison, the human subject is indicated to respond to a CDK2 inhibitor if the level (e.g., copy number of the CCNE1 gene, expression level of CCNE1, expression level of p16, or phosphorylation level of Rb at the serine corresponding to amino acid position 780 of SEQ ID NO:3) of the biomarker being analyzed is equal or comparable to (e.g., at least 85% but less than 115% of), the pre-established reference.

In certain embodiments, the "control" is a pre-established cut-off value. A cut-off value is typically a level (e.g., a copy number, an expression level, or a phosphorylation level) of a biomarker above or below which is considered predictive of responsiveness of a human subject to a therapy of interest. Thus, in accordance with the methods and compositions described herein, a reference level (e.g., of CCNE1 gene copy number, CCNE1 expression, p16 expression, or Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3) is identified as a cut-off value, above or below of which is predictive of responsiveness to a CDK2 inhibitor. Cut-off values determined for use in the methods described herein can be compared with, e.g., published ranges of

concentrations but can be individualized to the methodology used and patient population.

In some embodiments, the expression level of CCNE1 is increased as compared to the expression level of CCNE1 in a control. For example, the expression level of CCNE1 analyzed can be at least 1.5, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 25, at least 50, at least 75, or at least 100 times higher, or at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 300%, at least 400%, at least 500%, at least 600%, at least 700%, at least 800%, at least 900%, at least 1,000%, at least 1,500%, at least 2,000%, at least 2,500%, at least 3,000%, at least 3,500%, at least 4,000%, at least 4,500%, or at least 5,000% higher, than the expression level of CCNE1 in a control.

A p16 protein is present if the protein is detectable by any assay known in the art or described herein, such as, for example, western blot, immunohistochemistry, fluorescence-activated cell sorting, and enzyme-linked immunoassay. In some embodiments, a p16 protein is present at an expression level that is within at least 5%, at least 10%, at least 20%, or at least 30% of the p16 expression level in a healthy control.

In some embodiments, the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 being analyzed is reduced as compared to the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 in a control. For example, the level of the Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 being analyzed can be at least 1.5, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 25, at least 50, at least 75, or at least100 times lower, or at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% lower, than the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 in a control.

Biological Samples

Suitable biological samples for the methods described herein include any sample that contains blood or tumor cells obtained or derived from the human subject in need of treatment. For example, a biological sample can contain tumor cells from

biopsy from a patient suffering from a solid tumor. A tumor biopsy can be obtained by a variety of means known in the art. Alternatively, a blood sample can be obtained from a patients suffering from a hematological cancer.

A biological sample can be obtained from a human subject having, suspected of having, or at risk of developing, a disease or disorder associated with CDK2. In some embodiments, the disease or disorder associated with CDK2 is a cancer. In some embodiments, the disease or disorder associated with CDK2 is N-myc amplified neuroblastoma cells, K-Ras mutant lung cancers, and cancers with FBW7 mutation and CCNE1 overexpression. In some embodiments, the disease or disorder associated with CDK2 is lung squamous cell carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, bladder urothelial carcinoma, mesothelioma, or sarcoma. In some embodiments, the disease or disorder associated with CDK2 is lung adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, or stomach adenocarcinoma. In some embodiments, the disease or disorder associated with CDK2 is an adenocarcinoma, carcinoma, or cystadenocarcinoma. In some embodiments, the disease or disorder associated with CDK2 is uterine cancer, ovarian cancer, stomach cancer, esophageal cancer, lung cancer, bladder cancer, pancreatic cancer, or breast cancer.

In some embodiments, the breast cancer is chemotherapy or radiotherapy resistant breast cancer, endocrine resistant breast cancer, trastuzumab resistant breast cancer, or breast cancer demonstrating primary or acquired resistance to CDK4/6 inhibition. In some embodiments, the breast cancer is advanced or metastatic breast cancer.

Methods for obtaining and/or storing samples that preserve the activity or integrity of molecules (e.g., nucleic acids or proteins) in the sample are well known to those skilled in the art. For example, a biological sample can be further contacted with one or more additional agents such as buffers and/or inhibitors, including one or more of nuclease, protease, and phosphatase inhibitors, which preserve or minimize changes in the molecules in the sample.

Evaluating Biomarkers and Pharmacodynamic Markers

Expression levels of CCNE1 or p16 can be detected as, e.g., RNA expression of a target gene (i.e., the genes encoding CCNE1 or p16). That is, the expression

level (amount) of CCNE1 or p16 can be determined by detecting and/or measuring the level of mRNA expression of the gene encoding CCNE1. Alternatively, expression levels of CCNE1 or p16 can be detected as, e.g., protein expression of target gene (i.e., the genes encoding CCNE1 or p16). That is, the expression level (amount) of CCNE1 or p16 can be determined by detecting and/or measuring the level of protein expression of the genes encoding CCNE1 or p16.

In some embodiments, the expression level of CCNE1 or p16 is determined by measuring RNA levels. A variety of suitable methods can be employed to detect and/or measure the level of mRNA expression of a gene. For example, mRNA expression can be determined using Northern blot or dot blot analysis, reverse transcriptase-PCR (RT-PCR; e.g., quantitative RT-PCR), in situ hybridization (e.g., quantitative in situ hybridization), nucleic acid array (e.g., oligonucleotide arrays or gene chips) and RNA sequencing analysis. Details of such methods are described below and in, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual Second Edition vol. 1, 2 and 3. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, USA, Nov. 1989; Gibson et al. (1999) Genome Res., 6(10):995-1001; and Zhang et al. (2005) Environ. Sci. Technol., 39(8):2777-2785; U.S. Publication No. 2004086915; European Patent No. 0543942; and U.S. Patent No. 7,101,663; Kukurba et al. (2015) Cold Spring Harbor Protocols., 2015 (11): 951–69; the disclosures of each of which are incorporated herein by reference in their entirety.

In one example, the presence or amount of one or more discrete mRNA populations in a biological sample can be determined by isolating total mRNA from the biological sample (see, e.g., Sambrook et al. (supra) and U.S. Patent No. 6,812,341) and subjecting the isolated mRNA to agarose gel electrophoresis to separate the mRNA by size. The size-separated mRNAs are then transferred (e.g., by diffusion) to a solid support such as a nitrocellulose membrane. The presence or amount of one or more mRNA populations in the biological sample can then be determined using one or more detectably-labeled-polynucleotide probes, complementary to the mRNA sequence of interest, which bind to and thus render detectable their corresponding mRNA populations. Detectable-labels include, e.g., fluorescent (e.g., umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, allophycocyanin, or phycoerythrin), luminescent (e.g., europium, terbium, Qdot™ nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), radiological (e.g., 125I, 131I, 35S,

32P, 33P, or 3H), and enzymatic (horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase) labels.

In some embodiments, the expression level of CCNE1 or p16 is determined by measuring protein levels. A variety of suitable methods can be employed to detect and/or measure the level of protein expression of target genes. For example, CCNE1 or p16 protein expression can be determined using western blot, enzyme-linked immunosorbent assay ("ELISA"), fluorescence activated cell sorting, or immunohistochemistry analysis (e.g., using a CCNE1-specific or p16-specific antibody, respectively). Details of such methods are described below and in, e.g., Sambrook et al., supra.

In one example, the presence or amount of one or more discrete protein populations (e.g., CCNE1 or p16) in a biological sample can be determined by western blot analysis, e.g., by isolating total protein from the biological sample (see, e.g., Sambrook et al. (supra)) and subjecting the isolated protein to agarose gel electrophoresis to separate the protein by size. The size-separated proteins are then transferred (e.g., by diffusion) to a solid support such as a nitrocellulose membrane. The presence or amount of one or more protein populations in the biological sample can then be determined using one or more antibody probes, e.g., a first antibody specific for the protein of interest (e.g., CCNE1 or p16), and a second antibody, detectably labeled, specific for the first antibody, which binds to and thus renders detectable the corresponding protein population. Detectable-labels suitable for use in western blot analysis are known in the art.

Methods for detecting or measuring gene expression (e.g., mRNA or protein expression) can optionally be performed in formats that allow for rapid preparation, processing, and analysis of multiple samples. This can be, for example, in multi-welled assay plates (e.g., 96 wells or 386 wells) or arrays (e.g., nucleic acid chips or protein chips). Stock solutions for various reagents can be provided manually or robotically, and subsequent sample preparation (e.g., RT-PCR, labeling, or cell fixation), pipetting, diluting, mixing, distribution, washing, incubating (e.g., hybridization), sample readout, data collection (optical data) and/or analysis (computer aided image analysis) can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting the signal generated from the assay. Examples of such detectors include, but are not limited to, spectrophotometers, luminometers, fluorimeters, and devices

that measure radioisotope decay. Exemplary high-throughput cell-based assays (e.g., detecting the presence or level of a target protein in a cell) can utilize ArrayScan® VTI HCS Reader or KineticScan® HCS Reader technology (Cellomics Inc., Pittsburg, PA).

In some embodiments, the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 and/or the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is determined by evaluating the DNA sequence of the CDKN2A gene (e.g., genomic DNA or cDNA) or by evaluating the RNA sequence of the CDKN2A gene (e.g., RNA, e.g., mRNA). Methods of performing nucleic acid sequencing analyses are known in the art and described above. Nonlimiting examples of inactivating nucleic acid substitutions and/or deletions preventing the CDKN2A gene from encoding a protein comprising the amino acid sequence of SEQ ID NO:1 are described in Table 1, above. In specific embodiments, the one or more inactivating nucleic acid substitutions and/or deletions in the CDKN2A gene is as described in Yarbrough et al., Journal of the National Cancer Institute, 91(18):1569-1574, 1999; Liggett and Sidransky, Biology of Neoplasia, Journal of Oncology, 16(3):1197-1206, 1998, and Cairns et al., Nature Genetics, 11:210-212, 1995, each of which is incorporated by reference herein in its entirety.

In some embodiments, the expression level of a gene or the presence of a gene lacking one or more inactivating nucleic acid substitutions or deletions is determined by evaluating the copy number variation (CNV) of the gene. The CNV of genes (e.g., the CCNE1 gene and/or the CDKN2A gene) can be determined/identified by a variety of suitable methods. For example, CNV can be determined using fluorescent in situ hybridization (FISH), multiplex ligation dependent probe amplification (MLPA), array comparative genomic hybridization (aCGH), single-nucleotide polymorphisms (SNP) array, and next-generation sequencing (NGS) technologies.

In one example, the copy number variation of one or more discrete genes in a biological sample can be determined by MLPA, e.g., by extracting DNA specimens from the biological sample (see, e.g., Sambrook et al. (supra) and U.S. Patent No. 6,812,341), and amplifying DNA sequence of interest (e.g., CCNE1 or CDKN2A) using a mixture of MLPA probes. Each MLPA probe consists of two oligonucleotides that hybridize to immediately adjacent target DNA sequence (e.g., CCNE1 or CDKN2A) in order to be ligated into a single probe. Ligated probes are amplified

though PCR with one PCR primer fluorescently labeled, enabling the amplification products to be visualized during fragment separation by capillary electrophoresis. The presence, absence or amplification of one or more genes of interest in the biological sample is calculated by measuring PCR derived fluorescence, quantifying the amount of PCR product after normalization and comparing it with control DNA samples.

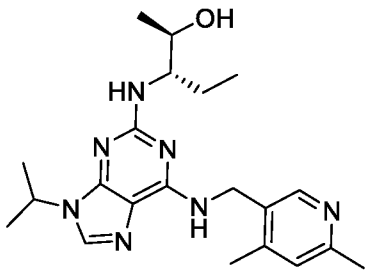
The level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 can be detected by a variety of suitable methods. For example, phosphorylation status can be determined using western blot, ELISA, fluorescence activated cell sorting, or immunohistochemistry analysis. Details of such methods are described below and in, e.g., Sambrook et al., supra.

As with the methods for detecting or measuring gene expression (above), methods for detecting or measuring the level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3 can optionally be performed in formats that allow for rapid preparation, processing, and analysis of multiple samples.

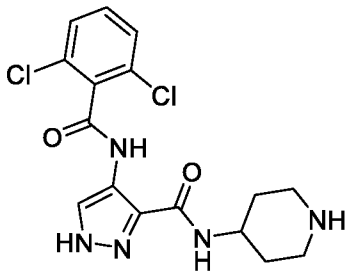
CDK2 Inhibitors

The compounds useful in the methods of the disclosure are CDK2 inhibitors. In some embodiments, the CDK2 inhibitor inhibits CDK2, CDK4, and CDK6. In some embodiments, the CDK2 inhibitor selectively inhibits CDK2 over CDK1 and CDK9. In some embodiments, the CDK2 inhibitor selectively inhibits CDK2 over CDK4 and CDK6. In some embodiments, the CDK2 inhibitor selectively inhibits CDK2 over CDK1, CDK9, CDK4, and CDK6. In some embodiments, the compounds are about 2-fold, 3-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold more selective for CDK2 over CDK1 and CDK9 as calculated by measuring IC_{50} according to the method in Examples A, B, and C. In some embodiments, the compounds are about 2-fold, 3-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold more selective for CDK2 over CDK1, CDK9, CDK4, or CDK6 as calculated by measuring by measuring IC_{50} according to the method in Examples A, B, C, D, and E. In some embodiments, the compounds are about 2-fold, 3-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold more selective for CDK2 over CDK4 and CDK6 as calculated by measuring IC_{50} according to the method in Examples A, D, and E.

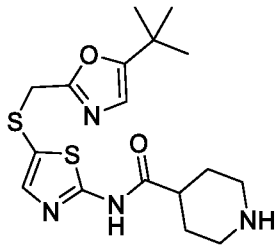
In some embodiments, the CDK2 inhibitor is dinaciclib (Merck), alvociclib (Tolero Pharmaceuticals), seliciclib (Cyclacel Pharmaceuticals), roniciclib (Bayer), milciclib (Nerviano), abemaciclib (Eli Lilly), trilaciclib (G1 Therapeutics), CYC065 (Cyclacel Pharmaceuticals), AT-7519 (Astex Therapeutics; *J Med. Chem.*, 2008, 51, 4986), BMS-387032/SNS032 (Sunesis; *J Med. Chem.*, 2004, 47, 1719), TG02 (Trajara Pharmaceuticals), R547 (Roche; *Mol. Can. Ther.* 2006, 2644), AZD5438 (AstraZeneca, *Mol. Can. Ther.* 2009, 1856), RGB-286638 (Agennix; *Leukemia*, 2013, 2366), AMG295 (Amgen; WO 2009/085185), PHA-793887 (Nerviano, BMC, 2010 18, 1844), ZK-304709 (*Biomed. Pharmacother.* 2006, 269), and AG-024322 (Pfizer; *Cancer Res.* 2005, 1045), or a pharmaceutically acceptable salt of any one of the foregoing. Provided below are the chemical structures of CYC065, AT7519, BMS-387032/SN032, TG02, R547, AZD5438, RGB-286638, AMG925, PHA-793887, ZK-304709, and AG-24322:



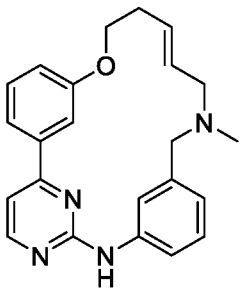
CYC065



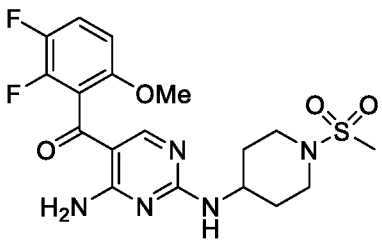
AT7519



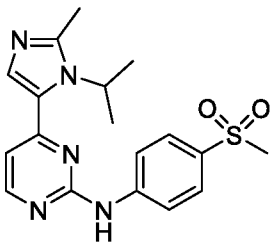
BMS-387032/SNS032



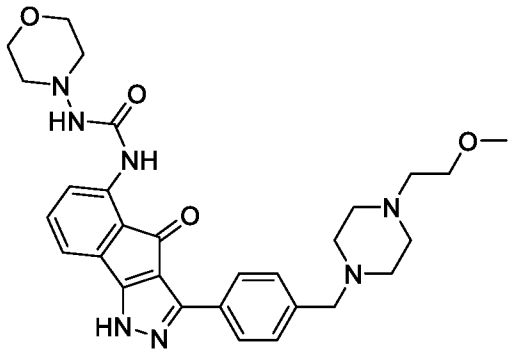
TG02



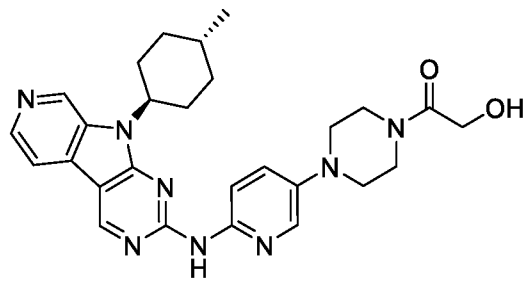
R547



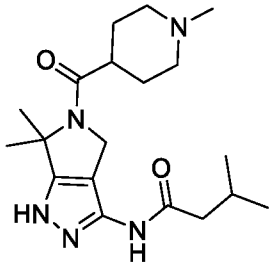
AZD5438



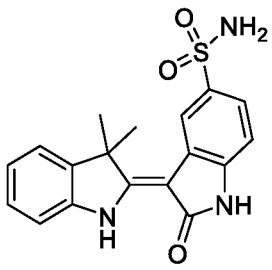
RGB-286638



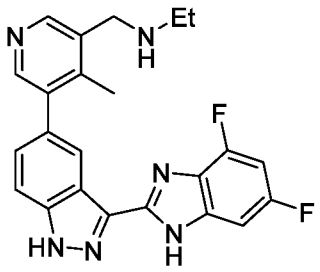
AMG925



PHA-793887

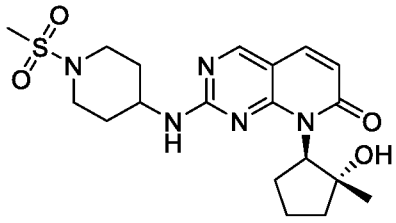


ZK-304709



AG-24322

In some embodiments, the CDK2 inhibitor is Compound A (8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one) having the structure below, or a pharmaceutically acceptable salt thereof:



Compound A

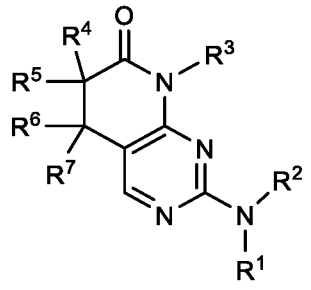
(8-[(1R,2R)-2-hydroxy-2-methylcyclo-pentyl]-2-{[1-(methylsulfonyl)piperidin-4-yl]amino}pyrido[2,3-d]pyrimidin-7(8H)-one; see US Patent Application Publication No. 2018/0044344 at page 51, paragraph [0987], which is incorporated by reference herein in its entirety).

In some embodiments, the compound is a compound in any of the embodiments or example compounds, or a pharmaceutically acceptable salt thereof, in US Patent Application Publication No. 2018/0044344, which is incorporated herein by reference in its entirety.

In some embodiments, the compound is a compound in any of the embodiments or example compounds, or a pharmaceutically acceptable salt thereof, in US Patent Appl. No. 16/598,777, filed Oct.10, 2019; or in US Provisional Appl. No.

62/806,269, filed Feb. 15, 2019, each of which is incorporated herein by reference in its entirety.

In a specific embodiment, the CDK2 inhibitor is a compound of Formula (A-I):



(A-I)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl;

R^2 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, 5-10 membered heteroaryl-C_{1-4} alkyl, $C(=O)R^b$, $C(=O)NR^cR^d$, $C(=O)OR^a$, $C(=NR^e)R^b$, $C(=NR^e)NR^cR^d$, $S(=O)R^b$, $S(=O)NR^cR^d$, $NR^cS(=O)_2R^b$, $NR^cS(=O)_2NR^cR^d$, $S(=O)_2R^b$, and $S(=O)_2NR^cR^d$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents;

each R^a, R^c, and R^d is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents;

each R^b is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10

membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which are optionally substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents;

each R^e is independently selected from H, CN, OH, C_{1-4} alkyl, and C_{1-4} alkoxy;

each R^f is independently selected from H, C_{1-4} alkyl, and C_{1-4} haloalkyl;

R^3 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{3A} substituents;

R^4, R^5, R^6, and R^7 have the definitions in Group (a) or (b):

Group (a):

R^4 and R^5 are independently selected from halo, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, alternatively, R^4 and R^5, together with the carbon atom to which they are attached, form a 3, 4, 5, 6, or 7 membered cycloalkyl ring or a 3, 4, 5, 6, or 7 membered heterocycloalkyl ring, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

R^6 and R^7 are independently selected from H, D, halo, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, alternatively, R^6 and R^7, together with the carbon atom to which they are attached, form a 3, 4, 5, 6, or 7 membered cycloalkyl ring or a 3, 4, 5, 6, or 7 membered heterocycloalkyl ring, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

Group (b):

R^4 and R^5 are independently selected from H, halo, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, alternatively, R^4 and R^5, together with the carbon atom to which they are attached, form a 3, 4, 5, 6, or 7 membered cycloalkyl ring or a 3, 4, 5, 6, or 7 membered heterocycloalkyl ring, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

R^6 and R^7 are independently selected from halo, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, and C_{3-6} cycloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, alternatively, R^6 and R^7, together with the carbon atom to which they are attached, form a 3, 4, 5, 6, or 7 membered cycloalkyl ring or a 3, 4, 5, 6, or 7 membered heterocycloalkyl ring, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{2A} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, 5-10 membered heteroaryl-C_{1-4} alkyl, OR^{a1}, SR^{a1}, $C(=O)R^{b1}$, $C(=O)NR^{c1}R^{d1}$, $C(=O)OR^{a1}$, $OC(=O)R^{b1}$, $OC(=O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(=O)R^{b1}$, $NR^{c1}C(=O)OR^{b1}$, $NR^{c1}C(=O)NR^{c1}R^{d1}$, $C(=NR^e)R^{b1}$, $C(=NR^e)NR^{c1}R^{d1}$, $NR^{c1}C(=NR^e)NR^{c1}R^{d1}$, $NHOR^{a1}$, $NR^{c1}S(=O)R^{b1}$, $NR^{c1}S(=O)NR^{c1}R^{d1}$, $S(=O)R^{b1}$, $S(=O)NR^{c1}R^{d1}$, $NR^{c1}S(=O)_2R^{b1}$, $NR^{c1}S(=O)_2NR^{c1}R^{d1}$, $S(=O)_2R^{b1}$, $S(=O)(=NR^f)R^{b1}$, and $S(=O)_2NR^{c1}R^{d1}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{2B} substituents;

each R^{a1}, R^{c1}, and R^{d1} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4}

alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{2B} substituents;

each R^{b1} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which are optionally substituted with 1, 2, 3, or 4 independently selected R^{2B} substituents;

each R^{3A} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, 5-10 membered heteroaryl-C_{1-4} alkyl, OR^{a2}, SR^{a2}, $C(=O)R^{b2}$, $C(=O)NR^{c2}R^{d2}$, $C(=O)OR^{a2}$, $OC(=O)R^{b2}$, $OC(=O)NR^{c2}R^{d2}$, $NR^{c2}R^{d2}$, $NR^{c2}C(=O)R^{b2}$, $NR^{c2}C(=O)OR^{b2}$, $NR^{c2}C(=O)NR^{c2}R^{d2}$, $C(=NR^{e})R^{b2}$, $C(=NR^{e})NR^{c2}R^{d2}$, $NR^{c2}C(=NR^{e})NR^{c2}R^{d2}$, $NHOR^{a2}$, $NR^{c2}S(=O)R^{b2}$, $NR^{c2}S(=O)NR^{c2}R^{d2}$, $S(=O)R^{b2}$, $S(=O)NR^{c2}R^{d2}$, $NR^{c2}S(=O)_2R^{b2}$, $NR^{c2}S(=O)_2NR^{c2}R^{d2}$, $S(=O)_2R^{b2}$, $S(=O)(=NR^{f})R^{b2}$, and $S(=O)_2NR^{c2}R^{d2}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{3B} substituents;

each R^{a2}, R^{c2}, and R^{d2} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{3B} substituents;

each R^{b2} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, C_{6-10} aryl-C_{1-4} alkyl, 4-10 membered

heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which are optionally substituted with 1, 2, 3, or 4 independently selected R^{3B} substituents;

each R^{2B} and R^{3B} is independently selected from H, D, halo, CN, NO_2, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl, OR^{a23}, SR^{a23}, $C(=O)R^{b23}$, $C(=O)NR^{c23}R^{d23}$, $C(=O)OR^{a23}$, $OC(=O)R^{b23}$, $OC(=O)NR^{c23}R^{d23}$, $NR^{c23}R^{d23}$, $NR^{c23}C(=O)R^{b23}$, $NR^{c23}C(=O)OR^{b23}$, $NR^{c23}C(=O)NR^{c23}R^{d23}$, $C(=NR^{e})R^{b23}$, $C(=NR^{e})NR^{c23}R^{d23}$, $NR^{c23}C(=NR^{e})NR^{c23}R^{d23}$, $NHOR^{a23}$, $NR^{c23}S(=O)R^{b23}$, $NR^{c23}S(=O)NR^{c23}R^{d23}$, $S(=O)R^{b23}$, $S(=O)NR^{c23}R^{d23}$, $NR^{c23}S(=O)_2R^{b23}$, $NR^{c23}S(=O)_2NR^{c23}R^{d23}$, $S(=O)_2R^{b23}$, $S(=O)(=NR^{f})R^{b23}$, and $S(=O)_2NR^{c23}R^{d23}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{G} substituents;

each R^{a23}, R^{c23}, and R^{d23} is independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{G} substituents;

each R^{b23} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which are optionally substituted with 1, 2, 3, or 4 independently selected R^{G} substituents; and

each R^{G} is independently selected from OH, NO_2, CN, halo, C_{1-3} alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{1-3} haloalkyl, cyano-C_{1-3} alkyl, HO-C_{1-3} alkyl, C_{1-3} alkoxy-C_{1-3} alkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy, amino, C_{1-3} alkylamino, di(C_{1-3} alkyl)amino, thio, C_{1-3} alkylthio, C_{1-3} alkylsulfinyl, C_{1-3} alkylsulfonyl, carbamyl, C_{1-3} alkylcarbamyl,

di(C_{1-3} alkyl)carbamyl, carboxy, C_{1-3} alkylcarbonyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkylcarbonyloxy, C_{1-3} alkylcarbonylamino, C_{1-3} alkoxycarbonylamino, C_{1-3} alkylaminocarbonyloxy, C_{1-3} alkylsulfonylamino, aminosulfonyl, C_{1-3} alkylaminosulfonyl, di(C_{1-3} alkyl)aminosulfonyl, aminosulfonylamino, C_{1-3} alkylaminosulfonylamino, di(C_{1-3} alkyl)aminosulfonylamino, aminocarbonylamino, C_{1-3} alkylaminocarbonylamino, and di(C_{1-3} alkyl)aminocarbonylamino.

In some embodiments, R^1 is H.

In some embodiments, R^2 is selected from C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents.

In some embodiments, R^2 is selected from 4-7 membered heterocycloalkyl and phenyl, each of which is substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents.

In some embodiments, R^2 is selected from piperidin-4-yl and phenyl, each of which is optionally substituted with 1 R^{2A} substituent.

In some embodiments, at least one R^{2A} is selected from $S(=O)_2R^{b1}$ and $S(=O)_2NR^{c1}R^{d1}$, wherein R^{b1} is C_{1-3} alkyl; and R^{c1} and R^{d1} are each independently selected from H and C_{1-3} alkyl.

In some embodiments, each R^{2A} is independently selected from $S(=O)_2CH_3$ and $S(=O)_2NH_2$.

In some embodiments, R^2 is piperidin-4-yl, substituted with $S(=O)_2R^{b1}$; or R^2 is phenyl substituted with $S(=O)_2NR^{c1}R^{d1}$.

In some embodiments, R^2 is piperidin-4-yl, substituted with $S(=O)_2CH_3$; or R^2 is phenyl substituted with $S(=O)_2NH_2$.

In some embodiments, R^3 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{3A} substituents.

In some embodiments, R^3 is selected from C_{1-6} alkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1 or 2 independently selected R^{3A} substituents.

In some embodiments, R^3 optionally substituted with 1, 2, 3, or 4 independently selected R^{3A} substituents is selected from 1,1-difluorobutan-2-yl, cyclopentyl, phenyl, tetrahydrofuran-3-yl, and (1-methyl-1*H*-pyrazol-5-yl)methyl.

In some embodiments, each R^{3A} is independently selected from H, halo, C_{1-6} alkyl, and C_{1-6} haloalkyl.

In some embodiments, R^4 and R^5 are each independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl; or, alternatively, R^4 and R^5, together with the carbon atom to which they are attached, form a 3, 4, 5, or 6 membered cycloalkyl ring.

In some embodiments, R^4 and R^5, together with the carbon atom to which they are attached, form a 3, 4, 5, 6, or 7 membered cycloalkyl ring.

In some embodiments, R^4 and R^5, together with the carbon atom to which they are attached, form a cyclopropyl ring.

In some embodiments, R^4 and R^5 are independently C_{1-3} alkyl or C_{1-3} haloalkyl.

In some embodiments, R^4 and R^5 are independently C_{1-3} alkyl.

In some embodiments, R^4 and R^5 are independently methyl.

In some embodiments, R^4 and R^5, together with the carbon atom to which they are attached, form a cyclopropyl ring; or R^4 and R^5 are independently C_{1-3} alkyl.

In some embodiments, R^6 and R^7 are each independently selected from H, C_{1-6} alkyl and C_{1-6} haloalkyl.

In some embodiments, R^6 and R^7 are each H.

In some embodiments:

R^1 is H;

R^2 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{2A} substituents;

R^3 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{3A} substituents;

R^4 and R^5 are each independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

or, alternatively, R^4 and R^5, together with the carbon atom to which they are attached form a 3, 4, 5, or 6 membered cycloalkyl ring;

R^6 and R^7 are each independently selected from H and C_{1-6} alkyl;

each R^{2A} is independently selected from halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OR^{a1}, SR^{a1}, $C(=O)R^{b1}$, $C(=O)NR^{c1}R^{d1}$, $C(=O)OR^{a1}$, $OC(=O)R^{b1}$, $OC(=O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(=O)R^{b1}$, $NR^{c1}C(=O)OR^{b1}$, $NR^{c1}C(=O)NR^{c1}R^{d1}$, $NHOR^{a1}$, $NR^{c1}S(=O)_2R^{b1}$, $NR^{c1}S(=O)_2NR^{c1}R^{d1}$, $S(=O)_2R^{b1}$, and $S(=O)_2NR^{c1}R^{d1}$;

each R^{a1}, R^{c1}, and R^{d1} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl;

each R^{b1} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{3A} is independently selected from halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OR^{a2}, SR^{a2}, $C(=O)R^{b2}$, $C(=O)NR^{c2}R^{d2}$, $C(=O)OR^{a2}$, $OC(=O)R^{b2}$, $OC(=O)NR^{c2}R^{d2}$, $NR^{c2}R^{d2}$, $NR^{c2}C(=O)R^{b2}$, $NR^{c2}C(=O)OR^{b2}$, $NR^{c2}C(=O)NR^{c2}R^{d2}$, $NHOR^{a2}$, $NR^{c2}S(=O)_2R^{b2}$, $NR^{c2}S(=O)_2NR^{c2}R^{d2}$, $S(=O)_2R^{b2}$, and $S(=O)_2NR^{c2}R^{d2}$;

each R^{a2}, R^{c2}, and R^{d2} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl; and

each R^{b2} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl.

In some embodiments:

R^1 is H;

R^2 is selected from 4-7 membered heterocycloalkyl and phenyl, each of which are substituted by 1 R^{2A} group;

R^{2A} is $S(=O)_2R^{b1}$ or $S(=O)_2NR^{c1}R^{d1}$;

R^{b1} is C_{1-3} alkyl;

R^{c1} and R^{d1} are each independently selected from H and C_{1-3} alkyl;

R^3 is selected from C_{1-6} alkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1, 2, 3, or 4 independently selected R^{3A} substituents;

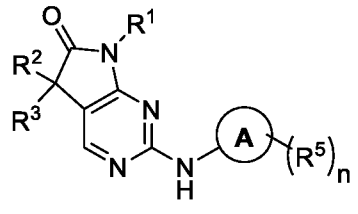
each R^{3A} is independently selected from H, halo, C_{1-6} alkyl, and C_{1-6} haloalkyl;

R^4 and R^5 are each methyl;

or R^4 and R^5, together with the carbon atom to which they are attached form, form a cyclopropyl ring; and

R^6 and R^7 are each H.

In a specific embodiment, the CDK2 inhibitor is a compound of Formula (B-I):



(B-I)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer selected from 0, 1, 2, 3, 4, 5, and 6;

Ring moiety **A** is a 3-14 membered cycloalkyl or 4-14 membered heterocycloalkyl, wherein Ring moiety **A** is attached to the NH group of Formula (I) at a saturated or partially saturated ring of said 3-14 membered cycloalkyl or 4-14 membered heterocycloalkyl;

R^1 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-14} cycloalkyl, 6-14 membered aryl, 4-14 membered heterocycloalkyl, 5-14 membered heteroaryl, C_{3-14} cycloalkyl-C_{1-4} alkyl, 6-14 membered aryl-C_{1-4} alkyl, 4-14 membered heterocycloalkyl-C_{1-4} alkyl, and 5-14 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-14} cycloalkyl, 6-14 membered aryl, 4-14 membered heterocycloalkyl, 5-14 membered heteroaryl, C_{3-14} cycloalkyl-C_{1-4} alkyl, 6-14 membered aryl-C_{1-4} alkyl, 4-14 membered heterocycloalkyl-C_{1-4} alkyl, and 5-14 membered heteroaryl-C_{1-4} alkyl are each optionally substituted by 1, 2, 3, 4, 5, or 6 independently selected R^4 substituents;

R^2 and R^3 are each independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl are each optionally substituted by 1, 2, 3, or 4 independently selected R^G substituents;

or R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-7 membered cycloalkyl ring or a 4-7 membered heterocycloalkyl ring, each of which is optionally substituted by 1, 2, 3, or 4 independently selected R^G substituents;

each R^4 is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, 5-10 membered heteroaryl-C_{1-4} alkyl, OR^{a4}, SR^{a4}, $NHOR^{a4}$, $C(O)R^{b4}$, $C(O)NR^{c4}R^{d4}$, $C(O)NR^{c4}(OR^{a4})$, $C(O)OR^{a4}$, $OC(O)R^{b4}$, $OC(O)NR^{c4}R^{d4}$, $NR^{c4}R^{d4}$, $NR^{c4}NR^{c4}R^{d4}$, $NR^{c4}C(O)R^{b4}$, $NR^{c4}C(O)OR^{a4}$, $NR^{c4}C(O)NR^{c4}R^{d4}$, $C(=NR^{e4})R^{b4}$, $C(=NR^{e4})NR^{c4}R^{d4}$, $NR^{c4}C(=NR^{e4})NR^{c4}R^{d4}$, $NR^{c4}C(=NR^{e4})R^{b4}$, $NR^{c4}S(O)NR^{c4}R^{d4}$, $NR^{c4}S(O)R^{b4}$, $NR^{c4}S(O)_2R^{b4}$, $NR^{c4}S(O)(=NR^{e4})R^{b4}$, $NR^{c4}S(O)_2NR^{c4}R^{d4}$, $S(O)R^{b4}$, $S(O)NR^{c4}R^{d4}$, $S(O)_2R^{b4}$, $S(O)_2NR^{c4}R^{d4}$, $OS(O)(=NR^{e4})R^{b4}$, $OS(O)_2R^{b4}$, $S(O)(=NR^{e4})R^{b4}$, SF_5, $P(O)R^{f4}R^{g4}$, $OP(O)(OR^{h4})(OR^{i4})$, $P(O)(OR^{h4})(OR^{i4})$, and $BR^{j4}R^{k4}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^5 is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, 5-10 membered heteroaryl-C_{1-4} alkyl, OR^{a5}, SR^{a5}, $NHOR^{a5}$, $C(O)R^{b5}$, $C(O)NR^{c5}R^{d5}$, $C(O)NR^{c5}(OR^{a5})$, $C(O)OR^{a5}$, $OC(O)R^{b5}$, $OC(O)NR^{c5}R^{d5}$, $NR^{c5}R^{d5}$, $NR^{c5}NR^{c5}R^{d5}$, $NR^{c5}C(O)R^{b5}$, $NR^{c5}C(O)OR^{a5}$, $NR^{c5}C(O)NR^{c5}R^{d5}$, $C(=NR^{e5})R^{b5}$, $C(=NR^{e5})NR^{c5}R^{d5}$, $NR^{c5}C(=NR^{e5})NR^{c5}R^{d5}$, $NR^{c5}C(=NR^{e5})R^{b5}$, $NR^{c5}S(O)NR^{c5}R^{d5}$, $NR^{c5}S(O)R^{b5}$, $NR^{c5}S(O)_2R^{b5}$, $NR^{c5}S(O)(=NR^{e5})R^{b5}$, $NR^{c5}S(O)_2NR^{c5}R^{d5}$, $S(O)R^{b5}$, $S(O)NR^{c5}R^{d5}$, $S(O)_2R^{b5}$, $S(O)_2NR^{c5}R^{d5}$, $OS(O)(=NR^{e5})R^{b5}$, $OS(O)_2R^{b5}$, $S(O)(=NR^{e5})R^{b5}$, SF_5, $P(O)R^{f5}R^{g5}$, $OP(O)(OR^{h5})(OR^{i5})$, $P(O)(OR^{h5})(OR^{i5})$, and $BR^{j5}R^{k5}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents;

each R^{4A} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered

heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl, OR^{a41}, SR^{a41}, $NHOR^{a41}$, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)NR^{c41}(OR^{a41})$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $C(=NR^{e41})R^{b41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})R^{b41}$, $NR^{c41}S(O)NR^{c41}R^{d41}$, $NR^{c41}S(O)R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $NR^{c41}S(O)(=NR^{e41})R^{b41}$, $NR^{c41}S(O)_2NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$, $OS(O)(=NR^{e41})R^{b41}$, $OS(O)_2R^{b41}$, $S(O)(=NR^{e41})R^{b41}$, SF_5, $P(O)R^{f41}R^{g41}$, $OP(O)(OR^{h41})(OR^{i41})$, $P(O)(OR^{h41})(OR^{i41})$, and $BR^{j41}R^{k41}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4B} substituents;

each R^{4B} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl, OR^{a42}, SR^{a42}, $NHOR^{a42}$, $C(O)R^{b42}$, $C(O)NR^{c42}R^{d42}$, $C(O)NR^{c42}(OR^{a42})$, $C(O)OR^{a42}$, $OC(O)R^{b42}$, $OC(O)NR^{c42}R^{d42}$, $NR^{c42}R^{d42}$, $NR^{c42}NR^{c42}R^{d42}$, $NR^{c42}C(O)R^{b42}$, $NR^{c42}C(O)OR^{a42}$, $NR^{c42}C(O)NR^{c42}R^{d42}$, $C(=NR^{e42})R^{b42}$, $C(=NR^{e42})NR^{c42}R^{d42}$, $NR^{c42}C(=NR^{e42})NR^{c42}R^{d42}$, $NR^{c42}C(=NR^{e42})R^{b42}$, $NR^{c42}S(O)NR^{c42}R^{d42}$, $NR^{c42}S(O)R^{b42}$, $NR^{c42}S(O)_2R^{b42}$, $NR^{c42}S(O)(=NR^{e42})R^{b42}$, $NR^{c42}S(O)_2NR^{c42}R^{d42}$, $S(O)R^{b42}$, $S(O)NR^{c42}R^{d42}$, $S(O)_2R^{b42}$, $S(O)_2NR^{c42}R^{d42}$, $OS(O)(=NR^{e42})R^{b42}$, $OS(O)_2R^{b42}$, $S(O)(=NR^{e42})R^{b42}$, SF_5, $P(O)R^{f42}R^{g42}$, $OP(O)(OR^{h42})(OR^{i42})$, $P(O)(OR^{h42})(OR^{i42})$, and $BR^{j42}R^{k42}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{G} substituents;

each R^{5A} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl,

OR^{a51}, SR^{a51}, $NHOR^{a51}$, $C(O)R^{b51}$, $C(O)NR^{c51}R^{d51}$, $C(O)NR^{c51}(OR^{a51})$, $C(O)OR^{a51}$, $OC(O)R^{b51}$, $OC(O)NR^{c51}R^{d51}$, $NR^{c51}R^{d51}$, $NR^{c51}NR^{c51}R^{d51}$, $NR^{c51}C(O)R^{b51}$, $NR^{c51}C(O)OR^{a51}$, $NR^{c51}C(O)NR^{c51}R^{d51}$, $C(=NR^{e51})R^{b51}$, $C(=NR^{e51})NR^{c51}R^{d51}$, $NR^{c51}C(=NR^{e51})NR^{c51}R^{d51}$, $NR^{c51}C(=NR^{e51})R^{b51}$, $NR^{c51}S(O)NR^{c51}R^{d51}$, $NR^{c51}S(O)R^{b51}$, $NR^{c51}S(O)_2R^{b51}$, $NR^{c51}S(O)(=NR^{e51})R^{b51}$, $NR^{c51}S(O)_2NR^{c51}R^{d51}$, $S(O)R^{b51}$, $S(O)NR^{c51}R^{d51}$, $S(O)_2R^{b51}$, $S(O)_2NR^{c51}R^{d51}$, $OS(O)(=NR^{e51})R^{b51}$, $OS(O)_2R^{b51}$, $S(O)(=NR^{e51})R^{b51}$, SF_5, $P(O)R^{f51}R^{g51}$, $OP(O)(OR^{h51})(OR^{i51})$, $P(O)(OR^{h51})(OR^{i51})$, and $BR^{j51}R^{k51}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents;

each R^{5B} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl, OR^{a52}, SR^{a52}, $NHOR^{a52}$, $C(O)R^{b52}$, $C(O)NR^{c52}R^{d52}$, $C(O)NR^{c52}(OR^{a52})$, $C(O)OR^{a52}$, $OC(O)R^{b52}$, $OC(O)NR^{c52}R^{d52}$, $NR^{c52}R^{d52}$, $NR^{c52}NR^{c52}R^{d52}$, $NR^{c52}C(O)R^{b52}$, $NR^{c52}C(O)OR^{a52}$, $NR^{c52}C(O)NR^{c52}R^{d52}$, $C(=NR^{e52})R^{b52}$, $C(=NR^{e52})NR^{c52}R^{d52}$, $NR^{c52}C(=NR^{e52})NR^{c52}R^{d52}$, $NR^{c52}C(=NR^{e52})R^{b52}$, $NR^{c52}S(O)NR^{c52}R^{d52}$, $NR^{c52}S(O)R^{b52}$, $NR^{c52}S(O)_2R^{b52}$, $NR^{c52}S(O)(=NR^{e52})R^{b52}$, $NR^{c52}S(O)_2NR^{c52}R^{d52}$, $S(O)R^{b52}$, $S(O)NR^{c52}R^{d52}$, $S(O)_2R^{b52}$, $S(O)_2NR^{c52}R^{d52}$, $OS(O)(=NR^{e52})R^{b52}$, $OS(O)_2R^{b52}$, $S(O)(=NR^{e52})R^{b52}$, SF_5, $P(O)R^{f52}R^{g52}$, $OP(O)(OR^{h52})(OR^{i52})$, $P(O)(OR^{h52})(OR^{i52})$, and $BR^{j52}R^{k52}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-

10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

or, any R^{c4} and R^{d4} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-10 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-10 membered heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{b4} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{e4} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{f4} and R^{g4} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{h4} and R^{i4} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{j4} and R^{k4} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j4} and R^{k4} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally

substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{a41}, R^{c41}, and R^{d41} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4B} substituents;

or, any R^{c41} and R^{d41} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-7 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-7 membered heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4B} substituents;

each R^{b41} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4B} substituents;

each R^{e41} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{f41} and R^{g41} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{h41} and R^{i41} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-

6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{j41} and R^{k41} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j41} and R^{k41} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{a42}, R^{c42}, and R^{d42} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, any R^{c42} and R^{d42} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-7 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-7 membered heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{b42} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{e42} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{f42} and R^{g42} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7

membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{h42} and R^{i42} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{j42} and R^{k42} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j42} and R^{k42} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{a5}, R^{c5}, and R^{d5} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents;

or, any R^{c5} and R^{d5} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-10 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-10 membered heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents;

each R^{b5} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents;

each R^{e5} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{f5} and R^{g5} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{h5} and R^{i5} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl;

each R^{j5} and R^{k5} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j5} and R^{k5} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{a51}, R^{c51}, and R^{d51} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents;

or, any R^{c51} and R^{d51} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-7 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-7 membered

heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents;

each R^{b51} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents;

each R^{e51} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{f51} and R^{g51} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{h51} and R^{i51} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{j51} and R^{k51} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j51} and R^{k51} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^{a52}, R^{c52}, and R^{d52} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl,

and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

or, any R^{c52} and R^{d52} attached to the same N atom, together with the N atom to which they are attached, form a 5- or 6-membered heteroaryl or a 4-7 membered heterocycloalkyl group, wherein the 5- or 6-membered heteroaryl and 4-7 membered heterocycloalkyl group are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{b52} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^G substituents;

each R^{e52} is independently selected from H, OH, CN, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{f52} and R^{g52} are independently selected from H, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{h52} and R^{i52} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl;

each R^{j52} and R^{k52} is independently selected from OH, C_{1-6} alkoxy, and C_{1-6} haloalkoxy;

or any R^{j52} and R^{k52} attached to the same B atom, together with the B atom to which they are attached, form a 5- or 6-membered heterocycloalkyl group optionally substituted with 1, 2, 3, or 4 substituents independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl; and

each R^G is independently selected from H, D, OH, NO_2, CN, halo, C_{1-3} alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{1-3} haloalkyl, cyano-C_{1-3} alkyl, HO-C_{1-3} alkyl, C_{1-3} alkoxy-

C_{1-3} alkyl, C_{3-7} cycloalkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy, amino, C_{1-3} alkylamino, di(C_{1-3} alkyl)amino, thio, C_{1-3} alkylthio, C_{1-3} alkylsulfinyl, C_{1-3} alkylsulfonyl, carbamyl, C_{1-3} alkylcarbamyl, di(C_{1-3} alkyl)carbamyl, carboxy, C_{1-3} alkylcarbonyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkylcarbonyloxy, C_{1-3} alkylcarbonylamino, C_{1-3} alkoxycarbonylamino, C_{1-3} alkylaminocarbonyloxy, C_{1-3} alkylsulfonylamino, aminosulfonyl, C_{1-3} alkylaminosulfonyl, di(C_{1-3} alkyl)aminosulfonyl, aminosulfonylamino, C_{1-3} alkylaminosulfonylamino, di(C_{1-3} alkyl)aminosulfonylamino, aminocarbonylamino, C_{1-3} alkylaminocarbonylamino, and di(C_{1-3} alkyl)aminocarbonylamino.

In some embodiments:

n is an integer selected from 0, 1, 2, 3, or 4;

Ring moiety **A** is a monocyclic 3-7 membered cycloalkyl or monocyclic 4-7 membered heterocycloalkyl;

R^1 is selected from C_{1-6} haloalkyl, C_{3-7} cycloalkyl, and phenyl, each of which is optionally substituted by 1 or 2 independently selected R^4 substituents;

R^2 is selected from C_{2-6} alkyl and C_{1-6} haloalkyl;

R^3 is selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

or R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-7 membered cycloalkyl ring;

each R^4 is independently selected from halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, OR^{a4}, and $NR^{c4}R^{d4}$;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H, C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^5 is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, $C(O)R^{b5}$, $C(O)NR^{c5}R^{d5}$, $C(O)OR^{a5}$, $S(O)_2R^{b5}$, and $S(O)_2NR^{c5}R^{d5}$;

each R^{5A} is independently selected from halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl;

each R^{a5}, R^{c5}, and R^{d5} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered

heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents; and

each R^{b5} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents.

In some embodiments:

n is an integer selected from 0, 1, 2, 3, or 4;

Ring moiety **A** is monocyclic 4-7 membered heterocycloalkyl;

R^1 is selected from C_{1-6} haloalkyl, C_{3-7} cycloalkyl, and phenyl, each of which is optionally substituted by 1 or 2 independently selected R^4 substituents;

R^2 is selected from ethyl, propyl, isopropyl, and C_{1-3} fluoroalkyl;

R^3 is selected from methyl, ethyl, propyl, isopropyl, and C_{1-3} fluoroalkyl;

or R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-4 membered cycloalkyl ring;

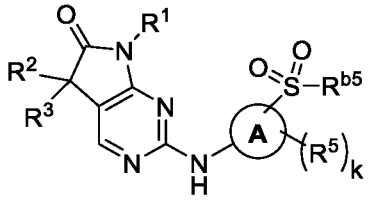
each R^4 is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl;

each R^5 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, and $S(O)_2R^{b5}$;

each R^{b5} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl, which are each optionally substituted by 1 or 2 independently selected R^{5A} substituents; and

each R^{5A} is independently selected from halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl.

In some embodiments, the compound is a compound of Formula (B-Ia)



(B-Ia)

or a pharmaceutically acceptable salt thereof, wherein k is n-1.

In some embodiments, R^1 is selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted by 1, 2, 3, 4, 5, or 6 independently selected R^4 substituents.

In some embodiments, R^1 is selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl, phenyl, 4-10 membered heterocycloalkyl, and 5-6 membered heteroaryl, each of which is optionally substituted by 1 or 2 independently selected R^4 substituents.

In some embodiments, each R^4 is independently selected from halo, CN, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OR^{a4}, $C(O)R^{b4}$, $C(O)NR^{c4}R^{d4}$, $C(O)OR^{a4}$, $OC(O)R^{b4}$, $OC(O)NR^{c4}R^{d4}$, $NR^{c4}R^{d4}$, $NR^{c4}C(O)R^{b4}$, $NR^{c4}C(O)OR^{a4}$, $NR^{c4}C(O)NR^{c4}R^{d4}$, $NR^{c4}S(O)_2R^{b4}$, $NR^{c4}S(O)_2NR^{c4}R^{d4}$, $S(O)_2R^{b4}$, and $S(O)_2NR^{c4}R^{d4}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, and C_{1-6} haloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents.

In some embodiments:

each R^{4A} is independently selected from halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}S(O)_2R^{b41}$, $NR^{c41}S(O)_2NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, and $S(O)_2NR^{c41}R^{d41}$;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{b4} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{a41}, R^{c41}, and R^{d41} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl; and

each R^{b41} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl.

In some embodiments:

each R^{4A} is independently selected from halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, OR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NNR^{c41}S(O)_2R^{b41}$, $S(O)_2R^{b41}$, and $S(O)_2NR^{c41}R^{d41}$;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H, C_{1-6} alkyl and C_{1-6} haloalkyl, wherein said C_{1-6} alkyl and C_{1-6} haloalkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{b4} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{4A} substituents;

each R^{a41}, R^{c41}, and R^{d41} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl; and

each R^{b41} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl.

In some embodiments, Ring moiety **A** is monocyclic 3-7 membered cycloalkyl or monocyclic 4-7 membered heterocycloalkyl.

In some embodiments, Ring moiety **A** is monocyclic 4-7 membered heterocycloalkyl.

In some embodiments, Ring moiety **A** is an azetidine ring, a pyrrolidine ring, a piperidine ring, or an azepane ring.

In some embodiments, Ring moiety **A** is a piperidine ring.

In some embodiments, n is 1 or 2.

In some embodiments, each R^5 is independently selected from halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, OR^{a5}, SR^{a5}, $C(O)R^{b5}$, $C(O)NR^{c5}R^{d5}$, $C(O)OR^{a5}$, $OC(O)R^{b5}$, $OC(O)NR^{c5}R^{d5}$, $NR^{c5}R^{d5}$, $NR^{c5}C(O)R^{b5}$, $NR^{c5}C(O)OR^{a5}$, $NR^{c5}C(O)NR^{c5}R^{d5}$, $NR^{c5}S(O)_2R^{b5}$, $NR^{c5}S(O)_2NR^{c5}R^{d5}$, $S(O)_2R^{b5}$, and $S(O)_2NR^{c5}R^{d5}$.

In some embodiments:

each R^{a5}, R^{c5}, and R^{d5} is independently selected from H and C_{1-6} alkyl; and

each R^{b5} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered

heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1 or 2 independently selected R^{5A} substituents.

In some embodiments, each R^5 is independently selected from halo and C_{1-6} alkyl.

In some embodiments, each R^{b5} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents.

In some embodiments, R^{b5} is selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, phenyl, 4-6 membered heterocycloalkyl, and 5-6 membered heteroaryl, each of which is optionally substituted by 1 or 2 R^{5A} substituents independently selected from halo, C_{1-6} alkyl, and 4-6 membered heterocycloalkyl, wherein said 4-6 membered heterocycloalkyl is optionally substituted by 1 or 2 R^{5B} substituents independently selected from C_{1-3} alkyl.

In some embodiments:

each R^5 is independently selected from halo, C_{1-3} alkyl, C_{1-3} haloalkyl, OR^{a5}, and $NR^{c5}R^{d5}$;

each R^{a5}, R^{c5}, and R^{d5} is independently selected from H and C_{1-6} alkyl;

R^{b5} is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1 or 2 independently selected R^{5A} substituents;

each R^{5A} is independently selected from halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, OR^{a51}, SR^{a51}, $C(O)R^{b51}$, $C(O)NR^{c51}R^{d51}$, $C(O)OR^{a51}$, $OC(O)R^{b51}$, $OC(O)NR^{c51}R^{d51}$, $NR^{c51}R^{d51}$, $NR^{c51}C(O)R^{b51}$, $NR^{c51}C(O)OR^{a51}$, $NR^{c51}C(O)NR^{c51}R^{d51}$, $NR^{c51}S(O)_2R^{b51}$, $NR^{c51}S(O)_2NR^{c51}R^{d51}$, $S(O)_2R^{b51}$, and $S(O)_2NR^{c51}R^{d51}$, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl are each optionally substituted with 1 or 2 independently selected R^{5B} substituents;

each R^{a51}, R^{c51}, and R^{d51} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl, wherein said C_{1-6} alkyl and C_{1-6} haloalkyl are each optionally substituted with 1 or 2 independently selected R^{5B} substituents;

each R^{b51} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl, which are each optionally substituted with 1 or 2 independently selected R^{5B} substituents; and

each R^{5B} is independently selected from halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl.

In some embodiments:

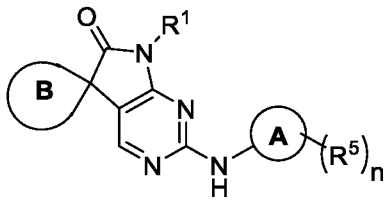
each R^5 is independently selected from halo and C_{1-3} alkyl;

R^{b5} is selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl, each of which is optionally substituted with 1 or 2 independently selected R^{5A} substituents;

each R^{5A} is independently selected from halo, C_{1-6} alkyl, and 4-7 membered heterocycloalkyl, wherein said C_{1-6} alkyl and 4-7 membered heterocycloalkyl are each optionally substituted with 1 or 2 independently selected R^{5B} substituents; and

each R^{5B} is independently selected from C_{1-6} alkyl.

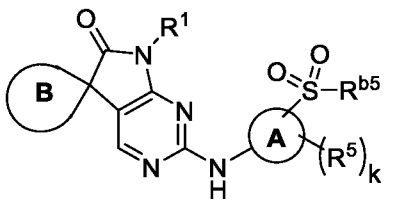
In some embodiments, the compound is a compound of Formula (B-II):



(B-II)

or a pharmaceutically acceptable salt thereof, wherein the variables are defined according to the definitions provided herein.

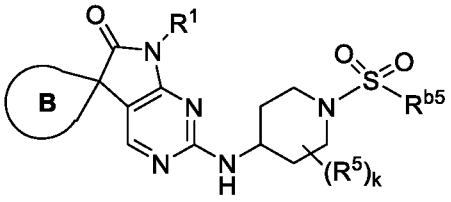
In some embodiments, the compound is a compound of Formula (B-IIa):



(B-IIa)

or a pharmaceutically acceptable salt thereof, wherein k is n-1, and the remaining variables are defined according to the definitions provided herein.

In some embodiments, the compound is a compound of Formula (B-IIb):

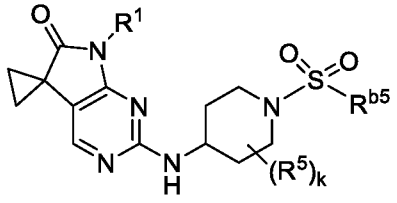


(B-IIb)

or a pharmaceutically acceptable salt thereof, wherein k is n-1, and the remaining variables are defined according to the definitions provided herein.

In some embodiments, Ring **B** is a 3-7 membered cycloalkyl ring.

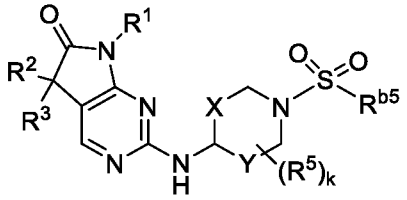
In some embodiments, the compound is a compound of Formula (B-IIc):



(B-IIc)

or a pharmaceutically acceptable salt thereof, wherein k is n-1, and the remaining variables are defined according to the definitions provided herein.

In some embodiments, the compound is a compound of Formula (B-IId):



(B-IId)

or a pharmaceutically acceptable salt thereof, wherein:

X is a bond or CH_2;

Y is a bond or CH_2; and

k is n-1.

In some embodiments, the compound has Formula (B-Ia), wherein:

k is n-1;

n is an integer selected from 1 and 2;

Ring moiety **A** is a monocyclic 4-6 membered heterocycloalkyl;

R^1 is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, phenyl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, phenyl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-

10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl are each optionally substituted by 1, 2, or 3 independently selected R^4 substituents;

R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-7 membered cycloalkyl ring;

each R^4 is independently selected from H, halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-4} cycloalkyl, OR^{a4}, $C(O)R^{b4}$, $C(O)NR^{c4}R^{d4}$, $C(O)OR^{a4}$, $OC(O)R^{b4}$, $OC(O)NR^{c4}R^{d4}$, $NR^{c4}R^{d4}$, $NR^{c4}C(O)R^{b4}$, $NR^{c4}C(O)OR^{a4}$, $NR^{c4}C(O)NR^{c4}R^{d4}$, $NR^{c4}S(O)_2R^{b4}$, $NR^{c4}S(O)_2NR^{c4}R^{d4}$, $S(O)_2R^{b4}$, and $S(O)_2NR^{c4}R^{d4}$;

each R^5 is independently selected from H, halo, CN, C_{1-3} alkyl, and C_{1-3} haloalkyl;

each R^{5A} is independently selected from H, D, halo, CN, NO_2, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, 5-6 membered heteroaryl-C_{1-4} alkyl, OR^{a51}, $C(O)R^{b51}$, $C(O)NR^{c51}R^{d51}C(O)OR^{a51}$, $OC(O)R^{b51}$, $OC(O)NR^{c51}R^{d51}$, $NR^{c51}R^{d51}$, $NR^{c51}C(O)R^{b51}$, $NR^{c51}C(O)OR^{a51}$, $NR^{c51}C(O)NR^{c51}R^{d51}$, $NR^{c51}S(O)_2R^{b51}$, $NR^{c51}S(O)_2NR^{c51}R^{d51}$, $S(O)_2R^{b51}$, and $S(O)_2NR^{c51}R^{d51}$, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents;

each R^{5B} is independently selected from H, halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, OH, NO_2, CN, halo, C_{1-3} alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{1-3} haloalkyl, cyano-C_{1-3} alkyl, HO-C_{1-3} alkyl, C_{1-3} alkoxy-C_{1-3} alkyl, C_{3-7} cycloalkyl, C_{1-3} alkoxy, C_{1-3} haloalkoxy, amino, C_{1-3} alkylamino, di(C_{1-3} alkyl)amino, thio, C_{1-3} alkylthio, C_{1-3} alkylsulfinyl, C_{1-3} alkylsulfonyl, carbamyl, C_{1-3} alkylcarbamyl, di(C_{1-3} alkyl)carbamyl, carboxy, C_{1-3} alkylcarbonyl, C_{1-3} alkoxycarbonyl, C_{1-3} alkylcarbonyloxy, C_{1-3} alkylcarbonylamino, C_{1-3} alkoxycarbonylamino, C_{1-3} alkylaminocarbonyloxy, C_{1-3} alkylsulfonylamino, aminosulfonyl, C_{1-3} alkylaminosulfonyl, di(C_{1-3} alkyl)aminosulfonyl, aminosulfonylamino, C_{1-3} alkylaminosulfonylamino, di(C_{1-3}

alkyl)aminosulfonylamino, aminocarbonylamino, C_{1-3} alkylaminocarbonylamino, and di(C_{1-3} alkyl)aminocarbonylamino;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl;

each R^{b5} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5A} substituents;

each R^{a51}, R^{c51}, and R^{d51} is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents; and

each R^{b51} is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, which are each optionally substituted with 1, 2, 3, or 4 independently selected R^{5B} substituents.

In some embodiments of compounds of Formula (B-Ia):

k is n-1;

n is 1 or 2;

Ring moiety **A** is 4-6 membered heterocycloalkyl;

R^1 is selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-10} cycloalkyl, 6-10 membered aryl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl-C_{1-4} alkyl, 6-10 membered aryl-C_{1-4} alkyl, 4-10 membered heterocycloalkyl-C_{1-4} alkyl, and 5-10 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted by 1, 2, or 3 independently selected R^4 substituents;

each R^4 is independently selected from halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, OR^{a4}, and $NR^{c4}R^{d4}$;

each R^{a4}, R^{c4}, and R^{d4} is independently selected from H and C_{1-6} alkyl;

R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-4 membered cycloalkyl ring;

each R^5 is independently selected from halo, C_{1-3} alkyl, C_{1-3} haloalkyl, OR^{a5}, and $NR^{c5}R^{d5}$;

each R^{a5}, R^{c5}, and R^{d5} is independently selected from H and C_{1-6} alkyl;

R^{b5} is selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, C_{3-7} cycloalkyl-C_{1-4} alkyl, phenyl-C_{1-4} alkyl, 4-7 membered heterocycloalkyl-C_{1-4} alkyl, and 5-6 membered heteroaryl-C_{1-4} alkyl, each of which is optionally substituted with 1 or 2 independently selected R^{5A} substituents;

each R^{5A} is independently selected from halo, CN, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, 5-6 membered heteroaryl, OR^{a51}, SR^{a51}, $C(O)R^{b51}$, $C(O)NR^{c51}R^{d51}$, $C(O)OR^{a51}$, $OC(O)R^{b51}$, $OC(O)NR^{c51}R^{d51}$, $NR^{c51}R^{d51}$, $NR^{c51}C(O)R^{b51}$, $NR^{c51}C(O)OR^{a51}$, $NR^{c51}C(O)NR^{c51}R^{d51}$, $NR^{c51}S(O)_2R^{b51}$, $NR^{c51}S(O)_2NR^{c51}R^{d51}$, $S(O)_2R^{b51}$, and $S(O)_2NR^{c51}R^{d51}$, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, phenyl, 4-7 membered heterocycloalkyl, and 5-6 membered heteroaryl are each optionally substituted with 1 or 2 independently selected R^{5B} substituents;

each R^{a51}, R^{c51}, and R^{d51} is independently selected from H, C_{1-6} alkyl, and C_{1-6} haloalkyl, wherein said C_{1-6} alkyl and C_{1-6} haloalkyl are each optionally substituted with 1 or 2 independently selected R^{5B} substituents;

each R^{b51} is independently selected from C_{1-6} alkyl and C_{1-6} haloalkyl, which are each optionally substituted with 1 or 2 independently selected R^{5B} substituents; and

each R^{5B} is independently selected from halo, CN, C_{1-6} alkyl, and C_{1-6} haloalkyl.

In some embodiments of compounds of Formula (B-Ia):

k is n-1;

n is 1 or 2;

Ring moiety **A** is a piperidine ring;

R^1 is selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl, phenyl, 4-10 membered heterocycloalkyl, and 5-6 membered heteroaryl, each of which is optionally substituted by 1 or 2 independently selected R^4 substituents;

each R^4 is independently selected from halo, OH, C_{1-3} alkyl, and C_{1-3} alkoxy;

R^2 and R^3, together with the carbon atom to which they are attached, form Ring **B**;

Ring **B** is a 3-4 membered cycloalkyl ring;

each R^5 is independently selected from halo and C_{1-3} alkyl; and

R^{b5} is selected from C_{1-6} alkyl, C_{3-6} cycloalkyl, phenyl, 4-6 membered heterocycloalkyl, and 5-6 membered heteroaryl, each of which is optionally substituted by 1 or 2 R^{5A} substituents independently selected from halo, C_{1-6} alkyl, and 4-6 membered heterocycloalkyl, wherein said 4-6 membered heterocycloalkyl is optionally substituted by 1 or 2 R^{5B} substituents independently selected from C_{1-3} alkyl.

In some embodiments, the compound is a compound selected from the compounds of the Examples, or a pharmaceutically acceptable salt thereof.

In some embodiments, 1, 2, 3, 4, 5, 6, 7, or 8 hydrogen atoms, attached to carbon atoms of "alkyl", "alkenyl", "alkynyl", "aryl", "phenyl", "cycloalkyl", "heterocycloalkyl", or "heteroaryl" substituents or "-C_{1-4} alkyl-" and "alkylene" linking groups, as described herein, are optionally replaced by deuterium atoms.

It is further appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination.

At various places in the present specification, divalent linking substituents are described. It is specifically intended that each divalent linking substituent include both the forward and backward forms of the linking substituent. For example, -$NR(CR'R'')_n$- includes both -$NR(CR'R'')_n$- and -$(CR'R'')_nNR$-. Where the structure clearly requires a linking group, the Markush variables listed for that group are understood to be linking groups.

When an embodiment that recites "one R^5 is $S(O)_2R^{b5}$; and each remaining R^5 is independently selected from" is combined through multiple dependencies with a

formula showing a floating -$S(O)_2R^{b5}$ substituent, then the floating -$S(O)_2R^{b5}$ substituent on the formula replaces the "one R^5 is $S(O)_2R^{b5}$" phrase. In the case of such an embodiment combined with a formula having the integer k, one of R^5 substituents (of n possible R^5 substituents) is replaced by the $S(O)_2R^{b5}$ substituent of the formula, wherein each of the remaining R^5 substituents (there being k remaining R^5 substituents) is independently selected from the "each remaining R^5" list.

The term "n-membered" where n is an integer typically describes the number of ring-forming atoms in a moiety where the number of ring-forming atoms is n. For example, piperidinyl is an example of a 6-membered heterocycloalkyl ring, pyrazolyl is an example of a 5-membered heteroaryl ring, pyridyl is an example of a 6-membered heteroaryl ring, and 1,2,3,4-tetrahydro-naphthalene is an example of a 10-membered cycloalkyl group.

As used herein, the phrase "optionally substituted" means unsubstituted or substituted. The substituents are independently selected, and substitution may be at any chemically accessible position. As used herein, the term "substituted" means that a hydrogen atom is removed and replaced by a substituent. A single divalent substituent, *e.g.*, oxo, can replace two hydrogen atoms. It is to be understood that substitution at a given atom is limited by valency, that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

As used herein, the phrase "each 'variable' is independently selected from" means substantially the same as wherein "at each occurrence 'variable' is selected from."

When any variable (e.g., R^S) occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 1, 2, 3, or 4 R^S, then said group may optionally be substituted with up to four R^S groups and R^S at each occurrence is selected independently from the definition of R^S. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds; for example the combination of a first M group and second M group in the combination of two R groups are permissible only if such combinations of M-M result in stable compounds (*e.g.*, M-M is not permissible if it will form highly reactive compounds such as peroxides having O-O bonds).

Throughout the definitions, the term "C_{n-m}" indicates a range which includes the endpoints, wherein n and m are integers and indicate the number of carbons. Examples include C_{1-3}, C_{1-4}, C_{1-6}, and the like.

As used herein, the term "C_{n-m} alkyl", employed alone or in combination with other terms, refers to a saturated hydrocarbon group that may be straight-chain or branched, having n to m carbons. Examples of alkyl moieties include, but are not limited to, chemical groups such as methyl (Me), ethyl (Et), *n*-propyl (*n*-Pr), isopropyl (iPr), *n*-butyl, *tert*-butyl, isobutyl, *sec*-butyl; higher homologs such as 2-methyl-1-butyl, *n*-pentyl, 3-pentyl, *n*-hexyl, 1,2,2-trimethylpropyl, and the like. In some embodiments, the alkyl group contains from 1 to 6 carbon atoms, from 1 to 4 carbon atoms, from 1 to 3 carbon atoms, or 1 to 2 carbon atoms.

As used herein, "C_{n-m} alkenyl" refers to an alkyl group having one or more double carbon-carbon bonds and having n to m carbons. Example alkenyl groups include, but are not limited to, ethenyl, *n*-propenyl, isopropenyl, *n*-butenyl, *sec*-butenyl, and the like. In some embodiments, the alkenyl moiety contains 2 to 6, 2 to 4, or 2 to 3 carbon atoms.

As used herein, "C_{n-m} alkynyl" refers to an alkyl group having one or more triple carbon-carbon bonds and having n to m carbons. Example alkynyl groups include, but are not limited to, ethynyl, propyn-1-yl, propyn-2-yl, and the like. In some embodiments, the alkynyl moiety contains 2 to 6, 2 to 4, or 2 to 3 carbon atoms. As used herein, the term "C_{n-m} alkoxy", employed alone or in combination with other terms, refers to a group of formula-O-alkyl, wherein the alkyl group has n to m carbons. Example alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy (e.g., *n*-propoxy and isopropoxy), butoxy (e.g., *n*-butoxy and *tert*-butoxy), and the like. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "amino" refers to a group of formula $-NH_2$.

As used herein, the term "aryl," employed alone or in combination with other terms, refers to an aromatic hydrocarbon group, which may be monocyclic or polycyclic (e.g., having 2 fused rings). The term "C_{n-m} aryl" refers to an aryl group having from n to m ring carbon atoms. Aryl groups include, e.g., phenyl, naphthyl, anthracenyl, phenanthrenyl, indanyl, indenyl, and the like. In some embodiments, the aryl group has from 6 to 10 carbon atoms. In some embodiments, the aryl group is phenyl or naphthyl. In some embodiments, the aryl is phenyl.

As used herein, "halo" refers to F, Cl, Br, or I. In some embodiments, halo is F, Cl, or Br. In some embodiments, halo is F or Cl. In some embodiments, halo is F. In some embodiments, halo is Cl.

As used herein, "C_{n-m} haloalkoxy" refers to a group of formula –O-haloalkyl having n to m carbon atoms. Example haloalkoxy groups include OCF_3 and $OCHF_2$. In some embodiments, the haloalkoxy group is fluorinated only. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} haloalkyl", employed alone or in combination with other terms, refers to an alkyl group having from one halogen atom to 2s+1 halogen atoms which may be the same or different, where "s" is the number of carbon atoms in the alkyl group, wherein the alkyl group has n to m carbon atoms. In some embodiments, the haloalkyl group is fluorinated only. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms. Example haloalkyl groups include CF_3, C_2F_5, CHF_2, CH_2F, CCl_3, $CHCl_2$, C_2Cl_5 and the like.

As used herein, the term "thio" refers to a group of formula -SH.

As used herein, the term "C_{n-m} alkylamino" refers to a group of formula -NH(alkyl), wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkoxycarbonyl" refers to a group of formula -C(O)O-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylcarbonyl" refers to a group of formula -C(O)-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylcarbonylamino" refers to a group of formula -NHC(O)-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkoxycarbonylamino" refers to a group of formula -NHC(O)O(C_{n-m} alkyl), wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylsulfonylamino" refers to a group of formula -NHS(O)$_2$-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "aminosulfonyl" refers to a group of formula $-S(O)_2NH_2$.

As used herein, the term "C_{n-m} alkylaminosulfonyl" refers to a group of formula $-S(O)_2NH(alkyl)$, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "di(C_{n-m} alkyl)aminosulfonyl" refers to a group of formula $-S(O)_2N(alkyl)_2$, wherein each alkyl group independently has n to m carbon atoms. In some embodiments, each alkyl group has, independently, 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "aminosulfonylamino" refers to a group of formula $-NHS(O)_2NH_2$.

As used herein, the term "C_{n-m} alkylaminosulfonylamino" refers to a group of formula $-NHS(O)_2NH(alkyl)$, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "di(C_{n-m} alkyl)aminosulfonylamino" refers to a group of formula $-NHS(O)_2N(alkyl)_2$, wherein each alkyl group independently has n to m carbon atoms. In some embodiments, each alkyl group has, independently, 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "aminocarbonylamino", employed alone or in combination with other terms, refers to a group of formula $-NHC(O)NH_2$.

As used herein, the term "C_{n-m} alkylaminocarbonylamino" refers to a group of formula $-NHC(O)NH(alkyl)$, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "di(C_{n-m} alkyl)aminocarbonylamino" refers to a group of formula $-NHC(O)N(alkyl)_2$, wherein each alkyl group independently has n to m carbon atoms. In some embodiments, each alkyl group has, independently, 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylcarbamyl" refers to a group of formula $-C(O)-NH(alkyl)$, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylthio" refers to a group of formula -S-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylsulfinyl" refers to a group of formula -S(O)-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylsulfonyl" refers to a group of formula -$S(O)_2$-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "cyano-C_{1-6} alkyl" refers to a group of formula -(C_{1-6} alkylene)-CN. As used herein, the term "cyano-C_{1-3} alkyl" refers to a group of formula -(C_{1-3} alkylene)-CN.

As used herein, the term "HO-C_{1-6} alkyl" refers to a group of formula -(C_{1-6} alkylene)-OH. As used herein, the term "HO-C_{1-3} alkyl" refers to a group of formula -(C_{1-3} alkylene)-OH.

As used herein, the term "C_{1-6} alkoxy-C_{1-6} alkyl" refers to a group of formula -(C_{1-6} alkylene)-O(C_{1-6} alkyl). As used herein, the term "C_{1-3} alkoxy-C_{1-3} alkyl" refers to a group of formula -(C_{1-3} alkylene)-O(C_{1-3} alkyl).

As used herein, the term "carboxy" refers to a group of formula -C(O)OH.

As used herein, the term "di(C_{n-m}-alkyl)amino" refers to a group of formula -$N(alkyl)_2$, wherein the two alkyl groups each has, independently, n to m carbon atoms. In some embodiments, each alkyl group independently has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "di(C_{n-m}-alkyl)carbamyl" refers to a group of formula –C(O)$N(alkyl)_2$, wherein the two alkyl groups each has, independently, n to m carbon atoms. In some embodiments, each alkyl group independently has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, the term "C_{n-m} alkylcarbonyloxy" is a group of formula -OC(O)-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, "aminocarbonyloxy" is a group of formula -OC(O)-NH_2.

As used herein, "C_{n-m} alkylaminocarbonyloxy" is a group of formula -OC(O)-NH-alkyl, wherein the alkyl group has n to m carbon atoms. In some embodiments, the alkyl group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein, "di(C_{n-m}alkyl)aminocarbonyloxy" is a group of formula -OC(O)-$N(alkyl)_2$, wherein each alkyl group has, independently, n to m carbon atoms.

In some embodiments, each alkyl group independently has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

As used herein C_{n-m} alkoxycarbonylamino refers to a group of formula -NHC(O)-O-alkyl, wherein the alkyl group has n to m carbon atoms.

As used herein, the term "carbamyl" to a group of formula $–C(O)NH_2$.

As used herein, the term "carbonyl", employed alone or in combination with other terms, refers to a -C(O)- group.

As used herein, "cycloalkyl" refers to non-aromatic cyclic hydrocarbons including cyclized alkyl and alkenyl groups. Cycloalkyl groups can include mono- or polycyclic (e.g., having 2, 3 or 4 fused rings) groups, spirocycles, and bridged rings (*e.g.*, a bridged bicycloalkyl group). Ring-forming carbon atoms of a cycloalkyl group can be optionally substituted by oxo or sulfido (e.g., C(O) or C(S)). Also included in the definition of cycloalkyl are moieties that have one or more aromatic rings fused (*i.e.*, having a bond in common with) to the cycloalkyl ring, for example, benzo or thienyl derivatives of cyclopentane, cyclohexane, and the like. A cycloalkyl group containing a fused aromatic ring can be attached through any ring-forming atom including a ring-forming atom of the fused aromatic ring. Cycloalkyl groups can have 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 ring-forming carbons (i.e., C_{3-14}). In some embodiments, the cycloalkyl is a C_{3-12} monocyclic or bicyclic cycloalkyl which is optionally substituted by CH_2F, CHF_2, CF_3, and CF_2CF_3. In some embodiments, the cycloalkyl is a C_{3-10} monocyclic or bicyclic cycloalkyl. In some embodiments, the cycloalkyl is a C_{3-7} monocyclic cycloalkyl. In some embodiments, the cycloalkyl is a C_{4-7} monocyclic cycloalkyl. In some embodiments, the cycloalkyl is a C_{4-14} spirocycle or bridged cycloalkyl (*e.g.*, a bridged bicycloalkyl group). Example cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclopentenyl, cyclohexenyl, cyclohexadienyl, cycloheptatrienyl, norbornyl, norpinyl, norcarnyl, cubane, adamantane, bicyclo[1.1.1]pentyl, bicyclo[2.1.1]hexyl, bicyclo[2.2.1]heptanyl, bicyclo[3.1.1]heptanyl, bicyclo[2.2.2]octanyl, spiro[3.3]heptanyl, and the like. In some embodiments, cycloalkyl is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

As used herein, "heteroaryl" refers to a monocyclic or polycyclic (e.g., having 2, 3, or 4 fused rings) aromatic heterocycle having at least one heteroatom ring member selected from N, O, S and B. In some embodiments, the heteroaryl ring has 1, 2, 3, or 4 heteroatom ring members independently selected from N, O, S and B. In

some embodiments, any ring-forming N in a heteroaryl moiety can be an N-oxide. In some embodiments, the heteroaryl is a 5-10 membered monocyclic or bicyclic heteroaryl having 1, 2, 3, or 4 heteroatom ring members independently selected from N, O, and S. In some embodiments, the heteroaryl is a 5-10 membered monocyclic or bicyclic heteroaryl having 1, 2, 3, or 4 heteroatom ring members independently selected from N, O, and S. In some embodiments, the heteroaryl is a 5-10 membered monocyclic or bicyclic heteroaryl having 1, 2, 3, or 4 heteroatom ring members independently selected from N, O, and S. In some embodiments, the heteroaryl is a 5-6 monocyclic heteroaryl having 1 or 2 heteroatom ring members independently selected from N, O, S and B. In some embodiments, the heteroaryl is a 5-6 monocyclic heteroaryl having 1 or 2 heteroatom ring members independently selected from N, O, and S. In some embodiments, the heteroaryl group contains 3 to 14, 3 to 10, 4 to 14, 4 to 10, 3 to 7, or 5 to 6 ring-forming atoms. In some embodiments, the heteroaryl group has 1 to 4 ring-forming heteroatoms, 1 to 3 ring-forming heteroatoms, 1 to 2 ring-forming heteroatoms or 1 ring-forming heteroatom. When the heteroaryl group contains more than one heteroatom ring member, the heteroatoms may be the same or different. Example heteroaryl groups include, but are not limited to, pyridine, pyrimidine, pyrazine, pyridazine, pyrrole, pyrazole, azolyl, oxazole, isoxazole, thiazole, isothiazole, imidazole, furan, thiophene, triazole, tetrazole, thiadiazole, quinoline, isoquinoline, indole, benzothiophene, benzofuran, benzisoxazole, imidazo[1, 2-b]thiazole, purine, triazine, thieno[3,2-*b*]pyridine, imidazo[1,2-*a*]pyridine, 1,5-naphthyridine, 1*H*-pyrazolo[4,3-*b*]pyridine, and the like.

A five-membered heteroaryl is a heteroaryl group having five ring-forming atoms wherein one or more (e.g., 1, 2, or 3) of the ring-forming atoms are independently selected from N, O, S or B. Exemplary five-membered ring heteroaryls are thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, isoxazolyl, 1,2,3-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-triazolyl, 1,2,4-thiadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-triazolyl, 1,3,4-thiadiazolyl, 1,3,4-oxadiazolyl, and 1,2-dihydro-1,2-azaborine.

A six-membered heteroaryl ring is a heteroaryl group having six ring-forming atoms wherein one or more (e.g., 1, 2, or 3) of the ring-forming atoms are independently selected from N, O, S, and B. Exemplary six-membered ring heteroaryls are pyridyl, pyrazinyl, pyrimidinyl, triazinyl, and pyridazinyl.

As used herein, "heterocycloalkyl" refers to monocyclic or polycyclic heterocycles having at least one non-aromatic ring (saturated or partially unsaturated ring), wherein one or more of the ring-forming carbon atoms of the heterocycloalkyl is replaced by a heteroatom selected from N, O, S, and B, and wherein the ring-forming carbon atoms and heteroatoms of the heterocycloalkyl group can be optionally substituted by one or more oxo or sulfido (e.g., C(O), S(O), C(S), or $S(O)_2$, etc.). Heterocycloalkyl groups include monocyclic and polycyclic (e.g., having 2 fused rings) systems. Included in heterocycloalkyl are monocyclic and polycyclic 12, 4-12, 3-10-, 4-10-, 3-7-, 4-7-, and 5-6-membered heterocycloalkyl groups. Heterocycloalkyl groups can also include spirocycles and bridged rings (*e.g.*, a 5-14 membered bridged biheterocycloalkyl ring having one or more of the ring-forming carbon atoms replaced by a heteroatom independently selected from N, O, S, and B). The heterocycloalkyl group can be attached through a ring-forming carbon atom or a ring-forming heteroatom. In some embodiments, the heterocycloalkyl group contains 0 to 3 double bonds. In some embodiments, the heterocycloalkyl group contains 0 to 2 double bonds.

Also included in the definition of heterocycloalkyl are moieties that have one or more aromatic rings fused (*i.e.*, having a bond in common with) to the non-aromatic heterocyclic ring, for example, benzo or thienyl derivatives of piperidine, morpholine, azepine, etc. A heterocycloalkyl group containing a fused aromatic ring can be attached through any ring-forming atom including a ring-forming atom of the fused aromatic ring. In some embodiments, the heterocycloalkyl group contains 3 to 14 ring-forming atoms, 4 to 14 ring-forming atoms, 3 to 10 ring-forming atoms, 4 to 10 ring-forming atoms, 3 to 7 ring-forming atoms, or 5 to 6 ring-forming atoms. In some embodiments, the heterocycloalkyl group has 1 to 4 heteroatoms, 1 to 3 heteroatoms, 1 to 2 heteroatoms or 1 heteroatom. In some embodiments, the heterocycloalkyl is a monocyclic 4-6 membered heterocycloalkyl having 1 or 2 heteroatoms independently selected from N, O, S, and B and having one or more oxidized ring members.

Example heterocycloalkyl groups include pyrrolidin-2-one, 1,3-isoxazolidin-2-one, pyranyl, tetrahydropyran, oxetanyl, azetidinyl, morpholino, thiomorpholino, piperazinyl, tetrahydrofuranyl, tetrahydrothienyl, piperidinyl, pyrrolidinyl, isoxazolidinyl, isothiazolidinyl, pyrazolidinyl, oxazolidinyl, thiazolidinyl, imidazolidinyl, azepanyl, benzazapene, 1,2,3,4-tetrahydroisoquinoline,

azabicyclo[3.1.0]hexanyl, diazabicyclo[3.1.0]hexanyl, oxabicyclo[2.1.1]hexanyl, azabicyclo[2.2.1]heptanyl, diazabicyclo[2.2.1]heptanyl, azabicyclo[3.1.1]heptanyl, diazabicyclo[3.1.1]heptanyl, azabicyclo[3.2.1]octanyl, diazabicyclo[3.2.1]octanyl, oxabicyclo[2.2.2]octanyl, azabicyclo[2.2.2]octanyl, azaadamantanyl, diazaadamantanyl, oxa-adamantanyl, azaspiro[3.3]heptanyl, diazaspiro[3.3]heptanyl, oxa-azaspiro[3.3]heptanyl, azaspiro[3.4]octanyl, diazaspiro[3.4]octanyl, oxa-azaspiro[3.4]octanyl, azaspiro[2.5]octanyl, diazaspiro[2.5]octanyl, azaspiro[4.4]nonanyl, diazaspiro[4.4]nonanyl, oxa-azaspiro[4.4]nonanyl, azaspiro[4.5]decanyl, diazaspiro[4.5]decanyl, diazaspiro[4.4]nonanyl, oxa-diazaspiro[4.4]nonanyl, and the like.

As used herein, "C_{o-p} cycloalkyl-C_{n-m} alkyl-" refers to a group of formula cycloalkyl-alkylene-, wherein the cycloalkyl has o to p carbon atoms and the alkylene linking group has n to m carbon atoms.

As used herein "C_{o-p} aryl-C_{n-m} alkyl-" refers to a group of formula aryl-alkylene-, wherein the aryl has o to p carbon atoms and the alkylene linking group has n to m carbon atoms.

As used herein, "heteroaryl-C_{n-m} alkyl-" refers to a group of formula heteroaryl-alkylene-, wherein alkylene linking group has n to m carbon atoms.

As used herein "heterocycloalkyl-C_{n-m} alkyl-" refers to a group of formula heterocycloalkyl-alkylene-, wherein alkylene linking group has n to m carbon atoms.

As used herein, the term "alkylene" refers a divalent straight chain or branched alkyl linking group. Examples of "alkylene groups" include methylene, ethan-1,1-diyl, ethan-1,2-diyl, propan-1,3-dilyl, propan-1,2-diyl, propan-1,1-diyl and the like.

As used herein, the term "alkenylene" refers a divalent straight chain or branched alkenyl linking group. Examples of "alkenylene groups" include ethen-1,1-diyl, ethen-1,2-diyl, propen-1,3-diyl, 2-buten-1,4-diyl, 3-penten-1,5-diyl, 3-hexen-1,6-diyl, 3-hexen-1,5-diyl, and the like.

As used herein, the term "alkynylene" refers a divalent straight chain or branched alkynyl linking group. Examples of "alkynylene groups" include propyn-1,3-diyl, 2-butyn-1,4-diyl, 3-pentyn-1,5-diyl, 3-hexyn-1,6-diyl, 3-hexyn-1,5-diyl, and the like.

As used herein, the term "oxo" refers to an oxygen atom (i.e., =O) as a divalent substituent, forming a carbonyl group when attached to a carbon (e.g., C=O

or C(O)), or attached to a nitrogen or sulfur heteroatom forming a nitroso, sulfinyl or sulfonyl group.

As used herein, the term "independently selected from" means that each occurrence of a variable or substituent are independently selected at each occurrence from the applicable list.

At certain places, the definitions or embodiments refer to specific rings (e.g., an azetidine ring, a pyridine ring, etc.). Unless otherwise indicated, these rings can be attached to any ring member provided that the valency of the atom is not exceeded. For example, an azetidine ring may be attached at any position of the ring, whereas a pyridin-3-yl ring is attached at the 3-position.

The compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically inactive starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. *Cis* and *trans* geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms. In some embodiments, the compound has the *(R)*-configuration. In some embodiments, the compound has the *(S)*-configuration. The Formulas (*e.g.*, Formula (A-I), (B-I), etc.) provided herein include stereoisomers of the compounds.

Resolution of racemic mixtures of compounds can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallization using a chiral resolving acid which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids such as β-camphorsulfonic acid. Other resolving agents suitable for fractional crystallization methods include stereoisomerically pure forms of α-methylbenzylamine (*e.g.*, *S* and *R* forms, or

diastereomerically pure forms), 2-phenylglycinol, norephedrine, ephedrine, N-methylephedrine, cyclohexylethylamine, 1,2-diaminocyclohexane, and the like.

Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent (*e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

Compounds provided herein also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond together with the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Example prototropic tautomers include ketone – enol pairs, amide-imidic acid pairs, lactam – lactim pairs, enamine – imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, for example, 1H- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, 1H- and 2H-isoindole, 2-hydroxypyridine and 2-pyridone, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. Compounds herein identified by name or structure as one particular tautomeric form are intended to include other tautomeric forms unless otherwise specified.

All compounds, and pharmaceutically acceptable salts thereof, can be found together with other substances such as water and solvents (e.g., hydrates and solvates) or can be isolated.

In some embodiments, preparation of compounds can involve the addition of acids or bases to affect, for example, catalysis of a desired reaction or formation of salt forms such as acid addition salts.

In some embodiments, the compounds provided herein, or salts thereof, are substantially isolated. By "substantially isolated" is meant that the compound is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compounds provided herein. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compounds provided herein, or salt thereof. Methods for isolating compounds and their salts are routine in the art.

In some embodiments, the CDK2 inhibitor can be an isotopically-labeled compound, or a pharmaceutically acceptable salt thereof. An "isotopically" or "radio-labeled" compound is a compound of the disclosure where one or more atoms are replaced or substituted by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature (i.e., naturally occurring). Suitable radionuclides that may be incorporated in compounds of the present disclosure include but are not limited to ^{2}H (also written as D for deuterium), ^{3}H (also written as T for tritium), ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{18}F, ^{35}S, ^{36}Cl, ^{82}Br, ^{75}Br, ^{76}Br, ^{77}Br, ^{123}I, ^{124}I, ^{125}I and ^{131}I. For example, one or more hydrogen atoms in a compound of the present disclosure can be replaced by deuterium atoms (e.g., one or more hydrogen atoms of a C_{1-6} alkyl group can be optionally substituted with deuterium atoms, such as –CD3 being substituted for –CH3).

One or more constituent atoms of the compounds described herein can be replaced or substituted with isotopes of the atoms in natural or non-natural abundance. In some embodiments, the compound includes at least one deuterium atom. In some embodiments, the compound includes two or more deuterium atoms. In some embodiments, the compound includes 1-2, 1-3, 1-4, 1-5, or 1-6 deuterium atoms. In some embodiments, all of the hydrogen atoms in a compound can be replaced or substituted by deuterium atoms.

Synthetic methods for including isotopes into organic compounds are known in the art (Deuterium Labeling in Organic Chemistry by Alan F. Thomas (New York, N.Y., Appleton-Century-Crofts, 1971; The Renaissance of H/D Exchange by Jens Atzrodt, Volker Derdau, Thorsten Fey and Jochen Zimmermann, Angew. Chem. Int. Ed. 2007, 7744-7765; The Organic Chemistry of Isotopic Labelling by James R. Hanson, Royal Society of Chemistry, 2011). Isotopically labeled compounds can be used in various studies such as NMR spectroscopy, metabolism experiments, and/or assays.

Substitution with heavier isotopes, such as deuterium, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances (see e.g., A. Kerekes et. al. J. Med. Chem. 2011, 54, 201-210; R. Xu et. al. J. Label Compd. Radiopharm. 2015, 58, 308-312). In particular, substitution at one or more metabolism sites may afford one or more of the therapeutic advantages.

Accordingly, in some embodiments, the CDK2 inhibitor is a compound, wherein one or more hydrogen atoms in the compound are replaced by deuterium atoms, or a pharmaceutically acceptable salt thereof.

The term "compound" as used herein is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted. Compounds herein identified by name or structure as one particular tautomeric form are intended to include other tautomeric forms unless otherwise specified.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The present disclosure also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, alcohols (e.g., methanol, ethanol, iso-propanol, or butanol) or acetonitrile (ACN) are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

The term "CDK2 inhibitor" includes any compound that inhibits CDK2, including its pharmaceutically acceptable salts, hydrates, solvates, and polymorphs.

Synthesis

Compounds of the invention, including salts thereof, can be prepared using known organic synthesis techniques and can be synthesized according to any of numerous possible synthetic routes, such as those in the Schemes below.

The reactions for preparing compounds of the invention can be carried out in suitable solvents which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially non-reactive with the starting materials (reactants), the intermediates or products at the temperatures at which the reactions are carried out, *e.g.*, temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected by the skilled artisan.

Preparation of compounds of the invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups is described, *e.g.*, in Kocienski, *Protecting Groups*, (Thieme, 2007); Robertson, *Protecting Group Chemistry*, (Oxford University Press, 2000); Smith *et al.*, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 6th Ed. (Wiley, 2007); Peturssion *et al.*, "Protecting Groups in Carbohydrate Chemistry," *J. Chem. Educ.*, 1997, *74*(11), 1297; and Wuts *et al.*, *Protective Groups in Organic Synthesis*, 4th Ed., (Wiley, 2006).

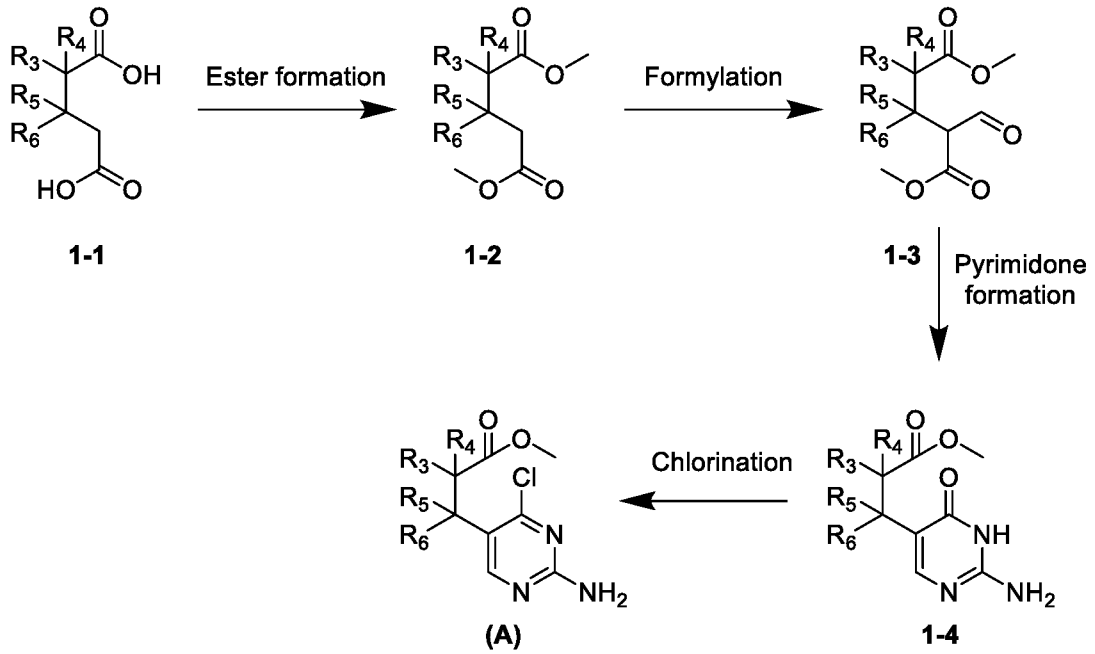
Reactions can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^{1}H or ^{13}C), infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), mass spectrometry or by chromatographic methods such as high performance liquid chromatography (HPLC) or thin layer chromatography (TLC).

The Schemes below provide general guidance in connection with preparing the compounds of the invention. One skilled in the art would understand that the preparations shown in the Schemes can be modified or optimized using general knowledge of organic chemistry to prepare various compounds of the invention.

Compounds of Formula (A-I) can be prepared, *e.g.*, using a process as illustrated in **Schemes 1** and **2** below.

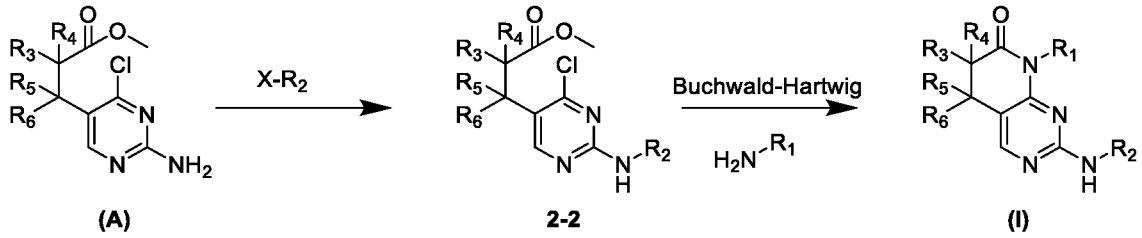
Compounds of Formula (A-I) can be prepared from an intermediate of general formula (A). Intermediate (A) can be prepared as shown in **Scheme 1**. **Scheme 1** shows that a diacid of formula 1-1 can be converted into a suitable diester, *e.g.*, a methyl or ethyl ester to provide compounds of formula 1-2, which can be formylated with an appropriate reagent (*e.g.*, methyl or ethyl formate) to provide compounds of formula 1-3. Reaction of compounds of formula 1-3 with an appropriate source of guanidine, such as guanidine carbonate or guanidine hydrochloride, can give compounds of formula 1-4. Finally, reaction of compounds of formula 1-4 with a suitable chlorinating reagent *e.g.*, phosphorus oxychloride can give structures of general formula (A).

Scheme 1



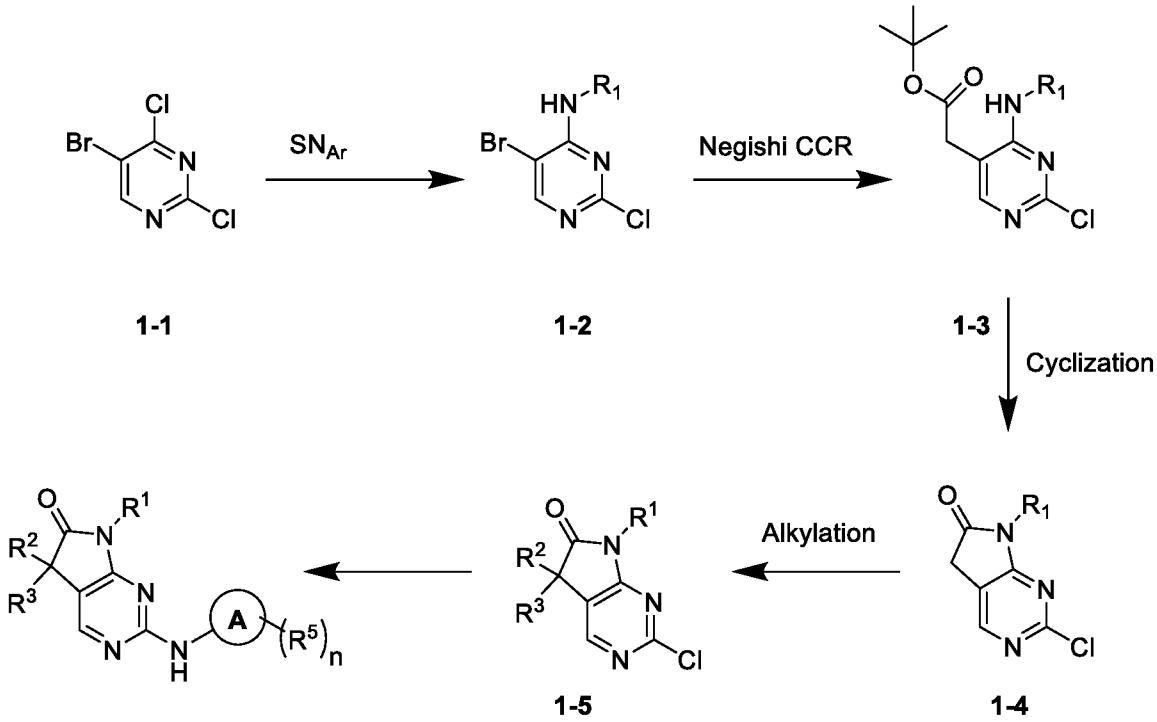
Intermediates of general formula A can be converted to compounds of formula (I) with various substituents at R_1 and as shown in **Scheme 2**. Compounds of formula (A) can be reacted with an appropriate R_2 substituent using a variety of methods (*e.g.*, reductive amination with an aldehyde or ketone, Buchwald-Hartwig amination, copper catalyzed amination, amide bond formation and others) to provide compounds of formula 2-2. The chloro group of compounds of formula 2-2 can be reacted with an appropriate amine under Buchwald-Hartwig amination conditions to provide compounds of formula (I).

Scheme 2



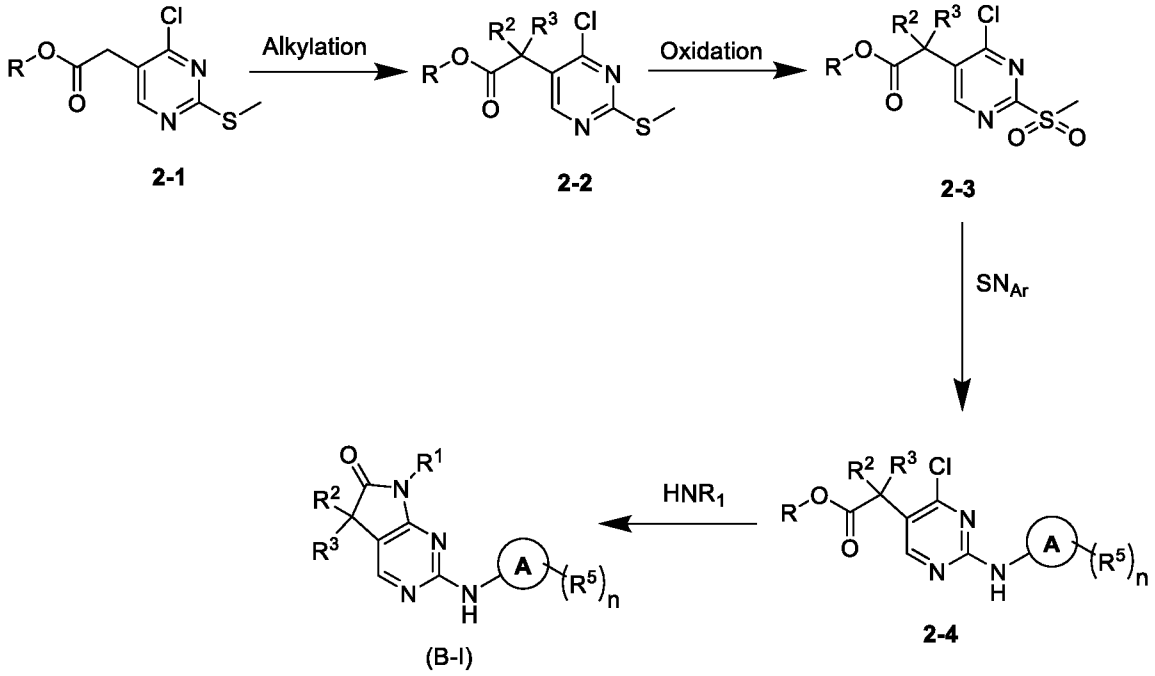
Compounds of Formula (B-I) can be prepared in a variety of manners depending on the position where variation is desired. For instance, compounds of Formula (B-I) with variation at Ring **A** can be prepared as shown in **Scheme 3.** In the process depicted in **Scheme 3**, selective displacement of the chloro group of the trihalo pyrimidine 1-1 with the desired amine provides compounds of formula 1-2. Intermediate 1-2 can be reacted via a selective Negishi cross coupling reaction (CCR) with an appropriate palladium precatalyst/ligand combination (*e.g.*, $Pd_2(dba)_3$ with QPhos or XPhos) to yield intermediate 1-3. Intermediate 1-3 can then be reacted via base promoted cyclization to provide a compound of formula 1-4. The desired substitution α to the amide of intermediate 1-4 can then be introduced (*e.g.*, via successive alkylation or Pd catalyzed arylation) to provide a compound of formula 1-5. Alternatively, reaction with a *bis* electrophile (*e.g.* 1,2-dibromoethane) under standard alkylation conditions provides compounds of formula 1-5 where R_2 and R_3 combined to form a cycle.) to provide a compound of formula 1-5. Finally, Buchwald-Hartwig amination with the appropriate amine provides compounds of Formula (B-I).

Scheme 3



Compounds of Formula (B-I) having different groups at R^1 can be formed as shown in **Scheme 4**. Thus, introduction of R^2 and R^3 of compound 2-1 as above provides compound 2-2, which can undergo selective oxidation of the sulfur (e.g., with m-CPBA) to provide intermediate 2-3. Selective SN_{Ar} reaction of intermediate 2-3 at the resulting sulfone with the appropriate N-formyl amine provides intermediate 2-4. Finally, reaction of intermediate 2-4 with the appropriate amine provides compounds of general formula (B-I). This coupling can be performed in one of two ways. First a tandem Buchwald-Hartwig amination and cyclization, catalyzed by the appropriate preformed catalyst (*e.g.*, RuPhos 2nd generation precatalyst or XantPhos 2nd generation precatalyst) can be used. Alternatively, a two-step protocol comprising of an SN_{Ar} reaction with the appropriate acidic (TFA) or basic (Hunig's base) catalyst and appropriate polar solvent (*i.e.*, 1,1,1-trifluoroethanol or 1-butanol) followed by a cyclization induced with the appropriate base (*i.e.*, sodium hydride).

Scheme 4



Methods of Treatment

The methods disclosed herein enable the assessment of whether or not a human subject having, suspected of having or at risk of developing a disease or disorder associated with CDK2 is likely to respond (e.g., likely to have greater improvement in disease as evidenced by disease remission/resolution, or have CDK2 inhibited) to a CDK2 inhibitor. A human subject having, suspected of having or at risk of developing a disease or disorder associated with CDK2 who is likely to respond to a CDK2 inhibitor can be administered a CDK2 inhibitor. Conversely, a human subject having, suspected of having or at risk of developing a disease or disorder associated with CDK2 who is less likely to respond to a CDK2 inhibitor can be administered an additional therapy that is suitable for treatment of the disease or disorder.

The methods of this disclosure also enable the stratification of human subjects having, suspected of having or at risk of developing a disease or disorder associated with CDK2 into groups of human subjects that are more likely to benefit, and groups of human subjects that are less likely to benefit, from treatment comprising a CDK2 inhibitor. The ability to select such human subjects from a pool of CDK2-associated disease or disorder human subjects who are being considered for treatment with a CDK2 inhibitor is beneficial for administering an effective treatment to the subject.

In one embodiment, the human subject to be treated with a CDK2 inhibitor has, is suspected of having, or is likely to develop a disease or disorder associated with CDK2. In certain embodiments, the human subject to be treated with a CDK2 inhibitor has, is suspected of having, or is likely to develop cancer.

If the human subject having a disease or disorder associated with CDK2 is more likely to respond to a CDK inhibitor (based on one or more of the markers described above (e.g., biomarkers or pharmacodynamics markers, e.g., CCNE1, p16, and Rb phosphorylation)), the human subject can then be administered an effective amount of the CDK2 inhibitor. An effective amount of the CDK2 inhibitor can suitably be determined by a health care practitioner taking into account, for example, the characteristics of the patient (age, sex, weight, race, etc.), the progression of the disease, and prior exposure to the drug. If the human subject is less likely to respond to a CDK2 inhibitor, the human subject can then be optionally administered a therapy that does not comprise a CDK2 inhibitor.

After stratifying or selecting a human subject based on whether the human subject will be more likely or less likely to respond to a CDK inhibitor, a medical practitioner (e.g., a doctor) can administer the appropriate therapeutic modality to the human subject. Methods of administering a CDK2 inhibitor are known in the art.

In cases where the human subject having a disease or disorder associated with CDK2 and predicted to respond to a CDK2 inhibitor has been previously administered one or more non-CDK2 inhibitor therapies, a CDK2 inhibitor can replace or augment a previously or currently administered therapy. For example, upon treating with a CDK2 inhibitor, administration of the one or more non-CDK2 inhibitor therapies can cease or diminish, e.g., be administered at lower levels. Administration of the previous therapy can be maintained while a CDK2 inhibitor is administered. In some embodiments, a previous therapy can be maintained until the level of a CDK2 inhibitor reaches a level sufficient to provide a therapeutic effect.

In a specific embodiment, provided herein is a method of treating a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2, comprising administering to the human subject a CDK2 inhibitor, wherein the human subject has been previously determined to (i) (a) have a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) have a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, and/or (c) express a p16 protein (e.g., a p16

protein comprising the amino acid sequence of SEQ ID NO:1), and (ii) (a) have an amplification of the CCNE1 gene and/or (b) an expression level of CCNE1 in a biological sample obtained from the subject that is higher than a control expression level of CCNE1. In certain embodiments, the biological sample was obtained from the human subject at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 2 months before the administering of the CDK2 inhibitor. In certain embodiments, the biological sample was obtained from the human subject at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, at most 4 weeks, or at most 2 months before the administering of the CDK2 inhibitor. In certain embodiments, the subject was determined to have a gene that encodes the p16 protein of SEQ ID NO:1 at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 2 months before the administering of the CDK2 inhibitor. In certain embodiments, the subject was determined to have a gene that encodes the p16 protein of SEQ ID NO:1 at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, at most 4 weeks, or at most 2 months before the administering of the CDK2 inhibitor. In a specific embodiment, the method further comprises:

(1) measuring, in a biological sample obtained from the subject after the administering the CDK2 inhibitor to the subject, a reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3; and

(2) after the measuring, continuing administering the CDK2 inhibitor to the human subject.

In certain embodiments, the biological sample obtained from the subject after the administering the CDK2 inhibitor to the subject was obtained at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks after the administering of the CDK2 inhibitor. In certain embodiments, the biological sample obtained from the subject after the administering the CDK2

inhibitor to the subject was obtained from the human subject at most 1 hour, at most 2 hours, at most 3 hours, at most 4 hours, at most 5 hours, at most 6 hours, at most 7 hours, at most 8 hours, at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, or at most 4 weeks after the administering of the CDK2 inhibitor. In certain embodiments, the continued administering of step (2) occurs at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 2 months after the measuring of step (1). In certain embodiments, the continued administering of step (2) occurs at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, at most 4 weeks, or at most 2 months after the measuring of step (1).

In another specific embodiment, provided herein is a method of treating a human subject having, suspected of having, or at risk of developing a disease or disorder associated with CDK2, comprising: (i) identifying, in a biological sample obtained from the human subject: (a) a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, (b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions, and/or (c) the presence of a p16 protein (e.g., a p16 protein comprising the amino acid sequence of SEQ ID NO:1), (ii) identifying, in a biological sample obtained from the human subject: (a) amplification of the CCNE1 gene and/or (b) an expression level of CCNE1 that is higher than a control expression level of CCNE1; and administering a CDK2 inhibitor to the human subject. In specific embodiments, the human subject has a disease or disorder associated with CDK2. In specific embodiments, the human subject is suspected of having or is at risk of developing a disease or disorder associated with CDK2. In certain embodiments, the administering of occurs at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 2 months after the identifying, in a biological sample obtained from the human subject, the CDKN2A gene, the p16 protein, and/or amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1. In certain embodiments, the administering occurs at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, at most 4 weeks, or at most 2 months after the identifying, in a

biological sample obtained from the human subject, the nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1, the CDKN2A gene lacking one or more inactivating nucleic acid substitutions, and/or the presence of a p16 protein, and/or amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1. In a specific embodiment, the method further comprises: measuring, in a biological sample obtained from the subject after the administering the CDK2 inhibitor to the subject, a reduced level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3, as compared to a control level of Rb phosphorylation at the serine corresponding to amino acid position 780 of SEQ ID NO:3; and, after the measuring, continuing administering the CDK2 inhibitor to the human subject. In certain embodiments, the biological sample obtained from the subject after the administering the CDK2 inhibitor to the subject was obtained from the human subject at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, or at least 4 weeks after the administering. In certain embodiments, the biological sample obtained from the subject after the administering the CDK2 inhibitor to the subject was obtained from the human subject at most 1 hour, at most 2 hours, at most 3 hours, at most 4 hours, at most 5 hours, at most 6 hours, at most 7 hours, at most 8 hours, at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, or at most 4 weeks after the administering. In certain embodiments, the continued administering occurs at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 2 weeks, at least 3 weeks, at least 4 weeks, or at least 2 months after the measuring. In certain embodiments, the continued administering occurs at most 1 day, at most 2 days, at most 3 days, at most 4 days, at most 5 days, at most 6 days, at most 7 days, at most 2 weeks, at most 3 weeks, at most 4 weeks, or at most 2 months after the measuring.

In some embodiments, the disease or disorder associated with CDK2 is N-myc amplified neuroblastoma cells (see Molenaar, et al., *Proc Natl Acad Sci USA* 106(31): 12968-12973) K-Ras mutant lung cancers (see Hu, S., et al., *Mol Cancer Ther,* 2015. 14(11): p. 2576-85, and cancers with FBW7 mutation and CCNE1 overexpression (see Takada, *et al.*, *Cancer Res*, 2017. 77(18): p. 4881-4893).

In some embodiments, the disease or disorder associated with CDK2 is lung squamous cell carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, bladder urothelial carcinoma, mesothelioma, or sarcoma.

In some embodiments, the disease or disorder associated with CDK2 is lung adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, or stomach adenocarcinoma.

In some embodiments, the disease or disorder associated with CDK2 is an adenocarcinoma, carcinoma, or cystadenocarcinoma.

In some embodiments, the disease or disorder associated with CDK2 is uterine cancer, ovarian cancer, stomach cancer, esophageal cancer, lung cancer, bladder cancer, pancreatic cancer, or breast cancer.

In some embodiments, the disease or disorder associated with CDK2 is a cancer.

In some embodiments, the cancer is characterized by amplification or overexpression of CCNE1. In some embodiments, the cancer is ovarian cancer or breast cancer, characterized by amplification or overexpression of CCNE1.

In some embodiments, the breast cancer is chemotherapy or radiotherapy resistant breast cancer, endocrine resistant breast cancer, trastuzumab resistant breast cancer, or breast cancer demonstrating primary or acquired resistance to CDK4/6 inhibition. In some embodiments, the breast cancer is advanced or metastatic breast cancer.

Examples of cancers that are treatable using the methods of the present disclosure include, but are not limited to, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, endometrial cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's disease, non-Hodgkin's lymphoma, 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, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder,

cancer of the kidney or urethra, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The methods of the present disclosure are also useful for the treatment of metastatic cancers.

In some embodiments, cancers treatable with methods of the present disclosure include melanoma (*e.g.*, metastatic malignant melanoma, BRAF and HSP90 inhibition-resistant melanoma), renal cancer (*e.g.*, clear cell carcinoma), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma), breast cancer, colon cancer, lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), squamous cell head and neck cancer, urothelial cancer (*e.g.*, bladder) and cancers with high microsatellite instability (MSI^{high}). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the methods of the disclosure.

In some embodiments, cancers that are treatable using the methods of the present disclosure include, but are not limited to, solid tumors (*e.g.*, prostate cancer, colon cancer, esophageal cancer, endometrial cancer, ovarian cancer, uterine cancer, renal cancer, hepatic cancer, pancreatic cancer, gastric cancer, breast cancer, lung cancer, cancers of the head and neck, thyroid cancer, glioblastoma, sarcoma, bladder cancer, etc.), hematological cancers (*e.g.*, lymphoma, leukemia such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), DLBCL, mantle cell lymphoma, non-Hodgkin lymphoma (including follicular lymphoma, including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma or multiple myeloma) and combinations of said cancers.

In some embodiments, cancers that are treatable using the methods of the present disclosure include, but are not limited to, cholangiocarcinoma, bile duct cancer, triple negative breast cancer, rhabdomyosarcoma, small cell lung cancer, leiomyosarcoma, hepatocellular carcinoma, Ewing's sarcoma, brain cancer, brain tumor, astrocytoma, neuroblastoma, neurofibroma, basal cell carcinoma, chondrosarcoma, epithelioid sarcoma, eye cancer, Fallopian tube cancer, gastrointestinal cancer, gastrointestinal stromal tumors, hairy cell leukemia, intestinal cancer, islet cell cancer, oral cancer, mouth cancer, throat cancer, laryngeal cancer, lip cancer, mesothelioma, neck cancer, nasal cavity cancer, ocular cancer, ocular melanoma, pelvic cancer, rectal cancer, renal cell

carcinoma, salivary gland cancer, sinus cancer, spinal cancer, tongue cancer, tubular carcinoma, urethral cancer, and ureteral cancer.

In some embodiments, the methods of the present disclosure can be used to treat sickle cell disease and sickle cell anemia.

In some embodiments, diseases and indications that are treatable using the methods of the present disclosure include, but are not limited to hematological cancers, sarcomas, lung cancers, gastrointestinal cancers, genitourinary tract cancers, liver cancers, bone cancers, nervous system cancers, gynecological cancers, and skin cancers.

Exemplary hematological cancers include lymphomas and leukemias such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), acute promyelocytic leukemia (APL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma, myeloproliferative diseases (*e.g.*, primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET)), myelodysplasia syndrome (MDS), T-cell acute lymphoblastic lymphoma (T-ALL) and multiple myeloma (MM).

Exemplary sarcomas include chondrosarcoma, Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma, angiosarcoma, fibrosarcoma, liposarcoma, myxoma, rhabdomyoma, rhabdosarcoma, fibroma, lipoma, harmatoma, and teratoma.

Exemplary lung cancers include non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), bronchogenic carcinoma, squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma, alveolar (bronchiolar) carcinoma, bronchial adenoma, chondromatous hamartoma, and mesothelioma.

Exemplary gastrointestinal cancers include cancers of the esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Kaposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), and colorectal cancer.

Exemplary genitourinary tract cancers include cancers of the kidney (adenocarcinoma, Wilm's tumor [nephroblastoma]), bladder and urethra (squamous

cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), and testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma).

Exemplary liver cancers include hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, and hemangioma.

Exemplary bone cancers include, for example, osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma, and giant cell tumors

Exemplary nervous system cancers include cancers of the skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma, glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), and spinal cord (neurofibroma, meningioma, glioma, sarcoma), as well as neuroblastoma and Lhermitte-Duclos disease.

Exemplary gynecological cancers include cancers of the uterus (endometrial carcinoma), cervix (cervical carcinoma, pre -tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), and fallopian tubes (carcinoma).

Exemplary skin cancers include melanoma, basal cell carcinoma, Merkel cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, and keloids. In some embodiments, diseases and indications that are treatable using the compounds of the present disclosure include, but are not limited to, sickle cell disease (*e.g.*, sickle cell anemia), triple-negative

breast cancer (TNBC), myelodysplastic syndromes, testicular cancer, bile duct cancer, esophageal cancer, and urothelial carcinoma.

Combination Therapy

A human subject treated with a CDK2 inhibitor according to the methods described herein can be treated in combination with one or more additional compositions or therapies that are effective for treatment of a disease or disorder associated with CDK2. In some embodiments, the CDK2 inhibitor is administered or used in combination with a BCL2 inhibitor or a CDK4/6 inhibitor.

I. Cancer therapies

Cancer cell growth and survival can be impacted by dysfunction in multiple signaling pathways. Thus, it is useful to combine different enzyme/protein/receptor inhibitors, exhibiting different preferences in the targets which they modulate the activities of, to treat such conditions. Targeting more than one signaling pathway (or more than one biological molecule involved in a given signaling pathway) may reduce the likelihood of drug-resistance arising in a cell population, and/or reduce the toxicity of treatment.

One or more additional pharmaceutical agents such as, for example, chemotherapeutics, anti-inflammatory agents, steroids, immunosuppressants, immune-oncology agents, metabolic enzyme inhibitors, chemokine receptor inhibitors, and phosphatase inhibitors, as well as targeted therapies such as Bcr-Abl, Flt-3, EGFR, HER2, JAK, c-MET, VEGFR, PDGFR, c-Kit, IGF-1R, RAF, FAK, and CDK4/6 kinase inhibitors such as, for example, those described in WO 2006/056399 can be used in combination with the compounds of the present disclosure for treatment of CDK2-associated diseases, disorders or conditions. Other agents such as therapeutic antibodies can be used in combination with the compounds of the present disclosure for treatment of CDK2-associated diseases, disorders or conditions. The one or more additional pharmaceutical agents can be administered to a patient simultaneously or sequentially.

In some embodiments, the CDK2 inhibitor is administered or used in combination with a BCL2 inhibitor or a CDK4/6 inhibitor.

The compounds as disclosed herein can be used in combination with one or more other enzyme/protein/receptor inhibitors therapies for the treatment of diseases,

such as cancer and other diseases or disorders described herein. Examples of diseases and indications treatable with combination therapies include those as described herein. Examples of cancers include solid tumors and non-solid tumors, such as liquid tumors, blood cancers. Examples of infections include viral infections, bacterial infections, fungus infections or parasite infections. For example, the compounds of the present disclosure can be combined with one or more inhibitors of the following kinases for the treatment of cancer: Akt1, Akt2, Akt3, BCL2, CDK4/6, TGF-βR, PKA, PKG, PKC, CaM-kinase, phosphorylase kinase, MEKK, ERK, MAPK, mTOR, EGFR, HER2, HER3, HER4, INS-R, IDH2, IGF-1R, IR-R, PDGFαR, PDGFβR, PI3K (alpha, beta, gamma, delta, and multiple or selective), CSF1R, KIT, FLK-II, KDR/FLK-1, FLK-4, flt-1, FGFR1, FGFR2, FGFR3, FGFR4, c-Met, PARP, Ron, Sea, TRKA, TRKB, TRKC, TAM kinases (Axl, Mer, Tyro3), FLT3, VEGFR/Flt2, Flt4, EphA1, EphA2, EphA3, EphB2, EphB4, Tie2, Src, Fyn, Lck, Fgr, Btk, Fak, SYK, FRK, JAK, ABL, ALK and B-Raf. In some embodiments, the compounds of the present disclosure can be combined with one or more of the following inhibitors for the treatment of cancer or infections. Non-limiting examples of inhibitors that can be combined with the compounds of the present disclosure for treatment of cancer and infections include an FGFR inhibitor (FGFR1, FGFR2, FGFR3 or FGFR4, e.g., pemigatinib (INCB54828), or INCB62079), an EGFR inhibitor (also known as ErB-1 or HER-1; e.g., erlotinib, gefitinib, vandetanib, orsimertinib, cetuximab, necitumumab, or panitumumab), a VEGFR inhibitor or pathway blocker (e.g., bevacizumab, pazopanib, sunitinib, sorafenib, axitinib, regorafenib, ponatinib, cabozantinib, vandetanib, ramucirumab, lenvatinib, ziv-aflibercept), a PARP inhibitor (e.g., olaparib, rucaparib, veliparib or niraparib), a JAK inhibitor (JAK1 and/or JAK2; e.g., ruxolitinib or baricitinib; or a JAK1 inhibitor; e.g., itacitinib (INCB039110), INCB052793, or INCB054707), a seitacitinib (INCB39110), an IDO inhibitor (e.g., epacadostat, NLG919, or BMS-986205, MK7162), an LSD1 inhibitor (e.g., GSK2979552, INCB59872 or INCB60003), a TDO inhibitor, a PI3K-delta inhibitor (e.g., parsaclisib (INCB50465) or INCB50797), a PI3K-gamma inhibitor such as PI3K-gamma selective inhibitor, a Pim inhibitor (e.g., INCB53914), a CSF1R inhibitor, a TAM receptor tyrosine kinases (Tyro-3, Axl, and Mer; e.g., INCB081776), an adenosine receptor antagonist (e.g., A2a/A2b receptor antagonist), an HPK1 inhibitor, a chemokine receptor inhibitor (e.g., CCR2 or CCR5 inhibitor), a SHP1/2 phosphatase inhibitor, a histone deacetylase inhibitor

(HDAC) such as an HDAC8 inhibitor, an angiogenesis inhibitor, an interleukin receptor inhibitor, bromo and extra terminal family members inhibitors (for example, bromodomain inhibitors or BET inhibitors such as INCB54329 and INCB57643), TAM receptor tyrosine kinases inhibitors (Tyro-3, Axl, and Mer; e.g., INCB81776); c-MET inhibitors (e.g., capmatinib); an anti-CD19 antibody (e.g., tafasitamab); an ALK2 inhibitor (e.g., INCB00928); or combinations thereof.

In some embodiments, the compound or salt described herein is administered with a PI3Kδ inhibitor. In some embodiments, the compound or salt described herein is administered with a JAK inhibitor. In some embodiments, the compound or salt described herein is administered with a JAK1 or JAK2 inhibitor (e.g., baricitinib or ruxolitinib). In some embodiments, the compound or salt described herein is administered with a JAK1 inhibitor. In some embodiments, the compound or salt described herein is administered with a JAK1 inhibitor, which is selective over JAK2.

Example antibodies for use in combination therapy include, but are not limited to, trastuzumab (*e.g.*, anti-HER2), ranibizumab (*e.g.*, anti-VEGF-A), bevacizumab (AVASTIN™, *e.g.*, anti-VEGF), panitumumab (*e.g.*, anti-EGFR), cetuximab (*e.g.*, anti-EGFR), rituxan (*e.g.*, anti-CD20), and antibodies directed to c-MET.

One or more of the following agents may be used in combination with the compounds of the present disclosure and are presented as a non-limiting list: a cytostatic agent, cisplatin, doxorubicin, taxotere, taxol, etoposide, irinotecan, camptosar, topotecan, paclitaxel, docetaxel, epothilones, tamoxifen, 5-fluorouracil, methotrexate, temozolomide, cyclophosphamide, SCH 66336, R115777, L778,123, BMS 214662, IRESSA™(gefitinib), TARCEVA™ (erlotinib), antibodies to EGFR, intron, ara-C, adriamycin, cytoxan, gemcitabine, uracil mustard, chlormethine, ifosfamide, melphalan, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, carmustine, lomustine, streptozocin, dacarbazine, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, oxaliplatin, leucovirin, ELOXATIN™ (oxaliplatin), pentostatine, vinblastine, vincristine, vindesine, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mithramycin, deoxycoformycin, mitomycin-C, L-asparaginase, teniposide 17.alpha.-ethinylestradiol, diethylstilbestrol, testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, testolactone, megestrolacetate, methylprednisolone, methyltestosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide,

estramustine, medroxyprogesteroneacetate, leuprolide, flutamide, toremifene, goserelin, carboplatin, hydroxyurea, amsacrine, procarbazine, mitotane, mitoxantrone, levamisole, navelbene, anastrazole, letrazole, capecitabine, reloxafine, droloxafine, hexamethylmelamine, avastin, HERCEPTIN™ (trastuzumab), BEXXAR™ (tositumomab), VELCADE™ (bortezomib), ZEVALIN™ (ibritumomab tiuxetan), TRISENOX™ (arsenic trioxide), XELODA™ (capecitabine), vinorelbine, porfimer, ERBITUX™ (cetuximab), thiotepa, altretamine, melphalan, trastuzumab, lerozole, fulvestrant, exemestane, ifosfomide, rituximab, C225 (cetuximab), Campath (alemtuzumab), clofarabine, cladribine, aphidicolon, rituxan, sunitinib, dasatinib, tezacitabine, Sml1, fludarabine, pentostatin, triapine, didox, trimidox, amidox, 3-AP, and MDL-101,731.

The compounds of the present disclosure can further be used in combination with other methods of treating cancers, for example by chemotherapy, irradiation therapy, tumor-targeted therapy, adjuvant therapy, immunotherapy or surgery. Examples of immunotherapy include cytokine treatment (e.g., interferons, GM-CSF, G-CSF, IL-2), CRS-207 immunotherapy, cancer vaccine, monoclonal antibody, bispecific or multi-specific antibody, antibody drug conjugate, adoptive T cell transfer, Toll receptor agonists, RIG-I agonists, oncolytic virotherapy and immunomodulating small molecules, including thalidomide or JAK1/2 inhibitor, PI3Kδ inhibitor and the like. The compounds can be administered in combination with one or more anti-cancer drugs, such as a chemotherapeutic agent. Examples of chemotherapeutics include any of: abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, anastrozole, arsenic trioxide, asparaginase, azacitidine, bevacizumab, bexarotene, baricitinib, bleomycin, bortezomib, busulfan intravenous, busulfan oral, calusterone, capecitabine, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, dalteparin sodium, dasatinib, daunorubicin, decitabine, denileukin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone propionate, eculizumab, epirubicin, erlotinib, estramustine, etoposide phosphate, etoposide, exemestane, fentanyl citrate, filgrastim, floxuridine, fludarabine, fluorouracil, fulvestrant, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin acetate, histrelin acetate, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib mesylate, interferon alfa 2a, irinotecan, lapatinib ditosylate, lenalidomide, letrozole, leucovorin, leuprolide acetate, levamisole, lomustine, meclorethamine, megestrol

acetate, melphalan, mercaptopurine, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone phenpropionate, nelarabine, nofetumomab, oxaliplatin, paclitaxel, pamidronate, panitumumab, pegaspargase, pegfilgrastim, pemetrexed disodium, pentostatin, pipobroman, plicamycin, procarbazine, quinacrine, rasburicase, rituximab, ruxolitinib, sorafenib, streptozocin, sunitinib, sunitinib maleate, tamoxifen, temozolomide, teniposide, testolactone, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, and zoledronate.

Additional examples of chemotherapeutics include proteasome inhibitors (*e.g.*, bortezomib), thalidomide, revlimid, and DNA-damaging agents such as melphalan, doxorubicin, cyclophosphamide, vincristine, etoposide, carmustine, and the like.

Example steroids include corticosteroids such as dexamethasone or prednisone.

Example Bcr-Abl inhibitors include imatinib mesylate (GLEEVAC™), nilotinib, dasatinib, bosutinib, and ponatinib, and pharmaceutically acceptable salts. Other example suitable Bcr-Abl inhibitors include the compounds, and pharmaceutically acceptable salts thereof, of the genera and species disclosed in U.S. Pat. No. 5,521,184, WO 04/005281, and U.S. Ser. No. 60/578,491.

Example suitable Flt-3 inhibitors include midostaurin, lestaurtinib, linifanib, sunitinib, sunitinib, maleate, sorafenib, quizartinib, crenolanib, pacritinib, tandutinib, PLX3397 and ASP2215, and their pharmaceutically acceptable salts. Other example suitable Flt-3 inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 03/037347, WO 03/099771, and WO 04/046120.

Example suitable RAF inhibitors include dabrafenib, sorafenib, and vemurafenib, and their pharmaceutically acceptable salts. Other example suitable RAF inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 00/09495 and WO 05/028444.

Example suitable FAK inhibitors include VS-4718, VS-5095, VS-6062, VS-6063, BI853520, and GSK2256098,and their pharmaceutically acceptable salts. Other example suitable FAK inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 04/080980, WO 04/056786, WO 03/024967, WO 01/064655, WO 00/053595, and WO 01/014402.

Example suitable CDK4/6 inhibitors include palbociclib, ribociclib, trilaciclib, lerociclib, and abemaciclib, and their pharmaceutically acceptable salts. Other

example suitable CDK4/6 inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 09/085185, WO 12/129344, WO 11/101409, WO 03/062236, WO 10/075074, and WO 12/061156.

In some embodiments, the compounds of the disclosure can be used in combination with one or more other kinase inhibitors including imatinib, particularly for treating patients resistant to imatinib or other kinase inhibitors.

In some embodiments, the compounds of the disclosure can be used in combination with a chemotherapeutic in the treatment of cancer, and may improve the treatment response as compared to the response to the chemotherapeutic agent alone, without exacerbation of its toxic effects. In some embodiments, the compounds of the disclosure can be used in combination with a chemotherapeutic provided herein. For example, additional pharmaceutical agents used in the treatment of multiple myeloma, can include, without limitation, melphalan, melphalan plus prednisone [MP], doxorubicin, dexamethasone, and Velcade (bortezomib). Further additional agents used in the treatment of multiple myeloma include Bcr-Abl, Flt-3, RAF and FAK kinase inhibitors. In some embodiments, the agent is an alkylating agent, a proteasome inhibitor, a corticosteroid, or an immunomodulatory agent. Examples of an alkylating agent include cyclophosphamide (CY), melphalan (MEL), and bendamustine. In some embodiments, the proteasome inhibitor is carfilzomib. In some embodiments, the corticosteroid is dexamethasone (DEX). In some embodiments, the immunomodulatory agent is lenalidomide (LEN) or pomalidomide (POM). Additive or synergistic effects are desirable outcomes of combining a CDK2 inhibitor of the present disclosure with an additional agent.

The agents can be combined with the present compound in a single or continuous dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms.

The compounds of the present disclosure can be used in combination with one or more other inhibitors or one or more therapies for the treatment of infections. Examples of infections include viral infections, bacterial infections, fungus infections or parasite infections.

In some embodiments, a corticosteroid such as dexamethasone is administered to a patient in combination with the compounds of the disclosure where the dexamethasone is administered intermittently as opposed to continuously.

The compounds as described herein, a compound as recited in any of the claims, or salts thereof can be combined with another immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines. Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MARTI and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF.

The compounds as described herein, a compound as recited in any of the claims, or salts thereof can be used in combination with a vaccination protocol for the treatment of cancer. In some embodiments, the tumor cells are transduced to express GM-CSF. In some embodiments, tumor vaccines include the proteins from viruses implicated in human cancers such as Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). In some embodiments, the compounds of the present disclosure can be used in combination with tumor specific antigen such as heat shock proteins isolated from tumor tissue itself. In some embodiments, the compounds as described herein, a compound as recited in any of the claims, or salts thereof can be combined with dendritic cells immunization to activate potent anti-tumor responses.

The compounds of the present disclosure can be used in combination with bispecific macrocyclic peptides that target Fe alpha or Fe gamma receptor-expressing effectors cells to tumor cells. The compounds of the present disclosure can also be combined with macrocyclic peptides that activate host immune responsiveness.

In some further embodiments, combinations of the compounds of the disclosure with other therapeutic agents can be administered to a patient prior to, during, and/or after a bone marrow transplant or stem cell transplant. The compounds of the present disclosure can be used in combination with bone marrow transplant for the treatment of a variety of tumors of hematopoietic origin.

The compounds as described herein, a compound as recited in any of the claims, or salts thereof can be used in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to, HIV,

Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas Aeruginosa.

Viruses causing infections treatable by methods of the present disclosure include, but are not limited to human papillomavirus, influenza, hepatitis A, B, C or D viruses, adenovirus, poxvirus, herpes simplex viruses, human cytomegalovirus, severe acute respiratory syndrome virus, ebola virus, measles virus, herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

Pathogenic bacteria causing infections treatable by methods of the disclosure include, but are not limited to, chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria.

Pathogenic fungi causing infections treatable by methods of the disclosure include, but are not limited to, Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Pathogenic parasites causing infections treatable by methods of the disclosure include, but are not limited to, Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

When more than one pharmaceutical agent is administered to a patient, they can be administered simultaneously, separately, sequentially, or in combination (*e.g.*, for more than two agents).

Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk

Reference" (PDR, e.g., 1996 edition, Medical Economics Company, Montvale, NJ), the disclosure of which is incorporated herein by reference as if set forth in its entirety.

II. Immune-checkpoint therapies

Compounds of the present disclosure can be used in combination with one or more immune checkpoint inhibitors for the treatment of diseases, such as cancer or infections. Exemplary immune checkpoint inhibitors include inhibitors against immune checkpoint molecules such as CBL-B, CD20, CD28, CD40, CD70, CD122, CD96, CD73, CD47, CDK2, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, HPK1, CD137 (also known as 4-1BB), ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, TLR (TLR7/8), TIGIT, CD112R, VISTA, PD-1, PD-L1 and PD-L2. In some embodiments, the immune checkpoint molecule is a stimulatory checkpoint molecule selected from CD27, CD28, CD40, ICOS, OX40, GITR and CD137. In some embodiments, the immune checkpoint molecule is an inhibitory checkpoint molecule selected from A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM3, TIGIT, and VISTA. In some embodiments, the compounds provided herein can be used in combination with one or more agents selected from KIR inhibitors, TIGIT inhibitors, LAIR1 inhibitors, CD160 inhibitors, 2B4 inhibitors and TGFR beta inhibitors.

In some embodiments, the compounds provided herein can be used in combination with one or more agonists of immune checkpoint molecules, e.g., OX40, CD27, GITR, and CD137 (also known as 4-1BB).

In some embodiments, the inhibitor of an immune checkpoint molecule is anti-PD1 antibody, anti-PD-L1 antibody, or anti-CTLA-4 antibody.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of PD-1 or PD-L1, e.g., an anti-PD-1 or anti-PD-L1 monoclonal antibody. In some embodiments, the anti-PD-1 or anti-PD-L1 antibody is nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab, cemiplimab, atezolizumab, avelumab, tislelizumab, spartalizumab (PDR001), cetrelimab (JNJ-63723283), toripalimab (JS001), camrelizumab (SHR-1210), sintilimab (IBI308), AB122 (GLS-010), AMP-224, AMP-514/MEDI-0680, BMS936559, JTX-4014, BGB-108, SHR-1210, MEDI4736, FAZ053, BCD-100, KN035, CS1001, BAT1306, LZM009,

AK105, HLX10, SHR-1316, CBT-502 (TQB2450), A167 (KL-A167), STI-A101 (ZKAB001), CK-301, BGB-A333, MSB-2311, HLX20, TSR-042, or LY3300054. In some embodiments, the inhibitor of PD-1 or PD-L1 is one disclosed in U.S. Pat. Nos. 7,488,802, 7,943,743, 8,008,449, 8,168,757, 8,217,149, or 10,308,644; U.S. Publ. Nos. 2017/0145025, 2017/0174671, 2017/0174679, 2017/0320875, 2017/0342060, 2017/0362253, 2018/0016260, 2018/0057486, 2018/0177784, 2018/0177870, 2018/0179179, 2018/0179201, 2018/0179202, 2018/0273519, 2019/0040082, 2019/0062345, 2019/0071439, 2019/0127467, 2019/0144439, 2019/0202824, 2019/0225601, 2019/0300524, or 2019/0345170; or PCT Pub. Nos. WO 03/042402, WO 2008/156712, WO 2010/089411, WO 2010/036959, WO 2011/066342, WO 2011/159877, WO 2011/082400, WO 2011/161699, or WO 2019/246110 which are each incorporated herein by reference in their entirety. In some embodiments, the inhibitor of PD-L1 is INCB086550.

In some embodiments, the antibody is an anti-PD-1 antibody, e.g., an anti-PD-1 monoclonal antibody. In some embodiments, the anti-PD-1 antibody is nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, cetrelimab toripalimab, sintilimab, AB122, AMP-224, JTX-4014, BGB-108, BCD-100, BAT1306, LZM009, AK105, HLX10, or TSR-042. In some embodiments, the anti-PD-1 antibody is nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, cetrelimab, toripalimab, or sintilimab. In some embodiments, the anti-PD-1 antibody is pembrolizumab. In some embodiments, the anti-PD-1 antibody is nivolumab. In some embodiments, the anti-PD-1 antibody is cemiplimab. In some embodiments, the anti-PD-1 antibody is spartalizumab. In some embodiments, the anti-PD-1 antibody is camrelizumab. In some embodiments, the anti-PD-1 antibody is cetrelimab. In some embodiments, the anti-PD-1 antibody is toripalimab. In some embodiments, the anti-PD-1 antibody is sintilimab. In some embodiments, the anti-PD-1 antibody is AB122. In some embodiments, the anti-PD-1 antibody is AMP-224. In some embodiments, the anti-PD-1 antibody is JTX-4014. In some embodiments, the anti-PD-1 antibody is BGB-108. In some embodiments, the anti-PD-1 antibody is BCD-100. In some embodiments, the anti-PD-1 antibody is BAT1306. In some embodiments, the anti-PD-1 antibody is LZM009. In some embodiments, the anti-PD-1 antibody is AK105. In some embodiments, the anti-PD-1 antibody is HLX10. In some embodiments, the anti-PD-1 antibody is TSR-042. In some embodiments, the anti-PD-1 monoclonal antibody is nivolumab or pembrolizumab. In some

embodiments, the anti-PD-1 monoclonal antibody is MGA012 (INCMGA0012; retifanlimab). In some embodiments, the anti-PD1 antibody is SHR-1210. Other anti-cancer agent(s) include antibody therapeutics such as 4-1BB (e.g., urelumab, utomilumab).

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of PD-L1, e.g., an anti-PD-L1 monoclonal antibody. In some embodiments, the anti-PD-L1 monoclonal antibody is atezolizumab, avelumab, durvalumab, tislelizumab, BMS-935559, MEDI4736, atezolizumab (MPDL3280A;also known as RG7446), avelumab (MSB0010718C), FAZ053, KN035, CS1001, SHR-1316, CBT-502, A167, STI-A101, CK-301, BGB-A333, MSB-2311, HLX20, or LY3300054. In some embodiments, the anti-PD-L1 antibody is atezolizumab, avelumab, durvalumab, or tislelizumab. In some embodiments, the anti-PD-L1 antibody is atezolizumab. In some embodiments, the anti-PD-L1 antibody is avelumab. In some embodiments, the anti-PD-L1 antibody is durvalumab. In some embodiments, the anti-PD-L1 antibody is tislelizumab. In some embodiments, the anti-PD-L1 antibody is BMS-935559. In some embodiments, the anti-PD-L1 antibody is MEDI4736. In some embodiments, the anti-PD-L1 antibody is FAZ053. In some embodiments, the anti-PD-L1 antibody is KN035. In some embodiments, the anti-PD-L1 antibody is CS1001. In some embodiments, the anti-PD-L1 antibody is SHR-1316. In some embodiments, the anti-PD-L1 antibody is CBT-502. In some embodiments, the anti-PD-L1 antibody is A167. In some embodiments, the anti-PD-L1 antibody is STI-A101. In some embodiments, the anti-PD-L1 antibody is CK-301. In some embodiments, the anti-PD-L1 antibody is BGB-A333. In some embodiments, the anti-PD-L1 antibody is MSB-2311. In some embodiments, the anti-PD-L1 antibody is HLX20. In some embodiments, the anti-PD-L1 antibody is LY3300054.

In some embodiments, the inhibitor of an immune checkpoint molecule is a small molecule that binds to PD-L1, or a pharmaceutically acceptable salt thereof. In some embodiments, the inhibitor of an immune checkpoint molecule is a small molecule that binds to and internalizes PD-L1, or a pharmaceutically acceptable salt thereof. In some embodiments, the inhibitor of an immune checkpoint molecule is a compound selected from those in US 2018/0179201, US 2018/0179197, US 2018/0179179, US 2018/0179202, US 2018/0177784, US 2018/0177870, US Ser. No. 16/369,654 (filed Mar. 29, 2019), and US Ser. No. 62/688,164, or a pharmaceutically

acceptable salt thereof, each of which is incorporated herein by reference in its entirety.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of KIR, TIGIT, LAIR1, CD160, 2B4 and TGFR beta.

In some embodiments, the inhibitor is MCLA-145.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CTLA-4, e.g., an anti-CTLA-4 antibody. In some embodiments, the anti-CTLA-4 antibody is ipilimumab, tremelimumab, AGEN1884, or CP-675,206.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of LAG3, e.g., an anti-LAG3 antibody. In some embodiments, the anti-LAG3 antibody is BMS-986016, LAG525, INCAGN2385, or eftilagimod alpha (IMP321).

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CD73. In some embodiments, the inhibitor of CD73 is oleclumab.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of TIGIT. In some embodiments, the inhibitor of TIGIT is OMP-31M32.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of VISTA. In some embodiments, the inhibitor of VISTA is JNJ-61610588 or CA-170.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of B7-H3. In some embodiments, the inhibitor of B7-H3 is enoblituzumab, MGD009, or 8H9.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of KIR. In some embodiments, the inhibitor of KIR is lirilumab or IPH4102.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of A2aR. In some embodiments, the inhibitor of A2aR is CPI-444.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of TGF-beta. In some embodiments, the inhibitor of TGF-beta is trabedersen, galusertinib, or M7824.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of PI3K-gamma. In some embodiments, the inhibitor of PI3K-gamma is IPI-549.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CD47. In some embodiments, the inhibitor of CD47 is Hu5F9-G4 or TTI-621.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CD73. In some embodiments, the inhibitor of CD73 is MEDI9447.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CD70. In some embodiments, the inhibitor of CD70 is cusatuzumab or BMS-936561.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of TIM3, e.g., an anti-TIM3 antibody. In some embodiments, the anti-TIM3 antibody is INCAGN2390, MBG453, or TSR-022.

In some embodiments, the inhibitor of an immune checkpoint molecule is an inhibitor of CD20, e.g., an anti-CD20 antibody. In some embodiments, the anti-CD20 antibody is obinutuzumab or rituximab.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of OX40, CD27, CD28, GITR, ICOS, CD40, TLR7/8, and CD137 (also known as 4-1BB).

In some embodiments, the agonist of CD137 is urelumab. In some embodiments, the agonist of CD137 is utomilumab.

In some embodiments, the agonist of an immune checkpoint molecule is an inhibitor of GITR. In some embodiments, the agonist of GITR is TRX518, MK-4166, INCAGN1876, MK-1248, AMG228, BMS-986156, GWN323, MEDI1873, or MEDI6469.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of OX40, e.g., OX40 agonist antibody or OX40L fusion protein. In some embodiments, the anti-OX40 antibody is INCAGN01949, MEDI0562 (tavolimab), MOXR-0916, PF-04518600, GSK3174998, BMS-986178, or 9B12. In some embodiments, the OX40L fusion protein is MEDI6383.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of CD40. In some embodiments, the agonist of CD40 is CP-870893, ADC-1013, CDX-1140, SEA-CD40, RO7009789, JNJ-64457107, APX-005M, or Chi Lob 7/4.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of ICOS. In some embodiments, the agonist of ICOS is GSK-3359609, JTX-2011, or MEDI-570.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of CD28. In some embodiments, the agonist of CD28 is theralizumab.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of CD27. In some embodiments, the agonist of CD27 is varlilumab.

In some embodiments, the agonist of an immune checkpoint molecule is an agonist of TLR7/8. In some embodiments, the agonist of TLR7/8 is MEDI9197.

The compounds of the present disclosure can be used in combination with bispecific antibodies. In some embodiments, one of the domains of the bispecific antibody targets PD-1, PD-L1, CTLA-4, GITR, OX40, TIM3, LAG3, CD137, ICOS, CD3 or TGFβ receptor. In some embodiments, the bispecific antibody binds to PD-1 and PD-L1. In some embodiments, the bispecific antibody that binds to PD-1 and PD-L1 is MCLA-136. In some embodiments, the bispecific antibody binds to PD-L1 and CTLA-4. In some embodiments, the bispecific antibody that binds to PD-L1 and CTLA-4 is AK104.

In some embodiments, the compounds of the disclosure can be used in combination with one or more metabolic enzyme inhibitors. In some embodiments, the metabolic enzyme inhibitor is an inhibitor of IDO1, TDO, or arginase. Examples of IDO1 inhibitors include epacadostat, NLG919, BMS-986205, PF-06840003, IOM2983, RG-70099 and LY338196. Inhibitors of arginase inhibitors include INCB1158.

As provided throughout, the additional compounds, inhibitors, agents, etc. can be combined with the present compound in a single or continuous dosage form, or they can be administered simultaneously or sequentially as separate dosage forms.

The following are examples of the practice of the invention. They are not to be construed as limiting the scope of the invention in any way.

EXAMPLES

Example 1. Characterization of cyclin E1 in ovarian and endometrial cancer cell lines

The cyclin E1 ("CCNE1") gene was evaluated in various ovarian and endometrial cancer cell lines (FIGs. 1A and 1B). CCNE1 was amplified in COV318, OVCAR3 OVARY, Fu-OV1, and KLE cells, each of which displayed a CCNE1 gain of function by copy number (copy number ("CN") > 2) (FIG. 1A). In contrast, CCNE1 was not amplified in COV504, OV56, or Igrov1 cells, each of which displayed copy neutral (2) or loss of function of the gene (CN ≤ 2). CN was obtained from the Broad Institute Cancer Cell Line Encyclopedia ("CCLE") database (Barretina, et al., *Nature*, 2012. 483(7391): p. 603-7, which is incorporated herein by reference in its entirety).

Western blot analysis was performed on protein samples from COV318, OVCAR3_OVARY, Fu-OV1, KLE, COV504, OV56, and Igrov1 cells to evaluate CCNE1 protein levels. CCNE1 protein levels were higher in cell lines with CCNE1 gain of function by copy number (CN > 2; i.e., COV318, OVCAR3 OVARY, Fu-OV1, and KLE cells) compared to cell lines with copy neutral or loss of function of the gene (CN ≤ 2; i.e., COV504, OV56, and Igrov1 cells).

Example 2. CDK2-knockdown by siRNA inhibits proliferation in CCNE1-amplified, but not CCNE1-non-amplified human cancer cell lines

The effect of CDK2-knockdown in CCNE1-amplified versus CCNE1-non-amplified cell lines was evaluated. CCNE1-amplified cell lines (Fu-OV1 and KLE) or CCNE1-non-amplified cell lines (COV504 and Igrov1) were treated with a control ("ctrl") or CDK2-specific small interfering RNAs ("siRNAs") ("CDK2 siRNA-1" and "CDK2 siRNA-2") (FIGs. 2A and 2B and 3A and 3B). Seventy-two hours after transfection with the siRNAs, the cells were harvested and subjected to cell cycle analysis by fluorescence activated cell sorting ("FACS") (FIGs. 2A and 3A). Knockdown of CDK2 was confirmed by western blot (FIGs. 2B and 3B). CDK2-knockdown inhibited proliferation in CCNE1-amplified cell lines, but not in CCNE1-non-amplified cell lines (FIGs. 2A and 3A).

A similar experiment was performed in additional CCNE1-amplified cell lines (COV318, OVCAR3, Fu-OV1, and KLE) and CCNE1-non-amplified cell lines

(COV504, OV56, and Igrov1) (FIG. 4). The percentage of cells at the S phase three days after treatment with CDK2-specific siRNAs was significantly decreased in CCNE1-amplified cell lines as compared to treatment with control siRNA (FIG. 4). Consistent with the results of FIGs. 2A and 3A, the percentage of cells at the S phase three days after treatment with CDK2-specific siRNAs was not significantly different in CCNE1-non-amplified cell lines as compared to treatment with control siRNA (FIG. 4).

Example 3. Proliferation in CCNE1 amplified and CCNE-non-amplified cell lines upon CDK4/6 inhibition

The effect of CDK4/6-inhibition in CCNE1-amplified versus CCNE1-non-amplified cell lines was evaluated. CCNE1-amplified cells (OVCAR3) or CCNE1-non-amplified cells (COV504) were treated with dimethyl sulfoxide ("DMSO") control or increasing concentrations of CDK4/6 inhibitor palbociclib (FIG. 5). Sixteen hours after treatment with DMSO or palbociclib, the cells were harvested and subjected to cell cycle analysis by FACS (FIG. 5). CDK4/6-inhibition resulted in dose-dependent inhibition of the proliferation in CCNE1-non-amplified cells, but not in CCNE1-amplified cells (FIG. 5).

A similar experiment was performed in a larger set of CCNE1-amplified cell lines (COV318 and OVCAR3) and CCNE1-non-amplified cell lines (COV504, OV56, and Igrov1) (FIG. 6). The percentage of cells at the S phase 16 hours after treatment with palbociclib was decreased in CCNE1-non-amplified cell lines in a dose-dependent fashion as compared to treatment with DMSO (FIG. 6). Consistent with the results of FIG. 5, the percentage of cells at the S phase 16 hours after treatment with palbociclib was not significantly different in CCNE1-amplified cell lines as compared to treatment with DMSO (FIG. 6).

Example 4. CDK2-knockdown blocks Rb phosphorylation at S780 in CCNE1-amplified, but not in CCNE1-non-amplified, cell lines

The effect of CDK2-knockdown on Rb phosphorylation at Ser-780 of SEQ ID NO:3 ("S780") in CCNE1-amplified versus CCNE1-non-amplified cell lines was evaluated. CCNE1-amplified cell lines (COV318, Fu-OV1 and KLE) or CCNE1-non-amplified cell lines (COV504, OV56 and Igrov1) were treated with ctrl or CDK2-specific siRNAs (FIGs. 7A and 7B). 72 hours after transfection with the

siRNAs, the cells were harvested and total protein was extracted and analyzed by western blot. Knockdown of CDK2 was confirmed by western blot. CDK2-knockdown blocked Rb phosphorylation at S780 in CCNE1-amplified cell lines (FIG. 7A), but not in CCNE1-non-amplified cell lines (FIG. 7B).

Example 5. Palbociclib blocks Rb phosphorylation at S780 in CCNE1 non-amplified, but not in CCNE1-amplified, cell lines

The effect of CDK4/6-inhibition on Rb phosphorylation at S780 in CCNE1-amplified versus CCNE1-non-amplified cell lines was evaluated. CCNE1-amplified cell lines (OVCAR3 and COV318) or CCNE1-non-amplified cell lines (COV504 and OV56) were treated with DMSO or various doses of palbociclib (FIGs. 8A and 8B). One or 15 hours after treatment, the cells were harvested and total protein was extracted and analyzed by western blot (FIG. 8). Palbociclib treatment blocked Rb phosphorylation at S780 in CCNE1-non-amplified cell lines (FIG. 8B), but not in CCNE1-amplified cell lines (FIG. 8A).

Example 6. CDK2 degradation by dTAG decreases Rb phosphorylation at S780

To further confirm that CDK2 knockdown decreases Rb phosphorylation at S780 in CCNE1-amplified cells (see Example 4), the dTAG system was used to degrade CDK2 and the level of S780-phosphorylated Rb was evaluated (Erb et al., *Nature*, 2017, 543(7644):270-274, which is incorporated herein by reference in its entirety). Briefly, OVCAR3 cells were engineered to express Cas9 by lentiviral transduction of Cas9 construct. The OVCAR3-Cas9 cells were then engineered to express CDK2-FKBP12F36V-HA fusion protein by lentiviral transduction of CDK2-FKBP12F36V-HA expression construct. Next, to engineer the line to have endogenous CDK2 inactivated, OVCAR3 (Cas9, CDK2-FKBP12F36V-HA) cells were transduced with CDK2 sgRNA ("CDK2-gRNA"); OVCAR3 (Cas9, CDK2-FKBP12F36V-HA) cells transduced with non-targeting sgRNA ("Ctl-gRNA"; Cellecta) served as a control cell line.

To degrade CDK2-FKBP12F36V-HA protein by dTAG (FIG. 9A), cells were treated with DMSO or with a titration of concentrations of dTAG for 14 hours. Cells were collected and processed for Western blot (FIG. 9B). A dose-responsive degradation of CDK2-FKBP12(F36V) was detected by western blot after treatment with dTAG in both control- and CDK2-gRNA treated cells (FIG. 9B). Degradation

was further confirmed by western blot for HA-Tag. Endogenous CDK2 protein was detected in OVCAR3 cells treated with control gRNA, but not with CDK2-gRNA (FIG. 9B). CDK2-FKBP12(F36V) degradation inhibited Rb phosphorylation at S780 in CDK2 knockout OVCAR3 cells, but not in OVCAR3 cells with endogenous CDK2 expression.

Example 7. p-Rb S780 HTRF cellular Assay for identification of CDK2 inhibitors

An in vitro CDK2/CCNE1 enzyme activity assay was used to measure phosphorylation of a peptide substrate using homogenous time-resolved energy transfer ("HTRF"). First, the specificity of 8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one (Compound A) for CDK2 inhibition was confirmed via a kinase activity assay (FIG. 10A). To this end, the LANCE® Ultra kinase assay was used with a ULight™-labeled EIF4E-binding protein 1 (Thr37/46) peptide (PerkinElmer, TRF0128-M) as substrate and an Europium-labeled anti-phospho-EIF4E binding protein1 (Thr37/46) antibody (PerkinElmer, TRF0216-M). A ratio of fluorescence transferred to the labeled substrate (665 nm) relative to fluorescence of the Europium donor (620 nm) represents the extent of phosphorylation. The IC_{50} for Compound A was determined to be 1.1 nM (FIG. 10A). In contrast, the IC_{50} for the CDK4/6 inhibitor palbociclib was 10,000 nM (FIG. 10A).

Next, a CDK2 pRb (S780) HTRF cellular assay was performed, enabling the quantitative detection of Rb phosphorylated on serine 780 in CCNE1 amplified COV318 cells upon treatment with Compound A or palbociclib (FIG. 10B). Treatment with Compound A, but not palbociclib, inhibited Rb phosphorylation on serine 780 in CCNE1 amplified cells (FIG. 10B). The IC_{50} for Compound A in this assay was 37 nM, while the IC_{50} for palbociclib was > 3,000 nM (FIG. 10B).

Example 8. Bioinformatics analysis of CCLE dataset reveals the sensitivity to CDK2 inhibition in CCNE1 amplified cells relies on functional p16

In an attempt to identify a biomarker for predicting sensitivity to CDK2-inhibition in CCNE1-amplified cells, 460 cell lines from CCLE were analyzed (Barretina, *supra*). First, the cell lines were filtered based on CCNE1 copy number and expression and CDK2 sensitive score based on shRNA knockdown data. A total

of 41 cell lines were identified as having CCNE1 copy number of > 3 and CCNE1 expression score (CCLE: > 3). Of these 41 cell lines, 18 (44%) were sensitive to CDK2 inhibition (CDK2 sensitive score ≤-3), while 23 (56%) were insensitive to CDK2 inhibition (CDK2 sensitive score >-3).

Next, the p16 status was evaluated in the CDK2-sensitive and CDK2-insensitive cell lines (FIG. 11). Of the 18 cell lines that were sensitive to CDK2-inhibition, 100% expressed normal p16 gene (FIG. 11). In contrast, only 4 of the 23 CDK2-insensitive cell lines expressed normal p16 gene (FIG. 11). The majority of the 23 CDK2-insensitive cell lines displayed dysfunctional p16 gene expression: the p16 gene was deleted in 10 of 23 cell lines; the p16 gene was silenced in 5 of the 23 cell lines, and the p16 gene was mutated in 4 of the 23 cell lines (FIG. 11).

A summary of CDK2 sensitivity and CDKN2A/p16 status in CCNE1 amplified cell lines is provided in Table 2, below.

Table 2. Cell lines with CDK2 sensitive Score ≤ 3 counted as CDK2 Sensitive lines; ≥3 as CDK2 insensitive line. Cell lines verified in experiments are in bold. NCIN87_STOMACH showed no CDKN2A/P16 protein expression in western blot. CCNE1 and CDKN2A/P16 copy number were calculated based on CCLE dataset. Expression Score <0 counted as gene silencing.

Cell Lines	CDK2 sensitive Score	CCNE1 Copy No.	CDKN2A Copy No.	CDKN2A/p16 mRNA Expression Score	CDKN2a/p16 Dysfunction
HCC1569_BREAST	-9.6	16	2	5.11	
OVISE_OVARY	-9.4	3	2	4.17	
MKN1_STOMACH	-8.9	5	1	4.28	
EFE184_ENDOMETRIUM	-8.7	3	2	3.97	
KURAMOCHI_OVARY	-8.2	3	2	3.60	
MKN7_STOMACH	-7.7	21	1	4.37	
MDAMB157_BREAST	-7.6	6	2	5.01	
HCC70_BREAST	-7.6	4	4	4.88	
N1HOVCAR3_OVARY	-7.4	10	2	4.15	
FUOV1_OVARY	-7	10	3	5.19	
KLE_ENDOMETRIUM	-7	7	2	6.24	
COV318_OVARY	-7	14	2	5.09	
CAOV4_OVARY	-6.7	3	2	3.59	
MFE280_ENDOMETRIUM	-6.3	4	2	4.97	
NCIH661_LUNG	-6.2	5	2	3.73	
OVCAR4_OVARY	-4.3	4	1	4.77	
SNU8_OVARY	-3.8	5	3	5.35	

OVCAR8_OVARY	-3.7	3	2	5.21	
RMUGS_OVARY	-2.8	4	1	-0.08	Silencing
NCCSTCK140_STOMACH	-2.7	3	0	-4.70	Deletion
NCIH2286_LUNG	-1.6	3	1	3.63	Mutation
HOP62_LUNG	-1.4	4	0	-1.21	Deletion
LN340_CENTRAL_NERVOUS_SYSTEM	-1.0	3	0	-5.47	Deletion
NCIH1339_LUNG	-0.8	3	2	2.42	Unknown
NC1N87_STOMACH	0.1	3	2	4.67	No protein
U2OS_BONE	0.4	3	1	-5.72	Silencing
SF172_CENTRAL_NERVOUS_SYSTEM	0.5	3	0	-2.35	Deletion
CAL120_BREAST	0.6	4	1	4.86	
RMGI_OVARY	0.9	3	0	-3.33	Deletion
OV90_OVARY	0.9	3	1	3.95	Mutation
SNU601_STOMACH	1.1	4	2	-3.79	Silencing
EW8_BONE	1.5	5	1	3.11	
JHESOAD1_OESOPHAGUS	1.7	5	0	-5.52	Deletion
HCC1806_BREAST	1.9	8	0	-4.61	Deletion
NCIH2170_LUNG	2.0	3	0	-3.73	Deletion
HCC1428_BREAST	2.3	3	2	2.28	
A549_LUNG	2.5	4	0	-6.13	Deletion
LXF289_LUNG	2.6	4	3	4.10	Mutation
AGS_STOMACH	3.0	3	2	-5.56	Silencing
NCIH647_LUNG	3.0	4	0	-5.07	Deletion
HLF_LIVER	3.9	3	2	3.40	

Example 9. CCNE1 amplified cells with dysfunctional p16 do not respond to CDK2 inhibition

To further evaluate the role of p16 in CDK2-sensitivity in CCNE1-amplified cells, p16 protein expression in three gastric cell lines with CCNE1-amplification was evaluated by western blot. AGS and NCI-N87 cells displayed absent or dramatically reduced levels of p16 (FIG. 12A). In contrast, p16 protein was detected in MKN1 cellular protein extracts (FIG. 12A).

Next, the impact of CDK2-knockdown in these cells was evaluated. Mkn1, Ags, and NCI-N87 cells were treated with control or CDK2-specific siRNA. Three days-post-siRNA transfection, cell cycle phase distribution of the cells was evaluated by FACS. The percentage of cells at the S phase in the Mkn1 cells (CCNE1-amplified, p16 protein detected) was significantly decreased in the CDK2 siRNA-treated cells as compared to control (FIG. 12B). In contrast, the percentage of cells at the S phase was not significantly decreased in Ags and NCI-N87 cells (CCNE1-

amplified, dysfunctional p16 protein levels) after treatment with CDK2 siRNA as compared to control (FIG. 12B).

Example 10. p16 knockdown by siRNA abolishes CDK2 inhibition induced cell cycle suppression in CCNE1 amplified cells

To confirm the role of p16 in CDK2-sensitivity of CCNE1-amplified cells, COV318 cells were treated with control or p16-specifict siRNA. Seventy-two hours after transfection, cells were treated with DMSO (control) or 100 nM of Compound A. Sixteen hours after treatment with DMSO or the CDK2-inhibitor, cells were harvested and subjected to cell cycle analysis by FACS. Consistent with the results described above, the percentage of S phase cells significantly decreased in the control siRNA-treated cells treated with CDK2-inhibitor (Compound A), but not with the DMSO control (FIG. 13). In contrast, the percentage of S phase cells was not significantly decreased after treatment with the CDK2-inhibitor (Compound A) in p16 knocked down cells as compared to DMSO control (FIG. 13).

Materials and Methods used in Examples 1-10

Cell culture and transfection

Human cyclin E1 (CCNE1) amplified ovarian cell lines OVCAR3, COV318, Fu-OV1, endometrial cell line KLE, gastric cell lines MKN1, AGS, NCIN87, and CCNE1 non-amplified ovarian cell lines COV504, OV56, Igrov1 were cultured in RPMI 1640 medium. The complete growth medium was supplemented with 10% FBS, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 100 units/mL penicillin G and 100 μg/mL streptomycin in 37° C humidified incubator and an atmosphere of 5% CO_2 in air. Fu-OV1 line was purchased from Leibniz-Institute DSMZ –German Collection of Microorganisms and Cell Cultures; MKN1 was purchased from Japanese Cancer Research Resources Bank; and the rest of cell lines were purchased from American Type Culture Collection. For transfection, cells were seeded into 6-well for 24 hours and transiently transfected by Lipofectamine 2000 Reagent (Thermo Fisher, 11668027). ON-TARGETplus Human CKD2 siRNAs (GE Healthcare Dharmacon, J-003236-11-0002 and J-003236-12-0002) and ON-TARGETplus Human CDKN2A/p16 siRNAs (GE Healthcare Dharmacon, J-011007-08-0002) were used to knockdown the endogenous CDK2 and CDKN2A/p16. ON-TARGETplus

Non-targeting Pool (GE Healthcare Dharmacon, D-001810-10-20) was used as a negative control.

Western blot analysis

Whole cell extracts were prepared using RIPA buffer (Thermo Scientific, 89900) with a Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, 78440). Protein concentration was quantified with a BCA Protein Assay Kit (Thermo Scientific, 23225) and 40 μg of protein lysates were loaded for SDS–PAGE using precast gradient gels (Bio-Rad, Hercules, No. 456-1094). Samples were diluted in 5X Laemmli buffer (300 mM Tris-HCl pH 6.8, 10% SDS (w/v), 5% 2-mercaptoethanol, 25% glycerol (v/v),0.1% bromphenol blue w/v) and boiled for 5 minutes. 35 μg of proteins were separated by 8–15%SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes. Unspecific binding sites on the PVDF membranes were blocked with 5% non-fat milk in TBST (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 1% Tween-20). Membranes were hybridized with antibodies against anti-CDKN2A/p16 (Cell Signaling Technology, 92803S), anti-Cas9 (Cell Signaling Technology, 97982S), anti-HA (Cell Signaling Technology, 3724S), anti-Rb (Cell Signaling Technology, 9309S), anti-phospho-Rb (Ser780) (Cell Signaling Technology, 8180S), anti-CDK2 (Cell Signaling Technology, 2546S), anti-CCNE1 (Cell Signaling Technology, 20808S) and anti-GAPDH (Cell Signaling Technology, 8884S) for overnight at 4° C, followed by incubation with horseradish peroxidase(HRP)-conjugated secondary antibodies for 1 hour at room temperature. The membranes were then developed using Immobilon Western chemiluminescence HRP substrates (Millipore, WBKLS0500). Images were captured by Luminescence/Fluorescence Imaging System Odyssey CLx Imager (LI-COR).

Cell cycle analysis

Cells were seeded in six-well tissue culture plates and 24 hours later were treated with a titration of concentrations of Palbociclib or Compound A. After overnight treatment, cells exposed to 10 μM EdU for 3 hours before detection of EdU-DNA by Click-iT AlexaFluor® 647 azide kit (Life Technology, C10424) following the manufacturer's instructions. Bulk DNA was stained with DAPI. Compound-treated and DMSO treated control cells were acquired with CytoFlex (Beckman Coulter) and were analyzed using the FlowJo software. For cell cycle

analysis of cells with siRNA knockdown, 72 hours after siRNA transfection, cells exposed to 10 μM EdU for 3 hours before detection of Click-iT Alexa Fluor® 647 azide kit.

Plasmids

LentiCas9 plasmid pRCCH-CMV-Cas9-2A (Cellecta, SVC9-PS) was used for Cas9 expression. sgRNA-CDK2 lentiviral construct, designed to target AAGCAGAGATCTCTCGGA (SEQ ID NO:8) of CDK2, was cloned into sgRNA expression vector pRSG-U6 and purchased from Cellecta (93661). For CDK2-FKBP12F36V-HA expression, a 1306 base pair DNA fragment encoding CDK2 and FKBP12F36V-2xHA tag at the C-terminus was synthesized and cloned into EcoRI and BamHI digested pCDH-EF1α-MCS-T2A-Puro lentivector (Systembio, CD527A-1).

Sequence of 1306 bp DNA fragment:

CCTCGAATTCAGCTGC**ATGGAGAACTTCCAAAAGGTGGAAAAGA**
TCGGAGAGGGCACGTACGGAGTTGTGTACAAAGCCAGAAACAAGTTG
ACGGGAGAGGTGGTGGCGCTTAAGAAAATCCGCCTGGACACTGAGAC
TGAGGGTGTGCCCAGTACTGCCATCCGAGAGATCTCTCTGCTTAAGGA
GCTTAACCATCCTAATATTGTCAAGCTGCTGGATGTCATTCACACAGAA
AATAAACTCTACCTGGTTTTTGAATTTCTGCACCAAGATCTCAAGAAAT
TCATGGATGCCTCTGCTCTCACTGGCATTCCTCTTCCCCTCATCAAGA
GCTATCTGTTCCAGCTGCTCCAGGGCCTAGCTTTCTGCCATTCTCATC
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AGGGGGCCATCAAGCTAGCAGACTTTGGACTAGCCAGAGCTTTTGGA
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GACTCTGGGGACCCCAGATGAGGTGGTGTGGCCAGGAGTTACTTCTA
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GTAAAGTTGTACCTCCCCTGGATGAAGATGGACGGAGCTTGTTATCGC
AAATGCTGCACTACGACCCTAACAAGCGGATTTCGGCCAAGGCAGCCC
TGGCTCACCCTTTCTTCCAGGATGTGACCAAGCCAGTACCCCATCTTC

GA*CTCGGAGTGCAGGTGGAAACCATCTCCCCAGGAGACGGGCGCACCTTCCC CAAGCGCGGCCAGACCTGCGTGGTGCACTACACCGGGATGCTTGAAGATGGAA AGAAAGTTGATTCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAA GCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTGGGT CAGAGAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCAC CCAGGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAA CTGGAAGGATACCCTTACGACGTTCCTGATTACGCTTACCCTTACGACGTTCCTG ATTACGCT*GGATCCTAATTCGAAAGC (SEQ ID NO:4)

GAATTC (SEQ ID NO:5; EcoRI), GGATCC (SEQ ID NO:6; BamHI) and TTCGAA (SEQ ID NO:7; BstBI) restriction enzyme sites were underlined. Sequence encoding CDK2 is in bold and sequence of FKBP12F36V-HA is in italics. 3 nucleic acids underlined within CDK2 sequence indicated modifications that abolished PAM sites to avoided CRISPR knockout effect. These changes did not change amino acids encoded.

Lentivirus production

Production of lentivirus was performed in 293T cells by co-transfection of Lentiviral Packaging Mix (Sigma, SHP001), and a given lentiviral expression plasmid using Lipofectamine 2000. Viral supernatants were collected 48 and 72 hours after transfection, filtered through a 0.22 μm membrane. All cells lines were transduced by spinoculation at 2000 revolutions per minute (rpm) for 1 hour at room temperature with 8 μg/mL polybrene (Santa Cruz, sc-134220).

CDK2-dTAG cells

OVCAR3 cells were first engineered to express Cas9 by lentiviral transduction of Cas9 construct. Cells were selected and maintained in 100 μg/mL hygromycin (Life Technologies, 10687010) and verified to express Cas9 by immunoblot. OVCAR3-Cas9 cells were then engineered to express CDK2-FKBP12F36V-HA fusion protein by lentiviral transduction of CDK2- FKBP12F36V-HA expression construct and selection with 2 μg/mL puromycin dihydrochloride (Life Technologies, A1113803). Expression of CDK2- FKBP12F36V-HA was verified by immunoblot using anti-CDK2 and anti-HA antibodies. Next, to engineer the line to have endogenous CDK2 inactivated, OVCAR3 (Cas9, CDK2-FKBP12F36V-HA) cells were transduced with CDK2 sgRNA and selected by 50 μg/mL Zeocin (Life

Technologies, R25001). Inactivated expression of endogenous CDK2 in the expanded clones was tested by immunoblotting. OVCAR3 (Cas9, CDK2-FKBP12F36V-HA) cells transduced with non-targeting sgRNA (Cellecta) were served as a control cell line.

To degrade CDK2-FKBP12F36V-HA protein by dTAG, 200,000 cells were seeded in 1 mL media in triplicate in a 24-well plate and treated with dimethyl sulfoxide (DMSO) or with a titration of concentrations of dTAG for 14 hours. Cells were collected and processed for Western blot.

CDK2 / CCNE1 enzymatic assay

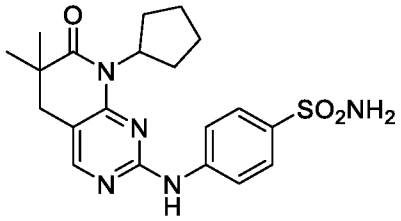
In vitro CDK2/CCNE1 enzyme activity assay measures phosphorylation of a peptide substrate using homogeneous time-resolved energy transfer (HTRF). The LANCE® Ultra kinase assay used a ULight™-labeled EIF4E-binding protein 1 (Thr37/46) peptide (PerkinElmer, TRF0128-M) as substrate and an Europium-labeled anti-phospho-EIF4E binding protein1 (Thr37/46) antibody (PerkinElmer, TRF0216-M). A ratio of fluorescence transferred to the labeled substrate (665 nm) relative to fluorescence of the Europium donor (620 nm) represents the extent of phosphorylation. Ratios for treated wells are normalized to DMSO only (100% activity) and no enzyme (0% activity) controls. Normalized data is analyzed using a four parameter dose response curve to determine IC_{50} for each compound.

CDK2 pRb (S780) HTRF cellular Assay

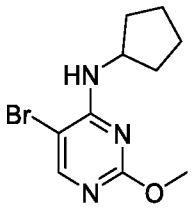
CDK2 pRb (S780) HTRF cellular assay enables the quantitative detection of Rb phosphorylated on serine 780 in CCNE1 amplified COV318 cells. The assay comprised two antibodies: Europium cryptate labeled anti-Phospho-Rb S780 antibody (donor) and d2 labeled anti-Rb antibody (acceptor). In brief, COV318 cells were seeded into the wells of 96-well plate at a density of 25,000 per well with 9-point, 3-fold serial diluted compounds and cultured overnight at 37 degree with 5% CO_2. The final concentrations of compounds start from 3 μM. The next day cells were lysed in 70 μL 1X Phospho-total protein lysis buffer #2 (Cisbio), supplemented with 0.7 μL blocking buffer (Cisbio) and 1.4 μL protease inhibitor cocktail set III, EDTA-free (Calbiochem, 539134). 16 μL of cell lysates were mixed with 4 μL of the fluorophore-conjugated antibodies to a final concentration of 0.188 nM cryptate-labeled anti-Phospho-Rb S780 antibody and 0.14 nM d2 labeled anti-Rb antibody.

After 2h of incubation at room temperature, HTRF signals were measured on the PHERAstar microplate reader (BMG Labtech), using 340 nm as excitation wavelength, a 620 nm filter for the Europium donor fluorescence, and a 665-nm filter for the acceptor fluorescence detection. HTRF signals were calculated as the HTRF ratio (ratio of fluorescence measured at 665 nm and 620 nm) × 10000.

Example A1. 4-((8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d[pyrimidin-2-yl)amino)benzenesulfonamide

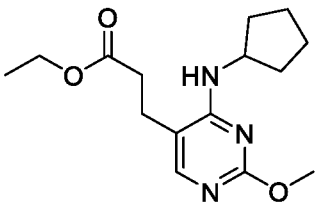


*Step 1. 5-bromo-*N*-cyclopentyl-2-methoxypyrimidin-4-amine*



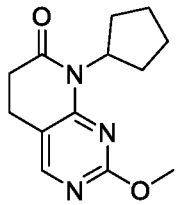
To a solution of 5-bromo-2,4-dichloropyrimidine (3.08 mL, 24.05 mmol) in THF (80 mL) was added cyclopentanamine (2.62 mL, 26.5 mmol) and the reaction mixture stirred at r.t. for 2 hr, then filtered. The filtrate was concentrated and dissolved in sodium methoxide in MeOH (21% w/w, 3 mL), then heated to reflux for 2 hr. The mixture was diluted with water and ethyl acetate and the layers were separated. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified by Biotage Isolera™ (0-50% ethyl acetate in hexanes) to provide the desired product as a white solid (4.7g, 72%). LCMS calculated for $C_{10}H_{15}BrN_3O$ $(M+H)^+$: m/z = 272.0/274.0; Found: 272.0/274.0.

Step 2. ethyl 3-(4-(cyclopentylamino)-2-methoxypyrimidin-5-yl)propanoate



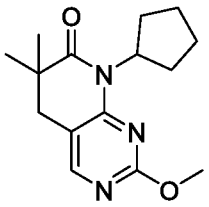
To a mixture of 5-bromo-*N*-cyclopentyl-2-methoxypyrimidin-4-amine (500 mg, 1.837 mmol), triethylamine (512 µL, 3.67 mmol), ethyl acrylate (300 µL, 2.76 mmol) and tetrakis(triphenylphosphine)palladium(0) (212 mg, 0.184 mmol) was added DMF (6 mL) and the reaction flask was evacuated, back filled with nitrogen, then stirred at 120 °C overnight. The mixture was then poured into ethyl acetate/water and the layers separated. The aqueous layer was extracted with ethyl acetate and the combined organics were washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (0-100% ethyl acetate in hexanes). The intermediate was dissolved in EtOH (6 mL) and palladium on carbon (10%, 391 mg, 0.367 mmol) was added. The reaction flask was evacuated, then back filled with hydrogen gas from a balloon. The reaction mixture was stirred at r.t. for 3 hr, then diluted with ethyl acetate and filtered through a plug of Celite. The filtrate was concentrated and the crude product used in the next step without further purification (340 mg, 63%). LCMS calculated for $C_{15}H_{24}N_3O_3$ $(M+H)^+$: m/z = 294.2; Found: 294.2.

*Step 3. 8-cyclopentyl-2-methoxy-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one*



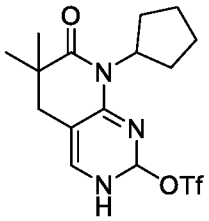
To a solution of ethyl 3-(4-(cyclopentylamino)-2-methoxypyrimidin-5-yl)propanoate (5.0 g, 17.04 mmol) in THF (28 mL)/Water (28 mL) was added lithium hydroxide hydrate (1.073 g, 25.6 mmol) and the reaction mixture was stirred at r.t. for 30 mins, then quenched with HCl (12 N, 2.13 mL, 25.6 mmol) and concentrated. The crude product was dissolved in DMF (4 mL) and HATU (7.13 g, 18.75 mmol) and Hunig's base (5.95 mL, 34.1 mmol) was added. The reaction was then stirred at r.t. for 2 hr, quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (20-100% ethyl acetate in hexanes) to provide the desired product (2.01g, 48%). LCMS calculated for $C_{13}H_{18}N_3O_2$ $(M+H)^+$: m/z = 248.2; Found: 248.2.

*Step 4. 8-cyclopentyl-2-methoxy-6,6-dimethyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one*



To a solution of 8-cyclopentyl-2-methoxy-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one (501 mg, 2.026 mmol) in DMF (10 mL) were added methyl iodide (380 µL, 6.08 mmol) and sodium hydride (60% in mineral oil, 284 mg, 7.09 mmol) and the reaction mixture was heated to 65 °C for 2 hr. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude residue was purified by Biotage Isolera™ (0-100% ethyl acetate in hexanes) to provide the desired product as a colorless oil (303 mg, 54%). LCMS calculated for $C_{15}H_{22}N_3O_2$ $(M+H)^+$: m/z = 276.2; Found: 276.2.

*Step 5. 8-cyclopentyl-6,6-dimethyl-7-oxo-2,3,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidin-2-yl trifluoromethanesulfonate*



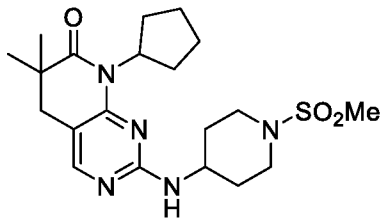
To a solution of 8-cyclopentyl-2-methoxy-6,6-dimethyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one (131 mg, 0.476 mmol) in acetonitrile (2.4 mL) were added sodium iodide (143 mg, 0.952 mmol) and TMS-Cl (122 µL, 0.952 mmol) and the reaction mixture was stirred at r.t. overnight, then quenched with water and extracted with ethyl acetate. The organic layer was washed with saturated aq. sodium thiosulfate, water and brine, dried over sodium sulfate and concentrated. The crude product was dissolved in DCM (2.5 mL) and pyridine (42.3 µL, 0.523 mmol) was added. The reaction mixture was cooled to 0 °C and trifluoromethanesulfonic anhydride (96 µL, 0.571 mmol) was added dropwise. The reaction mixture was then warmed to r.t. and stirred for 2 hr, then quenched with sat. sodium bicarbonate and extracted with DCM. The organic layer was dried over sodium sulfate and

concentrated. The crude product was used in the next step without further purification (141 mg, 75%). LCMS calculated for $C_{15}H_{21}F_3N_3O_4S$ $(M+H)^+$: m/z = 396.2; Found: 396.2.

Step 6. 4-((8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-2-yl)amino)benzenesulfonamide

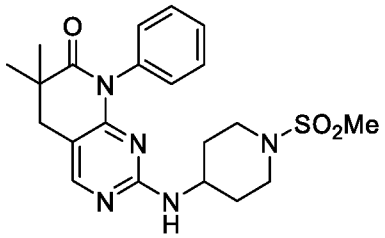
To a mixture of 8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-2-yl trifluoromethanesulfonate (20 mg, 0.051 mmol), 4-aminobenzenesulfonamide (17.51 mg, 0.102 mmol), XantPhos Pd G2 (4.52 mg, 5.08 μmol) and potassium carbonate (70.3 mg, 0.508 mmol) was added 1,4-dioxane (508 μL) and the reaction flask was evacuated, back filled with nitrogen, then stirred at 100 °C for 2 hr. The mixture was then diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min). LCMS calculated for $C_{20}H_{26}N_5O_3S$ $(M+H)^+$: m/z = 416.2; Found: 416.2.

Example A2. 8-cyclopentyl-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-d[pyrimidin-7(6H)-one

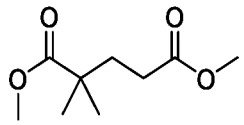


This compound was prepared in an analogous fashion to *Example A1, Step 6* using 1-(methylsulfonyl)piperidin-4-amine in place of 4-aminobenzenesulfonamide and RuPhos Pd G2 in place of XantPhos Pd G2. LCMS calculated for $C_{20}H_{32}N_5O_3S$ $(M+H)^+$: m/z = 422.2; Found: 422.2. ^{1}H NMR (600 MHz, DMSO) δ 8.01 (s, 1H), 5.44 – 5.22 (m, 1H), 3.85 (bs, 1H), 3.59 (d, *J* = 12.3 Hz, 1H), 2.9 (s, 3H), 2.85 (t, *J* = 12.2, 2.6 Hz, 1H), 2.60 (s, 2H), 2.05 (s, 1H), 1.98 (d, *J* = 16.3 Hz, 1H), 1.93 – 1.87 (m, 1H), 1.74 (s, 1H), 1.59 (m, 2H), 1.09 (s, 6H).

Example A3. 6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-phenyl-5,8-dihydropyrido[2,3-d[pyrimidin-7(6H)-one

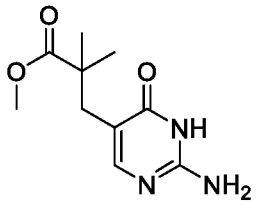


Step 1. dimethyl 2,2-dimethylpentanedioate



To a solution of 3,3-dimethyldihydro-2*H*-pyran-2,6(3*H*)-dione (10 g, 70.3 mmol) in methanol (100 mL) was added 10 drops of concentrated sulfuric acid and the reaction mixture heated to 60 °C overnight. The mixture was then concentrated. The residue was diluted with ethyl acetate and washed with sat. sodium bicarbonate and brine, then dried over sodium sulfate and concentrated. The crude product was used in the next step without further purification.

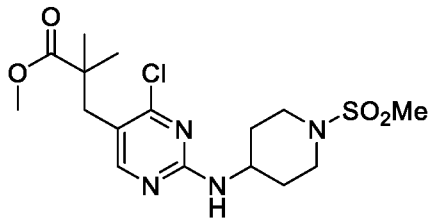
Step 2. methyl 3-(2-amino-6-oxo-1,6-dihydropyrimidin-5-yl)-2,2-dimethylpropanoate



To a solution of diisopropylamine (5.32 mL, 37.4 mmol) in THF (12 mL) at -78 °C was added *n*-BuLi (2.5M in hexanes, 14.94 mL, 37.4 mmol) dropwise and the reaction mixture stirred at -78 °C for 1 hr. A solution of dimethyl 2,2-dimethylpentanedioate (5.86 g, 31.1 mmol) in THF (20 mL) was then added dropwise and the reaction mixture stirred an additional 1.5 hr at -78 °C. Methyl formate (2.88 mL, 46.7 mmol) was then added and the reaction mixture stirred at -78 °C for 1 hr, then quenched with sat. ammonium chloride. After warming to r.t., the mixture was diluted with ethyl acetate/water and the layers separated. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The residue was dissolved in MeOH (10 mL) and guanidine carbonate (5.61 g, 31.1 mmol) was added. The reaction mixture was heated to 60 °C overnight, then concentrated and

purified by Biotage Isolera™ (2-12% methanol in dichloromethane) to provide the desired product as a white solid (2.45 g, 35%). LCMS calculated for $C_{10}H_{16}N_3O_3$ $(M+H)^+$: m/z = 226.2; Found: 226.2.

Step 3. methyl 3-(4-chloro-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrimidin-5-yl)-2,2-dimethylpropanoate



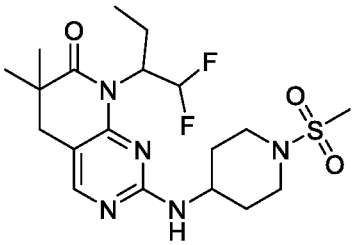
Methyl 3-(2-amino-6-oxo-1,6-dihydropyrimidin-5-yl)-2,2-dimethylpropanoate (2.45 g, 10.88 mmol) was dissolved in $POCl_3$ (10 mL) and heated to 100 °C overnight, then slowly added to sat. sodium bicarbonate. The mixture was extracted with DCM and the organic layer washed with sat. sodium bicarbonate and brine, dried over sodium sulfate and concentrated. To the intermediate were added DMF (36.3 mL), 1-(methylsulfonyl)piperidin-4-one (2.506 g, 14.14 mmol), TFA (5.03 mL, 65.3 mmol) and sodium triacetoxyborohydride (5.76 g, 27.2 mmol) and the reaction mixture was stirred at r.t. for 5 hr, then quenched with sat. sodium bicarbonate and extracted with DCM. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified by Biotage Isolera™ (2-12% methanol in DCM) to provide the desired product as a yellow solid (2.2 g, 50%). LCMS calculated for $C_{16}H_{26}ClN_4O_4S$ $(M+H)^+$: m/z = 404.2/406.2; Found: 404.2/406.2.

*Step 4. 6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(*6H*)-one*

To a mixture of methyl 3-(4-chloro-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrimidin-5-yl)-2,2-dimethylpropanoate (21 mg, 0.052 mmol), aniline (9.47 µL, 0.104 mmol), Ruphos Pd G2 (4.03 mg, 5.19 µmol) and cesium carbonate (50.7 mg, 0.156 mmol) was added 1,4-dioxane (519 µL) and the reaction flask was evacuated, back filled with nitrogen, then stirred at 100 °C overnight. The reaction mixture was diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow

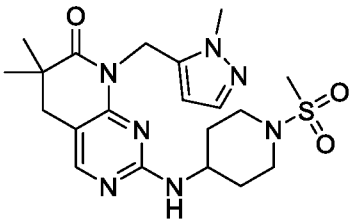
rate of 60 mL/min). LCMS calculated for $C_{21}H_{28}N_5O_3S$ $(M+H)^+$: m/z = 430.2; Found: 430.2.

Example A4. 8-(1,1-difluorobutan-2-yl)-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one



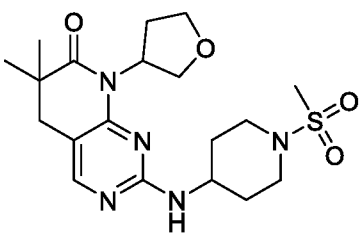
This compound was prepared in an analogous fashion to *Example A3, Step 4* using 1,1-difluorobutan-2-amine as the coupling partner. The product was isolated as a racemic mixture. LCMS calculated for $C_{19}H_{30}F_2N_5O_3S$ $(M+H)^+$: m/z = 446.2; Found: 446.2.

Example A5. 6,6-dimethyl-8-((1-methyl-1*H*-pyrazol-5-yl)methyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one



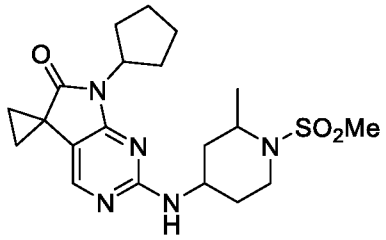
This compound was prepared in an analogous fashion to *Example A3, Step 4* using (1-methyl-1*H*-pyrazol-5-yl)methanamine as the coupling partner. LCMS calculated for $C_{20}H_{30}N_7O_3S$ $(M+H)^+$: m/z = 448.2; Found: 448.2.

Example A6. 6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-(tetrahydrofuran-3-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one

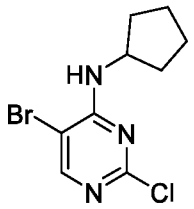


This compound was prepared in an analogous fashion to *Example A3, Step 4* using tetrahydrofuran-3-amine as the coupling partner. The product was obtained in racemic form. LCMS calculated for $C_{19}H_{30}N_5O_4S$ $(M+H)^+$: m/z = 424.2; Found: 424.2.

Example B1. 7'-cyclopentyl-2'-((2-methyl-1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one

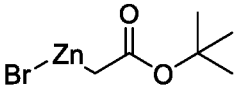


*Step 1. 5-bromo-2-chloro-*N*-cyclopentylpyrimidin-4-amine*



To a solution of 5-bromo-2,4-dichloropyrimidine (20 g, 88 mmol) and Hunig's base (22.99 mL, 132 mmol) in THF (219 mL) was added cyclopentanamine (9.56 mL, 97 mmol) and the reaction mixture stirred at r.t. overnight, then was quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified by Biotage Isolera™ (0-40% ethyl acetate in hexanes) to provide the desired product as a yellow solid (21.1 g, 87%). LCMS calculated for $C_9H_{12}BrClN_3$ $(M+H)^+$: m/z = 276.0/278.0; Found: 276.0/278.0.

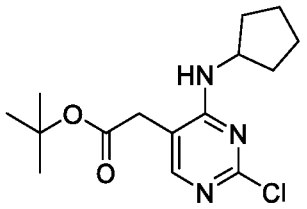
*Step 2. (2-(*tert*-butoxy)-2-oxoethyl)zinc(II) bromide*



Zinc was activated by washing zinc dust in 2% HCl for 1 hr, then decanting. To the solid was added water and the supernatant decanted three times. The solid was then collected by filtration, washed with water, ethanol, acetone and ether, then dried in the oven for 15 mins. To a portion of thus prepared zinc (4.87 g, 74.4 mmol) were added THF (65 mL) and TMS-Cl (0.865 mL, 6.77 mmol). The reaction mixture was

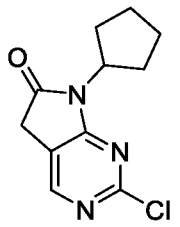
stirred at r.t for 1 hr then the *tert*-butyl 2-bromoacetate (10.00 mL, 67.7 mmol) was added dropwise. Addition was complete over ~15 mins. The mixture was then heated to 50 °C for 1 hr at which point most of the zinc metal had dissolved. The mixture was cooled to r.t and used as a ~ 0.9 M solution in subsequent steps.

Step 3. tert-*butyl 2-(2-chloro-4-(cyclopentylamino)pyrimidin-5-yl)acetate*



To a mixture of 5-bromo-2-chloro-*N*-cyclopentylpyrimidin-4-amine (10 g, 36.2 mmol), $Pd_2(dba)_3$ (0.993 g, 1.085 mmol) and 1,2,3,4,5-pentaphenyl-1'-(di-*t*-butylphosphino)ferrocene (QPhos, 0.771 g, 1.085 mmol) was added (2-(*tert*-butoxy)-2-oxoethyl)zinc(II) bromide (48.2 mL, 43.4 mmol) as a 0.9 M solution in THF, freshly prepared, and dioxane (72 mL). The mixture was evacuated, back filled with nitrogen, then stirred at r.t for 1 hr. The reaction was quenched with 1N HCl and extracted with ethyl acetate. The organic layer was washed with water and brine and concentrated. The crude product was purified by Biotage Isolera™ (0-50% ethyl acetate in hexanes) to provide the desired product as a pink solid (7.6 g, 67%). LCMS calculated for $C_{15}H_{23}ClN_3O_2$ $(M+H)^+$: m/z = 312.2; Found: 312.2.

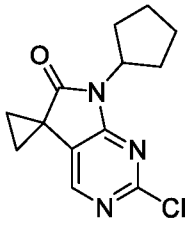
*Step 4. 2-chloro-7-cyclopentyl-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one*



To a solution of *tert*-butyl 2-(2-chloro-4-(cyclopentylamino)pyrimidin-5-yl)acetate (2.41 g, 7.73 mmol) in THF (25.8 mL) was added sodium hydride (60% in mineral oil, 0.618 g, 15.46 mmol) and the reaction mixture was heated to 60 °C for 1 hr, then cooled to r.t. and quenched with 1 N HCl. The mixture was extracted with ethyl acetate and the organic layer washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (0-

100% ethyl acetate in hexanes) to provide the desired product as a green solid (1.46 g, 79%). LCMS calculated for $C_{11}H_{13}ClN_3O$ $(M+H)^+$: m/z = 238.2; Found: 238.2.

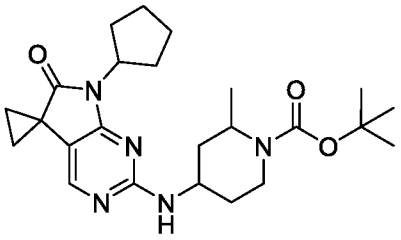
Step 5. 2'-chloro-7'-cyclopentylspiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one



To a suspension of sodium hydride (60% in mineral oil, 0.981 g, 24.54 mmol) in THF (20 mL)/HMPA (2 mL, 11.50 mmol) was added a solution of 2-chloro-7-cyclopentyl-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one (1.458 g, 6.13 mmol) in THF (2.5 mL) dropwise and the reaction mixture was stirred at r.t. for 10 mins. 1,2-dibromoethane (1.057 mL, 12.27 mmol) was added and the reaction mixture was heated to 50 °C for 1 hr, then quenched with 1N HCl and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (15-100% ethyl acetate in hexanes) to provide the desired product as an off-green solid (1.1 g, 68%).

LCMS calculated for $C_{13}H_{15}ClN_3O$ $(M+H)^+$: m/z = 264.2; Found: 264.2.

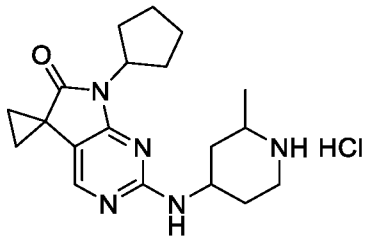
Step 6. tert-*butyl 4-((7'-cyclopentyl-6'-oxo-6',7'-dihydrospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-2'-yl)amino)-2-methylpiperidine-1-carboxylate*



A vial of 2'-chloro-7'-cyclopentylspiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (0.04 g, 0.152 mmol), *tert*-butyl 4-amino-2-methylpiperidine-1-carboxylate (0.098 g, 0.455 mmol), RuPhos Pd G2 (0.012 g, 0.015 mmol), and cesium carbonate (0.148 g, 0.455 mmol) was evacuated and back filled with nitrogen. 1,4-dioxane (1.996 mL) was added, and then the solution was stirred at 100 °C for 48 hr. The mixture was cooled, concentrated under reduced

pressure, and purified by Teledyne ISCO CombiFlash® Rf+ (0-100% ethyl acetate in hexanes) to provide the desired product as a red oil. LCMS calculated for $C_{24}H_{36}N_5O_3$ $(M+H)^+$: m/z = 442.3; Found: 442.3.

*Step 7. 7'-cyclopentyl-2'-((2-methylpiperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one hydrochloride*

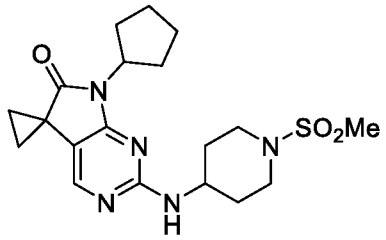


A solution of *tert*-butyl 4-((7'-cyclopentyl-6'-oxo-6',7'-dihydrospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-2'-yl)amino)-2-methylpiperidine-1-carboxylate (0.0163 g, 0.037 mmol) and 4M HCl in dioxane (0.157 mL, 0.628 mmol) in anhydrous methanol (0.159 mL) was stirred at room temperature (r.t.) for 1 hr. The solution was then concentrated under reduced pressure. Toluene was added and the solution was concentrated under reduced pressure to yield the desired product as an orange oil. LCMS calculated for $C_{19}H_{28}N_5O$ $(M+H)^+$: m/z = 342.2; Found: 342.2.

*Step 8. 7'-cyclopentyl-2'-((2-methyl-1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

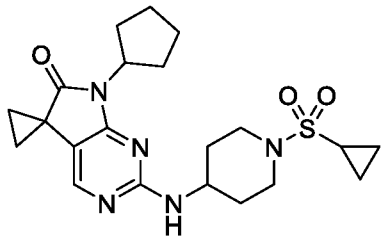
Methanesulfonyl chloride (6.06 µL, 0.078 mmol) was added dropwise to a solution of 7'-cyclopentyl-2'-((2-methylpiperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (0.022 g, 0.065 mmol) and Et_3N (10.84 µL, 0.078 mmol) in anhydrous CH_2Cl_2 (1.866 mL) at 0 °C. The solution was allowed to warm gradually to room temperature overnight. Then, the solution was diluted with MeOH and CH_3CN and purified by prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product as a white solid. LCMS calculated for $C_{20}H_{30}N_5O_3S$ $(M+H)^+$: m/z = 420.2; Found: 420.5.

Example B2. 7'-cyclopentyl-2'-((1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one

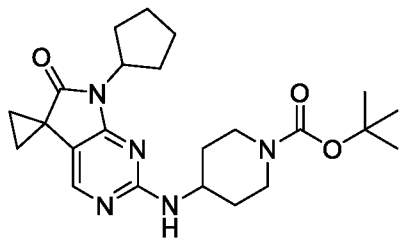


This compound was prepared in a similar fashion to *Example B1, Step 5* using 1-(methylsulfonyl)piperidin-4-amine as the amine coupling partner. LCMS calculated for $C_{19}H_{28}N_5O_3S$ $(M+H)^+$: m/z = 406.2; Found: 406.2.

Example B3. 7'-cyclopentyl-2'-((1-(cyclopropylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one

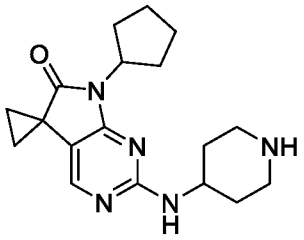


Step 1. tert-*butyl 4-((7'-cyclopentyl-6'-oxo-6',7'-dihydrospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-2'-yl)amino)piperidine-1-carboxylate*



This compound was prepared in a similar manner to *Example B1, Step 5*, using *tert*-butyl 4-aminopiperidine-1-carboxylate as the amine coupling partner. LCMS calculated for $C_{23}H_{34}N_5O_3$ $(M+H)^+$: m/z = 428.3; Found: 428.3.

Step 2. 7'-cyclopentyl-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one

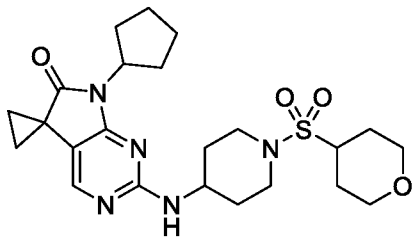


A solution of *tert*-butyl 4-((7'-cyclopentyl-6'-oxo-6',7'-dihydrospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-2'-yl)amino)piperidine-1-carboxylate (0.0599 g, 0.140 mmol) in 1:1 TFA (0.05 mL)/CH_2Cl_2 (0.050 mL) was stirred at room temperature for one hour. Then, the reaction was quenched with sat. $NaHCO_3$ and extracted into CH_2Cl_2 (2x). The organic layer was washed with brine, and the solution was concentrated under reduced pressure to yield the desired product as a brown solid, which was used without further purification. LCMS calculated for $C_{18}H_{26}N_5O$ $(M+H)^+$: m/z = 328.2; Found: 328.4.

*Step 3. 7'-cyclopentyl-2'-((1-(cyclopropylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'H)-one*

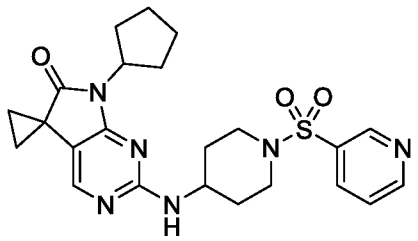
A solution of 7'-cyclopentyl-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (0.0115 g, 0.035 mmol), cyclopropanesulfonyl chloride (7.16 µL, 0.070 mmol), and Hunig's base (0.015 mL, 0.088 mmol) in anhydrous THF (0.702 mL) was stirred at room temperature overnight. Then, the solution was diluted with CH_3CN and purified by prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product as a white solid. LCMS calculated for $C_{21}H_{30}N_5O_3S$ $(M+H)^+$: m/z = 432.2; Found: 432.2.

Example B4. 7'-cyclopentyl-2'-((1-((tetrahydro-2H-pyran-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one



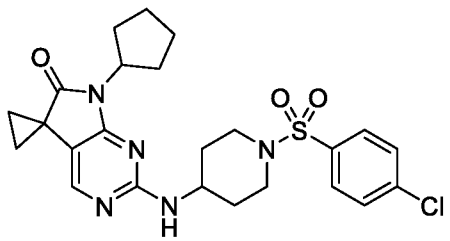
This compound was prepared in a similar fashion to *Example B3, Step 3* using tetrahydro-2*H*-pyran-4-sulfonyl chloride as the sulfonyl chloride. LCMS calculated for $C_{23}H_{34}N_5O_4S$ $(M+H)^+$: m/z = 476.2; Found: 476.2.

Example B5. 7'-cyclopentyl-2'-((1-(pyridin-3-ylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one



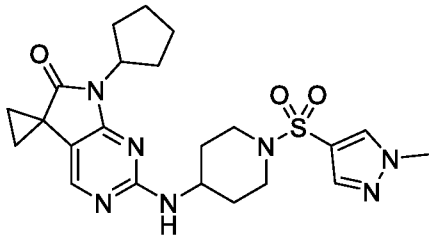
This compound was prepared in a similar fashion to *Example B3, Step 3* using pyridine-3-sulfonyl chloride hydrochloride as the sulfonyl chloride. LCMS calculated for $C_{23}H_{29}N_6O_3S$ $(M+H)^+$: m/z = 469.2; Found: 469.2.

Example B6. 2'-((1-((4-chlorophenyl)sulfonyl)piperidin-4-yl)amino)-7'-cyclopentylspiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one



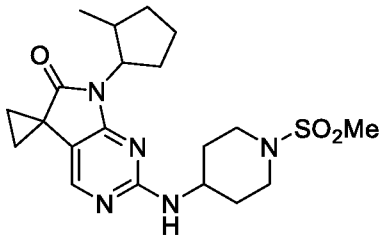
This compound was prepared in a similar fashion to *Example B3, Step 3* using 4-chlorobenzenesulfonyl chloride as the sulfonyl chloride. LCMS calculated for $C_{24}H_{29}ClN_5O_3S$ $(M+H)^+$: m/z = 502.2; Found: 502.2.

Example B7. 7'-cyclopentyl-2'-((1-((1-methyl-1H-pyrazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one

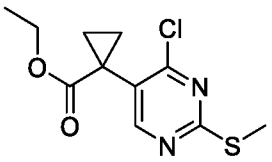


This compound was prepared in a similar fashion to *Example B3, Step 3* using 1-methyl-1*H*-pyrazole-4-sulfonyl chloride as the sulfonyl chloride. LCMS calculated for $C_{22}H_{30}N_7O_3S$ $(M+H)^+$: m/z = 472.2; Found: 472.4.

Example B8. 7'-(2-methylcyclopentyl)-2'-((1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one

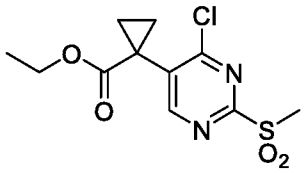


Step 1. ethyl 1-(4-chloro-2-(methylthio)pyrimidin-5-yl)cyclopropane-1-carboxylate



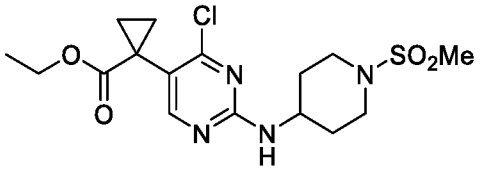
To a suspension of sodium hydride (2.006 g, 50.2 mmol) in DMF (60 mL) at 0 °C was added a solution of 1,2-dibromoethane (2.59 mL, 30.1 mmol) and ethyl 2-(4-chloro-2-(methylthio)pyrimidin-5-yl)acetate (4.95 g, 20.06 mmol) in DMF (40 mL) dropwise. The reaction mixture was warmed up to r.t .and stirred for 30 mins, then quenched with sat. ammonium chloride and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (0-50% ethyl acetate in hexanes) to provide the desired product as a yellow oil (3.2 g, 59%). LCMS calculated for $C_{11}H_{14}ClN_2O_2S$ $(M+H)^+$: m/z = 273.1; Found: 273.1.

Step 2. ethyl 1-(4-chloro-2-(methylsulfonyl)pyrimidin-5-yl)cyclopropane-1-carboxylate



To a solution of ethyl 1-(4-chloro-2-(methylthio)pyrimidin-5-yl)cyclopropane-1-carboxylate (3.1 g, 11.37 mmol) in DCM (60 mL) was added *m*-CPBA (5.88 g, 34.1 mmol) and the reaction mixture was stirred at r.t. for 3 hr, then quenched with sat. sodium bicarbonate and extracted with DCM. The organic layer was washed with sat. sodium bicarbonate and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (0-100% ethyl acetate in hexanes) to provide the desired product as a white solid. LCMS calculated for $C_{11}H_{14}ClN_2O_4S$ $(M+H)^+$: m/z = 305.1; Found: 305.1.

Step 3. ethyl 1-(4-chloro-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrimidin-5-yl)cyclopropane-1-carboxylate

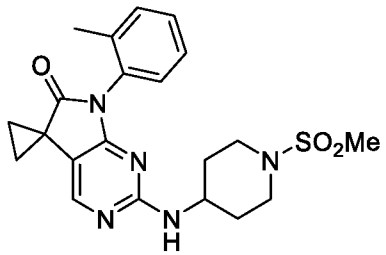


To a suspension of 1-(methylsulfonyl)piperidin-4-amine (2.226 g, 12.49 mmol) in tetrahydrofuran (56.8 mL) at 0 °C was added isopropylmagnesium chloride lithium chloride complex (10.48 mL, 13.62 mmol) and the reaction mixture was stirred at 0 °C for 30 mins. Then a solution of ethyl 1-(4-chloro-2-(methylsulfonyl)pyrimidin-5-yl)cyclopropane-1-carboxylate (3.46 g, 11.35 mmol) in THF was added dropwise and the reaction mixture was warmed to r.t, stirred 10 mins, then heated to 55 °C for 1 hr. The reaction was quenched with sat. ammonium chloride and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage Isolera™ (15-100% ethyl acetate in hexanes) to provide the desired product as a white solid. LCMS calculated for $C_{16}H_{24}ClN_4O_4S$ $(M+H)^+$: m/z = 403.2; Found:403.2

*Step 4. 7'-(2-methylcyclopentyl)-2'-((1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

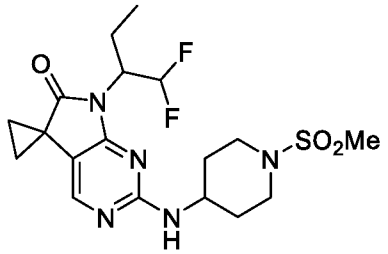
To a mixture of ethyl 1-(4-chloro-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrimidin-5-yl)cyclopropane-1-carboxylate (20 mg, 0.050 mmol), 2-methylcyclopenylamine (10 mg, 0.99 mmol), RuPhos Pd G2 (3.86 mg, 4.96 µmol) and cesium carbonate (48.5 mg, 0.149 mmol) was added 1,4-dioxane (496 µL) and the reaction flask was evacuated, back filled with nitrogen, then stirred at 140 °C for 1.5 hr. The mixture was diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product as a mixture of four diastereomers. LCMS calculated for $C_{20}H_{30}N_5O_3S$ $(M+H)^+$: m/z = 420.2; Found: 420.2.

Example B9. 2'-((1-(methylsulfonyl)piperidin-4-yl)amino)-7'-(o-tolyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one



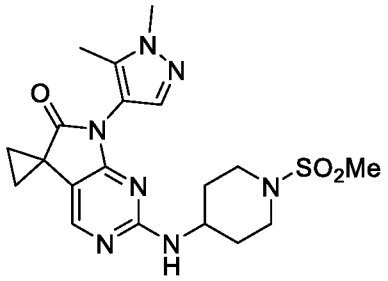
To a mixture of ethyl 1-(4-chloro-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrimidin-5-yl)cyclopropane-1-carboxylate (*Example B6, Step 2*, 20 mg, 0.050 mmol), XantPhos Pd G2 (4.41 mg, 4.96 µmol), *o*-toluidine (10.53 µL, 0.099 mmol) and cesium carbonate (81 mg, 0.248 mmol) was added 1,4-dioxane (165 µL) and the reaction flask was evacuated, back filled with nitrogen, then stirred at 120 degrees overnight. The mixture was diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min). LCMS calculated for $C_{21}H_{26}N_5O_3S$ $(M+H)^+$: m/z = 428.2; Found: 428.2.

Example B10. 7'-(1,1-difluorobutan-2-yl)-2'-((1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'H)-one



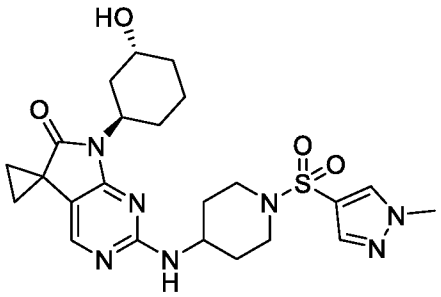
This compound was prepared in a similar manner to *Example B8*, using 1,1-difluorobutan-2-amine hydrochloride as the amine coupling partner. The product was isolated in racemic form. LCMS calculated for $C_{18}H_{26}F_2N_5O_3S$ $(M+H)^+$: m/z = 430.2; Found: 430.2.

Example B11. 7'-(1,5-dimethyl-1*H*-pyrazol-4-yl)-2'-((1-(methylsulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one

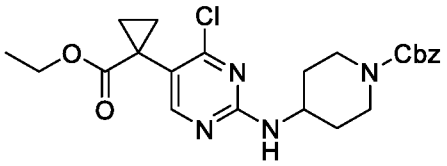


This compound was prepared in a similar manner to *Example B9*, using 1,5-dimethyl-1H-pyrazol-4-amine as the coupling partner. LCMS calculated for $C_{19}H_{26}N_7O_3S$ $(M+H)^+$: m/z = 432.2; Found: 432.2.

Example B12. 7'-((1*R*,3*R*)-3-hydroxycyclohexyl)-2'-((1-((1-methyl-1*H*-pyrazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one

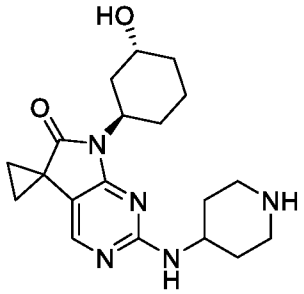


Step 1. benzyl 4-((4-chloro-5-(1-(ethoxycarbonyl)cyclopropyl)pyrimidin-2-yl)amino)piperidine-1-carboxylate



To a solution of ethyl 1-(4-chloro-2-(methylsulfonyl)pyrimidin-5-yl)cyclopropane-1-carboxylate (2.6 g, 8.53 mmol) and benzyl 4-formamidopiperidine-1-carboxylate (2.350 g, 8.96 mmol) in THF (28.4 ml) was added sodium hydride (0.512 g, 12.80 mmol, 60% in mineral oil) and the reaction mixture was stirred at 100 °C for 1 hr, then cooled to r.t. and quenched with sat. ammonium chloride. The mixture was diluted with water and ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was purified by Biotage™ (0-100% ethyl acetate in hexanes) to provide the desired product as an off white foam (2.65 g, 67%). LCMS calculated for $C_{23}H_{28}ClN_4O_4$ $(M+H)^+$: m/z = 459.2; Found: 459.2.

Step 2. 7'-((1R,3R)-3-hydroxycyclohexyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one



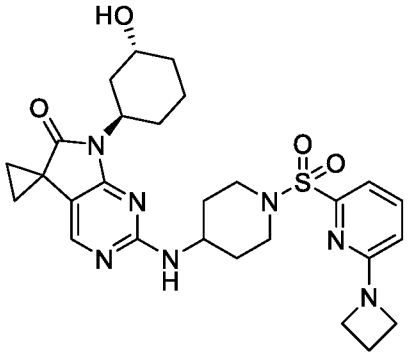
To a solution of benzyl 4-((4-chloro-5-(1-(ethoxycarbonyl)cyclopropyl)pyrimidin-2-yl)amino)piperidine-1-carboxylate (750 mg, 1.634 mmol) in trifluoroethanol (5.45 ml) in a microwave vial were added (1*R*,3*R*)-3-aminocyclohexan-1-ol (226 mg, 1.961 mmol) and TFA (151 µl, 1.961 mmol) and the reaction flask was sealed, then heated to 150 °C for 2 hr in the microwave. The mixture was cooled to r.t. and quenched with sat. sodium bicarbonate, then extracted with DCM. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was dissolved in THF (5 mL) and sodium hydride (131 mg, 3.27 mmol, 60% in mineral oil) was added.

The reaction mixture was heated to 70 °C for 1 hr, then quenched with sat. ammonium chloride and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was dissolved in MeOH (5 mL) and palladium on carbon (174 mg, 0.163 mmol) was added. The reaction flask was evacuated, back filled with hydrogen gas from a balloon, then stirred at r.t. overnight. The mixture was diluted with ethyl acetate and filtered through a plug of Celite. The filtrate was concentrated and the crude product was used in the next step without further purification (580 mg, 99%). LCMS calculated for $C_{19}H_{28}N_5O_2$ $(M+H)^+$: m/z = 358.2; Found: 358.2.

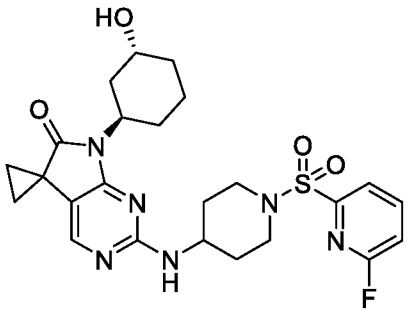
*Step 3. 7'-((1*R*,3*R*)-3-hydroxycyclohexyl)-2'-((1-((1-methyl-1*H*-pyrazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

To a solution of 7'-((1*R*,3*R*)-3-hydroxycyclohexyl)-2'-(piperidin-4-ylamino)spiro [cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (396 mg, 1.108 mmol) in DCM (5.54 ml) were added Hunig's base (232 µl, 1.329 mmol) and 1-methyl-1*H*-pyrazole-4-sulfonyl chloride (200 mg, 1.108 mmol) and the reaction mixture was stirred at r.t. for 15 mins, then quenched with sat. sodium bicarbonate and extracted with DCM. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The mixture was diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{23}H_{32}N_7O_4S$ $(M+H)^+$: m/z = 502.2; Found: 502.2. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.78 (s, 1H), 7.72 (s, 1H), 7.07 (d, J = 7.3 Hz, 1H), 4.62 (d, J = 13.3 Hz, 1H), 4.52 (d, J = 2.7 Hz, 1H), 4.07 (s, 1H), 3.92 (s, 3H), 3.66 (s, 1H), 3.47 (d, J = 11.6 Hz, 2H), 2.47 – 2.29 (m, 2H), 2.19 (q, J = 13.7, 12.6 Hz, 1H), 1.94 (d, J = 11.8 Hz, 2H), 1.78 – 1.67 (m, 1H), 1.67 – 1.48 (m, 6H), 1.42 (t, J = 3.7 Hz, 2H).

Example B13. 2'-((1-((6-(azetidin-1-yl)pyridin-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((1*R*,3*R*)-3-hydroxycyclohexyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one



*Step 1. 2'-((1-((6-fluoropyridin-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((1*R*,3*R*)-3-hydroxycyclohexyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one*



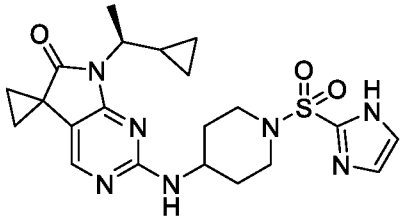
To a solution of 7'-((1*R*,3*R*)-3-hydroxycyclohexyl)-2'-(piperidin-4-ylamino)spiro [cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (Example 12, Step 2, 15 mg, 0.042 mmol) in THF (0.210 ml) were added 6-fluoropyridine-2-sulfonyl chloride (41.1 µl, 0.042 mmol) and Hunig's base (21.99 µl, 0.126 mmol) and the reaction mixture was stirred at r.t. for 30 mins, then quenched with water and extracted with DCM. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was used in the next step without further purification. LCMS calculated for $C_{24}H_{30}FN_6O_4S$ $(M+H)^+$: m/z = 517.2; Found: 517.2.

*Step 2. 2'-((1-((6-(azetidin-1-yl)pyridin-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((1*R*,3*R*)-3-hydroxycyclohexyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one*

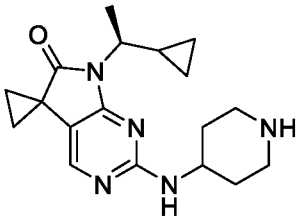
To a solution of 2'-((1-((6-fluoropyridin-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((1*R*,3*R*)-3-hydroxycyclohexyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (15 mg, 0.029 mmol) in 1,4-Dioxane (0.290 ml) were added azetidine

(4.97 mg, 0.087 mmol) and Hunig's base (15.21 µl, 0.087 mmol) and the reaction mixture was heated to 90 °C overnight, then diluted with MeOH purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{27}H_{36}N_7O_4S$ $(M+H)^+$: m/z = 554.2; Found: 554.2.

Example B14. (*S*)-2'-((1-((1*H*-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-(1-cyclopropylethyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one



Step 1. (S)-7'-(1-cyclopropylethyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one

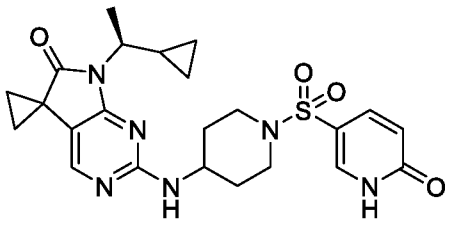


This compound was prepared in a similar manner to *Example B12, Step 2*, using (*S*)-1-cyclopropylethan-1-amine as the amine coupling partner. LCMS calculated for $C_{18}H_{26}N_5O$ $(M+H)^+$: m/z = 328.2; Found: 328.2.

Step 2. (S)-2'-((1-((1H-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-(1-cyclopropylethyl)spiro [cyclopropane-1,5'-pyrrolo[2,3-d]pyrimidin]-6'(7'H)-one

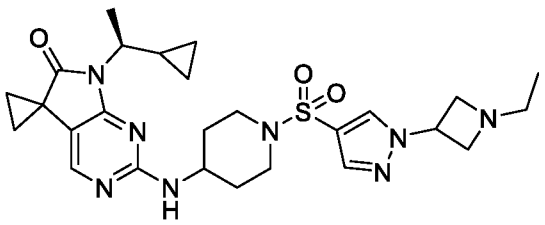
To a solution of (*S*)-7'-(1-cyclopropylethyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (10 mg, 0.031 mmol) in DCM (0.305 ml) was added Hunig's base (16.00 µl, 0.092 mmol) and 1*H*-imidazole-2-sulfonyl chloride (7.63 mg, 0.046 mmol) and the reaction mixture stirred at r.t. for 30 mins, then quenched with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{21}H_{28}N_7O_3S$ $(M+H)^+$: m/z = 458.2; Found: 458.2.

Example B15. (*S*)-7'-(1-cyclopropylethyl)-2'-((1-((6-oxo-1,6-dihydropyridin-3-yl)sulfonyl) piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one



To a solution of (*S*)-7'-(1-cyclopropylethyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (20 mg, 0.061 mmol) in DCM (0.611 ml) was added Hunig's base (21.34 µl, 0.122 mmol) and 6-methoxypyridine-3-sulfonyl chloride (12.68 mg, 0.061 mmol) and the reaction mixture was stirred at r.t. for 15 mins, then quenched with water and extracted with DCM. The organic layer was concentrated, then dissolved in acetonitrile and sodium iodide (36.6 mg, 0.244 mmol) and TMS-Cl (31.2 µl, 0.244 mmol) were added. The reaction mixture was stirred at 60 °C for 1 hr, then quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate and concentrated. The crude product was dissolved in MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{23}H_{29}N_6O_4S$ $(M+H)^+$: m/z = 485.2; Found: 485.2.

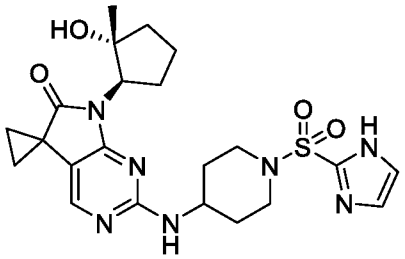
Example B16. (*S*)-7'-(1-cyclopropylethyl)-2'-((1-((1-(1-ethylazetidin-3-yl)-1*H*-pyrazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one



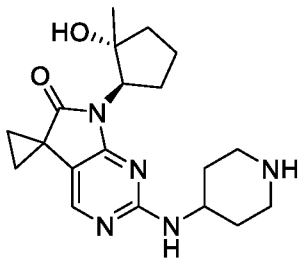
To a solution of (*S*)-7'-(1-cyclopropylethyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (30 mg, 0.092 mmol) in DCM (0.916 ml) was added Hunig's base (32.0 µl, 0.183 mmol) and 1*H*-pyrazole-4-sulfonyl chloride (15.26 mg, 0.092 mmol) and the reaction mixture was

stirred at r.t. for 15 mins, then quenched with water and extracted with DCM. The organic layer was concentrated, then dissolved in acetonitrile and *tert*-butyl 3-((methylsulfonyl)oxy)azetidine-1-carboxylate (69.1 mg, 0.275 mmol) and cesium carbonate (90 mg, 0.275 mmol) were added. The reaction mixture was stirred at 100 °C overnight, then quenched with 4N HCl in dioxane (1 mL) and stirred at r.t. for 30 mins. The mixture was washed with ethyl acetate and the organic layer was discarded. Solid sodium bicarbonate was added until the solution became basic, then the mixture was extracted with DCM. The organic layer was dried over sodium sulfate and concentrated. The crude product was dissolved in DCE (1 mL) and acetic acid (15.74 µl, 0.275 mmol), sodium triacetoxyborohydride (58.3 mg, 0.275 mmol) and acetaldehyde (7 µl, 0.275 mmol) were added. The reaction mixture was stirred at r.t. for 30 mins, then diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{26}H_{37}N_8O_3S$ $(M+H)^+$: m/z = 541.2; Found: 541.2.

Example B17. 2'-((1-((1*H*-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((*trans*)-2-hydroxy-2-methylcyclopentyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-d[pyrimidin[-6'(7'*H*)-one



*Step 1. 7'-((*trans*)-2-hydroxy-2-methylcyclopentyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

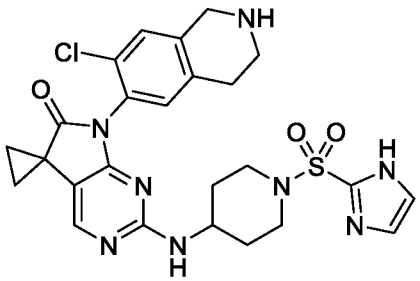


This compound was prepared in a similar manner to *Example B12, Step 2*, using (*trans*)-2-amino-1-methylcyclopentan-1-ol as the amine coupling partner. LCMS calculated for $C_{19}H_{28}N_5O_2$ $(M+H)^+$: m/z = 358.2; Found: 358.2.

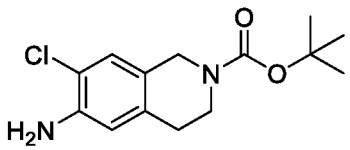
*Step 2. 2'-((1-((1*H*-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-((trans)-2-hydroxy-2-methylcyclopentyl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

To a solution of 7'-((*trans*)-2-hydroxy-2-methylcyclopentyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one, HCl (12 mg, 0.030 mmol) in DCM (0.609 ml) were added Hunig's base (5.32 µl, 0.030 mmol) and 1*H*-imidazole-2-sulfonyl chloride (6.05 mg, 0.034 mmol) and the reaction mixture was stirred at r.t. for 10 mins, then diluted with MeOH and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product. LCMS calculated for $C_{22}H_{30}N_7O_4S$ $(M+H)^+$: m/z = 488.2; Found: 488.2.

Example B18. 2'-((1-((1*H*-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-(7-chloro-1,2,3,4-tetrahydroisoquinolin-6-yl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one



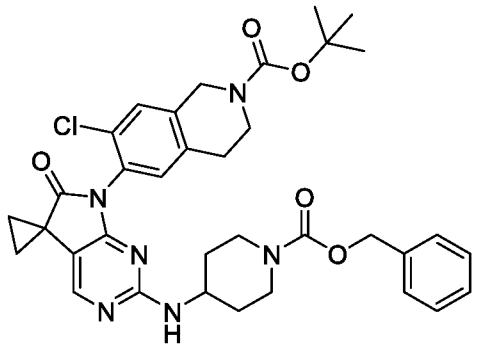
Step 1. tert-*butyl 6-amino-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate*



A solution of *tert*-butyl 7-chloro-6-nitro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (0.136 g, 0.434 mmol), iron (0.097 g, 1.736 mmol), and ammonium chloride (0.139 g, 2.60 mmol) in THF (0.723 mL)/methanol (0.723 mL)/water (0.723 mL) was stirred at 60 °C for 4 hr. Then, the solution was filtered through Celite and rinsed with ethyl acetate and methanol. The filtrate was washed with water and brine,

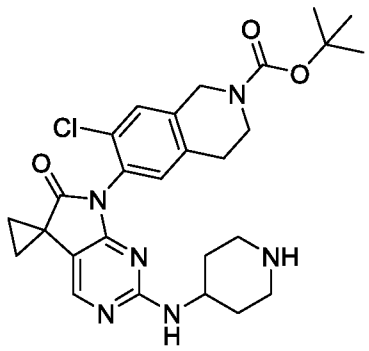
dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by Teledyne ISCO CombiFlash™ RF+ (0-100% ethyl acetate in hexanes) to provide the desired product as a brown solid (0.0762 g, 0.269 mmol, 62%). LCMS calculated for $C_{14}H_{20}ClN_2O_2$ $(M+H)^+$: m/z = 283.1; Found: 283.3.

Step 2. tert-*butyl 6-(2'-((1-((benzyloxy)carbonyl)piperidin-4-yl)amino)-6'-oxospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-7'(6'*H*)-yl)-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate*



Benzyl 4-((4-chloro-5-(1-(ethoxycarbonyl)cyclopropyl)pyrimidin-2-yl)amino)piperidine-1-carboxylate (Example 12, Step 1; 0.04 g, 0.087 mmol), cesium carbonate (0.085 g, 0.261 mmol), XantPhos-Pd G2 (7.75 mg, 8.72 µmol), and *tert*-butyl 6-amino-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (0.037 g, 0.131 mmol) were added to a 40-mL scintillation flask. The solution was vacuum/nitrogen purged 3x, and then anhydrous 1,4-dioxane (0.872 mL) was added. The solution was heated to 100 °C and stirred at 100 °C overnight. The solution was cooled and concentrated under reduced pressure. The crude product was purified by Teledyne ISCO CombiFlash™ RF+ (0-100% ethyl acetate in hexanes) to provide the desired product as a brown foam (0.023 g, 40%). LCMS calculated for $C_{35}H_{40}ClN_6O_5$ $(M+H)^+$: m/z = 659.3; Found: 659.5.

Step 3. tert-*butyl 7-chloro-6-(6'-oxo-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-7'(6'H)-yl)-3,4-dihydroisoquinoline-2(1H)-carboxylate*



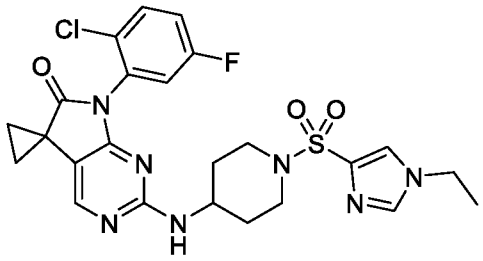
A solution of *tert*-butyl 6-(2'-((1-((benzyloxy)carbonyl)piperidin-4-yl)amino)-6'-oxospiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-7'(6'*H*)-yl)-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (0.0203 g, 0.035 mmol) and 10% palladium on carbon (6.40 mg, 6.02 µmol) in anhydrous methanol (0.301 mL) was stirred at r.t. under a hydrogen balloon for 1 hr. The reaction was filtered through Celite, washed with methanol, and concentrated under reduced pressure to provide the desired product as a white solid (0.016 g, 51 %). LCMS calculated for $C_{27}H_{34}ClN_6O_3$ $(M+H)^+$: m/z = 525.2; Found: 525.2.

*Step 4. 2'-((1-((1H-imidazol-2-yl)sulfonyl)piperidin-4-yl)amino)-7'-(7-chloro-1,2,3,4-tetrahydroisoquinolin-6-yl)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'H)-one*

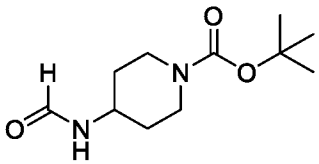
A solution of *tert*-butyl 7-chloro-6-(6'-oxo-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-7'(6'*H*)-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (0.0092 g, 0.018 mmol), 1*H*-imidazole-2-sulfonyl chloride (5.84 mg, 0.035 mmol), and Hunig's base (9.18 µl, 0.053 mmol) in anhydrous THF (0.350 mL) was stirred for 2 hr at r.t.. Then, the solution was washed with water, extracted into ethyl acetate 3x, dried over sodium sulfate, and concentrated under reduced pressure. The residue was dissolved in anhydrous methanol (0.1 mL), and 4M HCl in dioxane (0.074 mL, 0.298 mmol) was added. The solution was stirred at r.t. for 70 mins. Then, the solution was diluted with methanol and acetonitrile, purified by prep LCMS twice (Xbridge C18 column, eluting with a gradient of acetonitrile/water containing 0.15% NH_4OH in water, at flow rate of 60 mL/min, then Xbridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product as a

white solid. LCMS calculated for $C_{25}H_{28}ClN_8O_3S$ $(M+H)^+$: m/z = 555.2; Found: 555.2.

Example B19. 7'-(2-chloro-5-fluorophenyl)-2'-((1-((1-ethyl-1*H*-imidazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin[-6'(7'*H*)-one

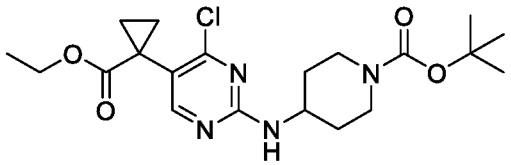


Step 1. tert-*butyl 4-formamidopiperidine-1-carboxylate*



A solution of ethyl formate (4.06 mL, 49.9 mmol) and *tert*-butyl 4-aminopiperidine-1-carboxylate (0.5 g, 2.49 mmol) was stirred at 70 °C for 2 hr. Then, the reaction was cooled and concentrated under reduced pressure to provide the desired product as a white solid, which was used in the next step without further purification. LCMS calculated for $C_7H_{13}N_2O_3$ $(M\text{-}tBu+H)^+$: m/z = 173.1; Found: 173.2.

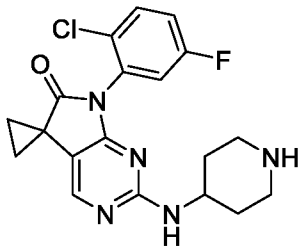
Step 2. tert-*butyl 4-((4-chloro-5-(1-(ethoxycarbonyl)cyclopropyl)pyrimidin-2-yl)amino)piperidine-1-carboxylate*



A slurry of ethyl 1-(4-chloro-2-(methylsulfonyl)pyrimidin-5-yl)cyclopropane-1-carboxylate (Example 8, Step 2, 0.9328 g, 3.06 mmol), *tert*-butyl 4-formamidopiperidine-1-carboxylate (0.699 g, 3.06 mmol), and 60% sodium hydride in mineral oil (0.122 g, 3.06 mmol) in anhydrous THF (15.3 mL) was stirred at 60 °C for 2 hr. Then the reaction was cooled and was quenched with 3 mL of 1M NaOH and the reaction was stirred over night at r.t.. Then, ethyl acetate and water were added to

the reaction, and the reaction was extracted into ethyl acetate 3x, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by Teledyne ISCO CombiFlash™ RF+ (0-100% ethyl acetate in hexanes) to provide the desired product as a light yellow oil (0.1652 g, 12%). LCMS calculated for $C_{16}H_{22}ClN_4O_4$ $(M\text{-}tBu+H)^+$: m/z = 369.1; Found: 369.2.

*Step 3. 7'-(2-chloro-5-fluorophenyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*



tert-Butyl 4-((4-chloro-5-(1-(ethoxycarbonyl)cyclopropyl)pyrimidin-2-yl)amino)piperidine-1-carboxylate (0.0763 g, 0.180 mmol), cesium carbonate (0.176 g, 0.539 mmol), XantPhos-Pd G2 (0.016 g, 0.018 mmol), and 2-chloro-5-fluoroaniline (0.039 g, 0.269 mmol) were added to a 40-mL scintillation flask. The solution was vacuum/nitrogen purged 3x, and then anhydrous 1,4-dioxane (1.796 mL) was added. The solution was heated to 100 °C and stirred at 100 °C overnight. The solution was cooled and concentrated under reduced pressure. The crude product was purified by Teledyne ISCO CombiFlash™ RF+ (0-100% ethyl acetate in hexanes) to provide the desired product as an orange oil. The residue was dissolved in anhydrous methanol (1 mL), and 4M HCl in dioxane (0.763 mL, 3.05 mmol) was added. The solution was stirred at r.t. for 70 minutes. Then, the solution was concentrated under reduced pressure to provide the desired product as a brown foam. LCMS calculated for $C_{19}H_{20}ClFN_5O$ $(M+H)^+$: m/z = 388.1; Found: 388.2.

*Step 4. 7'-(2-chloro-5-fluorophenyl)-2'-((1-((1-ethyl-1*H*-imidazol-4-yl)sulfonyl)piperidin-4-yl)amino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one*

A solution of 7'-(2-chloro-5-fluorophenyl)-2'-(piperidin-4-ylamino)spiro[cyclopropane-1,5'-pyrrolo[2,3-*d*]pyrimidin]-6'(7'*H*)-one (8.5 mg, 0.022 mmol), 1-ethyl-1*H*-imidazole-4-sulfonyl chloride (8.53 mg, 0.044 mmol), and Hunig's base (0.015 mL, 0.088 mmol) in anhydrous THF (0.438 mL) was stirred for 2

hr at r.t.. Then, the solution was diluted with acetonitrile and purified by prep LCMS twice (Xbridge C18 column, eluting with a gradient of acetonitrile/water containing 0.15% NH_4OH in water, at flow rate of 60 mL/min, then Xbridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% TFA, at flow rate of 60 mL/min) to provide the desired product as a white solid. LCMS calculated for $C_{24}H_{26}ClFN_7O_3S$ $(M+H)^+$: m/z = 546.2; Found: 546.2.

Example A. CDK2/Cyclin E1 HTRF Enzyme Activity Assay

CDK2/Cyclin E1 enzyme activity assays utilize full-length human CDK2 co-expressed as N-terminal GST-tagged protein with FLAG-Cyclin E1 in a baculovirus expression system (Carna Product Number 04-165). Assays are conducted in white 384-well polystyrene plates in a final reaction volume of 8 µL. CDK2/Cyclin E1 (0.25 nM) is incubated with compounds (40 nL serially diluted in DMSO) in the presence of ATP (50 µM or 1 mM) and 50 nM U*Light*™-labeled eIF4E-binding protein 1 (THR37/46) peptide (PerkinElmer) in assay buffer (containing 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 2 mM DTT, 0.05 mg/mL BSA, and 0.01% Tween 20) for 60 minutes at room temperature. The reactions are stopped by the addition of EDTA and Europium-labeled anti-phospho-4E-BP1 antibody (PerkinElmer), for a final concentration of 15 mM and 1.5 nM, respectively. HTRF signals are read after 1 hour at room temperature on a PHERAstar FS plate reader (BMG Labtech). Data is analyzed with IDBS XLFit and GraphPad Prism 5.0 software using a three or four parameter dose response curve to determine IC_{50} for each compound. The IC_{50} data as measured for the Examples at 1 mM ATP in the assay of Example A is shown in Table 3.

Table 3

Example	IC_{50} (nM)
A1	+
A2	+
A3	+++
A4	++
A5	+++
A6	+++
B1	+++
B2	+++
B3	+
B4	+++
B5	+

B6	++
B7	+
B8	+
B9	+++
B10	+
B11	++
B12	+
B13	+
B14	+
B15	+
B16	+
B17	+
B18	+
B19	+

+ refers to ≤ 50 nM
++ refers to >50 nM to 100 nM
+++ refers to >100 nM to 500 nM
++++ refers to >500 nM to 1000 nM

Example B: CDK1/Cyclin B1 HTRF Enzyme Activity Assay

CDK1/Cyclin B1 enzyme activity assays utilizes full-length human CDC2 [1-297(end) amino acids of accession number NP_001777.1] co-expressed as N-terminal GST-fusion protein (61 kDa) with CyclinB1 [1-433(end) amino acids of accession number NP_114172.1] using baculovirus expression system (Carna Product Number 04-102). Assays are conducted in white 384-well polystyrene plates in a final volume of 8 µL. CDK1/Cyclin B1 (0.25 nM) is incubated with compounds (40 nL serially diluted in DMSO) in the presence of ATP (25 µM or 1 mM) and 50 nM ULight™-labeled eIF4E-binding protein 1 (THR37/46) peptide (PerkinElmer) in assay buffer (containing 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl2, 2 mM DTT, 0.05mg/mL BSA, and 0.01% Tween 20) for 90 minutes at room temperature. The reactions are stopped by the addition of EDTA and Europium-labeled anti-phospho-4E-BP1 antibody (PerkinElmer), for a final concentration of 15 mM and 1.5 nM, respectively. HTRF signals are read after 1 hour at room temperature on a PHERAstar FS plate reader (BMG Labtech). Data is analyzed with IDBS XLFit and GraphPad Prism software using three or four parameter dose response curves to determine IC_{50} for each compound.

Example C: CDK9/Cyclin T1 HTRF Enzyme Activity Assay

CDK9/Cyclin T1 enzyme activity assays utilizes full-length human CDK9 [1-372(end) amino acids of accession number NP_001252.1] co-expressed as N-terminal GST-fusion protein (70 kDa) with His-CyclinT1 [1-726(end) amino acids of accession number NP_001231.2] in baculovirus expression system (Carna Product Number 04-110). Assays are conducted in white 384-well polystyrene plates in a final volume of 8 µL. CDK9/Cyclin T1 (0.2 nM) is incubated with compounds (40 nL serially diluted in DMSO) in the presence of ATP (7 µM or 1 mM) and 50 nM ULight™-labeled eIF4E-binding protein 1 (THR37/46) peptide (PerkinElmer) in assay buffer (containing 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl2, 2 mM DTT, 0.05mg/mL BSA, and 0.01% Tween 20) for 60 minutes at room temperature. The reactions are stopped by the addition of EDTA and Europium-labeled anti-phospho-4E-BP1 antibody (PerkinElmer), for a final concentration of 15 mM and 1.5 nM, respectively. HTRF signals are read after 1 hour at room temperature on a PHERAstar FS plate reader (BMG Labtech). Data is analyzed with IDBS XLFit and GraphPad Prism software using three or four parameter dose response curves to determine IC_{50} for each compound.

Example D: CDK4/Cyclin D1 HTRF Enzyme Activity Assay

CDK4/Cyclin D1 enzyme activity assays utilizes human CDK4, amino acids S4-E303 (as in NCBI/Protein entry NP_000066.1), N-terminal GST-fusion protein with a Thrombin cleavage site and human CycD1, amino acids Q4-I295 (as in NCBI/Protein entry NP_444284.1), N-terminal GST-fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells (ProQinase product # 0142-0143-1). Assays are conducted in white 384-well polystyrene plates in a final volume of 8 µL. CDK4/Cyclin D1 (1.0 nM) is incubated with compounds (40 nL serially diluted in DMSO) in the presence of ATP (1 mM) and 50 nM ULight™-labeled eIF4E-binding protein 1 (THR37/46) peptide (PerkinElmer) in assay buffer (containing 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl2, 2 mM DTT, 0.05mg/mL BSA, and 0.01% Tween 20) for 60 minutes at room temperature. The reactions are stopped by the addition of EDTA and Europium-labeled anti-phospho-4E-BP1 antibody (PerkinElmer), for a final concentration of 15 mM and 1.5 nM, respectively. HTRF signals are read after 1 hour at room temperature on a PHERAstar FS plate reader (BMG Labtech). Data is analyzed with IDBS XLFit and GraphPad Prism software

using three or four parameter dose response curves to determine IC_{50} for each compound.

Example E: CDK6/Cyclin D1 HTRF Enzyme Activity Assay

CDK6/Cyclin D1 enzyme activity assays utilizes full length human CDK6, M1-A326 (NCBI/Protein entry NP_001250.1), N-terminally fused to GST-Thrombin cleavage site and human CycD1, full length, amino acids Q4-I295 (NCBI/Protein entry NP_444284.1),, N-terminal GST-fusion protein with a Thrombin cleavage site, coexpressed in Sf9 insect cells (ProQinase product # 0051-0143-2). Assays are conducted in white 384-well polystyrene plates in a final volume of 8 μL. CDK6/Cyclin D1 (0.05 nM) is incubated with compounds (40 nL serially diluted in DMSO) in the presence of ATP (1 mM) and 50 nM ULight™-labeled eIF4E-binding protein 1 (THR37/46) peptide (PerkinElmer) in assay buffer (containing 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl2, 2 mM DTT, 0.05mg/mL BSA, and 0.01% Tween 20) for 60 minutes at room temperature. The reactions are stopped by the addition of EDTA and Europium-labeled anti-phospho-4E-BP1 antibody (PerkinElmer), for a final concentration of 15 mM and 1.5 nM.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An *in vitro* method of predicting the response of a human subject having a cyclin-dependent kinase 2 (CDK2)-associated cancer to a CDK2 inhibitor, comprising:

(i) determining the presence or absence of functional p16 gene expression by determining, from a biological sample obtained from the human subject by:

(a) the nucleotide sequence of a cyclin dependent kinase inhibitor 2A (CDKN2A) gene;

(b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and/or

(c) the presence of a p16 protein; and

(ii) determining the presence or absence of higher CCNE1 gene expression by determining, from a biological sample obtained from the human subject:

(a) the copy number of the cyclin E1 (CCNE1) gene; and/or

(b) the expression level of CCNE1,

wherein

(1)

(a) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1;

(b) the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and/or

(c) the presence of a p16 protein; and

(2) an amplification of the CCNE1 gene and/or an expression level of CCNE1 that is higher than a control expression level of CCNE1,

is predictive that the human subject will respond to the CDK2 inhibitor;

wherein the control expression level of CCNE1 is the expression level of CCNE1 in a sample or samples obtained from one or more subjects that have not responded to treatment with the CDK2 inhibitor; and

wherein the CDK2 inhibitor is:

4-((8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-2-

yl)amino)benzenesulfonamide,

8-cyclopentyl-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-phenyl-5,8-dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

8-(1,1-difluorobutan-2-yl)-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-8-((1-methyl-1*H*-pyrazol-5-yl)methyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-(tetrahydrofuran-3-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one,

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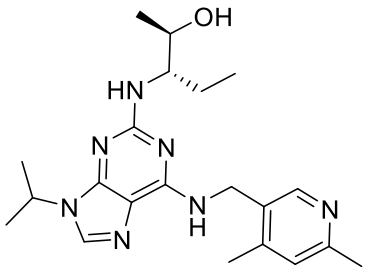
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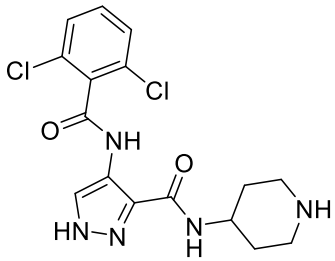
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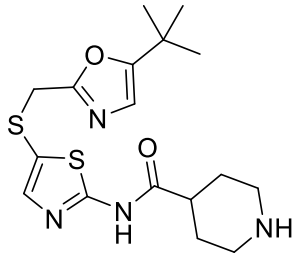
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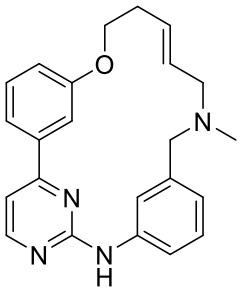
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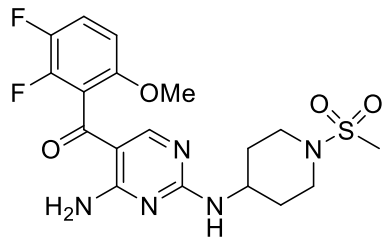
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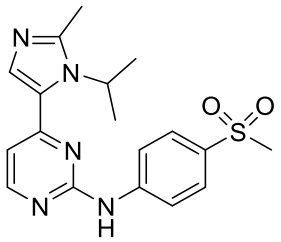
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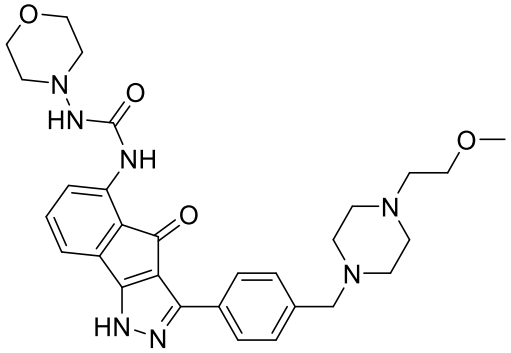
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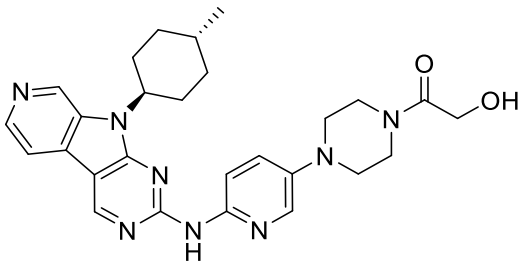
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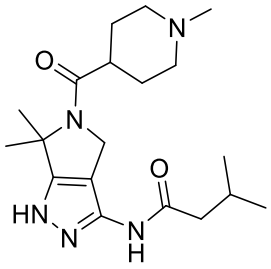
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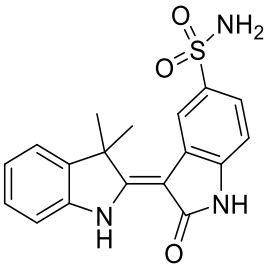
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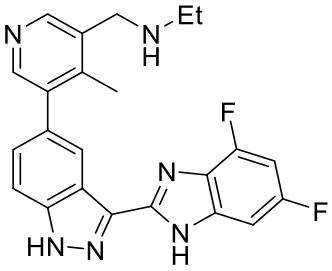
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or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, comprising:

(i) determining, from a biological sample obtained from the human subject:

(a) the nucleotide sequence of a CDKN2A gene and/or the presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and

(ii) determining, from a biological sample obtained from the human subject:

(a) the copy number of the CCNE1 gene,

wherein

(1)

(a) the presence of a CDKN2A gene encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1; and/or the

(b) presence of a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression, and

(2) an amplification of the CCNE1 gene,

is predictive that the human subject will respond to the CDK2 inhibitor.

3. Use of a CDK2 inhibitor in the manufacture of a medicament for the treatment of a human subject having a cyclin-dependent kinase 2 (CDK2)-associated cancer, wherein the human subject has been previously determined, according to the *in vitro* method of Claim 1 or Claim 2, to:

(i)

(a) have a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1;

(b) have a cyclin dependent kinase inhibitor 2A (CDKN2A) gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and/or

(c) express a p16 protein; and

(ii)

(a) have an amplification of the cyclin E1 (CCNE1) gene; and/or

(b) have an expression level of CCNE1 in a biological sample obtained from the human subject that is higher than a control expression level of CCNE1;

wherein the control expression level of CCNE1 is the expression level of CCNE1 in a sample or samples obtained from one or more subjects that have not responded to treatment with the CDK2 inhibitor; and

wherein the CDK2 inhibitor is:

4-((8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-2-yl)amino)benzenesulfonamide,

8-cyclopentyl-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-phenyl-5,8-dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

8-(1,1-difluorobutan-2-yl)-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-8-((1-methyl-1*H*-pyrazol-5-yl)methyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-(tetrahydrofuran-3-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one,

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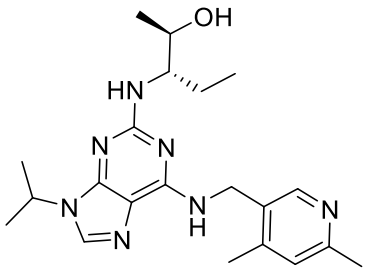
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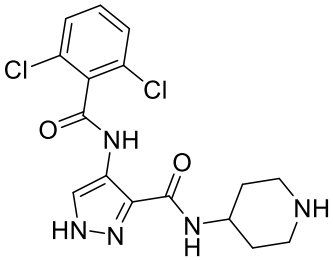
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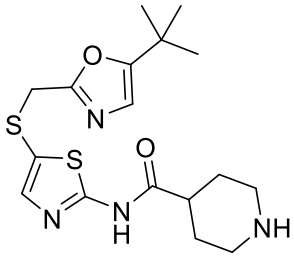
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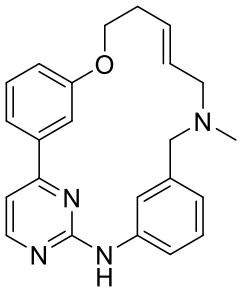
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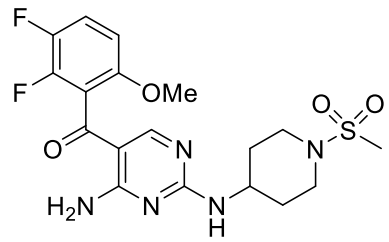
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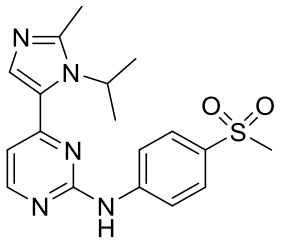
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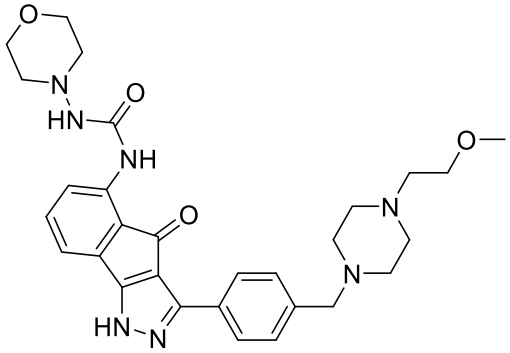
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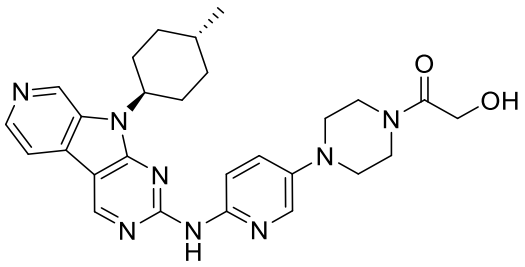
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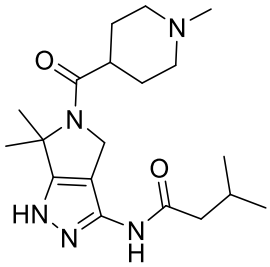
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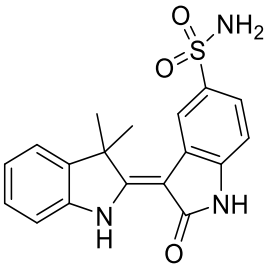
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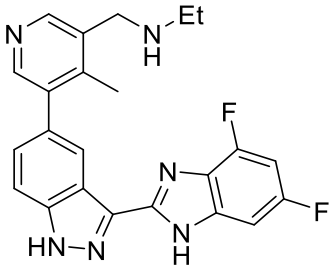
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or a pharmaceutically acceptable salt thereof.

4. The use of claim 3, wherein the human subject has been previously determined to:

(i)

(a) have a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1; and/or

(b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and

(ii) have an amplification of the CCNE1 gene in a biological sample obtained from the human subject.

5. The use of claim 3, wherein the expression level of CCNE1 in the biological sample is at least 1.5, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 25, at least 50, at least 75, or at least 100 times higher than the control expression level of CCNE1.

6. Use of a CDK2 inhibitor in the manufacture of a medicament for the treatment of a human subject having a cyclin-dependent kinase 2 (CDK2)-associated cancer, wherein, according to the *in vitro* method of Claim 1 or Claim 2:

(i) in a biological sample obtained from the human subject:

(a) a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present;

(b) a cyclin dependent kinase inhibitor 2A (CDKN2A) gene lacking one or more inactivating nucleic acid substitutions is present, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and/or

(c) a p16 protein is present; and

(ii) in a biological sample obtained from the human subject:

(a) there is present an amplification of the cyclin E1 (CCNE1) gene; and/or

(b) there is present an expression level of CCNE1 that is higher than a control expression level of CCNE1;

wherein the control expression level of CCNE1 is the expression level of CCNE1 in a sample or samples obtained from one or more subjects that have not responded to treatment with the CDK2 inhibitor; and

wherein the CDK2 inhibitor is:

4-((8-cyclopentyl-6,6-dimethyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-2-yl)amino)benzenesulfonamide,

8-cyclopentyl-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-phenyl-5,8-

dihydropyrido[2,3-d]pyrimidin-7(6H)-one,

8-(1,1-difluorobutan-2-yl)-6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-8-((1-methyl-1*H*-pyrazol-5-yl)methyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

6,6-dimethyl-2-((1-(methylsulfonyl)piperidin-4-yl)amino)-8-(tetrahydrofuran-3-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one,

8-((1R,2R)-2-hydroxy-2-methylcyclopentyl)-2-((1-(methylsulfonyl)piperidin-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one,

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alvociclib,

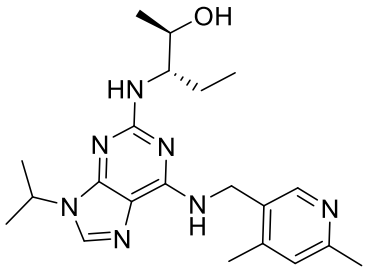
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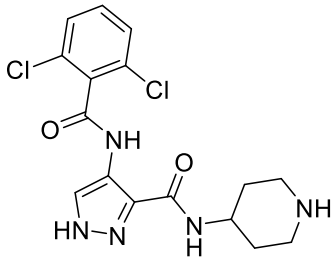
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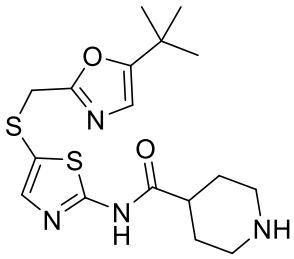
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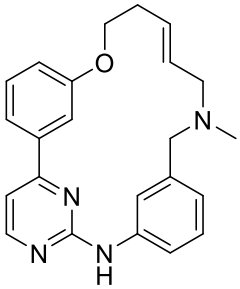
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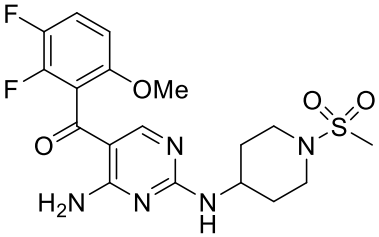
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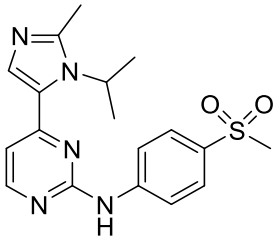
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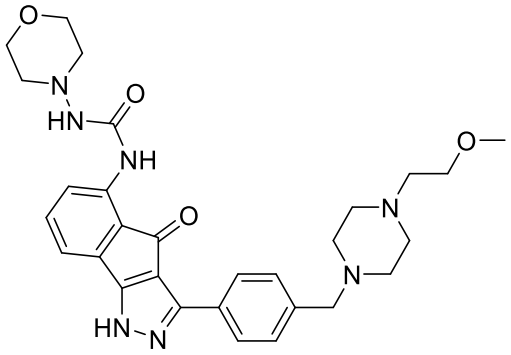
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R547

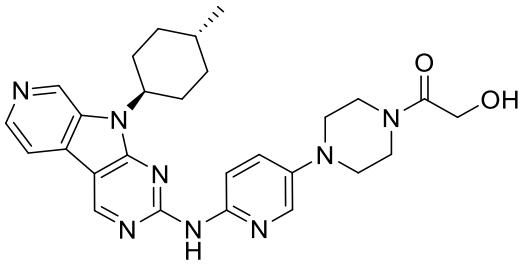


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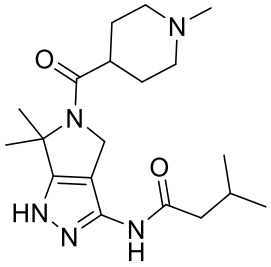
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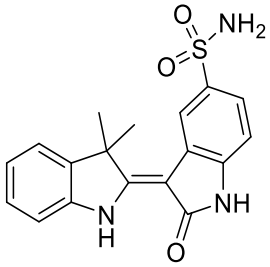
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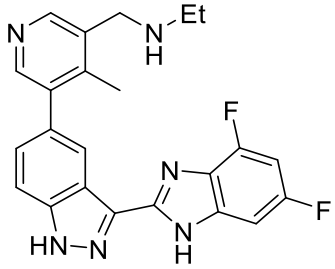
PHA-793887

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ZK-304709

, or



AG-24322

,

or a pharmaceutically acceptable salt thereof.

7. The use of claim 6, comprising:

(i) in a biological sample obtained from the human subject:

(a) a nucleotide sequence encoding a p16 protein comprising the amino acid sequence of SEQ ID NO:1 is present;

(b) a CDKN2A gene lacking one or more inactivating nucleic acid substitutions and/or deletions is present, which inactivating nucleic acid substitutions and/or deletions would, if present, result in dysfunctional p16 gene expression; and/or

(c) a p16 protein is present; and

(d) there is present an amplification of the CCNE1 gene.

8. The use of claim 6, wherein the expression level of CCNE1 in the biological sample is at least 1.5, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 25, at least 50, at least 75, or at least 100 times higher than the control expression level of CCNE1.

9. The use of any one of claims 3-7, wherein the medicament comprises a second therapeutic agent.

10. The use of claim 9, wherein the second therapeutic agent is a BCL2 inhibitor or a CDK4/6 inhibitor.

11. The *in vitro* method of claim 1 or claim 2 or the use of any one of claims 3-10 or, wherein the amplification of the CCNE1 gene comprises a gene copy number of at least 3; at least 5; or at least 21.

12. The *in vitro* method of any one of claims 1, 2, and 11 or the use of any one of claims 3-10, , wherein the expression level of CCNE1 is the expression level of CCNE1 mRNA; or wherein the expression level of CCNE1 is the expression level of CCNE1 protein.

13. The *in vitro* method of claim 14 or the use of claim 14, , wherein the expression level of CCNE1 is measured by RNA sequencing, quantitative polymerase chain reaction (PCR), in situ hybridization, nucleic acid array or RNA sequencing; or wherein the expression level of CCNE1 is measured by western blot, enzyme-linked immunosorbent assay, or immunohistochemistry staining.

14. The *in vitro* method of any one of claims 1, 2, and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is lung squamous cell carcinoma, lung adenocarcinoma, pancreatic adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, bladder urothelial carcinoma, mesothelioma, or sarcoma.

15. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is lung adenocarcinoma, breast invasive carcinoma, uterine carcinosarcoma, ovarian serous cystadenocarcinoma, or stomach adenocarcinoma.

16. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is an adenocarcinoma, carcinoma, or cystadenocarcinoma.

17. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is uterine cancer, ovarian cancer, stomach cancer, esophageal cancer, lung cancer, bladder cancer, pancreatic cancer, or breast cancer.

18. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is ovarian cancer, uterine carcinosarcoma, or breast cancer.

19. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is endometrial cancer.

20. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer comprises p27 inactivation.

21. The *in vitro* method of any one of claims 1, 2 and 11-13 or the use of any one of claims 3-13, wherein the CDK2-associated cancer is a N-myc amplified neuroblastoma, a K-Ras mutant lung cancer, or a cancer with a FBW7 mutation and CCNE1 overexpression.