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(54) **Title**: BCL-2BCL-XL INHIBITORS AND THERAPEUTIC METHODS USING THE SAME

(57) **Abstract**: Inhibitors of Bcl-2/Bcl-xL and compositions containing the same are disclosed. Methods of using the Bcl-2/Bcl-xL inhibitors in the treatment of diseases and conditions wherein inhibition of Bcl-2/Bcl-xL provides a benefit, like cancers, also are disclosed.

BCL-2/BCL-XL INHIBITORS AND THERAPEUTIC METHODS USING THE SAME

FIELD OF THE INVENTION

[0001] The present invention relates to Bcl-2/Bcl-xL inhibitors and to therapeutic methods of treating conditions and diseases wherein inhibition of Bcl-2/Bcl-xL provides a benefit.

BACKGROUND OF THE INVENTION

[0002] Apoptosis resistance is a hallmark of human cancer (1-3). Cancer cells must overcome a continual bombardment by cellular stresses, such as DNA damage, oncogene activation, aberrant cell cycle progression, and harsh microenvironments, that would cause normal cells to undergo apoptosis. One of the primary means by which cancer cells evade apoptosis is by up-regulation of anti-apoptotic proteins of the Bcl-2 family. Targeting key apoptosis regulators to overcome apoptosis-resistance and promote apoptosis of tumor cells is a new cancer therapeutic strategy (4,5).

[0003] Bcl-2 proteins function as critical regulators of apoptosis in both cancer and normal cells (6-10). Bcl-2 proteins serve as a check on apoptosis allowing healthy and useful cells to survive. This protein family includes anti-apoptotic proteins, such as Bcl-2, Bcl-xL, and Mcl-1, and pro-apoptotic molecules, including Bid, Bim, Bad, Bak and Bax (6-10). While normal cells have low expression levels of the anti-apoptotic Bcl-2 and Bcl-xL proteins, these proteins are found to be highly overexpressed in many different types of human tumors(6-10). This overexpression has been linked to poor prognosis in several types of cancer, and to clinical resistance to chemotherapeutic agents and radiation (6-10). Consistent with clinical observations, laboratory studies have established that overexpression of Bcl-2 or Bcl-xL causes cancer cells to become more resistant to chemotherapeutic agents *in vitro* and *in vivo* (6-10). Inhibition of apoptosis by Bcl-2 contributes to cancer by inhibiting cell death. Therefore, targeting Bcl-2 and/or Bcl-xL has been pursued as a cancer therapeutic strategy (11-34). Inhibiting Bcl-2 activity in cancer cells can reduce chemotherapeutic resistance and increase the killing of cancer cells.

[0004] Bcl-2 and Bcl-xL proteins inhibit apoptosis by heterodimerization with pro-apoptotic Bcl-2 family proteins, such as Bak, Bax, Bim, Bid, Puma, and Bad (6-10). Experimentally determined three-dimensional structures of Bcl-xL and Bcl-2 have shown that these proteins possess a well-defined groove, which interacts with the BH3 (Bcl-2 Homology 3) domain of the pro-apoptotic Bcl-2 proteins (38-42). It has been proposed that non-peptide small molecules designed to block the heterodimerization of Bcl-2/Bcl-xL proteins with their

pro-death binding partners may be effective as antagonists of Bcl-2/Bcl-xL, and that such small molecule inhibitors may have a great therapeutic potential for the treatment of human cancers in which Bcl-2 and/or Bcl-xL are highly expressed (18-37).

[0005] Although non-peptide, small molecule inhibitors of Bcl-2/Bcl-xL have been reported, most of the inhibitors have weak to modest affinities for these proteins and lack a well-defined mode of action for their cellular activity (18-37). The exceptions are ABT-737, ABT-263, and their analogues (26-34). ABT-737 and ABT-263 bind to Bcl-2, Bcl-xL, and Bcl-w with very high affinities (K_i <1 nM) and have high specificity over Mcl-1 and A1, two other anti-apoptotic Bcl-2 proteins (26, 32, 34). ABT-263 has advanced into Phase I/II clinical trials and shows promising antitumor activity in the clinic (45).

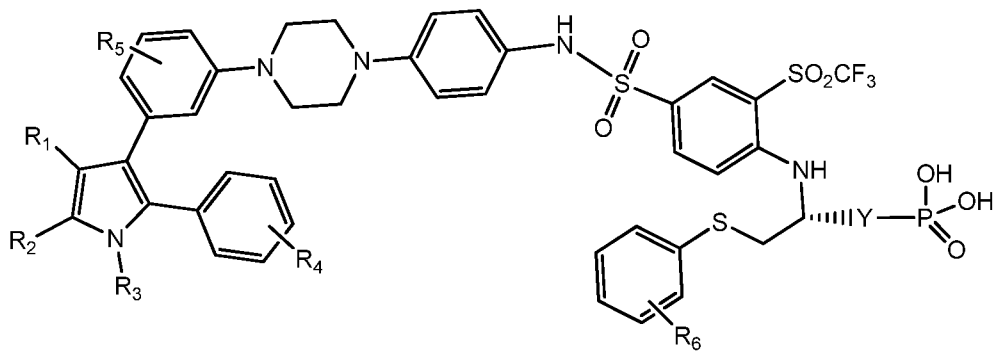
[0006] Despite the discovery of ABT-737 and ABT-263, the design of potent, non-peptide inhibitors of Bcl-2/Bcl-xL remains a significant challenge in modern drug discovery. Accordingly, a need still exists in the art for Bcl-2/Bcl-xL inhibitors having physical and pharmacological properties that permit use of the inhibitors in therapeutic applications. The present invention provides compounds designed to bind to Bcl-2/Bcl-xL and inhibit Bcl-2/Bcl-xL activity.

SUMMARY OF THE INVENTION

[0007] The present invention is directed to inhibitors of Bcl-2/Bcl-xL, to compositions comprising the inhibitors, and to methods of using the inhibitors in a therapeutic treatment of conditions and diseases wherein inhibition of Bcl-2/Bcl-xL activity provides a benefit. The present compounds are potent inhibitors of Bcl-2/Bcl-xL activation, and induce apoptosis of cancer cells that express Bcl-2 and/or Bcl-xL.

[0008] More particularly, the present invention is directed to compounds having a structural formula (I), (II), or (III):

(I)



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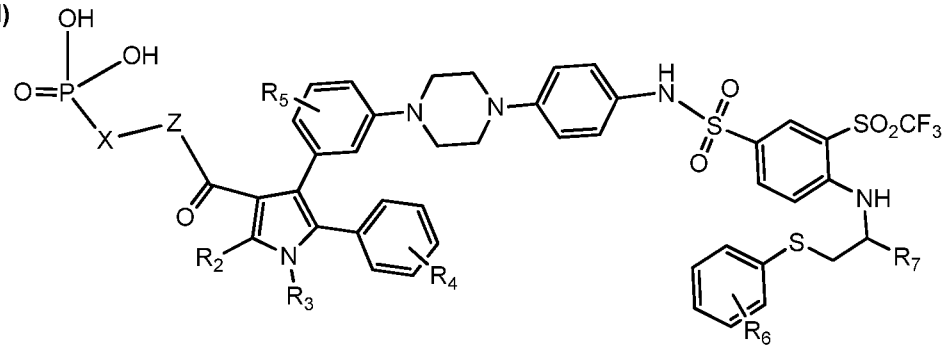

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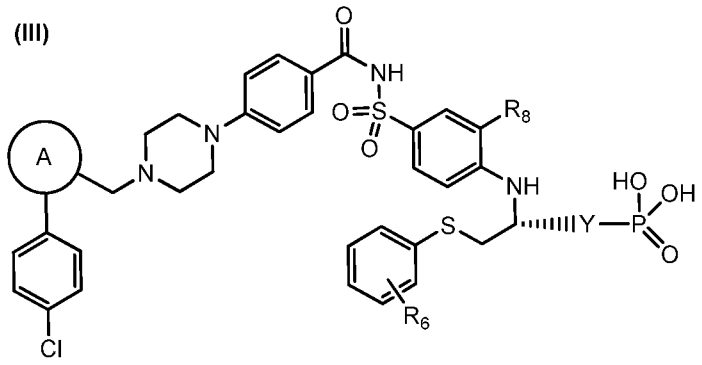
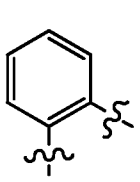
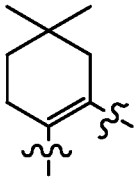
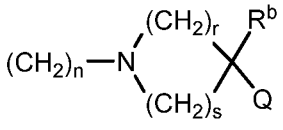


[0009] wherein the A ring is  or  ;

[0010] X, substituted or unsubstituted, is selected from the group consisting of alkylene, alkenylene, cycloalkylene, cycloalkenylene, and heterocycloalkylene;

[0011] Y is selected from the group consisting of $(CH_2)_n$-$N(R^a)_2$ and

[0012]  ;

[0013] Q is selected from the group consisting of O, $O(CH_2)_{1-3}$, NR^c, $NR^c(C_{1-3}alkylene)$, $OC(=O)(C_{1-3}alkylene)$, $C(=O)O$, $C(=O)O(C_{1-3}alkylene)$, $NHC(=O)(C_{1-3}alkylene)$, $C(=O)NH$, and $C(=O)NH(C_{1-3}alkylene)$;

[0014] Z is O or NR^c;

[0015] R_1 and R_2, independently, are selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", COR', CO_2R', OCOR', CONR'R", $CONR'SO_2R"$, NR'COR", NR'CONR"R'", NR'C=SNR"R'", $NR'SO_2R"$, SO_2R', and $SO_2NR'R"$;

[0016] R_3 is selected from a group consisting of H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', NR'R", OCOR', CO_2R', COR', CONR'R", CONR'SO_2R", C_{1-3}alkyleneCH(OH)CH_2OH, SO_2R', and $SO_2NR'R"$;

[0017] R', R", and R'", independently, are H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, C_{1-3}alkyleneheterocycloalkyl, or heterocycloalkyl;

[0018] R' and R", or R" and R'", can be taken together with the atom to which they are bound to form a 3 to 7 membered ring;

[0019] R_4 is hydrogen, halo, C_{1-3}alkyl, CF_3, or CN;

[0020] R_5 is hydrogen, halo, C_{1-3}alkyl, substituted C_{1-3}alkyl, hydroxyalkyl, alkoxy, or substituted alkoxy;

[0021] R_6 is selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", CO_2R', OCOR', CONR'R", CONR'SO_2R", NR'COR", NR'CONR"R'", NR'C=SNR"R'", NR'SO_2R", SO_2R', and $SO_2NR'R"$;

[0022] R_7, substituted or unsubstituted, is selected form the group consisting of hydrogen, alkyl, alkenyl, $(CH_2)_{0-3}$cycloalkyl, $(CH_2)_{0-3}$cycloalkenyl, $(CH_2)_{0-3}$heterocycloalkyl, $(CH_2)_{0-3}$aryl, and $(CH_2)_{0-3}$heteroaryl;

[0023] R_8 is selected form the group consisting of hydrogen, halo, NO_2, CN, CF_3SO_2, and CF_3;

[0024] R_a is selected from the group consisting of hydrogen, alkyl, heteroalkyl, alkenyl, hydroxyalkyl, alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, and heterocycloalkyl;

[0025] R_b is hydrogen or alkyl;

[0026] R_c is selected from the group consisting of hydrogen, alkyl, substituted alkyl, hydroxyalkyl, alkoxy, and substituted alkoxy; and

[0027] n, r, and s, independently, are 1, 2, 3, 4, 5, or 6;

[0028] or a pharmaceutically acceptable salt of (I), (II), or (III).

[0029] In some embodiments, R_1 and R_2 or R_2 and R_3 can be taken together to form a ring. In other embodiments, R' and R", or R" and R'", can be taken together with the atoms to which they are bound to form a 3 to 7 membered ring.

[0030] In one embodiment, the present invention provides a method of treating a condition or disease by administering a therapeutically effective amount of a compound of structural

formula (I), (II), or (III) to an individual in need thereof. The disease or condition of interest is treatable by inhibition of Bcl-2 and/or Bcl-xL, for example, a cancer.

[0031] Another embodiment of the present invention is to provide a composition comprising (a) a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and (b) an excipient and/or pharmaceutically acceptable carrier useful in treating diseases or conditions wherein inhibition of Bcl-2/Bcl-xL provides a benefit.

[0032] Another embodiment of the present invention is to utilize a composition comprising a compound of structural formula (I), (II), or (III) and a second therapeutically active agent in a method of treating an individual for a disease or condition wherein inhibition of Bcl-2/Bcl-xL provides a benefit.

[0033] In a further embodiment, the invention provides for use of a composition comprising a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and an optional second therapeutic agent for the manufacture of a medicament for treating a disease or condition of interest, e.g., a cancer.

[0034] Still another embodiment of the present invention is to provide a kit for human pharmaceutical use comprising (a) a container, (b1) a packaged composition comprising a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III), and, optionally, (b2) a packaged composition comprising a second therapeutic agent useful in the treatment of a disease or condition of interest, and (c) a package insert containing directions for use of the composition or compositions, administered simultaneously or sequentially, in the treatment of the disease or condition.

[0035] The Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and the second therapeutic agent can be administered together as a single-unit dose or separately as multi-unit doses, wherein the Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) is administered before the second therapeutic agent or *vice versa*. It is envisioned that one or more dose of a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and/or one or more dose of a second therapeutic agent can be administered.

[0036] In one embodiment, a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and a second therapeutic agent are administered simultaneously. In related embodiments, a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and second therapeutic agent are administered from a single composition or from separate compositions. In a further embodiment, the Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) and second therapeutic agent are administered sequentially. A Bcl-2/Bcl-xL inhibitor of structural

formula (I), (II), or (III), as used in the present invention, can be administered in an amount of about 0.005 to about 500 milligrams per dose, about 0.05 to about 250 milligrams per dose, or about 0.5 to about 100 milligrams per dose.

[0037] These and other embodiments and features of the present invention will become apparent from the following detailed description of the preferred embodiments.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0038] The present invention is described in connection with preferred embodiments. However, it should be appreciated that the invention is not limited to the disclosed embodiments. It is understood that, given the description of the embodiments of the invention herein, various modifications can be made by a person skilled in the art. Such modifications are encompassed by the claims below.

[0039] The term "Bcl-2/Bcl-xL" as used herein means Bcl-2, Bcl-xL, or Bcl-2 and Bcl-xL, i.e., Bcl-2 and/or Bcl-xL.

[0040] The term "a disease or condition wherein inhibition of Bcl-2 and/or Bcl-xL provides a benefit" pertains to a condition in which Bcl-2 and/or Bcl-xL, and/or an action of Bcl-2 and/or Bcl-xL, is important or necessary, e.g., for the onset, progress, expression of that disease or condition, or a disease or a condition which is known to be treated by a Bcl-2/Bcl-xL inhibitor, such as ABT-737 or ABT-263. An example of such a condition includes, but is not limited to, a cancer. One of ordinary skill in the art is readily able to determine whether a compound treats a disease or condition mediated by Bcl-2/Bcl-xL for any particular cell type, for example, by assays which conveniently can be used to assess the activity of particular compounds.

[0041] The term "second therapeutic agent" refers to a therapeutic agent different from a Bcl-2 and/or Bcl-xL inhibitor of structural formula (I), (II), and (III) and that is known to treat the disease or condition of interest. For example when a cancer is the disease or condition of interest, the second therapeutic agent can be a known chemotherapeutic drug, like taxol, or radiation, for example.

[0042] The term "disease" or “condition” denotes disturbances and/or anomalies that as a rule are regarded as being pathological conditions or functions, and that can manifest themselves in the form of particular signs, symptoms, and/or malfunctions. As demonstrated below, compounds of structural formula (I), (II), and (III) are potent inhibitors of Bcl-2/Bcl-

xL and can be used in treating diseases and conditions wherein inhibition of Bcl-2/Bcl-xL provides a benefit.

[0043] As used herein, the terms "treat," "treating," "treatment," and the like refer to eliminating, reducing, or ameliorating a disease or condition, and/or symptoms associated therewith. Although not precluded, treating a disease or condition does not require that the disease, condition, or symptoms associated therewith be completely eliminated. As used herein, the terms "treat," "treating," "treatment," and the like may include "prophylactic treatment," which refers to reducing the probability of redeveloping a disease or condition, or of a recurrence of a previously-controlled disease or condition, in a subject who does not have, but is at risk of or is susceptible to, redeveloping a disease or condition or a recurrence of the disease or condition. The term "treat" and synonyms contemplate administering a therapeutically effective amount of a compound of the invention to an individual in need of such treatment.

[0044] Within the meaning of the invention, "treatment" also includes relapse prophylaxis or phase prophylaxis, as well as the treatment of acute or chronic signs, symptoms and/or malfunctions. The treatment can be orientated symptomatically, for example, to suppress symptoms. It can be effected over a short period, be oriented over a medium term, or can be a long-term treatment, for example within the context of a maintenance therapy.

[0045] The term "therapeutically effective amount" or "effective dose" as used herein refers to an amount of the active ingredient(s) that is(are) sufficient, when administered by a method of the invention, to efficaciously deliver the active ingredient(s) for the treatment of condition or disease of interest to an individual in need thereof. In the case of a cancer or other proliferation disorder, the therapeutically effective amount of the agent may reduce (i.e., retard to some extent and preferably stop) unwanted cellular proliferation; reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., retard to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., retard to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; reduce Bcl-2/Bcl-xL signaling in the target cells; and/or relieve, to some extent, one or more of the symptoms associated with the cancer. To the extent the administered compound or composition prevents growth and/or kills existing cancer cells, it may be cytostatic and/or cytotoxic.

[0046] The term "container" means any receptacle and closure therefor suitable for storing, shipping, dispensing, and/or handling a pharmaceutical product.

[0047] The term "insert" means information accompanying a pharmaceutical product that provides a description of how to administer the product, along with the safety and efficacy data required to allow the physician, pharmacist, and patient to make an informed decision regarding use of the product. The package insert generally is regarded as the "label" for a pharmaceutical product.

[0048] "Concurrent administration," "administered in combination," "simultaneous administration," and similar phrases mean that two or more agents are administered concurrently to the subject being treated. By "concurrently," it is meant that each agent is administered either simultaneously or sequentially in any order at different points in time. However, if not administered simultaneously, it is meant that they are administered to an individual in a sequence and sufficiently close in time so as to provide the desired therapeutic effect and can act in concert. For example, a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) can be administered at the same time or sequentially in any order at different points in time as a second therapeutic agent. A present Bcl-2/Bcl-xL inhibitor and the second therapeutic agent can be administered separately, in any appropriate form and by any suitable route. When a present Bcl-2/Bcl-xL inhibitor and the second therapeutic agent are not administered concurrently, it is understood that they can be administered in any order to a subject in need thereof. For example, a present Bcl-2/Bcl-xL inhibitor can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapeutic agent treatment modality (e.g., radiotherapy), to an individual in need thereof. In various embodiments, a Bcl-2/Bcl-xL inhibitor of structural formula (I) and the second therapeutic agent are administered 1 minute apart, 10 minutes apart, 30 minutes apart, less than 1 hour apart, 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In one embodiment, the components of the combination therapies are administered at 1 minute to 24 hours apart.

[0049] The use of the terms "a", "an", "the", and similar referents in the context of describing the invention (especially in the context of the claims) are to be construed to cover

both the singular and the plural, unless otherwise indicated. Recitation of ranges of values herein are intended to merely serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended to better illustrate the invention and is not a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0050] Over the past decade, research into apoptosis has established that targeting Bcl-2 and/or Bcl-xL using small molecule inhibitors is a viable cancer therapeutic strategy (35-37). The discovery of ABT-737 and ABT-263, and the early clinical data on ABT-263, have demonstrated that non-peptide, small molecule inhibitors of Bcl-2 and/or Bcl-xL have great therapeutic potential for the treatment of many types of human cancer in which Bcl-2 and/or Bcl-xL are overexpressed and for which current anticancer agents are largely ineffective (26-36).

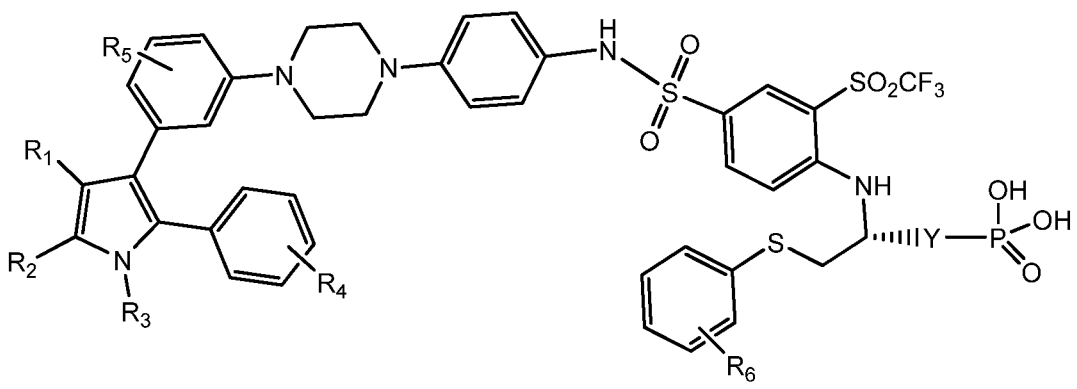
[0051] Despite the discovery of ABT-737 and ABT-263, few new classes of highly potent, small molecule inhibitors of Bcl-2/Bcl-xL with affinities to Bcl-2/Bcl-xL and cellular potencies approaching that achieved by ABT-737/ABT-263 have been reported. This is because the design of small molecule inhibitors of Bcl-2/Bcl-xL involves targeting and blocking the interactions of the Bcl-2/Bcl-xL proteins with their pro-apoptotic binding partners, a task which has been proven to be very challenging for at least three main reasons. First, compared to typical binding sites in enzymes and receptors, the interfaces between Bcl-2 or Bcl-xL and their binding partners are very large (38-42). The interaction of Bcl-2/Bcl-xL with its binding partners, such as BAD and Bim proteins, is mediated by a 20-25 residue BH3 domain in BAD and Bim and a large binding groove in Bcl-2/Bcl-xL. Second, the binding grooves in Bcl-2/Bcl-xL are very hydrophobic in nature, making it difficult to design druglike small molecules (26, 38-42). Third, Bcl-2 and Bcl-xL are extremely conformationally flexible and can adopt quite distinct conformations in the ligand-free structure and when bound to different ligands (26, 38-42). Some of the binding pockets observed for Bcl-xL in the crystal structures of its complexes with BAD (41), Bim (43), and ABT-737(44) are induced by ligand binding and are not presented in a ligand-free crystal structure (38). These three factors make the design of potent and druglike small molecule inhibitors of Bcl-2/Bcl-xL a paramount challenge in modern drug discovery.

[0052] The present invention is directed to new class of potent and specific inhibitors of Bcl-2/Bcl-xL. The present compounds can bind to Bcl-2 and/or Bcl-xL with K_i values <10 nM and function as potent antagonists of Bcl-2 and Bcl-xL in cell-free functional assays. The compounds potently induce apoptosis in cancer cells and have a mechanism of action that is highly consistent with targeting Bcl-2 and Bcl-xL. A tested compound demonstrates robust apoptosis induction *in vivo* in tumor tissues and shows strong antitumor activity against the H146 xenograft tumors.

[0053] The Bcl-2/Bcl-xL inhibitors of the present invention therefore are useful in the treatment of unwanted proliferating cells, including cancers and precancers, in subjects in need of such treatment. Also provided are methods of treating a subject having unwanted proliferating cells comprising administering a therapeutically effective amount of a present compound to a subject in need of such treatment. Also provided are methods of preventing the proliferation of unwanted proliferating cells, such as cancers and precancers, in a subject comprising the step of administering a therapeutically effective amount of a compound of structural formula (I) to a subject at risk of developing a condition characterized by unwanted proliferating cells. In some embodiments, the compounds of structural formula (I), (II), and (III) reduced the proliferation of unwanted cells by inducing apoptosis in those cells.

[0054] The present invention is directed to Bcl-2/Bcl-xL inhibitors having a structural formula (I), (II), or (III):

(I)



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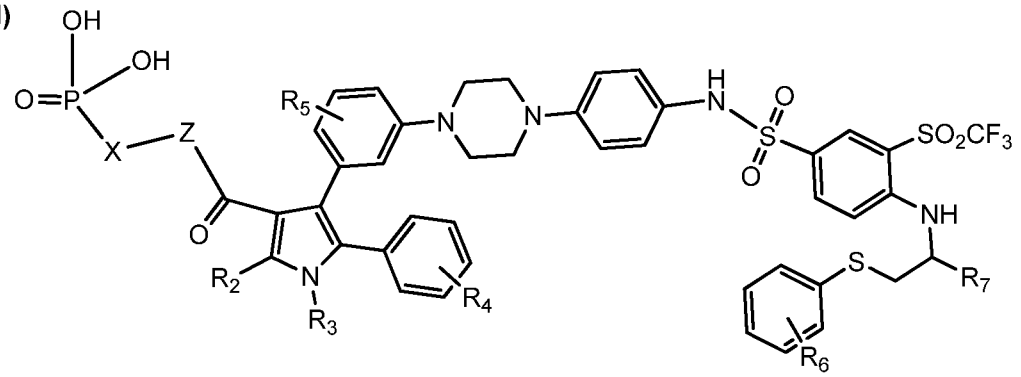
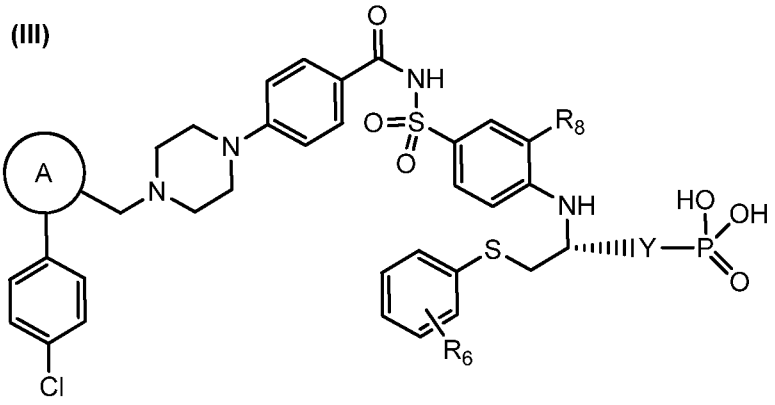
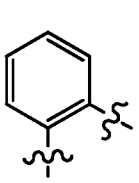
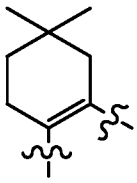


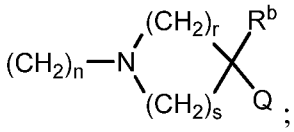
(III)



[0055] wherein the A ring is  or  ;

[0056] X, substituted or unsubstituted, is selected from the group consisting of alkylene, alkenylene, cycloalkylene, cycloalkenylene, and heterocycloalkylene;

[0057] Y is selected from the group consisting of $(CH_2)_n$-$N(R^a)_2$ and

[0058]  ;

[0059] Q is selected from the group consisting of O, $O(CH_2)_{1-3}$, NR^c, $NR^c(C_{1-3}alkylene)$, $OC(=O)(C_{1-3}alkylene)$, C(=O)O, $C(=O)O(C_{1-3}alkylene)$, $NHC(=O)(C_{1-3}alkylene)$, C(=O)NH, and $C(=O)NH(C_{1-3}alkylene)$;

[0060] Z is O or NR^c;

[0061] R_1 and R_2, independently, are selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", COR', CO_2R', OCOR', CONR'R", $CONR'SO_2R"$, NR'COR", NR'CONR"R'", NR'C=SNR"R'", $NR'SO_2R"$, SO_2R', and $SO_2NR'R"$;

[0062] R_3 is selected from a group consisting of H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', NR'R", OCOR', CO_2R', COR', CONR'R", $CONR'SO_2R"$, $C_{1-3}alkyleneCH(OH)CH_2OH$, SO_2R', and $SO_2NR'R"$;

[0063] R', R", and R'", independently, are H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, $C_{1-3}alkyleneheterocycloalkyl$, or heterocycloalkyl;

[0064] R' and R", or R" and R'", can be taken together with the atom to which they are bound to form a 3 to 7 membered ring;

[0065] R_4 is hydrogen, halo, C_{1-3}alkyl, CF_3, or CN;

[0066] R_5 is hydrogen, halo, C_{1-3}alkyl, substituted C_{1-3}alkyl, hydroxyalkyl, alkoxy, or substituted alkoxy;

[0067] R_6 is selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", CO_2R', OCOR', CONR'R", CONR'SO_2R", NR'COR", NR'CONR"R'", NR'C=SNR"R'", NR'SO_2R", SO_2R', and SO_2NR'R";

[0068] R_7, substituted or unsubstituted, is selected form the group consisting of hydrogen, alkyl, alkenyl, $(CH_2)_{0-3}$cycloalkyl, $(CH_2)_{0-3}$cycloalkenyl, $(CH_2)_{0-3}$heterocycloalkyl, $(CH_2)_{0-3}$aryl, and $(CH_2)_{0-3}$heteroaryl;

[0069] R_8 is selected form the group consisting of hydrogen, halo, NO_2, CN, CF_3SO_2, and CF_3;

[0070] R_a is selected from the group consisting of hydrogen, alkyl, heteroalkyl, alkenyl, hydroxyalkyl, alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, and heterocycloalkyl;

[0071] R_b is hydrogen or alkyl;

[0072] R_c is selected from the group consisting of hydrogen, alkyl, substituted alkyl, hydroxyalkyl, alkoxy, and substituted alkoxy; and

[0073] n, r, and s, independently, are 1, 2, 3, 4, 5, or 6;

[0074] or a pharmaceutically acceptable salt of (I), (II), or (III).

[0075] The compounds of structural formula (I), (II), and (III) inhibit Bcl-2/Bcl-xL and are useful in the treatment of a variety of diseases and conditions. In particular, the compounds of structural formula (I), (II), and (III) are used in methods of treating a disease or condition wherein inhibition of Bcl-2/Bcl-xL provides a benefit, for example, cancers. The method comprises administering a therapeutically effective amount of a compound of structural formula (I), (II), or (III) to an individual in need thereof. The present methods also encompass administering a second therapeutic agent to the individual in addition to the compound of structural formula (I), (II), or (III). The second therapeutic agent is selected from drugs known as useful in treating the disease or condition afflicting the individual in need thereof, e.g., a chemotherapeutic agent and/or radiation known as useful in treating a particular cancer.

[0076] As used herein, the term "alkyl" refers to straight chained and branched saturated C_{1-10} hydrocarbon groups, nonlimiting examples of which include methyl, ethyl, and straight chain and branched propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, and decyl groups. The term C_n means the alkyl group has "n" carbon atoms. The term C_{n-p} means that the alkyl group contains "n" to "p" carbon atoms. The term "alkylene" refers to an alkyl group having a substituent. An alkyl, e.g., methyl, or alkylene, e.g., —CH_2—, group can be unsubstituted or substituted with halo, trifluoromethyl, trifluoromethoxy, hydroxy, alkoxy, nitro, cyano, alkylamino, or amino groups, for example.

[0077] The term "alkenyl" is defined identically as "alkyl," except for containing a carbon-carbon double bond, e.g., ethenyl, propenyl, and butenyl. The term "alkenylene" is defined identically to "alkylene" except for containing a carbon-carbon double bond. The term "alkynyl" and "alkynylene" are defined identically as "alkyl" and "alkylene" except the group contains a carbon-carbon triple bond.

[0078] As used herein, the term "halo" is defined as fluoro, chloro, bromo, and iodo.

[0079] The term "hydroxy" is defined as —OH.

[0080] The term "alkoxy" is defined as —OR, wherein R is alkyl.

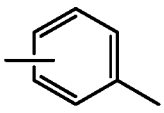
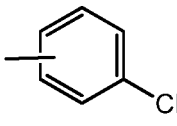
[0081] The term "amino" is defined as —NH_2, and the term "alkylamino" is defined as —NR_2, wherein at least one R is alkyl and the second R is alkyl or hydrogen.

[0082] The term "nitro" is defined as —NO_2.

[0083] The term "cyano" is defined as —CN.

[0084] The term "trifluoromethyl" is defined as —CF_3.

[0085] The term "trifluoromethoxy" is defined as —OCF_3.

[0086] As used herein, groups such as  is an abbreviation for 

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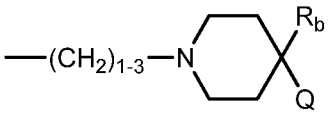
[0087] As used herein, the term "aryl" refers to a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl. Unless otherwise indicated, an aryl group can be unsubstituted or substituted with one or more, and in particular one to four, groups independently selected from, for example, halo, alkyl, alkenyl, —OCF_3, —CF_3, —NO_2, —CN, —NC, —OH, alkoxy, amino, alkylamino, —CO_2H, —CO_2alkyl, —OCOalkyl, aryl, and heteroaryl.

[0088] As used herein, the term "heteroaryl" refers to a monocyclic or bicyclic ring system containing one or two aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring. Unless otherwise indicated, a heteroaryl group can be unsubstituted or substituted with one or more, and in particular one to four, substituents selected from, for example, halo, alkyl, alkenyl, —OCF_3, —CF_3, —NO_2, —CN, —NC, —OH, alkoxy, amino, alkylamino, —CO_2H, —CO_2alkyl, —OCOalkyl, aryl, and heteroaryl.

[0089] As used herein, the term "cycloalkyl" means a monocyclic aliphatic ring containing three to eight carbon atoms. The term "heterocycloalkyl" means a monocyclic or bicyclic ring system containing at least one nitrogen, oxygen, or sulfur atom in the ring system. The terms "heteroaryl" and "heterocycloalkyl" encompass ring systems containing at least one oxygen atom, nitrogen atom, or sulfur atom, and includes ring systems containing oxygen and nitrogen atoms, oxygen and sulfur atoms, nitrogen and sulfur atoms, and nitrogen, oxygen, and sulfur atoms.

In some preferred embodiments, X is alkylene, and in preferred embodiments, is C_{1-3}alkylene.

[0090] In some embodiments, Y is

[0091] 

In preferred embodiments, n is 2. In other preferred embodiments, R_b is hydrogen or C_{1-3}alkyl.

[0092] In still other preferred embodiments, Q is O, $O(CH_2)_{1-3}$, $C(=O)O(CH_2)_{1-3}$, $OC(=O)(CH_2)_{1-3}$, or $C(=O)O(C_3H_7)_{1-3}$. In some embodiments, Q is O, OCH_2, $C(=O)OCH_2$, $C(=O)O(CH_2)_2$, $C(=O)O(CH_2)_3$, $OC(=O)CH_2$, or $C(=O)O(CH(CH_3)CH_2)$.

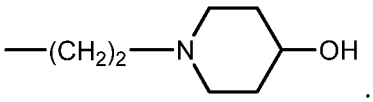
[0093] In some embodiments, Z is O, NH, or N(C_{1-3}alkyl). In preferred embodiments, Z is O, NH, or NCH_3.

[0094] In some embodiments, R_1 is SO_2R', $SO_2NR'R''$, NR'SOR'', H, or alkyl. In some preferred embodiments, R_1 is $SO_2(C_{1-3}alkyl)$, $SO_2N(C_{1-3}alkyl)_2$, $NHSO_2(C_{1-3}alkyl)$, H, or C_{1-3}alkyl. One preferred embodiment of R_1 is SO_2CH_3.

[0095] In some embodiments, R_2 and R_3, independently, are H, C_{1-3}alkyl, or cycloalkyl. R_2 also can be halo. In some preferred embodiments, R_2 and R_3, independently, are methyl, ethyl, n-propyl, isopropyl, cyclopentyl, or cyclohexyl. R_2 also can be Cl or F.

[0096] In some embodiments, R_4 is H, Cl, or F. In other embodiments, R_5 is H, methyl, ethyl, n-propyl, isopropyl, F, or Cl. In other embodiments, R_6 is H, halo, alkyl, or cycloalkyl. In some preferred embodiments, R_6 is H, F, Cl, C_{1-3}alkyl, cyclopentyl, or cyclohexyl.

[0097] In some embodiments, R_7 is $(CH_2)_{0-3}$cycloalkyl or $(CH_2)_{0-3}$heterocycloalkyl. In a preferred embodiment, R_7 is $(CH_2)_{0-3}$cycloalkyl, optionally substituted with –OH. In one embodiment, R_7 is

[0098]  .

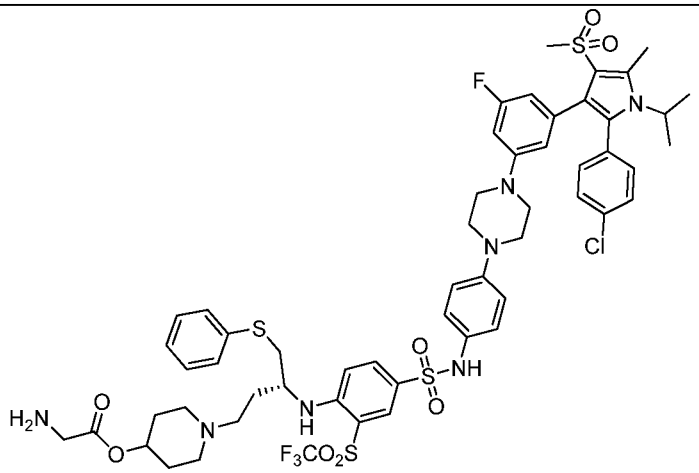
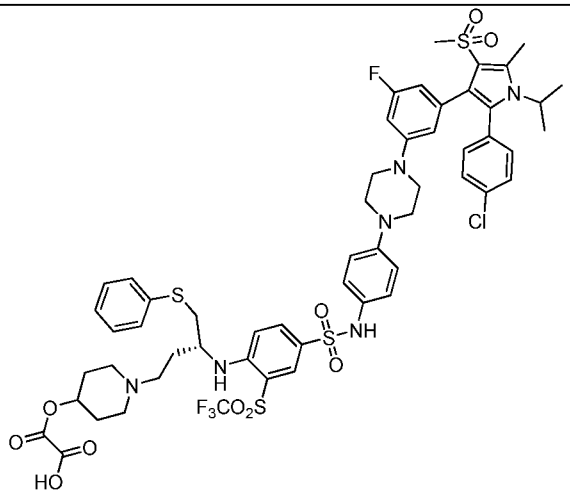
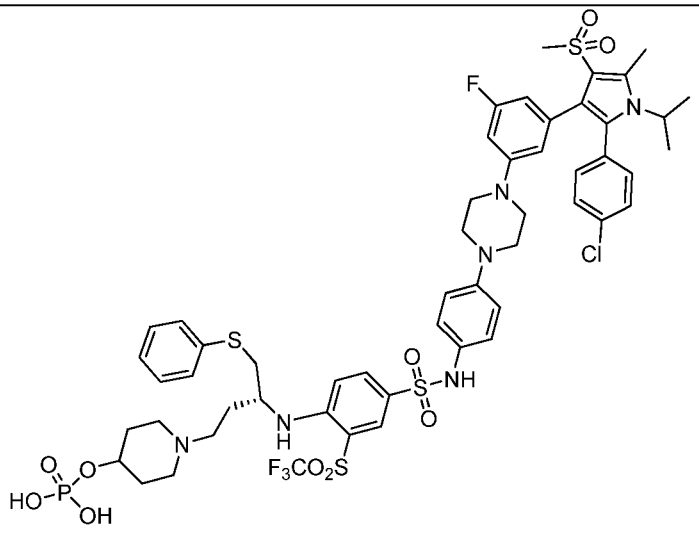
[0099] In some embodiments, R_8 is $CFSO_2$ or CF_3. In various embodiments, R_a, R_b, and R_c, independently, are H or C_{1-3}alkyl.

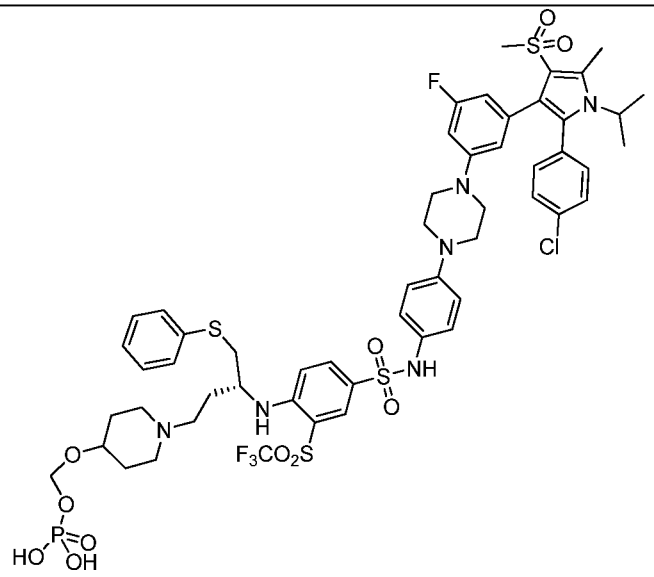
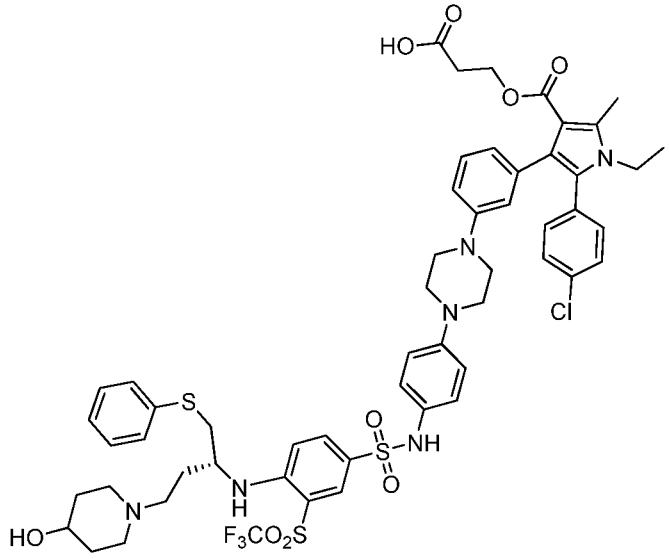
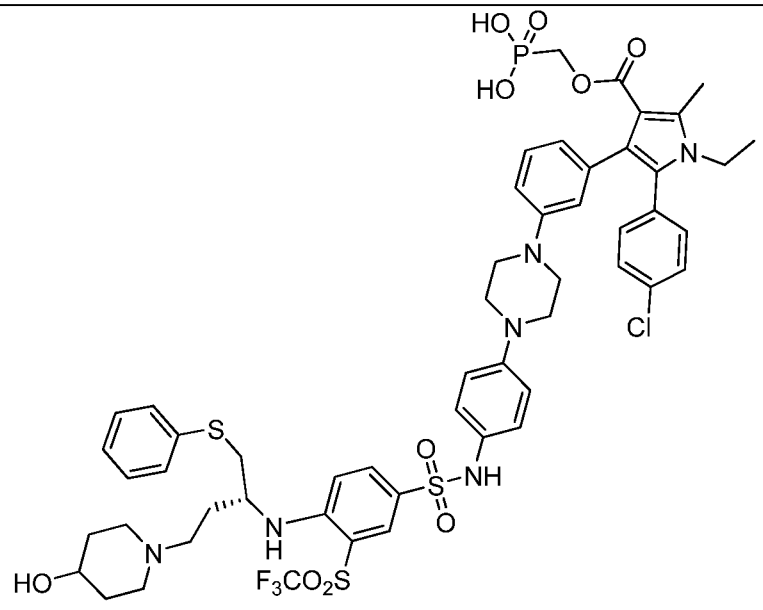
[0100] Additionally, salts, hydrates, and solvates of the present compounds also are included in the present invention and can be used in the methods disclosed herein. The present invention further includes all possible stereoisomers and geometric isomers of the compounds of structural formula (I), (II), and (III). The present invention includes both racemic compounds and optically active isomers. When a compound of structural formula (I), (II), or (III) is desired as a single enantiomer, it can be obtained either by resolution of the final product or by stereospecific synthesis from either isomerically pure starting material or use of a chiral auxiliary reagent, for example, see Z. Ma et al., *Tetrahedron: Asymmetry, 8(6)*, pages 883-888 (1997). Resolution of the final product, an intermediate, or a starting material can be achieved by any suitable method known in the art. Additionally, in situations where tautomers of the compounds of structural formula (I), (II), or (III) are possible, the present invention is intended to include all tautomeric forms of the compounds.

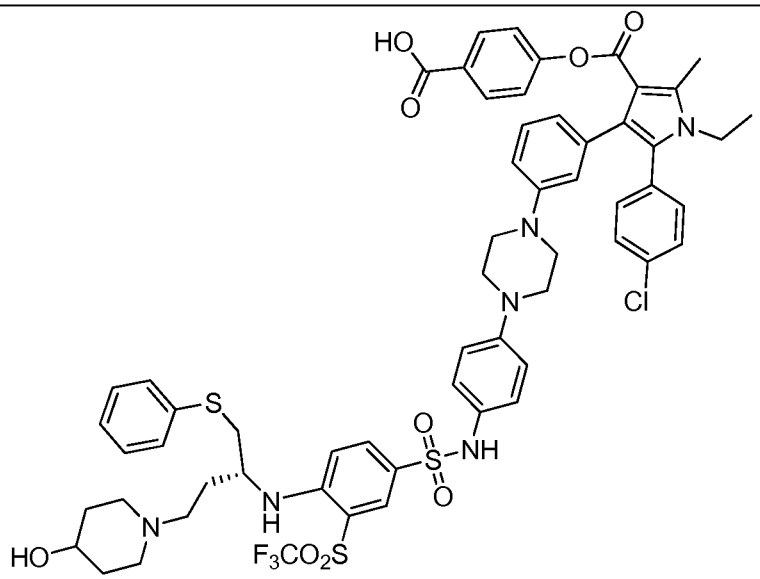
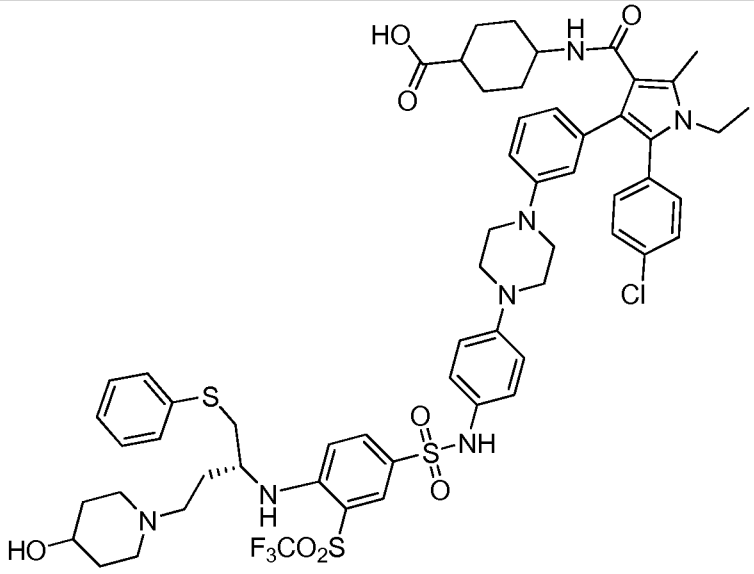
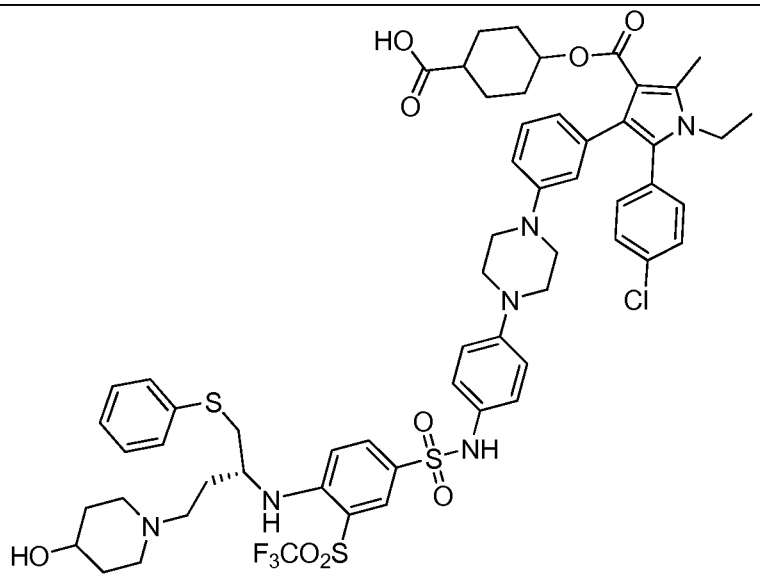
[0101] Compounds of the invention can exist as salts. Pharmaceutically acceptable salts of the compounds of the invention often are preferred in the methods of the invention. As used herein, the term "pharmaceutically acceptable salts" refers to salts or zwitterionic forms of the compounds of structural formula (I), (II), and (III). Salts of compounds of formula (I), (II), and (III) can be prepared during the final isolation and purification of the compounds or separately by reacting the compound with an acid having a suitable cation. The pharmaceutically acceptable salts of compounds of structural formula (I), (II), and (III) can be acid addition salts formed with pharmaceutically acceptable acids. Examples of acids which can be employed to form pharmaceutically acceptable salts include inorganic acids such as nitric, boric, hydrochloric, hydrobromic, sulfuric, and phosphoric, and organic acids such as oxalic, maleic, succinic, and citric. Nonlimiting examples of salts of compounds of

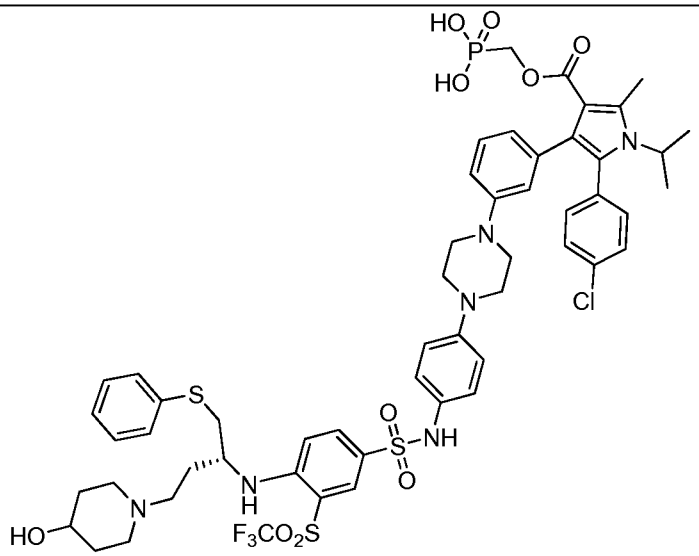
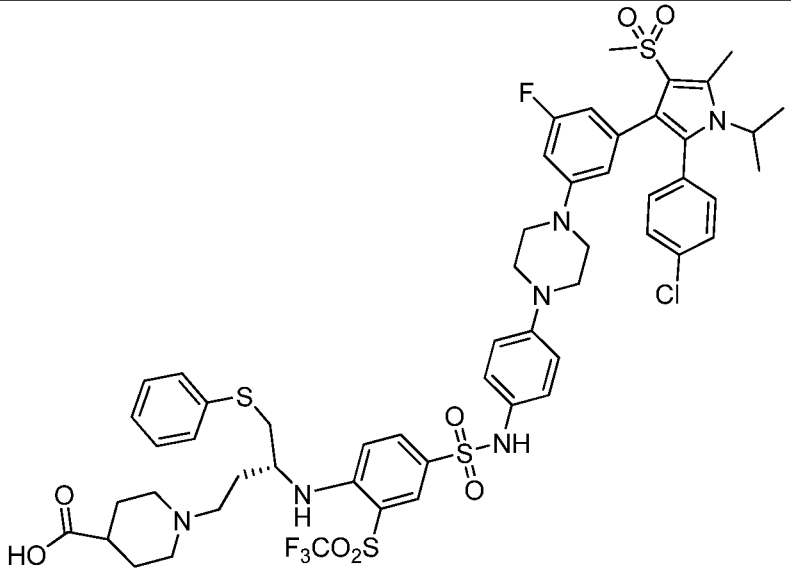
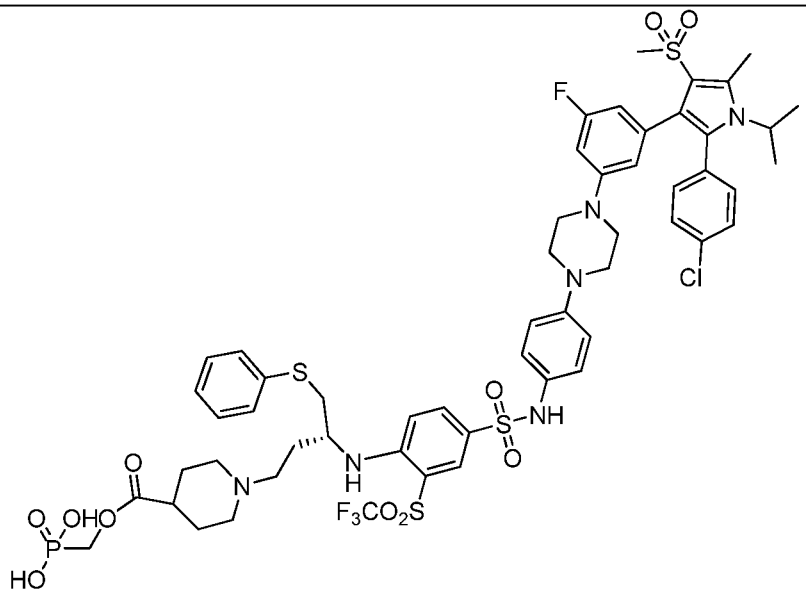
the invention include, but are not limited to, the hydrochloride, hydrobromide, hydroiodide, sulfate, bisulfate, 2-hydroxyethansulfonate, phosphate, hydrogen phosphate, acetate, adipate, alginate, aspartate, benzoate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerolphsphate, hemisulfate, heptanoate, hexanoate, formate, succinate, fumarate, maleate, ascorbate, isethionate, salicylate, methanesulfonate, mesitylenesulfonate, naphthylenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylproprionate, picrate, pivalate, propionate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, paratoluenesulfonate, undecanoate, lactate, citrate, tartrate, gluconate, methanesulfonate, ethanedisulfonate, benzene sulphonate, and p-toluenesulfonate salts. In addition, available amino groups present in the compounds of the invention can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and steryl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. In light of the foregoing, any reference to compounds of the present invention appearing herein is intended to include compounds of structural formula (I), (II), and (III), as well as pharmaceutically acceptable salts, hydrates, or solvates thereof.

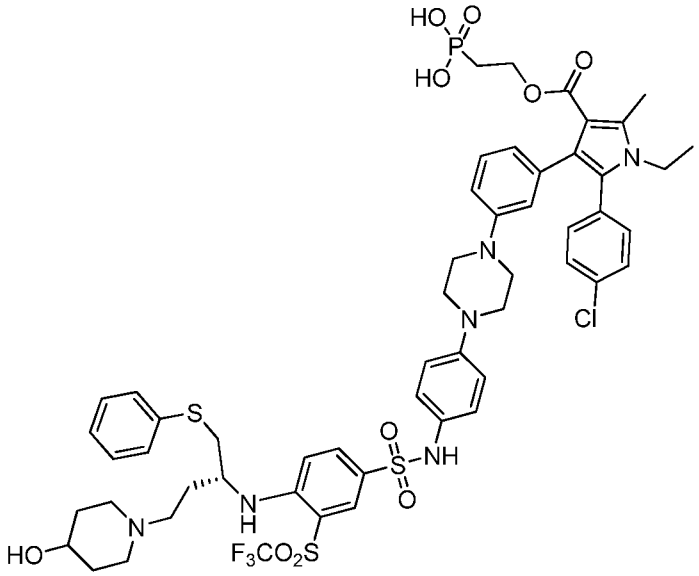
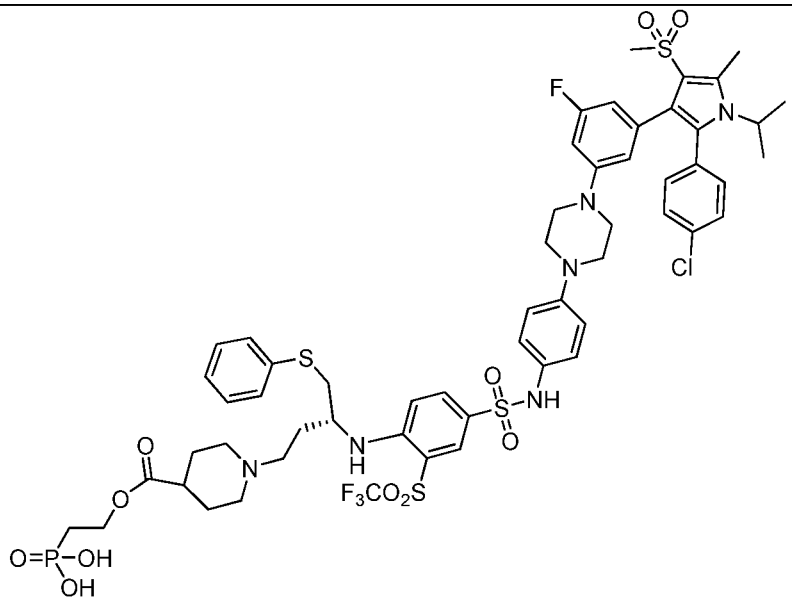
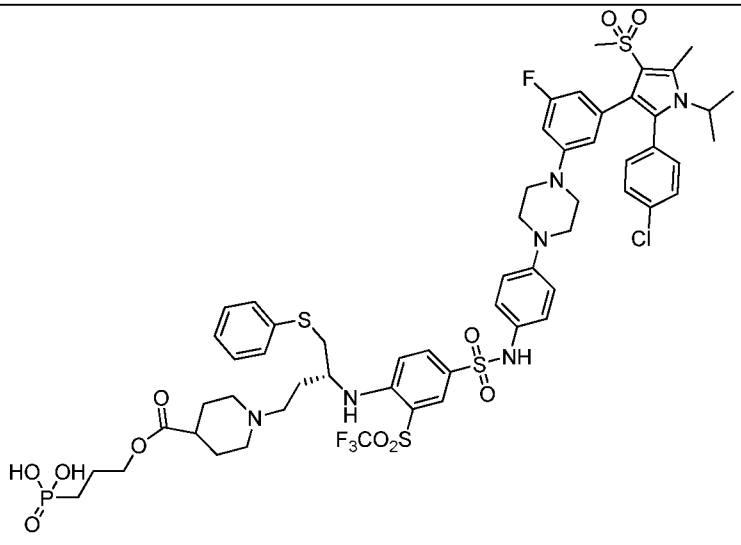
[0102] Specific compounds of the present invention include, but are not limited to, compounds having the structure set forth below.

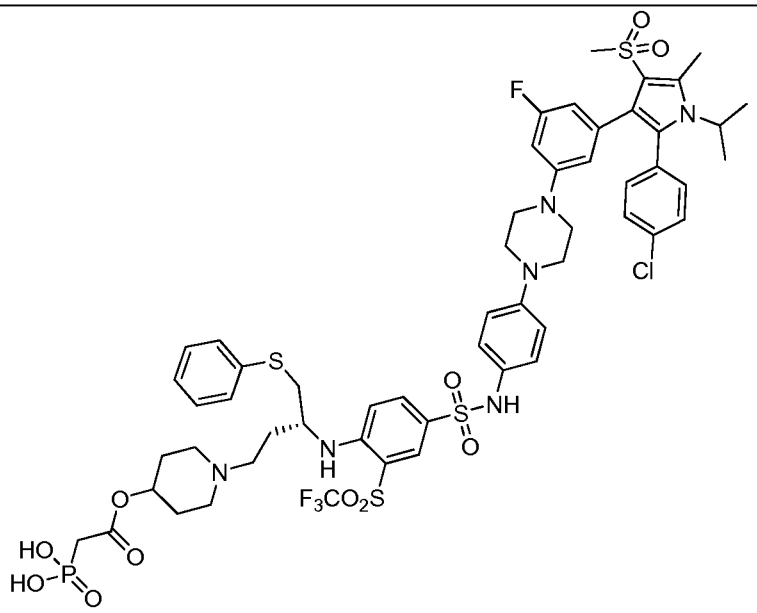
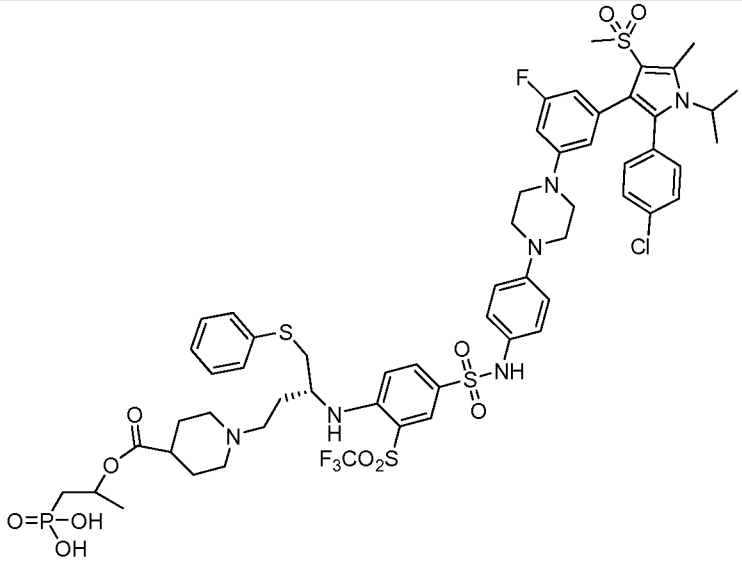
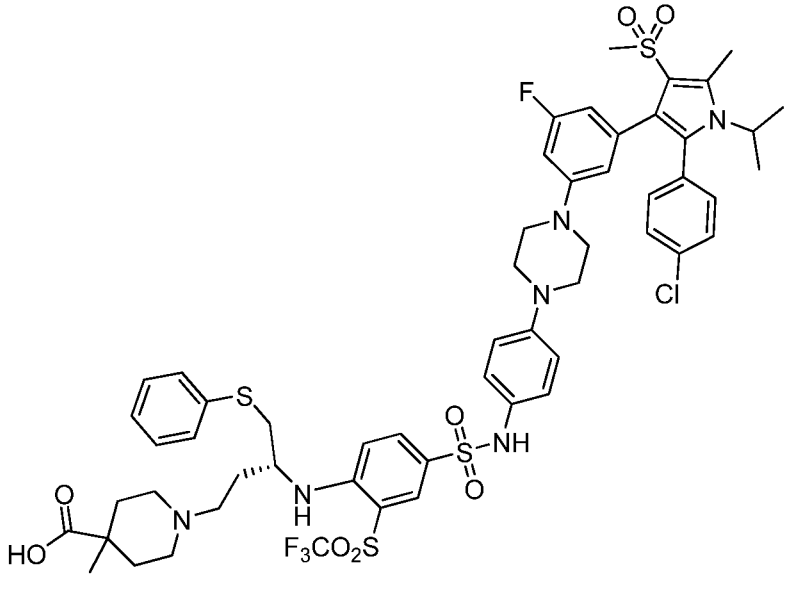
Compound No.	Structure
1	
2	
3	

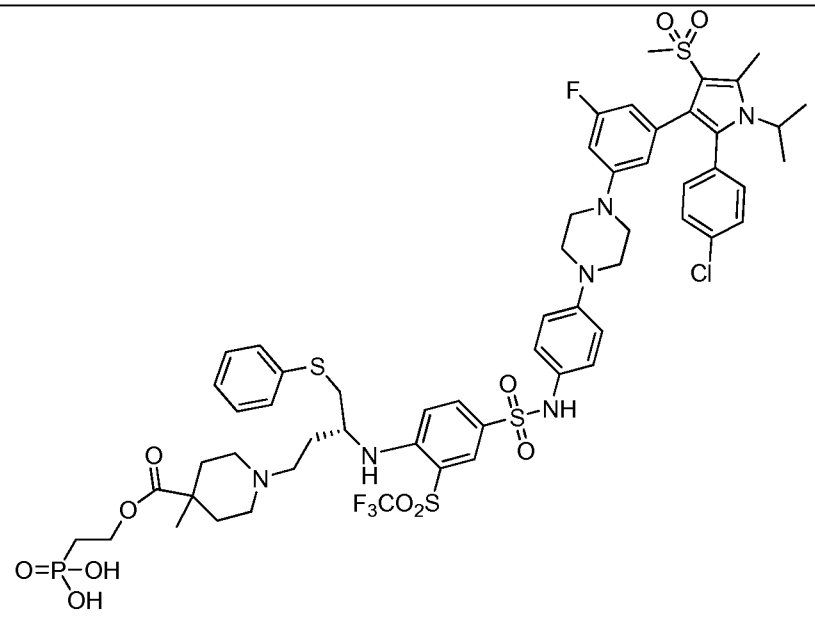
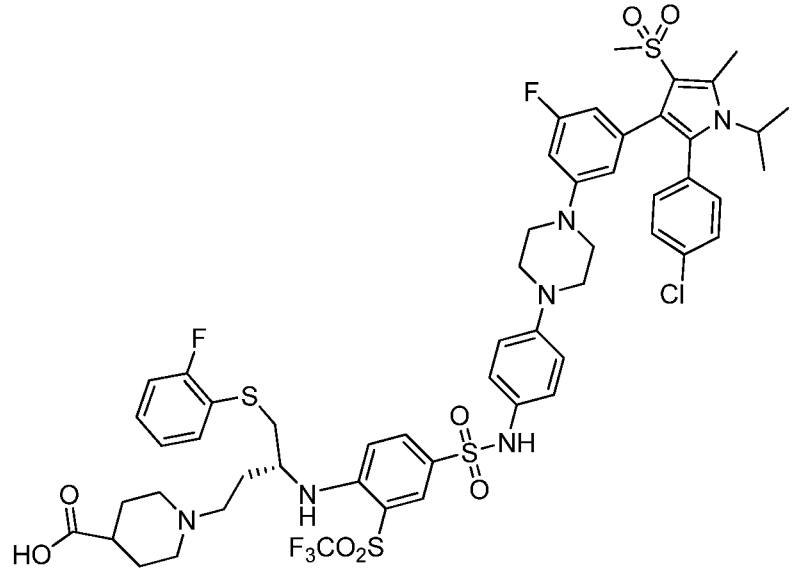
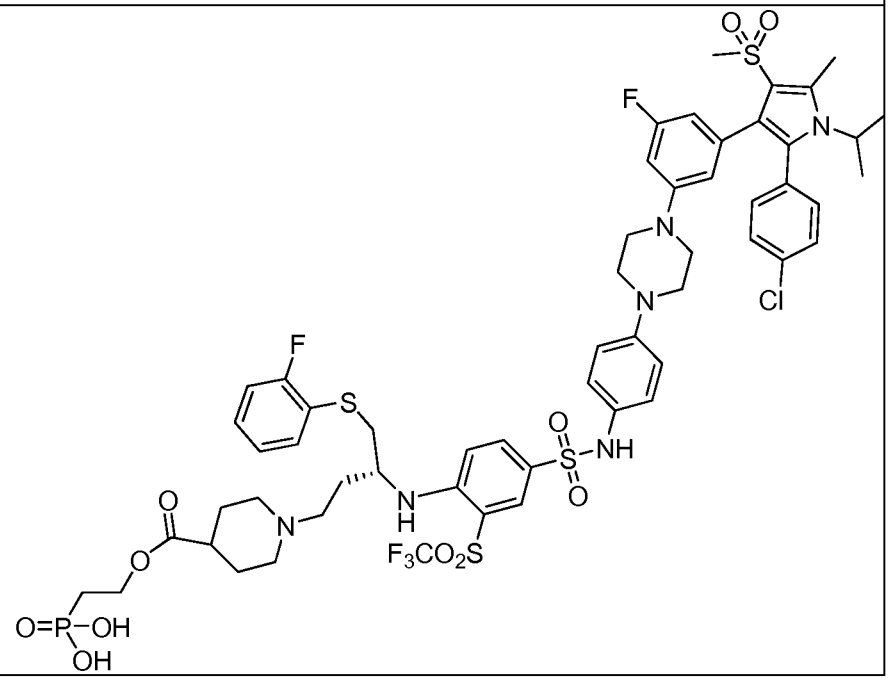
4	
5	
6	

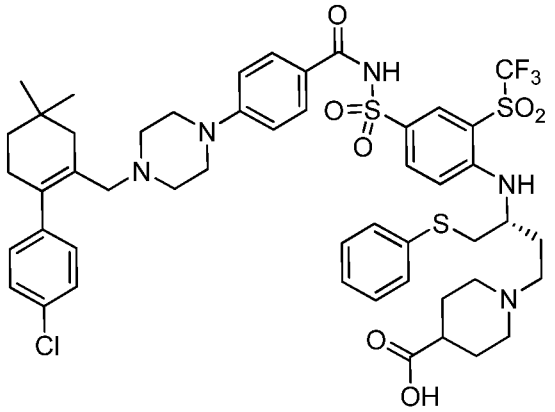
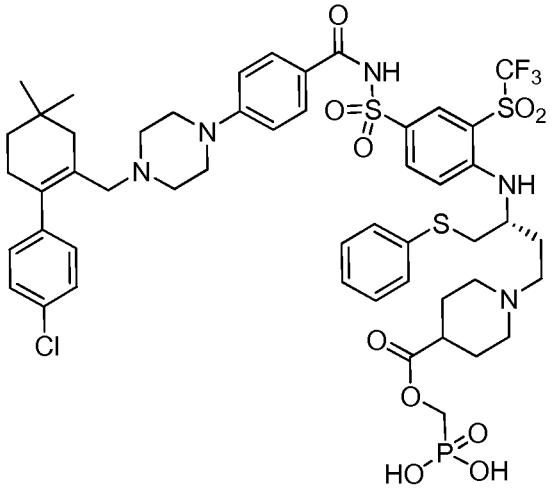
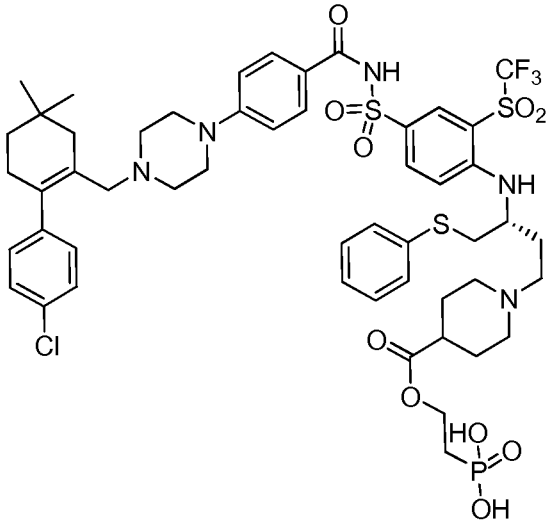
7	
8	
9	

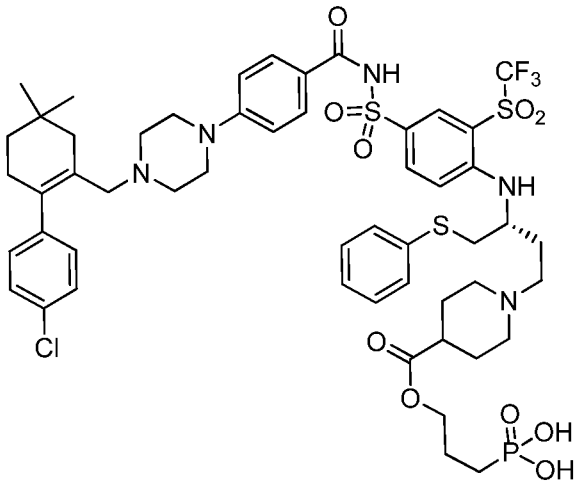
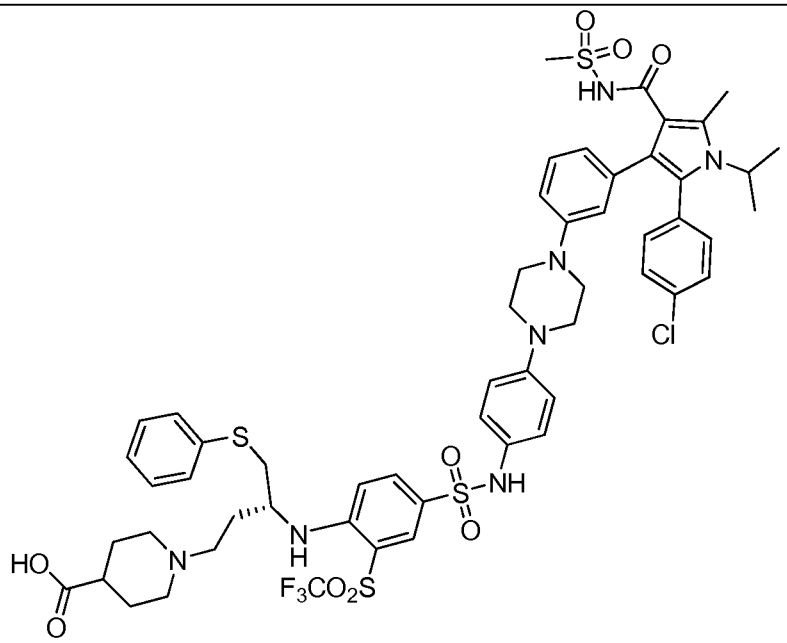
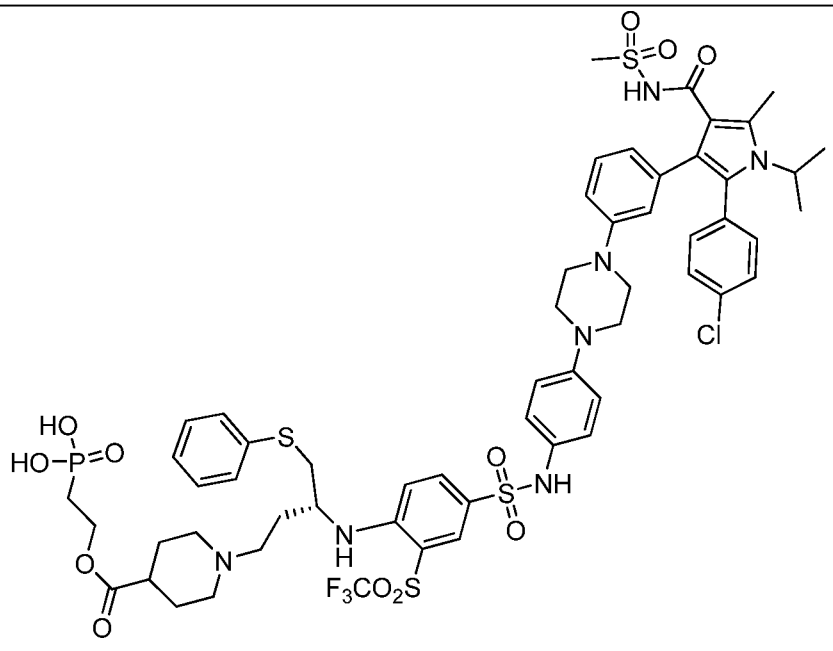
10	
11	
12	

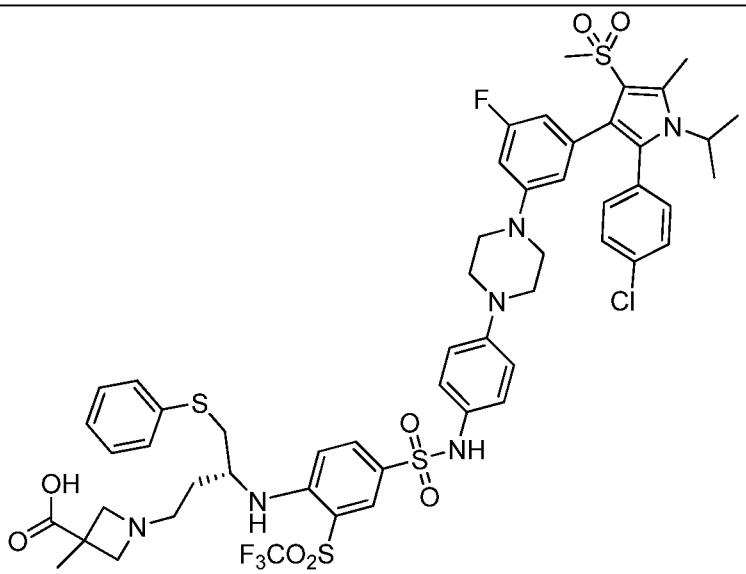
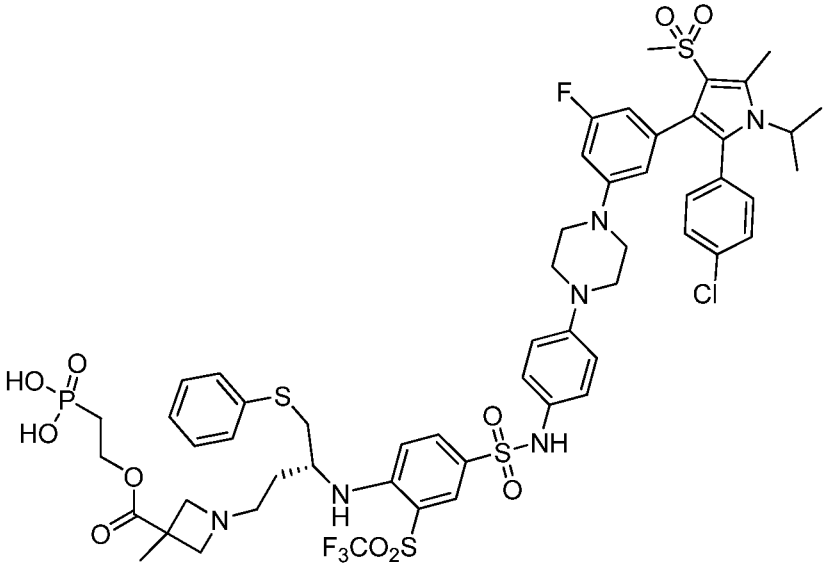
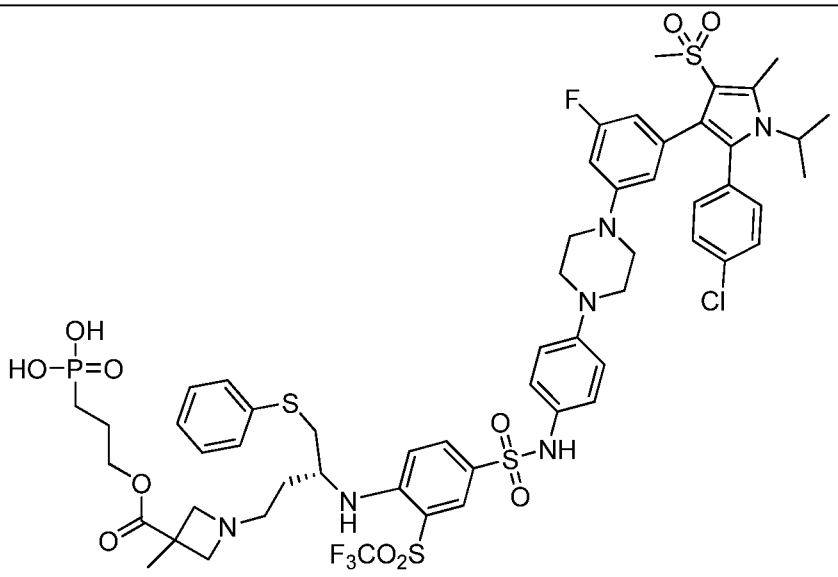
13	
14	
15	

16	
17	
18	

19	
20	
21	

22	
23	
24	

25	
26	
27	

28	
29	
30	

[0103] The present invention provides Bcl-2/Bcl-xL inhibitors, as exemplified by compounds of structural formula (I), (II), and (III), for the treatment of a variety of diseases and conditions wherein inhibition of Bcl-2 and/or Bcl-xL has a beneficial effect. In one embodiment, the present invention relates to a method of treating an individual suffering from a disease or condition wherein inhibition of the Bcl-2/Bcl-xL provides a benefit comprising administering a therapeutically effective amount of a compound of structural formula (I), (II), or (III) to an individual in need thereof.

[0104] The method of the present invention can be accomplished by administering a compound of structural formula (I), (II), or (III) as the neat compound or as a pharmaceutical composition. Administration of a pharmaceutical composition, or neat compound of structural formula (I), (II), or (III), can be performed during or after the onset of the disease or condition of interest. Typically, the pharmaceutical compositions are sterile, and contain no toxic, carcinogenic, or mutagenic compounds that would cause an adverse reaction when administered. Further provided are kits comprising a compound of structural formula (I), (II), or (III) and, optionally, a second therapeutic agent useful in the treatment of diseases and conditions wherein inhibition of Bcl-2/Bcl-xL provides a benefit, packaged separately or together, and an insert having instructions for using these active agents.

[0105] In many embodiments, a compound of structural formula (I), (II), or (III) is administered in conjunction with a second therapeutic agent useful in the treatment of a disease or condition wherein inhibition of Bcl-2/Bcl-xL provides a benefit. The second therapeutic agent is different from the compound of structural formula (I), (II), and (III). A compound of structural formula (I), (II), or (III) and the second therapeutic agent can be administered simultaneously or sequentially to achieve the desired effect. In addition, the compound of structural formula (I), (II), or (III) and second therapeutic agent can be administered from a single composition or two separate compositions.

[0106] The second therapeutic agent is administered in an amount to provide its desired therapeutic effect. The effective dosage range for each second therapeutic agent is known in the art, and the second therapeutic agent is administered to an individual in need thereof within such established ranges.

[0107] A compound of structural formula (I), (II), or (III) and the second therapeutic agent can be administered together as a single-unit dose or separately as multi-unit doses, wherein the compound of structural formula (I), (II), or (III) is administered before the second therapeutic agent or vice versa. One or more dose of the compound of structural formula (I),

(II), or (III) and/or one or more dose of the second therapeutic agent can be administered. The compounds of structural formula (I), (II), and (III) therefore can be used in conjunction with one or more second therapeutic agents, for example, but not limited to, anticancer agents.

[0108] The diseases and conditions that can be treated in accordance to the invention include, for example, cancers. A variety of cancers can be treated including, but not limited to: carcinomas, including bladder (including accelerated and metastatic bladder cancer), breast, colon (including colorectal cancer), kidney, liver, lung (including small and non-small cell lung cancer and lung adenocarcinoma), ovary, prostate, testes, genitourinary tract, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), esophagus, stomach, gall bladder, cervix, thyroid, renal, and skin (including squamous cell carcinoma); hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, histiocytic lymphoma, and Burketts lymphoma, hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias, myelodysplastic syndrome, myeloid leukemia, and promyelocytic leukemia; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarcoma, and osteosarcoma; and other tumors, including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, renal cell carcinoma (RCC), pancreatic cancer, myeloma, myeloid and lymphoblastic leukemia, neuroblastoma, and glioblastoma.

[0109] Additional forms of cancer treatable by the Bcl-2/Bcl-xL inhibitors of the present invention include, for example, adult and pediatric oncology, growth of solid tumors/malignancies, myxoid and round cell carcinoma, locally advanced tumors, metastatic cancer, human soft tissue sarcomas, including Ewing's sarcoma, cancer metastases, including lymphatic metastases, squamous cell carcinoma, particularly of the head and neck, esophageal squamous cell carcinoma, oral carcinoma, blood cell malignancies, including multiple myeloma, leukemias, including acute lymphocytic leukemia, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, and hairy cell leukemia, effusion lymphomas (body cavity based lymphomas), thymic lymphoma lung cancer (including small cell carcinoma, cutaneous T cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, cancer of the adrenal cortex, ACTH-producing tumors, nonsmall cell cancers, breast cancer, including small cell carcinoma and ductal carcinoma),

gastrointestinal cancers (including stomach cancer, colon cancer, colorectal cancer, and polyps associated with colorectal neoplasia), pancreatic cancer, liver cancer, urological cancers (including bladder cancer, such as primary superficial bladder tumors, invasive transitional cell carcinoma of the bladder, and muscle-invasive bladder cancer), prostate cancer, malignancies of the female genital tract (including ovarian carcinoma, primary peritoneal epithelial neoplasms, cervical carcinoma, uterine endometrial cancers, vaginal cancer, cancer of the vulva, uterine cancer and solid tumors in the ovarian follicle), malignancies of the male genital tract (including testicular cancer and penile cancer), kidney cancer (including renal cell carcinoma, brain cancer (including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, and metastatic tumor cell invasion in the central nervous system), bone cancers (including osteomas and osteosarcomas), skin cancers (including malignant melanoma, tumor progression of human skin keratinocytes, and squamous cell cancer), thyroid cancer, retinoblastoma, neuroblastoma, peritoneal effusion, malignant pleural effusion, mesothelioma, Wilms's tumors, gall bladder cancer, trophoblastic neoplasms, hemangiopericytoma, and Kaposi's sarcoma.

[0110] Additional diseases and conditions, including cancers, that can be treated by administration of a present Bcl-2/Bcl-xL inhibitor are disclosed in U.S. Patent Publication No. 2007/0027135; U.S. Patent No. 7,432,304; U.S. Patent Publication No. 2010/0278921; and WO 2012/017251, designating the U.S., each incorporated herein in its entirety.

[0111] In the present method, a therapeutically effective amount of one or more compound (I), (II), or (III), typically formulated in accordance with pharmaceutical practice, is administered to a human being in need thereof. Whether such a treatment is indicated depends on the individual case and is subject to medical assessment (diagnosis) that takes into consideration signs, symptoms, and/or malfunctions that are present, the risks of developing particular signs, symptoms and/or malfunctions, and other factors.

[0112] A compound of structural formula (I), (II), or (III) can be administered by any suitable route, for example by oral, buccal, inhalation, sublingual, rectal, vaginal, intracisternal or intrathecal through lumbar puncture, transurethral, nasal, percutaneous, i.e., transdermal, or parenteral (including intravenous, intramuscular, subcutaneous, intracoronary, intradermal, intramammary, intraperitoneal, intraarticular, intrathecal, retrobulbar, intrapulmonary injection and/or surgical implantation at a particular site) administration. Parenteral administration can be accomplished using a needle and syringe or using a high pressure technique.

[0113] Pharmaceutical compositions include those wherein a compound of structural formula (I), (II), or (III) is administered in an effective amount to achieve its intended purpose. The exact formulation, route of administration, and dosage is determined by an individual physician in view of the diagnosed condition or disease. Dosage amount and interval can be adjusted individually to provide levels of a compound of structural formula (I), (II), or (III) that is sufficient to maintain therapeutic effects.

[0114] Toxicity and therapeutic efficacy of the compounds of structural formula (I), (II), and (III) can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the maximum tolerated dose (MTD) of a compound, which defines as the highest dose that causes no toxicity in animals. The dose ratio between the maximum tolerated dose and therapeutic effects (e.g. inhibiting of tumor growth) is the therapeutic index. The dosage can vary within this range depending upon the dosage form employed, and the route of administration utilized. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0115] A therapeutically effective amount of a compound of structural formula (I), (II), or (III) required for use in therapy varies with the nature of the condition being treated, the length of time that activity is desired, and the age and the condition of the patient, and ultimately is determined by the attendant physician. Dosage amounts and intervals can be adjusted individually to provide plasma levels of the Bcl-2/Bcl-xL inhibitor that are sufficient to maintain the desired therapeutic effects. The desired dose conveniently can be administered in a single dose, or as multiple doses administered at appropriate intervals, for example as one, two, three, four or more subdoses per day. Multiple doses often are desired, or required. For example, a present Bcl-2/Bcl-xL inhibitor can be administered at a frequency of: one dose per day for 2 days with rest for 5 days for 2 weeks; one dose per day for 3 days with rest for 4 days for 3 weeks; weekly dosing for 2 weeks; weekly dosing for 4 weeks; or, any dose regimen determined to be appropriate for the circumstance.

[0116] A compound of structural formula (I), (II), or (III) used in a method of the present invention can be administered in an amount of about 0.005 to about 500 milligrams per dose, about 0.05 to about 250 milligrams per dose, or about 0.5 to about 100 milligrams per dose. For example, a compound of structural formula (I), (II), or (III) can be administered, per dose, in an amount of about 0.005, 0.05, 0.5, 5, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 milligrams, including all doses between 0.005 and 500 milligrams.

[0117] The dosage of a composition containing a Bcl-2/Bcl-xL inhibitor of structural formula (I), (II), or (III) or a composition containing the same, can be from about 1 ng/kg to about 200 mg/kg, about 1 μg/kg to about 100 mg/kg, or about 1 mg/kg to about 50 mg/kg. The dosage of a composition can be at any dosage including, but not limited to, about 1 μg/kg. The dosage of a composition may be at any dosage including, but not limited to, about 1 μg/kg, 10 μg/kg, 25 μg/kg, 50 μg/kg, 75 μg/kg, 100 μg/kg, 125 μg/kg, 150 μg/kg, 175 μg/kg, 200 μg/kg, 225 μg/kg, 250 μg/kg, 275 μg/kg, 300 μg/kg, 325 μg/kg, 350 μg/kg, 375 μg/kg, 400 μg/kg, 425 μg/kg, 450 μg/kg, 475 μg/kg, 500 μg/kg, 525 μg/kg, 550 μg/kg, 575 μg/kg, 600 μg/kg, 625 μg/kg, 650 μg/kg, 675 μg/kg, 700 μg/kg, 725 μg/kg, 750 μg/kg, 775 μg/kg, 800 μg/kg, 825 μg/kg, 850 μg/kg, 875 μg/kg, 900 μg/kg, 925 μg/kg, 950 μg/kg, 975 μg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, or 200 mg/kg. The above dosages are exemplary of the average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope of this invention. In practice, the physician determines the actual dosing regimen that is most suitable for an individual patient, which can vary with the age, weight, and response of the particular patient.

[0118] In the treatment of a cancer, a compound of structural formula (I), (II), or (III) can be administered with a chemotherapeutic agent and/or radiation.

[0119] Embodiments of the present invention employ electromagnetic radiation of: gamma-radiation (10^{-20} to 10^{-13} m), X-ray radiation (10^{-12} to 10^{-9} m), ultraviolet light (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation (700 nm to 1 mm), and microwave radiation (1 mm to 30 cm).

[0120] Many cancer treatment protocols currently employ radiosensitizers activated by electromagnetic radiation, e.g., X-rays. Examples of X-ray-activated radiosensitizers include, but are not limited to, metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FUdR), hydroxyurea, cis-platin, and therapeutically effective analogs and derivatives of the same.

[0121] Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the following, but are not limited to: hematoporphyrin derivatives, PHOTOFRIN®,

benzoporphyrin derivatives, NPe6, tin etioporphyrin (SnET2), pheoborbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective analogs and derivatives of the same.

[0122] Radiosensitizers can be administered in conjunction with a therapeutically effective amount of one or more compounds in addition to a present Bcl-2/Bcl-xL inhibitor, such compounds including, but not limited to, compounds that promote the incorporation of radiosensitizers to the target cells, compounds that control the flow of therapeutics, nutrients, and/or oxygen to the target cells, chemotherapeutic agents that act on the tumor with or without additional radiation, or other therapeutically effective compounds for treating cancer or other disease. Examples of additional therapeutic agents that can be used in conjunction with radiosensitizers include, but are not limited to, 5-fluorouracil (5-FU), leucovorin, oxygen, carbogen, red cell transfusions, perfluorocarbons (e.g., FLUOSOLW®-DA), 2,3-DPG, BW12C, calcium channel blockers, pentoxifylline, antiangiogenesis compounds, hydralazine, and L-BSO.

[0123] The chemotherapeutic agent can be any pharmacological agent or compound that induces apoptosis. The pharmacological agent or compound can be, for example, a small organic molecule, peptide, polypeptide, nucleic acid, or antibody. Chemotherapeutic agents that can be used include, but are not limited to, alkylating agents, antimetabolites, hormones and antagonists thereof, natural products and their derivatives, radioisotopes, antibodies, as well as natural products, and combinations thereof. For example, a Bcl-2/Bcl-xL inhibitor of the present invention can be administered with antibiotics, such as doxorubicin and other anthracycline analogs, nitrogen mustards, such as cyclophosphamide, pyrimidine analogs such as 5-fluorouracil, cis-platin, hydroxyurea, taxol and its natural and synthetic derivatives, and the like. As another example, in the case of mixed tumors, such as adenocarcinoma of the breast, where the tumors include gonadotropin-dependent and gonadotropin-independent cells, the compound can be administered in conjunction with leuprolide or goserelin (synthetic peptide analogs of LH-RH). Other antineoplastic protocols include the use of an inhibitor compound with another treatment modality, e.g., surgery or radiation, also referred to herein as "adjunct anti-neoplastic modalities." Additional chemotherapeutic agents useful in the invention include hormones and antagonists thereof, radioisotopes, antibodies, natural products, and combinations thereof.

[0124] Examples of chemotherapeutic agents useful in a method of the present invention are listed in the following table.

TABLE 1

Alkylating agents

Nitrogen mustards

mechlorethamine

cyclophosphamide

ifosfamide

melphalan

chlorambucil

uracil mustard

temozolomide

Nitrosoureas

carmustine (BCNU)

lomustine (CCNU)

semustine (methyl-CCNU)

chlormethine

streptozocin

Ethylenimine/Methyl-melamine

triethylenemelamine (TEM)

triethylene thiophosphoramide (thiotepa)

hexamethylmelamine (HMM, altretamine)

Alkyl sulfonates

busulfan

pipobroman

Triazines

dacarbazine (DTIC)

Antimetabolites

Folic Acid analogs

methotrexate

trimetrexate

pemetrexed (Multi-targeted antifolate)

Natural products

Antimitotic drugs

Taxanes

paclitaxel

Vinca alkaloids

vinblastine (VLB)

vincristine

vinorelbine

vindesine

Taxotere® (docetaxel)

estramustine

estramustine phosphate

Epipodophylotoxins

etoposide

teniposide

Antibiotics

actimomycin D

daunomycin (rubidomycin)

doxorubicin (adriamycin)

mitoxantroneidarubicin

bleomycin

splicamycin (mithramycin)

mitromycin-C

dactinomycin

aphidicolin

epirubicin

idarubicin

daunorubicin

mithramycin

deoxy co-formycin

Enzymes

L-asparaginase

L-arginase

Pyrimidine analogs

5-fluorouracil

fluorodeoxyuridine

gemcitabine

cytosine arabinoside

(AraC, cytarabine)

5-azacytidine

2,2´- difluorodeoxy-cytidine

floxuridine

pentostatine

Purine analogs

6-mercaptopurine

6-thioguanine

azathioprine

2'-deoxycoformycin

(pentostatin)

erythrohydroxynonyl-adenine (EHNA)

fludarabine phosphate

2-chlorodeoxyadenosine

(cladribine, 2-CdA)

Type I Topoisomerase Inhibitors

camptothecin

topotecan

irinotecan

Biological response modifiers

G-CSF

GM-CSF

Differentiation Agents

retinoic acid derivatives

Hormones and antagonists

Adrenocorticosteroids/ antagonists

prednisone and equivalents

dexamethasone

ainoglutethimide

Radiosensitizers

metronidazole

misonidazole

desmethylmisonidazole

pimonidazole

etanidazole

nimorazole

RSU 1069

EO9

RB 6145

Nonsteroidal antiandrogens

SR4233

flutamide

nicotinamide

5-bromodeozyuridine

5-iododeoxyuridine

bromodeoxycytidine

Miscellaneous agents

Platinium coordination complexes

cisplatin

carboplatin

oxaliplatin

anthracenedione

mitoxantrone

Substituted urea

hydroxyurea

Methylhydrazine derivatives

N-methylhydrazine (MIH)

procarbazine

Adrenocortical suppressant

mitotane (*o,p´*- DDD)

ainoglutethimide

Cytokines

interferon (α, β, γ)

Progestins
hydroxyprogesterone caproate
medroxyprogesterone acetate
megestrol acetate

Estrogens
diethylstilbestrol
ethynyl estradiol/ equivalents

Antiestrogen
tamoxifen

Androgens
testosterone propionate
fluoxymesterone/equivalents

Antiandrogens
flutamide
gonadotropin-releasing
hormone analogs
leuprolide

interleukin-2

Photosensitizers
hematoporphyrin derivatives
PHOTOFRIN®
benzoporphyrin derivatives
Npe6
tin etioporphyrin (SnET2)
pheoboride-a
bacteriochlorophyll-a
naphthalocyanines
phthalocyanines
zinc phthalocyanines

Radiation
X-ray
ultraviolet light
gamma radiation
visible light
infrared radiation
microwave radiation

[0125] Microtubule affecting agents interfere with cellular mitosis and are well known in the art for their cytotoxic activity. Microtubule affecting agents useful in the invention include, but are not limited to, allocolchicine (NSC 406042), halichondrin B (NSC 609395), colchicines (NSC 757), colchicines derivatives (e.g., NSC 33410), dolastatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (NSC 125973), TAXOL® derivatives (e.g., NSC 608832), thiocolchicine NSC 361792), trityl cysteine (NSC 83265), vinblastine sulfate (NSC 49842), vincristine sulfate (NSC 67574), natural and synthetic epothilones including but not limited to epothilone A, eopthilone B, and discodermolide (see Service, (1996) Science, 274:2009) estramustine, nocodazole, MAP4, and the like. Examples of such agents are also described in Bulinski (1997) *J. Cell Sci.* 110:3055 3064; Panda (1997) *Proc. Natl. Acad. Sci. USA* 94:10560-10564; Muhlradt (1997) *Cancer Res.* 57:3344-3346; Nicolaou (1997) *Nature* 397:268-272; Vasquez (1997) *Mol. Biol. Cell.* 8:973-985; and Panda (1996) *J. Biol. Chem.* 271:29807-29812.

[0126] Cytostatic agents that may be used include, but are not limited to, hormones and steroids (including synthetic analogs): 17-α-ethinylestadiol, diethylstilbestrol, testosterone, prednisone, fluoxymesterone, dromostanolone propionate, testolactone, megestrolacetate, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, hlorotrianisene, hydroxyprogesterone, aminogluthimide, estramustine, medroxyprogesteroneacetate, leuprolide, flutamide, toremifene, and zoladex.

[0127] Other cytostatic agents are antiangiogenics, such as matrix metalloproteinase inhibitors, and other VEGF inhibitors, such as anti-VEGF antibodies and small molecules such as ZD6474 and SU668. Anti-Her2 antibodies also may be utilized. An EGFR inhibitor is EKB-569 (an irreversible inhibitor). Also included are antibody C225 immunospecific for the EGFR and Src inhibitors.

[0128] Also suitable for use as a cytostatic agent is CASODEX® (bicalutamide, Astra Zeneca) which renders androgen-dependent carcinomas non-proliferative. Yet another example of a cytostatic agent is the antiestrogen TAMOXIFEN® which inhibits the proliferation or growth of estrogen dependent breast cancer. Inhibitors of the transduction of cellular proliferative signals are cytostatic agents. Representative examples include epidermal growth factor inhibitors, Her-2 inhibitors, MEK-1 kinase inhibitors, MAPK kinase inhibitors, PI3 inhibitors, Src kinase inhibitors, and PDGF inhibitors.

[0129] Additional second therapeutic agents that can be administered with a Bcl-2/Bcl-xL inhibitor of the present invention are disclosed in U.S. Patent Publication 2007/0027135; U.S. Patent No. 7,432,304; U.S. Patent Publication No. 2010/0278921; WO 2012/017251, designating the U.S., each incorporated herein by reference.

[0130] The compounds of the present invention typically are administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. Pharmaceutical compositions for use in accordance with the present invention are formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of compounds of structural formula (I), (II), and (III).

[0131] These pharmaceutical compositions can be manufactured, for example, by conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping, or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of the compound of structural formula (I), (II), or (III) is administered orally, the composition typically is in the

form of a tablet, capsule, powder, solution, or elixir. When administered in tablet form, the composition additionally can contain a solid carrier, such as a gelatin or an adjuvant. The tablet, capsule, and powder contain about 0.01% to about 95%, and preferably from about 1% to about 50%, of a compound of structural formula (I), (II), or (III). When administered in liquid form, a liquid carrier, such as water, petroleum, or oils of animal or plant origin, can be added. The liquid form of the composition can further contain physiological saline solution, dextrose or other saccharide solutions, or glycols. When administered in liquid form, the composition contains about 0.1% to about 90%, and preferably about 1% to about 50%, by weight, of a compound of structural formula (I), (II), or (III).

[0132] When a therapeutically effective amount of a compound of structural formula (I), (II), or (III) is administered by intravenous, cutaneous, or subcutaneous injection, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred composition for intravenous, cutaneous, or subcutaneous injection typically contains, an isotonic vehicle.

[0133] Compounds of structural formula (I), (II), and (III) can be readily combined with pharmaceutically acceptable carriers well-known in the art. Such carriers enable the active agents to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by adding the compound of structural formula (I), (II), or (III) to a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers and cellulose preparations. If desired, disintegrating agents can be added.

[0134] A compound of structural formula (I), (II), and (III) can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampules or in multidose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

[0135] Pharmaceutical compositions for parenteral administration include aqueous solutions of the active agent in water-soluble form. Additionally, suspensions of a compound of structural formula (I), (II), or (III) can be prepared as appropriate oily injection

suspensions. Suitable lipophilic solvents or vehicles include fatty oils or synthetic fatty acid esters. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds and allow for the preparation of highly concentrated solutions. Alternatively, a present composition can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0136] A compound of structural formula (I), (II), or (III) also can be formulated in rectal compositions, such as suppositories or retention enemas, e.g., containing conventional suppository bases. In addition to the formulations described previously, the compound of structural formula (I), (II), or (III) also can be formulated as a depot preparation. Such long-acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds of structural formula (I), (II), or (III) can be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins.

[0137] In particular, the compounds of structural formula (I), (II), and (III) can be administered orally, buccally, or sublingually in the form of tablets containing excipients, such as starch or lactose, or in capsules or ovules, either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavoring or coloring agents. Such liquid preparations can be prepared with pharmaceutically acceptable additives, such as suspending agents. The compounds of structural formula (I), (II), and (III) also can be injected parenterally, for example, intravenously, intramuscularly, subcutaneously, or intracoronarily. For parenteral administration, the Bcl-2/Bcl-xL inhibitors are best used in the form of a sterile aqueous solution which can contain other substances, for example, salts or monosaccharides, such as mannitol or glucose, to make the solution isotonic with blood.

[0138] As an additional embodiment, the present invention includes kits which comprise one or more compounds or compositions packaged in a manner that facilitates their use to practice methods of the invention. In one simple embodiment, the kit includes a compound or composition described herein as useful for practice of a method (e.g., a composition comprising a compound of structural formula (I), (II), or (III) and an optional second therapeutic agent), packaged in a container, such as a sealed bottle or vessel, with a label affixed to the container or included in the kit that describes use of the compound or composition to practice the method of the invention. Preferably, the compound or composition is packaged in a unit dosage form. The kit further can include a device suitable for administering the composition according to the intended route of administration.

[0139] In addition to its use in therapeutic medicine, compounds of structural formula (I), (II), and (III), and pharmaceutically acceptable salts thereof, also are useful as pharmacological tools in the development and standardization of *in vitro* and *in vivo* test systems for the evaluation of the effects of inhibitors of Bcl-2 and/or Bcl-X_L in laboratory animals, such as cats, dogs, rabbits, monkeys, rats, and mice, as part of the search for new therapeutic agents.

[0140] Prior Bcl-2/Bcl-xL inhibitors possessed properties that hindered their development as therapeutic agents. In accordance with an important feature of the present invention, compounds of structural formula (I), (II), and (III) were synthesized and evaluated as inhibitors for Bcl-2/Bcl-xL. For example, compounds of the present invention typically have a binding affinity (IC_{50}) to Bcl-2/Bcl-xL of less than 100 nM.

SYNTHESIS OF COMPOUNDS

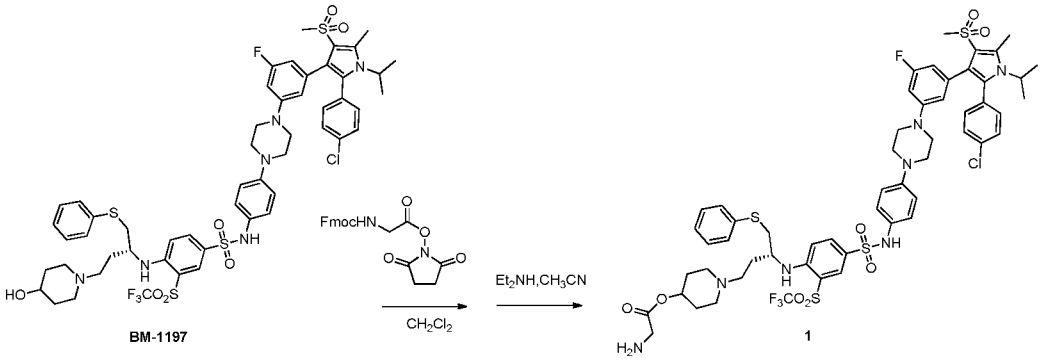
[0141] Compounds of the present invention were prepared as follows. The following synthetic schemes are representative of the reactions used to synthesize compounds of structural formula (I), (II), and (III). Modifications and alternate schemes to prepare Bcl-2/Bcl-xL inhibitors of the invention are readily within the capabilities of persons skilled in the art.

Solvents and reagents were obtained commercially and used without further purification. Chemical shifts (δ) of NMR spectra are reported as δ values (ppm) downfield relative to an internal standard, with multiplicities reported in the usual manner.

[0142] Unless otherwise stated all temperatures are in degrees Celsius.

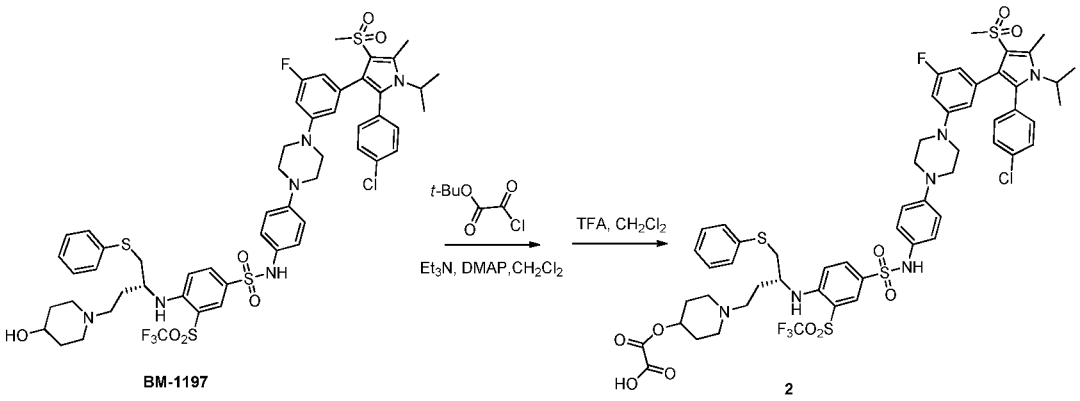
[0143] Certain key intermediates for the synthesis of the compounds of the present invention can be synthesized by the methods as set forth in WO 2012/103059, designating the U.S., and incorporated herein by reference in its entirety followed by conversion to its phosphate derivative as follows:

[0144] Scheme 1. Synthesis of compound 1



[0145] Experimental section: (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoro methylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidin-4-yl 2-aminoacetate (1). A solution of BM-1197 (113 mg, 0.10 mmol) and Fmoc-Gly-OSu (43 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 1 hour until no BM-1197 was observed by TLC. The solution was concentrated *in vacuo* to provide crude precursor of 1 which was used for next step without purification. The resulting residue was dissolved in Aceonitrile (5 mL) and followed by addition of diethyl amine (0.2 mL, 2 mmol). The mixture was stirred at room temperature for overnight until no starting material was observed by TLC and concentrated *in vacuo*. The residue was purified by HPLC to give the product 1 (salt with TFA, 83 mg, yield 70% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. MS (ESI) *m/z* 1189.08 $(M + H)^+$.

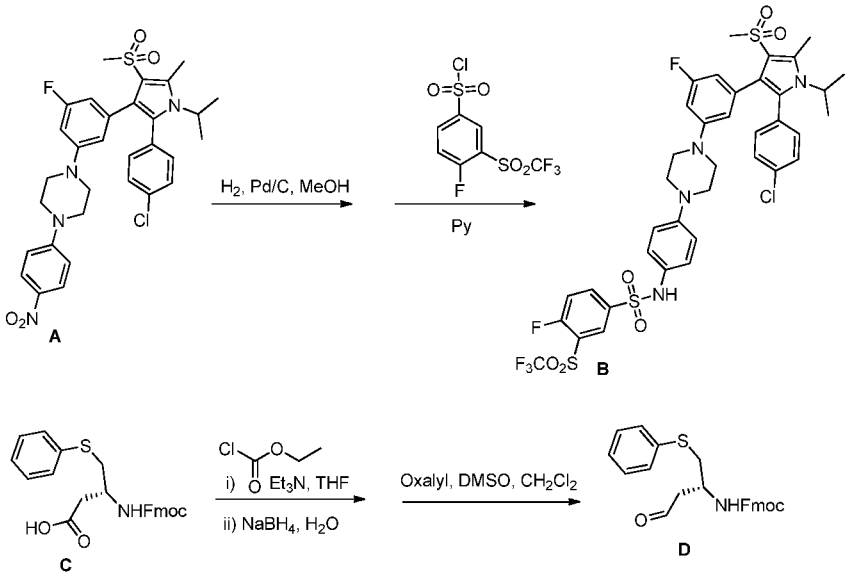
[0146] Scheme 2. Synthesis of 2



[0147] Experimental Section: (*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methyl sulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidin-4-yloxy)-2-oxoacetic acid (2). To a solution of BM-1197 (113

mg, 0.10 mmol), DMAP (2 mg, 0.02 mmol), Et_3N (42 uL, 0.3 mmol) in CH_2Cl_2 (2 mL) was added tert-butyl 2-chloro-2-oxoacetate (33 mg, 0.2 mmol). The solution was stirred at room temperature for 1 hour until no BM-1197 was observed by TLC and concentrated *in vacuo*. The crude residue was flash chromatographed on silica gel with 5% MeOH/CH_2Cl_2 to provide precursor of 2. The precursor was dissolved in CH_2Cl_2 (3 mL) and followed by addition of TFA (3 mL). The mixture was stirred at room temperature for 1 hour until no starting material was observed by TLC and concentrated *in vacuo*. The residue was purified by HPLC to give the product 2 (salt with TFA, 66 mg, yield 55% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. MS (ESI) *m/z* 1189.08 $(M + H)^+$.

[0148] **Scheme 3.** Preparation of key intermediate **B** and **D**

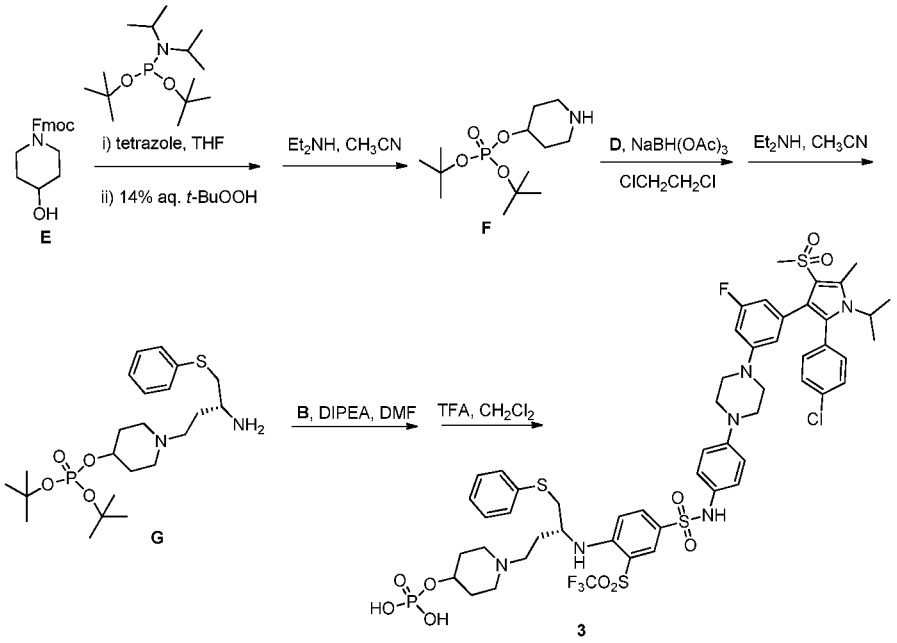


[0149] Experimental Section: N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)-4-fluoro-3-(trifluoromethylsulfonyl) benzenesulfonamide (B). To a solution of A (3.0 g, 4.9 mmol) in 150 mL of methanol was added 10% wt. Pd/C (300 mg, 0.1 eq. m/m). The solution was stirred under hydrogen atmosphere at room temperature for about 20 min until no A was observed by TLC. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was used for next step directly without purification. To the solution of this aniline in pyridine, 4-fluoro-3-(trifluoromethylsulfonyl)benzene-1-sulfonyl chloride (1.8 g, 5.4 mmol) was added at 0 °C. The mixture was stirred at 0 °C to room temprature for 1 hour until no aniline was observed by TLC. Water (10 mL) was added and extracted with ethyl acetate (200 mL * 2). The combined ethyl acetate solution was washed with brine (150 mL), dried over sodium sulfate and concentrated in vacuo. The concentrate was flash

chromatographed on silica gel with 40% EtOA/hexane to provide intermediate B (3.2 g, yield 75% over two steps). MS (ESI) *m/z* 931.75 $(M + K)^+$.

[0150] General procedure I. (*R*)-(9H-fluoren-9-yl)methyl 4-oxo-1-(phenylthio)butan-2-ylcarbamate (D). To a solution of **C** (5.0 g, 11.5 mmol) in THF (100 mL) was added triethylamine (4.8 mL, 34.5 mmol) and ethyl chloroformate (3.3 mL, 34.5 mmol) at -10 °C under argon atmosphere. The mixture was stirred at -10 °C for 1 h and $NaBH_4$ (1.7 g, 46.1 mmol) in water (60 mL) was added dropwise at -10 °C. The mixture was stirred at -10 °C for 1 h then at room temperature for 2 h. The reaction was quenched with 1 M aqueous $KHSO_4$ (200 mL) and the mixture was extracted EtOAc (3×200 mL). The extracts were washed with brine (200 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The concentrate was flash chromatographed on silica gel with 50% EtOA/hexane to provide corresponding alcohol (4.3 g, yield 90%). To a solution of oxalyl chloride (2.6 mL, 31.1 mmol) in DCM (100 mL) at -78 °C, was added dimethyl sulfoxide (3.7 mL, 51.8 mmol). The solution was warmed to -40 °C for 5 min and recooled to -78 °C, and then a solution of the resulting alcohol of previous step (4.3 g, 10.4 mmol) in DCM (50 mL) was added dropwise. The solution was stirred for additional 40 min and followed by excess triethylamine (25 mL) and stirred for another 30 min. The reaction mixture was warmed to room temperature followed by adding saturated aqueous ammonium chloride solution (100 mL), and extracted with DCM (2×200 mL). The combined DCM solution was washed with brine (150 mL), dried over sodium sulfate and concentrated in vacuo. The residue was flash chromatographed on silica gel with 20% EtOA/hexane to provide intermediate **D** (3.7 g, yield 85%). MS (ESI) *m/z* 418.25 $(M + H)^+$.

[0151] Scheme 4. Synthesis of 3



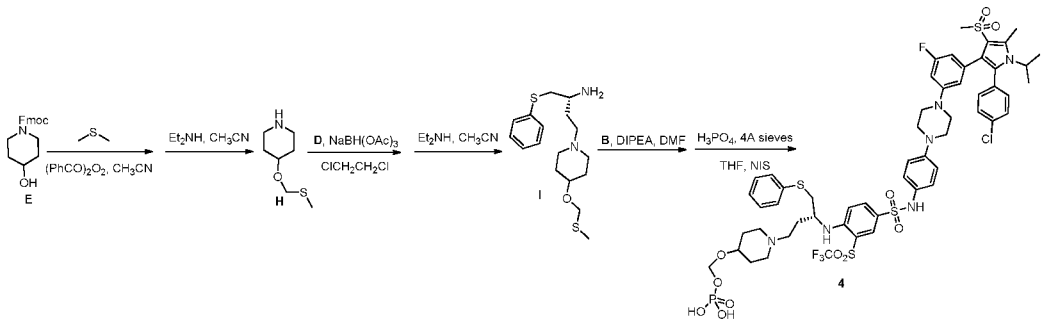
[0152] Experimental Section: **Di-tert-butyl piperidin-4-yl phosphate (F).** The solution of Di-t-butyl di-isopropyl phosphoramidite (832 mg, 3.0 mmol) and tetrazole (6.6 mL, 0.45 M in acetanitril) in THF (15 mL) was stirred under N_2 at room temperature for approximately 10 min. Compound **E** (626 mg, 2.0 mmol) in dry THF (2 mL) was then added to the reaction over 15 minutes and stirred at room temperature under N_2 for 2 hours until no **E** was observed by TLC. The reaction mixture was then cooled to 0 °C and a 14% aqueous solution of t-butyl peroxide (3.0 mL, 4.6 mmol) was added. The temperature was then allowed to rise to room temperature and the mixture was stirred overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (2 mL). Water (50 mL) was added into the reaction mixture, which was then extracted with ethyl acetate (2×50 mL). The combined ethyl acetate solution was washed with brine (50 mL), dried over sodium sulfate and concentrated in vacuo to give crude product which was used for the next step without purification. The resulting residue was dissolved in aceonitrile (20 mL) and followed by addition of diethyl amine (4.1 mL, 40 mmol). The mixture was stirred at room temperature for overnight until no starting material was observed by TLC and concentrated *in vacuo*. The residue was flash chromatographed on silica gel with 5% MeOH/DCM to provide intermediate **F** (452 mg, yield 77% over two steps). MS (ESI) *m/z* 295.17 $(M + H)^+$.

[0153] **General procedure II. (*R*)-1-(3-amino-4-(phenylthio)butyl)piperidin-4-yl di-tert-butyl phosphate (G).** To a solution of **F** (293 mg, 1.0 mmol) and intermediate **D** (500 mg, 1.2 mmol) in DCE (10 mL) was added $NaBH(OAc)_3$ (636 mg, 3.0 mmol), and the mixture was stirred at room temperature overnight until no **F** was observed by TLC. The

mixture was diluted with DCM (50 mL), washed with brine (50 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for the next step without purification. The resulting residue was dissolved in acetonitrile (10 mL) and followed by addition of diethyl amine (2.1 mL, 20 mmol). The mixture was stirred at room temperature for overnight until no starting material was observed by TLC and concentrated *in vacuo*. The residue was flash chromatographed on silica gel with 10% MeOH/DCM to provide intermediate **G** (307 mg, yield 65% over two steps). MS (ESI) *m/z* 474.00 $(M + H)^+$.

[0154] General procedure III. (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl) -1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl) sulfamoyl)-2-(trifluoro methylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidin-4-yl dihydrogen phosphate (3). To a solution of B (100 mg, 0.11 mmol) and G (65 mg, 0.14 mmol) in DMF (2 mL) was added DIPEA (1 mL). The solution was stirred for 4 hours at room temperature until no B was observed by TLC. The reaction mixture was concentrated in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in DCM (5 mL) and followed by adding TFA (2.5 mL). The solution was stirred at room temperature for 1 h until no material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was purified by HPLC to give the pure product 3 (salt with TFA, 88 mg, yield 66% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. ^1H NMR (300 M Hz, CD_3OD): δ 7.96 (s, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.32-7.07 (m, 13H), 6.93-6.41 (m, 4H), 4.61-4.41(m, 2H), 3.99 (s, 1H), 3.55-3.11 (m, 16H), 2.84 (s, 3H), 2.74 (s, 3H), 2.26-1.80 (m, 6H), 1.43 (d, *J* = 7.0 Hz, 6H). MS (ESI): *m/z* 1212.67 $(M + H)^+$.

[0155] Scheme 5. Synthesis of 4



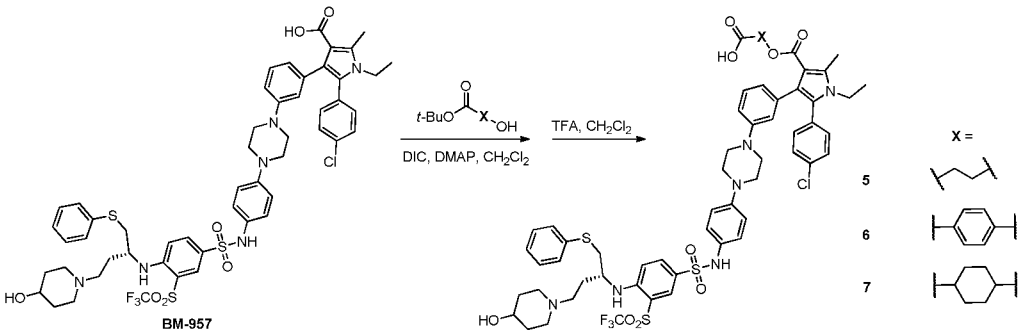
[0156] Experimental Section:**4-(Methylthiomethoxy)piperidine (H).** To a solution of alcohol **E** (1.0 g, 3.1 mmol) and methyl sulfide (1.8 mL, 24.8 mmol) in acetonitrile (31 mL) at 0 °C was added benzoyl peroxide (3.0 g, 12.4 mmol) in four equal portions over 10 min, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h until no E

was observed by TLC. The mixture was diluted with ethyl acetate (100 mL), washed with 10% Na_2CO_3 (100 mL) and then brine (100 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for the next step without purification. The resulting residue was dissolved in acetonitrile (10 mL) and followed by addition of diethyl amine (6.2 mL, 60 mmol). The mixture was stirred at room temperature for overnight until no starting material was observed by TLC and concentrated *in vacuo*. The residue was flash chromatographed on silica gel with 5% MeOH/DCM to provide intermediate H (270 mg, yield 54% over two steps). MS (ESI) *m/z* 162.83 $(M + H)^+$.

[0157] **(*R*)-4-(4-(methylthiomethoxy)piperidin-1-yl)-1-(phenylthio)butan-2-amine (I).** **I** was prepared from H and D according general procedure II. MS (ESI) *m/z* 341.58 $(M + H)^+$.

(*R*)-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl) -1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoro methylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidin-4-yloxy)methyl dihydrogen phosphate (4). To a solution of B (200 mg, 0.23 mmol) and I (86 mg, 0.25 mmol) in DMF (4 mL) was added DIPEA (2 mL). The solution was stirred for 4 hours at room temperature until no B was observed by TLC. The reaction mixture was concentrated in vacuo. The residue was flash chromatographed on silica gel with 5% MeOH/DCM to give corresponding thioether (241 mg, yield 88%). To a solution of the thioether from the first step (200 mg, 0.17 mmol), phosphoric acid (117 mg, 1.2 mmol), and molecular sieves (4 Å, 500 mg) in THF (6 mL) at 0 °C was added *N*-iodosuccinimide (57 mg, 0.26 mmol), and the mixture was stirred at room temperature for 1 h until no starting material was observed by TLC. The reaction mixture was filtered through Celite, and the solids were washed with methanol. The filtrate was concentrated in vacuo and the residue was purified by HPLC to give the pure product 4 (salt with TFA, 93 mg, yield 44%). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. MS (ESI): *m/z* 1242.08 $(M + H)^+$.

[0158] Scheme 6. Synthesis of compounds 5, 6, 7

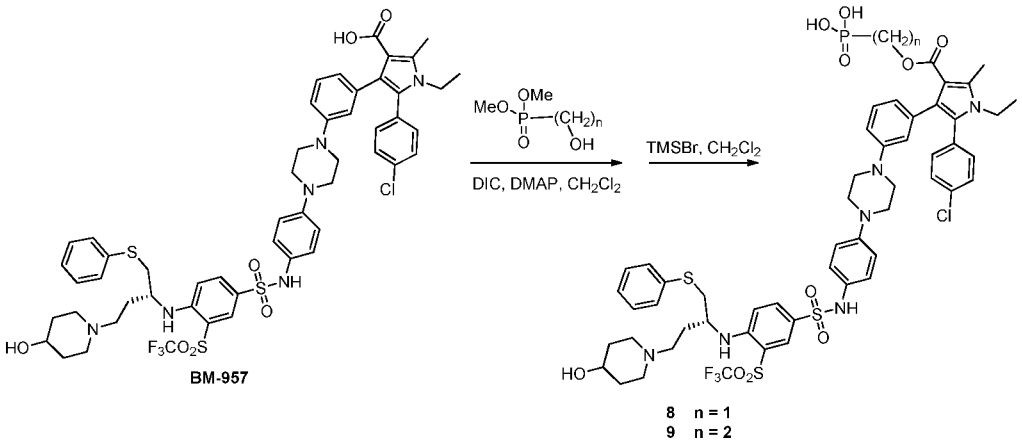


[0159] Experimental Section: General procedure IV. (*R*)-3-((5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl) sulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carbonyl)oxy)propanoic acid (5). To a solution of 957 (100 mg, 0.09 mmol), DIC (18 mg, 0.14 mmol) and DMAP (20 mg, 0.14 mmol) in DCM (2 mL) was added tert-butyl 3-hydroxypropanoate (41 mg, 0.28 mmol). The solution was stirred for 6 hours at room temperature until no BM-957 was observed by TLC. The reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in DCM (5 mL) and followed by adding TFA (2.5 mL). The solution was stirred at room temperature for 3 h until no starting material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was purified by HPLC to give the pure product 5 (salt with TFA, 75 mg, yield 70% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. MS (ESI): *m/z* 1238.17 $(M + H)^+$.

[0160] (*R*)-4-((5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenylsulfonamido) phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carbonyl)oxy)benzoic acid (6). 6 was prepared from BM-957 and tert-butyl 4-hydroxybenzoate according general procedure IV. MS (ESI): *m/z* 1186.00 $(M + H)^+$.

[0161] (*R*)-4-((5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenylsulfonamido) phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carbonyl)oxy)cyclohexane carboxylic acid (7). 7 was prepared from BM-957 and tert-butyl 4-hydroxycyclohexanecarboxylate according general procedure IV. MS (ESI): *m/z* 1192.25 $(M + H)^+$.

[0162] Scheme 7. Synthesis of 8, 9

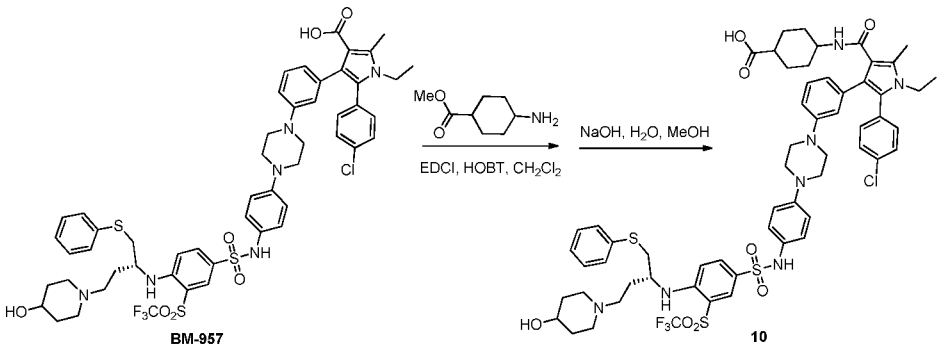


[0163] Experimental Section:

[0164] General procedure V. (*R*)-(((5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl) sulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carbonyl)oxy)methyl)phosphonic acid (8). To a solution of BM-957 (100 mg, 0.09 mmol), DIC (18 mg, 0.14 mmol) and DMAP (20 mg, 0.14 mmol) in DCM (2 mL) was added dimethyl (hydroxymethyl)phosphonate (40 mg, 0.28 mmol). The solution was stirred for 6 hours at room temperature until no BM-957 was observed by TLC. The reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in DCM (5 mL) and followed by adding TMSBr (248 uL, 1.9 mmol). The solution was stirred at room temperature for 20 h until no starting material was observed by MS. The reaction mixture was concentrated in vacuo and the residue was purified by HPLC to give the pure product 8 (salt with TFA, 74 mg, yield 68% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. ^1H NMR (300 M Hz, CD_3OD): δ 7.92 (s, 1H), 7.73-7.70 (m, 2H), 7.34-6.82 (m, 17H), 4.28 (d, J = 8.6 Hz, 2H), 4.06-3.35 (m, 14H), 3.20-2.92 (m, 5H), 2.65 (s, 3H), 2.24-1.67 (m, 6H), 1.10 (t, J = 7.0 Hz, 3H). MS (ESI): *m/z* 1259.50 $(M + H)^+$.

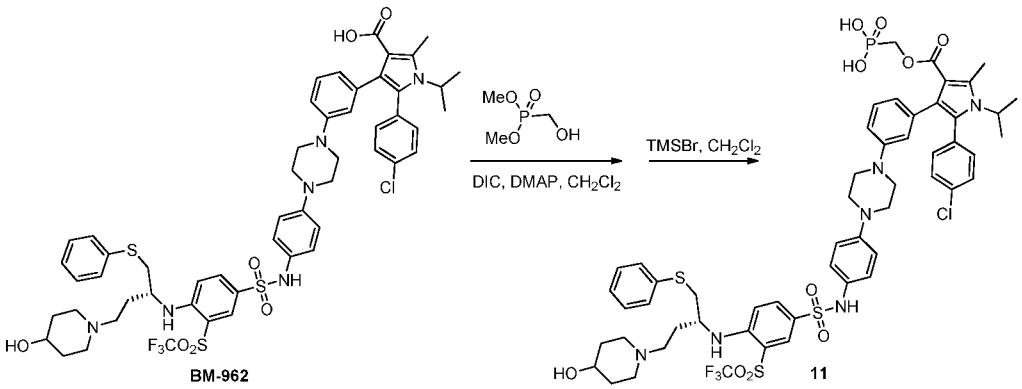
[0165] (*R*)-(2-((5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenylsulfonamido) phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carbonyl)oxy)ethyl) phosphonic acid (9). 9 was prepared from BM-957 and dimethyl (2-hydroxyethyl)phosphonate according general procedure V. MS (ESI): *m/z* 1173.42 $(M + H)^+$.

[0166] Scheme 8. Synthesis of 10



[0167] ((R)-4-(5-(4-chlorophenyl)-1-ethyl-4-(3-(4-(4-(4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1H-pyrrole-3-carboxamido)cyclohexanecarboxylic acid (10). To a solution of BM-957 (100 mg, 0.09 mmol), EDCI (27 mg, 0.14 mmol) and HOBT (19 mg, 0.14 mmol) in DCM (2 mL) was added methyl 4-aminocyclohexanecarboxylate (44 mg, 0.28 mmol). The solution was stirred for 2 hours at room temperature until no BM-957 was observed by TLC. The reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in H_2O and MeOH (5 mL and 5 mL respectively) and followed by adding NaOH (76 mg, 1.9 mmol). The solution was stirred at room temperature for 20 h until no starting material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was purified by HPLC to give the pure product 10 (salt with TFA, 61 mg, yield 55% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. MS (ESI): *m/z* 1191.17 $(M + H)^+$.

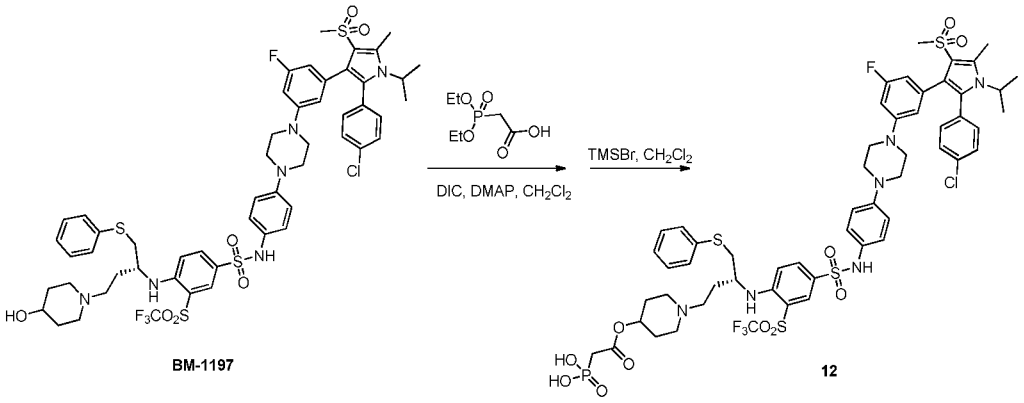
[0168] Scheme 9. Synthesis of 11



[0169] Experimental Section:

[0170] (*R*)-(((5-(4-chlorophenyl)-4-(3-(4-(4-(4-((4-(4-hydroxypiperidin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenylsulfonamido) phenyl)piperazin-1-yl)phenyl)-1-isopropyl-2-methyl-1H-pyrrole-3-carbonyl)oxy) methyl)phosphonic acid (11). 11 was prepared from BM-962 and dimethyl (hydroxymethyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 8.00 (s, 1H), 7.80-7.71 (m, 2H), 7.38-6.83 (m, 17H), 4.50-4.41 (m, 1H), 4.29 (d, J = 8.7 Hz, 2H), 4.11-3.59 (m, 12H), 3.25-3.01 (m, 6H), 2.77 (s, 3H), 2.28-1.70 (m, 6H), 1.47 (d, J = 7.1 Hz, 6H). MS (ESI): m/z 1174.25 $(M + H)^+$.

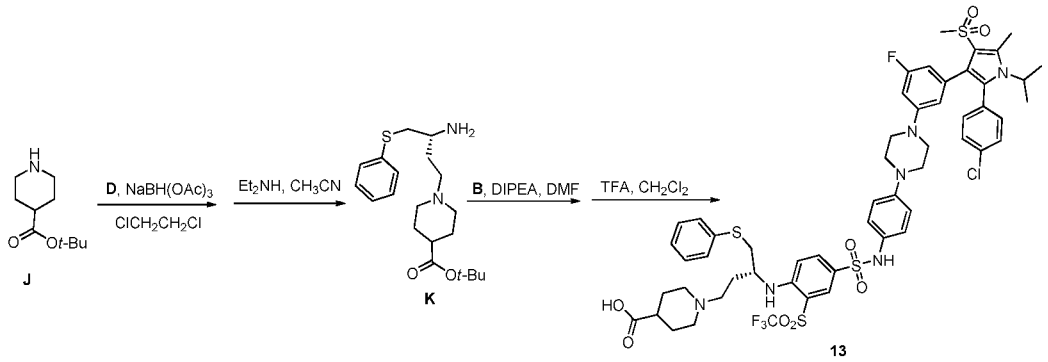
[0171] Scheme 9. Synthesis of 12



[0172] Experimental Section:

[0173] (*R*)-(2-((1-(3-((4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperidin-4-yl)oxy)-2-oxoethyl)phosphonic acid (12). 12 was prepared from BM-1197 and 2-(diethoxyphosphoryl)acetic acid according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.99 (s, 1H), 7.75 (d, J = 8.6 Hz, 1H), 7.36-7.13 (m, 12H), 6.92-6.43 (m, 5H), 5.10 (s, 1H), 4.51-4.44 (m, 1H), 4.10 (s, 1H), 3.56-2.93 (m, 18H), 2.87 (s, 3H), 2.76 (s, 3H), 2.29-1.90 (m, 6H), 1.46 (d, J = 7.3 Hz, 6H). MS (ESI): m/z 1253.36 $(M + H)^+$.

[0174] Scheme 10. Synthesis of 13

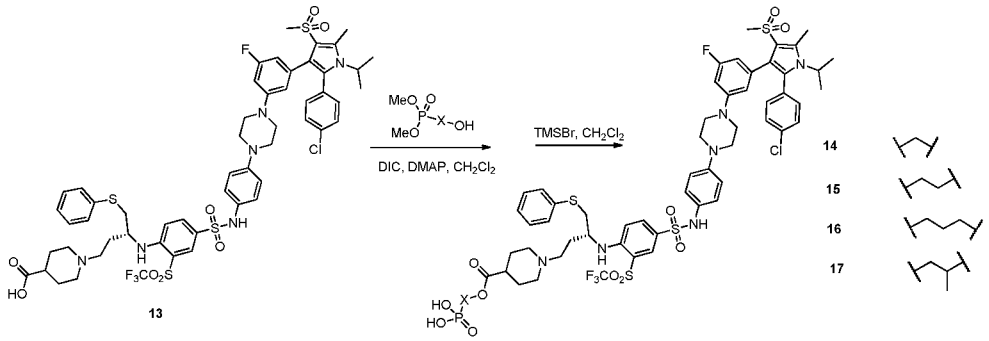


[0175] Experimental Section:

[0176] **(*R*)-tert-butyl 1-(3-amino-4-(phenylthio)butyl)piperidine-4-carboxylate (K).** **K** was prepared from tert-butyl piperidine-4-carboxylate and **D** according to general procedure II. MS (ESI): *m/z* 365.50 $(M + H)^+$.

[0177] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carboxylic acid (13). 13 was prepared from K and B according general procedure III. MS (ESI): *m/z* 365.50 $(M + H)^+$.

[0178] Scheme 11. Synthesis of 14, 15, 16, 17



[0179] Experimental Section:

[0180] (*R*)-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)methylphosphonic acid (14). 14 was prepared from 13 and dimethyl (2-hydroxymethyl)phosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (s, 1H), 7.72 (d, *J* = 9.1 Hz, 1H), 7.30-7.09 (m, 13H), 6.91-6.42 (m, 4H),

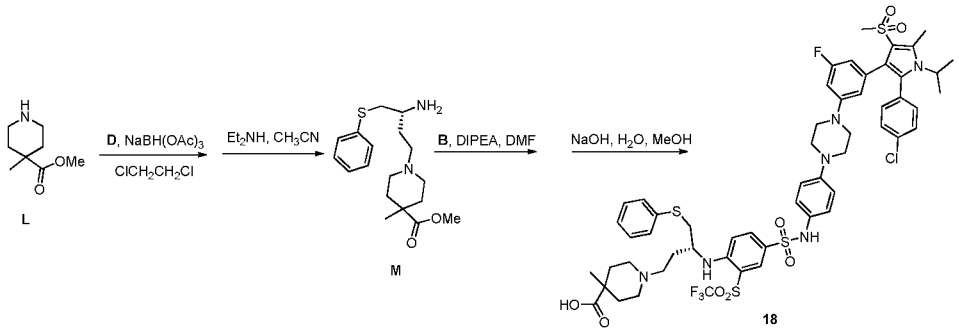
4.49-4.40 (m, 1H), 3.99 (s, 1H), 3.55-2.90 (m, 16H), 2.84 (s, 3H), 2.72 (s, 3H), 2.63-2.55 (m, 1H), 2.23-1.81 (m, 6H), 1.41 (d, J = 4.3 Hz, 6H). MS (ESI): m/z 1160.34 $(M + H)^+$.

[0181] (*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)ethylphosphonic acid (15). 15 was prepared from 13 and dimethyl (2-hydroxyethyl)phosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.93 (d, J = 1.9 Hz, 1H), 7.72 (dd, J = 9.2, 1.8 Hz, 1H), 7.30-7.12 (m, 12H), 6.83-6.42 (m, 5H), 4.46-4.33 (m, 3H), 3.96 (s, 1H), 3.54-2.93 (m, 16H), 2.82 (s, 3H), 2.72 (s, 3H), 2.71-2.55 (m, 1H), 2.24-1.65 (m, 8H), 1.41 (d, J = 7.1 Hz, 6H). MS (ESI): m/z 1268.58 $(M + H)^+$.

[0182] (*R*)-3-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)propylphosphonic acid (16). 16 was prepared from 13 and dimethyl 3-hydroxypropylphosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, J = 2.0 Hz, 1H), 7.73 (dd, J = 9.2, 2.1 Hz, 1H), 7.33-7.12 (m, 12H), 6.92-6.43 (m, 5H), 4.51-4.41 (m, 1H), 4.18-3.98 (m, 3H), 3.56-2.92 (m, 16H), 2.85 (s, 3H), 2.73 (s, 3H), 2.67-2.50 (m, 1H), 2.25-1.70 (m, 10H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): m/z 1282.34 $(M + H)^+$.

[0183] 2-(1-((*R*)-3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)propylphosphonic acid (17). 17 was prepared from 13 and dimethyl 2-hydroxypropylphosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.97 (d, J = 2.1 Hz, 1H), 7.73 (d, J = 9.2 Hz, 1H), 7.36-7.08 (m, 13H), 6.85-6.43 (m, 4H), 5.26 (s, 1H), 4.54-4.44 (m, 1H), 4.01 (s, 1H), 3.58-2.92 (m, 16H), 2.87 (s, 3H), 2.76 (s, 3H), 2.70-2.55 (m, 1H), 2.26-1.85 (m, 8H), 1.46 (d, J = 7.1 Hz, 6H), 1.38 (d, J = 5.9 Hz, 3H). MS (ESI): m/z 1281.34 $(M + H)^+$.

[0184] Scheme 12. Synthesis of 18

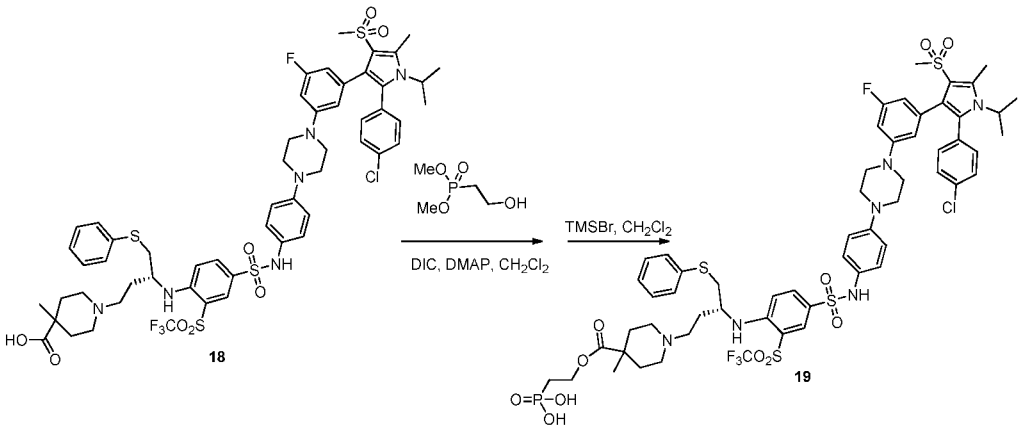


[0185] Experimental Section:

[0186] **(*R*)-methyl 1-(3-amino-4-(phenylthio)butyl)-4-methylpiperidine-4-carboxylate (M).** **M** was prepared from methyl 4-methylpiperidine-4-carboxylate and **D** according general procedure II. MS (ESI): *m/z* 337.55 $(M + H)^+$.

[0187] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-4-methylpiperidine-4-carboxylic acid (18). To a solution of B (100 mg, 0.11 mmol) and M (47 mg, 0.14 mmol) in DMF (2 mL) was added DIPEA (1 mL). The solution was stirred for 4 hours at room temperature until no B was observed by TLC. The reaction mixture was concentrated in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in H_2O and MeOH (5 mL and 5 mL respectively) and followed by adding NaOH (88 mg, 2.2 mmol). The solution was stirred at room temperature for 20 h until no starting material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was purified by HPLC to give the pure product 18 (salt with TFA, 75 mg, yield 58% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.99 (d, J = 1.6 Hz, 1H), 7.76 (dd, J = 9.1, 1.9 Hz, 1H), 7.37-6.84 (m, 14H), 6.68-6.45 (m, 3H), 4.55-4.45 (m, 1H), 4.02 (s, 1H), 3.58-2.92 (m, 17H), 2.88 (s, 3H), 2.77 (s, 3H), 2.41-1.86 (m, 5H), 1.47 (d, J = 7.1 Hz, 6H), 1.31 (s, 3H). MS (ESI): *m/z* 1173.73 $(M + H)^+$.

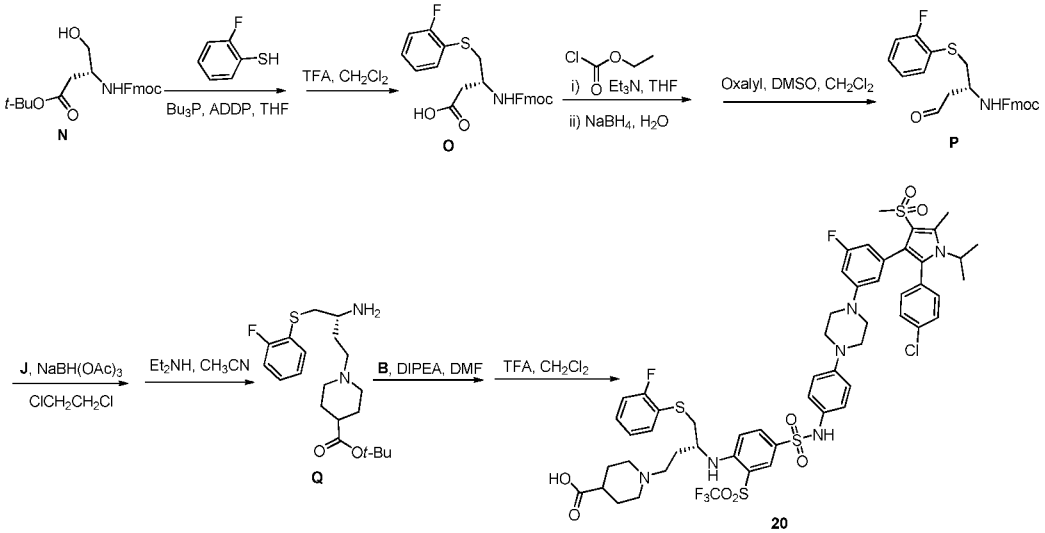
[0188] Scheme 13. Synthesis of 19



[0189] Experimental Section:

[0190] (*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-4-methylpiperidine-4-carbonyloxy)ethylphosphonic acid (19). 19 was prepared from 18 and dimethyl (2-hydroxyethyl)phosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.98 (d, *J* = 1.6 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.35-6.83 (m, 14H), 6.65-6.44 (m, 3H), 4.52-4.38 (m, 3H), 4.01 (s, 1H), 3.44-2.92 (m, 17H), 2.87 (s, 3H), 2.77 (s, 3H), 2.45-2.11 (m, 5H), 1.71 (t, *J* = 14.4 Hz, 2H), 1.46 (d, *J* = 7.1 Hz, 6H), 1.30 (s, 3H). MS (ESI): *m/z* 1281.92 $(M + H)^+$.

[0191] Scheme 14. Synthesis of compound 20



[0192] Experimental Section:

[0193] **(*R*)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(2-fluorophenylthio)butanoic acid (O)**. A solution of Bu_3P (0.8 mL, 3.3 mmol) and ADDP (833 mg, 3.3 mmol) in THF (30 mL) was treated with **N** (1.2 g, 3.0 mmol) and thiophenol

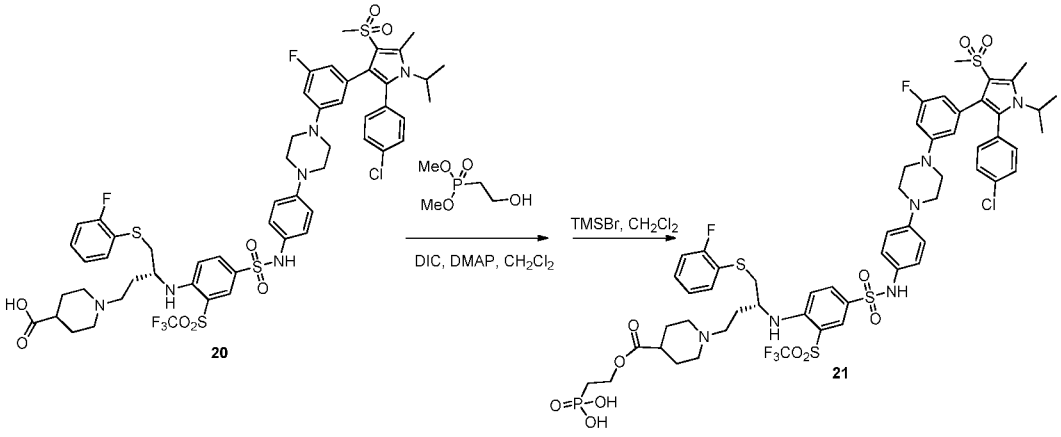
(320 uL, 3.0 mmol), stirred for 4 h until no N was observed by TLC. The mixture was diluted with ethyl acetate (100 mL), washed with 1M HCl aqueous (100 mL), brine (100 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in DCM (10 mL) and followed by adding TFA (5 mL). The solution was stirred at room temperature for 1 h until no starting material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was flash chromatographed on silica gel with 5% MeOH/DCM to provide intermediate **O** (840 mg, yield 62% over two steps). MS (ESI) *m/z* 452.86 $(M + H)^+$.

[0194] (*R*)-(9H-fluoren-9-yl)methyl 1-(2-fluorophenylthio)-4-oxobutan-2-ylcarbamate (P). P was prepared from O according to general procedure I. MS (ESI) *m/z* 437.00 $(M + H)^+$.

[0195] (*R*)-tert-butyl 1-(3-amino-4-(2-fluorophenylthio)butyl)piperidine-4-carboxylate (Q). Q was prepared from P and J according to general procedure II. MS (ESI) *m/z* 383.38 $(M + H)^+$.

[0196] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(2-fluorophenylthio)butyl)piperidine-4-carboxylic acid (20). 20 was prepared from Q and B according to general procedure III. ^1H NMR (300 M Hz, CD_3OD): δ 7.97 (d, *J* = 1.9 Hz, 1H), 7.76 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.39-6.87 (m, 13H), 6.65-6.43 (m, 3H), 4.54-4.45 (m, 1H), 4.01 (s, 1H), 3.67-2.93 (m, 17H), 2.87 (s, 3H), 2.77 (s, 3H), 2.29-1.86 (m, 6H), 1.46 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1177.92 $(M + H)^+$.

[0197] Scheme 15. Synthesis of 21

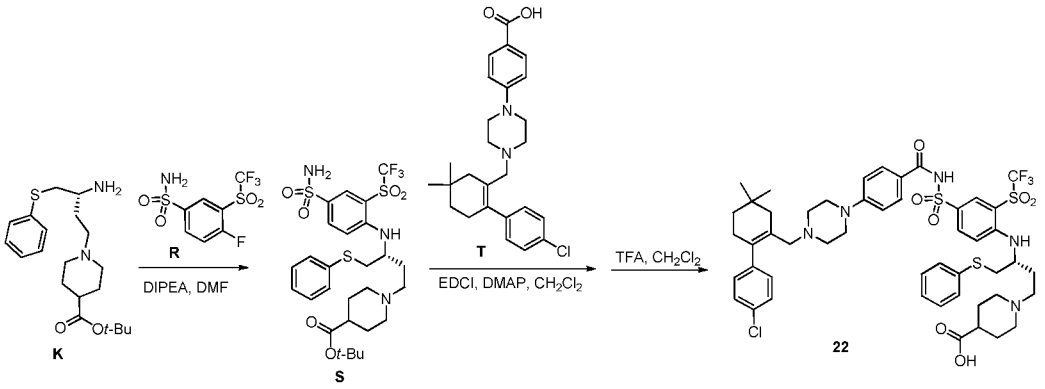


[0198] Experimental Section:

[0199] (*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1H-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-

(trifluoromethylsulfonyl)phenylamino)-4-(2-fluorophenylthio)butyl)piperidine-4-carbonyloxy)ethylphosphonic acid (21). 21 was prepared from 20 and dimethyl (2-hydroxyethyl)phosphonate according to general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, J = 1.7 Hz, 1H), 7.77 (dd, J = 9.0, 2.0 Hz, 1H), 7.36-6.86 (m, 13H), 6.66-6.44 (m, 3H), 4.51-4.33 (m, 3H), 4.01 (s, 1H), 3.58-2.93 (m, 16H), 2.85 (s, 3H), 2.74 (s, 3H), 2.70-2.58 (m, 1H), 2.27-1.84 (m, 8H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): m/z 1286.58 $(M + H)^+$.

[0200] Scheme 16. Synthesis of 22



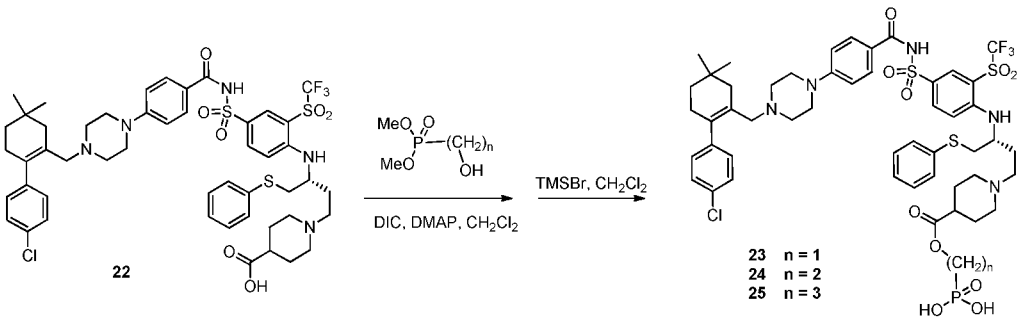
[0201] Experimental Section:

[0202] (*R*)-tert-butyl 1-(4-(phenylthio)-3-(4-sulfamoyl-2-(trifluoromethylsulfonyl)phenyl amino)butyl)piperidine-4-carboxylate (S). To a solution of K (1.1 g, 3.0 mmol) and R (922 mg, 3.0 mmol) in DMF (15 mL) was added DIPEA (3 mL). The solution was stirred for 4 hours at room temperature until no K was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was flash chromatographed on silica gel with 5% MeOH/DCM to provide intermediate S (1.7 g, yield 88% over two steps). MS (ESI) m/z 653.21 $(M + H)^+$.

[0203] (*R*)-1-(3-(4-(N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl) piperazin-1-yl)benzoyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carboxylic acid (22). To a solution of T (438 mg, 1.0 mmol), EDCI (386 mg, 2.0 mmol) and DMAP (121 mg, 1.0 mmol) in DCM (10 mL) was added S (718 mg, 1.1 mmol). The solution was stirred for 2 hours at room temperature until no T was observed by TLC. The reaction mixture was diluted with ethyl acetate (50 mL), washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over sodium sulfate. The solvent was removed in vacuo to give crude product which was used for next step without purification. The resulting residue was dissolved in DCM (10 mL) and followed by adding TFA (5 mL). The solution was stirred at room temperature for 1 h until no starting material was observed by TLC. The reaction mixture was concentrated in vacuo and the residue was

purified by HPLC to give the pure product 22 (salt with TFA, 742 mg, yield 73% over two steps). The gradient ran from 60% of solvent A and 40% of solvent B to 20% of solvent A and 80% of solvent B in 40 min. ^{1}H NMR (300 M Hz, CD_3OD): δ 8.30 (d, J = 2.1 Hz, 1H), 8.02 (dd, J = 9.2, 2.5 Hz, 1H), 7.70 (d, J = 8.9 Hz, 2H), 7.40-6.88 (m, 12H), 4.04 (s, 1H), 3.67-2.82 (m, 19H), 2.58 (t, J = 14.4 Hz, 1H), 2.37-1.81 (m, 10H), 1.53 (t, J = 6.2 Hz, 2H), 1.03 (s, 6H). MS (ESI): m/z 1017.50 $(M + H)^+$.

[0204] Scheme 17. Synthesis of 23, 24, 25



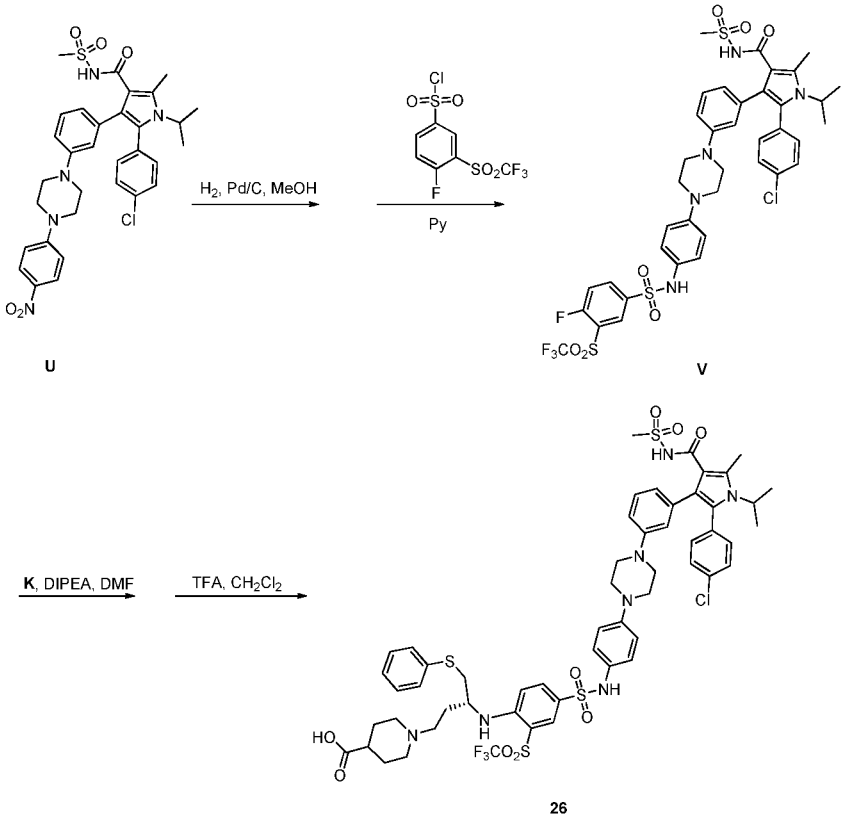
[0205] Experimental Section:

[0206] (*R*)-(1-(3-(4-(N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl) piperazin-1-yl)benzoyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)methylphosphonic acid (23). 23 was prepared from 22 and dimethyl (2-hydroxymethyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 8.35 (s, 1H), 8.09 (d, J = 6.7 Hz, 1H), 7.79 (d, J = 7.7 Hz, 2H), 7.44-6.82 (m, 12H), 4.30-4.10 (m, 3H), 3.74-2.73 (m, 19H), 2.43-1.44 (m, 12H), 1.10 (s, 6H). MS (ESI): m/z 1110.58 $(M + H)^+$.

[0207] (*R*)-2-(1-(3-(4-(N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl) piperazin-1-yl)benzoyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)ethylphosphonic acid (24). 24 was prepared from 22 and dimethyl (2-hydroxyethyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 8.29 (d, J = 2.0 Hz, 1H), 8.02 (dd, J = 9.2, 2.0 Hz, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.37-6.84 (m, 12H), 4.34-4.30 (m, 2H), 4.03 (s, 1H), 3.66-2.88 (m, 18H), 2.62 (t, J = 14.4 Hz, 1H), 2.36-1.82 (m, 12H), 1.53 (t, J = 6.1 Hz, 2H), 1.03 (s, 6H). MS (ESI): m/z 1025.64 $(M + H)^+$.

[0208] (*R*)-3-(1-(3-(4-(N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl) piperazin-1-yl)benzoyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)propylphosphonic acid (25). 25 was prepared from 22 and dimethyl 3-hydroxypropylphosphonate according general procedure V. ^{1}H NMR

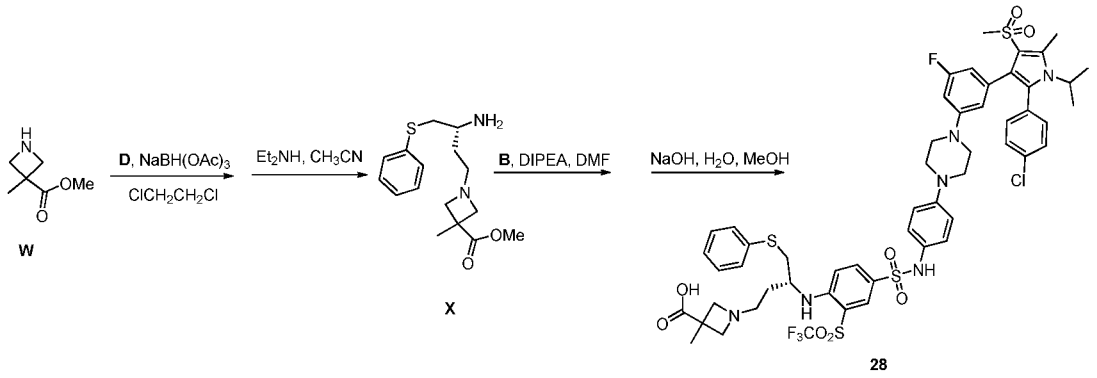
(300 M Hz, CD_3OD): δ 7.95 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.33-7.12 (m, 12H), 6.92-6.43 (m, 5H), 4.51-4.41 (m, 1H), 4.18-3.98 (m, 3H), 3.56-2.92 (m, 16H), 2.85 (s, 3H), 2.73 (s, 3H), 2.67-2.50 (m, 1H), 2.25-1.70 (m, 10H), 1.43 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1282.34 $(M + H)^+$.



[0209] 5-(4-chlorophenyl)-4-(3-(4-(4-(4-fluoro-3-(trifluoromethylsulfonyl)phenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-2-methyl-N-(methylsulfonyl)-1H-pyrrole-3-carboxamide (V). V was prepared from U according to the procedure described for the preparation of compound B.

[0210] (*R*)-1-(3-(4-(*N*-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonylcarbamoyl)-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carboxylic acid (26) (BM-1077): 26 was prepared from K and V according to general procedure III. ^1H NMR (300 M Hz, CD_3OD): δ 7.94 (d, J = 1.7 Hz, 1H), 7.71 (dd, J = 2.0, 9.2 Hz, 1H), 7.39-7.28 (m, 4H), 7.26-7.14 (m, 6H), 7.09-6.96 (m, 5H), 6.93-6.85 (m, 2H), 6.81 (d, J = 9.3 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 4.41 (quintet, J = 7.0 Hz, 1H), 4.06-3.88 (m, 1H), 3.66-3.33 (m, 8H), 3.25-2.79 (m, 10H), 2.63 (s, 3H), 2.36-1.71 (m, 8H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m*/z 1184.42 $(M + H)^+$.

[0211] (*R*)-2-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonylcarbamoyl)-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)piperidine-4-carbonyloxy)ethylphosphonic acid (27) (BM-1080): 27 was prepared from 26 and dimethyl (2-hydroxyethyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, J = 1.9 Hz, 1H), 7.69 (dd, J = 1.8, 9.3 Hz, 1H), 7.39-7.28 (m, 4H), 7.27-7.12 (m, 6H), 7.08-6.76 (m, 8H), 6.70 (d, J = 7.5 Hz, 1H), 4.49-4.27 (m, 3H), 4.04-3.89 (m, 1H), 3.65-3.48 (m, 2H), 3.29-2.84 (m, 15H), 2.63 (s, 3H), 2.37-1.74 (m, 11H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1292.00 $(M + H)^+$.



[0212] **(*R*)-methyl 1-(3-amino-4-(phenylthio)butyl)-3-methylazetidine-3-carboxylate (X).** **X** was prepared from methyl 3-methylazetidine-3-carboxylate (**W**), and **D** according to general procedure II.

[0213] (*R*)-1-(3-(4-(*N*-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1*H*-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-3-methylazetidine-3-carboxylic acid (28) (BM-1082): 28 was prepared from X and B according to the procedure described for the preparation of compound 18. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (d, J = 1.9 Hz, 1H), 7.70 (dd, J = 2.1, 9.1 Hz, 1H), 7.35-7.24 (m, 4H), 7.23-7.12 (m, 5H), 7.07-6.91 (m, 4H), 6.87 (d, J = 9.0 Hz, 1H), 6.81 (d, J = 9.3 Hz, 1H), 6.63-6.47 (m, 2H), 6.41 (d, J = 9.0 Hz, 1H), 4.55-4.38 (m, 2H), 3.97 (br. s., 3H), 3.29-3.08 (m, 13H), 2.84 (s, 3H), 2.74 (s, 3H), 2.12-1.81 (m, 2H), 1.56 (br. s., 3H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1144.75 $(M + H)^+$.

[0214] (*R*)-2-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1*H*-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-3-methylazetidine-3-carbonyloxy)ethylphosphonic acid (29) (BM-1083): 29 was prepared from 28 and dimethyl (2-hydroxyethyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD):

δ 7.94 (d, J =1.8 Hz, 1H), 7.72 (dd, J = 2.0, 9.1 Hz, 1H), 7.36-7.26 (m, 4H), 7.25-7.15 (m, 5H), 7.10-7.00 (m, 4H), 6.92-6.83 (m, 1H), 6.63 (s, 1H), 6.57 (d, J = 12.0 Hz, 1H), 6.42 (d, J = 9.2 Hz, 1H) 4.58-4.35 (m, 5H), 4.12-3.82 (m, 3H), 3.29-3.05 (m, 11H), 2.84 (s, 3H), 2.74 (s, 3H), 2.25-1.83 (m, 5H), 1.50 (br. s., 3H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1252.83 $(M + H)^+$.

[0215] (*R*)-3-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-chlorophenyl)-1-isopropyl-5-methyl-4-(methylsulfonyl)-1*H*-pyrrol-3-yl)-5-fluorophenyl)piperazin-1-yl)phenyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-3-methylazetidine-3-carbonyloxy)propylphosphonic acid (30) (BM-1084): 30 was prepared from 28 and dimethyl (3-hydroxypropyl)phosphonate according general procedure V. ^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (s, 1H), 7.71 (dd, 1.5, 9.0 Hz, 1H), 7.36-7.26 (m, 4H), 7.24-7.15 (m, 5H), 7.08-6.97 (m, 4H), 6.90-6.79 (m, 2H), 6.62 (s, 1H), 6.56 (d, J = 11.8 Hz, 1H), 6.41 (d, J = 8.8 Hz, 1H), 4.54-4.37 (m, 3H), 4.33-4.21 (m, 2H), 3.99 (br. s., 3H), 3.28-3.05 (m, 11H), 2.84 (s, 3H), 2.74 (s, 3H), 2.15-1.71 (m, 7H), 1.57 (s, 3H), 1.43 (d, J = 7.0 Hz, 6H). MS (ESI): *m/z* 1266.92 $(M + H)^+$.

Fluorescence polarization based binding assays for Bcl-2/Bcl-xL/Mcl-1 proteins

[0216] Sensitive and quantitative fluorescence polarization (FP)-based assays were developed and optimized to determine the binding affinities of Bcl-2 family protein inhibitors to the recombinant Bcl-2, Bcl-xL, and Mcl-1 proteins.

Determine K_d values of fluorescent probes to proteins

[0217] Homemade fluorescein labeled BIM (81-106), Bak (72-87) and BID (79-99) peptides, named as Flu-BIM, Flu-BAK, and Flu-BID were used as the fluorescent probes in FP assays for Bcl-2, Bcl-xL, and Mcl-1 respectively. By monitoring the total fluorescence polarization of mixtures composed with fluorescent probes at fixed concentrations and proteins with increasing concentrations up to the full saturation, the K_d values of Flu-BIM to Bcl-2, Flu-BAK to Bcl-xL, and Flu-BID to Mcl-1 were determined to be 0.55±0.15 nM, 4.4±0.8, and 6.8±1.5 nM, respectively. Fluorescence polarization values were measured using the Infinite M-1000 multi-mode plate reader (Tecan U.S., Research Triangle Park, NC) in Microfluor 2 96-well, black, round-bottom plates (Thermo Scientific). To each well, 1nM of Flu-BIM or 2nM of Flu-BAK or 2nM of Flu-BID and increasing concentrations of Bcl-2 or Bcl-xL or Mcl-1 were added to a final volume of 125 μl in the assay buffer (100mM potassium phosphate, pH 7.5, 100 μg/ml bovine γ-globulin, 0.02% sodium azide, Invitrogen, with 0.01% Triton X-100 and 4% DMSO). Plates were incubated at room temperature for 2

hours with gentle shaking to assure equilibrium. The polarization values in millipolarization units (mP) were measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Equilibrium dissociation constants (K_d) were then calculated by fitting the sigmoidal dose-dependent FP increases as a function of protein concentrations using Graphpad Prism 5.0 software (Graphpad Software, San Diego, CA).

Determine K_i values of Bcl-2 family protein inhibitors

[0218] K_i values of Bcl-2 family protein inhibitors to Bcl-2/Bcl-xL/Mcl-1 proteins were determined through an inhibitor dose-dependent competitive binding experiment in which serial dilutions of inhibitors competed against the fluorescent probe with fixed concentration for binding to a fixed concentration of the protein. Mixtures of 5 μl of the tested inhibitor in DMSO and 120 μl of pre-incubated protein/probe complex in the assay buffer were added into assay plates and incubated at room temperature for 2 hours with gentle shaking. Final concentrations of the protein and probe are 1.5nM and 1nM for the Bcl-2 assay, 10nM and 2nM for the Bcl-xL assay, and 20nM and 2nM for the Mcl-1 assay, respectively. Negative controls containing protein/probe complex only (equivalent to 0% inhibition), and positive controls containing free probe only (equivalent to 100% inhibition), were included in each assay plate. FP values were measured as described above. IC50 values were determined by nonlinear regression fitting of the competition curves. K_i values of inhibitors were calculated using the home derived equation described before (Z. Nikolovska-Coleska et al., *Analytical Biochemistry,* 2004**,** 332, 261-273.), based upon the IC50 values obtained, the K_d values of the probes to the proteins, and the concentrations of the proteins and probes in the competitive assays. K_i values were also calculated by using another very commonly used equation present in the literatures (X. Y. Huang, *Journal of Biomolecular Screening*, 2003, 8, 34-38.), results from which consisted with our results extremely well.

Cell Growth Assay

[0219] RS4;11 and H146 cells were seeded in 96-well cell culture plates at a density of 10,000 cells/well with serially diluted compounds and incubated at 37°C in an atmosphere of 95% air and 5% CO_2 for 4 days. Cell viability was determined using the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) based Cell Counting-8 Kit (Dojindo Molecular Technologies, Inc., Rockville, MD) according to the manufacture's instruction. Briefly, WST-8 was added to each well at a final concentration of 10% (v/v), and then the plates were incubated at 37°C for 1-2 hourrs for color development. The absorbance was measured at 450 nm using a

SPECTRAmax PLUS plate reader (Molecular Devices, Sunnyvale, CA). The half maximal inhibitory concentration (IC_{50}) was calculated using the GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

Cell Death Assay

[0220] Cell death assay was performed using a Trypan blue exclusion test of cell viability. One million cells were seeded in 6-well plates and incubated at 37°C in an atmosphere of 95% air and 5% CO_2 with or without compounds for the indicated time points. At the end of treatment, cells were collected and centrifuged at 1000 rpm for 5 minutes. The cell pellets were re-suspended in PBS and mixed with 0.4% Trypan blue (Invitrogen) at 1:1 dilution to determine cell viability using Olympus CKX41 microscope (Olympus, Center Valley, PA).

Apoptosis Assay

[0221] Apoptosis assay was performed using the Annexin-V-FLUOS Staining kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instruction. Briefly, cells were treated with compounds for the indicated time points, harvested and washed with PBS. Cells were stained with Annexin V-FITC and Propidium iodide for 15 minutes at room temperature in the dark before analyzed with a BD Biosciences FACSCaliburs (Becton Dickinson).

Western Blot Analysis

[0222] Cells were lysed with lysis buffer (PBS containing 1% NP40, 0.5% Na-deoxycholate, and 0.1% SDS) supplemented with protease inhibitors (α-complete, Roche). The protein extracts were quantified using a calorimetric assay (Bradford Reagent) (BioRad, Hercules, CA). Proteins were electrophoresed onto 4-20% SDS-PAGE gels (Invitrogen) and transferred onto polyvinylidene difluoride membranes (Bio-Rad). Following blocking in 5% milk, membranes were incubated with a specific primary antibody, washed, and incubated with horseradish peroxidase-linked secondary antibody (Pierce). The signals were visualized with the chemiluminescent horseradish peroxidase antibody detection reagent (Denville Scientific).

Cytochrome *c* and Smac Release Assay

[0223] Four million of H146 or RS4;11 cells were treated with compounds at 37°C in an atmosphere of 95% air and 5% CO_2 for the indicated time points, washed with PBS and re-suspended in 100 µl of digitonin buffer (75 mM NaCl, 8 mM Na2HPO4, 1 mM NaH2PO4, 1 mM EDTA, 350 µg/ml digitonin, and 250 mM sucrose). Cytosolic fractions were separated

from organelle membrane fraction by centrifugation at 13,000 rpm for 1 min. The cytosolic fractions were resolved on a 12% SDS-PAGE and probed using anti-cytochrome c antibody (BD Biosciences) and anti-Smac (Cell Signaling Technology, Danvers, MA) antibody.

[0224] In particular, a compound of the invention was assayed for affinity to Bcl-2, Bcl-xL, and Mcl-1. The assay results compared to assay results for ABT-737, a known, patent Bcl-2/Bcl-xL inhibitor, and to these peptides. The results are summarized in Table 1.

Table 1. Binding affinities to Bcl-2, Bcl-xL, and Mcl-1 proteins, as determined using established FP-based assays. 3-5 independent experiments were performed for each compound for each protein. ABT-737, BIM, BAD, and NOXA peptides were tested as controls.

Compound	**Binding Affinities**				
	Bcl-2		**Bcl-xL**		**Mcl-1**
	IC_{50} ± SD	K_i ± SD	IC_{50} ± SD	K_i ± SD	IC_{50} ± SD
ABT-737	2 ± 0.2 (nM)	<1 (nM)	6 ± 2 (nM)	1.6 ± 0.5 (nM)	> 1 (μM)
BIM	< 1(nM)	< 1(nM)	< 1(nM)	< 1(nM)	5 ± 1 (nM)
BAD	40 ± 8(nM)	10 ± 2(nM)	5 ± 0.3(nM)	1.5 ± 0.1(nM)	32 ± 2 (μM)
NOXA	17± 1 (μM)	3.6 (μM)	11 ±2 (μM)	3.4 (μM)	37 ± 3 (μM)

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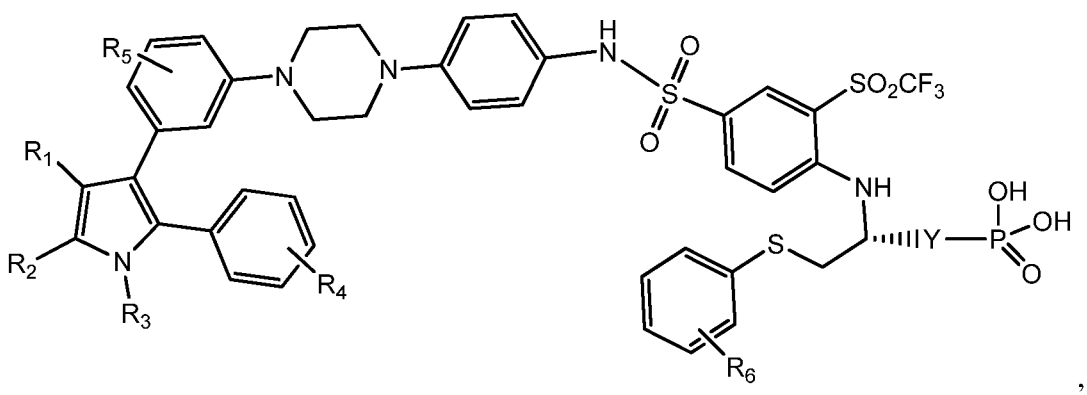
WHAT IS CLAIMED:

1. A compound having a structural formula:

(I)



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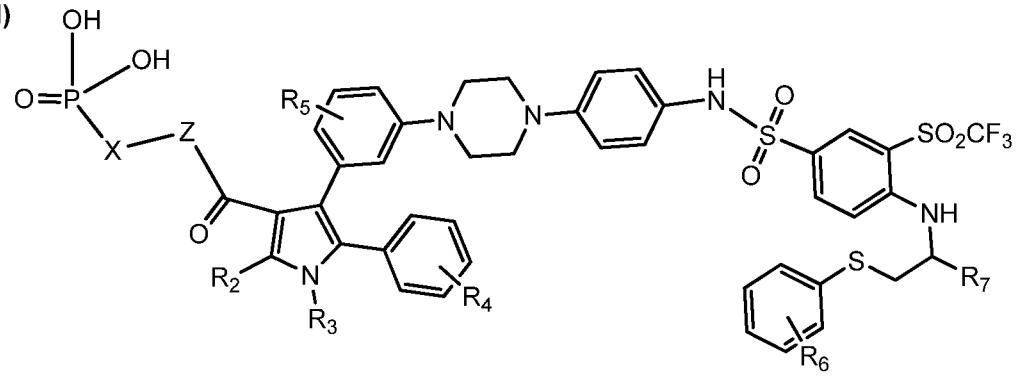
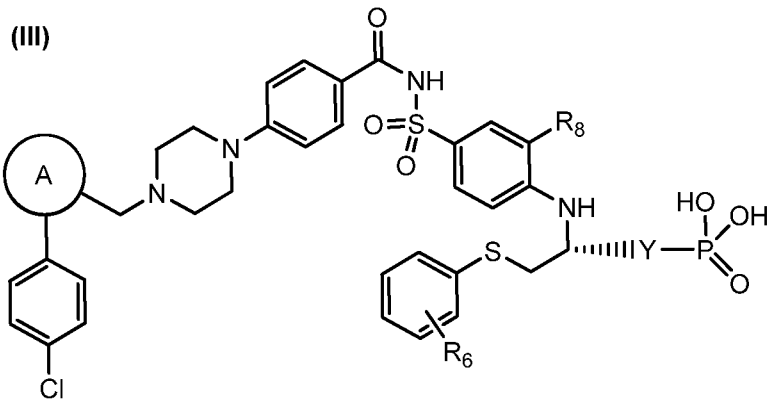
(II)



, or

(III)



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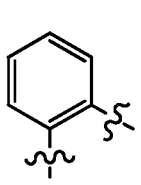
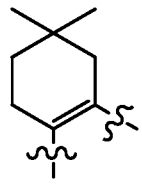
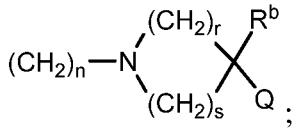
wherein the A ring is  or  ;

X, substituted or unsubstituted, is selected from the group consisting of alkylene, alkenylene, cycloalkylene, cycloalkenylene, and heterocycloalkylene;

Y is selected from the group consisting of $(CH_2)_n$-$N(R^a)$ and



Q is selected from the group consisting of O, $O(CH_2)_{1-3}$, NR^c, $NR^c(C_{1-3}alkylene)$, $OC(=O)(C_{1-3}alkylene)$, $C(=O)O$, $C(=O)O(C_{1-3}alkylene)$, $NHC(=O)(C_{1-3}alkylene)$, $C(=O)NH$, and $C(=O)NH(C_{1-3}alkylene)$;

Z is O or NR^c;

R_1 and R_2, independently, are selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", COR', CO_2R', OCOR', CONR'R", $CONR'SO_2R"$, NR'COR", NR'CONR"R'", NR'C=SNR"R'", $NR'SO_2R"$, SO_2R', and $SO_2NR'R"$;

R_3 is selected from a group consisting of H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', NR'R", OCOR', CO_2R', COR', CONR'R", $CONR'SO_2R"$, $C_{1-3}alkyleneCH(OH)CH_2OH$, SO_2R', and $SO_2NR'R"$;

R', R", and R'", independently, are H, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, $C_{1-3}alkyleneheterocycloalkyl$, or heterocycloalkyl;

R' and R", or R" and R'", can be taken together with the atom to which they are bound to form a 3 to 7 membered ring;

R_4 is hydrogen, halo, $C_{1-3}alkyl$, CF_3, or CN;

R_5 is hydrogen, halo, $C_{1-3}alkyl$, substituted $C_{1-3}alkyl$, hydroxyalkyl, alkoxy, or substituted alkoxy;

R_6 is selected from the group consisting of H, CN, NO_2, halo, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl, heterocycloalkyl, OR', SR', NR'R", CO_2R', OCOR', CONR'R", $CONR'SO_2R"$, NR'COR", NR'CONR"R'", NR'C=SNR"R'", $NR'SO_2R"$, SO_2R', and $SO_2NR'R"$;

R_7, substituted or unsubstituted, is selected form the group consisting of hydrogen, alkyl, alkenyl, $(CH_2)_{0-3}cycloalkyl$, $(CH_2)_{0-3}cycloalkenyl$, $(CH_2)_{0-3}heterocycloalkyl$, $(CH_2)_{0-3}aryl$, and $(CH_2)_{0-3}heteroaryl$;

R_8 is selected form the group consisting of hydrogen, halo, NO_2, CN, CF_3SO_2, and CF_3;

R_a is selected from the group consisting of hydrogen, alkyl, heteroalkyl, alkenyl, hydroxyalkyl, alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, and heterocycloalkyl;

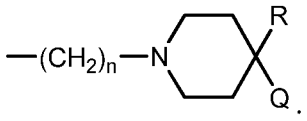
R_b is hydrogen or alkyl;

R_c is selected from the group consisting of hydrogen, alkyl, substituted alkyl, hydroxyalkyl, alkoxy, and substituted alkoxy; and

n, r, and s, independently, are 1, 2, 3, 4, 5, or 6;

or a pharmaceutically acceptable salt of (I), (II), or (III).

2. The compound of claim 1 wherein x is alkylene.

3. The compound of claim 1 or 2 wherein Y is  .

4. The compound of claim 3 wherein n is 1-3.

5. The compound of claim 3 wherein R_b is hydrogen or C_{1-3}alkyl.

6. The compound of any of claims 1-5 wherein Q is O, $O(CH_2)_{1-3}$, $C(=O)O(CH_2)_{1-3}$, $OC(=O)(CH_2)_{1-3}$, or $C(=O)O(C_3H_7)_{1-3}$.

7. The compound of any of claims 1-6 wherein Z is O, NH, or N(C_{1-3}alkyl).

8. The compound of any of claims 1-7 wherein R_1 is SO_2R', $SO_2NR'R''$, NR'SOR", H, or alkyl.

9. The compound of any of claims 1-8 wherein R_2 is H, C_{1-3}alkyl, cycloalkyl, or halo.

10. The compound of any of claims 1-9 wherein R_3 is H, C_{1-3}alkyl, or cycloalkylo.

11. The compound of any of claims 1-10 wherein R_4 is H or halo.

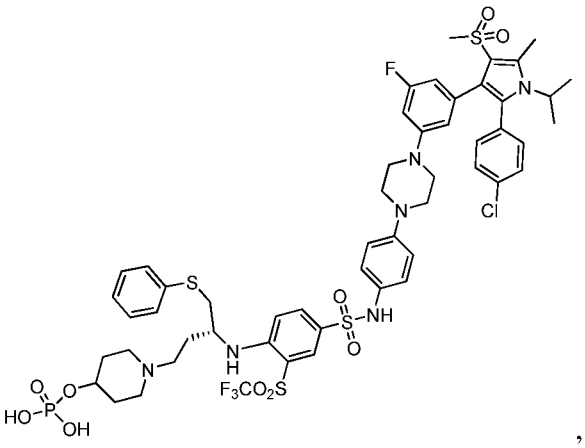
12. The compound of any of claims 1-11 wherein R_5 is H, halo, or C_{1-3}alkyl.

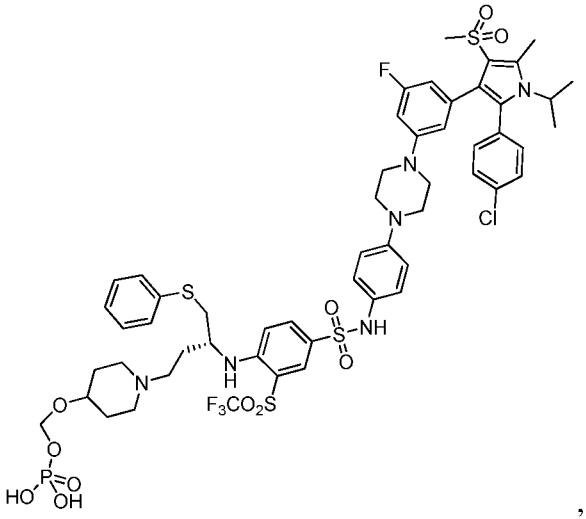
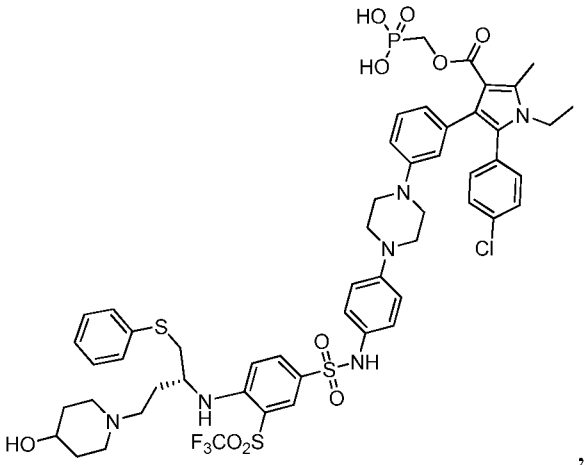
13. The compound of any of claims 1-12 wherein R_6 is H, halo, C_{1-3}alkyl, or cycloalkyl.

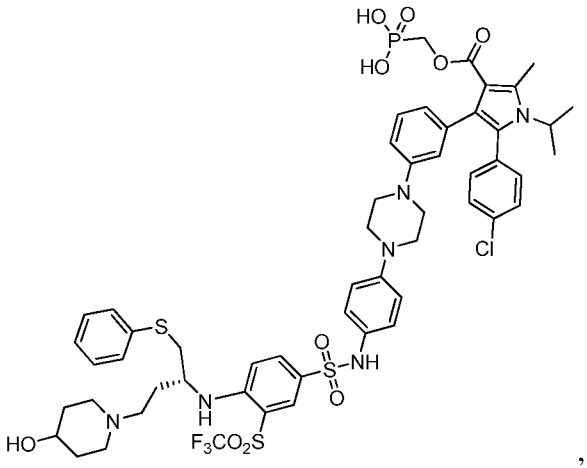
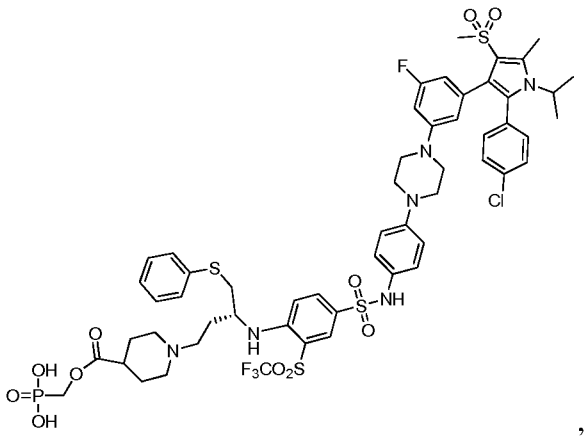
14. The compound of any of claims 1-13 wherein R_7 is $(CH_2)_{0-3}$cycloalkyl optionally substituted with –OH.

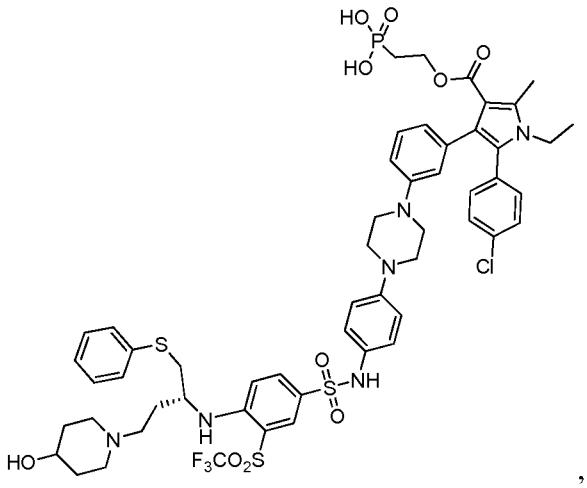
15. The compound of any of claims 1-14 wherein R_8 is CF_3SO_2 or CF_3.

16. The compound of any of claims 1-15 wherein R_b and R_c, independently, are H or C_{1-3}alkyl.

17. A compound having a structure  ,

 ,  ,

 ,  ,



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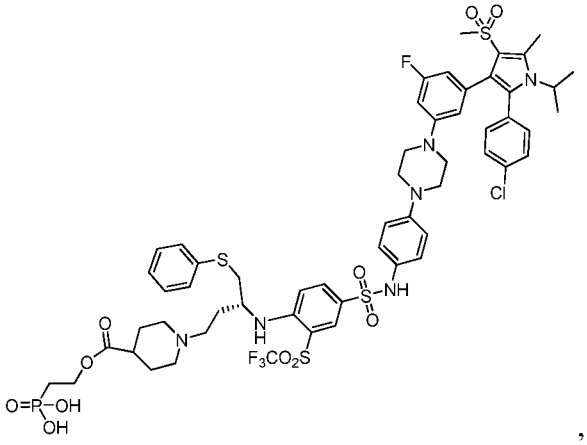

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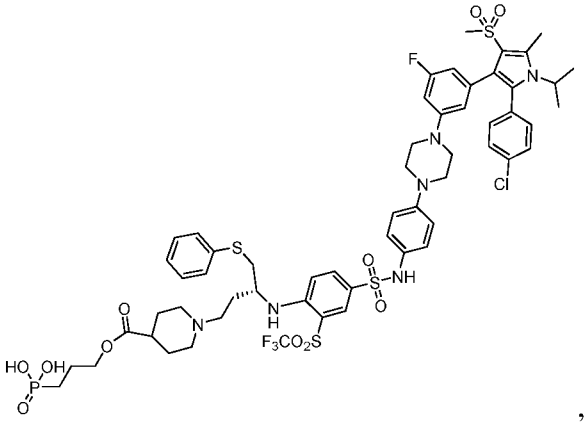

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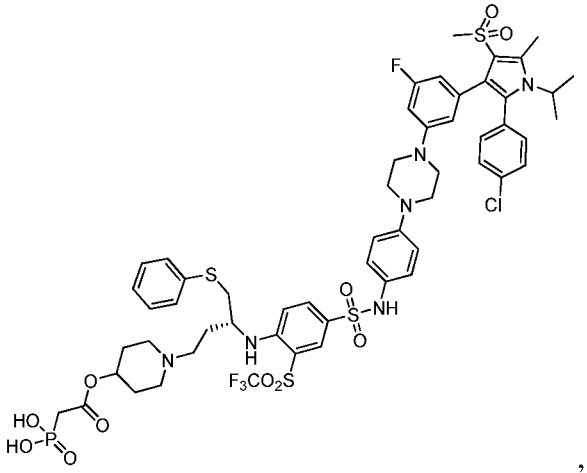

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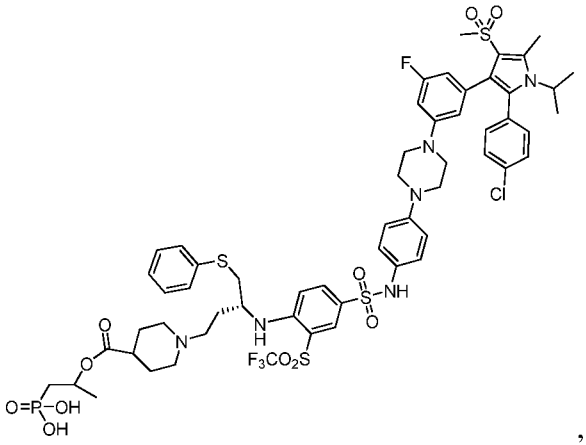

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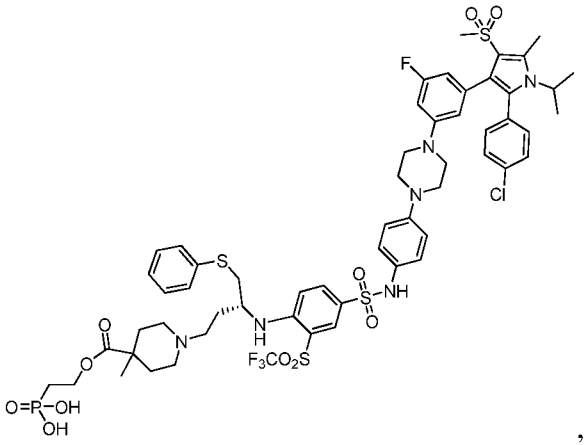

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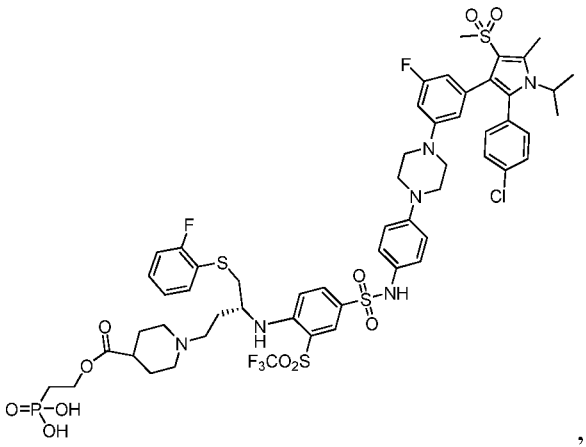

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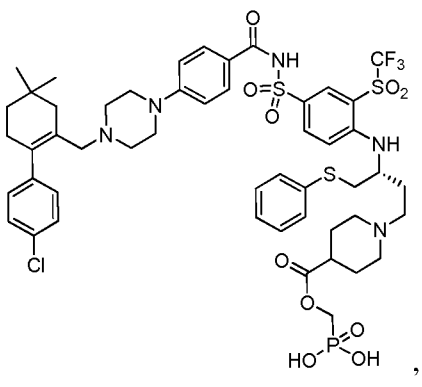

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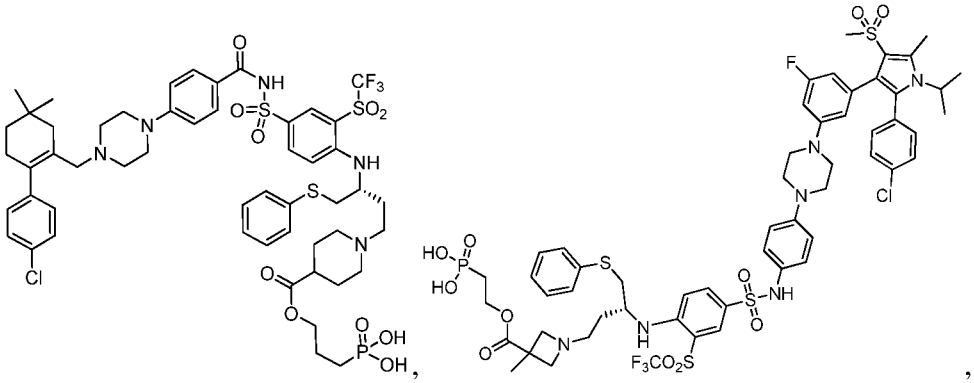
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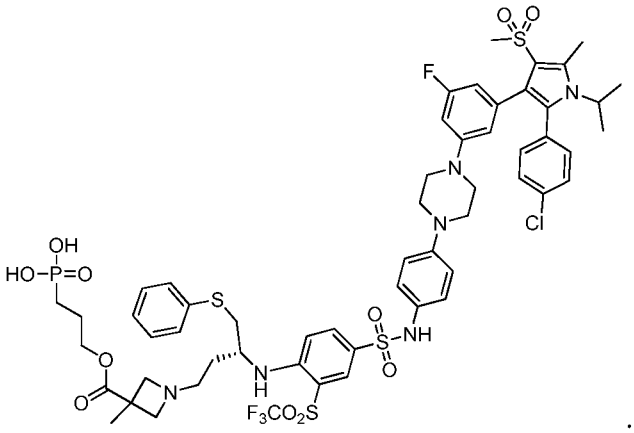
, and 
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18. A composition comprising (a) compound of claim 1, (b) a second therapeutic agent useful in the treatment of a disease or condition wherein inhibition of Bcl-2 or Bcl-xL provides a benefit, and (c) an optional excipient and/or pharmaceutically acceptable carrier.

19. The composition of claim 18 wherein the second therapeutic agent comprises a chemotherapeutic agent useful in the treatment of cancer.

20. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier or vehicle.

21. A method of treating a disease or condition wherein inhibition of Bcl-2 or Bcl-xL provides a benefit comprising administering a therapeutically effective amount of a compound of claim 1 to an individual in need thereof.

22. The method of claim 21 further comprising administering a therapeutically effective amount of a second therapeutic agent useful in the treatment of the disease or condition.

23. The method of claim 22 wherein the compound of claim 1 and the second therapeutic agent are administered simultaneously.

24. The method of claim 22 wherein the compound of claim 1 and the second therapeutic agent are administered separately.

25. The method of claim 21 wherein the disease or condition is a cancer.

26. The method of claim 22 wherein the disease is a cancer and the second therapeutic agent is one or more of a chemotherapeutic agent and radiation.

27. The method of claim 22 wherein the disease is a cancer and the second therapeutic agent is selected from the agents disclosed in paragraphs [0125] through [0131].

28. The method of claim 22 wherein the second therapeutic agent comprises radiation, and the radiation optionally is administered in conjunction with radiosensitizers and/or therapeutic agents disclosed in paragraphs [0122] through [0124] herein.

29. The method of claim 25 wherein the cancer is selected from a cancer disclosed in paragraphs [0110] through [0112] herein.

30. The method of claim 22 wherein the compound of claim 1 and the second therapeutic agent are administered from a single composition.

31. The method of claim 22 wherein the compound of claim 1 and the second therapeutic agent are administered from separate compositions.

32. The method of claim 24 wherein the compound of claim 1 is administered prior to the second therapeutic agent.

33. The method of claim 24 wherein the compound of claim 1 is administered after the second therapeutic agent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/011571

A. CLASSIFICATION OF SUBJECT MATTER

C07D 401/14(2006.01)i, A61K 31/496(2006.01)i, A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D 401/14; C07D 403/10; C07D 403/14; A61K 31/496; A61P 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: Bcl-2/Bcl-xL inhibitor , 4,5-diphenyl-1H-pyrrole-3-carboxylic acid, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012-103059 A2 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 02 August 2012 See claim 1; abstract and paragraph [0086].	1-5,17-20
X	ZHOU, H. et al, Structure-Based Design of Potent Bcl-2/Bcl-xL Inhibitors with Strong in Vivo Antitumor Activity. Journal of Medicinal Chemistry, 02 July 2012, Vol. 55, pages 6149-6161 See abstract; figures 1, 3 and 5; scheme 2.	1-5,17-20
X	CHEN, J. et al, Structure-Based Discovery of BM-957 as a Potent Small-Molecule Inhibitor of Bcl-2 and Bcl-xL Capable of Achieving Complete Tumor Regression. Journal of Medicinal Chemistry, 02 October 2012. Vol. 55, pages 8502-8514 See abstract; figure 1 and tables 1-3.	1-5,17-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2014 (29.05.2014)	**30 May 2014 (30.05.2014)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
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Telephone No. +82-42-481-8739

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/011571

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

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

1. ☒ Claims Nos.: 21-33
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 21-33 pertain to methods for diagnosing human diseases or treating the human body by therapy thus relate to a subject matter which this International Searching Authority is not required to search under the PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☒ Claims Nos.: 27-29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 27-29 are unclear, since it refers to the 'paragraph' (PCT Article 6).

3. ☒ Claims Nos.: 6-16, 32-33
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/011571

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012-103059 A2	02/08/2012	CA 2825306 A1	02/08/2012
		EP 2668180 A2	04/12/2013
		JP 2014-507421 A	27/03/2014
		KR 20140005984 A	15/01/2014
		SG 192126 A1	30/09/2013
		US 2012-189539 A1	26/07/2012
		WO 2012-103059 A3	18/10/2012

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105246882 A
(43)申请公布日 2016.01.13

(21)申请号 201480015733.0
(22)申请日 2014.01.15
(30)优先权数据
61/753066 2013.01.16 US
(85)PCT国际申请进入国家阶段日
2015.09.15
(86)PCT国际申请的申请数据
PCT/US2014/011571 2014.01.15
(87)PCT国际申请的公布数据
WO2014/113413 EN 2014.07.24
(71)申请人 密执安大学评议会
地址 美国密执安州
(72)发明人 S. 王 J. 陈 D. 麦凯彻恩 L. 白 L. 刘 D. 孙 X. 李 A. 阿圭拉
(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 黄登高 林森
(51)Int.Cl.
C07D 401/14(2006.01)
A61K 31/496(2006.01)
A61P 35/00(2006.01)

权利要求书5页 说明书49页

(54)发明名称

BCL-2/Bcl-xL抑制剂和使用所述抑制剂的治疗方法

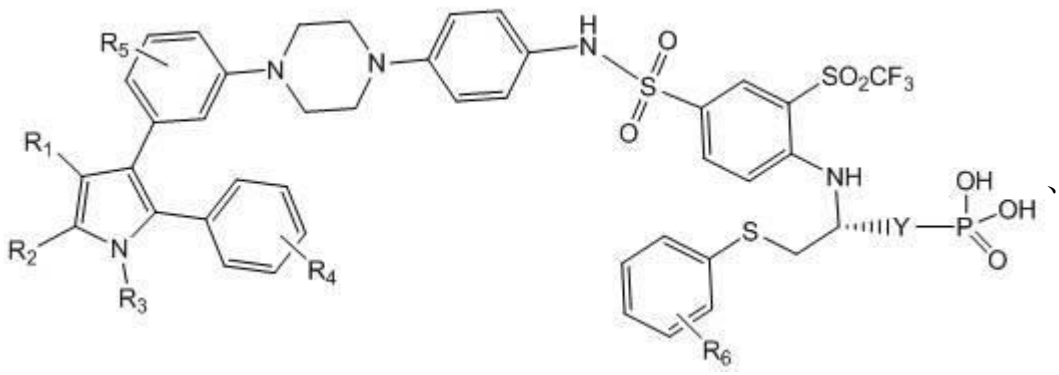
(57)摘要

公开了Bcl-2/Bcl-xL的抑制剂和含有所述抑制剂的组合物。还公开了在治疗其中Bcl-2/Bcl-xL的抑制提供益处的疾病和病况例如癌症中使用Bcl-2/Bcl-xL抑制剂的方法。

CN 105246882 A

1. 具有以下结构式的化合物,或 (I)、(II) 或 (III) 的药学上可接受的盐 :

(I)



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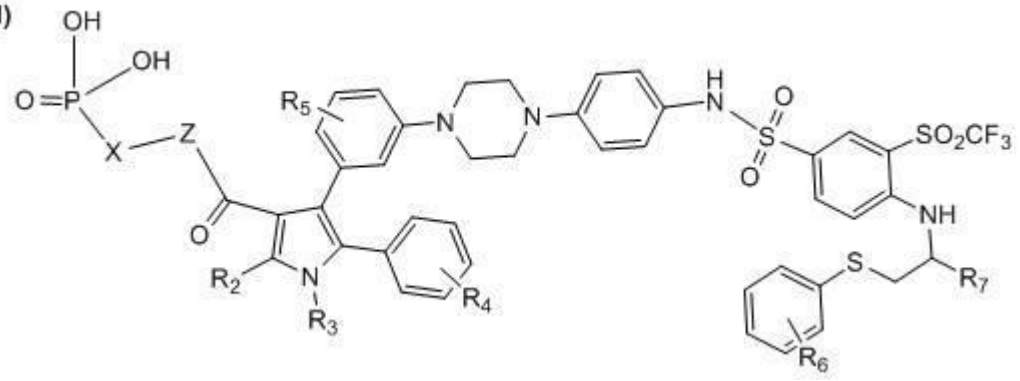
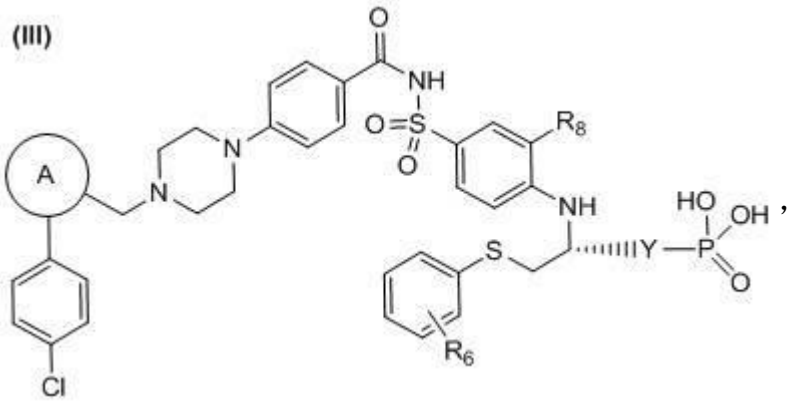
(II)



或

(III)



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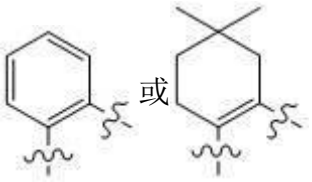
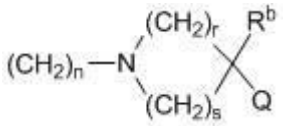
其中 A 环是  或 ;

取代或未取代的 X 选自亚烷基、亚烯基、环亚烷基、环亚烯基和杂环亚烷基 ;

Y 选自 $(CH_2)_n$-N(R^a) 和



;

Q 选自 O、O$(CH_2)_{1-3}$、NR^c、NR^c(C_{1-3}亚烷基)、OC(=O)(C_{1-3}亚烷基)、C(=O)O、C(=O)O(C_{1-3}亚烷基)、NHC(=O)(C_{1-3}亚烷基)、C(=O)NH 和 C(=O)NH(C_{1-3}亚烷基) ;

Z 是 O 或 NR^c;

R_1和 R_2独立地选自 H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、COR'、CO_2R'、OCOR'、CONR'R''、$CONR'SO_2R''$、NR'COR''、NR'CONR''R'''、NR'C=SNR''R'''、$NR'SO_2R''$、SO_2R' 和 $SO_2NR'R''$；

R_3选自 H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、NR'R''、OCOR'、CO_2R'、COR'、CONR'R''、$CONR'SO_2R''$、C_{1-3}亚烷基 CH(OH)CH_2OH、SO_2R' 和 $SO_2NR'R''$；

R'、R'' 和 R''' 独立地是 H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、C_{1-3}亚烷基杂环烷基或杂环烷基；

R' 和 R'' 或 R'' 和 R''' 可与它们所连接的原子一起形成 3-7 元环；

R_4是氢、卤素、C_{1-3}烷基、CF_3或 CN；

R_5是氢、卤素、C_{1-3}烷基、取代的 C_{1-3}烷基、羟基烷基、烷氧基或取代的烷氧基；

R_6选自 H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、CO_2R'、OCOR'、CONR'R''、$CONR'SO_2R''$、NR'COR''、NR'CONR''R'''、NR'C=SNR''R'''、$NR'SO_2R''$、SO_2R' 和 $SO_2NR'R''$；

取代或未取代的 R_7选自氢、烷基、烯基、$(CH_2)_{0-3}$环烷基、$(CH_2)_{0-3}$环烯基、$(CH_2)_{0-3}$杂环烷基、$(CH_2)_{0-3}$芳基和 $(CH_2)_{0-3}$杂芳基；

R_8选自氢、卤素、NO_2、CN、CF_3SO_2和 CF_3；

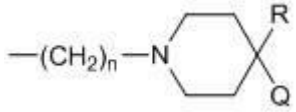
R_a选自氢、烷基、杂烷基、烯基、羟基烷基、烷氧基、取代的烷氧基、环烷基、环烯基和杂环烷基；

R_b是氢或烷基；

R_c选自氢、烷基、取代的烷基、羟基烷基、烷氧基和取代的烷氧基；和

n、r 和 s 独立地是 1、2、3、4、5 或 6。

2. 权利要求 1 的化合物，其中 x 是亚烷基。

3. 权利要求 1 或 2 的化合物，其中 Y 是 

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4. 权利要求 3 的化合物，其中 n 是 1-3。

5. 权利要求 3 的化合物，其中 R_b是氢或 C_{1-3}烷基。

6. 权利要求 1-5 中任一项的化合物，其中 Q 是 O、O$(CH_2)_{1-3}$、C(=O)O$(CH_2)_{1-3}$、OC(=O)$(CH_2)_{1-3}$或 C(=O)O$(C_3H_7)_{1-3}$。

7. 权利要求 1-6 中任一项的化合物，其中 Z 是 O、NH 或 N(C_{1-3}烷基)。

8. 权利要求 1-7 中任一项的化合物，其中 R_1是 SO_2R'、$SO_2NR'R''$、NR'SOR''、H 或烷基。

9. 权利要求 1-8 中任一项的化合物，其中 R_2是 H、C_{1-3}烷基、环烷基或卤素。

10. 权利要求 1-9 中任一项的化合物，其中 R_3是 H、C_{1-3}烷基或环烷基。

11. 权利要求 1-10 中任一项的化合物，其中 R_4是 H 或卤素。

12. 权利要求 1-11 中任一项的化合物，其中 R_5是 H、卤素或 C_{1-3}烷基。

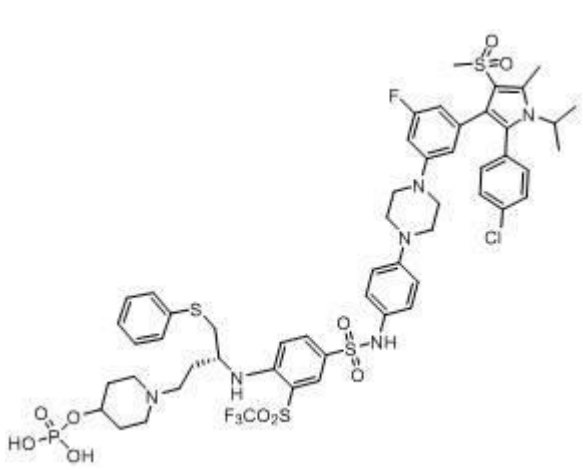
13. 权利要求 1-12 中任一项的化合物，其中 R_6是 H、卤素、C_{1-3}烷基或环烷基。

14. 权利要求 1-13 中任一项的化合物，其中 R_7是 $(CH_2)_{0-3}$环烷基，其任选被 -OH 取代。

15. 权利要求 1-14 中任一项的化合物，其中 R_8是 CF_3SO_2或 CF_3。

16. 权利要求 1-15 中任一项的化合物，其中 R_b和 R_c独立地为 H 或 C_{1-3}烷基。

17.具有以下结构的化合物：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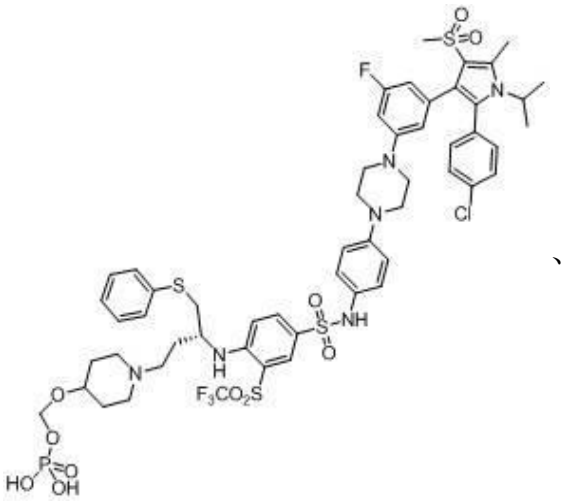
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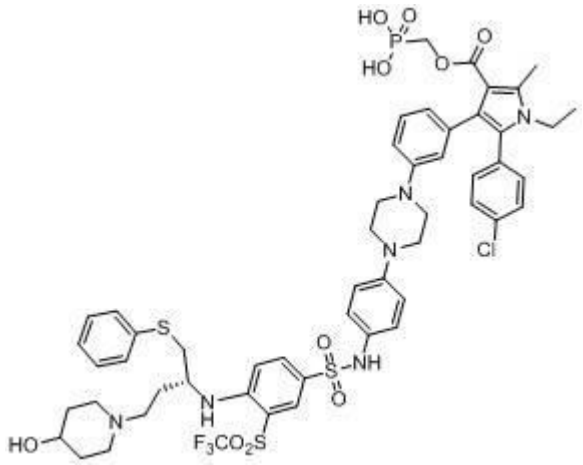
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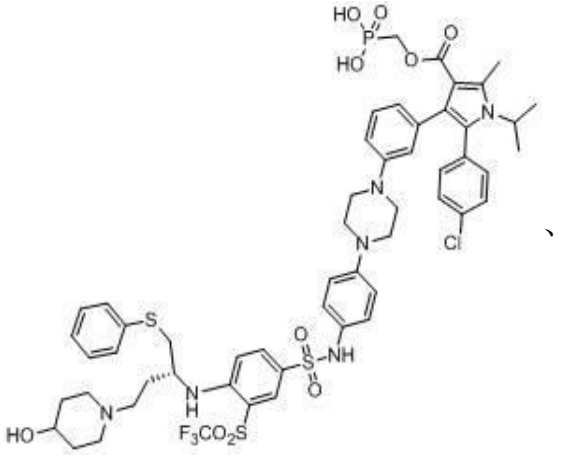
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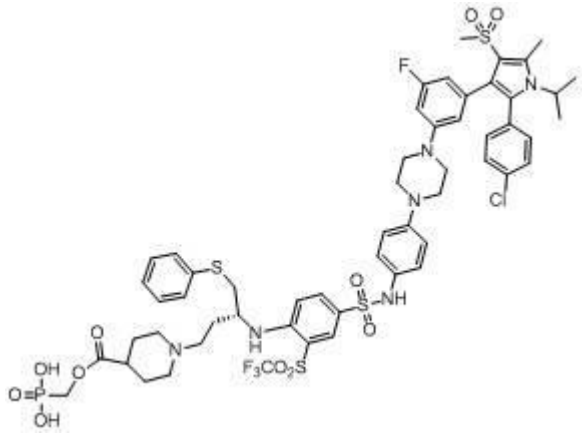
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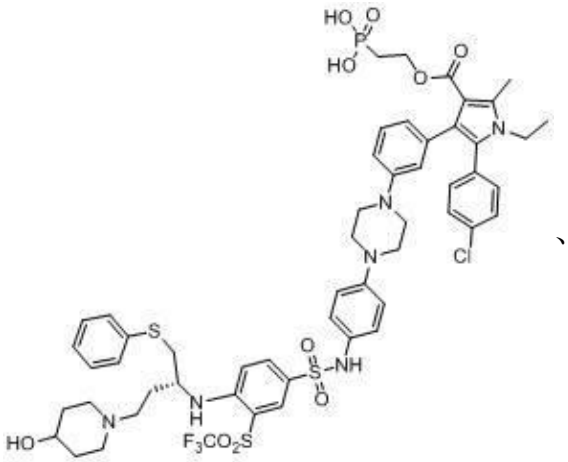
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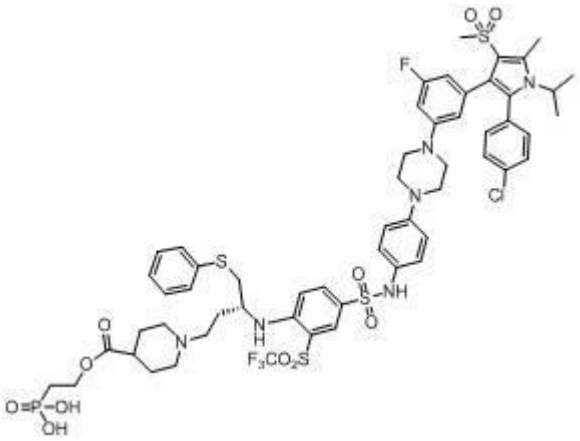
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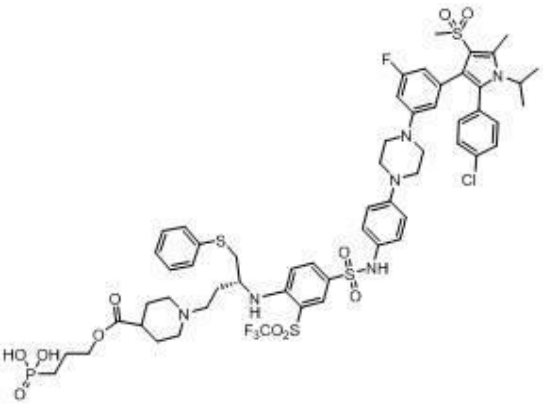
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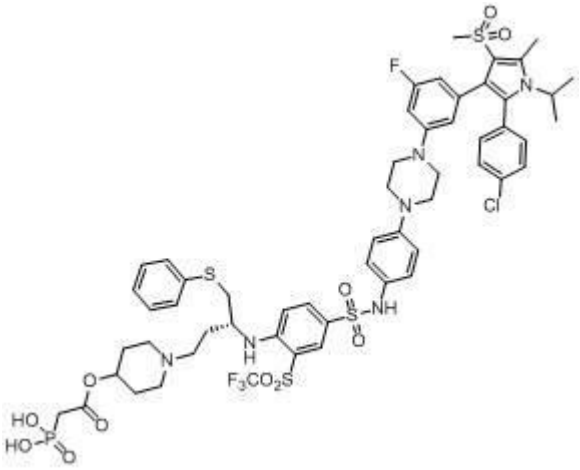
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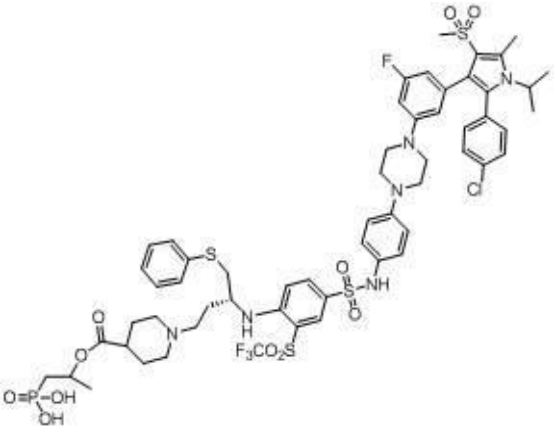
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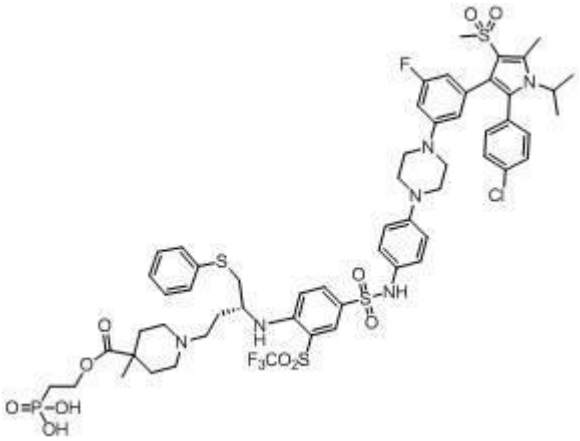
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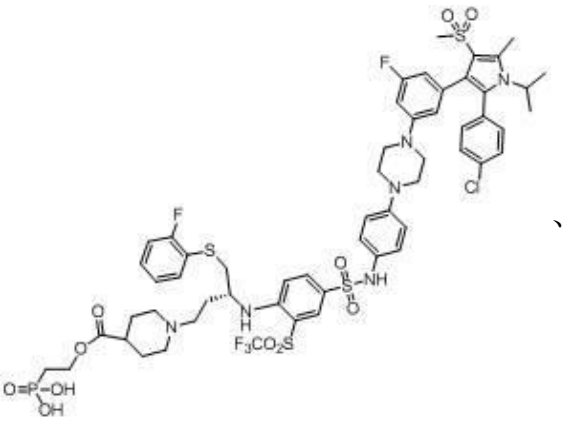
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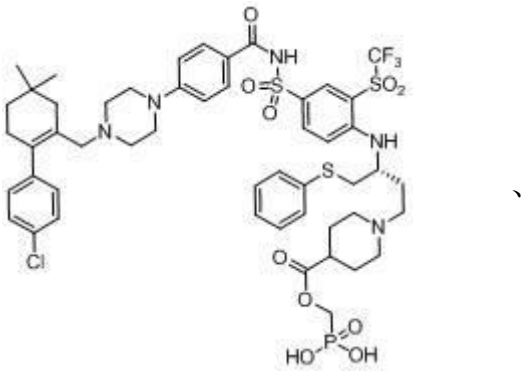
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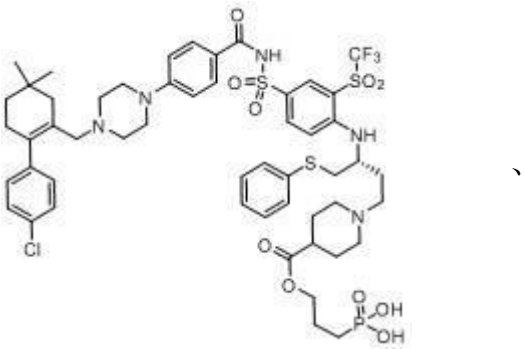
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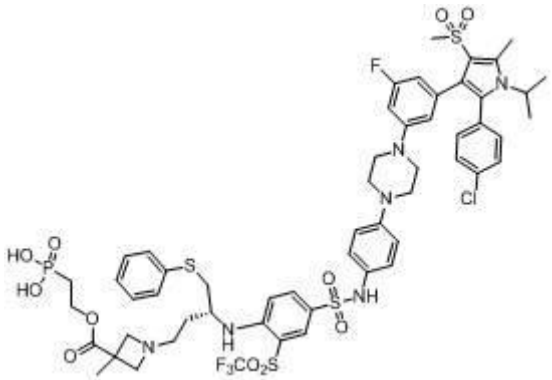
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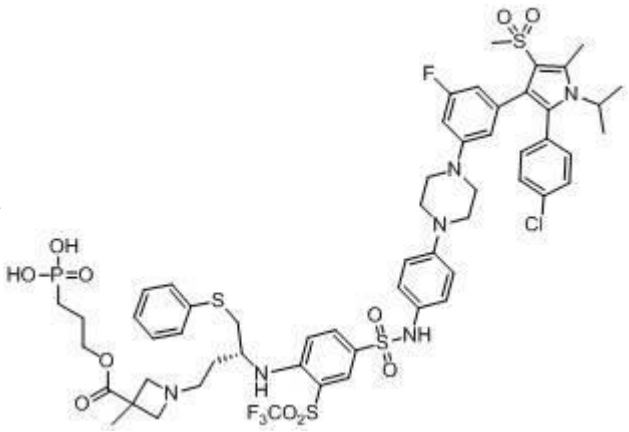
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18. 包含以下的组合物：(a) 权利要求 1 的化合物，(b) 用于治疗其中 Bcl-2 或 Bcl-xL 的抑制提供益处的疾病或病况的第二种治疗剂，和 (c) 任选的赋形剂和 / 或药学上可接受的载体。

19. 权利要求 18 的组合物，其中第二种治疗剂包括用于治疗癌症的化学治疗剂。

20. 包含权利要求 1 的化合物和药学上可接受的载体或溶媒的药物组合物。

21. 治疗其中 Bcl-2 或 Bcl-xL 的抑制提供益处的疾病或病况的方法，包括给予有需要的个体治疗有效量的权利要求 1 的化合物。

22. 权利要求 21 的方法，其进一步包括给予治疗有效量的用于治疗所述疾病或病况的第二种治疗剂。

23. 权利要求 22 的方法，其中同时给予权利要求 1 的化合物和第二种治疗剂。

24. 权利要求 22 的方法，其中分开给予权利要求 1 的化合物和第二种治疗剂。

25. 权利要求 21 的方法，其中所述疾病或病况是癌症。

26. 权利要求 22 的方法，其中所述疾病是癌症，和第二种治疗剂是化学治疗剂和辐射的一种或多种。

27. 权利要求 22 的方法，其中所述疾病是癌症，和第二种治疗剂选自段落 [0125]-[0131] 中公开的药剂。

28. 权利要求 22 的方法，其中所述第二种治疗剂包括辐射，和所述辐射任选与本文段落 [0122]-[0124] 中公开的辐射致敏剂和 / 或治疗剂结合给予。

29. 权利要求 25 的方法，其中所述癌症选自本文段落 [0110]-[0112] 中公开的癌症。

30. 权利要求 22 的方法，其中权利要求 1 的化合物和第二种治疗剂从单一组合物中给予。

31. 权利要求 22 的方法，其中权利要求 1 的化合物和第二种治疗剂从分开的组合物中给予。

32. 权利要求 24 的方法，其中权利要求 1 的化合物在第二种治疗剂之前给予。

33. 权利要求 24 的方法，其中权利要求 1 的化合物在第二种治疗剂之后给予。

BCL-2/Bcl-xL 抑制剂和使用所述抑制剂的治疗方法

发明领域

[0001] 本发明涉及 Bcl-2/Bcl-xL 抑制剂和治疗其中 Bcl-2/Bcl-xL 的抑制提供益处的病况和疾病的治疗方法。

[0002] 发明背景

细胞凋亡抵抗是人类癌症的标志 (1-3)。癌细胞必须克服通过细胞应激的持续轰击，所述细胞应激例如 DNA 损伤、癌基因激活、细胞周期进行异常和残酷的微环境，其将引起正常细胞遭受细胞凋亡。癌细胞逃避细胞凋亡的主要方式之一是通过增量调节抗 - 细胞凋亡的 Bcl-2 家族的蛋白。靶向关键的细胞凋亡调节物以克服细胞凋亡 - 抵抗和促进肿瘤细胞的细胞凋亡是新的癌症治疗策略 (4,5)。

[0003] Bcl-2 蛋白在癌细胞和正常细胞两者中起细胞凋亡的关键调节物的作用 (6-10)。Bcl-2 蛋白作为细胞凋亡的核查，允许健康和有用的细胞存活。该蛋白家族包括抗 - 细胞凋亡的蛋白，例如 Bcl-2、Bcl-xL 和 Mcl-1，和促 - 细胞凋亡的分子，包括 Bid、Bim、Bad、Bak 和 Bax (6-10)。尽管正常细胞具有低表达水平的抗 - 细胞凋亡的 Bcl-2 和 Bcl-xL 蛋白，但发现这些蛋白在许多不同类型的人类肿瘤中高度过表达 (6-10)。这些过表达与数种类型的癌症的预后差有关，和与对化学治疗剂和辐射的临床抵抗有关 (6-10)。与临床观察一致的是，实验室研究已经确定在体外和体内，Bcl-2 或 Bcl-xL 的过表达引起癌细胞变得对化学治疗剂更有抵抗力 (6-10)。Bcl-2 抑制细胞凋亡通过抑制细胞死亡而有助于癌症。因此，已寻求将靶向 Bcl-2 和 / 或 Bcl-xL 作为癌症治疗策略 (11-34)。抑制癌细胞的 Bcl-2 活性可降低化学治疗抵抗和增加杀死癌细胞。

[0004] Bcl-2 和 Bcl-xL 蛋白通过与促 - 细胞凋亡的 Bcl-2 家族蛋白例如 Bak、Bax、Bim、Bid、Puma 和 Bad 异二聚化而抑制细胞凋亡 (6-10)。实验上确定的 Bcl-xL 和 Bcl-2 的三维结构已经表明，这些蛋白具有充分定义的沟，其与促 - 细胞凋亡的 Bcl-2 蛋白的 BH3 (Bcl-2 同源性 3) 结构域相互作用 (38-42)。已经提出，经设计以阻断 Bcl-2/Bcl-xL 蛋白与其促 - 死亡结合配偶体的异二聚化的非肽小分子可有效作为 Bcl-2/Bcl-xL 的拮抗剂，并且所述小分子抑制剂可具有治疗其中 Bcl-2 和 / 或 Bcl-xL 高度表达的人类癌症的极大治疗潜力 (18-37)。

[0005] 尽管已经报道 Bcl-2/Bcl-xL 的非肽小分子抑制剂，但大部分抑制剂对这些蛋白具有弱至中等的亲和力，并对其细胞活性缺乏完全明确的作用模式 (18-37)。例外是 ABT-737、ABT-263 和其类似物 (26-34)。ABT-737 和 ABT-263 以非常高的亲和力结合 Bcl-2、Bcl-xL 和 Bcl-w (K_i <1 nM) 和具有比 Mcl-1 和 A1 (两种其它的抗 - 细胞凋亡的 Bcl-2 蛋白) 高的特异性 (26, 32, 34)。ABT-263 已经进入 I/II 期临床试验并显示临床上有前景的抗肿瘤活性 (45)。

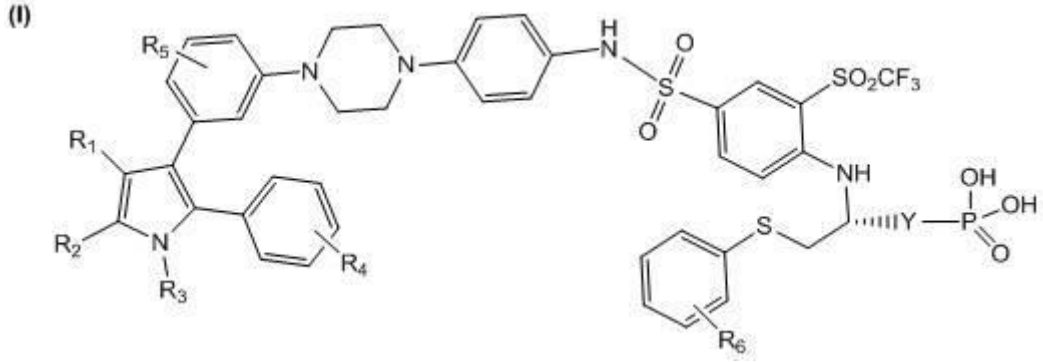
[0006] 尽管发现了 ABT-737 和 ABT-263，但 Bcl-2/Bcl-xL 的有效的非肽抑制剂的设计仍是现代药物发现中的重要挑战。因此，本领域仍对具有允许在治疗应用中使用抑制剂的物理和药理性质的 Bcl-2/Bcl-xL 抑制剂存在需要。本发明提供经设计以结合 Bcl-2/Bcl-xL 和抑制 Bcl-2/Bcl-xL 活性的化合物。

[0007] 发明简述

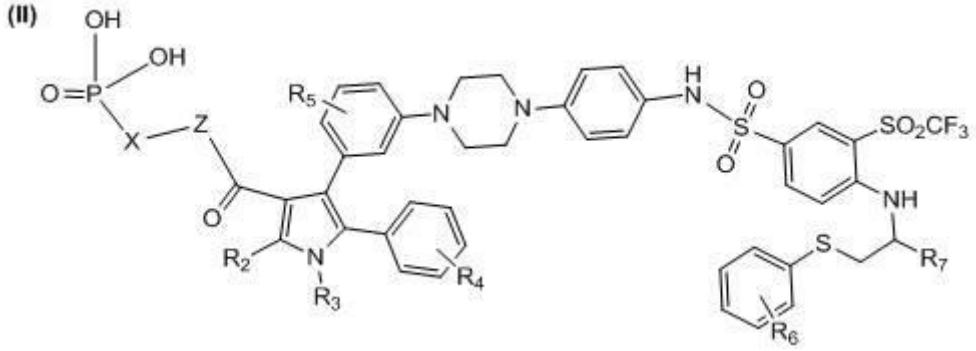
本发明涉及 Bcl-2/Bcl-xL 的抑制剂，涉及包含所述抑制剂的组合物，和涉及在治疗性治疗其中 Bcl-2/Bcl-xL 活性的抑制提供益处的病况和疾病中使用所述抑制剂的方法。本发明的化合物是 Bcl-2/Bcl-xL 激活的有效抑制剂，并诱导表达 Bcl-2 和 / 或 Bcl-xL 的癌细胞的细胞凋亡。

[0008] 更具体而言，本发明涉及具有结构式 (I)、(II) 或 (III) 的化合物或 (I)、(II) 或 (III) 的药学上可接受的盐：

(I)



(II)



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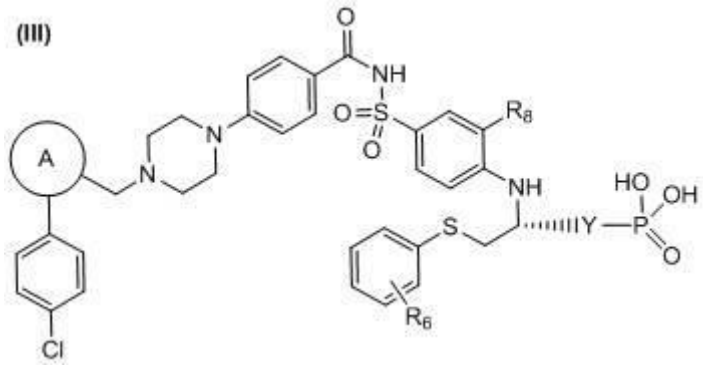
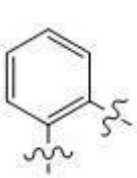
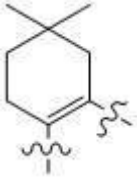
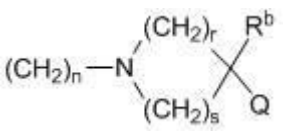


其中 A 环是  或  ；

取代或未取代的 X 选自亚烷基、亚烯基、环亚烷基、环亚烯基和杂环亚烷基；

Y 选自 $(CH_2)_n$-$N(R^a)_2$和

 ；

Q 选自 O、O(CH_2)$_{1-3}$、NR^c、NR^c(C_{1-3}亚烷基)、OC(=O)(C_{1-3}亚烷基)、C(=O)O、C(=O)O(C_{1-3}亚烷基)、NHC(=O)(C_{1-3}亚烷基)、C(=O)NH 和 C(=O)NH(C_{1-3}亚烷基)；

Z 是 O 或 NR^c；

R_1和 R_2独立选自 H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、COR'、CO_2R'、OCOR'、CONR'R''、CONR'SO_2R''、NR'COR''、NR'CONR''R'''、NR'C=SNR''R'''、NR'SO_2R''、SO_2R' 和 SO_2NR'R''；

R_3选自 H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、NR'R''、OCOR'、CO_2R'、COR'、CONR'R''、CONR'SO_2R''、C_{1-3}亚烷基 CH(OH)CH_2OH、SO_2R' 和 SO_2NR'R''；

R'、R'' 和 R''' 独立地为 H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、C_{1-3}亚烷基杂环烷基或杂环烷基；

R' 和 R'' 或 R'' 和 R''' 可与它们所连接的原子一起形成 3-7 元环；

R_4是氢、卤素、C_{1-3}烷基、CF_3或 CN；

R_5是氢、卤素、C_{1-3}烷基、取代的 C_{1-3}烷基、羟基烷基、烷氧基或取代的烷氧基；

R_6选自 H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、CO_2R'、OCOR'、CONR'R''、CONR'SO_2R''、NR'COR''、NR'CONR''R'''、NR'C=SNR''R'''、NR'SO_2R''、SO_2R' 和 SO_2NR'R''；

取代或未取代的 R_7选自氢、烷基、烯基、(CH_2)$_{0-3}$环烷基、(CH_2)$_{0-3}$环烯基、(CH_2)$_{0-3}$杂环烷基、(CH_2)$_{0-3}$芳基，和 (CH_2)$_{0-3}$杂芳基；

R_8选自氢、卤素、NO_2、CN、CF_3SO_2，和 CF_3；

R_a选自氢、烷基、杂烷基、烯基、羟基烷基、烷氧基、取代的烷氧基、环烷基、环烯基和杂环烷基；

R_b是氢或烷基；

R_c选自氢、烷基、取代的烷基、羟基烷基、烷氧基和取代的烷氧基；和

n、r 和 s 独立地为 1、2、3、4、5 或 6。

[0009] 在一些实施方案中，R_1和 R_2或 R_2和 R_3可一起形成环。在其它实施方案中，R' 和 R″ 或 R″ 和 R''' 可与它们所连接的原子一起形成 3-7 元环。

[0010] 在一个实施方案中，本发明提供通过给予有需要的个体治疗有效量的结构式 (I)、(II) 或 (III) 化合物来治疗病况或疾病的方法。目标疾病或病况可通过抑制 Bcl-2 和 / 或 Bcl-xL 治疗，例如癌症。

[0011] 本发明的另一个实施方案是提供用于治疗其中 Bcl-2/Bcl-xL 的抑制提供益处的疾病或病况的包含以下的组合物：(a) 结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和 (b) 赋形剂和 / 或药学上可接受的载体。

[0012] 本发明的另一个实施方案是在治疗个体的其中 Bcl-2/Bcl-xL 的抑制提供益处的疾病或病况的方法中使用包含结构式 (I)、(II) 或 (III) 的化合物和第二种治疗活性剂的组合物。

[0013] 在另一个实施方案中，本发明提供包含结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和任选的第二种治疗剂的组合物在制备用于治疗目标疾病或病况例如癌症的药物中的用途。

[0014] 本发明的又一个实施方案是提供用于人类药用的药盒，其包含 (a) 容器，(b1) 包

装的组合物，其包含结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂，和任选 (b2) 包装的组合物，其包含用于治疗目标疾病或病况的第二种治疗剂，和 (c) 包装插页 (package insert)，其包含在治疗疾病或病况中同时或序贯给予的所述一种或多种组合物的使用说明。

[0015] 结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和第二种治疗剂可作为单一单位剂量一起给予，或作为多个单位剂量分开给予，其中结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂在第二种治疗剂之前给予，或反之亦然。预期可给予一个或多个剂量的结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和 / 或一个或多个剂量的第二种治疗剂。

[0016] 在一个实施方案中，同时给予结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和第二种治疗剂。在相关的实施方案中，结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和第二种治疗剂从单一组合物中给予或从分开的组合物中给予。在进一步的实施方案中，序贯给予结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂和第二种治疗剂。本发明中使用的结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂可以约 0.005- 约 500 毫克 / 剂量、约 0.05- 约 250 毫克 / 剂量或约 0.5- 约 100 毫克 / 剂量的量给予。

[0017] 本发明的这些和其它实施方案和特征将从以下优选实施方案的详细描述中变得显而易见。

[0018] 优选实施方案的详细描述

本发明结合优选的实施方案进行描述。然而，应理解，本发明不限于所公开的实施方案。要理解，鉴于本文中本发明实施方案的描述，本领域的技术人员可进行各种修改。下文的权利要求包括这样的修改。

[0019] 本文所用的术语 "Bcl-2/Bcl-xL" 意指 Bcl-2、Bcl-xL、或 Bcl-2 和 Bcl-xL，即 Bcl-2 和 / 或 Bcl-xL。

[0020] 术语 " 其中 Bcl-2 和 / 或 Bcl-xL 的抑制提供益处的疾病或病况 " 属于其中 Bcl-2 和 / 或 Bcl-xL，和 / 或 Bcl-2 和 / 或 Bcl-xL 的作用对于例如所述疾病或病况的发作、进展、表现是重要或必要的病况，或已知通过 Bcl-2/Bcl-xL 抑制剂例如 ABT-737 或 ABT-263 治疗的疾病或病况。这样的病况的实例包括但不限于癌症。本领域的普通技术人员能够容易确定一种化合物是否治疗对于任何特定细胞类型由 Bcl-2/Bcl-xL 介导的疾病或病况，例如，通过可方便地用于评价特定化合物的活性的测定法。

[0021] 术语 " 第二种治疗剂 " 是指不同于结构式 (I)、(II) 和 (III) 的 Bcl-2 和 / 或 Bcl-xL 抑制剂的治疗剂，并且其已知治疗目标疾病或病况。例如，当癌症是目标疾病或病况时，第二种治疗剂可以是已知的化学治疗药，例如泰素或辐射。

[0022] 术语 " 疾病 " 或“病况”表示按惯例被视为病理学状况或功能，并且可以具体征兆、症状和 / 或机能障碍的形式表现自身的失调和 / 或异常。如下文所示，结构式 (I)、(II) 和 (III) 的化合物是 Bcl-2/Bcl-xL 的有效抑制剂，并且可用于治疗其中 Bcl-2/Bcl-xL 的抑制提供益处的疾病和病况。

[0023] 本文所用的术语 " 治疗 (treat)"、" 治疗 (treating)"、" 治疗 (treatment)" 等是指消除、减少或改善疾病或病况和 / 或与其相关的症状。尽管未排除，但治疗疾病或病况不要求所述疾病、病况或与其相关的症状被完全消除。本文所用的术语 " 治疗 (treat)"、" 治疗 (treating)"、" 治疗 (treatment)" 等可包括 " 预防性治疗 "，其是指在未患有疾病，但

有风险或易于重新发生疾病或病况或所述疾病或病况有复发风险或易于复发的受试者中，减少重新发生疾病或病况的可能性，或减少之前控制的疾病或病况的复发的可能性。术语″治疗″和同义词预期给予需要所述治疗的个体治疗有效量的本发明化合物。

[0024] 在本发明的含义内，″治疗″还包括复发预防或阶段预防，以及治疗急性或慢性征兆、症状和/或机能障碍。治疗可以是针对症状的，例如，抑制症状。其可在短时期内起作用，针对中等时期内，或可以是长期治疗，例如在维持疗法的情况下。

[0025] 本文所用的术语″治疗有效量″或″有效剂量″是指这样的活性成分的量，当通过本发明的方法给予时，其足以有效递送活性成分至有需要的个体用于治疗目标病况或疾病。在癌症或其它增殖病症的情况下，药剂的治疗有效量可减少（即，延缓至一定程度和优选停止）不需要的细胞增殖，减少癌细胞数，减少肿瘤大小；抑制（即，延缓至一定程度和优选停止）癌细胞侵润至周围器官；抑制（即，延缓至一定程度和优选停止）肿瘤转移；抑制肿瘤生长至一定程度；减少靶细胞中的Bcl-2/Bcl-xL信号转导；和/或缓解与癌症相关的一个或多个症状至一定程度。就所给予的化合物或组合物阻止生长和/或杀死存在的癌细胞的方面来说，它可以是细胞生长抑制的和/或细胞毒性的。

[0026] 术语″容器″意指任何贮器和其闭合体(closure)，适于储存、运输、分配和/或处理药物产品。

[0027] 术语″插页″意指伴随药物产品的信息，其提供如何给予产品的描述，以及允许医师、药剂师和患者作出关于使用产品的基于可靠信息的决定所需要的安全性和功效数据。包装插页通常被认为是药物产品的″标签″。

[0028] ″并发给予″、″组合给予″、″同时给予″和类似的词语意指两种或更多种药剂并发地给予待治疗的受试者。所谓″并发地″意指同时给予每种药剂，或者在不同的时间点以任何顺序序贯给予每种药剂。然而，如果不是同时给予，其意指将它们以某种顺序和足够接近的时间给予个体，以提供所需的治疗效果并可一致起作用。例如，结构式(I)、(II)或(III)的Bcl-2/Bcl-xL抑制剂可与第二种治疗剂同时给予或在不同时间点以任何顺序序贯给予。本发明的Bcl-2/Bcl-xL抑制剂和第二种治疗剂可以任何合适的形式和通过任何合适的途径分开给予。当本发明的Bcl-2/Bcl-xL抑制剂和第二种治疗剂不是并发给予时，应理解，它们可以任何顺序给予有需要的受试者。例如，本发明的Bcl-2/Bcl-xL抑制剂可在给予第二种治疗剂治疗形式（例如辐射疗法）之前（例如之前5分钟、15分钟、30分钟、45分钟、1小时、2小时、4小时、6小时、12小时、24小时、48小时、72小时、96小时、1周、2周、3周、4周、5周、6周、8周或12周）给予有需要的个体，与给予第二种治疗剂治疗形式（例如辐射疗法）一起并发地给予有需要的个体，或在给予第二种治疗剂治疗形式（例如辐射疗法）之后（例如之后5分钟、15分钟、30分钟、45分钟、1小时、2小时、4小时、6小时、12小时、24小时、48小时、72小时、96小时、1周、2周、3周、4周、5周、6周、8周或12周）给予有需要的个体。在各种实施方案中，结构式(I)的Bcl-2/Bcl-xL抑制剂和第二种治疗剂分开1分钟、分开10分钟、分开30分钟、分开小于1小时、分开1小时、分开1小时至2小时、分开2小时至3小时、分开3小时至4小时、分开4小时至5小时、分开5小时至6小时、分开6小时至7小时、分开7小时至8小时、分开8小时至9小时、分开9小时至10小时、分开10小时至11小时、分开11小时至12小时、分开不超过24小时或分开不超过48小时给予。在一个实施方案中，组合疗法的各组分分开1分钟至24小时给予。

[0029] 在描述本发明的背景下（尤其是在权利要求书的背景下），术语″一个″、″一种″、″所述″和类似的指示物的使用将解释为涵盖单数和复数两者，除非另有说明。本文数值范围的列举意指仅用作分别提及落入所述范围内的每个单独的值的速记方法，除非本文另有说明，和每个单独的值结合到本说明书中，如同其分别在本文中举出一样。本文提供的任何和所有实例或示例性语言（例如，″例如″）的使用，意欲更好地说明本发明，并非对本发明的范围进行限制，除非另有要求保护。本说明书中不应有语言被解释为指出任何非要求保护的要素为本发明的实施所必需的。

[0030] 在过去十年期间，对细胞凋亡的研究已确立，使用小分子抑制剂靶向 Bcl-2 和 / 或 Bcl-xL 是一种可行的癌症治疗策略 (35-37)。ABT-737 和 ABT-263 的发现以及关于 ABT-263 的早期临床数据已经证实，Bcl-2 和 / 或 Bcl-xL 的非肽的小分子抑制剂具有用于治疗许多类型的人类癌症的极大的治疗潜力，在所述癌症中 Bcl-2 和 / 或 Bcl-xL 过表达，并且对于所述癌症，当前的抗癌药在很大程度上无效 (26-36)。

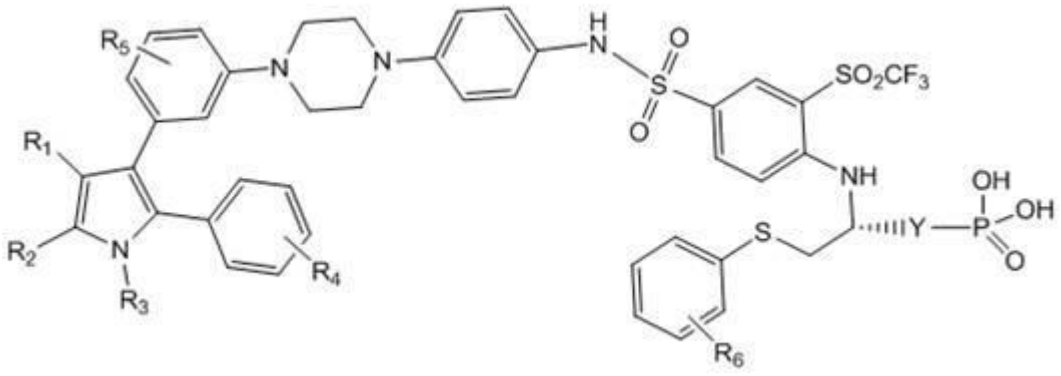
[0031] 尽管发现 ABT-737 和 ABT-263，但几乎没有报道新类型的高度有效的 Bcl-2/Bcl-xL 的小分子抑制剂，其对 Bcl-2/Bcl-xL 的亲和力和细胞效能接近 ABT-737/ABT-263 所实现的。这是因为 Bcl-2/Bcl-xL 的小分子抑制剂的设计包括靶向和阻断 Bcl-2/Bcl-xL 蛋白与它们的促 - 细胞凋亡的结合配偶体的相互作用，这是一个已被证明因为至少 3 个主要原因而极具挑战性的任务。第一，与酶和受体中的典型结合位点相比，Bcl-2 或 Bcl-xL 和它们的结合配偶体之间的界面非常大 (38-42)。Bcl-2/Bcl-xL 与其结合配偶体（例如 BAD 和 Bim 蛋白）的相互作用被 BAD 和 Bim 的 20-25 个残基 BH3 结构域以及 Bcl-2/Bcl-xL 的大的结合沟介导。第二，Bcl-2/Bcl-xL 的结合沟在性质上非常疏水，这使得难以设计药物样小分子 (26, 38-42)。第三，Bcl-2 和 Bcl-xL 在构象上非常柔韧，并且在无配体结构中和当结合至不同的配体时，可采用完全不同的构象 (26, 38-42)。在 Bcl-xL 与 BAD (41)、Bim (43) 和 ABT-737(44) 的复合物的晶体结构中对 Bcl-xL 所观察到的一些结合袋被配体结合诱导，并且不出现在无配体晶体结构中 (38)。这三个因素使得 Bcl-2/Bcl-xL 的有效和药物样小分子抑制剂的设计成为现代药物发现中至关重大的挑战。

[0032] 本发明涉及 Bcl-2/Bcl-xL 的新类型的有效和特异性抑制剂。本发明的化合物可结合 Bcl-2 和 / 或 Bcl-xL，K_i 值 <10 nM，和在无细胞功能测定法中作为 Bcl-2 和 Bcl-xL 的有效的拮抗剂起作用。所述化合物有效诱导癌细胞的细胞凋亡和具有与靶向 Bcl-2 和 Bcl-xL 高度一致的作用机制。所测试的化合物证实在肿瘤组织中稳健的体内细胞凋亡诱导和表明针对 H146 异种移植肿瘤的强的抗肿瘤活性。

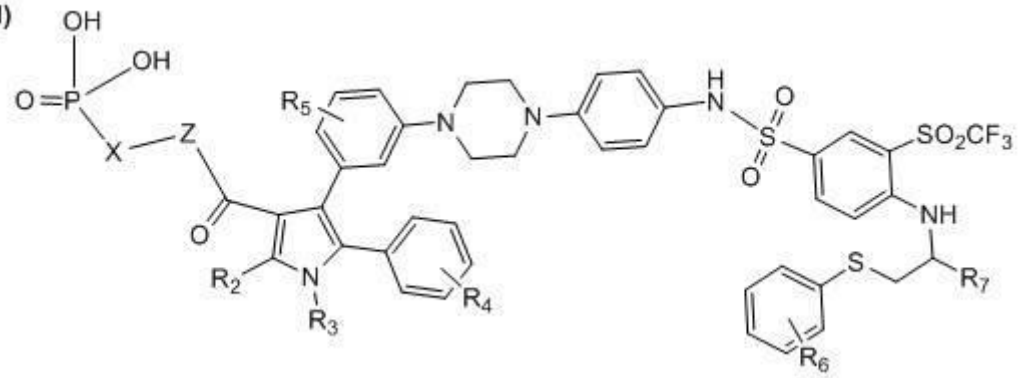
[0033] 因此，本发明的 Bcl-2/Bcl-xL 抑制剂可用于在需要所述治疗的受试者中治疗不需要的增值性细胞，包括癌症和初癌。还提供治疗具有不需要的增值性细胞的受试者的方法，包括给予需要所述治疗的受试者治疗有效量的本发明的化合物。还提供在受试者中预防不需要的增值性细胞（例如癌症和初癌）的增殖的方法，包括给予有发生特征为不需要的增值性细胞的病况的风险的受试者治疗有效量的结构式 (I) 的化合物的步骤。在一些实施方案中，结构式 (I)、(II) 和 (III) 的化合物通过诱导不需要的细胞的细胞凋亡来减少这些细胞的增殖。

[0034] 本发明涉及具有结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂：

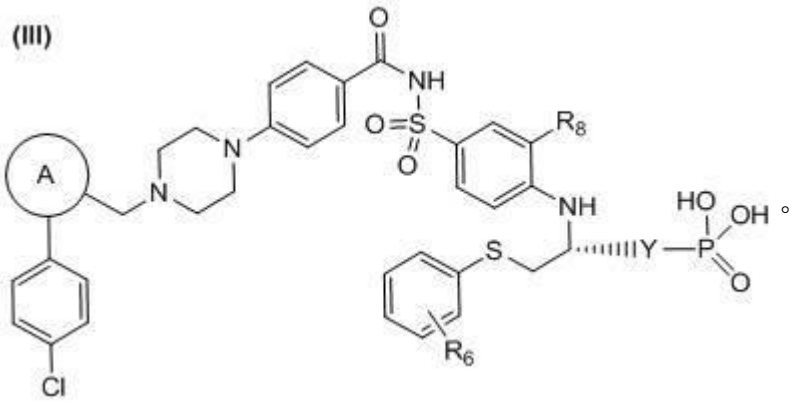
(I)

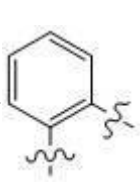
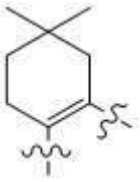


(II)



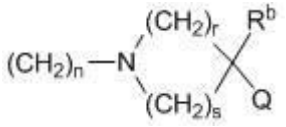
(III)



[0035] 其中 A 环是  或  ；

取代或未取代的 X 选自亚烷基、亚烯基、环亚烷基、环亚烯基和杂环亚烷基；

Y 选自 $(CH_2)_n$-$N(R^a)_2$和



；

Q 选自 O、$O(CH_2)_{1-3}$、NR^c、$NR^c(C_{1-3}$亚烷基）、$OC(=O)(C_{1-3}$亚烷基）、$C(=O)O$、$C(=O)O(C_{1-3}$亚烷基）、$NHC(=O)(C_{1-3}$亚烷基）、$C(=O)NH$ 和 $C(=O)NH(C_{1-3}$亚烷基）；

Z 是 O 或 NR^c；

R_1和 R_2独立地选自 H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、COR'、CO_2R'、OCOR'、CONR'R''、$CONR'SO_2R''$、NR'COR''、

NR'CONR''R'''、NR'C=SNR''R'''、NR'SO_2R''、SO_2R'和SO_2NR'R''；

R_3选自H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、NR'R''、OCOR'、CO_2R'、COR'、CONR'R''、CONR'SO_2R''、C_{1-3}亚烷基CH(OH)CH_2OH、SO_2R'和SO_2NR'R''；

R'、R''和R'''独立地是H、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、C_{1-3}亚烷基杂环烷基或杂环烷基；

R'和R''或R''和R'''可与它们所连接的原子一起形成3-7元环；

R_4是氢、卤素、C_{1-3}烷基、CF_3或CN；

R_5是氢、卤素、C_{1-3}烷基、取代的C_{1-3}烷基、羟基烷基、烷氧基或取代的烷氧基；

R_6选自H、CN、NO_2、卤素、烷基、环烷基、烯基、环烯基、炔基、芳基、杂芳基、杂环烷基、OR'、SR'、NR'R''、CO_2R'、OCOR'、CONR'R''、CONR'SO_2R''、NR'COR''、NR'CONR''R'''、NR'C=SNR''R'''、NR'SO_2R''、SO_2R'和SO_2NR'R''；

取代或未取代的R_7选自氢、烷基、烯基、$(CH_2)_{0-3}$环烷基、$(CH_2)_{0-3}$环烯基、$(CH_2)_{0-3}$杂环烷基、$(CH_2)_{0-3}$芳基和$(CH_2)_{0-3}$杂芳基；

R_8选自氢、卤素、NO_2、CN、CF_3SO_2和CF_3；

R_a选自氢、烷基、杂烷基、烯基、羟基烷基、烷氧基、取代的烷氧基、环烷基、环烯基和杂环烷基；

R_b是氢或烷基；

R_c选自氢、烷基、取代的烷基、羟基烷基、烷氧基和取代的烷氧基；和

n、r和s独立地是1、2、3、4、5或6；

或(I)、(II)或(III)的药学上可接受的盐。

[0036] 结构式(I)、(II)和(III)的化合物抑制Bcl-2/Bcl-xL和可用于治疗各种疾病和病况。具体而言，结构式(I)、(II)和(III)的化合物用于治疗其中Bcl-2/Bcl-xL的抑制提供益处的疾病或病况(例如癌症)的方法。所述方法包括给予有需要的个体治疗有效量的结构式(I)、(II)或(III)的化合物。除了结构式(I)、(II)或(III)的化合物之外，本发明的方法还包括给予个体第二种治疗剂。第二种治疗剂选自已知用于治疗累及有需要的个体的疾病或病况的药物，例如已知用于治疗特定癌症的化学治疗剂和/或辐射。

[0037] 本文所用的术语"烷基"是指直链和支链的饱和C_{1-10}烃基，其非限制性实例包括甲基、乙基、和直链和支链的丙基、丁基、戊基、己基、庚基、辛基、壬基和癸基。术语C_n意指烷基具有"n"个碳原子。术语C_{n-p}意指烷基包含"n"至"p"个碳原子。术语"亚烷基"是指具有取代基的烷基。烷基例如甲基，或亚烷基例如—CH_2—可以是未取代的或被例如卤素、三氟甲基、三氟甲氧基、羟基、烷氧基、硝基、氰基、烷基氨基或氨基取代。

[0038] 术语"烯基"与"烷基"相同定义，除了含有碳-碳双键，例如乙烯基、丙烯基和丁烯基。术语"亚烯基"与"亚烷基"相同定义，除了含有碳-碳双键。术语"炔基"和"亚炔基"与"烷基"和"亚烷基"相同定义，除了所述基团含有碳-碳三键。

[0039] 本文所用的术语"卤素"定义为氟、氯、溴和碘。

[0040] 术语"羟基"定义为—OH。

[0041] 术语"烷氧基"定义为—OR，其中R是烷基。

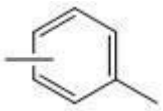
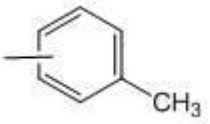
[0042] 术语"氨基"定义为—NH_2和术语"烷基氨基"定义为—NR_2，其中至少一个R是烷基和另一个R是烷基或氢。

[0043] 术语″硝基″定义为—NO_2。

[0044] 术语″氰基″定义为—CN。

[0045] 术语″三氟甲基″定义为—CF_3。

[0046] 术语″三氟甲氧基″定义为—OCF_3。

[0047] 本文所用的基团例如  是  的缩写。

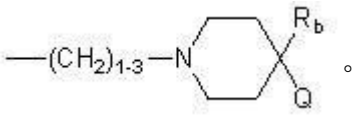
[0048] 本文所用的术语″芳基″是指单环或多环的芳族基团，优选单环或二环的芳族基团，例如苯基或萘基。除非另外说明，否则芳基可以是未取代的或被一个或多个（并且特别是 1-4 个）基团取代，所述基团独立选自例如卤素、烷基、烯基、—OCF_3、—CF_3、—NO_2、—CN、—NC、—OH、烷氧基、氨基、烷基氨基、—CO_2H、—CO_2烷基、—OCO 烷基、芳基和杂芳基。

[0049] 本文所用的术语″杂芳基″是指单环或二环的环系统，其包含一个或两个芳族环和在芳环中包含至少一个氮、氧或硫原子。除非另外说明，否则杂芳基可以是未取代的或被一个或多个（并且特别是 1-4 个）取代基取代，所述取代基选自例如卤素、烷基、烯基、—OCF_3、—CF_3、—NO_2、—CN、—NC、—OH、烷氧基、氨基、烷基氨基、—CO_2H、—CO_2烷基、—OCO 烷基、芳基和杂芳基。

[0050] 本文所用的术语″环烷基″意指含有 3-8 个碳原子的单环脂肪族环。术语″杂环烷基″意指单环或二环的环系统，在环系统中含有至少一个氮、氧或硫原子。术语″杂芳基″和″杂环烷基″包括包含至少一个氧原子、氮原子或硫原子的环系统，和包括包含氧和氮原子；氧和硫原子；氮和硫原子；和氮、氧和硫原子的环系统。

[0051] 在一些优选的实施方案中，X 是亚烷基，和在优选的实施方案中，是 C_{1-3}亚烷基。

[0052] 在一些实施方案中，Y 是



。

[0053] 在优选的实施方案中，n 是 2。在其它优选的实施方案中，R_b是氢或 C_{1-3}烷基。

[0054] 在仍其它优选的实施方案中，Q 是 O、$O(CH_2)_{1-3}$、$C(=O)O(CH_2)_{1-3}$、$OC(=O)(CH_2)_{1-3}$或 $C(=O)O(C_3H_7)_{1-3}$。在一些实施方案中，Q 是 O、OCH_2、$C(=O)OCH_2$、$C(=O)O(CH_2)_2$、$C(=O)O(CH_2)_3$、$OC(=O)CH_2$或 $C(=O)O(CH(CH_3)CH_2)$。

[0055] 在一些实施方案中，Z 是 O、NH 或 $N(C_{1-3}$烷基）。在优选的实施方案中，Z 是 O、NH 或 NCH_3。

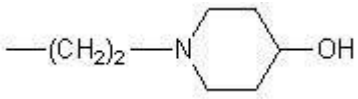
[0056] 在一些实施方案中，R_1是 SO_2R'、$SO_2NR'R''$、$NR'SOR''$、H 或烷基。在一些优选的实施方案中，R_1是 $SO_2(C_{1-3}$烷基）、$SO_2N(C_{1-3}$烷基$)_2$、$NHSO_2(C_{1-3}$烷基）、H 或 C_{1-3}烷基。R_1的一个优选的实施方案是 SO_2CH_3。

[0057] 在一些实施方案中，R_2和 R_3独立地是 H、C_{1-3}烷基或环烷基。R_2也可以是卤素。在一些优选的实施方案中，R_2和 R_3独立地是甲基、乙基、正丙基、异丙基、环戊基或环己基。R_2也可以是 Cl 或 F。

[0058] 在一些实施方案中，R_4是 H、Cl 或 F。在其它实施方案中，R_5是 H、甲基、乙基、正丙

基、异丙基、F 或 Cl。在其它实施方案中，R_6是 H、卤素、烷基或环烷基。在一些优选的实施方案中，R_6是 H、F、Cl、C_{1-3}烷基、环戊基或环己基。

[0059] 在一些实施方案中，R_7是 $(CH_2)_{0-3}$环烷基或 $(CH_2)_{0-3}$杂环烷基。在优选的实施方案，R_7是 $(CH_2)_{0-3}$环烷基，任选被 -OH 取代。在一个实施方案中，R_7是



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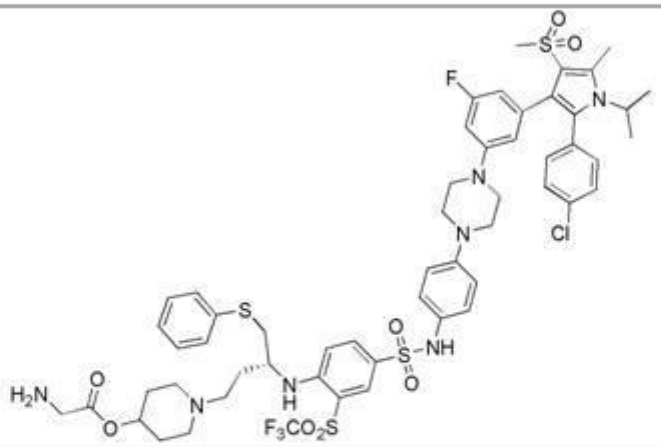
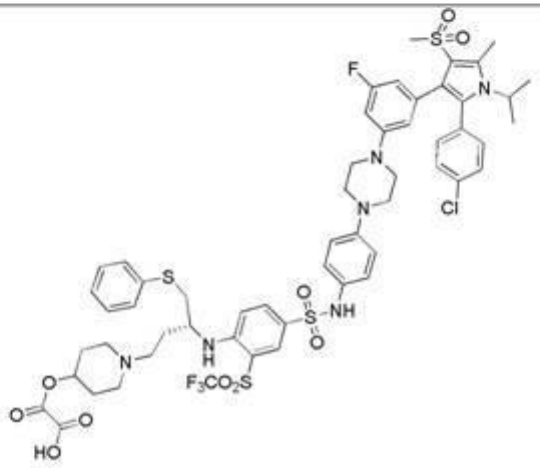
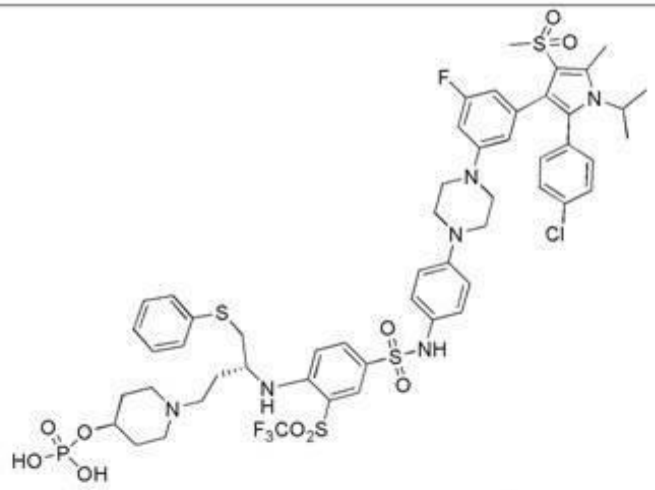
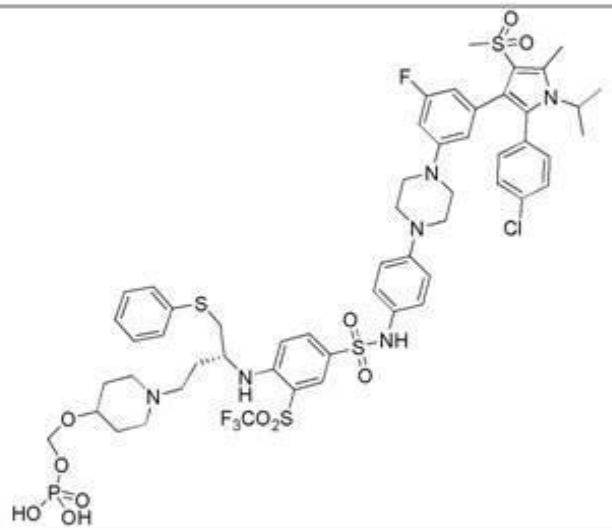
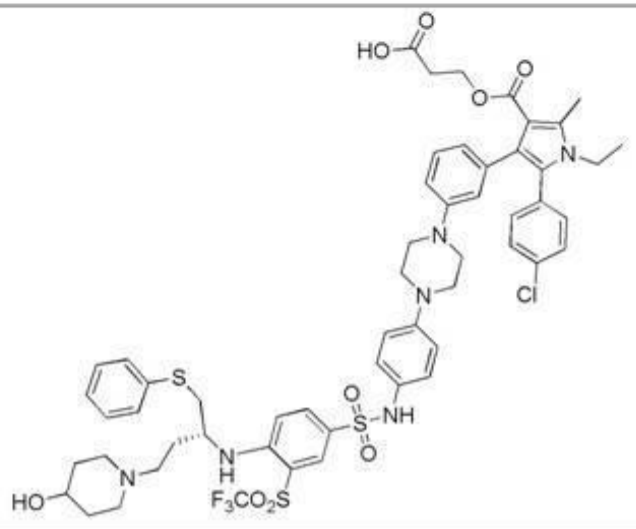
[0060] 在一些实施方案中，R_8是 $CFSO_2$或 CF_3。在各种实施方案中，R_a、R_b和 R_c独立地是 H 或 C_{1-3}烷基。

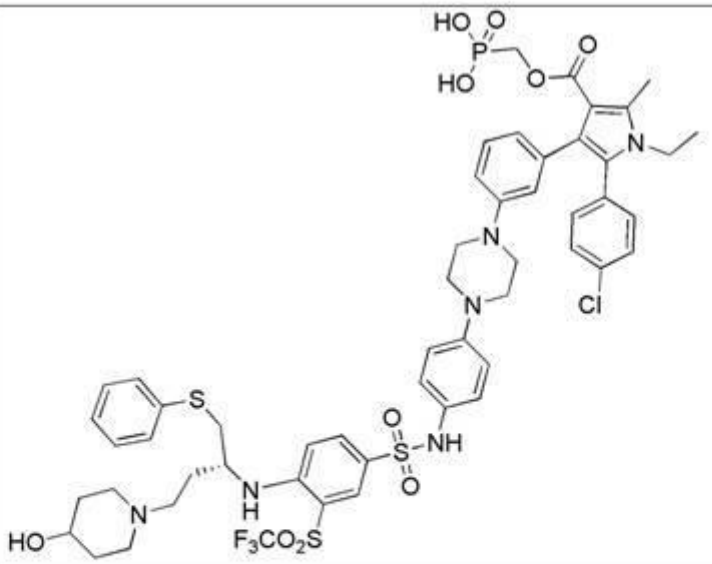
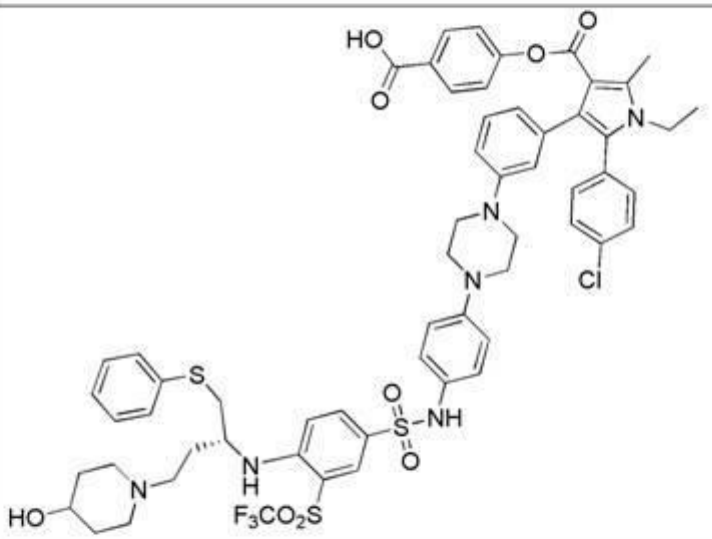
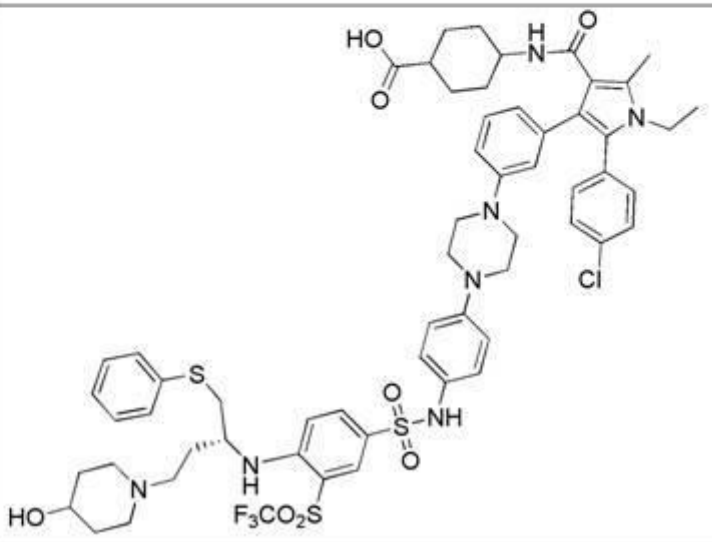
[0061] 另外，本发明的化合物的盐、水合物和溶剂合物也包括在本发明中，和可用于本文公开的方法。本发明还包括结构式 (I)、(II) 和 (III) 的化合物的所有可能的立体异构体和几何异构体。本发明包括外消旋化合物和光学活性异构体两者。当结构式 (I)、(II) 或 (III) 的化合物需要作为单一的对映体时，其可通过终产物的解析或通过自异构纯的原料或使用手性辅助试剂的立体特异性合成获得，例如参见 Z. Ma 等，*Tetrahedron: Asymmetry, 8(6)*, 第 883-888 页 (1997)。终产物、中间体或原料的解析可通过本领域已知的任何合适的方法实现。此外，在其中结构式 (I)、(II) 或 (III) 的化合物的互变异构体是可能的情况下，本发明意欲包括所述化合物的所有互变异构体形式。

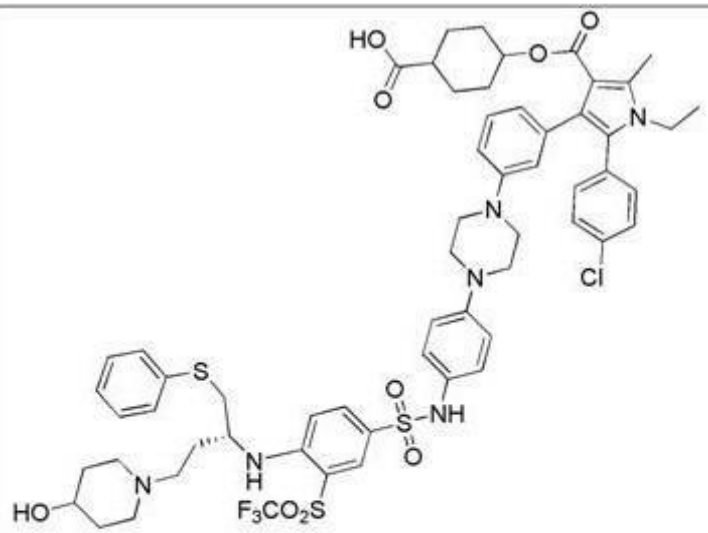
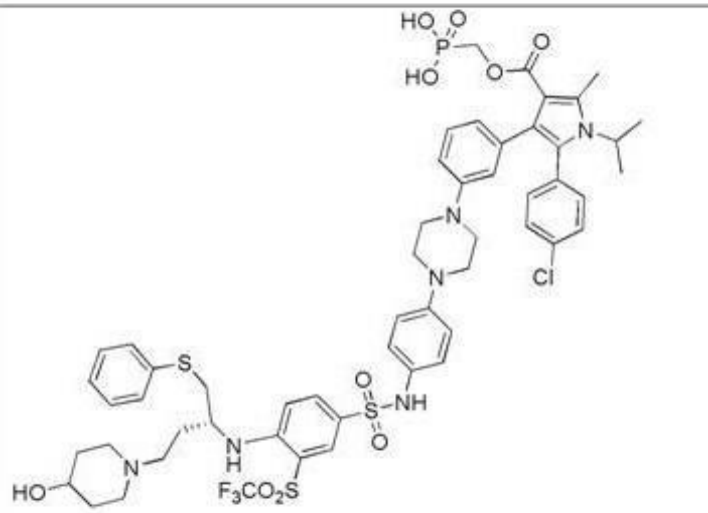
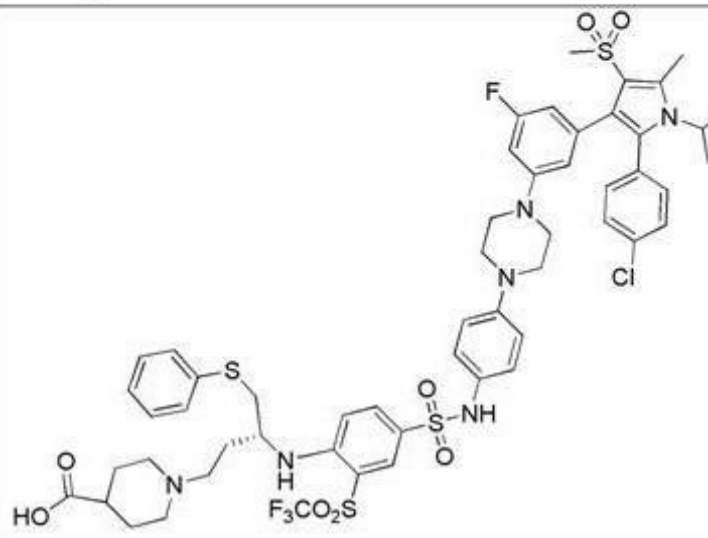
[0062] 本发明的化合物可作为盐存在。在本发明的方法中，本发明的化合物的药学上可接受的盐通常是优选的。本文所用的术语〝药学上可接受的盐〞是指结构式 (I)、(II) 和 (III) 的化合物的盐或两性离子形式。式 (I)、(II) 和 (III) 的化合物的盐可在化合物的最终分离和纯化期间制备，或分别通过将所述化合物与具有合适的阳离子的酸反应制备。结构式 (I)、(II) 和 (III) 的化合物的药学上可接受的盐可以是与药学上可接受的酸形成的酸加成盐。可用于形成药学上可接受的盐的酸的实例包括无机酸例如硝酸、硼酸、盐酸、氢溴酸、硫酸和磷酸，和有机酸例如草酸、马来酸、琥珀酸和柠檬酸。本发明的化合物的盐的非限制性实例包括但不限于盐酸盐、氢溴酸盐、氢碘酸盐、硫酸盐、硫酸氢盐、2- 羟基乙磺酸盐、磷酸盐、磷酸氢盐、乙酸盐、己二酸盐、藻酸盐、天冬氨酸盐、苯甲酸盐、硫酸氢盐、丁酸盐、樟脑酸盐、樟脑磺酸盐、二葡糖酸盐、甘油磷酸盐、半硫酸盐、庚酸盐、己酸盐、甲酸盐、琥珀酸盐、延胡索酸盐、马来酸盐、抗坏血酸盐、羟乙基磺酸盐、水杨酸盐、甲磺酸盐、均三甲苯磺酸盐、萘磺酸盐、烟酸盐、2- 萘磺酸盐、草酸盐、双羟萘酸盐、果胶酯酸盐、过硫酸盐、3- 苯基丙酸盐、苦味酸盐、特戊酸盐、丙酸盐、三氯乙酸盐、三氟乙酸盐、磷酸盐、谷氨酸盐、碳酸氢盐、对甲苯磺酸盐、十一酸盐、乳酸盐、柠檬酸盐、酒石酸盐、葡糖酸盐、甲磺酸盐、乙二磺酸盐、苯磺酸盐和对甲苯磺酸盐。此外，本发明的化合物中存在的可用的氨基可被甲基、乙基、丙基和丁基氯化物、溴化物和碘化物；二甲基、二乙基、二丁基和二戊基硫酸酯；癸基、月桂基、肉豆蔻基和硬脂基氯化物、溴化物和碘化物；和苄基和苯乙基溴化物季胺化。根据前述，对本文出现的本发明的化合物的任何提及意欲包括结构式 (I)、(II) 和 (III) 的化合物以及其药学上可接受的盐、水合物或溶剂合物。

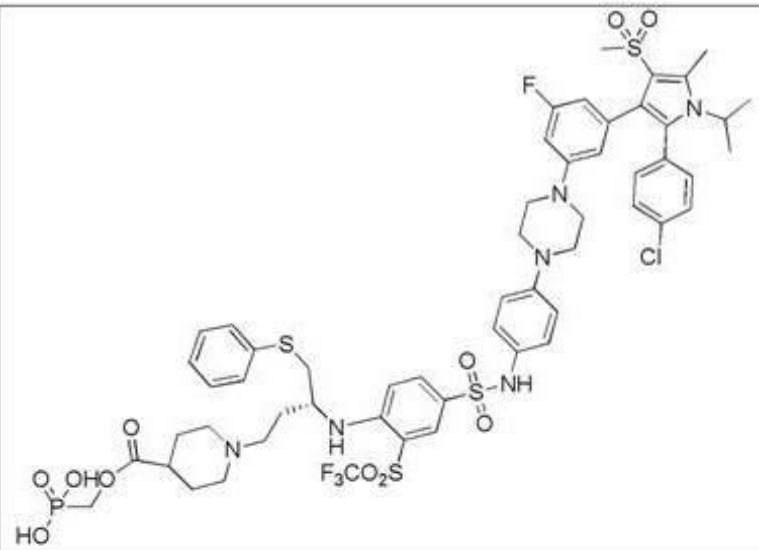
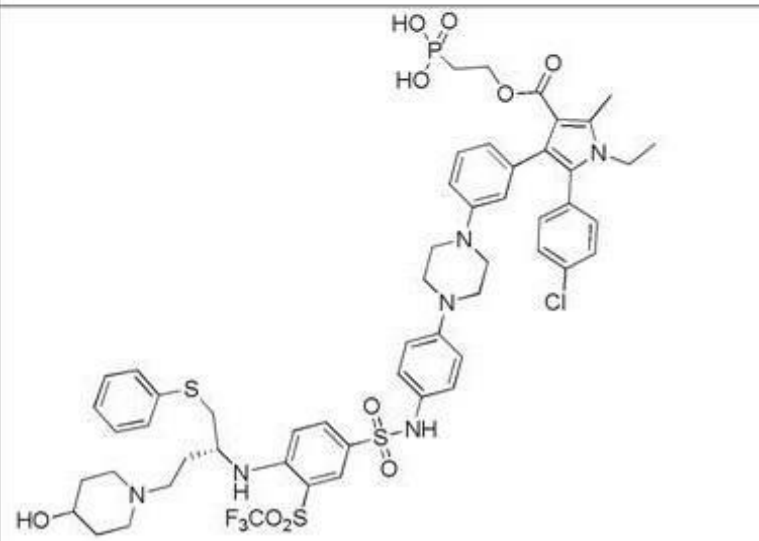
[0063] 本发明的具体化合物包括但不限于具有下文所述结构的化合物。

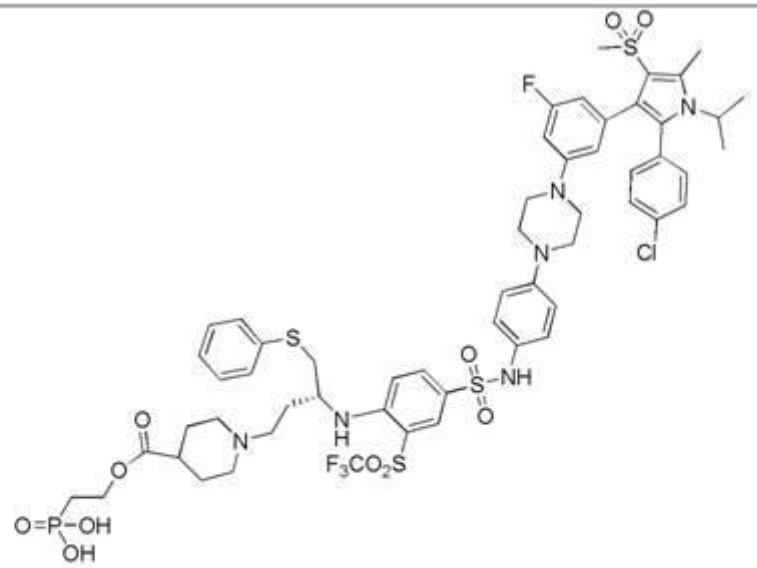
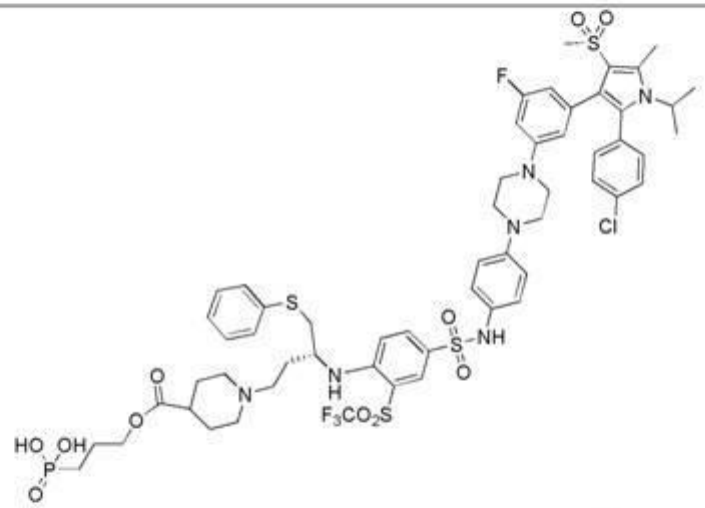
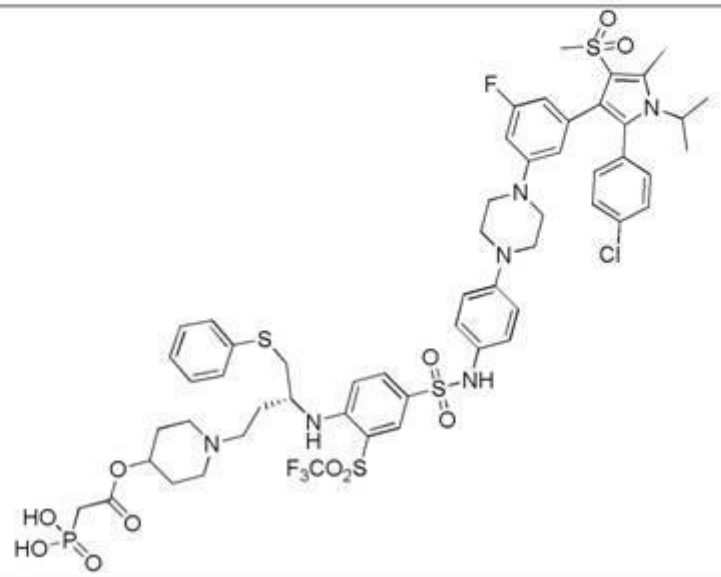
化合物编号	结构
1	
2	

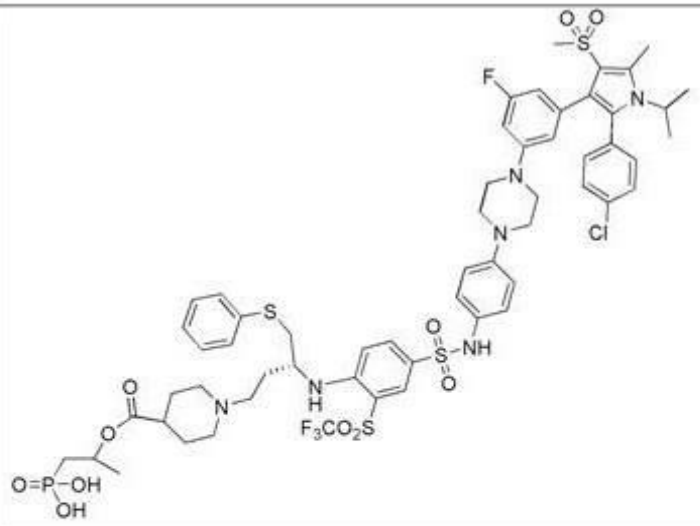
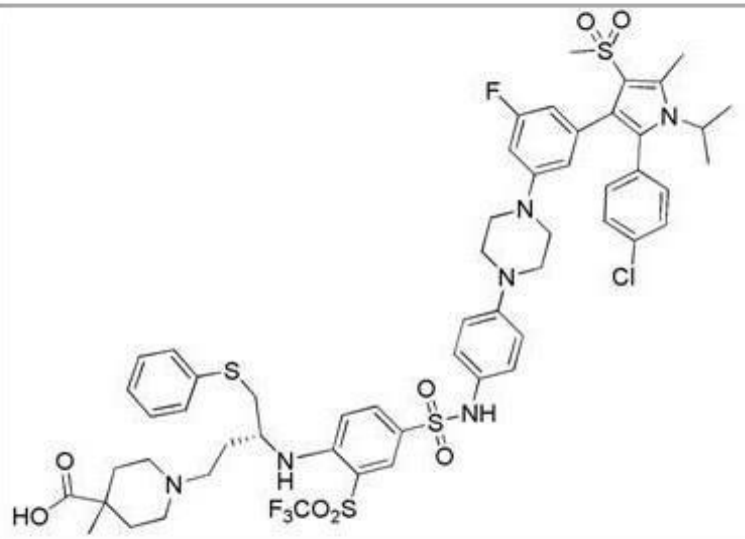
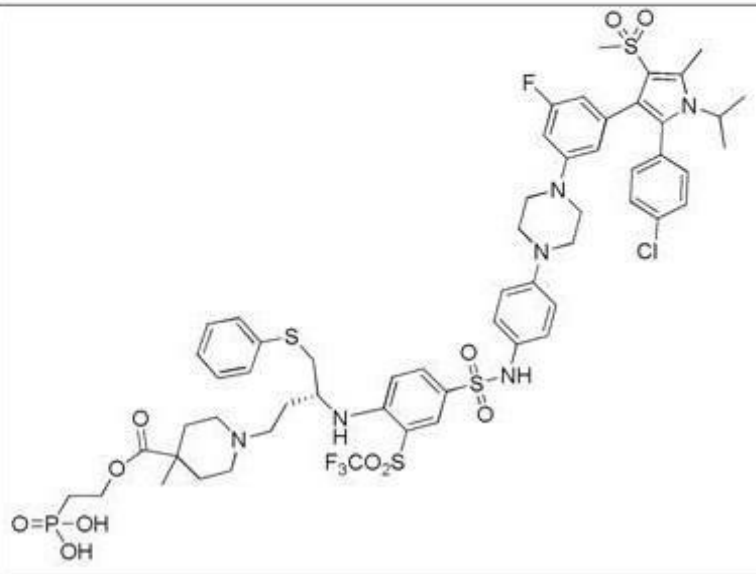
3	
4	
5	

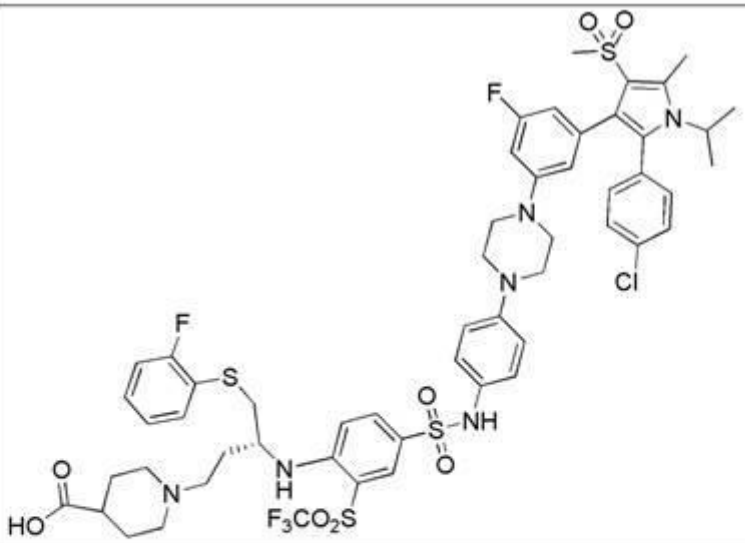
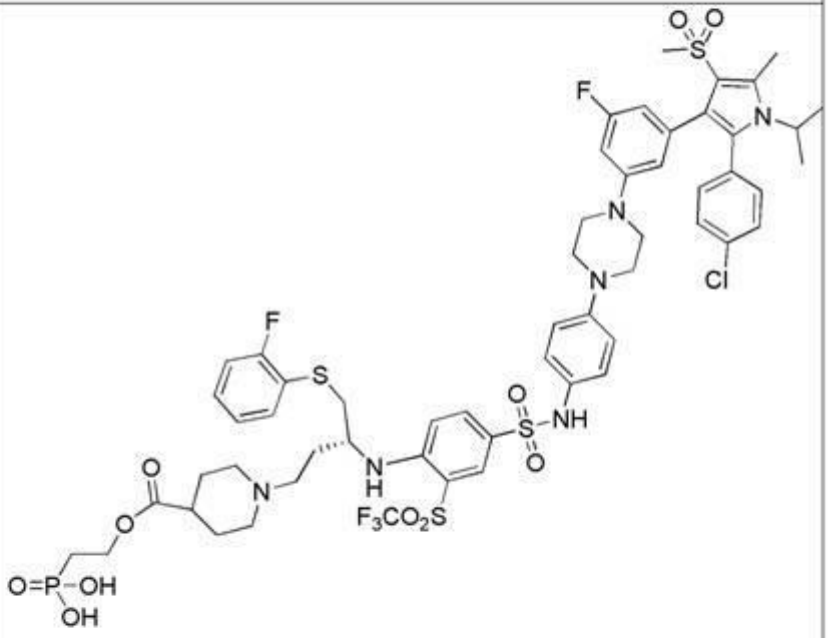
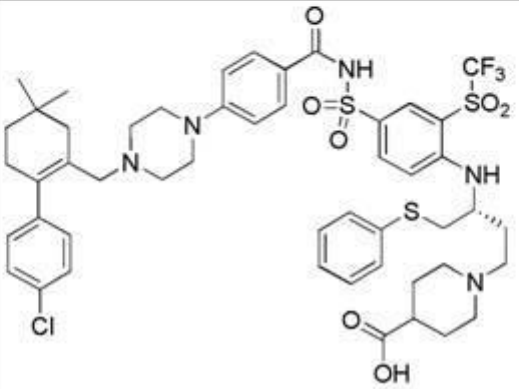
6	
7	
8	

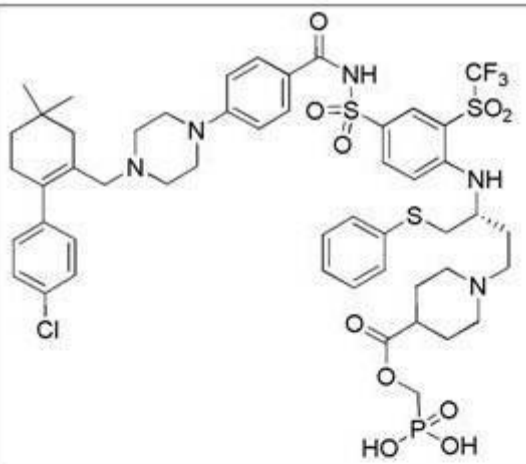
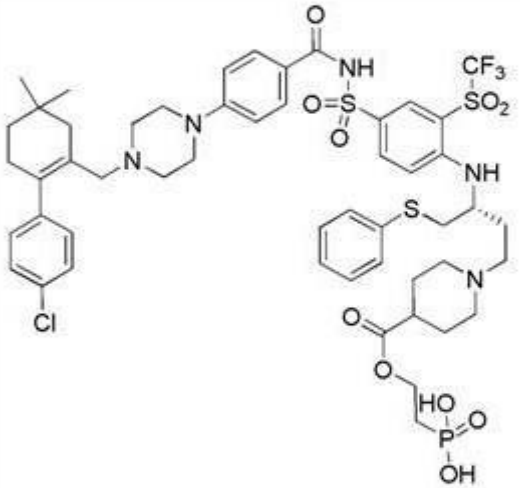
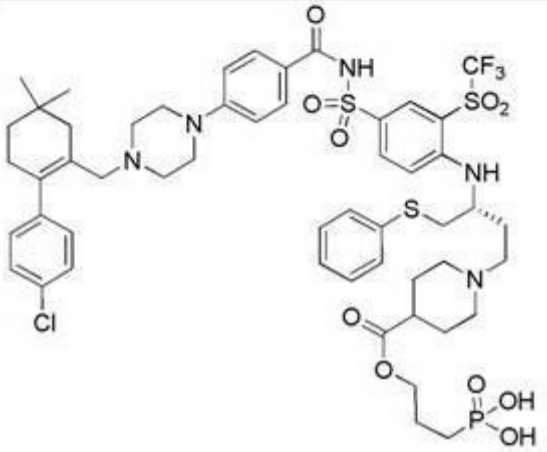
9	
10	
11	

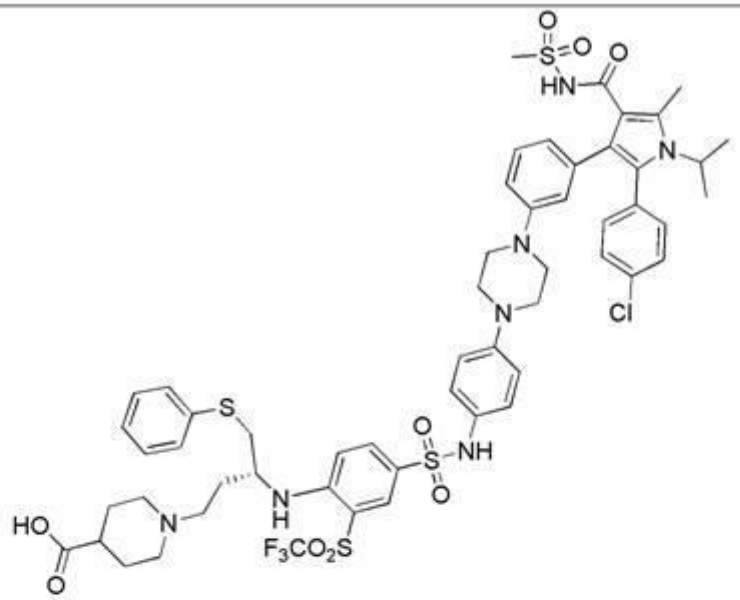
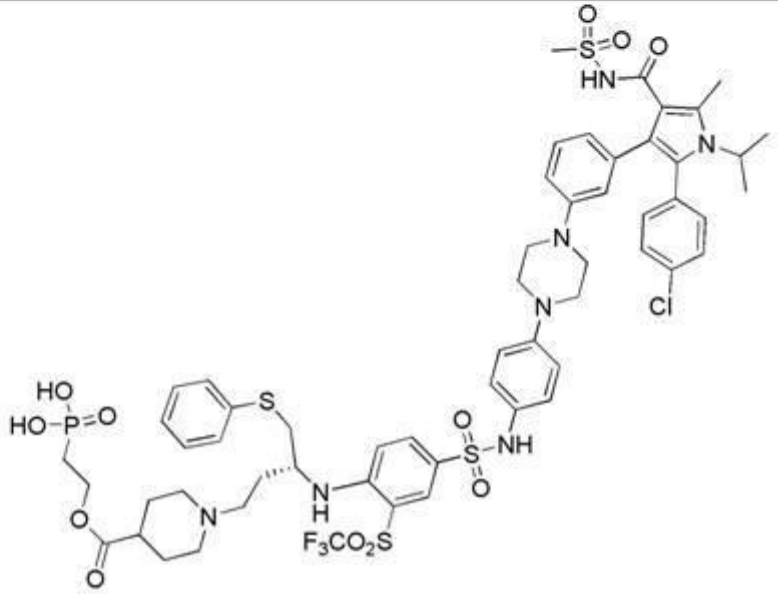
12	
13	

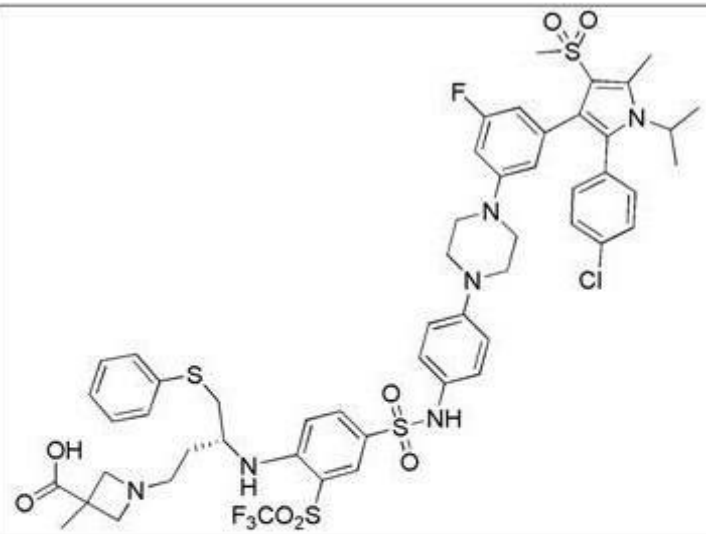
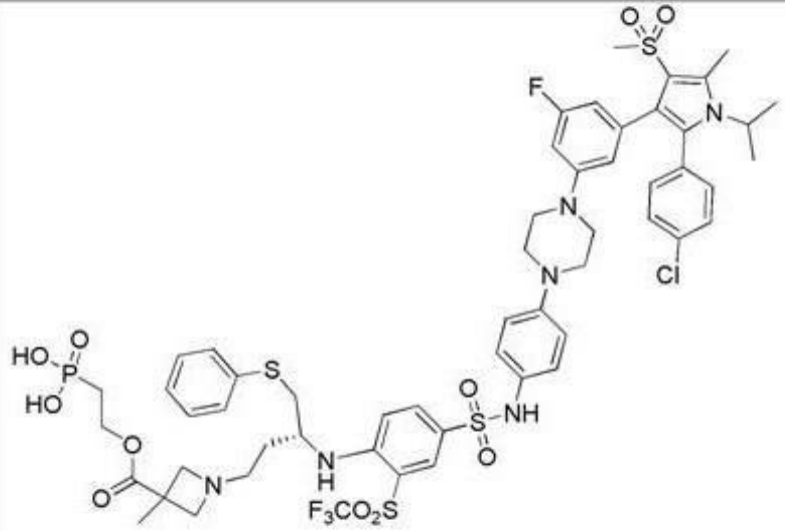
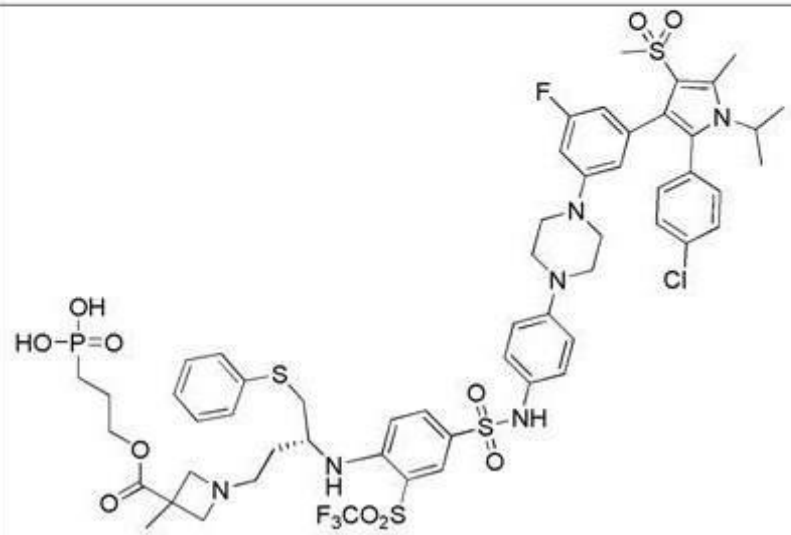
14	
15	
16	

17	
18	
19	

20	
21	
22	

23	
24	
25	

26	
27	

28	
29	
30	

[0064] 本发明提供Bcl-2/Bcl-xL抑制剂,例如结构式(I)、(II)和(III)的化合物,用于治疗其中Bcl-2和/或Bcl-xL的抑制具有有益作用的各种疾病和病况。在一个实施方案中,本发明涉及治疗患有其中Bcl-2/Bcl-xL的抑制提供益处的疾病或病况的个体的方法,包括给予有需要的个体治疗有效量的结构式(I)、(II)或(III)化合物。

[0065] 本发明的方法可通过给予作为纯的化合物或作为药物组合物的结构式 (I)、(II) 或 (III) 的化合物来实现。可在目标疾病或病况发作期间或之后进行药物组合物或纯的结构式 (I)、(II) 或 (III) 的化合物的给予。通常，药物组合物是无菌的，含有无毒性、无致癌性或无诱变性的化合物，当给予时所述化合物时将引起不良反应。还提供药盒，其包含分开或一起包装的结构式 (I)、(II) 或 (III) 的化合物和用于治疗其中 Bcl-2/Bcl-xL 的抑制提供益处的疾病和病况的任选第二种治疗剂，和带有这些活性剂的使用说明的插页。

[0066] 在许多实施方案中，结构式 (I)、(II) 或 (III) 的化合物与用于治疗其中 Bcl-2/Bcl-xL 的抑制提供益处的疾病或病况的第二种治疗剂结合给予。第二种治疗剂不同于结构式 (I)、(II) 和 (III) 的化合物。结构式 (I)、(II) 或 (III) 的化合物和第二种治疗剂可同时或序贯给予以实现所需效果。此外，结构式 (I)、(II) 或 (III) 的化合物和第二种治疗剂可自单一的组合物或两种分开的组合物给予。

[0067] 第二种治疗剂以提供其所需治疗效果的量给予。每种第二种治疗剂的有效剂量范围是本领域已知的，并且在这样的已确定的范围内给予有需要的个体第二种治疗剂。

[0068] 结构式 (I)、(II) 或 (III) 的化合物和第二种治疗剂可作为单一 - 单位剂量一起给予或作为多 - 单位剂量分开给予，其中结构式 (I)、(II) 或 (III) 的化合物在第二种治疗剂之前给予或反之亦然。可给予一个或多个剂量的结构式 (I)、(II) 或 (III) 的化合物和 / 或一个或多个剂量的第二种治疗剂。因此，结构式 (I)、(II) 和 (III) 的化合物可与一种或多种的第二种治疗剂 (例如但不限于抗癌剂) 一起使用。

[0069] 按照本发明可治疗的疾病和病况包括例如癌症。可治疗各种癌症，包括但不限于 :癌，包括膀胱癌 (包括加速的和转移的膀胱癌)、乳腺癌、结肠癌 (包括结肠直肠癌)、肾癌、肝癌、肺癌 (包括小和非小细胞肺癌以及肺腺癌)、卵巢癌、前列腺癌、睾丸癌、泌尿生殖道癌、淋巴系统癌、直肠癌、喉癌、胰腺癌 (包括外分泌胰腺癌)、食管癌、胃癌、胆囊癌、宫颈癌、甲状腺癌、肾癌和皮肤癌 (包括鳞状细胞癌) ;淋巴系的造血肿瘤，包括白血病、急性淋巴细胞性白血病、急性成淋巴细胞性白血病、B- 细胞淋巴瘤、T- 细胞淋巴瘤、霍奇金淋巴瘤、非霍奇金淋巴瘤、毛细胞淋巴瘤、组织细胞淋巴瘤和 Burketts 淋巴瘤 ;骨髓系的造血肿瘤，包括急性和慢性骨髓性白血病、骨髓发育不良综合征、骨髓性白血病和早幼粒细胞性白血病 ;中枢和外周神经系统的肿瘤，包括星形细胞瘤、成神经细胞瘤、神经胶质瘤和神经鞘瘤 ;间质来源的肿瘤，包括纤维肉瘤、横纹肌肉瘤和骨肉瘤 ;和其它肿瘤，包括黑素瘤、着色性干皮病、角化棘皮瘤、精原细胞瘤、甲状腺滤泡癌、畸胎癌、肾细胞癌 (RCC)、胰腺癌、骨髓瘤、骨髓性和成淋巴细胞性白血病、成神经细胞瘤和成胶质细胞瘤。

[0070] 可通过本发明的 Bcl-2/Bcl-xL 抑制剂治疗的其它形式的癌症包括例如成人和儿科肿瘤、实体瘤 / 恶性肿瘤的生长、粘液样和圆形细胞癌、局部晚期肿瘤、转移性癌、人软组织肉瘤 (包括尤因肉瘤)、癌转移 (包括淋巴转移)、鳞状细胞癌 (特别是头和颈、食管鳞状细胞癌)、口腔癌、血细胞恶性肿瘤 (包括多发性骨髓瘤)、白血病 (包括急性淋巴细胞性白血病、急性非淋巴细胞性白血病、慢性淋巴细胞性白血病、慢性髓细胞性白血病和毛细胞性白血病)、渗出淋巴瘤 (基于体腔的淋巴瘤)、胸腺淋巴瘤、肺癌 (包括小细胞癌)、皮肤 T 细胞淋巴瘤、霍奇金淋巴瘤、非霍奇金淋巴瘤、肾上腺皮质的癌症、产生 ACTH 的肿瘤、非小细胞癌、乳腺癌 (包括小细胞癌和管癌)、胃肠癌症 (包括胃癌、结肠癌、结肠直肠癌和与结肠直肠瘤形成有关的息肉)、胰腺癌、肝癌、泌尿道癌 (包括膀胱癌，例如原发性表面膀胱

肿瘤、膀胱的侵袭性移行细胞癌和肌肉侵袭性膀胱癌）、前列腺癌、女性生殖道的恶性肿瘤（包括卵巢癌、原发性腹膜上皮瘤、宫颈癌、子宫内膜癌、阴道癌、外阴癌症、子宫癌和卵泡中的实体瘤）、男性生殖道的恶性肿瘤（包括睾丸癌和阴茎癌）、肾癌（包括肾细胞癌）、脑癌（包括内在的脑肿瘤、成神经细胞瘤、星形细胞脑肿瘤、神经胶质瘤和中枢神经系统的转移性肿瘤细胞侵袭）、骨癌（包括骨瘤和骨肉瘤）、皮肤癌（包括恶性黑素瘤、人皮肤角质化细胞的肿瘤进展和鳞状细胞癌）、甲状腺癌、成视网膜细胞瘤、成神经细胞瘤、腹膜渗出物、恶性胸膜渗出物、间皮瘤、维尔姆斯肿瘤、胆囊癌、滋养层肿瘤、血管外皮细胞瘤和卡波西肉瘤。

[0071] 可通过给予本发明的 Bcl-2/Bcl-xL 抑制剂治疗的其它疾病和病况（包括癌症）公开于美国专利公开号 2007/0027135、美国专利号 7,432,304、美国专利公开号 2010/0278921 和指定国为美国的 WO 2012/017251，分别以其整体结合到本文中。

[0072] 在本发明的方法中，将通常按照药学实践配制的治疗有效量的一种或多种化合物 (I)、(II) 或 (III) 给予对其有需要的人。是否需要这样的治疗取决于个别的病例，并进行医学评估（诊断），其考虑存在的征兆、症状和 / 或机能障碍，发展特定征兆、症状和 / 或机能障碍的风险，和其它因素。

[0073] 结构式 (I)、(II) 或 (III) 的化合物可通过任何合适的途径给予，例如通过口服、含服、吸入、舌下、直肠、阴道、脑池内或经过腰椎刺穿的鞘内、经尿道、经鼻、经皮（即经皮肤）、或胃肠外（包括静脉内、肌肉内、皮下、冠状动脉内、皮内、乳房内、腹膜内、关节内、鞘内、眼球后、肺内注射和 / 或在特定位点的手术植入）给予。胃肠外给予可使用针头和注射器或使用高压技术完成。

[0074] 药物组合物包括其中结构式 (I)、(II) 或 (III) 的化合物以有效实现其预期目的量给予的那些。准确的配制、给药途径和剂量根据诊断的病况或疾病通过个体医师确定。可分别调整给药量和间隔以提供足以保持治疗效果的结构式 (I)、(II) 或 (III) 的化合物的水平。

[0075] 结构式 (I)、(II) 和 (III) 的化合物的毒性和治疗功效可通过标准制药程序在细胞培养物或实验动物中确定，例如用于确定化合物的最大耐受剂量 (MTD)，其定义为在动物中不引起毒性的最高剂量。在最大耐受剂量和治疗效果（例如抑制肿瘤生长）之间的剂量比是治疗指数。剂量可在该范围内变化，这取决于所用的剂型和所用的给药途径。治疗有效量的确定完全在本领域技术人员的能力范围内，特别是根据本文提供的详细公开内容。

[0076] 治疗用途所需的治疗有效量的结构式 (I)、(II) 或 (III) 的化合物随正治疗的病况的性质、所需活性的时间长度和患者的年龄以及病况而变化，和最终通过主治医师来确定。可分别调整给药量和间隔以提供足以保持所需治疗效果的 Bcl-2/Bcl-xL 抑制剂的血浆水平。可以单一剂量或按以合适间隔给予的多次剂量方便地给予所需剂量，例如按 1 次、2 次、3 次、4 次或更多次亚剂量 / 天。经常需要或要求多次剂量。例如，本发明的 Bcl-2/Bcl-xL 抑制剂可以以下频率给予 :1 次剂量 / 天，持续 2 天，休息 5 天，持续 2 周 ;1 次剂量 / 天，持续 3 天，休息 4 天，持续 3 周 ;每周 1 次给药，持续 2 周 ;每周 1 次给药，持续 4 周 ;或者，对情况适当确定的任何剂量方案。

[0077] 用于本发明的方法的结构式 (I)、(II) 或 (III) 的化合物可以约 0.005- 约 500 毫克 / 剂量、约 0.05- 约 250 毫克 / 剂量或约 0.5- 约 100 毫克 / 剂量的量给予。例如，结构式

(I)、(II) 或 (III) 的化合物可以每剂量约 0.005、0.05、0.5、5、10、20、30、40、50、100、150、200、250、300、350、400、450 或 500 毫克的量给予，包括在 0.005 和 500 毫克之间的所有剂量。

[0078] 包含结构式 (I)、(II) 或 (III) 的 Bcl-2/Bcl-xL 抑制剂的组合物或包含所述抑制剂的组合物的剂量可以是约 1ng/kg- 约 200 mg/kg、约 1 μg/kg- 约 100 mg/kg 或约 1 mg/kg- 约 50 mg/kg。组合物的剂量可以是以任何剂量，包括但不限于约 1 μg/kg。组合物的剂量可以是以任何剂量，包括但不限于约 1 μg/kg、10 μg/kg、25 μg/kg、50 μg/kg、75 μg/kg、100 μg/kg、125 μg/kg、150 μg/kg、175 μg/kg、200 μg/kg、225 μg/kg、250 μg/kg、275 μg/kg、300 μg/kg、325 μg/kg、350 μg/kg、375 μg/kg、400 μg/kg、425 μg/kg、450 μg/kg、475 μg/kg、500 μg/kg、525 μg/kg、550 μg/kg、575 μg/kg、600 μg/kg、625 μg/kg、650 μg/kg、675 μg/kg、700 μg/kg、725 μg/kg、750 μg/kg、775 μg/kg、800 μg/kg、825 μg/kg、850 μg/kg、875 μg/kg、900 μg/kg、925 μg/kg、950 μg/kg、975 μg/kg、1 mg/kg、5 mg/kg、10 mg/kg、15 mg/kg、20 mg/kg、25 mg/kg、30 mg/kg、35 mg/kg、40 mg/kg、45 mg/kg、50 mg/kg、60 mg/kg、70 mg/kg、80 mg/kg、90 mg/kg、100 mg/kg、125 mg/kg、150 mg/kg、175 mg/kg 或 200 mg/kg。上述剂量是平均情况的实例，但可存在个别情况，其中应该用更高或更低的剂量，并且这样的剂量在本发明的范围内。实践中，医师确定最适合于个体患者的实际的给药方案，其可随特定患者的年龄、体重和反应而改变。

[0079] 在癌症的治疗中，结构式 (I)、(II) 或 (III) 的化合物可与化学治疗剂和 / 或辐射一起给予。

[0080] 本发明的实施方案采用以下的电磁辐射：γ - 辐射 (10^{-20}至 10^{-13} m)、X- 射线辐射 (10^{-12}至 10^{-9} m)、紫外光 (10 nm 至 400 nm)、可见光 (400 nm 至 700 nm)、红外辐射 (700 nm 至 1 mm) 和微波辐射 (1 mm 至 30 cm)。

[0081] 当前，许多癌症治疗方案采用被电磁辐射例如 X- 射线激活的辐射致敏剂。X- 射线激活的辐射致敏剂的实例包括但不限于甲硝哒唑、迷索硝唑、去甲基醚醇硝唑、哌莫硝唑、依他硝唑、尼莫唑、丝裂霉素 C、RSU 1069、SR 4233、EO9、RB 6145、尼克酰胺、5- 溴脱氧尿苷 (BUdR)、5- 碘 - 脱氧尿苷 (IUdR)、溴脱氧胞苷、氟脱氧尿苷 (FUdR)、羟基脲、顺铂和其治疗有效的类似物和衍生物。

[0082] 癌症的光动力疗法 (PDT) 采用可见光作为致敏剂的辐射激活剂。光动力辐射致敏剂的实例包括以下，但不限于：血卟啉衍生物、PHOTOFRIN®、苯并卟啉衍生物、NPe6、本卟啉锡 (SnET2)、脱镁叶绿酸 -a、细菌叶绿素 -a、萘菁、酞菁、酞菁锌和其治疗有效量的类似物和衍生物。

[0083] 除了本发明的 Bcl-2/Bcl-xL 抑制剂之外，辐射致敏剂还可以与治疗有效量的一种或多种化合物联合给予，所述化合物包括但不限于促进辐射致敏剂掺入靶细胞的化合物；控制治疗剂、营养素和 / 或氧流向靶细胞的化合物；与另外的辐射一起或不与另外的辐射一起对肿瘤起作用的化学治疗剂；或用于治疗癌症或其它疾病的其它治疗有效化合物。可与辐射致敏剂联合使用的另外的治疗剂的实例包括但不限于 5- 氟尿嘧啶 (5-FU)、亚叶酸、氧、氧和 5% 二氧化碳的混合气 (carbogen)、红细胞输注、全氟化碳 (例如 FLUOSOLW®-DA)、2,3-DPG、BW12C、钙通道阻滞剂、己酮可可碱、血管生成抑制化合物、肼屈嗪

和 L-BSO。

[0084] 化学治疗剂可以是诱导细胞凋亡的任何药理学作用剂或化合物。所述药理学作用剂或化合物可以是例如小的有机分子、肽、多肽、核酸或抗体。可使用的化学治疗剂包括但不限于烷基化剂、抗代谢物、激素和其拮抗剂、天然产物和其衍生物、放射性同位素、抗体以及天然产物和其组合。例如，本发明的 Bcl-2/Bcl-xL 抑制剂可与抗生素例如多柔比星和其它蒽环类抗生素类似物、氮芥例如环磷酰胺、嘧啶类似物例如 5- 氟尿嘧啶、顺铂、羟基脲、泰素和其天然和合成衍生物等一起给予。作为另一个实例，在混合肿瘤例如乳腺的腺癌（其中所述肿瘤包括促性腺素依赖性细胞和促性腺素非依赖性细胞）的情况下，所述化合物可与亮丙瑞林或戈舍瑞林 (LH-RH 的合成肽类似物）联合给予。其它抗瘤方案包括使用抑制剂化合物与另一种治疗形式，例如手术或辐射，本文亦称为 ″ 辅助抗瘤形式 ″。可用于本发明的其它化学治疗剂包括激素和其拮抗剂、放射性同位素、抗体、天然产物和其组合。

[0085] 可用于本发明方法的化学治疗剂的实例列于下表中。

表 1

烷基化剂	天然产物
氮芥	抗有丝分裂药
双氯乙基甲胺	紫杉烷类
环磷酰胺	紫杉醇
异环磷酰胺	长春花碱
美法兰	长春碱(VLB)
苯丁酸氮芥	长春新碱
尿嘧啶芥	长春瑞滨
替莫唑胺	长春地辛
硝基脲	Taxotere® (多西他赛)
亚硝脲氮芥(BCNU)	雌莫司汀
环己亚硝脲(CCNU)	磷雌氮芥
甲基环己亚硝脲(甲基-CCNU)	表鬼臼毒素类
氮芥	依托泊苷
链唑霉素	替尼泊苷
氮丙啶/甲基-蜜胺	抗生素类
三亚胺嗪(TEM)	放线菌素D
三亚乙基硫代磷酰胺	道诺霉素(柔红霉素)
(硫替派)	多柔比星(阿霉素)
六甲蜜胺	米托蒽醌伊达比星
(HMM，六甲蜜胺)	博来霉素
烷基磺酸盐类	光神霉素(普卡霉素)
白消安	丝裂霉素C
哌血生	更生霉素
三嗪类	阿非迪霉素
氮烯唑胺(DTIC)	表柔比星
	伊达比星

抗代谢物

叶酸类似物

氨甲喋呤

曲美沙特

培美曲塞

(多靶向抗叶酸剂)

嘧啶类似物

5-氟尿嘧啶

氟脱氧尿苷

吉西他滨

阿糖胞苷

(AraC，阿糖胞苷)

5-氮杂胞苷

2,2′-二氟脱氧胞苷

氟尿苷

喷司他丁

嘌呤类似物

6-巯基嘌呤

6-硫代鸟嘌呤

硫唑嘌呤

2'-脱氧柯福霉素

(喷司他丁)

红-9-(2-羟基-3-壬基)腺嘌呤(EHNA)

磷酸氟达拉滨

2-氯脱氧腺苷

(克拉屈滨，2-CdA)

I型拓扑异构酶抑制剂

柔红霉素

普卡霉素

脱氧共生－间型霉素

酶

L-门冬酰胺酶

L-精氨酸酶

辐射致敏剂

甲硝唑

米索硝唑

脱甲基米索硝唑

哌莫硝唑

依他硝唑

尼莫唑

RSU 1069

EO9

RB 6145

非类固醇类抗雄激素

SR4233

氟他胺

尼克酰胺

5-溴脱氧尿苷

5-碘脱氧尿苷

溴脱氧胞苷

其它作用剂

铂配位络合物

顺铂

卡铂

奥沙利铂

喜树碱

托泊替康

依立替康

生物应答调节物

G-CSF

GM-CSF

分化剂

维甲酸衍生物

激素和拮抗剂

肾上腺皮质类固醇类/拮抗剂

泼尼松和等同物

地塞米松

氨鲁米特

孕酮类

己酸羟孕酮

醋酸甲羟孕酮

醋酸甲地孕酮

雌激素类

乙酚

炔雌醇/等同物

抗雌激素

他莫昔芬

雄激素类

丙酸睾酮

氟甲睾酮/等同物

抗雄激素

氟他胺

蒽二酮

米托蒽醌

取代的脲

羟基脲

甲基肼衍生物

N-甲基肼(MIH)

丙卡巴肼

肾上腺皮质抑制剂

米托坦(*o,p'*- DDD)

氨鲁米特

细胞因子

干扰素(α、β、γ)

白介素-2

光敏剂

血卟啉衍生物

PHOTOFRIN®

苯并卟啉衍生物

Npe6

本卟啉锡(SnET2)

脱镁叶绿酸-a

细菌叶绿素-a

萘菁

酞菁

酞菁锌

辐射

X-射线

紫外光

γ辐射

促性腺素释放激素类似物	可见光
亮丙瑞林	红外辐射
	微波辐射

[0086] 微管作用剂干扰细胞有丝分裂，并且其细胞毒素活性是本领域众所周知的。可用于本发明的微管作用剂包括但不限于别秋水仙碱 (NSC 406042)、软海绵素 B (NSC 609395)、秋水仙碱 (NSC 757)、秋水仙素衍生物 (例如 NSC 33410)、多拉司他汀 10 (NSC 376128)、美坦生 (NSC 153858)、根霉素 (NSC 332598)、紫杉醇 (NSC 125973)、泰素 ® 衍生物 (例如，NSC 608832)、硫代秋水仙碱 (NSC 361792)、三苯甲基半胱氨酸 (NSC 83265)、硫酸长春碱 (NSC 49842)、硫酸长春新碱 (NSC 67574)、天然和合成的 epothilone 包括但不限于 epothilone A、eopthilone B 和 discodermolide (参见 Service, (1996) Science, 274:2009) ；磷雌氮芥、诺考达唑、MAP4 等。这样的作用剂的实例还描述于 Bulinski (1997) *J. Cell Sci.* 110:3055 3064; Panda (1997) *Proc. Natl. Acad. Sci. USA* 94:10560-10564; Muhlradt (1997) *Cancer Res.* 57:3344-3346; Nicolaou (1997) *Nature* 397:268-272; Vasquez (1997) *Mol. Biol. Cell.* 8:973-985 ；和 Panda (1996) *J. Biol. Chem.* 271:29807-29812。

[0087] 可使用的细胞生长抑制剂包括但不限于激素和类固醇 (包括合成的类似物) ：17- α - 炔雌醇、己烯雌酚、睾酮、泼尼松、氟甲睾酮、丙酸屈他雄酮、睾内酯、醋酸甲地孕酮、甲泼尼龙、甲基 - 睾酮、泼尼松龙、曲安西龙、氯烯雌醚、羟孕酮、氨鲁米特、雌莫司汀、醋酸甲羟孕酮、亮丙瑞林、氟他胺、托瑞米芬和诺雷德。

[0088] 其它细胞生长抑制剂是抗血管生成药，例如基质金属蛋白酶抑制剂，和其它 VEGF 抑制剂，例如抗 -VEGF 抗体和小分子例如 ZD6474 和 SU668。还可使用抗 -Her2 抗体。EGFR 抑制剂是 EKB-569 (一种不可逆抑制剂) 。还包括对 EGFR 和 Src 抑制剂具有免疫特异性的抗体 C225。

[0089] 作为细胞生长抑制剂还适合使用的是 CASODEX® (比卡鲁胺，Astra Zeneca)，其赋予雄激素依赖性的癌非增值性。细胞生长抑制剂的又一个实例是抗雌激素 TAMOXIFEN®，其抑制雌激素依赖性乳腺癌的增殖或生长。细胞增殖信号转导的抑制剂是细胞生长抑制剂。代表性实例包括表皮生长因子抑制剂、Her-2 抑制剂、MEK-1 激酶抑制剂、MAPK 激酶抑制剂、PI3 抑制剂、Src 激酶抑制剂和 PDGF 抑制剂。

[0090] 可与本发明的 Bcl-2/Bcl-xL 抑制剂一起给予的另外的第二种治疗剂公开于美国专利公开号 2007/0027135、美国专利号 7,432,304、美国专利公开号 2010/0278921、指定国为美国的 WO 2012/017251，各自通过引用结合到本文中。

[0091] 本发明的化合物通常与药用载体混合给予，所述载体根据预期的给药途径和标准药学实践而选择。根据本发明使用的药物组合物使用一种或多种生理学可接受的载体以常规方式配制，所述载体包括促进结构式 (I)、(II) 和 (III) 的化合物处理的赋形剂和助剂。

[0092] 这些药物组合物可通过例如常规的混合、溶解、制粒、制锭、乳化、包胶、捕获或冻干过程制备。合适的制剂取决于所选的给药途径。当治疗有效量的结构式 (I)、(II) 或 (III) 的化合物口服给予时，所述组合物通常呈片剂、胶囊剂、粉剂、溶液剂或酏剂的形式。当以片剂形式给予时，所述组合物可另外包含固体载体，例如明胶或辅剂。片剂、胶囊剂和

粉剂含有约 0.01%- 约 95%、和优选约 1%- 约 50% 的结构式 (I)、(II) 或 (III) 的化合物。当以液体形式给予时，可加入液体载体例如水、石油或动物或植物来源的油。液体形式的组合物可进一步含有生理盐水溶液、葡萄糖或其它糖溶液或甘油。当以液体形式给予时，所述组合物含有约 0.1%- 约 90%、和优选约 1%- 约 50% 重量的结构式 (I)、(II) 或 (III) 的化合物。

[0093] 当治疗有效量的结构式 (I)、(II) 或 (III) 的化合物通过静脉内、皮肤或皮下注射给予时，所述组合物呈无热源的胃肠外可接受的水溶液的形式。适当考虑 pH、等渗性、稳定性等而制备这样的胃肠外可接受的溶液在本领域的技术范围内。用于静脉内、皮肤或皮下注射的优选的组合物通常含有等渗的溶媒。

[0094] 结构式 (I)、(II) 和 (III) 的化合物可容易地与本领域众所周知的药学上可接受的载体组合。这样的载体使得活性剂能够作为片剂、丸剂、锭剂、胶囊剂、液体剂、凝胶剂、糖浆剂、膏剂、混悬剂等配制，用于由待治疗的患者口服摄取。用于口服使用的药物制剂可通过添加结构式 (I)、(II) 或 (III) 的化合物至固体赋形剂，任选研磨所得的混合，和在加入合适的助剂之后 (如果需要)，加工粒料混合物以得到片剂或锭剂核心而获得。合适的赋形剂包括例如填充剂和纤维素制剂。如果需要，可加入崩解剂。

[0095] 结构式 (I)、(II) 和 (III) 的化合物可经配制通过注射用于胃肠外给予，例如通过推注或持续输注。注射制剂可以单位剂型提供，例如在安瓿中或在多剂量容器中，具有添加的防腐剂。所述组合物可呈这样的形式，例如在油性或水性溶媒中的混悬剂、溶液剂或乳剂，和可含有配制剂例如助悬剂、稳定剂和 / 或分散剂。

[0096] 用于胃肠外给予的药物组合物包括呈水溶形式的活性剂的水性溶液。此外，结构式 (I)、(II) 或 (III) 的化合物的混悬剂可制备为合适的油性注射混悬剂。合适的亲脂溶剂或溶媒包括脂肪油或合成的脂肪酸酯。水性注射混悬剂可包含增加混悬剂的粘度的物质。任选地，混悬剂还可包含合适的稳定剂或增加化合物的溶解度并允许制备高度浓缩溶液的作用剂。或者，本发明的组合物可以呈粉末形式，用于在使用前与合适的溶媒例如无菌无热源水配制。

[0097] 结构式 (I)、(II) 或 (III) 的化合物还可配制在直肠组合物中，例如栓剂或保留灌肠剂，例如含有常规的栓剂基料。除了前述制剂之外，结构式 (I)、(II) 或 (III) 的化合物还可配制为贮库制剂。这样的长效制剂可通过植入 (例如皮下或肌肉内) 或通过肌肉内注射给予。因此，例如，结构式 (I)、(II) 或 (III) 的化合物可与合适的聚合材料或疏水材料 (例如作为在可接受的油中的乳剂) 或离子交换树脂一起配制。

[0098] 具体而言，结构式 (I)、(II) 和 (III) 的化合物可以含有赋形剂例如淀粉或乳糖的片剂的形式，或以单独或与赋形剂混合的胶囊剂或卵形剂 (ovule) 的形式，或以含有矫味剂或着色剂的酏剂或混悬剂的形式，口服、含服或舌下给予。这样的液体制剂可与药学上可接受的添加剂 (例如助悬剂) 一起配制。结构式 (I)、(II) 和 (III) 的化合物还可经胃肠外注射，例如静脉内、肌肉内、皮下或冠状动脉内。对于胃肠外给予，Bcl-2/Bcl-xL 抑制剂最好以无菌水性溶液的形式使用，其可含有其它物质，例如盐或单糖，例如甘露糖醇或葡萄糖，以使所述溶液与血液等渗。

[0099] 作为另外的实施方案，本发明包括药盒，其包括以促进其用于实施本发明方法的方式包装的一种或多种化合物或组合物。在一个简单的实施方案中，所述药盒包括用于方法实践的本文所述的化合物或组合物 (例如，包含结构式 (I)、(II) 或 (III) 的化合物和任

选第二种治疗剂的组合物），其包装在容器中，例如密封的瓶或管，容器上附有标签或药盒内包括标签，其描述使用所述化合物或组合物以实施本发明的方法。优选地，所述化合物或组合物以单位剂型包装。所述试剂盒可进一步包括适于根据预期给药途径而给予所述组合物的装置。

[0100] 除了其在治疗药物中的用途之外，结构式（I）、（II）和（III）的化合物和其药学上可接受的盐还可用作在用于评价 Bcl-2 和 / 或 Bcl-X_L的抑制剂在实验动物（例如猫、狗、兔、猴、大鼠和小鼠）中的效果的体外和体内测试系统的开发和标准化中的药理学工具，作为新的治疗药的研究的一部分。

[0101] 在先的 Bcl-2/Bcl-xL 抑制剂具有妨碍其开发作为治疗药的性质。根据本发明的重要特征，结构式（I）、（II）和（III）的化合物作为 Bcl-2/Bcl-xL 的抑制剂进行合成和评价。例如，本发明的化合物通常具有小于 100 nM 的与 Bcl-2/Bcl-xL 的结合亲和力（IC_{50}）。

[0102] 化合物的合成

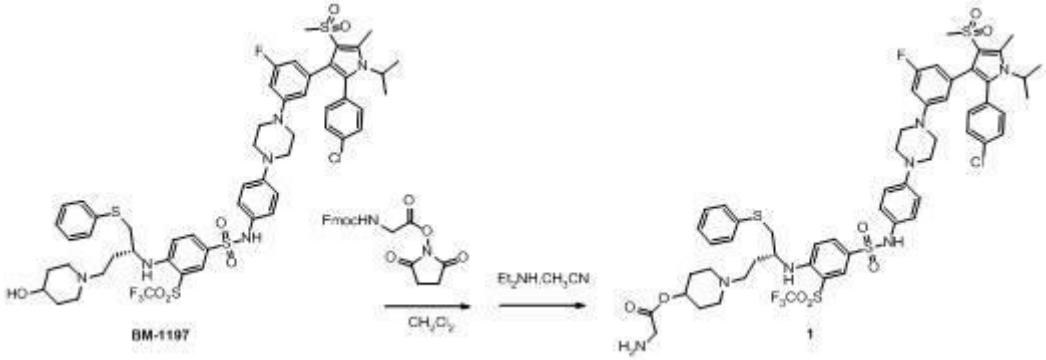
本发明的化合物如下制备。以下合成方案代表了用于合成结构式（I）、（II）和（III）的化合物的反应。制备本发明的 Bcl-2/Bcl-xL 抑制剂的变更和代替方案完全在本领域技术人员的能力范围内。

[0103] 溶剂和试剂可市售获得，无需进一步纯化而使用。NMR 光谱的化学位移（δ）报告为相对于内部标准的 δ 值（ppm）低磁场，以通常的方式报告多峰性。

[0104] 除非另有说明，否则所有的温度以摄氏度计。

[0105] 用于合成本发明的化合物的某些关键的中间体可通过指定国为美国的 WO 2012/103059 中所述的方法合成，所述专利通过引用以其整体结合到本文中，接着如下转化成其磷酸盐衍生物：

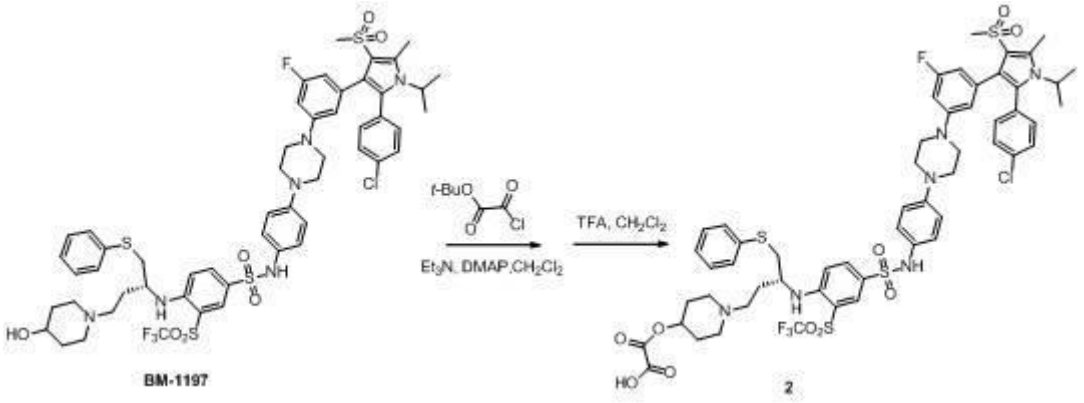
方案 1：化合物 1 的合成



实验部分：(*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4- 氯苯基）-1- 异丙基 -5- 甲基 -4-（甲基磺酰基）-1H- 吡咯 -3- 基）-5- 氟苯基）哌嗪 -1- 基）苯基）氨磺酰基）-2-（三氟甲基磺酰基）苯基氨基）-4-（苯基硫代）丁基）哌啶 -4- 基 2- 氨基乙酸酯（1）。将 BM-1197（113 mg, 0.10 mmol）和 Fmoc-Gly-OSu（43 mg, 0.11 mmol）在 CH_2Cl_2（2 mL）中的溶液在室温下搅拌 1 小时，直到通过 TLC 未观察到 BM-1197。将溶液真空浓缩，提供 1 的粗制前体，其用于下一步骤而无需纯化。将得到的残余物溶于乙腈（5 mL），接着加入二乙胺（0.2 mL, 2 mmol）。将混合物在室温下搅拌过夜，直到通过 TLC 未观察到原料，和真空浓缩。残余物通过 HPLC 纯化，得到产物 1（与 TFA 的盐，83 mg, 经 2 步收率 70%）。梯度为在 40 min 内 60% 的溶剂 A 和 40% 的溶剂 B 至 20% 的溶剂 A 和 80% 的溶剂 B。MS（ESI）*m/z* 1189.08（M

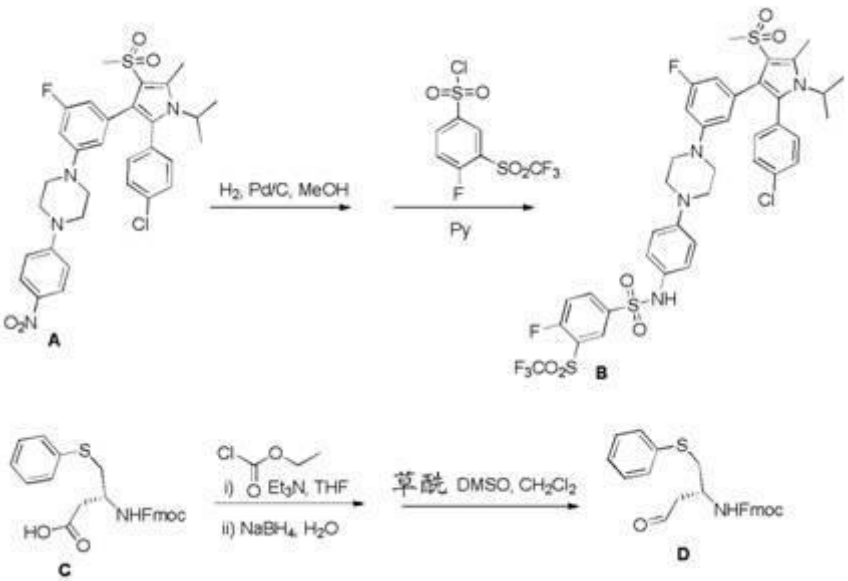
+ H)$^+$。

[0106] 方案 2 :2 的合成



实验部分 :(*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4- 氯苯基)-1- 异丙基 -5- 甲基 -4-(甲基磺酰基)-1H- 吡咯 -3- 基)-5- 氟苯基) 哌嗪 -1- 基) 苯基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基) 哌啶 -4- 基氧基)-2- 氧代乙酸 (2)。向 BM-1197 (113 mg, 0.10 mmol)、DMAP (2 mg, 0.02 mmol)、Et_3N (42 uL, 0.3 mmol) 在 CH_2Cl_2 (2 mL) 的溶液中加入 2- 氯 -2- 氧代乙酸叔丁酯 (33 mg, 0.2 mmol)。将溶液在室温下搅拌 1 小时,直到通过 TLC 未观察到 BM-1197,和真空浓缩。将粗制残余物在硅胶上用 5% MeOH/CH_2Cl_2快速色谱分离,提供 2 的前体。将前体溶于 CH_2Cl_2 (3 mL),接着加入 TFA (3 mL)。将混合物在室温下搅拌 1 小时,直到通过 TLC 未观察到原料,和真空浓缩。残余物通过 HPLC 纯化,得到产物 2 (与 TFA 的盐 , 66 mg, 经 2 步收率 55%)。梯度为在 40 min 内 60% 的溶剂 A 和 40% 的溶剂 B 至 20% 的溶剂 A 和 80% 的溶剂 B。MS (ESI) *m/z* 1189.08 (M + H)$^+$。

[0107] 方案 3 :关键中间体 B 和 D 的制备

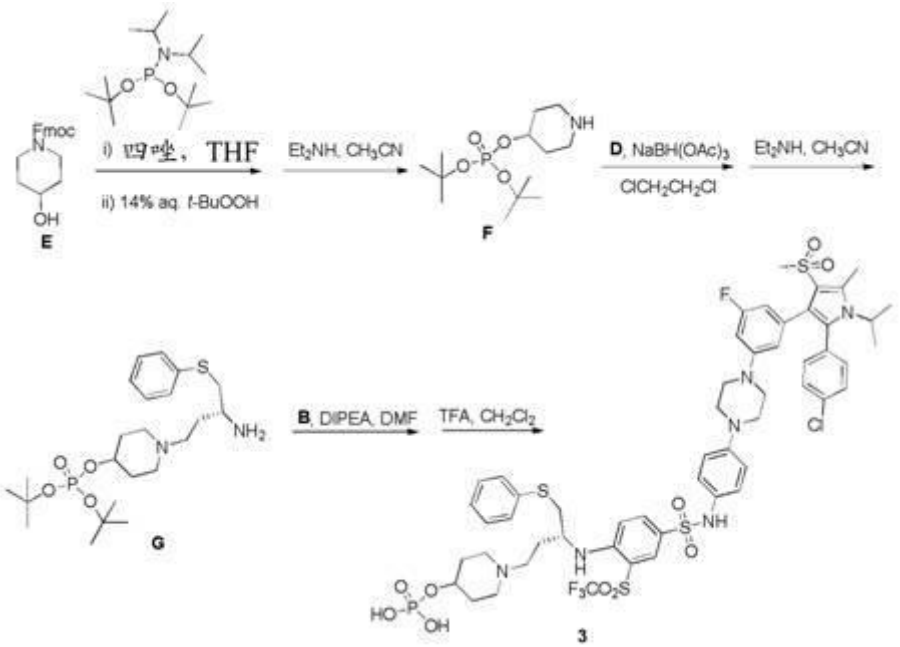


实验部分 :N-(4-(4-(3-(2-(4- 氯苯基)-1- 异丙基 -5- 甲基 -4-(甲基磺酰基)-1H- 吡咯 -3- 基)-5- 氟苯基) 哌嗪 -1- 基) 苯基)-4- 氟 -3-(三氟甲基磺酰基) 苯磺酰胺 (B)。向 A (3.0 g, 4.9 mmol) 在 150 mL 甲醇中的溶液加入 10% wt. Pd/C (300 mg, 0.1 eq. m/m)。将溶液在氢气氛下在室温下搅拌约 20 min,直到通过 TLC 未观察到 A。将反应混合物过滤,和将滤液真空浓缩。将残余物直接用于下一步骤而无需纯化。在 0℃向该苯胺在吡

啶中的溶液中加入 4- 氟 -3-(三氟甲基磺酰基) 苯 -1- 磺酰氯 (1.8 g, 5.4 mmol)。将混合物在 0℃至室温搅拌 1 小时,直到通过 TLC 未观察到苯胺。加入水 (10 mL) 和用乙酸乙酯 (200 mL * 2) 萃取。合并的乙酸乙酯溶液用盐水 (150 mL) 洗涤,经硫酸钠干燥和真空浓缩。浓缩物在硅胶上用 40% EtOA/ 己烷快速色谱分离,提供中间体 B (3.2 g, 经 2 步收率 75%)。MS (ESI) *m/z* 931.75 $(M + K)^+$。

[0108] 通用流程 I:(*R*)-(9H-芴 -9-基) 甲基 4-氧代 -1-(苯基硫代) 丁 -2-基氨基甲酸酯 (D)。在 -10℃在氩气氛下向 C (5.0 g, 11.5 mmol) 在 THF (100 mL) 中的溶液中加入三乙胺 (4.8 mL, 34.5 mmol) 和氯甲酸乙酯 (3.3 mL, 34.5 mmol)。将混合物在 -10℃搅拌 1 h,和在 -10℃滴加含 $NaBH_4$ (1.7 g, 46.1 mmol) 的水 (60 mL)。将混合物在 -10℃搅拌 1 h,然后在室温下搅拌 2 h。用 1 M 含水 $KHSO_4$ (200 mL) 将反应淬灭,混合物用 EtOAc (3×200 mL) 萃取。萃取物用盐水 (200 mL) 洗涤,经无水硫酸钠干燥,过滤和真空浓缩。浓缩物在硅胶上用 50% EtOA/ 己烷快速色谱分离,提供相应的醇 (4.3 g, 收率 90%)。在 -78℃向草酰氯 (2.6 mL, 31.1 mmol) 在 DCM (100 mL) 中的溶液中加入二甲基亚砜 (3.7 mL, 51.8 mmol)。将溶液升温至 -40℃ 5 min 和再次冷却至 -78℃,然后滴加前一步骤的所得醇 (4.3 g, 10.4 mmol) 在 DCM (50 mL) 中的溶液。将溶液搅拌另外 40 min,接着加入过量三乙胺 (25 mL) 和搅拌另外 30 min。将反应混合物升温至室温,接着加入饱和氯化铵水溶液 (100 mL),和用 DCM (2×200 mL) 萃取。合并的 DCM 溶液用盐水 (150 mL) 洗涤,经硫酸钠干燥和真空浓缩。残余物在硅胶上用 20% EtOA/ 己烷快速色谱分离,提供中间体 D (3.7 g, 收率 85%)。MS (ESI) *m/z* 418.25 $(M + H)^+$。

[0109] 方案 4 :3 的合成



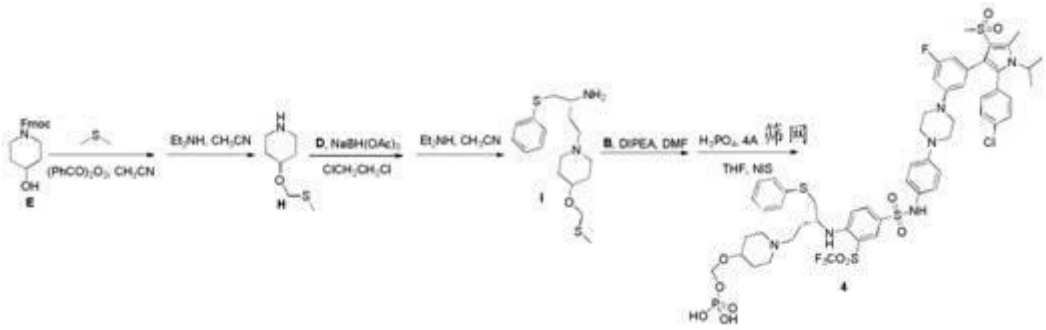
实验部分 :二 -叔丁基哌啶 -4-基磷酸酯 (F)。将二 - 叔丁基二 - 异丙基亚磷酰胺 (832 mg, 3.0 mmol) 和四唑 (6.6 mL, 0.45 M,在乙腈中) 在 THF (15 mL) 中的溶液在 N_2 下在室温下搅拌大约 10 min。然后经 15 分钟将在无水 THF (2 mL) 中的化合物 E (626 mg, 2.0 mmol) 加入至反应物,在室温下在 N_2 下搅拌 2 小时,直到通过 TLC 未观察到 E。然后将反应温度冷却至 0℃和加入叔丁基过氧化物 (3.0 mL, 4.6 mmol) 的 14% 水溶液。然后将温度升

至室温，将混合物搅拌过夜。用饱和 $NaHCO_3$ 水溶液 (2 mL) 淬灭反应。添加水 (50 mL) 至反应混合物中，然后将其用乙酸乙酯 (2×50 mL) 萃取。合并的乙酸乙酯溶液用盐水 (50 mL) 洗涤，经硫酸钠干燥和真空浓缩，得到粗制产物，其用于下一步骤而无需纯化。将得到的残余物溶于乙腈 (20 mL)，然后加入二乙胺 (4.1 mL, 40 mmol)。将混合物在室温下搅拌过夜，直到通过 TLC 未观察到原料，和真空浓缩。残余物在硅胶上用 5% MeOH/DCM 快速色谱分离，提供中间体 F (452 mg, 经 2 步收率 77%)。MS (ESI) m/z 295.17 $(M + H)^+$。

[0110] 通用程序 II：(*R*)-1-(3-氨基-4-(苯基硫代)丁基)哌啶-4-基二-叔丁基磷酸酯 (G)。向 F (293 mg, 1.0 mmol) 和中间体 D (500 mg, 1.2 mmol) 在 DCE (10 mL) 中的溶液中加入 $NaBH(OAc)_3$ (636 mg, 3.0 mmol)，将混合物在室温下搅拌过夜，直到通过 TLC 未观察到 F。混合物用 DCM (50 mL) 洗涤，用盐水 (50 mL) 洗涤和经硫酸钠干燥。真空除去溶剂，得到粗制产物，其用于下一步骤而无需纯化。将得到的残余物溶于乙腈 (10 mL)，接着加入二乙胺 (2.1 mL, 20 mmol)。将混合物在室温下搅拌过夜，直到通过 TLC 未观察到原料和真空浓缩。残余物在硅胶上用 10% MeOH/DCM 快速色谱分离，提供中间体 G (307 mg, 经 2 步收率 65%)。MS (ESI) m/z 474.00 $(M + H)^+$。

[0111] 通用程序 III：(*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-基二氢磷酸酯 (3)。向 B (100 mg, 0.11 mmol) 和 G (65 mg, 0.14 mmol) 在 DMF (2 mL) 中的溶液中加入 DIPEA (1 mL)。将溶液在室温下搅拌 4 小时，直到通过 TLC 未观察到 B。将反应混合物真空浓缩，得到粗制产物，其用于下一步骤而无需纯化。将得到的残余物溶于 DCM (5 mL)，接着加入 TFA (2.5 mL)。将溶液在室温下搅拌 1 h，直到通过 TLC 未观察到原料。将反应混合物真空浓缩和残余物通过 HPLC 纯化，得到纯的产物 3 (与 TFA 的盐，88 mg, 经 2 步收率 66%)。梯度为在 40 min 内 60% 的溶剂 A 和 40% 的溶剂 B 至 20% 的溶剂 A 和 80% 的溶剂 B。^1H NMR (300 M Hz, CD_3OD): δ 7.96 (s, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.32-7.07 (m, 13H), 6.93-6.41 (m, 4H), 4.61-4.41(m, 2H), 3.99 (s, 1H), 3.55-3.11 (m, 16H), 2.84 (s, 3H), 2.74 (s, 3H), 2.26-1.80 (m, 6H), 1.43 (d, J = 7.0 Hz, 6H). MS (ESI): m/z 1212.67 $(M + H)^+$。

[0112] 方案 5：4 的合成



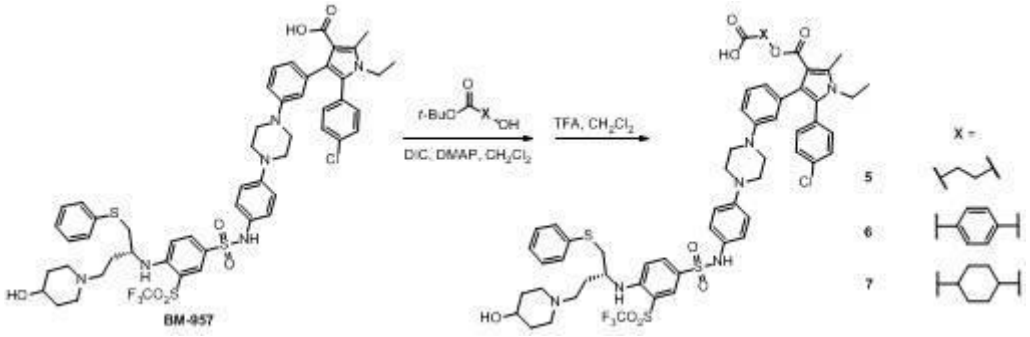
实验部分：4-(甲基硫代甲氧基)哌啶 (H)。在 0℃向醇 E (1.0 g, 3.1 mmol) 和硫化甲基 (1.8 mL, 24.8 mmol) 在乙腈 (31 mL) 中的溶液中经 10 min 分 4 等份加入苯甲酰过氧化物 (3.0 g, 12.4 mmol)，在 0℃将混合物搅拌 1 h，然后在室温下搅拌 1 h，直到通过 TLC 未观察到 E。混合物用乙酸乙酯 (100 mL) 稀释，用 10% Na_2CO_3 (100 mL) 洗涤，然后用盐水

(100 mL)洗涤,经硫酸钠干燥。真空除去溶剂,得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于乙腈(10 mL),接着加入二乙胺(6.2 mL, 60 mmol)。将混合物在室温下搅拌过夜,直到通过TLC未观察到原料和真空浓缩。残余物在硅胶上用5% MeOH/DCM快速色谱分离,提供中间体H (270 mg, 经2步收率54%)。MS (ESI) *m/z* 162.83 $(M + H)^+$。

[0113] (*R*)-4-(4-(甲基硫代甲氧基)哌啶-1-基)-1-(苯基硫代)丁-2-胺(I)。根据通用程序II自H和D制备I。MS (ESI) *m/z* 341.58 $(M + H)^+$。

[0114] (*R*)-(1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-基氧基)甲基二氢磷酸酯(4)。向B (200 mg, 0.23 mmol)和I (86 mg, 0.25 mmol)在DMF (4 mL)中的溶液中加入DIPEA (2 mL)。将溶液在室温下搅拌4小时,直到通过TLC未观察到B。将反应混合物在真空浓缩。残余物在硅胶上用5% MeOH/DCM快速色谱分离,得到相应的硫醚(241 mg, 收率88%)。在0℃向来自第一步骤的硫醚(200 mg, 0.17 mmol)、磷酸(117 mg, 1.2 mmol)和分子筛(4 Å, 500 mg)在THF (6 mL)中的溶液加入*N*-碘代琥珀酰亚胺(57 mg, 0.26 mmol),将混合物在室温下搅拌1 h,直到通过TLC未观察到原料。将反应混合物通过硅藻土过滤,固体用甲醇洗涤。滤液经真空浓缩,残余物通过HPLC纯化,得到纯的产物4 (与TFA的盐, 93 mg, 收率44%)。梯度为在40 min内60%的溶剂A和40%的溶剂B至20%的溶剂A和80%的溶剂B。MS (ESI): *m/z* 1242.08 $(M + H)^+$。

[0115] 方案6:化合物5、6、7的合成



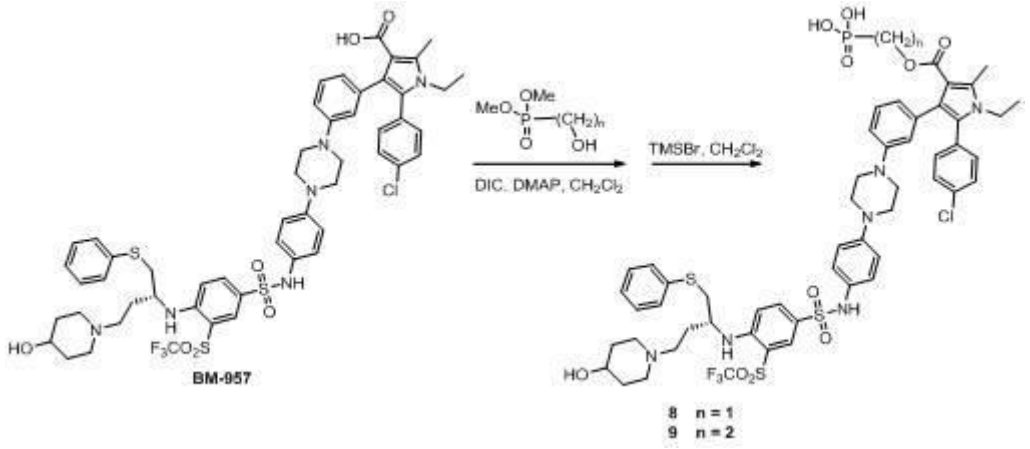
实验部分:通用程序IV。(*R*)-3-((5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基)磺酰胺基)苯基)哌嗪-1-基)苯基)-2-甲基-1H-吡咯-3-羰基)氧基)丙酸(5)。向957 (100 mg, 0.09 mmol)、DIC (18 mg, 0.14 mmol)和DMAP (20 mg, 0.14 mmol)在DCM (2 mL)中的溶液中加入3-羟基丙酸叔丁酯(41 mg, 0.28 mmol)。将溶液在室温下搅拌6小时,直到通过TLC未观察到BM-957。反应混合物用乙酸乙酯(50 mL)稀释,用饱和$NaHCO_3$溶液(50 mL)、盐水(50 mL)洗涤和经硫酸钠干燥。真空除去溶剂得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于DCM (5 mL),接着添加TFA (2.5 mL)。将溶液在室温下搅拌3 h,直到通过TLC未观察到原料。将反应混合物在真空浓缩,残余物通过HPLC纯化,得到纯的产物5 (与TFA的盐, 75 mg, 经2步收率70%)。梯度为在40 min内60%的溶剂A和40%的溶剂B至20%的溶剂A和80%的溶剂B。MS (ESI): *m/z* 1238.17 $(M + H)^+$。

[0116] (*R*)-4-((5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基磺酰胺基)

苯基）哌嗪-1-基）苯基)-2-甲基-1H-吡咯-3-羰基）氧基）苯甲酸(6)。6根据通用程序IV自BM-957和4-羟基苯甲酸叔丁酯制备。MS (ESI): *m/z* 1186.00 (M + H)$^+$。

[0117] (*R*)-4-((5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-2-甲基-1H-吡咯-3-羰基）氧基）环己烷甲酸(7)。7根据通用程序IV自BM-957和4-羟基环己烷甲酸叔丁酯制备。MS (ESI): *m/z* 1192.25 (M + H)$^+$。

[0118] 方案7:8、9的合成

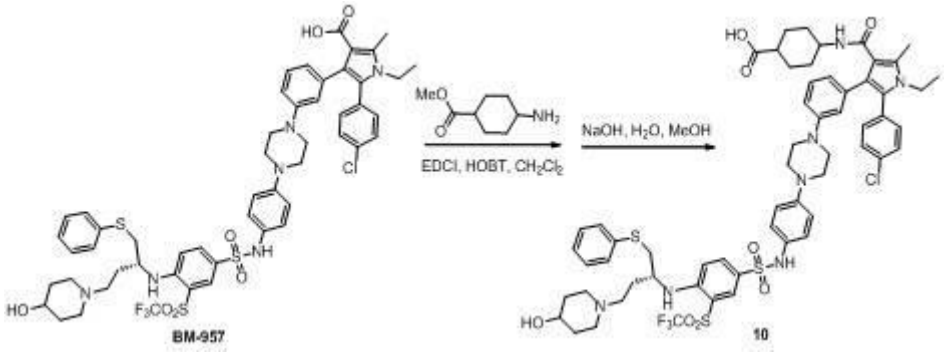


实验部分：

通用程序V:(*R*)-(((5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-2-甲基-1H-吡咯-3-羰基）氧基）甲基）膦酸(8)。向BM-957 (100 mg, 0.09 mmol)、DIC (18 mg, 0.14 mmol)和DMAP (20 mg, 0.14 mmol)在DCM (2 mL)中的溶液中加入二甲基(羟基甲基)膦酸酯(40 mg, 0.28 mmol)。将溶液在室温下搅拌6小时,直到通过TLC未观察到BM-957。将反应混合物用乙酸乙酯(50 mL)稀释,用饱和$NaHCO_3$溶液(50 mL)、盐水(50 mL)洗涤和经硫酸钠干燥。真空除去溶剂得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于DCM (5 mL),接着加入TMSBr (248 uL, 1.9 mmol)。将溶液在室温下搅拌20 h,直到通过MS未观察到原料。将反应混合物在真空浓缩,残余物通过HPLC纯化,得到纯的产物8 (与TFA的盐, 74 mg, 经2步收率68%)。梯度为在40 min内60%的溶剂A和40%的溶剂B至20%的溶剂A和80%的溶剂B。^{1}H NMR (300 MHz, CD_3OD): δ 7.92 (s, 1H), 7.73-7.70 (m, 2H), 7.34-6.82 (m, 17H), 4.28 (d, *J* = 8.6 Hz, 2H), 4.06-3.35 (m, 14H), 3.20-2.92 (m, 5H), 2.65 (s, 3H), 2.24-1.67 (m, 6H), 1.10 (t, *J* = 7.0 Hz, 3H). MS (ESI): *m/z* 1259.50 (M + H)$^+$。

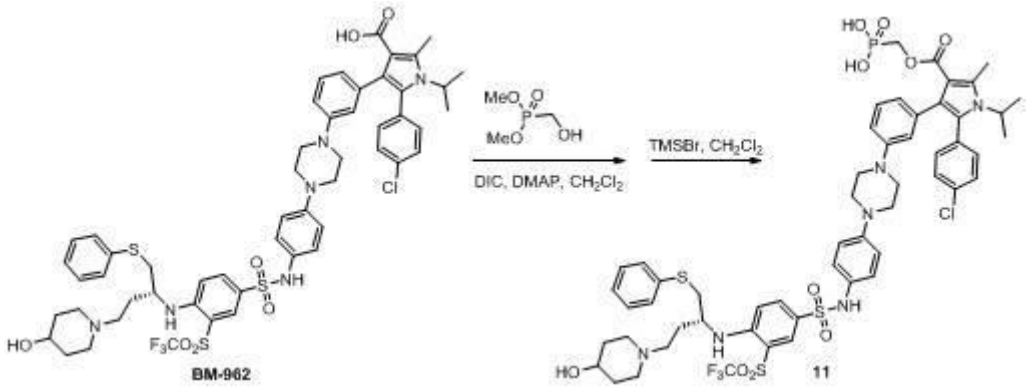
[0119] (*R*)-(2-((5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-2-甲基-1H-吡咯-3-羰基）氧基）乙基）膦酸(9)。9根据通用程序V自BM-957和二甲基(2-羟基乙基)膦酸酯制备。MS (ESI): *m/z* 1173.42 (M + H)$^+$。

[0120] 方案8:10的合成



((R)-4-(5-(4-氯苯基)-1-乙基-4-(3-(4-(4-(4-(4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基氨基)-3-(三氟甲基磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-2-甲基-1H-吡咯-3-甲酰胺基)环己烷甲酸(10)。向BM-957 (100 mg, 0.09 mmol)、EDCI (27 mg, 0.14 mmol)和HOBT (19 mg, 0.14 mmol)在DCM (2 mL)中的溶液中加入4-氨基环己烷甲酸甲酯(44 mg, 0.28 mmol)。将溶液在室温下搅拌2小时,直到通过TLC未观察到BM-957。反应混合物用乙酸乙酯(50 mL)稀释,用饱和$NaHCO_3$溶液(50 mL)、盐水(50 mL)洗涤和经硫酸钠干燥。真空除去溶剂,得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于H_2O和MeOH (分别5 mL和5 mL),接着加入NaOH (76 mg, 1.9 mmol)。将溶液在室温下搅拌20 h,直到通过TLC未观察到原料。将反应混合物在真空浓缩,残余物通过HPLC纯化,得到纯的产物10 (与TFA的盐, 61 mg, 经2步收率55%)。梯度为在40 min内60%的溶剂A和40%的溶剂B至20%的溶剂A和80%的溶剂B。MS (ESI): m/z 1191.17 $(M + H)^+$。

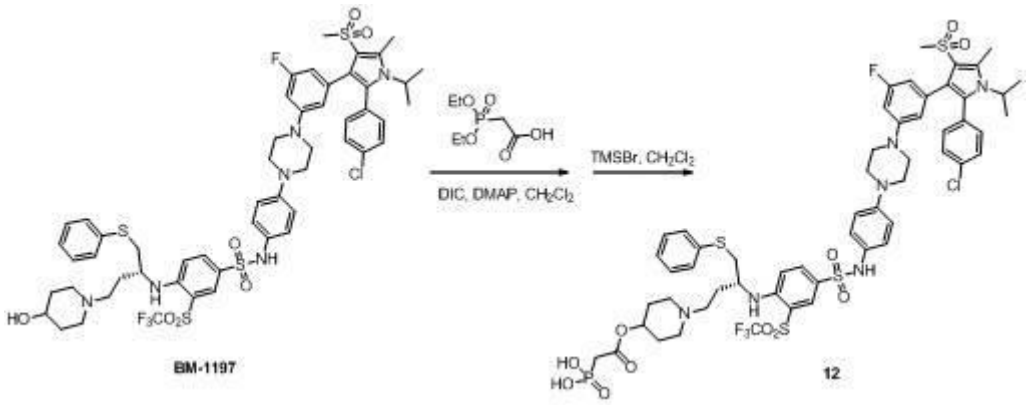
[0121] 方案9:11的合成



实验部分:

(*R*)-(((5-(4-氯苯基)-4-(3-(4-(4-(4-((4-(4-羟基哌啶-1-基)-1-(苯基硫代)丁-2-基)氨基)-3-((三氟甲基)磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-1-异丙基-2-甲基-1H-吡咯-3-羰基)氧基)甲基)膦酸(11)。11根据通用程序V自BM-962和二甲基(羟基甲基)膦酸酯制备。^1H NMR (300 M Hz, CD_3OD): δ 8.00 (s, 1H), 7.80-7.71 (m, 2H), 7.38-6.83 (m, 17H), 4.50-4.41 (m, 1H), 4.29 (d, J = 8.7 Hz, 2H), 4.11-3.59 (m, 12H), 3.25-3.01 (m, 6H), 2.77 (s, 3H), 2.28-1.70 (m, 6H), 1.47 (d, J = 7.1 Hz, 6H). MS (ESI): m/z 1174.25 $(M + H)^+$。

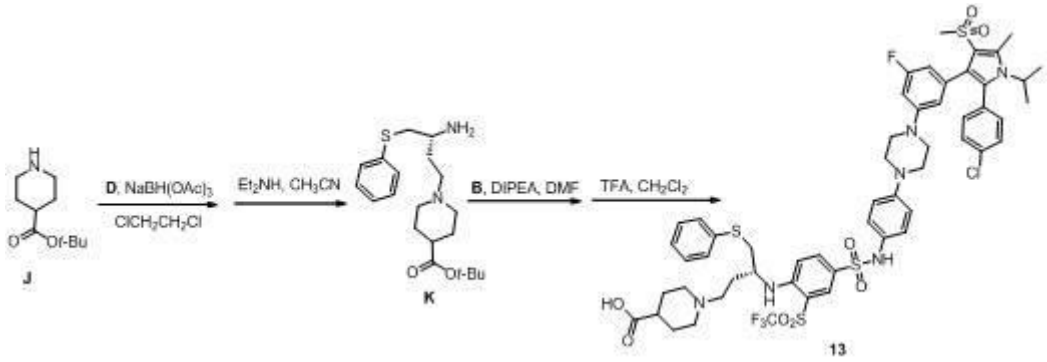
[0122] 方案9:12的合成



实验部分：

(*R*)-(2-((1-(3-((4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-((三氟甲基)磺酰基)苯基)氨基)-4-(苯基硫代)丁基)哌啶-4-基)氧基)-2-氧代乙基)膦酸(12)。12根据通用程序V自BM-1197和2-(二乙氧基磷酰基)乙酸制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.99 (s, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.36-7.13 (m, 12H), 6.92-6.43 (m, 5H), 5.10 (s, 1H), 4.51-4.44 (m, 1H), 4.10 (s, 1H), 3.56-2.93 (m, 18H), 2.87 (s, 3H), 2.76 (s, 3H), 2.29-1.90 (m, 6H), 1.46 (d, *J* = 7.3 Hz, 6H). MS (ESI): *m/z* 1253.36 $(M + H)^+$。

[0123] 方案10:13的合成

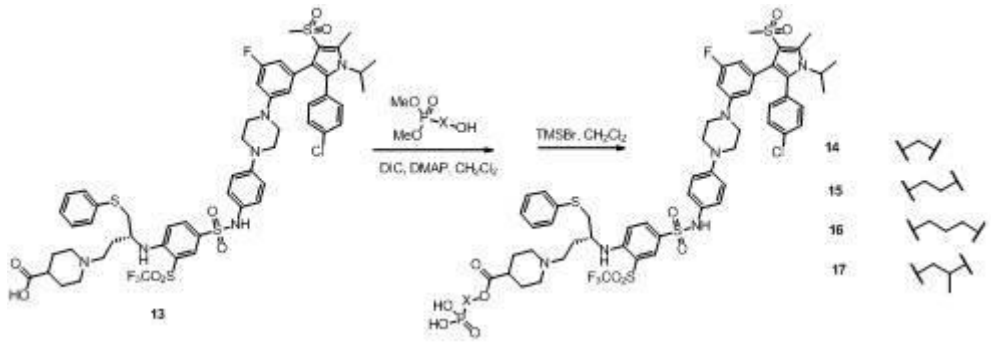


实验部分：

(*R*)-1-(3-氨基-4-(苯基硫代)丁基)哌啶-4-甲酸叔丁酯(K)。K根据通用程序II自哌啶-4-甲酸叔丁酯和D制备。MS (ESI): *m/z* 365.50 $(M + H)^+$。

[0124] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-甲酸(13)。13根据通用程序III自K和B制备。MS (ESI): *m/z* 365.50 $(M + H)^+$。

[0125] 方案11:14、15、16、17的合成



实验部分：

(*R*)-(1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-羰基氧基)甲基膦酸(14)。14根据通用程序V自13和二甲基(2-羟基甲基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (s, 1H), 7.72 (d, *J* = 9.1 Hz, 1H), 7.30-7.09 (m, 13H), 6.91-6.42 (m, 4H), 4.49-4.40 (m, 1H), 3.99 (s, 1H), 3.55-2.90 (m, 16H), 2.84 (s, 3H), 2.72 (s, 3H), 2.63-2.55 (m, 1H), 2.23-1.81 (m, 6H), 1.41 (d, *J* = 4.3 Hz, 6H). MS (ESI): *m/z* 1160.34 $(M + H)^+$。

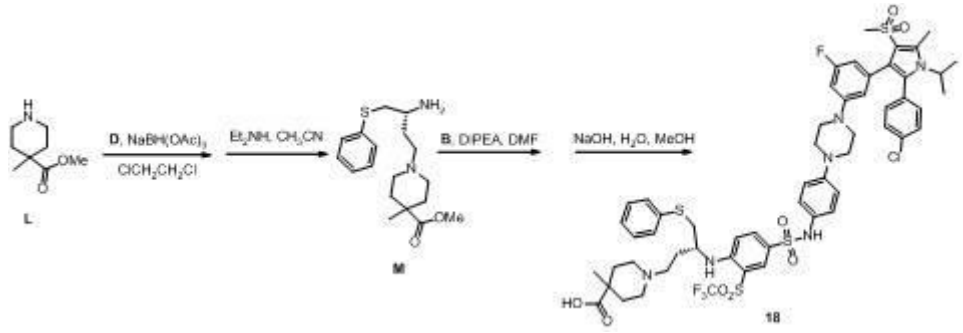
[0126] (*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-羰基氧基)乙基膦酸(15)。15根据通用程序V自13和二甲基(2-羟基乙基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.93 (d, *J* = 1.9 Hz, 1H), 7.72 (dd, *J* = 9.2, 1.8 Hz, 1H), 7.30-7.12 (m, 12H), 6.83-6.42 (m, 5H), 4.46-4.33 (m, 3H), 3.96 (s, 1H), 3.54-2.93 (m, 16H), 2.82 (s, 3H), 2.72 (s, 3H), 2.71-2.55 (m, 1H), 2.24-1.65 (m, 8H), 1.41 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1268.58 $(M + H)^+$。

[0127] (*R*)-3-(1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-羰基氧基)丙基膦酸(16)。16根据通用程序V自13和二甲基3-羟基丙基膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.33-7.12 (m, 12H), 6.92-6.43 (m, 5H), 4.51-4.41 (m, 1H), 4.18-3.98 (m, 3H), 3.56-2.92 (m, 16H), 2.85 (s, 3H), 2.73 (s, 3H), 2.67-2.50 (m, 1H), 2.25-1.70 (m, 10H), 1.43 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1282.34 $(M + H)^+$。

[0128] 2-(1-((*R*)-3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-羰基氧基)丙基膦酸(17)。17根据通用程序V自13和二甲基2-羟基丙基膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.97 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 9.2 Hz, 1H), 7.36-7.08 (m, 13H), 6.85-6.43 (m, 4H), 5.26 (s, 1H), 4.54-4.44 (m, 1H), 4.01 (s, 1H), 3.58-2.92 (m, 16H), 2.87 (s, 3H), 2.76 (s, 3H), 2.70-2.55 (m, 1H), 2.26-1.85 (m, 8H), 1.46 (d, *J* = 7.1

Hz, 6H), 1.38 (d, *J* = 5.9 Hz, 3H). MS (ESI): *m/z* 1281.34 $(M + H)^+$。

[0129] 方案 12 :18 的合成

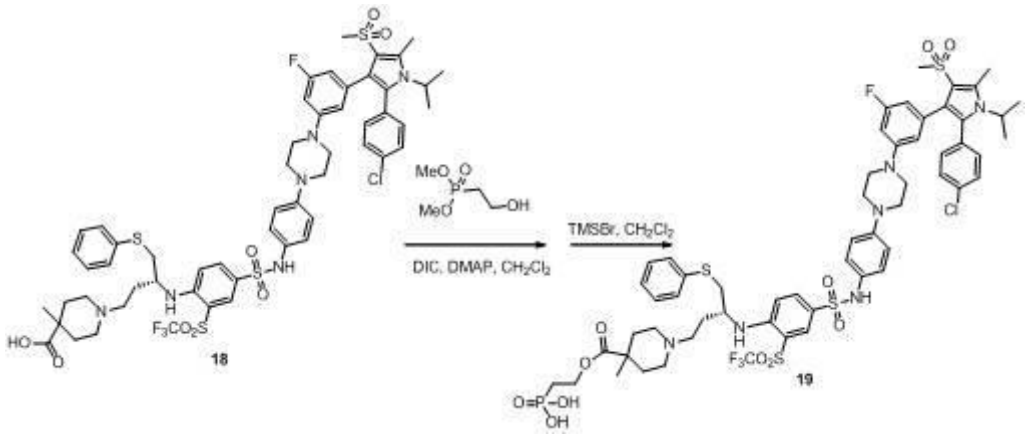


实验部分 :

(*R*)-1-(3-氨基 -4-(苯基硫代) 丁基)-4- 甲基哌啶 -4- 甲酸甲酯 (M)。M根据通用程序 II 自 4- 甲基哌啶 -4- 甲酸甲酯和 D制备。MS (ESI): *m/z* 337.55 $(M + H)^+$。

[0130] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4- 氯苯基)-1- 异丙基 -5- 甲基 -4-(甲基磺酰基)-1H- 吡咯 -3- 基)-5- 氟苯基) 哌嗪 -1- 基) 苯基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基)-4- 甲基哌啶 -4- 甲酸 (18)。向 B (100 mg, 0.11 mmol) 和 M (47 mg, 0.14 mmol) 在 DMF (2 mL) 中的溶液中加入 DIPEA (1 mL)。将溶液在室温下搅拌 4 小时,直到通过 TLC 未观察到 B。将反应混合物在真空浓缩,得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于 H_2O 和 MeOH (分别 5 mL 和 5 mL),接着加入 NaOH (88 mg, 2.2 mmol)。将溶液在室温下搅拌 20 h,直到通过 TLC 未观察到原料。将反应混合物在真空浓缩,残余物通过 HPLC 纯化得到纯的产物 18 (与 TFA 的盐, 75 mg, 经 2 步收率 58%)。梯度为在 40 min 内 60% 的溶剂 A 和 40% 的溶剂 B 至 20% 的溶剂 A 和 80% 的溶剂 B。^1H NMR (300 M Hz, CD_3OD): δ 7.99 (d, *J* = 1.6 Hz, 1H), 7.76 (dd, *J* = 9.1, 1.9 Hz, 1H), 7.37-6.84 (m, 14H), 6.68-6.45 (m, 3H), 4.55-4.45 (m, 1H), 4.02 (s, 1H), 3.58-2.92 (m, 17H), 2.88 (s, 3H), 2.77 (s, 3H), 2.41-1.86 (m, 5H), 1.47 (d, *J* = 7.1 Hz, 6H), 1.31 (s, 3H). MS (ESI): *m/z* 1173.73 $(M + H)^+$。

[0131] 方案 13 :19 的合成

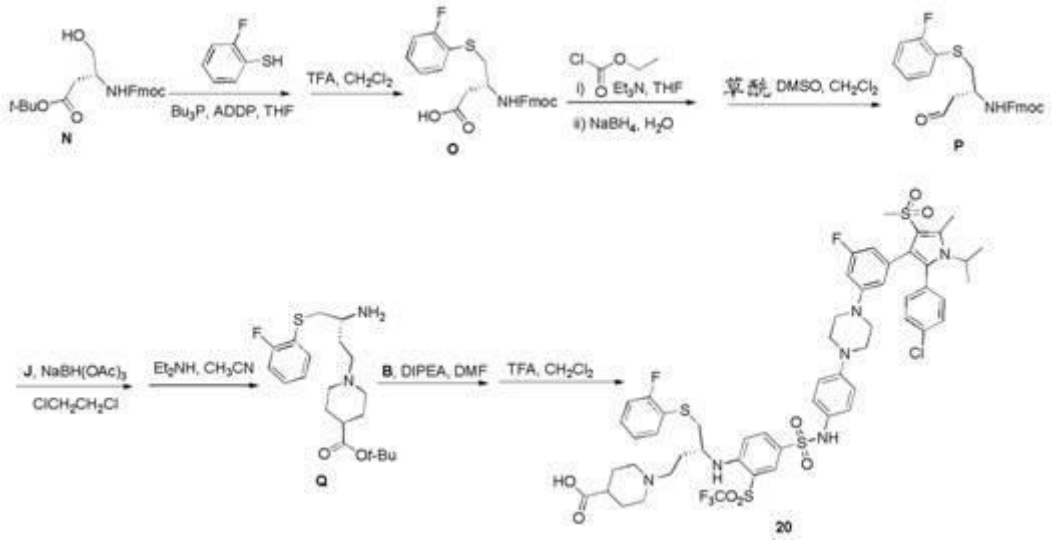


实验部分 :

(*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4- 氯苯基)-1- 异丙基 -5- 甲基 -4-(甲基磺酰基)-1H- 吡咯 -3- 基)-5- 氟苯基) 哌嗪 -1- 基) 苯基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基)-4- 甲基哌啶 -4- 羰基氧基) 乙基膦酸 (19)。19 根据

通用程序 V 自 18 和二甲基 (2- 羟基乙基) 膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.98 (d, *J* = 1.6 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.35-6.83 (m, 14H), 6.65-6.44 (m, 3H), 4.52-4.38 (m, 3H), 4.01 (s, 1H), 3.44-2.92 (m, 17H), 2.87 (s, 3H), 2.77 (s, 3H), 2.45-2.11 (m, 5H), 1.71 (t, *J* = 14.4 Hz, 2H), 1.46 (d, *J* = 7.1 Hz, 6H), 1.30 (s, 3H). MS (ESI): *m/z* 1281.92 (M + H)$^+$。

[0132] 方案 14 :化合物 20 的合成



实验部分 :

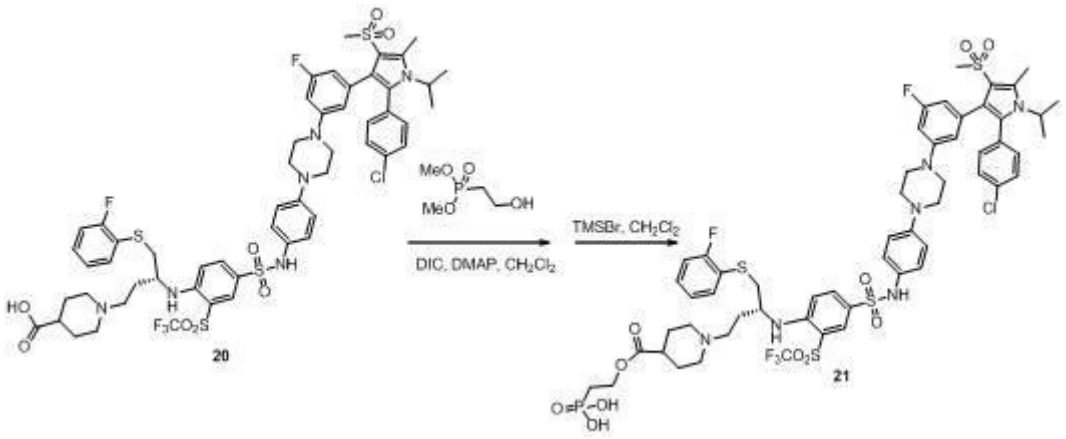
(*R*)-3-(((9H-芴 -9-基) 甲氧基) 羰基氨基)-4-(2-氟苯基硫代) 丁酸 (O)。Bu$_3$P (0.8 mL, 3.3 mmol) 和 ADDP (833 mg, 3.3 mmol) 在 THF (30 mL) 中的溶液用 N (1.2 g, 3.0 mmol) 和硫代苯酚 (320 uL, 3.0 mmol) 处理,搅拌 4 h 直到通过 TLC 未观察到 N。混合物用乙酸乙酯 (100 mL) 稀释,用 1M HCl 水溶液 (100 mL)、盐水 (100 mL) 洗涤和经硫酸钠干燥。真空除去溶剂得到粗制产物,其用于下一步骤而无需纯化。将得到的残余物溶于 DCM (10 mL),接着加入 TFA (5 mL)。将溶液在室温下搅拌 1 h,直到通过 TLC 未观察到原料。将反应混合物在真空浓缩,残余物在硅胶上用 5% MeOH/DCM 快速色谱分离,提供中间体 O (840 mg, 经 2 步收率 62%)。MS (ESI) *m/z* 452.86 (M + H)$^+$。

[0133] (*R*)-(9H- 芴 -9- 基) 甲基 1-(2- 氟苯基硫代)-4- 氧代丁 -2- 基氨基甲酸酯 (P)。P 根据通用程序 I 自 O 制备。MS (ESI) *m/z* 437.00 (M + H)$^+$。

[0134] (*R*)-1-(3- 氨基 -4-(2- 氟苯基硫代) 丁基) 哌啶 -4- 甲酸叔丁酯 (Q)。Q 根据通用程序 II 自 P 和 J 制备。MS (ESI) *m/z* 383.38 (M + H)$^+$。

[0135] (*R*)-1-(3-(4-(N-(4-(4-(3-(2-(4- 氯苯基)-1- 异丙基 -5- 甲基 -4-(甲基磺酰基)-1H- 吡咯 -3- 基)-5- 氟苯基) 哌嗪 -1- 基) 苯基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(2- 氟苯基硫代) 丁基) 哌啶 -4- 甲酸 (20)。20 根据通用程序 III 自 Q 和 B 制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.97 (d, *J* = 1.9 Hz, 1H), 7.76 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.39-6.87 (m, 13H), 6.65-6.43 (m, 3H), 4.54-4.45 (m, 1H), 4.01 (s, 1H), 3.67-2.93 (m, 17H), 2.87 (s, 3H), 2.77 (s, 3H), 2.29-1.86 (m, 6H), 1.46 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1177.92 (M + H)$^+$。

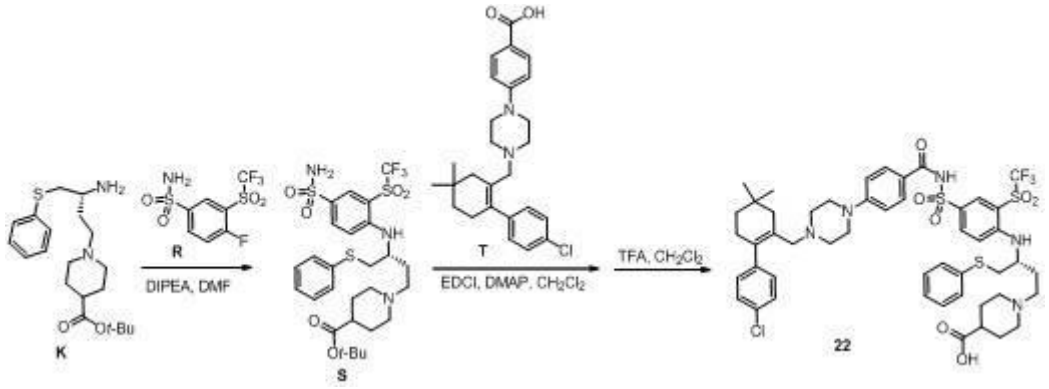
[0136] 方案 15 :21 的合成



实验部分：

(*R*)-2-(1-(3-(4-(N-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1H-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(2-氟苯基硫代)丁基)哌啶-4-羰基氧基)乙基膦酸(21)。21根据通用程序V自20和二甲基(2-羟基乙基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, *J* = 1.7 Hz, 1H), 7.77 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.36-6.86 (m, 13H), 6.66-6.44 (m, 3H), 4.51-4.33 (m, 3H), 4.01 (s, 1H), 3.58-2.93 (m, 16H), 2.85 (s, 3H), 2.74 (s, 3H), 2.70-2.58 (m, 1H), 2.27-1.84 (m, 8H), 1.43 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1286.58 $(M + H)^+$。

[0137] 方案16:22的合成



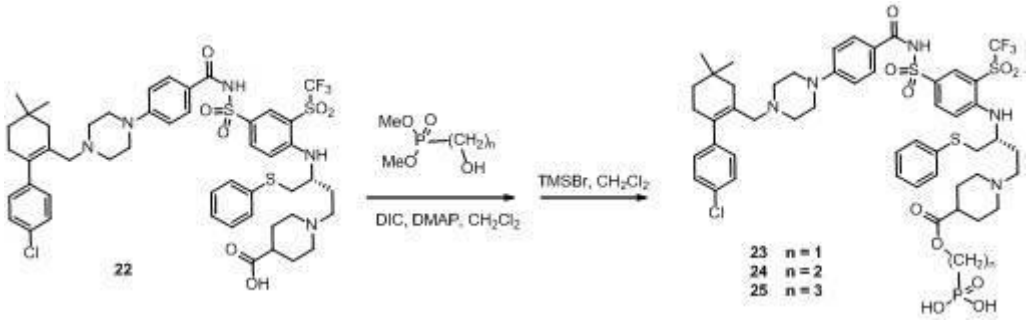
实验部分：

(*R*)-1-(4-(苯基硫代)-3-(4-氨磺酰基-2-(三氟甲基磺酰基)苯基氨基)丁基)哌啶-4-甲酸叔丁酯(S)。向K (1.1 g, 3.0 mmol)和R (922 mg, 3.0 mmol)在DMF (15 mL)中的溶液中加入DIPEA (3 mL)。将溶液在室温下搅拌4小时,直到通过TLC未观察到K。将反应混合物在真空浓缩,残余物在硅胶上用5% MeOH/DCM快速色谱分离,提供中间体S (1.7 g, 经2步收率88%)。MS (ESI) *m/z* 653.21 $(M + H)^+$。

[0138] (*R*)-1-(3-(4-(N-(4-(4-((2-(4-氯苯基)-5,5-二甲基环己-1-烯基)甲基)哌嗪-1-基)苯甲酰基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-甲酸(22)。向T (438 mg, 1.0 mmol)、EDCI (386 mg, 2.0 mmol)和DMAP (121 mg, 1.0 mmol)在DCM (10 mL)中的溶液中加入S (718 mg, 1.1 mmol)。将溶液在室温下搅拌2小时,直到通过TLC未观察到T。将反应混合物用乙酸乙酯(50 mL)稀释,用

饱和 $NaHCO_3$ 溶液 (50 mL)、盐水 (50 mL) 洗涤和经硫酸钠干燥。真空除去溶剂，得到粗制产物，其用于下一步骤而无需纯化。将得到的残余物溶于DCM (10 mL)，接着加入TFA (5 mL)。将溶液在室温下搅拌 1 h，直到通过 TLC 未观察到原料。将反应混合物在真空浓缩，残余物通过 HPLC 纯化，得到纯的产物 22 (与 TFA 的盐， 742 mg, 经 2 步收率 73%)。梯度为在 40 min 内 60% 的溶剂 A 和 40% 的溶剂 B 至 20% 的溶剂 A 和 80% 的溶剂 B。^{1}H NMR (300 M Hz, CD_3OD): δ 8.30 (d, *J* = 2.1 Hz, 1H), 8.02 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.40-6.88 (m, 12H), 4.04 (s, 1H), 3.67-2.82 (m, 19H), 2.58 (t, *J* = 14.4 Hz, 1H), 2.37-1.81 (m, 10H), 1.53 (t, *J* = 6.2 Hz, 2H), 1.03 (s, 6H). MS (ESI): *m/z* 1017.50 $(M + H)^+$。

[0139] 方案 17 :23、24、25 的合成



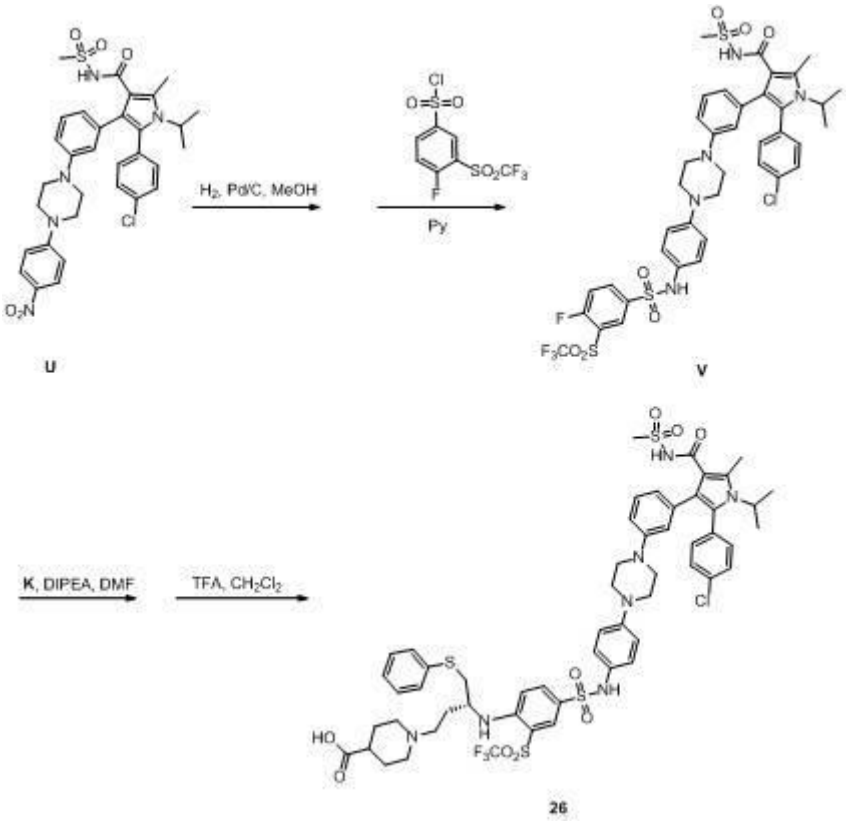
实验部分 :

(*R*)-(1-(3-(4-(N-(4-(4-((2-(4- 氯苯基)-5,5- 二甲基环己-1- 烯基) 甲基) 哌嗪-1- 基) 苯甲酰基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基) 哌啶-4- 羰基氧基) 甲基膦酸 (23)。23 根据通用程序 V 自 22 和二甲基 (2- 羟基甲基) 膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 8.35 (s, 1H), 8.09 (d, *J* = 6.7 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 2H), 7.44-6.82 (m, 12H), 4.30-4.10 (m, 3H), 3.74-2.73 (m, 19H), 2.43-1.44 (m, 12H), 1.10 (s, 6H). MS (ESI): *m/z* 1110.58 $(M + H)^+$。

[0140] (*R*)-2-(1-(3-(4-(N-(4-(4-((2-(4- 氯苯基)-5,5- 二甲基环己-1- 烯基) 甲基) 哌嗪-1- 基) 苯甲酰基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基) 哌啶-4- 羰基氧基) 乙基膦酸 (24)。24 根据通用程序 V 自 22 和二甲基 (2- 羟基乙基) 膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 8.29 (d, *J* = 2.0 Hz, 1H), 8.02 (dd, *J* = 9.2, 2.0 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.37-6.84 (m, 12H), 4.34-4.30 (m, 2H), 4.03 (s, 1H), 3.66-2.88 (m, 18H), 2.62 (t, *J* = 14.4 Hz, 1H), 2.36-1.82 (m, 12H), 1.53 (t, *J* = 6.1 Hz, 2H), 1.03 (s, 6H). MS (ESI): *m/z* 1025.64 $(M + H)^+$。

[0141] (*R*)-3-(1-(3-(4-(N-(4-(4-((2-(4- 氯苯基)-5,5- 二甲基环己-1- 烯基) 甲基) 哌嗪-1- 基) 苯甲酰基) 氨磺酰基)-2-(三氟甲基磺酰基) 苯基氨基)-4-(苯基硫代) 丁基) 哌啶-4- 羰基氧基) 丙基膦酸 (25)。25 根据通用程序 V 自 22 和二甲基 3- 羟基丙基膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.33-7.12 (m, 12H), 6.92-6.43 (m, 5H), 4.51-4.41 (m, 1H), 4.18-3.98 (m, 3H), 3.56-2.92 (m, 16H), 2.85 (s, 3H), 2.73 (s, 3H), 2.67-2.50 (m, 1H), 2.25-1.70 (m, 10H), 1.43 (d, *J* = 7.1 Hz, 6H). MS (ESI): *m/z* 1282.34 (M

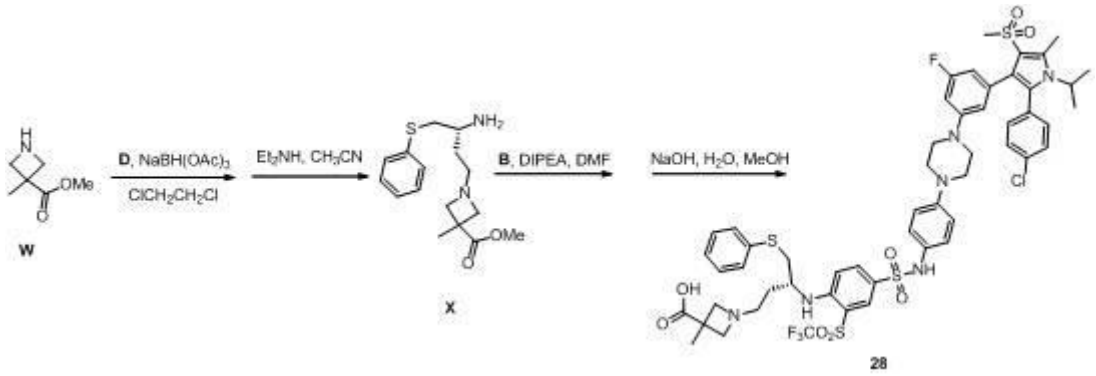
+ H)$^+$。



[0142] 5-(4-氯苯基)-4-(3-(4-(4-(4-氟-3-(三氟甲基磺酰基)苯基磺酰胺基)苯基)哌嗪-1-基)苯基)-1-异丙基-2-甲基-N-(甲基磺酰基)-1H-吡咯-3-甲酰胺(V)。V根据所述用于制备化合物B的流程自U制备。

[0143] (*R*)-1-(3-(4-(*N*-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基氨基甲酰基)-1*H*-吡咯-3-基)苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-甲酸(26)(BM-1077):26根据通用程序III自K和V制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (d, J = 1.7 Hz, 1H), 7.71 (dd, J = 2.0, 9.2 Hz, 1H), 7.39-7.28 (m, 4H), 7.26-7.14 (m, 6H), 7.09-6.96 (m, 5H), 6.93-6.85 (m, 2H), 6.81 (d, J = 9.3 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 4.41 (五重峰, J = 7.0 Hz, 1H), 4.06-3.88 (m, 1H), 3.66-3.33 (m, 8H), 3.25-2.79 (m, 10H), 2.63 (s, 3H), 2.36-1.71 (m, 8H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m*/z 1184.42 (M + H)$^+$。

[0144] (*R*)-2-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基氨基甲酰基)-1*H*-吡咯-3-基)苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)哌啶-4-羰基氧基)乙基膦酸(27)(BM-1080):27根据通用程序V自26和二甲基(2-羟基乙基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.95 (d, J = 1.9 Hz, 1H), 7.69 (dd, J = 1.8, 9.3 Hz, 1H), 7.39-7.28 (m, 4H), 7.27-7.12 (m, 6H), 7.08-6.76 (m, 8H), 6.70 (d, J = 7.5 Hz, 1H), 4.49-4.27 (m, 3H), 4.04-3.89 (m, 1H), 3.65-3.48 (m, 2H), 3.29-2.84 (m, 15H), 2.63 (s, 3H), 2.37-1.74 (m, 11H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1292.00 (M + H)$^+$。



[0145] (*R*)-1-(3-氨基-4-(苯基硫代)丁基)-3-甲基氮杂环丁烷-3-甲酸甲酯(X)。X根据通用程序II自3-甲基氮杂环丁烷-3-甲酸甲酯(W)和D制备。

[0146] (*R*)-1-(3-(4-(*N*-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1*H*-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)-3-甲基氮杂环丁烷-3-甲酸(28) (BM-1082):28根据所述用于制备化合物18的程序自X和B制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (d, J = 1.9 Hz, 1H), 7.70 (dd, J = 2.1, 9.1 Hz, 1H), 7.35-7.24 (m, 4H), 7.23-7.12 (m, 5H), 7.07-6.91 (m, 4H), 6.87 (d, J = 9.0 Hz, 1H), 6.81 (d, J = 9.3 Hz, 1H), 6.63-6.47 (m, 2H), 6.41 (d, J = 9.0 Hz, 1H), 4.55-4.38 (m, 2H), 3.97 (br. s., 3H), 3.29-3.08 (m, 13H), 2.84 (s, 3H), 2.74 (s, 3H), 2.12-1.81 (m, 2H), 1.56 (br. s., 3H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1144.75 $(M + H)^+$。

[0147] (*R*)-2-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1*H*-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)-3-甲基氮杂环丁烷-3-羰基氧基)乙基膦酸(29) (BM-1083):29根据通用程序V自28和二甲基(2-羟基乙基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (d, J =1.8 Hz, 1H), 7.72 (dd, J = 2.0, 9.1 Hz, 1H), 7.36-7.26 (m, 4H), 7.25-7.15 (m, 5H), 7.10-7.00 (m, 4H), 6.92-6.83 (m, 1H), 6.63 (s, 1H), 6.57 (d, J = 12.0 Hz, 1H), 6.42 (d, J = 9.2 Hz, 1H) 4.58-4.35 (m, 5H), 4.12-3.82 (m, 3H), 3.29-3.05 (m, 11H), 2.84 (s, 3H), 2.74 (s, 3H), 2.25-1.83 (m, 5H), 1.50 (br. s., 3H), 1.43 (d, J = 7.1 Hz, 6H). MS (ESI): *m/z* 1252.83 $(M + H)^+$。

[0148] (*R*)-3-(1-(3-(4-(*N*-(4-(4-(3-(2-(4-氯苯基)-1-异丙基-5-甲基-4-(甲基磺酰基)-1*H*-吡咯-3-基)-5-氟苯基)哌嗪-1-基)苯基)氨磺酰基)-2-(三氟甲基磺酰基)苯基氨基)-4-(苯基硫代)丁基)-3-甲基氮杂环丁烷-3-羰基氧基)丙基膦酸(30) (BM-1084):30根据通用程序V自28和二甲基(3-羟基丙基)膦酸酯制备。^{1}H NMR (300 M Hz, CD_3OD): δ 7.94 (s, 1H), 7.71 (dd, 1.5, 9.0 Hz, 1H), 7.36-7.26 (m, 4H), 7.24-7.15 (m, 5H), 7.08-6.97 (m, 4H), 6.90-6.79 (m, 2H), 6.62 (s, 1H), 6.56 (d, J = 11.8 Hz, 1H), 6.41 (d, J = 8.8 Hz, 1H), 4.54-4.37 (m, 3H), 4.33-4.21 (m, 2H), 3.99 (br. s., 3H), 3.28-3.05 (m, 11H), 2.84 (s, 3H), 2.74 (s, 3H), 2.15-1.71 (m, 7H), 1.57 (s, 3H), 1.43 (d, J = 7.0 Hz, 6H). MS (ESI): *m/z* 1266.92 $(M + H)^+$。

[0149] 用于 Bcl-2/Bcl-xL/Mcl-1蛋白的基于荧光偏振的结合测定法

灵敏和定量的基于荧光偏振 (FP) 的测定法经开发和优化以确定 Bcl-2 家族蛋白抑制剂与重组 Bcl-2、Bcl-xL 和 Mcl-1 蛋白的结合亲和力。

[0150] 确定荧光探针与蛋白的 K_d值

自制的荧光素标记的 BIM (81-106)、Bak (72-87) 和 BID (79-99) 肽,称为 Flu-BIM、Flu-BAK 和 Flu-BID,用作在 FP 测定法中分别针对 Bcl-2、Bcl-xL 和 Mcl-1 的荧光探针。通过监测由固定浓度的荧光探针和具有递增浓度直至完全饱和的蛋白组成的混合物的总荧光偏振,Flu-BIM 与 Bcl-2、Flu-BAK 与 Bcl-xL 和 Flu-BID 与 Mcl-1 的 K_d值分别确定为 0.55±0.15 nM、4.4±0.8 和 6.8±1.5 nM。使用 Infinite M-1000 多模式读板器 (Tecan U.S., Research Triangle Park, NC) 在Microfluor 2 96-孔黑色圆底板 (Thermo Scientific) 上测量荧光偏振值。向各孔中添加 1nM 的 Flu-BIM 或 2nM 的 Flu-BAK 或 2nM 的 Flu-BID 和递增浓度的 Bcl-2 或 Bcl-xL 或 Mcl-1 至在测定缓冲液 (100mM 磷酸钾, pH 7.5, 100 μg/ml 牛 γ-球蛋白, 0.02% 叠氮化钠, Invitrogen, 含 0.01% Triton X-100 和 4% DMSO) 中的 125 μl 终体积。将板在室温下孵育 2 小时,同时温和振荡以确保平衡。在激发波长为 485 nm 和发射波长为 530 nm 时测量以毫偏振单位 (mP) 计的偏振值。然后通过使用 Graphpad Prism 5.0 软件 (Graphpad Software, San Diego, CA) 拟合 S 形剂量依赖性 FP 增加作为蛋白浓度的函数,计算平衡离解常数 (K_d)。

[0151] 确定 Bcl-2家族蛋白抑制剂的 K_i值

Bcl-2 家族蛋白抑制剂与 Bcl-2/Bcl-xL/Mcl-1 蛋白的 K_i值通过抑制剂剂量依赖性竞争结合实验来确定,其中连续稀释的抑制剂针对固定浓度的荧光探针竞争结合至固定浓度的蛋白。将在 DMSO 中的 5 μl 受试抑制剂和在测定缓冲液中的 120 μl 预孵育蛋白 / 探针复合物的混合物加入至测定板并在室温下孵育 2 小时,同时温和振荡。对于 Bcl-2 测定,蛋白和探针的终浓度分别是 1.5nM 和 1nM,对于 Bcl-xL 测定为 10nM 和 2nM,和对于 Mcl-1 测定为 20nM 和 2nM。仅含蛋白 / 探针复合物的阴性对照 (等于 0% 抑制) 和仅含游离探针的阳性对照 (等于 100% 抑制) 包括在每个测定板中。如上所述测量 FP 值。通过竞争曲线的非线性回归拟合测定 IC50 值。基于得到的 IC50 值 (探针与蛋白的 K_d值) 和竞争测定中的蛋白与探针的浓度,使用前述的自推导的方程式计算抑制剂的 K_i值 (Z. Nikolovska-Coleska 等, *Analytical Biochemistry,* 2004, 332, 261-273.)。K_i值还通过使用文献中提供的另一经常使用的方程式计算 (X. Y. Huang, *Journal of Biomolecular Screening*, 2003, 8, 34-38.),其结果与我们的结果非常一致。

[0152] 细胞生长测定法

RS4;11 和 H146 细胞以 10,000 个细胞 / 孔的密度与连续稀释的化合物一起接种在 96- 孔细胞培养板中和在 37℃在 95% 空气和 5% CO_2的气氛中孵育 4 天。根据制造商的说明书,使用基于 WST-8 (2-(2- 甲氧基 -4- 硝基苯基)-3-(4- 硝基苯基)-5-(2,4- 二磺基苯基)-2H- 四唑单钠盐) 的 Cell Counting-8 Kit (Dojindo Molecular Technologies, Inc., Rockville, MD) 确定细胞成活力。简言之,将 WST-8 以终浓度 10% (v/v) 加入各孔,然后将板在 37℃孵育 1-2 小时以显色。使用 SPECTRAmax PLUS 读板器 (Molecular Devices, Sunnyvale, CA) 在 450 nm 测量吸光度。使用 GraphPad Prism 5 软件 (GraphPad Software, La Jolla, CA) 计算半最大抑制浓度 (IC_{50})。

[0153] 细胞死亡测定法

使用细胞成活力的锥虫蓝排除试验进行细胞死亡测定法。将一百万个细胞接种在6-孔板中，在37℃在95%空气和5% CO_2的气氛中与或不与化合物一起孵育指定的时间点。在处理结束时，收集细胞和在1000 rpm离心5分钟。将细胞沉淀重悬浮于PBS中，与0.4%锥虫蓝(Invitrogen)以1:1稀释混合，以使用Olympus CKX41显微镜(Olympus, Center Valley, PA)确定细胞成活力。

[0154] 细胞凋亡测定法

按照制造商的说明书，使用Annexin-V-FLUOS Staining试剂盒(Roche Diagnostics, Indianapolis, IN)进行细胞凋亡测定法。简言之，细胞用化合物处理指定的时间点，收获并用PBS洗涤。在室温下在暗室中，细胞用Annexin V-FITC和碘化丙锭染色15分钟，然后用BD Biosciences FACSCaliburs (Becton Dickinson)分析。

[0155] 蛋白印迹分析

细胞用补充蛋白酶抑制剂(α-完整, Roche)的裂解缓冲液(含1% NP40、0.5%脱氧胆酸钠和0.1% SDS的PBS)裂解。使用量热测定法(Bradford Reagent) (BioRad, Hercules, CA)定量测定蛋白提取物。蛋白在4-20% SDS-PAGE凝胶(Invitrogen)上进行电泳，然后转移到偏聚二氟乙烯膜(Bio-Rad)上。在5%牛奶中封闭后，将膜与特异性第一抗体一起孵育，洗涤，然后与辣根过氧化物酶连接的第二抗体(Pierce)一起孵育。将信号用化学发光辣根过氧化物酶抗体检测试剂(Denville Scientific)可视化。

[0156] 细胞色素 *c和* Smac释放测定法

将四百万个H146或RS4;11细胞在37℃用化合物在95%空气和5% CO_2的气氛中处理指定的时间点，用PBS洗涤和重悬浮于100 μl的毛地黄皂苷缓冲液(75 mM NaCl, 8 mM Na2HPO4, 1 mM NaH2PO4, 1 mM EDTA, 350 μg/ml毛地黄皂苷和250 mM蔗糖)中。通过在13,000 rpm离心1 min将细胞溶质级分与细胞器膜级分分离。将细胞溶质级分在12% SDS-PAGE上解析，使用抗-细胞色素c抗体(BD Biosciences)和抗-Smac (Cell Signaling Technology, Danvers, MA)抗体探测。

[0157] 具体而言，测定本发明的化合物与Bcl-2、Bcl-xL和Mcl-1的亲和力。测定结果与针对ABT-737（一种已知的专利Bcl-2/Bcl-xL抑制剂）的测定结果相比较，并与这些肽相比较。结果在表1中概述。

表 1. 与 Bcl-2、Bcl-xL 和 Mcl-1 蛋白的结合亲和力，如使用建立的基于 FP 的测定法所测定。对于每种化合物对于每种蛋白，进行 3-5 个独立实验。ABT-737、BIM、BAD 和 NOXA 肽作为对照进行测试。

化合物	结合亲和力				
	Bcl-2		Bcl-xL		Mcl-1
	IC_{50} ± SD	K_i ± SD	IC_{50} ± SD	K_i ± SD	IC_{50} ± SD
ABT-737	2 ± 0.2 (nM)	<1 (nM)	6 ± 2 (nM)	1.6 ± 0.5 (nM)	> 1 (μM)
BIM	< 1(nM)	< 1(nM)	< 1(nM)	< 1(nM)	5 ± 1 (nM)
BAD	40 ± 8(nM)	10 ± 2(nM)	5 ± 0.3(nM)	1.5 ± 0.1(nM)	32 ± 2 (μM)
NOXA	17± 1 (μM)	3.6 (μM)	11 ±2 (μM)	3.4 (μM)	37 ± 3 (μM)

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