CLAIMS

1 A peptide ligand specific for MT1-MMP comprising a polypeptide comprising three residues selected from cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap) and N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), the said three residues being separated by at least two loop sequences, and a molecular scaffold, the peptide being linked to the scaffold by covalent alkylamino linkages with the Dap or N-AlkDap or N-HAlkDap residues of the polypeptide and by thioether linkages with the cysteine residues of the polypeptide when the said three residues include cysteine, such that two polypeptide loops are formed on the molecular scaffold, wherein the peptide ligand comprises an amino acid sequence of formula (II):

- A_1-X_1-U/O_2-X_3-X_4-G_5-A_2-E_6-D_7-F_8-Y_9-X_{10}-X_{11}-A_3- (SEQ ID NO: 1)

(II)

or a pharmaceutically acceptable salt thereof;

wherein:

A_1, A_2, and A_3 are independently cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap), or N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), provided that at least one of A_1, A_2, and A_3 is Dap, N-AlkDap or N-HAlkDap;

X represents any amino acid residue;

U represents a polar, uncharged amino acid residue selected from N, C, Q, M, S and T; and

O represents a non-polar aliphatic amino acid residue selected from G, A, I, L, P and V.

2. The peptide ligand as defined in claim 1, wherein:

X_1 is selected from any one of the following amino acids: Y, M, F or V, such as Y, M or F, in particular, Y or M, more particularly Y; and/or

U/O_2 is selected from a U, such as an N, or an O, such as a G; and/or

X_3 is selected from U or Z, wherein U represents a polar, uncharged amino acid residue selected from N, C, Q, M, S and T and Z represents a polar, negatively charged amino acid residue selected from D or E, in particular the U at position 3 is selected from Q or the Z at position 3 is selected from E; and/or

X_4 is selected from J, wherein J represents a non-polar aromatic amino acid residue selected from F, W and Y; and/or

X_{10} is selected from Z, wherein Z represents a polar, negatively charged amino acid residue selected from D or E, such as D; and/or

X_{11} is selected from O, wherein O represents a non-polar aliphatic amino acid residue selected from G, A, I, L, P and V, such as I.

3. The peptide ligand as defined in any preceding claim, wherein the Bicycle of formula (II) is a compound of formula (IIa):

- A_1-Y/M/F/V-U/O-U/Z-J-G-A_2-E-D-F-Y-Z-O-A_3- (SEQ ID NO: 6) (IIa)

wherein U, O, J and Z are as defined hereinbefore; or

a compound of formula (IIb):

- A_1-Y/M/F/V-N/G-E/Q-F-G-A_2-E-D-F-Y-D-I-A_3- (SEQ ID NO: 7) (IIb); or

a compound of formula (IIc):

- A_1-Y/M/F-N/G-E/Q-F-G-A_2-E-D-F-Y-D-I-A_3- (SEQ ID NO: 8) (IIc); or

a compound of formula (IId):

- A_1-Y/M-N-E/Q-F-G-A_2-E-D-F-Y-D-I-A_3- (SEQ ID NO: 9) (IId); or

a compound of formula (IIe):

- A_1-Y-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-07) (SEQ ID NO: 2) (IIe).

4. The peptide ligand as defined in any preceding claim, wherein the Bicycle of formula (II) comprises a sequence selected from:

- A_1-Y-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-07) (SEQ ID NO: 2);
- A_1-M-N-Q-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-12) (SEQ ID NO: 10);
- A_1-F-G-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-02) (SEQ ID NO: 11);
- A_1-V-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-03) (SEQ ID NO: 12);
- A_1-F-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-04) (SEQ ID NO: 13);
- A_1-Y-N-E-Y-G-A_2-E-D-F-Y-D-I-A_3- (17-69-07-N057) (SEQ ID NO: 14); and
- A_1-Y-N-E-W-G-A_2-E-D-F-Y-D-I-A_3- (17-69-44-N002) (SEQ ID NO: 15),

such as:

- A_1-Y-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-07) (SEQ ID NO: 2); and
- A_1-M-N-Q-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-12) (SEQ ID NO: 10),

in particular:

- A_1-Y-N-E-F-G-A_2-E-D-F-Y-D-I-A_3- (17-69-07) (SEQ ID NO: 2)~~,~~

~~most particularly:~~

~~the Dap homologue designated as SEQ ID 16 ((bAla)-Sar10-AA_1(D-Ala)NE(1Nal)(D-Ala)A_2EDFYD(tBuGly)A_3-;~~

~~and the Dap homologue designated as SEQ ID 17 AA_1(D-Ala)NE(1Nal)(D-Ala)A_2EDFYD(tBuGly)A_3-~~.

5. The peptide ligand as defined in any preceding claim, wherein two of A_1, A_2 and A_3 are selected from Dap, N-AlkDap or N-HAlkDap, and the third one of A_1, A_2 and A_3 is cysteine, preferably wherein A_2 is cysteine.

6. The peptide ligand as defined in any of claims 1 to 4, wherein A_1, A_2 and A_3 are each N-AlkDap or N-HAlkDap~~;~~.

~~7. The peptide ligand as defined in any preceding claim, wherein one or more tyrosine residues is replaced by a phenylalanine residue.~~

~~8~~7. The peptide ligand as defined in any preceding claim, which additionally comprises one or more modifications selected from: N-terminal and/or C-terminal modifications; ~~replacement of one or more amino acid residues with one or more non-natural amino acid residues (such as replacement of one or more polar amino acid residues with one or more isosteric or isoelectronic amino acids; replacement of one or more hydrophobic amino acid residues with other non-natural isosteric or isoelectronic amino acids); replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues;~~ replacement of one or more L-amino acid residues with one or more D-amino acid residues; substitution of the hydrogen on the α-carbon of one or more amino acid residues with another chemical group, and post-synthetic bioorthogonal modification of amino acids such as cysteine, lysine, glutamate and tyrosine with suitable amine, thiol, carboxylic acid and phenol-reactive reagents.

~~9~~8. The peptide ligand as defined in claim 8, which comprises an N-terminal modification using suitable amino-reactive chemistry, and/or C-terminal modification using suitable carboxy-reactive chemistry; and/or

wherein the N-terminal modification comprises the addition of a molecular spacer group which facilitates the conjugation of effector groups and retention of potency of the bicyclic peptide to its target, such as an Ala, G-Sar10-A group or bAla-Sar10-A group; and/or

wherein said N-terminal and/or C-terminal modification comprises addition of a cytotoxic agent~~; and/or~~

~~which comprises a modification at amino acid position 1 and/or 9~~.

~~10. The peptide ligand as defined in claim 8 or 9, which comprises replacement of one or more amino acid residues with one or more non-natural amino acid residues.~~

~~11. The peptide ligand as defined in claim 10, wherein the non-natural amino acid residue is substituted at position 4 and is selected from: 1-naphthylalanine; 2-naphthylalanine; 3,4-dichlorophenylalanine; and homophenylalanine, such as 1-naphthylalanine; 2-naphthylalanine; and 3,4-dichlorophenylalanine, in particular 1-naphthylalanine.~~

~~12. The peptide ligand as defined in claim 10 or claim 11, wherein the non-natural amino acid residue is substituted at position 9 and/or 11 and is selected from: 4-bromophenylalanine or pentafluoro-phenylalanine for position 9 and/or tert-butylglycine for position 11; preferably wherein the non-natural amino acid residues, such as those present at position 9, is selected from: 4-bromophenylalanine, or the non-natural amino acid residues, such as those present at position 11, is selected from: tert-butylglycine.~~

~~13. The peptide ligand as defined in claim 8, wherein the amino acid residue at position 1 is substituted for a D-amino acid, such as D-alanine, wherein the amino acid residue at position 5 is substituted for a D-amino acid, such as D-alanine or D-arginine~~

~~14. The peptide ligand as defined in claim 10, which comprises a plurality of the above mentioned modifications, such as 2, 3, 4 or 5 or more of the following modifications, such as all of the following 5 modifications: D-alanine at position 1 and/or 5, a 1-naphthylalanine at position 4, a 4-bromophenylalanine at position 9 and a tert-butylglycine at position 11.~~

~~15~~9. The peptide ligand as defined in any one of claims 1 to ~~14~~8, wherein the Bicycle of formula (II) is a high affinity binder of the human, mouse and dog MT1-MMP hemopexin domain.

~~16~~10. The peptide ligand as defined in any one of claims 1 to ~~15~~9, wherein the Bicycle of formula (II) is selective for MT1-MMP, but does not cross-react with MMP-1, MMP-2, MMP-15 and MMP-16.

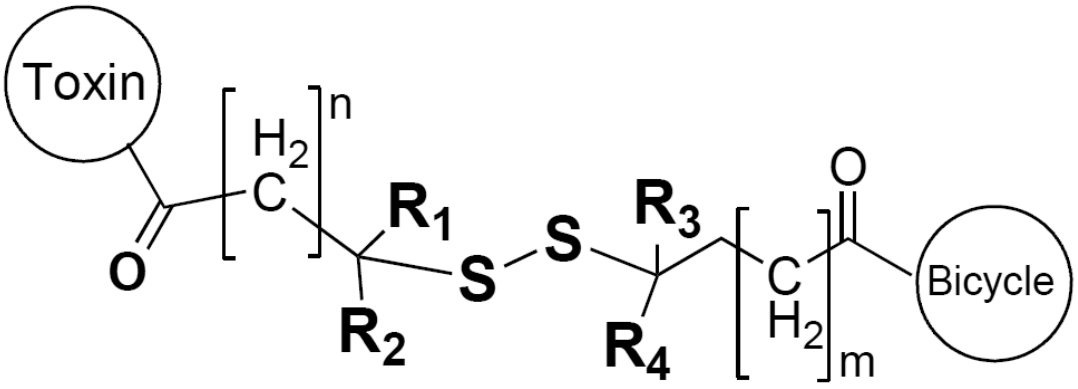
~~17~~11. A linear peptide comprising an amino acid sequence of formula (II) as defined in any of claims 1 to ~~14~~8.

~~18~~12. A method of making a peptide ligand according to any of claims 1 to ~~16~~10, the method comprising: providing a peptide according to claim 17; providing a scaffold molecule having at least three reactive sites for forming thioether and alkylamino linkages with the side chain –SH and amino

groups of the cysteine and diaminopropionic acid or β-N-Alkyldiaminopropionic acid residues of the peptide; and forming said thioether and alkylamino linkages between the peptide and the scaffold molecule.

~~19~~13. A drug conjugate comprising a peptide ligand as defined in any one of claims 1 to ~~16~~10, conjugated to one or more effector and/or functional groups, preferably wherein the effector and/or functional groups comprises a cytotoxic agent or a metal chelator or a cytotoxic agent such as DM1 or MMAE, preferably wherein the cytotoxic agent is linked to the bicyclic peptide by a cleavable bond, such as a disulphide bond.

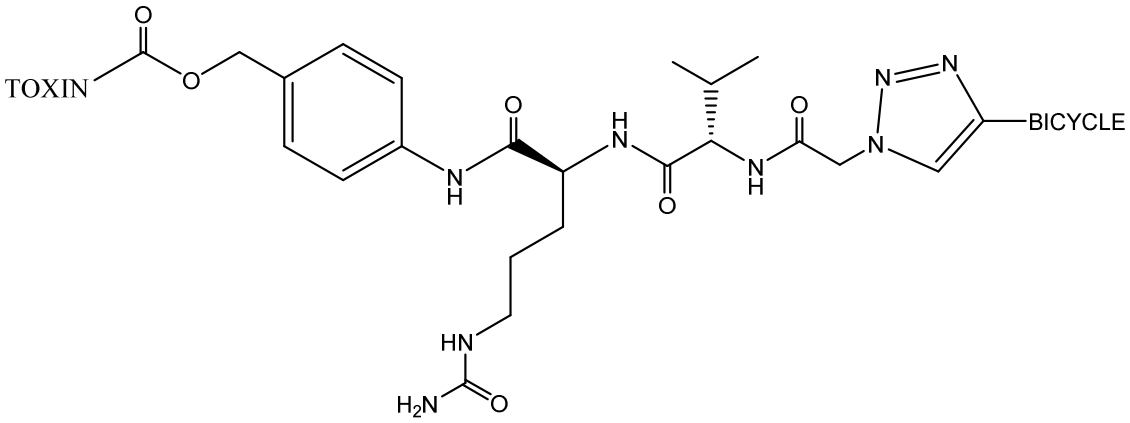
~~20~~14. The drug conjugate as defined in claim ~~19~~13, wherein the drug conjugate has the following structure:



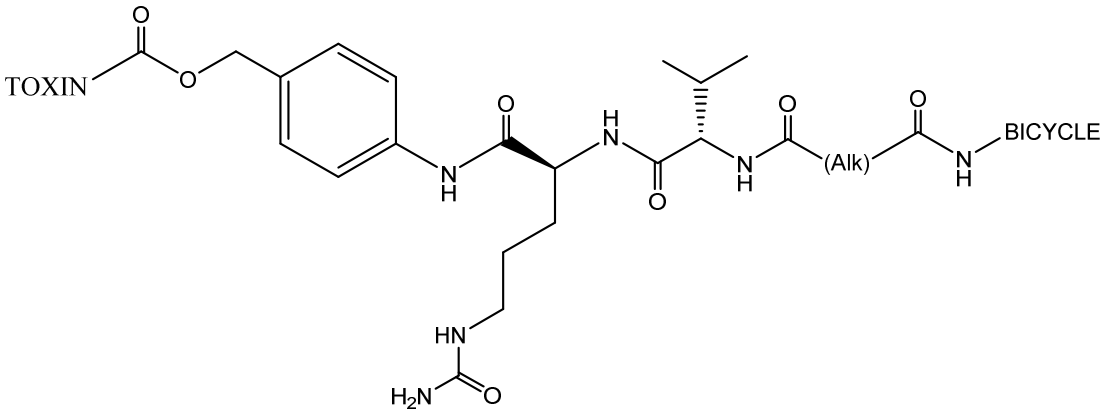
wherein R_1, R_2, R_3 and R_4 represent hydrogen or C1-C6 alkyl groups;
Toxin refers to any suitable cytotoxic agent defined herein;
Bicycle represents any suitable bicyclic peptide defined herein;
n represents an integer selected from 1 to 10; and
m represents an integer selected from 0 to 10.

~~21~~15. The drug conjugate as defined in claim ~~20~~14, wherein either: R_1, R_2, R_3 and R_4 are all H; or R_1, R_2, R_3 are all H and R_4 = methyl; or R_1, R_2 = methyl and R_3, R_4 = H; or R_1, R_3 = methyl and R_2, R_4 = H; or R_1, R_2 = H and R_3, R_4 = C1-C6 alkyl.

~~22~~16. The drug conjugate as defined in claim ~~19~~13, wherein the drug conjugate has the following structure:



~~23~~17. The drug conjugate as defined in claim ~~19~~13, wherein the drug conjugate has the following structure:



wherein (alk) is a linear or branched alkylene group of formula C_nH_{2n} wherein n is from 1 to 10.

18. A peptide ligand specific for MT1-MMP comprising a polypeptide comprising three residues selected from cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap) and N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), the said three residues being separated by at least two loop sequences, and a molecular scaffold, the peptide being linked to the scaffold by covalent alkylamino linkages with the Dap or N-AlkDap or N-HAlkDap residues of the polypeptide and by thioether linkages with the cysteine residues of the polypeptide when the said three residues include cysteine, such that two polypeptide loops are formed on the molecular scaffold, wherein the peptide ligand comprises an amino acid sequence of formula (II):

- A_1-X_1-U/O_2-X_3-X_4-G_5-A_2-E_6-D_7-F_8-Y_9-X_{10}-X_{11}-A_3- (SEQ ID NO: 1)

(II)

or a pharmaceutically acceptable salt thereof;

wherein:

A_1, A_2, and A_3 are independently cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap), or N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), provided that at least one of A_1, A_2, and A_3 is Dap, N-AlkDap or N-HAlkDap;
X represents any amino acid residue;
U represents a polar, uncharged amino acid residue selected from N, C, Q, M, S and T; and
O represents a non-polar aliphatic amino acid residue selected from G, A, I, L, P and V;
wherein one or more tyrosine residues is replaced by a phenylalanine residue.

19. A peptide ligand specific for MT1-MMP comprising a polypeptide comprising three residues selected from cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap) and N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), the said three residues being separated by at least two loop sequences, and a molecular scaffold, the peptide being linked to the scaffold by covalent alkylamino linkages with the Dap or N-AlkDap or N-HAlkDap residues of the polypeptide and by thioether linkages with the cysteine residues of the polypeptide when the said three residues include cysteine, such that two polypeptide loops are formed on the molecular scaffold, wherein the peptide ligand comprises an amino acid sequence of formula (II):
- A_1-X_1-U/O_2-X_3-X_4-G_5-A_2-E_6-D_7-F_8-Y_9-X_{10}-X_{11}-A_3- (SEQ ID NO: 1)
(II)
or a pharmaceutically acceptable salt thereof;
wherein:
A_1, A_2, and A_3 are independently cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap), or N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), provided that at least one of A_1, A_2, and A_3 is Dap, N-AlkDap or N-HAlkDap;
X represents any amino acid residue;
U represents a polar, uncharged amino acid residue selected from N, C, Q, M, S and T; and
O represents a non-polar aliphatic amino acid residue selected from G, A, I, L, P and V; which additionally comprises replacement of one or more amino acid residues with one or more non-natural amino acid residues (such as replacement of one or more polar amino acid residues with one or more isosteric or isoelectronic amino acids; replacement of one or more hydrophobic amino acid residues with other non-natural isosteric or isoelectronic amino acids); or replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues.

20. The peptide ligand as defined in claim 19, which comprises a modification at amino acid position 1 and/or 9.

21. The peptide ligand as defined in claim 19 or 20, which comprises replacement of one or more amino acid residues with one or more non-natural amino acid residues.

22. The peptide ligand as defined in claim 19, wherein the non-natural amino acid residue is substituted at position 4 and is selected from: 1-naphthylalanine; 2-naphthylalanine; 3,4-dichlorophenylalanine; and homophenylalanine, such as 1-naphthylalanine; 2-naphthylalanine; and 3,4-dichlorophenylalanine, in particular 1-naphthylalanine.

23. The peptide ligand as defined in claim 19 or claim 22, wherein the non-natural amino acid residue is substituted at position 9 and/or 11 and is selected from: 4-bromophenylalanine or pentafluoro-phenylalanine for position 9 and/or tert-butylglycine for position 11; preferably wherein the non-natural amino acid residues, such as those present at position 9, is selected from: 4-bromophenylalanine, or the non-natural amino acid residues, such as those present at position 11, is selected from: tert-butylglycine.

24. The peptide ligand as defined in claim 19, wherein the amino acid residue at position 1 is substituted for a D-amino acid, such as D-alanine, wherein the amino acid residue at position 5 is substituted for a D-amino acid, such as D-alanine or D-arginine

25. The peptide ligand as defined in claim 21, which comprises a plurality of the above mentioned modifications, such as 2, 3, 4 or 5 or more of the following modifications, such as all of the following 5 modifications: D-alanine at position 1 and/or 5, a 1-naphthylalanine at position 4, a 4-bromophenylalanine at position 9 and a tert-butylglycine at position 11.

26. A peptide ligand specific for MT1-MMP comprising a polypeptide comprising three residues selected from cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap) and N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), the said three residues being separated by at least two loop sequences, and a molecular scaffold, the peptide being linked to the scaffold by covalent alkylamino linkages with the Dap or N-AlkDap or N-HAlkDap residues of the polypeptide and by thioether linkages with the cysteine residues of the polypeptide when the said three residues include cysteine, such that two polypeptide loops are formed on the molecular scaffold, wherein the peptide ligand comprises an amino acid sequence of formula (II):

the Dap homologues of 17-69-07-N241 designated as SEQ ID NO: 16: ((bAla)-Sar10-AA_1(D-Ala)NE(1Nal)(D-Ala)A_2EDFYD(tBuGly)A_3 ; or

the Dap homologues of 17-69-07-N268 designated as SEQ ID NO: 17: AA_1(D-Ala)NE(1Nal)(D-Ala)A_2EDFYD(tBuGly)A_3 ;

or a pharmaceutically acceptable salt thereof;

wherein:

A_1, A_2, and A_3 are independently cysteine, L-2,3-diaminopropionic acid (Dap), N-beta-alkyl-L-2,3-diaminopropionic acid (N-AlkDap), or N-beta-haloalkyl-L-2,3-diaminopropionic acid (N-HAlkDap), provided that at least one of A_1, A_2, and A_3 is Dap, N-AlkDap or N-HAlkDap.