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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 February 2008 (21.02.2008)

PCT

(10) International Publication Number
WO 2008/021250 A2

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2007/017815

(22) International Filing Date: 10 August 2007 (10.08.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/836,918 10 August 2006 (10.08.2006) US

(71) Applicants *(for all designated States except US)*: **FRED HUTCHINSON CANCER RESEARCH CENTER** [US/US]; 1100 Fairview Avenue N., Mailstop J6-200, Seattle, Washington 98109-1024 (US). **VM DISCOVERY, INC.** [US/US]; 45535 Northport Loop East, 2nd Floor, Fremont, California 94538 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WU, Jay Jie-Qiang** [US/US]; 34069 Fredrick Lane, Fremont, California 94555 (US). **HOCKENBERY, David M.** [US/US]; 2817 32nd Avenue South, Seattle, Washington 98144 (US). **WANG, Ling** [CN/US]; 31404 San Ardo Court, Union City, California 94587 (US). **GUO, Jianxin** [CN/US]; 1301 West 24th, Suite D19, Lawrence, Kansas 66046 (US).

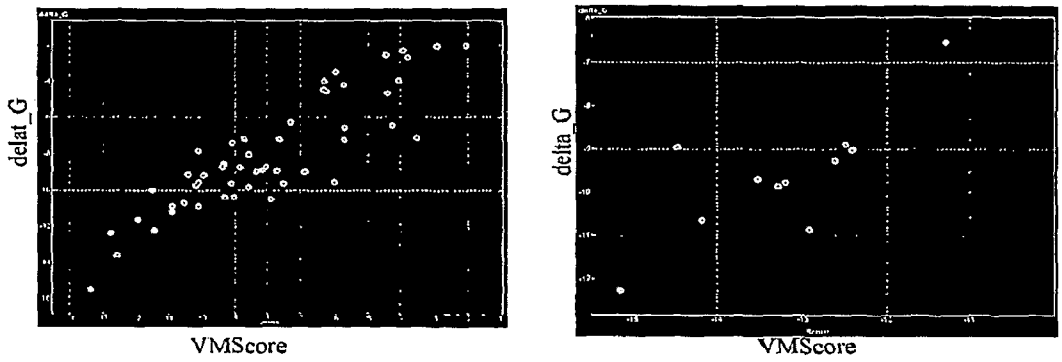
(74) Agent: **MYERS BIGEL SIBLEY & SAJOVEC, P.A.**; P.O. Box 37428, Raleigh, North Carolina 27627 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

(54) **Title:** COMPOSITIONS AND METHODS FOR MODULATING APOPTOSIS IN CELLS OVER-EXPRESSING BCL-2 FAMILY MEMBER PROTEINS



Left: VMScore result for the training set with 53 complex structures, $R^2 = 0.77$, with cross-validation prediction (leave-one-out) $Q^2 = 0.70$.
Right: VMScore prediction for the testing set of Endothiapepsin complex dataset, $R^2 = 0.62$.

(57) **Abstract:** The present invention relates to compounds for modulating apoptosis in cells over expressing Bcl-2 Family member proteins. The present invention also relates to pharmaceutical compositions containing these compounds, and methods of using the compounds.

WO 2008/021250 A2

COMPOSITIONS AND METHODS FOR MODULATING APOPTOSIS IN CELLS OVER-EXPRESSING Bcl-2 FAMILY MEMBER PROTEINS

Jay Jie-Qiang Wu, David Hockenbery, Ling Wang, Jianxin Guo

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of United States Provisional Application Number 60/836,918 (attorney docket no 9498-17Pr), filed August 10, 2006, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The present invention relates to compounds for modulating apoptosis in cells over expressing Bcl-2 Family member proteins. The present invention also relates to pharmaceutical compositions containing these compounds and methods of using the compounds.

BACKGROUND OF THE INVENTION

Many diseases are believed to be related to the down-regulation of apoptosis in the affected cells. For example, neoplasias may result, at least in part, from an apoptosis-resistant state in which cell proliferation signals inappropriately exceed cell death signals. Furthermore, some DNA viruses, such as Epstein-Barr virus, African swine fever virus and adenovirus, parasitize the host cellular machinery to drive their own replication and at the same time modulate apoptosis to repress cell death and allow the target cell to reproduce the virus. Moreover, certain diseases, such as lymphoproliferative conditions, cancer (including drug resistant cancer), arthritis, inflammation, autoimmune diseases, and the like, may result from a down regulation of cell death signals. In such diseases, it would be desirable to promote apoptotic mechanisms.

Most currently available chemotherapeutic agents target cellular DNA and induce apoptosis in tumor cells (Fisher *et al.*, *Cell* 78:539-542, 1994). A decreased sensitivity to apoptosis induction has emerged as an important mode of drug resistance. Members of the evolutionarily conserved Bcl-2 family are important regulators of apoptotic cell death and survival. The proteins Bcl-2, Bcl-x_L, Bcl-w, A1 and Mcl-1 are death antagonists while Bax, Bak, Bad, Bcl-xs, Bid, and Bik are death agonists (Kroemer *et al.*, *Nature Med.* 6:614-620, 1997). Over-expression of Bcl-2 and Bcl-x_L confers resistance to multiple chemotherapeutic

agents, including alkylating agents, antimetabolites, topoisomerase inhibitors, microtubule inhibitors and anti-tumor antibiotics, and may constitute a mechanism of clinical chemoresistance in certain tumors (Minn *et al.*, *Blood* 86:1903-1910, 1995; Decaudin *et al.*, *Cancer Res.* 57:62-67, 1997). Bcl-2/ Bcl-x_L-directed therapies, using either anti-sense oligonucleotides or novel protein-targeted drugs, can increase cellular sensitivity to standard agents *in vitro* or, in some cases, kill cells as single agents (Jansen *et al.*, *Nat. Med.* 4:232-234, 1998).

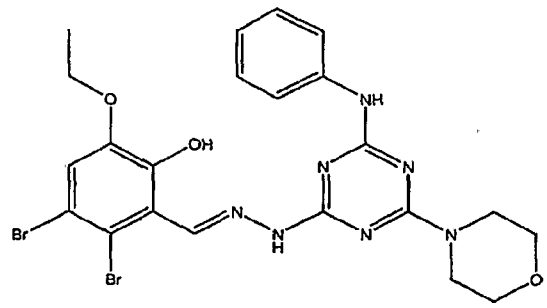
Structure solutions for Bcl-x_L and Bcl-2 have demonstrated the presence of a hydrophobic cleft at the surface of both proteins (Muchmore *et al.*, *Nature* 381:335-341, 1996). Functional studies implicate this groove as a binding surface for heterodimenc partners, including the related pro-apoptotic proteins Bax and Bak, and as a regulatory domain for an intrinsic membrane pore function (Sattler *et al.*, *Science* 275:983-986, 1997). Efforts to design small molecule inhibitors of Bcl-2/ Bcl-x_L have thus focused on this structural feature.

Two antimycins, antimycin A_1 and A_3, have been discovered to inhibit the activity of the anti-apoptotic Bcl-2 family member proteins, Bcl-2 or Bcl-x_L. These naturally obtained antimycins are toxic, however, because as discussed above, they also inhibit mitochondrial respiration. Therefore, antimycin derivatives that are effective in inducing apoptosis in cells where apoptosis is inappropriately regulated while exhibiting reduced inhibition of mitochondrial respiration have been identified.

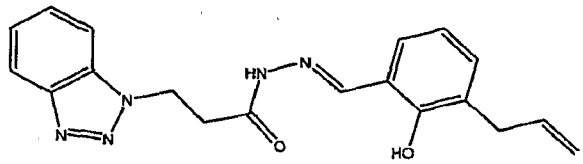
It would be desirable to identify other compounds that may be effective in inducing apoptosis in cells where apoptosis is inappropriately regulated while minimally inhibiting mitochondrial respiration. Furthermore, it would be desirable to use the identified compounds as to treat diseases related to the down regulation of apoptosis.

SUMMARY OF THE INVENTION

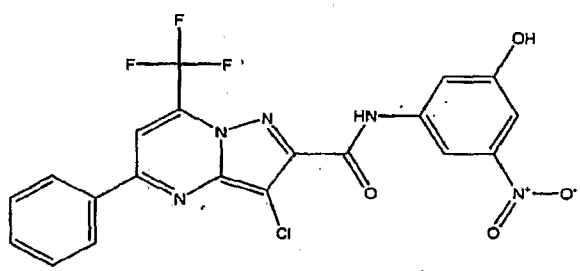
The present invention provides methods of treating an apoptosis-associated disease. Embodiments of the present invention provide methods for treating an apoptosis-associated disease (*e.g.*, cancer) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an active compound as described herein, such as a compound selected from the group consisting of:



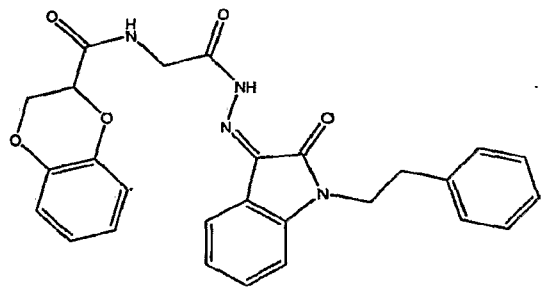
1



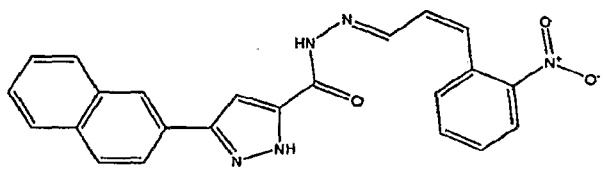
2



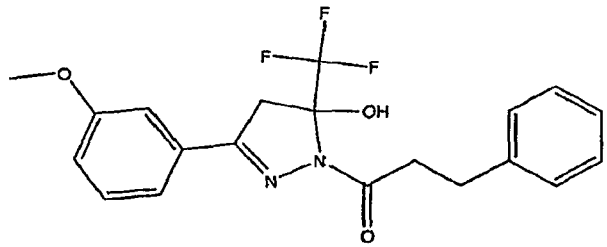
3



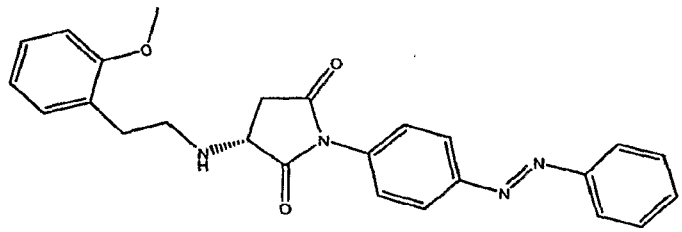
4



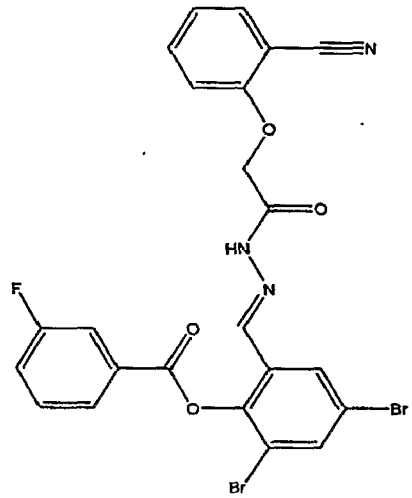
5



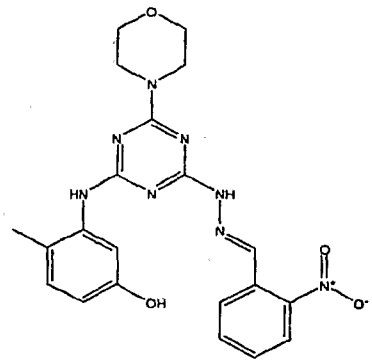
6



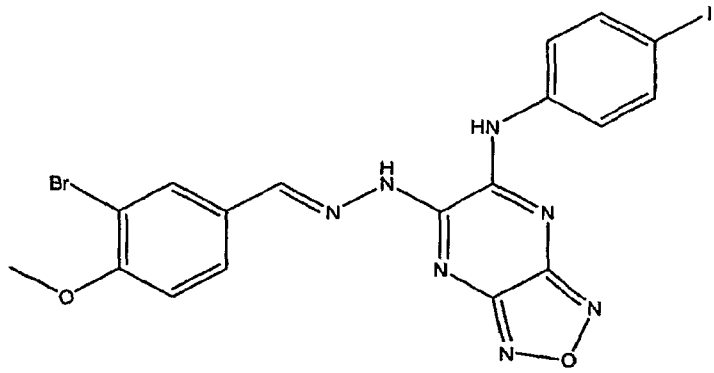
7



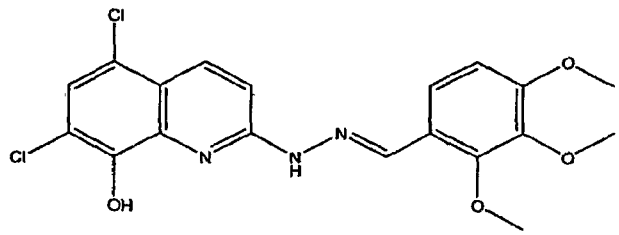
8



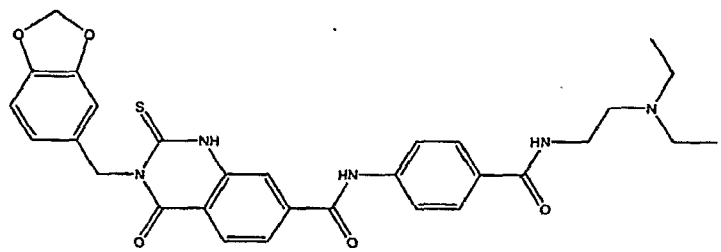
9



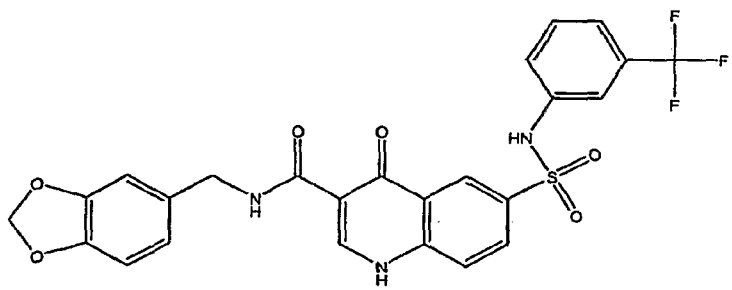
10



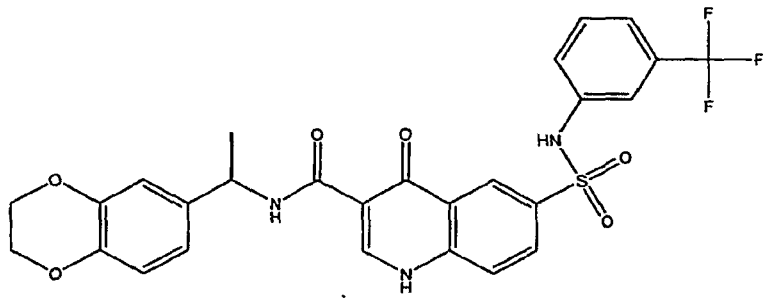
11



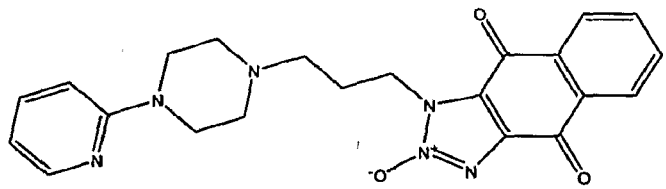
12



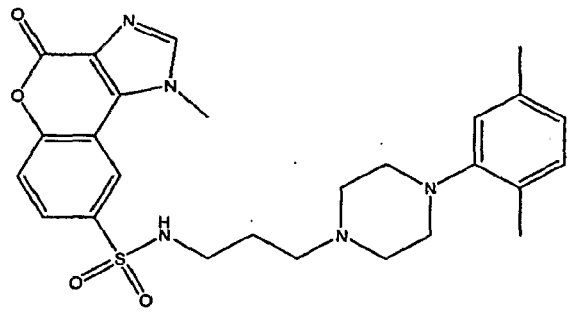
13



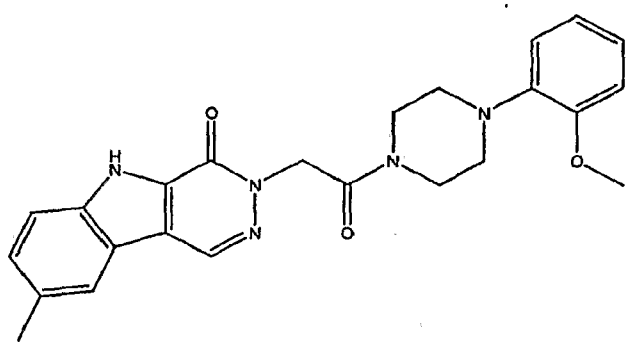
14



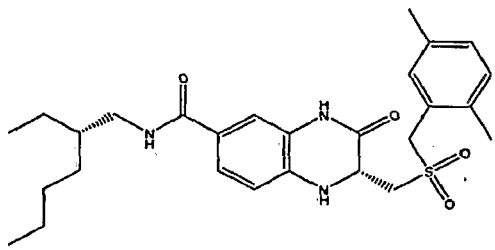
15



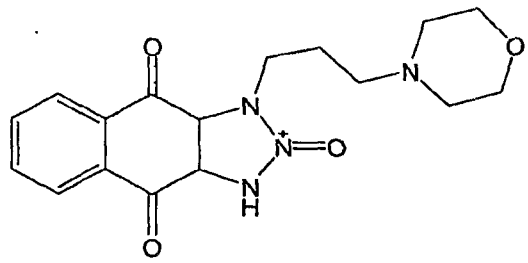
16



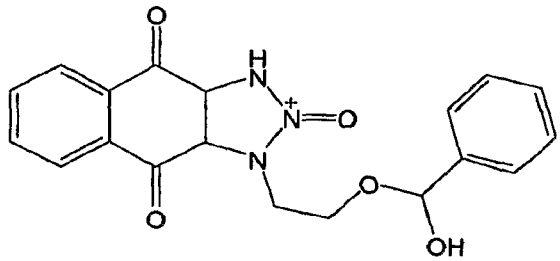
17



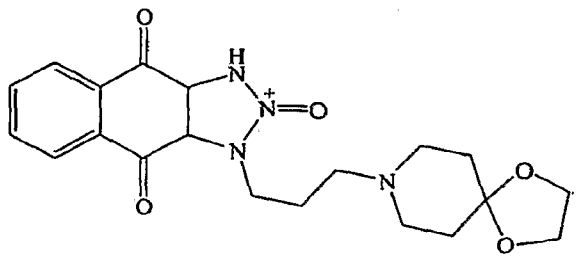
18



19



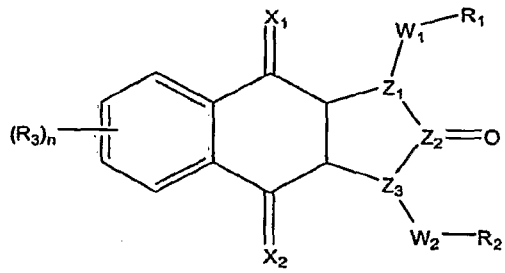
20



21

and pharmaceutically acceptable salts and prodrugs thereof.

Embodiments of the present invention provide methods for treating an apoptosis-associated disease (*e.g.*, cancer) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an active compound of Formula XI



XI

or a pharmaceutically acceptable salt or a prodrug thereof.

wherein:

n is from 0 to 4;

X_1 and X_2 are each independently O or S;

Z_1, Z_2, Z_3 are each independently C or N;

W_1 and W_2 are independently a bond or selected from the group consisting of C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkynyl, C_{1-10} alkoxy, C_{1-10} alkylsulfanyl, C_{1-10} haloalkyl, and C_{1-10} aminoalkyl;

R_1 and R_2 are each independently selected from the group consisting of C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-14} heteraryl and C_{3-10} heterocycle;

R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10}thioalkyl, C_{1-10}haloalkyl, C_{3-10}cycloalkyl, C_{3-10}cycloalkyl-C_{1-10}alkyl, C_{3-10}heterocycle, C_{3-10}heterocycle-C_{1-10}alkyl, C_{3-14} aryl, C_{6-12}aryl-C_{1-10}alkoxy, C_{3-10}heterocycle-C_{1-10}alkoxy and C_{3-14} heteroaryl;

wherein each of the foregoing alkyl, alkenyl, alkoxy, alkylsulfanyl, haloalkyl, aminoalkyl may be branched or unbranched, independently unsubstituted or substituted with one or more substituents selected from the group consisting of hydroxy, cyano, amine, nitro, halogen, mono- or dialkylamino, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl; and

wherein each of the foregoing C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-10} heterocycle, C_{3-14}aryl, C_{3-14} heteroaryl, may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, amino, cyano, nitro, -SH, C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkynyl, C_{1-10} alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl.

Embodiments of the present invention further provide a pharmaceutical formulation comprising, consisting of, or consisting essentially of an active compound as described herein in combination with a pharmaceutically acceptable carrier.

Embodiments of the present invention provide a method of treating an apoptosis-associated disease (*e.g.*, cancer) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an active compound as described herein.

Embodiments of the present invention further provide the use of an active compound as described herein for the preparation of a medicament for treating an apoptosis-associated disease (*e.g.*, cancer) in a subject in need thereof.

The foregoing and other objects and aspects of the present invention are explained in greater detail in the specification set forth below.

Brief Description of the Drawings

Figure 1 illustrates a test of the "VMScore" function. with a data set containing 53 complex (protein bound with compound) crystal structures with experimental binding affinity (Ki) data ($\Delta G \propto \log Ki$) as the training set. Results, Left, fit the experimental data with a squared correlation coefficient, $R^2 = 0.77$, and with LOO (leave-one-out) cross-validation, $Q^2 = 0.70$.

DETAIL DESCRIPTION OF THE INVENTION

The foregoing and other aspects of the present invention will now be described in more detail with respect to embodiments described herein. It should be appreciated that the invention can be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the embodiments of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Also, as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items. Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound, dose, time, temperature, and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount. Unless otherwise defined, all terms, including technical and scientific terms used in the description, have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent

may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms.

"Alkyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms. In some embodiments, the alkyl employed in the invention contains 1 to 6 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl, and the like. "Lower alkyl" as used herein, is a subset of alkyl, in some embodiments preferred, and refers to a straight or branched chain hydrocarbon group containing from 1 to 4 carbon atoms. Representative examples of lower alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, and the like. The term "akyl" or "loweralkyl" is intended to include both substituted and unsubstituted alkyl or loweralkyl unless otherwise indicated and these groups may be substituted with groups selected from halo (*e.g.*, haloalkyl), alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl, hydroxyl, alkoxy (thereby creating a polyalkoxy such as polyethylene glycol), alkenyloxy, alkynyloxy, haloalkoxy, cycloalkoxy, cycloalkylalkyloxy, aryloxy, arylalkyloxy, heterocyclooxy, heterocyclolalkyloxy, mercapto, alkyl-$S(O)_m$, haloalkyl-$S(O)_m$, alkenyl-$S(O)_m$, alkynyl-$S(O)_m$, cycloalkyl-$S(O)_m$, cycloalkylalkyl-$S(O)_m$, aryl-$S(O)_m$, arylalkyl-$S(O)_m$, heterocyclo-$S(O)_m$, heterocycloalkyl-$S(O)_m$, amino, carboxy, alkylamino, alkenylamino, alkynylamino, haloalkylamino, cycloalkylamino, cycloalkylalkylamino, arylamino, arylalkylamino, heterocycloamino, heterocycloalkylamino, disubstituted-amino, acylamino, acyloxy, ester, amide, sulfonamide, urea, alkoxyacylamino, aminoacyloxy, nitro or cyano where m= 0, 1, 2 or 3.

"Alkenyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms (or in loweralkenyl 1 to 4 carbon atoms) which include 1 to 4 double bonds in the normal chain. In some embodiments, the alkenyl employed in the invention contain 1 to 6 carbonatoms. Representative examples of alkenyl include, but are not limited to, vinyl, 2-propenyl, 3-butenyl, 2-butenyl, 4-pentenyl,

3-pentenyl, 2-hexenyl, 3-hexenyl, 2,4-heptadiene, and the like. The term "alkenyl" or "loweralkenyl" is intended to include both substituted and unsubstituted alkenyl or loweralkenyl unless otherwise indicated and these groups may be substituted with groups as described in connection with alkyl and loweralkyl above.

"Alkynyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms (or in loweralkynyl 1 to 4 carbon atoms) which include 1 to 4 triple bond in the normal chain. In some embodiments, the alkynyl employed in the invention contain 1 to 6 carbonatoms. Representative examples of alkynyl include, but are not limited to, 2-propynyl, 3-butynyl, 2- butynyl, 4-pentynyl, 3-pentynyl, and the like. The term "alkynyl" or "loweralkynyl" is intended to include both substituted and unsubstituted alkynyl or loweralknynyl unless otherwise indicated and these groups may be substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Cycloalkyl", as used herein alone or as part of another group, refers to groups having 3 to 10 carbon atoms. In some embodiments, the cycloalkyl employed in the invention have 3 to 8 carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or hetercyclic moieties, may optionally be substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Heterocycloalkyl" or "heterocycle", as used herein alone or as part of another group, refers to a non-aromatic 3-, 4-, 5-, 6-, 7-, or 8- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and four heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) the nitrogen and sulfur heteroatoms may be optionally oxidized, (ii) the nitrogen heteroatom may optionally be quaternized, and (iv) may form a spiro ring or be fused with an cycloalkyl, aryl, heterocyclic ring, benzene or a heteroaromatic ring. In some embodiments, the heterocycle employed in the invention have 3 to 10 carbon atoms. Representative heterocycles include, but are not limited to, 1,4-dioxa-8-azaspiro[4.5]decane, morpholine, azetidine, azepine, aziridine, diazepine, 1,3-dioxolane, dioxane, dithiane, furan, imidazole, imidazoline, imidazolidine, isothiazole, isothiazoline, isothiazolidine, isoxazole, isoxazoline, isoxazolidine, morpholine, oxadiazole, oxadiazoline, oxadiazolidine, oxazole, oxazoline, oxazolidine, piperazine, piperidine, pyran, pyrazine, pyrazole, pyrazoline, pyrazolidine, pyridine, pyrimidine, pyridazine, pyrrole, pyrroline, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, tetrazine, tetrazole, thiadiazole, thiadiazoline,

thiadiazolidine, thiazole, thiazoline, thiazolidine, thiophene, thiomorpholine, thiomorpholine sulfone, thiopyran, triazine, triazole, trithiane, benzimidazole, benzothiazole, benzothiadiazole, benzothiophene, benzoxadiazole, benzoxazole, benzofuran, benzopyran, benzothiopyran, benzodioxine, 1,3-benzodioxole, cinnoline, indazole, indole, indoline, indolizine, naphthyridine, isobenzofuran, isobenzothiophene, isoindole, isoindoline, isoquinoline, phthalazine, purine, pyranopyridine, quinoline, quinolizine, quinoxaline, quinazoline, tetrahydroisoquinoline, tetrahydroquinoline, thiopyranopyridine, and the like. These rings include quaternized derivatives thereof and may be optionally substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Aryl" as used herein alone or as part of another group, refers to a monocyclic carbocyclic ring system or a bicyclic carbocyclic fused ring system having one or more aromatic rings. In some embodiments, the aryl employed in the invention have 3 to 14 carbon atoms. Representative examples of aryl include, azulenyl, indanyl, indenyl, naphthyl, phenyl, tetrahydronaphthyl, and the like. The term "aryl" is intended to include both substituted and unsubstituted aryl unless otherwise indicated and these groups may be optionally substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Arylalkyl" as used herein alone or as part of another group, refers to an aryl group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of arylalkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, 2-naphth-2-ylethyl, and the like.

"Heteroaryl" as used herein alone or as part of another group, refers to a cyclic, aromatic hydrocarbon in which one or more carbon atoms have been replaced with heteroatoms such as O, N, and S. If the heteroaryl group contains more than one heteroatom, the heteroatoms may be the same or different. In some embodiments, the heteroaryl employed in the invention have 3 to 14 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidinyl, imidazolyl, thienyl, furyl, pyrazinyl, pyrrolyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, indolyl, isoindolyl, indolizinyl, triazolyl, pyridazinyl, indazolyl, purinyl, quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, isothiazolyl, and benzo[b]thienyl. In some embodiments, heteroaryl groups are five and six membered rings and contain from one to three heteroatoms independently selected from O, N, and S. The heteroaryl group, including each heteroatom, can be unsubstituted or substituted with from 1 to 4 substituents, as chemically feasible. For example, the heteroatom N or S may be substituted with one or two oxo groups, which may be shown as

=O.

"Alkoxy" (or "alkyloxy"), or "thioalkyl", as used herein alone or as part of another group, refers to an alkyl or loweralkyl group, as defined herein (and thus including substituted versions such as polyalkoxy), appended to the parent molecular moiety through an oxy group, -O-, through a sulfur atom. In some embodiments, the alkoxy or thioalkyl group contains 1-10 carbon atoms. In other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 carbon atoms. In still other embodiments, the alkyl group contains 1-6 carbon atoms. In yet other embodiments, the alkyl group contains 1-4 carbon atoms. Representative examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy and the like. Representative examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

"Halo" as used herein alone or as part of another group, refers to any suitable halogen, including –F, -Cl, -Br, and –I.

"Amine" or "amino group", as used herein alone or as part of another group, refers to the radical $-NH_2$. An "optionally substituted" amines refers to $-NH_2$ groups wherein none, one or two of the hydrogens is replaced by a suitable substituent. Disubstituted amines may have substituents that are bridging, i.e., form a heterocyclic ring structure that includes the amine nitrogen.

"Aminoalkyl group" is intended to mean the radical $-NHR_a$, where R_a is an alkyl group.

"Haloalkyl", as used herein alone or as part of another group, refers to an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

The term "apoptosis" refers to a regulated network of biochemical events which lead to a selective form of cell suicide, and is characterized by readily observable morphological and biochemical phenomena, such as the fragmentation of the deoxyribo-nucleic acid (DNA), condensation of the chromatin, which may or may not be associated with endonuclease activity, chromosome migration, margination in cell nuclei, the formation of apoptotic bodies, mitochondrial swelling, widening of the mitochondrial cristae, opening of the mitochondrial permeability transition pores and/or dissipation of the mitochondrial proton gradient and the like.

The term "preferentially induce" apoptosis refers to at least a 5-fold greater stimulation of apoptosis, at a given concentration an agent, including a 2-methoxy antimycin

derivative, in cells that over-express a Bcl-2 family member protein as compared with cells that do not over-express the Bcl-2 family member protein (*e.g.*, a 5-fold lower LD_{50} or IC_{50}).

The term "substantially non-toxic" refers to an agent including a 2-methoxyantimycin that induces apoptosis in at least about 50 percent of cells that over-express a Bcl-2 family member protein, but does not induce apoptosis in more than about 5%, more preferably less than 1%, of cells that do not over-express the Bcl-2 family member protein.

The term "Bcl-2 family member protein(s)" refers to an evolutionarily conserved family of proteins characterized by having one or more amino acid homology domains, BH1, BH2, BH3, and/or BH4. The Bcl-2 family member proteins include Bcl-2, Bcl-x_L, Bcl-w, A1, Mcl-1, Bax, Bak, Bad, Bcl-xs, Bid and the like. The "Bcl-2 family member proteins" further include those proteins, or their biologically active fragments, that have at least 70%, preferably at least 80%, and more preferably at least 90% amino acid sequence identity with a Bcl-2 family member protein.

The term "anti-apoptotic Bcl-2 family member protein" refers to Bcl-2, Bcl-x_L, Bcl-w, A1, Mcl-1, and other proteins characterized by having one or more amino acid homology domains, BH1, BH2, BH3, and/or BH4, and that promote cell survival by attenuating or inhibiting apoptosis. The "anti-apoptotic Bcl-2 family member proteins" further include those proteins, or their biologically active fragments, that have at least 70%, preferably at least 80%, and more preferably at least 90% amino acid sequence identity with an anti-apoptotic Bcl-2 family member protein.

The terms "identity" or "percent identity" in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using either a PILEUP or BLAST sequence comparison algorithm (*see, e.g., J. Mol. Evol.* 35:351-360, 1987; Higgins and Sharp, *CABIOS* 5:151-153, 1989; Altschul *et al., J. Mol. Biol.* 215:403-410, 1990; Zhang *et al., Nucleic Acid Res.* 26:3986-3990, 1998; Altschul *et al., Nucleic Acid Res.* 25:3389-33402, 1997). Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson and Lipman, *Proc. Nat. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and

TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see, generally,* Ausubel *et al., supra*).

In the context of Bcl-2 family member proteins, "correspondence" of one polypeptide sequence to another sequence (*e.g.*, regions, fragments, nucleotide or amino acid positions, or the like) is based on the convention of numbering according to nucleotide or amino acid position number, and then aligning the sequences in a manner that maximizes the number of nucleotides or amino acids that match at each position, as determined by visual inspection or by using a sequence comparison algorithm such as, for example, PILEUP (*see, e.g., supra*; Higgins & Sharp, *supra*) or BLAST (*see, e.g.*, Altschul *et al., supra*; Zhang *et al., supra*; Altschul *et al., supra*). For example, a mutant Bcl-2 family member amino acid sequence having one or more amino acid substitutions, additions, or deletions as compared to the wild-type protein may correspond to a second Bcl-2 family member amino acid sequence (*e.g.*, the wild-type sequence or a functionally equivalent variant thereof) according to the convention for numbering the second Bcl-2 family member sequence, whereby the mutant sequence is aligned with the second Bcl-2 family member sequence such that at least 50%, typically at least 60%, more typically at least 70%, preferably at least 80%, more preferably at least 90%, and even more preferably at least 95% of the amino acids in a given sequence of at least 20 consecutive amino acids are identical. Because not all positions with a given "corresponding region" need be identical, non-matching positions within a corresponding region are herein regarded as "corresponding positions."

As used herein, a single amino acid substitution in one ("first") mutant Bcl-2 family member protein "corresponds" to a single amino acid substitution in a second mutant Bcl-2 family member protein (*e.g.*, Bcl-x_L) where the corresponding substituted amino acid positions of the first and second mutant proteins are identical.

In the context of Bcl-2 family member protein mutants, the phrase "no substantial effect on tertiary protein structure relative to the corresponding wild-type Bcl-2 family member protein" or "no substantial alteration of tertiary protein structure relative to the corresponding wild-type Bcl-2 family member protein" means that, when a Cα trace providing a position for each Cα carbon of the mutant protein is superimposed onto a Cα trace of the corresponding wild-type protein and an α carbon root mean square (RMS) difference root mean square deviation (RMSD) is calculated; *i.e.*, the deviation of the mutant structure from that of the wild-type structure), the RMSD value is no more than about 1.0 Å when calculated using the same structural modeling method, typically no more than about

0.75Å, even more typically no more than about 0.5 Å, preferably no more than about 0.35 Å, and even more preferably no more than about 0.25 Å.

The terms "biologically active" or "biological activity" refer to the ability of a molecule to modulate apoptosis, such as by binding to a Bcl-2 family member protein. A biologically active molecule can modulate apoptosis by causing a change in the mitochondrial protonmotive force gradient (*see, e.g.*, Example 2); by causing a change in mitochondrial swelling or the morphological characteristics of mitochondria (*see, e.g.*, Example 2); by affecting the release of a reporter molecule, such as, for example, rhodamine 123 or calcein, from mitochondria or vesicles (*see, e.g.*, Examples 4 and 8) comprising a pore-forming anti-apoptotic Bcl-2 family member protein (*see, e.g.*, Example 8); or by causing any other morphological change associated with apoptosis.

The term "effective amount" or "effective" is intended to designate a dose that causes a relief of symptoms of a disease or disorder as noted through clinical testing and evaluation, patient observation, and/or the like. "Effective amount" or "effective" further can further designate a dose that causes a detectable change in biological or chemical activity. The detectable changes may be detected and/or further quantified by one skilled in the art for the relevant mechanism or process. Moreover, "effective amount" or "effective" can designate an amount that maintains a desired physiological state, i.e., reduces or prevents significant decline and/or promotes improvement in the condition of interest. For example, an amount of an agent that effectively modulates the apoptotic state of an individual cell such that apoptosis is induced and/or the inappropriately regulated cell death cycle in the cell returns to a normal state. As is generally understood in the art, the dosage will vary depending on the administration routes, symptoms and body weight of the patient but also depending upon the compound being administered.

The terms "therapeutically useful" and "therapeutically effective" refer to an amount of an agent that effectively modulates the apoptotic state of an individual cell such that apoptosis is induced and/or the inappropriately regulated cell death cycle in the cell returns to a normal state.

The terms "diagnostically useful" and "diagnostically effective" refer to an agent (*e.g.*, an antimycin derivative) for detecting the induction or inhibition of apoptosis in a subject. These terms further include molecules useful for detecting diseases associated with apoptosis, or the susceptibility to such diseases, and for detecting over-expression or under-expression of a Bcl-2 family member protein.

The terms "over-expression" and "under-expression" refer to an increase or decrease, respectively, in the levels of a Bcl-2 family member protein in a cell relative to the level of such a protein found in the same cell or a closely related non-malignant cell under normal physiological conditions.

The term "apoptosis-associated disease" includes diseases, disorders, and conditions that are linked to an increased or decreased state of apoptosis in at least some of the cells of a subject. Such diseases include neoplastic disease (*e.g.*, cancer and other proliferative diseases), tumor formation, arthritis, inflammation, autoimmune disease, human immunodeficiency virus (HIV) immunodeficiency syndrome, neurodegenerative diseases, myelodysplastic syndromes (such as aplastic anemia), ischaemic syndromes (such as myocardial infarction), liver diseases which are induced by toxins (such as alcohol), alopecia, damage to the skin due to UV light, lichen planus, atrophy of the skin, cataract, and graft rejections and other premalignant and noneoplastic hyperproliferative disorders. Apoptosis-associated diseases further include drug resistance associated with increased or decreased levels of an anti-apoptotic Bcl-2 family member protein as well as multiple chemotherapeutic drug resistance.

A "combinatorial library" is a collection of compounds in which the compounds comprising the collection are composed of one or more types of subunits. The subunits can be selected from natural or unnatural moieties, including dienes, benzene compounds, cycloalkanes, lactones, dilactones, amino acids, alkanes, and the like. The compounds of the combinatorial library differ in one or more ways with respect to the number, order, type or types of modifications made to one or more of the subunits comprising the compounds. Alternatively, a combinatorial library may refer to a collection of "core molecules" which vary as to the number, type or position of R groups they contain and/or the identity of molecules composing the core molecule. The collection of compounds is generated in a systematic way. Any method of systematically generating a collection of compounds differing from each other in one or more of the ways set forth above is a combinatorial library.

A combinatorial library can be synthesized on a solid support from one or more solid phase-bound resin starting materials. The library can contain five (5) or more, preferably ten (10) or more, organic molecules, which are different from each other (*i.e.*, five (5) different molecules and not five (5) copies of the same molecule). Each of the different molecules (different basic structure and/or different substituents) will be present in an amount such that its presence can be determined by some means (*e.g.*, can be isolated, analyzed,

detected with a binding partner or suitable probe). The actual amounts of each different molecule needed so that its presence can be determined can vary due to the actual procedures used and can change as the technologies for isolation, detection and analysis advance. When the molecules are present in substantially equal molar amounts, an amount of about 100 picomoles or more can be detected. Preferred libraries comprise substantially equal molar amounts of each desired reaction product and do not include relatively large or small amounts of any given molecules so that the presence of such molecules dominates or is completely suppressed in any assay.

Combinatorial libraries are generally prepared by derivatizing a starting compound onto a solid-phase support (such as a bead). In general, the solid support has a commercially available resin attached, such as a Rink or Merrifield Resin, and the like. After attachment of the starting compound, substituents are attached to the starting compound. For example, the starting compound can comprise the dilactone moiety, or a precursor thereof. Substituents are added to the starting compound, and can be varied by providing a mixture of reactants comprising the substituents. Examples of suitable substituents include, but are not limited to, the following:

(1) hydrocarbon substituents, that is, aliphatic (*e.g.*, alkyl or alkenyl), alicyclic (*e.g.*, cycloalkyl, cycloalkenyl) substituents, aromatic, aliphatic and alicyclic-substituted aromatic nuclei, and the like, as well as cyclic substituents;

(2) substituted hydrocarbon substituents, that is, those substituents containing nonhydrocarbon radicals which do not alter the predominantly hydrocarbon substituent; those skilled in the art will be aware of such radicals (*e.g.*, halo (especially chloro and fluoro), alkoxy, mercapto, alkylmercapto, nitro, nitroso, sulfoxy, and the like);

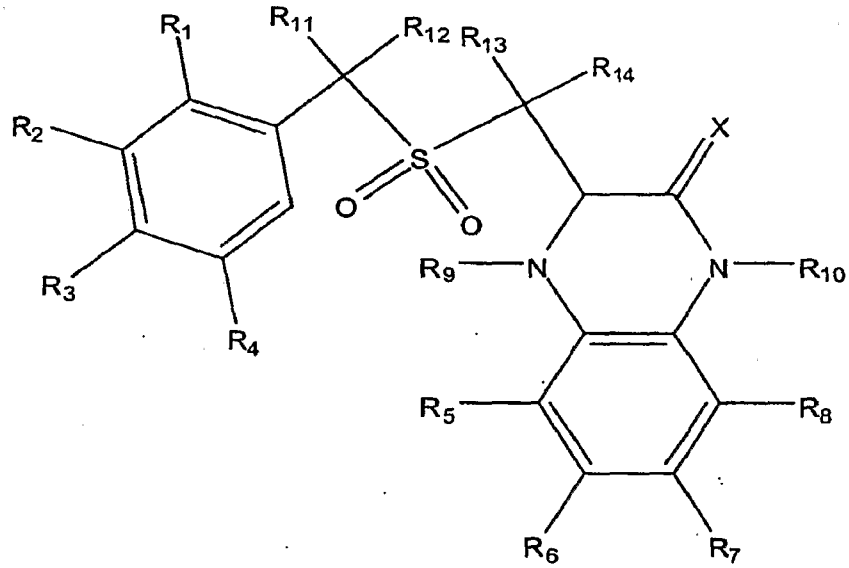
(3) hetero substituents, that is, substituents which will, while having predominantly hydrocarbyl character, contain other than carbon atoms. Suitable heteroatoms will be apparent to those of ordinary skill in the art and include, for example, sulfur, oxygen, nitrogen, and such substituents as pyridyl, furanyl, thiophenyl, imidazolyl, and the like. Heteroatoms, and typically no more than one, will be present for each carbon atom in the hydrocarbon-based substituents. Alternatively, there may be no such radicals or heteroatoms in the hydrocarbon-based substituent and it will, therefore, by purely hydrocarbon.

Methods of making combinatorial libraries are known in the art, and include for example, the following: U.S. Patent Nos. 5,958,792; 5,807,683; 6,004,617; 6,077,954.

Active Compounds

Embodiments of the present invention provide active compounds are provided. In some embodiments, the active compounds may be used to modulate apoptosis in cells that over-express a Bcl-2 family member protein, which may be used to treat apoptosis-associated diseases. Examples of active compounds include compounds of **Formula I** through **Formula XI** as set forth below, and including the pharmaceutically acceptable salts and prodrugs thereof.

Formula I



I

wherein:

X = NR_{17}, O, S;

R_1, R_2, R_3, and R_4 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy and heteroaryl;

R_5, R_6, R_7, and R_8 are independently selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, heteroaryl and $-CO-NR_{15}R_{16}$;

R_9 and R_{10} are independently selected from the group of substituents consisting of: hydrogen, C_{1-8}-alkyl;

R_{11}, R_{12}, R_{13}, and R_{14} are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-heterocycloalkyl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, and C_{1-8}-carbonyloxy;

R_{15} and R_{16} are independently selected from the group consisting of C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-8}-alkoxy, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, and heteroaryl; or

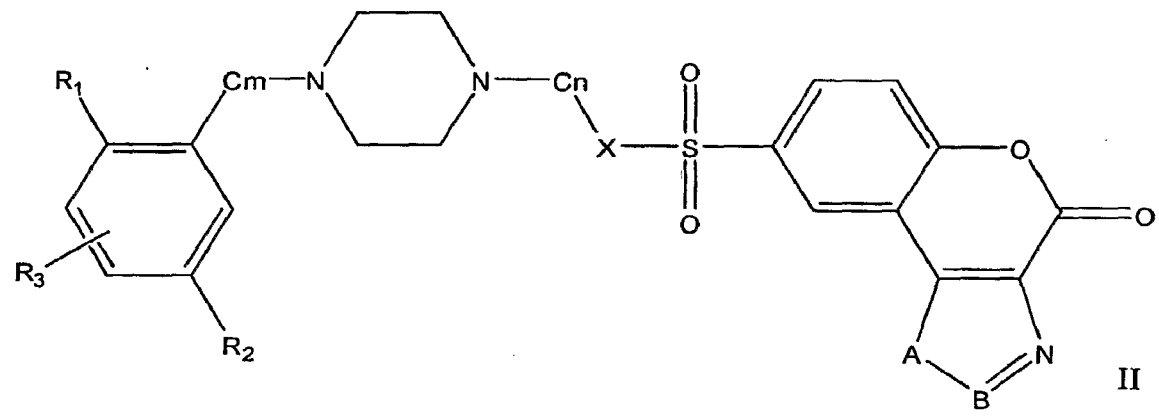
R_{15} and R_{16} together with nitrogen atom to which are attached, form a ring selected from the group consisting of imidazolyl, morpholinyl, piperazinyl, piperidinyl, pyrrolindinyl, pyrrolyl, thiomorpholinyl, phenylpiperazinyl, and pyrindylpiperazinyl;

R_{17} is selected from the group consisting of: hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, and C_{1-10}-alkylsulfanyl; and

pharmaceutically acceptable salts and prodrugs thereof.

An example of compounds of Formula I is compound 18 herein, and Formula I is to be construed as encompassing compound 18 herein.

Formula II



II

wherein:

A = O, S, NR_4; B = C, N; X = NH, O, S, or is absent; m, n = 0-8;

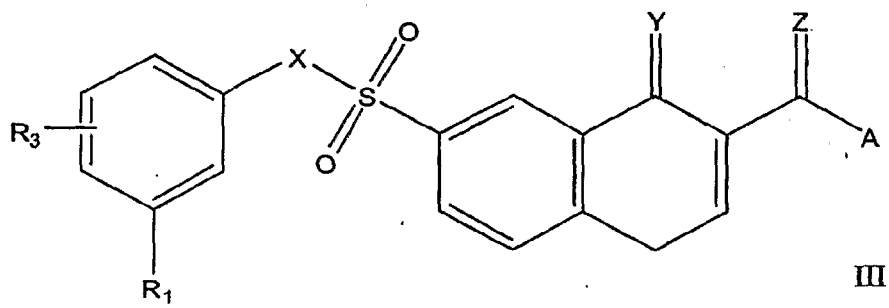
R_1, R_2 and R_3 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy and heteroaryl;

R_4 is selected from the group consisting of hydrogen, cyano, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, and aryl; and

pharmaceutically acceptable salts and prodrugs thereof.

An example of compounds of Formula II herein is compound 16, and Formula II is to be construed as encompassing compound 16 herein.

Formula III



III

wherein:

X = O, S, NR_4; Y, Z = O, S; A = OR_2, NR_2R_5;

R_1 and R_3 are independently selected from the group of substituents consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

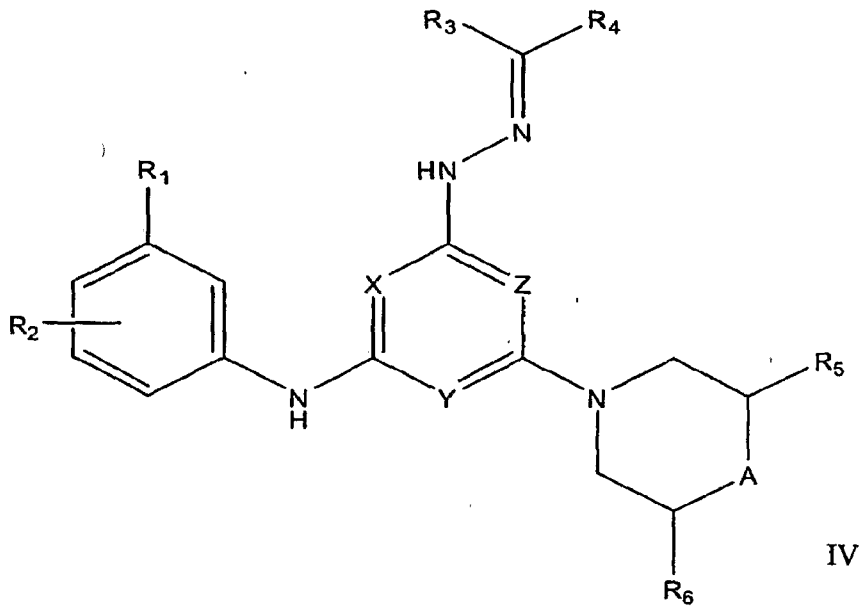
R_2 and R_5 are independently selected from the group consisting of hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, heteroaryl and benzyl-dioxole;

R_4 is selected from the group of substituents consisting of hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; and

pharmaceutically acceptable salts and prodrugs thereof.

An example of compounds of Formula III herein is compound 13, and Formula III is to be construed as encompassing compound 13 herein.

Formula IV



IV

wherein:

X, Y, Z = C, N; A = NH, O, S;

R_1 and R_2 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; or

R_1 and R_2 together form a five- to seven-membered aromatic carbocyclic ring wherein from one to three carbon atoms are replaced by heteroatom – nitrogen, oxygen and sulfur.

R_3 and R_4 are independently selected from the group consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-

heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; or

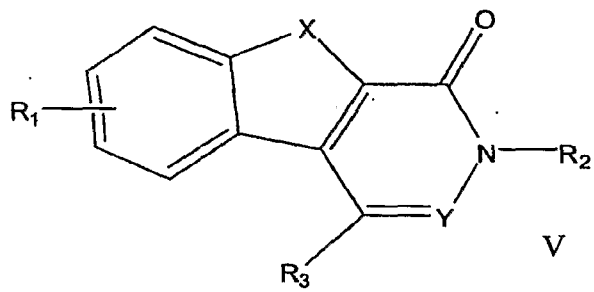
R_3 and R_4 together form an aromatic carbocyclic ring system containing two rings. Each ring contains five- to seven-members wherein from one to three carbon atoms are replaced by heteroatom – nitrogen, oxygen and sulfur. One example is indolin-2-one.

R_5 and R_6 are independently selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, and C_{1-8}-haloalkyl; and

pharmaceutically acceptable salts and prodrugs thereof.

Examples of compounds of Formula IV are compounds 1 and 9 herein, and Formula IV is to be construed as encompassing compounds 1 and 9 herein.

Formula V



V

wherein:

X = O, S, NR_4; Y = C, N;

R_1 and R_3 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

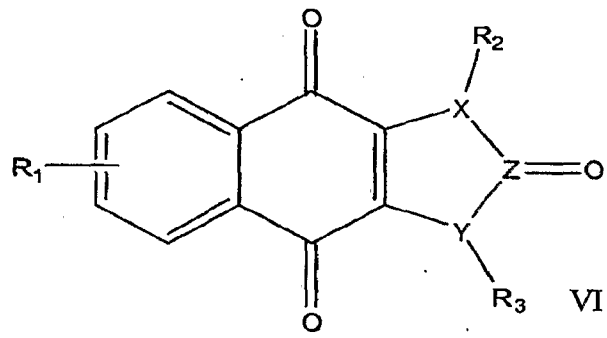
R_2 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

R_4 is selected from the group consisting of: hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy; and

pharmaceutically acceptable salts and prodrugs thereof.

An example of a compound of Formula V is compound 17 herein, and Formula V is to be construed as encompassing compound 17 herein.

Formula VI



VI

wherein:

X, Y, Z = C, N;

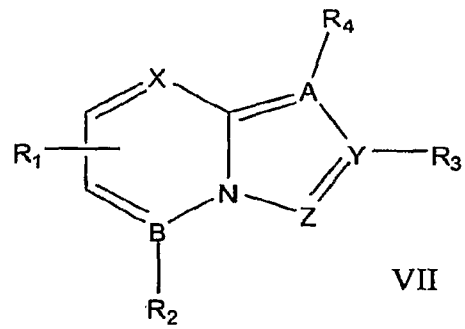
R_1 is selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

R_2 and R_3 are independently selected from the group consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; and

pharmaceutically acceptable salts and prodrugs thereof.

An example of a comound of Formula VI is compound 15 herein, and Formula VI is to be construed as encompassing compound 15 herein.

Formula VII



VII

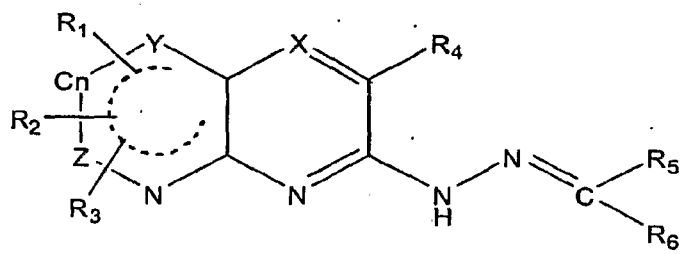
wherein:

X, Y, Z, A, B = C, N;

R_1, R_2 R_3, and R_4 are independently selected from the group of substituents consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; and pharmaceutically acceptable salts and prodrugs thereof.

An example of compounds of formula VII are compounds 2 and 3 herein, and formula VII is to be construed as encompassing compounds 2 and 3 herein.

Formula VIII



VIII

wherein:

X = C, N; Y, Z = O, S, C, N; n = 0-2;

R_1, R_2, and R_3 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

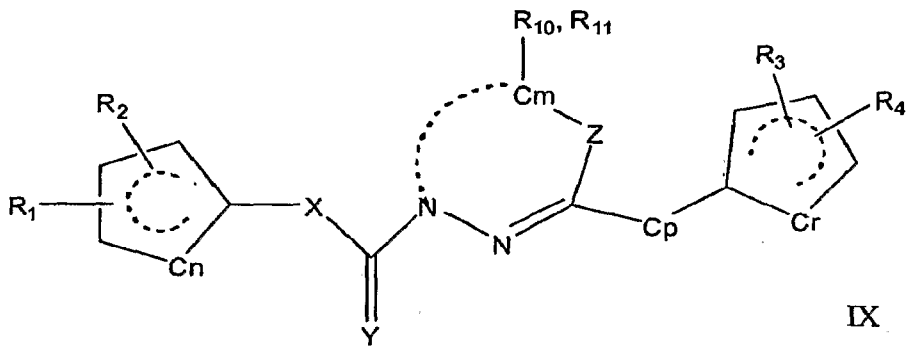
R_4 is selected from the group consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, heteroaryl, C_{1-8}-alkenylene-amino, C_{1-8}-alkynylene-amino, aryl-amino, aryl-C_{1-8}-akyl-amino, aryl-C_{1-8}-akylene-amino, aryl-C_{1-8}-akenylene-amino, aryl-C_{1-8}-akynylene-amino, C_{3-7}-cycloalkyl-amino, heteroaryl-amino, heteroaryl-C_{1-8}-akyl-amino, heteroaryl-C_{1-8}-akylene-amino, heteroaryl-C_{1-8}-akenylene-amino, heteroaryl-C_{1-8}-akynylene-amino, and C_{3-7}-cycloalkyl-amino;

R_5 and R_6 are independently selected from the group of substituents consisting of: hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl; and

pharmaceutically acceptable salts and prodrugs thereof.

Examples of compounds of Formula VIII are compounds 2, 5, 10 and 11 herein, and Formula VIII is to be construed as encompassing compounds 2, 5, 10 and 11 herein.

Formula IX



IX

wherein:

X = Cq , O-Cq, S-Cq; Y = O, S; Z = H, C, N, O, S; m,n,p,q,r = 0-4;

R_1, R_2, R_3 and R_4 are independently selected from the group of substituents consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, heteroaryl, C_{1-8}-alkenylene-

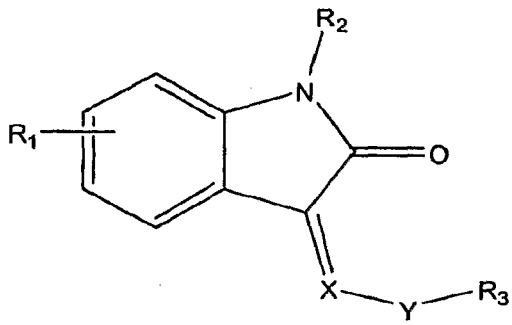
amino, C_{1-8}-alkynylene-amino, aryl-amino, aryl-C_{1-8}-akyl-amino, aryl-C_{1-8}-akylene-amino, aryl-C_{1-8}-akenylene-amino, aryl-C_{1-8}-akynylene-amino, C_{3-7}-cycloalkyl-amino, heteroaryl-amino, heteroaryl-C_{1-8}-akyl-amino, heteroaryl-C_{1-8}-akylene-amino, heteroaryl-C_{1-8}-akenylene-amino, heteroaryl-C_{1-8}-akynylene-amino, and C_{3-7}-cycloalkyl-amino;

R_{10} and R_{11} are independently selected from the group consisting of: hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

and pharmaceutically acceptable salts and prodrugs thereof.

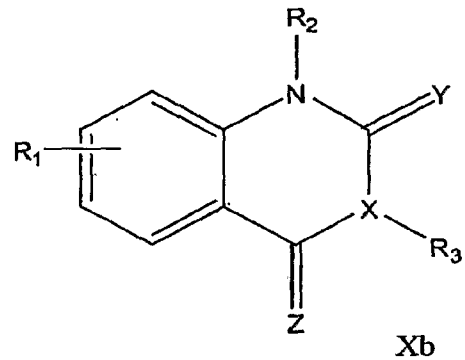
Examples of compounds of Formula IX herein are compounds 2, 6, 7 and 8 herein, and Formula IX is to be construed as encompassing compounds 2, 6, 7 and 8 herein.

Formulae X(a) and X(b)



Xa

or



Xb

wherein:

X = C, N; Y, Z = O, S;

R_1 is selected from the group of substituents consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-10}-alkylsulfonylalkyl, C_{5-10}-heterocyclo-sulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

R_2 is selected from the group of substituents consisting of hydrogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, and heteroaryl;

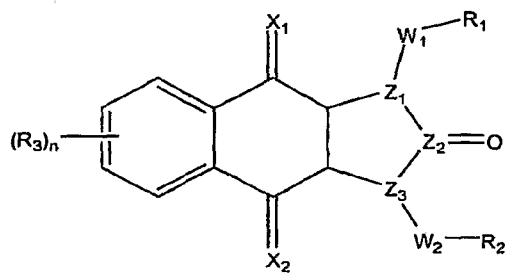
R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-8}-alkyl, C_{1-8}-alkenyl, C_{1-10}-alkoxy, C_{1-10}-alkylsulfanyl, C_{1-8}-haloalkyl, C_{3-7}-cycloalkyl, C_{3-7}-cycloalkyl-C_{1-8}-alkyl, C_{3-7}-heterocycloalkyl, C_{3-7}-heterocycloalkyl-C_{1-8}-alkyl, aryl, C_{6-12}-arylalkoxy, C_{6-12}-aryloxyalkoxy, C_{1-8}-carbonyloxy, C_{3-7}-heterocycle-C_{1-8}-alkoxy, heteroaryl, C_{1-8}-alkenylene-amino, C_{1-8}-alkynylene-amino, aryl-amino, aryl-C_{1-8}-akyl-amino, aryl-C_{1-8}-akylene-amino, aryl-C_{1-8}-akenylene-amino, aryl-C_{1-8}-akynylene-amino, C_{3-7}-cycloalkyl-amino, heteroaryl-amino, heteroaryl-C_{1-8}-akyl-amino, heteroaryl-C_{1-8}-akylene-amino, heteroaryl-C_{1-8}-akenylene-amino, heteroaryl-C_{1-8}-akynylene-amino, and C_{3-7}-cycloalkyl-amino;

and pharmaceutically acceptable salts and prodrugs thereof.

An example of a compound of Formula Xa is compound 4 herein, and Formula Xa is to be construed as encompassing compound 4 herein.

An example of a compound of Formula Xb is compound 12 herein, and Formula Xb is to be construed as encompassing compound 12 herein.

Formula XI



XI

or a pharmaceutically acceptable salt or a prodrug thereof;

wherein:

n is from 0 to 4;

X_1 and X_2 are each independently O or S;

Z_1, Z_2, Z_3 are each independently C or N;

W_1 and W_2 are independently a bond or selected from the group consisting of C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkoxy, C_{1-10} alkylsulfanyl, C_{1-10} haloalkyl, and C_{1-10} aminoalkyl;

R_1 and R_2 are each independently selected from the group consisting of C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-14} heteraryl and C_{3-10} heterocycle;

R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10}alkoxy, C_{1-10}thioalkyl, C_{1-10}haloalkyl, C_{3-10}cycloalkyl, C_{3-10}cycloalkyl-C_{1-10}alkyl, C_{3-10}heterocycle, C_{3-10}heterocycle-C_{1-10}alkyl, C_{3-14} aryl, C_{6-12}aryl-C_{1-10}alkoxy, C_{3-10}heterocycle-C_{1-10}alkoxy and C_{3-14} heteroaryl;

wherein each of the foregoing alkyl, alkenyl, alkoxy, alkylsulfanyl, haloalkyl, aminoalkyl may be branched or unbranched, independently unsubstituted or substituted with one or more substituents selected from the group consisting of hydroxy, cyano, amine, nitro, halogen, mono- or dialkylamino, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl; and

wherein each of the foregoing C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-10} heterocycle, C_{3-14}aryl, C_{3-14} heteroaryl, may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, amino, cyano, nitro, -SH, C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkynyl, C_{1-10} alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl.

In some embodiments, n is 0.

In some embodiments, X_1 and X_2 are O.

In some embodiments, Z_1, Z_2 and Z_3 are N.

In some embodiments, W_1 and W_2 are independently C_{1-10} alkyl or C_{1-10} alkoxy.

In some embodiments, R_1 and R_2 are C_{3-14} aryl or C_{3-10} heterocycle.

In some embodiments, n is 0; X_1 and X_2 are O; Z_1, Z_2 and Z_3 are N; W_1 and W_2 are independently C_{1-6}alkyl or C_{1-6}alkoxyl; R_1 and R_2 are C_{3-14}aryl or C_{3-10}heterocycle;

or pharmaceutically acceptable salts or prodrugs thereof.

An example of a compound of Formula XI is compound 15 herein, and Formula XI is to be construed as encompassing compound 15 herein.

The compounds used in the methods of the invention may be synthesized by any suitable method known to one of skill in the art. See the examples, *infra*, for commercial sources for some of the compounds useful in embodiments of the invention.

Methods of Identifying Compounds that Modulate Apoptosis

Compounds that modulate apoptosis and are substantially non-toxic to cells that do not over-express a Bcl-2 family member may be identified by a method generally comprises the steps of contacting a candidate compound with a cell that over-expresses a Bcl-2 family member protein; contacting the compound with another cell that does not over-express the

Bcl-2 family member protein; and determining whether the compound modulates the activity of the Bcl-2 family member protein to produce a physiological change in the cell that over-expresses the Bcl-2 family member protein, but does not produce a substantial physiological change in the cell which does not over-express that protein. Physiological changes that are indicative of binding of the candidate compound to the Bcl-2 family member protein (*e.g.*, in the hydrophobic pocket) include an affect on cell death, cell shrinkage, chromosome condensation and migration, mitochondria swelling, and/or disruption of mitochondrial transmembrane potential (*i.e.*, the mitochondrial proton gradient), and/or cell death (*e.g.*, as measured by trypan dye exclusion).

Specific assay methods for identifying apoptosis-modulating compounds may be found in PCT Application No. PCT/US00/22891, the content of which is herein incorporated by reference in its entirety. Biologically active compounds can also be identified by evaluating the ability of the agents to modulate glucose uptake and/or lactate production in cells expressing an anti-apoptotic Bcl-2 family member protein. Apoptosis-modulating compounds increase cellular glucose uptake or lactate production in proportion to the level of expression of a Bcl-2 family member target protein. Methods for assaying glucose production or lactate production are well known in the art.

Combinatorial libraries of test compounds can be screened for biological activity using any of the assay methods described above. Combinatorial libraries and processes are described, for example, in PCT/US00/22891. We deposited chemical structures for commercially available small organic molecules from two-dozen chemical vendors in a single screening database. This database consists of over 5.7 million small organic molecules, with the following filters applied: (a) deprotonation of acids and protonatation of bases; (b) omission of compounds with transition metals; (c) omission compounds with 9-membered rings or larger; (d) omission compounds with d-hybridized atoms. Some of the larger chemical vendors that provide these compounds are Asinex (347,725 compounds), ChemBridge (411,532), ChemDiv (493,524 compounds), SPECS (261,875 compounds), Timtec (216,277 compounds), and CNC-2D (4,062,088 compounds).

Computer-based methods may also be used to identify biologically active compounds by using a "molecular docking" algorithm to score test compounds for binding to each of a Bcl-2 family member protein and a corresponding mutant protein as described *supra*. Those test compounds that demonstrate a lower score for binding to the mutant protein relative to the corresponding Bcl-2 family member protein (*e.g.*, a mutant Bcl- x_L protein (having a E92L, F97W, L130A, A142L, F146L, or Y195G mutation) and the wild-

type Bcl- x_L protein, respectively) can be further evaluated in biological assays as described *supra* to verify biological activity.

Computer-based techniques for examining potential ligands (*e.g.*, candidate compounds) for binding to target molecules are well known in the art. (*See, e.g.*, Kuntz *et al.*, *J. Mol. Biol.* 161:269-288, 1982; Kuntz, *Science* 257:1078-1082, 1992; Ewing and Kuntz, *J. Comput. Chem.* 18:1175-1189, 1997). For example, the DOCK suite of programs is designed to find possible orientations of a ligand in a receptor site. *See, e.g.*, Ewing and Kuntz, *supra.* The orientation of the ligand is evaluated with a shape scoring function (an empirical function resembling the van der Waals attractive energy) and/or a function approximating the ligand-receptor binding energy (which is taken to be approximately the sum of the van der Waals and electrostatic interaction energies). After an initial orientation and scoring evaluation, a grid-based rigid body minimization is carried out for the ligand to locate the nearest local energy minimum within the receptor binding site. The position and conformation of each docked molecule can be optimized, for example, using the single anchor search and torsion minimization method of DOCK4.0. (*See, e.g.*, Ewing and Kuntz, *supra*; Kuntz, *supra.*)

In addition, heuristic docking and consensus scoring strategies can be used in the computer-based identification of biologically active compounds (*i.e.*, different docking and scoring methods can be applied to evaluate the screening results). For example, following a primary screening using, *e.g.*, DOCK4.0 (*supra*), top-scoring compounds can be re-scored using other docking algorithms such as, for example, GOLD, FlexX, PM (*see* Muegge and Martin, *J. Med. Chem.* 42:791, 1999, and/or AutoDock3.0 (*see* Morris *et al.*, *J. Comput. Chem.* 19:1639-1662, 1998). Optionally, following a primary and any subsequent screen(s) using individual docking algorithms, a consensus score (Cscore) can be determined by combining results from any of the individual docking programs used to score the candidate compounds (*see* Clark *et al.*, *J. Mol. Graph Model* 20:281-295, 2002). Based on the scoring results from a secondary or other subsequent screen, a subset, for example, of the top-scoring molecules from the primary screen can be selected for further analyses (*e.g.*, a tertiary virtual screen or, alternatively or additionally, biological screening assays such as, for example, any of the assays described herein or otherwise known in the art).

Similarity Searching/ Pharmacophore Mapping

2D database similarity searching may be performed using BITMACCS within MOE, and 2Dph, an in-house 2D-pharmacophore-based program, to search for compounds similar to 2-MeAA1, NSC310343 and troglitazone (setting the threshold for the percentage of

similarity at >65%) from our screening database. The final "hits" of commercially available compounds may then be saved into the separate databases. Anther approach may focus on partial and whole 3D pharmaophore mapping/searching with potential bioisosteric replacements of sub-group(s) of our identified initial hits based on a topological pharmacophore description of the fragments. For examples, the 5-benzylthiazolidine-2, 4-dione moiety of troglitazone can be substituted by various 2-oxy-3-arylcarboxylic acids, and the 9-membered ring of 2-MeAA1 could be replaced by two 5- or 6-merbered rings. The Bioster database (Accelrys, San Diego, CA) can be used to search for the most promising replacements, and focused virtual libraries can be built. This method has been used in the pharmaceutical industry to improve the ADME/Tox properties of initial promising lead compounds and for broadening patentability (40).

Docking

The BH3 peptide-bound Bcl-xL structure, PDB Code: 1BXL and unliganded Bcl-xL structure, PDB Code: 1R2D are used as target receptors. The Flexx 2.0.1 (41) docking program, a fully automatic docking tool for flexible ligands is used to dock the ligands into their binding sites within the receptor structures. All small molecule compounds are prepared by Corina and MOE. After removing all water molecules, the active sites are dissected from the NMR structure of the protein complexes by including all residues that have at least one single (heavy) atom within a distance of 6.5 Å from any heavy atoms of the ligand. Residues are kept fixed in their NMR coordinates in all docking experiments. This docking method consists of three steps: the selection of a part of the molecule, the base fragment, the placement of the base fragment into the active site of a protein, and the subsequent reconstruction of the complete drug molecule by linking the remaining components step by step. For placing base fragments, two algorithms are in use. The first one superposes triples of interaction centers of a base fragment with triples of compatible interaction points in the active site. If a base fragment has fewer than three interaction centers or if the number of placements is too low, the second algorithm, called line matching, is started. This algorithm matches pairs of interaction centers with pairs of interaction points. Because of geometric ambiguity, multiple placements are generated by rotation around the axis defined by the interaction points and centers. Both base placement algorithms generate a large number of solutions. Sampling is done with 400 solutions per partial solution at each iteration of incremental construction. A reduction by clash tests and clustering follows. All FlexX parameters are set to their default standard values, and only the 10 best scored poses by

FlexX are used for further ranking. The final ranking of docking results is performed with an in-house novel scoring-function, VMScore (see below).

Novel score-function, VMScore, in target structure-based modeling and prediction.

The docking-score is the key component in structure-based drug design. The score-function is used for estimating the binding affinity of the ligand with docked conformation within the binding packet. It will help to decide which compound(s) should be selected for experimental development. Score-functions which derived directly from molecular mechanics are not often used in practice largely due to the time-consuming nature of the process and high demand for computational power for a large set of molecules. In the past years, several (semi) empirically based score-functions have been used in the ranking of docked structures. "Score" (30) and "Chemscore" (31) use contributions from hydrogen bonding, metal-ligand contacts, lipophilic contact area and frozen rotatable bonds. The "HINT Score" use contributions representing different types of surface areas (32). Score-functions based on the probability of pair-wise contact of heavy atoms such as "PMF" (potential of mean force) (33) and "Smog" (34) have reported. However, these score-functions do not consider all possible energy contributions for binding. For examples, "Score" and "Chemscore" do not consider the contribution of the restricted conformation of the bound ligand compared to the free molecule; "PMF" and "Smog" do not consider contributions from hydrogen bonding. In addition, most of the current score-functions do not take desolvation energy into account.

We have developed a novel score-function –"VMScore" by a method of considering all possible energy contributions including those from electrostatic, van de waal's, hydrogen bonding, conformation change, desolvation and entropy. It is given as,

$$\Delta G = A^*\Delta G_{ele} + B^*\Delta G_{vdw} + C^*\Delta G_{hb} + D^*\Delta G_{conf} + E^*\Delta G_{desol} + F^*\Delta G_{ent}$$

Where, ΔG_{ele} is free energy contribution from electrostatic interaction. ΔG_{vdw} is free energy contribution from van de waal's interaction. ΔG_{hb} is free energy contribution from hydrogen bonding, ΔG_{conf} is free energy contribution from conformation change, ΔG_{desol} is free energy contribution from desolvation energy difference between ligand, protein and their complex, ΔG_{ent} is entropy contribution from translation and rotation, and A to F are coefficients.

To test and validate our new score-function, "VMScore", we have taken a well-described data set containing 53 complex (protein bound with compound) crystal structures with experimental binding affinity (Ki) data ($\Delta G \propto \log Ki$) as training set (32). Our results, shown in **Figure1**, Left, fit the experimental data with a squared correlation coefficient, $R^2 = 0.77$, and with LOO (leave-one-out) cross-validation, $Q^2 = 0.70$.. These results are much better than the reported "HINT Score" with $R^2 = 0.54$ for the same training set.

To further validate the predictive ability of "VMScore", we have tested one additional diverse dataset from public sources, i.e. Endothiapepsin complex (11 data). The small molecules in this data set contain multiple rotatable bonds, and both hydrogen bond donors and acceptors and are thus very flexible. This dataset has been widely used as testing set for currently available score-functions. The prediction results based on currently available score-functions are listed in Table-4. None of these (squared) correlation coefficients is larger than 0.3. The poor predictions mainly derive from lack of consideration of either conformation change and/or hydrogen bonding. By using our "VMScore" score-function, a (squared) correlation coefficient, $R^2 = 0.62$ has been achieved for the same dataset (Fig.10, Right and Table-4), which represents a significant improvement over the current available score-functions.

Table-4. The prediction results of different docking score-functions for the same Endothiapepsin complex testing set.

	"Score"	"ChemScore"	"DrugScore"	"PMF"	"Smog1996"	"Smog2000"	**"VMScore"**
R^2	0.39	-0.30	0.30	0.22	0.05	0.19	**0.62**
Ref.	(30)	(31)	(35)	(33)	(34)	(34)	-

Methods of Using the Apoptosis-Modulating Compounds

The compounds of the present invention are useful for treating cells in which the cell death signal is down-regulated and the affected cell has an inappropriately diminished propensity for cell death, which is referred to herein as being in a "decreased apoptotic state." The invention further provides methods for the administration to a subject of a therapeutically effective amount of an apoptosis-modulating compound of the invention to treat an ap optosis-associated disease in which it is desirable to induce apoptosis in certain types of cells, such as virus-infected or autoantibody-expressing cells.

In a specific embodiment, a method of treating a cancer characterized by the over-expression of a Bcl-2 family member is provided. Examples of cancers known to be associated with over-expression of a Bcl-2 family member and which can be treated according to the methods provided herein are shown in Table 4.

Table 4. Percentages of common human cancers with elevated levels of Bcl-2 or Bcl-x_L expression

Tumor	Bcl-2	Bcl-x_L
Lymphoma	Hodgkin's - 47-65% NHL - 9-57%	Hodgkin's - 48-94% NHL - 25-45%
Leukemia	AML - 13-20% ALL - 89-92% CML - 33-54% CLL - 70-95%	AML - 38% ALL - 13%
Myeloma	43%	29%
Lung	NSCLC-squamous - 25% adenoca - 12% SCLC - 83-90%	Most NSCLC, SCLC
Colorectal	Adenoma - 65-98% Carcinoma - 46-60%	Adenoma - 50% Carcinoma - 60%
Breast	80%	43-75%
Pancreas	23%	88%
Urogenital	Bladder - 24% Renal - 53%	Bladder - 80.9% Renal - 38%
Liver	Rare	95+%
Ovary	30-39%	62%
Brain	Medulloblastoma - 5-25% Glioma - 28-92% Oligodendroglioma -16-60%	Medulloblastoma - 56% Glioma - 98% Oligodendroglioma - <5%
Esophagus	SCC - 45% Adenocarcinoma - 20-40%	Adenocarcinoma - 90%

In some cases, the treatment of the cancer can include the treatment of solid tumors or the treatment of leukemias. For example, the cancer can be of the skin, breast, brain, cervix, testis, and the like. More particularly, cancers may include, but are not limited to, the following organs or systems: cardiac, lung, gastrointestinal, genitourinary tract, liver, bone, nervous system, gynecological, hematologic, skin, and adrenal glands. More particularly, the methods herein can be used for treating gliomas (Schwannoma, glioblastoma, astrocytoma), neuroblastoma, pheochromocytoma, paraganlioma, meningioma, adrenal cortical carcinoma, kidney cancer, vascular cancer of various types, osteoblastic osteocarcoma, prostate cancer, ovarian cancer, uterine leiomyomas, salivary gland cancer, choroid plexus carcinoma,

mammary cancer, pancreatic cancer, colon cancer, B and T cell lymphomas, acute and chronic myeloid or lymphoid leukemias, and multiple myeloma. Further, treatment may include pre-malignant conditions associated with any of the above cancers (*e.g.*, colon adenomas, myelodysplastic syndrome).

In other embodiments, methods of treating a neurodegenerative disease characterized by the over-expression of a Bcl-2 family member are provided. Neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis and other diseases linked to degeneration of the brain, such as Creutzfeldt-Jakob disease and expanded polyglutamine repeat diseases. Expanded polyglutamine repeat diseases with which the present invention is concerned include, but are not limited to, Huntington's disease, dentatorubral pallidoluysian atrophy, spinobulbar muscular atrophy, and spinocerebellar ataxia types 1, 2, 3, 6 and 7. *See, e.g.*, US Patent No. 6,632,616 to Burke et al.

In other embodiments, methods of treating arthritis, inflammation, autoimmune diseases, human immunodeficiency virus (HIV) immunodeficiency syndrome, myelodysplastic syndromes (such as aplastic anemia), ischaemic syndromes (such as myocardial infarction), liver diseases which are induced by toxins (such as alcohol), alopecia, damage to the skin due to UV light, lichen planus, atrophy of the skin, cataract, and graft rejections are provided.

Typically, the compounds used in embodiments of the invention will be substantially purified prior to administration. The subject can be an animal, including, but not limited to, cows, pigs, horses, chickens, cats, dogs, and the like, and is typically a mammal, and in a particular embodiment human. In another specific embodiment, a non-human mammal is the subject.

Various delivery systems are known and can be used to administer a compound of the invention, such as, for example, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of producing the derivative, receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, *J. Biol. Chem.* 262:4429-4432, 1987), and the like. The apoptosis-modulating compounds are administered as therapeutic or pharmaceutical compositions by any suitable route known to the skilled artisan including, for example, intravenous, subcutaneous, intramuscular, intradermal, transdermal, intrathecal, intracerebral, intraperitoneal, intransal, epidural, and oral routes. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulations. For treating tissues in the central nervous system, administration can be by

injection or infusion into the cerebrospinal fluid (CSF). When it is intended that a compound be administered to cells in the central nervous system, administration can be with one or more other components capable of promoting penetration of the derivative across the blood-brain barrier. In addition, it can be desirable to introduce a compound into the target tissue by any suitable route, including intravenous and intrathecal injection. Pulmonary administration can also be employed, such as, for example, by use of an inhaler or nebulizer, and formulation of the compound with an aerosolizing agent. In certain embodiments, the compound is co-administered with an inhibitor of esterase activity to further stabilize the compound.

Pharmaceutical compositions can also be administered orally in any orally acceptable dosage form including, but not limited to, capsules, tablets, caplets, lozenges, aqueous suspensions or solutions. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating aids, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required, the agent can be combined with emulsifying and suspending aids. If desired, certain sweeteners, flavorants, or colorants can also be used.

Further, the compounds of the present invention can be combined with any other tumor and/or cancer therapy. The therapy can include, for example and not by way of limitation, surgery, radiation, and chemotherapy either individually or in any combination. Chemotherapy can include any current known or yet to be discovered chemotherapeutic agent including but are not limited to Aceglatone; Aclarubicin; Altretamine; Aminoglutethimide; 5-Aminogleavulinic Acid; Amsacrine; Anastrozole; Ancitabine Hydrochloride; 17-1A Antibody; Antilymphocyte Immunoglobulins; Antineoplaston A10; Asparaginase; Pegaspargase; Azacitidine; Azathioprine; Batimastat; Benzoporphyrin Derivative; Bicalutamide; Bisantrene Hydrochloride; Bleomycin Sulphate; Brequinar Sodium; Broxuridine; Busulphan; Campath-IH; Caracemide; Carbetimer; Carboplatin; Carboquone; Carmofur; Carmustine; Chlorambucil; Chlorozotocin; Chromomycin; Cisplatin; Cladribine; Corynebacterium parvum; Cyclophosphamide; Cyclosporin; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Diaziquone; Dichlorodiethylsulphide; Didemnin B.; Docetaxel; Doxifluridine; Doxorubicin Hychloride; Droloxifene; Echinomycin; Edatrexate; Elliptinium; Elmustine; Enloplatin; Enocitabine; Epirubicin Hydrochloride; Estramustine Sodium Phosphate; Etanidazole; Ethoglucid; Etoposide; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flutamide; Formestane; Fotemustine; Gallium Nitrate; Gencitabine;

Gusperimus; Homoharringtonine; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Improsulfan Tosylate; Inolimomab; Interleukin-2; Irinotecan; JM-216; Letrozole; Lithium Gamolenate; Lobaplatin; Lomustine; Lonidamine; Mafosfamide; Meiphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Miboplatin; Miltefosine; Misonidazole; Mitobronitol; Mitoguazone Dihydrochioride; Mitolactol; Mitomycin; Mitotane; Mitozanetrone Hydrochloride; Mizoribine; Mopidamol; Muitlaichilpeptide; Muromonab-CD3; Mustine Hydrochloride; Mycophenolic Acid; Mycophenolate Mofetil; Nedaplatin; Nilutamide; Nimustine Hydrochloride; Oxaliplatin; Paclitaxel; PCNU; Penostatin; Peplomycin Sulphate; Pipobroman; Pirarubicin; Piritrexim Isethionate; Piroxantrone Hydrochloride; Plicamycin; porfimer Sodium; Prednimustine; Procarbazine Hydrochloride; Raltitrexed; Ranimustine; Razoxane; Rogletimide; Roquinimex; Sebriplatin; Semustine; Sirolimus; Sizofiran; Sobuzoxane; Sodium Bromebrate; Sparfosic Acid; Sparfosate Sodium; Sreptozocin; Sulofenur; Tacrolimus; Tamoxifen; Tegafur; Teloxantrone Hydrochloride; Temozolomide; Teniposide; Testolactone; Tetrasodium Mesotetraphenylporphine-sulphonate; Thioguanine; Thioinosine; Thiotepa; Topotecan; Toremifene; Treosulfan; Trimetrexate; Trofosfamide; Tumor Necrosis Factor; Ubenimex; Uramustine; Vinblastine Sulphate; Vincristine Sulphate; Vindesine Sulphate; Vinorelbine Tartrate; Vorozole; Zinostatin; Zolimomab Aritox; and Zorubicin Hydrochloride, and the like, either individually or in any combination. *See, e.g.*, US Patent No. 7,071,158.

In some embodiments, the compounds of the present invention can be administered locally to the area in need of treatment; this administration can be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (*e.g.*, in conjunction with a wound dressing after surgery), by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes such as silastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the compounds of the invention can be delivered in a vesicle, in particular a liposome (*see, e.g.*, Langer, *Science* 249:1527-1533, 1990; Treat *et al.*, In *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365, 1989; Lopez-Berestein, *supra*, pp. 317-327).

In yet another embodiment, the compounds of the invention can be delivered in a controlled release system. In one embodiment, a pump can be used (*see, e.g.*, Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201, 1987; Buchwald *et al.*, *Surgery* 88:507, 1980;

Saudek *et al.*, *N. Engl. J. Med.* 321:574, 1989). In another embodiment, polymeric materials can be used (*see, e.g.*, *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida, 1974; *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61, 1983; *see also* Levy *et al.*, *Science* 228:190, 1985; During *et al.*, *Ann. Neurol.* 25:351, 1989; Howard *et al.*, *J. Neurosurg.* 71:105, 1989). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, *Medical Applications of Controlled Release*, *supra*, Vol. 2, pp. 115-138, 1984). Other controlled release systems are discussed in, for example, the review by Langer (*Science* 249:1527-1533, 1990).

The present invention also provides pharmaceutical compositions. Such compositions comprise a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of the invention. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more typically in humans. The term "carrier" refers to a diluent, adjuvant, excipient, stabilizer, vehicle, or any combination thereof, with which the agent is formulated for administration. Pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water is a typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Pharmaceutical compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. In addition, in certain embodiments, the pharmaceutical composition includes an inhibiter of esterase activity as a stabilizing agent.

Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium

carbonate, and the like. Examples of suitable pharmaceutical carriers are described in, for example, *Remington's Pharmaceutical Sciences,* by E.W. Martin. Such compositions will contain a therapeutically effective amount of a compound of the invention, typically in purified form, together with a suitable amount of carrier so as to provide a formulation proper for administration to the subject. The formulation should suit the mode of administration.

In one embodiment, the compound of the present invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, pharmaceutical compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition can also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form. For example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. A "pharmaceutically acceptable salt" as used herein refers to a salt form of a compound permitting its use or formulation as a pharmaceutical and which retains the biological effectiveness of the free acid and base of the specified compound and that is not biologically or otherwise undesirable. Examples of such salts are described in *Handbook of Pharmaceutical Salts: Properties, Selection, and Use,* Wermuth, C.G. and Stahl, P.H. (eds.), Wiley-Verlag Helvetica Acta, Zürich, 2002 [ISBN 3-906390-26-8]. Examples of pharmaceutically acceptable salts, without limitation, include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, and the like, and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine and the like. Examples of salts also include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogen phosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates,

methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ-hydroxybutyrates, glycollates, tartrates, methanesulfonates, ethane sulfonates, propanesulfonates, toluenesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates. In some embodiments, pharmaceutically acceptable salt includes sodium, potassium, calcium, ammonium, trialkylarylammonium and tetraalkylammonium salts.

Furthermore, "pharmaceutically acceptable prodrugs" of the compounds may be used in embodiments of the invention. Pharmaceutically acceptable prodrugs as used herein refers to those prodrugs of the active compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, commensurate with a reasonable risk/benefit ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formula, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Prodrugs as Novel delivery Systems, Vol. 14 of the A.C.S. Symposium Series and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated by reference herein. *See also* US Patent No. 6,680,299. Examples include a prodrug that is metabolized in vivo by a subject to an active drug having an activity of active compounds as described herein, wherein the prodrug is an ester of an alcohol or carboxylic acid group, if such a group is present in the compound; an acetal or ketal of an alcohol group, if such a group is present in the compound; an N-Mannich base or an imine of an amine group, if such a group is present in the compound; or a Schiff base, oxime, acetal, enol ester, oxazolidine, or thiazolidine of a carbonyl group, if such a group is present in the compound, such as described in US Patent No. 6,680,324 and US Patent No. 6,680,322.

The amount of the compound of the invention that is combined with the carrier to produce a single dosage form will vary, depending upon the nature of that agent and the composition of the dosage form. It should be understood, however, that a specific dosage and treatment regime for any particular patient or disease state will depend upon a variety of factors, including the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease being treated. The amount of active agent will also depend upon the specific activity of the compound and whether that agent is co-administered with any other

therapeutic or prophylactic ingredients. Determination of therapeutically effective dosages is typically based on animal model studies and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the apoptosis-associated disease in model subjects (*e.g.*, in the case of treatment of malignancies, a tumor xenograft model in mice can be used (*see, e.g.*, Example 20). For treatment of human subjects, such animal model studies are typically followed up by human clinical trials. A non-limiting range for a therapeutically effective amount of the compounds is about 0.001 mg/kg and about 100 mg/kg body weight per day, and in more specific embodiments between about 0.001 mg/kg and about 50 mg/kg, between about 0.01 mg/kg and about 20 mg/kg. between about 0.1 and about 10 mg/kg, or between about 0.1 mg/kg and about 5 mg/kg body weight per day.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

The following examples are provided merely as illustrative of various aspects of the invention and shall not be construed to limit the invention in any way.

EXAMPLES

Example 1

The selectivity and IC_{50} for the following compounds was determined (in Example 2):

1: 3,4-Dibromo-6-ethoxy-2-[(4-morpholin-4-yl-6-phenylamino-[1,3,5]triazin-2-yl)-hydrazonomethyl]-phenol (ChemDiv., Inc., 0927-0006)

2: 3-Benzotriazol-1-yl-propionic acid (3-allyl-2-hydroxy-benzylidene)-hydrazide (ChemDiv., Inc., 1761-1783)

3: 3-Chloro-5-phenyl-7-trifluoromethyl-pyrazolo[1,5-a]pyrimidine-2-carboxylic acid (3-hydroxy-5-nitro-phenyl)-amide (ChemDiv., Inc., 1762-0212)

4: 2,3-Dihydro-benzo[1,4]dioxine-2-carboxylic acid (2-oxo-1-phenethyl-1,2-dihydro-indol-3-ylidene-hydrazinocarbonylmethyl)-amide (ChemDiv., Inc., 1805-1390)

5: 5-Naphthalen-2-yl-2H-pyrazole-3-carboxylic acid [3-(2-nitro-phenyl)-allylidene]-hydrazide (ChemDiv., Inc., 3254-3290)

6: 1-[5-Hydroxy-3-(3-methoxy-phenyl)-5-trifluoromethyl-4,5-dihydro-pyrazol-1-yl]-3-phenyl-propan-1-one (ChemDiv., Inc., 3852-0615)

7: 3-[2-(2-Methoxy-phenyl)-ethylamino]-1-(4-phenylazo-phenyl)-pyrrolidine-2,5-dione (ChemDiv., Inc., 4295-0309)

8: 3-Fluoro-benzoic acid 2,4-dibromo-6-{[2-(2-cyano-phenoxy)-acetyl]-hydrazonomethyl}-phenyl ester (ChemDiv., Inc., 8005-7568)

9: 4-Methyl-3-{4-morpholin-4-yl-6-[N'-(2-nitro-benzylidene)-hydrazino]-[1,3,5]triazin-2-ylamino}-phenol (ChemDiv., Inc., 8008-5380)

10: {6-[N'-(3-Bromo-4-methoxy-benzylidene)-hydrazino]-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl}-(4-iodo-phenyl)-amine (ChemDiv., Inc., 8008-5406)

11: 2-Oxy-1-[3-(4-pyridin-2-yl-piperazin-1-yl)-propyl]-1H-naphtho[2,3-d][1,2,3]triazole-4,9-dione (ChemDiv., Inc., 8017-6394)

12: 1-Methyl-4-oxo-1,4-dihydro-chromeno[3,4-d]imidazole-8-sulfonic acid {3-[4-(2,5-dimethyl-phenyl)-piperazin-1-yl]-propyl}-amide (ChemDiv., Inc., E783-0144)

13: 2-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-2-oxo-ethyl}-6-methyl-2,9-dihydro-2,3,9-triaza-fluoren-1-one (ChemDiv., Inc., E902-033)

14: 5,7-Dichloro-2-[N'-(2,3,4-trimethoxy-benzylidene)-hydrazino]-quinolin-8-ol (ChemDiv., Inc., KD72-0095)

15: 3-Benzo[1,3]dioxol-5-ylmethyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-quinazoline-7-carboxylic acid [4-(2-diethylamino-ethylcarbamoyl)-phenyl]-amide (ChemDiv., Inc., K288-0948)

16: 4-Oxo-6-(3-trifluoromethyl-phenylsulfamoyl)-1,4-dihydro-quinoline-3-carboxylic acid (benzo[1,3]dioxol-5-ylmethyl)-amide (ChemDiv., Inc., K788-0964)

17: 4-Oxo-6-(3-trifluoromethyl-phenylsulfamoyl)-1,4-dihydro-quinoline-3-carboxylic acid [1-(2,3-dihydro-benzo[1,4]dioxin-6-yl)-ethyl]-amide (ChemDiv., Inc., K788-0981)

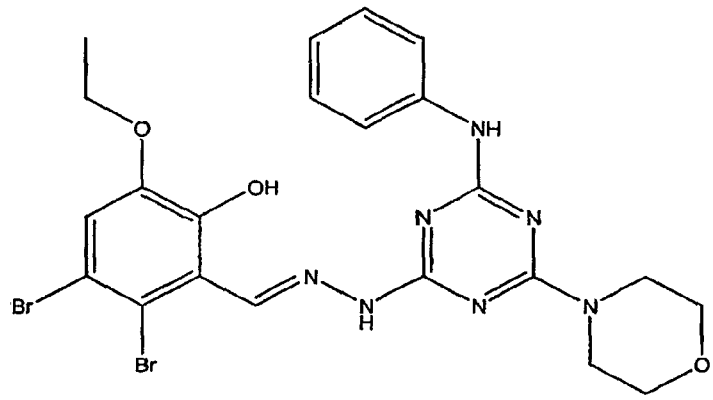
18: 2-(2,5-Dimethyl-phenylmethanesulfonylmethyl)-3-oxo-1,2,3,4-tetrahydro-quinoxaline-6-carboxylic acid (2-ethyl-hexyl)-amide (ChemDiv., Inc., K977-0410)

19: 1-(3-Morpholin-4-yl-propyl)-2,4,9-trioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium (ChemDiv., Inc.,)

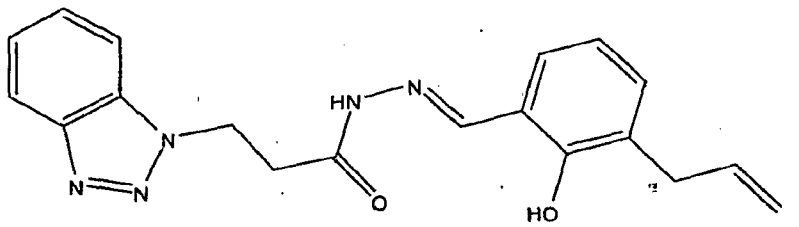
20: 1-[2-(Hydroxy-phenyl-methoxy)-ethyl]-2,4,9-trioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium (ChemDiv., Inc.,)

21: 1-[3-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-propyl]-2,9-dioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium (ChemDiv., Inc.,)

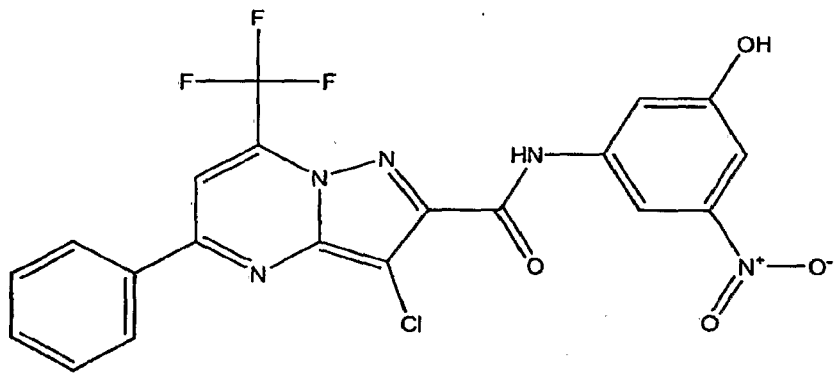
The structures for the compounds described above are provided below.



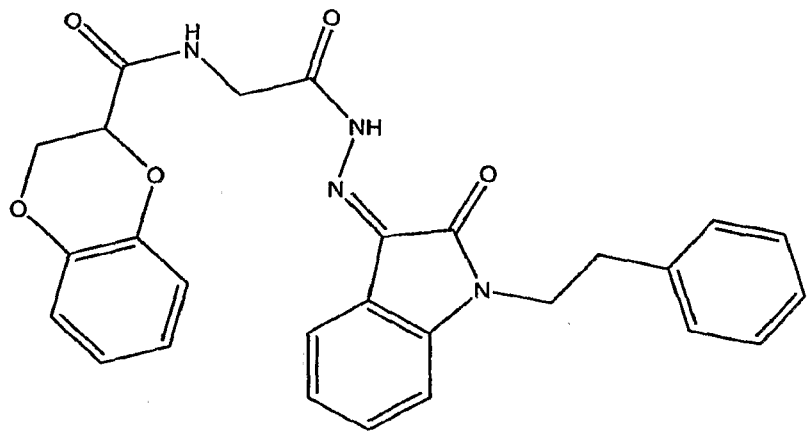
1



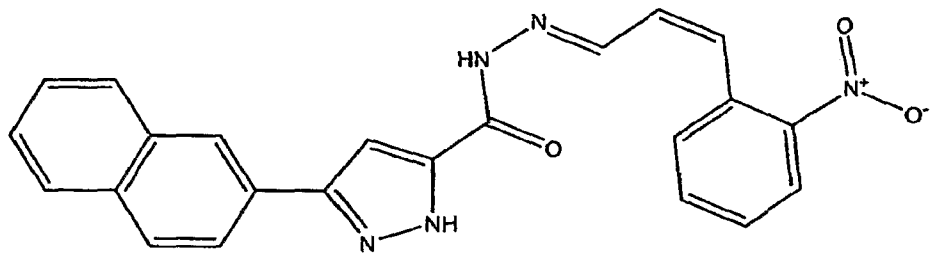
2



3

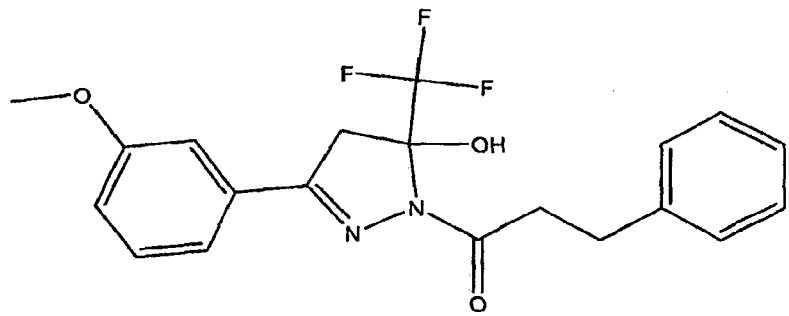


4

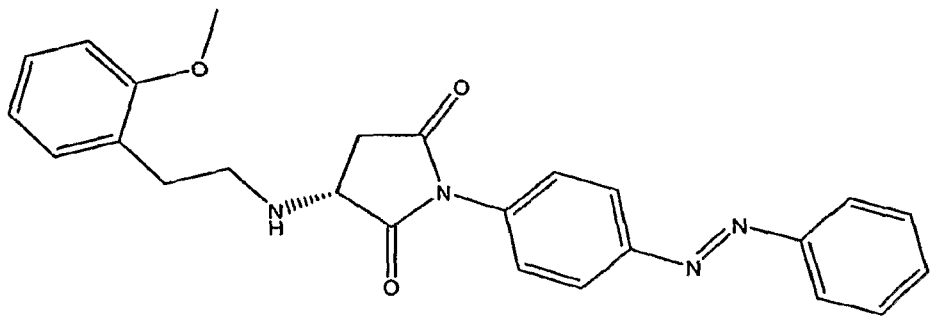


5

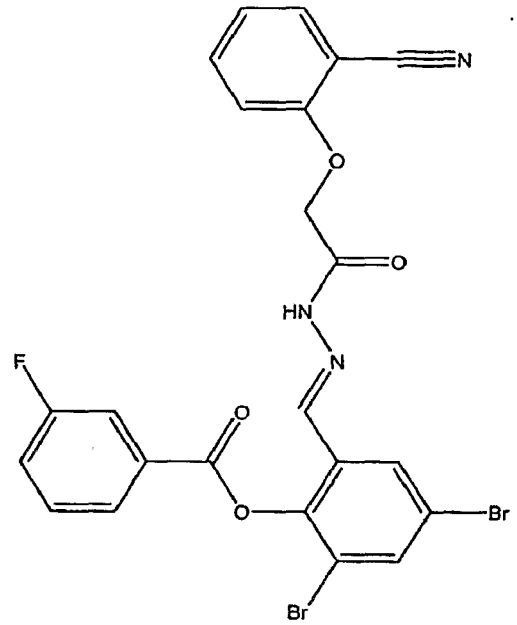
5



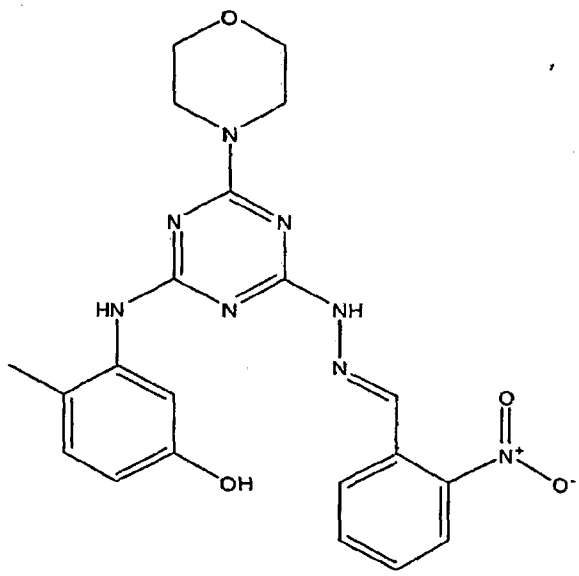
6



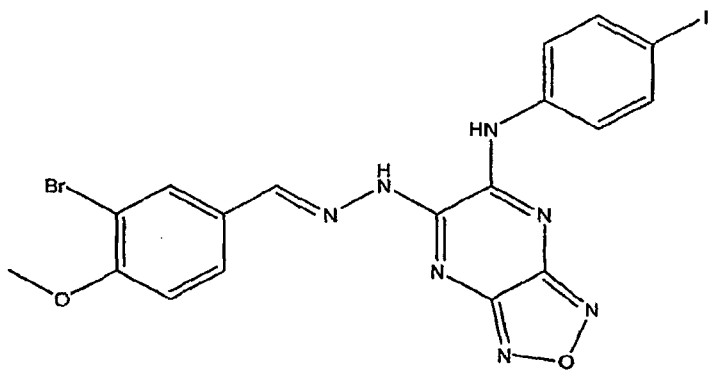
7



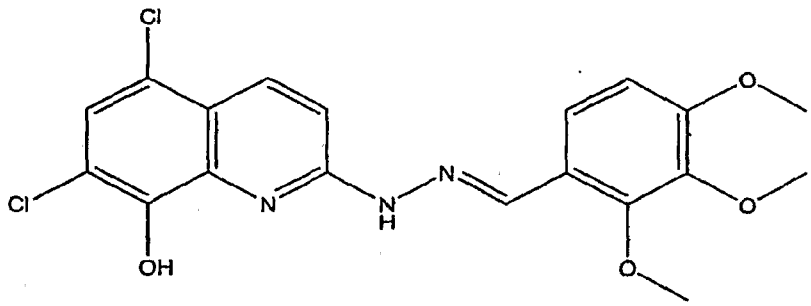
8



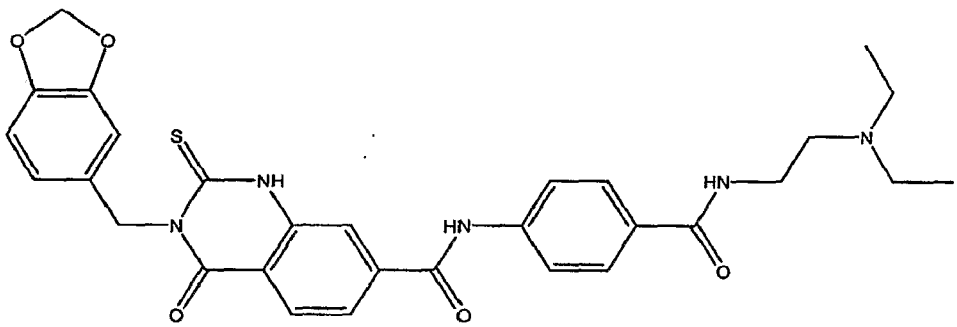
9



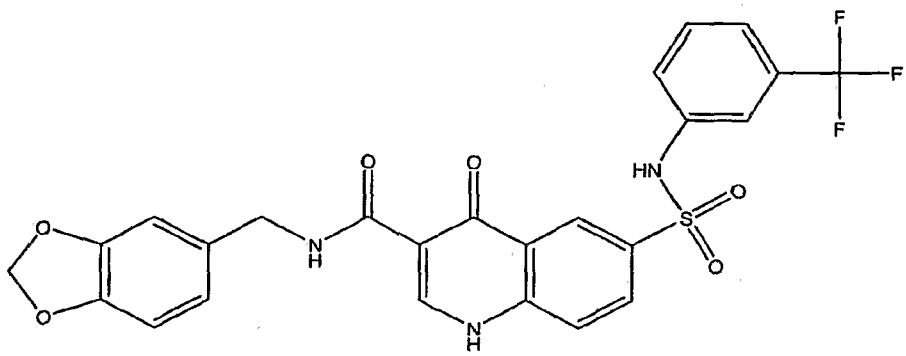
10



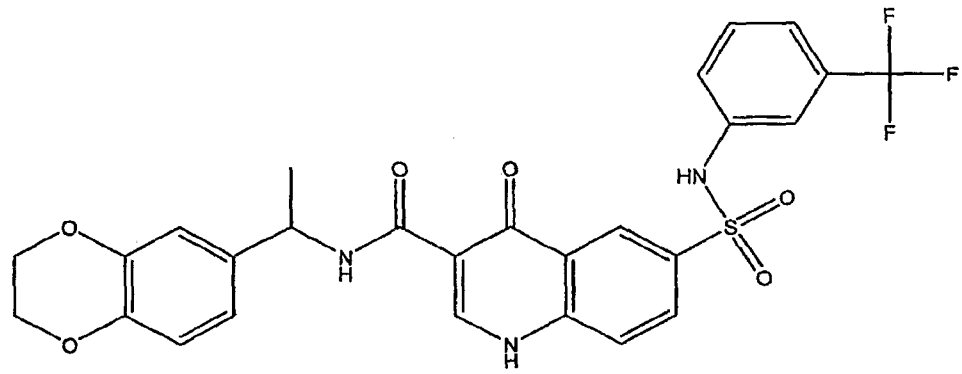
11



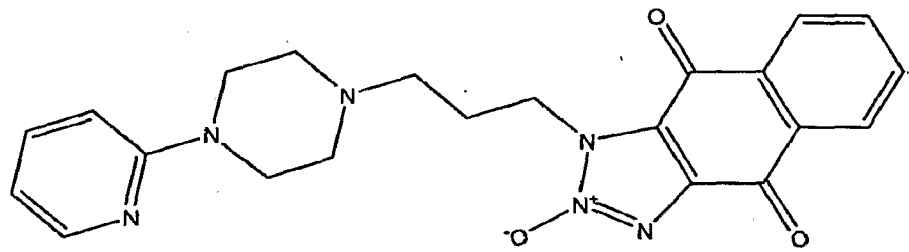
12



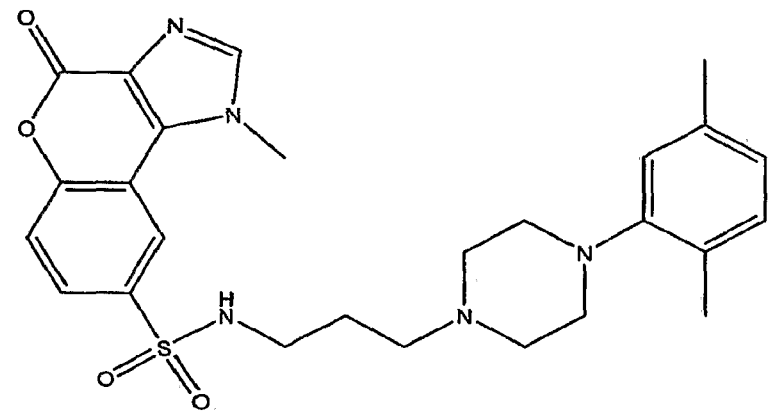
13



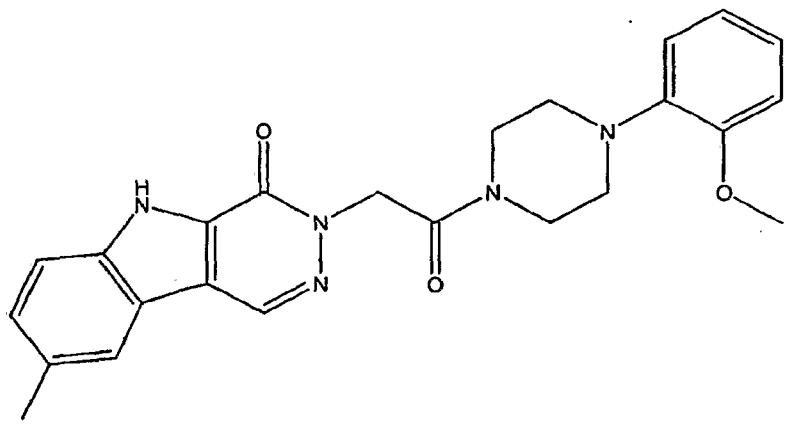
14



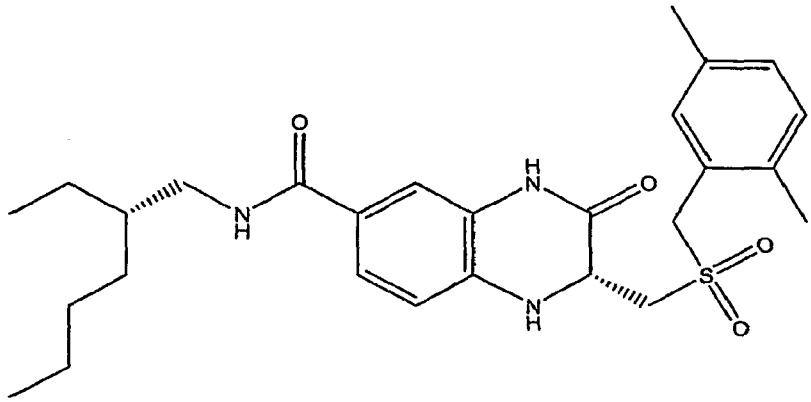
15



16



17



18

Example 2

The effect of the compounds described in Example 1 on TABX2S cells (cells over-expressing Bcl-X_L) and TAMH.neo (control) cells are summarized below in Table 5. Table 5 includes dose dependence of TABX2S cell survival (IC_{50}) and the selective induction of apoptosis in TABX2S cells over the TAMH.neo control.

Table 5

Compound	IC50 (TABX2S)	Selectivity (IC50_TABX2S/IC50_Neo)
1	15	1.3
2	12	3.4
3	13.5	1.8
4	20	>20
5	19	1.7
6	15	7.5
7	56	3.3
8	16	2.1
9	5	3.4
10	19	2.7
11	0.3	2.7
12	21	7.9
13	47	3.5
14	12	2.4
15	24	2.9
16	9	4.7
17	9	4.2
18	7	6.6

Example 3

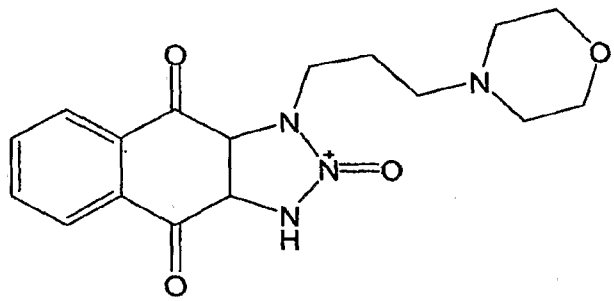
The selectivity and EC_{50} for the following compounds were determined (in Example 4):

19: 1-(3-Morpholin-4-yl-propyl)-2,4,9-trioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium

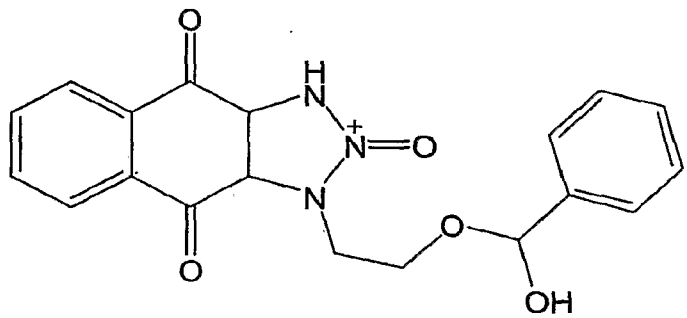
20: 1-[2-(Hydroxy-phenyl-methoxy)-ethyl]-2,4,9-trioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium

21: 1-[3-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-propyl]-2,9-dioxo-2,3,3a,4,9,9a-hexahydro-1H-naphtho[2,3-d][1,2,3]triazol-2-ium

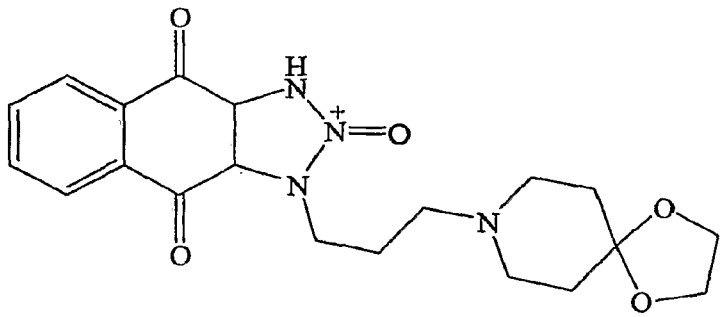
The structures for the compounds described above are provided below.



19



20



21

Example 4

The effect of the compounds described in Example 3 on TABX2S cells (cells over-expressing Bcl-x_L) and TAMH.neo (control) cells are summarized below in Table 6. Table 6 includes dose dependence of TABX2S cell survival (EC_{50}) and the selective induction of apoptosis in TABX2S cells over the TAMH.neo control.

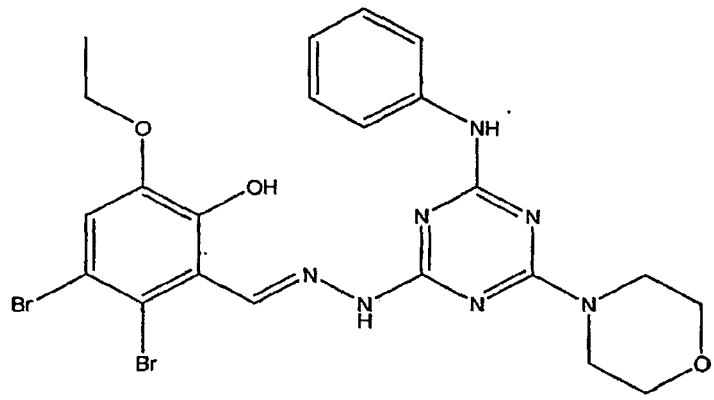
Table 6

	2S (EC50, μm)	Neo (EC50, μm)	Selectivity (EC50_ Neo/EC50_TABX2S 2S)
19	1.9	2.2	1.1
20	1.7	3.6	2.1
21	0.5	1.6	3.2

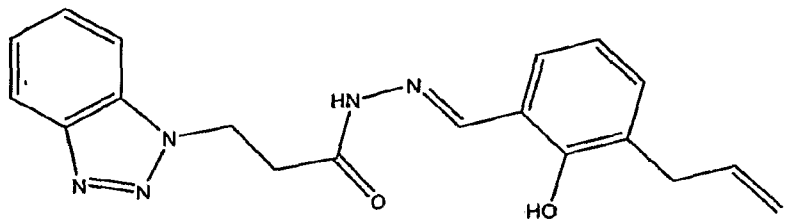
The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the claims. Therefore, it is to be understood that the foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims. The invention is defined by the following claims, with equivalents of the claims to be included therein.

WHAT IS CLAIMEd is:

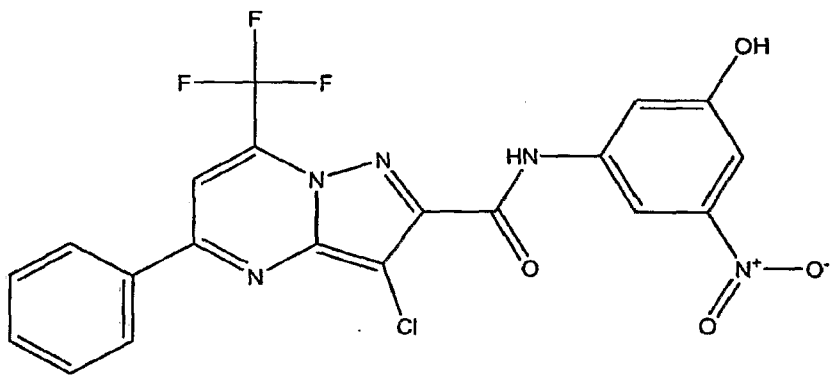
1. A method for treating an apoptosis-associated disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an active compound selected from the group consisting of:



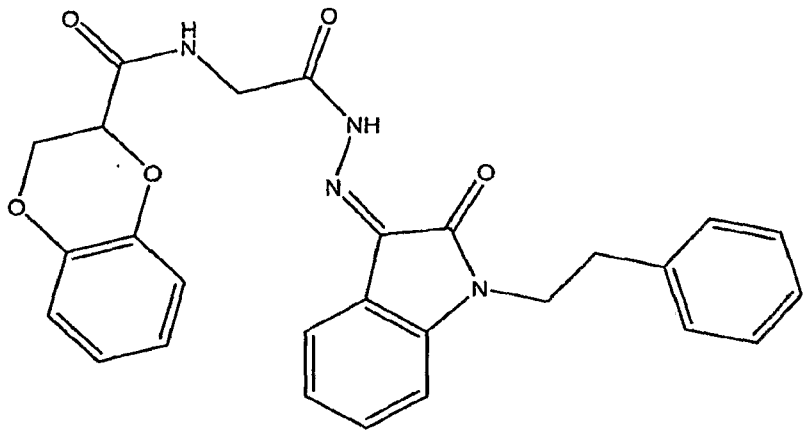
1



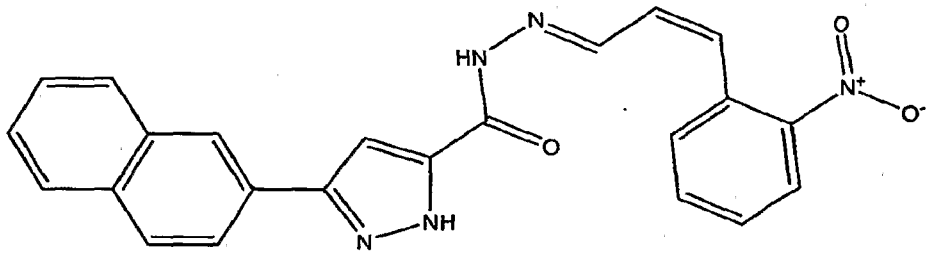
2



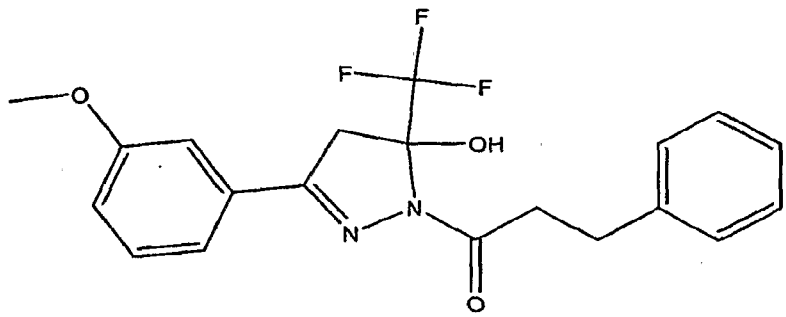
3



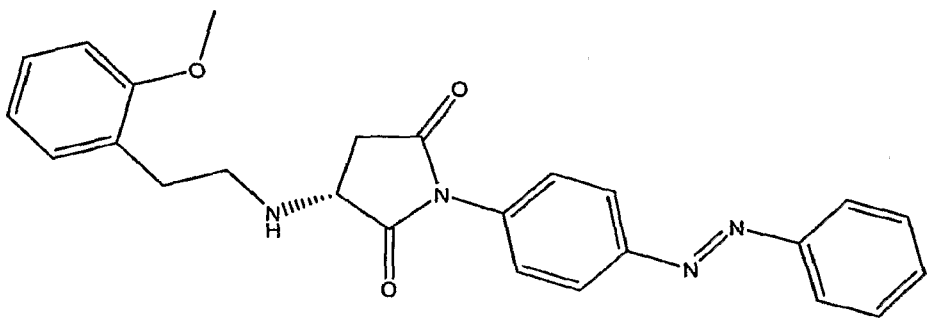
4



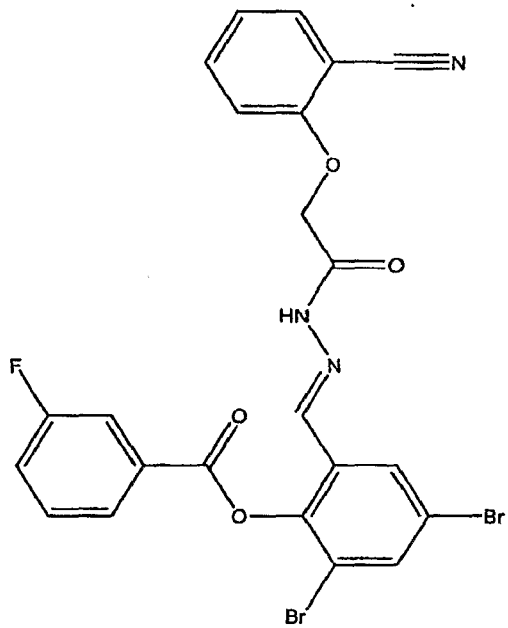
5



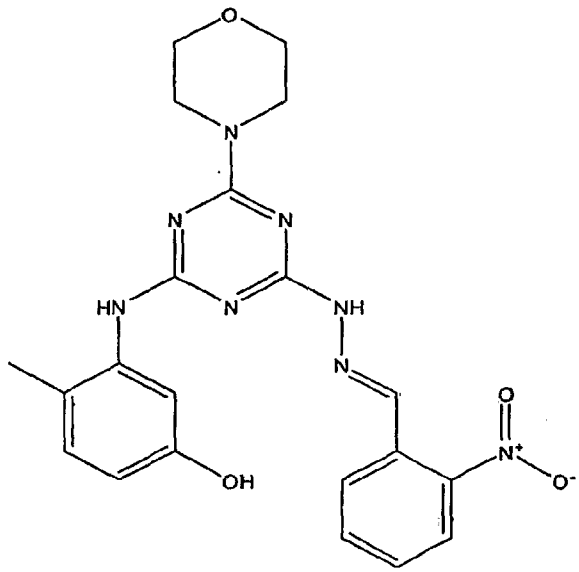
6



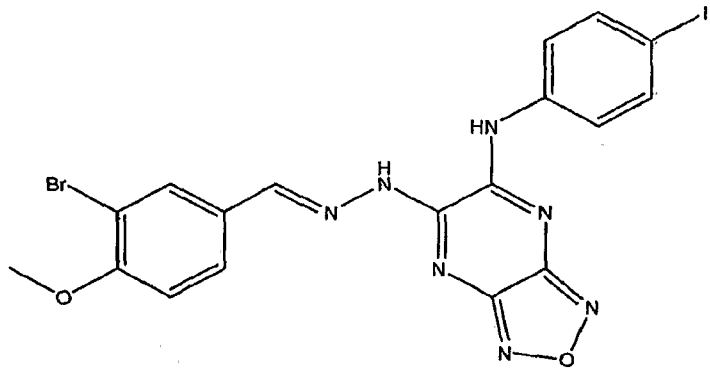
7



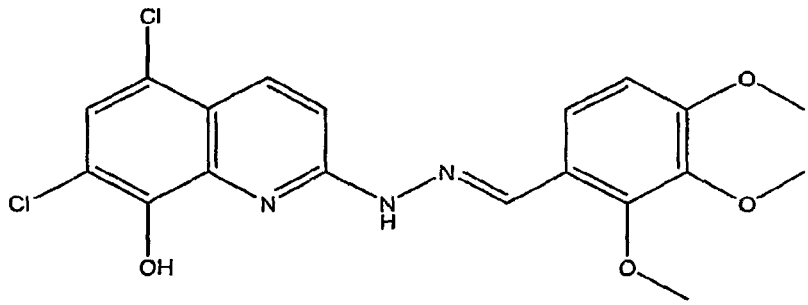
8



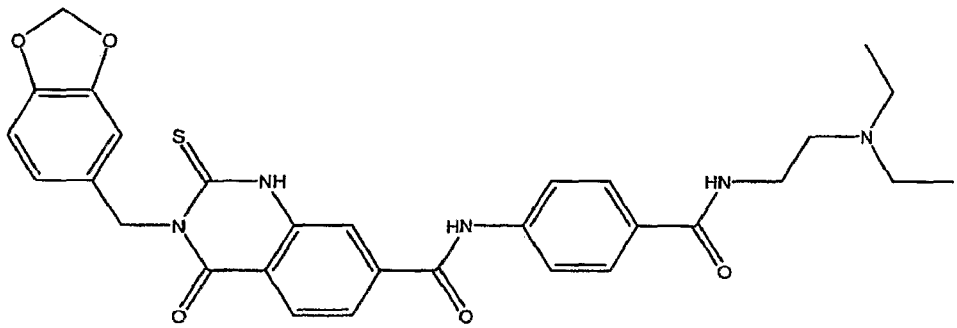
9



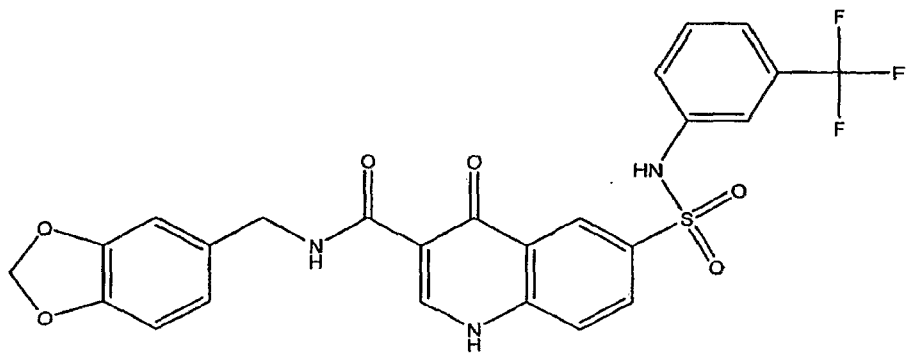
10



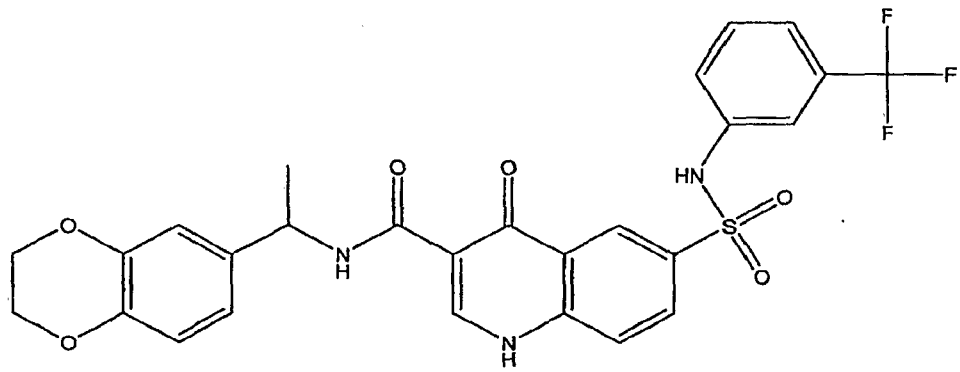
11



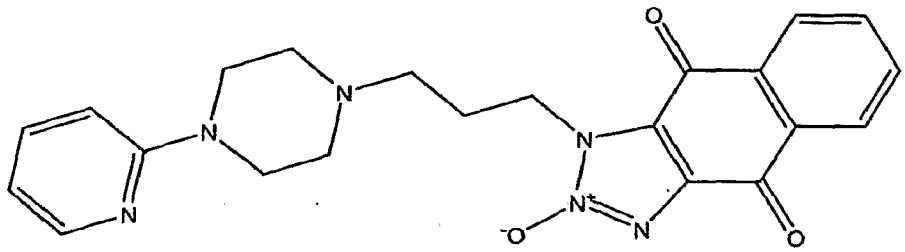
12



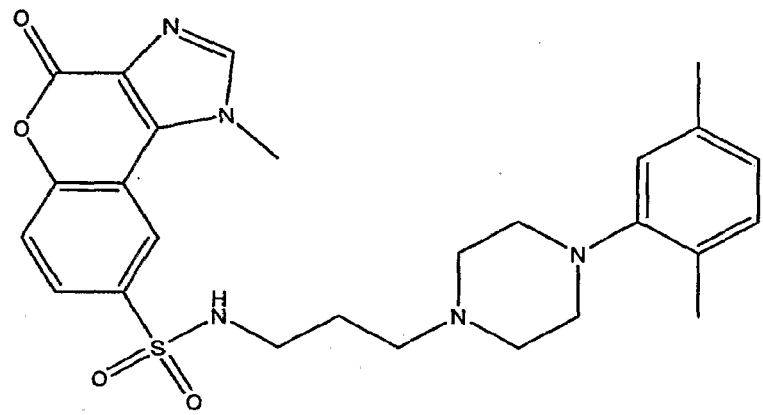
13



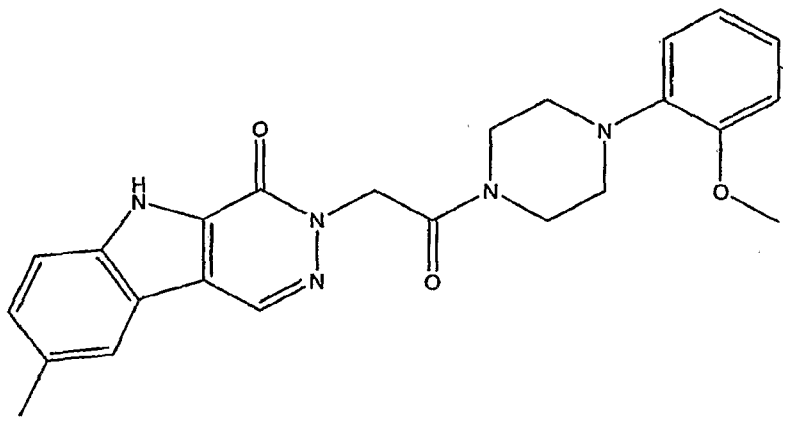
14



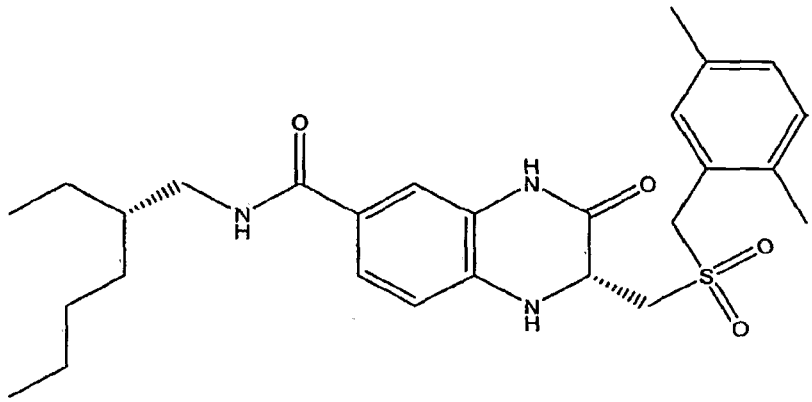
15



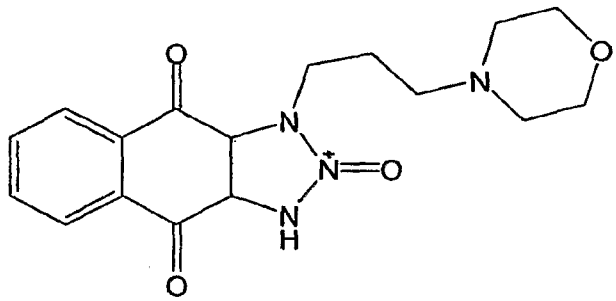
16



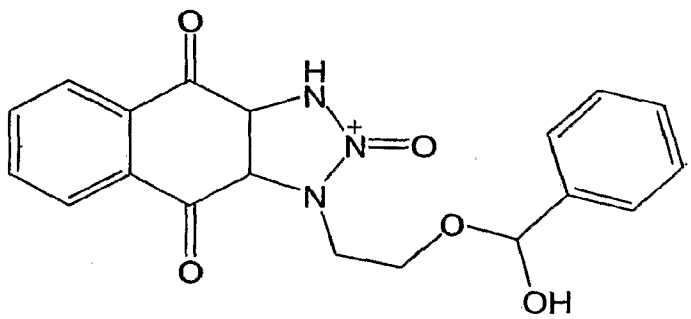
17



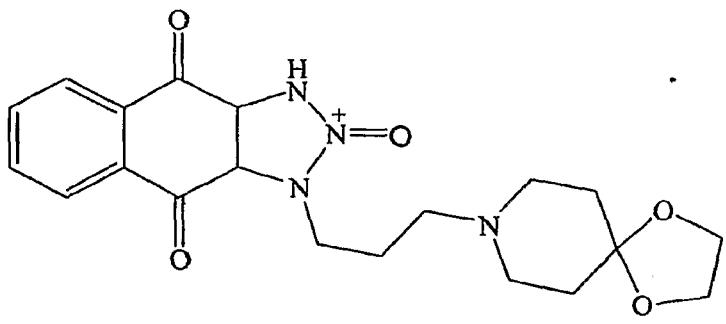
18



19



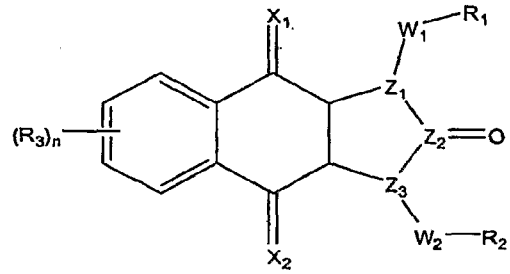
20



21

and pharmaceutically acceptable salts and prodrugs thereof.

2. A method for treating an apoptosis-associated disease in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an active compound of Formula XI



XI

or a pharmaceutically acceptable salt or a prodrug thereof

wherein:

n is from 0 to 4;

X_1 and X_2 are each independently O or S;

Z_1, Z_2, Z_3 are each independently C or N;

W_1 and W_2 are independently a bond or selected from the group consisting of C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkoxy, C_{1-10} alkylsulfanyl, C_{1-10} haloalkyl, and C_{1-10} aminoalkyl;

R_1 and R_2 are each independently selected from the group consisting of C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-14} heteraryl and C_{3-10} heterocycle;

R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10}alkoxy, C_{1-10}thioalkyl, C_{1-10}haloalkyl, C_{3-10}cycloalkyl, C_{3-10}cycloalkyl-C_{1-10}alkyl, C_{3-10}heterocycle, C_{3-10}heterocycle-C_{1-10}alkyl, C_{3-14} aryl, C_{6-12}aryl-C_{1-10}alkoxy, C_{3-10}heterocycle-C_{1-10}alkoxy and C_{3-14} heteroaryl;

wherein each of the foregoing alkyl, alkenyl, alkoxy, alkylsulfanyl, haloalkyl, aminoalkyl may be branched or unbranched, independently unsubstituted or substituted with one or more substituents selected from the group consisting of hydroxy, cyano, amine, nitro, halogen, mono- or dialkylamino, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl; and

wherein each of the foregoing C_{3-10}cycloalkyl, C_{3-14} aryl, C_{3-10} heterocycle, C_{3-14}aryl, C_{3-14} heteroaryl, may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, amino, cyano, nitro, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl.

3. The method of Claim 2, wherein n is 0; X_1 and X_2 are O; Z_1, Z_2 and Z_3 are N; W_1 and W_2 are independently C_{1-6} alkyl or C_{1-6}alkoxyl; R_1 and R_2 are C_{3-14} aryl or C_{3-10} heterocycle.

4. The method of Claims 1 to 3, wherein said compound modulates apopotosis by binding to a Bc-2 family member protein.

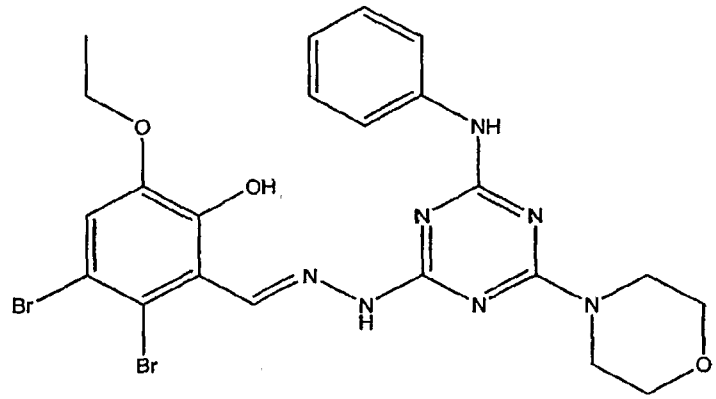
5. The method of Claim 4, wherein said Bcl-2 family member protein is Bcl-2 or Bcl-x_L.

6. The method of Claims 1 to 3, wherein the apoptosis-associated disease is a neoplastic disease.

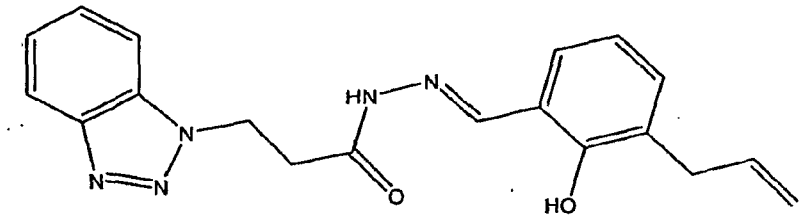
7. The method of Claims 6, wherein the neoplastic disease is a cancer.

8. The method of Claims 7, wherein the cancer comprises a solid tumor.

9. A pharmaceutical composition comprising an active compound selected from the group consisting of:

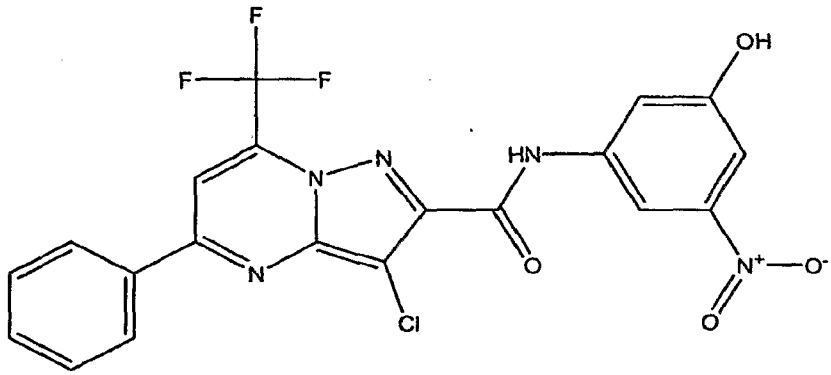


1

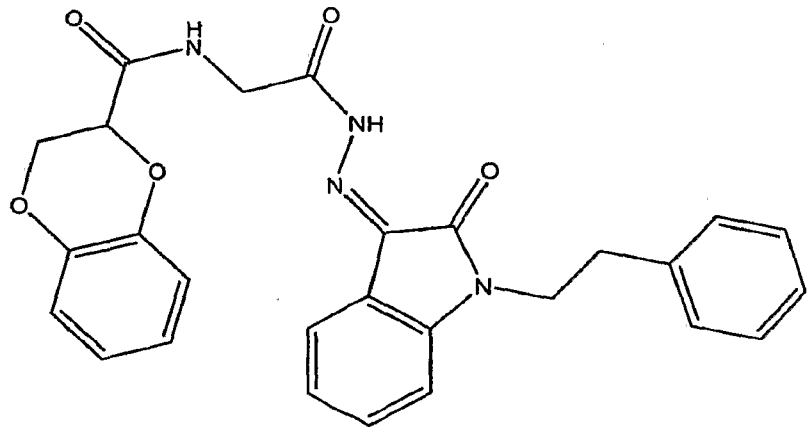


2

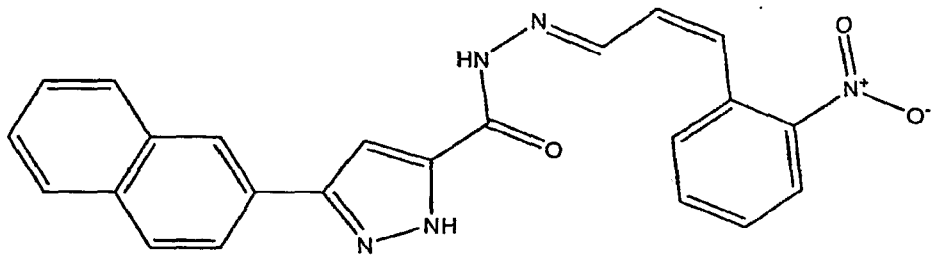
5



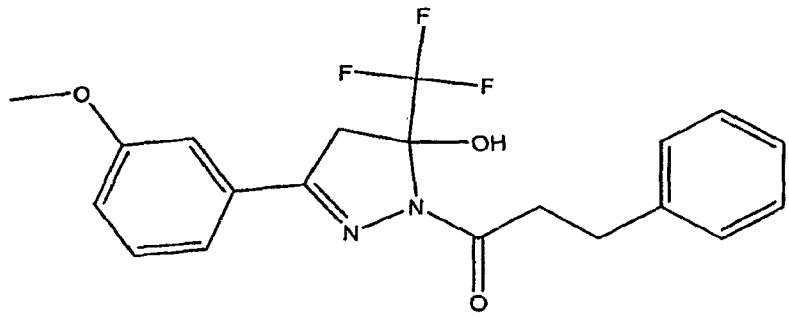
3



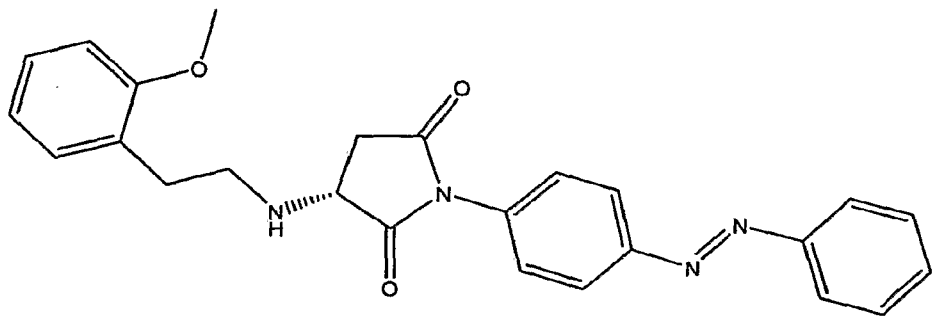
4



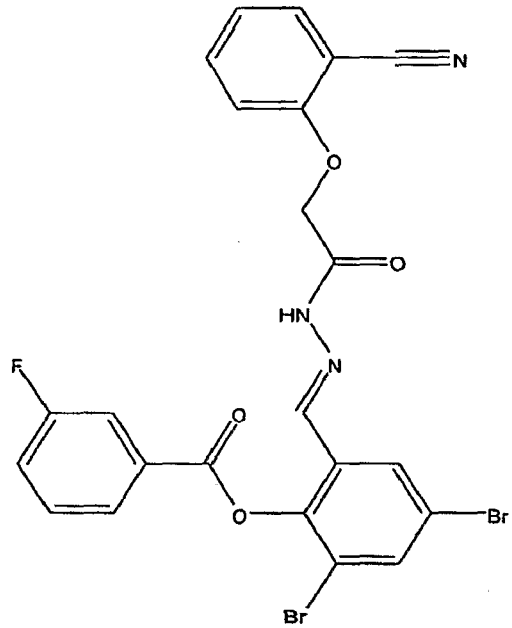
5



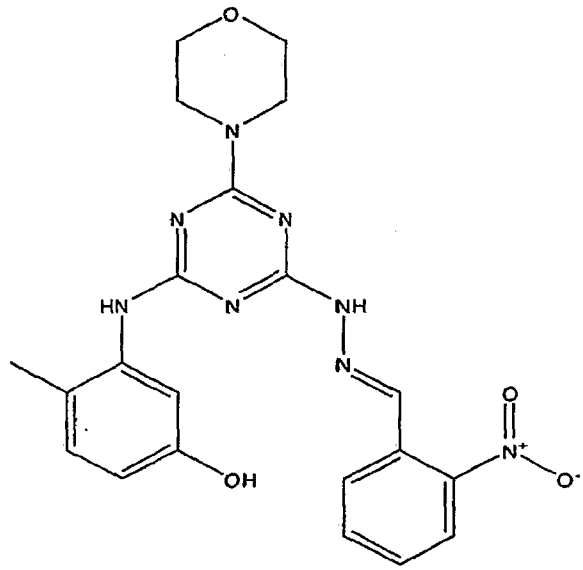
6



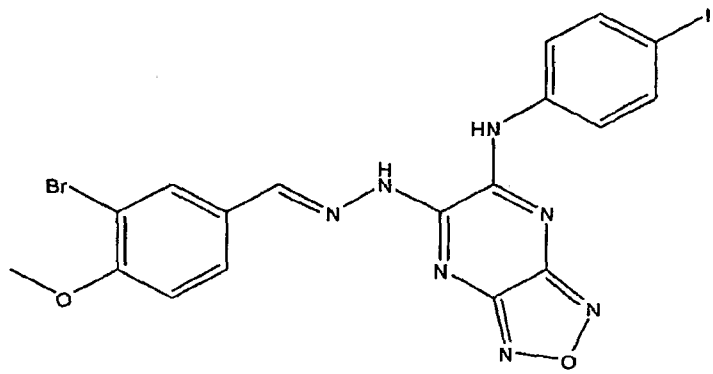
7



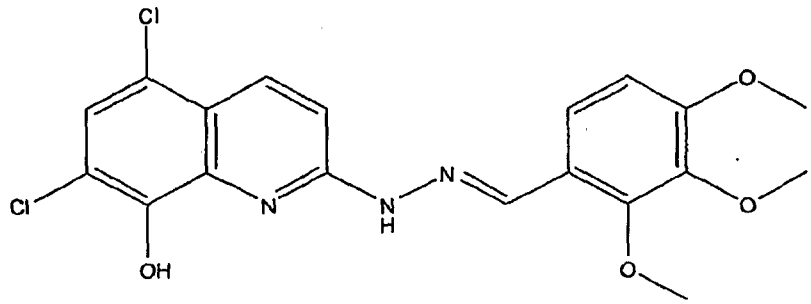
8



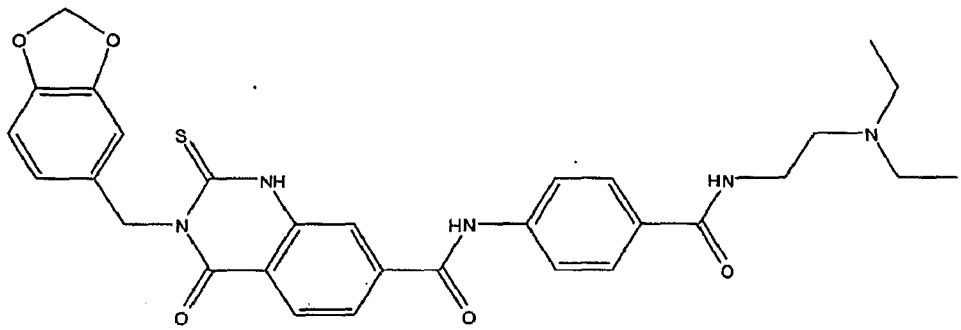
9



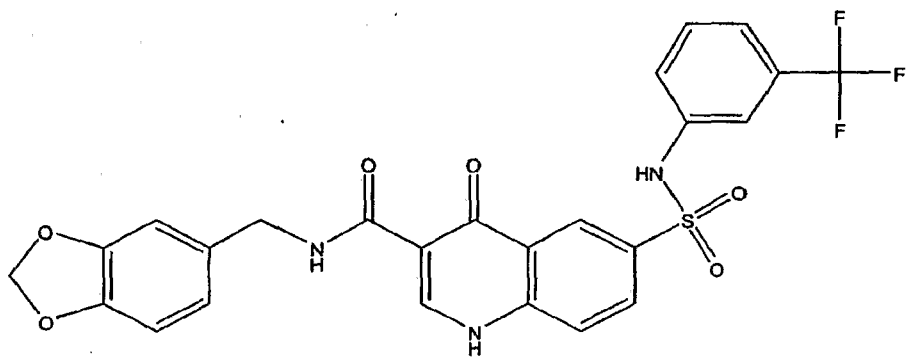
10



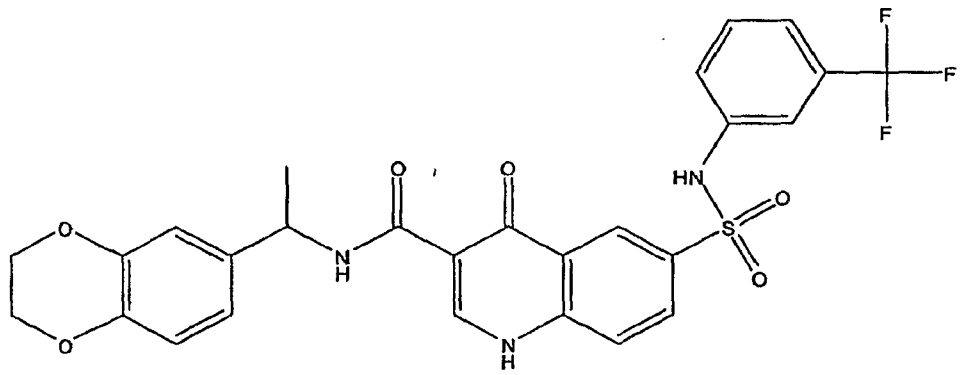
11



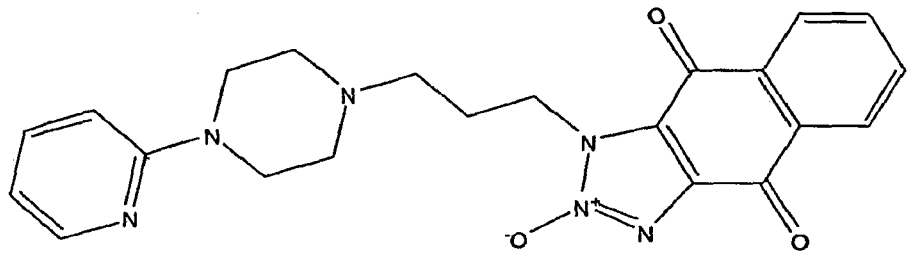
12



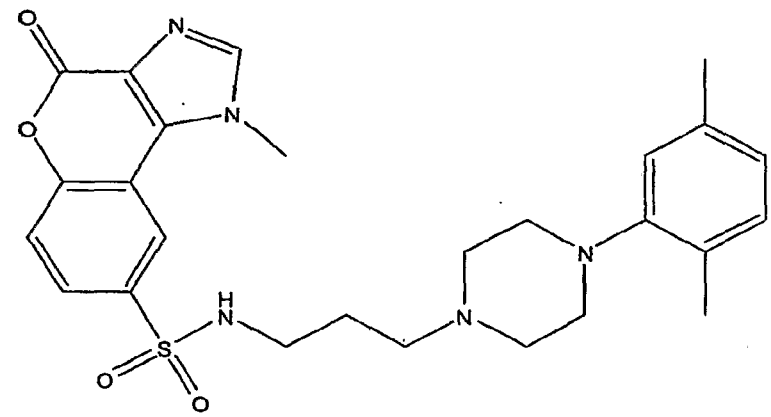
13



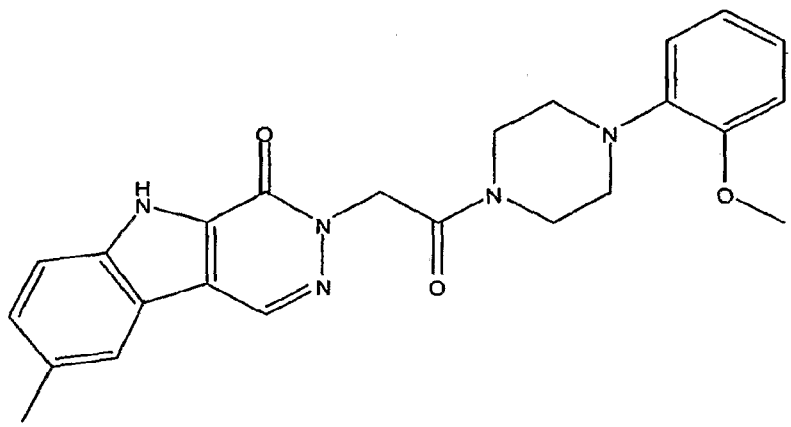
14



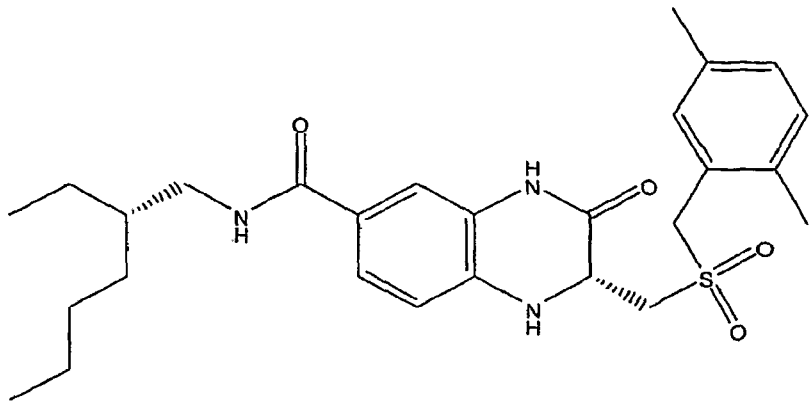
15



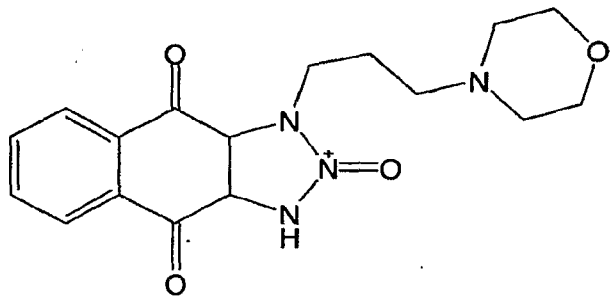
16



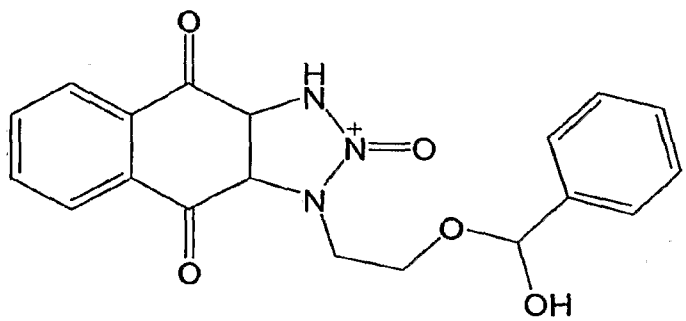
17



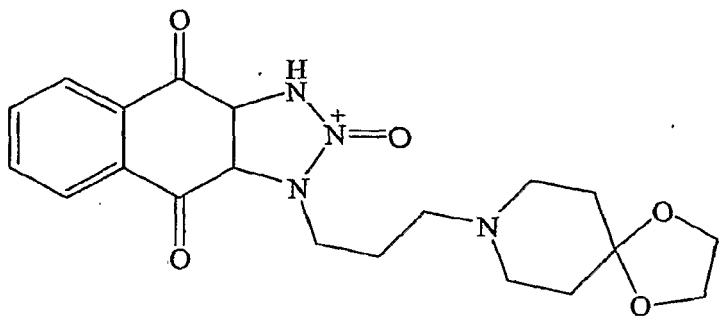
18



19



20

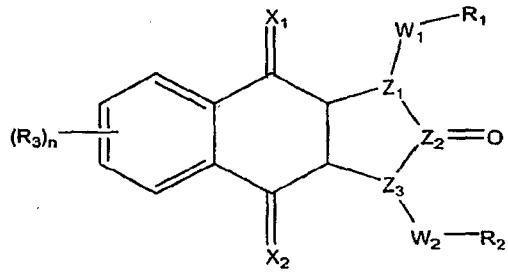


21

and pharmaceutically acceptable salts and prodrugs thereof;

and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition comprising an active compound of Formula XI



XI

or a pharmaceutically acceptable salt or prodrug thereof

wherein:

n is from 0 to 4;

X_1 and X_2 are each independently O or S;

Z_1, Z_2, Z_3 are each independently C or N;

W_1 and W_2 are independently a bond or selected from the group consisting of C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkoxy, C_{1-10} alkylsulfanyl, C_{1-10} haloalkyl, and C_{1-10} aminoalkyl;

R_1 and R_2 are each independently selected from the group consisting of C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-14} heteraryl and C_{3-10} heterocycle;

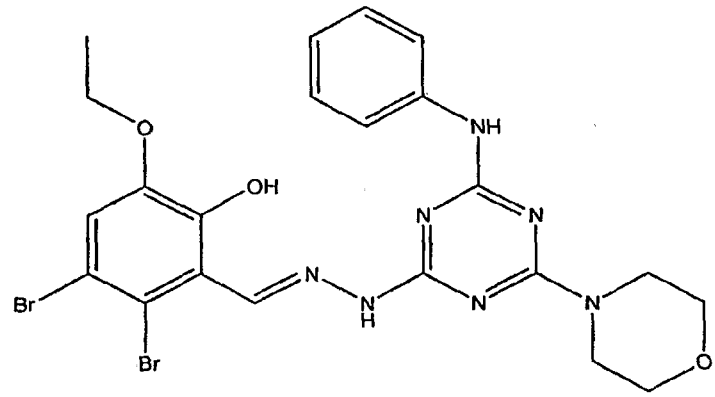
R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10}alkoxy, C_{1-10}thioalkyl, C_{1-10}haloalkyl, C_{3-10}cycloalkyl, C_{3-10}cycloalkyl-C_{1-10}alkyl, C_{3-10}heterocycle, C_{3-10}heterocycle-C_{1-10}alkyl, C_{3-14} aryl, C_{6-12}aryl-C_{1-10}alkoxy, C_{3-10}heterocycle-C_{1-10}alkoxy and C_{3-14} heteroaryl;

wherein each of the foregoing alkyl, alkenyl, alkoxy, alkylsulfanyl, haloalkyl, aminoalkyl may be branched or unbranched, independently unsubstituted or substituted with one or more substituents selected from the group consisting of hydroxy, cyano, amine, nitro, halogen, mono- or dialkylamino, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl; and

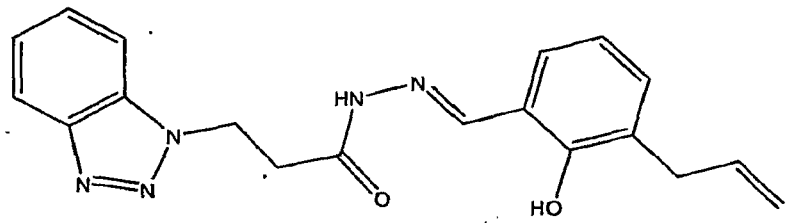
wherein each of the foregoing C_{3-10}cycloalkyl, C_{3-14} aryl, C_{3-10} heterocycle, C_{3-14}aryl, C_{3-14} heteroaryl, may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, amino, cyano, nitro, -

SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl.

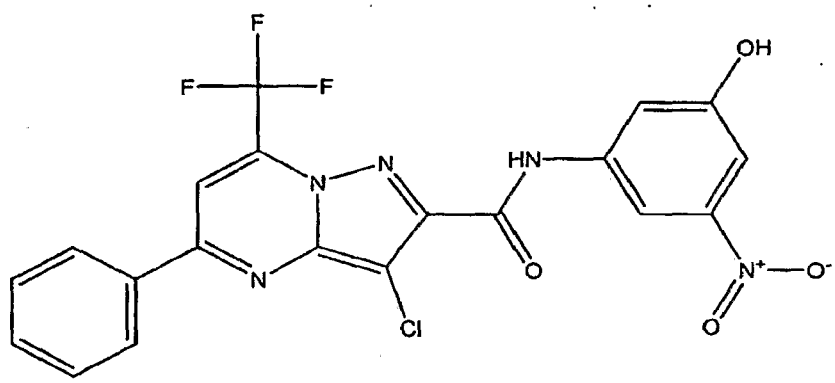
11. The use of an active compound selected from the group consisting of:



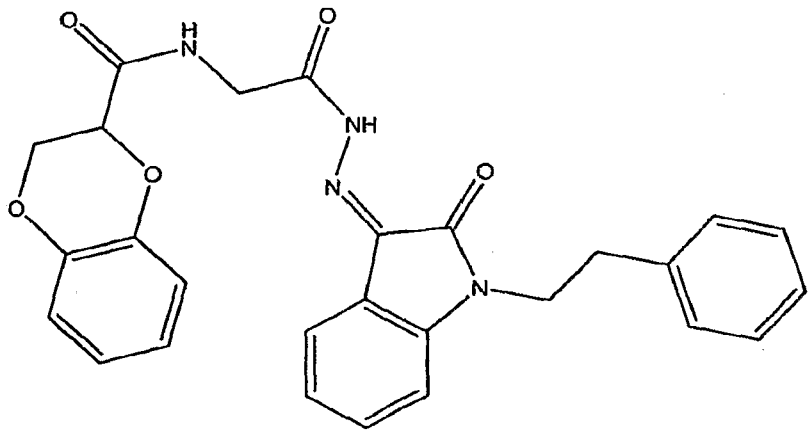
1



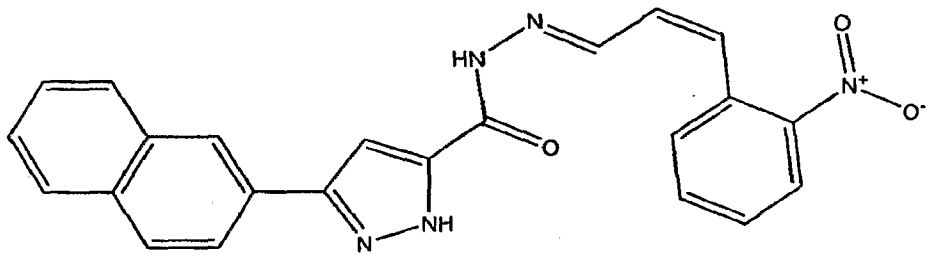
2



3

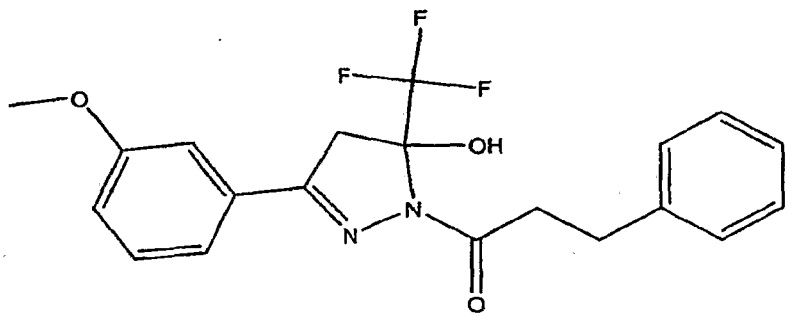


4

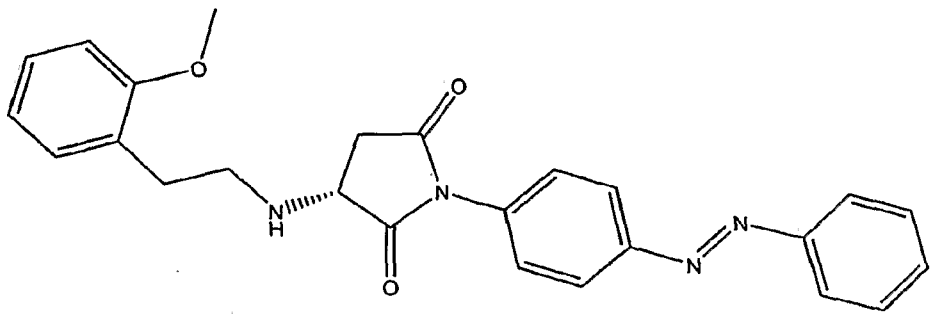


5

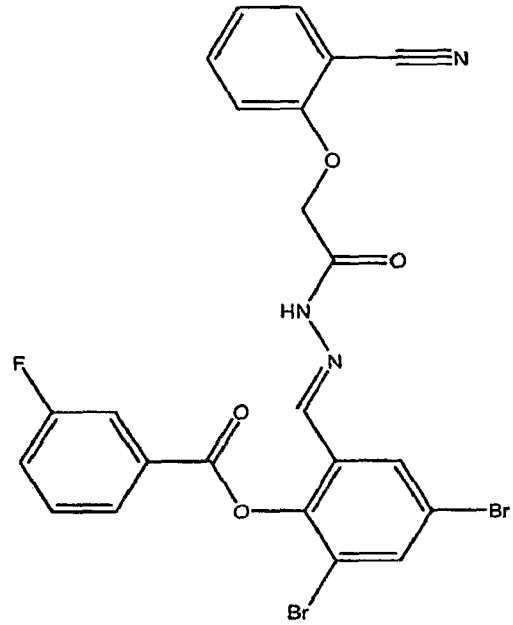
5



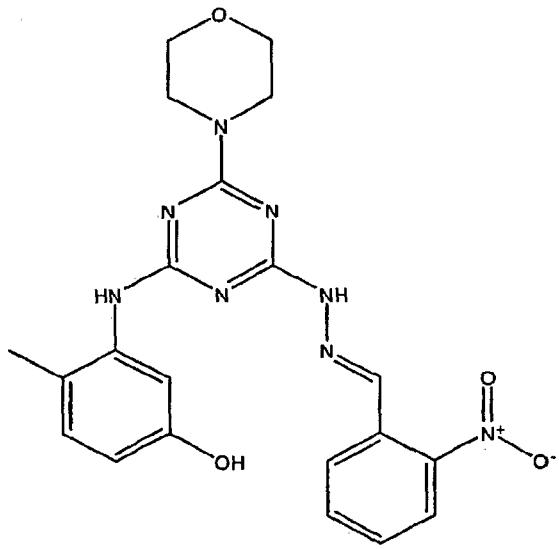
6



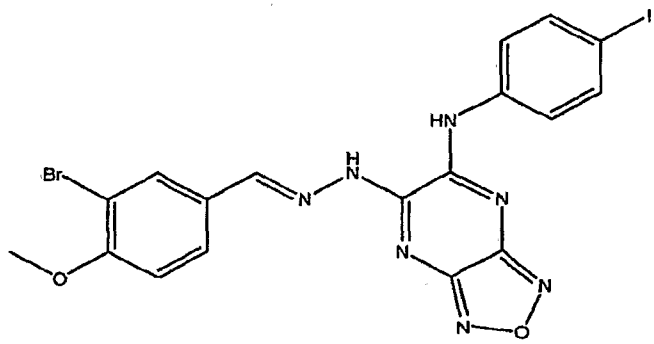
7



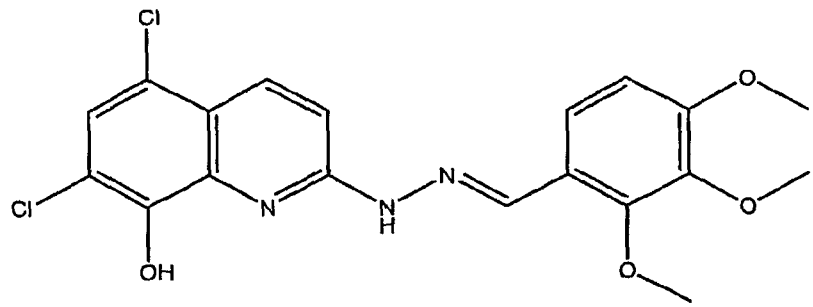
8



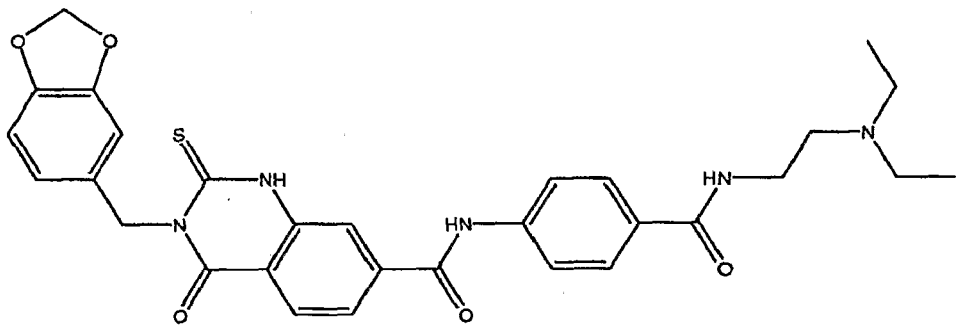
9



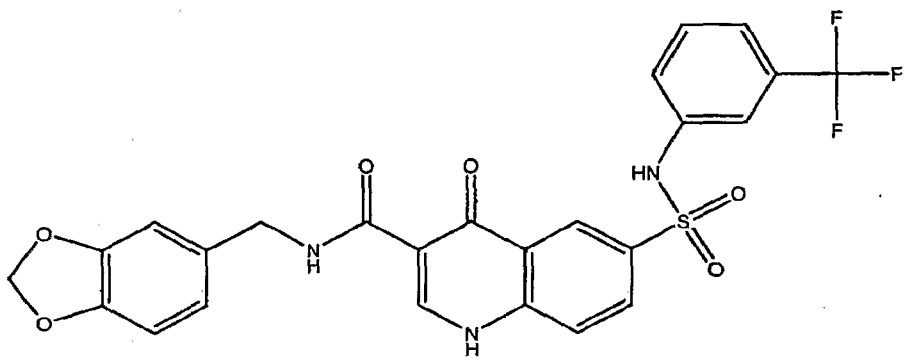
10



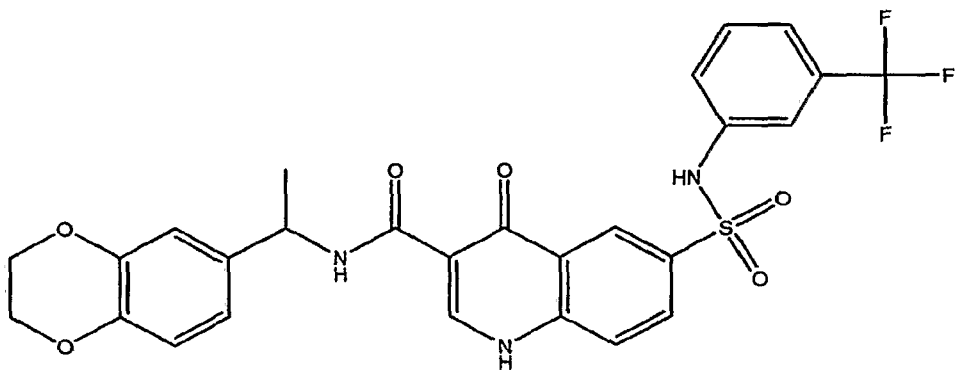
11



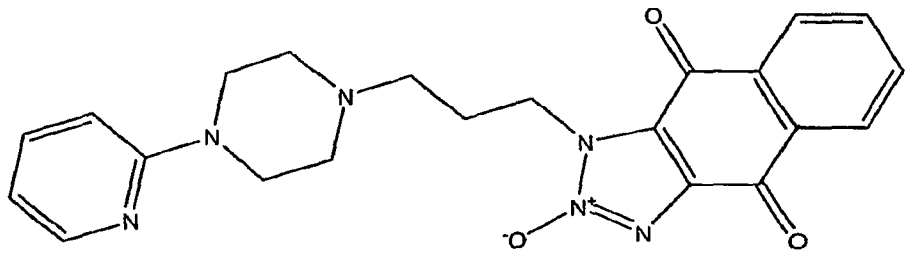
12



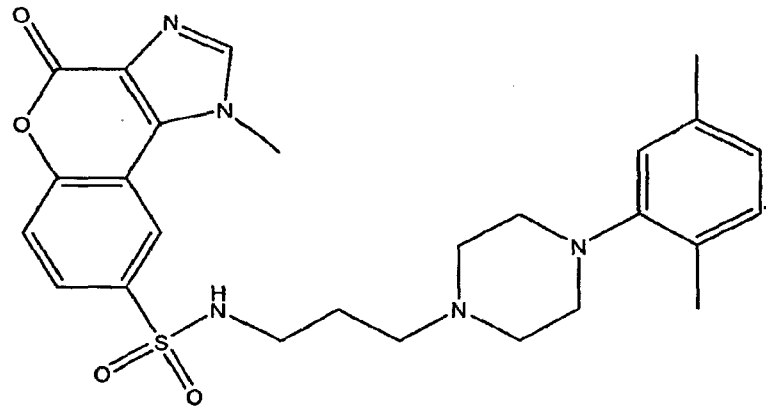
13



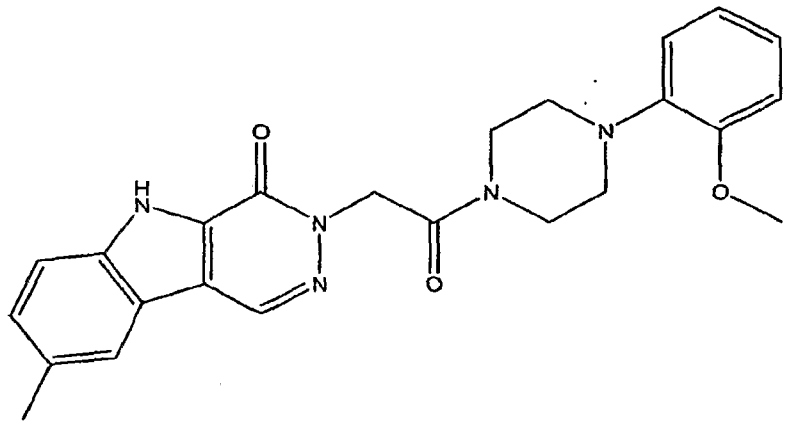
14



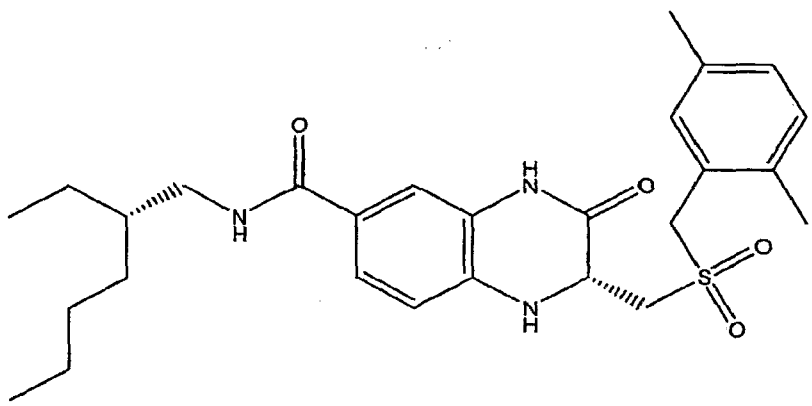
15



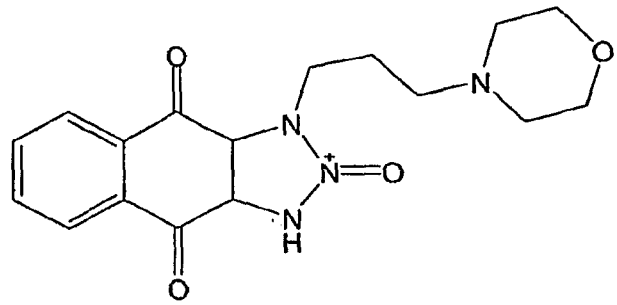
16



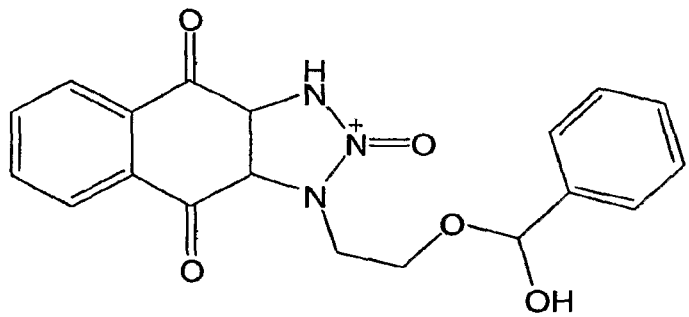
17



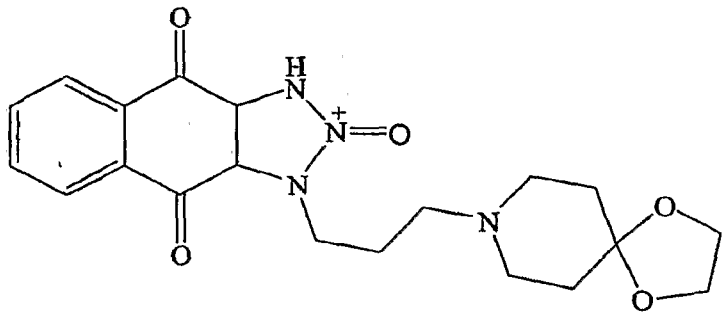
18



19



20

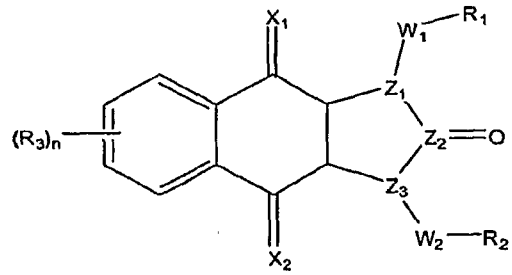


21

and pharmaceutically acceptable salts and prodrugs thereof

in the manufacture of a medicament for the treatment of apoptosis-associated disease in a subject in need thereof.

12. The use of an active compound of Formula XI:



XI

or a pharmaceutically acceptable salt or a prodrug thereof;

wherein:

n is from 0 to 4;

X_1 and X_2 are each independently O or S;

Z_1, Z_2, Z_3 are each independently C or N;

W_1 and W_2 are independently a bond or selected from the group consisting of C_{1-10} alkyl, C_{1-10} alkenyl, C_{1-10} alkoxy, C_{1-10} alkylsulfanyl, C_{1-10} haloalkyl, and C_{1-10} aminoalkyl;

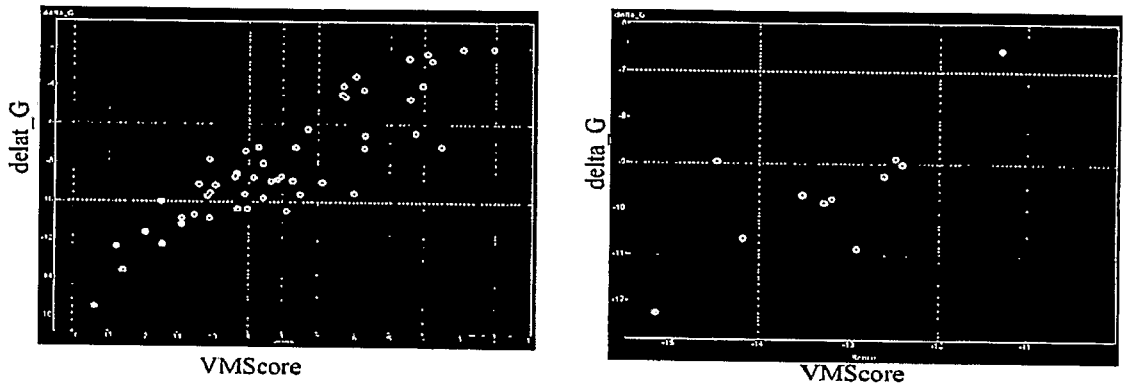
R_1 and R_2 are each independently selected from the group consisting of C_{3-10} cycloalkyl, C_{3-14} aryl, C_{3-14} heteraryl and C_{3-10} heterocycle;

R_3 is selected from the group consisting of hydrogen, hydroxy, cyano, amine, nitro, halogen, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10}alkoxy, C_{1-10}thioalkyl, C_{1-10}haloalkyl, C_{3-10}cycloalkyl, C_{3-10}cycloalkyl-C_{1-10}alkyl, C_{3-10}heterocycle, C_{3-10}heterocycle-C_{1-10}alkyl, C_{3-14} aryl, C_{6-12}aryl-C_{1-10}alkoxy, C_{3-10}heterocycle-C_{1-10}alkoxy and C_{3-14} heteroaryl;

wherein each of the foregoing alkyl, alkenyl, alkoxy, alkylsulfanyl, haloalkyl, aminoalkyl may be branched or unbranched, independently unsubstituted or substituted with one or more substituents selected from the group consisting of hydroxy, cyano, amine, nitro, halogen, mono- or dialkylamino, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl; and

wherein each of the foregoing C_{3-10}cycloalkyl, C_{3-14} aryl, C_{3-10} heterocycle, C_{3-14}aryl, C_{3-14} heteroaryl, may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, amino, cyano, nitro, -SH, C_{1-10} alkyl, C_{1-10}alkenyl, C_{1-10} alkynyl, C_{1-10}alkoxy, C_{1-10} thioalkyl, C_{1-10} haloalkyl, C_{1-10} aminoalkyl,

in the manufacture of a medicament for the treatment of apoptosis-associated disease in a subject in need thereof.



Left: VMScore result for the training set with 53 complex structures, $R^2 = 0.77$, with cross-validation prediction (leave-one-out) $Q^2 = 0.70$.
Right: VMScore prediction for the testing set of Endothiapepsin complex dataset, $R^2 = 0.62$.

Figure 1