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(54) **Titre : METHODE DE TRAITEMENT D'UN LYMPHOME A L'AIDE DE COMPOSES THIENOTRIAZOLODIAZEPINE**
(54) **Title: METHOD OF TREATING LYMPHOMA USING THIENOTRIAZOLODIAZEPINE COMPOUNDS**

(57) **Abrégé/Abstract:**
A method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising (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. The B-cell malignant cancers include diffuse large B-cell lymphoma and splenic marginal zone lymphoma. The T-cell malignant cancers include anaplastic large T-cell lymphoma.

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(54) **Title**: METHOD OF TREATING LYMPHOMA USING THIENOTRIAZOLODIAZEPINE COMPOUNDS

(57) **Abstract**: A method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising (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. The B-cell malignant cancers include diffuse large B-cell lymphoma and splenic marginal zone lymphoma. The T-cell malignant cancers include anaplastic large T-cell lymphoma.

METHOD OF TREATING LYMPHOMA USING THIENOTRIAZOLODIAZEPINE COMPOUNDS

FIELD OF THE INVENTION

[0001] The present invention relates to methods of treating B-cell malignant cancers and T-cell malignant cancers using pharmaceutically acceptable amounts of a composition comprising a thienotriazolodiazepine compound.

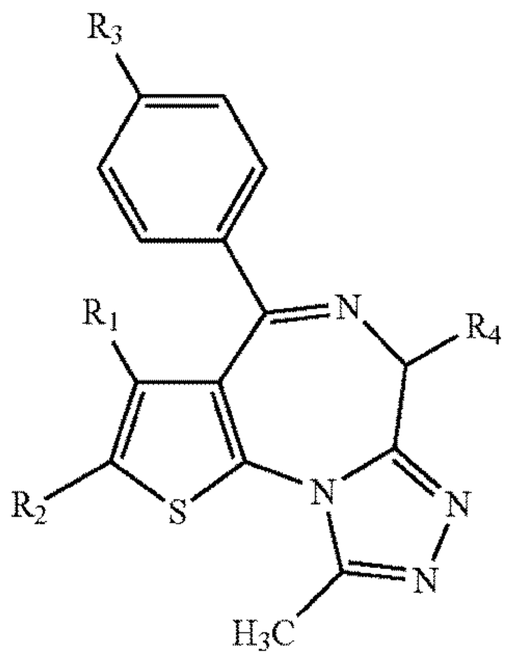
BACKGROUND OF THE INVENTION

[0002] Bromodomain-containing proteins play an important role in gene expression regulation, via chromatin structure remodelling. Antitumor activity has been reported in acute and chronic hematological malignancies, including B-cell and T-cell malignancies, using inhibitors of BRD2/3/4, members of the Bromodomain and Extraterminal (BET) family. B-cell malignancies, which are also known as B-cell neoplasms or B-cell lymphomas, are cancers that occur when B-cells are overproduced or are malignant. B-cell malignancies include for example diffuse large B-cell lymphoma (DLBCL), mantel cell lymphoma (MCL), splenic marginal zone lymphoma (SMZL), and multiple myeloma (MM). T-cell malignancies, such as anaplastic large T-cell lymphoma, are a heterogeneous group of lymphoid neoplasms representing malignant transformation of the T lymphocytes. The present disclosure presents methods of treating certain B-cell malignant cancers and T-cell malignant cancers

SUMMARY OF THE INVENTION

[0003] In one embodiment, the invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1:

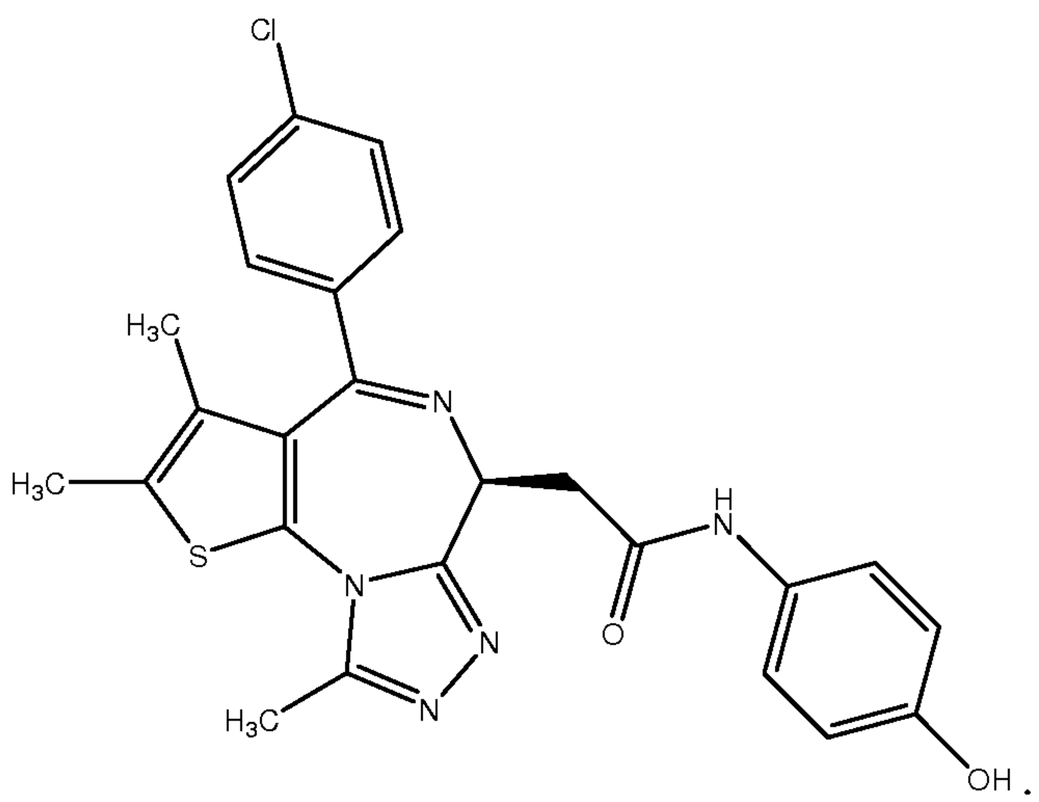
(I)



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, or a pharmaceutically acceptable salt thereof or a hydrate or solvate thereof.

[0004] In one embodiment, the present invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound independently selected from the group 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.

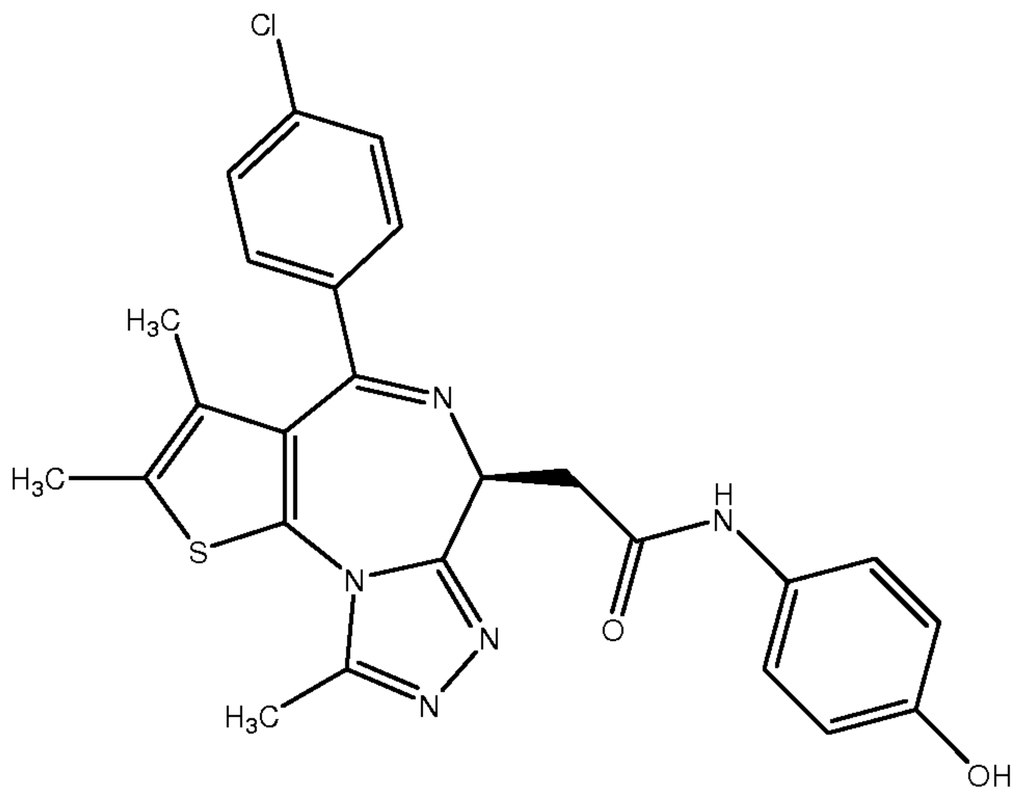
[0005] In one embodiment, the present invention provides for a method of trea malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising (S)-2-[4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide having the structure of Formula 2:



[0006] In one embodiment, the present invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1 wherein the patient is a human.

[0007] In one embodiment for treating B-cell malignant cancers using a pharmaceutically acceptable amount of Formula (1), the B-cell malignant cancers include diffuse large B-cell lymphoma and splenic marginal zone lymphoma. In another embodiment treating T-cell malignant cancers using a pharmaceutically acceptable amount of Formula (1), the T-cell malignant cancers include anaplastic large T-cell lymphoma.

[0008] In one embodiment, the present invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising (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 having the structure of Formula 2:



[0009] In one embodiment, the present invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers in a patient by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 2 wherein the patient is a human.

[0010] In one embodiment for treating B-cell malignant cancers using a pharmaceutically acceptable amount of Formula (2), the B-cell malignant cancers include diffuse large B-cell lymphoma and splenic marginal zone lymphoma. In another embodiment for treating T-cell malignant cancers using a pharmaceutically acceptable amount of Formula (2), the T-cell malignant cancers include anaplastic large T-cell lymphoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

[0012] Figure 1 illustrates cell cycle alterations induced by various concentrations of Formula 2 in DLBLC cell lines, $DoHH_2$, U-2932, Karpas 422, SU-DHL-6 and Val. X-axis, cell lines. Y-axis, percentage of cells in each cell cycle phase.

[0013] Figures 2A-2C illustrate the induction of cellular senescense in $DoHH_2$ DLBLC cell line and L-82 ALCL cell line after 48 hours exposure to Formula 2. Y-axis is percentage of cells positive to • -galactosidase.

[0014] Figures 3A nd 3B illustrate the expression levels of BRD2, BRD3 and I cell lines, SU-DHL-2, SU-DHL-4, SU-DHL-5, SU-DHL-6, SU-DHL-7, Val, OCI-Ly7, U-2932, $DoHH_2$ and Karpas 422. X-axis, cell lines. Y-axis, mRNA quantities, relative to GAPDH.

[0015] Figures 4A and 4B illustrate the expression levels of BRD2, BRD3 and BRD4 in ALCL cell lines, MAC1, FE-PD, Karpas 299, SU-DHL-1, SUPM-2, L82, JB6 and TS. X-axis, cell lines. Y-axis, mRNA quantities, relative to GAPDH.

[0016] Figure 5A-5F illustrate MYC mRNA levels after increasing doses of Formula 2 in DLBCL cell lines, SU-DHL-2, OCI-Ly3, U-2932, $DoHH_2$, Karpas 422 and SU-DHL-6. X-axis, cell lines. Y-axis, mRNA quantities, relative to un-treated sample.

[0017] Figures 6A-6D illustrate MYC mRNA levels after increasing doses of Formula 2 in ALCL cell lines, L82, Karpas 299, FE-PD and SU-DHL-1. X-axis, cell lines. Y-axis, mRNA quantities, relative to un-treated sample.

[0018] Figures 7A-7C illustrate MYC mRNA levels for DLBCL cell lines, $DoHH_2$, Karpas 422 and SU-DHL-2, after 2 hour exposure of 1 μM Formula 2 followed by wash-out.

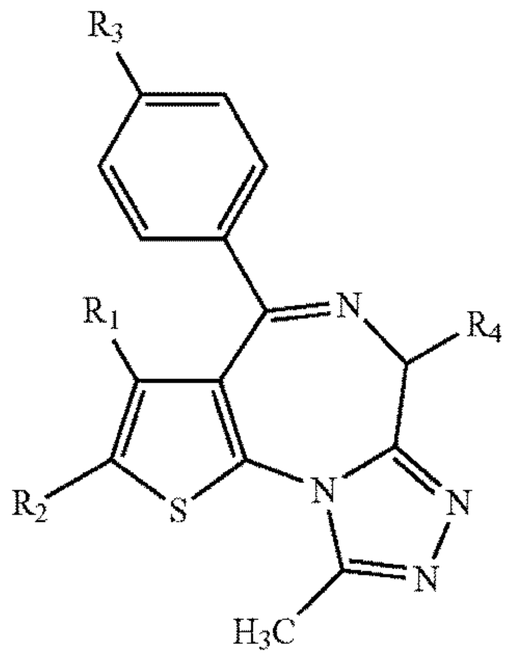
[0019] Figures 8A-8C illustrate the effect of Formula 2 on the proliferation of DLBCL cell lines, $DoHH_2$, U-2932 and SU-DHL-6, with time after 24 hour treatment with IC50 dose of Formula 2 followed by wash-out.

[0020] Figures 9A-9B illustrate NF•B targets mRNA levels (IRF4, A20, BIRC3) in ABC-DLBCL cell lines, SU-DHL-2 and U-2932, after increasing doses of Formula 2. X-axis, cell lines. Y-axis, fold change, relative to un-treated sample.

DETAILED DESCRIPTION OF THE INVENTION

[0021] In one embodiment, the invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers with a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound. In one such embodiment, the thienotriazolodiazepine compound is represented by the following Formula (1):

(I)



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, or a pharmaceutically acceptable salt thereof or a hydrate or solvate thereof. In one such embodiment, the patient is a human.

[0022] In one embodiment, the invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers with a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula (1) wherein the B-cell malignant cancers or T-cell malignant cancers is independently selected from Hodgkin's lymphoma or non-Hodgkin's lymphoma. In one such embodiment, the patient is a human.

[0023] In one such embodiment, the Hodgkin's lymphoma is independently selected from nodular sclerosis classical Hodgkin's lymphoma (CHL), mixed cellularity CHL, lymphocyte-depletion CHL, lymphocyte-rich CHL and nodular lymphocyte predominant Hodgkin's lymphoma.

[0024] In another such embodiment, the non-Hodgkin's lymphoma is indepenc AIDS-related lymphomas, anaplastic large-cell lymphoma, angioimmunoblastic lymphoma, blastic NK-cell lymphoma, chronic lymphocytic leukemia/small lymphocyti lymphoma, cutaneous T-cell lymphoma, diffuse large B-cell lymphoma, eteropathy-type T-cell lymphoma, follicular lymphoma, hepatosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, marginal zone lymphoma, nasal T-cell lymphoma, pediatric lymphoma, peripheral T-cell lymphomas, primary central nervous system lymphoma, T-cell leukemia, transformed lymphomas, treatment-related T-cell lymphoma and Waldenstrom's macroglobulinemia.

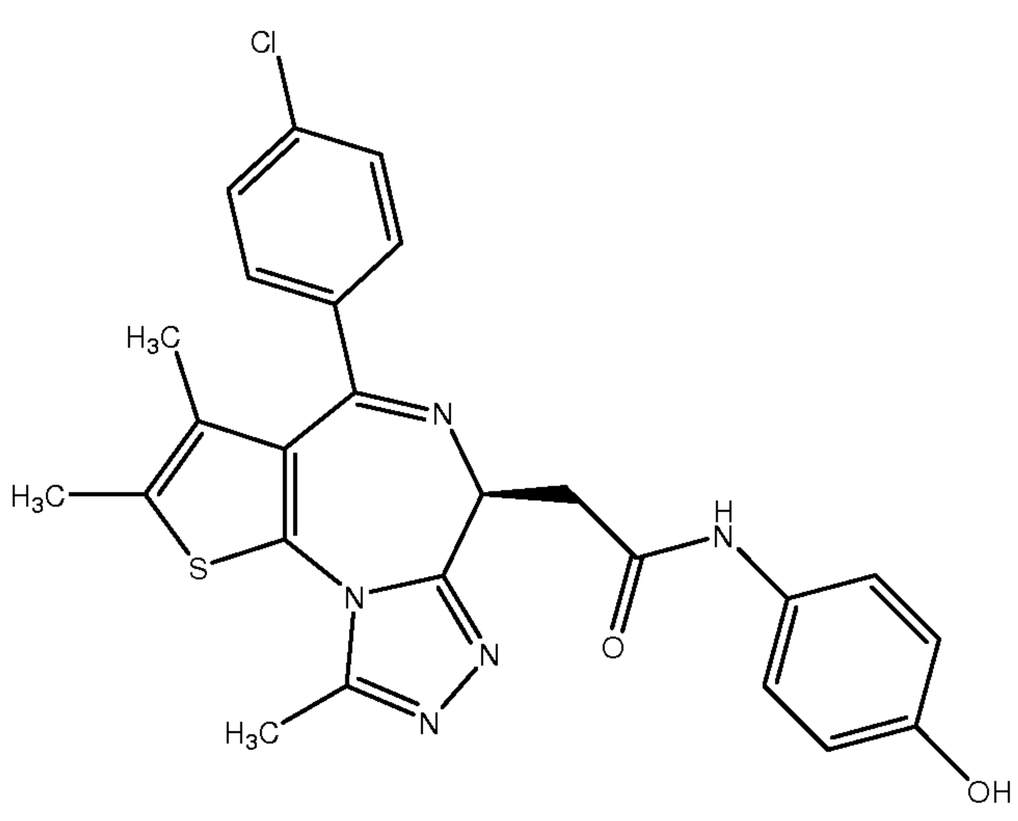
[0025] In one embodiment, the present invention provides for a method of treating diffuse large B-cell lymphoma by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1. In one such embodiment, the patient is a human.

[0026] In one embodiment, the present invention provides for a method of treating splenic marginal zone lymphoma in a patient by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1. In one such embodiment, the patient is a human.

[0027] In one embodiment, the present invention provides for a method of treating anaplastic large T-cell lymphoma by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1. In one such embodiment, the patient is a human.

[0028] In another embodiment the invention comprises a method of treating B-cell malignant cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 1 that 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, the patient is a human.

[0029] In another embodiment the invention comprises a method of treating B-cancers or T-cell malignant cancers by administering to a patient a pharmaceutically acceptable amount of a composition comprising (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 (Formula 2), also known as Y-803 and OTX-015:



Formula (2)

In one such embodiment, the patient is a human.

[0030] In one embodiment, the invention provides for a method of treating B-cell malignant cancers or T-cell malignant cancers with a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula (2) wherein the B-cell malignant cancers or T-cell malignant cancers is independently selected from Hodgkin's lymphoma or non-Hodgkin's lymphoma. In one such embodiment, the patient is a human.

[0031] In one such embodiment, the Hodgkin's lymphoma is independently selected from nodular sclerosis classical Hodgkin's lymphoma (CHL), mixed cellularity CHL, lymphocyte-depletion CHL, lymphocyte-rich CHL and nodular lymphocyte predominant Hodgkin's lymphoma.

[0032] In another such embodiment, the non-Hodgkin's lymphoma is independently selected from AIDS-related lymphomas, anaplastic large-cell lymphoma, angioimmunoblastic lymphoma, blastic NK-cell lymphoma, chronic lymphocytic leukemia/small lymphocyti lymphoma, cutaneous T-cell lymphoma, diffuse large B-cell lymphoma, eteropathy-type T-cell lymphoma, follicular lymphoma, hepatosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, marginal zone lymphoma, nasal T-cell lymphoma, pediatric lymphoma, peripheral T-cell lymphomas, primary central nervous

system lymphoma, T-cell leukemia, transformed lymphomas, treatment-related T and Waldenstrom's macroglobulinemia.

[0033] In another embodiment, the invention comprises a method of treating diffuse large B-cell lymphoma by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 2. In one such embodiment, the patient is a human.

[0034] In one embodiment, the present invention provides for a method of treating splenic marginal zone lymphoma by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 2. In one such embodiment, the patient is a human.

[0035] In one embodiment, the present invention provides for a method of treating anaplastic large T-cell lymphoma by administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound represented by Formula 2. In one such embodiment, the patient is a human.

[0036] The preparation of the compounds represented by Formula 1 and Formula 2 can be accomplished by chemical synthesis by those of ordinary skill in the art according to the methods previously described in the art, including those described in U.S. Patent No. 5,712,274, which is incorporated by reference here in its entirety.

[0037] The compounds represented by Formula 1 or Formula 2 can be mixed with pharmaceutically-acceptable carriers for oral delivery. The carriers can include binders, lubricants, disintegrants, and other functional and non-functional excipients.

[0038] The dose of the compounds represented by Formula 1 or Formula 2 can be determined by one of ordinary skill in the art by taking into consideration the body mass, age, health condition, diet, and other relevant factors presented by a patient as well as the bioavailability of Formula 1 or Formula 2 and the Formula 1 or Formula 2 product formulation. In one embodiment, the oral dose of compound of Formula 1 or Formula 2 may range from 40 to 100 mg.

[0039] The invention is further described by the following non-limiting examples, which illustrate the unexpected results of the methods of treatment.

EXAMPLES

[0040] EXAMPLE 1: EFFECTS OF FORMULA 2 ON LYMPHOMA CELL PROLIFERATION

[0041] The antiproliferative activity of Formula 2 was evaluated in twenty-two (22) diffuse large B-cell lymphoma (DLBCL), eight (8) anaplastic large T-cell lymphoma (ALCL), four (4) mantle cell lymphoma (MCL), and three (3) splenic marginal zone lymphoma (SMZL) established human cell lines. Cell lines were cultured in RPMI-1640 (GIBCO Invitrogen, Basel, Switzerland) or DMEM (GIBCO Invitrogen, Basel, Switzerland) medium supplemented with 10% fetal calf serum, 1% L-glutamine, and penicillin-streptomycin-neomycin (~5,000 units penicillin, 5 mg/mL streptomycin, 10 mg/mL neomycin).

[0042] For proliferation assays, cells were seeded into 96-well plates at a density of 10^4 cells per well. Formula 2 (OncoEthix SA, Lausanne, Switzerland) was dissolved in DMSO as a stock solution of 10 mM, then divided into aliquots and stored at -80°C. For each experiment, an aliquot of the stock solution was thawed, diluted serially into culture medium, and used within 2 to 3 days. Cells were treated with DMSO (control) or increasing doses of Formula 2 (in five replicates) for 72 hours at 37°C. To detect cell proliferation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Buchs, Switzerland) was prepared as a stock solution of 5 mg/mL in phosphate-buffered saline (PBS) and filter-sterilized. An amount of MTT solution equal to 0.5 mg/mL was then added to each well and incubated in the dark at 37°C for 4 hours. Cells were then lysed with 25% sodium dodecylsulfate (SDS) lysis buffer and absorbance was read at 570 nm on a Beckman-Coulter AD340 instrument. The doses corresponding to the concentration that produced 50% growth inhibition (GI_{50}) were estimated by fitting a sigmoidal model through the dose response curve using the R statistical package (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria).

[0043] Results, summarized in Table 1, showed that 68% (15/22) of DLBCL lines, 100% (3/3) of SMZL lines, 62% (5/8) of ALCL lines, but no (0/3) MCL lines were sensitive to growth inhibition by Formula 2, where sensitivity was defined by $GI_{50} < 500$ nM. Of interest, there was no apparent difference in sensitivity between cell lines derived from DLBCL of the germinal center type (GBC-DLBCL) and those derived from DLBCL of the activated B-cell like type (ABC-DLBCL).

[0045] Table 1: Effects of Compound 2 on Proliferation of Human Lymphoma

Lymphoma Subtype	Cell Line	GI_{50} (nM)	Cell Death, basal/treated (%)	Lymphoma Subtype	Cell Line	GI_{50} (nM)
Diffuse large B-cell lymphoma, activated B-cell like subtype (ABC-DLBCL)	SU-DHL-2	69	23 / 87	Anaplastic large T-cell lymphoma (ALCL)	L82	36
	TMD8	131	51 / 75		FE-PD	158
	OCI-Ly3	179	7 / 49		MAC1	311
	U2932	202	7 / 5		Karpas 299	411
	OCI-Ly10	380	7 / 4		SUPM2	546
	HBL1	704	2 / 2		T5	1173
	RIVA	2280	6 / 11		JB6	1944
	RCK8	>10,000	12 / 10		SU-DHL-1	9109
Diffuse large B-cell lymphoma, germinal center subtype (GBC-DLBCL)	SU-DHL-10	77	26 / 32			
	DoHH2	90	16 / 11	Mantle cell lymphoma (MCL)	Rec-1	1224
	OCI-Ly2	92	6 / 6		MAVER-1	1224
	SU-DHL-6	110	9 / 10		Jeko-1	2787
	SU-DHL-7	132	5 / 9		Granta 519	>10,000
	OCI-Ly19	170	14 / 14			
	SU-DHL-5	189	1 / 6	Splenic marginal zone lymphoma (SMZL)	V151	105
	Karpas 422	277	13 / 5		K1718	165
	OCI-Ly8	527	15 / 13		SSK41	240
	WSU-DLCL2	552	2 / 1			
	SU-DHL-4	607	5 / 9			
	OCI-Ly7	1387	14 / 27			
	OCI-Ly1	1550	1 / 1			
	VAL	>10,000	7 / 17			

GI_{50}: Concentration which inhibited proliferation of 50% of cells

[0046] To examine the possible cytotoxic effect of Formula 2 on DLBCL cell lines, the degree of cell death was evaluated after exposure to the compound for 72 hours at doses ranging from 100 to 1500 nM (covering the range of GI_{50} values). To assess for cell death, cells were treated with

DMSO or different doses of Formula 2 for 72 hours, harvested, washed once with stained with propidium iodide (1 µg/mL, Sigma) in PBS. Absorbance was read at 535 nm on a Beckman-Coulter AD340 instrument. The analysis of percentage of cell death was performed using CellQuest Pro software (Becton Dickinson). Results (Table 1) showed that Formula 2 induced cell death in only a small percentage of DLBCL lines with low GI_{50} values (SU-DHL-2, TMD8, OCI-Ly3).

[0047] Additionally, the effect of Formula 2 on induction of apoptosis was evaluated in four DLBCL cell lines (Karpas 422, SU-DHL-2, SU-DHL-6, U2932) and four ALCL cell lines (FE-PD, K299, L82, SU-DHL-1). Cells were treated with DMSO (control) or different doses of Formula 2 for 72 hours, then stained with Click-iT EdU Flow Cytometry Assay Kits (Invitrogen) and 7-ADD (BD Pharmingen) and analyzed for DNA content using a FACScan Flow Cytometer. The analysis of percentage apoptosis was performed using FlowJo 7.6.3 software (Cytek Development, Fremont, California, USA). Results showed no induction of apoptosis in either DLBCL or ALCL cell lines.

[0048] Since Formula 2 did not induce massive cell death or apoptosis despite markedly reducing cell viability, the effect of Formula 2 on cell cycle was evaluated in 5 DLBCL cell lines (DohH2, Karpas 422, SU-DHL-6, VAL, U-2932). Cells were treated with DMSO (control) or different doses of Formula 2 for 24 hours, then harvested, washed once in PBS and fixed in 80% ethanol at 4°C for at least one hour. Fixed cells were stained with propidium iodide (50 µg/mL, Sigma) in PBS containing RNase-A (75 kU/mL, Sigma) and analyzed for DNA content using a FACScan flow cytometer (Becton Dickinson). Cell cycle analysis was performed using the ModFit LT software package (Verity Software House, Inc., Topsham, Maine, USA). As illustrated in Figure 1, the results showed that Formula 2 induced G1-arrest in a dose-dependent manner.

[0049] Because Formula 2 induced a marked decrease in cell viability and G1-arrest without induction of apoptosis, induction of cell senescence was evaluated in a representative DLBCL and ALCL cell line. Cells were treated with Formula 2 for 48 hours and then stained for galactosidase. As illustrated in Figure 2, the results showed a marked increase in senescent cells in both the DLBLC and ALCL cell lines suggesting that Formula 2 has mainly a cytostatic effect on lymphoma cells.

[0050] EXAMPLE 2: EFFECTS OF FORMULA 2 ON DOWN-REGULATION OF c-MYC ONCOGENE

[0051] Formula 2 previously has been shown to be a competitive inhibitor of tl bromodomain proteins 2, 3, and 4 (BRD2, BRD3, BRD4) as disclosed in U.S. Patent Appl. Publ. No. 20100286127 which is incorporated by reference in its entirety herein. Inhibition of BRD4 has been shown to result in down-regulation of the c-MYC oncogene *[Delmore JE, Issa GC, LemieuxME, et al: BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011; 146:1-14]*. Accordingly, basal levels of BRD2, BRD3, and BRD4 mRNA and protein and effects of Formula 2 on c-MYC mRNA and protein levels were evaluated in selected DLBCL and ALCL cell lines.

[0052] To assess protein levels, Western blotting analysis was performed as follows. Cells were solubilized in hot SDS lysis buffer (2.5% SDS in pH 7.4 Tris-HCl) and sonicated for 15 seconds. The protein content in the different samples was determined using a bicinchonininic acid (BCA) protein assay (Pierce Chemical Co., Rockford, Illinois, USA). Lysates (40 µg) were fractionated by SDS-PAGE using 8% polyacrylamide gels, based upon the expected molecular weight. The resolved proteins were blotted onto a nitrocellulose membrane by electric transfer. The membranes were blocked in TBS-T buffer (20 mM Tris-HCl, pH 7.6 containing 137 mM NaCl, 0.1% Tween 20, and 5% bovine serum albumin) for one hour. Membranes were then incubated overnight with primary antibodies diluted in TBS-T. The following antibodies were used: anti-BRD2 (ab37633, AbCam, Cambridge, UK), anti-BRD3 (ab56342, AbCam), anti-BRD4 (ab75898, AbCam), and anti-GAPDH (MAB374, Millipore, Billerica, Massachusetts, USA). Membranes were washed in TBS-T three times for ten minutes each and then incubated for one hour in TBS-T containing the appropriate horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies (Amersham Life Sciences, Arlington Heights, Massachusetts, USA). The membranes were washed three time in TBS-T for ten minutes each and then processed for enhanced chemiluminescence detection according to the manufacturer's instructions (Amersham Life Science). Equal loading of samples was confirmed by probing for GAPDH.

[0053] mRNA analysis was performed as follows. RNA was extracted from cells using the RNA easy kit (Qiagen AG, Hombrechtikon, Switzerland). The concentration of total RNA was determined spectrophotometrically at 260 nm using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Deleware, USA). One microgram of total RNA was reverse-transcribed using the Superscript First-Strand Synthesis System for real-time PCR kit (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. PCR amplification was performed using the

Fast SYBR Green Master Mix on a StepOnePlus real-time PCR System (Applied City, California, USA). Primer sets for BRD2, BRD3, and BRD4 (Table 2) were designed using the Primer3 software package (Rozen S, Skaletsky H: Primer3 on the WWW for general users and for biologist programmers. In: Misener S, Krawetz SA (Eds) Methods in Molecular Biology, Vol 132: Bioinformatics Methods and Protocols. Towota, New Jersey, USA; Humana Press, Inc., 2000, pp 365-386) and primer sets for c-MYC were from published studies. All samples were analyzed in triplicate. The relative quantity of the specific mRNA for each sample was calculated based on mean cycle threshold (Ct) values using the delta-delta Ct with a correction for experimental variations by normalization to the housekeeping gene GAPDH.

[0054] Table 2. Sequences of Used Primers

BRD2-F	5'-ACTTGGCCTGCATGACTACC-3'
BRD2-R	5'-CTGTAGCTTTCGTGCCATTG-3'
BRD3-F	5'-CAACCATCACTGCAAACGTC-3'
BRD3-R	5'-GGGAGTGGTTGTGTCTGCTT-3'
BRD4-F	5'-AGTCATCCAGCACCACCATT-3'
BRD4-R	5'-TCTTAGGCTGGACGTTTTGC-3'
MYC-F	5'-GGTGCTCCATGAGGAGACA-3'
MYC-R	5'-CCTGCCTCTTTTCCACAGAA-3'

[0055] Results showed that basal levels of BRD2 mRNA and protein varied widely among DLBCL cell lines and ALCL cell lines, as illustrated in Figures 3 and 4, respectively, while all cell lines tested had low levels of BRD4 mRNA and protein and only trace levels of BRD3 mRNA and protein. Interestingly, basal levels of BRD2 mRNA and protein were not correlated with sensitivity to growth inhibition by Formula 2. Among the DLBCL lines tested, similar GI_{50} values were obtained for the line with the highest BRD2 mRNA levels (SU-DHL-6 GI_{50} = 110 nM) and lowest BRD2 mRNA levels (DoHH2 GI_{50} = 90 nM). Similarly, among the ALCL lines tested similar GI_{50} values were obtained for the line with the highest BRD2 mRNA levels (L82 GI_{50} = 36 nM) and lowest BRD2 mRNA levels (FE-PD GI_{50} = 158 nM).

[0056] Results of mRNA analysis showed that 24 hour exposure to Formula 2 : down-regulation of c-MYC mRNA in five of six (5 of 6) DLBCL cell lines tested and four of four (4 of 4) ALCL cell lines tested, as illustrated in Figures 5 and 6, respectively. Interestingly, the DLBCL line that showed minimal down-regulation of c-MYC was very sensitive growth inhibition by Formula 2 (SU-DHL-6 GI_{50} = 110 nM).

[0057] To evaluate whether the Formula 2-induced down-regulation of c-MYC was reversible, three DLBCL lines (DoHH2, Karpas 422, SU-DHL-2) were treated with Formula 2 for two hours and then the medium containing Formula 2 was changed to a medium not containing Formula 2 ("wash-out"). Following wash-out, a time-dependent restoration of c-MYC mRNA expression was observed in all three cell lines, with different kinetics, as illustrated in Figure 7. In a related experiment, DLBCL cells treated with Formula 2 at the GI_{50} concentration for 24 hours started to re-grow after "wash out" as illustrated in Figure 8.

[0058] EXAMPLE 3: EFFECTS FOR FORMULA 2 ON DOWN-REGUATION OF NFκB

[0059] Formula 2 previously has been shown to be a competitive inhibitor of the BET bromodomain proteins 2, 3, and 4 (BRD2, BRD3, BRD4), as disclosed in U.S. Patent Appl. Publ. No. 20100286127, and BRD4 has been reported to be involved in the regulation of the transcription factor NFκB, which can act as a tumor suppressor in certain settings *[Huang B, Yang XD, Zhou MM, Ozato K, Chen LF: Brd4 coactivates transcriptional activation of NF-κB via specific binding to acetylated RelA. Mol Cell Biol 2009; 29:1375-1387]*. Accordingly, effects of Formula 2 on mRNA expression of NFκB targets (IRF4, A20, BIRC3) were evaluated in five DLBCL ($DoHH_2$, Karpas 422, SU-DHL-2, SU-DHL-6, U2932) cell lines. Results showed that Formula 2 induced down-regulation of NFκB targets; representative results are shown in Figure 9.

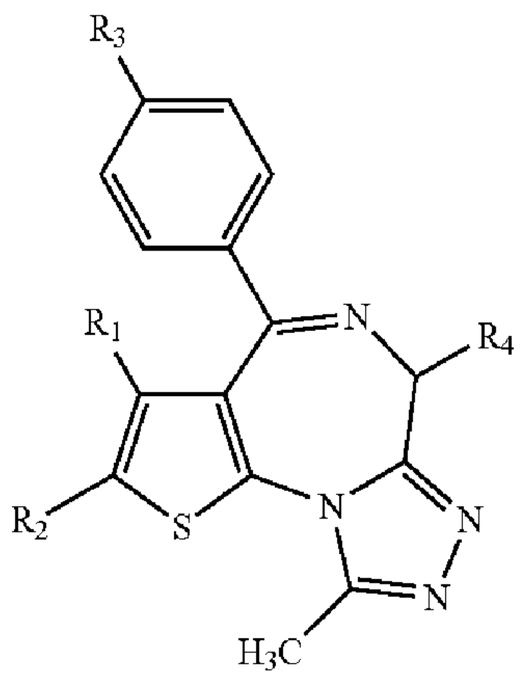
The present disclosure may be embodied in other specific forms without departing from the spirit or essential attributes of the disclosure. Accordingly, reference should be made to the appended claims, rather than the foregoing specification, as indicating the scope of the disclosure. Although the foregoing description is directed to the preferred embodiments of the disclosure, it is noted that other variations and modification will be apparent to those skilled in the art, and may be made without departing from the spirit or scope of the disclosure.

What is claimed is:

1. A method of treating B-cell malignant cancer or T-cell malignant cancer comprising:

administering to a patient a pharmaceutically acceptable amount of a composition comprising a thienotriazolodiazepine compound, said thienotriazolodiazepine compound being represented by the following Formula (1):

(I)



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, or a pharmaceutically acceptable salt thereof or a hydrate or solvate thereof.

2. The method of claim 1, wherein the patient is a human.

3. The method of claim 1, wherein the B-cell malignancy is diffuse large B-cell ly

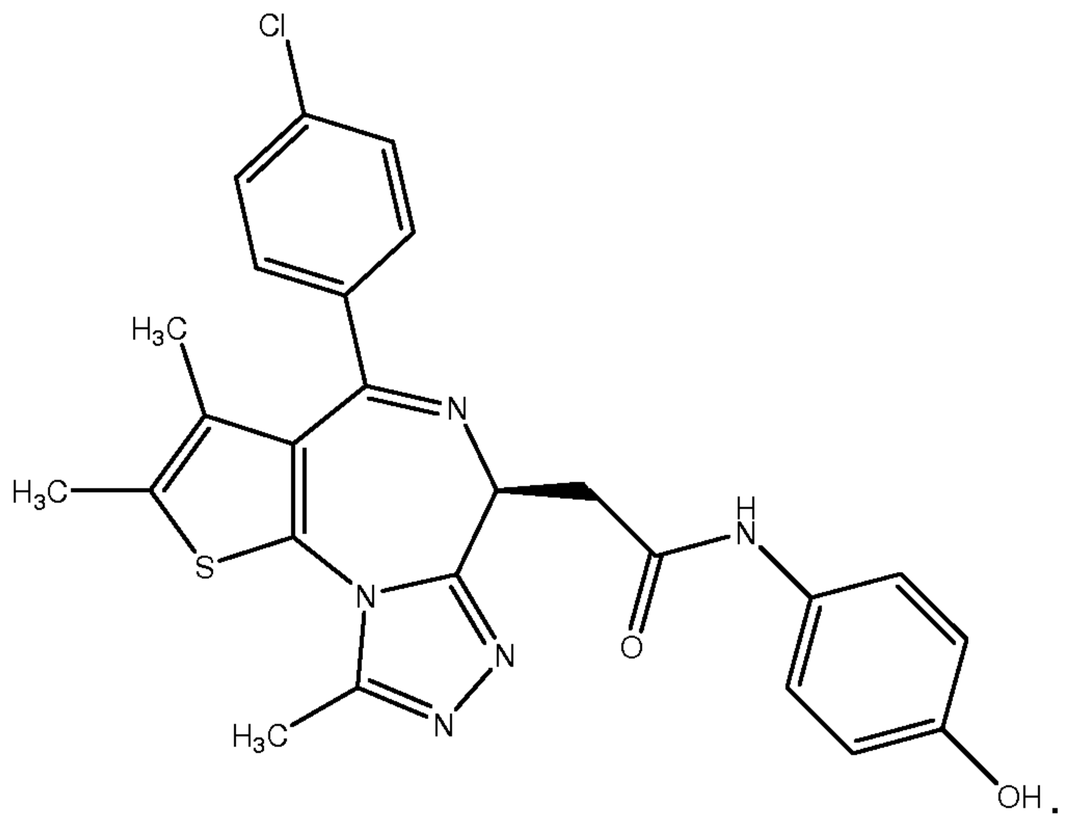
4. The method of claim 1, wherein the B-cell malignant cancer is splenic marginal zone lymphoma.

5. The method of claim 1, wherein the T-cell malignant cancer is anaplastic large T-cell lymphoma.

6. The method of claim 1, wherein the thienotriazolodiazepine compound represented by Formula 1 is independently 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.

7. The method of claim 1, wherein the thienotriazolodiazepine compound 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 represented by the following Formula (2)



8. The method of claim 7, wherein the patient is a human.

9. The method of claim 7, wherein the B-cell malignancy is diffuse large B-cell lymphoma.

10. The method of claim 7, wherein the B-cell malignant cancer is splenic marginal zone lymphoma.

11. The method of claim 7, wherein the T-cell malignant cancer is anaplastic large lymphoma.

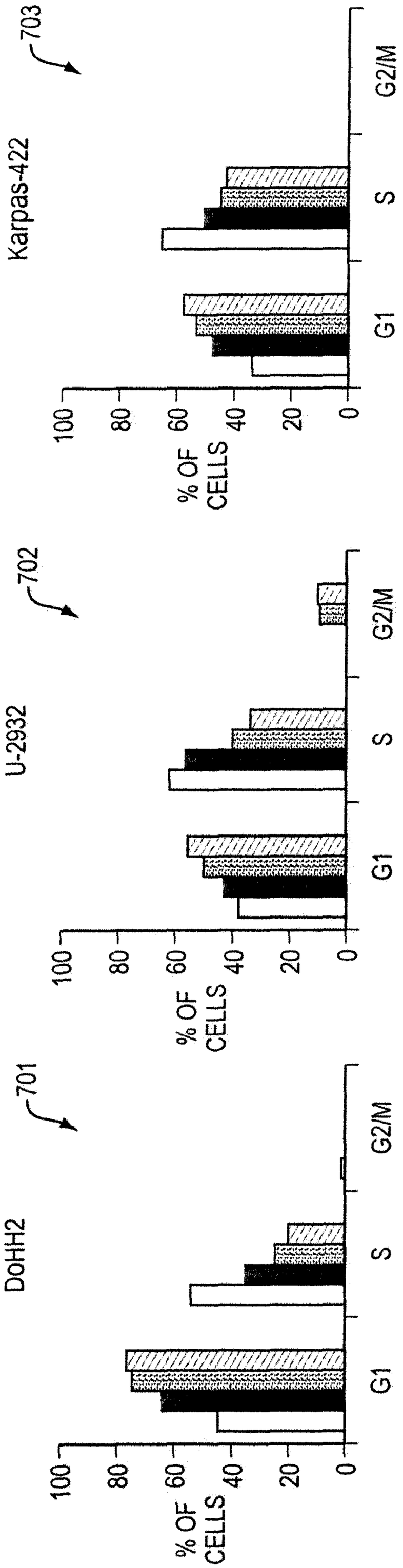
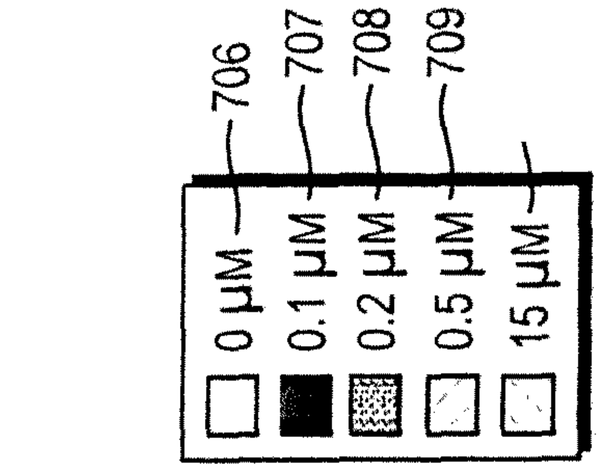


FIG. 1A

FIG. 1B



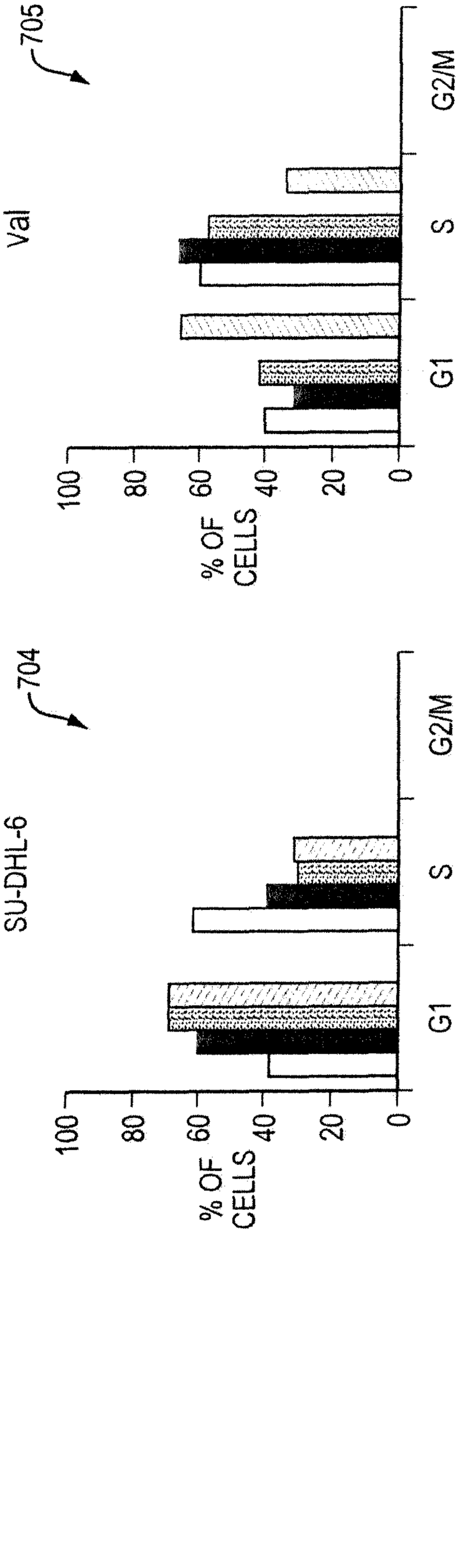


FIG. 1D

FIG. 1E

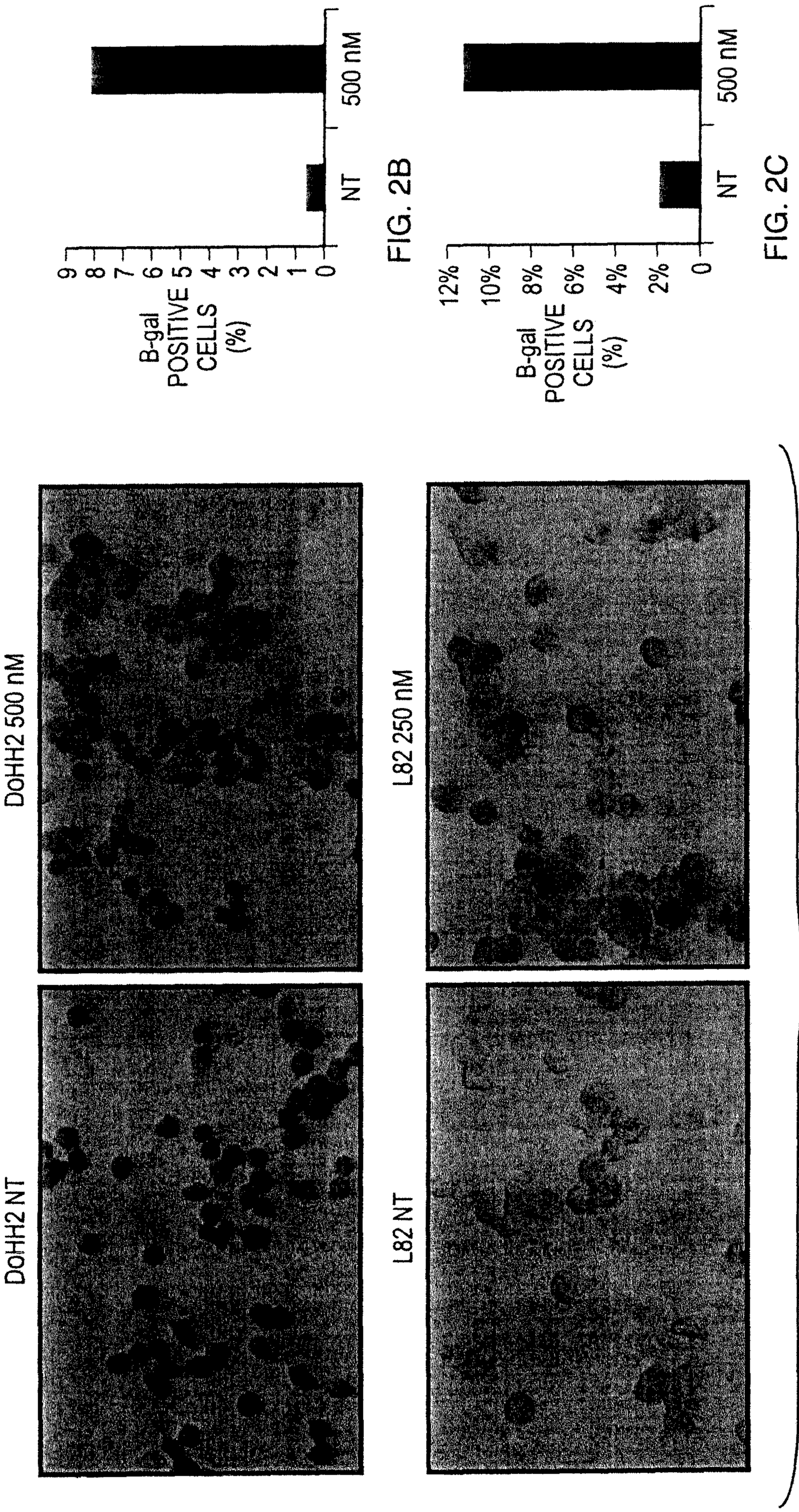


FIG. 2A

FIG. 2B

FIG. 2C

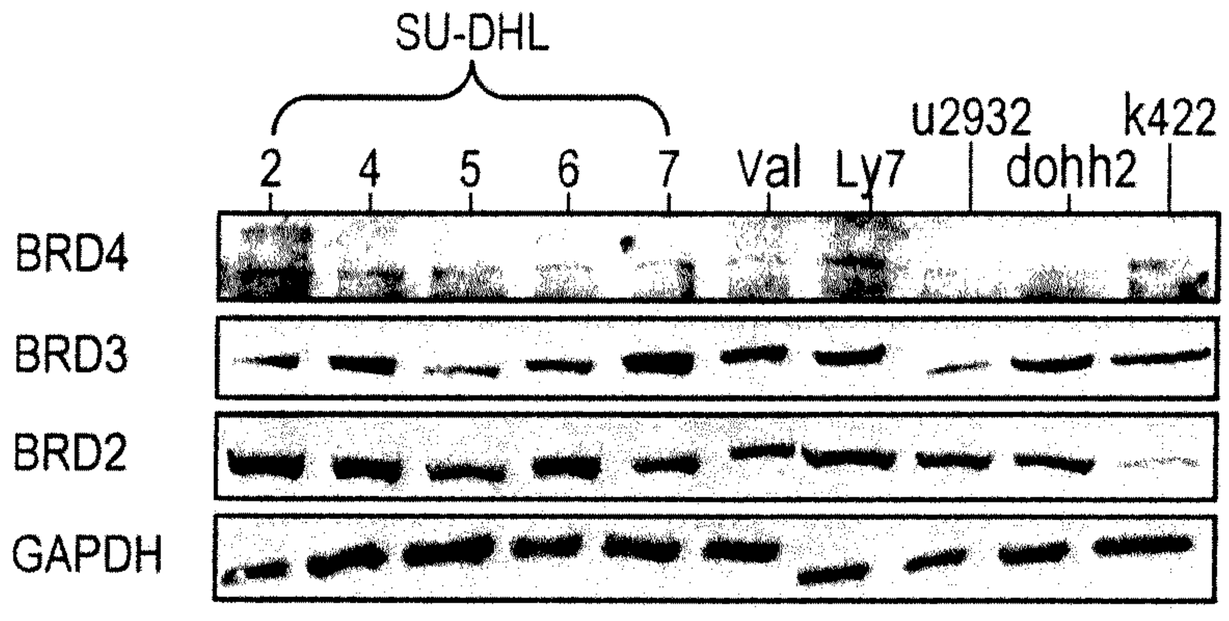

SU-DHL
2
4
5
6
7
Val
Ly7
u2932
k422
dohh2
BRD4
BRD3
BRD2
GAPDH

FIG. 3A

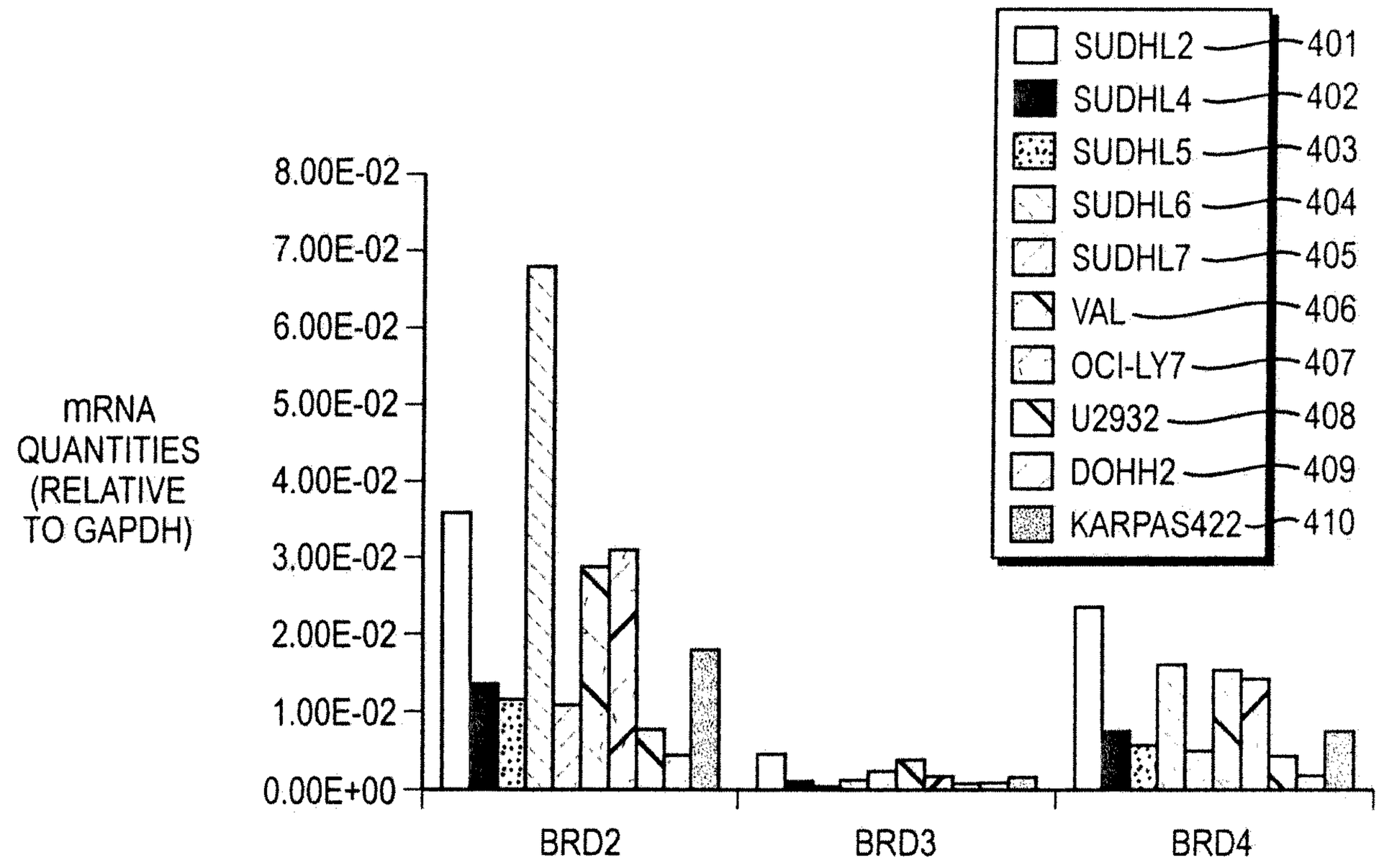
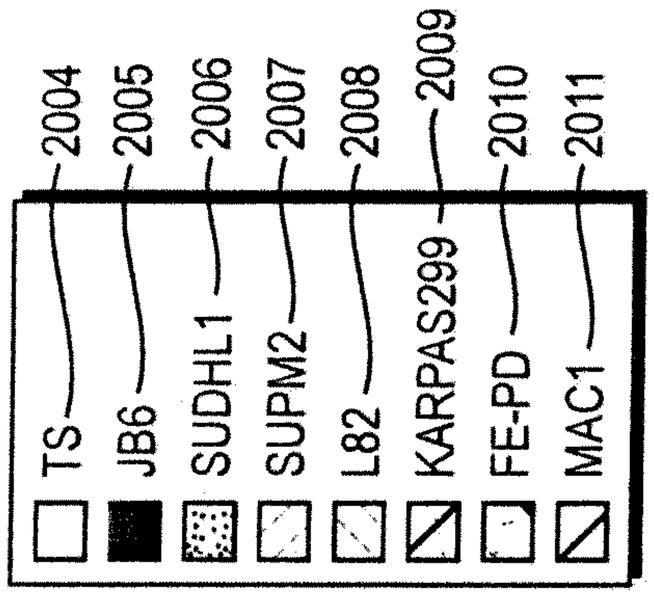

mRNA QUANTITIES (RELATIVE TO GAPDH)
8.00E-02
7.00E-02
6.00E-02
5.00E-02
4.00E-02
3.00E-02
2.00E-02
1.00E-02
0.00E+00
SUDHL2
SUDHL4
SUDHL5
SUDHL6
SUDHL7
VAL
OCI-LY7
U2932
DOHH2
KARPAS422
401
402
403
404
405
406
407
408
409
410
BRD2
BRD3
BRD4

FIG. 3B


TS
JB6
SUDHL1
SUPM2
L82
KARPAS299
FE-PD
MAC1
2004
2005
2006
2007
2008
2009
2010
2011

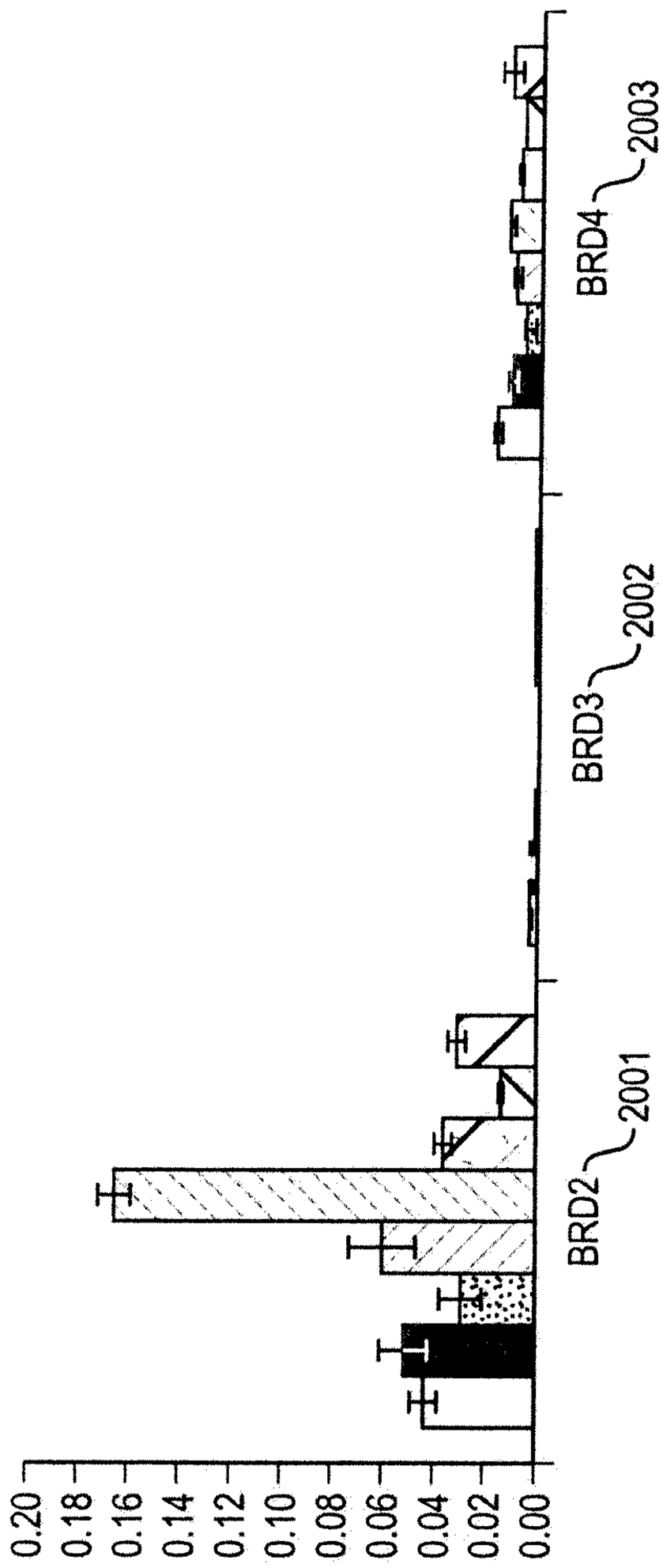

mRNA QUANTITIES (RELATIVE TO GAPDH)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
BRD2
BRD3
BRD4
2001
2002
2003

FIG. 4A

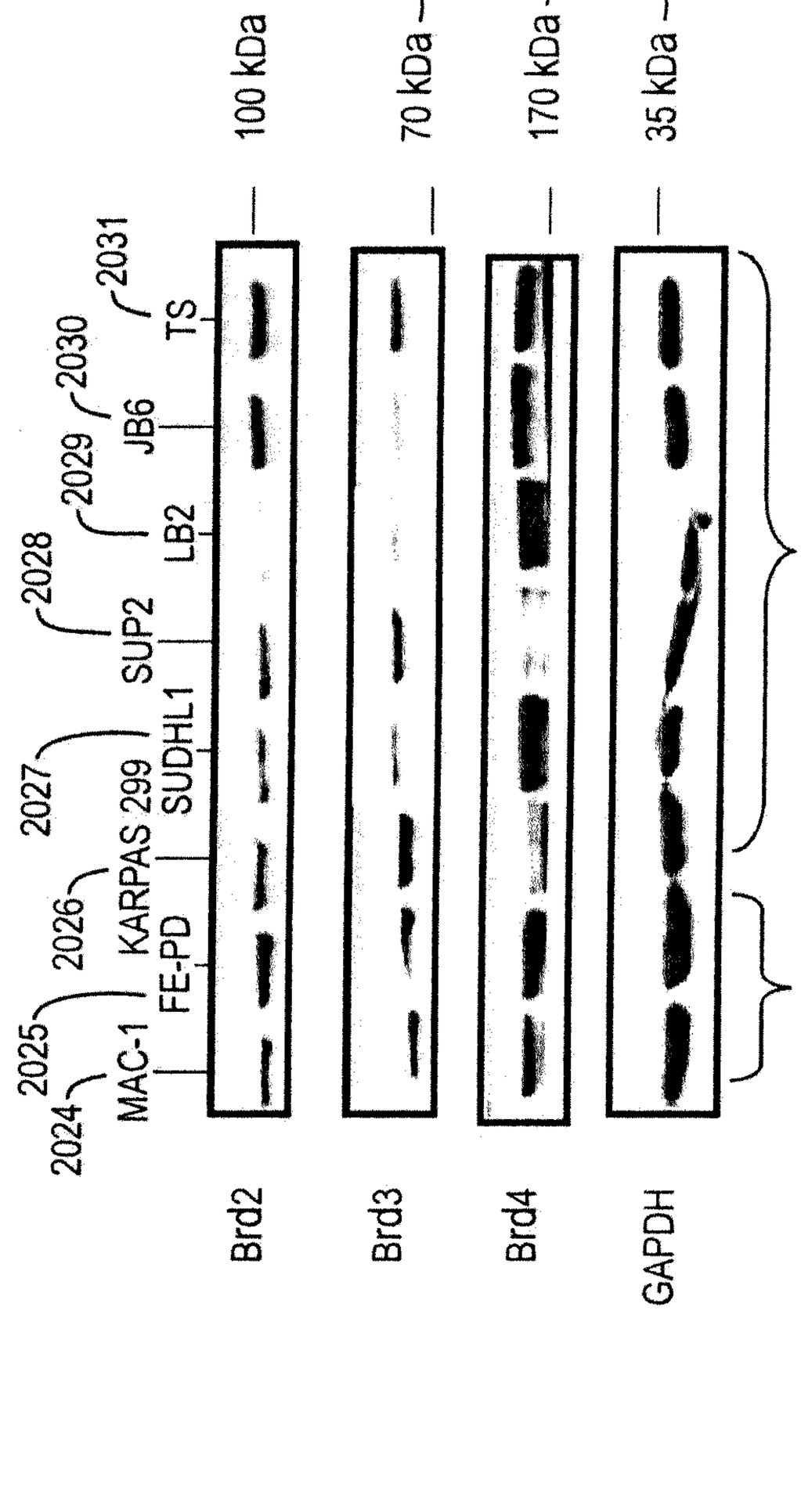

Brd2
Brd3
Brd4
GAPDH
2024
2025
2026
2027
2028
2029
2030
2031
MAC-1
FE-PD
KARPAS 299
SUDHL1
SUP2
LB2
JB6
TS
100 kDa
70 kDa
170 kDa
35 kDa
ALKneg
ALKpos

FIG. 4B

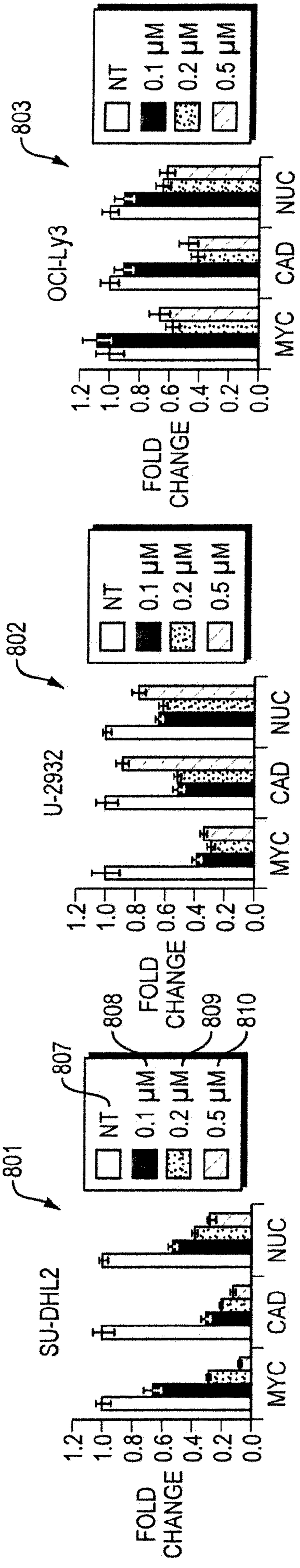


FIG. 5C

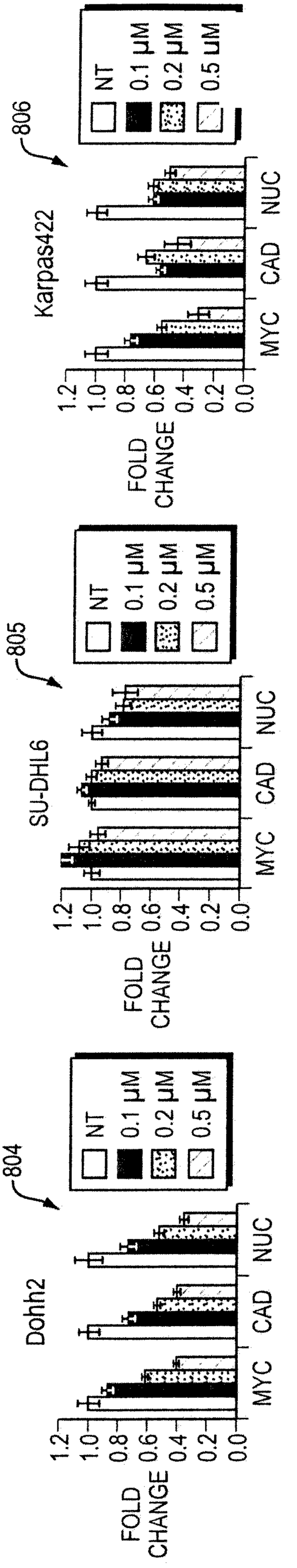


FIG. 5F

FIG. 5B

FIG. 5E

FIG. 5A

FIG. 5D

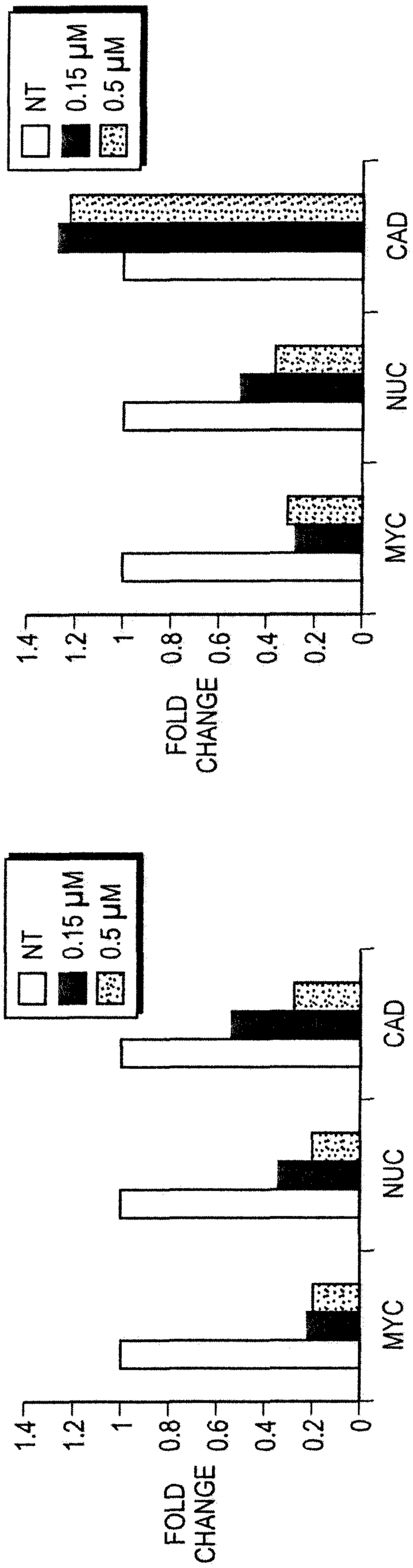


FIG. 6B

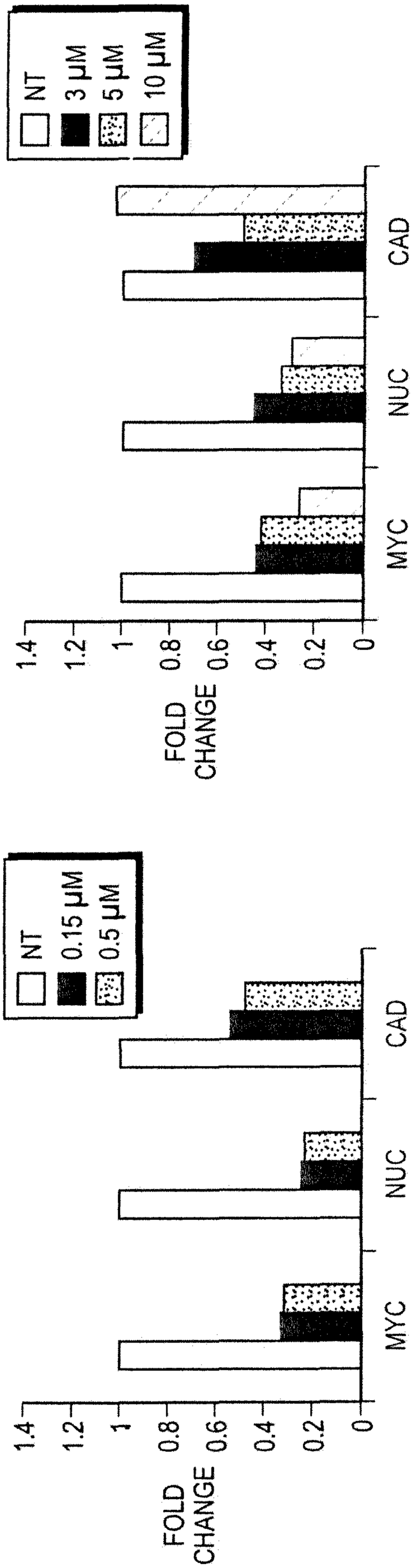


FIG. 6D

FIG. 6A

FIG. 6C

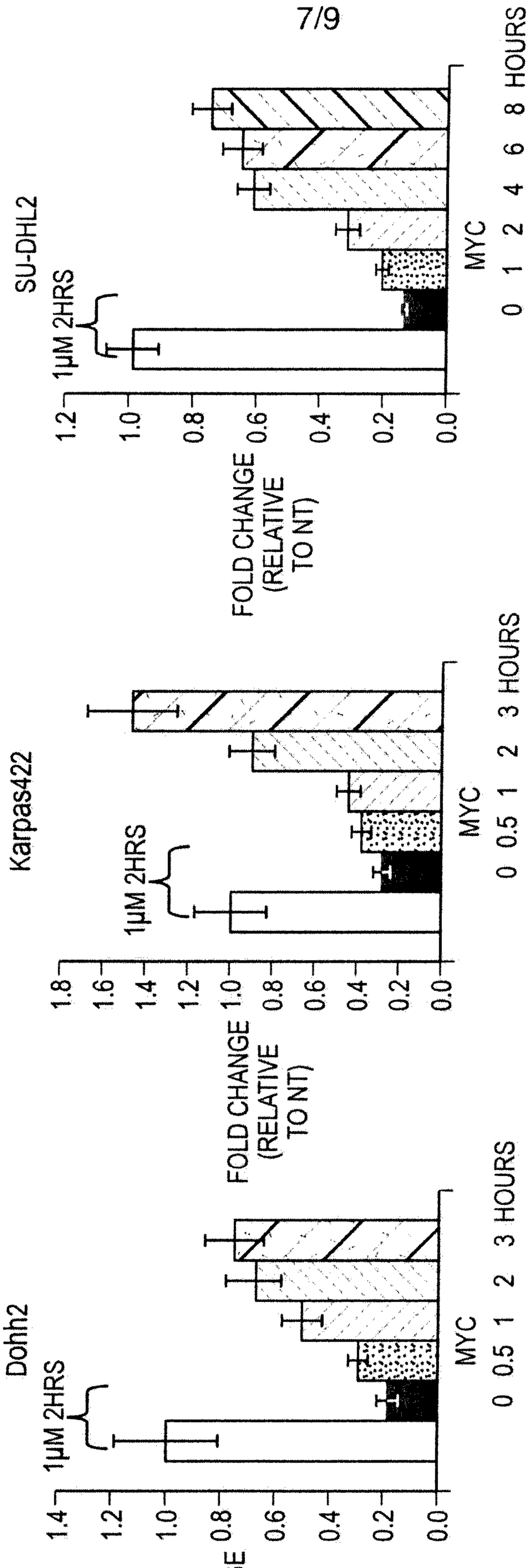


FIG. 7C

FIG. 7B

FIG. 7A

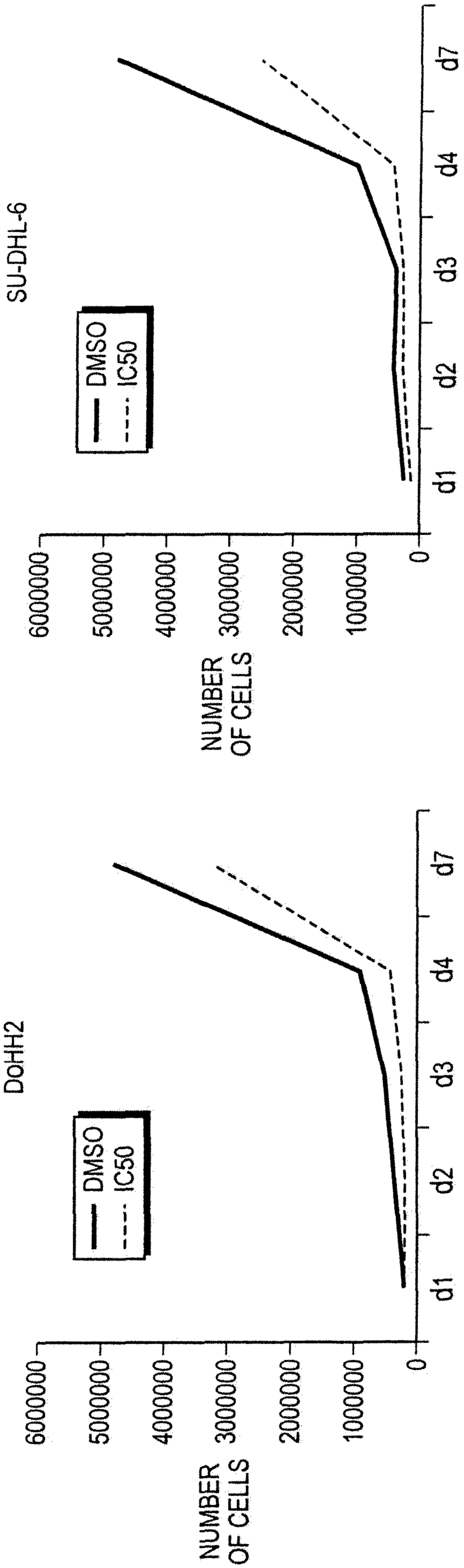


FIG. 8B

FIG. 8C

FIG. 8A

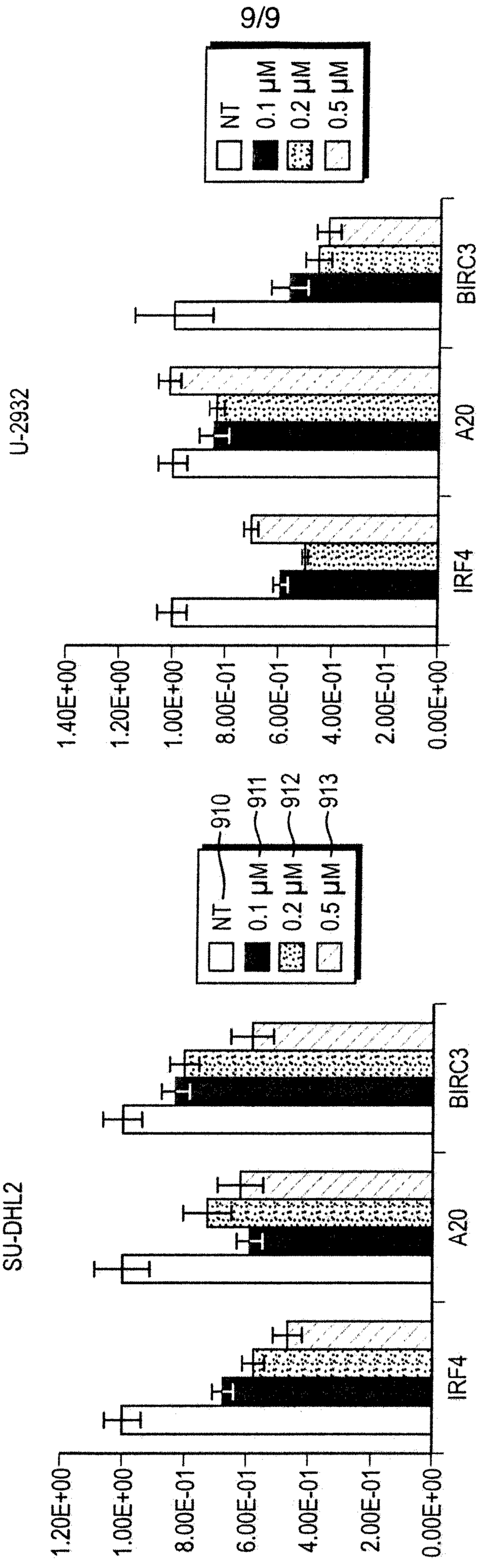

NT
0.1 µM
0.2 µM
0.5 µM
BIRC3
A20
IRF4
U-2932
1.40E+00
1.20E+00
1.00E+00
8.00E-01
6.00E-01
4.00E-01
2.00E-01
0.00E+00
910
911
912
913
SU-DHL2

FIG. 9B

FIG. 9A