

These compounds have been shown to be highly useful cytotoxic agents (Howard, P.W., *et al.*, *Bioorg. Med. Chem.* (2009), 19 (22), 6463-6466, doi: 10.1016/j.bmcl.2009.09.012).

Due to the manner in which these highly potent compounds act in cross-linking DNA, these molecules have been made symmetrically. This provides for straightforward synthesis, either by constructing the PBD moieties simultaneously having already formed the dimer linkage, or by reacting already constructed PBD moieties with the dimer linking group.

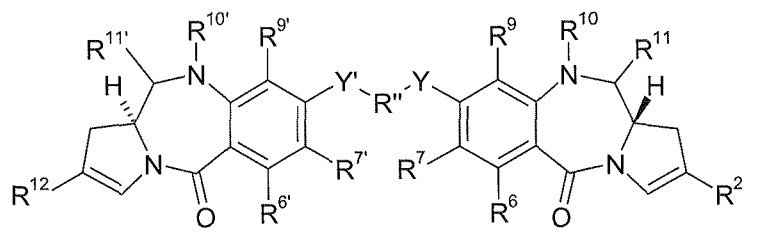
WO 2010/043880 discloses unsymmetrical dimeric PBD compound bearing aryl groups in the C2 position of each monomer, where one of these aryl groups bears a substituent designed to provide an anchor for linking the compound to another moiety. Co-pending International application PCT/US2011/032664, filed 15 April 2011, published as WO 2011/130613, discloses the inclusion of these PBD dimer compounds in targeted conjugates. Co-pending International application PCT/US2011/032668, filed 15 April 2011, published as WO 2011/130616, discloses unsymmetrical dimeric PBD compound bearing an aryl group in the C2 position of one monomer bearing a substituent designed to provide an anchor for linking the compound to another moiety, the other monomer bearing a non-aromatic group in the C2 position. The inclusion of these compounds in targeted conjugates is also disclosed.

Summary Disclosure of the invention

The present inventors have developed further unsymmetrical dimeric PBD compounds for inclusion in targeted conjugates, eliminating the need for an aryl group bearing an anchor. The C2 group bearing a substituent designed to provide an anchor for linking the compound to another moiety in the present ~~invention-disclosure~~ is a propylene group.

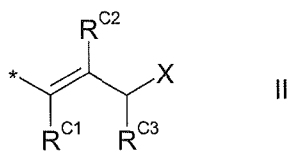
These compounds may be easier to synthesise and have advantages in their use, particularly in their biological properties and the synthesis of conjugates, and the biological properties of these conjugates.

In an aspect, there is provided a compound with the formula I:

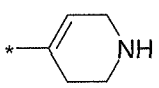
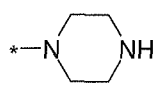


wherein:

R^2 is of formula II:



where X is selected from the group comprising: OH, SH, CO₂H, COH, N=C=O, NHHNH₂,

CONHHNH₂, , , NHR^N, wherein R^N is selected from the group comprising H and C₁₋₄ alkyl;

R^{C1}, R^{C2} and R^{C3} are independently selected from H and unsubstituted C₁₋₂ alkyl;

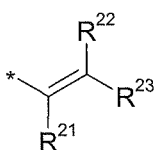
and either:

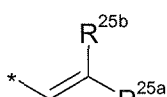
R¹² is selected from the group consisting of:

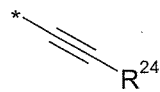
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

(ic) C₃₋₆ saturated cycloalkyl;

(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;

(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



(if) R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2 , NHR, NRR' , nitro, Me_3Sn and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2 , NHR, $NHRR'$, nitro, Me_3Sn and halo; either:

(a) R^{10} is H, and R^{11} is OH, OR^A , where R^A is C_{1-4} alkyl;

(b) R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) R^{10} is H and R^{11} is SO_2M , where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

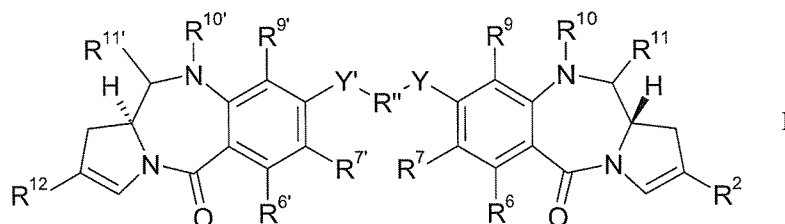
R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, and/or aromatic rings;

Y and Y' are selected from O, S, or NH;

R^6 , R^7 , R^9 are selected from the same groups as R^6 , R^7 and R^9 respectively and $R^{10'}$ and

$R^{11'}$ are the same as R^{10} and R^{11} , wherein if R^{11} and $R^{11'}$ are SO_2M , M may represent a divalent pharmaceutically acceptable cation.

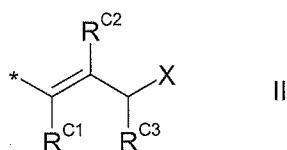
The present invention-disclosure comprises a compound with the formula I:



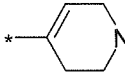
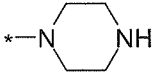
or a pharmaceutically acceptable salt or solvate thereof,

wherein:

5 R^2 is of formula II:



where X is selected from the group comprising: OH, SH, CO₂H, COH, N=C=O, NHNH₂,

CONHNH₂, , , NHR^N, wherein R^N is selected from the group comprising H and C₁₋₄ alkyl;

10 R^{C1} , R^{C2} and R^{C3} are independently selected from H and unsubstituted C₁₋₂ alkyl;

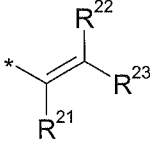
and either:

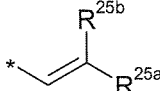
R^{12} is selected from the group consisting of:

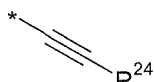
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

(ic) C₃₋₆ saturated cycloalkyl;

(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;

(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and



(if) R^{24} , where R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2 , NHR, NRR' , nitro, Me_3Sn and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2 , NHR, $NHRR'$, nitro, Me_3Sn and halo; either:

(a) R^{10} is H, and R^{11} is OH, OR^A , where R^A is C_{1-4} alkyl; or

(b) R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) R^{10} is H and R^{11} is SO_zM , where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

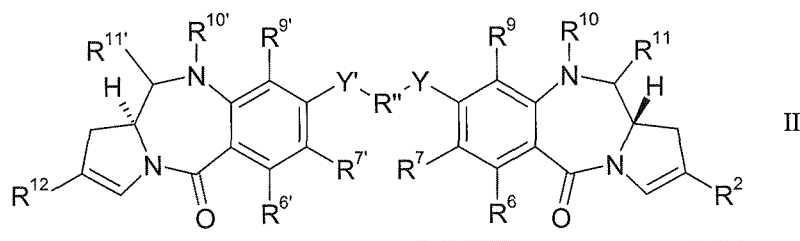
Y and Y' are selected from O, S, or NH;

R^6 , R^7 , R^9 are selected from the same groups as R^6 , R^7 and R^9 respectively and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11} , wherein if R^{11} and $R^{11'}$ are SO_2M , M may represent a divalent pharmaceutically acceptable cation.

A second aspect of the present ~~invention-disclosure~~ provides the use of a compound of the first aspect of the ~~invention-disclosure~~ in the manufacture of a medicament for treating a proliferative disease. ~~The second~~ Another aspect also provides a compound of the first aspect of the ~~invention-disclosure~~ for use in the treatment of a proliferative disease.

One of ordinary skill in the art is readily able to determine whether or not a candidate conjugate treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

In a third aspect, there is provided a compound of formula II:



wherein:

R^2 , R^6 , R^7 , R^9 , Y , R'' , Y' , R^{12} , $R^{6'}$, $R^{7'}$ and $R^{9'}$ are as defined for a compound of formula I

herein;

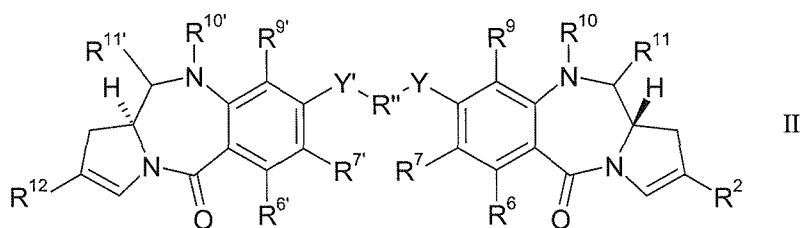
and either:

(a) R^{10} is carbamate nitrogen protecting group, and R^{11} is $O\text{-Prot}^O$, wherein Prot^O is an oxygen protecting group;

(b) R^{10} is a hemi-aminal nitrogen protecting group and R^{11} is an oxo group;

and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11} .

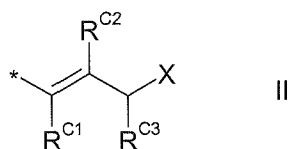
A third aspect embodiment of the present invention disclosure comprises a compound of formula II:



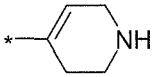
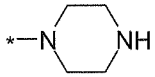
or a pharmaceutically acceptable salt or solvate thereof,

wherein:

R² is of formula II:



where X is selected from the group comprising: OH, SH, CO₂H, COH, N=C=O, NHNH₂,

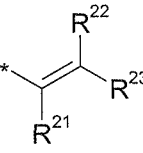
CONHNH₂, , , NHR^N, wherein R^N is selected from the group comprising H and C₁₋₄ alkyl, and either:

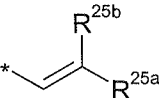
R¹² is selected from the group consisting of:

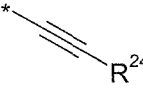
(ia) C₅₋₁₀ aryl group, optionally substituted by one or more substituents selected from the group comprising: halo, nitro, cyano, ether, C₁₋₇ alkyl, C₃₋₇ heterocyclyl and bis-oxy-C₁₋₃ alkylene;

(ib) C₁₋₅ saturated aliphatic alkyl;

(ic) C₃₋₆ saturated cycloalkyl;

(id) , wherein each of R²¹, R²² and R²³ are independently selected from H, C₁₋₃ saturated alkyl, C₂₋₃ alkenyl, C₂₋₃ alkynyl and cyclopropyl, where the total number of carbon atoms in the R¹² group is no more than 5;

(ie) , wherein one of R^{25a} and R^{25b} is H and the other is selected from: phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl; and

(if)  R^{24} , where R^{24} is selected from: H; C_{1-3} saturated alkyl; C_{2-3} alkenyl; C_{2-3} alkynyl; cyclopropyl; phenyl, which phenyl is optionally substituted by a group selected from halo, methyl, methoxy; pyridyl; and thiophenyl;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2 , NHR, NRR' , nitro, Me_3Sn and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2 , NHR, $NHRR'$, nitro, Me_3Sn and halo; either:

(a) R^{10} is carbamate nitrogen protecting group, and R^{11} is $O-Prot^O$, wherein $Prot^O$ is an oxygen protecting group; or

(b) R^{10} is a hemi-aminal nitrogen protecting group and R^{11} is an oxo group;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

R^6 , R^7 , R^9 are selected from the same groups as R^6 , R^7 and R^9 respectively and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11} .

A fourth ~~aspect-embodiment~~ of the present ~~invention-disclosure~~ comprises a method of making a compound of formula I, or a pharmaceutically acceptable salt or solvate thereof, from a compound of formula II, or a pharmaceutically acceptable salt or solvate thereof, by deprotection of the imine bond.

The unsymmetrical dimeric PBD compounds of the present ~~invention-disclosure~~ are made by different strategies to those previously employed in making symmetrical dimeric PBD compounds. In particular, the present inventors have developed a method which involves adding each each C2 substituent to a symmetrical PBD dimer core in separate method steps. Accordingly, a fifth ~~aspect-embodiment~~ of the present ~~invention-disclosure~~ provides a method of making a compound of the first or third aspect of the ~~invention-disclosure~~, comprising at least one of the method steps set out below.

In an ~~embodiment~~ ~~sixth aspect~~, the present ~~invention~~ ~~disclosure~~ relates to Conjugates comprising dimers of PBDs linked to a targeting agent, wherein the PBD dimer is of formula I, or a pharmaceutically acceptable salt or solvate thereof (supra).

5 In an aspect, there is provided a Conjugate having formula III:



wherein L is a Ligand unit, LU is a Linker unit, p is 1 to 20; and D is a Drug unit which is a PBD dimer as disclosed herein, wherein LU is connected to D via the X substituent of R².

10 In another aspect, there is provided the use of a Conjugate of the aspect above, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease.

In some embodiments, the Conjugates have the following formula III:



or a pharmaceutically acceptable salt or solvate thereof, wherein L is a Ligand unit (i.e., a targeting agent), LU is a Linker unit and D is a Drug unit that is a PBD dimer (see below).

The subscript p is an integer of from 1 to 20. Accordingly, the Conjugates comprise a Ligand unit covalently linked to at least one Drug unit by a Linker unit. The Ligand unit,

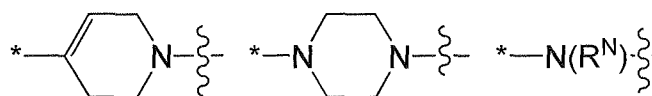
20 described more fully below, is a targeting agent that binds to a target moiety. The Ligand unit can, for example, specifically bind to a cell component (a Cell Binding Agent) or to

other target molecules of interest. Accordingly, the present ~~invention~~ ~~disclosure~~ also provides methods for the treatment of, for example, various cancers and autoimmune

disease. These methods encompass the use of the Conjugates wherein the Ligand unit is

25 a targeting agent that specifically binds to a target molecule. The Ligand unit can be, for example, a protein, polypeptide or peptide, such as an antibody, an antigen-binding fragment of an antibody, or other binding agent, such as an Fc fusion protein.

30 In conjugates of the present ~~invention~~ ~~disclosure~~, the PBD dimer D is of formula I, or a pharmaceutically acceptable salt or solvate thereof, except that X is selected from the group comprising: *-O⁺-, *-S⁺-, *-CO₂⁺-, *-CO⁺-, *-NH(C=O)-⁺, *-NHNH⁺-, *-CONHNH⁺-,

, wherein R^N is selected from the group comprising H and C₁₋₄ alkyl, and the asterix indicates the point of attachment to the

remainder of the Drug unit and the wavy line or ⁺ indicates the point of attachment to the Linker Unit.

5 The drug loading is represented by p, the number of drug molecules per Ligand unit (e.g., an antibody). Drug loading may range from 1 to 20 Drug units (D) per Ligand unit (e.g., Ab or mAb). For compositions, p represents the average drug loading of the Conjugates in the composition, and p ranges from 1 to 20.

10 In some embodiments, p is from about 1 to about 8 Drug units per Ligand unit. In some embodiments, p is 1. In some embodiments, p is 2. In some embodiments, p is from

about 2 to about 8 Drug units per Ligand unit. In some embodiments, p is from about 2 to about 6, 2 to about 5, or 2 to about 4 Drug units per Ligand unit. In some embodiments, p is about 2, about 4, about 6 or about 8 Drug units per Ligand unit.

The average number of Drugs units per Ligand unit in a preparation from a conjugation reaction may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Conjugates in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous Conjugates, where p is a certain value, from Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

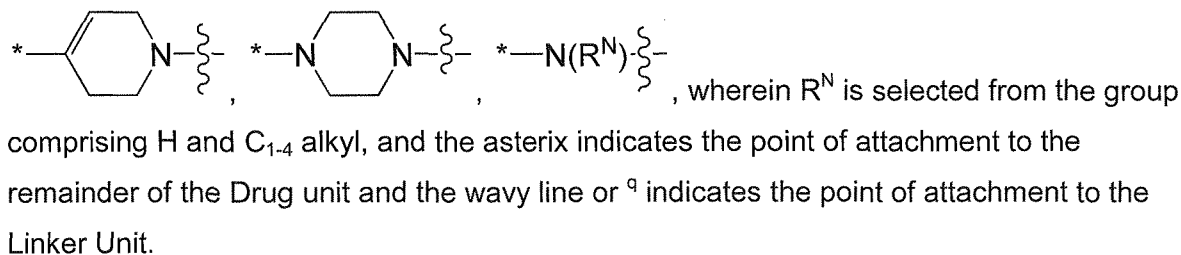
~~In a seventh aspect aspect embodiment, the present invention-disclosure~~ relates to Linker-Drug compounds (i.e., Drug-Linkers) comprising dimers of PBDs (see above) linked to a linking unit. These Drug-linkers can be used as intermediates for the synthesis of Conjugates comprising dimers of PBDs linked to a targeting agent.

~~In an aspect, there is provided a These Drug-Linkers have of~~ the following formula V:



or a pharmaceutically acceptable salt or solvate thereof, wherein LU is a Linker unit and D is a Drug unit that is a PBD dimer as disclosed herein.

In the Drug-Linkers of the present ~~invention~~disclosure, the PBD dimer D is of formula I, or a pharmaceutically acceptable salt or solvate thereof, except that X is selected from the group comprising: $^*\text{-O}^q$, $^*\text{-S}^q$, $^*\text{-CO}_2^q$, $^*\text{-CO}^q$, $^*\text{-NH(C=O)}^q$, $^*\text{-NHNH}^q$, $^*\text{-CONHNH}^q$,



Definitions

Pharmaceutically acceptable cations

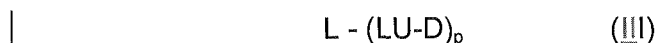
Examples of pharmaceutically acceptable monovalent and divalent cations are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977), which is incorporated herein by reference.

37. A compound according to claim 36, wherein R¹⁰ is Troc.

38. A compound according to either claim 36 or claim 37, wherein R¹¹ is OTBS.

5 39. A compound according to 36, wherein R¹¹ is oxo and R¹⁰ is SEM.

40. A Conjugate having formula III:



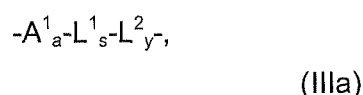
wherein L is a Ligand unit,

10 LU is a Linker unit,

p is 1 to 20; and

D is a Drug unit which is a PBD dimer according to any one of claims 1 to 33,
wherein LU is connected to D via the X substituent of R².

15 41. The Conjugate according to claim 40, wherein the Linker unit (LU) has the formula IIIa or IIIb:



wherein:

20 -A¹- is a Stretcher unit,

a is 1 or 2,

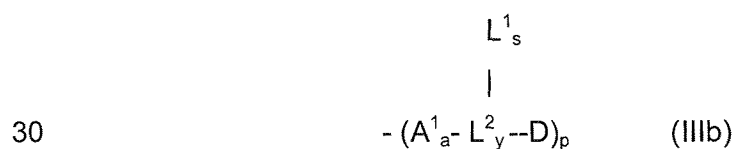
L¹- is a Specificity unit,

s is an integer ranging from 0 to 12,

-L²- is a Spacer unit, and

25 y is 0, 1 or 2, and

p is from 1- 20; or

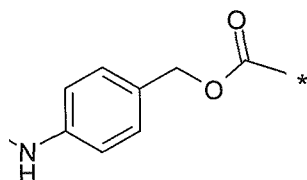


wherein:

-A¹- is a Stretcher unit linked to a Stretcher unit (L²),

a is 1 or 2,

35 L¹- is a Specificity unit linked to a Stretcher unit (L²),



53. The use of a Conjugate of according to any one of claims 40 to 52, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease.

~~54. The use of a Conjugate according to any one of the claims 40 to 52 for treating a proliferative disease or an autoimmune disease.~~

~~55. A method of treating a mammal having a proliferative disease or an autoimmune disease, comprising administering an effective amount of the Conjugate of any one of claims 40 to 52.~~

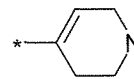
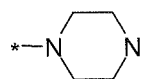
~~56~~54. A drug linker of formula V:

LU-D (V)

or a pharmaceutically acceptable salt or solvate thereof, wherein LU is a Linker unit and

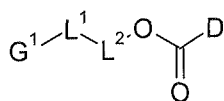
where D is a Drug unit which is a PBD dimer according to any one of claims 1 to 33, but

where X is selected from O, S, CO₂, CO, NH(C=O), NHHH, CONHHH,



, NR^N, wherein R^N is selected from the group comprising H and C₁₋₄ alkyl.

~~57~~55. A drug linker according to claim ~~56~~54 of formula:



(a) , where G¹ is a Stretcher group to form a connection to a Ligand unit, L¹ is a Specificity unit, L² is a covalent bond or together with -OC(=O)- forms a self-immolative group; or

(b) G¹-L¹-L²-D, where G¹ is a Stretcher unit to form a connection to a Ligand unit, L¹ is a Specificity unit, L² is a covalent bond or a self-immolative group;

where L¹ and L² are as defined in any one of claims 41 to 52.