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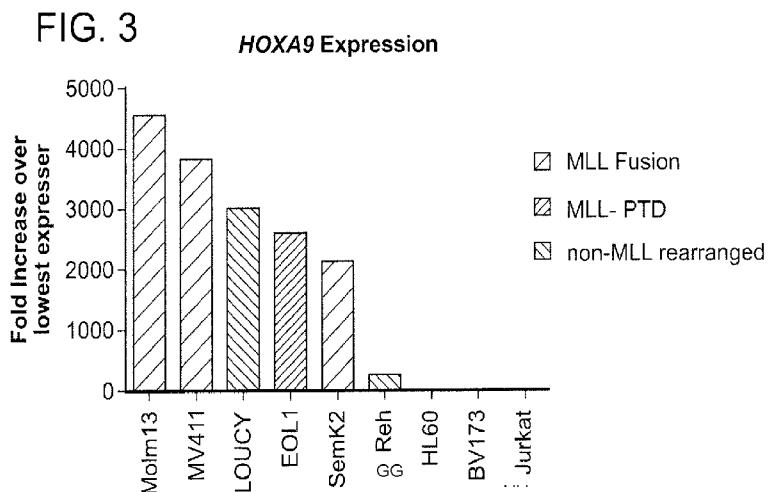
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(54) Title: DOT1 L INHIBITORS FOR USE IN THE TREATMENT OF LEUKEMIA



(57) Abstract: The present invention relates to DOT1L inhibitors. The present invention also relates to pharmaceutical compositions containing these compounds and methods of treating disorders in which DOT1L-mediated protein methylation plays a part, such as cancer, by administering these compounds and pharmaceutical compositions to subjects in need thereof.

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DOT1L INHIBITORS FOR USE IN THE TREATMENT OF LEUKEMIA

RELATED APPLICATION

[001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 61/745,294, filed December 21, 2012, the contents of which are incorporated herein in their entireties.

FIELD OF INVENTION

[002] The present invention relates generally to the field of cancer treatment, and in particular, the treatment of leukemia associated with the HOXA9, Flt3, MEIS1 and/or DOT1L. More particularly, the present invention provides methods and compositions which treat, alleviate, prevent, diminish or otherwise ameliorate the symptoms of leukemia associated with the HOXA9, Flt3, MEIS1 and/or DOT1L.

BACKGROUND OF THE INVENTION

[003] In eukaryotic cells, DNA is packaged with histones to form chromatin. Approximately 150 base pairs of DNA are wrapped twice around an octamer of histones (two each of histones 2A, 2B, 3, and 4) to form a nucleosome, the basic unit of chromatin. Changes in the ordered structure of chromatin can lead to alterations in transcription of associated genes. This process is highly controlled because changes in gene expression patterns can profoundly affect fundamental cellular processes such as differentiation, proliferation, and apoptosis. Control of changes in chromatin structure (and hence of transcription) is mediated by covalent modifications to histones, most notably of their N-terminal tails. These modifications are often referred to as epigenetic because they can lead to heritable changes in gene expression, but do not affect the sequence of the DNA itself. Covalent modifications (for example, methylation, acetylation, phosphorylation, and ubiquitination) of the side chains of amino acids are enzymatically mediated.

[004] The selective addition of methyl groups to specific amino acid sites on histones is controlled by the action of a unique family of enzymes known as histone methyltransferases (HMTs). The level of expression of a particular gene is influenced by the presence or absence of a methyl group at a relevant histone site. The specific effect of a methyl group at a particular histone site persists until the methyl group is removed by a histone demethylase, or until the modified histone is replaced through nucleosome turnover. In a like manner,

other enzyme classes can decorate DNA and histones with other chemical species and still other enzymes can remove these species to provide temporal control of gene expression.

[005] The orchestrated collection of biochemical systems behind transcriptional regulation must be tightly controlled in order for cell growth and differentiation to proceed optimally. Disease states result when these controls are disrupted by aberrant expression and/or activity of the enzymes responsible for DNA and histone modification. In human cancers, for example, there is a growing body of evidence to suggest that dysregulated epigenetic enzyme activity contributes to the uncontrolled cell proliferation associated with cancer as well as other cancer-relevant phenotypes such as enhanced cell migration and invasion. Beyond cancer, there is growing evidence for a role of epigenetic enzymes in a number of other human diseases, including metabolic diseases (such as diabetes), inflammatory diseases (such as Crohn's disease), neurodegenerative diseases (such as Alzheimer's disease) and cardiovascular diseases. Therefore, selectively modulating the aberrant action of epigenetic enzymes holds great promise for the treatment of a range of diseases.

[006] There is an ongoing need for new agents which modulate the aberrant action of epigenetic enzymes.

SUMMARY OF THE INVENTION

[007] The present invention provides a method for treating or alleviating a symptom of leukemia comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4. In one aspect, the leukemia is characterized by a chromosomal rearrangement. In one aspect, the chromosomal rearrangement is chimeric fusion of mixed lineage leukemia gene (MLL) or partial tandem duplication of the MLL gene (MLL-PTD). In another aspect, the subject has an increased level of HOXA9, Fms-like tyrosine kinase 3 (FLT3), MEIS1, and/or DOT1L.

[008] The present invention provides a method for treating or alleviating a symptom of leukemia comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4, wherein the subject has an increased level of HOXA9, FLT3, MEIS1, and/or DOT1L.

[009] The present invention provides a method for treating or alleviating a symptom of leukemia in a subject comprising: obtaining a sample from the subject; detecting the level of HOXA9, FLT3, MEIS1, and/or DOT1L, wherein an increased level of HOXA9, FLT3, MEIS1, and/or DOT1L indicates the subject is responsive to a compound listed in any of Tables 1-4; and administering to the subject a therapeutically effective amount of said

compound when said subject is responsive to said compound.

[010] The present invention provides a method for treating or alleviating a symptom of leukemia in a subject comprising: obtaining a sample from the subject; detecting the presence of a genetic lesion of MLL in the sample; and administering to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4 when said genetic lesion is present in the sample. In one aspect, the genetic lesion is chimeric fusion of MLL or MLL-PTD.

[011] In any of the foregoing methods, the sample is selected from bone marrow, peripheral blood cells, blood, plasma, serum, urine, saliva, a cell, or a tumor tissue.

[012] The present invention provides a method for treating a disorder mediated by translocation, deletion and/or duplication of a gene on chromosome 11q23, comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4.

[013] In another aspect, the invention features a method of selecting a therapy for a subject having leukemia. The method includes the steps of: detecting the presence of a partial tandem duplication of the MLL gene (MLL-PTD) in a sample from the subject; and selecting, based on the presence of the MLL-PTD, a therapy for treating leukemia. In one embodiment, the therapy includes administering to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the method further includes administrating to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the leukemia is characterized by partial tandem duplication of the MLL gene.

[014] In another aspect, a method of treatment is provided for a subject in need thereof, the method comprising the steps of : detecting presence of a partial tandem duplication of the MLL gene (MLL-PTD) in a sample from the subject; and treating the subject based on the presence of MLL-PTD with a therapy that includes administrating to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the subject in need thereof has leukemia that is characterized by partial tandem duplication of the MLL gene.

[015] In another aspect, the invention features a method of selecting a therapy for a subject having leukemia. The method includes the steps of: detecting the level of HOXA9, FLT3, MEIS1, and/or DOT1L in a sample from the subject; and selecting, based on the presence of the increased level of HOXA9, FLT3, MEIS1, and/or DOT1L a therapy for treating leukemia. In one embodiment, the therapy includes administering to the subject a

therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the method further includes administrating to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the leukemia is characterized by partial tandem duplication of the MLL gene. In another embodiment, the leukemia is characterized by overexpression of HOXA9, FLT3, MEIS1 and/or DOT1L.

[016] In yet another aspect, a method of treatment is provided for a subject in need thereof, the method comprising the steps of : detecting the the level of HOXA9, FLT3, MEIS1, and/or DOT1L in a sample from the subject; and treating the subject based on the presence of the increased level of HOXA9, FLT3, MEIS1, and/or DOT1L with a therapy that includes administrating to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4. In one embodiment, the subject in need thereof has leukemia that is characterized by partial tandem duplication of the MLL gene. In another embodiment, the subject in need thereof has leukemia that is characterized by overexpression of HOXA9, FLT3, MEIS1 and/or DOT1L.

[017] In any of the foregoing methods, the compound is Compound A2.

[018] In any of the foregoing methods, the compound is Compound T (also referred to as Compound D16 in Table 4).

[019] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods and examples are illustrative only and are not intended to be limiting.

[020] Other features and advantages of the invention will be apparent from the following detailed description and claims.

DESCRIPTION OF THE FIGURES

[021] Figures 1A and 1B are respectively a plot and a Western blot image demonstrating inhibition of cell growth (A) and H3K79me2 status (B) in MLL PTD cell line EOL-1 after

treatment with Compound A2.

[022] Figures 2A and 2B are respectively a plot and a table demonstrating that the potency of the tested compounds correlates with EOL-1 cell growth inhibition.

[023] Figure 3 is a graph showing HOXA9 expression in a panel of MLL fusion, MLL PTD and non-MLL rearranged human leukemia cell lines.

[024] Figure 4 is a plot showing reduction of HOXA9 and FLT3 expression after Compound A2 treatment in a dose-dependent manner.

[025] Figure 5 is a plot showing reduction of HOXA9, FLT3, MEIS1 and DOT1L after Compound T treatment in a dose-dependent manner.

DETAILED DESCRIPTION OF THE INVENTION

[026] One aspect of the present invention is based in part upon the surprising discovery that DOT1L inhibitors can effectively treat leukemia that is characterized by partial tandem duplication of the MLL gene. Another aspect of the present invention is based in part upon the surprising discovery that DOT1L inhibitors can effectively treat leukemia that is characterized by overexpression of HOXA9, FLT3, MEIS1 and/or DOT1L. Specifically, tumors or tumor cells having increased mRNA or protein level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L are sensitive to the DOT1L inhibitors of the present invention. Accordingly, the present invention provides methods of treating or alleviating a symptom of leukemia in a subject by administering a therapeutically effective amount of a DOT1L inhibitor of any of Tables 1-4 to the subject, particular leukemia associated with overexpression of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L.

[027] The compounds of the present invention inhibit the histone methyltransferase activity of DOT1L or a mutant thereof. Based upon the surprising discovery that methylation regulation by DOT1L involves in tumor formation, particular tumors bearing an increased mRNA, protein and/or activity (function) level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, the compounds described herein are suitable candidates for treating cancers, *i.e.*, to decrease methylation or restore methylation to roughly its level in counterpart normal cells.

[028] The present invention features a method for treating or alleviating a symptom of cancer. The method includes administering to a subject in need thereof, a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph, solvate, or stereoisomer thereof.

[029] The present invention provides methods for the treatment of a cancer mediated by DOT1 (e.g., DOT1L)-mediated protein methylation in a subject in need thereof by administering to a subject in need of such treatment, a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof. The present invention further provides the use of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, for the preparation of a medicament useful for the treatment of a cancer mediated by DOT1L-mediated protein methylation.

[030] The present invention provides methods for the treatment of a cancer the course of which is influenced by modulating the methylation status of histones or other proteins, wherein said methylation status is mediated at least in part by the activity of DOT1L. Modulation of the methylation status of histones can in turn influence the level of expression of target genes activated by methylation, and/or target genes suppressed by methylation. The method includes administering to a subject in need of such treatment, a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph, solvate, or stereoisomeror thereof.

[031] The present invention also provides methods of protecting against or preventing a cancer in which DOT1L-mediated protein methylation plays a part in a subject in need thereof by administering a therapeutically effective amount of compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, to a subject in need of such treatment. The present invention also provides the use of compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph, solvate, or stereoisomeror thereof, for the preparation of a medicament useful for the prevention of a cell proliferative disorder.

[032] In one aspect, the cancer is a cancer selected from the group consisting of brain and CNS cancer, kidney cancer, ovarian cancer, pancreatic cancer, lung cancer, breast cancer, colon cancer, prostate cancer, or a hematological cancer. For example, the hematological cancer is leukemia or lymphoma. Preferably the cancer is leukemia.

[033] The present invention further provides the use of a compound of the present invention, or a pharmaceutically acceptable salt, ester, prodrug, metabolite, polymorph or solvate thereof in the treatment of leukemia, or, for the preparation of a medicament useful for the treatment of such leukemia. The leukemia can be acute or chronic leukemia. Preferably, the leukemia is acute myeloid leukemia, acute lymphocytic leukemia or mixed lineage leukemia. Exemplary leukemia that may be treated is mixed lineage leukemia (MLL).

Preferably, the MLL that can be treated by the compound of the present invention is chimeric fusion of MLL, partial tandem duplication of the MLL gene (MLL-PTD) or non-rearranged MLL.

[034] Mixed lineage leukemia (MLL) is a genetically distinct form of acute leukemia that constitutes over 70% of infant leukemias and approximately 10% of adult acute myeloid leukemias (AML) (Hess, J. L. (2004), Trends Mol Med 10, 500-507; Krivtsov, A. V., and Armstrong, S. A. (2007), Nat Rev Cancer 7, 823-833). MLL represents a particularly aggressive form of leukemia and patients with this disease generally have poor prognoses; these patients often suffer from early relapse after treatment with current chemotherapies. There is thus a great and present need for new treatment modalities for patients suffering with MLL.

[035] A universal hallmark of MLL disease is a chromosomal translocation affecting the MLL gene on chromosome 11q23 (Hess, 2004; Krivtsov and Armstrong, 2007). Normally, the MLL gene encodes for a SET-domain histone methyltransferase that catalyzes the methylation of lysine 4 of histone H3 (H3K4) at specific gene loci (Milne et al. (2002) Mol Cell 10, 1107-1117; Nakamura et al. (2002), Mol Cell 10, 1119-1128). Gene localization is conferred by specific interactions with recognition elements within MLL, external to the SET-domain (Ayton et al. (2004) Mol Cell Biol 24, 10470-10478; Slany et al., (1998) Mol Cell Biol 18, 122-129; Zeleznik-Le et al. (1994) Proc Natl Acad Sci U S A 91, 10610-10614). In the disease-linked translocations, the catalytic SET-domain is lost and the remaining MLL protein is fused to a variety of partners, including members of the AF and ENL family of proteins such as AF4, AF9, AF10 and ENL (Hess, 2004; Krivtsov and Armstrong, 2007; Slany (2009) Haematologica 94, 984-993). These fusion partners are capable of interacting directly, or indirectly, with another histone methyltransferase, DOT1L (Bitoun et al. (2007) Hum Mol Genet 16, 92-106; Mohan et al. (2010) Genes Dev. 24, 574-589; Mueller et al. (2007) Blood 110, 4445-4454; Mueller et al. (2009) PLoS Biol 7, e1000249; Okada et al. (2005) Cell 121, 167-178; Park et al. (2010) Protein J 29, 213-223; Yokoyama et al. (2010) Cancer Cell 17, 198-212; Zhang et al. (2006) J Biol Chem 281, 18059-18068). As a result, translocation products retain gene-specific recognition elements within the remainder of the MLL protein, but also gain the ability to recruit DOT1L, to these locations (Monroe et al. (2010) Exp Hematol. 2010 Sep 18. [Epub ahead of print] Pubmed PMID: 20854876; Mueller et al., 2007; Mueller et al., 2009; Okada et al., 2005). DOT1L catalyzes the methylation of H3K79, a chromatin modification associated with actively transcribed genes (Feng et al. (2002) Curr Biol 12, 1052-1058; Steger et al. (2008) Mol Cell Biol 28, 2825-2839). The

ectopic H3K79 methylation that results from MLL fusion protein recruitment of DOT1L leads to enhanced expression of leukemogenic genes, including HOXA9 and MEIS1 (Guenther et al. (2008) *Genes & Development* 22, 3403-3408; Krivtsov et al. (2008) *Nat Rev Cancer* 7, 823-833; Milne et al. (2005) *Cancer Res* 65, 11367-11374; Monroe et al., 2010; Mueller et al., 2009; Okada et al., 2005; Thiel et al. (2010) *Cancer Cell* 17, 148-159). Hence, while DOT1L is not genetically altered in the disease *per se*, its mislocated enzymatic activity is a direct consequence of the chromosomal translocation affecting MLL patients; thus, DOT1L has been proposed to be a catalytic driver of leukemogenesis in this disease (Krivtsov et al., 2008; Monroe et al., 2010; Okada et al., 2005; Yokoyama et al. (2010) *Cancer Cell* 17, 198-212). Further support for a pathogenic role of DOT1L in MLL comes from studies in model systems that demonstrate a requirement for DOT1L in propagating the transforming activity of MLL fusion proteins (Mueller et al., 2007; Okada et al., 2005).

[036] Evidence indicates that the enzymatic activity of DOT1L is critical to pathogenesis in MLL and inhibition of DOT1L may provide a pharmacologic basis for therapeutic intervention in this disease. Compound treatment results in selective, concentration-dependent killing of leukemia cells bearing the MLL-translocation without effect on non-MLL transformed cells. Gene expression analysis of inhibitor treated cells shows downregulation of genes aberrantly over expressed in MLL-rearranged leukemias and similarities with gene expression changes caused by genetic knockout of the DOT1L gene in a mouse model of MLL-AF9 leukemia.

[037] MLL can be characterized by the genetic lesions of the MLL gene. Such genetic lesions include chromosomal rearrangements, such as translocations, deletions, and/or duplications of the MLL gene. MLL has been categorized or characterized as having a chimeric fusion of MLL, partial tandem duplication of the MLL gene (MLL-PTD), or nonrearranged MLL. Chromosomal rearrangements or translocations can be identified by methods known in the art. For example, chromosomal rearrangements resulting in chimeric fusions can be detected by probe-based assays, such as FISH (fluorescence in situ hybridization) or sequence amplification by PCR. Those chromosomal rearrangements that result in partial tandem duplications are often difficult to detect by probe-based assays, and therefore, other DNA sequencing methods known in the art may be used, such as Sanger sequencing, de novo sequencing, shotgun sequencing, or next generation sequencing methods. MLL-PTD can be identified by DNA sequencing. MLL chimeric fusions can be identified by FISH. Diagnosis of MLL can be performed by detection of rearrangements of the MLL gene, or increased mRNA, protein, and/or activity level of at least one protein

selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, as further described herein.

[038] Compounds of the present invention can selectively inhibit proliferation of tumor or tumor cells characterized with an increased mRNA, protein and/or activity (function) level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L (as shown in Figures 1-5).

[039] Accordingly, the present invention provides methods for treating or alleviating a symptom of leukemia characterized with an increased mRNA, protein and/or activity (function) level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L by a compound of the present invention, or a pharmaceutically acceptable salt, ester, prodrug, metabolite, polymorph or solvate thereof. Exemplary leukemia that may be treated is mixed lineage leukemia (MLL). Preferably, MLL that can be treated by the compound of the present invention is chimeric fusion of MLL, partial tandem duplication of MLL (MLL-PTD) or nonrearranged MLL.

[040] The present invention also provides methods for treating or alleviating a symptom of leukemia characterized by the presence of a genetic lesion of MLL. For example, this method comprises obtaining sample from the subject; detecting the presence of a genetic lesion of MLL in the sample; and when the genetic lesion is present in the sample, administering to the subject a therapeutically effective amount of a DOT1L inhibitor (*i.e.*, a compound listed in any one of Tables 1-4). The genetic lesion is chimeric fusion of MLL or MLL-PTD.

[041] The present invention also provides methods for treating a disorder mediated by translocation, deletion and/or duplication of a gene on chromosome 11q23, comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any one of Tables 1-4.

[042] In other aspects, the present invention provides personalized medicine, treatment and/or cancer management for a subject by genetic screening of increased gene expression (mRNA or protein), and/or increased function or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L in the subject. For example, the present invention provides methods for treating, preventing or alleviating a symptom of cancer or a precancerous condition by determining responsiveness of the subject to a DOT1L inhibitor and when the subject is responsive to the DOT1L inhibitor, administering to the subject a therapeutically effective amount of the DOT1L inhibitor, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph, solvate, or stereoisomer or thereof. The responsiveness is determined by obtaining a sample from the subject and

detecting increased mRNA or protein, and/or increased activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, and the presence of such gain of expression and/or function indicates that the subject is responsive to the DOT1L inhibitor. Once the responsiveness of a subject is determined, a therapeutically effective amount of a DOT1L inhibitor, for example, any compound from Tables 1-4, can be administered. The therapeutically effective amount of a DOT1L inhibitor can be determined by one of ordinary skill in the art.

[043] As used herein, the term “responsiveness” is interchangeable with terms “responsive”, “sensitive”, and “sensitivity”, and it is meant that a subject is showing therapeutic responses when administered an DOT1L inhibitor, *e.g.*, tumor cells or tumor tissues of the subject undergo apoptosis and/or necrosis, and/or display reduced growing, dividing, or proliferation. This term is also meant that a subject will or has a higher probability, relative to the population at large, of showing therapeutic responses when administered an DOT1L inhibitor, *e.g.*, tumor cells or tumor tissues of the subject undergo apoptosis and/or necrosis, and/or display reduced growing, dividing, or proliferation.

[044] As used herein, a “subject” is interchangeable with a “subject in need thereof”, both of which refers to a subject having a disorder in which DOT1L-mediated protein methylation plays a part, or a subject having an increased risk of developing such disorder relative to the population at large. A subject in need thereof may be a subject having a disorder associated DOT1L. A subject in need thereof can have a precancerous condition. Preferably, a subject in need thereof has cancer. A subject in need thereof can have cancer associated with DOT1L. A subject in need thereof can have cancer associated with increased expression (mRNA or protein) and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L. In a preferred aspect, a subject in need thereof has one or more cancers selected from the group consisting of brain and central nervous system (CNS) cancer, head and neck cancer, kidney cancer, ovarian cancer, pancreatic cancer, leukemia, lung cancer, lymphoma, myeloma, sarcoma, breast cancer, prostate cancer and a hematological cancer. Preferably, a subject in need thereof has a hematologic cancer, wherein the hematologic cancer is leukemia or lymphoma. Exemplary leukemia is MLL. Other hematologic cancers of the present invention can include multiple myeloma, lymphoma (including Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, childhood lymphomas, and lymphomas of lymphocytic and cutaneous origin), leukemia (including childhood leukemia, hairy-cell leukemia, acute lymphocytic leukemia, acute myelocytic

leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, chronic myelogenous leukemia, and mast cell leukemia), myeloid neoplasms and mast cell neoplasms.

[045] As used herein, a “subject” includes a mammal. The mammal can be *e.g.*, a human or appropriate non-human mammal, such as primate, mouse, rat, dog, cat, cow, horse, goat, camel, sheep or a pig. The subject can also be a bird or fowl. In one embodiment, the mammal is a human. A subject can be male or female.

[046] A subject in need thereof can be one who has been previously diagnosed or identified as having cancer or a precancerous condition. A subject in need thereof can also be one who is having (suffering from) cancer or a precancerous condition. Alternatively, a subject in need thereof can be one who is having an increased risk of developing such disorder relative to the population at large (*i.e.*, a subject who is predisposed to developing such disorder relative to the population at large).

[047] Optionally a subject in need thereof has already undergone, is undergoing or will undergo, at least one therapeutic intervention for the cancer or precancerous condition.

[048] A subject in need thereof may have refractory cancer on most recent therapy. “Refractory cancer” means cancer that does not respond to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment.

Refractory cancer is also called resistant cancer. In some embodiments, the subject in need thereof has cancer recurrence following remission on most recent therapy. In some embodiments, the subject in need thereof received and failed all known effective therapies for cancer treatment. In some embodiments, the subject in need thereof received at least one prior therapy.

[049] In some embodiments, a subject in need thereof may have a secondary cancer as a result of a previous therapy. “Secondary cancer” means cancer that arises due to or as a result from previous carcinogenic therapies, such as chemotherapy. In some embodiments, the secondary cancer is a hematologic cancer, such as leukemia.

[050] In any method of the present invention, a subject in need thereof may have increased mRNA, protein, and/or activity level of at least of at least one signaling component downstream of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L. Such downstream components are readily known in the art, and can include other transcription factors, or signaling proteins.

[051] As used herein, the term “increase in activity” refers to increased or a gain of function of a gene product/protein compared to the wild type. In one aspect of the present invention, increased activity can be caused by increased mRNA and/or increased protein levels.

Increased mRNA levels can be caused by gene amplification and increased transcription, for example. Increased protein levels can be caused by increased stability, inhibition of degradation pathways, or increased transcription. Alternatively, increased activity levels can be caused by a gain of function mutation resulting from a point mutation (*e.g.*, a substitution, a missense mutation, or a nonsense mutation), an insertion, and/or a deletion, or a rearrangement in a polypeptide selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, or a nucleic acid sequence encoding a polypeptide selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L. The mutations referred herein are somatic mutations. The term "somatic mutation" refers to a deleterious alteration in at least one gene allele that is not found in every cell of the body, but is found only in isolated cells. A characteristic of the somatic mutations as used herein is, that they are restricted to particular tissues or even parts of tissues or cells within a tissue and are not present in the whole organism harboring the tissues or cells. The term "wild-type" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

[052] Accordingly, an increase in mRNA or protein expression and/or activity levels can be detected using any suitable method available in the art. For example, an increase in activity level can be detected by measuring the biological function of a gene product, such as the histone methyltransferase activity of DOT1L (*i.e.*, methylation of histone substrates such as H3K79 by immunoblot); transcriptional activity of HOXA9 or MEIS1 (*i.e.*, expression levels of HOXA9 or MEIS1 target genes by RT-PCR); or phosphorylation activity of FLT3 (*i.e.*, phosphorylation status of FLT3 targets by immunoblot or radioimmunoassay). Alternatively, a gain of function mutation can be determined by detecting any alteration in a nucleic acid sequence encoding a protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L. For example, a nucleic acid sequence encoding HOXA9, FLT3, MEIS1 and DOT1L having a gain of function mutation can be detected by whole-genome resequencing or target region resequencing (the latter also known as targeted resequencing) using suitably selected sources of DNA and polymerase chain reaction (PCR) primers in accordance with methods well known in the art. The method typically and generally entails the steps of genomic DNA purification, PCR amplification to amplify the region of interest, cycle sequencing, sequencing reaction cleanup, capillary electrophoresis, and/or data analysis. Alternatively or in addition, the method may include the use of microarray-based targeted region genomic DNA capture and/or sequencing. Kits, reagents, and methods for selecting

appropriate PCR primers and performing resequencing are commercially available, for example, from Applied Biosystems, Agilent, and NimbleGen (Roche Diagnostics GmbH). Detection of mRNA expression can be detected by methods known in the art, such as Northern blot, nucleic acid PCR, and quantitative RT-PCR. Detection of polypeptide expression (*i.e.*, wild-type or mutant) can be carried out with any suitable immunoassay in the art, such as Western blot analysis.

[053] By "sample" it means any biological sample derived from the subject, includes but is not limited to, cells, tissues samples, body fluids (including, but not limited to, mucus, blood, plasma, serum, urine, saliva, and semen), tumor cells, and tumor tissues. Preferably, the sample is selected from bone marrow, peripheral blood cells, blood, plasma and serum. Samples can be provided by the subject under treatment or testing. Alternatively samples can be obtained by the physician according to routine practice in the art.

[054] The present invention also provides methods for diagnosing leukemia in a subject by obtaining a sample from the subject and detecting an increased mRNA, protein and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, and the presence of such increased mRNA, protein and/or activity level indicates that the subject has or is at risk for developing leukemia compared to a subject without such increased mRNA, protein and/or activity level, or a subject that does not have leukemia.

[055] The present invention also provides methods for determining predisposition of a subject to leukemia by obtaining a sample from the subject and detecting an increased mRNA, protein and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, and the presence of such increased mRNA, protein and/or activity level indicates that the subject is predisposed to (*i.e.*, having higher risk of) developing leukemia compared to a subject without such increased mRNA, protein and/or activity level.

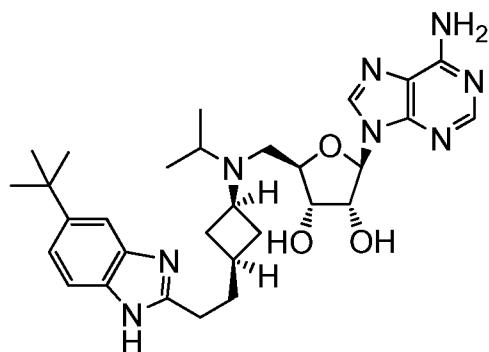
[056] The term "predisposed" as used herein in relation to cancer or a precancerous condition is to be understood to mean the increased probability (*e.g.*, at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, or more increase in probability) that a subject with an increased mRNA, protein and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, will suffer leukemia, as compared to the probability that another subject not having an increased mRNA, protein and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, will suffer leukemia, under circumstances where

other risk factors (*e.g.*, chemical/environment, food, and smoking history, etc.) for having leukemia between the subjects are the same.

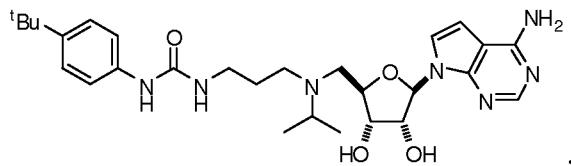
[057] “Risk” in the context of the present invention, relates to the probability that an event will occur over a specific time period and can mean a subject’s “absolute” risk or “relative” risk. Absolute risk can be measured with reference to either actual observation post-measurement for the relevant time cohort, or with reference to index values developed from statistically valid historical cohorts that have been followed for the relevant time period. Relative risk refers to the ratio of absolute risks of a subject compared either to the absolute risks of low risk cohorts or an average population risk, which can vary by how clinical risk factors are assessed. Odds ratios, the proportion of positive events to negative events for a given test result, are also commonly used (odds are according to the formula $p/(1-p)$ where p is the probability of event and $(1-p)$ is the probability of no event) to no-conversion.

[058] In other example, the present invention provides methods of cancer management in a subject by determining predisposition of the subject to a cancer or a precancerous condition periodically. The methods comprise steps of obtaining a sample from the subject and detecting increased mRNA or protein, and/or increased activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L, and the presence of such gain of expression and/or function indicates that the subject is predisposed to developing the cancer or the precancerous condition compared to a subject without such gain of mRNA or protein expression and/or function of the at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L.

[059] Any compounds (*e.g.*, DOT1L inhibitor) of the present invention can be used for the methods described above. In one embodiment, the DOT1L inhibitor used in any preceding methods is Compound A2 having the formula:



[060] Alternatively, the DOT1L inhibitor used in any preceding methods is Compound T (*i.e.*, Compound D16) having the formula



[061] As used herein, the term “cell proliferative disorder” refers to conditions in which unregulated or abnormal growth, or both, of cells can lead to the development of an unwanted condition or disease, which may or may not be cancerous. Exemplary cell proliferative disorders of the invention encompass a variety of conditions wherein cell division is deregulated. Exemplary cell proliferative disorder include, but are not limited to, neoplasms, benign tumors, malignant tumors, pre-cancerous conditions, *in situ* tumors, encapsulated tumors, metastatic tumors, liquid tumors, solid tumors, immunological tumors, hematological tumors, cancers, carcinomas, leukemias, lymphomas, sarcomas, and rapidly dividing cells. The term “rapidly dividing cell” as used herein is defined as any cell that divides at a rate that exceeds or is greater than what is expected or observed among neighboring or juxtaposed cells within the same tissue.

[062] A cell proliferative disorder includes a precancer or a precancerous condition. A cell proliferative disorder includes cancer. Preferably, the methods provided herein are used to treat or alleviate a symptom of cancer. The term “cancer” includes solid tumors, as well as, hematologic tumors and/or malignancies. A “precancer cell” or “precancerous cell” is a cell manifesting a cell proliferative disorder that is a precancer or a precancerous condition. A “cancer cell” or “cancerous cell” is a cell manifesting a cell proliferative disorder that is a cancer. Any reproducible means of measurement may be used to identify cancer cells or precancerous cells. Cancer cells or precancerous cells can be identified by histological typing or grading of a tissue sample (e.g., a biopsy sample). Cancer cells or precancerous cells can be identified through the use of appropriate molecular markers.

[063] Exemplary non-cancerous conditions or disorders include, but are not limited to, rheumatoid arthritis; inflammation; autoimmune disease; lymphoproliferative conditions; acromegaly; rheumatoid spondylitis; osteoarthritis; gout, other arthritic conditions; sepsis; septic shock; endotoxic shock; gram-negative sepsis; toxic shock syndrome; asthma; adult respiratory distress syndrome; chronic obstructive pulmonary disease; chronic pulmonary inflammation; inflammatory bowel disease; Crohn’s disease; psoriasis; eczema; ulcerative colitis; pancreatic fibrosis; hepatic fibrosis; acute and chronic renal disease; irritable bowel syndrome; pyresis; restenosis; cerebral malaria; stroke and ischemic injury; neural trauma; Alzheimer’s disease; Huntington’s disease; Parkinson’s disease; acute and chronic pain; allergic rhinitis; allergic conjunctivitis; chronic heart failure; acute coronary syndrome;

cachexia; malaria; leprosy; leishmaniasis; Lyme disease; Reiter's syndrome; acute synovitis; muscle degeneration, bursitis; tendonitis; tenosynovitis; herniated, ruptures, or prolapsed intervertebral disk syndrome; osteopetrosis; thrombosis; restenosis; silicosis; pulmonary sarcosis; bone resorption diseases, such as osteoporosis; graft-versus-host reaction; Multiple Sclerosis; lupus; fibromyalgia; AIDS and other viral diseases such as Herpes Zoster, Herpes Simplex I or II, influenza virus and cytomegalovirus; and diabetes mellitus.

[064] Exemplary cancers include, but are not limited to, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, anorectal cancer, cancer of the anal canal, appendix cancer, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, basal cell carcinoma, skin cancer (non-melanoma), biliary cancer, extrahepatic bile duct cancer, intrahepatic bile duct cancer, bladder cancer, urinary bladder cancer, bone and joint cancer, osteosarcoma and malignant fibrous histiocytoma, brain cancer, brain tumor, brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas/carcinoids, carcinoid tumor, gastrointestinal, nervous system cancer, nervous system lymphoma, central nervous system cancer, central nervous system lymphoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, lymphoid neoplasm, mycosis fungoides, Sezary Syndrome, endometrial cancer, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, ovarian germ cell tumor, gestational trophoblastic tumor glioma, head and neck cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, ocular cancer, islet cell tumors (endocrine pancreas), Kaposi Sarcoma, kidney cancer, renal cancer, kidney cancer, laryngeal cancer, acute lymphoblastic leukemia, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, AIDS-related lymphoma, non-Hodgkin lymphoma, primary central nervous system lymphoma, Waldenstram macroglobulinemia, medulloblastoma, melanoma, intraocular (eye) melanoma, merkel cell carcinoma, mesothelioma malignant, mesothelioma, metastatic squamous neck cancer, mouth cancer, cancer of the tongue, multiple endocrine neoplasia syndrome, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/ myeloproliferative

diseases, chronic myelogenous leukemia, acute myeloid leukemia, multiple myeloma, chronic myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, oral cancer, oral cavity cancer, oropharyngeal cancer, ovarian cancer, ovarian epithelial cancer, ovarian low malignant potential tumor, pancreatic cancer, islet cell pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, prostate cancer, rectal cancer, renal pelvis and ureter, transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, ewing family of sarcoma tumors, Kaposi Sarcoma, soft tissue sarcoma, uterine cancer, uterine sarcoma, skin cancer (non-melanoma), skin cancer (melanoma), merkel cell skin carcinoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, testicular cancer, throat cancer, thymoma, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter and other urinary organs, gestational trophoblastic tumor, urethral cancer, endometrial uterine cancer, uterine sarcoma, uterine corpus cancer, vaginal cancer, vulvar cancer, and Wilm's Tumor.

[065] A “cell proliferative disorder of the hematologic system” is a cell proliferative disorder involving cells of the hematologic system. A cell proliferative disorder of the hematologic system can include lymphoma, leukemia, myeloid neoplasms, mast cell neoplasms, myelodysplasia, benign monoclonal gammopathy, lymphomatoid granulomatosis, lymphomatoid papulosis, polycythemia vera, chronic myelocytic leukemia, agnogenic myeloid metaplasia, and essential thrombocythemia. A cell proliferative disorder of the hematologic system can include hyperplasia, dysplasia, and metaplasia of cells of the hematologic system. Preferably, compositions of the present invention may be used to treat a cancer selected from the group consisting of a hematologic cancer of the present invention or a hematologic cell proliferative disorder of the present invention. A hematologic cancer of the present invention can include multiple myeloma, lymphoma (including Hodgkin's lymphoma, non-Hodgkin's lymphoma, childhood lymphomas, and lymphomas of lymphocytic and cutaneous origin), leukemia (including childhood leukemia, hairy-cell leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, chronic myelogenous leukemia, and mast cell leukemia), myeloid neoplasms and mast cell neoplasms.

[066] A “cell proliferative disorder of the lung” is a cell proliferative disorder involving cells of the lung. Cell proliferative disorders of the lung can include all forms of cell

proliferative disorders affecting lung cells. Cell proliferative disorders of the lung can include lung cancer, a precancer or precancerous condition of the lung, benign growths or lesions of the lung, and malignant growths or lesions of the lung, and metastatic lesions in tissue and organs in the body other than the lung. Preferably, compositions of the present invention may be used to treat lung cancer or cell proliferative disorders of the lung. Lung cancer can include all forms of cancer of the lung. Lung cancer can include malignant lung neoplasms, carcinoma *in situ*, typical carcinoid tumors, and atypical carcinoid tumors. Lung cancer can include small cell lung cancer (“SCLC”), non-small cell lung cancer (“NSCLC”), squamous cell carcinoma, adenocarcinoma, small cell carcinoma, large cell carcinoma, adenosquamous cell carcinoma, and mesothelioma. Lung cancer can include “scar carcinoma,” bronchioalveolar carcinoma, giant cell carcinoma, spindle cell carcinoma, and large cell neuroendocrine carcinoma. Lung cancer can include lung neoplasms having histologic and ultrastructural heterogeneity (e.g., mixed cell types).

[067] Cell proliferative disorders of the lung can include all forms of cell proliferative disorders affecting lung cells. Cell proliferative disorders of the lung can include lung cancer, precancerous conditions of the lung. Cell proliferative disorders of the lung can include hyperplasia, metaplasia, and dysplasia of the lung. Cell proliferative disorders of the lung can include asbestos-induced hyperplasia, squamous metaplasia, and benign reactive mesothelial metaplasia. Cell proliferative disorders of the lung can include replacement of columnar epithelium with stratified squamous epithelium, and mucosal dysplasia. Individuals exposed to inhaled injurious environmental agents such as cigarette smoke and asbestos may be at increased risk for developing cell proliferative disorders of the lung. Prior lung diseases that may predispose individuals to development of cell proliferative disorders of the lung can include chronic interstitial lung disease, necrotizing pulmonary disease, scleroderma, rheumatoid disease, sarcoidosis, interstitial pneumonitis, tuberculosis, repeated pneumonias, idiopathic pulmonary fibrosis, granulomata, asbestosis, fibrosing alveolitis, and Hodgkin's disease.

[068] A “cell proliferative disorder of the colon” is a cell proliferative disorder involving cells of the colon. Preferably, the cell proliferative disorder of the colon is colon cancer. Preferably, compositions of the present invention may be used to treat colon cancer or cell proliferative disorders of the colon. Colon cancer can include all forms of cancer of the colon. Colon cancer can include sporadic and hereditary colon cancers. Colon cancer can include malignant colon neoplasms, carcinoma *in situ*, typical carcinoid tumors, and atypical carcinoid tumors. Colon cancer can include adenocarcinoma, squamous cell carcinoma, and

adenosquamous cell carcinoma. Colon cancer can be associated with a hereditary syndrome selected from the group consisting of hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, Gardner's syndrome, Peutz-Jeghers syndrome, Turcot's syndrome and juvenile polyposis. Colon cancer can be caused by a hereditary syndrome selected from the group consisting of hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, Gardner's syndrome, Peutz-Jeghers syndrome, Turcot's syndrome and juvenile polyposis.

[069] Cell proliferative disorders of the colon can include all forms of cell proliferative disorders affecting colon cells. Cell proliferative disorders of the colon can include colon cancer, precancerous conditions of the colon, adenomatous polyps of the colon and metachronous lesions of the colon. A cell proliferative disorder of the colon can include adenoma. Cell proliferative disorders of the colon can be characterized by hyperplasia, metaplasia, and dysplasia of the colon. Prior colon diseases that may predispose individuals to development of cell proliferative disorders of the colon can include prior colon cancer. Current disease that may predispose individuals to development of cell proliferative disorders of the colon can include Crohn's disease and ulcerative colitis. A cell proliferative disorder of the colon can be associated with a mutation in a gene selected from the group consisting of *p53*, *ras*, *FAP* and *DCC*. An individual can have an elevated risk of developing a cell proliferative disorder of the colon due to the presence of a mutation in a gene selected from the group consisting of *p53*, *ras*, *FAP* and *DCC*.

[070] A "cell proliferative disorder of the pancreas" is a cell proliferative disorder involving cells of the pancreas. Cell proliferative disorders of the pancreas can include all forms of cell proliferative disorders affecting pancreatic cells. Cell proliferative disorders of the pancreas can include pancreas cancer, a precancer or precancerous condition of the pancreas, hyperplasia of the pancreas, and dysplasia of the pancreas, benign growths or lesions of the pancreas, and malignant growths or lesions of the pancreas, and metastatic lesions in tissue and organs in the body other than the pancreas. Pancreatic cancer includes all forms of cancer of the pancreas. Pancreatic cancer can include ductal adenocarcinoma, adenosquamous carcinoma, pleomorphic giant cell carcinoma, mucinous adenocarcinoma, osteoclast-like giant cell carcinoma, mucinous cystadenocarcinoma, acinar carcinoma, unclassified large cell carcinoma, small cell carcinoma, pancreatoblastoma, papillary neoplasm, mucinous cystadenoma, papillary cystic neoplasm, and serous cystadenoma. Pancreatic cancer can also include pancreatic neoplasms having histologic and ultrastructural heterogeneity (e.g., mixed cell types).

[071] A “cell proliferative disorder of the prostate” is a cell proliferative disorder involving cells of the prostate. Cell proliferative disorders of the prostate can include all forms of cell proliferative disorders affecting prostate cells. Cell proliferative disorders of the prostate can include prostate cancer, a precancer or precancerous condition of the prostate, benign growths or lesions of the prostate, and malignant growths or lesions of the prostate, and metastatic lesions in tissue and organs in the body other than the prostate. Cell proliferative disorders of the prostate can include hyperplasia, metaplasia, and dysplasia of the prostate.

[072] A “cell proliferative disorder of the skin” is a cell proliferative disorder involving cells of the skin. Cell proliferative disorders of the skin can include all forms of cell proliferative disorders affecting skin cells. Cell proliferative disorders of the skin can include a precancer or precancerous condition of the skin, benign growths or lesions of the skin, melanoma, malignant melanoma and other malignant growths or lesions of the skin, and metastatic lesions in tissue and organs in the body other than the skin. Cell proliferative disorders of the skin can include hyperplasia, metaplasia, and dysplasia of the skin.

[073] A “cell proliferative disorder of the ovary” is a cell proliferative disorder involving cells of the ovary. Cell proliferative disorders of the ovary can include all forms of cell proliferative disorders affecting cells of the ovary. Cell proliferative disorders of the ovary can include a precancer or precancerous condition of the ovary, benign growths or lesions of the ovary, ovarian cancer, malignant growths or lesions of the ovary, and metastatic lesions in tissue and organs in the body other than the ovary. Cell proliferative disorders of the skin can include hyperplasia, metaplasia, and dysplasia of cells of the ovary.

[074] A “cell proliferative disorder of the breast” is a cell proliferative disorder involving cells of the breast. Cell proliferative disorders of the breast can include all forms of cell proliferative disorders affecting breast cells. Cell proliferative disorders of the breast can include breast cancer, a precancer or precancerous condition of the breast, benign growths or lesions of the breast, and malignant growths or lesions of the breast, and metastatic lesions in tissue and organs in the body other than the breast. Cell proliferative disorders of the breast can include hyperplasia, metaplasia, and dysplasia of the breast.

[075] A cell proliferative disorder of the breast can be a precancerous condition of the breast. Compositions of the present invention may be used to treat a precancerous condition of the breast. A precancerous condition of the breast can include atypical hyperplasia of the breast, ductal carcinoma *in situ* (DCIS), intraductal carcinoma, lobular carcinoma *in situ* (LCIS), lobular neoplasia, and stage 0 or grade 0 growth or lesion of the breast (e.g., stage 0 or grade 0 breast cancer, or carcinoma *in situ*). A precancerous condition of the breast can be

staged according to the TNM classification scheme as accepted by the American Joint Committee on Cancer (AJCC), where the primary tumor (T) has been assigned a stage of T0 or Tis; and where the regional lymph nodes (N) have been assigned a stage of N0; and where distant metastasis (M) has been assigned a stage of M0.

[076] The cell proliferative disorder of the breast can be breast cancer. Preferably, compositions of the present invention may be used to treat breast cancer. Breast cancer includes all forms of cancer of the breast. Breast cancer can include primary epithelial breast cancers. Breast cancer can include cancers in which the breast is involved by other tumors such as lymphoma, sarcoma or melanoma. Breast cancer can include carcinoma of the breast, ductal carcinoma of the breast, lobular carcinoma of the breast, undifferentiated carcinoma of the breast, cystosarcoma phyllodes of the breast, angiosarcoma of the breast, and primary lymphoma of the breast. Breast cancer can include Stage I, II, IIIA, IIIB, IIIC and IV breast cancer. Ductal carcinoma of the breast can include invasive carcinoma, invasive carcinoma *in situ* with predominant intraductal component, inflammatory breast cancer, and a ductal carcinoma of the breast with a histologic type selected from the group consisting of comedo, mucinous (colloid), medullary, medullary with lymphocytic infiltrate, papillary, scirrhous, and tubular. Lobular carcinoma of the breast can include invasive lobular carcinoma with predominant *in situ* component, invasive lobular carcinoma, and infiltrating lobular carcinoma. Breast cancer can include Paget's disease, Paget's disease with intraductal carcinoma, and Paget's disease with invasive ductal carcinoma. Breast cancer can include breast neoplasms having histologic and ultrastructural heterogeneity (e.g., mixed cell types).

[077] Preferably, compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph, or solvate thereof, may be used to treat breast cancer. A breast cancer that is to be treated can include familial breast cancer. A breast cancer that is to be treated can include sporadic breast cancer. A breast cancer that is to be treated can arise in a male subject. A breast cancer that is to be treated can arise in a female subject. A breast cancer that is to be treated can arise in a premenopausal female subject or a postmenopausal female subject. A breast cancer that is to be treated can arise in a subject equal to or older than 30 years old, or a subject younger than 30 years old. A breast cancer that is to be treated has arisen in a subject equal to or older than 50 years old, or a subject younger than 50 years old. A breast cancer that is to be treated can arise in a subject equal to or older than 70 years old, or a subject younger than 70 years old.

[078] A breast cancer that is to be treated can be typed to identify a familial or spontaneous mutation in BRCA1, BRCA2, or p53. A breast cancer that is to be treated can be typed as having a HER2/neu gene amplification, as overexpressing HER2/neu, or as having a low, intermediate or high level of HER2/neu expression. A breast cancer that is to be treated can be typed for a marker selected from the group consisting of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2, Ki-67, CA15-3, CA 27-29, and c-Met. A breast cancer that is to be treated can be typed as ER-unknown, ER-rich or ER-poor. A breast cancer that is to be treated can be typed as ER-negative or ER-positive. ER-typing of a breast cancer may be performed by any reproducible means. ER-typing of a breast cancer may be performed as set forth in *Onkologie* 27: 175-179 (2004). A breast cancer that is to be treated can be typed as PR-unknown, PR-rich, or PR-poor. A breast cancer that is to be treated can be typed as PR-negative or PR-positive. A breast cancer that is to be treated can be typed as receptor positive or receptor negative. A breast cancer that is to be treated can be typed as being associated with elevated blood levels of CA 15-3, or CA 27-29, or both.

[079] A breast cancer that is to be treated can include a localized tumor of the breast. A breast cancer that is to be treated can include a tumor of the breast that is associated with a negative sentinel lymph node (SLN) biopsy. A breast cancer that is to be treated can include a tumor of the breast that is associated with a positive sentinel lymph node (SLN) biopsy. A breast cancer that is to be treated can include a tumor of the breast that is associated with one or more positive axillary lymph nodes, where the axillary lymph nodes have been staged by any applicable method. A breast cancer that is to be treated can include a tumor of the breast that has been typed as having nodal negative status (*e.g.*, node-negative) or nodal positive status (*e.g.*, node-positive). A breast cancer that is to be treated can include a tumor of the breast that has metastasized to other locations in the body. A breast cancer that is to be treated can be classified as having metastasized to a location selected from the group consisting of bone, lung, liver, or brain. A breast cancer that is to be treated can be classified according to a characteristic selected from the group consisting of metastatic, localized, regional, local-regional, locally advanced, distant, multicentric, bilateral, ipsilateral, contralateral, newly diagnosed, recurrent, and inoperable.

[080] A compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, may be used to treat or prevent a cell proliferative disorder of the breast, or to treat or prevent breast cancer, in a subject having an increased risk of developing breast cancer relative to the population at large. A subject with an

increased risk of developing breast cancer relative to the population at large is a female subject with a family history or personal history of breast cancer. A subject with an increased risk of developing breast cancer relative to the population at large is a female subject having a germ-line or spontaneous mutation in BRCA1 or BRCA2, or both. A subject with an increased risk of developing breast cancer relative to the population at large is a female subject with a family history of breast cancer and a germ-line or spontaneous mutation in BRCA1 or BRCA2, or both. A subject with an increased risk of developing breast cancer relative to the population at large is a female who is greater than 30 years old, greater than 40 years old, greater than 50 years old, greater than 60 years old, greater than 70 years old, greater than 80 years old, or greater than 90 years old. A subject with an increased risk of developing breast cancer relative to the population at large is a subject with atypical hyperplasia of the breast, ductal carcinoma *in situ* (DCIS), intraductal carcinoma, lobular carcinoma *in situ* (LCIS), lobular neoplasia, or a stage 0 growth or lesion of the breast (e.g., stage 0 or grade 0 breast cancer, or carcinoma *in situ*).

[081] A breast cancer that is to be treated can histologically graded according to the Scarff-Bloom-Richardson system, wherein a breast tumor has been assigned a mitosis count score of 1, 2, or 3; a nuclear pleiomorphism score of 1, 2, or 3; a tubule formation score of 1, 2, or 3; and a total Scarff-Bloom-Richardson score of between 3 and 9. A breast cancer that is to be treated can be assigned a tumor grade according to the International Consensus Panel on the Treatment of Breast Cancer selected from the group consisting of grade 1, grade 1-2, grade 2, grade 2-3, or grade 3.

[082] A cancer that is to be treated can be staged according to the American Joint Committee on Cancer (AJCC) TNM classification system, where the tumor (T) has been assigned a stage of TX, T1, T1mic, T1a, T1b, T1c, T2, T3, T4, T4a, T4b, T4c, or T4d; and where the regional lymph nodes (N) have been assigned a stage of NX, N0, N1, N2, N2a, N2b, N3, N3a, N3b, or N3c; and where distant metastasis (M) can be assigned a stage of MX, M0, or M1. A cancer that is to be treated can be staged according to an American Joint Committee on Cancer (AJCC) classification as Stage I, Stage IIA, Stage IIB, Stage IIIA, Stage IIIB, Stage IIIC, or Stage IV. A cancer that is to be treated can be assigned a grade according to an AJCC classification as Grade GX (e.g., grade cannot be assessed), Grade 1, Grade 2, Grade 3 or Grade 4. A cancer that is to be treated can be staged according to an AJCC pathologic classification (pN) of pNX, pN0, PN0 (I-), PN0 (I+), PN0 (mol-), PN0 (mol+), PN1, PN1(mi), PN1a, PN1b, PN1c, pN2, pN2a, pN2b, pN3, pN3a, pN3b, or pN3c.

[083] A cancer that is to be treated can include a tumor that has been determined to be less than or equal to about 2 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be from about 2 to about 5 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be greater than or equal to about 3 centimeters in diameter. A cancer that is to be treated can include a tumor that has been determined to be greater than 5 centimeters in diameter. A cancer that is to be treated can be classified by microscopic appearance as well differentiated, moderately differentiated, poorly differentiated, or undifferentiated. A cancer that is to be treated can be classified by microscopic appearance with respect to mitosis count (e.g., amount of cell division) or nuclear pleiomorphism (e.g., change in cells). A cancer that is to be treated can be classified by microscopic appearance as being associated with areas of necrosis (e.g., areas of dying or degenerating cells). A cancer that is to be treated can be classified as having an abnormal karyotype, having an abnormal number of chromosomes, or having one or more chromosomes that are abnormal in appearance. A cancer that is to be treated can be classified as being aneuploid, triploid, tetraploid, or as having an altered ploidy. A cancer that is to be treated can be classified as having a chromosomal translocation, or a deletion or duplication of an entire chromosome, or a region of deletion, duplication or amplification of a portion of a chromosome.

[084] A cancer that is to be treated can be evaluated by DNA cytometry, flow cytometry, or image cytometry. A cancer that is to be treated can be typed as having 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of cells in the synthesis stage of cell division (e.g., in S phase of cell division). A cancer that is to be treated can be typed as having a low S-phase fraction or a high S-phase fraction.

[085] As used herein, a “normal cell” is a cell that cannot be classified as part of a “cell proliferative disorder”. A normal cell lacks unregulated or abnormal growth, or both, that can lead to the development of an unwanted condition or disease. Preferably, a normal cell possesses normally functioning cell cycle checkpoint control mechanisms.

[086] As used herein, “contacting a cell” refers to a condition in which a compound or other composition of matter is in direct contact with a cell, or is close enough to induce a desired biological effect in a cell.

[087] As used herein, “candidate compound” refers to a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, that has been or will be tested in one or more *in vitro* or *in vivo* biological assays, in order to determine if that compound is likely to elicit a desired biological or medical response in a

cell, tissue, system, animal or human that is being sought by a researcher or clinician. A candidate compound is a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof. The biological or medical response can be the treatment of cancer. The biological or medical response can be treatment or prevention of a cell proliferative disorder. *In vitro* or *in vivo* biological assays can include, but are not limited to, enzymatic activity assays, electrophoretic mobility shift assays, reporter gene assays, *in vitro* cell viability assays, and the assays described herein.

[088] For example, an *in vitro* biological assay that can be used includes the steps of (1) mixing a histone substrate (*e.g.*, an isolated histone sample for a histone or modified histone of interest, or an isolated oligonucleosome substrate) with recombinant DOT1L enzyme (*e.g.*, recombinant protein containing amino acids 1-416); (2) adding a candidate compound of the invention to this mixture; (3) adding non-radioactive and 3 H-labeled S-Adenosyl methionine (SAM) to start the reaction; (4) adding excessive amount of non-radioactive SAM to stop the reaction; (4) washing off the free non-incorporated 3 H-SAM; and (5) detecting the quantity of 3 H-labeled histone substrate by any methods known in the art (*e.g.*, by a PerkinElmer TopCount platereader).

[089] For example, an *in vitro* cell viability assay that can be used includes the steps of (1) culturing cells (*e.g.*, EOL-1 cells) in the presence of increasing concentration of candidate compound (*e.g.*, Compound A2, Compound D16); (2) determining viable cell number every 3-4 days by methods known in the art (*e.g.*, using the Millipore Guava Viacount assay); (3) plotting concentration-dependence growth curves; and optionally (4) calculating IC₅₀ values from the concentration-dependence growth curves using methods known in the art (*e.g.*, using GraphPad Prism Software).

[090] For example, a histone methylation assay that can be used includes the steps of (1) culturing cells (*e.g.*, EOL-1 cells) in the presence of candidate compound (*e.g.*, Compound A2 or Compound D16); (2) harvesting the cells; (3) extracting histone proteins, using methods known in the art (*e.g.*, sulfuric acid precipitation); (4) fractionating histone extracts by SDS-PAGE electrophoresis and transferring to a filter; (5) probing the filter with antibodies specific to a protein or methylated-protein of interest (*e.g.*, H3K79me2-specific antibody and total histone H3-specific antibody); and (6) detecting the signal of the antibodies using methods known in the art (*e.g.*, Li-cor Odyssey infrared imager).

[091] For example, a gene expression assay that can be used includes the steps of (1) culturing cells (*e.g.*, EOL-1, Molm13, MV411, LOUCY, SemK2, Reh, HL60, BV173, or Jurkat cells) in the presence or absence of candidate compound (*e.g.*, Compound A2 or

Compound D16); (2) harvesting the cells; (3) extracting the RNA using methods known in the art (e.g., Qiagen RNeasy Kit); (4) synthesizing cDNA from the extracted RNA (e.g., Applied Biosystems reverse transcriptase kit); (5) preparing qPCR reactions using, for example, primers and probes (e.g., predesigned labeled primer and probe sets for HOXA9, MEIS1, FLT3, DOT1L, and β2-microglobulin from Applied Biosystems), synthesized sample cDNA, and qPCR master mix reagent (e.g., Applied Biosystems Taqman universal PCR master mix); (6) running samples on PCR machine (e.g., Applied Biosystems); (7) analysis of the data and calculation of relative gene expression.

[092] As used herein, “monotherapy” refers to the administration of a single active or therapeutic compound to a subject in need thereof. Preferably, monotherapy will involve administration of a therapeutically effective amount of a single active compound. For example, cancer monotherapy with one of the compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, analog or derivative thereof, to a subject in need of treatment of cancer. In one aspect, the single active compound is a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof.

[093] As used herein, “treating” or “treat” describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, to alleviate the symptoms or complications of a disease, condition or disorder, or to eliminate the disease, condition or disorder.

[094] A compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, can also be used to prevent a disease, condition or disorder. As used herein, “preventing” or “prevent” describes reducing or eliminating the onset of the symptoms or complications of the disease, condition or disorder.

[095] As used herein, the term “alleviate” is meant to describe a process by which the severity of a sign or symptom of a disorder is decreased. Importantly, a sign or symptom can be alleviated without being eliminated. In a preferred embodiment, the administration of pharmaceutical compositions of the invention leads to the elimination of a sign or symptom, however, elimination is not required. Effective dosages are expected to decrease the severity of a sign or symptom. For instance, a sign or symptom of a disorder such as cancer, which can occur in multiple locations, is alleviated if the severity of the cancer is decreased within at least one of multiple locations.

[096] As used herein, the term "severity" is meant to describe the potential of cancer to transform from a precancerous, or benign, state into a malignant state. Alternatively, or in addition, severity is meant to describe a cancer stage, for example, according to the TNM system (accepted by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC)) or by other art-recognized methods. Cancer stage refers to the extent or severity of the cancer, based on factors such as the location of the primary tumor, tumor size, number of tumors, and lymph node involvement (spread of cancer into lymph nodes). Alternatively, or in addition, severity is meant to describe the tumor grade by art-recognized methods (see, National Cancer Institute, www.cancer.gov). Tumor grade is a system used to classify cancer cells in terms of how abnormal they look under a microscope and how quickly the tumor is likely to grow and spread. Many factors are considered when determining tumor grade, including the structure and growth pattern of the cells. The specific factors used to determine tumor grade vary with each type of cancer. Severity also describes a histologic grade, also called differentiation, which refers to how much the tumor cells resemble normal cells of the same tissue type (see, National Cancer Institute, www.cancer.gov). Furthermore, severity describes a nuclear grade, which refers to the size and shape of the nucleus in tumor cells and the percentage of tumor cells that are dividing (see, National Cancer Institute, www.cancer.gov).

[097] In another aspect of the invention, severity describes the degree to which a tumor has secreted growth factors, degraded the extracellular matrix, become vascularized, lost adhesion to juxtaposed tissues, or metastasized. Moreover, severity describes the number of locations to which a primary tumor has metastasized. Finally, severity includes the difficulty of treating tumors of varying types and locations. For example, inoperable tumors, those cancers which have greater access to multiple body systems (hematological and immunological tumors), and those which are the most resistant to traditional treatments are considered most severe. In these situations, prolonging the life expectancy of the subject and/or reducing pain, decreasing the proportion of cancerous cells or restricting cells to one system, and improving cancer stage/tumor grade/histological grade/nuclear grade are considered alleviating a sign or symptom of the cancer.

[098] As used herein the term "symptom" is defined as an indication of disease, illness, injury, or that something is not right in the body. Symptoms are felt or noticed by the individual experiencing the symptom, but may not easily be noticed by others. Others are defined as non-health-care professionals.

[0099] As used herein the term "sign" is also defined as an indication that something is not right in the body. But signs are defined as things that can be seen by a doctor, nurse, or other health care professional.

[0100] Cancer is a group of diseases that may cause almost any sign or symptom. The signs and symptoms will depend on where the cancer is, the size of the cancer, and how much it affects the nearby organs or structures. If a cancer spreads (metastasizes), then symptoms may appear in different parts of the body.

[0101] As a cancer grows, it begins to push on nearby organs, blood vessels, and nerves. This pressure creates some of the signs and symptoms of cancer. If the cancer is in a critical area, such as certain parts of the brain, even the smallest tumor can cause early symptoms.

[0102] But sometimes cancers start in places where it does not cause any symptoms until the cancer has grown quite large. Pancreas cancers, for example, do not usually grow large enough to be felt from the outside of the body. Some pancreatic cancers do not cause symptoms until they begin to grow around nearby nerves (this causes a backache). Others grow around the bile duct, which blocks the flow of bile and leads to a yellowing of the skin known as jaundice. By the time a pancreatic cancer causes these signs or symptoms, it has usually reached an advanced stage.

[0103] A cancer may also cause symptoms such as fever, fatigue, or weight loss. This may be because cancer cells use up much of the body's energy supply or release substances that change the body's metabolism. Or the cancer may cause the immune system to react in ways that produce these symptoms.

[0104] Sometimes, cancer cells release substances into the bloodstream that cause symptoms not usually thought to result from cancers. For example, some cancers of the pancreas can release substances which cause blood clots to develop in veins of the legs. Some lung cancers make hormone-like substances that affect blood calcium levels, affecting nerves and muscles and causing weakness and dizziness.

[0105] Cancer presents several general signs or symptoms that occur when a variety of subtypes of cancer cells are present. Most people with cancer will lose weight at some time with their disease. An unexplained (unintentional) weight loss of 10 pounds or more may be the first sign of cancer, particularly cancers of the pancreas, stomach, esophagus, or lung.

[0106] Fever is very common with cancer, but is more often seen in advanced disease. Almost all patients with cancer will have fever at some time, especially if the cancer or its treatment affects the immune system and makes it harder for the body to fight infection. Less often, fever may be an early sign of cancer, such as with leukemia or lymphoma.

[0107] Fatigue may be an important symptom as cancer progresses. It may happen early, though, in cancers such as with leukemia, or if the cancer is causing an ongoing loss of blood, as in some colon or stomach cancers.

[0108] Pain may be an early symptom with some cancers such as bone cancers or testicular cancer. But most often pain is a symptom of advanced disease.

[0109] Along with cancers of the skin (see next section), some internal cancers can cause skin signs that can be seen. These changes include the skin looking darker (hyperpigmentation), yellow (jaundice), or red (erythema); itching; or excessive hair growth.

[0110] Alternatively, or in addition, cancer subtypes present specific signs or symptoms. Changes in bowel habits or bladder function could indicate cancer. Long-term constipation, diarrhea, or a change in the size of the stool may be a sign of colon cancer. Pain with urination, blood in the urine, or a change in bladder function (such as more frequent or less frequent urination) could be related to bladder or prostate cancer.

[0111] Changes in skin condition or appearance of a new skin condition could indicate cancer. Skin cancers may bleed and look like sores that do not heal. A long-lasting sore in the mouth could be an oral cancer, especially in patients who smoke, chew tobacco, or frequently drink alcohol. Sores on the penis or vagina may either be signs of infection or an early cancer.

[0112] Unusual bleeding or discharge could indicate cancer. Unusual bleeding can happen in either early or advanced cancer. Blood in the sputum (phlegm) may be a sign of lung cancer. Blood in the stool (or a dark or black stool) could be a sign of colon or rectal cancer. Cancer of the cervix or the endometrium (lining of the uterus) can cause vaginal bleeding. Blood in the urine may be a sign of bladder or kidney cancer. A bloody discharge from the nipple may be a sign of breast cancer.

[0113] A thickening or lump in the breast or in other parts of the body could indicate the presence of a cancer. Many cancers can be felt through the skin, mostly in the breast, testicle, lymph nodes (glands), and the soft tissues of the body. A lump or thickening may be an early or late sign of cancer. Any lump or thickening could be indicative of cancer, especially if the formation is new or has grown in size.

[0114] Indigestion or trouble swallowing could indicate cancer. While these symptoms commonly have other causes, indigestion or swallowing problems may be a sign of cancer of the esophagus, stomach, or pharynx (throat).

[0115] Recent changes in a wart or mole could be indicative of cancer. Any wart, mole, or freckle that changes in color, size, or shape, or loses its definite borders indicates the potential development of cancer. For example, the skin lesion may be a melanoma.

[0116] A persistent cough or hoarseness could be indicative of cancer. A cough that does not go away may be a sign of lung cancer. Hoarseness can be a sign of cancer of the larynx (voice box) or thyroid.

[0117] While the signs and symptoms listed above are the more common ones seen with cancer, there are many others that are less common and are not listed here. However, all art-recognized signs and symptoms of cancer are contemplated and encompassed by the instant invention.

[0118] Treating cancer can result in a reduction in size of a tumor. A reduction in size of a tumor may also be referred to as “tumor regression”. Preferably, after treatment, tumor size is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor size is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Size of a tumor may be measured by any reproducible means of measurement. The size of a tumor may be measured as a diameter of the tumor.

[0119] Treating cancer can result in a reduction in tumor volume. Preferably, after treatment, tumor volume is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor volume is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Tumor volume may be measured by any reproducible means of measurement.

[0120] Treating cancer results in a decrease in number of tumors. Preferably, after treatment, tumor number is reduced by 5% or greater relative to number prior to treatment; more preferably, tumor number is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. Number of tumors may be measured by any reproducible means of measurement. The number of tumors may be measured by counting tumors visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[0121] Treating cancer can result in a decrease in number of metastatic lesions in other tissues or organs distant from the primary tumor site. Preferably, after treatment, the number of metastatic lesions is reduced by 5% or greater relative to number prior to treatment; more preferably, the number of metastatic lesions is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. The number of metastatic lesions may be measured by any reproducible means of measurement. The number of metastatic lesions may be measured by counting metastatic lesions visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[0122] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population receiving carrier alone. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0123] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population of untreated subjects. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0124] Treating cancer can result in increase in average survival time of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, analog or derivative thereof. Preferably, the average survival time is increased by

more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[0125] Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving carrier alone. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, analog or derivative thereof. Preferably, the mortality rate is decreased by more than 2%; more preferably, by more than 5%; more preferably, by more than 10%; and most preferably, by more than 25%. A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means. A decrease in the mortality rate of a population may be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with an active compound. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with an active compound.

[0126] Treating cancer can result in a decrease in tumor growth rate. Preferably, after treatment, tumor growth rate is reduced by at least 5% relative to number prior to treatment; more preferably, tumor growth rate is reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Tumor growth rate may be measured by any reproducible means of measurement. Tumor growth rate can be measured according to a change in tumor diameter per unit time.

[0127] Treating cancer can result in a decrease in tumor regrowth. Preferably, after treatment, tumor regrowth is less than 5%; more preferably, tumor regrowth is less than 10%; more preferably, less than 20%; more preferably, less than 30%; more preferably, less than 40%;

more preferably, less than 50%; even more preferably, less than 50%; and most preferably, less than 75%. Tumor regrowth may be measured by any reproducible means of measurement. Tumor regrowth is measured, for example, by measuring an increase in the diameter of a tumor after a prior tumor shrinkage that followed treatment. A decrease in tumor regrowth is indicated by failure of tumors to reoccur after treatment has stopped.

[0128] Treating or preventing a cell proliferative disorder can result in a reduction in the rate of cellular proliferation. Preferably, after treatment, the rate of cellular proliferation is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%. The rate of cellular proliferation may be measured by any reproducible means of measurement. The rate of cellular proliferation is measured, for example, by measuring the number of dividing cells in a tissue sample per unit time.

[0129] Treating or preventing a cell proliferative disorder can result in a reduction in the proportion of proliferating cells. Preferably, after treatment, the proportion of proliferating cells is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%.

The proportion of proliferating cells may be measured by any reproducible means of measurement. Preferably, the proportion of proliferating cells is measured, for example, by quantifying the number of dividing cells relative to the number of nondividing cells in a tissue sample. The proportion of proliferating cells can be equivalent to the mitotic index.

[0130] Treating or preventing a cell proliferative disorder can result in a decrease in size of an area or zone of cellular proliferation. Preferably, after treatment, size of an area or zone of cellular proliferation is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Size of an area or zone of cellular proliferation may be measured by any reproducible means of measurement. The size of an area or zone of cellular proliferation may be measured as a diameter or width of an area or zone of cellular proliferation.

[0131] Treating or preventing a cell proliferative disorder can result in a decrease in the number or proportion of cells having an abnormal appearance or morphology. Preferably,

after treatment, the number of cells having an abnormal morphology is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. An abnormal cellular appearance or morphology may be measured by any reproducible means of measurement. An abnormal cellular morphology can be measured by microscopy, *e.g.*, using an inverted tissue culture microscope. An abnormal cellular morphology can take the form of nuclear pleiomorphism.

[0132] As used herein, the term “selectively” means tending to occur at a higher frequency in one population than in another population. The compared populations can be cell populations. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, acts selectively on a cancer or precancerous cell but not on a normal cell. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, acts selectively to modulate one molecular target (*e.g.*, a target protein methyltransferase) but does not significantly modulate another molecular target (*e.g.*, a non-target protein methyltransferase). The invention also provides a method for selectively inhibiting the activity of an enzyme, such as a protein methyltransferase. Preferably, an event occurs selectively in population A relative to population B if it occurs greater than two times more frequently in population A as compared to population B. An event occurs selectively if it occurs greater than five times more frequently in population A. An event occurs selectively if it occurs greater than ten times more frequently in population A; more preferably, greater than fifty times; even more preferably, greater than 100 times; and most preferably, greater than 1000 times more frequently in population A as compared to population B. For example, cell death would be said to occur selectively in cancer cells if it occurred greater than twice as frequently in cancer cells as compared to normal cells.

[0133] A compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, can modulate the activity of a molecular target (*e.g.*, a target protein methyltransferase). Modulating refers to stimulating or inhibiting an activity of a molecular target. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, modulates the activity of a molecular target if it stimulates or inhibits the activity of the molecular target by at least 2-fold relative to the activity of the molecular target under the

same conditions but lacking only the presence of said compound. More preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, modulates the activity of a molecular target if it stimulates or inhibits the activity of the molecular target by at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to the activity of the molecular target under the same conditions but lacking only the presence of said compound. The activity of a molecular target may be measured by any reproducible means. The activity of a molecular target may be measured *in vitro* or *in vivo*. For example, the activity of a molecular target may be measured *in vitro* by an enzymatic activity assay or a DNA binding assay, or the activity of a molecular target may be measured *in vivo* by assaying for expression of a reporter gene.

[0134] A compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, does not significantly modulate the activity of a molecular target if the addition of the compound does not stimulate or inhibit the activity of the molecular target by greater than 10% relative to the activity of the molecular target under the same conditions but lacking only the presence of said compound.

[0135] As used herein, the term “isozyme selective” means preferential inhibition or stimulation of a first isoform of an enzyme in comparison to a second isoform of an enzyme (e.g., preferential inhibition or stimulation of a protein methyltransferase isozyme alpha in comparison to a protein methyltransferase isozyme beta). Preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, demonstrates a minimum of a fourfold differential, preferably a tenfold differential, more preferably a fifty fold differential, in the dosage required to achieve a biological effect. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, demonstrates this differential across the range of inhibition, and the differential is exemplified at the IC₅₀, *i.e.*, a 50% inhibition, for a molecular target of interest.

[0136] Administering a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, to a cell or a subject in need thereof can result in modulation (*i.e.*, stimulation or inhibition) of an activity of a protein methyltransferase of interest.

[0137] The present invention provides methods to assess biological activity of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof or methods of identifying a test compound as a modulator (e.g., an

inhibitor) of DOT1L. DOT1L polypeptides and nucleic acids can be used to screen for compounds that bind to and/or modulate (e.g., increase or decrease) one or more biological activities of DOT1L, including but not limited to H3K79 HMTase activity, SAM binding activity, histone and/or nucleosome binding activity, AF10 binding activity, AF10-MLL or other MLL fusion protein binding activity, and/or any other biological activity of interest. A DOT1L polypeptide can be a functional fragment of a full-length DOT1L polypeptide or functional equivalent thereof, and may comprise any DOT1 domain of interest, including but not limited to the catalytic domain, the SAM binding domain and/or the positively charged domain, the AF10 interaction domain and/or a nuclear export signal.

[0138] Methods of assessing DOT1L binding to histones, nucleosomes, nucleic acids or polypeptides can be carried out using standard techniques that will be apparent to those skilled in the art (see the Exemplification for exemplary methods). Such methods include yeast and mammalian two-hybrid assays and co-immunoprecipitation techniques.

[0139] For example, a compound that modulates DOT1L H3K79 HMTase activity can be verified by: contacting a DOT1L polypeptide with a histone or peptide substrate comprising H3 in the presence of a test compound; detecting the level of H3K79 methylation of the histone or peptide substrate under conditions sufficient to provide H3K79 methylation, wherein an elevation or reduction in H3K79 methylation in the presence of the test compound as compared with the level of histone H3K79 methylation in the absence of the test compound indicates that the test compound modulates DOT1L H3K79 HMTase activity.

[0140] The screening methods of the invention can be carried out in a cell-based or cell-free system. As a further alternative, the assay can be performed in a whole animal (including transgenic non-human animals). Further, with respect to cell-based systems, the DOT1L polypeptide (or any other polypeptide used in the assay) can be added directly to the cell or can be produced from a nucleic acid in the cell. The nucleic acid can be endogenous to the cell or can be foreign (e.g., a genetically modified cell).

[0141] In some assays, immunological reagents, *e.g.*, antibodies and antigens, are employed. Fluorescence can be utilized in the measurement of enzymatic activity in some assays. As used herein, “fluorescence” refers to a process through which a molecule emits a photon as a result of absorbing an incoming photon of higher energy by the same molecule. Specific methods for assessing the biological activity of the disclosed compounds are described in the examples.

[0142] Administering a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, to a cell or a subject in need thereof

results in modulation (*i.e.*, stimulation or inhibition) of an activity of an intracellular target (*e.g.*, substrate). Several intracellular targets can be modulated with the compounds of the present invention, including, but not limited to, protein methyltransferase.

[0143] Activating refers to placing a composition of matter (*e.g.*, protein or nucleic acid) in a state suitable for carrying out a desired biological function. A composition of matter capable of being activated also has an unactivated state. An activated composition of matter may have an inhibitory or stimulatory biological function, or both.

[0144] Elevation refers to an increase in a desired biological activity of a composition of matter (*e.g.*, a protein or a nucleic acid). Elevation may occur through an increase in concentration of a composition of matter.

[0145] As used herein, “a cell cycle checkpoint pathway” refers to a biochemical pathway that is involved in modulation of a cell cycle checkpoint. A cell cycle checkpoint pathway may have stimulatory or inhibitory effects, or both, on one or more functions comprising a cell cycle checkpoint. A cell cycle checkpoint pathway is comprised of at least two compositions of matter, preferably proteins, both of which contribute to modulation of a cell cycle checkpoint. A cell cycle checkpoint pathway may be activated through an activation of one or more members of the cell cycle checkpoint pathway. Preferably, a cell cycle checkpoint pathway is a biochemical signaling pathway.

[0146] As used herein, “cell cycle checkpoint regulator” refers to a composition of matter that can function, at least in part, in modulation of a cell cycle checkpoint. A cell cycle checkpoint regulator may have stimulatory or inhibitory effects, or both, on one or more functions comprising a cell cycle checkpoint. A cell cycle checkpoint regulator can be a protein or not a protein.

[0147] Treating cancer or a cell proliferative disorder can result in cell death, and preferably, cell death results in a decrease of at least 10% in number of cells in a population. More preferably, cell death means a decrease of at least 20%; more preferably, a decrease of at least 30%; more preferably, a decrease of at least 40%; more preferably, a decrease of at least 50%; most preferably, a decrease of at least 75%. Number of cells in a population may be measured by any reproducible means. A number of cells in a population can be measured by fluorescence activated cell sorting (FACS), immunofluorescence microscopy and light microscopy. Methods of measuring cell death are as shown in Li *et al.*, *Proc Natl Acad Sci U S A.* 100(5): 2674-8, 2003. In an aspect, cell death occurs by apoptosis.

[0148] Preferably, an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, is not

significantly cytotoxic to normal cells. A therapeutically effective amount of a compound is not significantly cytotoxic to normal cells if administration of the compound in a therapeutically effective amount does not induce cell death in greater than 10% of normal cells. A therapeutically effective amount of a compound does not significantly affect the viability of normal cells if administration of the compound in a therapeutically effective amount does not induce cell death in greater than 10% of normal cells. In an aspect, cell death occurs by apoptosis.

[0149] Contacting a cell with a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, can induce or activate cell death selectively in cancer cells. Administering to a subject in need thereof a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, can induce or activate cell death selectively in cancer cells. Contacting a cell with a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, can induce cell death selectively in one or more cells affected by a cell proliferative disorder. Preferably, administering to a subject in need thereof a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, induces cell death selectively in one or more cells affected by a cell proliferative disorder.

[0150] The present invention relates to a method of treating or preventing cancer by administering a compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, to a subject in need thereof, where administration of the compound of the present invention, or a pharmaceutically acceptable salt, prodrug, metabolite, polymorph or solvate thereof, results in one or more of the following: accumulation of cells in G1 and/or S phase of the cell cycle, cytotoxicity via cell death in cancer cells without a significant amount of cell death in normal cells, antitumor activity in animals with a therapeutic index of at least 2, and activation of a cell cycle checkpoint. As used herein, “therapeutic index” is the maximum tolerated dose divided by the efficacious dose.

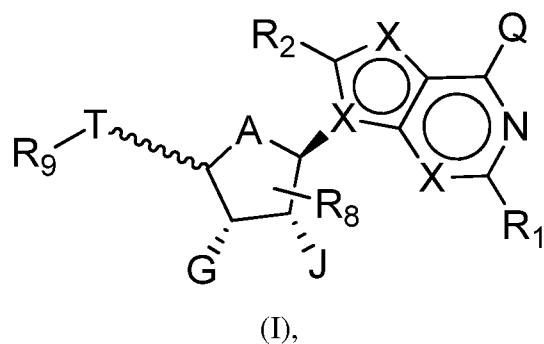
[0151] One skilled in the art may refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. (2005); Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (3rd edition), Cold Spring Harbor Press, Cold Spring Harbor, New York (2000); Coligan *et al.*, *Current Protocols in Immunology*, John Wiley & Sons, N.Y.; Enna *et al.*, *Current Protocols in Pharmacology*, John Wiley & Sons,

N.Y.; Fingl *et al.*, *The Pharmacological Basis of Therapeutics* (1975), *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 18th edition (1990). These texts can, of course, also be referred to in making or using an aspect of the invention

[0152] The compounds of the instant invention can also be utilized to treat or prevent neurologic diseases or disorders. Neurologic diseases or disorders that may be treated with the compounds of this invention include epilepsy, schizophrenia, bipolar disorder or other psychological and/or psychiatric disorders, neuropathies, skeletal muscle atrophy, and neurodegenerative diseases, e.g., a neurodegenerative disease. Exemplary neurodegenerative diseases include: Alzheimer's, Amyotrophic Lateral Sclerosis (ALS), and Parkinson's disease. Another class of neurodegenerative diseases includes diseases caused at least in part by aggregation of poly-glutamine. Diseases of this class include: Huntington's Diseases, Spinalbulbar Muscular Atrophy (SBMA or Kennedy's Disease) Dentatorubropallidolusian Atrophy (DRPLA), Spinocerebellar Ataxia 1 (SCA1), Spinocerebellar Ataxia 2 (SCA2), Machado-Joseph Disease (MJD; SCA3), Spinocerebellar Ataxia 6 (SCA6), Spinocerebellar Ataxia 7 (SCA7), and Spinocerebellar Ataxia 12 (SCA12).

[0153] Any other disease in which epigenetic methylation, which is mediated by DOT1, plays a role may be treatable or preventable using compounds and methods described herein.

[0154] In one aspect, the compound suitable for the method of the invention, e.g., a DOT1L inhibitor, is a compound of Formula (I) below or a pharmaceutically acceptable salt or ester thereof:



wherein,

T is a linker group of a 6-10 carbon atoms, in which one or more carbon atoms are optionally replaced with a heteroatom and T is optionally substituted;

R₉ comprises a C₆-C₁₀ aryl or 5 to 10-membered heteroaryl optionally substituted with one or more substituents selected from the group consisting of unsubstituted or substituted t-butyl, CF₃, cyclohexyl, C₆-C₁₀ aryl, and 5 to 10-membered heteroaryl;

A is O or CH₂;

each of G and J, independently, is H, halo, C(O)OH, C(O)O-C₁-C₆ alkyl or OR_a, R_a

being H, C₁-C₆ alkyl, C(O)-C₁-C₆ alkyl, or silyl, wherein C(O)O-C₁-C₆ alkyl, C₁-C₆ alkyl or C(O)-C₁-C₆ alkyl is optionally substituted with one or more substituents selected from the group consisting of halo, cyano hydroxyl, carboxyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl;

each X independently is N or CR_x, in which R_x is H, halo, hydroxyl, carboxyl, cyano, or R_{S1}, R_{S1} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_{S1} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

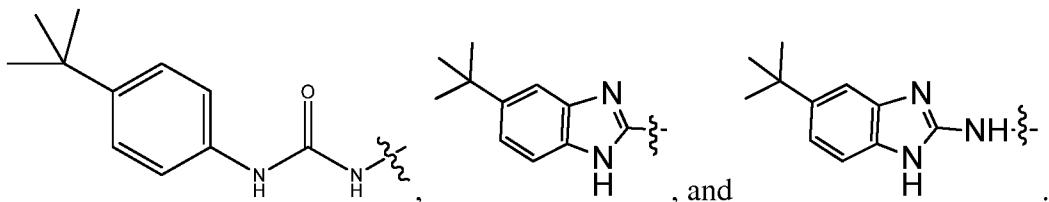
each of R₁ and R₂, independently is H, halo, hydroxyl, carboxyl, cyano, or R_{S2}, R_{S2} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, or C₃-C₈ cycloalkyl, and each R_{S2} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

R₈ is H, halo or R_{S3}, R_{S3} being C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and R_{S3} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano amino, C₁-C₆ alkoxy, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl; and

Q is H, NH₂, NHR_b, NR_bR_c, R_b, =O, OH, or OR_b, in which each of R_b and R_c independently is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5 to 10-membered heteroaryl, or -M₁-T₁ in which M₁ is a bond or C₁-C₆ alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy and T₁ is C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, or R_b and R_c, together with the N atom to which they attach, form 4 to 7-membered heterocycloalkyl having 0 or 1 additional heteroatoms to the N atom optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and each of R_b, R_c, and T₁ is optionally substituted with one or more substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano,

C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl.

[0155] For example, in Formula (I), R₉ is selected from the group consisting of



[0156] For example, in Formula (I), T is -CH₂-L₁-L₂-L₃-, with L₃ connected to R₉, wherein:

L₁ is N(Y), S, SO, or SO₂;

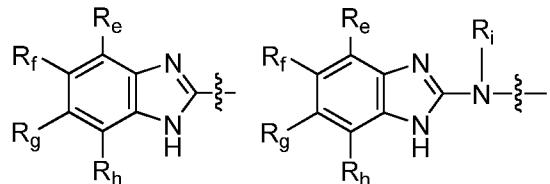
L₂ is CO or absent when L₁ is N(Y), or L₂ is absent when L₁ is S, SO, or SO₂, in which Y is H, R_d, SO₂R_d, or COR_d when L₂ is absent, or Y is H or R_d when L₂ is CO, R_d being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_d being optionally substituted with one or more substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, carboxyl, cyano, C₁-C₆ alkoxy, C₁-C₆ alkylsulfonyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 8-membered heterocycloalkyl, 5 to 6-membered heteroaryl, OR_d, OCOR_d, and N(R_d)₂, and with C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl further optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl; each R_d independently being H, C₁-C₆ alkyl, silyl, C₁-C₆ alkyl-C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 5 to 6-membered heteroaryl, aralkyl, or heteroaralkyl;

L₃ is -(CR₄R₅)_n(CR₆R₇)_m- or -(CR₄R₅)_n-unsubstituted or substituted C₃-C₈ cycloalkyl-(CR₆R₇)_m-, with (CR₆R₇)_m connected to R₉;

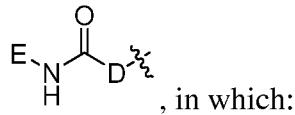
each of R₄, R₅, R₆, and R₇, independently, is H, halo, hydroxyl, carboxyl, cyano, or R_{S2}, R_{S2} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and each R_{S2} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl; or two geminal R₄ and R₅ or two geminal R₆ and R₇ taken together are ethylene, propylene or butylene;

m is 0, 1, or 2; and

n is 0, 1, or 2.



[0157] For example, in Formula (I) R₉ is



, in which:

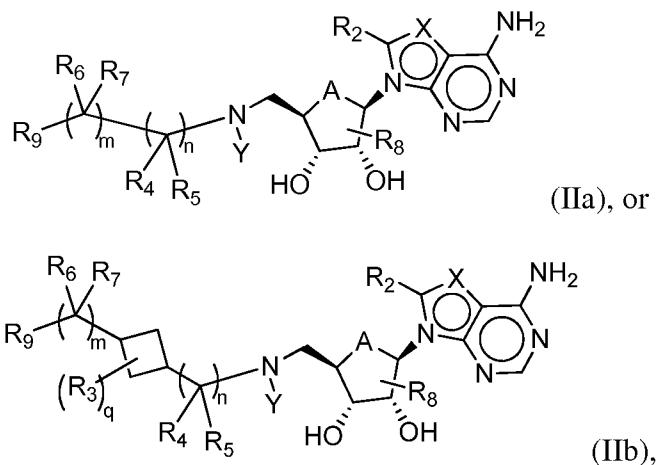
each of R_e, R_f, R_g, and R_h, independently is -M₂-T₂, in which M₂ is a bond, SO₂, SO, S, CO, CO₂, O, O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, NH, or N(R_t), R_t being C₁-C₆ alkyl, and T₂ is H, halo, or R_{S4}, R_{S4} being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 8-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, and each of O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, R_t, and R_{S4} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl, R_i is H or C₁-C₆ alkyl optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl,

D is O, NR_j, or CR_jR_k, each of R_j and R_k independently being H or C₁-C₆ alkyl, or R_j and R_k taken together, with the carbon atom to which they are attached, form a C₃-C₁₀ cycloalkyl ring, and

E is-M₃-T₃, M₃ being a bond or C₁-C₆ alkyl linker optionally substituted with halo or cyano, T₃ being C₃-C₁₄ carbocycle or 4 to 14-membered heterocycle, and T₃ being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, thiol, carboxyl, cyano, nitro, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, C₁-C₆ haloalkoxyl, C₁-C₆ alkylthio, C₁-C₆ alkylsulfonyl, C₁-C₆ haloalkylsulfonyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, oxo, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₄-C₁₂ alkylcycloalkyl, C₆-C₁₀ aryl, C₆-C₁₀ aryloxy, C₇-C₁₄ alkylaryl, C₆-C₁₀ aminoaryloxy, C₆-C₁₀ arylthio, 4 to 6-membered heterocycloalkyl optionally substituted with halo, C₁-C₄ alkyl, C₁-C₄

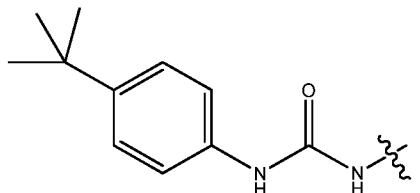
haloalkyl, 5 to 6-membered heteroaryl optionally substituted with halo, C₁-C₄ alkyl, and C₁-C₆ alkyl that is substituted with hydroxy, halo, C₁-C₆ alkoxy carbonyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl optionally further substituted with halo, hydroxyl, or C₁-C₆ alkoxy.

[0158] For example, the compound of Formula (I) is of formula (IIa) or (IIb):



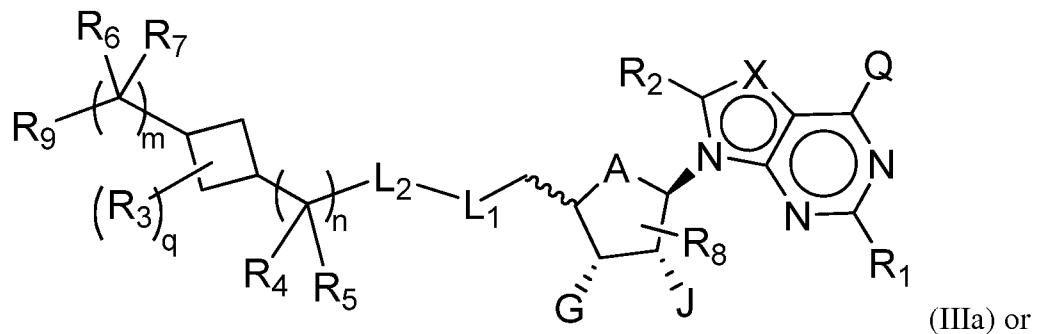
wherein R₃ is H, halo, hydroxyl, carboxyl, cyano, or R_{S2}, and q is 0, 1, 2, 3, or 4.

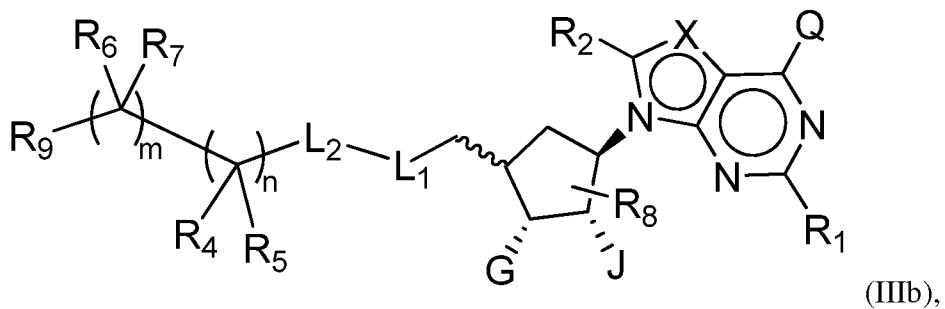
[0159] For example, the compound is of formula (IIa) and R₉ is



[0160] For example, the compound is of formula (IIb) and R₉ is

[0161] Compounds of Formula (I) also include those of Formula (IIIa) or (IIIb)





or a pharmaceutically acceptable salt or ester thereof, wherein:

A is O or CH₂;

each of G and J, independently, is H, halo, C(O)OH, C(O)O-C₁-C₆ alkyl or OR_a, R_a being H, C₁-C₆ alkyl or C(O)-C₁-C₆ alkyl, wherein C(O)O-C₁-C₆ alkyl, C₁-C₆ alkyl or C(O)-C₁-C₆ alkyl is optionally substituted with one or more substituents selected from the group consisting of halo, cyano hydroxyl, carboxyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl;

Q is H, NH₂, NHR_b, NR_bR_c, R_b, =O, OH, or OR_b, in which each of R_b and R_c independently is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5 to 10-membered heteroaryl, or -M₁-T₁ in which M₁ is a bond or C₁-C₆ alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy and T₁ is C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, or R_b and R_c, together with the N atom to which they attach, form 4 to 7-membered heterocycloalkyl having 0 or 1 additional heteroatoms to the N atom optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and each of R_b, R_c, and T₁ is optionally substituted with one or more substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

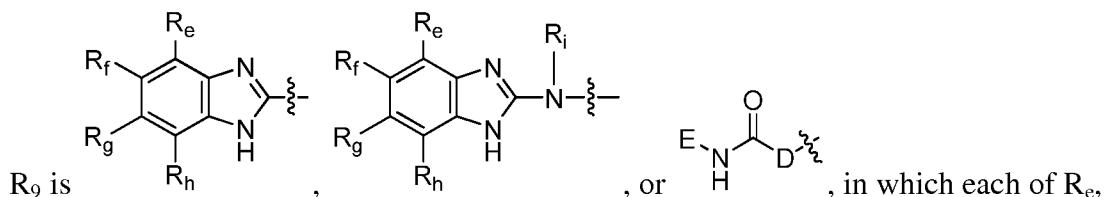
X is N or CR_x, in which R_x is H, halo, hydroxyl, carboxyl, cyano, or R_{S1}, R_{S1} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_{S1} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

L_1 is N(Y), S, SO, or SO_2 ;

L_2 is CO or absent when L_1 is N(Y) or L_2 is absent when L_1 is S, SO, or SO_2 , in which Y is H, R_d , SO_2R_d , or COR_d when L_2 is absent, or Y is H or R_d when L_2 is CO, R_d being C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_d being optionally substituted with one or more substituents selected from the group consisting of C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, cyano, C_1 - C_6 alkoxy, C_1 - C_6 alkylsulfonyl, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl and with C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl further optionally substituted with C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, $C(O)OH$, $C(O)O-C_1-C_6$ alkyl, $OC(O)-C_1-C_6$ alkyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl;

each of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 , independently, is H, halo, hydroxyl, carboxyl, cyano, R_{S2} , R_{S2} being amino, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, or C_2 - C_6 alkynyl, and each R_{S2} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

R_8 is H, halo or R_{S3} , R_{S3} being C_1 - C_6 alkyl, C_2 - C_6 alkenyl, or C_2 - C_6 alkynyl, and R_{S3} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano amino, C_1 - C_6 alkoxy, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, and C_3 - C_8 cycloalkyl;



, or , in which each of R_e , R_f , R_g , and R_h , independently is $-M_2-T_2$, in which M_2 is a bond, SO_2 , SO, S, CO, CO_2 , O, $O-C_1-C_4$ alkyl linker, C_1-C_4 alkyl linker, NH, or $N(R_t)$, R_t being C_1 - C_6 alkyl, and T_2 is H, halo, or R_{S4} , R_{S4} being C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 8-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, and each of $O-C_1-C_4$ alkyl linker, C_1-C_4 alkyl linker, R_t , and R_{S4} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C_1 - C_6

alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl, R_i is H or C₁-C₆ alkyl optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl, D is O, NR_j, or CR_jR_k, each of R_j and R_k independently being H or C₁-C₆ alkyl, or R_j and R_k taken together, with the carbon atom to which they are attached, form a C₃-C₁₀ cycloalkyl ring, and E is—M₃-T₃, M₃ being a bond or C₁-C₆ alkyl linker optionally substituted with halo or cyano, T₃ being C₃-C₁₀ cycloalkyl, C₆-C₁₀ aryl, 5 to 10-membered heteroaryl, or 4 to 10-membered heterocycloalkyl, and T₃ being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, thiol, carboxyl, cyano, nitro, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, C₁-C₆ haloalkoxy, C₁-C₆ alkylthio, C₁-C₆ alkylsulfonyl, C₁-C₆ haloalkylsulfonyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, o xo, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₄-C₁₂ alkylcycloalkyl, C₆-C₁₀ aryl, C₆-C₁₀ aryloxy, C₇-C₁₄ alkylaryl, C₆-C₁₀ aminoaryloxy, C₆-C₁₀ arylthio, 4 to 6-membered heterocycloalkyl optionally substituted with halo, C₁-C₄ alkyl, C₁-C₄ haloalkyl, 5 to 6-membered heteroaryl optionally substituted with halo, C₁-C₄ alkyl, and C₁-C₆ alkyl that is substituted with hydroxy, halo, C₁-C₆ alkoxy carbonyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl optionally further substituted with halo, hydroxyl, or C₁-C₆ alkoxy;

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

m is 0, 1, or 2; and

n is 0, 1, or 2.

[0162] For example, the sum of m and n is at least 1.

[0163] For example, m is 1 or 2 and n is 0.

[0164] For example, m is 2 and n is 0

[0165] For example, A is CH₂.

[0166] For example, A is O.

[0167] For example, L₁ is N(Y).

[0168] For example, L₁ is SO or SO₂.

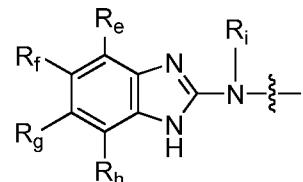
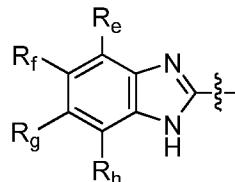
[0169] For example, Y is R_d.

[0170] For example, R_d is C₁-C₆ alkyl.

[0171] For example, L₂ is absent.

[0172] For example, each of G and J independently is OR_a.

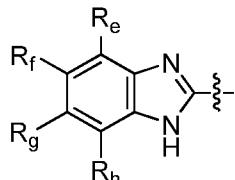
[0173] For example, R_a is H.



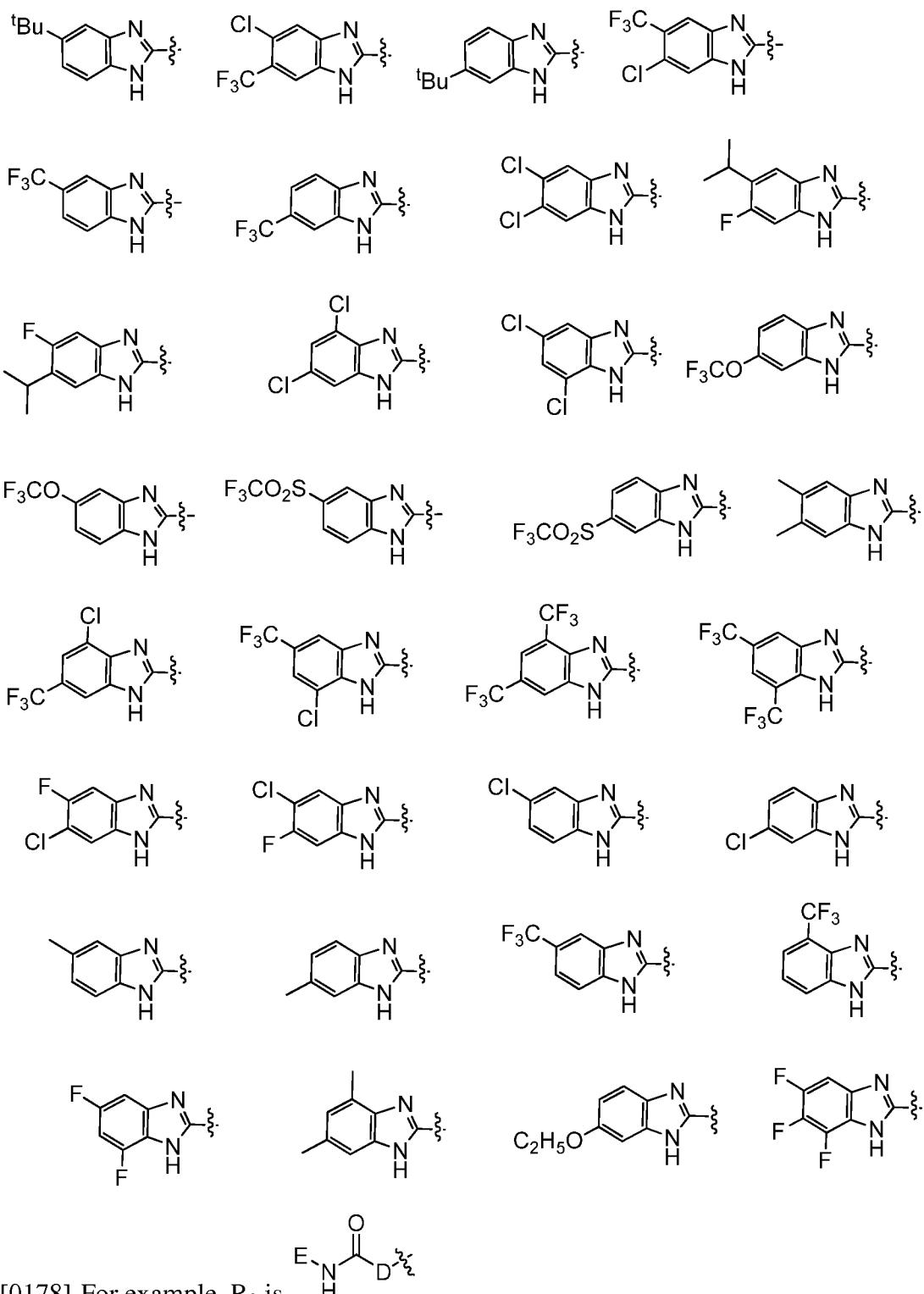
[0174] For example, R₉ is . For example, R₉ is .

[0175] For example, at least one of R_e, R_f, R_g, and R_h is halo (such as F, Cl, and Br), C₁-C₆ alkoxy optionally substituted with one or more halo (such as OCH₃, OCH₂CH₃, O-iPr, and OCF₃), C₁-C₆ alkylsulfonyl optionally substituted with one or more halo (such as SO₂CF₃), or C₁-C₆ alkyl optionally substituted with one or more halo (such as CH₃, i-propyl, n-butyl, and CF₃).

[0176] For example, R_i is H or C₁-C₆ alkyl (e.g., methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, s-pentyl and n-hexyl).



[0177] For example, is unsubstituted benzimidazolyl or one of the following groups:



[0178] For example, R₉ is

[0179] For example, D is O.

[0180] For example, D is NR_j.

[0181] For example, R_j is H.

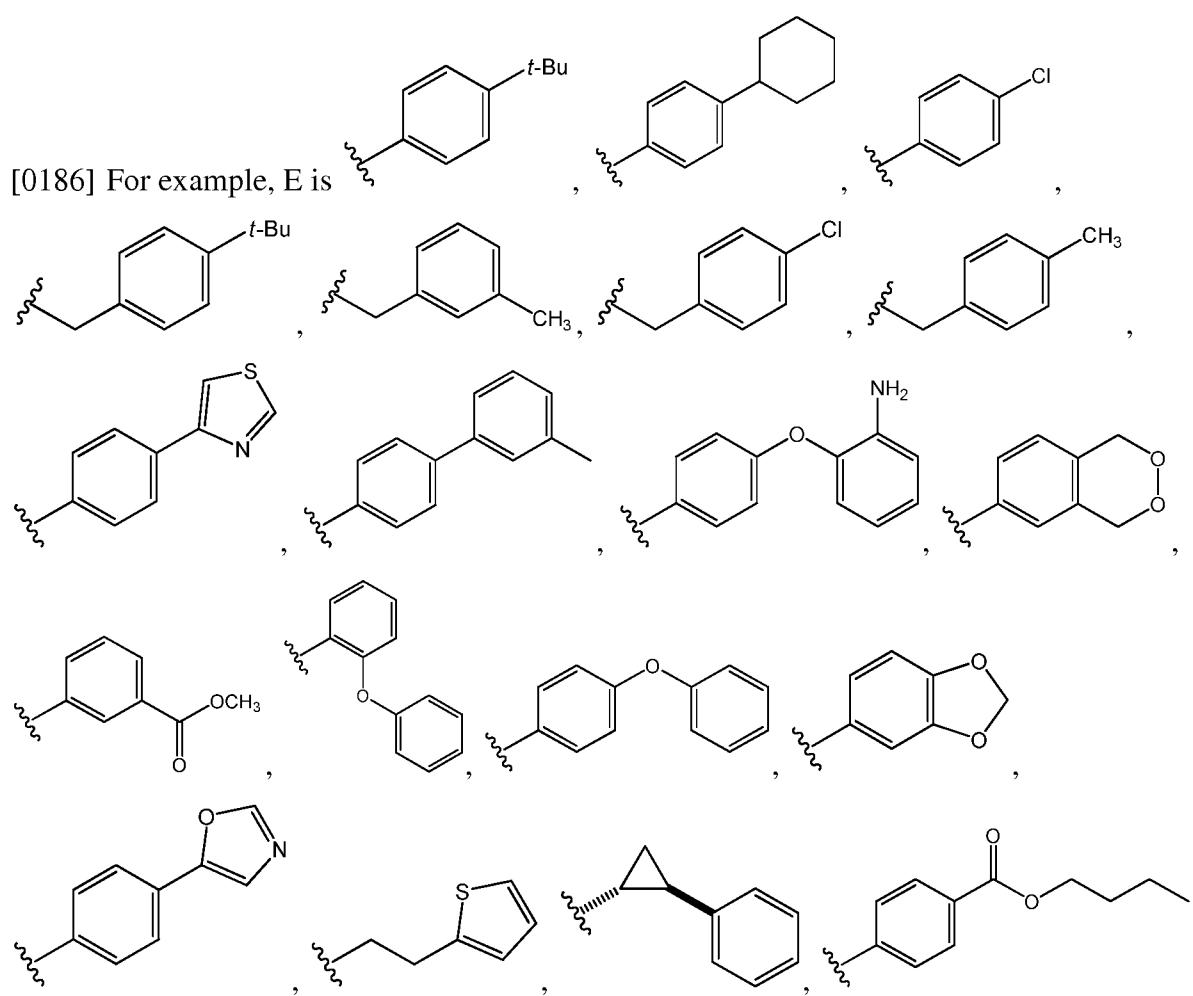
[0182] For example, D is CR_jR_k.

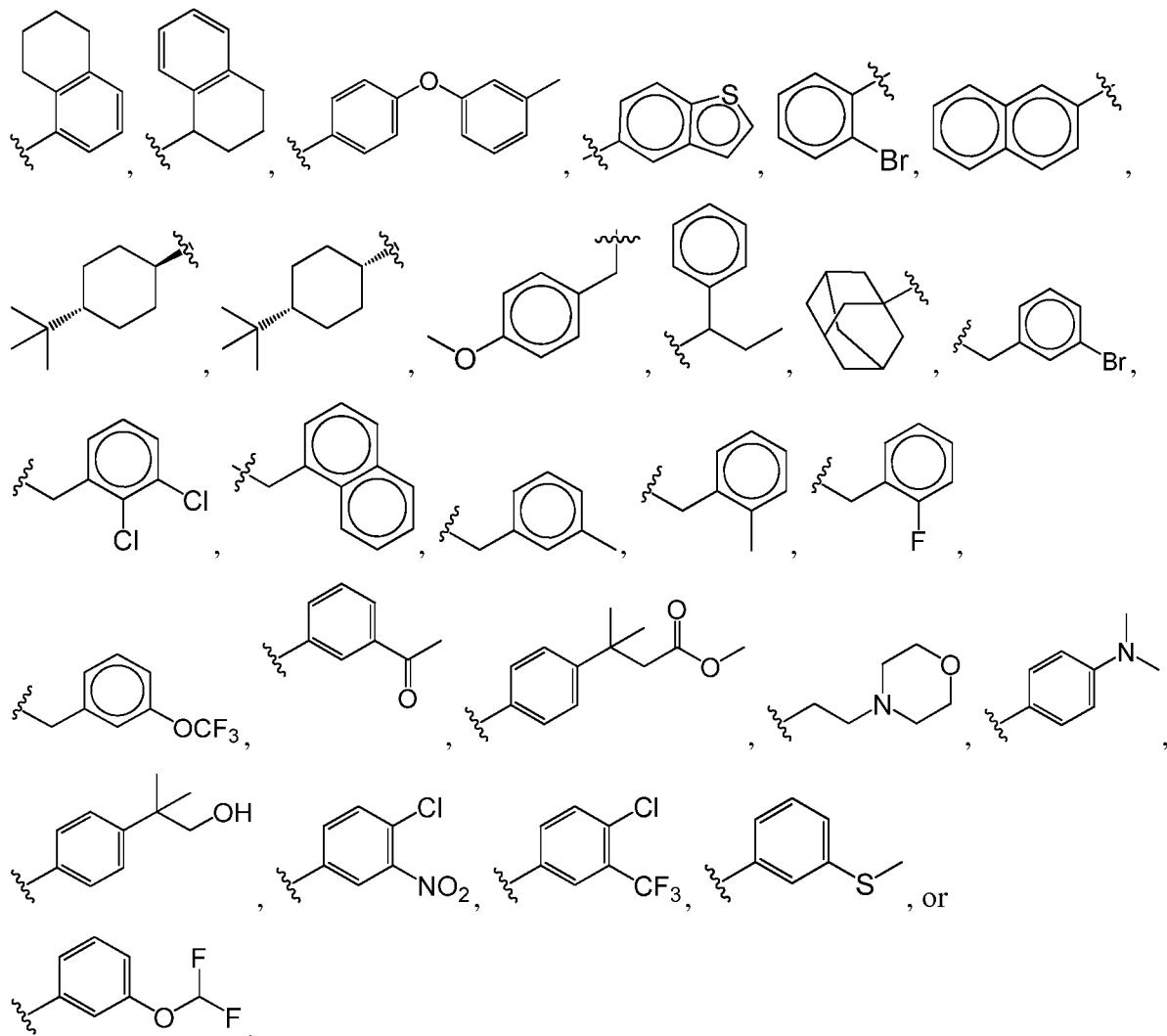
[0183] For example, each of R_j and R_k is H.

[0184] For example, E is $-M_3-T_3$, in which M_3 is a bond or C_1-C_3 alkyl linker, T_3 is phenyl, naphthyl, thienyl, cyclopropyl, or cyclohexyl, and T_3 is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, thiol, carboxyl, cyano, nitro, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 alkoxy, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkoxy carbonyl, oxo, amino, mono- C_1-C_6 alkylamino, di- C_1-C_6 alkylamino, C_3-C_8 cycloalkyl, C_4-C_{12} alkylcycloalkyl, C_6-C_{10} aryl, C_6-C_{10} aryloxy, C_7-C_{14} alkylaryl, C_6-C_{10} aminoaryloxy, C_6-C_{10} arylthio, 4 to 6-membered heterocycloalkyl optionally substituted with C_1-C_4 alkyl, 5 to 6-membered heteroaryl optionally substituted with C_1-C_4 alkyl, and C_1-C_6 alkyl that is substituted with hydroxy, C_1-C_6 alkoxy carbonyl, C_3-C_8 cycloalkyl, C_6-C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl.

[0185] For example, T_3 is phenyl optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, nitro, C_1 - C_6 alkyl (e.g., methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, s-pentyl and n-hexyl), C_1 - C_6 alkoxy, C_1 - C_6 haloalkyl, C_1 - C_6 haloalkoxy, C_1 - C_6 alkylsulfonyl, C_6 - C_{10} aryl (e.g., phenyl or naphthyl), and C_6 - C_{10} aryloxy, and C_7 - C_{14} alkylaryl.

[0186] For example, E is





[0187] For example, X is N.

[0188] For example, X is CR_x.

[0189] For example, X is CH.

[0190] For example, Q is NH₂ or NHR_b, in which R_b is -M₁-T₁, M₁ being a bond or C₁-C₆ alkyl linker and T₁ being C₃-C₈ cycloalkyl.

[0191] For example, Q is H.

[0192] For example, R₁, R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are each H.

[0193] For example, when R₈ is halo and is attached to the same carbon atom as J, then J is not hydroxyl.

[0194] For example, when R₈ is halo and is attached to the same carbon atom as G, then G is not hydroxyl.

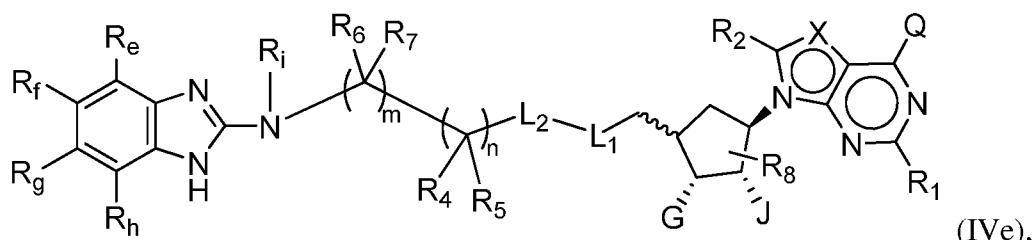
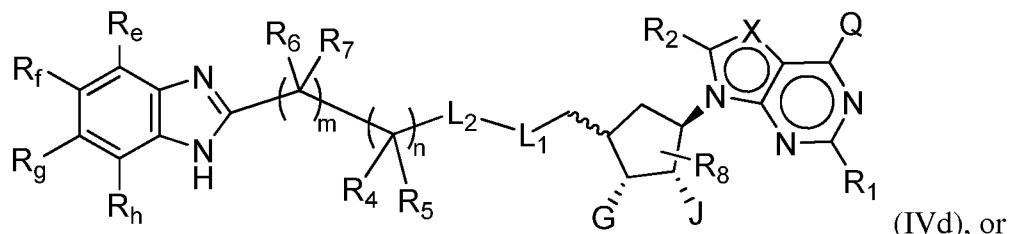
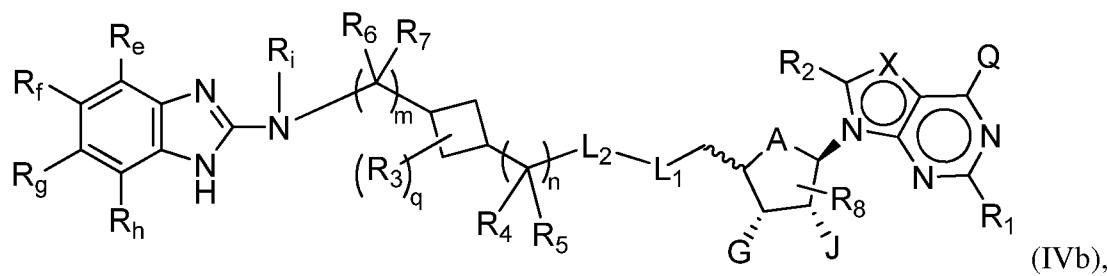
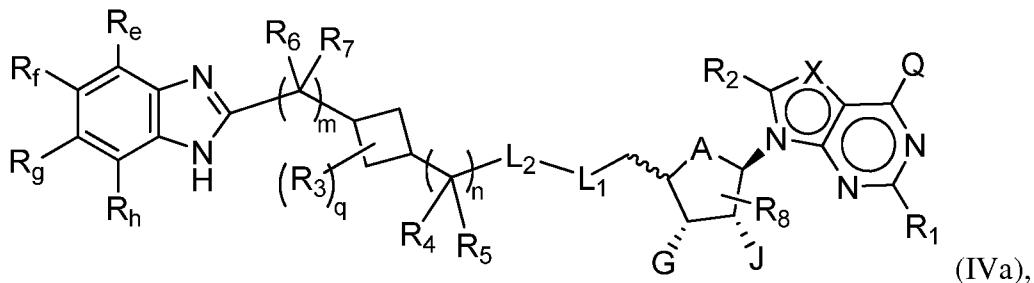
[0195] For example, T₂ is not halo when M₂ is SO₂, SO, S, CO or O.

[0196] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a heteroatom.

[0197] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a N atom.

[0198] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a C atom.

[0199] The present invention provides the compounds of Formula (IVa), (IVb), (IVd), or (IVe):



or a pharmaceutically acceptable salt or ester thereof, wherein:

A is O or CH₂;

each of G and J, independently, is H, halo, C(O)OH, C(O)O-C₁-C₆ alkyl or OR_a,

R_a being H, C₁-C₆ alkyl or C(O)-C₁-C₆ alkyl, wherein C(O)O-C₁-C₆ alkyl, C₁-C₆ alkyl or C(O)-C₁-C₆ alkyl is optionally substituted with one or more substituents selected from the group consisting of halo, cyano, hydroxyl, carboxyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl;

Q is H, NH₂, NHR_b, NR_bR_c, R_b, =O, OH, or OR_b, in which each of R_b and R_c independently is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5 to 10-membered heteroaryl, or -M₁-T₁ in which M₁ is a bond or C₁-C₆ alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy and T₁ is C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, or R_b and R_c, together with the N atom to which they attach, form 4 to 7-membered heterocycloalkyl having 0 or 1 additional heteroatoms to the N atom optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and each of R_b, R_c, and T₁ is optionally substituted with one or more substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

X is N or CR_x, in which R_x is H, halo, hydroxyl, carboxyl, cyano, or R_{S1}, R_{S1} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_{S1} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

L₁ is N(Y), S, SO, or SO₂;

L₂ is CO or absent when L₁ is N(Y) or L₂ is absent when L₁ is S, SO, or SO₂, in which Y is H, R_d, SO₂R_d, or COR_d when L₂ is absent, or Y is H or R_d when L₂ is CO, R_d being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_d being optionally substituted with one or more substituents selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, C₁-C₆ alkylsulfonyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl and with C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl further optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆

alkoxyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl;

each of R₁, R₂, R₃, R₄, R₅, R₆, and R₇, independently, is H, halo, hydroxyl, carboxyl, cyano, R_{S2}, R_{S2} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and each R_{S2} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

R₈ is H, halo or R_{S3}, R_{S3} being C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and R_{S3} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano amino, C₁-C₆ alkoxy, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl;

each of R_e, R_f, R_g, and R_h, independently is -M₂-T₂, in which M₂ is a bond, SO₂, SO, S, CO, CO₂, O, O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, NH, or N(R_t), R_t being C₁-C₆ alkyl, and T₂ is H, halo, or R_{S4}, R_{S4} being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 8-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, and each of O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, R_t, and R_{S4} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl,

R_i is H or C₁-C₆ alkyl optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

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

m is 0, 1, or 2; and

n is 0, 1, or 2.

[0200] For example, the sum of m and n is at least 1.

[0201] For example, m is 1 or 2 and n is 0.

[0202] For example, m is 2 and n is 0

[0203] For example, A is CH₂.

[0204] For example, A is O.

[0205] For example, L₁ is N(Y).

[0206] For example, L₁ is SO or SO₂.

[0207] For example, Y is R_d.

[0208] For example, R_d is C₁-C₆ alkyl.

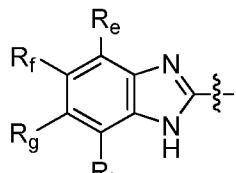
[0209] For example, L₂ is absent.

[0210] For example, each of G and J independently is OR_a.

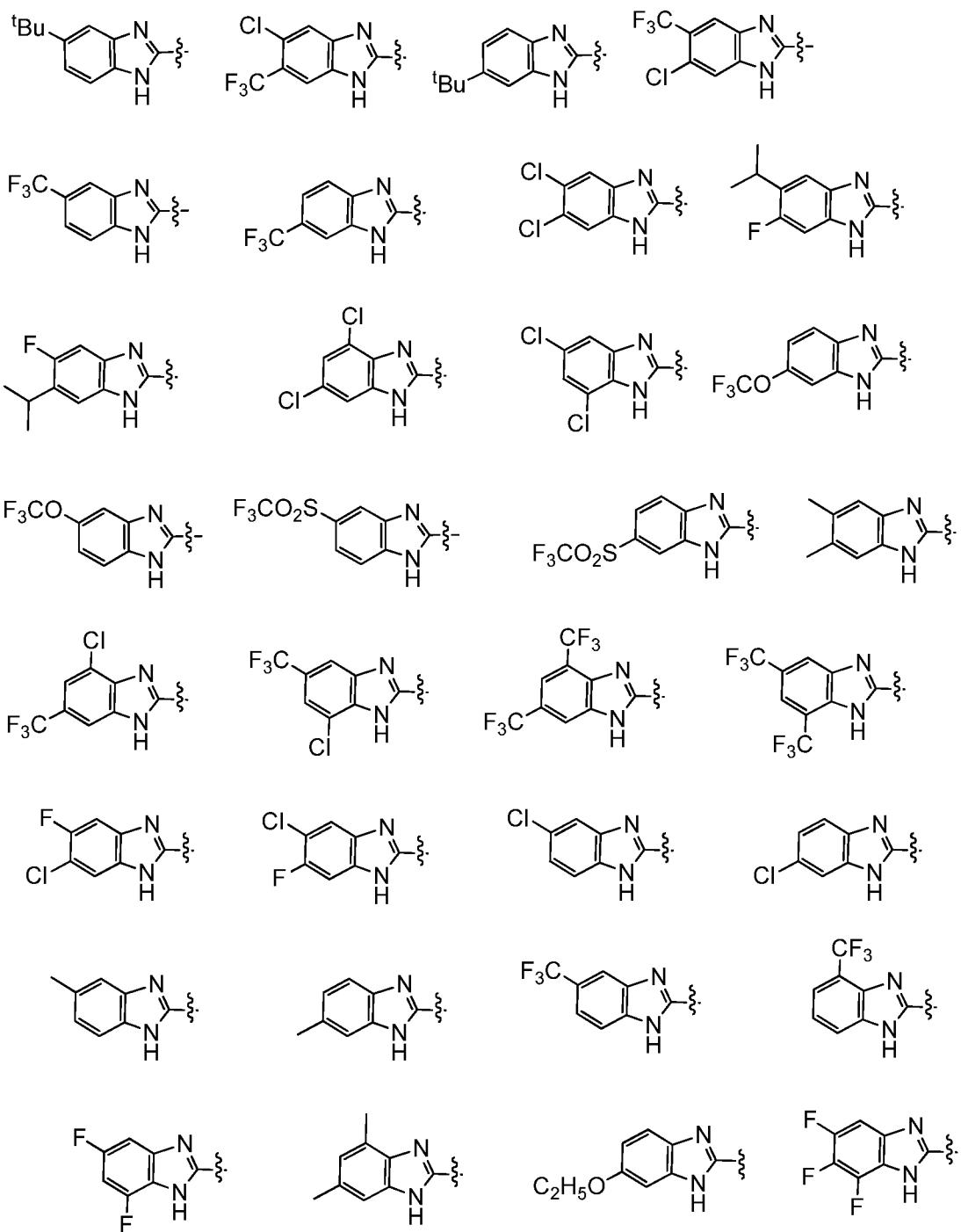
[0211] For example, R_a is H.

[0212] For example, at least one of R_e, R_f, R_g, and R_h is halo (such as F, Cl, and Br), C₁-C₆ alkoxyl optionally substituted with one or more halo (such as OCH₃, OCH₂CH₃, O-iPr, and OCF₃), C₁-C₆ alkylsulfonyl optionally substituted with one or more halo (such as SO₂CF₃), or C₁-C₆ alkyl optionally substituted with one or more halo (such as CH₃, i-propyl, n-butyl, and CF₃).

[0213] For example, R_i is H or C₁-C₆ alkyl (e.g., methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, s-pentyl and n-hexyl).



[0214] For example, is unsubstituted benzimidazolyl or one of the following groups:



[0215] For example, X is N.

[0216] For example, X is CR_x.

[0217] For example, X is CH.

[0218] For example, Q is NH₂ or NHR_b, in which R_b is -M₁-T₁, M₁ being a bond or C₁-C₆ alkyl linker and T₁ being C₃-C₈ cycloalkyl.

[0219] For example, Q is H.

[0220] For example, R₁, R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are each H.

[0221] For example, when R₈ is halo and is attached to the same carbon atom as J, then J is not hydroxyl.

[0222] For example, when R₈ is halo and is attached to the same carbon atom as G, then G is not hydroxyl.

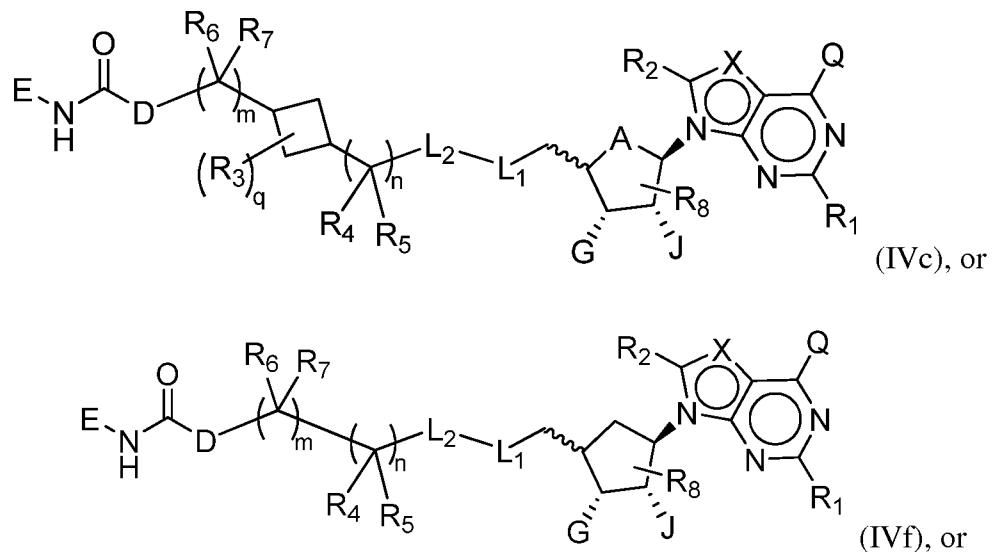
[0223] For example, T_2 is not halo when M_2 is SO_2 , SO , S , CO or O .

[0224] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a heteroatom.

[0225] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a N atom.

[0226] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a C atom.

[0227] The present invention provides the compounds of Formula (IVc) or (IVf):



a pharmaceutically acceptable salt or ester thereof, wherein:

A is O or CH_2 ;

each of G and J, independently, is H, halo, C(O)OH, C(O)O-C₁-C₆ alkyl or OR_a,

R_a being H, C_1 - C_6 alkyl or $C(O)$ - C_1 - C_6 alkyl, wherein $C(O)O$ - C_1 - C_6 alkyl, C_1 - C_6 alkyl or $C(O)$ - C_1 - C_6 alkyl is optionally substituted with one or more substituents selected from the group consisting of halo, cyano hydroxyl, carboxyl, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, and C_3 - C_8 cycloalkyl;

Q is H, NH₂, NHR_b, NR_bR_c, R_b, =O, OH, or OR_b, in which each of R_b and R_c independently is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5 to 10-membered heteroaryl, or -M₁-T₁ in which M₁ is a bond or C₁-C₆ alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy and T₁ is C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5

to 10-membered heteroaryl, or R_b and R_c , together with the N atom to which they attach, form 4 to 7-membered heterocycloalkyl having 0 or 1 additional heteroatoms to the N atom optionally substituted with C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, $C(O)OH$, $C(O)O-C_1-C_6$ alkyl, $OC(O)-C_1-C_6$ alkyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and each of R_b , R_c , and T_1 is optionally substituted with one or more substituents selected from the group consisting of C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

X is N or CR_x , in which R_x is H, halo, hydroxyl, carboxyl, cyano, or RS_1 , RS_1 being amino, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and RS_1 being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

L_1 is $N(Y)$, S, SO, or SO_2 ;

L_2 is CO or absent when L_1 is $N(Y)$ or L_2 is absent when L_1 is S, SO, or SO_2 , in which Y is H, R_d , SO_2R_d , or COR_d when L_2 is absent, or Y is H or R_d when L_2 is CO, R_d being C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_d being optionally substituted with one or more substituents selected from the group consisting of C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, cyano, C_1 - C_6 alkoxy, C_1 - C_6 alkylsulfonyl, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl and with C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl further optionally substituted with C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, halo, hydroxyl, carboxyl, $C(O)OH$, $C(O)O-C_1-C_6$ alkyl, $OC(O)-C_1-C_6$ alkyl, cyano, C_1 - C_6 alkoxy, amino, mono- C_1 - C_6 alkylamino, di- C_1 - C_6 alkylamino, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl;

each of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and R_7 , independently, is H, halo, hydroxyl, carboxyl, cyano, RS_2 , RS_2 being amino, C_1 - C_6 alkoxy, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, or C_2 - C_6 alkynyl, and each RS_2 being optionally substituted with one or more substituents

selected from the group consisting of halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

R₈ is H, halo or R_{S3}, R_{S3} being C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and R_{S3} being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano amino, C₁-C₆ alkoxy, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, and C₃-C₈ cycloalkyl;

D is O, NR_j, or CR_jR_k, each of R_j and R_k independently being H or C₁-C₆ alkyl, or R_j and R_k taken together, with the carbon atom to which they are attached, form a C₃-C₁₀ cycloalkyl ring;

E is -M₃-T₃, M₃ being a bond or C₁-C₆ alkyl linker optionally substituted with halo or cyano, T₃ being C₃-C₁₀ cycloalkyl, C₆-C₁₀ aryl, 5 to 10-membered heteroaryl, or 4 to 10-membered heterocycloalkyl, and T₃ being optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, thiol, carboxyl, cyano, nitro, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, C₁-C₆ haloalkoxyl, C₁-C₆ alkylthio, C₁-C₆ alkylsulfonyl, C₁-C₆ haloalkylsulfonyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, oxo, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₄-C₁₂ alkylcycloalkyl, C₆-C₁₀ aryl, C₆-C₁₀ aryloxy, C₇-C₁₄ alkylaryl, C₆-C₁₀ aminoaryloxy, C₆-C₁₀ arylthio, 4 to 6-membered heterocycloalkyl optionally substituted with halo, C₁-C₄ alkyl, C₁-C₄ haloalkyl, 5 to 6-membered heteroaryl optionally substituted with halo, C₁-C₄ alkyl, and C₁-C₆ alkyl that is substituted with hydroxy, halo, C₁-C₆ alkoxy carbonyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl optionally further substituted with halo, hydroxyl, or C₁-C₆ alkoxy;

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

m is 0, 1, or 2; and

n is 0, 1, or 2.

[0228] For example, the sum of m and n is at least 1.

[0229] For example, m is 1 or 2 and n is 0.

[0230] For example, m is 2 and n is 0

[0231] For example, A is CH₂.

[0232] For example, A is O.

[0233] For example, L₁ is N(Y).

[0234] For example, L₁ is SO or SO₂.

[0235] For example, Y is R_d.

[0236] For example, R_d is C₁-C₆ alkyl.

[0237] For example, L₂ is absent.

[0238] For example, each of G and J independently is OR_a.

[0239] For example, R_a is H.

[0240] For example, D is O.

[0241] For example, D is NR_j.

[0242] For example, R_j is H.

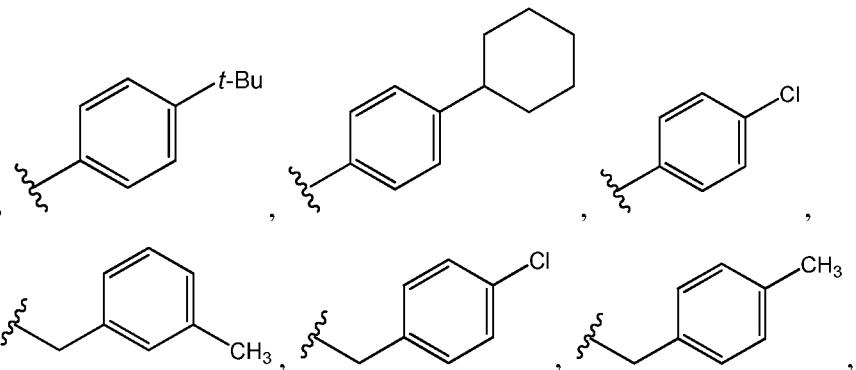
[0243] For example, D is CR_jR_k.

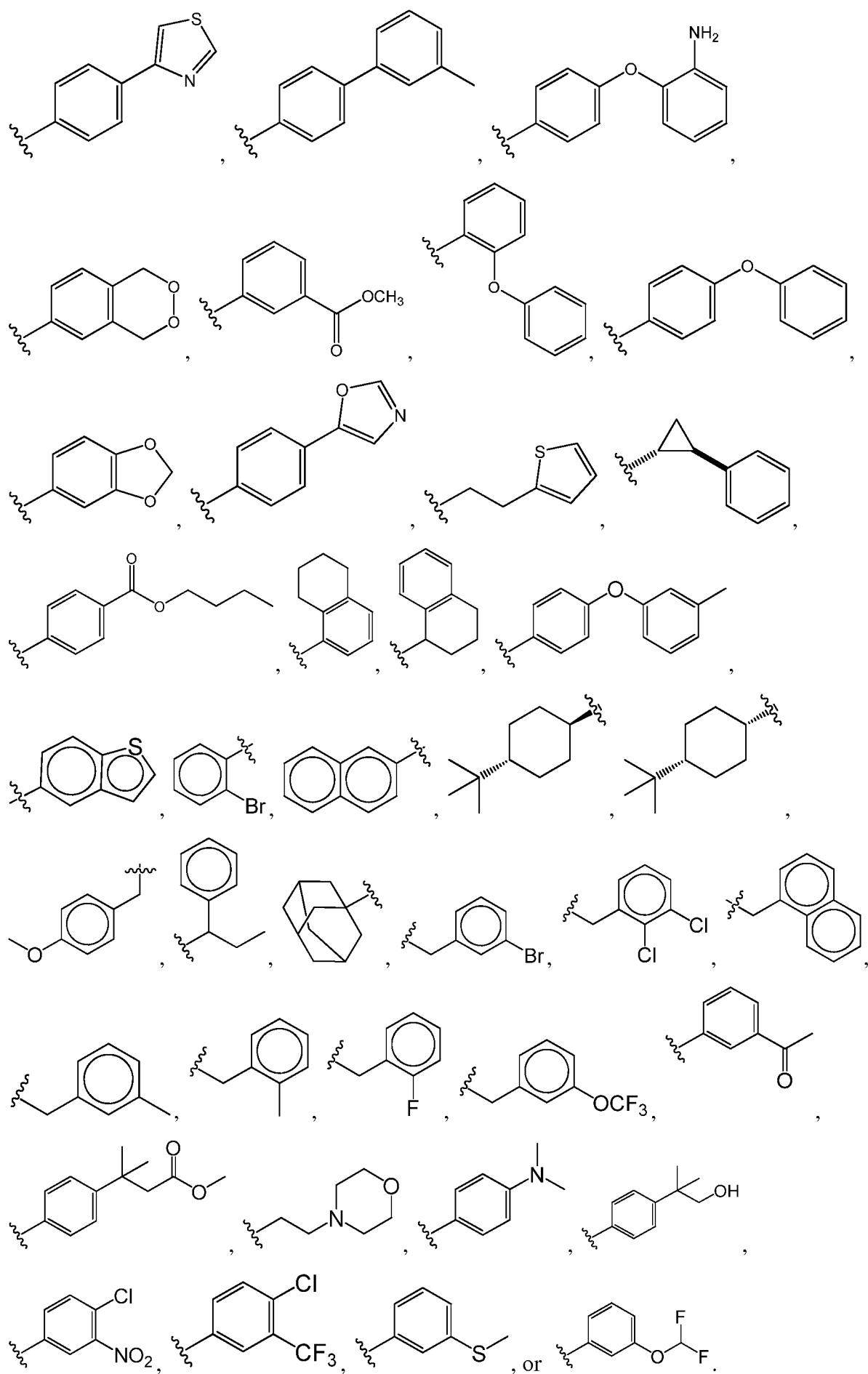
[0244] For example, each of R_j and R_k is H.

[0245] For example, E is $-M_3-T_3$, in which M₃ is a bond or C₁-C₃ alkyl linker, T₃ is phenyl, naphthyl, thienyl, cyclopropyl, or cyclohexyl, and T₃ is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, thiol, carboxyl, cyano, nitro, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, C₁-C₆ haloalkoxy, C₁-C₆ alkylthio, C₁-C₆ alkylsulfonyl, C₁-C₆ alkylcarbonyl, C₁-C₆ alkoxy carbonyl, oxo, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₄-C₁₂ alkylcycloalkyl, C₆-C₁₀ aryl, C₆-C₁₀ aryloxy, C₇-C₁₄ alkylaryl, C₆-C₁₀ aminoaryloxy, C₆-C₁₀ arylthio, 4 to 6-membered heterocycloalkyl optionally substituted with C₁-C₄ alkyl, 5 to 6-membered heteroaryl optionally substituted with C₁-C₄ alkyl, and C₁-C₆ alkyl that is substituted with hydroxy, C₁-C₆ alkoxy carbonyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl.

[0246] For example, T₃ is phenyl optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, carboxyl, cyano, nitro, C₁-C₆ alkyl (e.g., methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, s-pentyl and n-hexyl), C₁-C₆ alkoxy, C₁-C₆ haloalkyl, C₁-C₆ haloalkoxy, C₁-C₆ alkylsulfonyl, C₆-C₁₀ aryl (e.g., phenyl or naphthyl), and C₆-C₁₀ aryloxy, and C₇-C₁₄ alkylaryl.

[0247] For example, E is





[0248] For example, X is N.

[0249] For example, X is CR_x.

[0250] For example, X is CH.

[0251] For example, Q is NH₂ or NHR_b, in which R_b is –M₁–T₁, M₁ being a bond or C₁–C₆ alkyl linker and T₁ being C₃–C₈ cycloalkyl.

[0252] For example, Q is H.

[0253] For example, R₁, R₂, R₃, R₄, R₅, R₆, R₇, and R₈ are each H.

[0254] For example, when R₈ is halo and is attached to the same carbon atom as J, then J is not hydroxyl.

[0255] For example, when R₈ is halo and is attached to the same carbon atom as G, then G is not hydroxyl.

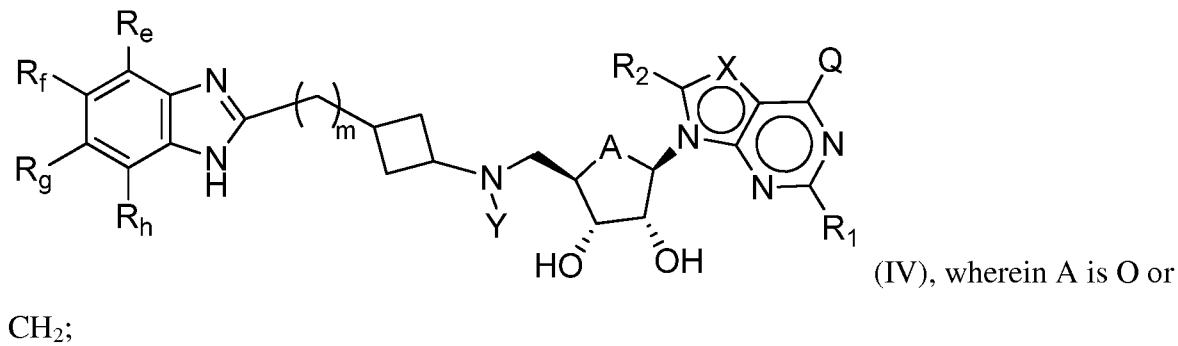
[0256] For example, T₂ is not halo when M₂ is SO₂, SO, S, CO or O.

[0257] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a heteroatom.

[0258] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a N atom.

[0259] For example, T₂ is a 4-8 membered heterocycloalkyl which is bound to M₂ via a C atom.

[0260] The invention also relates to a compound of Formula (IV) or its N-oxide or a pharmaceutically acceptable salt thereof:



Q is H, NH₂, NHR_b, NR_bR_c, R_b, =O, OH, or OR_b, in which each of R_b and R_c independently is C₁–C₆ alkyl, C₂–C₆ alkenyl, C₂–C₆ alkynyl, C₃–C₈ cycloalkyl, C₆–C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5 to 10-membered heteroaryl, or –M₁–T₁ in which M₁ is a bond or C₁–C₆ alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁–C₆ alkoxy and T₁ is C₃–C₈ cycloalkyl, C₆–C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, or R_b and R_c, together with the N atom to which they attach, form 4 to 7-membered heterocycloalkyl having 0 or 1 additional heteroatoms to the N atom optionally

substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and each of R_b, R_c, and T₁ is optionally substituted with one or more substituents selected from C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

X is N or CR_x, in which R_x is H, halo, hydroxyl, carboxyl, cyano, or R_{S1}, R_{S1} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_{S1} being optionally substituted with one or more substituents selected from halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

Y is H, R_d, SO₂R_d, or COR_d, R_d being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl, and R_d being optionally substituted with one or more substituents selected from C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, C₁-C₆ alkylsulfonyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl and with C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl further optionally substituted with C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, halo, hydroxyl, carboxyl, C(O)OH, C(O)O-C₁-C₆ alkyl, OC(O)-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, or 5 to 6-membered heteroaryl;

each of R₁ and R₂ independently, is H, halo, hydroxyl, carboxyl, cyano, R_{S2}, R_{S2} being amino, C₁-C₆ alkoxy, C₁-C₆ alkyl, C₂-C₆ alkenyl, or C₂-C₆ alkynyl, and each R_{S2} being optionally substituted with one or more substituents selected from halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl;

each of R_e, R_f, R_g, and R_h, independently is -M₂-T₂, in which M₂ is a bond, SO₂, SO, S, CO, CO₂, O, O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, NH, or N(R_t), R_t being C₁-C₆ alkyl, and T₂ is H, halo, or R_{S4}, R_{S4} being C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-

C₁₀ aryl, 4 to 8-membered heterocycloalkyl, or 5 to 10-membered heteroaryl, and each of O-C₁-C₄ alkyl linker, C₁-C₄ alkyl linker, R_t, and R_{S4} being optionally substituted with one or more substituents selected from halo, hydroxyl, carboxyl, cyano, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₆ alkoxy, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 6-membered heterocycloalkyl, and 5 to 6-membered heteroaryl, and

m is 0, 1, or 2.

[0261] For example, A is O. In certain compounds of Formula (IV), A is O and m is 2.

[0262] In certain compounds of Formula (IV), X is N.

[0263] For example, in certain compounds, Q is NH₂ or NHR_b, in which R_b is -M₁-T₁, M₁ being a bond or C₁-C₆ alkyl linker and T₁ being C₃-C₈ cycloalkyl

[0264] For example, in certain compounds of Formula (IV), R₁ and R₂ are each H.

[0265] In certain compounds of Formula (IV), Y is R_d. For example, R_d is C₁-C₆ alkyl optionally substituted with C₃-C₈ cycloalkyl or halo. For example, R_d is C₃-C₈ cycloalkyl optionally substituted with C₁-C₆ alkyl or halo.

[0266] The invention also relates to a compound of Formula (IV), wherein at least one of R_e, R_f, R_g, and R_h is halo, C₁-C₆ alkoxy optionally substituted with one or more halo; C₁-C₆ alkylsulfonyl optionally substituted with one or more halo; C₁-C₆ alkyl optionally substituted with one or more substituents selected from CN, halo, C₃-C₈ cycloalkyl, hydroxy, and C₁-C₆ alkoxy; C₃-C₈ cycloalkyl optionally substituted with one or more C₁-C₆ alkyl or CN; or 4 to 8-membered heterocycloalkyl optionally substituted with one or more substituents selected from CN, halo, hydroxy, C₁-C₆ alkyl and C₁-C₆ alkoxy. For example, the compound of Formula (IV) has at least one of R_e, R_f, R_g, and R_h selected from F; Cl; Br; CF₃; OCF₃; SO₂CF₃; oxetanyl optionally substituted with one or more substituents selected from CN, halo, hydroxy, C₁-C₆ alkyl and C₁-C₆ alkoxy; C₃-C₈ cycloalkyl optionally substituted with one or more substituents selected from C₁-C₄ alkyl; and C₁-C₄ alkyl optionally substituted with one or more substituents selected from halo, C₃-C₈ cycloalkyl, hydroxy and C₁-C₆ alkoxy.

[0267] For example, the invention relates to compounds of Formula (IV) where at least one of R_f and R_g is alkyl, optionally substituted with hydroxyl. For example, the invention relates to compounds where at least one of R_f and R_g is *t*-butyl substituted with hydroxyl.

[0268] The invention relates to a compound selected from Tables 1-4. The invention also

relates to a salt of a compound selected from Tables 1-4. The invention also relates to an N-oxide of compound selected from Tables 1-4. The invention also relates to a salt of an N-oxide of compound selected from Tables 1-4. For example, the invention relates to a compound selected from Compounds A1-A7, A9-A109, and A111-A140.

[0269] Other compounds suitable for the methods of the invention are described in WO2012/075381, WO2012/075492, WO2012/082436, and WO2012/75500 the contents of which are hereby incorporated by reference in their entireties.

[0270] The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a compound of any of the Formulae disclosed herein and a pharmaceutically acceptable carrier.

[0271] The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a salt of a compound of any of the Formulae disclosed herein and a pharmaceutically acceptable carrier.

[0272] The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a hydrate of a compound of any of the Formulae disclosed herein and a pharmaceutically acceptable carrier.

[0273] The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a compound selected from Tables 1-4 and a pharmaceutically acceptable carrier. The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a salt of a compound selected from Tables 1-4 and a pharmaceutically acceptable carrier. The invention also relates to a pharmaceutical composition of a therapeutically effective amount of an N-oxide of a compound selected from Tables 1-4 and a pharmaceutically acceptable carrier. The invention also relates to a pharmaceutical composition of a therapeutically effective amount of an N-oxide of salt of a compound selected from Tables 1-4 and a pharmaceutically acceptable carrier. The invention also relates to a pharmaceutical composition of a therapeutically effective amount of a hydrate of a compound selected from Tables 1-4 and a pharmaceutically acceptable carrier.

[0274] The present invention provides methods of treating or preventing cancer. The present invention provides methods of treating cancer. The present invention also provides methods of preventing cancer. The method includes administering to a subject in need thereof a therapeutically effective amount of the compound of any of the Formulae disclosed herein. The cancer can be a hematological cancer. Preferably, the cancer is leukemia. More

preferably, the cancer is acute myeloid leukemia, acute lymphocytic leukemia or mixed lineage leukemia.

[0275] The present invention provides methods of treating or preventing a disease or disorder mediated by translocation of a gene on chromosome 11q23. The present invention provides methods of treating a disease or disorder mediated by translocation of a gene on chromosome 11q23. The present invention also provides methods of preventing a disease or disorder mediated by translocation of a gene on chromosome 11q23. The method includes administering to a subject in need thereof a therapeutically effective amount of the compound of any of the Formulae disclosed herein.

[0276] The present invention provides methods of treating or preventing a disease or disorder in which DOT1L-mediated protein methylation plays a part or a disease or disorder mediated by DOT1L-mediated protein methylation. The present invention provides methods of treating a disease or disorder in which DOT1L-mediated protein methylation plays a part or a disease or disorder mediated by DOT1L-mediated protein methylation. The present invention also provides methods of preventing a disease or disorder in which DOT1L-mediated protein methylation plays a part or a disease or disorder mediated by DOT1L-mediated protein methylation. The method includes administering to a subject in need thereof a therapeutically effective amount of the compound of any of the Formulae disclosed herein.

[0277] The present invention provides methods of inhibiting DOT1L activity in a cell. The method includes contacting the cell with an effective amount of one or more of the compound of any of the Formulae disclosed herein.

[0278] Still another aspect of the invention relates to a method of reducing the level of Histone H3 Lysine residue 79 (H3-K79) methylation in a cell. The method includes contacting a cell with a compound of the present invention. Such method can be used to ameliorate any condition which is caused by or potentiated by the activity of DOT1L through H3-K79 methylation.

[0279] The present invention relates to use of the compounds disclosed herein in preparation of a medicament for treating or preventing cancer. The use includes a compound of any of the Formulae disclosed herein for administration to a subject in need thereof in a therapeutically effective amount. The cancer can be a hematological cancer. Preferably, the cancer is leukemia. More preferably, the cancer is acute myeloid leukemia, acute lymphocytic leukemia or mixed lineage leukemia.

[0280] The present invention provides use of the compounds disclosed herein in preparation of a medicament for treating or preventing a disease or disorder mediated by translocation of a gene on chromosome 11q23. The use includes a compound of any of the Formulae disclosed herein for administration to a subject in need thereof in a therapeutically effective amount.

[0281] The present invention provides use of the compounds disclosed herein in preparation of a medicament for treating or preventing a disease or disorder in which DOT1L-mediated protein methylation plays a part or a disease or disorder mediated by DOT1L-mediated protein methylation. The use includes a compound of any of the Formulae disclosed herein for administration to a subject in need thereof in a therapeutically effective amount.

[0282] The present invention provides use of the compounds disclosed herein for inhibiting DOT1L activity in a cell. The use includes contacting the cell with an effective amount of one or more of the compound of any of the Formulae disclosed herein.

[0283] Still another aspect of the invention relates to a use of the compounds disclosed herein for reducing the level of Histone H3 Lysine residue 79 (H3-K79) methylation in a cell. The use includes contacting a cell with a compound of the present invention. Such use can ameliorate any condition which is caused by or potentiated by the activity of DOT1L through H3-K79 methylation.

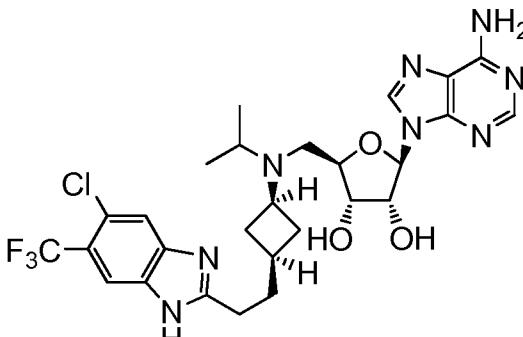
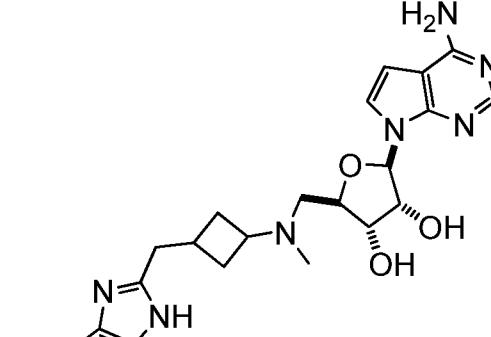
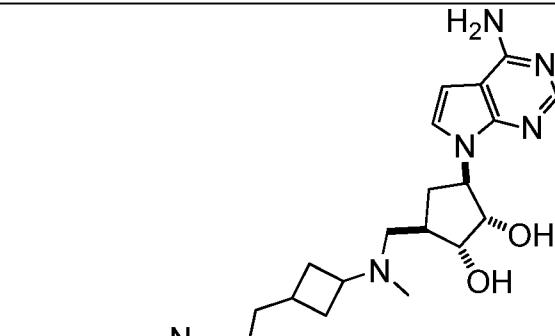
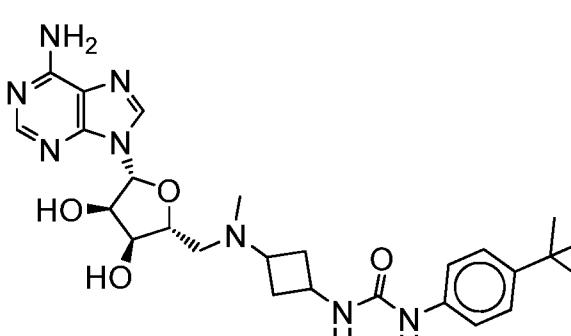
[0284] In the formulae presented herein, the variables can be selected from the respective groups of chemical moieties later defined in the detailed description.

[0285] In addition, the invention provides methods of synthesizing the foregoing compounds. Following synthesis, a therapeutically effective amount of one or more of the compounds can be formulated with a pharmaceutically acceptable carrier for administration to a mammal, particularly humans, for use in modulating an epigenetic enzyme. In certain embodiments, the compounds of the present invention are useful for treating, preventing, or reducing the risk of cancer or for the manufacture of a medicament for treating, preventing, or reducing the risk of cancer. Accordingly, the compounds or the formulations can be administered, for example, *via* oral, parenteral, otic, ophthalmic, nasal, or topical routes, to provide an effective amount of the compound to the mammal.

[0286] Representative compounds of the present invention include compounds listed in Tables 1-4.

Table 1

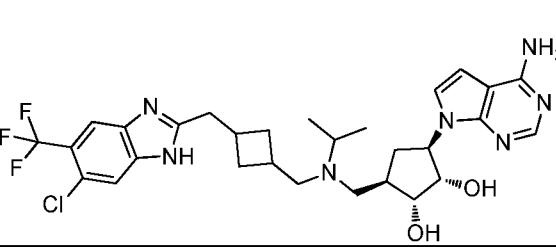
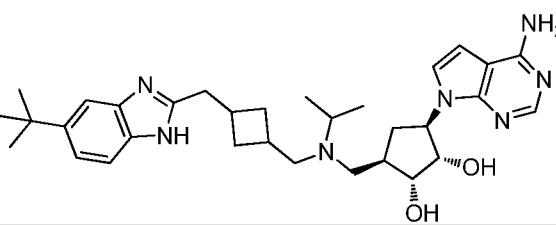
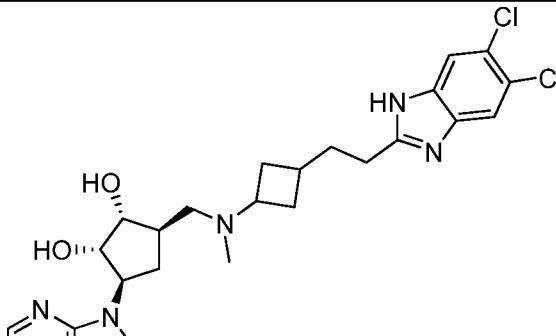
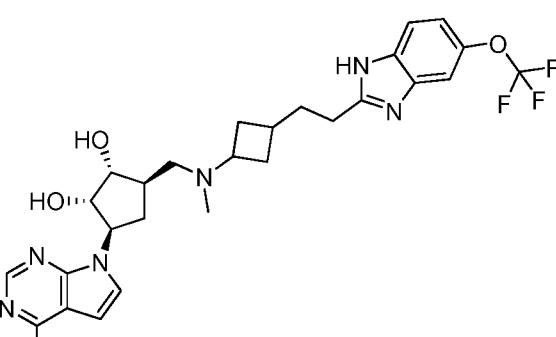
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A1		(2R,3S,4R,5R)-2-(((3-(2-(1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)-5-(6-amino-9H-purin-9-yl)tetrahydrofuran-3,4-diol	
A2		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	563.4 (M+H) ⁺
A3		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	563.5 (M+H) ⁺
A4		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	609.2 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A5		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	609.2 (M+H) ₊
A6		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-((5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)methyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	520.4 (M+H) ₊
A7		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(2-(6-chloro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	579.7 (M+H) ₊
A8		1-(3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(methyl)amino)cyclobutyl)-3-(4-tert-butylphenyl)urea	525.5 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A9		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(6-chloro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	578.3 (M+H) ₊
A10		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1r,3S)-3-(2-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.3 (M+H) ₊
A11		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1s,3R)-3-(2-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.3 (M+H) ₊
A12		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(6-chloro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	578.3 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A13		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl(3-(2-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.5 (M+H) ⁺
A14		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	532.3 (M+H) ⁺
A15		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl((3-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)cyclobutyl)methyl)amino)methyl)cyclopentane-1,2-diol	572.4 (M+H) ⁺
A16		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	550.3 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A17		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-((3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentanol	562.3 (M+H) ⁺
A18		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((3-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)cyclobutyl)methyl)amino)methyl)cyclopentane-1,2-diol	544 (M+H) ⁺
A19		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A20		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(6-chloro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol	NMR data

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A21		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-((6-chloro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)cyclobutyl)methyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol	606.3 (M+H) ⁺
A22		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-((5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)methyl)cyclobutyl)methyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol	560.4 (M+H) ⁺
A23		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(2-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)methyl)(methyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A24		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)methyl)(methyl)amino)methyl)cyclopentane-1,2-diol	558.2 (M-H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A25		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(ethyl)amino)methyl)cyclopentane-1,2-diol	546.3 (M+H) ₊
A26		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-bromo-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	554.1 (M+H) ₊
A27		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl(3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydropuran-3,4-diol	575.5 (M+H) ₊
A28		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydropuran-3,4-diol	575.5 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A29		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl(3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.4 (M+H) ⁺
A30		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1r,3S)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	547.6 (M+H) ⁺
A31		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	575.6 (M+H) ⁺
A32		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	532.4 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A33		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl(3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	547.3 (M+H) ₊
A34		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1s,3R)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	547.5 (M+H) ₊
A35		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A36		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1r,3S)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.4 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A37		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	558.3 (M-H) -
A38		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)ethylamino)methyl)cyclopentane-1,2-diol	546.3 (M+H) +
A39		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1s,3R)-3-(2-(5-(1-methylcyclobutyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	544.3 (M+H) +

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A40		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclopropylmethyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A41		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropylamino)methyl)cyclopentane-1,2-diol	NMR data
A42		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclobutylmethyl)amino)methyl)cyclopentane-1,2-diol	586.3 (M+H) ⁺
A43		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	572.2 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A44		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclopropylmethyl)amino)methyl)cyclopentane-1,2-diol	572.6 (M+H) ⁺
A45		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isobutyl)amino)methyl)cyclopentane-1,2-diol	574.6 (M+H) ⁺
A46		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclobutyl)amino)methyl)cyclopentane-1,2-diol	572.6 (M+H) ⁺
A47		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-bromo-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	556.0 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A48		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isobutyl)amino)methylcyclopentane-1,2-diol	572.3 (M-H) -
A49		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(ethyl)amino)methylcyclopentane-1,2-diol	546.3 (M+H) +
A50		(1R,2S,3R,5R)-3-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol	561.4 (M+H) +
A51		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)cyclobutylamino)methylcyclopentane-1,2-diol	572.7 (M+H) +

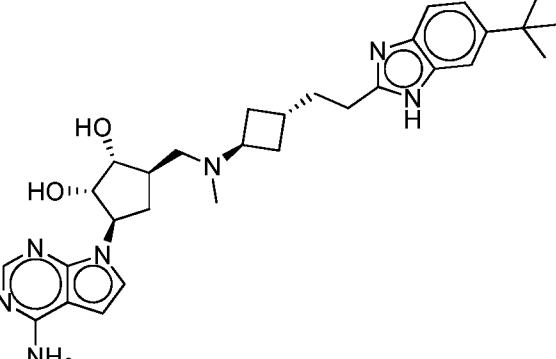
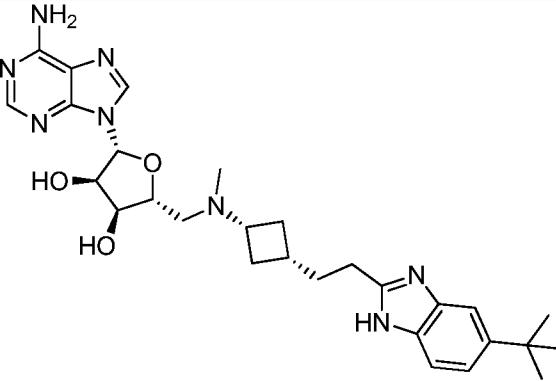
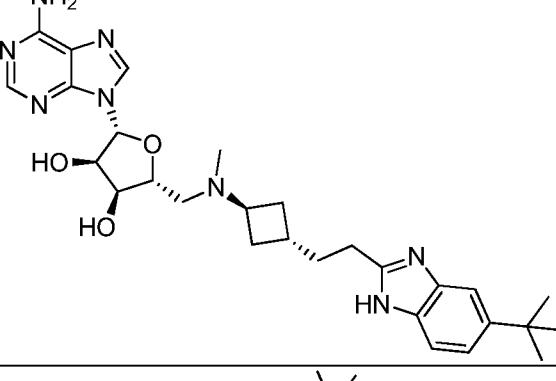
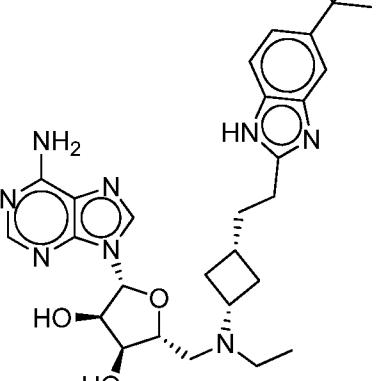
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A52		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A53		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isobutyl)amino)methyl)cyclopentane-1,2-diol	572.3 (M-H)
A54		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclopropylmethyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A55		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-bromo-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	NMR data

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A56		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl(3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	588.2 (M+H) ⁺
A57		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	560.1 (M+H) ⁺
A58		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	NMR data

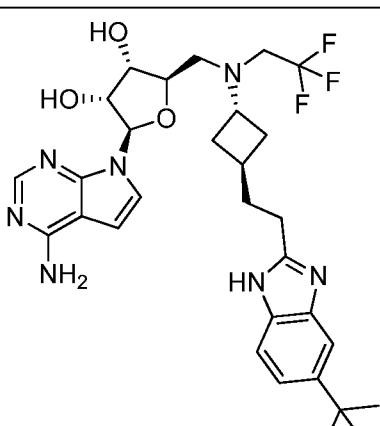
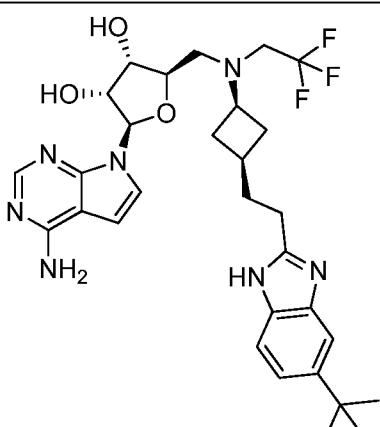
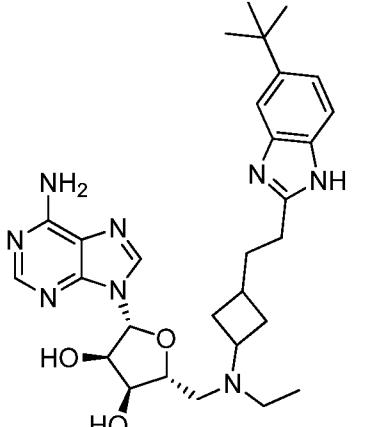
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A59		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(cyclobutylmethyl)amino)methyl)cyclopentane-1,2-diol	586.4 (M+H) ⁺
A60		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	588.2 (M+H) ⁺
A61		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	588.7 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A62		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl(3-(5-(oxetan-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)cyclopentane-1,2-diol	NMR data
A63		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1r,3S)-3-(2-(5-(oxetan-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydron-3,4-diol	535.4 (M+H) ₊
A64		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl(3-(2-(5-(oxetan-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydron-3,4-diol	535.3 (M+H) ₊
A65		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1s,3R)-3-(2-(5-(oxetan-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydron-3,4-diol	535.4 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A66		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methylcyclopentane-1,2-diol	600.2 (M+H) ⁺
A67		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	561.5 (M+H) ⁺
A68		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((3-(2-(5-(1-methoxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	565.4 (M+H) ⁺
A69		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methylcyclopentane-1,2-diol	532.3 (M+H) ⁺

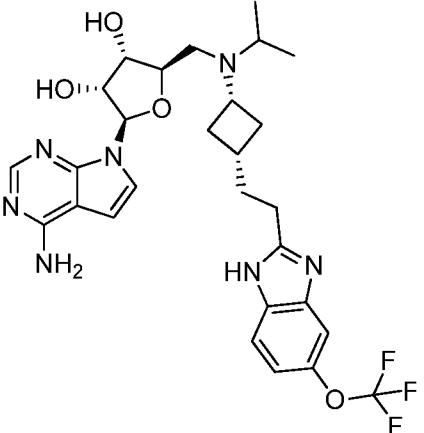
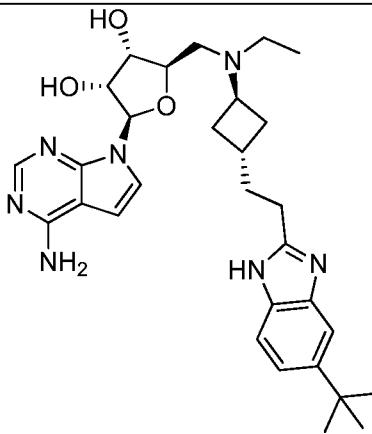
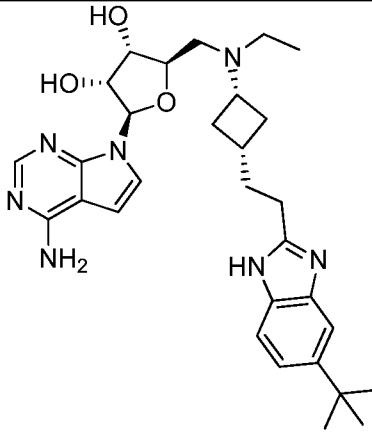
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A70		(1R,2S,3R,5R)-3-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(6-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)cyclopentane-1,2-diol	532.3 (M+H) ⁺
A71		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	535.3 (M+H) ⁺
A72		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	535.3 (M+H) ⁺
A73		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(ethyl)amino)methyltetrahydrofuran-3,4-diol	549.3 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A74		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(ethyl)amino)methyl)tetrahydrofuran-3,4-diol	549.3 (M+H) ₊
A75		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyl)tetrahydrofuran-3,4-diol	562.5 (M+H) ₊
A76		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrofuran-3,4-diol	590.3 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A77		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methyltetrahydrofuran-3,4-diol	602.3 (M+H) ₊
A78		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methyltetrahydrofuran-3,4-diol	602.3 (M+H) ₊
A79		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(ethyl)amino)methyl)tetrahydrofuran-3,4-diol	549.3 (M+H) ₊

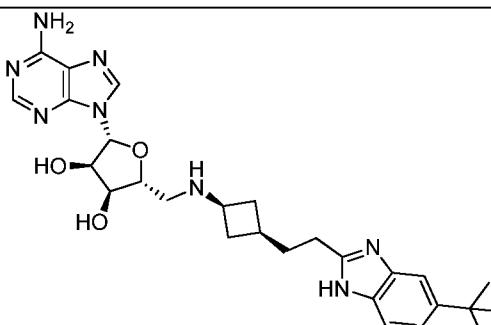
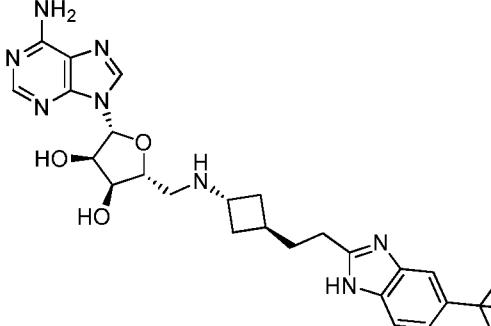
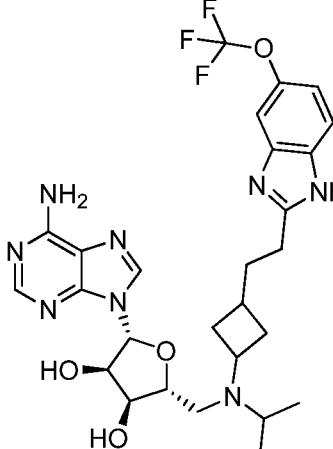
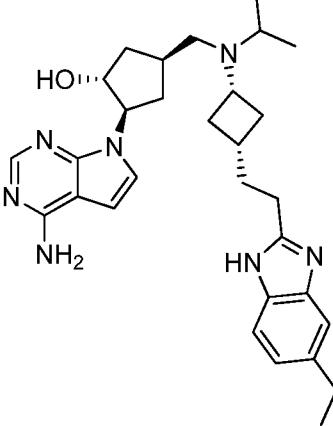
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A80		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methyltetrahydrofuran-3,4-diol	603.3 (M+H) ₊
A81		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1r,3R)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methylcyclopentanol	562.3 (M+H) ₊
A82		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methyltetrahydrofuran-3,4-diol	603.3 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A83		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-((3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methylcyclopentanol	544.5 (M+H) ⁺
A84		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-((3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methylcyclopentanol	590.3 (M+H) ⁺
A85		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1s,3S)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methylcyclopentanol	562.3 (M+H) ⁺
A86		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	534.3 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A87		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrofuran-3,4-diol	590.3 (M+H) ₊
A88		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)ethyl)amino)methyl)tetrahydrofuran-3,4-diol	548.3 (M+H) ₊
A89		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)ethyl)amino)methyl)tetrahydrofuran-3,4-diol	548.3 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A90		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	562.5 (M+H) ⁺
A91		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	591.2 (M+H) ⁺
A92		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	534.3 (M+H) ⁺
A93		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	591.3 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A94		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	562.2 (M+H) ⁺
A95		(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((methyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	562.3 (M+H) ⁺
A96		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1r,3S)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	563.3 (M+H) ⁺
A97		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((methyl((1s,3R)-3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	563.3 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A98		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	521.3 (M+H) ⁺
A99		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	521.3 (M+H) ⁺
1A00		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl(3-(2-(5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	591.3 (M+H) ⁺
A101		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1r,3R)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyl)cyclopentanol	544.1 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A102		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(2,2,2-trifluoroethyl)amino)methyltetrahydrofuran-3,4-diol	603.3 (M+H) ₊
A103		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1s,3S)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methylcyclopentanol	589.9 (M+H) ₊
A104		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1s,3S)-3-(2-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methylcyclopentanol	544.1 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A105		(1R,2R,4S)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-(((1r,3R)-3-(2-(5-chloro-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methylcyclopentanol	589.9 (M+H) ₊
A106		(1r,3S)-N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)-N-isopropylcyclobutanamine oxide	579.4 (M+H) ₊
A107		(R,1s,3R)-N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-3-(2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)ethyl)-N-isopropylcyclobutanamine oxide	579.4 (M+H) ₊

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A108		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-(1-hydroxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	579.4 (M+H) ⁺
A109		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-(1-hydroxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	579.4 (M+H) ⁺
A110		1-((3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(methyl)amino)cyclobutyl)methyl-3-(4-(tert-butyl)phenyl)urea	539.3 (M+H) ⁺

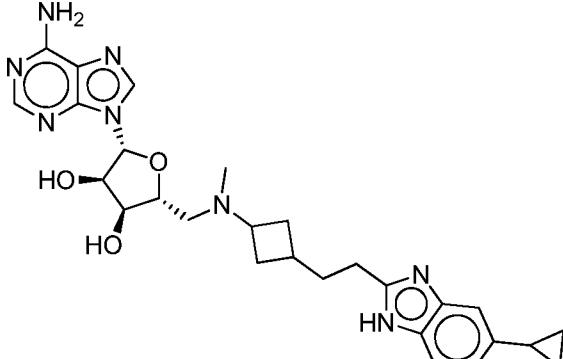
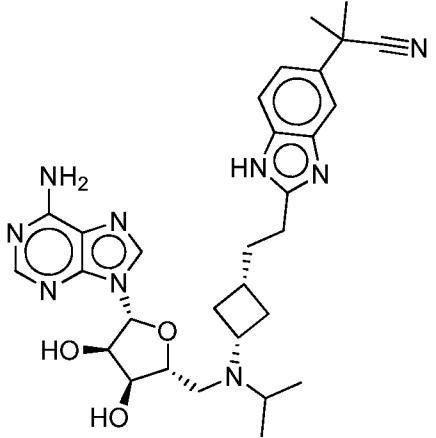
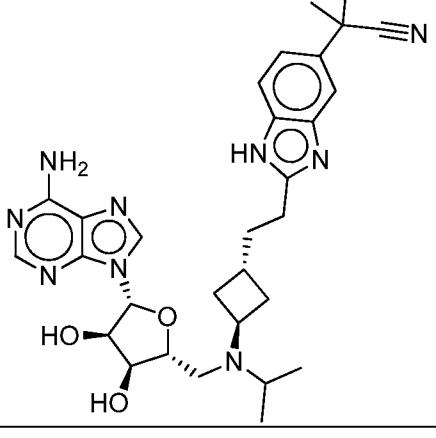
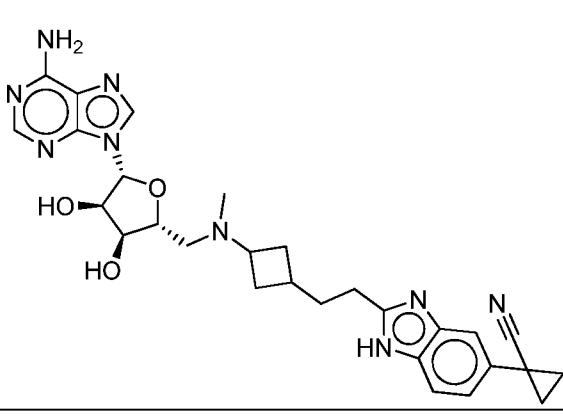
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A111		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	561 (M+H) ⁺
A112		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	547 (M+H) ⁺
A113		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl(3-(2-(5-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyltetrahydrofuran-3,4-diol	589 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A114		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	561 (M+H) ⁺
A115		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	561 (M+H) ⁺
A116		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrofuran-3,4-diol	547 (M+H) ⁺

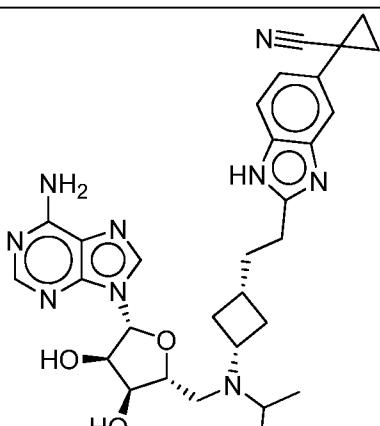
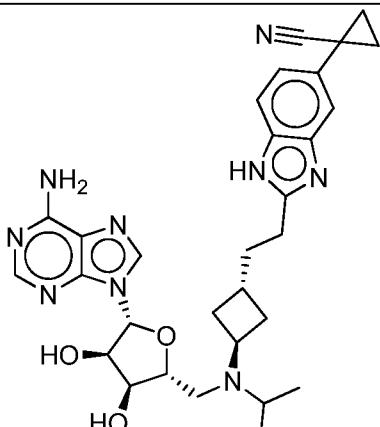
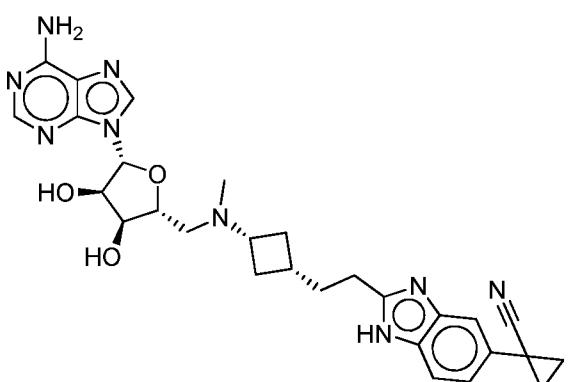
Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A117		1-(2-(2-(3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclobutanecarbonitrile	586 (M+H) ⁺
A118		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl(3-(2-(5-(1-methoxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	593 (M+H) ⁺
A119		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(isopropyl)amino)methyltetrahydrafuran-3,4-diol	547 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A120		2-(2-(2-(3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)-2-methylpropanenitrile	574 (M+H) ⁺
A121		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(1-methoxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	593 (M+H) ⁺
A122		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(1-methoxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	593 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A123		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1s,3R)-3-(2-(5-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	589 (M+H) ⁺
A124		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl((1r,3S)-3-(2-(5-(2,2,2-trifluoroethyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahyd rofuran-3,4-diol	589 (M+H) ⁺
A125		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)tetrahyd rofuran-3,4-diol	533 (M+H) ⁺
A126		1-(2-(2-(3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahyd rofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-ylcyclopropanecarbonitrile	572 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A127		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)tetrahydrofuran-3,4-diol	519 (M+H) ⁺
A128		2-(2-(2-((1S,3r)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)-2-methylpropanenitrile	574 (M+H) ⁺
A129		2-(2-(2-((1R,3s)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)-2-methylpropanenitrile	574 (M+H) ⁺
A130		1-(2-(2-(3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(methyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclopropanecarbonitrile	544 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A131		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	533 (M+H) ⁺
A132		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-cyclobutyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	533 (M+H) ⁺
A133		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1r,3S)-3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	519 (M+H) ⁺
A134		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((1s,3R)-3-(2-(5-cyclopropyl-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyltetrahydrofuran-3,4-diol	519 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A135		1-(2-(2-((1S,3r)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclopropanecarbonitrile	572 (M+H) ⁺
A136		1-(2-(2-((1R,3s)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclopropanecarbonitrile	572 (M+H) ⁺
A137		1-(2-(2-((1S,3r)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(methyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclopropanecarbonitrile	544 (M+H) ⁺

Cmpd No.	Structure	Chemical Name	Data (MS or NMR)
A138		1-(2-(2-((1R,3S)-3-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(methyl)amino)cyclobutyl)ethyl)-1H-benzo[d]imidazol-5-yl)cyclopropanecarbonitrile	544 (M+H) ⁺
A139		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-((isopropyl(3-(2-(5-(1-methylcyclopropyl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)amino)methyl)tetrahydrafuran-3,4-diol	561 (M+H) ⁺
A140		(2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((3-(2-(5-(1-methoxy-2-methylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)ethyl)cyclobutyl)(methyl)amino)methyl)tetrahydrafuran-3,4-diol	565 (M+H) ⁺

Table 2

Cmpd. No.	Structures	Chemical Name
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B1		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-amino-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-((4-(5- <i>tert</i> -butyl-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)butyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol
B2		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>S</i>)-3-(4-amino-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-((4-(5- <i>tert</i> -butyl-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)butyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol
B3		1-(3-(((1 <i>S</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(6-amino-9 <i>H</i> -purin-9-yl)-2,3-dihydroxycyclopentyl)methyl)(methyamino)propyl)-3-(4-(<i>tert</i> -butyl)phenyl)urea
B4		(1 <i>S</i> ,2 <i>R</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(((4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)butyl)(isopropyl)amino)methyl)-5-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)cyclopentane-1,2-diol
B5		(1 <i>S</i> ,2 <i>R</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(((4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)butyl)(methyl)amino)methyl)-5-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)cyclopentane-1,2-diol
B6		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-((isopropyl(4-(6-(trifluoromethyl)-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)butyl)amino)methyl)cyclopentane-1,2-diol

B7		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-((methyl(4-(6-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)amino)methyl)cyclopentane-1,2-diol
B8		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-(((4-(5,6-dichloro-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)(methyl)amino)methyl)cyclopentane-1,2-diol
B9		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-(((4-(5,6-dichloro-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)(isopropyl)amino)methyl)cyclopentane-1,2-diol trihydrochloride
B10		1-((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(6-amino-9 <i>H</i> -purin-9-yl)-2,3-dihydroxycyclopentyl)methyl)(methyl)amino)propyl-3-(4-(<i>tert</i> -butyl)phenyl)urea
B11		N-(4-(5-(tert-butyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)-N-(((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-2,3-dihydroxycyclopentyl)methyl)methanesulfonamide
B12		N-(4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)-N-(((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-2,3-dihydroxycyclopentyl)methyl)methanesulfonamide

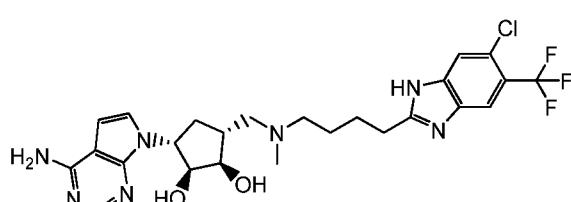
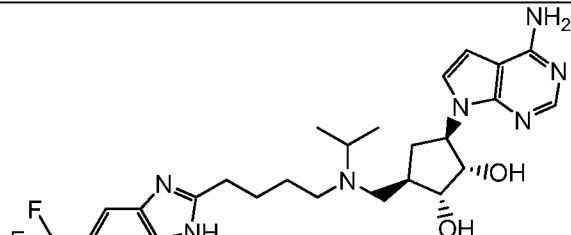
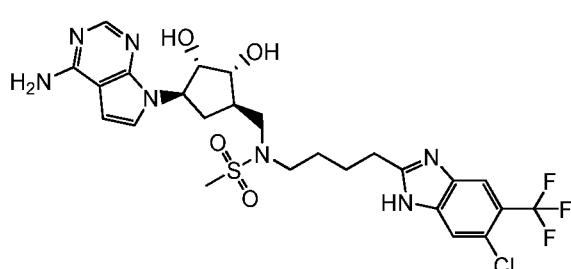
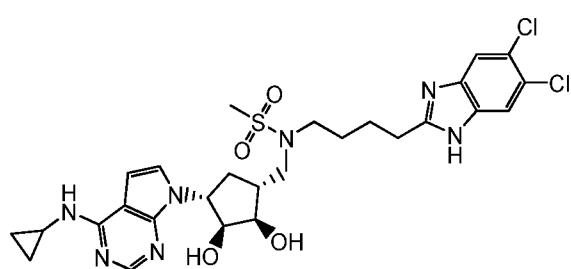
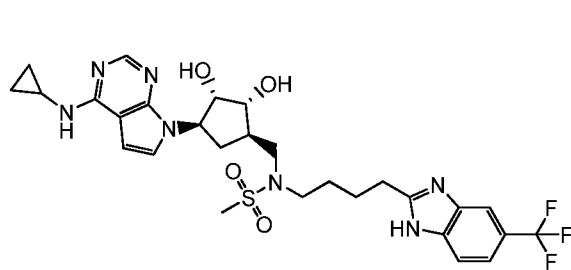
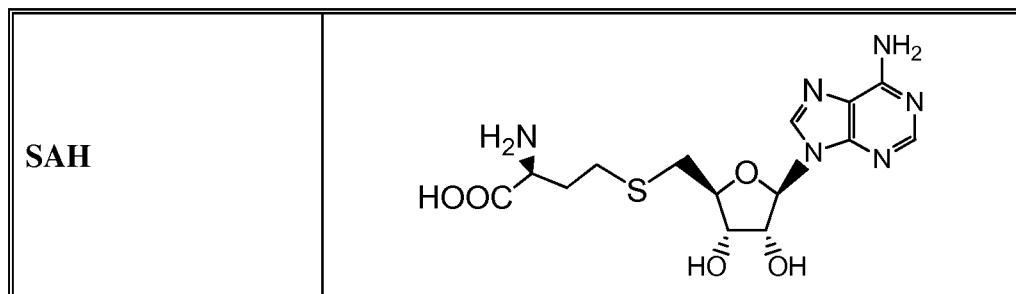
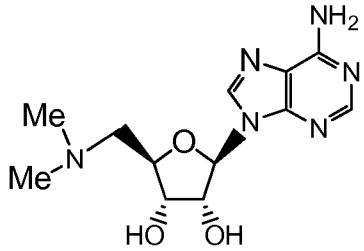
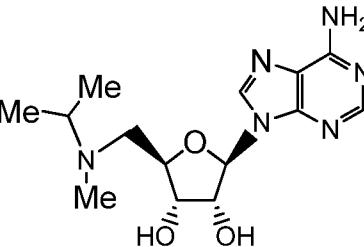
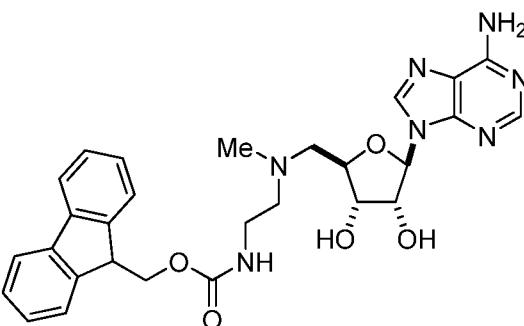
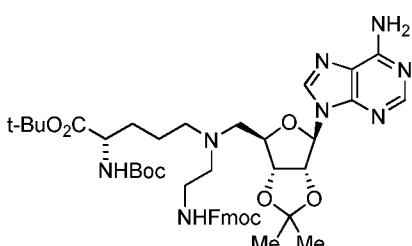
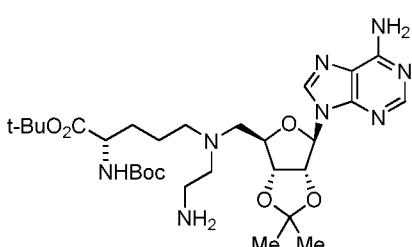
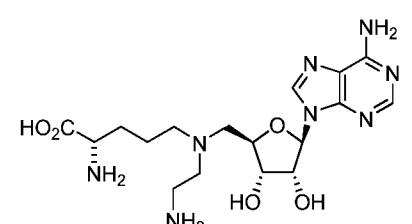
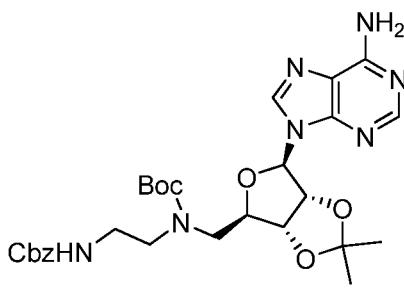
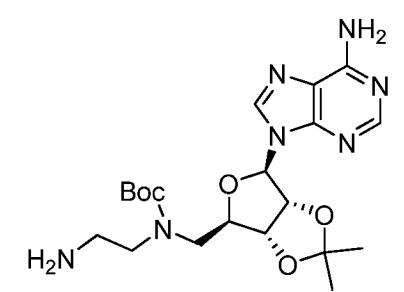
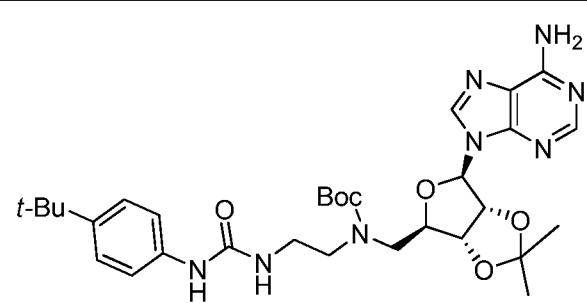
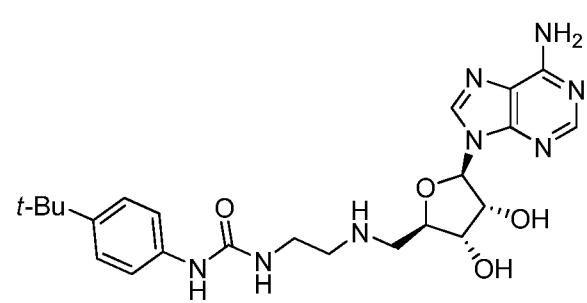
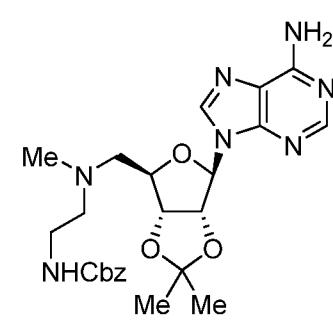
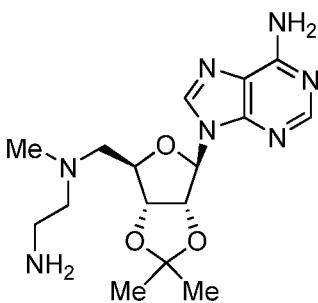
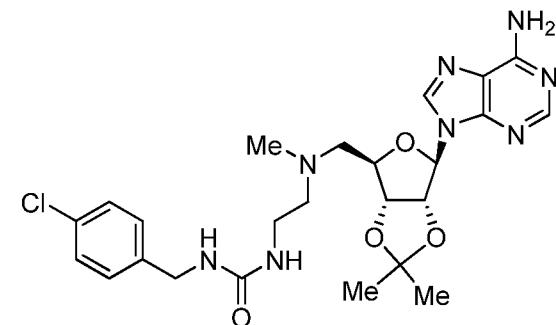
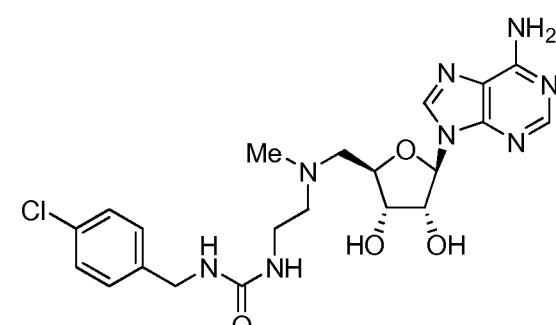
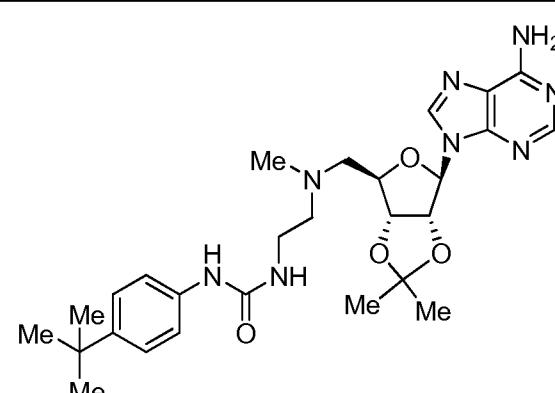
B13		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-amino-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-(((4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)(methyl)amino)methyl)cyclpentane-1,2-diol
B14		(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i> ,5 <i>R</i>)-3-(4-amino-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-5-(((4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)(isopropyl)amino)methyl)cyclpentane-1,2-diol
B15		N-((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(4-amino-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-2,3-dihydroxycyclopentyl)methyl)-N-(4-(6-chloro-5-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)methanesulfonamide
B16		N-((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-2,3-dihydroxycyclopentyl)methyl)-N-(4-(5,6-dichloro-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)methanesulfonamide
B17		N-((1 <i>R</i> ,2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4-(4-(cyclopropylamino)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-7-yl)-2,3-dihydroxycyclopentyl)methyl)-N-(4-(5-(trifluoromethyl)-1 <i>H</i> -benzo[d]imidazol-2-yl)butyl)methanesulfonamide

Table 3



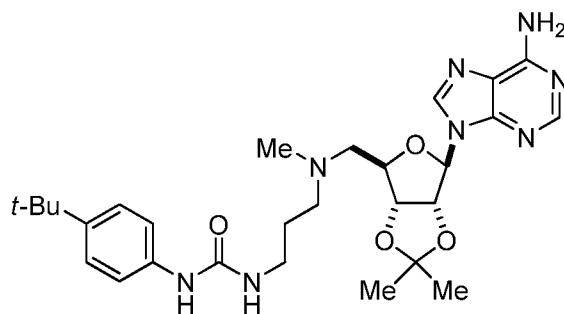
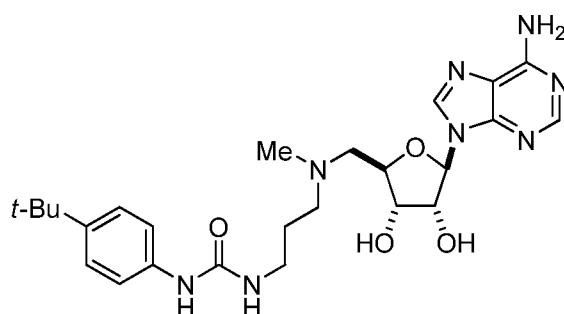
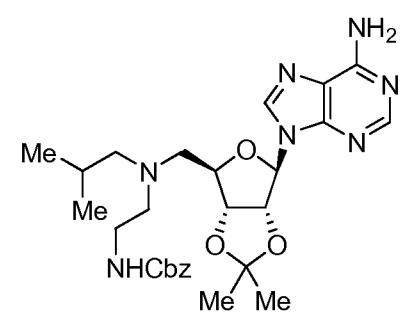
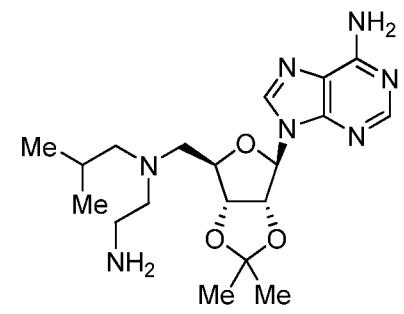
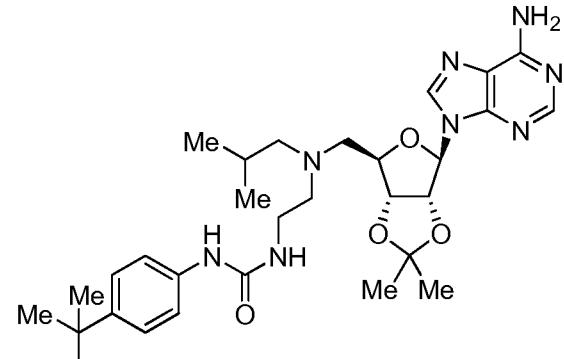
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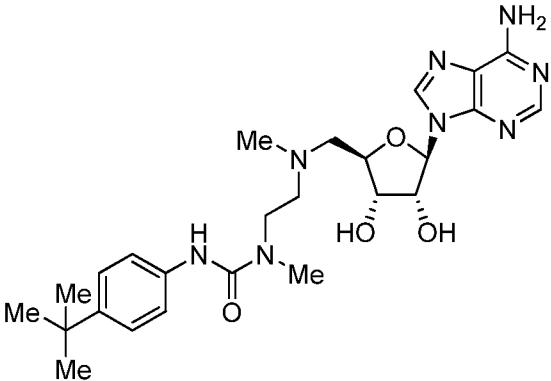
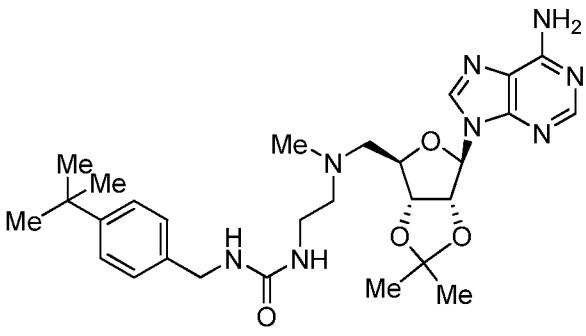
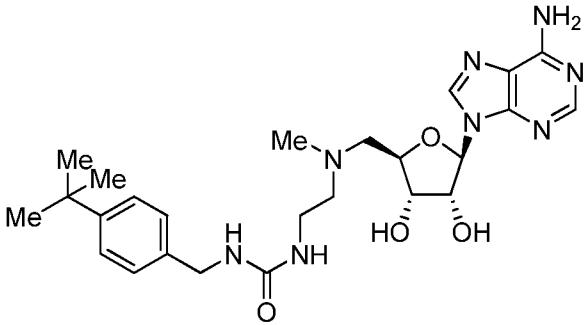
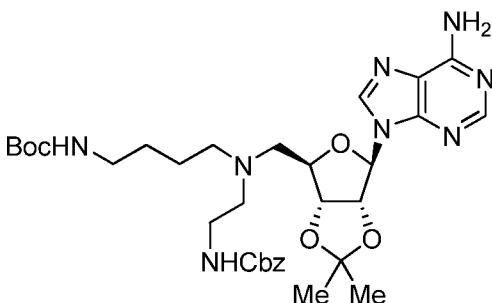
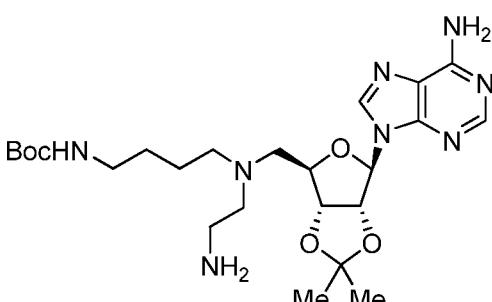
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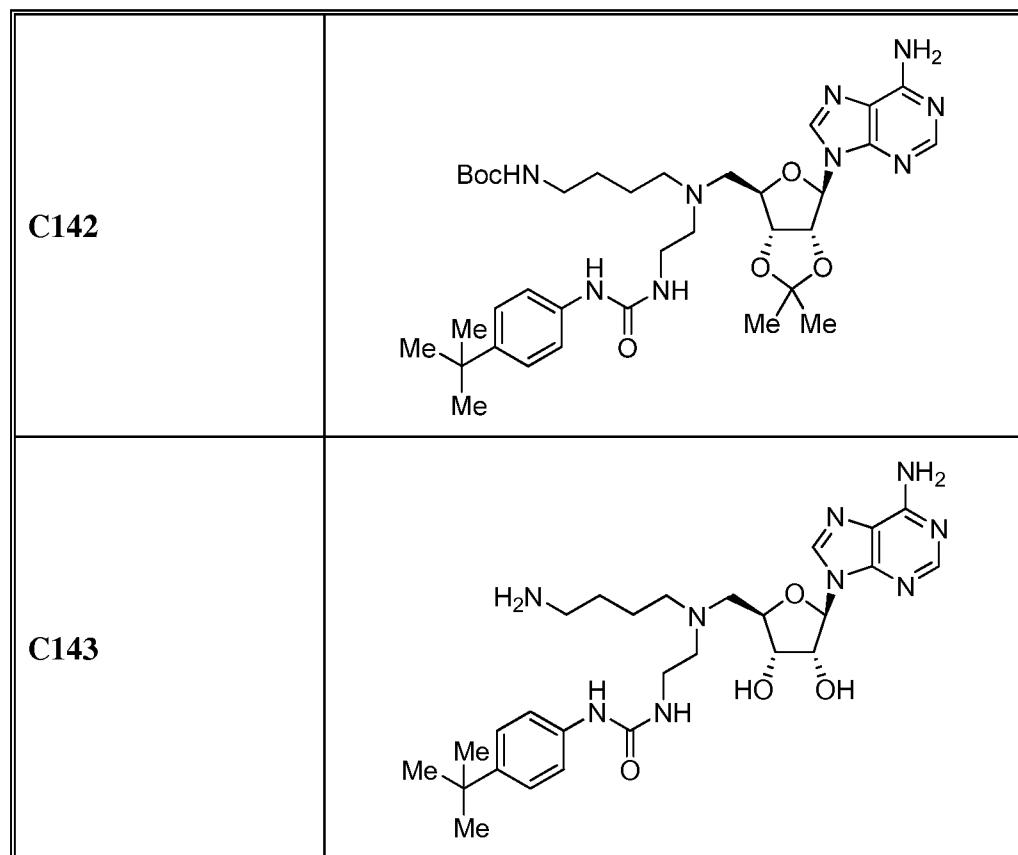
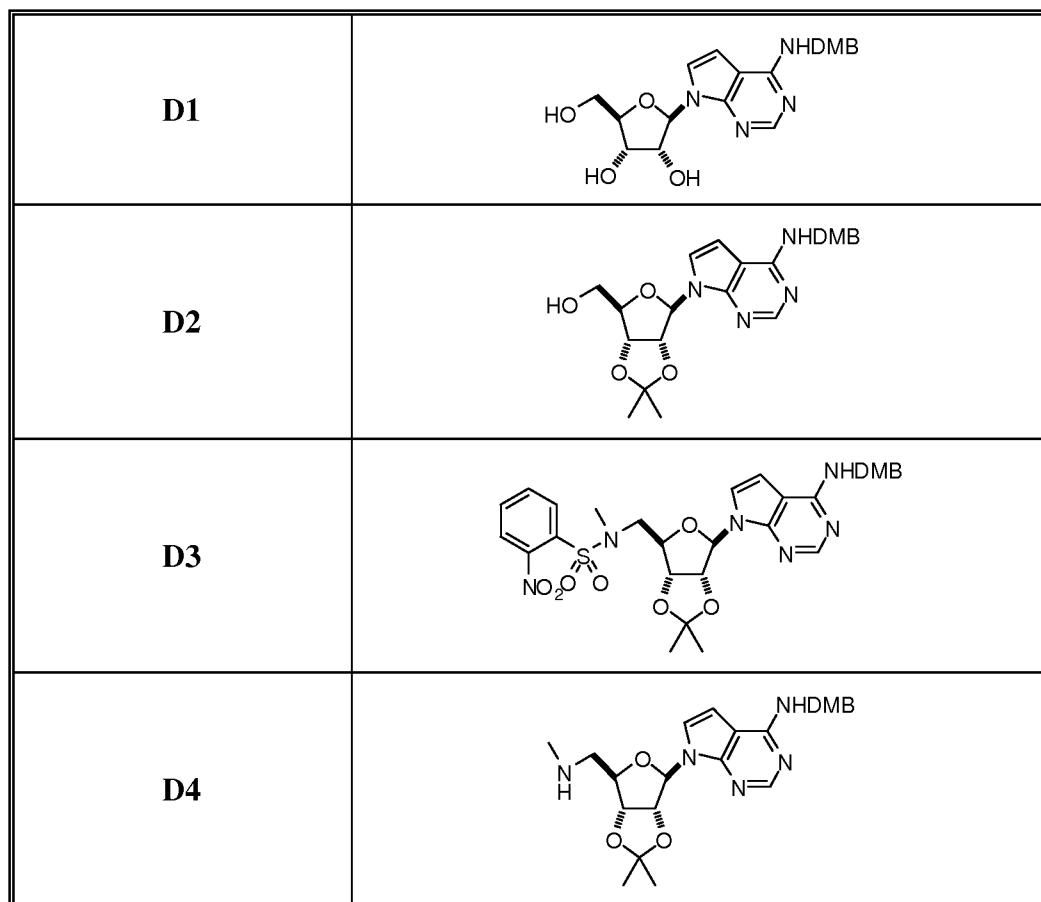
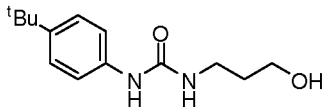
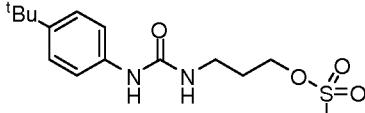
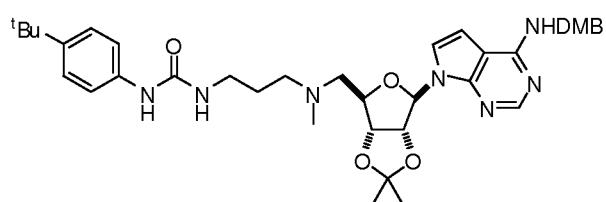
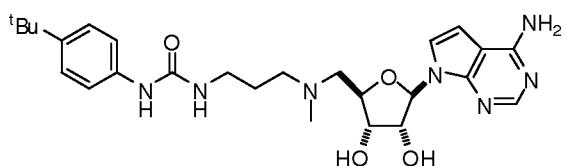
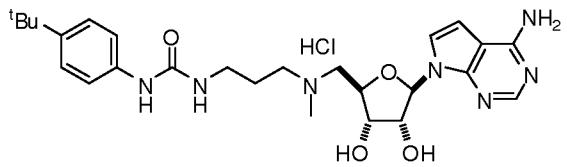
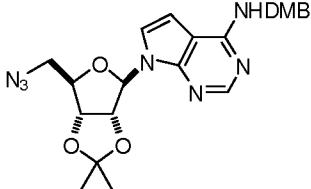
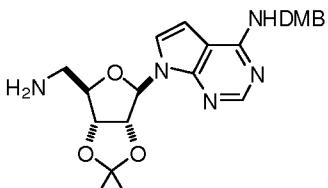
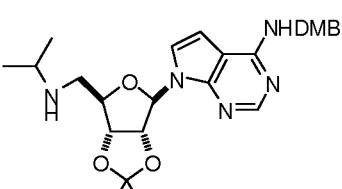
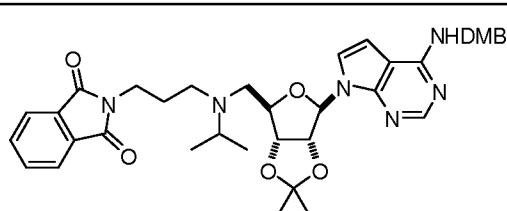
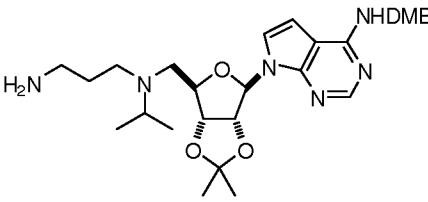
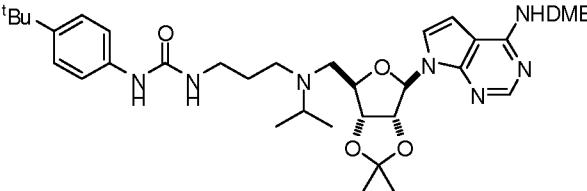
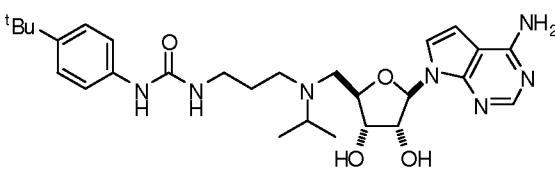
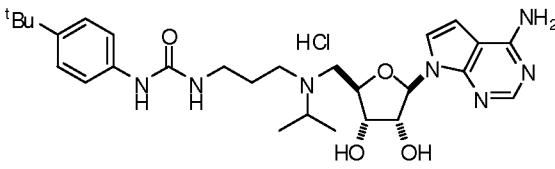


Table 4



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[0287] As used herein, "alkyl", "C₁, C₂, C₃, C₄, C₅ or C₆ alkyl" or "C₁-C₆ alkyl" is intended to include C₁, C₂, C₃, C₄, C₅ or C₆ straight chain (linear) saturated aliphatic hydrocarbon groups and C₃, C₄, C₅ or C₆ branched saturated aliphatic hydrocarbon groups. For example, C₁-C₆ alkyl is intended to include C₁, C₂, C₃, C₄, C₅ and C₆ alkyl groups. Examples of alkyl include, moieties having from one to six carbon atoms, such as, but not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, s-pentyl or n-hexyl.

[0288] In certain embodiments, a straight chain or branched alkyl has six or fewer carbon atoms (e.g., C₁-C₆ for straight chain, C₃-C₆ for branched chain), and in another embodiment, a straight chain or branched alkyl has four or fewer carbon atoms.

[0289] As used herein, the term "cycloalkyl" refers to a saturated or unsaturated nonaromatic hydrocarbon mono-or multi-ring system having 3 to 30 carbon atoms (e.g., C₃-C₁₀). Examples of cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, and adamantyl. The term "heterocycloalkyl" refers to a saturated or unsaturated nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having one or more heteroatoms (such as O, N, S, or Se). Examples of heterocycloalkyl

groups include, but are not limited to, piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, and tetrahydrofuranyl.

[0290] The term “optionally substituted alkyl” refers to unsubstituted alkyl or alkyl having designated substituents replacing one or more hydrogen atoms on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

[0291] An “arylalkyl” or an “aralkyl” moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)). An “alkylaryl” moiety is an aryl substituted with an alkyl (e.g., methylphenyl).

[0292] As used herein, “alkyl linker” is intended to include C₁, C₂, C₃, C₄, C₅ or C₆ straight chain (linear) saturated divalent aliphatic hydrocarbon groups and C₃, C₄, C₅ or C₆ branched saturated aliphatic hydrocarbon groups. For example, C₁-C₆ alkyl linker is intended to include C₁, C₂, C₃, C₄, C₅ and C₆ alkyl linker groups. Examples of alkyl linker include, moieties having from one to six carbon atoms, such as, but not limited to, methyl (-CH₂-), ethyl (-CH₂CH₂-), n-propyl (-CH₂CH₂CH₂-), i-propyl (-CHCH₃CH₂-), n-butyl (-CH₂CH₂CH₂CH₂-), s-butyl (-CHCH₃CH₂CH₂-), i-butyl (-C(CH₃)₂CH₂-), n-pentyl (-CH₂CH₂CH₂CH₂CH₂-), s-pentyl (-CHCH₃CH₂CH₂CH₂-) or n-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₂-).

[0293] “Alkenyl” includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double bond. For example, the term “alkenyl” includes straight chain alkenyl groups (e.g., ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl), and branched alkenyl groups. In certain embodiments, a straight chain or branched alkenyl group has six or fewer carbon atoms in its backbone (e.g., C₂-C₆ for straight chain, C₃-C₆ for branched chain). The term “C₂-C₆” includes alkenyl groups containing two to six carbon atoms. The term “C₃-C₆” includes alkenyl groups containing three to six carbon atoms.

[0294] The term “optionally substituted alkenyl” refers to unsubstituted alkenyl or alkenyl having designated substituents replacing one or more hydrogen atoms on one or more hydrocarbon backbone carbon atoms. Such substituents can include, for example, alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

[0295] “Alkynyl” includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one triple bond. For example, “alkynyl” includes straight chain alkynyl groups (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl), and branched alkynyl groups. In certain embodiments, a straight chain or branched alkynyl group has six or fewer carbon atoms in its backbone (*e.g.*, C₂-C₆ for straight chain, C₃-C₆ for branched chain). The term “C₂-C₆” includes alkynyl groups containing two to six carbon atoms. The term “C₃-C₆” includes alkynyl groups containing three to six carbon atoms.

[0296] The term “optionally substituted alkynyl” refers to unsubstituted alkynyl or alkynyl having designated substituents replacing one or more hydrogen atoms on one or more hydrocarbon backbone carbon atoms. Such substituents can include, for example, alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

[0297] Other optionally substituted moieties (such as optionally substituted cycloalkyl, heterocycloalkyl, aryl, or heteroaryl) include both the unsubstituted moieties and the moieties having one or more of the designated substituents.

[0298] “Aryl” includes groups with aromaticity, including “conjugated,” or multicyclic systems with at least one aromatic ring and do not contain any heteroatom in the ring structure. Examples include phenyl, benzyl, 1,2,3,4-tetrahydronaphthalenyl, etc.

[0299] “Heteroaryl” groups are aryl groups, as defined above, except having from one to four heteroatoms in the ring structure, and may also be referred to as “aryl heterocycles” or “heteroaromatics.” As used herein, the term “heteroaryl” is intended to include a stable 5- or 6-membered monocyclic or 7-, 8-, 9-, 10-, 11- or 12-membered bicyclic aromatic heterocyclic ring which consists of carbon atoms and one or more heteroatoms, *e.g.*, 1 or 1-2 or 1-3 or 1-4 or 1-5 or 1-6 heteroatoms, or *e.g.*, 1, 2, 3, 4, 5, or 6 heteroatoms, independently selected from the group consisting of nitrogen, oxygen and sulfur. The nitrogen atom may be substituted or unsubstituted (*i.e.*, N or NR wherein R is H or other substituents, as defined). The nitrogen and sulfur heteroatoms may optionally be oxidized (*i.e.*, N \rightarrow O and S(O)_p, where p = 1 or 2). It is to be noted that total number of S and O atoms in the aromatic heterocycle is not more than 1.

[0300] Examples of heteroaryl groups include pyrrole, furan, thiophene, thiazole, isothiazole, imidazole, triazole, tetrazole, pyrazole, oxazole, isoxazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like.

[0301] Furthermore, the terms “aryl” and “heteroaryl” include multicyclic aryl and heteroaryl groups, *e.g.*, tricyclic, bicyclic, *e.g.*, naphthalene, benzoxazole, benzodioxazole, benzothiazole, benzoimidazole, benzothiophene, methylenedioxyphenyl, quinoline, isoquinoline, naphthrydine, indole, benzofuran, purine, benzofuran, deazapurine, indolizine.

[0302] In the case of multicyclic aromatic rings, only one of the rings needs to be aromatic (*e.g.*, 2,3-dihydroindole), although all of the rings may be aromatic (*e.g.*, quinoline). The second ring can also be fused or bridged.

[0303] The aryl or heteroaryl aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkylaminocarbonyl, aralkylaminocarbonyl, alkenylaminocarbonyl, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, alkenylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl,

alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings, which are not aromatic so as to form a multicyclic system (e.g., tetralin, methylenedioxyphenyl).

[0304] As used herein, “carbocycle” or “carbocyclic ring” is intended to include any stable monocyclic, bicyclic or tricyclic ring having the specified number of carbons, any of which may be saturated, unsaturated, or aromatic. For example, a C₃-C₁₄ carbocycle is intended to include a monocyclic, bicyclic or tricyclic ring having 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 carbon atoms. Examples of carbocycles include, but are not limited to, cyclopropyl, cyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclohexyl, cycloheptenyl, cycloheptyl, cycloheptenyl, adamanyl, cyclooctyl, cyclooctenyl, cyclooctadienyl, fluorenyl, phenyl, naphthyl, indanyl, adamanyl and tetrahydronaphthyl. Bridged rings are also included in the definition of carbocycle, including, for example, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane and [2.2.2]bicyclooctane. A bridged ring occurs when one or more carbon atoms link two non-adjacent carbon atoms. In one embodiment, bridge rings are one or two carbon atoms. It is noted that a bridge always converts a monocyclic ring into a tricyclic ring. When a ring is bridged, the substituents recited for the ring may also be present on the bridge. Fused (e.g., naphthyl, tetrahydronaphthyl) and spiro rings are also included.

[0305] As used herein, “heterocycle” includes any ring structure (saturated or partially unsaturated) which contains at least one ring heteroatom (e.g., N, O or S). Examples of heterocycles include, but are not limited to, morpholine, pyrrolidine, tetrahydrothiophene, piperidine, piperazine and tetrahydrofuran.

[0306] Examples of heterocyclic groups include, but are not limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-*b*]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,4-oxadiazol5(4H)-one, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl,

phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolanyl, tetrahydroquinolanyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl and xanthenyl.

[0307] The term “substituted,” as used herein, means that any one or more hydrogen atoms on the designated atom is replaced with a selection from the indicated groups, provided that the designated atom’s normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is oxo or keto (*i.e.*, =O), then 2 hydrogen atoms on the atom are replaced. Keto substituents are not present on aromatic moieties. Ring double bonds, as used herein, are double bonds that are formed between two adjacent ring atoms (*e.g.*, C=C, C=N or N=N). “Stable compound” and “stable structure” are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

[0308] When a bond to a substituent is shown to cross a bond connecting two atoms in a ring, then such substituent may be bonded to any atom in the ring. When a substituent is listed without indicating the atom via which such substituent is bonded to the rest of the compound of a given formula, then such substituent may be bonded via any atom in such formula. Combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0309] When any variable (*e.g.*, R₃) 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 0-2 R₃ moieties, then the group may optionally be substituted with up to two R₃ moieties and R₃ at each occurrence is selected independently from the definition of R₃. Also, combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0310] The term “hydroxy” or “hydroxyl” includes groups with an -OH or -O⁻.

[0311] As used herein, “halo” or “halogen” refers to fluoro, chloro, bromo and iodo. The term “perhalogenated” generally refers to a moiety wherein all hydrogen atoms are replaced

by halogen atoms. The term “haloalkyl” or “haloalkoxyl” refers to an alkyl or alkoxy substituted with one or more halogen atoms.

[0312] The term “carbonyl” includes compounds and moieties which contain a carbon connected with a double bond to an oxygen atom. Examples of moieties containing a carbonyl include, but are not limited to, aldehydes, ketones, carboxylic acids, amides, esters, anhydrides, etc.

[0313] The term “carboxyl” refers to –COOH or its C₁-C₆ alkyl ester.

[0314] “Acyl” includes moieties that contain the acyl radical (R-C(O)-) or a carbonyl group. “Substituted acyl” includes acyl groups where one or more of the hydrogen atoms are replaced by, for example, alkyl groups, alkynyl groups, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulphydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

[0315] “Aroyl” includes moieties with an aryl or heteroaromatic moiety bound to a carbonyl group. Examples of aroyl groups include phenylcarboxy, naphthyl carboxy, etc.

[0316] “Alkoxyalkyl,” “alkylaminoalkyl,” and “thioalkoxyalkyl” include alkyl groups, as described above, wherein oxygen, nitrogen, or sulfur atoms replace one or more hydrocarbon backbone carbon atoms.

[0317] The term “alkoxy” or “alkoxyl” includes substituted and unsubstituted alkyl, alkenyl and alkynyl groups covalently linked to an oxygen atom. Examples of alkoxy groups or alkoxy radicals include, but are not limited to, methoxy, ethoxy, isopropoxy, propoxy, butoxy and pentoxy groups. Examples of substituted alkoxy groups include halogenated alkoxy groups. The alkoxy groups can be substituted with groups such as alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulphydryl, alkylthio, arylthio, or an aromatic or heteroaromatic moiety.

thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moieties. Examples of halogen substituted alkoxy groups include, but are not limited to, fluoromethoxy, difluoromethoxy, trifluoromethoxy, chloromethoxy, dichloromethoxy and trichloromethoxy.

[0318] The term “ether” or “alkoxy” includes compounds or moieties which contain an oxygen bonded to two carbon atoms or heteroatoms. For example, the term includes “alkoxyalkyl,” which refers to an alkyl, alkenyl, or alkynyl group covalently bonded to an oxygen atom which is covalently bonded to an alkyl group.

[0319] The term “ester” includes compounds or moieties which contain a carbon or a heteroatom bound to an oxygen atom which is bonded to the carbon of a carbonyl group. The term “ester” includes alkoxy carboxy groups such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, pentoxy carbonyl, etc.

[0320] The term “thioalkyl” includes compounds or moieties which contain an alkyl group connected with a sulfur atom. The thioalkyl groups can be substituted with groups such as alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, carboxyacid, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moieties.

[0321] The term “thiocarbonyl” or “thiocarboxy” includes compounds and moieties which contain a carbon connected with a double bond to a sulfur atom.

[0322] The term “thioether” includes moieties which contain a sulfur atom bonded to two carbon atoms or heteroatoms. Examples of thioethers include, but are not limited to alkthioalkyls, alkthioalkenyls, and alkthioalkynyls. The term “alkthioalkyls” include moieties with an alkyl, alkenyl, or alkynyl group bonded to a sulfur atom which is bonded to an alkyl group. Similarly, the term “alkthioalkenyls” refers to moieties wherein an alkyl, alkenyl or alkynyl group is bonded to a sulfur atom which is covalently bonded to an alkenyl group; and alkthioalkynyls” refers to moieties wherein an alkyl, alkenyl or alkynyl group is bonded to a sulfur atom which is covalently bonded to an alkynyl group.

[0323] As used herein, “amine” or “amino” refers to unsubstituted or substituted -NH₂. “Alkylamino” includes groups of compounds wherein nitrogen of -NH₂ is bound to at least one alkyl group. Examples of alkylamino groups include benzylamino, methylamino, ethylamino, phenethylamino, etc. “Dialkylamino” includes groups wherein the nitrogen of -NH₂ is bound to at least two additional alkyl groups. Examples of dialkylamino groups include, but are not limited to, dimethylamino and diethylamino. “Arylamino” and “diarylamino” include groups wherein the nitrogen is bound to at least one or two aryl groups, respectively. “Aminoaryl” and “aminoaryloxy” refer to aryl and aryloxy substituted with amino. “Alkylarylamino,” “alkylaminoaryl” or “arylaminoalkyl” refers to an amino group which is bound to at least one alkyl group and at least one aryl group. “Alkaminoalkyl” refers to an alkyl, alkenyl, or alkynyl group bound to a nitrogen atom which is also bound to an alkyl group. “Acylamino” includes groups wherein nitrogen is bound to an acyl group. Examples of acylamino include, but are not limited to, alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido groups.

[0324] The term “amide” or “aminocarboxy” includes compounds or moieties that contain a nitrogen atom that is bound to the carbon of a carbonyl or a thiocarbonyl group. The term includes “alkaminocarboxy” groups that include alkyl, alkenyl or alkynyl groups bound to an amino group which is bound to the carbon of a carbonyl or thiocarbonyl group. It also includes “arylaminocarboxy” groups that include aryl or heteroaryl moieties bound to an amino group that is bound to the carbon of a carbonyl or thiocarbonyl group. The terms “alkylaminocarboxy”, “alkenylaminocarboxy”, “alkynylaminocarboxy” and “arylaminocarboxy” include moieties wherein alkyl, alkenyl, alkynyl and aryl moieties, respectively, are bound to a nitrogen atom which is in turn bound to the carbon of a carbonyl group. Amides can be substituted with substituents such as straight chain alkyl, branched alkyl, cycloalkyl, aryl, heteroaryl or heterocycle. Substituents on amide groups may be further substituted.

[0325] Compounds of the present invention that contain nitrogens can be converted to N-oxides by treatment with an oxidizing agent (*e.g.*, 3-chloroperoxybenzoic acid (*m*CPBA) and/or hydrogen peroxides) to afford other compounds of the present invention. Thus, all shown and claimed nitrogen-containing compounds are considered, when allowed by valency and structure, to include both the compound as shown and its N-oxide derivative (which can be designated as N→O or N⁺-O⁻). Furthermore, in other instances, the nitrogens in the compounds of the present invention can be converted to N-hydroxy or N-alkoxy compounds. For example, N-hydroxy compounds can be prepared by oxidation of the parent amine by an

oxidizing agent such as *m*-CPBA. All shown and claimed nitrogen-containing compounds are also considered, when allowed by valency and structure, to cover both the compound as shown and its N-hydroxy (*i.e.*, N-OH) and N-alkoxy (*i.e.*, N-OR, wherein R is substituted or unsubstituted C₁-C₆ alkyl, C₁-C₆ alkenyl, C₁-C₆ alkynyl, 3-14-membered carbocycle or 3-14-membered heterocycle) derivatives.

[0326] In the present specification, the structural formula of the compound represents a certain isomer for convenience in some cases, but the present invention includes all isomers, such as geometrical isomers, optical isomers based on an asymmetrical carbon, stereoisomers, tautomers, and the like. In addition, a crystal polymorphism may be present for the compounds represented by the formula. It is noted that any crystal form, crystal form mixture, or anhydride or hydrate thereof is included in the scope of the present invention. Furthermore, so-called metabolite which is produced by degradation of the present compound *in vivo* is included in the scope of the present invention.

[0327] “Isomerism” means compounds that have identical molecular formulae but differ in the sequence of bonding of their atoms or in the arrangement of their atoms in space. Isomers that differ in the arrangement of their atoms in space are termed “stereoisomers.”

Stereoisomers that are not mirror images of one another are termed “diastereoisomers,” and stereoisomers that are non-superimposable mirror images of each other are termed “enantiomers” or sometimes optical isomers. A mixture containing equal amounts of individual enantiomeric forms of opposite chirality is termed a “racemic mixture.”

[0328] A carbon atom bonded to four nonidentical substituents is termed a “chiral center.”

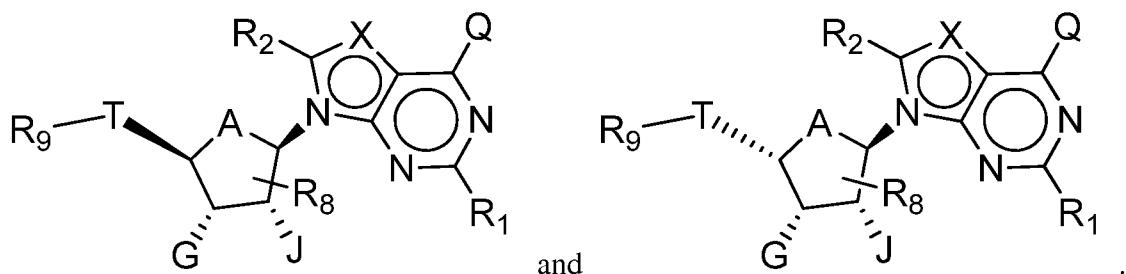
[0329] “Chiral isomer” means a compound with at least one chiral center. Compounds with more than one chiral center may exist either as an individual diastereomer or as a mixture of diastereomers, termed “diastereomeric mixture.” When one chiral center is present, a stereoisomer may be characterized by the absolute configuration (R or S) of that chiral center. Absolute configuration refers to the arrangement in space of the substituents attached to the chiral center. The substituents attached to the chiral center under consideration are ranked in accordance with the *Sequence Rule* of Cahn, Ingold and Prelog. (Cahn *et al.*, *Angew. Chem. Inter. Edit.* 1966, 5, 385; errata 511; Cahn *et al.*, *Angew. Chem.* 1966, 78, 413; Cahn and Ingold, *J. Chem. Soc.* 1951 (London), 612; Cahn *et al.*, *Experientia* 1956, 12, 81; Cahn, *J. Chem. Educ.* 1964, 41, 116).

[0330] “Geometric isomer” means the diastereomers that owe their existence to hindered rotation about double bonds or a cycloalkyl linker (e.g., 1,3-cyclobutyl). These configurations are differentiated in their names by the prefixes cis and trans, or Z and E,

which indicate that the groups are on the same or opposite side of the double bond in the molecule according to the Cahn-Ingold-Prelog rules.

[0331] It is to be understood that the compounds of the present invention may be depicted as different chiral isomers or geometric isomers. It should also be understood that when compounds have chiral isomeric or geometric isomeric forms, all isomeric forms are intended to be included in the scope of the present invention, and the naming of the compounds does not exclude any isomeric forms.

[0332] For example, compounds of Formula (I) include those of the following chiral isomers and geometric isomers.

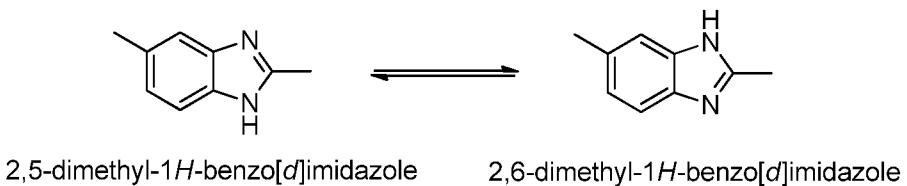


[0333] Furthermore, the structures and other compounds discussed in this invention include all atropic isomers thereof. “Atropic isomers” are a type of stereoisomer in which the atoms of two isomers are arranged differently in space. Atropic isomers owe their existence to a restricted rotation caused by hindrance of rotation of large groups about a central bond. Such atropic isomers typically exist as a mixture, however as a result of recent advances in chromatography techniques, it has been possible to separate mixtures of two atropic isomers in select cases.

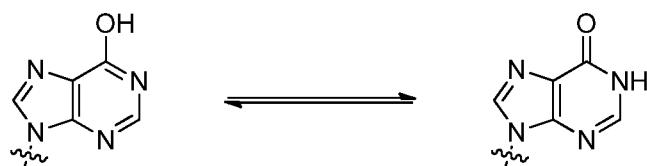
[0334] “Tautomer” is one of two or more structural isomers that exist in equilibrium and is readily converted from one isomeric form to another. This conversion results in the formal migration of a hydrogen atom accompanied by a switch of adjacent conjugated double bonds. Tautomers exist as a mixture of a tautomeric set in solution. In solutions where tautomerization is possible, a chemical equilibrium of the tautomers will be reached. The exact ratio of the tautomers depends on several factors, including temperature, solvent and pH. The concept of tautomers that are interconvertable by tautomerizations is called tautomerism.

[0335] Of the various types of tautomerism that are possible, two are commonly observed. In keto-enol tautomerism a simultaneous shift of electrons and a hydrogen atom occurs. Ring-chain tautomerism arises as a result of the aldehyde group (-CHO) in a sugar chain molecule reacting with one of the hydroxy groups (-OH) in the same molecule to give it a cyclic (ring-shaped) form as exhibited by glucose.

[0336] Common tautomeric pairs are: ketone-enol, amide-nitrile, lactam-lactim, amide-imidic acid tautomerism in heterocyclic rings (e.g., in nucleobases such as guanine, thymine and cytosine), amine-enamine and enamine-enamine. Benzimidazoles also exhibit tautomerism, when the benzimidazole contains one or more substituents in the 4, 5, 6 or 7 positions, the possibility of different isomers arises. For example, 2,5-dimethyl-1H-benzo[d]imidazole can exist in equilibrium with its isomer 2,6-dimethyl-1H-benzo[d]imidazole via tautomerization.



[0337] Another example of tautomerism is shown below.



[0338] It is to be understood that the compounds of the present invention may be depicted as different tautomers. It should also be understood that when compounds have tautomeric forms, all tautomeric forms are intended to be included in the scope of the present invention, and the naming of the compounds does not exclude any tautomer form.

[0339] The term “crystal polymorphs”, “polymorphs” or “crystal forms” means crystal structures in which a compound (or a salt or solvate thereof) can crystallize in different crystal packing arrangements, all of which have the same elemental composition. Different crystal forms usually have different X-ray diffraction patterns, infrared spectral, melting points, density hardness, crystal shape, optical and electrical properties, stability and solubility. Recrystallization solvent, rate of crystallization, storage temperature, and other factors may cause one crystal form to dominate. Crystal polymorphs of the compounds can be prepared by crystallization under different conditions.

[0340] Compounds of the invention may be crystalline, semi-crystalline, non-crystalline, amorphous, mesomorphous, etc.

[0341] The compounds of any of the Formulae disclosed herein include the compounds themselves, as well as their N-oxides, salts, their solvates, and their prodrugs, if applicable. A salt, for example, can be formed between an anion and a positively charged group (e.g., amino) on the compound or inhibitor (e.g., a substituted nucleoside compound such as a substituted purine or 7-deazapurine compound). Suitable anions include chloride, bromide,

iodide, sulfate, bisulfate, sulfamate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, glutamate, glucuronate, glutarate, malate, maleate, succinate, fumarate, tartrate, tosylate, salicylate, lactate, naphthalenesulfonate, and acetate. Likewise, a salt can also be formed between a cation and a negatively charged group (e.g., carboxylate) on the compound or inhibitor (e.g., a substituted nucleoside compound such as a substituted purine or 7-deazapurine compound). Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. The compound or inhibitor (e.g., a substituted nucleoside compound such as a substituted purine or 7-deazapurine compound) also include those salts containing quaternary nitrogen atoms. Examples of prodrugs include esters and other pharmaceutically acceptable derivatives, which, upon administration to a subject, are capable of providing active substituted nucleoside compound such as a substituted purine or 7-deazapurine.

[0342] Additionally, the compounds of the present invention, for example, the salts of the compounds, can exist in either hydrated or unhydrated (the anhydrous) form or as solvates with other solvent molecules. Nonlimiting examples of hydrates include hemihydrates, monohydrates, dihydrates, trihydrates, etc. Nonlimiting examples of solvates include ethanol solvates, acetone solvates, etc.

[0343] “Solvate” means solvent addition forms that contain either stoichiometric or non stoichiometric amounts of solvent. Some compounds have a tendency to trap a fixed molar ratio of solvent molecules in the crystalline solid state, thus forming a solvate. If the solvent is water the solvate formed is a hydrate; and if the solvent is alcohol, the solvate formed is an alcoholate. Hydrates are formed by the combination of one or more molecules of water with one molecule of the substance in which the water retains its molecular state as H_2O . A hemihydrate is formed by the combination of one molecule of water with more than one molecule of the substance in which the water retains its molecular state as H_2O .

[0344] As used herein, the term “analog” refers to a chemical compound that is structurally similar to another but differs slightly in composition (as in the replacement of one atom by an atom of a different element or in the presence of a particular functional group, or the replacement of one functional group by another functional group). Thus, an analog is a compound that is similar or comparable in function and appearance, but not in structure or origin to the reference compound.

[0345] As defined herein, the term “derivative” refers to compounds that have a common core structure, and are substituted with various groups as described herein. For example, all

of the compounds represented by Formula (I) are substituted purine compounds or substituted 7-deazapurine compounds, and have Formula (I) as a common core.

[0346] The term “bioisostere” refers to a compound resulting from the exchange of an atom or of a group of atoms with another, broadly similar, atom or group of atoms. The objective of a bioisosteric replacement is to create a new compound with similar biological properties to the parent compound. The bioisosteric replacement may be physicochemically or topologically based. Examples of carboxylic acid bioisosteres include, but are not limited to, acyl sulfonimides, tetrazoles, sulfonates and phosphonates. See, *e.g.*, Patani and LaVoie, *Chem. Rev.* 96, 3147-3176, 1996.

[0347] The present invention is intended to include all isotopes of atoms occurring in the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium, and isotopes of carbon include C-13 and C-14.

[0348] The present invention also provides methods for the synthesis of the compounds of any of the Formulae disclosed herein. The present invention also provides detailed methods for the synthesis of various disclosed compounds of the present invention according to the schemes and the Examples described in WO2012/075381, WO2012/075492, WO2012/082436, WO2012/75500, and U.S. Provisional Application No. 61/682,090, the contents of which are hereby incorporated by reference in their entireties.

[0349] Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[0350] The synthetic processes of the invention can tolerate a wide variety of functional groups, therefore various substituted starting materials can be used. The processes generally provide the desired final compound at or near the end of the overall process, although it may be desirable in certain instances to further convert the compound to a pharmaceutically acceptable salt, ester, or prodrug thereof.

[0351] Compounds of the present invention can be prepared in a variety of ways using commercially available starting materials, compounds known in the literature, or from readily

prepared intermediates, by employing standard synthetic methods and procedures either known to those skilled in the art, or which will be apparent to the skilled artisan in light of the teachings herein. Standard synthetic methods and procedures for the preparation of organic molecules and functional group transformations and manipulations can be obtained from the relevant scientific literature or from standard textbooks in the field. Although not limited to any one or several sources, classic texts such as Smith, M. B., March, J., *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 5th edition, John Wiley & Sons: New York, 2001; Greene, T.W., Wuts, P.G. M., *Protective Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons: New York, 1999; R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), incorporated by reference herein, are useful and recognized reference textbooks of organic synthesis known to those in the art. The following descriptions of synthetic methods are designed to illustrate, but not to limit, general procedures for the preparation of compounds of the present invention.

[0352] One of ordinary skill in the art will note that, during the reaction sequences and synthetic schemes described herein, the order of certain steps may be changed, such as the introduction and removal of protecting groups.

[0353] One of ordinary skill in the art will recognize that certain groups may require protection from the reaction conditions via the use of protecting groups. Protecting groups may also be used to differentiate similar functional groups in molecules. A list of protecting groups and how to introduce and remove these groups can be found in Greene, T.W., Wuts, P.G. M., *Protective Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons: New York, 1999.

[0354] Preferred protecting groups include, but are not limited to:

[0355] For the hydroxyl moiety: TBS, benzyl, THP, Ac

[0356] For carboxylic acids: benzyl ester, methyl ester, ethyl ester, allyl ester

[0357] For amines: Cbz, BOC, DMB

[0358] For diols: Ac (x2) TBS (x2), or when taken together acetonides

[0359] For thiols: Ac

[0360] For benzimidazoles: SEM, benzyl, PMB, DMB

[0361] For aldehydes: di-alkyl acetals such as dimethoxy acetal or diethyl acetyl.

[0362] In the reaction schemes described herein, multiple stereoisomers may be produced. When no particular stereoisomer is indicated, it is understood to mean all possible stereoisomers that could be produced from the reaction. A person of ordinary skill in the art will recognize that the reactions can be optimized to give one isomer preferentially, or new schemes may be devised to produce a single isomer. If mixtures are produced, techniques such as preparative thin layer chromatography, preparative HPLC, preparative chiral HPLC, or preparative SFC may be used to separate the isomers.

[0363] The following abbreviations are used throughout the specification and are defined below:

[0364] AA	ammonium acetate
[0365] Ac	acetyl
[0366] ACN	acetonitrile
[0367] AcOH	acetic acid
[0368] atm	atmosphere
[0369] Bn	benzyl
[0370] BOC	tert-butoxy carbonyl
[0371] BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
[0372] Cbz	benzyloxycarbonyl
[0373] COMU	(1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate
[0374] d	days
[0375] DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
[0376] DCE	1,2 dichloroethane
[0377] DCM	dichloromethane
[0378] DEA	diethylamine
[0379] DEAD	diethyl azodicarboxylate
[0380] DIAD	diisopropyl azodicarboxylate
[0381] DiBAL-H	diisobutylalumininium hydride
[0382] DIPEA	N,N-diisopropylethylamine (Hunig's base)
[0383] DMAP	N,N-dimethyl-4-aminopyridine
[0384] DMB	2,4 dimethoxybenzyl
[0385] DMF	dimethylformamide
[0386] DMSO	dimethylsulfoxide

[0387] DPPA	diphenylphosphoryl azide
[0388] EA or EtOAc	ethylacetate
[0389] EDC or EDCI	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
[0390] ELS	Evaporative Light Scattering
[0391] ESI-	Electrospray negative mode
[0392] ESI+	Electrospray positive mode
[0393] Et ₂ O	diethyl ether
[0394] Et ₃ N or TEA	triethylamine
[0395] EtOH	ethanol
[0396] FA	formic acid
[0397] FC	flash chromatography
[0398] h	hours
[0399] H ₂ O	water
[0400] HATU	O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
[0401] HCl	hydrochloric acid
[0402] HOAT	1-hydroxy-7-azabenzotriazole
[0403] HOBr	1-hydroxybenzotriazole
[0404] HOSu	N-hydroxysuccinimide
[0405] HPLC	high performance liquid chromatography
[0406] Inj. Vol.	injection volume
[0407] I.V. or IV	intravenous
[0408] KHMDs	potassium hexamethyldisilazide
[0409] LC/MS or LC-MS	liquid chromatography mass spectrum
[0410] LDA	lithium diisopropylamide
[0411] LG	leaving group
[0412] LiHMs	lithium hexamethyldisilazide
[0413] M	Molar
[0414] m/z	mass/charge ratio
[0415] m-CPBA	meta-chloroperbenzoic acid
[0416] MeCN	acetonitrile
[0417] MeOD	d ₄ -methanol
[0418] MeOH	methanol
[0419] MgSO ₄	magnesium sulfate

[0420]	min	minutes
[0421]	MS	mass spectrometry or mass spectrum
[0422]	Ms	mesyl
[0423]	MsCl	methanesulfonyl chloride
[0424]	MsO	mesylate
[0425]	MWI	microwave irradiation
[0426]	Na ₂ CO ₃	sodium carbonate
[0427]	NaHCO ₃	sodium bicarbonate
[0428]	NaHMDs	sodium hexamethyldisilazide
[0429]	NaOH	sodium hydroxide
[0430]	NIS	N-iodosuccinimide
[0431]	NMR	Nuclear Magnetic Resonance
[0432]	o/n or O/N	overnight
[0433]	PE	petroleum ether
[0434]	PG	protecting group
[0435]	PKMT	protein lysine methyltransferase
[0436]	PMB	para-methoxybenzyl
[0437]	PMT	protein methyltransferase
[0438]	PPAA	1-propanephosphonic acid cyclic anhydride
[0439]	ppm	parts per million
[0440]	prep HPLC	preparative high performance liquid chromatography
[0441]	prep TLC	preparative thin layer chromatography
[0442]	p-TsOH	para-toluenesulfonic acid
[0443]	rt or RT	room temperature
[0444]	SAH	<i>S</i> -adenosylhomocysteine
[0445]	SAM	<i>S</i> -adenosylmethionine
[0446]	SAR	structure activity relationship
[0447]	SEM	2-(trimethylsilyl)ethoxymethyl
[0448]	SEMCl	(trimethylsilyl)ethoxymethyl chloride
[0449]	SFC	supercritical chromatography
[0450]	SGC	silica gel chromatography
[0451]	SPR	surface plasmon resonance
[0452]	STAB	sodium triacetoxyborohydride
[0453]	TBAF	tetra-n-butylammonium fluoride

[0454] TFA	trifluoroacetic acid
[0455] TfO	triflate
[0456] THF	tetrahydrofuran
[0457] THP	tetrahydropyran
[0458] TLC	thin layer chromatography
[0459] Ts	tosyl
[0460] TsOH	tosic acid
[0461] UV	ultraviolet

[0462] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes are described as having, including, or comprising specific process steps, it is contemplated that compositions of the present invention also consist essentially of, or consist of, the recited components, and that the processes of the present invention also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions are immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[0463] Compounds suitable for the methods of the invention, once produced, can be characterized using a variety of assays known to those skilled in the art to determine whether the compounds have biological activity. For example, the molecules can be characterized by conventional assays, including but not limited to those assays described below, to determine whether they have a predicted activity, binding activity and/or binding specificity.

[0464] Furthermore, high-throughput screening can be used to speed up analysis using such assays. As a result, it can be possible to rapidly screen the molecules described herein for activity, using techniques known in the art. General methodologies for performing high-throughput screening are described, for example, in Devlin (1998) *High Throughput Screening*, Marcel Dekker; and U.S. Patent No. 5,763,263. High-throughput assays can use one or more different assay techniques including, but not limited to, those described herein.

[0465] To further assess a compound's drug-like properties, measurements of inhibition of cytochrome P450 enzymes and phase II metabolizing enzyme activity can also be measured either using recombinant human enzyme systems or more complex systems like human liver microsomes. Further, compounds can be assessed as substrates of these metabolic enzyme activities as well. These activities are useful in determining the potential of a compound to cause drug-drug interactions or generate metabolites that retain or have no useful

antimicrobial activity.

[0466] To get an estimate of the potential of the compound to be orally bioavailable, one can also perform solubility and Caco-2 assays. The latter is a cell line from human epithelium that allows measurement of drug uptake and passage through a Caco-2 cell monolayer often growing within wells of a 24-well microtiter plate equipped with a 1 micron membrane. Free drug concentrations can be measured on the basolateral side of the monolayer, assessing the amount of drug that can pass through the intestinal monolayer. Appropriate controls to ensure monolayer integrity and tightness of gap junctions are needed. Using this same system one can get an estimate of P-glycoprotein mediated efflux. P-glycoprotein is a pump that localizes to the apical membrane of cells, forming polarized monolayers. This pump can abrogate the active or passive uptake across the Caco-2 cell membrane, resulting in less drug passing through the intestinal epithelial layer. These results are often done in conjunction with solubility measurements and both of these factors are known to contribute to oral bioavailability in mammals. Measurements of oral bioavailability in animals and ultimately in man using traditional pharmacokinetic experiments will determine the absolute oral bioavailability.

[0467] Experimental results can also be used to build models that help predict physical-chemical parameters that contribute to drug-like properties. When such a model is verified, experimental methodology can be reduced, with increased reliance on the model predictability.

[0468] The present invention also provides pharmaceutical compositions comprising a compound of any of the Formulae disclosed herein in combination with at least one pharmaceutically acceptable excipient or carrier.

[0469] A “pharmaceutical composition” is a formulation containing the compounds of the present invention in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler or a vial. The quantity of active ingredient (*e.g.*, a formulation of the disclosed compound or salt, hydrate, solvate or isomer thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular,

intraperitoneal, inhalational, buccal, sublingual, intrapleural, intrathecal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active compound is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[0470] As used herein, the phrase “pharmaceutically acceptable” refers to those compounds, materials, compositions, carriers, 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.

[0471] “Pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable excipient” as used in the specification and claims includes both one and more than one such excipient.

[0472] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), and transmucosal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0473] A compound or pharmaceutical composition of the invention can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, a compound of the invention may be injected directly into tumors, injected into the blood stream or body cavities or taken orally or applied through

the skin with patches. The dose chosen should be sufficient to constitute effective treatment but not as high as to cause unacceptable side effects. The state of the disease condition (*e.g.*, cancer, precancer, and the like) and the health of the patient should preferably be closely monitored during and for a reasonable period after treatment.

[0474] The term “therapeutically effective amount”, as used herein, refers to an amount of a pharmaceutical agent to treat, ameliorate, or prevent an identified disease or condition, or to exhibit a detectable therapeutic or inhibitory effect. The effect can be detected by any assay method known in the art. The precise effective amount for a subject will depend upon the subject’s body weight, size, and health; the nature and extent of the condition; and the therapeutic selected for administration. Therapeutically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician. In a preferred aspect, the disease or condition to be treated is cancer. In another aspect, the disease or condition to be treated is a cell proliferative disorder.

[0475] For any compound, the therapeutically effective amount can be estimated initially either in cell culture assays, *e.g.*, of neoplastic cells, or in animal models, usually rats, mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic/prophylactic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The dosage may vary within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0476] Dosage and administration are adjusted to provide sufficient levels of the active agent(s) or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug interaction(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0477] The pharmaceutical compositions containing active compounds of the present invention may be manufactured in a manner that is generally known, *e.g.*, by means of

conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and/or auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[0478] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol and sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0479] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0480] Oral compositions generally include an inert diluent or an edible pharmaceutically acceptable carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0481] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0482] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0483] The active compounds can be prepared with pharmaceutically acceptable carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0484] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

[0485] In therapeutic applications, the dosages of the pharmaceutical compositions used in accordance with the invention vary depending on the agent, the age, weight, and clinical condition of the recipient patient, and the experience and judgment of the clinician or practitioner administering the therapy, among other factors affecting the selected dosage. Generally, the dose should be sufficient to result in slowing, and preferably regressing, the growth of the tumors and also preferably causing complete regression of the cancer. Dosages can range from about 0.01 mg/kg per day to about 5000 mg/kg per day. In preferred aspects, dosages can range from about 1 mg/kg per day to about 1000 mg/kg per day. In an aspect, the dose will be in the range of about 0.1 mg/day to about 50 g/day; about 0.1 mg/day to about 25 g/day; about 0.1 mg/day to about 10 g/day; about 0.1 mg to about 3 g/day; or about 0.1 mg to about 1 g/day, in single, divided, or continuous doses (which dose may be adjusted for the patient's weight in kg, body surface area in m², and age in years). An effective amount of a pharmaceutical agent is that which provides an objectively identifiable improvement as noted by the clinician or other qualified observer. For example, regression of a tumor in a patient may be measured with reference to the diameter of a tumor. Decrease in the diameter of a tumor indicates regression. Regression is also indicated by failure of tumors to reoccur after treatment has stopped. As used herein, the term "dosage effective manner" refers to amount of an active compound to produce the desired biological effect in a subject or cell.

[0486] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0487] The compounds of the present invention are capable of further forming salts. All of these forms are also contemplated within the scope of the claimed invention.

[0488] As used herein, "pharmaceutically acceptable salts" refer to derivatives of the compounds of the present invention wherein the parent compound is modified by making acid or base salts thereof. 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 include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include, but are not limited to, those derived from inorganic and organic acids selected from 2-acetoxybenzoic, 2-hydroxyethane sulfonic, acetic, ascorbic, benzene sulfonic, benzoic, bicarbonic, carbonic, citric, edetic, ethane disulfonic, 1,2-ethane sulfonic, fumaric, glucoheptonic, gluconic, glutamic, glycolic, glycollyarsanilic, hexylresorcinic, hydrabamic, hydrobromic, hydrochloric, hydroiodic, hydroxymaleic, hydroxynaphthoic, isethionic, lactic, lactobionic, lauryl sulfonic, maleic, malic, mandelic, methane sulfonic, napsylic, nitric, oxalic, pamoic, pantothenic, phenylacetic, phosphoric, polygalacturonic, propionic, salicyclic, stearic, subacetic, succinic, sulfamic, sulfanilic, sulfuric, tannic, tartaric, toluene sulfonic, and the commonly occurring amine acids, *e.g.*, glycine, alanine, phenylalanine, arginine, etc.

[0489] Other examples of pharmaceutically acceptable salts include hexanoic acid, cyclopentane propionic acid, pyruvic acid, malonic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo-[2.2.2]-oct-2-ene-1-carboxylic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, muconic acid, and the like. The present invention also encompasses salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

[0490] It should be understood that all references to pharmaceutically acceptable salts include solvent addition forms (solvates) or crystal forms (polymorphs) as defined herein, of the same salt.

[0491] The compounds of the present invention can also be prepared as esters, for example, pharmaceutically acceptable esters. For example, a carboxylic acid function group in a compound can be converted to its corresponding ester, *e.g.*, a methyl, ethyl or other ester. Also, an alcohol group in a compound can be converted to its corresponding ester, *e.g.*, acetate, propionate or other ester.

[0492] The compounds of the present invention can also be prepared as prodrugs, for example, pharmaceutically acceptable prodrugs. The terms “pro-drug” and “prodrug” are used interchangeably herein and refer to any compound which releases an active parent drug

in vivo. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.), the compounds of the present invention can be delivered in prodrug form. Thus, the present invention is intended to cover prodrugs of the presently claimed compounds, methods of delivering the same and compositions containing the same. “Prodrugs” are intended to include any covalently bonded carriers that release an active parent drug of the present invention *in vivo* when such prodrug is administered to a subject. Prodrugs in the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound. Prodrugs include compounds of the present invention wherein a hydroxy, amino, sulfhydryl, carboxy or carbonyl group is bonded to any group that may be cleaved *in vivo* to form a free hydroxyl, free amino, free sulfhydryl, free carboxy or free carbonyl group, respectively.

[0493] Examples of prodrugs include, but are not limited to, esters (e.g., acetate, dialkylaminoacetates, formates, phosphates, sulfates and benzoate derivatives) and carbamates (e.g., N,N-dimethylaminocarbonyl) of hydroxy functional groups, esters (e.g., ethyl esters, morpholinoethanol esters) of carboxyl functional groups, N-acyl derivatives (e.g., N-acetyl) N-Mannich bases, Schiff bases and enaminones of amino functional groups, oximes, acetals, ketals and enol esters of ketone and aldehyde functional groups in compounds of the invention, and the like, See Bundgaard, H., *Design of Prodrugs*, p1-92, Elsevier, New York-Oxford (1985).

[0494] The compounds, or pharmaceutically acceptable salts, esters or prodrugs thereof, are administered orally, nasally, transdermally, pulmonary, inhalationally, buccally, sublingually, intraperitoneally, subcutaneously, intramuscularly, intravenously, rectally, intrapleurally, intrathecally and parenterally. In one embodiment, the compound is administered orally. One skilled in the art will recognize the advantages of certain routes of administration.

[0495] The dosage regimen utilizing the compounds is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

[0496] Techniques for formulation and administration of the disclosed compounds of the invention can be found in *Remington: the Science and Practice of Pharmacy*, 19th edition, Mack Publishing Co., Easton, PA (1995). In an embodiment, the compounds described

herein, and the pharmaceutically acceptable salts thereof, are used in pharmaceutical preparations in combination with a pharmaceutically acceptable carrier or diluent. Suitable pharmaceutically acceptable carriers include inert solid fillers or diluents and sterile aqueous or organic solutions. The compounds will be present in such pharmaceutical compositions in amounts sufficient to provide the desired dosage amount in the range described herein.

[0497] All percentages and ratios used herein, unless otherwise indicated, are by weight. Other features and advantages of the present invention are apparent from the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

[0498] In the synthetic schemes described herein, compounds may be drawn with one particular configuration for simplicity. Such particular configurations are not to be construed as limiting the invention to one or another isomer, tautomer, regioisomer or stereoisomer, nor does it exclude mixtures of isomers, tautomers, regioisomers or stereoisomers.

[0499] Compounds described herein are assayed for modulation of activity, for example, histone methylation, modulation of cell growth and/or IC_{50} , described in the examples below. IC_{50} values for DOT1L inhibition for select DOT1L inhibitors were determined as described in Example 1 and are listed below.

Compound	DOT1L IC_{50} (μ M)
A2	0.00074
A3	0.00073
A5	0.00059
A69	0.00251
A75	0.00059
A86	0.00062
A87	0.0008
A91	0.00218
A93	0.00292

[0500] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of

written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

[0501] Example 1: General Methods

[0502] Cell Culture:

[0503] Human leukemia cell line EOL-1 (Catalog # ACC-386) was purchased from DSMZ and were grown in Roswell Park Memorial Institute medium (RPMI) with 10% Fetal Bovine Serum (FBS). Cells were kept in log growth as outlined in the technical data sheet provided by the vendor.

[0504] Cell Growth and Viability Assay

[0505] Exponentially growing EOL-1 cells were plated in 96-well plates at a density of 3×10^4 viable cells/well. Each treatment was seeded in triplicate with a final well volume of 150 μ Ls. Cells were incubated with increasing concentrations of DOT1L inhibitor up to 50 μ M. Viable cell number was determined every 3 – 4 days for 11 days using the Guava Viacount assay (Millipore # 4000-0040) and analyzed on a Guava EasyCyte Plus instrument according to the manufacturer's protocol. On the days of cell counts, growth media and inhibitor were replenished and cells maintained in log phase culture by reseeding at a density of 5×10^4 viable cells/well. Total cell number was expressed as split-adjusted viable cells per well. For each cell inhibitor IC₅₀ values were determined from concentration-dependence curves at day 11. All calculations were done using GraphPad Prism, version 5.00 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

[0506] Histone Extraction of Cell Pellets:

[0507] Frozen pellets were allowed to thaw briefly on ice and then lysed by a 5 minute incubation on ice with 250 μ l nuclear extraction buffer (10 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 25 mM KCl, 1% Triton X-100, 8.6% Sucrose, plus a Roche protease inhibitor tablet 1836153001). Nuclei were collected by centrifugation at 600 g for 5 minutes at 4°C and washed once in Tris/EDTA buffer (pH 7.4). Supernatant was removed and histones extracted for one hour with 60 μ l 0.4 N cold sulfuric acid. Extracts were clarified by centrifugation at 10,000 g for 10 minutes at 4°C and transferred to a fresh microcentrifuge tube containing 600 μ l ice cold acetone. Histones were precipitated at -20° C for 2 hours, pelleted by centrifugation at 10,000 g for 10 minutes and resuspended in 60 μ l distilled water (DI water). Total protein of the acid extracts was assessed using a bicinchoninic acid (BCA) protein quantification assay with a bovine serum albumin (BSA) standard (Pierce Biotechnology).

[0508] H3K79me2 Immunoblot:

[0509] For immunoblot analysis of the H3K79me2 inhibition by DOT1L inhibitor, exponentially growing EOL-1 cells were seeded at 2×10^5 cells/mL and incubated in the presence of increasing concentrations of DOT1L inhibitor for 4 days. Following incubation, cells ($2-3 \times 10^6$) were harvested and histones extracted as described. Histones (400 ng) were fractionated on a 10-20% Tris HCl gels (Bio-Rad) with Tris-Glycine SDS running buffer (Invitrogen) under denaturing conditions and transferred to a nitrocellulose filter. The filter was incubated for 1 hour in blocking buffer (Odyssey blocking buffer, Li-cor, 927-40000) at RT and then incubated overnight at 4°C in blocking buffer containing a antibody specific for H3K79me2 (1:5000 dilution, abcam ab3594). Filters were washed 3 times for 5 minutes with wash buffer (PBST) and incubated with infrared tagged secondary antibody (Alexa Flour 680 goat anti-rabbit IgG (1:20,000), Invitrogen A-21076) at RT for 1 hour. Filters were washed in PBST and reprobed for 1 hour at RT with the appropriate total histone antibody control (mouse anti-histone H3 (1:20,000), CST 3638, or mouse anti-histone H4 (1:10,000), CST 2935). Filters were washed again in PBST and incubated with infrared tagged secondary antibody (IRDye 800Cw donkey-anti-mouse IgG (1:20,000), Li- Cor 926-32212) at RT for 1 hour. After a final wash in PBST, filters were scanned using the Odyssey infrared imager (Li-cor). Signal intensities specific for each methyl-specific antibody was quantified using Odyssey software and normalized to that of the appropriate total histone control signal on the same filter by dividing the methyl-specific antibody signal intensity by the total histone control signal intensity.

[0510] Quantitative Real-Time PCR:

[0511] Exponentially growing EOL-1 cells were plated in a 12 well plate at 2×10^5 cells/mL. Cells were incubated in the presence of increasing concentrations of Compound A2 up to 10 μ M. On day 4, cells were maintained in log phase culture by reseeding at 5×10^5 cells/mL and compound was replenished. At day 6 cells were washed twice with PBS and pelleted by centrifugation at 200 X g. Cell pellets were lysed in 300 μ L RLT buffer (Qiagen) and total RNA was isolated using the RNeasy total RNA isolation kit (Qiagen 74106). Total RNA (1 μ g) was reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems 4368813). RNA isolation and cDNA synthesis were carried out according to the manufacturer's protocol. Predesigned labeled primer and probe sets for *HOXA9* (Hs00365956), *MEIS1* (Hs00180020) and *FLT3* (Hs00975659) were purchased from Applied Biosystems. Quantitative real-time PCR (qPCR) reactions contained 50 ng cDNA, 1X labeled primer and probe set, and 1X Taqman universal PCR master mix (Applied

Biosystems 4304437). Samples were run on a 7900 HT Fast Real Time PCR machine (Applied Biosystems 4351405) with cycling conditions of 2 min 50°C, 10 min 95°C, 40 cycles at 15 sec 95°C and 1 min 60°C. Target gene cycle numbers were normalized to the house keeping gene β 2-microglobulin (Applied Biosystems 4333766) to get a Δ CT value. Percent of DMSO control was calculated with the equation $(2^{-\Delta\Delta\text{CT}})^{*}100$ where the $\Delta\Delta\text{CT}$ is the difference between normalized target gene and DMSO control (ΔCT sample – ΔCT control = $\Delta\Delta\text{CT}$).

[0512] **Determination of IC₅₀.** Test compounds were serially diluted 3 fold in DMSO for 10 points and 1 μ l was plated in a 384 well microtiter plate. Positive control (100% inhibition standard) was 2.5 μ M final concentration of S-adenosyl-L-homocysteine and negative control (0% inhibition standard) contained 1 μ l of DMSO. Compound was then incubated for 30 minutes with 40 μ l per well of DOT1L(1-416) (0.25 nM final concentration in assay buffer: 20 mM TRIS, pH 8.0, 10 mM NaCl, 0.002% Tween20, 0.005% Bovine Skin Gelatin, 100 mM KCl, and 0.5 mM DTT). 10 μ l per well of substrate mix (same assay buffer with 200 nM S-[methyl-³H]-adenosyl-L methionine, 600 nM of unlabeled S-[methyl-³H]-adenosyl-L methionine, and 20 nM oligonucleosome) was added to initiate the reaction. Reaction was incubated for 120 minutes at room temperature and quenched with 10 μ l per well of 100 μ M S-methyl-adenosyl –L methionine. For detection, substrate from 50 μ l of reaction was immobilized on a 384 well Streptavidin coated Flashplate (Perkin Elmer) (also coated with 0.2% polyethyleneimine) and read on a Top Count scintillation counter (Perkin Elmer).

[0513] Other related general procedures and specific preparation procedures can also be found in the PCT publication No. WO2012/075381 and U.S. Provisional Application No. 61/682,090, the contents of each are incorporated herein by reference in their entireties.

[0514] Example 2: Effect of DOT1L Inhibition on Cell Growth and Viability

[0515] The effect of DOT1L inhibitors on leukemia cell growth and viability was investigated. EOL-1 cells, a leukemia cell line characterized by MLL PTD, were plated in 96-well plates at a density of 3×10^4 viable cells/well. Cells were incubated with increasing concentrations of DOT1L inhibitor between the 0.003 μ M - 50 μ M. The number of viable cells was determined every 3-4 days for 11 days. Cells were maintained in log phase by reseeding and replenishing growth media and the indicated concentration of DOT1L inhibitor on each day of cell counts (Day 0, Day 4, Day 7, and Day 11). Total cell number was expressed as split-adjusted viable cells per well. DMSO-treated cells were used as a control.

[0516] Figure 1A depicts the results of the cell growth and viability assay after treatment with Compound A2 for 11 days. DMSO-treated cells continued to grow exponentially.

However, cell growth was significantly inhibited under treatment with Compound A2. As expected, cell proliferation was inhibited in a dosage-dependent manner, with the highest concentration (50 μ M) having the most pronounced inhibitory effect on cell proliferation and viability. Even the cells treated with the lowest concentrations of Compound A2 (for example, 0.003 and 0.012 μ M) resulted in some inhibition of cell proliferation and/or affected cell viability after 11 days.

[0517] Other compounds, such as Compound T and control compound, can also be assessed for the effect on cell proliferation and viability, and compared to a control compound or Compound A2, in a growth assay as described.

[0518] Example 3: Inhibition of DOT1L methyltransferase activity

[0519] Inhibition of methylation of H3K79 was assessed after 4 days of treatment of DOT1L compounds in exponentially growing EOL-1 cells.

[0520] H3K79 methylation status after Compound A2 treatment was first examined by immunoblot. Following treatment, cells were harvested and histones were extracted. Western blot analysis was performed using antibodies specific for H3K79me2 and total histone 3 (as a control). Signal intensities specific to H3K79me2 was quantified and normalized to that of the total histone 3 signal. The results of the western blot analysis demonstrate a dose-dependent inhibition of methyltransferase activity of DOT1L (Figure 1B). Treatment with Compound A2 within the range of 0.009 and 10 μ M resulted in some inhibitory effect on DOT1L methyltransferase activity, as evidenced by reduced signal of di-methylated H3K79me2. The IC50 was determined to be at 0.027 μ M. Thus, 0.027 μ M is required to inhibit the methylation of H3K79 by half.

[0521] Example 4: Potency of DOT1L inhibition

[0522] The IC50 and inhibition constants of the DOT1L inhibitors described herein can be determined from concentration-dependence growth curves, as described in Example 2. The concentration-dependence curves at day 11 for Compound A2, Compound T, and a control compound were plotted on a single log graph and compared (Figure 2A). IC50 values, or maximal inhibitory concentration, for each DOT1L inhibitor can be determined from the curves. Specifically, IC50 values for Compound A2, Compound T and a control compound that has no or little DOT1L inhibition are listed in Figure 2B. Compound A2 has the lowest IC50 value, therefore demonstrating that a very low concentration of Compound A2 (specifically, 0.002 μ M) can reduce biological activity (*i.e.*, cell growth) by half. In contrast, the control compound which has little or no DOT1L inhibitory activity has requires a high concentration (50 μ M) to reduce biological activity (*i.e.*, cell growth) by half.

[0523] The inhibition constant (Ki) can also be determined for the DOT1L inhibitors described herein. For example, Ki values for Compound A2, Compound T, and a control compound is listed in Figure 2B. Compound A2 having the lowest Ki, which indicates that a very low concentration (0.08 nM) of Compound A2 is required to decrease the maximal rate of the reaction to half of the uninhibited value, in the presence of a low substrate concentration. As expected, the control compound has a very high Ki (more than 50,000 nM), showing that the control compound requires a very little inhibitory activity.

[0524] Example 5: Gene Overexpression in Leukemias

[0525] HOXA9 expression levels were assessed in a panel of leukemia cells lines, demonstrating that HOXA9 is often overexpressed in various hematologic cancers. The leukemia cells assessed include MolM13 (acute monocytic leukemia cell line), MV411 (acute myelocytic leukemia), LOUCY (T-cell acute lymphoblastic leukemia), EOL-1 (eosinophilic leukemia) SemK2 (B-cell acute lymphoblastic leukemia), Reh (acute lymphoblastic leukemia), HL60 (promyelocytic leukemia) and BV173 (pre-B-cell leukemia). Molm13, MV411, and SemK2 cell lines are characterized as having MLL fusions. LOUCY and Reh cell lines are characterized as having non-MLL chromosomal rearrangements. EOL-1 cells are characterized as having MLL-PTD.

[0526] Cells were harvested, RNA was extracted, and cDNA was prepared as described in Example 1. The expression level of HOXA9 was determined by quantitative real-time PCR (Figure 3). HOXA9 expression level was normalized to the lowest HOXA9 expresser. Jurkat cells, an immortalized T-lymphocyte cell line, were used as control. HL60, BV173, and Reh cell lines had very low overexpression of HOXA9. SemK2, EOL-1, LOUCY, MV411, and Molm13 had extremely high overexpression of HOXA9, with at least a 2000-fold increase over the lowest expresser. Overexpression of other cancer-associated genes, such as FLT3, MEIS1, or DOT1L can be determined using similar methods as those described herein.

[0527] Example 6: Gene Expression after DOT1L Inhibition

[0528] Leukemia cell lines can be treated with DOT1L inhibitors and the expression of select genes can be examined to assess the effects of DOT1L inhibition on cancer-associated gene overexpression. Leukemia cell lines were treated with increasing concentrations of DOT1L inhibitors (Compound A2 and Compound T) up to 10 μ M for 6 days. For the vehicle control, cells were treated with DMSO. Cells were then harvested, RNA was extracted, and cDNA was prepared as described in Example 1.

[0529] Expression levels of select genes, including HOXA9, FLT3, MEIS1, and DOT1L, were determined by quantitative real-time PCR. Figure 4 shows the expression of HOXA9

and FLT3 after treatment with varying concentrations of Compound A2. Expression of both HOXA9 and FLT3 was reduced in a dose-dependent manner. Figure 5 shows the expression of HOXA9, FLT3, MEIS1 and DOT1L after treatment with varying concentrations of Compound T. As expected, DOT1L gene expression was not reduced after treatment of DOT1L inhibitor and can be considered useful for control purposes. HOXA9, FLT3, and MEIS1 expression was also reduced in a dosage-dependent manner after treatment with Compound T.

[0530] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

[0531] The invention can be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

What is claimed is:

1. A method for treating or alleviating a symptom of leukemia comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4.

2. The method of claim 1, wherein said leukemia is characterized by a chromosomal rearrangement.

3. The method of claim 2, wherein said chromosomal rearrangement is chimeric fusion of mixed lineage leukemia gene (MLL) or partial tandem duplication of MLL (MLL-PTD).

4. The method of claim 1, wherein said subject has an increased level of HOXA9, Fms-like tyrosine kinase 3 (FLT3), MEIS1, and/or DOT1L.

5. A method for treating or alleviating a symptom of leukemia comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4, wherein the subject has an increased level of HOXA9, FLT3, MEIS1, and/or DOT1L.

6. A method for treating or alleviating a symptom of leukemia in a subject comprising

- 1) obtaining a sample from the subject;
- 2) detecting the level of HOXA9, FLT3, MEIS1, and/or DOT1L, wherein an increased level of HOXA9, FLT3, MEIS1, and/or DOT1L indicates the subject is responsive to a compound listed in any of Tables 1-4; and
- 3) administering to the subject a therapeutically effective amount of said compound when said subject is responsive to said compound.

7. A method for treating or alleviating a symptom of leukemia in a subject comprising

- 1) obtaining a sample from the subject;
- 2) detecting the presence of a genetic lesion of MLL in the sample; and

3) administering to the subject a therapeutically effective amount of a compound listed in any of Tables 1-4 when said genetic lesion is present in the sample.

8. The method of claim 5 or claim 7, wherein said sample is selected from bone marrow, peripheral blood cells, blood, plasma, serum, urine, saliva, a cell, or a tumor tissue.

9. The method of claim 7, wherein said genetic lesion is chimeric fusion of MLL or MLL-PTD.

10. A method for treating a disorder mediated by translocation, deletion and/or duplication of a gene on chromosome 11q23, comprising administering to a subject in need thereof a therapeutically effective amount of a compound listed in any of Tables 1-4.

11. The method of claim 1, wherein said compound is Compound A2.

12. The method of claim 1, wherein said compound is Compound D16.

13. The method of claim 6, wherein said compound is Compound A2.

14. The method of claim 6, wherein said compound is Compound D16.

15. The method of claim 7, wherein said compound is Compound A2.

16. The method of claim 7, wherein said compound is Compound D16.

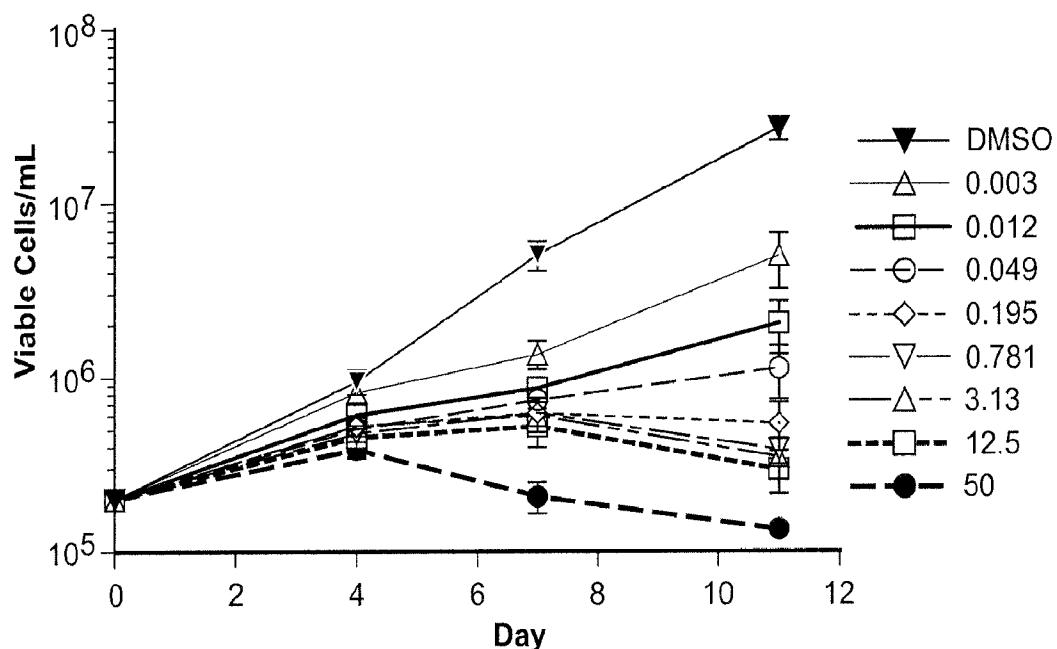
17. The method of claim 10, wherein said compound is Compound A2.

18. The method of claim 10, wherein said compound is Compound D16.

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FIG. 1

A Eol-1 14 Day Growth with Compound A2



B

H3K79me2 (Day 4)

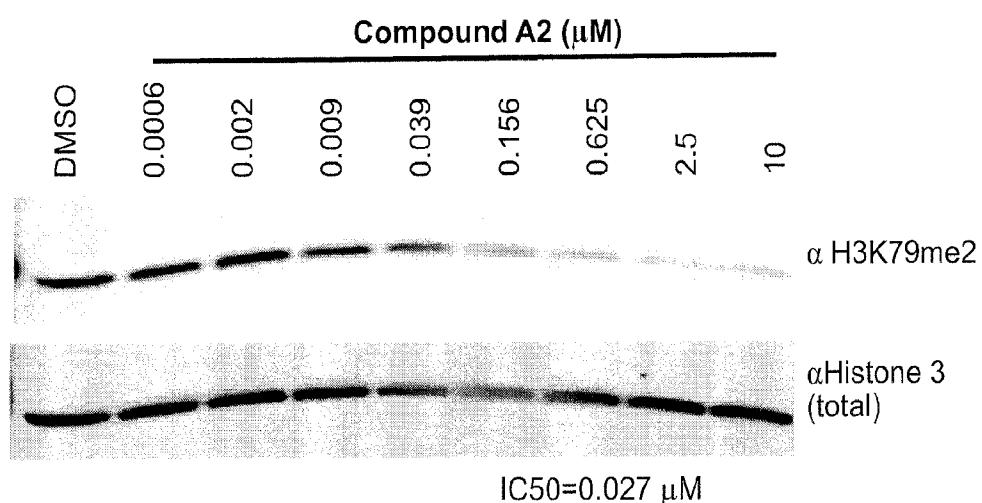
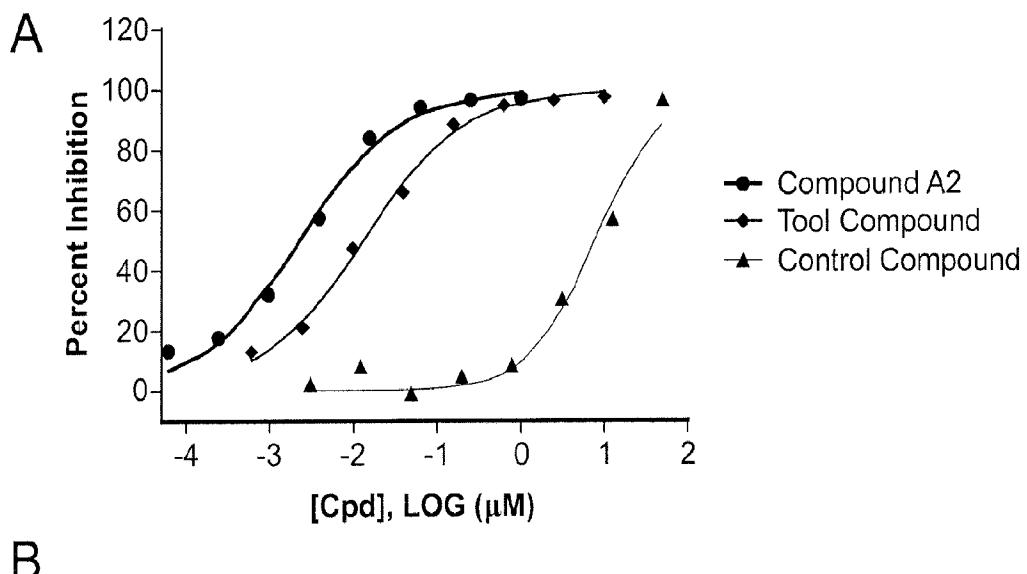


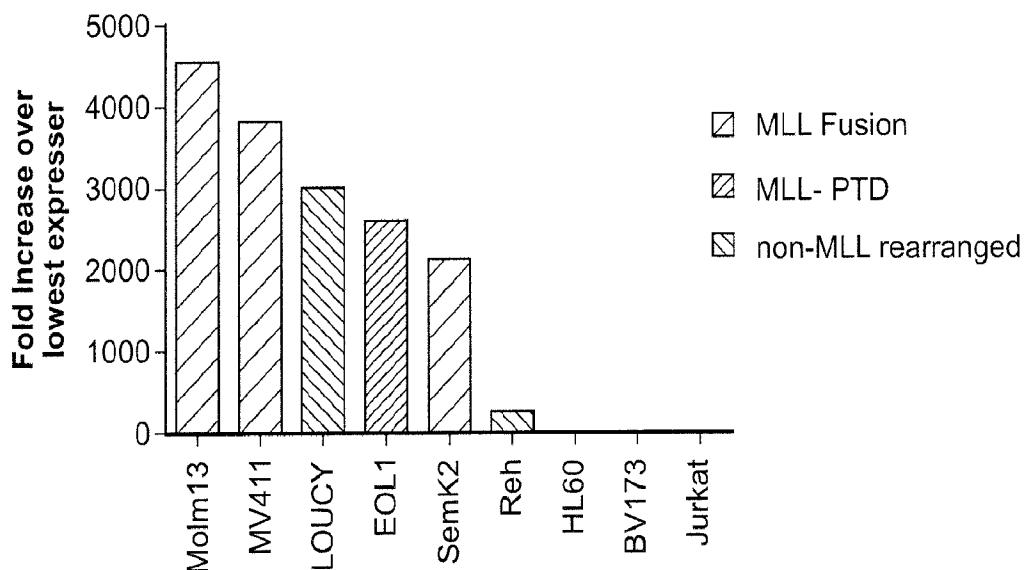
FIG. 2

2/3



B

Compound	K _i (nM)	EOL-1 11 Day Cell Growth IC ₅₀ (μM)
Control Compound	>50,000	>50
Compound T	0.3	0.012
Compound A2	0.08	0.002

FIG. 3
HOXA9 Expression

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FIG. 4

qPCR (6 Day incubation)

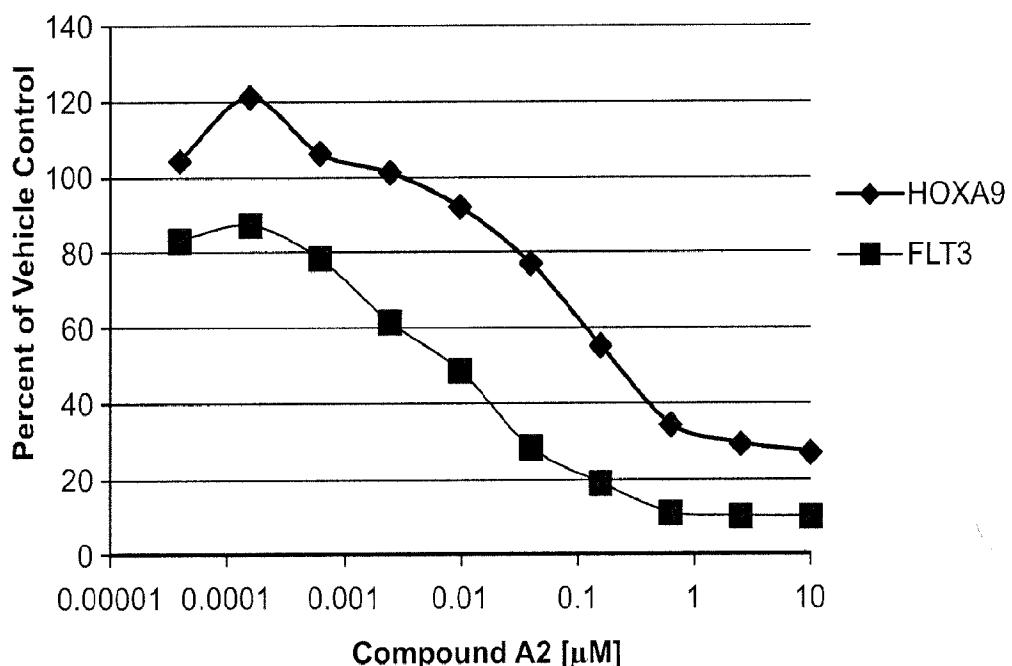
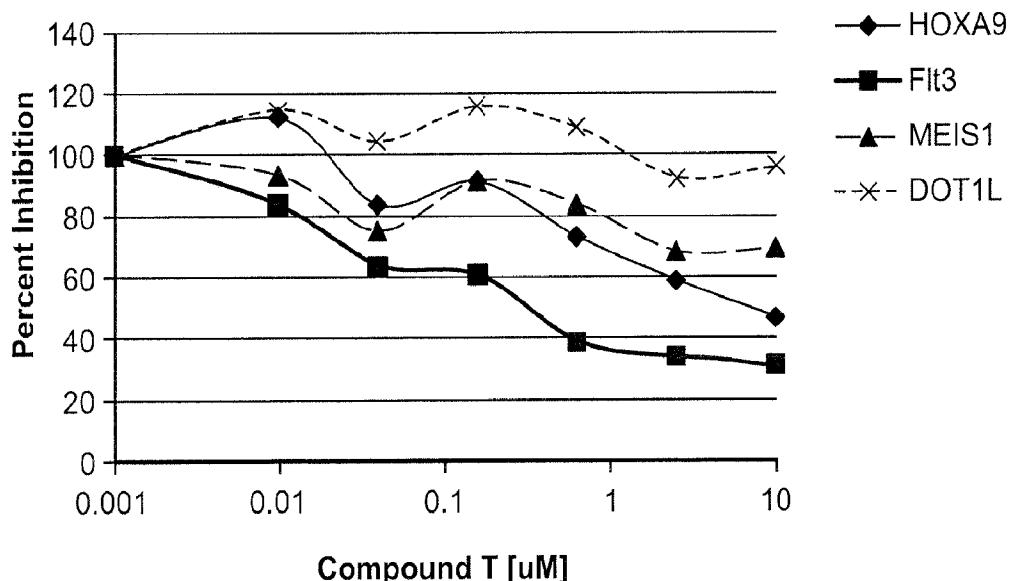


FIG. 5

Day 6 Gene Expression



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/077082

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7076 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SCOTT R DAIGLE ET AL: "Selective Killing of Mixed Lineage Leukemia Cells by a Potent Small-Molecule DOT1L Inhibitor", CANCER CELL, CELL PRESS, US, vol. 20, no. 1, 16 June 2011 (2011-06-16), pages 53-65, XP028099809, ISSN: 1535-6108, DOI: 10.1016/J.CCR.2011.06.009 [retrieved on 2011-06-20] figure 1A page 53, abstract page 59, left-hand column, paragraph 2</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/-</p>	1-18



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2014	25/03/2014
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Baurand, Petra

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/077082

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COPELAND R A: "Protein methyltransferase inhibitors as personalized cancer therapeutics", DRUG DISCOVERY TODAY: THERAPEUTIC STRATEGIES 2012 ELSEVIER LTD GBR, vol. 9, no. 2-3, October 2012 (2012-10), pages e83-e90, XP002721606, ISSN: 1740-6773 page e86, left-hand column, paragraph 1 table 3 -----	1-18
X	BASAVAPATHRUNI ARAVIND ET AL: "Conformational Adaptation Drives Potent, Selective and Durable Inhibition of the Human Protein Methyltransferase DOT1L", CHEMICAL BIOLOGY & DRUG DESIGN, vol. 80, no. 6, 9 October 2012 (2012-10-09), pages 971-980, XP002721607, ISSN: 1747-0277 figure 1 page 979, left-hand column, paragraph 1 page 979, left-hand column, paragraph 2 -----	1-18
X	CHEN LIYING ET AL: "Abrogation of MLL-AF10 and CALM-AF10 Mediated Transformation Through Genetic Inactivation or Pharmacological Inhibition of the H3K79 Methyltransferase DOT1L", BLOOD, vol. 120, no. 21, November 2012 (2012-11), page 2384, XP002721608, & 54TH ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ATLANTA, GA, USA; DECEMBER 08 -11, 2012 abstract -----	1-18
Y	JIANG XI ET AL: "Blockade of Mir-150 Maturation by MLL-Fusion/MYC/Lin-28 Is Required for MLL-Associated Leukemia", BLOOD, vol. 120, no. 21, November 2012 (2012-11), page 3499, XP002721609, & 54TH ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ATLANTA, GA, USA; DECEMBER 08 -11, 2012 abstract ----- -/-	1-18

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/077082

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>BERNT KATHRIN M ET AL: "Demonstration of a Role for Dot11 In MLL-Rearranged Leukemia Using a Conditional Loss of Function Model", BLOOD, vol. 116, no. 21, November 2010 (2010-11), page 34, XP002721610, & 52ND ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ORLANDO, FL, USA; DECEMBER 04 -07, 2010 abstract</p> <p>-----</p>	1-18