

NON-MEMBRANE DISRUPTIVE p53 ACTIVATING STAPLED PEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of Singapore provisional application No. 10201401119W, filed 13 February 2015, the contents of it being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[002] The present invention relates generally to the field of protein chemistry. In particular, the present invention relates to the use of stapled peptides.

BACKGROUND

[003] Inhibition of the p53:Mdm2 interaction is a therapeutic target. Molecules able to block the interaction can activate the p53 response by blocking the two inhibitory activities of Mdm2, namely its occlusion of the N-terminal p53 transactivation domain and its targeting of p53 for ubiquitination and proteasomal degradation. Such molecules could be used to re-activate p53 function in p53 wild-type tumor cells.

[004] Several classes of molecules that inhibit this interaction between p53 and Mdm2 have been developed (e.g. Nutlin, MI-219). These molecules mimic the conserved residues from a section of sequence in the p53 N-terminal that are essential for the interaction with the N-terminal p53 binding domain of Mdm2. However, these peptides exhibit off-target toxicity to p53-null cell and are not functional in the presence of serum.

[005] There is therefore a need to provide improved peptides that are capable of inhibiting the p53:Mdm2 interaction.

SUMMARY

[005a] In one aspect, the present invention provides a peptide comprising or consisting of the amino acid sequence of:



wherein

Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof; and

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Xaa₂ and Xaa₃ are independently any type of amino acid or modified amino acid, and the peptide further comprises a sequence comprising the amino acids –ENXaa₅ bound to its C-terminus, resulting in



5 wherein Xaa₅ is F or Y.

[005b] In another aspect, the present invention provides a peptide comprising or consisting of the amino acid sequence of:



wherein

10 Xaa₁ and Xaa₃ are any type of amino acid or modified amino acid;

Xaa₂ is S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine; and wherein Xaa₅ is F or Y.

[005c] In yet another aspect, the present invention provides a peptide comprising or consisting of the amino acid sequence of:

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$$\text{TSFXaa}_1\text{EYW Xaa}_3\text{LLXaa}_2$$

wherein: Xaa₁, Xaa₂ and Xaa₃ are independently selected from any type of amino acid or modified amino acid, and

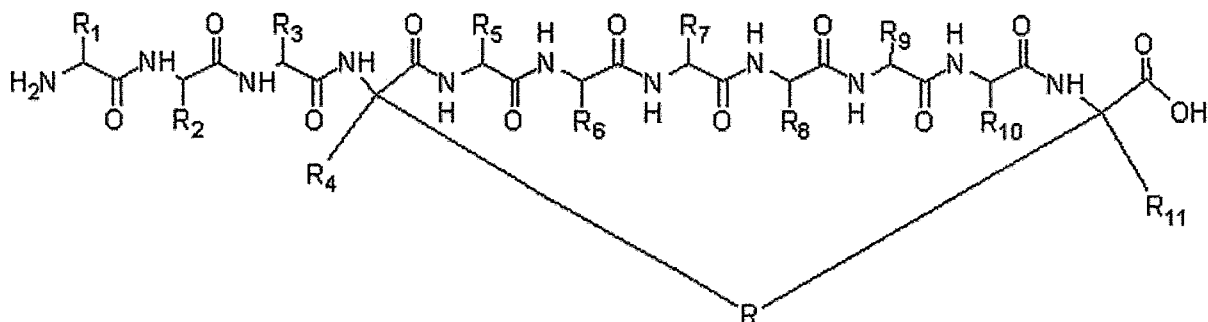
the N-terminus of the peptide is bound to a linker that results in a molecule not perturbing cell membrane integrity; and

20 wherein the peptide further comprises a sequence comprising the amino acids –ENXaa₅ bound to its C-terminus, resulting in



wherein Xaa₅ is F or Y; optionally,

wherein the peptide comprises the formula:

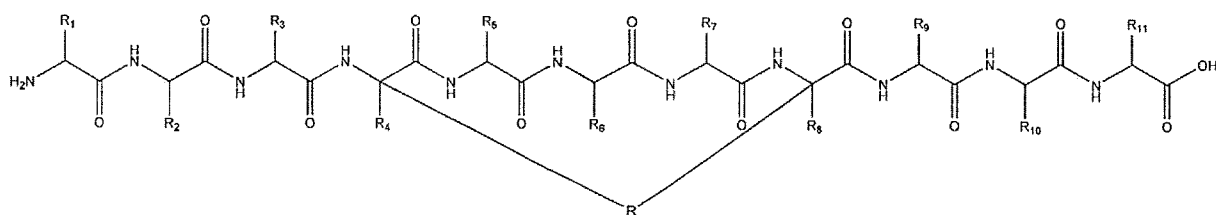


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or

wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₂; or wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₃, and wherein the peptide comprises

the formula:



wherein:

R₁ is -C(OH)CH₃ [T];

5 R₂ is -CH₂OH [S];

R₃ is benzyl [F];

R₄ and R₁₁ are independently H or a C₁ to C₁₀ alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclalkyl;

R₅ is - (CH₂)₂C(O)OH [E];

10 R₆ is -CH₂-Phenyl-OH [Y];

R₇ is the side chain of Trp, wherein C₆ of Trp is substituted with a hydrogen or a halogen and/or wherein Trp is independently an L or D optical isomer;

R₈ is the side chain of any amino acid;

R₉ and R₁₀ are -CH₂CH(CH₃)₂ [L];

15 R is alkyl, alkenyl, alkynyl; [R'-B-R'']_n; each of which is substituted with 0-6 R₁₂;

R' and R'' are independently alkylene, alkenylene or alkynylene;

each R₁₂ is independently halo, alkyl, OR₁₃, N(R₁₃)₂, SR₁₃, SOR₁₃, SO₂R₁₃, CO₂R₁₃, R₁₃, a fluorescent moiety, or a radioisotope;

B is independently O, S, SO, SO₂, CO, CO₂, CONR₁₃ or ;

20 each R₁₃ is independently H, alkyl, or a therapeutic agent;

n is an integer from 1-4.

[005d] In yet another aspect, the present invention provides a pharmaceutical composition comprising a peptide disclosed herein.

25 [005e] In yet another aspect, the present invention provides use of a peptide disclosed herein, in the manufacture of a medicament for treating or preventing cancer in a patient.

[006] Disclosed is a peptide comprising or consisting of the amino acid sequence of TSFXaa₁EYWXaa₃LLXaa₂, wherein Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof; and Xaa₂ and Xaa₃ are independently any type of amino acid or modified amino acid. Also disclosed is a peptide comprising or consisting of the amino acid sequence of TSFXaa₁EYW Xaa₃LLXaa₂ENXaa₅, wherein Xaa₁ and Xaa₃ are any type of amino acid or modified amino acid; Xaa₂ is S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine; and wherein Xaa₅ is F or Y. Also disclosed is

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a peptide comprising or consisting of the amino acid sequence of TSFXaa₁EYW Xaa₃LLXaa₂ wherein Xaa₁, Xaa₂ and Xaa₃ are independently selected from any type of amino acid or modified amino acid; and wherein the N-terminus of the peptide (i.e. T) is bound to a linker that results in a molecule not perturbing cell membrane integrity.

[007] Also disclosed is an isolated nucleic acid molecule encoding a peptide according any of the preceding claims. Also disclosed is a vector comprising an isolated nucleic acid molecule as described herein. Also disclosed is a host cell comprising a nucleic acid molecule as described herein or a vector as described herein.

[008] Also disclosed is a pharmaceutical composition comprising a peptide as described herein, an isolated nucleic acid molecule as described herein, or a vector as described herein. Also disclosed is the use of the peptide as described herein in the manufacture of a medicament for treating or preventing cancer. Also disclosed is a method of treating or preventing cancer in a patient comprising administering a pharmaceutically effective amount of the peptide as described herein or the isolated nucleic acid molecule as described herein, or the vector as described herein.

BRIEF DESCRIPTION OF DRAWINGS

[009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0010] Fig. 1 is an image showing auxiliary binding sites (circled) identified on the Mdm2 surface. Benzene occupancy maps (mesh) derived from ligand-mapping simulations of apo Mdm2 are overlaid on crystal structures of its complexes with a) p53 peptide (PDB 1YCR) and b) nutlin-2 (PDB 1RV1).

[0011] Fig. 2 shows images depicting the design of extended stapled peptides. a) shows a phenyl (Phe) residue appended to the C-terminus of the p53 peptide (PDB 1YCR), such that it overlaps with the benzene bound in the second nutlin interaction site. b) shows a phenyl (Phe) residue was appended to the C-terminus of SAH-p53-8 (PDB 3V3B) such that it overlaps with the benzene bound in the proximal Pro27 site. c) shows the conformation of YS-01 after 50 nanoseconds of molecular dynamic (MD) simulation. d) shows the conformation of YS-03 after 50 nanoseconds of molecular dynamic (MD) simulation.

[0012] Fig. 3 shows the results of a murine T22 p53 reporter screen of YS-01 to -04 stapled peptides performed in A) the absence and B) the presence of 10% fetal calf serum

CLAIMS

1. A peptide comprising or consisting of the amino acid sequence of:



wherein

- 5 Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof; and

Xaa₂ and Xaa₃ are independently any type of amino acid or modified amino acid, and the peptide further comprises a sequence comprising the amino acids –ENXaa₅ bound to its C-terminus, resulting in

- 10
$$\text{TSFXaa}_1\text{EYW Xaa}_3\text{LLXaa}_2\text{ENXaa}_5$$

wherein Xaa₅ is F or Y.

2. The peptide of claim 1, wherein Xaa₃ is A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine, and/or

- 15 wherein Xaa₂ is S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine.

3. A peptide comprising or consisting of the amino acid sequence of:



- 20 wherein

Xaa₁ and Xaa₃ are any type of amino acid or modified amino acid;

Xaa₂ is S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine; and

wherein Xaa₅ is F or Y.

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4. The peptide of claim 34, wherein Xaa₃ is N, or A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine; and wherein if Xaa₃ is N, Xaa₁ is not A and/or Xaa₂ is not S, and/or

- 30 wherein Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof; optionally

wherein Xaa₃ is A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine.

5. A peptide comprising or consisting of the amino acid sequence of:



wherein: Xaa₁, Xaa₂ and Xaa₃ are independently selected from any type of amino acid or modified amino acid, and

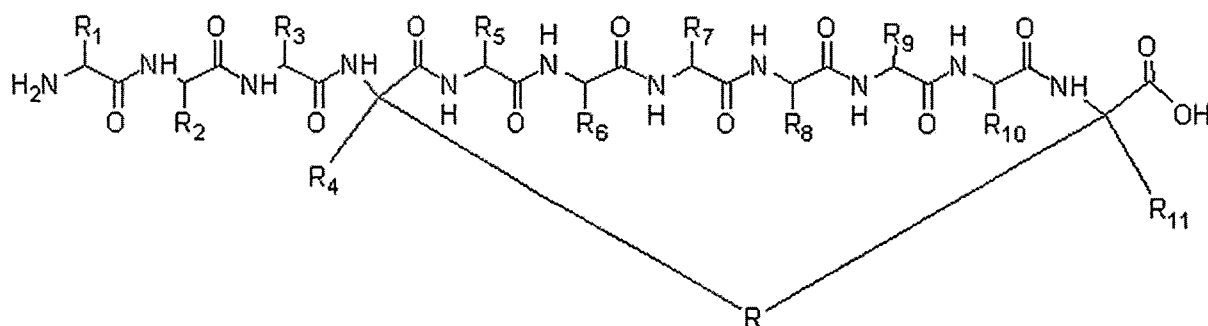
the N-terminus of the peptide is bound to a linker that results in a molecule not perturbing cell membrane integrity; and

wherein the peptide further comprises a sequence comprising the amino acids –ENXaa₅ bound to its C-terminus, resulting in



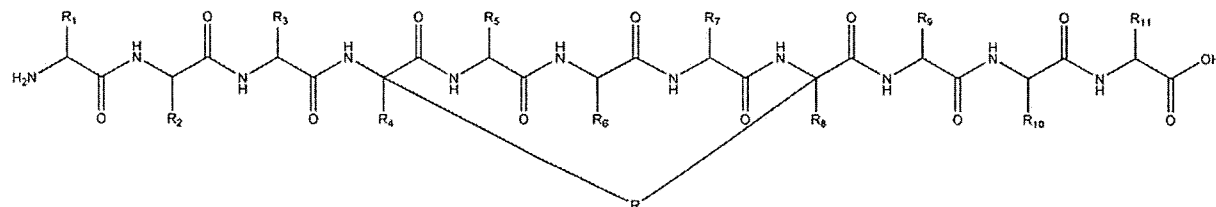
wherein Xaa₅ is F or Y; optionally,

wherein the peptide comprises the formula:



or

wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₂, or wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₃, and wherein the peptide comprises the formula:



wherein:

R₁ is –C(OH)CH₃ [T];

R₂ is –CH₂OH [S];

R₃ is benzyl [F];

R₄ and R₁₁ are independently H or a C₁ to C₁₀ alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclalkyl;

R₅ is –(CH₂)₂C(O)OH [E];

R₆ is –CH₂-Phenyl-OH [Y];

5 R₇ is the side chain of Trp, wherein C₆ of Trp is substituted with a hydrogen or a halogen and/or wherein Trp is independently an L or D optical isomer;

R₈ is the side chain of any amino acid;

R₉ and R₁₀ are –CH₂CH(CH₃)₂ [L];

R is alkyl, alkenyl, alkynyl; [R'–B–R'']_n; each of which is substituted with 0-6 R₁₂;

10 R' and R'' are independently alkylene, alkenylene or alkynylene;

each R₁₂ is independently halo, alkyl, OR₁₃, N(R₁₃)₂, SR₁₃, SOR₁₃, SO₂R₁₃, CO₂R₁₃, R₁₃, a fluorescent moiety, or a radioisotope;

B is independently O, S, SO, SO₂, CO, CO₂, CONR₁₃ or ;

each R₁₃ is independently H, alkyl, or a therapeutic agent;

15 n is an integer from 1-4.

6. The peptide of claim 58, wherein Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof; and/or,

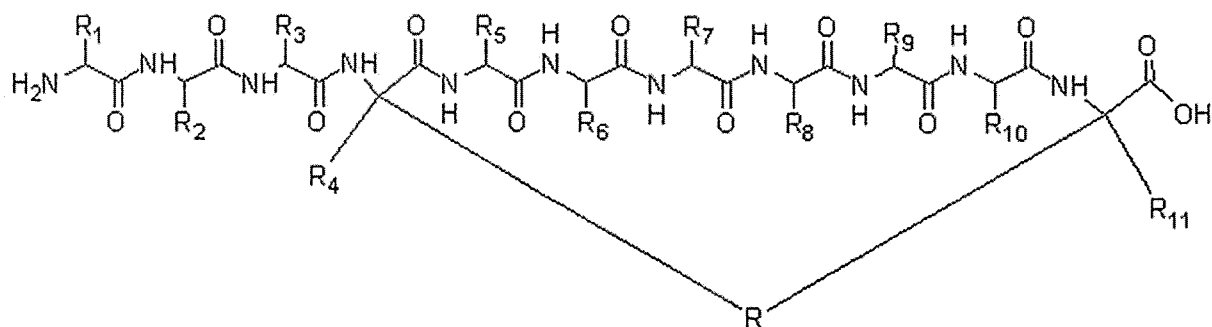
20 wherein Xaa₂ is S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine; and/or,

wherein Xaa₃ is N, or A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine; and wherein if Xaa₃ is N, Xaa₁ is not A and/or Xaa₂ is not S; optionally,

25 wherein Xaa₃ is A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine.

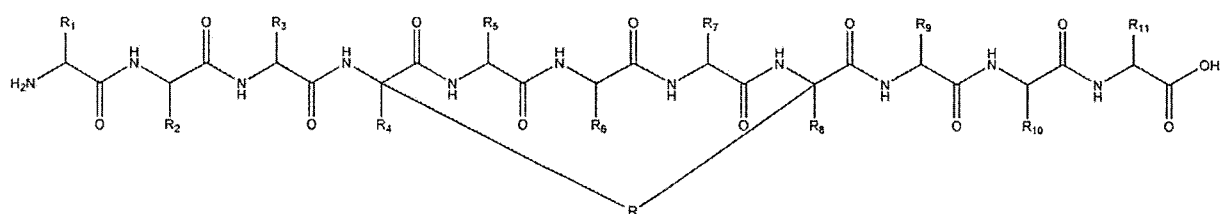
7. The peptide of any one of claims 1 to 6, wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₂; or wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a
30 second amino acid Xaa₃.

8. The peptide of claim 1, wherein the peptide comprises the formula:



or

the peptide of claim 14, wherein the peptide comprises the formula:



5 wherein:

R₁ is -C(OH)CH₃ [T];

R₂ is -CH₂OH [S];

R₃ is benzyl [F];

10 R₄ and R₁₁ are independently H or a C₁ to C₁₀ alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclylalkyl;

R₅ is -(CH₂)₂C(O)OH [E];

R₆ is -CH₂-Phenyl-OH [Y];

R₇ is the side chain of Trp, wherein C₆ of Trp is substituted with a hydrogen or a halogen and/or wherein Trp is independently an L or D optical isomer;

15 R₈ is the side chain of any amino acid;

R₉ and R₁₀ are -CH₂CH(CH₃)₂ [L];

R is alkyl, alkenyl, alkynyl; [R'-B-R']_n; each of which is substituted with 0-6 R₁₂;

R' and R'' are independently alkylene, alkenylene or alkynylene;

20 each R₁₂ is independently halo, alkyl, OR₁₃, N(R₁₃)₂, SR₁₃, SOR₁₃, SO₂R₁₃, CO₂R₁₃, R₁₃, a fluorescent moiety, or a radioisotope;

B is independently O, S, SO, SO₂, CO, CO₂, CONR₁₃ or ;

each R₁₃ is independently H, alkyl, or a therapeutic agent;

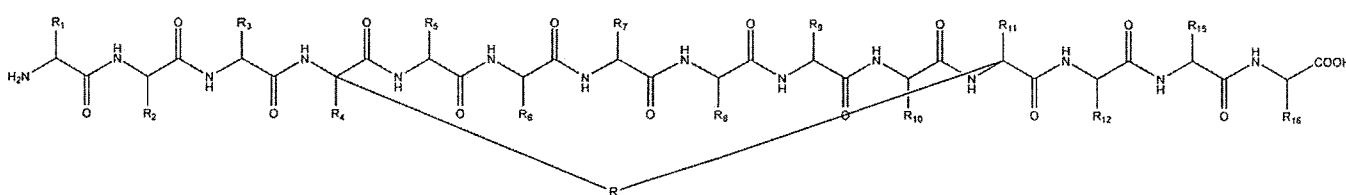
n is an integer from 1-4.

9. The peptide according to claim 8, wherein R_4 and R_{11} are independently H or C_1 - C_6 alkyl, and/or

wherein R is selected from the group consisting of C_8 alkyl, C_{11} alkyl, alkenyl, C_8 alkenyl, C_{11} alkenyl, linear chain alkyl, alkenyl and alkynyl; and/or

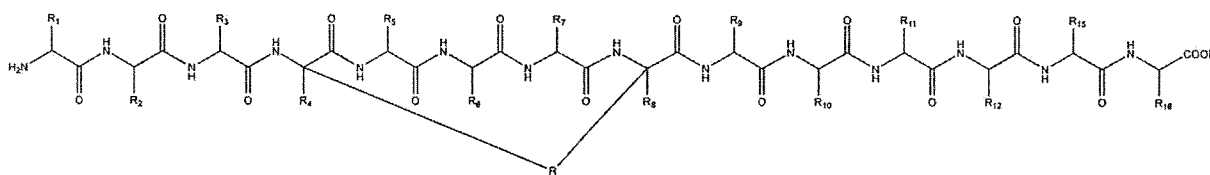
5 wherein R_1 , R_2 , R_3 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} are as defined in claim 10; wherein R_4 and R_{11} are H; and wherein R is C_{11} alkenyl.

10. The peptide of claim 9, wherein the peptide comprises the formula:



10 or

the peptide of claim 9, wherein the peptide comprises the formula:



wherein:

R_1 is $-C(OH)CH_3$ [T];

15 R_2 is $-CH_2OH$ [S];

R_3 is benzyl [F];

R_4 and R_{11} are independently H or a C_1 to C_{10} alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclalkyl;

R_5 is $-(CH_2)_2C(O)OH$ [E];

20 R_6 is $-CH_2$ -Phenyl-OH [Y];

R_7 is the side chain of Trp, wherein C_6 of Trp is substituted with a hydrogen or a halogen and/or wherein Trp is independently an L or D optical isomer;

R_8 is independently the side chain of any amino acid;

R_9 and R_{10} are $-CH_2CH(CH_3)_2$ [L];

25 R is alkyl, alkenyl, alkynyl; $[R'-B-R'']_n$; each of which is substituted with 0-6 R_{13} ;

R' and R'' are independently alkylene, alkenylene or alkynylene;

each R_{13} is independently halo, alkyl, OR_{14} , $N(R_{14})_2$, SR_{14} , SOR_{14} , SO_2R_{14} , CO_2R_{14} , R_{14} , a fluorescent moiety, or a radioisotope;

B is independently O, S, SO, SO_2 , CO, CO_2 , $CONR_{14}$ or ;

each R_{14} is independently H, alkyl, or a therapeutic agent;

5 n is an integer from 1-4;

R_{12} is $-(CH_2)_2C(O)OH$ [E];

R_{15} is $-CH_2C(O)NH_2$ [N];

R_{16} is independently either benzyl [F] or $-CH_2$ -Phenyl-OH [Y].

10 11. The peptide according to claim 10, wherein R_4 and R_{11} are independently H or C_1 - C_6 alkyl; and/or

wherein R is selected from the group consisting of C_8 alkyl, C_{11} alkyl, alkenyl, C_8 alkenyl, C_{11} alkenyl, linear chain alkyl, alkenyl and alkynyl; and/or

15 wherein R_1 , R_2 , R_3 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} are as defined in claim 12; wherein R_4 and R_{11} are H; and wherein R is C_{11} alkenyl.

12. The peptide according to claim 4, wherein R_4 and R_{11} are independently H or C_1 - C_6 alkyl; and/or,

20 wherein R is selected from the group consisting of C_8 alkyl C_{11} alkyl, alkenyl, C_{11} alkenyl, C_8 alkenyl, a linear chain alkyl, alkenyl and alkynyl; and/or,

wherein R_1 , R_2 , R_3 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} are as defined in claim 7; wherein R_4 and R_{11} are H; and wherein R is C_{11} alkenyl.

25 13. The peptide of any one of claims 1-12, wherein the peptide comprises a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

14. A pharmaceutical composition comprising a peptide according to any one of claims 1 to 13.

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15. The pharmaceutical composition according to claim 14, wherein the pharmaceutical composition comprises a further therapeutic compound; optionally

wherein the further therapeutic compound is an apoptosis promoting compound.

16. Use of a peptide of any one of claims 1 to 13, in the manufacture of a medicament for treating or preventing cancer in a patient.