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(54) **Title:** THERAPEUTIC COMPOUNDS

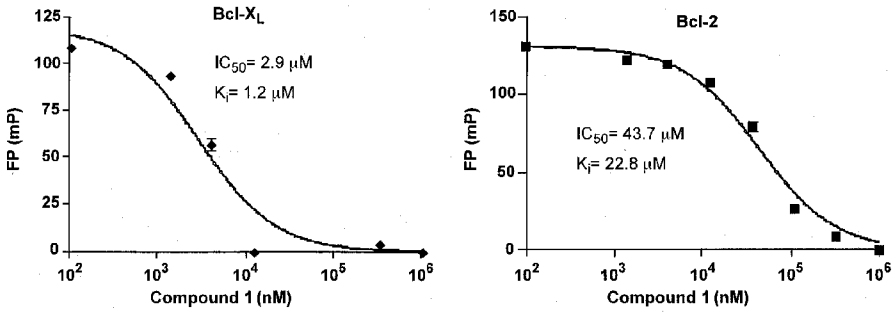
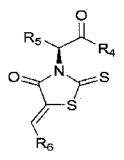


Fig. 2



I

(57) **Abstract:** The invention provides a compound of formula I: wherein R_4, R_5 and R_6 have any of the values described herein or a salt thereof, as well as synthetic processes and intermediates useful for preparing such compounds. The compounds have anti-cancer activity.

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5-BENZYLIDENE-4-OXO-2-THIOOXOTHIAZOLIDIN-3-YLACETIC ACID COMPOUNDS AS BCL-2 PROTEIN ANTAGONISTS

Government Funding

The invention described herein was made with government support under Grant Number CA114294 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

Background of the Invention

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths among males in the United States of America with about 218,890 new cases and 27,050 prostate cancer-related deaths expected for 2007 (Jemal A, et al., CA Cancer J. Clin. 2007;57:43-66). This rate translates to one in six men being diagnosed with prostate cancer in their lifetime. While treatment for localized therapy can be highly effective, current treatment strategies for metastatic prostate cancer are temporizing as the disease following initial remission in response to androgen ablation relapses to a hormone-refractory phenotype (HRPC), a generally fatal form of prostate cancer. It was estimated that a median expected survival for patients with HRPC is only 10 months (Sciarra A, Salciccia S., Eur. Urol. 2007;52:964-72). Therefore, the search for novel agents and approaches for the treatment of HRPC must be considered a high priority.

While HRPC was traditionally thought to be a chemoresistant disease, docetaxel-based chemotherapy has recently been demonstrated to modestly improve the overall survival of HRPC patients by approximately 6 months (Petrylak DP., Rev. Urol. 2006;8:S48-55). These results have now made docetaxel the standard of care for the treatment of HPRC. Mechanistically, docetaxel is a microtubule-stabilizing agent – one subfamily of anti-microtubule agents which halts the cell cycle at the metaphase-anaphase transition, eventually resulting in apoptosis.

Although docetaxel has been demonstrated to have moderate success in the treatment of HRPC patients, most of these patients develop resistance to docetaxel and die from the drug-resistant phenotype (Mancuso A, et al., Crit. Rev. Oncol. Hematol. 2007;61:176-85). Drug-resistant HRPC is often genetically associated with the over-expression of Bcl-2 protein, a key regulator in the prohibition of apoptosis and strongly implicated in the development of the drug-resistant phenotype of multiple cancers (Mancuso A, et al., Crit. Rev. Oncol. Hematol. 2007;61:176-85; Oliver CL, et al., Mol. Cancer Ther. 2005;4:23-31; McConkey DJ, et al., Cancer Res. 1996;56:5594-9; and Li X, et al., Cancer Res. 2001;61:1699-706.). Since Bcl-2

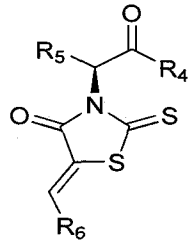
protein is generally not expressed in the normal prostate, inhibition of the anti-apoptotic Bcl-2 protein represents a promising and potentially safe strategy to overcome the resistance of prostate cancer to docetaxel treatment (McDonnell TJ , et al., Cancer Res. 1992;52:6940-4). This concept is supported by the results of recent clinical trial that combined docetaxel with oblimersen sodium (Genasense – an antisense for Bcl-2 protein) in the treatment of patients with HRPC (Tolcher AW, et al., Clin. Cancer Res. 2004;10:5048-57; and Tolcher AW, et al., Clin. Cancer Res. 2005;11:3854-61). Other than Bcl-2 antisense, small-molecule antagonists against anti-apoptotic Bcl-2 proteins are also being intensely investigated (Wang J, et al., Proc. Natl. Acad. Sci. 2000;97:7124-9; Degterev A, et al., Nat. Cell Biol. 2001;3:173-82; Kitada S, et al., J. Med. Chem. 2003;46:4259-64; Leone M, et al., Cancer Res. 2003;63:8118-21; Oltersdorf T , et al., Nature 2005;435:677-81; and Tang G, et al., J. Med. Chem. 2007;50:1723-6). (-)-Gossypol, for example, has been demonstrated to enhance the response of PC-3 cells to radiation therapy via targeted inhibition of Bcl-X_L (Xu L, et al., Mol. Cancer Ther. 2005;4:197-205).

In spite of the above reports, there is currently a need for additional agents that are useful for treating cancer. In particular, there is a need for single agents that effectively target drug-resistant cancers and that are capable of circumventing drug resistance. There is also a need for pharmacological tools for the further study of the physiological processes associated with cancer (e.g. HRPC).

Summary of the Invention

Applicant has identified compounds with activity as dual antagonists against anti-apoptotic Bcl-2 proteins and microtubule. These compounds have been shown to have activity against HRPC. Considering the pivotal function of anti-apoptotic Bcl-2 proteins in the development and metastasis of HRPC and the promising future of antimicrotubule agents against HRPC, a compound possessing dual antagonism provides a new opportunity for the effective treatment of HRPC. The ability to obtain dual activity within a single molecule eliminates the complicated dosing requirements associated with the administration of two or more agents.

Accordingly, in one embodiment the invention provides a compound of the invention which is a compound of formula I:



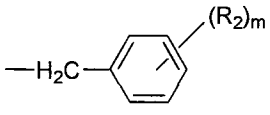
I

wherein:

R_4 is OH or $NHSO_2R_1$;

R_1 is aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, aryl$(C_1\text{-}C_6)$alkyl, heteroaryl$(C_1\text{-}C_6)$alkyl, or $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl is optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, $-C(=O)NR_aR_b$, trifluoromethoxy, and nitro;

R_5 is $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, or

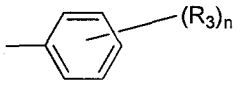


;

m is 0, 1, 2, 3, 4, or 5;

each R_2 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b,

-C(=O)NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl(C_1-C_6)alkyl, or heteroaryl(C_1-C_6)alkyl, wherein any aryl or heteroaryl of R_2 is optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_cR_d, -C(=O) NR_cR_d, and trifluoromethoxy;

R_6 is 

;

each R_3 is independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl(C_1-C_6)alkyl, heteroaryl(C_1-C_6)alkyl, or -A-B-C-D wherein A is pyrrolidino, piperidino, piperazino, or morpholino, B is (C_1-C_6)alkyl, C is aryl or absent and D is aryl or absent and wherein any aryl or heteroaryl of R_3, C and D is optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy;

n is 0, 1, 2, 3, 4, or 5;

each R_a and R_b is independently hydrogen, (C_1-C_6)alkyl, or (C_1-C_6)alkanoyl wherein any (C_1-C_6)alkyl is optionally substituted with -S-aryl or -O-aryl; or R_a and R_b taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

each R_c and R_d is independently hydrogen, (C_1-C_6)alkyl, or (C_1-C_6)alkanoyl; or R_c and R_d taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring; and

each R_e and R_f is independently hydrogen, (C_1-C_6)alkyl, or (C_1-C_6)alkanoyl; or R_e and R_f taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

or a salt thereof.

In another embodiment the invention also provides a composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

In another embodiment the invention also provides a therapeutic method for treating cancer (e.g. lymphoma, prostate cancer, hormone-refractory phenotype prostate cancer and leukemia) in an animal (e.g. a mammal such as a human) comprising, administering to the animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof as described herein for use in medical therapy.

In another embodiment the invention also provides the use of a compound of formula I or a pharmaceutically acceptable salt thereof as described herein for the manufacture of a medicament useful for the treatment of cancer (e.g. lymphoma, prostate cancer, hormone-refractory phenotype prostate cancer and leukemia) in an animal (e.g. a mammal such as a human).

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of cancer (e.g. lymphoma, prostate cancer, hormone-refractory phenotype prostate cancer and leukemia) in an animal (e.g. a mammal such as a human).

In another embodiment the invention also provides a method to antagonize anti-apoptotic Bcl-2 proteins in an animal (e.g. a mammal such as a human) comprising administering to the animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

In another embodiment the invention also provides a method to antagonize microtubule formation in an animal (e.g. a mammal such as a human) comprising administering to the animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

In another embodiment the invention also provides a method to antagonize anti-apoptotic Bcl-2 proteins and to antagonize microtubule formation in an animal (e.g. a mammal such as a human) comprising administering to the animal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing anti-apoptotic Bcl-2 proteins in an animal (e.g. a mammal such as a human).

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing microtubule formation in an animal (e.g. a mammal such as a human).

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing anti-apoptotic Bcl-2 proteins and for antagonizing microtubule formation in an animal (e.g. a mammal such as a human).

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal (e.g. a mammal such as a human) wherein the antagonism of anti-apoptotic Bcl-2 proteins is desired.

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal (e.g. a mammal such as a human)wherein the antagonism of microtubule formation is desired.

In another embodiment the invention also provides a compound of formula I or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal (e.g. a mammal such as a human) wherein the antagonism of anti-apoptotic Bcl-2 proteins and the antagonism of microtubule formation is desired.

The invention also provides processes and intermediates disclosed herein that are useful for preparing compounds of formula (I) or salts thereof.

Brief Description of the Figures

Figure 1. The chemical structures of the representative Bcl-2 antagonists screened for microtubule interactions.

Figure 2. Competition of Compound 1 against Bak peptides to Bcl-2 and Bcl-X_L proteins. Analysis was carried out as described in Materials and Methods. In brief, Bcl-2 or Bcl-X_L was first mixed with a fluorescein-labeled Bak peptide to form a protein-peptide complex, which resulted in fluorescence polarization (FP) increase. Upon the addition of Compound 1, FP was measured, which reflects the amount of remaining protein-peptide complex.

Figure 3. The effect of Compound 1 on microtubule and cell cycle. A. Dose-dependent

Compound 1 induction of microtubule depolymerization. B. Compound 1 (80 μM) induction of cell cycle arrest. C. Effects of Compound 1 on cytoskeletal organization in cultured mammalian cells. CHO cells expressing GFP-tagged centrin2 were treated with (B to D, E-E') or without (A-A') Compound 1 at 75 μM for 10-12 h and immunofluorescently stained with anti-α-tubulin and filamin antibodies. Merged images of microtubules (red), centrosomes (green), and DAPI-stained nuclei (blue) are shown in A-E, and distribution of filamin-containing microfilaments is also shown in the same cells (A' and E'). Compound 1 caused partial depolymerization of cytoplasmic microtubules (B-E) originated from the centrosome in interphase cells (A). Microtubules tended to bundle around the nucleus (A, C) and at the cell periphery (D, E), and stress fibers became less prominent in drug treated cells (A' and E'). Bar, 10 μm.

Figure 4. *in vitro* cytotoxicity and apoptotic induction by Compound 1. A. Dose-dependent effect of Compound 1 on the cell viability of PC-3, DU145, LNCaP and TRAMP cells. These cells were exposed to Compound 1 at the indicated concentrations in 96-well plates for 48 hours and cell viability was assessed by a CellTiter Blue Viability Kit. Points, mean; bars, SD (n=3). B and C, Evidence of apoptotic death in drug-treated PC-3 cells. B. Levels of caspase-3/-7 induced by different doses of Compound 1. Columns, mean, normalized to untreated cell sample; bars, SD (n=3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). PC-3 cells were treated with Compound 1 at the indicated concentration for 24 hours and caspase-3/-7 activity was evaluated by Apo-ONE® Caspase-3/-7 reagent. C, DNA fragmentation in PC-3 cells upon Compound 1 treatment. PC-3 cells were treated with Compound 1 at the indicated concentration for 6 hours and DNA fragmentation was assessed by using Apoptotic DNA Ladder Extraction Kit.

Figure 5. Overcoming drug resistance by Compound 1. A. Sensitivity of various anticancer agents and Compound 1 to Jurkat cells with varied level of Bcl-2 and Bcl-X_L. Jurkat cells were exposed to the drugs at the indicated concentrations for 24 hours and cell viability was assessed by a CellTiter Blue Viability Kit. Points, mean; bars, SD (n=3). B. Sensitivities of drug-resistant PC-3 cells against the corresponding anticancer agents used for the resistance and Compound 1. PC-3 cells were exposed the drugs at the indicated concentrations for 24 hours. Cell viability was assessed by a CellTiter Blue Viability Kit. Columns, mean, normalized to untreated cell sample; bars, SD (n=3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 6. Effect of p.o. Compound 1 at 50 and 100 mg/kg on the growth of PC-3 tumors in nude mice. Each mouse was inoculated s.c. in the right flank with 2 x 10^6 PC3-LN4 cells

suspended in 0.2 mL PBS. Seventy-two hours later, mice were randomized into three groups (n = 8) and were given daily Compound 1 at 50 and 100 mg/kg body weight per day with intraperitoneal saline for 12 days. Controls received saline. A. Tumor volumes. Points, mean; bars, SE (n = 8; * < 0.05 compared with the control group). B. Serum level of Compound 1. Points, mean; bars, SE (n = 4; * < 0.05 between the two treated groups).

Detailed Description

The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, etc. denote both straight and branched groups; but reference to an individual radical such as propyl embraces only the straight chain radical, a branched chain isomer such as isopropyl being specifically referred to. Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Heteroaryl encompasses a radical of a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(X) wherein X is absent or is H, O, (C_1-C_4)alkyl, phenyl or benzyl, as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine anti-cancer activity using the standard tests described herein, or using other similar tests which are well known in the art.

Specific values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents

Specifically, (C_1-C_6)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C_3-C_6)cycloalkyl can be cyclopropyl, cyclobutyl, cyclopentyl,

or cyclohexyl; (C_1-C_6)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C_1-C_6)alkanoyl can be acetyl, propanoyl or butanoyl; (C_1-C_6)alkoxycarbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C_2-C_6)alkanoyloxy can be acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy; aryl can be phenyl, indenyl, or naphthyl; and heteroaryl can be furyl, imidazolyl, triazolyl, triazinyl, oxazoyl, isoxazoyl, thiazolyl, isothiazoyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl, (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide) or quinolyl (or its N-oxide).

A specific value for R_4 is $NHSO_2R_1$.

A specific value for R_4 is OH.

A specific value for R_1 is aryl optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O)NR_aR_b, trifluoromethoxy, and nitro.

A specific value for R_1 is phenyl, optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O)NR_aR_b, trifluoromethoxy, and nitro.

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A specific value for R_1 is aryl optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from (C_1-C_6)alkyl, NR_aR_b and nitro.

A specific value for R_1 is phenyl optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from nitro and NR_aR_b.

A specific value for R_1 is phenyl optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from nitro and NR_aR_b wherein each R_a and R_b is independently hydrogen or (C_1-C_6)alkyl wherein any (C_1-C_6)alkyl is optionally substituted with SAryl or OAryl.

A specific value for R_1 is 3-nitro-4-(2-phenylthioethylamino)phenyl.

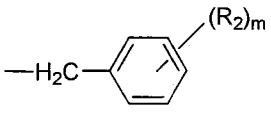
A specific value for R_1 is aryl optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy.

A specific value for R_1 is phenyl, optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy.

A specific value for R_1 is aryl optionally substituted with one or more (e.g. 1, 2, 3, or 4) $(C_1\text{-}C_6)$alkyl.

A specific value for R_1 is phenyl optionally substituted with one or more (e.g. 1, 2, 3, or 4) $(C_1\text{-}C_6)$alkyl.

A specific value for R_1 is 4-methylphenyl.

A specific value for R_5 is 

.

A specific value for m is 0, 1 or 2.

A specific value for m is 0.

A specific value for R_5 benzyl.

A specific value for R_5 is $(C_1\text{-}C_6)$alkyl.

A specific value for R_5 is isobutyl.

A specific value for n is 1 or 2.

A specific value for n is 1, 2, or 3.

A specific value for R_3 is hydroxy, aryl, heteroaryl or -A-B-C-D, wherein any aryl, heteroaryl of R_3, C or D is optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy .

A specific value for R_3 is hydroxy, phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}$

C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

A specific value for R_3 is hydroxy, phenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl, wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from hydroxy and (C_1-C_6)alkoxy.

A specific value for R_3 is hydroxy, phenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl, wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally substituted with one or two groups independently selected from hydroxy and (C_1-C_6)alkoxy.

A specific value for R_6 is 4-biphenylyl, 4'-hydroxy-biphenyl-4-yl, 3'-hydroxy-4'-methoxybiphenyl-4-yl, 3'-hydroxybiphenyl-4-yl, 3', 5'-dihydroxybiphenyl-4-yl, 2'-hydroxybiphenyl-4-yl, 3-hydroxybiphenyl-4-yl, 2-hydroxybiphenyl-4-yl, 4-(4-pyridyl)phenyl, 4-(2-pyridyl)phenyl or 4-(3-pyridyl)phenyl.

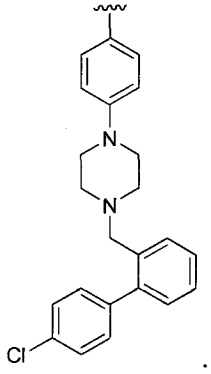
A specific value for R_3 is -A-B-C-D.

A specific value for R_3 is -A-B-C-D wherein C is aryl and D is aryl.

A specific value for R_3 is -A-B-C-D wherein A is piperazino, B is methylene, C is aryl and D is aryl.

A specific value for R_3 is -A-B-C-D wherein A is piperazino, B is methylene, C is phenyl and D is phenyl.

A specific value for R_6 is



.

A specific group of compounds are compounds wherein n is 1 and R_3 is independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl(C_1-C_6)alkyl, or heteroaryl(C_1-C_6)alkyl, wherein any aryl or heteroaryl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano,

hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

A specific group of compounds are compounds wherein n is 1 and R_3 is aryl or aryl(C_1-C_6)alkyl, wherein any aryl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

A specific group of compounds are compounds wherein n is 1 and R_3 is phenyl or phenyl(C_1-C_6)alkyl, wherein any phenyl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy;

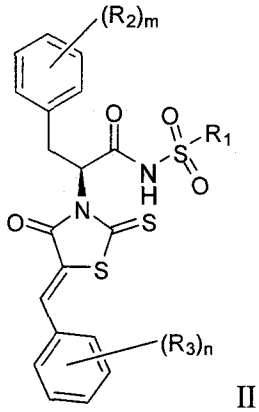
A specific group of compounds are compounds wherein n is 1 and R_3 is phenyl, optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

A specific group of compounds are compounds wherein n is 1 and R_3 is phenyl.

In one specific embodiment the invention provides compounds of formula I wherein the group R_5-CH-C(O)-R_4 has the absolute stereochemistry corresponding to a natural L amino acid. However, in another embodiment the invention also provides compounds of formula I wherein the group R_5-CH-C(O)-R_4 has the absolute stereochemistry corresponding to a D amino acid.

In one specific embodiment of the invention n is 1 and R_3 is on the 4-position of the phenyl ring in Formula I.

In another embodiment the invention provides a compound of formula II:



II

wherein:

R_1 is aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, aryl$(C_1\text{-}C_6)$alkyl, heteroaryl$(C_1\text{-}C_6)$alkyl, or $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl is optionally substituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy;

m is 0, 1, 2, 3, 4, or 5;

n is 0, 1, 2, 3, 4, or 5;

each R_2 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1\text{-}C_6)$alkyl, or heteroaryl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl of R_2 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_cR_d, -C(=O) NR_cR_d, and trifluoromethoxy;

each R_3 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1\text{-}C_6)$alkyl, or heteroaryl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl of R_3 is optionally susbtituted with one or more (e.g. 1, 2, 3, or 4) groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl,

$(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy;

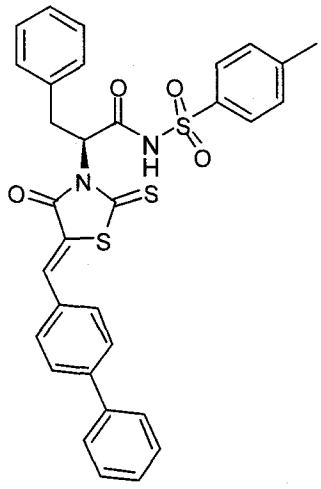
each R_a and R_b is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_a and R_b taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

each R_c and R_d is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_c and R_d taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring; and

each R_e and R_e is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_e and R_f taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

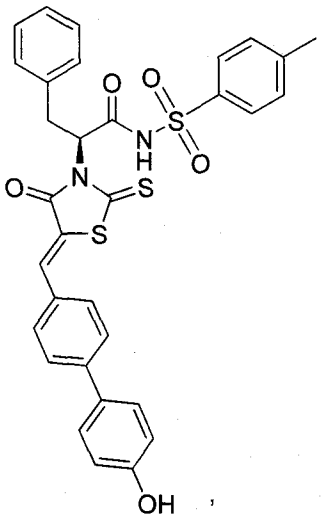
or a salt thereof.

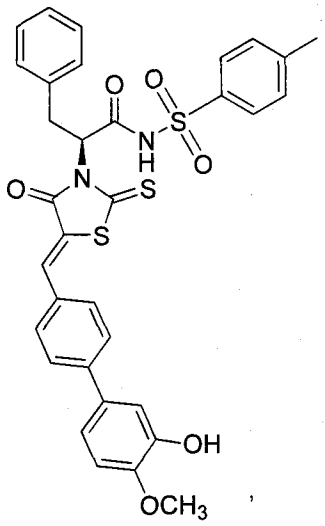
A specific compound of formula I is a compound of the following formula:

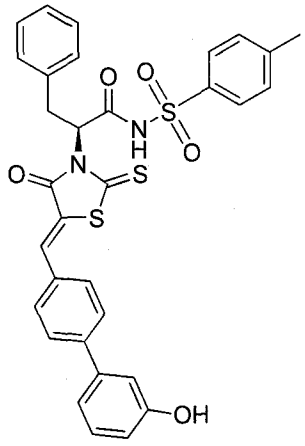


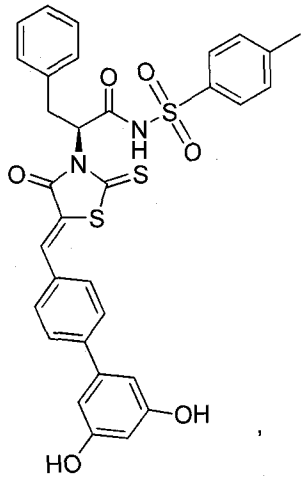
or a salt thereof.

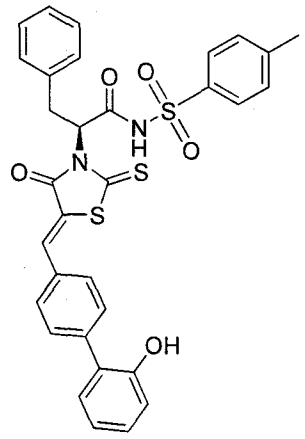
A specific compound of formula I is a compound of the following formula:

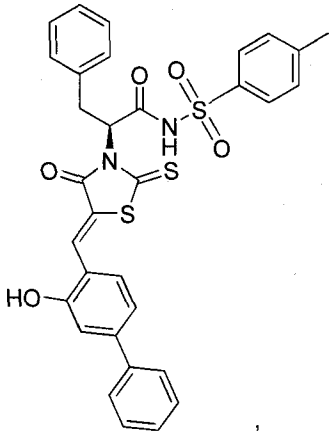




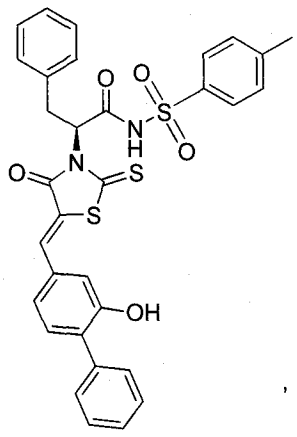
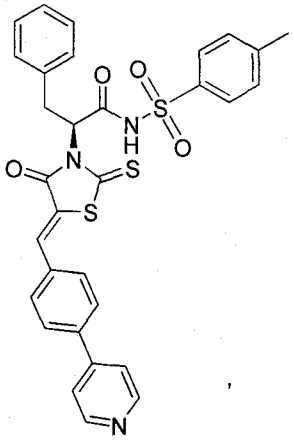
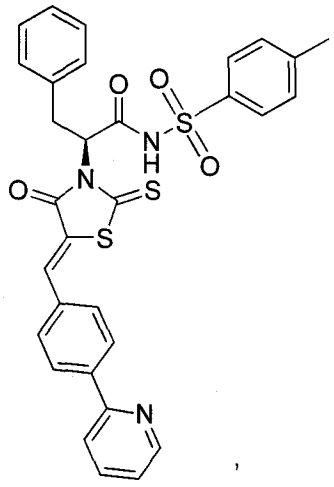
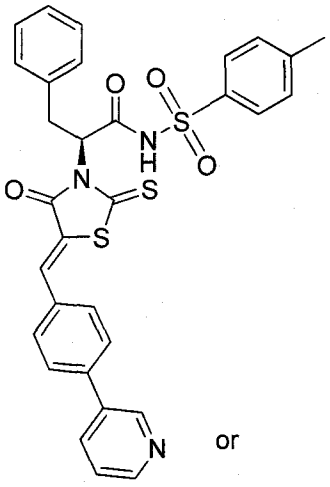
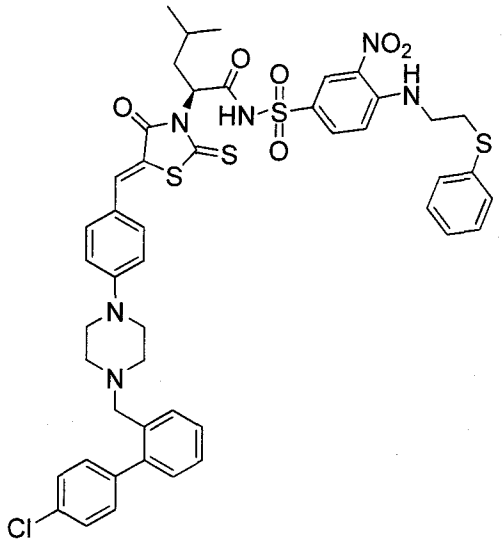






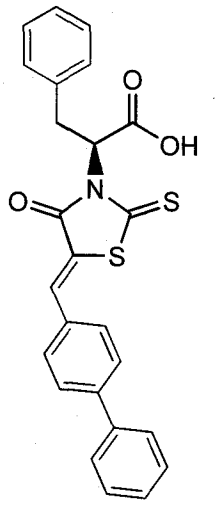


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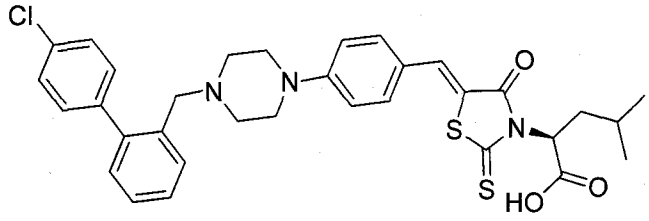

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or


or a salt thereof.

A specific compound of formula I is a compound of the following formula:



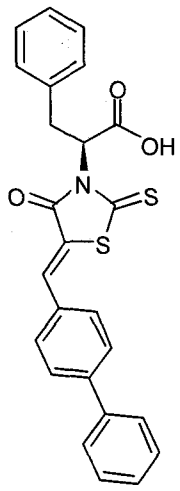
or



or a salt thereof.

Processes for preparing compounds of formula I are provided as further embodiments of the invention and are illustrated in Scheme 1 below.

For example, a compound of formula I wherein R_4 is $NHSO_2R_1$ can be prepared by converting a corresponding acid of formula **3:**



3

to the compound of formula I. Thus, an intermediate acid of formula 3 is useful for preparing a compound of formula I.

The invention also provides a method for preparing a salt of a compound of formula I comprising converting a corresponding compound of formula I to the salt.

In cases where compounds are sufficiently basic or acidic, a salt of a compound of formula I can be useful as an intermediate for isolating or purifying a compound of formula I. Additionally, administration of a compound of formula I as a pharmaceutically acceptable acid or base salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartrate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids and sulfonimides can also be made.

The compounds of formula I can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations will typically contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In one embodiment the compound can be conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

Ideally, the active ingredient should typically be administered to achieve peak plasma concentrations of the active compound of from about 0.25 to about 75 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

Compounds of the invention can also be administered in combination with other therapeutic agents, for example, other agents that are useful for the treatment of cancer. Examples of such agents include anti-microtubule agents such as docetaxel, paclitaxel, vincrystine and vinblastine. Accordingly, in one embodiment the invention also provides a composition comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, at least one other therapeutic agent, and a pharmaceutically acceptable diluent or carrier. The invention also provides a kit comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, at least one other therapeutic agent, packaging material, and instructions for administering the compound of formula I or the pharmaceutically acceptable salt thereof and the other therapeutic agent or agents to an animal to treat cancer (e.g. HRPC).

Test A. **Binding of Compound 1 with recombinant Bcl-2 or Bcl-X_L protein.**

The ability of a compound of the invention to bind with recombinant Bcl-2 or Bcl-X_L protein may be determined using pharmacological models which are well known to the art, or using Test A described below.

The binding interaction of Compound 1 with recombinant Bcl-2 or Bcl-X_L protein was evaluated by following an established procedure (Doshi JM, et al., J. Med. Chem. 2006;49:7731-9). Briefly, recombinant Bcl-2 protein (1 μM) or Bcl-X_L protein (130 nM) was incubated with Flu-Bak peptide (10 nM) for 1 hour at room temperature to form the protein-peptide complex. Such a complex was then mixed with varying concentrations of Compound 1. Fluorescence polarization (FP) of the solution was determined by using a Tecan GENios Pro multi-well plate reader from Tecan US (Durham, NC). The binding of Compound 1 to the recombinant proteins would release the Flu-Bak peptide from the protein-peptide complex, resulting in the decrease of FP. Controls included dose-response measurements in the absence of proteins to assess for any interactions between Compound 1 and the Flu-Bak peptide with such effects taken into account by subtraction. Inhibitory constant (Ki) was determined by fitting the FP values to the concentrations of the small molecule using a single-site competition model in GraphPad (Doshi JM, et al., J. Med. Chem. 2006;49:7731-9).

The ability of Compound 1 to disrupt the binding interaction of anti-apoptotic Bcl-2 proteins with a BH3-domain Bak peptide was evaluated as described above. (Fig. 2). Compared to BHI-1 (K_i = 44 μM for Bcl-2 and K_i = 133 μM for Bcl-X_L), Compound 1 has greatly improved its potency to disrupt the binding interaction of the Bak peptide with the anti-apoptotic Bcl-2 proteins (K_i = 23 μM for Bcl-2 and K_i = 1.2 μM for Bcl-X_L), especially against Bcl-X_L

protein (> 100 fold improvement).

The ability of a compound of the invention to induce microtubule depolymerization may be determined using pharmacological models which are well known to the art, or using Test B described below.

Test B. **Induction of microtubule depolymerization.**

Microtubule Stabilization Destabilization Assay Kit was purchased from Cytoskeleton (Denver, CO). Cell Lysis Buffer, 2 × Reaction Buffer, Dithiothreitol (DTT) and Apoptotic DNA Ladder Extraction Kit were purchased from Biovision (Mountain View, CA). CellTilter-Blue® Cell Viability Assay kit and Apo-ONE® Homogeneous Caspase-3/7 Assay kit were purchased from Promega (Madison, WI).

The influence of the Bcl-2 antagonists on microtubules was evaluated using the Microtubule Stabilization/Destabilization Assay Kit from Cytoskeleton (Denver, CO) by following the manufacture procedures. Briefly, the buffers and tubulin were prepared and stored according to manufacture's protocol. The compounds to be tested were dissolved in DMSO to 3 mM and further diluted to varying concentrations in Buffer 3 with 10 % DMSO in the final solution. The solutions of 2 x 0.5 ml of Buffer 1, 12 µl of GTP stock, 10 µl of paclitaxel were defrosted. The following components was mixed in a tube placed on ice: Buffer 1 (1 ml), Buffer 2 (88 µl), GTP stock solution (12 µl), and Paclitaxel (2.8 µl, 200 µM) and the solution was labeled as MT BUFFER. Tubulin (170 µl) was suspended with 800 µl of MT BUFFER by pipetting up and down for one minute. The solution was then incubated for exactly 30 min at 37 °C in a water bath and labeled as microtubule stock (MT STOCK). MT STOCK was incubated at room temperature for two hours. Buffer 3 (5 µl) was pipetted into the control wells and the small molecules of varying concentrations (5 µl) were pipetted into the other wells as appropriate. 2 mM $CaCl_2$ (5 µl) was used as a destabilization control and 200 µM paclitaxel (5 µl) was used as a stabilization control. MT STOCK (45 µl) was then slowly pipetted into each well and the solution was mixed through gentle shaking. The fluorescence intensity in each well was measured by using the GENios Pro multi-well plate reader from Tecan with excitation at 360 nm and emission at 420 nm for 15 min with recording every 30 sec. The small-molecule controls included dose-response measurements in the absence of tubulin to assess for any fluorescence background from the small molecules. Eventual effects were taken into account by subtraction.

Compound 1 was found to induce microtubule depolymerization at a single concentration

of 30 μM. The dose-dependent effect of Compound 1 towards microtubule depolymerization was then assessed (Fig. 3 A). Over the concentrations ranging from 5 – 60 μM, Compound 1 enhanced the depolymerization rate of microtubule in a dose-dependent manner. Compound 1 at 20 μM was of equal potency as Ca^{2+} (the standard depolymerizer) at 300 μM in inducing the microtubule depolymerization, suggesting that Compound 1 is a potent destabilizing agent against microtubule. Next the effect of Compound 1 towards cell cycle process was evaluated since anti-microtubule agents would prevent cell division upon disrupting the dynamics of microtubule. As shown in Fig. 3 B, Compound 1 changed the distribution of cells among the different phases of the cell cycle. With 80 μM Compound 1 treatment, more cells were accumulated at G2/M phase (34.4% vis 13.3%) and less cells were at the S phase (11.9% vis 38.7%) compared to the control cell samples, further supporting that Compound 1 may interfere with microtubule. The *in vitro* effect of Compound 1 towards microtubule was further explored by using microscopy analysis of cytoskeleton of CHO (Chinese hamster ovary) cells upon Compound 1 treatment (Fig. 3C). Control cells included a well-developed network of interphase microtubules emanating from the centrosome at the juxanuclear position (arrows in Fig. 3CA). Those cells also revealed the presence of prominent actin-containing stress fibers as shown in Figure 3CA'. Compound 1 treatment caused disappearance of microtubules in a dose-dependent manner. Although little difference in microtubule organization between control cells and cells treated with the drug below 20-25 μM was noted, administration of 75 μM Compound 1 for 12-18 hr resulted in partial depolymerization of cytoplasmic microtubules (Fig. 3CB-CE). Microtubules tended to be bundled around the nuclei (Fig. 3CC) or at the periphery of cells (Fig. 3CD and CE). Concomitantly, stress fibers became disorganized in drug-treated cells (Fig. 3CE'). Besides CHO cells, similar effects of Compound 1 were noted in HeLa cells. We were unable to detect complete depolymerization of microtubule in cells treated with Compound 1 at higher concentrations. This is mainly due to the change of cell morphology caused by drug treatment: as a result of quick entrance into apoptotic pathways, the cells became round-up which made it difficult to detect microtubule architectures. Thus, Compound 1 caused partial depolymerization of interphase microtubules *in vitro*. Together, these *ex vitro* and *in vitro* data suggest that Compound 1 interferes with cell cycle likely through its ability to induce microtubule depolymerization.

The effects of a compound of the invention on cell viability may be determined using pharmacological models which are well known to the art, or using Test described below.

Test C. **Cell viability analysis**

Bcl-2 over-expressing and Bcl-X_L over-expressing Jurkat cells were provided by Dr. Claus Belka at University of Tuebingen and Dr. Daniel Johnson at the University of Pittsburgh respectively and characterized as described (22). Jurkat cells and various prostate cancer cells were maintained in RPMI 1640 medium with 10% fetal bovine serum (V/V), 100 units/ml penicillin G, 100 μg/ml streptomycin, and 5 % CO_2 at 37 °C.

For Jurkat cells, 1 $\times 10^4$ cells / well were plated in a 96-well plate. For prostate cancer cells, 3000 cells / well were plated in a 96-well plate. The cells were treated with either a vehicle control or various concentrations of Compound 1 for 24 or 48 hours. At the end of each treatment, cell viability was measured by using CellTilter-Blue® Cell Viability Assay kit from Promega. Briefly, 20 μl of the dye was added to each well with 100 μl of culture media and incubated for 1 hour at 37 °C. The fluorescence intensity was recorded with excitation at 560 nm and emission at 590 nm, and normalized to the vehicle-treated control.

The *in vitro* cytotoxicity of Compound 1 was evaluated against several prostate cancer cell line, along with its ability to induce apoptosis. The prostate cancer cells evaluated included human androgen-dependent (LNCaP), human androgen independent (PC-3 and DU-145), and transgenic adenocarcinoma mouse prostate (TRAMP) cell lines. These four prostate cancer cell lines were selected for evaluation because they represent different stages along the development and progression of prostate malignancy. The IC_{50}s of Compound 1 against these four malignancies were all in the low micromolar concentration (10 – 20 μM, Fig. 4 A), suggesting that Compound 1 was effective against prostate cancer of different stages of progression. This growth inhibition was attributable to apoptotic cell death, as evidenced by the activation of caspase-3/-7 and DNA fragmentation in PC-3 cells by Compound 1 in a dose-dependent manner (Fig. 4 B and C).

Next the effects of Compound 1 on anti-apoptotic Bcl-2 drug resistance was evaluated. To test this, two Jurkat cells stably transfected with either Bcl-2 or Bcl-X_L were acquired and characterized as detailed before (Doshi JM, et al., J. Med. Chem. 2006;49:7731-9). They showed extensive resistance to anticancer agents with varying mechanisms of action, including doxorubicin, taxol, and thioguanine (Fig. 5 A). However, when these two cells were evaluated against Compound 1, they both showed the same sensitivity as the parent Jurkat cells (Fig. 5 A), demonstrating that over-expressing anti-apoptotic Bcl-2 proteins was not effective in inducing resistance to Compound 1.

Compound 1 was studied to determine if it could overcome drug resistance induced through prolonged drug exposure, which mimics the natural development of drug resistance. To test this, three drug-resistant PC-3 cells were developed by culturing parent PC-3 cells in the presence of sublethal dosage of cisplatin, doxorubicin, or taxol respectively for at least 6 months. The PC-3 cells all acquired resistance to the corresponding agents though the extent of resistance varied, with relative high drug resistance for cisplatin and taxol (Fig. 5 B). All three drug-resistant PC-3 cells were more sensitive to Compound 1 compared to the parent PC-3 cells (Fig. 5 C), indicating that Compound 1 would be potentially an effective anticancer agent against drug-resistant prostate malignancies. WL-240, the analog of Compound 1 with similar potency against Bcl-2 proteins and no activity to induce microtubule depolymerization, however, did not demonstrate selective toxicity against these drug resistant PC-3 cells, implicating the potential requirement of the dual antagonism to Bcl-2 protein and microtubule for the selective targeting of drug resistant cells.

The effects of a compound of the invention on DNA fragmentation may be determined using pharmacological models which are well known to the art, or using Test D described below.

Test D. **DNA Fragmentation**

DNA fragmentation was assessed by Apoptotic DNA Ladder Extraction Kit from Biovision. Briefly, PC-3 cells were treated by Compound 1 for 6 hours. 2.0×10^6 cells were harvested and washed with PBS. The cells were suspended in 50 µl DNA Ladder Extraction Buffer. After incubation at 23 °C for 10 seconds with gentle pipetting, the mixture was centrifuged for 5 min at 1600 × *g*. The supernatant was transferred to a fresh tube and the cell pellet was extracted again with DNA Ladder Extraction Buffer (50 µl). The supernatants were combined and 5 µl Enzyme A solution was added into the supernatant. The solution was mixed by gentle vortex and incubated at 37 °C for 10 min. Enzyme B solution (5 µl) was then added into the mixture and further incubated overnight at 50 °C. Ammonium acetate solution from Biovision (5 µl) was added to the sample and mixed well. Isopropanol (100 µl) was added and the solution was mixed well and kept at - 20 °C for 20 min. DNA pellet was obtained by centrifugation at 13,000 × *g* for 10 min. The pellet was washed twice with cold 75 % ethanol, dried, and re-suspended in 20 µl DNA Suspension Buffer. Samples were loaded onto a 1.2 % agarose gel containing 0.5 µg/ml Ethidium bromide in both gel and running buffer. Electrophoresis was run at 50 V for 1 hour. DNA was visualized with UV light and photographed.

The effects of a compound of the invention on caspase activity may be determined using pharmacological models which are well known to the art, or using Test E described below.

Test E. **Measurement of caspase-3/-7 activity**

Apo-ONE® Homogeneous Caspase-3/-7 Assay kit from Promega was used to measure the caspase-3/-7 activity according to the manufacturer's instructions. Briefly, after Compound 1 treatment, the cell culture medium was removed and fresh RPMI cell culture medium (50 µl) with Apo-ONE® Caspase-3/-7 reagent (50 µl) was added to each well. The solution was mixed gently and incubated at 37 °C for 45 min. The fluorescence intensity of each well was measured with excitation at 485 nm and emission at 530 nm. Caspase-3/-7 activity was normalized to the vehicle-treated control. Results are shown in Figures 4B and 4C.

The effects of a compound of the invention on cell cycle arrest may be determined using pharmacological models which are well known to the art, or using Test F described below.

Test F. **Cell cycle arrest**

The effect of Compound 1 to cell cycle was evaluated by following a reported procedure from Nicoletti et al (Nicoletti I, et al., J. Immunol. Methods. 1991;139:271-9). Briefly, Jurkat cells were treated with varying concentrations of Compound 1 for 24 hours. The cells were pelleted and suspended in hypotonic fluorochrome solution to stain cellular DNA with propidium iodide. Upon overnight incubation, the nuclei of the cells were analyzed by flow cytometry and the distribution of cells along the cell cycle was determined by the relative amount of cellular DNA.

The effects of a compound of the invention on microtubule depolymerization may be determined using pharmacological models which are well known to the art, or using Test G described below.

Test G. **Induction of microtubule depolymerization by Compound 1 *in vitro*.**

Effects of Compound 1 on the cytoskeleton were analyzed by fluorescence staining of cultured mammalian cells with antibodies specific to a microtubule subunit protein (α-tubulin) and an actin-binding protein (filamin). CHO (Chinese hamster ovary) cells constitutively expressing GFP-tagged centrin2 (24) were prepared as reported previously (25) and grown as monolayers in Ham's F-10 medium containing 10% fetal bovine serum. Cells on coverslips were treated with 75 µM Compound 1 for 10-12 h and fixed with methanol for 5 min at - 20°C. After rehydration with 0.05% Tween-20-containing PBS (PBS-Tw20), samples were incubated with primary antibodies that contained monoclonal mouse anti-α-tubulin (Sigma-Aldrich) and

polyclonal rabbit anti-filamin (Kuriyama, unpublished) antibodies. Excess antibodies were washed by PBS-Tw20, and cells were further treated with a mixture of secondary antibodies (Texas-red-conjugated anti-mouse and Cy5-conjugated anti-rabbit antibodies) for 30 min at 37°C. Microscopic observation was made on a Nikon Eclipse microscope to visualize microtubules, microfilaments, and centrosomes in a single cell using SlideBook imaging softwares.

The effects of a compound of the invention on xenograph tumor growth may be determined using pharmacological models which are well known to the art, or using Test H described below.

Test H. **Xenograft Tumor Growth**

Male athymic BALB/c nude mice (obtained from the Frederick Cancer Research Facility, National Cancer Institute, Frederick, MD) were maintained in a laminar airflow cabinet under pathogen-free conditions and used at 8 to 12 weeks of age. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care in accordance with the current regulations and standards of the U.S. Department of Agriculture, U.S. Department of Health and Human Services, and NIH.

Cultured PC3-LN4 cells (60-70% confluent) were prepared for injection as described previously (26). Mice were anesthetized with isoflurane. Viable tumor cells (2×10^6 per 0.2 mL) in PBS were implanted subcutaneously into the flank. Formation of a bulla indicated a satisfactory injection. Beginning on day three after injection, groups of mice were then treated with intraperitoneal saline, 50 mg/kg Compound 1, or 100 mg/kg Compound 1 for 12 days. Tumor size and volume were assessed every 2 days. Mice were subjected to necropsy 24 hours after the last Compound 1 treatment. The tumors were removed and weighed. The tumors were quickly frozen in liquid nitrogen or fixed in 10% buffered formalin for additional analysis. At the time of sacrifice, the serum from each animal was collected for Compound 1 quantification.

The *in vivo* effect of daily i.p. Compound 1 at two different doses, 50 and 100 mg/kg, on the growth of PC-3 xenograft tumors was assessed (Fig.6 A). All animals tolerated the treatments well without observable signs of toxicity and were characterized by stable body weights throughout the course of study. No gross pathologic abnormalities were noted at necropsy after 12 days of treatment. As shown, both treatments displayed a significant inhibitory effect ($P < 0.05$) compared to the control group. In fact, Compound 1 at the dose of 100 mg/kg induced near complete inhibition of tumor growth. Even at the dose of 50 mg/kg,

Compound 1 inhibited tumor growth to ~ 30 % of the tumor volume in the control group.

Upon sacrifice, serum of each mouse was collected and stored at -80 °C till usage. The serum level of Compound 1 was determined by recovering Compound 1 from the serum and quantifying the recovered Compound 1 through HPLC at 395 nm with 4-phenylphenol as the internal standard. Briefly, serum (200 μL) was mixed a mixture of methanol and acetonitrile (600 μL, v:v 1 : 1) and the mixture was mixed through vortex for 10 sec. The suspension was centrifuged at 14,000 x *g* for 10 min. The supernatant was recovered, 500 μL of which was mixed with a solution of 4-phenylphenol (100 μM, 500 μL of H_2O : MeOH : acetonitrile 2 : 1 : 1). The solution was then analyzed by HPLC. HPLC analysis was performed on a Beckman Coulter System Gold 126 solvent module and 168 detector. A Phenomenex Polar RP column (5 μm, 250 x 4.6 mm) was used for the analyses. The flow rate used was 0.6 mL/min. The mobile phase A was water with 0.1% acetic acid while B was acetonitrile with 0.1% acetic acid. The time program used for the analyses was 40% A (0-5 min), 60-95% B (5-20 min), 95% B (20-25 min), 5-40% A (25-27 min).

The serum stability of a compound of the invention may be determined using pharmacological models which are well known to the art, or using Test I described below.

Test I. **Serum Stability**

Briefly, authentic Compound 1 was dissolved in serum collected from the control mice. The serum and Compound 1 mixture was incubated at room temperature for varying periods of time followed by the recovery of Compound 1, the quantity and purity of which were analyzed by HPLC. Based on the HPLC profile, no new peaks were detected other than Compound 1 (Authentic compounds **2** and **3** in Scheme 1 below as the potential metabolites were analyzed under the same HPLC conditions that confirmed no compounds **2** or **3** present in the serum, Supplementary Data). In addition, quantitative recovery of Compound 1 was achieved, suggesting that Compound 1 was likely to be stable *in vivo*.

Additionally, the serum level of Compound 1 in the mice treated with the two different dosages of Compound 1 in Test H was evaluated by following the procedure above (Fig. 6 B). Other than Compound 1, no potential metabolites from Compound 1 were detected in the serum by HPLC (representative HPLC trace in Supplementary Data). 0.27 μM Compound 1 was detected in the mice treated at the dose of 50 mg/kg and 0.34 μM Compound 1 was detected in the mice treated at the dose of 100 mg/kg. The relatively low level of Compound 1 in serum may result from its body clearance as the serum was collected 24 hours after the final Compound 1 administration. Because of the absence of metabolites of Compound 1 *in vivo* and the different

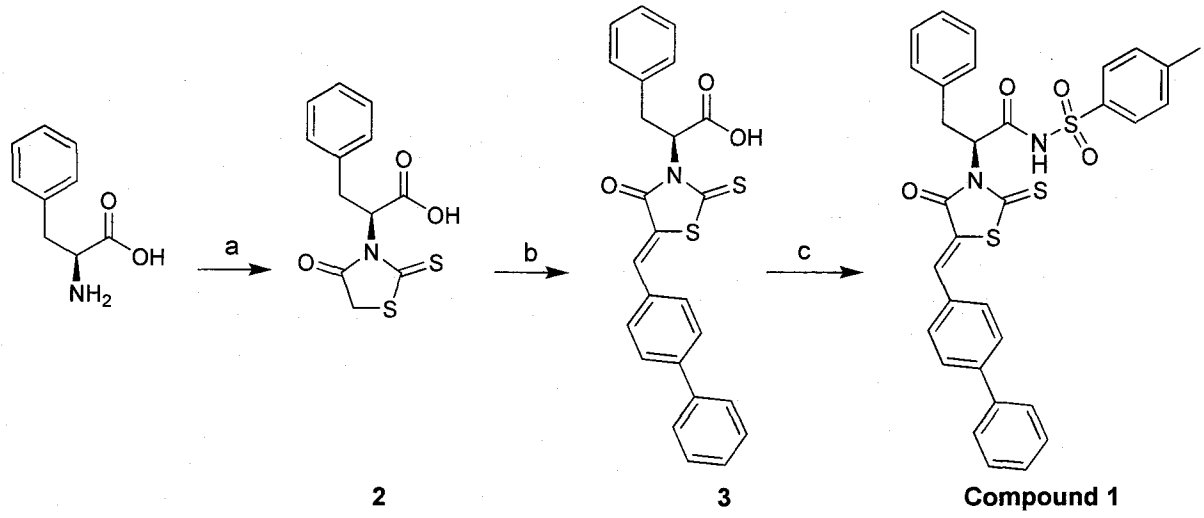
abundance of Compound 1 in serum among the two groups of treated mice, the different extent of tumor suppression between these two groups is likely due to the different levels of Compound 1.

For the above tests, most of the biological experiments, including the binding assays, microtubule destabilization assays, cell viability assays, cell cycle analyses, caspase-3/-7 assays, and HPLC quantification of serum Compound 1 were performed at least twice with triplicates in each experiment. For DNA fragmentation, western blot analyses, *in vitro* cytoskeleton analyses, and *in vivo* xenograft tumor growth, at least two independent assays were performed. Representative results are depicted in this report. Data were analyzed using the GraphPad software. Student's *t*-test was applied for comparison between groups using the GraphPad software. Differences were considered statistically significant at $p \leq 0.05$.

The invention will now be illustrated by the following non-limiting Examples.

Example 1.

Scheme 1



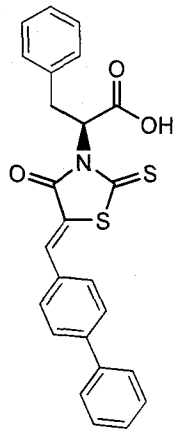
Reagents and conditions: (a) CS_2, NaOH; $ClCH_2COONa$; 5.5 M HCl, reflux, overnight;
(b) 4- biphenylcarboxaldehyde, NH_4OAc in Toluene, reflux, 16h;
(c) p-Tolunenesulfonamide, EDC, DMAP in DCM, rt, overnight.

Compound 1 was synthesized by the following procedures (Scheme 1). Briefly, to the suspension of L-phenylalanine (1.65 g, 100 mmol) in water (150 mL) was added NaOH (0.8 g, 200 mmol). The mixture was stirred to complete dissolution. Carbon disulfide (0.6 mL) was then added and the mixture was stirred vigorously overnight. Aqueous solution of $ClCH_2CO_2Na$ (100 mL, 1M) was added and the mixture was stirred at room temperature for 8 h. Hydrochloric acid solution (5.5M) was then added to adjust the pH of the solution to 2-3, followed by refluxing the solution overnight. The reaction mixture was quenched by saturated $NaHCO_3$ solution. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography eluting with 30% ethyl acetate in hexanes plus 0.2% HOAc to give 1.282 g (45%) of the cyclized product (**2**). The solution of **2** (980 mg, 3.487 mmol) in dry toluene (70 mL) was mixed with 4-biphenylcarboxaldehyde (952 mg, 5.231 mmol) and NH_4OAc (537 mg, 6.794 mmol) followed by refluxing for 16 h. Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography eluting with 0-30% ethyl acetate in hexanes plus 0.2 % HOAc to give 1.413 g of desired product **3** (90%). The solution of **3** (445 mg, 1 mmol) in DCM (60 mL) was treated with p-tolunenesulfonamide (188 mg, 1.1 mmol), EDC (229 mg, 1.2 mmol) and DMAP (61 mg, 0.5 mmol), stirred at room temperature overnight.

Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography eluting with 0-15% ethyl acetate in hexanes plus 0.2% HOAc to give the desired product Compound 1 as yellow powder 430 mg (71%). TLC (ethyl acetate/hexanes = 1: 3), R_f = 0.37. Mp: 195-196 °C. ^{1}H NMR(300MHz, $CDCl_3$) δ 9.16 (d, J = 8.7 Hz, 1H), 7.76–7.72 (m, 2H), 7.65–7.60 (m, 4H), 7.50–7.26 (m, 6H), 7.23–7.20 (m, 2H), 7.18–7.7.10 (m, 3H), 7.01–6.99 (m, 2H), 5.64 (bs, 1H), 3.43 (d, J = 7.5Hz, 2H), 2.35 (d, J = 2.7 Hz, 3H). HRMS ($C_{32}H_{25}N_2O_4S_3$) [M – H^+]: found *m/z* 597.0994, calcd *m/z* 597.0976.

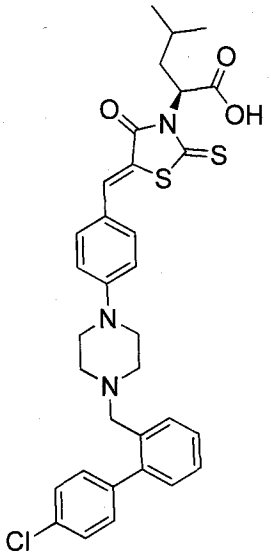
Example 2.

Compounds 4, 5 and 6 of formula I were prepared utilizing the procedure outlined in Scheme 1 of Example 1. The amino acid, aldehyde and sulfonamide starting materials for compounds 4, 5 and 6 were available from commercial sources or prepared by the method of Oltersdorf (T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, R.C. Armstrong, D.J. Augeri, B.A. Belli, M. Bruncko, T.L. Deckwerth, J. Dinges, P.J. Hajduk, M.K. Joseph, S. Kitada, S.J. Korsmeyer, A.R. Kunzer, A. Letai, C. Li, M.J. Mitten, D.J. Nettesheim, S. Ng, P.M. Nimmer, J.M. O'Connor, A. Oleksijew, A.M. Petros, J.C. Reed, W. Shen, S.K. Tahir, C.B. Thompson, K.J. Tomaselli, B. Wang, M.B. Wendt, H. Zhang, S.W. Fesik and S.H. Rosenberg, *An inhibitor of Bcl-2 family proteins induces regression of solid tumours*. Nature *435*, 677-681 (2005)).



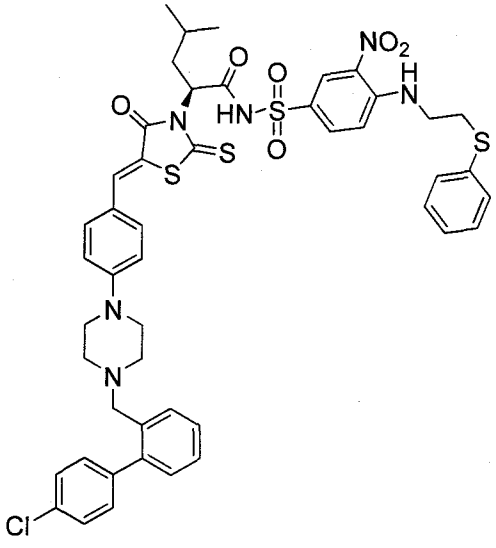
Compound 4

^{1}H NMR(300MHz, $CDCl_3$) δ 12.16 (bs,1H), 7.76–7.72 (m, 2H), 7.65–7.60 (m, 4H), 7.50–7.26 (m, 6H), 7.18–7.7.10 (m, 3H), 5.64 (bs, 1H), 3.43 (d, J = 7.5Hz, 2H).



Compound 5

(S)-4-4-((4'-chloro-1'1-biphenyl-2-yl)methyl)-1-piperazinyl)benzylidene-2-(4-oxo-2-thiooxothiazolidin)-4-methylpentanoic acid. ^{1}H NMR(600MHz, CDCl3) δ 7.69 (d, *J* = 7.8 Hz, 1H), 7.42–7.16 (m, 10H), 6.78(d, *J* = 9.0 Hz, 2H), 5.63 (bs, 1H), 4.18 (d, *J* = 12.6 Hz, 1H), 3.78 (d, *J* = 13.2 Hz, 1H), 3.29 (bs, 2H), 3.21 (bs, 2H), 2.72 (bs, 2H), 2.63(bs, 2H), 2.07 (bs, 2H), 1.38–1.35 (m, 1H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.88 (d, *J* = 6.0 Hz, 3H). Orange powder. TLC(ethyl acetate/hexanes = 2: 1), Rf 0.20. Mp: 210-212°C. HRMS (C33H33ClN3O3S2) [M - H]- : found *m/z* 618.1662, calcd *m/z* 618.1652.



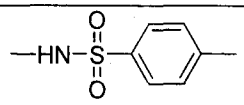
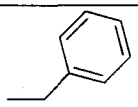
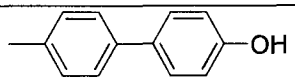
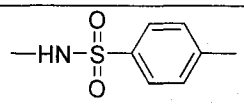
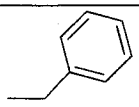
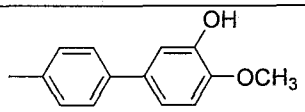
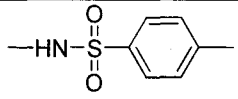
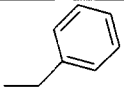
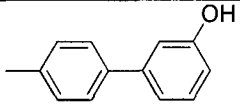
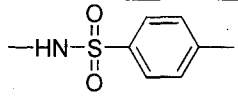
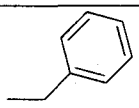
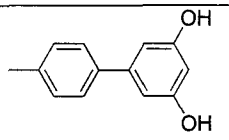
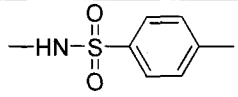
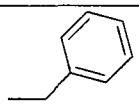
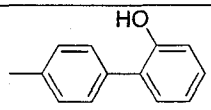
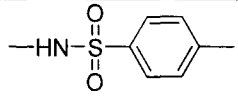
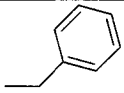
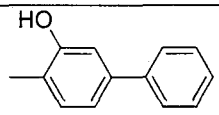
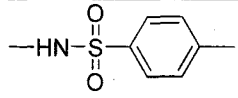
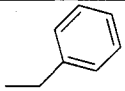
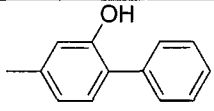
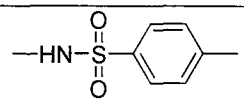
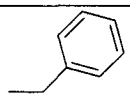
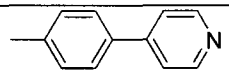
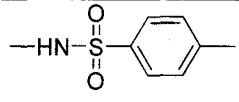
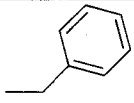
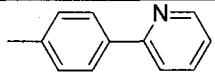
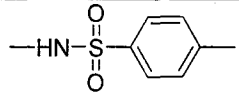
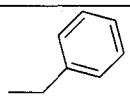
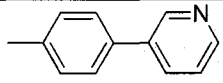
Compound 6

(S)-N-(4-4-((4'-chloro-1'1-biphenyl-2-yl)methyl)-1-piperazinyl)benzylidene-2-(4-oxo-2-thiooxothiazolidin)-4-methylpentanyl)-3-nitro-4-(2-phenylsulfanylethylamino)-

benzenesulfonamide. TLC(ethyl acetate/hexanes = 2: 1), Rf 0.62. Mp: 215-217°C. HRMS (C47H46ClN6O6S4) [M - H]- : found *m/z* 953.2079, calcd *m/z* 953.2050. RP-^{1}H NMR(600MHz, CDCl3) δ 8.65 – 8.56 (m, 2H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.42 – 7.25 (m, 13H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.74 – 6.70 (m, 3H), 5.53 (bs, 1H), 3.78 – 3.73 (m, 2H), 3.53 (t, *J* = 6.6 Hz, 2H), 3.34(bs, 4H), 3.20 (t, *J* = 6.6 Hz, 2H), 2.63 (bs, 4H), 1.51–1.47 (m, 1H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.88 (d, *J* = 6.0 Hz, 3H).

Example 3.

The following compounds of formula I were prepared utilizing the procedure outlined in Scheme 1 of Example 1. The required amino acid, aldehyde and sulfonamide starting materials were commercially available

Compound	R_4	R_5	R_6
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

Example 4.

Compounds 4, 5 and 6 were evaluated against human androgen independent PC-3 cell lines as described in Test C above. The GI_{50}s were determined to be 23.7 μM, 7.2 μM and 12.5 μM, respectively.

Example 5. The following illustrate representative pharmaceutical dosage forms, containing a compound of formula I ('Compound X'), for therapeutic or prophylactic use in humans.

(i) Tablet 1	mg/tablet
Compound X=	100.0
Lactose	77.5
Povidone	15.0
Croscarmellose sodium	12.0
Microcrystalline cellulose	92.5
Magnesium stearate	3.0
	300.0

(ii) Tablet 2	mg/tablet
Compound X=	20.0
Microcrystalline cellulose	410.0
Starch	50.0
Sodium starch glycolate	15.0
Magnesium stearate	5.0
	500.0

(iii) Capsule	mg/capsule
Compound X=	10.0
Colloidal silicon dioxide	1.5
Lactose	465.5
Pregelatinized starch	120.0
Magnesium stearate	3.0
	600.0

(iv) Injection 1 (1 mg/ml)	mg/ml
Compound X= (free acid form)	1.0
Dibasic sodium phosphate	12.0
Monobasic sodium phosphate	0.7
Sodium chloride	4.5
1.0 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(v) Injection 2 (10 mg/ml)	mg/ml
Compound X= (free acid form)	10.0
Monobasic sodium phosphate	0.3
Dibasic sodium phosphate	1.1
Polyethylene glycol 400	200.0
01 N Sodium hydroxide solution (pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(vi) Aerosol	mg/can
Compound X=	20.0
Oleic acid	10.0
Trichloromonofluoromethane	5,000.0
Dichlorodifluoromethane	10,000.0
Dichlorotetrafluoroethane	5,000.0

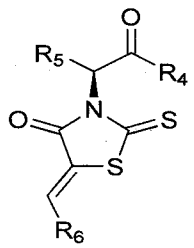
The above formulations may be obtained by conventional procedures well known in the pharmaceutical art.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A compound of formula I:



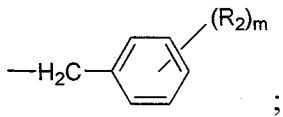
I

wherein:

R_4 is OH or $NHSO_2R_1$;

R_1 is aryl, heteroaryl, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, aryl$(C_1\text{-}C_6)$alkyl, heteroaryl$(C_1\text{-}C_6)$alkyl, or $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, $-C(=O)NR_aR_b$, trifluoromethoxy, and nitro;

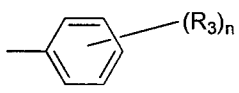
R_5 is $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, or



;

m is 0, 1, 2, 3, 4, or 5;

each R_2 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, $-C(=O)NR_aR_b$, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1\text{-}C_6)$alkyl, or heteroaryl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl of R_2 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_cR_d, $-C(=O)NR_cR_d$, and trifluoromethoxy;

R_6 is  ;

each R_3 is independently selected from halo, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_3$-$C_6)$cycloalkyl, $(C_3$-$C_6)$cycloalkyl$(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_1$-$C_6)$alkoxycarbonyl, $(C_1$-$C_6)$alkanoyloxy, $(C_3$-$C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1$-$C_6)$alkyl, heteroaryl$(C_1$-$C_6)$alkyl, or -A-B-C-D wherein A is pyrrolidino, piperidino, piperazino, or morpholino, B is $(C_1$-$C_6)$alkyl, C is aryl or absent and D is aryl or absent and wherein any aryl or heteroaryl of R_3, C and D is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_1$-$C_6)$alkoxycarbonyl, $(C_1$-$C_6)$alkanoyloxy, $(C_3$-$C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy;

n is 0, 1, 2, 3, 4, or 5;

each R_a and R_b is independently hydrogen, $(C_1$-$C_6)$alkyl, or $(C_1$-$C_6)$alkanoyl wherein any $(C_1$-$C_6)$alkyl is optionally substituted with -S-aryl or -O-aryl; or R_a and R_b taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

each R_c and R_d is independently hydrogen, $(C_1$-$C_6)$alkyl, or $(C_1$-$C_6)$alkanoyl; or R_c and R_d taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring; and

each R_e and R_f is independently hydrogen, $(C_1$-$C_6)$alkyl, or $(C_1$-$C_6)$alkanoyl; or R_e and R_f taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

or a salt thereof.

2. The compound of claim 1 wherein R_4 is $NHSO_2R_1$.

3. The compound of claim 1 wherein R_4 is OH.

4. The compound of claim 1or 2 wherein R_1 is aryl optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_1$-$C_6)$alkoxycarbonyl, $(C_1$-$C_6)$alkanoyloxy, $(C_3$-$C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O)NR_aR_b, trifluoromethoxy, and nitro.

5. The compound of claim 1or 2 wherein R_1 is phenyl, optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, $-C(=O)NR_aR_b$, trifluoromethoxy, and nitro.

6. The compound of claim 1 or 2 wherein R_1 is phenyl, optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, $-C(=O)$ NR_aR_b, and trifluoromethoxy.

7. The compound of claim 1or 2 wherein R_1 is aryl optionally substituted with one or more groups independently selected from (C_1-C_6)alkyl, NR_aR_b and nitro.

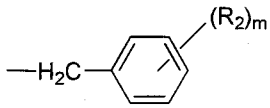
8. The compound of claim 1or 2 wherein R_1 is phenyl optionally substituted with one or more (C_1-C_6)alkyl.

9. The compound of claim 1or 2 wherein R_1 is 4-methylphenyl.

10. The compound of claim 1or 2 wherein R_1 is phenyl optionally substituted with one or more groups independently selected from nitro and NR_aR_b.

11. The compound of claim 1 or 2 wherein R_1 is phenyl optionally substituted with one or more groups independently selected from nitro and NR_aR_b wherein each R_a and R_b is independently hydrogen or (C_1-C_6)alkyl wherein any (C_1-C_6)alkyl is optionally substituted with -S-aryl or -O-aryl.

12. The compound of claim1 or 2 wherein R_1 is 3-nitro-4-(2-phenylthioethylamino)phenyl.

13. The compound of any one of claims 1-12 wherein R_5 is 

.

14. The compound of claim 13 wherein m is 0, 1 or 2.

15. The compound of claim 13 wherein m is 0.

16. The compound of any one of claims 1-12 wherein R_5 is $(C_1$-$C_6)$alkyl.

17. The compound of claim 16 wherein R_5 is isobutyl.

18. The compound of any one of claims 1-17 wherein n is 1, 2 or 3.

19. The compound of any one of claims 1-17 wherein n is 1 or 2.

20. The compound of claim 19 wherein each R_3 is independently hydroxy, aryl, heteroaryl or -A-B-C-D, wherein any aryl or heteroaryl of R_3, C and D is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_1$-$C_6)$alkoxycarbonyl, $(C_1$-$C_6)$alkanoyloxy, $(C_3$-$C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

21. The compound of claim 19 wherein each R_3 is independently hydroxy, phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl , wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkoxy, $(C_1$-$C_6)$alkanoyl, $(C_1$-$C_6)$alkoxycarbonyl, $(C_1$-$C_6)$alkanoyloxy, $(C_3$-$C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

22. The compound of claim 19 wherein each R_3 is independently hydroxy, phenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl, wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally susbtituted with one or more groups independently selected from hydroxy and $(C_1$-$C_6)$alkoxy.

23. The compound of claim 19 wherein each R_3 is independently hydroxy, phenyl, 2-pyridyl, 3-pyridyl, or 4-pyridyl, wherein any phenyl, 2-pyridyl, 3-pyridyl or 4-pyridyl of R_3 is optionally substituted with one or two groups independently selected from hydroxy and $(C_1$-$C_6)$alkoxy.

24. The compound of any one of claims 1-17 wherein R_6 is 4-biphenylyl, 4'-hydroxy-biphenyl-4-yl, 3'-hydroxy-4'-methoxybiphenyl-4-yl, 3'-hydroxybiphenyl-4-yl, 3', 5'-

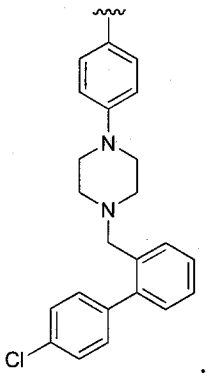
dihydroxybiphenyl-4-yl, 2'-hydroxybiphenyl-4-yl, 3-hydroxybiphenyl-4-yl, 2-hydroxybiphenyl-4-yl, 4-(4-pyridyl)phenyl, 4-(2-pyridyl)phenyl or 4-(3-pyridyl)phenyl.

25. The compound of claim 19 wherein R_3 is -A-B-C-D.

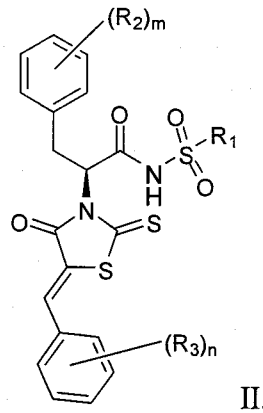
26. The compound of claim 19 wherein R_3 is -A-B-C-D wherein C is aryl and D is aryl.

27. The compound of claim 19 wherein R_3 is -A-B-C-D wherein A is piperazino, B is methylene, C is phenyl and D is phenyl.

28. The compound of any one of claims 1-17 wherein R_6 is



29. The compound of claim 1 wherein the compound of formula I is a compound of formula II:



II.

30. The compound of claim 1 or 29 wherein R_1 is aryl, heteroaryl, (C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, aryl(C_1-C_6)alkyl, heteroaryl(C_1-C_6)alkyl, or (C_3-C_6)cycloalkyl(C_1-C_6)alkyl,

wherein any aryl or heteroaryl is optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy;

m is 0, 1, 2, 3, 4, or 5;

n is 0, 1, 2, 3, 4, or 5;

each R_2 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1\text{-}C_6)$alkyl, or heteroaryl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl of R_2 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_cR_d, -C(=O) NR_cR_d, and trifluoromethoxy;

each R_3 is independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl$(C_1\text{-}C_6)$alkyl, or heteroaryl$(C_1\text{-}C_6)$alkyl, wherein any aryl or heteroaryl of R_3 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkoxycarbonyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_3\text{-}C_6)$cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy;

each R_a and R_b is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_a and R_b taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

each R_c and R_d is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_c and R_d taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring; and

each R_e and R_e is independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $(C_1\text{-}C_6)$alkanoyl; or R_e and R_f taken together with the nitrogen to which they are attached form a pyrrolidino, piperidino, piperazino, or morpholino ring;

or a salt thereof.

31. The compound of claim 30 wherein m is 0.

32. The compound of claim 30 or 31 wherein R_1 is aryl optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy.

33. The compound of claim 30 or 31 wherein R_1 is phenyl, optionally substituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, and trifluoromethoxy.

34. The compound of claim 30 or 31 wherein R_1 is aryl optionally substituted with one or more (C_1-C_6)alkyl.

35. The compound of claim 30 or 31 wherein R_1 is phenyl optionally substituted with one or more (C_1-C_6)alkyl.

36. The compound of claim 30 or 31 wherein R_1 is 4-methylphenyl.

37. The compound of any one of claims 30-36 wherein n is 1, 2, or 3.

38. The compound of any one of claims 30-36 wherein n is 1 and R_3 is independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_3-C_6)cycloalkyl, (C_3-C_6)cycloalkyl(C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_aR_b, -C(=O) NR_aR_b, trifluoromethoxy, aryl, heteroaryl, aryl(C_1-C_6)alkyl, or heteroaryl(C_1-C_6)alkyl, wherein any aryl or heteroaryl of R_3 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

39. The compound of any one of claims 30-36 wherein n is 1 and R_3 is aryl or aryl(C_1-C_6)alkyl, wherein any aryl of R_3 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

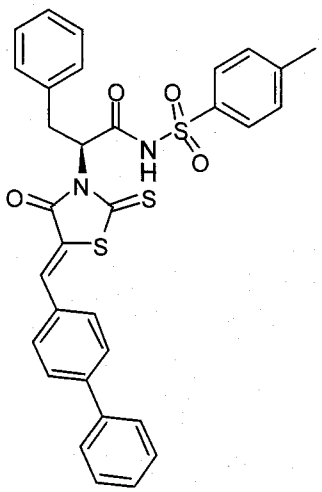
40. The compound of any one of claims 30-36 wherein n is 1 and R_3 is phenyl or phenyl(C_1-C_6)alkyl, wherein any phenyl of R_3 is optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

41. The compound of any one of claims 30-36 wherein n is 1 and R_3 is phenyl, optionally susbtituted with one or more groups independently selected from halo, cyano, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, (C_1-C_6)alkanoyl, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkanoyloxy, (C_3-C_6)cycloalkyl, trifluoromethyl, NR_eR_f, -C(=O) NR_eR_f, and trifluoromethoxy.

42. The compound of any one of claims 30-36 wherein n is 1 and R_3 is phenyl.

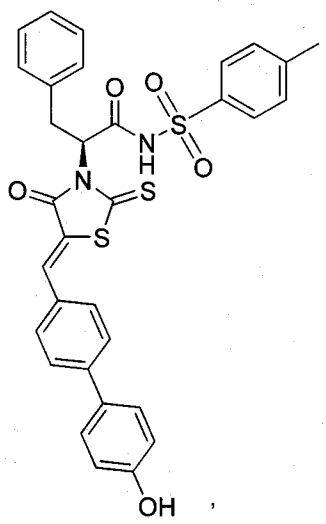
43. The compound of any one of claims 38-42 wherein R_3 is on the 4-position of the phenyl ring to which it is attached.

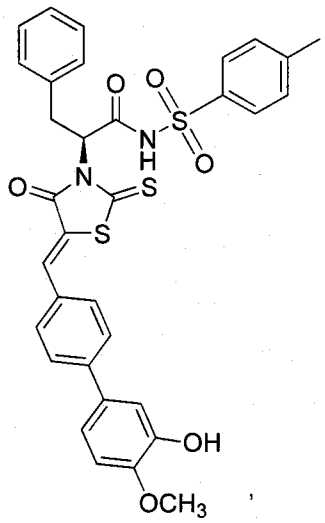
44. The compound of claim 1 which is a compound of the following formula:

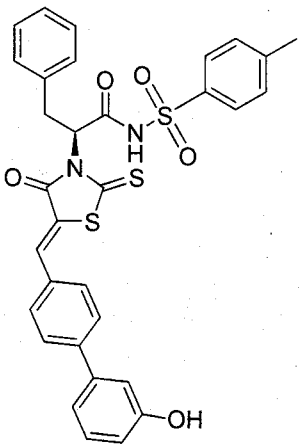


or a salt thereof.

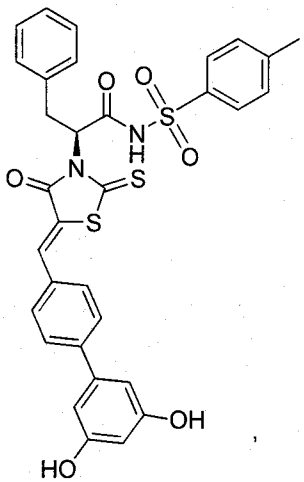
45. The compound of claim 1 which is a compound of the following formula:



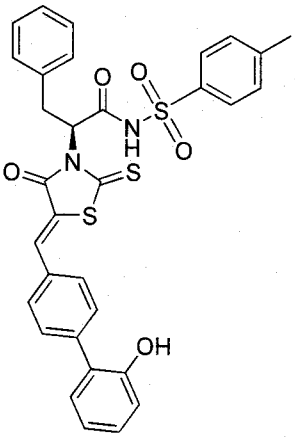




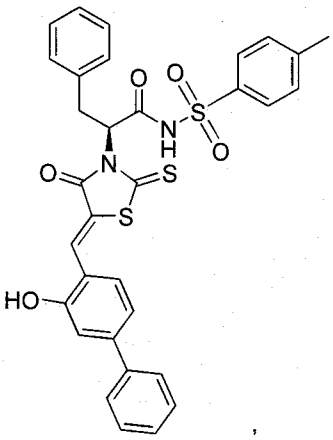
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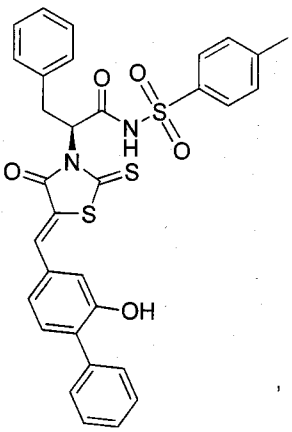
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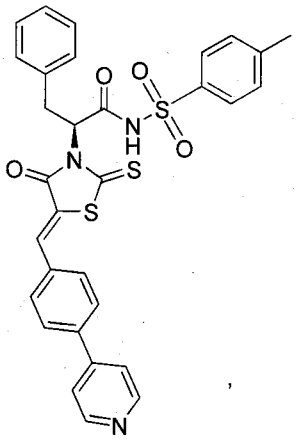
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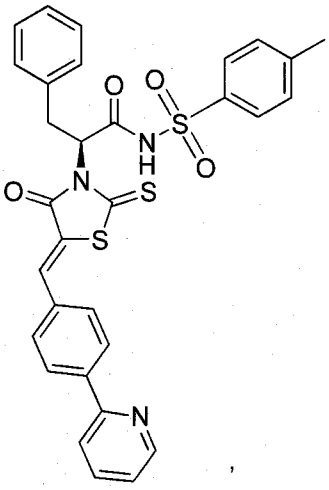


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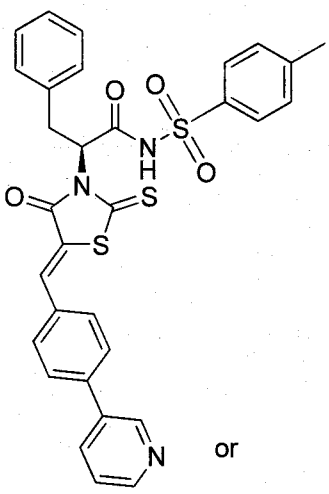


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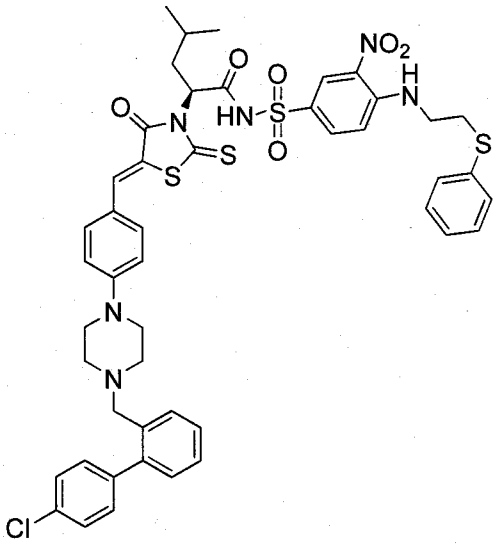
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,

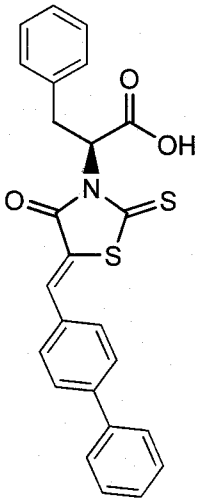


or

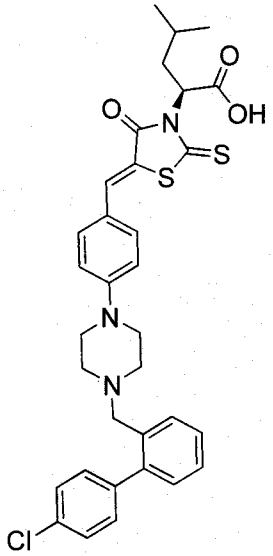


or a salt thereof.

46. The compound of claim 1 which is a compound of the following formula:



or



or a salt thereof.

47. A composition comprising a compound as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

48. A therapeutic method for treating cancer in a animal (e.g. a mammal) comprising, administering to the animal an effective amount of a compound as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof.

49. The method of claim 48 wherein the cancer is lymphoma.

50. The method of claim 48 wherein the cancer is prostate cancer.

51. The method of claim 50 wherein the prostate cancer is hormone-refractory phenotype.

52. The method of claim 48 wherein the cancer is leukemia.

53. A compound of formula I as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof, for use in medical therapy.

54. The use of a compound of formula I as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament useful for the treatment of cancer in a mammal.

55. The use of claim 54 wherein the cancer is prostate cancer.

56. The use of claim 55 wherein the prostate cancer is hormone-refractory phenotype.

57. The use of claim 54 wherein the cancer is lymphoma.

58. The use of claim 54 wherein the cancer is leukemia.

59. A compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of cancer in a mammal.

60. The compound of claim 59 wherein the cancer is prostate cancer.

61. The compound of claim 60 wherein the prostate cancer is hormone-refractory phenotype.

62. The compound of claim 59 wherein the cancer is lymphoma.

63. The compound of claim 59 wherein the cancer is leukemia.

64. A method to antagonize anti-apoptotic Bcl-2 proteins in an animal comprising administering to the animal an amount of a compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof that is effective to antagonize the anti-apoptotic Bcl-2 proteins in the animal.

65. A method to antagonize microtubule formation in an animal comprising administering to the animal an amount of a compound of formula I as described in any one of claims 1-46 or a

pharmaceutically acceptable salt thereof that is effective to antagonize microtubule formation in the animal.

66. A method to antagonize anti-apoptotic Bcl-2 proteins and to antagonize microtubule formation in an animal comprising administering to the animal an amount of a compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof that is effective to antagonize the anti-apoptotic Bcl-2 proteins and microtubule formation in the animal.

67. The use of a compound of formula I as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing anti-apoptotic Bcl-2 proteins in an animal.

68. The use of a compound of formula I as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing microtubule formation in an animal.

69. The use of a compound of formula I as described in any one of claims 1-46, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament useful for antagonizing anti-apoptotic Bcl-2 proteins and for antagonizing microtubule formation in an animal.

70. A compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal wherein the antagonism of anti-apoptotic Bcl-2 proteins is desired.

71. A compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal wherein the antagonism of microtubule formation is desired.

72. A compound of formula I as described in any one of claims 1-46 or a pharmaceutically acceptable salt thereof for use in the prophylactic or therapeutic treatment of a pathological condition or symptom in an animal wherein the antagonism of anti-apoptotic Bcl-2 proteins and the antagonism of microtubule formation is desired.

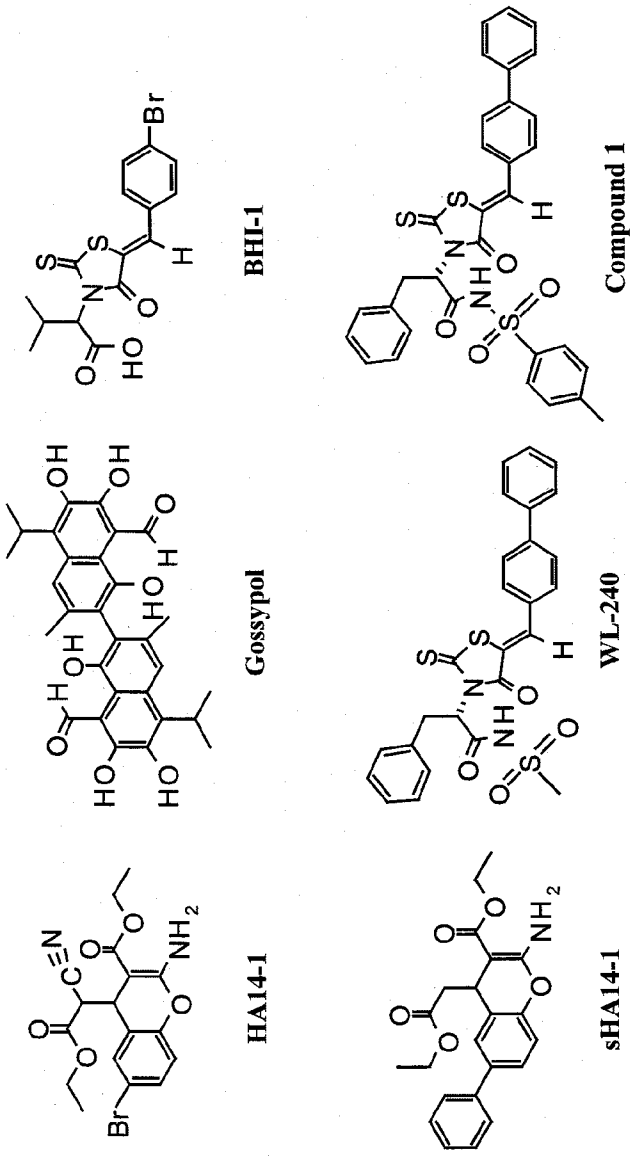


Fig. 1

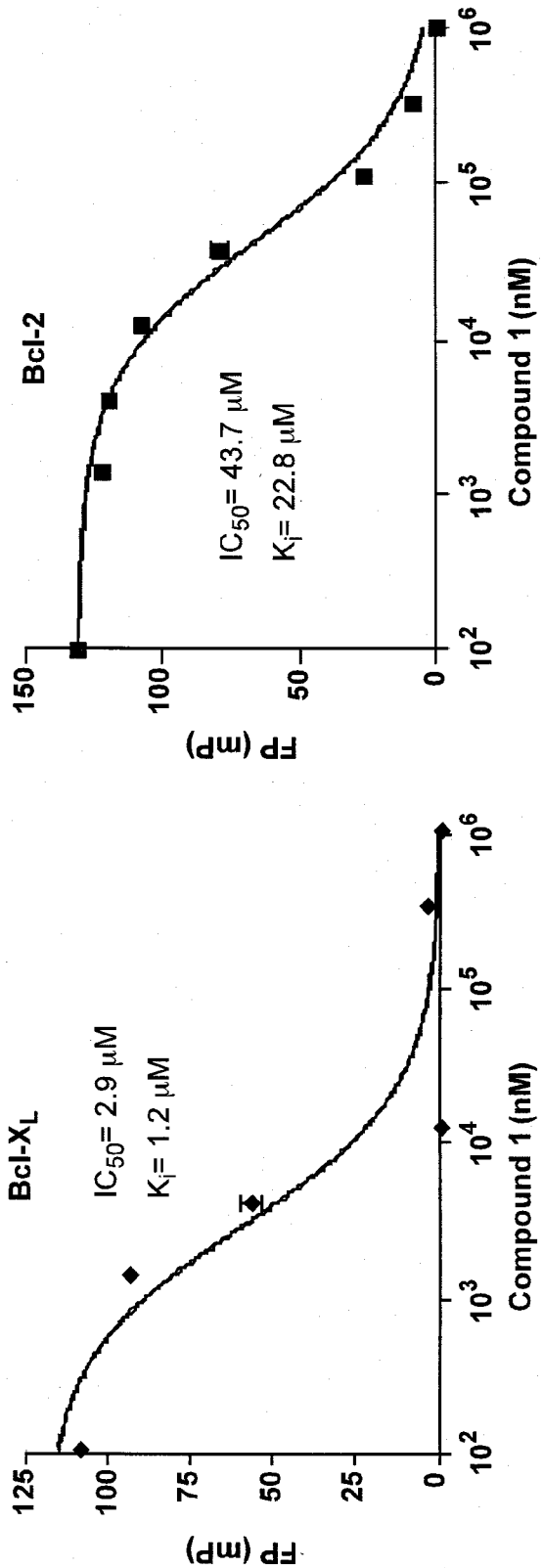

Bcl-2
IC_{50}= 43.7 μM
K_i= 22.8 μM
FP (mP)
Compound 1 (nM)
Bcl-X_L
IC_{50}= 2.9 μM
K_i= 1.2 μM
FP (mP)
Compound 1 (nM)

Fig. 2

Fig. 3A

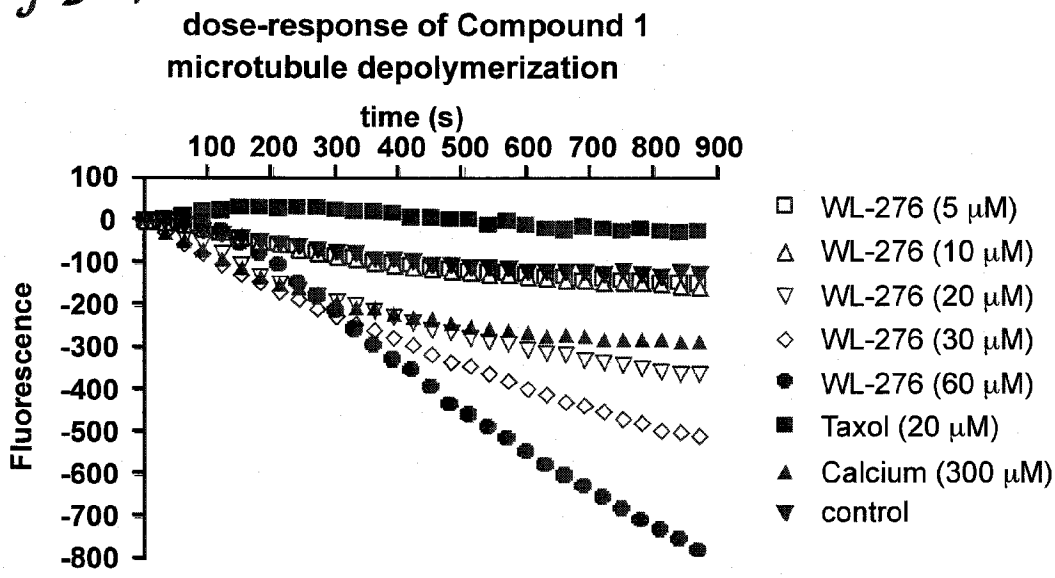
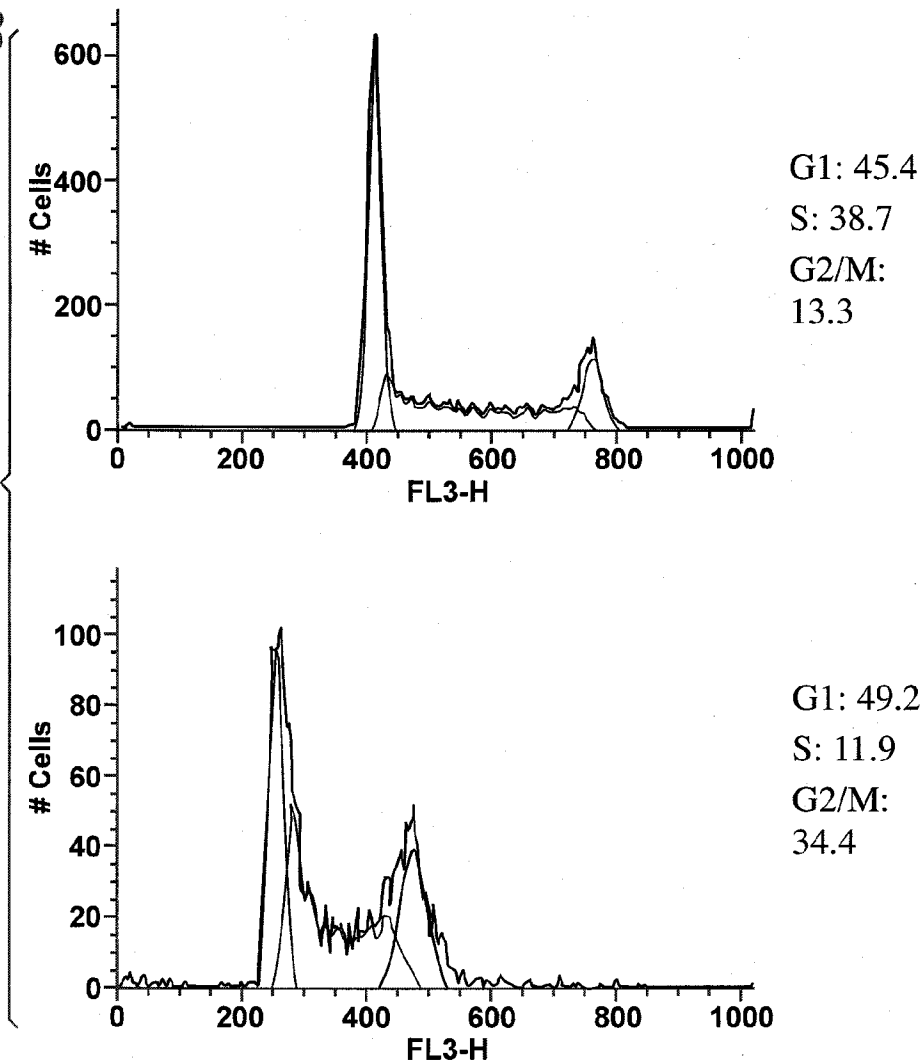

dose-response of Compound 1
microtubule depolymerization
time (s)
100 200 300 400 500 600 700 800 900
100
0
-100
-200
-300
-400
-500
-600
-700
-800
Fluorescence
WL-276 (5 μM)
WL-276 (10 μM)
WL-276 (20 μM)
WL-276 (30 μM)
WL-276 (60 μM)
Taxol (20 μM)
Calcium (300 μM)
control

Fig. 3B


Cells
FL3-H
G1: 45.4
S: 38.7
G2/M: 13.3
G1: 49.2
S: 11.9
G2/M: 34.4

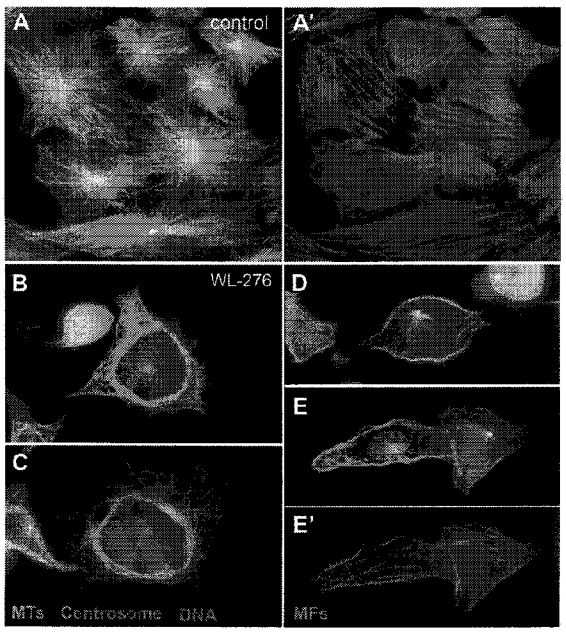
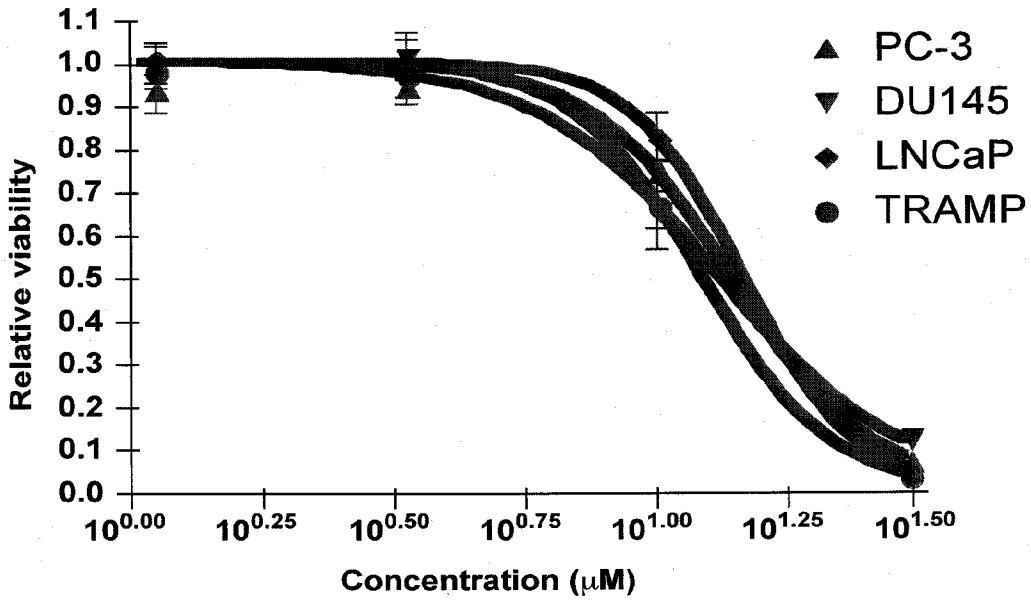


Fig. 3C



	PC-3	DU145	LNCaP	TRAMP
IC_{50} (μM)	13.6	12.9	14.4	12.0

Fig. 4A

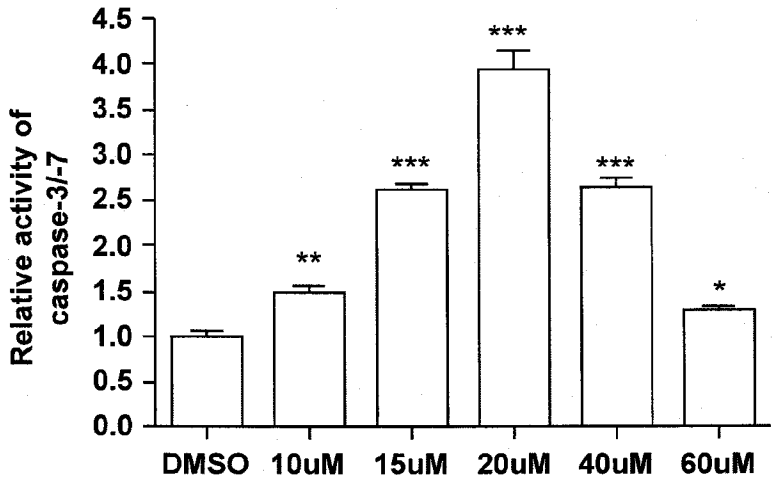

Relative activity of caspase-3/-7
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
***
***
***
***
*
DMSO
10uM
15uM
20uM
40uM
60uM

Fig. 4B

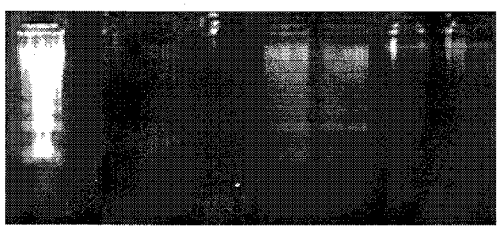


Fig. 4C

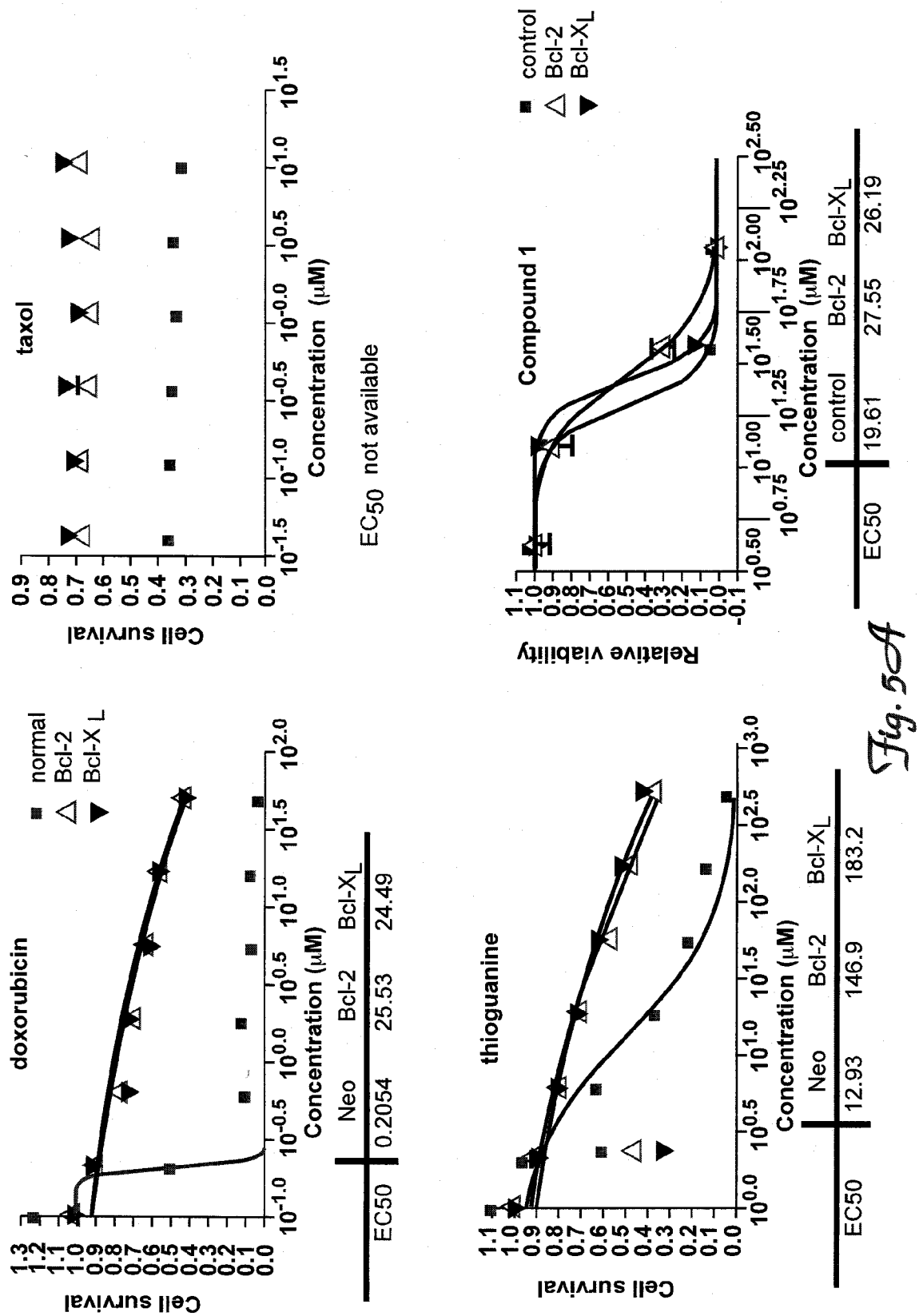

taxol
Cell survival
Concentration (μM)
EC50 not available
doxorubicin
normal
Bcl-2
Bcl-X L
Cell survival
Concentration (μM)
Neo Bcl-2 Bcl-XL
EC50 0.2054 25.53 24.49
Compound 1
control
Bcl-2
Bcl-XL
Relative viability
Concentration (μM)
control Bcl-2 Bcl-XL
EC50 19.61 27.55 26.19
thioguanine
Cell survival
Concentration (μM)
Neo Bcl-2 Bcl-XL
EC50 12.93 146.9 183.2

Fig. 5A

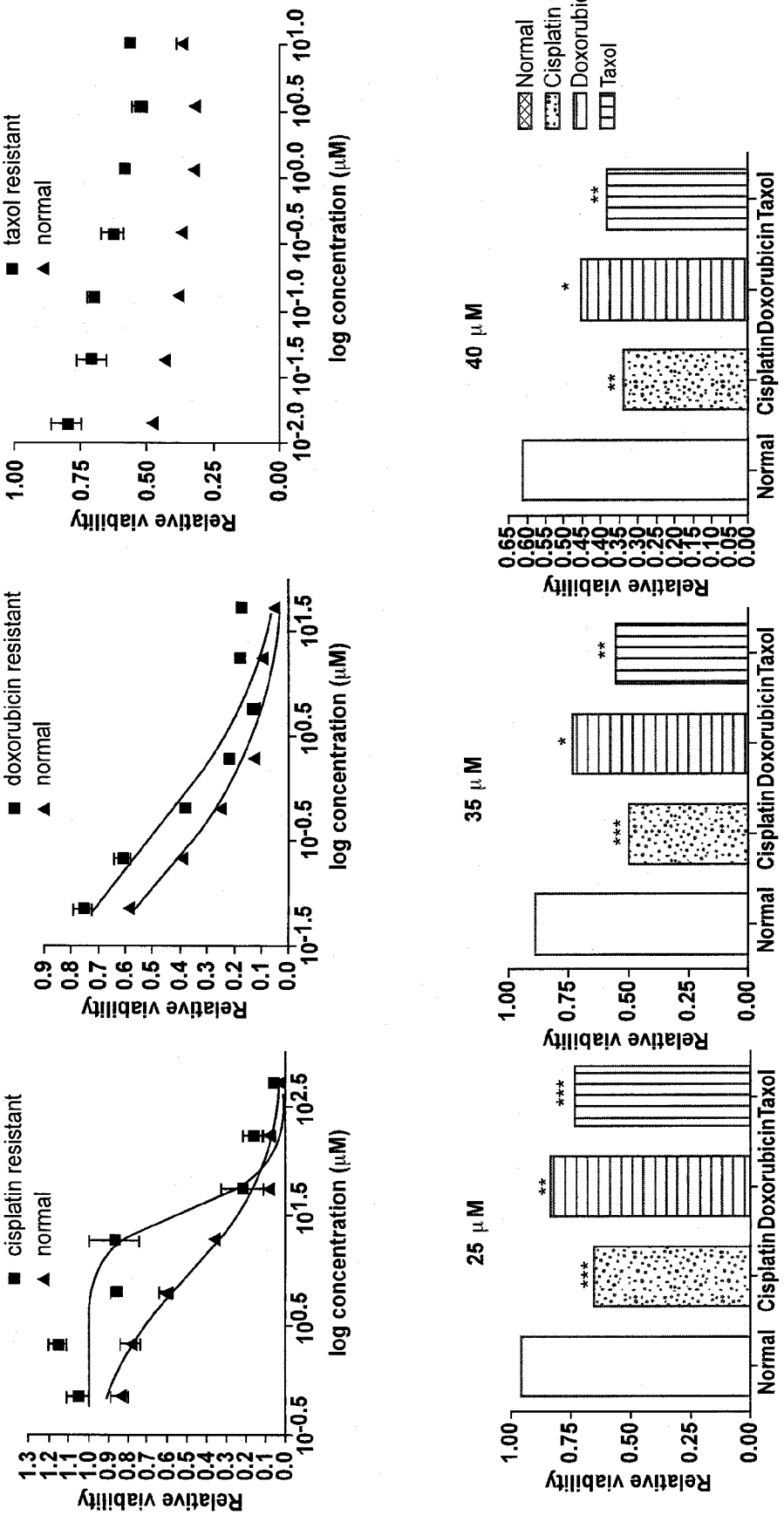

cisplatin resistant
normal
Relative viability
log concentration (μM)
doxorubicin resistant
normal
Relative viability
log concentration (μM)
taxol resistant
normal
Relative viability
log concentration (μM)
25 μM
35 μM
40 μM
Normal
Cisplatin
Doxorubicin
Taxol
Relative viability

Fig. 5B

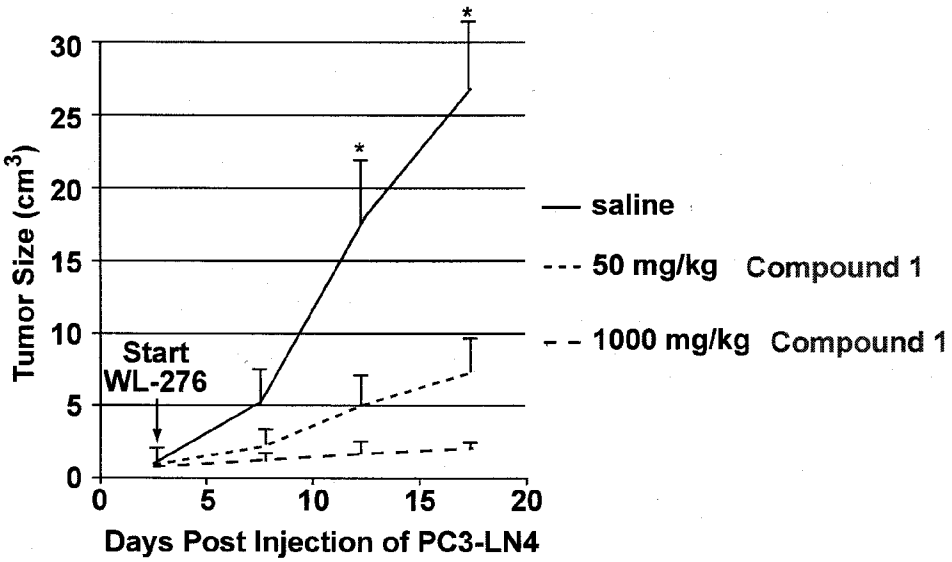
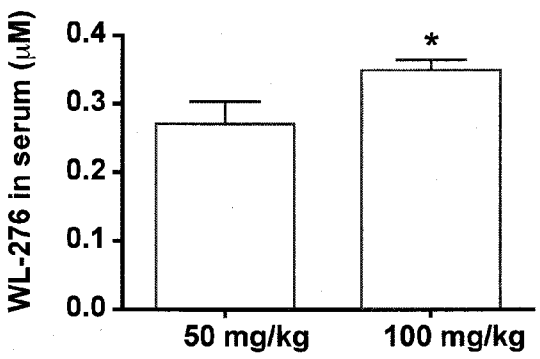


Fig. 6A



	50 mg/kg	100 mg/kg
Mean	0.2678	0.3451
Std. Deviation	0.1212	0.06053

Fig. 6B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/084409

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D277/36 C07D417/10 A61K31/427 A61P35/00

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)
C07D

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. XING ET AL.: "Development of selective inhibitors for anti-apoptotic Bcl-2 proteins from BHI-1" BIOORGANIC & MEDICINAL CHEMISTRY, vol. 15, no. 5, 14 December 2006 (2006-12-14), pages 2167-2176, XP002515398 abstract; table 3 -----	1-72
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 February 2009	10/03/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Herz, Claus

INTERNATIONAL SEARCH REPORT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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