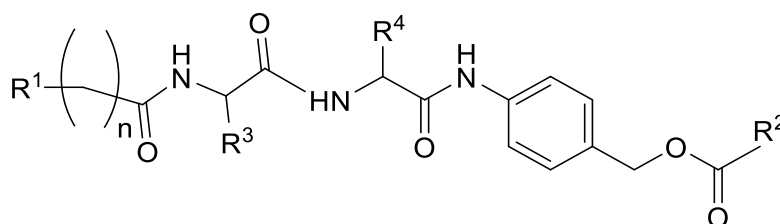


Drug Conjugates active across multiple cancer cell lines and demonstrate broad utility to be conjugated to several antibodies or other targeting moieties while still retaining favorable properties and efficacy. Thus, this disclosure relates to Ligand-Drug Candidates having linker dipeptide sequences that provide enhanced exposure of released free cytotoxic compound to tumor tissue in comparison to normal tissue and thus selective entry into targeted cells and decreased exposure of normal tissue in comparison to tumor tissue to the cytotoxic compound.

[0005] Exemplary linker-payload compounds include, but are not limited to, the structures shown throughout this disclosure. Exemplary Ligand-Drug Conjugates (e.g, ADCs) formed using the linker-payloads described herein are also described. Other embodiments, examples, aspects and features of the present disclosure are either further described in or will be apparent from the ensuing description, examples and appended claims.

[0006] In each of the examples described herein, each variable is selected independently of the other unless otherwise noted.

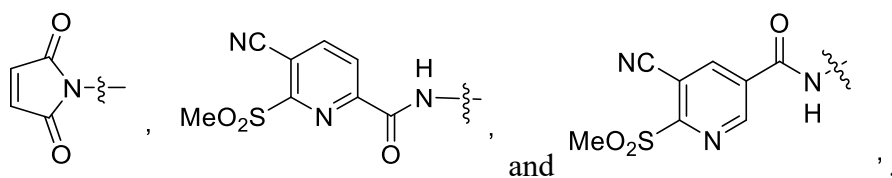
[0007] The present disclosure provides a dipeptide linker-payload (drug), and pharmaceutically acceptable salts, solvates, and stereoisomers thereof, comprising a structure of Formula I:



Formula I

wherein:

R¹ is selected from:



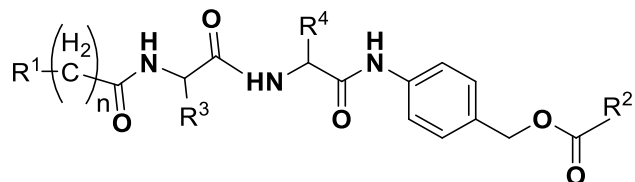
~~~~ wavy line indicates the site of covalent attachment;

R<sup>2</sup> is a cytotoxic drug;

R<sup>3</sup> and R<sup>4</sup> independently represent C<sub>1-3</sub> alkyl or a naturally occurring or unnatural amino acid side chain; and

n is an integer from 1 to 4.

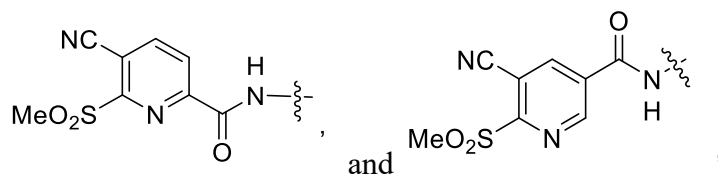
**[0007a]** An aspect of the disclosure relates to a compound of Formula I, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof:



I

wherein:

R<sup>1</sup> is selected from:



~ wavy line indicates the site of covalent attachment;

R<sup>2</sup> is a cytotoxic drug;

R<sup>3</sup> and R<sup>4</sup> independently represent C<sub>1-3</sub> alkyl or a naturally occurring or unnatural amino acid acid side chain; and

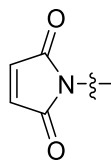
n is an integer from 1 to 4.

[0008] An example of Formula I is realized when n is 1.

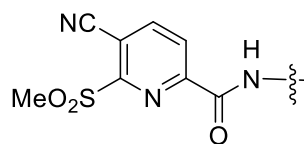
[0009] An example of Formula I is realized when n is 2.

[0010] An example of Formula I is realized when n is 3.

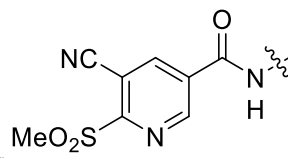
[0011] An example of Formula I is realized when n is 4.



[0012] An example of Formula I is realized when R<sup>1</sup> is



[0013] Another example of Formula I is realized when R<sup>1</sup> is



[0014] Another example of Formula I is realized when R<sup>1</sup> is

[0015] Another example of Formula I is realized when R<sup>2</sup> is a cytotoxic drug or payload selected from anthracyclines, auristatins, camptothecins, duocarmycins, etoposides, maytansinoids, pyrrolobenzodiazepine dimers, DNA minor groove binders, taxanes, vinca alkaloids, enediynes, anti-tubulins, and vinca alkaloids. A subexample of this is realized when R<sup>2</sup> is selected from auristatin T, auristatin E, auristatin F phenylenediamine, AEB, AEVB, monomethyl auristatin F (MMAF), lipophilic monomethyl auristatin F, monomethyl auristatin E (MMAE)), lexitropsins, duocarmycins, paclitaxel and docetaxel, T67 (Tularik), vincristine, vinblastine, vindesine, vinorelbine, nicotinamide phosphoribosyltransferase inhibitor (NAMPTi), tubulysin M, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, melphalan, methotrexate, mitomycin C, etoposide, CC-1065 analogue, calicheamicin, maytansine, an analog of dolastatin 10, rhizoxin, palytoxin, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermoide and eleuthrobin. The cytotoxic or cytostatic agent can be an anti-tubulin agent. Examples of anti-tubulin agents include taxanes (e.g., Taxol® (paclitaxel), Taxotere® (docetaxel)), T67 (Tularik), vinca alkyls (e.g., Other suitable antitubulin agents include, for example, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine

and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermoide and eleuthrobin.

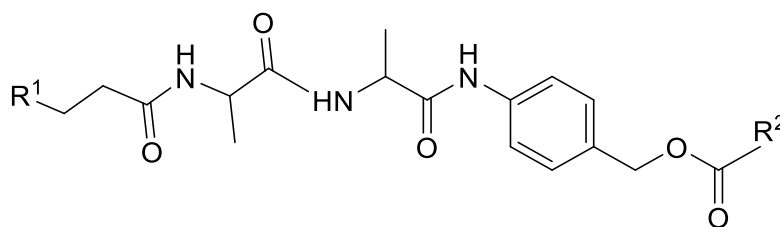
**[0016]** Another subexample of this is realized when  $R^2$  is an auristatin drug. A subexample of this is realized when  $R^2$  is an auristatin drug selected from AE, auristatin F phenylenediamine (AFP), AEB, AEVB, MMAF, and MMAE. An subexample of this is realized when  $R^2$  is MMAE. A further example is realized when  $R^2$  is MMAF.

**[0017]** Another example of Formula I is realized when  $R^2$  is a pyrrolobenzodiazepine dimer. A subexample of this is realized when  $R^2$  is tesirine.

**[0018]** An example of Formula I is realized when  $R^3$  and  $R^4$  are independently selected from  $C_{1-3}$  alkyl. A subexample of this is realized when  $R^3$  and  $R^4$  are independently selected from  $-CH_3$ ,  $-CH_2CH_3$ ,  $-(CH_2)_2CH_3$ . A subexample of this is realized when  $R^3$  and  $R^4$  are both  $CH_3$ . A subexample of this is realized when  $R^3$  and  $R^4$  are both  $-CH_2CH_3$ . A subexample of this is realized when  $R^3$  and  $R^4$  are both  $-(CH_2)_2CH_3$ . A subexample of this is realized when one of  $R^3$  and  $R^4$  is  $CH_3$  and the other is selected from  $-CH_2CH_3$ , and  $-(CH_2)_2CH_3$ .

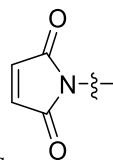
**[0019]** An example of Formula I is realized when  $R^3$  and  $R^4$  are independently a naturally occurring or non-naturally occurring amino acid side chain. A subexample of this is realized when  $R^3$  and  $R^4$  are independently selected from arginine, glutamine, phenylalanine, tyrosine, tryptophan, lysine, glycine, alanine, histidine, serine, proline, glutamic acid, aspartic acid, threonine, cysteine, methionine, leucine, asparagine, isoleucine, and valine or side chains, in the L or D-configuration. Another example of Formula I is realized when  $R^3$  and  $R^4$  are independently selected from a naturally occurring or non-naturally occurring amino acid side chain selected from the side chains of alanine, valine, leucine and methionine.

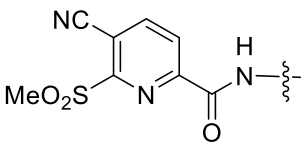
**[0020]** In another example, the present disclosure provides a compound of Formula II and pharmaceutically acceptable salts, solvates, and stereoisomers thereof, comprising a structure of :

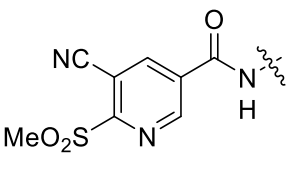


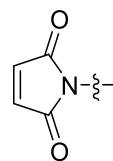
II

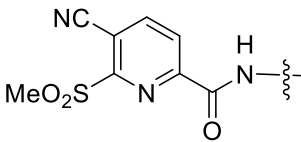
wherein  $R^1$  and  $R^2$  are as described herein. An example of Formula II is realized when  $R^2$  is an auristatin drug. A subexample of this is realized when  $R^2$  is an auristatin drug selected from AE, auristatin F phenylenediamine (AFP), AEB, AEVB, MMAF, and MMAE. A subexample of this is realized when  $R^2$  is MMAE. A further subexample is realized when  $R^2$  is MMAF.

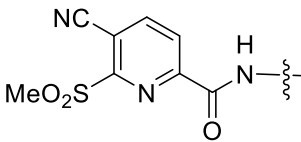


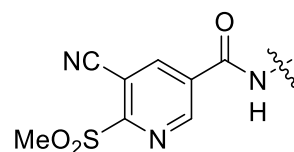
**[0021]** An example of Formula II is realized when  $R^1$  is . An example of Formula II is

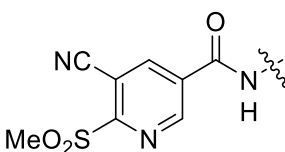
realized when  $R^1$  is . An example of Formula II is realized when  $R^1$  is



**[0022]** Another example of Formula II is realized when  $R^1$  is  and  $R^2$  is MMAE.

**[0023]** Another example of Formula II is realized when  $R^1$  is  and  $R^2$  is MMAE.



**[0024]** Another example of Formula II is realized when  $R^1$  is , and  $R^2$  is MMAE.

**[0025]** Another example of Formula II is realized when  $R^2$  is a pyrrolobenzodiazepine dimer. A subexample of this is realized when  $R^2$  is tesirine.

**[0026]** Another example of Formula II is represented by structural Formula II':


$$\text{L}' - \left( \text{R}' - \left( \text{CH}_2 \right)_n - \text{C}(=\text{O}) - \text{NH} - \text{CH}(\text{R}^3) - \text{C}(=\text{O}) - \text{NH} - \text{CH}(\text{R}^4) - \text{C}(=\text{O}) - \text{NH} - \text{C}_6\text{H}_4 - \text{CH}_2 - \text{O} - \text{C}(=\text{O}) - \text{R}^2 \right)$$

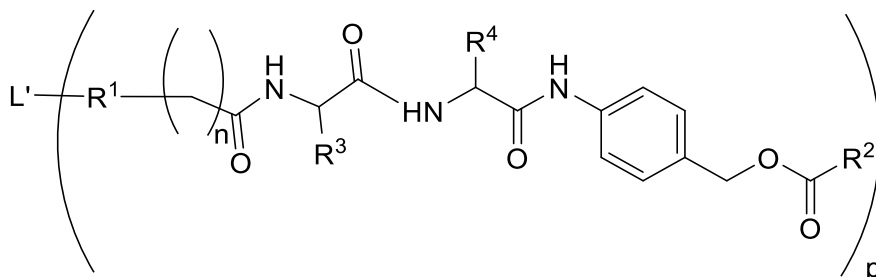




-6-

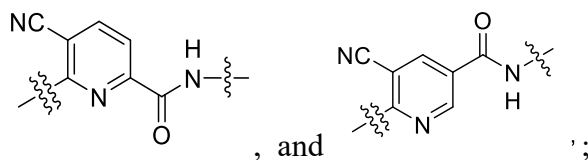
some examples, p is 2, 4, 6, or 8, or p is 1 to 2, including fraction and decimal values between 2 to 8.

**[0027a]** Another aspect of the disclosure relates to a compound of Formula III or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof:



Formula III

wherein



$R^1$  is selected from:

~~~~ single wavy line indicates the site of covalent attachment to  $-(CH_2)_n$ ;

~~~~ double wavy line indicates the site of covalent attachment to a sulfur of a cysteine residue of  $L'$

$R^2$  is a cytotoxic drug;

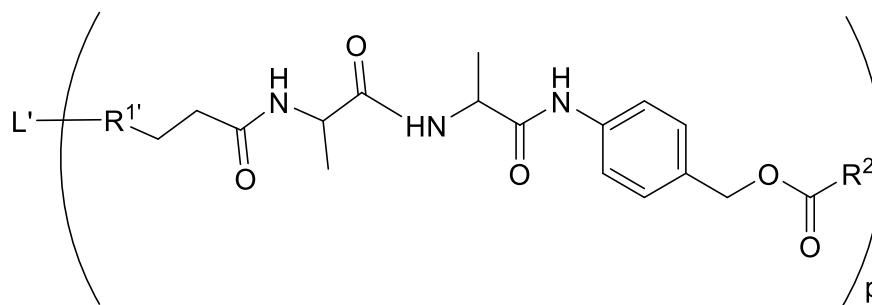
$R^3$  and  $R^4$  independently represent  $C_{1-3}$  alkyl or a naturally occurring or unnatural amino acid side chain;

n is an integer from 1 to 4;

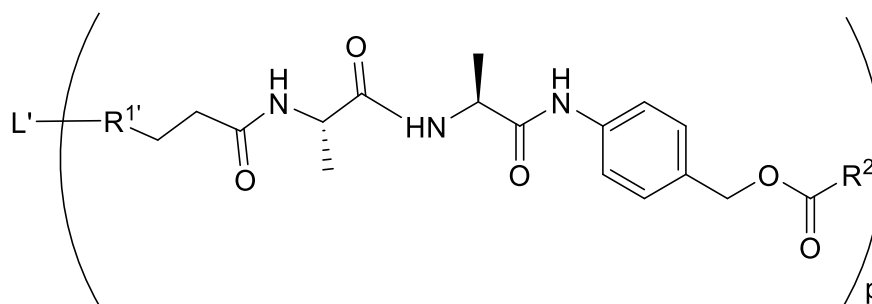
$L'$  is a ligand that is an antibody, or an antigen-binding fragment of an antibody); and

p is a positive rational number from 1 to 24, including fractions and decimals.

**[0028]** Another example of Formula III is represented by structural Formula III' or III'':



Formula III'



III''

wherein L', R¹', and R² are as described herein.

**[0029]** An example of Formula III, III' and III'' is realized when L' includes intact monoclonal antibodies, polyclonal antibodies, monospecific antibodies, multi-specific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity. It is further envisioned that the antibody drug conjugates of this disclosure might be replaced with anything that specifically binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population. For example, instead of containing an antibody, conjugates of the disclosure might contain a targeting molecule that binds to complexes with, or reacts with a receptor, antigen or other receptive moiety of a cell population sought to be therapeutically or otherwise biologically modified (e.g., small molecular weight proteins, polypeptide or peptides, lectins, glycoproteins, non-peptides, vitamins, nutrient-transport molecules (e.g., transferrin)) and any other cell binding molecule or substance.

**[0030]** Examples of ligand L' useful in the present disclosure are disclosed herein. selected from trastuzumab, or mutants thereof, oregovomab, edrecolomab, cetuximab, a humanized monoclonal antibody to the vitronectin receptor (α<sub>v</sub>β<sub>3</sub>), alemtuzumab, anti-HLA-DR

antibodies including a humanized anti-HLA-DR antibody for the treatment of non-Hodgkin's lymphoma, 121I Lym-1, anti-HLA-Dr10 antibodies including a murine anti-DLA-Dr10 antibody for the treatment of non-Hodgkin's lymphoma, anti-CD33 antibodies, anti-CD22 antibodies including a humanized anti-CD22 mAb for the treatment of Hodgkin's Disease or non-Hodgkin's lymphoma, labetuzumab, bevacizumab, ibritumomab tiuxetan, ofatumumab, panitumumab, rituximab, tositumamab, ipilimumab, sacituzumab, cetuximab (ERBITUX), and gentuzumab.

**[0031]** This disclosure also relates to a pharmaceutical composition comprising a therapeutically effective amount of a compound of Formula I, II, II', III, III', III'' or a pharmaceutically acceptable salt or solvate thereof and one or more pharmaceutically acceptable carrier(s), diluent(s) or excipients(s). Another aspect of the disclosure relates to a pharmaceutical composition comprising a compound as defined herein, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier.

**[0032]** The disclosure also relates to a pharmaceutical composition comprising a therapeutically effective amount of a compound of Formula I, II, II', III, III', or III'' as described herein, or a tautomer, mesomere, racemate, enantiomer, diastereomer thereof, or mixture thereof, or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carrier(s), diluent(s) or excipient(s).

**[0033]** The disclosure also relates to a compound of Formula I, II, II', III, III', or III'' as described herein, or a tautomer, mesomere, racemate, enantiomer, diastereomer thereof, or mixture thereof, or a pharmaceutically acceptable salt thereof for use as a drug or drug component.

**[0034]** The disclosure also relates to a compound of Formula I, II, II', III, III', or III'' as described herein, or a tautomer, mesomere, racemate, enantiomer, diastereomer thereof, or mixture thereof, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition in the preparation of a medicament for treating or preventing a tumor.

**[0034a]** Another aspect of the disclosure relates to use of a compound as defined herein, or a pharmaceutically acceptable salt or solvate thereof, or of the pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment or prevention of cancers or tumors.

**[0034b]** Another aspect of the disclosure relates to use of a compound as defined herein, or a pharmaceutically acceptable salt or solvate thereof, or a pharmaceutical composition comprising said compound, salt or solvate thereof in the manufacture of a medicament for treating or preventing a cancer selected from breast cancer, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, kidney cancer, urethral cancer, bladder cancer, liver cancer, stomach cancer, endometrial cancer, salivary gland cancer, esophageal cancer, melanoma, glioma, neuroblastoma, sarcoma, lung cancer (for example, small cell lung cancer and non-small cell lung cancer) colon cancer, rectal cancer, colorectal cancer, leukemia, acute promyelocytic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia), bone cancer, skin cancer, thyroid cancer, pancreatic cancer, and lymphoma (for example, Hodgkin's lymphoma, non-Hodgkin's lymphoma, or recurrent anaplastic large cell lymphoma).

**[0034c]** Another aspect of the disclosure relates to a compound or pharmaceutical composition comprising the compound as defined herein, or a pharmaceutically acceptable salt or solvate thereof, or the pharmaceutical composition as defined herein for use in therapy.

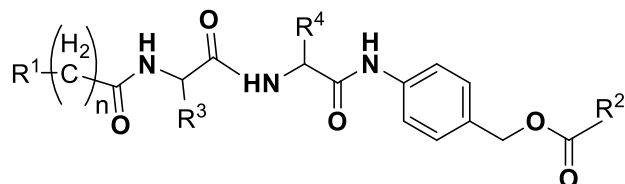
**[0035]** In another example, the compounds of the disclosure include those identified herein as Examples in the tables below, and pharmaceutically acceptable salts thereof.

#### DETAILED DESCRIPTION OF THE DISCLOSURE

**[0036]** For each of the following embodiments, any variable not explicitly defined in the embodiment is as defined in Formula (I). In each of the embodiments described herein, each variable is selected independently of the other unless otherwise noted.

WHAT IS CLAIMED:

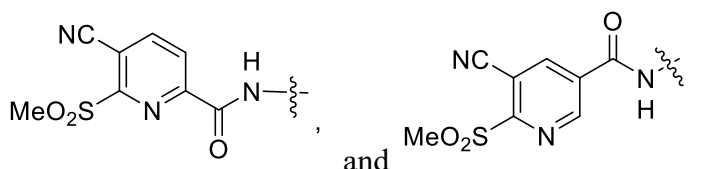
1. A compound of Formula I, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof:



I

wherein:

R<sup>1</sup> is selected from:



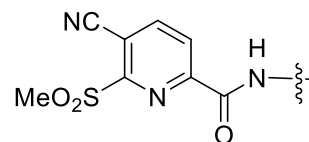
~~~~ wavy line indicates the site of covalent attachment;

R² is a cytotoxic drug;

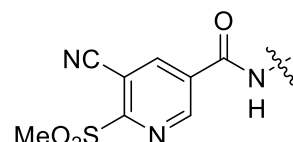
R³ and R⁴ independently represent C₁₋₃ alkyl or a naturally occurring or unnatural amino acid acid side chain; and

n is an integer from 1 to 4.

2. The compound according to claim 1 wherein R¹ is



3. The compound according to claim 1 wherein R¹ is



4. The compound according to any of claims 1 to 3 wherein R² is a cytotoxic drug selected from anthracyclines, auristatins, camptothecins, duocarmycins, etoposides,

maytansinoids, pyrrolobenzodiazepine dimers, DNA minor groove binders, taxanes, vinca alkaloids, enediyne, anti-tubulins, and vinca alkaloids.

5. The compound according to any of claims 1 to 4 wherein R^2 is selected from auristatin T, auristatin E, auristatin F phenylenediamine, benzoyl-auristatin E ester, 5-benzoylvaleric acid-AE ester, monomethyl auristatin F, lipophilic monomethyl auristatin F, monomethyl auristatin E, lexitropsins, duocarmycins, paclitaxel and docetaxel, T67 (Tularik), vincristine, vinblastine, vindesine, vinorelbine, nicotinamide phosphoribosyltransferase inhibitor (NAMPTi), tubulysin M, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, melphalan, methotrexate, mitomycin C, etoposide, CC-1065 analogue, calicheamicin, maytansine, an analog of dolastatin 10, rhizoxin, palytoxin, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine and colcemid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermoide, tesirine and eleuthrobin.

6. The compound according to any of claims 1 to 5 wherein R^2 is an auristatin drug.

7. The compound according to claims any of 1 to 6 wherein R^2 is an auristatin drug selected from auristatin E auristatin F phenylenediamine, benzoyl-auristatin E ester, 5-benzoylvaleric acid-auristatin E ester, monomethyl auristatin F, and monomethyl auristatin E.

8. The compound according to any of claims 1 to 7 wherein R^2 is monomethyl auristatin E.

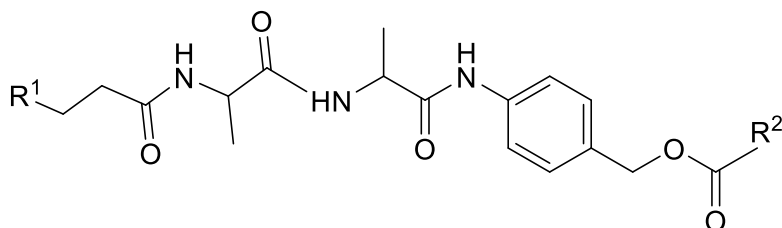
9. The compound according to claims an of 1-4 wherein R^2 is a pyrrolobenzodiazepine dimer.

10. The compound according to any of claims 1 to 9 wherein R^3 and R^4 are independently selected from C_{1-3} alkyl.

11. The compound according to any of claims 1 to 10 wherein R^3 and R^4 are both CH_3 .

12. The compound according to any of claims 1 to 9 wherein R³ and R⁴ are independently a naturally occurring or non-naturally occurring amino acid residue.

13. The compound according to claim 1 and pharmaceutically acceptable salts, solvates, or stereoisomer thereof, comprising a structure of Formula II:

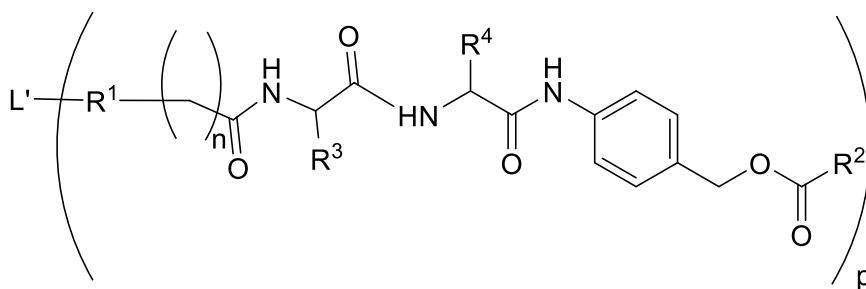


II

wherein R¹ and R² are as described herein.

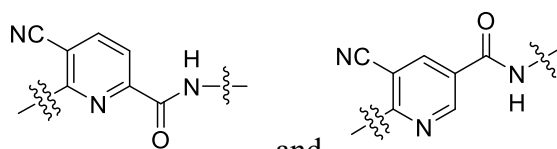
14. The compound according to claim 13 wherein R² is an auristatin drug selected from auristatin E, auristatin F phenylenediamine, benzoyl-auristatin E ester, 5-benzoylvaleric acid-AE ester, monomethyl auristatin F, monomethyl auristatin E, or a pyrrolobenzodiazepine dimer.

15. A compound of Formula III or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof:



Formula III

wherein



R¹ is selected from:

, and

;

~~~~ single wavy line indicates the site of covalent attachment to  $-(CH_2)_n$ ;

⋈ double wavy line indicates the site of covalent attachment to a sulfur of a cysteine residue of L'

R<sup>2</sup> is a cytotoxic drug;

R<sup>3</sup> and R<sup>4</sup> independently represent C<sub>1-3</sub> alkyl or a naturally occurring or unnatural amino acid acid side chain;

n is an integer from 1 to 4;

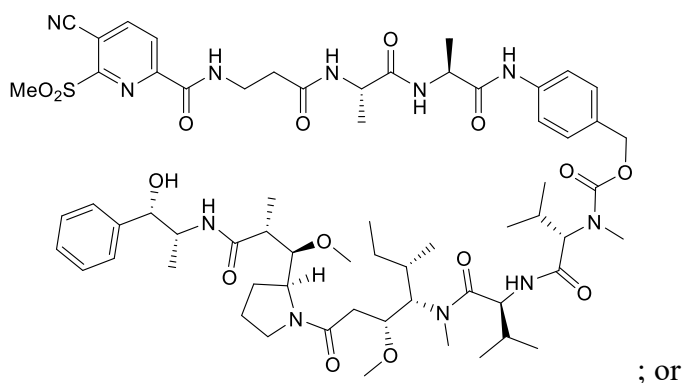
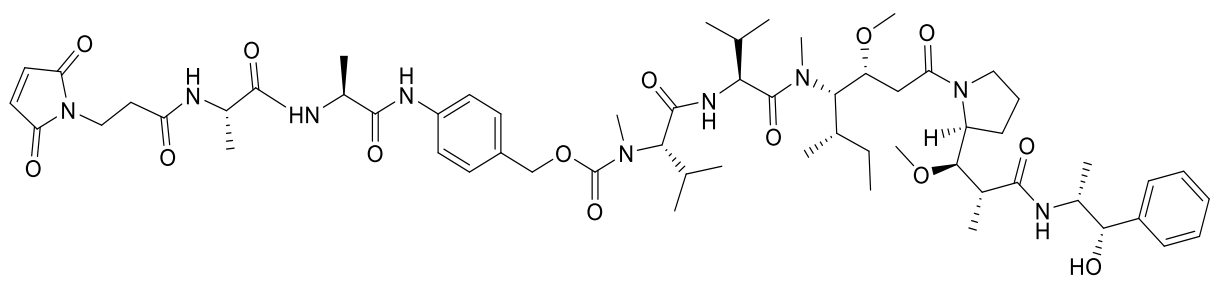
L' is a ligand that is an antibody, or an antigen-binding fragment of an antibody); and

p is a positive rational number from 1 to 24, including fractions and decimals.

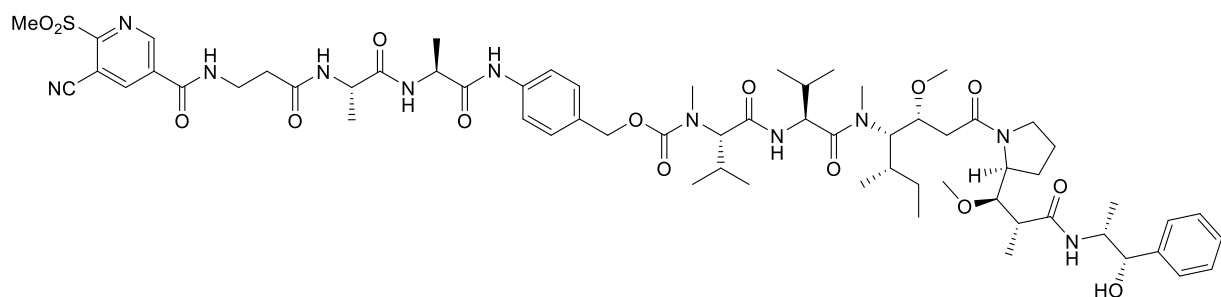
16. The compound according to claim 15 wherein and wherein R<sup>2</sup> is an auristatin drug selected from auristatin E, auristatin F phenylenediamine, benzoyl-auristatin E ester, 5-benzoylvaleric acid-AE ester, monomethyl auristatin F, monomethyl auristatin E, or a pyrrolobenzodiazepine dimer, and R<sup>3</sup> and R<sup>4</sup> both are CH<sub>3</sub>.

17. The compound according to any of claims 15 and 16 wherein R<sup>2</sup> is monomethyl auristatin E.

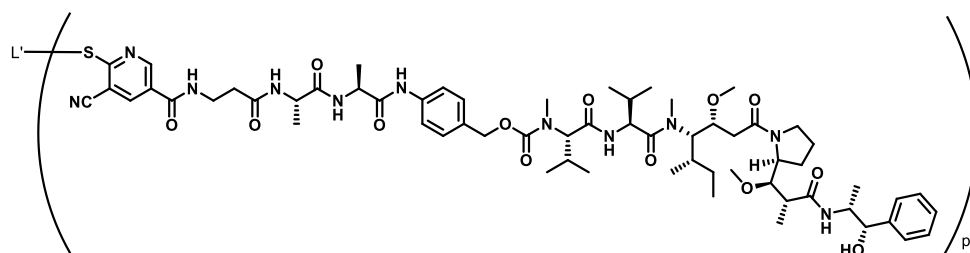
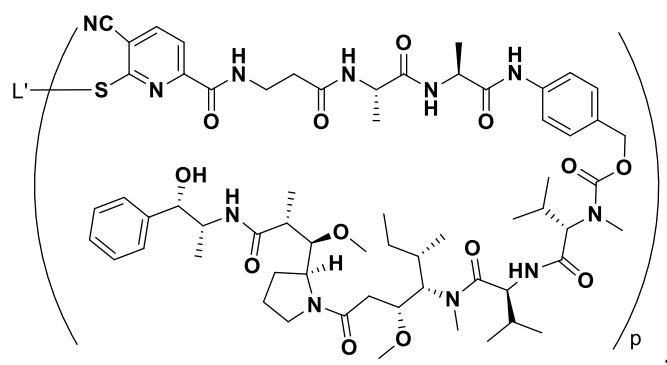
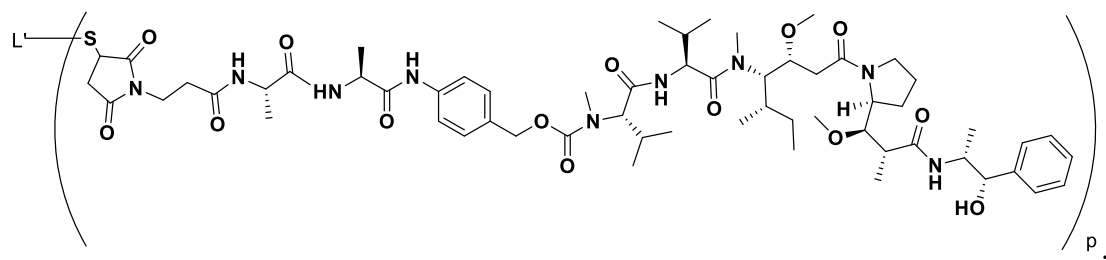
18. The compound according to claim 1, or a pharmaceutically acceptable salt thereof which is



; or



19. The compound according to claim 15, or pharmaceutically acceptable salts, or solvates, selected from:



wherein L' is an antibody and p is an integer from 1 to 8.

20. The compound according to claim 18, or pharmaceutically acceptable salts, or solvates, thereof wherein p is an integer from 1 to 2.

21. A pharmaceutical composition comprising a compound of any of claims 1 to 20, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier.

22. Use of a compound of any of claims 1 to 20, or a pharmaceutically acceptable salt or solvate thereof, or of the pharmaceutical composition of claim 21, in the manufacture of a medicament for the treatment or prevention of cancers or tumors.

23. Use of a compound of claims 1-20, or a pharmaceutically acceptable salt or solvate thereof, or a pharmaceutical composition comprising said compound, salt or solvate thereof in the manufacture of a medicament for treating or preventing a cancer selected from breast cancer, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, kidney cancer, urethral cancer, bladder cancer, liver cancer, stomach cancer, endometrial cancer, salivary gland cancer, esophageal cancer, melanoma, glioma, neuroblastoma, sarcoma, lung cancer (for example, small cell lung cancer and non-small cell lung cancer) colon cancer, rectal cancer, colorectal cancer, leukemia, acute promyelocytic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia), bone cancer, skin cancer, thyroid cancer, pancreatic cancer, and lymphoma (for example, Hodgkin's lymphoma, non-Hodgkin's lymphoma, or recurrent anaplastic large cell lymphoma).

24. A compound or pharmaceutical composition comprising the compound of claims 1-20, or a pharmaceutically acceptable salt or solvate thereof, or the pharmaceutical composition of claim 21 for use in therapy.