

BICYCLIC PEPTIDE LIGANDS SPECIFIC FOR MT1-MMP

FIELD OF THE INVENTION

The present invention relates to polypeptides which are covalently bound to non-aromatic molecular scaffolds such that two or more peptide loops are subtended between attachment points to the scaffold. In particular, the invention describes peptides which are high affinity binders of membrane type 1 metalloprotease (MT1-MMP), such as the collagen binding site of MT1-MMP. The invention also describes drug conjugates comprising said peptides, conjugated to one or more effector and/or functional groups which have utility in imaging and targeted cancer therapy.

BACKGROUND OF THE INVENTION

Cyclic peptides are able to bind with high affinity and target specificity to protein targets and hence are an attractive molecule class for the development of therapeutics. In fact, several cyclic peptides are already successfully used in the clinic, as for example the antibacterial peptide vancomycin, the immunosuppressant drug cyclosporine or the anti-cancer drug octreotide (Driggers *et al.* (2008), Nat Rev Drug Discov 7 (7), 608-24). Good binding properties result from a relatively large interaction surface formed between the peptide and the target as well as the reduced conformational flexibility of the cyclic structures. Typically, macrocycles bind to surfaces of several hundred square angstrom, as for example the cyclic peptide CXCR4 antagonist CVX15 (400 Å²; Wu *et al.* (2007), Science 330, 1066-71), a cyclic peptide with the Arg-Gly-Asp motif binding to integrin αVβ3 (355 Å²) (Xiong *et al.* (2002), Science 296 (5565), 151-5) or the cyclic peptide inhibitor upain-1 binding to urokinase-type plasminogen activator (603 Å²; Zhao *et al.* (2007), J Struct Biol 160 (1), 1-10).

Due to their cyclic configuration, peptide macrocycles are less flexible than linear peptides, leading to a smaller loss of entropy upon binding to targets and resulting in a higher binding affinity. The reduced flexibility also leads to locking target-specific conformations, increasing binding specificity compared to linear peptides. This effect has been exemplified by a potent and selective inhibitor of matrix metalloproteinase 8 (MMP-8) which lost its selectivity over other MMPs when its ring was opened (Cherney *et al.* (1998), J Med Chem 41 (11), 1749-51). The favorable binding properties achieved through macrocyclization are even more pronounced in multicyclic peptides having more than one peptide ring as for example in vancomycin, nisin and actinomycin.

Different research teams have previously tethered polypeptides with cysteine residues to a synthetic molecular structure (Kemp and McNamara (1985), J. Org. Chem; Timmerman *et*

al. (2005), ChemBioChem). Melen and co-workers had used tris(bromomethyl)benzene and related molecules for rapid and quantitative cyclisation of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces (Timmerman *et al.* (2005), ChemBioChem). Methods for the generation of candidate drug compounds wherein said
5 compounds are generated by linking cysteine containing polypeptides to a molecular scaffold as for example TATA (1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one, Heinis *et al.* Angew Chem, Int Ed. 2014; 53:1602–1606).

Phage display-based combinatorial approaches have been developed to generate and screen
10 large libraries of bicyclic peptides to targets of interest (Heinis *et al.* (2009), Nat Chem Biol 5 (7), 502-7 and WO 2009/098450). Briefly, combinatorial libraries of linear peptides containing three cysteine residues and two regions of six random amino acids (Cys-(Xaa)₆-Cys-(Xaa)₆-Cys) were displayed on phage and cyclised by covalently linking the cysteine side chains to a small molecule (tris-(bromomethyl)benzene).

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a peptide ligand specific for the collagen binding site of MT1-MMP comprising a polypeptide comprising at least three cysteine residues, separated by at least two loop sequences, and a non-aromatic molecular scaffold
20 which forms covalent bonds with the cysteine residues of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.

According to a further aspect of the invention, there is provided a drug conjugate comprising a peptide ligand as defined herein conjugated to one or more effector and/or functional groups.

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising a peptide ligand or a drug conjugate as defined herein in combination with one or more pharmaceutically acceptable excipients.

According to a further aspect of the invention, there is provided a peptide ligand or drug conjugate as defined herein for use in preventing, suppressing or treating a disease or disorder mediated by MT1-MMP.

DETAILED DESCRIPTION OF THE INVENTION

35 In one embodiment, said loop sequences comprise 6 amino acids.

In a further embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences both of which consists of 6 amino acids.

In one embodiment, the peptide ligand comprises an amino acid sequence selected from:

5 C_i-P-F/I/Y-D/S-W-H-T-C_{ii}-L-F-G-D/E-Y-T/S-C_{iii} (SEQ ID NO: 1)

wherein C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively or a pharmaceutically acceptable salt thereof.

10 In one embodiment, the peptide ligand of C_i-P-F/I/Y-D/S-W-H-T-C_{ii}-L-F-G-D/E-Y-T/S-C_{iii} (SEQ ID NO: 1) is selected from:

CPYSWETCLFGDYRC (SEQ ID NO: 2);

CPFDWHTCLFGDYTC (SEQ ID NO: 3);

CPFDWHTCLFGEYSC (SEQ ID NO: 4);

CPIDWHTCLFGDYTC (SEQ ID NO: 5);

15 CPFSWHTCLFGEYSC (SEQ ID NO: 6);

CPFSWHTCLFGDYTC (SEQ ID NO: 7);

CPISWHTCLFGDYSC (SEQ ID NO: 8); and

CPYSWHTCLFGDYSC (SEQ ID NO: 9).

20 In a further embodiment, the peptide ligand of C_i-P-F/I/Y-D/S-W-H-T-C_{ii}-L-F-G-D/E-Y-T/S-C_{iii} (SEQ ID NO: 1) is selected from:

A-(SEQ ID NO: 2)-A (BCY1026);

A-(SEQ ID NO: 3)-A (BCY1057);

A-(SEQ ID NO: 4)-A (BCY1065);

25 A-(SEQ ID NO: 5)-A (BCY1067);

A-(SEQ ID NO: 6)-A (BCY1073);

A-(SEQ ID NO: 7)-A (BCY1074);

A-(SEQ ID NO: 8)-A (BCY1075); and

A-(SEQ ID NO: 9)-A (BCY1076).

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In one embodiment, the molecular scaffold is TATA and the peptide ligand of C_i-P-F/I/Y-D/S-W-H-T-C_{ii}-L-F-G-D/E-Y-T/S-C_{iii} (SEQ ID NO: 1) is selected from:

A-(SEQ ID NO: 2)-A (BCY1026);

A-(SEQ ID NO: 3)-A (BCY1057);

35 A-(SEQ ID NO: 4)-A (BCY1065);

A-(SEQ ID NO: 5)-A (BCY1067);

A-(SEQ ID NO: 6)-A (BCY1073);

A-(SEQ ID NO: 7)-A (BCY1074);
 A-(SEQ ID NO: 8)-A (BCY1075); and
 A-(SEQ ID NO: 9)-A (BCY1076).

- 5 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art, such as in the arts of peptide chemistry, cell culture and phage display, nucleic acid chemistry and biochemistry. Standard techniques are used for molecular biology, genetic and biochemical methods (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel *et al.*, Short Protocols in Molecular Biology 10 (1999) 4th ed., John Wiley & Sons, Inc.), which are incorporated herein by reference.

Nomenclature

Numbering

- 15 When referring to amino acid residue positions within the peptides of the invention, cysteine residues (C_i, C_{ii} and C_{iii}) are omitted from the numbering as they are invariant, therefore, the numbering of amino acid residues within the peptides of the invention is referred to as below:

C_i-P₁-Y₂-S₃-W₄-E₅-T₆-C_{ii}-L₇-F₈-G₉-D₁₀-Y₁₁-R₁₂-C_{iii} (SEQ ID NO: 2).

20

For the purpose of this description, all bicyclic peptides are assumed to be cyclised with 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)tripropan-1-one (TATA) and yielding a tri-substituted structure. Cyclisation with TATA occurs on C_i, C_{ii}, and C_{iii}.

- 25 Molecular Format

N- or C-terminal extensions to the bicycle core sequence are added to the left or right side of the sequence, separated by a hyphen. For example, an N-terminal βAla-Sar10-Ala tail would be denoted as:

βAla-Sar10-A-(SEQ ID NO: X).

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Inversed Peptide Sequences

- In light of the disclosure in Nair *et al* (2003) J Immunol 170(3), 1362-1373, it is envisaged that the peptide sequences disclosed herein would also find utility in their retro-inverso form. For example, the sequence is reversed (i.e. N-terminus becomes C-terminus and *vice versa*) and their stereochemistry is likewise also reversed (i.e. D-amino acids become L-amino acids and *vice versa*).
- 35

Peptide Ligands

A peptide ligand, as referred to herein, refers to a peptide covalently bound to a molecular scaffold. Typically, such peptides comprise two or more reactive groups (i.e. cysteine residues) which are capable of forming covalent bonds to the scaffold, and a sequence
5 subtended between said reactive groups which is referred to as the loop sequence, since it forms a loop when the peptide is bound to the scaffold. In the present case, the peptides comprise at least three cysteine residues (referred to herein as C_i, C_{ii} and C_{iii}), and form at least two loops on the scaffold.

Advantages of the Peptide Ligands

10 Certain bicyclic peptides of the present invention have a number of advantageous properties which enable them to be considered as suitable drug-like molecules for injection, inhalation, nasal, ocular, oral or topical administration. Such advantageous properties include:

15 - Species cross-reactivity. Certain ligands demonstrate cross-reactivity across PBPs from different bacterial species and hence are able to treat infections caused by multiple species of bacteria. Other ligands may be highly specific for the PBPs of certain bacterial species which may be advantageous for treating an infection without collateral damage to the beneficial flora of the patient;

20 - Protease stability. Bicyclic peptide ligands should ideally demonstrate stability to plasma proteases, epithelial ("membrane-anchored") proteases, gastric and intestinal proteases, lung surface proteases, intracellular proteases and the like. Protease stability should be maintained between different species such that a bicycle lead candidate can be
25 developed in animal models as well as administered with confidence to humans;

- Desirable solubility profile. This is a function of the proportion of charged and hydrophilic versus hydrophobic residues and intra/inter-molecular H-bonding, which is important for formulation and absorption purposes;

30 - An optimal plasma half-life in the circulation. Depending upon the clinical indication and treatment regimen, it may be required to develop a bicyclic peptide for short exposure in an acute illness management setting, or develop a bicyclic peptide with enhanced retention in the circulation, and is therefore optimal for the management of more chronic disease states.
35 Other factors driving the desirable plasma half-life are requirements of sustained exposure for maximal therapeutic efficiency versus the accompanying toxicology due to sustained exposure of the agent; and

- Selectivity. Certain peptide ligands of the invention demonstrate selectivity for MT1-MMP, but does not cross-react with MMP isoforms, such as MMP-1, MMP-2, MMP-15 and MMP-16.

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Pharmaceutically Acceptable Salts

It will be appreciated that salt forms are within the scope of this invention, and references to peptide ligands include the salt forms of said ligands.

- 10 The salts of the present invention can be synthesized from the parent compound that contains a basic or acidic moiety by conventional chemical methods such as methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with the
- 15 appropriate base or acid in water or in an organic solvent, or in a mixture of the two.

- Acid addition salts (mono- or di-salts) may be formed with a wide variety of acids, both inorganic and organic. Examples of acid addition salts include mono- or di-salts formed with an acid selected from the group consisting of acetic, 2,2-dichloroacetic, adipic, alginic,
- 20 ascorbic (e.g. L-ascorbic), L-aspartic, benzenesulfonic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulfonic, (+)-(1S)-camphor-10-sulfonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulfuric, ethane-1,2-disulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, formic, fumaric, galactaric, gentisic, glucoheptonic, D-gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α -oxoglutaric, glycolic, hippuric,
- 25 hydrohalic acids (e.g. hydrobromic, hydrochloric, hydriodic), isethionic, lactic (e.g. (+)-L-lactic, ($\pm$)-DL-lactic), lactobionic, maleic, malic, (-)-L-malic, malonic, ($\pm$)-DL-mandelic, methanesulfonic, naphthalene-2-sulfonic, naphthalene-1,5-disulfonic, 1-hydroxy-2-naphthoic, nicotinic, nitric, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, pyruvic, L-pyroglutamic, salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulfuric, tannic, (+)-L-tartaric, thiocyanic, *p*-toluenesulfonic, undecylenic and valeric acids, as well as acylated amino
- 30 acids and cation exchange resins.

- One particular group of salts consists of salts formed from acetic, hydrochloric, hydriodic, phosphoric, nitric, sulfuric, citric, lactic, succinic, maleic, malic, isethionic, fumaric,
- 35 benzenesulfonic, toluenesulfonic, sulfuric, methanesulfonic (mesylate), ethanesulfonic, naphthalenesulfonic, valeric, propanoic, butanoic, malonic, glucuronic and lactobionic acids. One particular salt is the hydrochloride salt. Another particular salt is the acetate salt.

If the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be -COO⁻), then a salt may be formed with an organic or inorganic base, generating a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Li⁺, Na⁺ and K⁺, alkaline earth metal cations such as Ca²⁺ and Mg²⁺, and other cations such as Al³⁺ or Zn⁺. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH₄⁺) and substituted ammonium ions (e.g., NH₃R⁺, NH₂R₂⁺, NHR₃⁺, NR₄⁺). Examples of some suitable substituted ammonium ions are those derived from: methylamine, ethylamine, diethylamine, propylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is N(CH₃)₄⁺.

Where the peptides of the invention contain an amine function, these may form quaternary ammonium salts, for example by reaction with an alkylating agent according to methods well known to the skilled person. Such quaternary ammonium compounds are within the scope of the peptides of the invention.

Modified Derivatives

It will be appreciated that modified derivatives of the peptide ligands as defined herein are within the scope of the present invention. Examples of such suitable modified derivatives include 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 non-polar amino acid residues with other non-natural isosteric or isoelectronic amino acids); addition of a spacer group; replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues; replacement of one or more amino acid residues with an alanine, replacement of one or more L-amino acid residues with one or more D-amino acid residues; N-alkylation of one or more amide bonds within the bicyclic peptide ligand; replacement of one or more peptide bonds with a surrogate bond; peptide backbone length modification; substitution of the hydrogen on the alpha-carbon of one or more amino acid residues with another chemical group, modification of amino acids such as cysteine, lysine, glutamate/aspartate and tyrosine with suitable amine, thiol, carboxylic acid and phenol-reactive reagents so as to functionalise said amino acids, and introduction or replacement of amino acids that introduce orthogonal reactivities that are suitable for functionalisation, for

example azide or alkyne-group bearing amino acids that allow functionalisation with alkyne or azide-bearing moieties, respectively.

5 In one embodiment, the modified derivative comprises an N-terminal and/or C-terminal modification. In a further embodiment, wherein the modified derivative comprises an N-terminal modification using suitable amino-reactive chemistry, and/or C-terminal modification using suitable carboxy-reactive chemistry. In a further embodiment, said N-terminal or C-terminal modification comprises addition of an effector group, including but not limited to a cytotoxic agent, a radiochelator or a chromophore.

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In a further embodiment, the modified derivative comprises an N-terminal modification. In a further embodiment, the N-terminal modification comprises an N-terminal acetyl group. In this embodiment, the N-terminal cysteine group (the group referred to herein as C_i) is capped with acetic anhydride or other appropriate reagents during peptide synthesis leading to a molecule
15 which is N-terminally acetylated. This embodiment provides the advantage of removing a potential recognition point for aminopeptidases and avoids the potential for degradation of the bicyclic peptide.

20 In an alternative embodiment, 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.

In a further embodiment, the modified derivative comprises a C-terminal modification. In a further embodiment, the C-terminal modification comprises an amide group. In this
25 embodiment, the C-terminal cysteine group (the group referred to herein as C_{iii}) is synthesized as an amide during peptide synthesis leading to a molecule which is C-terminally amidated. This embodiment provides the advantage of removing a potential recognition point for carboxypeptidase and reduces the potential for proteolytic degradation of the bicyclic peptide.

30 In one embodiment, the modified derivative comprises replacement of one or more amino acid residues with one or more non-natural amino acid residues. In this embodiment, non-natural amino acids may be selected having isosteric/isoelectronic side chains which are neither recognised by degradative proteases nor have any adverse effect upon target potency.

35 Alternatively, non-natural amino acids may be used having constrained amino acid side chains, such that proteolytic hydrolysis of the nearby peptide bond is conformationally and sterically impeded. In particular, these concern proline analogues, bulky sidechains, C α -

disubstituted derivatives (for example, aminoisobutyric acid, Aib), and cyclo amino acids, a simple derivative being amino-cyclopropylcarboxylic acid.

In one embodiment, the modified derivative comprises the addition of a spacer group. In a
5 further embodiment, the modified derivative comprises the addition of a spacer group to the N-terminal cysteine (C_i) and/or the C-terminal cysteine (C_{iii}).

In one embodiment, the modified derivative comprises replacement of one or more oxidation sensitive amino acid residues with one or more oxidation resistant amino acid residues.

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In one embodiment, the modified derivative comprises replacement of one or more charged amino acid residues with one or more hydrophobic amino acid residues. In an alternative embodiment, the modified derivative comprises replacement of one or more hydrophobic amino acid residues with one or more charged amino acid residues. The correct balance of
15 charged versus hydrophobic amino acid residues is an important characteristic of the bicyclic peptide ligands. For example, hydrophobic amino acid residues influence the degree of plasma protein binding and thus the concentration of the free available fraction in plasma, while charged amino acid residues (in particular arginine) may influence the interaction of the peptide with the phospholipid membranes on cell surfaces. The two in combination may
20 influence half-life, volume of distribution and exposure of the peptide drug, and can be tailored according to the clinical endpoint. In addition, the correct combination and number of charged versus hydrophobic amino acid residues may reduce irritation at the injection site (if the peptide drug has been administered subcutaneously).

25 In one embodiment, the modified derivative comprises replacement of one or more L-amino acid residues with one or more D-amino acid residues. This embodiment is believed to increase proteolytic stability by steric hindrance and by a propensity of D-amino acids to stabilise β -turn conformations (Tugyi *et al* (2005) PNAS, 102(2), 413–418).

30 In one embodiment, the modified derivative comprises removal of any amino acid residues and substitution with alanines. This embodiment provides the advantage of removing potential proteolytic attack site(s).

It should be noted that each of the above mentioned modifications serve to deliberately
35 improve the potency or stability of the peptide. Further potency improvements based on modifications may be achieved through the following mechanisms:

- Incorporating hydrophobic moieties that exploit the hydrophobic effect and lead to lower off rates, such that higher affinities are achieved;
- Incorporating charged groups that exploit long-range ionic interactions, leading to faster on rates and to higher affinities (see for example Schreiber *et al*, *Rapid, electrostatically assisted association of proteins* (1996), Nature Struct. Biol. 3, 427-31); and
- Incorporating additional constraint into the peptide, by for example constraining side chains of amino acids correctly such that loss in entropy is minimal upon target binding, constraining the torsional angles of the backbone such that loss in entropy is minimal upon target binding and introducing additional cyclisations in the molecule for identical reasons.

(for reviews see Gentilucci *et al*, Curr. Pharmaceutical Design, (2010), 16, 3185-203, and Nestor *et al*, Curr. Medicinal Chem (2009), 16, 4399-418).

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Isotopic variations

The present invention includes all pharmaceutically acceptable (radio)isotope-labeled peptide ligands of the invention, wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature, and peptide ligands of the invention, wherein metal chelating groups are attached (termed "effector") that are capable of holding relevant (radio)isotopes, and peptide ligands of the invention, wherein certain functional groups are covalently replaced with relevant (radio)isotopes or isotopically labelled functional groups.

Examples of isotopes suitable for inclusion in the peptide ligands of the invention comprise isotopes of hydrogen, such as ^2H (D) and ^3H (T), carbon, such as ^{11}C , ^{13}C and ^{14}C , chlorine, such as ^{36}Cl , fluorine, such as ^{18}F , iodine, such as ^{123}I , ^{125}I and ^{131}I , nitrogen, such as ^{13}N and ^{15}N , oxygen, such as ^{15}O , ^{17}O and ^{18}O , phosphorus, such as ^{32}P , sulfur, such as ^{35}S , copper, such as ^{64}Cu , gallium, such as ^{67}Ga or ^{68}Ga , yttrium, such as ^{90}Y and lutetium, such as ^{177}Lu , and Bismuth, such as ^{213}Bi .

Certain isotopically-labelled peptide ligands of the invention, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The peptide ligands of the invention can further have valuable diagnostic properties in that they can be used for detecting or identifying the formation of a complex between a labelled compound and other molecules, peptides, proteins, enzymes or receptors. The detecting or identifying methods can use compounds that are labelled with labelling agents such as

radioisotopes, enzymes, fluorescent substances, luminous substances (for example, luminol, luminol derivatives, luciferin, aequorin and luciferase), etc. The radioactive isotopes tritium, *i.e.* ^3H (T), and carbon-14, *i.e.* ^{14}C , are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

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Substitution with heavier isotopes such as deuterium, *i.e.* ^2H (D), may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

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Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining target occupancy.

Isotopically-labeled compounds of peptide ligands of the invention can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

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Non-Aromatic Molecular scaffold

References herein to the term “non-aromatic molecular scaffold” refer to any molecular scaffold as defined herein which does not contain an aromatic (*i.e.* unsaturated) carbocyclic or heterocyclic ring system.

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Suitable examples of non-aromatic molecular scaffolds are described in *Heinis et al (2014) Angewandte Chemie, International Edition 53(6) 1602-1606*.

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As noted in the foregoing documents, the molecular scaffold may be a small molecule, such as a small organic molecule.

In one embodiment the molecular scaffold may be a macromolecule. In one embodiment the molecular scaffold is a macromolecule composed of amino acids, nucleotides or carbohydrates.

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In one embodiment the molecular scaffold comprises reactive groups that are capable of reacting with functional group(s) of the polypeptide to form covalent bonds.

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The molecular scaffold may comprise chemical groups which form the linkage with a peptide, such as amines, thiols, alcohols, ketones, aldehydes, nitriles, carboxylic acids, esters, alkenes, alkynes, azides, anhydrides, succinimides, maleimides, alkyl halides and acyl halides.

5

An example of an $\alpha\beta$ unsaturated carbonyl containing compound is 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) (Angewandte Chemie, International Edition (2014), 53(6), 1602-1606).

10 ***Effector and Functional Groups***

According to a further aspect of the invention, there is provided a drug conjugate comprising a peptide ligand as defined herein conjugated to one or more effector and/or functional groups.

Effector and/or functional groups can be attached, for example, to the N and/or C termini of the polypeptide, to an amino acid within the polypeptide, or to the molecular scaffold.

15

Appropriate effector groups include antibodies and parts or fragments thereof. For instance, an effector group can include an antibody light chain constant region (CL), an antibody CH1 heavy chain domain, an antibody CH2 heavy chain domain, an antibody CH3 heavy chain domain, or any combination thereof, in addition to the one or more constant region domains. An effector group may also comprise a hinge region of an antibody (such a region normally being found between the CH1 and CH2 domains of an IgG molecule).

20

In a further embodiment of this aspect of the invention, an effector group according to the present invention is an Fc region of an IgG molecule. Advantageously, a peptide ligand-effector group according to the present invention comprises or consists of a peptide ligand Fc fusion having a $t_{1/2}$ half-life of a day or more, two days or more, 3 days or more, 4 days or more, 5 days or more, 6 days or more or 7 days or more. Most advantageously, the peptide ligand according to the present invention comprises or consists of a peptide ligand Fc fusion having a $t_{1/2}$ half-life of a day or more.

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Functional groups include, in general, binding groups, drugs, reactive groups for the attachment of other entities, functional groups which aid uptake of the macrocyclic peptides into cells, and the like.

35

The ability of peptides to penetrate into cells will allow peptides against intracellular targets to be effective. Targets that can be accessed by peptides with the ability to penetrate into cells include transcription factors, intracellular signalling molecules such as tyrosine kinases and molecules involved in the apoptotic pathway. Functional groups which enable the penetration of cells include peptides or chemical groups which have been added either to the peptide or the molecular scaffold. Peptides such as those derived from such as VP22, HIV-Tat, a homeobox protein of *Drosophila* (Antennapedia), e.g. as described in Chen and Harrison, Biochemical Society Transactions (2007) Volume 35, part 4, p821; Gupta *et al.* in Advanced Drug Discovery Reviews (2004) Volume 57 9637. Examples of short peptides which have been shown to be efficient at translocation through plasma membranes include the 16 amino acid penetratin peptide from *Drosophila* Antennapedia protein (Derossi *et al.* (1994) J Biol. Chem. Volume 269 p10444), the 18 amino acid 'model amphipathic peptide' (Oehlke *et al.* (1998) Biochim Biophys Acts Volume 1414 p127) and arginine rich regions of the HIV TAT protein. Non peptidic approaches include the use of small molecule mimics or SMOCs that can be easily attached to biomolecules (Okuyama *et al.* (2007) Nature Methods Volume 4 p153). Other chemical strategies to add guanidinium groups to molecules also enhance cell penetration (Elson-Scwab *et al.* (2007) J Biol Chem Volume 282 p13585). Small molecular weight molecules such as steroids may be added to the molecular scaffold to enhance uptake into cells.

One class of functional groups which may be attached to peptide ligands includes antibodies and binding fragments thereof, such as Fab, Fv or single domain fragments. In particular, antibodies which bind to proteins capable of increasing the half-life of the peptide ligand *in vivo* may be used.

In one embodiment, a peptide ligand-effector group according to the invention has a $t_{1/2}$ half-life selected from the group consisting of: 12 hours or more, 24 hours or more, 2 days or more, 3 days or more, 4 days or more, 5 days or more, 6 days or more, 7 days or more, 8 days or more, 9 days or more, 10 days or more, 11 days or more, 12 days or more, 13 days or more, 14 days or more, 15 days or more or 20 days or more. Advantageously a peptide ligand-effector group or composition according to the invention will have a $t_{1/2}$ half-life in the range 12 to 60 hours. In a further embodiment, it will have a $t_{1/2}$ half-life of a day or more. In a further embodiment still, it will be in the range 12 to 26 hours.

In one particular embodiment of the invention, the functional group is selected from a metal chelator, which is suitable for complexing metal radioisotopes of medicinal relevance.

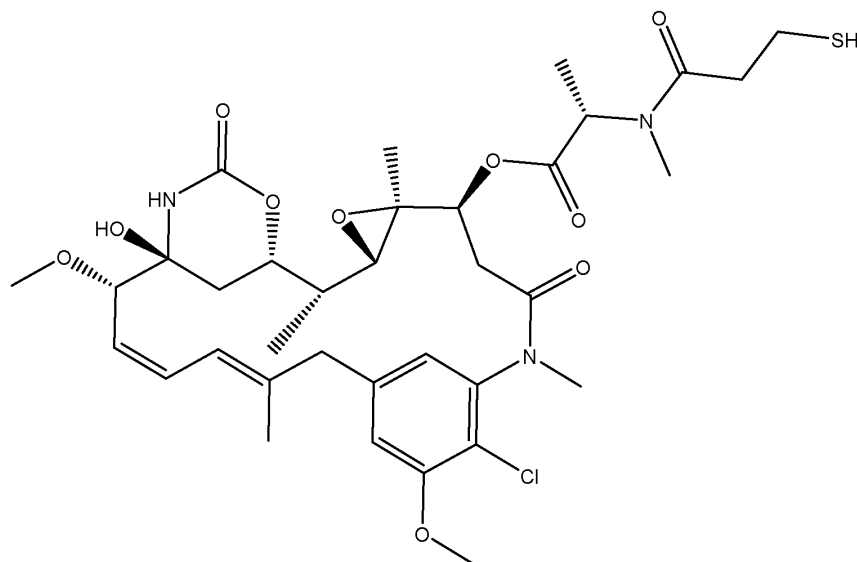
Possible effector groups also include enzymes, for instance such as carboxypeptidase G2 for use in enzyme/prodrug therapy, where the peptide ligand replaces antibodies in ADEPT.

- 5 In one particular embodiment of the invention, the functional group is selected from a drug, such as a cytotoxic agent for cancer therapy. Suitable examples include: alkylating agents such as cisplatin and carboplatin, as well as oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide; Anti-metabolites including purine analogs azathioprine and mercaptopurine or pyrimidine analogs; plant alkaloids and terpenoids including vinca alkaloids
- 10 such as Vincristine, Vinblastine, Vinorelbine and Vindesine; Podophyllotoxin and its derivatives etoposide and teniposide; Taxanes, including paclitaxel, originally known as Taxol; topoisomerase inhibitors including camptothecins: irinotecan and topotecan, and type II inhibitors including amsacrine, etoposide, etoposide phosphate, and teniposide. Further agents can include antitumour antibiotics which include the immunosuppressant dactinomycin
- 15 (which is used in kidney transplantations), doxorubicin, epirubicin, bleomycin, calicheamycins, and others.

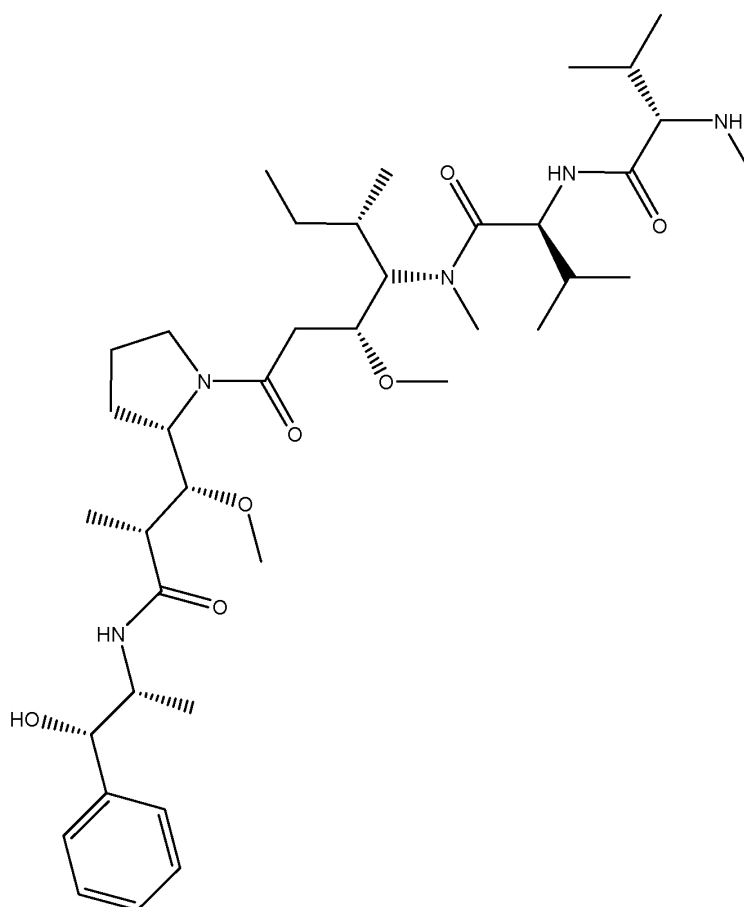
In one further particular embodiment of the invention, the cytotoxic agent is selected from maytansinoids (such as DM1) or monomethyl auristatins (such as MMAE).

20

DM1 is a cytotoxic agent which is a thiol-containing derivative of maytansine and has the following structure:



Monomethyl auristatin E (MMAE) is a synthetic antineoplastic agent and has the following structure:



In one yet further particular embodiment of the invention, the cytotoxic agent is selected from monomethyl auristatin E (MMAE). Data is presented herein in Figure 1 and Tables 3 and 4 which demonstrates the effects of peptide ligands conjugated to a toxin containing MMAE.

In one embodiment, the cytotoxic agent is linked to the bicyclic peptide by a cleavable bond, such as a disulphide bond or a protease sensitive bond. In a further embodiment, the groups
10 adjacent to the disulphide bond are modified to control the hindrance of the disulphide bond, and by this the rate of cleavage and concomitant release of cytotoxic agent.

Published work established the potential for modifying the susceptibility of the disulphide bond to reduction by introducing steric hindrance on either side of the disulphide bond (Kellogg *et al* (2011) *Bioconjugate Chemistry*, 22, 717). A greater degree of steric hindrance reduces the rate of reduction by intracellular glutathione and also extracellular (systemic) reducing agents, consequentially reducing the ease by which toxin is released, both inside and outside the cell.

Thus, selection of the optimum in disulphide stability in the circulation (which minimises undesirable side effects of the toxin) versus efficient release in the intracellular milieu (which maximises the therapeutic effect) can be achieved by careful selection of the degree of hindrance on either side of the disulphide bond.

5

The hindrance on either side of the disulphide bond is modulated through introducing one or more methyl groups on either the targeting entity (here, the bicyclic peptide) or toxin side of the molecular construct.

- 10 In one embodiment, the cytotoxic agent and linker is selected from any combinations of those described in WO 2016/067035 (the cytotoxic agents and linkers thereof are herein incorporated by reference).

Synthesis

- 15 The peptides of the present invention may be manufactured synthetically by standard techniques followed by reaction with a molecular scaffold *in vitro*. When this is performed, standard chemistry may be used. This enables the rapid large scale preparation of soluble material for further downstream experiments or validation. Such methods could be accomplished using conventional chemistry such as that disclosed in Timmerman *et al*
20 (*supra*).

- Thus, the invention also relates to manufacture of polypeptides selected as set out herein, wherein the manufacture comprises optional further steps as explained below. In one embodiment, these steps are carried out on the end product polypeptide made by chemical
25 synthesis.

Peptides can also be extended, to incorporate for example another loop and therefore introduce multiple specificities.

- 30 To extend the peptide, it may simply be extended chemically at its N-terminus or C-terminus or within the loops using orthogonally protected lysines (and analogues) using standard solid phase or solution phase chemistry. Standard (bio)conjugation techniques may be used to introduce an activated or activatable N- or C-terminus. Alternatively additions may be made by fragment condensation or native chemical ligation e.g. as described in (Dawson *et al.* 1994.
35 Synthesis of Proteins by Native Chemical Ligation. Science 266:776-779), or by enzymes, for example using subtiligase as described in (Chang *et al.* Proc Natl Acad Sci U S A. 1994 Dec

20; 91(26):12544-8 or in Hikari *et al*/ Bioorganic & Medicinal Chemistry Letters Volume 18, Issue 22, 15 November 2008, Pages 6000-6003).

Alternatively, the peptides may be extended or modified by further conjugation through
5 disulphide bonds. This has the additional advantage of allowing the first and second peptide
to dissociate from each other once within the reducing environment of the cell. In this case,
the molecular scaffold (e.g. TATA) could be added during the chemical synthesis of the first
peptide so as to react with the three cysteine groups; a further cysteine or thiol could then be
appended to the N or C-terminus of the first peptide, so that this cysteine or thiol only reacted
10 with a free cysteine or thiol of the second peptide, forming a disulfide –linked bicyclic peptide-
peptide conjugate.

Similar techniques apply equally to the synthesis/coupling of two bicyclic and bispecific
macrocycles, potentially creating a tetraspecific molecule.

15 Furthermore, addition of other functional groups or effector groups may be accomplished in
the same manner, using appropriate chemistry, coupling at the N- or C-termini or via side
chains. In one embodiment, the coupling is conducted in such a manner that it does not block
the activity of either entity.

20

Pharmaceutical Compositions

According to a further aspect of the invention, there is provided a pharmaceutical composition
comprising a peptide ligand as defined herein in combination with one or more
pharmaceutically acceptable excipients.

25

Generally, the present peptide ligands will be utilised in purified form together with
pharmacologically appropriate excipients or carriers. Typically, these excipients or carriers
include aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline
and/or buffered media. Parenteral vehicles include sodium chloride solution, Ringer's
30 dextrose, dextrose and sodium chloride and lactated Ringer's. Suitable physiologically-
acceptable adjuvants, if necessary to keep a polypeptide complex in suspension, may be
chosen from thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin and
alginates.

35 Intravenous vehicles include fluid and nutrient replenishers and electrolyte replenishers, such
as those based on Ringer's dextrose. Preservatives and other additives, such as

antimicrobials, antioxidants, chelating agents and inert gases, may also be present (Mack (1982) Remington's Pharmaceutical Sciences, 16th Edition).

The compounds of the invention can be used alone or in combination with another agent or
5 agents. The other agent for use in combination may be for example another antibiotic, or an antibiotic 'adjuvant' such as an agent for improving permeability into Gram-negative bacteria, an inhibitor of resistance determinants or an inhibitor of virulence mechanisms.

Suitable antibiotics for use in combination with the compounds of the invention include but
10 are not limited to:
Beta lactams, such as penicillins, cephalosporins, carbapenems or monobactams. Suitable penicillins include oxacillin, methicillin, ampicillin, cloxacillin, carbenicillin, piperacillin, ticarcillin, flucloxacillin, and nafcillin; suitable cephalosporins include cefazolin, cefalexin, cefalothin, ceftazidime, cefepime, ceftobiprole, ceftaroline, ceftolozane and cefiderocol;
15 suitable carbapenems include meropenem, doripenem, imipenem, ertapenem, biapenem and tebipenem; suitable monobactams include aztreonam;
Lincosamides such as clindamycin and lincomycin;
Macrolides such as azithromycin, clarithromycin, erythromycin, telithromycin and solithromycin;
20 Tetracyclines such as tigecycline, omadacycline, eravacycline, doxycycline, and minocycline;
Quinolones such as ciprofloxacin, levofloxacin, moxifloxacin, and delafloxacin;
Rifamycins such as rifampicin, rifabutin, rifalazil, rifapentine, and rifaximin;
Aminoglycosides such as gentamycin, streptomycin, tobramycin, amikacin and plazomicin;
25 Glycopeptides such as vancomycin, teichoplanin, telavancin, dalbavancin, and oritavancin,
Pleuromutilins such as lefamulin
Oxazolidinones such as linezolid or tedizolid
Polymyxins such as polymyxin B or colistin;
Trimethoprim, iclaprim, sulfamethoxazole;
30 Metronidazole;
Fidaxomicin;
Mupirocin;
Fusidic acid;
Daptomycin;
35 Murepavidin;
Fosfomicin; and
Nitrofurantoin.

Suitable antibiotic 'adjuvants' include but are not limited to:

agents known to improve uptake into bacteria such as outer membrane permeabilisers or efflux pump inhibitors; outer membrane permeabilisers may include polymyxin B

- 5 nonapeptide or other polymyxin analogues, or sodium edetate;
inhibitors of resistance mechanisms such as beta-lactamase inhibitors; suitable beta-lactamase inhibitors include clavulanic acid, tazobactam, sulbactam, avibactam, relebactam and nacubactam; and
inhibitors of virulence mechanisms such as toxins and secretion systems, including
10 antibodies.

The compounds of the invention can also be used in combination with biological therapies such as nucleic acid based therapies, antibodies, bacteriophage or phage lysins.

- 15 The route of administration of pharmaceutical compositions according to the invention may be any of those commonly known to those of ordinary skill in the art. For therapy, the peptide ligands of the invention can be administered to any patient in accordance with standard techniques. Routes of administration include, but are not limited to, oral (e.g., by ingestion); buccal; sublingual; transdermal (including, e.g., by a patch, plaster, etc.); transmucosal
20 (including, e.g., by a patch, plaster, etc.); intranasal (e.g., by nasal spray); ocular (e.g., by eyedrops); pulmonary (e.g., by inhalation or insufflation therapy using, e.g., via an aerosol, e.g., through the mouth or nose); rectal (e.g., by suppository or enema); vaginal (e.g., by pessary); parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular,
25 subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot or reservoir, for example, subcutaneously or intramuscularly. Preferably, the pharmaceutical compositions according to the invention will be administered parenterally. The dosage and frequency of administration will depend on the age, sex and condition of the patient, concurrent administration of other
30 drugs, counterindications and other parameters to be taken into account by the clinician.

- The peptide ligands of this invention can be lyophilised for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective and art-known lyophilisation and reconstitution techniques can be employed. It will be appreciated by those
35 skilled in the art that lyophilisation and reconstitution can lead to varying degrees of activity loss and that levels may have to be adjusted upward to compensate.

The compositions containing the present peptide ligands or a cocktail thereof can be administered for therapeutic treatments. In certain therapeutic applications, an adequate amount to accomplish at least partial inhibition, suppression, modulation, killing, or some other measurable parameter, of a population of selected cells is defined as a "therapeutically-effective dose". Amounts needed to achieve this dosage will depend upon the severity of the disease and the general state of the patient's own immune system, but generally range from 10 µg to 250 mg of selected peptide ligand per kilogram of body weight, with doses of between 100 µg to 25 mg/kg/dose being more commonly used.

- 10 A composition containing a peptide ligand according to the present invention may be utilised in therapeutic settings to treat a microbial infection or to provide prophylaxis to a subject at risk of infection eg undergoing surgery, chemotherapy, artificial ventilation or other condition or planned intervention. In addition, the peptide ligands described herein may be used extracorporeally or *in vitro* selectively to kill, deplete or otherwise effectively remove a target
- 15 cell population from a heterogeneous collection of cells. Blood from a mammal may be combined extracorporeally with the selected peptide ligands whereby the undesired cells are killed or otherwise removed from the blood for return to the mammal in accordance with standard techniques.

20 ***Therapeutic Uses***

- The bicyclic peptides of the invention have specific utility as high affinity binders of membrane type 1 metalloprotease (MT1-MMP, also known as MMP14). More specifically to the collagen binding region of the hemopexin domain (Arkadash *et al* J. Biol. Chem. 2017, 292(8), 3481-3495). MT1-MMP is a transmembrane metalloprotease that plays a major role
- 25 in the extracellular matrix remodeling, directly by degrading several of its components and indirectly by activating pro-MMP2. MT1-MMP is crucial for tumor angiogenesis (Sounni *et al* (2002) FASEB J. 16(6), 555-564) and is over-expressed on a variety of solid tumours, therefore the drug conjugates comprising MT1-MMP-binding bicycle peptides of the present invention have particular utility in the targeted treatment of cancer, in particular solid tumours
- 30 such as non-small cell lung carcinomas. In one embodiment, the bicyclic peptide of the invention is specific for human MT1-MMP. In a further embodiment, the bicyclic peptide of the invention is specific for mouse MT1-MMP. In a yet further embodiment, the bicyclic peptide of the invention is specific for human and mouse MT1-MMP. In a yet further embodiment, the bicyclic peptide of the invention is specific for human, mouse and dog MT1-
- 35 MMP.

Polypeptide ligands of the invention may be employed in *in vivo* therapeutic and prophylactic applications, *in vitro* and *in vivo* diagnostic applications, *in vitro* assay and reagent applications, and the like. Ligands having selected levels of specificity are useful in applications which involve testing in non-human animals, where cross-reactivity is desirable, or in diagnostic applications, where cross-reactivity with homologues or paralogues needs to be carefully controlled. In some applications, such as vaccine applications, the ability to elicit an immune response to predetermined ranges of antigens can be exploited to tailor a vaccine to specific diseases and pathogens.

Substantially pure peptide ligands of at least 90 to 95% homogeneity are preferred for administration to a mammal, and 98 to 99% or more homogeneity is most preferred for pharmaceutical uses, especially when the mammal is a human. Once purified, partially or to homogeneity as desired, the selected polypeptides may be used diagnostically or therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings and the like (Lefkovite and Pernis, (1979 and 1981) Immunological Methods, Volumes I and II, Academic Press, NY).

The conjugates of the peptide ligands of the present invention will typically find use in preventing, suppressing or treating cancer, in particular solid tumours such as non-small cell lung carcinomas.

Thus, according to a further aspect of the invention, there are provided drug conjugates of the peptide ligand as defined herein for use in preventing, suppressing or treating cancer, in particular solid tumours such as non-small cell lung carcinomas.

According to a further aspect of the invention, there is provided a method of preventing, suppressing or treating cancer, in particular solid tumours such as non-small cell lung carcinomas which comprises administering to a patient in need thereof a drug conjugate of the peptide ligand as defined herein.

Examples of cancers (and their benign counterparts) which may be treated (or inhibited) include, but are not limited to tumours of epithelial origin (adenomas and carcinomas of various types including adenocarcinomas, squamous carcinomas, transitional cell carcinomas and other carcinomas) such as carcinomas of the bladder and urinary tract, breast, gastrointestinal tract (including the esophagus, stomach (gastric), small intestine, colon, rectum and anus), liver (hepatocellular carcinoma), gall bladder and biliary system, exocrine pancreas, kidney, lung (for example adenocarcinomas, small cell lung carcinomas,

- non-small cell lung carcinomas, bronchioalveolar carcinomas and mesotheliomas), head and neck (for example cancers of the tongue, buccal cavity, larynx, pharynx, nasopharynx, tonsil, salivary glands, nasal cavity and paranasal sinuses), ovary, fallopian tubes, peritoneum, vagina, vulva, penis, cervix, myometrium, endometrium, thyroid (for example thyroid follicular carcinoma), adrenal, prostate, skin and adnexae (for example melanoma, basal cell carcinoma, squamous cell carcinoma, keratoacanthoma, dysplastic naevus); haematological malignancies (i.e. leukemias, lymphomas) and premalignant haematological disorders and disorders of borderline malignancy including haematological malignancies and related conditions of lymphoid lineage (for example acute lymphocytic leukemia [ALL], chronic lymphocytic leukemia [CLL], B-cell lymphomas such as diffuse large B-cell lymphoma [DLBCL], follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, T-cell lymphomas and leukaemias, natural killer [NK] cell lymphomas, Hodgkin's lymphomas, hairy cell leukaemia, monoclonal gammopathy of uncertain significance, plasmacytoma, multiple myeloma, and post-transplant lymphoproliferative disorders), and haematological malignancies and related conditions of myeloid lineage (for example acute myelogenous leukemia [AML], chronic myelogenous leukemia [CML], chronic myelomonocytic leukemia [CMML], hypereosinophilic syndrome, myeloproliferative disorders such as polycythaemia vera, essential thrombocythaemia and primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, and promyelocytic leukemia);
- tumours of mesenchymal origin, for example sarcomas of soft tissue, bone or cartilage such as osteosarcomas, fibrosarcomas, chondrosarcomas, rhabdomyosarcomas, leiomyosarcomas, liposarcomas, angiosarcomas, Kaposi's sarcoma, Ewing's sarcoma, synovial sarcomas, epithelioid sarcomas, gastrointestinal stromal tumours, benign and malignant histiocytomas, and dermatofibrosarcoma protuberans; tumours of the central or peripheral nervous system (for example astrocytomas, gliomas and glioblastomas, meningiomas, ependymomas, pineal tumours and schwannomas); endocrine tumours (for example pituitary tumours, adrenal tumours, islet cell tumours, parathyroid tumours, carcinoid tumours and medullary carcinoma of the thyroid); ocular and adnexal tumours (for example retinoblastoma); germ cell and trophoblastic tumours (for example teratomas, seminomas, dysgerminomas, hydatidiform moles and choriocarcinomas); and paediatric and embryonal tumours (for example medulloblastoma, neuroblastoma, Wilms tumour, and primitive neuroectodermal tumours); or syndromes, congenital or otherwise, which leave the patient susceptible to malignancy (for example Xeroderma Pigmentosum).
- References herein to the term "prevention" involves administration of the protective composition prior to the induction of the disease. "Suppression" refers to administration of the composition after an inductive event, but prior to the clinical appearance of the disease.

"Treatment" involves administration of the protective composition after disease symptoms become manifest.

Animal model systems which can be used to screen the effectiveness of the drug conjugates in protecting against or treating the disease are available. The use of animal model systems is facilitated by the present invention, which allows the development of polypeptide ligands which can cross react with human and animal targets, to allow the use of animal models.

The invention is further described below with reference to the following examples.

10

Examples

Materials and Methods

Peptide Synthesis

Peptide synthesis was based on Fmoc chemistry, using a Symphony peptide synthesiser manufactured by Peptide Instruments and a Syro II synthesiser by MultiSynTech. Standard Fmoc-amino acids were employed (Sigma, Merck), with appropriate side chain protecting groups: where applicable standard coupling conditions were used in each case, followed by deprotection using standard methodology.

Alternatively, peptides were purified using HPLC and following isolation they were modified with 1,3,5-triacryloylhexahydro-1,3,5-triazine (TATA, Sigma). For this, linear peptide was diluted with 50:50 MeCN:H₂O up to ~35 mL, ~500 µL of 100 mM TATA in acetonitrile was added, and the reaction was initiated with 5 mL of 1 M NH₄HCO₃ in H₂O. The reaction was allowed to proceed for ~30 -60 min at RT, and lyophilised once the reaction had completed (judged by MALDI). Once completed, 1ml of 1M L-cysteine hydrochloride monohydrate (Sigma) in H₂O was added to the reaction for ~60 min at RT to quench any excess TATA.

Following lyophilisation, the modified peptide was purified as above, while replacing the Luna C8 with a Gemini C18 column (Phenomenex), and changing the acid to 0.1% trifluoroacetic acid. Pure fractions containing the correct TATA-modified material were pooled, lyophilised and kept at -20°C for storage.

All amino acids, unless noted otherwise, were used in the L- configurations.

In some cases peptides are converted to activated disulfides prior to coupling with the free thiol group of a toxin using the following method; a solution of 4-methyl(succinimidyl 4-(2-pyridylthio)pentanoate) (100mM) in dry DMSO (1.25 mol equiv) was added to a solution of peptide (20mM) in dry DMSO (1 mol equiv). The reaction was well mixed and DIPEA (20 mol equiv) was added. The reaction was monitored by LC/MS until complete.

BIOLOGICAL DATA

Human Fluorescence Polarisation Competition Binding Assay

Due to its high affinity to the MT1-MMP Hemopexin domain (PEX), the fluoresceinated derivative of 17-88-N006 (SEQ ID NO: 10) can be used for competition experiments (using FP for detection). Here, a preformed complex of PEX with the fluorescent PEX-binding tracer is titrated with free, non-fluoresceinated bicyclic peptide. Since all 17-69-based peptides are expected to bind at the same site, the titrant will displace the fluorescent tracer from PEX. Dissociation of the complex can be measured quantitatively, and the K_d of the competitor (titrant) to the target protein determined. The advantage of the competition method is that the affinities of non-fluoresceinated bicyclic peptides can be determined accurately and rapidly. Concentrations of tracer are usually at the K_d or below (here, 1 nM), and the binding protein (here, hemopexin of MT1-MMP) is at a 15-fold excess such that >90% of the tracer is bound. Subsequently, the non-fluorescent competitor bicyclic peptide (usually just the bicycle core sequence) is titrated, such that it displaces the fluorescent tracer from the target protein. The displacement of the tracer is measured and associated with a drop in fluorescence polarisation. The drop in fluorescence polarisation is proportional to the fraction of target protein bound with the non-fluorescent titrant, and thus is a measure of the affinity of titrant to target protein.

The raw data is fit to the analytical solution of the cubic equation that describes the equilibria between fluorescent tracer, titrant, and binding protein. The fit requires the value of the affinity of fluorescent tracer to the target protein, which can be determined separately by direct binding FP experiments (see next section). The curve fitting was performed using Sigmaplot 12.0 and used as an adapted version of the equation described by Zhi-Xin Wang (FEBS Letters (1995) 360, 111-114).

Selected peptides of the invention were tested in the above mentioned Human Fluorescence Polarisation competition binding assay and the results are shown in Table 1:

Table 1: Human MT1-MMP Fluorescence Polarisation Competition Binding

Bicyclic Peptide	Molecular Scaffold	Tracer	K_i	n
BCY1026	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	2801.67 ± 1040.54	3
BCY1057	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	436.5 ± 81.34	2
BCY1065	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	567 ± 137.2	2
BCY1067	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	429.5 ± 44.1	2
BCY1073	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	554.5 ± 238.14	2
BCY1074	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	356 ± 45.08	2
BCY1075	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	441 ± 113.68	2
BCY1076	TATA	ACPYSWETCLFGDYRCA[Sar] ₆ [KFI] (17-88-N006) (SEQ ID NO: 10)	903.5 ± 12.74	2

CLAIMS

1. A peptide ligand specific for the collagen binding site of MT1-MMP comprising a polypeptide comprising at least three cysteine residues, separated by at least two loop sequences, and a non-aromatic molecular scaffold which forms covalent bonds with the cysteine residues of the polypeptide such that at least two polypeptide loops are formed on the molecular scaffold.
2. The peptide ligand as defined in claim 1, wherein said loop sequences comprise 6 amino acids.
3. The peptide ligand as defined in claim 1 or claim 2, wherein said loop sequences comprise three cysteine residues separated by two loop sequences both of which consists of 6 amino acids.
4. The peptide ligand as defined in any one of claims 1 to 3, wherein the peptide ligand comprises an amino acid sequence selected from:
 C_i -P-F/I/Y-D/S-W-H-T- C_{ii} -L-F-G-D/E-Y-T/S- C_{iii} (SEQ ID NO: 1)
 wherein C_i , C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively or a pharmaceutically acceptable salt thereof.
5. The peptide ligand as defined in claim 4, wherein the peptide ligand of C_i -P-F/I/Y-D/S-W-H-T- C_{ii} -L-F-G-D/E-Y-T/S- C_{iii} (SEQ ID NO: 1) is selected from:
 $CPYSWETCLFGDYRC$ (SEQ ID NO: 2);
 $CPFDWHTCLFGDYTC$ (SEQ ID NO: 3);
 $CPFDWHTCLFGEYSC$ (SEQ ID NO: 4);
 $CPIDWHTCLFGDYTC$ (SEQ ID NO: 5);
 $CPFSWHTCLFGEYSC$ (SEQ ID NO: 6);
 $CPFSWHTCLFGDYTC$ (SEQ ID NO: 7);
 $CPISWHTCLFGDYSC$ (SEQ ID NO: 8); and
 $CPYSWHTCLFGDYSC$ (SEQ ID NO: 9);
 such as:
 A -(SEQ ID NO: 2)- A (BCY1026);
 A -(SEQ ID NO: 3)- A (BCY1057);
 A -(SEQ ID NO: 4)- A (BCY1065);
 A -(SEQ ID NO: 5)- A (BCY1067);

A-(SEQ ID NO: 6)-A (BCY1073);
 A-(SEQ ID NO: 7)-A (BCY1074);
 A-(SEQ ID NO: 8)-A (BCY1075); and
 A-(SEQ ID NO: 9)-A (BCY1076).

5

6. The peptide ligand as defined in any one of claims 1 to 5, wherein the molecular scaffold is TATA.

7. The peptide ligand as defined in claim 6, wherein the molecular scaffold is TATA and the peptide ligand of C_i-P-F/I/Y-D/S-W-H-T-C_{ii}-L-F-G-D/E-Y-T/S-C_{iii} (SEQ ID NO: 1) is selected from:

A-(SEQ ID NO: 2)-A (BCY1026);
 A-(SEQ ID NO: 3)-A (BCY1057);
 A-(SEQ ID NO: 4)-A (BCY1065);
 A-(SEQ ID NO: 5)-A (BCY1067);
 A-(SEQ ID NO: 6)-A (BCY1073);
 A-(SEQ ID NO: 7)-A (BCY1074);
 A-(SEQ ID NO: 8)-A (BCY1075); and
 A-(SEQ ID NO: 9)-A (BCY1076).

20

8. The peptide ligand as defined in any one of claims 1 to 7, wherein the pharmaceutically acceptable salt is selected from the free acid or the sodium, potassium, calcium, ammonium salt.

9. The peptide ligand as defined in any one of claims 1 to 8, wherein the MT1-MMP is human MT1-MMP.

10. A drug conjugate comprising a peptide ligand as defined in any one of claims 1 to 9, conjugated to one or more effector and/or functional groups.

30

11. The drug conjugate as defined in claim 10, conjugated to one or more cytotoxic agents.

12. The drug conjugate as defined in claim 11, wherein said cytotoxic agent is selected from MMAE or DM1.

35

13. A pharmaceutical composition which comprises the peptide ligand of any one of claims 1 to 9 or the drug conjugate of any one of claims 10 to 12, in combination with one or more pharmaceutically acceptable excipients.

5 14. The pharmaceutical composition as defined in claim 13, which additionally comprises one or more therapeutic agents.

15. The drug conjugate as defined in any one of claims 10 to 12, for use in preventing, suppressing or treating a disease or disorder mediated by MT1-MMP.

10