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

(19) World Intellectual Property Organization

International Bureau





(10) International Publication Number WO 2014/062720 A2

- (43) International Publication Date 24 April 2014 (24.04.2014)
- (21) International Application Number:

(51) International Patent Classification:

A61K 31/5377 (2006.01)

PCT/US2013/065112

(22) International Filing Date:

15 October 2013 (15.10.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/714,140	15 October 2012 (15.10.2012)	US
61/714,045	15 October 2012 (15.10.2012)	US
61/714,145	15 October 2012 (15.10.2012)	US
61/758,972	31 January 2013 (31.01.2013)	US
61/780,703	13 March 2013 (13.03.2013)	US
61/786,277	14 March 2013 (14.03.2013)	US

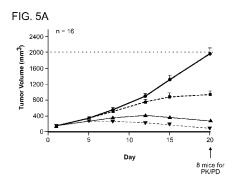
- (71) Applicant: EPIZYME, INC. [US/US]; 400 Technology Square, 4th Floor, Cambridge, MA 02139 (US).
- (72) Inventors: KNUTSON, Sarah, K.; 24 Bay State Road, Unit 22, Cambridge, MA 02138 (US). WARHOLIC, Natalie; 81 Strathmore Road, Apartment 32, Brighton, MA 02135 (US). KEILHACK, Heike; 3 Falmouth Street, Belmont, MA 02478 (US).
- (74) Agents: ELRIFI, Ivor, R. et al.; Mintz Levin Cohn Ferris Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

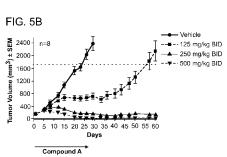
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

 without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: METHODS OF TREATING CANCER





(57) Abstract: The present invention relates to methods of treating cancer by administering the EZH2 inhibitor compounds and pharmaceutical compositions to subjects in need thereof. The present invention also relates to the use of such compounds for research or other non-therapeutic purposes.





METHODS OF TREATING CANCER

RELATED APPLICATIONS

[001] This application claims priority to, and the benefit of U.S. Provisional Application Nos. 61/714,045, field October 15, 2012, 61/758,972, filed January 31, 2013, 61/714,140, filed October 15, 2012, 61/714,145, filed October 15, 2012, 61/780,703, filed March 13, 2013, and 61/786,277, filed March 14, 2013. The entire contents of each of these provisional applications are incorporated herein by reference in their entireties.

FIELD OF INVENTION

[002] The present invention relates generally to the field of cancer treatment, and in particular, the treatment of cancer associated with the SWI/SNF complex (*i.e.*, SWI/SNF mediated cancer). More particularly, the present invention provides methods and compositions which treat, alleviate, prevent, diminish or otherwise ameliorate the symptoms of cancer associated with the SWI/SNF complex.

BACKGROUND OF THE INVENTION

[003] Disease-associated chromatin-modifying enzymes (*e.g.*, EZH2) play a role in diseases such as proliferative disorders, metabolic disorders, and blood disorders. Thus, there is a need for the development of small molecules that are capable of modulating the activity of EZH2.

SUMMARY OF THE INVENTION

[004] The present invention provides a method for treating or alleviating a symptom of a SWI/SNF-associated cancer in a subject by administering to a subject in need thereof a therapeutically effective amount of an EZH2 inhibitor, where the subject has a cancer selected from the group consisting of brain and central nervous system cancer, head and neck cancer, kidney cancer, ovarian cancer, pancreatic cancer, leukemia, lung cancer, lymphoma, myeloma,

sarcoma, breast cancer, and prostate cancer. For example, the SWI/SNF-associated cancer is characterized by reduced expression and/or loss of function of the SWI/SNF complex or one or more components of the SWI/SNF complex.

- [005] For example, the subject has a cancer selected from the group consisting of medulloblastoma, malignant rhabdoid tumor, and atypical teratoid/rhabdoid tumor.
- [006] For example, the one or more components are selected from the group consisting of SNF5, ATRX, and ARID1A.
- [007] For example, the loss of function is caused by a loss of function mutation resulting from a point mutation, a deletion, and/or an insertion.
- [008] For example, the subject has a deletion of SNF5.
- [009] For example, the subject has a mutation of ATRX selected from the group consisting of a substitution of asparagine (N) for the wild type residue lysine (K) at amino acid position 688 of SEQ ID NO: 5 (K688N), and a substitution of isoleucine (I) for the wild type residue methionine (M) at amino acid position 366 of SEQ ID NO: 5 (M366I).
- For example, subject has a mutation of ARID1A selected from the group consisting [010] of a nonsense mutation for the wild type residue cysteine (C) at amino acid position 884 of SEQ ID NO: 11 (C884*), a substitution of lysine (K) for the wild type residue glutamic acid (E) at amino acid position 966 (E966K), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1411 of SEQ ID NO: 11 (Q1411*), a frame shift mutation at the wild type residue phenylalanine (F) at amino acid position 1720 of SEQ ID NO: 11 (F1720fs), a frame shift mutation after the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue cysteine (C) at amino acid position 1874 of SEQ ID NO: 11 (C1874fs), a substitution of glutamic acid (E) for the wild type residue aspartic acid (D) at amino acid position 1957 (D1957E), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1430 of SEQ ID NO: 11 (Q1430*), a frame shift mutation at the wild type residue arginine (R) at amino acid position 1721 of SEQ ID NO: 11 (R1721fs), a substitution of glutamic acid (E) for the wild type residue glycine (G) at amino acid position 1255 (G1255E), a frame shift mutation at the wild type residue glycine (G) at amino acid position 284 of SEQ ID NO: 11 (G284fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1722 of SEQ ID NO: 11 (R1722*), a frame shift mutation at the wild type residue methionine (M) at amino acid

position 274 of SEQ ID NO: 11 (M274fs), a frame shift mutation at the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue P at amino acid position 559 of SEQ ID NO: 11 (P559fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1276 of SEQ ID NO: 11 (R1276*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 2176 of SEQ ID NO: 11 (Q2176fs), a frame shift mutation at the wild type residue histidine (H) at amino acid position 203 of SEQ ID NO: 11 (H203fs), a frame shift mutation at the wild type residue alanine (A) at amino acid position 591 of SEQ ID NO: 11 (A591fs), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1322 of SEQ ID NO: 11 (Q1322*), a nonsense mutation for the wild type residue serine (S) at amino acid position 2264 of SEQ ID NO: 11 (S2264*), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 586 of SEQ ID NO: 11 (Q586*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 548 of SEQ ID NO: 11 (Q548fs), and a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 756 of SEQ ID NO: 11 (N756fs).

- [011] The present invention also provides a method of treating or alleviating a symptom of a SWI/SNF-associated cancer in a subject in need thereof by (a) determining the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes and tumor suppressor genes in a sample obtained from the subject; (b) selecting the subject having a decreased expression level of at least one gene in step a; and (c) administering to the subject selected in step b an effective amount of an EZH2 inhibitor, thereby treating or alleviating a symptom of cancer in the subject.
- [012] The present invention further provides a method of treating or alleviating a symptom of a SWI/SNF-associated cancer in a subject in need thereof by (a) determining the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genesin a sample obtained from the subject; (b) selecting the subject having an increased expression level of at least one gene in step a; and (c) administering to the subject selected in step b an effective amount of an EZH2 inhibitor, thereby treating or alleviating a symptom of cancer in the subject.
- [013] For example, the cancer can be medulloblastoma, malignant rhabdoid tumor or atypical teratoid rhabdoid tumor.

[014] For example, the neuronal differentiation gene is CD133, DOCK4, or PTPRK.

- [015] For example, the cell cycle inhibition gene is CKDN1A or CDKN2A.
- [016] For example, the tumor suppressor gene is BIN1.
- [017] For example, the hedgehog pathway gene is GLI1 or PTCH1.
- [018] For example, the myc pathway gene is MYC.
- [019] For example, the histone methyltransferase gene is EZH2.
- [020] The present invention also provides a method of inducing neuronal differentiation, cell cycle inhibition or tumor suppression by contacting a cell with an EZH2 inhibitor. The EZH2 inhibitor may be in an amount sufficient to increase expression of at least one gene selected from the group consisting of CD133, DOCK4, PTPRK, CKDN1A, CDKN2A andBIN1.
- [021] The present invention also provides a method of inhibiting hedgehog signaling by contacting a cell with an EZH2 inhibitor. The EZH2 inhibitor can be in an amount sufficient to reduce expression of GLI1 and/or PTCH1.
- [022] The present invention also provides a method of inducing gene expression by contacting a cell with an EZH2 inhibitor. The EZH2 inhibitor can be in an amount sufficient to induce neuronal differentiation, cell cycle inhibition and/or tumor suppression. For example, the gene can be CD133, DOCK4, PTPRK, CKDN1A, CKDN2A or BIN1.
- [023] The present invention also provides a method of inhibiting gene expression by contacting a cell with an EZH2 inhibitor. The EZH2 inhibitor is in an amount sufficient to inhibit hedgehog signaling. For example, the gene can be GLI1 or PTCH1.
- [024] For example, the cell may have loss of function of SNF5, ARID1A, ATRX, and/or a component of the SWI/SNF complex.
- [025] For example, the loss of function is caused by a deletion of SNF5.
- [026] For example, the cell is a cancer cell. The cancer can be medulloblastoma, malignant rhabdoid tumor or atypical teratoid rhabdoid tumor.
- [027] For example, the EZH2 inhibitor is Compound A having the following formula:

(A), stereoisomers thereof, or pharmaceutically acceptable salts

or solvates thereof.

[028] For example, the EZH2 inhibitor is Compound B having the following formula:

(B), stereoisomers thereof, or pharmaceutically acceptable salts or

solvates thereof.

[029] For example, the EZH2 inhibitor is Compound C having the following formula:

(C), stereoisomers thereof, or pharmaceutically acceptable salts

or solvates thereof.

[030] For example, the EZH2 inhibitor is Compound D having the following formula:

(D), stereoisomers thereof, or pharmaceutically acceptable salts

or solvates thereof.

[031] For example, the EZH2 inhibitor is Compound E having the following formula:

(E), stereoisomers thereof, or pharmaceutically

acceptable salts or solvates thereof.

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.

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

BRIEF DESCRIPTIONS OF FIGURES

- [034] Figures 1A and 1B are a series of Western blot analyses of cell lines with wild type (RD and SJCRH30) and mutant SNF5.
- [035] Figures 2A-2E are a series of graphs establishing that SNF5 mutant cell lines A204 (C), G401 (D) and G402 (E) selectively respond to EZH2 compound (Compound E) compared to wild type cell lines RD (A) and SJCRH30 (B).
- [036] Figures 3A-3D are a series of bar graphs showing that G401 SNF mutant cell line is responding to Compound E after 7 days in soft agar compared to wild type cells RD. A shows cell line RD (5,000 cells/well). B shows G401 cells (5,000 cells/well). C shows G401 cells in 2D growth. D shows G401 cells (10,000 cells/well).
- [037] Figures 4A-4D are four graphs showing that G401 SNF5 mutant cell line is sensitive to Compound A in vitro. Wild type cell line SJCRH30 (A) and RD (C) and SNF5 mutant cell line G401 (B) and A204 (D) were pretreated for 7 days with indicated concentrations of Compound A and replated on day 0. Cell viability was determined by CellTiter-Glo® Luminescent Cell Viability Assay.
- [038] Figures 5A-5D are a series of graphs showing durable regressions in G401 xenografts (malignant rhabdoid tumor model) with Compound A treatment. (A) Tumor regressions induced by Compound A at the indicated doses. (B) Tumor regressions induced by twice daily administration of Compound A at the indicated doses. Data represent the mean values \pm SEM (n=8). Compound administration was stopped on day 28. (C) EZH2 target inhibition in G401 xenograft tumor tissue collected from a parallel cohort of mice on day 21. Each point shows the ratio of H3K27Me3 to total H3. Horizontal lines represent group mean values. BLLQ = below lower limit of quantification. (D, E) Immunohistochemical staining of tumor histone methylation of tumor samples from the vehicle treated (D) and Compound A treated (E) (at 125 mg/kg) mice.
- [039] Figure 6 is a graph showing the locations of ATRX mutations identified in SCLC cell lines.
- [040] Figure 7A is a graph showing that LNCAP prostate cancer cells display dose-dependent cell growth inhibition with Compound E treatment *in vitro*.
- [041] Figure 7B is a graph showing IC50 value of Compound E at day 11 and day 14 for WSU-DLCL2 and LNCAP cells.

[042] Figures 8A-8C are three graphs establishing that ATRX mutant SCLC lines NCI-H446 (A), SW1271 (B) and NCI-H841 (C) are responding to Compound E.

- [043] Figures 9A-9C are three microscopy images showing that SCLC line NCI-H841 changes morphology after treatment with vehicle (A) or Compound E at concentration of 4.1E-02 uM (B) or 3.3 uM (C).
- [044] Figures 10A-10C are a series of graphs showing effects of Compound A on cellular global histone methylation and cell viability. (A) Chemical structure of Compound A. (B) Concentration-dependent inhibition of cellular H3K27Me3 levels in G401 and RD cells. (C) Selective inhibition of proliferation of *SMARCB1*-deleted G401 cells by Compound A in vitro (measured by ATP content). G401 (panels a and b) and RD cells (panels c and d) were replated at the original seeding densities on day 7. Each point represents the mean for each concentration (n=3).
- [045] Figures 11A and 11B are a series of graphs showing biochemical mechanism of action studies. The IC₅₀ value of Compound A increases with increasing SAM concentration (A) and is minimally affected by increasing oligonucleosome concentration (B), indicating SAM-competitive and nucleosome-noncompetitive mechanism of action.
- [046] Figures 12A and 12B are a series of panels demonstrating verification of SMARCB1 and EZH2 expression in cell lines and specificity of Compound A for inhibition of cellular histone methylation. (A) Cell lysates were analyzed by immunoblot with antibodies specific to SMARCB1, EZH2 and Actin (loading control). (B) Selective inhibition of cellular H3K27 methylation in G401 and RD cells. Cells were incubated with Compound A for 4 days, and acid-extracted histones were analyzed by immunoblot.
- Figures 13A and 13B are a series of bar graphs demonstrating that Compound A induces G_1 arrest and apoptosis in *SMARCB1*-deleted MRT cells. Cell cycle analysis (by flow cytometry) and determination of apoptosis (by TUNEL assay) in RD (panel A) or G401 cells (panel B) during incubation with either vehicle or 1 μ M Compound A for up to 14 days. G_1 arrest was observed as of day 7 and apoptosis was induced as of day 11. Data are represented as mean values \pm SEM (n=2). The DMSO control values shown are the average \pm SEM from each time point. Cells were split and re-plated on days 4, 7 and 11 at the original seeding density.

[048] Figures 14A-14B are a series of graphs showing that Compound A induces changes in expression of SMARCB1 regulated genes and cell morphology. (A) Basal expression of SMARCB1 regulated genes in G401 *SMARCB1*-deleted cells, relative to RD control cells (measured by qPCR, n=2). (B) G401 and RD cells were incubated with either DMSO or 1 μM Compound A for 2, 4 and 7 days. Gene expression was determined by qPCR (n=2) and is expressed relative to the DMSO control of each time point. Panels a-j correspond to genes GLI1, PTCh1, DOCK4, CD133, PTPRK, BIN1, CDKN1A, CDKN2A, EZH2, and MYC, respectively. (C) G402 cells were incubated with either DMSO (left panel) or 1 μM Compound A (right panel) for 14 days. Cells were split and re-plated to the original seeding density on day 7.

[049] Figures 15A-15D are series of graphs demonstrating body weights, tumor regressions and plasma levels in G401 xenograft bearing mice treated with Compound A. (A) Body weights were determined twice a week for animals treated with Compound A on a BID schedule for 28 days. Data are presented as mean values \pm SEM (n=16 until day 21, n=8 from day 22 to 60). (B) Tumor regressions induced by twice daily (BID) administration of Compound A for 21 days at the indicated doses (mean values \pm SEM, n=16). * p <0.05, ** p < 0.01, repeated measures ANOVA, Dunnett's post-test vs. vehicle. (C) Tumor weights of 8 mice euthanized on day 21. **** p < 0.0001, Fisher's exact test. (D) Plasma was collected 5 min before and 3 h after dosing of Compound A on day 21, and compound levels were measured by LC-MS/MS. Animals were euthanized, and tumors were collected 3 h after dosing on day 21. Tumor homogenates were generated and subjected to LC-MS/MS analysis to determine Compound A concentrations. Note that tumor compound levels could not be determined from all animals especially in the higher dose groups because the xenografts were too small on day 21. Dots represent values for the individual animals; horizontal lines represent group mean values.

[050] Figures 16A-16C are a series of graphs showing that Compound A eradicates SMARCB1-deleted MRT xenografts in SCID mice. (A) Tumor regressions induced by twice daily (BID) administration of Compound A for 28 days at the indicated doses. Compound administration was stopped on day 28 and tumors were allowed to re-grow until they reached 2000 mm³ (data shown as mean values \pm SEM, n=8). (B) EZH2 target inhibition in G401 xenograft tumor tissue collected from mice euthanized on day 21. Each point shows the ratio

of H3K27Me3 to total H3, measured by ELISA. Horizontal lines represent group mean values; grey symbols are values outside of the ELISA standard curve. (C) Change in gene expression in G401 xenograft tumor tissue collected from mice treated with Compound A for 21 days. Panels a-d correspond to genes CD133, PTPRK, DOCK4, and GLI1, respectively. Data are presented as fold change compared to vehicle \pm SEM (n=6, n=4 for 500 mg/kg group). * p < 0.05, ** p < 0.01, **** p < 0.0001, vs. vehicle, Fisher's exact test.

DETAILED DESCRIPTION OF THE INVENTION

- effectively treat SWI/SNF-associated cancers that are characterized by altered expressions and/or loss of function of certain biomarkers or genes. Specifically, tumors or tumor cells having altered expressions and/or loss of function of selected biomarkers or genesare sensitive to the EZH2 inhibitors of the present invention. Accordingly, the present invention provides methods of treating or alleviating a symptom of cancers in a subject by administering a therapeutically effective amount of an EZH2 inhibitor to the subject, particular treating cancers associated with altered expression and/or loss of function of certain biomarkers or genes. For example, the biomarker is one component of the SWI/SNF complex. For example, the gene is selected from the group consisting of neuronal differentiation genes, cell cycle gene inhibition genes, tumor suppressor genes, hedgehog pathway genes, myc pathway genes and histone methyltransferase genes.
- The SWI/SNF complex in human includes at least evolutionarily conserved core subunits and variant subunits. Evolutionarily conserved core subunits include SNF5 (also called SMARCB1, INI1 or BAF47), SMARCA4 (also known as BRM/SWI2-related gene 1, BRG1), BAF155, and BAF170. Variant subunits include BAF53 (A or B), BAF60 (A, B or C), BAF 57, BAF45 (A, B, C, or D). Other subunits include ARIDI1A (also known as SMARCF1), ARID1B, SMARCA2 (also known as brahma homologue, BRM), ATRX, BAF200, BAF180 (also known as PBRM1), and bromodomain-containing 7 (BRD7). The at least one component of the SWI/SNF complex can by any component of the complex, for example, the component/subunit described herein or known in the art.

In any methods presented herein, neuronal differentiation gene may be, but is not limited to, CD133 (also called PROM1), DOCK4, PTPRK, PROM2, LHX1, LHX6, LHX9, PAX6, PAX7, VEFGA, FZD3B, FYN, HIF1A, HTRA2, EVX1, CCDC64, or GFAP. [054] In any methods presented herein, cell cycle inhibition gene may be, but is not limited to, CKDN1A, CDKN2A, MEN1, CHEK1, IRF6, ALOX15B, CYP27B1, DBC1, NME6, GMNN, HEXIM1, LATS1, MYC, HRAS, TGFB1, IFNG, WNT1, TP53, THBS1, INHBA, IL8, IRF1, TPR, BMP2, BMP4, ETS1, HPGD, BMP7, GATA3, NR2F2, APC, PTPN3, CALR, IL12A, IL12B, PML, CDKN2B, CDKN2C, CDKN1B, SOX2, TAF6, DNA2, PLK1, TERF1, GAS1, CDKN2D, MLF1, PTEN, TGFB2, SMAD3, FOXO4, CDK6, TFAP4, MAP2K1, NOTCH2, FOXC1, DLG1, MAD2L1, ATM, NAE1, DGKZ, FHL1, SCRIB, BTG3, PTPRK, RPS6KA2, STK11, CDKN3, TBRG1, CDC73, THAP5, CRLF3, DCUN1D3, MYOCD, PAF1, LILRB1, UHMK1, PNPT1, USP47, HEXIM2, CDK5RAP1, NKX3-1, TIPIN, PCBP4, USP44, RBM38, CDT1, RGCC, RNF167, CLSPN, CHMP1A, WDR6, TCF7L2, LATS2, RASSF1, MLTK, MAD2L2, FBXO5, ING4, or TRIM35.

- [055] In any methods presented herein, tumor suppressor gene may be, but is not limited to, BIN1. As used herein, the term "tumor suppressor gene" has its commonly understood meaning in the art, i.e. a gene whose expression and normal function act to suppress the neoplastic phenotype or induce apoptosis, or both. In some embodiments, tumor suppressor genes include cell cycle inhibition genes. Exemplary categories of tumor suppressors based on their functions include, but not limited to:
- (1) genes that inhibit cell cycles;

[053]

- (2) genes that are coupling the cell cycle to DNA damage. When there is damaged DNA in the cell, the cell should not divide. If the damage can be repaired, the cell cycle can continue. If the damage cannot be repaired, the cell should initiate apoptosis (programmed cell death);
- (3) genes that prevent tumor cells from dispersing, block loss of contact inhibition, and inhibit metastasis. These genes and their encoded proteins are also known as metastasis suppressors;
- (4) DNA repair proteins. Mutations in these genes increase the risk of cancer.
- In any methods presented herein, hedgehog signaling pathway gene may be, but is [056] not limited to, GLI1, PTCH1, SUFU, KIF7, GLI2, BMP4, MAP3K10, SHH, TCTN3, DYRK2, PTCHD1, or SMO.

In any methods presented herein, myc pathway gene may be, but is not limited to, MYC NMI, NFYC, NFYB, Cyclin T1, RuvB-like 1, GTF2I, BRCA1, T-cell lymphoma invasion and metastasis-inducing protein 1, ACTL6A, PCAF, MYCBP2, MAPK8, Bcl-2, Transcription initiation protein SPT3 homolog, SAP130, DNMT3A, mothers against decapentaplegic homolog 3, MAX, mothers against decapentaplegic homolog 2, MYCBP, HTATIP, ZBTB17, Transformation/transcription domain-associated protein, TADA2L, PFDN5, MAPK1, TFAP2A, P73, TAF9, YY1, SMARCB1, SMARCA4, MLH1, EP400 or let-7.

[058] In any methods presented herein, histone methyltransferase gene may be, but is not limited to, EZH2.

Compounds of the present invention inhibit the histone methyltransferase activity [059] of EZH2 or a mutant thereof and, accordingly, in one aspect of the invention, compounds disclosed herein are candidates for treating or preventing certain conditions and diseases. The present invention provides methods for treating, preventing or alleviating a symptom of cancer or a precancerous condition. 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, polymorph, solvate, or stereoisomeror thereof. Exemplary cancers that may be treated include medulloblastoma, oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and not otherwise specified (NOS) sarcoma. Alternatively, cancers to be treated by the compounds of the present invention are non NHL cancers.

[060] The present invention further provides the use of a compound of the present invention, or a pharmaceutically acceptable salt, polymorph or solvate thereof in the treatment of cancer or precancer, or, for the preparation of a medicament useful for the treatment of such cancer or pre-cancer. Exemplary cancers that may be treated include medulloblastoma,

oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and not otherwise specified (NOS) sarcoma. Alternatively, the compound of the present invention can be used for the treatment of non NHL cancers, or, for the preparation of a medicament useful for the treatment of non NHL cancers.

[061] The compounds of this invention can be used to modulate protein (*e.g.*, histone) methylation, *e.g.*, to modulate histone methyltransferase or histone demethylase enzyme activity. The compounds of the invention can be used *in vivo* or *in vitro* for modulating protein methylation. Based upon the surprising discovery that methylation regulation by EZH2 involves in tumor formation, particular tumors bearing altered expression and/or loss of function of selected biomarkers/genes, the compounds described herein are suitable candidates for treating these diseases, *i.e.*, to decrease methylation or restore methylation to roughly its level in counterpart normal cells.

In some embodiments, compounds of the present invention can selectively inhibit proliferation of the SWI/SNF complex associated tumor or tumor cells (as shown in Figures 1-9). Accordingly, the present invention provides methods for treating, preventing or alleviating a symptom of the SWI/SNF complex associated cancer or a precancerous condition by a compound of the present invention, or a pharmaceutically acceptable salt, polymorph or solvate thereof. The present invention further provides the use of a compound of the present invention, or a pharmaceutically acceptable salt, polymorph or solvate thereof in the treatment of the SWI/SNF complex associated cancer or a precancer condition, or, for the preparation of a medicament useful for the treatment of such cancer or pre-cancer.

[063] Also provided in the present invention are methods for determining responsiveness of a subject having a cancer to an EZH2 inhibitor. The method includes the steps of obtaining

a sample (a nucleic acid sample or a protein sample) from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex, detecting the expression and/or function of this component, and the presence of such reduced expression, haploinsufficiency, and/or loss of function indicates that the subject is responsive to the EZH2 inhibitor. The term "sample" 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. 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.

[064] The present invention also provides methods for determining predisposition of a subject to a cancer or a precancerous condition by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex, and the presence of such reduced expression, haploinsufficiency, and/or loss of function indicates that the subject is predisposed to (*i.e.*, having higher risk of) developing the cancer or the precancerous condition compared to a subject without such loss of function of the at least one component of the SWI/SNF complex.

[065] 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 reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex, will suffer cancer or a precancerous condition, as compared to the probability that another subject not having reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex, will suffer cancer or a precancerous condition, under circumstances where other risk factors (*e.g.*, chemical/environment, food, and smoking history, etc.) for having cancer or a precancerous condition between the subjects are the same.

[066] "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.

[067] Accordingly, the present invention provides personalized medicine, treatment and/or cancer management for a subject by genetic screening of reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex 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 an EZH2 inhibitor and when the subject is responsive to the EZH2 inhibitor, administering to the subject a therapeutically effective amount of the EZH2 inhibitor, or a pharmaceutically acceptable salt, solvate, or stereoisomeror thereof. The responsiveness is determined by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex (such as SNF5, ARID1A or ATRX), and the presence of such loss of function indicates that the subject is responsive to the EZH2 inhibitor.

[068] 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 includesteps of obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of at least one component of the SWI/SNF complex, and the presence of such reduced expression, haploinsufficiency, and/orloss of function indicates that the subject is predisposed to developing the cancer or the precancerous condition compared to a subject without such reduced expression, haploinsufficiency, and/orloss of function of the at least one component of the SWI/SNF complex.

[069] In merely illustrative embodiments, the methods of treatment presented herein include steps of (a) collecting a nucleic acid sample or a protein sample from a biological sample obtained from a subject, (b) measuring the expression level or function level of a component of the SWI/SNF complex in the sample, (c) measuring the expression level or

function level of the component of the SWI/SNF in a control sample; (d) comparing the expression level or the function level of the component measured in step (b) in the tested sample to the expression level or the function level of the component measured in step (c) in the control sample (or a reference value); (e) identifying the subject as a candidate for treatment when the expression level or the function level of the component measured in step (b) is reduced or lost (e.g.,haploinsufficiency or loss of function) compared to the expression level or the function level of the component measured in step (c); and (f) administering a therapeutically effective amount of an EZH2 inhibitor to the subject identified in step (e) or selecting a treatment regimen for the subject identified in step (e). The expression level or the functionlevel of component in the subject sample is reduced, for example, 10%, 25%, 50% or 1-, 2-, 5- or more fold compared to the expression level or the functionlevel of the component in the control sample. Any suitable methods known in the art can be utilized to measure the expressionlevel or the function level of the component of the SWI/SNF complex. In some embodiments, the subject has malignant rhabdoid tumor, medulloblastoma or atypical teratoid rhabdoid tumor. In some embodiments, the component is SNF5, ARID1A or ATRX.

[070] For example, the identified subject can be treated with the standard of care treatment as described in the most current National Comprehensive Cancer Network (NCCN) guidelines.

[071] For example, a control sample is obtained from a healthy, normal subject. Alternatively, a control sample is obtained from a subject who is not suffering, has not been diagnosed, or isnot at risk of developing cancer associated with the SWI/SNF complex.

[072] In one preferred aspect, the present invention provides a method for treating or alleviating a symptom of cancer in a subject by determining responsiveness of the subject to an EZH2 inhibitor and administering to the subject a therapeutically effective amount of the EZH2 inhibitor if the subject is responsive to the EZH2 inhibitor and the subject has a cancer selected from the group consisting of brain and CNS cancer, kidney cancer, ovarian cancer, pancreatic cancer, leukemia, lymphoma, myeloma, and/or sarcoma. Such responsiveness is determined by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of SNF5, ARID1A, and/or ATRX, and the presence of the reduced expression, haploinsufficiency, and/or loss of function indicates the subject is responsive to the EZH2 inhibitor.

[073] In another preferred aspect, the present invention provides a method for treating or alleviating a symptom of malignant rhabdoid tumor in a subject by determining responsiveness of the subject to an EZH2 inhibitor and administering to the subject a therapeutically effective amount of the EZH2 inhibitor if the subject is responsive to the EZH2 inhibitor. Such responsiveness is determined by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of SNF5, ARID1A, and/or ATRX, and the presence of the reduced expression, haploinsufficiency, and/or loss of function indicates the subject is responsive to the EZH2 inhibitor.

In another preferred aspect, the present invention provides a method for treating or alleviating a symptom of medulloblastoma in a subject by determining responsiveness of the subject to an EZH2 inhibitor and administering to the subject a therapeutically effective amount of the EZH2 inhibitor if the subject is responsive to the EZH2 inhibitor. Such responsiveness is determined by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of SNF5, ARID1A, and/or ATRX, and the presence of the reduced expression, haploinsufficiency, and/or loss of function indicates the subject is responsive to the EZH2 inhibitor.

In another preferred aspect, the present invention provides a method for treating or alleviating a symptom of atypical teratoid rhabdoid tumor in a subject by determining responsiveness of the subject to an EZH2 inhibitor and administering to the subject a therapeutically effective amount of the EZH2 inhibitor if the subject is responsive to the EZH2 inhibitor. Such responsiveness is determined by obtaining a sample from the subject and detecting reduced expression, haploinsufficiency, and/or loss of function of SNF5, ARID1A, and/or ATRX, and the presence of the reduced expression, haploinsufficiency, and/or loss of function indicates the subject is responsive to the EZH2 inhibitor.

[076] Malignant rhabdoid tumors (MRTs) and atypical teratoid rhabdoid tumors (ATRTs) are extremely aggressive pediatric cancers of the brain, kidney, and soft tissues that are highly malignant, locally invasive, frequently metastatic, and particularly lethal. They are typically diploid and lack genomic aberrations; however, they are characterized by an almost complete penetrance of loss of SMARCB1 (also called SNF5, INI1 or BAF47), a core component of the SWI/SNF chromatin remodeling complex. The biallelic inactivation of *SMARCB1* is in

essence the sole genetic event in MRTs and ATRTs which suggests a driver role for this genetic aberration.

[077] Without being bound by any theory, a compound of the present invention specifically inhibits cellular H3K27 methylation leading to selective apoptotic killing of SMARCB1 mutant MRT cells. For example, *in vitro* treatment of *SMARCB1*-deleted MRT cell lines with Compound A induced strong anti-proliferative effects with IC₅₀ values in the nM range; while the control (wild-type) cell lines were minimally affected (Figure 10C and table 6). Furthermore, the compound of the present invention induces genes of neuronal differentiation, cell cycle inhibition and tumor suppression while suppressing expression of hedgehog pathway genes, MYC and EZH2. For example, Compound A treatment of G401 *SMARCB1*-deleted cells for up to 7 days strongly induced expression of *CD133*, *DOCK4* and *PTPRK* and upregulated cell cycle inhibitors *CDKN1A* and *CDKN2A* and tumor suppressor *BIN1*, all in a time-dependent manner (Figure 14B). Simultaneously, the expression of hedgehog pathway genes, *MYC* and *EZH2* were reduced. Notably, G402 *SMARCB1*-deleted cells exposed to Compound A for 14 days assumed a neuron-like morphology (Figure 14C).

[078] Accordingly, the present invention further provides methods of treating or alleviating a symptom of cancer in a subject in need thereof by (a) determining the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes and tumor suppressor genes in a sample obtained from the subject; (b) selecting a subject having a decreased expression level of at least one gene in step (a); and (c) administering to the subject selected in step (b) an effective amount of a compound of the invention, thus treating or alleviating a symptom of cancer in the subject.

[079] The present invention also provides methods of treating or alleviating a symptom of cancer in a subject in need thereof by (a) determining the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes in a sample obtained from the subject; (b) selecting a subject having an increased expression level of at least one gene in step (a); and (c) administering to the subject selected in step (b) an effective amount of a compound of the invention, thus treating or alleviating a symptom of cancer in the subject.

[080] Also provided herein are methods of selecting a cancer therapy for a subject in need thereof by (a) determining the expression level of at least one gene selected from the group

consisting of neuronal differentiation genes, cell cycle inhibition genes, and tumor suppressor genes in a sample obtained from the subject, and (b) selecting a cancer therapy when the subject has a decreased expression level of at least one gene in step (a), where the cancer therapy includes the administration of an effective amount of a compound of the invention to the subject.

[081] The present invention further provides methods of selecting a cancer therapy for a subject in need thereof by (a) determining the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes in a sample obtained from the subject, and (b) selecting a cancer therapy when the subject has an increased expression level of at least one gene in step (a), where the cancer therapy includes the administration of an effective amount of a compound of the invention to the subject.

In merely illustrative embodiments, the methods presented herein may include the steps of (a) collecting a nucleic acid or a protein sample from a biological sample obtained from a subject, (b) measuring the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes, and tumor suppressor genes in the sample, (c) measuring the expression levelof the same gene(s) in a control sample; (d) comparing the expression level of the gene measured in step (b) in the tested sample to the expression level of the gene measured in step (c) in the control sample (or to a reference value); (e) identifying the subject as a candidate for treatment when the expression level of the component measured in step (b) is reduced compared to the expression level of the gene measured in step (c); and (f) administering a therapeutically effective amount of an EZH2 inhibitor to the subject identified in step (e) or selecting a treatment regimen for the subject identified in step (e). The expression level of the gene in the tested subject is reduced, for example, 10%, 25%, 50% or 1-, 2-, 5- or more fold compared to the expression level of the gene in the control sample.

[083] In merely illustrative embodiments, the methods presented herein may include the steps of (a) collecting a nucleic acid or a protein sample from a biological sample obtained from a subject, (b) measuring the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes in the sample, (c) measuring the expression level of the same gene(s) in a control sample;

(d) comparing the expression level of the gene measured in step (b) in the tested sample to the expression level of the gene measured in step (c) in the control sample (or to a reference value); (e) identifying the subject as a candidate for treatment when the expression level of the component measured in step (b) is increased compared to the expression level of the gene measured in step (c); and (f) administering a therapeutically effective amount of an EZH2 inhibitor to the subject identified in step (e) or selecting a treatment regimen for the subject identified in step (e). The expression level of the gene in the tested subject is increased, for example, 10%, 25%, 50% or 1-, 2-, 5- or more fold compared to the expression level of the gene in the control sample.

The term "expression level" refers to protein, RNA, or mRNA level of a particular gene of interest. Any methods known in the art can be utilized to determine the expression level of a particular gene of interest. Examples include, but are not limited to, reverse transcription and amplification assays (such as PCR, ligation RT-PCR or quantitative RT-PCT), hybridization assays, Northern blotting, dot blotting, *in situ* hybridization, gel electrophoresis, capillary electrophoresis, column chromatography, Western blotting, immunohistochemistry, immunostaining, or mass spectrometry. Assays can be performed directly on biological samples or on protein/nucleic acids isolated from the samples. It is routine practice in the relevant art to carry out these assays. For example, the measuring step in any method described herein includes contacting the nucleic acid sample from the biological sample obtained from the subject with one or more primers that specifically hybridize to the gene of interest presented herein. Alternatively, the measuring step of any method described herein includes contacting the protein sample from the biological sample obtained from the subject with one or more antibodies that bind to the biomarker of the interest presented herein.

[085] A decreased expression level of a particular gene means a decrease in its expression level by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 1000%, 1500%, or more compared to a reference value or the expression level of this gene measured in a different (or previous) sample obtained from the same subject.

[086] An increased expression level of a particular gene means an increase in its expression level by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 1000%, 1500%, or

more compared to a reference value or the expression level of this gene measured in a different (or previous) sample obtained from the same subject.

A "reference or baseline level/value" as used herein can be used interchangeably and is meant to be relative to a number or value derived from population studies, including without limitation, such subjects having similar age range, disease status (e.g., stage), subjects in the same or similar ethnic group, or relative to the starting sample of a subject undergoing treatment for cancer. Such reference values can be derived from statistical analyses and/or risk prediction data of populations obtained from mathematical algorithms and computed indices of cancer. Reference indices can also be constructed and used using algorithms and other methods of statistical and structural classification.

[088] In some embodiments of the present invention, the reference or baseline value is the expression level of a particular gene of interest in a control sample derived from one or more healthy subjects or subjects who have not been diagnosed with any cancer.

[089] In some embodiments of the present invention, the reference or baseline value is the expression level of a particular gene of interest in a sample obtained from the same subject prior to any cancer treatment. In other embodiments of the present invention, the reference or baseline value is the expression level of a particular gene of interest in a sample obtained from the same subject during a cancer treatment. Alternatively, the reference or baseline value is a prior measurement of the expression level of a particular gene of interest in a previously obtained sample from the same subject or from a subject having similar age range, disease status (e.g., stage) to the tested subject.

In some embodiments, an effective amount means an amount sufficient to increase the expression level of at least one gene which is decreased in the subject prior to the treatment or an amount sufficient to alleviate one or more symptoms of cancer. For example, an effective amount is an amount sufficient to increase the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes, and tumor suppressor genes by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%,1000%, 1500%, or more compared to a reference value or the expression level without the treatment of any compound.

In some embodiments, an effective amount means an amount sufficient to decrease the expression level of at least one gene which is increased in the subject prior to the treatment or an amount sufficient to alleviate one or more symptoms of cancer. For example, an effective amount is an amount sufficient to decrease the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, MYC and EZH2 by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 1000%, 1500%, or more compared to a reference value or the expression level without the treatment of any compound.

- [092] 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. An effective amount for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician.
- [093] The present invention further provides a method of determining efficacy of a cancer treatment in a subject in need thereof by (a) measuring the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes, and tumor suppressor genes in a sample obtained from the subject, (b) comparing the expression level of at least one gene in step (a) to a reference value or a prior measurement, and (c) determining the efficacy of the cancer treatment based on the comparison step. An exemplary cancer treatment is administering a compound of the invention to the tested subject.
- [094] The treatment is effective when the tested subject has an increased expression of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes and tumor suppressor genes 1) compared to a reference value or a prior measurement; or 2) over the period of time being monitored, such as 1, 2, 3, 4, 5, 6, or 7 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or longer. When the existing treatment is not effective, a new treatment or an increased dosage of the existing treatment (for example, increasing the dosage of the compound administered to the subject) should be sought for the tested subject.
- [095] The present invention also provides a method of determining efficacy of a cancer treatment in a subject in need thereof by (a) measuring the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes in a sample obtained from the subject, (b) comparing the expression

level of at least one gene in step (a) to a reference value or a prior measurement, and (c) determining the efficacy of the cancer treatment based on the comparison step. An exemplary cancer treatment is administering an EZH2 inhibitor of the invention to the tested subject.

[096] For example, the treatment is effective when the tested subject has a decreased expression of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes 1) compared to a reference value or a prior measurement; or 2) over the period of time being monitored, such as 1, 2, 3, 4, 5, 6, or 7 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or longer. When the existing treatment is not effective, a new treatment or an increased dosage of the existing treatment (for example, increasing the dosage of the compound administered to the subject) should be sought for the tested subject.

[097] In any methods presented herein, cancer is 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, and prostate cancer. Preferably, cancer is selected from the group consisting of medulloblastoma, oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and not otherwise specified (NOS) sarcoma. More preferably, cancer is medulloblastoma, malignant rhabdoid tumor, or atypical teratoid rhabdoid tumor.

[098] 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 EZH 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 EZH inhibitor, *e.g.*, tumor cells or tumor tissues of the subject undergo apoptosis and/or necrosis, and/or display reduced growing, dividing, or proliferation.

[099] As used herein, a "subject" is interchangeable with a "subject in need thereof", both of which refer to a subject having a disorder in which EZH2-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 with SWI/SNF complex. 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 SWI/SNFcomplex. A subject in need thereof can have cancer associated with loss of function in at least one component of SWI/SNF complex. 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, and prostate cancer. Preferably, a subject in need thereof has medulloblastoma, oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and not otherwise specified (NOS) sarcoma. Alternatively, a subject in need thereof has a non NHL cancer.

[0100] 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.

[0101] A subject in need thereof can be one who has not been previously diagnosed or identified as having cancer or a precancerous condition. 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 has 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).

[0102] 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.

[0103] 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.

A subject in need thereof may be one who had, is having or is predisposed to [0104] developing a cancer or a precancerous condition associated with the SWI/SNF complex. A subject in need thereof may be one who had, is having or is predisposed to developing cancer or a precancerous condition associated with loss of function of at least one component of the SWI/SNF complex. In a preferred aspect, a subject in need thereof is one who had, is having or is predisposed to developing 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, and prostate cancer. Preferably, a subject in need thereof is one who had, is having or is predisposed to developing brain and CNS cancer, kidney cancer, ovarian cancer, pancreatic cancer, leukemia, lymphoma, myeloma, and/or sarcoma. Exemplary brain and central CNS cancer includes medulloblastoma, oligodendroglioma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, and pineoblastoma. Exemplary ovarian cancer includes ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, and ovarian serous adenocarcinoma. Exemplary pancreatic cancer includes pancreatic ductal adenocarcinoma and pancreatic endocrine tumor. Exemplary sarcoma

includes chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, and not otherwise specified (NOS) sarcoma. Alternatively, cancers to be treated by the compounds of the present invention are non NHL cancers.

Alternatively, a subject in need thereof is one who had, is having or is predisposed [0105] to developing one or more cancers selected from the group consisting of medulloblastoma, oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, and not otherwise specified (NOS) sarcoma. Preferably, a subject is one who had, is having or is predisposed to developing medulloblastoma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, pancreatic ductal adenocarcinoma, malignant rhabdoid tumor, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, glioblastoma, meningioma, pineoblastoma, carcinosarcoma, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, ewing sarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and/or NOS sarcoma. More preferably, a subject in need thereof is one who had, is having or is predisposed to developing malignant rhabdoid tumor, medulloblastoma and/or atypical teratoid rhabdoid tumor.

[0106] In some embodiments of the present invention, a subject in need thereof has a decreased expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes, and tumor suppressor genes.

[0107] In some embodiments, a subject in need thereof has an increased expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes.

[0108] In some embodiments of the present invention, a subject in need thereof has loss of function of at least one component/subunit of the SWI/SNF complex. Alternatively, a subject

in need thereof has reduced expression or haploinsufficiency of at least one component/subunit of the SWI/SNF complex. In certain embodiments, a subject in need thereof has loss of function of SNF5 subunit.

[0109] In any method of the present invention, a subject in need thereof may have reduced expression, haploinsufficiency or loss of function of at least one signaling component downstream of SWI/SNF complex. Such downstream component includes, but is not limited to, polycomb complex (PcG) and its targets.

[0110] As used herein, the term "loss of function" refers to less or no function of a gene product/protein compared to the wild type. Loss of function of a SWI/SNF complex component means the component/subunit or the entire SWI/SNF complex has less or no biological function compared to the wild type component/subunit or the entire SWI/SNF complex, respectively. Loss of function can be caused by transcriptional, post-transcription, or post translational mechanisms. In one aspect of the present invention, loss of function is caused by loss of function mutation resulted from a point mutation (e.g., a substitution, a missense mutation, or a nonsense mutation), an insertion, and/or a deletion in a polypeptide of a SWI/SNF complex component or a nucleic acid sequence encoding a polypeptide of a SWI/SNF complex component. 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.

[0111] Accordingly, a loss of function mutation or a reduced expression can be detected using any suitable method available in the art. For example, a loss of function mutation can be detected by measuring the biological function of a gene product, such as the ATP-dependent chromatin remodeling activity of the SWI/SNF complex. Alternatively, a loss of function mutation can be determined by detecting any alternation in a nucleic acid sequence encoding a component of the SWI/SNF complex. For example, a nucleic acid sequence encoding a

component of the SWI/SNF complex having a loss 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). Alternatively or in addition, a nucleic acid sequence encoding a SWI/SNF polypeptide having a loss of function mutation may be detected using a Southern blot in accordance with methods well known in the art. Optionally, a loss of function mutation can be detected by measuring the absence of the expression of a component polypeptide or by measuring the expression of the mutant component polypeptide. Detection of (mutant) polypeptide expression can be carried out with any suitable immunoassay in the art, such as Western blot analysis.

- [0112] Human nucleic acid and amino acid sequence of components of the SWI/SNF complex have previously been described. *See*, *e.g.*, GenBank Accession Nos NP_003064.2, NM_003073.3, NP_001007469.1, andNM_001007468.1 for SNF5, GenBank Accession Nos NM_000489.3, NP_000480.2, NM_138270.2, andNP_612114.1 for ATRX, GenBank Accession Nos NP_006006.3, NM_006015.4, NP_624361.1, and NM_139135.2 for ARID1A, each of which is incorporated herein by reference in its entirety.
- [0113] Spectrum of hSNF5 somatic mutations in human has also been described in Sevenet *et al.*, Human Molecular Genetics, 8: 2359-2368, 1999, which is incorporated herein by reference in its entirety.
- [0114] A subject in need thereof may have reduced expression, haploinsufficiency, and/or loss of function of SNF5. For example, a subject can comprise a deletion of SNF5 in SNF5 polypeptide or a nucleic acid sequence encoding a SNF5 polypeptide.

SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 isoform a (SMARCB1, also called SNF5) [Homo sapiens] (SEQ ID NO: 1)

- 1 mmmmalsktf gqkpvkfqle ddgefymigs evgnylrmfr gslykrypsl wrrlatveer
- 61 kkivasshgk ktkpntkdhg yttlatsvtl lkaseveeil dgndekykav sistepptyl
- 121 reqkakrnsq wvptlpnssh hldavpcstt inrnrmgrdk krtfplcfdd hdpavihena

```
181 sqpevlvpir ldmeidgqkl rdaftwnmne klmtpemfse ilcddldlnp ltfvpaiasa
241 irqqiesypt dsiledqsdq rviiklnihv gnislvdqfe wdmsekensp ekfalklcse
301 lglggefvtt iaysirgqls whqktyafse nplptveiai rntgdadqwc plletltdae
361 mekkirdqdr ntrrmrrlan tapaw
```

Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 (SMARCB1, also called SNF5), transcript variant 1, mRNA (SEQ ID NO: 2)

```
1 aacgccagcg cctgcgcact gagggcggcc tggtcgtcgt ctgcggcggc ggcggcgct
  61 gaggageeeg getgaggege eagtaeeegg eeeggteege atttegeett eeggettegg
 121 tttccctcgg cccagcacgc cccggccccg ccccagccct cctgatccct cgcagcccgg
 181 ctccggccgc ccgcctctgc cgccgcaatg atgatgatgg cgctgagcaa gaccttcggg
 241 cagaageeeg tgaagtteea getggaggae gaeggegagt tetacatgat eggeteegag
 301 gtgggaaact acctccgtat gttccgaggt tctctgtaca agagataccc ctcactctgg
 361 aggegaetag eeactgtgga agagaggaag aaaatagttg categteaca tggtaaaaaa
 421 acaaaaccta acactaagga tcacggatac acgactctag ccaccagtgt gaccctgtta
 481 aaagcctcgg aagtggaaga gattctggat ggcaacgatg agaagtacaa ggctgtgtcc
 541 atcagcacag agcccccac ctacctcagg gaacagaagg ccaagaggaa cagccagtgg
 601 gtacccacce tgcccaacag ctcccaccac ttagatgccg tgccatgctc cacaaccatc
 661 aacaggaacc gcatgggccg agacaagaag agaaccttcc ccctttgctt tgatgaccat
 721 gacccagctg tgatccatga gaacgcatct cagcccgagg tgctggtccc catccggctg
 781 gacatggaga tegatgggca gaagetgega gacgeettea eetggaacat gaatgagaag
 841 ttgatgacgc ctgagatgtt ttcagaaatc ctctgtgacg atctggattt gaacccgctg
 901 acgtttgtgc cagccatcgc ctctgccatc agacagcaga tcgagtccta cccacggac
961 agcatectgg aggaceagte agaceagege gteateatea agetgaaeat eeatgtggga
1021 aacattteee tggtggacca gtttgagtgg gacatgteag agaaggagaa eteaceagag
1081 aagtttgeee tgaagetgtg eteggagetg gggttgggeg gggagtttgt caccaccate
1141 gcatacagca tccggggaca gctgagctgg catcagaaga cctacgcctt cagcgagaac
1201 cctctgccca cagtggagat tgccatccgg aacacgggcg atgcggacca gtggtgccca
1261 ctgctggaga ctctgacaga cgctgagatg gagaagaaga tccgcgacca ggacaggaac
1321 acgaggcgga tgaggcgtct tgccaacacg gccccggcct ggtaaccagc ccatcagcac
1381 acggctccca cggagcatct cagaagattg ggccgcctct cctccatctt ctggcaagga
1441 cagaggegag gggacageee agegeeatee tgaggategg gtgggggtgg agtggggget
1501 tecaggtgge cettecegge acacatteca tttgttgage eccagteetg ecceecacee
1561 caccetecet acceetecee agtetetggg gteaggaaga aacettattt taggttgtgt
1621 tttgtttttg tataggagcc ccaggcaggg ctagtaacag tttttaaata aaaggcaaca
1681 ggtcatgttc aatttcttca acaaaaaaa aaaaaaa
```

SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 isoform b [Homo sapiens] (SMARCB1, also called SNF5) (SEQ ID NO: 3)

```
1 mmmmalsktf gqkpvkfqle ddgefymigs evgnylrmfr gslykrypsl wrrlatveer 61 kkivasshdh gyttlatsvt llkaseveei ldgndekyka vsisteppty lreqkakrns 121 qwvptlpnss hhldavpcst tinrnrmgrd kkrtfplcfd dhdpavihen asqpevlvpi 181 rldmeidgqk lrdaftwnmn eklmtpemfs eilcddldln pltfvpaias airqqiesyp 241 tdsiledqsd qrviiklnih vgnislvdqf ewdmsekens pekfalklcs elglggefvt 301 tiaysirgql swhqktyafs enplptveia irntgdadqw cplletltda emekkirdqd 361 rntrrmrla ntapaw
```

Homo sapiens SWI/SNF related, matrix associated, actin dependent regulator of chromatin,

subfamily b, member 1 (SMARCB1, also called SNF5), transcript variant 2, mRNA (SEQ ID NO: 4)

```
1 aacgccagcg cctgcgcact gagggcggcc tggtcgtcgt ctgcggcggc ggcggcgt
  61 gaggageeeg getgaggege eagtaeeegg eeeggteege atttegeett eeggettegg
 121 tttccctcgg cccagcacgc cccggccccg ccccagccct cctgatccct cgcagcccgg
181 ctccqqccqc ccqcctctqc cqccqcaatq atqatqatqq cqctqaqcaa qaccttcqqq
241 cagaagcccg tgaagttcca gctggaggac gacggcgagt tctacatgat cggctccgag
301 gtgggaaact acctccgtat gttccgaggt tctctgtaca agagataccc ctcactctgg
361 aggcgactag ccactgtgga agagaggaag aaaatagttg catcgtcaca tgatcacgga
421 tacacgactc tagccaccag tgtgaccctg ttaaaagcct cggaagtgga agagattctg
481 gatggcaacg atgagaagta caaggctgtg tccatcagca cagagccccc cacctacctc
541 agggaacaga aggccaagag gaacagccag tgggtaccca ccctgcccaa cagctcccac
601 cacttagatg ccgtgccatg ctccacaacc atcaacagga accgcatggg ccgagacaag
661 aagagaacct tccccctttg ctttgatgac catgacccag ctgtgatcca tgagaacgca
721 teteageeeg aggtgetggt ecceateegg etggacatgg agategatgg geagaagetg
781 cgagacgcct tcacctggaa catgaatgag aagttgatga cgcctgagat gttttcagaa
841 atcctctgtg acgatctgga tttgaacccg ctgacgtttg tgccagccat cgcctctgcc
901 atcagacage agategagte etaccecacg gacageatee tggaggacea gtcagaceag
961 cgcgtcatca tcaagctgaa catccatgtg ggaaacattt ccctggtgga ccagtttgag
1021 tgggacatgt cagagaagga gaactcacca gagaagtttg ccctgaagct gtgctcggag
1081 ctggggttgg gcggggagtt tgtcaccacc atcgcataca gcatccgggg acagctgagc
1141 tggcatcaga agacctacgc cttcagcgag aaccctctgc ccacagtgga gattgccatc
1201 cggaacacgg gcgatgcgga ccagtggtgc ccactgctgg agactctgac agacgctgag
1261 atggagaaga agateegega eeaggaeagg aacaegagge ggatgaggeg tettgeeaae
1321 acggccccgg cctggtaacc agcccatcag cacacggctc ccacggagca tctcagaaga
1381 ttgggccgcc tctcctccat cttctggcaa ggacagaggc gaggggacag cccagcgcca
1441 teetgaggat egggtggggg tggagtgggg getteeaggt ggeeetteee ggeacacatt
1501 ccatttgttg agecccagte etgeccecca ecceaecete ectaececte eccagtetet
1621 gggctagtaa cagtttttaa ataaaaggca acaggtcatg ttcaatttct tcaacaaaaa
1681 aaaaaaaaaa
```

[0115] A subject in need thereof may have reduced expression, haploinsufficiency, and/or loss of function of ATRX. For example, a subject can comprise a mutation selected from the group consisting of a substitution of asparagine (N) for the wild type residue lysine (K) at amino acid position 688 of SEQ ID NO: 5 (K688N), and a substitution of isoleucine (I) for the wild type residue methionine (M) at amino acid position 366 of SEQ ID NO: 5 (M366I).

```
Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (ATRX) isoform 1 (SEQ ID NO: 5)
```

```
1 mtaepmsesk lntlvqklhd flahsseese etsspprlam nqntdkisgs gsnsdmmens
61 keegtsssek skssgsrsk rkpsivtkyv esddekpldd etvnedasne nsenditmqs
121 lpkgtvivqp epvlnedkdd fkgpefrsrs kmktenlkkr gedglhgivs ctacgqqvnh
181 fqkdsiyrhp slqvlicknc fkyymsddis rdsdgmdeqc rwcaeggnli ccdfchnafc
241 kkcilrnlgr kelstimden nqwycyichp eplldlvtac nsvfenleql lqqnkkkikv
301 dseksnkvye htsrfspkkt ssncngeekk lddscsgsvt ysysalivpk emikkakkli
361 ettanmnssy vkflkqatdn seissatklr qlkafksvla dikkahlale edlnsefram
421 davnkekntk ehkvidakfe tkarkgekpc alekkdisks eaklsrkqvd sehmhqnvpt
481 eeqrtnkstg gehkksdrke epqyepants edldmdivsv pssvpedife nletamevqs
541 svdhqgdgss gteqevesss vklnisskdn rggiksktta kvtkelyvkl tpvslsnspi
601 kgadcqevpq dkdgykscgl npklekcglg qensdnehlv enevslllee
```

```
661 ttplrrptet npvtsnsdee cnetvkekqk lsvpvrkkdk rnssdsaidn pkpnklpksk
 721 qsetvdqnsd sdemlailke vsrmshssss dtdineihtn hktlydlktq agkddkgkrk
 781 rksstsgsdf dtkkgksaks siiskkkrgt gsessnydse lekeiksmsk igaarttkkr
 841 ipntkdfdss edekhskkqm dngqhknlkt sqegssddae rkqeretfss aegtvdkdtt
 901 imelrdrlpk kggasastdg vdklsgkegs ftslevrkva etkekskhlk tktckkvgdg
 961 lsdiaekflk kdgsdetsed dkkqskkqte ekkkpsdfkk kvikmeqqye sssdqteklp
1021 ereeichfpk gikgikngtt dgekkskkir dktskkkdel sdyaekstgk gdscdssedk
1081 kskngaygre kkrckligks srkrgdcsss dtekysmked gcnssdkrlk rielrerrnl
1141 sskrntkeig sgssssdaee ssednkkkkg rtsskkkavi vkekkrnslr tstkrkgadi
1201 tsssssdied ddqnsigegs sdeqkikpvt enlvlsshtg fcqssgdeal sksvpvtvdd
1261 ddddndpenr iakkmlleei kanlssdedg ssddepeegk krtgkgneen pgdeeakngv
1321 nsesdsdsee skkpryrhrl lrhkltvsdg esgeekktkp kehkevkgrn rrkvssedse
1381 dsdfqesgvs eevsesedeq rprtrsakka eleenqrsyk qkkkrrrikv qedsssenks
1441 nseeeeeeke eeeeeeeee eeeedendds kspgkgrkki rkilkddklr tetqnalkee
1501 eerrkriaer erereklrev ieiedasptk cpittklvld edeetkeplv gvhrnmvikl
1561 kphqvdgvqf mwdcccesvk ktkkspgsgc ilahcmglgk tlqvvsflht vllcdkldfs
1621 talvvcplnt alnwmnefek wqeglkddek levselatvk rpqersymlq rwqedggvmi
1681 igyemyrnla qgrnvksrkl keifnkalvd pgpdfvvcde ghilkneasa vskamnsirs
1741 rrriiltgtp lqnnlieyhc mvnfikenll gsikefrnrf inpiqngqca dstmvdvrvm
1801 kkrahilyem lagcvqrkdy taltkflppk heyvlavrmt siqcklyqyy ldhltgvgnn
1861 seggrgkaga klfqdfqmls riwthpwclq ldyiskenkg yfdedsmdef iasdsdetsm
1921 slssddytkk kkkqkkqkkd ssssqsqsdn dvevikvwns rsrqqqeqnv detqnnpsvs
1981 lkleeskats ssnpsspapd wykdfvtdad aevlehsgkm vllfeilrma eeigdkvlvf
2041 sqslisldli edflelasre ktedkdkpli ykgegkwlrn idyyrldgst taqsrkkwae
2101 efndetnvrg rlfiistkag slginlvaan rviifdaswn psydigsifr vyrfgqtkpv
2161 yvyrflaggt medkiydrqv tkqslsfrvv dqqqverhft mneltelytf epdllddpns
2221 ekkkkrdtpm lpkdtilael lqihkehivg yhehdslldh keeeelteee rkaawaeyea
2281 ekkgltmrfn iptgtnlppv sfnsqtpyip fnlgalsams nqqledling grekvveatn
2341 svtavriqpl ediisavwke nmnlseaqvq alalsrqasq eldvkrreai yndvltkqqm
2401 liscvqrilm nrrlqqqynq qqqqqmtyqq atlghlmmpk ppnlimnpsn yqqidmrgmy
2461 qpvaggmqpp plqrapppmr sknpgpsqgk sm
```

Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (ATRX), transcript variant 1, mRNA (SEQ ID NO: 6)

```
1 aatteteetg eetgageete ggeecaacaa aatggeggeg geageggtgt egetttgttt
  61 ccgcggctcc tgcggcggtg gcagtggtag cggcctttga gctgtgggga ggttccagca
 121 gcagctacag tgacgactaa gactccagtg catttctatc gtaaccgggc gcgggggagc
 181 gcagategge geceageaat caeagaagee gacaaggegt teaagegaaa acatgaeege
 241 tgagcccatg agtgaaagca agttgaatac attggtgcag aagcttcatg acttccttgc
 301 acactcatca gaagaatctg aagaaacaag ttctcctcca cgacttgcaa tgaatcaaaa
 361 cacagataaa atcagtggtt ctggaagtaa ctctgatatg atggaaaaca gcaaggaaga
 421 gggaactage tetteagaaa aateeaagte tteaggateg teaegateaa agaggaaace
 481 ttcaattgta acaaagtatg tagaatcaga tgatgaaaaa cctttggatg atgaaactgt
 541 aaatgaagat gegtetaatg aaaatteaga aaatgatatt aetatgeaga gettgeeaaa
601 aggtacagtg attgtacagc cagagccagt gctgaatgaa gacaaagatg attttaaagg
661 gcctgaattt agaagcagaa gtaaaatgaa aactgaaaat ctcaaaaaac gcggagaaga
 721 tgggcttcat gggattgtga gctgcactgc ttgtggacaa caggtcaatc attttcaaaa
 781 agattccatt tatagacacc cttcattgca agttcttatt tgtaagaatt gctttaagta
841 ttacatgagt gatgatatta gccgtgactc agatggaatg gatgaacaat gtaggtggtg
901 tgcggaaggt ggaaacttga tttgttgtga cttttgccat aatgctttct gcaagaaatg
961 cattctacgc aaccttggtc gaaaggagtt gtccacaata atggatgaaa acaaccaatg
1021 gtattgctac atttgtcacc cagageettt gttggaettg gtcactgcat gtaacagegt
1081 atttgagaat ttagaacagt tgttgcagca aaataagaag aagataaaag ttgacagtga
1141 aaagagtaat aaagtatatg aacatacatc cagattttct ccaaagaaga ctagttcaaa
```

```
1201 ttqtaatqqa qaaqaaaaqa aattaqatqa ttcctqttct qqctctqtaa cctactctta
1261 ttccgcacta attgtqccca aaqaqatqat taagaaqqca aaaaaactga ttgaqaccac
1321 agccaacatg aactccagtt atgttaaatt tttaaagcag gcaacagata attcagaaat
1381 cagttctqct acaaaattac qtcaqcttaa qqcttttaaq tctqtqttqq ctqatattaa
1441 gaaggeteat ettgeattgg aagaagaett aaatteegag tttegagega tggatgetgt
1501 aaacaaaqaq aaaaatacca aaqaqcataa aqtcataqat qctaaqtttq aaacaaaaqc
1561 acqaaaaqqa qaaaaacctt qtqctttqqa aaaqaaqqat atttcaaaqt caqaaqctaa
1621 actttcaaga aaacaggtag atagtgagca catgcatcag aatgttccaa cagaggaaca
1681 aagaacaaat aaaagtaccg gtggtgaaca taagaaatct gatagaaaag aagaacctca
1741 atatqaacct qccaacactt ctqaaqattt aqacatqqat attqtqtctq ttccttcctc
1801 agttccagaa gacatttttg agaatcttga gactgctatg gaagttcaga gttcagttga
1861 tcatcaaqqq qatqqcaqca qtqqaactqa acaaqaaqtq qaqaqttcat ctqtaaaatt
1921 aaatatttct tcaaaagaca acagaggagg tattaaatca aaaactacag ctaaagtaac
1981 aaaagaatta tatgttaaac tcactcctgt ttccctttct aattccccaa ttaaaggtgc
2041 tgattgtcag gaagttccac aagataaaga tggctataaa agttgtggtc tgaaccccaa
2101 gttagagaaa tgtggacttg gacaggaaaa cagtgataat gagcatttgg ttgaaaatga
2161 agtttcatta cttttagagg aatctgatct tcgaagatcc ccacgtgtaa agactacacc
2221 cttgaggcga ccgacagaaa ctaaccctgt aacatctaat tcagatgaag aatgtaatga
2281 aacagttaag gagaaacaaa aactatcagt tccagtgaga aaaaaggata agcgtaattc
2341 ttctgacagt gctatagata atcctaagcc taataaattg ccaaaatcta agcaatcaga
2401 gactgtggat caaaattcag attctgatga aatgctagca atcctcaaag aggtgagcag
2461 gatgagtcac agttcttctt cagatactga tattaatgaa attcatacaa accataagac
2521 tttgtatgat ttaaagactc aggcggggaa agatgataaa ggaaaaagga aacgaaaaag
2581 ttctacatct qqctcaqatt ttqatactaa aaaqqqcaaa tcaqctaaqa qctctataat
2641 ttctaaaaag aaacgacaaa cccagtctga gtcttctaat tatgactcag aattagaaaa
2701 agagataaag agcatgagta aaattggtgc tgccagaacc accaaaaaaa gaattccaaa
2761 tacaaaagat tttgactctt ctgaagatga gaaacacagc aaaaaaggaa tggataatca
2821 agggcacaaa aatttgaaga cctcacaaga aggatcatct gatgatgctg aaagaaaaca
2881 agagagaga actttctctt cagcagaagg cacagttgat aaagacacga ccatcatgga
2941 attaaqaqat cqacttccta aqaaqcaqca aqcaaqtqct tccactqatq qtqtcqataa
3001 gctttctggg aaagagcaga gttttacttc tttggaagtt agaaaagttg ctgaaactaa
3061 agaaaagagc aagcatctca aaaccaaaac atgtaaaaaa gtacaggatg gcttatctga
3121 tattqcaqaq aaattcctaa aqaaaqacca qaqcqatqaa acttctqaaq atqataaaaa
3181 gcagagcaaa aagggaactg aagaaaaaaa gaaaccttca gactttaaga aaaaagtaat
3241 taaaatggaa caacagtatg aatcttcatc tgatggcact gaaaagttac ctgagcgaga
3301 agaaatttgt cattttccta agggcataaa acaaattaag aatggaacaa ctgatggaga
3361 aaagaaaagt aaaaaaataa gagataaaac ttctaaaaag aaggatgaat tatctgatta
3421 tgctgagaag tcaacaggga aaggagatag ttgtgactct tcagaggata aaaagagtaa
3481 gaatggagca tatggtagag agaagaaaag gtgcaagttg cttggaaaga gttcaaggaa
3541 gagacaagat tgttcatcat ctgatactga gaaatattcc atgaaagaag atggttgtaa
3601 ctcttctgat aagagactga aaagaataga attgagggaa agaagaaatt taagttcaaa
3661 gagaaatact aaggaaatac aaagtggctc atcatcatct gatgctgagg aaagttctga
3721 agataataaa aagaagaagc aaagaacttc atctaaaaag aaggcagtca ttgtcaagga
3781 gaaaaagaga aactccctaa gaacaagcac taaaaggaag caagctgaca ttacatcctc
3841 atcttcttct gatatagaag atgatgatca gaattctata ggtgagggaa gcagcgatga
3901 acagaaaatt aagcctgtga ctgaaaattt agtgctgtct tcacatactg gattttgcca
3961 atcttcagga gatgaagcct tatctaaatc agtgcctgtc acagtggatg atgatgatga
4021 cgacaatgat cctgagaata gaattgccaa gaagatgctt ttagaagaaa ttaaagccaa
4081 tettteetet gatgaggatg gatetteaga tgatgageea gaagaaggga aaaaaagaae
4141 tggaaaacaa aatgaagaaa acccaggaga tgaggaagca aaaaatcaag tcaattctga
4201 atcagattca gattctgaag aatctaagaa gccaagatac agacataggc ttttgcggca
4261 caaattgact gtgagtgacg gagaatctgg agaagaaaaa aagacaaagc ctaaagagca
4321 taaagaagtc aaaggcagaa acagaagaaa ggtgagcagt gaagattcag aagattctga
4381 ttttcaggaa tcaggagtta gtgaagaagt tagtgaatcc gaagatgaac agcggcccag
4441 aacaaggtct gcaaagaaag cagagttgga agaaaatcag cggagctata aacagaaaaa
4501 gaaaaggcga cgtattaagg ttcaagaaga ttcatccagt gaaaacaaga gtaattctga
```

4621	ggaagatgaa	aatgatgatt	ccaagtctcc	tggaaaaggc	agaaagaaaa	ttcggaagat	
		gataaactga					
4741	acgaaaacgt	attgctgaga	gggagcgtga	gcgagaaaaa	ttgagagagg	tgatagaaat	
4801	tgaagatgct	tcacccacca	agtgtccaat	aacaaccaag	ttggttttag	atgaagatga	
4861	agaaaccaaa	gaacctttag	tgcaggttca	tagaaatatg	gttatcaaat	tgaaacccca	
4921	tcaagtagat	ggtgttcagt	ttatgtggga	ttgctgctgt	gagtctgtga	aaaaaacaaa	
4981	gaaatctcca	ggttcaggat	gcattcttgc	ccactgtatg	ggccttggta	agactttaca	
5041	ggtggtaagt	tttcttcata	cagttctttt	gtgtgacaaa	ctggatttca	gcacggcgtt	
5101	agtggtttgt	cctcttaata	ctgctttgaa	ttggatgaat	gaatttgaga	agtggcaaga	
5161	gggattaaaa	gatgatgaga	agcttgaggt	ttctgaatta	gcaactgtga	aacgtcctca	
5221	ggagagaagc	tacatgctgc	agaggtggca	agaagatggt	ggtgttatga	tcataggcta	
5281	tgagatgtat	agaaatcttg	ctcaaggaag	gaatgtgaag	agtcggaaac	ttaaagaaat	
5341	atttaacaaa	gctttggttg	atccaggccc	tgattttgtt	gtttgtgatg	aaggccatat	
5401	tctaaaaaat	gaagcatctg	ctgtttctaa	agctatgaat	tctatacgat	caaggaggag	
5461	gattattta	acaggaacac	cacttcaaaa	taacctaatt	gagtatcatt	gtatggttaa	
5521	ttttatcaag	gaaaatttac	ttggatccat	taaggagttc	aggaatagat	ttataaatcc	
5581	aattcaaaat	ggtcagtgtg	cagattctac	catggtagat	gtcagagtga	tgaaaaaacg	
5641	tgctcacatt	ctctatgaga	tgttagctgg	atgtgttcag	aggaaagatt	atacagcatt	
5701	aacaaaattc	ttgcctccaa	aacacgaata	tgtgttagct	gtgagaatga	cttctattca	
5761	gtgcaagctc	tatcagtact	acttagatca	cttaacaggt	gtgggcaata	atagtgaagg	
5821	tggaagagga	aaggcaggtg	caaagctttt	ccaagatttt	cagatgttaa	gtagaatatg	
5881	gactcatcct	tggtgtttgc	agctagacta	cattagcaaa	gaaaataagg	gttattttga	
5941	tgaagacagt	atggatgaat	ttatagcctc	agattctgat	gaaacctcca	tgagtttaag	
		tatacaaaaa					
		ggcagtgaca					
		gaaggaaatg					
		aaagctactt					
		acagatgctg					
	_	cttcgaatgg			_		
		ctggacttga					
		aaacccctta				_	
	_	gatggttcca	_			_	
		aatgtgagag					
		gtagctgcta					
	-	agtatattca				_	
		gctcagggaa		_		_	
		tttcgagttg ctttatactt					
		gatactccca		_	-		
		cacattgtag				_	
	-	actgaagaag		-			
		atgcgtttca					
		ccttatattc			_	_	
		ctcattaatc					
		attcaacctc					
		gcccaagtac		_			
		agagaagcaa					
		cgaatactta					
		acttatcaac			_		
		aatccttcta	_			_	
		atgcagccac					
		tcccaaggga			_		
		aagatctttt		_	_		
		ggtcccctta					
		taccatttct					
		tacagaagat	_				
		ccacagattt			_		
							•

```
8041 ttgtcataaa attaataaat ttaggaaaga ataaagattt atatattcat tctttacata
 8101 taaaaacaca caqctqaqtt cttaqaqttq attcctcaaq ttatqaaata cttttqtact
 8161 taatccattt cttgattaaa gtgattgaaa tggttttaat gttcttttga ctgaagtctg
 8221 aaactgggct cctgctttat tgtctctgtg actgaaagtt agaaactgag ggttatcttt
 8281 gacacagaat tgtgtgcaat attettaaat actaetgete taaaagttgg agaagtettg
 8341 cagttatett ageattgtat aaacageett aagtatagee taagaagaga atteettttt
 8401 cttctttagt ccttctgcca ttttttattt tcagttatat gtgctgaaat aattactggt
 8461 aaaatttcag ggttgtggat tatcttccac acatgaattt tctctctcct ggcacgaata
 8521 taaagcacat ctcttaactg catggtgcca gtgctaatgc ttcatcctgt tgctggcagt
 8581 qqqatqtqqa cttaqaaaat caaqttctaq cattttaqta qqttaacact qaaqttqtqq
 8641 ttqttaqqtt cacaccctqt tttataaaca acatcaaaat qqcaqaacca ttqctqactt
 8701 taggttcaca tgaggaatgt acttttaaca attcccagta ctatcagtat tgtgaaataa
 8761 tteetetgaa agataagaat eactggette tatgegette ttttetetea teateatgtt
8821 cttttacccc agtttcctta catttttta aattgtttca gagtttgttt ttttttagt
8881 ttagattgtg aggcaattat taaatcaaaa ttaattcatc caatacccct ttactagaag
8941 ttttactaga aaatgtatta cattttattt tttcttaatc cagttctgca aaaatgacct
9001 ataaatttat tcatgtacaa ttttggttac ttgaattgtt aaagaaaaca ttgtttttga
9061 ctatgggagt caactcaaca tggcagaacc atttttgaga tgatgataca acaggtagtg
9121 aaacagctta agaattccaa aaaaaaaaaa aaaaaaaaa aaaagaaaac tgggtttggg
9181 ctttgcttta ggtatcactg gattagaatg agtttaacat tagctaaaac tgctttgagt
9241 tqtttqqatq attaaqaqat tqccattttt atcttqqaaq aactaqtqqt aaaacatcca
9301 agagcactag gattgtgata cagaatttgt gaggtttggt ggatccacgc ccctctccc
9361 cactttccca tqatqaaata tcactaataa atcctqtata tttaqatatt atqctaqcca
9421 tgtaatcaga tttatttaat tgggtggggc aggtgtgtat ttactttaga aaaaatgaaa
9481 aagacaagat ttatgagaaa tatttgaagg cagtacactc tggccaactg ttaccagttg
9541 gtatttctac aagttcagaa tattttaaac ctgatttact agacctggga attttcaaca
9601 tggtctaatt atttactcaa agacatagat gtgaaaattt taggcaacct tctaaatctt
9661 tttcaccatg gatgaaacta taacttaaag aataatactt agaagggtta attggaaatc
9721 agagtttgaa ataaaacttg gaccactttg tatacactct tctcacttga cattttagct
9781 atataatatg tactttgagt ataacatcaa gctttaacaa atatttaaag acaaaaaaat
9841 cacqtcaqta aaatactaaa aqqctcattt ttatatttqt tttaqatqtt ttaaataqtt
9901 gcaatggatt aaaaatgatg atttaaaatg ttgcttgtaa tacagttttg cctgctaaat
9961 tetecacatt ttgtaacetg ttttatttet ttgggtgtaa agegtttttg ettagtattg
10021 tgatattgta tatgttttgt cccagttgta tagtaatgtt tcagtccatc atccagcttt
10081 ggctgctgaa atcatacagc tgtgaagact tgcctttgtt tctgttagac tgcttttcag
10141 ttctgtattg agtatcttaa gtactgtaga aaagatgtca cttcttcctt taaggctgtt
10201 ttgtaatata tataaggact ggaattgtgt ttttaaagaa aagcattcaa gtatgacaat
10261 atactatctg tgttttcacc attcaaagtg ctgtttagta gttgaaactt aaactattta
10321 atgtcattta ataaagtgac caaaatgtgt tgtgctcttt attgtatttt cacagctttg
10381 aaaatctgtg cacatactgt ttcatagaaa atgtatagct tttgttgtcc tatataatgg
10441 tggttctttt gcacatttag ttatttaata ttgagaggtc acgaagtttg gttattgaat
10501 ctgttatata ctaaattctg taaagggaga tctctcatct caaaaagaat ttacatacca
10561 ggaagtccat gtgtgtttgt gttagttttg gatgtctttg tgtaatccag ccccatttcc
10621 tgtttcccaa cagctgtaac actcatttta agtcaagcag ggctaccaac ccacacttga
10681 tagaaaagct gcttaccatt cagaagcttc cttattacct ggcctccaaa tgagctgaat
10741 attttgtagc cttcccttag ctatgttcat tttccctcca ttatcataaa atcagatcga
10801 tatttatgtg ccccaaacaa aactttaaga gcagttacat tctgtcccag tagcccttgt
10861 ttcctttgag agtagcatgt tgtgaggcta tagagactta ttctaccagt aaaacaggtc
10921 aatcctttta catgtttatt atactaaaaa ttatgttcag ggtatttact actttatttc
10981 accagactca gtctcaagtg acttggctat ctccaaatca gatctaccct tagagaataa
11041 acatttttct acceptattt tttttcaagt ctataatctg agccagtccc aaaggagtga
11101 tcaagtttca gaaatgcttt catcttcaca acattttata tatactatta tatggggtga
```

Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (ATRX) isoform 2

(SEO ID NO: 7) 1 mtaepmsesk lntlvqklhd flahsseese etsspprlam ngntdkisgs gsnsdmmens 61 keegtsssek skssgssrsk rkpsivtkyv esddekpldd etvnedasne nsenditmqs 121 lpkedglhgi vsctacgqqv nhfqkdsiyr hpslqvlick ncfkyymsdd isrdsdgmde 181 gcrwcaeggn liccdfchna fckkcilrnl grkelstimd enngwycyic hpeplldlvt 241 acnsvfenle qllqqnkkki kvdseksnkv yehtsrfspk ktssncngee kklddscsgs 301 vtysysaliv pkemikkakk liettanmns syvkflkqat dnseissatk lrqlkafksv 361 ladikkahla leedlnsefr amdavnkekn tkehkvidak fetkarkgek pcalekkdis 421 kseaklsrkq vdsehmhqnv pteeqrtnks tggehkksdr keepqyepan tsedldmdiv 481 svpssvpedi fenletamev qssvdhqgdg ssgteqeves ssvklnissk dnrggikskt 541 takvtkelyv kltpvslsns pikgadcqev pqdkdgyksc glnpklekcg lgqensdneh 601 lvenevslll eesdlrrspr vkttplrrpt etnpvtsnsd eecnetvkek qklsvpvrkk 661 dkrnssdsai dnpkpnklpk skqsetvdqn sdsdemlail kevsrmshss ssdtdineih 721 tnhktlydlk tqagkddkgk rkrksstsgs dfdtkkgksa kssiiskkkr qtqsessnyd 781 selekeiksm skigaarttk kripntkdfd ssedekhskk gmdnqghknl ktsqegssdd 841 aerkqeretf ssaegtvdkd ttimelrdrl pkkqqasast dgvdklsgke qsftslevrk 901 vaetkekskh lktktckkvq dglsdiaekf lkkdqsdets eddkkqskkg teekkkpsdf 961 kkkvikmeqq yesssdgtek lpereeichf pkgikqikng ttdgekkskk irdktskkkd 1021 elsdyaekst gkgdscdsse dkkskngayg rekkrckllg kssrkrqdcs ssdtekysmk 1081 edgcnssdkr lkrielrerr nlsskrntke iqsgssssda eessednkkk kqrtsskkka 1141 vivkekkrns lrtstkrkqa ditsssssdi edddqnsige gssdeqkikp vtenlvlssh 1201 tgfcqssgde alsksvpvtv ddddddndpe nriakkmlle eikanlssde dgssddepee 1261 gkkrtgkqne enpgdeeakn qvnsesdsds eeskkpryrh rllrhkltvs dgesgeekkt 1321 kpkehkevkg rnrrkvssed sedsdfqesg vseevsesed eqrprtrsak kaeleengrs 1381 ykqkkkrrri kvqedsssen ksnseeeeee keeeeeeee eeeeedend dskspgkgrk 1441 kirkilkddk lrtetqnalk eeeerrkria ererereklr evieiedasp tkcpittklv 1501 ldedeetkep lvqvhrnmvi klkphqvdgv qfmwdccces vkktkkspgs gcilahcmgl 1561 gktlqvvsfl htvllcdkld fstalvvcpl ntalnwmnef ekwqeqlkdd eklevselat 1621 vkrpgersym lgrwgedggv milgyemyrn laggrnvksr klkeifnkal vdpgpdfvvc 1681 deghilknea savskamnsi rsrrriiltg tplqnnliey hcmvnfiken llgsikefrn 1741 rfinpiqngg cadstmvdvr vmkkrahily emlagcvqrk dytaltkflp pkheyvlavr 1801 mtsiqcklyq yyldhltgvg nnseggrgka gaklfqdfqm lsriwthpwc lqldyisken 1861 kgyfdedsmd efiasdsdet smslssddyt kkkkkgkkgk kdssssgsgs dndvevikvw 1921 nsrsrgggeg nvdetgnnps vslkleeska tsssnpsspa pdwykdfvtd adaevlehsg 1981 kmvllfeilr maeeigdkvl vfsqslisld liedflelas rektedkdkp liykgegkwl 2041 rnidyyrldg sttagsrkkw aeefndetnv rgrlfiistk agslginlva anrviifdas 2101 wnpsydigsi frvyrfggtk pvyvyrflag gtmedkiydr gvtkgslsfr vvdgggverh 2161 ftmneltely tfepdllddp nsekkkkrdt pmlpkdtila ellqihkehi vqyhehdsll 2221 dhkeeeelte eerkaawaey eaekkqltmr fniptqtnlp pvsfnsqtpy ipfnlqalsa 2281 msnqqledli nqgrekvvea tnsvtavriq plediisavw kenmnlseaq vqalalsrqa 2341 sqeldvkrre aiyndvltkq qmliscvqri lmnrrlqqqy nqqqqqqmty qqatlghlmm 2401 pkppnlimnp snyqqidmrg myqpvaggmq ppplqrappp mrsknpgpsq gksm

Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (ATRX), transcript variant 2, mRNA (SEQ ID NO: 8)

```
1 aatteteetg eetgageete ggeecaacaa aatggeggeg geageggtgt egetttgtt 61 cegeggetee tgeggeggtg geagtggtag eggeetttga getgtgggga ggtteeagea 121 geagetacag tgaegaetaa gaeteeagtg eattetate gtaaceggee gegggggage 181 geagategge geecageaat eacagaagee gaeaaggegt teaagegaaa acatgaeege 241 tgageeeatg agtgaaagea agttgaatae attggtgeag aagetteatg aetteettge 301 acaeteatea gaagaatetg aagaaacaag tteteeteea egaettgeaa tgaateaaaa 361 caeagataaa ateagtggtt etggaagtaa etetgatatg atggaaaaca geaaggaaga 421 gggaaetage tetteagaaa aateeaagte tteaggateg teaegateaa agaggaaace 481 tteaattgta aeaaagtatg tagaateaga tgatgaaaaa eetttggatg atgaaactgt
```

```
541 aaatgaagat gegtetaatg aaaatteaga aaatgatatt aetatgeaga gettgeeaaa
 601 agaagatggg cttcatggga ttgtgagctg cactgcttgt ggacaacagg tcaatcattt
 661 tcaaaaaqat tccatttata qacacccttc attqcaaqtt cttatttqta aqaattqctt
 721 taagtattac atgagtgatg atattagccg tgactcagat ggaatggatg aacaatgtag
 781 gtggtgtgcg gaaggtggaa acttgatttg ttgtgacttt tgccataatg ctttctgcaa
 841 qaaatqcatt ctacqcaacc ttqqtcqaaa qqaqttqtcc acaataatqq atqaaaacaa
 901 ccaatggtat tgctacattt gtcacccaga gcctttgttg gacttggtca ctgcatgtaa
 961 cagcgtattt gagaatttag aacagttgtt gcagcaaaat aagaagaaga taaaagttga
1021 cagtgaaaag agtaataaag tatatgaaca tacatccaga ttttctccaa agaagactag
1081 ttcaaattqt aatqqaqaaq aaaaqaaatt aqatqattcc tqttctqqct ctqtaaccta
1141 ctcttattcc qcactaattq tqcccaaaqa qatqattaaq aaqqcaaaaa aactqattqa
1201 gaccacagcc aacatgaact ccagttatgt taaattttta aagcaggcaa cagataattc
1261 agaaatcagt totgotacaa aattacgtca gottaaggot tttaagtotg tgttggotga
1321 tattaagaag gctcatcttg cattggaaga agacttaaat tccgagtttc gagcgatgga
1381 tgctgtaaac aaagagaaaa ataccaaaga gcataaagtc atagatgcta agtttgaaac
1441 aaaagcacga aaaggagaaa aaccttgtgc tttggaaaag aaggatattt caaagtcaga
1501 agctaaactt tcaagaaaac aggtagatag tgagcacatg catcagaatg ttccaacaga
1561 ggaacaaaga acaaataaaa gtaccggtgg tgaacataag aaatctgata gaaaagaaga
1621 acctcaatat gaacctgcca acacttctga agatttagac atggatattg tgtctgttcc
1681 ttcctcagtt ccagaagaca tttttgagaa tcttgagact gctatggaag ttcagagttc
1741 agttgatcat caaggggatg gcagcagtgg aactgaacaa gaagtggaga gttcatctgt
1801 aaaattaaat atttcttcaa aagacaacag aggaggtatt aaatcaaaaa ctacagctaa
1861 agtaacaaaa gaattatatg ttaaactcac tcctgtttcc ctttctaatt ccccaattaa
1921 aggtgctgat tgtcaggaag ttccacaaga taaagatggc tataaaagtt gtggtctgaa
1981 ccccaagtta gagaaatgtg gacttggaca ggaaaacagt gataatgagc atttggttga
2041 aaatgaagtt tcattacttt tagaggaatc tgatcttcga agatccccac gtgtaaagac
2101 tacaccettg aggegacega cagaaactaa ceetgtaaca tetaatteag atgaagaatg
2161 taatgaaaca gttaaggaga aacaaaaact atcagttcca gtgagaaaaa aggataagcg
2221 taattettet gacagtgeta tagataatee taageetaat aaattgeeaa aatetaagea
2281 atcagagact gtggatcaaa attcagattc tgatgaaatg ctagcaatcc tcaaagaggt
2341 gagcaggatg agtcacagtt cttcttcaga tactgatatt aatgaaattc atacaaacca
2401 taagactttg tatgatttaa agactcaggc ggggaaagat gataaaggaa aaaggaaacg
2461 aaaaagttct acatctggct cagattttga tactaaaaag ggcaaatcag ctaagagctc
2521 tataatttct aaaaagaaac gacaaaccca gtctgagtct tctaattatg actcagaatt
2581 agaaaaagag ataaagagca tgagtaaaat tggtgctgcc agaaccacca aaaaaagaat
2641 tccaaataca aaagattttg actcttctga agatgagaaa cacagcaaaa aaggaatgga
2701 taatcaaggg cacaaaaatt tgaagacctc acaagaagga tcatctgatg atgctgaaag
2761 aaaacaagag agagagactt tctcttcagc agaaggcaca gttgataaag acacgaccat
2821 catggaatta agagatcgac ttcctaagaa gcagcaagca agtgcttcca ctgatggtgt
2881 cgataagett tetgggaaag ageagagttt taettetttg gaagttagaa aagttgetga
2941 aactaaagaa aagagcaagc atctcaaaac caaaacatgt aaaaaagtac aggatggctt
3001 atctgatatt gcagagaaat tcctaaagaa agaccagagc gatgaaactt ctgaagatga
3061 taaaaagcag agcaaaaagg gaactgaaga aaaaaagaaa ccttcagact ttaagaaaaa
3121 agtaattaaa atggaacaac agtatgaatc ttcatctgat ggcactgaaa agttacctga
3181 gcgagaagaa atttgtcatt ttcctaaggg cataaaacaa attaagaatg gaacaactga
3241 tggagaaaag aaaagtaaaa aaataagaga taaaacttct aaaaagaagg atgaattatc
3301 tgattatgct gagaagtcaa cagggaaagg agatagttgt gactcttcag aggataaaaa
3361 gagtaagaat ggagcatatg gtagagagaa gaaaaggtgc aagttgcttg gaaagagttc
3421 aaggaagaga caagattgtt catcatctga tactgagaaa tattccatga aagaagatgg
3481 ttgtaactct tctgataaga gactgaaaag aatagaattg agggaaagaa gaaatttaag
3541 ttcaaagaga aatactaagg aaatacaaag tggctcatca tcatctgatg ctgaggaaag
3601 ttctgaagat aataaaaaga agaagcaaag aacttcatct aaaaagaagg cagtcattgt
3661 caaggagaaa aagagaaact ccctaagaac aagcactaaa aggaagcaag ctgacattac
3721 atcctcatct tcttctgata tagaagatga tgatcagaat tctataggtg agggaagcag
3781 cgatgaacag aaaattaagc ctgtgactga aaatttagtg ctgtcttcac atactggatt
3841 ttgccaatct tcaggagatg aagccttatc taaatcagtg cctgtcacag tggatgatga
3901 tgatgacgac aatgatcctg agaatagaat tgccaagaag atgcttttag aagaaattaa
```

3961	agccaatctt	tcctctgatg	aggatggatc	ttcagatgat	gagccagaag	aagggaaaaa	
4021	aagaactgga	aaacaaaatg	aagaaaaccc	aggagatgag	gaagcaaaaa	atcaagtcaa	
4081	ttctgaatca	gattcagatt	ctgaagaatc	taagaagcca	agatacagac	ataggctttt	
4141	gcggcacaaa	ttgactgtga	gtgacggaga	atctggagaa	gaaaaaaaga	caaagcctaa	
4201	agagcataaa	gaagtcaaag	gcagaaacag	aagaaaggtg	agcagtgaag	attcagaaga	
4261	ttctgatttt	caggaatcag	gagttagtga	agaagttagt	gaatccgaag	atgaacagcg	
	-	aggtctgcaa					
		aggcgacgta					
		gaagaggagg					
		gatgaaaatg					
		aaagatgata	_			_	
		aaacgtattg					
		gatgcttcac					
		accaaagaac					
		gtagatggtg				_	
		tctccaggtt	-				
	-	gtaagttttc		-			
		gtttgtcctc					
		ttaaaagatg	_				
		agaagctaca					
		atgtatagaa					
		aacaaagctt	_				
	_	aaaaatgaag					
		attttaacag					
		atcaaggaaa	_			_	
		caaaatggtc		-		-	
		cacattctct					
		aaattcttgc				_	
		aagctctatc					
		agaggaaagg					
		catccttggt					
		gacagtatgg		_			
		gatgattata	_				
	_	ggaagtggca	_				
		ggtggtgaag					
		gaaagtaaag					
		tttgttacag					
		gaaattcttc					
		atatctctgg					
	_	aaagataaac		_			
		cgtttagatg					
	_	gaaactaatg	_				
	_	aatctggtag					
		atccagagta		_			
		ttcttagctc					
		ctgtcttttc			_		
		actgaacttt					
		aagagggata	_			_	
		aaagaacaca	_		_		
		gagttgactg					
		ctgaccatgc					
		caaactcctt	-				
		gaggacctca					
		gtgaggattc					
		tcagaggccc					
		aaacgaagag					
		gttcagcgaa					
	<u> </u>	<u> </u>			<u> </u>		-

```
7381 gcaacagcaa caaatgactt atcaacaagc aacactgggt cacctcatga tgccaaagcc
 7441 cccaaatttq atcatqaatc cttctaacta ccaqcaqatt qatatqaqaq qaatqtatca
 7501 gccagtggct ggtggtatgc agccaccacc attacagcgt gcaccacccc caatgagaag
 7561 caaaaatcca qqaccttccc aaqqqaaatc aatqtqattt tqcactaaaa qcttaatqqa
 7621 ttgttaaaat catagaaaga tcttttattt ttttaggaat caatgactta acagaactca
 7681 actqtataaa taqtttqqtc cccttaaatq ccaatcttcc atattaqttt tactttttt
 7741 ttttttaaat agggcatacc atttcttcct gacatttgtc agtgatgttg cctagaatct
 7801 tettacacae getgagtaca gaagatattt caaattgttt teagtgaaaa caagteette
 7861 cataatagta acaactccac agatttcctc tctaaatttt tatgcctgct tttagcaacc
 7921 ataaaattqt cataaaatta ataaatttaq qaaaqaataa aqatttatat attcattctt
 7981 tacatataaa aacacacaqc tqaqttctta qaqttqattc ctcaaqttat qaaatacttt
 8041 tgtacttaat ccatttcttg attaaagtga ttgaaatggt tttaatgttc ttttgactga
 8101 agtetgaaae tgggeteetg etttattgte tetgtgaetg aaagttagaa aetgagggtt
 8161 atctttgaca cagaattgtg tgcaatattc ttaaatacta ctgctctaaa agttggagaa
 8221 gtcttgcagt tatcttagca ttgtataaac agccttaagt atagcctaag aagagaattc
 8281 ctttttcttc tttagtcctt ctgccatttt ttattttcag ttatatgtgc tgaaataatt
 8341 actggtaaaa tttcagggtt gtggattatc ttccacacat gaattttctc tctcctggca
 8401 cgaatataaa gcacatctct taactgcatg gtgccagtgc taatgcttca tcctgttgct
 8461 ggcagtggga tgtggactta gaaaatcaag ttctagcatt ttagtaggtt aacactgaag
 8521 ttgtggttgt taggttcaca ccctgtttta taaacaacat caaaatggca gaaccattgc
 8581 tqactttagq ttcacatqaq qaatqtactt ttaacaattc ccaqtactat caqtattqtq
 8641 aaataattcc tctgaaagat aagaatcact ggcttctatg cgcttctttt ctctcatcat
 8701 catqttcttt taccccaqtt tccttacatt tttttaaatt qtttcaqaqt ttqtttttt
 8761 tttagtttag attgtgaggc aattattaaa tcaaaattaa ttcatccaat acccctttac
 8821 tagaagtttt actagaaaat gtattacatt ttattttttc ttaatccagt tctgcaaaaa
 8881 tgacctataa atttattcat gtacaatttt ggttacttga attgttaaag aaaacattgt
 8941 ttttgactat gggagtcaac tcaacatggc agaaccattt ttgagatgat gatacaacag
9061 tttgggcttt gctttaggta tcactggatt agaatgagtt taacattagc taaaactgct
9121 ttgagttgtt tggatgatta agagattgcc atttttatct tggaagaact agtggtaaaa
9181 catccaagag cactaggatt gtgatacaga atttgtgagg tttggtggat ccacgcccct
9241 ctcccccact ttcccatgat gaaatatcac taataaatcc tgtatattta gatattatgc
9301 tagccatgta atcagattta tttaattggg tggggcaggt gtgtatttac tttagaaaaa
9361 atgaaaaaga caagatttat gagaaatatt tgaaggcagt acactctggc caactgttac
 9421 cagttggtat ttctacaagt tcagaatatt ttaaacctga tttactagac ctgggaattt
 9481 tcaacatggt ctaattattt actcaaagac atagatgtga aaattttagg caaccttcta
 9541 aatcttttc accatggatg aaactataac ttaaagaata atacttagaa gggttaattg
 9601 gaaatcagag tttgaaataa aacttggacc actttgtata cactcttctc acttgacatt
 9661 ttagctatat aatatgtact ttgagtataa catcaagctt taacaaatat ttaaagacaa
 9721 aaaaatcacg tcagtaaaat actaaaaggc tcatttttat atttgtttta gatgttttaa
9781 atagttgcaa tggattaaaa atgatgattt aaaatgttgc ttgtaataca gttttgcctg
9841 ctaaattctc cacattttgt aacctgtttt atttctttgg gtgtaaagcg tttttgctta
9901 gtattgtgat attgtatatg ttttgtccca gttgtatagt aatgtttcag tccatcatcc
9961 agetttgget getgaaatea tacagetgtg aagaettgee tttgtttetg ttagaetget
10021 tttcagttct gtattgagta tcttaagtac tgtagaaaag atgtcacttc ttcctttaag
10081 gctgttttgt aatatatata aggactggaa ttgtgttttt aaagaaaagc attcaagtat
10141 gacaatatac tatctgtgtt ttcaccattc aaagtgctgt ttagtagttg aaacttaaac
10201 tatttaatgt catttaataa agtgaccaaa atgtgttgtg ctctttattg tattttcaca
10261 gctttgaaaa tctgtgcaca tactgtttca tagaaaatgt atagcttttg ttgtcctata
10321 taatggtggt tcttttgcac atttagttat ttaatattga gaggtcacga agtttggtta
10381 ttgaatctgt tatatactaa attctgtaaa gggagatctc tcatctcaaa aagaatttac
10441 ataccaggaa gtccatgtgt gtttgtgtta gttttggatg tctttgtgta atccagccc
10501 atttcctgtt tcccaacage tgtaacacte attttaagte aagcaggget accaacceae
10561 acttgataga aaagctgctt accattcaga agcttcctta ttacctggcc tccaaatgag
10621 ctgaatattt tgtagccttc ccttagctat gttcattttc cctccattat cataaaatca
10681 gategatatt tatgtgeece aaacaaaact ttaagageag ttacattetg teecagtage
10741 ccttgtttcc tttgagagta gcatgttgtg aggctataga gacttattct accagtaaaa
```

```
10801 caggtcaatc cttttacatg tttattatac taaaaattat gttcagggta tttactactt 10861 tatttcacca gactcagtct caagtgactt ggctatctcc aaatcagatc tacccttaga 10921 gaataaacat ttttctaccg ttatttttt tcaagtctat aatctgagcc agtcccaaag 10981 gagtgatcaa gtttcagaaa tgctttcatc ttcacaacat tttatatata ctattatatg 11041 gggtgaataa agttttaaat ccgaaatata aaaaaaaaa aaaaaaaa
```

[0116] A subject in need thereof may have reduced expression, haploinsufficiency, and/or loss of function of ARID1A. For example, a subject may comprise a mutation selected from the group consisting of a nonsense mutation for the wild type residue cysteine (C) at amino acid position 884 of SEQ ID NO: 11 (C884*), a substitution of lysine (K) for the wild type residue glutamic acid (E) at amino acid position 966 (E966K), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1411 of SEQ ID NO: 11 (Q1411*), a frame shift mutation at the wild type residue phenylalanine (F) at amino acid position 1720 of SEQ ID NO: 11 (F1720fs), a frame shift mutation after the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue cysteine (C) at amino acid position 1874 of SEQ ID NO: 11 (C1874fs), a substitution of glutamic acid (E) for the wild type residue aspartic acid (D) at amino acid position 1957 (D1957E), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1430 of SEQ ID NO: 11 (Q1430*), a frame shift mutation at the wild type residue arginine (R) at amino acid position 1721 of SEQ ID NO: 11 (R1721fs), a substitution of glutamic acid (E) for the wild type residue glycine (G) at amino acid position 1255 (G1255E), a frame shift mutation at the wild type residue glycine (G) at amino acid position 284 of SEQ ID NO: 11 (G284fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1722 of SEQ ID NO: 11 (R1722*), a frame shift mutation at the wild type residue methionine (M) at amino acid position 274 of SEQ ID NO: 11 (M274fs), a frame shift mutation at the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue P at amino acid position 559 of SEQ ID NO: 11 (P559fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1276 of SEQ ID NO: 11 (R1276*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 2176 of SEQ ID NO: 11 (Q2176fs), a frame shift mutation at the wild type residue histidine (H) at amino acid position 203 of SEQ ID NO: 11 (H203fs), a frame shift mutation at the wild type residue alanine (A) at amino acid position 591 of SEQ ID NO: 11 (A591fs), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position

1322 of SEQ ID NO: 11 (Q1322*), a nonsense mutation for the wild type residue serine (S) at amino acid position 2264 of SEQ ID NO: 11 (S2264*), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 586 of SEQ ID NO: 11 (Q586*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 548 of SEQ ID NO: 11 (Q548fs), and a frame shift mutation at the wild type residue asparagine (N) at amino acid position 756 of SEQ ID NO: 11 (N756fs). "*" used herein refers to a stop codon. "fs" used herein refers to a frame shift.

AT-rich interactive domain-containing protein 1A (ARID1A) isoform a [Homo sapiens] (SEQ ID NO: 9)

```
1 maaqvapaaa sslgnppppp pselkkaeqq qreeaggeaa aaaaaergem kaaagqeseg
  61 pavgppqplg kelqdgaesn gggggggags gggpgaepdl knsngnagpr palnnnltep
 121 pggggggssd gvgapphsaa aalpppaygf gqpygrspsa vaaaaaavfh qqhggqqspg
 181 laalqsgggg glepyagpqq nshdhgfpnh qynsyypnrs aypppapaya lssprggtpg
 241 sgaaaaagsk pppsssasas sssssfaqqr fgamggggps aagggtpqpt atptlnqllt
 301 spssargyqg ypggdysggp qdggagkgpa dmasqcwgaa aaaaaaaaas ggaqqrshha
 361 pmspgssggg gqplartpqp sspmdqmgkm rpqpyggtnp ysqqqgppsg pqqghgypgq
 421 pygsqtpqry pmtmqgraqs amgglsytqq ippygqqgps gygqqgqtpy ynqqsphpqq
 481 qqppysqqpp sqtphaqpsy qqqpqsqppq lqssqppysq qpsqpphqqs papypsqqst
 541 tqqhpqsqpp ysqpqaqspy qqqqpqqpap stlsqqaayp qpqsqqsqqt aysqqrfppp
601 qelsqdsfgs qassapsmts skggqedmnl slqsrpsslp dlsgsiddlp mgtegalspg
 661 vstsgisssq geqsnpaqsp fsphtsphlp girgpspspv gspasvaqsr sgplspaavp
721 gnqmpprpps gqsdsimhps mnqssiaqdr gymqrnpqmp qysspqpgsa lsprqpsggq
 781 ihtgmgsyqq nsmgsygpqg gqygpqggyp rqpnynalpn anypsagmag ginpmgaggq
 841 mhgqpgippy gtlppgrmsh asmgnrpygp nmanmppqvg sgmcpppggm nrktqetava
901 mhvaansiqn rppgypnmnq ggmmgtgppy gqginsmagm inpqgppysm ggtmannsag
961 maaspemmgl gdvkltpatk mnnkadgtpk teskskksss stttnekitk lyelggeper
1021 kmwvdrylaf teekamgmtn lpavgrkpld lyrlyvsvke iggltqvnkn kkwrelatnl
1081 nvgtsssaas slkkqyiqcl yafeckierg edpppdifaa adskksqpki qppspagsgs
1141 mggpqtpqst sssmaeggdl kpptpastph sqipplpgms rsnsvgiqda fndgsdstfq
1201 krnsmtpnpg yqpsmntsdm mgrmsyepnk dpygsmrkap gsdpfmssgq gpnggmgdpy
1261 sraagpglgn vamgprqhyp yggpydrvrt epgigpegnm stgapqpnlm psnpdsgmys
1321 psryppqqqq qqqqrhdsyg nqfstqgtps gspfpsqqtt myqqqqqnyk rpmdgtygpp
1381 akrhegemys vpystgqgqp qqqqlppaqp qpasqqqaaq pspqqdvynq ygnaypatat
1441 aaterrpagg pqnqfpfqfg rdrvsappgt naqqnmppqm mggpiqasae vaqqgtmwqg
1501 rndmtynyan rqstgsapqg payhgvnrtd emlhtdqran hegswpshgt rqppygpsap
1561 vppmtrppps nyqpppsmqn hipqvsspap lprpmenrts pskspflhsg mkmqkagppv
1621 pashiapapv qppmirrdit fppgsveatq pvlkqrrrlt mkdigtpeaw rvmmslksgl
1681 laestwaldt inillyddns imtfnlsqlp gllellveyf rrclieifgi lkeyevgdpg
1741 qrtlldpgrf skvsspapme ggeeeeellg pkleeeeee vvendeeiaf sgkdkpasen
1801 seekliskfd klpvkivqkn dpfvvdcsdk lgrvqefdsg llhwrigggd ttehiqthfe
1861 sktellpsrp hapcppaprk hvttaegtpg ttdqegpppd gppekritat mddmlstrss
1921 tltedgakss eaikesskfp fgispaqshr nikiledeph skdetplctl ldwqdslakr
1981 cvcvsntirs lsfvpgndfe mskhpgllli lgklillhhk hperkqaplt yekeeeqdqg
2041 vscnkvewww dclemlrent lvtlanisgq ldlspypesi clpvldgllh wavcpsaeaq
2101 dpfstlgpna vlspqrlvle tlsklsiqdn nvdlilatpp fsrleklyst mvrflsdrkn
2161 pvcremavvl lanlaqqdsl aaraiavqkg signllgfle dslaatqfqq sqasllhmqn
2221 ppfeptsvdm mrraaralla lakvdenhse ftlyesrlld isvsplmnsl vsqvicdvlf
2281 liggs
```

Homo sapiens AT rich interactive domain 1A (SWI-like) (ARID1A), transcript variant 1, mRNA (SEQ ID NO: 10)

1 cagaaagcgg agagtcacag cggggccagg ccctggggag cggagcctcc accgccccc 61 tcattcccag gcaagggctt ggggggaatg agccgggaga gccgggtccc gagcctacag 121 agccgggagc agctgagccg ccggcgcctc ggccgccgcc gccgcctcct cctcctccgc 181 cgccgccagc ccggagcctg agccggcggg gcgggggga gaggagcgag cgcagcgcag 241 cageggagee eegeegggee ggtggggagg geageeeggg ggaetgggee 301 ccggggcggg gtgggagggg gggagaagac gaagacaggg ccgggtctct ccgcggacga 361 gacagegggg ateatggeeg egeaggtege eeeegeegee geeageagee tgggeaacee 421 geogeogeog eegeeetegg agetgaagaa ageegageag cageageggg aggaggeggg 481 gggcgaggcg gcggcggcgg cagcggccga gcgcggggaa atgaaggcag ccgccgggca 541 ggaaagggag ggcccgccg tggggccgcc gcagccgctg ggaaaggagc tgcaggacgg 601 ggccgagage aatgggggtg gcggcggcg cggagccggc agcggcggcg ggcccggcgc 661 ggagccggac ctgaagaact cgaacgggaa cgcgggccct aggcccgccc tgaacaataa 721 cctcacqqaq ccqcccqqcq qcqqcqqtqq cqqcaqcaqc qatqqqqtqq qqqcqctcc 781 teacteagee geggeegeet tgeegeeece ageetaegge ttegggeaac eetaeggeeg 841 gagecegtet geegtegeeg eegeegege egeegtette caccaacaac atggeggaca 901 acaaageeet ggeetggeag egetgeagag eggeggegge gggggeetgg ageeetaege 961 ggggccccag cagaactctc acgaccacgg cttccccaac caccagtaca actcctacta 1021 ccccaaccgc agcgcctacc ccccgcccgc cccggcctac gcgctgagct ccccgagagg 1081 tggcactccg ggctccggcg cggcggcggc tgccggctcc aagccgcctc cctcctccag 1141 cgcctccgcc tcctcgtcgt cttcgtcctt cgctcagcag cgcttcgggg ccatgggggg 1201 aggcggccc tccgcggccg gcgggggaac tccccagccc accgccaccc ccaccctcaa 1261 ccaactgete acgtegecea geteggeeeg gggetaceag ggetaceeeg ggggegaeta 1321 cagtggcggg ccccaggacg ggggcgccgg caagggcccg gcggacatgg cctcgcagtg 1381 ttggggggct gcggcggcgg cagctgcggc ggcggccgcc tcgggagggg cccaacaaag 1441 gagccaccac gcgcccatga gccccgggag cagcggcggc ggggggcagc cgctcgcccg 1501 gacccctcag ccatccagtc caatggatca gatgggcaag atgagacctc agccatatgg 1561 cgggactaac ccatactcgc agcaacaggg acctccgtca ggaccgcagc aaggacatgg 1621 gtacccaggg cagccatacg ggtcccagac cccgcagcgg tacccgatga ccatgcaggg 1681 ccgggcgcag agtgccatgg gcggcctctc ttatacacag cagattcctc cttatggaca 1741 acaaggeece agegggtatg gteaacaggg ceagaeteea tattacaace ageaaagtee 1801 teacceteag cageageage caccetacte ceageaacea cegteecaga ecceteatge 1861 ccaaccttcg tatcagcagc agccacagtc tcaaccacca cagctccagt cctctcagcc 1921 tecatactee cageageeat eccageetee acateageag tecceggete cataceeete 1981 ccaqcaqtcq acqacacaqc aqcacccca qaqccaqccc ccctactcac aqccacaqqc 2041 teagteteet taccageage ageaacetea geageeagea eeetegaege teteecagea 2101 ggctgcgtat cctcagcccc agtctcagca gtcccagcaa actgcctatt cccagcagcg 2161 cttccctcca ccgcaggagc tatctcaaga ttcatttggg tctcaggcat cctcagcccc 2221 ctcaatgacc tccagtaagg gagggcaaga agatatgaac ctgagccttc agtcaagacc 2281 ctccagcttg cctgatctat ctggttcaat agatgacctc cccatgggga cagaaggagc 2341 tetgagteet ggagtgagea cateagggat ttecageage caaggagage agagtaatee 2401 ageteagtet cetttetete etcatacete eceteacetg cetggeatee gaggecette 2461 cccgtcccct gttggctctc ccgccagtgt tgctcagtct cgctcaggac cactctcgcc 2521 tgctgcagtg ccaggcaacc agatgccacc tcggccaccc agtggccagt cggacagcat 2581 catgcatect tecatgaace aateaageat tgeecaagat egaggttata tgeagaggaa 2641 cccccagatg ccccagtaca gttcccccca gcccggctca gccttatctc cgcgtcagcc 2701 ttccggagga cagatacaca caggcatggg ctcctaccag cagaactcca tggggagcta 2761 tggtccccag gggggtcagt atggcccaca aggtggctac cccaggcagc caaactataa 2821 tgccttgccc aatgccaact accccagtgc aggcatggct ggaggcataa accccatggg 2881 tgccggaggt caaatgcatg gacagcctgg catcccacct tatggcacac tccctccagg 2941 gaggatgagt cacqcctcca tgggcaaccg gccttatggc cctaacatgg ccaatatgcc 3001 acctcaggtt gggtcaggga tgtgtccccc accagggggc atgaaccgga aaacccaaga 3061 aactgetgte gecatgeatg ttgetgeeaa etetateeaa aacaggeege caggetaeee 3121 caatatgaat caagggggca tgatgggaac tggacctcct tatggacaag ggattaatag 3181 tatggctggc atgatcaacc ctcagggacc cccatattcc atgggtggaa ccatggccaa

3241 caattetga ggatagaa eeaaccaaa garaatagaa ettaggatag taaagtaaa 3301 tecaacaaca aaaatagaaa aaaaataaca aagtagtaga gagaagaagaa 3421 tecaacaaa aatetgactg ettagatgat gagaacactaa gegaaaaaaaa gagagaaa 3601 tecaacaaa etcaatagaa gacaataaa agtagaagaa aacaaaaaat gagagaaaa 3601 tecaacaaa etcattgag gacaataaag agtagaaagaa aecaacaaaa aacaacaaaa aasaa gagagaagaa 3601 tecaacaaa etcattgaga gacaataaag eagagaagaa etcaacacaa aacaacaaaa eccacaacaa 3911 gaataagaaa gacaagaagaa atecaacaaca eacagaagaa atecaacacaa aasaa tecaacacaa 3911 gecaagaaga aateaagaaa atecaacacaa aagaaagagaa tecaagaaga decaacaacaa aaagaaacaa decaacaacaa 3911 gecaagaagagaa atecaatagaa tecaacacaa aagaaagagaa tecaagaaga gacaagaaga atecaacaca aagaacaaga decaagaaga gagagaagaa atecaagaaca aagaacaaga 4021 tacetetgaa atagatgaga atecatteaa tagacaaca gagagaagaa atecaacaca gagaacaaga aagaacaca 4021 aagacacat eecaatgaga gacacacaa gacagaacaa aagaacaca dagaacaagaa dagaacaaga gagaagaaca agaagaagaa atecaacaca 2021 aaagacata eecaatgaaga gacacacaa aagaacaca aagaacaca 4221 gagaagaata tecacacaca gacaacaca aagaacaca aacaacaca 4221 gagaagaata tecacacaca gacaacaca aacaacaca 4221 gagaagaata tecacacaca gacaacaca aacaacaca 4221 gagaagaata tecacacaca gacaacaca aacaacaca 4221 gagaagaaca cacaatgaaca gacaacacaca 4221 gagaagaaca cacaatgaaca gacaacaca aacaatgaaca 4221 tecaagagaaca cacaatgaaca gacaacacacaa 4221 gagaagaaca cacaacacaca gagaacaca aacaacacacac								
3361 gaaatccagt tettetaeta caaccatga gaagtacac aagtegtata gaegagca 3481 gggcatgac aatetgeet etgggtaag gaaaccet ggcetetate geetetate 3541 gtetetagaag agaagtegg gacatcaag aaccataga gagaagteg gaaaccet gacetetate geetetate 3591 gtecaccaag acteatatgag gaaagtega 3661 tatecagtgt etetataget tegaatgaag aatetgaget gagaagaaga 3661 tatecagtgt etetataget tegaatgaag aatetgaaged gagaagaaga 3721 catetttega getgetgatt caaagaaga caagceaga accagcagt etececaga 3781 ggaatcagga tetatgeag ggeeccagae tececagaa accagcagt eccecaga 3781 ggaatcagga tetatgeagg ggeeccagae tececagaa accagcagt eccecatagaga gagaagaaga attaagtag gaatgaagaagaagaagaagaagaagaagaagaagaagaa	3241	caattctgca	gggatggcag	ccagcccaga	gatgatgggc	cttggggatg	taaagttaac	
3421 tgagoctgaa agaagatgt gggtggacg ttatctgcc ttcactgag agaagaccaa 3481 gggcatgaca aatctgcctg cgtggtatg agaacctctg gactctate gcctctatg 3541 gtctgtgaag gagattggtg gattgatca ggtcacaag acaaaaaa ggcgggaac 3601 tgcaaccaac ctcaatgtg gacaataaag cagtgctgcc agctccttga aaaagagat 3601 tatcagtgt ctctatgcct ttgaatgcaa gattgaagg ggagaagac ctccccaga 3721 catctttgca gctgctgatt ccaagaagatc cacaccaag atccagcatc categocag 3781 gggatcagga tctatagcag ggccccagac tccccagtc accacgagt ccategocag 3841 aggagaagac ttatagcag ggccccagac tcccagca accacagat ccategocag 3841 aggagaagac ttaagacgag attcatgtag gatccagaat ccategocag 3841 aggagaagac atcatgagg gactgtcta tgagccagaa acctttaatg atggaagtg 3861 ctcacattc cagagaagga atcacttag tgagccaaaa aaggatcctt atggaagtg 3861 ctcacattc cagagaagga atccctcag gctcagaa aatggaccca aggagagag 4881 gagaaagat cagagaggag atcccttaatg gagccagaa aaggaccca aggagagag 4881 gagaaagat accattgag gtccttatga cagagtgaga aatgtggca tgagaacca 4891 gagaaagat ccctatgag gtccttatga cagagtgaga aatgtggca tgagaaccac 4821 gaggatgtat tctcctagc gctacccac gcagaagaag cagaagacga gaaaagaac 4881 tgagttcat gcaacagat tccaacacc gcagaagaag cagaagaga gaagattat atgagcact ctcgcaaag gacagaaga gagaagtaa agaaggac ctccacagact 4891 atatggcct tccgcaaca gcagaacaag cagaagacag cagaacgac 4801 acaagagac cctcagaag agaagttgcc ccagaacaga gaagagac cctcacagact 4801 acaagagac cctcagaaga gacagatgaa gagaagaa agaacgac 4801 gcaagagaa cctcagaaga gagaagtaga gagaagaa agaacgac 4801 gcaagagaa cctcagaaga gacagaaga gagaagaa agaacgac 4801 gcaagagaa cctcagaaga gacagaagaa gagaagaa agaacgac 4801 gcaacacaa atatggagag gcacaataga gagaagaa agagagac 4801 gcaacacaa atatgagaga gacagaaga gagaagaa agaacgac 4801 gcaacacaa atatgagaga gcccaataa agaacgaaga gacaagaaga 4801 gcaacacaa atatgagaga gcccaataa agaacacaa agaaagaaa 4801 gcaacacaa atatgagaga gcccaataa agaacacaa agaaagaaa 4801 gcaacacaa atatgatgaga gacagaagag gaacaagaa gacacaagaa 4801 gcaacacaa gagaacaagaa cctaacaaga gaacaagaa 4801 gcaacacaa gagaacaagaa cctaacaaga gaacaagaa 4801 gcaacacaa gaacacaaga gccaacaagaa gaacaagaa 4801 gaacacaaa ga								
3841 ggcatgaca aatctgoctg cigtgggtag gaaacactctg gacctatatc gcotctatg 3541 gtctgtgaag gagattggtg gattgactca ggtcacacag aacaaaaaat ggcgggaac 3661 tatccagtgt ctctatgct ttgaatgcaa gattgaceg agctccttga aacagcagt 3661 tatccagtgt ctctatgct ttgaatgcaa gattgaceg agctccttga aacagcagt 3781 gagatcagga tctatgcagt ggccccagac tccccatgca accagcagt ccctccctg 3781 gggatcagga tctatgcagg ggccccagac tccccagtca accagcagt ccctccctg 3781 ggcaggagac tataagcaca cacactcage atccaccac cacagtag tcccccat 3891 gccaggatg agcaggaga attcattga gatccaggat gcctttaatg atggaagtg 3861 ctccacattc cagaagcga attcattga gatccaggat gcctttaatg atggaagtg 3861 ctccacattc cagaagcgg catttccat tagaccagat agcagtcct atggacgac 4021 tacccttgac atgatggagg gcatgtccta tgagccaat aaggatcct atggacgac 4021 acaccttgac atgatggagg gatgtctat gtcctaagg caggaccca acggaggac 4261 tgagggaac ttcctatgag gtccttatga cagagtgag acggagcac 4261 tgagggaaca atgacacat gccgaatctc atgcctcca accagact 4271 acagggatgt ttctctagc gctaccccc gagacagaa acgagagacg agcaaggac 4381 tgattcctat ggcaatcagt tctccacca agcagaatca caccagact 4381 tgattcctat ggcaatcagt tctccacca agcagcacct tctggcagc ccttccca 4501 atatggcct cctgccaag ggcacgaagg ggagatgta agcgtgcat acagcaga 4501 atatggcct cctgccaag ggcacgaagg ggagatgta agcgtgcat acagcaga 4501 atatggcct cctgccaag ggcacgaagg ggagatgta agcgtgcca tagagagca 4501 atatggcct cctgccaag ggcacgaagg ggaagtgta agcgtgcca acagcaga 4501 gccaccaca atgatggc gcccataca ggcaccacca cagttagg cagcactut 461 taccagttt ggcagaacc stgatctctc cctcagcaaga ggcaccaca 4801 gccaccaca atgatgggag gcccataca ggcatacac 4801 gccaccaca atgatggag gcccataca ggcacacac 4801 gccaccaca atgatggag gcccataca ggcacacac 4801 gccaccaca atgatggag gcccataca ggcacacac 4801 gacaccaca atgatggag gcccataca ggcacacac 4801 gacaccaca atgatggag gccccataca ggcacacac 4801 gacaccaca atgatggag gccccataca ggcacacac 4801 gacaccaca aggacacact ctcacacacacacacacacacacacacacaca	3361	gaaatccagt	tcttctacta	caaccaatga	gaagatcacc	aagttgtatg	agctgggtgg	
3541 gtotgtgaag gagattggtg gattgattea ggteaacaag aacaaaaaat ggegggaac 3601 tgcaaccaac cteaatgtgg gcacatcaag cagtgctgc agtccttga aaaagcagt 3601 tatccagtgt ctctatgct ttgaatgcaa gattgaacgg ggagaagac ctccccaga 3721 catctttgca gctgctgatt ccaagaagt ccaagcacag atccacagat ccctatggaag 3781 ggagtacagga tctatagaag ggccccaaca atccacaca cacagtcaga tcccccatagaag 3841 agaggagaac ttaaagcaca caactcaga atccacaca cacagtcaga tcccccatagaag 38901 gccaggcatg agcaggagga attcatgtgg gatccaggat gccttaatg atggaagtg 4021 tacctctgac atgatgggg gcatgtcctt tgaaccacat agggatccca acgaggag 4021 tacctctgac atgatgggg gcatgtcctt tgaaccacat agggaccca acgaggggg 4021 tacctctgac atgatgggg gcatgtcctt tgaaccacat agggaccca acgaggggg 4141 gggtgaccc tacagtcgtg ctgccggcc tgggctagga aatgtggcga tgggaccac 4201 acagcactat ccttatgaag gtcctttatga acgaggaggac tgggagccca acgaggaga 4211 ggggatgat tctcctcagcc gctaccccc gcagcagaag aggaggccca accagatag 4221 ggggatgat tctcctagcc gctaccccc gcagcagaag aggaggacac 4231 gaggatgat tctcccacaca gcagcagaag aggaagtcat 4231 gaggatgat tctcccacaca aggacaccat tctgcagacc 4241 caagcagac acaagtgat acaagcagaa gcagaagaag aggaagcaca 4381 tgattcctat gcaatcagt tctccacaca aggacacct tctgcagac 4381 tatgcactcc cttgcaaga ggacagaag gagaattac aggtggcaa tggatggca 4381 tacaggaggaa gctagaaga gagaagtac acaagaagaaga 4381 agatccata ggaatcagt ctccacaca aggacagaag 4381 tacaggaggaa ctagaagaag gagaagtac acaagaagaagaagaagaagaagaagaagaagaagaagaa	3421	tgagcctgag	aggaagatgt	gggtggaccg	ttatctggcc	ttcactgagg	agaaggccat	
3661 taccagtac ctcatatgcg gacatcaaq cagtqctqcc agctccttga aaaaqcagt sa661 tatccagtqt ctctatqcct ttgaatqcaa gattqaacqg gagaaqaacc ctccccaaq 3721 catctttqca gctqctqatt ccaaqaaqt caaqccaaq atccaqcatc cctcccaqq 3781 gagatcaqqq tctatqcaqq gqcccaqac tccccaqaa atccaqcctc cctctcctq 3781 gagatqaaqqa tctatqcaqq gqcccaqac tccccaatca accaqcaqtt ccatqqcaq 3841 agqaqaqact tcaaqacqqq atccaqqat tccacqatca 3901 gccaqcattq agaaqqaqa atccatqqq atccaqqat gcctttaatq atggaaqtq 4021 tacctctqcac atgatqqqqq atccatqqq tccaqqacq 4081 gagqaaqqt ccaqqqqqq atccatqqq tccaqqqq 4081 gagqaaqqt ccaqqqqqq atccatqqq tqqqqqqqqqqqq								
3661 tatocatgt ctotatgoot togatgaga gargaagag gagaagagoc ctococcag 3721 catotttgoa getgetgatt coaagaagat coaagocaaga atcoagoct coctoctog 3781 gagatoagga totatagacga gagococagac tococaga atcoagocat coctoctog 3781 gagatoagga totatagacoa caactocaga atcoacoac cacagocagat tococaga 3841 aggagagaga thaaagocac caactocaga atcoacoac cacagocagat tococaga 3901 gocagocatta gagaagagga attocattga gatocacaca cacagocagat coccocat 4921 tacottotgac atgatgagga atcoatoac tocaaacoct gagatatcag coagocaga 4021 tacottotgac atgatgagga gatoctata tgagocaaat aggagocaca aggagogga 4181 gaggaagaac cacagogagg atcoctaat gagcacaat aggagoccac acagocagaga 4261 tgaggaaac accatagag gtocttatag aggatgaga aaggagocca aggagogaga 4261 tgaggaaca atgaggacat gtocataga gagaagagagagagagagagagagagagagagag	3541	gtctgtgaag	gagattggtg	gattgactca	ggtcaacaag	aacaaaaaat	ggcgggaact	
3781 gggatcagga tetatgcagg ggececagae tececagtea atcaagette cetetetgg 3891 aggagtagga tetatgcagg ggececagae tececagtea accaqeagt tecececat 3901 gecaggeatg aggaggaga atteatgtigg gatecaggat geetttaatg atggaagt 3961 decacactte cagaagggag attecatgae tecaaacect gggatataage ceafataga 4021 tacetetgae atgatgggg geatgteeta tgagecaaat aaggateett atggaagga 4081 gaggaaaget ecagggagtg atceettgat tgeetaagae gaggaceca acggaggaga 4141 gggtgaacece tacaagtegg decettaaga gaggacecae aggaggaga 4261 tgagggaaaa atgaggacatg gggeecaaca geegaatea atgeetteea accagaatg 4261 tgagggaaaa atgaggacatg gggeecaaca geegaatea atgeetteea acaagtegga 4381 tgatteetat ggaaataagt tetecaceae aggacaggag aaggaaggag 4381 tgatteetat ggaaataagt tetecaaceaa gagaaataca agaggageca tgagagecaa tagagacaga 4381 tgatteetat ggaaataagt tetecaaceaa aggaaataca aggagaceae tetagagagea 4361 acaaggagac ecteagaaga ggacagaagg ggaagatgaa aggaggeca tetagagagac 4561 geaggggaaa ectagaaga ggaaagagg ggaagatgaa aggaggaa aggaagga	3601	tgcaaccaac	ctcaatgtgg	gcacatcaag	cagtgctgcc	agctccttga	aaaagcagta	
3841 aggagacaga totatacaga geococagac tococagtoa accagotaga tocaccocasta 3841 aggagagaa totaaagocaa caactocaga atcacacca cacagtoaga tococcocata 3901 geoaggaata gacaagagaca attoaataga atcacacca geotataataga tococcocataga 4021 tococactto cagaagogga attocatagac tocacaccaca daggaccacaca aaggaccata 4021 tococcacto cagagagaga attocatagac tocacacacaca daggaccacaca aggaccacaca atagacacacacacacacacacacacacacacacacacac	3661	tatccagtgt	ctctatgcct	ttgaatgcaa	gattgaacgg	ggagaagacc	ctccccaga	
3841 agagagajac ttaaagccac caactacagc atcacacca cacagtcaga tcccccat 3901 gccaggcatg agcaggagca attcattgg gatccaggat gcctttaatg atggaatgg 3961 ctccacattc cagaagcgga attcattgac tccaaaccct gggtatcaga ccagtatga 4021 tacctctgac atgatggggg gcatgtccta tgagccaaat aaggatcct atggaggaggatgg gatgtcctat gtgctcaggg cagggccca acggcggga 4141 gggtgaccc tacagtcgtg ctgccggcc tgggtatgga aatgtgggcg tgggacaca 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg gaatagggc 4261 tgagggaaaa atgagcactg gggcccacaa gcggaattct atgccttcaa accaagact 4221 ggggatgat tctcctagcc gctaccccc gcagcaattc atgccttcaa accaagact 4321 ggggatgat tctcctagcc gctaccccc gcagaattc atgccttcaa accaagact 4381 tgattcctat ggcaatcagt tctccaccca agggaagcac cagcagcac cttcccacaca 4381 tgattcctat ggcaatcagt tctccaccca aggaagatta aaggggccaa tggatggca 4501 atatggccct cctgccaagc gcacagagg ggaagtata acggtgccat acgacagca 4501 acaagtctgc cagccttccc ctcagcaaga ggaagttac acggtgccat acaagcatgc 4621 acaagctgcc acagctgcta ctgagcagca gccaccaga cccaagccag 4621 acaagctgcc acagctgcta ctgagcagca accagcagc gcccccaga accaactgc 4621 acaagctgcc acagctgcta ctgagcagca gcccccaga accaactgc 4621 acaagctgca gggcgtaattg actgagcag ggcccccaag accaactgcag 4621 acaagctgca gggcggatatg actgagcag ggcccccaag accaacaga 4801 gccaccacaa atgatgggg gccccataca ggcatcagc gaggttgct 4801 gccaccaag aggcccgcct atcatgggcg accagaag gaggttgct 4801 gccaccaag aggcccgcct atcatggcg gccccctaca ggcaacag 4801 gccaccaag agcaccgcc tactagcag gcccccaca gccacagagag 4801 tccatggcag ggcccgcca acagagag gcccccaag accaacaga 4801 gccaccaag aaccacagaag gccaccaca gccacatag 4801 tccatggca gccccgcct acatgaca gccacatac 4801 tccatgcc cctgtgccc cctggacct tccatgacac gccacatac 4801 tccacatgc gaccacatac ccagaaag gccacatac 4801 tccacacac gacacagaa gcacacatac 4801 tcagaagaaca accaataca cctagaaca gccacacac 4801 tcagaagaaca accacataca accacacac 5801 aagccacaca gacacacaca accacacac 5801 aagccacaca gacacacaca agcacacaca accacacaca	3721	catctttgca	gctgctgatt	ccaagaagtc	ccagcccaag	atccagcctc	cctctcctgc	
3901 gecaggaeta ageagagaga atteagttag gatecaggat gectttaatag atggaagtg 3961 etecacatte cagaagegga attecatae tecaaaceet gggtateae cagatatga 4021 tacetetgae atgatggage geatgteeta tagageeaaat aaggateet atggeagea 4081 gaggaaaget eacaggagtg atecetteat gteeteagg cagggeeea aeggegga 4141 gggtgaeeee tacagtegtg etgeeggee taggetagga aatgtggega taggaeeae 4261 tagaggaaaa atgageaetg ggeeeeaea geegaateet atgeetteea accaagage 4261 tagaggaaaa atgageaetg ggeeeeeee geegaatee atgeetteea accaagage 4261 tagaggaaaa atgageaetg gggeeeeaea geegaateet atgeetteea accaagage 4381 tyatteetat ggeaateagt tetecaceea aggeaeeeet tetggeagee cetteeea 4381 tyatteetat ggeaateagt tetecaceea aggeaeeet tetggeagee cettecea 4361 aatsggeeet ectgeeaage gagaagtgae aggaggeeaa tggatgeea 4561 geaggggaag ceteageag gacagatgae geagagtgae acaggeegae 4561 geaggggeag ceteageag ageagttgee eccaageegg 4661 geaggggeag ceteageag ageagttgee eccaageegg 4621 acaagetege eageetteee eteageaaga gagaagtgae accaagtgea 4681 tyeeaetygee acagtggtae eteagageag accaagage ggeeceaaa 4801 geeaeeaaa atgatgggeg geeceaaaa ggeateagage gggeeceaaa 4801 geeaeeaaa atgatgggeg geeceaaaa ggeateagage accaagageag 4801 tecaeetgee acagtgggee geeceaaaa ggeateagage 4801 gageaeaaa aacaagaaaa gtyteetetge 4801 geeeeeaaa atgatgggeg geeeaaaaa ggeateagag 4801 geeeeeaaa atgatgggeg geeeaaaaa ggeateagag 4801 tecaeetgea gggeeeaaaa gaetgaeea gagaagagaa 4801 geeeeeaaa atgatgggeg geeeaaaa ggeateagag 4801 tecaeetgea ggeeeagaaga 4801 tecetegee ectgtgeeee ectggeeaaa gagaagaaaa 4801 tecetegee etetgeeee ectggeaaaa 4801 tecetegee etetgeeee ectggeeaaaa 4801 gagaagaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	3781	gggatcagga	tctatgcagg	ggccccagac	tccccagtca	accagcagtt	ccatggcaga	
3961 otecacatte cagaagega attecatgae tecaaaacet gggtateage cagtatga 4021 tacetetgae atgatgggge geatgteeta tgagecaaat aaggateett atgageagea 4081 gaggaaaaget ceaggagtg atecetteat gteetaagg cagggeecea acggeggga 4141 gggtgacee tacagtegtg etgeeggee tgggetagga aatgtggea tgggacea 4201 acageactat ecetatggag gteetataga cagagtagg acggageetg gaataggge 4261 tgagggaaac atgageactg gggeeceaca geegaatete atgeetteea acecagaet 4321 ggggatgtat tetectagee getaeceee geageaceet etggeeage cageageag 4381 tgatteetat ggeaateagt tetecaceae aggeaceeet etggeeage cetteceea 4441 ceageagaet acaatgtate aacageacaa geagaattae aageggeea 4501 atatggeeet etgeeagag gggaatgtae ageggeeaa tggatggea 4501 atatggeeet etgeeagag ageagtgee eeageaceag eecageeage 4501 atatggeeet eageetteee etcageaaga ggaagatgtae agegtgeea tacageagag 4501 atatggeeet eageetteee etcageaaga gtgattaeaa eageagaea 4501 atatggeea geetgeta etgagegee aceageagg ggeeeceaa atgaetatee 4681 tgeeactgee eageetgeta etgagegee aceageagge ggeeecaaaagagagaagaga	3841	aggaggagac	ttaaagccac	caactccagc	atccacacca	cacagtcaga	tccccccatt	
4021 tacctctgac atgatgggg gatgtcta tgagcaaat aaggatcct atggcaga 4081 gaggaaagct ccagggagtg atccttcat gtcctcaggg caggggccca acgggggga 4141 gggtgaaccc tacagtcgtg ctgccggcc tgggctagga aatgtggga tgggacca 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg gataagggc 4261 tgagggaaaa atgagcactg ggcccaca gccgaatcct atgccttcca acccagact 4321 ggggatgtat tctcctagcc gctaccccc gcagcagacgac agcagagca cagcagcag 4381 tgattcctat ggcaatcagt tctccacaca agcacacct tctggcagc ccttccca 4341 ccagcagact acaatgtatc acaagcaaca gcagaattac aagcggcaa tggatggca 4501 atatggccct cctgccaagc ggcacgaagg ggagtgtac agcgtgccat acagcatgc 4561 gcagggggaa cctcagcag agcagtgcc cccagccag cccagccag 4621 acaagatcgc cagccttcc ctcagcaaga ggagtgtac agcgtgccat acagcatgc 4681 tgccactgc acagctgcta ctgagcgc accagcagg ggccccaga accaattc 4681 tgccactgc acagctgcta ctgagcgcg accacacag ggccccaga accaattc 4741 attccagttt ggccgagacc gtgtctctgc accacctggc accaatgcc agcaaagca 4861 catgtggcag ggcgtaatg acatgaccta taattatgcc acaatgccc agcaaagca 4861 catgtggcag ggcccaca atcatggcgt gaaccgaaca gaggtgctc agcaagca 4861 tcagagggcc accacagag gctgtctggcc ttccatagc agactgcagc accacacag 4981 tcagagggcc accacagag gctgtctggcc ttccatagc acacacacag 5101 aagcatcacaa atcacattc ctcaggtat cagcaccacac 5101 aagcagcaca tctcctaga agctccatt cctgcactct 5281 gcgggatatc accttcccac ctgaccatac gccctccc ggcacacaca 5341 gaggcggctc acaatgaag acattggaac acctgcccct gggatgaaaa tgcagagag 5221 aggtcccca gtacctgcac ctcctagaag acctgacacac aggatgagaa 5341 gaggcagctc acaatgaag acattggaa acctgaccct 5401 caagtctggt ctcctgaag agagacaatg ggaatagac 5401 agaagaagag gaacaaga gccgctatag acctgacct gggatgaaa tgagagaga 5401 agaagaagag agaatagatg acctgagac ccgaatagac 5401 agatgacaca agaacaaga gctgattga gagaagaa 5401 agatgacaca agaacaaga gccgcatagac 5401 agatgacaca agaacaaga gccgaataga 5401 agatgacaca agaacaaga gaacaatag 5401 agatgacaca agaacaaga gaacaatag 5401 agatgacaca agaacaaga gaacaatag 5401 agatgacaca agaacaaga gaagaagaa agaacatca agaagaga 5401 agatcacat gaagagaa acaagagaa gaaaacatca agaagagag 5401 agaccacaca agaa	3901	gccaggcatg	agcaggagca	attcagttgg	gatccaggat	gcctttaatg	atggaagtga	
4081 gaggaaaget caaggagtg atcecttcat gtectagga aatgtggega agggaccaa 4201 acagcactat cetatggag gtecttatga cagagtgaga aatgtggega tgggaccac 4201 tgaggaaaa atgagcactg ggaccacaa geggaactet atgaggagaaa atgagaactg ggaccacaa geggaactet atgaggagaaa atgagaactg ggaccacaa geggaactet atgaggagaaagaa tgagaacaga ggaccacaa gegaagaacgac atgatteca tgcaagagaga ggaccacaa gagaagaacagac 4321 ggggatgat tectatage ggaccacaa gagaagaacac acgaagaga aaaaggacaa acaatgatat aaaaggaacaa gagaagaacacat ttggaagagaa gagaagaacagac 4381 tgattectat ggcaatcagt tetteacacca aggaacacac ttggaggaa atgaggagaa cetgaaggag ggaagatgaa aaagggacaa tggatggaa 4561 atatggeet cetgacaaga ggaagatgac accaagcacaga gacagagaaga gacagaagaa gacagaagaa gacagaagaa gacagaagaa gacagaagaa gacagaagaa gacagaagaa gacaagaacaa 4681 tgecactgac aaagctgeta etgagegeg accaagaagg ggceccaagaa atgatggaa atgatgaa atgactate 4681 tgecacacaa atgatggag gcccataca ggaatagac acagaaggaa ggacaggaa 4801 gacaacaaa atgatgggg gccccataca ggaatagac aacagagaga ggcecgggatatg acatgacca taattatgac aacagagaag aggacaggac	3961	ctccacattc	cagaagcgga	attccatgac	tccaaaccct	gggtatcagc	ccagtatgaa	
4141 gggtgaccc tacagtegt ctgccggccc tgggctagga aatgtggcga tgggaccac 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagctg gaatagggc 4261 tgagggaaac atgagcactg ggcaccaca gccgaatctc atgcctcca acccagact 4321 ggggatgtat tctcctacgc gctaccccc gcagcagcag cagcagcagc acgcaagcag 4381 tgattcctat ggcaatcagt tctccacca aggcaccct tctggcaagc acgcaagcag 4441 ccagcagact acaatgtatc aacagcaaca gcagaattac aagcggccaa tggatggca 4501 atatggccct cctcacaagc ggcacaagg ggaagattac aagcgccaa tggatggca 4561 gcaggggcag cctcagcagc agcagtagc cccaagcagc acccaagcagc 4621 acaagctgcc cagccttccc ctcagcaaga tgtatacaac 4681 tgccactgcc acagctgcc ctcagcagc ggccccaaga ggccccaaga 4861 gcaccacacaa atgatgggg gcccataca ggcatcagct 4741 attccagttt ggccgaacc gtgtctctgc 4861 gccaccacaa atgatgggg gcccataca ggcatcagct 4861 gccaccacaa atgatgggg gcccataca ggcatcaagc 4861 catgtggcag gggcqtaatg acatgaccta taattatgcc 4861 catgtggcag ggcccgcct atcatggcgt gaaccgaaca gatgaaatgc gacaagaca 4861 catgtggcag acccacaagag gtctgtgcc 4921 tgccccccag ggccccgcct atcatgagct 4921 tgccccccag ggccccgcct atcatggcgt 5041 tcccttdgcc ccttgtgccc cctagcacag gccccctca tctaactacc agcccaag 5041 tcactctgcc cttcctagca agtctcact cctgcacct gggatgaaaag 5161 gaaccgcacc tctcctagca agtctcact cctgcactct 5101 aagcagcacc tctcctagca agtctccat cctgcacct gggatgaaaa tgcaagaag 5221 aggtcccca gtacctgct cgcaatagc acctgccct gggatgaaaa tgcaagaag 5341 gaggcggtc acaatgaag acattggac acctgccct gggatgaaaa tgcaagag 5341 gaggcggct acaatgaag acattggac acctgacctg	4021	tacctctgac	atgatggggc	gcatgtccta	tgagccaaat	aaggatcctt	atggcagcat	
4201 acagcactat coctatggag gtocttatga cagagtagag acgagactg gaatagggc 4261 tagaggaaac atgagacatg gggcoccaca gocgaattct atgocttoca accagact 4321 ggggatgat totoctago ggcaccaca gocgaattca atgocttoca accagact 4321 tagattcctat ggcaatcagt totocacca aggcaccct totggcago cottoccca 4441 cocagcagact acaatgtata cacagcacag gagaattac aagggaccaa tagatgagoc 4561 gcaggggag cotcagcag ggcacgaagag gagaattac aagggaccaa tagatgagoc 4561 gcaggggag cotcagcag ggcacgaagag gagaattac aagggaccaa tagatgagoc 4621 acaaggtgcc acagcottocc ctocagcaaga tytatacaac cagtatgga accaatttc 4741 attocagtt ggcogagac gtgtototgc accagcagg ggcoccaga accaatttc 4741 attocagtt ggcogagac gtgtototgc accaccagag accaattcagt ggcacacaaa ggcatgagag ggcoccaga accaatttc 4741 attocagtt ggcogagac gtgtototgc accectgg accaatgcc agcaagac 4801 gccaccacaa atgatggggg ggcoccataca ggcatcacag ggcataagag ggcoccagaa gcaagaga 4921 tgccocccag ggcocgcct atcatggcg tatatacaaca ggcatcagag gacaggaga gcacaggggt 4921 tgccocccag ggcocgcct atcatggcg taccatgag accaatgac agcagagaga gcacaggggt 5041 tocctottgoc cottggcoc cottgacaag gcccottoca tottaatcacc agcoccaca 5101 aagcatgcag aatcacattc ctcaggat cacagcagc coccatatgc cacagcaga 5221 aggtoccca gtacctgcc catagcaag gcccctoca tottaatcacc agcoccaca 5101 aagcatgcag taccattcacc ctcagcatag cactgccct ggatgaaaa tgcagaaga 5341 gaggcogaca taccacagaa gtttocatt cottgacact gggatgaaaa tagcacacag 5221 aggtocccca gtacctgcc ctcagcaataga acctgcccct gtgacagccc catagtatc 5401 caagtottggt ctcctgaca gagacacatg ggcattagat accatcacaca tcctgctgt 5461 tgatgacaca agcatcatga ccttcaacct cagtcagtc ccagggttg tagagcaga 5381 gggtgaccac ggacagaaga cgctactga gacattgga ttctagaaga ggtatgagaga agcatataga cactacaca agcatcatga ggtgagaaa agcatcagag ggtgagaaaa agcatcagaag ggagaaaaa agcatcaaga gggagaaaaa agcatcaaga ggtgagaaaa agcatcaaga gagaagaaga gaaagaaga gaaagaaga gaaagaag	4081	gaggaaagct	ccagggagtg	atcccttcat	gtcctcaggg	cagggcccca	acggcgggat	
4261 tgagggaata atgagcactg gggcccaca gccgaatct atgcttcca acccagact 4321 ggggatytat tetectagee getaceceee gcagcagcag cagcaagcag cagcaagcag 4381 tgattectat ggcaatcagt etecacee aggacacgae ceteceeee 4441 ccagcagaet acaatgtate acaagcacag ggagattae aggagecee teteggaage ceteceeee 4441 ccagcagaet acaatgtate acaagcagg ggagattate aaggegecaa tggatggea 4561 gaaggggaag ceteagcaga ggagattgae ccagcectage acagcatge 4621 acaagetgee cagcetteee eteagcaaga tgtatacaae cagtatggea atgeetate 4681 tgecactgee acagetgeta etgagegeg accagcagge ggeccecaga accaattee 4741 attecagttt ggecgagaee gtgeteteg acccettgge accaatgeee agaagged 4861 catgtggeag ggggtaatag acatgaceta taattatgee acaaggcaga ggacaggget 4921 tgececeag ggececegeet atcatggegt gaccectaea ggactgagag ggccccaataea 4801 gecececag ggececegeet atcatggegt gaccectaea gacaaggag gacaggget 4921 tgececeag ggececegeet atcatggegt gaccectaea gacaggage 4981 tcagagggge aaccagaag getegtggge ttecetage acaaggagag gcagagget 5041 teceetegee cettgtgeee catgacaag gececetea tetaactaee agececeae 5101 aagcatgaga aaccacatte etcaggtate cagcectget eceetggeee gecaattgg 5221 aggtececa gtacetgeet egcacatage acctteceet gggagtaaaa tgcagagag 5341 gaggeggata acctteceae etggetetgt tgaagcaca cagcetgtt tgaaggaga tgaggagaag 5341 gaggeggete acaattgaaa acttggaae ecegacetget tgagaggea tgaggggtaa tgaaggaga 5341 gaggeggete acaatagaa accttecaee etggetetgt tgaagcaea cagcetgtgt tgaaggaga 5381 gggtgacaa aggacataga accttacaea ggcatataga accttaagaa aggatataga ggatatgagag 5581 gggtgaccaa ggagagaga gaagaagaa gatatttga ggtgagaaa tteagagaga ggaagaagaa agaactteta ggtetaagaa tecagagagagaa ggaagaagaa agaactteta ggtetaagaa tecagagagagaa agaagaagaa agaactteta ggtetaagaa actaagaaga agaagaagaa agaactteta ggtetaagaa tegagaggagaa agaagaagaa agaactteta ggtetaagaa acaagagagaa agaagaagaa agaactteta ggtetaagaa acaagagaagaa agaagaagaa agaactteta ggtetaagaa acaagagagaa agaagaagaa agaactteta ggaagaaga agaagaagaa agaactteta ggacaacaa agaagaagaa agaagaagaa agaacttea agaagaagaagaagaagaagaagaagaagaagaagaag	4141	gggtgacccc	tacagtcgtg	ctgccggccc	tgggctagga	aatgtggcga	tgggaccacg	
4321 ggggatgtat totoctago gotaccoco goagcagoag cagcagoag agcaacgac 4381 tgattoctat gocaatcagt totocacoca agcaaccoct totogoago cottococa 4441 ccagcagact acaatgtato acaagcaaca goagaattac aagcggocaa tgataggca 4501 atatggocot cotgocaago goacgaagg ggagatgtac agcgtgocaa tgataggca 4561 goaggagoag ccagcateco cagcatgo cocagcocag coccagoctg ccagcago 4621 acaagotgoc cagcottoco ctocagcaaga ggtatatacaac cagtatggoa atgoctate 4681 tgocactgoc acagctgota ctgagogog accagcago ggococcaga accaattto 4741 attocagttt goccagaago ggottototgo accactgog accaatgoco agcaaacaca 4801 gocaccacaa atgatggog gocccataca ggoatcagot gaggttgoto agcaagoga 4861 catgtggoag gggcctaatg acatgacota taattatgoc acacagogog 4921 tgococcocag ggococgoct atcatggogt gaaccgaaca gatgaaatgo tgocacaga 4881 tocagaggoc aaccacgaag gotogtgoc ttoccatgoc accacgocago coccatatag 5041 tocototgoc octgtgococ catgagogoc ttoccatgo acaacgocago coccatatag 5041 tocototgoc octgtgococ catgagoag goccetoca totaactaco agcocago 5101 aagcatgoag aatcacatto ctocagotata cagcoctgoc tocotagocago 5221 aggtococca gtacotgoct occatgagoag goccetoca totaactaco agcocago 5221 aggtococca gtacotgoct occatgagoag goccetoca 5341 gaggoggot accatgaag accttocat octgocottg gggatgaaag 5341 gaggoggot accatgaag accttocat octgocottg gggatgaaag 5341 gaggoggot accatgaa accttocac octgocottg tgaagocac cagcottgt tgaagocac 5401 caagtottgot otoctggoag agaagaacat ggcagtatga accttocacoc caggottgt tgaagocac 5521 tgtagaatat ttocgacgat goctgattga gatctttgo attttaagaa gattgttoc 5521 tgtagaatat ttocgacgat goctgattga gatctttgo attttaagaa gatagagog 5701 agaaggaaga gaagtagtt aaaatgaaga agaactoct aggaagaaga gagatagago 5701 agaaggaaga gaagtagtta aaaatgaaga gagaagaaga tttaagaaga gagatagaga 5821 cgtacagaag aatagttga aaaatgatga gagaatagoc ttttcaagoc agaagaga 5821 cgtacagaag aatagttga aaaatgagaga ggagatagac cctagagag ttgagaagag 5821 cgtacagaag aatagttga aaaatgagaga ggagaagaaga cgtacacaca gagagagag 5821 cgtacagaag aatagatcat tgggggga tggagagaga tggtagaagag 6821 cgtacaaca gagagatgaa caacagaaga ggaacacaca agaagaagaga 6821 cgacacaca gagagaaga	4201	acagcactat	ccctatggag	gtccttatga	cagagtgagg	acggagcctg	gaatagggcc	
4381 tgattcctat ggcaatcagt tetecaceca aggeacecet tetggaage cettececa 4441 ceagcagaet acaatgtate acaatgaaca geagaattac aagegeeaa tggatggea 4501 aatatggeece cetecageage ageagaagg ggagatgtae agegtgeeat acaagactge 4561 geagggeag ceteageage ageagtgee eecageeag ceceageeg 4621 acaagetgee cageetteee etcageaaga tgtatacaae cagtatggea atgeettate 4741 attecagttt ggeegagaee gtgtetetge accaetagee ggeeceeaga aceaattte 4741 attecagttt ggeegagaee gtgtetetge accaetagee ageaaacaa 4801 gecaceacaa atgatgggeg geceeataca ggeateaget gaggttgete ageaagage 4821 tecgeoceeag ggeecegeet atcatggege gaacegaaca gatgaaatge tgeacacag 4861 catgtggeag ggegtaatg acatgaceta taattatgee acaaggeeag 4821 tecgeoceeag ggeecegeet atcatggege teceatagae gatgaaatge tgeacacag 4831 teagagggee aaceacatae etcaggaace gaceeceae eccatage 5041 tecetetgee eetgtgeee ecatgacaag geeceeteea tetaaacae ageeceeae 5101 aageatgeag aateacatte etcaggtaate cageeceteet gggaatgaaaa tgeagaagg 5221 aggteeceea gtaeetgeet egeacatage acetgeeet gggaatgaaa tgeagaagg 5221 aggteeceea gtaeetgeet egeacatage acetgeeet gggaatgaaa tgeagagag 5241 gaggggatate acetteceae etggetetgt tgaageacae cageetgtgt tgaageaga 5341 gaggeggete acetteceae etggetetgt tgaageacae cageetgtgt tgaageaga 5341 gaggeggete acetteceae etggetetgt tgaageacae aggetgtgt tgaageaga 5341 gaggagaate tecetagaa agaacatag ggeattagat aceatcaacae tecetgetgt 5461 tgatgacaaa agaataatga ecttecaacet eagteagete ecagggtgt tagatgtge 5521 tgtagaatat tteegacaga ggetgatga gaattttgag attettagge atteagaga ggaatagag 5701 agaagaagaga gaagtagtga aaaagaagaa agaactteta ggeeggate tagagaege 5761 agettecagag gaagtagtga acaagaega ggaagaega ggaagaega ggaagaaga 5881 gtttgacaag gaggtgggg aaaagaagaagaagaagaagaagaagaagaagaag	4261	tgagggaaac	atgagcactg	gggccccaca	gccgaatctc	atgccttcca	acccagactc	
4441 ccagcagact acaatgtate aacagcaaca gcagaattac aagcgccaa tggatggca 4501 atatggccet cctgccaage ggacagaagg ggagatgtac agcgtgcca acagcactg 4561 gcaggggcag cctcagcag cccagccag cccagcctg ccagccag ccagcctg ccagccag 4621 acaagctgcc cagccttcc ctcagcaaga tgtatacaac cagtatggca atgcctate 4681 tgccactgcc cagcctgctac ctgagcgcc accagcaggc ggccccaga accaattgcc 4741 attccagttt ggccgagacc gtgtctctgc acccctggc accaatgccc agcaagca 4801 gccaccacaa atgatgggcg gccccataca ggcatcagct gagttgctc agcaagga 4861 catgtggcag ggccgcatt gagaccta taattatgcc aacagcagga gcacgggct 4921 tgcccccaag ggcccgcct atcatgggg gaaccgaaca gatgaaatgc tgcaacacag 4881 tcagaggggc aaccaatgac cccatagacag gtcctctgc accagcagc ccccatagc 4921 tgccccccag ggcccgcct atcatgggg gaaccgaaca gatgaaatgc tgcaacacag 5041 tccctctgcc ccttgtgccc ccttgagcac cccatagacag gcccctcca ttcaactaca agccccacc 5101 aagcatgcag aatcacattc ctcaggtatc cagccctgct cccttgacccc ggccaattgg 5221 aggtcccca gtacctgcct cccatagacaag gccccttct ggggatgaaa tgcagaagga 5221 aggtcccca gtacctgcct cccacataga accttcccat ggggggatat accttccacc ctggctctgt tgaagcaca cagctgtgt tgaagcaga 5341 gagggggtc accaatgaaa acattcgaa accttgcct gggatgaa ttgatgtccc 5521 tgtagaatat ttccgacgat gcctgattga gactttgga acctacaaca tcctgctgt 5461 tgatgacaca agcatcataga ccttcaacct cagtcagtc ccaggggta tgatgagag 5581 gggtgacca ggagagaagaa ccttcaacct cagtcagtt gaggttgcctaaga agaagtagtg gaagaagaag agaacttta ggctcaaac tagaagag 5701 agaagaagag gaagtagtg aaaatgaag gaagatgat gaggtgagaagaagaagaagaagaagaagaagaagaagaa	4321	ggggatgtat	tctcctagcc	gctacccccc	gcagcagcag	cagcagcagc	agcaacgaca	
4501 atatggocct cctgocaagc ggaacgaagg ggagatgtac aggtgocat acagcacge 4561 gcaggggoag cctcagcagc agcagttgoc cccagccagc cccagccagc cctcagcagcagc accagcagc cccagcagcagc cccagcagcagc cccagcagcagc cccagcagcagc cccagcagcagc cccagcagcagc accagcagc accagcagc ggcaccacaa atgatggca atgatgagca atgoctatca ctgagcagca ggcaccacaa caattca attatatatcagca accaattca aggatgagca atgatggca aggatgata acatgatggaggggt aggatgata acatgatggagggggggtaatgaggagggggggggg	4381	tgattcctat	ggcaatcagt	tctccaccca	aggcacccct	tctggcagcc	ccttccccag	
4561 gcagggcag cctcagcage agcagttgce cccagccag ccccagcctg ccagcage 4621 acaagctgce cagcettcec ctcagcaaga tgtatacaac cagtatggca atgectate 4681 tgccactgce cagcetgcta ctgagegeg accagcage ggecccaaga accaattte 4741 attccagttt ggccgagace gtgtetctge accecetgge accaatagece agcaagace 4801 gccaccacaa atgatgggeg gccccataca ggcatcaget gaggttgcte agcaaggca 4861 catgttggcag gggcgtaatg acatgaccta taattatgec aacaaggcaag gaggggt 4921 tgccccccag ggccccgcct atcatggggg gaaccgaaca gatgaaattge tgcacacag 4981 tcagagggc aaccacgaag gctcgtgge ttcccatgge acacggccggc 5041 tcccttgce cctgtgccc ccatgacaag gccccctcca tctaactacc agcccatg 5101 aagcatgcag aatcacatte ctcaggtate cagccctget cccctggccc ggccaatag 5221 aggtcccca gtacctgcct cgcactatag acctgccct gtggaggagg 5221 aggtcccca gtacctgcct cgcacatage acctgccct gtggaggagag 5341 gagggggtc acaatgaaag acattgaa accttgcact cgcacatage acctgccct gtgagagcaca cagcctgtt tgaagcaga 5341 gagggggtc cacaatgaa acattgaac cccgaggga ttggaggaa tgagtacc 5401 caagtctggt ctcctggaag agagcacatg ggcattagat accatcaaca tcctggct 5221 tgtagaaata ttccgacgat gcctgattg ggaattagat accatcaaca tcctgctg 5521 tgtagaatat ttccgacgat gcctgattga gatctttgg attttaaaga ggatagag 5581 gggtgacca ggacagagaa gcctactgga tcctggagg ttcagagag ggacacag 5701 agaagaagag gaagtagttg aaaaagaga gaagaagaaga gaagaagaaga ggagatagag 5881 gtttgacagt ggcctgctga acaagaga ggaagatagc ttttcaggca aggacaaga 5881 gtttgacagt ggcctgctga acaagaga ggaagatagc ttttcaggca ggcaaagag 5881 gtttgacagt ggcctgctga acaagagag ggaacacact ggaacaagag 5881 gttgacaga gagacaca caagaagag agaagtgga tggtggggg gacacacatg aggacaagag 5881 gttgacaga aacgacaaga acgacagag aggacagaga ggaacaagag 5881 gttgacaaga atgatcaa ttgtggggg tggtggggg gacacacaca acaagaagag 5881 gttcaagaag atgatcaa ttgtggggga acaagagag ggacacaca acaagaagag 5881 gcccaacac ggacagaaga agaagtaga caagagagagagagagagagagagagagagagagag	4441	ccagcagact	acaatgtatc	aacagcaaca	gcagaattac	aagcggccaa	tggatggcac	
4621 acaagetgee cageetteee etcageaga tytaatacaac cagtatggea atgeetate 4681 tyeeactgee acagetgeta etgagegeeg accageagege ggeeceaga accaattee 4741 attecagtt ggeegagaee gtgtetetge acceeetgge accaatgeee ageaaaca 4801 geecaccaaa atgatggeeg geeceataca ggeateaget gaggttgete ageaagaga 4861 catgtggeag ggeegeetata acatgaeeta taattatgee aacaaggeaga geaeggget 4921 tyeeceeeag ggeecegeet atcatggegg gaacegaaca gatgaaatge tyeacacaga 4981 teagagggee aacaacagaag getegtggee tteecatgge accageage eccetatatg 5041 teecettgee etgtgeeee catgacaaag geeceeteea tetaactaca ageeceeae 5101 agacetgaag aatacaatte etcaggtate eageeceee gggeaatatg gaggteeee ggaggteee etcaggaaca acctgeeee ggeeaatagg 5221 agggeeee accatgaeea agteteeat eetgeacete gggatgaaaa tyeagaagag 5221 agggegetee accatgaeea acctgeeeet gtggaggeee eccatgatge 5341 gaggeggete acaatgaaag acattggaae eetgeeee gggaggaaa acattggaae eetgeeee 5401 caagtetggt etcetggeag agagaeaatg ggeattagat accateaaca teetgeete 5401 caagtetggt etcetggeag agagaeaatg ggeattagat accateaaca teetgetgt 5461 tgatgacaac agaateatga eetteaacet eagteagete eetgagggg5581 gggtgacea ggaagagaa egetattgga gacttttgge attttaaaagg attttgagg5581 gggtgacea ggaagagaa egetattgga gactettgga attttaaaagg attttagge 5581 gggtgacea ggaagagga agaagaagaa ggaagaaga ggaagaa	4501	atatggccct	cctgccaagc	ggcacgaagg	ggagatgtac	agcgtgccat	acagcactgg	
4681 tgccactgcc acagctgcta ctgagcgcg accagcaggc ggccccaga accaattcc 4741 attccagttt ggccgagacc gtgtctctgc accactgcc accaatgccc agacaacaca 4801 gccaccacaa atgatgggcg gccccataca ggcatcagct gaggttgctc agcaacacaca 4861 catgtggcag ggcccgcct acatggccta taattatgcc aacaaggcaag acaggcagagag ggccccagcc accaggaggt gaaccgaaca gatgaaatgc tgcacacag 4921 tgcccccag ggccccccc accatgacaag gcccctcaattatgccaacaggagagagagagagagagag	4561	gcaggggcag	cctcagcagc	agcagttgcc	cccagcccag	ccccagcctg	ccagccagca	
4741 attocagitt ggccgagacc gtgttotige accectage accaatgece agcaaaaca 4801 gccaccacaa atgatggggg gccccataca ggcatcaget gaggttgete agcaaggca 4861 catgtggcag gggcgtaatg acatgaccta taattatgee aacaggcaga gcacggget 4921 tgcccccag ggcccgcct atcatggcgg gaaccgaaca gatgaaatge tgcacacag 4981 tcagagggce aaccacgaag gctcgtggce ttcccatgge acacgccage ccccatatg 5041 tccctctgce cctgtgccce catgacaag gccccctca tctaactace agccccac 5101 aagcatgcag aatcacatte ctcaggtate cagcctget cccttgccce gggcaatgg 5161 gaaccgcace tctcctagca agtcccatt cctgcactet gggatgaaaa tgcagaagg 5221 aggtcccca gtacctgcc ccgacatage acctgccct gtgcagcce ccatgacag 5341 gaggcggcte acaatgaaaa acattggaac cctgccct gtgaggccc ccatgattc 5281 gcgggatate accttcccac ctggctctgt tgaagccaca cagcctggt tgaagcaga 5341 gaggcggcte acaatgaaaa acattggaac cccggaggca tggcgggtaa tgatgtccc 5401 caagtctgt ctcctggcag agagcacatg ggcattagat accatacaca tcctgctgt 5461 tgatgacaac agcatcatga ccttcaacct cagtcagte ccagggttge tagagctcc 5521 tgtagaatat ttccgacgat gcctgattga gacttttgg agctccaaac agctctgtg 5701 agctcccatg gagggtgggg aagaagaaga agaacttcta ggcctaaac tagaagag 5701 agctccagag aatagttg aaaaatgtga gagatagget ttttcaggca aggacaagg 5761 agctccagag aatagttg aaaaatgtga gagatagtt gaccaacte cagtaagat ccgtacagaa aatagtgag agaagactgat cagtaagtt gaccaacte gagacaaga cagacagag cgctgattg gaccacactg agcatatec 5941 gaccactc gaggacaag cagagactgat cagtaagtt gaccaacac aggacaage 5761 agctccagag aatagtcga cagaagacga cagaagagg gacaccactg agcatatcc 6001 agcccctcg aagcaagac cagaagacga cagaagacga cagaagacga acaacacaca ggacaacac ggacaacac ggacaacac ggacaacac gaagacaga cagaacacac gaagacacacac	4621	acaagctgcc	cagccttccc	ctcagcaaga	tgtatacaac	cagtatggca	atgcctatcc	
4801 gccaccaca atgatgggg gccccataca ggcatcagct gaggttgctc agcaaggca 4861 catgtgcag gggcgtaatg acatgaccta taattatgcc aacaggcaga gcacgggct 4921 tgcccccag ggccccgcct atcatggcg gaaccgaaca gatgaatgc tgcacacag 4861 tcagagggc aaccacgaag gctcgtggcc ttcccatggc acacggcagc cccatatgc 5041 tcccttctgc cctgtgccc ccatgacaag gcccctcca tctaactacc agccccac 5101 aagcatgcag aatcacattc ctcaggtatc cagccctgct cccctgcccc ggccaatgg 5161 gaaccgcacc tctcctagca agtctccatt cctgcactct gggatgaaaa tgcagagg 5221 aggtcccca gtacctgcc cgcactaga acctgccct gggatgaaaa tgcagagg 5341 gaggcggtc acaatgaag acattggaac cccgagagga tgggggtaa tgagtgccc 5401 caagtctggt ctcctggcag agagacacag ggcattagat accttccaac ctggcttgt tgaagcaca agcatgtgt tgaagcaga 5341 tgatgacaac agcatcatga ccttcaacct cagtcagtc ccagggttgc tagagctcc 5401 caagtctggt ctcctggcag agagacacag ggcattagat accttccaact cagtcagtc ccagggttgc tagagctcc 5521 tgatgacaac agcatcatga ccttcaacct cagtcagct ccagggttgc tagagctcc 5521 tgatgacaac agcatcatga ccttcaacct cagtcagct ccagggttgc tagagctcc 5521 tgatgacaca ggacagaga cgctactgga tcctggaggg ttcagcaagg tgtctagtc 5541 agctcccatg gaggtgggg aagaagaaga agaacttcta ggtcctaaac tagaagagg 5701 agaagaagag gaagtagtg aaaaatgatga ggagatagcc ttttcaggca aggacaagg 5761 agcttccaaga aatgatgag aaaagagaaga agaacttcta ggtcctaaac tagaagagg 5821 cggacagaa aatgatcact ttgtggtga ctgctcaga agcacacctg ggtgcagg 5881 gtttgacaga aatgatcat ttgtggtga ctgctcaga agcacacatg ggtgcagg 5881 gttgacaga agcatgtga cacaggagg tggtgggg gacacacat gggacaacac ccaggaagg 6061 gccccccacc gatggacaa ccagagagtg cctgccaac agcacact gagaacacac agcacact gagacacac cagaagaga gacacacac gggacaacac ccaggaagg 6181 cagcaagtt ccatttggac ttagccaga agcacacac agcacacac ccaggaagg 6181 cagcaacact gagacccac acagaagag agcacacaca gggacaacac ccaggaaga agcacacaca	4681	tgccactgcc	acagctgcta	ctgagcgccg	accagcaggc	ggcccccaga	accaatttcc	
4861 catgtggcag gggcgtaatg acatgaccta taattatgcc aacaggaga ggacgggct 4921 tgcccccag ggccccgcct atcatgggct gaaccgaaca gatgaaatgc tgcacacag 4981 tcagagggcc aaccacagaag gctcgtggcc ttcccatggc accacacaga gcccctca tctaactacc tgcaccacaga gcccctcaca tctaactacc agccccacacacacacacacacacacacacacacac	4741	attccagttt	ggccgagacc	gtgtctctgc	accccctggc	accaatgccc	agcaaaacat	
4921 tgececeag ggeceget ateatggegt gaacegaaca gatgaaatge tgeacacag 4981 teagaggge aacacagaag getegtggee tteceatgge acacgeage eccetatage 5041 teeetetgee eetgtgeeee eatgacaag geeeeteea tetaactaee ageeeeae 5101 aageatgeag aateacatte eteaggtate eageeetget ggeaagaag 5161 gaacegeace teteetagea agteteeatt eetgeacte gggaatgaaaa tgeagaagg 5221 aggteeeea gtaeetgeet egacaatage acetgeeeet gtgeageee ecatgatte 5281 gegggatate acetteeeae etggetetgt tgaageeaea eageetgtgt tgaageaga 5341 gaggeggete acaatgaaag acattggaae eeggaggea tggegggtaa tgatgteee 5401 eaagtetggt eteetggeag aggacacatg ggeattagat aceateaaea teetgeetgt 5461 tgatgacaae aggateatga eetteaacet eagteagete eeagggttge tagagetee 5521 tgtagaatat teeggacga teetagaag gatetttgga attetaaga gatetagg 5581 gggtgaceea ggacagagaa egetactgga teetgggagg tteaggagg 5581 aggetgacea ggacagagaa egetactgga teetgggagg teetagge 5701 agaagaagag gaagtagttg aaaaatgatga ggagatagee tteeaggaag tgtetagte 57101 agetteagag aatagtagg agaagetgat eegtacatga gacaaagee eagtaagge 5701 agetteagag aatagtagag agaagetgat eegtacagaa aggacaaage 5701 agetteagag aatagtagag agaagetgat eegteeaga ageacaacte eagtaagge 5701 ageeceacte gaaggeaga eegtgetge eegteegga tggtggggg gacaacacte eagtaagag 5821 egttacagaa aatagtagag agaagetgat etgeteaga ageacacte eagtaagag 5821 getteagaag eagagtagtg acacaggaga tegeteega eagtagggg 5701 ageeceacte gaagageaga eagagetget ggeteeceag eegtacagee eetgeega 5711 ageeceacte gaagageaga eagagetget ggeteegag eetgeega ageacacactg ageacacactg 5711 ageeceacte gaagageaga eagagetget ggeteegag eetgeegagaagaageegaagaagaagaagaagaagaagaaga	4801	gccaccacaa	atgatgggcg	gccccataca	ggcatcagct	gaggttgctc	agcaaggcac	
4981 tcagagggcc aaccacgaag getegtggcc tteccatggc acacgccagc ceccatatgg 5041 tccctetgec cctgtgcccc ccatgacaag gecceteca tctaactacc agecceacc 5101 aagcatgcag aatcacattc ctcaggtatc cagecetget ccctgcccc ggcaattgg 5161 gaaccgcacc tetectagca agtetecatt cetgcactet gggatgaaaa tgcagaagg 5221 aggtcccca gtactgcct cgcacataga acctgcccct gggatgaaaa tgcagaagg 5221 agggggtata accttccac ctggetetgt tgaagccaca cagcetgtt tgaagcaga 5341 gagggggtc acaatgaaag acattggaac cccggaggca tggegggtaa tgatgtccc 5401 caagtctggt ctcctggcag agagcacatg ggcattagat accatcaaca tcctgctgt 5461 tgatgacaac agcatcatga cettcaacct cagtcagtc ccagggttge tagagctcc 5521 tgtagaatat ttccgacgat gcctgattga gatctttggc attttaaagg agtatgagg 5581 gggtgacca ggacgagaa cgctactgga tcctgggagg ttcaggagg ttcaggag 55641 agctccatg gagggtggg aagaagaaga agaactcta ggctactaac tagaaggg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagcc ttttcaggca aggacaagc 5321 cgtacagaag aatagtagag agaagctgat cagtaagttt gacaagcttc cagtaaaga 5821 cgtacagaag aatagtacat ttgtggtgga ctgatagtt gacaagcttc cagtaaaga 5821 cgtacagaag aatagtcat ttgtggtgga ctgctcagat tggtggggg gacaccacta ggagacaaga 6061 gccccacct gatggacct cagaaaaacag gggtacacac ggacaagac cacacgaagg 6061 gccccacct gatggacct cagaaaaacag aggacaagac agaaccact aggacaagac 6241 ggacgaaccc cacagtaagg atgagccac agaagccac cacaggagga 6241 ggacgaaccc cacagtaagg atgagccac acaaggagga acacacac ggacaagtt cagaaggcca tcaagaggaa 6241 ggacgaaccc cacagtaagg atgagccac acaaggaaga 6241 ggacgaaccc cacagtaagg atgagccac acaaggagaa 6241 ggacgaaccc cacagtaagg atgagaccc acaagaagaca cacacaagaaga acaagacaca agacacacaa agacaccaa aacacacaag gctgctgctc atacctggac accatta ttgtgccag 6421 gctgcacaa agacaccaa aacaccaag gctgctgct ataccatcaa agacaccaa aacacacaag gctgcacacaa aacacacaag gctgcacacaa aacacacaag gctgcacacaa aacacacaag gctgcacacacaa aacacacaag gctgcacacacaa aacacacaag gctgcacacacacaacaagaagaacaacaacaagaagaa acacacac	4861	catgtggcag	gggcgtaatg	acatgaccta	taattatgcc	aacaggcaga	gcacgggctc	
5041 tecetetgee cetgtgeece ceatgacaaaa geeceeteea tetaactace ageeceeae 5101 aageatgeaa aateacatte eteaagtate cageeetget eecetgeece ggecaatga 5161 gaacegeace teteetagea agteteeatt eetgeactet ggagatgaaaa tgeagaagg 5221 aggteeceea gtaeetgeet eegacataage acetgeecet gtgeageece ceatgatte 5281 gegggatate acetteecae etggetetgt tgaageeaa eageetgtgt tgaageaga 5341 gaggeegete acaatgaaaaa acattggaae eegagagaa tggegggtaa tgatgteee 5401 caagtetggt eteetggaa gageeaatg ggeattagat aceateaaca teetgetgt 5461 tgatgacaae ageateatga eetteaaeet eagteagete eeagggttge tagagetee 5521 tgtagaatat tteegaegat geetgattga gatetttgge attitaaagg agtatgagg 5581 gggtgaceea ggacaagaaa egetaggaa teetgggagg teegacaaagg 5701 agaagaagaag gaagtagttg aaaatgatga ggagatagee ttetteaggea ggagatagae 5761 ageteeagaa gaagtagttg aaaaatgatga ggagatagee ttetteaggea aggacaaage 5761 ageteeagaa aatagtgaag agaagetgat eagtaaget ggeetaaaaa atgeteagag 5821 egtacagaaa aatgateeat ttgtggtgaa etgeteagat gacaaagete eagtaaagag 5821 egtacagaaa aatgateeat ttgtggtgaa etgeteagat aagettggee gtgtgeagg 5881 gtttgacagt ggeetgetge actggeggat tggtgggggg gacaccactg ageatatee 6001 ageeceacte gagageaaga eagagetget geetteeegg eetcaagaa ageatatee 6001 ageeceacte gagageaaga eagagetget geetteeegg eetcaagaaaaaa geaacteeg 6061 geececacet gatggacete eagaaaaaaa gggataaaaaa ageaagagaa 6181 cacteggtet ageacettga eegagatga aaaaagagaa eegaagaaaaaaaaaa	4921	tgccccccag	ggccccgcct	atcatggcgt	gaaccgaaca	gatgaaatgc	tgcacacaga	
5101 aagcatgcag aatcacatte etcaggtate eagecetget eccetgeece ggecaatgg 5161 gaacegeace tetectagea agtetecatt ectgeactet gggatgaaaa tgeagaagg 5221 aggteecea gtacetgeet egeacatage acetgeecet gtgeageece ecatgatte 5281 gegggatate acetteecae etggetetgt tgaageeaca eageetgtgt tgaageaga 5341 gaggeggete acaatgaaag acattggaac ecceggaggea tggegggtaa tgatgteee 5401 caagtetggt etcetggeag agageacatg ggeattagat aceateaaca teetgetgt 5461 tgatgacaac ageateatga ectteaacet eagteagete ecaggggtaa tgatgagg 5521 tgtagaatat tteegacgat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgaceca ggacagagaa egetactgga teetgggagg tteagacaac tagaagagg 5501 agaagaaga gaagtagttg aaaatgatga ggagatagee tttteagea aggacaage 5701 agaagaagag gaagtagttg aaaatgatga ggagatagee tttteaggea aggacaage 5701 agaagaagag gaagtagtg aaaatgatga ggagatagee tttteaggea aggacaage 5761 agetteagag aatagtgagg agaagetgat eagtaagtt gacaagette eagtaagag 5881 gtttgacagt ggeetgetg actggeggat tggtggggg gacaacaetg ggeetgeagg 5881 gtttgacagt ggeetgetg actggeggat tggtggggg gacaacaetg ageatatee 5941 gacecaette gagageaaga eagagetget geetteeegg ectacagea ectgeeae 6001 ageeeetegg aageatgtga eagagetget geetteegg ectacageae ectgeeae 6001 ageeeetegg aageatgtga eagagetgg gggtacacea gggacaacae ectgeeae 601 ageeeeteg aageacttga eagagetgg ggtacacea gggacaacae ectgeeae 6021 tacteggtet ageacettga ecaggagatgg agetaagagt teagaggeea teaaggaga 6181 eageaagtt eeatttggea ttageeeaga acaggageae ectteggaea ageacatea agacetetag 6241 ggacgaacee eacagtaagg atgagaeee aacagageea ectteggaea ageagagae 6241 ggacgaacee eacagtaagg atgagaeee aacaecaag getgetgete ateetgggaa ageagagae 6241 ggacgaacee aacaecaag aacaecaagg getgetgee acettggaea ageetgetee 62421 getgeacaa aagaaceeaa aacaecaagg getgetgete ateetgggea agetgatee 62421 getgeacaa aagaaceeaa aacaecaagg getgetgete acettggaea ageagagagagagagagaeaacaagaacaaagagaagaa aacaecaaaaga ggagtgagagagagagagagagagaacaaaagaacaaaagaagaa	4981	tcagagggcc	aaccacgaag	gctcgtggcc	ttcccatggc	acacgccagc	ccccatatgg	
5161 gaaccgcacc tetectagca agtetecatt cetgcactet gggatgaaaa tgcagaagg 5221 aggteccca gtacetget egcacatage acetgeecet gtgcagece ceatgatte 5281 gegggatate acetteccae etggetetgt tgaagecaca cageetgtgt tgaageaga 5341 gaggegget acaatgaaag acattggaac eeceggaggea tggegggtaa tgatgteec 5401 caagtetggt eteetggeag agagcacatg ggeattagat accateacaa teetgetgg 5461 tgatgacaac agcateatga eetteaacet cagteagete ecagggttge tagagetee 5521 tgtagaatat tteegacgat geetgattga gatetttgge atttaaagg agtatgagg 5581 gggtgacca ggacagagaa egetactga teetgggagg tteageaagg tgtetagte 5641 ageteccatg gagggtggg aagaagaaga agaactetea ggteetaaac tagaagagg 5701 agaagaagag gaagtagtg aaaatgatga ggagatagee ttteagea ggeetgatga 5761 agetteagaa aatagtagag agaagetgat eagtaaget ggtetaagaa atagateeat ttgtggtgga eetggetge actggetgetg eetggetge actggeggat tggtggggg gaagaagee ggttgacaga gagacatee gagageaga eetggeggat tggtggggg gaaccacatg ageactee gagagaagaa eagagetget geetteegg eetcacee eetgeecac 6001 ageeceacete gagagacate eagaagetg ggtacacaa gggacaacaa ageattegg 6121 tacteggtet ageacettga eegagaaga gagaaaacg agaacacaca aggagagag6181 eagagaaget eacattggaa agaacete eacagaagag atageece acagagaga atageece acagagaga atageece acagagaga agaaceece acagagaga agaacacacaca agaacetga fagacacacacacacacacacacacacacacacacacaca	5041	tccctctgcc	cctgtgcccc	ccatgacaag	gccccctcca	tctaactacc	agcccccacc	
5221 aggteccca gtacetget egeacatage acetgecet gtgeageec ceatgatte 5281 gegggatate acetteceae etggetetgt tgaageeaa eageetgtgt tgaageaga 5341 gaggeggete acaatgaaag acattggaae eeeggaggea tggegggtaa tgatgteee 5401 caagtetggt etcetggeag agageacatg ggeattagat aceateaaca teetgetgt 5461 tgatgacaae ageateatga eetteaaeet eagteagete eeagggttge tagagetee 5521 tgtaagaatat teegaegat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgacea gaagaggaa egetaetgga teetgggagg teagaagagg 5701 agaagaagag gaagtagtg aaaaatgatga ggagatagee tttteagea aggaeaagg 5761 agetteagag gaagtagtg aaaaatgatgag gaagatagee tttteaggea aggaeaage 5761 agetteagag aatagtgag agaagetgat eagtaagttt gaeaagette eagtaagag 5821 egtaeagaa aatgateeat ttgtggtga etgeteagat aagettgge gtgtgeagg 5881 gtttgaeagt ggeetgetge actgeggat tggtgggggg gaeaceaetg ggeetgetge 6001 ageeeeteeg gatggaeete eagaaaaaeg gggtaeaeea gggaeaaaga eagaegggg 6061 geeeeeaeet gatggaeete eagaaaaaeg gateaeeae gggaeaaeae eagagggg 6061 eageaagtte eeatttggea taggaeee eagagagaga agaagaeee eagagagge 6361 caatgaette eeatttggea taggaeeee aetaggaee eeteeggaee 6361 eaatgaette gagageeee aaeaeeeag aggaeeee eagagagae 6361 eaatgaette gagagtgee aaeaeeeag aggaeeee eacaggaega aggaeaeee eacaggaegae 6361 eaatgaette gagagtgee aaeaeeeag aggaeeeee 6421 getgeaeea aageaeeeag aaegaeeea aaegaeeee aaeaeeeag agetgaeee 6481 aeaggaeeaa agggtgaee geaaeaaag ggagtggtgg tgggaetget tgggagaee 6541 eegggaaaae aeettggtta eaceteggae aaeetteggae ageagaaga 6541 eegggaaaae aeettggtta eaceteggae aaeetteggae ageagaagae 6541 eegggaaaae aeettggtta eaceteggeae aaeggagagg 6541 eegggaaaae aaeettggtta eaceteggae ageagaagae 6541 eegggaaaae aeettggtta eaceteggae aaeetteggae aggaggaggg 6541 eegggaaaae aeettggtta eaceteggae aaeetteggae ageagagae 6541 eegggaaaae aaeettggtta eaceteggae aaeeteggae aaeeeggaeggae 6541 eegggaaaae aeettggtta eaceteggae aaeeteggae aaeeteggae aaeeeggaegae 6541 eegggaaaae aeettggtta eaceteggae aaeeteggae aaeeegeae 6541 eegggaaaae aeettggtta eaceteggae 6541 eegggaaaae aeettggtae eaceteggae 6541 eegggaaaae aeettggta eacetegae 6	5101	aagcatgcag	aatcacattc	ctcaggtatc	cagccctgct	cccctgcccc	ggccaatgga	
5281 gegggatate acetteceae etggetetgt tgaagecaea eageetgtgt tgaageaga 5341 gaggeggete acaatgaaag acattggaae eeeggaggea tggegggtaa tgatgteee 5401 caagtetggt eteetggeag agageacatg ggeattagat aceateaaea teetgetgt 5461 tgatgacaae ageateatga eetteaaeet eagteagete eeagggttge tagagetee 5521 tgtagaatat tteegaegat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgaceea ggacagagaa egetactgga teetgggggt teageaagg tgtetagte 5641 ageteecatg gagggtgggg aaaatgatgag gaagaagaag gaagtagttg aaaatgatgag ggagatagee tttteaggea aggacaagag 5701 agaagaagag gaagtagttg aaaatgatga ggagatagee tttteaggea aggacaage 5761 agetteagag aatagtgagg agaagetgat eagtaagett gacaagette eagtaaggs 5821 egtacagaa aatgateeat ttgtggtgga etggteagg gacaecaetg gggetgeegg 5881 gtttgacagt ggeetgete actggeggat tggtggggg gacaecaetg ageatatee 5941 gaceeaette gagageaaga eagagetget geetteeegg eettacegg eettacegg 6061 geeeceaeet gatgacete eagaaaaaeg gataacaea gggacaaeae eetaggagg 6061 geeeceaeet gatggacete eagagaaaaae gggtacaeae gggacaaeae eetaggagg 6181 eageaagttt eeatttggea ttageecage aeagageeae eagagaeee eacagtaagga 6301 tettgeeaag egetgetet gtgtgteeaa taceattega ageetgtea ttggtgegg 6361 eaatgaettt gagatgteea aaeaeeeag gggeaeeae eacagtaea ggegggtggeg 6421 gegegaeaea aageaeeeag aaeaeeeag gggeaeeaea aaeageaega 6481 acaggaeeaa aggggtgaget geaaeaaag ggggtgggg geaeeaeata aetttggaa aggaggagg 6481 acaggaeaaa acettggtta eactegeeaa eactteggge ageetgeee 6541 eegggaaaae acettggtta eactegeeaa eactteggge eagttggaee 6541 eegggaaaaa acettggtta eactegeeaa eactteggge eagttggaee ttggagatge 6541 eegggaaaaa acettggtta eactegeeaa eactteggge eagttggaee ttggagatge 6541 eegggaaaaa acettggtta eactegeeaa eacteggga eagttggge 6541 eegggaaaaa acettggtta eactegeeaa eacteggga eacteeta eggagaage 6541 eegggaaaaa acettggtta eactegeeaa eacteggga eacteetagge agetggaget eactegggaaaea eacttgggea aaceteggaaeee eggaaeaea aacettgggea ageaeaeae eactggaaeae eactggaaeae eacaeeaga ggeaeaeaea aacettgggea ageaeaeaeaea ageaeeaeaeaeaeaeaeaea	5161	gaaccgcacc	tctcctagca	agtctccatt	cctgcactct	gggatgaaaa	tgcagaaggc	
5341 gaggeggete acaatgaaag acattggaac ceeggaggea tggegggtaa tgatgteee 5401 caagtetggt eteetggeag agageacatg ggeattagat aceateaaca teetgetg 5461 tgatgacaac ageateatga eetteaacet eagteagete eeagggttge tagagetee 5521 tgtagaatat tteegaegat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgaceca ggacagagaa egetaetgga teetgggagg tteageaagg tgtetagte 5641 ageteecatg gagggtggg aagaagaaga agaaetteta ggteetaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagee tttteaggea aggacaage 5761 agetteagag aatagtgagg agaagetgat eagtaagttt gacaagette eagtaaaga 5821 egtacagaag aatgateeat ttgtggtgga etgeteagat aagettggge gtgtgeagg 5881 gtttgacagt ggeetgetge actggeggat tggtggggg gacaceactg ageatatee 5941 gaceeactte gagageaaga eagagetget geetteeegg eetteeegg eetteeege 6001 ageeeetegg aageatgtga eagaagetget gggacaacaag accaggagg 6061 geeeecaeet gatgacete eagaaaaeeg gateacagae eactatggaga 6181 eageaagtt eeatttggea ttageeeage aggacaacae eggaacatea agateetag 6241 ggacgaaeee eacagtaagg atgageeee eagageeee eeggaeeee eeggaaeatee 6301 tettgeeaag egetgetet gtgtgteeaa taceattega ageetgteat ttgtgeeag 6361 eaatgaettt gagatgteea aacaceeag gegaeeeee ettetggea agetggagg 6481 acaggaceaa agggtgaget geaacaaagt ggagtggtgg tgggactget ttggagatge 6541 eegggaaaac acettggtta eacteggeaa eacteteggg eagttggaee tateteea	5221	aggtccccca	gtacctgcct	cgcacatagc	acctgcccct	gtgcagcccc	ccatgattcg	
5401 caagtetggt eteetggeag agageacatg ggeattagat accateaaca teetgetgt 5461 tgatgacaac ageateatga cetteaacet cagteagete ecagggttge tagagetee 5521 tgtagaatat tteegacgat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgaceca ggacagagaa egetactgga teetgggagg tteageaagg tgtetagte 5641 ageteecatg gagggtggg aagaagaaga agaaetteta ggteetaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagee tttteaggea aggacaage 5761 agetteagag aatagtgag agaagetgat eagtaagttt gacaagette eagtaagag 5821 egtacagaag aatgatecat ttgtggtga etgeteagat aagettggge gtgtgeagg 5881 gtttgacagt ggeetgetge actggeggat tggtggggg gacaceactg ageatatee 5941 gacecactte gagageaga eagagetget geetteeegg eettacegg eettacegg eettacegg eettacegg 6061 geeecacet gatggacet eagaagagg gateaacaag gggtacacaa ageagggg 6181 eageaagtte eacattggac tegaggatgg ageaagaage eacaggagga 6241 ggacgaacee cacagtaagg atgageee acagageee eacaggagea eacagageee eacagtagga eacacatg ageagaate eacagtagga atgageacee acaggaggat teagaggeea eacaggaga ageagaacee eacagtagga atgagaceee actatggate teagaggea teagaggaga 6361 eaatgactt gagatgeea aacaceagg getgetee accattegga agetgatee 6421 getgeacea aagaacecaa aacaceagg getgetgete ateetggea agetgageg 6481 acaggacaa agegtgaget geaacaaagt ggagtggtgg tgggactget tggagatge 6541 eegggaaaac accttggtta eacteggea accteggga eactteeat tggagatge 6541 eegggaaaac accttggtta eactteggea acctteggga eactteeat tggagatge	5281	gcgggatatc	accttcccac	ctggctctgt	tgaagccaca	cagcctgtgt	tgaagcagag	
5461 tgatgacaac agcatcatga cetteaacet cagteagete ceagggttge tagagetee 5521 tgtagaatat tteegaegat geetgattga gatetttgge attttaaagg agtatgagg 5581 gggtgaceca ggacagagaa egetaetgga teetgggagg tteageaagg tgtetagte 5641 ageteecatg gagggtgggg aagaagaaga agaaetteta ggteetaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagee tttteaggea aggacaage 5761 agetteagag aatagtgagg agaagetgat eagtaagttt gacaagette eagtaaaga 5821 egtacagaag aatgateeat ttgtggtgga etgeteagat aagettggge gtgtgeagg 5881 gtttgacagt ggeetgetge aetggeggat tggtggggg gacaecaetg ageatatee 5941 gacecaette gagageaaga eagagetget geetteeegg eeteaegae eetgeeae 6001 ageecetegg aageatgtga eaacageaga gggtacaeca gggacaacag aceaggagg 6061 geececaeet gatggacete eagagaatgg agetaaegee actatggatg acatgttgt 6121 taeteggtet ageaecettga eegaggatgg agetaagagt teagaggeea teaaggaga 6181 eageaagttt eeatttggea ttageecage acagageeae eggaaecatea agateetag 6241 ggaegaaeee eacagtaagg atgagaeeee aetgtgtaee ettetggaet ggeaggatt 6301 tettgeeaag egetgegtet gtgtgteeaa taeeattega ageetgteat ttgtgeeag 6361 eaatgaettt gagatgteea aacaeceagg getgetgete ateetgggea agetgatee 6421 getgeaeeae aageaeeeag aaeggaagea ggeaeeaeta aettatgaaa aggaggagg 6481 acaggaeeaa ageggtagget geaaeaaagt ggagtggtgg tgggaetget tggagatge 6541 eegggaaaae aeettggtta eaeteggeaa eateteggge eagttggaee tateteeat	5341	gaggcggctc	acaatgaaag	acattggaac	cccggaggca	tggcgggtaa	tgatgtccct	
5521 tgtagaatat ttccgacgat gcctgattga gatctttggc attttaaagg agtatgagg 5581 gggtgaccca ggacagagaa cgctactgga tcctgggagg ttcagcaagg tgtctagtc 5641 agctcccatg gagggtggg aagaagaaga agaacttcta ggtcctaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagcc ttttcaggca aggacaagc 5761 agcttcagag aatagtgag agaagctgat cagtaagttt gacaagcttc cagtaaaga 5821 cgtacagaag aatgatccat ttgtggtgga ctgctcagat aagcttgggc gtgtgcagg 5881 gtttgacagt ggcctgctgc actggcggat tggtggggg gacaccactg agcatatcc 5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgcac cctgccac 6001 agcccctcgg aagcatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gccccacct gatggacctc cagaaaaacg gatcacagcc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagttt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatgc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggaccgc tatctccat	5401	caagtctggt	ctcctggcag	agagcacatg	ggcattagat	accatcaaca	tcctgctgta	
5581 gggtgacca ggacagagaa cgctactgga tcctggagg ttcagcaagg tgtctagtc 5641 agctccatg gagggtggg aagaagaaga agaacttcta ggtcctaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagcc ttttcaggca aggacaagc 5761 agcttcagag aatagtgagg agaagctgat cagtaagttt gacaagcttc cagtaaaga 5821 cgtacagaag aatgatccat ttgtggtgga ctgctcagat aagcttgggc gtgtgcagg 5881 gtttgacagt ggcctgctgc actggcggat tggtgggggg gacaccactg agcatatcc 5941 gacccacttc gagagacaaga cagagctgct gccttcccgg cctcacgcac cctgcccac 6001 agcccctcgg aagcatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gccccacct gatggacctc cagaaaaacg gatcacagc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagttt ccatttggca ttagcccage acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcaccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggacaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgcaa catctcgggg cagttggacc tatctccat	5461	tgatgacaac	agcatcatga	ccttcaacct	cagtcagctc	ccagggttgc	tagagctcct	
5641 agctccatg gagggtggg aagaagaaga agaacttcta ggtcctaaac tagaagagg 5701 agaagaagag gaagtagttg aaaatgatga ggagatagcc ttttcaggca aggacaagc 5761 agcttcagag aatagtgagg agaagctgat cagtaagttt gacaagcttc cagtaaaga 5821 cgtacagaag aatgatccat ttgtggtgga ctgctcagat aagcttgggc gtgtgcagg 5881 gtttgacagt ggcctgctgc actggcggat tggtgggggg gacaccactg agcatatcc 5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgac cctgccacc 6001 agcccctcgg aagcatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gccccacct gatgacctc cagaaaaacg gatcacagca actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagttt ccatttggca ttagcccagc actatggat tcagaggcca tcaaggaga 6241 ggacgaaccc cacagtaagg atgagaccc actggtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgcaa catctcggg cagttggacc tatctccat	5521	tgtagaatat	ttccgacgat	gcctgattga	gatctttggc	attttaaagg	agtatgaggt	
5701 agaagaagag gaagtagttg aaaatgatga ggagatagcc ttttcaggca aggacaagcc 5761 agcttcagag aatagtgagg agaagctgat cagtaagttt gacaagcttc cagtaaaga 5821 cgtacagaag aatgatccat ttgtggtgga ctgctcagat aagcttgggc gtgtgcagg 5881 gtttgacagt ggcctgctgc actggcggat tggtgggggg gacaccactg agcatatcc 5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgcac cctgccacc 6001 agcccctcgg aagcatgtga caacagcaga gggtacacca gggacaacaag accaggagg 6061 gccccacct gatggacctc cagaaaaacg gatcacagcc actatggatg acatgttgt tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggagac 6181 cagcaagttt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgctct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat	5581	gggtgaccca	ggacagagaa	cgctactgga	tcctgggagg	ttcagcaagg	tgtctagtcc	
5761 agetteagag aatagtgagg agaagetgat eagtaagttt gacaagette eagtaaaga 5821 egtacagaag aatgatecat ttgtggtgga etgeteagat aagettggge gtgtgeagg 5881 gtttgacagt ggeetgetge aetggeggat tggtgggggg gacaecaetg ageatatee 5941 gacecaette gagageaaga eagagetget geetteeegg eeteaegae eetggeegae 6001 ageeeetegg aageatgtga eaacageaga gggtacaeca gggacaacaag aceaggagg 6061 geeeeaeet gatggacete eagaaaaaeg gateaeagee actatggatg acatgttgt 6121 taeteggtet ageaeettga eegaggatgg agetaagagt teagaggeea teaaggagae 6181 eageaagttt eeatttggea ttageeeage acaggageeae eggaaeatea agateetag 6241 ggaegaaeee eacagtaagg atgagaeee actgtgtaee ettetggaet ggeaggatt 6301 tettgeeaag egetgegtet gtgtgteeaa taeeattega ageetgteat ttgtgeeag 6361 eaatgaettt gagatgteea aacaeceagg getgetgete ateetggeea agetggaegg 6421 getgeaeea aageaeeeag aacggaagea ggeaeeaeta aettatgaaa aggaggagg 6481 acaggaeeaa ggggtgaget geaaeaaagt ggagtggtgg tgggaeetget tggagatgee 6541 eegggaaaae aeettggtta eacteggeaa eateteeggg eagttggaee tateteeat	5641	agctcccatg	gagggtgggg	aagaagaaga	agaacttcta	ggtcctaaac	tagaagagga	
5821 cgtacagaag aatgatccat ttgtggtgga ctgctcagat aagcttgggc gtgtgcagg 5881 gtttgacagt ggcctgctgc actggcggat tggtgggggg gacaccactg agcatatcc 5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgcac cctgccacc 6001 agcccctcgg aagcatgtga caacagcaga gggtacacca gggacaacaag accaggagg 6061 gccccacct gatggacctc cagaaaaaacg gatcacagcc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggagac 6181 cagcaagttt ccatttggca ttagcccagc acagaggccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagacccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgcaa catctcgggg cagttggacc tatctccat	5701	agaagaagag	gaagtagttg	aaaatgatga	ggagatagcc	ttttcaggca	aggacaagcc	
5881 gtttgacagt ggcctgctgc actggcggat tggtgggggg gacaccactg agcatatcc 5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgcac cctgccac 6001 agccctcgg aagcatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gccccacct gatggacctc cagaaaaacg gatcacagcc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagttt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgcaa catctcgggg cagttggacc tatctccat	5761	agcttcagag	aatagtgagg	agaagctgat	cagtaagttt	gacaagcttc	cagtaaagat	
5941 gacccacttc gagagcaaga cagagctgct gccttcccgg cctcacgcac cctgccac 6001 agccctcgg aagcatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gccccacct gatggacctc cagaaaaacg gatcacagcc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagtt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgcaa catctcgggg cagttggacc tatctccat	5821	cgtacagaag	aatgatccat	ttgtggtgga	ctgctcagat	aagcttgggc	gtgtgcagga	
6001 agecectegg aageatgtga caacagcaga gggtacacca gggacaacag accaggagg 6061 gececacet gatggacete cagaaaaacg gateacagee actatggatg acatgttgt 6121 tacteggtet ageacettga eegaggatgg agetaagagt teagaggeea teaaggaga 6181 cageaagtt ceatttggea ttageecage acagageeae eggaacatea agateetag 6241 ggacgaacee cacagtaagg atgagacee actgtgtace ettetggaet ggeaggatt 6301 tettgeeaag egetgegtet gtgtgteeaa taceattega ageetgteat ttgtgeeag 6361 caatgaettt gagatgteea aacaeceagg getgetgete ateetgggea agetgatee 6421 getgeaceae aageaceeag aacggaagea ggeaceacta acttatgaaa aggaggagg 6481 acaggaecaa ggggtgaget geaacaaagt ggagtggtgg tgggaetget tggagatgee 6541 eegggaaaae acettggtta eactegeeaa catetegggg eagttggaee tateteeat	5881	gtttgacagt	ggcctgctgc	actggcggat	tggtggggg	gacaccactg	agcatatcca	
6061 gccccacct gatggacctc cagaaaaacg gatcacagcc actatggatg acatgttgt 6121 tactcggtct agcaccttga ccgaggatgg agctaagagt tcagaggcca tcaaggaga 6181 cagcaagttt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat	5941	gacccacttc	gagagcaaga	cagagctgct	gccttcccgg	cctcacgcac	cctgcccacc	
6121 tacteggtet ageacettga cegaggatgg agetaagagt teagaggeea teaaggaga 6181 cageaagttt ceatttggea ttageeeage acagageeae eggaacatea agateetag 6241 ggaegaacee cacagtaagg atgagacee actgtgtace ettetggaet ggeaggatt 6301 tettgeeaag egetgegtet gtgtgteeaa taceattega ageetgteat ttgtgeeag 6361 caatgaettt gagatgteea aacaceeagg getgetgete ateetgggea agetgatee 6421 getgeaceae aageaceeag aacggaagea ggeaceacta acttatgaaa aggaggagg 6481 acaggaeeaa ggggtgaget geaacaaagt ggagtggtgg tgggaetget tggagatge 6541 eegggaaaae acettggtta eactegeeaa catetegggg eagttggaee tateteeat	6001	agcccctcgg	aagcatgtga	caacagcaga	gggtacacca	gggacaacag	accaggaggg	
6181 cagcaagttt ccatttggca ttagcccagc acagagccac cggaacatca agatcctag 6241 ggacgaaccc cacagtaagg atgagaccc actgtgtacc cttctggact ggcaggatt 6301 tcttgccaag cgctgcgtct gtgtgtccaa taccattcga agcctgtcat ttgtgccag 6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat	6061	gcccccacct	gatggacctc	cagaaaaacg	gatcacagcc	actatggatg	acatgttgtc	
6241 ggacgaacce cacagtaagg atgagacce actgtgtace ettetggact ggeaggatt 6301 tettgccaag egetgegtet gtgtgtecaa taceattega ageetgteat ttgtgccag 6361 caatgacttt gagatgteca aacacceagg getgetgete ateetgggea agetgatee 6421 getgeaccae aageacceag aacggaagea ggeaccacta aettatgaaa aggaggagg 6481 acaggaccaa ggggtgaget geaacaaagt ggagtggtgg tgggactget tggagatge 6541 cegggaaaac acettggtta cactegceaa catetegggg cagttggace tatetecat	6121	tactcggtct	agcaccttga	ccgaggatgg	agctaagagt	tcagaggcca	tcaaggagag	
6301 tettgecaag egetgegtet gtgtgtecaa taceattega ageetgteat ttgtgecag 6361 caatgaettt gagatgteca aacaceeagg getgetgete ateetgggea agetgatee 6421 getgeaceae aageaceeag aacggaagea ggeaceaeta aettatgaaa aggaggagg 6481 acaggaecaa ggggtgaget geaacaaagt ggagtggtgg tgggaetget tggagatge 6541 cegggaaaae acettggtta eactegeeaa eatetegggg eagttggaee tateteeat	6181	cagcaagttt	ccatttggca	ttagcccagc	acagagccac	cggaacatca	agatcctaga	
6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat	6241	ggacgaaccc	cacagtaagg	atgagacccc	actgtgtacc	cttctggact	ggcaggattc	
6361 caatgacttt gagatgtcca aacacccagg gctgctgctc atcctgggca agctgatcc 6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat								
6421 gctgcaccac aagcacccag aacggaagca ggcaccacta acttatgaaa aggaggagg 6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat								
6481 acaggaccaa ggggtgagct gcaacaaagt ggagtggtgg tgggactgct tggagatgc 6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat								
6541 ccgggaaaac accttggtta cactcgccaa catctcgggg cagttggacc tatctccat								
								_

6661 agctgaagcc caggaccct tttccaccct gggccccaat gccgtccttt cccgcagag 6721 actggtettg gaaaceetea geaaacteag cateeaggae aacaatgtgg acetgattet 6781 ggccacacce ceetteagee geetggagaa gttgtatage actatggtge getteeteag 6841 tgaccgaaag aacccggtgt gccgggagat ggctgtggta ctgctggcca acctggctca 6901 gggggacage etggcagete gtgccattge agtgcagaag ggcagtateg gcaaceteet 6961 gggcttccta gaggacagce ttgccgccac acagttccag cagagccagg ccagcctcct 7021 ccacatgcag aacccacct ttgagccaac tagtgtggac atgatgcggc gggctgcccg 7081 egegetgett geettggeea aggtggaega gaaccactea gagtttacte tgtacgaate 7141 acggctgttg gacatctcgg tatcaccgtt gatgaactca ttggtttcac aagtcatttg 7201 tgatgtactg tttttgattg gccagtcatg acagccgtgg gacacctccc cccccgtgt 7261 gtgtgtgcgt gtgtggagaa cttagaaact gactgttgcc ctttatttat gcaaaaccac 7321 ctcagaatcc agtttaccct gtgctgtcca gcttctccct tgggaaaaag tctctcctgt 7381 ttctctccc tccttccacc tcccctccct ccatcacctc acgcctttct gttccttgtc 7441 ctcaccttac tcccctcagg accctacccc accctctttg aaaagacaaa gctctgccta 7501 catagaagac tttttttatt ttaaccaaag ttactgttgt ttacagtgag tttggggaaa 7561 aaaaataaaa taaaaatggc tttcccagtc cttgcatcaa cgggatgcca catttcataa 7621 ctgtttttaa tggtaaaaaa aaaaaaaaa aatacaaaaa aaaattctga aggacaaaaa 7681 aggtgactgc tgaactgtgt gtggtttatt gttgtacatt cacaatcttg caggagccaa 7741 gaagttcgca gttgtgaaca gaccctgttc actggagagg cctgtgcagt agagtgtaga 7801 ccctttcatg tactgtactg tacacctgat actgtaaaca tactgtaata ataatgtctc 7861 acatggaaac agaaaacgct gggtcagcag caagctgtag tttttaaaaa tgtttttagt 7921 taaacqttqa qqaqaaaaaa aaaaaaqqct tttcccccaa aqtatcatqt qtqaacctac 7981 aacaccctga cctctttctc tcctccttga ttgtatgaat aaccctgaga tcacctctta 8041 gaactggttt taacctttag ctgcagcggc tacgctgcca cgtgtgtata tatatgacgt 8101 tgtacattgc acataccett ggatececae agtttggtee teeteecage tacceettta 8161 tagtatgacg agttaacaag ttggtgacct gcacaaagcg agacacagct atttaatctc 8221 ttgccagata tcgcccctct tggtgcgatg ctgtacaggt ctctgtaaaa agtccttgct 8341 tetttetaat egaggtgtga aaaagtteta ggtteagttg aagttetgat gaagaaacae 8401 aattgagatt ttttcagtga taaaatctgc atatttgtat ttcaacaatg tagctaaaac 8461 ttgatgtaaa tteeteettt tttteetttt ttggettaat gaatateatt tatteagtat 8521 gaaatettta taetatatgt teeaegtgtt aagaataaat gtacattaaa tettggtaag 8581 acttt

AT-rich interactive domain-containing protein 1A (ARID1A) isoform b (SEQ ID NO: 11)

1 maaqvapaaa sslgnppppp pselkkaeqq qreeaggeaa aaaaaergem kaaagqeseg 61 pavgppgplg kelgdgaesn ggggggags gggpgaepdl knsngnagpr palnnnltep 121 pggggggssd gygapphsaa aalpppaygf ggpygrspsa vaaaaaavfh gghggggspg 181 laalqsqqqq qlepyaqpqq nshdhqfpnh qynsyypnrs aypppapaya lssprqqtpq 241 sqaaaaaqsk pppsssasas sssssfaqqr fqamqqqqps aaqqqtpqpt atptlnqllt 301 spssargygg ypggdysggp gdggagkgpa dmasgcwgaa aaaaaaaaas ggaggrshha 361 pmspgssggg gqplartpqp sspmdqmgkm rpqpyggtnp ysqqqgppsg pqqghgypgq 421 pygsqtpqry pmtmqgraqs amgglsytqq ippygqqgps gygqqgqtpy ynqqsphpqq 481 qqppysqqpp sqtphaqpsy qqqpqsqppq lqssqppysq qpsqpphqqs papypsqqst 541 tqqhpqsqpp ysqpqaqspy qqqqpqqpap stlsqqaayp qpqsqqsqqt aysqqrfppp 601 qelsqdsfgs qassapsmts skggqedmnl slqsrpsslp dlsgsiddlp mgtegalspg 661 vstsgisssq geqsnpaqsp fsphtsphlp girgpspspv gspasvaqsr sgplspaavp 721 gnqmpprpps gqsdsimhps mnqssiaqdr gymqrnpqmp qysspqpgsa lsprqpsggq 781 ihtgmgsyqq nsmgsygpqg gqygpqggyp rqpnynalpn anypsagmag ginpmgaggq 841 mhgqpgippy gtlppgrmsh asmgnrpygp nmanmppqvg sgmcpppggm nrktqetava 901 mhvaansiqn rppgypnmnq ggmmgtgppy gqginsmagm inpqgppysm ggtmannsag 961 maaspemmgl gdvkltpatk mnnkadgtpk teskskksss stttnekitk lyelggeper 1021 kmwvdrylaf teekamgmtn lpavgrkpld lyrlyvsvke iggltqvnkn kkwrelatnl 1081 nvgtsssaas slkkqyiqcl yafeckierg edpppdifaa adskksqpki qppspagsgs 1141 mqgpqtpqst sssmaeggdl kpptpastph sqipplpgms rsnsvgiqda fndgsdstfq

```
1201 krnsmtpnpg yqpsmntsdm mgrmsyepnk dpygsmrkap gsdpfmssgq gpnggmgdpy
1261 sraagpglgn vamgprqhyp yggpydrvrt epgigpegnm stgapqpnlm psnpdsgmys
1321 psryppqqqq qqqqrhdsyg nqfstqgtps gspfpsqqtt myqqqqqvss paplprpmen
1381 rtspskspfl hsgmkmqkag ppvpashiap apvqppmirr ditfppgsve atqpvlkqrr
1441 rltmkdigtp eawrvmmslk sgllaestwa ldtinillyd dnsimtfnls qlpgllellv
1501 eyfrrcliei fgilkeyevg dpgqrtlldp grfskvsspa pmeggeeeee llgpkleeee
1561 eeevvendee iafsgkdkpa senseeklis kfdklpvkiv qkndpfvvdc sdklgrvqef
1621 dsgllhwrig ggdttehiqt hfesktellp srphapcppa prkhvttaeg tpgttdqegp
1681 ppdgppekri tatmddmlst rsstltedga ksseaikess kfpfgispaq shrnikiled
1741 ephskdetpl ctlldwqdsl akrcvcvsnt irslsfvpgn dfemskhpgl llilgklill
1801 hhkhperkqa pltyekeeeq dqgvscnkve wwwdclemlr entlvtlani sqqldlspyp
1861 esiclpvldg llhwavcpsa eaqdpfstlg pnavlspqrl vletlsklsi qdnnvdlila
1921 tppfsrlekl ystmvrflsd rknpvcrema vvllanlaqg dslaaraiav qkgsignllg
1981 fledslaatq fqqsqasllh mqnppfepts vdmmrraara llalakvden hseftlyesr
2041 lldisvsplm nslvsqvicd vlfligqs
```

Homo sapiens AT rich interactive domain 1A (SWI-like) (ARID1A), transcript variant 2, mRNA (SEQ ID NO: 12)

```
1 cagaaagcgg agagtcacag cggggccagg ccctggggag cggagcctcc accgccccc
  61 tcattcccag gcaagggctt ggggggaatg agccgggaga gccgggtccc gagcctacag
 121 ageogggage agetgageeg eeggegeete ggeegeegee geegeeteet eeteeteege
 181 cgccgccagc ccggagcctg agccggcggg gcggggggga gaggagcgag cgcagcgcag
 241 cageggagee eegegaggee egeeegggeg ggtggggagg geageeeggg ggaetgggee
 301 ccggggcggg gtgggagggg gggagaagac gaagacaggg ccgggtctct ccgcggacga
 361 gacagegggg ateatggeeg egeaggtege eecegeegee geeageagee tgggeaacee
 421 gccgccgccg ccgccctcgg agctgaagaa agccgagcag cagcagcggg aggaggcggg
 481 gggcgaggcg gcggcggg cagcggccga gcgcggggaa atgaaggcag ccgccgggca
 541 ggaaagcgag ggccccgccg tggggccgcc gcagccgctg ggaaaggagc tgcaggacgg
601 ggccgagagc aatgggggtg gcggcggcg cggagccggc agcggcggcg ggcccggcgc
 661 ggagccggac ctgaagaact cgaacgggaa cgcgggccct aggcccgccc tgaacaataa
 721 cctcacggag ccgcccggcg gcggcggtgg cggcagcagc gatggggtgg gggcgctcc
 781 teacteagee geggeegeet tgeegeeeee ageetaegge ttegggeaac eetaeggeeg
 841 gagecegtet geogtegeeg eegeegege egeegtette caccaacaac atggeggaca
901 acaaageeet ggeetggeag egetgeagag eggeggegge gggggeetgg ageeetaege
961 ggggccccag cagaactete acgaccacgg ettececaae caccagtaca actectaeta
1021 ecceaacege agegeetace eccegeeege eccggeetae gegetgaget eccegagagg
1081 tggcactccg ggctccggcg cggcggcggc tgccggctcc aagccgcctc cctcctccag
1141 egecteegee teetegtegt ettegteett egeteageag egettegggg ceatgggggg
1201 aggeggeece teegeggeeg gegggggaae teeceageee acegeeacee ceaceeteaa
1261 ccaactgete aegtegeeea geteggeeeg gggetaeeag ggetaeeeeg ggggegaeta
1321 cagtggcggg ccccaggacg ggggcgccgg caagggcccg gcggacatgg cctcgcagtg
1381 ttgggggget geggeggegg eagetgegge ggeggeegee tegggagggg eecaacaaag
1441 gagccaccac gcgcccatga gccccgggag cagcggcggc ggggggcagc cgctcgcccg
1501 gacccctcag ccatccagtc caatggatca gatgggcaag atgagacctc agccatatgg
1561 cgggactaac ccatactcgc agcaacaggg acctccgtca ggaccgcagc aaggacatgg
1621 gtacccaggg cagccatacg ggtcccagac cccgcagcgg tacccgatga ccatgcaggg
1681 ccgggcgcag agtgccatgg gcggcctctc ttatacacag cagattcctc cttatggaca
1741 acaaggeece agegggtatg gteaacaggg ceagacteea tattacaace ageaaagtee
1801 tcaccctcag cagcagcagc caccctactc ccagcaacca ccgtcccaga cccctcatgc
1861 ccaaccttcg tatcagcagc agccacagtc tcaaccacca cagctccagt cctctcagcc
1921 tocatactoc cagcagocat cocagoctoc acatoagoag tococggoto catacocoto
1981 ccagcagtcg acgacacagc agcacccca gagccagccc ccctactcac agccacaggc
2041 teagteteet taccageage ageaacetea geageeagea ceetegaege teteceagea
2101 ggctgcgtat cctcagcccc agtctcagca gtcccagcaa actgcctatt cccagcagcg
```

211 checatogac tecaquaga tatetcaaga tecattegg tetcaggaat cotaaqace 2221 checaquaga tecaquaga qagqaaqaa aqaataqaa cuqaqeette aqteaqaqae 22341 tetgaqteet gagqaqaaqa cataqaqaa etacaqqaqa tecaqqaqaa caaqqaaqa 2341 tetgaqteet gagqaqaaqa cataqaqaa tecaqqaqaa tecaqqaqaa teqaqaaqaa qaqataatee 2401 ageteaqte tettetete etacaacate coctoacate cettaggaa aqaqtaatee 2521 tyetqeaqty caaqqaaca agatqacace tegaqaaca aqaqaacatee 2521 tyetqeaqty caaqqaaca agatqacace tegaqaaca agatqacaca cagtqacaaqaa aqatqaatace 2521 tyetqeaqty cacaqaaca atcaaqqaa tyqcacaaqaa qaqataataca caqqaaqaqa aqatqacaca adqacatqqq tecatacaq caqaactoa tyqqaqata 2281 tyqctcaaq qaqqataa taqqacaaca aqqaaqaqa qaqatqaa aacccatqqq daqaacaca caqqaaqqqq aaqatqaqa qaqaaqaaqa qaaqaacqqq qaaqaada acccaaqqa 2941 tyqctcaaq qaqqataa aacccaaqqa qaqaaqaaqaa acccaaqqa 2941 qaqqatqaa caaqcctcaa tyqqqaaqaa atqaaccqaq caaactaataa 2821 tyqcttqacaa acccaaqqa qaaqaacqqq qaaqaadaqa caadqaaqa taqqacaaca tatqqaqaa aaccaaqqa 2941 qaqqaataa aacqctcaa tyqqqaaqaa atqaacaqaa accaaqqa 2941 qaqqaataaqa caaqcctcaa tyqqqaaqaa atqaacqqa qaqaataaca aacqaqaaqa taqqaaqaa aaccaaqqa 2941 qaqqaataa aacqaqaaqa tyqqaaqaa atqaacqaa aacqaaqaa qaqaadaa aacqaaqaaqa taqqaaqaa qaqaataqaa aacqaaqaaqa qaqaataqaa aacqaaqaaqa qaqaataqaa aacqaaqaaqa qaqaataca aaqqaaqaa taqqaaqaa qaqaaqaaqa qaqaaqaaqa qaqaaqaaq							
2281 ctcagette octyacteat etgyteaat agatyacete occatggga cagaagagae 2240 ageteagtet octtocte otcatacete occeacete octyacagaa agatyaatee 2401 ageteagtet octtocte otcatacete occeacete octygeace gaggeotte 2461 ceceptecete yttygetete occapatyt tycetoagtet octygeageae agatyacete 2521 tyctycagtyg occagaaa gatyaceae toggeeaee agtyceageae 2521 catycateet tocatyaace aatcaageat tycecaagea gaggetage occagatyaa occagatyaa occagatyaa tycecaagea goottaceae gaggatyaa tycecageae goottaceae yoo occagatyaa gaggatyaa occagatyaa occagadyaa occagaaceae aaccaatya 2821 tycectygee aaatycaaty gagagetya agggatyaa occagatyaa occagaggaa occagatyaa occagaggaa occagatyaa occagaggaa ocaaccaga 301 acctcagyt yggtcagga tygtycecco accagggge octatacea aaatycgetya occagaagaa tygtycecco accagaggaa aaaccaaga aaatycaaa occagagaa occagatyaa occagaagaa occaaagaagaa occaaagaaa occaaagaagaa occaaagaaaa occ	2161	cttccctcca	ccgcaggagc	tatctcaaga	ttcatttggg	tctcaggcat	cctcagcccc
2341 tetgagtect gagtgagea eateagggat theagagea caaggage agagtaatee 2401 ageteagtet eetstletete eetstaetee eetsteetge eetgeetee getgeetee 2521 tygtgeagtg eeaggeaace agatgeeace teggeeaget geteaggae eactetegee 2521 tygtgeagtg eeaggeaace agatgeeaget tygeeagat gegegeaget 2521 tygtgeagtg eeaggeaace agatgeeaget gegegeete 2521 tygtgeagtg eeaggeaace agatgeeaget 2521 tygtgeagtg eeaggeaace aggtgeeaget 2521 tygtgeagtg eeaggeaace aggtgeeaget 2521 tygtgeagtg eeaggeaace 2521 tygtgeagtg eeaggeaace 2521 tygtgeagtg eeaggeaace 2521 tygtgeagtg eeaggeaggg 2521 tygtgeaggg eeaggeaggg 2521 tygtgeagggg eeaggagggg 2521 tygtgeagggg eeaggagggg 2521 tygtgeagggg eeaggagggg 2521 tygtgeaggggggggggggggggggggggggggggggggg	2221	ctcaatgacc	tccagtaagg	gagggcaaga	agatatgaac	ctgagccttc	agtcaagacc
2401 ageteaptet extitetee eteataecte cocteacetg ectogeatee gaggecette 2461 eccopteceet gittgetotee cogecagigt tigeteaptet eqeteagage cacteracee 2521 tigetgeagtg ecaggeace agatgeace tigegeacee agitgeacee agitgeage eacteracee 2581 catgeateet teatagaace asteageate tigegeage egaggetate 2581 catgeateet teatagaace asteageage eccacagate geoggetaet gaggateage 2701 titeegagagg cagatacea gitteeceea geoggetae geoggetae eccacagagge 2701 titeegagagg cagatacea cagagaatgga etigeteecae gitteeceae gaggagateae eccacagagge 2701 titeegagagg cagatacea ageoggetae aggagagaacea cacataaa 2821 tigeettgee aatgeeaaet acceagtge aggaatgget gagagagaacea acceataga 2821 tigeettgee aatgeeaaet acceagtge aggaatgget gagagagaacea acceatagaggga 1941 gaggatagat eacgeeteea tiggeaaee gacteaceaet tatggaacaa teetecaaggagga 2941 gaggatagat eacgeeteea tiggeaaee gacteacea 3121 caatatagat caaggaggaa tiggtgeaaa eccatagagagaa tiggagaacea cacatataa 33061 aactgeetig egagtagaace tetagggaac tiggagaacea cacatataa 33061 aactgeetig aggatagaacea teagaggaga tiggagaacea cacatataa 33061 aactgeetig aggatagaacea taggagaace cacatataa agagagaaa 3181 tiggecaaa aggagagaa tiggagaacea cacatagaa 33061 aactgeetigaacea agaaagagaa tiggagaacea agagaagaa tiggagaacea agaacaaaa 33061 aactgeetigaa aactgeetigaacea aacaagagagaa tiggagaacea agagaagaacea agagaagaacea agaacaaaa 33061 aacteagagaa aactagaaca acaagaagaa tiggagaacea aagacagaa tiggagaacea agagaagaa tiggagaacea aacaagaagaa aacaaaaaaa 33061 aacteagaa aactagacea gagaagaacea aagacagaa caagacagaa 3401 ticaagaacaa aactagacea gagaagaacea agagaagaacea aagacagaa aacaaaaaaa 33061 aacaatagaa aactagaacea aactagaacea aactagaacea aacaaaaaa 33061 aacaaacaa aacaaaaaaa 33061 aacaaacaa aacaaaaaaaa agagaacaaaaaaaaaa	2281	ctccagcttg	cctgatctat	ctggttcaat	agatgacctc	cccatgggga	cagaaggagc
2461 cogtocott gttggototo cogocagtgt tottoagto cagtogaca castotaga castotaga castotaga castotaga tottoagaca tottgagaa castotaga castotaga castotaga cattotaga selectato totagaaga gattgacaga totatagaaga castotaga coccagata gttcococa gocogotota gottatoto cogototaga cagtala coccagataga castotaga cagatacaaa acoccagataga gatgataga coccagaga castotagaga tagtgotota coccagataga caccactagag sent tococcagatagaga caccagagataga caccagagaga caccagagatagatagatagatagatagatagatagatag	2341	tctgagtcct	ggagtgagca	catcagggat	ttccagcagc	caaggagagc	agagtaatcc
2521 tgotgoagtg coagocaaca agatgocaac toggocacca agtgocagt toggaagaa 2681 catgocatcct tecatgaaca attcaagcat togcocaagat coagottata togcoagaa 2681 coccoagatg coccagtaca gettatect coccytoagac 2701 ttcoggaaga cagatacaca cagacatgga tectacacag cagacatcca taggagagcta 2761 tgotcoccaa gagagtcacaca cagacatgga tectacacag cagacaccacacacacacacacacacacacaca	2401	agctcagtct	cctttctctc	ctcatacctc	ccctcacctg	cctggcatcc	gaggcccttc
2521 tgotgoagtg coagocaaca agatgocaac toggocacca agtgocagt toggaagaa 2681 catgocatcct tecatgaaca attcaagcat togcocaagat coagottata togcoagaa 2681 coccoagatg coccagtaca gettatect coccytoagac 2701 ttcoggaaga cagatacaca cagacatgga tectacacag cagacatcca taggagagcta 2761 tgotcoccaa gagagtcacaca cagacatgga tectacacag cagacaccacacacacacacacacacacacaca	2461	cccgtcccct	gttggctctc	ccgccagtgt	tgctcagtct	cgctcaggac	cactctcgcc
2581 catgcatect tecatgaace astecageat tegecaagat egagettata tegagaggaa 2641 ececcagatg ececagtaca gttececcae gecegetea geettatete egegteagee 2701 teceggaaga cagatacaa atggecaca aggegetea geettatete egegteagee 2701 teceggaaga caagatacaa atggecaca aggegeteag eceagagae caaacatataa 2821 tegectecca gggggeteagt acecageaga gagageataa acecaatgg 2881 tegecgaagat caaatecaatg gacageetga gagagatgat ecacactagg 2881 tegecgaagat caaatecaatg gacageetga gagagatgat ecacactagg 2941 gagagatagat cacagetea tegegaagae gacacecaacgg 2941 gagagatgagt caacactagag gatgateaacg geettatagge cetacacatgg caaatetgaaga 3061 aceteaggt gggteagga tegtgeece aceagggge atgaacegga aaaceaaga 3061 acetegtetg egeatgeatg tegtgeece aceagggge atgaacegg aaaceaaga 3181 tatggetgea atgateaace ecagggace ecatattee atgggtegaa ecatggeeaa 3181 teagecace aggatagaca ecaaggagaa tegageacec aagacagaat ecaaategaa 3301 tecageace aaaatgaaca acaaggagaa tegagacace aagacagaat caaatecaa 3301 tecageace aaaatgaaca acaacaatga gaagatagge ettggggat tagaaggaat 3481 gagaatgaca tettetacta caacaataga gaagataca aagttgtatag agctggggggat 3421 tagageetgag aggaatgatgg gattgaaceg tetatetgge tetactgaga gaagagacat 3481 gagaatgaca aatetgeetg ettggggaag gaaacetetg gacetetate geetettatgaf 3541 gteetgaaga gagattggg gattgaacea gagtegeaca 3601 tecacacaca etcaaatgag gacaatecaag acatgeacaaga aceacaaaaa 3601 tecacacaca etcaaatgag gacaatecaag acatgeacaaga aceacaaaaa 3601 tecacaatea etcaatgeetg gacaatecaag acatgeaga aceacacaa aceacaaaaaa 3601 tecacaatea etcaaatgeg gacaatecaag acatgeaga ateaagacea acaacacaa aceacaagaa aceacaaaaaa 3601 tecacaatea etcaaatgaga gacacaagaa tecaagaca etcacaagaagaa 4021 acatettgaa gagaagagaa ateaagaaca caaateaaga gagaagaaca tecaagaagaa ateaagaagaa ateaagaca acaacaagaagaa ateaagaagaagaa ateaagaagaa aaaagaagaa aaaagaagaa aaaagaaga							
2641 coccagatg coccagtaca gttcoccca gocogoctca gocttatcto cogotagec 2701 ttcoggaga agastacas cagacatoga ctoctaccag cagaactcca tgggagacta 2761 tggtcoccag gggggtcagt atggccaca atggcagac cacactataa 2821 tgccttgocc aatgccaact acccagtga aggcatgact ggaggcataa acccatgag 2841 tgcctgagag caaatccaat gacagcctg catccacct tatggacaac tcccaagg 2941 gaggatgagt cacaccctca tgggcaacc gccttatggc coctaacatgg ccaatatgcc 3001 acctcaggt gggccacat tgggcacac tcctccaag 3061 acctcaggt gggcagag tgtgtcccc accaggggc atgaaccaga aaacccaaga 3061 acctgctgt gccacact ttggcacac tctctccaag 3181 tatggctggc atgatcacac tctaggagac ccctaattcc atggggaag agattatata 3181 tatggctggc atgatcacac accagagagac cccatattcc atggggaag cagattacac 3301 tocagccac aaaatgaaca acaaggcaga tgggacacc aagacagaat ccaaatcaa 3361 gaaatccagt tcttctacta caaccaatga gagaatacac aagttgtat aaagttaac 3361 gaaatccagt tcttctacta caaccaatga gagaatacac aagttgtatg aactgggtga 2421 tgagcctga aggaagtgggggggggggggggggggggg							
2821 tgoctocca gagagtocat atgaccaca aggtagtota cocagogago caaactataa 2821 tgoctocaco aatgocaact accocatgo aggactagot gagagcataa accocatgog 2881 tgocogaagt caaactocat accocatgog 2941 gagagtagat cacacoctca taggcaacog gocttatgo cotaacatgo cocatactoca cacagogaga tagagacoga accocacaga 3061 aactgotot gagtcagaga tgottoccac accagogago atgaccoga cagactacacaga 3061 aactgotot gocatgotot tgottocaca cocatactoca tatggacaaga 3061 aactgototo gocatgotot cotaacatgo cacatattoca aactgocoga 3121 caaatatgaat caaaggaggaa tgatggaaco cocatattoc atggacaag gagtataaca 3241 caattotga gagatgaca caaccacaga agatgatggoga ctaaccagaa 3361 tatagcacac aaaatagaaca acaaggagaa tggacacoc aagacagaat cacaatcacaa 3361 gaaatccaga tottotacta caaccaatga gaagatcacc aagtgatag taaagttaac 3361 gaaatccaga tottotacta caaccaatga gaagatcacc aagtgatag agatggggg 3421 tagaccaga agagaatgdg gagtgagacog tatactggoc thatctgaga gagattagat gagtgggaga ctatactggoc thatctgaga gagattgggg gattgacca tatactgac taccacaga agagagacac aaccaaaga agagagaga tgagagaga totaacaga aacaaaaaaa gagagagaga 3481 ggocatgaca aatotocag gattgatca gattgacta gacaccaaga accacaaaga accacaaaga 3661 tatocagaga totaaccat ttgaaagaca cacacaaagaga totaacaaga 3721 catotttgaa gagatggga ctaagaagaga gattgagaga tatacatgaa gattgacaga gagaagagac coccacaga 3721 catotttgaa gacagagaa accacaagaaga aacaacaa 3841 aggagagaga ttaagacca caaccaagaaga tocacacaa acaagaagaga 3841 aggagagaga ttaagacag gacacagaa tocacacaa cacagacagat coccacagagaga 3841 aggagagaga ttaagacaa caaccaagaaga tocacacaa acaagaagaga 3841 aggagagaga ttaagacaa aacacaagaa tocacacaa cacagaagaga atcacacaa atgagaagaa atcaacaca cacacacaa cacagaagaa acaatgagaa atcacacaa atgagaagaagaa atcacacaa acaacacaa gagaagagaa							
2821 tgoctocca gagagtocat atgaccaca aggtagtota cocagogago caaactataa 2821 tgoctocaco aatgocaact accocatgo aggactagot gagagcataa accocatgog 2881 tgocogaagt caaactocat accocatgog 2941 gagagtagat cacacoctca taggcaacog gocttatgo cotaacatgo cocatactoca cacagogaga tagagacoga accocacaga 3061 aactgotot gagtcagaga tgottoccac accagogago atgaccoga cagactacacaga 3061 aactgotot gocatgotot tgottocaca cocatactoca tatggacaaga 3061 aactgototo gocatgotot cotaacatgo cacatattoca aactgocoga 3121 caaatatgaat caaaggaggaa tgatggaaco cocatattoc atggacaag gagtataaca 3241 caattotga gagatgaca caaccacaga agatgatggoga ctaaccagaa 3361 tatagcacac aaaatagaaca acaaggagaa tggacacoc aagacagaat cacaatcacaa 3361 gaaatccaga tottotacta caaccaatga gaagatcacc aagtgatag taaagttaac 3361 gaaatccaga tottotacta caaccaatga gaagatcacc aagtgatag agatggggg 3421 tagaccaga agagaatgdg gagtgagacog tatactggoc thatctgaga gagattagat gagtgggaga ctatactggoc thatctgaga gagattgggg gattgacca tatactgac taccacaga agagagacac aaccaaaga agagagaga tgagagaga totaacaga aacaaaaaaa gagagagaga 3481 ggocatgaca aatotocag gattgatca gattgacta gacaccaaga accacaaaga accacaaaga 3661 tatocagaga totaaccat ttgaaagaca cacacaaagaga totaacaaga 3721 catotttgaa gagatggga ctaagaagaga gattgagaga tatacatgaa gattgacaga gagaagagac coccacaga 3721 catotttgaa gacagagaa accacaagaaga aacaacaa 3841 aggagagaga ttaagacca caaccaagaaga tocacacaa acaagaagaga 3841 aggagagaga ttaagacag gacacagaa tocacacaa cacagacagat coccacagagaga 3841 aggagagaga ttaagacaa caaccaagaaga tocacacaa acaagaagaga 3841 aggagagaga ttaagacaa aacacaagaa tocacacaa cacagaagaga atcacacaa atgagaagaa atcaacaca cacacacaa cacagaagaa acaatgagaa atcacacaa atgagaagaagaa atcacacaa acaacacaa gagaagagaa	2701	ttccggagga	cagatacaca	caggcatggg	ctcctaccag	cagaactcca	tggggagcta
2821 tgccttgcc aatgccaat acccaatgc agcatggct gaggcataa acccatgg 2821 tgcctggggt caaatgcatg gacagcctgg catccacct tatggcacac tcctccagg 2941 gaggatgagt cacgctcca tgggcaaccg gcttatggc cctaacatgg ccaatatgcc 3001 acctcaggtt gggtcaggga ttgtgtcccca accagggggc atgaaccgga aaacccaaga 3061 aactgctggte gccatgcatg ttgtgtccca acctatccaa aacaggccgc aggataccc 3121 caatatgaat caaggggga tgatgggaac ccaatatcca acaggcccaa 3241 caattctgca gggatggaag ccaggcagaa tgaggacctct tatggacaag ggattaatag 3181 tatggctgg aggatggaag ccaggcaga tgaggacccc aagacagaat ccaaatccaa 3361 gaaatccagt tcttctacta caaccaatga gaagtatggg cttggggtgg taaagttaac 3301 tccagccaca aaatgacaa acaaggagaa tgaggacacc aagacagaat ccaaatccaa 3361 gaaatccagt tcttctacta caaccaatga gaagatgag cttggggtgg 3421 tqagcctgag aggaagatgt gggtgaccg ttatctggcc ttcactaag gagatgggggggggg							
2881 tgccggaggt caatgcatg gacagctgg catccaact tatggcaaca tecetecagg 2941 gaggatggqt cacgecteca tgggcaacg gecttatgge ectaacatgg coatatatgee 3001 acctcaggt tggtcaggg tgttsceec accaggggge atgaaccgg aaacccaaga 3061 aactgctgte gecatgcatg ttgctgccae etctatecaa accagggge caggataccc 3121 caatatgaat caagggggga tgatggaac tggaactect tatggacaag ggattaatag 3181 tatggctgge atgatcaace ctcagggace eccatattee atgggtggaa coatggcaa 3241 caattctgca gggatggcag eccaggaggat tggagcacce aagacggaac caaaatgaaa acaaggagaa tggagcacce aagacggaat caaaatgaaa acaaggagaa tggagcacce aagacggaat caaaatgaaca acaaggagga tggagcacce aagacggaat caaatccaa 3361 gaaatccagt tettetacta caaccaatga gaagatcacc aagttgtatg agctggtgg 3421 tqagcctgaa gagaattgtgg gattgacceg ttatctggcg ttactgagggggggggggggggggggg							
2941 gaggatgagt cacgoctoca toggocacco goottateggo cotaacatg coaatatgoc 3001 acotcaggt gggtcagga tytytococo accaggggge atgaaccgag aaacccaaga 3061 aactgottot gocatgcatg ttgotgocaa ctotatocaa aacaggocgo caggetacco 3121 caatatgaat caaggggga tytytogocaa ctotatocaa tagggtogaa coatggocaa 3241 caattotgoca gggatggaa coaggocaca coatatoca tagggtogaa coatggocaa 3241 caattotgoca aaaatgaaca acaaggaaga toggaacacca aagacagaat cocaagcaaa 3361 tocagocaca aaaatgaaca acaaggaaga toggaacacca aagacagaat cocaagtocaa 3361 gaaatccagt tottotacta caaccaatga gaagatacca aagattytata agotgggtgg 3421 tagagcotgaa aatotgoctg cotggggtag gaaacctotg gacotctat goctctatgt gtottotagoca attoggaacga ttatotgoc tocatgagg agaaagagga totacaaga gagaatagga accaagaa accaagaa 3361 gtocaccaa cocaatgga gagaataggt gattgacca gagtcaccaaga aacaaaaaaa ggcgggaact 3481 gyggatgaca atotgoctg gacaacaaga gagtcaccaaga acacaaaga 3721 catotttoca gotgotgat cocaagaac caattcaaga gagaagaacc cocatggaaga 3721 catottotga gotgotgat cocaagaac cocaagacaga atocaccaca aagaagaga taacagcaa atacagcagaa 3881 aggaggagaac taaagacca caactcaaga caaccaccaca acaagacagat cocaccacaca aagagagaga atocaccacaca caagatagaa gagaagacc tocaccacta agaagagaa atocaccacaa acaagtaaga stocaccacata caagagaagaa atocaccacaa acaagtaaga stocaccacata agagagagaa atocaccacaa acagtcagat tocaccacata 4021 tacottotgaa agaagagaa attocatcaaga gacacacacac gaggaagaac atocaccacacacacacacacacacacacacacacacaca							
3001 acctcaggt ggcatagga tygtptocca accaggggg atgaacogga aaaccaaga 3061 aactgctgtc gccatgatg tygtgcaa ctctatccaa aacaggcag caggctaccc 3121 caatatgaat caaggggga tyatgaggaac tygagcatcct tatggacaag ggattaatag 3181 tatggctgc atgatcaca ctcagggacc cccatattcc atgggtggaa cattggcaa 3241 caatctcga gggatggaac ccaagaccaga gatgatggc cttggggatg taaagttaac 3301 tocagcaaca aaaatgaaca acaaggcaga tygagacaccc aagacagaat ccaaatccaag 3361 gaaatccagt tottotacta caaccaatga gaagatcacc aagacagaat ccaaatccag 3481 gggcatgaca aactcgctg ctgtgggtag gaaacctctg gacctctatc ggcctctattg 3481 ggcatgaac aactcgctg ctgtgggtag gaaacctctg gacctctatc ggcctctattg 3541 gtctgtgaag gaagattggtg gattgactca ggtcaacaag agacagaac ctcaatgtg gattgatcac agttcacaag agacagaac ctcaatgtgg gacaacaag agttgaacgg agtcacttag aaaagagaa 3661 tatccatgtg ctctatgct ttgaatgcaa gattgaacgg gggagaagac ctccccaga 3721 catctttgca gctcgtgtatt ccaagaagtc ccaagccaag							
3061 aactgctgtc gccatgcatg ttgctgcaa ctctatccaa aacaggccgc caggataccc 3121 caatatgaat caagggggca tgatgggaac tgagacctcct tatggacaag ggattaatag 3181 tatggctggc atgatcaacc ctcagggacc cccatattcc atgggtgga ccatggcaa 3241 caattctgca ggattgacac caacagcaag atgagacacc aagacagaat caaagcaca aaaatgaaca acaaggacag tgggacacca aagtgtgtat taaagttaac 3301 tccagccaca aaaatgaaca acaaggacag tgggacacca aagttgtat gacaagaat cacaa 3361 gaaatccagt tcttctacta caaccaatga gaagatcacc aagttgtat gactggggg 3421 tgagcctga aggaagatgt gggtggacg ttatctggc ttcactgag aggaagacgt gggtggaagacgt ggcactcatc gcctctatc gcactcatc ggctcatatg stottgtggaag agaattggtg gattgactca ggtcaacaaa aacaaaaat ggcggggaact 3481 ggcgatgaca aatctgcctg ctgtgggtag gaaacctctg gacctcatc gcctcatctg stottgggaag gaagacgacacacaaccaa cacaagcagt ctctatcgc ctcaatgtgg gacaacaaa caagtgctgc agaccacaa 3601 tgcaaccaac ctcaatgtgg gacaacaaag cagtgctgcc agccctctag aaaagcagaa 3661 tatccagtgt ctctatgcct ttgaatgcaa gattgaacag gggagaagac ccccccaga 3721 catctttgca gctgctgatt ccaagaagtc ccagccaaga atccaagcagt cccccaaga 3721 catctttgca gctgctgatt ccaagaagtc ccacacaca acaagcagtt cccccaga 3841 aggaggagaa ttaaagcaac caacaccaag atccaacaca acaagcagtt ccctcaga 3841 aggaggagac ttaaagcaac acacaccaa accaagcagtt cccccagt 3901 gccaagaagaa attcaagcaa atccaaga ggtccaaaaaca 4021 tacctctgaa atgaagggg gattccata tgagcaaat aggagaccat atgagaagaa 4021 tacctctgaa atgatgggg gattccata tgagcaaaca aggagacca acagacagaca 4021 acagcacata ccctatgga gtcctataga gacgagaaca acagacagaca 4201 acagcacata tcctaagtag ggccccaaa gcggacaagaa aatgagacaa atgaggaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaca atgagagaaca acaagagaa atgagagaca acaagacaaca aggagagaca acaagacaaca aggagagaca acaagacaaca acaagaacaa acaagaacaa acaagaacaa acaagacaaca acaagaacaa acaagacaaca acaagaacaa acaagaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaacaacaa acaagaacaacaa acaagaacaacaa acaagaacaaa acaagaacaacaa acaagaacaaa acaagaacaaa acaagaacaacaa							
3121 caatatgaat caaggggca tgatgggaac tggacctcct tatggacaag ggattaatag 3181 tatggctggc atgatcaacc ctcagggacc cccatattcc atgggtggaa ccatggccaa 3241 caattctgca gggatggcag ccagcccaga gatgatgggc cttgggggatg taaagttaac 3301 tccagccacc aaaatgacaa acaaggcaga tgggacaccc aagaccagaat ccaaatccaa 3361 gaaatccagt tcttctacta caaccaatga gaagatcacc aagttgatag agetgggtgg 3421 tgagcctgaq aggaagatg tggtggaccg ttatctggcc ttcactgagg agaaggccat 3481 gggcatgaca aatctgcctg ctgtgggtag gaaacctctg gacctctatc gcctctattg 3541 gtctgtgaag gagattggtg gattgactca ggtcaaccaag acacaacaaa ggggggaact 3601 tgcaaccaac ctcaatgtgg gaccatcaag cagtcgtgcc agctccttaa ggggggaact 3661 tatccatgtg ctctatgcct ttgaatgcaa gattgaacg gggagaagac ctcccccaga 3721 catctttgga getgctgatt ccaagaagtc ccagccaaga atccagccct ccttctctgc 3781 gggatcagga tctatgcag ggccccagac tccccagtca accagcagt ccatggaaga 3841 aggaggagac ttaaagccac caactccagc atccaccacca cacagtcaga tccatccatt 3901 gccaggcatg agcaggaagaa attcatgttg gatccaggat gcctttaatg atggaagtga 3961 ctccacattc cagaagggg gatgtcta tgaagccaact acaagtcaga tccaccatt 4081 gaggaaaggt ccaggaggag gtctttatg atggaagtga 4021 tacctcttgac atgatggggg gcatgtcta tgagccaaca aaggcgggat 4141 gggtgaccc tacagtcgtg ctgcggccc tgggctagga aatgtggga gaggaccac 4261 tgagggaaac atgagagga gtccttatag caggaccaca acaggcgggat 4271 tagaggaaac atgagacatg ggccccaca gccgaatctc atggccagcat 4381 tgattcctat ggcaatcag gtccttatag caggagacca aggagaccg 4261 tgagggaac atggaactg ggtccccaa ggcagaactg aggagaccac 4381 tgattcctat ggcaatcag cacaccacca gagaagaac aggagaccac 4381 tgattcctat ggcaatcag gacaccac agcagaacac aggagagac 4381 tcaagcagac acaagaaca agaagaacac agaagaacacacac							
3181 tatggetage atgateaace etcaggaace eccatattee atgggtagaa ecatgecaa 3241 caattetgea gagatgeaga ceageceaga gatgatgage ettaggaatg aagattaace 3301 tecagecaace aaaatgaaca acaageagaa teggacacee aagatagaat ecaaatecaa 3361 gaaatecagt tettetacta eaaceaatga gaagateace aagttgtatg gagetgggg 3421 tgageetgga aggaagatgt gggtggaceg ttatetggee tecatgagg aggeetatgg 3421 tgageetgag aggaatgtg ggttggaceg ttatetggee tecatgaggggageet 3481 ggeatgaca aatetgeetg etgtgggtag gaaceetetg gacetetate geetetatgt 3541 gtetgtgaag gagattggtg gattgacea ggteacaaag aacaaaaaat ggegggaact 3601 tgeacacaac eccatatgtgg geacateaag eagtgetgee ageteettga aagacagta 3661 tatecagtgt etetatgeet ttgaatgeag ggtagaacg gggagaagace etceceaga 3721 eatetttgea getgetgatt ecaagaagte ecaagecaag atecaageet ecteetega 3781 gggateagag tetatgagg ggeeceagae tececagaa acaagaageagaa 3841 aggaggagaa tetatgagag ggeeceagae tececagaa acaageagta ecceceatt 3901 gecaggaat deaagaegaa attecatgag gatecaggat geetttaatg atggaags 3961 etcecacatte cagaageaga attecatgae tecaaaceet gggtataage ecatgaega 3961 etcecacatte cagaagaegag attecatgae tecaaaceet gggtataage ecatgaegaa 4021 tacetetgae atgatgggg gattgteeta tgagecaaat aaggateett atggeagaat 4081 gaggaaaage tecaagtggg geetgteeta tgagecaaat aaggaeceta aeggeggaat 4141 gggtgaceec tacagtetg etgeeggeee tgggtataga aatgggeeca aeggegaecaagaa 4201 acageacatat ecetatggag gtgeeceaa gegaagteet atggeecaagae 4201 acageacatat ecetatggag gtgeeceaa gegaagaeagaagaeagaeagaagaata tecatagaa atgagaacatat eecaagaagaa ggeeceaaa gagaagaagaa aagagaacaagaa 4381 tgatteetat ggeaateaga tecteacee geaaceeee tteggaagaa aegaagaeagaaaagaaaagaaaa							
3241 caattctgca gggatggcag ccagccaga gatgatgggc cttggggatg taaagttaac 3301 tecagcacc aaaatgaaca acaaggcaga tgggacacc aagactgata tecaaatcaa 3361 gaaatccagt tettetacta caaccaatga gaagatcacc aagttgtatg agctgggtg 3421 tgagcctgag aggaagatg gggtgaccg ttatetggc ttcactgagg agaaggccat 3481 ggcatgaca aatctgcctg ctgtgggtag gaaactctctg gacctctate gectetatgt tgtctggagaa gaagtgggag gtcactcaag gacctctatg gectetatgt ftgetggaga gaagtgggagacg gggaagaagac cetcaatgtgg gacaatcaag cagtgctgcc agctccttatg gectetatgt tgtatgactca ggtcaacaag aacaaaaaat ggcgggaact 3661 tatecagtgt ctctatgcct ttgaatgcaa gattgaacgg gagaagacc ctcccccaga 3781 gggatcagga tctatgcag ggccccagac tecccaatga gagagagac tecatgcaga ggggacagac taatgcaga ggggacagac tecatgcaga ggggacagac tecatgcaga ggcccagac tecccagta accagcagt tecatggaga 3841 aggaggaga tetaagcaga attcaagtgg gatccaacacca cacagtcaga teccccatt 3901 gecaggcatg agcaggaga attcatgtagg gatccaggat gcctttaatg atggaagtga 3961 etccacatte cagaagcaga attcatgtagg gatccaggat gcctttaatg atggaagtga 3961 etccacatte cagaagcaga attcatgtag gatccaggat gcctttaatg atggaagtga 4021 tacctttgac atgatgggg gattgtecta tgagccaaat aaggatectt atggcagcat 4081 gaggaaagct ccaaggaggt atcettcat gtcctaagga gaggaccac acggggggat 41141 gggtgacccc tacagtcggg gtcttatga cagagtgaga aatgtggga tgggaacacq 4201 acagcactat ecctatggag gtccttatga cagagtgaga aatgtgggac gaatagggec 4261 tgagggaac atgacactg gggeccaca gecgaactet atgcetteca accagaccq 4201 gggaattat tetectagca ggtceccaca gecgaaccct tetggcagcc ectteccaaga 4441 ecagcagaa acaatgtate acaagaaa gattgaaga acgaagcacc decagacac 4381 tgattectat ggcaatcagt tetecacccc gagcagcacc tetggeagac ectteccaaga 4441 ecagcagaa acaatgtate acaatgaaca gattgaagac ecttggeagac ectteccaaga 4441 ecagcagaga aggagcacca etcetagaaa gttecatte etcagaaga ggccaccacca teteggagaata gagagagaa ggtggggatatea ectteccaca tagaagaa ettggagac ecttggagaata 4561 geagaagaga aggagaccac etcatgaaga gagagaaca ggccaccaca aggagaacaccaca aggcacccc 4621 catgattegg gaggaacaca gacagaagaa gagagaagaa gagagaagaa gagagaga							
3301 tecagecace aaaatgaaca acaaggcaga tgggacacec aagacagaat ccaaatecaa 3361 gaaatecaagt tettetaeta caaceaatga gaagateace aagtetgatag agtaggatgg taggatgateg tataetggec tecatgagg agaaggacat 3481 gggcatgaca aatetgeetg etgtgggtag gaaacetetg gacetetate geetetatg 3541 gtetgtgaag gaagatggtg gattgaetea ggtcaacaag aacaaaaaaa ggegggaact 3601 tgcaaceaac etcaatgtgg gattgaetea ggtcaacaag aacaaaaaaat ggegggaact 3601 tgcaaceaac etcaatgtgg gacaateaag cagtgetgee ageteettga aaaagcagta 3661 tatecagtgt etetatgeet ttgaatgcaa gattgaacgg ggagaagace etceccaga 3721 catetttgaa getgetgatt ecaagaagte ecagecaag atecagecace etcectatgg 3781 gggatcagga tetatgcagg ggececagae tececagaa atecagecate ectetetetge 3781 gggatcagga tataaggeag ggececagae tececagtea accageagte ecatggaag 3961 etceacatte eagaagggga atteagtgg gatecaggat geetttaatg atggaagtga 3961 etceacatte eagaagggga attecatgae tecaaaceca geggtateage ecagtatgaa 4021 tacetetggae atgatggggg egattgeeta tgagecaaat aggatectat atggeagaat 4141 gggtgacee tacagtegtg etgeeggeee tgggetaaga aatgtgggga tgggacacag 4201 acageactat ecetatggag gteettatga etgaggagga acggageetg gaaggacacag 4201 acageactat ecetatggag gteettatga eagaggagga aggaggeed atggggeedaaga atggggeeda atggggeedaagaa atggggeeda 4201 acageactat ecetatggag gteettatga eagaggagga acggaggeed acagaagaa 4381 tgattectat ggeaateagt tetecacea ggegaatete atgeetteea accagaaca 4381 tgattectat ggeaateagt tetecacea aggeaceet tetggaagae eetteecaga 4401 ecageagaat acaatgtate aacageaaaa gacaacaaca eetteecaca 421 gaaggaagg aggegetaa acaatgtate acaagaaaaa gaagaacaca eetteecaca 421 gaaggaagga aggeggatataa eettecacea aggeaceet tetggaagae eetteecaga 4401 ecageagaaga aggeggetea eacatgaac ettegaaga eetteecaca agceetgtgtt 4881 gaagcaagag aggeggatataa eettecacaca ggecaceet tetggaagae eetteecacaa agceetgtgtt 4881 gaagcaagag aggeggatataa eettecacaca ggecacacaa agceetgtgtt 4881 gaagcagagg aggagaacaa gaagaagaa gaagaagaa gaagaagaa gaagaag			_				
3361 gaaatccagt tettetata caaccaatga gaagatcace aagttgatg agetggggg 3421 tgagectgag agagaagatgt gggtggaceg ttatetggee tetcactgag agaagaceat 3481 gggcatgaac aatetgeetg etgtgggtag gaaacetetg gacetetate geetectatgt 3541 gtetgtgaag gagattggtg gattgactea ggteaacaag aacaaaaaat ggegggaact 3601 tgeaaceaac eteaatgtgg gacaatcaag cagtgetgee ageteettga aaaagcagta 3661 tatecagtgt etetatgeet ttgaatgeaa gattgaaegg ggagaagace etececcaga 3721 catetttgea getgetgatt ecaagaagte ecagecaaga atecageete eeteteetge 3781 gggatcaga tetatgeagg ggeeceagae teeceagaa atecageete eeteteetge 3781 gggatcaga tetatgeagg ggeeceagae teeceagaa atecageete eeteteetge 3781 gggatcagae ttaaagceac eaactecage atecaeacea acaagtagaa teeceecatt 3901 geeaggaaga atteaatgaeg gaatecagga gggtateage eeagtaga 3841 aggaggagaa etaaagcaga atteaatgaeg gaatecaggat geettaatga daggaggaga atteeceeatgag ggatacaga teeceecatt 3901 geeaggaatg ageagggggggggggggggggggggggg		_					_
3421 tgagcctgag aggaagatgt gggtggaccg ttatetggcc ttcactgagg agaaggccat 3481 ggccatgaca aatctgcctg ctgtgggtag gaaaacctctg gacctctatc gcctctatct gtctgtgaag gagattggtca ggttagaccaa gaccaaaga aacaaaaaa ggcggaact 3601 tgcaaccaac ctcaatgtgg gcacatcaag cagtgctgcc agctccttga aaaagcagta 3661 tatccagtgt ctctatgcct ttgaatgcaa gattgaacgg gagaagacc ctcccccaga 3721 catctttgca gctgctgatt ccaagaagtc ccagccaag atccagcac ctcccccaga 3781 gggatcagga tctatgcagg ggcccaagac tccccagtca accagcagtt ccatggcaga 3841 aggaggagac ttaaagccac caactccagc atccacacca cacagtcaga tcccccatt 3901 gccaggcatg agcaggagaca attcatgcac ttgaccagga gcctttaatg atggaagtga 3961 ctcacaattc cagaagcgga attcatgacg gatccaggac tccacagca aggaggaga 4021 tacctctgac atgatgggc gcatgtcta tgagccaaat aaggatcctt atggcagcat 4141 gggtgacccc tacagtcgtg ctgccggacc tgggctagag aatgggggat 4141 gggtgacccc tacagtcgtg ctgccggccc tgggctagg aatggggacca aggggggat 4261 tgagggaaac atgagcactg gggcccaaca gcggaatctc atggctgac aggggacca 4261 tgagggaaca atgagcactg gggcccaaca gcgaatcca atgactgga ggggatgat tctcctagc gctaccccc gcagcagcag cagcagcagc agcaacgaca 4381 tgattcctat ggcaatcagt tctcacacac gcagaaccac accagaccc 4321 ggggatgat tctcctagc gctaccccc gcagcagcag cagcagcag agcaacgaca 4441 ccagcagac acaatgtatc aacaacgacac agcaacgaca 4561 gcaaatggag agccccaca gccaacacc tctggcagcc ccttcccag 4501 gcaaatggag agcccccag tctcacacac agcaacccct tctggcagcc ccttcccag 4621 catgattcg cgggatatac acatgacac acctgcccc gcacaatgga agcaggaa agcggcacc acaatgaaca gcacacacac agcaacacac agcacacacaca		_	_	22 2	2 2 2		
3481 gggaatgaca aatctgcctg ctgtgggtag gaaacctctg gacctcatc gcctctatgt 3541 gtctgtgaag gagattggtg gattgactca ggtcaacaaa aacaaaaaaa ggggggaact 3601 tgcaaccaac ctcaatgtgg gacaatcaag cagtgctgcc agctccttga aaaagcagta 3661 tatccagtgt ctctatgct ttgaatgcaa gattgaacgg ggagaagacc ctccccaga 3721 catctttgca gctgctgatt ccaagaagtc ccagccaaga atccaagcct cctctcctgc 3781 gggatcagga tctatgcagg ggcccaagac tccccagtca accaagcagt ccatggcaga 3841 aggaggagac ttaaagccac caactccagc atccacacca cacagtcaga tcccccatt gccaggat agcaggagac attcatgcag ggcccaaga atccacacca cacagtcaga tcccccatt gccaggat gccagatga agcaggagac attcatgatgg gatccaggat gcctttaatg atggaagtga 3961 ctccacattc cagaagcgga attccatgac tccaaaaccct gggtatcaga ccagtgaga 4021 tacctctgac atgatggggg gatgtccat tgagccaaaa aaggatcctt atggaagtga 3961 ctccacattc cagaagcgga atcccttcat gtacccaaaacct gggtatcaga ccagtgaga 4021 tacctctgac atgatggggg gatgtcctat tgagccaaaa aaggaccct atggagaatt41 gggtgaaccc tacagtgggg gatgtcctat tgagccaaaa aaggaccct atggagaacacg 4201 acagcacata ccctatggag gtccttatga cagaagtgag acggagccca acggaggat 4201 acagcacata ccctatggag gtccttatga cagaagtgag acggagccca aggaaccag 4261 tgagggaaac atgagcaccg ggccccaca gccgaatctc atgccttcca acccagaccc 4331 ggggatgtat tctcctagcc gctaccccc gcagcagcag cagcagcaga agaacgaca 4381 tgattcctat ggcaatcagt tctccacca aggcaaccct tctggaagac ccttcccag 4441 ccagcagaac acaatgtatc aacagcaaca gcaggagacca acaaggaca 4561 gcagaaggag agacgcact ctcctagcaa gtctccattc ctggcagcc ccttcccag 4501 gccaatggag agacgacct ctcctagcaa gtctccattc ctggcagcc cttccccag 4561 gaagcaagag agacggatatac accttccaccc ggacataga cctgccccc 4621 catgattcg cgggatatac accttccaccc tggcccttt gaagcccacc 4621 catgattcg cgggatatac accttccaccc tggcccttt gaagcccacc 4621 aagattgg gggggatatac accttccacac tggcctgtt gaagccacca agcctgtgtt 4861 agagctcctt gtagaacaa gcacataga cttccaacct agcagcaca agcctgtgtt 4881 gatcagaga gatgaccaaga agaagaga agagggga agagagaga gagaggga agagagaga gagaggga agagaggag							
3541 gtctgtgaag gagattggtg gattgactca ggtcaacaag aacaaaaaat ggcgggaact 3601 tgcaaccaac ctcaatgtgg gcacatcaag cagtgctgcc agctccttga aaaagcagta 3661 tatccagtgt ctctatgcct ttgaatgcaa gattgaacgg ggagaagacc ctccccaga 3721 catctttgca gctgctgatt ccaagaagtc ccagccaaga atccagcctc ctctcctgc 3781 gggatcagga tctatgcagg ggccccagac tccccagtca accagcagtt ccatggaaga 3841 aggaggagac ttaaagccac caactccagc atccacacca cacagtcaga tcccccatt 3901 gccaggcatg agcaggaga attcattgg gatccaggat gcctttaatg atggaagtga 3961 ctccacattc cagaagcgga attcattgag gatccaggat gcctttaatg atggaagtga 4021 tacctctgac atgatggggc gcatgtccta tgagccaaat aaggatcctt atggcagcat 4141 gggtgacccc tacagtcgtg ctgccggccc tgggctagga aatgggggat 4141 gggtgacccc tacagtcgtg ctgccggccc tgggctagga aatgggggg gaatggccaagg cagggcccaa acggggggat 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcct gggaaccacg 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg ggaataggcc 4261 tgagggaaac atggacactg gctaccccca gacgaagaac agcagcaga agcaagaca 4381 tgattcctat ggcaatcagt tctccaccca ggcagaacc atccaagaacc 4381 tgattcctat ggcaatcagt tctccaccca aggcaccct tctggaagcac acaatggaac 4401 ccagcaagaga aaccgcacct ctcctagca gtctccattc ctgcactctc ggagaaaat 4561 gcaagaagga agcggccct tcctagaa gtctccattc ctgcacctcg ggatgaaaat 4561 gcaagaagga agcggccca tacctgcccc gaagcaatgaac acceccccc 4621 catgattcg ggggatatca ccttccacc tggctctgtt gaagccacc agcctgttt 4681 gaagcaggag agcgggcta caatgaaga cattggaac ccggaggaaat 4741 gatgtccctc aagtctggtc tcctggcaga gagacaatgg gcattagaat ccatcaacat 4801 cctgctgtat gaagaatat tcccaggaagaa gacagaaga gacagaagaa gacaagaaa agaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaagaagaa gacaacacc ctacaggaagaa							
3601 tgcaaccaac ctcaatgtgg gcacatcaag cagtgctgcc agctccttga aaaagcagta 3661 tatccagtgt ctctatgcct ttgaatgcaa gattgaacgg ggagaagaacc ctccccaga 3721 catctttgca gctgctgatt ccaagaagtc cagcaccaag atccagcacc ctctcctgc 3781 gggatcagga tctatgcagg ggcccaaga tccaccagca accagcagct ccattgcagg 3841 aggaggagac ttaaagcac caactccagc atccacacca cacagtcaga tcccccatt 3901 gccaggcatg agcaggaga attcagttgg gatccaggat gcctttaatg atggaagtga 3961 ctccacattc cagaagcgga attccatgac tccaaaccct gggtatcaga caggatgaa 4021 tacctctgac atgatgggc gcatgtccta tgagccaaat aaggatcctt atggcagcat 4081 gaggaaagct ccagggagtg atccttcat gtcctcaggg cagggccca acggggggat 4141 gggtgacccc tacagtcgtg ctgccggccc tgggctagga aatgtggcg ggaatagggc 4261 tgaaggaaaca atgagcactg ggccccaaa gccgaatct atgccttcaa ggggaccacag 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcccg ggaatagggcc 4321 ggggaatgat tctcctagcc gctaccccc gcagcagcag acgaagcag agcaacgaca 4381 tgattcctat ggcaatcagt tctccaacca aggcagcacc ttctggaagc ccttcccag 4501 gccaatggag accgacact tctctcaacca aggcaccct tctggcagcc ccttccccag 4501 gccaatggag accgacacc tctctagcaa gtctccattc ctggaagcc ccttccccag 4501 gccaatggag agcgcccaa tacctgccc gaacatagac actggacccc 4621 catgattcgg cgggatatca ccttcccacc tggctctgtt gaagcacaca agcctgtgtt 4681 gaagcagagg aggcggcta caatgaaca cttccaacc tggctctgtt gaagcacaca agcctgtgtt 4681 gaagcagagg agggggctca caatgaaga cattggaac cctggcccctg tgcagcccc 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacct agtcagcac cctggctgtt 4861 agaagctcctt gtagaacaa gcatcatgac cttcaacct agtcagcac cagggttgct 4861 agaagctcct gatgaacaa gcacacaa gacagagaa gacacacaa gacacacaa gacacacaacaacaacaacaacaacaacaacaacaacaac							
3661 tatccagtgt ctctatgeet ttgaatgeaa gattgaaegg gagaaagaec cteeceegga 3721 catctttgea getgetgatt caagaagte ceagecaaag atecageete eteteteegga 3781 gggateagga tetatgeagg ggeeceagae teeceagtea aceageagtt ceatggeaga 3841 aggaaggagae tetatgeagg ggeeceagae teeceagea aceageagtt ceatggeaga 3841 aggaaggagae tetatgeagga caacteeage atecacacea caeagteaga teececeatt 3901 geeaggeatg ageaggagaa attecatgee teeaaaecet gggtateage eeagtatgaa 4021 taceetetgae atgatgggge geatgteeta tgageeaaat aaggateett atggeagaat 4081 gaggaaagae eeaggaagtg atecetteat gteeteaggg eagggeeeaa aeggegggat 4141 gggtgaeeee tacagtegtg etgeeggeee tgggetagga aatgtggega tgggaeeaeg 4261 tgagggaaae atgageaetg gteettatga eagaatgagg aeggageetg aggaaeeaeg 4261 tgagggaaae atgageaetg gggeeeaaa gegaateete atgeeetteea aceaggaete 4321 ggggatgtat teteetagee getaeeeeee geagaaeee acaaggaega acaaatgaat eacaatgaat eacaageaaea ggagtatee ageeetgeee eetteeeag 4441 ceageagae acaaatgtate aacaageaaea ggagtatee ageeetgete eetteeeag 4461 geagaaggaa ggaceeee eetteeeae geaaatagaa eetteeeae 4561 geagaaggaa gggeeeeaag eetteeeaa gteteeea 4681 gaageagga gagegeeee eetteeeae geaaatagaa eetteeeee 4681 gaageagga agggegeee eetteeeae geaaatagaa eetteeeee 4881 gaageagga ggggeeeea aageaeae eetteeeae 4881 gaageagga ggggeeeea acaatgaa eetteeeae ggagtatee ageeetgete eetgeeeeg 4881 gaageagaag aggeggeee eetteeeae ggagtatee ageeetgete eetgeeeeg 4881 gaageagga gggggeee eeteeeae ggagtatee ageeetgeee eetgeeeeg 4881 gaageagaa ggggggeee eetgeeeg ggaatatee eetgeeeg ggagtate 4881 gaageagaag agggggeee eetgeeeg ggaagaagaa eetgeeeg 4881 gaageagaagaa gaggaggaa ggagaagaagaa ggaggaagaa							
3721 catetttgca getgetgatt ceaagaagte ceageceag atceagete cetteetge 3781 gggateagga tetatgeagg ggeeceagae tececagtea aceageaggt ceatggeaga 3841 aggaggage teaageace caacteage atceacace aceagteaga tececeatt 3901 geeaggeatg ageaggagea atteagtgg gatecaggat geetttaatg atggaagtga 3961 etceacatte cagaagegga attecatgae tecaaaceet gggtateage ceagtatgaa 4021 tacetetgae atgatgggg geatgteeta tgagecaaat aaggateett atggeageat 4081 gaggaaaget ecagggagt atceetteat gteeteagg eagggeecea aeggegggat 4141 gggtgaceee tacagteegg etggedeaga atggeageaet 4201 acageactat ecetatggag gteettatga eaggatgagg aeggageecea aeggagggee 4201 acageactat ecetatggag gteettatga eagagtgagg aeggageetg gaatagggee 4261 tgagggaaac atggeacetg gggeeceaea geegaatete atgeetteea aeceagaete 4321 gggagtgata teteetagee getaeceee geagaageag eageagaega ecetaeceag 4381 tgatteetat ggeaateagt teteeaceea aggeaceet tetggeagee eetteeeag 4381 tgatteetat ggeaateagt teteeaceea aggeaceet tetggeagee eetteeeag 4361 geeagaagga aacegeacet eteetageaa gteeteatte etggeagee eetteeeag 4361 geagaaggag aggegeeta eacetgeete geaatagae eetgeeetg ggatgaaaat 4561 geagaaggag aggegetea eetteeete geacatagea eetgeeetg tgeageeee 4621 eatgattegg egggatatea eetteeace tggetetgtt gaagecaeae ageetgttt 4681 gaageagaag aggeggete eaatgaaga eattggaac eetgeeetg tgeageeee 4621 eatgattegg egggatatea eetteeace tggetetgtt gaagecaeae ageetgtgtt 4861 agageceet aagtetggte eaatgaaga eattggaae eetggaggtaat 4741 gatgteeete aagtetggte eaatgaaga eattggaae eetggaggtgaat 4881 eetggetga gatgaeaaa gaagaagaa gaagaagaa gaagaagaa geataatga eetteaacete ageeggtgtgt 4881 getaagata gatgaaaaa gaagaagaa gaagaagaa gaagaagaa gaagaa		_		-			
3781 gggatcagga tetatgcagg ggecccagac tececagtea accagcagt tecatgcaga 3841 aggaggagac ttaaagccac caactecage atccacaca cacagtcaga tececcett 3901 gecaggagag aggaggaga atteagttgg gatcaggaga geetttaatg atggaagtga 3961 etceacatte cagaaggaga atteagteg gatcagagat geetttaatg atggaagtga 3961 etceacatte cagaaggagga attecatgac tecaaaacect gggtatcage cagtatgaa 4021 tacetetgac atgatggggg geatgteeta tgagcaaat aaggateett atggacgaat 4081 gaggaaaget ecatggggg geetteat gteetcaggg cagggeecea acggggggat 4141 gggtgacece tacagteggg gteettatga cagggtgggg acgggggggggggggggggggg							
3841 aggaggagac ttaaagcac caactccagc atccacaca cacagtcaga tcccccatt 3901 gccaggcatg agcaggagga attcatgac tccaaaccca gggtatcagg ccctttaatg atggaagtga 3961 ctccacattc cagaagcgga attcatgac tccaaaccct gggtatcagc ccagtatgaa 4021 tacctctgac atgatgggg gcatgtccta tgagccaaat aaggatcctt atggcagcat 4081 ggggaaagct ccagggggt atcccttcat gtcctcaggg cagggccca acggcgggat 4141 gggtgacccc tacagtcgg ctgccggccc tgggctagga aatgggggg tgggggacacg 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagccg 4201 tgagggaaac atgagcactg gggcccaaca gcgaatctc atgcctcca accagactc 4321 ggggatgtat tctcctagcc gctaccccc gcagaaccct tctggcagc cccttcccag 4401 caagcagact acaatgtatc acacgcaaca gcagaaccac atgcctccag 4381 tgattcctat ggcaatcagt tctccaacca aggcacccct tctggcagc ccttcccag 4401 ccagcaagca accagcact tctccaacca aggcaccct tctggcagc ccttcccag 4501 gccaatggag accgcacct ctcctagcaa gtctccattc ggaagaagaa agaagaaga 4561 gaagaaggag aggcgccaa tacctgcctc gcaatagaa cctggcccc 4621 catgattcgg ggggatatca ccttccaacc tggctctgt gaagccacac agcctgttt 4681 gaagcagagg aggcggcta caatgaaaga cattggaacc ccggaggcat 4741 gatgtccctc aggtctggtc tcctggcaga gacacatgg gattagacaca agcctgtgtt 4861 agagctcct tgagaatatt tccgacga gacacatgg cttaagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacct agtcagtcc cagggttgct 4861 agagctcct tgagaatatt tccgacgat gctactggat cctggaggat ggtgggggatatc agacagagaa gacagagaa agaagaagaa gacacatgg cttaagata ccttaaacct 5041 agaagagga ggtgaccag aaggaggagaa agagagaaga gaagaagaa gaagaa		_				_	_
3901 gccaggcatg agcaggagca attcagttgg gatccaggat gcctitaatg atggaagtga 3961 ctccacattc cagaagcgga attccatgac tccaaaccct gggtatcagc ccagtatgaa 4021 tacctctgac atgatgggg gcatgtccta tgagccaaat aaggatcctt atggacagcat 4081 gaggaaagct ccagggagtg atcccttcat gtcctcaggg cagggcccca acggggggat 4141 gggtgacccc tacagtcgtg ctgccggccc tggggtagga aatgtggga tgggaccacg 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg gaatagggcc 4261 tgagggaacc atgagcactg gggccccaca gccgaatccc atgcctcca acccagactc 4321 ggggatgtat tctcctagcc gctaccccc gcagcagcag cagcagcagc accagaacaca 4381 tgattcctat ggcaatcagt tctccaccca aggcaccct tctccagca 4441 ccagcagact acaatgtatc accagcaac acaatgaaca acaatgtatc acaatgaaca ggcacccct tctccaagca gccaatggag aaccgagca ccttccccag 4501 gccaatggag aaccgcact ctcctagcaa gtctccattc ctgcacccc 4621 catgattcgg ggggatatac ccttccaccc tggctctgtt gaagccacac agcctggtt catgattcgg ggggatataca ccttccaccc tggctctgtt gaagccacac agcctggtt catgattcgg ggggatataca accttccacc tggctctgtt gaagccacac agcctggttt aagtgccccc aagtgaaaga aggagggctca aagtgaaga ggacaatgg ggattagaaca agctgtgtt cctggcaga gagcacatgg ggaggatataga ccttcaaccc tggctctgtt gaagccacac agcctgtgtt 4861 agagcaccc ggagaatatt tccgacgag agagcacatgg gagcacatag gcattagaac agcaggtgat gagagaaga gaagagaga ggagacacaga gaagagaaga gaagagaga gctcccatga gagagagaa gaagaagaga gaagaagaga gacacatgg cctggatgat tcaagcacat 4801 cctgctgtat gatgacacaa gcatcatgac cttcaacct agtcagctc caggggtat fccaacacat 4801 gagaagaga gagaagaga gaagaagaga gaagaaga					_		
4021 tacctctgac atgatggga attecatgac tecaaacect gggtateage ceagtatgaa 4021 tacctctgac atgatgggg geatgtecta tgagecaaat aaggateett atggeageat 4081 gaggaaaget eacagtegtg atceetteat gteeteaggg cagggeecea aeggegggat 4141 gggtgaecee tacagtegtg etgeeggeee tgggetagga aatgtggga tgggaecaeg 4201 acageactat ecetatggag gteettatga eagagtgagg aeggageetg gaatagggee 4261 tgagggaaac atgageactg gggeeceaea geegaatete atgeetteea aeceagaete 4321 ggggatgtat teteetagee getaeceee geageageag eageageage ageaaegaea 4381 tgatteetat ggeaateagt teteeaeeea aggeaeeeet tetggeagee eetteeceag 4441 ceageagaet acaatgate acaegeaaea geaggtatee atgeetteee eetteeceag 4501 geeaatggag aacegeaeet eteetageag gteeteeag 4501 geaaatggag aggeeeeet eteetageag gteeteeag teteetagee getaeceee tggeageatee eetteeceag 4501 geaaatggag aggegeeet eetteecaee tggetetgtt gaagecaeee ggaggagaat 4561 gaagaagga gggeggtaa eetteecaee tggetetgtt gaagecaeee ageetgtgtt 4681 gaageagaag aggeggetea eaatgaaaga eattggaaee eetggaggatat eageeggtata 4741 gatgteeete aagetetggte teetggeaga gageacatag geattagaat eetteaaeet aggeagtatagae eetggaggtaat 4741 gatgteeete aagetetggte teetggeaga gageacatag geattagaat eetteaaeet aggeagtatgagaggagggtaat 4861 agageteett gtagaatatt teegaegag eetgattgag atetttggea teetggagaggt gatagaggag ggtagaaeaea geateatgae eetggaggtat eetggaggtgaga 4921 gtatgaggtg ggtgaeceag gacagagaa geatatgag eetggggtaa agaagaagag gaattetagge teetggaaggt ggagaagaga gaaagaagaa gaacttetag gteetaaaet 5041 agaagaagga gaagaagag agagggggaa aagaaga			_	_			
4021 tacetetgac atgatgggc geatgteeta tgagecaaat aaggateett 4081 gaggaaaget ceagggagtg ateeetteat gteeteaggg eagggeeee aeggegggat 4141 gggtgaceee tacagtegtg etgeeggeee tgggetagga aatgtggega tgggaeeagg 4201 acageactat eectatggag gteettatga eagagtgagg aeggageegg gaatagggee 4261 tgagggaaae atgageaetg gggeeeeaea geegaateete atgeetteea aeceagaete 4321 ggggatgat teteetagee getaeeeee geageageag eageageage ageaaegaea 4381 tgatteetat ggeaateagt teteeaeea aggeaeeeet tetggeagee eetteeea 4321 gegaateagt teteeaeea aggeaeeet tetggeagee eetteeea 4321 geeaatggag aacegeaeet eteetageaa gteteeatte etggeagee eetteeeag 4441 eeageaggag aacegeaeet eteetageaa gteteeatte etggeaeee eetteeeag 4501 geeaatggag aacegeaeet eteetageaa gteteeatte etggeaetetg ggatgaaaat 4561 geagaaggea aggeggetea eattgeete gaageeeeet ggageeeee 4621 eatgattegg egggatatea eetteeeaee tggetetgt gaageeeee aggeggtat 4681 gaageaggag aggeggetea eatagaaaga eattggaaee eetggegggatat 4741 gatgteeete aagtetggte teetggeaga gageaeatgg geatagata eetteggaeee eaggggggtaat 4861 agaageteett gaagaeaea geateatgae etteaaeete ageeagtge eaggggtgat 4861 agaageteett gtagaatatt teegaegatg eetgattgag atetttgga tttaaaagga 4921 gtatgaggag ggtgaeeeag gaeaggaae getaetggat eetggggggat teetagaeag 4981 gtetagteea geteeeatg agggtggga agaagaagaa agaagaagaa gaagaagaaga aagatgtga gaaattetag gteetaaaet 5041 agaagaggaa gaagaagaag aagatggga agaagaagaa gaaatteeag gaaatteeag 5221 tgtegaggaa tttgaaagaa atgateeat tgtgggggat tgeteagata agettggegg 5221 tgtgeaggag tttgaaagaaga agaatgagaa agaagatgaa eetggggggg aeaeeeacee 5341 etgeeeaea ageeeteega ageaettgae eagaagaaga gaaaaagaa agaatgeee etggeggatt ggtgggggg aeaeeaeae 53401 eeagagagg eeeeectegaa ageaettgae eagaagaaga ggtaeaeea ggaaaaagaa 5401 eeagagagg eeeeectegaa ageaettgae eagaagaaga getaaaeaea 5401 eeagagagg eeeeectegaa ageaettgae eagaagaaga ggaaaaaga eetaeegea etaaegaaga 5401 eeagagaggg eeeeeectegaa ageaettgae eagaagaaga ggaaaaagaa eetaeegea etaaegaaga 5401 eeagagaggg eeeeeectega ageaeettgae eagaaaaaaga eagaaaaaaaga eaceaettga agaaaaaaa 6401 eagaggaggg eeeee							
4081 gaggaaagct ccagggagtg atccettcat gtcctcaggg cagggccca acggcgggat 4141 gggtgacccc tacagtcgtg ctgccggccc tggggctagga aatgtggcga tggggaccacg 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg gaatagggcc 4261 tgagggaaac atgagcactg gggcccacaa gccgaatctc atgccttcca acccagactc 4321 ggggatgtat tctcctagcc gctacccccc gcagcaggag cagcagga agcaacgaca 4381 tgattcctat ggcaatcagt tctccaccca aggacccct tctggcagcc ccttccccag 4441 ccagcagact acaatgtatc aacagcaaca gcaggtatcc agccctgctc ccctgccccg 4501 gccaatggag aaccgcacct ctcctagcaa gtctccattc ctgcactct ggatgaaaat 4561 gcagaagga agccgcact ctcctagcaa gtctccattc ctgcactct ggatgaaaat 4561 gaagcagaga aggcggctaa caatgaaaga cattggaac ccggaggcac aggcgggtat 4681 gaagcagagg aggcgctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcactcc cagggttgct 4861 agagctcct gtagaaatat tccgacgag cctgattgag atctttgga ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctggagggt tcagcaaggt 4981 gtctagtca gctccatgg agggtggga agaagagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagag aagtagttga aatgtgagg agaagagaa gaagaagag agagagag				_			
4141 gggtgacccc tacagtcgtg ctgccggccc tgggctagga aatgtggcga tgggaccacg 4201 acagcactat ccctatggag gtccttatga cagagtgagg acggagcctg gaatagggcc 4261 tgagggaaac atgagcactg gggcccaca gccgaatctc atgccttcca acccagactc 4321 ggggatgtat tctcctagcc gctacccccc gcagcagcag cagcagcagc agcaacgaca 4381 tgattcctat ggcaatcagt tctccaccca aggcacccct tctggcagcc ccttccccag 4441 ccagcagact acaatgtatc aacagcaaca gcaggtatcc agcctgctc ccctgccccg 4501 gccaatggag aaccgcacct ctcctagcaa gtctccattc ctgcactctg ggatgaaaat 4561 gcagaaggca ggtccccag tacctgctc gcacatagca cctgcccctg tgcagcccc 4621 catgattcgg cgggatatca ccttccacc tggctctgtt gaagccaca agcctgttt 4681 gaagcagagg aggcggctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgag gacacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacacac gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcct gtagaatatt tccgacgatg cctgattgga atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gcacatgga cctggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtggga agaagagaag							
4201 acagcactat coctatggag gtccttatga cagagtgagg acggagcctg gaatagggcc 4261 tgagggaaac atgagcactg gggcccaca gccgaatctc atgccttcca acccagactc 4321 ggggatgtat tetectagec getacecee gcagcageag cagcagcage agcaacgaca 4381 tgattectat ggcaatcagt tetecacea aggcacecet tetggcagec cetteceagg 4441 ccagcagact acaatgtate aacagcaaca gcaggtatee agcectgete cectgcceeg 4501 gccaatggag aaccgacet eteetagcaa gtetecatte etggcactet ggatgaaaat 4561 gcagaaggea ggtececcag tacetgcee gcacatagea eetgeceet teteggcaee eetgeceet tacetageae eetgeceet tacetgeet ggatgaaaat 4561 gaagcagagg aggcgetea cactgcee gcacatagea eetgeceet tagagccee 4621 catgattegg egggatatea eetteccace tggetetgtt gaagccacae agcetgtgtt 4681 gaagcagagg aggeggetea caatgaaaga cattggaace eeggaggcat ggegggtaat 4741 gatgteeete aagtetggte teetggcaga gagcacatgg gcattagata ecatcaacat 4801 cetgetgtat gatgacaaca gcatcatgae etteaacet agteagetee eaggggttget 4861 agageteett gtagaatatt teegacgatg eetgattgag actettggea tettaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat eetgggaggt teagcaaggt 4981 gtetagteca geteccatgg agggtgggaa agaagaagaa gaacttetag gteetaaact 5041 agaaagagaa gaagaagaga gaagtagtga aagtagttga aatgtagaga ggaagaagac gttacagaa atagtgaga gaagaagac atgtaggtggaa agaagaagaa gaacttetag gteetaaact 5041 agaaagaca gettecaagaa atagtgaga gaagtagte ttteaggcaa 5101 ggacaagee gettecagaa atagtgaga gaagtagte agtaagtttg acaagettee 5161 agtaaagate gttagaagag gettgeetgea etggtggga ttggcagaa atagtgaga getgetgee etgetgegg 5221 tgtgcaggag ttgacagtg geetgetgea etggtgggat eetecegge etcacgaace 5341 etgeccaca geecetegga aggacagaa agagtggtg eetecegga etcacecega 5341 etgeccacaa geecetegga atggacetee agaaaaacga ggtacaccaa ggacaacaga 5401 ecaggagggg ececcacttgg aggacetee agaaaaacga gtacaccaa etatggatga 6461 eatgttgtet acteggteta gcacettga ecgagatgga ggtacaccaa etatggatga 6461 eatgttgtet acteggteta gcacettga ecgagataga ggtacaccaa etatggatga 6461 eatgttgtet acteggteta gcacettga ecgagataga ggtacaccaa etatggatga 6461 eatgttgtet acteggteta gcacettga ecgagataga ggtacaccaa etatggatga 6461 eatgttgtet acteg							
4261 tgagggaaac atgagcactg gggcccaca gccgaatctc atgccttca acccagactc 4321 ggggatgtat tctcctagcc gctaccccc gcagcagcag cagcagcagc agcaacgaca 4381 tgattcctat ggcaatcagt tctccacca aggcacccct tctggcagcc ccttccccag 4441 ccagcagact acaatgtatc aacagcaaca gcaggtatcc agcctgctc ccctgccccg 4501 gccaatggag aaccgcact ctctagcaa gtctccattc ctgcactct ggatgaaaat 4561 gcagaaggca ggtccccag tacctgctc gcacatagca cctgcccct tcgagcaccc 4621 catgattcgg cgggatatca ccttcccacc tggctctgtt gaagccaca agcctgtgtt 4681 gaagcagagg aggcgctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcacact 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcacccc 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttagata ccatcacacat 4801 cctgctgtat gatgacacac gcatcatgac cttcaacctc agtcagctc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtca gctccatgg agggtggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagag aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgaga gaagctgatc agtaagttt acagagaa 5101 ggacaagca gctcagaga atagtgaga gaagctgatc agtaagttt acagagcag 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctc cttcccggc ctcacgcacc 5341 ctgcccacca gccctctcga agagcagac agaagaagag ggtacaccag ggtacaccag accacctga 5461 cagttgtct actggtcta acccactt ggacgatgga gctaagagt cataggagg 5461 catgttgtct actggtcta acccacttga cgaggatgga gctaagagt cataggagg accacactga 5461 catgttgtct actggtcta acccacttga cgaggatgga gctaagagt cataggagg accacactga 5461 catgttgtct actggtcta acccacttga ggacaccac agagcacacacacacacacacacacac							
4321 ggggatgtat tetectagec getaceceee geageageag cageageage ageaaegaea 4381 tgatteetat ggeaateagt tetecaeeea aggeaeeeet tetggeagee cetteceeag 4441 ceageagaet acaatgtate aacageaaea geaggtatee ageeetgete eeetgeeeg 4501 geeaatggag aacegeaeet eteetageaa gtetecatte etgeaetetg ggatgaaaaat 4561 geagaaggea ggteeeeag taeetgeete geacatagea eetgeeeetg tgeageeeee 4621 catgattegg egggatatea eetteeeaee tggetetgtt gaageeaeae ageetgtgtt 4681 gaageagagg aggeggetea eaatgaaaga eattggaaee eeggaggeat ggegggtaat 4741 gatgteeete aagtetggte teetggeaga gageaeatgg geattagata eeateaaeat 4801 eetgetgtat gatgaeaea geateatgae etteaaeete agteagetee eagggttget 4861 agaageteett gtagaatatt teegaegatg eetgattgag atettttggea tittaaagga 4921 gtatgaggtg ggtgaeeeag gaeagagaae getaetggat eetgaggaggt teageaaggt 4981 gtetagteea geteeeatgg agggtgggga aagaagaagaa gaaettetaag gteetaaaea 5101 ggaeaagea geteeaagaa atagtgagga gaagetgate agtaagttt acaagaetee 5161 agtaaagate gtacagaaga atagtgaga gaagetgate agtaagtttg acaagettee 5161 agtaaagate gtacagaaga atagteetat tgtggtggae tgtgaegggg acaaceaetga 5221 tgtgeaggag tttgaeagta geetgetgea etggeggatt ggtgggggg acaaceaetga 5281 geatateeag aceeetegga ageatgtgae agaagaagag ggtaeaeeag 5341 etgeeeaeea geeeteegga ageatgtgae aacageagag ggtaeaeeag 5361 eegagagggg eeeeeeetegga ageatgtgae aacageagag ggtaeaeeag 5361 eegaggaggg eeeeeeetegga ageatgtgae aacageagag ggtaeaeeag 5401 eeaggagggg eeeeeeetegga ageatgtgae agaaaaaagg ateaeagea etatggatga 5461 eetgeeeeee geeeeeetega ageatgtgae eegagaagaga ggtaeaeeag 5461 eetgeeeeea geeeetegga ageatgtgae agaaaaaaegg ateaeagea etatggatga aacageaeaga 5461 eetgeeeeea geeeetegga ageatgtgae eegagaagaga ggtaeaeeag 5461 eetgeeeaea geeeetegga ageatgtgae eegagaagaga gaaaaaaegg ateaeaega eetatggatga aacageaeaega 6461 eetgeeeaea geeeetegga ageatgtgae eetgeegaa eetatggaega eetatggaega 6461 eetgeeeaea geeeetegga ageatgtgae agaaaaaaagg ggtaeaeeag 6461 eetgeeeaea geeeetegga ageatggae eegagaaaaaagga eetgeeagaagae eegagaaaaaagagaa eacaeeetga 6461 eetgeeaeaea geeeeteggaaaaaaaaaagaa eacaeeaeaagaa 6461 eetge							
4381 tgattcctat ggcaatcagt tctccacca aggcaccct tctggcagc ccttcccag 4441 ccagcagact acaatgtatc aacagcaaca gcaggtatcc agcctgctc ccctgcccg 4501 gccaatggag aaccgcacct ctcctagcaa gtctccattc ctgcactctg ggatgaaaat 4561 gcagaaggca ggtcccccag tacctgcctc gcacatagca cctgcccctg tgcagcccc 4621 catgattcgg cgggatatca ccttcccacc tggctctgtt gaagccacac agcctgtgtt 4681 gaagcagagg aggcggctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtgggga agaacgaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagag aatgagttga aaatgatgag gagatagcc tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagttg acaagctcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggc tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag accacttcg agagcaagac agagctgctg ccttcccggc ctcacgcac 5341 ctgcccacca gcccctcgga agcatgtgac agaaaaacgg ggtacaccag ggacaacaga 5401 ccaggagggg cccccactg atggacctc agaaaaacgg atcacagca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagggt cagagccat						_	_
4441 ccagcagact acaatgtate aacagcaaca gcaggtatee agecetgete eeetgeeeg 4501 gccaatggag aacegcact etectageaa gtetecatte etgeactetg ggatgaaaat 4561 gcagaaggea ggteeeeag tacetgeete geacatagea eetgeeeeg 4621 catgattegg egggatatea eetteeeae tggetetgtt gaagceaeae ageetgtgtt 4681 gaagcagagg aggeggetea eaatgaaaga eattggaace eeggaggeat ggegggtaat 4741 gatgteeete aagtetggte teetggeaga gagcacatgg geattagata eeateaaeat 4801 eetgetgtat gatgacaaea geateatgae etteaaeete agteagetee eagggttget 4861 agageteett gtagaatatt teegacgatg eetgattgag atetttggea ttetaaagga 4921 gtatgaggtg ggtgaceeag gacagagaae getactggat eetggaggt teageaaggt 4981 gtetagteea geteeeatga agggtggga agaagaagaa gaaettetag gteetaaaet 5041 agaagaggaa gaagaagag aagtagttga aaatgatgag gagatageet ttteaggeaa 5101 ggacaageea geteeagaa atagtgagga gaagetgate agtaagttg acaagettee 5161 agtaaagate gtacagaaga atgateeatt tgtggtggae tgeteagata agettgggeg 5221 tgtgeaggag tttgacagtg geetgetgea etggeggatt ggtgggggg acaeeaetga 5281 geatateeag aceeaetteg agageaagae agagetgetg eetteeegga tetaegeae 5341 etgeeeaea geeeetegga ageatgtgae agaaaaaegg ggtacaeeag ggacaaeaga 5401 eeaggagggg eeeeeaettg aggaeetta eegaggatga getaaggat eagaggeeat 6261 eatgttgtet acteggteta geaeettgae eggagatgga getaaagagt eatatggatga 5461 eatgttgtet acteggteta geaeettgae eggagatgga getaaagagt eatatggatga			-	-			
4501 gccaatggag aaccgcacct ctcctagcaa gtctccattc ctgcactctg ggatgaaaat 4561 gcagaaggca ggtccccag tacctgcctc gcacatagca cctgcccctg tgcagcccc 4621 catgattcgg cgggatatca ccttcccacc tggctctgtt gaagccacac agcctgtgtt 4681 gaagcagagg aggcggctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagaag aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agcatgtgac agagatgggg ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atgacctcc agaaaaacgg atcacagca ctatggatga 5461 catgttgtct actcggtcta acccacttgac cgaggatgga gcaagaggt cagaggcatt		_					_
4561 gcagaaggca ggtccccag tacctgctc gcacatagca cctgccctg tgcagcccc 4621 catgattcgg cgggatatca ccttcccacc tggctctgtt gaagccacac agcctgtgtt 4681 gaagcagagg aggcggctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtccctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtgggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgaga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgcccacca gccctcgga agcatgtgac agaaaaacgg ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcac ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat			_	_			
4621 catgattegg egggatatea cetteceace tggetetgtt gaageeacae ageetgtgtt 4681 gaageagagg aggeggetea caatgaaaga cattggaace eeggaggeat ggegggtaat 4741 gatgteeete aagtetggte teetggeaga gageacatgg geattagata eeateaacat 4801 cetgetgtat gatgacaaca geateatgae etteaacete agteagetee eagggttget 4861 agageteett gtagaatatt teegaegatg eetgattgag atetttggea ttttaaagga 4921 gtatgaggtg ggtgaceeag gacagagaae getaetggat eetgggaggt teageaaggt 4981 gtetagteea geteeeatgg agggtgggga agaagaagaa gaacttetag gteetaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatageet ttteaggeaa 5101 ggacaageea getteagaga atagtgagga gaagetgate agtaagtttg acaagettee 5161 agtaaagate gtacagaaga atgateeatt tgtggtggae tgeteagata ageettgggeg 5221 tgtgeaggag tttgacagtg geetgetgea etggeggatt ggtgggggg acaceaetga 5281 geatateeag acceaetteg agageaagae agagetgetg eetteeegge etcaegeae 5341 etgeecacea geeeetegga ageatgtgae aacageagag ggtacaceag ggacaacaga 5401 eeaggaggg eeeecacetg atggacetee agaaaaaegg ateaeageea etatggatga 5461 eatgttgtet acteggteta geaeettgae egaggatgga getaagagt eagaggeeat							
4681 gaagcagagg aggcggctca caatgaaaga cattggaacc ccggaggcat ggcgggtaat 4741 gatgtcctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtgggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgaga gaagctgatc agtaagttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg ccccacctg atggacctc agaaaaacgg atcacagcac ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaaagatt cagaggccat							
4741 gatgtcctc aagtctggtc tcctggcaga gagcacatgg gcattagata ccatcaacat 4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
4801 cctgctgtat gatgacaaca gcatcatgac cttcaacctc agtcagctcc cagggttgct 4861 agagctcctt gtagaatatt tccgacgatg cctgattgag atctttggca ttttaaagga 4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaaagagt cagaggccat							
4861 agageteett gtagaatatt teegaegatg eetgattgag atetttggea tittaaagga 4921 gtatgaggtg ggtgaeeeag gacagagaac getaetggat eetgggaggt teageaaggt 4981 gtetagteea geteeeatgg agggtggga agaagaagaa gaaettetag gteetaaaet 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatageet titteaggeaa 5101 ggaeaageea getteagaga atagtgagga gaagetgate agtaagttg acaagettee 5161 agtaaagate gtacagaaga atgateeatt tgtggtggae tgeteagata agettgggeg 5221 tgtgeaggag tittgaeagtg geetgetgea etggeggatt ggtgggggg acaeeactga 5281 geatateeag acceaetteg agageaagae agagetgetg eetteeegge eteaegeaee 5341 etgeeacea geeeetegga ageatgtgae aacageagag ggtaeaceag ggaeaacaga 5401 eeaggaggg eeeecaectg atggaeetee agaaaaaegg ateaeageea etatggatga 5461 eatgttgtet aeteggteta geaeettgae egaggatgga getaagagtt eagaggeeat							
4921 gtatgaggtg ggtgacccag gacagagaac gctactggat cctgggaggt tcagcaaggt 4981 gtctagtcca gctcccatgg agggtggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcac ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
4981 gtctagtcca gctcccatgg agggtgggga agaagaagaa gaacttctag gtcctaaact 5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttggggg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtgggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcac ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5041 agaagaggaa gaagaagagg aagtagttga aaatgatgag gagatagcct tttcaggcaa 5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtggggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5101 ggacaagcca gcttcagaga atagtgagga gaagctgatc agtaagtttg acaagcttcc 5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtggggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5161 agtaaagatc gtacagaaga atgatccatt tgtggtggac tgctcagata agcttgggcg 5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtggggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgcccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5221 tgtgcaggag tttgacagtg gcctgctgca ctggcggatt ggtggggggg acaccactga 5281 gcatatccag acccacttcg agagcaagac agagctgctg ccttcccggc ctcacgcacc 5341 ctgcccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5281 gcatatecag acceaetteg agageaagae agagetgetg cettecegge etcaegeace 5341 etgeeeacea geeeetegga ageatgtgae aacageagag ggtacaeeag ggaeaacaga 5401 eeaggagggg eeeeeacetg atggaeetee agaaaaaegg ateaeageea etatggatga 5461 eatgttgtet aeteggteta geaeettgae egaggatgga getaagagtt eagaggeeat							
5341 ctgcccacca gcccctcgga agcatgtgac aacagcagag ggtacaccag ggacaacaga 5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5401 ccaggagggg cccccacctg atggacctcc agaaaaacgg atcacagcca ctatggatga 5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
5461 catgttgtct actcggtcta gcaccttgac cgaggatgga gctaagagtt cagaggccat							
			_			_	
- Jy. J. J J							
			<u> </u>		- , , , 	. , . , . ,	<u> </u>

```
5581 gatectagag gaegaaceee acagtaagga tgagaceeca etgtgtacee ttetggaetg
5641 gcaggattet ettgecaage getgegtetg tgtgtecaat accattegaa geetgteatt
5701 tgtgccaggc aatgactttg agatgtccaa acacccaggg ctgctgctca tcctgggcaa
5761 getgateetg etgeaceaca ageaceeaga aeggaageag geaceactaa ettatgaaaa
5821 ggaggaggaa caggaccaag gggtgagctg caacaaagtg gagtggtggt gggactgctt
5881 ggagatgete egggaaaaca cettggttac actegecaac atetegggge agttggaeet
5941 atotocatac coogagagea titigootgoo tgtootggac ggactootac actgggcagt
6001 ttgcccttca gctgaagccc aggacccctt ttccaccctg ggccccaatg ccgtcctttc
6061 cccgcagaga ctggtcttgg aaaccctcag caaactcagc atccaggaca acaatgtgga
6121 cctgattctg gccacacccc ccttcagccg cctggagaag ttgtatagca ctatggtgcg
6181 cttcctcagt gaccgaaaga acccggtgtg ccgggagatg gctgtggtac tgctggccaa
6241 cctggctcag ggggacagcc tggcagctcg tgccattgca gtgcagaagg gcagtatcgg
6301 caaceteetg ggetteetag aggacageet tgeegeeaca cagtteeage agageeagge
6361 cagectecte caeatgeaga acceaecett tgagecaact agtgtggaca tgatgeggeg
6421 ggctgccgc gcgctgcttg ccttggccaa ggtggacgag aaccactcag agtttactct
6481 gtacgaatca cggctgttgg acatctcggt atcaccgttg atgaactcat tggtttcaca
6541 agtcatttgt gatgtactgt ttttgattgg ccagtcatga cagccgtggg acacctcccc
6601 cccccgtgtg tgtgtgcgtg tgtggagaac ttagaaactg actgttgccc tttatttatg
6661 caaaaccacc tcagaatcca gtttaccctg tgctgtccag cttctccctt gggaaaaagt
6721 ctctcctgtt tctctctcct ccttccacct ccctccctc catcacctca cgcctttctg
6781 ttccttgtcc tcaccttact cccctcagga ccctacccca ccctctttga aaagacaaag
6841 ctctgcctac atagaagact ttttttattt taaccaaagt tactgttgtt tacagtgagt
6901 ttggggaaaa aaaataaaat aaaaatggct ttcccagtcc ttgcatcaac gggatgccac
6961 atttcataac tgtttttaat ggtaaaaaaa aaaaaaaaa atacaaaaaa aaattctgaa
7021 ggacaaaaaa ggtgactgct gaactgtgtg tggtttattg ttgtacattc acaatcttgc
7081 aggagccaag aagttcgcag ttgtgaacag accctgttca ctggagaggc ctgtgcagta
7141 gagtgtagac cctttcatgt actgtactgt acacctgata ctgtaaacat actgtaataa
7201 taatgtetea eatggaaaca gaaaacgetg ggteageage aagetgtagt ttttaaaaat
7261 gtttttagtt aaacgttgag gagaaaaaaa aaaaaggctt ttcccccaaa gtatcatgtg
7321 tgaacctaca acaccctgac ctctttctct cctccttgat tgtatgaata accctgagat
7381 cacctcttag aactggtttt aacctttage tgeagegget aegetgeeae gtgtgtatat
7441 atatgacgtt gtacattgca cataccettg gatececaca gtttggteet eeteecaget
7501 accectttat agtatgaega gttaacaagt tggtgaeetg cacaaagega gacacageta
7561 tttaatctct tgccagatat cgcccctctt ggtgcgatgc tgtacaggtc tctgtaaaaa
7621 gtccttgctg tctcagcagc caatcaactt atagtttatt tttttctggg tttttgtttt
7681 gttttgtttt ctttctaatc gaggtgtgaa aaagttctag gttcagttga agttctgatg
7741 aagaaacaca attgagattt tttcagtgat aaaatctgca tatttgtatt tcaacaatgt
7801 agctaaaact tgatgtaaat tcctcctttt tttccttttt tggcttaatg aatatcattt
7861 attcagtatg aaatctttat actatatgtt ccacgtgtta agaataaatg tacattaaat
7921 cttggtaaga cttt
```

[0117] The present invention also provides methods of inducing neuronal differentiation by contacting a cell with a compound (i.e., an EZH2 inhibitor) of the invention. Preferably, the compound is in an amount sufficient to increase expression of at least one gene selected from the group consisting of CD133 (also called PROM1), DOCK4, PTPRK, PROM2, LHX1, LHX6, LHX9, PAX6, PAX7, VEFGA, FZD3B, FYN, HIF1A, HTRA2, EVX1, CCDC64, and GFAP.

[0118] The term "inducing neuronal differentiation" used herein refers to causing a cell to develop into a cell of the neuronal lineage as a result of a direct or intentional effect on the cell.

[0119] The present invention also provides methods of inducing cell cycle inhibition by contacting a cell with a compound of the invention. Preferably, the compound is in an amount sufficient to increase expression of at least one gene selected from the group consisting of CKDN1A, CDKN2A, MEN1, CHEK1, IRF6, ALOX15B, CYP27B1, DBC1, NME6, GMNN, HEXIM1, LATS1, MYC, HRAS, TGFB1, IFNG, WNT1, TP53, THBS1, INHBA, IL8, IRF1, TPR, BMP2, BMP4, ETS1, HPGD, BMP7, GATA3, NR2F2, APC, PTPN3, CALR, IL12A, IL12B, PML, CDKN2B, CDKN2C, CDKN1B, SOX2, TAF6, DNA2, PLK1, TERF1, GAS1, CDKN2D, MLF1, PTEN, TGFB2, SMAD3, FOXO4, CDK6, TFAP4, MAP2K1, NOTCH2, FOXC1, DLG1, MAD2L1, ATM, NAE1, DGKZ, FHL1, SCRIB, BTG3, PTPRK, RPS6KA2, STK11, CDKN3, TBRG1, CDC73, THAP5, CRLF3, DCUN1D3, MYOCD, PAF1, LILRB1, UHMK1, PNPT1, USP47, HEXIM2, CDK5RAP1, NKX3-1, TIPIN, PCBP4, USP44, RBM38, CDT1, RGCC, RNF167, CLSPN, CHMP1A, WDR6, TCF7L2, LATS2, RASSF1, MLTK, MAD2L2, FBXO5, ING4, and TRIM35.

- [0120] The term "inducing cell cycle inhibition" used herein refers to causing an accumulation or an arrest at any phase during cell division and/or duplication.
- [0121] The present invention also provides methods of inducing tumor suppression by contacting a cell with a compound of the invention. Preferably, the compound is in an amount sufficient to increase expression of BIN1 or any tumor suppressors.
- [0122] The term "inducing tumor suppression" may include, but is not limited to, a reduction in size of a tumor, a reduction in tumor volume, a decrease in number of tumors, a decrease in number of metastatic lesions in other tissues or organs distant from the primary tumor site, an increase in average survival time of a population of treated subjects in comparison to a population receiving carrier alone, an increase in average survival time of a population of treated subjects in comparison to a population of untreated subjects, an 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, a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving carrier alone, a decrease in tumor growth rate, or a decrease in tumor regrowth rate.
- [0123] The present invention also provides methods of inhibiting hedgehog signaling by contacting a cell with a compound of the invention. Preferably, the compound is in an amount

sufficient to reduce expression of at least one gene selected from the group consisting of GLI1, PTCH1, SUFU, KIF7, GLI2, BMP4, MAP3K10, SHH, TCTN3, DYRK2, PTCHD1, and SMO. [0124] The phrase "inhibiting hedgehog signaling" means the hedgehog signaling strength (intensity) with a compound treatment is reduced by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%,1000%, 1500%, or more compared to the hedgehog signaling strength (intensity) without any compound treatment.

The present invention also provides methods of inducing a gene expression by [0125] contacting a cell with a compound of the invention. Preferably, the compound is in an amount sufficient to induce neuronal differentiation, cell cycle inhibition and/or tumor suppression. Such gene is selected from the group consisting of CD133 (also called PROM1), DOCK4, PTPRK, PROM2, LHX1, LHX6, LHX9, PAX6, PAX7, VEFGA, FZD3B, FYN, HIF1A, HTRA2, EVX1, CCDC64, GFAP, CKDN1A, CDKN2A, MEN1, CHEK1, IRF6, ALOX15B, CYP27B1, DBC1, NME6, GMNN, HEXIM1, LATS1, MYC, HRAS, TGFB1, IFNG, WNT1, TP53, THBS1, INHBA, IL8, IRF1, TPR, BMP2, BMP4, ETS1, HPGD, BMP7, GATA3, NR2F2, APC, PTPN3, CALR, IL12A, IL12B, PML, CDKN2B, CDKN2C, CDKN1B, SOX2, TAF6, DNA2, PLK1, TERF1, GAS1, CDKN2D, MLF1, PTEN, TGFB2, SMAD3, FOXO4, CDK6, TFAP4, MAP2K1, NOTCH2, FOXC1, DLG1, MAD2L1, ATM, NAE1, DGKZ, FHL1, SCRIB, BTG3, PTPRK, RPS6KA2, STK11, CDKN3, TBRG1, CDC73, THAP5, CRLF3, DCUN1D3, MYOCD, PAF1, LILRB1, UHMK1, PNPT1, USP47, HEXIM2, CDK5RAP1, NKX3-1, TIPIN, PCBP4, USP44, RBM38, CDT1, RGCC, RNF167, CLSPN, CHMP1A, WDR6, TCF7L2, LATS2, RASSF1, MLTK, MAD2L2, FBXO5, ING4, TRIM35, BIN1 and any tumor suppressors.

[0126] The phrase "inducing a gene expression" means the expression level of a particular gene of interest is increased by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 1000%, 1500%, or more compared to the expression level of this gene without any compound treatment. [0127] The present invention also provides methods of inhibiting a gene expression comprising contacting a cell with a compound of the invention. Preferably, the compound is in an amount sufficient to inhibit hedgehog signaling. Such gene is GLI1, PTCH1, SUFU, KIF7, GLI2, BMP4, MAP3K10, SHH, TCTN3, DYRK2, PTCHD1, or SMO.

[0128] The phrase "inhibiting a gene expression" means the expression level of a particular gene of interest is reduced by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%,1000%, 1500%, or more compared to the expression level of this gene without any compound treatment.

- [0129] Neuronal differentiation, cell cycle inhibition, tumor suppression and hedgehog signaling inhibition can be determined by any methods known in the art.
- [0130] As used herein, a cell refers to any cell that can be obtained and used by a method described herein. For example, a cell may be obtained from a cell culture. Alternatively, a cell may be isolated from a subject. A cell may also refer to a cell of a subject.
- [0131] A cell may comprise loss of function of SNF5, ARID1A, ATRX, and/or a component of the SWI/SNF complex. Preferably, a cell may comprise a deletion of SNF5.
- [0132] A cell may be a cancer cell, where the cancer is selected from the group consisting of medulloblastoma, oligodendroglioma, ovarian clear cell adenocarcinoma, ovarian endomethrioid adenocarcinoma, ovarian serous adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic endocrine tumor, malignant rhabdoid tumor, astrocytoma, atypical teratoid rhabdoid tumor, choroid plexus carcinoma, choroid plexus papilloma, ependymoma, glioblastoma, meningioma, neuroglial tumor, oligoastrocytoma, oligodendroglioma, pineoblastoma, carcinosarcoma, chordoma, extragonadal germ cell tumor, extrarenal rhabdoid tumor, schwannoma, skin squamous cell carcinoma, chondrosarcoma, clear cell sarcoma of soft tissue, ewing sarcoma, gastrointestinal stromal tumor, osteosarcoma, rhabdomyosarcoma, epitheloid sarcoma, renal medullo carcinoma, diffuse large B-cell lymphoma, follicular lymphoma and not otherwise specified (NOS) sarcoma. More preferably a cell is a cancer cell of medulloblastoma, malignant rhabdoid tumor, or atypical teratoid rhabdoid tumor.
- [0133] 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.

- [0134] 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.
- [0135] 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.
- [0136] 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.
- [0137] 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 an active compound. For example, cancer monotherapy with one of the compound of the present invention, or a pharmaceutically acceptable salt, polymorph, solvate, analog or derivative thereof, to a subject in need of treatment of cancer. Monotherapy may be contrasted with combination therapy, in which a combination of multiple active compounds is administered, preferably with each component of the combination present in a therapeutically effective amount. In one aspect, monotherapy with a compound of the present invention, or a pharmaceutically acceptable salt, polymorph or solvate thereof, is more effective than combination therapy in inducing a desired biological effect.
- [0138] 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, polymorph or

solvate thereof, to alleviate one or more symptoms or complications of a disease, condition or disorder, or to eliminate the disease, condition or disorder. The term "treat" can also include treatment of a cell *in vitro* or an animal model.

[0139] A compound of the present invention, or a pharmaceutically acceptable salt, polymorph or solvate thereof, can also be used to prevent a disease, condition or disorder, or used to identify suitable candidates for such purposes. As used herein, "preventing" or "prevent" describes reducing or eliminating the onset of the symptoms or complications of the disease, condition or disorder.

[0140] 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.

[0141] 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).

[0142] 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.

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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 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.

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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, polymorph, solvate, 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.

[0153] 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, polymorph, solvate, 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 on 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.

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.

[0155] 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.

[0156] Treating cancer 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.

[0157] Treating cancer 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.

[0158] Treating cancer 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.

[0159] Treating cancer 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 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.

[0160] Treating cancer 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 50%; most 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.

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, 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, 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.

[0162] A compound of the present invention, or a pharmaceutically acceptable salt, 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, 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, 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.

[0163] A compound of the present invention, or a pharmaceutically acceptable salt, 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.

[0164] 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, 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, 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.

[0165] Administering a compound of the present invention, or a pharmaceutically acceptable salt, 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.

[0166] Detection of methylation of H3-K27, formation of trimethylated H3-K27, conversion of monomethylated H3-K27 to dimethylated H3-K27, or conversion of dimethylated H3-K27 to trimethylated H3-K27 can be accomplished using any suitable method. Exemplary methods can be found in US20120071418, the contents of which are incorporated herein by reference.

[0167] Administering a compound of the present invention, or a pharmaceutically acceptable salt, 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 methyltrasferase.

[0168] Preferably, an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, 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.

[0169] Contacting a cell with a compound of the present invention, or a pharmaceutically acceptable salt, 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, 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, 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, polymorph or solvate thereof, induces cell death selectively in one or more cells affected by a cell proliferative disorder.

[0170] 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.

[0171] A compound (i.e., an EZH2 inhibitor) that can be used in any methods described herein may have the following Formula I:

(I) or a pharmaceutically acceptable salt thereof; wherein

 R^{701} is H, F, OR^{707} , NHR^{707} , $-(C\equiv C)$ - $(CH_2)_{n7}$ - R^{708} , phenyl, 5- or 6-membered heteroaryl, C_{3-8} cycloalkyl, or 4-7 membered heteroaryl, C_{3-8} cycloalkyl or 4-7 membered heteroayl, C_{3-8} cycloalkyl or 4-7 membered heterocycloalkyl each independently is optionally substituted with one or more groups selected from halo, C_{1-3} alkyl, OH, $O-C_{1-6}$ alkyl, $NH-C_{1-6}$ alkyl, and, C_{1-3} alkyl substituted with C_{3-8} cycloalkyl or 4-7 membered heterocycloalkyl containing 1-3 heteroatoms, wherein each of the $O-C_{1-6}$ alkyl and $NH-C_{1-6}$ alkyl is optionally substituted with hydroxyl, $O-C_{1-3}$ alkyl or $NH-C_{1-3}$ alkyl, each of the $O-C_{1-3}$ alkyl and $NH-C_{1-3}$ alkyl being optionally further substituted with $O-C_{1-3}$ alkyl or $NH-C_{1-3}$ alkyl;

each of R^{702} and R^{703} , independently is H, halo, C_{1-4} alkyl, C_{1-6} alkoxyl or C_6 - C_{10} aryloxy, each optionally substituted with one or more halo;

each of R^{704} and R^{705} , independently is C_{1-4} alkyl;

 R^{706} is cyclohexyl substituted by $N(C_{1-4}$ alkyl)₂ wherein one or both of the C_{1-4} alkyl is substituted with C_{1-6} alkoxy; or R^{706} is tetrahydropyranyl;

 R^{707} is C_{1-4} alkyl optionally substituted with one or more groups selected from hydroxyl, C_{1-4} alkoxy, amino, mono- or di- C_{1-4} alkylamino, C_{3-8} cycloalkyl, and 4-7 membered heterocycloalkyl containing 1-3 heteroatoms, wherein the C_{3-8} cycloalkyl or 4-7 membered heterocycloalkyl each independently is further optionally substituted with C_{1-3} alkyl;

 R^{708} is $C_{1\text{--}4}$ alkyl optionally substituted with one or more groups selected from OH, halo, and $C_{1\text{--}4}$ alkoxy, 4-7 membered heterocycloalkyl containing 1-3 heteroatoms, or O-C₁₋₆ alkyl, wherein the 4-7 membered heterocycloalkyl can be optionally further substituted with OH or $C_{1\text{--}6}$ alkyl; and

 n_7 is 0, 1 or 2.

[0172] For example, R^{706} is cyclohexyl substituted by $N(C_{1-4}$ alkyl)₂ wherein one of the C_{1-4} alkyl is unsubstituted and the other is substituted with methoxy.

[0173] For example, R^{706} is

[0174] For example, the compound is of Formula II:

[0175] For example, R^{702} is methyl or isopropyl and R^{703} is methyl or methoxyl.

[0176] For example, R⁷⁰⁴ is methyl.

[0177] For example, R^{701} is OR^{707} and R^{707} is C_{1-3} alkyl optionally substituted with OCH_3 or morpholine.

[0178] For example, R^{701} is H or F.

[0179] For example, R^{701} is tetrahydropyranyl, phenyl, pyridyl, pyrimidyl, pyrazinyl, imidazolyl, or pyrazolyl, each of which is optionally substituted with methyl, methoxy, ethyl substituted with morpholine, or $-OCH_2CH_2OCH_3$.

[0180] For example, R^{708} is morpholine, piperidine, piperazine, pyrrolidine, diazepane, or azetidine, each of which is optionally substituted with OH or C_{1-6} alkyl.

[0181] For example, R⁷⁰⁸ is morpholine

[0182] For example, R^{708} is piperazine substituted with C_{1-6} alkyl.

[0183] For example, R^{708} is methyl, t-butyl or $C(CH_3)_2OH$.

[0184] A compound (i.e., an EZH2 inhibitor) that can be used in any methods described herein may have the following Formula III:

(III) or a pharmaceutically acceptable salt thereof.

[0185] In this formula:

 R^{801} is C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-8} cycloalkyl, 4-7 membered heterocycloalkyl containing 1-3 heteroatoms, phenyl or 5- or 6-membered heteroaryl, each of which is substituted with O- C_{1-6} alkyl- R_x or NH- C_{1-6} alkyl- R_x , wherein R_x is hydroxyl, O- C_{1-3} alkyl or NH- C_{1-3} alkyl, and R_x is optionally further substituted with O- C_{1-3} alkyl or NH- C_{1-3} alkyl except when R_x is hydroxyl; or R^{801} is phenyl substituted with $-Q_2-T_2$, wherein Q_2 is a bond or C_1-C_3 alkyl linker optionally substituted with halo, cyano, hydroxyl or C_1-C_6 alkoxy, and T_2 is optionally substituted 4- to 12-membered heterocycloalkyl; and R^{801} is optionally further substituted;

each of R^{802} and R^{803} , independently is H, halo, $C_{1\text{-}4}$ alkyl, $C_{1\text{-}6}$ alkoxyl or $C_6\text{-}C_{10}$ aryloxy, each optionally substituted with one or more halo;

each of R^{804} and R^{805} , independently is C_{1-4} alkyl; and

 R^{806} is $-Q_x$ - T_x , wherein Q_x is a bond or $C_{1\text{-}4}$ alkyl linker, T_x is H, optionally substituted $C_{1\text{-}4}$ alkyl, optionally substituted C_3 - C_8 cycloalkyl or optionally substituted 4- to 14-membered heterocycloalkyl.

[0186] For example, each of Q_x and Q_2 independently is a bond or methyl linker, and each of T_x and T_2 independently is tetrahydropyranyl, piperidinyl substituted by 1, 2, or 3 C_{1-4} alkyl groups, or cyclohexyl substituted by $N(C_{1-4}$ alkyl)₂ wherein one or both of the C_{1-4} alkyl is optionally substituted with C_{1-6} alkoxy;

[0187] For example, R^{806} is cyclohexyl substituted by $N(C_{1-4}$ alkyl)₂ or R^{806} is tetrahydropyranyl.

[0188] For example, R^{806} is

[0189] For example, R^{801} is phenyl or 5- or 6-membered heteroaryl substituted with O-C₁₋₆ alkyl-R_x, or R^{801} is phenylsubstituted with CH₂-tetrahydropyranyl.

[0190] For example, a compound of the present invention is of Formula IVa or IVb:

$$R^{802}$$
 R^{803}
 R^{804}
 R^{804}
 R^{804}
 R^{804}
 R^{804}
 R^{803}
 R^{803}

Z' is CH or N, and R^{807} is C_{2-3} alkyl- R_x .

 $[0191] \qquad \text{For example, } R^{807} \text{ is } -\text{CH}_2\text{CH}_2\text{OH, } -\text{CH}_2\text{CH}_2\text{OCH}_3, \text{ or-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3.$

[0192] For example, R^{802} is methyl or isopropyl and R^{803} is methyl or methoxyl.

[0193] For example, R⁸⁰⁴ is methyl.

[0194] A compound of the present invention may have the following Formula (V):

$$R_8$$
 R_{12}
 R_{12}
 R_{12}
 R_{12}
 R_{12}
 R_{12}
 R_{12}

(V), or a pharmaceutically acceptable salt or ester thereof.

[0195] In this formula:

 R_2 , R_4 and R_{12} are each, independently $C_{1\text{-}6}$ alkyl;

 R_6 is C_6 - C_{10} aryl or 5- or 6-membered heteroaryl, each of which is optionally substituted with one or more $-Q_2-T_2$, wherein Q_2 is a bond or C_1-C_3 alkyl linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy, and T₂ is H, halo, cyano, -OR_a, -NR_aR_b, $-(NR_aR_bR_c)^+A^-$, $-C(O)R_a$, $-C(O)OR_a$, $-C(O)NR_aR_b$, $-NR_bC(O)R_a$, $-NR_bC(O)OR_a$, $-S(O)_2R_a$, $-S(O)_2NR_aR_b$, or R_{S2} , in which each of R_a , R_b , and R_c , independently is H or R_{S3} , A^- is a pharmaceutically acceptable anion, each of R_{S2} and R_{S3}, independently, is C₁-C₆ alkyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 12-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, or R_a and R_b, together with the N atom to which they are attached, form a 4 to 12-membered heterocycloalkyl ring having 0 or 1 additional heteroatom, and each of R_{S2}, R_{S3}, and the 4 to 12-membered heterocycloalkyl ring formed by R_a and R_b, is optionally substituted with one or more $-Q_3$ - T_3 , wherein Q_3 is a bond or C_1 - C_3 alkyl linker each optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy, and T₃ is selected from the group consisting of halo, cyano, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 12-membered heterocycloalkyl, 5- or 6membered heteroaryl, OR_d, COOR_d, -S(O)₂R_d, -NR_dR_e, and -C(O)NR_dR_e, each of R_d and R_e independently being H or C_1 - C_6 alkyl, or $-Q_3$ - T_3 is oxo; or any two neighboring $-Q_2$ - T_2 , together with the atoms to which they are attached form a 5- or 6-membered ring optionally containing 1-4 heteroatoms selected from N, O and S and optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, COOH, C(O)O-C₁-C₆

alkyl, cyano, C_1 - C_6 alkoxyl, 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 12-membered heterocycloalkyl, and 5- or 6-membered heteroaryl;

 R_7 is $-Q_4$ - T_4 , in which Q_4 is a bond, C_1 - C_4 alkyl linker, or C_2 - C_4 alkenyl linker, each linker optionally substituted with halo, cyano, hydroxyl or C₁-C₆ alkoxy, and T₄ is H, halo, $cyano, NR_fR_g, -OR_f, -C(O)R_f, -C(O)OR_f, -C(O)NR_fR_g, -C(O)NR_fOR_g, -NR_fC(O)R_g, -S(O)_2R_f, -C(O)NR_fR_g, -C(O)NR_fOR_g, -NR_fC(O)R_g, -NR_fC(O)R_g, -NR_fC(O)R_f, -NR_fC(O)R_f, -NR_fC(O)R_f, -NR_fC(O)R_f, -NR_fC(O)R_f, -NR_fC(O)R_f, -NR_fC(O)R_g, -NR_fC(O)R_f, -NR_fC(O)R_f$ or R_{S4}, in which each of R_f and R_g, independently is H or R_{S5}, each of R_{S4} and R_{S5}, independently is 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 12-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, and each of R_{S4} and R_{S5} is optionally substituted with one or more $-Q_5$ - T_5 , wherein Q_5 is a bond, C(O), $C(O)NR_k$, $NR_kC(O)$, $S(O)_2$, or C_1 - C_3 alkyl linker, R_k being H or C_1 - C_6 alkyl, and T_5 is H, halo, C_1 - C_6 alkyl, hydroxyl, cyano, C₁-C₆ alkoxyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 12-membered heterocycloalkyl, 5- or 6-membered heteroaryl, or $S(O)_q R_q$ in which q is 0, 1, or 2 and R_q is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 12-membered heterocycloalkyl, or 5- or 6membered heteroaryl, and T₅ is optionally substituted with one or more substituents selected from the group consisting of halo, C₁-C₆ alkyl, hydroxyl, cyano, C₁-C₆ alkoxyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 12-membered heterocycloalkyl, and 5- or 6-membered heteroaryl except when T₅ is H, halo, hydroxyl, or cyano; or $-Q_5$ - T_5 is oxo; and

 R_8 is H, halo, hydroxyl, COOH, cyano, R_{S6} , OR $_{S6}$, or COOR $_{S6}$, in which R_{S6} is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_8 cycloalkyl, 4 to 12-membered heterocycloalkyl, amino, mono- C_1 - C_6 alkylamino, or di- C_1 - C_6 alkylamino, and R_{S6} is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, COOH, C(O)O- C_1 - C_6 alkyl, cyano, C_1 - C_6 alkoxyl, amino, mono- C_1 - C_6 alkylamino, and di- C_1 - C_6 alkylamino; or R_7 and R_8 , together with the N atom to which they are attached, form a 4 to 11-membered heterocycloalkyl ring having 0 to 2 additional heteroatoms, and the 4 to 11-membered heterocycloalkyl ring formed by R_7 and R_8 is optionally substituted with one or more $-Q_6$ - T_6 , wherein Q_6 is a bond, C(O), $C(O)NR_m$, $NR_mC(O)$, $S(O)_2$, or C_1 - C_3 alkyl linker, R_m being H or C_1 - C_6 alkyl, and T_6 is H, halo, C_1 - C_6 alkyl, hydroxyl, cyano, C_1 - C_6 alkoxyl, 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 12-membered heterocycloalkyl, 5- or 6-membered heteroaryl, or $S(O)_pR_p$ in which p is 0, 1, or 2 and R_p is 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 12-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, and T_6 is optionally substituted with one or more substituents selected from the group consisting of halo, C_1 - C_6 alkyl, hydroxyl, cyano, C_1 - C_6 alkoxyl, 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 12-membered heterocycloalkyl, and 5- or 6-membered heteroaryl except when T_6 is C_6 - $C_$

[0196] For example, R_6 is C_6 - C_{10} aryl or 5- or 6-membered heteroaryl, each of which is optionally, independently substituted with one or more $-Q_2$ - T_2 , wherein Q_2 is a bond or C_1 - C_3 alkyl linker, and T_2 is H, halo, cyano, $-OR_a$, $-NR_aR_b$,

-(NR_aR_bR_c)⁺A⁻, -C(O)NR_aR_b, -NR_bC(O)R_a, -S(O)₂R_a, or R_{S2}, in which each of R_a and R_b, independently is H or R_{S3}, each of R_{S2} and R_{S3}, independently, is C₁-C₆ alkyl, or R_a and R_b, together with the N atom to which they are attached, form a 4 to 7-membered heterocycloalkyl ring having 0 or 1 additional heteroatom, and each of R_{S2}, R_{S3}, and the 4 to 7-membered heterocycloalkyl ring formed by R_a and R_b, is optionally, independently substituted with one or more $-Q_3$ -T₃, wherein Q₃ is a bond or C₁-C₃ alkyl linker and T₃ is selected from the group consisting of halo, C₁-C₆ alkyl, 4 to 7-membered heterocycloalkyl, OR_d, -S(O)₂R_d, and -NR_dR_e, each of R_d and R_e independently being H or C₁-C₆ alkyl, or $-Q_3$ -T₃ is oxo; or any two neighboring $-Q_2$ -T₂, together with the atoms to which they are attached form a 5- or 6-membered ring optionally containing 1-4 heteroatoms selected from N, O and S.

[0197] For example, the compound of the present invention is of Formula (VI):

(VI)or a pharmaceutically acceptable salt

thereof, wherein Q_2 is a bond or methyl linker, T_2 is H, halo, $-OR_a$, $-NR_aR_b$, $-(NR_aR_bR_c)^+A^-$, or $-S(O)_2NR_aR_b$, R_7 is piperidinyl, tetrahydropyran, cyclopentyl, or cyclohexyl, each optionally substituted with one $-Q_5$ - T_5 and R_8 is ethyl.

[0198] A compound of the present invention may have the following Formula (VIa):

each of R_a and R_b , independently is H or R_{S3} , R_{S3} being C_1 - C_6 alkyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 12-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, or R_a and R_b , together with the N atom to which they are attached, form a 4 to 12-membered heterocycloalkyl ring having 0 or 1 additional heteroatom, and each of R_{S3} and the 4 to 12-membered heterocycloalkyl ring formed by R_a and R_b , is optionally substituted with one or more $-Q_3$ - T_3 , wherein Q_3 is a bond or C_1 - C_3 alkyl linker each optionally substituted with halo, cyano, hydroxyl or C_1 - C_6 alkoxy, and T_3 is selected from the group consisting of halo, cyano, C_1 - C_6 alkyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, 4 to 12-membered heterocycloalkyl, 5- or 6-membered heteroaryl, OR_d , $COOR_d$, $-S(O)_2R_d$, $-NR_dR_e$, and $-C(O)NR_dR_e$, each of R_d and R_e independently being H or C_1 - C_6 alkyl, or $-Q_3$ - T_3 is oxo;

 R_7 is $-Q_4$ - T_4 , in which Q_4 is a bond, C_1 - C_4 alkyl linker, or C_2 - C_4 alkenyl linker, each linker optionally substituted with halo, cyano, hydroxyl or C_1 - C_6 alkoxy, and T_4 is H, halo, cyano, NR_fR_g , $-OR_f$, $-C(O)R_f$, $-C(O)OR_f$, $-C(O)NR_fR_g$, $-C(O)NR_fOR_g$, $-NR_fC(O)R_g$, $-S(O)_2R_f$, or R_{S4} , in which each of R_f and R_g , independently is H or R_{S5} , each of R_{S4} and R_{S5} , independently is 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 7-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, and each of R_{S4} and R_{S5} is optionally substituted with one or more $-Q_5$ - T_5 , wherein Q_5 is a bond, C(O), $C(O)NR_k$, $NR_kC(O)$, $S(O)_2$, or C_1 - C_3 alkyl linker, R_k being H or C_1 - C_6 alkyl, and T_5 is H, halo, C_1 - C_6 alkyl, hydroxyl, cyano, C_1 - C_6 alkoxyl, 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 7-membered heterocycloalkyl, 5- or 6-membered heteroaryl, or $S(O)_qR_q$ in which q is 0, 1, or 2 and R_q is 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 7-membered heterocycloalkyl, or 5- or 6-membered heteroaryl, and T_5 is optionally substituted with one or more substituents selected from the group consisting of halo, C_1 - C_6 alkyl, hydroxyl, cyano, C_1 - C_6 alkoxyl, 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 7-membered heterocycloalkyl, and 5- or 6-membered heteroaryl except when T_5 is H, halo, hydroxyl, or cyano; or $-Q_5$ - T_5 is oxo; provided that R_7 is not H; and

R₈ is H, halo, hydroxyl, COOH, cyano, R_{S6}, OR_{S6}, or COOR_{S6}, in which R_{S6} is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, amino, mono-C₁-C₆ alkylamino, or di-C₁-C₆ alkylamino, and R_{S6} is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, COOH, C(O)O-C₁-C₆ alkyl, cyano, C₁-C₆ alkoxyl, amino, mono-C₁-C₆ alkylamino, and di-C₁-C₆ alkylamino; or R₇ and R₈, together with the N atom to which they are attached, form a 4 to 11-membered heterocycloalkyl ring which has 0 to 2 additional heteroatoms and is optionally substituted with one or more $-Q_6$ - T_6 , wherein Q_6 is a bond, C(O), C(O)NR_m, NR_mC(O), S(O)₂, or C₁-C₃ alkyl linker, R_m being H or C₁-C₆ alkyl, and T₆ is H, halo, C₁-C₆ alkyl, hydroxyl, cyano, C₁-C₆ alkoxyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, 5- or 6membered heteroaryl, or $S(O)_pR_p$ in which p is 0, 1, or 2 and R_p is C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C₂-C₆ alkynyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, or 5- or 6membered heteroaryl, and T₆ is optionally substituted with one or more substituents selected from the group consisting of halo, C₁-C₆ alkyl, hydroxyl, cyano, C₁-C₆ alkoxyl, amino, mono-C₁-C₆ alkylamino, di-C₁-C₆ alkylamino, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, 4 to 7-membered heterocycloalkyl, and 5- or 6-membered heteroaryl except when T₆ is H, halo, hydroxyl, or cyano; or $-Q_6$ - T_6 is oxo.

[0199] For example, R_a and R_b , together with the N atom to which they are attached, form a 4 to 7-membered heterocycloalkyl ring having 0 or 1 additional heteroatoms to the N atom and the ring is optionally substituted with one or more $-Q_3$ - T_3 , wherein the heterocycloalkyl is azetidinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, oxazolidinyl, isoxazolidinyl, triazolidinyl, piperidinyl, 1,2,3,6-tetrahydropyridinyl, piperazinyl, or morpholinyl.

[0200] For example, R_7 is C_3 - C_8 cycloalkyl or 4 to 7-membered heterocycloalkyl, each

[0201] For example, R_7 is piperidinyl, tetrahydropyran, tetrahydro-2H-thiopyranyl, cyclopentyl, cyclohexyl, pyrrolidinyl, or cycloheptyl, each optionally substituted with one or more $-Q_5$ - T_5 .

[0202] For example, R_8 is H or C_1 - C_6 alkyl which is optionally substituted with one or more substituents selected from the group consisting of halo, hydroxyl, COOH, C(O)O- C_1 - C_6 alkyl, cyano, C_1 - C_6 alkoxyl, amino, mono- C_1 - C_6 alkylamino, and di- C_1 - C_6 alkylamino.

[0203] In some embodiments, a compound that can be used in any methods presented here is:

(Compound A), stereoisomers thereof or pharmaceutically

acceptable salt or solvate thereof.

[0204] In some embodiments, a compound that can be used in any methods presented here is:

, stereoisomers thereof or pharmaceutically acceptable salts

and solvates thereof.

In some embodiments, a compound that can be used in any methods presented here [0205] is:

(F), stereoisomers thereof or pharmaceutically acceptable

salts and solvates thereof.

[0206] In some embodiments, the compounds suitable for use in the method of this invention include compounds of Formula (VII):

wherein,

 V^1 is N or CR^7 ,

 V^2 is N or CR^2 , provided when V^1 is N, V^2 is N,

X and Z are selected independently from the group consisting of hydrogen, (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, unsubstituted or substituted (C_3-C_8) cycloalkyl, unsubstituted or substituted (C_5-C_8) cycloalkenyl, unsubstituted or substituted (C_5-C_8) cycloalkenyl, (C_6-C_{10}) bicycloalkyl, unsubstituted or substituted heterocycloalkyl, unsubstituted or substituted heterocycloalkyl, unsubstituted or substituted aryl, unsubstituted or substituted aryl, unsubstituted or substituted aryl, unsubstituted or substituted aryl, unsubstituted

or substituted heteroaryl, unsubstituted or substituted heteroaryl- $(C_1$ - $C_8)$ alkyl or - $(C_2$ - $C_8)$ alkenyl, halo, cyano,

 $-COR^a, -CO_2R^a, -CONR^aR^b, -CONR^aNR^aR^b, -SR^a, -SOR^a, -SO_2R^a, -SO_2NR^aR^b, nitro, -NR^aR^b, -NR^aC(O)R^b, -NR^aC(O)NR^aR^b, -NR^aC(O)OR^a, -NR^aSO_2R^b, -NR^aSO_2NR^aR^b, -NR^aNR^aR^b, -NR^aNR^aC(O)R^b, -NR^aNR^aC(O)NR^aR^b, -NR^aNR^aC(O)OR^a, -OR^a, -OC(O)R^a, and -OC(O)NR^aR^b;$

Y is H or halo;

 R^1 is (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, unsubstituted or substituted (C_3-C_8) cycloalkyl, unsubstituted or substituted (C_3-C_8) cycloalkyl- (C_1-C_8) alkyl or - (C_2-C_8) alkenyl, unsubstituted or substituted (C_5-C_8) cycloalkenyl, unsubstituted or substituted (C_5-C_8) cycloalkenyl- (C_1-C_8) alkyl or - (C_2-C_8) alkenyl, unsubstituted or substituted (C_6-C_{10}) bicycloalkyl, unsubstituted or substituted heterocycloalkyl or - (C_2-C_8) alkenyl, unsubstituted or substituted aryl, unsubstituted or substituted aryl- (C_1-C_8) alkyl or - (C_2-C_8) alkenyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted heteroaryl- (C_1-C_8) alkyl or - (C_2-C_8) alkenyl, - (C_2-C_8) alkenyl, - (C_2-C_8) alkenyl, - (C_3-C_8) alkenyl

 R^2 is hydrogen, (C_1-C_8) alkyl, trifluoromethyl, alkoxy, or halo, in which said (C_1-C_8) alkyl is optionally substituted with one to two groups selected from amino and (C_1-C_3) alkylamino;

 R^7 is hydrogen, (C_1-C_3) alkyl, or alkoxy;

 $R^3 \ is \ hydrogen, \ (C_1\text{-}C_8) alkyl, \ cyano, \ trifluoromethyl, \ \text{-}NR^aR^b, \ or \ halo;$

 R^6 is selected from the group consisting of hydrogen, halo, (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) alkynyl, unsubstituted or substituted (C_3-C_8) cycloalkyl, unsubstituted or substituted (C_3-C_8) cycloalkyl- (C_1-C_8) alkyl, unsubstituted or substituted (C_5-C_8) cycloalkenyl, unsubstituted or substituted or substituted or substituted or substituted heterocycloalkyl, unsubstituted or substituted heterocycloalkyl, unsubstituted or substituted heterocycloalkyl, unsubstituted or substituted aryl- (C_1-C_8) alkyl, unsubstituted or substituted or substituted or substituted heteroaryl, unsubstituted or substituted heteroaryl- (C_1-C_8) alkyl, cyano, $-COR^a$, $-CO_2R^a$,

 $-CONR^aR^b, -CONR^aNR^aR^b, -SR^a, -SOR^a, -SO_2R^a, -SO_2NR^aR^b, nitro, -NR^aR^b, -NR^aC(O)R^b, \\$

 $-NR^aC(O)NR^aR^b, -NR^aC(O)OR^a, -NR^aSO_2R^b, -NR^aSO_2NR^aR^b, -NR^aNR^aR^b, -NR^aNR^aC(O)R^b, \\ -NR^aNR^aC(O)NR^aR^b, -NR^aNR^aC(O)OR^a, -OR^a, -OC(O)R^a, -OC(O)NR^aR^b;$

wherein any (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkynyl, cycloalkyl, cycloalkenyl, bicycloalkyl, heterocycloalkyl, aryl, or heteroaryl group is optionally substituted by 1, 2 or 3 groups independently selected from the group consisting of $-O(C_1-C_6)$ alkyl $(R^c)_{1-2}$, $-S(C_1-C_6)$ C_6)alkyl(R^c)₁₋₂, -(C_1 - C_6)alkyl(R^c)₁₋₂, -(C_1 - C_8)alkyl-heterocycloalkyl, (C_3 - C_8)cycloalkylheterocycloalkyl, halo, (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, -COR^a, -CO₂R^a, -CONR^aR^b, -SR^a, -SOR^a, -SO₂R^a, -SO₂NR^aR^b, nitro, -NR^aR^b, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, $OC(O)NR^aR^b$, heterocycloalkyl, aryl, heteroaryl, aryl(C_1 - C_4)alkyl, and heteroaryl(C_1 - C_4)alkyl; wherein any aryl or heteroaryl moiety of said aryl, heteroaryl, $aryl(C_1-C_4)alkyl$, or heteroaryl(C_1 - C_4)alkyl is optionally substituted by 1, 2 or 3 groups independently selected from the group consisting of halo, (C₁-C₆)alkyl, (C₃-C₈)cycloalkyl, (C₅-C₈)cycloalkenyl, (C₁-C₆)haloalkyl, cyano, -COR^a, -CO₂R^a, -CONR^aR^b,-SR^a, -SOR^a., -SO₂R^a, -SO₂NR^aR^b, nitro, -NR^aR^b, -NR^aC(O)R^b,-NR^aC(O)NR^aR^b, -NR^aC(O)OR^a, -NR^aSO2R^b, -NR^aSO₂NR^aR^b, -OR^a, -OC(O)R^a, and -OC(O)NR^aR^b;

 $R^a \ and \ R^b \ are each independently hydrogen, (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, (C_3-C_8)cycloalkyl, (C_5-C_8)cycloalkenyl, (C_6-C_{10})bicycloalkyl, heterocycloalkyl, aryl, or heteroaryl, wherein said (C_1-C_8)alkyl, (C_2-C_8)alkenyl, (C_2-C_8)alkynyl, cycloalkyl, cycloalkyl, bicycloalkyl,heterocycloalkyl, aryl or heteroaryl group is optionally substituted by 1, 2 or 3 groups independently selected from halo, hydroxyl, (C_1-C_4)alkoxy, amino, (C_1-C_4)alkylamino, ((C_1-C_4)alkyl)((C_1-C_4)alkyl)amino, -CO_2H, -CO_2(C_1-C_4)alkyl, -CONH_2, -CONH(C_1-C_4)alkyl, (C_1-C_4)alkyl,$

 $-CON((C_1-C_4)alkyl)((C_1-C_4)alkyl), -SO_2(C_1-C_4)alkyl, -SO_2NH_2, -SO_2NH(C_1-C_4)alkyl, and \\SO_2N((C_1-C_4)alkyl)((C_1-C_4)alkyl); \\$

or R^a and R^b taken together with the nitrogen to which they are attached represent a 5-8 membered saturated or unsaturated ring, optionally containing an additional heteroatom selected from oxygen, nitrogen, and sulfur, wherein said ring is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_4) alkyl, (C_1-C_4) haloalkyl, amino, (C_1-C_4)

 C_4)alkylamino, ((C_1 - C_4)alkyl)((C_1 - C_4)alkyl)amino, hydroxyl, oxo, (C_1 - C_4)alkoxy, and (C_1 - C_4)alkoxy(C_1 - C_4)alkyl, wherein said ring is optionally fused to a (C_3 - C_8)cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring;

or R^a and R^b taken together with the nitrogen to which they are attached represent a 6-to 10-membered bridged bicyclic ring system optionally fused to a (C₃-C₈)cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring;

each R^c is independently (C_1-C_4) alkylamino, $-NR^aSO2R^b$, $-SOR^a$., $-SO_2R^a$, $-NR^aC(O)OR^a$, $-NR^aR^b$, or $-CO_2R^a$;

or a salt thereof.

Subgroups of the compounds encompassed by the general structure of Formula (I) are represented as follows:

Subgroup A of Formula (VII)

X and Z are selected from the group consisting of (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, heterocycloalkyl, aryl, heteroaryl, $-NR^aR^b$, and $-OR^a$;

Y is H or F;

 R^1 is selected from the group consisting of (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, heterocycloalkyl, aryl, and heteroaryl;

 R^2 is hydrogen, (C₁-C₈)alkyl, trifluoromethyl, alkoxy, or halo, in which said (C₁-C₈)alkyl is optionally substituted with one to two groups selected from amino and (C₁-C₃)alkylamino;

 R^7 is hydrogen, (C₁-C₃)alkyl, or alkoxy;

 R^3 is selected from the group consisting of hydrogen, (C₁-C₈)alkyl, cyano, trifluoromethyl,-NR^aR^b, and halo;

 R^6 is selected from the group consisting of hydrogen, halo, cyano, trifluoromethyl, amino, (C₁-C₈)alkyl, (C₃-C₈)cycloalkyl;, aryl, heteroaryl, acylamino; (C₂-C₈)alkynyl, arylalkynyl, heteroarylalkynyl; -SO₂R^a; -SO₂NR^aR^b and -NR^aSO₂R^b;

wherein any (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_8) alkynyl, arylalkynyl, heteroarylalkynyl group is optionally substituted by 1, 2 or 3 groups independently selected from $-O(C_1-C_6)$ alkyl $(R^c)_{1-2}$, $-S(C_1-C_6)$ alkyl $(R^c)_{1-2}$, $-(C_1-C_6)$ alkyl $(R^c)_{1-2}$, $-(C_1-C_6)$ alkyl $(R^c)_{1-2}$, $-(C_1-C_8)$ alkyl-heterocycloalkyl, (C_3-C_8) cycloalkyl-heterocycloalkyl, halo, (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl,

> (C₁-C₆)haloalkyl, cvano. -COR^a, -CO₂R^a, -CONR^aR^b, -SR^a, -SOR^a, -SO₂R^a, -SO₂NR^aR^b, nitro, -NR^aR^b, -NR^aC(O)R^b, -NR^aC(O)NR^aR^b, -NR^aC(O)OR^a, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, $-OC(O)NR^aR^b$, heterocycloalkyl, aryl, heteroaryl, aryl(C_1 - C_4)alkyl, and heteroaryl(C_1 - C_4)alkyl;

 R^a and R^b are each independently hydrogen, (C_1-C_8) alkyl, (C_2-C_8) alkenyl, (C_2-C_8) C₈)alkynyl, (C₃-C₈)cycloalkyl, (C₅-C₈)cycloalkenyl, (C₆-C₁₀)bicycloalkyl, heterocycloalkyl, aryl, or heteroaryl, wherein said (C₁-C₈)alkyl, (C₂-C₈)alkenyl, (C₂-C₈)alkynyl, cycloalkyl, cycloalkenyl, bicycloalkyl, heterocycloalkyl ,aryl or heteroaryl group is optionally substituted by 1, 2 or 3 groups independently selected from halo, hydroxyl, (C₁-C₄)alkoxy, amino, (C₁- C_4)alkylamino, $((C_1-C_4)alkyl)((C_1-C_4)alkyl)$ amino, $-CO_2H$, $-CO_2(C_1-C_4)alkyl$, $-CONH_2$, - $CONH(C_1-C_4)alkyl$, $-CON((C_1-C_4)alkyl)((C_1-C_4)alkyl)$, $-SO_2(C_1-C_4)alkyl$, $-SO_2NH_2$, $SO_2NH(C_1-C_4)$ alkyl, and

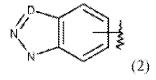
-SO₂N((C₁-C₄)alkyl)((C₁-C₄)alkyl);

or Ra and Rb taken together with the nitrogen to which they are attached represent a 5-8 membered saturated or unsaturated ring, optionally containing an additional heteroatom selected from oxygen, nitrogen, and sulfur, wherein said ring is optionally substituted by 1, 2 or 3 groups independently selected from (C₁-C₄)alkyl, (C₁-C₄)haloalkyl, amino, (C₁- C_4)alkylamino, ((C_1 - C_4)alkyl)((C_1 - C_4)alkyl)amino, hydroxyl, oxo, (C_1 - C_4)alkoxy, and (C_1 - C_4)alkoxy(C_1 - C_4)alkyl, wherein said ring is optionally fused to a (C_3 - C_8)cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring;

or R^a and R^b taken together with the nitrogen to which they are attached represent a 6to 10-membered bridged bicyclic ring system optionally fused to a (C₃-C₈)cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring. An aryl or heteroaryl group in this particular subgroup A is selected independently from the group consisting of furan, thiophene, pyrrole, oxazole, thiazole, imidazole, pyrazole, oxadiazole, thiadiazole, triazole, tetrazole, benzofuran, benzothiophene, benzoxazole, benzothiazole, phenyl, pyridine, pyridazine, pyrimidine, pyrazine, triazine, tetrazine, quinoline, cinnoline, quinazoline, quinoxaline, and naphthyridine or another aryl or heteroaryl group as follows:

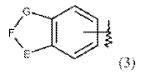
wherein in (1),

A is O, NH, or S; B is CH or N, and C is hydrogen or C₁-C₈ alkyl; or



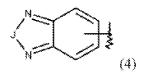
wherein in (2),

D is N or C optionally substituted by hydrogen or C₁-C₈ alkyl; or



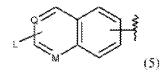
wherein in (3),

E is NH or CH₂; F is O or CO; and G is NH or CH₂; or



wherein in (4),

J is O, S or CO; or



wherein in (5),

Q is CH or N;

M is CH or N; and

 $L/(5) \quad \text{is hydrogen, halo, amino, cyano, } (C_1-C_8)\text{alkyl, } (C_3-C_8)\text{cycloalkyl, } -\text{COR}^a, -\text{CO}_2\text{R}^a, -\text{CONR}^a\text{R}^b, -\text{CONR}^a\text{NR}^a\text{R}^b, -\text{SO}_2\text{R}^a, -\text{SO}_2\text{NR}^a\text{R}^b, -\text{NR}^a\text{C}(O)\text{R}^b, -\text{NR}^a\text{SO}_2\text{R}^b, \\ -\text{NR}^a\text{SO}_2\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{NR}^a\text{R}^b, \text{or } -\text{OR}^a, \\ -\text{NR}^a\text{SO}_2\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{NR}^a\text{R}^b, \text{or } -\text{OR}^a, \\ -\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^b, -\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^a\text{C}(O)\text{NR}^a, -\text{NR}^a\text{NR}^a\text{NR}^a\text{C}(O)\text{NR}^a, -\text{NR}^a\text{NR}^a\text{NR}^a, -\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^a\text{NR}^a, -\text{NR}^a\text{NR}$

wherein any (C_1-C_8) alkyl or (C_3-C_8) cycloalkyl group is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$,

 $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, and $-OC(O)NR^aR^b$; wherein R^a and R^b are defined as above; or

wherein in (6),

L/(6) is NH or CH_2 ; or

wherein in 7,

 $M/(7) \ is \ hydrogen, \ halo, \ amino, \ cyano, \ (C_1-C_8)alkyl, \ (C_3-C_8)cycloalkyl,$ $heterocycloalkyl, \ -COR^a, \ -CO_2R^a, \ -CONR^aR^b, \ -CONR^aNR^aR^b, \ -SO_2R^a, \ -SO_2NR^aR^b,$ $-NR^aR^b, \ -NR^aC(O)R^b, -NR^aSO_2R^b, \ -NR^aSO_2NR^aR^b, \ -NR^aNR^aR^b, \ -NR^aNR^aC(O)R^b,$ $-NR^aNR^aC(O)NR^aR^b, \ or \ -OR^a,$

wherein any (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, or heterocycloalkyl group is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, $-SO_2R^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, and $-OC(O)NR^aR^b$; wherein R^a and R^b are defined as above; or

wherein in (8),

P is CH_2 , NH, O, or S; Q/(8) is CH or N; and n is 0-2; or

wherein in (9),

S/(9) and T/(9) is C, or S/(9) is C and T/(9) is N, or S/(9) is N and T/(9) is C;

R is hydrogen, amino, methyl, trifluoromethyl, or halo;

 $\label{eq:continuous} U \ is \ hydrogen, \ halo, \ amino, \ cyano, \ nitro, \ trifluoromethyl, \ (C_1-C_8)alkyl, \ (C_3-C_8)cycloalkyl, \ -COR^a, \ -CO_2R^a, \ -CONR^aR^b, \ -SO_2R^a, \ -SO_2NR^aR^b, \ -NR^aR^b, \ -NR^aC(O)R^b, -NR^aSO_2R^b,$

-NR^aSO₂NR^aR^b, -NR^aNR^aR^b, -NR^aNR^aC(O)R^b, -OR^a, or 4-(1H-pyrazol-4-y1),

wherein any (C_1-C_8) alkyl or (C_3-C_8) cycloalkyl group is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, SOR^a , $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, and $-OC(O)NR^aR^b$; wherein R^a and R^b are defined as above.

Subgroup B of Formula (VII)

X and Z are selected independently from the group consisting of (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, heterocycloalkyl, aryl, heteroaryl, $-NR^aR^b$, and $-OR^a$;

Y is H;

 R^1 is (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, or heterocycloalkyl;

 R^2 is hydrogen, (C_1-C_3) alkyl, or halo, in which said (C_1-C_3) alkyl is optionally substituted with one to two groups selected from amino and (C_1-C_3) alkylamino;

 R^7 is hydrogen, (C_1-C_3) alkyl, or alkoxy;

R³ is hydrogen, (C₁-C₈)alkyl or halo;

 $R^6 \ is \ hydrogen, \ halo, \ cyano, \ trifluoromethyl, \ amino, \ (C_1-C_8)alkyl, \ (C_3-C_8)cycloalkyl, \ aryl, \ heteroaryl, \ acylamino; \ (C_2-C_8)alkynyl, \ arylalkynyl, \ heteroarylalkynyl, \ -SO_2R^a, \ -SO_2NR^aR^b, \ or$

 $-NR^aSO_2R^b$;

wherein any (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, (C_2-C_8) alkynyl, arylalkynyl, or heteroarylalkynyl group is optionally substituted by 1, 2 or 3 groups independently selected from halo, (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^aR^b$,

$$\begin{split} & \text{nitro, -NR}^aR^b, \text{-NR}^aC(O)R^b, \text{-NR}^aC(O)NR^aR^b, \text{-NR}^aC(O)OR^a, \text{-NR}^aSO_2R^b,} \\ & \text{-NR}^aSO_2NR^aR^b, \text{-OR}^a, \text{-OC}(O)R^a, \text{-OC}(O)NR^aR^b, \text{heterocycloalkyl, aryl, heteroaryl,} \\ & \text{aryl}(C_1\text{-}C_4)\text{alkyl, and heteroaryl}(C_1\text{-}C_4)\text{alkyl;} \end{split}$$

 R^a and R^b are each independently hydrogen, $(C_1\text{-}C_8)$ alkyl, $(C_2\text{-}C_8)$ alkenyl, $(C_3\text{-}C_8)$ cycloalkyl, $(C_5\text{-}C_8)$ cycloalkenyl, $(C_6\text{-}C_{10})$ bicycloalkyl, heterocycloalkyl, aryl, or heteroaryl, wherein said $(C_1\text{-}C_8)$ alkyl, $(C_2\text{-}C_8)$ alkenyl, $(C_2\text{-}C_8)$ alkynyl, cycloalkyl, cycloalkyl, bicycloalkyl, heterocycloalkyl ,aryl or heteroaryl group is optionally substituted by 1, 2 or 3 groups independently selected from halo, hydroxyl, $(C_1\text{-}C_4)$ alkoxy, amino, $(C_1\text{-}C_4)$ alkylamino, $((C_1\text{-}C_4)$ alkyl) $((C_1\text{-}C_4)$ alkyl)amino, $(C_1\text{-}C_4)$ alkyl, $(C_1\text{-}C_4)$ alkyl,

 $-CON((C_1-C_4)alkyl)((C_1-C_4)alkyl), -SO_2(C_1-C_4)alkyl, -SO_2NH_2, -SO_2NH(C_1-C_4)alkyl, and -SO_2N((C_1-C_4)alkyl)((C_1-C_4)alkyl);\\$

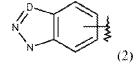
or R^a and R^b taken together with the nitrogen to which they are attached represent a 5-8 membered saturated or unsaturated ring, optionally containing an additional heteroatom selected from oxygen, nitrogen, and sulfur, wherein said ring is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_4) alkyl, (C_1-C_4) haloalkyl, amino, (C_1-C_4) alkylamino, $((C_1-C_4)$ alkyl) $((C_1-C_4)$ alkyl)amino, hydroxyl, oxo, (C_1-C_4) alkoxy, and (C_1-C_4) alkoxy (C_1-C_4) alkyl, wherein said ring is optionally fused to a (C_3-C_8) cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring;

or R^a and R^b taken together with the nitrogen to which they are attached represent a 6-to 10-membered bridged bicyclic ring system optionally fused to a (C₃-C₈)cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring. Aryl and heteroaryl in this definition are selected from the group consisting of furan, thiophene, pyrrole, oxazole, thiazole, imidazole, pyrazole, oxadiazole, thiadiazole, triazole, tetrazole, benzofuran, benzothiophene, benzoxazole, benzothiazole, phenyl, pyridine, pyridazine, pyrimidine, pyrazine, triazine, tetrazine, quinoline, cinnoline, quinazoline, quinoxaline, and naphthyridine or a compound of another aryl or heteroaryl group as follows:



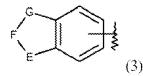
wherein in (1),

A is O, NH, or S; B is CH or N, and C is hydrogen or C₁-C₈ alkyl; or



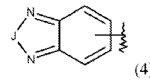
wherein in (2),

D is N or C optionally substituted by hydrogen or C₁-C₈ alkyl; or



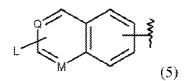
wherein in (3),

E is NH or CH₂; F is O or CO; and G is NH or CH₂; or



wherein in (4),

J is O, S or CO; or



wherein in (5),

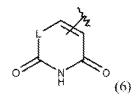
Q is CH or N;

M is CH or N; and

 $L/(5) \text{ is hydrogen, halo, amino, cyano, } (C_1-C_8)\text{alkyl, } (C_3-C_8)\text{cycloalkyl, } -\text{COR}^a, -\text{CO}_2\text{R}^a, \\ -\text{CONR}^a\text{R}^b, -\text{CONR}^a\text{NR}^a\text{R}^b, -\text{SO}_2\text{R}^a, -\text{SO}_2\text{NR}^a\text{R}^b, -\text{NR}^a\text{R}^b, -\text{NR}^a\text{C}(O)\text{R}^b, -\text{NR}^a\text{SO}_2\text{R}^b, \\ -\text{NR}^a\text{SO}_2\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{R}^b, -\text{NR}^a\text{NR}^a\text{C}(O)\text{NR}^a\text{R}^b, \text{ or } -\text{OR}^a, \\ \end{array}$

wherein any (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, group is optionally substituted by 1,2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, and $-OC(O)NR^aR^b$,

wherein R^a and R^b are defined as above; or



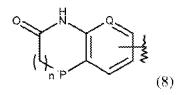
wherein in (6),

L/(6) is NH or CH_2 ; or

wherein in (7),

 $M/(7) \ is \ hydrogen, \ halo, \ amino, \ cyano, \ (C_1-C_8)alkyl, \ (C_3-C_8)cycloalkyl, \\ heterocycloalkyl, \ -COR^a, \ -CO_2R^a, \ -CONR^aR^b, \ -CONR^aNR^aR^b, \ -SO_2R^a, \ -SO_2NR^aR^b, \\ -NR^aR^b, \ -NR^aC(O)R^b, -NR^aSO_2R^b, \ -NR^aSO_2NR^aR^b, \ -NR^aNR^aR^b, \ -NR^aNR^aC(O)R^b, \\ -NR^aNR^aC(O)NR^aR^b, \ or \ -OR^a, \\ \end{cases}$

wherein any (C_1-C_8) alkyl, (C_3-C_8) cycloalkyl, heterocycloalkyl group is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SR^a$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, $-OC(O)NR^aR^b$; wherein R^a and R^b are defined as above; or



wherein in (8),

P is CH_2 , NH, O, or S; Q/(8) is CH or N; and n is 0-2; or

wherein in (9),

S/(9) and T/(9) is C, or S/(9) is C and T/(9) is N, or S/(9) is N and T/(9) is C;

R is hydrogen, amino, methyl, trifluoromethyl, halo;

 $\label{eq:continuous} U \ is \ hydrogen, \ halo, \ amino, \ cyano, \ nitro, \ trifluoromethyl, \ (C_1-C_8)alkyl, \ (C_3-C_8)cycloalkyl, \ -COR^a, \ -CO_2R^a, \ -CONR^aR^b, \ -SO_2R^a, \ -SO_2NR^aR^b, \ -NR^aR^b, \ -NR^aC(O)R^b, -NR^aSO_2R^b,$

-NR^aSO₂NR^aR^b, -NR^aNR^aR^b, -NR^aNR^aC(O)R^b, -OR^a, or 4-(1H-pyrazol-4-y1),

wherein any (C_1-C_8) alkyl, or (C_3-C_8) cycloalkyl group is optionally substituted by 1, 2 or 3 groups independently selected from (C_1-C_6) alkyl, (C_3-C_8) cycloalkyl, (C_5-C_8) cycloalkenyl, (C_1-C_6) haloalkyl, cyano, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, nitro, $-NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)NR^aR^b$, $-NR^aC(O)OR^a$, $-NR^aSO_2R^b$, $-NR^aSO_2NR^aR^b$, $-OR^a$, $-OC(O)R^a$, and $-OC(O)NR^aR^b$, wherein Ra and Rb are defined as above.

[0207] In some embodiments, the EZH2 inhibitor is:

(G), stereoisomers thereof or pharmaceutically

acceptable salt or solvate thereof.

[0208] In some embodiments, the EZH2 inhibitor is

(H), stereoisomers thereof or pharmaceutically acceptable

salt or solvate thereof.

[0209] The compounds described herein can be synthesized according to any method known in the art. For example, the compounds having the Formula (VII) can be synthesized according to the method described in WO 2011/140325; WO 2011/140324; and WO 2012/005805, each of which is incorporated by reference in its entirety.

[0210] As used herein, "alkyl", " C_1 , C_2 , C_3 , C_4 , C_5 or C_6 alkyl" or " C_1 - C_6 alkyl" is intended to include C_1 , C_2 , C_3 , C_4 , C_5 or C_6 straight chain (linear) saturated aliphatic hydrocarbon groups and C_3 , C_4 , C_5 or C_6 branched saturated aliphatic hydrocarbon groups. For example, C_1 - C_6 alkyl is intended to include C_1 , C_2 , C_3 , C_4 , C_5 and C_6 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.

[0211] In certain embodiments, a straight chain or branched alkyl has six or fewer carbon atoms (e.g., C_1 - C_6 for straight chain, C_3 - C_6 for branched chain), and in another embodiment, a straight chain or branched alkyl has four or fewer carbon atoms.

[0212] As used herein, the term "cycloalkyl" refers to a saturated or unsaturated nonaromatic hydrocarbon mono-or multi-ring (e.g., fused, bridged, or spiro rings) 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, cyclopentyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, and adamantyl. The term "heterocycloalkyl" refers to a saturated or unsaturated nonaromatic 3-8 membered monocyclic, 7-12 membered bicyclic (fused, bridged, or spiro rings), or 11-14 membered tricyclic ring system (fused, bridged, or spiro rings) having one or more heteroatoms (such as O, N, S, or Se), unless specified otherwise. Examples of heterocycloalkyl groups include, but are not limited to,piperidinyl, piperazinyl, pyrrolidinyl, dioxanyl, tetrahydrofuranyl, isoindolinyl, indolinyl, imidazolidinyl, pyrazolidinyl,

oxazolidinyl, isoxazolidinyl, triazolidinyl, tetrahyrofuranyl, oxiranyl, azetidinyl, oxetanyl, thietanyl, 1,2,3,6-tetrahydropyridinyl, tetrahydropyranyl, dihydropyranyl, pyranyl, morpholinyl, 1,4-diazepanyl, 1,4-oxazepanyl, 2-oxa-5-azabicyclo[2.2.1]heptanyl, 2,5-diazabicyclo[2.2.1]heptanyl, 2-oxa-6-azaspiro[3.3]heptanyl, 2,6-diazaspiro[3.3]heptanyl, 1,4-dioxa-8-azaspiro[4.5]decanyl and the like.

- [0213] 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, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, 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.
- [0214] An "arylalkyl" or an "aralkyl" moiety isan alkyl substituted with an aryl (*e.g.*, phenylmethyl (benzyl)). An "alkylaryl" moiety isan aryl substituted with an alkyl (*e.g.*, methylphenyl).
- [0215] As used herein, "alkyl linker" is intended to include C_1 , C_2 , C_3 , C_4 , C_5 or C_6 straight chain (linear) saturated divalent aliphatic hydrocarbon groups and C_3 , C_4 , C_5 or C_6 branched saturated aliphatic hydrocarbon groups. For example, C_1 - C_6 alkyl linker is intended to include C_1 , C_2 , C_3 , C_4 , C_5 and C_6 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) $_2$ CH₂-), n-pentyl (-CH₂CH₂CH₂CH₂CH₂-), s-pentyl (-CHCH₃CH₂CH₂CH₂-) or n-hexyl (-CH₂CH₂CH₂CH₂CH₂-).
- [0216] "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., C_2 - C_6 for straight chain, C_3 - C_6 for branched chain). The term " C_2 - C_6 " includes alkenyl groups containing two to six carbon atoms. The term " C_3 - C_6 " includes alkenyl groups containing three to six carbon atoms.

[0217] 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, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, 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, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

"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.

[0219] 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, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylcarbonylamino, carbamoyl 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.

[0220] 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. For example, substituted heterocycloalkyl includes those substituted with one or more alkyl groups, such as 2,2,6,6-tetramethyl-piperidinyl and 2,2,6,6-tetramethyl-1,2,3,6-tetrahydropyridinyl.

[0221] "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.

[0222] "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-, 6-, or 7-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.

[0223] Examples of heteroaryl groups include pyrrole, furan, thiophene, thiazole, isothiazole, imidazole, triazole, tetrazole, pyrazole, oxazole, isoxazole, pyridine, pyridine, pyridine, and the like.

[0224] 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.

[0225] 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.

[0226] The cycloalkyl, heterocycloalkyl, aryl, or heteroaryl ring can be substituted at one or more ring positions (e.g., the ring-forming carbon or heteroatom such as N) with such substituents as described above, for example, alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkylaminocarbonyl, aralkylaminocarbonyl, alkenylaminocarbonyl, alkylcarbonyl, arylcarbonyl, aralkylcarbonyl, alkenylcarbonyl, alkoxycarbonyl, 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 and heteroaryl 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).

[0227] 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. Carbocycle includes cycloalkyl and aryl. 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, cycloheptenyl, adamantyl, cyclooctyl, cyclooctenyl, cyclooctadienyl, fluorenyl, phenyl, naphthyl, indanyl, adamantyl 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.

[0228] As used herein, "heterocycle" or "heterocyclic group" includes any ring structure (saturated, unsaturated, or aromatic) which contains at least one ring heteroatom (*e.g.*, N, O or S). Heterocycle includes heterocycloalkyl and heteroaryl. Examples of heterocycles include, but are not limited to, morpholine, pyrrolidine, tetrahydrothiophene, piperidine, piperazine, oxetane, pyran, tetrahydropyran, azetidine, and tetrahydrofuran.

Examples of heterocyclic groups include, but are not limited to, acridinyl, azocinyl, [0229] 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, 1*H*-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, 1,2,4oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,4-oxadiazol5(4H)-one, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenazinyl, phenoxathinyl, phenoxazinyl, phenoxazinyl, piperazinyl, piperidinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyrazyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4thiadiazolyl, thianthrenyl, thiazolyl, thienolyl, thienothiazolyl, thienoxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl and xanthenyl.

[0230] 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.

[0231] 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.

[0232] When any variable $(e.g., R_1)$ 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 R_1 moieties, then the group may optionally be substituted with up to two R_1 moieties and R_1 at each occurrence is selected independently from the definition of R_1 . Also, combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

- [0233] The term "hydroxy" or "hydroxyl" includes groups with an -OH or -O.
- [0234] 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 alkoxyl substituted with one or more halogen atoms.
- [0235] 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.
- [0236] The term "carboxyl" refers to -COOH or its C_1-C_6 alkyl ester.
- [0237] "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, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, 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.

[0238] "Aroyl" includes moieties with an aryl or heteroaromatic moiety bound to a carbonyl group. Examples of aroyl groups include phenylcarboxy, naphthyl carboxy, etc.

[0239] "Alkoxyalkyl," "alkylaminoalkyl," and "thioalkoxyalkyl" include alkyl groups, as described above, wherein oxygen, nitrogen, or sulfur atoms replace one or more hydrocarbon backbone carbon atoms.

The term "alkoxy" or "alkoxyl" includes substituted and unsubstituted alkyl, T02401 alkenyl and alkynyl groups covalently linked to an oxygen atom. Examples of alkoxy groups or alkoxyl radicals include, but are not limited to, methoxy, ethoxy, isopropyloxy, 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, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, 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 moieties. Examples of halogen substituted alkoxy groups include, but are not limited to, fluoromethoxy, difluoromethoxy, trifluoromethoxy, chloromethoxy, dichloromethoxy and trichloromethoxy.

[0241] 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.

[0242] 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 alkoxycarboxy groups such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, etc.

[0243] 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, arylcarbonyloxy, alkoxycarbonyloxy, carboxylate, carboxyacid, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, 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.

[0244] The term "thiocarbonyl" or "thiocarboxy" includes compounds and moieties which contain a carbon connected with a double bond to a sulfur atom.

[0245] 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.

[0246] 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.

[0247] 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" 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.

[0248] 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, it being understood that not all isomers may have the same level of activity. 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.

[0249] "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."

[0250] A carbon atom bonded to four nonidentical substituents is termed a "chiral center."

"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).

[0252] "Geometric isomer" means the diastereomers that owe their existence to hindered rotation about double bonds or a cycloalkyl linker (e.g., 1,3-cylcobutyl). 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.

[0253] 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, it being understood that not all isomers may have the same level of activity.

[0254] Furthermore, the structures and other compounds discussed in this invention include all atropic isomers thereof, it being understood that not all atropic isomers may have the same level of activity. "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.

"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.

[0256] 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.

[0257] 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), imine-enamine and enamine-enamine. An example of keto-enol equilibria is between pyridin-2(1H)-ones and the corresponding pyridin-2-ols, as shown below.

pyridin-2(1
$$H$$
)-one pyridin-2-ol

[0258] 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. It will be understood that certain tautomers may have a higher level of activity than others.

[0259] 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.

[0260] The compounds of any of Formulae disclosed herein include the compounds themselves, as well as their salts or their solvates, if applicable. A salt, for example, can be formed between an anion and a positively charged group (e.g., amino) on an aryl- or heteroaryl-substituted benzene 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 (e.g., trifluoroacetate). The term "pharmaceutically acceptable anion" refers to an anion suitable for forming a pharmaceutically acceptable salt. Likewise, a salt can also be formed between a cation and a negatively charged group (e.g., carboxylate) on an aryl- or heteroaryl-substituted benzene compound. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. The aryl- or heteroaryl-substituted benzene compounds also include those salts containing quaternary nitrogen atoms.

[0261] 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 monohydrates, dihydrates, etc. Nonlimiting examples of solvates include ethanol solvates, acetone solvates, etc.

[0262] "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 .

[0263] 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.

[0264] 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 aryl- or heteroaryl-substituted benzene compounds, and have Formula (I) as a common core.

[0265] 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.

[0266] 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.

[0267] The present invention provides methods for the synthesis of the compounds of any Formula disclosed herein. The present invention also provides detailed methods for the synthesis of various disclosed compounds of the present invention according to the following schemes as shown in the Examples.

[0268] 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.

[0269] 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, polymorph or solvate thereof.

[0270] 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. [0271] Compounds of the present invention can be conveniently prepared by a variety of methods familiar to those skilled in the art. The compounds of this invention with any Formula disclosed herein may be prepared according to the procedures illustrated in Schemes 1-10 below, from commercially available starting materials or starting materials which can be prepared using literature procedures. The Z and R groups (such as R₂, R₃, R₄, R₆, R₇, R₈, and R₁₂) in Schemes 1-10 are as defined in any of Formulae disclosed herein, unless otherwise specified.

[0272] 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.

[0273] 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.

- [0274] Preferred protecting groups include, but are not limited to:
- [0275] For a hydroxyl moiety: TBS, benzyl, THP, Ac
- [0276] For carboxylic acids: benzyl ester, methyl ester, ethyl ester, allyl ester
- [0277] For amines: Cbz, BOC, DMB
- [0278] For diols: Ac (x2) TBS (x2), or when taken together acetonides
- [0279] For thiols: Ac
- [0280] For benzimidazoles: SEM, benzyl, PMB, DMB
- [0281] For aldehydes: di-alkyl acetals such as dimethoxy acetal or diethyl acetyl.
- [0282] 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.

[0283] The following abbreviations are used throughout the specification and are defined below:

[0284] Ac acetyl

[0285] AcOH acetic acid

[0286] aq. aqueous

[0287] BID or b.i.d. bis in die (twice a day)

[0288] BOC tert-butoxy carbonyl

[0289] Cbz benzyloxy carbonyl

[0290]	CDCl ₃	deuterated chloroform
[0291]	CH ₂ Cl ₂	dichloromethane
[0292]	DCM	dichloromethane
[0293]	DMB	2,4 dimethoxy benzyl
[0294]	DMF	N,N-Dimethylformamide
[0295]	DMSO	Dimethyl sulfoxide
[0296]	EA or EtOAc	Ethyl acetate
[0297]	EDC or EDCI	$N\hbox{-}(3\hbox{-}Dimethylaminopropyl)\hbox{-}N'\hbox{-}ethylcarbodiimide}$
[0298]	ESI-	Electrospray negative mode
[0299]	ESI+	Electrospray positive mode
[0300]	EtOH	ethanol
[0301]	h	hours
[0302]	H_2O	water
[0303]	HOBt	1-Hydroxybenzotriazole
[0304]	HC1	hydrogen chloride or hydrochloric acid
[0305]	HPLC	High performance liquid chromatography
[0306]	K_2CO_3	potassium carbonate
[0307]	LC/MS or LC-MS	Liquid chromatography mass spectrum
[0308]	M	Molar
[0309]	MeCN	Acetonitrile
[0310]	min	minutes
[0311]	Na_2CO_3	sodium carbonate
[0312]	Na_2SO_4	sodium sulfate
[0313]	NaHCO ₃	sodium bicarbonate
[0314]	NaHMDs	Sodium hexamethyldisilazide
[0315]	NaOH	sodium hydroxide
[0316]	NaHCO ₃	sodium bicarbonate
[0317]	Na_2SO_4	sodium sulfate
[0318]	NMR	Nuclear Magnetic Resonance
[0319]	$Pd(OH)_2$	Palladium dihydroxide
[0320]	PMB	para methoxybenzyl

[0321]	p.o.	per os (oral adinsitration)
[0322]	ppm	parts per million
[0323]	prep HPLC	preparative High Performance Liquid Chromatography
[0324]	PYBOP	(Benzotriaz ol-1-yloxy) tripyrrolidin ophosphonium
		hexafluorophosphate
[0325]	Rt or RT	Room temperature
[0326]	TBME	tert-Butyl methyl ether
[0327]	TFA	trifluoroacetic acid
[0328]	THF	tetrahydrofuran
[0329]	THP	tetrahydropyran

[0330] The present invention also provides pharmaceutical compositions comprising a compound of any Formula disclosed herein in combination with at least one pharmaceutically acceptable excipient or carrier.

T03311 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.

[0332] As used herein, the phrase "pharmaceutically acceptable" refers to those compounds, anions, cations, 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.

[0333] "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.

[0334] 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.

[0335] 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 so 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.

[0336] 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 or combination of therapeutics 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.

[0337] 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.

[0338] 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 combination(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.

[0339] 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.

[0340] 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 ELTM (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.

[0341] 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.

[0342] 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.

[0343] 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.

[0344] 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.

[0345] 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.

[0346] 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.

In therapeutic applications, the dosages of the pharmaceutical compositions used in [0347] 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.

[0348] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0349] 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.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the [0350] 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.

[0351] 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. In the salt form, it is understood that the ratio of the compound to the cation or anion of the salt can be 1:1, or any ration other than 1:1, e.g., 3:1, 2:1, 1:2, or 1:3.

[0352] 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.

[0353] 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.

[0354] The compounds, or pharmaceutically acceptable salts or solvates thereof, are administered orally, nasally, transdermally, pulmonary, inhalationally, buccally, sublingually, intraperintoneally, 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.

[0355] 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.

[0356] 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.

[0357] 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.

[0358] 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; however, it will be understood that a given isomer, tautomer, regioisomer or stereoisomer may have a higher level of activity than another isomer, tautomer, regioisomer or stereoisomer.

[0359] Compounds designed, selected and/or optimized by methods described above, 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.

[0360] 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 below.

[0361] An EZH2 inhibitor of the present invention may, if desired, be presented in a kit (*e.g.*, a pack or dispenser device) which may contain one or more unit dosage forms containing the EZH2 inhibitor. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising an EZH2 inhibitor of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Instructions for use may also be provided.

[0362] Also provided herein are kits comprising a plurality of methylation detection reagents that detect the methylated H3-K27. For example, the kit includes mono-methylated H3-K27, di-methylated H3-K27 and tri-methylated H3-K27 detection reagents. The detection reagent is for example antibodies or fragments thereof, polypeptide or aptamers.

[0363] A kit may also include reagents for detecting loss of function of at least one component of the SWI/SNF complex, *e.g.*, nucleic acids that specifically identify a mutant

component nucleic acid sequence by having homologous nucleic acid sequences, such as oligonucleotide sequences, complementary to a portion of the mutant component nucleic acid sequence or antibodies to proteins encoded by the wild type and/or mutant component nucleic acids packaged together in the form of a kit. The oligonucleotides can be fragments of the component gene. For example the oligonucleotides can be 200, 150, 100, 50, 25, 10 or less nucleotides in length. The kit may contain in separate containers an aptamer or an antibody, control formulations (positive and/or negative), and/or a detectable label such as fluorescein, green fluorescent protein, rhodamine, cyanine dyes, Alexa dyes, luciferase, radiolabels, among others. In addition, reagents for detecting the biological activity of the SWI/SNF complex (such as its chromatin remodeling activity) may be included in the kit.

[0364] Instructions (*e.g.*, written, tape, VCR, CD-ROM, etc.) for carrying out the assay may be included in the kit. The assay may for example be in the form of a Western Blot analysis, Immunohistochemistry (IHC), immunofluorescence (IF), sequencing and Mass spectrometry (MS) as known in the art.

Example 1: Durable Tumor Regression in Genetically Altered Lymphomas and Malignant Rhabdoid Tumors by Inhibition of EZH2

[0365] Compound A is a potent and selective inhibitor of EZH2: Cell free biochemical assays that included radiolabeled SAM and either chicken erythrocyte oligonucleosomes or peptides corresponding to H3K27 as substrates showed that Compound A selectively inhibited the activity of human PRC2 containing wild-type EZH2 with an inhibition constant (Ki) value of 2.5 ± 0.5 nmol/L and IC50 values of 11 ± 5 nM (nucleosome assay) or 16 ± 12 nM (peptide assay). The IC50 values were similar for human and rat EZH2 enzymes as well as for EZH2 proteins bearing all known lymphoma change-of-function mutations. The IC50 value of Compound A increased with increasing concentration of SAM, but was minimally affected by increasing the amount of oligonucleosome which is consistent with a SAM-competitive and nucleosome-noncompetitive modality of inhibition. In order to demonstrate HMT selectivity, inhibition by Compound A against a panel of HMTs other than EZH2 encompassing both lysine and arginine HMTs was assessed. Compound A displayed a 35-fold selectivity versus EZH1 and greater than 4500-fold selectivity relative to the 14 other HMTs tested.

[0366] Compound A specifically inhibits cellular H3K27 methylation in cells: When WSU-DLCL2 EZH2 Y641F mutant lymphoma cells were incubated with Compound A for 4 days, a concentration-dependent reduction in global H3K27Me3 levels was observed with an average IC₅₀ value of 0.26 μM (H3K27Me3 levels determined by ELISA). When studying the kinetics of methylation inhibition, the half-life of H3K27Me3 was approximately 1 day as 90% inhibition was only achieved after 3 to 4 days of incubation. When OCI-LY19 EZH2 wild-type lymphoma cells were incubated with 2.7 μM Compound A for 4 days, the only methyl marks affected were the H3K27Me1, H3K27Me2 and H3K27Me3, the three known products of PRC2 catalysis. Incubation with Compound A also resulted in an increase in H3K27 acetylation. The ability of Compound A to reduce global H3K27 trimethylation levels was further tested in several other human lymphoma cell lines including lines expressing either wild-type or mutant EZH2. Compound A reduced H3K27Me3 with similar potency in all cell lines independent of the EZH2 status (Table 1).

Compound A leads to selective killing of lymphoma cell lines bearing EZH2 T03671 point mutations: Incubation of WSU-DLCL2 EZH2 Y641F mutant cells with Compound A lead to anti-proliferative effects with an average IC₅₀ value of $0.28 \pm 0.14 \,\mu\text{M}$ in a 6 day proliferation assay. The kinetics of the effect of Compound A on viable cell number was further tested over an extended period of 11 days. The antiproliferative effect of Compound A was apparent after WSU-DLCL2 cells had been exposed to compound for longer than 4 days, consistent with the kinetics of Compound A-mediated cellular H3K27 methylation inhibition. The IC₅₀ value for Compound A inhibition of proliferation of WSU-DLCL2 cells in the 11-day assay (0.0086 µM, Table 1) was lower when compared with results obtained with a 6-day proliferation assay, suggesting increased sensitivity with longer incubation periods. In contrast to the WSU-DLCL2 cells, the growth of OCI-LY19 human lymphoma cells (EZH2 wild type for residue Y641) over 11 days was not significantly affected, despite comparable IC₅₀ values for H3K27Me3 inhibition for both cell lines (Table 1). In order to identify a concentration at which cells stop proliferating considering the entire incubation period of 11 days, the lowest cytotoxic concentration (LCC) for a particular cell line was calculated. The LCC value for WSU-DLCL2 EZH2 Y641F mutant human lymphoma cells was significantly lower when compared with OCI-LY19 cells that are wild type for EZH2 (Table 1). This context specific cell killing was further supported by results from 11-day proliferation assays with an extended

lymphoma cell line panel. All cell lines harboring an EZH2 mutation, with the exception of the RL cell line (EZH2 Y641N), were more sensitive to the antiproliferative effects of Compound A when compared with cell lines with wild-type EZH2 (Table 1). The Pfeiffer cell line (EZH2 A677G) showed a 20 to 300 fold increase in sensitivity to Compound A, as measured by IC₅₀ value and LCC, respectively, over the Y641 mutant cell lines. Next the minimum time of compound exposure necessary for sustained cell killing was investigated by washout experiments. The LCC values on day 11 or 14 for WSU-DLCL2cells that were either incubated with Compound A for 7 days (followed by 7 days of compound washout) or continuously for 14 days were similar (Table 2). Drug exposure for only 4 days, however, was not sufficient to induce LCC values similar to continuous incubation.

Compound A induces G₁ arrest and apoptosis in EZH2 mutant lymphoma cells: Next, the effects of incubation with Compound A (1 μM) for 7 days on cell cycle progression and apoptosis in WSU-DLCL2 cells were assessed. An increase in the percentage of cells in G₁ phase, and a decrease in the percentage of cells in S phase and G₂/M phase was apparent after 2 days of Compound A incubation. The maximum effect was achieved after 4 days. There was no apparent increase in the sub-G₁ fraction suggesting that apoptosis was not induced by Compound A incubation for 7 days. This is in agreement with the growth curves of WSU-DLCL2 cells in the presence of Compound A indicating that cytotoxic effects were observed only after 7 days of incubation. Following incubation of WSU-DLCL2 cells with Compound A for up to 14 days, the fraction of apoptotic cells determined by TUNEL assay was significantly increased on day 14 compared to vehicle, indicating that Compound A-mediated cell death occurred through the induction of apoptosis.

mutant xenograft models in mice: The effect of oral dosing of Compound A on systemic compound exposure and *in vivo* target inhibition in mice bearing EZH2 mutant lymphoma xenografts was investigated. First, SCID mice implanted subcutaneously with WSU-DLCL2 xenografts were orally dosed with Compound A for 4 or 7 days. Measuring Compound A plasma levels either 5 minutes before or 3 hours after the last dose revealed a clear dose dependent increase in exposure. Only animals dosed at 160 mg/kg TID or 213 mg/kg BID maintained mean compound levels in plasma above the LCC for WSU-DLCL2 cells throughout a dosing cycle (1652 ng/mL, with mouse plasma protein binding considered). Compound

determination in homogenates from tumors collected 3 hours after the last dose revealed that only for the highest dose groups compound levels in the 2 compartments were similar. When H3K27Me3 levels in tumors were analyzed, dose dependent EZH2 target inhibition was observed. H3K27Me3 inhibition was less in tumors from mice dosed at 213 mg/kg QD, suggesting that maintaining a plasma concentration above LCC throughout a dosing cycle is required for optimal target inhibition. Dosing for 4 days at 160 mg/kg TID resulted in slightly lower target inhibition than dosing for 7 days at the same dose and schedule, indicating that prolonged dosing increased the degree of target inhibition in WSU-DLCL2 tumors. A similar 7-day study in nude mice implanted subcutaneously with KARAPS-422 xenografts assessing both BID and QD schedules was performed. Compound A induced a dose-dependent reduction of tumor H3K27Me3 levels at both regimens.

Compound A induces significant antitumor effects in several EZH2 mutant lymphoma xenografts: When WSU-DLCL2 EZH2 Y641F mutant xenograft tumor bearing SCID mice were treated with Compound A for 28 days, dose-dependent tumor growth inhibition, 58% at the highest dose of 150 mg/kg TID, was observed. Only animals administered the highest dose maintained mean Compound A plasma levels above LCC for WSU-DLCL2 cells throughout the dosing cycle. Dosing of Compound A for 28 days led to a relative compound accumulation in tumor tissue compared with plasma, in contrast to what was observed with 7-day dosing. ELISA analysis of histones from tumors collected on day 28 indicated dose-dependent target inhibition. H3K27Me3 levels in WSU-DLCL2 xenografts were lower in mice dosed for 28 days compared with 7 days indicating that prolonged administration of Compound A increased the degree of target inhibition. In KARPAS-422 EZH2 Y461N mutant xenografts, 28-day dosing of Compound A on a BID schedule had much more dramatic effects. Tumor growth inhibition was observed at doses as low as 80.5 mg/kg BID, but higher doses eradicated the xenografts, and no re-growth was observed for up to 90 days after cessation of dosing. When intermittent dosing schedules were investigated in KARPAS-422 xenograft bearing mice, Compound A again showed significant dose-dependent antitumor effects with two cycles of 7-day on/7-day off and 21 day on/7 day off schedules. For all dosing schedules, tumor growth inhibition and complete regressions were observed at 90 and 361 mg/kg BID, respectively. The Pfeiffer EZH2 A677G mutant xenograft model was the most sensitive tumor model, as suggested by the potent anti-proliferative effects of Compound

A on this cell line in vitro. All Compound A dose groups (QD schedule) except the lowest one (30 mg/kg QD) showed complete tumor regressions in all animals. Again, tumor re-growth was not observed until the end of the study (36 days after stopping Compound A administration). Although tumor re-growth was observed at 30 mg/kg QD, this very low dose induced tumor stasis during the administration period. Due to tolerability issues dosing was stopped on day 12 for mice administered 1140 mg/kg QD; still, durable complete regressions were observed in this group that were only exposed to Compound A for 12 days.

[0371] Compound A selectively kills SMARCB1 mutant MRT cells *in vitro* and *in vivo*: Whether EZH2 inhibition had any effects on the growth and survival of SMARCB1-deleted MRT cells was tested. Incubating SMARCB1-deleted MRT cell lines G401 and A204 with Compound A in a 14-day proliferation assay *in vitro* induced strong anti-proliferative effects with IC₅₀ values in the nM range while the control cell lines RD and SJCRH30 which expressed SMARCB1 were minimally affected (Table 3). Dosing of SCID mice bearing subcutaneous G401 xenografts with Compound A at 266 or 532 mg/kg BID for 28 days eliminated those extremely fast growing tumors. Similar to the KARPAS-422 and Pfeiffer EZH2 mutant NHL xenograft models re-growth was not observed at study end, 32 days after dosing stop. Compound A dosed at 133 mg/kg induced stasis during the administration period, and produced a significant tumor growth delay compared to vehicle after dosing stop. Tumors that were harvested from subsets of mice from each group on day 21 showed strong EZH2 target inhibition at all doses.

[0372] Compound A inhibits H3K27 methylation in nontumor tissues in a dose dependent manner: The data described above demonstrate that Compound A represents a new treatment modality for SWI/SNF driven cancers and MRTs. Measuring pharmacodynamic biomarker modulation post-dose is often performed in early clinical trials to assess the degree of target inhibition that is predicted to produce a response based on data from preclinical models. Since the collection of post-dose tumor biopsies is often not possible, easier accessible surrogate tissues such as peripheral blood mononuclear cells (PBMCs), skin or bone marrow are often collected instead. To test EZH2 target inhibition in surrogate tissues male and female Sprague Dawley rats were orally administered 100, 300, or 1000 mg/kg Compound A for 28 days, and PBMCs, bone marrow and skin samples were collected at study end. Plasma levels of Compound A increased dose-dependently in both male and female rats, and the plasma

levels were generally higher in females compared with those in males. Due to tolerability issues, females in the 1000 mg/kg group had to be euthanized on day 23. Dose-dependent target inhibition was observed in PBMCs and bone marrow from rats dosed with Compound A, as measured by ELISA. The degree of target inhibition was less pronounced for PBMCs from females that were dosed for 22 days compared with males that were dosed for 28 days (same dose of 1000 mg/kg). A dose dependent reduction in H3K27Me3 positive cells was observed in the epidermis of skin of Compound A-dosed rats, as assessed by an IHC assay. The maximum effect was observed at the highest dose, and was already evident after 22 days of Compound A administration.

Compound A displayed similar properties as other EZH2 inhibitors in vitro, such as [0373] very high specificity for EZH2 in biochemical assays when compared with other HMTs and specific inhibition of cellular H3K27 methylation leading to context specific killing of EZH2 mutated NHL cell lines. However, this compound achieved an approximately 10-fold increase in potency, reflected by decreased K_i and IC₅₀ values determined in biochemical and cellfunctional assays. In addition, Compound A showed excellent oral bioavailability when administered to rodents which lead to dose dependent EZH2 target inhibition in xenograft tumor and nontumor tissues. Importantly, dosing of Compound A induced significant antitumor effects in mice bearing EZH2 mutant lymphoma xenografts. The responses ranged from tumor eradication (no regrowth after dosing cessation) to dose-dependent tumor growth inhibition. The delayed onset of antitumor activity (after 4 to 7 days) was consistent with the kinetics of methylation inhibition and antiproliferative activity induced by incubation of cells with Compound A in vitro. Keeping Compound A plasma levels above LCC throughout a dosing cycle was necessary for the WSU-DLCL2 xenograft model to induce maximal target inhibition and antitumor response. The other two lymphoma xenograft models (KARPAS-422 and Pfeiffer), however, were extremely sensitive to Compound A administration, and keeping plasma levels above LCC was not necessary. Pfeiffer EZH2 A677G mutant xenograft tumors disappeared permanently with very low doses or short dosing periods, suggesting that patients with this type of genetically defined NHL would have a significant treatment effect with Compound A.

[0374] MRTs are extremely aggressive pediatric cancers of the brain, kidney, and soft tissues that are highly malignant, locally invasive, frequently metastatic, and particularly lethal,

but they are typically diploid and lack genomic aberrations. They are, however, characterized by an almost complete penetrance of loss of expression of the SMARCB1, a core component of the SWI/SNF chromatin remodeling complex. The biallelic inactivation of SMARCB1, for instance induced by mutations, is in essence the sole genetic event in MRTs which suggests a driver role for this genetic aberration. Through genetic studies it has been suggested that PRC2 and SWI/SNF antagonistically regulate gene expression around the RB, Cyclin D1 and MYC pathways. Here, it has been demonstrated pharmacological EZH2 inhibition induced antiproliferative effects in SMARCB1 deleted MRT cell lines and permanently eradicated MRT xenografts in mice. This confirms the dependency of such cancers, in which EZH2 itself is not genetically altered, on PRC2 activity.

[0375] Compound A represents a new treatment modality for genetically defined subsets of NHL and for MRTs. The ability to measure dose-dependent changes in H3K27Me3 levels in skin, PBMCs and bone marrow portends the use of signal from these surrogate tissues as a non-invasive pharmacodynamics biomarker in human clinical trials.

[0376] Table 1: IC_{50} Values for Methylation and Proliferation as well as LCC Values for Compound A in Human Lymphoma Cell Lines

Cell Line	EZH2 Status	Methylation IC ₅₀ (nmol/L) ^a	Proliferation IC ₅₀ (µmol/L) ^b	LCC (µmol/L) ^b
DOHH-2	Wild Type	ND	1.7	>10
Farage	Wild Type	ND	0.099	>10
OCI-LY19	Wild Type	8	6.2	10 – 25
Toledo	Wild Type	ND	7.6	>10
Karpas-422	Y641N	90	0.0018	0.12
Pfeiffer	A677G	2	0.00049	0.0005
RL	Y641N	22	5.8	>25
SU-DHL-10	Y641F	ND	0.0058	0.14
SU-DHL-6	Y641N	20	0.0047	0.21
WSU-DLCL2	Y641F	9	0.0086	0.17

a: Derived after incubation for 4 days by immunoblot. Values represent the result from one experiment.

[0377] Table 2: LCC Values for Compound A for WSU-DLCL2 Human Lymphoma Cells Dosed Either Continuously or After Compound Washout

WSU-DLCL2 Washout	Day 11	Day 14
WSU-DLCL2 Washout	LCC (µM)	LCC (µM)
No Washout	0.17	0.11

b: Derived after incubation for 11 days. Compound incubations for each experiment were performed in triplicate, and values represent one experiment for all cell lines except OCI-LY19, Pfeiffer, and WSU-DLCL2. For the remaining three cell lines, values represent the mean from the following number of experiments: OCI-LY19 n=9; Pfeiffer n=2 and WSU-DLCL2 n=15.

4-day Compound A; 11-day Washout	0.36	0.42
7-day Compound A; 7-day Washout	0.19	0.075

Values represent the mean of duplicate experiments with three replicates per incubation concentration within the experiments.

[0378] Table 3: IC₅₀ Values for Compound A for SMARCB1 Negative MRT Cell Lines and SMARCB1 Positive Control Cell Lines

Cell Line	SMARCB1 Status	Proliferation IC ₅₀ (μM), day 7	Proliferation IC ₅₀ (μM), day 14
RD	Wild Type	9.2	5.2
SJCRH30	Wild Type	6.1	8.8
G401	Mutant	0.087	0.042
A204	Mutant	3.2	0.14

Values represent the mean of duplicate experiments with three replicates per incubation concentration within the experiments.

Example 2: Durable Tumor Regression in Genetically Altered Malignant Rhabdoid Tumors by Inhibition of EZH2

[0379] Compound A is a potent and selective inhibitor of EZH2: Compound A was developed through iterative medicinal chemistry (Figure 10A). Compound A inhibited the activity of human PRC2 containing wild-type EZH2 with an inhibition constant (Ki) value of 2.5 ± 0.5 nM, and similar potency was observed for EZH2 proteins bearing all known lymphoma change-of-function mutations (Table 5). The compound was found to be SAM-competitive and nucleosome-noncompetitive by steady state kinetic studies (Figure 11). Inhibition by Compound A against a panel of HMTs other than EZH2 encompassing both lysine and arginine HMTs was also assessed. Compound A displayed a 35-fold selectivity versus EZH1 and > 4500-fold selectivity relative to 14 other HMTs tested (Table 5).

[0380] Table 4: Histone Methyltransferase Inhibition by Compound A

Enzyme Assay	IC ₅₀ (nM)	% Inhibition at 1 μM Compound A ^a
CARM1	>50,000 ^b	5 ± 3
DOT1L	>50,000°	2 ± 8
EHMT1	>50,000°	6 ± 6
EHMT2	>50,000°	7 ± 3
EZH1 ^{d,e}	$392 \pm 72^{\rm f}$	98 ± 1
EZH2 Peptide Assay ^{d,e}	$11 \pm 5^{\rm f}$	ND
EZH2 Nucleosome Assay ^d	$16 \pm 12^{\rm f}$	100 ± 1
A677G EZH2 ^{d,e}	2 ^b	ND
A687V EZH2 ^{d,e}	2 ^b	ND
Y641F EZH2 ^{d,e}	$14 \pm 5^{\rm f}$	ND

Y641C EZH2 ^{d,e}	16°	ND
Y641H EZH2 ^{d,e}	6°	ND
Y641N EZH2 ^{d,e}	38 ^b	ND
Y641S EZH2 ^{d,e}	6°	ND
rat EZH2 ^{d,e}	4 ^c	ND
PRMT1	>50,000°	5 ± 4
PRMT3	ND	2 ± 2
PRMT5/MEP50	>50,000°	2 ± 6
PRMT6	ND	3 ± 3
PRMT8	>50,000°	7 ± 3
SETD7	ND	4 ± 3
SMYD2	>50,000°	1 ± 2
SMYD3	ND	0 ± 5
WHSC1	>100,000°	8 ± 3
WHSC1L1	>100,000°	9 ± 8

a: Values represent the mean and standard deviation of duplicate experiments determined at $10~\mu\text{mol/L}$ Compound A.

Compound A specifically inhibits cellular H3K27 methylation leading to T03811 selective apoptotic killing of SMARCB1 mutant MRT cells: A panel of SMARCB1 deficient MRT cells and SMARCB1 wild-type control cells (confirmed by immunoblot, Figure 12A) were treated with Compound A for 4 days, resulting in concentration-dependent reductions in global H3K27Me3 levels (Figure 10B and table 6). Treatment of either wild-type or mutant cells resulted in diminution only of methyl marks on H3K27, with no other histone methyl marks being affected (Figure 12B). *In vitro* treatment of *SMARCB1*-deleted MRT cell lines with Compound A induced strong anti-proliferative effects with IC₅₀ values in the nM range; while the control (wild-type) cell lines were minimally affected (Figure 10C and table 6). Antiproliferative effects were apparent in SMARCB1-deleted MRT cells after 7 days of compound exposure, but required 14 days of exposure for maximal activity. The effects of incubation with Compound A (1 µM) for 14 days on cell cycle progression and apoptosis in G401 and RD cells were also assessed. Compound A incubation of RD SMARCB1 wild-type cells showed no changes in cell cycle or apoptosis compared to the DMSO control (Figure 13A). In contrast, G401 SMARCB1-deleted cells showed an increase in the percentage of cells in G_1 phase, and a concomitant decrease in S phase and G_2/M phase after 7 days (Figure 13B). There was no apparent increase in the sub-G₁ fraction through day 7, suggesting that apoptosis

b: Values represent the mean of duplicate experiments with two replicates per experiment.

c: Values represent one experiment with two replicates per experiment.

d: All EZH1 and EZH2 proteins were assayed in the context of 4 PRC2 components (EZH1/2, SUZ12, RBAP48, EED).

e: Assayed with H3K27 peptides as substrates.

was not yet induced by that time. This coincides with the growth curves of G401 cells in the presence of Compound A that display cytotoxicity only after 7 days of incubation (Figure 10C). Following Compound A treatment of G401 cells for up to 14 days, the fraction of cells in sub-G₁ as well as apoptotic cells determined by TUNEL assay increased in a time dependent manner through days 11 and 14, indicating that Compound A-mediated cell death occurred through the induction of apoptosis (Figure 13B).

Table 6

Cell Line	SMARCB1 Status	Methylation IC ₅₀ (nM) ^a	Proliferation IC ₅₀ on Day 14 (nM) ^b
G401	mutant	2.7	135
A204	mutant	1.4	590
G402	mutant	1.7	144
KYM-1	mutant	4.3	32
RD	wild-type	5.6	6100, > 10000°
293	wild-type	2.4	> 10000
SJCRH30	wild-type	4.9	5100, >10000°

a: Derived after incubation for 4 days, extraction of histones, immunoblot and densitometry. Values represent the mean from two experiments.

[0382] Compound A induces genes of neuronal differentiation and cell cycle inhibition while suppressing expression of hedgehog pathway genes, MYC and EZH2: It has been suggested that SMARCB1 loss drives cancer formation through simultaneous epigenetic perturbation of key cancer pathways. The present data confirmed the previously described reduced expression of genes important for neuronal differentiation (CD133, DOCK4, PTPRK), cell cycle inhibition (CDKN2A) and tumor suppression (BIN1), as well as increased expression of the hedgehog pathway gene GL11 in SMARCB1-deleted G401 cells compared to control cells (Figure 14A). Compound A treatment of G401 cells for up to 7 days strongly induced expression of CD133, DOCK4 and PTPRK and up-regulated cell cycle inhibitors CDKN1A and CDKN2A and tumor suppressor BIN1, all in a time-dependent manner (Figure 14B). Simultaneously, the expression of hedgehog pathway genes, MYC and EZH2 were reduced. Notably, G402 SMARCB1-deleted cells exposed to Compound A for 14 days assumed a neuron-like morphology (Figure 14C). In contrast, Compound A incubation of RD control cells had minimal effect on expression of the above-mentioned genes.

b: Compound incubations for each experiment were performed in triplicate, and values represent the mean of 2 experiments for all cell lines.

c: Mean calculation of duplicate experiment not possible.

Compound A eradicates SMARCB1 mutant MRT xenografts: Oral dosing of [0383] Compound A led to systemic compound exposure, in vivo target inhibition and antitumor activity in mice bearing SMARCB1-deleted MRT xenografts. A study in SCID mice bearing subcutaneous G401 xenografts was performed where animals were dosed for 21 days with Compound A. Half of the mice per group were euthanized on day 21 to collect blood and tissues, while the remaining animals were treated for an additional 7 days and then left without dosing for another 32 days. Compound A was well tolerated at all doses with minimal effect on body weight (Figure 15A). Dosing at 250 or 500 mg/kg twice daily (BID) for 21 to 28 days practically eliminated the fast-growing G401 tumors (Figures 15B, 14C and 16A). Re-growth was not observed for 32 days after dose cessation. Compound A dosed at 125 mg/kg induced tumor stasis during the administration period, and produced a significant tumor growth delay compared to vehicle after the dosing period. Measuring Compound A plasma levels either 5 min before or 3 h after dosing on day 21 revealed a clear dose-dependent increase in systemic exposure (Figure 15D). Tumors that were harvested from subsets of mice from each group on day 21 showed strong inhibition of H3K27me3, correlating with the antitumor activity (maximum effect achieved at 250 mg/kg, Figure 16B). In addition, dose-dependent changes in the expression of CD133, PTPRK, DOCK4 and GLI1 were detected in the G401 xenograft tumors (Figure 16C).

[0384] The present data demonstrate that pharmacological inhibition of EZH2 induced antiproliferative effects specifically in *SMARCB1*-deleted MRT cell lines and permanently eradicated MRT xenografts in mice. This confirms the dependency of such cancers on PRC2 activity, despite the fact that EZH2 itself is not genetically altered in this context. Data presented herein show that in the context of *SMARCB1*-deleted MRT, inhibition of EZH2 functions as a SMARCB1 surrogate and de-represses neuronal differentiation genes, cell cycle inhibitors and tumor suppressors while reducing *GLI1*, *PTCH1*, *MYC* and *EZH2*. The sum of the effects of Compound A mediated EZH2 inhibition on several cancer pathways is the cause for the dramatic and permanent anti-tumor activity seen in MRT models. Thus, Compound A represents a new treatment modality for these lethal childhood tumors.

[0385] Furthermore, since several members of the SWI/SNF complex are genetically altered in other cancer types besides MRT, it is conceivable that EZH2 also plays a role in tumor maintenance and survival in a spectrum of cancer types. Combined with recent reports

demonstrating the effectiveness of EZH2 inhibitors in selective killing of EZH2 mutant bearing non-Hodgkin lymphomas, the present data demonstrate that small molecule-based inhibition of EZH2 is an effective mechanism of therapeutic intervention in a variety of hematologic and solid tumors for which genetic alterations – either target-directed or indirect – confer a proliferative dependency on EZH2 enzymatic activity.

Example 3: Material and Methods

[0386] Cell Culture: Cell lines 293T, RD, SJCRH30, A204, G401, G402, and KYM-1. 293T (CRL-11268), RD (CRL-136), SJCRH30 (CRL-2061), A204 (HTB-82), G401 (CRL-1441), and G402 (CRL-1440) were obtained from ATCC. KYM-1 (JCRB0627) was obtained from JCRB. 293T and RD cells were cultured in DMEM+10% FBS. SJCRH30 cells were cultured in RPMI+10% FBS. A204, G401, and G402 cells were cultured in McCoys 5a+10% FBS. KYM-1 cells were cultured in DMEM/Ham's F12+10% FBS.

[0387] Western blots analysis: Histones were acid extracted as previously described (Daigle et al., Blood. 2013 Aug 8;122(6):1017-25). Western blots for acid extracted histones were performed as previously described (Knutson et al., Proc Natl Acad Sci U S A. 2013 May 7;110(19):7922-7). Whole cell lysates (WCL) were prepared using a modified RIPA buffer (10x RIPA Lysis Buffer (Millipore #20-188), 0.1% SDS (Invitrogen AM9823), protease minitablet (Roche #1836153)). Cells were pelleted, washed with ice cold PBS, resuspended in ice cold RIPA buffer, and incubated on ice for 5 minutes. Lysates were sonicated 3x for 10sec at 50% power, then incubated on ice for 10 minutes. Lysates were then centrifuged at max speed for 15 minutes at 4 degrees in a table top centrifuge. Clarified lysates were aliquoted to a fresh tube, and protein concentrations for WCL were determined by BCA assay (Pierce). Ten micrograms of each lysate was fractionated on 10-20% Tris-Glycine gel (Biorad), transferred using iBlot (7 minutes on program 3, using Nitrocellulose transfer stacks), and probed with the following antibodies in Odyssey blocking buffer: SNF5 (CST #8745), EZH2 (CST #5246), and Beta-actin (CST #3700).

[0388] *In vitro* **cell assays**: For the adherent cell line proliferation assays (all cell lines except KYM-1, which was analyzed as previously described for suspension cell lines (Daigle et al., Blood. 2013 Aug 8;122(6):1017-25), plating densities for each cell line were determined based on growth curves (measured by ATP viability) and density over a 7 day timecourse. On the day before compound treatment, cells were plated in either 96-well plates in triplicate (for

the day 0-7 timecourse) or 6-well plates (for replating on day 7 for the remainder of the timecourse). On Day 0, cells were either untreated, DMSO-treated, or treated with Compound A starting at 10uM and decreasing in either 3- or 4-fold dilutions. Plates were read on Day 0, Day 4, and Day 7 using CellTiter-Glo® (Promega), with compound/media being replenished on Day 4. On Day 7, the 6-well plates were trypsinized, centrifuged, and resuspended in fresh media for counting by Vi-Cell. Cells from each treatment were replated at the original density in 96-well plates in triplicate. Cells were allowed to adhere to the plate overnight, and cells were treated as on Day 0. On Day 7, 11 and 14, plates were read using CellTiter-Glo®, with compound/media being replenished on Day 11. Averages of triplicates were used to plot proliferation over the timecourse, and calculate IC50 values. For cell cycle and apoptosis, G401 and RD cells were plated in 15 cm dishes in duplicate at a density of 1x10⁶ cells per plate. Cells were incubated with Compound A at 1 uM, in a total of 25 mL, over a course of 14 days, with cells being split back to original plating density on day 4, 7, and 11. Cell cycle analysis and TUNEL assay were performed using a Guava® flow cytometer, following the manufacturer's protocol.

[0389] Gene Expression Analysis: G401 and RD cells were plated in T-75 flasks at 175,000 cells/flask and 117,000 cells/flask respectively and allowed to adhere overnight. On Day 0, cells were treated in duplicates with DMSO or 1 uM Compound A. Cells were harvested and pelleted on Day 2, 4, and 7 with media and compound being replenished on Day 4. Tumor tissue from the G401 xenograft animals dosed for 21 days (vehicle, 125 mg/kg, and 250 mg/kg (6 animals each) and 500 mg/kg (4 animals) Compound A dose groups) were used for gene expression analysis. Total mRNA was extracted from cell pellets and tumor tissue using the RNeasy Mini Kit (Qiagen #74106) and reverse transcribed by the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems (AB) #4368813). RT-PCR was performed by ViiATM 7 Real-Time PCR Systems (AB) using TaqMan Fast Advanced Master Mix (AB #4444964) and TaqMan primer/probe sets in table below. Gene expression was normalized to 18S (AB #Hs99999901_s1) and fold change was calculated using the ΔΔCt method. For the in vivo samples, the average Ct value +/- SD was determined for each dose group and fold change compared to vehicle dose group was calculated using the ΔΔCt method.

Gene	<u>AB#</u>
MYC	Hs00153408_m1

EZH2	Hs00172783_m1
PTCH1	Hs00181117_m1
PROM1 (CD133)	Hs01009250_m1
GLI1	Hs01110766_m1
DOCK4	Hs00206807_m1
PTPRK	Hs00267788_m1
BIN1	Hs00184913 m1

[0390] **ELISA**: Histones were isolated from tumors as previously described (Daigle et al) and were prepared in equivalent concentrations (0.5 ng/ul for H3 and 4 ng/ul for H3K27Me3) in coating buffer (PBS with 0.05% BSA). Sample or standard (100 µL) was added in duplicate to two 96-well ELISA plates (Thermo Labsystems, Immulon 4HBX #3885). Histones isolated from G401 cells that were treated with DMSO or 10 µmol/L Compound A for 4 days were added to control wells at the same histone concentration as the tumor histone samples. The plates were sealed and incubated overnight at 4°C. The following day, plates were washed 3 times with 300 µL/well PBST (PBS with 0.05% Tween 20; 10x PBST, KPL #51-14-02) on a Bio Tek plate washer. Plates were blocked with 300 μL/well of diluent (PBS + 2% BSA + 0.05% Tween 20), incubated at room temperature for 2 hours, and washed 3 times with PBST. All antibodies were diluted in diluent. 100 uL/well of anti-H3K27Me3 (CST #9733, 50%) glycerol stock 1:1000) or anti-total H3 (Abcam #ab1791, 50% glycerol stock 1:10,000) was added to each plate. Plates were incubated for 90 minutes at room temperature and washed 3 times with PBST. 100 µL/well of anti-Rb-IgG-HRP (Cell Signaling Technology, 7074) was added 1:2000 to the H3K27Me3 plate and 1:6000 to the H3 plate and incubated for 90 minutes at room temperature. Plates were washed 4 times with PBST. For detection, 100 µL/well of TMB substrate (BioFx Laboratories, #TMBS) was added and plates incubated in the dark at room temperature for 5 minutes. Reaction was stopped with 100 μL/well 1N H₂SO₄. Absorbance at 450 nm was read on SpectraMax M5 Microplate reader.

[0391] **Xenograft study**: All the procedures related to animal handling, careand the treatment in this study were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Chemparner following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). For

the *in vivo* study, mice were inoculated subcutaneously at the right flank with G-401 tumor cells (5x10⁶ /mouse) in 0.2 ml mixture of base media and Matrigel (McCoy's 5A: Matrigel=1:1) for tumor development. The treatments were started when the tumor size reached approximately 157 mm3 for the tumor efficacy study (n=16 mice per group). Compound A or vehicle (0.5% NaCMC+0.1% Tween-80 in water) was administered orally BID at a dose volume of 10µL/g for either 21 or 28 days. Animal body weights were measured every day during the first week, then twice weekly for the remainder of the study. Tumor size was measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm³. For PK/PD analysis, 8 mice with the largest tumor burden were euthanized for tumor and blood collection after 21 days of dosing. The remaining mice continued dosing for one more week, and from day 29, treatment was stopped and the mice were enrolled in a tumor growth delay study. Mice were observed as individuals until they reached the tumor weight endpoint (2000mm³) or until day 60 (whichever came first).

[0392] **Pharmacokinetic analyses:** Dexamethasone was used as internal standard. An aliquot of 30 μ L plasma sample was added with 30 μ L IS (Dexamethasone, 1000 ng/mL) and 150 μ L ACN. The mixture was vortexed for 5 min and centrifuged at 14000 rpm for 5 min. An aliquot of 2 μ L supernatant was injected for LC-MS/MS analysis (Q-trap 3200). For 10-fold diluted plasma samples an aliquot of 3 μ L plasma sample was added with 27 μ L blank plasma, the dilution factor was 10, then added with 30 μ L IS (Dexamethasone, 1000 ng/mL) and 150 μ L ACN. The mixture was vortexed for 5 min and centrifuged at 14000 rpm for 5 min. An aliquot of 2 μ L supernatant was injected for LC-MS/MS analysis. Tumor samples were homogenized on Beadbeater® for 30 seconds with 3 x PBS (w/v) to obtain a tumor homogenate. An aliquot of 30 μ L tumor homogenate sample was added with 30 μ L IS (Dexamethasone, 1000 ng/mL) and 150 μ L ACN. The mixture was vortexed for 5 min and centrifuged at 14000 rpm for 5 min. An aliquot of 2 μ L supernatant was injected for LC-MS/MS analysis.

Example 4: General experimental procedures

NMR

[0393] ¹H-NMR spectra were taken using CDCl₃ unless otherwise stated and were recorded at 400 or 500 MHz using a Varian or Oxford instruments magnet (500 MHz) instruments. Multiplicities indicated are s=singlet, d = doublet, t = triplet, q = quartet, quint =

quintet, sxt = sextet, m = multiplet, dd =doublet of doublets, dt = doublet of triplets; br indicates a broad signal.

LCMS and HPLC

[0394] Shimadzu LC-Q, Shimadzu LCMS-2010EV or Waters Acquity Ultra Performance LC. HPLC: Products were analyzed by Shimadzu SPD-20A with 150 x 4.5mm YMC ODS-M80 column or 150 x 4.6mm YMC-Pack Pro C18 column at 1.0ml/min.

[0395] Mobile phase was MeCN:H2O=3:2 (containing 0.3% SDS and 0.05% H₃PO₄),

[0396] 0.05% TFA in water, 0.05% TFA in acetonitrile (gradient Initial 20 %, then 0.05% TFA/MeCN to conc. to 95 % in 3 min. holds for 0.5 min. at 3.51 to 4.50 min then 0.05% TFA/MeCN conc. 20 %).

[0397] Alternatively the LCMS, 2 different methods were used; the one we use the most is the high pH (METCR1600) and the other one for more standard compounds (METCR1416).

[0398] 0.1% Formic acid in water – Mobile phase "A" 0.1% Formic acid in acetonitrile – Mobile phase "B" utilizing Waters Atlantis dC18, 2.1 mm x 100 mm, 3μ m column, with a flow rate = 0.6 ml/min Column temperature = 40° C; Time (mins) %B 0.00 min 5% B. 5.0 mins 100% B, 5.4 mins 100% B and .42 mins 5%B

[0399] 3.5 minute method refers to Atlantis dC18, 2.1 mm x 50 mm, 3μm column, flow rate of 1ml/min at 40C. Mobile phase A Formic acid (aq.) 0.1% mobile phase B formic acid (MeCN) 0.1%, injection 3 μL, gradient 0 mins (5% organic), 2.5 min (100 % organic), 2.7 mins (100 % organic), 2.71 min (5% organic), 3.5 min (5% organic)

[0400] 7.0 minute method refers to Atlantis dC18, 2.1 mm x 100 mm, 3 μ m column, flow rate of 0.6ml/min at 40C. Mobile phase A Formic acid (aq.) 0.1% mobile phase B formic acid (MeCN) 0.1%, injection 3 μ L, gradient 0 mins (5% organic), 5 min (100 % organic), 5.4 mins (100 % organic), 5.42 min (5% organic), 7 min (5% organic)

[0401] Both the 3. 5 and 7 minute methods were performed on a MS18 Shimadzu LCMS-2010EV or a MS19 Shimadzu LCMS-2010EV system utilizing LC-20AB pumps and SPD-M20A PDA detectors.

[0402] Products were purified by HPLC/MS using Waters AutoPurification System with 3100 Mass Detector.

[0403] HPLC analyses may also be performed on a Shimdazu LC-2010CHT using an YMC ODS-A, C18, (150x4.6 x5 μ m) column at ambient temperature with a flow Rate of 1.4

ml/min. An injection volume of $10~\mu l$ is utilized and detection occurs via UV/PDA. Mobile Phase A is 0.05~% TFA in water and Mobile Phase B is 0.05~% TFA in acetonitrile with a gradient program of Initial 5~% B to 95~% B in 8min, hold for 1.5 min, at 9.51 to 12 min B. conc. 0.5~%. The diluent is the mobile phase

Other

[0404] Automated flash column chromatography was performed on a Biotage Isolera version 4. 10g SNAP cartridge running at 12 ml/min or a 25g SNAP cartridge running at 25 ml/min and detecting at 254 nm and 280 nm.

[0405] Select Nitrile reductions may be performed on a ThalesNano H-Cube® according to the conditions described in the experimental procedure.

[0406] Other related general procedures can also be found in PCT publication No. WO12/118812, PCT application No. PCT/US2012/033648 and PCT application No. PCT/US2012/033662, each of which is incorporated herein by reference in its entirety.

Example 5: Synthesis of N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(ethyl (tetrahydro-2H-pyran-4-yl)amino)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide

Compound A

[0407] Step 1: Synthesis of 5-bromo-2-methyl-3-nitrobenzoic acid

[0408] To stirred solution of 2-methyl-3-nitrobenzoic acid (100 g, 552 mmol) in conc. H_2SO_4 (400 mL), 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione (88 g, 308 mmol) was added in a portion wise manner at room temperature and the reaction mixture was then stirred

at room temperature for 5 h. The reaction mixture was poured onto ice cold water, the precipitated solid was filtered off, washed with water and dried under vacuum to afford the desired compoundas a solid (140 g, 98%). The isolated compound was taken directly into the next step. 1 H NMR (DMSO- d_{6} , 400 MHz) δ 8.31 (s, 1H), 8.17 (s, 1H), 2.43 (s, 3H).

[0409] Step 2: Synthesis of methyl 5-bromo-2-methyl-3-nitrobenzoate

[0410] To a stirred solution of 5-bromo-2-methyl-3-nitrobenzoic acid (285 g, 1105 mmol) in DMF (2.8L) at room temperature was added sodium carbonate (468 g, 4415 mmol) followed by addition of methyl iodide (626.6 g, 4415 mmol). The resulting reaction mixture was heated at 60 °C for 8 h. After completion (monitored by TLC), the reaction mixture was filtered (to remove sodium carbonate) and washed with ethyl acetate (1L X 3). The combined filtrate was washed with water (3L X 5) and the aqueous phase was back extracted with ethyl acetate (1L X 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the title compound as a solid (290g, 97% yield). The isolated compound was taken directly into the next step. 1 H NMR (CDCl₃, 400 MHz) δ 8.17 (s, 1H), 7.91 (s, 1H), 3.96 (s, 3H), 2.59 (s, 3H).

[0411] Step 3: Synthesis of methyl 3-amino-5-bromo-2-methylbenzoate

[0412] To a stirred solution of methyl 5-bromo-2-methyl-3-nitrobenzoate (290 g, 1058 mmol) in ethanol (1.5L) was added aqueous ammonium chloride (283 g, 5290 mmol dissolved in 1.5L water). The resulting mixture was stirred at 80°C to which iron powder (472 g, 8451 mmol) was added in a portion wise manner. The resulting reaction mixture was heated at 80 °C for 12 h. Upon completion as determined by TLC, the reaction mixture was hot filtered over celite® and the celite bed was washed with methanol (5L) followed by washing with 30% MeOH in DCM (5L). The combined filtrate was concentrated in-vacuo, the residue obtained was diluted with aqueous sodium bicarbonate solution (2L) and extracted with ethyl

acetate (5L X 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the title compound as a solid (220 g, 85%). The compound was taken directly into the next step. 1 H NMR (CDCl₃, 400 MHz) δ 7.37 (s, 1H), 6.92 (s, 1H), 3.94 (s, 3H), 3.80 (bs, 2H), 2.31 (s, 3H).

[0413] Step 4: Synthesis of methyl 5-bromo-2-methyl-3-((tetrahydro-2H-pyran-4-yl) amino) benzoate

[0414] To a stirred solution of methyl 3-amino-5-bromo-2-methylbenzoate(15 g, 61.5 mmol) and dihydro-2H-pyran-4(3)-one (9.2 g, 92 mmol) in dichloroethane (300 mL) was added acetic acid (22 g, 369 mmol) and the reaction mixture stirred at room temperature for 15 minutes, then the reaction mixture was cooled to 0°C and sodium triacetoxyborohydride (39 g, 184 mmol) was added. The reaction mixture was stirred overnight at room temperature. Upon completion of the reaction as determined by TLC, aqueous sodium bicarbonate solution was added to the reaction mixture until a pH of 7-8 was obtained. The organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude compound was purified by column chromatography (100-200 mesh silica gel) eluting with ethyl acetate: hexane to afford the desired compound as a solid (14 g, 69%). ¹H NMR (DMSO- d_6 , 400 MHz) δ 7.01 (s, 1H), 6.98 (s, 1H), 5.00 (d, 1H, J=7.6 Hz), 3.84-3.87 (m, 2H), 3.79 (s, 3H), 3.54-3.56 (m, 1H), 3.43 (t, 2H, J=12 Hz), 2.14 (s, 3H), 1.81-1.84 (m, 2H), 1.47-1.55 (m, 2H).

[0415] Step 5: Synthesis of methyl 5-bromo-3-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-2-methylbenzoate

[0416] To a stirred solution of methyl 5-bromo-2-methyl-3-((tetrahydro-2H-pyran-4-yl) amino) benzoate (14 g, 42.7 mmol) in dichloroethane (150 mL) was added acetaldehyde (3.75 g, 85.2 mmol) and acetic acid (15.3 g, 256 mmol). The resulting reaction mixture was stirred at room temperature for 15 minutes. The mixture was cooled to 0 °C and sodium triacetoxyborohydride (27 g, 128 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours. Upon completion of the reaction as determined by TLC, aqueous sodium bicarbonate solution was added to the reaction mixture until a pH 7-8 was obtained, the organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude compound was purified by column chromatography (100-200 mesh silica gel) eluting with ethyl acetate: hexane to afford the desired compound as a viscous liquid (14 g, 93%). 1 H NMR (DMSO- 2 6, 400 MHz) δ 7.62 (s, 1H), 7.52 (s, 1H), 3.80 (bs, 5H), 3.31 (t, 2H), 2.97-3.05 (m, 2H), 2.87-2.96 (m, 1H), 2.38 (s, 3H), 1.52-1.61 (m, 2H), 1.37-1.50 (m, 2H), 0.87 (t, 3H, J=6.8 Hz).

[0417] Step 6: Synthesis of 5-bromo-N-((4, 6-dimethyl-2-oxo-1, 2-dihydropyridin-3-yl) methyl)-3-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-2-methylbenzamide

[0418] To a stirred solution of 5-bromo-3-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-2-methylbenzoate (14 g, 39.4 mmol) in ethanol (100 mL) was added aqueous NaOH (2.36 g, 59.2 mmol in 25mL water) and the resulting mixture was stirred at 60 °C for 1 h. Upon completion of the reaction as determined by TLC, the solvent was removed under reduced pressure and the residue obtained was acidified with 1N HCl until a pH 7 was obtained and then aqueous citric acid solution was added until a pH 5-6 was obtained. The aqueous layer was extracted with 10% MeOH in DCM (200mL X 3), the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the respective acid (14 g, 100%).

[0419] The above acid (14 g, 40.9 mmol) was then dissolved in DMSO (70 mL) and 3-(amino methyl)-4, 6-dimethylpyridin-2(1H)-one (12.4 g, 81.9 mmol) was added to it. The reaction mixture was stirred at room temperature for 15 minutes, then PYBOP (31.9 g, 61.4 mmol) was added and stirring was continued for overnight at room temperature. Upon completion of the reaction as determined by TLC, the reaction mixture was poured onto ice-cold water (700 mL), stirred for 30 minutes and the precipitated solid was collected by filtration, washed with water (500 mL) and air dried. The solid obtained was stirred with acetonitrile (75mL X 2), filtered and air dried. The solid obtained was again stirred with 5% MeOH in DCM (100mL), filtered and dried completely under vacuum to afford the title compound as a solid (14 g, 74 %). 1 H NMR (DMSO- d_6 , 400 MHz) δ 11.47 (s, 1H), 8.23 (t, 1H), 7.30 (s, 1H), 7.08 (s, 1H), 5.85 (s, 1H), 4.23 (d, 2H, J=4.4 Hz), 3.81 (d, 2H, J=10.4 Hz), 3.20-3.26 (m, 2H), 3.00-3.07 (m, 1H), 2.91-2.96 (m, 2H), 2.18 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 1.58-1.60 (m, 2H), 1.45-1.50 (m, 2H), 0.78 (t, 3H, J=6.8 Hz).

[0420] Step 7: Synthesis of N-((4, 6-dimethyl-2-oxo-1, 2-dihydropyridin-3-yl) methyl)-5-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-4-methyl-4'-(morpholinomethyl)-[1, 1'-biphenyl]-3-carboxamide

[0421] To a stirred solution of 5-bromo-N-((4, 6-dimethyl-2-oxo-1, 2-dihydropyridin-3-yl) methyl)-3-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-2-methylbenzamide (14 g, 29.5 mmol) in dioxane/ water mixture (70 mL/14 mL) was added 4-(4-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl) benzyl) morpholine (13.4 g, 44.2 mmol) followed by addition of Na₂CO₃ (11.2 g, 106.1 mmol). The solution was purged with argon for 15 minutes and then Pd (PPh₃)₄ (3.40 g, 2.94 mmol) was added and the solution was again purged with argon for a further 10 min. The reaction mixture was heated at 100°C for 4 h. After completion (monitored by TLC), the reaction mixture was diluted with water and extracted with 10% MeOH/DCM. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated

under reduced pressure. The crude compound was purified by column chromatography (100-200 mesh silica gel) eluting with methanol: DCM to the title compound as a solid (12 g, 71 %). **Analytical Data:** LCMS: 573.35 (M + 1) $^+$; HPLC: 99.5% (@ 254 nm) (R_t;3.999; **Method:** Column: YMC ODS-A 150 mm x 4.6 mm x 5 μ; Mobile Phase: A; 0.05% TFA in water/ B; 0.05% TFA in acetonitrile; Inj. Vol: 10 μL, Col. Temp.: 30 °C; Flow rate: 1.4 mL/min.; Gradient: 5% B to 95% B in 8 min, Hold for 1.5 min, 9.51-12 min 5% B); 1 H NMR (DMSO- d_6 , 400 MHz) δ 11.46 (s, 1H), 8.19 (t, 1H), 7.57 (d, 2H, J=7.2 Hz), 7.36-7.39 (m, 3H), 7.21 (s, 1H), 5.85 (s, 1H), 4.28 (d, 2H, J=2.8 Hz), 3.82 (d, 2H, J=9.6 Hz), 3.57 (bs, 4H), 3.48 (s, 2H), 3.24 (t, 2H, J=10.8Hz), 3.07-3.09 (m, 2H), 3.01 (m, 1H), 2.36 (m, 4H), 2.24 (s, 3H), 2.20 (s, 3H), 2.10 (s, 3H), 1.64-1.67 (m, 2H), 1.51-1.53 (m, 2H), 0.83 (t, 3H, J=6.4 Hz).

[0422] Step 8: Synthesis of N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(ethyl (tetrahydro-2H-pyran-4-yl)amino)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide trihydrochloride

[0423] N-((4, 6-dimethyl-2-oxo-1, 2-dihydropyridin-3-yl) methyl)-5-(ethyl (tetrahydro-2H-pyran-4-yl) amino)-4-methyl-4'-(morpholinomethyl)-[1, 1'-biphenyl]-3-carboxamide (12 g, 21.0 mmol) was dissolved in methanolic HCl (200 mL) and stirred at room temperature for 3 h. After three hours of stirring, the reaction mixture was concentrated under reduced pressure. The solid obtained was stirred with ether (100mL X 2) to afford the desired salt as a solid (11 g, 77 %). **Analytical Data of the tri-HCl salt:** LCMS: 573.40 (M + 1)⁺; HPLC: 99.1% (@ 254 nm) (R_i;3.961; **Method:** Column: YMC ODS-A 150 mm x 4.6 mm x 5 μ ; Mobile Phase: A; 0.05% TFA in water/ B; 0.05% TFA in acetonitrile; Inj. Vol: 10 μ L, Col. Temp.: 30 °C; Flow rate: 1.4 mL/min.; Gradient: 5% B to 95% B in 8 min, Hold for 1.5 min, 9.51-12 min 5% B); ¹H NMR (D₂O 400 MHz) δ 7.92 (bs, 1H,) 7.80 (s, 1H), 7.77 (d, 2H, J=8 Hz), 7.63 (s, 1H), 7.61 (s, 1H), 6.30 (s, 1H), 4.48 (s, 2H), 4.42 (s, 2H), 4.09-4.11 (m, 4H), 3.95-3.97 (m, 2H), 3.77 (t,

3H, J=10.4 Hz), 3.44-3.47 (m, 3H), 3.24-3.32 (m, 3H), 2.42 (s, 3H), 2.35 (s, 3H), 2.26 (s, 3H), 2.01 (m, 2H), 1.76 (m, 2H), 1.04 (t, 3H, J=6.8 Hz).

Example 6: N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(((1r,4r)-4-(dimethylamino)cyclohexyl)(ethyl)amino)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide

Compound E

[0424] Step 1: 5-bromo-2-methyl-3-nitrobenzoic acid

[0425] To stirred solution of 2-methyl-3-nitrobenzoic acid (100 g, 552.48 mmol) in conc. H_2SO_4 (400 mL), 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione (87.98 g, 307.70 mmol) was added in a portion-wise manner at room temperature. The reaction mixture was then stirred at room temperature for 5 h. The reaction mixture was poured into ice cold water, the precipitated solid collected by filtration, washed with water and dried under vacuum to afford desired 5-bromo-2-methyl-3-nitrobenzoic acidas off-white solid (140 g, 97.90% yield). ¹H NMR (DMSO- d_6 , 400 MHz) δ 8.31 (s, 1H), 8.17 (s, 1H), 2.43 (s, 3H).

[0426] Step 2: methyl 5-bromo-2-methyl-3-nitrobenzoate

[0427] To a stirred solution of 5-bromo-2-methyl-3-nitrobenzoic acid (285 g, 1104.65 mmol) in DMF (2.8L) was added sodium carbonate (468 g, 4415.09 mmol) followed by addition of methyl iodide (626.63 g, 4415 mmol) at room temperature. The resulting reaction mixture was stirred at 60 °C for 8 h. The reaction mixture was then filtered to remove suspended solids which were washed well with ethyl acetate (3 x 1 L). The combined filtrates were washed well with water (5 x 3 L) and the aqueous phase back extracted with ethyl acetate (3 x 1 L). The combined organic extracts dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford methyl 5-bromo-2-methyl-3-nitrobenzoate as an off-white solid (290g, 97% yield). 1 H NMR (CDCl₃, 400 MHz) δ 8.17 (s, 1H), 7.91 (s, 1H), 3.96 (s, 3H), 2.59 (s, 3H).

[0428] Step 3: methyl 3-amino-5-bromo-2-methylbenzoate

[0429] To a stirred solution of methyl 5-bromo-2-methyl-3-nitrobenzoate (290 g, 1058.39 mmol) in ethanol (1.5 L) was added aqueous ammonium chloride (283 g, 5290 mmol dissolved in 1.5 L water). The resulting mixture was stirred and heated at 80 °C followed by addition of iron powder (472 g, 8451 mmol) in portions at 80 °C. The resulting reaction mixture was heated at 80 °C for 12 h. The reaction mixture was then hot filtered through Celite® and the Celite® bed washed well methanol (5 L) and then with 30% MeOH in DCM (5 L). The combined filtrates were concentrated in vacuo and the residue obtained was diluted with aqueous bicarbonate (2 L) and extracted with ethyl acetate (3 x 5 L). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford methyl 3-amino-5-bromo-2-methylbenzoate as a brown solid (220 g, 89.41% yield).

[0430] A portion of the product (5 g) was dissolved in hot ethanol (20mL), insoluble residue filtered off and mother liquor concentrated to obtain methyl 3-amino-5-bromo-2-methylbenzoate (3.5g, 70% yield) with HPLC purity 93.81% as light brown solid. 1 H NMR (CDCl₃, 400 MHz) δ 7.37 (s, 1H), 6.92 (s, 1H), 3.94 (s, 3H), 3.80 (bs, 2H), 2.31 (s, 3H).

[0431] Step 4: methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-2-methylbenzoate

[0432] To a stirred solution of methyl 3-amino-5-bromo-2-methylbenzoate(5 g, 20.5 mmol) and tert-butyl (4-oxocyclohexyl)carbamate (5.69 g, 26.7 mmol) in dichloroethane (50 mL), acetic acid (7.4 g, 123 mmol) was added and the reaction was stirred at room temperature for 10 minutes. Sodium triacetoxyborohydride (13.1 g, 61.7 mmol) was then added at 0 °C and reaction was stirred at room temperature for 16 hours. The reaction was quenched with aqueous sodium bicarbonate, the organic phase separated and the aqueous phase extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by silica gel column chromatography (100-200 mesh size) eluting with 10% ethyl acetate in hexane to afford 3.5 g of the more polar (trans) isomer, methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-2-methylbenzoate, as solid (38.46%). ¹H NMR (CDCl₃, 400 MHz) δ 7.21 (s, 1H), 6.80 (s, 1H), 4.41 (bs, 1H), 3.85 (s, 3H), 3.60 (m, 1H), 3.45 (m, 1H), 3.20 (m, 1H), 2.22 (s, 3H), 2.15 (bs, 2H), 2.05 (bs, 2H), 1.45 (s, 9H), 1.30 (m, 4H).

[0433] Step 5: methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexyl)-(ethyl)amino)-2-methylbenzoate

[0434] To a stirred solution of methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)-cyclohexyl)(ethyl)amino)-2-methylbenzoate (55 g, 0.124 mol) and acetaldehyde (11 g, 0.25 mol) in dichloroethane (550 mL), acetic acid (44.64 g, 0.744 mol) was added and the reaction mixture stirred at room temperature for 10 minutes. Sodium triacetoxyborohydride (79 g, 0.372 mol) was then added at 0 °C and the reaction mixture was stirred at room temperature for 16 hours. The reaction was quenched with aqueous sodium bicarbonate, the organic phase separated and the aqueous phase extracted with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate and concentrated in-vacuo. The crude compound was purified by silica gel column chromatography (100-200 mesh size) eluting with 10% ethyl acetate in hexane to afford 44 g of methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexyl)-(ethyl)amino)-2-methylbenzoate (75.2%) as solid. ¹H NMR (DMSO-d6, 400 MHz) δ 7.55 (s, 1H), 7.45 (s, 1H), 6.65 (d, 1H), 3.80 (s, 3H), 3.15 (bs, 1H), 3.05 (q, 2H), 2.60 (m, 1H), 2.30 (s, 3H), 1.75 (m, 4H), 1.40 (m, 2H), 1.35 (s, 9H), 1.10 (m, 2H), 0.80 (t, 3H).

[0435] Step 6: tert-butyl ((1r,4r)-4-((5-bromo-3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2-methylphenyl)(ethyl)amino)cyclohexyl)carbamate

[0436] Aqueous NaOH (3.5 g, 0.08 mol in 10 mL H_2O) was added to a solution of methyl 5-bromo-3-(((1r,4r)-4-((tert-butoxycarbonyl)amino)cyclohexyl)-(ethyl)amino)-2-methylbenzoate (25 g, 0.053 mol) in EtOH (100 mL) and stirred at 60 °C for 1 h. The ethanol was then removed under reduced pressure and acidified to pH 8 with dilute HCl and to pH 6 with citric acid. The mixture was extracted with 10% methanol in DCM (3 x 200 mL). The combined organic layers were dried and concentrated giving the respective acid (24.2 g, 99.0 %). 1 H NMR (DMSO-d6, 400 MHz) δ 13.13 (s, 1H), 7.54 (s, 1H), 7.43 (s, 1H), 6.68 (d, 1H), 3.14 (bs, 1H), 3.03 (q, 2H), 2.56 (m, 1H), 2.33 (s, 3H), 1.80-1.65 (m, 4H), 1.40 (m, 2H), 1.35 (s, 9H), 1.10 (m, 2H), 0.77 (t, 3H).

[0437] The acid (24 g, 0.053 mol) was dissolved in DMSO (100 mL) and 3-(aminomethyl)-4,6-dimethylpyridin-2(1H)-one (16 g, 0.106 mol) and triethylamine (5.3 g, 0.053 mol) was added. The reaction mixture was stirred at room temperature for 15 min before PyBop (41 g, 0.079 mmol) was added and stirring was then continued for overnight at room

temperature. The reaction mixture was poured into ice water (1L). The resulting precipitate was collected by filtration, washed well with water (2 x 1L) and dried. The product obtained was further purified by washings with acetonitrile (3 x 200 mL) and DCM (100 mL) to afford tert-butyl ((1r,4r)-4-((5-bromo-3-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2-methylphenyl)(ethyl)amino)cyclohexyl)-carbamate (24 g, 77 %). 1 H NMR (DMSO-d6, 400 MHz) δ 11.47 (s, 1H), 8.24 (t, 1H), 7.25 (s, 1H), 7.04 (s, 1H), 6.67 (d, 1H), 5.85 (s, 1H), 4.24 (d, 2H), 3.13 (bs, 1H), 3.01 (q, 2H), 2.53 (m, 1H), 2.18 (s, 3H), 2.10 (s, 6H), 1.80-1.65 (m, 4H), 1.40 (m, 2H), 1.35 (s, 9H), 1.10 (m, 2H), 0.77 (t, 3H). [0438] Step 7: tert-butyl ((1r,4r)-4-((5-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)(ethyl)amino)cyclohexyl)carbamate

To a stirred solution of tert-butyl ((1r,4r)-4-((5-bromo-3-(((4,6-dimethyl-2-oxo-1,2-[0439] dihydropyridin-3-yl)methyl)carbamoyl)-2-methylphenyl)(ethyl)amino)cyclohexyl)-carbamate (24 g, 0.041 mol) and 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)morpholine (18 g, 0.061 mol) in dioxane/ water mixture (160 mL + 40 mL), Na₂CO₃ (15 g, 0.15 mol) was added and solution purged with argon for 15 min. Pd(PPh₃)₄ (4.7 g, 0.041 mol) was then added and the reaction mixture again purged with argon for 10 min. The reaction mixture was heated at 100 °C for 4 h. The reaction mixture was then diluted with 10% MeOH/ DCM (500 mL) and filtered. The filtrate was concentrated, diluted with water (500 mL) and extracted with 10% MeOH in DCM (3 x 500mL). The combined organic layers were dried over Na₂SO₄ and solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography (100-200 mesh) eluting with 7% MeOH in DCM to afford tert-butyl ((1r,4r)-4-((5-(((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)(ethyl)amino)cyclohexyl)carbamate (20 g, 71.43 %). ¹H NMR (DMSO-d6, 400 MHz) δ 11.46 (s, 1H), 8.20 (t, 1H), 7.56 (d, 2H), 7.36 (m, 3H), 7.17 (s, 1H), 6.66 (d, 1H), 5.85 (s, 1H), 4.28 (d, 2H), 3.57 (bs, 4H), 3.48 (s, 2H), 3.20-3.05 (m, 3H), 2.62 (m, 1H), 2.36 (bs, 4H), 2.20 (s, 6H), 2.10 (s, 3H), 1.75 (m, 4H), 1.42 (m, 2H), 1.35 (s, 9H), 1.10 (m, 2H), 0.82 (t, 3H).

[0440] Step 8: 5-(((1r,4r)-4-aminocyclohexyl)(ethyl)amino)-N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide

[0441] To a stirred solution of tert-butyl ((1r,4r)-4-((5-(((4,6-dimethyl-2-oxo-1,2-dimethyl-2dihydropyridin-3-yl)methyl)carbamoyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3yl)(ethyl)amino)cyclohexyl)carbamate (20 g, 0.03 mol) in DCM (200 mL) at 0 °C, TFA (75 mL) was added and reaction was stirred for 2 h at room temperature. The reaction mixture was then concentrated to dryness and the residue basified with aqueous saturated bicarbonate solution (300 mL) to pH 8. The mixture was extracted with 20% methanol in DCM (4 x 200 m). The combined extracts were dried over Na₂SO₄ and the solvent removed under reduced pressure to afford 5-(((1r,4r)-4-aminocyclohexyl)(ethyl)amino)-N-((4,6-dimethyl-2-oxo-1,2dihydropyridin-3-yl)methyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide (15.5 g, 91%) which was used as is in the next reaction. 1H NMR (DMSO-d6, 400 MHz) $\delta\,8.18$ (bs, 1H), 7.57 (d, 2H), 7.38 (m, 3H), 7.20 (s, 1H), 5.85 (s, 1H), 4.29 (d, 2H), 3.57 (bs, 4H), 3.48 (s, 2H), 3.31 (bs, 2H), 3.10 (m, 2H), 2.91 (m, 1H), 2.67 (m, 1H), 2.36 (bs, 4H), 2.21 (s, 3H), 2.20 (s, 3H), 2.10 (s, 3H), 1.90 (m, 2H), 1.83 (m, 2H), 1.45 (m, 2H), 1.23 (m, 2H), 0.83 (t, 3H). Step 9: N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(((1r,4r)-4-(dimethylamino)cyclohexyl)(ethyl)amino)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3carboxamide

[0443] To a stirred solution of 5-(((1r,4r)-4-aminocyclohexyl)(ethyl)amino)-N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-4-methyl-4'-(morpholinomethyl)-[1,1'-biphenyl]-3-carboxamide(14g, 0.023 mol) in dichloromethane (150 mL) was added aqueous 35% formaldehyde solution (2.4g, 0.080 mol) at 0° C. After stirring for 20 min, Na(OAc)₃BH (12.2 g, 0.057 mol) was added and stirring continued for 2h at 0° C. Water (100 mL) was then added to the reaction mixture and the mixture extracted with 20% methanol in DCM (3 x 200 mL). The combined extracts were dried over Na₂SO₄ and the solvent removed under reduced pressure. The crude product was purified by basic alumina column chromatography eluting with 6-7% MeOH in DCM to afford the title compound (10 g, 63.6%). LCMS: 614.65 (M + 1)⁺; HPLC: 98.88% (@ 210-370 nm) (R_t;3.724; Method: Column: YMC ODS-A 150 mm x 4.6 mm x 5 μ; Mobile Phase: A; 0.05% TFA in water/ B; 0.05% TFA in acetonitrile; Inj. Vol: 10 μL, Col. Temp.: 30 °C; Flow rate: 1.4 mL/min.; Gradient: 5% B to 95% B in 8 min, Hold for 1.5 min, 9.51-12 min 5% B); ¹H NMR (DMSO-d6, 400 MHz) δ 11.45 (s, 1H), 8.17 (t, 1H), 7.56 (d, 2H, J=8 Hz), 7.36 (m, 3H), 7.17 (s, 1H), 5.85 (s, 1H), 4.29 (d, 2H, J=4.4 Hz), 3.57 (bs,

4H), 3.48 (s, 2H), 3.09 (q, 2H), 2.66 (m, 1H), 2.36 (bs, 4H), 2.21 (s, 3H), 2.20 (s, 3H), 2.11 (s, 9H), 1.79 (m, 4H), 1.36 (m, 2H), 1.11 (m, 2H), 0.82 (t, 3H, J=6.4&6.8 Hz).

[0444] **Example 7:** Bioassay protocol and General Methods Protocol for Wild-Type and Mutant PRC2 Enzyme Assays

[0445] **General Materials.** *S*-adenosylmethionine (SAM), *S*-adenosylhomocyteine (SAH), bicine, KCl, Tween20, dimethylsulfoxide (DMSO) and bovine skin gelatin (BSG) were purchased from Sigma-Aldrich at the highest level of purity possible. Dithiothreitol (DTT) was purchased from EMD. ³H-SAM was purchased from American Radiolabeled Chemicals with a specific activity of 80 Ci/mmol. 384-well streptavidin Flashplates were purchased from PerkinElmer.

Substrates. Peptides representative of human histone H3 residues 21 – 44 containing either an unmodified lysine 27 (H3K27me0) or dimethylated lysine 27 (H3K27me2) were synthesized with a C-terminal G(K-biotin) linker-affinity tag motif and a C-terminal amide cap by 21st Century Biochemicals. The peptides were high-performance liquid chromatography (HPLC) purified to greater than 95% purity and confirmed by liquid chromatography mass spectrometry (LC-MS). The sequences are listed below.

H3K27me0: ATKAARKSAPATGGVKKPHRYRPGGK(biotin)-amide (SEQ ID NO: 7)

H3K27me2: ATKAARK(me2)SAPATGGVKKPHRYRPGGK(biotin)-amide (SEQ ID NO: 8)

[0447] Chicken erythrocyte oligonucleosomes were purified from chicken blood according to established procedures.

[0448] **Recombinant PRC2 Complexes.** Human PRC2 complexes were purified as 4-component enzyme complexes co-expressed in *Spodoptera frugiperda* (sf9) cells using a baculovirus expression system. The subunits expressed were wild-type EZH2 (NM_004456) or EZH2 Y641F, N, H, S or C mutants generated from the wild-type EZH2 construct, EED (NM_003797), Suz12 (NM_015355) and RbAp48 (NM_005610). The EED subunit contained an N-terminal FLAG tag that was used to purify the entire 4-component complex from sf9 cell lysates. The purity of the complexes met or exceeded 95% as determined by SDS-PAGE and Agilent Bioanalyzer analysis. Concentrations of enzyme stock concentrations (generally 0.3 –

1.0 mg/mL) was determined using a Bradford assay against a bovine serum albumin (BSA) standard.

[0449] General Procedure for PRC2 Enzyme Assays on Peptide Substrates. The assays were all performed in a buffer consisting of 20 mM bicine (pH = 7.6), 0.5 mM DTT, 0.005% BSG and 0.002% Tween20, prepared on the day of use. Compounds in 100% DMSO (1 μL) were spotted into polypropylene 384-well V-bottom plates (Greiner) using a Platemate 2 X 3 outfitted with a 384-channel pipet head (Thermo). DMSO (1 μL) was added to columns 11, 12, 23, 24, rows A – H for the maximum signal control, and SAH, a known product and inhibitor of PRC2 (1 µL) was added to columns 11,12, 23, 24, rows I – P for the minimum signal control. A cocktail (40 μL) containing the wild-type PRC2 enzyme and H3K27me0 peptide or any of the Y641 mutant enzymes and H3K27me2 peptide was added by Multidrop Combi (Thermo). The compounds were allowed to incubate with PRC2 for 30 min at 25 °C, then a cocktail (10 µL) containing a mixture of non-radioactive and ³H-SAM was added to initiate the reaction (final volume = $51 \mu L$). In all cases, the final concentrations were as follows: wild-type or mutant PRC2 enzyme was 4 nM, SAH in the minimum signal control wells was 1 mM and the DMSO concentration was 1%. The final concentrations of the rest of the components are indicated in Table 7, below. The assays were stopped by the addition of non-radioactive SAM (10 μL) to a final concentration of 600 μM, which dilutes the ³H-SAM to a level where its incorporation into the peptide substrate is no longer detectable. 50 µL of the reaction in the 384-well polypropylene plate was then transferred to a 384-well Flashplate and the biotinylated peptides were allowed to bind to the streptavidin surface for at least 1h before being washed three times with 0.1% Tween20 in a Biotek ELx405 plate washer. The plates were then read in a PerkinElmer TopCount platereader to measure the quantity of ³H-labeled peptide bound to the Flashplate surface, measured as disintegrations per minute (dpm) or alternatively, referred to as counts per minute (cpm).

[0450] Table 7: Final concentrations of components for each assay variation based upon EZH2 identity (wild-type or Y641 mutant EZH2)

PRC2 Enzyme (denoted by EZH2 identity)	Peptide (nM)	Non-radioactive SAM (nM)	³ H-SAM (nM)
Wild-type	185	1800	150
Y641F	200	850	150
Y641N	200	850	150

Y641H	200	1750	250
Y641S	200	1300	200
Y641C	200	3750	250

General Procedure for Wild-Type PRC2 Enzyme Assay on Oligonucleosome T04511 **Substrate.** The assays was performed in a buffer consisting of 20 mM bicine (pH = 7.6), 0.5mM DTT, 0.005% BSG, 100 mM KCl and 0.002% Tween20, prepared on the day of use. Compounds in 100% DMSO (1 µL) were spotted into polypropylene 384-well V-bottom plates (Greiner) using a Platemate 2 X 3 outfitted with a 384-channel pipet head (Thermo). DMSO (1 μL) was added to columns 11, 12, 23, 24, rows A – H for the maximum signal control, and SAH, a known product and inhibitor of PRC2 (1 µL) was added to columns 11,12, 23, 24, rows I – P for the minimum signal control. A cocktail (40 μL) containing the wild-type PRC2 enzyme and chicken erythrocyte oligonucleosome was added by Multidrop Combi (Thermo). The compounds were allowed to incubate with PRC2 for 30 min at 25 °C, then a cocktail (10 μL) containing a mixture of non-radioactive and ³H-SAM was added to initiate the reaction (final volume = $51 \mu L$). The final concentrations were as follows: wild-type PRC2 enzyme was 4 nM, non-radioactive SAM was 430 nM, ³H-SAM was 120 nM, chicken erythrocyte olignonucleosome was 120 nM, SAH in the minimum signal control wells was 1 mM and the DMSO concentration was 1%. The assay was stopped by the addition of non-radioactive SAM (10 μ L) to a final concentration of 600 μ M, which dilutes the ³H-SAM to a level where its incorporation into the chicken erythrocyte olignonucleosome substrate is no longer detectable. 50 μL of the reaction in the 384-well polypropylene plate was then transferred to a 384-well Flashplate and the chicken erythrocyte nucleosomes were immobilized to the surface of the plate, which was then washed three times with 0.1% Tween20 in a Biotek ELx405 plate washer. The plates were then read in a PerkinElmer TopCount platereader to measure the quantity of ³H-labeled chicken erythrocyte oligonucleosome bound to the Flashplate surface, measured as disintegrations per minute (dpm) or alternatively, referred to as counts per minute (cpm).

[0452] **% Inhibition Calculation**

% inh=100-
$$\left(\frac{dpm_{cmpd}-dpm_{min}}{dpm_{max}-dpm_{min}}\right) x100$$

[0453] Where dpm = disintegrations per minute, cmpd = signal in assay well, and min and max are the respective minimum and maximum signal controls.

[0454] Four-parameter IC₅₀ fit

$$Y=Bottom+ \frac{(Top\text{-Bottom})}{1+(\frac{X}{IC_{50}})^{Hill\ Coefficient}}$$

[0455] Where top and bottom are the normally allowed to float, but may be fixed at 100 or 0 respectively in a 3-parameter fit. The Hill Coefficient normally allowed to float but may also be fixed at 1 in a 3-parameter fit. Y is the % inhibition and X is the compound concentration.

[0456] IC₅₀ values for the PRC2 enzyme assays on peptide substrates (e.g., EZH2 wild type and Y641F) are presented in Table 8 below.

[0457] WSU-DLCL2 Methylation Assay

[0458] WSU-DLCL2 suspension cells were purchased from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). RPMI/Glutamax Medium, Penicillin-Streptomycin, Heat Inactivated Fetal Bovine Serum, and D-PBS were purchased from Life Technologies, Grand Island, NY, USA. Extraction Buffer and Neutralization Buffer(5X) were purchased from Active Motif, Carlsbad, CA, USA. Rabbit anti-Histone H3 antibody was purchased from Abcam, Cambridge, MA, USA. Rabbit anti-H3K27me3 and HRP-conjugated anti-rabbit-IgG were purchased from Cell Signaling Technology, Danvers, MA, USA. TMB "Super Sensitive" substrate was sourced from BioFX Laboratories, Owings Mills, MD, USA. IgG-free Bovine Serum Albumin was purchased from Jackson ImmunoResearch, West Grove, PA, USA. PBS with Tween (10X PBST) was purchased from KPL, Gaithersburg, MD, USA. Sulfuric Acid was purchased from Ricca Chemical, Arlington, TX, USA. Immulon ELISA plates were purchased from Thermo, Rochester, NY, USA. Vbottom cell culture plates were purchased from Corning Inc., Corning, NY, USA.V-bottom polypropylene plates were purchased from Greiner Bio-One, Monroe, NC, USA. WSU-DLCL2 suspension cells were maintained in growth medium (RPMI 1640 [0459]

supplemented with 10% v/v heat inactivated fetal bovine serum and 100 units/mL penicillin-streptomycin) and cultured at 37 °C under 5% CO₂. Under assay conditions, cells were

incubated in Assay Medium (RPMI 1640 supplemented with 20% v/v heat inactivated fetal bovine serum and 100 units/mL penicillin-streptomycin) at 37 °C under 5% CO₂ on a plate shaker.

[0460] WSU-DLCL2 cells were seeded in assay medium at a concentration of 50,000 cells per mL to a 96-well V-bottom cell culture plate with 200 µL per well. Compound (1µL) from 96 well source plates was added directly to V-bottom cell plate. Plates were incubated on a titer-plate shaker at 37°C, 5% CO2 for 96 hours. After four days of incubation, plates were spun at 241 x g for five minutes and medium was aspirated gently from each well of cell plate without disturbing cell pellet. Pellet was resuspended in 200 µL DPBS and plates were spun again at 241 x g for five minutes. The supernatant was aspirated and cold (4°C) Extraction buffer (100 µL) was added per well. Plates were incubated at 4°C on orbital shaker for two hours. Plates were spun at 3427 x g x 10 minutes. Supernatant (80 µL per well) was transferred to its respective well in 96 well V-bottom polypropylene plate. Neutralization Buffer 5X (20 µL per well) was added to V-bottom polypropylene plate containing supernatant. V-bottom polypropylene plates containing crude histone preparation (CHP) were incubated on orbital shaker x five minutes. Crude Histone Preparations were added (2µL per well) to each respective well into duplicate 96 well ELISA plates containing 100 µL Coating Buffer (1X PBS + BSA 0.05% w/v). Plates were sealed and incubated overnight at 4°C. The following day, plates were washed three times with 300 µL per well 1X PBST. Wells were blocked for two hours with 300 µL per well ELISA Diluent ((PBS (1X) BSA (2% w/v) and Tween20 (0.05%) v/v)). Plates were washed three times with 1X PBST. For the Histone H3 detection plate, 100 μL per well were added of anti-Histone-H3 antibody (Abcam, ab1791) diluted 1:10,000 in ELISA Diluent. For H3K27 trimethylation detection plate, 100 µL per well were added of anti-H3K27me3 diluted 1:2000 in ELISA diluent. Plates were incubated for 90 minutes at room temperature. Plates were washed three times with 300 µL 1X PBST per well. For Histone H3 detection, 100 µL of HRP-conjugated anti-rabbit IgG antibody diluted to 1:6000 in ELISA diluent was added per well. For H3K27me3 detection, 100 µL of HRP conjugated anti-rabbit IgG antibody diluted to 1:4000 in ELISA diluent was added per well. Plates were incubated at room temperature for 90 minutes. Plates were washed four times with 1X PBST 300 μL per well. TMB substrate 100 µL was added per well. Histone H3 plates were incubated for five minutes at room temperature. H3K27me3 plates were incubated for 10 minutes at room

temperature. The reaction was stopped with sulfuric acid 1N (100 μ L per well). Absorbance for each plate was read at 450 nm.

[0461] First, the ratio for each well was determined by: $\left(\frac{H3K27me3\ OD450\ value}{Histore\ H3\ OD450\ value}\right)$

[0462] Each plate included eight control wells of DMSO only treatment (Minimum Inhibition) as well as eight control wells for maximum inhibition (Background wells).

[0463] The average of the ratio values for each control type was calculated and used to determine the percent inhibition for each test well in the plate. Test compound was serially diluted three-fold in DMSO for a total of ten test concentrations, beginning at 25 μ M. Percent inhibition was determined and IC₅₀ curves were generated using duplicate wells per concentration of compound. IC₅₀ values for this assay are presented in Table 8 below.

[0464] Percent Inhibition = 100-

$$\left(\left(\frac{\text{(Individual Test Sample Ratio)} - \text{(Background Avg Ratio)}}{\text{(Minimum Inhibition Ratio)} - \text{(Background Average Ratio)}} \right) * 100 \right)$$

[0465] Cell proliferation analysis

[0466] WSU-DLCL2 suspension cells were purchased from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). RPMI/Glutamax Medium, Penicillin-Streptomycin, Heat Inactivated Fetal Bovine Serum were purchased from Life Technologies, Grand Island, NY, USA. V-bottom polypropylene 384-well plates were purchased from Greiner Bio-One, Monroe, NC, USA. Cell culture 384-well white opaque plates were purchased from Perkin Elmer, Waltham, MA, USA. Cell-Titer Glo® was purchased from Promega Corporation, Madison, WI, USA. SpectraMax M5 plate reader was purchased from Molecular Devices LLC, Sunnyvale, CA, USA.

[0467] WSU-DLCL2 suspension cells were maintained in growth medium (RPMI 1640 supplemented with 10% v/v heat inactivated fetal bovine serum and cultured at 37 °C under 5% CO₂. Under assay conditions, cells were incubated in Assay Medium (RPMI 1640 supplemented with 20% v/v heat inactivated fetal bovine serum and 100 units/mL penicillin-streptomycin) at 37 °C under 5% CO₂.

For the assessment of the effect of compounds on the proliferation of the WSU-DLCL2 cell line, exponentially growing cells were plated in 384-well white opaque plates at a density of 1250 cell/ml in a final volume of 50 μ l of assay medium. A compound source plate was prepared by performing triplicate nine-point 3-fold serial dilutions in DMSO, beginning at 10

mM (final top concentration of compound in the assay was 20 μ M and the DMSO was 0.2%). A 100 nL aliquot from the compound stock plate was added to its respective well in the cell plate. The 100% inhibition control consisted of cells treated with 200 nM final concentration of staurosporine and the 0% inhibition control consisted of DMSO treated cells. After addition of compounds, assay plates were incubated for 6 days at 37°C, 5% CO₂, relative humidity > 90% for 6 days. Cell viability was measured by quantization of ATP present in the cell cultures, adding 35 μ l of CellTiter-Glo®® reagent to the cell plates. Luminescence was read in the SpectraMax M5.The concentration inhibiting cell viability by 50% was determined using a 4-parametric fit of the normalized dose response curves.

INCORPORATION BY REFERENCE

[0468] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

[0469] 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 above are for purposes of illustration and not limitation of the claims that follow.

EQUIVALENTS

[0470] 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 a SWI/SNF-associated cancer in a subject comprising administering to a subject in need thereof a therapeutically effective amount of an EZH2 inhibitor, wherein the subject has a cancer selected from the group consisting of brain and central nervous system cancer, head and neck cancer, kidney cancer, ovarian cancer, pancreatic cancer, leukemia, lung cancer, lymphoma, myeloma, sarcoma, breast cancer, and prostate cancer.

- 2. The method of claim 1, wherein the SWI/SNF-associated cancer is characterized by reduced expression or loss of function of the SWI/SNF complex or one or more components of the SWI/SNF complex.
- 3. The method of claim 1, wherein the subject has a cancer selected from the group consisting of medulloblastoma, malignant rhabdoid tumor and atypical teratoid/rhabdoid tumor.
- 4. The method of claim 2, wherein the one or more components are selected from the group consisting of SNF5, ATRX and ARID1A.
- 5. The method of claim 2, wherein the loss of function is caused by a loss of function mutation resulting from a point mutation, a deletion and/or an insertion.
- 6. The method of claim 1, wherein the subject has a deletion of SNF5.
- 7. The method of claim 1, wherein the subject has a mutation of ATRX selected from the group consisting of a substitution of asparagine (N) for the wild type residue lysine (K) at amino acid position 688 of SEQ ID NO: 5 (K688N), and a substitution of isoleucine (I) for the wild type residue methionine (M) at amino acid position 366 of SEQ ID NO: 5 (M366I).
- 8. The method of claim 1, wherein said subject has a mutation of ARID1A selected from the group consisting of a nonsense mutation for the wild type residue cysteine (C) at amino acid position 884 of SEQ ID NO: 11 (C884*), a substitution of lysine (K) for the wild type

residue glutamic acid (E) at amino acid position 966 (E966K), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1411 of SEQ ID NO: 11 (Q1411*), a frame shift mutation at the wild type residue phenylalanine (F) at amino acid position 1720 of SEQ ID NO: 11 (F1720fs), a frame shift mutation after the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue cysteine (C) at amino acid position 1874 of SEQ ID NO: 11 (C1874fs), a substitution of glutamic acid (E) for the wild type residue aspartic acid (D) at amino acid position 1957 (D1957E), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1430 of SEQ ID NO: 11 (Q1430*), a frame shift mutation at the wild type residue arginine (R) at amino acid position 1721 of SEQ ID NO: 11 (R1721fs), a substitution of glutamic acid (E) for the wild type residue glycine (G) at amino acid position 1255 (G1255E), a frame shift mutation at the wild type residue glycine (G) at amino acid position 284 of SEQ ID NO: 11 (G284fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1722 of SEQ ID NO: 11 (R1722*), a frame shift mutation at the wild type residue methionine (M) at amino acid position 274 of SEQ ID NO: 11 (M274fs), a frame shift mutation at the wild type residue glycine (G) at amino acid position 1847 of SEQ ID NO: 11 (G1847fs), a frame shift mutation at the wild type residue P at amino acid position 559 of SEQ ID NO: 11 (P559fs), a nonsense mutation for the wild type residue arginine (R) at amino acid position 1276 of SEQ ID NO: 11 (R1276*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 2176 of SEQ ID NO: 11 (Q2176fs), a frame shift mutation at the wild type residue histidine (H) at amino acid position 203 of SEQ ID NO: 11 (H203fs), a frame shift mutation at the wild type residue alanine (A) at amino acid position 591 of SEQ ID NO: 11 (A591fs), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 1322 of SEQ ID NO: 11 (Q1322*), a nonsense mutation for the wild type residue serine (S) at amino acid position 2264 of SEQ ID NO: 11 (S2264*), a nonsense mutation for the wild type residue glutamine (Q) at amino acid position 586 of SEQ ID NO: 11 (Q586*), a frame shift mutation at the wild type residue glutamine (Q) at amino acid position 548 of SEQ ID NO: 11 (Q548fs), and a frame shift mutation at the wild type residue asparagine (N) at amino acid position 756 of SEQ ID NO: 11 (N756fs).

9. A method of treating or alleviating a symptom of a SWI/SNF-associated cancer in a subject in need thereof comprising:

- a. determining the expression level of at least one gene selected from the group consisting of neuronal differentiation genes, cell cycle inhibition genes and tumor suppressor genes in a sample obtained from the subject;
- b. selecting a subject having a decreased expression level of at least one gene in step a; and
- c. administering to the subject selected in step b an effective amount of an EZH2 inhibitor, thereby treating or alleviating a symptom of cancer in the subject.
- 10. A method of treating or alleviating a symptom of a SWI/SNF-associated cancer in a subject in need thereof comprising:
 - a. determining the expression level of at least one gene selected from the group consisting of hedgehog pathway genes, myc pathway genes and histone methyltransferase genes in a sample obtained from the subject,
 - b. selecting a subject having an increased expression level of at least one gene in step a; and
 - c. administering to the subject selected in step b an effective amount of an EZH2 inhibitor, thereby treating or alleviating a symptom of cancer in the subject.
- 11. The method of claim 9, wherein the cancer is selected from the group consisting of medulloblastoma, malignant rhabdoid tumor, and atypical teratoid rhabdoid tumor.
- 12. The method of claim 10, wherein the cancer is selected from the group consisting of medulloblastoma, malignant rhabdoid tumor, and atypical teratoid rhabdoid tumor.
- 13. The method of claim 9, wherein the neuronal differentiation gene is CD133, DOCK4, or PTPRK.
- 14. The method of claim 9, wherein the cell cycle inhibition gene is CKDN1A or CDKN2A.

- 15. The method of claim 9, wherein the tumor suppressor gene is BIN1.
- 16. The method of claim 10, wherein the hedgehog pathway gene is GLI1 or PTCH1.
- 17. The method of claim 10, wherein the myc pathway gene is MYC.
- 18. The method of claim 10, wherein the histone methyltransferase gene is EZH2.
- 19. A method of inducing neuronal differentiation, cell cycle inhibition or tumor suppression comprising contacting a cell with an EZH2 inhibitor.
- 20. The method of claim 19, wherein the EZH2 inhibitor is in an amount sufficient to increase expression of at least one gene selected from the group consisting of CD133, DOCK4,PTPRK, CKDN1A,CDKN2A, andBIN1.
- 21. A method of inhibiting hedgehog signaling comprising contacting a cell with an EZH2 inhibitor.
- 22. The method of claim 21, wherein the EZH2 inhibitor is in an amount sufficient to reduce expression of GLI1 and/or PTCH1.
- 23. A method of inducing gene expression comprising contacting a cell with an EZH2 inhibitor.
- 24. The method of claim 23, wherein the EZH2 inhibitor is in an amount sufficient to induce neuronal differentiation, cell cycle inhibition and/or tumor suppression.
- 25. The method of claim 23, wherein the gene is selected from the group consisting of CD133, DOCK4, PTPRK, CKDN1A, CKDN2A, and BIN1.
- 26. A method of inhibiting gene expression comprising contacting a cell with an EZH2 inhibitor.
- 27. The method of claim 26, wherein the EZH2 inhibitor is in an amount sufficient to inhibit hedgehog signaling.

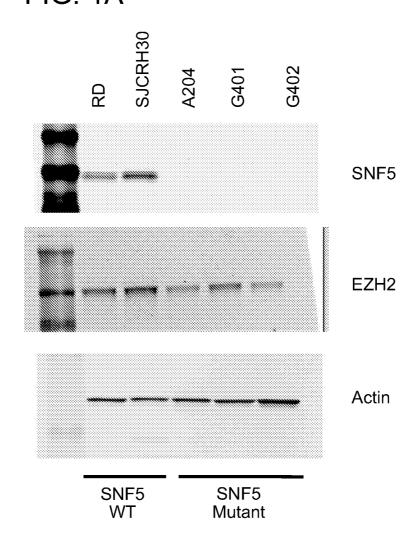
- 28. The method of claim 26, wherein the gene is GLI1 or PTCH1.
- 29. The method of any one of claims 19-28, wherein the cell comprises loss of function of SNF5, ARID1A, ATRX, and/or a component of the SWI/SNF complex.
- 30. The method of claim 29, wherein the loss of function is caused by a deletion of SNF5.
- 31. The method of any one of 19-28, wherein the cell is a cancer cell.
- 32. The method of claim 31, wherein the cancer is selected from the group consisting of medulloblastoma, malignant rhabdoid tumor, and atypical teratoid rhabdoid tumor.
- 33. The method of any one of the preceding claims, wherein the EZH2 inhibitor is:

(A) or pharmaceutically acceptable salt thereof.

34. The method of any one of the preceding claims, wherein the EZH2 inhibitor is:

148

FIG. 1A



2/29

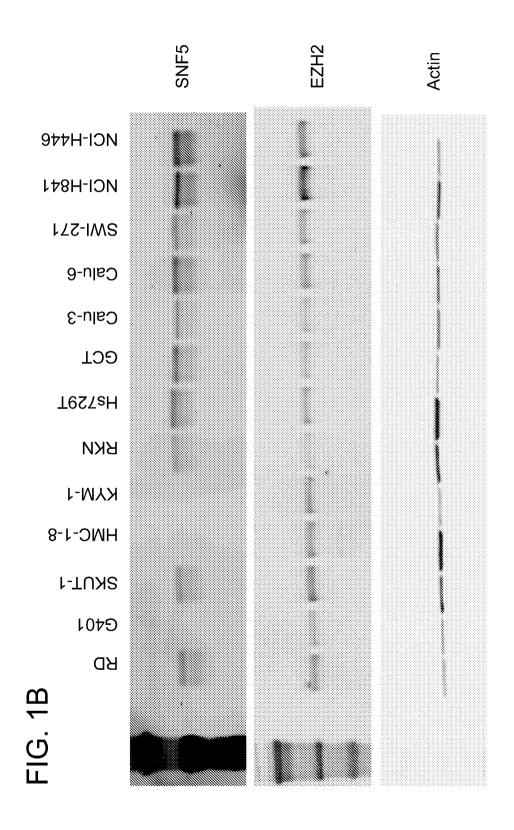
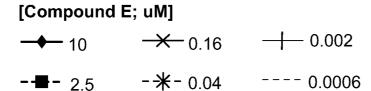


FIG. 2A

SNF5 WT



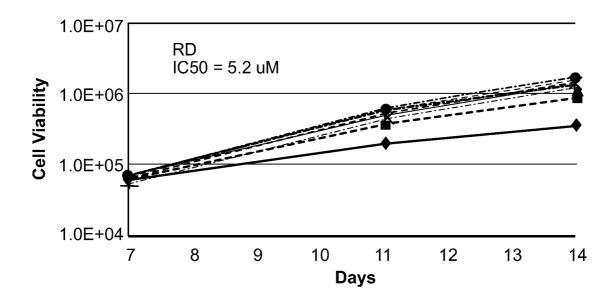
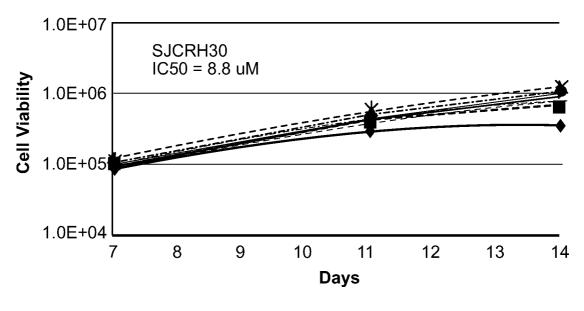


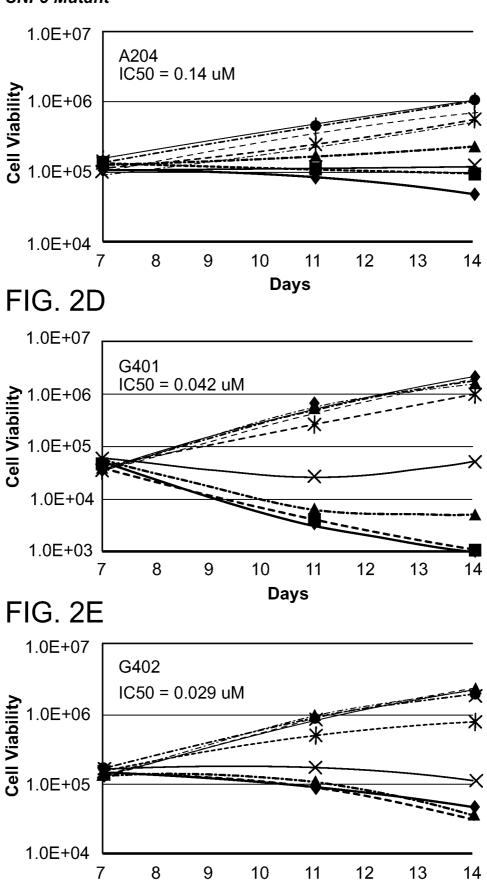
FIG. 2B



SUBSTITUTE SHEET (RULE 26)

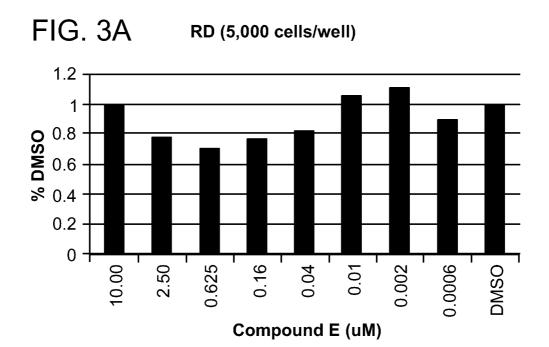


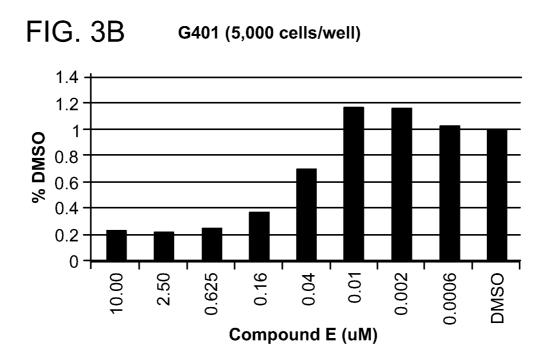
SNF5 Mutant



SUBSTITUTE SHEET (RULE 26)

Days





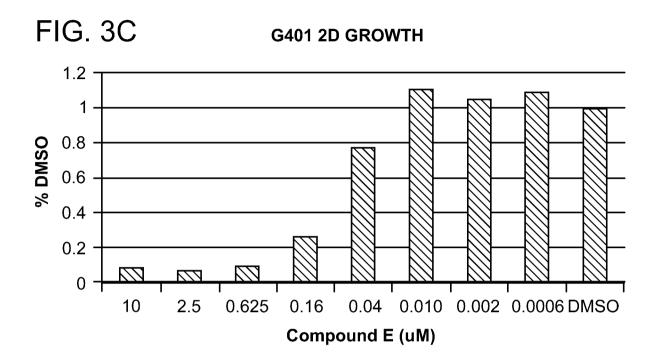
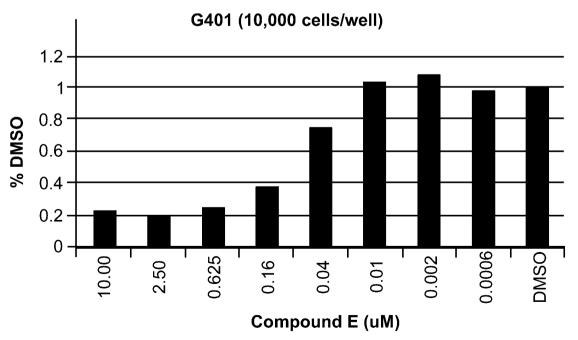
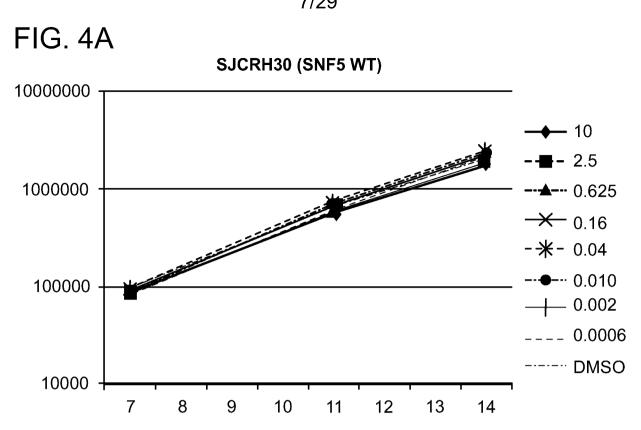
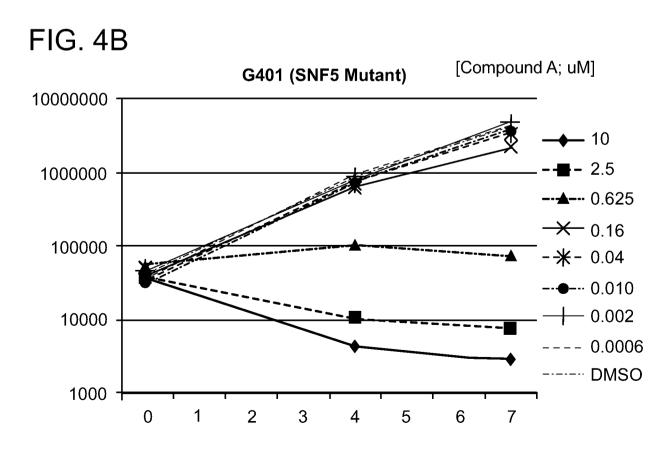


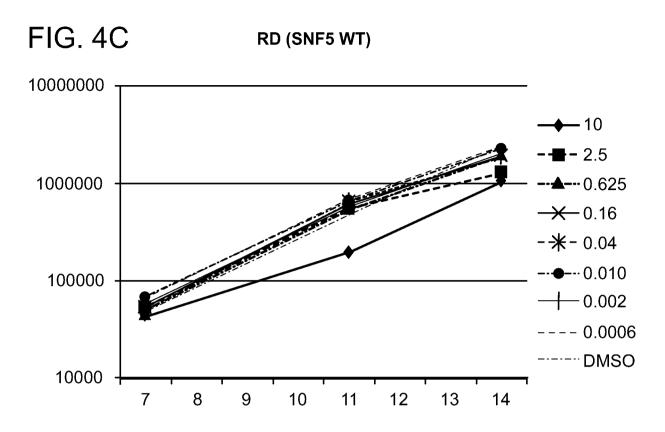
FIG. 3D

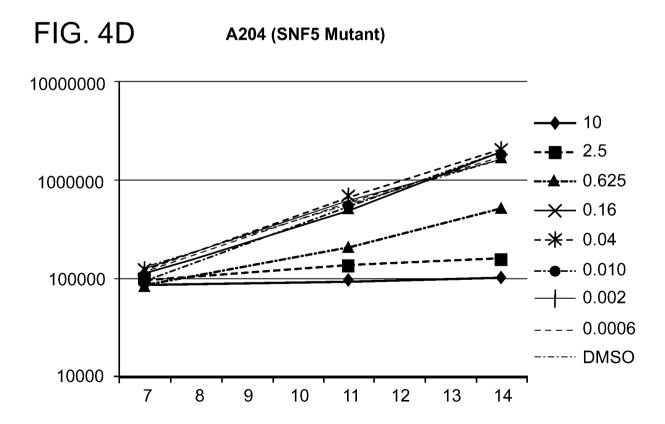






SUBSTITUTE SHEET (RULE 26)





SUBSTITUTE SHEET (RULE 26)

FIG. 5A

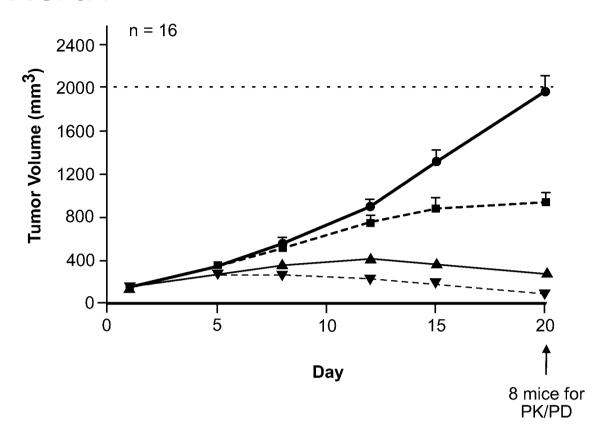
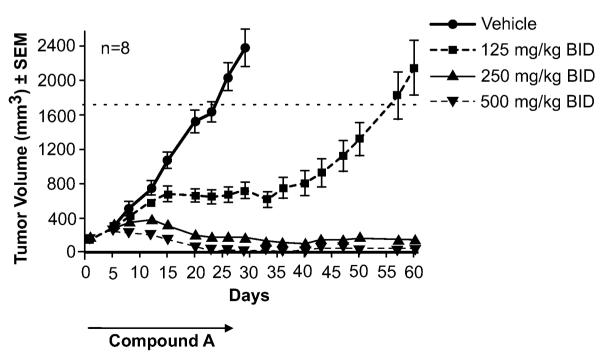
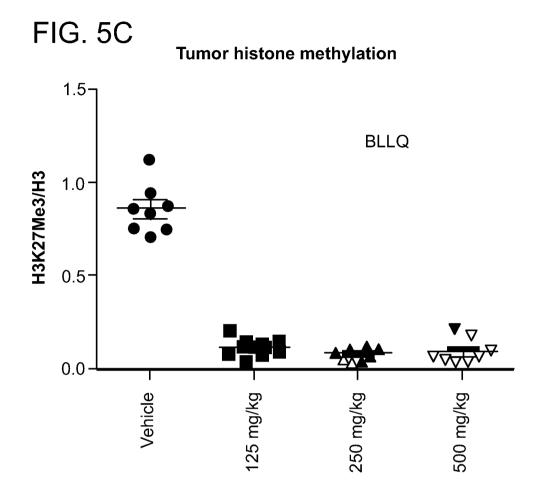
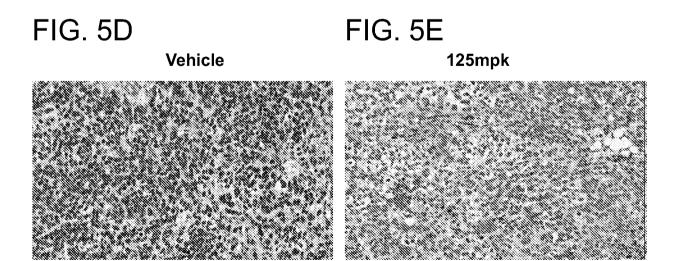
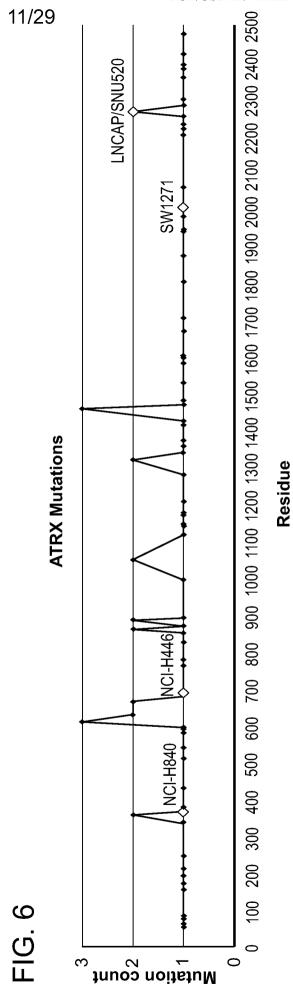


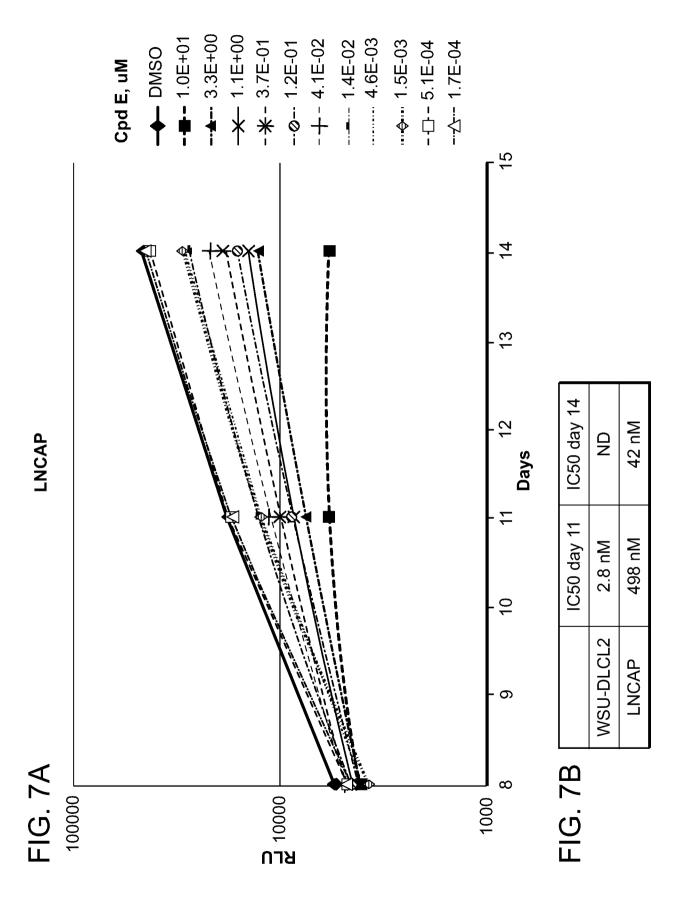
FIG. 5B











SUBSTITUTE SHEET (RULE 26)

PCT/US2013/065112

FIG. 8A



13/29

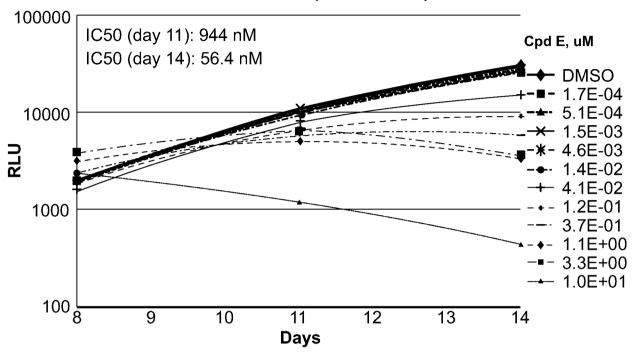
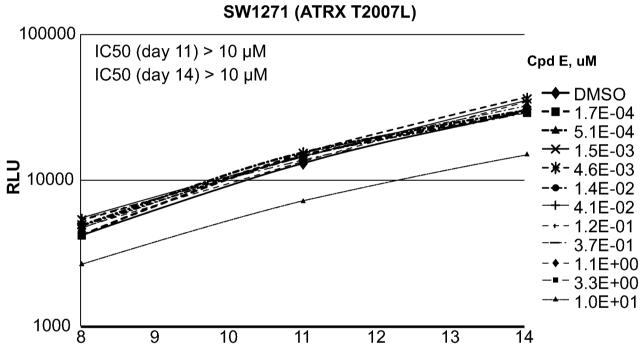


FIG. 8B

NCI-H841 (ATRX M366I) 100000 IC50 (day 11): 486 nM Cpd E, uM IC50 (day 11): 64.8 nM - DMSO - 1.7E-04 ·▲· 5.1E-04 × 1.5E-03 -*-4.6E-03 10000 ●- 1.4E-02 +- 4.1E-02 -+-1.2E-01 -- 3.7E-01 - **+**- 1.1E+00 -•-3.3E+00 --- 1.0E+01 1000 9 10 11 12 13 14 **Days**

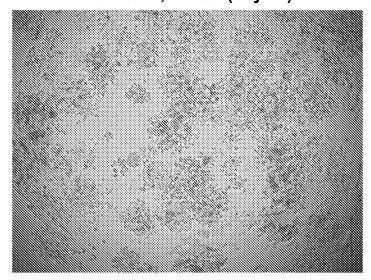
FIG. 8C



Days

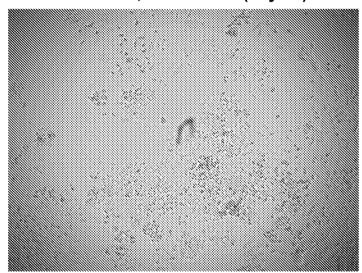
NCI-H841; DMSO (day 14)

FIG. 9A



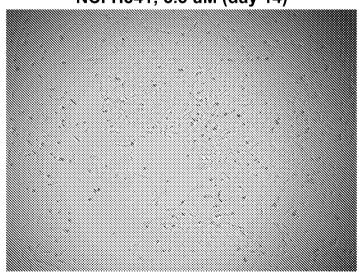
NCI-H841; 4.1E-02 uM (day 14)

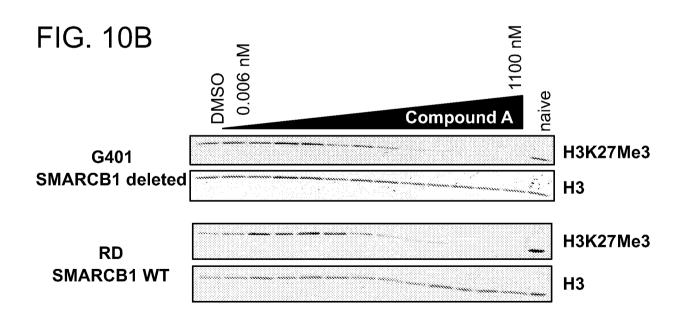
FIG. 9B



NCI-H841; 3.3 uM (day 14)

FIG. 9C





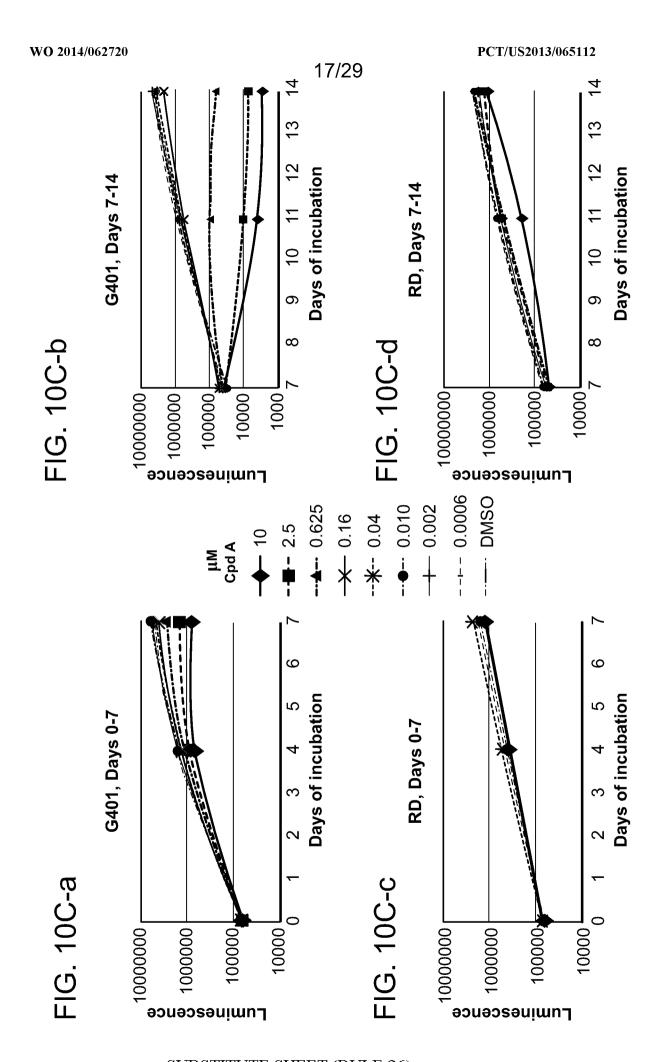


FIG. 11A

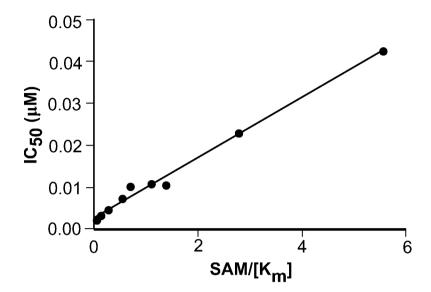
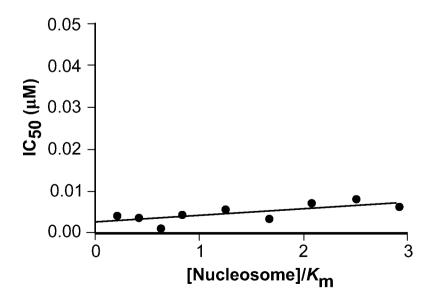


FIG. 11B



PCT/US2013/065112

19/29

FIG. 12A

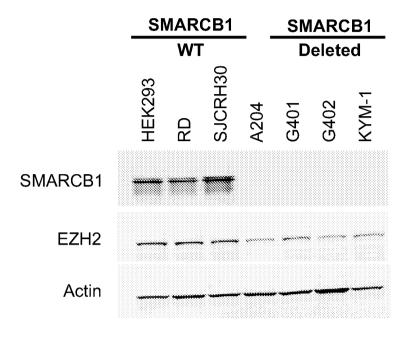
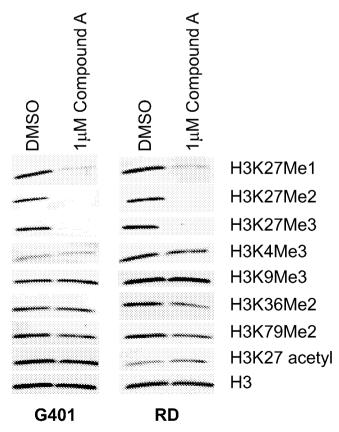
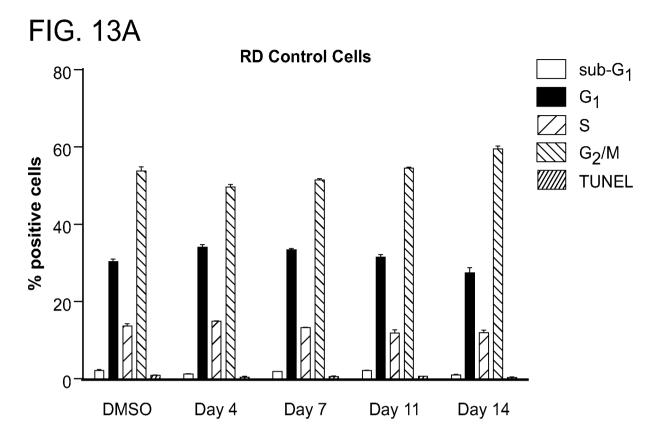
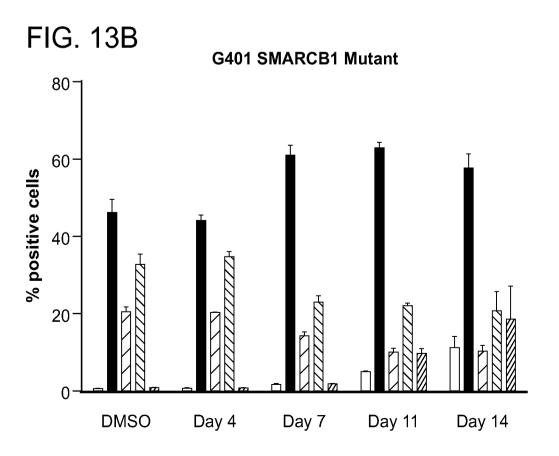


FIG. 12B



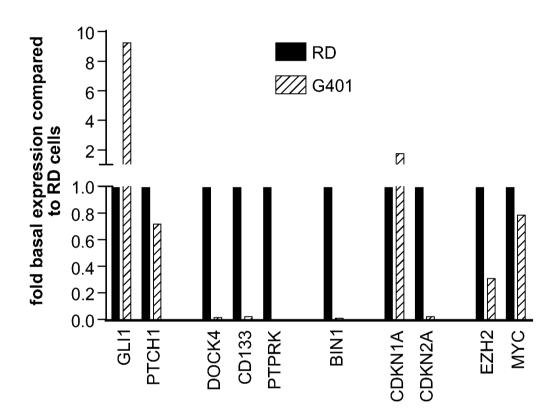


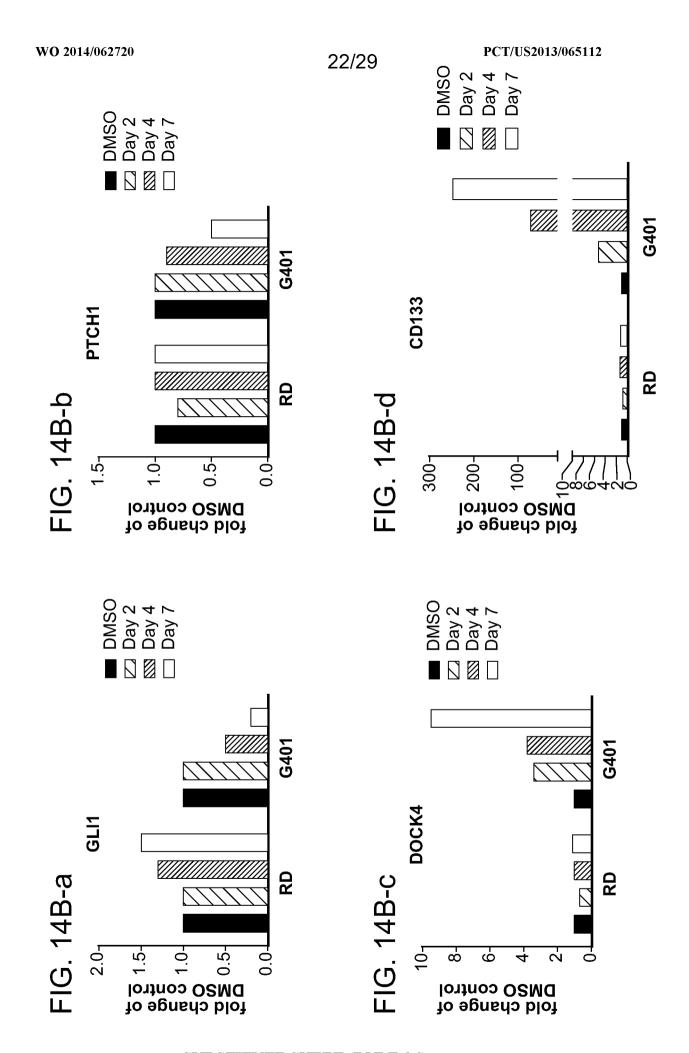


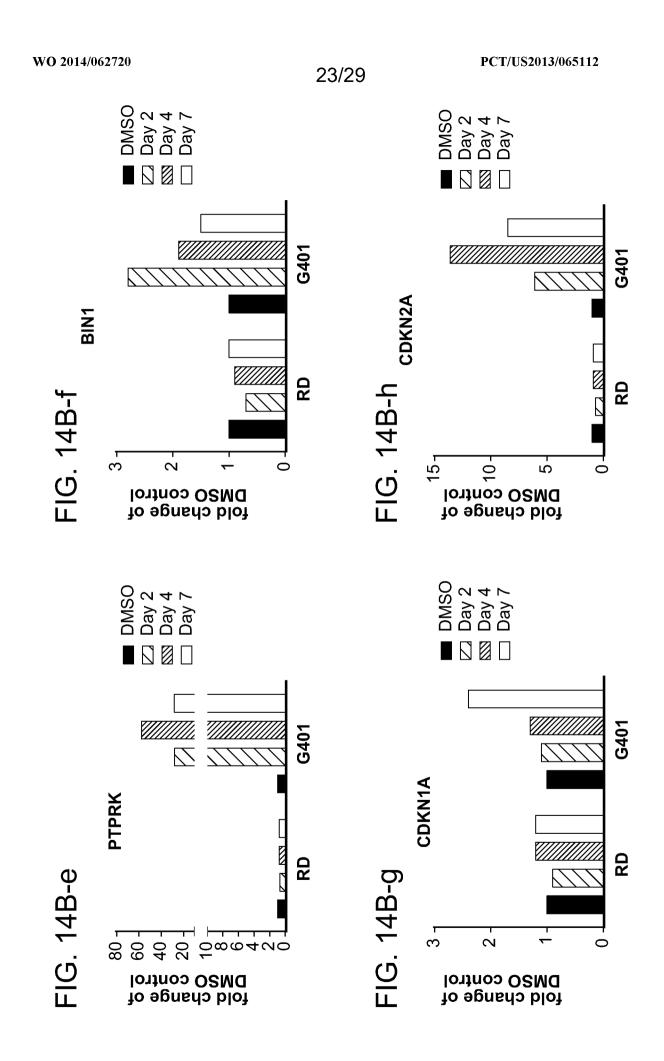


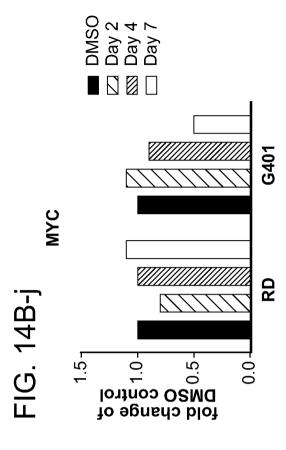
SUBSTITUTE SHEET (RULE 26)

FIG. 14A









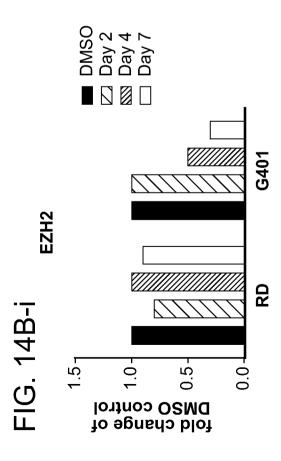


FIG. 14C

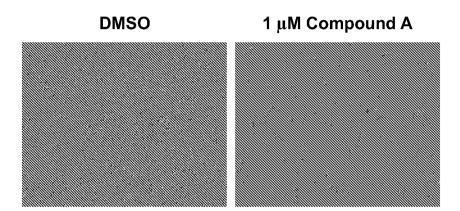


FIG. 15A

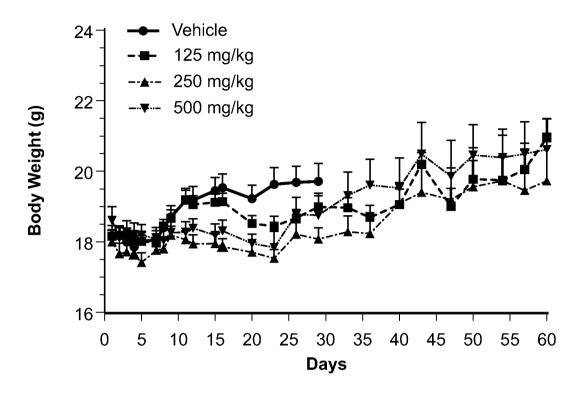
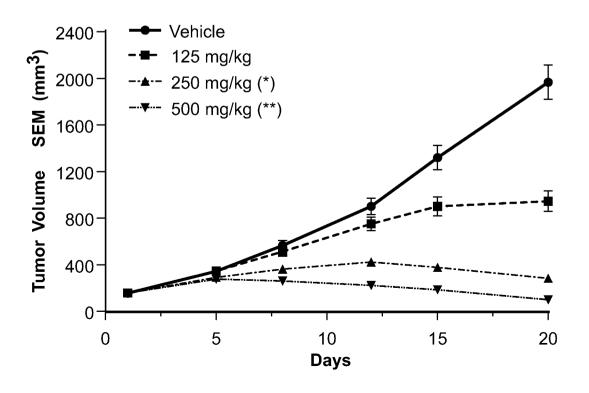


FIG. 15B



SUBSTITUTE SHEET (RULE 26)

FIG. 15C

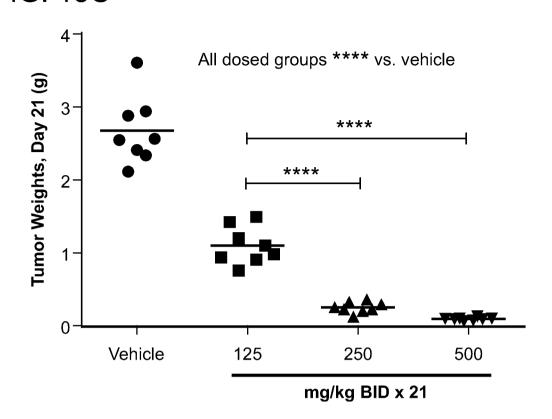


FIG. 15D

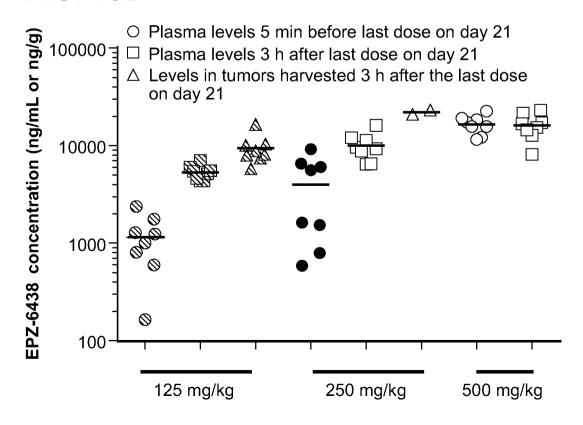


FIG. 16A

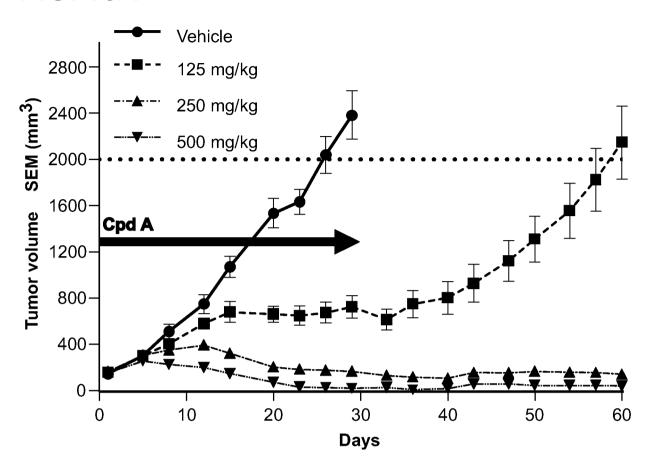


FIG. 16B

