

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 disclosure refers to a peptide comprising or consisting of the amino acid sequence of:

TSFX_{a1}EYWX_{a3}LLX_{a2}ENX_{a5}

wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid X_{a1} to a second amino acid X_{a2}; X_{a1} is (R)-2-(7'-octenyl)alanine, X_{a2} is (S)-2-(4'-pentenyl)alanine, X_{a3} is A, X_{a5} is F or Y;

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, Xaa₁ is (R)-2-(4'-pentenyl)alanine or (S)-2-(4'-pentenyl)alanine, Xaa₃ is (R)-2-(4'-pentenyl)alanine or (S)-2-(4'-pentenyl)alanine; and Xaa₂ is S or P, Xaa₅ is F or Y.

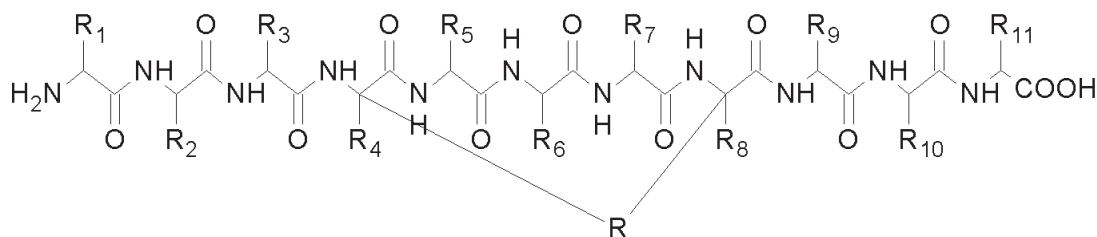
5 [005d] In yet another aspect, the present invention provides a pharmaceutical composition comprising a peptide disclosed herein.

[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.

10 [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
15 wherein Xaa₅ is F or Y. Also disclosed is

(I)

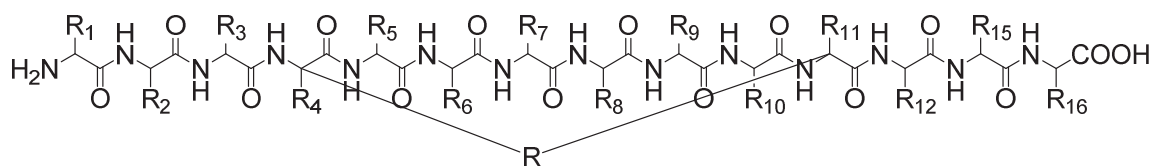
or formula II



(II),

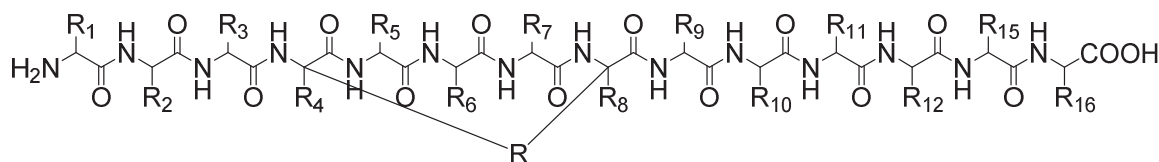
- 5 wherein the substituents are defined as follows. This definition is understood to also include combinations of the substituents described herein. In one example, R₁ is $-\text{C}(\text{OH})\text{CH}_3$ [T]. In another example, R₂ is $-\text{CH}_2\text{OH}$ [S]. In yet another example, R₃ is benzyl [F]. In another example, R₄ and R₁₁ are independently selected from, but not limited to H, or a C₁ to C₁₀ alkyl, or alkenyl, or alkynyl, or arylalkyl, or cycloalkylalkyl, or heteroarylalkyl, or heterocyclylalkyl. In another
- 10 example, R₅ is $-(\text{CH}_2)_2\text{C}(\text{O})\text{OH}$ [E]. In a further example, R₆ is $-\text{CH}_2\text{-Phenyl-OH}$ [Y]. In yet another example, R₇ is the side chain of Trp (tryptophan). In another example, R₇ is a side chain of Trp (tryptophan), wherein C₆ of Trp (tryptophan) is substituted with a hydrogen or a halogen. In another example, Trp can be independently an L or D optical isomer. In yet another example, R₈ is the side chain of any amino acid. In one example, R₉ and R₁₀ are $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ [L]. In
- 15 another example, R is alkyl, alkenyl, alkynyl; $[\text{R}'-\text{B}-\text{R}']_n$; each of which may be substituted with 0-6 R₁₂. Thus in one example, R' and R'' may be independently, but are not limited to, alkylene, alkenylene or alkynylene. In another example, each R₁₂ may be independently, but is not limited to, halo, alkyl, OR₁₃, N(R₁₃)₂, SR₁₃, SOR₁₃, SO₂R₁₃, CO₂R₁₃, R₁₃, a fluorescent moiety, or a radioisotope. In yet another example, B is independently selected from, but not limited to O, S,
- 20 SO, SO₂, CO, CO₂, or CONR₁₃. In another example, each R₁₃ may independently be H, alkyl, or a therapeutic agent. In yet another example, n is an integer from 1 to 4 (e.g. 1, 2, 3 or 4). In another example, R₄ and R₁₁ are independently H or C₁-C₆ alkyl. In another example, R may be a linear chain alkyl, alkenyl or alkynyl. In yet another example, R can be C₈ alkyl or C₁₁ alkyl. In a further example, R is alkenyl. In a further example, R can be C₈ alkenyl or C₁₁ alkenyl. In yet another
- 25 example, R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₉, R₁₀ are as previously defined, wherein R₄ and R₁₁ are H, and wherein R is C₁₁ alkenyl.

[0069] In a further example, the present disclosure describes peptides comprising the sequence as shown in formula III



(III)

or formula IV



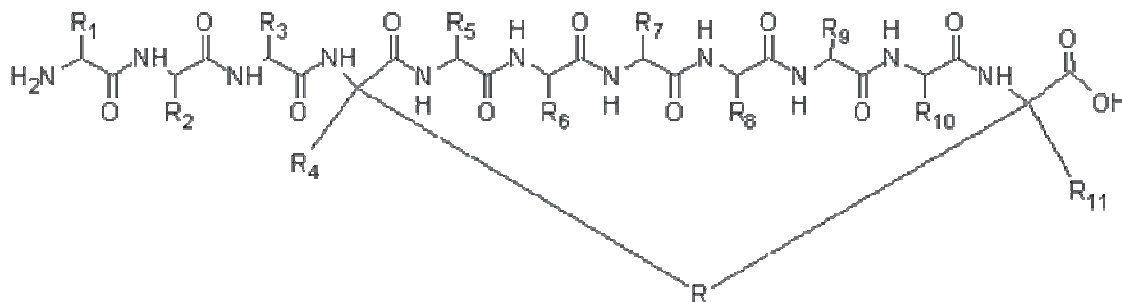
(IV),

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wherein the substituents are defined as follows. This definition is understood to also include combinations of the substituents described. In one example, R_1 to R_{11} are as described herein. In another example, each R_{13} is independently selected from, but not limited to, 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. In another example, B is independently selected from, but not limited to, O, S, SO, SO_2 , CO, CO_2 , $CONR_{14}$. In yet another example, each R_{14} can be independently H, alkyl, or a therapeutic agent. In yet another example, n is an integer from 1 to 4. In a further example, R_{12} is $-(CH_2)_2C(O)OH$ [E]. In yet another example, R_{15} is $-CH_2C(O)NH_2$ [N]. In one example, R_{16} is independently either benzyl [F] or $-CH_2$ -Phenyl-OH [Y]. In another example, the peptide comprises a sequence selected from, but not limited to, 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, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

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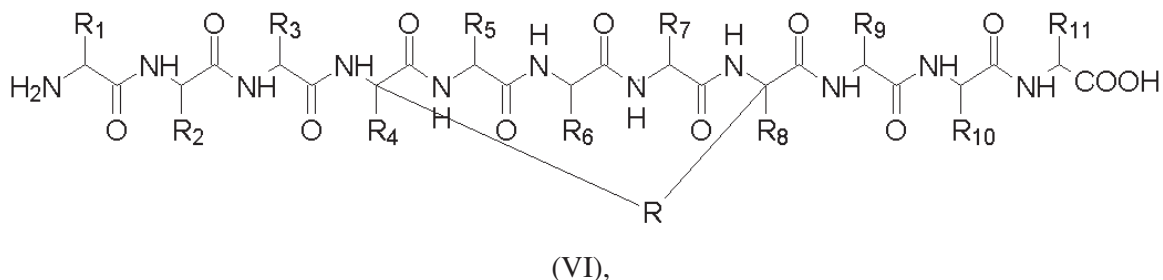
[0070] In yet another example, the present disclosure describes a peptide comprising the sequence of formula V



(V)

20

Or formula VI



wherein the substituents are defined as follows. This definition is understood to also include combinations of the substituents described, whereby the substituents are as described herein.

[0071] In one example, the peptide as disclosed herein may comprise at least 1, at least 2 or more halogens. In another example, the peptide comprises about 1, about 2, about 3, about 4, or about 5 halogens. In one example, W of the peptide as disclosed herein can be modified by addition of one or more halogen independently selected from, but not limited to, F, or Cl, or Br, or I. In a further example, the halogen is Cl. In another example, W is at position 7 of the peptide as defined herein. In another example, W, at position 7 of the peptide as described herein, is modified by the addition of, for example, a halogen. In another example, W is at position 7 of the peptide as described herein is modified by the addition of, for example, a halogen at position C₆ of W. In another example, W may be independently an L or D optical isomer.

[0072] The present disclosure also describes peptides that are, for example, 14 amino acids in length. In one example, the peptide comprises or consists of the amino acid sequence of TSFXaa₁EYW Xaa₃LLXaa₂ENXaa₅. In one example, Xaa₁ and Xaa₃ are any type of amino acid or modified amino acid. In another example, Xaa₂ is selected from, but not limited to, S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine. In yet another example, Xaa₅ can be F or Y. In a further example, the peptide comprises or consists of the amino acid sequence of TSFXaa₁EYW Xaa₃LLXaa₂ENXaa₅, wherein Xaa₁ and Xaa₃ are any type of amino acid or modified amino acid, Xaa₂ is selected from, but not limited to, S, or P, or (S)-2-(4'-pentenyl)alanine or a derivative of (S)-2-(4'-pentenyl)alanine, and Xaa₅ is F or Y. In one example, Xaa₃ is selected from, but not limited to, N, or A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine. In yet another example, if Xaa₃ is N, Xaa₁ is not A and/or Xaa₂ is not S. In another example, 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. In one example, Xaa₁ is (R)-2-(7'-octenyl)alanine or derivative thereof, or is (R)-2-(4'-pentenyl)alanine or derivative thereof. In another example, Xaa₃ is A, or (R)-2-(4'-pentenyl)alanine or a derivative of (R)-2-(4'-pentenyl)alanine.

determined apparent K_d values (shown in the table below) were used in determining the apparent K_d values in subsequent competition assays, for both Mdm4 and Mdm2, against the respective competing ligands:

Mdm4 K_d	Mdm2 K_d	Peptide
4.2 nM	13.0 nM	(FAM)-RFMDYWEGL-NH ₂

5 [00166] Apparent dissociation constant (K_d) values were determined for a variety of molecules via competitive fluorescence anisotropy experiments. Titrations were carried out with the concentrations of Mdm2 and Mdm4 held constant at 250 nM and 75 nM, respectively, and the labelled peptide at 50 nM. The competing molecules were then titrated against the complex of the FAM labelled peptide and protein. Apparent dissociation constant (K_d) values were determined by
10 fitting the experimental data to the equations shown below:

$$r = r_o + (r_b + r_o) \times \frac{2\sqrt{(d^2 - 3e)} \cos(\theta/3) - 9}{3K_{d1} + 2\sqrt{(d^2 - 3e)} \cos(\theta/3) - d} \quad (\text{Eq. 6-1})$$

$$d = K_{d1} + K_{d2} + [L]_{st} + [L]_t - [P]_t \quad (\text{Eq. 6-2})$$

$$e = ([L]_t - [P]_t)K_{d1} + ([L]_{st} - [P]_t)K_{d2} + K_{d1}K_{d2} \quad (\text{Eq. 6-3})$$

$$f = -K_{d1}K_{d2}[P]_t \quad (\text{Eq. 6-4})$$

$$\theta = \arccos \left[\frac{-2d^3 + 9de - 27f}{2\sqrt{(d^2 - 3e)^3}} \right] \quad (\text{Eq. 6-5})$$

15 [00167] $[L]_{st}$ and $[L]_t$ denote labelled ligand and total unlabelled ligand input concentrations, respectively. K_{d2} is the dissociation constant of the interaction between the unlabelled ligand and the protein. In all competitive types of experiments, it is assumed that $[P]_t > [L]_{st}$, otherwise considerable amounts of free labelled ligand would always be present and would interfere with
20 measurements. K_{d1} is the apparent K_d for the labelled peptide used in the respective experiment, which has been experimentally determined as described in the previous paragraph. The FAM-labelled peptide was dissolved in dimethyl sulfoxide (DMSO) at 1 mM and diluted into

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

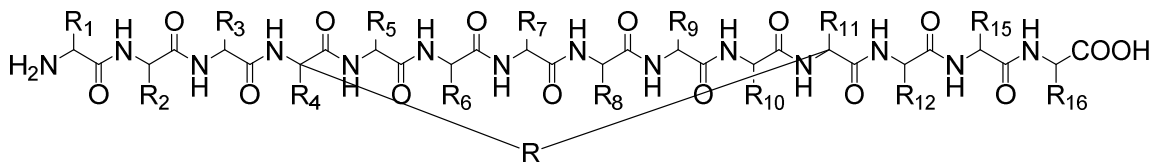


wherein the peptide is a cross-linked peptide with a cross-linker to connect a first amino acid Xaa₁ to a second amino acid Xaa₂; Xaa₁ is (R)-2-(7'-octenyl)alanine, Xaa₂ is (S)-2-(4'-pentenyl)alanine, Xaa₃ is A, Xaa₅ is F or Y;

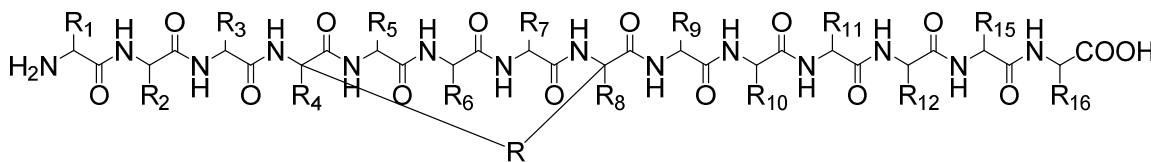
or

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, Xaa₁ is (R)-2-(4'-pentenyl)alanine or (S)-2-(4'-pentenyl)alanine, Xaa₃ is (R)-2-(4'-pentenyl)alanine or (S)-2-(4'-pentenyl)alanine; and Xaa₂ is S or P, Xaa₅ is F or Y.

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



or:



wherein:

R₁ is -C(OH)CH₃ [T];

R₂ is -CH₂OH [S];

R₃ is benzyl [F];

R₄ is -CH₃ [(R)-2-(7'-octenyl)alanine, (R)-2-(4'-pentenyl)alanine, or (S)-2-(4'-pentenyl)alanine];

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;

R₈ is -CH₃ [A or (R)-2-(4'-pentenyl)alanine];

R₉ and R₁₀ are $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ [L];

R₁₁ is $-\text{CH}_2\text{OH}$ [S] or $-(\text{CH}_2)_3-$ [P] or $-\text{CH}_3$ [(S)-2-(4'-pentenyl)alanine];

R is C₈ alkenyl or C₁₁ alkenyl

R₁₂ is $-(\text{CH}_2)_2\text{C}(\text{O})\text{OH}$ [E];

5 R₁₅ is $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$ [N];

R₁₆ is benzyl [F] or $-\text{CH}_2\text{-Phenyl-OH}$ [Y].

10 3. The peptide of claim 1 or 2, 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.

4. A pharmaceutical composition comprising a peptide according to any one of claims 1 to 3.

15 5. The pharmaceutical composition according to claim 4, wherein the pharmaceutical composition comprises a further therapeutic compound.

6. The pharmaceutical composition of claim 5, wherein the further therapeutic compound is an apoptosis promoting compound.

20 7. Use of a peptide of any one of claims 1 to 3, in the manufacture of a medicament for treating or preventing cancer in a patient.