low affinity for Mdm2/Mdm4 (452 ± 11 nM and 646 ± 26 nM, respectively) and comes from a region of p53 that is known to interact with many other proteins.

[0006] Extensive studies have used phage display to select for linear peptides that bind Mdm2 with high affinity. However, the proline at position P12 is not observed in the electron density map in the crystal structure of the Mdm2: peptide complex and is not critical for binding to Mdm 2.

[0007] There is therefore a need to provide improved peptides or staple peptides that overcomes the disadvantages mentioned above to improve efficiency of binding to Mdm2/Mdm4 and reduce off target toxicity.

SUMMARY

[0008] In one aspect, the present invention refers to a peptide comprising or consisting of the amino acid sequence of TSFXaa1EYWXaa3LLXaa2 Xaa4; wherein Xaa1, and Xaa2 are independently any type of amino acid; wherein Xaa4 is any type of amino acid other than P; wherein Xaa3 is N or A and wherein in case Xaa3 is N, Xaa1 is not A and/or Xaa2 is not S; wherein the peptide is a crosslinked peptide with a cross-linker to connect a first amino acid Xaa1 to a second amino acid Xaa2; and wherein Xaa1 and Xaa2 is independently any type of amino acid. In one example, the present disclosure is directed to a peptide comprising or consisting of the amino acid sequence of: TSFXaa$_1$EYW Xaa$_3$LLXaa$_2$ wherein Xaa$_1$ and Xaa$_2$ are any type of amino acid; and wherein Xaa$_3$ is N or A and wherein in case Xaa$_3$ is N, Xaa$_1$ is not A and/or Xaa$_2$ is not S. In one aspect, the present invention refers to a peptide comprising or consisting of the amino acid sequence of TSFXaa$_1$EYWXaa$_3$LLXaa$_2$ wherein Xaa$_1$, Xaa$_2$ and Xaa$_3$ is independently any type of amino acid; and wherein the peptide is a crosslinked peptide with a cross-linker to connect a first amino acid Xaa$_1$ to a second amino acid Xaa$_2$, and wherein in case Xaa$_3$ is N, Xaa$_1$ is not A and/or Xaa$_2$ is not S. In one example, the present disclosure describes a peptide comprising or consisting of the amino acid sequence of: TSFXaa$_1$EYWXaa$_3$LLXaa$_2$ wherein Xaa$_1$, Xaa$_2$ and Xaa$_3$ is independently any type of amino acid; and wherein the peptide is a crosslinked peptide with a cross-linker to connect a first amino acid Xaa$_1$ to a second amino acid Xaa$_2$. In another example, the present invention is directed to a peptide comprising or consisting of the amino acid sequence of: TSFXaa$_1$EYWXaa$_3$LLXaa$_2$ Xaa$_4$ wherein Xaa$_1$, and Xaa$_2$ are independently any type of amino acid; wherein Xaa$_4$ is any type of amino acid other than P; wherein Xaa$_3$ is N or A and wherein in case Xaa$_3$ is N, Xaa$_1$ is not A and/or Xaa$_2$ is not S. In one aspect, the present invention is directed to a use of the peptide according to the present invention or the isolated nucleic acid molecule according to the present invention or the vector according to the present invention in the manufacture of a medicament for treating or preventing cancer in a patient. According to one example, there is described a peptide comprising or consisting of the amino acid sequence of:

TSFXaa$_1$EYW Xaa$_3$LLXaa$_2$

wherein Xaa$_1$ and Xaa$_2$ are any type of amino acid; and wherein Xaa$_3$ is N or A and wherein in case Xaa$_3$ is N, Xaa$_1$ is not A and/or Xaa$_2$ is not S.

[0009] According to another example, there is described a peptide comprising or consisting of the amino acid sequence of:

Claims

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

$TSFXaa_1EYWXaa_3LLXaa_2$

wherein Xaa_1, Xaa_2 and Xaa_3 is independently any type of amino acid; and
wherein the peptide is a crosslinked peptide with a cross-linker to connect a first amino acid Xaa_1 to a second amino acid Xaa_2, and wherein in case Xaa_3 is N, Xaa_1 is not A and/or Xaa_2 is not S.

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

$TSFXaa_1EYWXaa_3LLXaa_2\ Xaa_4$

wherein Xaa_1, and Xaa_2 are independently any type of amino acid;
wherein Xaa_4 is any type of amino acid other than P;
wherein Xaa_3 is N or A and wherein in case Xaa_3 is N, Xaa_1 is not A and/or Xaa_2 is not S;
wherein the peptide is a crosslinked peptide with a cross-linker to connect a first amino acid Xaa_1 to a second amino acid Xaa_2; and wherein Xaa_1 and Xaa_2 is independently any type of amino acid.

3. The peptide of any one of claims 1 to 3, wherein the peptide has a length of between about 11 to 15 amino acids.

4. The peptide of any one of claims 1 to 3, wherein the peptide which has undergone a post-translational modification selected from the group consisting of addition of one or more phosphoryl groups.

5. The peptide of any one of claims 1 to 4, wherein the peptide is modified to include one or more ligands selected from the group consisting of: hydroxyl, phosphate, amine, amide, sulphate, sulphide, a biotin moiety, a carbohydrate moiety, a fatty acid-derived acid group, a fluorescent moiety, a chromophore moiety, a radioisotope, a PEG linker, an affinity label, a targeting moiety, an antibody, a cell penetrating peptide and a combination of the aforementioned ligands.

6. The peptide of any one of claims 1 to 5, wherein the nitrogen of the backbone of the peptide is methylated.

7. The peptide of any of claims 1 to 6, wherein the peptide is fused to a heterologous polypeptide sequence.

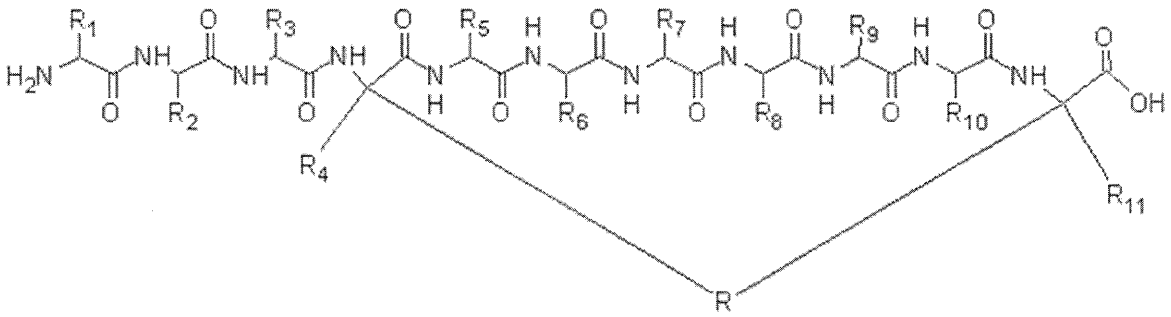
8. The peptide according to any one of the preceding claims, wherein W at position 7 of the peptide is modified by addition of one or more halogen independently selected from the group consisting of F, Cl, Br, and I.

9. The peptide of claim 8, wherein the peptide comprises 1, 2, 3, 4, or 5 halogens.

10. The peptide of claim 8, wherein W at position 7 is modified by addition of a halogen at position C_6 of W and/or wherein W is independently an L or D optical isomer.

11. The peptide of any one of claims 8 to 10, wherein the halogen is Cl.

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



wherein:

R_1 is –C(OH)CH_3 [T];

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 heterocyclylalkyl;

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

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 the side chain of any amino acid;

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

R is alkyl, alkenyl, alkynyl; $[R'–K–R"]_n$; each of which is substituted with 0-6 R_{12};

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

each R_{12} is independently halo, alkyl, OR_{13}, $N(R_{13})_2$, SR_{13}, SOR_{13}, SO_2R_{13}, CO_2R_{13}, R_{13}, a fluorescent moiety, or a radioisotope;

K is independently O, S, SO, SO_2, CO, CO_2, $CONR_{13}$ or ;

each R_{13} is independently H, alkyl, or a therapeutic agent;

n is an integer from 1-4.

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

14. The peptide of claim 12, wherein R is C_8 alkyl.

15. The peptide of claim 12, wherein R is C_{11} alkyl.

16. The peptide of claim 12, wherein R is alkenyl.

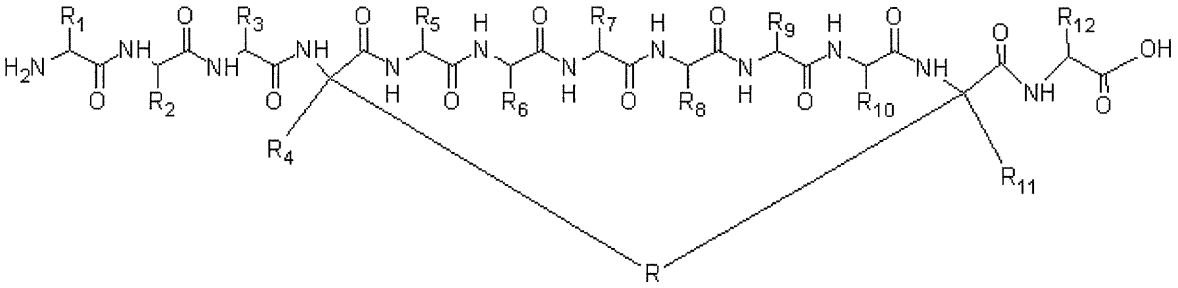
17. The peptide of claim 12, wherein R is C_8 alkenyl.

18. The peptide of claim 12, wherein R is C_{11} alkenyl.

19. The peptide of claim 12, wherein R_1, R_2, R_3, R_5, R_6, R_7, R_8, R_9, R_{10} are as defined in claim 13; wherein R_4 and R_{11} are H; and wherein R is C_{11} alkenyl.

20. The peptide of claim 12, wherein R is a linear chain alkyl, alkenyl or alkynyl.

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



wherein:

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

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 heterocyclylalkyl;

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

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 $-CH_2$-$C(O)NH_2$ [N] or $-CH_3$ [A] and

R_{12} is any type of amino acid other than P;

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

R is alkyl, alkenyl, alkynyl; $[R'-K-R'']_n$; 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;

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

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

n is an integer from 1-4.

22. The peptide according to claim 21, wherein R_4 and R_{11} are independently H or C_1-C_6 alkyl.

23. The peptide of claim 21, wherein R is C_8 alkyl.

24. The peptide of claim 21, wherein R is C_{11} alkyl.

25. The peptide of claim 21, wherein R is alkenyl.

26. The peptide of claim 21, wherein R is C_8 alkenyl.

27. The peptide of claim 21, wherein R is C_{11} alkenyl.

28. The peptide of claim 21, wherein R_1, R_2, R_3, R_5, R_6, R_7, R_8, R_9, R_{10} and R_{12} are as defined in claim 21; wherein R_4 and R_{11} are H; and wherein R is C_{11} alkenyl

29. The peptide of claim 21, wherein R is a linear chain alkyl, alkenyl or alkynyl.

30. An isolated nucleic acid molecule encoding a peptide according to claim 2.

31. A vector comprising an isolated nucleic acid molecule according to claim 30.

32. A host cell comprising a nucleic acid molecule of claim 30 or a vector of claim 31.

33. A pharmaceutical composition comprising a peptide according to any one of claims 1 to 29, an isolated nucleic acid molecule according to claim 30, or a vector according to claim 31.

34. The pharmaceutical composition according to claim 33 further comprising one or more pharmaceutically acceptable excipients, vehicles or carriers.

35. The pharmaceutical composition according to claim 33 or 34, wherein the pharmaceutical composition comprises a further therapeutic compound.

36. The pharmaceutical composition of claim 35, wherein the further therapeutic compound is an apoptosis promoting compound.

37. The pharmaceutical composition of claim 36, wherein the apoptosis promoting compound is selected from the group consisting of Cyclin-dependent Kinase (CDK) inhibitors, Receptor Tyrosine Kinase (RTK) inhibitors, BCL (B-cell lymphoma) family BH3 (Bcl-2 homology domain 3)-mimetic inhibitors and Ataxia Telangiectasia Mutated (ATM) inhibitors.

38. The pharmaceutical composition of claim 37, wherein the CDK inhibitors comprise inhibitors selected from the group consisting of:

2-(*R*)-(1-Ethyl-2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine (CYC202; Roscovitine; Seliciclib);

4-[[5-Amino-1-(2,6-difluorobenzoyl)-1H-1,2,4-triazol-3-yl]amino]benzenesulfonamide (JNJ-7706621);

N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide (AT-7519);

N-(5-(((5-(1,1-dimethylethyl)-2-oxazolyl)methyl)thio)-2-thiazolyl)-4-piperidinecarboxamide (SNS-032);

8,12-Epoxy-1H,8H-2,7b,12a-triazadibenzo(a,g)cyclonona(cde)triinden-1-one, 2,3,9,10,11,12-hexahydro-3-hydroxy-9-methoxy-8-methyl-10-(methylamino)- (UCN-01; 7-Hydroxystaurosporine; KRX-0601);

N,1,4,4-tetramethyl-8-((4-(4-methylpiperazin-1-yl)phenyl)amino)-4,5-dihydro-1H-pyrazolo[4,3-h]quinazoline-3-carboxamide (PHA-848125; milciclib);

2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methylpiperidin-4-yl]chromen-4-one hydrochloride (flavopiridol; alvocidib);

6-acetyl-8-cyclopentyl-5-methyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one hydrochloride (PD 0332991);

4-(1-isopropyl-2-methyl-1H-imidazol-5-yl)-N-(4-(methylsulfonyl)phenyl)pyrimidin-2-amine (AZD5438);

(S)-3-(((3-ethyl-5-(2-(2-hydroxyethyl)piperidin-1-yl)pyrazolo[1,5-a]pyrimidin-7-yl)amino)methyl)pyridine 1-oxide (Dinaciclib; SCH 727965);

N-(4-Piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide hydrochloride (AT-7519); and pharmaceutically acceptable salts thereof.

39. The pharmaceutical composition of claim 37, wherein the RTK inhibitors comprise inhibitors selected from the group consisting of:

N-[3-chloro-4-[(3-fluorophenyl)methoxy] phenyl]-6- [5-[(2-methylsulfonylethylamino) methyl]-2-furyl]quinazolin-4-amine (lapatinib);

N1'-[3-fluoro-4-[[6-methoxy-7-(3-morpholinopropoxy)-4-quinolyl]oxy]phenyl]-N1-(4-fluorophenyl) cyclopropane-1,1-dicarboxamide (foretinib);

N-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-*N*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (cabozantinib(XL184));

N-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-*N*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (cabozantinib(XL184));

3-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine (crizotinib (Xalkori));

(3Z)-N-(3-Chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl}methylene)-N-methyl-2-oxo-2,3-dihydro-1H-indole-5-sulfonamide (SU11274);

(3Z)-5-[[(2,6-Dichlorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[[(2R)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1H-pyrrol-2-yl]methylene]-1,3-dihydro-2H-indol-2-one hydrate (PHA-665752);

6-[[6-(1-Methylpyrazol-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-3-yl]sulfanyl]quinoline (SGX-523);

4-[1-(6-Quinolinylmethyl)-1H-1,2,3-triazolo[4,5-b]pyrazin-6-yl]-1H-pyrazole-1-ethanol methanesulfonate (1:1) (PF-04217903);

2-Fluoro-N-methyl-4-[7-[(quinolin-6-yl)methyl]imidazo[1,2-b]-[1,2,4]triazin-2-yl]benzamide (INCB28060);

N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3*S*)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide (afatinib);

3-(5,6-Dihydro-4H-pyrrolo[3,2,1-ij]quinolin-1-yl)-4-(1H-indol-3-yl)-pyrrolidine-2,5-dione (ARQ-197 (Tivantinib));

N-[(2R)-1,4-dioxan-2-ylmethyl]-N-methyl-N'-[3-(1-methyl-1H-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[1,2-b]pyridin-7-yl]sulfuric diamide (MK-2461);

N-[4-(3-Amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea (Linifanib(ABT 869));
4-[[(3S)-3-Dimethylaminopyrrolidin-1-yl]methyl]-N-[4-methyl-3-[(4-pyrimidin-5-ylpyrimidin-2-yl)amino]phenyl]-3-(trifluoromethyl)benzamide (Bafetinib (INNO-406)); and pharmaceutical salts thereof.

40. The pharmaceutical composition of claim 37, wherein the BCL family BH3-mimetic inhibitors comprise inhibitors selected from the group consisting of:
4-[4-[[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-N-[[4-[[(1R)-3-(4-morpholinyl)-1-[(phenylthio)methyl]propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl]sulfonyl]benzamide (ABT 263;Navitoclax);
2-[2-[(3,5-Dimethyl-1H-pyrrol-2-yl)methylene]-3-methoxy-2H-pyrrol-5-yl]-1H-indole methanesulfonate (Obatoclax mesylate (GX15-070));
4-[4-[(4'-chloro[1,1'-biphenyl]-2-yl)methyl]-1-piperazinyl]-N-[[4-[[(1R)-3-(dimethylamino)-1-[(phenylthio)methyl]propyl]amino]-3-nitrophenyl]sulfonyl]-Benzamide (ABT-737); and pharmaceutically acceptable salts thereof.

41. The pharmaceutical composition of claim 37, wherein the ATM inhibitors comprise inhibitors selected from the group consisting of:
2-Morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU-55933);
(2R,6S)-2,6-Dimethyl-N-[5-[6-(4-morpholinyl)-4-oxo-4H-pyran-2-yl]-9H-thioxanthen-2-yl]-4-morpholineacetamide (KU-60019);
1-(6,7-Dimethoxy-4-quinazolinyl)-3-(2-pyridinyl)-1H-1,2,4-triazol-5-amine (CP466722);
α-Phenyl-N-[2,2,2-trichloro-1-[[[(4-fluoro-3-nitrophenyl)amino]thioxomethyl]amino] ethyl]benzene acetamide (CGK 733) and pharmaceutically acceptable salts thereof.

42. Use of the peptide according to any one of claims 1 to 29 or the isolated nucleic acid molecule according to claim 30, or the vector according to claim 31 in the manufacture of a medicament for treating or preventing cancer in a patient.

43. The use according to claim 42, wherein cancer comprises a tumor comprising a non-mutant p53 sequence.

44. The use according to claim 42 or 43, wherein cancer is selected from a group comprising or consisting of gastric cancer, colon cancer, lung cancer, breast cancer, bladder cancer, neuroblastoma, melanoma, and leukemia.

45. The use according to any one of claims 42 to 44 wherein one or more further therapeutic agent(s) is/are to be administered to the patient, wherein said one or more further therapeutic agent(s) is/are to be administered simultaneous, sequential or separate.

46. The use of any one of claims 42, wherein the patient suffering or suspected to suffer from cancer comprises a tumor with p53 deficient tumor cells or p53 genes comprising a mutation which causes the cancer.