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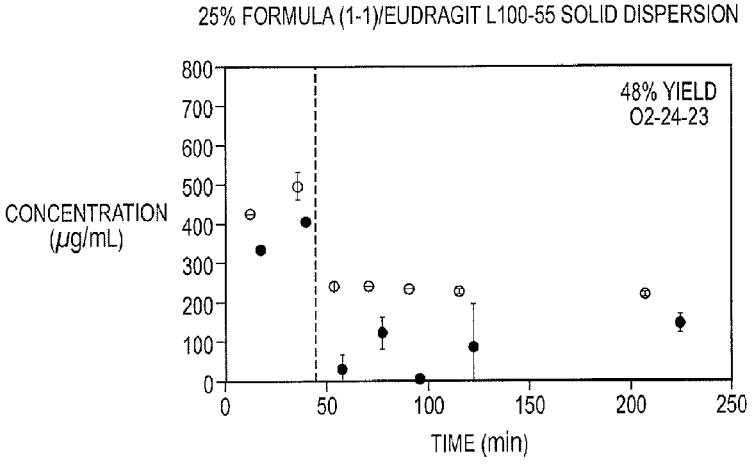
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(54) Title: METHOD OF TREATING ACUTE MYELOID LEUKEMIA AND/OR ACUTE LYMPHOBLASTIC LEUKEMIA USING THIENOTRIAZOLODIAZEPINE COMPOUNDS




(57) Abstract: A method of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal by administering a solid dispersion comprising an amorphous thienotriazolodiazepine compound of the Formula (1) wherein R1 is alkyl having a carbon number of 1-4, R2 is a hydrogen atom; a halogen atom; or alkyl having a carbon number of 1-4 optionally substituted by a halogen atom or a hydroxyl group, R3 is a halogen atom; phenyl optionally substituted by a halogen atom, alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4 or cyano; -NR 5 -(CH 2)m R6 wherein R5 is a hydrogen atom or alkyl having a carbon number of 1-4, m is an integer of 0-4, and R6 is phenyl or pyridyl optionally substituted by a halogen atom.

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# TITLE OF THE INVENTION

# METHOD OF TREATING ACUTE MYELOID LEUKEMIA AND/OR ACUTE LYMPHOBLASTIC LEUKEMIA USING THIENOTRIAZOLODIAZEPINE COMPOUNDS

## FIELD OF INVENTION

**[0001]** The present disclosure describes methods of treating acute myeloid leukemia and/or acute lymphoblastic leukemia using thienotriazolodiazepine compounds that have improved solubility and bioavailability and may be provided in the form of solid dispersions.

## BACKGROUND OF THE INVENTION

**[0002]** The compound of Formula (1), described herein below, has been shown to inhibit the binding of acetylated histone H4 to the tandem bromodomain (BRD)-containing family of transcriptional regulators known as the BET (bromodomains and extraterminal) proteins, which include BRD2, BRD3, and BRD4. *See* U.S. Patent Application Publication No. 2010/0286127 A1, which is incorporated herein by reference in its entirety. The BET proteins have emerged as major epigenetic regulators of proliferation and differentiation and also have been associated with predisposition to dyslipidemia or improper regulation of adipogenesis, elevated inflammatory profile and risk for cardiovascular disease and type 2 diabetes, and increased susceptibility to autoimmine diseases such as rheumatoid arthritis and systemic lupus erythematosus as reported by Denis, G.V. "Bromodomain coactivators in cancer, obesity, type 2 diabetes, and inflammation," *Discov Med* 2010; 10:489-499, which is incorporated herein by reference in its entirety. Accordingly, the compound of formula (II) may be useful for treatment of various cancers, cardiovascular disease, type 2 diabetes, and autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus.

## BRIEF SUMMARY OF THE INVENTION

**[0003]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, chronic acute myeloid leukemia, high risk myelodysplastic syndrome, and/or high risk myelodysplastic syndrome together with acute myeloid leukemia (referred to as HR-MDS/AML) using the compositions described herein.

**[0004]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising: administering to a patient in need a pharmaceutically

acceptable amount of a composition comprising a solid dispersion according to any of the compositions described in Sections III, IV, V and VI described herein. In some embodiments, the present disclosure provides for methods of treatment wherein the composition described in Sections III, IV, V and VI described herein is formed as a solid dispersion.

**[0005]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia wherein the acute myeloid leukemia can be mixed lineage leukemia (MLL) fusion psoitive, post-JAK2 mutated polycythemia vera, or post-mylodysplastic syndrome. In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia wherein the acute myeloid leukemia can be acute basophilic leukemia, acute panmyelosis with myelofibrosis, or myeloid sarcoma. In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia wherein the acute myeloid leukemia can be acute myeloblastic leukemia (minimally differentiated), acute myeloblastic leukemia (without maturation), acute myeloblastic leukemia (with granulocytic maturation), acute promyelocyytic leukemia, acute myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia, acute monocytic leukemia, acute erythroid leukemia, or acute megakaryoblastic leukemia. In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia wherein the acute myeloid leukemia can be acute myeloid leukemia with recurrent genetic abnormalities, acute myeloid leukemia with multilineage displasia, or therapy -related acute myeloid leukemia. In some embodiments the acute myeloid leukemia with recurrent genetic abnormalities is acute myeloid leukemia with recurrent genetic abnormalities is acute myeloid leukemia with t(8; 21)(q22; q22), acute myeloid leukemia with inv(16)(p13q22) or t(16; 16)(pl3; q22), acute myeloid leukemia with t(15; 17)(q22; q12), or acute myeloid leukemia with 11q23 abnormalities. In some embodiments the therapy related acute myeloid leukemia is alkylating agent-related acute myeloid leukemia or topoisomerase II inhibitor-related acute myeloid leukemia.

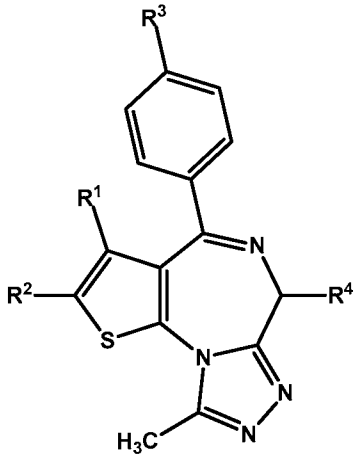
**[0006]** In some embodiments the present disclosure provides for methods of treating acute lymphoblastic leukemia wherein the acute lymphoblastic leukemia can be classified as ALL-Ll. In other embodiments the acute lymphoblastic leukemia can be classified as ALL-L2. In still other embodiments the acute lymphoblastic leukemia can be classified as ALL-L3. In some embodiments the acute lymphoblastic leukemia can be classified as precursor B acute lymphoblastic leukemia or pre-B cell. In other embodiments the acute lymphoblastic leukemia can be classified as precursor T acute lymphoblastic leukemia or pre-T cell. In some embodiments precursor B acute lymphoblastic leukemia can have the cytogenetic subtype t(12;21)(p12,q22) TEL/ AML-1; t(l ;19)(q23;p13)

PBX/E2A; t(9;22)(q34;qll) ABLIBCR; or T(V,ll)(V;q23) V/MLL. In some embodiments the acute lymphoblastic leukemia is Burkitt's leukemia. In some embodiments the acute lymphoblastic leukemia is Biphenotypic acute leukemia.

**[0007]** In some embodiments the present disclosure provides for methods of treating chronic acute myeloid leukemia wherein the chronic acute myeloid leukemia is in a chronic phase. In other embodiments the chronic acute myeloid leukemia is in an accelerated phase. In still othe rembodiments the chronic acute myeloid leukemia is a blast crisis phase.

**[0008]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a DNA methyltransferase inhibitor, an HDAC inibitor, an mTOR inhibitor, a glucocorticoid, an epigenetic modulator, cytotoxic agent, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the DNA methyltransferase inhibitor is azacitidine. In some embodiments the HDAC-inhibitor is panobinostat. In some embodiments the mTOR inhibitor is everolimus. In some embodiments the glucocorticoid is dexamethasone. In some embodiments the cytotoxic agent is selected from the group consisting of daunorubicin, cytarabine, methotrexate, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof. The thienotriazolodiazepine compound of Formula (1), and the DNA methyltransferase inhibitor, HDAC inibitor, mTOR inhibitor, glucocorticoid, epigenetic modulator, or ester, derivative, prodrug, salt, or complex thereof can be administered either simultaneously or sequentially. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1), and a DNA methyltransferase inhibitor, HDAC inibitor, mTOR inhibitor, glucocorticoid, epigentic modulator, or ester, derivative, prodrug, salt, or complex thereof can produce a synergistic effect.

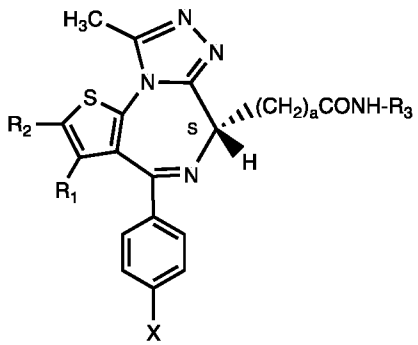
**[0009]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML using thienotriazolodiazepine compounds of the Formula (1)



**[0010]** wherein

$R^1$ is alkyl having a carbon number of 1-4, $R^2$ is a hydrogen atom; a halogen atom; or alkyl having a carbon number of 1-4 optionally substituted by a halogen atom or a hydroxyl group, $R^3$ is a halogen atom; phenyl optionally substituted by a halogen atom, alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4 or cyano; $-NR^5-(CH_2)_m-R^6$ wherein $R^5$ is a hydrogen atom or alkyl having a carbon number of 1-4, m is an integer of 0-4, and $R^6$ is phenyl or pyridyl optionally substituted by a halogen atom; or $-NR^7-CO-(CH_2)_n-R^8$ wherein $R^7$ is a hydrogen atom or alkyl having a carbon number of 1-4, n is an integer of 0-2, and $R^8$ is phenyl or pyridyl optionally substituted by a halogen atom, and $R^4$ is $-(CH_2)_a-CO-NH-R^9$ wherein a is an integer of 1-4, and $R^9$ is alkyl having a carbon number of 1-4; hydroxyalkyl having a carbon number of 1-4; alkoxy having a carbon number of 1-4; or phenyl or pyridyl optionally substituted by alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4, amino or a hydroxyl group or $-(CH_2)_b-COOR^{10}$ wherein b is an integer of 1-4, and $R^{10}$ is alkyl having a carbon number of 1-4, including any salts, isomers, enantiomers, racemates, hydrates, solvates, metabolites, and polymorphs thereof.

**[0011]** In some embodiments, Formula (1) is selected from Formula (1A):



**[0012]**

wherein X is a halogen, $R^1$ is $C_1$-$C_4$ alkyl, $R^2$ is $C_1$-$C_4$ alkyl, a is an integer of 1-4, $R^3$ is $C_1$-$C_4$ alkyl, $C_1$-$C_4$ hydroxyalkyl, $C_1$-$C_4$ alkoxy, phenyl optionally having substituent(s), or heteroaryl optionally having substituent(s), a pharmaceutically acceptable salt thereof or a hydrate thereof; and a pharmaceutically acceptable polymer. In one such embodiment, the thienotriazolodiazepine compound is formulated as a solid dispersion comprising an amorphous thienotriazolodiazepine compound.

**[0013]** In one embodiment, Formula (1A) is selected from the group consisting of: (i) (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo- [4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide or a dihydrate thereof, (ii) methyl (S)-{4-(3'-cyanobiphenyl-4-yl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]tri- azolo[4,3-a][1,4]diazepin-6-yl}acetate, (iii) methyl (S)-{2,3,9-trimethyl-4-(4-phenylaminophenyl)-6H-thieno[3,2-f][1,2,4]triaz- olo[4,3-a][1,4]diazepin-6-yl}acetate; and (iv) methyl (S)-{2,3,9-trimethyl-4-[4-(3-phenylpropionylamino)phenyl]-6H-thieno[3,2-f- ][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl}acetate. In one such embodiment, Formula (1A) is (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,- 4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide.

**[0014]** In some embodiments, the pharmaceutically acceptable polymer is hydroxypropylmethylcellulose acetate succinate. In some such embodiments, the solid dispersion has a thienotriazolodiazepine compound to hydroxypropylmethylcellulose acetate succinate (HPMCAS), weight ratio of 1:3 to 1:1. In some such embodiments, the solid dispersion exhibits a single glass transition temperature (Tg) inflection point ranging from about 130 °C to about 140 °C. In some such embodiments, a concentration of the thienotriazolodiazepine compound after exposure to the relative humidity of 75 % at 40 °C for at least one month is at least 90 % of the concentration the amorphous thienotriazolodiazepine compound prior to such exposure.

**[0015]** In other embodiments, the pharmaceutically acceptable polymer is PVP. In some such embodiments, the solid dispersion has a thienotriazolodiazepine compound to PVP weight ratio of 1:3 to 1:1. In some such embodiments, the solid dispersion exhibits a single glass transition temperature (Tg) inflection point ranging from about 175 °C to about 185 °C. In some such embodiments, a concentration of the thienotriazolodiazepine compound after exposure to the relative humidity of 75 % at 40 °C for at least one month is at least 90 % of the concentration the amorphous thienotriazolodiazepine compound prior to such exposure.

**[0016]** In another embodiment, the solid dispersion is obtained by spray drying.

**[0017]** In another embodiment, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1A).

**[0018]** In yet another embodiment, the solid dispersion provides an area under the curve (AUC) value that is at least 0.5 times that of a corresponding AUC value provided by a control composition administered intravenously, wherein the control composition comprises an equivalent quantity of a crystalline thienotriazolodiazepine compound of Formula (1A).

**[0019]** In still yet another embodiment, the solid dispersion provides a concentration, of the amorphous thienotriazolodiazepine compound, in an aqueous in vitro test medium at pH between 5.0 to 7.0, of at least 5-fold greater than a concentration of a crystalline thienotriazolodiazepine compound of Formula (1A) without polymer, in a control in vitro test medium at pH between 5.0 to 7.0 test medium.

**[0020]** In yet another embodiment, a concentration of the amorphous thienotriazolodiazepine compound, from the solid dispersion, in an aqueous in vitro test medium having a pH of 1.0 to 2.0, is at least 50% higher than a concentration of a crystalline thienotriazolodiazepine compound of Formula (1A) without polymer in an in vitro test medium having a pH between 5.0 and 7.0.

**[0021]** In one embodiment, the concentration of the amorphous thienotriazolodiazepine compound, is at least 50% higher compared to a concentration of thienotriazolodiazepine compound of Formula (1A), from a solid dispersion of thienotriazolodiazepine compound of the Formula (1A) and a pharmaceutically acceptable polymer selected from the group consisting of: hypromellose phthalate and ethyl acrylate-methyl methacrylate-trimethylammonioethyl methacrylate chloride copolymer, wherein each solid dispersion was placed in an aqueous in vitro test medium having a pH of 1.0 to 2.0.

**[0022]** In one embodiment, the concentration of the amorphous thienotriazolodiazepine compound of Formula (1A), is at least 50% higher compared to a concentration of thienotriazolodiazepine compound of Formula (1A), from a solid dispersion of thienotriazolodiazepine compound of the Formula (1A) and a pharmaceutically acceptable polymer selected from the group consisting of: hypromellose phthalate and ethyl acrylate-methyl methacrylate-trimethylammonioethyl methacrylate chloride copolymer, wherein each solid dispersion was placed in an aqueous in vitro test medium having a pH of 1.0 to 2.0.

**[0023]** The present disclosure further provides for a pharmaceutical formulation, used to treat acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML, comprising a spray dried dispersion, as described herein, and one or more

pharmaceutically acceptable excipients selected from the group consisting of: lactose monohydrate; microcrystalline cellulose; croscarmellose sodium; colloidal silicon dioxide; magnesium stearate; and combinations thereof. In some embodiments, the pharmaceutical formulation has a bulk density ranging from 0.55 g/cc to 0.60 g/cc. In some embodiments, the pharmaceutical formation may be a pharmaceutical capsule. In some embodiments, the pharmaceutical formation may be a pharmaceutical tablet.

**[0024]** The present disclosure further provides for a pharmaceutical formulation, used to treat acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML, comprising 10-15 wt. % of a spray dried solid dispersion, as described herein, and hydroxypropylmethylcellulose acetate succinate (HPMCAS), wherein the thienotriazolodiazepine compound is amorphous in the dispersion and has a thienotriazolodiazepine compound to hydroxypropylmethylcellulose acetate succinate (HPMCAS), weight ratio of 1:3 to 1:1; 45 -50 wt. % of lactose monohydrate; 35-40 wt. % of microcrystalline cellulose; 4-6 wt. % of croscarmellose sodium; 0.8-1.5 wt. % of colloidal silicon dioxide; and 0.8-1.5 wt. % of magnesium stearate.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0025]** The foregoing summary, as well as the following detailed description of embodiments of the pharmaceutical compositions including thienotriazolodiazepine formulations and methods of the present invention, will be better understood when read in conjunction with the appended drawings of exemplary embodiments. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

**[0026]** In the drawings:

**[0027]** Figure 1A illustrates dissolution profile of a comparator formulation comprising a solid dispersion comprising 25% compound (1-1) and Eudragit L100-55;

**[0028]** Figure 1B illustrates dissolution profile of a comparator formulation comprising a solid dispersion comprising 50% compound (1-1) and Eudragit L100-55;

**[0029]** Figure 1C illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 25% compound (1-1) and polyvinylpyrrolidone (PVP);

**[0030]** Figure 1D illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 50% compound (1-1) and PVP;

**[0031]** Figure 1E illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 25% compound (1-1) and PVP-vinyl acetate (PVP-VA);

**[0032]** Figure 1F illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 50% compound (1-1) and PVP-VA;

**[0033]** Figure 1G illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 25% compound (1-1) and hypromellose acetate succinate (HPMCAS-M);

**[0034]** Figure 1H illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 50% compound (1-1) and HPMCAS-M;

**[0035]** Figure 1I illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 25% compound (1-1) and hypromellose phthalate (HPMCP-HP55);

**[0036]** Figure 1J illustrates dissolution profile of an exemplary formulation comprising a solid dispersion comprising 50% compound (1-1) and HMCP-HP55;

**[0037]** Figure 2A illustrates results of *in vivo* screening of an exemplary formulation comprising a solid dispersion of 25% compound (1-1) and PVP;

**[0038]** Figure 2B illustrates results of an *in vivo* screening of an exemplary formulation comprising a solid dispersion of 25% compound (1-1) and HPMCAS-M;

**[0039]** Figure 2C illustrates results of an *in vivo* screening of an exemplary formulation comprising a solid dispersion of 50% compound (1-1) and HPMCAS-M;

**[0040]** Figure 3 illustrates powder X-ray diffraction profiles of solid dispersions of compound (1-1);

**[0041]** Figure 4A illustrates modified differential scanning calorimetry trace for a solid dispersion of 25% compound (1-1) and PVP equilibrated under ambient conditions;

**[0042]** Figure 4B illustrates modified differential scanning calorimetry trace for a solid dispersion of 25% compound (1-1) and HPMCAS-M equilibrated under ambient conditions;

**[0043]** Figure 4C illustrates modified differential scanning calorimetry trace for a solid dispersion of 50% compound (1-1) and HPMCAS-M equilibrated under ambient conditions;

**[0044]** Figure 5 illustrates plot of glass transition temperature (Tg) versus relative hunidity (RH) for solid dispersions of 25% compound (1-1) and PVP or HMPCAS-M and 50% compound (1-1) and HPMCAS-MG;

**[0045]** Figure 6 illustrates modified differential scanning calorimetry trace for a solid dispersion of 25% compound (1-1) and PVP equilibrated under 75% relative humidity;

**[0046]** Figure 7 illustrates plasma concentration versus time curves for Compound (1-1) after 1 mg/kg intravenous dosing (solid rectangles) and 3 mg/kg oral dosing as 25% Compound (1-1):PVP

(open circles), 25% Compound (1-1):HPMCAS-MG (open triangles), and 50% Compound (1-1):HPMCAS-MG (open inverted triangles). The inset depicts the same data plotted on a semilogarithmic scale;

**[0047]** Figure 8 illustrates plasma concentration versus time curves for Compound (1-1) after 3 mg/kg oral dosing as 25% Compound (1-1):PVP (open circles), 25% Compound (1-1):HPMCAS-MG (open triangles), and 50% Compound (1-1):HPMCAS-MG (open inverted triangles). The inset depicts the same data plotted on a semi-logarithmic scale;

**[0048]** Figure 9 illustrates a powder X-ray diffraction profile of solid dispersions of compound (1-1) in HPMCAS-MG at time zero of a stability test;

**[0049]** Figure 10 illustrates a powder X-ray diffraction profile of solid dispersions of compound (1-1) in HPMCAS-MG after 1 month at 40 °C and 75 % relative humidity;

**[0050]** Figure 11 illustrates a powder X-ray diffraction profile of solid dispersions of compound (1-1) in HPMCAS-MG after 2 months at 40 °C and 75 % relative humidity;

**[0051]** Figure 12 illustrates a powder X-ray diffraction profile of solid dispersions of compound (1-1) in HPMCAS-MG after 3 month at 40 °C and 75 % relative humidity;

**[0052]** Figure 13A illustrates the 48 hours combination index values for HL60, U937, Jurkat and K562 cell lines treated with a concomitant combination of compound (1-1) and various compounds;

**[0053]** Figure 13B illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a concomitant combination of Azacytidine and compound (1-1) for 48 hours;

**[0054]** Figure 13C illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a concomitant combination of panobinostat and compound (1-1) for 48 hours;

**[0055]** Figure 14A illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of Azacytidine for 48 hours followed by compound (1-1) for 48 hours;

**[0056]** Figure 14B illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of Azacytidine for 72 hours followed by compound (1-1) for 24 hours;

**[0057]** Figure 14C illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of Azacytidine for 72 hours followed by compound (1-1) for 48 hours;

**[0058]** Figure 14D illustrates a summary of the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of Azacytidine followed by compound (1-1);

**[0059]** Figure 15A illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of panobinostat for 48 hours followed by compound (1-1) for 48 hours;

**[0060]** Figure 15B illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination of panobinostat for 72 hours followed by compound (1-1) for 24 hours;

**[0061]** Figure 15C illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination ofpanobinostat for 72 hours followed by compound (1-1) for 48 hours;

**[0062]** Figure 15D illustrates a summary ofthe combination index value for HL60, U937, Jurkat and K562 cell lines treated with a sequential combination ofpanobinostat followed by compound (1-1 );

**[0063]** Figure 16A illustrates the cell percentage of subG1 for HL60 cell line treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0064]** Figure 16B illustrates the cell percentage of subG1 for K562 cell line treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0065]** Figure 17A illustrates the Western blot profiles of cMYC, cleaved caspase 3, cleaved PARP, p21 and β-actin for HL60 cell line treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0066]** Figure 17B illustrates the Western blot profiles of cMYC, cleaved caspase 3, cleaved PARP, p21 and β-actin for Jurkat cell line treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0067]** Figure 17C illustrates the Western blot profiles of cMYC, cleaved caspase 3, cleaved PARP, p21 and β-actin for U937 cell line treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0068]** Figure 17D illustrates the Western blot profiles of cMYC, cleaved caspase 3, cleaved PARP, p21 and β-actin for K562 cell lines treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours;

**[0069]** Figure 18 illustrates the GI50 and Emax for HL60, U937, Jm·kat, Molt3 and K562 cell lines for treatment with compound ( 1-1) for 72 hours;

**[0070]** Figure 19A illustrates the extracellular levels of compound (1-1) versus time for HL60, U937, Jurkat, Molt3 and K562 cell lines;

**[0071]** Figure 19B illustrates the intracellular levels of compound (1-1) versus time for HL60, U937, Jurkat, Molt3 and K562 cell lines;

**[0072]** Figure 20A illustrates the Western blot profile for c-MYC and tublin for HL60 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0073]** Figure 20B illustrates the fluorescence intensity for c-MYC levels for HL60 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0074]** Figure 20C illustrates the extra and intracellular levels of compound (1-1) for HL60 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0075]** Figure 21A illustrates the Western blot profile for c-MYC and tublin for Jurkat cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0076]** Figure 21B illustrates the fluorescence intensity for c-MYC levels for Jurkat cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0077]** Figure 21C illustrates the extra and intracellular levels of compound (1-1) for Jurkat cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0078]** Figure 22A illustrates the Western blot profile for c-MYC and tublin for U937 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0079]** Figure 22B illustrates the fluorescence intensity for c-MYC levels for U937 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0080]** Figure 22C illustrates the extra and intracellular levels of compound (1-1) for U93 7 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0081]** Figure 23A illustrates the Western blot profile for c-MYC and tublin for K562 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0082]** Figure 23B illustrates the fluorescence intensity for c-MYC levels for K562 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0083]** Figure 23C illustrates the extra and intracellular levels of compound (1-1) for K562 cell line treated with compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0084]** Figure 24A illustrates the fluorescence intensity for Bcl2 for HL60, U937, Jurkat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0085]** Figure 24B illustrates the fluorescence intensity for p21 for HL60, U937, Jurkat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0086]** Figure 24C illustrates the fluorescence intensity for BRD2 for HL60, U937, Jurkat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0087]** Figure 24D illustrates the fluorescence intensity for BRD3 for HL60, U937, Jurkat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes;

**[0088]** Figure 24E illustrates the fluorescence intensity for BRD4 for HL60, U937, Jm·kat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes; and

**[0089]** Figure 24F illustrates the fluorescence intensity for HEXIM1 for HL60, U937, Jurkat, Molt3 and K562 treated with 500 nM compound (1-1) for 5, 15, 30, 60, 120 and 360 minutes.

## DETAILED DESCRIPTION OF THE INVENTION

**[0090]** The present subject matter will now be described more fully hereinafter with reference to the accompanying Figures and Examples, in which representative embodiments are shown. The present subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided to describe and enable one of skill in the art. 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 the subject matter pertains. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entireties.

### I. Definitions:

**[0091]** The term "alkyl group" as used herein refers to a saturated straight or branched hydrocarbon.

**[0092]** The term "substituted alkyl group" refers to an alkyl moiety having one or more substituents replacing a hydrogen or one or more carbons of the hydrocarbon backbone.

**[0093]** The term "alkenyl group" whether used alone or as part of a substituent group, for example, "$C_{1-4}$alkenyl(aryl)," refers to a partially unsaturated branched or straight chain monovalent hydrocarbon radical having at least one carbon—carbon double bond, whereby the double bond is derived by the removal of one hydrogen atom from each of two adjacent carbon atoms of a parent alkyl molecule and the radical is derived by the removal of one hydrogen atom from a single carbon atom. Atoms may be oriented about the double bond in either the cis (Z) or trans (E) conformation. Typical alkenyl radicals include, but are not limited to, ethenyl, propenyl, allyl(2-propenyl), butenyl and the like. Examples include $C_{2-8}$alkenyl or $C_{2-4}$alkenyl groups.

**[0094]** The term "$C_{(j-k)}$" (where *j* and *k* are integers referring to a designated number of carbon atoms) refers to an alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl radical or to the alkyl portion of a

radical in which alkyl appears as the prefix root containing from j to k carbon atoms inclusive. For example, $C_{(1-4)}$ denotes a radical containing 1, 2, 3 or 4 carbon atoms.

**[0095]** The terms "halo" or "halogen" as used herein refer to F, Cl, Br, or I.

**[0096]** The term "pharmaceutically acceptable salts" is art-recognized and refers to the relatively non-toxic, inorganic and organic acid addition salts, or inorganic or organic base addition salts of compounds, including, for example, those contained in compositions of the present invention.

**[0097]** The term "solid dispersion" as used herein refers to a group of solid products consisting of at least two different components, generally a hydrophilic carrier and a hydrophobic drug (active ingredient).

**[0098]** The term "chiral" is art-recognized and refers to molecules That have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner. A "prochiral molecule" is a molecule that has the potential to be converted to a chiral molecule in a particular process.

**[0099]** The symbol "------" is used to denote a bond that may be a single, a double or a triple bond.

**[00100]** The term "enantiomer" as it used herein, and structural formulas depicting an enantiomer are meant to include the "pure" enantiomer free from its optical isomer as well as mixtures of the enantiomer and its optical isomer in which the enantiomer is present in an enantiomeric excess, e.g., at least 10%, 25%, 50%, 75%, 90%, 95%, 98%, or 99% enantiomeric excess.

**[00101]** The term "stereoisomers" when used herein consist of all geometric isomers, enantiomers or diastereomers. The present invention encompasses various stereoisomers of these compounds and mixtures thereof. Conformational isomers and rotamers of disclosed compounds are also contemplated.

**[00102]** The term "stereoselective synthesis" as it is used herein denotes a chemical or enzymatic reaction in which a single reactant forms an unequal mixture of stereoisomers during the creation of a new stereocenter or during the transformation of a pre-existing one, and are well known in the art. Stereoselective syntheses encompass both enantioselective and diastereoselective transformations. For examples, see Carreira, E. M. and Kvaerno, L., *Classics in Stereoselective Synthesis*, Wiley-VCH: Weinheim, 2009.

**[00103]** The term "spray drying" refers to processes which involve the atomization of the feed suspension or solution into small droplets and rapidly removing solvent from the mixture in a processor chamber where there is a strong driving force for the evaporation (e.g., hot dry gas or partial vacuum or combinations thereof).

**[00104]** The term "therapeutically effective amount" as used herein refers to any amount of a thienotriazolodiazepine of the present invention or any other pharmaceutically active agent which, as compared to a corresponding a patient who has not received such an amount of the thienotriazolodiazepine or the other pharmaceutically active agent, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder.

**[00105]** The term "about" means +/- 10%.

**[00106]** Throughout this application and in the claims that follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", should be understood to imply the inclusion of a stated integer step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

**[00107]** It has now been found that thienotriazolodiazepine compound of Formula (1), described herein below, can be formulated as a solid dispersion with pharmaceutically acceptable polymers, to provide an oral formulation that provides high absorption of the pharmaceutical ingredient into the circulation from the gastrointestinal tract. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate (also called hydroxypropylmethylcellulose acetate succinate or HPMCAS). In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone (PVP).

**[00108]** In some embodiments, the hydroxypropylmethyl cellulose acetate succinates (HPMCAS), may include M grade having 9% acetyl/11% succinoyl (e.g., HPMCAS having a mean particle size of 5 μm (i.e., HPMCAS-MF, fine powder grade) or having a mean particle size of 1 mm (i.e., HPMCAS-MG, granular grade)), H grade having 12% acetyl/6% succinoyl (e.g., HPMCAS having a mean particle size of 5 μm (i.e., HPMCAS-HF, fine powder grade) or having a mean particle size of 1 mm (i.e., HPMCAS-HG, granular grade)), and L grade having 8% acetyl/15% succinoyl (e.g., HPMCAS having a mean particle size of 5 μm (i.e., HPMCAS-LF, fine powder grade) or having a mean particle size of 1 mm (i.e., HPMCAS-LG, granular grade).

**[00109]** In some embodiments, the polyvinyl pyrrolidones may have molecular weights of about 2,500 (Kollidon ®12 PF, weight-average molecular weight between 2,000 to 3,000), about 9,000 (Kollidon® 17 PF, weight-average molecular weight between 7,000 to 11,000), about 25,000 (Kollidon® 25, weight-average molecular weight between 28,000 to 34,000), about 50,000 (Kollidon® 30, weight-average molecular weight between 44,000 to 54,000), and about 1,250,000

(Kollidon® 90 or Kollidon® 90F, weight-average molecular weight between 1,000,000 to 1,500,000).

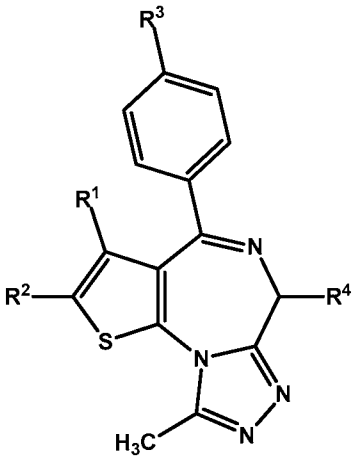
## II. Methods of Treatment

**[00110]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML comprising admnistering a pharmaceutically acceptable amount of the compositions described herein.

**[00111]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a solid dispersion according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00112]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a pharmaceutical formulation according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00113]** In some embodiments, methods of treating acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML use a thienotriazolodiazepine compound of the Formula (1)

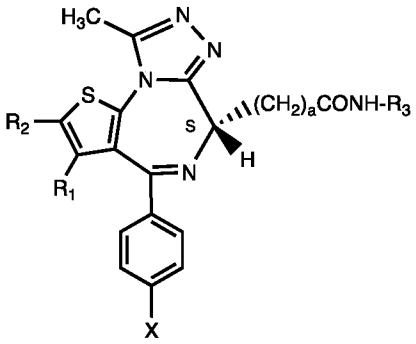



**[00114]** wherein

$R^1$ is alkyl having a carbon number of 1-4, $R^2$ is a hydrogen atom; a halogen atom; or alkyl having a carbon number of 1-4 optionally substituted by a halogen atom or a hydroxyl group, $R^3$ is a halogen

atom; phenyl optionally substituted by a halogen atom, alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4 or cyano; —$NR^5$—$(CH_2)_m$—$R^6$ wherein $R^5$ is a hydrogen atom or alkyl having a carbon number of 1-4, m is an integer of 0-4, and $R^6$ is phenyl or pyridyl optionally substituted by a halogen atom; or –$NR^7$—CO—$(CH_2)_n$—$R^8$ wherein $R^7$ is a hydrogen atom or alkyl having a carbon number of 1-4, n is an integer of 0-2, and $R^8$ is phenyl or pyridyl optionally substituted by a halogen atom, and $R^4$ is —$(CH_2)_a$—CO—NH—$R^9$ wherein a is an integer of 1-4, and $R^9$ is alkyl having a carbon number of 1-4; hydroxyalkyl having a carbon number of 1-4; alkoxy having a carbon number of 1-4; or phenyl or pyridyl optionally substituted by alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4, amino or a hydroxyl group or —$(CH_2)_b$—$COOR^{10}$ wherein b is an integer of 1-4, and $R^{10}$ is alkyl having a carbon number of 1-4, including any salts, isomers, enantiomers, racemates, hydrates, solvates, metabolites, and polymorphs thereof.

**[00115]** In some embodiments, Formula (1) is selected from Formula (1A):




**[00116]** wherein X is a halogen, $R^1$ is $C_1$-$C_4$ alkyl, $R^2$ is $C_1$-$C_4$ alkyl, a is an integer of 1-4, $R^3$ is $C_1$-$C_4$ alkyl, $C_1$-$C_4$ hydroxyalkyl, $C_1$-$C_4$ alkoxy, phenyl optionally having substituent(s), or heteroaryl optionally having substituent(s), a pharmaceutically acceptable salt thereof or a hydrate thereof; and a pharmaceutically acceptable polymer. In one such embodiment, the thienotriazolodiazepine compound is formulated as a solid dispersion comprising an amorphous thienotriazolodiazepine compound.

**[00117]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a pharmaceutical formulation according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00118]** Acute myeloid leukemia includes cancer of the blood and bone marrow, in which the bone marrow makes abnormal myeloblasts, red blood cells, or platelets. Acute myeloid leukemia includes, but is not limited to, subclasses of acute myeloid leukemia as classified by the World Health Organization as

well as subclasses of acute myeloid leukemia as classified by the FrenchAmerican-British (FAB) cooperative group. In some embodiments acute myeloid leukemia can be mixed lineage leukemia (MLL) fusion positive, post-JAK2 mutated polycythemia vera, or postmyelodysplastic syndrome. In some embodiments acute myeloid leukemia can be acute basophilic leukemia, acute panmyelosis with myelofibrosis, or myeloid sarcoma. In some embodiments acute myeloid leukemia is acute myeloblastic leukemia (minimally differentiated), acute myeloblastic leukemia (without maturation), acute myeloblastic leukemia (with granulocytic maturation), acute promyelocytic leukemia, acute myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia, acute monocytic leukemia, acute erythroid leukemia, or acute megakaryoblastic leukemia. In some embodiments, acute myeloid leukemia is acute myeloid leukemia with recurrent genetic abnormalities, acute myeloid leukemia with multilineage displasia, or therapy -related acute myeloid leukemia. AML with recurrent genetic abnormalities includes, but is not limited to, AML with t(8; 21)(q22; q22), AML with inv(16)(p13q22) or t(16; 16)(p13; q22), AML with t(15; 17)(q22; q12), and AML with 11q23 abnormalities. Therapy related AML includes, but is not limited to, alkylating agent-related AML and topoisomerase II inhibitor-related AML.

**[00119]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof.

**[00120]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof and further comprising administering pharmaceutically acceptable amount a second agent selected from the group consisting of a DNA methyltransferase inhibitor, an HDAC-inhibitor, a glucocorticoid, an mTOR inhibitor, an epigenetic modulator, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof.

**[00121]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a DNA methyltransferase inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the

DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the DNA methyltransferase inhibitor is azacitidine. In some embodiments the DNA methyltransferase inhibitor is decitabine.

**[00122]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an HDAC inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the HDAC inhibitor is panobinostat.

**[00123]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an mTOR inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and an mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the mTOR inhibitor is everolimus.

**[00124]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a glucocorticoid, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments

the thienotriazolodiazepine compound of Formula (1) and the glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the glucocorticoid is dexamethasone.

**[00125]** In some embodiments, the present disclosure provides for methods of treating acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a cytotoxic agent, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the cytotoxic agent is selected from daunorubicin, cytarabine, and methotrexate.

**[00126]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a pharmaceutical formulation according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00127]** In some embodiments the acute lymphoblastic leukemia can be classified as ALL-Ll. In other embodiments the acute lymphoblastic leukemia can be classified as ALL-L2. In still other embodiments the acute lymphoblastic leukemia can be classified as ALL-L3. In some embodiments the acute lymphoblastic leukemia can be classified as precursor B acute lymphoblastic leukemia or pre-B cell. In other embodiments the acute lymphoblastic leukemia can be classified as precursor T acute lymphoblastic leukemia or pre-T cell. In some embodiments precursor B acute lymphoblastic leukemia can have the cytogenetic subtype t(12; 21)(p12, q22) TEL/AML-1; t(1; 19)(q23; p3) PBX/E2A; t(9;22)(q34;q11) ABL/BCR; or T(V,11)(V;q23) V/MLL. In some embodiments the acute lymphoblastic leukemia is Burkitt's leukemia. In some embodiments the acute lymphoblastic leukemia is Biphenotypic acute leukemia.

**[00128]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising administering a pharmaceutically acceptable

amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof.

**[00129]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof and further comprising administering pharmaceutically acceptable amount a second agent selected from the group consisting of a DNA methyltransferase inhibitor, an HDAC-inhibitor, a glucocorticoid, an mTOR inhibitor, an epigenetic modulator, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof.

**[00130]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a DNA methyltransferase inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the DNA methyltransferase inhibitor is azacitidine. In some embodiments the DNA methyltransferase inhibitor is decitabine.

**[00131]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an HDAC inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the HDAC inhibitor is panobinostat.

**[00132]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an mTOR inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and an mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the mTOR inhibitor is everolimus.

**[00133]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a glucocorticoid, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the glucocorticoid is dexamethasone.

**[00134]** In some embodiments, the present disclosure provides for methods of treating acute lymphoblastic leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a cytotoxic agent, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the cytotoxic agent is selected from daunorubicin, cytarabine, and methotrexate.

**[00135]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a pharmaceutical formulation according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00136]** In some embodiments the chronic acute myeloid leukemia can be in a chronic phase. In other embodiments the chronic acute myeloid leukemia can be in an accelerated phase. In still other embodiments the chronic acute myeloid leukemia can be in a blast crisis phase.

**[00137]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof.

**[00138]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof and further comprising administering pharmaceutically acceptable amount a second agent selected from the group consisting of a DNA methyltransferase inhibitor, an HDAC-inhibitor, a glucocorticoid, an mTOR inhibitor, an epigenetic modulator, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof.

**[00139]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a DNA methyltransferase inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the DNA methyltransferase inhibitor is azacitidine. In some embodiments the DNA methyltransferase inhibitor is decitabine.

**[00140]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further

comprising administering an HDAC inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the HDAC inhibitor is panobinostat.

**[00141]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an mTOR inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and an mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the mTOR inhibitor is everolimus.

**[00142]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a glucocorticoid, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the glucocorticoid is dexamethasone.

**[00143]** In some embodiments, the present disclosure provides for methods of treating chronic acute myeloid leukemia in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further

comprising administering a cytotoxic agent, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the cytotoxic agent is selected from daunorubicin, cytarabine, and methotrexate.

**[00144]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising: administering to a patient in need a pharmaceutically acceptable amount of a composition comprising a pharmaceutical formulation according to any of the compositions described in Sections III, IV, V and VI described herein.

**[00145]** In some embodiments, the present disclosure provides for methods of treating treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof.

**[00146]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of Formula (1), or a salt, ester, derivative, prodrug, or complex thereof and further comprising administering pharmaceutically acceptable amount a second agent selected from the group consisting of a DNA methyltransferase inhibitor, an HDAC-inhibitor, a glucocorticoid, an mTOR inhibitor, an epigenetic modulator, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof.

**[00147]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a DNA methyltransferase inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the DNA methyltransferase inhibitor (or

ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a DNA methyltransferase inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the DNA methyltransferase inhibitor is azacitidine. In some embodiments the DNA methyltransferase inhibitor is decitabine.

**[00148]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an HDAC inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a HDAC inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the HDAC inhibitor is panobinostat.

**[00149]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering an mTOR inhibitor, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and an mTOR inhibitor (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the mTOR inhibitor is everolimus.

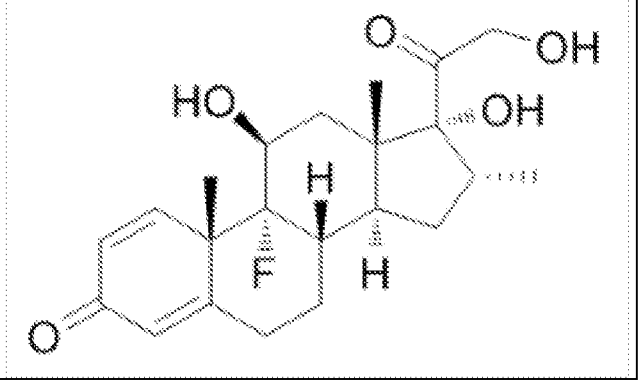
**[00150]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a glucocorticoid, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered

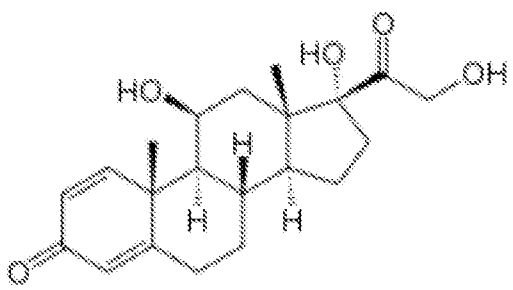
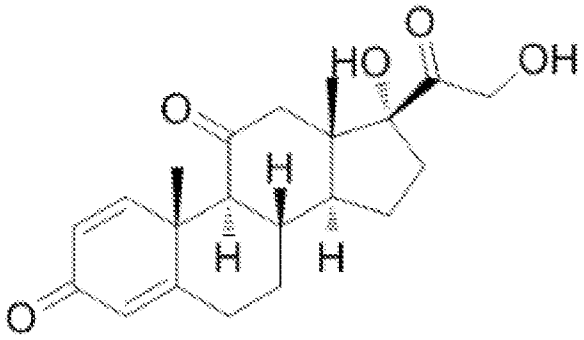
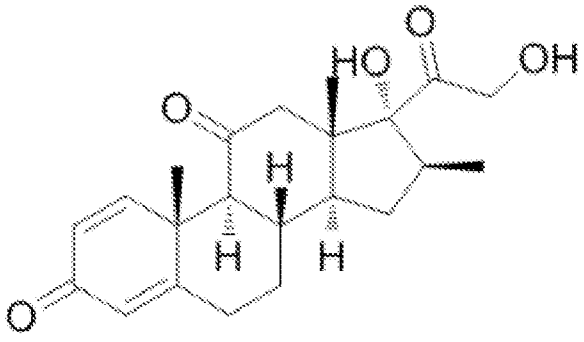
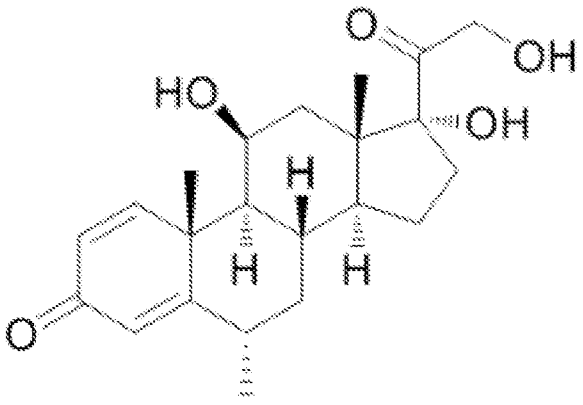
sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the glucocorticoid is dexamethasone.

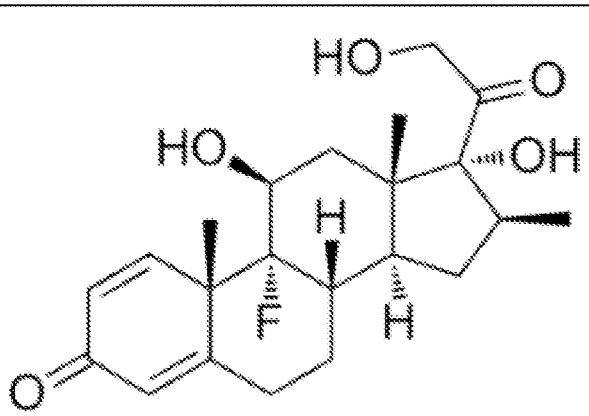
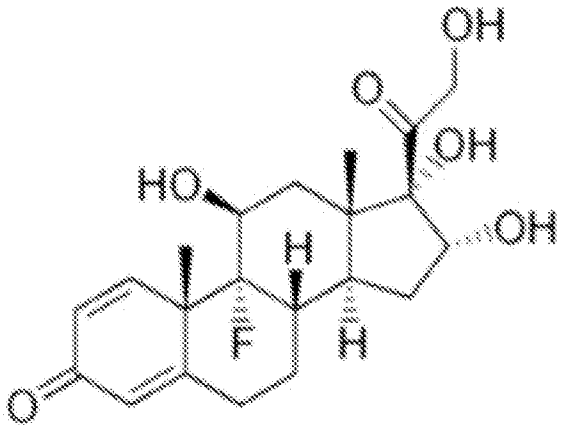
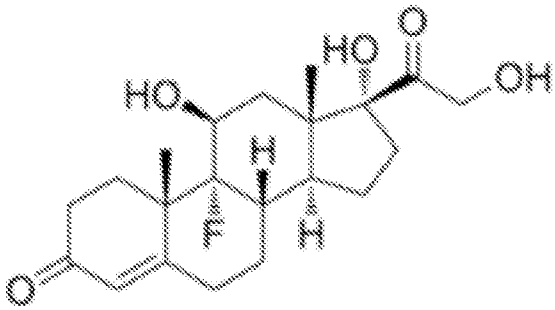
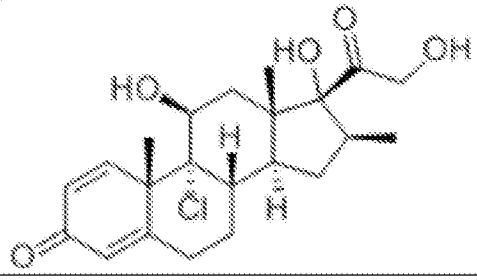
**[00151]** In some embodiments, the present disclosure provides for methods of treating high risk myelodysplastic syndrome, and/or HR-MDS/AML in a mammal comprising the step of administering a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1); and further comprising administering a cytotoxic agent, or an ester, derivative, prodrug, salt, or complex thereof. In some embodiments the thienotriazolodiazepine compound of Formula (1) and glucocorticoid (or ester, derivative, prodrug, salt, or complex thereof) are administered sequentially. In other embodiments the thienotriazolodiazepine compound of Formula (1) and the cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) are administered concomitantly. In some embodiments administration of a thienotriazolodiazepine compound of Formula (1) and a cytotoxic agent (or ester, derivative, prodrug, salt, or complex thereof) produce a synergistic effect. In some embodiments the cytotoxic agent is selected from daunorubicin, cytarabine, and methotrexate.

**[00152]** Suitable glucocorticoids for use in combinations with the thienotriazolodiazepine of Formula (1) in the methods of the present invention include, but are not limited to, the glucocorticoids listed in the below Table A.

**[00153]** Table A:

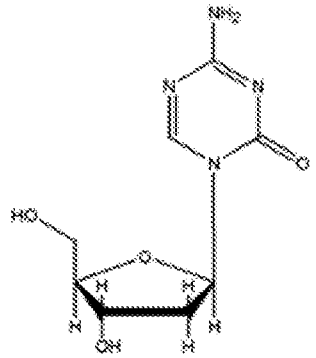
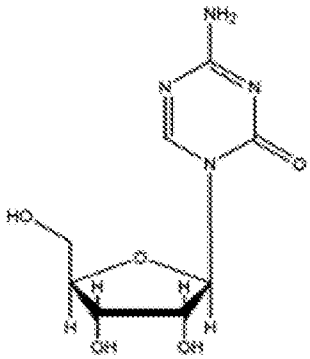
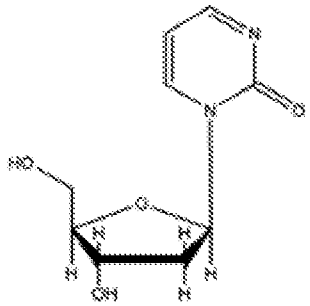
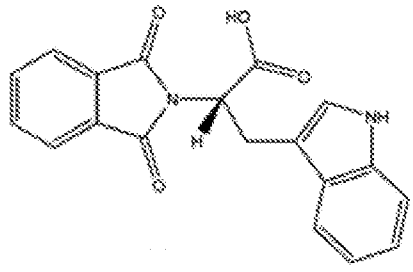
| No. | Glucocorticoid Name | Glucocorticoid Structure |
|---|---|---|
| 1 | dexamethasone |  |

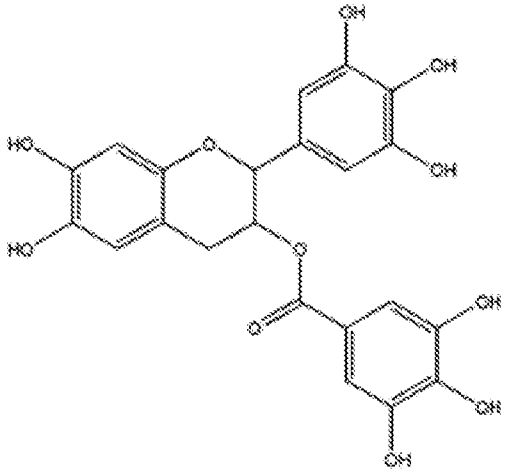
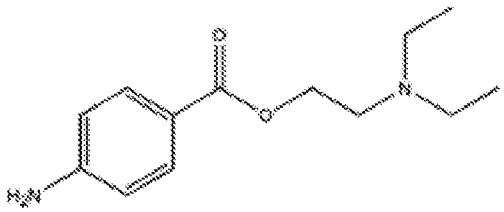
| No. | Glucocorticoid Name | Glucocorticoid Structure |
|---|---|---|
| 2 | prednisolone |  |
| 3 | prednisone |  |
| | meprednisone |  |
| 4 | methylprednisolone |  |

| No. | Glucocorticoid Name | Glucocorticoid Structure |
|---|---|---|
| 5 | betamethasone |  |
| 6 | triamicinolone |  |
| 7 | fludrocortisone |  |
| 8 | beclamethasone |  |

**[00154]** Example DNA methyltransferase inhibitors for use in combinations with the thienotriazolodiazepine compounds of Formula (1) in the methods of the present invention include the compounds listed in the below Table B.

**[00155]** Table B:

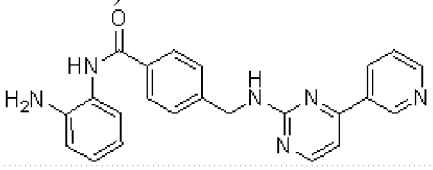
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
| decitabine (5-aza-2'-deoxycytidine)  | | J Natl Cancer Inst 2005;97:1498-1506 |
| 5-azacytidine  | | Experientia 1964; 20:202-3; Cell 1980; 20:85-93; J Natl Cancer Inst 2005;97:1498-1506 |
| zebularin  | | J Natl Cancer Inst 2005;97:1498-1506 |
| RG108  | | J Natl Cancer Inst 2005;97:1498-1506 |
| epigallocatechin-3-gallate (EGCG) | | J Natl Cancer Inst 2005;97:1498-1506 |

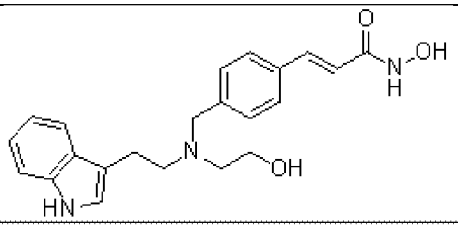
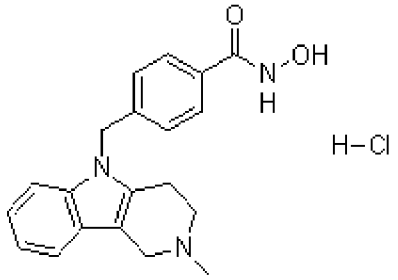
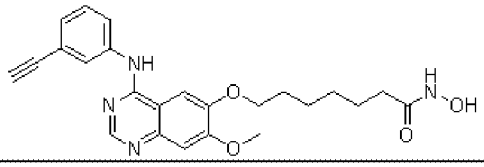
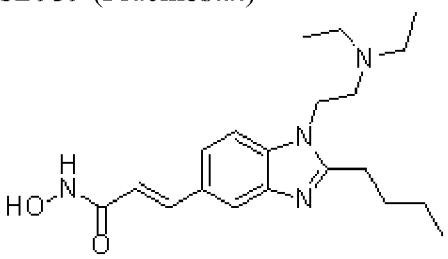
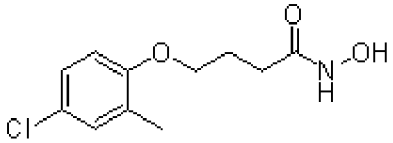
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
|  | | |
| procaine  | | J Natl Cancer Inst 2005;97:1498-1506 |

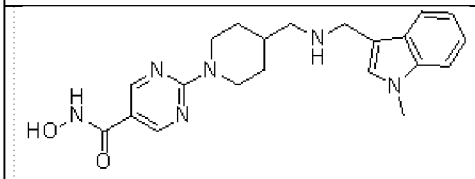
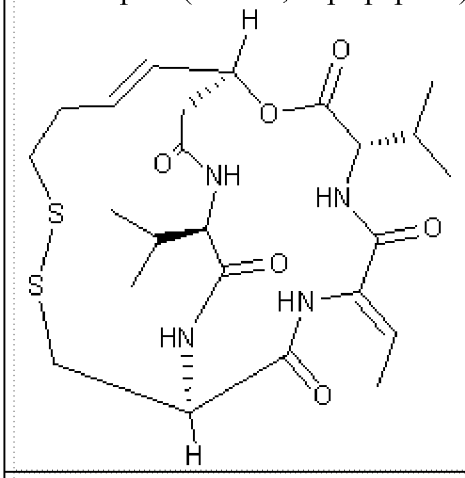
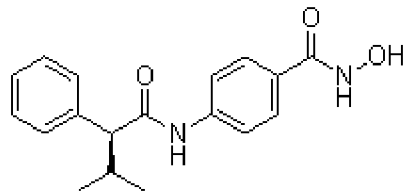
**[00156]** Example histone deacetylase (HDAC) inhibitors for use in combinations with the thienotriazolodiazepine of Formula (1) in the methods of the present invention include, but are not limited to, the HDAC inhibitors listed in the below Table C.

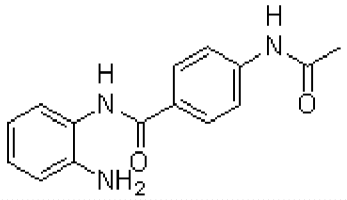
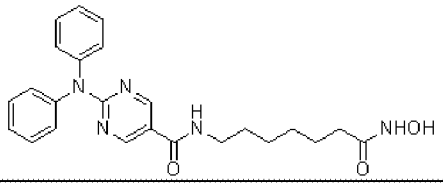
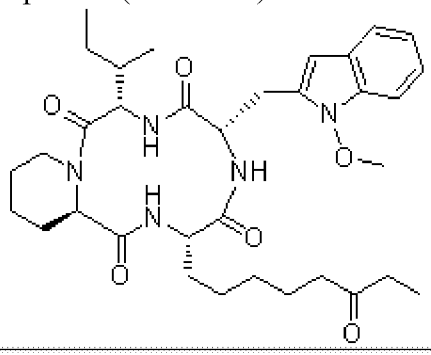
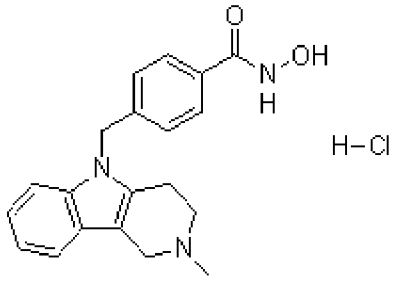
**[00157]** Table C:

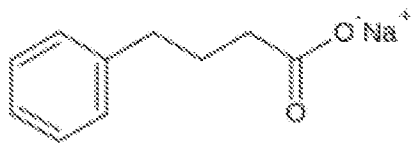
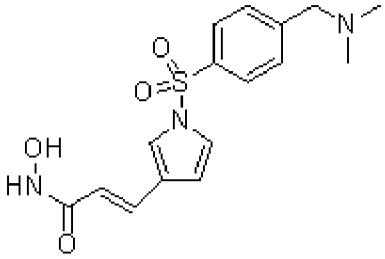
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
| Vorinostat (SAHA, MK0683) | Vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza) is an HDAC inhibitor with IC50 of ~10 nM. | Nature, 2011, 471(7337):235-9; Nat Biotechnol, 2011, 29(3), 255-265; J Exp Med, 2012, 209(1):35-50. |
| Entinostat (MS-275,SNDX-275) | MS-275 is an HDAC inhibitor of HDAC1 and HDAC3 with IC50 of 0.51 μM and 1.7 μM, respectively. | Nat Biotechnol, 2011, 29(3), 255-265; Proc Natl Acad Sci U S A, 2011, 108(49):19629-34; Circ Res, 2012, 110(5):739-48. |

| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
| Panobinostat (LBH589, NVP-LBH589) | LBH589 (Panobinostat) is a novel broad-spectrum HDAC inhibitor with IC50 of 5 nM and 20 nM in MOLT-4 and Reh cells, respectively. | Nat Biotechnol, 2011, 29(3), 255-265; Blood, 2012, 119(6):1450-8; Acta Neuropathol, 2011, 122(5):637-50 |
| Trichostatin A (TSA) | Trichostatin A (TSA) is an HDAC inhibitor with IC50 of ~1.8 nM. | Plant J, 2013, 74(5), 815-828; Epigenetics, 2012, 7(10), 1161-1172. |
| Mocetinostat (MGCD0103, MG0103)  | MGCD0103 (Mocetinostat) is a potent HDAC inhibitor for HDAC1, HDAC2 and HDAC3 with IC50 of 0.15 μM, 0.29 μM and 1.66 μM, respectively. | Nat Struct Mol Biol, 2013, 20(3):317-25; Circ Res, 2012, 110(5):739-48; Oncogene, 2011, 30(27), 3062-3072. |
| Belinostat (PXD101) | Belinostat (PXD101) is a novel HDAC inhibitor with IC50 of 27 nM in HeLa cell extracts. | Nat Biotechnol, 2011, 29(3), 255-265; Breast Cancer Res Treat, 2011, 131(3), 777-789; PLoS One, 2011, 6(2), e17138. |
| MC1568 | MC1568 is a selective HDAC inhibitor with IC50 of 220 nM. | Proc Natl Acad Sci U S A, 2012, 109(34):E2284-93; Oncogene, 2013, ; J Biol Chem, 2011, 286(27), 23842-23851. |
| LAQ824 (NVP-LAQ824,Dacinostat) | LAQ824 (NVP-LAQ824) is a novel HDAC inhibitor with IC50 of 32 nM. | Nat Biotechnol, 2011, 29(3), 255-265; Diabetologia, 2012, 55(9):2421-31; |

| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
|  | | Mol Pain, 2010, 6, 51. |
| ITF2357 (Givinostat) | ITF2357 (Givinostat) is a potent inhibitor of HDAC with IC50 of 7.5-16 nM. | J Neurosci, 2013, 33(17), 7535-7547. |
| Tubastatin A HCl  | Tubastatin A is a potent HDAC6 inhibitor with IC50 of 15 nM. | |
| CUDC-101  | CUDC-101 is a potent multi-target inhibitor targeting HDAC, EGFR and HER2 with IC50 of 4.4 nM, 2.4 nM, and 15.7 nM, respectively. | |
| SB939 (Pracinostat)  (E)-3-(2-butyl-1-(2-(diethylamino)ethyl)-1H-benzo[d]imidazol-5-yl)-N-hydroxyacrylamide | SB939 is a potent HDAC inhibitor with IC50 of 40-140 nM. | Antimicrob Agents Chemother, 2012, 56(7), 3849-3856 |
| Droxinostat  4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide | Droxinostat (CMH, 5809354) is a selective inhibitor of HDAC3, HDAC6 and HDAC8 with IC50 of 16.9 μM, 2.47μM and 1.46 μM, respectively. | Nat Struct Mol Biol, 2013, 20(3):317-25 |
| JNJ-26481585 (Quisinostat) | JNJ-26481585 (Quisinostat) is an HDAC inhibitor for HDAC1, HDAC2, HDAC4, HDAC10 and HDAC11 with IC50 of 0.11 nM, 0.33 nM, 0.64 nM, 0.46 nM and 0.37 | |

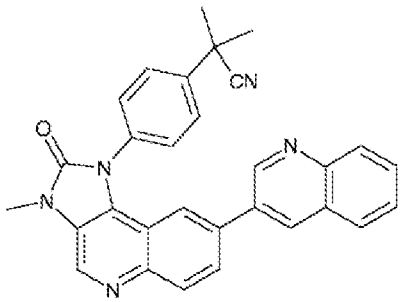
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
|  N-hydroxy-2-(4-((((1-methyl-1H-indol-3-yl)methyl)amino)methyl)piperidin-1-yl)pyrimidine-5-carboxamide | nM, respectively. | |
| PCI-24781(CRA-024781) | PCI-24781 (CRA-024781) is a novel broad spectrum HDAC inhibitor targeting HDAC1, HDAC2, HDAC3, HDAC6, HDAC8 and HDAC10 with Ki of 7 nM, 19 nM, 8.2 nM, 17 nM, 280 nM, 24 nM, respectively. | PLoS One, 2013, 8(5), e65369; Nat Biotechnol, 2011, 29(3), 255-265. |
| Romidepsin (FK228, depsipeptide)  | Romidepsin (FK228, FR901228, depsipeptide, NSC 630176) is a potent HDAC1 and HDAC2 inhibitor with IC50 of 36 nM and 47 nM, respectively. (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(1methylethyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16ene-3,6,9,19,22-pentone | J Neurosci, 2013, 33(17):7535-7547; Br J Haematol, 2013. |
| AR-42 (OSU-HDAC42)  (S)-N-hydroxy-4-(3-methyl-2-phenylbutanamido)benzamide | AR-42 (HDAC-42, OSU-HDAC42) is a pan-HDAC inhibitor with IC50 30 nM. | |
| Valproic acid sodium salt (Sodium valproate) | Valproic acid sodium salt (Sodium valproate) is a HDAC inhibitor with IC50 of 0.4 mM and also inhibits GABA-transaminase or succinic semialdehyde dehydrogenase. | J Neurosci, 2013, 33(17), 7535-7547. |
| PCI-34051 | PCI-34051 is a potent and specific HDAC8 inhibitor with IC50 of 10 nM. | |
| CI994 (Tacedinaline) | CI994 (Tacedinaline) is an anti-cancer | |

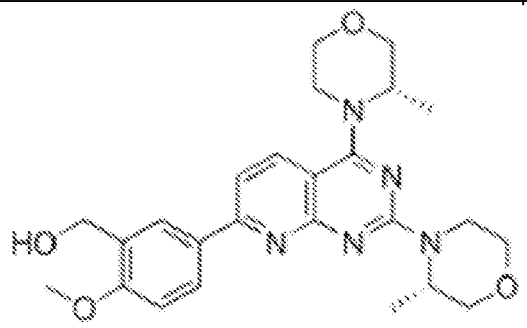
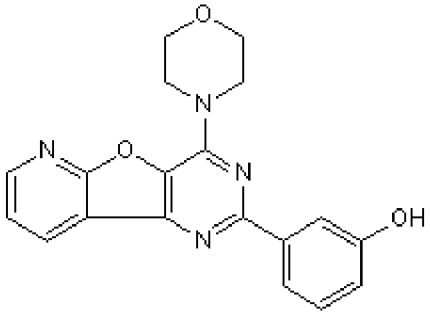
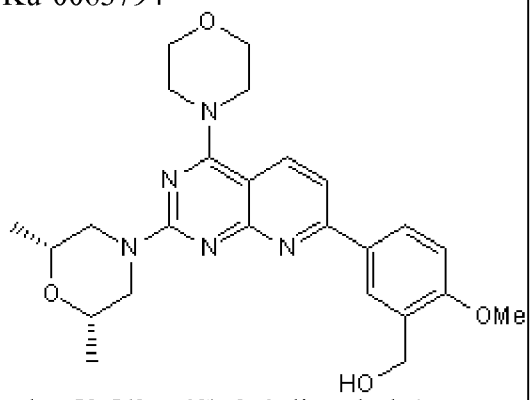
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
|  | drug which inhibits HDAC1 with IC50 of 0.57 μM. | |
| M344 | M344 is a potent HDAC inhibitor with IC50 of 100 nM. | |
| PI3K/HDAC Inhibitor I | PI3K/HDAC Inhibitor I is a dual PI3K and HDAC inhibitor for PI3Kα, HDAC1, HDAC2, HDAC3 and HDAC10 with IC50 of 19 nM, 1.7 nM, 5 nM, 1.8 nM and 2.8 nM, respectively. | |
| Rocilinostat (ACY-1215)  | Rocilinostat (ACY-1215) is a selective HDAC6 inhibitor with IC50 of 5 nM. | |
| Apicidin (OSI-2040)  | Apicidin is a potent HDAC inhibitor with IC50 of 0.7 nM.<br><br>(3S,6S,9S,15aR)-9-((R)-sec-butyl)-6-((1-methoxy-1H-indol-2-yl)methyl)-3-(6-oxooctyl)decahydro-1H-pyrido[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10(12H)-tetraone | |
| Scriptaid | Scriptaid is an inhibitor of HDAC. | |
| Tubastatin A  N-hydroxy-4-((2-methyl-3,4-dihydro-1H-pyrido[4,3-b]indol-5(2H)-yl)methyl)benzamide hydrochloride | Tubastatin A is a potent and selective inhibitor of HDAC6 with IC50 of 15 nM. | J Biol Chem, 2013, 288(20), 14400-7. |
| Sodium Phenylbutyrate | Sodium Phenylbutyrate is a transcriptional | |

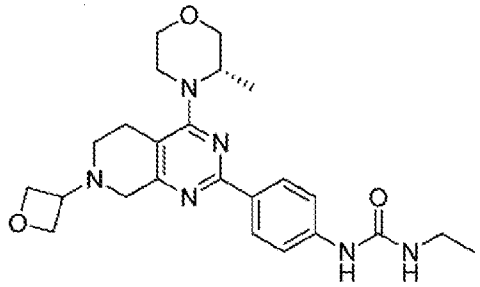
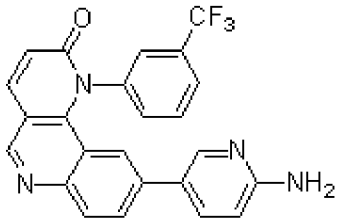
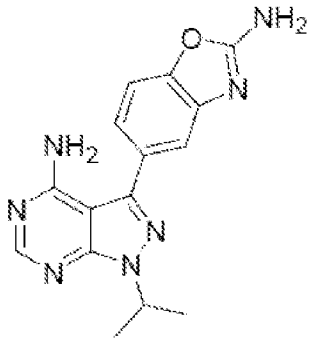
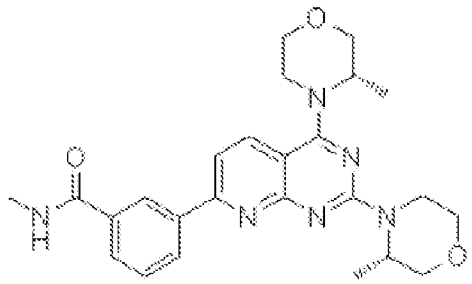
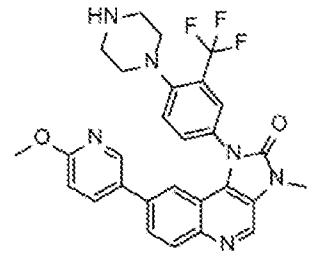
| Inhibitor Name | Inhibitor Information | Literature Citations |
|---|---|---|
|  | regulators that act by altering chromatin structure via the modulation of HDAC activity. | |
| Resminostat (RAS2410)  | (E)-3-(1-((4-((dimethylamino)methyl)phenyl)sulfonyl)-1H-pyrrol-3-yl)-N-hydroxyacrylamide | |

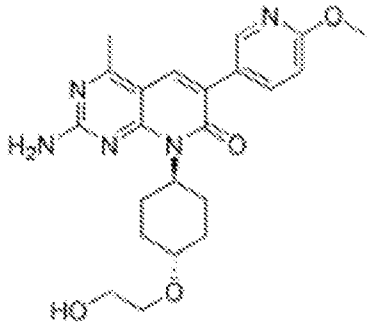
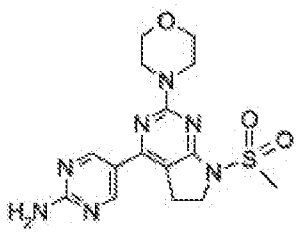
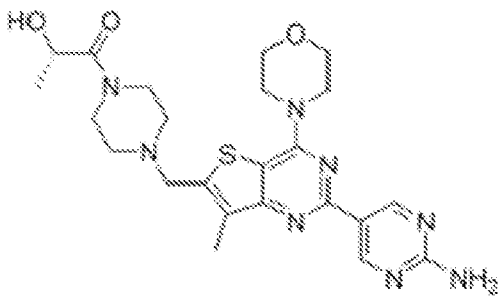
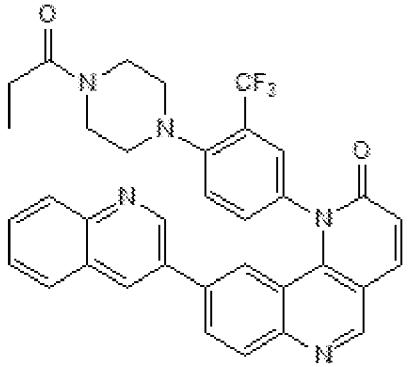
**[00158]** Example mammalian target of rapamycin (mTOR) inhibitors for use in combinations with the thienotriazolodiazepine of Formula (1) in the methods of the present invention include, but are not limited to, the mTOR inhibitors listed in the below Table D.

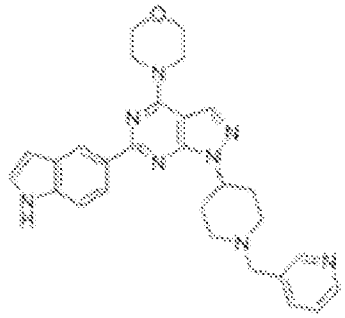
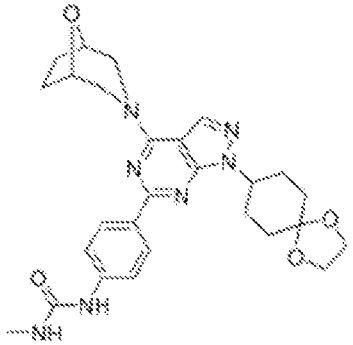
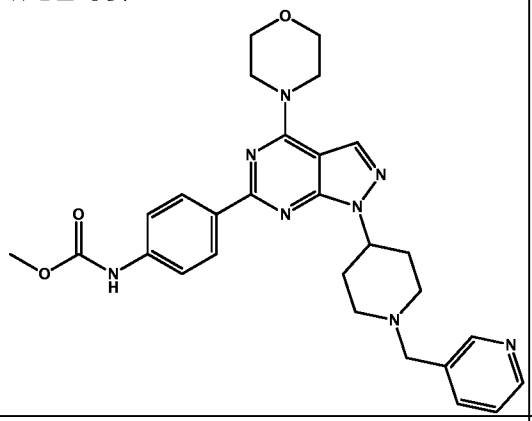
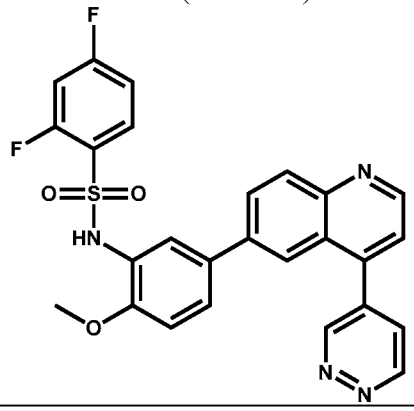
**[00159]** Table D:

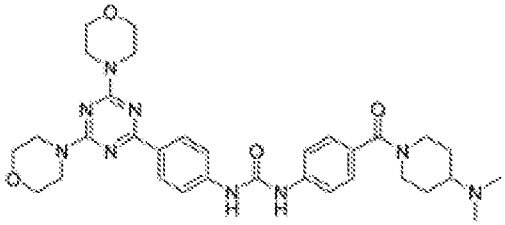
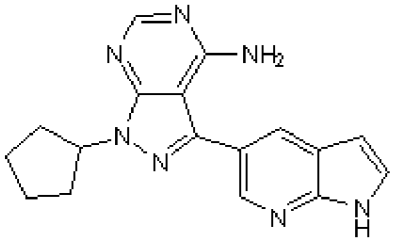
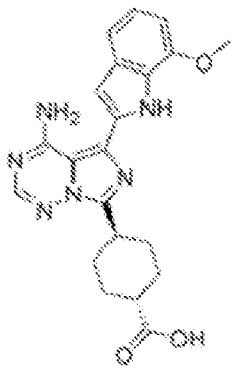
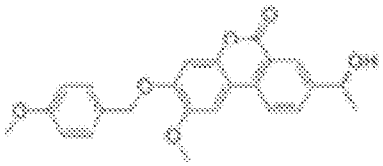
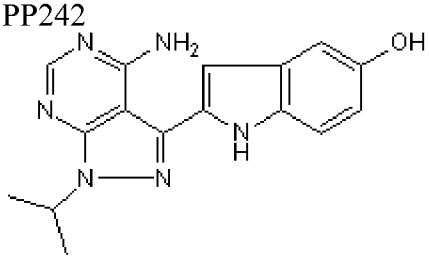
| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| 1 | BEZ235 (NVP-BEZ235)  | BEZ235 (NVP-BEZ235) is a dual ATP-competitive PI3K and mTOR inhibitor of p110α, p110γ, p110δ and p110β with IC50 of 4 nM, 5 nM, 7 nM and 75 nM, respectively, and also inhibits ATR with IC50 of 21 nM. | Nature, 2012, 487(7408):505-9;Blood, 2011, 118(14), 3911-3921;Cancer Res, 2011, 71(15), 5067-5074. |
| 2 | Everolimus (RAD001) | Everolimus (RAD001) is an mTOR inhibitor of FKBP12 with IC50 of 1.6-2.4 nM. | Cell, 2012, 149(3):656-70;;Cancer Cell, 2012, 21(2), 155-167; Clin Cancer Res, 2013, 19(3):598-609. |
| 3 | Rapamycin (Sirolimus, AY22989, NSC226080) | Rapamycin (Sirolimus, AY-22989, WY-090217) is a specific mTOR inhibitor with IC50 of ~0.1 nM. | Cancer Cell, 2011, 19(6), 792-804;;Cancer Res, 2013, ;Cell Res, 2012, 22(6):1003-21. |
| 4 | AZD8055 | AZD8055 is a novel ATP-competitive | Autophagy, 2012, Am J Transplant, |

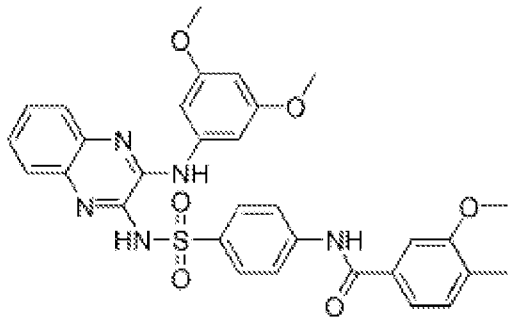
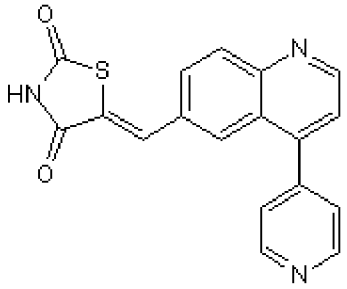
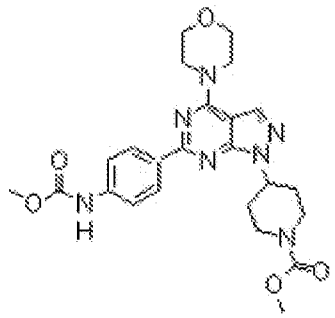
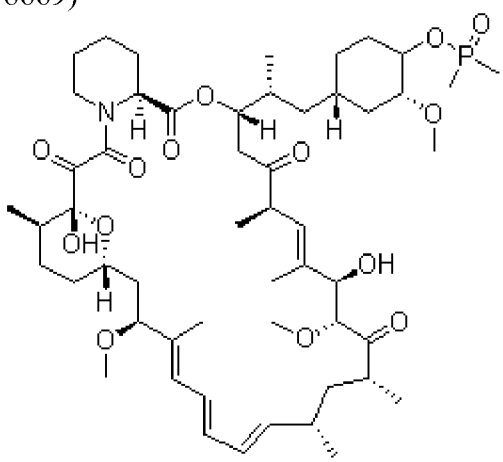
| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| |  | inhibitor of mTOR with IC50 of 0.8 nM. | 2013, ;Biochem Pharmacol, 2012, 83(9), 1183-1194 |
| 5 | PI-103  3-[4-(4-Morpholinylpyrido [3',2':4,5]f-uro[3,2-d]pyrimidin-2-yl] phenol | PI-103 is a potent, ATP-competitive PI3K inhibitor of DNA-PK, p110α, mTORC1, PI3KC2β, p110δ, mTORC2, p110β, and p110γ with IC50 of 2 nM, 8 nM, 20 nM, 26 nM, 48 nM, 83 nM, 88 nM and 150 nM, respectively. | Leukemia, 2013, 27(3):650-60;Leukemia, 2012, 26(5):927-33;Biochem Pharmacol, 2012, 83(9), 1183-1194. |
| 6 | Temsirolimus (CCI-779, NSC-683864) | Temsirolimus (CCI-779, Torisel) is a specific mTOR inhibitor with IC50 of 1.76 μM. | Autophagy, 2011, 7(2), 176-187;Tuberc Respir Dis (Seoul), 2012, 72(4), 343-351;PLoS One, 2013, 8(5):e62104. |
| 7 | Ku-0063794  rel-5-[2-[(2R,6S)-2,6-dimethyl-4-mo-rpholinyl]-4-(4-morpholinyl)pyrido[2,3-d]pyrimidin--7-yl]-2-methoxybenzenemethanol | KU-0063794 is a potent and highly specific mTOR inhibitor for both mTORC1 and mTORC2 with IC50 ~10 nM. | Cell Stem Cell, 2012, 10(2):210-7;Circ Res, 2010, 107(10), 1265-1274;J Immunol, 2013, 190(7), 3246-55. |
| 8 | GDC-0349 | GDC-0349, is a potent and selective ATP-competitive inhibitor of | |

| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| |  | mTOR with Ki of 3.8 nM. | |
| 9 | Torin 2  9-(6-Amino-3-pyridinyl)-1-[3-(trifl-uoromethyl)phenyl]-benzo[h]-1,6-naphthyridin-2(1H)--one | Torin 2 is a highly potent and selective mTOR inhibitor with IC50 of 0.25 nM, and also exhibits potent cellular activity against ATM/ATR/DNA-PK with EC50 of 28 nM, 35 nM and 118 nM, respectively. | |
| 10 | INK 128 (MLN-0128)  | INK 128 is a potent and selective mTOR inhibitor with IC50 of 1 nM. | |
| 11 | AZD2014  | AZD2014 is a novel dual mTORC1 and mTORC2 inhibitor with potential antineoplastic activity. | |
| 12 | NVP-BGT226(BGT226)  | NVP-BGT226 is a novel dual PI3K/mTOR inhibitor with IC50 of 1 nM. | |
| 13 | PF-04691502 | PF-04691502 is an | |

| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| |  | ATP-competitive, selective inhibitor of PI3K(α/β/δ/γ)/mTOR with Ki of 1.8 nM/2.1 nM/1.6 nM/1.9 nM and 16 nM, also inhibits Akt phosphorylation on T308/S473 with IC50 of 7.5 nM/3.8 nM. | |
| 14 | CH5132799  | CH5132799 exhibits a strong inhibitory activity especially against PI3Kα with IC50 of 14 nM and also inhibits mTOR with IC50 of 1.6 μM. | |
| 15 | GDC-0980 (RG7422)  | GDC-0980 (RG7422) is a potent, selective inhibitor of PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ with IC50 of 5 nM, 27 nM, 7 nM, and 14 nM, and also a mTOR inhibitor with Ki of 17 nM. | |
| 16 | Torin 1  1-[4-[4-(1-Oxopropyl)-1-piperazinyl-]-3-(trifluoromethyl)phenyl]-9-(3-quinolinyl)-benz-o[h]-1,6-naphthyridin-2(1H)-one | Torin1 is a potent inhibitor of mTOR with IC50 of 2-10 nM. | |
| 17 | WAY-600 | WAY-600 is a potent, ATP-competitive and selective inhibitor of mTOR with IC50 of 9 | |

| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| |  | nM. | |
| 18 | WYE-125132(WYE-132)  | WYE-125132 is a highly potent, ATP-competitive and specific mTOR inhibitor with IC50 of 0.19 nM. | |
| 19 | WYE-687  | WYE-687 is an ATP-competitive and selective inhibitor of mTOR with IC50 of 7 nM. | |
| 20 | GSK2126458(GSK458)  | GSK2126458 is a highly selective and potent inhibitor of p110α, p110β, p110γ, p110δ, mTORC1 and mTORC2 with Ki of 0.019 nM, 0.13 nM, 0.024 nM, 0.06 nM, 0.18 nM and 0.3 nM, respectively. | |
| 21 | PF-05212384 (PKI-587) | PKI-587 is a highly potent dual inhibitor of PI3Kα, PI3Kγ and mTOR with IC50 of 0.4 | |

| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| |  | nM, 5.4 nM and 1.6 nM, respectively. | |
| 22 | PP-121  1-Cyclopentyl-3-(1H-pyrrolo[2,3-b]p-yridin-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine | PP-121 is a multi-target inhibitor of PDGFR, Hck, mTOR, VEGFR2, Src and Abl with IC50 of 2 nM, 8 nM, 10 nM, 12 nM, 14 nM and 18 nM, respectively, and also inhibits DNA-PK with IC50 of 60 nM. | |
| 23 | OSI-027(ASP4786)  | OSI-027 is a selective and potent dual inhibitor of mTORC1 and mTORC2 with IC50 of 22 nM and 65 nM, respectively. | Exp Eye Res, 2013, 113C, 9-18 |
| 24 | Palomid 529(P529)  | Palomid 529 inhibits both the mTORC1 and mTORC2 complexes, reduces phosphorylation of pAktS473, pGSK3βS9, and pS6 but neither pMAPK nor pAktT308. Phase 1. | |
| 25 | PP242  2-[4-Amino-1-(1-methylethyl)-1H-pyr-azolo[3,4-d]pyrimidin-3-yl]-1H-indol-5-ol | PP242 is a selective mTOR inhibitor with IC50 of 8 nM. | Autophagy, 2012, 8(6), 903-914 |

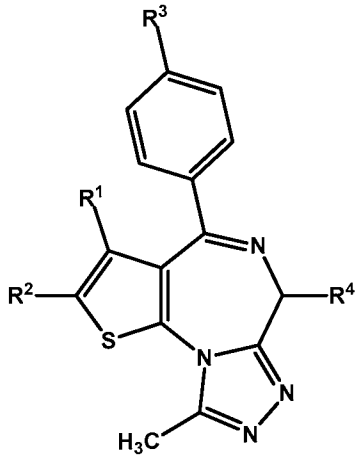
| No. | Inhibitor Name | Description | Literature Citations |
|---|---|---|---|
| 26 | XL765(SAR245409)  | XL765 is a dual inhibitor of mTOR/PI3k for mTOR, p110α, p110β, p110γ and p110δ with IC50 of 157 nM, 39 nM, 113 nM, 9 nM and 43 nM, respectively. | Endocrinology, 2013, 154(3):1247-59 |
| 27 | GSK1059615  5-[[4-(4-Pyridinyl)-6-quinolinyl]me-thylene]-2,4-thiazolidenedione | GSK1059615 is a novel and dual inhibitor of PI3Kα, PI3Kβ, PI3Kδ, PI3Kγ and mTOR with IC50 of 0.4 nM, 0.6 nM, 2 nM, 5 nM and 12 nM, respectively. | Nature, 2012, 486(7404), 532-536 |
| 28 | WYE-354  | WYE-354 is a potent, specific and ATP-competitive inhibitor of mTOR with IC50 of 5 nM. | Mol Cancer Res, 2012, 10(6), 821-833. |
| 29 | Deforolimus (Ridaforolimus, MK-8669)  | Deforolimus (Ridaforolimus; AP23573; MK-8669; 42-(Dimethylphosphinate) rapamycin; Ridaforolimus ) is a selective mTOR inhibitor with IC50 of 0.2 nM. | Mol Genet Meta, 2010, 100(4), 309-315. |

**[00160]** A mammalian subject as used herein can be any mammal. In one embodiment, the mammalian subject includes, but is not limited to, a human; a non-human primate; a rodent such as a mouse, rat, or guinea pig; a domesticated pet such as a cat or dog; a horse, cow, pig, sheep, goat, or rabbit. In one embodiment, the mammalian subject includes, but is not limited to, a bird such as a duck, goose, chicken, or turkey. In one embodiment, the mammalian subject is a human. In one embodiment, the mammalian subject can be either gender and can be any age.

**[00161]** In the present invention, "treatment" or "treat" refers to an act or the action of administration of the active ingredient of the present invention to a person diagnosed by a doctor to acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML or be at risk of developing acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML (patient), which aims, for example, to alleviate the acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML (or symptom of the same), prevent the onset of the acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML (or symptom of the same), or restore the state before onset of the acute myeloid leukemia, acute lymphoblastic leukemia, high risk myelodysplastic syndrome, and/or HR-MDS/AML.

## III. Thienotriazolodiazepine Compounds:

**[00162]** In one embodiment, the thienotriazolodiazepine compounds, used in the formulations of the present invention, are represented by Formula (1):




(1)

**[00163]** wherein

$R^1$ is alkyl having a carbon number of 1-4, $R^2$ is a hydrogen atom; a halogen atom; or alkyl having a carbon number of 1-4 optionally substituted by a halogen atom or a hydroxyl group, $R^3$ is a halogen

atom; phenyl optionally substituted by a halogen atom, alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4 or cyano; —$NR^5$—$(CH_2)_m$—$R^6$ wherein $R^5$ is a hydrogen atom or alkyl having a carbon number of 1-4, m is an integer of 0-4, and $R^6$ is phenyl or pyridyl optionally substituted by a halogen atom; or –$NR^7$—CO—$(CH_2)_n$—$R^8$ wherein $R^7$ is a hydrogen atom or alkyl having a carbon number of 1-4, n is an integer of 0-2, and $R^8$ is phenyl or pyridyl optionally substituted by a halogen atom, and $R^4$ is —$(CH_2)_a$—CO—NH—$R^9$ wherein a is an integer of 1-4, and $R^9$ is alkyl having a carbon number of 1-4; hydroxyalkyl having a carbon number of 1-4; alkoxy having a carbon number of 1-4; or phenyl or pyridyl optionally substituted by alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4, amino or a hydroxyl group or —$(CH_2)_b$—$COOR^{10}$ wherein b is an integer of 1-4, and $R^{10}$ is alkyl having a carbon number of 1-4, including any salts, isomers, enantiomers, racemates, hydrates, solvates, metabolites, and polymorphs thereof.

**[00164]** In one embodiment, a suitable alkyl group includes linear or branched akyl radicals including from 1 carbon atom up to 4 carbon atoms. In one embodiment, a suitable alkyl group includes linear or branched akyl radicals including from 1 carbon atom up to 3 carbon atoms. In one embodiment, a suitable alkyl group includes linear or branched akyl radicals include from 1 carbon atom up to 2 carbon atoms. In one embodiment, exemplary alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl. In one embodiment, exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, and 2-methyl-2-propyl.

**[00165]** In some embodiments, the present invention provides pharmaceutically acceptable salts, solvates, including hydrates, and isotopically-labeled forms of the thienotriazolodiazepine compounds described herein. In one embodiment, pharmaceutically acceptable salts of the thienotriazolodiazepine compounds include acid addition salts formed with inorganic acids. In one embodiment, pharmaceutically acceptable inorganic acid addition salts of the thienotriazolodiazepine include salts of hydrochloric, hydrobromic, hydroiodic, phosphoric, metaphosphoric, nitric and sulfuric acids. In one embodiment, pharmaceutically acceptable salts of the thienotriazolodiazepine compounds include acid addition salts formed with organic acids. In one embodiment, pharmaceutically acceptable organic acid addition salts of the thienotriazolodiazepine include salts of tartaric, acetic, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, formic, propionic, glycolic, gluconic, maleic, succinic, camphorsulfuric, isothionic, mucic, gentisic, isonicotinic, saccharic, glucuronic, furoic, glutamic, ascorbic, anthranilic, salicylic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, pantothenic, stearic, sulfinilic,

alginic, galacturonic and arylsulfonic, for example benzenesulfonic and 4-methyl benzenesulfonic acids.

**[00166]** The present invention provides pharmaceutically acceptable isotopically-labeled forms of the thienotriazolodiazepine compounds, described herein, wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in the thienotriazolodiazepine compounds include isotopes of hydrogen, e.g., $^{2}H$ and $^{3}H$, carbon, e.g., $^{11}C$, $^{13}C$ and $^{14}C$, chlorine, e.g., $^{36}Cl$, fluorine, e..g., $^{18}F$, iodine, e.g., $^{123}I$ and $^{125}I$, nitrogen, e.g., $^{13}N$ and $^{15}N$, oxygen, e.g., $^{15}O$, $^{17}O$ and $^{18}O$, and sulfur, e.g., $^{35}S$. Isotopically-labeled forms of the thienotriazolodiazepine compounds generally can be prepared by conventional techniques known to those skilled in the art.

**[00167]** Certain isotopically-labeled forms of the compound of Formula (1), for example those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium ($^{3}H$) and carbon-14 ($^{14}C$) are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Substitution with heavier isotopes such as deuterium ($^{2}H$) may afford certain therapeutic advantages that result from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances. Substitution with positron emitting isotopes, such as $^{11}C$, $^{18}F$, $^{15}O$, and $^{13}N$ can be used in Positron Emission Tomography (PET) studies for examining substrate receptor occupancy.

**[00168]** In some embodiments, the thienotriazolodiazepine compounds disclosed herein can exist in solvated as well as unsolvated forms with pharmaceutically acceptable solvents. It will be understood by those skilled-in the art that a solvate is a complex of variable stoichiometry formed by a solute (in this case, the thienotriazolodiazepine compounds described herein) and a solvent. It is preferred that such solvents not interfere with the biological activity of the solute (the thienotriazolodiazepine compounds). Examples of suitable solvents for solvate formation include, but are not limited to, water, methanol, dimethyl sulfoxide, ethanol and acetic acid. Suitably the solvent used is a pharmaceutically acceptable solvent. Suitably the solvent used is water. In one embodiment, pharmaceutically acceptable solvates of the thienotriazolodiazepine compounds, described herein, include ethanol solvate, a isopropanol solvate, a dioxolane solvate, a tetrahydrofuran solvate, a dimethyl sulfoxide solvate, tert-butanol solvate, 2-butanol solvate, dioxolane solvate, 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone ("DMPU") solvate, 1,3-

dimethylimidazolidinone ("DMI") solvate, and 1,3-dimethylimidazolidinone ("DMP") solvate, or mixtures thereof.

**[00169]** In some embodiments, the thienotriazolodiazepine compounds, described herein, may contain one or more chiral centers and/or double bonds and, therefore, may exist as geometric isomers, enantiomers or diastereomers. The enantiomer and diastereomers of the thienotriazolodiazepine compounds may be designated in accordance with the Cahn–Ingold–Prelog convention, which assigns an *"R"* or *"S"* descriptor to each stereocenter (also sometimes referred to as a chiral center) and an *E* or *Z* descriptor to each carbon-carbon double bond (to designate geometric isomers) so that the configuration of the entire molecule can be specified uniquely by including the descriptors in its systematic name.

**[00170]** In some embodiments, the thienotriazolodiazepine compounds, described herein, may exist as a racemic mixture, or racemate, which includes equal amounts of left- and right-handed enantiomers of a chiral molecule. Such a racemic mixture may be denoted by the prefix (±)- or dl-, indicating an equal (1:1) mixture of dextro and levo isomers. Also, the prefix *rac-* (or *racem-*) or the symbols *RS* and *SR* may be used to designate the racemic mixture.

**[00171]** Geometric isomers, resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a cycloalkyl or heterocyclic ring, can also exist in the compounds of the present invention. In some embodiments, the symbol ------ may be used to denote a bond that may be a single, double or triple bond. Substituents around a carbon-carbon double bond are designated as being in the "*Z*" or "*E*" configuration wherein the terms "*Z*" and "*E*" are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the "*E"* and "*Z"* isomers. Substituents around a carbon-carbon double bond alternatively can be referred to as "*cis*" or "*trans*," where "*cis*" represents substituents on the same side of the double bond and "*trans*" represents substituents on opposite sides of the double bond. The arrangement of substituents around a carbocyclic ring can also be designated as "*cis*" or "*trans*." The term "*cis*" represents substituents on the same side of the plane of the ring and the term "*trans*" represents substituents on opposite sides of the plane of the ring. Mixtures of compounds wherein the substituents are disposed on both the same and opposite sides of a plane of a ring are designated "*cis/trans*" or "*Z/E*."

**[00172]** In some embodiments, thienotriazolodiazepine compounds disclosed herein may exist in single or multiple crystalline forms or polymorphs. In one embodiment, a thienotriazolodiazepine compound disclosed herein comprises an amorphous form thereof. In one embodiment, a thienotriazolodiazepine compound disclosed herein comprises a single polymorph thereof. In

another embodiment, a thienotriazolodiazepine compound disclosed herein comprises a mixture of polymorphs thereof. In another embodiment, the compound is in a crystalline form.

**[00173]** In some embodiments, thienotriazolodiazepine compounds disclosed herein may exist as a single enantiomers or in enatiomerically enriched forms. In one embodiment, a thienotriazolodiazepine compound disclosed herein exists in an entiomeric excess of more than 80%. In one embodiment, a thienotriazolodiazepine compound disclosed herein exists in an entiomeric excess of more than 90%. In one embodiment, a thienotriazolodiazepine compound disclosed herein exists in an entiomeric excess of more than 98%. In one embodiment, a thienotriazolodiazepine compound disclosed herein exists in an entiomeric excess of more than 99%. In some embodiments, a thienotriazolodiazepine compound disclosed herein exists in an entiomeric excess selected from the group consisting of at least 10%, at least 25%, at least 50%, at least 75%, at least 90%, at least 95%, at least 98%, at least and at least 99% enantiomeric excess.

**[00174]** For a pair of enantiomers, enantiomeric excess (ee) of enantiomer *E1* in relation to enantiomer *E2* can be calculated using the following equation eq. (1):

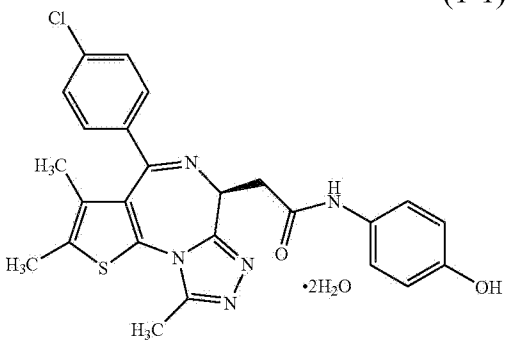
$$\%\ \text{enantiomeric excess of}\ E1 = \frac{(E1 - E2)\text{x}100\%}{(E1 + E2)} \qquad \text{eq. (1)}$$

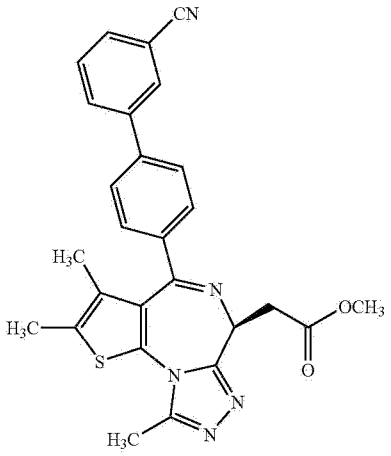
Relative amounts of *E1* and *E2* can be determined by chiral high performance liquid chromatography (HPLC ), nuclear magnetic resonance (NMR) or any other suitable methods. In some embodiments, purity of an entiormeric compound may refer to the amount of the enantiomers *E1* and *E2*, relative to the amount of other materials, which may notably include by-products and/or unreacted reactants or reagents.

**[00175]** In some embodiments, thienotriazolodiazepine compounds of Formula (1) include, but are not limited to, the thienotriazolodiazepine compounds (1-1) to (1-18), which are listed in the following Table A.

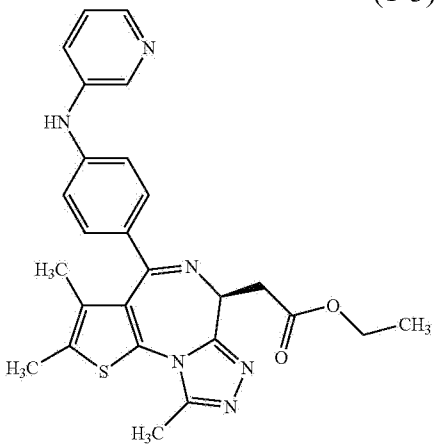
**[00176]** Table A: Exemplary compounds which may be used in the formulations described herein:

(1-1) (1-2)

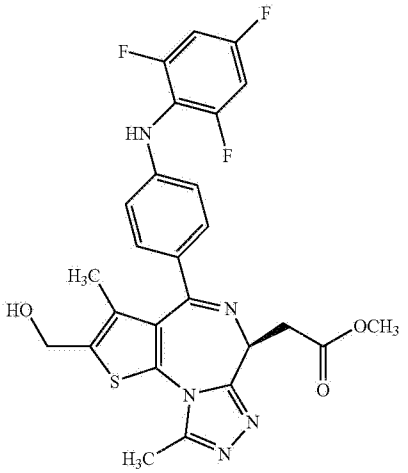




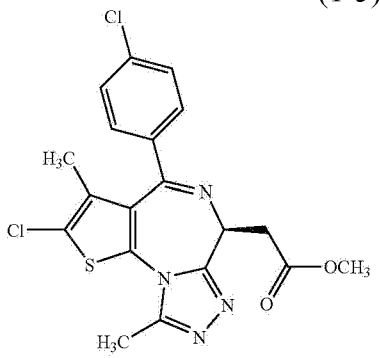
(1-3)



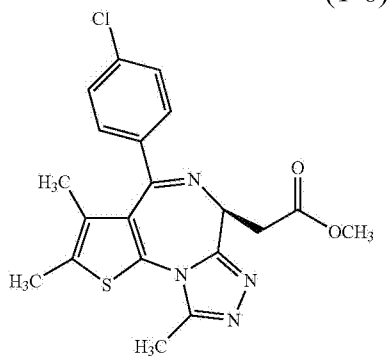
(1-4)



(1-5)



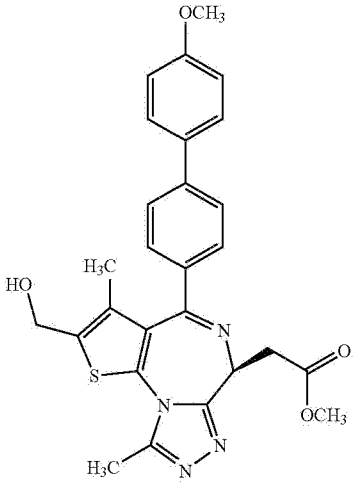
(1-6)



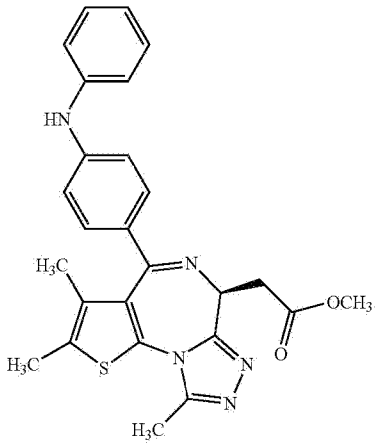
**[00177]** Table A (continued):

(1-7)

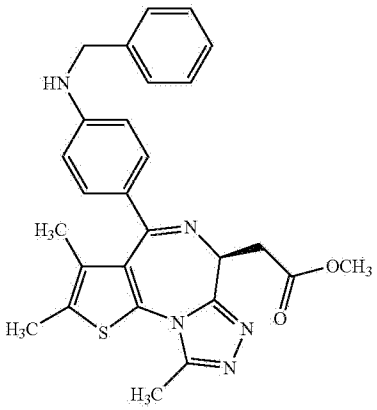
(1-8)



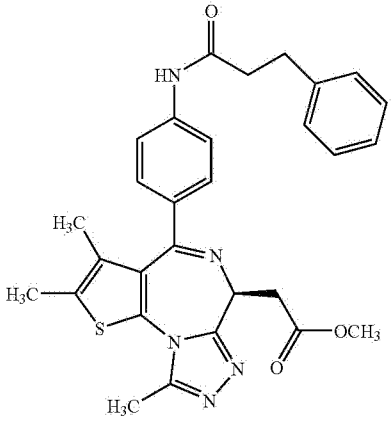
(1-9)



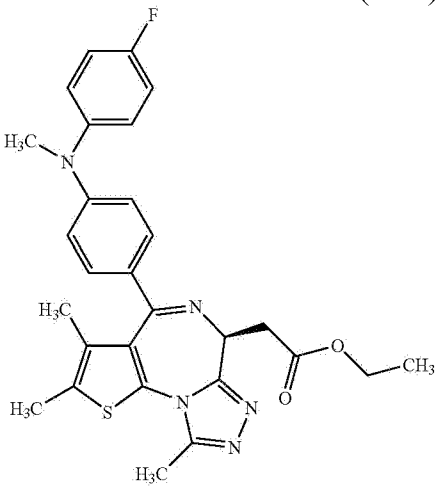
(1-10)



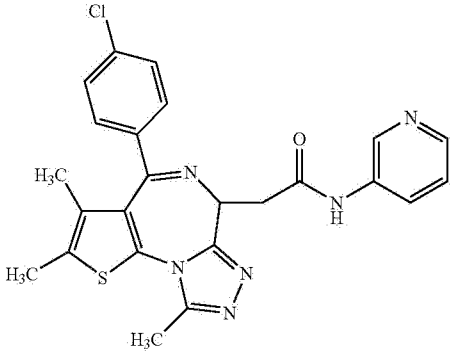
(1-11)



(1-12)

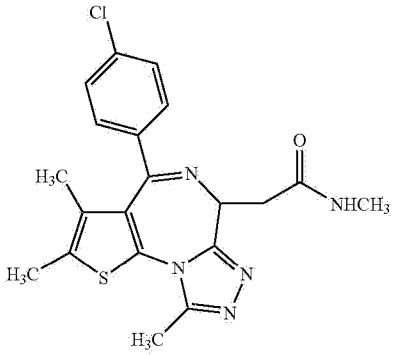


(1-13)

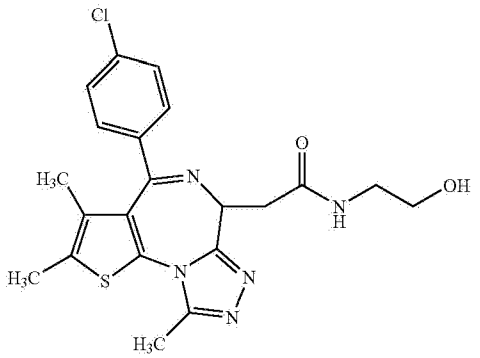


(1-14)

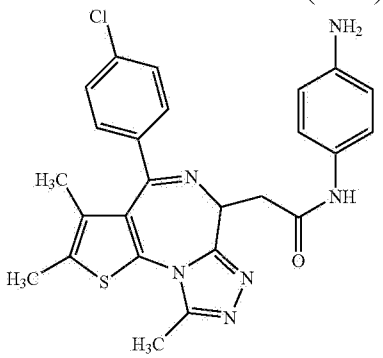
**[00178]** Table A (continued):



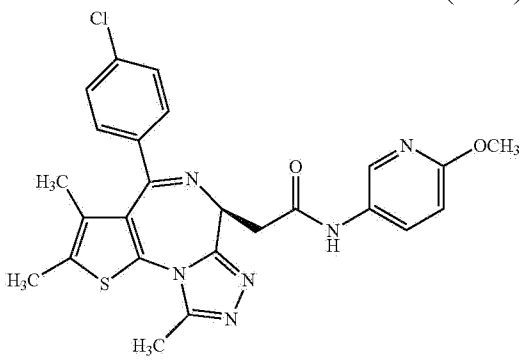
(1-15)



(1-16)



(1-17)



(1-18)

**[00179]** In some embodiments, thienotriazolodiazepine compounds of Formula (1) include (i) (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo- [4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide or a dihydrate thereof, (ii) methyl (S)-{4-(3'-cyanobiphenyl-4-yl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]tri- azolo[4,3-a][1,4]diazepin-6-yl}acetate, (iii) methyl (S)-{2,3,9-trimethyl-4-(4-phenylaminophenyl)-6H-thieno[3,2-f][1,2,4]triaz- olo[4,3-a][1,4]diazepin-6-yl}acetate; and (iv) methyl (S)-{2,3,9-trimethyl-4-[4-(3-phenylpropionylamino)phenyl]-6H-thieno[3,2-f- ][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl}acetate.

**[00180]** In some embodiments, thienotriazolodiazepine compounds of Formula (1) include (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,- 4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide.

## IV. Formulations:

**[00181]** The compound of Formula (1) presents highly specific difficulties in relation to administration generally and the preparation of galenic compositions in particular, including the particular problems of drug bioavailability and variability in inter- and intra-patient dose response, necessitating development of a non-conventional dosage form with respect to the practically water-insoluble properties of the compound.

**[00182]** Previously, it had been found that the compound of Formula (1) could be formulated as a solid dispersion with the carrier ethyl acrylate-methyl methacrylate-trimethylammonioethyl methacrylate chloride copolymer (Eudragit RS, manufactured by Rohm) to provide an oral formulation that preferentially released the pharmaceutical ingredient in the lower intestine for treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (US Patent Application 20090012064 A1, published Jan 8, 2009). It was found, through various experiments, including animal tests, that in inflammatory bowel diseases drug release in a lesion and a direct action thereof on the inflammatory lesion were more important than the absorption of the drug into circulation from the gastrointestinal tract.

**[00183]** It has now been unexpectedly found that thienotriazolodiazepine compounds, according to Formula (1), pharmaceutically acceptable salts, solvates, including hydrates, racemates, enantiomers isomers, and isotopically-labeled forms thereof, can be formulated as a solid dispersion with pharmaceutically acceptable polymers to provide an oral formulation that provides high absorption of the pharmaceutical ingredient into the circulation from the gastrointestinal tract for treatment of diseases other than inflammatory bowel diseases. Studies in both dogs and humans have confirmed high oral bioavailability of these solid dispersions compared with the Eudragit solid dispersion formulation previously developed for the treatment of inflammatory bowel disease.

**[00184]** Solid dispersions are a strategy to improve the oral bioavailability of poorly water soluble drugs.

**[00185]** The term "solid dispersion" as used herein refers to a group of solid products including at least two different components, generally a hydrophilic carrier and a hydrophobic drug, the thienotriazolodiazepine compounds, according to Formula (1). Based on the drug's molecular arrangement within the dispersion, six different types of solid dispersions can be distinguished. Commonly, solid dispersions are classified as simple eutectic mixtures, solid solutions, glass solution and suspension, and amorphous precipitations in a crystalline carrier. Moreover, certain

combinations can be encountered, for example, in the same sample some molecules may be present in clusters while some are molecularly dispersed.

**[00186]** In one embodiment, the thienotriazolodiazepine compounds, according to Formula (1) can be dispersed molecularly, in amorphous particles (clusters). In another embodiment, the thienotriazolodiazepine compounds, according to Formula (1) can be dispersed as crystalline particles. In one embodiment, the carrier can be crystalline. In another embodiment, the carrier can be amorphous.

**[00187]** In one embodiment, the present invention provides a pharmaceutical composition comprising a solid dispersion of a thienotriazolodiazepine compound, in accordance with Formula (1), or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate (also called hydroxypropylmethylcellulose acetate succinate or HPMCAS). In one embodiment, the dispersion has a thienotriazolodiazepine compound to hydroxypropylmethylcellulose acetate succinate (HPMCAS) weight ratio of 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In other such embodiments, the single Tg occurs at about 135 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00188]** In one embodiment, the present invention provides a pharmaceutical composition comprising a solid dispersion of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof in a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone (also called povidone or PVP). In one embodiment, the dispersion has a thienotriazolodiazepine compound to PVP weight ratio of 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine

compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to about 185 °C. In other such embodiments, the single Tg occurs at about 179 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00189]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of an amorphous form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to hypromellose acetate succinate ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In other such embodiments, the single Tg occurs at about 135 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00190]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of an amorphous form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one

embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to polyvinylpyrrolidone ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to about 185 °C. In other such embodiments, the single Tg occurs at about 179 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00191]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of a crystalline form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to hypromellose acetate succinate ranges from 1:3 to 1:1.

**[00192]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of a crystalline form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to polyvinylpyrrolidone ranges from 1:3 to 1:1.

**[00193]** In some embodiments, a pharmaceutical composition comprising a solid dispersion is prepared by spray drying.

**[00194]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an

isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate. In one embodiment, the weight ratio of compound (1) to hypromellose acetate succinate ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In other such embodiments, the single Tg occurs at about 135 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00195]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone. In one embodiment, the weight ratio of compound (1) to polyvinylpyrrolidone ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to 185 °C. In other such embodiments, the single Tg occurs at about 179 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00196]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of an amorphous form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to hypromellose acetate succinate ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In other such embodiments, the single Tg occurs at about 135 °C. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

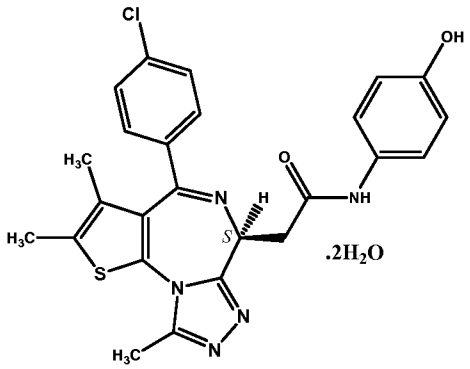
**[00197]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of an amorphous form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to polyvinylpyrrolidone ranges from 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to 185 °C. In other such embodiments, the single Tg occurs at about 179 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound

of Formula (1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound of Formula (1).

**[00198]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of a crystalline form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is hypromellose acetate succinate. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to hypromellose acetate succinate ranges from 1:3 to 1:1.

**[00199]** In one embodiment, a pharmaceutical composition of the present invention comprises a spray dried solid dispersion of a crystalline form of a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is polyvinylpyrrolidone. In one embodiment, the weight ratio of thienotriazolodiazepine compound of Formula (1) to polyvinylpyrrolidone ranges from 1:3 to 1:1.

**[00200]** In one preferred embodiment, the present invention provides a pharmaceutical composition comprising a solid dispersion of 2-[(6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thienol[3,2-f]-[1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide dihydrate, compound (1-1):




(1-1)

or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is HPMCAS. In one embodiment, the dispersion has compound (1-1) and HPMCAS in a weight ratio of 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout

the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In one embodiment, the solid dispersion is spray dried. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In other such embodiments, the single Tg occurs at about 135 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound (1-1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound (1-1).

**[00201]** In another embodiment, the pharmaceutical composition comprises a solid dispersion compound (1-1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is PVP. In one embodiment, the dispersion has compound (1-1) and PVP in weight ratio 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In one embodiment, the solid dispersion is spray dried. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to 185 °C. In other such embodiments, the single Tg occurs at about 179 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound (1-1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound (1-1).

**[00202]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of an amorphous form of a thienotriazolodiazepine compound (1-1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is HPMCAS. In one embodiment, the

dispersion has compound (1-1) and HPMCAS in a weight ratio of 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In one embodiment, the solid dispersion is spray dried. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 130 °C to 140 °C. In other such embodiments, the single Tg occurs at about 135 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound (1-1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound (1-1).

**[00203]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of an amorphous form of a thienotriazolodiazepine compound (1-1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is PVP. In one embodiment, the dispersion has compound (1-1) and PVP in weight ratio 1:3 to 1:1. In one embodiment, at least some portion of the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In another embodiment, the thienotriazolodiazepine compound is homogeneously dispersed throughout the solid dispersion. In one embodiment, the solid dispersion is spray dried. In some embodiments, the solid dispersion exhibits a single inflection for the glass transition temperature (Tg). In some embodiments, the single Tg occurs between 175 °C to 185 °C. In other such embodiments, the single Tg occurs at about 189 °C. In some such embodiments, the solid dispersion was exposed to a relative humidity of 75 % at 40 °C for at least one month. In some embodiments, the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound (1-1). For the purpose of this application "substantially free" shall mean the absence of a diffraction line, above the amorphous halo, at about 21° 2-theta associated with crystalline thienotriazolodiazepine compound (1-1).

**[00204]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of a crystalline form of a thienotriazolodiazepine compound (1-1) or a

pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is HPMCAS. In one embodiment, the dispersion has compound (1-1) and HPMCAS in a weight ratio of 1:3 to 1:1. In one embodiment, the solid dispersion is spray dried.

**[00205]** In one embodiment, a pharmaceutical composition of the present invention comprises a solid dispersion of a crystalline form of a thienotriazolodiazepine compound (1-1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof; and a pharmaceutically acceptable polymer. In one embodiment, the pharmaceutically acceptable polymer is PVP. In one embodiment, the dispersion has compound (1-1) and PVP in weight ratio 1:3 to 1:1. In one embodiment, the solid dispersion is spray dried.

**[00206]** The solid dispersions of the invention, described herein, exhibit especially advantageous properties when administered orally. Examples of advantageous properties of the solid dispersions include, but are not limited to, consistent and high level of bioavailability when administered in standard bioavailability trials in animals or humans. The solid dispersions of the invention can include a solid dispersion comprising thienotriazolodiazepine compound of Formula (1) and a polymer and additives. In some embodiments, the solid dispersions can achieve absorption of the thienotriazolodiazepine compound of Formula (1) into the bloodstream that cannot be obtained by merely admixing the thienotriazolodiazepine compound of Formula (1) with additives since the thienotriazolodiazepine compound of Formula (1) drug has negligible solubility in water and most aqueous media. The bioavailability, of thienotriazolodiazepine compound of Formula (1) or of thienotriazolodiazepine compound (1-1) may be measured using a variety of in vitro and/or in vivo studies. The in vivo studies may be performed, for example, using rats, dogs or humans.

**[00207]** The bioavailability may be measured by the area under the curve (AUC) value obtained by plotting a serum or plasma concentration, of the thienotriazolodiazepine compound of Formula (1) or thienotriazolodiazepine compound (1-1), along the ordinate (Y-axis) against time along the abscissa (X-axis). The AUC value of the thienotriazolodiazepine compound of Formula (1) or thienotriazolodiazepine compound (1-1) from the solid dispersion, is then compared to the AUC value of an equivalent concentration of crystalline thienotriazolodiazepine compound of Formula (1) or crystalline thienotriazolodiazepine compound (1-1) without polymer. In some embodiments, the solid dispersion provides an area under the curve (AUC) value, when administered orally to a dog, that is selected from: at least 0.4 times, 0.5 times, 0.6 time, 0.8 time, 1.0 times, a corresponding

AUC value provided by a control composition administered intravenously to a dog, wherein the control composition comprises an equivalent quantity of a crystalline thienotriazolodiazepine compound of Formula (1).

**[00208]** The bioavailability may be measured by in vitro tests simulating the pH values of a gastric environment and an intestine environment. The measurements may be made by suspending a solid dispersion of the thienotriazolodiazepine compound of Formula (1) or thienotriazolodiazepine compound (1-1), in an aqueous in vitro test medium having a pH between 1.0 to 2.0, and the pH is then adjusted to a pH between 5.0 and 7.0, in a control in vitro test medium. The concentration of the amorphous thienotriazolodiazepine compound of Formula (1) or amorphous thienotriazolodiazepine compound (1-1) may be measured at any time during the first two hours following the pH adjustment. In some embodiments, the solid dispersion provides a concentration, of the amorphous thienotriazolodiazepine compound of Formula (1) or amorphous thienotriazolodiazepine compound (1-1), in an aqueous in vitro test medium at pH between 5.0 to 7.0 that is selected from: at least 5-fold greater, at least 6 fold greater, at least 7 fold greater, at least 8 fold greater, at least 9 fold greater or at least 10 fold greater, compared to a concentration of a crystalline thienotriazolodiazepine compound of Formula (1) or crystalline thienotriazolodiazepine compound (1-1), without polymer.

**[00209]** In other embodiments, the concentration of the amorphous thienotriazolodiazepine compound of Formula (1) or amorphous thienotriazolodiazepine compound (1-1), from the solid dispersion placed in an aqueous in vitro test medium having a pH of 1.0 to 2.0, is: at least 40%, at least 50% higher, at least 60 %, at least 70 %; at least 80 %, than a concentration of a crystalline thienotriazolodiazepine compound of Formula (1) without polymer. In some such embodiments, the polymer of the solid dispersion is HPMCAS. In some such embodiments, the polymer of the solid dispersion is PVP.

**[00210]** In other embodiments, a concentration of the amorphous thienotriazolodiazepine compound of Formula (1) or amorphous thienotriazolodiazepine compound (1-1), from the solid dispersion, is: at least 40%, at least 50% higher, at least 60 %, at least 70 %; at least 80 %, compared to a concentration of thienotriazolodiazepine compound of Formula (1), from a solid dispersion of thienotriazolodiazepine compound of the Formula (1) and a pharmaceutically acceptable polymer selected from the group consisting of: hypromellose phthalate and ethyl acrylate-methyl methacrylate-trimethylammonioethyl methacrylate chloride copolymer, wherein each solid dispersion was placed in an aqueous in vitro test medium having a pH of 1.0 to 2.0. In some such

embodiments, the polymer of the solid dispersion is HPMCAS. In some such embodiments, the polymer of the solid dispersion is PVP.

**[00211]** In some embodiments, the solid dispersions, described herein, exhibit stability against recrystallization of the thienotriazolodiazepine compound of the Formula (1) or the thienotriazolodiazepine compound (1-1) when exposed to humidity and temperature over time. In one embodiment, the concentration of the amorphous thienotriazolodiazepine compound of the Formula (1) or the thienotriazolodiazepine compound (1-1) which remains amorphous is selected from: at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% and at least 99%.

## V. Dosage Forms:

**[00212]** Suitable dosage forms that can be used with the solid dispersions of the present invention include, but are not limited to, capsules, tablets, mini-tablets, beads, beadlets, pellets, granules, granulates, and powder. Suitable dosage forms may be coated, for example using an enteric coating. Suitable coatings may comprise but are not limited to cellulose acetate phthalate, hydroxypropylmethylcellulose (HPMC), hydroxypropylmethylcellulose phthalate, a polymethylacrylic acid copolymer, or hydroxylpropylmethylcellulose acetate succinate (HPMCAS). In some embodiments, certain combinations can be encountered, for example, in the same sample some molecules of the thienotriazolodiazepine of the present invention may be present in clusters while some are molecularly dispersed with a carrier.

**[00213]** In some embodiments, the solid dispersions of the invention may be formulated as tablets, caplets, or capsules. In one some embodiments, the solid dispersions of the invention may be formulated as mini-tablets or pour-into-mouth granules, or oral powders for constitution. In some embodiments, the solid dispersions of the invention are dispersed in a suitable diluent in combination with other excipients (e.g., re-crystallization/precipitation inhibiting polymers, taste-masking components, etc.) to give a ready-to-use suspension formulation. In some embodiments, the solid dispersions of the invention may be formulated for pediatric treatment.

**[00214]** In one embodiment, the pharmaceutical composition of the present invention is formulated for oral administration. In one embodiment, the pharmaceutical composition comprises a solid dispersion, according to the various embodiments described herein, comprising a thienotriazolodiazepine compound of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof;

and a polymer carrier. In one embodiment, the pharmaceutical composition further includes one or more additives such as disintegrants, lubricants, glidants, binders, and fillers.

**[00215]** Examples of suitable pharmaceutically acceptable lubricants and pharmaceutically acceptable glidants for use with the pharmaceutical composition include, but are not limited to, colloidal silica, magnesium trisilicate, starches, talc, tribasic calcium phosphate, magnesium stearate, aluminum stearate, calcium stearate, magnesium carbonate, magnesium oxide, polyethylene glycol, powdered cellulose, glyceryl behenate, stearic acid, hydrogenated castor oil, glyceryl monostearate, and sodium stearyl fumarate.

**[00216]** Examples of suitable pharmaceutically acceptable binders for use with the pharmaceutical composition include, but are not limited to starches; celluloses and derivatives thereof, e.g., microcrystalline cellulose (e.g., AVICEL PH from FMC), hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxylpropylmethylcellulose (HPMC, e.g., METHOCEL from Dow Chemical); sucrose, dextrose, corn syrup; polysaccharides; and gelatin.

**[00217]** Examples of suitable pharmaceutically acceptable fillers and pharmaceutically acceptable diluents for use with the pharmaceutical composition include, but are not limited to, confectioner's sugar, compressible sugar, dextrates, dextrin, dextrose, lactose, mannitol, microcrystalline cellulose (MCC), powdered cellulose, sorbitol, sucrose, and talc.

**[00218]** In some embodiments, excipients may serve more than one function in the pharmaceutical composition. For example, fillers or binders may also be disintegrants, glidants, anti-adherents, lubricants, sweeteners and the like.

**[00219]** In some embodiments, the pharmaceutical compositions of the present invention may further include additives or ingredients, such as antioxidants (e.g., ascorbyl palmitate, butylated hydroxylanisole (BHA), butylated hydroxytoluene (BHT), -tocopherols, propyl gallate, and fumaric acid), antimicrobial agents, enzyme inhibitors, stabilizers (e.g., malonic acid), and/or preserving agents.

**[00220]** Generally, the pharmaceutical compositions of the present invention may be formulated into any suitable solid dosage form. In some embodiments, the solid dispersions of the invention are compounded in unit dosage form, e.g., as a capsule, or tablet, or a multi-particulate system such as granules or granulates or a powder, for administration.

**[00221]** In one embodiment, a pharmaceutical compositions includes a solid dispersion of a thienotriazolodiazepine compound of Formula (1), according to the various embodiments of solid dispersions described herein, and hydroxypropylmethylcellulose acetate succinate (HPMCAS), wherein the thienotriazolodiazepine compound is amorphous in the solid dispersion and has a

thienotriazolodiazepine compound to hydroxypropylmethylcellulose acetate succinate (HPMCAS), weight ratio of 1:3 to 1:1; 45 -50 wt. % of lactose monohydrate; 35-40 wt. % of microcrystalline cellulose; 4-6 wt. % of croscarmellose sodium; 0.8-1.5 wt. % of colloidal silicon dioxide; and 0.8-1.5 wt. % of magnesium stearate.

## VI. Dosage:

**[00222]** In one embodiment, the present invention provides a pharmaceutical composition that maybe formulated into any suitable solid dosage form. In one embodiment, a pharmaceutical composition in accordance with the present invention comprises one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) as described herein in a dosage amount ranging from about 10 mg to about 100 mg. In one embodiment, the pharmaceutical composition of the present invention includes one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) as described herein in a dosage amount selected from the group consisting of from about 10 mg to about 100 mg, about 10 mg to about 90 mg, about 10 mg to about 80 mg, about 10 mg to about 70 mg, about 10 mg to about 60 mg, about 10 mg to about 50 mg, about 10 mg to about 40 mg, about 10 mg to about 30 mg, and about 10 mg to about 20 mg. In one embodiment, the pharmaceutical composition of the present invention includes one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) as described herein in a dosage amount selected from the group consisting of about 10 mg, about 50 mg, about 75 mg, about 100 mg.

**[00223]** In one embodiment, the pharmaceutical composition of the present invention includes administering to a subject in need thereof one or more of the various embodiments of the thienotriazolodiazepine of Formula (I) as described herein in a dosage amount selected from the group consisting of about 1 mg, about 2 mg, about 2.5 mg, about 3 mg, about 4 mg, about 5 mg, about 7.5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, and about 150 mg, and in a dosage form selected from the group consisting of once weekly, once daily every sixth day, once daily every fifth day, once daily every fourth day, once daily every third day, once daily every other day, once daily, twice daily, three times daily, four times daily, and five times daily. In another embodiment, any of the foregoing dosage amounts or dosage forms is decreased periodically or increased periodically. In

one embodiment, the pharmaceutical composition of the present invention includes administering to a subject in need thereof a thienotriazolodiazepine selected from the group consisting of compounds (1-1), (1-2), (1-3), (1-4), (1-5), (1-6), (1-7), (1-8), (1-9), (1-10), (1-11), (1-12), (1-13), (1-14), (1-15), (1-16), (1-17), and (1-18), in a dosage amount selected from the group consisting of about 1 mg, about 2 mg, about 2.5 mg, about 3 mg, about 4 mg, about 5 mg, about 7.5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, and about 150 mg, and in a dosage form selected from the group consisting of once weekly, once daily every sixth day, once daily every fifth day, once daily every fourth day, once daily every third day, once daily every other day, once daily, twice daily, three times daily, four times daily, and five times daily. In another embodiment, any of the foregoing dosage amounts or dosage forms is decreased periodically or increased periodically.

**[00224]** Such unit dosage forms are suitable for administration 1 to 5 times daily depending on the particular purpose of therapy, the phase of therapy, and the like. In one embodiment, the dosage form may be administered to a subject in need thereof at least once daily for at least two successive days. In one embodiment, the dosage form may be administered to a subject in need thereof at least once daily on alternative days. In one embodiment, the dosage form may be administered to a subject in need thereof at least weekly and divided into equal and/or unequal doses. In one embodiment, the dosage form may be administered to a subject in need thereof weekly, given either on three alternate days and/or 6 times per week. In one embodiment, the dosage form may be administered to a subject in need thereof in divided doses on alternate days, every third day, every fourth day, every fifth day, every sixth day and/or weekly. In one embodiment, the dosage form may be administered to a subject in need thereof two or more equally or unequally divided doses per month.

**[00225]** The dosage form used, e.g., in a capsule, tablet, mini-tablet, beads, beadlets, pellets, granules, granulates, or powder may be coated, for example using an enteric coating. Suitable coatings may comprise but are not limited to cellulose acetate phthalate, hydroxypropylmethylcellulose (HPMC), hydroxypropylmethylcellulose phthalate, a polymethylacrylic acid copolymer, or hydroxylpropylmethylcellulose acetate succinate (HPMCAS).

## VII. Process:

**[00226]** The thienotriazolodiazepine compounds disclosed herein can exist as free base or as acid addition salt can be obtained according to the procedures described in US Patent Application Publication No. 2010/0286127, incorporated by reference in its entirety herein, or in the present application. Individual enantiomers and diastereomers of the thienotriazolodiazepine compounds of the present invention can be prepared synthetically from commercially available starting materials that contain asymmetric or stereogenic centers, or by preparation of racemic mixtures followed by resolution methods well known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary, (2) salt formation employing an optically active resolving agent, (3) direct separation of the mixture of optical enantiomers on chiral liquid chromatographic columns or (4) kinetic resolution using stereoselective chemical or enzymatic reagents. Racemic mixtures can also be resolved into their component enantiomers by well-known methods, such as chiral-phase gas chromatography or crystallizing the compound in a chiral solvent.

**[00227]** If desired, a particular enantiomer of the thienotriazolodiazepine compounds disclosed herein may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers, thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers. Various methods well known in the art may be used to to prepare the thienotriazolodiazepine compounds of Formula (1) with an enantiomeric excess of generally more than about 80%. Advantageously, preferred enantiomeric excess is of more than 80%, preferably of more than 90%, more preferably of more than 95%, and most preferably of 99% and more.

**[00228]** The solid dispersions of the present invention can be prepared by a number of methods, including by melting and solvent evaporation. The solid dispersions of the present invention can also be prepared according to the procedures described in: Chiou WL, Riegelman S: "Pharmaceutical applications of solid dispersion systems", *J. Pharm. Sci.* 1971; 60:1281-1302; Serajuddin ATM: "Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recentbreakthroughs", *J. Pharm. Sci.* 1999; 88:1058-1066; Leuner C, Dressman J: "Improving drug solubility for oral delivery using solid dispersions", *Eur. J. Pharm. Biopharm.*

2000; 50:47-60; and Vasconcelos T, Sarmento B, Costa P: "Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs", *Drug Discovery Today* 2007; 12:1068-1075, all of which are incorporated herein by reference in their entireties.

**[00229]** In one embodiment, solid dispersions of the present invention are prepared by a melting process. In one embodiment, the melting process comprises melting one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) within a carrier. In one embodiment, the melting process includes cooling a melted compound of the present invention and a carrier. In one embodiment, the melting process comprises pulverization of the melted compound and the carrier. In one embodiment, a melted compound of the present invention and a carrier are pulverized following the cooling step.

**[00230]** In some embodiments in which the thienotriazolodiazepine of Formula (1) or a pharmaceutically acceptable salt, a solvate, including a hydrate, a racemate, an enantiomer, an isomer, or an isotopically-labeled form thereof and the carrier are incompatible, a surfactant may be added during the melting step to prevent formation of two liquid phases or a suspension in the heated mixture. In some embodiments, one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) is suspended in a previously melted carrier, instead of using both drug and carrier in the melted state, thereby reducing the process temperature. In one embodiment, melted drug and carrier mixture is cooled an ice bath agitation. In one embodiment, melted drug and carrier mixture is cooled and solidified by spray cooling (alternatively spray congealing).

**[00231]** In one embodiment, melted drug and carrier mixture is cooled and solidified by forming the melt into particles by spraying the melt into a cooling chamber through which ambient or cooled, low temperature air is passing. In one embodiment, melted drug and carrier mixture is cooled and solidified by atomization and re-solidification of the molten dispersion in a suitable fluid bed processor. In one embodiment, melted drug and carrier mixture is cooled and solidified by melt-granulation in a heatable high-shear mixer.

**[00232]** In some embodiments, hot-stage extrusion or melt agglomeration may be used to avoid melting limitations of the drug. Hot-stage extrusion consists of the extrusion, at high rotational speed, of the drug and carrier, previously mixed, at melting temperature for a short period of time; the resulting product is collected after cooling at room temperature and milled.

**[00233]** In one embodiment, one or more of the various embodiments of the thienotriazolodiazepine of Formula (1) is processed at a reduced processing temperature to avoid degradation of any thermally labile compound. In one embodiment, the reduced processing temperature is achieved by associating a hot-stage extrusion with a temporary plasticizer such as carbon dioxide. In one embodiment, melt agglomeration is used in the preparation of solid dispersions in accordance with the present invention in conventional high shear mixers or in a rotary processors. In one embodiment, the solid dispersion in accordance with the present invention is prepared by adding a molten carrier containing a thienotriazolodiazepine compound in accordance with the present invention to a heated excipient. In one embodiment, the solid dispersion in accordance with the present invention is prepared by adding by adding a molten carrier to a heated mixture of the thienotriazolodiazepine in accordance with the present invention and one or more excipients. In one embodiment, the solid dispersion in accordance with the present invention is prepared by heating a mixture of a thienotriazolodiazepine compound in accordance with the present invention, a carrier and one or more excipients to a temperature within or above the melting range of the carrier.

**[00234]** In some embodiments, a one or more of the various embodiments for the formulation of the thienotriazolodiazepine, according to Formula (1), is prepared by a solvent evaporation method. In one embodiment, the solvent evaporation method comprises solubilization of a thienotriazolodiazepine compound, according to Formula (1), carrier in a volatile solvent that is subsequently evaporated. In one embodiment, the volatile solvent may one or more excipients. In one embodiment, the one or more excipients include, but are not limited to anti-sticking agents, inert fillers, surfactants wetting agents, pH modifiers and additives. In one embodiment, the excipients may dissolved or in suspended or swollen state in the volatile solvent.

**[00235]** In one embodiment, preparation of solid dispersions in accordance with the present invention includes drying one or more excipients suspended in a volatile solvent. In one embodiment, the drying includes vacuum drying, slow evaporation of the volatile solvent at low temperature, use of a rotary evaporator, spray-drying, spray granulation, freeze-drying, or use of supercritical fluids.

**[00236]** In one embodiment, spray drying preparation of a formulation for the thienotriazolodiazepine composition, according to Formula (1), is used which involves atomization of a suspension or a solution of the composition into small droplets, followed by rapid removal

solvent from the formulation. In one embodiment, preparation of a formulation in accordance with the present invention involves spray granulation in which a solution or a suspension of the composition in a solvent is sprayed onto a suitable chemically and/or physically inert filler, such as lactose or mannitol. In one embodiment, spray granulation of the solution or the suspension of the composition is achieved via two-way or three-way nozzles.

**[00237]** In some embodiments, preparation of solid dispersions in accordance with the present invention includes use of supercritical fluids. The term "supercritical fluids" refers to substances existing as a single fluid phase above their critical temperature and critical pressure. In one embodiment, preparation of a formulation, in accordance with the present invention, includes use a supercritical carbon dioxide fluid. In one embodiment, preparation of a formulation, in accordance with the present invention, using the supercritical fluid technique comprises dissolving a thienotriazolodiazepine compound, according to Formula (1), and carrier in a common solvent that is introduced into a particle formation vessel through a nozzle, simultaneously with carbon dioxide; and spraying the solution to allow the solvent be rapidly extracted by the supercritical fluid, thereby resulting in the precipitation of solid dispersion particles on the walls of the vessel.

**[00238]** In some embodiments, preparation of solid dispersions in accordance with the present invention includes use of a co-precipitation method. In one embodiment, a non-solvent is added dropwise to a thienotriazolodiazepine composition, according to Formula (1), and a carrier solution, under constant stirring. In one embodiment, the thienotriazolodiazepine composition, according to Formula (1), and the carrier are co-precipitated to form microparticles during the addition of the non-solvent. In one embodiment, the resulting microparticles are filtered and dried to provide the desired solid dispersion.

**[00239]** The proportion of compound of Formula (1) and polymeric carrier(s) to be mixed is not particularly limited, as long as it can improve the bioavailability of the compound of Formula (1) and varies depending on the kind of polymer.

**[00240]** The invention is illustrated in the following non-limiting examples.

## VIII. Examples:

**[00241]** The invention is illustrated in the following non-limiting examples.

Example 1: *in vitro* screening of solid dispersions of compound (1-1)

**[00242]** Ten solid dispersions were prepared using compound (1-1) and one of five polymers, including hypromellose acetate succinate (HPMCAS-M), hypromellose phthalate (HPMCP-HP55), polyvinylpyrrolidone (PVP), PVP-vinyl acetate (PVP-VA), and Euragit L100-55, at both 25% and 50% of compound (1-1) loading, for each polymer. Solid dispersions were prepared by a solvent evaporation method, using spray-drying followed by secondary drying in a low-temperature convection oven. The performance of each solid dispersion was assessed via a non-sink dissolution performance test which measured both the total amount of drug and the amount of free drug present in solution over time. Non-sink dissolution was chosen because it best represents the in vivo situation for low soluble compounds. This test included a "gastric transfer" of dispersion from gastric pH (0.1N NaCl, pH 1.0) to intestinal pH (FaFSSIF, pH 6.5) approximately 30 to 40 minutes after the introduction of dispersion to the test medium, simulating *in vivo* conditions. [FaFSSIF is Fasted State Simulated Intestinal Fluid, comprised of 3 mM sodium taurocholate, 0.75 mM lechithin, 0.174 g NaOH pellets, 1.977 g $NaH_2PO_4 \cdot H_2O$, 3.093 g NaCl, and purified water qs 500 mL.] The amount of dissolved drug was quantified using a high-performance liquid chromatrography (HPLC) method and an Agilent 1100 series HPLC. The dissolution profiles of the formulations (Figures 1A-1J) showed large increases in drug solubility in all dispersion candidates relative to the unformulated compound in the same media. Of the solid dispersions, the 25% compound (1-1) in PVP, 25% compound (1-1) in HPMCAS-M, and 50% compound (1-1) in HPMCAS-M dispersions provided enhanced oral absorption as compared to the unformulated compound, based on finding higher levels of free drug released at intestinal pH.

Example 2: *in vivo* screening of solid dispersions of compound (1-1)

**[00243]** The solid dispersions of compound (1-1), namely the 25% compound (1-1) in PVP, 25% compound (1-1) in HPMCAS-MG, and 50% compound (1-1) in HPMCAS-M dispersions, were prepared at larger scale for *in vivo* studies. Each formulation was assessed in the in vitro dissolution test described in Example 1. To ensure that these dispersions were both amorphous and homogeneous, each dispersion was assessed by powder x-ray diffraction (PXRD) and modulated differential scanning calorimetry (mDSC). The x-ray diffractomer was a Bruker D-2 Phaser. Additionally, to understand the effect of water on the glass transition temperature (Tg) for each dispersion, mDSC was performed on samples first equilibrated at a set relative humidity (i.e., 25%, 50%, and 75% RH) for at least 18 hours. [Water can act as a plasticizer for solid dispersions and the hygroscopicity of the system due to the active compound or polymer can affect the amount of water uptake by these systems.]

**[00244]** The non-sink dissolution results (Figures 2A-2C) were comparable to those found for the dispersions in Example 1. PXRD results (Figure 3) showed no evidence of crystalline compound in any of the dispersions and mDSC results (Figures 4A-4C) showed a single glass transition temperature (Tg) for each dispersion, indicating that each dispersion was homogeneous. An inverse relationship between Tg and relative humidity was observed for each (Figure 5). Notably, for the 25% compound (1-1) in PVP solid dispersion equilibrated at 75% RH, there appeared to be two Tgs, indicating that phase separation was occurring, and this dispersion also showed a melt event at 75% RH, suggesting that crystallization occurred during the RH equilibration (Figure 6). This finding suggests that the 25% compound (1-1) in PVP dispersion may be less stable than the HPMCAS-M dispersions.

**[00245]** To assess the bioavailability of the three dispersions, groups of male beagle dogs (three per group) were given a 3 mg/kg dose of an aqueous suspension of solid dispersion of compound (1-1) administered by oral gavage or a 1 mg/kg dose of compound (1-1) dissolved in water:ethanol:polyethylene glycol (PEG) 400 (60:20:20) and administered as an intravenous bolus into the cephalic vein. Blood samples were collected from the jugular vein of each animal at 0 (pre-dose), 5, 15, and 30 minutes and 1, 2, 4, 8, 12, and 24 hours following intravenous administration and at 0 (pre-dose), 15 and 30 minutes and 1, 2, 4, 8, 12, and 24 hours following oral gavage administration. The amount of compound (1-1) present in each sample was detected using a qualified LC-MS/MS method with a lower limit of quantification of 0.5 ng/mL. The area under the plasma concentration-time curve (AUC) was determined by use of the linear trapezoidal rule up to the last measurable concentration without extrapolation of the terminal elimination phase to infinity. The elimination half-life ($t_{1/2}$) was calculated by least-squares regression analysis of the terminal linear part of the log concentration-ime curve. The maximum plasma concentration ($C_{max}$) and the time to $C_{max}$ ($t_{max}$) were derived directly from the plasma concentration data. The oral bioavailability (F) was calculated by dividing the dose normalized AUC after oral administration by the dose normalized AUC after intravenous administration and reported as percentages (%). Results, summarized in Table 1 below, gave mean oral bioavailabilities of the 25% compound (1-1) in PVP, 25% compound (1-1) in HPMCAS-M, and 50% compound (1-1) in HPMCAS-M solid dispersions of 58%, 49%, and 74%, respectively.

Table 1: pharmacokinetic parameters of compound (1-1) after oral (po) and intravenous (iv) administrations to dogs (the values are averages from three dogs)

| Compound (1-1) | Dose & | $C_{max}$ | $t_{max}$ | AUC | $t_{1/2}$ | F |
|---|---|---|---|---|---|---|

| formulation | Route | (ng/L) | (hr) | (ng•min/mL) | (hr) | (%) |
|---|---|---|---|---|---|---|
| Solution in water:ethanol: PEG400 (60:20:20) | 1 mg/kg IV | 769 | 0.083 | 53,312 | 1.5 | ---- |
| Aqueous suspension of 25% compound (1-1)/PVP solid dispersion | 3 mg/kg PO | 487 | 1.0 | 93,271 | 1.6 | 58 |
| Aqueous suspension of 25% compound (1-1)/HPMCAS-M solid dispersion | 3 mg/kg PO | 228 | 0.5 | 78,595 | 2.0 | 49 |
| Aqueous suspension of 50% compound (1-1)/HPMCAS-M solid dispersion | 3 mg/kg PO | 371 | 1.0 | 118,174 | 1.5 | 74 |

AUC: area under the plasma concentration-time curve; $C_{max}$: maximum plasma concentration; F: bioavailability; HPMCAS: hypromellose acetate sodium; IV: intravenous; PEG: polyethylene glycon; PO; *per os*, oral; PVP: polyvinylpyrrolidone; $t_{max}$: time of $C_{max}$; $t_{1/2}$: plasma elimination half-life

Example 3: preparation and clincial use of capsules containing a solid dispersion of compound (1-1)

**[00246]** A gelatin capsule of 10 mg strength was prepared for initial clinical studies in patients with hematologic malignancies. Based on results of *in vitro* and *in vivo* testing of solid dispersions of compound (1-1), as described in Examples 1 and 2, a 50% compound (1-1) in HPMCAS-M solid dispersion was selected for capsule development. Capsule development was initiated targeting a fill weight of 190 mg in a size 3 hard gelatin capsule, as this configuration would potentially allow increasing the capsule strength by filling a larger size capsule while maintaining the pharmaceutical composition. Based on experience, four capsule formulations were designed with different amounts of disintegrant and with and without wetting agent. Since all four formulations showed similar disintegration test and dissolution test results, the simplest formulation (without wetting agent and minimum disintegrant) was selected for manufacturing. Manufacturing process development and scale-up studies were performed to confirm the spray drying process and post-drying times for the solid dispersion; blending parameters; roller compaction and milling of the blend to achieve target bulk density of approximately 0.60 g/cc; and capsule filling conditions.

**[00247]** Crystalline compound (1-1) and the polymer hypromellose actate succinate (HPMCAS-M) were dissolved in acetone and spray-dried to produce solid dispersion intermediate (SDI) granules containing a 50% compound (1-1) loading. The SDI was shown by PXRD analysis to be amorphous and by mDSC analysis to be homogeneous (i.e., single Tg under ambient conditions). The 50% compound (1-1) in HPMCAS-M solid dispersion (1000 g) and excipients, including

microcrystalline cellulose filler-binder (4428 g), croscarmellose sodium disintegrant (636 g), colloidal silicon dioxide dispersant/lubricant 156 g), magnesium stearate dispersant/lubricant (156 g), and lactose monohydrate filler (5364 g) were blended in stages in a V-blender. The blend was them compacted and granulated to obtain a bulk density of approximately 0.6 g/mL. The blend was dispensed into size 3 hard gelatin capsules (target fill weight: 190 mg) using an automated filling machine and finished capsules were polished using a capsule polisher machine.

**[00248]** Pharmacokinetic assessments were performed following oral dosing of 10 mg capsules containing the 50% compound (1-1) in HPMCAS solid dispersion and results were compared with pharmacokinetic assessments performed following oral dosing of administration of 4 x 10 mg capsules containing the Eudragit solid dispersion of compound (1-1) to healthy volunteers

**[00249]** A comparison of the two pharmaceutical compositions is provided in Tables 2A and 2B below. The Eudragit formulation previously was described in Example 5 in US Patent Application 2009/0012064 A1, published January 8, 2009. That application noted that the Eudragit solid dispersion formulation was made by dissolving and/or dispersing the thienotriazolodiazepine of formula (A) and coating excipients, including ammonio methacrylate copolymer type B (Eudragit RS), methacrylic acid copolymer type C (Eudragit L100-55), talc, and magnesium aluminosilicate, in a mixture of water and ethanol. This heterogeneous mixture then was applied to microcrystalline cellulose spheres (Nonpareil 101, Freund) using a centrifugal fluidizing bed granulator to produce granules that were dispensed into size 2 hydroxypropyl methylcellulose capsules.

**[00250]** In both clinical studies, blood levels of compound (1-1) were determined using validated LC-MS/MS methods and pharmacokinetic analyses were performed based on plasma concentrations of compound (1-1) measured at various time points over 24 hours after capsule administration. Results, summarized in Table 3 below, showed that the HPMCAS-M solid dispersion formulation had over 3-fold higher bioavailability in humans than the Eudragit solid dispersion formulation based on AUCs (924*4 / 1140, adjusting for difference in doses administered). Additionally, based on the observed $T_{max}$, the HPMCAS formulation is more rapidly absorbed than the Eudragit formulation ($T_{max}$ of 1 h vs 4-6 h). The marked improvement in systemic exposure with the HPMCAS-M solid dispersion formulation is unexpected.

Table 2A: solid dispersion capsules of compound (1-1) for clinical use

pharmaceutical composition containing 50% HPMCAS solid dispersion of compound (1-1): 10 mg strength, size 3 hard gelatin capsule

| Ingredient | Function | Capsule Content | |
|---|---|---|---|
| | | mg | Wt % |

| | | | |
|---|---|---|---|
| Compound of formula (II) | active agent | 10.0* | 5.56 |
| Hypromellose acetate succinate (HPMCAS-M) | carrier for solid dispersion | 10.0 | 5.56 |
| Lactose monohydrate | filler | 85.0 | 47.22 |
| Microcrystalline cellulose | filler-binder | 70.0 | 38.89 |
| Croscarmellose sodium | disintegrant | 10.0 | 5.56 |
| Collidal silicon dioxide | dispersant/lubricant | 2.5 | 1.39 |
| Magnesium stearate | dispersant/lubricant | | |
| | Total | 190.0 | 100.0 |

Table 2B: pharmaceutical composition containing Eudragit L100-55solid dispersion of compound (1-1): 10 mg strength, size 2 hard gelatin capsule

| Ingredient | Function | Capsule Content | |
|---|---|---|---|
| | | mg | Wt % |
| Compound (1-1) | active agent | 10.0* | 3.8 |
| Core: | | | |
| Microcrystalline cellulose spheres (Nonpareil 101, Freund, Inc) | vehicle | 100.0 | 38.5 |
| Compound/polymer layer: | | | |
| Ammonio methacrylate copolymer, type B (NF. PhEur) (Edragit RS, Evonik) | coating agent | 10.8 | 4.2 |
| Methacrylic acid copolymer, type C (NF)/ Methacrylic acid-ethyl acrylate copolymer (1:1) type A (PhEur) (Eudragit L100-55, Evonik) | coating agent | 25.2 | 9.7 |
| Talc | coating agent | 88.2 | 33.9 |
| Magnesium aluminometasilicate (Neuslin, Fuji Chemical) | coating agent | 20.0 | 7.7 |
| Triethyl citrate | plasticizer | 5.0 | 1.9 |
| Silicon dioxide | fluidizing agent | 0.8 | 0.3 |
| | | 260.0 | 100.0 |

* as anhydrate

Table 3: pharmacokinetic parameters following oral administration of solid dispersions of compound (1-1) to humans

| Compound (1-1) formulation | # Patients | Dose and Route | $C_{max}$ (ng/mL) | $T_{max}$ (hr) | $AUC_{0-24h}$ (ng•h/mL) |
|---|---|---|---|---|---|
| Eudragit solid dispersion formulation | 7 | 40 mg PO | 83 | 4 to 6 | 1140 |
| 50% HMPCAS-M solid dispersion formulation | 7 | 10 mg PO | 286 | 1 | 925 |

$AUC_{0-24h}$: area under the OTX015 plasma concentration vs. time curve over 24 hours
$C_{max}$: maximum concentration in plasma
hr: hour

HPMCAS: hypromellose acetate succinate
mL: milliliter
ng: nanogram
PO: *per os*, oral
$T_{max}$: time of $C_{max}$

**[00251]** Example 4. Oral exposure in the rat

**[00252]** The oral bioavailability of three formulations of solid dispersions of compound (1-1) was determined in rats. The three dispersions chosen were the 25% dispersion of compound (1-1) in PVP, the 25% dispersion of compound (1-1) in HPMCAS-MG, and the 50% dispersion of compound (1-1) in HPMCAS-MG. The animals used in the study were Specific Pathogen Free (SPF) Hsd:Sprague Dawley rats obtained from the Central Animal Laboratory at the University of Turku, Finland. The rats were originally purchased from Harlan, The Netherlands. The rats were female and were ten weeks of age, and 12 rats were used in the study. The animals were housed in polycarbonate Makrolon II cages (three animals per cage), the animal room temperature was 21 +/- 3 °C, the animal room relative humidity was 55 +/- 15%, and the animal room lighting was artificial and was cycled for 12 hour light and dark periods (with the dark period between 18:00 and 06:00 hours). Aspen chips (Tapvei Oy, Estonia) were used for bedding, and bedding was changed at least once per week. Food and water was provided prior to dosing the animals but was removed during the first two hours after dosing.

**[00253]** The oral dosing solutions containing the 25% dispersion of compound (1-1) in PVP, the 25% dispersion of compound (1-1) in HPMCAS-MG, and the 50% dispersion of compound (1-1) in HPMCAS-MG were prepared by adding a pre-calculated amount of sterile water for injection to containers holding the dispersion using appropriate quantities to obtain a concentration of 0.75 mg/mL of compound (1-1). The oral dosing solutions were subjected to vortex mixing for 20 seconds prior to each dose. The dosing solution for intravenous administration contained 0.25 mg/mL of compound (1-1) and was prepared by dissolving 5 mg of compound (1-1) in a mixture containing 4 mL of polyethylene glycol with an average molecular weight of 400 Da (PEG400), 4 mL of ethanol (96% purity), and 12 mL of sterile water for injection. The dosing solution containing the 25% dispersion of compound (1-1) in PVP was used within 30 minutes after the addition of water. The dosing solutions containing the 25% dispersion of compound (1-1) in HPMCAS-MG and the 50% dispersion of compound (1-1) in HPMCAS-MG were used within 60 minutes of after the addition of water. A dosing volume of 4 mL/kg was used to give dose levels of

compound (1-1) of 1 mg/kg for intravenous administration and 3 mg/kg for oral administration. The dosing scheme is given in Table 4.

**[00254]** Table 4. Dosing scheme for rat oral exposure study.

| Rat | Weight | Dose (mL) | Test Item | Route |
|---|---|---|---|---|
| 1 | 236.5 | 0.95 | Compound (1-1) | intravenous |
| 2 | 221 | 0.88 | Compound (1-1) | intravenous |
| 3 | 237.5 | 0.95 | Compound (1-1) | intravenous |
| | | | | |
| 4 | 255.5 | 1.02 | 25% dispersion of compound (1-1) in PVP | oral |
| 5 | 224.2 | 0.90 | 25% dispersion of compound (1-1) in PVP | oral |
| 6 | 219.2 | 0.88 | 25% dispersion of compound (1-1) in PVP | oral |
| | | | | |
| 7 | 251.6 | 1.01 | 25% dispersion of compound (1-1) in HPMCAS-MG | oral |
| 8 | 240.4 | 0.96 | 25% dispersion of compound (1-1) in HPMCAS-MG | oral |
| 9 | 238 | 0.95 | 25% dispersion of compound (1-1) in HPMCAS-MG | oral |
| | | | | |
| 10 | 226.6 | 0.91 | 50% dispersion of compound (1-1) in HPMCAS-MG | oral |
| 11 | 228.4 | 0.91 | 50% dispersion of compound (1-1) in HPMCAS-MG | oral |
| 12 | 228.5 | 0.91 | 50% dispersion of compound (1-1) in HPMCAS-MG | oral |

**[00255]** Blood samples of approximately 50 μL were collected into Eppendorf tubes containing 5 μL of ethylenediaminetetraacetic acid (EDTA) solution at time points of 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing, with each sample collected within a window of 5 minutes from the prescribed time point. From each sample, 20 μL of plasma was obtained and stored at dry ice temperatures for analysis. Analysis of each sample for the concentration of compound (1-1) was performed using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method with a lower limit of quantitation of 0.5 ng/mL.

**[00256]** Pharmacokinetic parameters were calculated with the Phoenix WinNonlin software package (version 6.2.1, Pharsight Corp., CA, USA) with standard noncompartmental methods. The elimination phase half-life ($t_{1/2}$) was calculated by least-squares regression analysis of the terminal linear part of the log concentration-time curve. The area under the plasma concentration-time curve (AUC) was determined by use of the linear trapezoidal rule up to the last measurable concentration and thereafter by extrapolation of the terminal elimination phase to infinity. The mean residence time (MRT), representing the average amount of time a compound remains in a compartment or system, was calculated by extrapolating the drug concentration profile to infinity. The maximum plasma concentration ($C_{max}$) and the time to $C_{max}$ ($t_{max}$) were derived directly from the plasma concentration data. The tentative oral bioavailability (F) was calculated by dividing the dose normalised AUC after oral administration by the dose normalised AUC after intravenous administration, i.e. F = (AUC(oral)/Dose(oral))/(AUC(intravenous) / Dose(intravenous))] and is reported as percentage (%).

**[00257]** The pharmacokinetic parameters are given in Table 5, and the plasma concentration versus time plots are shown in Figures 7 and 8.

Table 5. Pharmacokinetic parameters of compound (1-1) after oral and intravenous administrations. The values are an average from three animals.

| Compound | Parameter | 1 mg/kg intravenous | 3 mg/kg oral | F(%) |
|---|---|---|---|---|
| Compound (1-1) water:ethanol:PEG 400 (60:20:20) | AUC (min*ng/ml)<br>$C_{max}$ (ng/ml)<br>$T_{max}$ (hr)<br>$t_{1/2}$ (hr) 8.5<br>CI/F (ml/min/kg)<br>MRT (hr) | 74698<br>730<br>0.25<br>8.5<br>13.4<br>7.4 | | |
| 25% dispersion of compound (1-1) in PVP | AUC (min*ng/ml)<br>$C_{max}$ (ng/ml)<br>$T_{max}$ (hr)<br>$t_{1/2}$ (hr) 8.5<br>CI/F (ml/min/kg)<br>MRT (hr) | | 39920<br>77.9<br>1<br>13.8<br>75.2<br>18.0 | 18 |
| 25% dispersion of compound (1-1) in HPMCAS-MG | AUC (min*ng/ml)<br>$C_{max}$ (ng/ml)<br>$T_{max}$ (hr)<br>$t_{1/2}$ (hr) 8.5<br>CI/F (ml/min/kg)<br>MRT (hr) | | 35306<br>48.3<br>0.5<br>11.0<br>85.0<br>17.1 | 16 |
| 50% dispersion of | AUC (min*ng/ml) | | 40238 | 18 |

| | | | | |
|---|---|---|---|---|
| compound (1-1) in HPMCAS-MG | $C_{max}$ (ng/ml)<br>$T_{max}$ (hr)<br>$t_{1/2}$ (hr) 8.5<br>CI/F (ml/min/kg)<br>MRT (hr) | | 67.0<br>2<br>9.5<br>74.6<br>12.8 | |

**[00258]** Example 5. Preparation of spray dried dispersions.

**[00259]** Spray dried dispersions of compound (1-1) were prepared using five selected polymers: HPMCAS-MG (Shin Etsu Chemical Co., Ltd.), HPMCP-HP55 (Shin Etsu Chemical Co., Ltd.), PVP (ISP, a division of Ashland, Inc.), PVP-VA (BASF Corp.), and Eudragit L100-55 (Evonik Industries AG). All spray dried solutions were prepared at 25% and 50% by weight with each polymer. All solutions were prepared in acetone, with the exception of the PVP solutions, which were prepared in ethanol. For each solution, 1.0 g of solids (polymer and compound (1-1)) were prepared in 10 g of solvent. The solutions were spray dried using a Büchi B-290, PE-024 spray dryer with a 1.5 mm nozzle and a Büchi B-295, P-002 condenser. The spray dryer nozzle pressure was set to 80 psi, the target outlet temperature was set to 40 °C, the chiller temperature was set to -20 °C, the pump speed was set to 100%, and the aspirator setting was 100%. After spray drying, the solid dispersions were collected and dried overnight in a low temperature convection oven to remove residual solvents.

**[00261]** Example 6: Stability with humidity and temperature.

**[00262]** Table 6

| Test | Procedure | Acceptance Criteria | T=0 (Initial) | | T-1 month (storage at 40°C/75%RH) | | T-- 2 month (storage at 40°C/75%RH) | | T= 3 month (storage at 40°C/75%RH) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Appearance | AM-0002 | White to off-white powder | Test Date/Ref: 06Aug2012/02-41-2 | | Test Date/Ref: 24Sep2012/02-41-59 | | Test Date/Ref:24Oct2012/02-37-106 | | Test Date/Ref:17Dec2012/02-37-119 | |
| | | | White Powder | | White Powder | | White Powder | | White Powder | |
| Potency (HPLC) | AM-0028 | 45.0 - 55.0 wt% | Test Date/Ref: 25Jul2012/02-37-21 | | Test Date/Ref: 25Sep2012/02-4H10 | | Test Date/Ref:24Oct2012/02-37-105 | | Test Date/Ref: 29Nov2012/02-34-107 | |
| | | | 50.0 | | 49.4 | | 49.8 | | 49.2 | |
| Individual Related Substances (HPLC) | AM-0029 | Report results | Test Date/Ref: 25Jul2012/02-34-49 | | Test Date/Ref: 26Sep2012/02-41-64 | | Test Date/Ref: 24Oct2012/02-37-105 | | Test Date/Ref: 29Nov2012/02-34-107 | |
| | | | RRT | % Area | RRT | % Area | RRT | % Area | RRT | % Area |
| | | | No reportable related substances | | No reportable related substances | | 0.68 | 0.06 | 0.68 | 0.07 |
| | | | | | | | 0.77 | 0.06 | 0.77 | 0.09 |
| Total Related Substances (HPLC) | AM-0029 | Report results | Test Date/Ref: 25Jul2012/02-34-49 | | Test Date/Ref: 26Sep2012/02-41-64 | | Test Date/Ref: 24Oct2012/02-37-105 | | Test Date/Ref: 29Nov2012/02-34-107 | |
| | | | No reportable related substances | | No reportable related substances | | 0.12% | | 0.16% | |
| Water Content (KF) | AM-0030 USP <921> | Report results (wt%) | Test Date/Ref: 02Aug2012/02-41-1 | | Test Date/Ref: 27Sep2012/02-37-99 | | Test Date/Ref: 25Oct2012102-37-110 | | Test Date/Ref: 29Nov2012/02-37-116 | |
| | | | 1.52 | | 2.53 | | 2.70 | | 3.43 | |
| X-Ray Powder Diffraction (XRPD) | USP <941> | Consistent with an amorphous form | Test Date/Ref: 24Jul2012/02-24-131 | | Test Date/Ref: 01Oct2012/02-41-73 | | Test Date/Ref: 24Oct2012/02-37-107 | | Test Date/Ref: 17Dec2012/02-37-120 | |
| | | | Consistent with an amorphous form | | Consistent with an amorphous form | | Consistent with an amorphous form | | Consistent with an amorphous form | |

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| | | | See Figure 9 | See Figure 10 | See Figure 11 | See Figure 12 |
|---|---|---|---|---|---|---|
| Modulated Differential Scanning Calorimetry (mDSC) | USP <891> (n = 2 replicates) | Report individual and average glass transtion temperatures ($T_g$, °C) | Test Dale/Ref: 24Jul2012/02-24-130 | Test Date/Ref: 26Sep2012/02-37-98 | Test Date/Ref: 24Oct2012/02-37-108 | Test Date/Ref: 17Dec2012/02-37-121 |
| | | | Replicate 1 = 134.30°C,Replicate 2 = 134.23°C,Replicate 3 = 135.28°C, Average = 134.60°C | Replicate 1 = 134.65°C, Replicate 2 = 134.43°C, Average=134.54°C | Replicate 1 = 135.35°C, Replicate 2 = 134.93°C, Average = 135.14°C | Replicate 1 = 134.36°C, Replicate 2 = 137.16°C, Average= 135.76°C |



**[00263]** Spray dried dispersions of compound (1-1) in HPMCAS-MG were assessed for stability by exposure to moisture at elevated temperature. The glass transition temperature (Tg) as a function of relative humidity was determined at 75% relative humidity, 40 °C for 1, 2 and 3 months. The spray dried dispersion was stored in an LDPE bag inside a HDPE bottle to simulate bulk product packaging. The results are summarized in Table 6. At time zero, the Tg was 134 °C, at 1 month the Tg was 134 °C, at 2 months the Tg was 135 °C and at 3 months the Tg was 134 °C and only a single inflection point was observed for each measurement. X-ray diffraction patterns were also obtained for each sample. Figure 9 illustrates a powder X-ray diffraction profile of solid dispersions of compound (1-1) in HPMCAS-MG at time zero of a stability test. Figures 10, 11 and 12 illustrate powder X-ray diffraction profiles of solid dispersions of compound (1-1) in

**[00264]** Example 7. *in vitro* studies

**[00265]** Eight human cell lines from acute (HL-60, U-937) and chronic (K-562, NALM-1) acute myeloid leukemia, and acute lymphoblastic leukemia (Jurkat, CCRF-CEM, MOLT-3/-4) were treated with increasing compound (1-1) doses and MTT assays were performed after 72 hours. Protein was analyzed by Western blot and qRT-PCR was performed with Fast SYBR Green on a StepOnePlus Real-Time PCR System. Simultaneous and sequential schedules of compound (1-1) combined with DNA methyltransferase inhibitors (azacitidine, decitabine), a broad-spectrum HDAC inhibitor (panobinostat), mTOR inhibitor (everolimus), glucocorticoids (dexamethasone), and conventional cytotoxic drugs (daunorubicin, cytarabine and methotrexate) were evaluated. Combination Index (CI) was determined using Chou & Talalay analysis; CI<1 reflects synergy, CI=1 additivity and CI > 1 reflects antagonism.

**[00266]** Antiproliferative effects of single-agent compound (1-1) gave GI50s between 230 and 384nM in HL60, U937 and Jurkat, and 2: 6,000nM for other cell lines. Combination studies were performed in 3 compound (1-1) -sensitive cell lines (HL60, U937, Jurkat) and one resistant (K-562). 48h-concomitant combination of compound (1-1) with azacytine (CI=0.6; 0.6-0.7), decitabine (CI=0.6; 0.3-0.9), cytarabine (CI=0.8; 0.4-0.9), daunorubicin (CI=0.8; 0.4-0.9) and methotrexate (CI=0.2; 0.1-0.3) was synergistic in all4 cell lines. Dexamethasone, everolimus and panobinostat were synergystic with compound (1-1) in HL60, K562 and Jurkat cell lines. Sequential combination of azacytidine or panobinostat followed by compound (1-1) for 48-48h, 72h-24h or 72-48h displayed stronger synergy than concomitant treatment, with lowest CI values after 72-24h combination in the 4 cell lines. In HL-60 cells, 24h-combination of compound (1-1) (500nM) with azacytidine (311M) or panobinostat (20nM) increased caspase-3 and PARP cleavage, whereas in K562 cells the combination increased P21 expression and enhanced C-MYC downregulation.

**[00267]** Example 8. *in vitro studies*

**[00268]** Four established human cell lines derived from acute (HL60, U937) or chronic (K562) myeloid leukemia cells, and acute lymphoblastic leukemia cells (Jurkat) were treated with increasing doses of compound (1-1). MTT assays were performed after 72 hours exposure. GI50 and Emax values were calculated with the equation for sigmoidal dose response using Prism 5.00 and cell lines were classified as sensitive or resistant. Cell lines with GI50 values< 500 nM were considered compound (1-1)-sensitive. Anti proliferative effects of single-agent compound (1-1) gave GI50s between 230 and 384nM in HL60, U937 and Jurkat, and 2': 6,000nM for other cell lines.

**[00269]** Concomitant combination of compound (1-1) with demethylating agents (azacitidine, decitabine), a broad-spectrum HDAC inhibitor (panobinostat), mTOR inhibitor (everolimus), glucocorticoids (dexamethasone), and conventional cytotoxic drugs (daunorubicin, cytarabine and methotrexate) were evaluated. Combination Index (CI) was determined using Chou & Talay analysis; CI<1 reflects synergy, CI=1 additivity and CI> 1 reflects antagonism. The results are shown in Figure 13A.

**[00270]** Compound (1-1) exerts strong synergistic effects in concomitant combination with demethylating agents, HDAC and mTOR inhibitors, glucocorticoids, and conventional cytotoxic drugs in both compound (1-1 )-sensitive and resistant leukemic cells. As shown in Figure 13A, 8h concomitant combination of compound (1-1) had CI values of with azacytine (CI=0.6;0.6-0.7), decitabine CI=0.6;0.3-0.9), cytarabine (CI=0.8;0.4-0.9), daunorubicin (CI=0.8;0.4-0.9) and methotrexate (CI=0.2;0.1-0.3) was synergistic in all 4 cell lines. Dexamethasone, everolimus and panobinostat were synergytic with compound (1-1) in HL60, K562 and Jurkat cell lines. Further, Figure 13B illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a concomitant combination of Azacytidine and compound (1-1) for 48 hours. Figure 13C illustrates the combination index value for HL60, U937, Jurkat and K562 cell lines treated with a concomitant combination ofpanobinostat and compound (1-1) for 48 hours.

**[00271]** Sequential combination studies were performed in three compound (1-1) -sensitive cell lines (HL60, U937, Jurkat) and one resistant (K-562). Sequential combination of azacytidine followed by compound (1-1) for 48-48h, 72h-24h or 72-48h displayed stronger synergy than concomitant treatment, with lowest CI values after 72-24h combination in the 4 cell lines as illustrated in Figures 14A-14D. The lowest CI values were observed after the 72 hours-48 hours combination. Sequential combination of panobinostat followed by compound (1-1) for 48-48h, 72h-24h or 72-48h displayed stronger synergy than concomitant treatment, with lowest CI values after 72-24h combination in the 4 cell lines as illustrated in Figures 15A-15D.

**[00272]** Cell cycle studies of HL-60 cell line were conducted for a 24 hour combination of compound (1-1) (500nM) with azacytidine (3f.LM) or panobinostat (20nM). Figures 16A and 16B illustrate the cell percentage of subG 1 for HL60 and K562 cell lines treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours. In HL60 cells, concomitant exposure to compound (1-1) and azacytidine increased the percentage of cells in the sub G1 phase, whereas in K562 cells, the combination increased the percentage of cells in the G1 phase.

**[00273]** Figures 17 A-D illustrates the Western blot profiles of cMYC, cleaved caspase 3, cleaved PARP, p21 and β-actin for HL60, Jurkat, U937, and K562 cell lines, respectively, treated with concomitant combination of compound (1-1) and azacytidine or panobinostat for 24 hours. In HL-60 cells, 24h-combination of compound (1-1) (500nM) with azacytidine (3f.LM) or panobinostat (20nM) increased caspase-3 and PARP cleavage, whereas in K562 cells the combination increased P21 expression and enhanced C-MYC downregulation.

**[00274]** Example 9. *in vitro* studies

**[00275]** Five established human cell lines from acute and chronic acute myeloid leukemia (HL-60, U937, K562) and acute lymphoblastic leukemia (Jurkat, MOLT-3) were used. Anti-proliferative effect of compound 1-1 was assessed by MTT and GI50 values after 72h-exposure. For cellular PK studies, cells were seeded at 2.106 cells/ml and exposed to 500 nM compound 1-1 for 0, 5, 15, 30, 60, 120 and 360 minutes. At each time point, compound 1-1 extracellular and intracellular concentrations were analyzed in cell supernatant and pellets respectively, using an Ultra Performance Liquid Chromatography with tandem Mass Spectrometry (concentration range 1-250 ng/mL). Experiments were performed in quadruplicate. In addition, in cell pellets protein modulation was analyzed by Western blot using commercial antibodies and qRT-PCR was performed using Fast SYBR Green on a StepOnePlus RT-PCR System.

**[00276]** GI50 values were between 230 and 384 nM in HL60, U937 and Jurkat, and were 2: 6,000 nM for other cell lines. Cellular uptake of compound 1-1 was rapid ( <5 min) in both sensitive and resistant leukemic cell lines, with a mean concentration of~ 15 ng/106cells (3-23ng.mL-1/106cells), whereas extracellular levels of compound 1-1 were stable up to 6h of incubation (~ 200ng.mL-1). In the resistant cell line, K562, we observed CMYC mRNA levels downregulation after 2h of exposure, even though compound 1-1 intracellular levels are detected after 5 min of exposure. In the sensitive cell lines, HL60 and Jurkat, a rapid downregulation of CMYC protein and mRNA levels were observed correlating with the rapid compound 1-1 uptake, whereas other compound 1-1-targeted genes, such as HEXIM, P21 and BCL2 mRNA levels were modulated after longer exposure time points in sensitive cell lines.

**[00277]** Compound 1-1 intracellular accumulation occurred to a similar extent and timing in compound 1-1 sensitive and resistant leukemic cell lines. Rapid regulation of compound 1-1-target genes was observed at mRNA and protein levels only in sensitive cell lines.

**[00278]** Example 10. Pharmacokinetics of Compound (1-1) in a phase Ib dose-finding study of patients with hematologic malignancies

**[00279]** An oncology phase Ib trial of compound (1-1) was conducted by the inventors with the primary objective of investigating escalation of the dose of compound (1-1) administered to patients with acute leukemia or other hematologic malignancies. The study enrolled patients with acute leukemia or other hematologic malignancies. The patients received several active dose levels of compound (1-1) ranging from 10 mg to 160 mg according to different dosing schedules. Blood samples from 7 time points were collected from each patient over 24 hours post-administration on Day 1 for leukemia patients ("complete PK") and 4 blood samples were collected from each patient over 8 hours post-administration for patients with other hematologic malignancies ("limited PK"). compound (1-1) plasma concentrations were measured on the basis of the blood samples using validated ultra performance liquid chromatography with tandem mass spectrometry detection with a concentration range 1–250 ng/mL. Analyses and population pharmacokinetics (PPK) modeling were performed with the nonlinear mixed effect modeling software program Monolix version 4.3. Pharmacokinetic Parameters, including absorption constant (Ka); apparent distribution volume (V/F), apparent clearance (CL/F) and lean body mass (LBM; calculated calculated considering patient sex, weight and height) were calculated.

**[00280]** 85 patients enrolled in the phase Ib trial of compound (1-1) and were treated from January 2013 to August 2014. The patients were randomized to six dose levels: 10 mg, 20 mg, 40 mg, 80 mg, 120 mg and 160 mg once a day dosing (QD); and 40 mg twice a day dosing (BID). Among them, 81 patients with 630 plasma concentrations (607 + 23 BLQ) were evaluable for PK assessment. A 1-compartment open model adequately described the total compound (1-1) concentration-time curve. The population pharmacokinetics (PPK) parameters obtained for the structural model were Ka=0.74 h-1 (12%); V/F=71.7 L (6.0%) and CL/F=8.45 L/h (5.0%). The best correlation between compound (1-1) AUC values and dose was observed from 10 mg to 120 mg dose levels (R2=0.84). The absorption phase was linear and Tmax was between 1 h and 4 h. Mean elimination half-life of compound (1-1) for all patients 30 was 5.8 h (± 1.1). In the PPK study, the best simulation model was obtained when LBM was considered in the analysis. A correlation between CL/F and V/F was also observed for compound (1-1).

**[00281]** It will be appreciated by those skilled in the art that changes could be made to the exemplary embodiments shown and described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the exemplary embodiments shown and described, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the claims. For example, specific features of the exemplary embodiments may or may not be part of the claimed invention and features of the

disclosed embodiments may be combined. Unless specifically set forth herein, the terms "a", "an" and "the" are not limited to one element but instead should be read as meaning "at least one".

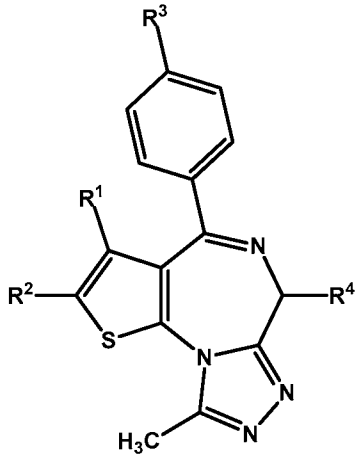
**[00282]** It is to be understood that at least some of the figures and descriptions of the invention have been simplified to focus on elements that are relevant for a clear understanding of the invention, while eliminating, for purposes of clarity, other elements that those of ordinary skill in the art will appreciate may also comprise a portion of the invention. However, because such elements are well known in the art, and because they do not necessarily facilitate a better understanding of the invention, a description of such elements is not provided herein.

**[00283]** Further, to the extent that the method does not rely on the particular order of steps set forth herein, the particular order of the steps should not be construed as limitation on the claims. The claims directed to the method of the present invention should not be limited to the performance of their steps in the order written, and one skilled in the art can readily appreciate that the steps may be varied and still remain within the spirit and scope of the present invention.

# CLAIMS

I/we claim:

1. A method of treating acute myeloid leukemia and/or acute lymphoblastic leukemia in a mammal comprising the step of: administering to a patient a pharmaceutically acceptable amount of a thienotriazolodiazepine compound of the Formula (1)




(1)

wherein $R^1$ is alkyl having a carbon number of 1-4, $R^2$ is a hydrogen atom; a halogen atom; or alkyl having a carbon number of 1-4 optionally substituted by a halogen atom or a hydroxyl group, $R^3$ is a halogen atom; phenyl optionally substituted by a halogen atom, alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4 or cyano; $-NR^5-(CH_2)_m-R^6$ wherein $R^5$ is a hydrogen atom or alkyl having a carbon number of 1-4, m is an integer of 0-4, and $R^6$ is phenyl or pyridyl optionally substituted by a halogen atom; or $-NR^7-CO-(CH_2)_n-R^8$ wherein $R^7$ is a hydrogen atom or alkyl having a carbon number of 1-4, n is an integer of 0-2, and $R^8$ is phenyl or pyridyl optionally substituted by a halogen atom, and $R^4$ is $-(CH2)a-CO-NH-R^9$ wherein a is an integer of 1-4, and $R^9$ is alkyl having a carbon number of 1-4; hydroxyalkyl having a carbon number of 1-4; alkoxy having a carbon number of 1-4; or phenyl or pyridyl optionally substituted by alkyl having a carbon number of 1-4, alkoxy having a carbon number of 1-4, amino or a hydroxyl group or $-(CH2)_b-COOR_{10}$ wherein b is an integer of 1-4, and $R^{10}$ is alkyl having a carbon number of 1-4, or a pharmaceutically acceptable salt, hydrate or solvate thereof.

2. The method according to claim 1, wherein the thienotriazolodiazepine compound represented by Formula (1) is selected from the group consisting of: (i) (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo- [4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide or a dihydrate thereof, (ii) methyl (S)-{4-(3'-cyanobiphenyl-4-yl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl}acetate, (iii) methyl (S)-{2,3,9-

trimethyl-4-(4-phenylaminophenyl)-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl}acetate; and (iv) methyl (S)-{2,3,9-trimethyl-4-[4-(3-phenylpropionylamino)phenyl]-6H-thieno[3,2-f- ][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl}acetate.

3. The method according to claim 2, wherein the thienotriazolodiazepine compound represented by Formula (1) is (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,-4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide dihydrate.

4. The method according to claim 2, wherein the thienotriazolodiazepine compound represented by Formula (1) is (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,-4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide.

5. The method according to claim any one of claims 1-4, wherein the thienotriazolodiazepine compound is formed as a solid dispersion.

6. The method according to claim 5, wherein the solid dispersion exhibits a single glass transition temperature (Tg) inflection point ranging from about 130 °C to about 140 °C.

7. The method according to claim 5, wherein the solid dispersion exhibits a single glass transition temperature (Tg) inflection point ranging from about 175 °C to about 185 °C.

8. The method according to claim 5, wherein the solid dispersion comprises an amorphous thienotriazolodiazepine compound of (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,- 4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide dihydrate and a pharmaceutically acceptable salt thereof or a hydrate thereof; and a pharmaceutically acceptable polymer.

9. The method according to claim 8, wherein the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,- 4]triazolo[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide dihydrate.

10. The method according to claim 9, wherein the solid dispersion exhibits an X-ray powder diffraction pattern substantially free of diffraction lines associated with crystalline thienotriazolodiazepine compound of Formula (1).

11. The method according to claim 10, wherein the solid dispersion is obtained by spray drying.

12. The method according to claim 10, wherein the solid dispersion comprises an amorphous thienotriazolodiazepine compound of the Formula (1) and a pharmaceutically acceptable salt thereof or a hydrate thereof; and a pharmaceutically acceptable polymer.

13. The solid dispersion according to claim 12, wherein the pharmaceutically acceptable polymer is PVP.

14. The solid dispersion according to claim 13, wherein the solid dispersion has a thienotriazolodiazepine compound to PVP weight ratio of 1:3 to 1:1.

15. The method according to claim 12, wherein the pharmaceutically acceptable polymer is hydroxypropylmethylcellulose acetate succinate (HPMCAS).

16. The method according to claim 15, wherein the solid dispersion has a thienotriazolodiazepine compound to HPMCAS weight ratio of 1:3 to 1:1.

17. The method according to any one of claims 1-16, wherein acute myeloid leukemia is mixed lineage leukemia (MLL) fusion positive, post-JAK2 mutated polycythemia vera, or post-myelodysplastic syndrome.

18. The method according to any one of claims 1-16, wherein acute myeloid leukemia is acute basophilic leukemia, acute panmyelosis with myelofibrosis, or myeloid sarcoma.

19. The method according to any one of claims 1-16, wherein acute myeloid leukemia is acute myeloblastic leukemia (minimally differentiated), acute myeloblastic leukemia (without maturation), acute myeloblastic leukemia (with granulocytic maturation), acute promyelocyytic leukemia, acute

myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia, acute monocytic leukemia, acute erythroid leukemia, or acute megakaryoblastic leukemia.

20. The method according to any one of claims 1-16, wherein acute myeloid leukemia is acute myeloid leukemia with recurrent genetic abnormalities, acute myeloid leukemia with multilineage displasia, or therapy -related acute myeloid leukemia.

21. The method according to claim 20, wherein acute myeloid leukemia with recurrent genetic abnormalities is AML with t(8; 21)(q22; q22), AML with inv(16)(p13q22) or t(16; 16)(pl3; q22), AML with t(15; 17)(q22; q12), or AML with 11q23 abnormalities.

22. The method according to claim 20, wherein therapy related AML is alkylating agent-related AML or topoisomerase II inhibitor-related AML.

23. The method according to any one of claims 1-16, where the thienotriazolodiazepine compound of the Formula (1) is administered to treat acute lymphoblastic leukemia.

24. The method according to claim 23, wherein the acute lymphoblastic leukemia is classified as ALL-L1, ALL-L2, or ALL-L3.

25. The method according to claim 23, wherein the acute lymphoblastic leukemia is classified as precursor B acute lymphoblastic leukemia or precursor T acute lymphoblastic leukemia.

26. The method according to claim 25, wherein the precursor B acute lymphoblastic leukemia is characterized by the cytogenetic subtype t(12;21)(p12,q22) TEL/ AML-1; t(l ;19)(q23;p13) PBX/E2A; t(9;22)(q34;qll) ABLIBCR; or T(V,ll)(V;q23) V/MLL.

27. The method according to claim 23, wherein the acute lymphoblastic leukemia is Burkitt's leukemia or Biphenotypic acute leukemia.

28. The method according to claim 23, wherein a one-compartment pharmacokinetic model for the thienotriazolodiazepine compound or a pharmaceutically acceptable salt, a hydrate or a solvate

thereof is parameterized in terms of apparent systemic clearance (CL/F), apparent central compartment volume (V/F), an absorption rate constant (Ka), and lean body mass (LBM).

29. The method according to claims 23, wherein population pharmacokinetics (PPK) parameters obtained for the one-compartment pharmacokinetic model are Ka = 0.74 h-1, V/F = 71.7 L, and CL/F = 8.45 L/h.

30. The method according to claim 28 or claim 29, wherein interindividual variability is about 5.0% for CL/F, about 6.0% for V/F and about 12% for Ka.

31. The method according to claim 23, wherein the thienotriazolodiazepine compound or a pharmaceutically acceptable salt, a hydrate or a solvate thereof is administered in an amount selected from the group consisting of about 10 mg, about 20 mg, about 40 mg, about 80 mg, about 120 and about 160 mg.

32. The method according to claim 23, wherein the pharmaceutical composition is administered once a day.

33. The method according to claim 23, wherein the pharmaceutical composition is administered twice a day.

34. The method according to any one of claims 1-33, further comprising administering pharmaceutically acceptable amount a second agent selected from the group consisting of a DNA methyltransferase inhibitor, a HDAC-inhibitor, a glucocorticoid, an mTOR inhibitor, an epigenetic modulator, an ester thereof, a derivative thereof, a prodrug thereof, a salt thereof, a complex thereof, and combinations thereof.

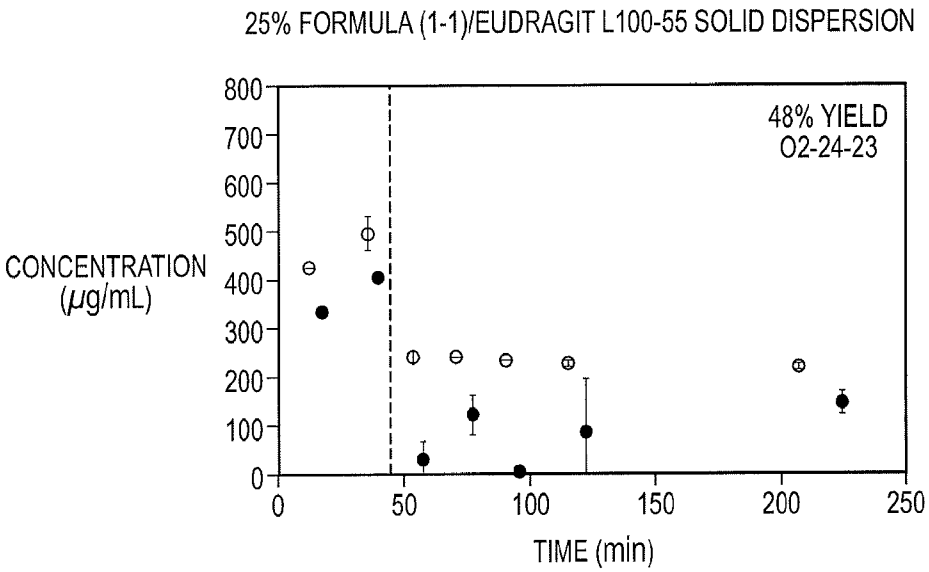

25% FORMULA (1-1)/EUDRAGIT L100-55 SOLID DISPERSION
48% YIELD
O2-24-23
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1A

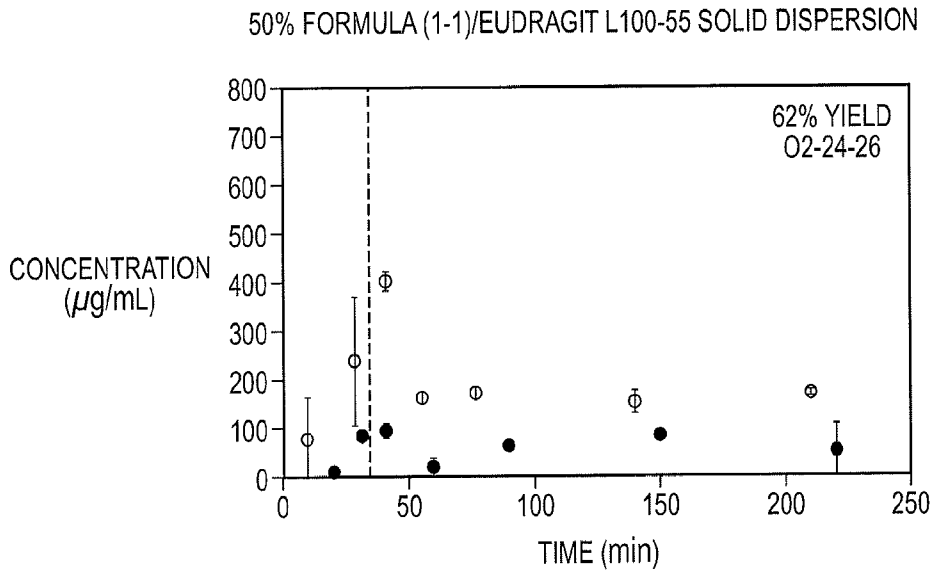

50% FORMULA (1-1)/EUDRAGIT L100-55 SOLID DISPERSION
62% YIELD
O2-24-26
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1B

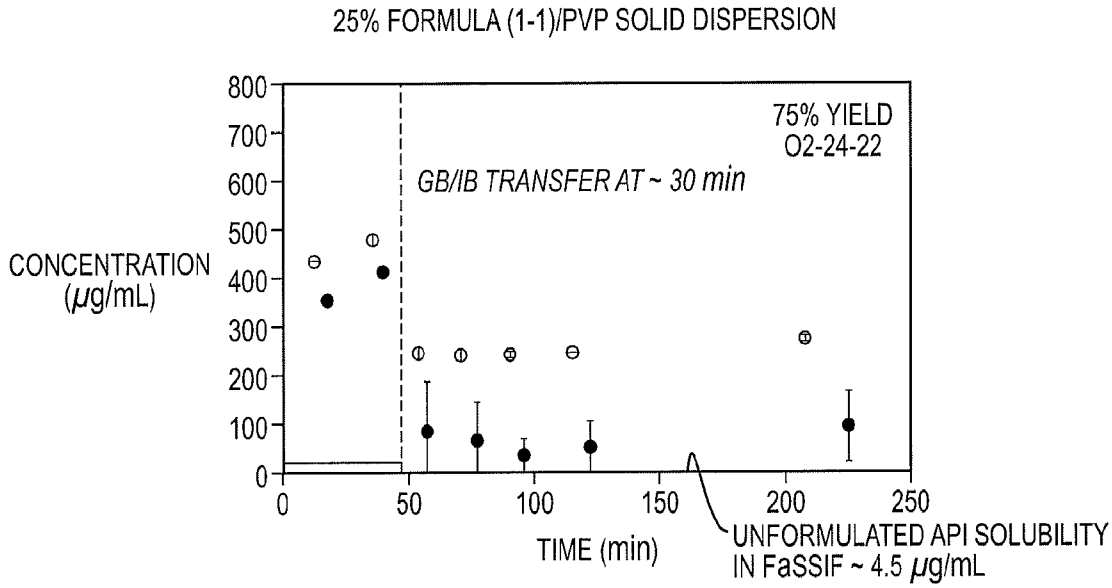

25% FORMULA (1-1)/PVP SOLID DISPERSION
75% YIELD
O2-24-22
GB/IB TRANSFER AT ~ 30 min
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)
UNFORMULATED API SOLUBILITY
IN FaSSIF ~ 4.5 μg/mL

FIG. 1C

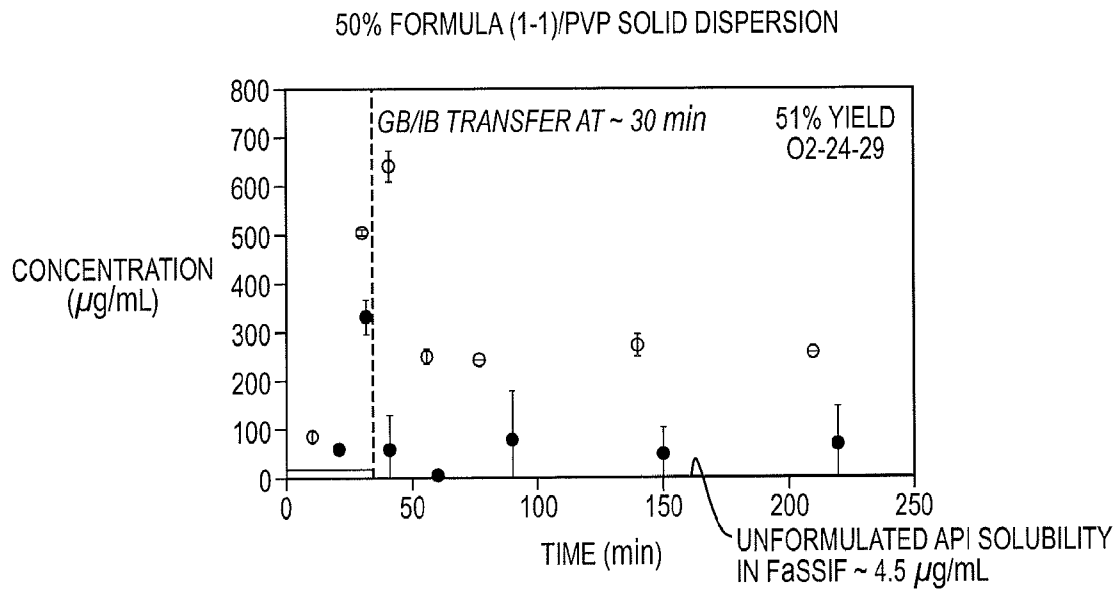

50% FORMULA (1-1)/PVP SOLID DISPERSION
GB/IB TRANSFER AT ~ 30 min
51% YIELD
O2-24-29
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)
UNFORMULATED API SOLUBILITY
IN FaSSIF ~ 4.5 μg/mL

FIG. 1D

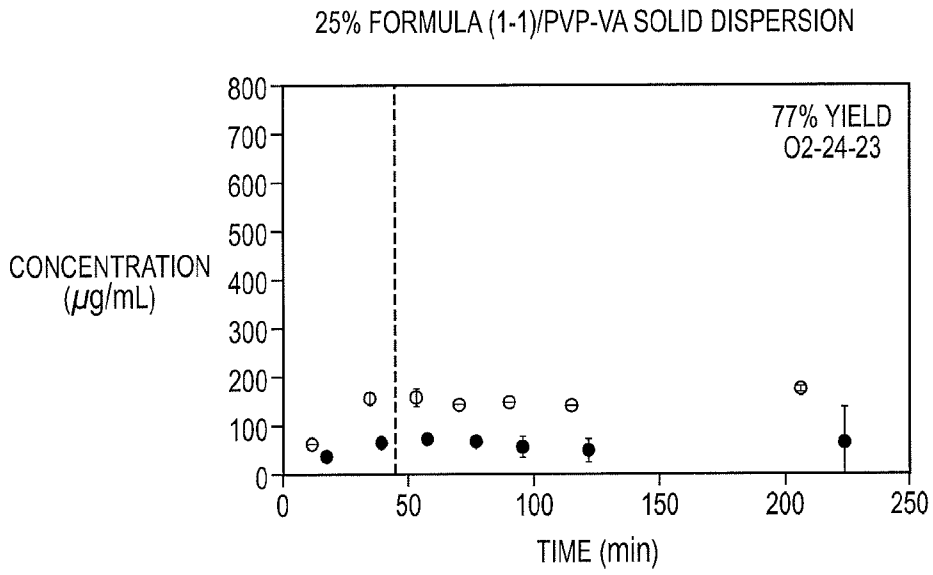

25% FORMULA (1-1)/PVP-VA SOLID DISPERSION
77% YIELD
O2-24-23
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1E

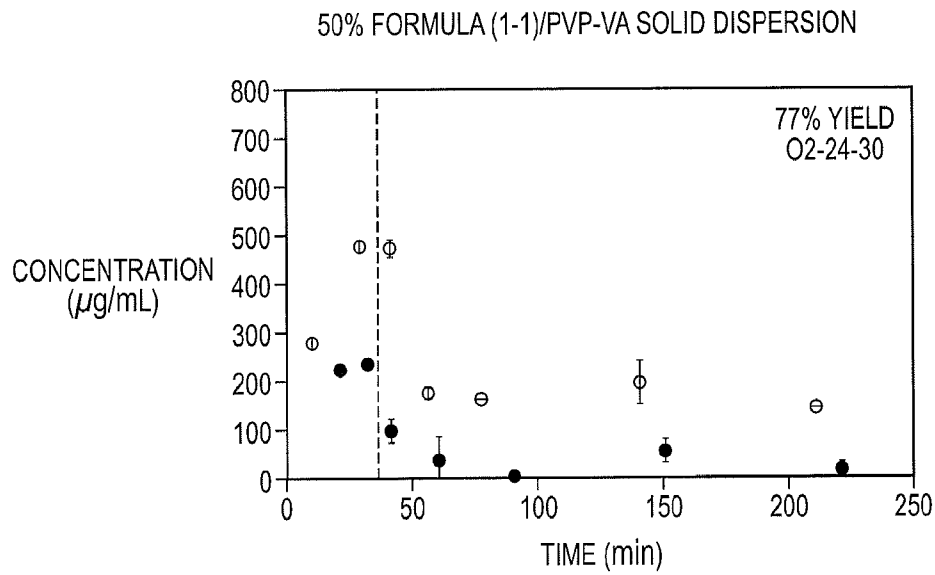

50% FORMULA (1-1)/PVP-VA SOLID DISPERSION
77% YIELD
O2-24-30
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1F

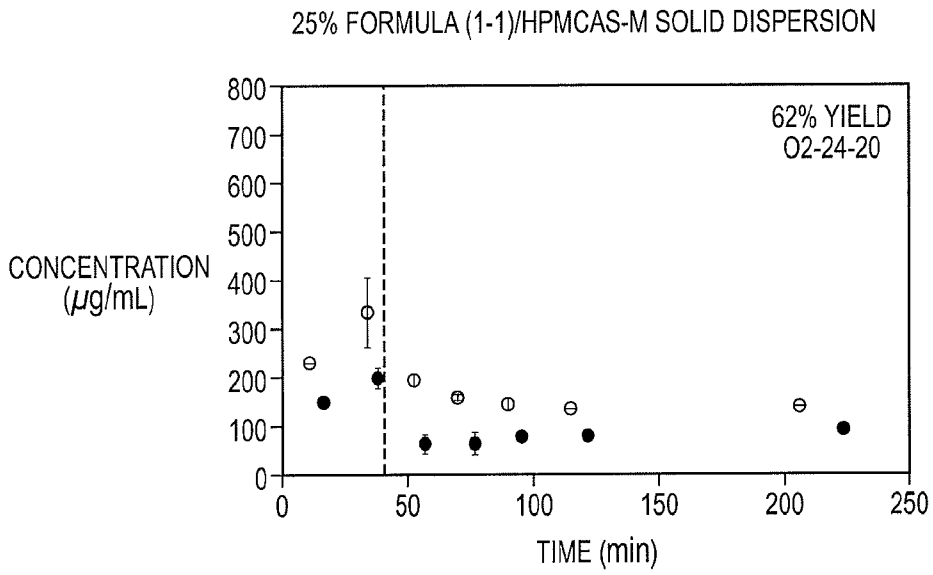

25% FORMULA (1-1)/HPMCAS-M SOLID DISPERSION
62% YIELD
O2-24-20
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1G

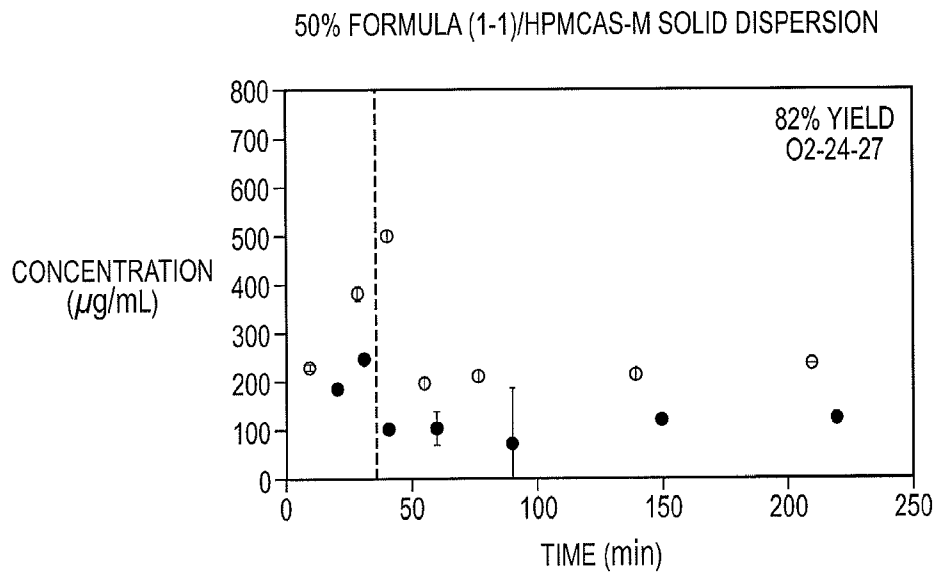

50% FORMULA (1-1)/HPMCAS-M SOLID DISPERSION
82% YIELD
O2-24-27
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1H

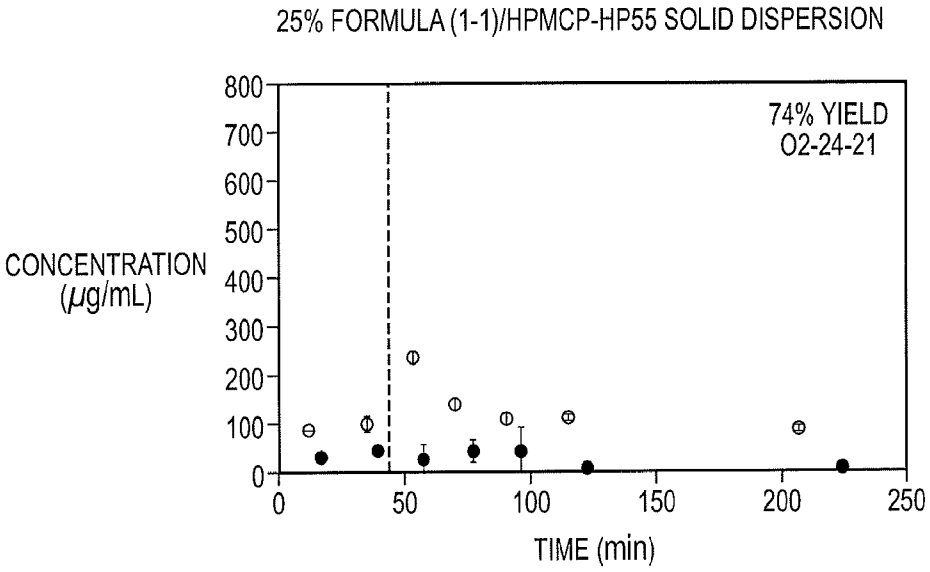

25% FORMULA (1-1)/HPMCP-HP55 SOLID DISPERSION
74% YIELD
O2-24-21
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1I

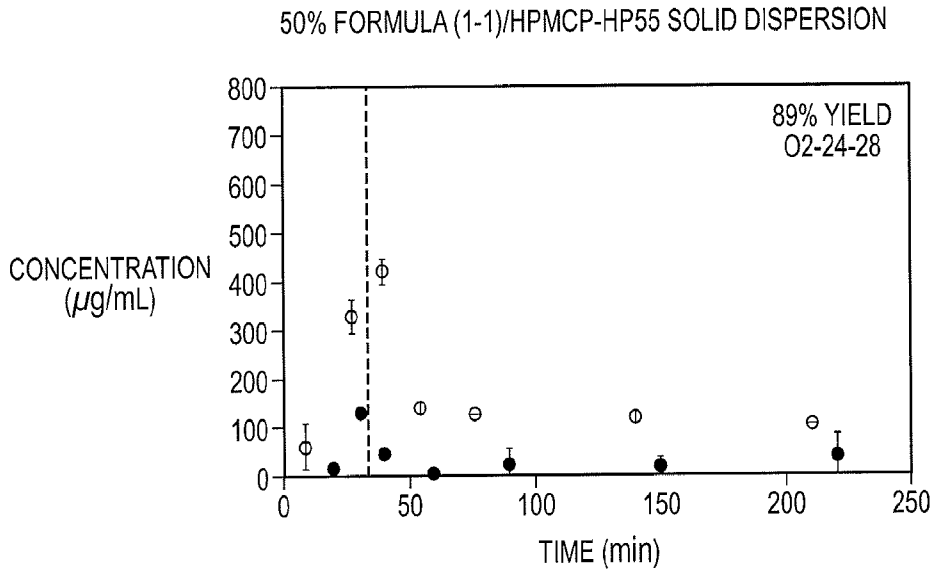

50% FORMULA (1-1)/HPMCP-HP55 SOLID DISPERSION
89% YIELD
O2-24-28
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 1J

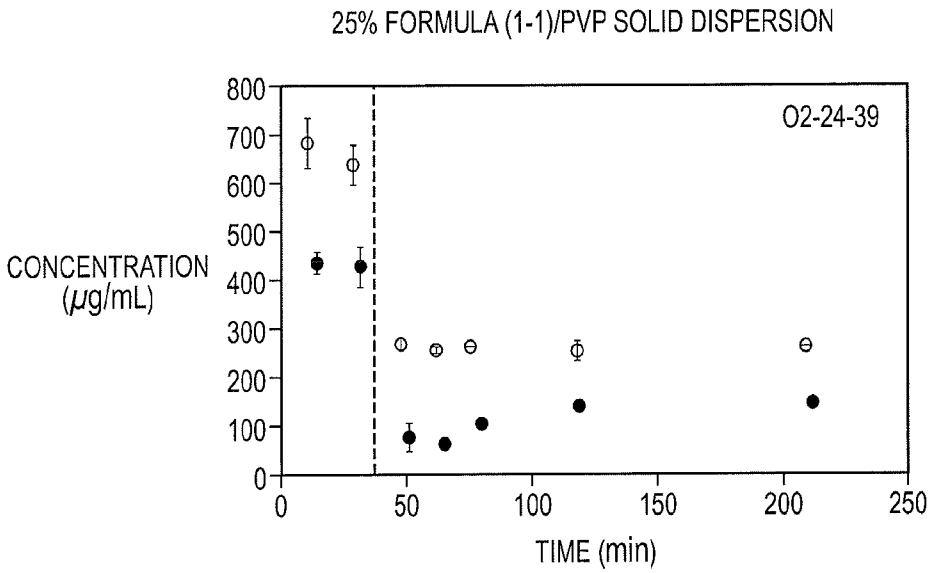

25% FORMULA (1-1)/PVP SOLID DISPERSION
O2-24-39
CONCENTRATION (μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 2A

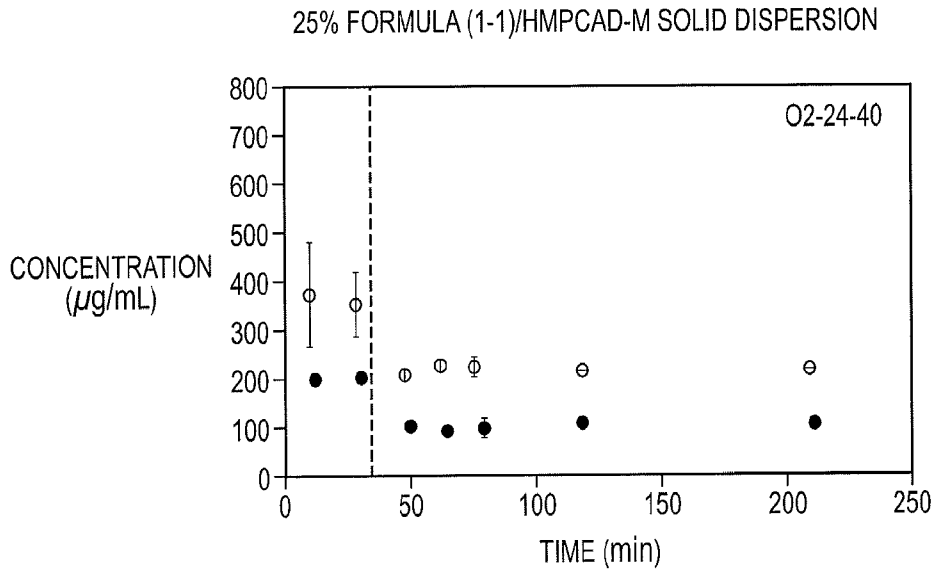

25% FORMULA (1-1)/HMPCAD-M SOLID DISPERSION
O2-24-40
CONCENTRATION (μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 2B

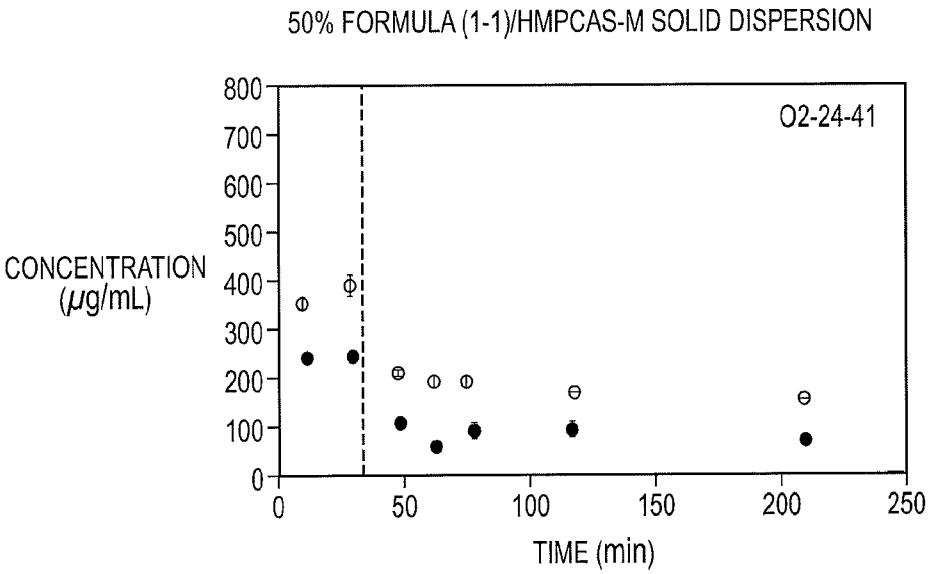

50% FORMULA (1-1)/HMPCAS-M SOLID DISPERSION
O2-24-41
CONCENTRATION
(μg/mL)
800
700
600
500
400
300
200
100
0
0
50
100
150
200
250
TIME (min)

FIG. 2C

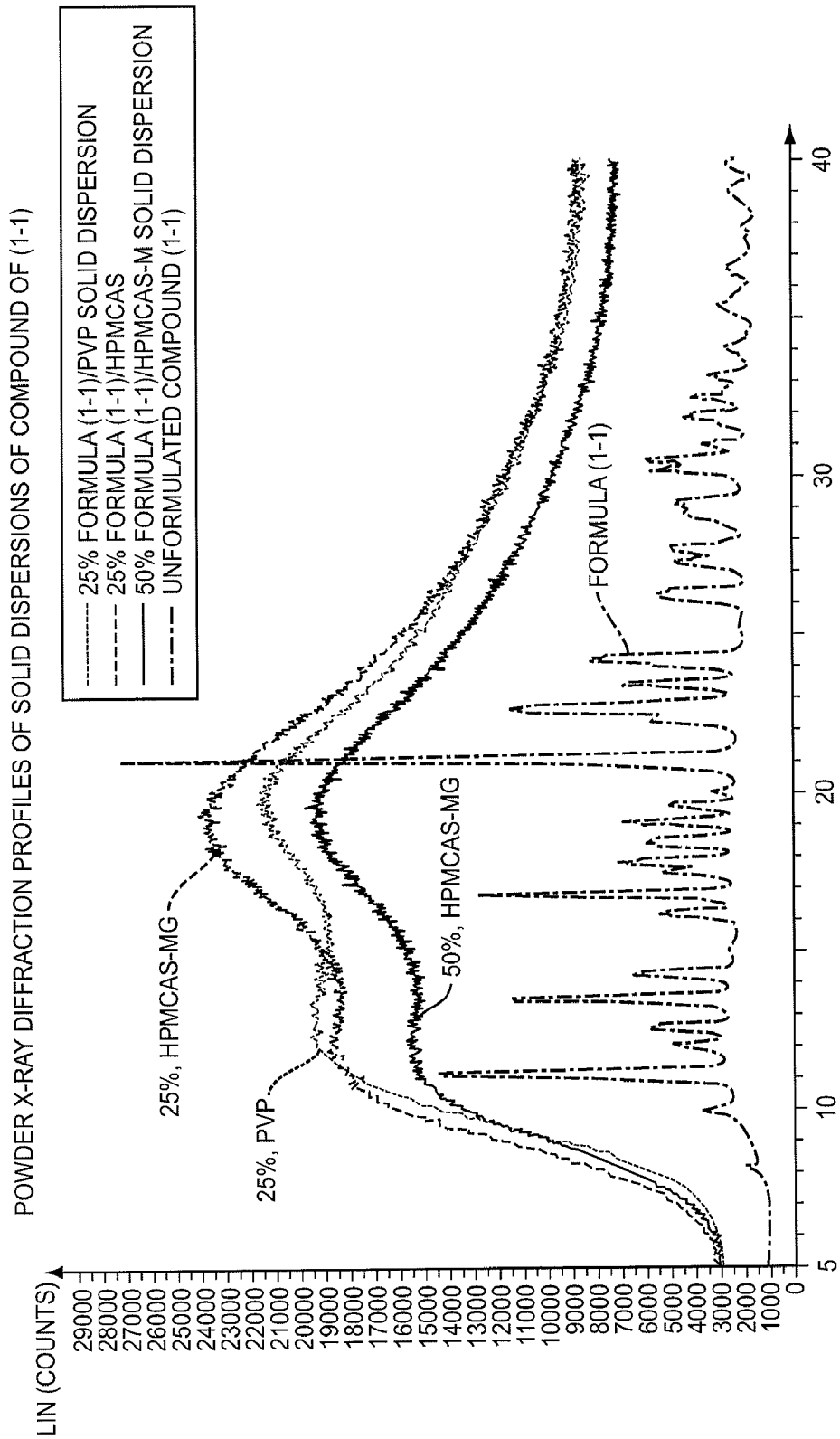

POWDER X-RAY DIFFRACTION PROFILES OF SOLID DISPERSIONS OF COMPOUND OF (1-1)
25% FORMULA (1-1)/PVP SOLID DISPERSION
25% FORMULA (1-1)/HPMCAS
50% FORMULA (1-1)/HPMCAS-M SOLID DISPERSION
UNFORMULATED COMPOUND (1-1)
LIN (COUNTS)
2-THETA SCALE
25%, HPMCAS-MG
25%, PVP
50%, HPMCAS-MG
FORMULA (1-1)

FIG. 3

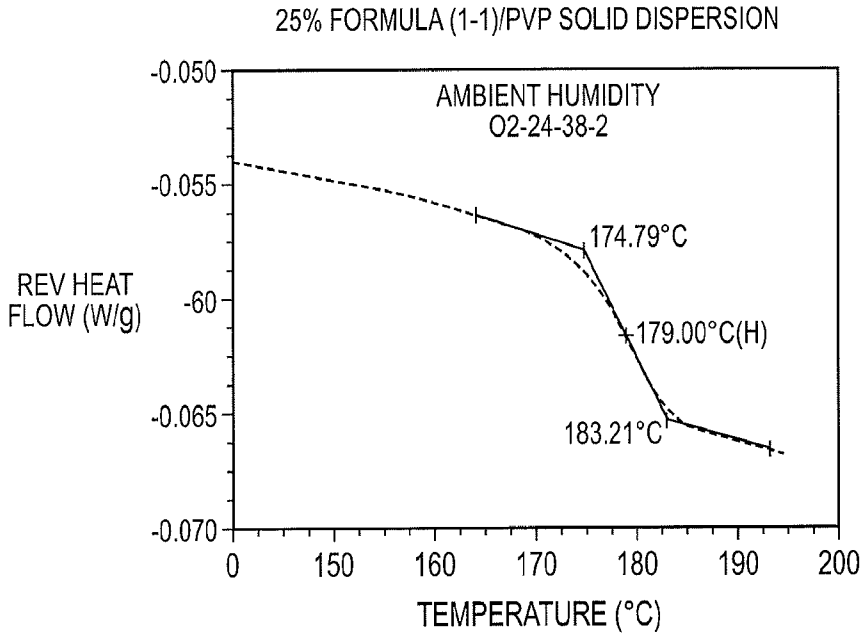

25% FORMULA (1-1)/PVP SOLID DISPERSION
AMBIENT HUMIDITY
O2-24-38-2
174.79°C
179.00°C(H)
183.21°C
REV HEAT FLOW (W/g)
-0.050
-0.055
-60
-0.065
-0.070
0
150
160
170
180
190
200
TEMPERATURE (°C)

FIG. 4A

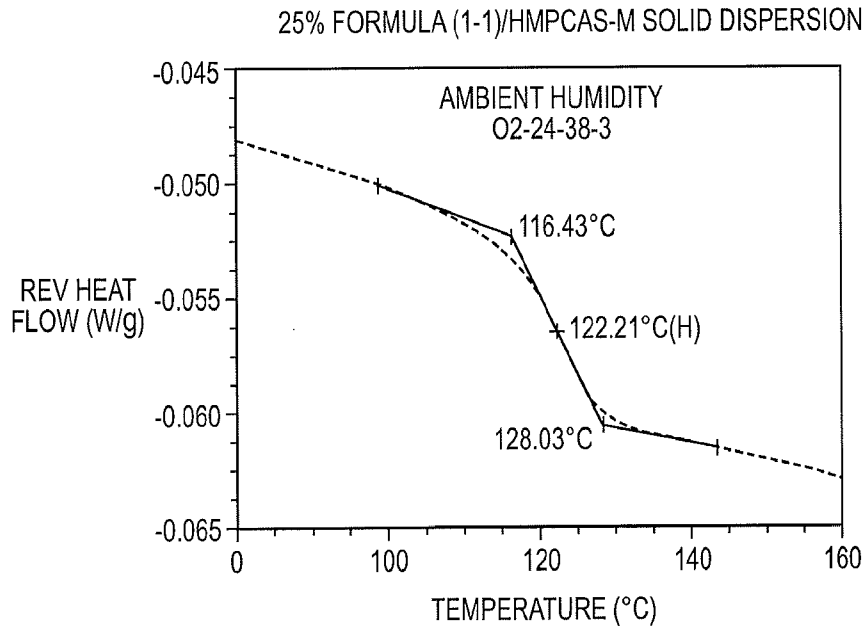

25% FORMULA (1-1)/HMPCAS-M SOLID DISPERSION
AMBIENT HUMIDITY
O2-24-38-3
116.43°C
122.21°C(H)
128.03°C
REV HEAT FLOW (W/g)
-0.045
-0.050
-0.055
-0.060
-0.065
0
100
120
140
160
TEMPERATURE (°C)

FIG. 4B

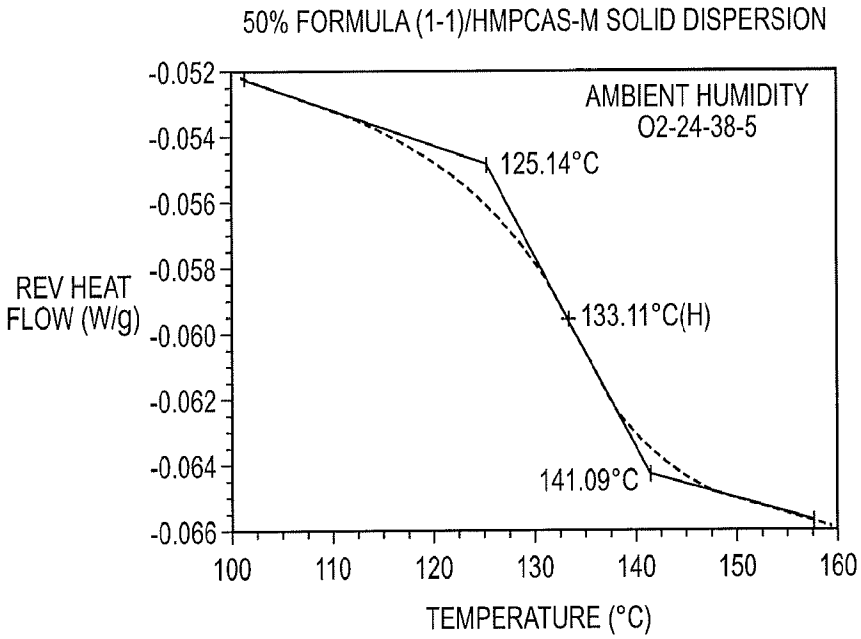

50% FORMULA (1-1)/HMPCAS-M SOLID DISPERSION
AMBIENT HUMIDITY
O2-24-38-5
125.14°C
133.11°C(H)
141.09°C
REV HEAT FLOW (W/g)
-0.052
-0.054
-0.056
-0.058
-0.060
-0.062
-0.064
-0.066
100
110
120
130
140
150
160
TEMPERATURE (°C)

FIG. 4C

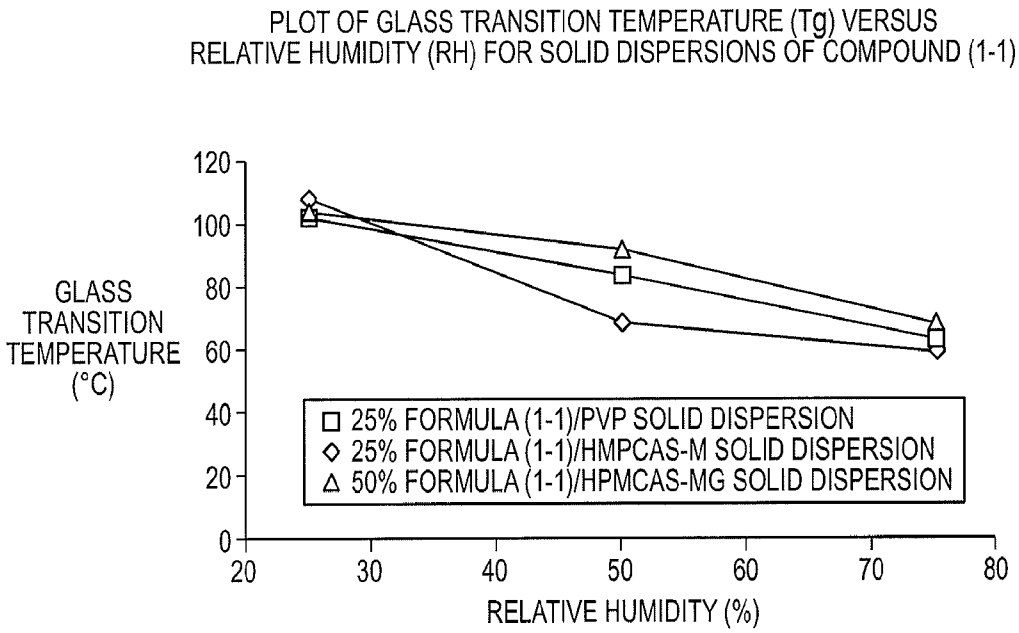

PLOT OF GLASS TRANSITION TEMPERATURE (Tg) VERSUS
RELATIVE HUMIDITY (RH) FOR SOLID DISPERSIONS OF COMPOUND (1-1)
GLASS TRANSITION TEMPERATURE (°C)
120
100
80
60
40
20
0
20
30
40
50
60
70
80
RELATIVE HUMIDITY (%)
25% FORMULA (1-1)/PVP SOLID DISPERSION
25% FORMULA (1-1)/HMPCAS-M SOLID DISPERSION
50% FORMULA (1-1)/HPMCAS-MG SOLID DISPERSION

FIG. 5

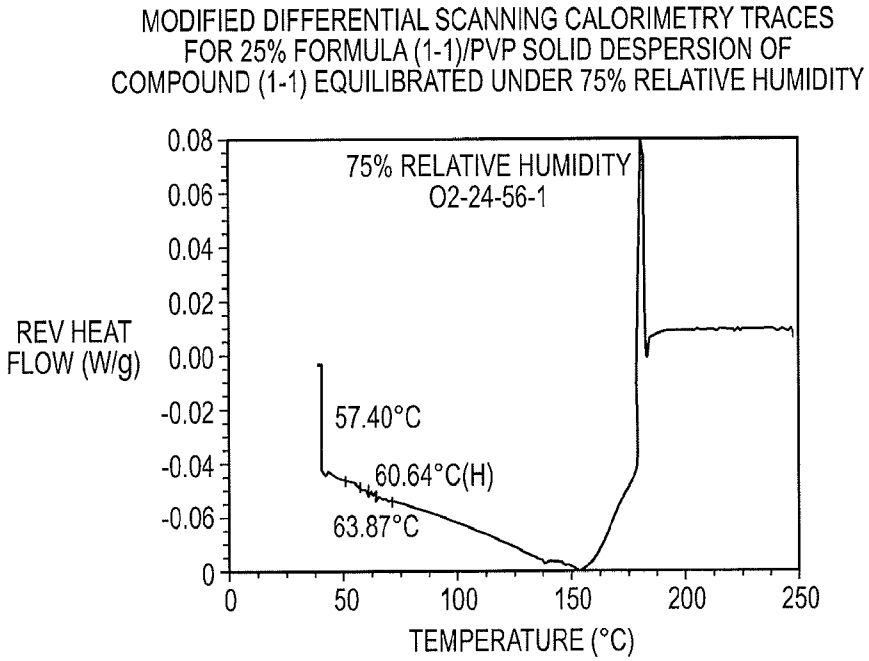

MODIFIED DIFFERENTIAL SCANNING CALORIMETRY TRACES
FOR 25% FORMULA (1-1)/PVP SOLID DESPERSION OF
COMPOUND (1-1) EQUILIBRATED UNDER 75% RELATIVE HUMIDITY
REV HEAT FLOW (W/g)
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
0
75% RELATIVE HUMIDITY
O2-24-56-1
57.40°C
60.64°C(H)
63.87°C
0
50
100
150
200
250
TEMPERATURE (°C)

FIG. 6

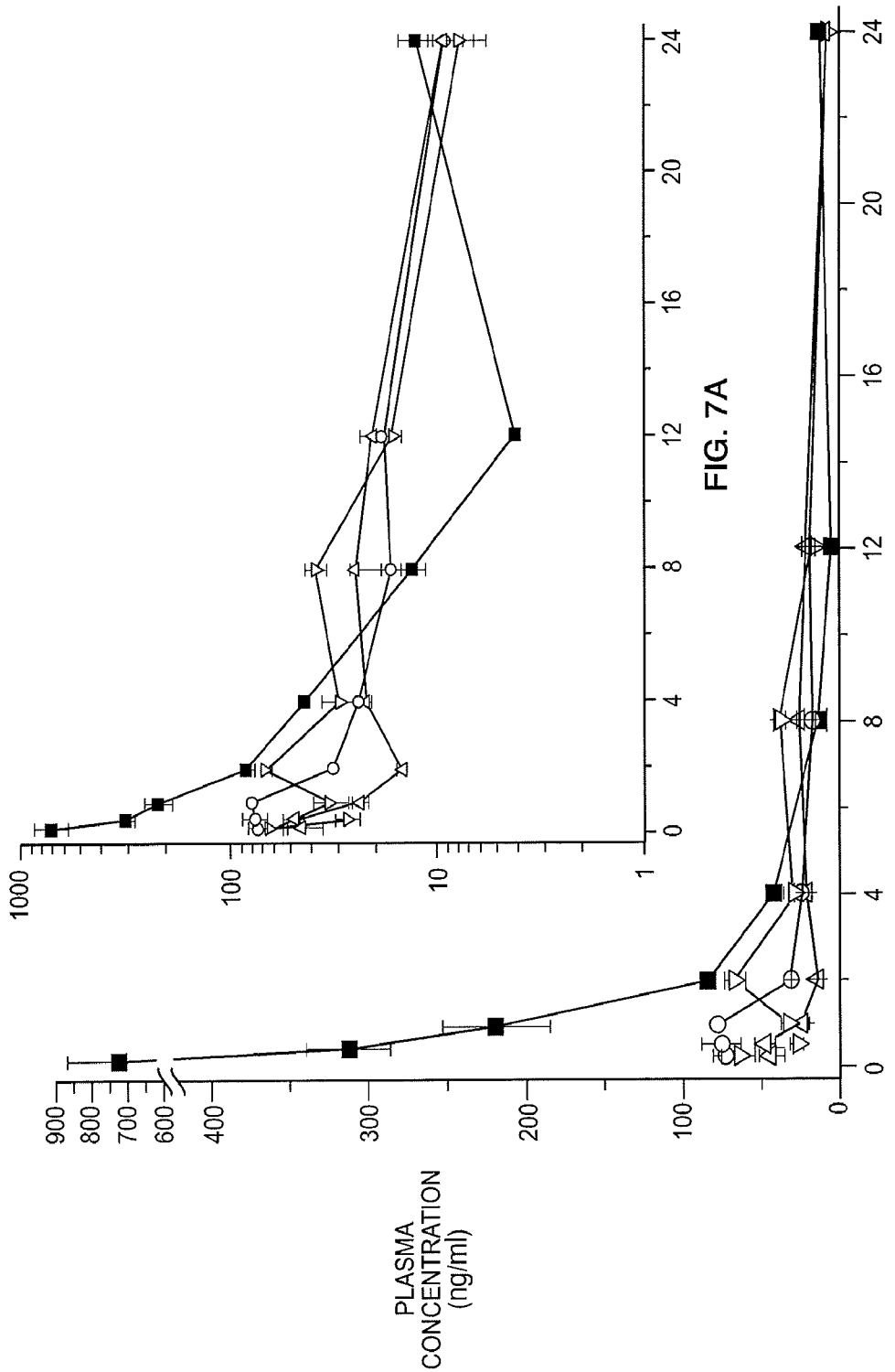

PLASMA CONCENTRATION (ng/ml)
TIME (h)
0
100
200
300
400
600
700
800
900
0
4
8
12
16
20
24
1
10
100
1000

FIG. 7A

FIG. 7B

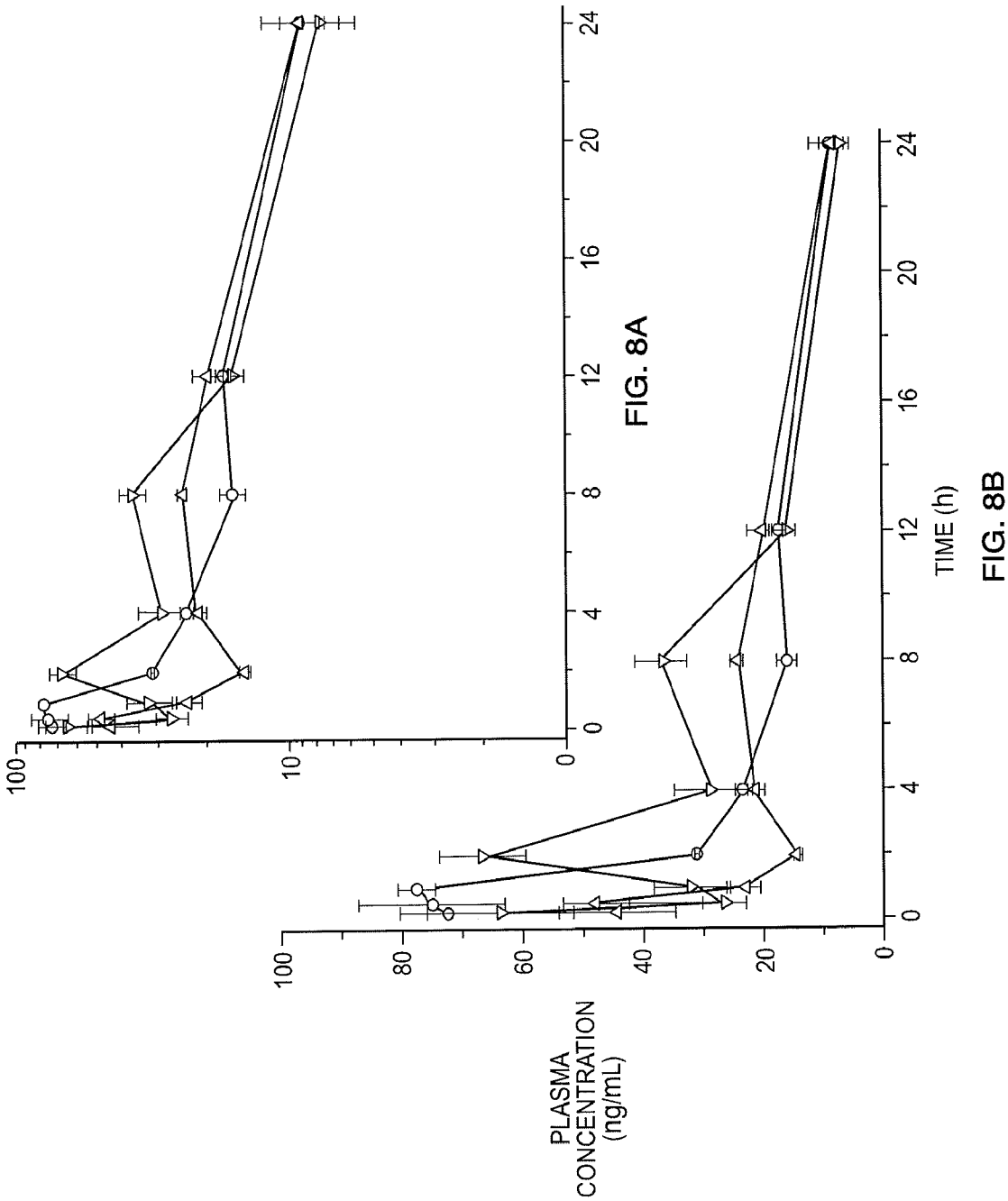

PLASMA CONCENTRATION (ng/mL)
TIME (h)
0
20
40
60
80
100
0
4
8
12
16
20
24
10
100

FIG. 8A

FIG. 8B

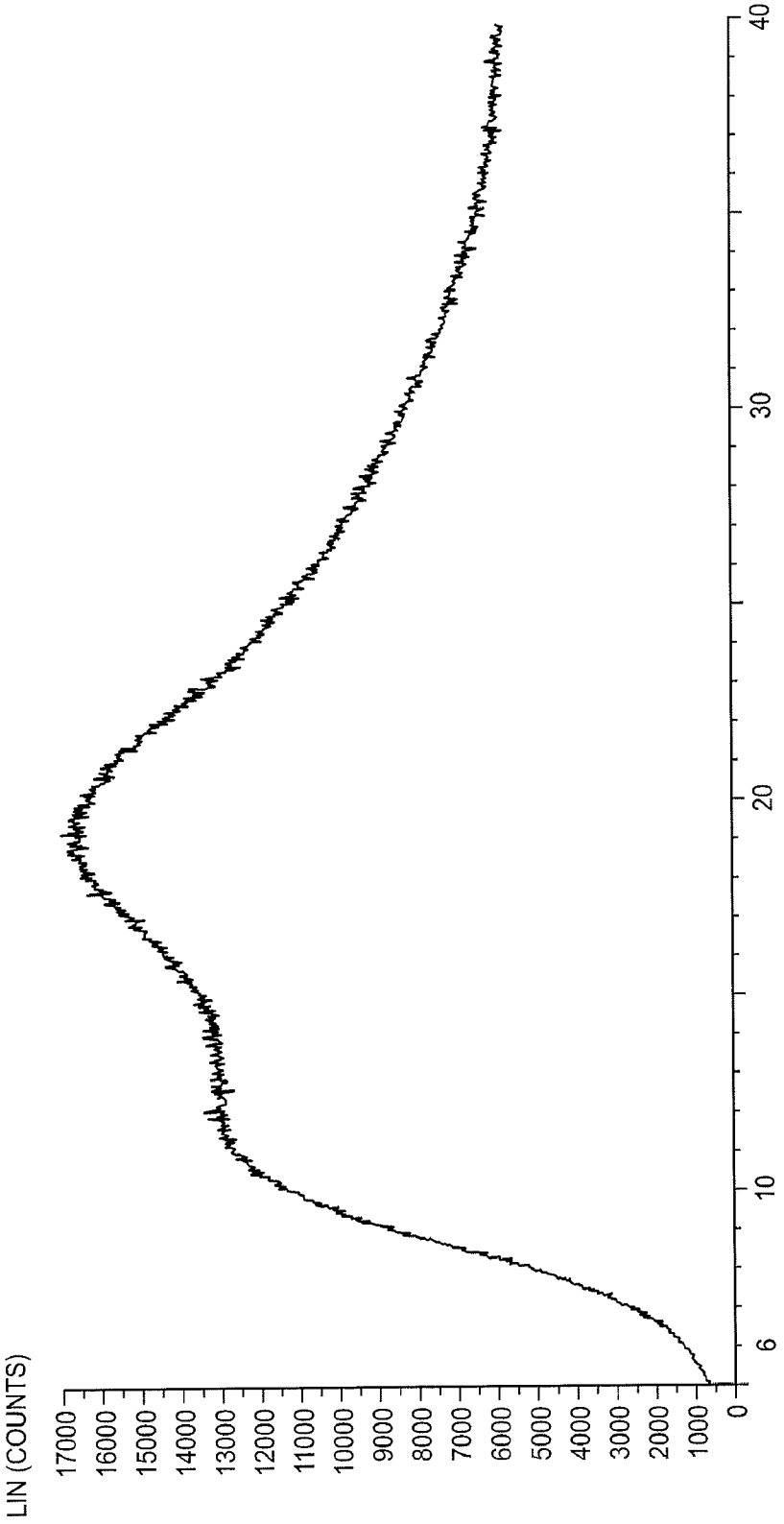

PXRD RESULTS FOR T = 0 SAMPLE (LNR O2-24-131)
LIN (COUNTS)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
6
10
20
30
40
2-THETA-SCALE

FIG. 9

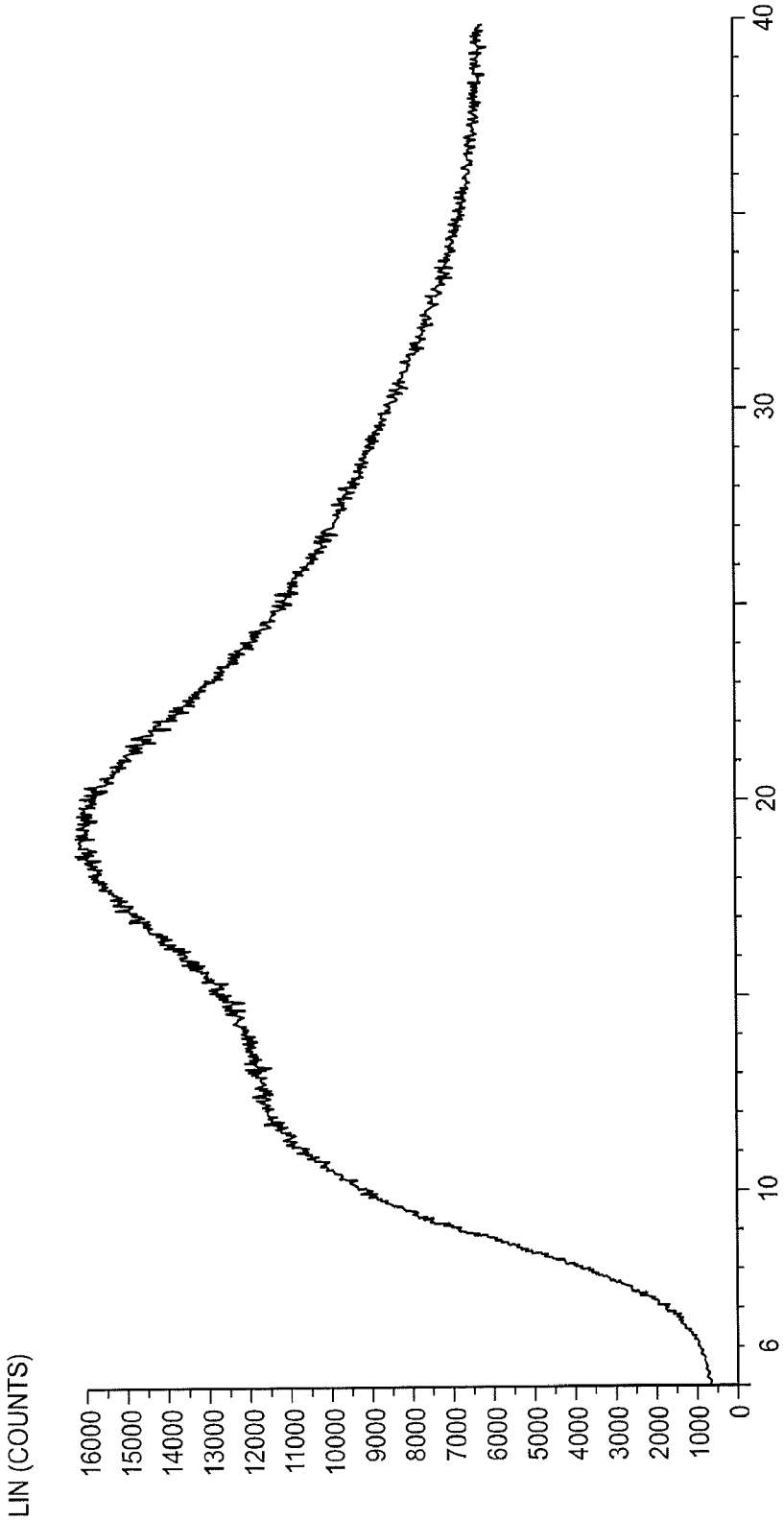

PXRD RESULTS FOR 1 MONTH STABILITY SAMPLE (LNR O2-41-73)
LIN (COUNTS)
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
6
10
20
30
40
2-THETA-SCALE

FIG. 10

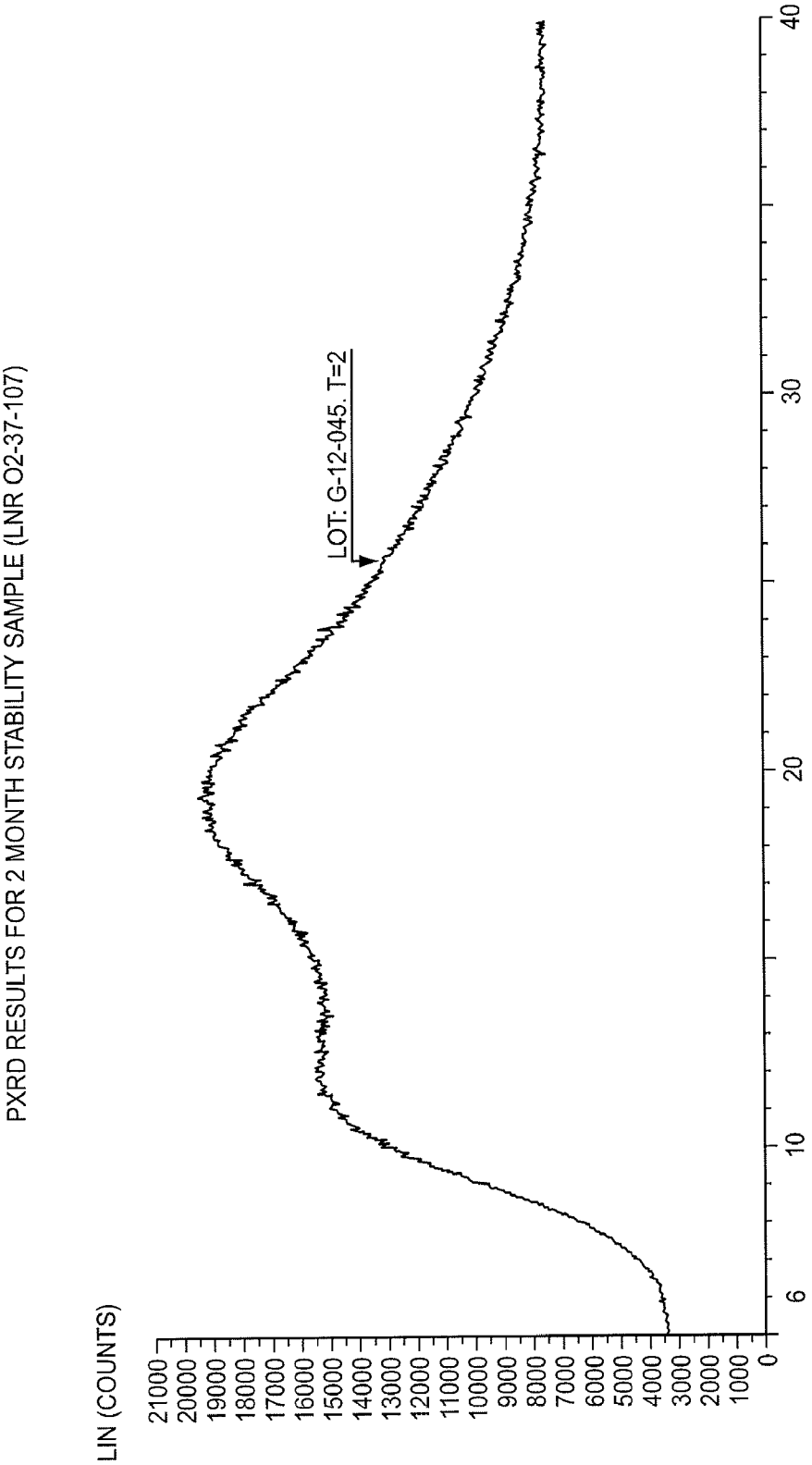

PXRD RESULTS FOR 2 MONTH STABILITY SAMPLE (LNR O2-37-107)
LOT: G-12-045. T=2
LIN (COUNTS)
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
6
10
20
30
40
2-THETA-SCALE

FIG. 11

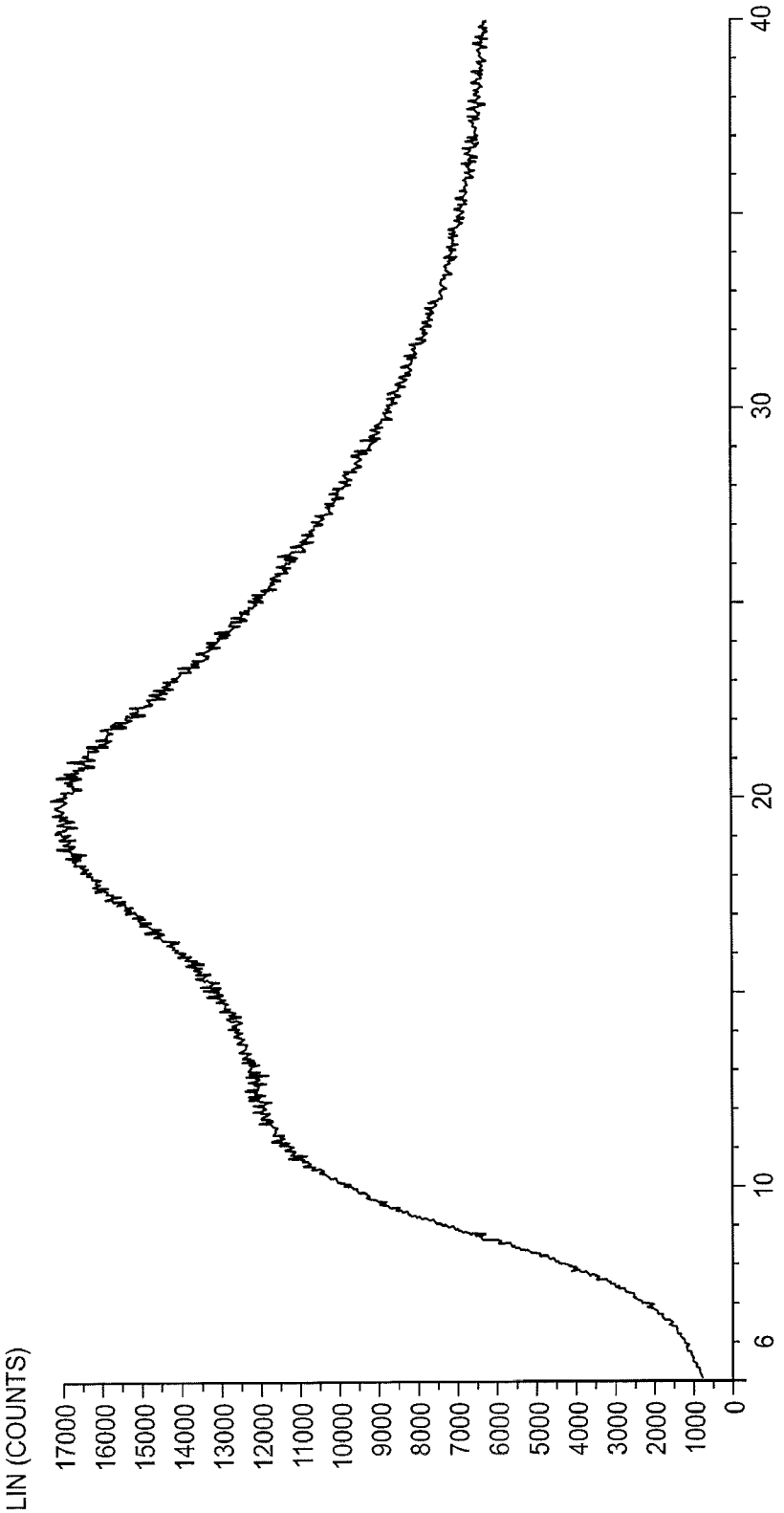

PXRD RESULTS FOR 3 MONTH STABILITY SAMPLE (LNR O2-37-120)
LIN (COUNTS)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
6
10
20
30
40
2-THETA-SCALE

FIG. 12

48h-CONCOMITANT COMBINATIONS

| DRUG | CI VALUES | OTX015-SENSITIVE CELL LINES | | | OTX015-RESISTANT CELL LINE |
|---|---|---|---|---|---|
| | | HL60 | U937 | JURKAT | K562 |
| DECITABINE | MEDIAN<br>MIN<br>MAX | 0.5<br>0.1<br>1.8 | 0.9<br>0.001<br>8.7 | 0.5<br>0.04<br>7.7 | 0.3<br>0.1<br>1.9 |
| CYTARABINE | MEDIAN<br>MIN<br>MAX | 0.9<br>0.1<br>2.5 | 1.0<br>0.3<br>1.7 | 0.7<br>0.5<br>1.8 | 1.1<br>0.2<br>7.8 |
| DAUNORUBICIN | MEDIAN<br>MIN<br>MAX | 0.9<br>0.4<br>2.8 | 0.8<br>0.3<br>3.2 | 0.9<br>0.4<br>1.8 | 0.4<br>0.1<br>1.7 |
| METHOTREXATE | MEDIAN<br>MIN<br>MAX | 0.1<br>0.0<br>4.9 | 0.3<br>0.0<br>2.4 | 0.1<br>0.0<br>1.8 | 0.1<br>0.0<br>5.1 |
| DEXAMETHASONE | MEDIAN<br>MIN<br>MAX | 0.3<br>0.1<br>1.2 | 1.9<br>0.5<br>9.3 | 0.2<br>0.0<br>2.0 | 0.7<br>0.3<br>2.6 |
| EVEROLIMUS | MEDIAN<br>MIN<br>MAX | 0.8<br>0.2<br>4.6 | 2.0<br>0.6<br>6.2 | 0.7<br>0.1<br>5.7 | 0.6<br>0.1<br>3.3 |
| AZACYTIDINE | MEDIAN<br>MIN<br>MAX | 1.6<br>0.4<br>8.0 | 0.8<br>0.3<br>2.0 | 0.7<br>0.4<br>2.4 | 0.6<br>0.1<br>9.1 |
| PANOBINOSTAT | MEDIAN<br>MIN<br>MAX | 0.7<br>0.2<br>3.3 | 1.3<br>0.4<br>6.5 | 0.5<br>0.2<br>0.8 | 0.5<br>0.1<br>2.7 |

FIG. 13A

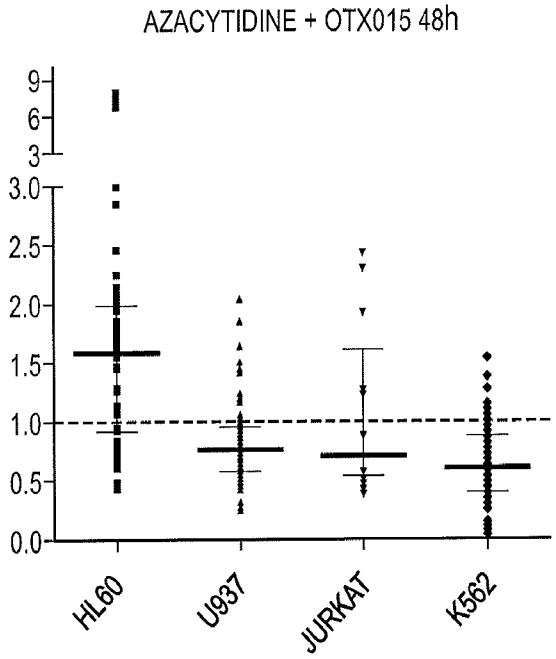



FIG. 13B

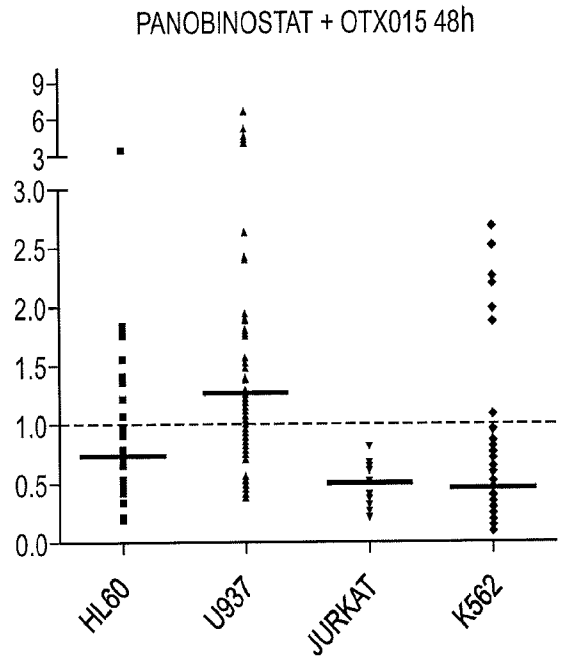



FIG. 13C

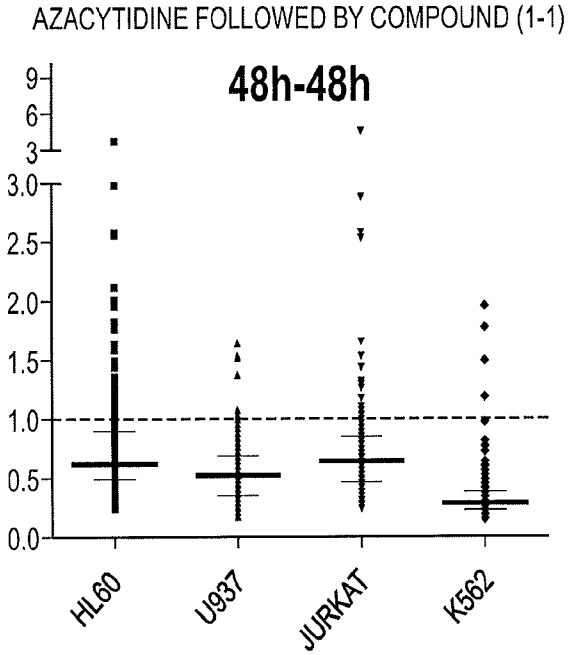



FIG. 14A

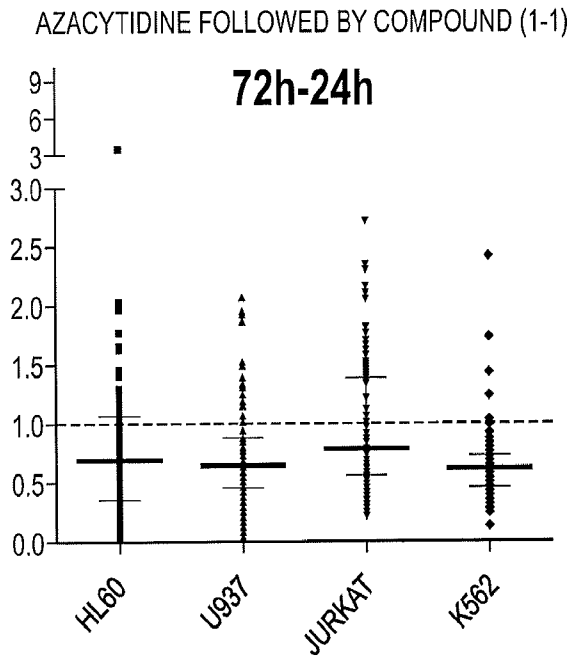



FIG. 14B

AZACYTIDINE FOLLOWED BY COMPOUND (1-1)

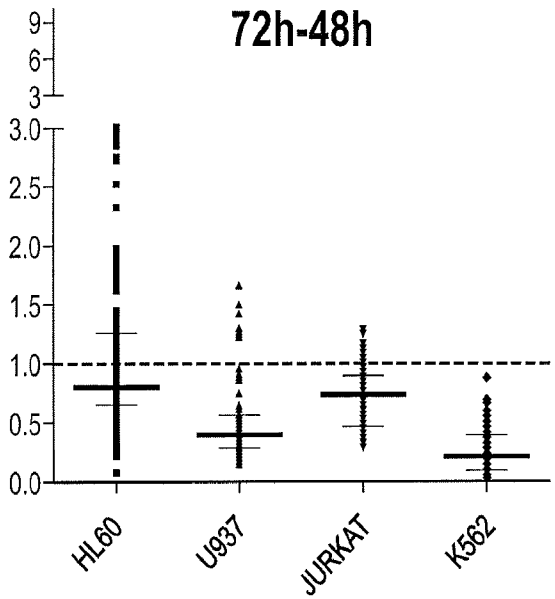



FIG. 14C

AZACYTIDINE FOLLOWED BY COMPOUND (1-1)

| SCHEDULE | CI VALUES | HL60 | U937 | JURKAT | K562 |
|---|---|---|---|---|---|
| Aza 48h-OTX015 48h | MEDIAN<br>MIN<br>MAX | 0.6<br>0.2<br>3.7 | 0.5<br>0.02<br>1.6 | 0.6<br>0.2<br>4.4 | 0.3<br>0.1<br>4.6 |
| Aza 72h-OTX015 24h | MEDIAN<br>MIN<br>MAX | 0.7<br>0.02<br>2.0 | 0.6<br>0.06<br>2.0 | 0.8<br>0.2<br>2.7 | 0.6<br>0.1<br>2.4 |
| Aza 72h-OTX015 48h | MEDIAN<br>MIN<br>MAX | 0.8<br>0.1<br>3.0 | 0.4<br>0.2<br>1.7 | 0.7<br>0.3<br>1.3 | 0.2<br>0.01<br>0.9 |

FIG. 14D

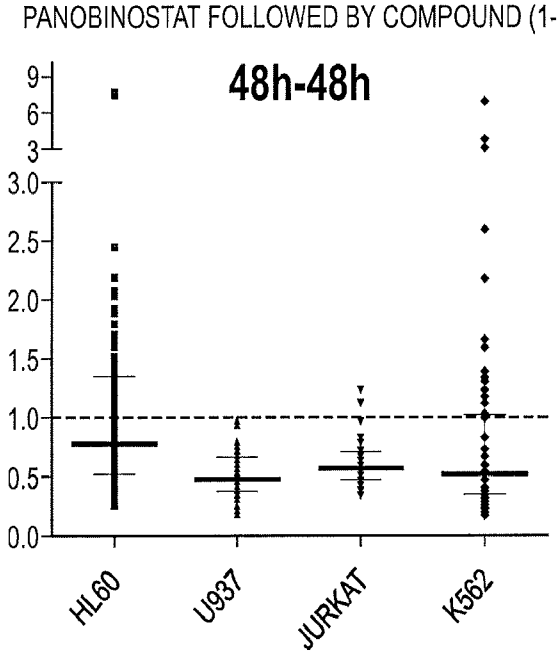



FIG. 15A

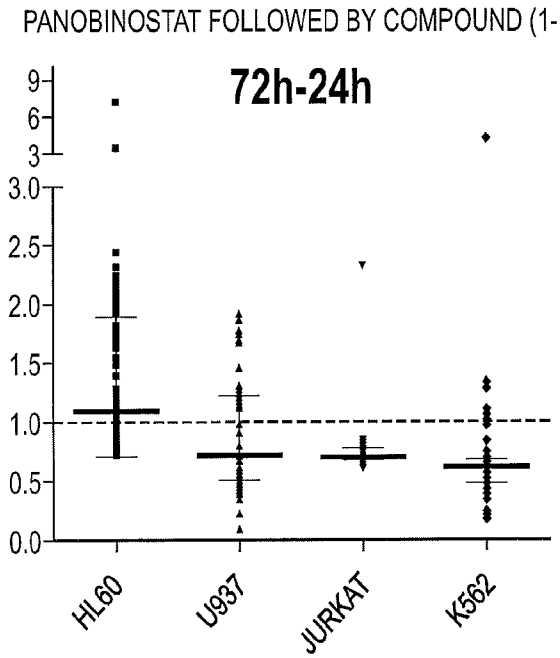



FIG. 15B

PANOBINOSTAT FOLLOWED BY COMPOUND (1-1)

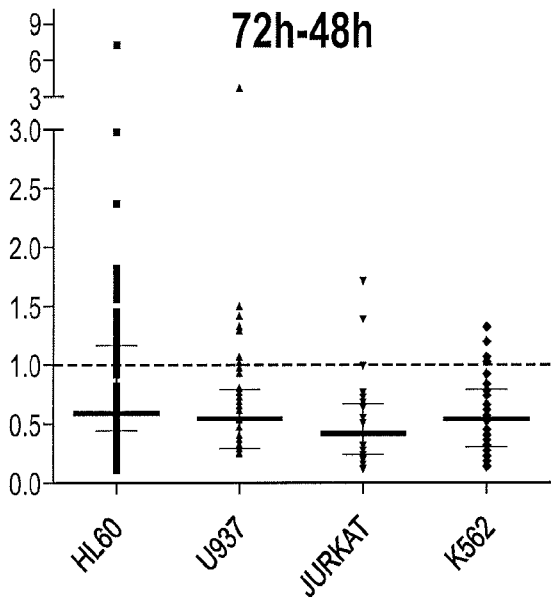



FIG. 15C

PANOBINOSTAT FOLLOWED BY COMPOUND (1-1)

| SCHEDULE | CI VALUES | HL60 | U937 | JURKAT | K562 |
|---|---|---|---|---|---|
| Pano 48h-OTX015 48h | MEDIAN<br>MIN<br>MAX | 0.8<br>0.3<br>7.4 | 0.5<br>0.2<br>1.0 | 0.6<br>0.3<br>1.2 | 0.5<br>0.2<br>6.9 |
| Pano 72h-OTX015 24h | MEDIAN<br>MIN<br>MAX | 1.1<br>0.2<br>7.1 | 0.7<br>0.1<br>1.9 | 0.7<br>0.6<br>2.3 | 0.6<br>0.2<br>4.2 |
| Pano 72h-OTX015 48h | MEDIAN<br>MIN<br>MAX | 0.6<br>0.1<br>7.2 | 0.5<br>0.2<br>3.8 | 0.4<br>0.1<br>1.7 | 0.5<br>0.1<br>1.3 |

FIG. 15D

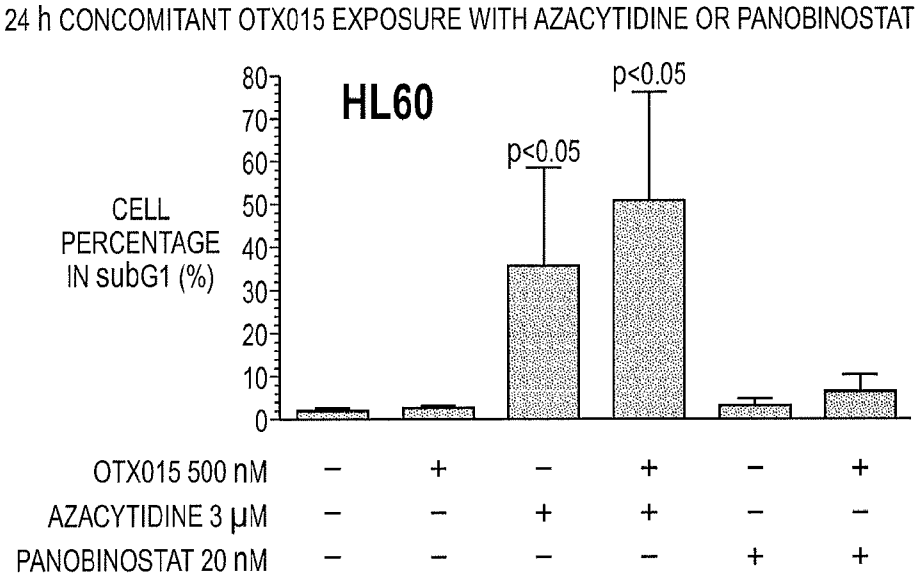



FIG. 16A

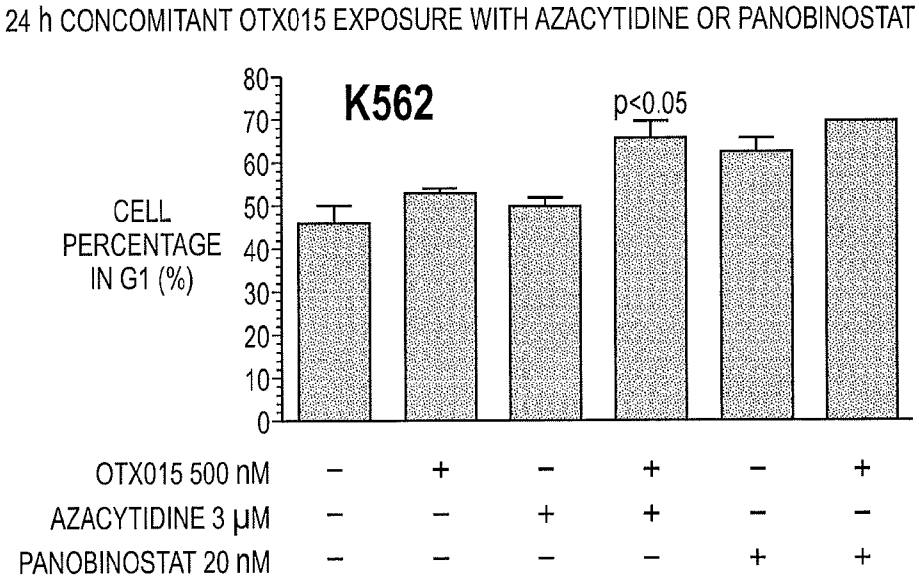



FIG. 16B

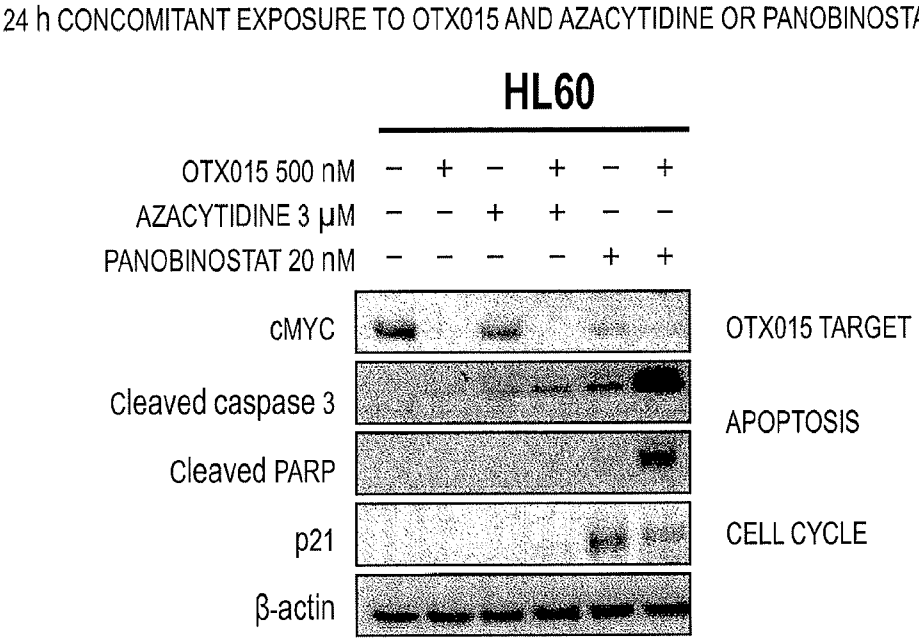



FIG. 17A

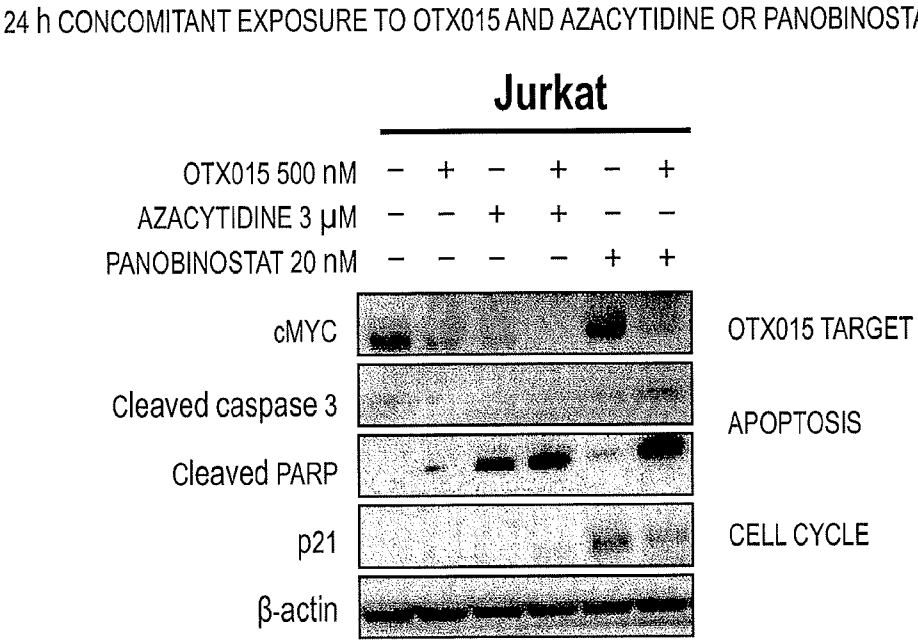



FIG. 17B

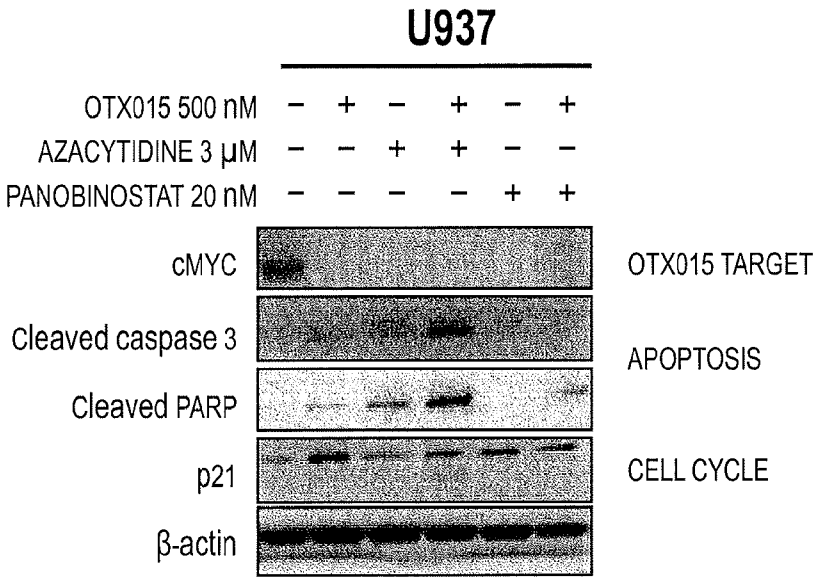



FIG. 17C

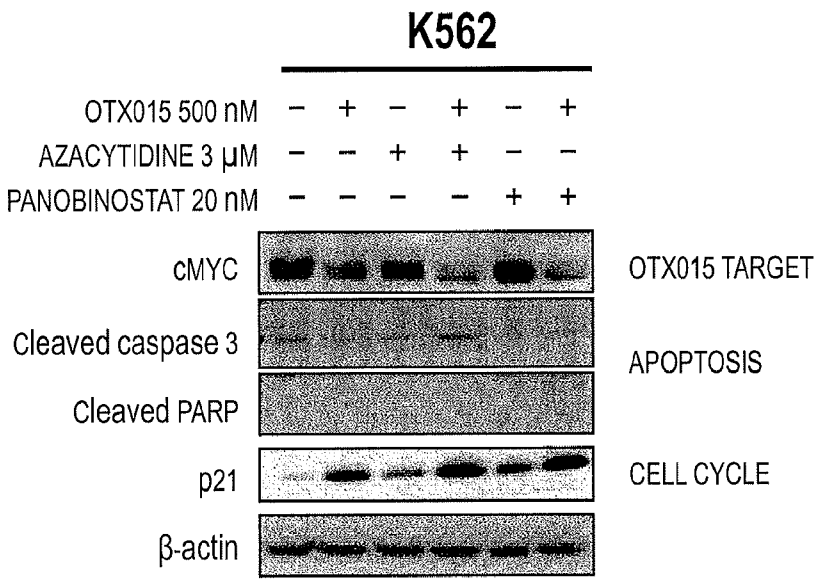



FIG. 17D

IN VITRO ANTIPROLIFERATIVE EFFECT OF OTX015 AFTER 72-h EXPOSURE

| TUMOR TYPE | CELL LINE | OTX015 | |
|---|---|---|---|
| | | GI50 [nM] (95%IC) | Emax AT 6µM (%) |
| AML | HL60 | 285.0 (204.1-397.8) | 12.8 |
| | U937 | 383.5 (176.9-831.2) | 39.9 |
| ALL | Jurkat | 282.2 (217.3-366.6) | 63.2 |
| | Molt3 | >6000 | --- |
| CML | K562 | >6000 | --- |

FIG. 18

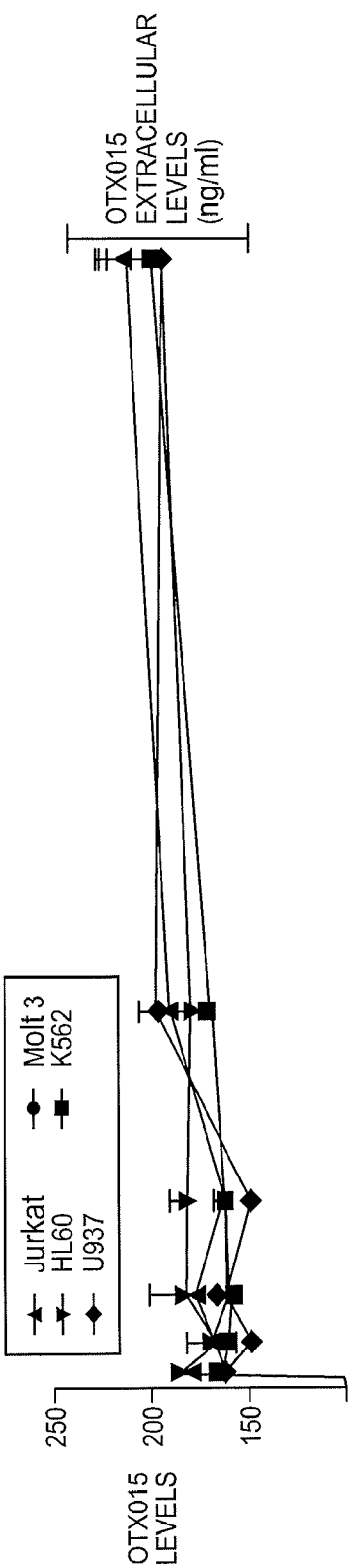



FIG. 19A

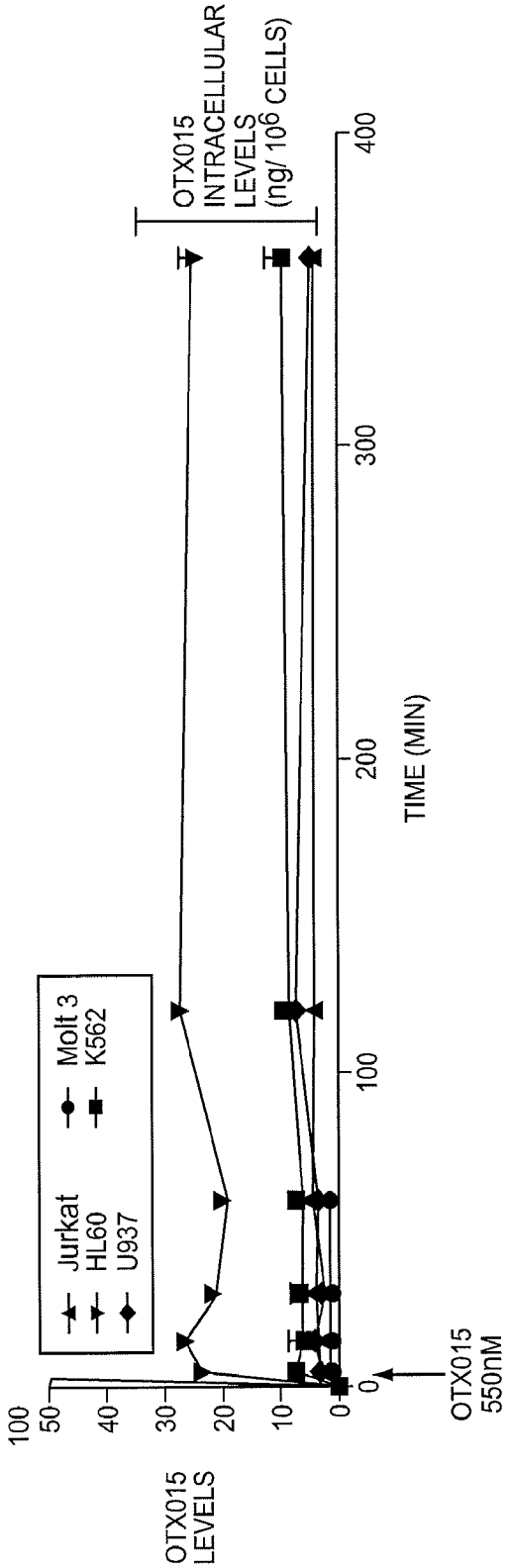



FIG. 19B

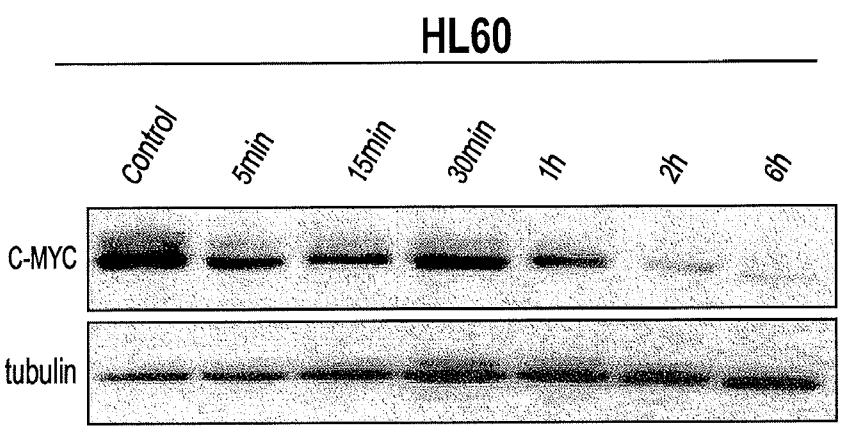



FIG. 20A

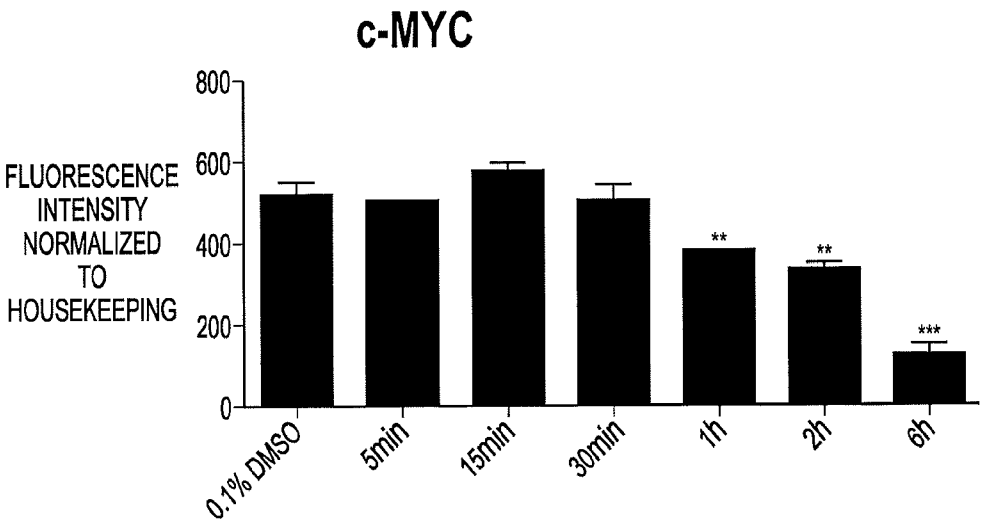



FIG. 20B

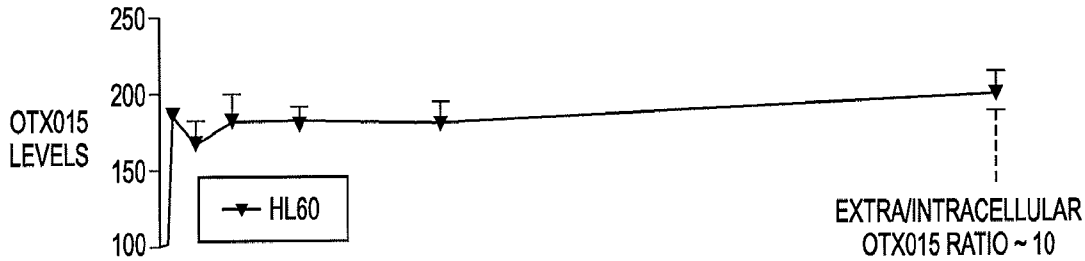



FIG. 20C

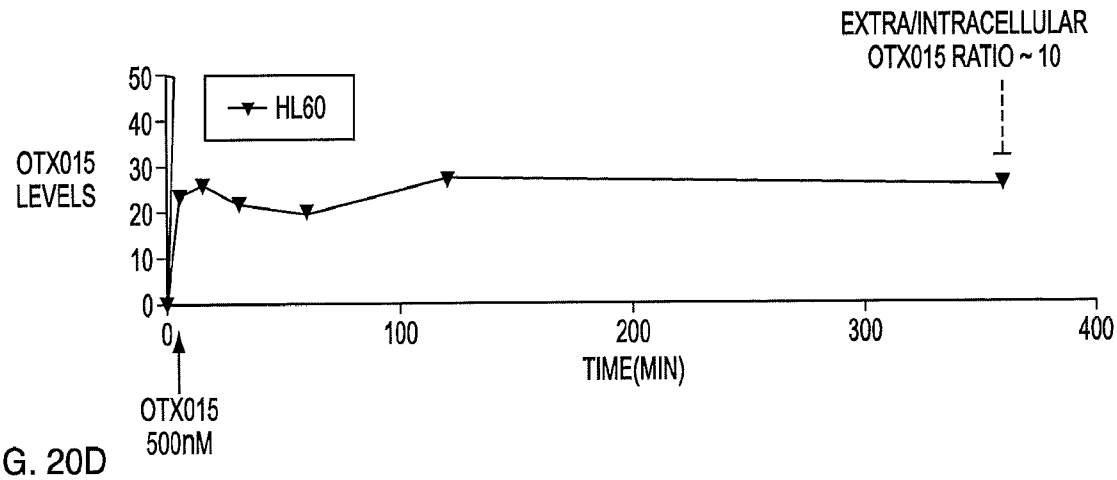



FIG. 20D

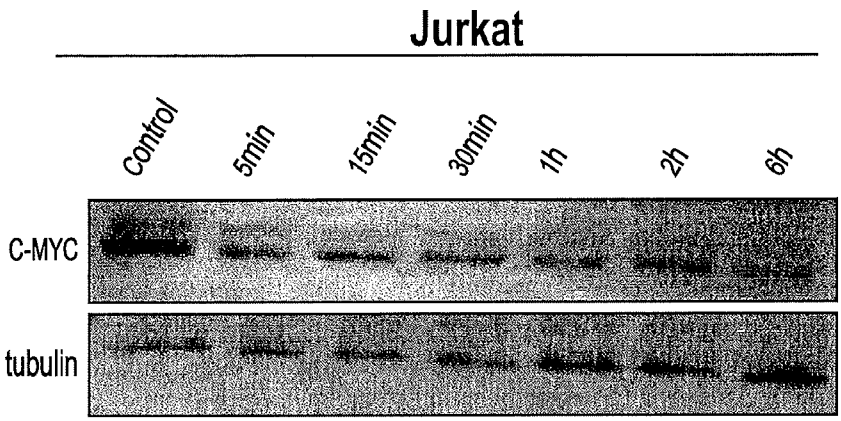



FIG. 21A

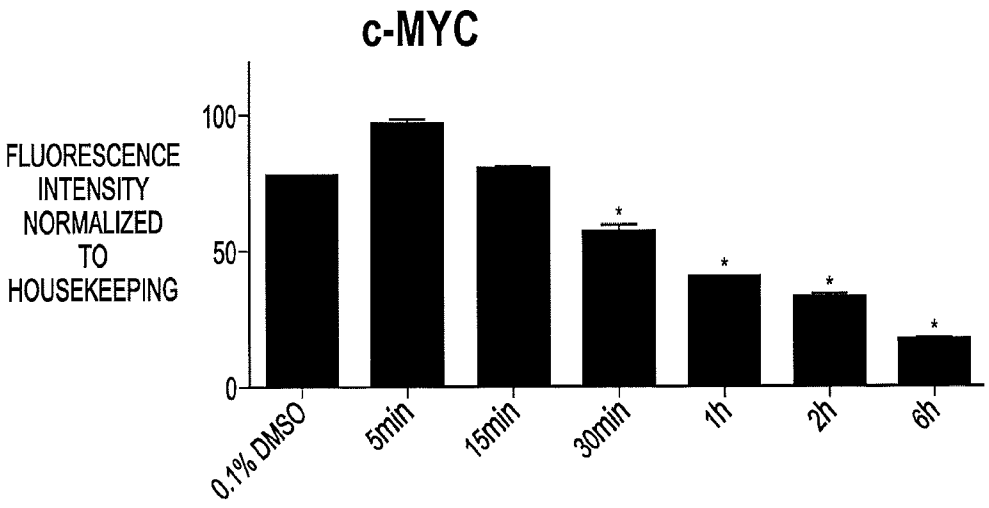



FIG. 21B

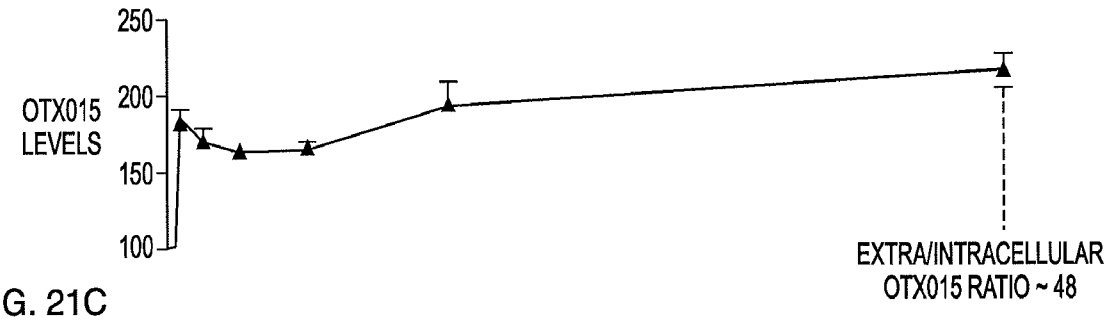



FIG. 21C

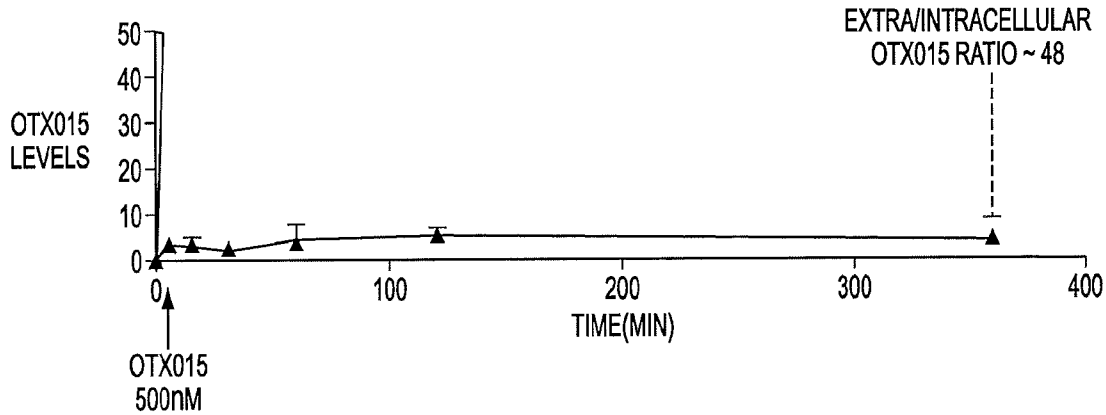



FIG. 21D

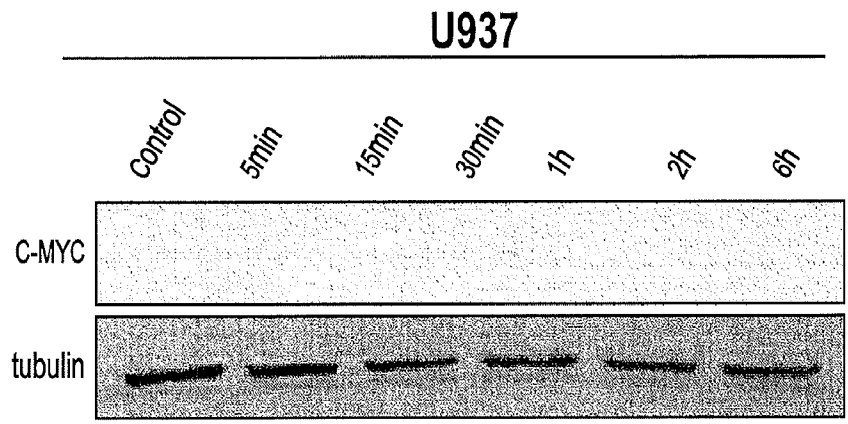



FIG. 22A

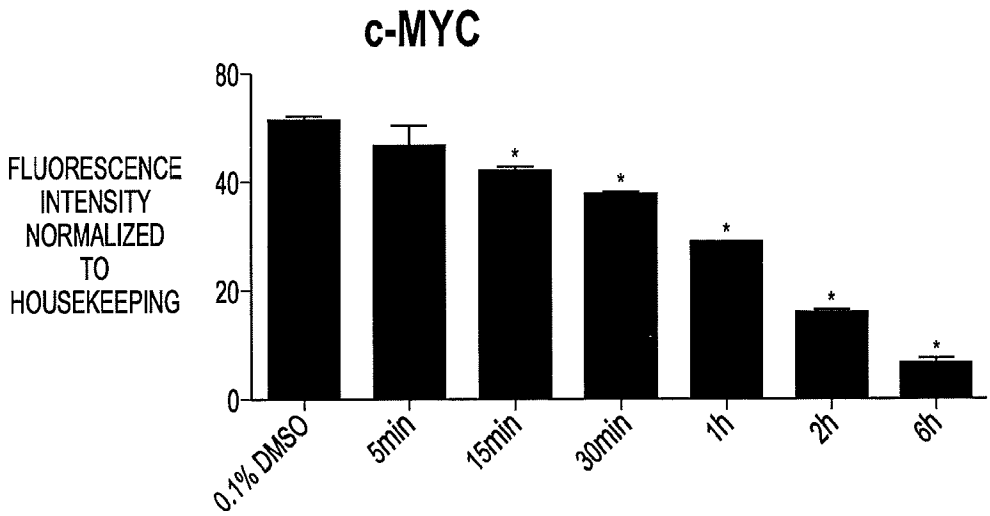



FIG. 22B

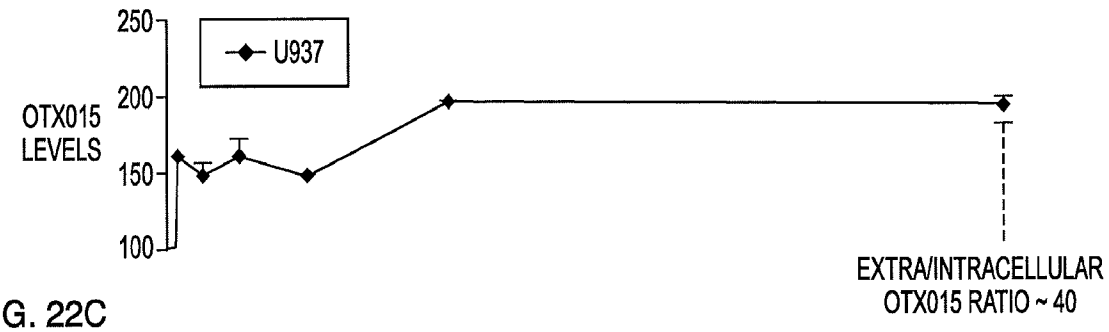



FIG. 22C

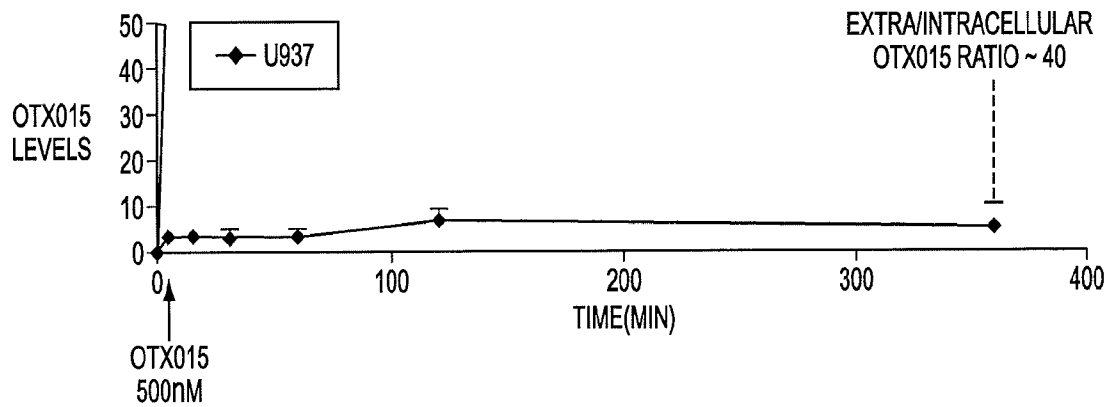



FIG. 22D

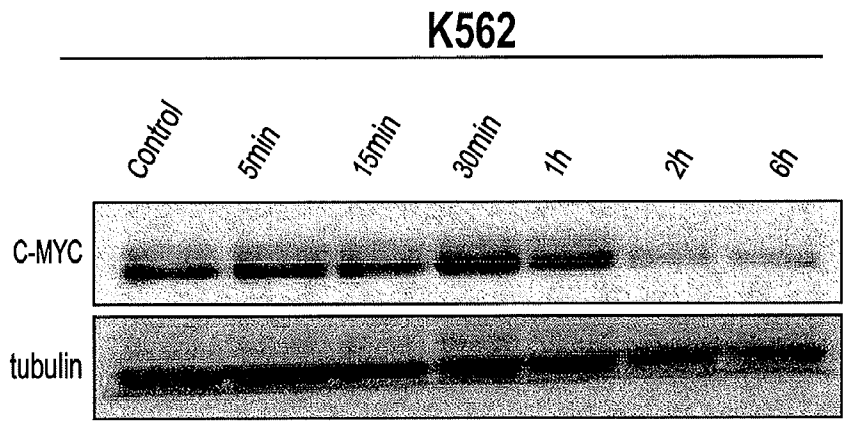



FIG. 23A

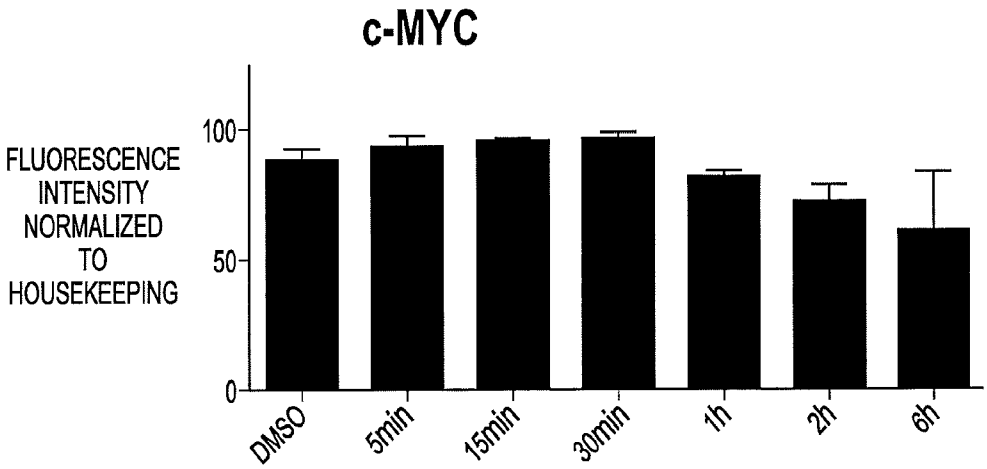



FIG. 23B

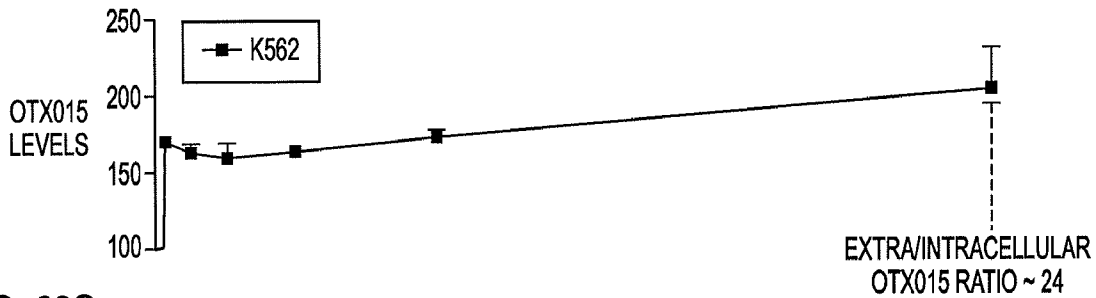



FIG. 23C

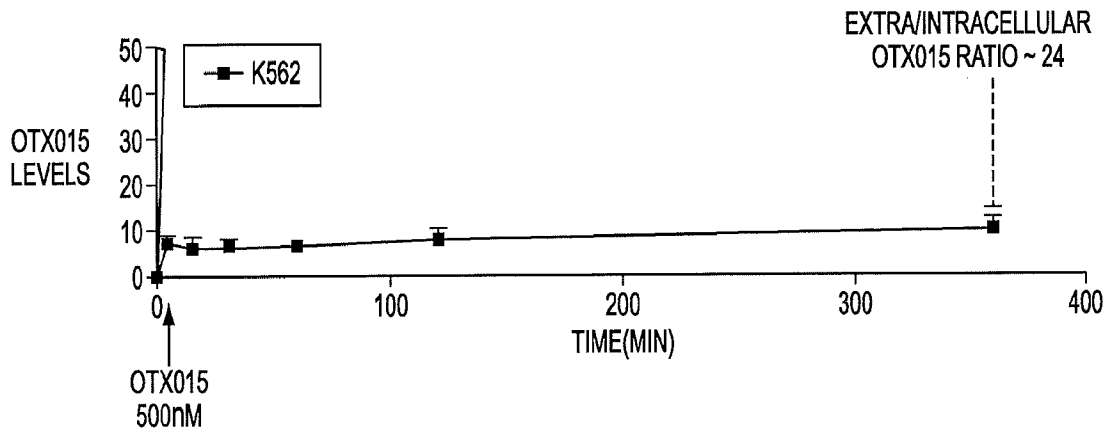



FIG. 23D

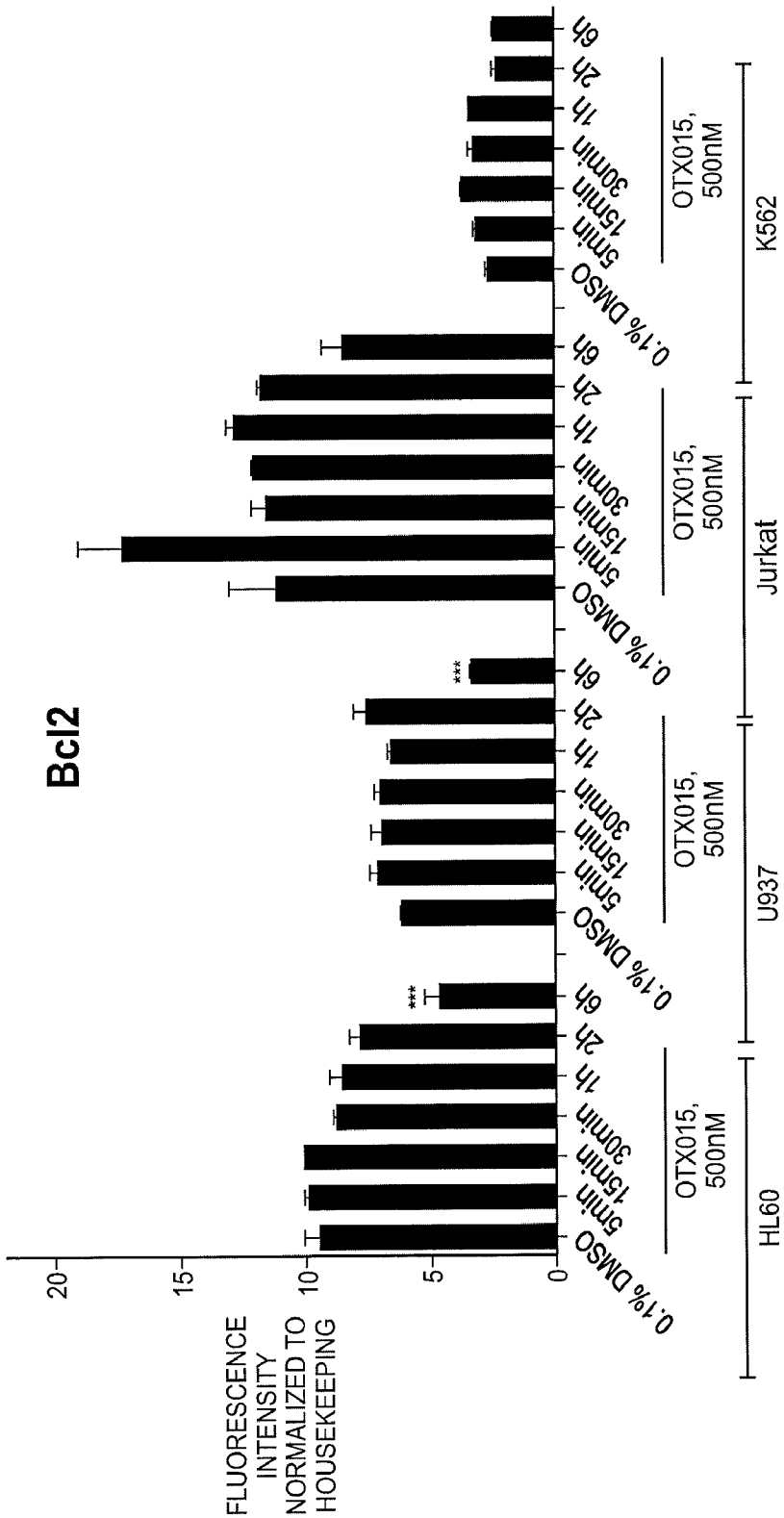

Bcl2
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
20
15
10
5
0
***
***
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562

FIG. 24A

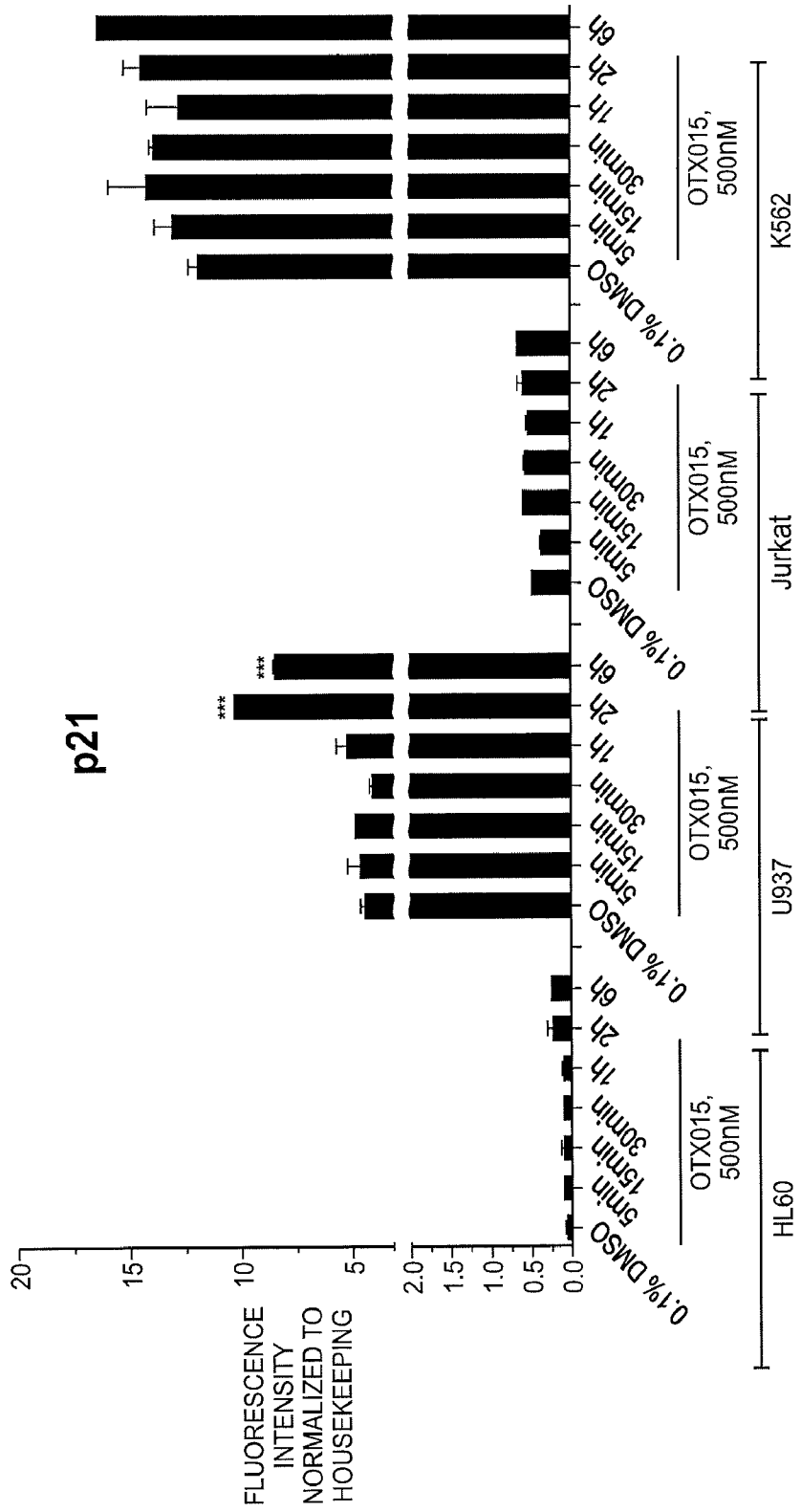

p21
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
20
15
10
5
2.0
1.5
1.0
0.5
0.0
***
***
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562

FIG. 24B

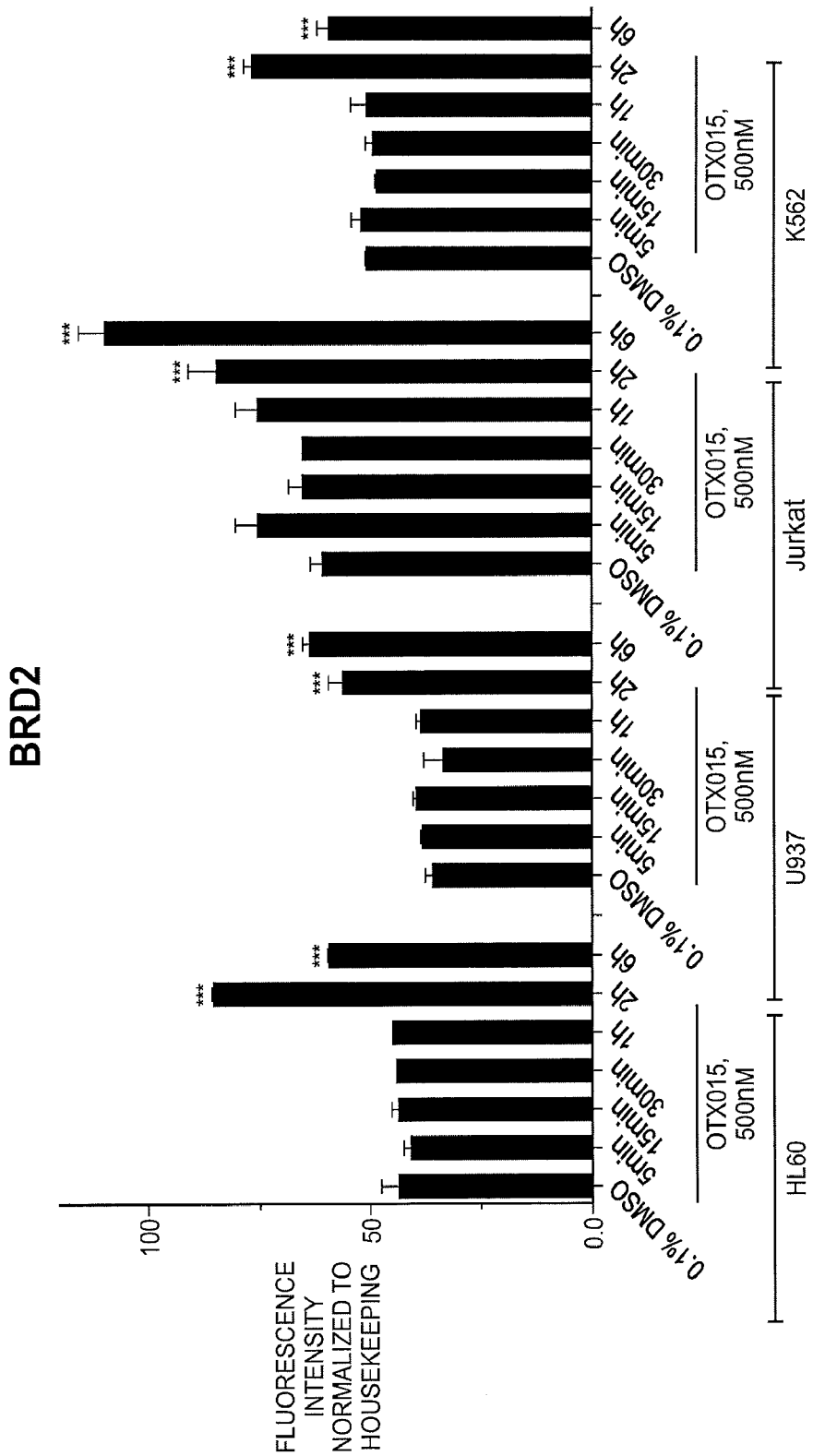

BRD2
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
100
50
0.0
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562
***

FIG. 24C

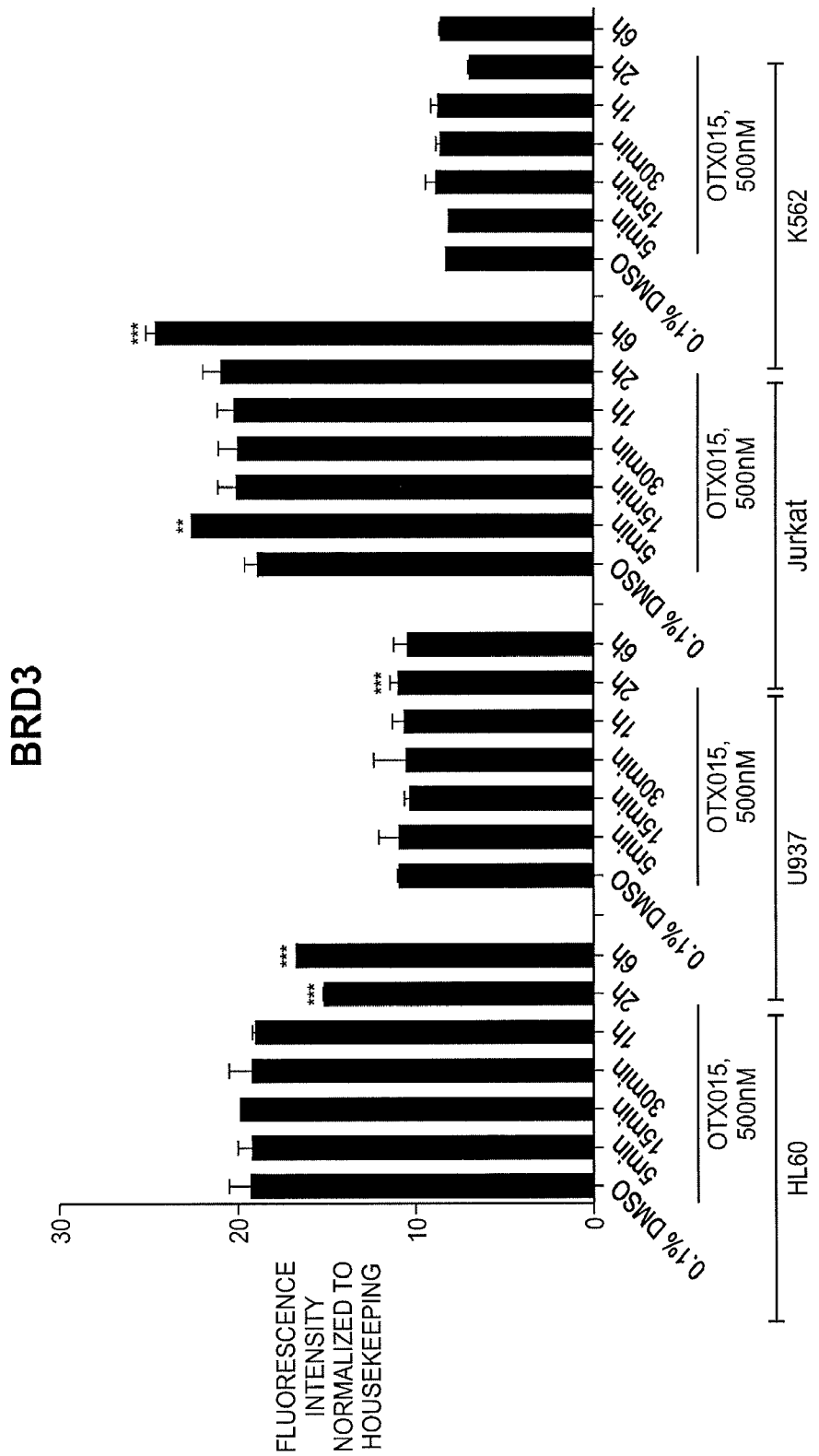

BRD3
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
30
20
10
0
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562
***
**

FIG. 24D

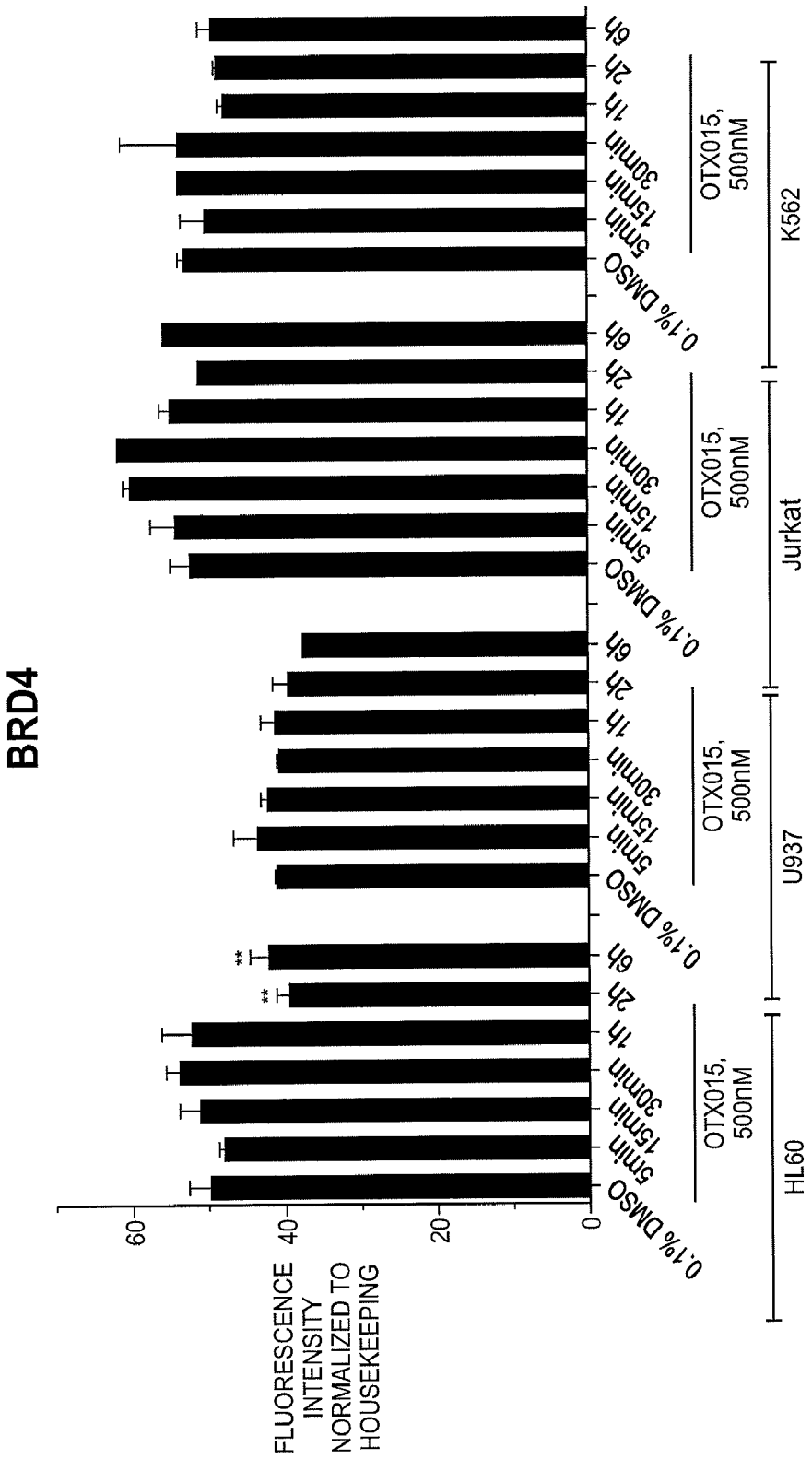

BRD4
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
60
40
20
0
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562
**
**

FIG. 24E

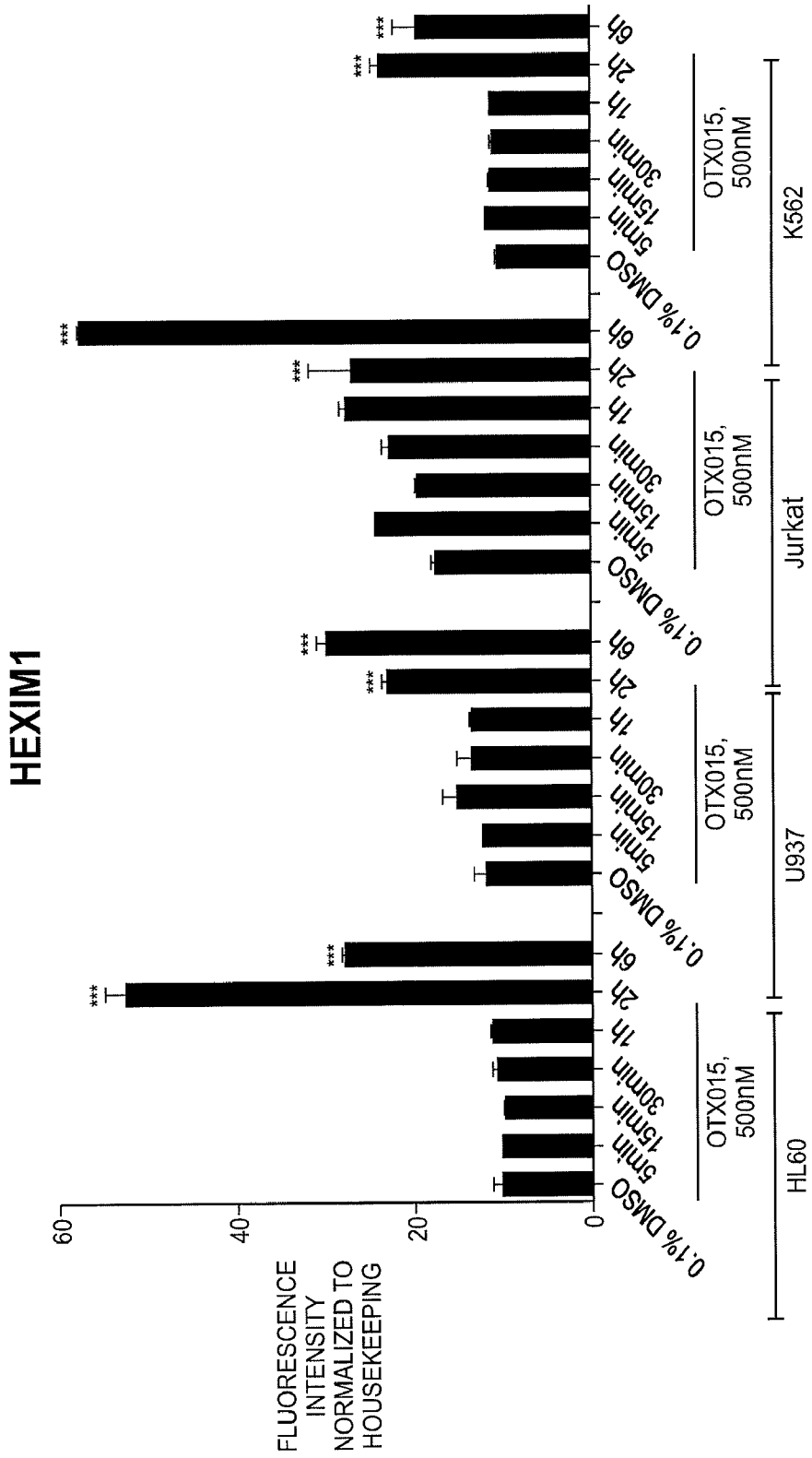

HEXIM1
FLUORESCENCE INTENSITY NORMALIZED TO HOUSEKEEPING
60
40
20
0
0.1% DMSO
5min
15min
30min
1h
2h
6h
OTX015, 500nM
HL60
U937
Jurkat
K562
***

FIG. 24F