PEPTIDOMIMETIC MACROCYCLES AND USES THEREOF

CROSS-REFERENCE

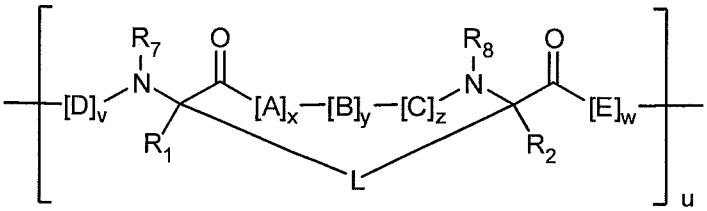
[0001] This application claims priority to U.S. Provisional Application No. 62/214,142, filed September 3, 2015; U.S. Provisional Application No. 62/310,254, filed March 18, 2016; U.S. Provisional Application No. 62/344,651, filed June 2, 2016; and U.S. Provisional Application No. 62/344,791, filed June 2, 2016, which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] The human transcription factor protein p53 induces cell cycle arrest and apoptosis in response to DNA damage and cellular stress, and thereby plays a critical role in protecting cells from malignant transformation. The E3 ubiquitin ligase MDM2 (also known as HDM2) negatively regulates p53 function through a direct binding interaction that neutralizes the p53 transactivation activity, leads to export from the nucleus of p53 protein, and targets p53 for degradation via the ubiquitylation-proteasomal pathway. Loss of p53 activity, either by deletion, mutation, or MDM2 overexpression, is the most common defect in human cancers. Tumors that express wild type p53 are vulnerable to pharmacologic agents that stabilize or increase the concentration of active p53. In this context, inhibition of the activities of MDM2 has emerged as a validated approach to restore p53 activity and resensitize cancer cells to apoptosis *in vitro* and *in vivo.* MDMX (MDM4) has more recently been identified as a similar negative regulator of p53, and studies have revealed significant structural homology between the p53 binding interfaces of MDM2 and MDMX. The p53-MDM2 and p53-MDMX protein-protein interactions are mediated by the same 15-residue alpha-helical transactivation domain of p53, which inserts into hydrophobic clefts on the surface of MDM2 and MDMX. Three residues within this domain of p53 (F19, W23, and L26) are essential for binding to MDM2 and MDMX. There remains a considerable need for compounds capable of binding to and modulating the activity of p53, MDM2 and/or MDMX. Provided herein are p53-based peptidomimetic macrocycles that modulate an activity of p53. Also provided herein are p53-based peptidomimetic macrocycles that inhibit the interactions between p53, MDM2 and/or MDMX proteins. Further, provided herein are p53-based peptidomimetic macrocycles that can be used for treating diseases including but not limited to cancer and other hyperproliferative diseases.

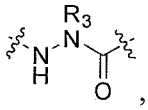
SUMMARY OF THE INVENTION

[0003] In one aspect, there is provided use of a therapeutically-effective amount of a peptidomimetic macrocycle or a pharmaceutically acceptable salt thereof and at least one additional pharmaceutically active agent in the manufacture of a medicament for modulating the activity of MDM2 and MDMX in a subject with cancer, wherein the peptidomimetic macrocycle comprises an amino acid sequence which is at least about 60% identical to an amino acid sequence in any of Table 1, Table 1a, Table 1b, and Table 1c, and wherein the peptidomimetic macrocycle has the formula:



wherein:

each A, C, D, and E is independently an amino acid;

each B is independently an amino acid, , [–NH–L_3–CO–], [–NH–L_3–SO_2–], or [–NH–L_3–];

each R_1 and R_2 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–; or forms a macrocycle–forming linker L' connected to the alpha position of one of said D or E amino acids;

each R_3 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R_5;

each L and L' is independently a macrocycle–forming linker of the formula $–L_1–L_2–$;

each L_1, L_2, and L_3 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[–R_4–K–R_4–]_n$, each being optionally substituted with R_5;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO_2, CO, CO_2, or $CONR_3$;

each R_5 is independently halogen, alkyl, $–OR_6$, $–N(R_6)_2$, $–SR_6$, $–SOR_6$, $–SO_2R_6$, $–CO_2R_6$, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_7 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with a D residue;

each R_8 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

each v is independently an integer from 1–1000;

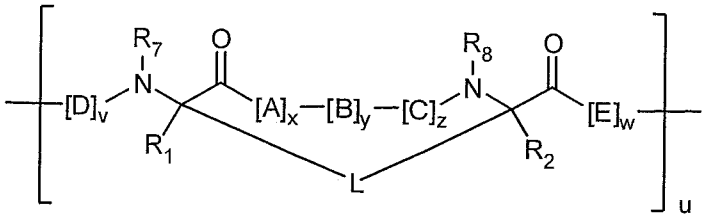
each w is independently an integer from 1–1000;

u is an integer from 1–10;

each x, y and z is independently an integer from 0–10; and

each n is independently an integer from 1–5;

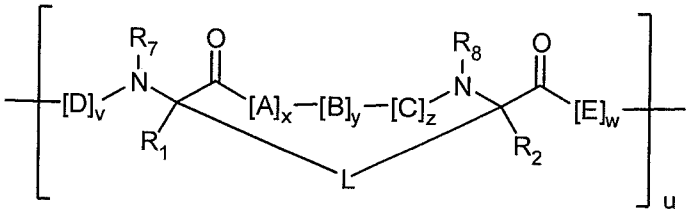
wherein the peptidomimetic macrocycle is not a peptidomimetic macrocycle of Tables 2a or 2b, wherein the at least one additional pharmaceutically active agent is fulvestrant, everolimus, romidepsin, palbociclib, trametinib, rituximab, dabrafenib, vemurafenib, azacitidine, decitabine, midostaurin, vincristine, cyclophosphamide or cytarabine. In one example, the disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effectiveamount of a peptidomimetic macrocycle and at least one additional pharmaceutically active agent, wherein the peptidomimetic macrocycle has a Formula:



CLAIMS

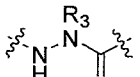
WHAT IS CLAIMED IS:

1. Use of a therapeutically-effective amount of a peptidomimetic macrocycle or a pharmaceutically acceptable salt thereof and at least one additional pharmaceutically active agent in the manufacture of a medicament for modulating the activity of MDM2 and MDMX in a subject with cancer, wherein the peptidomimetic macrocycle comprises an amino acid sequence which is at least about 60% identical to an amino acid sequence in any of Table 1, Table 1a, Table 1b, and Table 1c, and wherein the peptidomimetic macrocycle has the formula:



wherein:

each A, C, D, and E is independently an amino acid;

each B is independently an amino acid,  , [–NH–L_3–CO–], [–NH–L_3–SO_2–], or [–NH–L_3–];

each R_1 and R_2 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–; or forms a macrocycle–forming linker L' connected to the alpha position of one of said D or E amino acids;

each R_3 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, aryl, or heteroaryl, optionally substituted with R_5;

each L and L' is independently a macrocycle–forming linker of the formula –L_1–L_2–;

each L_1, L_2, and L_3 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or [–R_4–K–R_4–]$_n$, each being optionally substituted with R_5;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO_2, CO, CO_2, or $CONR_3$;

each R_5 is independently halogen, alkyl, $-OR_6$, $-N(R_6)_2$, $-SR_6$, $-SOR_6$, $-SO_2R_6$, $-CO_2R_6$, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_7 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with a D residue;

each R_8 is independently hydrogen, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

each v is independently an integer from 1–1000;

each w is independently an integer from 1–1000;

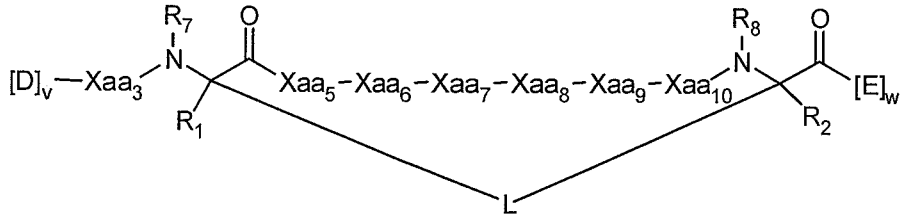
u is an integer from 1–10;

each x, y and z is independently an integer from 0–10; and

each n is independently an integer from 1–5;

wherein the peptidomimetic macrocycle is not a peptidomimetic macrocycle of Tables 2a or 2b, wherein the at least one additional pharmaceutically active agent is fulvestrant, everolimus, romidepsin, palbociclib, trametinib, rituximab, dabrafenib, vemurafenib, azacitidine, decitabine, midostaurin, vincristine, cyclophosphamide or cytarabine.

2. The use of claim 1, wherein the peptidomimetic macrocycle has a Formula:



wherein:

each of Xaa_3, Xaa_5, Xaa_6, Xaa_7, Xaa_8, Xaa_9, and Xaa_{10} is individually an amino acid, wherein at least three of Xaa_3, Xaa_5, Xaa_6, Xaa_7, Xaa_8, Xaa_9, and Xaa_{10} are the same amino acid as the amino acid at the corresponding position of the sequence Phe_3-X_4-His_5-Tyr_6-Trp_7-Ala_8-Gln_9-Leu_{10}-X_{11}-Ser_{12} or Phe_3-X_4-Glu_5-Tyr_6-Trp_7-Ala_8-Gln_9-Leu_{10}/Cba_{10}-X_{11}-Ala_{12} wherein each X_4 and X_{11} is independently an amino acid;

each D and E is independently an amino acid;

R_1 and R_2 are independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroalkyl, or heterocycloalkyl, unsubstituted or substituted with halo–; or at least one of R_1 and R_2 forms a macrocycle-forming linker L' connected to the alpha position of one of said D or E amino acids;

each L and L' is independently a macrocycle-forming linker of the formula $–L_1–L_2–$;

each L_1 and L_2 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, or $[-R_4\text{-}K\text{-}R_4-]_n$, each being optionally substituted with R_5;

each R_4 is independently alkylene, alkenylene, alkynylene, heteroalkylene, cycloalkylene, heterocycloalkylene, arylene, or heteroarylene;

each K is independently O, S, SO, SO_2, CO, CO_2, or $CONR_3$;

each R_5 is independently halogen, alkyl, $-OR_6$, $-N(R_6)_2$, $-SR_6$, $-SOR_6$, $-SO_2R_6$, $-CO_2R_6$, a fluorescent moiety, a radioisotope or a therapeutic agent;

each R_6 is independently –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heterocycloalkyl, a fluorescent moiety, a radioisotope or a therapeutic agent;

R_7 is –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with a D residue;

R_8 is –H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkyl, heteroalkyl, cycloalkylalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted with R_5, or part of a cyclic structure with an E residue;

v is an integer from 1-1000;

w is an integer from 3-1000; and

n is an integer from 1-5.

3. The use of claim 1, wherein w is more than 2.

4. The use of claim 3, wherein each of the first two amino acid represented by E comprises an uncharged side chain or a negatively charged side chain.

5. The use of claim 4, wherein each E is independently an amino acid selected from Ala (alanine), D-Ala (D-alanine), Aib (α-aminoisobutyric acid), Sar (N-methyl glycine), and Ser (serine).

6. The use of claim 1, wherein the peptidomimetic macrocycle:

(i) comprises less than 20 amino acids;

(ii) has a single cross link spanning amino acids in the i position to the i+7 position of the amino acid sequence of the Formula in claim 2;

(iii) has more than three amino acids between the i+7 position and the carboxyl terminus of the amino acid sequence of the Formula in claim 2;

(iv) binds to human MDM2 and MDM4; and

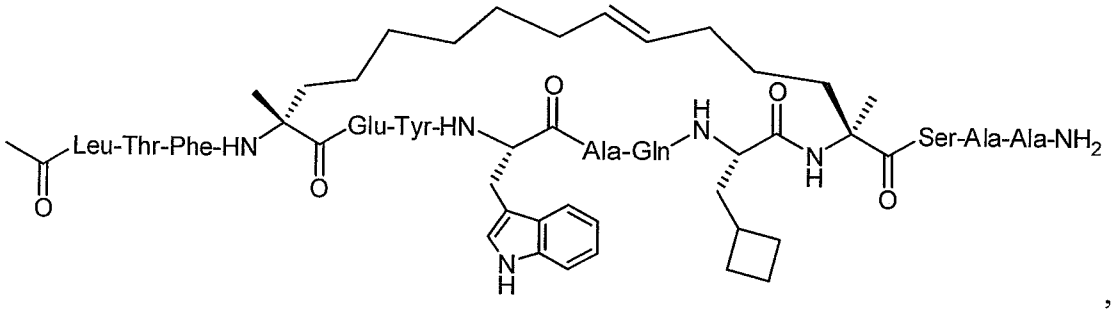
(v) has an observed mass of 950-975 m/e as measured by electrospray ionization-mass spectrometry.

7. The use of claim 1, wherein the at least one additional pharmaceutically active agent is palbociclib.

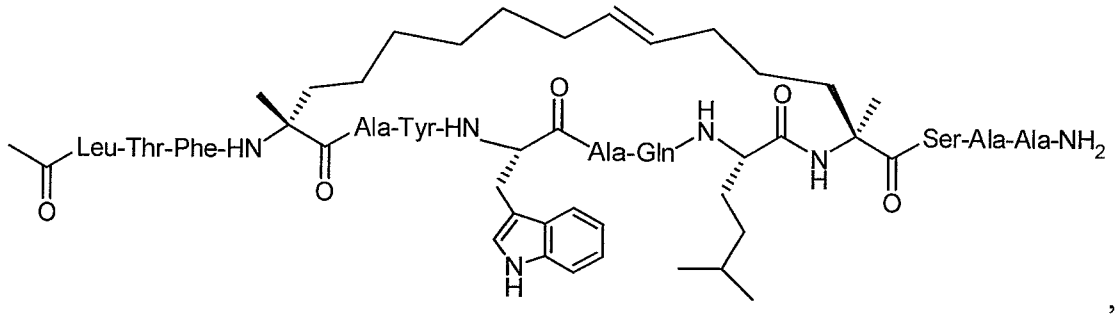
8. The use of claim 1, wherein the cancer is selected from the group consisting of head and neck cancer, melanoma, lung cancer, breast cancer, colon cancer, ovarian cancer, NSCLC, stomach cancer, prostate cancer, leukemia, lymphoma, mesothelioma, renal cancer, non-Hodgkin lymphoma (NHL), and glioma.

9. The use of claim 1, wherein the subject comprises cancer cells that overexpress PD-L1, PD-1, miR-34, or any combination thereof.

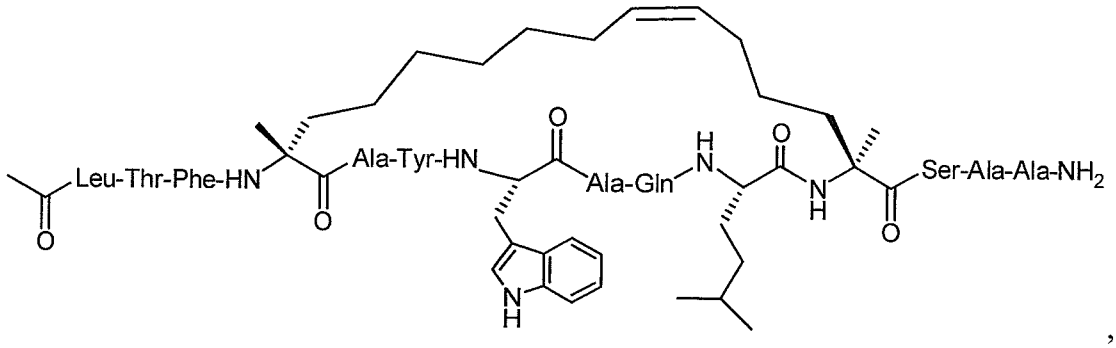
10. The use of claim 1, wherein the peptidomimetic macrocycle is selected from the group consisting of:



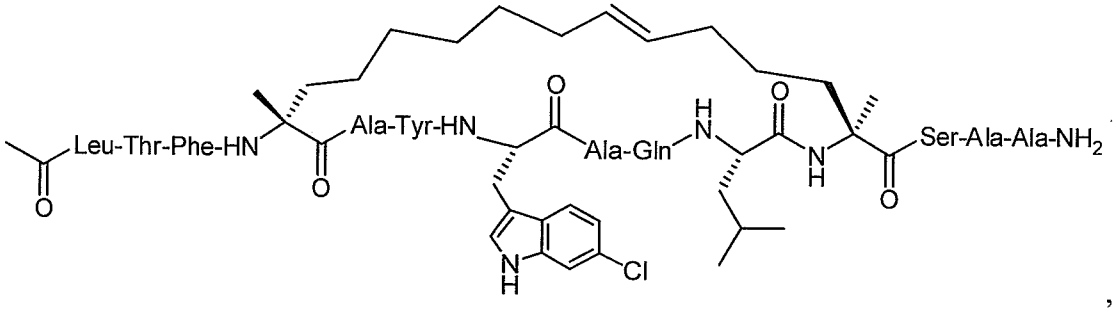
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Ala-Gln
Ser-Ala-Ala-NH2

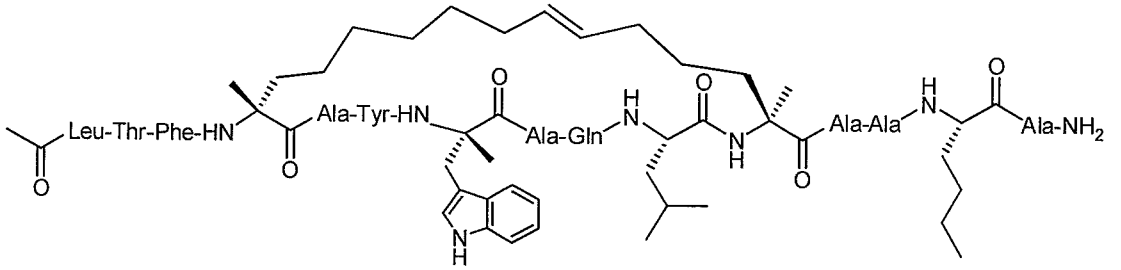
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Ala-Gln
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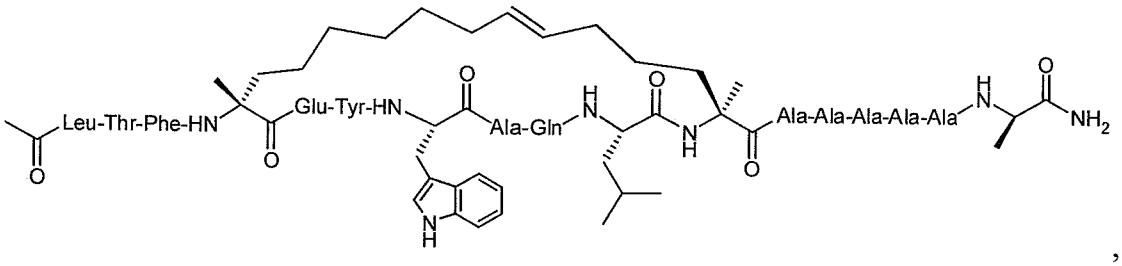
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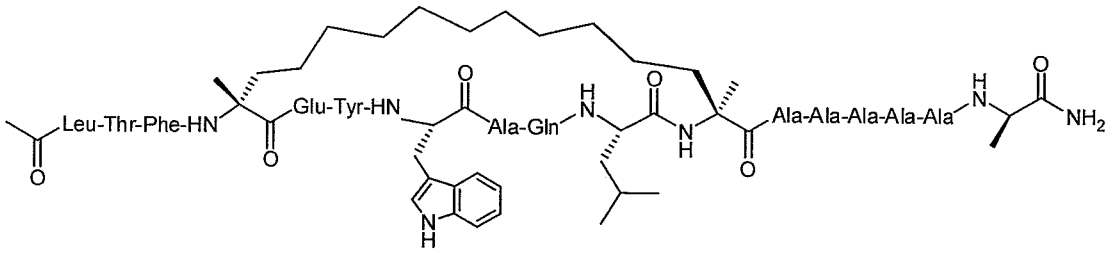
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Ala-Ala
Ala-NH2

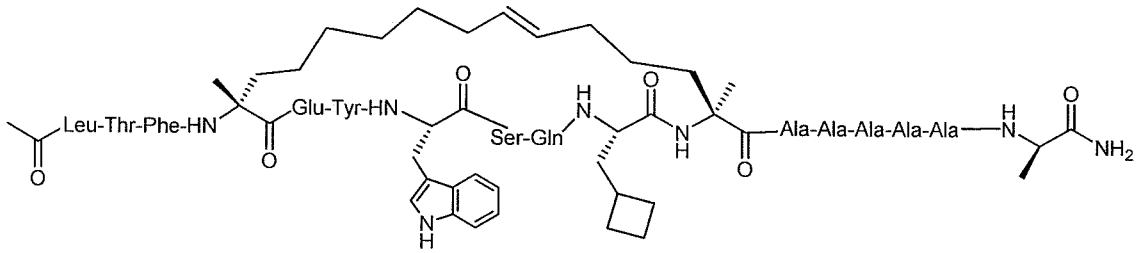
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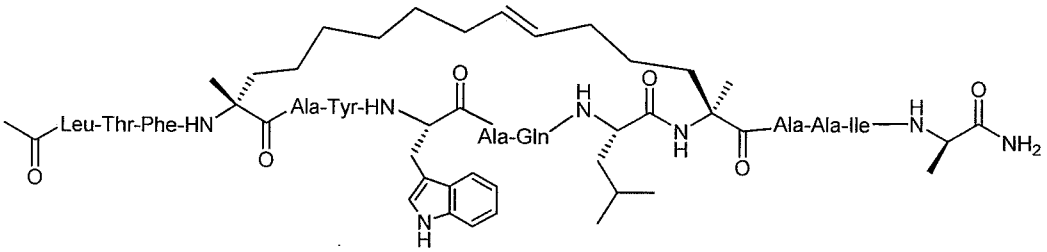

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Ala-Ala-Ala-Ala-Ala
NH2

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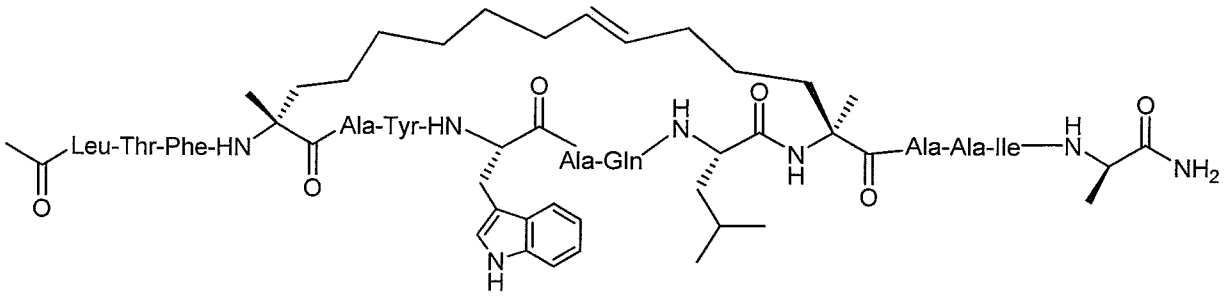

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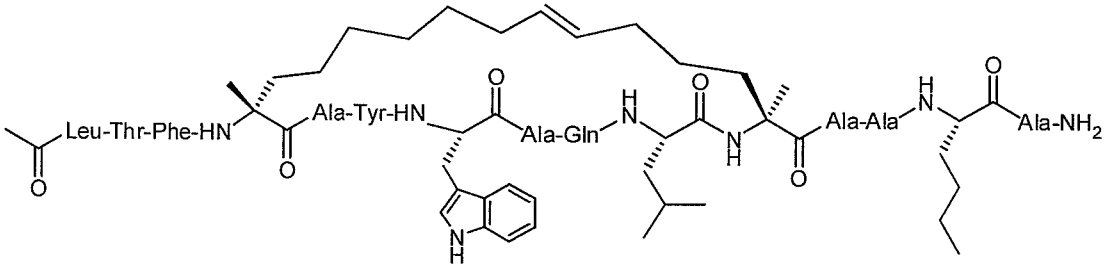

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NH2

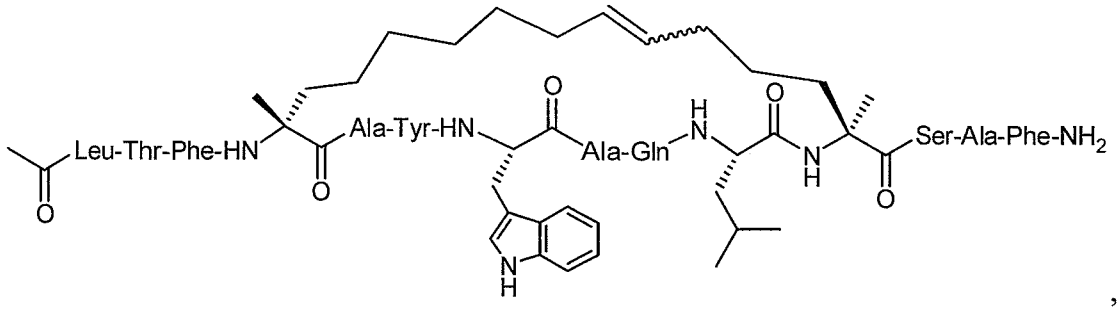
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Ala-Ala-Ile
NH2

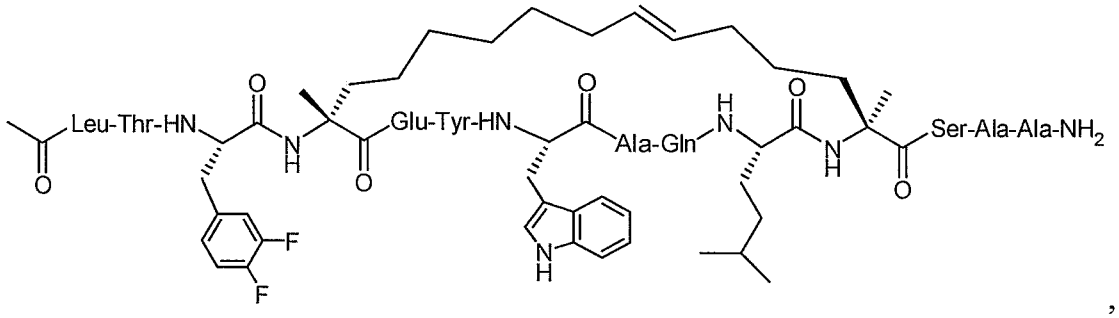
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Ala-Ala
Ala-NH2

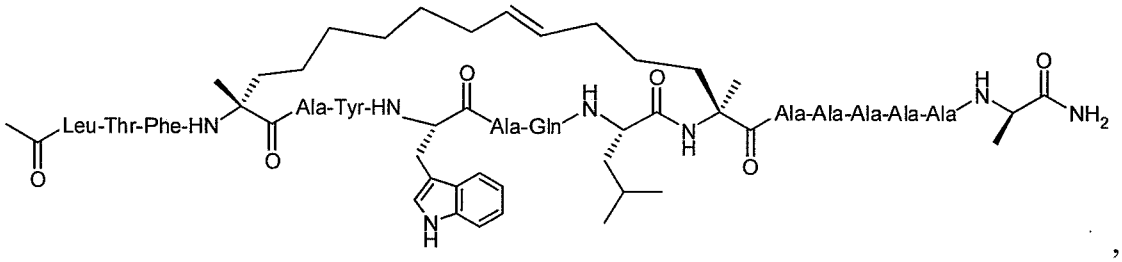
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Ser-Ala-Phe-NH2

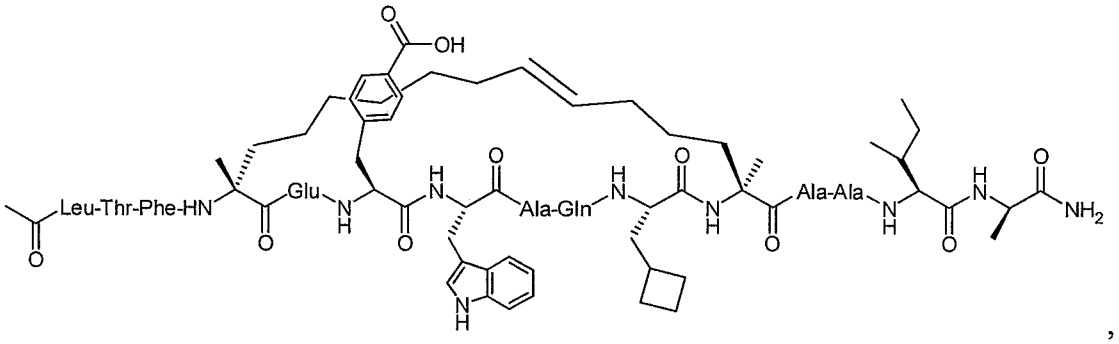
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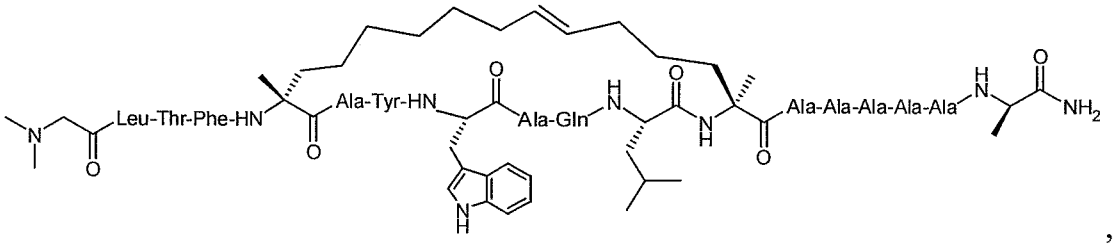
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NH2

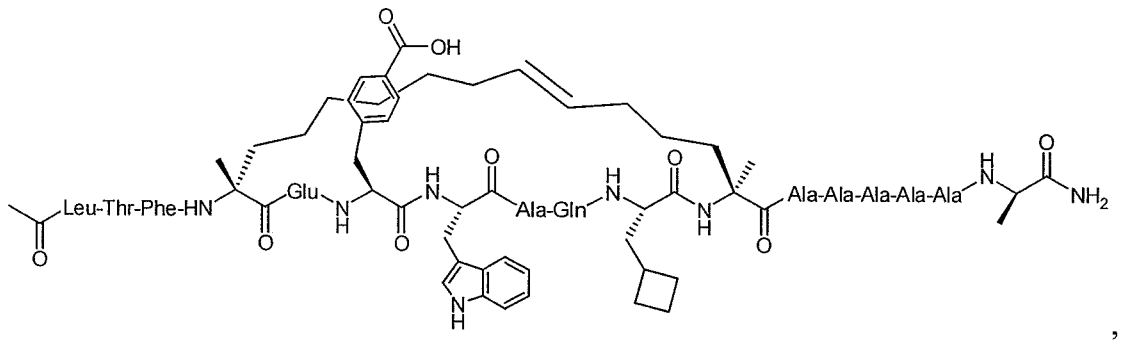
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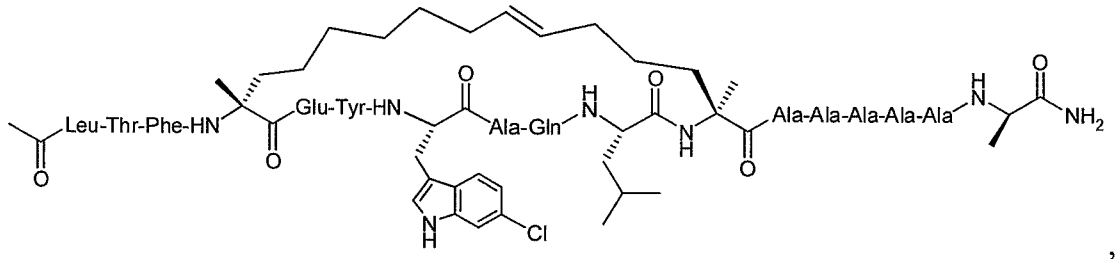
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NH2

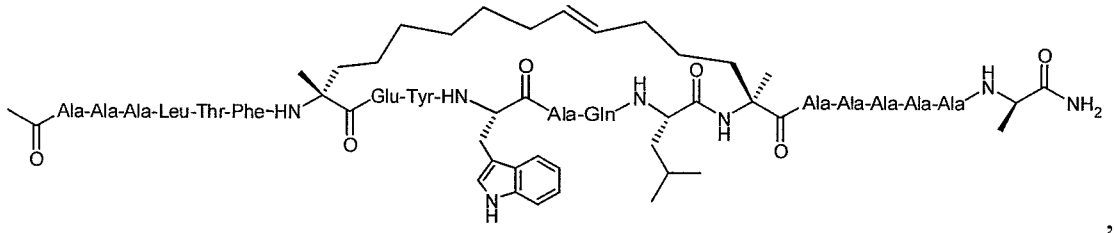
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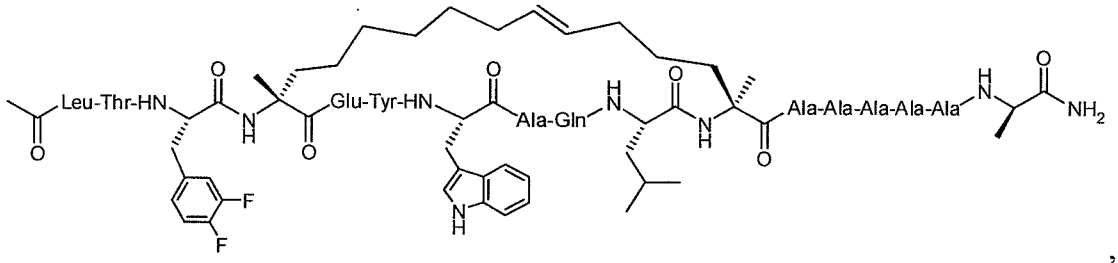
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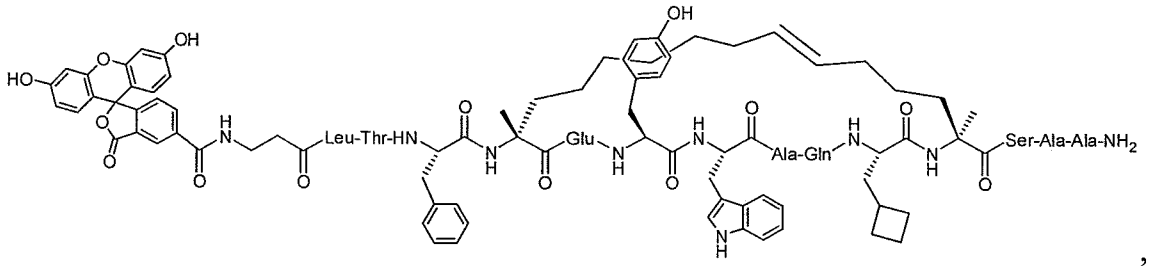
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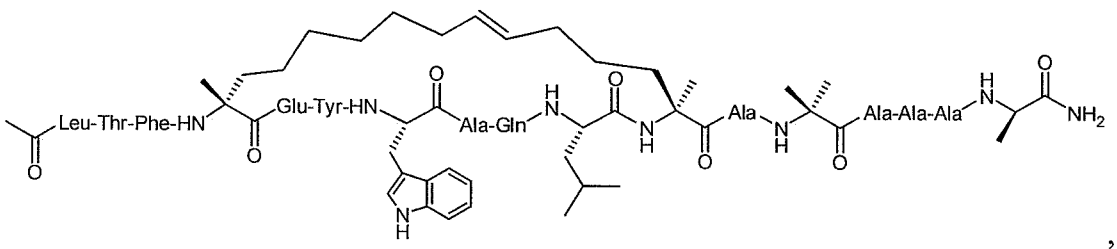
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NH2
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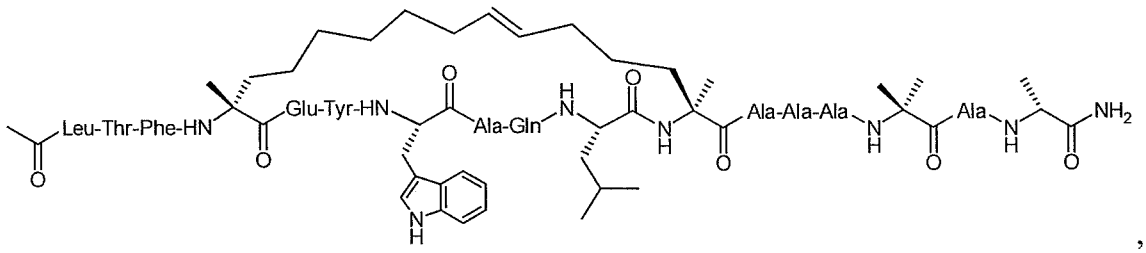
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OH
HO
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Glu
Ala-Gln
Ser-Ala-Ala-NH2

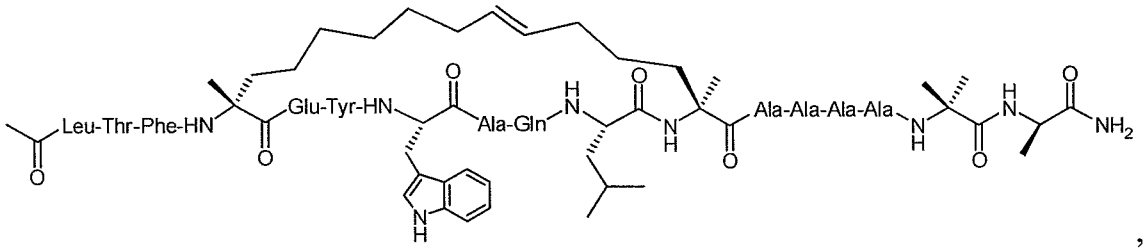
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Leu-Thr-Phe-HN
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Ala-Gln
Ala
Ala-Ala-Ala
NH2

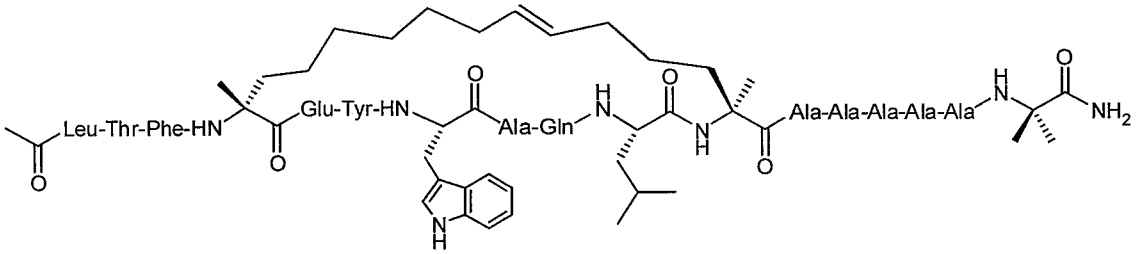
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Ala-Gln
Ala-Ala-Ala
Ala
NH2

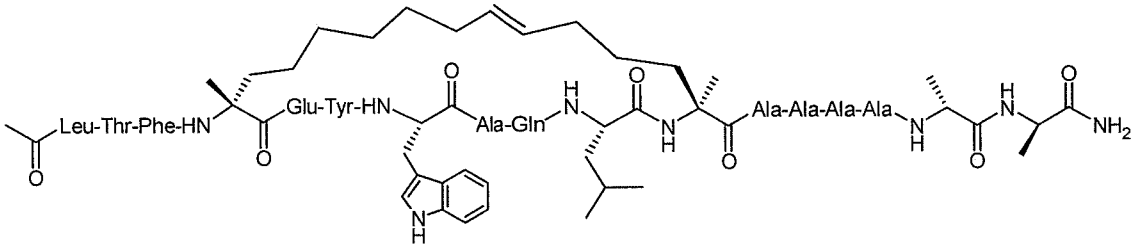
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Ala-Gln
Ala-Ala-Ala-Ala
NH2

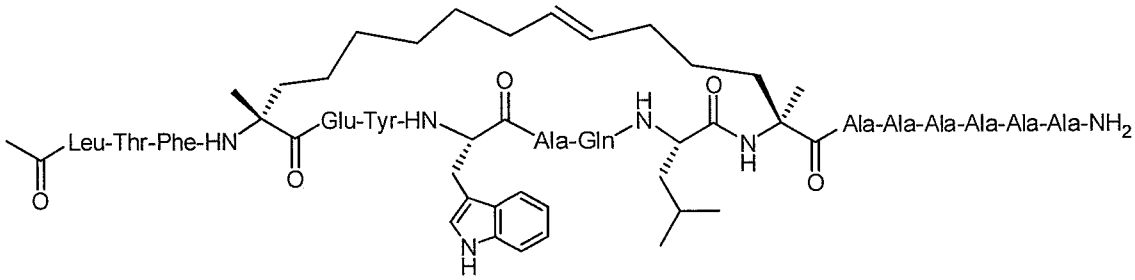
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Leu-Thr-Phe-HN
Glu-Tyr-HN
Ala-Gln
Ala-Ala-Ala-Ala-Ala
NH2

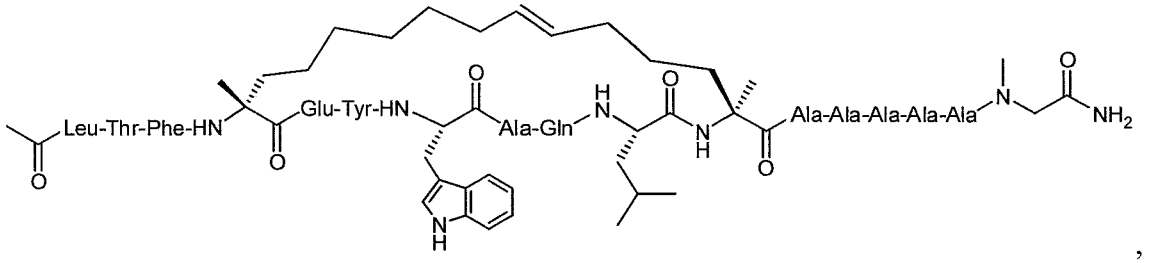
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Leu-Thr-Phe-HN
Glu-Tyr-HN
Ala-Gln
Ala-Ala-Ala-Ala
NH2

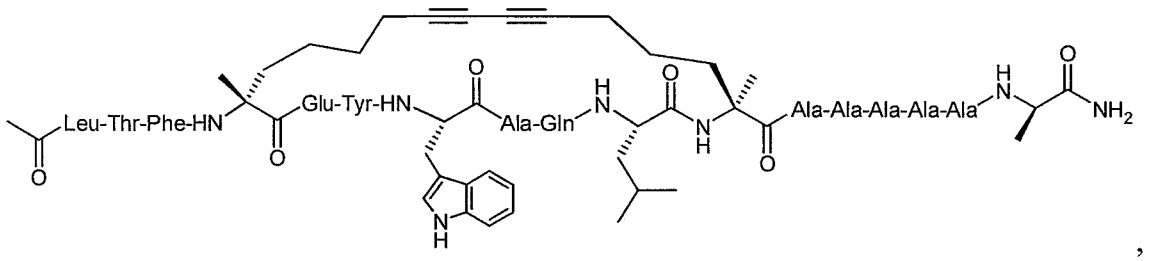
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Leu-Thr-Phe-HN
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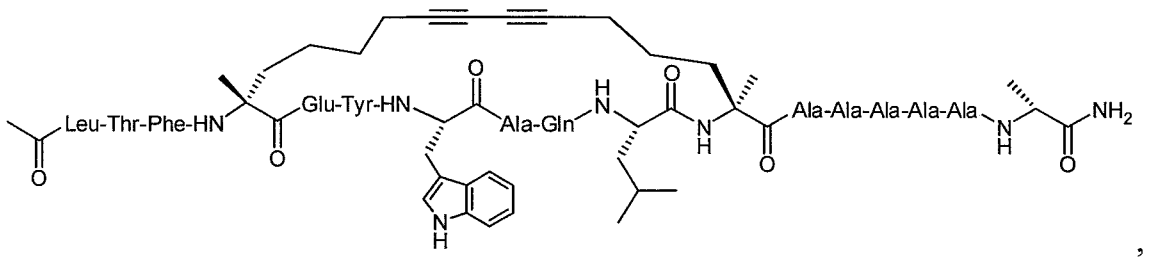
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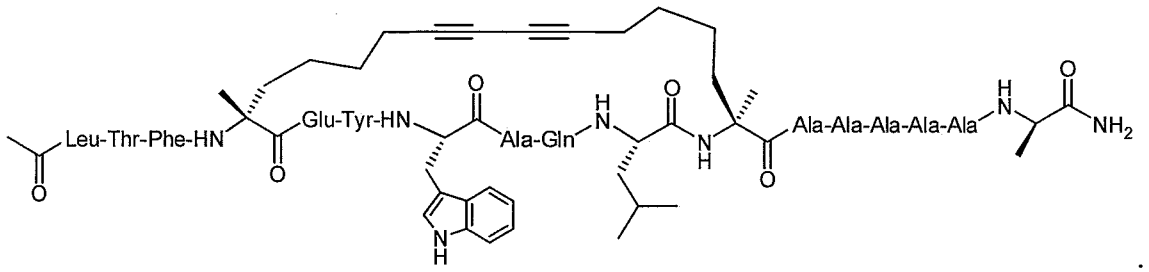


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and



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11. The use of claim 1, wherein the peptidomimetic macrocycle comprises an amino acid sequence Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}, wherein:

Xaa_3 is Phe or an analog thereof;

Xaa_4 and Xaa_{11} are independently a cross-linking amino acid;

Xaa_5 is Glu, His, or an analog thereof;

Xaa_6 is Tyr, or an analog thereof;

Xaa_7 is Trp, or an analog thereof;

Xaa_8 is Ala, or an analog thereof;

Xaa_9 is Gln, or an analog thereof;

Xaa_{10} is Leu, Cba, or an analog thereof; and

Xaa_{12} is Ser, Ala or an analog thereof.

12. The use of claim 1, wherein the cancer is AML.

13. The use of claim 1, wherein the cancer is myelodysplastic syndrome.

14. The use of claim 1, wherein the cancer is peripheral T cell lymphoma.

15. The use of claim 1, wherein the cancer is breast cancer.

16. The use of claim 1, wherein the cancer is sarcoma, liposarcoma, osteosarcoma, chondrosarcoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, chordoma, synovioma, angiosarcoma, glioblastoma, medulla blastema, mesothelioma, epithelial carcinoma, embryonal carcinoma, bladder carcinoma, non-small-cell lung carcinoma (NSCLC), testicular cancer, invasive (or infiltrating) ductal carcinoma, invasive (or infiltrating) lobular carcinoma, esophageal cancer, diffuse large B-cell lymphoma, hepatocellular cancer, prostate cancer, stomach cancer, squamous cell carcinoma, cystadenocarcinoma, renal cell carcinoma, colon cancer, papillary carcinoma, pancreatic cancer, uterine cancer, uveal melanoma, or tumors involving other tissues including peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites.

17. The use of claim 1, wherein the at least one additional pharmaceutically active agent is a cytarabine.

18. The use of claim 1, wherein the cancer is AML and the at least one additional pharmaceutically active agent is cytarabine.

19. The use of claim 1, wherein the combination of the peptidomimetic macrocycle or a pharmaceutically acceptable salt thereof and the at least one additional pharmaceutically active agent is synergistic.

20. The use of claim 19, wherein the at least one additional pharmaceutically active agent is midostaurin, vemurafenib, fluvestant, everolimus, cytarabine, azacitidine, vincristine, or cyclophosphamide.