[0008] One E3 ligase with therapeutic potential is the von Hippel-Lindau (VHL) tumor suppressor, the substrate recognition subunit of the E3 ligase complex VCB, which also consists of elongins B and C, Cul2 and Rbx1. The primary endogenous substrate of VHL is Hypoxia Inducible Factor 1α (HIF-1α), a transcription factor that upregulates genes such as the pro-angiogenic growth factor VEGF and the red blood cell inducing cytokine erythropoietin in response to low oxygen levels. While HIF-1α is constitutively expressed, its intracellular levels are kept very low under normoxic conditions via its hydroxylation by prolyl hydroxylase domain (PHD) proteins and subsequent VHL-mediated ubiquitination.

[0009] Although CIDEs address many of the issues associated with small molecule inhibitors in targeted therapy of intracellular proteins, they introduce a new set of challenges. For example, the potency of small molecule CIDEs often depends on the stability of the ternary complex formed between target protein, CIDE, and ubiquitin system. Therefore, a critical aspect of efficient CIDE design is the stabilization of the ternary complex between the target protein and the ubiquitin system. Typical (linear) CIDE molecules contain long flexible linker elements. This leads to dynamic molecules that exhibit multiple conformations and a high entropic binding penalty and are thus not ideally suited for stabilization of a ternary structure between two proteins.

[0010] There is therefore a need for treatments for diseases and conditions involving unwanted or defective proteins such as bromodomain proteins like BRD4 in diseases such as cancer, fibrosis, immunological disorders and for compounds that can inhibit or degrade those proteins.

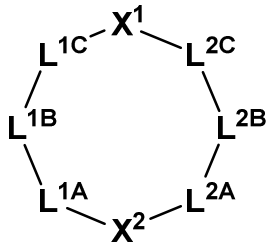
SUMMARY

[0011] Provided herein are macrocyclic compounds comprising an E3 ubiquitin ligase binding motif (EULBM) and at least one amino acid, and methods of making and using such macrocyclic EULBMs. Also provided herein are macrocyclic heterobifunctional chemical inducers of degradation (CIDEs) comprising an E3 ubiquitin ligase binding motif (EULBM) and a target protein binding motif (TPBM) linked by at least one amino acid, and methods of making and using such macrocyclic heterobifunctional CIDEs.

[0012] In one aspect, the disclosure provides a macrocyclic compound, or a pharmaceutically acceptable salt thereof, comprising a VHL binding motif comprising a hydroxyproline and two or more amino acids, wherein the VHL binding motif and the two or more amino acids form a cyclic polypeptide. In one example, provided herein is a macrocyclic compound comprising an EULBM and at least one amino acid. In examples, the EULBM and three or more amino acids form a

cyclic polypetide. In examples, the EULBM is a VHL binding motif. In examples, the macrocyclic compound further comprises a TPBM conjugated to at least one amino acid in said macrocyclic compound.

[0013] In one example, provided herein is a compound having the formula:



(I)

wherein X^1, X^2, L^{1A}, L^{1B}, L^{1C}, L^{2A}, L^{2B}, L^{2C} are each independently a bond or an amino acid.

[0014] In another example, X^1 is an EULBM, and L^{1A}, L^{1B}, L^{1C}, X^2, L^{2A}, L^{2B}, L^{2C} are each independently a bond or an amino acid.

[0015] In yet another example, X^1 is an EULBM, X^2 is a TPBM, and L^{1A}, L^{1B}, L^{1C}, L^{2A}, L^{2B}, L^{2C} are each independently a bond or an amino acid.

[0016] In other examples, X^2 is a D-α amino acid or a D-δ amino acid, L^{2C} is a D-α amino acid or a D-β amino acid or a bond. L^{2A} and L^{2B} are each independently a bond or an amino acid. L^{1A}, L^{1B} and L^{1C} are each independently a bond or an amino acid.

[0017] In one aspect, the disclosure provides a compound comprising a cyclic peptide comprising a sequence selected from the group consisting of SEQ ID NOs. 1-68 and 112-120, wherein the amine end of the first amino acid in said sequence is covalently bonded to the carboxyl end of the last amino acid in said sequence. In an example, provided herein is a cyclic VHL binding motif oligopeptide including a sequence selected from the group consisting of SEQ ID NOs. 1-68.

[0018] In one aspect, the disclosure provides a compound comprising a cyclic oligopeptide having comprising an E3 ubiquitin ligase binding motif (EULBM) wherein the cyclic oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 69-111. In an eample, provided herein is a cyclic oligopeptide linked to an EULBM such as a VHL binding motif to form a macrocyclic EULBM including a sequence selected from the group consisting of SEQ ID NOs. 69-111.

[0019] In one aspect, the disclosure provides a pharmaceutical composition comprising a macrocyclic compound as disclosed herein, or a pharmaceutically acceptable salt thereof, or a compound as disclosed herein, and a pharmaceutically acceptable excipient. In an example, provided herein are pharmaceutical compositions including a compound as described herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, including examples.

[0020] In one aspect, the disclosure provides use of a macrocyclic compound as disclosed herein, or a pharmaceutically acceptable salt thereof, or a compound as disclosed herein, in the manufacture of medicament for treatment of a cancer. In an example, provided herein is a method of treating cancer, including administering to a subject in need thereof a therapeutically effective amount of a compound as described herein, or a pharmaceutically acceptable salt thereof.

[0021] In an example, provided herein is a method of treating a fibrotic condition such as idiopathic pulmonary fibrosis (IPF) including administering to a subject in need thereof a

therapeutically effective amount of a compound as described herein, or a pharmaceutically acceptable salt thereof.

[0022] In an example, provided herein is a macrocyclic EULBM which can be incorporated into a CIDE.

DETAILED DESCRIPTION

I. Definitions

[0023] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0024] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., $-CH_2O-$ is equivalent to $-OCH_2-$.

[0025] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include mono-, di- and multivalent radicals. The alkyl may include a designated number of carbons (e.g., C_1-C_{10} means one to ten carbons). Alkyl is an uncyclized chain. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. An alkoxy is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-). An alkyl moiety may be an alkenyl moiety. An alkyl moiety may be an alkynyl moiety. An alkyl moiety may be fully saturated. An alkenyl may include more than one double bond and/or one or more triple bonds in addition to the one or more double bonds. An alkynyl may include more than one triple bond and/or one or more double bonds in addition to the one or more triple bonds.

[0026] The term "alkylene," by itself or as part of another substituent, means, unless otherwise stated, a divalent radical derived from an alkyl, as exemplified, but not limited by,

WHAT IS CLAIMED IS:

1. A macrocyclic compound, or a pharmaceutically acceptable salt thereof, comprising a VHL binding motif comprising a hydroxyproline and two or more amino acids, wherein the VHL binding motif and the two or more amino acids form a cyclic polypeptide.

2. The macrocyclic compound of claim 1, further comprising a target protein binding motif (TPBM) conjugated to at least one amino acid in said macrocyclic compound.

3. The macrocyclic compound of claim 1 or 2, wherein at least one amino acid is a D-α amino acid, a D-β amino acid or a D-δ amino acid.

4. The macrocyclic compound of claim 1 or 2, wherein at least one amino acid is a D-α amino acid.

5. The macrocyclic compound of claim 4, wherein the D-α amino acid is selected from the group consisting of D-Cys(S-ac), Gly, D-hCys(S-ac), NMe-D-Cys(S-ac), O1Pen, NMe-O1Pen, GABA, Ava, AEP, Ahx, Ahp, S1Pen, NMe-Ava, 2-AminoMePheAc, Nme-Ahx, δMe-Ava, αMeAva, βMe-Ava and 4PipAc.

6. The macrocyclic compound of claim 1 or 2, wherein the VHL binding motif comprises an L-hydroxyproline or an L-fluorohydroxyproline.

7. The macrocyclic compound of claim 6, wherein the VHL binding motif further comprises two amino acids.

8. The macrocyclic compound of claim 7, wherein at least one of the two amino acids is an L-α amino acid or an L-β amino acid.

9. The macrocyclic compound of claim 8, wherein the L-α amino acid or L-β amino acid is selected from the group consisting of L-Tle, L-bMe-Ile, L-Val, L-Ala, L-Abu, L-Pen, L-Cha, L-Cpa, L-Cba, L-bMe2AllylGly, L-AdaGly, L-ThpGly, L-Tle, L-Tle-Tria, NMe-LTle-Tria and L-Tle-TriaCyP.

10. The macrocyclic compound of claim 7, wherein at least one of the two amino acids is a D-α amino acid or a D-β amino acid.

11. The macrocyclic compound of claim 10, wherein the D-α amino acid or a D-β amino acid is selected from the group consisting of D-MTPG, D-BiPhe, D-Ala, Aib, D-Bta, D-bMtpg, D-MtPhe and D-Phe(4I).

12. The macrocyclic compound of claim 2, wherein the TPBM is conjugated to a D-α amino acid or D-δ amino acid.

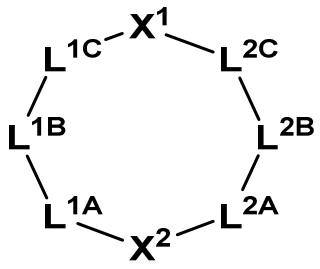
13. The macrocyclic compound of claim 12, wherein the D-α amino acid or D-δ amino acid is selected from the group consisting of D-Dap, D-Dap-NMe, D-b2Orn, D-Dab, L-Dap, D-Pip, D-bLys, D-Dap(Peg3), D-diaminoacetic acid, D-Orn and NMe-D-Dap.

14. The macrocyclic compound of claim 2, wherein the TPBM comprises a triazolodiazepine or an isoxazole azepine.

15. The macrocyclic compound of claim 1 or 2, wherein at least one of the two or more amino acids is Gly, a D-α amino acid or a D-β amino acid.

16. The macrocyclic compound of claim 15, wherein the D-α amino acid or a D-β amino acid is selected from the group consisting of D-Ala, bAla, DPyrAla, D-Phe, D-BiPhe, D-Val, D-Gln, D-Lys and D-Lys(N3).

17. The macrocyclic compound of claim 1, wherein the macrocyclic compound is a compound of formula (I):



(I)

wherein

X^1 is the VHL binding motif;

X^2 is a D-α amino acid or a D-δ amino acid;

L^{2C} is a D-α amino acid or a D-β amino acid or a bond;

L^{2B} is a bond or an amino acid;

L^{2A} is a bond or an amino acid; and

L^{1A}, L^{1B} and L^{1C} are each independently a bond or an amino acid.

18. The macrocyclic compound of claim 17, wherein L^{2B} is a bond.

19. The macrocyclic compound of claim 17, wherein L^{2A} is a bond.

20. The macrocyclic compound of claim 17, wherein L^{1A} is a bond.

21. The macrocyclic compound of claim 17, wherein L^{1B} is a bond or an L-α amino acid.

22. The macrocyclic compound of claim 17, wherein L^{1B} is L-Gln or L-Ala.

23. The macrocyclic compound of claim 17, wherein L^{1C} is a D-α amino acid.

24. The macrocyclic compound of claim 17, wherein L^{1C} is selected from the group consisting of D-Cys(S-ac), Gly, D-hCys(S-ac), NMe-D-Cys(S-ac), O1Pen, NMe-O1Pen, GABA, Ava, AEP, Ahx, Ahp, S1Pen, NMe-Ava, 2-AminoMePheAc, Nme-Ahx, δMe-Ava, αMe-Ava, βMe-Ava and 4PipAc.

25. The macrocyclic compound of any one of claims 17 to 24, wherein X^1 has the formula $–X^{1A}\text{-}X^{1B}\text{-}X^{1C}–$, wherein

X^{1A} is an L-α amino acid or an L-β amino acid attached to L^{1C};

X^{1B} is an L-hydroxyproline or an L-fluorohydroxyproline; and

X^{1C} is a D-α amino acid or a D-β amino acid attached to L^{2C}.

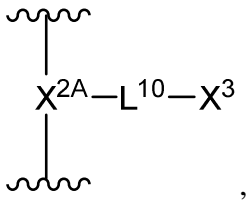
26. The macrocyclic compound of claim 25, wherein X^{1A} is selected from the group consisting of L-Tle, L-bMe-Ile, L-Tle-Tria, L-Val, L-Ala, L-Abu, L-Pen, L-Cha, L-Cpa, L-Cba, L-bMe2AllylGly, L-AdaGly and L-ThpGly.

27. The macrocyclic compound of claim 25, wherein X^{1C} is selected from the group consisting of D-MTPG, D-BiPhe, D-Ala, Aib, D-Bta, L-Bta, D-bMtpg, L-bMtpg, D-MtPhe, L-BiPhe, L-Tyr(O-Me), D-bBiPhe, D-bRMeBiPhe, D-bSMeBiPhe, D-Phe(4Br), D-Phe(4Cl), D-Phe(4F), D-Phe(4CN) and D-Phe(4I).

28. The macrocyclic compound of claim 17, wherein L^{2C} is selected from the group consisting of Gly, D-Ala, L-Ala, bAla, D-PyrAla, D-Phe, D-BiPhe, D-Val, D-Gln, D-Lys and D-Lys(N3).

29. The macrocyclic compound of claim 17, wherein X^2 is a target protein binding motif (TPBM) comprising the D-α amino acid or D-δ amino acid.

30. The macrocyclic compound of claim 29, wherein X^2 has the formula



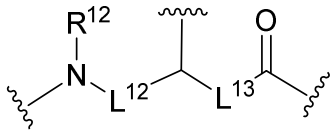
, wherein

X^{2A} is at least one natural or unnatural amino acid that forms a bond with L^{1A} and L^{2A};

L^{10} is a bond, a peptide linker or a non-peptide linker; and

X^3 is a targeting moiety.

31. The macrocyclic compound of claim 30, wherein X^{2A} has the formula



, wherein

the X^{2A} carbonyl is attached to L^{1A} amino, and the X^{2A} amine is attached to L^{2A} carbonyl, and the third attachment point is attached to L^{10};

L^{12} and L^{13} are each independently a bond or substituted or unsubstituted, saturated, unsaturated or partially unsaturated C_1-C_{10} alkyl; and

R^{12} is hydrogen or an unsubstituted C_1-C_5 alkyl.

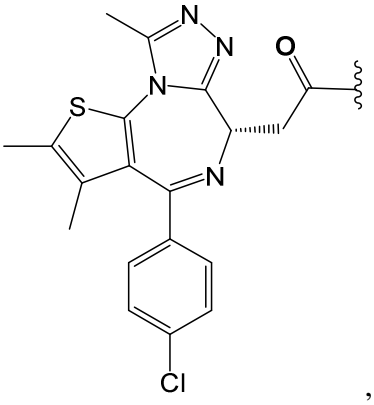
32. The macrocyclic compound of claim 30 or 31, wherein L^{10} is $–(CH(R^{112}))_{n12}–N(R^{110})–$, wherein R^{110} and R^{112} are each independently hydrogen or C_1-C_6 alkyl, and n12 is an integer from 0 to 6.

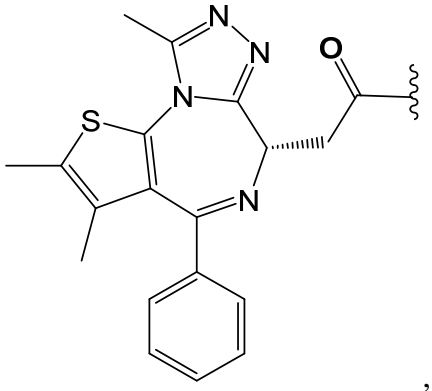
33. The macrocyclic compound of claim 30 or 31, wherein $-X^{2A}–L^{10}-$ is selected from the group consisting of D-Dap, D-Dap-NMe, D-b2Orn, D-Dab, L-Dap, D-Pip, D-bLys, D-Dap(Peg3), (D/L)-diaminoacetic acid, D-Orn, L-Orn and NMe-D-Dap.

34. The macrocyclic compound of claim 30 or 31, wherein X^3 is a triazolodiazepine or an isoxazole azepine.

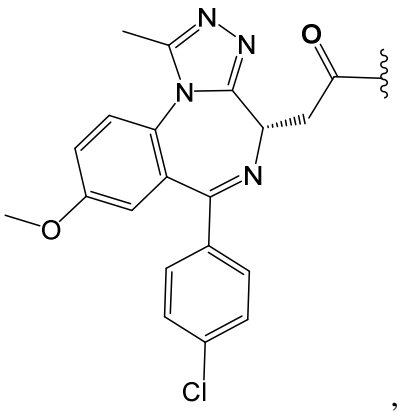
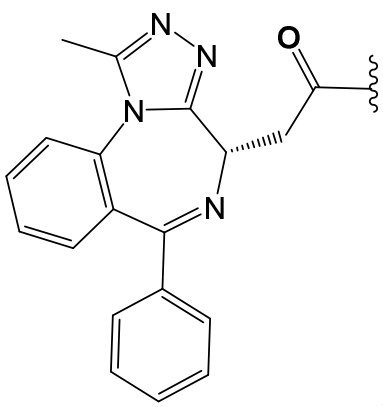
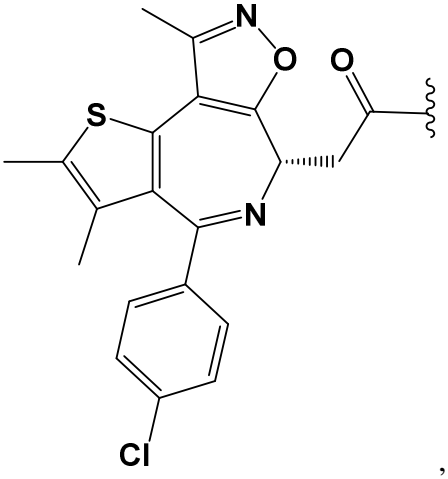
35. The macrocyclic compound of claim 34, wherein X^3 is selected from the group consisting of a thienotriazolodiazepine, benzotriazolodiazepine, thienoisoxazoloazepine and benzoisoxazoloazepine.

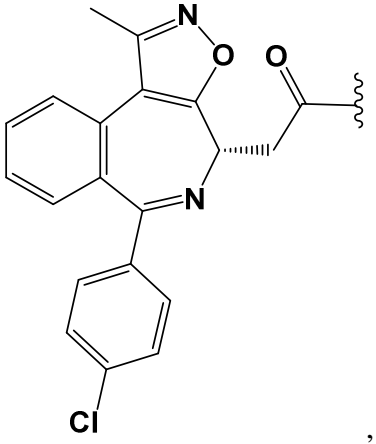
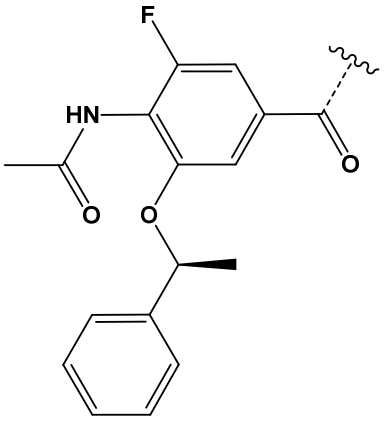
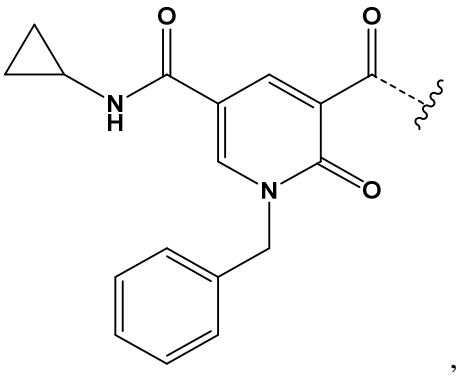
36. The macrocyclic compound of claim 34, wherein X^3 is selected from the group consisting of

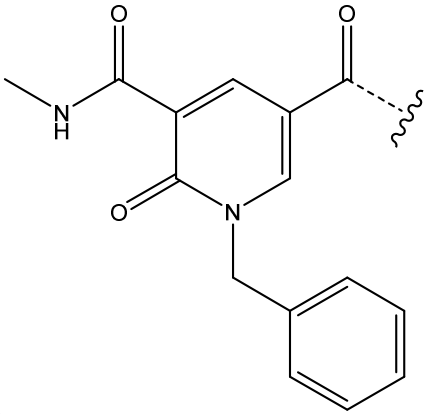


,


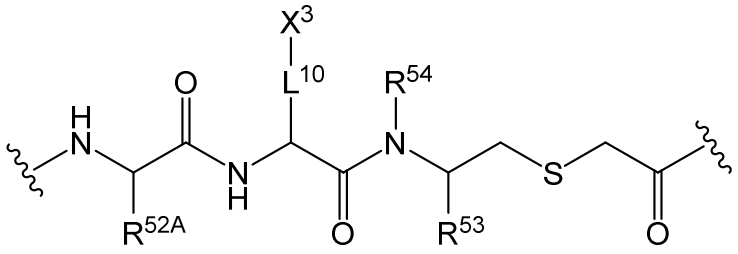
,

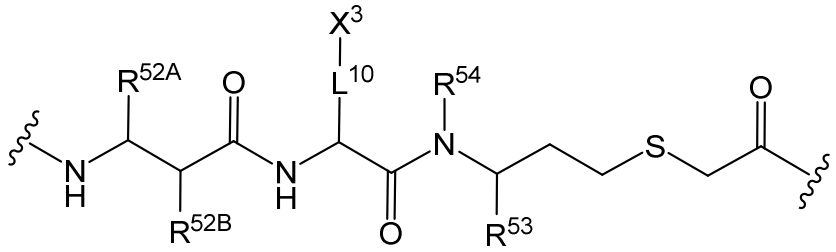

, 
, 
,

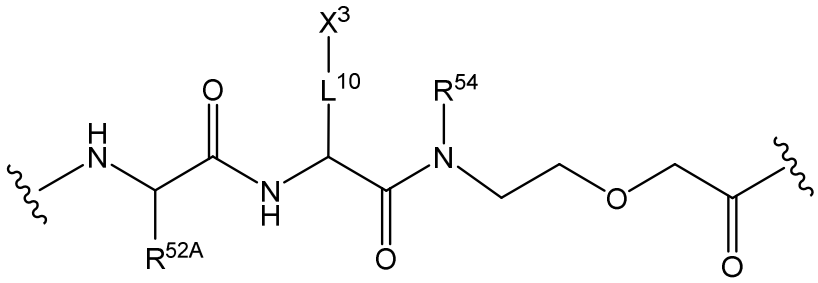

, 
, 
,

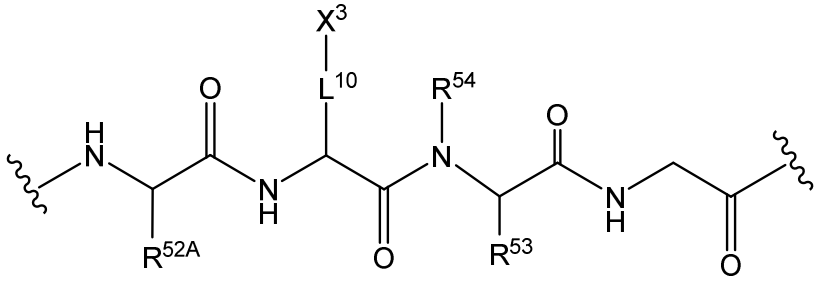

and .

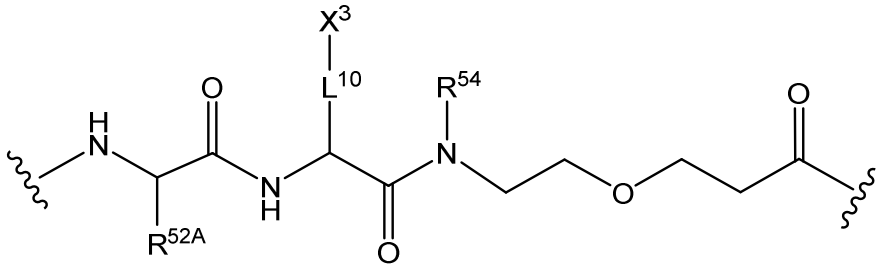
37. The macrocyclic compound of claim 17, wherein L^{2C}-L^{2B}-L^{2A}-X^2-L^{1A}-L^{1B}-L^{1C} is selected from the group consisting of

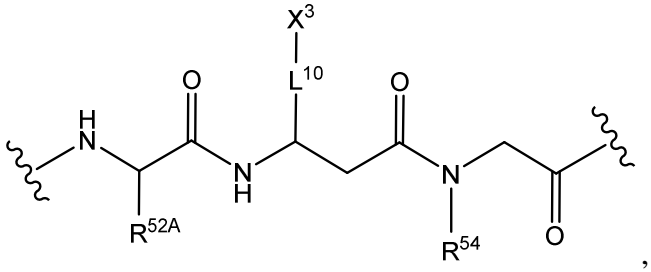




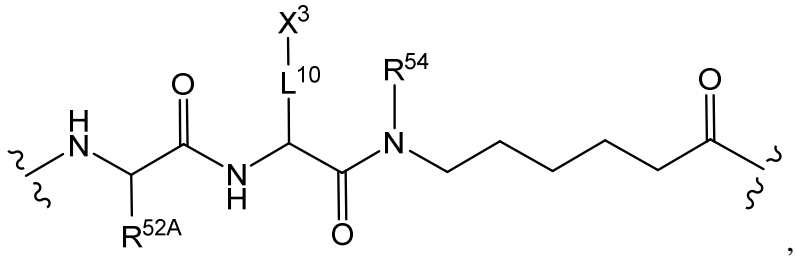




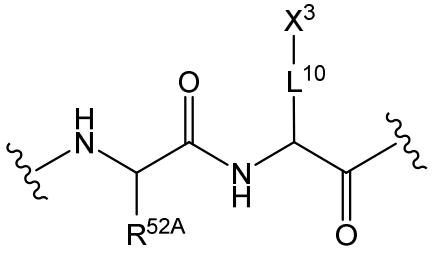




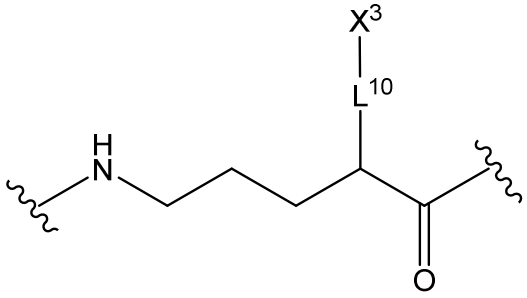
,

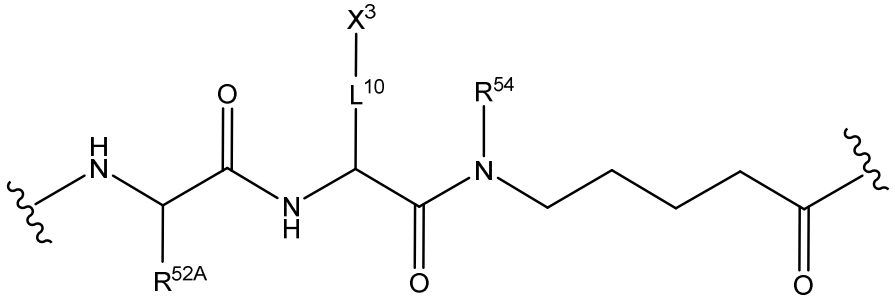


,

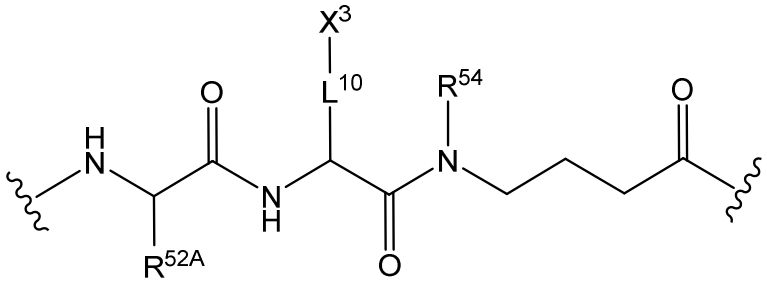


,

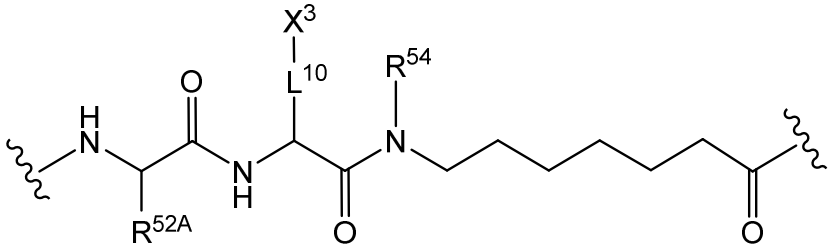




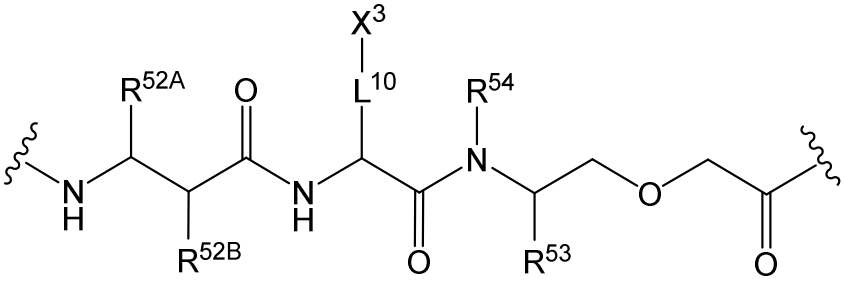
,



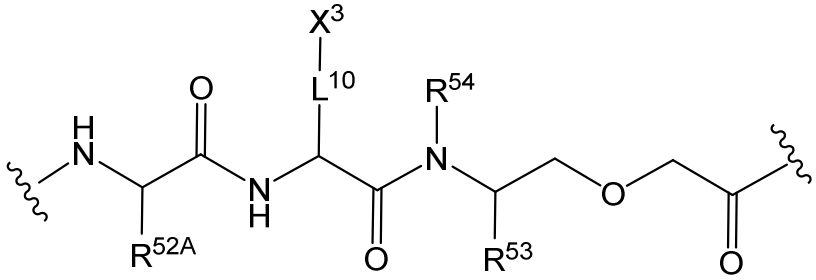
,



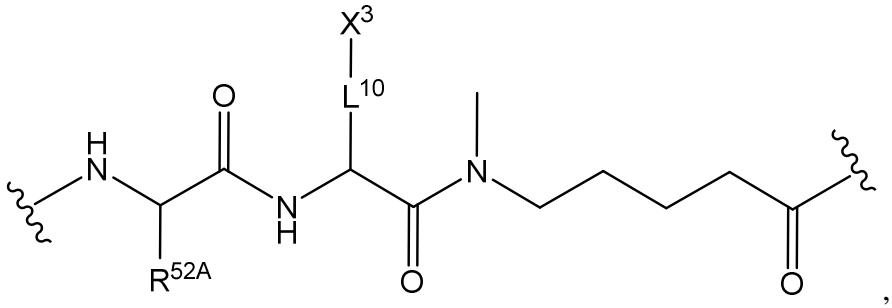
,



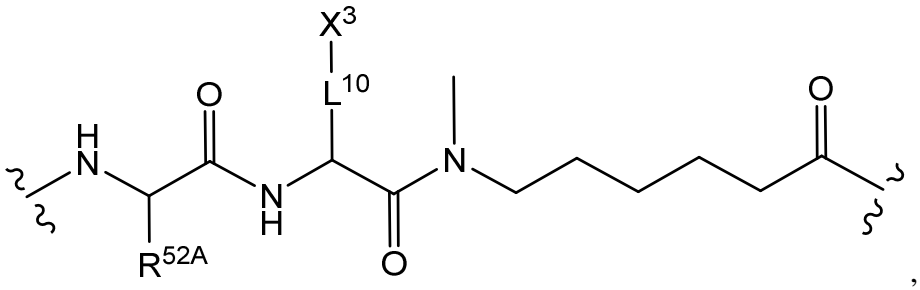
,

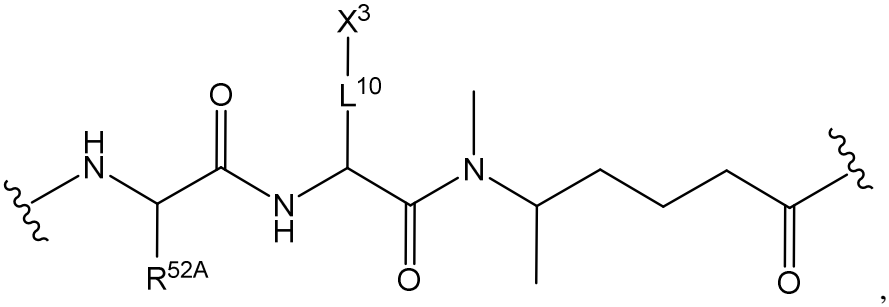


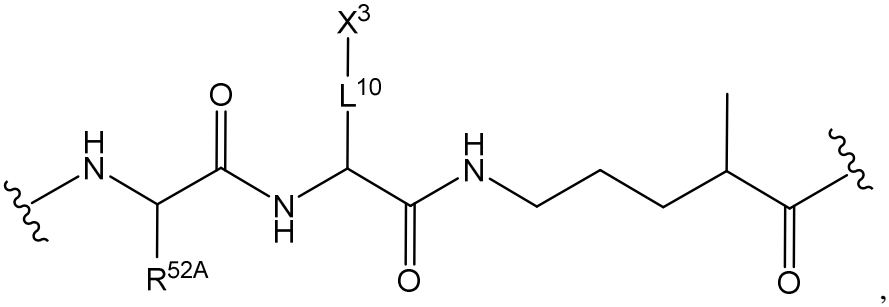
,

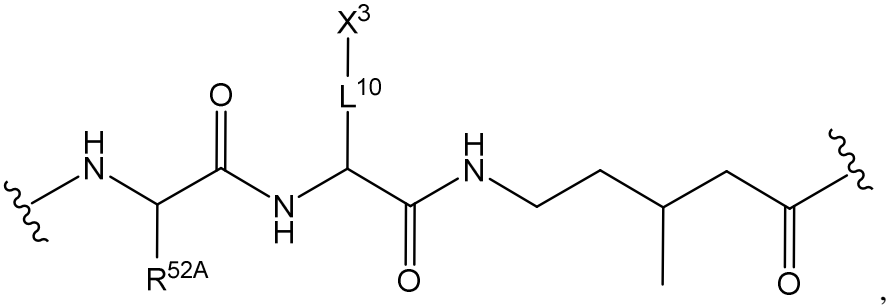


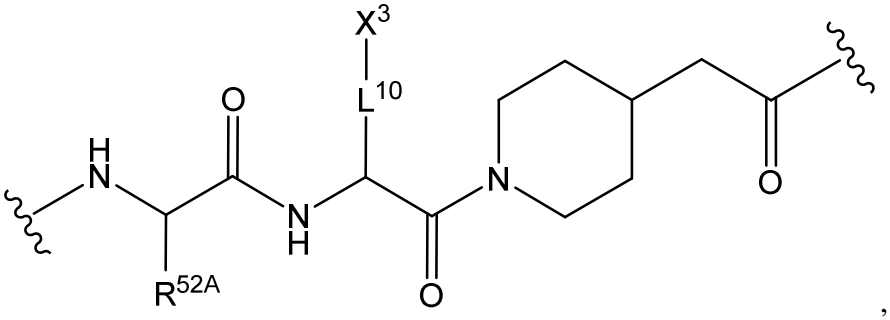
,

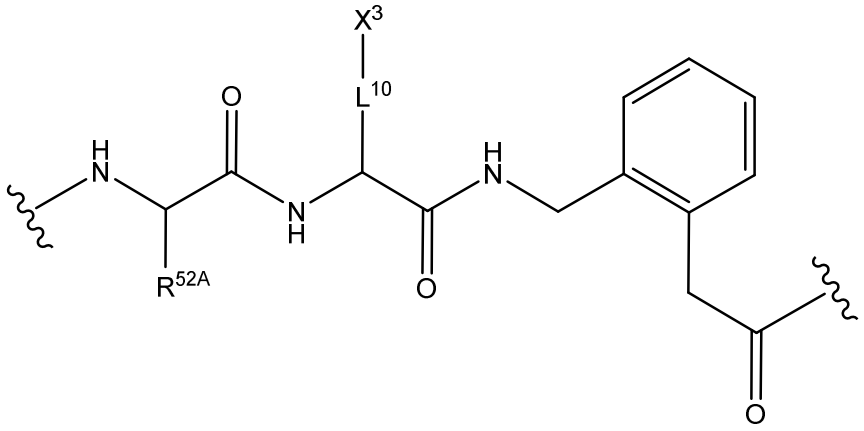

,


,


,


,


, and



wherein

R^{52A} and R^{52B} are independently selected from the group consisting of hydrogen, C_1-C_4 alkyl, -CH_2-phenyl, -CH_2-biphenyl, -CH_2-pyridyl, -CH_2-CH_2-C(O)-NH_2, and -$(CH_2)_{n15}$-R^{111}, wherein n15 is an integer from 1 to 4, and R^{111} is selected from the group consisting of -NH_2, N_3, and -C(O)-NH_2;

R^{53} is selected from the group consisting of hydrogen, –C(O)NH_2, -$[CH_2]_{n16}$-NH_2–, and –$[C(O)NH$-$CH_2]_{n17}$-C(O)NH_2–, wherein each of n16 and n17 are independently an integer from 1 to 3;

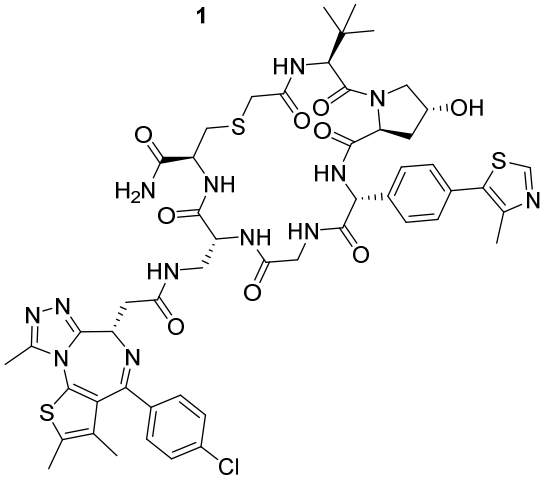
R^{54} is hydrogen or unsubstituted C_1-C_6 alkyl;

L^{10} is a bond, a peptide linker or a non-peptide linker; and

X^3 is a targeting moiety.

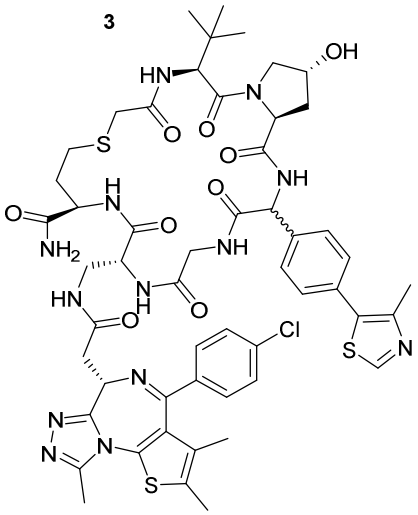
38. The macrocyclic compound of claim 1, wherein said compound is selected from the group consisting of:

1



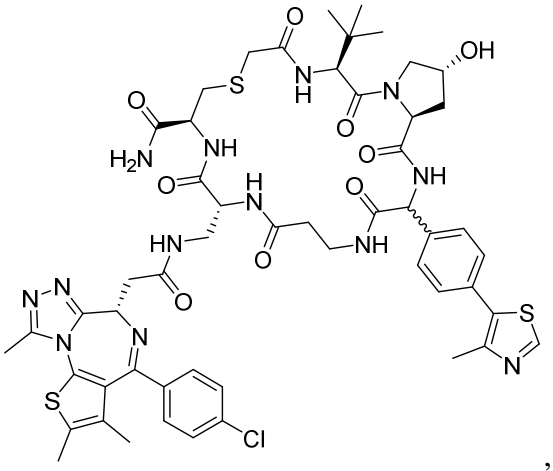
,

3



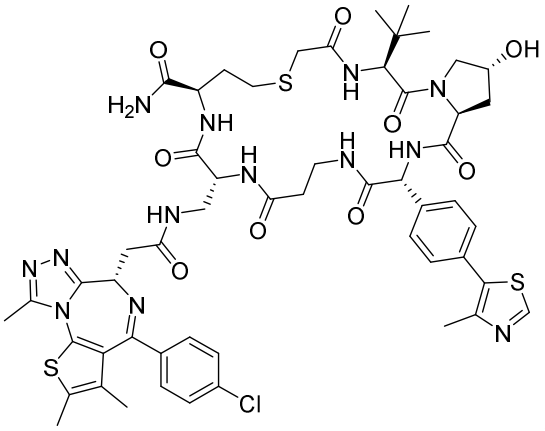
,

4



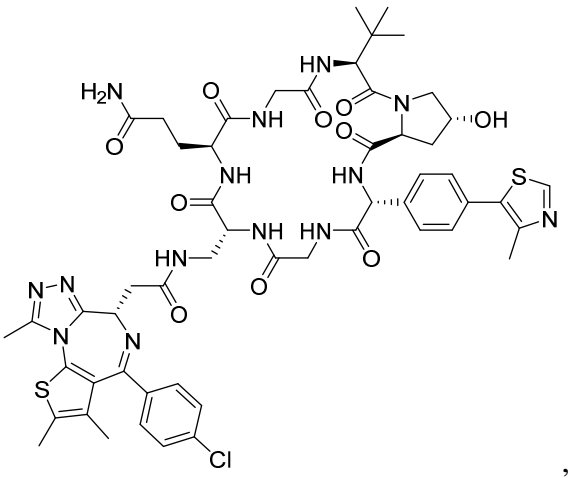
,

5



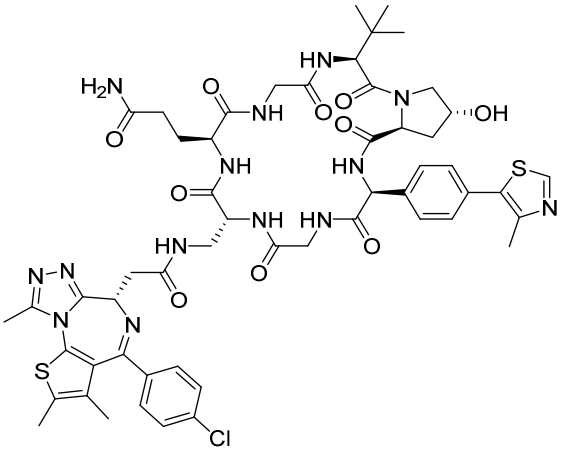
,

6



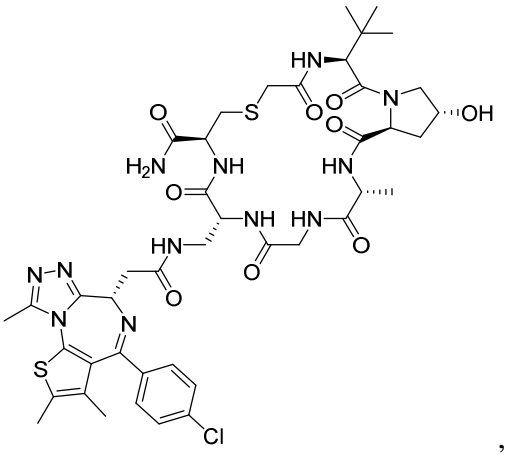
,

7



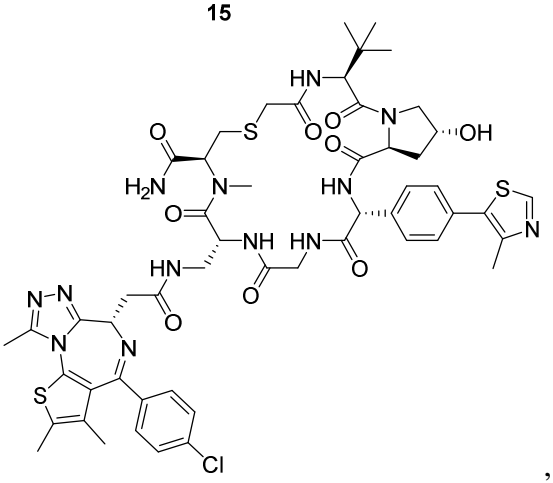
,

8



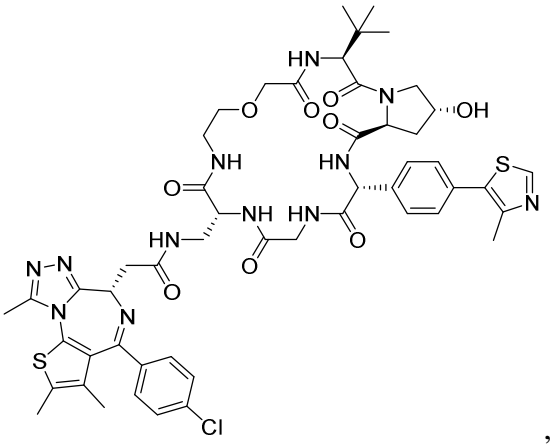
,

15



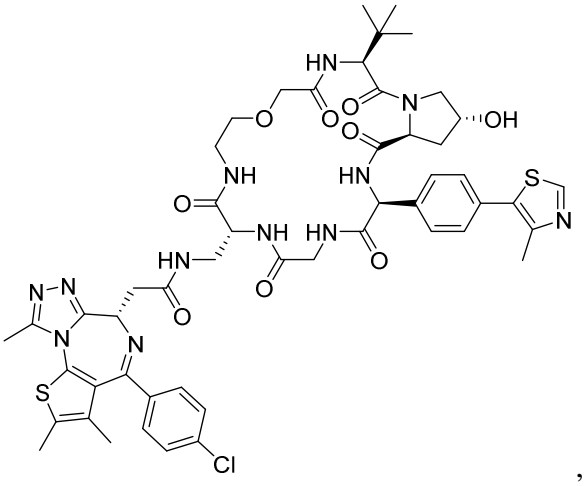
,

16



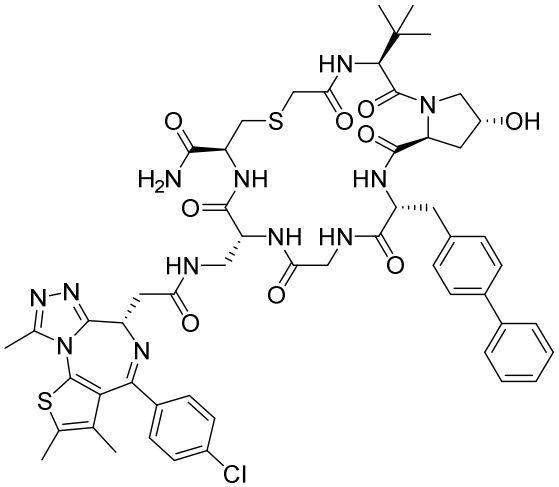
,

17



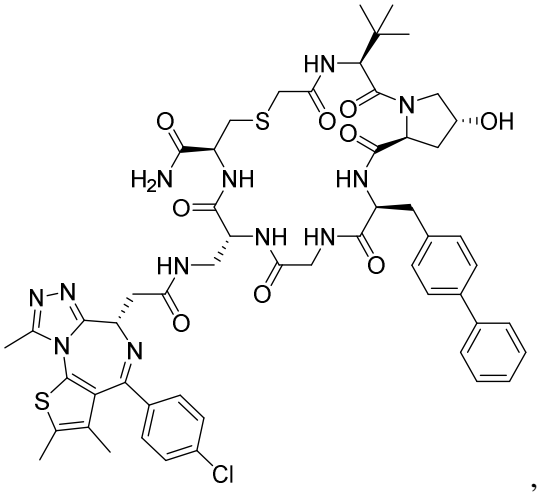
,

18



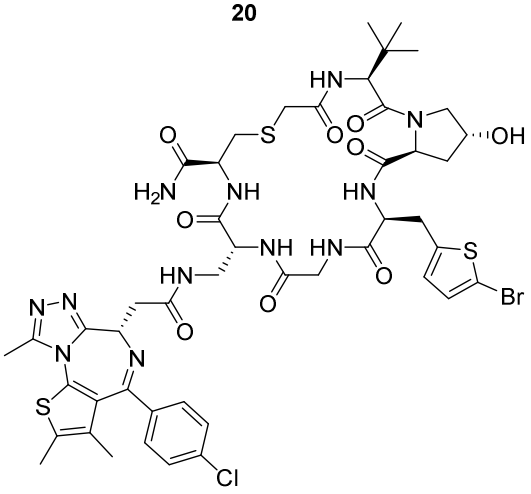
,

19



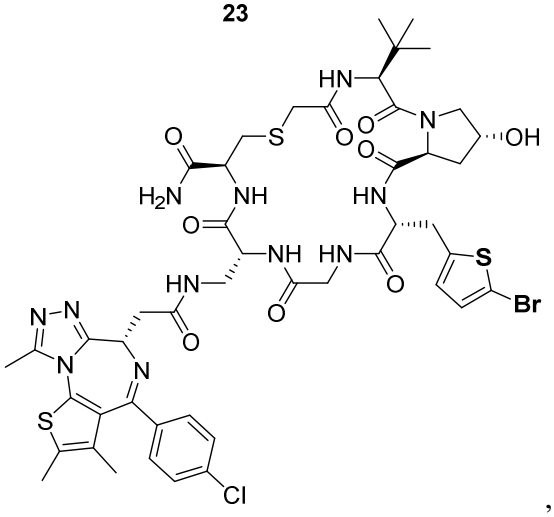
,

20



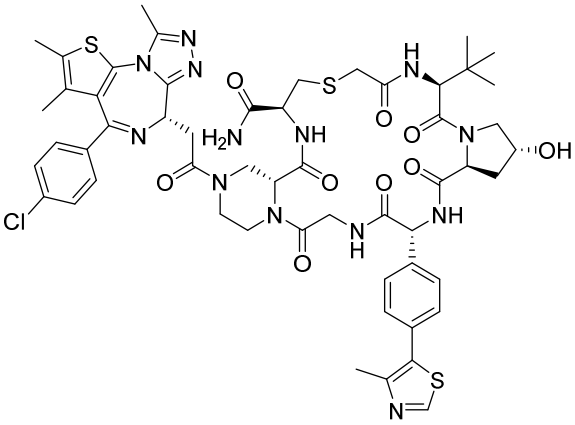
,

23



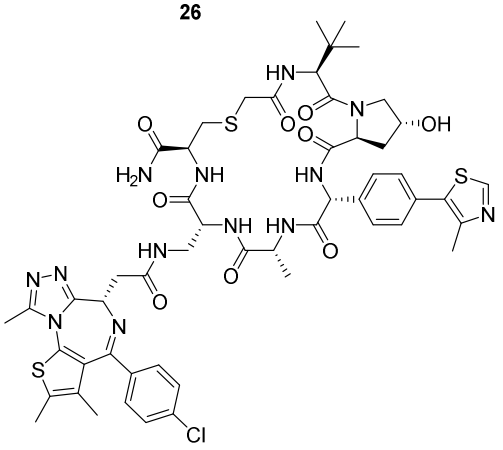
,

25



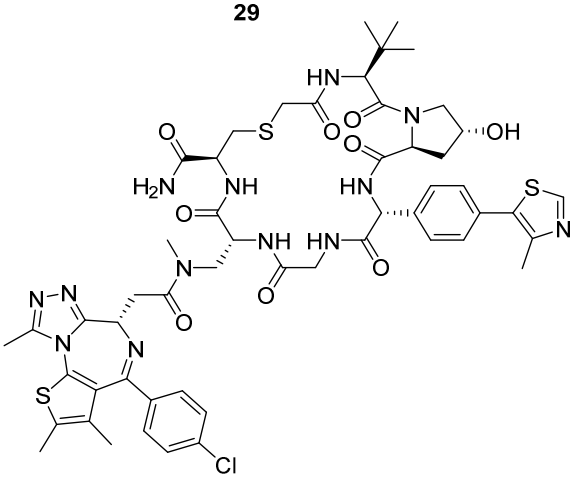
,

26



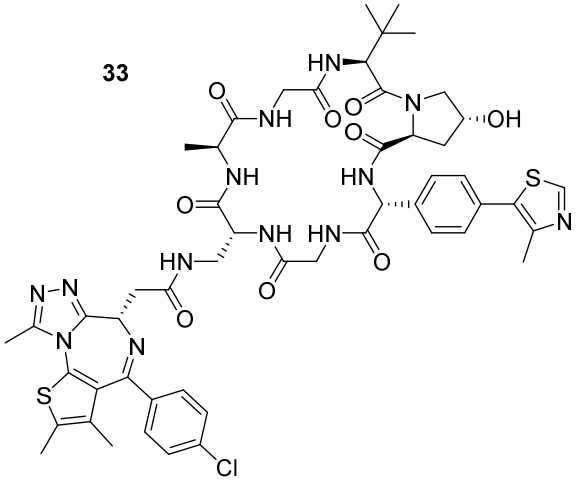
,

29



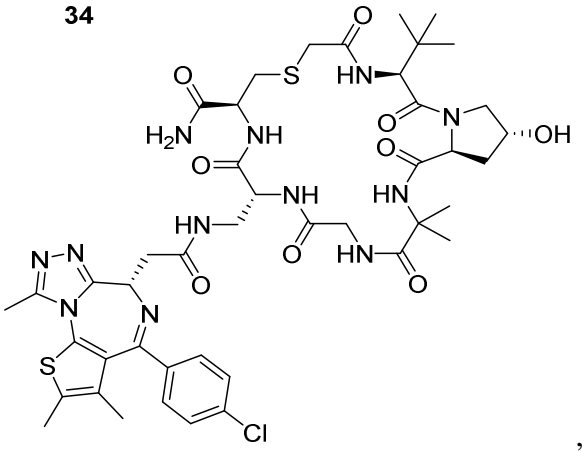
,

33



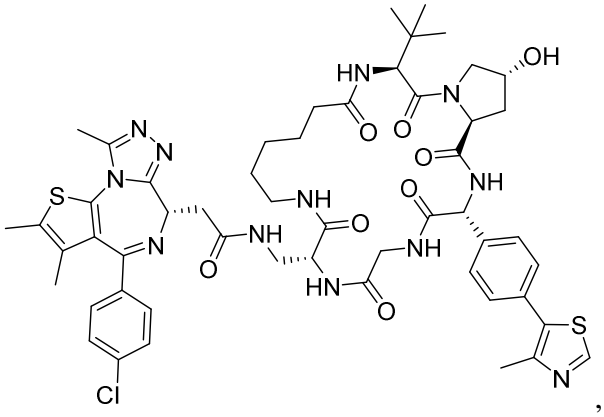
,

34



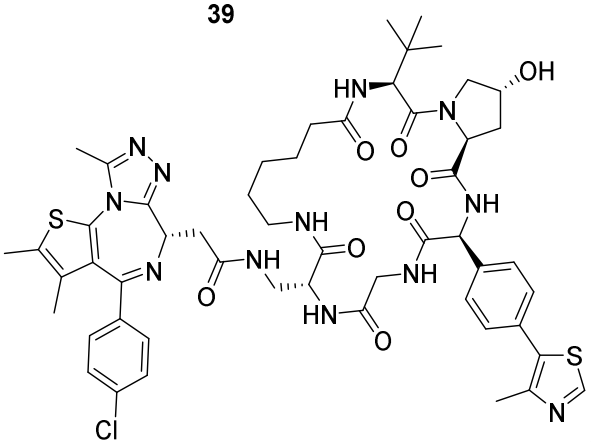
,

38



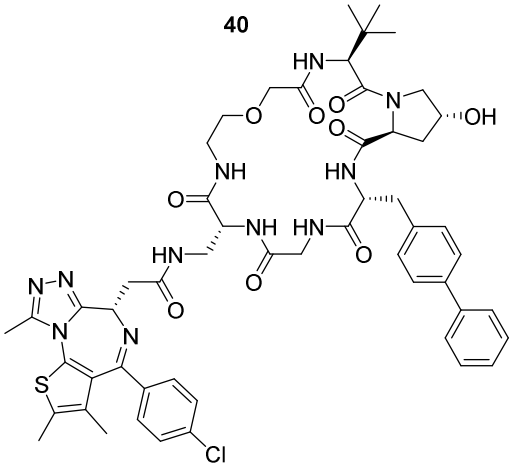
,

39



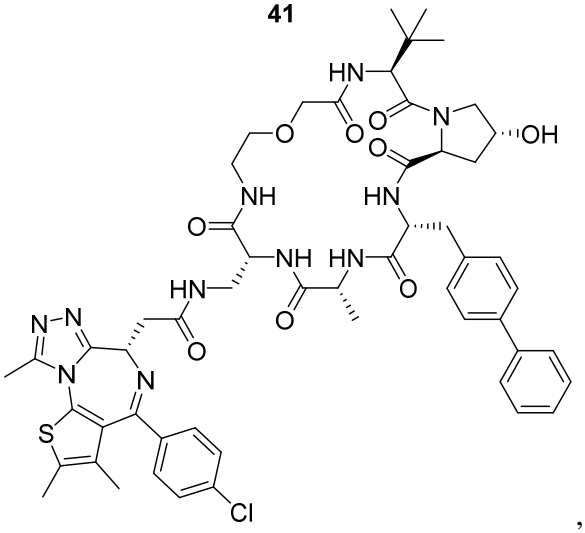
,

40



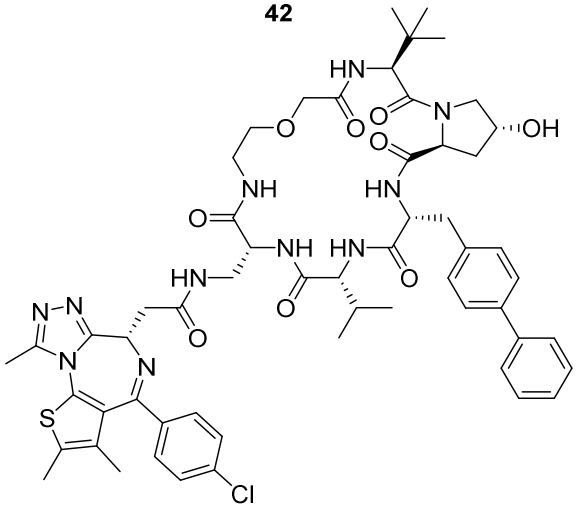
,

41



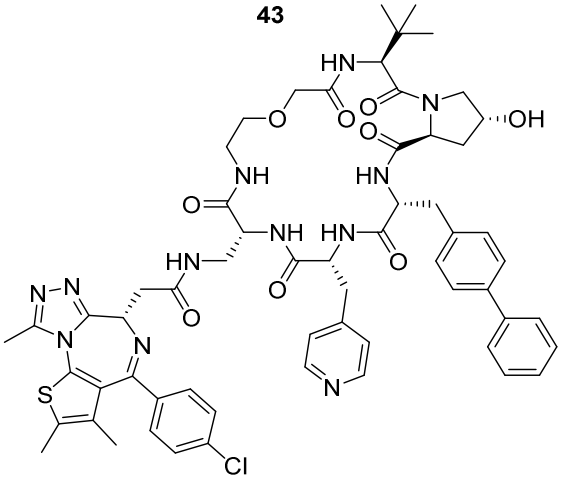
,

42



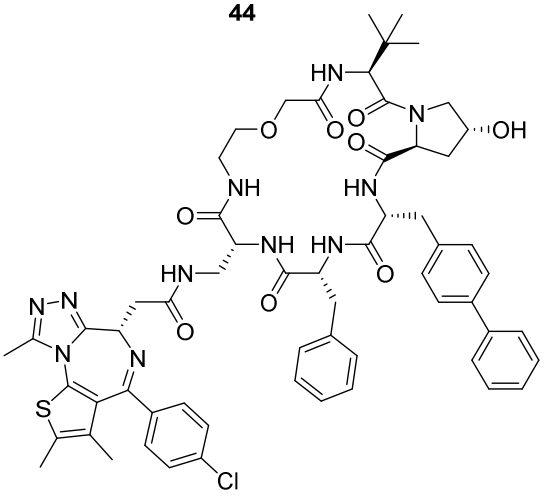
,

43



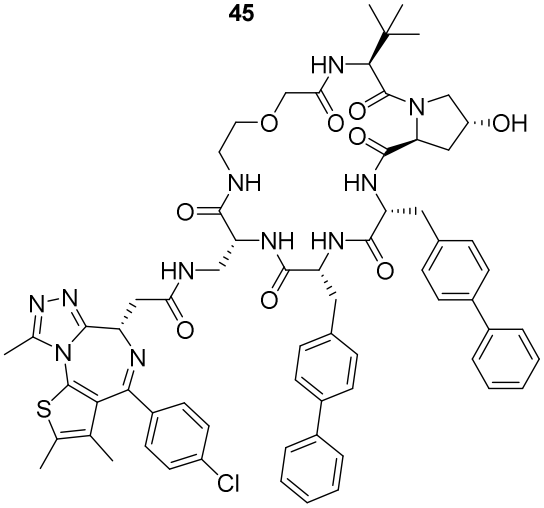
,

44



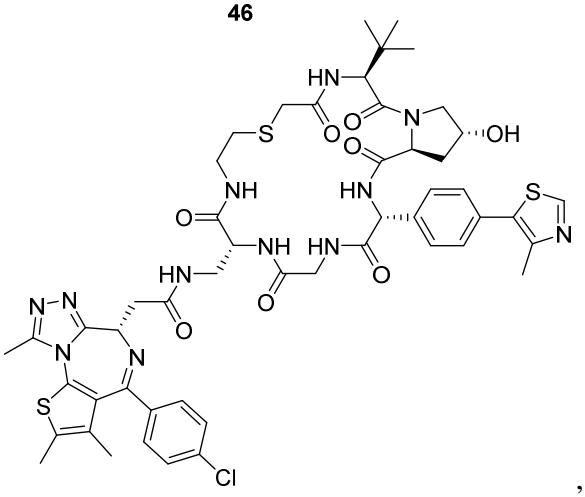
,

45



,

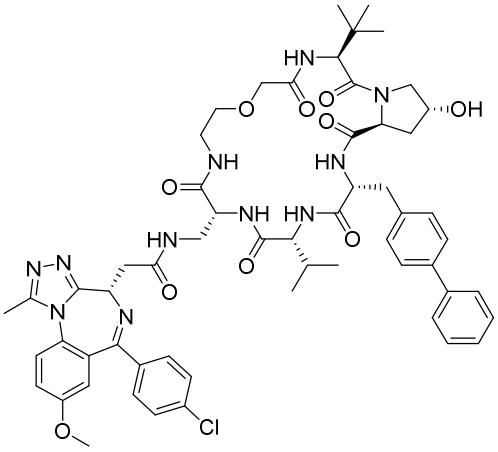
46



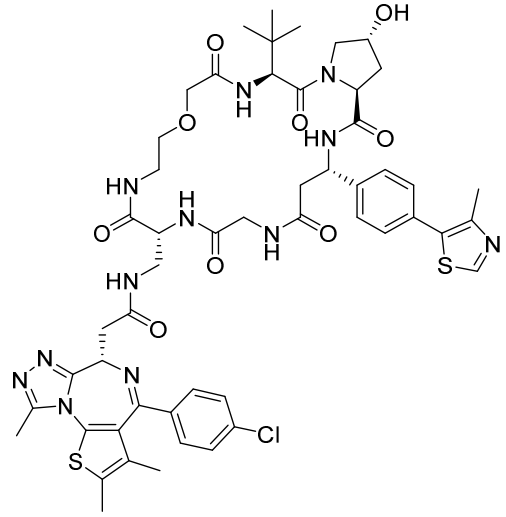
,

54

52

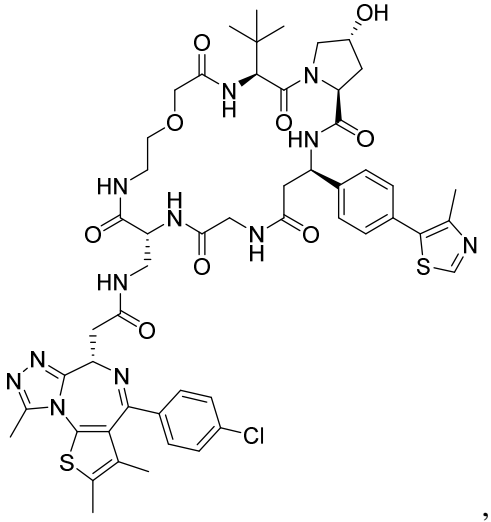


,



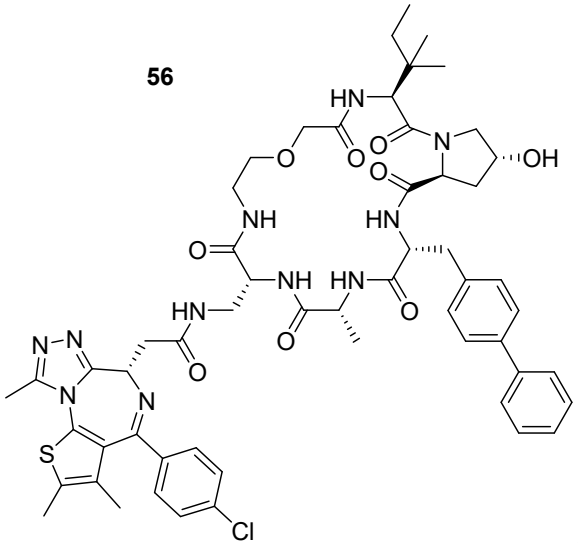
,

55



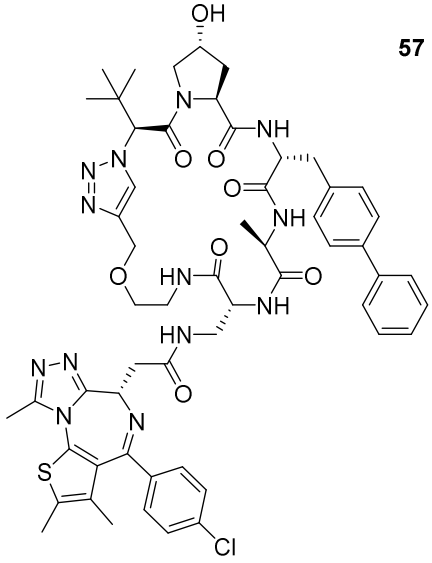
,

56



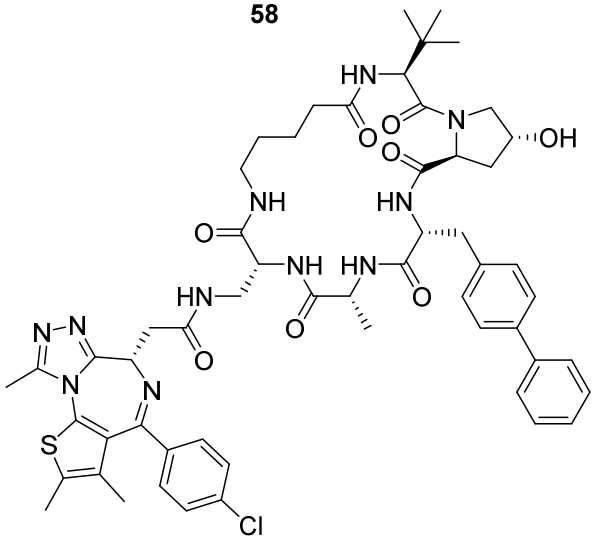
,

57



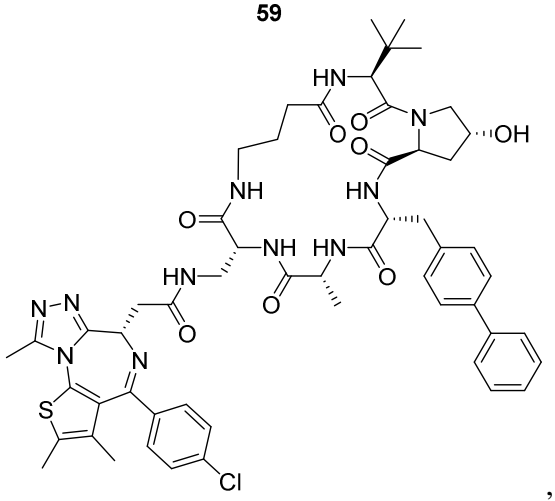
,

58



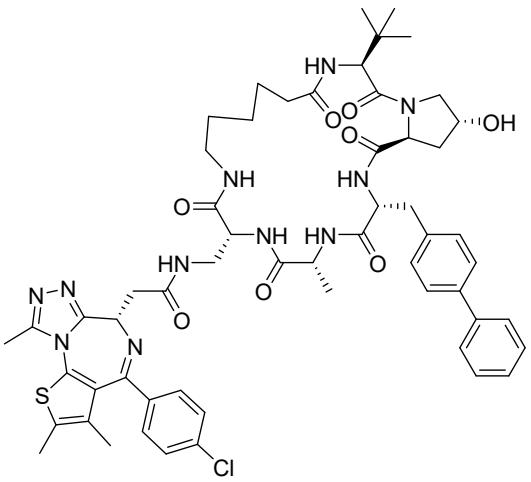
,

59



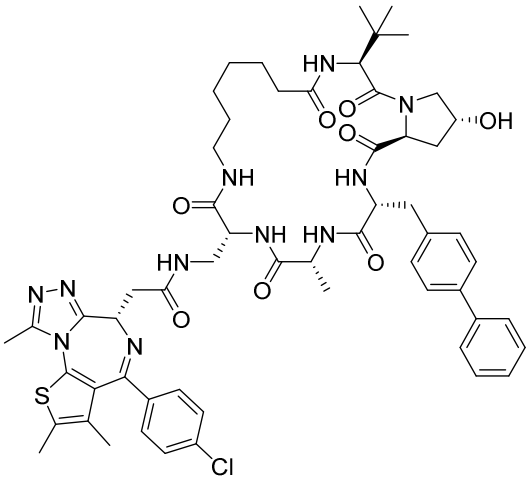
,

60



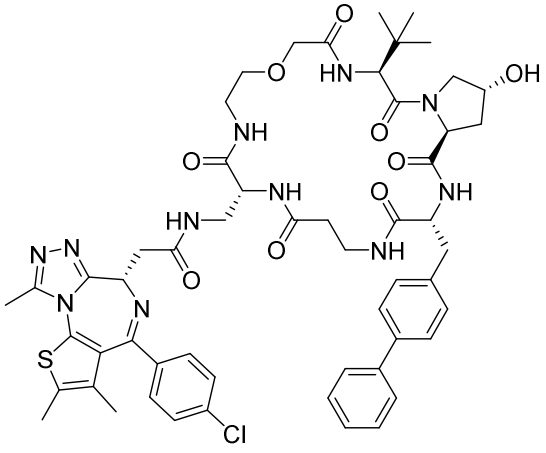
,

61



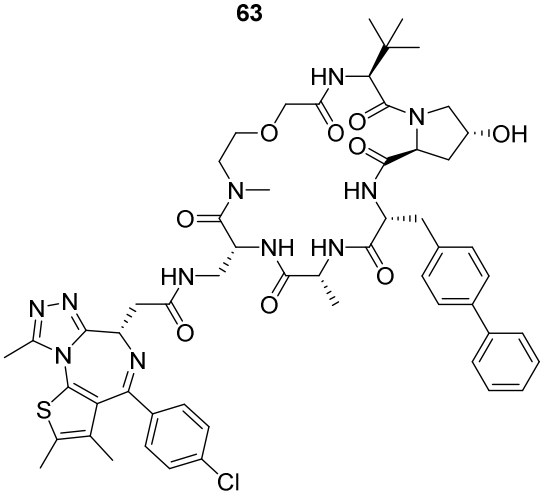
,

62



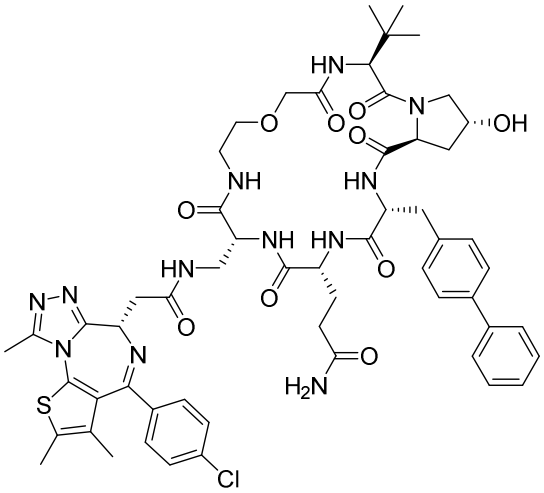
,

63



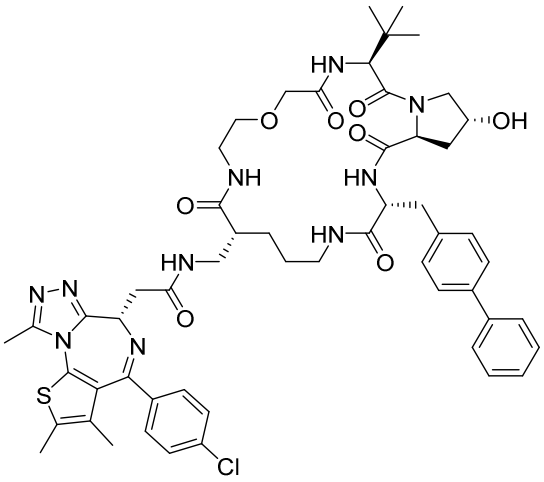
,

66



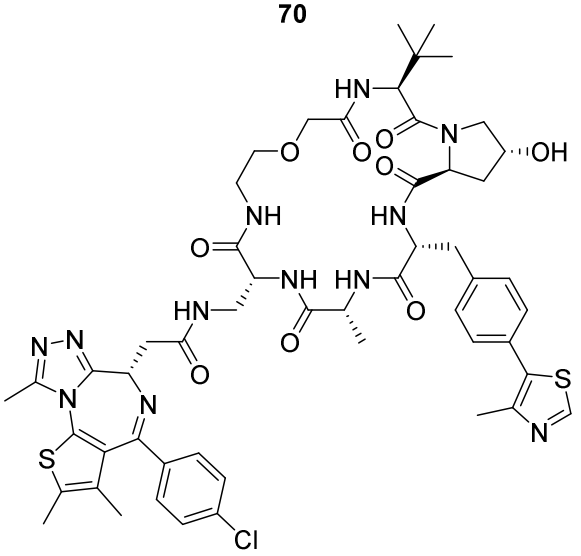
,

67

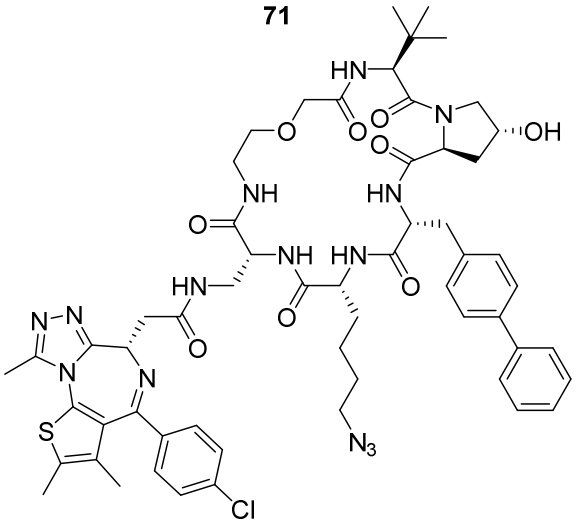


,

70

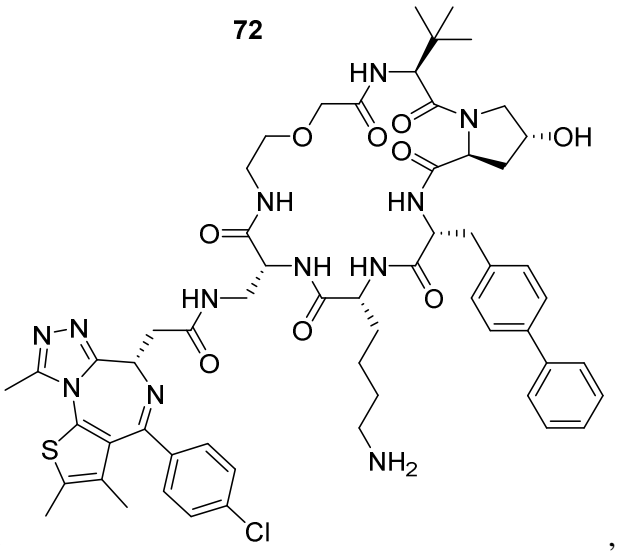


71



and

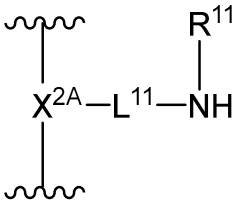
72



,

or a pharmaceutically acceptable salt thereof.

39. The macrocyclic compound of claim 29, wherein X^2 has the formula



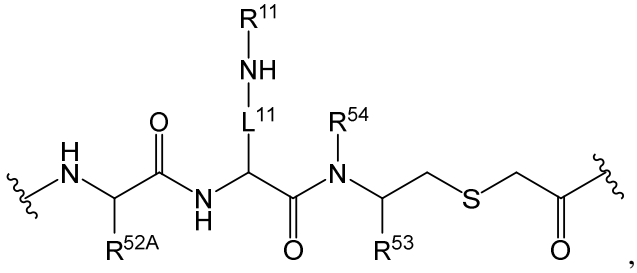
, wherein

X^{2A} is at least one natural or unnatural amino acid that forms a bond with L^{1A} and L^{2A};

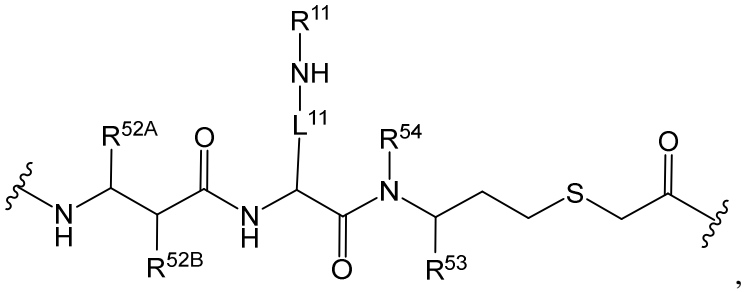
L^{11} is a bond or a substituted or unsubstituted C_1-C_{20} alkylene; and

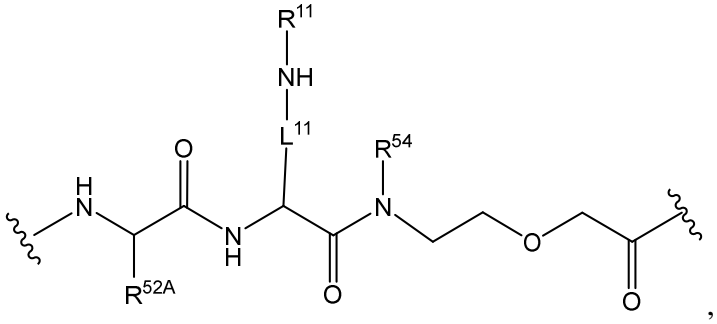
R^{11} is hydrogen, an unsubstituted $C_{1\text{-}5}$ alkyl or a protecting group.

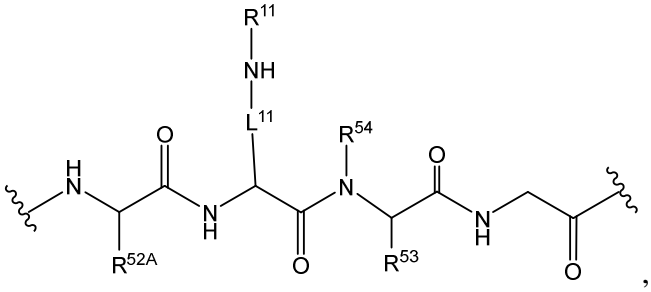
40. The macrocyclic compound of claim 39, wherein $-L^{2C}-L^{2B}-L^{2A}-X^2-L^{1A}-L^{1B}-L^{1C}-$ is selected from the group consisting of

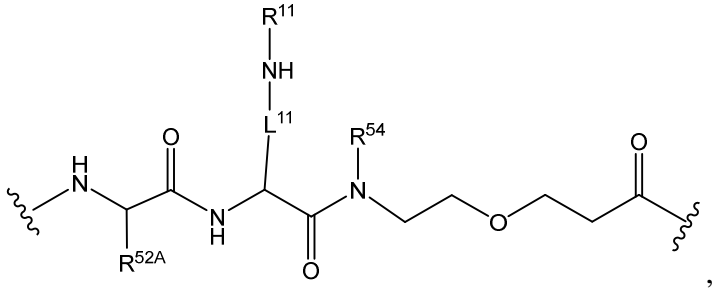


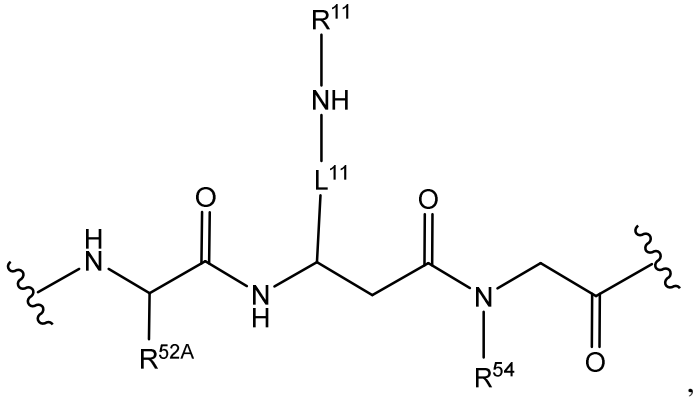
,

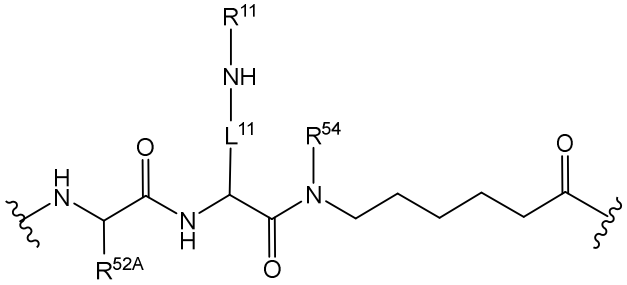


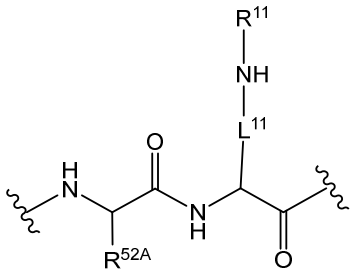


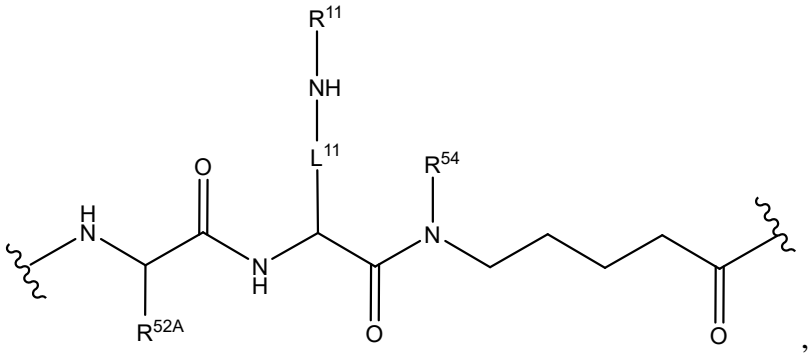


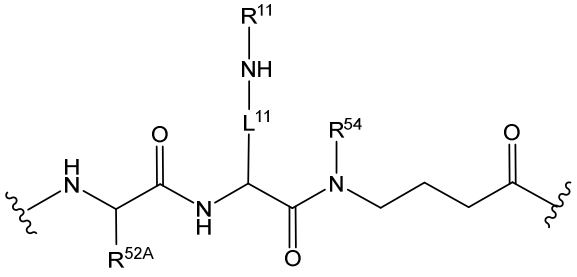


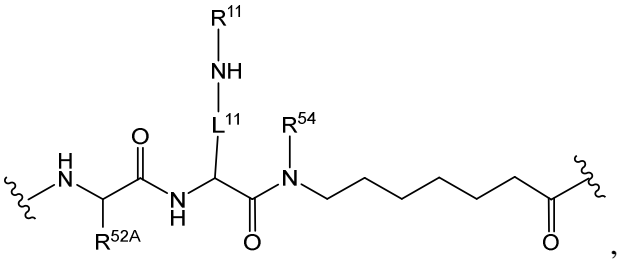


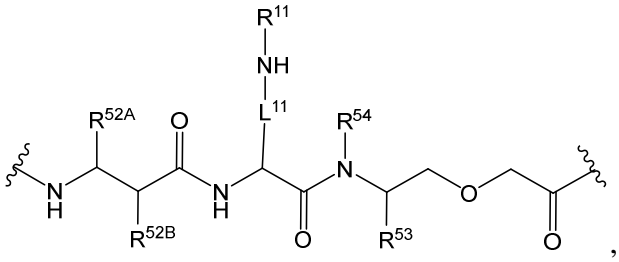


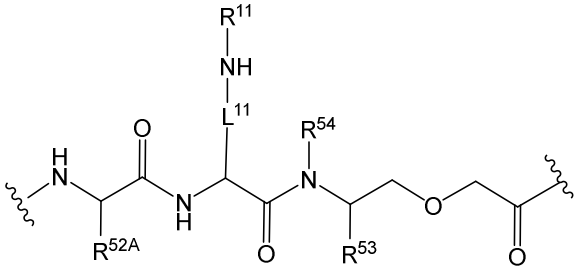


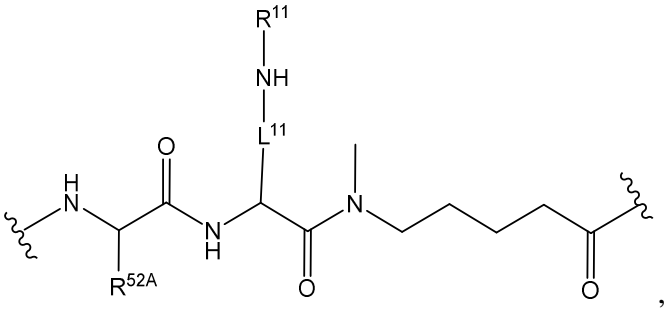


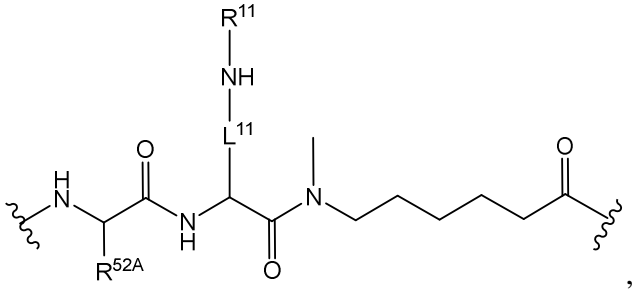


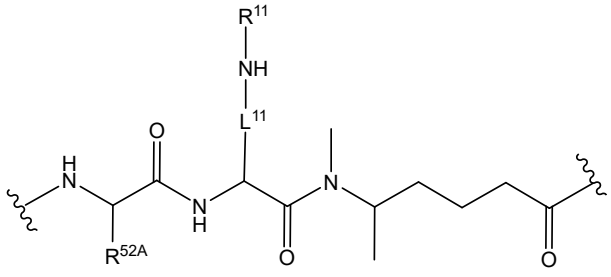


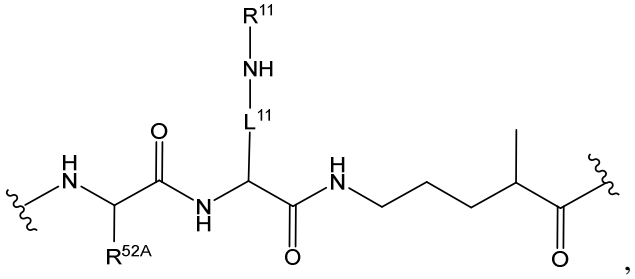


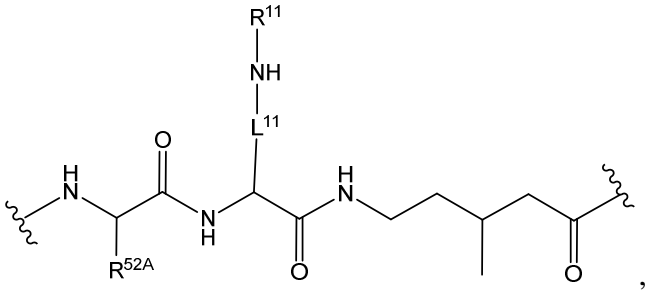


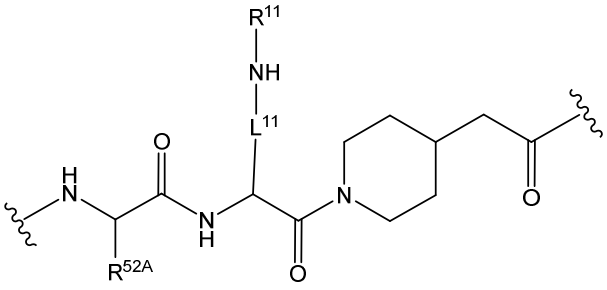


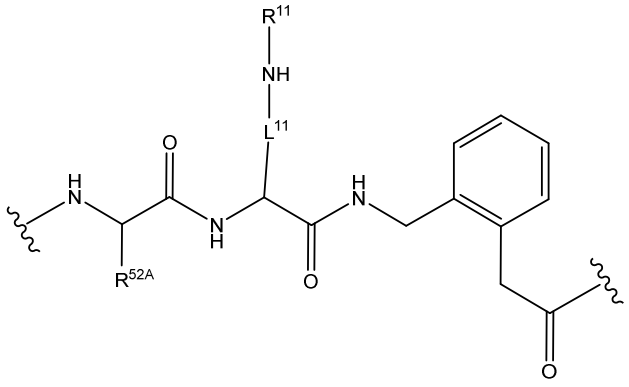

,


,


,


,


, or



wherein

the carbonyl group of L^{1C} and the amino group of L^{2C} are linked to X1;

R^{52A} and R^{52B} are each independently selected from the group consisting of hydrogen, C_1-C_4 alkyl, -CH_2-phenyl, -CH_2-biphenyl, -CH_2-pyridyl, -CH_2-CH_2-C(O)-NH_2 and -$(CH_2)_{n15}$-R^{111}, wherein n15 is an integer from 1 to 4 and R^{111} is -NH_2, N_3, or -C(O)-NH_2;

R^{53} is hydrogen, –C(O)NH_2, -$[CH_2]_{n16}$-NH_2–, or –$[C(O)NH$-$CH_2]_{n17}$-C(O)NH_2–, wherein n16 and n17 are each independently an integer from 1 to 3;

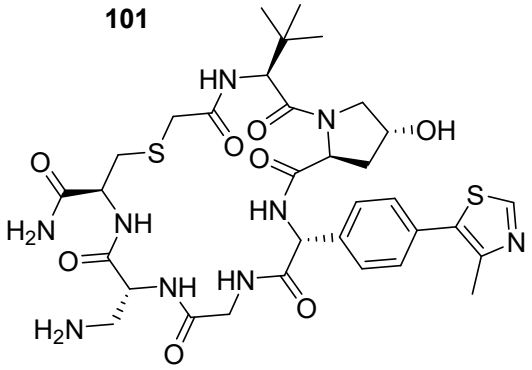
R^{54} is hydrogen or unsubstituted C_1-C_6 alkyl;

L^{11} is a bond or a substituted or unsubstituted C_1-C_{20} alkylene; and

R^{11} is hydrogen, an unsubstituted $C_{1\text{-}5}$ alkyl or a protecting group.

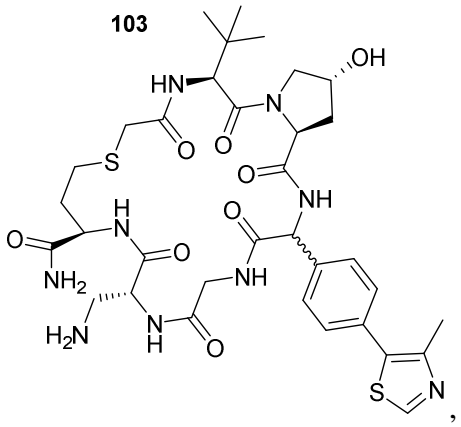
41. The macrocyclic compound of claim 39 or 40, wherein said compound is selected from the group consisting of:

101



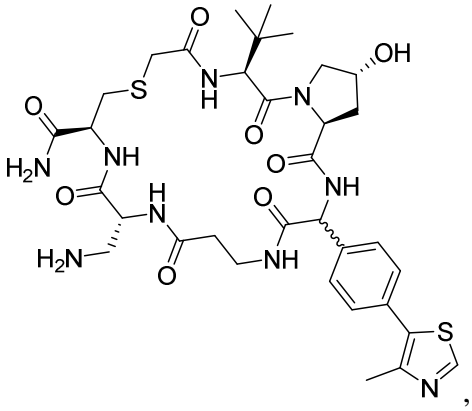
,

103



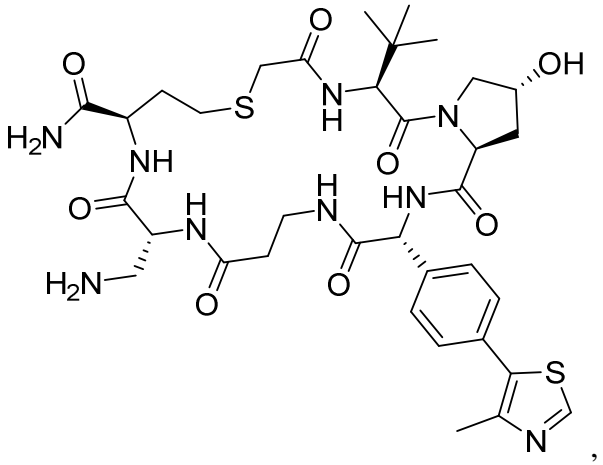
,

104



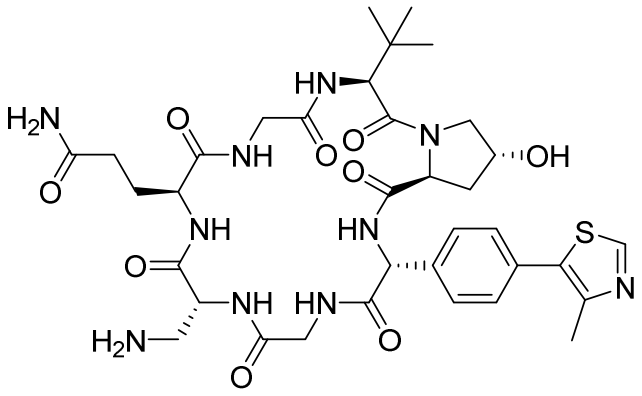
,

105



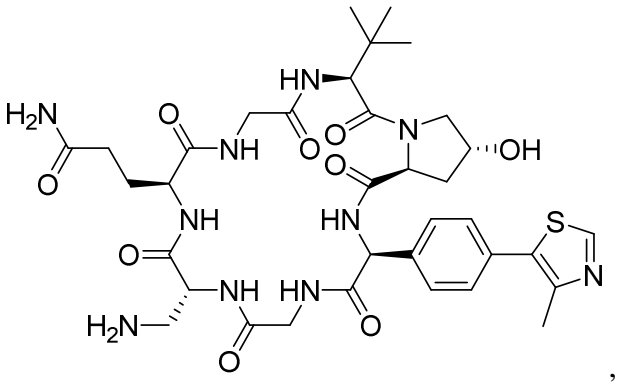
,

106



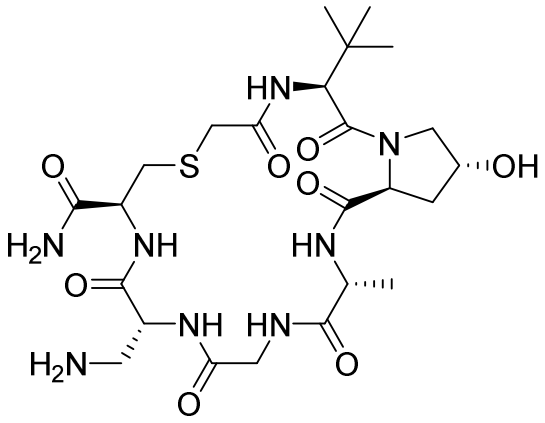
,

107



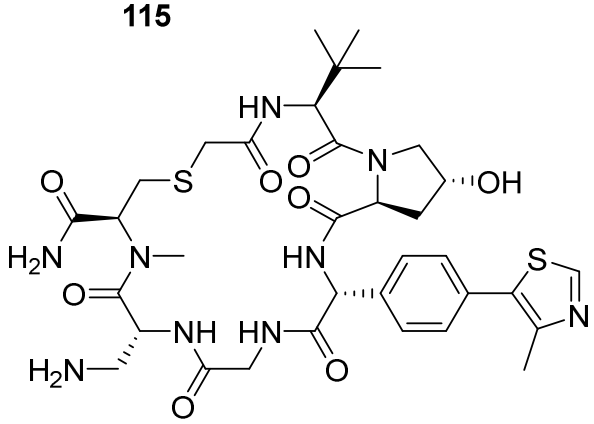
,

108



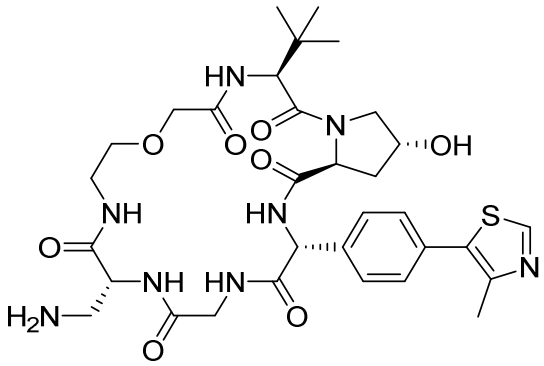
,

115

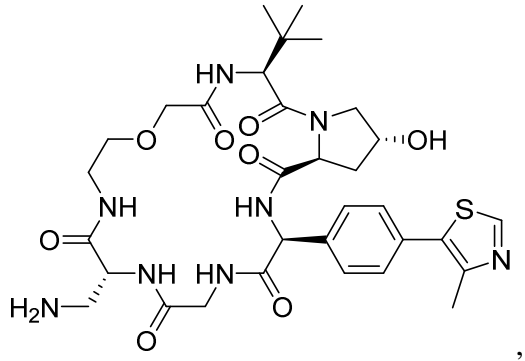


,

116

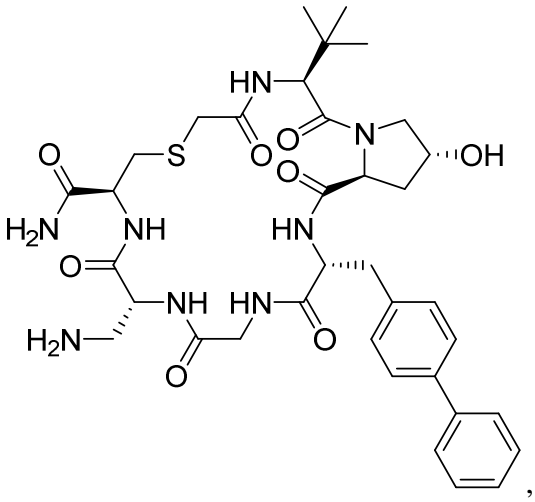


117



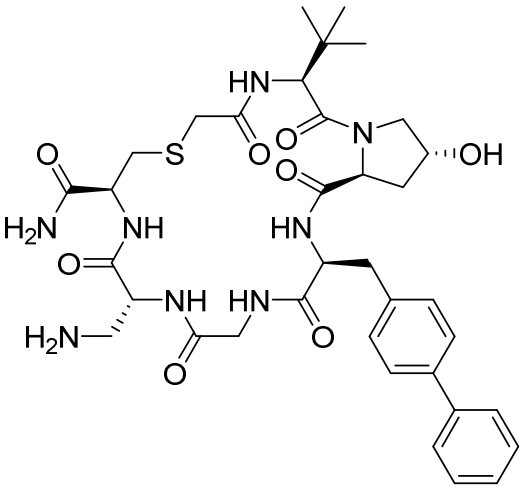
,

118



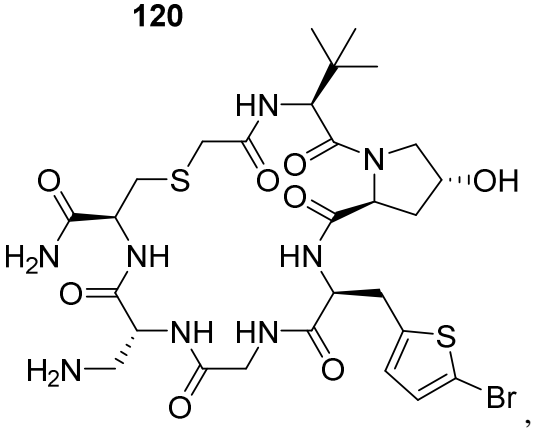
,

119



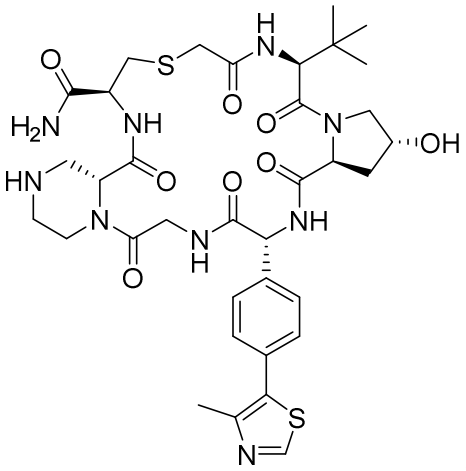
,

120



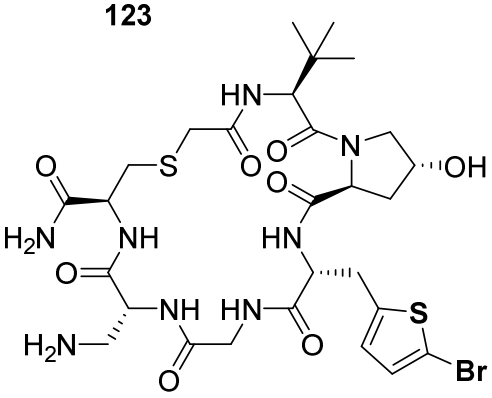
,

125



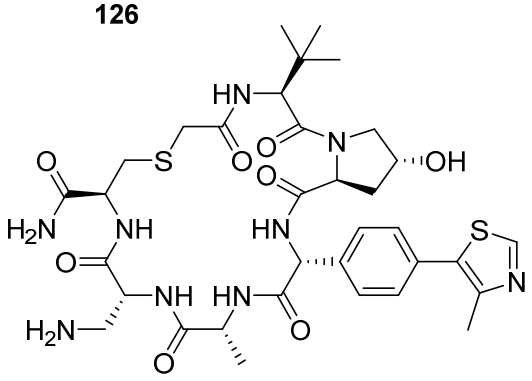
,

123



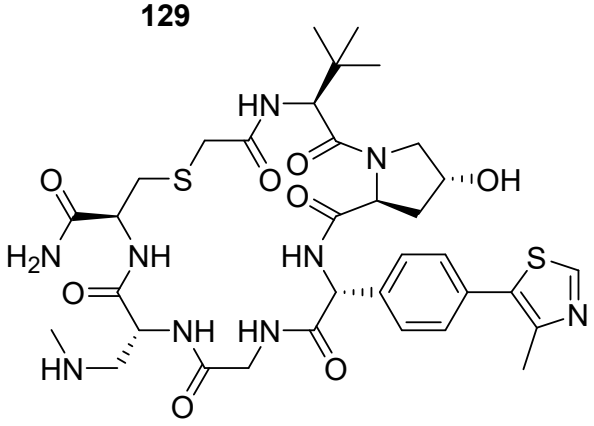
,

126



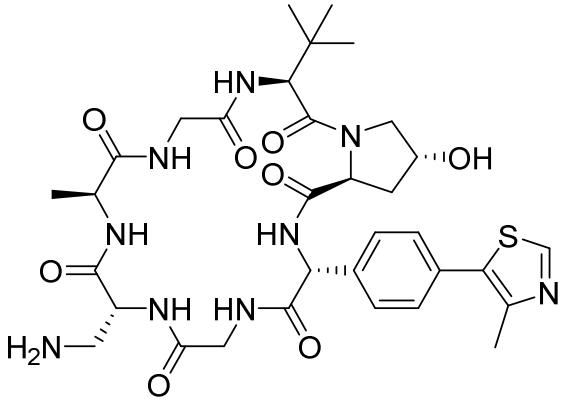
,

129



,

133

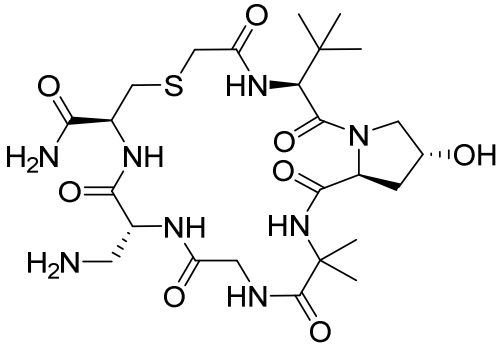


,

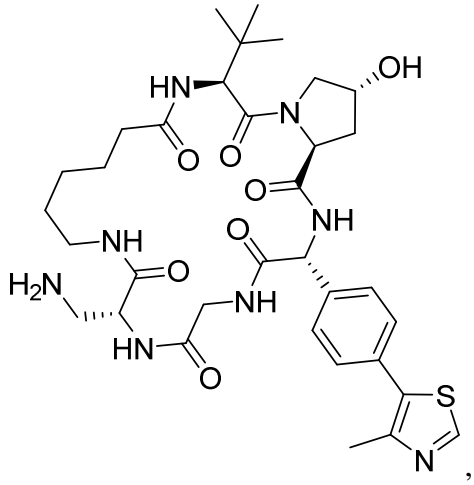
,

138

134

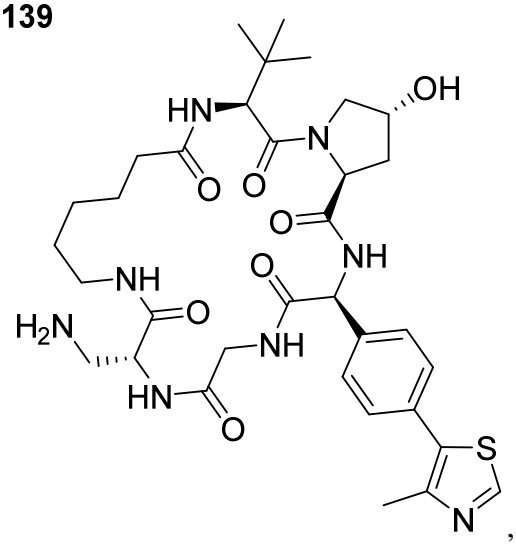


,



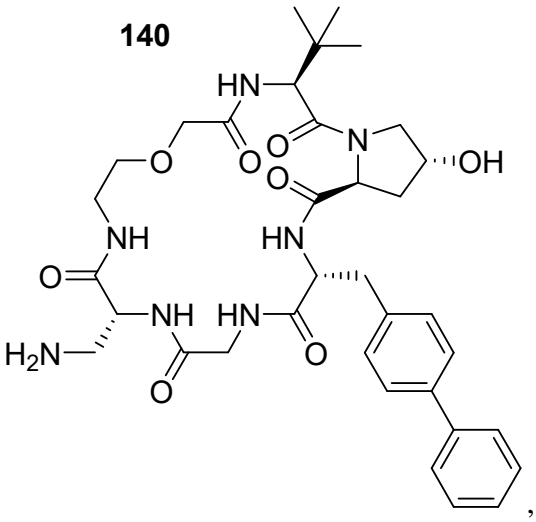
,

139



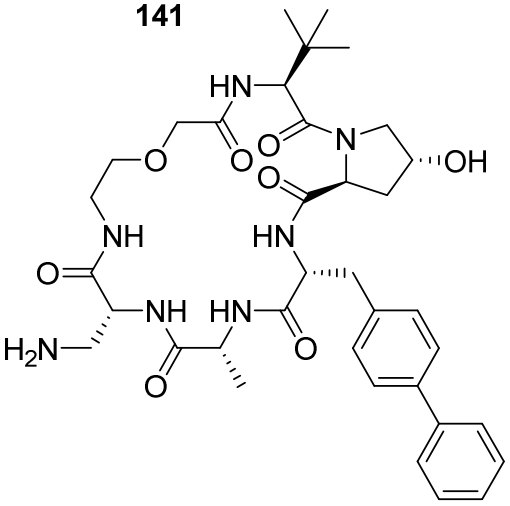
,

140



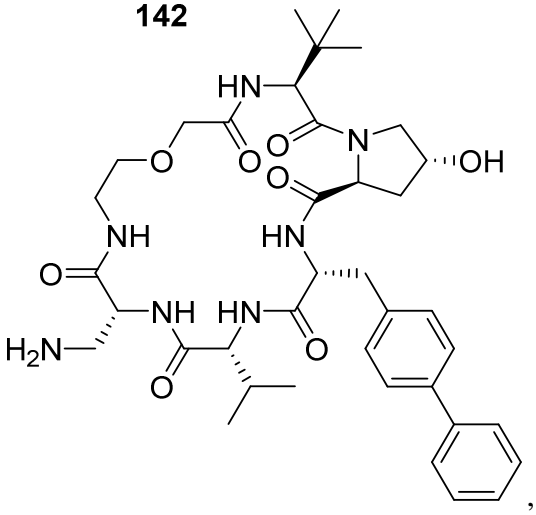
,

141



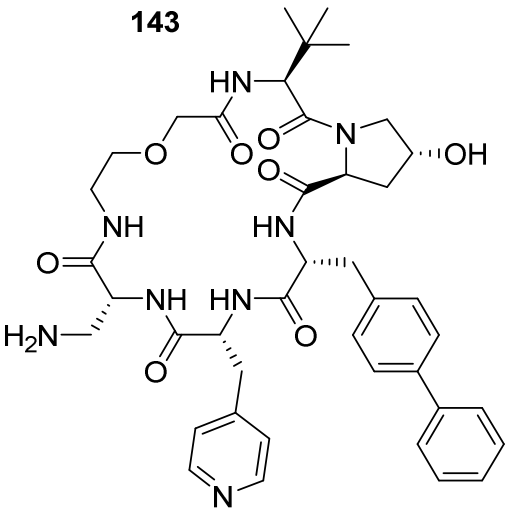
,

142



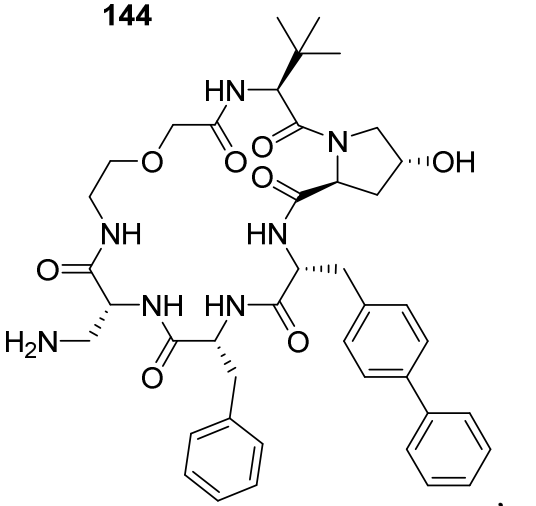
,

143



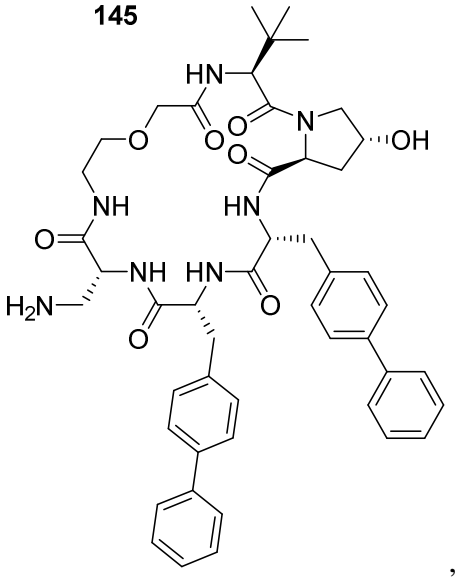
,

144

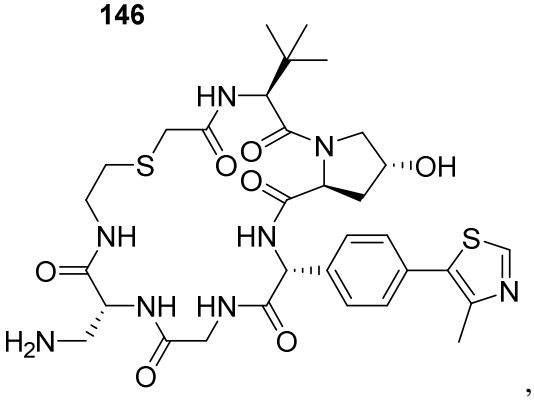


,

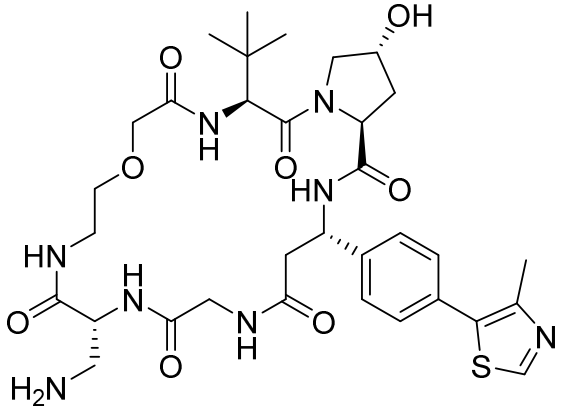
145



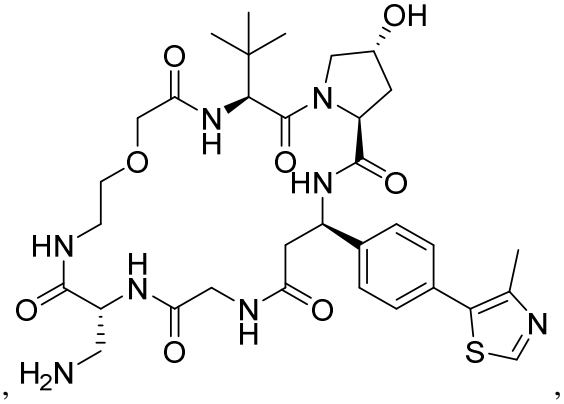
146



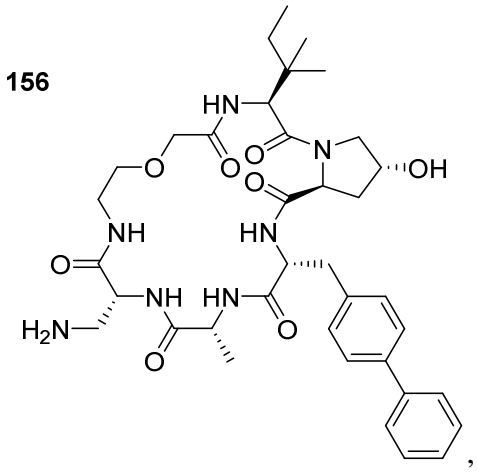
154



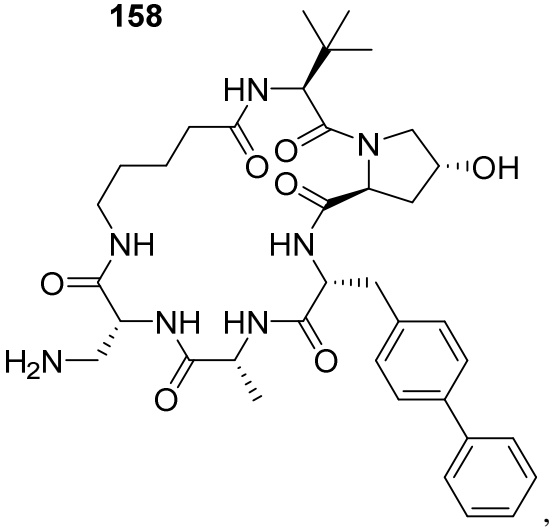
155



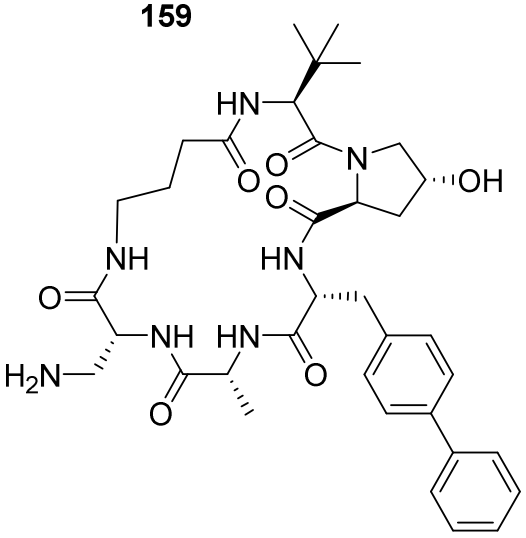
156



158

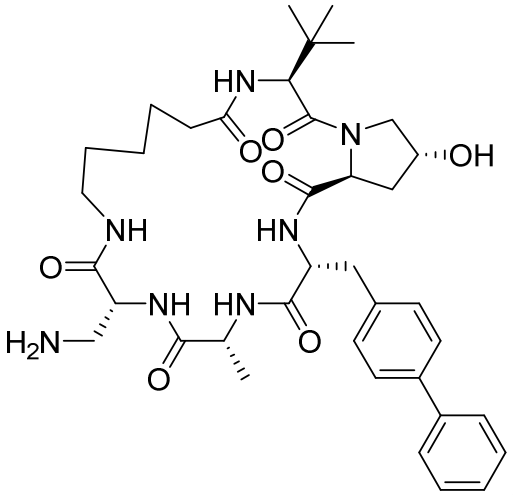


159



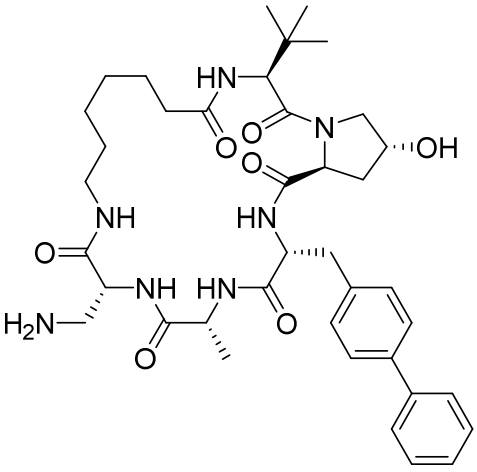
,

160



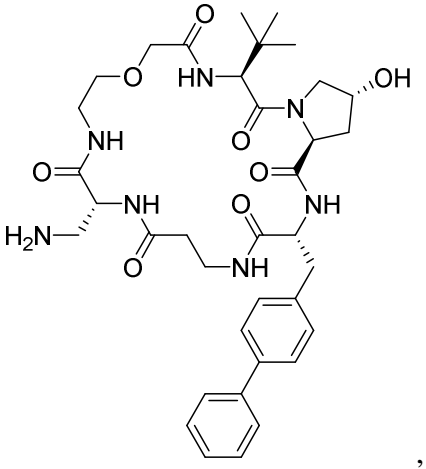
,

161



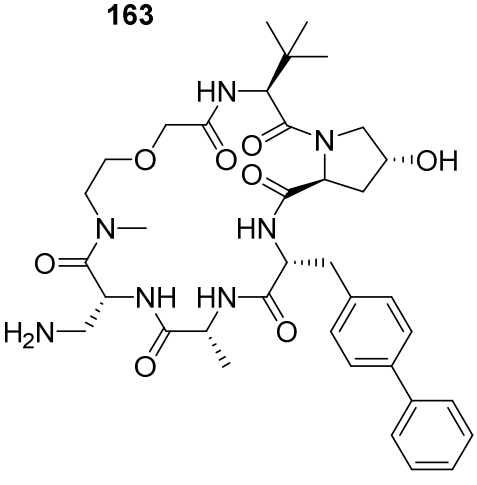
,

162



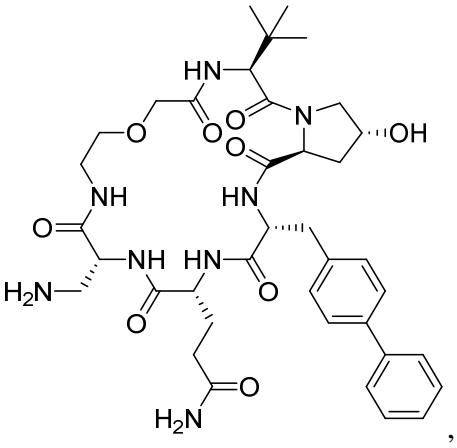
,

163



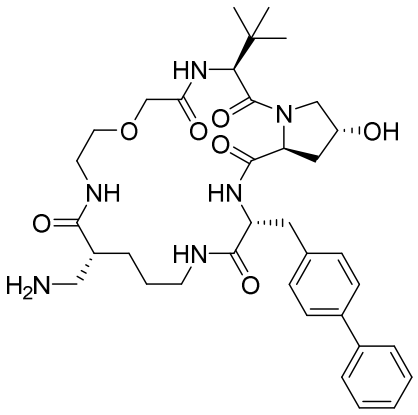
,

166



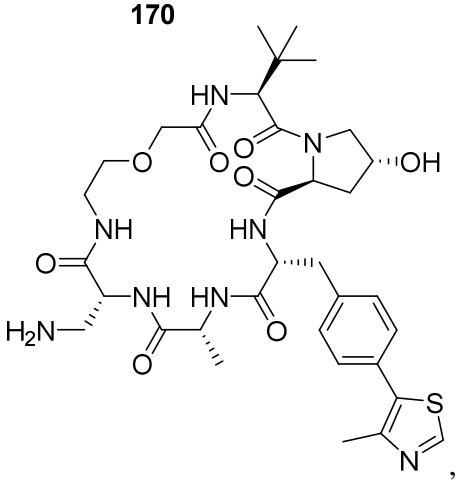
,

167



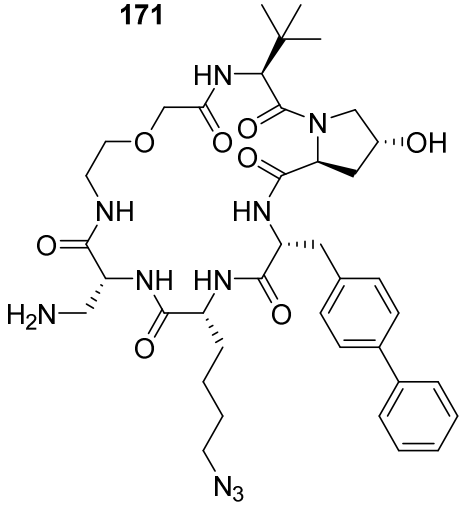
,

170



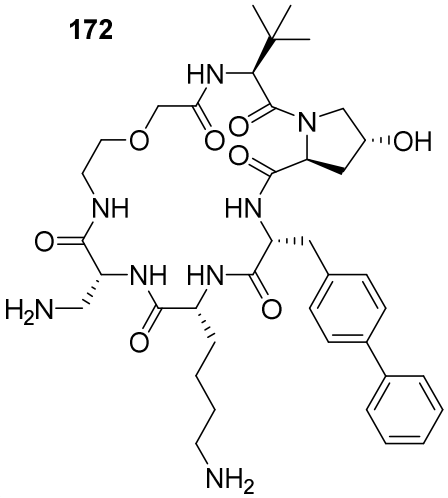
,

171



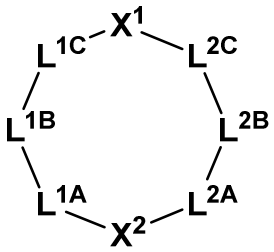
, and

172



, or a pharmaceutically acceptable salt thereof.

42. The macrocyclic compound of claim 1, wherein the macrocyclic compound has formula (I):



(I)

wherein

X^1 is a VHL binding motif, having the formula

–X^{1A}-X^{1B}-X^{1C}–, wherein

X^{1A} is selected from the group consisting of L-Tle, L-bMe-Ile, L-Tle-Tria, L-Val, L-Ala, L-Abu, L-Pen, L-Cha, L-Cpa, L-Cba, L-bMe2AllylGly, L-AdaGly and L-ThpGly;

X^{1B} is an L-Hyp or an F-L-Hyp; and

X^{1C} is selected from the group consisting of D-MTPG, D-BiPhe, D-Ala, Aib, D-Bta, D-MtPhe, D-bRMeBiPhe, D-bSMeBiPhe, D-Phe(4Br), D-Phe(4Cl), D-Phe(4F), D-Phe(4CN) and D-Phe(4I);

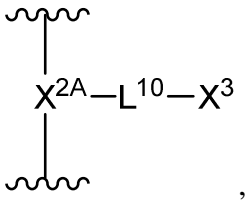
L^{2C} is selected from the group consisting of Gly, D-Ala, bAla, D-PyrAla, D-Phe, D-BiPhe, D-Val, D-Gln, D-Lys and D-Lys(N3);

L^{2A} and L^{2B} form a single bond between L^{2C} and X^2;

L^{1A} and L^{1B} form a single bond between L^{1C} and X^2;

L^{1C} is selected from the group consisting of D-Cys(S-ac), Gly, D-hCys(S-ac), NMe-D-Cys(S-ac), O1Pen, NMe-O1Pen, GABA, Ava, AEP, Ahx, Ahp, S1Pen, NMe-Ava, 2-AminoMePheAc, Nme-Ahx, αMe-Ava, βMe-Ava, γMe-Ava and 4PipAc; and

X^2 is a target protein binding motif having the formula



, wherein

X^{2A}–L^{10}- is selected from the group consisting of D-Dap, D-Dap-NMe, NMe-D-Dap, D-b2Orn and D-Pip, and

X^3 is selected from the group consisting of tert-butyl (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate, tert-butyl (S)-2-(2,3,9-trimethyl-4-phenyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate, benzyl *N*-(1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl)carbamate, 2-[(4S)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]-N-ethylacetamide, 8-chloro-1,4-dimethyl-6-phenyl-4h-[1,2,4]triazolo[4,3-A][1,3,4]benzotriazepine, (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-isoxazolo[5,4-c]thieno[2,3-e]azepin-6-yl)acetamide, 2-[(4*S*)-6-(4-chlorophenyl)-1-methyl-4*H*-[1,2]oxazolo[5,4-d][2]benzazepin-4-yl]acetamide, 4-acetamido-3-fluoro-N-((1r,4S)-4-hydroxycyclohexyl)-5-((S)-1-phenylethoxy)benzamide, 1-benzyl-N5-

cyclopropyl-N3-methyl-2-oxo-1,2-dihydropyridine-3,5-dicarboxamide and 1-benzyl-N3,N5-dimethyl-2-oxo-1,2-dihydropyridine-3,5-dicarboxamide.

43. A compound comprising a cyclic peptide comprising a sequence selected from the group consisting of SEQ ID NOs. 1-68 and 112-120, wherein the amine end of the first amino acid in said sequence is covalently bonded to the carboxyl end of the last amino acid in said sequence.

44. A compound comprising a cyclic oligopeptide having comprising an E3 ubiquitin ligase binding motif (EULBM) wherein the cyclic oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 69-111.

45. Use of a macrocyclic compound of any one of claims 1-42, or a pharmaceutically acceptable salt thereof, or a compound of any one of claims 43-44, in the manufacture of medicament for treatment of a cancer.

46. A pharmaceutical composition comprising a macrocyclic compound of any one of claims 1-42, or a pharmaceutically acceptable salt thereof, or a compound of any one of claims 43-44, and a pharmaceutically acceptable excipient.