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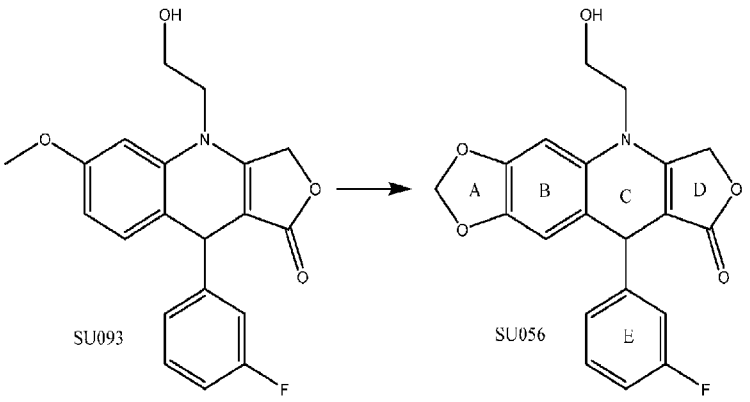
(54) Title: Y BOX BINDING PROTEIN 1 INHIBITORS

FIG. 1A



(57) Abstract: Provided herein are novel azopodophyllotoxin analog compounds, pharmaceutical compositions comprising them, and their use as inhibitors of Y box protein 1 (YB1 or YBX1) in treatments for conditions including gynecological, breast, bladder, kidney, head and neck, neuronal, and prostate cancers, lymphomas, and leukemias. Methods of their use in sensitizing resistant cancers to treatment with anticancer agents and radiation are also provided.

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[Continued on next page]

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Y BOX BINDING PROTEIN 1 INHIBITORS

FIELD OF THE INVENTION

The present invention concerns novel compounds, pharmaceutical compositions, and their use as inhibitors of Y box protein 1 (YB1 or YBX1) to treat conditions including gynecological, breast, bladder, kidney, head and neck, neuronal, and prostate cancers, lymphomas, and leukemias.

BACKGROUND OF THE INVENTION

Y box binding protein 1 (YB-1) is a multifunctional protein associated with tumor progression and the emergence of treatment resistance (TR). Here, we report an azopodophyllotoxin small molecule, SU056, that potently inhibits tumor growth and progression *via* YB-1 inhibition. This first-in-class YB-1 inhibitor inhibits cell proliferation, resistance to apoptosis in ovarian cancer (OC) cells and arrests in the G1 phase. Inhibitor treatment leads to enrichment of proteins associated with apoptosis and RNA degradation pathways while downregulating spliceosome pathway. *In vivo*, SU056 independently restrains OC progression and exerts a synergistic effect with paclitaxel to further reduce disease progression with no observable liver toxicity. Moreover, *in vitro* mechanistic studies showed delayed disease progression *via* inhibition of drug efflux and multi-drug resistance 1 (MDR1), and significantly lower neurotoxicity as compared to Etoposide. These data suggest that YB-1 inhibition may be an effective strategy to reduce OC progression, antagonize TR, and decrease patient mortality.

Y box binding protein 1 (YB-1, YBX1) is a multifunctional cold shock protein that binds to DNA and RNA. It regulates DNA and RNA associated cellular events including mRNA transcription, splicing, packaging, stability, and translation (Lyabin et al., 2014). mRNA stabilization is an important event for sustained expression of any gene and YB-1 robustly stabilizes the mRNA via blocking the 5' end from mRNA degradation (Evdokimova et al., 2001). It was first described by Didier et al. as a negative regulator of the MHC class II molecule (Didier et al., 1988). The oncogenic role of YB-1 is well-characterized in many cancers and its amplified levels have been found in a large number of cancer

cases (Goodarzi et al., 2015). It increases the stability of short-lived mRNAs for multiple oncogenic proteins including c-myc (Laird-Offringa et al., 1990), c-fos (Blattner et al., 2000), cyclin B1 (Maity et al., 1995), HIF1α (Goodarzi et al., 2015), Snail (Evdokimova et al., 2009), and MDR1 (Bargou et al., 1997), which are associated with disease progression and treatment resistance. Genetic knockdown studies have demonstrated that inhibition of YB-1 significantly arrests proliferation and induces apoptosis in many cancer models, demonstrating its essential role in disease progression (Evdokimova et al., 2009, El-Naggar et al., 2015). YB-1 is associated with the development of treatment resistance (TR) *via* its role in activating proliferation, promoting cancer cell stemness, responding to growth factors, cytokines, cellular stress responses, and promoting drug efflux via the membrane P-glycoprotein ATP-dependent efflux pump ABCB1 (MDR1) (Bargou et al., 1997, Saupe et al., 2015, Mo et al., 2016). It is also associated with alternative splicing of CD44 exon via binding to the A/C-rich region (Stickeler et al., 2001). The YB-1 gene is highly conserved and only ~1% of cancer patients show the mutation, although it is nonetheless overexpressed in a wide range of cancers *via* alternative gene regulatory networks.

Ovarian cancer (OC) accounts for only 3% of all cancer cases in women, but nonetheless causes disproportionate mortality (Dietl, 2014, Jayson et al., 2014, Agarwal and Kaye, 2003). Surgical resection followed by chemotherapy is the main treatment strategy for OC patients. Platinum- and taxol-based drugs and their combination are the first-line treatment for the majority of OC patients (Seifter, 1997). The majority of women are diagnosed with OC at Stage III+ and frequently develop TR and disease relapse. BRCA1/2 mutations, amplification of MYC, and upregulation MDR1 (ABCB1/P-gp) are the most common known causes of TR in OC (Zeng et al., 2018, Christie and Bowtell, 2017, Sun et al., 2015). Patient-based studies have shown that MYC amplification is associated with disease progression and TR in many high-grade epithelial OC lesions (Jung et al., 2017, Jung et al., 2018). Nuclear localization of YB-1 plays an important role in the regulation of MYC, MDR1, and CD44 (Kang et al., 2013, Sobocan et al., 2020). Analysis of high-grade ovarian serous carcinoma samples suggests that patients with higher YB-1

expression (median survival 48.5 months) had shorter term survival compared to lower YB-1 expression (median survival 65 months) (Kang et al., 2013). Primary surgical and chemotherapy treatments for OC may be followed by maintenance therapy, which includes prolonged drug usage such as paclitaxel and PARP inhibitors (olaparib, pazopanib, and niraparib), but unfortunately still with limited outcomes (Franzese et al., 2019). Literature from the last three decades strongly suggests that YB-1 could be a potential target to treat ovarian and other cancers including those in which treatment resistance has developed. Even after an extensive investigation, there were no significant efforts had been made to develop small molecule inhibitors that can directly inhibit the YB-1. Y-box-binding protein 1 (YB-1), encoded by the YBX1 gene, has been noted as modulating or regulating cellular signaling pathways and may be seen as a molecular marker for cancer progression and as a target for cancer therapies.

Lasham et al. describe in their review article *YB-1: oncoprotein, prognostic marker and therapeutic target?*, Biochem. J. (2013) 449, 11-13, how "YB-1 regulates multiple proliferation pathways, overrides cell-cycle check points, promotes replicative immortality and genomic instability, may regulate angiogenesis, has a role in invasion and metastasis, and promotes inflammation." They further describe cell lines in which YB-1 reduction induced apoptosis or inhibited cell proliferation, including melanoma, fibrosarcoma, liver cancer, lung cancer, bladder cancer, multiple myeloma, paediatric glioblastoma, breast cancer (ER-negative), breast cancer (ER-positive), prostate cancer, and colon cancer cell lines.

Sobočan et al. (Cancers, 2020, 12, 205) describe dual targeting of Y-box-protein 1 (YB-1) and mTOR as improving the inhibition of carcinogenic activity in gynecological cancers, including ovarian, endometrial, fallopian tube, and cervical cancers.

The article *Oncogenic Y-box binding protein-1 as an effective therapeutic target in drug-resistant cancer*, Kuwano et al., Cancer Science, 2019, 110:1536-1543, describes the function of YBX2 in promoting transcriptional activation of the ABCB1 transporter gene, which has been associated as a transcriptional mechanism of how tumor multidrug resistance is acquired during chemotherapeutic treatments in human malignancies, including breast, lung, ovarian, prostate, colorectal, and gastric cancers.

The relationship between increased expression of YBX1 and melanoma is discussed in the article *The increased expression of Y box-binding protein 1 in melanoma stimulates proliferation and tumor invasion, antagonizes apoptosis and enhances chemoresistance*, Schittek et al., Int. J. Cancer: 120, 2110-

2118 (2007). YB1 overexpression has also been associated with radio-resistance in colorectal cancer cells, as discussed by Kim et al., Mol. Cancer Ther., 30 Oct 2019, 19(2), 479-89.

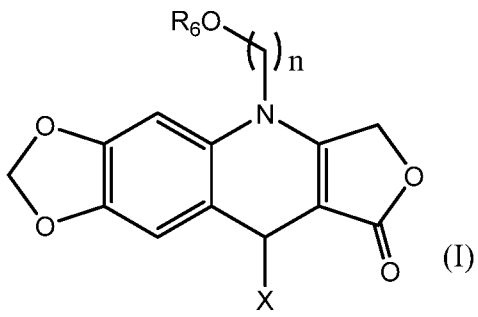
WO 2019/178091 A1 (Malholtra et al., The Board of Trustees of the Leland Stanford Junior University) teaches novel N-hydroxyethyl didehydroazapodophyllotoxins as GBP1 inhibitors and methods for their use in overcoming treatment resistance in cancers. Included in the disclosure are the specific compounds 9-(3-fluorophenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (SU056); 5-(2-hydroxyethyl)-9-(3-(trifluoromethyl)phenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one; 3-(5-(2-hydroxyethyl)-8-oxo-5,6,8,9-tetrahydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-9-yl)benzonitrile; and 5-(2-hydroxyethyl)-9-(pyridin-4-yl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one.

There remains a need for small molecule inhibitors of YB-1 for pharmaceutical use.

SUMMARY OF THE INVENTION

Bioisosterism is considered a crucial tool for rational drug design as medicinal chemists can rapidly manipulate a lead structure to optimize potency and selectivity, absorption, distribution, metabolism, and excretion (ADME) properties. Herein, we used bioisostere replacement to optimize the anti-OC effects of a lead compound using structure-guided approaches followed by target identification *via* Cellular Thermal Shift Assay (CETSA) (Savitski et al., 2014). Through *in vitro* and *in vivo* studies, we showed that the azopodophyllotoxin (AzP) small molecule SU056 potently inhibits YB-1 and reduces OC progression while sensitizing to chemotherapy-mediated cytotoxicity.

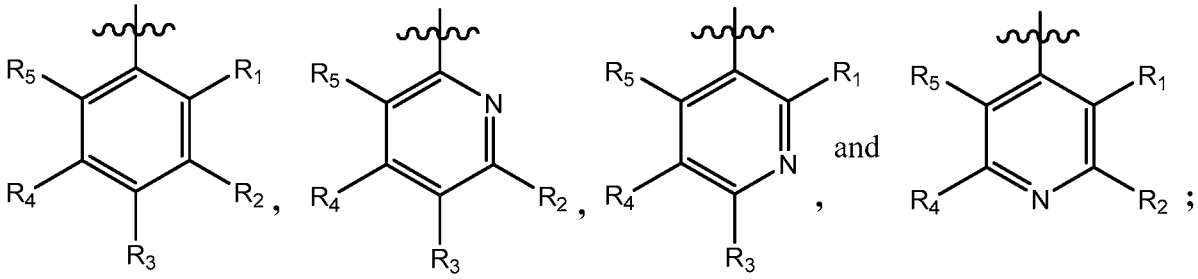
One embodiment provides a compound of Formula (I),



(I)

wherein:

X is selected from the group of:

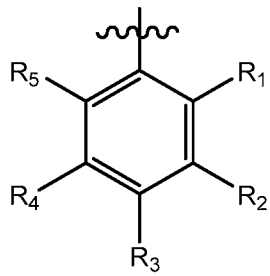


R_1, R_2, R_3, R_4, and R_5 are each independently selected in each instance from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_6 is selected from the group of H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl; wherein the C_1-C_6 alkyl group is substituted with 0, 1, 2, 3, or 4 substituents selected from F, Cl, Br, I, OH, CN, NO_2, and OH; and the C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl groups are substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when X is  and R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystals, pharmaceutically acceptable esters, pharmaceutically acceptable solvates, hydrates, isomers (including optical isomers, racemates, or other mixtures thereof), tautomers, isotopes, polymorphs, and pharmaceutically acceptable prodrugs thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A depicts conversion of SU093 to SU056.

FIGURE 1B charts IC_{50} values of SU093 and SU056 on various ovarian cancer cells.

FIGURE 1C presents a photo of colony formation from respective wells.

FIGURE 1D graphs the number of colonies formed after SU093 and SU056 treatment.

FIGURE 1E provides a table representing % inhibition values of etoposide, SU093, and SU056 treatment in neuronal (SH-SY5Y, N27) and HEK293 cells.

FIGURE 1F presents a table of cell cycle distribution of propidium iodide (PI)-stained OVCAR8, SKOV3, and ID8 cells.

FIGURE 1G graphs effects of SU093 and SU056 on apoptotic cell death.

FIGURE 1H graphs results of a cell migration assay.

FIGURE 2A presents representative images of tumor regression compared to control in mice after 42 days of drug treatment.

FIGURE 2B graphs tumor volume/mouse as a function of time.

FIGURE 2C graphs tumor weight/mouse at the end of the study.

FIGURE 2D provides a graph of liver toxicity parameters at the end of 42 days showing no significant difference between control, SU093, and SU056.

FIGURE 2E presents an image of H&E staining from a lung metastasis assay.

FIGURE 2F provides a graph of the number of lung metastatic nodules.

FIGURE 3A provides a heat map representation of the thermal stability of 804 soluble proteins in ovarian cancer cells treated with vehicle-DMSO (left) and SU056 (right).

FIGURE 3B graphs density distributions of protein Tm values calculated in SU056 treated cells and vehicle cells.

FIGURE 3C presents a graph of density distributions of Tm shifts between SU056 and vehicle treatment.

FIGURE 3D provides a scatter plot of Tm calculated in SU056 and vehicle treatment.

FIGURE 3E presents melting curves for six proteins with and without SU056 treatment.

FIGURE 3F provides a chart of change in melting temperature (T_m) of the top six proteins upon SU056 treatment.

FIGURE 4A depicts a Western blot analysis was performed for the top three targets identified by CETSA.

FIGURE 4B provides subset images for immunohistochemistry of tumor samples from ID8 tumor xenograft study.

FIGURE 4C shows a graph of % inhibition in respective OC cells treated with SU056 and YB-1.

FIGURE 4D presents a graph of YB-1 (IC50) of SU056 for OC cell.

FIGURE 4E presents graphs depicting YB-1 inhibition time kinetics study for SU056 effect on OC cell lines.

FIGURE 5A presents the structure of biotinylated SU056.

FIGURE 5B depicts a pulldown assay using biotinylated SU056.

FIGURE 5C provides a representative sensogram for SU093.

FIGURE 5D provides a representative sensogram for S SU056.

FIGURE 5E presents a Western blot analysis conforming YB-1 expression in transduced cells.

FIGURE 5F presents an image demonstrating the cellular effect of SU056 is dependent on YB-1 expression.

FIGURE 5G provides a graph of IC_{50} values of SU056 on different transduced OVCAR8 cells expressing SC, YBX1 shRNA1, YBX1 shRNA2.

FIGURE 6A depicts a cycloheximide chase assay to determine the effect of SU056 on YB-1 protein stability and a graph of fold change over time.

FIGURE 6B depicts a SDS-PAGE and Western blot analysis performed for YB-1, cell cycle, and apoptosis-associated markers.

FIGURE 6C and FIGURE 6D represent observed enrichment in the Apoptosis and RNA degradation pathway in proteins that increase in abundance upon treatment with SU056.

FIGURE 6E provides a graph of enrichment in the Spliceosome pathways observed in proteins that decrease in abundance upon treatment with SU056.

FIGURE 7A provides a chart representing the sensitizing effects of SU056 on the viability of OVCAR8 and SKOV3 cells in combination with paclitaxel treatment.

FIGURE 7B graphs an Alexa Fluor-488-tagged paclitaxel efflux assay showing SU056 cotreatment inhibits paclitaxel efflux.

FIGURE 7C presents an SDS-PAGE gel for immunoblotting of YB-1 and MDR1. OVCAR8 cells treated with either vehicle (C), paclitaxel, SU056 and paclitaxel + SU056.

FIGURE 7D provides microscopic images of spheroid at 10X magnification

FIGURE 7E provides graphs of spheroid formation quantified after 7 days of incubation

FIGURE 7F) presents representative images of mice after 28 days of drug treatment showing tumor regression compared to control.

FIGURE 7G graphs tumor volume/mouse as a function of time.

FIGURE 7H tumor weight/mouse at the end of the study. I) Immunohistochemistry staining. Tumor sections were stained with Ki67, and slides were scored for KI67 staining. Data shown are mean ± SD from 5 mice in each group. * $P< 0.05$, ** $P<0.01$, *** $P<0.001$ compared with respective control.

FIGURE 8A depicts a western blot analysis for cell lysates from control and SU056 treated cells exposed at 37 and 53°C temperature and analyzed for expression of YB-1, TMSB10 and PSMB2.

FIGURE 8B depicts micrographs of cells imaged using confocal microscope for the mCherry-YB1 after 3 h treatment of SU056 (2.5 & 5 μM) at 10X magnification.

FIGURE 8C presents a graph representing 2500 cells treated with SU056

FIGURE 9 presents graphs representing the sensitizing effects of SU056 on the viability of OVCAR8 and SKOV3 cells in combination with paclitaxel treatment.

FIGURE 10 presents a graph representing pharmacokinetics of SU056.

FIGURE 11A provides graphs representing the growth inhibitory effect of SU056 evaluated using MTT assay.

FIGURE 11B provides an image representing colony formation from cells treated with SU056 and incubated further to 7-10 days.

FIGURE 11C depicts graphs representing the number of colonies formed after SU056 treatment.

FIGURE 11D provides graphs representing the effect of SU056 on cell cycle distribution in TNBC cells.

FIGURE 12A presents SDS-PAGE and western blot analysis for SU056 treatment inhibition of protein translation associated molecules among MDA-MB-231b cells.

FIGURE 12B presents SDS-PAGE and western blot analysis for SU056 treatment inhibition of protein translation associated molecules among MDA-MB-468 cells

FIGURE 12C presents SDS-PAGE and western blot analysis for SU056 treatment inhibition of protein translation associated molecules among SUM 159 cells.

FIGURE 13A presents a graph of tumor volume (MDA-MB-231) as a function of time.

FIGURE 13B presents a graph of tumor weight (MDA-MB-231) at the end of the study.

FIGURE 13C presents a graph of body weight (MDA-MB-231) as a function of time.

FIGURE 13D presents a graph of tumor volume (MDA-MB-468) as a function of time.

FIGURE 13E presents a graph of tumor weight (MDA-MB-468) at the end of the study.

FIGURE 13F presents a graph of body weight (MDA-MB-468) as a function of time.

FIGURE 13G provides representative images of tumor (MDA-MB-231) at the end of study.

FIGURE 13H provides representative images of tumor (MDA-MB-468) at the end of study.

FIGURE 13I presents a graph representing tumor volume (SUTI151-PDX) as a function of time.

FIGURE 13J presents a graph representing tumor weight (SUTI151-PDX) at the end of the study.

FIGURE 13K presents a graph representing Body weight (SUTI151-PDX) as a function of time.

FIGURE 14A presents a graph of tumor volume (4T1) as a function of time in SU056 inhibition in a 4T1 tumor xenograft in BALB/c.

FIGURE 14B presents a graph of tumor weight (4T1) at the end of the study.

FIGURE 14C presents a graph of body weight (4T1) as a function of time.

FIGURE 14D provides representative images of tumor (4T1) at the end of study.

FIGURE 14E presents a graph of tumor volume (4T1) as a function of time.

FIGURE 14F presents a graph of tumor weight (4T1) at the end of the study.

FIGURE 14G presents a graph of body weight (4T1) as a function of time.

FIGURE 15A present a graph depicting SU056 treatment is well tolerated in mice and rat as reflected by change in body weight in mice.

FIGURE 15B present a graph depicting SU056 treatment is well tolerated in mice and rat as reflected by change in body weight in rat.

FIGURE 15C presents a table of data indicating SU056 treatment of different concentration did not cause death among mice.

FIGURE 15D presents a table of data indicating SU056 treatment of different concentration did not cause death among rats.

FIGURE 15E presents a graph showing SU056 had a mean half-life of 40 minutes.

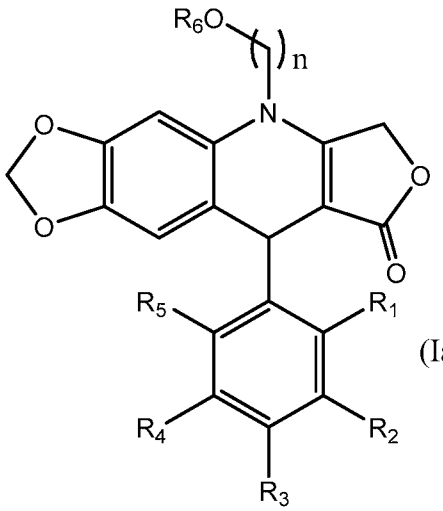
FIGURE 16 presents normalized expression measurements of YBX1 in depicted cell lines.

FIGURES 17, 18, and 19 represent SDS-PAGE and western blots demonstrating SU056 treatment inhibited translation initiation factors in TNBC cells.

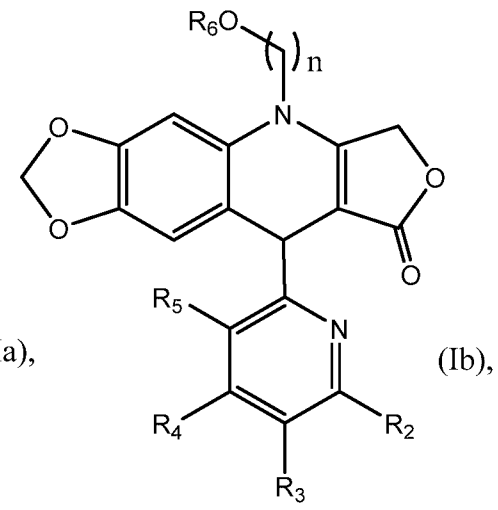
DETAILED DESCRIPTION OF THE INVENTION

It is understood that, in the proviso above, that the rotational nature of phenyl rings that a compound in which R_2 is F or CF_3 and R_1, R_3, R_4, and R_5 are each H indicates the same substitution pattern as when R_4 is F or CF_3 and R_1, R_2, R_3, and R_5 are each H. Viewing the compound as having a F or CF_3 group at R_4 would indicate a proviso in which at least one of R_1, R_2, R_3, and R_5 is not H.

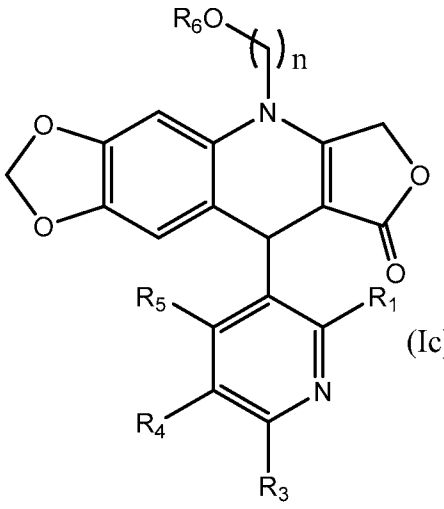
It is also understood that, within the scope of Formula (I) above, there are four additional and separate embodiments, each comprising a compound of Formula (Ia), Formula (Ib), Formula (Ic), or Formula (Id), respectively. In each embodiment, all variables (including n, R_1, R_2, R_3, R_4, R_5, and R_6), when present are as defined for Formula (I) above, with the compound of Formula (Ia) having the proviso that, when R_2 is F or CF_3, at least one of R_1, R_3, R_4, and R_5 is not H.



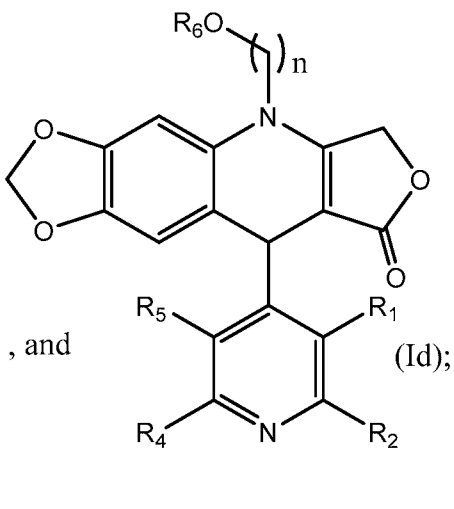
(Ia),



(Ib),



(Ic), and



(Id);

Another embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, 3, 4, and 5.

Still another embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, 3, and 4.

A further embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, and 3.

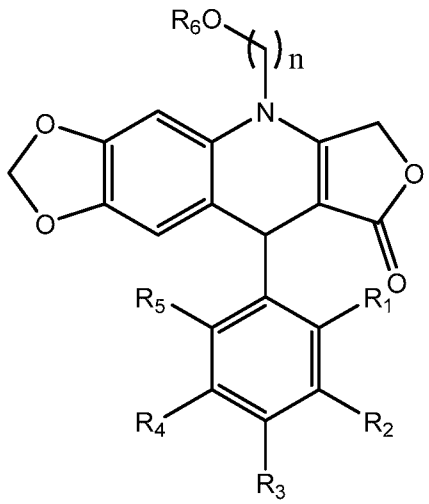
A further embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 2 and 3.

A further embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is 2.

A further embodiment comprises a compound of Formula (I) defined otherwise as above, wherein X, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is 3.

Within each of the embodiments above comprising a compound of Formula (I), there is a further embodiment wherein X, R_1, R_2, R_3, R_4, R_5, and n are as defined for the particular embodiment, wherein R_6 is H.

One embodiment provides a compound of Formula (II):



(II)

wherein:

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_6 is selected from the group of H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl; wherein the C_1-C_6 alkyl group is substituted with 0, 1, 2, 3, or 4 substituents selected from F, Cl, Br, I, OH, CN, NO_2, and OH; and the C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl groups are substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt thereof.

Another embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, 3, 4, and 5.

Still another embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, 3, and 4.

A further embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 1, 2, and 3.

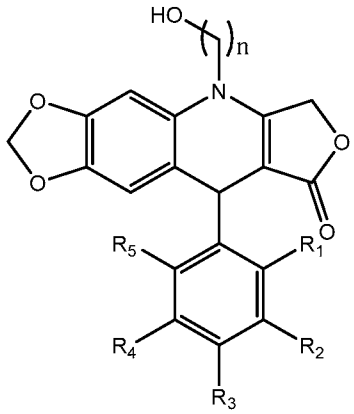
A further embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is an integer independently in each instance from the group of 2 and 3.

A further embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is 2.

A further embodiment comprises a compound of Formula (II), wherein R_1, R_2, R_3, R_4, R_5, and R_6 are as defined and subject to the provisos above, and n is 3.

Within each of the embodiments above comprising a compound of Formula (II), there is a further embodiment wherein R_1, R_2, R_3, R_4, R_5, and n are as defined for the particular embodiment, wherein R_6 is H.

A further embodiment provides a compound of Formula (III),



(III)

wherein: n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt thereof.

Another embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6; R_1 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH.

Yet another embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6; R_3 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and R_1, R_2, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH.

A still further embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein:

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5.

A still further embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein:

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_3 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F, C_1-C_3 fluoroalkyl.

A still further embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein:

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, CF_3, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F and CF_3.

A still further embodiment provides a compound of Formula (III), or a pharmaceutically acceptable salt thereof, wherein:

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, CF_3, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are F.

Another embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is an integer independently in each instance from the group of 1, 2, 3, 4, and 5.

Still another embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is an integer independently in each instance from the group of 1, 2, 3, and 4.

A further embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is an integer independently in each instance from the group of 1, 2, and 3.

A further embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is an integer independently in each instance from the group of 2 and 3.

A further embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is 2.

A further embodiment comprises a compound of Formula (III), wherein R_1, R_2, R_3, R_4, and R_5 are as defined and subject to the proviso above, and n is 3.

Pharmaceutical Compositions

Also provided is a pharmaceutical composition comprising a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof, and a pharmaceutically acceptable carrier or excipient.

Methods of Treatment

Each of the methods of treatment below will reference the use of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystals, pharmaceutically acceptable esters, pharmaceutically acceptable solvates, hydrates, isomers (including optical isomers, racemates, or other mixtures thereof), tautomers, isotopes, polymorphs, and pharmaceutically acceptable prodrugs thereof. It is understood that each method provides additional embodiments wherein the compound or compounds used is of Formula (Ia), Formula (Ib), Formula (Ic), Formula (Id), Formula (II), Formula (III), SU056, etc., or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

Provided is a method of inhibiting YB1 protein activity in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of

Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

Provided is a method of inhibiting YB1 protein activity in a subject experiencing a cancer, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof. In different embodiments, the cancer experienced by the subject is selected from the group of a gynecological cancer (including ovarian, endometrial, fallopian tube, and cervical cancers), breast cancers, lung cancers, ovarian cancer, prostate cancer, colorectal cancer, and gastric cancer.

Methods of inhibiting YB1 protein activity may be used in treating other cancers that have been associated with YBX1 expression, including acute myeloid leukemia (Zhou et al., Journal of Experimental & Clinical Cancer Research (2021) 40:353), renal cell carcinoma (Ruan et al., Oncogene (2020) 39:6113-6128), bladder cancer (Xu et al., Oncotarget, 2017, Vol. 8, No. 39, pp. 65946-65956, osteosarcoma (Fujiwara-Okada et al., British Journal of Cancer (2013) 108, pp. 836-847), head and neck cancer (Kolk et al., British Journal of Cancer (2011) 105, pp. 1864-1873, nasopharyngeal carcinoma (Zhou et al., Experimental Cell Research 361 (2017) pp. 126-134 and Ban et al., Journal of Cancer 2021, Vol. 12 (11), pp. 3315-3324).

Also provided is a method of sensitizing cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof. It is understood that included herein are separate methods for sensitizing each of the cancers referenced herein to their relevant anticancer agents.

Also provided is a method of sensitizing cancer cells expressing YB1 (YBX1) protein in a subject to treatment with radiation, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof. It is understood that included herein are separate methods for sensitizing each of the cancers referenced herein to radiation therapy.

It is also understood that the pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be administered in a regimen concurrently with an additional anticancer agent or radiation. In other embodiments, the pharmaceutically effective amount

of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be administered to a subject in need thereof in a dose or regimen prior to subsequent administration of a designated cancer agent or agents and/or radiation therapy.

In some embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be administered for an initial period of time, such as from 1 to 7 days, followed in sequence by administration to the subject in need thereof of a designated cancer agent or agents and/or radiation therapy.

In further embodiments, the compound of Formula (I), or a pharmaceutically acceptable salt thereof, and one or more designated cancer agent or agents and/or radiation therapy may be administered to the subject in need thereof in repeating sequential periods of time, such as from 1 to 14 days each, with or without a refractory period involving neither treatment in between each pair of administrations.

In other embodiments, the compound of Formula (I) may be administered for an initial period of time, such as from 1 to 7 days, followed by a second period of co-administration to the subject in need thereof of both a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of a designated cancer agent or agents and/or radiation therapy.

In different embodiments, the cancer cells expressing YB1 (YBX1) protein in a subject sensitized to the treatments described herein are selected from the group of a gynecological cancer (including ovarian, endometrial, fallopian tube, and cervical cancers), leukemias, lymphomas, kidney cancer, bladder cancer, pancreatic cancer, head and neck cancer, breast cancers (including triple negative, ER-negative, ER-positive breast cancers, and progesterone-positive), lung cancers, ovarian cancer, prostate cancer, colorectal cancer, gastric cancer, and neuronal cancer (including gliomas).

Gynecological Cancers

Provided is a method of treatment of gynecological cancers expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of gynecological cancers expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a

pharmaceutically effective amount of an mTOR inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the mTOR inhibitor is selected from the group of sirolimus, everolimus, deforolimus, and temsirolimus.

Also provided is a method of enhancing the effect of an anticancer agent in a subject experiencing a gynecological cancer expressing YB1 (YBX1) protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the treatment with a compound of Formula (I) sensitizes gynecological cancer cells expressing YB1 (YBX1) protein in the subject to the treatment of the anticancer agent.

In some embodiments, the anticancer agent used to treat the gynecological cancer is an inhibitor or antagonist of phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt).

In some embodiments, the gynecological cancer expressing YB1 (YBX1) protein to be treated is ovarian cancer. In other embodiments, the gynecological cancer to be treated is endometrial cancer. In still other embodiments, the gynecological cancer to be treated is cervical cancer.

As such, also provided is a method of treatment of ovarian cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of cisplatin, or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of ovarian cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a taxane compound, or a pharmaceutically acceptable salt thereof.

In some embodiments, the taxane compound used in the method of treatment of the gynecological cancers expressing YB1 (YBX1) protein discussed herein is selected from the group of paclitaxel, docetaxel, and cabazitaxel.

For each of the methods of sensitizing cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent and/or radiation or inhibiting YB1 protein activity in a subject

experiencing a cancer, there is a corresponding method with the initial step of detecting the presence or absence of expressed YB1 (YBX1) protein in sample cells of the cancer and, when YB1 (YBX1) protein is determined to be present in the sample cells, treating the subject experiencing the cancer in question as described for each method with a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof, and any other agent or agents indicated by the particular method.

As a non-limiting example, also provided is a method of treatment of ovarian cancer expressing YB1 (YBX1) protein in a subject, the method comprising the steps of:

a) determining the presence or absence of expressed YB1 protein in an ovarian cancer tumor sample collected from the subject in need thereof; and
b) when expressed YB1 protein is determined to be present in the ovarian cancer tumor sample, administering to the subject in need thereof:
    i) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
    ii) a pharmaceutically effective amount of a taxane compound, or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of fallopian tube cancer (fallopian tube carcinoma) expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a taxane compound, or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of fallopian tube cancer (fallopian tube carcinoma) expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof;

b) a pharmaceutically effective amount of a taxane compound, or a pharmaceutically acceptable salt thereof; and

c) a pharmaceutically effective amount of carboplatin, or a pharmaceutically acceptable salt thereof.

In some embodiments, the taxane compound in the methods of treating fallopian tube cancer is selected from the group of paclitaxel, albumin-bound paclitaxel, docetaxel, and cabazitaxel.

Prostate Cancer

Also provided is a method of treatment of prostate cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting prostate cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing prostate cancer expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments, the administration of a compound of Formula (I) sensitizes the prostate cancer expressing YB1 (YBX1) protein in the subject to treatment with a taxane anticancer agent. In some embodiments, the taxane anticancer agent is selected from the group of paclitaxel, docetaxel, and cabazitaxel, or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of prostate cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of a taxane compound selected from the group of paclitaxel, docetaxel, and cabazitaxel, or a pharmaceutically acceptable salt thereof.

In some embodiments, the administration of a compound of Formula (I) sensitizes the prostate cancer expressing YB1 (YBX1) protein in the subject to treatment with an androgen receptor inhibitor anticancer agent. In some embodiments the androgen receptor inhibitor is selected from the group of apalutamide, enzalutamide, darolutamide, and abiraterone acetate.

As such, also provided is a method of treatment of prostate cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an androgen receptor inhibitor compound selected from the group of apalutamide, enzalutamide, darolutamide, and abiraterone acetate , or a pharmaceutically acceptable salt thereof.

In some embodiments, the apalutamide is administered to the subject in need thereof at a daily dosage of from about 100 mg to about 300 mg. In some embodiments, the apalutamide is administered at a dosage of about 240 mg per day.

In some embodiments, the anticancer agent is a luteinizing hormone-releasing hormone (LHRH) agonist. In some embodiments, the LHRH agonist is selected from the group of leuprolide/leuprorelin, goserelin, triptorelin, buserelin, and histrelin.

As such, also provided is a method of treatment of prostate cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a luteinizing hormone-releasing hormone (LHRH) agonist compound selected from the group of leuprolide/leuprorelin, goserelin, triptorelin, buserelin, and histrelin, or a pharmaceutically acceptable salt thereof.

In other embodiments, the anticancer agent is a luteinizing hormone-releasing hormone (LHRH) antagonist. In some embodiments, the LHRH agonist is degarelix.

As such, also provided is a method of treatment of prostate cancer in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of degarelix, or a pharmaceutically acceptable salt thereof.

In other embodiments, the anticancer agent is anti-androgen agent. In some embodiments, the anti-androgen agent is selected from the group of flutamide, bicalutamide, and nilutamide.

As such, also provided is a method of treatment of prostate cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anti-androgen compound selected from the group of flutamide, bicalutamide, and nilutamide, or a pharmaceutically acceptable salt thereof.

In some embodiments concerning the methods herein for treating prostate cancer and/or inhibiting prostate cancer metastasis, the prostate cancer in question is an androgen-independent prostate cancer. In other embodiments, the prostate cancer in question is castration-sensitive prostate cancer. In other embodiments, the prostate cancer in question is metastatic castration-sensitive prostate cancer. In additional embodiments, the prostate cancer expressing YB1 (YBX1) protein to be treated is non-metastatic castration-resistant prostate cancer. In other embodiments, the prostate cancer is hormone-refractory prostate cancer (HRPC).

Melanoma

Also provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting melanoma metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing melanoma cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in

need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

c) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
d) a pharmaceutically effective amount of a PD-1 inhibitor agent selected from the group of pembrolizumab and nivolumab, or a pharmaceutically acceptable salt thereof.

Further provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of atezolizumab, or a pharmaceutically acceptable salt thereof.

Further provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof;
b) a pharmaceutically effective amount of atezolizumab, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of a third agent selected from the group of cobimetinib and vemurafenib, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a CTLA-4 inhibitor (such as ipilimumab).

As such, also provided is a method of treatment of melanoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of interleukin-2 (IL-2).

Cisplatin resistance

YB-1 expression or overexpression has also been associated with resistance to cisplatin treatments in some cancers, including breast, bladder, and ovarian cancers.

In some embodiments, the breast cancer to be treated is refractory to endocrine therapeutics, such as selective estrogen receptor modulators (SERMs), including tamoxifen and toremifene. In some embodiments, the breast cancer is refractory to selective estrogen receptor degrader (SERDs), such as fulvestrant and elacestrant. In other embodiments, the breast cancer to be treated is refractory to aromatase inhbitors, such as letrozole, anastrozole, exemestane, and testolactone.

Provided herein is a method of sensitizing a cancer expressing YB1 (YBX1) protein in a subject to treatment with cisplatin, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Provided herein is a method of sensitizing a cancer expressing YB1 (YBX1) protein in a subject to treatment with a taxane compound, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Breast Cancer

Also provided is a method of treatment of breast cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting breast cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing breast cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the method sensitizes breast cancer cells expressing YB1 (YBX1) protein in the subject in need thereof to treatment with one or more agents selected from the group of anthracyclines (such as doxorubicin, pegylated liposomal doxorubicin, and epirubicin), taxane compounds (such as paclitaxel, albumin-bound paclitaxel, docetaxel, and cabazitaxel), 5-fluorouracil, capecitabine, cyclophosphamide, vinarelbine, gemcitabine, ixabepilone, eribulin, and platinum agents (such as carboplatin and cisplatin). In other embodiments, the method sensitizes breast cancer cells expressing YB1 (YBX1) to treatment with radiation therapy.

An embodiment provides a method of treatment of breast cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of doxorubicin, pegylated liposomal doxorubicin, epirubicin, paclitaxel, docetaxel, 5-fluorouracil, capecitabine, cyclophosphamide, and carboplatin, or a pharmaceutically acceptable salt thereof.

Another embodiment provides a method of treatment of breast cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of paclitaxel, albumin-bound paclitaxel, docetaxel, doxorubicin, pegylated liposomal doxorubicin, epirubicin, cisplatin, carboplatin, vinorelbine, capecitabine, gemcitabine, ixabepilone, and eribulin, or a pharmaceutically acceptable salt thereof.

Colorectal Cancer

Also provided is a method of treatment of colorectal cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting colorectal cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing colorectal cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of colorectal cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anti-cancer agent selected from the group of 5-fluorouracil, capecitabine, irinotecan, oxaliplatin, and trifluridine and tipiracil, or a pharmaceutically acceptable salt thereof.

Bladder Cancer

Also provided is a method of treatment of bladder cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting bladder cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing bladder cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Provided is a method of treatment of bladder cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

d) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof;

e) a pharmaceutically effective amount of an anticancer agent selected from the group of cisplatin, cisplatin plus 5-fluorouracil, and mitomycin with 5-fluorouracil, or a pharmaceutically acceptable salt thereof;

f) a therapeutically effective dose of radiation.

Also provided is a method of treatment of bladder cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of an anticancer agent selected from the group of

i) gemcitabine and cisplatin;

ii) Dose-dense methotrexate, vinblastine, doxorubicin (Adriamycin), and cisplatin (DDMVAC);

iii) Cisplatin, methotrexate, and vinblastine (CMV); and

iv) Gemcitabine and paclitaxel

Also provided is a method of treatment of bladder cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof;

b) a pharmaceutically effective amount of an anticancer agent selected from the group of docetaxel, paclitaxel, doxorubicin, methotrexate, ifosfamide, and pemetrexed, or a pharmaceutically acceptable salt thereof.

Liver Cancer

Also provided is a method of treatment of liver cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting liver cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing liver cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject

in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of liver cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anti-cancer agent selected from the group of gemcitabine, oxaliplatin, cisplatin, doxorubicin, 5-fluorouracil, capecitabine, and mitoxantrone, or a pharmaceutically acceptable salt thereof.

Lung Cancer

Also provided is a method of treatment of small cell lung cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting small cell lung cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing small cell lung cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of small cell lung cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anti-cancer agent selected from the group of cisplatin and etoposide, carboplatin and etoposide, cisplatin and irinotecan, and carboplatin and irinotecan, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of non-small cell lung cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a

pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting non-small cell lung cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing non-small cell lung cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of non-small cell lung cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of cisplatin, carboplatin, paclitaxel, albumin-bound paclitaxel, docetaxel, gemcitabine, vinarelbine, etoposide, and premetrexed, or a pharmaceutically acceptable salt thereof.

Multiple Myeloma

Also provided is a method of treatment of multiple myeloma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting multiple myeloma metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing multiple myeloma cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of multiple myeloma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anti-cancer agent selected from the group of Melphalan, vincristine, cyclophosphamide, etoposide, doxorubicin, liposomal doxorubicin, and bendamustine, or a pharmaceutically acceptable salt thereof.

Soft Tissue Sarcomas

Also provided herein are methods of treatment of soft tissue sarcomas expressing YB1 (YBX1) protein, including angiosarcoma, dermatofibrosarcoma protuberans, epitheloid sarcoma, gastrointestinal stromal tumor (GIST), Kaposi's sarcoma, Leiomyosarcoma, liposarcoma, malignant peripheral nerve sheath tumors, myxofibrosarcoma, rhabdomyosarcoma, solitary fibrous tumors, synovial sarcoma, and undifferentiated pleomorphic sarcoma.

Also provided is a method of treatment of soft tissue sarcomas expressing YB1 (YBX1) protein, such as fibrosarcoma expressing YB1 (YBX1) protein, in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting soft tissue sarcomas expressing YB1 (YBX1) protein, such as fibrosarcoma expressing YB1 (YBX1) protein, in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing soft tissue sarcoma cells expressing YB1 (YBX1) protein, such as fibrosarcoma cells expressing YB1 (YBX1) protein, cells in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of soft tissue sarcomas expressing YB1 (YBX1) protein, such as fibrosarcoma expressing YB1 (YBX1) protein, in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of ifosfamide, doxorubicin, dacarbazine (DTIC), epirubicin, temozolomide, docetaxel,

gemcitabine, vinorelbine, trabectedin, and eribulin, or a pharmaceutically acceptable salt thereof.

In some embodiments, when the anti-cancer agent ifosfamide is used, the drug mesna is also given to protect the bladder from the toxic effects of ifosfamide. In other embodiments, the anti-cancer agent is a combination of mesna, Adriamycin [doxorubicin], ifosfamide, and dacarbazine, sometimes referred to by the acronym MAID. In other embodiments, the anti-cancer agent is a combination of Adriamycin [doxorubicin], ifosfamide, and mesna, sometimes referred to by the acronym AIM. In some embodiments in the methods of treating soft tissue sarcomas herein, the anti-cancer agent or agents are administered to the subject in need thereof using isolated limb perfusion.

Osteosarcomas: Also provided is a method of treatment of osteosarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting osteosarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing osteosarcoma cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of osteosarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of methotrexate, doxorubicin, cisplatin, carboplatin, Ifosfamide, cyclophosphamide, etoposide, and gemcitabine, or a pharmaceutically acceptable salt thereof.

In some embodiments in the method of treating osteosarcoma expressing YB1 (YBX1) protein above, the anti-cancer agent is a combination of High-dose methotrexate, doxorubicin, and cisplatin (the MAP regimen). In other embodiments, a combination of doxorubicin and cisplatin are

administered. In other embodiments, a combination of ifosfamide and etoposide are used. In still other embodiments, a combination is administered of ifosfamide and epirubicin with either cisplatin or carboplatin.

Ewing's Sarcoma

Also provided is a method of treatment of Ewing's sarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting Ewing's sarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing Ewing's sarcoma cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of Ewing's sarcoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of cyclophosphamide, doxorubicin, etoposide, Ifosfamide, and vincristine, or a pharmaceutically acceptable salt thereof.

In some embodiments in the method of treating Ewing's sarcoma expressing YB1 (YBX1) protein above, the anti-cancer agent is a combination of vincristine, doxorubicin, and cyclophosphamide, alternating with ifosfamide and etoposide, the regimen referred to as VDC/IE.

Gastric cancers

Also provided is a method of treatment of gastric (stomach) cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting gastric cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing gastric cancer cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of gastric cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of 5-fluorouracil, capecitabine, carboplatin, cisplatin, docetaxel, epirubicin, irinotecan, oxaliplatin, paclitaxel, and trifluridine + tipracil (LONSURF®) or a pharmaceutically acceptable salt thereof.

In some embodiments in the method of treating gastric cancer expressing YB1 (YBX1) protein above, the anti-cancer agent is a combination of epirubicin, cisplatin, and 5-fluorocil, sometimes referred to by the acronym ECF. In other embodiments, the combination of docetaxel or paclitaxel with either 5-FU or capecitabine, sometimes combined with radiation. In another embodiment cisplatin is administered with either 5-FU or capecitabine, sometimes combined with radiation. In further embodiments paclitaxel and carboplatin are administered, sometimes combined with radiation. In other embodiments the combination of docetaxel, cisplatin, and 5-fluoruracil (DCF) are administered. In others, irinotecan is administered along with cisplatin, 5-flourouracil, or capecitabine. In still others, oxaliplatin is administered with 5-fluorouracil or capecitabine. In still others, trifluridine + tipracil (LONSURF®) is given.

Glioblastoma

Also provided is a method of treatment of glioblastoma multiforme (GBM or glioblastoma) expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting glioblastoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing glioblastoma cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of glioblastoma expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anti-cancer agents selected from the group of temozolomide, bevacizumab, lomustine, carmustine, fluzoparil, pembrolizumab, nivolumab, ipilimumab, anlotinib, glasdegib, and bavituximab, or a pharmaceutically acceptable salt thereof.

In some embodiments, the glioblastoma in the methods above is a pediatric glioblastoma expressing YB1 (YBX1) protein. In some embodiments the glioblastoma is a primary glioblastoma expressing YB1 (YBX1) protein. In others, it is a secondary glioblastoma expressing YB1 (YBX1) protein.

Head and Neck Cancer

Reference to "head and neck cancer" herein refers to any of the cancers of the oral cavity, throat (pharynx, including the nasopharynx, oropharynx, and hypopharynx), larynx, paranasal sinuses, nasal cavity, and salivary glands. The head and neck cancers include Hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer, nasopharyngeal cancer, oropharyngeal cancer, paranasal sinus and nasal cavity cancer, and salivary gland cancer. It is understood that, for each of the methods of treatment of head and neck cancer described herein, disclosed also is the corresponding method for each of the head and neck cancers listed in this paragraph.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting head and neck cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing head and neck cancer expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

In some embodiments concerning the treatment of head and neck cancer expressing YB1 (YBX1), the anticancer agent used to treat the subject is radiation therapy. In some embodiments, the radiation utilized is external-beam radiation therapy. In some embodiments, the treatment comprises administering a pharmaceutically effective amount of one or more EGFR inhibitors to the subject in need thereof. Other embodiments concern respectively administering a pharmaceutically effective amount of larotrectinib (Vitrakvi) and/or larotrectinib to the subject in need thereof.

Other methods of treating head and neck cancer comprise the use of immunotherapy, such as the administration a pharmaceutically effective amount of pembrolizumab and/or nivolumab to the subject in need thereof.

Provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anticancer agent selected from the group of paclitaxel, docetaxel, cisplatin, carboplatin, 5-fluorouracil, methotrexate, and capecitabine, or a pharmaceutically acceptable salt thereof.

As such, also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a taxane compound selected from the group of paclitaxel and docetaxel, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a cisplatin, or a pharmaceutically acceptable salt thereof.

In some embodiments, the cisplatin is administered to the subject in need thereof at a dose of from about 20 mg/m^2 to about 100 mg/m^2 delivered every 3 weeks × 3.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of a carboplatin, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of a drug selected from the group of 5-fluorouracil (5FU) and cetuximab.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of cisplatin, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of a drug selected from the group of 5-fluorouracil (5FU) and cetuximab.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of cisplatin, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of paclitaxel, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of carboplatin, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of paclitaxel, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of head and neck cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of hydroxyurea, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of a drug selected from the group of 5-fluorouracil (5FU) and cetuximab.

Further provided is a method of treatment of nasopharyngeal cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of hydroxyurea, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of a drug selected from the group of carboplatin, doxorubicin, epirubicin, paclitaxel, docetaxel, gemcitabine, bleomycin, and methotrexate.

Pancreatic Cancer

Provided is a method of treatment of pancreatic cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting pancreatic cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a

pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing pancreatic cancer expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Provided is a method of treatment of pancreatic cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of gemcitabine, 5-fluoruracil, oxaliplatin, paclitaxel, albumin-bound paclitaxel, docetaxel, capecitabine, cisplatin, and irinotecan, or a pharmaceutically acceptable salt thereof.

Neuronal Cancers

The compounds of Formula (I) may also be used to treat and/or sensitize to treatment neuronal cancers (brain and spinal cord cancers), including medulloblastoma, glioblastoma multiforme (GBM), astrocytomas (anapastic astrocytomas and pilocytic astrocytomas), ependymomas, and oligodendrogliomas. It is understood for each of the following methods for treating a neuronal cancer or sensitizing a neuronal cancer to treatment described herein, included separate methods of each type for each of the neuronal cancers listed in this paragraph.

Provided is a method of treatment of neuronal cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting neuronal cancer metastasis expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally provided is a method of sensitizing neuronal cancer expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Provided is a method of treatment of neuronal cancer expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of carboplatin, carmustine (BCNU), cisplatin, irinotecan, cyclophosphamide, etoposide, lomustine, methotrexate, procarbazine, temozolomide, and vincristine, or a pharmaceutically acceptable salt thereof.

In some embodiments, the pharmaceutically effective amount of carmustine administered to the subject in need thereof is in the form of a carmustine wafer or implant, such as that in the GLIADEL® Wafer (carmustine implant) product available from Arbor Pharmaceuticals, LLC.

Leukemias

Methods of the present invention also include those for the treatment of leukemias, wherein the leukemia cells in question express YB-1 protein, including acute myeloid leukemia (AML), Chronic myelogenous leukemia (CML), acute lymphoblastic (or lymphocytic) leukemia (ALL), and chronic lymphocytic leukemia (CLL). It is understood that for each method described herein for a treatment for leukemia or of sensitizing a leukemia to a treatment, separate corresponding methods are understood for each of the referenced leukemias (AML, CML, ALL, and CLL).

Additionally provided is a method of sensitizing leukemia cells expressing YB1 (YBX1) protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. In some embodiments, the anticancer agent used to treat the subject is radiation therapy.

Also provided is a method of treatment of acute myeloid leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of an anthracycline drug selected from the group of daunorubicin and idarubicin, or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of cytarabine, or a pharmaceutically acceptable salt thereof

Also provided is a method of treatment of acute myeloid leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of cladribine (2-CdA), fludarabine, mitoxantrone, etoposide, 6-thioguanine, hydroxyurea, prednisone, dethamexasone, methotrexate, 6-mercaptopurine, azacitidine, and decitabine, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of chronic myeloid leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of hydroxyurea, cytarabine (Ara-C), busulfan, cyclophosphamide (CYTOXAN®), and vincristine (ONCOVIN®), or a pharmaceutically acceptable salt thereof

Also provided is a method of treatment of chronic myeloid leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more tyrosine kinase inhibitor anticancer agents selected from the group of imatinib (GLEEVEC®), dasatinib (SPRYCEL®), nilotinib (TASIGNA®), bosutinib (BOSULIF®), ponatinib (ICLUSIG®), and asciminib (SCEMBLIX®), or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of chronic myeloid leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of interferon-alpha, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of acute lymphoblastic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of vincristine, dexamethasone, imatinib, prednisone, doxorubicin and daunorubicin, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of acute lymphoblastic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of methotrexate, 6-mercaptopurine, vincristine, prednisone, and imatinib, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of acute lymphoblastic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of vincristine, dexamethasone, prednisone, doxorubicin, and daunorubicin, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of acute lymphoblastic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of methotrexate, 6-mercaptopurine (6-MP), vincristine, prednisone, and imatinib, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of chronic lymphocytic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of one or more anticancer agents selected from the group of ibrutinib, acalabrutinib, idelalisib, and duvelisib, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of chronic lymphocytic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
b) a pharmaceutically effective amount of venetoclax, or a pharmaceutically acceptable salt thereof.

Also provided is a method of treatment of chronic lymphocytic leukemia expressing YB1 (YBX1) protein in a subject, the method comprising administering to the subject in need thereof:

a) a pharmaceutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof; and
c) a pharmaceutically effective amount of one or more anticancer agents selected from the group of rituximab, ofatumumab, and obinutuzumab, or a pharmaceutically acceptable salt thereof.

Definitions

The terms “YB1” and “YBX1” refer to Y box binding protein 1, also known as Y-box transcription factor or nuclease-sensitive element-binding protein 1, a protein that in humans is encoded by the YBX1 gene.

The wavy line ( ) in chemical structures indicates a bond through which the structure shown is bound to another chemical moiety or group.

A “heterocycle” or “heterocyclic group” herein refers to a chemical ring containing carbon atoms and at least one ring heteroatom selected from O, S, and N. The terms “heterocycle” and

"heterocyclic" include groups with saturated rings, partially unsaturated rings, and aromatic rings (i.e., heteroaromatic rings). Examples of 5-membered and 6-membered heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, 4-piperidinyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, morpholinyl, and oxazolidinyl.

The term "alkyl" refers to a straight or branched hydrocarbon. For example, an alkyl group can include those having 1 to 6 carbon atoms (i.e, C_1-C_6 alkyl), 1 to 4 carbon atoms (i.e., C_1-C_4 alkyl), or 1 to 3 carbon atoms (i.e., C_1-C_3 alkyl). Examples of suitable alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl (-$CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, --$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, --$CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, --$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, --$C(CH_3)_3$), 1-pentyl (n-pentyl, --$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (--$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (--$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (-$C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl (--$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (--$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (-$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (--$CH_2CH_2CH2CH_2CH_2CH_3$), 2-hexyl (--$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (-$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl (-$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (-$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (-$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (--$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (--$C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl (-$CH(CH_3)C(CH_3)_3$, and the like.

The term "alkoxy" refers to a group having the formula -O-alkyl, in which an alkyl group, as defined above, is attached to the parent molecule via an oxygen atom. The alkyl portion of an alkoxy group can have 1 to 6 carbon atoms (i.e., C_1-C_6 alkoxy), 1 to 4 carbon atoms (i.e., C_1-C_4 alkoxy), or 1 to 3 carbon atoms (i.e., C_1-C_3 alkoxy). Examples of suitable alkoxy groups include, but are not limited to, methoxy (-O-CH_3 or -OMe), ethoxy (-OCH_2CH_3 or -OEt), *n*-propoxy (-CH_2-CH_2-CH_3), isopropoxy (-$CH(CH_3)_2$), *n*-butyl (-CH_2-CH_2-CH_2-CH_3), isobutoxy (-CH_2-$CH(CH_3)_2$), sec-butoxy (–$CH(CH_3)CH_2$-CH_3), t-butoxy (-O-$C(CH_3)_3$ or -OtBu), and the like.

The term "cycloalkyl" refers to a saturated ring having 3 to 6 carbon atoms as a monocycle, including cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl groups.

The term "subject" refers to an animal, such as a mammal, that has been or will be the object of treatment, observation or experiment. The methods described herein may be useful in both human

therapy and veterinary applications. In some embodiments, the subject is a mammal; in some embodiments the subject is human; and in some embodiments the subject is chosen from cats and dogs. "Subject in need thereof" or "human in need thereof" refers to a subject, such as a human, who may have or is suspected to have diseases or conditions that would benefit from certain treatment; for example treatment with a compound of Formula (I), Formula (II), or Formula (III), or a pharmaceutically acceptable salt or co-crystal thereof, as described herein. This includes a subject who may be determined to be at risk of or susceptible to such diseases or conditions, such that treatment would prevent the disease or condition from developing.

In some embodiments, a “subject in need thereof” concerning a method of treatment herein is a patient from whom a tumor sample, such as from a tumor biopsy, is taken and the presence of expressed YBX1 protein is identified in the sampled material, such as through immunohistochemical or Western Blotting techniques known in the art.

The terms “effective amount,” "therapeutically effective amount," or "pharmaceutically effective amount" refer to an amount that is sufficient to effect treatment, as defined below, when administered to a subject (e.g., a mammal, such as a human) in need of such treatment. The therapeutically or pharmaceutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. For example, an “effective amount,” "therapeutically effective amount," or a "pharmaceutically effective amount" of a compound of Formula (I), Formula (II), or Formula (III), or a pharmaceutically acceptable salt or co-crystal thereof, is an amount sufficient to modulate YXB1 expression or activity, and thereby treat a subject (e.g., a human) suffering an indication, or to ameliorate or alleviate the existing symptoms of the indication. For example, a therapeutically or pharmaceutically effective amount may be an amount sufficient to decrease a symptom of a disease or condition responsive to inhibition of YXB1 activity.

In some embodiments, an “effective amount” is an amount of a subject compound that, when administered to an individual in one or more doses, in monotherapy or in combination therapy, is effective to inhibit YB-1 by about 20% (20% inhibition), at least about 30% (30% inhibition), at least about 40% (40% inhibition), at least about 50% (50% inhibition), at least about 60% (60% inhibition), at least about 70% (70% inhibition), at least about 80% (80% inhibition), or at least about 90% (90% inhibition), compared to the YB-1 activity in the individual in the absence of treatment with the

compound, or alternatively, compared to the YB-1 activity in the individual before or after treatment with the compound.

In some embodiments, an "effective amount" is an amount of a subject compound that, when administered to an individual in one or more doses, in monotherapy or in combination therapy, is effective to decrease tumor burden in the subject by about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, compared to tumor burden in the individual in the absence of treatment with the compound, or alternatively, compared to the tumor burden in the subject before or after treatment with the compound. As used herein the term "tumor burden" refers to the total mass of tumor tissue carried by a subject with cancer. In some embodiments, an "effective amount" is an amount of a subject compound that, when administered to an individual in one or more doses, in monotherapy or in combination therapy, is effective to reduce the dose of radiotherapy required to observe tumor shrinkage in the subject by about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, compared to the dose of radiotherapy required to observe tumor shrinkage in the individual in the absence of treatment with the compound. In some embodiments, an "effective amount" of a compound is an amount that, when administered in one or more doses to an individual having cancer, is effective to achieve a 1.5- log, a 2-log, a 2.5-log, a 3 -log, a 3.5-log, a 4-log, a 4.5-log, or a 5 -log reduction in tumor size.

In some embodiments, a compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be administered at a daily dose of from about 0.2 mg/kg to about 10 mg/kg. In other embodiments, it may be administered at a daily dose of from about 0.2 mg/kg to about 5 mg/kg.

In some embodiments, an effective amount of a compound is an amount that ranges from about 50 ng/kg body weight to about 50 pg/kg body weight (e.g., from about 50 ng/kg body weight to about 40 pg/kg body weight, from about 30 ng/kg body weight to about 20 pg/kg body weight, from about 50 ng/kg body weight to about 10 pg/kg body weight, from about 50 ng/kg body weight to about 1 pg/kg body weight, from about 50 ng/kg body weight to about 800 ng/kg body weight, from about 50 ng/kg body weight to about 700 ng/kg body weight, from about 50 ng/kg body weight to about 600 ng/kg body weight, from about 50 ng/kg body weight to about 500 ng/kg body weight, from about 50 ng/kg body weight to about 400 ng/kg body weight, from about 60 ng/kg body weight to about 400 ng/kg body weight, from about 70 ng/kg body weight to about 300 ng/kg body weight, from about 60 ng/kg body weight to about 100 ng/kg body weight, from about 65 ng/kg body weight to about 85 ng/kg body weight, from about 70 ng/kg body weight to about 90 ng/kg body weight, from about 200 ng/kg

body weight to about 900 ng/kg body weight, from about 200 ng/kg body weight to about 800 ng/kg body weight, from about 200 ng/kg body weight to about 700 ng/kg body weight, from about 200 ng/kg body weight to about 600 ng/kg body weight, from about 200 ng/kg body weight to about 500 ng/kg body weight, from about 200 ng/kg body weight to about 400 ng/kg body weight, or from about 200 ng/kg body weight to about 300 ng/kg body weight).

In some embodiments, an effective amount of a compound is an amount that ranges from about 10 pg to about 100 mg, e.g., from about 10 pg to about 50 pg, from about 50 pg to about 150 pg, from about 150 pg to about 250 pg, from about 250 pg to about 500 pg, from about 500 pg to about 750 pg, from about 750 pg to about 1 ng, from about 1 ng to about 10 ng, from about 10 ng to about 50 ng, from about 50 ng to about 150 ng, from about 150 ng to about 250 ng, from about 250 ng to about 500 ng, from about 500 ng to about 750 ng, from about 750 ng to about 1 pg, from about 1 pg to about 10 pg, from about 10 pg to about 50 pg, from about 50 mg to about 150 gg, from about 150 gg to about 250 gg, from about 250 gg to about 500 gg, from about 500 gg to about 750 gg, from about 750 gg to about 1 g, from about 1 mg to about 50 mg, from about 1 mg to about 100 mg, or from about 50 mg to about 100 mg. The amount can be a single dose amount or can be a total daily amount. The total daily amount can range from 10 pg to 100 mg, or can range from 100 mg to about 500 mg, or can range from 500 mg to about 1000 mg.

In some embodiments, a single dose of a compound is administered. In other embodiments, multiple doses are administered. Where multiple doses are administered over a period of time, the compound can be administered twice daily (qid), daily (qd), every other day (qod), every third day, three times per week (tiw), or twice per week (biw) over a period of time. For example, a compound is administered qid, qd, qod, tiw, or biw over a period of from one day to about 2 years or more. For example, a compound is administered at any of the aforementioned frequencies for one week, two weeks, one month, two months, six months, one year, or two years, or more, depending on various factors.

Administration of an effective amount of a subject compound to an individual with cancer can result in one or more of: 1) a reduction in tumor burden; 2) a reduction in the dose of radiotherapy required to effect tumor shrinkage (e.g. resulting from sensitization to radiotherapy); 3) a reduction in the spread of a cancer from one cell to another cell in an individual; 4) a reduction of morbidity or mortality in clinical outcomes; 5) shortening the total length of treatment when combined with other anti -cancer agents (e.g. resulting from sensitization to other anti-cancer agents); and 6) an improvement in an indicator of disease response (e.g., a reduction in one or more symptoms of cancer).

Any of a variety of methods can be used to determine whether a treatment method is effective. For example, a biological sample obtained from an individual who has been treated with a subject method can be assayed.

The terms “inhibiting” or "inhibition" indicates a decrease, such as a significant decrease, in the baseline activity of a biological activity or process. "Inhibition of YB-1 activity" refers to a decrease in YB-1 activity as a direct or indirect response to the presence of a compound of Formula I, or a pharmaceutically acceptable salt or co-crystal thereof, relative to the activity of YB-1 in the absence of such compound or a pharmaceutically acceptable salt or co-crystal thereof. The decrease in activity may be due to the direct interaction of the compound with YB-1, or due to the interaction of the compound(s) described herein with one or more other factors that in turn affect YB-1 activity. For example, the presence of the compound(s) may decrease YB-1 activity by directly binding to the YB-1, by causing (directly or indirectly) another factor to decrease YB-1 activity, or by (directly or indirectly) decreasing the amount of YB-1 present in the cell or organism. In some embodiments, the inhibition of YB-1 activity may be compared in the same subject prior to treatment, or other subjects not receiving the treatment. The term “inhibitor” is understood to refer to a compound or agent that, upon administration to a human in need thereof at a pharmaceutically or therapeutically effective dose, provides the inhibition activity desired.

The term “pharmaceutical composition” refers to a composition containing a pharmaceutically effective amount of one or more of the isotopic compounds described herein, or a pharmaceutically acceptable salt thereof, formulated with a pharmaceutically acceptable carrier, which can also include other additives, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (*e.g.*, a tablet, capsule, caplet, gelcap, or syrup); for topical administration (*e.g.*, as a cream, gel, lotion, or ointment); for intravenous administration (*e.g.*, as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in *Remington: The Science and Practice of Pharmacy*, 21st Ed., Gennaro, Ed., Lippencott Williams & Wilkins (2005) and in The United States Pharmacopeia: The National Formulary (USP 36 NF31), published in 2013.

As used herein, "pharmaceutically acceptable excipient" is a pharmaceutically acceptable vehicle that includes, without limitation, any and all carriers, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term “pharmaceutically acceptable carrier” refers to any ingredient in a pharmaceutical composition other than the disclosed pharmaceutically active or therapeutic compounds, or a pharmaceutically acceptable salt thereof (*e.g.*, a carrier capable of suspending or dissolving the active isotopic compound) and having the properties of being nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

The term "pharmaceutically acceptable salt" includes, for example, salts with inorganic acids and salts with an organic acid. Examples of salts may include hydrochloride, phosphate, diphosphate, hydrobromide, sulfate, sulfinate, nitrate, malate, maleate, fumarate, tartrate, succinate, citrate, acetate, lactate, methanesulfonate (mesylate), benzenesuflonate (besylate), p-toluenesulfonate (tosylate), 2-hydroxyethylsulfonate, benzoate, salicylate, stearate, and alkanoate (such as acetate, $HOOC-(CH_2)_n-COOH$ where n is 0-4). In addition, if the compounds described herein are obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those

skilled in the art will recognize various synthetic methodologies that may be used to prepare nontoxic pharmaceutically acceptable addition salts.

Also described for a compound of Formula I are the pharmaceutically acceptable salts, pharmaceutically acceptable co-crystals, pharmaceutically acceptable esters, pharmaceutically acceptable solvates, hydrates, isomers (including optical isomers, racemates, or other mixtures thereof), tautomers, isotopes, polymorphs, and pharmaceutically acceptable prodrugs of such compounds. For the sake of brevity, the list of forms in the prior sentence may not be listed in all references to compounds herein, including those of Formula (I), Formula (Ia), Formula (Ib), Formula (Ic), Formula (Id), Formula (II), Formula (III), SU056, etc., but each is understood to be disclosed and included herein, even if only pharmaceutically acceptable salts are included in a description applied anywhere herein, including in association with descriptions of chemical compounds, pharmaceutical compositions, methods of use/treatment, or other references.

The term "crystal forms" and related terms herein refer to the various crystalline modifications of a given substance, including, but not limited to, polymorphs, solvates, hydrates, co-crystals, and other molecular complexes, as well as salts, solvates of salts, hydrates of salts, other molecular complexes of salts, and polymorphs thereof. Crystal forms of a substance can be obtained by a number of methods, as known in the art. Such methods include, but are not limited to, melt recrystallization, melt cooling, solvent recrystallization, recrystallization in confined spaces such as, e.g., in nanopores or capillaries, recrystallization on surfaces or templates, such as, e.g., on polymers, recrystallization in the presence of additives, such as, e.g., co-crystal counter-molecules, desolvation, dehydration, rapid evaporation, rapid cooling, slow cooling, vapor diffusion, sublimation, grinding and solvent-drop grinding.

The term “refractory” used herein in regard to a cancer refers to a cancer that does not respond to one or more treatments. In some embodiments, the cancer does not respond to one or more chemotherapeutic agents. In other embodiments, the cancer does not respond to radiation therapy. In other embodiments, the refractory cancer does not respond to one or more chemotherapeutic agents and radiation therapy. A refractory cancer may be resistant at the beginning of such treatments or may become resistant over the course of one or more treatments. In cases of chemotherapy, refractory cancers may also be referred to as “chemotherapy resistant cancers” or “chemo-resistant cancers.”

“Androgen-independent prostate cancer” or “hormone-refractory prostate cancer (AIPC)” is prostate cancer that progresses after primary androgen-ablation therapy, either from orchiectomy or a gonadotropin-releasing hormone (LHRH) agonist, followed by addition and subsequent withdrawal of an antiandrogen. In some embodiments hormone-refractory prostate cancer is defined as 2-3 consecutive

rises in prostate-specific antigen (PSA) levels obtained at intervals of greater than 2 weeks and/or documented disease progression based on findings from CT scan and/or bone scan, bone pain, or obstructive voiding symptoms. In some embodiments, the PSA level does not rise at diagnosis or throughout the entire course of the disease. In some embodiments, the prostate cancer is an advanced prostate cancer. In some embodiments, the prostate cancer or metastatic prostate cancer is resistant to treatments with hormone-blocking therapies, such as abiraterone (ZYTIGA®), enzalutamide (XTANDI®), bicalutamide (CASODEX®), flutamide (DROGENIL®), or cyproterone acetate (CYPROSTAT®).

Cancer cells that are resistant to radiation treatment, whether intrinsically or acquired over time, are referred to as “radiation resistant cancers” or “radioresistant cancers.” The terms are intended to describe cancer cells that are less responsive to radiation treatments than non-resistant cancer cells.

All ranges disclosed and/or claimed herein are inclusive of the recited endpoint and independently combinable. For example, the ranges of "from 2 to 10" and “2-10” are inclusive of the endpoints, 2 and 10, and all the intermediate values between in context of the units considered. For instance, reference to “Claims 2-10” or “C_2-C_{10} alkyl” includes units 2, 3, 4, 5, 6, 7, 8, 9, and 10, as claims and atoms are numbered in sequential numbers without fractions or decimal points, unless described in the context of an average number. The context of “pH of from 5-9” or “a temperature of from 5˚C to 9˚C”, on the other hand, includes whole numbers 5, 6, 7, 8, and 9, as well as all fractional or decimal units in between, such as 6.5 and 8.24.

By "significant" is meant any detectable change that is statistically significant in a standard parametric test of statistical significance such as Student's T-test, where $p<0.05$.

The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., includes the degree of error associated with measurement of the particular quantity). In some embodiments the term “about” refers to the amount indicated, plus or minus 10%. In some embodiments the term “about” refers to the amount indicated, plus or minus 5%.

The descriptions herein set forth exemplary methods, parameters and the like. It should be recognized, however, that such descriptions are not intended as a limitation on the scope of the present disclosure but is instead provided as a description of exemplary embodiments.

Results

Drug Design and Synthesis

We previously discovered an effective, azopodophyllotoxin-based small molecule that blocks GBP1:PIM1 activity *in vitro*, 9-(3-fluorophenyl)-4-(2-hydroxyethyl)-6-methoxy-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one, since dubbed SU093 (Andreoli et al., *J Med Chem,* 57**,** 7916-32, 2014). This study revealed that SU093 was more active in cell lines that were more resistant to microtubule-targeting agents, such as paclitaxel. Motivated by this work, we have now designed a SU093 analog incorporating fluorine (F) bioisostere group to develop an optimized lead compound SU056 with improved inhibitory properties capable of reducing OC disease progression and sensitizing to OC chemotherapy.

The introduction and manipulation of bioisostere groups offer several advantages in the drug design and development process, such as enhancing the desired biological or physical properties, reducing toxicity, and even altering the metabolism of a given drug compound. Fluorine, considered a classical bioisostere group for hydrogen, has been studied extensively since the first approval of a fluorine-containing drug (9α-fluorocortisol) in 1955 (Fried and Sabo, 1954). Over the years several strategies have been developed to introduce fluorine in drug design. Incorporation of fluorine can influence the lipophilicity of the drug due to the introduction of a strong dipole moment that is closely aligned with carbonyl groups and, compared to that of hydrogen, increases the van der Waals radii of the active sites (Gillis et al., 2015). Direct replacement of hydrogen or even methyl (CH_3) groups in known drugs could lead to improved potency due to the alteration of substrate susceptibility to intracellular oxidative metabolism. This has been seen in the case of paclitaxel, where the substitution of two methyl groups for fluorine in the taxol derivative (3'-difluorovinyl taxoid) enhanced the potency by at least 1,000-fold (Kuznetsova et al., 2012). Additionally, this difluoro-taxol derivative was resistant to metabolic modification by CYP 450 enzymes. Based on these observations, we modified our previously reported compound SU093 by introducing Fluorine as a bioisotere group in the ring E to obtain a new AzP derivative SU056 (Figure 1A), which was synthesized using a multicomponent reaction similar to SU093 (Andreoli et al., 2014) as described in supplementary information.

AzP Treatment Inhibits Ovarian Cancer Cell Proliferation

AzP analogs were screened against an ovarian cancer cell line panel (OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SKOV-3) with an additional screening on HEK293, SH-SY5Y, and N27 cell lines. We tested the relationship between AzPs dose and the viability of human ovarian cancer cell lines and the ID8 murine ovarian cancer cell line. As shown in Figure 1B, SU056 showed decreased IC_{50} values after 48 h of treatment compared to SU093 (In OVCAR4, OVCAR5 and ID8 cells, this decrease was up to 2 fold), a trend which was also reflected in the clonogenic assay shown in Figures 1C and 1D, where both inhibitors decreased OC colony formation in a dose-dependent and significant manner in OVCAR-8 and ID8 cells. On the other hand, SU093 moderately affect SKOV-3 at 0.5 & 1 μM concentrations.

Etoposide is natural podophyllotoxin and an approved chemotherapy drug used to treat many cancers. A major limitation of etoposide is the potential for neuropathy and neurotoxicity in long-term treatment. We tested the cytotoxic effect of etoposide, SU093, and SU056 on SH-SYSY and N27 neuronal cell lines at 10 μM dose for 48 h to compare neurotoxicity profiles. Cell viability assayed by MTT found that SU056 was markedly less cytotoxic at 49.54% (N27) and 28.51% (SH-SY5Y) less toxic than etoposide (Figure 1E). These results suggest that bioisostere modification improved overall efficacy in inhibiting OC proliferation with reduced neurotoxic side effects.

AzP Treatment Causes Cell Cycle Arrest, Apoptosis, and Cell Migration Inhibition

To determine whether proliferation inhibition was the dominant factor driving the reduction in OC viability seen with AzP treatment, we used propidium iodide and annexin-FITC stains with flow cytometry to measure the distribution of cell cycle phases after treatment. Data showed a significant, dose-dependent arrest of OC lines in the sub-G1 and G1 phases and concomitant decreases in the proportion of cells in the S or G2/M phases of the cell cycle (Figure 1F). SU056 demonstrated greater cell

cycle arrest than SU093. This trend was again mirrored in the proportion of apoptotic cells in each treated culture, where AzP treatment significantly increased apoptosis at all doses and in each OC line studied (Figure 1G). Furthermore, in the Boyden chamber assay for cell migration, SU093 inhibited cell migration by 40-46% (1 μM, $P<0.05$) in different OC cell lines. However, SU056 treatment was the most effective as it caused 78-87% (1 μM, $P<0.05$) inhibition in the same cell lines (Figure 1H). These data show that SU056 treatment-mediates cell cycle arrest, significantly decreases proliferation and migration.

AzP Inhibits Tumor Progression & Metastasis *In Vivo*

These promising results led us to test SU093 and SU056 *in vivo*. C57BL/6 mice were implanted with $2x10^6$ firefly luciferase (luc+)-expressing ID-8 syngeneic ovarian cancer cells subcutaneously. Once the tumors reached 100 mm^3 in size, we began daily intraperitoneal treatment with SU093 and SU056 at 20 mg/kg (vehicle control 30% PEG-300 in saline). Tumor growth was monitored *via* bioluminescence imaging (IVIS). SU093 and SU056 treatment significantly delay the tumor progression than vehicle control through the treatment period (Figures 2A and 2B). At 42 days, a final assessment of the tumor weight showed that SU056 treatment resulted in a 2-fold reduction in the tumor weight, whereas SU093 shrunk tumors by 1.5-fold (Figure 2C). This was accompanied by no significant impact on blood chemistry indicators such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (Figure 2D), showing that daily SU056 and SU093 are well-tolerated treatment regimens.

Due to prior observation of a significant reduction in the migratory ability of OC cells treated with the two drugs (Figure 1H), we also measured the effect of drug treatment on ovarian cancer metastases. At the end of above experiment, lungs were fixed and metastatic foci were counted under the dissecting microscope followed by H&E staining. Measurement of the metastasis in SU056-treated

mice demonstrated a 3-fold reduction in lung metastases (Figures 2E and 2F), as assessed *via* histological scoring. Based on these data and superior performance in all assays, we selected SU056 for further study.

Target Identification: SU056 Interacts with Proteins Associated with Oncogenesis

The cellular thermal shift assay (CETSA) is based on the principle of protein-ligand interactions causing a shift in protein thermal stability (Savitski et al., 2014). When a protein interacts with ligand, its thermal stability increases, and this principle allows identification of which protein targets that are engaged by small molecules or drug candidates. We used this assay to identify protein targets of SU056. OVCAR8 cells were treated with SU056 (2.5 μM) for 1.5 h, and protein lysates were analyzed for a thermal shift. The heat maps represent the thermal stability of 804 soluble proteins in the presence and absence of SU056 compare with 37°C (Figure 3A). 77% of proteins were identified in both vehicle control and SU056 treated cells and 97% of these proteins passed criteria to be adjusted to the melting curves. The compression of thermal stability between DMSO- and SU056-treated proteomes suggests that SU056 treatment increases the overall stability of cellular proteome (Figures 3A, 3B, and 3C). Out of 804 soluble proteins, SU056 treatment significantly increased the thermal stability of six proteins whose p-value < 0.01 and RSQ > 0.7 (Figure 3D). These six proteins are YBX1 (YB-1), TMSB10, SUMO2, PSMB2, TMSB4X, and CALM3 (Figure 3D). Melting curves of these proteins with and without SU056 suggest that SU056 increases thermal stability by 5.92 ± 0.86, 5.89 ± 1.18, 5.4 ± 1.08, 5.36 ± 0.76, 4.43 ± 1.31, and 4.03 ± 1.07 °C of YB-1, TMSB10, SUMO2, PSMB2, TMSB4X, and CALM3, respectively (Figures 3E and 3F). This result was further validated using western blotting (WB) (Figure 8A). All six of these proteins have previously reported to contribute to carcinogenesis.

SU056 Treatment Inhibit the YB-1

Out of six targets identified, we selected proteins whose thermal shift is above 5° in the presence of SU056 for further study of protein expression after treatment. Western blot analysis of OVCAR8 cell treated with DMSO and SU056 (1, 2.5 and 5 μM) for 12h suggests that SU056 treatment inhibits the YB-1, TMSB10, SUMO2 and PMSB2 proteins in a dose-dependent manner (Figure 4A). SU056 strongly inhibits YB-1 expression and, moreover considering the role of YB-1 in interacting with many oncogenic proteins treatment resistance factors led us to select YB-1 for further validation. To study the *in vivo* effects of SU056 treatment, we analyzed ID8 tumor xenograft samples *via* immunohistochemistry (IHC). IHC for YB-1 and MDR1 expression in vehicle and SU056 (20 mg/kg) treated tumors suggests that SU056 strongly inhibits YB-1 expression followed by downregulation of downstream MDR1 (Figure 4B). To identify the broader effects of SU056 on YB-1 and its downstream protein/activity in different ovarian cancer cell lines, we selected six different cell lines and treated them with SU056 (2.5 μM) for 12 h. Cells were analyzed for YB-1 and CD44 expression and Multidrug resistance 1 (MDR1, ABC pump mediated efflux). Results suggest that this treatment inhibits YB-1 expression by 28-56% and is accompanied by a 36-70% decrease in CD44 expression and 41-63% decrease in MDR expression (Figure 4C). This result suggests that SU056 broadly inhibits YB-1 across different OC cell lines irrespective of their genetic background and stage of cancer. To calculate the 50% YB-1 inhibitory concentration (IC_{50}) of SU056, we treated OVCAR4, OVCAR8, and SKOV3 cell lines with SU056 (0.01, 0.1,1,10 μM) for 12 h and YB-1 expression was analyzed using YB-1 ELISA. The IC_{50} values for OVCAR4, OVCAR8, and SKOV3 were 5.6±0.36 μM, 3.2 ± 0.19 μ M, and for 3.7 ± 0.21 μ M, respectively (Figure 4D). Finally, we tested the time kinetics and dose-dependent effects of SU056 on YB-1 expression. OVCAR4, OVCAR8, and SKOV3 cells were treated with SU056 (1, 2.5, and 5 μM) for 3, 6, 12, and 24 h and YB-1 expression was measured using YB-1 ELISA. Results suggest that SU056 treatment inhibits YB-1 in a time- and dose-dependent manner (Figure 4E). 5 μM treatment for 24 h inhibited YB-1 completely (100%) in all three OC cells (Figure 4E). This result was further conformed using OVCAR8 cells stably expressing mCherry tagged YB-

1. Confocal imaging and fluorescence intensity measurement of YB-1 OVCAR8 cells also suggest that SU056 treatment inhibit the YB-1 expression time and dose dependent manner (Figures 8B and 8C). Overall, results of YB-1 expression and its downstream factors (Figure 4) suggest that SU056 is a strong inhibitor of YB-1 protein and its activity.

SU056 biophysically binds to YB-1 and its cellular activity is YB-1 dependent

Previously, our group reported that SU093 interacts with GBP1 and inhibits the GBP1:PIM1 interaction (Andreoli et al., 2014). SU056 is the second generation small molecule derivative of SU093 and, to confirm whether it has the same molecular target as SU093, we performed a pulldown assay using biotinylated SU056 (Figure 5A). Pulldown from cells treated with Biotinylated-SU056 and from protein lysate indicates that SU056 physically interacts with YB-1 but not with GBP1 (Figure 5B). This result suggests that, although SU093 and SU056 have structural similarity, their targets are quite different. In further support this, we performed a Surface Plasma Resonance (SPR) screen to measure the orthogonal biophysical binding between YB-1 and SU093 or SU056. His-tagged YB-1 protein was immobilized on a GE NTA chip and different concentrations (1-100 µM) of SU093 and SU056 were tested for binding (Figure 5CD). SPR results also support that SU056:YB-1 has a strong biophysical interaction (Figure 5D) compared to SU093 (Figure 5C). To check the dependency of SU056 activity on YB-1 expression, we also tested OVCAR8 cells transfected with YB-1 knockdown using transduction of lentiviral shRNA vectors. Two different shRNA sequences (YBX1-shRNA1 and YBX1-shRNA2) were used and both yielded 80-90% inhibition in the expression of YB-1 compared to scrambled control (SC)-transduced cells (Figure 5E). A clonogenic assay was performed using these three cell lines with and without SU056 (1 µM). Results suggest that YB-1 knockdown cells are more resistant to SU056 treatment compared to SC cells (Figure 5F). Furthermore, we also performed a cell viability assay using knockdown cells to calculate IC_{50} values . The IC_{50} of SU056 for SC cells was 3.54 µM (±0.21) and for YBX1-shRNA1 and YBX1-shRNA2 was 15.84

(±0.13) and 19.15 (±0.34), respectively (Figure 5G). YB-1 knockdown increased the IC_{50} value ~5 fold compared to SC. These results show that the effect of SU056 is dependent on cellular expression of YB-1.

SU056 Modulates the YB-1 Associated Proteins and Pathways

CETSA results show that SU056 physically interacts with YB-1 and inhibits it and its activity. To study the effect of SU056 on YB-1 protein stability, we used the Cycloheximide chase (CHX) assay. Cycloheximide is a protein translation inhibitor and is used as a molecular biology tool to determine the half-life of proteins. OVCAR8 cells were treated with CHX and DMSO or SU056 (2.5 μM), and protein lysates were collected at different time points (0, 30, 60, 120, 180 min) followed by YB-1 immunoblotting. Results suggest that SU056 treatment lead the proteasomal degradation and reduces the half-life of YB-1 to from ~130 minutes to ~40 minutes (Figure 6A). YB-1 is associated with the transcription and translation of many oncogenic proteins including CD44, ABCB1/MDR1, c-Myc, and Bcl-2. Immunoblotting of whole-cell lysates from DMSO- or SU056- (2.5 μM, 12 h) treated SKOV3 and OVCAR8 cells indicate that SU056 treatment inhibits YB-1 protein (Figure 6B). This was followed by a decrease in the expression of CDK2, CDC25A, MDR1, CD44, c-Myc, and Bcl-2 and an increase in the expression of pro-apoptotic protein Bax (Figure 6B). To evaluate proteome changes lead by SU056, we undertook proteome profiling of DMSO- and SU056- (2.5 μM, 12 h) treated OVCAR8 cells. GSEA KEGG analysis of the resulting proteomics data suggest that SU056 treatment significantly induces apoptosis (ES=0.792, p-value-0.01), RNA degradation (ES=0.783, p-value-0.03), Alanine, aspartate, and glutamate metabolism (p-value-0.02), Arginine and proline metabolism (p-value-0.04), FcεRI signaling pathway (p-value-0.01), T-cell receptor signaling pathway (p-value-0.02), Natural killer cell-mediated cytotoxicity (p-value-0.03), Epithelial cell signaling in Helicobacter pylori infection (p-value-0.04), and FcγR mediated phagocytosis pathways (p-value-0.05) (Figure 6C-D, Table 1). On the other hand, SU056 treatment also inhibits the spliceosome pathway (ES=-0.413, p-value-0.02) (Figure 6CE, Table 1). YB-1 protects and stabilizes the mRNA via 5' end capping

(Evdokimova et al., 2001) and enhances exon splicing (ex. Alternative splicing of CD44) (Stickeler et al., 2001). YB-1 inhibition *via* SU056 treatment inhibits the Spliceosome pathway and induces RNA degradation and apoptosis. These results suggest that SU056 primarily targets YB-1, in turn inhibits various YB-1 associated proteins and pathways involved in cancer progression and TR.

YB-1 Inhibition *via* SU056 Treatment Sensitizes OC to Paclitaxel

YB-1 is involved in the emergence of cisplatin and taxane drug resistance (Kang et al., 2013, Mo et al., 2016). Therefore, we tested whether SU056 synergizes with chemotherapies such as paclitaxel to modulate YB-1. To measure this, we performed a dose dependent combination study of SU056 and paclitaxel. Using the MTT assay, we found that SU056 treatment significantly potentiates the cytotoxic effects of paclitaxel at 0.1, 0.5, and 1 nM doses (Figure S2, 7A). Treatments with 0.5 or 1.0 μM SU056 significantly reduced cell viability in both OVCAR8 and SKOV-3 OC cell lines. We calculated the combination index (CI) using the Chou-Talalay method and found a CI value of <1 for SU056 and paclitaxel combination. CI<1 suggests significant synergy of SU056 with the growth inhibitory effect of paclitaxel (Figure 7A). ABCB1/MDR1 drug efflux pumps are also known to play an important downstream role with YB-1 in increased taxol efflux and subsequent TR in cancer. To evaluate whether SU056 affects drug efflux, we used Alexa Fluor-488-tagged paclitaxel to measure taxol efflux *in vitro*. Results suggested that SU056 co-treatment significantly inhibits the efflux of paclitaxel in comparison to only paclitaxel-treated cells (Figure 7B). Efflux is primarily driven by the ATP-binding cassette transporters (ABC pumps) present on the cell surface (Genovese et al., 2017). We examined the expression of MDR1 and YB-1 in OVCAR8 cells treated with vehicle, SU056, and paclitaxel combinations *via* Western blot. Immunoblot results show that paclitaxel treatment alone is sufficient to upregulate the expression of YB-1 and MDR1, which is then reversed by the combined treatment of SU056 and

paclitaxel (Figure 7C). These results strongly suggest that SU056 synergizes with the efficacy of paclitaxel.

3D spheroids are an alternative tumor model that closely mimic the TR phenotype via increased drug efflux. We cultured OVCAR8 and SKOV3 cells in ultralow attachment plates with growth factor-defined media in the presence or absence of paclitaxel and SU056. Results show that a combination of SU056 and paclitaxel significantly inhibits the formation by OC cell spheroids (Figures 7D and 7E). Combination-treated cells form 78% (OVACR8) and 83% (SKOV3) fewer spheroids than the vehicle-treated cells (Figure 7E).

In a pharmacokinetics study *in vivo*, injection of 20 mg/kg SU056 led to a maximum serum concentration of 28.19 μg/mL with a $T_{1/2}$ of ~45 min. We then tested the synergism of paclitaxel and SU056 co-treatment *in vivo* in NOD-SCID mice implanted with OVCAR8 OC tumors as described above. Mice were given daily treatment of SU056 (10 mg/kg) and weekly treatment of paclitaxel (5 mg/kg) were administered intraperitoneally. Both paclitaxel and SU056 treatment independently showed significant reduction in OC tumor growth. However, the combination of paclitaxel and SU056 demonstrated a much greater reduction in OC tumor growth, effectively stabilizing disease progression over the treatment period (Figures 7F and 7G). The drug combination showed sustained inhibitory effects on tumor growth until the experiment terminated (Figure 7H). We also measured the proliferative index within the excised tumor using Ki-67 staining (Figure 7I), which showed that both SU056 and paclitaxel exerted independent anti-proliferative effects that synergized to produce a greater therapeutic effect when combined. This likely resulted from decreased paclitaxel efflux from OVCAR8 cells in the presence of SU056.

Discussion

Ovarian cancer (OC) is the fifth most common cancer in women with an overall 5-year survival rate of just 47.6% in the United States of America (USA). Surgical resection and chemotherapy are the primary treatments for OC but are limited by surgical difficulties due to the abdominal spread of metastasizing OC and a high 2-year relapse rate of 80-90% after taxane or platinum chemotherapy treatment (Jayson et al., 2014, Agarwal and Kaye, 2003). While paclitaxel offers some effect, relapsed disease is frequently TR, in which tumor cells bypass or overcome the molecular mechanisms of cytotoxicity despite ongoing treatment (Singh and Settleman, 2010, Blagosklonny and Fojo, 1999, Horwitz et al., 1986). An investigation into the mechanisms of TR in taxane-resistant OC by the Sood group revealed significantly upregulated YB-1 in treated OC patients where patients with high YB-1 expression had significantly shorter overall survival (Kang et al., 2013). Here, we report fluorine-based derivatives of podophyllotoxins as new potent and highly effective YB-1 inhibitors capable of restraining disease progression and synergizing with chemotherapy.

Overexpression of βIII-tubulin is a prominent signature of paclitaxel-TR and the GBP1:PIM1 interaction may help activate its function (De Donato et al., 2012, Mariani et al., 2011). Our group previously reported a small molecule podophyllotoxins (SU093) as an inhibitor of the GBP1:PIM1 interaction capable of overcoming taxane resistance *in vitro* (Andreoli et al., 2014). To improve the potency of SU093, we constructed a second-generation compound library through a structure-guided and bioisostere replacement strategy. Screening of this library found that a fluorine-based derivative, SU056, has markedly improved potency and safety with a different mechanism of action. We screened SU056 for its cytotoxic effects and found improved efficacy compared to SU093. SU093 and SU056 both caused G1 cell cycle phase arrest, increased apoptotic cell death, and inhibited cell migration. Both compounds also inhibited tumor progression and metastasis in the ID8 xenograft model. During treatment, neither compound caused any liver toxicity. In each assay, SU056 proved more potent than SU093 or other AzP derivatives, and therefore selected for further study.

We used the Cellular Thermal Shift Assay (CETSA) to identify targets of SU056 and found that this compound interacts with YB-1, TMSB10, SUMO2, PSMB2, TMSB4X, and CALM3. Target identification suggested that SU056 inhibits proteins that have an oncogenic role in ovarian and /or other cancers. SU056 treatment was found to decrease the expression of YB-1 in a dose and time-dependent manner in different OC cell lines. Our experiments validated that SU056 treatment strongly interact with YB-1 and inhibits and its associated downstream proteins and pathways. SU056 arrests the OC cells in the G1 phase and also inhibits the key drivers of G1/S phase (CDK2 and CDC25A). On the other hand, YB-1 plays a role in the phosphorylation and activation of CDC25A to drive G1/S phase progression (Zhao et al., 2016), and the knockdown of YB-1 leads the G0/G1 phase arrest (Harada et al., 2014). CD44, c-Myc, and MDR1 are the most prominent oncogenic downstream proteins regulated by YB-1, and SU056 treatment significantly inhibits their expression. YB-1 is a mRNA binding protein involved in nucleoprotein filament formation in cytoplasm (Kretov et al., 2019). Together with YB-2 and YB-3, YB-1 binds with the cold-shock domain of single-stranded RNA/DNA (Graumann and Marahiel, 1998). It is involved in shuttling nucleic acids in both cytoplasm and nucleus (Matsumoto and Wolffe, 1998). In the cytoplasm, it regulates RNA stability, translation activity, and alternative splicing (Chansky et al., 2001). In the nucleus, it binds to the specific promoter sequences to regulate the transcription of oncogenic proteins, including MDR1 (Bargou et al., 1997). SU056-mediated YB-1 inhibition significantly upregulates the RNA degradation pathway, while inhibiting the spliceosome pathway; a result which concords with the literatures about YB-1 function. Collectively, SU056:YB-1 interaction inhibits the YB-1 activity in different OC cell line *via* proteasomal degradation and inhibits various downstream factors involved in tumor progression and TR. These results confirm YB-1 inhibition-specific activity of SU056 as a new drug candidate.

Furthermore, we also investigated the effect of SU056 in combination with paclitaxel, and we found that lower doses of 10 mg/kg of SU056 potentiate the cytotoxic effect of paclitaxel treatment. This effect

may be because co-treatment with SU056 lowers the efflux rate of paclitaxel in OC cells. Drug efflux by cancer cells is one of the important mechanisms for the development of TR (Li and Nikaido, 2009, Gottesman and Pastan, 2015). Efflux is primarily driven by ATP binding cassette (ABC) transporters present on cell surfaces. This superfamily includes ABCB1, also known as multidrug resistance 1 (MDR1), P-glycoprotein, ABCC1 (MRP1), and ABCG2 (BCRP/MXR) (Fletcher et al., 2010). Paclitaxel is a substrate for ABCB1, and paclitaxel treatment upregulates its expression in various cancer types (Gottesman and Pastan, 1993). ABC transporters are a hallmark of both cancer progression, and TR and has been viewed as a potential therapeutic target. ABCB1 chemical inhibitors have been developed, but first, second, and third-generation compounds failed in clinical trials thus far. First-generation compounds had toxicity issues, whereas second-generation compounds such as Valspodar, showed no treatment benefit in combination with paclitaxel or carboplatin in ovarian or peritoneal cancer patients (Lhomme et al., 2008). The third-generation inhibitor Zosuquidar also failed to show any benefits (Ruff et al., 2009), leaving the inhibition of MDR1 and TR phenotype an unmet clinical goal. In testing the effects of paclitaxel, SU056, and their combination on the expression of MDR1 and YB-1, we found that paclitaxel treatment upregulates the expression of both YB-1 and MDR1, whereas SU056 alone or in combination with paclitaxel significantly decreases MDR1 and YB-1. This is supported by paclitaxel efflux data showing that SU056 treatment inhibits MDR1 and leads to higher intracellular concentrations and activity of paclitaxel.

Tumor spheroids, a common 3D cell culture model, have been used to examine stemness, efflux, and MDR1 expression changes after treatment (Wartenberg et al., 2005, Wartenberg et al., 1998, Chen et al., 2017). In our study, treating OC cells with a combination of paclitaxel and SU056 significantly inhibited their spheroid formation capability, which indicates decreased tumorigenic potential. Previous transcriptome analysis of taxane-resistant ovarian cancer cell lines has revealed elevated levels of both YB-1 and MDR1(Sun et al., 2015, Kuwano et al., 2004, Shiota et al., 2014, Wu et al., 2007). YB-1 is

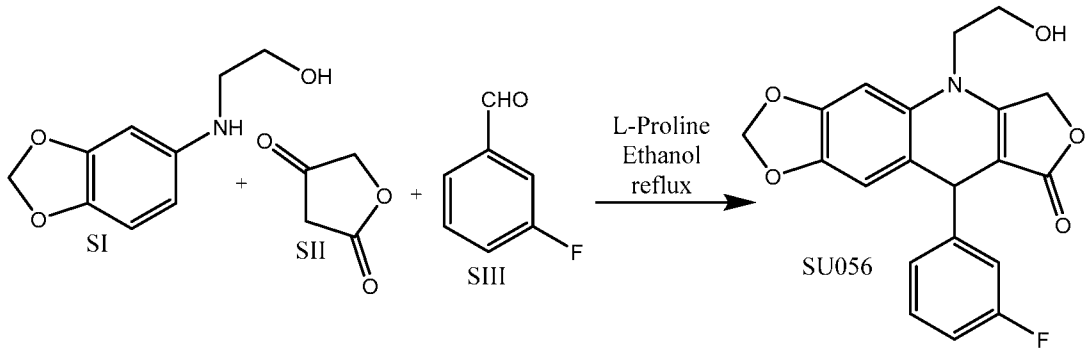
associated with transcriptional regulation of ABC transporters and epithelial-to-mesenchymal transition-associated proteins implicated in disease progression and TR (Lim et al., 2018, Wu et al., 2014, Evdokimova et al., 2009). SU056 co-treatment with paclitaxel was sufficient to halt tumor progression in the OVCAR8 xenograft model. This further suggests that SU056 and the analogs thereof described herein provide a promising strategy for OC, rescuing treatment efficacy, and increasing patient survival.

In summary, bioisostere replacement was a highly effective chemical strategy to optimize SU093 for improved efficacy and reduced toxicity, resulting in the development of SU056 and the corresponding analogs discussed herein. These AzP derivatives are the first inhibitors reported for YB-1, ultimately decreasing cell proliferation and migration, while sensitizing OC cells to the cytotoxic effects of paclitaxel *in vitro* and *in vivo*. Additional work to characterize the impact of SU056 treatment on different animal models, other cancer types, and the immune microenvironment will provide further insight into the potential of SU056 and its analogs to combat treatment resistance in the clinic.

Materials and Methods

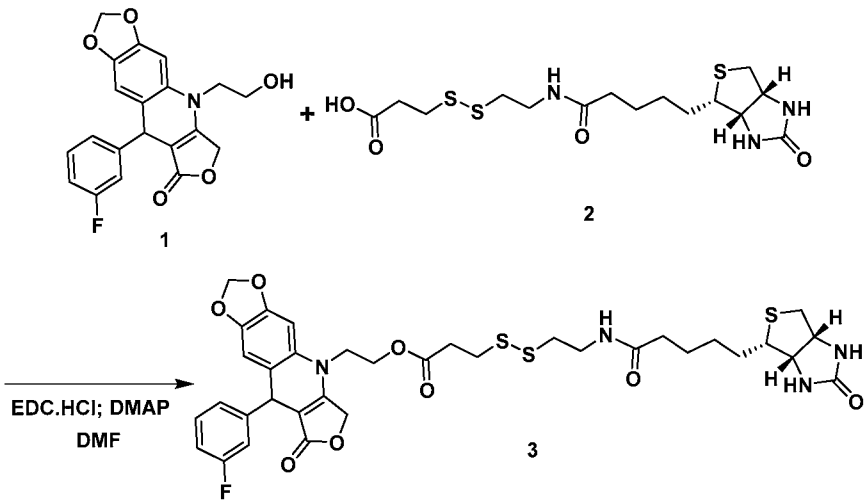
Compound synthesis: AzP derivative SU093 was synthesized and characterized as reported previously (Andreoli et al., 2014). SU056 and biotinylated SU056 were synthesized following protocol described below.

Synthesis of SU056



A solution of aryl amino alcohol SI (Kumar *et al*., 2010) (1 mmol), 3-fluorobenzaldeyde (SIII) (1.2 mmol), L-proline (0.1 mmol, 10 mol %), and tetronic acid (SII) (1.2 mmol) was prepared in anhydrous ethanol (4 mL) and the reaction mixture was refluxed for 3-4 h. Upon consumption of amino alcohol component and appearance of a fluorescent spot via TLC (9:1 of 50% EtOAc/Hex:MeCN), a slurry of silica gel was prepared and purified by flash chromatography to give SU056 as a solid (0.203Gm 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (td, *J* = 7.9, 6.1 Hz, 2H), 7.10 – 6.98 (m, 4H), 7.01 – 6.91 (m, 1H), 6.96 (s, 2H), 6.67 – 6.62 (m, 2H), 5.97 (d, *J* = 1.1 Hz, 2H), 5.91 (d, *J* = 1.1 Hz, 2H), 5.15 – 5.00 (m, 4H), 5.04 – 4.96 (m, 2H), 4.94 (s, 2H), 3.81 (d, *J* = 10.5 Hz, 1H), 3.71 – 3.58 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.59, 161.11, 150.24, 147.52, 143.76, 131.50, 130.66, 130.58, 124.02, 118.94, 114.77, 114.56, 113.69, 110.48, 101.88, 96.83, 94.82, 66.31, 58.38, 48.64. MS-ESI m/z calculated for $C_{20}H_{16}FNO_5$ $[M+H]^+$: 370.1, found 370.1.

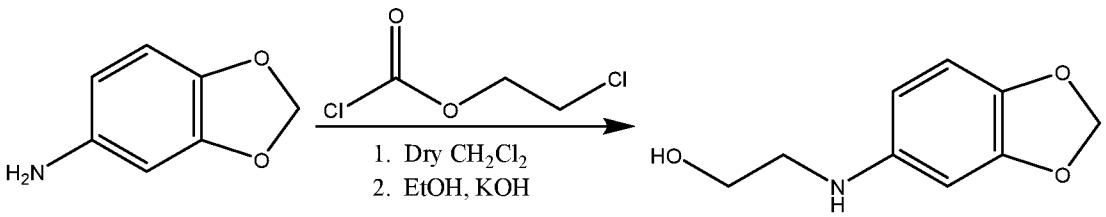
Synthesis of Biotinylated SU056



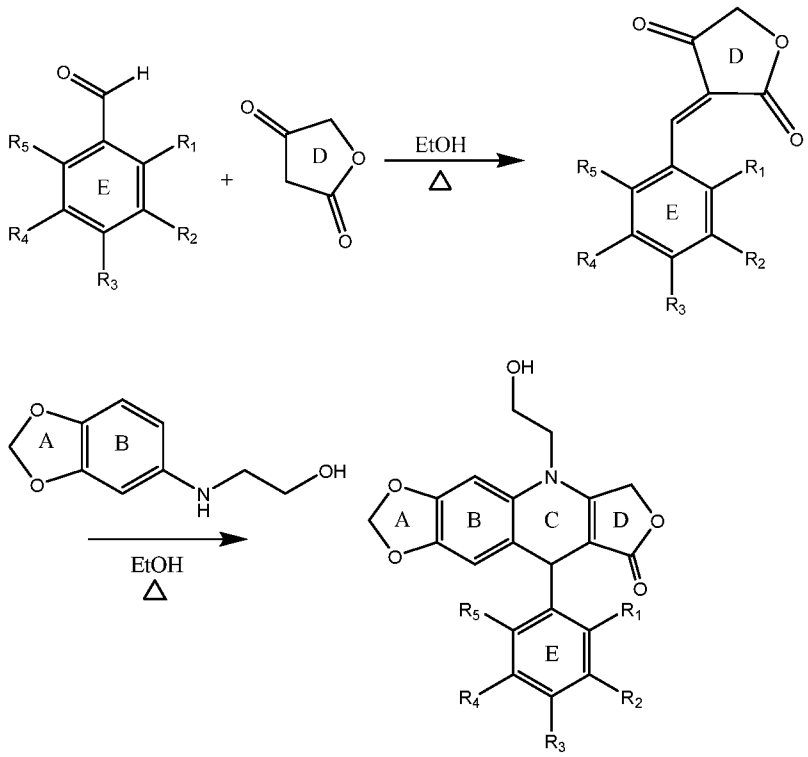
2-(9-(3-fluorophenyl)-8-oxo-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-5(8H)-yl)ethyl 3-((2-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethyl)disulfaneyl) propanoate: Under a nitrogen atmosphere, a solution of 9-(3-fluorophenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (40.0 mg, 0.108 mmol) in dry DMF (10.0 mL) was treated with 3-[2-N-(Biotinyl)aminoethyldithio]propanoic acid (44.0 mg, 0.108 mmol), EDC.HCl (31.1 mg, 0.162 mmol) and DMAP (19.8 mg, 0.162 mmol). The reaction mixture was stirred at rt for 18 h then diluted

with water (~10 mL). The mixture was then extracted with ethyl acetate (2 X 25 mL). Combined organic phase was washed with water followed by brine solution. The organic phase was dried over Na2SO4, filtered, and evaporated to dryness. The crude was purified with CombiFlash chromatography on silica gel using 0-10% of methanol in dichloromethane as an eluent. Combined pure fractions was evaporated to dryness to afford the desired product as an off-white solid (15.0 mg, 19%). 1H NMR (400 MHz, Chloroform-d) δ 7.28 – 7.21 (m, 1H), 7.14 – 6.98 (m, 1H), 6.97 – 6.78 (m, 2H), 6.65 – 6.50 (m, 2H), 6.42 – 6.21 (m, 1H), 5.98 (dt, J = 10.5, 1.3 Hz, 2H), 5.38 – 5.23 (m, 1H), 5.15 – 4.88 (m, 3H), 4.71 – 4.30 (m, 3H), 4.14 – 3.91 (m, 1H), 3.90 – 3.79 (m, 1H), 3.75 (q, J = 7.0 Hz, 1H), 3.61 – 3.32 (m, 2H), 3.19 (td, J = 7.4, 4.6 Hz, 1H), 2.95 (ddd, J = 12.8, 5.0, 1.3 Hz, 1H), 2.85 (dd, J = 8.3, 6.2 Hz, 2H), 2.80 – 2.65 (m, 2H), 2.34 – 2.13 (m, 4H), 1.70 (dq, J = 14.7, 8.1, 7.4 Hz, 3H), 1.49 (q, J = 7.8 Hz, 3H), 1.36 – 1.13 (m, 2H). LC-MS (ESI-QQQ): m/z 759.2 ([C35H39FN4O8S3 + H]+ calcd. 759.2). Purity >98% (rt 4.64 min).

General Synthesis: Compounds described herein may also be prepared by methods known in the art, including those described in the article *Synthesis of Novel Functionalized 4-Aza-2,3-didehydropodophyllotoxin Derivatives with Potential Antitumor Activity*, Kumar et al., J. Heterocyclic Chem., 47(6), 1275-1282, November 2010.



2-(benzo[d][1,3]dioxol-5-ylamino)ethan-1-ol can be prepared by slowly adding 2-chloroethyl carbonochloridate to benzo[d][1,3]dioxol-5-amine in dry dichloromethane and pyridine. The mixture is stirred at room temperature for 2.5 hours, washed with water, and dried over anhydrous magnesium sulfate and concentrated under vacuum prior to additional washing, concentration, and drying.



A benzaldehyde compound of desired substitution can be reacted with tetronic acid in ethanol and refluxed for 30 to 90 minutes to form the comparably substituted (Z)-3-benzylidenefuran-2,4(3H,5H)-dione, which then may be reacted with 2-(benzo[d][1,3]dioxol-5-ylamino)ethan-1-ol to obtain the final substituted 5-(2-hydroxyethyl)-9-phenyl-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one of interest. Synthesis of corresponding chloro analog 9-(3-chlorophenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one can also be seen in U.S. Pat. Appln. Publication No. 2017/0342086 (Kumar).

Method:

Pharmacokinetics study: SU056 Plasma concentration was determined by mass quantification analysis using an Agilent 6490 iFunnel triple quadrupole (QQQ) mass spectrometer equipped with an Agilent 1290 infinity II UHPLC. An analytical C18 column, ZORBAX C18 (Eclipse Plus, 2.1x 50 mm, 1.8 µm particle size) was used. The mobile phase was composed of 60% water buffered with 0.1% formic acid and 4mM ammonium formate and 40% acetonitrile buffered with 0.1% formic acid. The flow rate of mobile phase

was set at 0.4 mL/min and column temperature was adjusted at 30oC. The electrospray ionization source was operated in positive ion mode. Mass spectrometer parameters were optimized as: source temperature 550 oC, nebulizer gas (nitrogen) 20 psi, ion spray (IS) voltage 5000 V, collision energy 21 V. Multiple reaction monitoring (MRM) method was used for the detection of SU056 and an internal standard (IS), 4-(2-hydroxyethyl)-6-methoxy-9-phenyl-4,9-dihydrofuro[3,4-b]quinolin-1(3H)-one, a similar analogue of SU056. The precursor ion [M+H]+ and product ion for SU056 were monitored at m/z 370.0 and m/z 274.2, respectively. And the precursor ion [M+H]+ and product ion for IS were monitored at m/z 338.1 and m/z 260, respectively. A calibration curve was generated using known concentrations of SU056 and IS, and this curve was used to calculate unknown concentrations of SU056 in the plasma at different time points. SU056 was injected at the dose of 20 mg/kg to each mouse at time zero intraperitonially. Blood was collected retro-orbitally at 5, 15, 30, 60, 120, 240 and 360 minutes after injection. Blood plasma was separated via centrifugation at 7,000 RPM for 10 minutes. A 5 µL plasma was taken from each sample and mixed with a 10 µL of IS solution and 990 µL of MS-grade acetonitrile then vortexed for 30 seconds followed by a 5 minutes incubation at RT. Mixture was centrifuged at 11,000 RPM for 15 min at 4°C and supernatant was collected and further cleaned by re-centrifugation. Each protein-free plasma fraction (n=3) was used to determine the concentration of SU056 using the HPLC/MS MRM method as described above.

Table 1. Most significant (average $p < 0.05$) processes found to be enriched after 12 hours treatment with SU056 according to GSEA using the KEGG pathway database. Proteins shown in bold were enriched in each pathway.

KEGG Pathway Increase	p-value	Proteins in pathway detected (bold are enriched)
Apoptosis	0.01	AIFM1, PRKAR2A, PIK3CG, CAPN1, CAPN2, CYCS, DFFA, PRKAR1A
Alanine, aspartate, and glutamate metabolism	0.02	ADSS, GLUD1, ADSL, GFPT1, GOT1, GOT2, GLS, CAD
RNA degradation	0.03	PAPOLB, LSM2, DDX6, ENO2, HSPA9, HSPD1, ENO1

Arginine and proline metabolism	0.04	ALDH18A1, GLUD1, GOT1, SMS, OAT, ALDH9A1, GOT2, P4HA1, CKB, SRM, GLS, LAP3, ALDH7A1
FcεRI signaling pathway	0.01	VAV2, MAPK14, PIK3CG, RAC1, GRB2
T-cell receptor signaling pathway	0.02	VAV2, MAPK14, PIK3CG, RHOA, GRB2, CDC42, PAK2
Natural killer cell mediated cytotoxicity	0.03	VAV2, PTPN11, PIK3CG, RAC1, GRB2, ICAM1
Epithelial cell signaling in helicobacter pylori infection	0.04	MAPK14, ATP6V1G1, PTPN11, RAC1, EGFR, ATP6V1A, CDC42
FcγR mediated phagocytosis	0.05	MARCKSL1, VAV2, ARPC4, PIK3CG, RAC1, CFL2, VASP, ARPC2, ARPC3, ARPC5, GSN, ARPC1B, MARCKS, CDC42, CFL1, DNM2, ARPC5L
KEGG Pathway Decrease	p-value	Proteins in pathway detected (bold are enriched)
Spliceosome	0.02	SF3A3, SF3B2, CWC15, SF3B1, SF3A2, SF3B3, SNRPA1, RBM25, SRSF7, SNW1, EFTUD2, DDX42, SF3B4, HNRNPA3, PCBP1, ACIN1, SRSF10, NHP2L1, PRPF40A, CDC5L, DDX39B, SF3A1, U2SURP, U2AF1, PPIL1, HSPA8, RBM8A, SNRNP200, RBMX, PRPF8, SNRPD2, HNRNPA1, CTNNBL1, MAGOH, SRSF1, HSPA1B, SRSF4, HNRNPM, HSPA6, SRSF3, HNRNPK, SNRPC, SNRNP70, SRSF9, PUF60, DDX5, PRPF19, DHX15, HNRNPC, HNRNPU, SNRPD3, U2AF2, SNRPB, LSM2, EIF4A3, SRSF2

Cell lines: Human ovarian cancer (OC) OVCAR3, OVCAR4, OVCAR5, OVCAR8, and SKOV-3 cell lines were obtained from the NCI cell line repository (DTP). SH-SY5Y and N27 cell lines were obtained from Dr. Manish Chamoli (Buck Institute, CA, USA). ID8 and Luciferase-tagged ID8 cells were obtained from Dr. Erinn Rankin (Stanford University, CA, USA). Scrambled control (SC), YBX1 knockdown (1 & 2) and mCherry-YBX1 OVCAR8 cell lines were created using lentiviral based transduction and selected using puromycin resistance followed by cell shorting. All OVCAR cells were maintained in RPMI-1640 (Corning, USA; #10-040-CV) supplemented with 10% FBS (Corning, USA; #35-015-CV) and 1% Antibiotic-Antimycotic solution (Gibco, USA; #15240062). SKOV3 and ID8 cells were maintained in DMEM media (Corning, USA; #10-013-CV) supplemented with 10% FBS and 1% Antibiotic-Antimycotic solution. All cells were maintained at 37°C and 5% CO_2. SH-SY5Y and N27 cells were maintained in DMEM/F12 media (Hyclone, #SH30525.01) supplemented with 10% FBS and 1% Antibiotic-Antimycotic solution. All cells

were maintained at 37°C and 5% CO_2. OVCAR8 cells were tagged with Luciferase using lentiviral vector-based plasmid pLenti PGK Blast V5-LUC (w528-1) (gifted by Eric Campeau & Paul Kaufman, Addgene # 19166) and selected using blasticidin. Luciferase-tagged cells were maintained as described above.

Cell viability assay: Cell viability was assessed using the standard MTT assay protocol. In brief, 5,000 cells were plated in each well of 96 well plates (Corning-Costar, #3598) and allowed to attach for 24 h. Cells were treated with a respective concentration of compounds for respective time points. A stock solution of each compound was prepared in DMSO. DMSO concentration was kept constant and maintained below 0.1%. After each incubation, 50 μL of 0.5 mg/ml MTT solution prepared in 1X PBS was added to each well followed by 1h incubation at 37°C and 5% CO_2. MTT solution and media were then removed, and MTT formazan crystals were dissolved in 100 μL of DMSO per well. Absorbance at 570 nm for each well was recorded using a multimode plate reader to quantitate MTT crystallization. Each absorbance value was normalized to controls and converted into percent cell viability.

Clonogenic assay: 300 suspended OC cells were plated in each well of 12 well plates (Corning-Costar, #3598) and incubated for 24 h for attachment. The media was replaced after 24 h with media containing a respective concentration of the test compound and incubated for 5-8 days until visible colonies appeared in vehicle-treated wells. Cells were then washed and fixed with 2% paraformaldehyde, followed by washing and staining with 0.5% Crystal violet for 1h. Cells were de-stained using DI water and allowed to dry. Colonies were counted under the microscope at 100X magnification.

Cell cycle analysis: 30,000 OC cells were plated in each well of 12 well plates and incubated for 24 h. Cells were treated with a respective concentration of test compounds for 6 h. Both live and dead cells were collected via trypsinization and cell pellets were fixed with 70% ethanol. Fixed cells were stained

using propidium iodide (PI) cocktail (80 µg/mL RNase A and 50 µg/mL PI in saponin-EDTA solution) and incubated at 4°C overnight. Each sample was analyzed using a Guava easyCyte Flow Cytometer (Millipore, Burlington, MA). FlowJo software calculated the % cells in each cell cycle phase.

Apoptotic cell death assay: Cells were plated and treated as described in cell cycle analysis assay above. Treated cells were incubated for 24 h and both live and dead cells were collected. Cells were stained with Annexin V and PI using FITC Annexin V apoptosis detection kit (BD Pharmingen, San Jose, CA) by following the manufacturer's protocol. Guava easyCyte Flow Cytometer was used to analyze the stained cells.

Cell migration assay: 1 x 10^5 cells were plated in 60 mm cell culture dishes (Corning-Falcon, #353002) and incubated for 24 h. Cells were treated with a respective concentration of each compound for 12 h. Cells were trypsinized and collected in a conical tube for each plate. Pellets of cells were resuspended, and live cells were counted using a hemocytometer and trypan blue staining. 40,000 live cells were then plated in the upper chamber of 8-micron transwell (Corning-Falcon, #353097) with 0.2% FBS media. The lower chamber contained 10% FBS complete media. Cells were incubated at 37°C and 5% CO_2 for 16h. Each transwell was washed, swiped for non-migrated cells and fixed using 75% ethanol. Fixed transwells were stained with 0.5% crystal violet for 1h. De-stained transwell membranes were cut and mounted on a slide using DPX mounting media. Migrated cells were counted under the microscope at 100X magnification.

Cellular Thermal Shift Assay (CETSA): The assay was performed as previously described (Savitski et al., 2014). Briefly, OVCAR8 cells were treated with vehicle (DMSO) or SU056 (2.5 µM) at 70-80% confluency for 1.5 h. Cells were harvested and washed with 1x PBS twice. Cells were pelleted and resuspended in PBS. 10 different PCR tubes with 1 x 10^6 cells/tube (in 100 µL PBS) were prepared for both the groups. Tubes were exposed to respective temperatures (37, 41, 44, 47, 50, 53, 56, 59, 63, 67°C) for 3 min using a thermal cycler (Biorad, CA, USA) followed by 2 min incubation at room

temperature. Each tube was snap-frozen in liquid nitrogen. Cells were lysed using freeze/thaw cycle, and soluble and insoluble fractions were separated by centrifugation at 14000 RPM for 30 min at 4°C. An equal amount of soluble fraction for each temperature of both groups was labeled with Tandem Mass Tag (TMT) using manufacturer's protocol (TMT10plex™ Isobaric Label Reagent Set, # 90110, Thermo Fisher Scientific, Waltham, MA). TMT labeled samples were analyzed using LC-MS/MS in triplicates, as previously described by our group (Going et al., 2018).

CETSA protein quantification, normalization, curve fitting, estimation of slope, and melting point and statistical analysis: Proteins were quantified from individual peptide spectra by a sum-based bootstrap algorithm using each corresponding TMT reporter ion intensity after correcting for isotope impurities using MaxQuant (Cox and Mann, 2008) in both vehicle and SU056 treated samples. In each sample, the lowest temperature was used as reference to calculate the log2 ratio of signal of the soluble fraction in each temperature, and each signal was compared with the highest temperature to estimate the percentage of signal lost in the soluble fraction. A fitting model of the curves to the S-curves with the Boltzmann Equation was used to describe the statistical behavior in a thermodynamic system not in a state of equilibrium, like a denaturalization curve, using the same principles as previously described (Savitski et al., 2014). To this end, the sum of squares difference between the original and Bolztmann adjusted curve was minimized using a brute-force algorithm with R, solving the Bolztmann equation to calculate the slope of the melting curve and the half-value and the temperature in which half of the protein has been denatured. The melting point differences between the fitted curves with correlations above 0.75 and p-value less than 0.01 were considered as specific interactors of the drug.

Immunoblotting: Once 70% confluency of OC cells plated in 100 mm cell culture was obtained, dishes were treated with a respective concentration of SU056 for a respective time. At the end of treatment, cells were collected and lysed using M-PER™ lysis solution (Thermo Scientific, #78503), supplemented

with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, #78440). An equal amount (40-60 µg) of proteins was resolved using 8%/10%/12% SDS-PAGE gel electrophoresis. Proteins were then transferred onto a PVDF membrane (BioRad, #162-01277). Blocked membranes were incubated with respective primary antibody solution prepared in 5% nonfat milk TPBS at 4°C overnight with gentle rocking. Primary antibody-probed membranes were washed and probed with respective HRP-conjugated secondary antibody. Proteins were detected using Immobilon® Crescendo Western HRP Substrate (Millipore, Germany) and visualized on an IVIS Lumina Imaging System (Perkin Elmer, Waltham, MA). The following primary and secondary antibodies were used: YB-1 (Cell signaling technology (CST), #8475; 1:2000), TMSB10 (R&D Systems, #AF6429; 1:2000), SUMO2/3 (CST, #4971; 1:1000), PSMB2 (Bethyl Laboratories, #A303817AT; 1:1000), MDR1 (CST, #13978; 1:2000), CD44 (CST, #37259; 1:1000), c-Myc (Novusbio, #NB600-302SS; 1:2000), CDK2 (CST, #2546; 1:2000), CDC25A (CST, # 3652; 1:1000), Cyclin E (CST, #4132; 1:2000), Bax (CST, #5023; 1:1000), Bcl-2 (CST, #2876; 1:1000), GBP1 (Abnova, #H00002633-PW1, 1:2000), β-actin (Novusbio, #NB600-501SS; 1:10000), anti-mouse IgG HRP-linked antibody (CST, #7076, 1:5000), and anti-rabbit IgG HRP-linked antibody (CST, #7074, 1:5000).

Total YB-1 sandwich ELISA: Total YB-1 protein level was analyzed using PathScan® Total YB1 Sandwich ELISA Kit (Cell Signaling, #12543) by following the manufacturer's protocol.

Multidrug Resistance Assay: The effect of SU056 on Multidrug Resistance of different OC cells was assayed by using the Multidrug Resistance Assay Kit (Fluorometric MDR Assay) (Sigma-Aldrich, #MAK161), following the manufacturer's protocol.

CD44 ELISA: The effect of SU056 on CD44 expression of different OC cells was assayed by using Human CD44 ELISA Kit (Colorimetric) (Novusbio, #NBP1-86819), following the manufacturer's protocol.

Pulldown assay using biotinylated SU056: protein pulldown assay was performed using biotinylated SU056. 1. Pulldown from cells: OVCAR8 cells were treated with 2.5 µM biotinylated SU056

for 1.5 h. Treated cells were collected and lysed using M-PER™ lysis solution (Thermo Scientific, #78503), supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, #78440). 300 µg protein was incubated with magnetic conjugate streptavidin bead (CST, #5947) at 4°C on rocker overnight. 2. Pulldown from cell lysates: OVCAR8 cells were collected and lysed using M-PER™ lysis solution (Thermo Scientific, #78503), supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, #78440). 1000 µg protein was incubated with 10 µM biotinylated SU056 at 4°C on rocker overnight followed by overnight incubation with magnetic conjugate streptavidin bead (CST, #5947) at 4°C. After both the pulldown, biotin-streptavidin conjugates were pulldown and washed using magnetic rack. After three washing, beads were resuspended in 2X SDS sample buffer followed by heating at 90-100°C for 5 min. Samples were resolved and probed with GBP1 (Abnova, #H00002633-PW1, 1:2000) and YB-1 (CST, #8475; 1:2000) as described above in immunoblotting. Samples of protein lysates from OVCAR8 with and without biotinylated SU056 (2.5 µM) treatment (input), samples from pulldown using only biotin and only streptavidin beads were also resoled as experimental controls. Surface Plasmon Resonance (SPR): experiments were performed using a Biacore T200 (GE Healthcare) instrument at 25°C. The His-tagged (N-Terminal) YB-1 protein (Novusbio, # NBP2-30101) was captured via the His-tag on an NTA chip (GE Healthcare) and immobilized through amine coupling amine coupling chemistry using N-hydroxysuccinimide (NHS) and N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (GE Healthcare). All small molecule (SU093 and SU056) analysis experiments were performed in PBS (10 mM Phosphate buffer, 2.7 mM KCl, 0.137 NaCl) running buffer pH adjusted to yield pH 7.4 when supplemented with 5% DMSO. To investigate binding of the compounds or at 6 different (1-100 µM) concentrations prepared from a 10 mM stock solution, with a final concentration of 5% DMSO were injected over the different immobilized ligands at a flow rate of 30 µl/min for either 75 or 120 seconds with a regeneration time of 600 seconds. After each injection the flow delivery system was washed with 50% DMSO. DMSO solvent correction curves were generated by injecting the running

buffers with serial concentrations of DMSO ranging from 4.5 to 5.8%. All data were corrected for non-specific binding by subtracting the signal measured in a control cell lacking immobilized ligand.

Global proteome profiling: OVCAR8 cells were treated with vehicle or SU056 (2.5 μM) for 12 h. Cells were washed and lysed (100 mM triethylammonium bicarbonate (TEAB, Thermo Fisher Scientific) with 1% sodium dodecyl sulfate (SDS)). Samples were processed and labeled with TMT using manufacturer's protocol (TMTsixplex™ Isobaric Label Reagent Set, # 90061, Thermo Fisher Scientific, Waltham, MA). Samples were analyzed using LC-MS/MS in triplicates as previously described by our group (Going et al., 2018).

Combination index calculation: The combination index (CI) for paclitaxel and SU093 or SU056 was calculated using the Chou-Talalay Method (Chou, 2010). CI values were computed via CampuSyn software. CI<1, CI=1, and CI>1 indicate the synergistic, additive and antagonistic effect of the combination.

Paclitaxel efflux assay: OC cells were treated with 5 nM Oregon Green™ 488-conjugated paclitaxel (Molecular Probes, # P22310) for 1 h. Cells were washed and incubated in phenol red-free DMEM media supplemented with 10% FBS. After each respective time point (30, 60, 120, 180 minutes), the media was collected and centrifuged to remove floating cells. The fluorescence intensity of efflux paclitaxel in media was read at Ex 496 and Em 524 using a multimode plate reader.

Spheroid culture: OC cells at a density of 100 per well were plated in an ultra-low attachment 24 well plate (Corning, #3473) in MEGM media (Lonza, #CC-3150) supplemented with hEGF, insulin, hydrocortisone BPE, and 2-mercaptoethanol. Cells were treated with each respective compound and incubated for 6-8 days. The number of spheroids was counted under the microscope at 40X magnification. 5 different fields from each well were imaged at 100X magnification.

In vivo xenograft model and drug efficacy study: All animal experiments were reviewed and approved by the Animal Care and Use Committee of Stanford University, CA, USA. Luciferase tagged ID8

(2×10^6) or OVCAR8 (5×10^6) cells were implanted into the right flank of 6-7-week-old female C57BL/6 mice and NOD/SCID mice, respectively. Respective treatment began after tumors grew to 100-200 mm^3 diameters. The ID8 syngeneic mice model was treated with a vehicle (30% PEG-300 in saline), 20 mg/kg SU093, and 20 mg/kg SU056 intraperitoneally (IP) daily for 42 days. At the end of treatment, blood was collected from each mouse and analyzed for liver toxicity parameters, including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALKP). OVCAR8 xenograft mice model was treated with vehicle (30% PEG-300 in saline), 5 mg/kg paclitaxel (once a week), 10 mg/kg SU056 (daily), a combination of paclitaxel (5 mg/kg, once a week) and SU056 (10 mg/kg, daily) for 4 weeks. Mice from both studies were euthanized, the tumor and different organs were collected and fixed in neutral buffered formalin and further processed for immunohistochemical analysis.

Immunohistochemistry: Fixed tumors and organs were embedded in paraffin. Each block was cut in 5 μm sections and fixed on poly-L lysine-coated slides. Paraffin sections were deparaffinized and rehydrated. Antigen retrieved sections were incubated with primary antibody followed by HRP conjugated secondary antibody and Dab staining using ImmPACT™ DAB kit (Vectorlabs, CA). Counterstained sections were dehydrated and mounted using VectaMount™ (Vector labs). Biotinylated horse anti-mouse IgG (Vector Labs, CA) and horse anti-rabbit IgG (Vector Labs, CA) were used as secondary antibodies. The following primary antibodies were used: YB-1 (Cell signaling, #8475; 1:100) and anti-MDR1 (Cell signaling, #13978; 1:500) and Ki67 (Biolegend, #350502; 1:500).

Statistical analysis: Each set of data was analyzed for its statistical significance using GraphPad Prism 6 software. Each result is represented in Mean ± SD. P-values are denoted using * as follows: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Presence of expressed YBX1 protein in cancer cells referenced herein may be identified by methods known in the art. Non-limiting examples include the YBX1 ELISA Kit available from antibodies-online Inc, Jones Blvd 321, Limerick, PA 19464 (Catalog No. ABIN6975583).

In immunohistochemical methods for human testing, YB1 (D2B12) Rabbit mAb #8475 (available from Cell Signal Technology online at https://www.cellsignal.com/products/primary-antibodies/yb1-d2b12-rabbit-mab/8475?site-search-type=Products&N=4294956287&Ntt=ybx1&fromPage=plp .

Significance

New chemotherapeutic strategies are urgently needed to improve the treatment of cancer, where late diagnosis and high risk of relapse collude with treatment resistance (TR) to cause high mortality despite intensive chemotherapy treatment regimens. Y box binding protein 1 (YB1 or YBX1) is the multifunction protein which binds to the DNA and RNA and is associated with tumor progression and the emergence of TR. YB-1 plays the important role in transcription, translation and RNA stabilization of various oncogenic proteins. Role of YB-1 is very well established in various cancers but there is no small molecule inhibitor available/reported so far. Herein, we report a novel azopodophyllotoxin (AzP) derivative, SU056, that inhibits disease progression *via* YB1 inhibition. This first-in class YB-1 inhibitor potently inhibits the ovarian cancer (OC) cell proliferation and resistance to apoptosis and arrests the cells in G1 phase. This treatment leads to an enrichment of proteins associated with apoptosis and RNA degradation pathway and downregulates the spliceosome pathway. *In vivo*, SU056 independently restrains ovarian cancer progression and exerts a synergistic effect with paclitaxel to further reduce disease progression with no liver toxicity. Moreover, *in vitro* mechanistic studies showed delayed disease progression via inhibition of drug efflux and multi-drug resistance 1 (MDR1) and significantly low neural toxicity as compared to Etoposide. These data suggest that YB-1 inhibitor may be an effective strategy to reduce OC progression, TR and decrease patient mortality.

Data availability

The mass spectrometry proteomics data have been deposited to the PRIDE Archive (http://www.ebi.ac.uk/pride/archive/) via the PRIDE partner repository with the data set identifier PXD022332.

References

AGARWAL et al., 2003. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer,* 3**,** 502-16.

ANDREOLI et al., 2014. Identification of the first inhibitor of the GBP1:PIM1 interaction. Implications for the development of a new class of anticancer agents against paclitaxel resistant cancer cells. *J Med Chem,* 57**,** 7916-32.

BARGOU et al., 1997. Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic MDR1 gene expression. *Nat Med,* 3**,** 447-50.

BLAGOSKLONNY et al., 1999. Molecular effects of paclitaxel: myths and reality (a critical review). *Int J Cancer,* 83**,** 151-6.

BLATTNER et al., 2000. UV-Induced stabilization of c-fos and other short-lived mRNAs. *Mol Cell Biol,* 20**,** 3616-25.

CHANSKY et al., 2001. Oncogenic TLS/ERG and EWS/Fli-1 fusion proteins inhibit RNA splicing mediated by YB-1 protein. *Cancer Res,* 61**,** 3586-90.

CHEN et al., 2017. The STAT3-miRNA-92-Wnt Signaling Pathway Regulates Spheroid Formation and Malignant Progression in Ovarian Cancer. *Cancer Res,* 77**,** 1955-1967.

CHOU, T. C. 2010. Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res,* 70**,** 440-6.

CHRISTIE et al., 2017. Acquired chemotherapy resistance in ovarian cancer. *Ann Oncol,* 28**,** viii13-viii15.

COX et al., 2008. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol,* 26**,** 1367-72.

DE DONATO et al., 2012. Class III beta-tubulin and the cytoskeletal gateway for drug resistance in ovarian cancer. *J Cell Physiol,* 227**,** 1034-41.

DIDIER et al., 1988. Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. *Proc Natl Acad Sci U S A,* 85**,** 7322-6.

DIETL, J. 2014. Revisiting the pathogenesis of ovarian cancer: the central role of the fallopian tube. *Arch Gynecol Obstet,* 289**,** 241-6.

EL-NAGGAR et al., 2015. Translational Activation of HIF1alpha by YB-1 Promotes Sarcoma Metastasis. *Cancer Cell,* 27**,** 682-97.

EVDOKIMOVA et al., 2001. The major mRNA-associated protein YB-1 is a potent 5' cap-dependent mRNA stabilizer. *EMBO J,* 20**,** 5491-502.

EVDOKIMOVA et al., 2009. Translational activation of snail1 and other developmentally regulated transcription factors by YB-1 promotes an epithelial-mesenchymal transition. *Cancer Cell,* 15**,** 402-15.

FLETCHER et al., 2010. ABC transporters in cancer: more than just drug efflux pumps. *Nat Rev Cancer,* 10**,** 147-56.

FRANZESE et al., 2019. PARP inhibitors in ovarian cancer. *Cancer Treat Rev,* 73**,** 1-9.

FRIED et al., 1954. 9α-Fluoro Derivatives of Cortisone and Hydrocortisone. *Journal of the American Chemical Society,* 76**,** 1455-1456.

GENOVESE et al., 2017. Not only P-glycoprotein: Amplification of the ABCB1-containing chromosome region 7q21 confers multidrug resistance upon cancer cells by coordinated overexpression of an assortment of resistance-related proteins. *Drug Resist Updat,* 32**,** 23-46.

GILLIS et al., 2015. Applications of Fluorine in Medicinal Chemistry. *Journal of Medicinal Chemistry,* 58, 8315-8359.

GOING et al., 2018. Quantitative Proteomic Profiling Reveals Key Pathways in the Anticancer Action of Methoxychalcone Derivatives in Triple Negative Breast Cancer. *J Proteome Res,* 17, 3574-3585.

GOODARZI et al., 2015. Endogenous tRNA-Derived Fragments Suppress Breast Cancer Progression via YBX1 Displacement. *Cell,* 161, 790-802.

GOTTESMAN et al.,1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem,* 62, 385-427.

GOTTESMAN et al., 2015. The Role of Multidrug Resistance Efflux Pumps in Cancer: Revisiting a JNCI Publication Exploring Expression of the MDR1 (P-glycoprotein) Gene. *J Natl Cancer Inst,* 107.

GRAUMANN et al., 1998. A superfamily of proteins that contain the cold-shock domain. *Trends Biochem Sci,* 23, 286-90.

HARADA et al., 2014. YB-1 promotes transcription of cyclin D1 in human non-small-cell lung cancers. *Genes Cells,* 19, 504-16.

HORWITZ et al., 1986. Taxol: mechanisms of action and resistance. *Ann N Y Acad Sci,* 466, 733-44.

JAYSON et al., 2014. Ovarian cancer. *Lancet,* 384, 1376-88.

JUNG et al., 2018. Clinical Importance of Myc Family Oncogene Aberrations in Epithelial Ovarian Cancer. *JNCI Cancer Spectrum,* 2.

JUNG et al., 2017. A Myc Activity Signature Predicts Poor Clinical Outcomes in Myc-Associated Cancers. *Cancer Res,* 77, 971-981.

KANG et al., 2013. Role of focal adhesion kinase in regulating YB-1-mediated paclitaxel resistance in ovarian cancer. *J Natl Cancer Inst,* 105, 1485-95.

KRETOV et al., 2019. YB-1, an abundant core mRNA-binding protein, has the capacity to form an RNA nucleoprotein filament: a structural analysis. *Nucleic Acids Res,* 47, 3127-3141.

KUWANO et al., 2004. The role of nuclear Y-box binding protein 1 as a global marker in drug resistance. *Mol Cancer Ther,* 3**,** 1485-92.

KUZNETSOVA et al.,2012. Synthesis and Biological Evaluation of Novel 3'-Difluorovinyl Taxoids. *J Fluor Chem,* 143**,** 177-188.

LAIRD-OFFRINGA et al., 1990. Poly(A) tail shortening is the translation-dependent step in c-myc mRNA degradation. *Mol Cell Biol,* 10**,** 6132-40.

LHOMME et al., 2008. Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer. *J Clin Oncol,* 26**,** 2674-82.

LI et al., 2009. Efflux-mediated drug resistance in bacteria: an update. *Drugs,* 69 (12)**,** 1555-623.

LIM et al.,2018. Targeting metabolic flexibility via angiopoietin-like 4 protein sensitizes metastatic cancer cells to chemotherapy drugs. *Mol Cancer,* 17**,** 152.

LYABIN et al., 2014. YB-1 protein: functions and regulation. *Wiley Interdiscip Rev RNA,* 5**,** 95-110.

MAITY et al., 2011. Class III beta-tubulin (TUBB3): more than a biomarker in solid tumors? *Curr Mol Med,* 11**,** 726-31.

MATSUMOTO et al., P. 1998. Gene regulation by Y-box proteins: coupling control of transcription and translation. *Trends Cell Biol,* 8**,** 318-23.

MO et al., 2016. Human Helicase RECQL4 Drives Cisplatin Resistance in Gastric Cancer by Activating an AKT-YB1-MDR1 Signaling Pathway. *Cancer Res,* 76**,** 3057-66.

RUFF et al., 2009. A randomized, placebo-controlled, double-blind phase 2 study of docetaxel compared to docetaxel plus zosuquidar (LY335979) in women with metastatic or locally recurrent breast cancer who have received one prior chemotherapy regimen. *Cancer Chemother Pharmacol,* 64**,** 763-8.

SAUPE et al., 2015. Differential expression of the multidrug resistance 1 (MDR1) protein in prostate cancer cells is independent from anticancer drug treatment and Y box binding protein 1 (YB-1) activity. *World J Urol,* 33**,** 1481-6.

SAVITSKI et al., 2014. Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science,* 346**,** 1255784.

SEIFTER, E. J. 1997. Cancer: Principles and Practice of Oncology, 5th EditionVincent T. DeVita, Jr., Samuel Hellman, Steven A. Rosenberg, eds. Philadelphia:Lippincott-Raven Publishers, 1997.3125 pp., illus. ISBN 0-397-51573-4. *JNCI: Journal of the National Cancer Institute,* 89**,** 353-353.

SHIOTA et al., 2014. Targeting ribosomal S6 kinases/Y-box binding protein-1 signaling improves cellular sensitivity to taxane in prostate cancer. *Prostate,* 74**,** 829-38.

SINGH, A. & SETTLEMAN, J. 2010. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene,* 29**,** 4741-51.

SOBOCAN et al., 2020. The Communication Between the PI3K/AKT/mTOR Pathway and Y-box Binding Protein-1 in Gynecological Cancer. *Cancers (Basel),* 12.

STICKELER et al., 2001. The RNA binding protein YB-1 binds A/C-rich exon enhancers and stimulates splicing of the CD44 alternative exon v4. *EMBO J,* 20**,** 3821-30.

SUN et al., 2015. Integrative transcriptomics-based identification of cryptic drivers of taxol-resistance genes in ovarian carcinoma cells: Analysis of the androgen receptor. *Oncotarget,* 6**,** 27065-82.

WARTENBERG et al., 1998. Development of an intrinsic P-glycoprotein-mediated doxorubicin resistance in quiescent cell layers of large, multicellular prostate tumor spheroids. *Int J Cancer,* 75**,** 855-63.

WARTENBERG et al., 2005. Regulation of the multidrug resistance transporter P-glycoprotein in multicellular prostate tumor spheroids by hyperthermia and reactive oxygen species. *Int J Cancer,* 113**,** 229-40.

WU et al.,. 2007. YB-1 is a Transcription/Translation Factor that Orchestrates the Oncogenome by Hardwiring Signal Transduction to Gene Expression. *Transl Oncogenomics,* 2**,** 49-65.

WU et al., 2014. Cell fate factor DACH1 represses YB-1-mediated oncogenic transcription and translation. *Cancer Res,* 74**,** 829-39.

ZENG et al., 2018. Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13. *Elife,* 7.

ZHAO et al., 2016. YBX1 regulates tumor growth via CDC25a pathway in human lung adenocarcinoma. *Oncotarget,* 7**,** 82139-82157.

Figure 1: A) Lead optimization of SU093 to obtain SU056. B) IC_{50} values of SU093 and SU056 on various ovarian cancer cells. Clonogenic survival of OC cells treated with SU093 or SU056. 300-500 ovarian cancer cells were plated/well of 12 well plate and allow to attach for 24h. Cells were treated with SU093 and SU056 and incubated further for 7 days. Each well was stained with crystal violet and colonies were counted under 10X microscope. C) Representative colony formation from respective wells. D) Number of colonies formed after SU093 and SU056 treatment. E) % inhibition values of etoposide, SU093, and SU056 treatment at 10 μM concentration for 48 h in neuronal (SH-SY5Y, N27) and HEK293 cells. F) Cell cycle distribution of propidium iodide (PI)-stained OVCAR8, SKOV3, and ID8 cells. Effects of SU093 and SU056 on cell cycle distribution showing G1 phase arrest after 12 h treatment. G) Effects of SU093 and SU056 on apoptotic cell death analyzed by Annexin-FITC staining. Both the compounds induce apoptotic cell death in ovarian cancer cells after 24 h treatment. H) Cell migration assay. Imaging of cells after 16 h through Boyden chambers showed that treatment by SU093 and SU056 significantly reduces the cell migration property of ovarian cancer cells. Data are shown as mean ± SD of triplicate samples. * $P<0.05$, significantly different compared with respective controls by one-way ANOVA followed by Dunnett's test.

Figure 2. SU093 and SU056 inhibit mice ovarian ID-8 tumor xenograft growth in C57BL/6 mice. Mice were subcutaneously injected with ID-8 cells mixed with Matrigel in 1:1 ratio and the drug treatment started when tumors reached 100mm^3. Mice were intraperitoneally (IP) injected with either vehicle (30% PEG300 in saline) or 20 mg/kg SU093 or SU056 for 42 days daily. A) Representative images of mice after 42 days of drug treatment showed tumor regression compared to control. B) Tumor volume/mouse as a function of time. Data shown are mean ± SD from 5 mice in each group. * $P< 0.05$, compared with respective control. C) Tumor weight/mouse at the end of the study. Data shown are mean ± SD from 5 mice in each group. * $P< 0.05$, ** $P<0.01$, *** $P<0.001$, compared with respective control. D) Liver toxicity parameters at the end of 42 days showing no significant difference between control, SU093, and SU056. E-F) Lung metastasis assay. E) H&E staining of the lung (red arrow indicates the metastasis from ID8 xenograft). Scale bar, 250 μm. F) No. of lung metastatic nodules. Data shown are mean ± SD from 5 mice in each group. * $P< 0.05$, ** $P<0.01$, *** $P<0.001$, compared with respective control.

Figure 3: Target identification using Cellular Thermal Shift Assay (CETSA). Differential profiling of SU056 on the thermal proteome profile of OVCAR8 cells. OVCAR8 cells were treated with DMSO or SU056 (2.5 μM) for 1.5 h. Cells were collected and 10^6 cells in each PCR tube incubated at different temperatures (37, 41, 44, 47, 50, 53, 56, 59, 63, 67°C). Cells were lysed and an equal quantity of soluble protein was labeled with TMT, followed by LC-MS/MS analysis. A) Heat map representation of the thermal stability of 804 soluble proteins in ovarian cancer cells treated with vehicle-DMSO (left) and SU056 (right). B) Density distributions of protein Tm values calculated in SU056 treated cells (red) and vehicle cells (blue). C) Density distributions of Tm shifts between SU056 and vehicle treatment. D) A scatter plot of Tm calculated in SU056 and vehicle treatment. Proteins that passed the significant values (p-value < 0.01, RSQ > 0.7) and identification criteria are highlighted in red. E) Melting curves for

identified top six proteins (YB-1, TMSB10, SUMO-2, PSMB2, TMSB4X, and CALM3) with and without SU056 treatment. F) Change in melting temperature (T_m) of the top six proteins upon SU056 treatment.

Figure 4: SU056 inhibits YB-1. A) OVCAR8 cells were treated with SU056 (1, 2.5 and 5 μM) for 12 h and total cell lysates were prepared as described in the 'Methods' section. SDS-PAGE and Western blot analysis was performed for the top three targets identified by CETSA (YB-1, TMSB10, SUMO-2 and PSMB2). Membranes were stripped and re-probed with an anti-beta-actin antibody to ensure equal protein loading. B) Immunohistochemistry of tumor samples from ID8 tumor xenograft study from control and SU056 -a related group for YB-1 and MDR1 expression. Subset images are at 20x magnification: scale bar, 50 μm. C-E) Effect of SU056 on YB-1 and its associated proteins in different ovarian cancer cell lines. C) Respective OC cells were treated with 2.5 μM SU056 for 12 h and YB-1 & CD44 expression and multidrug resistance activity was measured as described in the 'method' section. The % inhibition was calculated and compared to the control for the respective cell line. D) YB-1 50% inhibitory concentration (IC50) of SU056 for OC cell lines was determined after 12 h of treatment using PathScan® Total YB1 Sandwich ELISA Kit. E) YB-1 inhibition time kinetics study for SU056 effect on OC cell lines. Cells were treated with SU056 (1, 2.5 and 5 μM) for 3, 6, 12, and 24 h. YB-1 was assayed using PathScan® Total YB1 Sandwich ELISA Kit.

Figure 5: SU056 physically interact with YB-1. A) Structure of biotinylated SU056. B) Pulldown assay using biotinylated SU056. Pulldown were carried out from OVCAR8 cells and OVCAR8 cell lysates as described in 'Methods' secession. Both the pulldowns were run in duplicate. C-D) Representative sensograms for C) SU093 and D) SU056. His-tagged YB-1 protein was immobilized on NTA chip and different concentration of SU093 and SU056 (1-100 μM) were tested for physical interaction as described in 'Methods' secession. E-G) Cellular effect of SU056 is dependent on YB-1 expression. OVCAR8 cells were stably express with Scrambled control (SC), YBX1 shRNA1 and YBX1 shRNA2 using lentiviral vector. E) Western blot analysis was performed to conform the YB-1 expression in transduced

cells. F) 500 cells (SC, shRNA1, shRNA2) were plated/well of 12 well plate and allow to attach for 24h. Transduced cells were treated with SU056 and incubated further for 7 days. Each well was stained with crystal violet and colonies were counted under 10X microscope. Representative colony formation from respective wells. G) IC_{50} values of SU056 on different transduced OVCAR8 cells expressing SC, YBX1 shRNA1, YBX1 shRNA2.

Figure 6: SU056 modulates the YB-1 associated proteins and pathways. A) Cycloheximide chase assay (CHX) to determine the effect of SU056 on YB-1 protein stability. Data are shown as mean ± SD of triplicate samples. * $P<0.05$, significantly different compared with respective controls by one-way ANOVA followed by Dunnett's test. B) Total cell lysates were prepared as described in the 'Methods' section. SDS-PAGE and Western blot analysis was performed for YB-1, cell cycle, and apoptosis-associated markers. Membranes were stripped and re-probed with an anti-beta-actin antibody to ensure equal protein loading. C-F) GSEA was performed on the proteomics results to determine the enrichment of KEGG pathways upon treatment on the OVCAR8 cell line with SU056. C) Enrichment plot of pathways modulated by SU056 treatment. D-E) Enrichment in the Apoptosis and RNA degradation pathway was observed in proteins that increase in abundance upon treatment with SU056. F) Enrichment in the Spliceosome pathways was observed in proteins that decrease in abundance upon treatment with SU056.

Figure 7: SU056 treatment sensitizes the ovarian cancer cell for taxane treatment. A) Sensitizing effects of SU056 on the viability of OVCAR8 and SKOV3 cells in combination with paclitaxel treatment. Cells treated with 0.1, 0.5, and 1 μM SU056 followed by 0.1, 0.5 and 1 nM paclitaxel treatment for 48 h showed a synergistic cytotoxic effect. Combination index values for paclitaxel and SU056. B) Alexa Fluor-488-tagged paclitaxel efflux assay showed that SU056 cotreatment inhibits paclitaxel efflux. C) Immunoblotting of YB-1 and MDR1. OVCAR8 cells were treated with either vehicle (C), paclitaxel (0.5 nM, P), SU056 (0.5 μM, 56) and paclitaxel + SU056 (P+56) for 12 h and cell lysates were prepared.

Proteins were resolved on SDS-PAGE gel and blotted for respective antibodies as described in the method section. Membranes were stripped and re-probed for loading control actin. Spheroid formation assay. 500 cells were cultured in ultra-low attachment plates and treated with each drug and their combinations. The cells incubated for 7 days to form spheroids. D) Microscopic images of spheroid at 10X magnification. Scale bar, 250 μm E) Spheroid formation was quantified after 7 days of incubation. Data are shown as mean ± SD of triplicate samples. * *P*< 0.05, ** P<0.01, *** P<0.001, significantly different compared with respective controls by one-way ANOVA followed by Dunnett's test. F-I) Combination study of SU056 and paclitaxel on the OVCAR8 xenograft model. NOD-SCID female mice were subcutaneously injected with OVCAR8 cells mixed with Matrigel in 1:1 ratio. Drug treatment started when tumors grew to 200mm^3. Mice were intraperitoneally (IP) injected with either vehicle (30% PEG300 in saline) or 10 mg/kg SU056 daily and/or 5 mg/kg Paclitaxel once a week for 28 days (4 weeks). F) Representative images of mice after 28 days of drug treatment showing tumor regression compared to control. G) Tumor volume/mouse as a function of time. H) tumor weight/mouse at the end of the study. I) Immunohistochemistry staining. Tumor sections were stained with Ki67, and slides were scored for KI67 staining. Data shown are mean ± SD from 5 mice in each group. * *P*< 0.05, ** P<0.01, *** P<0.001 compared with respective control.

Figure 8: A) Validation of CETSA results using western blot analysis. Cell lysates from control and SU056 treated cells exposed at 37 and 53°C temperature were analyzed for expression of YB-1, TMSB10 and PSMB2. B-C) SU056 treatment inhibit the expression of YB-1 in OVCAR8 cells. OVCAR8 cells stably expressing with mCheery tagged YB-1 were used for these assays. B) Cells were imaged using confocal microscope for the mCherry-YB1 after 3 h treatment of SU056 (2.5 & 5 μM) at 10X magnification. C) 2500 cells were plated in 96 well plate (black well clear bottom) and treated with SU056 (1-5 μM) for 3 and 6 h. Relative fluorescence intensity was measured as using multimode plate reader. Data was

normalized with % viable cells and presented in percentage compare to non-treated cells. Data shown are mean ± SD from 5 replicates in each group. * $P< 0.05$ compared with respective control.

Figure 9: Sensitizing effects of SU056 on the viability of OVCAR8 and SKOV3 cells in combination with paclitaxel treatment. Cells treated with 0.1, 0.5 and 1 μM SU056 followed by 0.1, 0.5 and 1 nM paclitaxel treatment for 48 h showed synergistic cytotoxic effect.

Figure 10: Pharmacokinetics of SU056. Determination of SU056 plasma concentration as a function of time (min) after administration of the drug intraperitonially at a dose of 20 mg/kg (n=3). After injection plasma was collected at respective time point and was analyzed using LC MS/MS as described in method.

Figure 11: A). The growth inhibitory effect of SU056 was evaluated using MTT assay. TNBC cell lines (MDA-MB-231, MDA-MB-468, SUM159, 4T1, E0771 and EMT6) were plated in 96 well plate. Next day, treated with vehicle (DMSO) alone or 0.005-50 μM of SU056 in fresh medium. After 48 hr of treatments, cell viability was measured using MTT assay. B). A total of 300-600 cells were plated per well of a 12-well plate and allowed to attach for 24 hr. Next day, cells were treated with SU056 and incubated further to 7-10 days. Each well stained with crystal violet and colonies were counted under a 10x microscope. Representative colony formations from respective wells of cell. C) Number of colonies formed after SU056 treatment. D) Effect of SU056 on cell cycle distribution in TNBC cells. MDA-MB-231, MDA-MB-468 and SUM 159 cells were treated with vehicle or SU056 for 12 and 24 hr. At the end of treatment, cells were collected and analysed using flow cytometry. SU056 treatment showing G2/M phase arrest. SU056 induces level of phosphor-Histone H3 in cells at 12 and 24 hr.

Figure 12. TNBC cells were treated for 12 and 24 hr and total cell lysate were prepared. SDS-PAGE and western blot analyses were performed for translation associated molecules. Beta-actin were probed to ensure equal protein loading. SU056 treatment inhibited the protein translation associated molecules among all TNBC cells A) MDA-MB-231, B) MDA-MB-468 and C) SUM 159.

Figure 13. SU056 inhibit tumor xenograft of TNBC models. Mice were subcutaneously injected with MDA-MB-231 (2 x 10^6 cells), MDA-MB-468 (5 x 10^6 cells) and patient derived xenograft SUTI151-PDX (2 x 10^6 cells) and drug treatment started when tumor reached 100 mm^3. Mice were given either vehicle (40% PEG in saline) or SU056 (50 mg/kg) through oral route using oral gavage. A) Tumor volume (MDA-MB-231) as a function of time. B) Tumor weight (MDA-MB-231) at the end of the study. C) Body weight (MDA-MB-231) as a function of time. D) Tumor volume (MDA-MB-468) as a function of time. E) Tumor weight (MDA-MB-468) at the end of the study. F) Body weight (MDA-MB-468) as a function of time. G) Representative images of tumor (MDA-MB-231) at the end of study. H) Representative images of tumor (MDA-MB-468) at the end of study. I) Tumor volume (SUTI151-PDX) as a function of time. B) Tumor weight (SUTI151-PDX) at the end of the study. C) Body weight (SUTI151-PDX) as a function of time.

Figure 14. SU056 inhibit 4T1 tumor xenograft in BALB/c. Mice were subcutaneously injected with 4T1 cells and drug treatment started after three day of implantation. Mice were given either vehicle (40% PEG in saline) or SU056 (50 mg/kg) through oral route using oral gavage. A) Tumor volume (4T1) as a function of time. B) Tumor weight (4T1) at the end of the study. C) Body weight (4T1) as a function of time. D) Representative images of tumor (4T1) at the end of study. Combination of Paclitaxel and SU056 in 4T1 xenograft model. E) Tumor volume (4T1) as a function of time. F) Tumor weight (4T1) at the end of the study. G) Body weight (4T1) as a function of time.

Figure 15. SU056 treatment is well tolerated in mice and rat. SU056 treatment at increasing concentrations dose not challenge the dietary behavior as reflected by change in body weight A) Mice and B) Rat. SU056 treatment of different concentration did not cause death among C) mice and D) Rat. Liver microsomes were used for the purpose of metabolic profiling. E) SU056 had a mean half-life of 40 minutes.

Figure 16 depicts reference-normalized expression levels of YBX1 for the cancer cell lines: a) Lung – IMR90, A549, MRC5, H1299, NHBE, NCIH460, and BEAS2; b) Lymphocyte – JURKAT, MT4, BJAB, HEL CELLS, HL60, and RAJI; c) Breast/Mammary – MCF7, MCF10, MDAMB231, SKBR3, MDAMB468, and

MDAMB453; d) Fibroblast – BJ, KB, HT1080, NHDF, and TIG; e) Prostate – LNCAP, PC3, DU145, and C42; f) Other – NALM6, DAOY, JEG3, and BEWO; g) Kidney – 293T, HEK293, 293F, and FLPIN TREX 293; h) Blood – THP1, PLB985, CEM, and HELAT4; i) Ovary – SKOV3, 2008, and OVCAR3; j) Skin – HNSCC and A431; k) Sarcoma – LHCNM2 and U2OS; l) Lymphoid – REH and RPMI8226; m) Colon – HCT116 and HT29; n) Cervix – HELA and HELA S3; o) Brain – SHSY5Y and SKNMC; p) Bone – K562 and U2OS; q) Uterus – BEWO; r) Pancreas – PANC1; s) Neuroblastoma – IMR32; t) Macrophage U937; u) Liver – HEPG2; v) Keratinocyte – HACAT; w) Glial – H4; x) Connective – G401; y) Bone Marrow – KG1 CELLS; and a) Bladder – T24. This information is provided online by the Ma'ayan Lab Data Coordination and Integration Center at the Mount Sinai Center for Bioinformatics and may be accessed at:

https://maayanlab.cloud/archs4/gene/YBX1#correlation .

Figure 17: Assay of SU056 in NCI-60 cell line panel. SU056 was assayed against the NCI-60 cell line panel at five dose (100 to 0.01 µM) for 48 hours, providing the IC50 values in the table below. Growth inhibition was measured and displayed as a heatmap from no inhibition (black), to growth inhibition (white), as seen in Figure 17.

NCI60-IC50 values

Cell Lines	IC50 (µM)
CCRF-CEM	2.37
HL-60(TB)	1.26
K-562	0.353
MOLT-4	3.02
RPMI-8226	2.28
SR	0.294
A549/ATCC	8.74
EKVX	3.03
HOP-62	2.19
HOP-92	3.1
NCI-H226	50
NCI-H23	3.96
NCI-H322M	4.01
NCI-H460	2.08
NCI-H522	2.59
COLO 205	1.46
HCC-2998	7.06
HCT-116	1.53
HCT-15	1.08
HT29	1.52
KM12	1.48
SW-620	1.65

SF-268	3.2
SF-295	1.2
SF-539	1.13
SNB-19	3
SNB-75	0.82
U251	2.5
LOX IMVI	2.35
M14	0.357
MDA-MB-435	0.187
SK-MEL-2	2.46
SK-MEL-28	20.3
SK-MEL-5	1.26
UACC-257	50
UACC-62	2.25
IGROV1	4.19
OVCAR-3	1.27
OVCAR-4	46.8
OVCAR-5	4.33
OVCAR-8	3.18
NCI/ADR-RES	2.02
SK-OV-3	1.73
786-0	1.86
A498	1.11
ACHN	3.03
CAKI-1	1.24
RXF 393	1.51
SN12C	3.05
TK-10	15.6
UO-31	2.88
PC-3	1.46
DU-145	2.71
MCF7	1.44
MDA-MB-231/ATCC	3.09
HS 578T	1.42
BT-549	1.46
T-47D	2.18
MDA-MB-468	1.12

In each of Figures 18, 19, and 20: TNBC cells were treated for 12 and 24 hr and total cell lysate were prepared. SDS-PAGE and western blot analyses were performed for translation

associated molecules. Beta-actin were probed to ensure equal protein loading. SU056 treatment inhibited the translation initiation factors in TNBC cells.

STAR Methods

Key Resources Table

Reagent or Resource	Source	Identifier
Antibodies		
YB-1	Cell signaling technology (CST)	#8475
TMSB10	R&D Systems	#AF6429
SUMO2/3	CST	#4971
PSMB2	Bethyl Laboratories	#A303817AT
MDR1	CST	#13978
CD44	CST	#37259
GBP1	Abnova	#H00002633-PW1
c-Myc	Novesbio	# NB600-302SS
CDK2	CST	#2546
CDC25A	CST	#3652
Cyclin E	CST	#4132
Bax	CST	#5023
Bcl-2	CST	#2876
β-actin	Novusbio	#NB600-501SS
Ki67	Biolegend	#350502
Anti-mouse IgG HRP-linked antibody	CST	#7076
Anti-rabbit IgG HRP-linked antibody	CST	#7074
Recombinant Proteins		
Human YB-1 protein (His Tag)	Novusbio	# NBP2-30101
Chemicals		
RPMI-1640	Corning	#10-040-CV
DMEM	Corning	#10-013-CV
DMEM/F12	Hyclone	#SH30525.01
MEGM media	Lonza	#CC-3150
FBS	Corning	#35-015-CV
Antibiotic-Antimycotic solution	Gibco	15240062
M-PER™ lysis solution	Thermo Scientific	#78503
Halt protease and phosphatase inhibitor cocktail	Thermo Scientific	#78440
Magnetic conjugate streptavidin bead	CST	#5947
Oregon Green™ 488-conjugated paclitaxel	Molecular Probes	# P22310

Cell Lines		
OVCAR3	NCI cell line repository (DTP)	
OVCAR4	NCI cell line repository (DTP)	
OVCAR5	NCI cell line repository (DTP)	
OVCAR8	NCI cell line repository (DTP)	
SKOV3	NCI cell line repository (DTP)	
SH-SY5Y	Dr. Manish Chamoli (Buck Institute, CA, USA)	
ID8	Dr. Erinn Rankin (Stanford University, CA, USA)	
Assay kits		
Tandem Mass Tag (TMT)	Thermo Fisher Scientific	# 90110, #90061
PathScan® Total YB1 Sandwich ELISA Kit	CST	#12543
Multidrug Resistance Assay Kit	Sigma-Aldrich	#MAK161
Human CD44 ELISA Kit (Colorimetric)	Novusbio	#NBP1-86819
Recombinant DNA		
pLenti PGK Blast V5-LUC (w528-1)	Eric Campeau & Paul Kaufman, Addgene	Addgene # 19166
pLV[Exp]-Puro-EF1A>hYBX1[NM_004559.5]/mCherry	VectorBuilder Inc	
pLV[shRNA]-EGFP:T2A:Puro-U6>hYBX1[shRNA#1] shRNA sequence: CCTGTTAATAAAGGTCTTAAA	VectorBuilder Inc	
pLV[shRNA]-EGFP:T2A:Puro-U6>hYBX1[shRNA#2] shRNA sequence: CCAGTTCAAGGCAGTAAATAT	VectorBuilder Inc	
pLV[shRNA]-EGFP:T2A:Puro-U6>Scramble[shRNA#1] shRNA sequence: CCTAAGGTTAAGTCGCCCTCG	VectorBuilder Inc	

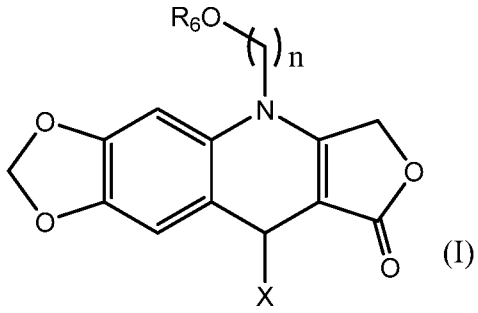
Human YB-1 protein (His Tag) (Novus Biological Catalog No. NBP2-30101) is a recombinant protein with a N-terminal His-tag and corresponding to the amino acids 1-324 of Human YB1. Source: E. Coli. Gene: YBX1. Amino Acid Sequence: MGSSHHHHHH SSGLVPRGSH MGSMSSEAET QQPPAAPPAA PASLSAADTKP GTTGSGAGSG GPGGLETSAAP AGGDKKVIAT KVLGTVKWFN VRNGYGFINR NDTKEDVFVH QTAIKKNNPR KYLRSVGDGE TVEFDVVEGE KGAEAANVTG PGGVPVQGSK YAADRNHYRR YPRRRGPPRN YQQNYQNSES GEKNEGSESA PEGQAQQRRP YRRRFPPYY MRRPYGRRPQ YSNPPVQGEV MEGADNQGAG EQGRPVRQNM

YRGYRPRFRR GPPRQRQPRE DGNEEDKENQ GDETQGQQPP QRRYRRNFNY RRRRPENPKP QDGKETKAAD

PPAENSSAPE AEQGGAE

What is claimed:

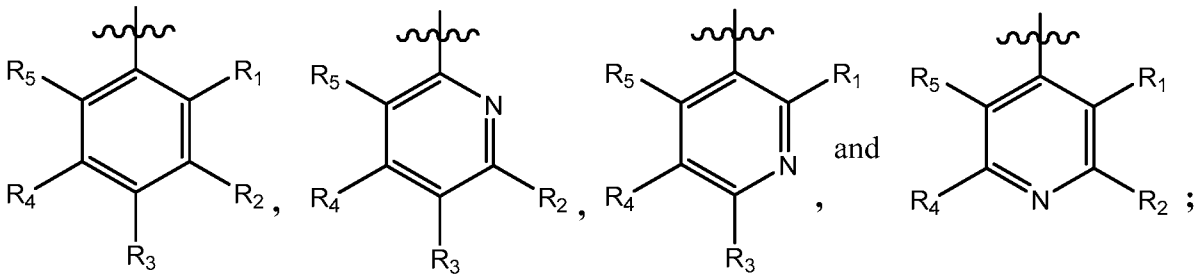
1. A method of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I),



(I)

wherein:

X is selected from the group of:

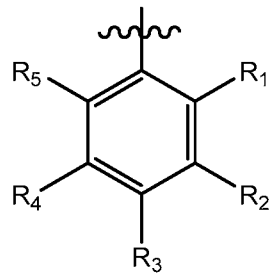


R_1, R_2, R_3, R_4, and R_5 are each independently selected in each instance from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

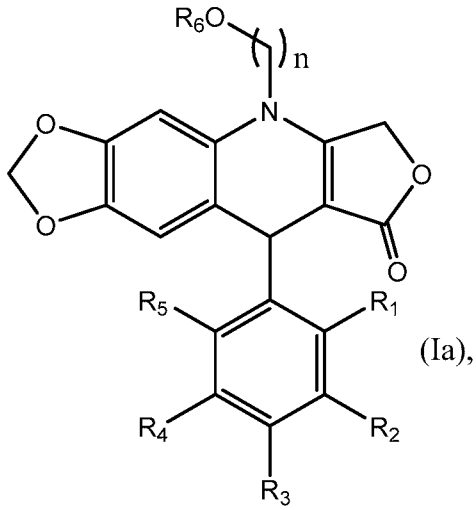
n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_6 is selected from the group of H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl; wherein the C_1-C_6 alkyl group is substituted with 0, 1, 2, 3, or 4 substituents selected from F, Cl, Br, I, OH, CN, NO_2, and OH; and the C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl groups are substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when X is  and R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

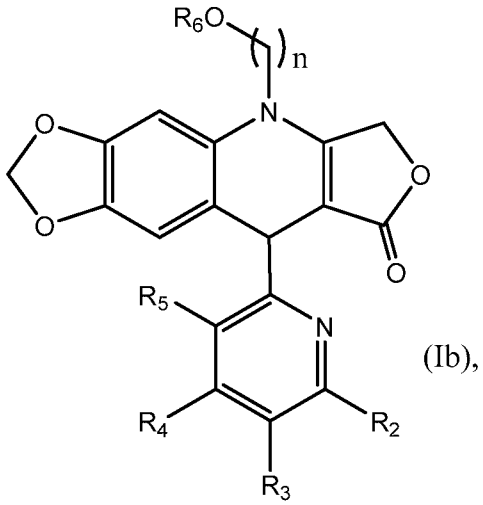
2. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (Ia):



(Ia),

wherein n, R_1, R_2, R_3, R_4, R_5, and R_6 are as defined in Claim 1; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

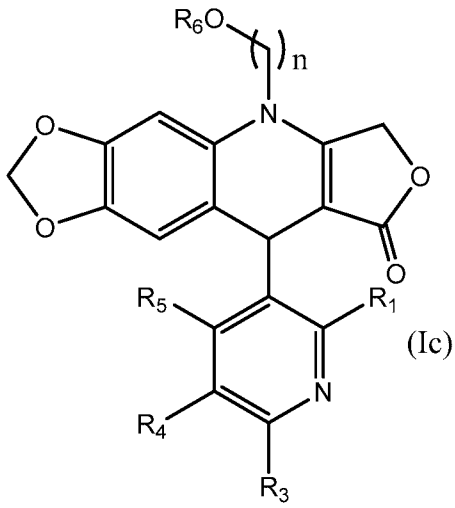
3. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (Ib):



(Ib),

wherein n, R_2, R_3, R_4, R_5, and R_6 are as defined in Claim 1; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

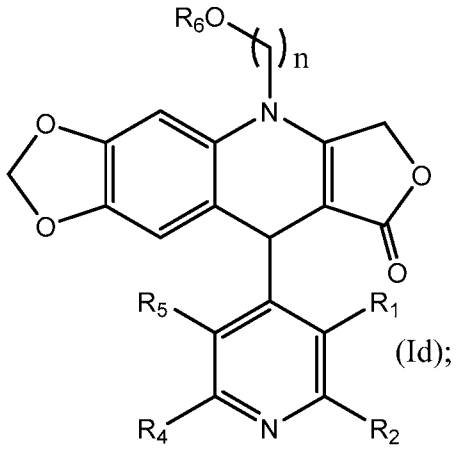
4. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (Ic):



(Ic)

wherein n, R_1, R_3, R_4, R_5, and R_6 are as defined in Claim 1; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

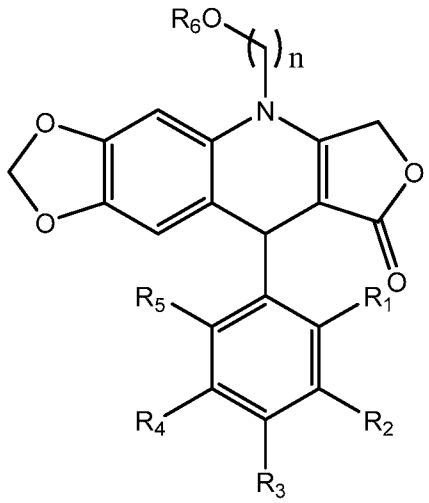
5. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (Id):



(Id);

wherein n, R_1, R_2, R_4, R_5, and R_6 are as defined in Claim 1; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

6. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (II):



(II)

wherein:

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

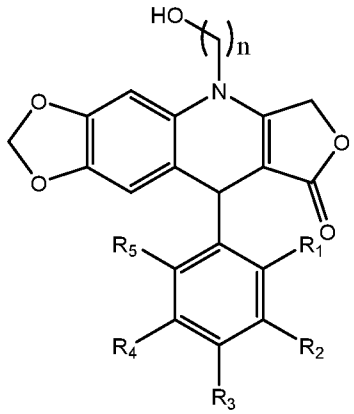
R_6 is selected from the group of H, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered heterocycle, phenyl, and -$(CH_2)_n$-phenyl; wherein the C_1-C_6 alkyl group is substituted with 0, 1, 2, 3, or 4 substituents selected from F, Cl, Br, I, OH, CN, NO_2, and OH; and the C_3-C_6 cycloalkyl, -$(CH_2)_n$-C_3-C_6 cycloalkyl, 3-6-membered heterocycle, -$(CH_2)_n$-3-6-membered

heterocycle, phenyl, and -$(CH_2)_n$-phenyl groups are substituted with 0, 1, 2, 3, or 4 substituents C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

7. The method of Claim 1 wherein the compound of Formula (I) is a compound of Formula (III),



(III)

wherein: n is an integer selected independently in each instance from the group of 1, 2, 3, 4, 5, and 6;

R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least one of R_1, R_2, R_3, R_4, and R_5 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and

with the proviso that, when R_2 is F or CF_3, then at least one of R_1, R_3, R_4, and R_5 is not H; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

8. The method of Claim 7, wherein R_1 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

9. The method of any of Claims 7 and 8, wherein R_3 is selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5; and R_1, R_2, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4

fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

10. The method of any of Claims 7, 8, and 9, wherein R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_4 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5;

or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

11. The method of any of Claims 7, 8, 9, and 10, wherein R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, C_1-C_3 fluoroalkyl, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F, C_1-C_3 fluoroalkyl;

or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

12. The method of any of Claims 7, 8, 9, 10, and 11, wherein R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, CF_3, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are selected from the group of F and CF_3;

or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

13. The method of any of Claims 7, 8, 9, 10, 11, and 12, wherein R_1, R_2, R_3, R_4, and R_5 are each independently selected from the group of H, F, CF_3, SF_5, Cl, Br, I, OH, C_1-C_4 alkyl, C_1-C_4 alkoxy, CN, NO_2, and OH;

with the proviso that at least two of R_1, R_2, R_3, R_4, and R_5 are F;

or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

14. The method of any of Claims 1-13, wherein n is an integer independently in each instance from the group of 1, 2, 3, 4, and 5; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

15. The method of any of Claims 1-13, wherein n is an integer independently in each instance from the group of 1, 2, 3, and 4; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

16. The method of any of Claims 1-13, wherein n is an integer independently in each instance from the group of 1, 2, and 3; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

17. The method of any of Claims 1-13, wherein n is an integer independently in each instance from the group of 2 and 3; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

18. The method of any of Claims 1-13, wherein n is 1; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

19. The method of any of Claims 1-13, wherein n is 2; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

20. The method of any of Claims 1-13, wherein n is 3; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

21. The method of any of Claims 1-13, wherein n is 4; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

22. The method of any of Claims 1-13, wherein n is 5; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

23. The method of any of Claims 1-13, wherein n is 6; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

24. The method of any of Claims 1-23, wherein R_6 is H; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

25. The method of any of Claims 1-25, wherein R_6 is C_1-C_6 alkyl substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

26. The method of any of Claims 1-25, wherein R_6 is C_3-C_6 cycloalkyl, wherein the C_3-C_6 cycloalkyl substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

27. The method of any of Claims 1-25, wherein R_6 is -$(CH_2)_n$-C_3-C_6 cycloalkyl, wherein the C_3-C_6 cycloalkyl ring is substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

28. The method of any of Claims 1-25, wherein R_6 is a 3-6-membered heterocycle, wherein the heterocycle ring is substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

29. The method of any of Claims 1-25, wherein R_6 is a -$(CH_2)_n$-3-6-membered heterocycle, wherein the heterocycle ring is substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl,

C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

30. The method of any of Claims 1-25, wherein R_6 is phenyl, wherein the phenyl ring is substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

31. The method of any of Claims 1-25, wherein R_6 is -$(CH_2)_n$-phenyl, wherein the phenyl ring is substituted with 0, 1, 2, 3, or 4 substituents selected from the group of C_1-C_3 alkyl, C_1-C_3 alkoxy, F, Cl, Br, I, OH, CN, NO_2, and OH; or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

32. The method of any of Claims 1-31, wherein two of R_1, R_2, R_3, R_4, and R_5, when present, are selected from the group of F, C_1-C_4 fluoroalkyl, and SF_5.

33. The method of any of Claims 1-32, wherein two of R_1, R_2, R_3, R_4, and R_5, when present, are selected from the group of F, C_1-C_3 fluoroalkyl.

34. The method of any of Claims 1-33, wherein two of R_1, R_2, R_3, R_4, and R_5, when present, are selected from the group of F and CF_3.

35. The method of any of Claims 1-31, wherein three of R_1, R_2, R_3, R_4, and R_5, when present, are selected from the group of F and CF_3.

36. The method of any of Claims 1-31, wherein four of R_1, R_2, R_3, R_4, and R_5, when present, are selected from the group of F and CF_3.

37. The method of any of Claims 1-36, wherein two of R_1, R_2, R_3, R_4, and R_5, when present, are F.

38. The method of any of Claims 1-36, wherein three of R_1, R_2, R_3, R_4, and R_5, when present, are F.

39. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound of Formula (I) of any of Claims 1-38, or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof, and a pharmaceutically acceptable carrier or excipient.

40. The use of a compound of Formula (I) as described in any of Claims 1-38, or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof, in the preparation of a medicament for the treatment of a cancer expressing YB1 protein.

41. A method of inhibiting YB1 protein activity in a subject experiencing a cancer expressing the YB1 protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I) as described in any of Claims 1-38, or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

41. A method of inhibiting YB1 protein activity in a subject experiencing a cancer, wherein the cancer is expressing the YB1 protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I) as described in any of Claims 1-38, or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

42. A method of sensitizing cancer cells expressing the YB1 protein in a subject to treatment with an anticancer agent, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I) as described in any of Claims 1-38, or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

43. A method of sensitizing cancer cells expressing the YB1 protein in a subject to treatment with radiation, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound of Formula (I) as described in any of Claims 1-38, or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

44. The method of any of Claims 41, 42, and 43, wherein the cancer expressing YB1 protein is selected from the group of a gynecological cancer (including ovarian, endometrial, fallopian tube, and cervical cancers), breast cancers, lung cancers, prostate cancer, colorectal cancer, bladder cancer, melanoma, liver cancer, multiple myeloma, soft tissue sarcoma, osteosarcoma, Ewing's sarcoma, glioblastoma, acute myeloid leukemia, Chronic myelogenous leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphoma, kidney cancer, renal cell carcinoma, osteosarcoma, pancreatic cancer, head and neck cancer, nasopharyngeal carcinoma, and gastric cancer.

45. The method of any of Claims 1-44, wherein the compound of Formula (I) is 9-(3-fluorophenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (SU056), or a pharmaceutically acceptable salt, pharmaceutically acceptable co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof.

FIG. IA

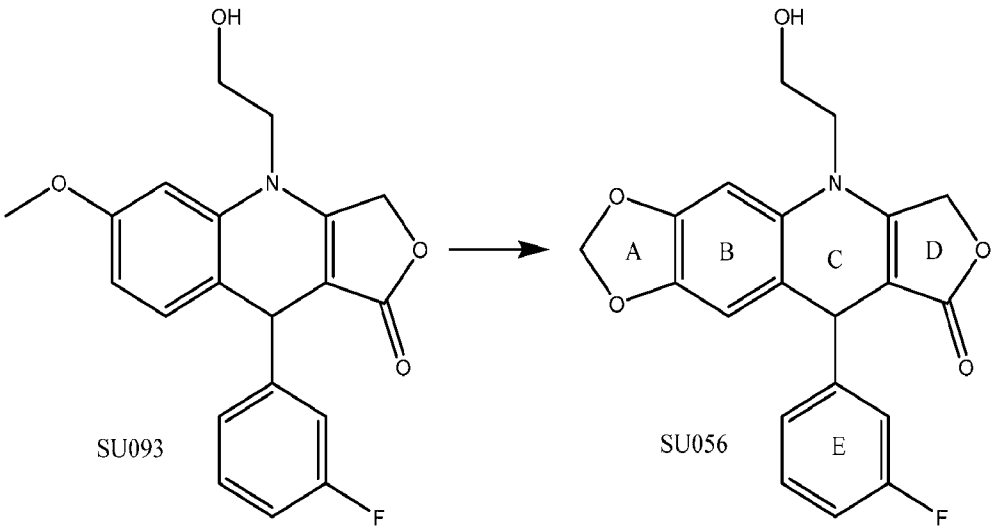


FIG. IB

Drug	IC_{50} values at 48 hours (μM)					
	OVCAR3	OVCAR4	OVCAR5	OVCAR8	SKOV3	ID8
SU093	1.18 ± 0.03	13.69 ± 1.04	14.96 ± 1.16	2.03 ± 0.43	1.93 ± 0.06	6.95 ± 0.76
SU056	1.27 ± 0.14	6.8 ± 0.53	4.33 ± 0.2	3.18 ± 0.07	1.73 ± 0.16	3.75 ± 0.03

FIG. 1C

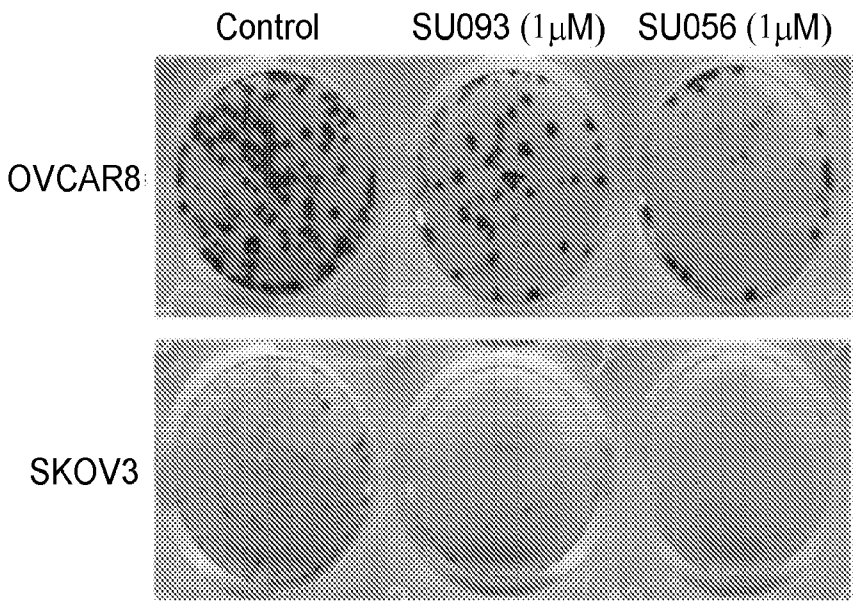


FIG. 1D

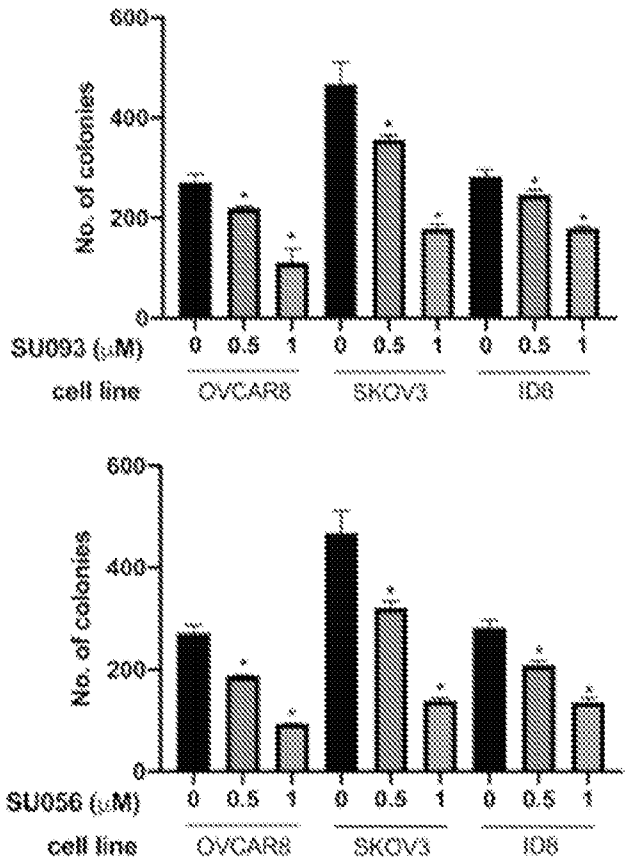


FIG. 1E

Drug	% Inhibition at 10 μM for 48 h		
	N27	SN-SYSY	HEK293
Etoposide	62.23 ± 1.23	24.53 ± 1.18	58.22 ± 2.31
SU093	33.17 ± 0.89	20.01 ± 0.67	52.52 ± 3.41
SU056	31.40 ± 1.43	17.53 ± 0.72	50.2 ± 3.21

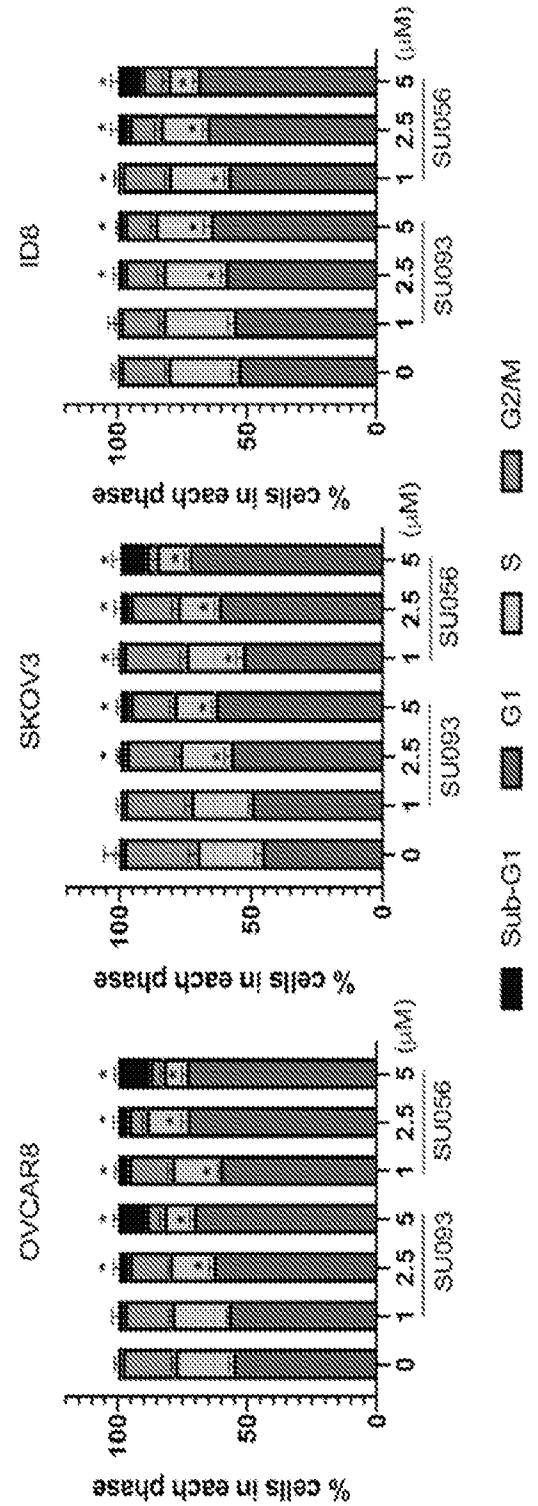

OVCAR8
SKOV3
ID8
% cells in each phase
100
50
0
0 1 2.5 5 1 2.5 5 (μM)
SU093
SU056
Sub-G1
G1
S
G2/M

FIG. 1F

FIG. IG

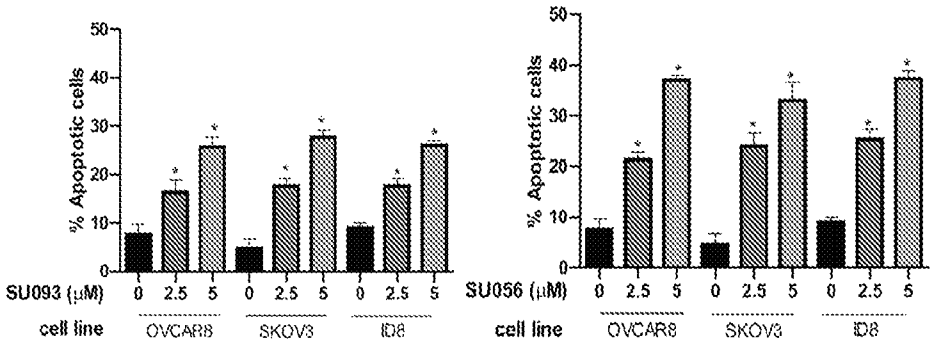


FIG. IH

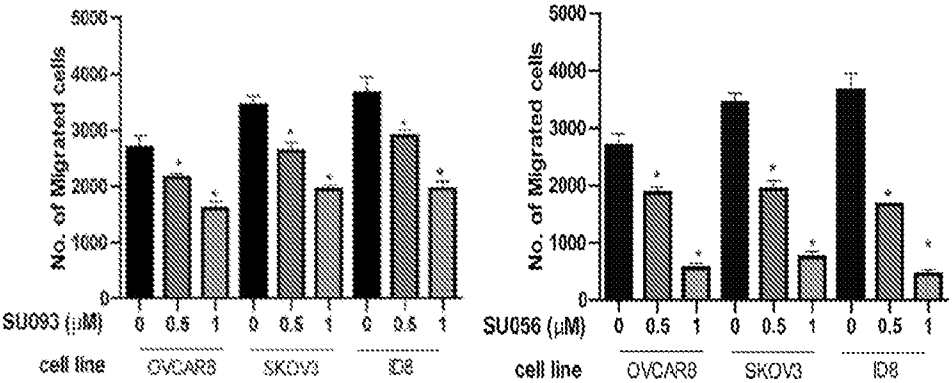


FIG. 2A

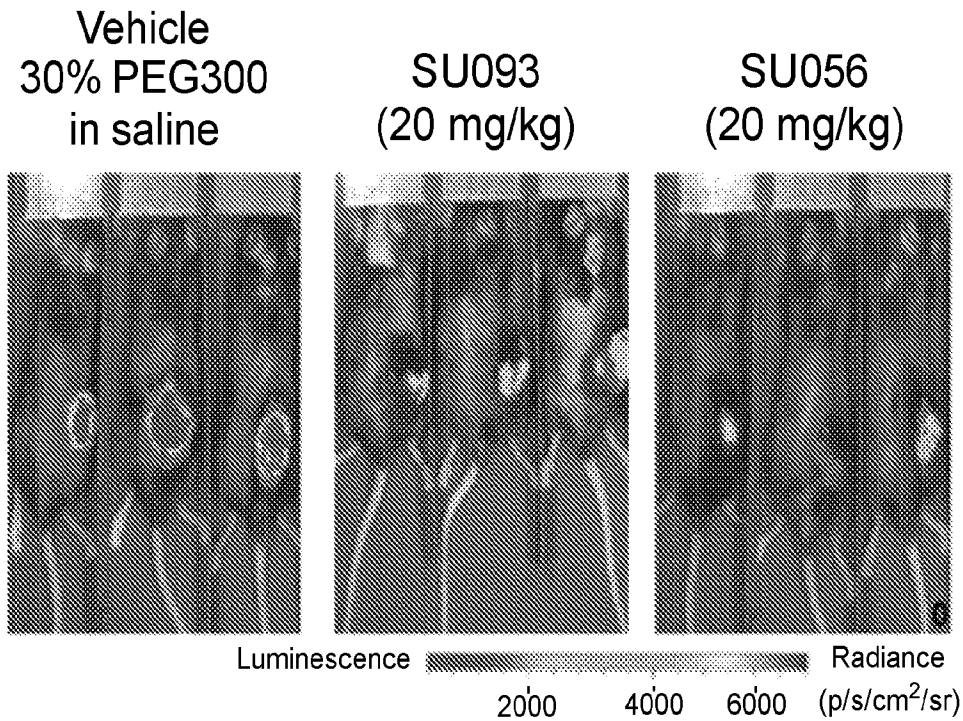


FIG. 2B

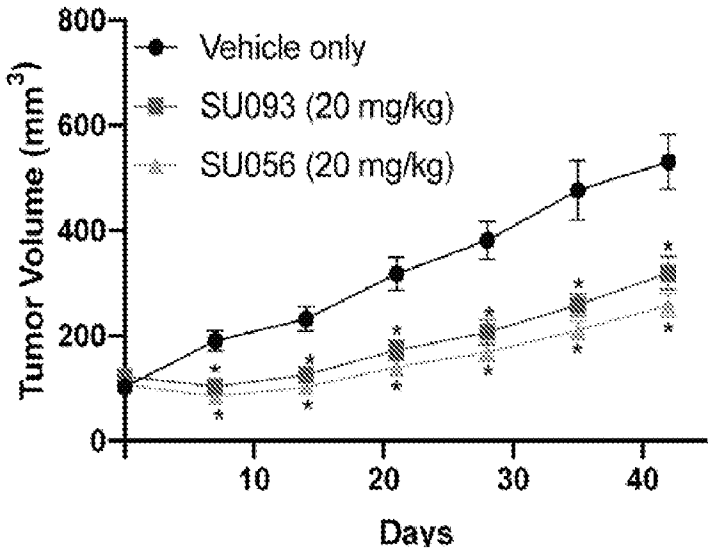

Vehicle only
SU093 (20 mg/kg)
SU056 (20 mg/kg)
Tumor Volume (mm3)
800
600
400
200
0
10
20
30
40
Days

FIG. 2C

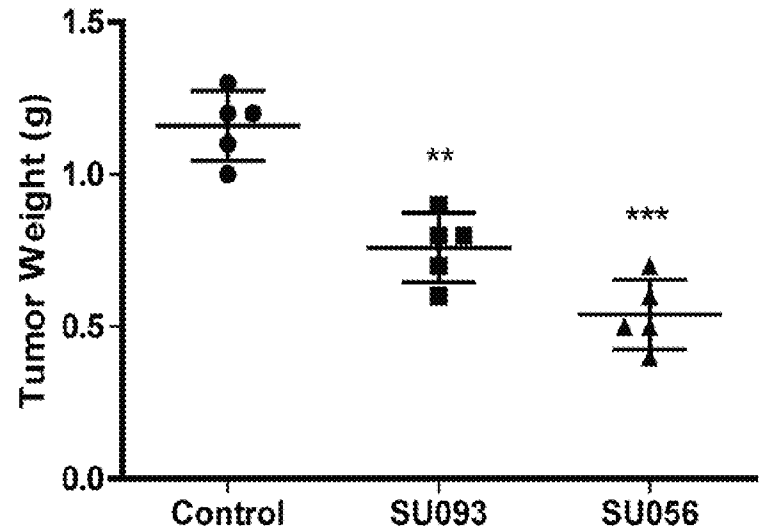

Tumor Weight (g)
1.5
1.0
0.5
0.0
**
***
Control
SU093
SU056

FIG. 2D

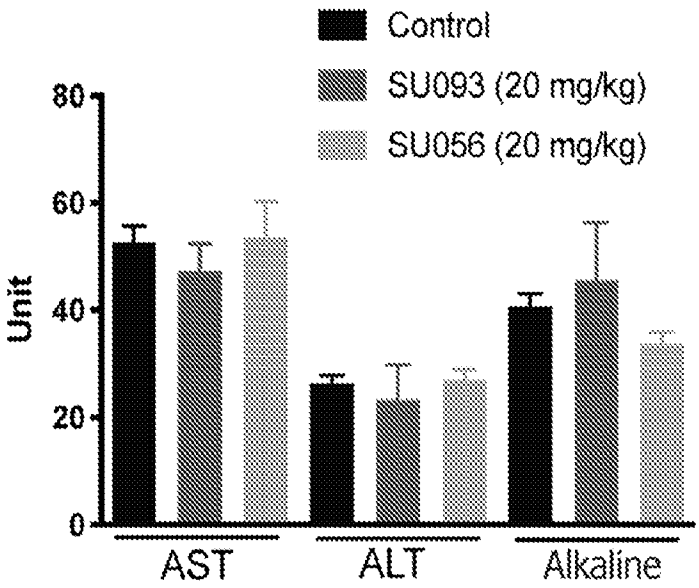

Control
SU093 (20 mg/kg)
SU056 (20 mg/kg)
Unit
80
60
40
20
0
AST
ALT
Alkaline

FIG. 2E

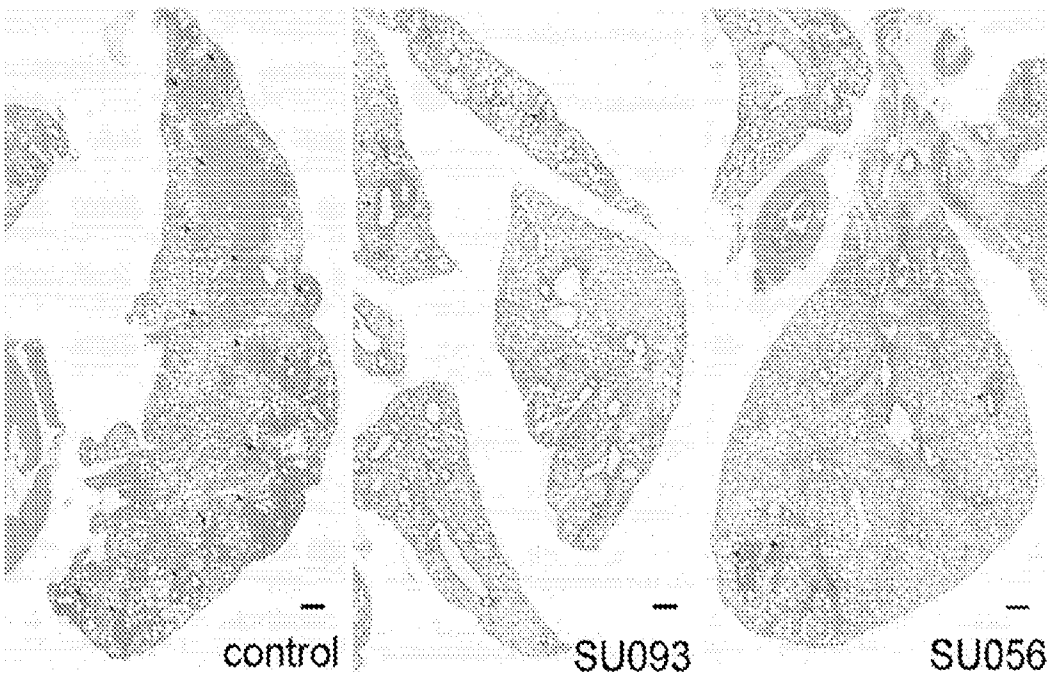

control
SU093
SU056

FIG. 2F

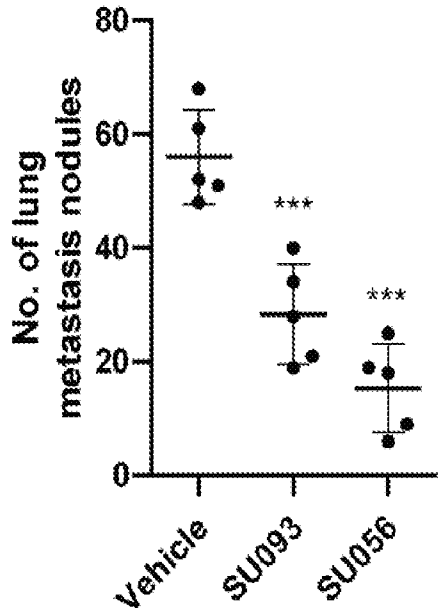

No. of lung metastasis nodules
80
60
40
20
0
***
***
Vehicle
SU093
SU056

FIG. 3A

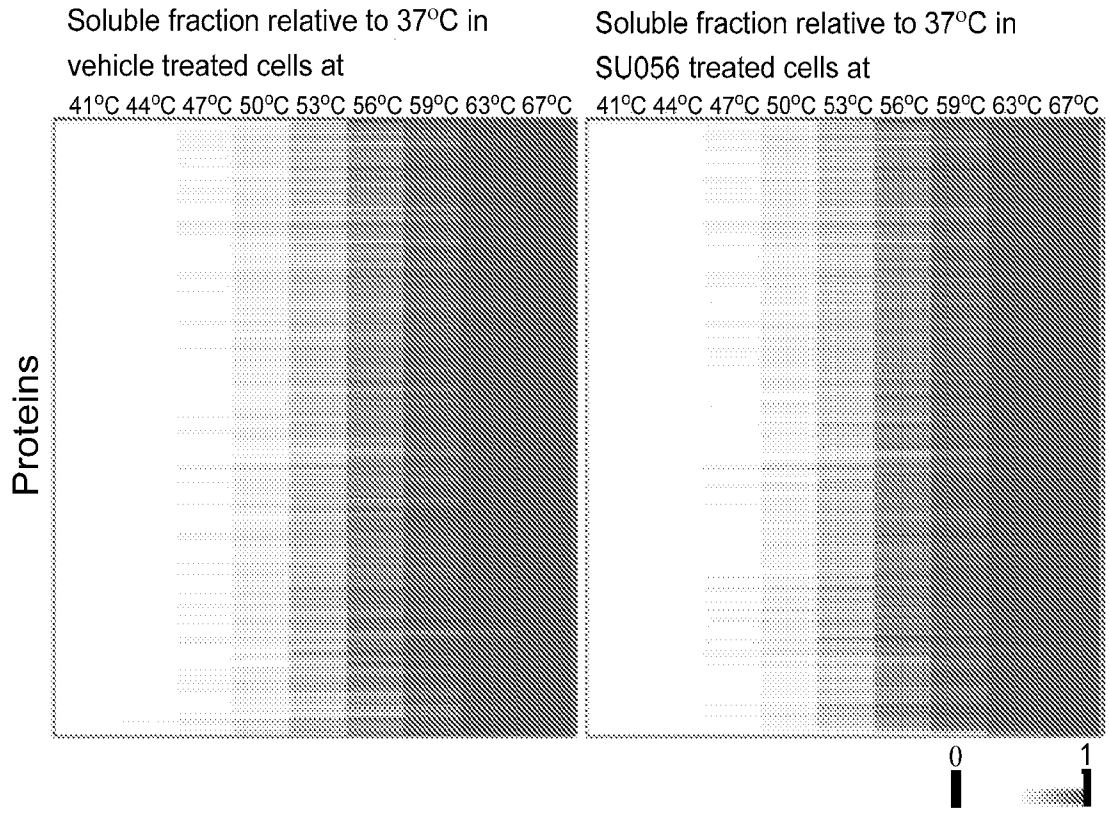

Soluble fraction relative to 37°C in vehicle treated cells at
41°C 44°C 47°C 50°C 53°C 56°C 59°C 63°C 67°C
Soluble fraction relative to 37°C in SU056 treated cells at
41°C 44°C 47°C 50°C 53°C 56°C 59°C 63°C 67°C
Proteins
0
1

FIG. 3B

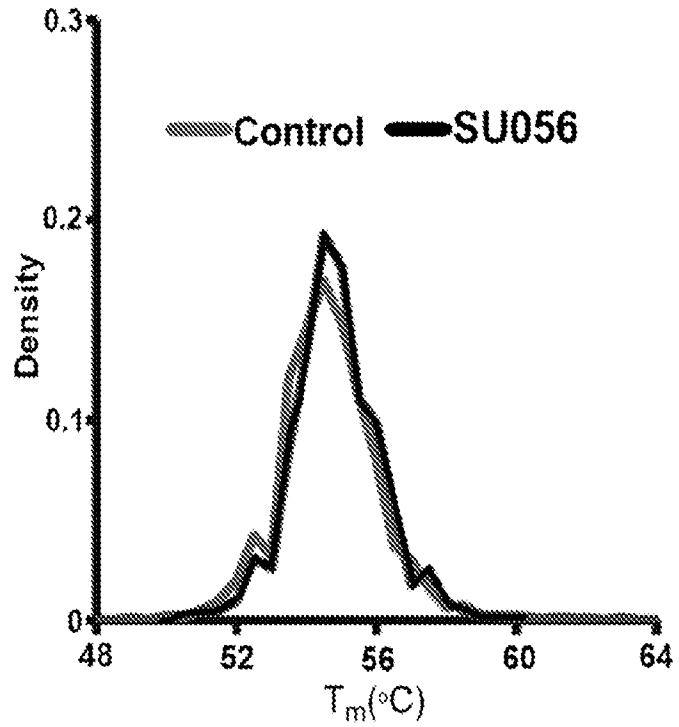


FIG. 3C

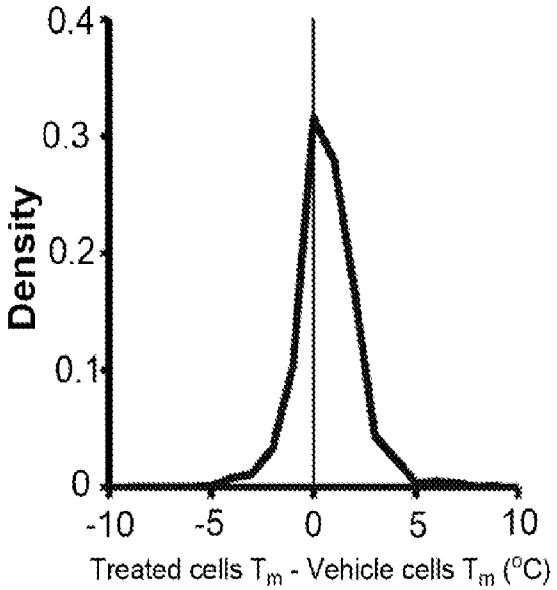


FIG. 3D

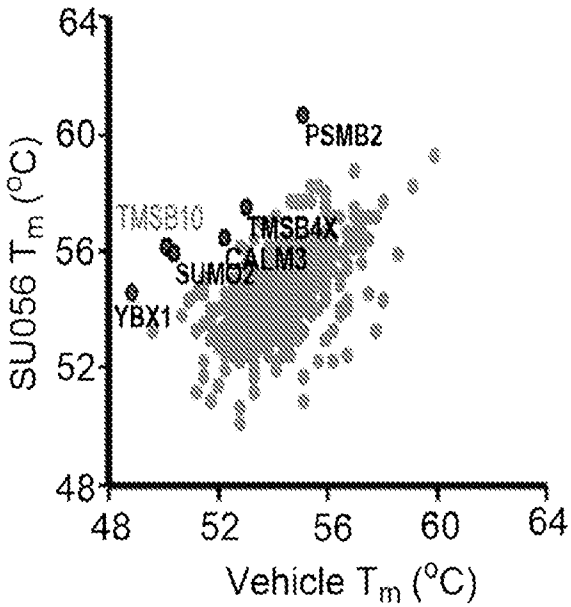


FIG. 3E

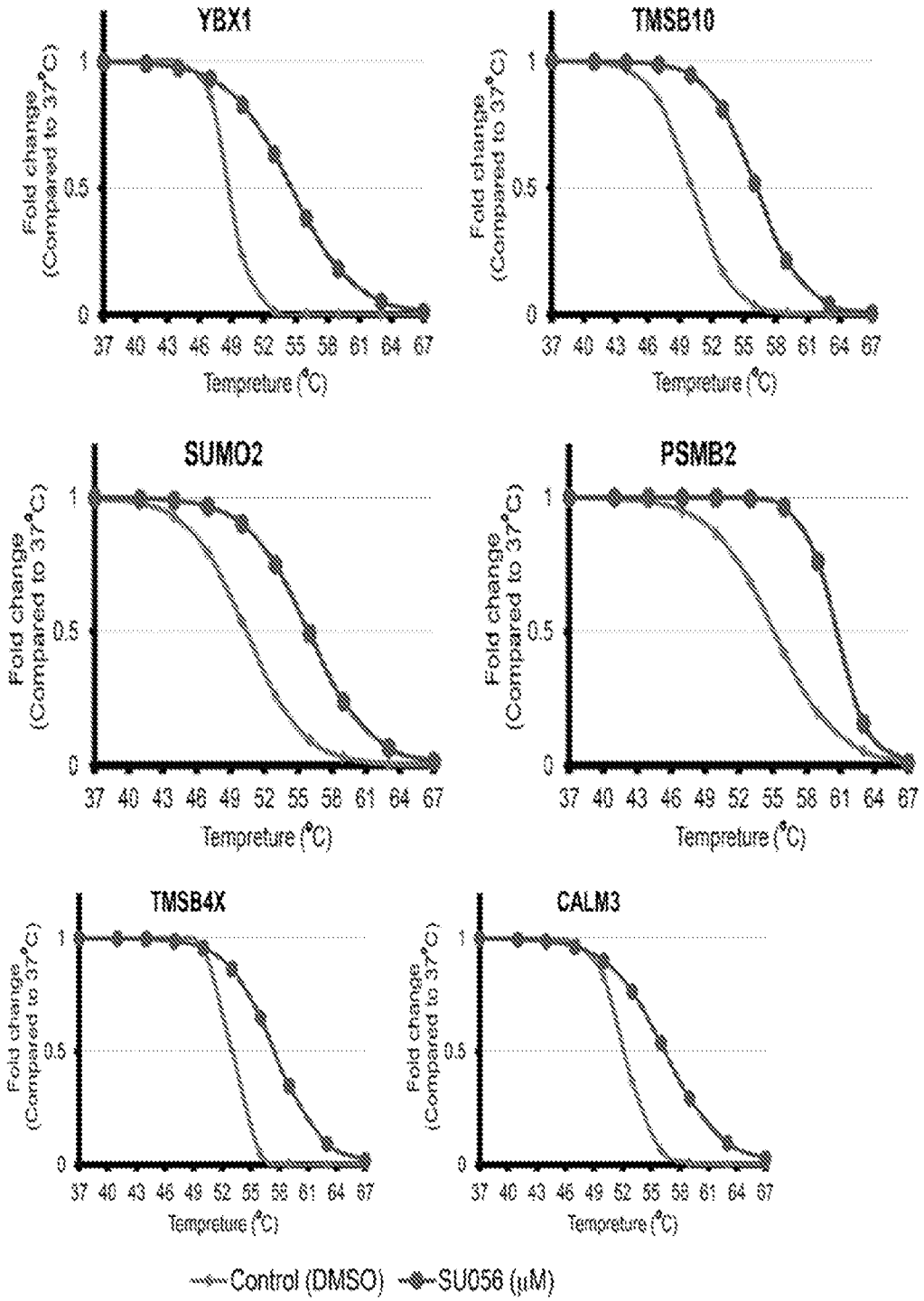

YBX1
TMSB10
SUMO2
PSMB2
TMSB4X
CALM3
Fold change (Compared to 37°C)
Tempreture (°C)
Control (DMSO)
SU056 (μM)

FIG. 3F

No.	Targeted Proteins	Thermal Shift (°C)
1	YBX1/YB-1 (Y box binding protein 1)	5.92 ± 0.86
2	TMSB10 (Thymosin beta-10)	5.89 ± 1.18
3	SUMO2 (Small Ubiquitin Like Modifier 2)	5.4 ± 1.08
4	PSMB2 (Proteasome subunit beta type 2)	5.36 ± 0.76
5	TMSB4X (Thymosin Beta 4 X-Linked)	4.43 ± 1.31
6	CALM3 (Calmodulin 3)	4.03 ± 1.07

FIG. 4A

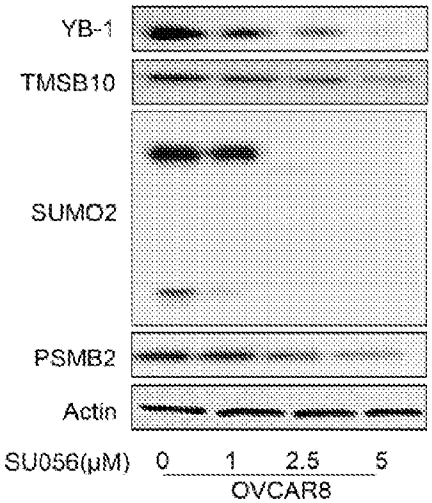


FIG. 4B

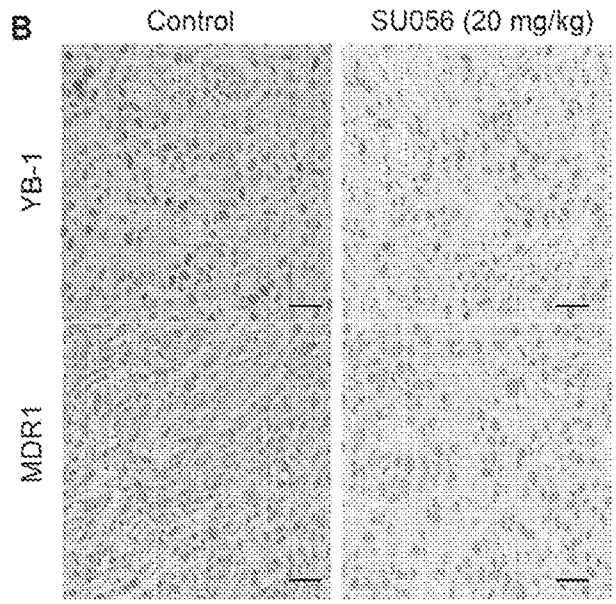


FIG. 4C

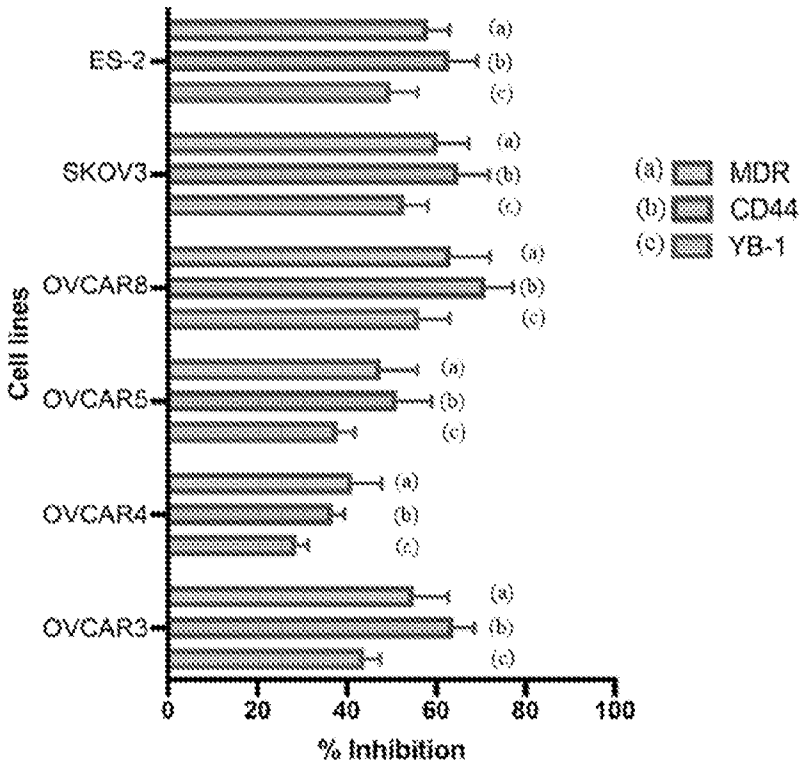

ES-2
SKOV3
OVCAR8
OVCAR5
OVCAR4
OVCAR3
Cell lines
(a) MDR
(b) CD44
(c) YB-1
0
20
40
60
80
100
% Inhibition

FIG. 4D

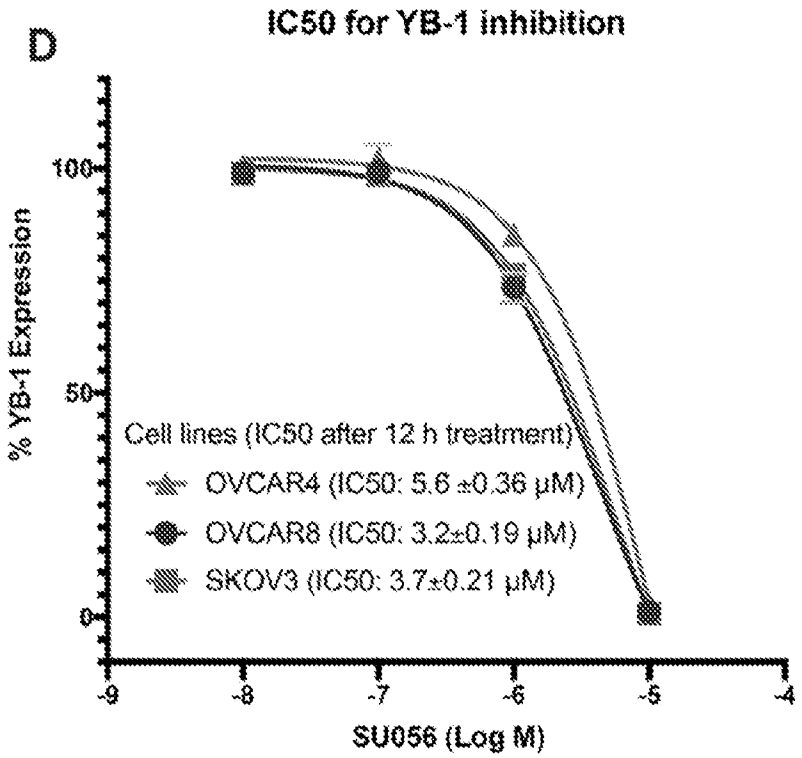

D
IC50 for YB-1 inhibition
% YB-1 Expression
100
50
0
Cell lines (IC50 after 12 h treatment)
OVCAR4 (IC50: 5.6 ±0.36 μM)
OVCAR8 (IC50: 3.2±0.19 μM)
SKOV3 (IC50: 3.7±0.21 μM)
-9
-8
-7
-6
-5
-4
SU056 (Log M)

FIG. 4E

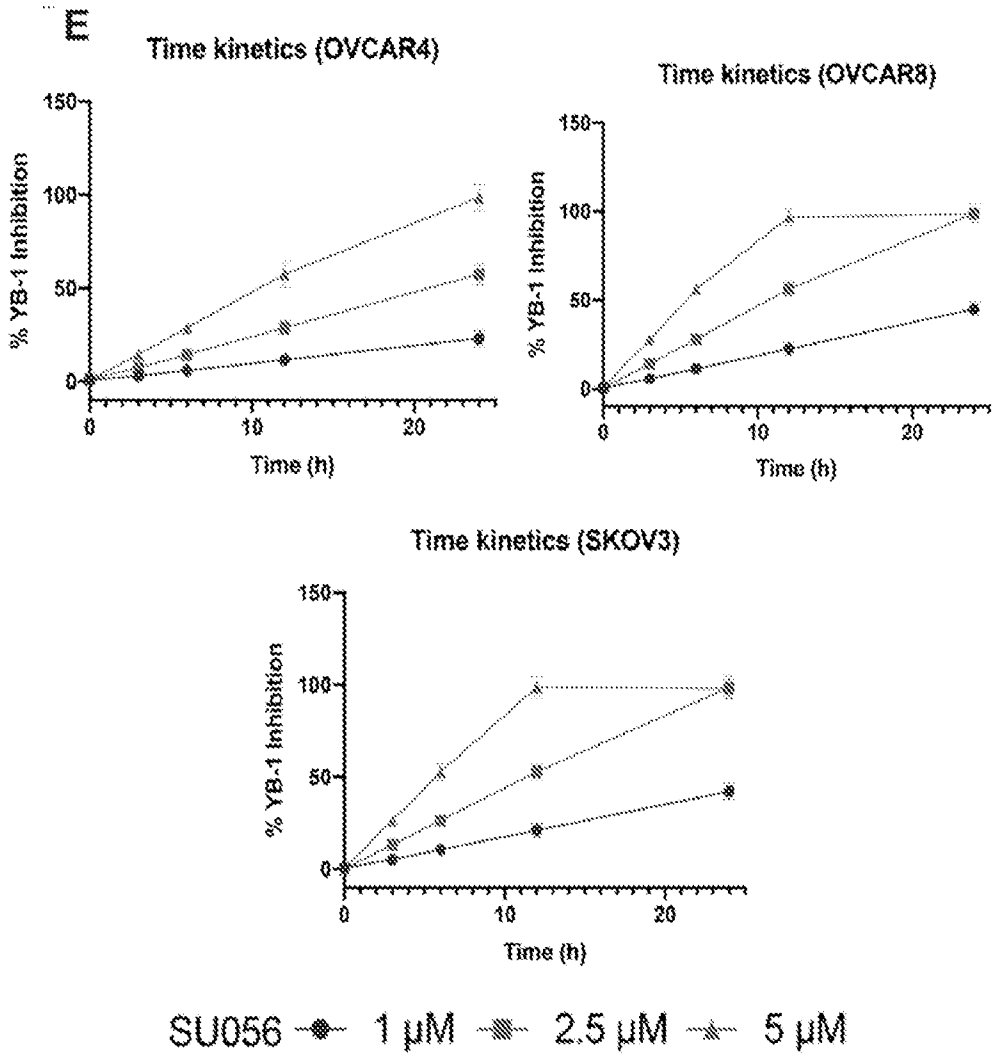

E
Time kinetics (OVCAR4)
Time kinetics (OVCAR8)
Time kinetics (SKOV3)
% YB-1 Inhibition
Time (h)
SU056 1 µM 2.5 µM 5 µM

FIG. 5A

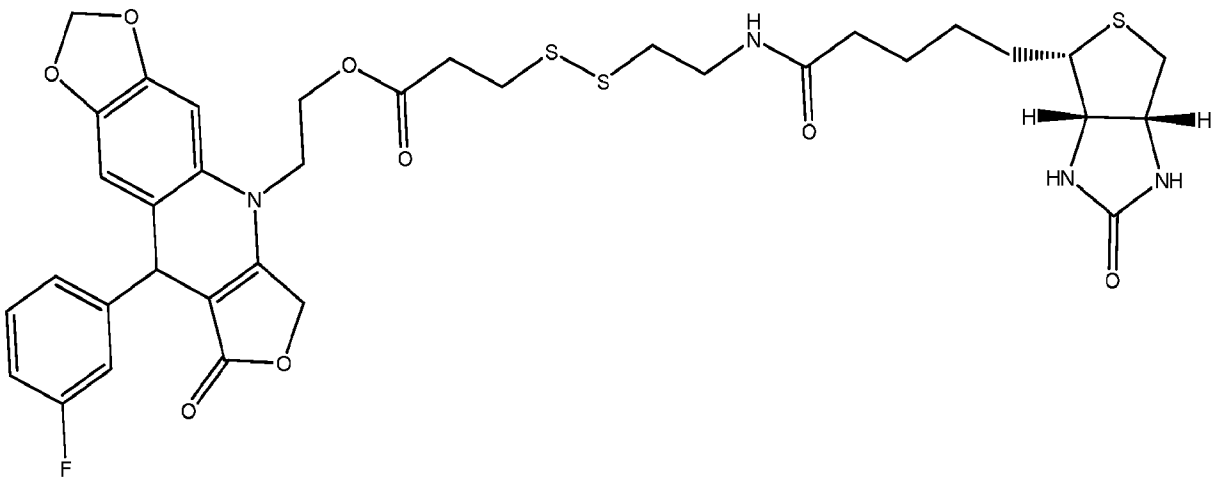


FIG. 5B

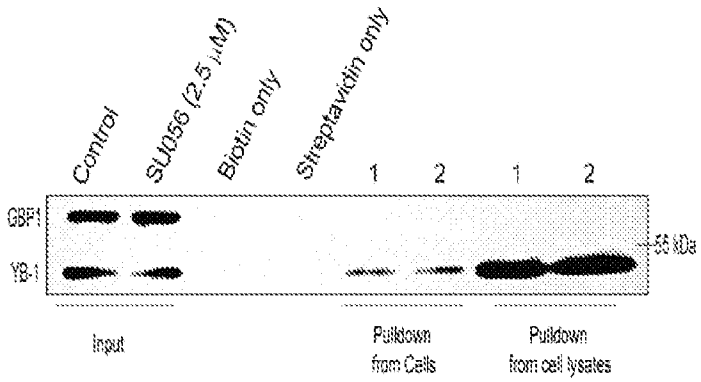


FIG. 5C

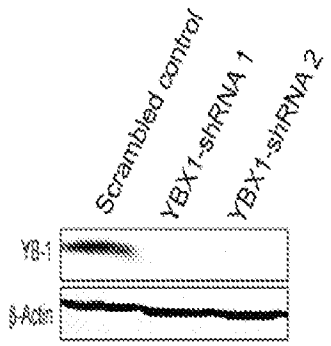


FIG. 5F

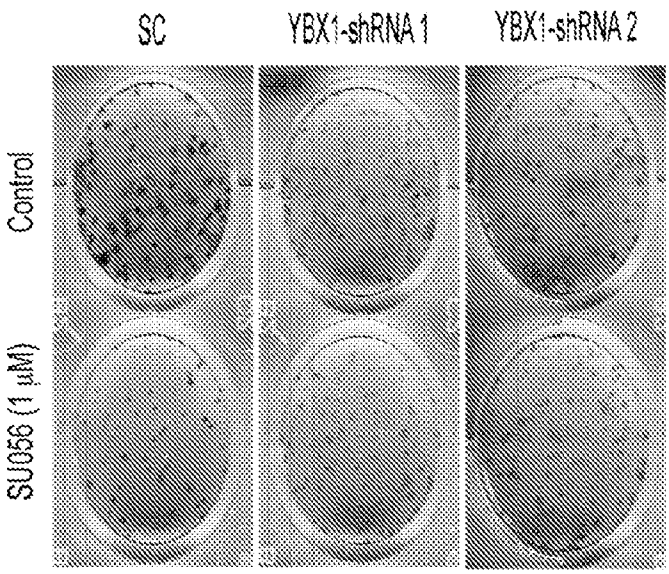


FIG. 5G

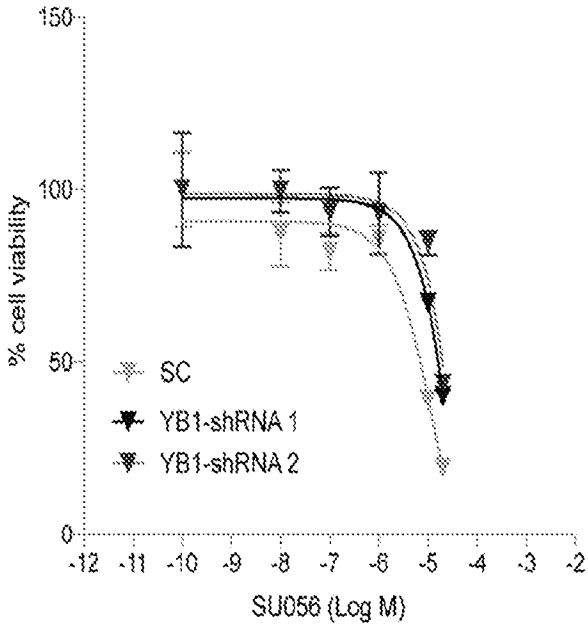


FIG. 6A

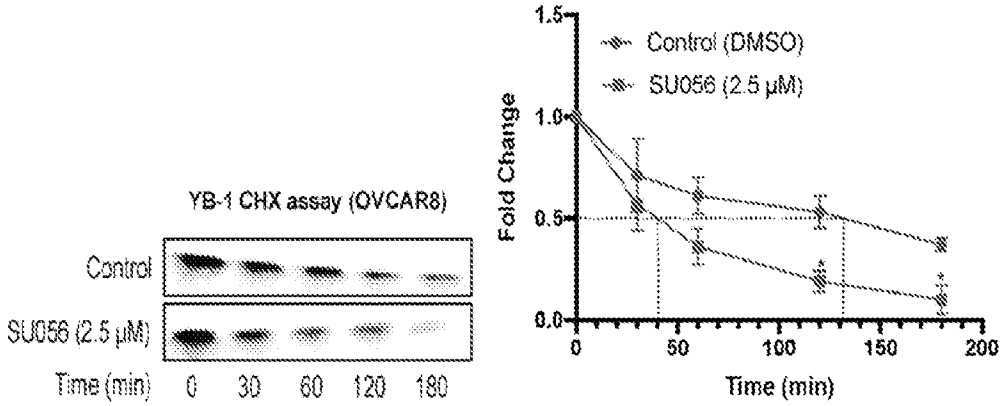


FIG. 6B

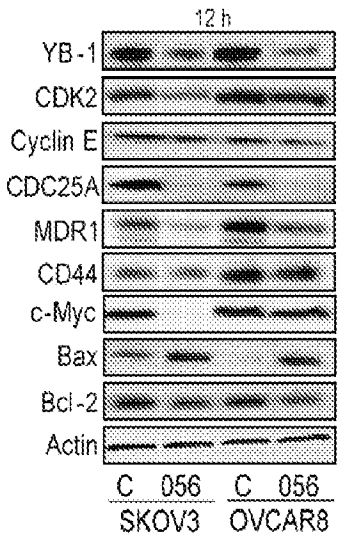


FIG. 6C

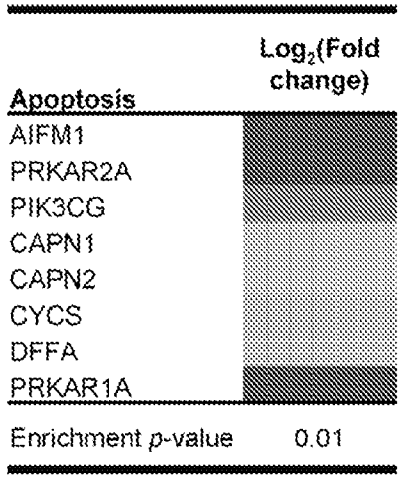
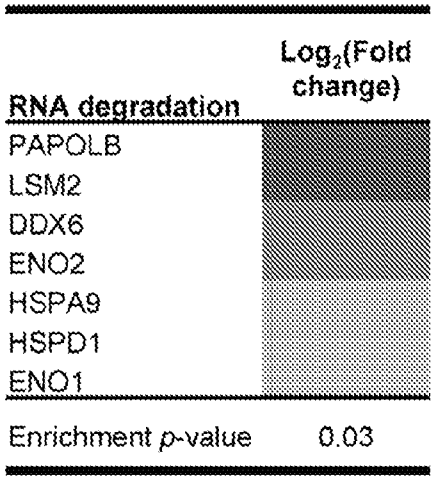


FIG. 6d



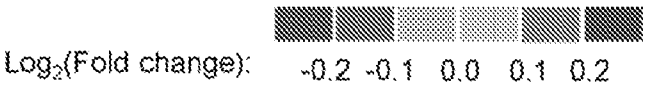


FIG. 6E

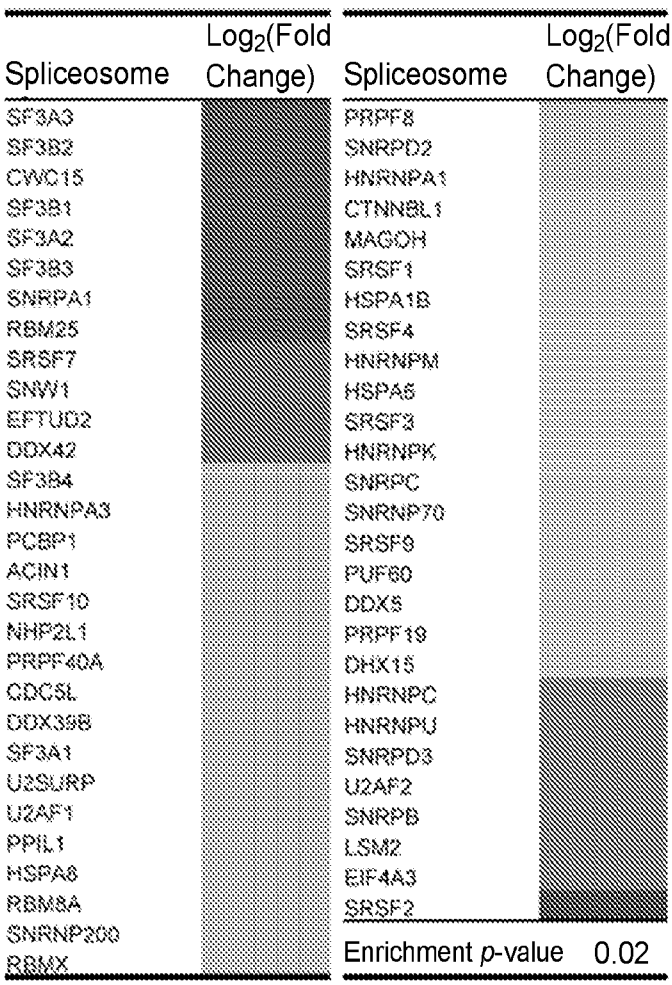


FIG. 7A

Combination Index

Cell Line	SU056 (μM)	Paclitaxel (nM)		
		0.1	0.5	1
OVCAR8	0.1	0.318	0.548	0.582
	0.5	0.647	0.655	0.584
	1	1.16	1.006	0.711
SKOV3	0.1	0.425	0.425	0.574
	0.5	0.711	0.61	0.516
	1	0.97	0.877	0.612

FIG. 7B

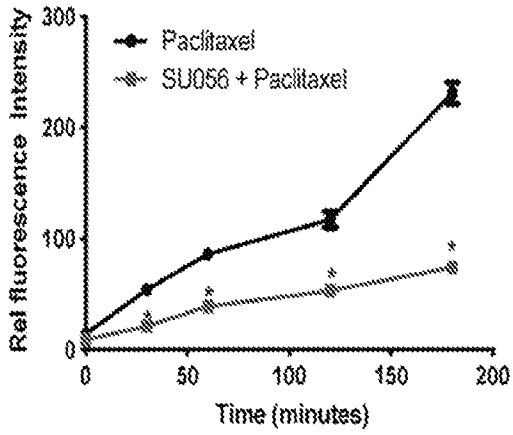


FIG. 7C

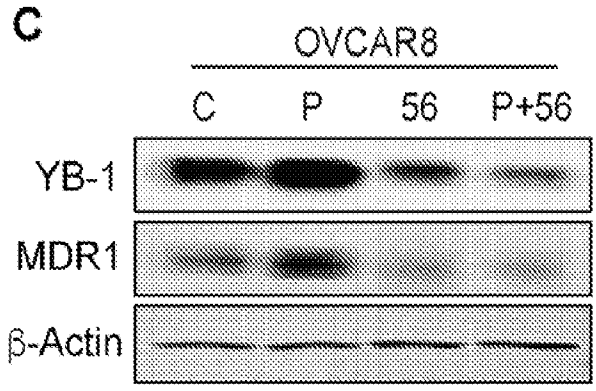


FIG. 7D

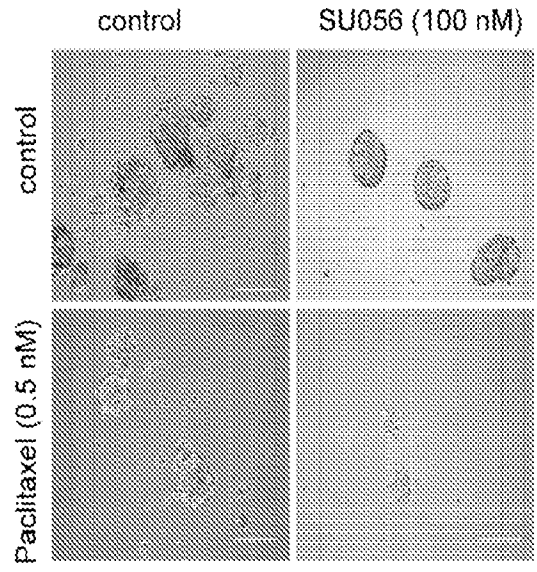


FIG. 7E

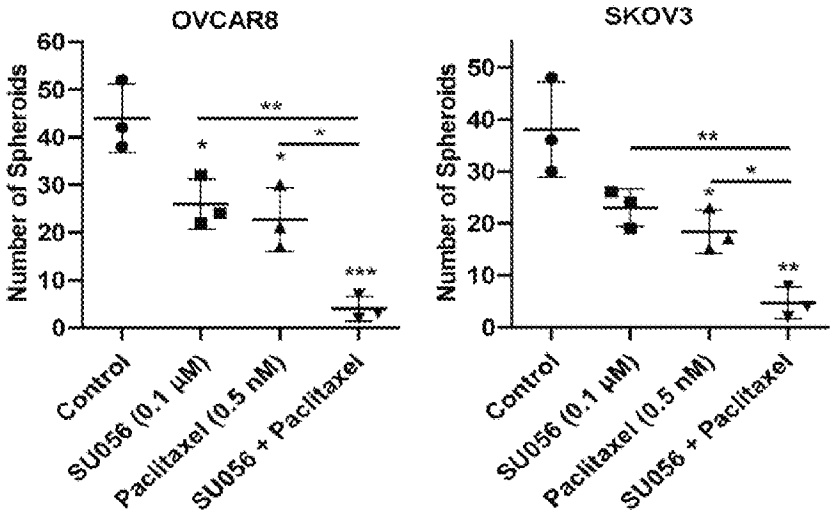


FIG. 7F

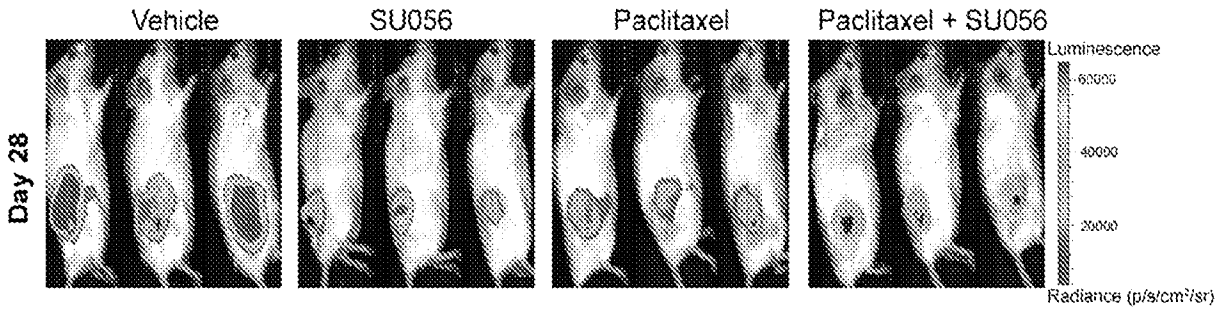


FIG. 7G

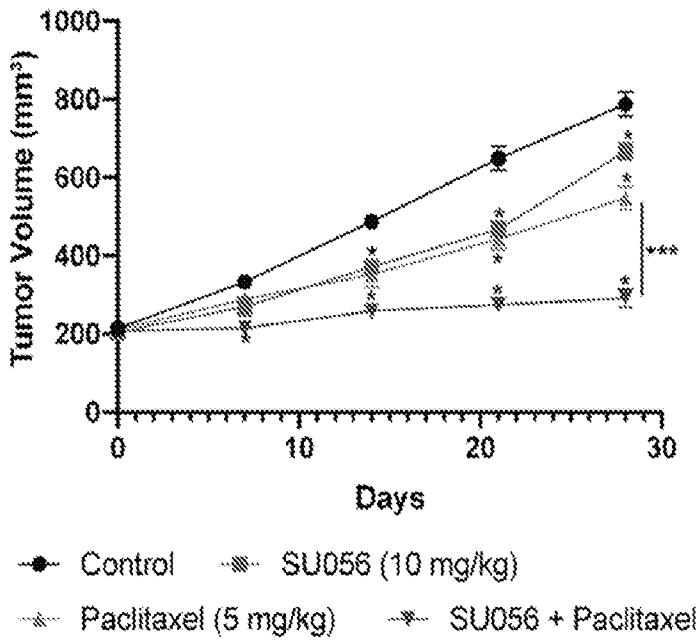


FIG. 7H

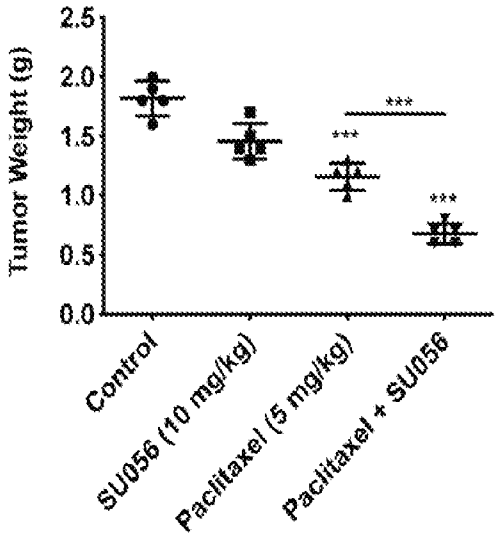


FIG. 7I

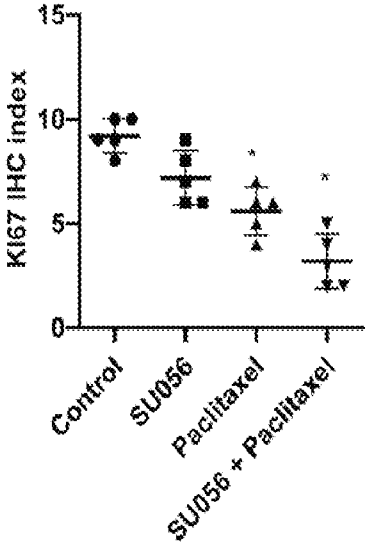


FIG. 8A

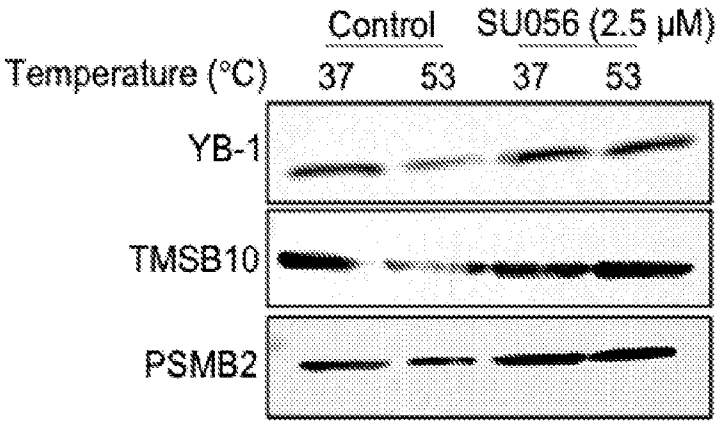


FIG. 8B

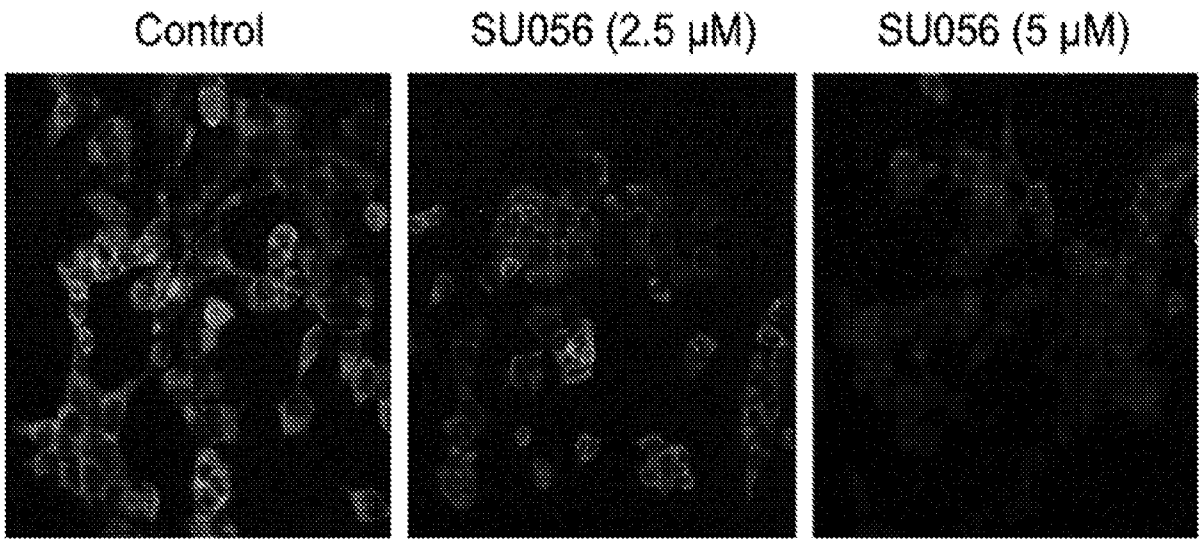


FIG. 8C

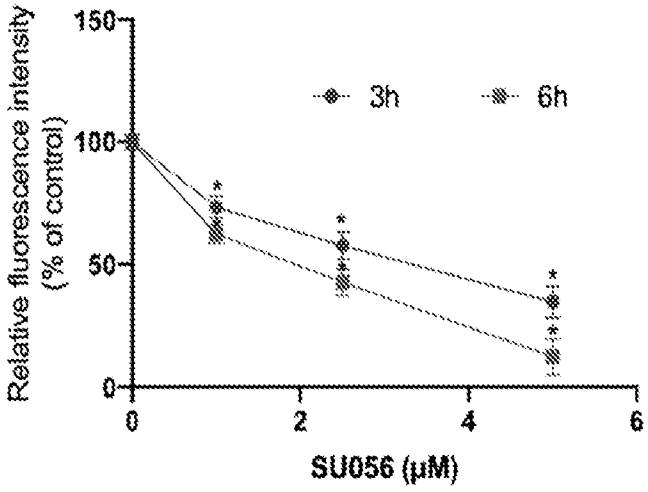


FIG. 9

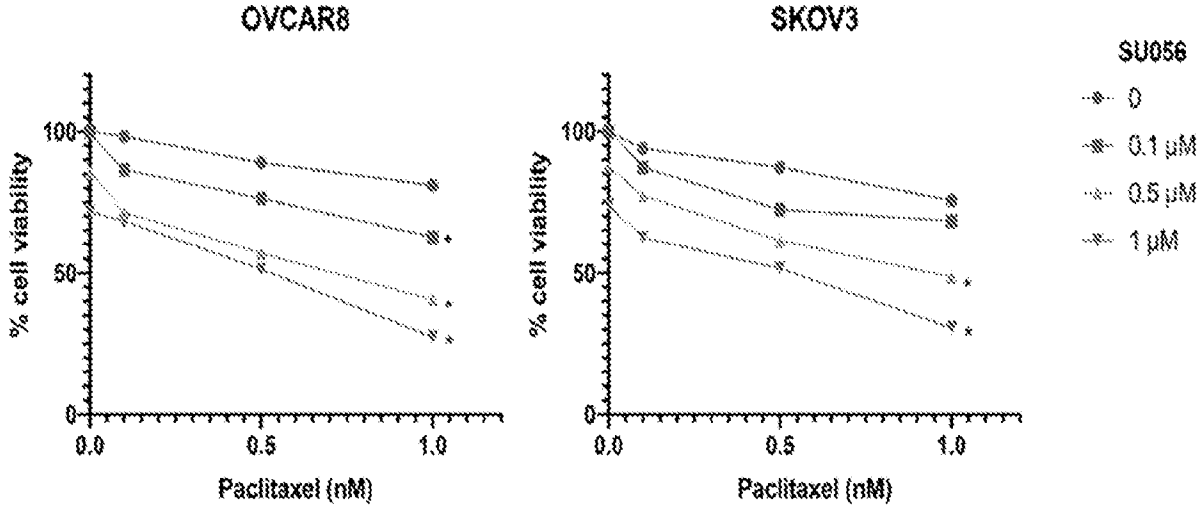


FIG. 10

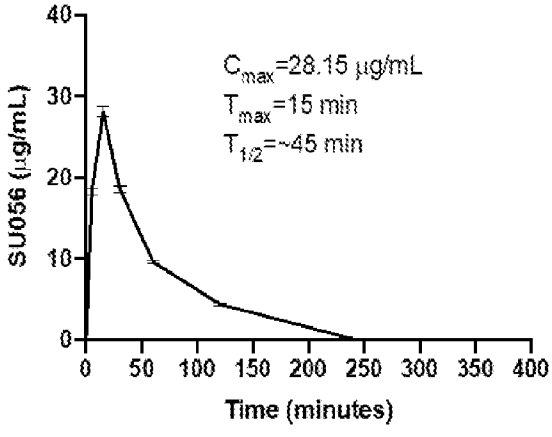


FIG. 11A

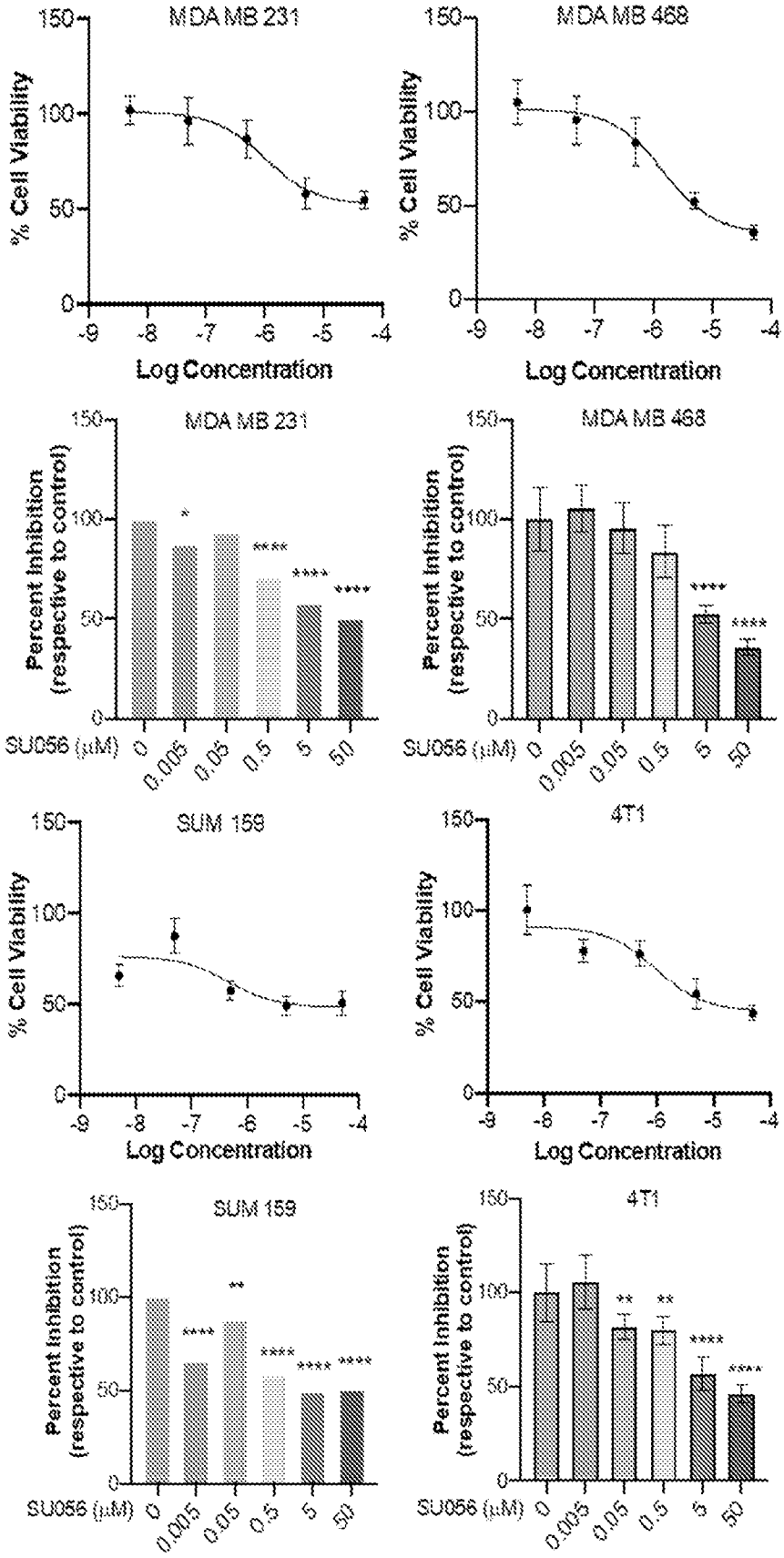
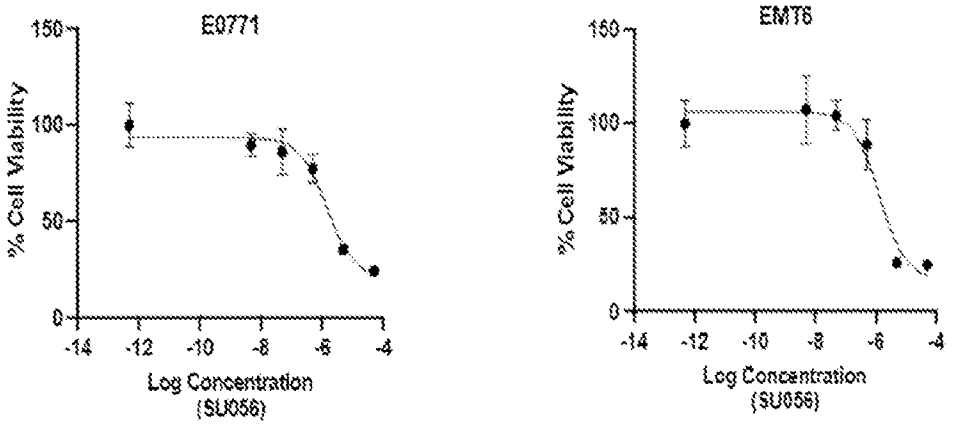

MDA MB 231
MDA MB 468
% Cell Viability
Log Concentration
Percent Inhibition (respective to control)
SU056 (μM)
0
0.005
0.05
0.5
5
50
SUM 159
4T1

FIGURE 11A (continued)



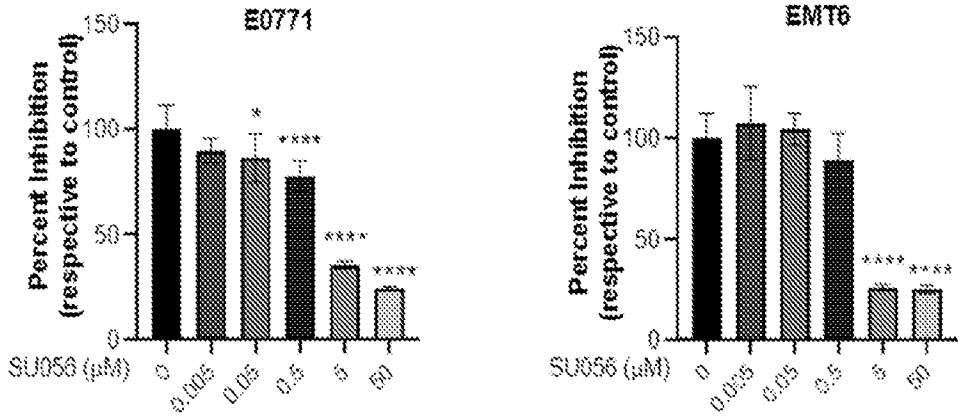


FIG. 11B

FIG. 11C

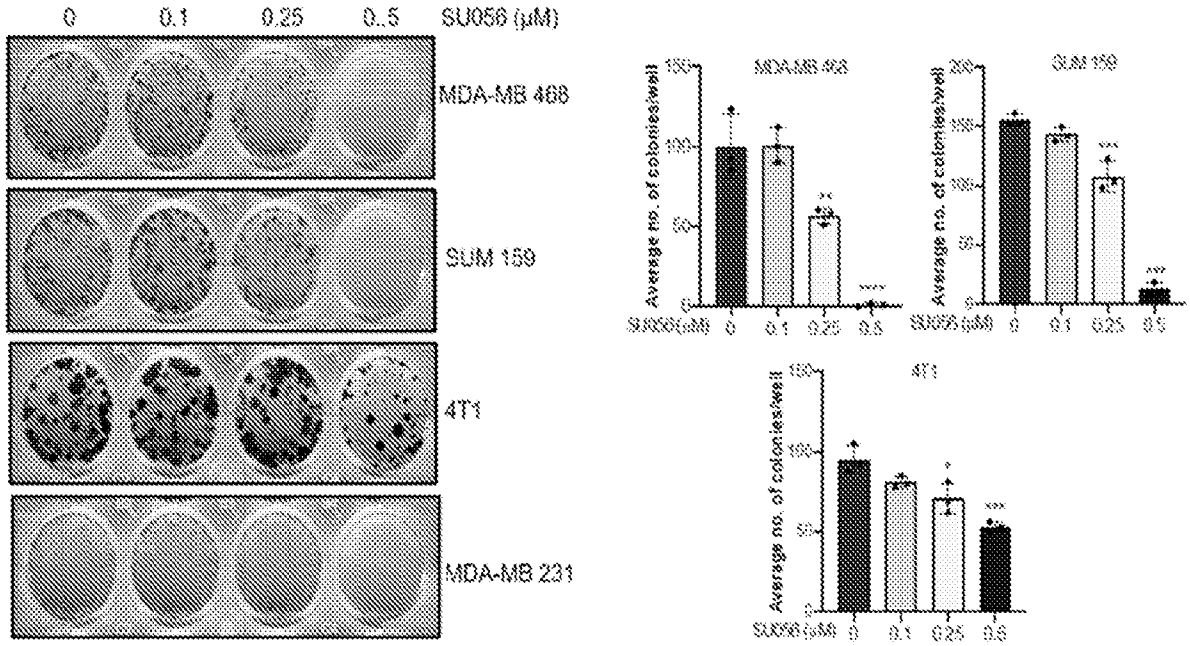


FIG. 11D

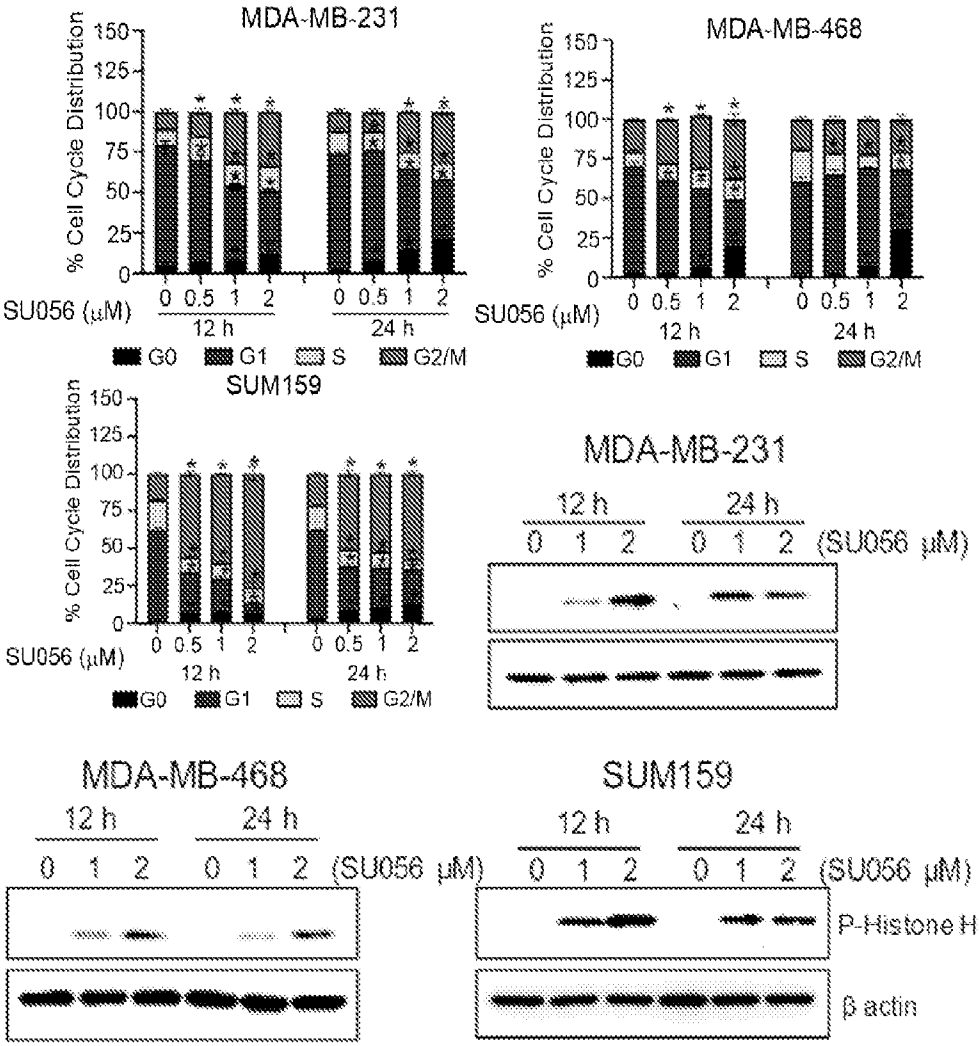


FIG. 12A

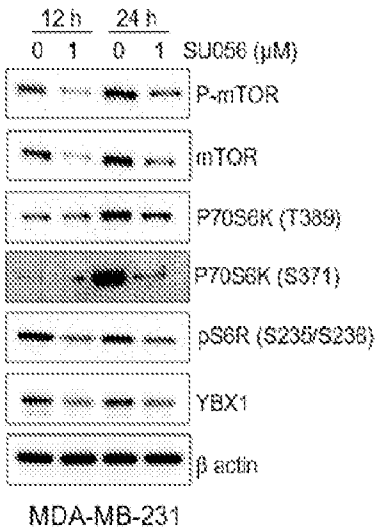


FIG. 12B

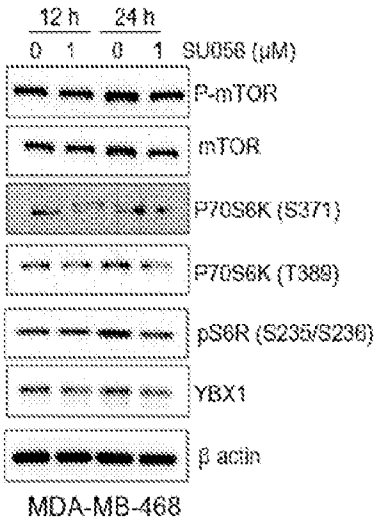


FIG. 12C

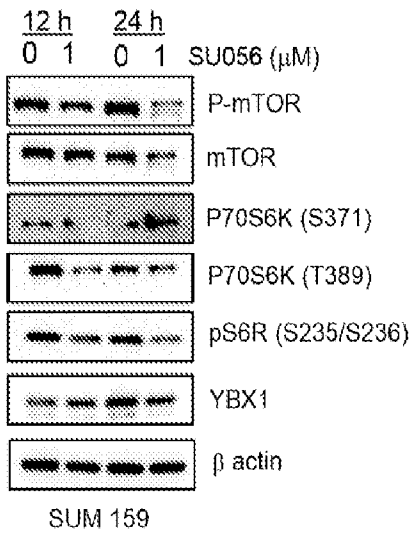


FIG. 13A

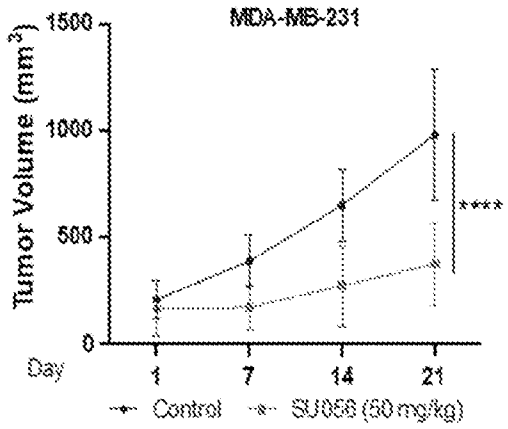


FIG. 13B

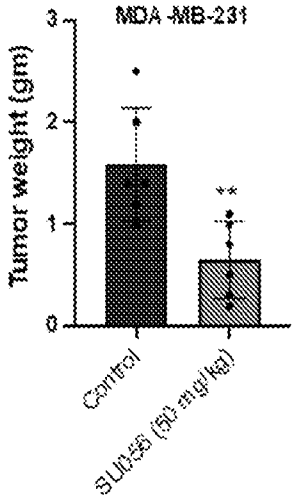


FIG. 13C

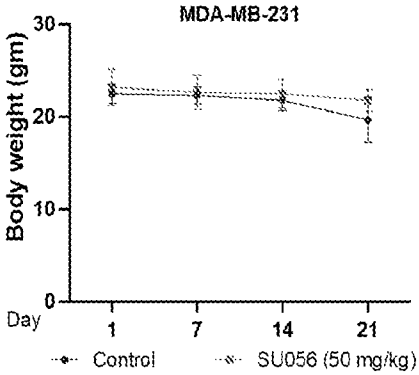


FIG. 13D

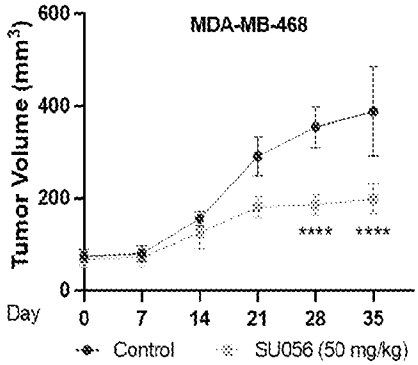


FIG. 13E

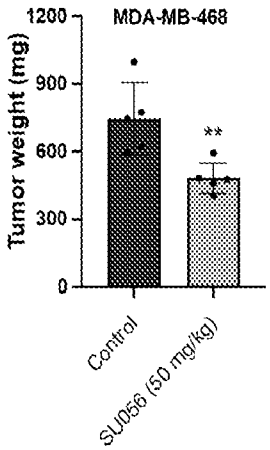


FIG. 13F

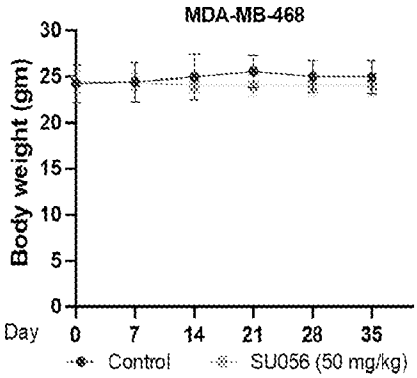


FIG. 13G

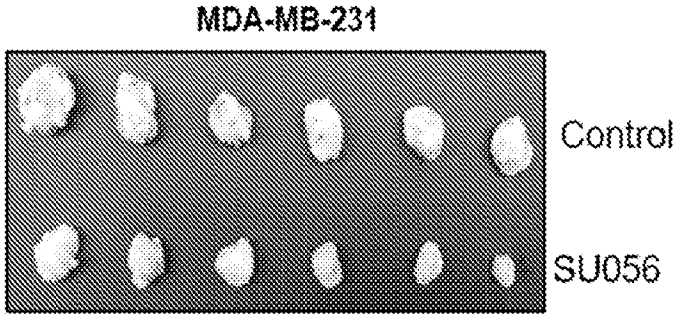


FIG. 13H

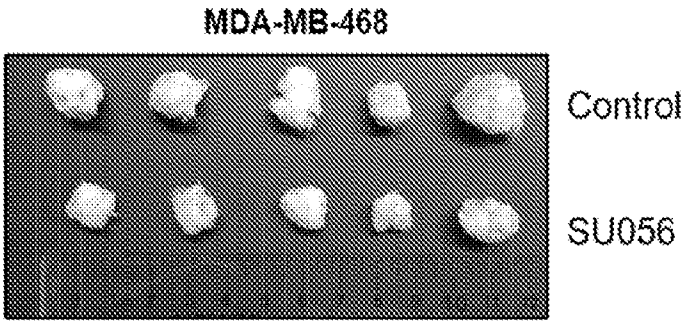


FIG. 13I

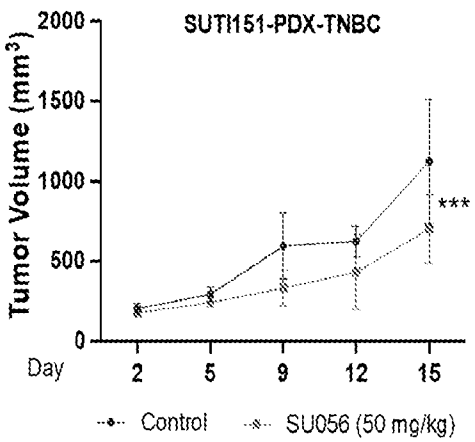


FIG. 13J

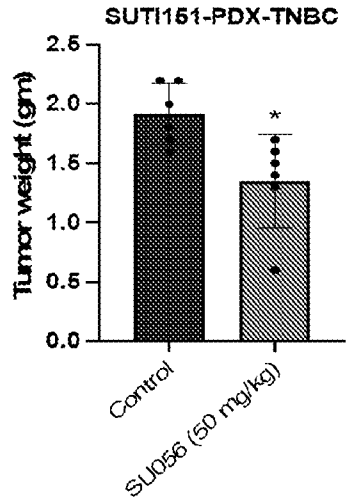


FIG. 13K

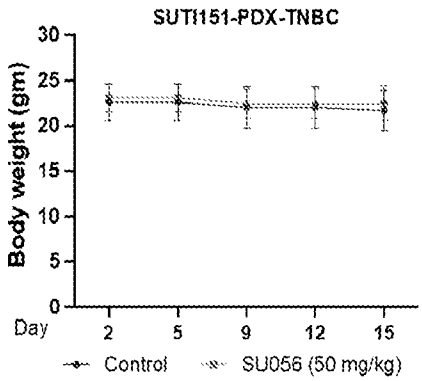


FIG. 14A

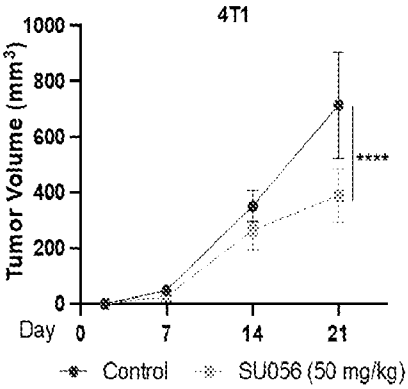


FIG. 14B

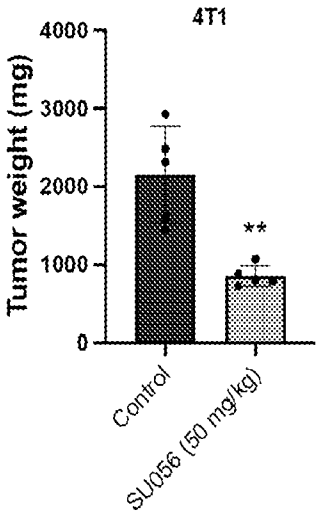


FIG. 14C

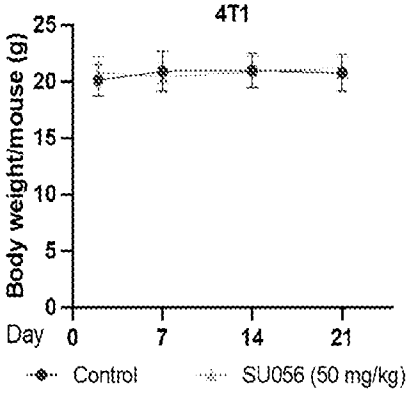


FIG. 14D

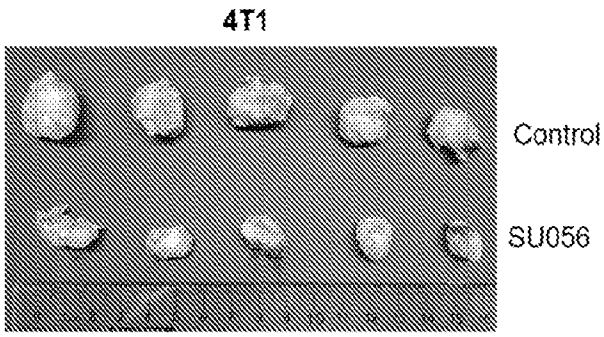


FIG. 14E

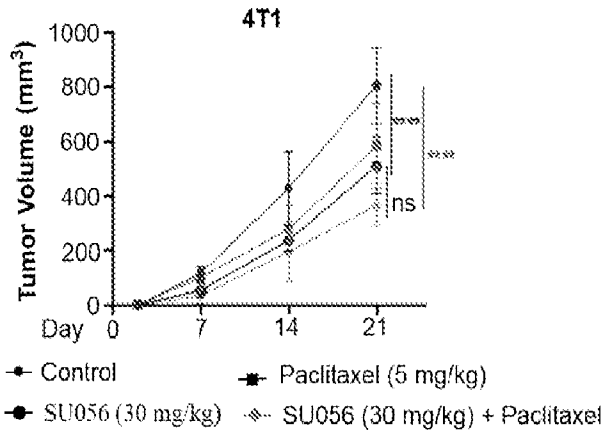


FIG. 14F

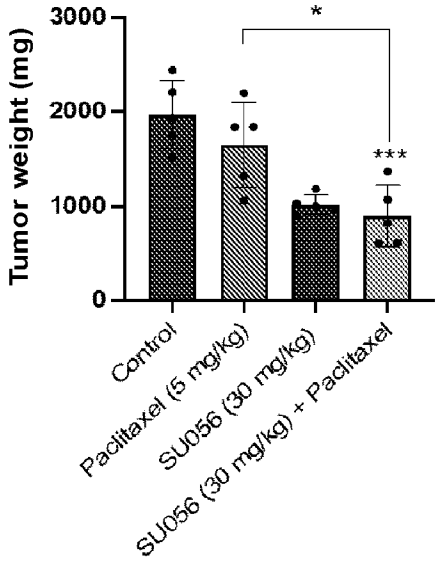


FIG. 14 G

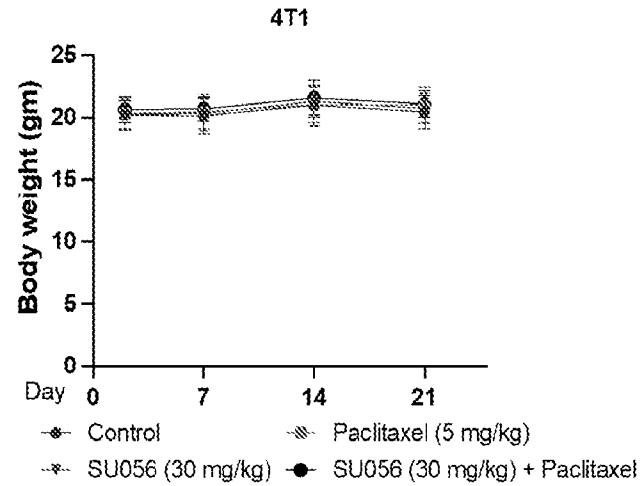


FIG. 15A

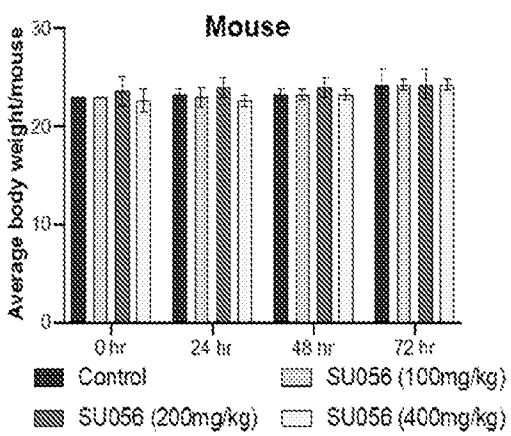


FIG. 15B

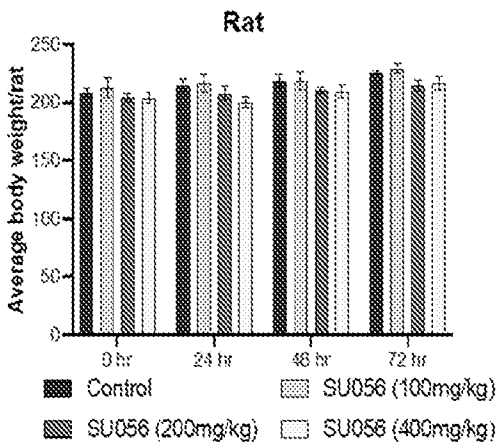


FIG. 15C

Compound	Route	Dose (mg/kg)	Toxicity (death / test) Strain: ICR (female)					
			15 min	1 hr	2 hr	24 hr	48 hr	72 hr
Vehicle (5% DMSO/45% PEG300/ 50% Saline)	IP	5 mL/kg	0/3	0/3	0/3	0/3	0/3	0/3
(SU056)	PO	100	0/3	0/3	0/3	0/3	0/3	0/3
		200	0/3	0/3	0/3	0/3	0/3	0/3
		400 (10 mL/kg)	0/3	0/3	0/3	0/3	0/3	0/3

FIG. 15D

Compound	Route	Dose (mg/kg)	Toxicity (death / test) Strain: Female SD rat				
			15 min	1 hr	24 hr	48 hr	72 hr
Vehicle (5% DMSO/45% PEG300/ 50% Saline)	IP	5 mL/kg	0/3	0/3	0/3	0/3	0/3
(SU056)	PO	100	0/3	0/3	0/3	0/3	0/3
		200	0/3	0/3	0/3	0/3	0/3
		400 (10 mL/kg)	0/3	0/3	0/3	0/3	0/3

FIG. 15E

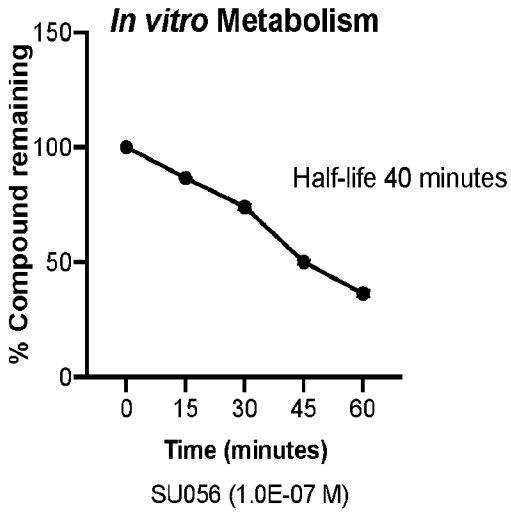


FIGURE 16

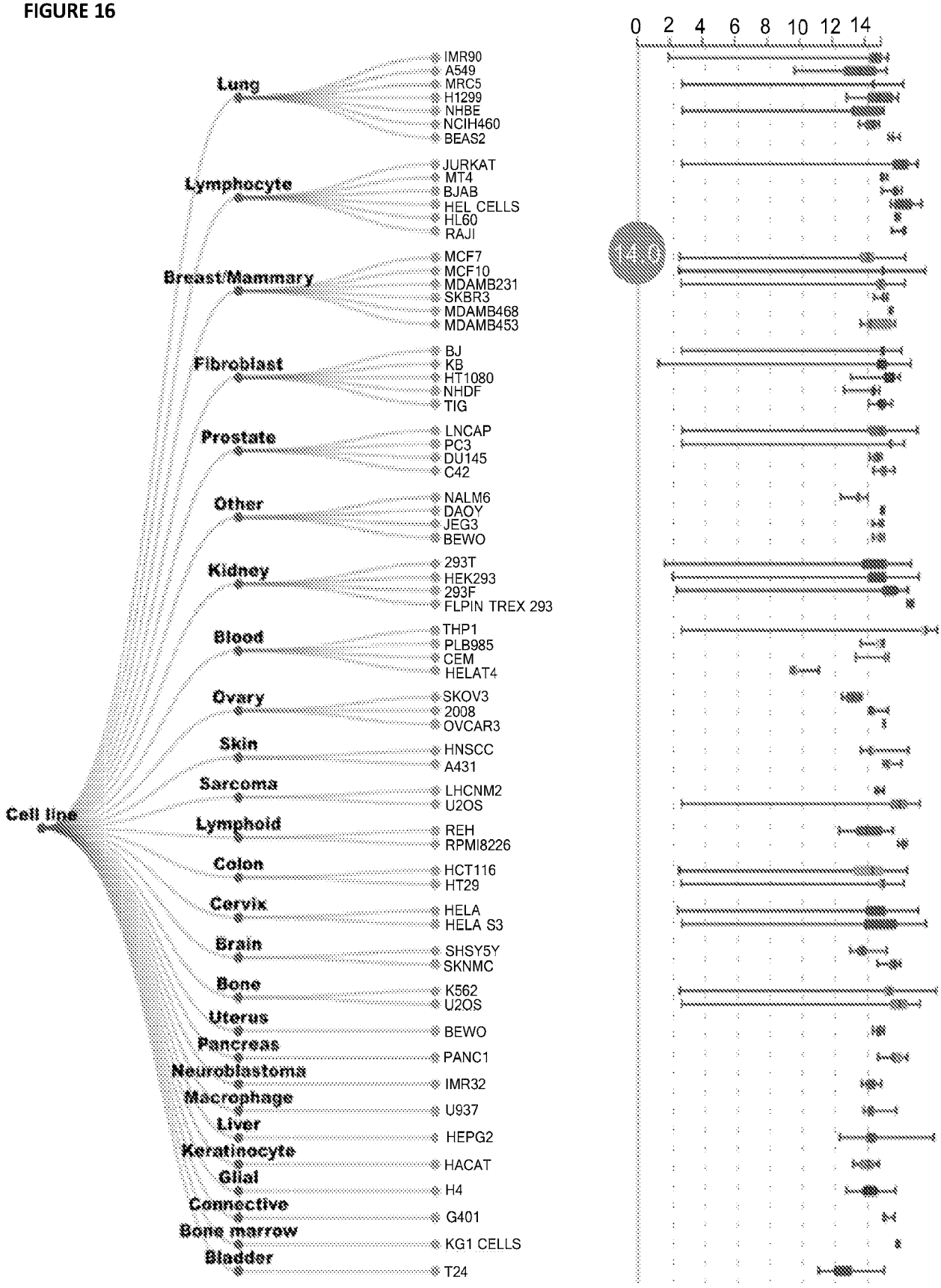

0 2 4 6 8 10 12 14
Cell line
Lung
IMR90
A549
MRC5
H1299
NHBE
NCIH460
BEAS2
Lymphocyte
JURKAT
MT4
BJAB
HEL CELLS
HL60
RAJI
Breast/Mammary
MCF7
MCF10
MDAMB231
SKBR3
MDAMB468
MDAMB453
Fibroblast
BJ
KB
HT1080
NHDF
TIG
Prostate
LNCAP
PC3
DU145
C42
Other
NALM6
DAOY
JEG3
BEWO
Kidney
293T
HEK293
293F
FLPIN TREX 293
Blood
THP1
PLB985
CEM
HELAT4
Ovary
SKOV3
2008
OVCAR3
Skin
HNSCC
A431
Sarcoma
LHCNM2
U2OS
Lymphoid
REH
RPMI8226
Colon
HCT116
HT29
Cervix
HELA
HELA S3
Brain
SHSY5Y
SKNMC
Bone
K562
U2OS
Uterus
BEWO
Pancreas
PANC1
Neuroblastoma
IMR32
Macrophage
U937
Liver
HEPG2
Keratinocyte
HACAT
Glial
H4
Connective
G401
Bone marrow
KG1 CELLS
Bladder
T24

FIGURE 17

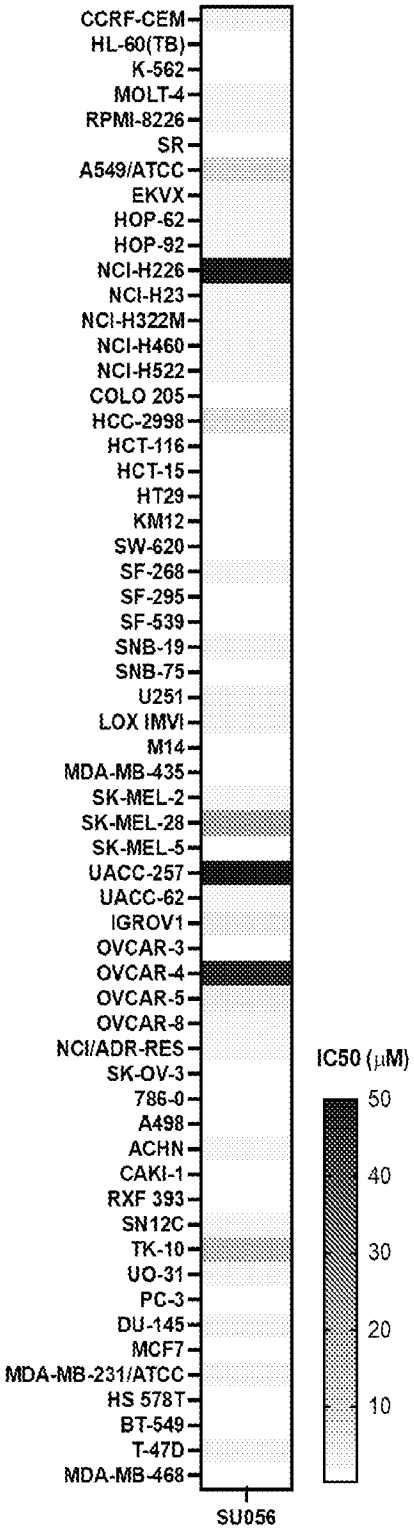

CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251
LOX IMVI
M14
MDA-MB-435
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
NCI/ADR-RES
SK-OV-3
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
PC-3
DU-145
MCF7
MDA-MB-231/ATCC
HS 578T
BT-549
T-47D
MDA-MB-468
SU056
IC50 (μM)
50
40
30
20
10

FIGURE 18

Translation initiation factors

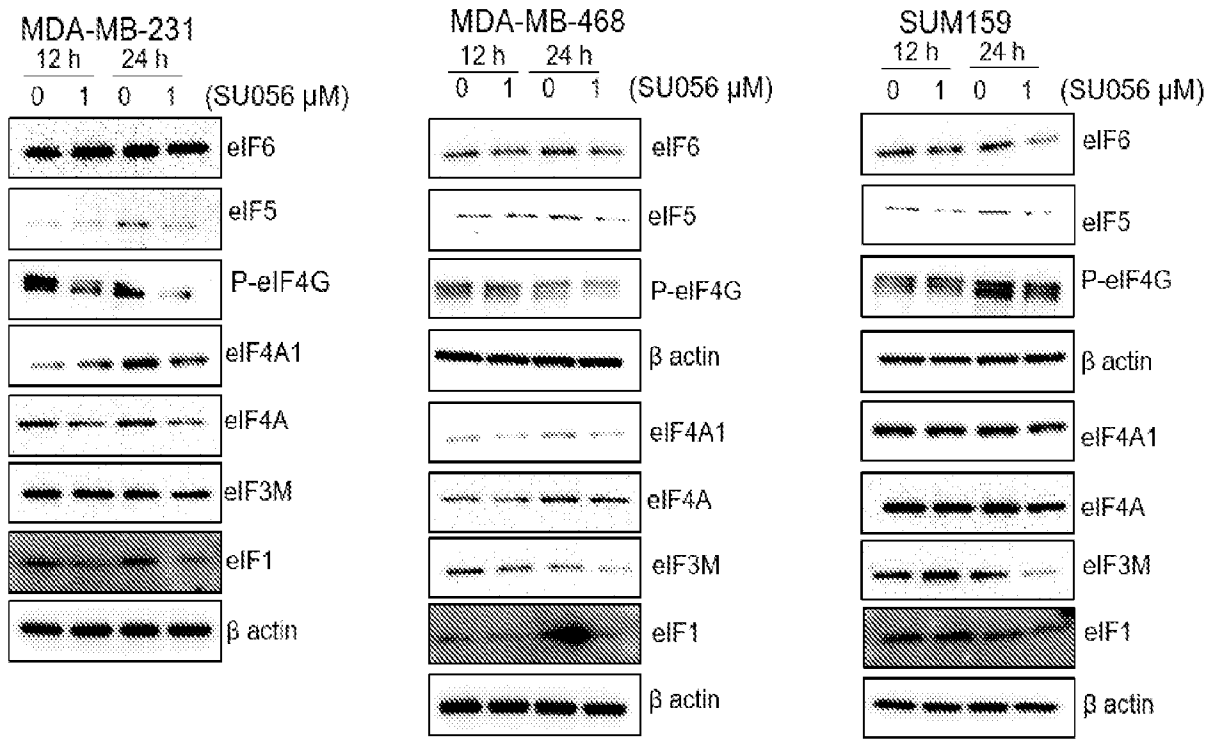


FIGURE 19

Ribosomal large subunit proteins

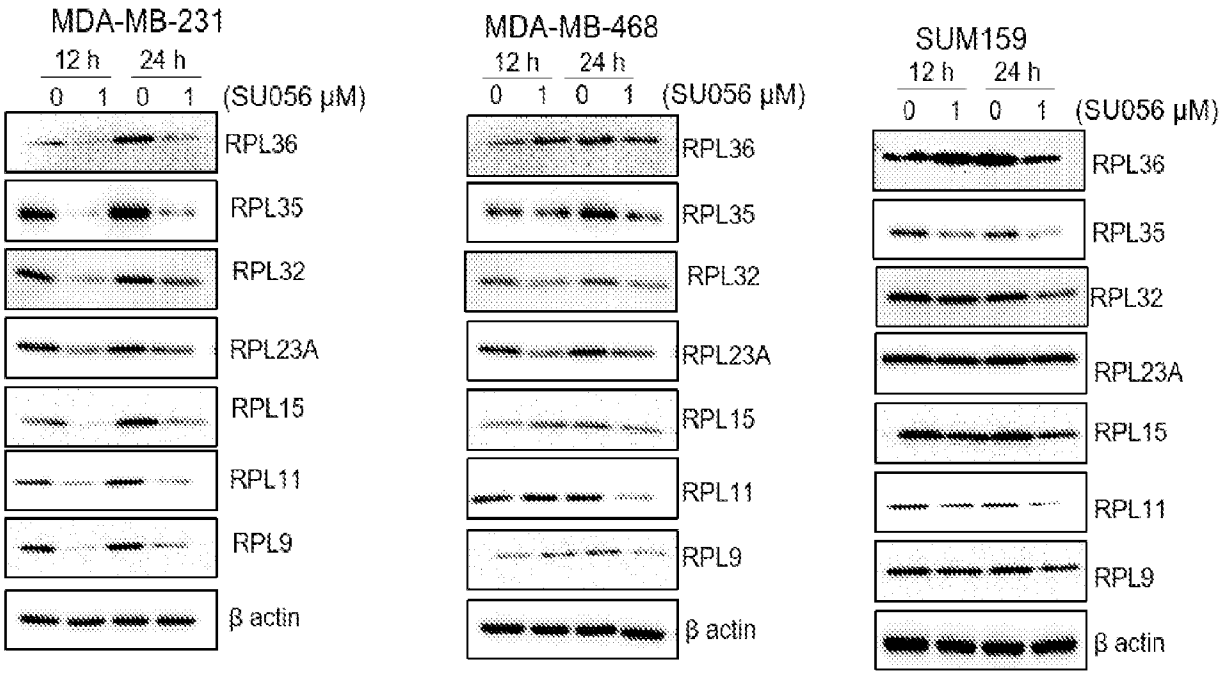
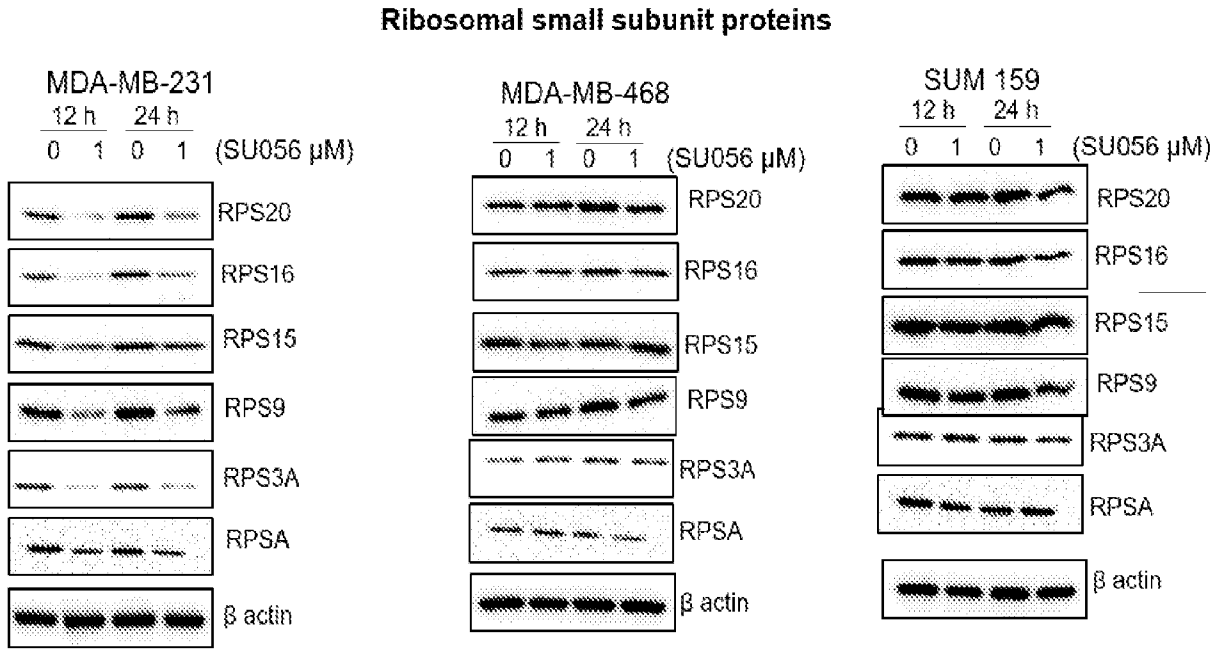


FIGURE 20


Ribosomal small subunit proteins
MDA-MB-231
12 h
24 h
0 1 0 1 (SU056 µM)
RPS20
RPS16
RPS15
RPS9
RPS3A
RPSA
β actin
MDA-MB-468
12 h
24 h
0 1 0 1 (SU056 µM)
RPS20
RPS16
RPS15
RPS9
RPS3A
RPSA
β actin
SUM 159
12 h
24 h
0 1 0 1 (SU056 µM)
RPS20
RPS16
RPS15
RPS9
RPS3A
RPSA
β actin

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061906

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/435; A61K 31/4375; C07D 215/00 (2022.01)
CPC - A61K 31/435; A61K 31/4375; C07D 215/00 (2022.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PUBCHEM, SID 319685906, Available Date: 08 December 2016 [retrieved on 19 January 2022].,Retrieved from the Internet <URL: https://pubchem.ncbi.nlm.nih.gov/substance/319685906> entire document	1, 2, 6-8
A	SETOGUCHI et al., Antisense Oligonucleotides Targeting Y-Box Binding Protein-1 Inhibit Tumor Angiogenesis by Downregulating Bcl-xL-VEGFR2/-Tie Axes, Molecular Therapy: Nucleic Acids, Vol. 9, December 2017 [retrieved on 19 January 2022]. Retrieved from the Internet: <URL: https://www.sciencedirect.com/science/article/pii/S2162253117302536>. Pgs. 170-181	1, 2, 6-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 March 2022	MAR 30 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061906

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061906

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-40, 41a, 41b, 42-45
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 6-8

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061906

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-9 are drawn to compounds having a structure of Formula (I), or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof, and methods of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein thereof.

The first invention of Group I+ is restricted based on the proviso that at least one of R1, R2, R3, R4, and R5 is selected from the group of F, C1-C4 fluoroalkyl, and SF5, and with the proviso that, when X is the shown phenyl moiety and R2 is F or CF3, then at least one of R1 R3, R4, and R5 is not H; and is restricted to a compound having a structure of Formula (I) wherein X is the first shown moiety; R1 is F; R2, R3, R4, and R5 are each H; n is 1; and R6 is H, or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof; and methods of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein thereof. It is believed that claims 1, 2, and 6-8 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a compound having a structure of Formula (I) wherein X is the first shown moiety; R1, R2, R4, and R5 are each H; R3 is F; n is 1; and R6 is H, or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof; and methods of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein thereof. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables, X, R1, R2, R3, R4, R5, n, R6, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of a compound having the core structure of Formula (I), or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof; and a method of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound, these shared technical features do not represent a contribution over the prior art as disclosed by the publication entitled "SID 319685906" by PubChem (hereinafter, "PubChem") and the publication entitled "Antisense Oligonucleotides Targeting Y-Box Binding Protein-1 Inhibit Tumor Angiogenesis by Downregulating Bcl-xL-VEGFR2/-Tie Axes" by Setoguchi et al. (hereinafter, "Setoguchi").

PubChem teaches a compound having the core structure of Formula (I), or a pharmaceutically acceptable salt, co-crystal, ester, solvate, hydrate, isomer, tautomer, isotope, polymorph, or prodrug thereof (Pg. 2, compound as shown).

Setoguchi teaches a method of inhibiting YB1 protein activity in a subject experiencing a cancer expressing YB1 protein, the method comprising administering to the subject in need thereof a pharmaceutically effective amount of a compound (Abstract, Y-box binding protein-1 (YB-1), involved in cancer progression and chemoradiation resistance, is overexpressed in not only cancer cells but also tumor blood vessels. In this study, we investigated the potential value of amido-bridged nucleic acid (AmNA)-modified antisense oligonucleotides (ASOs) targeting YB-1 (YB-1 ASOA) as an antiangiogenic cancer therapy; Abstract, YB-1 ASOA administered i.v. significantly inhibited YB-1 expression in CD31-positive angiogenic endothelial cells...YB-1 ASOA significantly suppressed tumor growth).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.