and poor pharmacological parameters. Accordingly, the development of new ADCs that overcome these problems and can selectively deliver Bcl-xL to target cancer cells would be a significant discovery.

3. SUMMARY

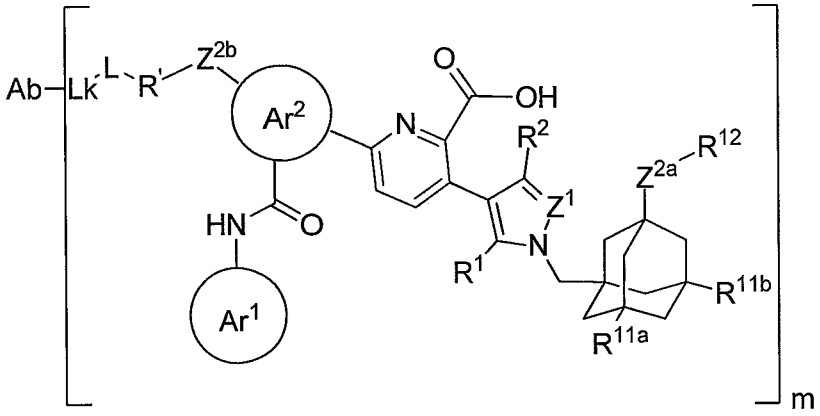
[00010] It has now been discovered that small molecule inhibitors of Bcl-xL are efficacious when administered in the form of antibody drug conjugates (ADCs; also called immunoconjugates) that bind to antigens expressed on the surface of cells where inhibition of Bcl-xL and consequent induction of apoptosis would be beneficial. This discovery provides, for the first time, the ability to target Bcl-xL inhibitory therapies to specific cells and/or tissues of interest, potentially lowering serum levels necessary to achieve desired therapeutic benefit and/or avoiding and/or ameliorating potential side effects associated with systemic administration of the small molecule Bcl-xL inhibitors *per se*.

[00011] Disclosed are ADCs comprising Bcl-xL inhibitors useful for, among other things, inhibiting anti-apoptotic Bcl-xL proteins as a therapeutic approach towards the treatment of diseases that involve a dysregulated apoptosis pathway (*e.g.*, cancer). The ADCs generally comprise small molecule inhibitors of Bcl-xL (referred to herein as Bcl-xL inhibitors) linked by way of linkers to an antibody that specifically binds an antigen expressed on a target cell of interest.

[00012] Disclosed are Bcl-xL inhibitors that have low cell-permeability. The Bcl-xL inhibitors may be used therapeutically as a component of an ADC or may be used independently from the ADCs. The Bcl-xL inhibitors described herein include solubilizing hydrophilic groups that increase water solubility and decrease the cell permeability as compared to similar inhibitors without the solubilizing groups. In certain embodiments, solubilizing group comprises a moiety capable of hydrogen bonding, dipole-dipole interactions, and/or that contains a polyol, a polyethylene glycol polymeric moiety, a salt or a moiety that is charged at physiological pH. In certain embodiments, the Bcl-xL inhibitors of the disclosure have very low cell permeability.

[00013] In embodiments where the Bcl-xL inhibitor is a component of an ADC, the use of a low cell-permeable Bcl-xL inhibitor can have benefits in that, once released from the antibody within a cell, it will have limited ability to permeate other cells and cause effects other than the intended anti-tumor effect. For instance, following internalization by ADC delivery, the Bcl-xL inhibitors of the disclosure are less likely to diffuse out of the cell than cell-permeable inhibitors, likely decreasing or

(Id)



[00031] Disclosed are intermediate synthons useful for synthesizing the ADCs described herein, as well as methods for synthesizing the ADCs. The intermediate synthons generally comprise Bcl-xL inhibitors linked to a linker moiety that includes a functional group capable of linking the synthon to an antibody. The synthons are generally compounds according to structural formula (III), below, or salts thereof, where D is a Bcl-xL inhibitor as previously described herein, L is a linker as previously described and R^x comprises a functional group capable of conjugating the synthon to a complementary functional group on an antibody:

(III) D——L——R^x

[00032] In certain specific embodiments, the intermediate synthons are compounds according to structural formulae (IIIa), (IIIb), (IIIc) and (IIId), below, or salts thereof, where the various substituents Ar^1, Ar^2, Z^1, Z^{2a}, Z^{2b}, R', R^1, R^2, R^4, R^{11a}, R^{11b}, R^{12} and R^{13} are as previously defined for structural formulae (IIa), (IIb), (IIc) and (IId), respectively, L is a linker as previously described and R^x is a functional group as described above:

[00033] To synthesize an ADC, intermediate synthons according to structural formulae (III) or (IIIa)-(IIId), or salts thereof, are contacted with an antibody of interest under conditions in which functional group R^x reacts with a complementary functional group on the antibody to form a covalent linkage. The identity of group R^x will depend upon the desired coupling chemistry and the complementary groups on the antibody to which the synthons will be attached. Numerous groups suitable for conjugating molecules to antibodies are known in the art. Any of these groups may be suitable for R^x. Non-limiting exemplary functional groups (R^x) include NHS-esters, maleimides, haloacetyls, isothiocyanates,vinyl sulfones and vinyl sulfonamides. In certain embodiments, R^x comprises a functional group selected from the group consisting of NHS-esters, maleimides, haloacetyls, and isothiocyanates.

[00034] Disclosed are compositions including the Bcl-xL inhibitors or ADCs described herein. The compositions generally comprise one or more Bcl-xL inhibitors or ADCs as described herein, and/or salts thereof, and one or more excipients, carriers or diluents. The compositions may be formulated for pharmaceutical use, or other uses. In a specific embodiment, the composition is formulated for pharmaceutical use and comprises a Bcl-xL inhibitor according to structural formula (IIa), (IIb), (IIc) or (IId), or a pharmaceutically acceptable salt thereof, where # is hydrogen. In another embodiment, the composition is formulated for pharmaceutical use and comprises an ADC according to structural formula (Ia), (Ib), (Ic) or (IIId), or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable excipients, carriers or diluents.

[00035] Bcl-xL inhibitory compositions formulated for pharmaceutical use may be packaged in bulk form suitable for multiple administrations, or may be packaged in the term of unit doses, such as for example tablets or capsules, suitable for a single administration. Likewise, ADC compositions formulated for pharmaceutical use may be packaged in bulk form suitable for multiple administrations, or may be packaged in the form of unit doses suitable for a single administration. Whether packaged in bulk or in the form of unit doses, the ADC composition may be a dry composition, such as a lyophilate, or a liquid composition. Unit dosage liquid ADC compositions may be conveniently packaged in the form of syringes pre-filled with an amount of ADC suitable for a single administration.

[00036] Disclosed are methods of inhibiting anti-apoptotic Bcl-xL proteins. The method generally involves contacting an ADC as described herein, for example, an ADC according to structural formula (Ia), (Ib), (Ic) or (Id), or a salt thereof, with a target cell that

expresses or overexpresses Bcl-xL and an antigen for the antibody of the ADC under conditions in which the antibody binds the antigen on the target cell. Depending upon the antigen, the ADC may become internalized into the target cell. The method may be carried out *in vitro* in a cellular assay to inhibit Bcl-xL activity, or *in vivo* as a therapeutic approach towards the treatment of diseases in which inhibition of Bcl-xL activity is desirable. The method may alternatively involve contacting a cell that expresses or over-expresses Bcl-xL with a Bcl-xL inhibitor, such as an inhibitor according to structural formula (IIa), (IIb), (IIc) or (IId), where # is hydrogen, or a salt thereof.

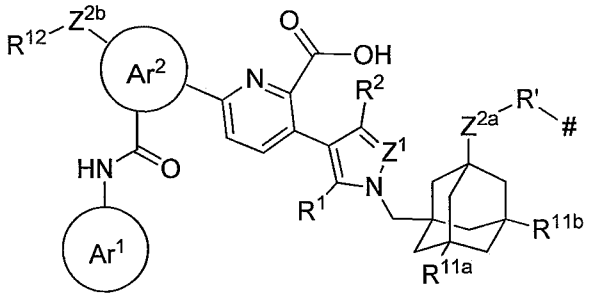
[00037] Disclosed are methods of inducing apoptosis in cells. The method generally involves contacting an ADC as described herein, for example, an ADC according to structural formula (Ia), (Ib), (Ic) or (Id), or a salt thereof, with a target cell that expresses or overexpresses Bcl-xL and an antigen for the antibody of the ADC under conditions in which the antibody binds the antigen on the target cell. Depending upon the antigen, the ADC may become internalized into the target cell. The method may be carried out *in vitro* in a cellular assay to induce apoptosis, or *in vivo* as a therapeutic approach towards the treatment of diseases in which induction of apoptosis in specific cells would be beneficial. The method may alternatively involve contacting a cell that expresses or over-expresses Bcl-xL with a Bcl-xL inhibitor, for example an inhibitor according to structural formula (IIa), (IIb), (IIc) or (IId), where # is hydrogen, or a salt thereof.

[00038] Disclosed are methods of treating disease in which inhibition of Bcl-xL and/or induction of apoptosis would be desirable. As will be discussed more thoroughly in the Detailed Description section, a wide variety of diseases are mediated, at least in part, by dysregulated apoptosis stemming, at least in part, by expression or over-expression of anti-apoptotic Bcl-xL proteins. Any of these diseases may be treated or ameliorated with the Bcl-xL inhibitors or ADCs described herein.

[00039] The methods include administering to a subject suffering from a disease mediated, at least in part by expression or over-expression of Bcl-xL, an amount of a Bcl-xL inhibitor or ADC described herein effective to provide therapeutic benefit. For ADCs, the identity of the antibody of the ADC administered will depend upon the disease being treated. The therapeutic benefit achieved with the Bcl-xL inhibitors and ADCs described herein will also depend upon the disease being treated. In certain instances, the Bcl-xL inhibitory or ADC may treat or ameliorate the specific disease when administered as monotherapy. In other instances, the Bcl-xL inhibitor or ADC may be

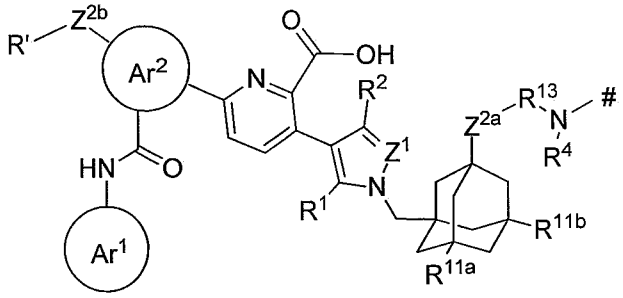
[00042] ADCs will provide a means of delivering Bcl-xL inhibitors that would be difficult to deliver in unconjugated form. Due to their low cell permeability, once inside the cell, the Bcl-xL inhibitors will be unlikely to "leak" out of the cell.

[00042a] In one aspect, there is provided a method of making an ADC, comprising contacting a synthon according to structural formula D-L-R^x, or a pharmaceutically acceptable salt thereof, wherein: **D** is a Bcl-xL inhibitor according to structural formulae (IIa), (IIb), (IIc) or (IId), or a pharmaceutically acceptable salt thereof,

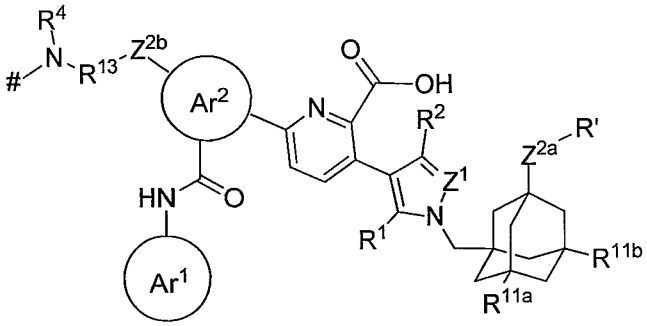


(IIa)

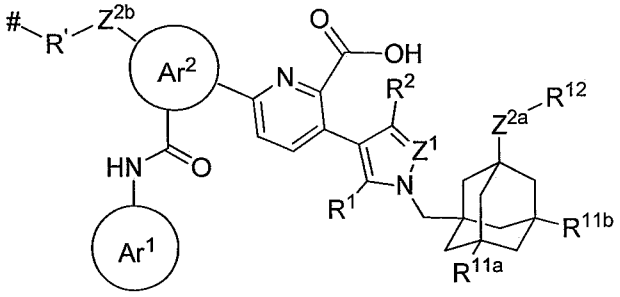
(IIb)



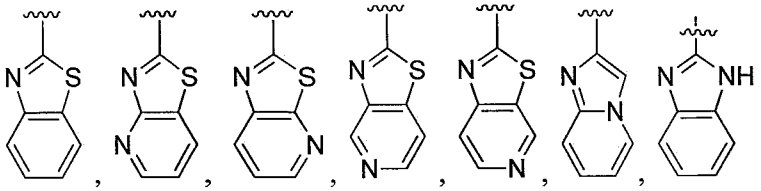
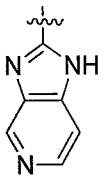
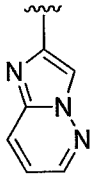
(IIc)

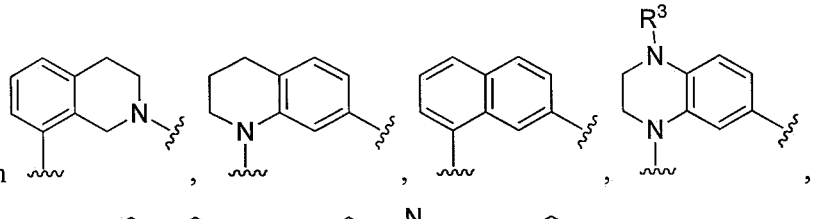
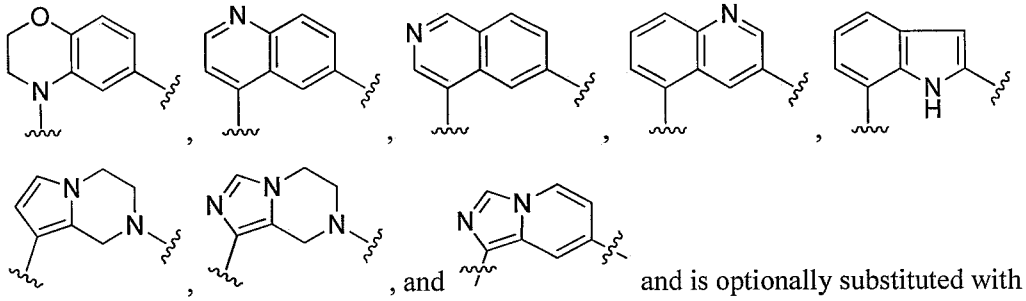


(IId)



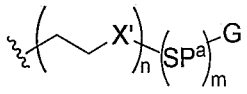
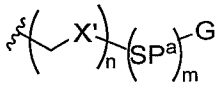
wherein:

Ar^1 is selected from   and , and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, alkoxy, amino, cyano and halomethyl;

Ar^2 is selected from   and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, alkoxy, amino, cyano and halomethyl, wherein the R^{12}-Z^{2b}-, R'-Z^{2b}-, #-N(R^4)-R^{13}-Z^{2b}-, or #-R'-Z^{2b}- substituents are attached to Ar^2 at any Ar^2 atom capable of being substituted;

Z^1 is selected from N, CH, C-halo, C-CH_3 and C-CN;

Z^{2a} and Z^{2b} are each , independently from one another, selected from a bond, NR^6, $CR^{6a}R^{6b}$, O, S, S(O), SO_2, -NR^6C(O)-,-NR^{6a}C(O)NR^{6b}-, and –NR^6C(O)O-;

R' is  or , wherein #, where attached to R', is attached to R' at any R' atom capable of being substituted;

X' is selected at each occurrence from -N(R^{10})- , -N(R^{10})C(O)-, -N(R^{10})S(O)$_2$-, -S(O)$_2$N(R^{10})-, and -O-;

n is selected from 0-3;

R^{10} is independently selected at each occurrence from hydrogen, alkyl, heterocycle, aminoalkyl, G-alkyl, heterocycle, and -$(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$-NH_2;

G at each occurrence is independently selected from a polyol, a polyethylene glycol with between 4 and 30 repeating units, a salt and a moiety that is charged at physiological pH;

SP^a is independently selected at each occurrence from oxygen, -S(O)$_2$N(H)-, -N(H)S(O)$_2$-, -N(H)C(O)-, -C(O)N(H) -, -N(H)- , arylene, heterocyclene, and optionally substituted methylene; wherein methylene is optionally substituted with one or more of –NH$(CH_2)_2$G, NH_2, alkyl, and carbonyl;

m is selected from 0-12;

R^1 is selected from hydrogen, methyl, halo, halomethyl, ethyl, and cyano;

R^2 is selected from hydrogen, methyl, halo, halomethyl and cyano;

R^3 is selected from hydrogen, methyl, ethyl, halomethyl and haloethyl;

R^4 is selected from hydrogen, lower alkyl and lower heteroalkyl or is taken together with an atom of R^{13} to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms;

R^6, R^{6a} and R^{6b} are each, independent from one another, selected from hydrogen, optionally substituted lower alkyl, optionally substituted lower heteroalkyl, optionally substituted cycloalkyl and optionally substituted heterocyclyl, or are taken together with an atom from R^4 and at atom from R^{13} to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH_3;

R^{12} is optionally R' or is selected from hydrogen, halo, cyano, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted heterocyclyl, and optionally substituted cycloalkyl;

R^{13} is selected from optionally substituted alkylene, optionally substituted heteroalkylene, optionally substituted heterocyclene, and optionally substituted cycloalkylene; and

represents the point of attachment to a linker L;

L is a linker; and

$\mathbf{R^x}$ is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,

with an antibody under conditions in which the synthon covalently links to the antibody.

4. DETAILED DESCRIPTION

[00043] The present disclosure concerns Bcl-xL inhibitors having low cell permeability, ADCs comprising the inhibitors, synthons useful for synthesizing the ADCs, compositions comprising the inhibitors or ADCs, and various methods of using the inhibitors and ADCs.

[00044] As will be appreciated by skilled artisans, the ADCs disclosed herein are "modular" in nature. Throughout the instant disclosure, various specific embodiments of the various "modules" comprising the ADCs, as well as the synthons useful for synthesizing the ADCs, are described. As specific non-limiting examples, specific embodiments of antibodies, linkers, and Bcl-xL inhibitors that may comprise the ADCs and synthons are described. It is intended that all of the specific embodiments described may be combined with each other as though each specific combination were explicitly described individually.

It will also be appreciated by skilled artisans that the various Bcl-xL inhibitors, ADCs and/or ADC synthons described herein may be in the form of salts, and in certain embodiments, particularly pharmaceutically acceptable salts. The compounds of the present disclosure that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion, e.g., a halide such as a bromide, chloride, or fluoride.

[00046] Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, *etc.* Base addition salts include those derived from inorganic bases, such as ammonium and alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like.

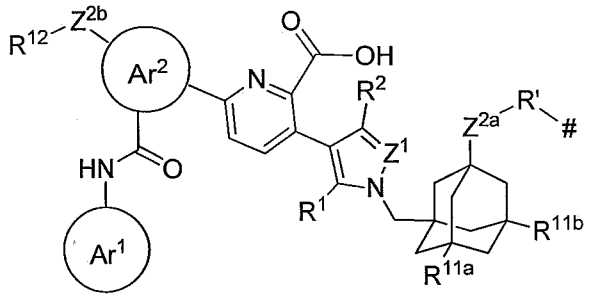
[00047] In the disclosure below, if both structural diagrams and nomenclature are included and if the nomenclature conflicts with the structural diagram, the structural diagram controls.

WHAT IS CLAIMED IS:

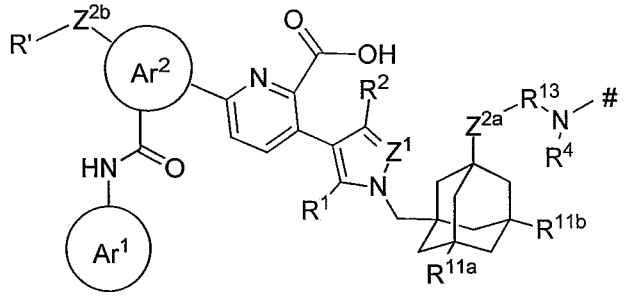
1. A method of making an ADC, comprising contacting a synthon according to structural formula D-L-R^x, or a pharmaceutically acceptable salt thereof, wherein:

D is a Bcl-xL inhibitor according to structural formulae (IIa), (IIb), (IIc) or (IId), or a pharmaceutically acceptable salt thereof,

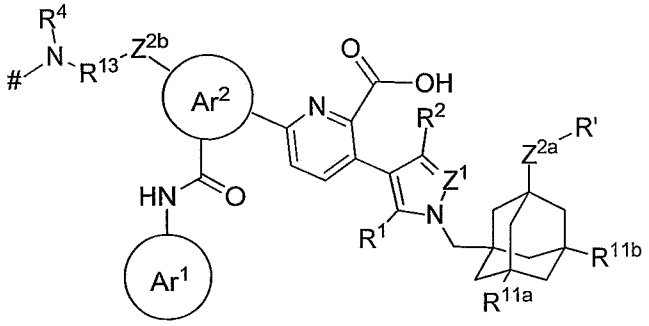
(IIa)

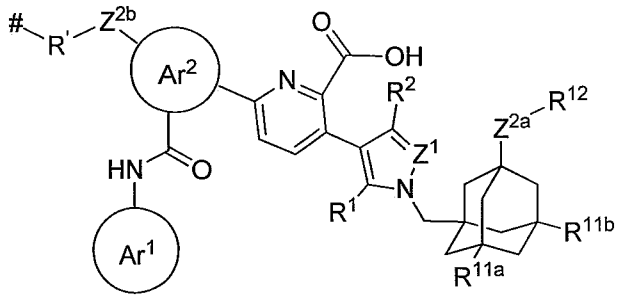


(IIb)

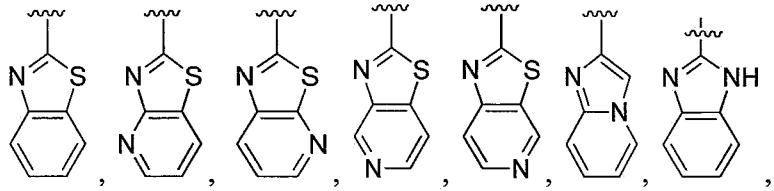
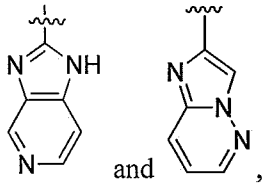


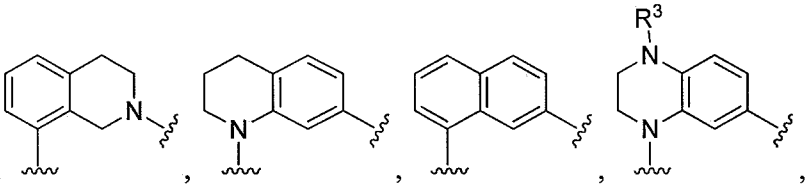
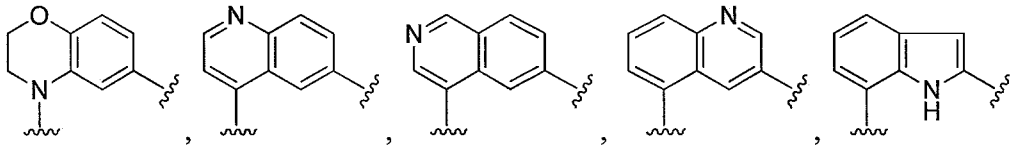
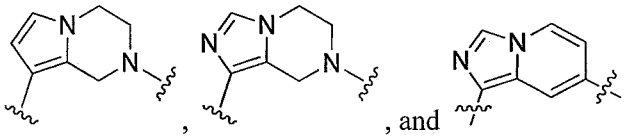
(IIc)



(IId) 

wherein:

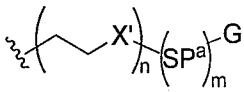
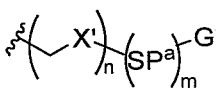
Ar^1 is selected from , and , and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, alkoxy, amino, cyano and halomethyl;

Ar^2 is selected from , , and  and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, alkoxy,

amino, cyano and halomethyl, wherein the R^{12}-Z^{2b}-, R'-Z^{2b}-, #-N(R^4)-R^{13}-Z^{2b}-, or #-R'-Z^{2b}- substituents are attached to Ar^2 at any Ar^2 atom capable of being substituted;

Z^1 is selected from N, CH, C-halo, C-CH_3 and C-CN;

Z^{2a} and Z^{2b} are each , independently from one another, selected from a bond, NR^6, $CR^{6a}R^{6b}$, O, S, S(O), SO_2, -NR^6C(O)-,-NR^{6a}C(O)NR^{6b}-, and –NR^6C(O)O-;

R' is  or  , wherein #, where attached to R', is attached to R' at any R' atom capable of being substituted;

X' is selected at each occurrence from -N(R^{10})- , -N(R^{10})C(O)-, -N(R^{10})S(O)$_2$-, -S(O)$_2$N(R^{10})-, and -O-;

n is selected from 0-3;

R^{10} is independently selected at each occurrence from hydrogen, alkyl, heterocycle, aminoalkyl, G-alkyl, heterocycle, and -$(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$-NH_2;

G at each occurrence is independently selected from a polyol, a polyethylene glycol with between 4 and 30 repeating units, a salt and a moiety that is charged at physiological pH;

SP^a is independently selected at each occurrence from oxygen, -S(O)$_2$N(H)-, -N(H)S(O)$_2$-, -N(H)C(O)-, -C(O)N(H) -, -N(H)- , arylene, heterocyclene, and optionally substituted methylene; wherein methylene is optionally substituted with one or more of –$NH(CH_2)_2$G, NH_2, alkyl, and carbonyl;

m is selected from 0-12;

R^1 is selected from hydrogen, methyl, halo, halomethyl, ethyl, and cyano;

R^2 is selected from hydrogen, methyl, halo, halomethyl and cyano;

R^3 is selected from hydrogen, methyl, ethyl, halomethyl and haloethyl;

R^4 is selected from hydrogen, lower alkyl and lower heteroalkyl or is taken together with an atom of R^{13} to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms;

R^6, R^{6a} and R^{6b} are each, independent from one another, selected from hydrogen, optionally substituted lower alkyl, optionally substituted lower heteroalkyl, optionally substituted cycloalkyl and optionally substituted heterocyclyl, or are taken together with an atom from R^4 and at atom from R^{13} to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms;

R^{11a} and R^{11b} are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH_3;

R^{12} is optionally R' or is selected from hydrogen, halo, cyano, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted heterocyclyl, and optionally substituted cycloalkyl;

R^{13} is selected from optionally substituted alkylene, optionally substituted heteroalkylene, optionally substituted heterocyclene, and optionally substituted cycloalkylene; and

represents the point of attachment to a linker L;

L is a linker; and

$\mathbf{R^x}$ is a moiety comprising a functional group capable of covalently linking the synthon to an antibody,

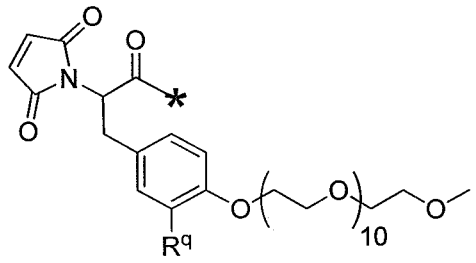
with an antibody under conditions in which the synthon covalently links to the antibody.

2. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which the linker is cleavable by a lysosomal enzyme.

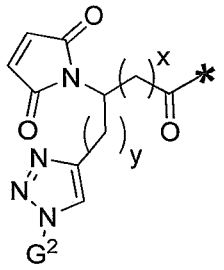
3. The method of **claim 2**, or a pharmaceutically acceptable salt thereof, in which the lysosomal enzyme is Cathepsin B.

4. The method of **claim 1** in which the linker comprises a segment according to structural formula (VIIa), (VIIb), or (VIIc):

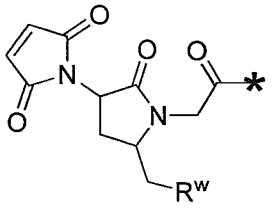
(VIIa)



(VIIb)



(VIIc)



or salts thereof, wherein:

R^q is H or $-O-(CH_2CH_2O)_{11}-CH_3$;

x is 0 or 1;

y is 0 or 1;

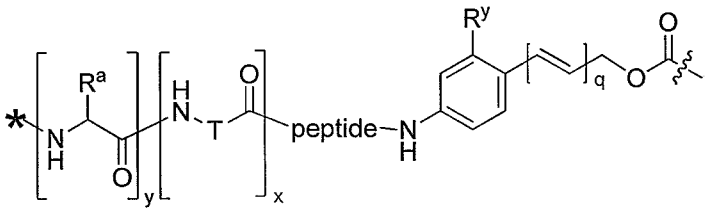
G^2 is $-CH_2CH_2CH_2SO_3H$ or $-CH_2CH_2O-(CH_2CH_2O)_{11}-CH_3$;

R^w is $-O-CH_2CH_2SO_3H$ or $-NH(CO)-CH_2CH_2O-(CH_2CH_2O)_{12}-CH_3$;

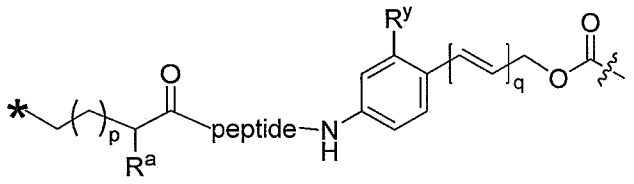
* represents the point of attachment to the remainder of the linker.

5. The method of **claim 2** in which the linker comprises a segment according to structural formula (IVa), (IVb), (IVc), or (Vd):

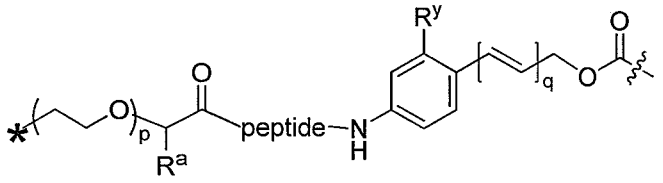
(IVa)



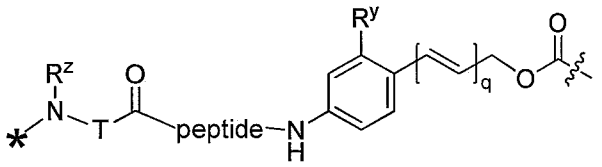
(IVb)



(IVc)



(IVd)



or a pharmaceutically acceptable salt thereof, wherein:

peptide represents a peptide (illustrated N→C, wherein peptide includes the amino and carboxy "termini") cleavable by a lysosomal enzyme;

T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof;

R^a is selected from hydrogen, alkyl, sulfonate and methyl sulfonate;

R^y is hydrogen or C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^1 or C_{1-4} alkyl-(N)-[(C_{1-4} alkylene)-G^1]$_2$;

R^z is C_{1-4} alkyl-$(O)_r$-(C_{1-4} alkylene)$_s$-G^2;

G^1 is SO_3H, CO_2H, PEG 4-32, or sugar moiety;

G^2 is SO_3H, CO_2H, or PEG 4-32 moiety;

r is 0 or 1;

s is 0 or 1;

p is an integer ranging from 0 to 5;

q is 0 or 1;

x is 0 or 1;

y is 0 or 1;

$\overset{\S}{\sim}$ represents the point of attachment of the linker to the Bcl-xL inhibitor; and

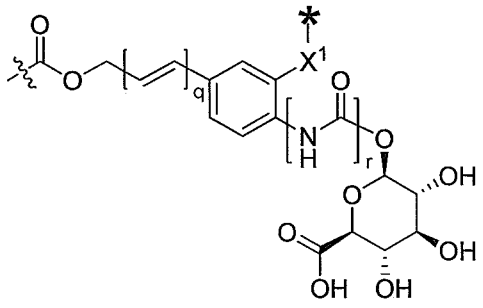
* represents the point of attachment to the remainder of the linker.

6. The method of **claim 5**, or a pharmaceutically acceptable salt thereof, in which peptide is selected from the group consisting of Val-Cit; Cit-Val; Ala-Ala; Ala-Cit; Cit-Ala; Asn-Cit; Cit-Asn; Cit-Cit; Val-Glu; Glu-Val; Ser-Cit; Cit-Ser; Lys-Cit; Cit-Lys; Asp-Cit; Cit-Asp; Ala-Val; Val-Ala; Phe-Lys; Lys-Phe; Val-Lys; Lys-Val; Ala-Lys; Lys-Ala; Phe-Cit; Cit-Phe; Leu-Cit; Cit-Leu; Ile-Cit; Cit-Ile; Phe-Arg; Arg-Phe; Cit-Trp; and Trp-Cit, and salts thereof.

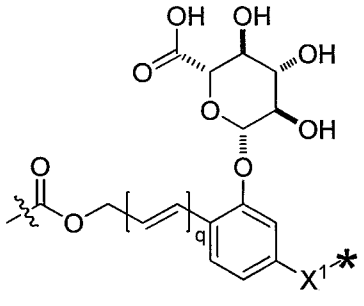
7. The method of **claim 2**, or a pharmaceutically acceptable salt thereof, in which the lysosomal enzyme is β-glucuronidase or β-galactosidase.

8. The method of **claim 7** in which the linker comprises a segment according to structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

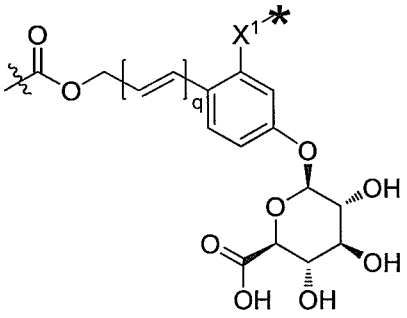
(Va)



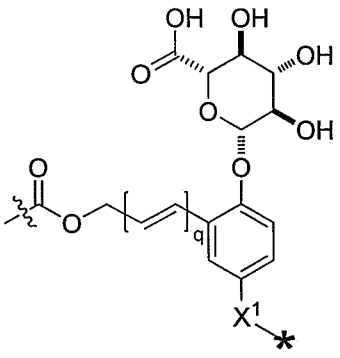
(Vb)

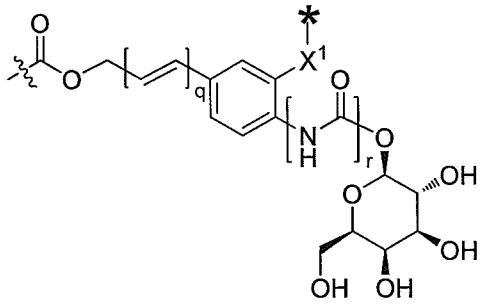


(Vc)



(Vd)



(Ve) 

or a pharmaceutically acceptable salt thereof, wherein:

q is 0 or 1;

r is 0 or 1;

X^1 is CH_2, O or NH;

$\sim\!\!\sim$ represents the point of attachment of the linker to the drug; and

* represents the point of attachment to the remainder of the linker.

9. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

10. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which linker **L** is selected from (IVa), (IVb), (IVc), (IVd) or salts thereof.

11. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which $\mathbf{R^x}$ comprises a functional group capable of linking the synthon to an amino group on an antibody.

12. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which $\mathbf{R^x}$ comprises an NHS-ester or an isothiocyanate.

13. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which $\mathbf{R^x}$ comprises a functional group capable of linking the synthon to a sulfhydryl group on an antibody.

14. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which $\mathbf{R^x}$ comprises a haloacetyl or a maleimide.

15. The method of **claim 1**, or a pharmaceutically acceptable salt thereof, in which $\mathbf{R^x}$ comprises a functional group selected from the group consisting of NHS-ester, isothiocyanate, haloacetyl and maleimide.