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(54) Title: BICYCLIC PEPTIDE LIGANDS SPECIFIC FOR CD38

(57) Abstract: 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 CD38.The invention also includes drug conjugates comprising said peptides, conjugated to one or more effector and/or functional groups, to pharmaceutical compositions comprising said peptide ligands and drug conjugates and to the use of said peptide ligands and drug conjugates in preventing, suppressing or treating a disease or disorder mediated by CD38.

BICYCLIC PEPTIDE LIGANDS SPECIFIC FOR CD38

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 CD38. The invention also includes drug conjugates comprising said peptides, conjugated to one or more effector and/or functional groups, to pharmaceutical compositions comprising said peptide ligands and drug conjugates and to the use of said peptide ligands and drug conjugates in preventing, suppressing or treating a disease or disorder mediated by CD38.

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 $Å^2$; Wu *et al.* (2007), Science 330, 1066-71), a cyclic peptide with the Arg-Gly-Asp motif binding to integrin αVb3 (355 $Å^2$) (Xiong *et al.* (2002), Science *296* (5565), 151-5) or the cyclic peptide inhibitor upain-1 binding to urokinase-type plasminogen activator (603 $Å^2$; 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). Meloen 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 compounds are generated by linking cysteine containing polypeptides to a molecular scaffold as for example 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) (Heinis *et al* (2014) Angewandte Chemie, International Edition 53(6) 1602-1606).

Phage display-based combinatorial approaches have been developed to generate and screen 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 scaffold.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a peptide ligand specific for CD38 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.

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 CD38.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, said loop sequences comprise 2 or 7 amino acids.

In a further embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences one of which consists of 2 amino acids and the other of which consists of 7 amino acids.

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

C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23);

wherein X_1-X_5 represent any amino acid residue, and C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively or a pharmaceutically acceptable salt thereof.

In one embodiment, said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 7 amino acids and the second of which consists of 2 amino acids, and said peptide ligand comprises an amino acid sequence selected from:

C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23);

wherein X_1-X_6 represent any amino acid residue, and C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively or a pharmaceutically acceptable salt thereof.

In a further embodiment, the peptide ligand of C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23) comprises a peptide ligand of C_i-X_1-W-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 24).

In a further embodiment, the peptide ligand of C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23) comprises an amino acid sequence selected from any one of SEQ ID NOS: 1-22:

C_iYWNPFMGC_{ii}YTC_{iii} (SEQ ID NO: 1);
C_iYWNPFTGC_{ii}YSC_{iii} (SEQ ID NO: 2);
C_iYWNPFIQC_{ii}SPC_{iii} (SEQ ID NO: 3);
C_iYWNPFTAC_{ii}YMC_{iii} (SEQ ID NO: 4);
C_iYWNPFTAC_{ii}YTC_{iii} (SEQ ID NO: 5);
C_iYWNPFAAC_{ii}YDC_{iii} (SEQ ID NO: 6);
C_iYWNPFSGC_{ii}YSC_{iii} (SEQ ID NO: 7);
C_iMWNPFTGC_{ii}YAC_{iii} (SEQ ID NO: 8);
C_iLYNPFTGC_{ii}YDC_{iii} (SEQ ID NO: 9);
C_iYWNPFSGC_{ii}WDC_{iii} (SEQ ID NO: 10);
C_iYWNPFMAC_{ii}FDC_{iii} (SEQ ID NO: 11);
C_iYWNPFMGC_{ii}YSC_{iii} (SEQ ID NO: 12);
C_iYWNPFMAC_{ii}YVC_{iii} (SEQ ID NO: 13);

C_iYWNPFMGC_{ii}YVC_{iii} (SEQ ID NO: 14);
C_iYWNPFTGC_{ii}YAC_{iii} (SEQ ID NO: 15);
C_iYWNPFTAC_{ii}WSC_{iii} (SEQ ID NO: 16);
C_iFWNPFTGC_{ii}YSC_{iii} (SEQ ID NO: 17);
C_iYWNPFTAC_{ii}FSC_{iii} (SEQ ID NO: 18);
C_iYWNPFTAC_{ii}YVC_{iii} (SEQ ID NO: 19);
C_iYWNPFTAC_{ii}YSC_{iii} (SEQ ID NO: 20);
C_iYWNPFTAC_{ii}WAC_{iii} (SEQ ID NO: 21); and
C_iYWNPFTAC_{ii}FAC_{iii} (SEQ ID NO: 22);

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

In a further embodiment, the peptide ligand of C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23) comprises an amino acid sequence selected from:

A-(SEQ ID NO: 1)-A (herein referred to as 66-50-01-N001);
A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);
A-(SEQ ID NO: 3)-A (herein referred to as 66-50-03-N001);
A-(SEQ ID NO: 4)-A (herein referred to as 66-50-04-N001);
A-(SEQ ID NO: 5)-A (herein referred to as 66-50-05-N001);
A-(SEQ ID NO: 6)-A (herein referred to as 66-50-06-N001);
A-(SEQ ID NO: 7)-A (herein referred to as 66-50-07-N001);
A-(SEQ ID NO: 8)-A (herein referred to as 66-50-08-N001);
A-(SEQ ID NO: 9)-A (herein referred to as 66-50-09-N001);
A-(SEQ ID NO: 10)-A (herein referred to as 66-50-10-N001);
A-(SEQ ID NO: 11)-A (herein referred to as 66-50-11-N001);
A-(SEQ ID NO: 12)-A (herein referred to as 66-50-12-N001);
A-(SEQ ID NO: 13)-A (herein referred to as 66-50-13-N001);
A-(SEQ ID NO: 14)-A (herein referred to as 66-50-14-N001);
A-(SEQ ID NO: 15)-DST (herein referred to as 66-50-15-T01-N001);
A-(SEQ ID NO: 15)-EAD (herein referred to as 66-50-15-T02-N001);
A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);
A-(SEQ ID NO: 16)-DTS (herein referred to as 66-50-22-T01-N001).
ASDN-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T01-N001);
ARNE-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T02-N001);
A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001);
A-(SEQ ID NO: 19)-DVP (herein referred to as 66-50-19-T01-N001);

A-(SEQ ID NO: 20)-TEN (herein referred to as 66-50-19-T02-N001);
A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001);
A-(SEQ ID NO: 22)-EEP (herein referred to as 66-50-21-T01-N001); and

In one embodiment, the molecular scaffold is selected from 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises an amino acid sequence selected from:

A-(SEQ ID NO: 1)-A (herein referred to as 66-50-01-N001);
A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);
A-(SEQ ID NO: 3)-A (herein referred to as 66-50-03-N001);
A-(SEQ ID NO: 4)-A (herein referred to as 66-50-04-N001);
A-(SEQ ID NO: 5)-A (herein referred to as 66-50-05-N001);
A-(SEQ ID NO: 6)-A (herein referred to as 66-50-06-N001);
A-(SEQ ID NO: 7)-A (herein referred to as 66-50-07-N001);
A-(SEQ ID NO: 8)-A (herein referred to as 66-50-08-N001);
A-(SEQ ID NO: 9)-A (herein referred to as 66-50-09-N001);
A-(SEQ ID NO: 10)-A (herein referred to as 66-50-10-N001);
A-(SEQ ID NO: 11)-A (herein referred to as 66-50-11-N001);
A-(SEQ ID NO: 12)-A (herein referred to as 66-50-12-N001);
A-(SEQ ID NO: 13)-A (herein referred to as 66-50-13-N001);
A-(SEQ ID NO: 14)-A (herein referred to as 66-50-14-N001);
A-(SEQ ID NO: 15)-DST (herein referred to as 66-50-15-T01-N001);
A-(SEQ ID NO: 15)-EAD (herein referred to as 66-50-15-T02-N001);
A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);
A-(SEQ ID NO: 16)-DTS (herein referred to as 66-50-22-T01-N001).
ASDN-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T01-N001);
ARNE-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T02-N001);
A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001);
A-(SEQ ID NO: 19)-DVP (herein referred to as 66-50-19-T01-N001);
A-(SEQ ID NO: 20)-TEN (herein referred to as 66-50-19-T02-N001);
A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001);
A-(SEQ ID NO: 22)-EEP (herein referred to as 66-50-21-T01-N001); and

In a further embodiment, the molecular scaffold is selected from 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises an amino acid sequence selected from:

A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);
A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);
A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001); and
A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001).

The scaffold/peptide ligands of this embodiment demonstrated superior CD38 competition binding as shown herein in Table 1.

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 (1999) 4th ed., John Wiley & Sons, Inc.), which are incorporated herein by reference.

Nomenclature

Numbering

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:

$$-C_i-Y_1-W_2-N_3-P_4-F_5-M_6-G_7-C_{ii}-Y_8-T_9-C_{iii}- \text{ (SEQ ID NO: 1).}$$

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)triprop-2-en-1-one (TATA) and yielding a tri-substituted structure. Cyclisation with TATA occurs on C_i, C_{ii}, and C_{iii}.

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).

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*).

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 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

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:

- Species cross-reactivity. This is a typical requirement for preclinical pharmacodynamics and pharmacokinetic evaluation;

- 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 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;

- 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. 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 good selectivity over other CDs.

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.

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 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, ascorbic (e.g. L-ascorbic), L-aspartic, benzenesulfonic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulfonic, (+)-(1*S*)-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, hydrohalic acids (e.g. hydrobromic, hydrochloric, hydriodic), isethionic, lactic (e.g. (+)-L-lactic, (±)-DL-lactic), lactobionic, maleic, malic, (-)-L-malic, malonic, (±)-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 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,

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 Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+} or Zn^+. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). 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 $N(CH_3)_4^+$.

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.

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.

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 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.

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 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.

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.

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, Cα-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 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. In a further embodiment, the modified derivative comprises replacement of a tryptophan residue with a naphthylalanine or alanine residue. This embodiment provides the advantage of improving the pharmaceutical stability profile of the resultant bicyclic peptide ligand.

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 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 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).

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).

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 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).

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 ^{2}H (D) and ^{3}H (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, and to clinically assess the presence and/or absence of the CD38 target on diseased tissues. 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.* ^{3}H (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.

Substitution with heavier isotopes such as deuterium, *i.e.* ^{2}H (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.

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.

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.

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

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.

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.

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.

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).

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.

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).

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 tβ 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 tβ half-life of a day or more.

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.

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 tβ 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 tβ half life in the range 12 to 60 hours. In a further embodiment, it will have a tβ half-life of a day or more. In a further embodiment still, it will be in the range 12 to 26 hours.

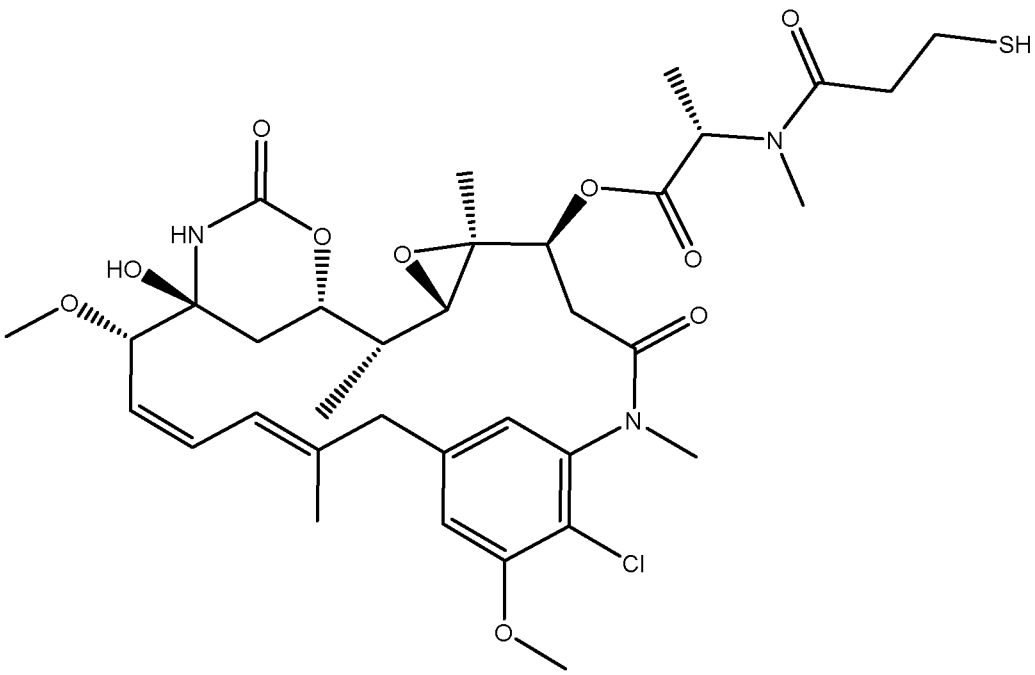
In one particular embodiment of the invention, the functional group is selected from a metal chelator, which is suitable for complexing metal radioisotopes of medicinal relevance.

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.

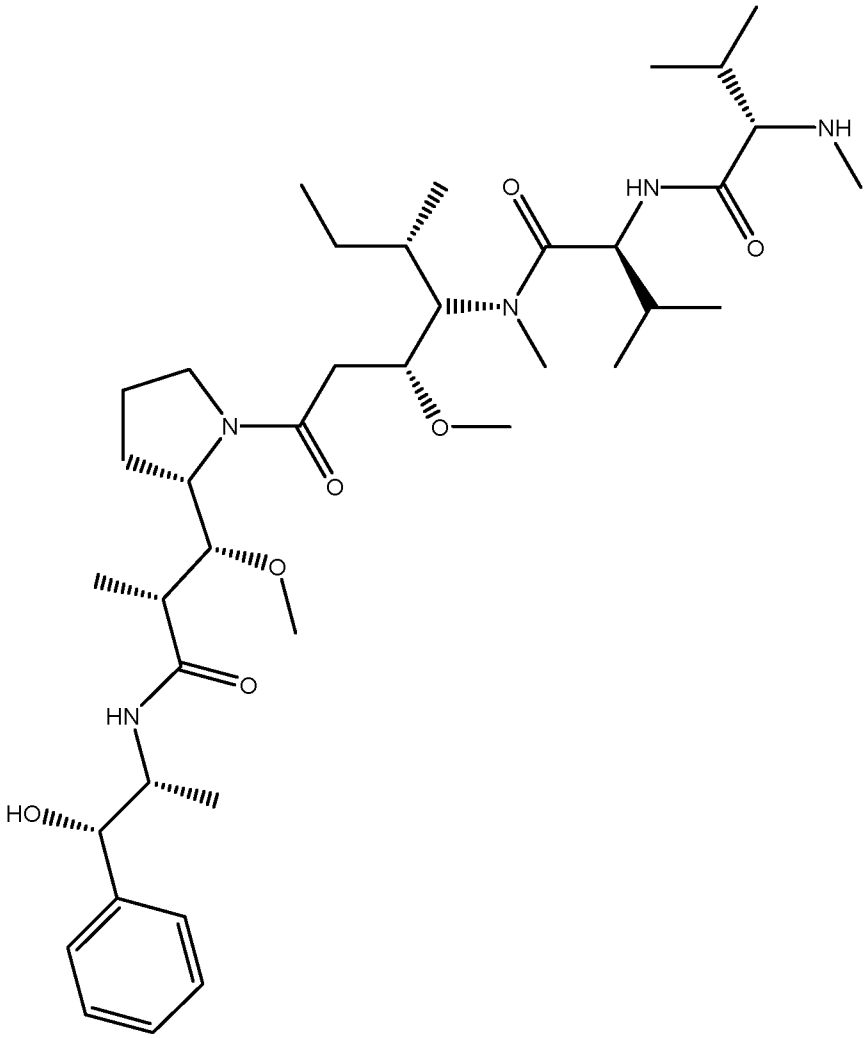
In one particular embodiment of the invention, the functional group is selected from a drug, such as a cytotoxic agent for cancer therapy. Suitable examples include: alkylating agents such as cisplatin and carboplatin, as well as oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide; Anti-metabolites including purine analogs azathioprine and mercaptopurine or pyrimidine analogs; plant alkaloids and terpenoids including vinca alkaloids 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 (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).

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 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 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.

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.

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

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* (*supra*).

Thus, the invention also relates to manufacture of polypeptides or conjugates 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/conjugate made by chemical synthesis.

Optionally amino acid residues in the polypeptide of interest may be substituted when manufacturing a conjugate or complex.

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

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. 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 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 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 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.

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.

Pharmaceutical Compositions

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.

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 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.

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 peptide ligands of the present invention may be used as separately administered compositions or in conjunction with other agents. These can include antibodies, antibody fragments and various immunotherapeutic drugs, such as cyclosporine, methotrexate, adriamycin or cisplatinum and immunotoxins. Pharmaceutical compositions can include "cocktails" of various cytotoxic or other agents in conjunction with the protein ligands of the present invention, or even combinations of selected polypeptides according to the present invention having different specificities, such as polypeptides selected using different target ligands, whether or not they are pooled prior to administration.

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. The administration can be by any appropriate mode, including parenterally, intravenously, intramuscularly, intraperitoneally, transdermally, via the pulmonary route, or also, appropriately, by direct infusion with a catheter. Preferably, the pharmaceutical compositions according to the invention will be administered by inhalation. The dosage and frequency of administration will depend on the age, sex and condition of the patient, concurrent administration of other 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 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 prophylactic and/or 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 0.005 to 5.0 mg of selected peptide ligand per kilogram of body weight, with doses of 0.05 to 2.0 mg/kg/dose being more commonly used. For

prophylactic applications, compositions containing the present peptide ligands or cocktails thereof may also be administered in similar or slightly lower dosages.

A composition containing a peptide ligand according to the present invention may be utilised in prophylactic and therapeutic settings to aid in the alteration, inactivation, killing or removal of a select target cell population in a mammal. In addition, the peptide ligands described herein may be used extracorporeally or *in vitro* selectively to kill, deplete or otherwise effectively remove a target 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.

Therapeutic Uses

The bicyclic peptides of the invention have specific utility as CD38 binding agents.

CD38 is a 45 kD type II transmembrane glycoprotein with a long C-terminal extracellular domain and a short N-terminal cytoplasmic domain. The CD38 protein is a bifunctional ectoenzyme that can catalyze the conversion of NAD+ into cyclic ADP-ribose (cADPR) and also hydrolyze cADPR into ADP-ribose. During ontogeny, CD38 appears on CD34+ committed stem cells and lineage-committed progenitors of lymphoid, erythroid and myeloid cells. CD38 expression persists mostly in the lymphoid lineage with varying expression levels at different stages of T and B cell development.

CD38 is upregulated in many hematopoeitic malignancies and in cell lines derived from various hematopoietic malignancies, including non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma (BL), multiple myeloma (MM), B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), T cell lymphoma (TCL), acute myeloid leukemia (AML), hairy cell leukemia (HCL), Hodgkin's Lymphoma (HL), and chronic myeloid leukemia (CML). On the other hand, most primitive pluripotent stem cells of the hematopoietic system are CD38−. CD38 expression in hematopoietic malignancies and its correlation with disease progression makes CD38 an attractive target for antibody therapy.

CD38 has been reported to be involved in Ca^{2+} mobilization (Morra *et al.* (1998) FASEB J. 12; 581-592; Zilber *et al.* (2000) Proc Natl Acad Sci USA 97, 2840-2845) and in the signal transduction through tyrosine phosphorylation of numerous signaling molecules, including phospholipase C-γ, ZAP-70, syk, and c-cbl, in lymphoid and myeloid cells or cell lines

(Funaro *et al.* (1993) Eur J Immunol 23, 2407-2411; Morra *et al.* (1998), *supra*; Funaro *et al.* (1990) J Immunol 145, 2390-2396; Zubiaur *et al.* (1997) J Immunol 159, 193-205; Deaglio *et al.* (2003) Blood 102, 2146-2155; Todisco *et al.* (2000) Blood 95, 535-542; Konopleva *et al.* (1998) J Immunol 161, 4702-4708; Zilber *et al.* (2000) Proc Natl Acad Sci USA 97, 2840-2845; Kitanaka *et al.* (1997) J Immunol 159, 184-192; Kitanaka *et al.* (1999) J Immunol 162, 1952-1958; Mallone *et al.* (2001) Int Immunol 13, 397-409). On the basis of these observations, CD38 was proposed to be an important signaling molecule in the maturation and activation of lymphoid and myeloid cells during their normal development.

The exact role of CD38 in signal transduction and hematopoiesis is still not clear, especially since most of these signal transduction studies have used cell lines ectopically overexpressing CD38 and anti-CD38 monoclonal antibodies, which are non-physiological ligands. Because the CD38 protein has an enzymatic activity that produces cADPR, a molecule that can induce Ca^{2+} mobilization (Lee *et al.* (1989) J Biol Chem 264, 1608-1615; Lee and Aarhus (1991) Cell Regul 2, 203-209), it has been proposed that CD38 ligation by monoclonal antibodies triggers Ca^{2+} mobilization and signal transduction in lymphocytes by increasing production of cADPR (Lee *et al.* (1997) Adv Exp Med Biol 419, 411-419). Contrary to this hypothesis, the truncation and point-mutation analysis of CD38 protein showed that neither its cytoplasmic tail nor its enzymatic activity is necessary for the signaling mediated by anti-CD38 antibodies (Kitanaka *et al.* (1999) J Immunol 162, 1952-1958; Lund *et al.* (1999) J Immunol 162, 2693-2702; Hoshino *et al.* (1997) J Immunol 158, 741-747).

The best evidence for the function of CD38 comes from CD38−/− knockout mice, which have a defect in their innate immunity and a reduced T-cell dependent humoral response due to a defect in dendritic cell migration (Partida-Sanchez *et al.* (2004) Immunity 20, 279-291; Partida-Sanchez *et al.* (2001) Nat Med 7, 1209-1216). Nevertheless, it is not clear if the CD38 function in mice is identical to that in humans since the CD38 expression pattern during hematopoiesis differs greatly between human and mouse: a) unlike immature progenitor stem cells in humans, similar progenitor stem cells in mice express a high level of CD38 (Randall *et al.* (1996) Blood 87, 4057-4067; Dagher *et al.* (1998) Biol Blood Marrow Transplant 4, 69-74), b) while during the human B cell development, high levels of CD38 expression are found in germinal center B cells and plasma cells (Uckun (1990) Blood 76, 1908-1923; Kumagai *et al.* (1995) J Exp Med 181, 1101-1110), in the mouse, the CD38 expression levels in the corresponding cells are low (Oliver *et al.* (1997) J Immunol 158, 1108-1115; Ridderstad and Tarlinton (1998) J Immunol 160, 4688-4695).

Several anti-human CD38 antibodies with different proliferative properties on various tumor cells and cell lines have been described in the literature. For example, a chimeric OKT10 antibody with mouse Fab and human IgG1 Fc mediates antibody-dependent cell-mediated cytotoxicity (ADCC) very efficiently against lymphoma cells in the presence of peripheral blood mononuclear effector cells from either MM patients or normal individuals (Stevenson *et al.* (1991) Blood 77, 1071-1079). A CDR-grafted humanized version of the anti-CD38 antibody AT13/5 has been shown to have potent ADCC activity against CD38-positive cell lines (U.S. Patent Application No. 09/797,941). Human monoclonal anti-CD38 antibodies have been shown to mediate the *in vitro* killing of CD38-positive cell lines by ADCC and/or complement-dependent cytotoxicity (CDC), and to delay the tumor growth in SCID mice bearing MM cell line RPMI-8226 (WO 2005/103083). On the other hand, several anti-CD38 antibodies, IB4, SUN-4B7, and OKT10, but not IB6, AT1, or AT2, induced the proliferation of peripheral blood mononuclear cells (PBMC) from normal individuals (Ausiello *et al.* (2000) Tissue Antigens 56, 539-547).

Some of the antibodies of the prior art have been shown to be able to trigger apoptosis in CD38+ B cells. However, they can only do so in the presence of stroma cells or stroma-derived cytokines. An agonistic anti-CD38 antibody (IB4) has been reported to prevent apoptosis of human germinal center (GC) B cells (Zupo *et al.* (1994) Eur J Immunol 24, 1218-1222), and to induce proliferation of KG-1 and HL-60 AML cells (Konopleva *et al.* (1998) J Immunol 161, 4702-4708), but induces apoptosis in Jurkat T lymphoblastic cells (Morra *et al.* (1998) FASEB J 12, 581-592). Another anti-CD38 antibody T16 induced apoptosis of immature lymphoid cells and leukemic lymphoblast cells from an ALL patient (Kumagai *et al.* (1995) J Exp Med 181, 1101-1110), and of leukemic myeloblast cells from AML patients (Todisco *et al.* (2000) Blood 95, 535-542), but T16 induced apoptosis only in the presence of stroma cells or stroma-derived cytokines (IL-7, IL-3, stem cell factor).

Polypeptide ligands selected according to the method of the present 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).

According to a further aspect of the invention, there is provided a peptide ligand or a drug conjugate as defined herein, for use in preventing, suppressing or treating a disease or disorder mediated by CD38.

According to a further aspect of the invention, there is provided a method of preventing, suppressing or treating a disease or disorder mediated by CD38, which comprises administering to a patient in need thereof an effector group and drug conjugate of the peptide ligand as defined herein.

In one embodiment, the CD38 is mammalian CD38. In a further embodiment, the mammalian CD38 is human CD38 (hCD38).

In one embodiment, the disease or disorder mediated by CD38 is selected from cancer.

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 myelogenousleukemia [AML], chronic myelogenousleukemia [CML], chronic myelomonocyticleukemia [CMML], hypereosinophilic syndrome, myeloproliferative disorders such as polycythaemia vera, essential thrombocythaemia and primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, and promyelocyticleukemia); 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 dermatofibrosarcomaprotuberans; 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).

In a further embodiment, the cancer is selected from a hematopoietic malignancy such as selected from: non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma (BL), multiple myeloma (MM), B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), T cell lymphoma (TCL), acute myeloid leukemia (AML), hairy cell leukemia (HCL), Hodgkin's Lymphoma (HL), and chronic myeloid leukemia (CML).

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 peptide ligands 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.

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. 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:H_2O 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 NH_4HCO_3 in H_2O. 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 H_2O 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 -20ºC 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

1. CD38 Competition Binding Assay

Affinity of the peptides of the invention for human CD38 (Ki) was determined using a fluorescence polarisation assay, using the method reported by Lea *et al* (Expert Opin Drug Discov. 2011 6(1): 17–3) and using the following fluorescently labelled peptides ACYWNPFTGCYTCA-Sar_6-K(Fl) ((SEQ ID NO: 25)-Sar_6-K(Fl)) for TATA derivatives where Fl is a fluorescein molecule.

The peptide ligands of the invention were tested in the above mentioned CD38 competition binding assay and the results are shown in Table 1:

Table 1: Biological Assay Data for Peptide Ligands of the Invention

Peptide	***Molecular Scaffold***	***Ki (nM)***
66-50-01-N001	TATA	393 ± 141.12
66-50-02-N001	TATA	97.8 ± 22.67
66-50-03-N001	TATA	1703 ± 872.18
66-50-04-N001	TATA	811.5 ± 187.18
66-50-05-N001	TATA	438 ± 192.08
66-50-06-N001	TATA	935 n=1
66-50-07-N001	TATA	497.5 ± 198.94
66-50-08-N001	TATA	158 ± 68.24
66-50-09-N001	TATA	749 ± 64.68
66-50-10-N001	TATA	335 ± 56.84
66-50-11-N001	TATA	684.5 ± 157.78
66-50-12-N001	TATA	315 ± 362.59
66-50-13-N001	TATA	784.5 ± 87.22
66-50-14-N001	TATA	939.5 ± 285.17

66-50-15-T01-N001	TATA	104 ± 90.16
66-50-15-T02-N001	TATA	179 ± 125.44
66-50-16-T01-N001	TATA	90 ± 49
66-50-17-T01-N001	TATA	358.5 ± 187.18
66-50-17-T02-N001	TATA	260 ± 160.72
66-50-18-T01-N001	TATA	77.5 ± 51.94
66-50-19-T001-N001	TATA	575 ± 219.52
66-50-19-T002-N001	TATA	315.5 ± 4.9
66-50-20-T001-N001	TATA	85 ± 115.64
66-50-21-T001-N001	TATA	337 ± 229.32
66-50-22-T001-N001	TATA	182.5 ± 46.06

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CLAIMS

1. A peptide ligand specific for CD38 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 2 or 7 amino acids.

3. The peptide ligand as defined in claim 1 or claim 2, which comprises an amino acid sequence selected from:

C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23);

wherein X_1-X_5 represent any amino acid residue, and C_i, C_{ii} and C_{iii} represent first, second and third cysteine residues, respectively or a pharmaceutically acceptable salt thereof.

4. The peptide ligand as defined in any one of claims 1 to 3, wherein said loop sequences comprise three cysteine residues separated by two loop sequences the first of which consists of 7 amino acids and the second of which consists of 2 amino acids, and said peptide ligand comprises an amino acid sequence selected from:

C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23);

wherein X_1-X_5 represent any amino acid residue, and 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 3 or claim 4, wherein the peptide ligand of C_i-X_1-W/Y-N-P-F-X_2-X_3-C_{ii}-X_4-X_5-C_{iii} (SEQ ID NO: 23) comprises an amino acid sequence selected from any one of SEQ ID NOS: 1-22:

C_iYWNPFMGC_{ii}YTC_{iii} (SEQ ID NO: 1);

C_iYWNPFTGC_{ii}YSC_{iii} (SEQ ID NO: 2);

C_iYWNPFIQC_{ii}SPC_{iii} (SEQ ID NO: 3);

C_iYWNPFTAC_{ii}YMC_{iii} (SEQ ID NO: 4);

C_iYWNPFTAC_{ii}YTC_{iii} (SEQ ID NO: 5);

C_iYWNPFAAC_{ii}YDC_{iii} (SEQ ID NO: 6);

C_iYWNPFSGC_{ii}YSC_{iii} (SEQ ID NO: 7);

C_iMWNPFTGC_{ii}YAC_{iii} (SEQ ID NO: 8);

C_iLYNPFTGC_{ii}YDC_{iii} (SEQ ID NO: 9);

C_iYWNPFSGC_{ii}WDC$_{iii}$ (SEQ ID NO: 10);
C_iYWNPFMAC$_{ii}$FDC$_{iii}$ (SEQ ID NO: 11);
C_iYWNPFMGC$_{ii}$YSC$_{iii}$ (SEQ ID NO: 12);
C_iYWNPFMAC$_{ii}$YVC$_{iii}$ (SEQ ID NO: 13);
C_iYWNPFMGC$_{ii}$YVC$_{iii}$ (SEQ ID NO: 14);
C_iYWNPFTGC$_{ii}$YAC$_{iii}$ (SEQ ID NO: 15);
C_iYWNPFTAC$_{ii}$WSC$_{iii}$ (SEQ ID NO: 16);
C_iFWNPFTGC$_{ii}$YSC$_{iii}$ (SEQ ID NO: 17);
C_iYWNPFTAC$_{ii}$FSC$_{iii}$ (SEQ ID NO: 18);
C_iYWNPFTAC$_{ii}$YVC$_{iii}$ (SEQ ID NO: 19);
C_iYWNPFTAC$_{ii}$YSC$_{iii}$ (SEQ ID NO: 20);
C_iYWNPFTAC$_{ii}$WAC$_{iii}$ (SEQ ID NO: 21); and
C_iYWNPFTAC$_{ii}$FAC$_{iii}$ (SEQ ID NO: 22);

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

such as:

an amino acid sequence selected from:

A-(SEQ ID NO: 1)-A (herein referred to as 66-50-01-N001);
A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);
A-(SEQ ID NO: 3)-A (herein referred to as 66-50-03-N001);
A-(SEQ ID NO: 4)-A (herein referred to as 66-50-04-N001);
A-(SEQ ID NO: 5)-A (herein referred to as 66-50-05-N001);
A-(SEQ ID NO: 6)-A (herein referred to as 66-50-06-N001);
A-(SEQ ID NO: 7)-A (herein referred to as 66-50-07-N001);
A-(SEQ ID NO: 8)-A (herein referred to as 66-50-08-N001);
A-(SEQ ID NO: 9)-A (herein referred to as 66-50-09-N001);
A-(SEQ ID NO: 10)-A (herein referred to as 66-50-10-N001);
A-(SEQ ID NO: 11)-A (herein referred to as 66-50-11-N001);
A-(SEQ ID NO: 12)-A (herein referred to as 66-50-12-N001);
A-(SEQ ID NO: 13)-A (herein referred to as 66-50-13-N001);
A-(SEQ ID NO: 14)-A (herein referred to as 66-50-14-N001);
A-(SEQ ID NO: 15)-DST (herein referred to as 66-50-15-T01-N001);
A-(SEQ ID NO: 15)-EAD (herein referred to as 66-50-15-T02-N001);
A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);
A-(SEQ ID NO: 16)-DTS (herein referred to as 66-50-22-T01-N001).
ASDN-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T01-N001);

ARNE-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T02-N001);
A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001);
A-(SEQ ID NO: 19)-DVP (herein referred to as 66-50-19-T01-N001);
A-(SEQ ID NO: 20)-TEN (herein referred to as 66-50-19-T02-N001);
A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001);
A-(SEQ ID NO: 22)-EEP (herein referred to as 66-50-21-T01-N001); and

6. The peptide ligand as defined in claim 1, wherein the molecular scaffold is selected from 1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one (TATA) and the peptide ligand comprises an amino acid sequence selected from:

A-(SEQ ID NO: 1)-A (herein referred to as 66-50-01-N001);
A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);
A-(SEQ ID NO: 3)-A (herein referred to as 66-50-03-N001);
A-(SEQ ID NO: 4)-A (herein referred to as 66-50-04-N001);
A-(SEQ ID NO: 5)-A (herein referred to as 66-50-05-N001);
A-(SEQ ID NO: 6)-A (herein referred to as 66-50-06-N001);
A-(SEQ ID NO: 7)-A (herein referred to as 66-50-07-N001);
A-(SEQ ID NO: 8)-A (herein referred to as 66-50-08-N001);
A-(SEQ ID NO: 9)-A (herein referred to as 66-50-09-N001);
A-(SEQ ID NO: 10)-A (herein referred to as 66-50-10-N001);
A-(SEQ ID NO: 11)-A (herein referred to as 66-50-11-N001);
A-(SEQ ID NO: 12)-A (herein referred to as 66-50-12-N001);
A-(SEQ ID NO: 13)-A (herein referred to as 66-50-13-N001);
A-(SEQ ID NO: 14)-A (herein referred to as 66-50-14-N001);
A-(SEQ ID NO: 15)-DST (herein referred to as 66-50-15-T01-N001);
A-(SEQ ID NO: 15)-EAD (herein referred to as 66-50-15-T02-N001);
A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);
A-(SEQ ID NO: 16)-DTS (herein referred to as 66-50-22-T01-N001).
ASDN-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T01-N001);
ARNE-(SEQ ID NO: 17)-A (herein referred to as 66-50-17-T02-N001);
A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001);
A-(SEQ ID NO: 19)-DVP (herein referred to as 66-50-19-T01-N001);
A-(SEQ ID NO: 20)-TEN (herein referred to as 66-50-19-T02-N001);
A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001);
A-(SEQ ID NO: 22)-EEP (herein referred to as 66-50-21-T01-N001); and

such as:

an amino acid sequence selected from:

A-(SEQ ID NO: 2)-A (herein referred to as 66-50-02-N001);

A-(SEQ ID NO: 16)-END (herein referred to as 66-50-16-T01-N001);

A-(SEQ ID NO: 18)-FSCDDD (herein referred to as 66-50-18-T01-N001); and

A-(SEQ ID NO: 21)-EPD (herein referred to as 66-50-20-T01-N001).

7. The peptide ligand as defined in any one of claims 1 to 6, wherein the pharmaceutically acceptable salt is selected from the free acid or the sodium, potassium, calcium, ammonium salt.

8. The peptide ligand as defined in any one of claims 1 to 7, wherein the CD38 is human CD38.

9. A drug conjugate comprising a peptide ligand as defined in any one of claims 1 to 8, conjugated to one or more effector and/or functional groups.

10. The drug conjugate comprising a peptide ligand as defined in any one of claims 1 to 8, conjugated to one or more cytotoxic agents.

11. A pharmaceutical composition which comprises the peptide ligand of any one of claims 1 to 8 or the drug conjugate of claim 9 or claim 10, in combination with one or more pharmaceutically acceptable excipients.

12. The peptide ligand as defined in any one of claims 1 to 8 or the drug conjugate as defined in claim 9 or claim 10, for use in preventing, suppressing or treating a disease or disorder mediated by CD38.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/050073

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/64 A61P35/00 C07K7/08
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Liuhong Chen: "The Bicycle platform: an efficient technology to generate high affinity, high selectivity molecules (Bicycles) with unique drug like properties that are amenable to conjugation", , 26 April 2017 (2017-04-26), XP055622533, Retrieved from the Internet: URL:https://www.bicycletherapeutics.com/wp-content/uploads/16_PEGS-Bicycle_-30-04-2017-poster.pdf [retrieved on 2019-09-16]	1,2,7-12
A	abstract page 1, column 1, last paragraph figure 3 page 1, column 3, paragraph 3 figure 7	3-6

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2020	07/04/2020

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/050073

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHRISTIAN HEINIS ET AL: "Phage-encoded combinatorial chemical libraries based on bicyclic peptides", NATURE CHEMICAL BIOLOGY, vol. 5, no. 7, 31 May 2009 (2009-05-31), pages 502-507, XP055562241, Basingstoke ISSN: 1552-4450, DOI: 10.1038/nchembio.184 the whole document	1-12
A	CURRAN A. RHODES ET AL: "Bicyclic Peptides as Next-Generation Therapeutics", CHEMISTRY - A EUROPEAN JOURNAL, vol. 23, no. 52, 18 September 2017 (2017-09-18), pages 12690-12703, XP055479791, DE ISSN: 0947-6539, DOI: 10.1002/chem.201702117 the whole document	1-12
X,P	WO 2019/243353 A1 (BICYCLETX LTD [GB]) 26 December 2019 (2019-12-26) page 53; table 2 claim 17	1-12

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2020/050073

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019243353 A1	26-12-2019	NONE	