On the other hand, all-D configuration α-amino acid peptides are hyper-stable against proteolysis as most proteases are chiral, they distinguish between L- and D-enantiomeric versions of the substrate; as a result, all-D configuration α-amino acid peptides are able to resist the activity of proteases. All-D configuration α-amino acid peptides have been engineered with strong binding affinity against a variety of targets including p53–MDM2 [41-42], VEGF–VEGF-receptor [43], PD-1–PD-L1 [44], and human immunodeficiency virus type 1 (HIV-1) entry [45]. Unfortunately, although all-D configuration α-amino acid peptides are intrinsically hyper-stable to proteolysis, the generally lack membrane permeability and cellular activity.

For example, ^{D}PMI-δ, is an all-D configuration α-amino acid linear peptide (PMI refers to *p*53-*M*DM2/MDMX *i*nhibitor) that was derived from a mirror image phage display screen reported by Liu et al. [41] and in U.S. Pub. Patent No. 20120328692. However, this peptide lacked cell permeability, but did activate p53 in cells when delivered using nano-carriers [42].

Peptide-based inhibitors hold great potential for targeted modulation of intracellular protein-protein interactions (PPIs) since they can access a huge range of chemical space including sequence diversity as well as a host of different secondary and tertiary structures. Currently, however, liabilities hinder the development of peptide therapeutics including poor conformational stability, proteolytic sensitivity and cell permeability. In addition, translation of these in vitro binders into intracellularly active and in vivo active compounds with on target selectivity and specificity is difficult. Several contemporary peptide design strategies address these issues to different degrees. Strategic macrocyclization through optimally placed chemical braces such as an olefin hydrocarbon crosslinks, commonly referred to as staples, can address these issues by i) restricting conformational freedom to give improved target affinities, ii) improving proteolytic resistance, and iii) enhancing cell permeability. Conversely, molecules constructed entirely from D-amino acids are hyper-resistant to proteolytic cleavage but generally lack conformational stability and membrane permeability.

SUMMARY

We have developed p53 peptidomimetic macrocycles that resolve many of the issues with peptide inhibitors identified in the art. The present disclosure provides p53 peptidomimetic macrocycles, each p53 peptidomimetic macrocycle comprising an i, i + 4 olefin staple and a polypeptide tail covalently linked to the p53 peptidomimetic macrocycle; an i, i + 7 olefin staple and a polypeptide tail covalently linked to the p53 peptidomimetic macrocycle; or

an i, i + 7 di-alkyne staple and optionally a polypeptide tail covalently linked to the p53 peptidomimetic macrocycle; wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids (D-amino acids) and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail has a D-configuration. In one aspect, there is provided a p53 peptidomimetic macrocycle, comprising: an i, i + 4 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle; wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration, or each amino acid in the polypeptide tail having a D-configuration. These p53 peptidomimetic macrocycles (i) are protease resistant, (ii) have conformational stability, (iii) are cell-permeable without inducing membrane disruption, and (iv) have low or no cellular toxicity (the disclosed p53 peptidomimetic macrocycles reduced cellular activity with counter-screen activity and lactate dehydrogenase (LDH) release). Furthermore, the p53 peptidomimetic macrocycles of the present disclosure bind mouse double minute 2 (MDM2 aka E3 ubiquitin-protein ligase) and MDMX (aka MDM4) and intracellularly activate p53 by binding MDM2 and MDMX, thereby antagonizing MDM2 and MDMX binding to p53.

In particular examples, the present disclosure provides a p53 peptidomimetic macrocycle comprising an i, i + 4 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle; an i, i+7 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle; or, an i, i+7 di-alkyne staple and optionally a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle; wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In particular examples, the aforementioned p53 peptidomimetic macrocycle comprises an i, i+4 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail

comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In particular examples, the aforementioned p53 peptidomimetic macrocycle comprises an i, i+7 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three

to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration. In specific examples, the olefin staple is an alkyne staple.

In particular examples, the aforementioned p53 peptidomimetic macrocycle comprises an i, i+7 di-alkyne staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In further examples, the present disclosure provides a p53 peptidomimetic macrocycle comprising an i, i+4 olefin staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 6 and 10 of the p53 peptidomimetic macrocycle (such staple a "6-10 olefin staple") and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration. In particular examples, the α,α-disubstituted amino acids at positions 6 and 10 comprise (R)-2-amino-2-methylhept-6-enoic acid.

In further examples, the present disclosure provides a p53 peptidomimetic macrocycle comprising 12 amino acids and an i, i+4 olefin staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 6 and 10 of the p53 peptidomimetic macrocycle and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration. In particular examples, the α,α-disubstituted amino acids at amino acid positions 6 and 10 comprise ((R)-2-amino-2-methylhept-6-enoic acid.

In particular examples of the p53 peptidomimetic macrocycle comprising the 6-10 olefin staple, the p53 peptidomimetic macrocycle further comprises D-6-fluoro-tryptophane at position 3 and D-p-CF_3-phenylalanine at position 7.

In a further still example of the p53 peptidomimetic macrocycle comprising the 6-10 olefin staple, the amino acid at position 1 comprises threonine, the amino acid at position 2 comprises alanine, the amino acid at position 4 comprises tyrosine, the amino acid at position 5 comprises alanine, the amino acid at position 8 comprises glutamic acid, the amino acid at position 9 comprises lysine or glutamine, the amino acid at position 11 comprises leucine, and the amino acid at position 12 comprises arginine or serine.

In a further example of the p53 peptidomimetic macrocycle comprising the 6-10 olefin staple, the amino acid at position 9 comprises glutamine and the amino acid at position 12 comprises serine.

In further examples, the present disclosure provides p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 5 and 12 of the p53 peptidomimetic macrocycle and optionally a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In particular examples of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the α,α-disubstituted amino acid at amino acid position 5 comprises (S)-2-amino-2-methylhept-6-ynoic acid and the α,α-disubstituted amino acid at amino acid position 12 comprises (R)-2-amino-2-methyloct-7-ynoic acid.

In particular examples of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the above p53 peptidomimetic macrocycle is covalently linked at the C-terminal amino acid to the N-terminus of a polypeptide tail, wherein the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In further examples, the present disclosure provides a p53 peptidomimetic macrocycle that comprises 12 amino acids and an i, i+7 di-alkyne staple formed between the α-

carbons of two α,α-disubstituted amino acids located at amino acid positions 5 and 12 of the p53 peptidomimetic macrocycle (such staple a 5-12 di-alkyne staple'), and optionally a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration.

In particular examples of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the α,α-disubstituted amino acid at amino acid position 5 comprises (S)-2-amino-2-methylhept-6-ynoic acid and the α,α-disubstituted amino acid at amino acid position 12 comprises (R)-2-amino-2-methyloct-7-ynoic acid.

In particular examples of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the p53 peptidomimetic macrocycle is covalently linked at the C-terminal amino acid to the N-terminus of a polypeptide tail, wherein the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In particular examples of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple further comprises D-6-fluoro-tryptophane at amino acid position 3 and D-p-CF_3-phenylalanine at amino acid position 7.

In a further still example of the p53 peptidomimetic macrocycle comprising a 5-12 di-alkyne staple, the amino acid at position 1 comprises threonine, the amino acid at position 2 comprises alanine, the amino acid at position 4 comprises tyrosine, the amino acid at position 6 comprises asparagine, the amino acid at position 8 comprises glutamic acid, the amino acid at position 9 comprises lysine or glutamine, the amino acid at position 10 comprises leucine, and the amino acid at position 11 comprises leucine. In a further example, the amino acid at position 9 comprises glutamine.

In further examples, the present disclosure provides a p53 peptidomimetic macrocycle comprising an i, i+7 olefin staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 5 and 12 of the p53 peptidomimetic macrocycle (such staple a "5-12 olefin staple") and optionally a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the

polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration, and wherein the α,α-disubstituted amino acid at amino position 5 is (S)-2-amino-2-methyldec-9-enoic acid and the α,α-disubstituted amino acid at amino acid position 12 is (R)-2-amino-2-methylhept-6-enoic acid.

In particular examples of the p53 peptidomimetic macrocycle comprising the 5-12 olefin linkage, the p53 peptidomimetic macrocycle is covalently linked at the C-terminal amino acid to the N-terminus of a polypeptide tail, wherein the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In further examples, the present disclosure provides a p53 peptidomimetic macrocycle that comprises 12 amino acids and an i, i+7 olefin staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 5 and 12 of the p53 peptidomimetic macrocycle and optionally a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration, and wherein the α,α-disubstituted amino acid at amino position 5 is 2-amino-2-methyldec-9-enoic acid and the α,α-disubstituted amino acid at amino acid position 12 is (R)-2-amino-2-methylhept-6-enoic acid.

In particular examples of the p53 peptidomimetic macrocycle comprising the 5-12 olefin linkage, the p53 peptidomimetic macrocycle is covalently linked at the C-terminal amino acid to the N-terminus of a polypeptide tail, wherein the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration.

In particular examples of the p53 peptidomimetic macrocycle comprising the 5-12 olefin linkage, the p53 peptidomimetic macrocycle further comprises D-6-fluoro-tryptophane at amino acid position 3 and D-p-CF_3-phenylalanine at amino acid position 7.

In a further still example of the p53 peptidomimetic macrocycle comprising the 5-12 olefin linkage, the amino acid at position 1 comprises threonine, the amino acid at position 2 comprises alanine, the amino acid at position 4 comprises tyrosine, the amino acid at

position 6 comprises asparagine, the amino acid at position 8 comprises glutamic acid, the amino acid at position 9 comprises lysine or glutamine, the amino acid at position 10 comprises leucine, and the amino acid at position 11 comprises leucine. In a further example, the amino acid at position 9 comprises glutamine.

In particular example of the p53 peptidomimetic macrocycles disclosed herein, one substituent of the α,α-disubstituted amino acids is an alkenyl and the other substituent of the α,α-disubstituted amino acids is a methyl. In particular examples, the alkenyl is a C^{3-10} alkenyl, a C^{4-7} alkenyl, or a C^{5-6} alkenyl.

The present disclosure further provides a method for converting a p53 peptidomimetic macrocycle and an i, i +4 olefin linkage into a p53 peptidomimetic macrocycle having improved pharmacological properties of low or undetectable toxicity, conformational stability, and membrane permeability comprising covalently linking to the C-terminal amino acid of the p53 peptidomimetic macrocycle a polypeptide tail comprising three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration. In a further example, the α-carbons of two α,α-disubstituted amino acids at positions 6 and 10 of the p53 peptidomimetic macrocycles are linked in the i, i +4 olefin linkage. In particular examples, the α,α-disubstituted amino acids at amino acid positions 6 and 10 comprise ((R)-2-amino-2-methylhept-6-enoic acid.

The present disclosure further provides a method for converting a p53 peptidomimetic macrocycle and an i, i + 7 olefin linkage or i, i + 7 di-alkyne linkage into a macrocycle having improved pharmacological properties of low or undetectable toxicity, conformational stability, and membrane permeability comprising linking to the C-terminal amino acid of the p53 peptidomimetic macrocycle a polypeptide tail comprising three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration. In a further example, the α-carbons of two α,α-disubstituted amino acids at positions 5 and 12 of the p53 peptidomimetic macrocycles are linked in the i, i +7 olefin linkage. In particular examples, the α,α-disubstituted amino acid at amino acid position 5 comprises (S)-2-amino-2-methylhept-6-ynoic acid and the α,α-disubstituted amino acid at amino acid position 12 comprises (R)-2-amino-2-methyloct-7-ynoic acid.

In a further example of the method, a p53 peptidomimetic macrocycle is converted into a p53 peptidomimetic macrocycle having improved pharmacological properties of

low or undetectable toxicity, conformational stability, and membrane permeability by linking the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 5 and 12 of the p53 peptidomimetic macrocycle in an olefin linkage and covalently linking to the C-terminal amino acid of the p53 peptidomimetic macrocycle a polypeptide tail comprising three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific examples, each amino acid in the polypeptide tail having a D-configuration, wherein the α,α-disubstituted amino acid at amino position 5 is (S)-2-amino-2-methyldec-9-enoic acid and the α,α-disubstituted amino acid at amino acid position 12 is (R)-2-amino-2-methylhept-6-enoic acid.

In examples in which the objective is to improve binding of the p53 peptidomimetic macrocycle to MDM2 and/or MDMX and improve cellular activity in a p53 cellular reporter gene assay, the amino acid at each position of the polypeptide tail comprising three to nine amino acids is independently selected from natural or unnatural L- or D-amino acids. In particular examples of the disclosed p53 peptidomimetic macrocycles, the amino acid at each position of the polypeptide tail comprises alanine. In a further example, the amino acids at positions 3 and 6 of the polypeptide tail are each independently selected from alkyl or aromatic amino acids. In particular examples of the polypeptide tail, the amino acids at positions 3 and 6 of the polypeptide tail are each independently selected from alkyl or Phenylalanine. In particular examples of the polypeptide tail, the polypeptide tail comprises or consists of six amino acids. In particular examples of the polypeptide tail, the alkyl amino acid of the polypeptide tail comprises alanine. In particular examples of the polypeptide tail, the polypeptide tail comprises six amino acids. In further examples of the polypeptide tail, each amino acid of the polypeptide tail is in the D configuration.

In examples in which the objective is to improve amphipathicity, solubility and/or cellular activity of the p53 peptidomimetic macrocycle, the amino acid at each position of the polypeptide tail comprising three to nine amino acids is independently selected from natural or unnatural L- or D-negatively charged amino acids with the proviso that at least one amino acid is negatively charged. In a further example, of the polypeptide tail, the amino acid at position 1 of the polypeptide tail is independently selected from alkyl, glutamic acid, or gamma-carboxylic glutamic acid (Gla); the amino acid at position 3 of the polypeptide tail is independently selected from alkyl or glutamic acid; the amino acid at position 5 of the polypeptide tail is independently selected from alkyl and alpha-methyl glutamic acid, with the proviso that at least one of the amino acids at positions 1, 3, or 5 comprises glutamic acid or

alpha-methyl glutamic acid. In particular examples of the polypeptide tail, the polypeptide tail comprises or consists of six amino acids. In further examples of the polypeptide tail, the alkyl amino acid of the polypeptide tail comprises alanine. In further examples of the polypeptide tail, each amino acid of the polypeptide tail is in the D configuration.

The present disclosure further provides a p53 peptidomimetic macrocycle comprising:

$TAX^3YAX^6X^7EKX^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}$-$X^{19}X^{20}X^{21}$ (SEQ ID NO: 17)

wherein X^3 is D-Trp, D-6-Fluoro-Trp, D-6-Chloro-Trp, D-6-Bromo-Trp, D-6-Iodo-Trp, D-6-methyl-Trp, D-6-cyano-Trp, D-6-hydroxy-Trp, D-6-NO_2-Trp, D-7-Fluoro-Trp, D-7-Chloro-Trp, D-7-Bromo-Trp, D-7-Iodo-Trp, D-7-methyl-Trp, D-7-cyano-Trp, D-7-hydroxy-Trp, D-7-NO_2-Trp, D-6,7-Fluoro-Trp, D-6,7-Chloro-Trp, D-6,7-Bromo-Trp, D-6,7-Iodo-Trp, D-6,7-methyl-Trp, D-6,7-cyano-Trp, D-6,7-hydroxy-Trp, or D-6,7-NO_2-Trp; X^6 is (R)-2-amino-2-methylhept-6-enoic acid; X^7 is D-p-Fluoro-Phe, D-p-Chloro-Phe, D-p-Bromo-Phe, D-p-Iodo-Phe, D-p-methyl-Phe, D-p-cyano-Phe, D-p-hydroxy-Phe, D-p-NO_2-Phe, or D-p-CF_3-Phe; X^{10} is (R)-2-amino-2-methylhept-6-enoic acid; X^{11} is D-Leu; X^{12} is D-Arg or D-Ser; X^{13} is D-Ala, D-Glu, or D-Gla (γ-carboxylic glutamic acid); X^{14} is D-Ala; X^{15} is D-Ala, D-Phe, or D-Glu; X^{16} is D-Ala or absent; X^{17} is D-Ala, D-α-methyl-Glu, or absent; X^{18} is D-Ala or absent; X^{19} is an D-Ala or absent; X^{20} is D-Ala or absent; X^{21} is D-Ala or absent; the N-terminal amino group of the Thr at position 1 is conjugated to an acyl group having the formula RCO-; R is an alkane having the formula C_nH_{2n+1}; n is an integer from 1 to 10; the C-terminal amino acid optionally comprises an amino group; and the staple is an olefin obtained through ring-closing metathesis between X^6 and X^{10}.

In particular examples, amino acids 1-12 are all D amino acids and amino acids 13-21 are each independently an L-amino acid or D-amino acid or are each a D-amino acid.

In a further example of the p53 peptidomimetic macrocycle, X^{12} is D-Ser. In further examples of the p53 peptidomimetic macrocycle, X^3 is D-6-Fluoro-Trp; X^7 is D-p-CF_3-Phe; or X^3 is D-6-Fluoro-Trp and X^7 is D-p-CF_3-Phe. In a further example, the acyl group is an acetyl group.

The present disclosure provides a p53 peptidomimetic macrocycle comprising:

$TAX^3YX^5NX^7EX^9LX^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}$-$X^{19}X^{20}X^{21}$ (SEQ ID NO: 18)

wherein X^3 is D-Trp, D-6-Fluoro-Trp, D-6-Chloro-Trp, D-6-Bromo-Trp, D-6-Iodo-Trp, D-6-methyl-Trp, D-6-cyano-Trp, D-6-hydroxy-Trp, D-6-NO_2-Trp, D-7-Fluoro-Trp, D-7-Chloro-Trp, D-7-Bromo-Trp, D-7-Iodo-Trp, D-7-methyl-Trp, D-7-cyano-Trp, D-7-hydroxy-Trp, D-7-NO_2-Trp, D-6,7-Fluoro-Trp, D-6,7-Chloro-Trp, D-6,7-Bromo-Trp, D-6,7-Iodo-Trp, D-6,7-methyl-Trp, D-6,7-cyano-Trp, D-6,7-hydroxy-Trp, or D-6,7-NO_2-Trp; X^5 is (S)-2-amino-2-methylhept-6-ynoic acid; X^7 is D-p-Fluoro-Phe, D-p-Chloro-Phe, D-p-Bromo-Phe, D-p-Iodo-Phe, D-p-methyl-Phe, D-p-cyano-Phe, D-p-hydroxy-Phe, D-p-NO_2-Phe, or D-p-CF_3-Phe; X^9 is D-Lys or D-Gln; X^{11} is D-Leu; X^{12} is (R)-2-amino-2-methyloct-7-ynoic acid; X^{13} is D-Ala, D-Glu, D-Gla (γ-carboxylic glutamic acid) or absent; X^{14} is D-Ala or absent; X^{15} is D-Ala, D-Phe, or D-Glu or absent; X^{16} is D-Ala or absent; X^{17} is D-Ala, D-α-methyl-Glu, or absent; X^{18} is D-Ala or absent; X^{19} is D-Ala or absent; X^{20} is D-Ala or absent; X^{21} is D-Ala or absent; the N-terminus amino group of the Thr at position 1 is conjugated to an acyl group having the formula RCO-; R is an alkane having the formula C_nH_{2n+1}; n is an integer from 1 to 10; the C-terminal amino acid optionally comprises an amino group; and the staple is a di-alkyne obtained through alkyne cross-coupling between X^5 and X^{12}.

In particular examples, amino acids 1-12 are all D amino acids and amino acids 13-21 are each independently an L-amino acid or D-amino acid or are each a D-amino acid.

In a further example of the p53 peptidomimetic macrocycle, X^9 is D-Gln. In a further example of the p53 peptidomimetic macrocycle, X^3 is D-6-Fluoro-Trp; X^7 is D-p-CF_3-Phe; or X^3 is D-6-Fluoro-Trp and X^7 is D-p-CF_3-Phe. In a further example, the acyl group is an acetyl group.

The present disclosure provides a p53 peptidomimetic macrocycle comprising:

$$TAX^3YX^5NX^7EX^9LX^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}X^{19}X^{20}X^{21}$$ (SEQ ID NO: 27)

wherein X^3 is D-Trp, D-6-Fluoro-Trp, D-6-Chloro-Trp, D-6-Bromo-Trp, D-6-Iodo-Trp, D-6-methyl-Trp, D-6-cyano-Trp, D-6-hydroxy-Trp, D-6-NO_2-Trp, D-7-Fluoro-Trp, D-7-Chloro-Trp, D-7-Bromo-Trp, D-7-Iodo-Trp, D-7-methyl-Trp, D-7-cyano-Trp, D-7-hydroxy-Trp, D-7-NO_2-Trp, D-6,7-Fluoro-Trp, D-6,7-Chloro-Trp, D-6,7-Bromo-Trp, D-6,7-Iodo-Trp, D-6,7-methyl-Trp, D-6,7-cyano-Trp, D-6,7-hydroxy-Trp, or D-6,7-NO_2-Trp; X^5 is (S)-2-amino-2-methyldec-9-enoic acid; X^7 is D-p-Fluoro-Phe, D-p-Chloro-Phe, D-p-Bromo-Phe, D-p-Iodo-Phe, D-p-methyl-

Phe, D-p-cyano-Phe, D-p-hydroxy-Phe, D-p-NO_2-Phe, or D-p-CF_3-Phe; X^9 is D-Lys or D-Gln; X^{11} is D-Leu; X^{12} is (R)-2-amino-2-methylhept-6-enoic acid; X^{13} is D-Ala, D-Glu, D-Gla (γ-carboxylic glutamic acid); X^{14} is D-Ala; X^{15} is D-Ala, D-Phe, or D-Glu; X^{16} is D-Ala; X^{17} is D-Ala or D-α-methyl-Glu; X^{18} is D-Ala; X^{19} is D-Ala or absent; X^{20} is D-Ala or absent; X^{21} is D-Ala or absent; the N-terminus amino group of the Thr at position 1 is conjugated to an acyl group having the formula RCO-; R is an alkane having the formula C_nH_{2n+1}; n is an integer from 1 to 10; the C-terminal amino acid optionally comprises an amino group; amino acids 1-12 are D amino acids; and the staple is an olefin obtained through ring-closing metathesis between X^5 and X^{12}.

In particular examples, amino acids 1-12 are all D amino acids and amino acids 13-21 are each independently an L-amino acid or D-amino acid or are each a D-amino acid.

In a further example of the p53 peptidomimetic macrocycle, X^9 is D-Gln. In a further example of the p53 peptidomimetic macrocycle, X^3 is D-6-Fluoro-Trp; X^7 is D-p-CF_3-Phe; or X^3 is D-6-Fluoro-Trp and X^7 is D-p-CF_3-Phe. In a further example, the acyl group is an acetyl group.

The present disclosure provides a p53 peptidomimetic macrocycle comprising:

$TAX^3YX^5NX^7EX^9LX^{11}X^{12}$ (SEQ ID NO: 19)

wherein X^3 is D-Trp, D-6-Fluoro-Trp, D-6-Chloro-Trp, D-6-Bromo-Trp, D-6-Iodo-Trp, D-6-methyl-Trp, D-6-cyano-Trp, D-6-hydroxy-Trp, D-6-NO_2-Trp, D-7-Fluoro-Trp, D-7-Chloro-Trp, D-7-Bromo-Trp, D-7-Iodo-Trp, D-7-methyl-Trp, D-7-cyano-Trp, D-7-hydroxy-Trp, D-7-NO_2-Trp, D-6,7-Fluoro-Trp, D-6,7-Chloro-Trp, D-6,7-Bromo-Trp, D-6,7-Iodo-Trp, D-6,7-methyl-Trp, D-6,7-cyano-Trp, D-6,7-hydroxy-Trp, or D-6,7-NO_2-Trp; X^5 is (S)-2-amino-2-methylhept-6-ynoic acid; X^7 is D-p-Fluoro-Phe, D-p-Chloro-Phe, D-p-Bromo-Phe, D-p-Iodo-Phe, D-p-methyl-Phe, D-p-cyano-Phe, D-p-hydroxy-Phe, D-p-NO_2-Phe, or D-p-CF_3-Phe; X^9 is D-Lys or D-Gln; X^{11} is D-Leu; X^{12} is (R)-2-amino-2-methyloct-7-ynoic acid; the N-terminus amino group of the Thr at position 1 is conjugated to an acyl group having the formula RCO-; R is an alkane having the formula C_nH_{2n+1}; n is an integer from 1 to 10; the C-terminal amino acid optionally comprises an amino group; amino acids 1-12 are D amino acids; and, the staple is a di-alkyne obtained through alkyne cross-coupling between X^5 and X^{12}.

In a further example of the p53 peptidomimetic macrocycle, X^9 is D-Gln. In a further example of the p53 peptidomimetic macrocycle, X^3 is D-6-Fluoro-Trp; X^7 is D-p-CF_3-Phe; or X^3 is D-6-Fluoro-Trp and X^7 is D-p-CF_3-Phe. In a further example, the acyl group is an acetyl group.

In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

The present disclosure, in another aspect, provides a composition comprising a p53 peptidomimetic macrocycle disclosed herein and a pharmaceutically acceptable carrier. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

The present disclosure further provides a method for treating cancer in a subject in need thereof comprising administering to the subject a p53 peptidomimetic macrocycle disclosed herein or a composition comprising said p53 peptidomimetic macrocycle. The present disclosure, in another aspect, provides the p53 peptidomimetic macrocycle disclosed herein or the composition disclosed herein for use in therapy. The present disclosure further provides a use of a peptidomimetic macrocycle disclosed herein for the preparation of a medicament for treating cancer. The present disclosure, in another aspect, provides use of the p53 peptidomimetic macrocycle disclosed herein or the composition disclosed herein in the preparation of a medicament for treating cancer. The present disclosure further provides a peptidomimetic macrocycle disclosed herein for the treatment of cancer. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

In further examples of any disclosed method, use or composition for use, the cancer is selected from the group consisting of melanoma, non-small cell lung cancer, head and neck cancer, urothelial cancer, breast cancer, gastrointestinal cancer, multiple myeloma, hepatocellular cancer, non-Hodgkin lymphoma, renal cancer, Hodgkin lymphoma, mesothelioma, ovarian cancer, small cell lung cancer, esophageal cancer, anal cancer, biliary tract cancer, colorectal cancer, cervical cancer, thyroid cancer, salivary cancer, pancreatic cancer,

bronchus cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, and cancer of hematological tissues.

The present disclosure further provides a method of modulating the activity of p53 and/or MDM2 and/or MDMX in a subject comprising administering to the subject a p53 peptidomimetic macrocycle disclosed herein, or a composition comprising said p53 peptidomimetic macrocycle. The present disclosure further provides the use of a peptidomimetic macrocycle disclosed herein for the preparation of a medicament for modulating said activity. The present disclosure further provides a peptidomimetic macrocycle disclosed herein for the modulation of said activity.

In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

The present disclosure further provides a method of antagonizing the interaction between p53 and MDM2 and/or between p53 and MDMX in a subject comprising administering to the subject a p53 peptidomimetic macrocycle disclosed herein, or a composition comprising said p53 peptidomimetic macrocycle. The present disclosure further provides the use of a p53 peptidomimetic macrocycle disclosed herein for the preparation of a medicament for antagonizing the interaction between p53 and MDM2 and/or between p53 and MDMX.

The present disclosure further provides a peptidomimetic macrocycle disclosed herein for antagonizing the interaction between p53 and MDM2 and/or between p53 and MDMX. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

The present disclosure further provides a combination therapy for treating cancer comprising administering to a subject a therapeutically effective amount of a p53 peptidomimetic macrocycle disclosed herein or a composition comprising said p53 peptidomimetic macrocycle

and a therapeutically effective dose of a chemotherapy agent or radiation. In a further example, the chemotherapy agent or radiation is administered to the subject followed by administration of the p53 peptidomimetic macrocycle; the p53 peptidomimetic macrocycle is administered to the subject followed by administration of the chemotherapy agent or radiation; or the chemotherapy agent or radiation is administered to the subject simultaneously with administration of the p53 peptidomimetic macrocycle. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

The present disclosure further provides a combination therapy for the treatment of a cancer comprising a therapeutically effective amount of a p53 peptidomimetic disclosed herein or a composition comprising said p53 peptidomimetic macrocycle and a therapeutically dose of a chemotherapy agent or radiation. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

In particular examples, the chemotherapy agent is selected from the group consisting of actinomycin, all-trans retinoic acid, alitretinoin, azacitidine, azathioprine, bexarotene, bleomycin, bortezomib, carmofur, carboplatin, capecitabine, cisplatin, chlorambucil, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, docetaxel, doxifluridine, doxorubicin, epirubicin, epothilone, etoposide, fluorouracil, gemcitabin, hydroxyurea, idarubicin, imatinib, ixabepilone, irinotecan, mechlorethamine, melphalan, mercaptopurine, methotrexate, mitoxantrone, nitrosoureas, oxaliplatin, paclitaxel, pemetrexed, romidepsin, tegafur, temozolomide(oral dacarbazine), teniposide, tioguanine, topotecan, utidelone, valrubicin, vemurafenib, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat.

The present disclosure further provides a combination therapy for treating cancer comprising administering to a subject a therapeutically effective amount of a p53 peptidomimetic macrocycle disclosed herein or a composition comprising said p53 peptidomimetic macrocycle and a therapeutically effective amount of a checkpoint inhibitor. In particular examples, the checkpoint inhibitor is an anti-PD1 antibody or an anti-PD-L1 antibody. In a further

example, the combination therapy further includes administering to the subject a therapeutically effective dose of a chemotherapy agent or radiation. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO:

The present disclosure further provides a treatment for cancer comprising administering to a subject having the cancer a vector comprising a nucleic acid molecule encoding a wildtype p53 or p53 variant or analog with transcriptional activation activity followed by one or more administrations of a therapeutically effective amount of a p53 peptidomimetic macrocycle disclosed herein or a composition comprising said p53 peptidomimetic macrocycle. In particular examples, the vector is a plasmid, a retrovirus, adenovirus, or adeno-associated virus. In particular examples, the subject is administered a chemotherapy or radiation treatment prior to administering the vector to the subject or subsequent to administering the vector to the subject. In further examples, the therapy further includes administering to the subject a checkpoint inhibitor. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10. In a further example, the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 9.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows a crystal structure of native human p53 peptide:MDM2 (Protein Data Bank (PDB) ID:1YCR) complex (Baek et al., JACS 134: 103-106 (2012)). MDM2 is shown as surface and bound native p53 peptide (SEQ ID NO: 15) is shown as a ribbon cartoon with interacting residues L-Phe^{19}, L-Trp^{23}, and L-Leu^{26} of the p53 peptide are highlighted in sticks. Hydrogen bond interactions are shown as dotted lines. The amino acid numbering of the p53 peptide corresponds to amino acids 15-29 of native human p53 having the amino acid sequence set forth in SEQ ID NO: 16. H96, K94, Q72, and Y100 are amino acids in the MDM2.

Fig. 1B shows a crystal structure of ^{D}PMI-δ p53 peptidomimetic peptide:MDM2 (PDB ID: 3PTX) complex (Zhan et al., J. Med. Chem. 55: 6237-6241 (2012)). MDM2 is shown as surface and bound ^{D}PMI-δ peptidomimetic macrocycle (SEQ ID NO: 11) is shown as a ribbon cartoon with interacting residues ^{D}Leu^{11}, pCF$_3$-^{D}Phe^{7}, and 6-F-^{D}Trp^{3} highlighted in sticks.

WHAT IS CLAIMED:

1. A p53 peptidomimetic macrocycle, comprising:

an i, i + 4 olefin staple and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle;

wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration, or each amino acid in the polypeptide tail having a D-configuration.

2. The p53 peptidomimetic macrocycle of claim 1, wherein the p53 peptidomimetic macrocycle comprises 12 amino acids and an i, i + 4 olefin staple formed between the α-carbons of two α,α-disubstituted amino acids located at amino acid positions 6 and 10 of the p53 peptidomimetic macrocycle and a polypeptide tail covalently linked at its N-terminus to the C-terminal amino acid of the p53 peptidomimetic macrocycle, wherein the p53 peptidomimetic macrocycle comprises all D-configuration amino acids and the polypeptide tail comprises three to nine amino acids, each amino acid of the polypeptide tail independently having a D-configuration or an L-configuration or, in specific embodiments, each amino acid in the polypeptide tail having a D-configuration.

3. The p53 peptidomimetic macrocycle of claim 2, wherein the α,α-disubstituted amino acids at amino acid positions 6 and 10 of the p53 peptidomimetic macrocycle comprise (R)-2-amino-2-methylhept-6-enoic acid.

4. The p53 peptidomimetic macrocycle of claim 3, wherein the p53 peptidomimetic macrocycle further comprises D-6-fluoro-tryptophane at amino acid position 3 and D-p-CF_3-phenylalanine at amino acid position 7.

5. The p53 peptidomimetic macrocycle of claim 4, wherein the p53 peptidomimetic macrocycle further comprises threonine at amino acid position 1, alanine at amino acid position 2, tyrosine at amino acid position 4, alanine at amino acid position 5, glutamic acid at amino acid position 8, lysine or glutamine at amino acid position 9, leucine at amino acid position 11, and arginine or serine at amino acid position 12.

6. The p53 peptidomimetic macrocycle of claim 5, wherein the p53 peptidomimetic macrocycle comprises glutamine at amino acid at position 9 and serine at amino acid at position 12.

7. The p53 peptidomimetic macrocycle of claim 1, wherein the p53 peptidomimetic macrocycle comprises an amino acid sequence set forth in 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: 9, or SEQ ID NO: 10.

8. A composition comprising the p53 peptidomimetic macrocycle of any one of claims 1-7 and a pharmaceutically acceptable carrier.

9. The p53 peptidomimetic macrocycle of any one of claims 1-7 or the composition of claim 8 for use in therapy.

10. Use of the p53 peptidomimetic macrocycle of any one of claims 1-7 or the composition of claim 8 in the preparation of a medicament for treating cancer.

11. The use of claim 10, wherein the cancer is selected from the group consisting of melanoma, non-small cell lung cancer, head and neck cancer, urothelial cancer, breast cancer, gastrointestinal cancer, multiple myeloma, hepatocellular cancer, non-Hodgkin lymphoma, renal cancer, Hodgkin lymphoma, mesothelioma, ovarian cancer, small cell lung cancer, esophageal cancer, anal cancer, biliary tract cancer, colorectal cancer, cervical cancer, thyroid cancer, salivary cancer, pancreatic cancer, bronchus cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, and cancer of hematological tissues.