PEPTIDE CONJUGATES OF MICROTUBULE-TARGETING AGENTS AS THERAPEUTICS

FIELD OF THE INVENTION

The present invention relates to peptide conjugates of microtubule-targeting agents such as maytansinoid derivatives which are useful for the treatment of diseases such as cancer.

BACKGROUND OF THE INVENTION

Cancer is a group of diseases characterized by aberrant control of cell growth. The annual incidence of cancer is estimated to be in excess of 1.6 million in the United States alone. While surgery, radiation, chemotherapy, and hormones are used to treat cancer, it remains the second leading cause of death in the U.S. It is estimated that about 600,000 Americans will die from cancer each year.

Treatment of cancer in humans by systemic administration of pharmaceutical agents often functions by slowing or terminating the uncontrolled replication that is a characteristic of cancer cells. One class of such agents is microtubule-targeting agents. Cell division requires formation of an intact mitotic spindle apparatus, composed of microtubules undergoing random length changes. The random length changes of microtubules is referred to as dynamic instability. Disruption of the dynamic instability of microtubules can lead to the suppression of further cell division. Drugs that target microtubules to suppress dynamic instability are currently used in the clinic as effective anticancer agents for a wide variety of cancers. See Lopus, M, Cancer Lett., 2011, 307(2): 113-118.

The maytansinoids, (e.g., mertansine, DM1, or DM4) are a class of microtubule-targeting agents that have emerged as potential clinical chemotherapeutics. See Lopus, M, Cancer Lett., 2011, 307(2): 113-118; and Widdison, W., J. Med. Chem. 2006, 49:4392-4408 Although DM1 has been shown to be effective in the treatment of several types of cancer, including lymphoma and breast cancer, toxic side effects such as peripheral neuropathy have hindered the clinical development of tubulin-targeting agents such as the maytansinoids. Preferential delivery of maytansinoid compounds, such as DM1, to diseased tissues could avoid these serious side effects. Thus, there is a need for more selective delivery of maytansinoid compounds to diseased tissue.

SUMMARY

The present disclosure provides, *inter alia*, a compound of Formula (I):

$$R^2—L—R^1 \quad (I)$$

or a pharmaceutically acceptable salt thereof, wherein constituent variables are defined herein.

The present disclosure further provides a pharmaceutical composition comprising a compound of the disclosure, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier or excipient.

The present disclosure also provides methods of treating a disease or condition (e.g., cancer) by administering to a human or other mammal in need of such treatment a therapeutically effective amount of a compound of the disclosure. In some embodiments, the disease or condition is characterized by acidic or hypoxic diseased tissues.

The present disclosure also provides use of a compound described herein in the manufacture of a medicament for use in therapy. The present disclosure also provides the compounds described herein for use in therapy.

The present disclosure also provides methods for synthesizing the compounds of the disclosure and intermediates useful in these methods.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a plot of the effect of free DM4 and Compound 5 on in vitro β-tubulin polymerization (in terms of relative fluorescence units) at various concentrations.

FIG. 2 depicts the kinetic analysis of Compound 5 binding to β-tubulin in vitro as determined by Biacore surface plasmon resonance.

FIG. 3A shows a plot of the mean tumor volume in nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5.

FIG. 3B shows the percent change in body weight of nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5 relative to day 0.

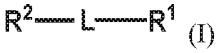
FIG. 4 depicts a Kaplan-Meier plot of nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5.

FIG. 5A depicts the ventral view and extracted lungs of nude mice inoculated with 4T1-RFP fluorescent cells via tail vein injection and imaged 11 days after inoculation and after 3 doses of vehicle or Compound 6.

FIG. 5B depicts a graph of the fluorescent signal from extracted lungs of 4T1-RFP inoculated mice after 3 doses of vehicle or Compound 6.

DETAILED DESCRIPTION

Provided herein is a compound of Formula (I):



(I)

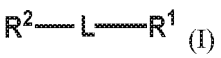
or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide;

R^2 is a small molecule microtubule targeting moiety; and

L is a linker, which is covalently linked to moiety R^1 and R^2.

Provided herein is a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide having 5 to 50 amino acids;

R^2 is a small molecule microtubule targeting moiety; and

L is a linker, which is covalently linked to moiety R^1 and R^2.

Also provided herein is a compound of Formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide capable of selectively delivering R^2L- across a cell membrane having an acidic or hypoxic mantle;

R^2 is a small molecule microtubule targeting moiety; and

L is a linker, which is covalently linked to moiety R^1 and R^2.

In some embodiments, R^2 is a maytansine-derived microtubule targeting moiety.

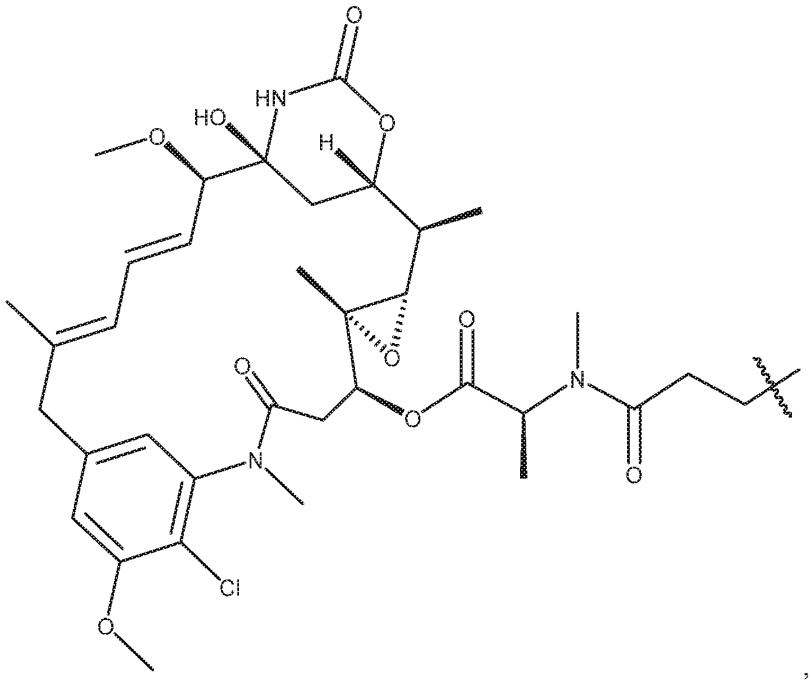
Provided herein is a compound of Formula (I):

$$R^2—L—R^1 \quad (I)$$

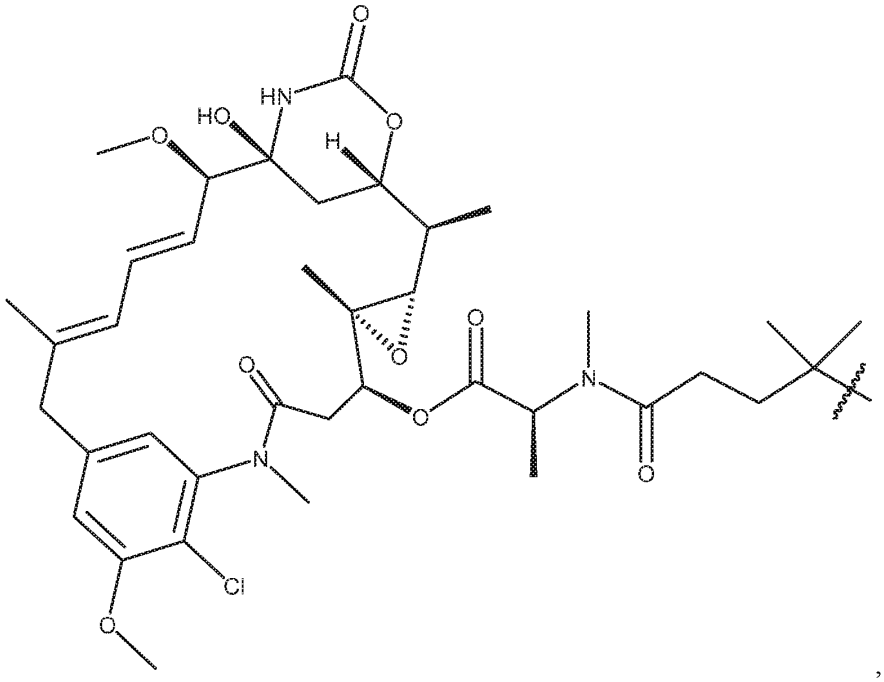
or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide capable of selectively delivering R^2L- across a cell membrane having an acidic or hypoxic mantle;

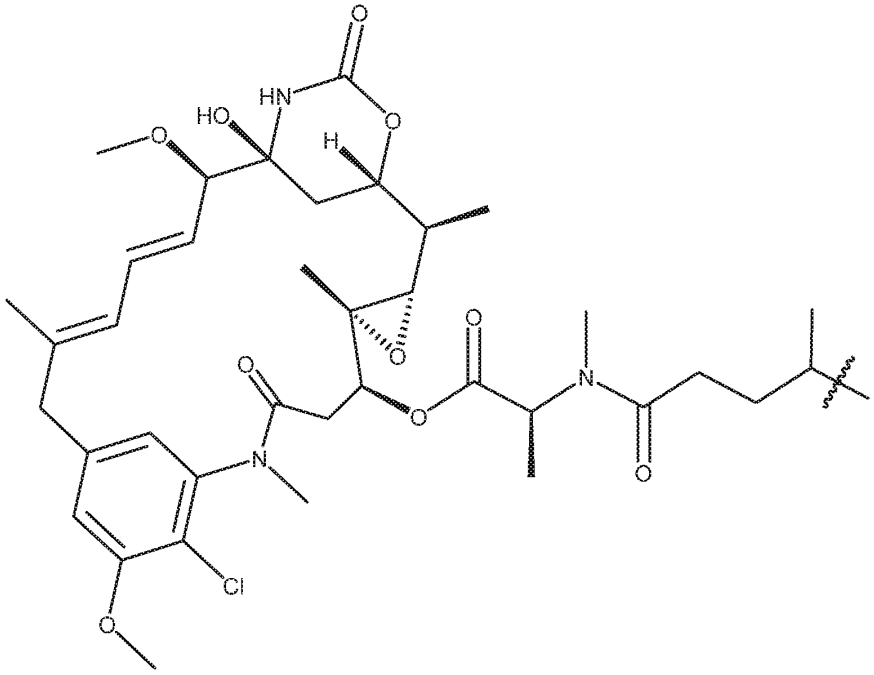
R^2 is selected from the group consisting of:



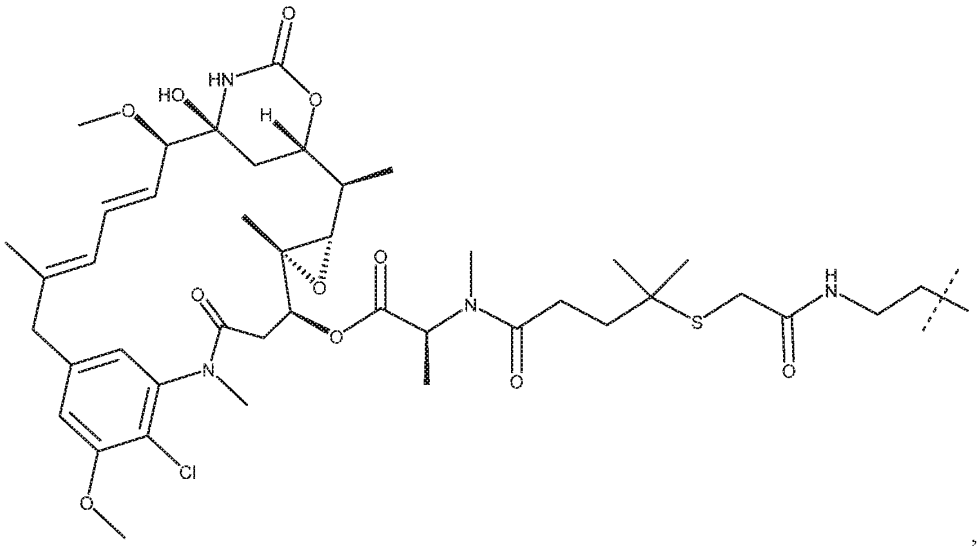
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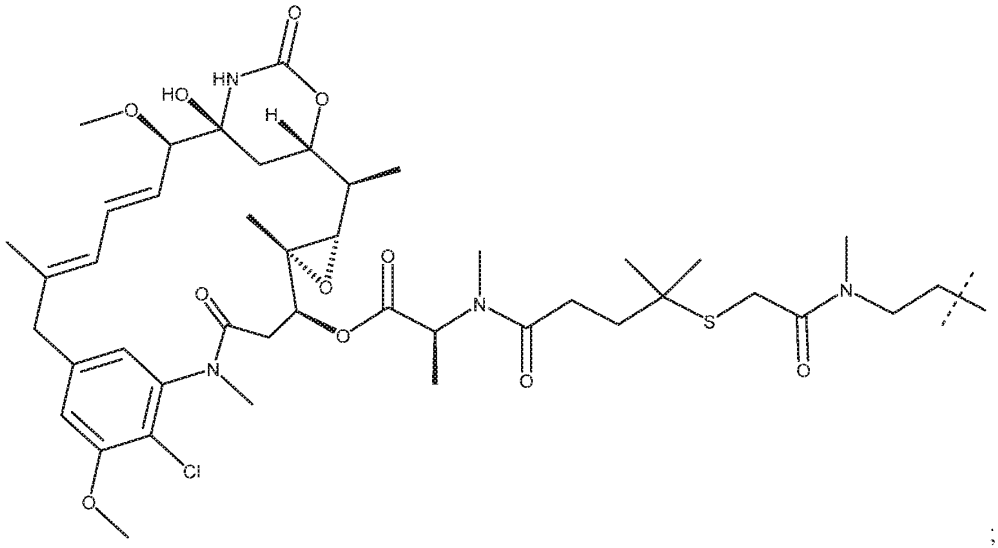
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; and

L is a linker, which is covalently linked to moiety R^1 and R^2.

Provided herein is a compound of Formula (I):

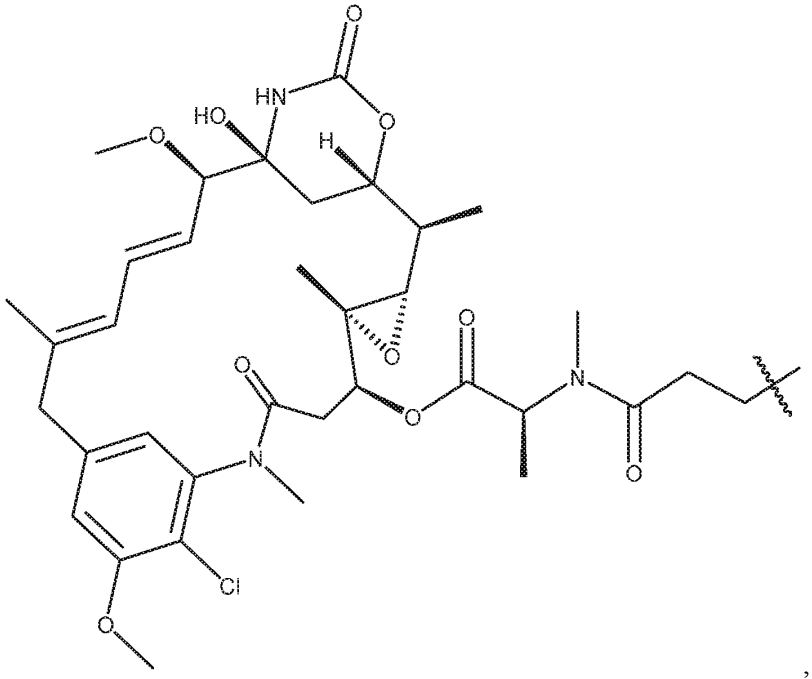


(I)

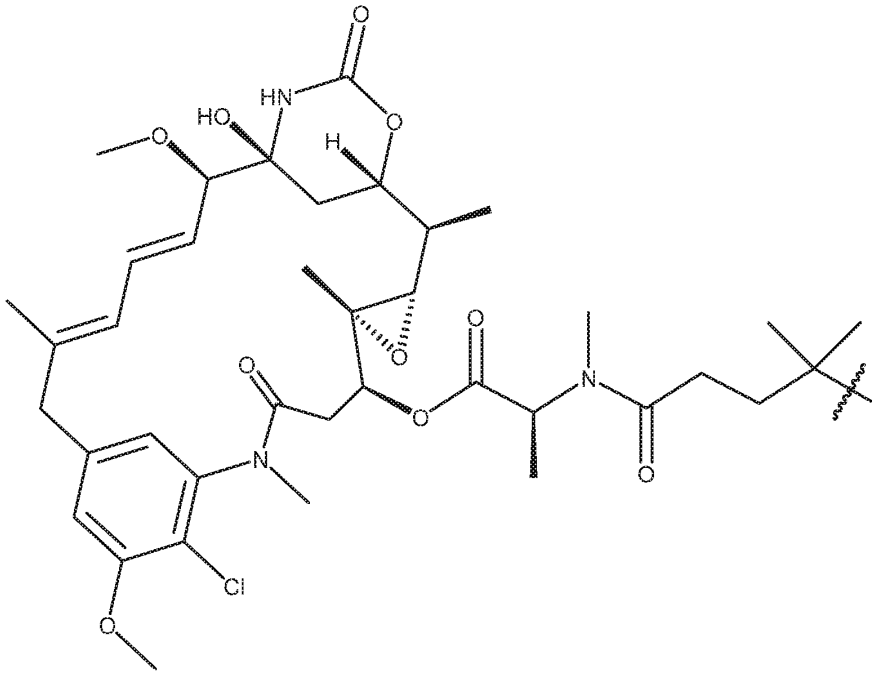
or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide capable of selectively delivering R^2L- across a cell membrane having an acidic or hypoxic mantle;

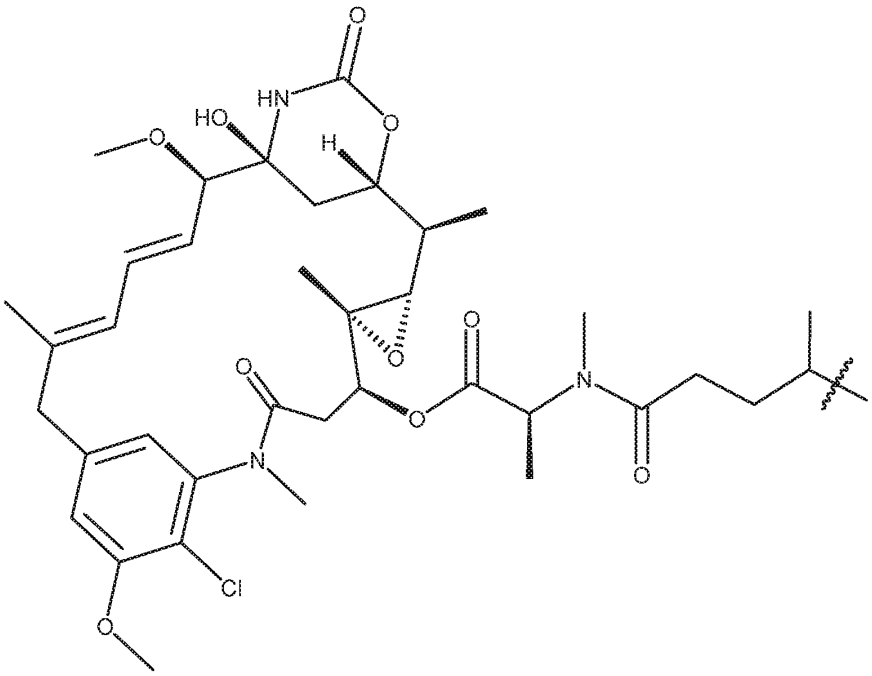
R^2 is selected from the group consisting of:



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, and



; and

L is a linker, which is covalently linked to moiety R^1 and R^2.

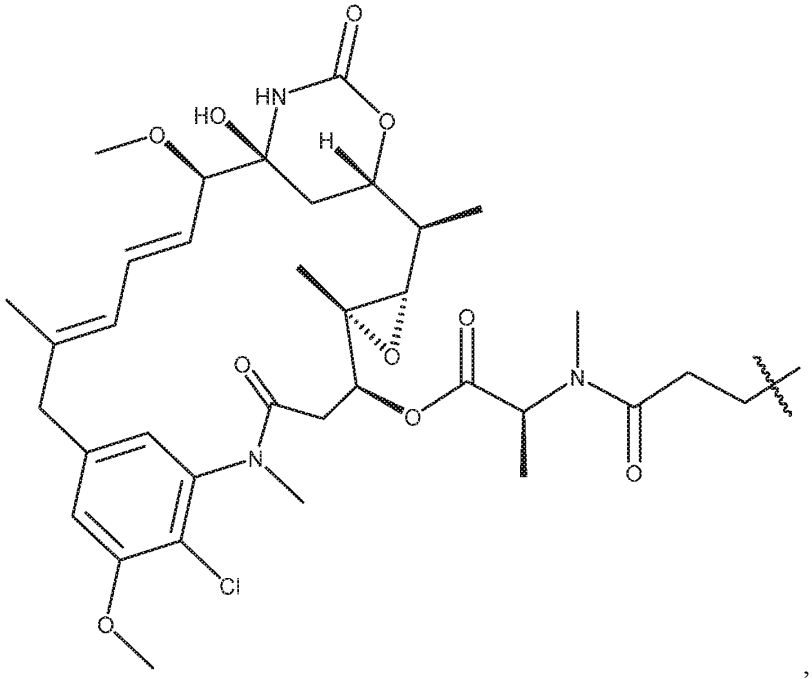
Provided herein is a compound of Formula (I):

$$R^2—L—R^1 \quad (I)$$

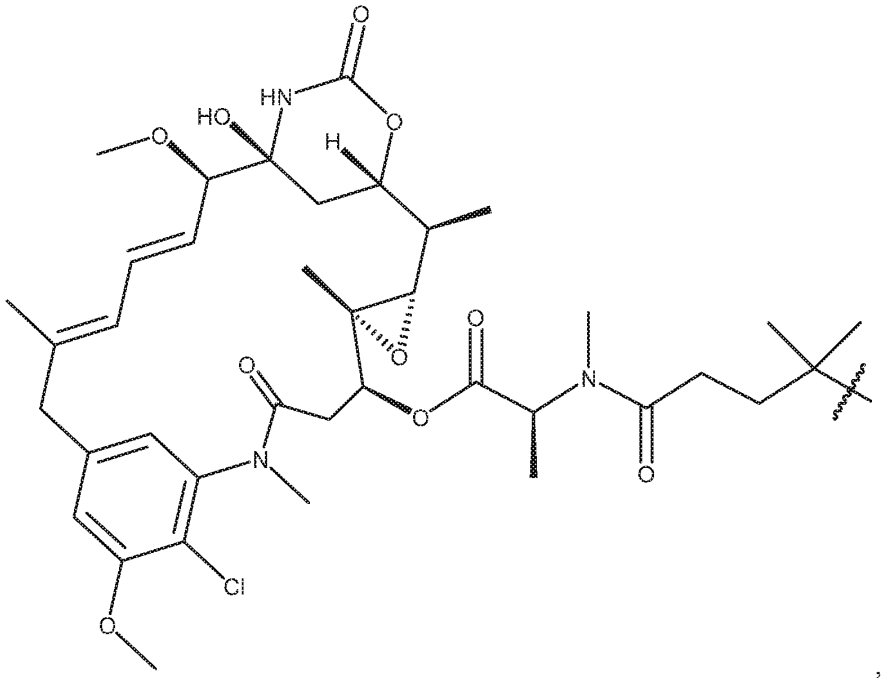
or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide capable of selectively delivering R^2L- across a cell membrane having an acidic or hypoxic mantle;

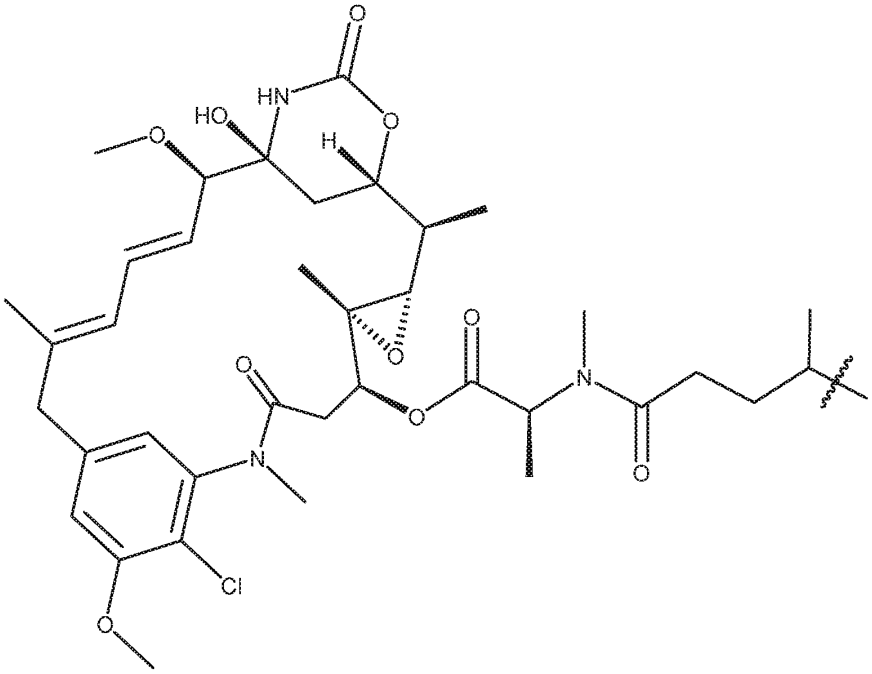
R^2 is selected from the group consisting of:



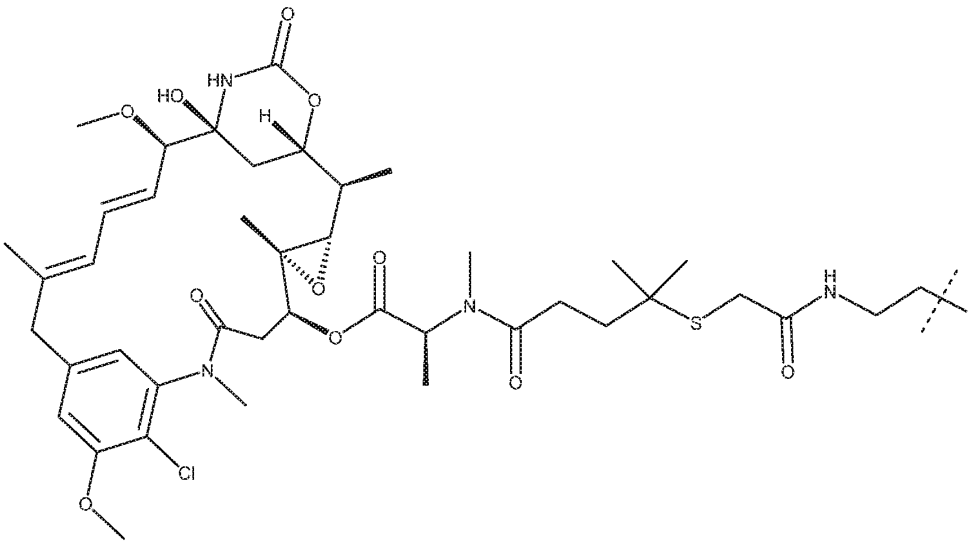
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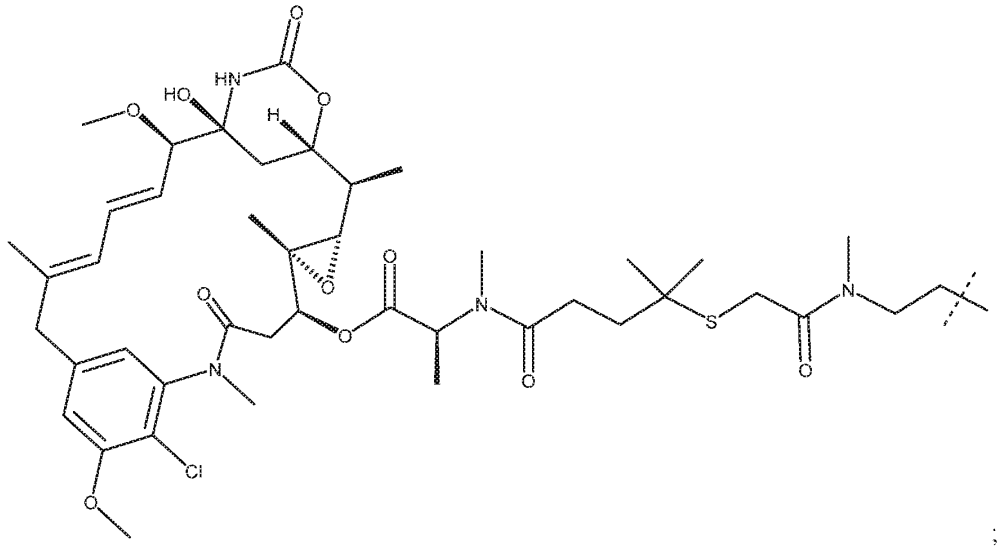
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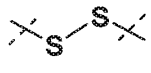


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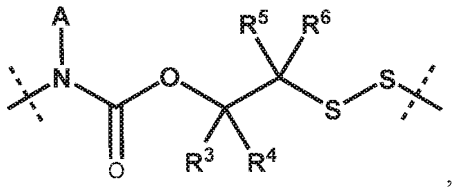


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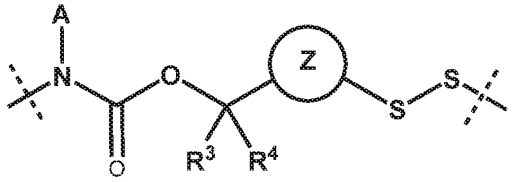
L is a group selected from:



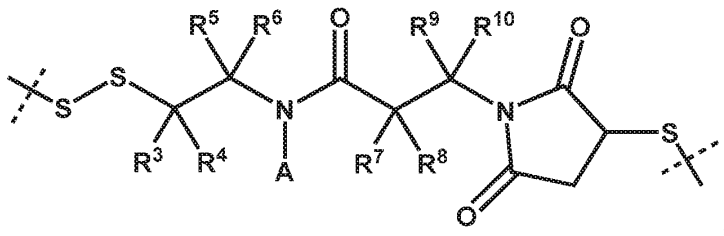
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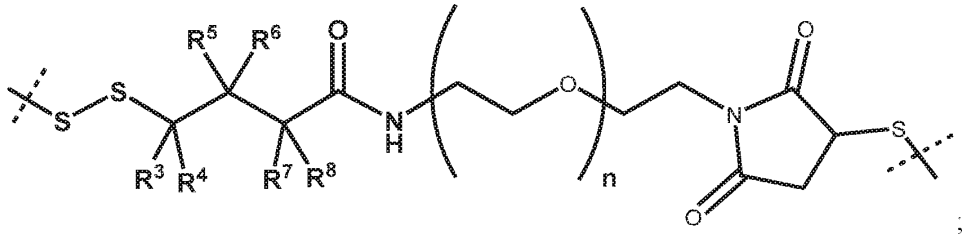
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wherein

R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are each independently selected from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, C_{3-10} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$, wherein said C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, and 5-10 membered heteroaryl are each optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^3 and R^4 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^3 and R^5 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^4 and R^6 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1},

$C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^5 and R^6 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^7 and R^8 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^7 and R^9 together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^8 and R^{10} together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^9 and R^{10} together with the carbon atoms to which they are attached form a C_{3-14} cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

Z is C_{6-10} aryl or 5-10 membered heteroaryl; wherein the 5-10 membered heteroaryl has at least one ring-forming carbon atom and 1, 2, 3, or 4 ring-forming heteroatoms independently selected from N, O, and S, wherein the C_{6-10} aryl and 5-10 membered heteroaryl are each

optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

A is H or C_{1-4} alkyl;

R^{a1}, R^{b1}, R^{c1}, and R^{d1} are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OH, CN, NO_2, and CO_2CH_3; wherein said C_{1-6} alkyl and C_{2-6} alkenyl are each optionally substituted with OH, CN, NO_2, or CO_2CH; and

n is 0, 1, or 2.

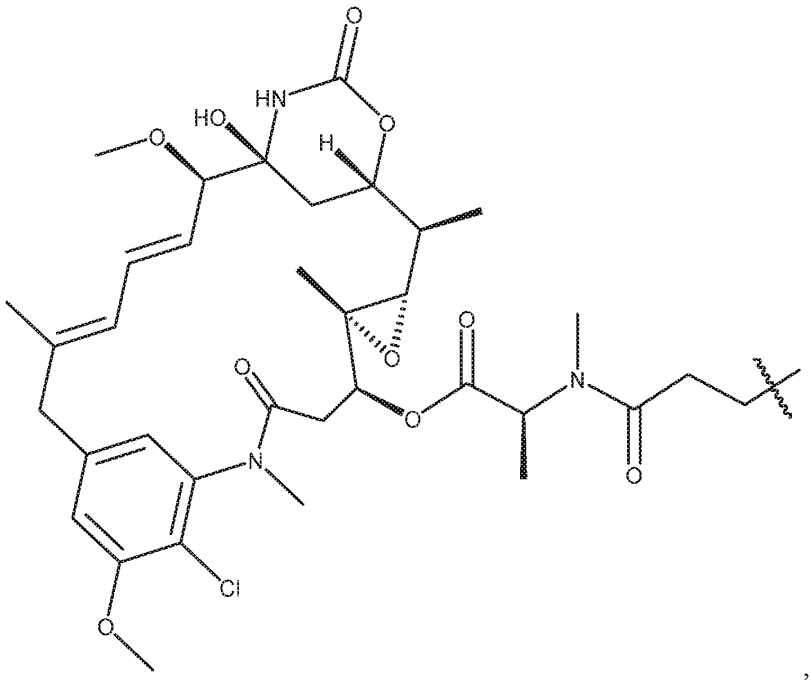
Provided herein is a compound of Formula (I):

$$R^2—L—R^1 \quad (I)$$

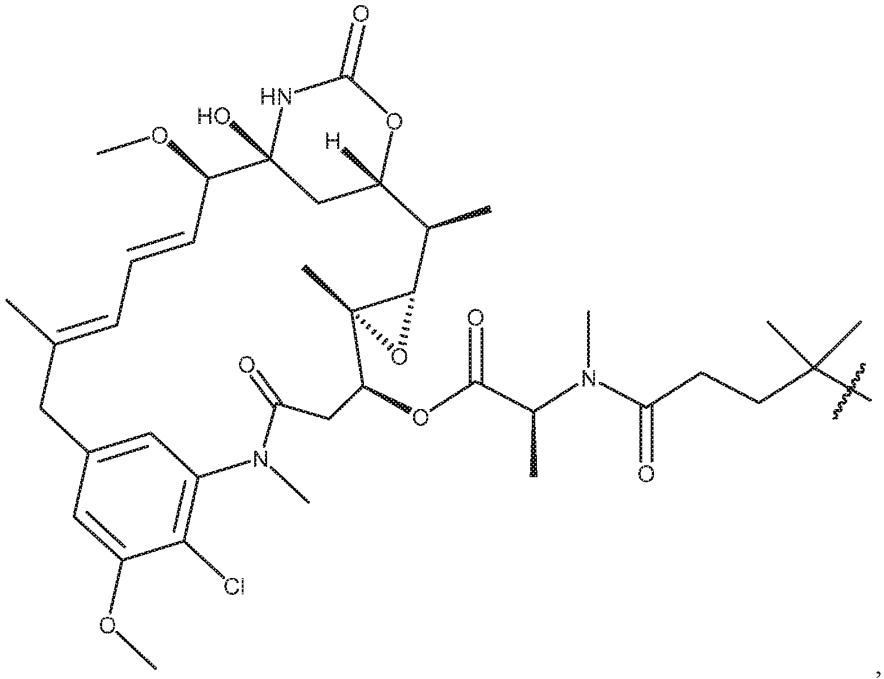
or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide capable of selectively delivering R^2L- across a cell membrane having an acidic or hypoxic mantle;

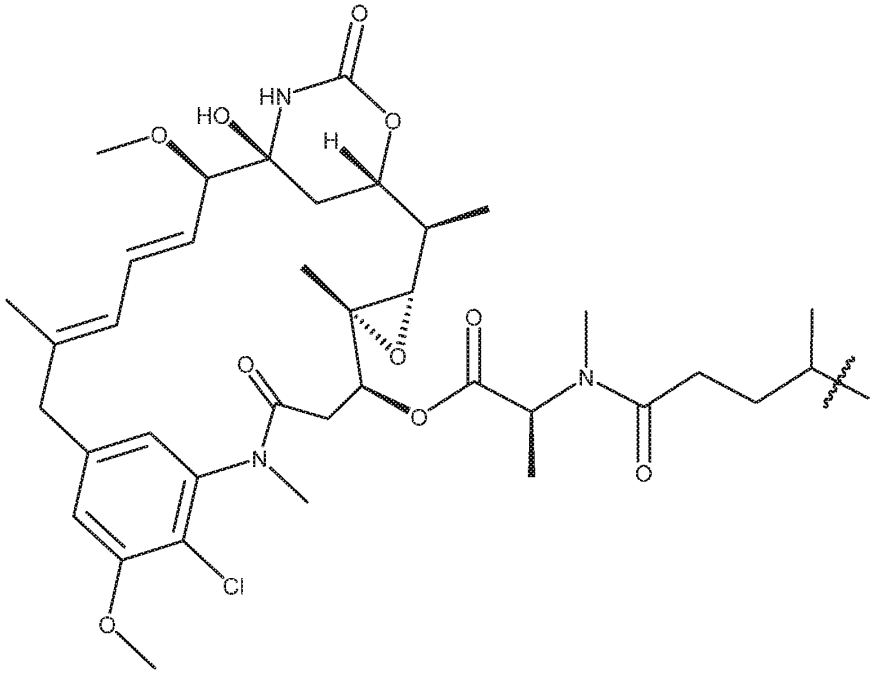
R^2 is selected from the group consisting of:



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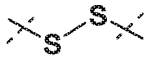


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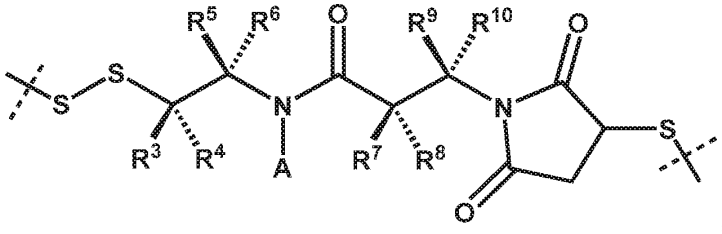


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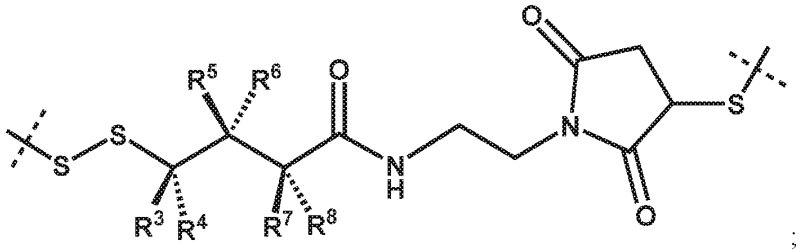
L is a group selected from:



,



, and



; wherein

R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are each independently selected from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, 5-10 membered heteroaryl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$, wherein said C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, and 5-10 membered heteroaryl are each optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^3 and R^4 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^3 and R^5 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^4 and R^6 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^5 and R^6 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^7 and R^8 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^7 and R^9 together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^8 and R^{10} together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or R^9 and R^{10} together with the carbon atom to which they are attached form an C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

A is H or C_{1-4} alkyl; and

R^{a1}, R^{b1}, R^{c1}, and R^{d1} are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, OH, CN, NO_2, and CO_2CH_3; wherein said C_{1-6} alkyl and C_{2-6} alkenyl are each optionally substituted with OH, CN, NO_2, or CO_2CH.

In some embodiments, the lefthand side of L attaches to R^2 and the righthand side of L attaches to R^1.

In some embodiments, a sulfur atom of the disulfide moiety of L is part of a cysteine residue of R^1.

As used herein, "peptide" refers to a targeting moiety comprising a 10-50 amino acid sequence, made up of naturally-occurring amino acid residues and optionally one or more non-naturally-occurring amino acids. In some embodiments, the peptide of R^1 is a peptide of 20 to 40, 20 to 30 amino acids, or 30 to 40 residues. Peptides suitable for use in the compounds of the invention are those that can insert across a cell membrane via a conformational change or a

change in secondary structure in response to environmental pH changes. In this way, the peptide can target acidic tissue and selectively translocate polar, cell-impermeable molecules across cell membranes in response to low extracellular pH. In some embodiments, the peptide is capable of selectively delivering a conjugated moiety (e.g., R^2L-) across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.0. In some embodiments, the peptide is capable of selectively delivering a conjugated moiety (e.g., R^2L-) across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.5. In some embodiments, the peptide is capable of selectively delivering a conjugated moiety (e.g., R^2L-) across a cell membrane having an acidic or hypoxic mantle having a pH less than about 5.5. In some embodiments, the peptide is capable of selectively delivering a conjugated moiety (e.g., R^2L-) across a cell membrane having an acidic or hypoxic mantle having a pH between about 5.0 and about 6.0.

In certain embodiments, the peptide of R^1 includes a cysteine residue which can form the site of attachment to a payload moiety (e.g., R^2L-) to be delivered across a cell membrane. In some embodiments, R^1 is attached to L through a cysteine residue of R^1. In some embodiments, the sulfur atom of the cysteine residue can form part of the disulfide bond of the disulfide bond-containing linker L.

Suitable peptides, that can conformationally change based on pH and insert across a cell membrane, are described, for example, in United States patents 8,076,451 and 9,289,508 (each of which is incorporated herein by reference in its entirety). Other suitable peptides are described, for example, in Weerakkody, *et al.*, PNAS 110 (15), 5834-5839 (April 9, 2013), which is also incorporated herein by reference in its entirety.

In some embodiments, R^1 is a peptide comprising at least one of the following sequences:

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1),

AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2), and

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3);

Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG (SEQ ID NO. 4; Pv4);

AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID No. 5; Pv5); and

AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG (SEQ ID No. 6; Pv6);

wherein R^1 is attached to L through a cysteine residue of R^1.

In some embodiments, $R^1$is a peptide comprising at least one of the following sequences: ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1), AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2), and ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3); and AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG (SEQ ID No. 6; Pv6); wherein R^1 is attached to L through a cysteine residue of R^1.

In some embodiments, R^1 is a peptide comprising the sequence ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1).

In some embodiments, R^1 is a peptide comprising the sequence AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2).

In some embodiments, R^1 is a peptide comprising the sequence ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3).

In some embodiments, R^1 is a peptide comprising the sequence Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG (SEQ ID NO. 4; Pv4).

In some embodiments, R^1 is a peptide comprising the sequence AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID NO. 5; Pv5).

In some embodiments, R^1 is a peptide comprising the sequence AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG (SEQ ID NO. 6; Pv6).

In some embodiments, R^1 is a peptide consisting of the sequence ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1).

In some embodiments, R^1 is a peptide consisting of the sequence AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2).

In some embodiments, R^1 is a peptide consisting of the sequence ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3).

In some embodiments, R^1 is a peptide consisting of the sequence Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG (SEQ ID NO. 4; Pv4).

In some embodiments, R^1 is a peptide consisting of the sequence AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID NO. 5; Pv5).

In some embodiments, R^1 is a peptide consisting of the sequence AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG (SEQ ID NO. 6; Pv6).

In some embodiments, R^1 is a peptide comprising at least one sequence selected from SEQ ID NO: 7 to SEQ ID NO: 311 as shown in Table 1.

In some embodiments, R^1 is a peptide consisting of a sequence selected from SEQ ID NO: 7 to SEQ ID NO: 311 as shown in Table 1.

Table 1. Additional R^1 Sequences

SEQ ID NO.	Sequence
7	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
8	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
9	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
10	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
11	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
12	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
13	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
14	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
15	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
16	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADECT
17	ACEQNPIYWARYANWLFTTPLLLLNLALLVDADEGTG
18	ACEQNPIYWARYAKWLFTTPLLLLKLALLVDADEGTG
19	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
20	AAEQNPIYWARYADWLFTTPLLLLALALLVDADEGT
21	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGT
22	AAEQNPIYWARYADWLFTTALLLLDLALLVDADEGT
23	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGT
24	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGT
25	AAEQNPIIYWARYADWLFTDLPLLLLDLLALLVDADEGT
26	GEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
27	GGEQNPIYWARYADWLFTTPLLLDLLALLVDADEGTCG
28	GGEQNPIYWARYADWLFTTPLLLLLDALLVDADEGTCG

29	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
30	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCG
31	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
32	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
33	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADEGCT
34	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
35	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
36	AEQNPIYWARYADFLFTTPLLLLDLALLVDADET
37	AEQNPIYFARYADWLFTTPLLLLDLALLVDADEGT
38	AEQNPIYFARYADFLFTTPLLLLDLALLWDADET
39	AKEDQNPYWARYADWLFTTPLLLLDLALLVDG
40	ACEDQNPYWARYADWLFTTPLLLLDLALLVDG
41	AEDQNPYWARYADWLFTTPLLLLDLALLVDCG
42	AEDQNPYWARYADWLFTTPLLLLELALLVECG
43	AKEDQNPYWRAYADLFTPLTLLDLLALWDG
44	ACEDQNPYWRAYADLFTPLTLLDLLALWDG
45	ACDDQNPWRAYLDLLFPTDTLLLDLLW
46	TEDADVLLALDLLLLPTTFLWD
47	AEQNPIYWARYADWLFTTPL
48	AEQNPIYWARYADWLFTTPCL
49	ACEQNPIYWARYADWLFTTPL
50	AEQNPIYFARYADWLFTTPL
51	KEDQNPWARYADLLFPTTLAW
52	ACEDQNPWARYADLLFPTTLAW
53	ACEDQNPWARYADWLFPTTLLLLD
54	ACEEQNPWARYAELLFPTTLAW
55	ACEEQNPWARYAEWLFPTTLLLLE
56	ACEEQNPWARYLEWLFPTETLLLEL
57	GGEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT
58	ACEQNPIY WARYADWLFTTPLLLLDLALLV
59	WARYADWLFTTPLLLLDLALLV DADEGTCG
60	WARYADWLFTTPLLLLDLALLV DADEGCT
61	GGEQNPIY WARYADWLFTTPLLLLDLALLV DADEGTCG

62	ACEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT
63	AKEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT
64	AKEQNPIY WARYADWLFTTPLLLLDLALLV DADECT
65	AAEQNPIY WARYADWLFTTALLLLDLALLV DADEGT
66	ACAEQNPIY WARYADWLFTTGLLLLDLALLV DADEGT
67	AEQNPIY WARYADFLFTTALLLLDLALLV DADE_T
68	AEQNPIY FARYADWLFTTPLLLLDLALLV DADEGT
69	AEQNPIY FARYADFLFTTPLLLLDLALLW DADE_T
70	AKEDQNP_Y WARYADWLFTTPLLLLDLALLV DG_____
71	ACEDQNP_Y WARYADWLFTTPLLLLDLALLV DG_____
72	AEDQNP_Y WARYADWLFTTPLLLLDLALLV DG_____
73	AEDQNP_Y WARYADWLFTTPLLLLELALLV ECG_____
74	AKEDQNP_Y WRAYAD_LFT_PLTLLDLLALW DG_____
75	ACEDQNP_Y WRAYAD_LFT_PLTLLDLLALW DG_____
76	AKEDQNDP_Y WARYADWLFTTPLLLLDLALLV G_____
77	TEDADVLLALDLLLLPTTFLWDAYRAWYPNQECA
78	GGEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT
79	AEQNPIY WARYADWLFTTPL
80	AEQNPIY WARYADWLFTTPCL
81	ACEQNPIY WARYADWLFTTPL
82	ACEQNPIY FARYADWLFTTPL
83	ACDDQNP WRAYLDLLFPTDTLLLDLLW
84	ACEEQNP WRAYLELLFPTETLLLELLW
85	ACDDQNP WARYLDWLFPTDTLLLDL
86	CDNNNP WRAYLDLLFPTDTLLLDW
87	ACEEQNP WARYLEWLFPTETLLLEL
88	ACEDQNP WARYADWLFPTTLLLLD
89	ACEEQNP WARYAEWLFPTTLLLLE
90	ACEDQNP WARYADLLFPTTLAW
91	ACEDQNP WARYAELLFPTTLW
92	KEDQNP WARYADLLFPTTLW
93	DDDEDNP IYWARYAHWLFTTPLLLLHGALLVDADECT
94	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT

95	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
96	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
97	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
98	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGIG
99	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
100	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
101	GGEQNPIYWARYADWLFTTPLLLDLLALLVDADEGTCG
102	GGEQNPIYWARYADWLFTTPLLLLLDALLVDADEGTCG
103	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCG
104	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGTCG
105	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
106	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
107	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
108	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
109	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
110	ACEQNPIYWARYANWLFTTPLLLLNLALLVDADEGTG
111	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
112	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
113	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
114	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADECT
115	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT
116	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
117	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCG
118	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
119	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
120	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
121	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
122	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
123	GGEQNPIYWARYADWLFTTPLLLLDALLVNANQGT
124	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
125	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
126	ACEQNPIYWARYAKWLFTTPLLLLKLALLVDADEGTG
127	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG

128	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
129	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
130	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
131	AAEQNPIYWARYADWLFTDLPLLLLDLLALLVDADEGT
132	GGEQNPIYWARYADWLFTTPLLLLLDALLVDADEGTCG
133	GGEQNPIYWARYADWLFTTPLLLDLLALLVDADEGTCG
134	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
135	AEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
136	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
137	GGEQNPIYWAQDYAWLFTTPLLLLDLALLDADEGTCG
138	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
139	AAEQNPIYWARYADWLFTTPLLLLALALLVDADEGTCG
140	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG .....EGTK(rhodamine)C(phalloidin)G
141	AAEQNPIYWARYADWLFTTPLLLLELALLDADEGTKCG
142	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
143	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
144	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
145	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
146	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
147	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
148	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
149	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
150	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
151	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
152	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
153	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
154	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
155	DDDEDNPIYWARYAHWLFTTPLLLLBGALLVDADECT
156	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT
157	DDDEDNPIYWARYAHWLFTTPLLLLBGALLVNADECT
158	DDDEDNPIYWARYAHWLFTTPLLLLBGALLVNANECT
159	DDDEDNPIYWARYADWLFTTPLLLLIBGALLVDADECT
160	DDDEDNPIYWARYADWTFTTPLLLLHGALLVDADECT

161	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT
162	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADECT
163	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
164	DDDEDNPIYWARYHWLFTTPLLLLHGALLVNANECT
165	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
166	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
167	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
168	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADECT
169	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT
170	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
171	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
172	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
173	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADECT
174	DDDEDNPIYWARYAHMLFTTPLLLLDGALLVDADECT
175	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
176	DDDEDNPIYWARYAHWLFTTPLLLLDGALLVDADECT
177	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
178	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADECT
179	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADECT
180	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANECT
181	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
182	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCG
183	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
184	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
185	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
186	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
187	GGEQNPIYWARYADWLFTTPLLLLDALLVDADEGTCG
188	GGEQNPIYWARYADWLFTTPLLLDLLALLVDADEGTCG
189	GGEQNPIYWARYADWLFTTPLLLDLLALLVDADEGTCG
190	GGEQNPIYWARYADWLFTTPLLLLLDALLVDADEGTCG
191	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
192	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
193	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG

194	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
195	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
196	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
197	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
198	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
199	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
200	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
201	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
202	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
203	.....EGTK(rhidamine)C(phalloidin)G
204	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
205	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
206	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
207	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
208	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
209	AAEQNPIYWARYADWLFTDLPLLLLDLLALLVDADEGT
210	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
211	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
212	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
213	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
214	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGTCG
215	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
216	AAEQNPIYWARYADWLFTTPLLLLALALLVDADEGTCG
217	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCG
218	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
219	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
220	ACEQNPIYWARYAKWLFTTPLLLLKLALLVDADEGTG
221	ACEQNPIYWARYANWLFTTPLLLLNLALLVDADEGTG
222	AAEQNPIYWARYADWLFTTALLLLDLALLVDADEGT
223	AEQNPIYFARYADLLFPTTLAW
224	AEQNPIYWARYADLLFPTTLAF
225	AEQNPIYWARYADLLFPTTLAW
226	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET

227	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
228	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
229	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
230	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADECT
231	CCTCTTACCTCAGTTACA
232	D-Arg8 D-Arg8-CCTCTTACCTCAGTTACA
233	D-Lys4 D-Lys4-CCTCTTACCTCAGTTACA
234	S-S-CCTCTTACCTCAGTTACA
235	S-S-CCTCTGACCTCATTTACA
236	D-Arg8-Deca D-Arg8-Deca-CCTCTTACCTCAGTTACA
237	D-Arg8-Deca-mismatch D-Arg8-Deca-CCTCTGACCTCATTTACA
238	S-S-CCTCTTACCTCAGTTACA
239	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
240	AEDQNPYWARYDWLFTTPLLLLDLALLVDCG
241	AEDQNPYWARYADWLFTTPLLLLELALLVECG
242	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGCT
243	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
244	AE-QN-PI YWARYADWLFTTPLLLLDLALLV DADEGT-COOH
245	AEDQN-P- YWARYADWLFTTPLLLLDLALLV D---G--COOH
246	AEDQNDP-YWARYADWLFTTPLLLLDLALLV-----G--COOH
247	AEQNPI YWARYADFLFTTPLLLLDLALLV DADET-COOH
248	AEQNPI YFARYADWLFTTPLLLLDLALLV DADET-COOH
249	AEQNPI YFARYADFLFTTPLLLLDLALLW DADET-COOH
250	AE-QN-PI YWARYADWLFTTPLLLLDLALLV DADEGCT-COOH
251	AEDQN-PI YWARYADWLFTTPLLLLDLALLV DC--G-T-COOH
252	AEDQNDPI YWARYADWLFTTPLLLLELALLV EC--G-T-COOH
253	Chelate-ACEEQNPWARYLEWLFPTETLLLEL
254	AEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT-COOH
255	AKEDQNPY WARYADWLFTTPLLLLDLALLV DG-COOH
256	AKEDQNDPY WARYADWLFTTPLLLLDLALLV G-COOH
257	AEQNPI YWARYADWLFTTPLLLLDLALLV DADEGC-Biotin-T-COO H
258	AEDQNP YWARYADWLFTTPLLLLDLALLV DC-Biotin-G-COOH
259	AEDQNP YWARYADWLFTTPLLLLELALLV EC-Biotin-G-COOH

260	ACEQNPIY WARYADWLFTTPLLLLDLALLV DADEGT
261	ACEDQNPY WARYADWLFTTPLLLLDLALLV DG
262	ACEDQNPY WRAYADLFTPLTLLDLLALW DG
263	ACDDQNP WRAYLDLLFPTDTLLLDLLW
264	WRAYLELLFPTETLLLELLW
265	WARYLDWLFPTDTLLLDL
266	WRAYLDLLFPTDTLLLDW
267	WARYLEWLFPTETLLLEL
268	WAQYLELLFPTETLLLEW
269	WRAYLELLFPTETLLLEW
270	WARYADWLFPTTLLLLD
271	WARYAEWLFPTTLLLLE
272	ACEDQNP WARYADLLFPTTLAW
273	ACEEQNP WARYAELLFPTTLAW
274	Ac-TEDAD VLLALDLLLLPTTFLWDAYRAW YPNQECA-Am
275	CDDDDDNPNY WARYANWLFTTPLLLLNGALLV EAEET
276	CDDDDDNPNY WARYAPWLFTTPLLLLPGALLV EAEET
277	Ac-AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGCT
278	Ac-AKEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
279	ACEQNPIYWARYANWLFTTPLLLLNLALLVDADEGT
280	Ac-AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
281	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADET
282	CDDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADET
283	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADEGT
284	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADEGT
285	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANEGT
286	AKEDQNDPYWARYADWLFTTPLLLLDLALLVG
287	AEDQNPYWARYADWLFTTPLLLLELALLVCG
288	AKDDQNPWRAYLDLLFPTDTLLLDLLWC
289	ACEEQNPWRAYLELLFPTETLLLELLW
290	ACDDQNPWARYLDWLFPTDTLLLDL
291	CDNNNPWRAYLDLLFPTDTLLLDW
292	CEEQQPWAQYLELLFPTETLLLEW

293	EEQQPWRAYLELLFPTETLLLEW
294	CDDDDDNPNYWARYANWLFTTPLLLLNGALLVEAEET
295	CDDDDDNPNYWARYAPWLFTTPLLLLPGALLVEAEE
296	AEQNPIYFARYADLLFPTTLAW
297	AEQNPIYWARYADLLFPTTLAF
298	AEQNPIYWARYADLLFPTTLAW
299	KEDQNPWARYADLLFPTTLW
300	ACEEQNPQAEYAEWLFPTTLLLLE
301	AAEEQNPWARYLEWLFPTETLLLEL
302	AKEEQNPWARYLEWLFPTETLLLEL
303	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
304	XXEXNPIYWAXXXXXLFTXXLLLXXXALLVXAXXXTXG
305	DAAEQNPIYWARYADWLFTTLPLLLLDLLALLVDADEGTKGG
306	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
307	XXEXNPIYWAXXXXXLFTXXLLLXXXALLVXAXXXTGG
308	DGGEQNDPIYWARYADWLFTTLPLLLLDLLALLVDADEGCTXGG
309	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
310	AEDQNPYWARYDWLFTTPLLLLDLALLVDCG
311	GLAGLAGLLGLEGLLGLPLGLLEGLWLGLELEGN

Any of the recited peptides useful in the present invention can be modified to include a cysteine residue by replacing a non-cysteine residue with cysteine, or appending a cysteine residue to either the N-terminus or C-terminus.

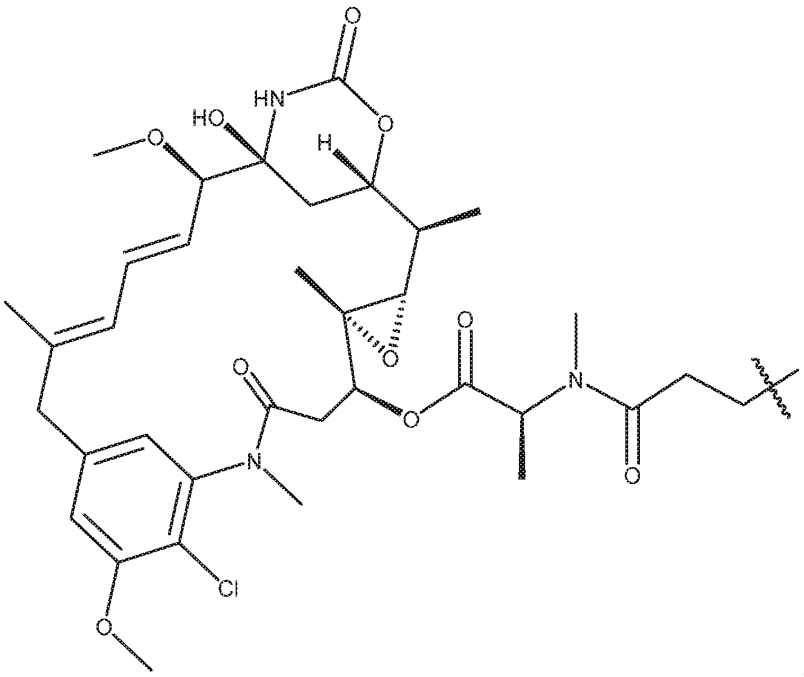
In some embodiments, the peptide of R^1 is a conformationally restricted peptide. A conformationally restricted peptide can include, for example, macrocyclic peptides and stapled peptides. A stapled peptide is a peptide constrained by a covalent linkage between two amino acid side-chains, forming a peptide macrocycle. Conformationally restricted peptides are described, for example, in Guerlavais et al., Annual Reports in Medicinal Chemistry 2014, 49, 331-345; Chang et al., Proceedings of the National Academy of Sciences of the United States of America (2013), 110(36), E3445-E3454; Tesauro et al., Molecules 2019, 24, 351-377; Dougherty et al., Journal of Medicinal Chemistry (2019), 62(22), 10098-10107; and Dougherty

et al., Chemical Reviews (2019), 119(17), 10241-10287, each of which is incorporated herein by reference in its entirety.

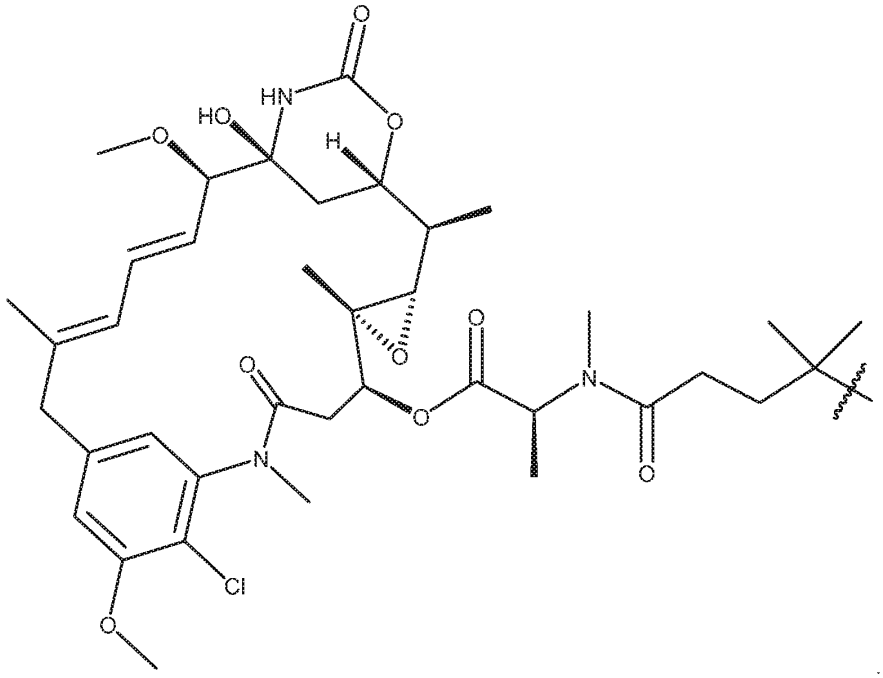
In some embodiments, R^1 is a peptide having 10 to 50 amino acids. In some embodiments, R^1 is a peptide having 20 to 40 amino acids. In some embodiments, R^1 is a peptide having 20 to 40 amino acids. In some embodiments, R^1 is a peptide having 10 to 20 amino acids. In some embodiments, R^1 is a peptide having 20 to 30 amino acids. In some embodiments, R^1 is a peptide having 30 to 40 amino acids.

Suitable small molecule microtubule targeting moieties (e.g., R^2) can be cytotoxic compounds like maytansinoids that may have undesirable side effects when delivered systemically because of their possible deleterious effect on normal tissue. Small molecule microtubule targeting agents include, but are not limited to, maytansinoids, aclitaxel, docetaxel, epothilones, discodermolide, the vinca alkaloids, colchicine, combretastatins, and derivatives and analogues of the aforementioned. Microtubule targeting agents are described in Tangutur, A. D., Current Topics in Medicinal Chemistry, 2017 17(22): 2523-2537. Microtubule-targeting agents also include maytansinoids, such as maytansine (DM1) and derivatives and analogues thereof, which are described in Lopus, M, Cancer Lett., 2011, 307(2): 113-118; and Widdison, W., J. Med. Chem. 2006, 49:4392-4408.

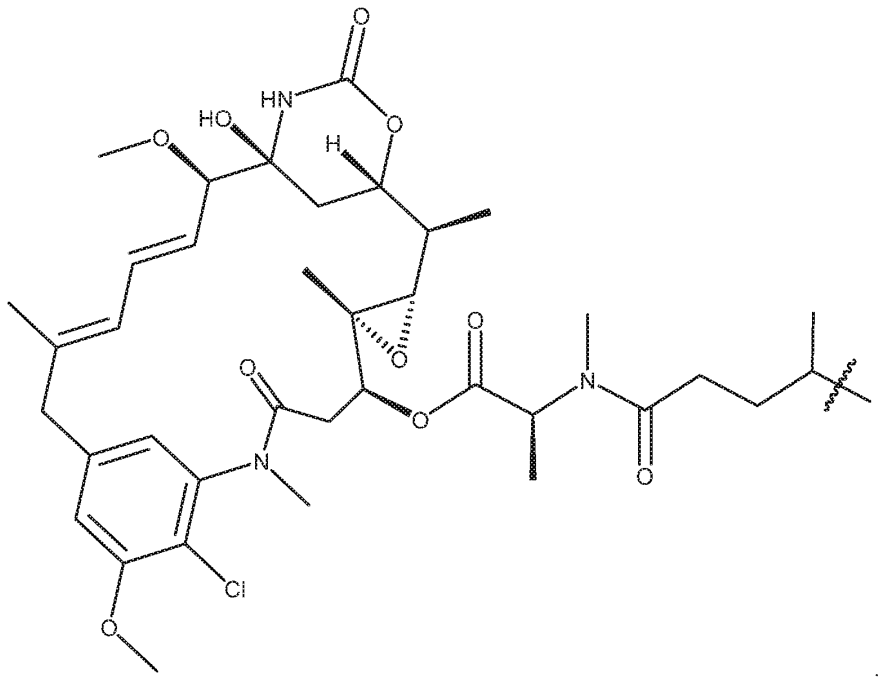
In some embodiments, R^2 is the following group:



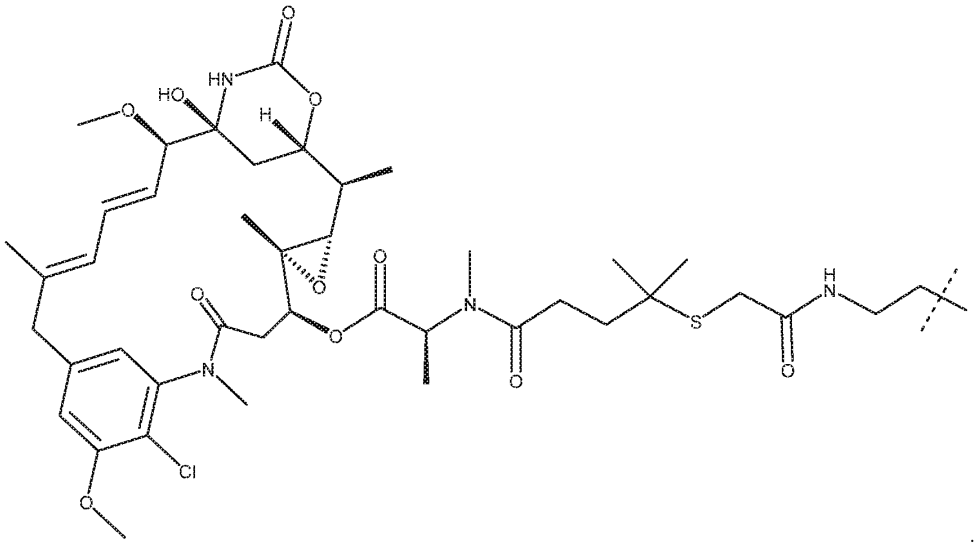
In some embodiments, R^2 is the following group:



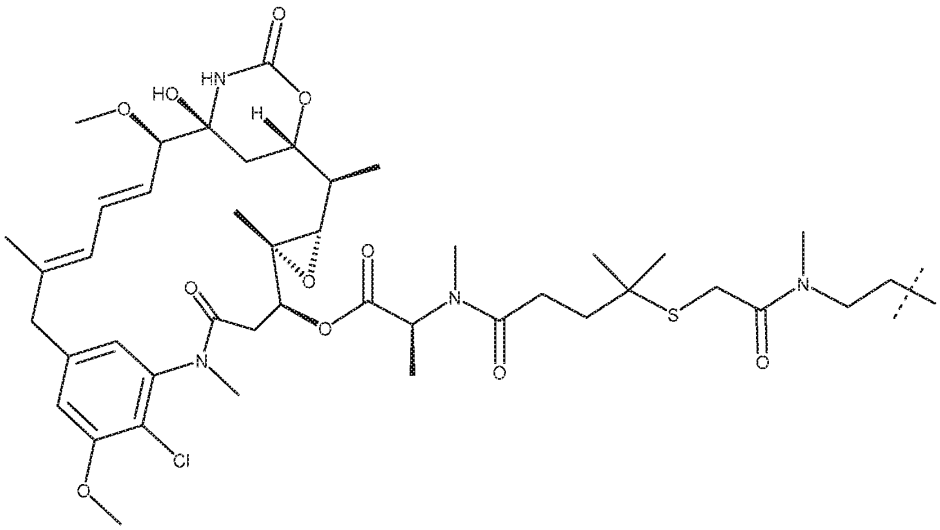
In some embodiments, R^2 is the following group:



In some embodiments, R^2 is the following group:



In some embodiments, R^2 is the following group:



In some embodiments, R^2 is a maytansinoid. In some embodiments, R^2 is DM1 or DM4. In some embodiments, R^2 is DM1. In some embodiments, R^2 is DM4.

In some embodiments, L is a linking moiety that covalently connects R^1 and R^2, and functions to release a moiety containing R^2 in the vicinity of acidic or hypoxic tissue, such as inside a cell of diseased tissue.

In some embodiments, L is a linking chain of 1 to 40, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 to 5 chain atoms (including both carbon and heteroatoms), which is optionally substituted with 1-10 R^q substituents, and wherein one or more chain carbon atoms of L can be oxidized to form a carbonyl (C=O), and wherein one or more N and S chain atoms can each be optionally oxidized to form an amine oxide, sulfoxide or sulfonyl group; wherein

each R^q is independently selected from OH, CN, -COOH, NH_2, halo, C_{1-6} haloalkyl, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkylthio, phenyl, 5-6 membered heteroaryl, 4-6 membered heterocycloalkyl, C_{3-6} cycloalkyl, $NH(C_{1-6}$ alkyl) and $N(C_{1-6}$ alkyl)$_2$, wherein the C_{1-6} alkyl, phenyl, C_{3-6} cycloalkyl, 4-6 membered heterocycloalkyl, and 5-6 membered heteroaryl of R^q are each optionally substituted with halo, OH, CN, -COOH, NH_2, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, phenyl, C_{3-10} cycloalkyl, 5- or 6-membered heteroaryl or 4-6 membered heterocycloalkyl; and

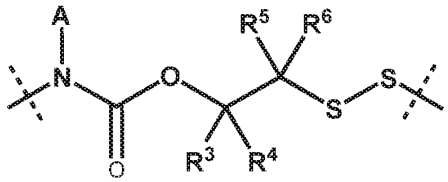
two R^q groups together with the chain atoms to which they are attached can form a phenyl, 5-6 membered heteroaryl, 4-6 membered heterocycloalkyl, or C_{3-6} cycloalkyl ring.

In some embodiments, R^q is independently selected from OH, CN, -COOH, NH_2, halo, C_{1-6} haloalkyl, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, $NH(C_{1-6}$ alkyl) and $N(C_{1-6}$ alkyl)$_2$.

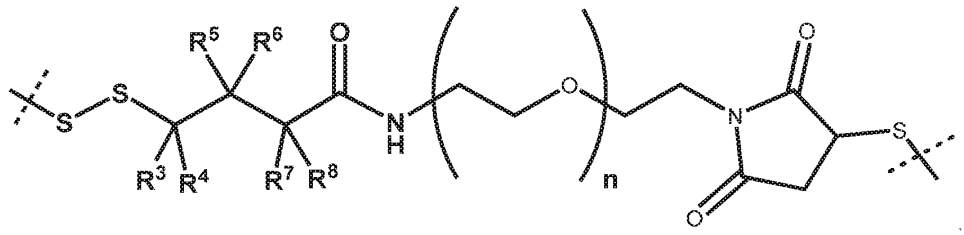
In some embodiments, L is the following group:



In some embodiments, L is the following group:

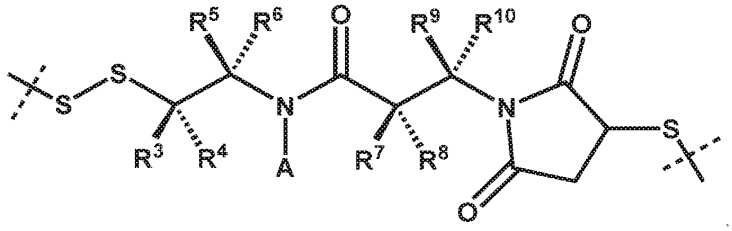


In some embodiments, L is the following group:

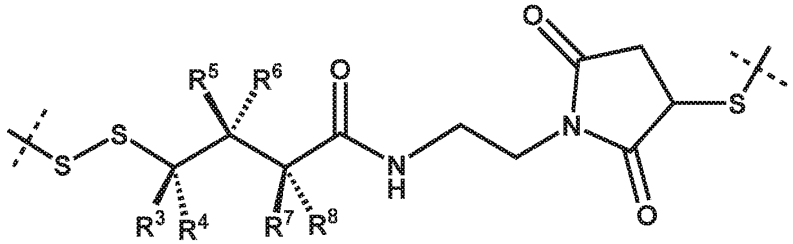


In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2.

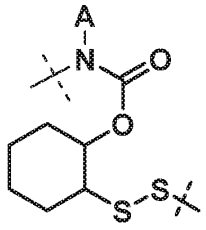
In some embodiments, L is the following group:



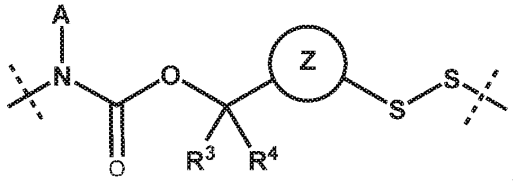
In some embodiments, L is the following group:



In some embodiments, L is the following group:



In some embodiments, L is the following group:



In some embodiments, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are each independently selected from H and C_{1-4} alkyl. In some embodiments, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are each H.

In some embodiments, R^3 and R^4 are each independently selected from H and C_{1-4} alkyl. In some embodiments, R^3 and R^4 are each H.

In some embodiments, R^5 and R^6 are each independently selected from H and C_{1-4} alkyl. In some embodiments, R^5 and R^6 are each H.

In some embodiments, R^7 and R^8 are each independently selected from H and C_{1-4} alkyl. In some embodiments, R^7 and R^8 are each H.

In some embodiments, R^9 and R^{10} are each independently selected from H and C_{1-4} alkyl. In some embodiments, R^9 and R^{10} are each H.

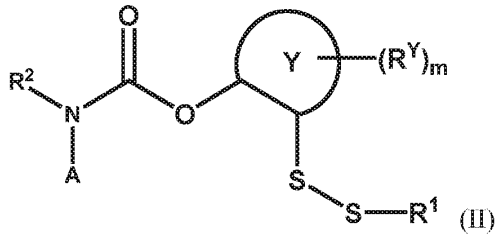
In some embodiments, A is H. In some embodiments, A is C_{1-4} alkyl. In some embodiments, A is CH_3.

In some embodiments, Z is C_{6-10} aryl, optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$.

In some embodiments, Z is phenyl, optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$.

In some embodiments, Z is phenyl.

In some embodiments, the compound of the invention is a compound of Formula (II):



(II)

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a peptide;

R^2 is a a small molecule microtubule targeting moiety;

A is H or C_{1-4} alkyl;

Ring Y is a monocyclic C_{5-7} cycloalkyl ring or a monocyclic 5-7 membered heterocycloalkyl ring;

each R^Y is independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

or two adjacent R^Y together with the atoms to which they are attached form a fused monocyclic C_{5-7} cycloalkyl ring, a fused monocyclic 5-7 membered heterocycloalkyl ring, a fused C_{6-10} aryl ring, or a fused 6-10 membered heteroaryl ring, each of which is optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$;

R^{a1}, R^{b1}, R^{c1}, and R^{d1} are each independently selected from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, each optionally substituted with 1, 2, or 3 substituents independently selected from halo, OH, CN, and NO_2; and

m is 0, 1, 2, or 3.

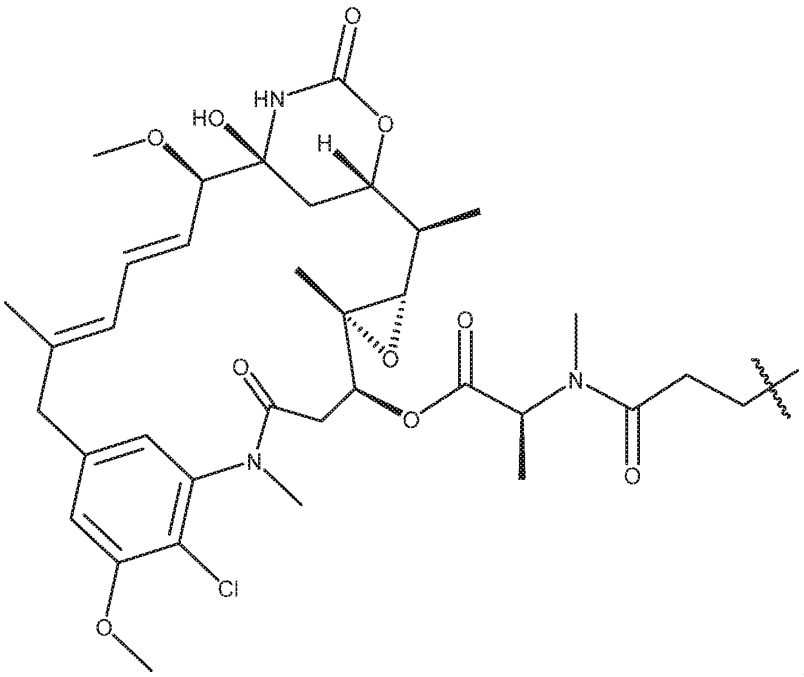
In some embodiments of compounds of Formula (II), R^1 is a peptide comprising the sequence of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5.

In some embodiments of compounds of Formula (II), R^1 is Pv1, Pv2, Pv3, Pv4, or Pv5.

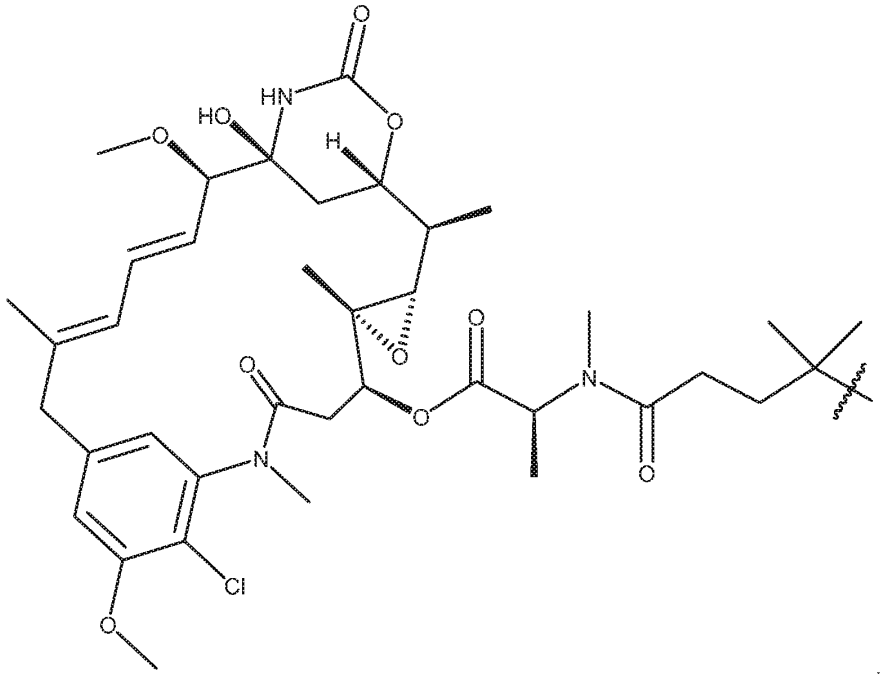
In some embodiments of compounds of Formula (II), R^1 is attached to the core via a cysteine residue of R^1 wherein one of the sulfur atoms of the disulfide moiety in Formula II is dervied from the cysteine residue.

In some embodiments of compounds of Formula (II), R^2 is a maytansinoid. In some embodiments of Formula (II), R^2 is DM1 or DM4. In some embodiments of Formula (II), R^2 is DM1. In some embodiments of Formula (II), R^2 is DM4.

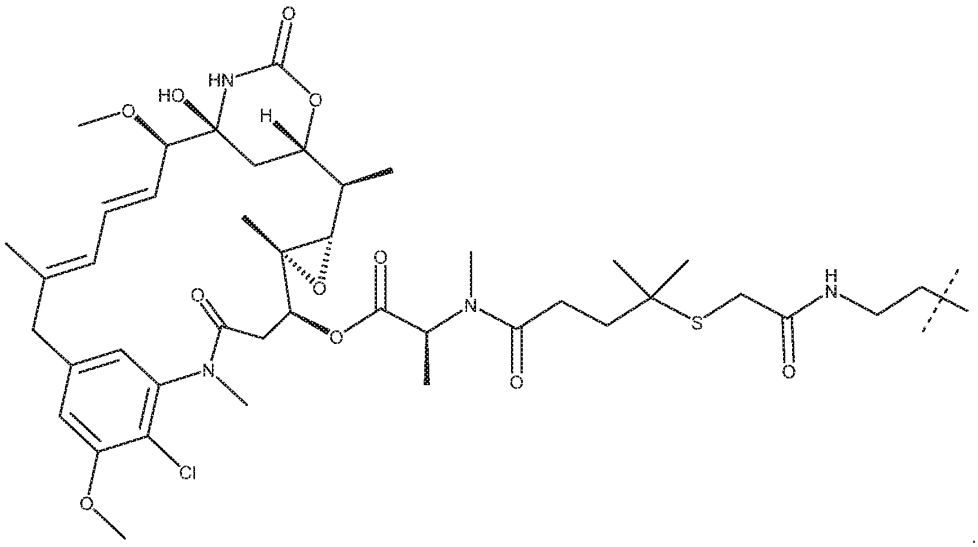
In some embodiments of compounds of Formula (II), R^2 is the following group:



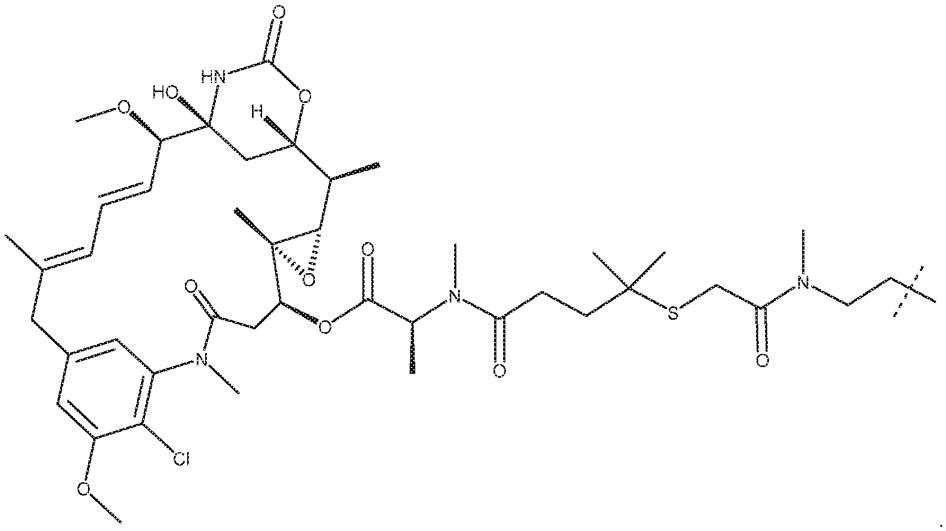
In some embodiments of compounds of Formula (II), R^2 is the following group:



In some embodiments of compounds of Formula (II), R^2 is the following group:



In some embodiments of compounds of Formula (II), R^2 is the following group:



In some embodiments of compounds of Formula (II), A is H. In some embodiments of compounds of Formula (II), A is C_{1-4} alkyl. In some embodiments of compounds of Formula (II), A is CH_3.

In some embodiments of compounds of Formula (II), Ring Y is a monocyclic C_{5-7} cycloalkyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a cyclopentyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a cyclohexyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a cycloheptyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a monocyclic 5-7 membered heterocycloalkyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a 5-membered heterocycloalkyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a 6-membered heterocycloalkyl ring.

In some embodiments of compounds of Formula (II), Ring Y is a 7-membered heterocycloalkyl ring.

In some embodiments of compounds of Formula (II), two adjacent R^Y together with the atoms to which they are attached form a fused monocyclic C_{5-7} cycloalkyl ring, a fused monocyclic 5-7 membered heterocycloalkyl ring, a fused C_{6-10} aryl ring, or a fused 6-10

membered heteroaryl ring, each of which is optionally substituted with 1, 2, or 3 substituents independently selected from C_{1-4} alkyl, halo, CN, NO_2, OR^{a1}, SR^{a1}, $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$.

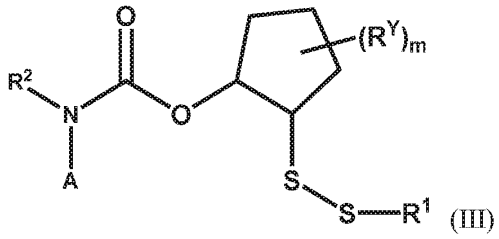
In some embodiments of compounds of Formula (II), m is 0.

In some embodiments of compounds of Formula (II), m is 1.

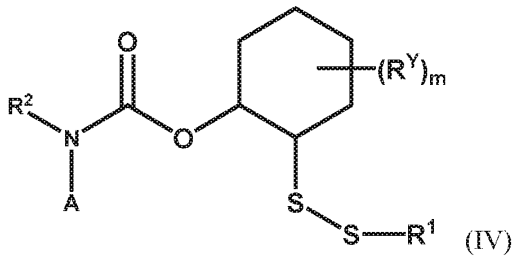
In some embodiments of compounds of Formula (II), m is 2.

In some embodiments of compounds of Formula (II), m is 3.

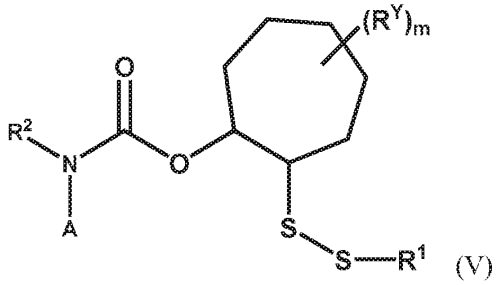
In some embodiments, the compounds of the invention is a compound of Formula (III), Formula (IV), or Formula (V):



(III)



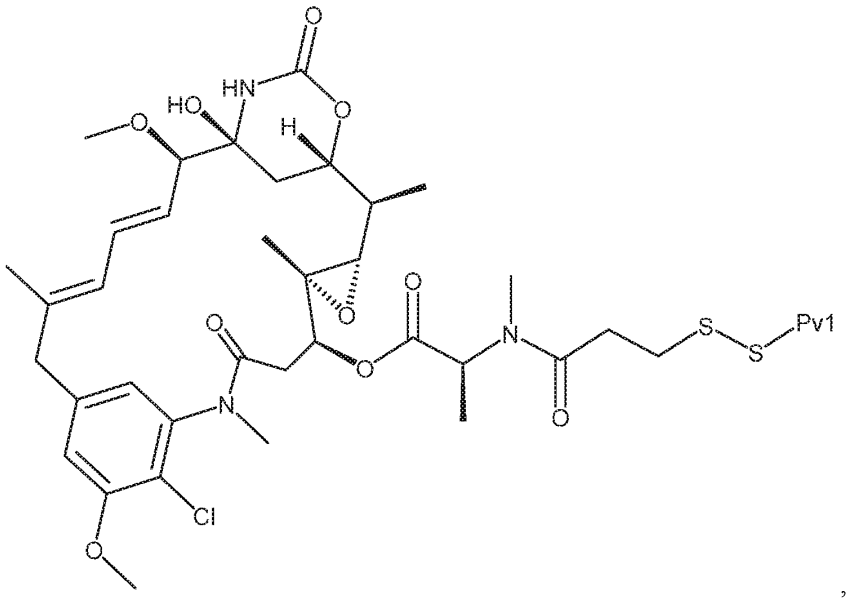
(IV)



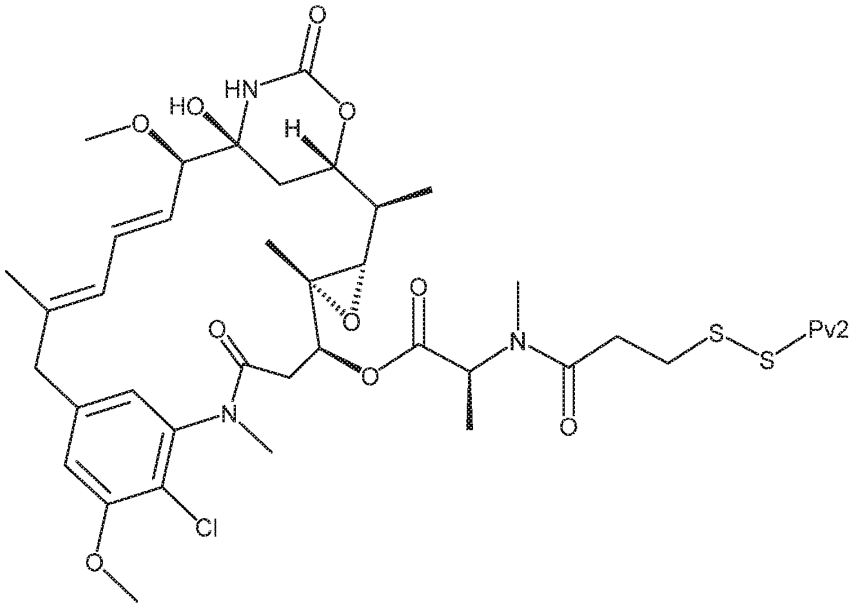
(V)

or a pharmaceutically acceptable salt thereof, wherein R^1, R^2, R^Y, A, and m are defined as in any of the embodiments above for Formula (II).

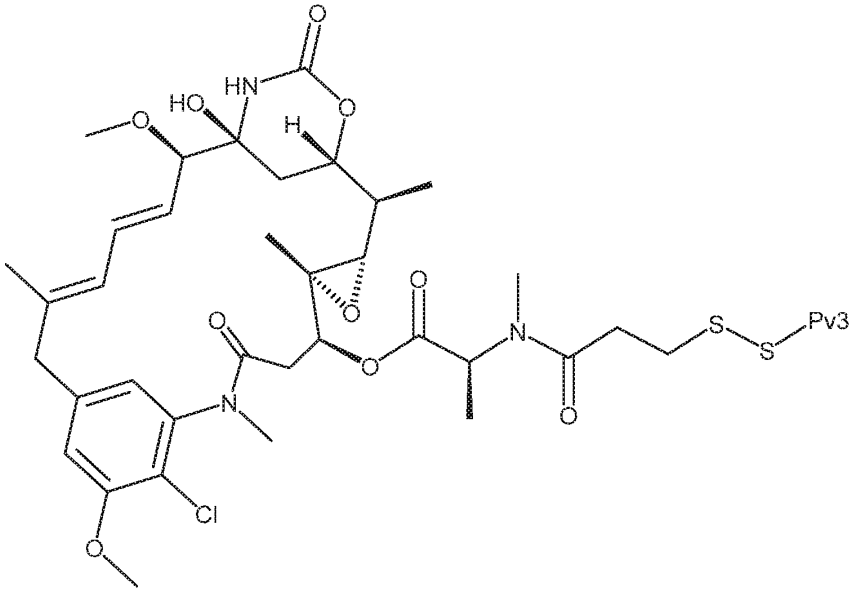
In some embodiments, the compound of formula (I) is selected from:



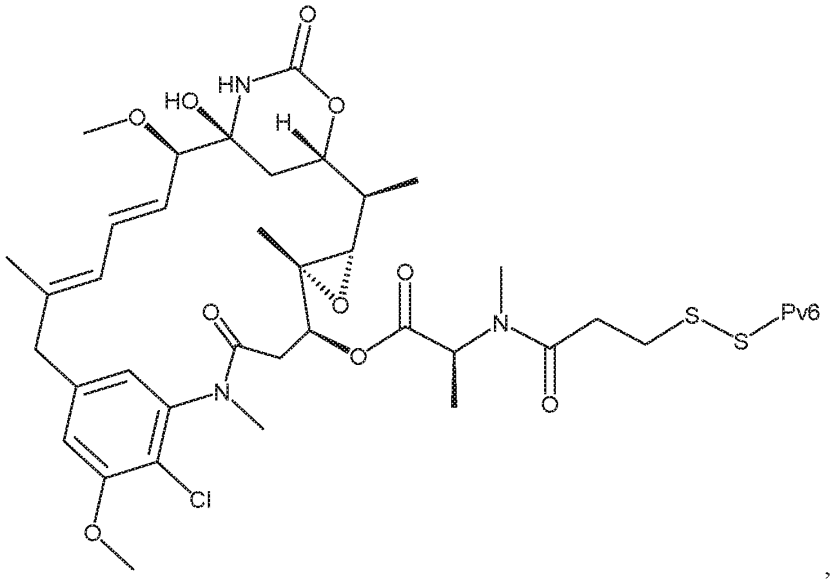
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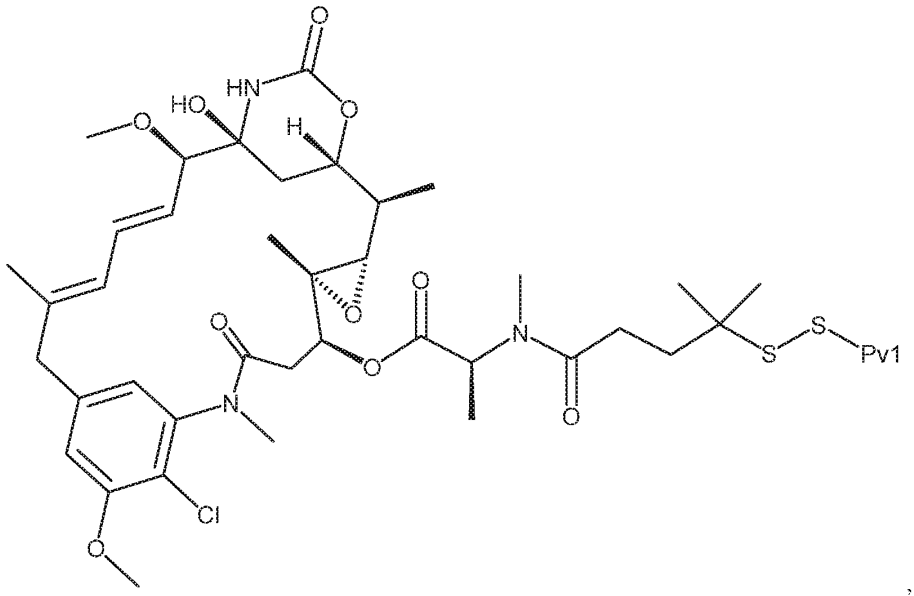
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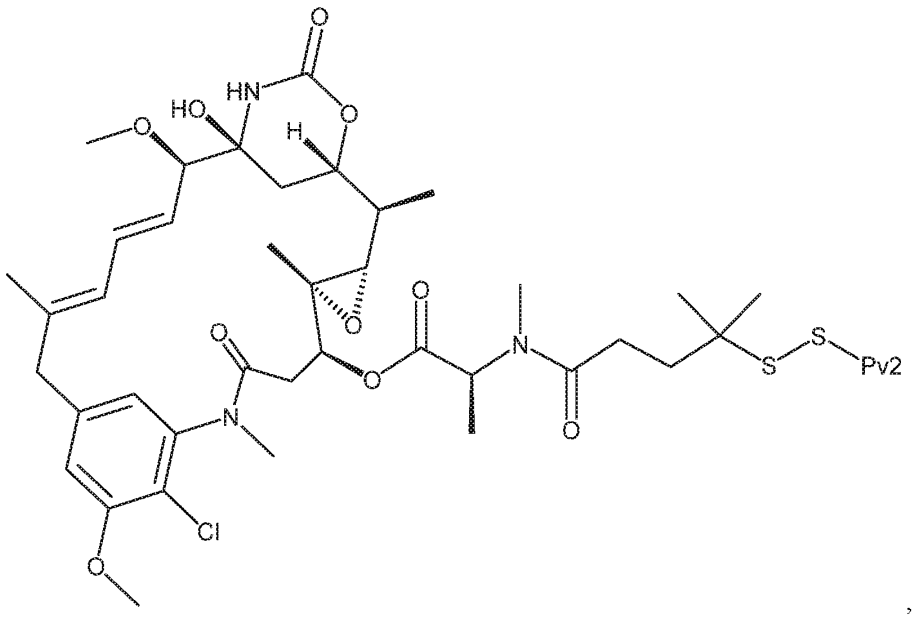
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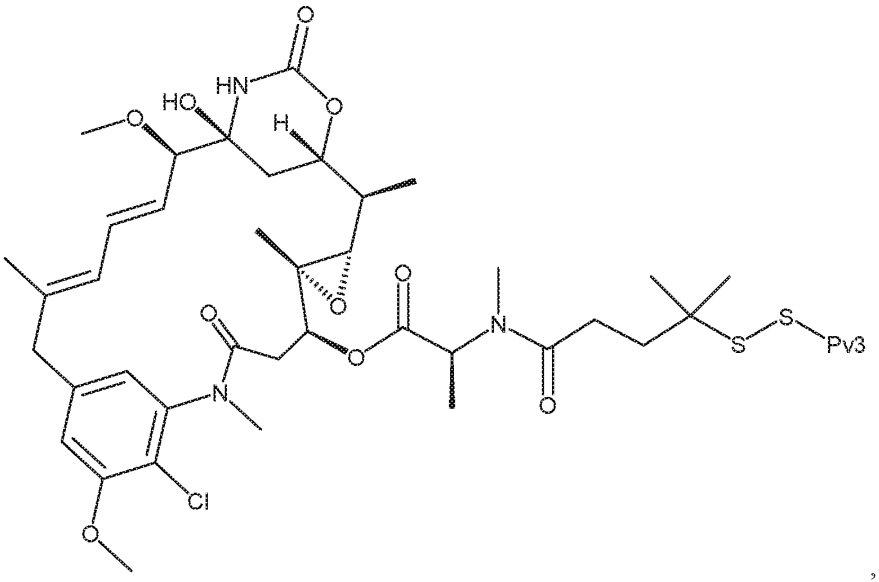
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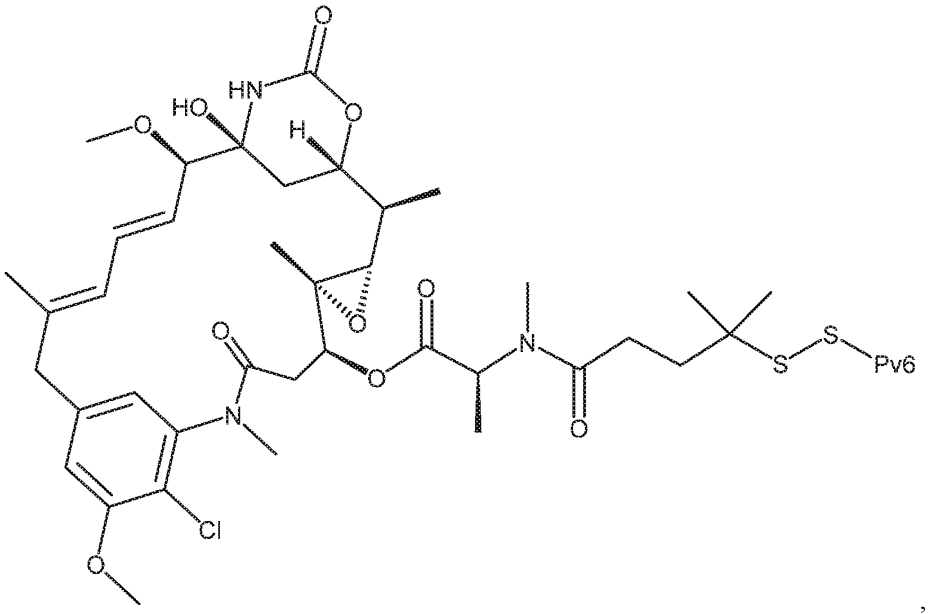
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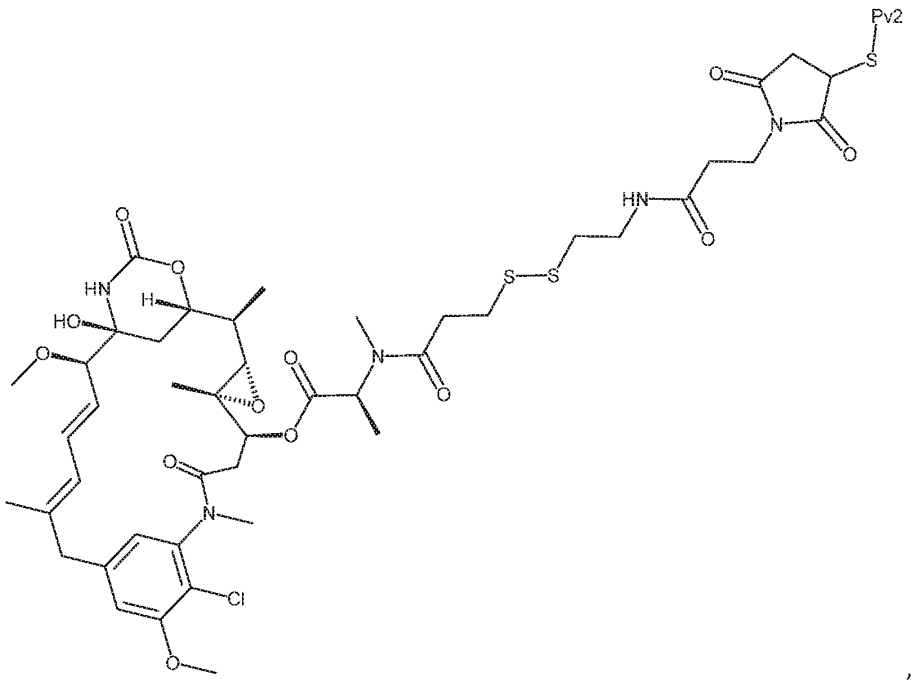
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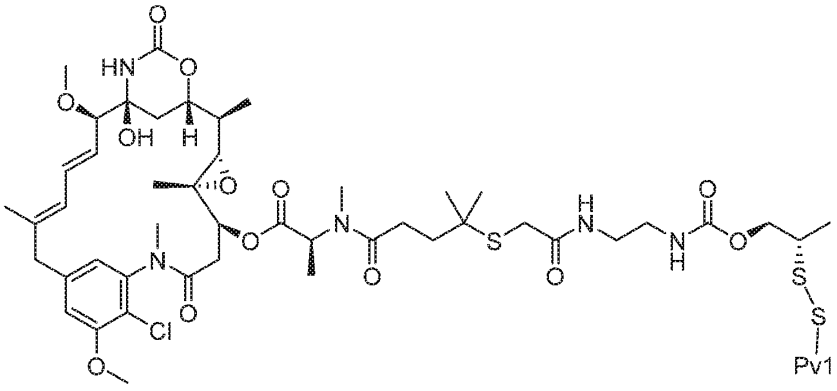

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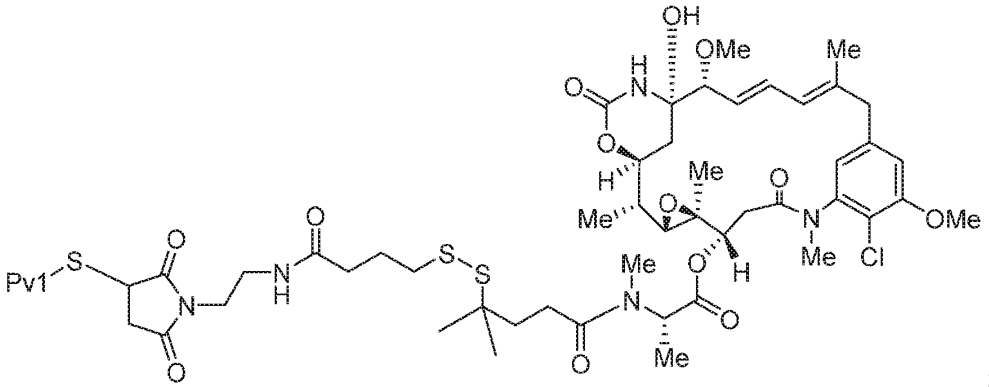
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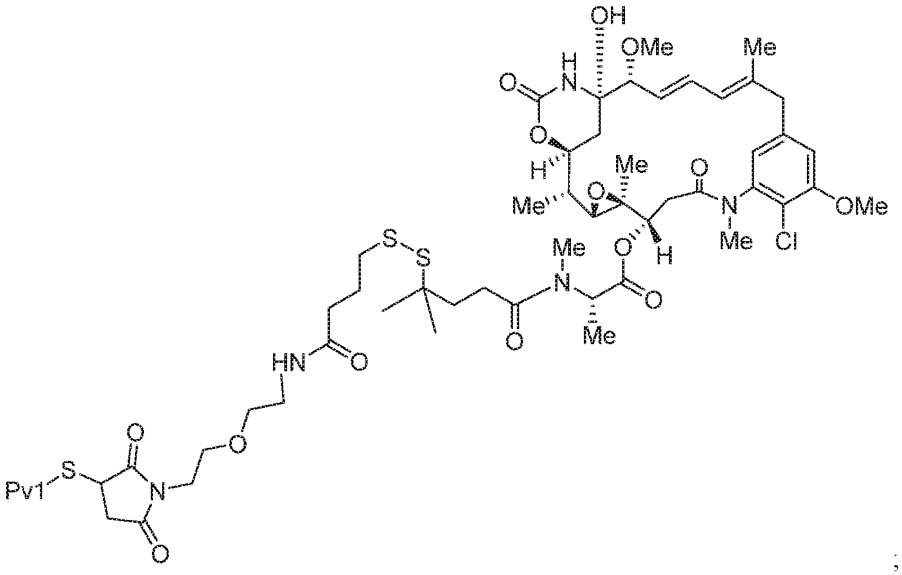
or a pharmaceutically acceptable salt of any of the aforementioned.

In some embodiments, the compound of Formula (I) is selected from:

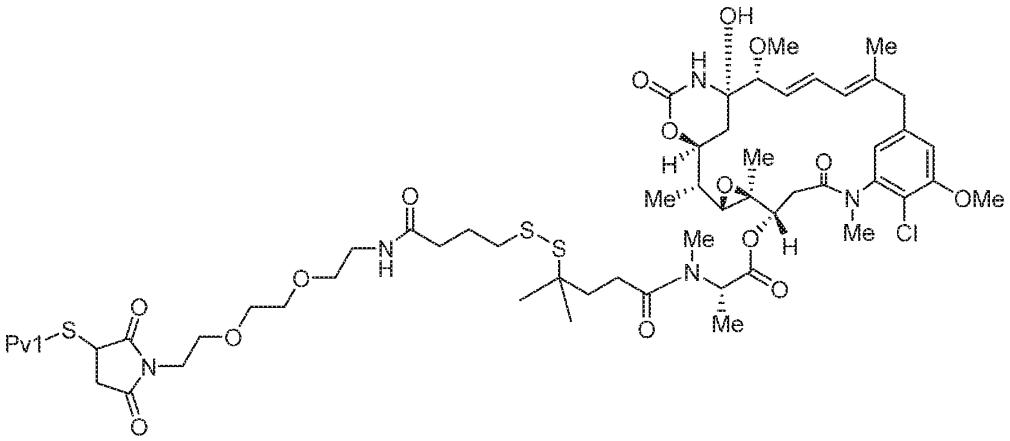



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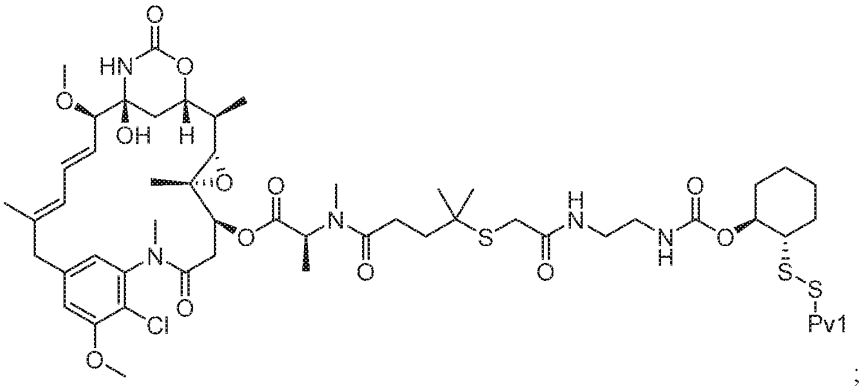
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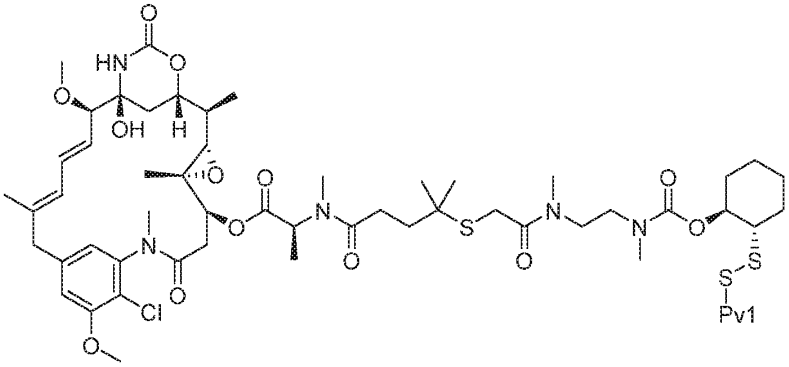
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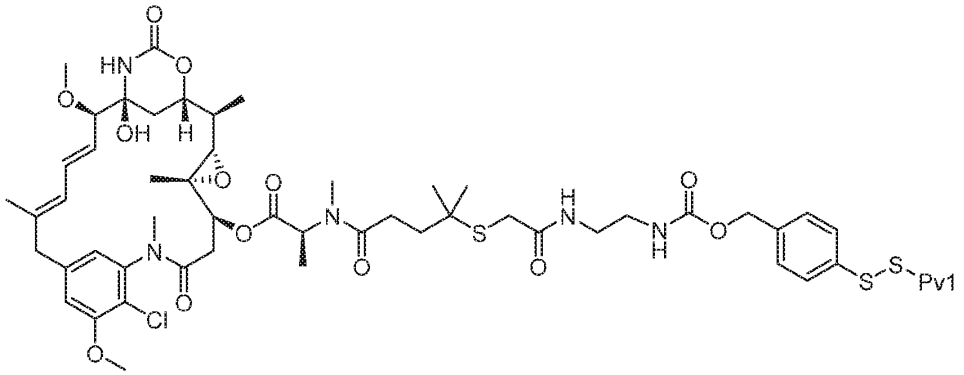
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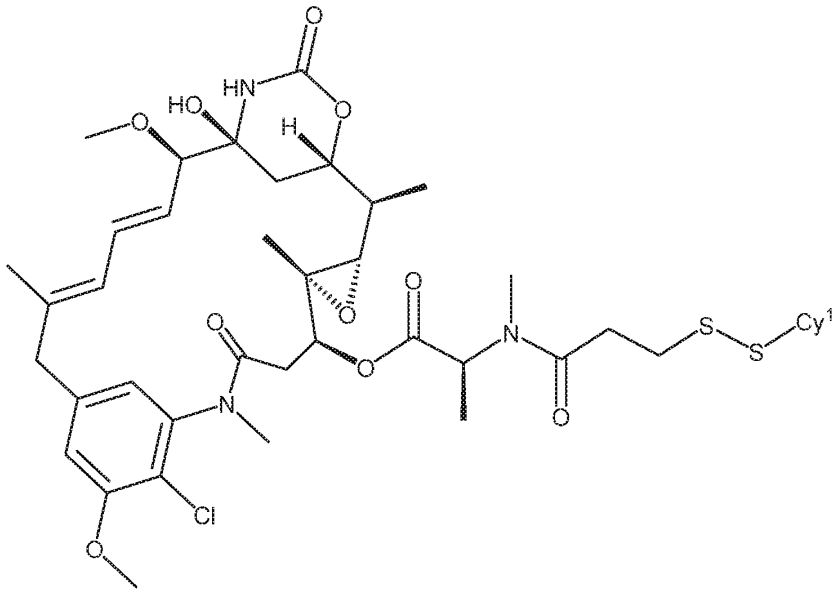
; and



,

or a pharmaceutically acceptable salt of any of the aforementioned.

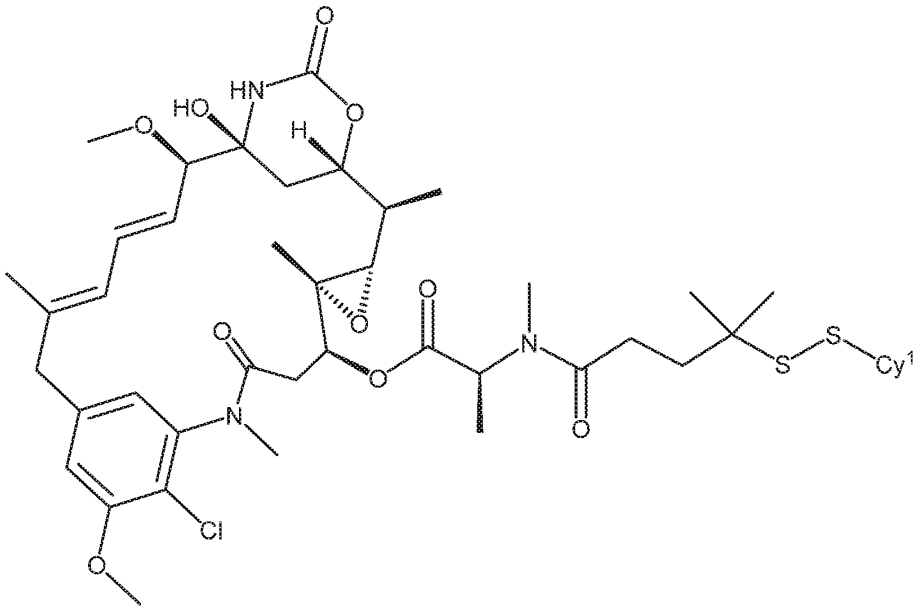
In some embodiments, provided herein is a compound having Formula (I-A):



(I-A),

or a salt thereof, wherein Cy^1 is C_{6-10} aryl or 5-10 membered heteroaryl. In some embodiments, Cy^1 is pyridyl.

In some embodiments, provided herein is a compound having Formula (I-B):



(I-B),

or a salt thereof, wherein Cy^1 is C_{6-10} aryl or 5-10 membered heteroaryl. In some embodiments, Cy^1 is pyridyl.

The molecules of the invention can be tagged, for example, with a probe such as a fluorophore, radioisotope, and the like. In some embodiments, the probe is a fluorescent probe, such as LICOR. A fluorescent probe can include any moiety that can re-emit light upon light excitation (e.g., a fluorophore).

The Amino acids are represented by the IUPAC abbreviations, as follows: Alanine (Ala; A), Arginine (Arg; R), Asparagine (Asn; N), Aspartic acid (Asp; D), Cysteine (Cys; C), Glutamine (Gln; Q), Glutamic acid (Glu; E), Glycine (Gly; G), Histidine (His; H), Isoleucine (Ile; I), Leucine (Leu; L), Lysine (Lys; K), Methionine (Met; M), Phenylalanine (Phe; F), Proline (Pro; P), Serine (Ser; S), Threonine (Thr; T), Tryptophan (Trp; W), Tyrosine (Tyr; Y), Valine (Val; V).

The term "Pv1" means ADDQNPWRAYLDLLFPTDTLLLDLLWCG, which is the the peptide of SEQ ID No. 1.

The term "Pv2" means AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG, which is the peptide of SEQ ID No. 2.

The term "Pv3" means ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG, which is the peptide of SEQ ID No. 3.

The term "Pv4" means Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG, which is the peptide of SEQ ID NO. 4.

The term "Pv5" means AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC, which is the peptide of SEQ ID NO. 5. The term "Pv6" means AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG, which is the peptide of SEQ ID NO. 6. In the compounds of the invention, the peptides R^1 are attached to the disulfide linker by a cysteine moiety.

The term "acidic and/or hypoxic mantle" refers to the environment of the cell in the diseased tissue in question having a pH lower than 7.0 and preferably lower than 6.5. An acidic or hypoxic mantle more preferably has a pH of about 5.5 and most preferably has a pH of about 5.0. The compounds of formula (I) insert across a cell membrane having an acidic and/or hypoxic mantle in a pH dependent fashion to insert R^2L into the cell, whereupon the disulfide bond of the linker is cleaved to deliver free R^2L (or R^2L^*, wherein L* is a product of degradation). Since the compounds of formula (I) are pH-dependent, they preferentially insert across a cell membrane only in the presence of an acidic or hypoxic mantle surrounding the cell and not across the cell membrane of "normal" cells, which do not have an acidic or hypoxic mantle.

The terms "pH-sensitive" or "pH-dependent" as used herein to refer to the peptide R^1 or to the mode of insertion of the peptide R^1 or of the compounds of the invention across a cell membrane, means that the peptide has a higher affinity to a cell membrane lipid bilayer having an acidic or hypoxic mantle than a membrane lipid bilayer at neutral pH. Thus, the compounds of the invention preferentially insert through the cell membrane to insert R^2L to the interior of the cell (and thus deliver R^2H as described above) when the cell membrane lipid bilayer has an acidic or hypoxic mantle (a "diseased" cell) but does not insert through a cell membrane when the mantle (the environment of the cell membrane lipid bilayer) is not acidic or hypoxic (a "normal" cell). It is believed that this preferential insertion is achieved as

a result of the peptide R^1 forming a helical configuration, which facilitates membrane insertion.

The term "small molecule microtubule targeting moiety" refers to a chemical group that binds to microtubules. The small molecule microtubule targeting moiety can be a group derived from a compound that inhibits the activity of microtubules. For example, the small molecule microtubule targeting moiety may suppress the dynamic stability of microtubules. In some embodiments, the small molecule microtubule targeting moiety has a molecular weight (Da) of about 100-1500, about 100-800, about 500-1,000, about 600-1,000, about 100-500, about 700-900, or about 250-500.

It is further appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment (while the embodiments are intended to be combined as if written in multiply dependent form). Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination. Thus, it is contemplated as features described as embodiments of the compounds of Formula (I) can be combined in any suitable combination.

At various places in the present specification, certain features of the compounds are disclosed in groups or in ranges. It is specifically intended that such a disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_{1-6} alkyl" is specifically intended to individually disclose (without limitation) methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl and C_6 alkyl.

The term "n-membered," where n is an integer, typically describes the number of ring-forming atoms in a moiety where the number of ring-forming atoms is n. For example, piperidinyl is an example of a 6-membered heterocycloalkyl ring, pyrazolyl is an example of a 5-membered heteroaryl ring, pyridyl is an example of a 6-membered heteroaryl ring and 1,2,3,4-tetrahydro-naphthalene is an example of a 10-membered cycloalkyl group.

At various places in the present specification, variables defining divalent linking groups may be described. It is specifically intended that each linking substituent include both the forward and backward forms of the linking substituent. For example, $-NR(CR'R'')_n-$ includes both $-NR(CR'R'')_n-$ and $-(CR'R'')_nNR-$ and is intended to disclose each of the forms individually.

Where the structure requires a linking group, the Markush variables listed for that group are understood to be linking groups. For example, if the structure requires a linking group and the Markush group definition for that variable lists "alkyl" or "aryl" then it is understood that the "alkyl" or "aryl" represents a linking alkylene group or arylene group, respectively.

The term "substituted" means that an atom or group of atoms formally replaces hydrogen as a "substituent" attached to another group. The term "substituted", unless otherwise indicated, refers to any level of substitution, *e.g.*, mono-, di-, tri-, tetra- or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. It is to be understood that substitution at a given atom is limited by valency. It is to be understood that substitution at a given atom results in a chemically stable molecule. The phrase "optionally substituted" means unsubstituted or substituted. The term "substituted" means that a hydrogen atom is removed and replaced by a substituent. A single divalent substituent, *e.g.*, oxo, can replace two hydrogen atoms.

The term "C_{n-m}" indicates a range which includes the endpoints, wherein n and m are integers and indicate the number of carbons. Examples include C_{1-4}, C_{1-6} and the like.

The term "alkyl" employed alone or in combination with other terms, refers to a saturated hydrocarbon group that may be straight-chained or branched. The term "C_{n-m} alkyl", refers to an alkyl group having n to m carbon atoms. An alkyl group formally corresponds to an alkane with one C-H bond replaced by the point of attachment of the alkyl group to the remainder of the compound. In some embodiments, the alkyl group contains from 1 to 6 carbon atoms, from 1 to 4 carbon atoms, from 1 to 3 carbon atoms, or 1 to 2 carbon atoms. Examples of alkyl moieties include, but are not limited to, chemical groups such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, isobutyl, *sec*-butyl; higher homologs such as 2-methyl-1-butyl, *n*-pentyl, 3-pentyl, *n*-hexyl, 1,2,2-trimethylpropyl and the like.

The term "alkenyl" employed alone or in combination with other terms, refers to a straight-chain or branched hydrocarbon group corresponding to an alkyl group having one or more double carbon-carbon bonds. An alkenyl group formally corresponds to an alkene with one C-H bond replaced by the point of attachment of the alkenyl group to the remainder of the compound. The term "C_{n-m} alkenyl" refers to an alkenyl group having n to m carbons. In some embodiments, the alkenyl moiety contains 2 to 6, 2 to 4, or 2 to 3 carbon atoms. Example

alkenyl groups include, but are not limited to, ethenyl, *n*-propenyl, isopropenyl, *n*-butenyl, *sec*-butenyl and the like.

The term "alkynyl" employed alone or in combination with other terms, refers to a straight-chain or branched hydrocarbon group corresponding to an alkyl group having one or more triple carbon-carbon bonds. An alkynyl group formally corresponds to an alkyne with one C-H bond replaced by the point of attachment of the alkyl group to the remainder of the compound. The term "C_{n-m} alkynyl" refers to an alkynyl group having n to m carbons. Example alkynyl groups include, but are not limited to, ethynyl, propyn-1-yl, propyn-2-yl and the like. In some embodiments, the alkynyl moiety contains 2 to 6, 2 to 4, or 2 to 3 carbon atoms.

The term "alkylene", employed alone or in combination with other terms, refers to a divalent alkyl linking group. An alkylene group formally corresponds to an alkane with two C-H bond replaced by points of attachment of the alkylene group to the remainder of the compound. The term "C_{n-m} alkylene" refers to an alkylene group having n to m carbon atoms. Examples of alkylene groups include, but are not limited to, ethan-1,2-diyl, ethan-1,1-diyl, propan-1,3-diyl, propan-1,2-diyl, propan-1,1-diyl, butan-1,4-diyl, butan-1,3-diyl, butan-1,2-diyl, 2-methyl-propan-1,3-diyl and the like.

The term "amino" refers to a group of formula $-NH_2$.

The term "carbonyl", employed alone or in combination with other terms, refers to a -C(=O)- group, which also may be written as C(O).

The term "cyano" or "nitrile" refers to a group of formula $-C\equiv N$, which also may be written as -CN.

The terms "halo" or "halogen", used alone or in combination with other terms, refers to fluoro, chloro, bromo and iodo. In some embodiments, "halo" refers to a halogen atom selected from F, Cl, or Br. In some embodiments, halo groups are F.

The term "haloalkyl" as used herein refers to an alkyl group in which one or more of the hydrogen atoms has been replaced by a halogen atom. The term "C_{n-m} haloalkyl" refers to a C_{n-m} alkyl group having n to m carbon atoms and from at least one up to {2(n to m)+1} halogen atoms, which may either be the same or different. In some embodiments, the halogen atoms are fluoro atoms. In some embodiments, the haloalkyl group has 1 to 6 or 1 to 4 carbon atoms.

Example haloalkyl groups include CF_3, C_2F_5, CHF_2, CH_2F, CCl_3, $CHCl_2$, C_2Cl_5 and the like. In some embodiments, the haloalkyl group is a fluoroalkyl group.

The term "haloalkoxy", employed alone or in combination with other terms, refers to a group of formula -O-haloalkyl, wherein the haloalkyl group is as defined above. The term "C_{n-m} haloalkoxy" refers to a haloalkoxy group, the haloalkyl group of which has n to m carbons. Example haloalkoxy groups include trifluoromethoxy and the like. In some embodiments, the haloalkoxy group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

The term "oxo" refers to an oxygen atom as a divalent substituent, forming a carbonyl group when attached to carbon, or attached to a heteroatom forming a sulfoxide or sulfone group, or an *N*-oxide group. In some embodiments, heterocyclic groups may be optionally substituted by 1 or 2 oxo (=O) substituents.

The term "oxidized" in reference to a ring-forming N atom refers to a ring-forming N-oxide.

The term "oxidized" in reference to a ring-forming S atom refers to a ring-forming sulfonyl or ring-forming sulfinyl.

The term "aromatic" refers to a carbocycle or heterocycle having one or more polyunsaturated rings having aromatic character (*i.e.*, having (4n + 2) delocalized □ (pi) electrons where n is an integer).

The term "aryl," employed alone or in combination with other terms, refers to an aromatic hydrocarbon group, which may be monocyclic or polycyclic (*e.g.*, having 2 fused rings). The term "C_{n-m} aryl" refers to an aryl group having from n to m ring carbon atoms. Aryl groups include, *e.g.*, phenyl, naphthyl, and the like. In some embodiments, aryl groups have from 6 to about 10 carbon atoms. In some embodiments aryl groups have 6 carbon atoms. In some embodiments aryl groups have 10 carbon atoms. In some embodiments, the aryl group is phenyl.

The term "heteroaryl" or "heteroaromatic," employed alone or in combination with other terms, refers to a monocyclic or polycyclic aromatic heterocycle having at least one heteroatom ring member selected from sulfur, oxygen and nitrogen. In some embodiments, the heteroaryl ring has 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, any ring-forming N in a heteroaryl moiety can be an N-oxide. In

some embodiments, the heteroaryl has 5-14 ring atoms including carbon atoms and 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl has 5-10 ring atoms including carbon atoms and 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl has 5-6 ring atoms and 1 or 2 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl is a five-membered or six-membered heteroaryl ring. In other embodiments, the heteroaryl is an eight-membered, nine-membered or ten-membered fused bicyclic heteroaryl ring.

A five-membered heteroaryl ring is a heteroaryl group having five ring atoms wherein one or more (*e.g.*, 1, 2 or 3) ring atoms are independently selected from N, O and S.

A six-membered heteroaryl ring is a heteroaryl group having six ring atoms wherein one or more (*e.g.*, 1, 2 or 3) ring atoms are independently selected from N, O and S.

The term "cycloalkyl," employed alone or in combination with other terms, refers to a non-aromatic hydrocarbon ring system (monocyclic, bicyclic or polycyclic), including cyclized alkyl and alkenyl groups. The term "C_{n-m} cycloalkyl" refers to a cycloalkyl that has n to m ring member carbon atoms. Cycloalkyl groups can include mono- or polycyclic (*e.g.*, having 2, 3 or 4 fused rings) groups and spirocycles. Cycloalkyl groups can have 3, 4, 5, 6 or 7 ring-forming carbons (C_{3-7}). In some embodiments, the cycloalkyl group has 3 to 6 ring members, 3 to 5 ring members, or 3 to 4 ring members. In some embodiments, the cycloalkyl group is monocyclic. In some embodiments, the cycloalkyl group is monocyclic or bicyclic. In some embodiments, the cycloalkyl group is a C_{3-6} monocyclic cycloalkyl group. Ring-forming carbon atoms of a cycloalkyl group can be optionally oxidized to form an oxo or sulfido group. Cycloalkyl groups also include cycloalkylidenes. In some embodiments, cycloalkyl is cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. Also included in the definition of cycloalkyl are moieties that have one or more aromatic rings fused (*i.e.*, having a bond in common with) to the cycloalkyl ring, *e.g.*, benzo or thienyl derivatives of cyclopentane, cyclohexane and the like. A cycloalkyl group containing a fused aromatic ring can be attached through any ring-forming atom including a ring-forming atom of the fused aromatic ring. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclopentenyl, cyclohexenyl,

cyclohexadienyl, and the like. In some embodiments, the cycloalkyl group is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

The term "heterocycloalkyl," employed alone or in combination with other terms, refers to a non-aromatic ring or ring system, which may optionally contain one or more alkenylene groups as part of the ring structure, which has at least one heteroatom ring member independently selected from nitrogen, sulfur, oxygen and phosphorus, and which has 4-10 ring members, 4-7 ring members, or 4-6 ring members. Included within the term "heterocycloalkyl" are monocyclic 4-, 5-, 6- and 7-membered heterocycloalkyl groups. Heterocycloalkyl groups can include mono- or bicyclic (*e.g.*, having two fused or bridged rings) or spirocyclic ring systems. In some embodiments, the heterocycloalkyl group is a monocyclic group having 1, 2 or 3 heteroatoms independently selected from nitrogen, sulfur and oxygen. Ring-forming carbon atoms and heteroatoms of a heterocycloalkyl group can be optionally oxidized to form an oxo or sulfido group or other oxidized linkage (*e.g.*, C(O), S(O), C(S) or $S(O)_2$, *N*-oxide *etc.*) or a nitrogen atom can be quaternized. The heterocycloalkyl group can be attached through a ring-forming carbon atom or a ring-forming heteroatom. In some embodiments, the heterocycloalkyl group contains 0 to 3 double bonds. In some embodiments, the heterocycloalkyl group contains 0 to 2 double bonds. Also included in the definition of heterocycloalkyl are moieties that have one or more aromatic rings fused (*i.e.*, having a bond in common with) to the heterocycloalkyl ring, *e.g.*, benzo or thienyl derivatives of piperidine, morpholine, azepine, *etc.* A heterocycloalkyl group containing a fused aromatic ring can be attached through any ring-forming atom including a ring-forming atom of the fused aromatic ring. Examples of heterocycloalkyl groups include 2-pyrrolidinyl, morpholinyl, azetidinyl, tetrahydrofuranyl, tetrahydropyranyl, and piperazinyl.

At certain places, the definitions or embodiments refer to specific rings (*e.g.*, an azetidine ring, a pyridine ring, *etc.*). Unless otherwise indicated, these rings can be attached to any ring member provided that the valency of the atom is not exceeded. For example, an azetidine ring may be attached at any position of the ring, whereas an azetidin-3-yl ring is attached at the 3-position.

The compounds described herein can be asymmetric (*e.g.*, having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless

otherwise indicated. Compounds of the present invention that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically inactive starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. *Cis* and *trans* geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms.

Resolution of racemic mixtures of compounds can be carried out by any of numerous methods known in the art. One method includes fractional recrystallization using a chiral resolving acid which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, *e.g.*, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids such as α-camphorsulfonic acid. Other resolving agents suitable for fractional crystallization methods include stereoisomerically pure forms of α-methylbenzylamine (*e.g.*, *S* and *R* forms, or diastereomerically pure forms), 2-phenylglycinol, norephedrine, ephedrine, *N*-methylephedrine, cyclohexylethylamine, 1,2-diaminocyclohexane and the like.

Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent (*e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

In some embodiments, the compounds of the invention have the (*R*)-configuration. In other embodiments, the compounds have the (*S*)-configuration. In compounds with more than one chiral centers, each of the chiral centers in the compound may be independently (*R*) or (*S*), unless otherwise indicated.

Compounds of the invention also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond together with the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Example prototropic tautomers include ketone – enol pairs, amide - imidic acid pairs, lactam – lactim pairs, enamine

– imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, *e.g.*, 1*H*- and 3*H*-imidazole, 1*H*-, 2*H*- and 4*H*- 1,2,4-triazole, 1*H*- and 2*H*-isoindole and 1*H*- and 2*H*-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

Compounds of the invention can also include all isotopes of atoms occurring in the intermediates or final compounds. Isotopes include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium. One or more constituent atoms of the compounds of the invention can be replaced or substituted with isotopes of the atoms in natural or non-natural abundance. In some embodiments, the compound includes at least one deuterium atom. For example, one or more hydrogen atoms in a compound of the present disclosure can be replaced or substituted by deuterium. In some embodiments, the compound includes two or more deuterium atoms. In some embodiments, the compound includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 deuterium atoms. Synthetic methods for including isotopes into organic compounds are known in the art (Deuterium Labeling in Organic Chemistry by Alan F. Thomas (New York, N.Y., Appleton-Century-Crofts, 1971; The Renaissance of H/D Exchange by Jens Atzrodt, Volker Derdau, Thorsten Fey and Jochen Zimmermann, Angew. Chem. Int. Ed. 2007, 7744-7765; The Organic Chemistry of Isotopic Labelling by James R. Hanson, Royal Society of Chemistry, 2011). Isotopically labeled compounds can used in various studies such as NMR spectroscopy, metabolism experiments, and/or assays.

Substitution with heavier isotopes such as deuterium, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances. (A. Kerekes et.al. *J. Med. Chem.* **2011**, *54*, 201-210; R. Xu et.al. *J. Label Compd. Radiopharm.* **2015**, *58*, 308-312).

The term, "compound," as used herein is meant to include all stereoisomers, geometric isomers, tautomers and isotopes of the structures depicted. The term is also meant to refer to compounds of the inventions, regardless of how they are prepared, e.g., synthetically, through biological process (e.g., metabolism or enzyme conversion), or a combination thereof.

All compounds, and pharmaceutically acceptable salts thereof, can be found together with other substances such as water and solvents (*e.g.*, hydrates and solvates) or can be isolated. When in the solid state, the compounds described herein and salts thereof may occur in various forms and may, *e.g.*, take the form of solvates, including hydrates. The compounds may be in any solid state form, such as a polymorph or solvate, so unless clearly indicated otherwise, reference in the specification to compounds and salts thereof should be understood as encompassing any solid state form of the compound.

In some embodiments, the compounds of the invention, or salts thereof, are substantially isolated. By "substantially isolated" is meant that the compound is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, *e.g.*, a composition enriched in the compounds of the invention. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compounds of the invention, or salt thereof.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The expressions, "ambient temperature" and "room temperature," as used herein, are understood in the art, and refer generally to a temperature, *e.g.*, a reaction temperature, that is about the temperature of the room in which the reaction is carried out, *e.g.*, a temperature from about 20 °C to about 30 °C.

The present invention also includes pharmaceutically acceptable salts of the compounds described herein. The term "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present invention include the non-toxic salts of the parent compound formed, *e.g.*,

from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, alcohols (*e.g.*, methanol, ethanol, iso-propanol or butanol) or acetonitrile (MeCN) are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th Ed., (Mack Publishing Company, Easton, 1985), p. 1418, Berge *et al.*, *J. Pharm. Sci.*, **1977**, *66*(1), 1-19 and in Stahl *et al.*, *Handbook of Pharmaceutical Salts: Properties, Selection, and Use*, (Wiley, 2002). In some embodiments, the compounds described herein include the N-oxide forms.

Synthesis

Compounds of the invention, including salts thereof, can be prepared using known organic synthesis techniques and can be synthesized according to any of numerous possible synthetic routes, such as those in the Schemes below.

The reactions for preparing compounds of the invention can be carried out in suitable solvents which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially non-reactive with the starting materials (reactants), the intermediates or products at the temperatures at which the reactions are carried out, *e.g.*, temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected by the skilled artisan.

Preparation of compounds of the invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups is described, *e.g.*, in Kocienski, *Protecting Groups*, (Thieme, 2007); Robertson, *Protecting Group Chemistry*, (Oxford University Press, 2000); Smith *et al.*, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 6th Ed. (Wiley, 2007);

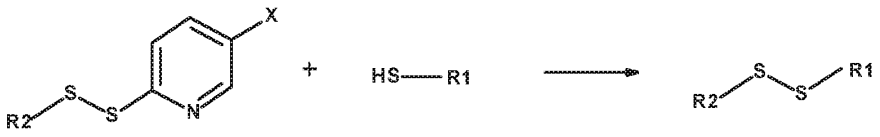
Peturssion *et al.*, "Protecting Groups in Carbohydrate Chemistry," *J. Chem. Educ.*, 1997, *74*(11), 1297; and Wuts *et al.*, *Protective Groups in Organic Synthesis*, 4th Ed., (Wiley, 2006).

Reactions can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^{1}H or ^{13}C), infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), mass spectrometry or by chromatographic methods such as high performance liquid chromatography (HPLC) or thin layer chromatography (TLC).

The Schemes below provide general guidance in connection with preparing the compounds of the invention. One skilled in the art would understand that the preparations shown in the Schemes can be modified or optimized using general knowledge of organic chemistry to prepare various compounds of the invention.

Compounds of Formula (I) can be prepared, *e.g.*, using a process as illustrated in the schemes below.

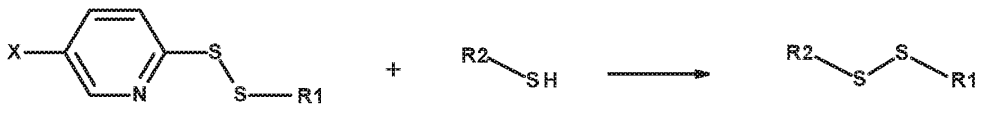
Scheme 1: Synthesis of Direct Conjugates



I

A small molecule microtubule targeting moietywhich contains an inherent thiol (R^2-SH), such as DM1 or DM4, can be activated by the formation of pyridyl disulfide (wherein X is, for example, H, halo, etc.), which can be displaced with a thiolcontaining R^1 peptide in a disulfide exchange reaction to give the desired conjugate where –S-S- is the linking moiety L.

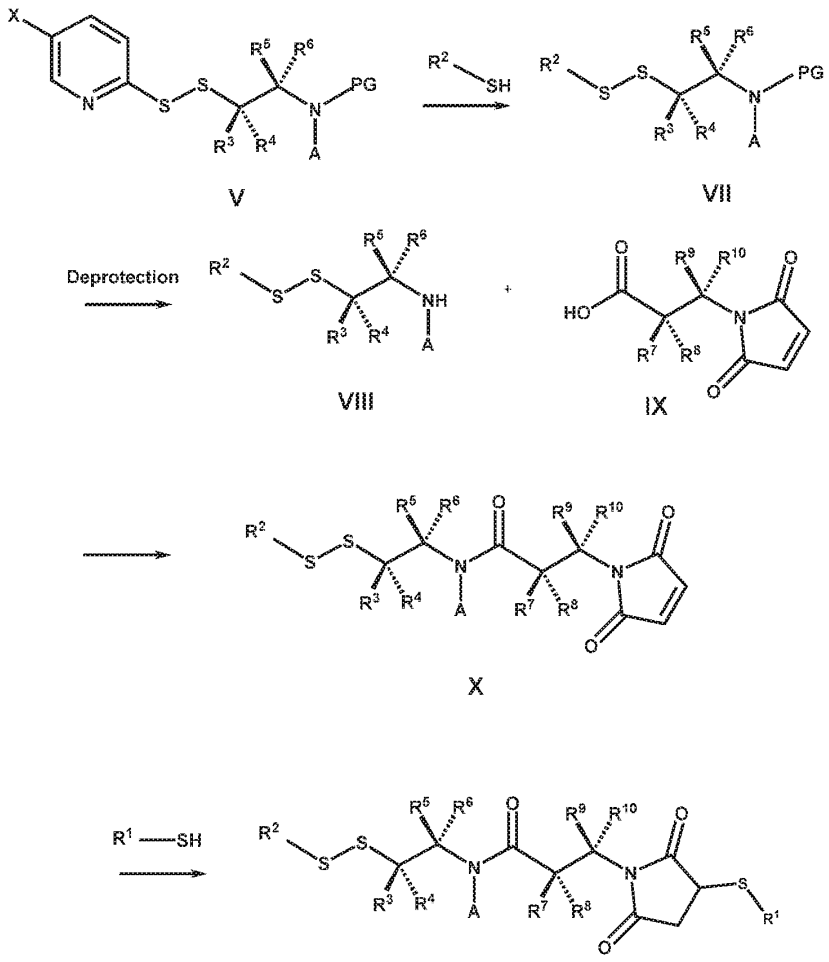
Scheme 2: Synthesis of Direct Conjugates



II

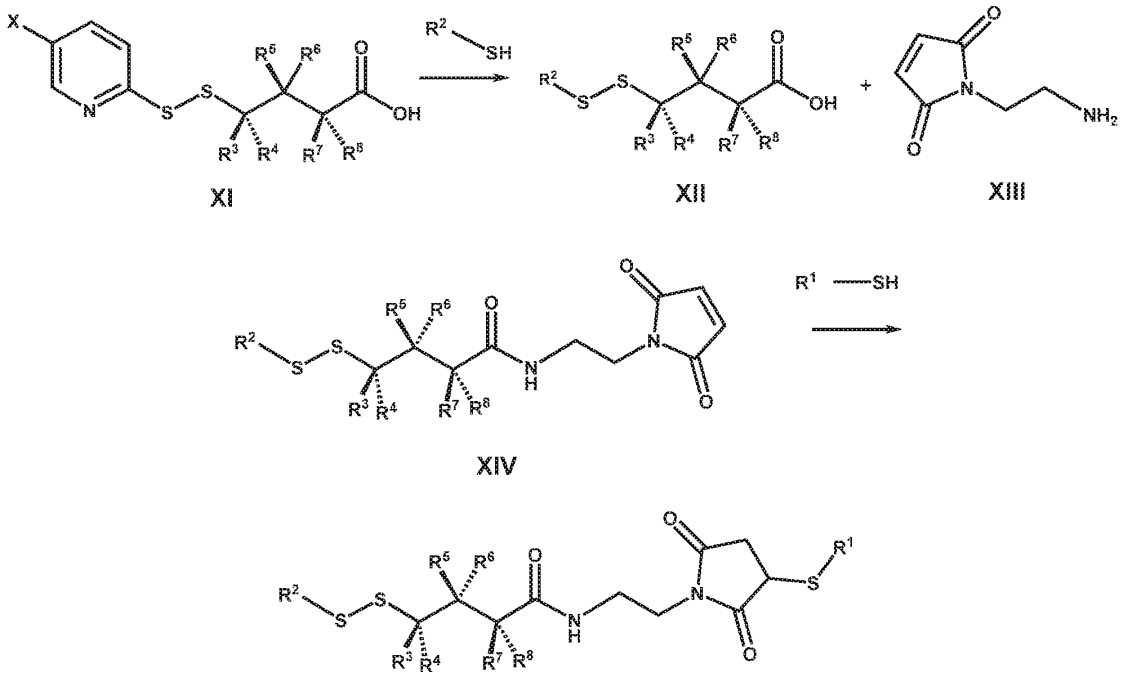
Alternatively, the R^1 peptide, which has an inherent thiol, can be activated by the formation of pyridyl disulfide (II) which can be displaced with a thiol-containing R^2 in a disulfide exchange reaction to give the desired direct conjugate where L is –S-S-.

Scheme 3: Synthesis of Thioethyl Amine Amide Conjugates



A protected ethyl amine, containing a thiol group that has been activated as pyridyl disulfide V can be reacted with thiol-containing R^2 in a disulfide exchange reaction to give VII. Disulfide VII can be deprotected to give VIII and further reacted with a propionic maleimide IX in an acid coupling reaction to provide amide X. Amide X can be reacted with a thiol containing peptide in a Michael addition to give the desired conjugate.

Scheme 4: Synthesis of Thiobutyric Amide Conjugates



A thiol-containing butyric acid that has been activated as a pyridyl disulfide XI can be reacted with thiol containing R^2 in a disulfide exchange reaction to give XII. Disulfide acid XII can be reacted with ethylaminomaleimide XIII in an acid coupling reaction to provide amide XIV. Amide XIV can be reacted with a thiol-containing peptide in a Michael addition to give the desired conjugate.

The peptides R^1 may be prepared using the solid-phase synthetic method first described by Merrifield in J.A.C.S., Vol. 85, pgs. 2149-2154 (1963), although other art-known methods may also be employed. The Merrifield technique is well understood and is a common method for preparation of peptides. Useful techniques for solid-phase peptide synthesis are described in several books such as the text "Principles of Peptide Synthesis" by Bodanszky, Springer Verlag 1984. This method of synthesis involves the stepwise addition of protected amino acids to a growing peptide chain which was bound by covalent bonds to a solid resin particle. By this

procedure, reagents and by-products are removed by filtration, thus eliminating the necessity of purifying intermediates. The general concept of this method depends on attachment of the first amino acid of the chain to a solid polymer by a covalent bond, followed by the addition of the succeeding protected amino acids, one at a time, in a stepwise manner until the desired sequence is assembled. Finally, the protected peptide is removed from the solid resin support and the protecting groups are cleaved off.

The amino acids may be attached to any suitable polymer. The polymer must be insoluble in the solvents used, must have a stable physical form permitting ready filtration, and must contain a functional group to which the first protected amino acid can be firmly linked by a covalent bond. Various polymers are suitable for this purpose, such as cellulose, polyvinyl alcohol, polymethylmethacrylate, and polystyrene.

Methods of Use

Provided herein is the use of the compounds of formula (I) in the treatment of diseases, such as cancer or neurodegenerative disease. Another aspect of the present invention is the use of the compounds of formula (I) in the treatment of diseases involving acidic or hypoxic diseased tissue, such as cancer. Hypoxia and acidosis are physiological markers of many disease processes, including cancer. In cancer, hypoxia is one mechanism responsible for development of an acid environment within solid tumors. As a result, hydrogen ions must be removed from the cell (e.g., by a proton pump) to maintain a normal pH within the cell. As a consequence of this export of hydrogen ions, cancer cells have an increased pH gradient across the cell membrane lipid bilayer and a lower pH in the extracellular milieu when compared to normal cells. One approach to improving the efficacy and therapeutic index of cytotoxic agents is to leverage this physiological characteristic to afford selective delivery of compound to hypoxic cells over healthy tissue.

In these methods of treatment, a therapeutically-effective amount of a compound of formula (I) or a pharmaceutically-acceptable salt thereof may be administered as a single agent or in combination with other forms of therapy, such as ionizing radiation or cytotoxic agents in the case of cancer. In combination therapy, the compound of formula (I) may be administered before, at the same time as, or after the other therapeutic modality, as will be appreciated by

those of skill in the art. Either method of treatment (single agent or combination with other forms of therapy) may be administered as a course of treatment involving multiple doses or treatments over a period of time.

Examples of cancers that are treatable using the compounds of the present disclosure include, but are not limited to, colorectal cancer, gastric cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, endometrial cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or urethra, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T -cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

In some embodiments, cancers treatable with compounds of the present disclosure include bladder cancer, bone cancer, glioma, breast cancer (e.g., triple-negative breast cancer), cervical cancer, colon cancer, colorectal cancer, endometrial cancer, epithelial cancer, esophageal cancer, Ewing's sarcoma, pancreatic cancer, gallbladder cancer, gastric cancer, gastrointestinal tumors, head and neck cancer (upper aerodigestive cancer), intestinal cancers, Kaposi's sarcoma, kidney cancer, laryngeal cancer, liver cancer (e.g., hepatocellular carcinoma), lung cancer (e.g., non-small cell lung cancer, adenocarcinoma), melanoma, prostate cancer, rectal cancer, renal clear cell carcinoma, skin cancer, stomach cancer, testicular cancer, thyroid cancer, and uterine cancer.

In some embodiments, cancers treatable with compounds of the present disclosure include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell

carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), breast cancer, triple-negative breast cancer, colon cancer and lung cancer (e.g. non-small cell lung cancer and small cell lung cancer). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the compounds of the disclosure.

In some embodiments, cancers that are treatable using the compounds of the present disclosure include, but are not limited to, solid tumors (*e.g.*, prostate cancer, colon cancer, esophageal cancer, endometrial cancer, ovarian cancer, uterine cancer, renal cancer, hepatic cancer, pancreatic cancer, gastric cancer, breast cancer, lung cancer, cancers of the head and neck, thyroid cancer, glioblastoma, sarcoma, bladder cancer, etc.), hematological cancers (*e.g.*, lymphoma, leukemia such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), DLBCL, mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma or multiple myeloma) and combinations of said cancers.

In certain embodiments, a compound of formula (I) or a pharmaceutically-acceptable salt thereof may be used in combination with a chemotherapeutic agent, a targeted cancer therapy, an immunotherapy or radiation therapy. The agents can be combined with the present compounds in a single dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms. In some embodiments, the chemotherapeutic agent, targeted cancer therapy, immunotherapy or radiation therapy is less toxic to the patient, such as by showing reduced bone marrow toxicity, when administered together with a compound of formula (I), or a pharmaceutically acceptable salt thereof, as compared with when administered in combination with the corresponding microtubule targeting agent (e.g., R^2-H).

Suitable chemotherapeutic or other anti-cancer agents include, for example, alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes) such as uracil mustard, chlormethine, cyclophosphamide (Cytoxan™), ifosfamide, melphalan, chlorambucil, pipobroman, triethylene-melamine, triethylenethiophosphoramine, busulfan, carmustine, lomustine, streptozocin, dacarbazine, and temozolomide.

Other suitable agents for use in combination with the compounds of the present invention include: dacarbazine (DTIC), optionally, along with other chemotherapy drugs such as carmustine (BCNU) and cisplatin; the "Dartmouth regimen," which consists of DTIC, BCNU, cisplatin and tamoxifen; a combination of cisplatin, vinblastine, and DTIC; or temozolomide. Compounds according to the invention may also be combined with immunotherapy drugs, including cytokines such as interferon alpha, interleukin 2, and tumor necrosis factor (TNF).

Suitable chemotherapeutic or other anti-cancer agents include, for example, antimetabolites (including, without limitation, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors) such as methotrexate, 5-fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable chemotherapeutic or other anti-cancer agents further include, for example, certain natural products and their derivatives (for example, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins) such as vinblastine, vincristine, vindesine, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, ara-C, paclitaxel (TAXOL™), mithramycin, deoxycoformycin, mitomycin-C, L-asparaginase, interferons (especially IFN-a), etoposide, and teniposide.

Other cytotoxic agents that can be administered in combination with the compounds of the invention include, for example, navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

Also suitable are cytotoxic agents such as, for example, epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes such as cis-platin and carboplatin; biological response modifiers; growth inhibitors; antihormonal therapeutic agents; leucovorin; tegafur; and haematopoietic growth factors.

Other anti-cancer agent(s) include antibody therapeutics such as trastuzumab (Herceptin), antibodies to costimulatory molecules such as CTLA-4, 4-1BB and PD-1, or antibodies to cytokines (IL-10, TGF-α, etc.).

Other anti-cancer agents also include those that block immune cell migration such as antagonists to chemokine receptors, including CCR2 and CCR4.

Other anti-cancer agents also include those that augment the immune system such as adjuvants or adoptive T cell transfer.

Anti-cancer vaccines that can be administered in combination with the compounds of the invention include, for example, dendritic cells, synthetic peptides, DNA vaccines and recombinant viruses.

Other suitable agents for use in combination with the compounds of the present invention include chemotherapy combinations such as platinum-based doublets used in lung cancer and other solid tumors (cisplatin or carboplatin plus gemcitabine; cisplatin or carboplatin plus docetaxel; cisplatin or carboplatin plus paclitaxel; cisplatin or carboplatin plus pemetrexed) or gemcitabine plus paclitaxel bound particles (Abraxane®).

Compounds of this invention may be effective in combination with anti-hormonal agents for treatment of breast cancer and other tumors. Suitable examples are anti-estrogen agents including but not limited to tamoxifen and toremifene, aromatase inhibitors including but not limited to letrozole, anastrozole, and exemestane, adrenocorticosteroids (e.g. prednisone), progestins (e.g. megastrol acetate), and estrogen receptor antagonists (e.g. fulvestrant). Suitable anti-hormone agents used for treatment of prostate and other cancers may also be combined with compounds of the present invention. These include anti-androgens including but not limited to flutamide, bicalutamide, and nilutamide, luteinizing hormone-releasing hormone (LHRH) analogs including leuprolide, goserelin, triptorelin, and histrelin, LHRH antagonists (e.g. degarelix), androgen receptor blockers (e.g. enzalutamide) and agents that inhibit androgen production (e.g. abiraterone).

Compounds of the present invention may be combined with or administered in sequence with other agents against membrane receptor kinases especially for patients who have developed primary or acquired resistance to the targeted therapy. These therapeutic agents include inhibitors or antibodies against EGFR, Her2, VEGFR, c-Met, Ret, IGFR1, or Flt-3 and against cancer-associated fusion protein kinases such as Bcr-Abl and EML4-Alk. Inhibitors against EGFR include gefitinib and erlotinib, and inhibitors against EGFR/Her2 include but are not limited to dacomitinib, afatinib, lapitinib and neratinib. Antibodies against the EGFR

include but are not limited to cetuximab, panitumumab and necitumumab. Inhibitors of c-Met may be used in combination with the compounds of the invention. These include onartumzumab, tivantnib, and INC-280. Agents against Abl (or Bcr-Abl) include imatinib, dasatinib, nilotinib, and ponatinib and those against Alk (or EML4-ALK) include crizotinib.

Angiogenesis inhibitors may be efficacious in some tumors in combination with compounds of the invention. These include antibodies against VEGF or VEGFR or kinase inhibitors of VEGFR. Antibodies or other therapeutic proteins against VEGF include bevacizumab and aflibercept. Inhibitors of VEGFR kinases and other anti-angiogenesis inhibitors include but are not limited to sunitinib, sorafenib, axitinib, cediranib, pazopanib, regorafenib, brivanib, and vandetanib

Activation of intracellular signaling pathways is frequent in cancer, and agents targeting components of these pathways have been combined with receptor targeting agents to enhance efficacy and reduce resistance. Examples of agents that may be combined with compounds of the present invention include inhibitors of the PI3K-AKT-mTOR pathway, inhibitors of the Raf-MAPK pathway, inhibitors of JAK-STAT pathway, and inhibitors of protein chaperones and cell cycle progression.

Agents against the PI3 kinase include but are not limited topilaralisib, idelalisib, buparlisib. Inhibitors of mTOR such as rapamycin, sirolimus, temsirolimus, and everolimus may be combined with compounds of the invention. Other suitable examples include but are not limited to vemurafenib and dabrafenib (Raf inhibitors) and trametinib, selumetinib and GDC-0973 (MEK inhibitors). Inhibitors of one or more JAKs (e.g., ruxolitinib, baricitinib, tofacitinib), Hsp90 (e.g., tanespimycin), cyclin dependent kinases (e.g., palbociclib), HDACs (e.g., panobinostat), PARP (e.g., olaparib), and proteasomes (e.g., bortezomib, carfilzomib) can also be combined with compounds of the present invention. A further example of a PARP inhibitor that can be combined with a compound of the invention is talazoparib.

Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR, e.g., 1996 edition, Medical Economics

Company, Montvale, NJ), the disclosure of which is incorporated herein by reference as if set forth in its entirety.

The phrase "therapeutically effective amount" of a compound (therapeutic agent, active ingredient, drug, etc.) refers to an amount of the compound to be administered to a subject in need of therapy or treatment which alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions, according to clinically acceptable standards for the disorder or condition to be treated. For instance, a therapeutically effective amount can be an amount which has been demonstrated to have a desired therapeutic effect in an *in vitro* assay, an *in vivo* animal assay, or a clinical trial. The therapeutically effective amount can vary based on the particular dosage form, method of administration, treatment protocol, specific disease or condition to be treated, the benefit/risk ratio, etc., among numerous other factors.

Said therapeutically effective amount can be obtained from a clinical trial, an animal model, or an *in vitro* cell culture assay. It is known in the art that the effective amount suitable for human use can be calculated from the effective amount determined from an animal model or an *in vitro* cell culture assay. For instance, as reported by Reagan-Shaw et al., FASEB J. 2008: 22(3) 659-61, "μg/ml" (effective amount based on *in vitro* cell culture assays) = "mg/kg body weight/day" (effective amount for a mouse). Furthermore, the effective amount for a human can be calculated from the effective amount for a mouse based on the fact that the metabolism rate of mice is 6 times faster than that of humans.

As an example of treatment using a compound of formula (I) in combination with a cytotoxic agent, a therapeutically-effective amount of a compound of formula (I) may be administered to a patient suffering from cancer as part of a treatment regimen also involving a therapeutically-effective amount of ionizing radiation or a cytotoxic agent. In the context of this treatment regimen, the term "therapeutically-effective" amount should be understood to mean effective in the combination therapy. It will be understood by those of skill in the cancer-treatment field how to adjust the dosages to achieve the optimum therapeutic outcome.

Similarly, the appropriate dosages of the compounds of the invention for treatment of non-cancerous diseases or conditions (such as cardiovascular diseases) may readily be determined by those of skill in the medical arts.

The term "treating" as used herein includes the administration of a compound or composition which reduces the frequency of, delays the onset of, or reduces the progression of symptoms of a disease involving acidic or hypoxic diseased tissue, such as cancer, stroke, myocardial infarction, or long-term neurodegenerative disease, in a subject relative to a subject not receiving the compound or composition. This can include reversing, reducing, or arresting the symptoms, clinical signs, or underlying pathology of a condition in a manner to improve or stabilize a subject's condition (e.g., regression of tumor growth, for cancer or decreasing or ameliorating myocardial ischemia reperfusion injury in myocardial infarction, stroke, or the like cardiovascular disease). The terms "inhibiting" or "reducing" are used for cancer in reference to methods to inhibit or to reduce tumor growth (e.g., decrease the size of a tumor) in a population as compared to an untreated control population.

All publications (including patents) mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the constructs and methodologies that are described in the publications, which might be used in connection with the disclosure herein described. The publications discussed throughout the text are provided solely for their disclosure prior to the filing date of the present application.

Disclosed herein are several types of ranges. When a range of any type is disclosed or claimed, the intent is to disclose or claim individually each possible number that such a range could reasonably encompass, including end points of the range as well as any sub-ranges and combinations of sub-ranges encompassed therein. When a range of therapeutically effective amounts of an active ingredient is disclosed or claimed, for instance, the intent is to disclose or claim individually every possible number that such a range could encompass, consistent with the disclosure herein. For example, by a disclosure that the therapeutically effective amount of a compound can be in a range from about 1 mg/kg to about 50 mg/kg (of body weight of the subject).

Formulation, Dosage Forms and Administration

To prepare the pharmaceutical compositions of the present invention, a compound of Formula (I) or a pharmaceutically-acceptable salt thereof is combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical

compounding techniques, which carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations such as for example, suspensions, elixirs, and solutions; or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like in a case of oral solid preparations, such as for example, powders, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar coated or enteric coated by standard techniques. For parenterals, the carrier will usually comprise sterile water, although other ingredients, for example, to aid solubility or for preservative purposes, may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents, and the like may be employed. One of skill in the pharmaceutical and medical arts will be able to readily determine a suitable dosage of the pharmaceutical compositions of the invention for the particular disease or condition to be treated.

EXAMPLES

As used herein, all abbreviations, symbols and conventions are consistent with those used in the contemporary scientific literature. See, e.g., Janet S. Dodd, ed., *The ACS Style Guide: A Manual for Authors and Editors*, 2nd Ed., Washington, D.C.: American Chemical Society, 1997. The following definitions describe terms and abbreviations used herein:

- Brine: a saturated NaCl solution in water
- DCM: dichloromethane
- TFA: trifluoroacetic acid
- DIPEA: diisopropylethylamine
- DMA: dimethylacetamide
- DME: dimethoxyethane
- DMF: dimethylformamide
- DMSO: methylsulfoxide

- DTT: dithiothreitol
- MSD: mass spec detector
- Et_2O: ethyl ether
- EtOAc: ethyl acetate
- EtOH: ethyl alcohol
- HATU: O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate
- HOBt: 1-hydroxybenzotriazole
- RP: reverse phase
- HPLC: high performance liquid chromatography
- IPA: isopropanol
- LAH: lithium aluminum hydride
- N-BuLi: n-butyl lithium
- LC-MS: liquid chromatography-mass spectrometry
- LDA: lithium diisoproylethylamide
- Me: methyl
- MeOH: methanol
- MTBE: methyl t-butyl ether
- NMP: N-methylpyrrolidine
- Ph: phenyl
- PNPC: para-nitrophenylchloroformate
- RT or rt: room temperature
- SFC: supercritical fluid chromatography
- TBAI: tetrabutylammonium iodide
- TBME: tert-butylmethyl ether
- tBu: tertiary butyl
- THF: tetrahydrofuran
- TEA: triethylamine
- TMEDA: tetramethylethylenediamine
- GSH: Glutathione

- GS: Glutathione bonded at sulfur
- LiOH: lithium hydroxide
- DPPA: diphenyl phosphoryl azide
- $Sn(Bu)_2(Laurate)_2$: dibutyltin dilaurate
- PBS: phosphate buffered saline
- ACN: acetonitrile
- AcOH: acetic acid
- EEDQ: *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
- DMAP: 4-dimethylaminopyridine
- EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

The HPLC methods employed are set forth below.

HPLC Methods

A: Sunfire C18 150x4.6mm; H_2O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 1ml/min; Wavelength = 217 nM.

B: Ace Equivalence 250x4.6mm; H_2O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 1ml/min; Wavelength = 217 nM.

C: Sunfire C18 150x30mm; H_2O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 30ml/min; Wavelength = 217 nM.

D: Sunfire C18 150x4.6mm; H_2O/Acetonitrile w/ AcOH modifier (0.5%); Flow rate: 1ml/min; Wavelength = 217 nM.

E: Sunfire C18 150x30mm; H_2O/Acetonitrile w/ AcOH modifier (0.5%); Flow rate: 30ml/min; Wavelength = 217 nM.

F: Agilent 1100/1200/1260 or 1290 systems (coupled or uncoupled with MS).

HPLC Parameters	
Mobile Phase A	0.1% AcOH in water
Mobile Phase B	0.1% AcOH in ACN
Column	Merck Chromolith RP-18e
Column Temperature	rt

Autosampler Temperature	rt		
Injection Volume	5 µL		
Flow Rate	1 mL/minute		
Wavelength	Agilent diode array detector at λ = 254, 220 or 280 nm		
Gradient Program	Time (min)	% A	% B
	Initial	95.00	5.00
	4.00	5.00	95.00
	4.99	5.00	95.00
	5.00	95.00	5.00
	6.00	95.00	5.00
Run Time	6.00		

G: Agilent 1100/1200/1260 or 1290 systems (coupled or uncoupled with MS).

HPLC Parameters			
Mobile Phase A	0.1% TFA in water		
Mobile Phase B	0.1% TFA in ACN		
Column	Agilent Eclipse XDB C8 column (3.5 µm, 4.6 x 150 mm)		
Column Temperature	40 °C		
Autosampler Temperature	rt		
Injection Volume	5 µL		
Flow Rate	1.5 mL/minute		
Wavelength	Agilent diode array detector at λ = 254, 220 or 280 nm		
Gradient Program	**Time (min)**	**% A**	**% B**
	Initial	80.00	20.00
	0.20	80.00	20.00
	7.50	20.00	80.00
	8.00	0.00	100.00
	9.00	0.00	100.00
	10.00	80.00	20.00
Run Time	10.00		

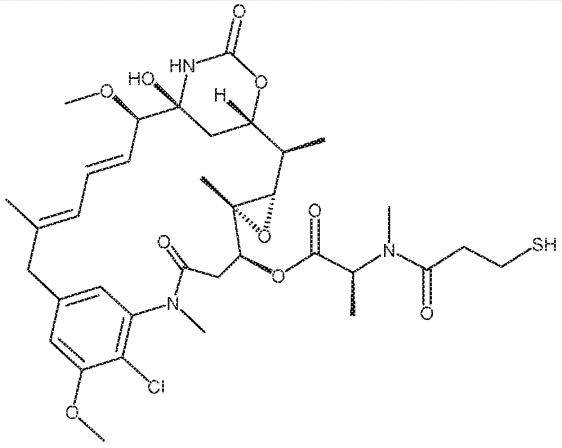
Mass Spectrometry Methods

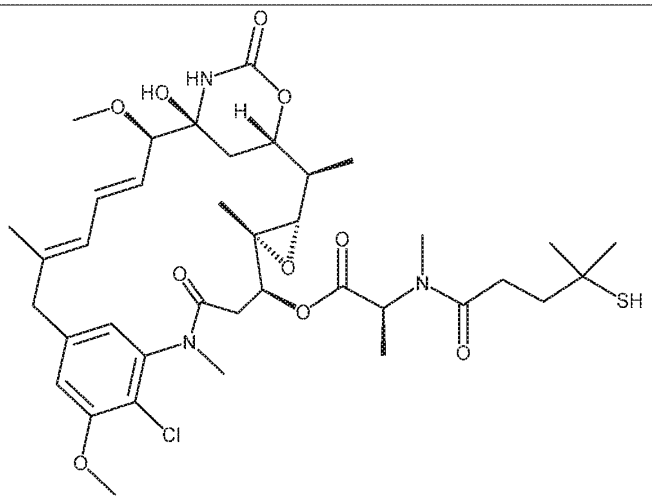
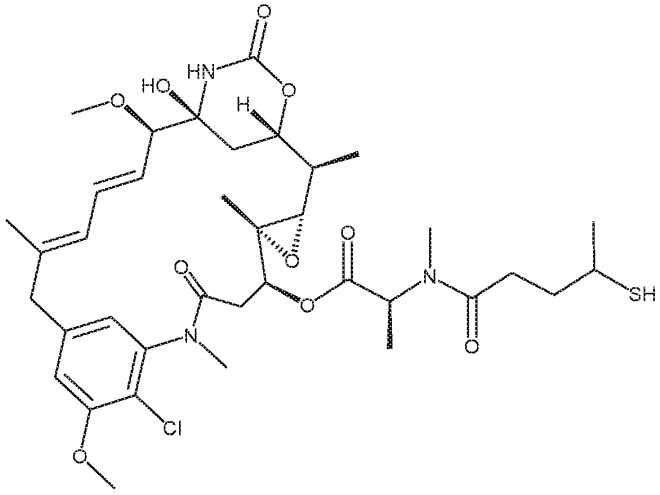
Maldi-TOF (Matrix-assisted laser desorption/ionization-Time of Flight) mass spectrometry was measured on an Applied Biosystems Voyager System 6268. The sample was prepared as a matrix of α-cyano hydroxy cinnamic acid on an AB Science plate (Part# V700666).

ESI (Electrospray Ionization) mass spectrometry was measured on either an Agilent 1100 series LC-MS with a 1946 MSD or a Waters Xevo Qtof high-resolution MS, both providing a mass/charge species (m/z=3).

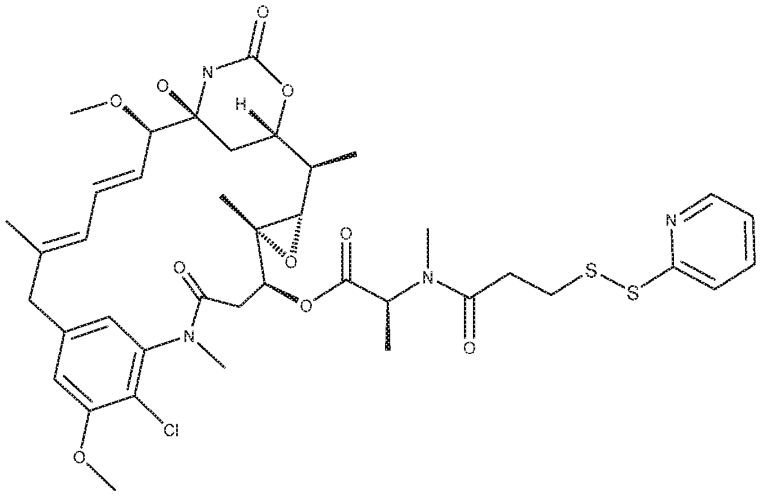
The source of the starting materials employed in the Examples are set forth below in the following tables.

Table 2. Starting materials for R^2

R^2 Code	R^2H Structure	Synthesis, Reference or Purchased
R^2SH-1		MedKoo 123212

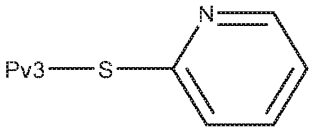
R^2SH-2		MedKoo 206839
R^2SH-3		US 20040235840 A1

Synthesis of Intermediate I (R^2S-S-Pyr)



To [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[methyl(3-sulfanylpropanoyl)amino]propanoate (46.7 mg, 0.06 mmol) in 1 mL of CH_3CN was added 2-(2-pyridyldisulfanyl)pyridine (20.0 mg, 0.09 mmol). The mixture was concentrated and purified (SiO_2, 0-10% MeOH/CH_2Cl_2) to give [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[methyl-[3-(2-pyridyldisulfanyl)propanoyl]amino]propanoate (53.6 mg, yield: 100 %). MS m/z 847.1 $[M+H]^+$.

Synthesis of Pv3-S-Pyr (Intermediate II-3)



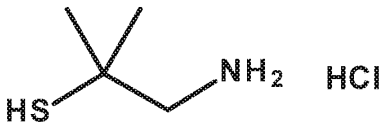
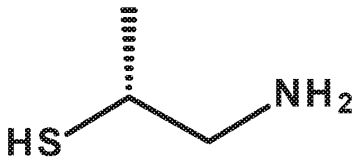
Pv3 (250 mg, 0.06 mmol; as a free flowing solid) and 2-(2-pyridyldisulfanyl)pyridine (0.110 g, 0.5 mol) were dissolved in MeOH (10 mL) and the reaction stirred overnight at room temperature. LC-MS indicates the desired product was formed. The reaction mixture

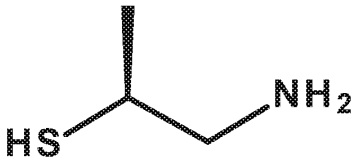
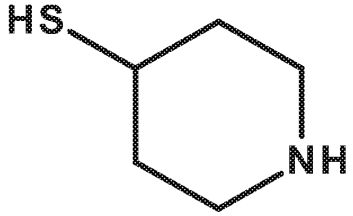
was concentrated and the residue taken up in DMSO and purified by reverse phase column chromatography (40-65% CH_3CN/H_2O (0.5% AcOH), 13 min) to give 212 mg of the desired product (187 mg, yield: 74.9 %). MS m/z=3 1273.4.

Intermediates II-1, II-2 and II-6 were prepared analogously to II-3, using Pv1, Pv2, and Pv6, as shown below:

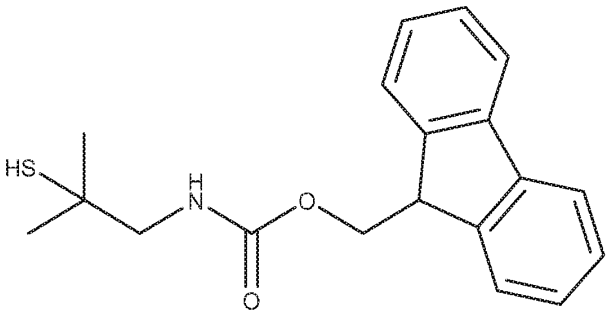
Intermediate	Structure	**MS** **A: Maldi-TOF** **B: m/z=3**
II-1	Pv1-SPyr	B: 1130.1
II-2	Pv2-SPyr	B: 1373.9
II-3	Pv3-SPyr	B: 1273.4
II-6	Pv6-SPyr	B: 1450.3

Table 3. Starting materials for L groups

Intermediate	Structure	Purchased, Reference or Synthesized
III-1		Enamine EN3000-33931
III-2		Astatech 39541
III-3		Enamine EN3000-6731388

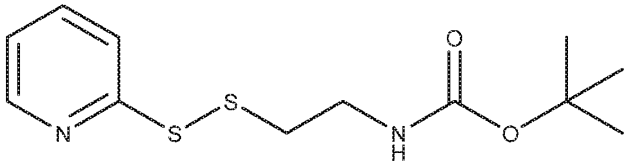
III-4		Enamine EN3000-6731596
III-5		Astatech 39018

Synthesis of Intermediate VI-2



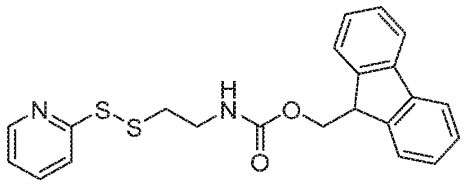
1-Amino-2-methyl-propane-2-thiol hydrochloride (100 mg, 0.706 mmol) was dissolved in CH_2Cl_2 (7 mL) and to it was added 9H-fluoren-9-ylmethyl carbonochloridate (274 mg, 1.06 mmol) and *N,N*-diisopropylethylamine (182 mg, 1.41 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with water and concentrated. The residue was purified by column chromatography (0-50% EtOAc/hexanes) to give 9H-fluoren-9-ylmethyl N-(2-methyl-2-sulfanyl-propyl)carbamate (213 mg, yield: 92.2 %). MS m/z 350.1 $[M+Na]^+$.

Synthesis of Intermediate V-1



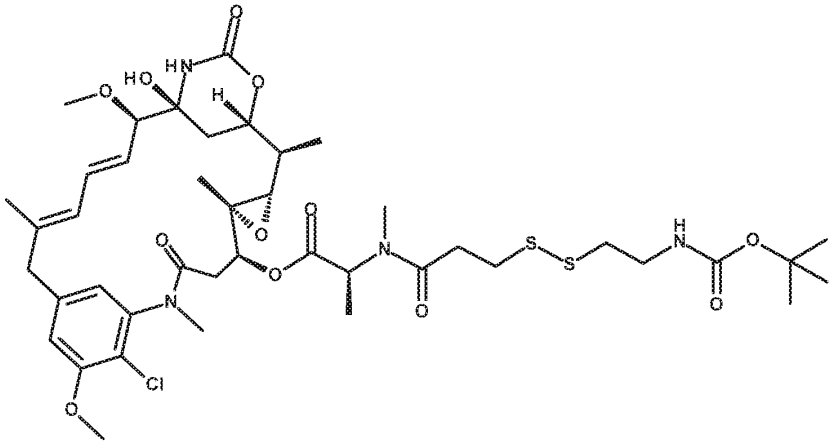
2-(2-Pyridyldisulfanyl)pyridine (746 mg, 3.38 mmol) was dissolved in MeOH (15 mL) and to it was added tert-butyl N-(2-sulfanylethyl)carbamate (200 mg, 1.13 mmol). The reaction was stirred for 3 h at room temperature. The mixture was concentrated and the residue purified by column chromatography (0-50% EtOAc/hexanes) to give tert-butyl N-[2-(2-pyridyldisulfanyl)ethyl]carbamate (200 mg, yield: 61.9 %). MS m/z 287.1 $[M+H]^+$.

Synthesis of Intermediate V-2



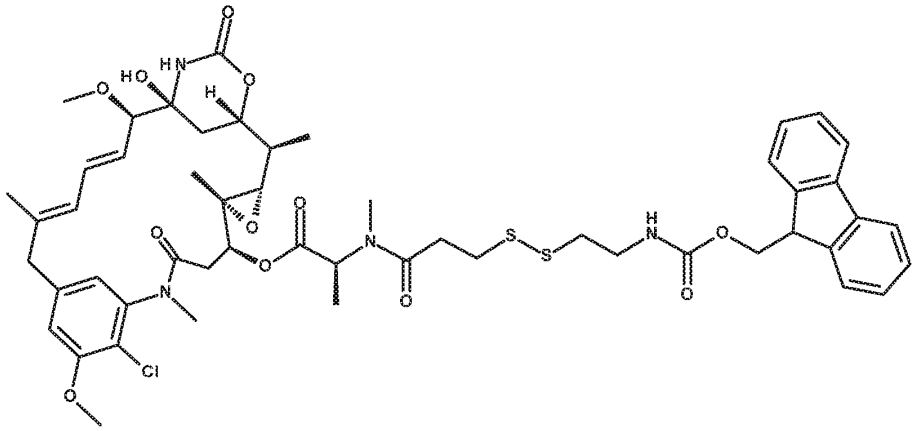
2-(2-Pyridyldisulfanyl)ethanamine hydrochloride (200 mg, 0.898 mmol) was dissolved in CH_2Cl_2 and to it was added 9H-fluoren-9-ylmethyl carbonochloridate (348 mg, 1.35 mmol) and *N,N*-diisopropylethylamine (232 mg, 1.80 mmol). The reaction mixture was stirred at RT for 2h, washed with water and concentrated. The residue was purified by column chromatography (0-50% EtOAc/hexanes) to give 9H-fluoren-9-ylmethyl N-[2-(2-pyridyldisulfanyl)ethyl]carbamate (288 mg, yield: 78.5 %). MS m/z 409.1 $[M+H]^+$.

Synthesis of Intermediate VII-1



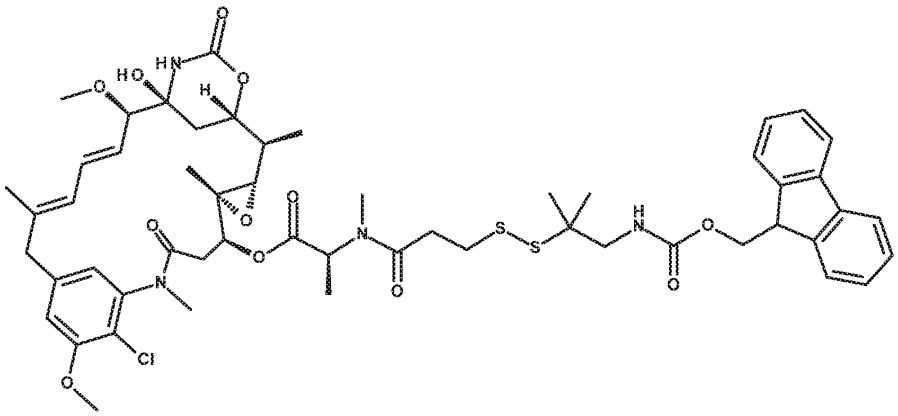
To a vial containing [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[methyl(3-sulfanylpropanoyl)amino]propanoate (25.0 mg, 0.03 mmol) in 1 mL of CH_3CN was added tert-butyl N-[2-(2-pyridyldisulfanyl)ethyl]carbamate (Intermediate V-1, 14.5 mg, 0.051 mmol) and 4-methylmorpholine (0.138 mL, 1.25 mmol). The mixture was stirred for 16h. LC-MS analysis indicates the desired material. The mixture was concentrated, dissolved in 50 mL of EtOAc and washed with 1x25 mL of sat. NH_4Cl and 1x25 mL of sat. brine. The organic phase was dried with $MgSO_4$, filtered and concentrated. The crude resifue was purified (SiO_2, 0-100% EtOAc/hexanes) to give [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-(tert-butoxycarbonylamino)ethyldisulfanyl]propanoyl-methyl-amino]propanoate (30.9 mg, yield: 100 %). MS m/z 913.2 $[M+H]^+$.

Synthesis of Intermediate VII-2



Intermediate VII-2 was prepared analogously to VII-1, using Intermediate V-2 in place of Intermediate V-1.

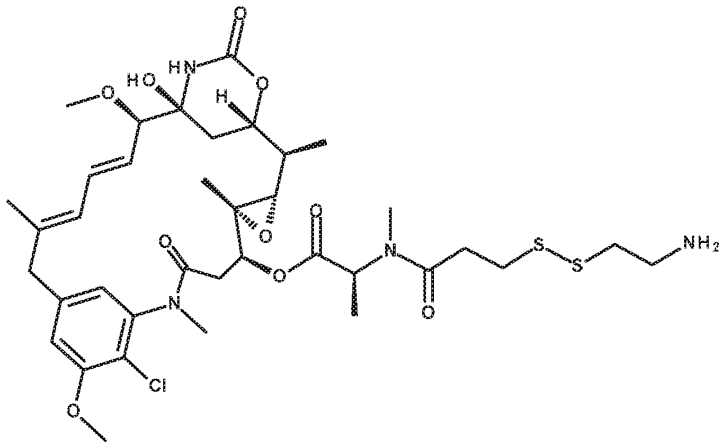
Synthesis of Intermediate VII-3



To a vial containing [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyldisulfanyl]propanoyl-methyl-amino]propanoate (25.0 mg, 0.03 mmol) in 1 mL of CH_3CN was added 9H-fluoren-9-ylmethyl N-(2-methyl-2-

sulfanyl-propyl)carbamate (14.5 mg, 0.044 mmol) and 4-methylmorpholine (0.120 mL, 1.09 mmol). The mixture was stirred for 16h. LC-MS analysis indicated the desired material was formed. The mixture was concentrated, dissolved in 50 mL of EtOAc and washed with 1x25 mL of sat. NH_4Cl and 1x25 mL of sat. brine. The organic phase was dried with $MgSO_4$, filtered and concentrated. The crude residue was purified (SiO_2, 0-100% EtOAc/hexanes) to [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)-1,1-dimethyl-ethyl]disulfanyl]propanoyl-methyl-amino]propanoate (0.0313 g, yield: 100 %). MS m/z 1085.0 $[M+Na]^+$.

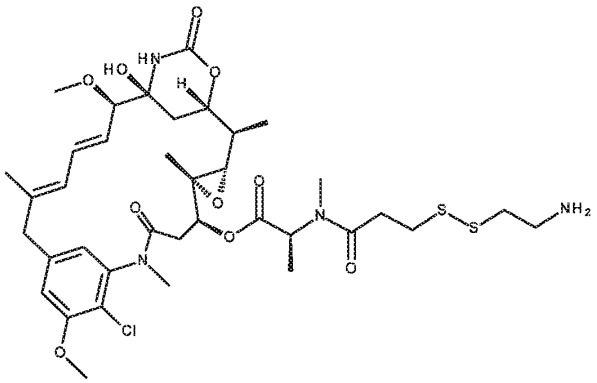
Synthesis of Intermediate VIII-1 (BOC Deprotection)



[(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-(tert-butoxycarbonylamino)ethyldisulfanyl]propanoyl-methyl-amino]propanoate (31.9 mg, 0.05 mmol) was dissolved in 0.3/0.1/0.1 mL of CH_3CN/H_2O/TFA. The mixture was stirred for 36 h. LC-MS indicated complete deprotection. The mixture was purified by prep HPLC (20-95% CH_3CN/H_2O w/ 0.05%TFA) to give [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-

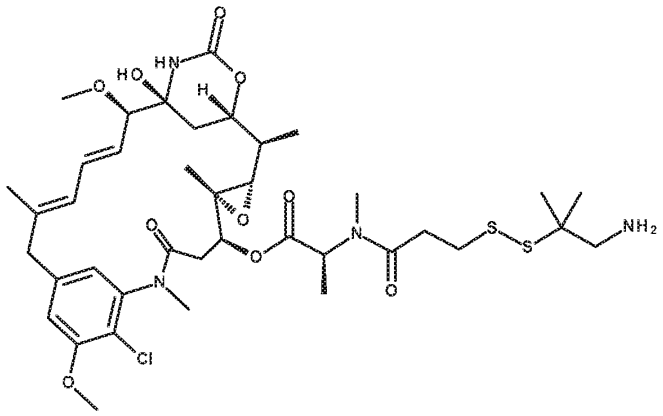
diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-(2-aminoethyldisulfanyl)propanoyl-methyl-amino]propanoate;2,2,2-trifluoroacetic acid (22.9 mg, yield: 70.7 %). MS m/z 813.2 $[M+H]^+$.

Alternative Synthesis of Intermediate VIII-1 (FMOC Deprotection)



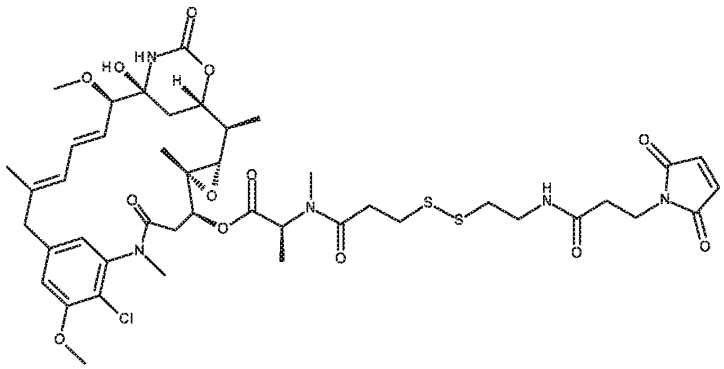
To a vial containing [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-(9H-fluoren-9-ylmethoxycarbonylamino)ethyldisulfanyl]propanoyl-methyl-amino]propanoate (Intermediate VII-2; 29.6 mg, 0.03 mmol) was added 0.5 mL of DMF and 4-methylmorpholine (0.120 mL, 1.09 mmol). The mixture was heated for 16 h at 40 °C. LC-MS confirmed complete deprotection. The mixture was purified by (20-95% CH_3CN/H_2O w/ 0.05%TFA) to give [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-(2-aminoethyldisulfanyl)propanoyl-methyl-amino]propanoate trifluoroacetate (22.9 mg, yield: 86.4 %). MS m/z 813.2 $[M+H]^+$.

Synthesis of Intermediate VIII-2



Intermediate VIII-2 was prepared analogously to Intermediate VIII-1. MS m/z 841.2 $[M+H]^+$.

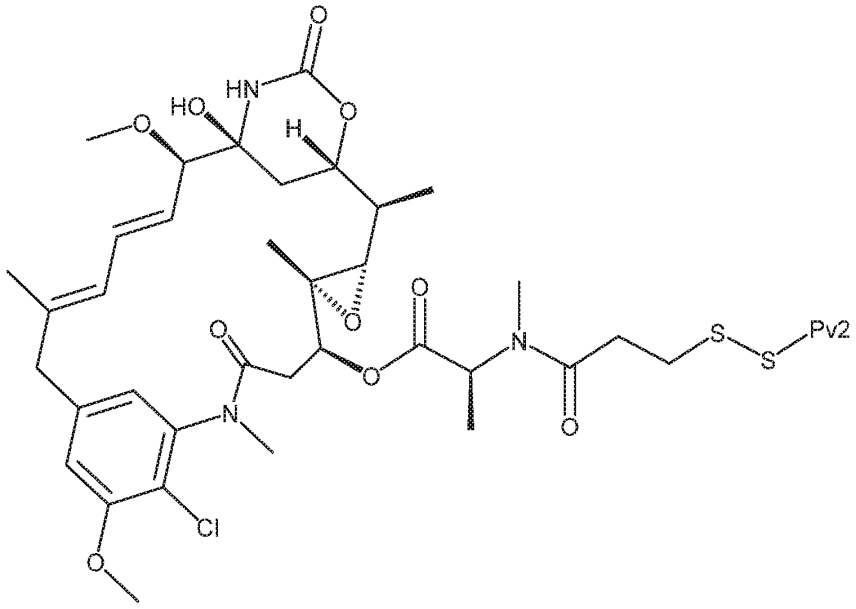
Synthesis of Intermediate X-1



To [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-(2-aminoethyldisulfanyl)propanoyl-methyl-amino]propanoate trifluoroacetate (Intermediate VIII-1; 45.8 mg, 0.05 mmol) in 1 mL of DMF was added 3-(2,5-dioxopyrrol-1-yl)propanoic acid (12.5 mg, 0.074 mmol), TBTU (23.8 g, 0.074 mmol) and DIPEA (0.0169 mL, 0.1 mmol). LC-MS indicated complete conversion to the product. The mixture was diluted with 50 mL of EtOAc. This was washed with 1x25mL sat

NH_4Cl, 4x25mL H_2O and 1x25 mL of H_20. The organic phase was dried with $MgSO_4$, filtered and concentrated. The crude product was purified (SiO_2, 0-10% MeOH/CL_2Cl_2) to give [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethyldisulfanyl]propanoyl-methyl-amino]propanoate (17.3 mg, yield: 36.5 %) MS m/z 986.1 $[M+Na]^+$.

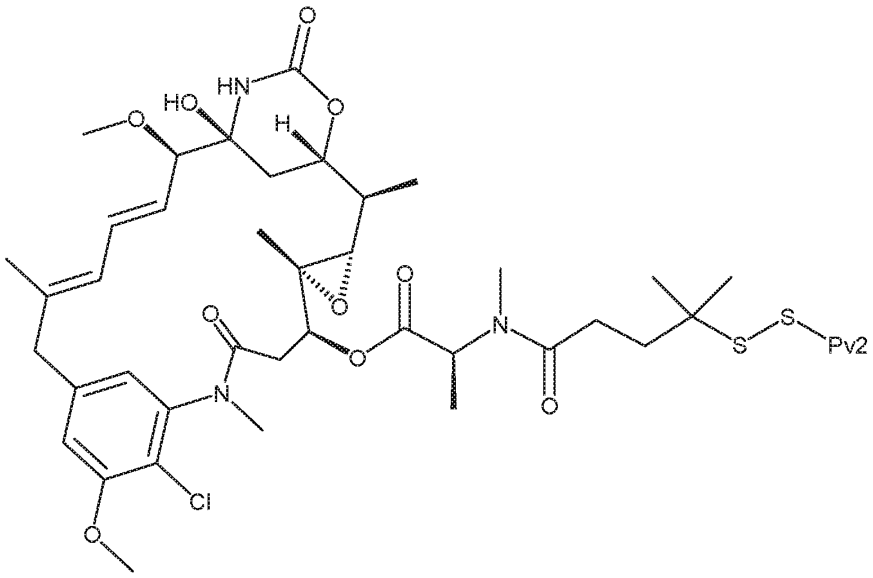
Example 2: Synthesis of Compound 2



To a vial containing Pv2 (25.0 mg, 0.006 mmol; as a free flowing solid) and [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[methyl-[3-(2-pyridyldisulfanyl)propanoyl]amino]propanoate (7.70 mg, 0.009 mmol) was added 1 mL of degassed DMF and 0.5 mL degassed H_2O. To this was added CH_3CO_2H (0.0103 mL, 0.180 mmol). The mixture was stirred for 72 h. LC-MS indicated formation of desired product. The

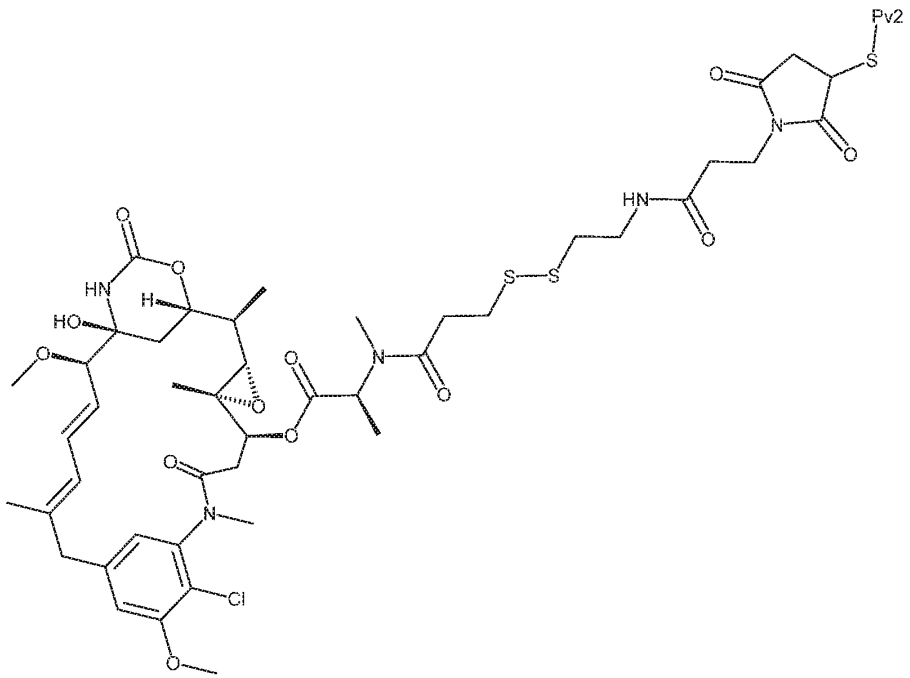
mixture was purified by prep HPLC (Sunfire C18 150x30 mm; 20-77% H_2O/Acetonitrile w/ 0.5% AcOH modifier; 15 min run; Flow rate: 30ml/min; Wavelength = 217 nM) to give the desired conjugate (17.0 mg, yield: 59.1 %).

Example 6: Synthesis of Compound 6



To a vial containing Pv2-SPyr (Intermediate II-2; 27.0 mg, 6.55e-6 mol) and [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10,12,14(26),16,18-pentaen-6-yl] (2S)-2-[methyl-(4-methyl-4-sulfanyl-pentanoyl)amino]propanoate (7.67 mg, 0.01 mmol). To this was added 1 mL of degassed DMF and 0.5 mL degassed H_2O. To this was added CH_3CO_2H (0.015 mL, 0.262 mmol). The mixture was stirred for 72 h. LC-MS indicated formation of desired product. The mixture was purified by prep HPLC (Sunfire C18 150x30 mm; 20-80% H_2O/Acetonitrile w/ 0.5% AcOH modifier; 16 min run; Flow rate: 30ml/min; Wavelength = 217 nM) to give the desired conjugate (11.4 g, yield: 36.6 %).

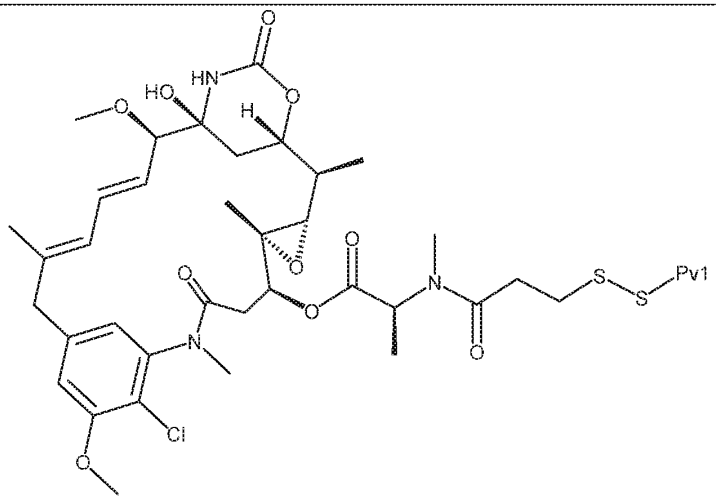
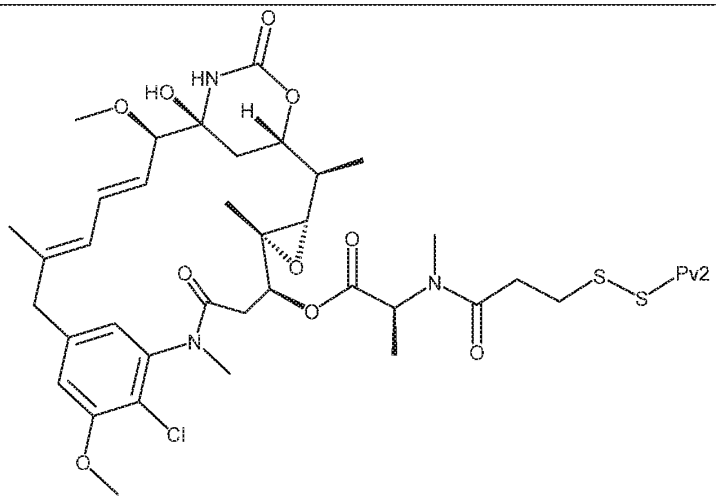
Example 9. Synthesis of Compound 9

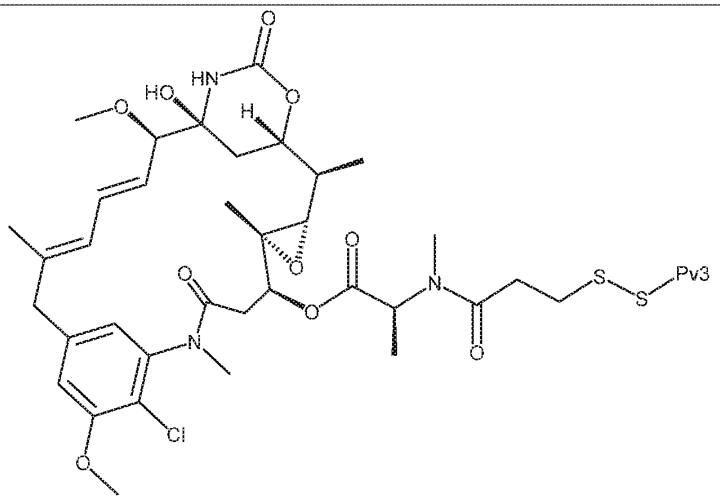
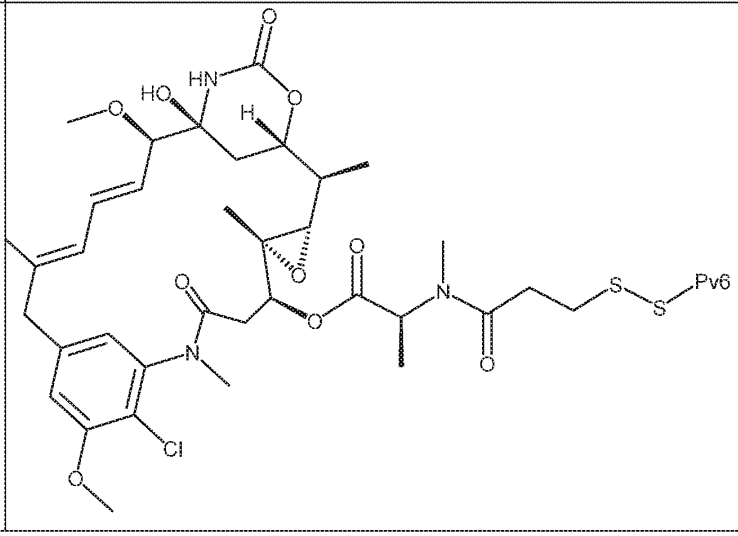


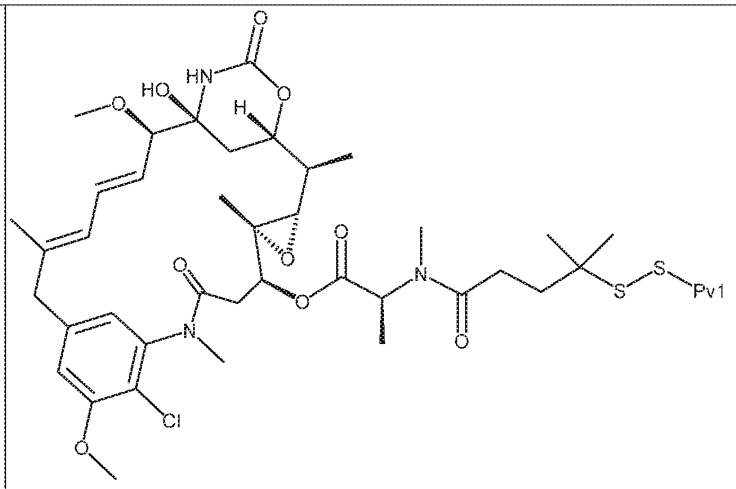
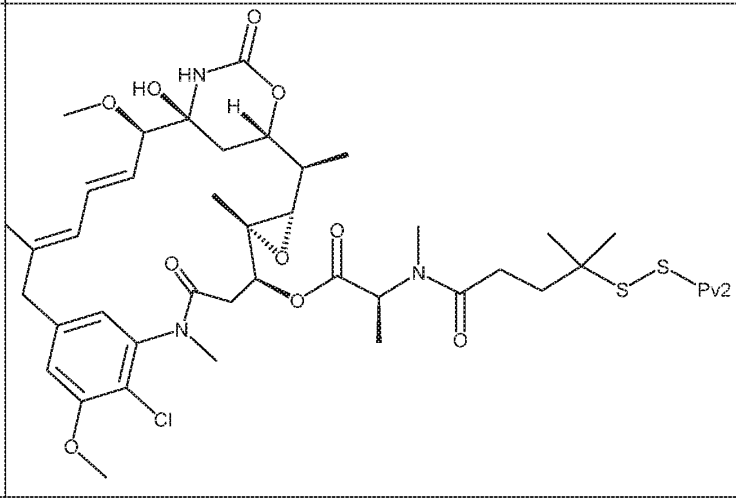
To a vial containing Pv2 (25.0 mg, 0.006 mol; as a free flowing solid) and [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10(26),11,13,16,18-pentaen-6-yl] (2S)-2-[3-[2-[3-(2,5-dioxopyrrol-1-yl)propanoylamino]ethyldisulfanyl]propanoyl-methyl-amino]propanoate (Intermediate X-1; 0.00877 g, 0.01 mmol) was added 1 mL of CH_3CN. The mixture was heterogeneous. To this was added 0.5 mL of CH_3CN, 0.5 mL of H_2O and 0.5 mL of MeOH. Homogeneity was not achieved. The mixture was stirred rigorously for 72 h. LC-MS indicated formation of desired product. The mixture was purified by prep HPLC (Sunfire C18 150x30 mm; 45-61% H_2O/Acetonitrile w/ 0.05% TFA modifier; 13 min run; Flow rate: 30ml/min; Wavelength = 217 nM) to give desired conjugate (21.1 mg, yield: 70.0 %).

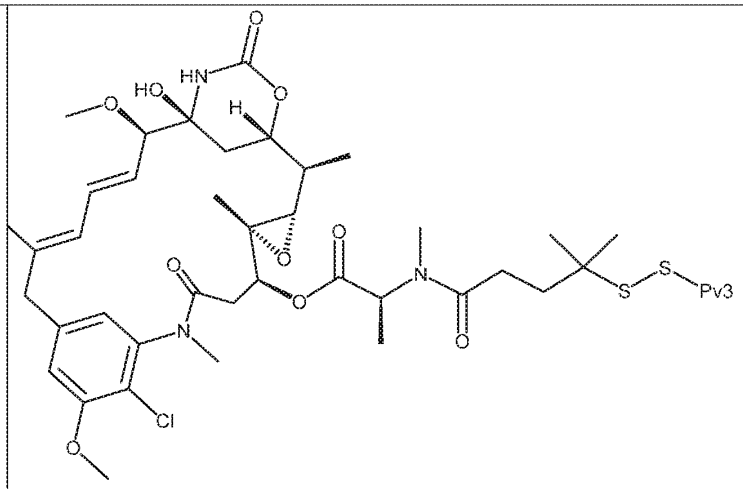
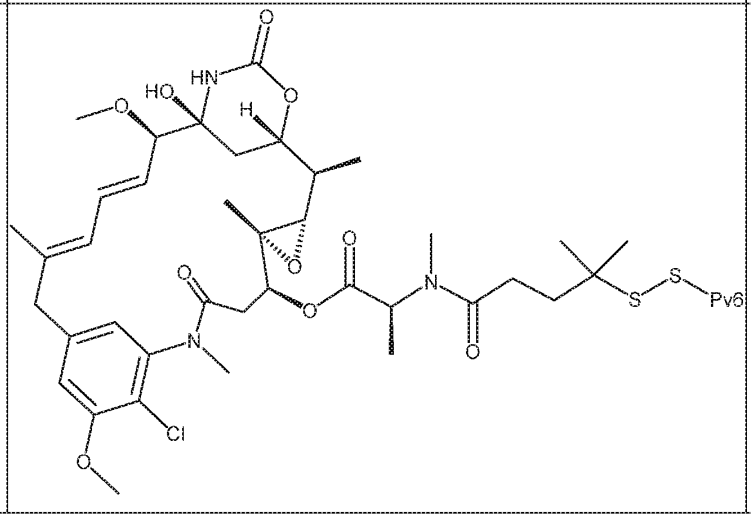
Compounds 1, 3, and 4 were synthesized analogously to the compound of Compound 2, using Pv1, Pv3, and Pv4, respectively. Compounds 5, 7, and 8 were synthesized analogously to Compound 6, using Intermediates II-1, II-3, and II-6, respectively.

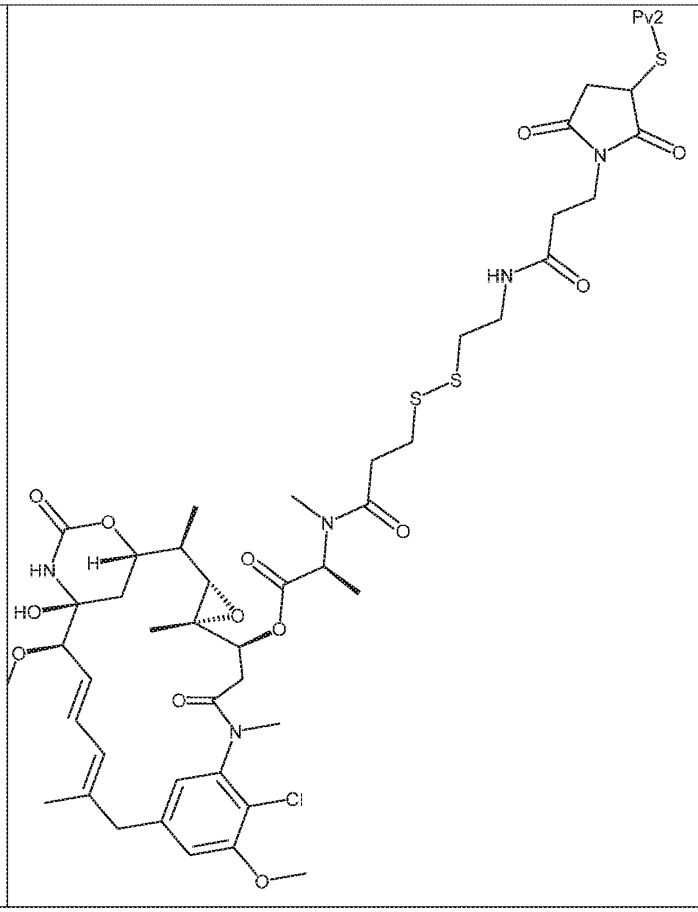
Table 4. Example Compounds

Comp-ound	Structure	MS A: Maldi-TOF (M+) B: ESI (m/z=3)	Conditions %ACN/H_2O Run Time RT
1		B: 1339.3	D 20-95% 11min 7.28min
2		B: 1582.7	A 20-95% 11min 7.0min

3		B: 1483.8	D 20-95% 11min 7.82min
4		B: 1659.4	A 20-95% 11min 6.92min

5		B: 1352.8	A 20-95% 11min 6.81min
6		B: 1597.0	A 20-95% 11min 7.44min

7		**B:** **1487.1**	**A** **20-95%** **11min** **6.60min**
8		**B:** **1673.4**	**A** **20-95%** **11min** **7.14min**

9		B: 1659.4	A 20-95% 11min 7.16min

Example 5: Detailed Synthesis of Compound 5

To Pv1 (50.0 mg, 1.48e-5 mol) and [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10,12,14(26),16,18-pentaen-6-yl] (2S)-2-[methyl-(4-methyl-4-sulfanyl-pentanoyl)amino]propanoate (0.0150 g, 1.92e-5 mol) in 3 mL of 2:1 CH_3CN/H_2O was added N-methylmorpholine (0.0600 mL, 0.000546 mol). The mixture was stirred for 36 h. LC-MS analysis indicated formation of the desired material. The mixture was purified by Gilson prep HPLC (Sunfire C18 30x150mm; 20-80% CH_3CN/H_2O w/ 0.05% TFA; 16 min run; 13.5 min) to give desired conjugate. The mixture was purified by Gilson prep HPLC (Sunfire C18 30x150mm; 20-72% CH_3CN/H_2O w/ 0.05% TFA; 15 min run; 12.5 min; retention

time: 6.847 min) to give Compound 5 (0.0322 g, 7.94e-6 mol, yield: 53.8 %). ESI (m/z=3): 1352.8.

Example 5a: Alternative synthesis of Compound 5

Step 1. Preparation of Pv1-S-Pyridyl

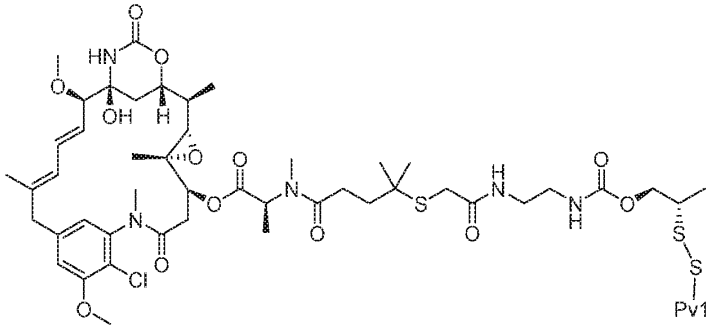
Peptide Pv1 and 2,2'-dipyridyl disulfide were dissolved in MeOH and the reaction was stirred overnight. LC-MS indicated the desired product was formed. The reaction mixture was concentrated and the residue was taken up in DMSO and purified by reverse phase column chromatography (40-75% ACN/H2O (0.5% AcOH), 15 min) to give 212 mg of the desired product.

Step 2. Preparation of Compound 5

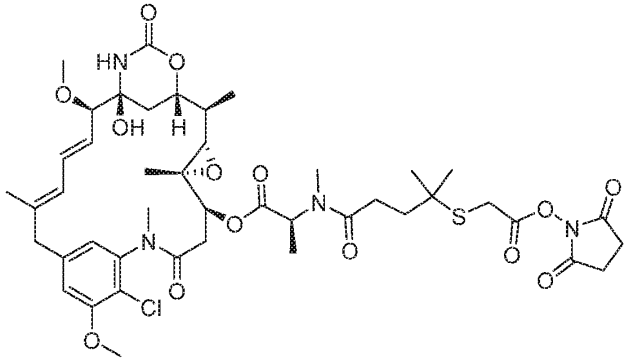
To a vial containing Pv1-SPyr (25.0 mg, 736e-6 mol) and [(1S,2R,3S,6S,16E,18E,20R,21S)-11-chloro-21-hydroxy12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa10,12,14(26),16,18-pentaen-6-yl] (2S)-2-[methyl-(4-methyl-4-sulfanyl-pentanoyl)amino]propanoate (0.00864 g, 1.11e-5 mol). To this mixture was added 1 mL of degassed DMF and 0.5 mL degassed H_2O. CH_3CO_2H (0.017 mL, 0.000295 mol) was added. The mixture was stirred for 72 h. LC-MS indicated formation of the desired product. The mixture was purified by Gilson prep HPLC

(Sunfire C18 30x150mm; 20-80 CH3CN/H2O w/ 0.5% AcOH; 16 min run; 12.9 min) to give Compound 5 (0.00750 g, 1.85e-6 mol, yield: 25.1 %).

Example 10: Synthesis of Compound 10



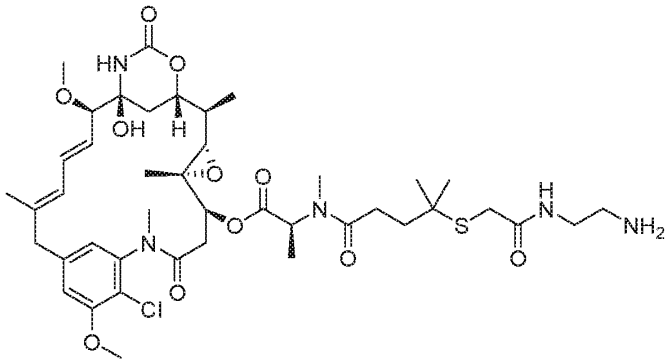
Step 1. (1⁴S,1⁶S,3²S,3³S,2R,4S,10E,12E,14R)-8⁶-chloro-1⁴-hydroxy-8⁵,14-dimethoxy-3³,2,7,10-tetramethyl-1²,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate



180 mg of DM4 (0.23 mmol) and 57 mg of bromoacetic acid N-hydroxysuccinimide ester (0.24 mmol) were dissolved in DMF (4.6 mL) and cooled in ice-water bath. 36.2 µL of DBU (0.24 mmol) was added at once and the mixture was allowed to warm to RT. At that moment LC/MS indicated nearly 95% conversion and the reaction was quenched with addition of 0.1 mL AcOH. Crude reaction mixture was directly loaded onto a 50 g C18Aq column and purified via standard 10-100% B gradient (A: water w. 0.05% AcOH; B: water w. 0.05% AcOH). Product

containing fractions were lyophilized to afford 160 mg of product (77% yield). HPLC purity at 254 nm: 96%. Retention time: 2.83 min (Method F). LCMS: 935.4 MH^+.

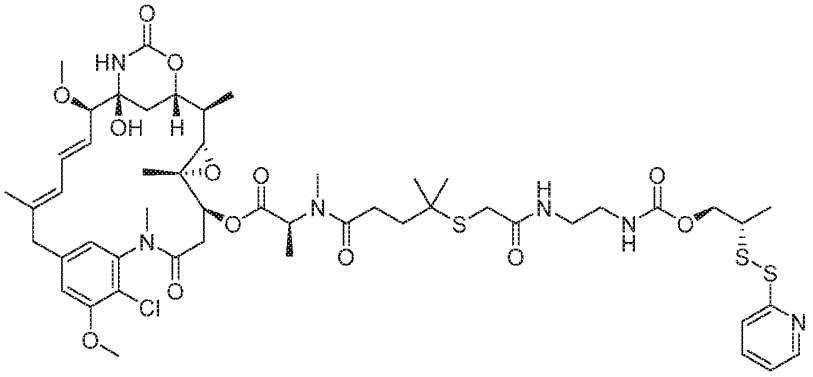
Step 2. $1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)$-$8^6$-chloro-1+-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2-aminoethyl)amino)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate



25 mg of $1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)$-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate (0.027 mmol) and 36 mg of N1-((4-methoxyphenyl)diphenylmethyl)ethane-1,2-diamine (0.11 mmol, 4 eq) were dissolved in dioxane (1 mL). After 3 h, the reaction appeared to be complete on LC/MS. The mixture was concentrated to dryness and dissolved in 80% AcOH in water (2 mL). LC/MS showed complete deprotection of the intermediate and the mixture was directly freeze-dried. The residue was dissolved in DMSO (1 mL) and loaded onto 15.5 g C18Aq column and purified via standard 5-100% B gradient (A: water w. 0.05% AcOH; B: water w. 0.05% AcOH). Product containing fractions were lyophilized to afford 18 mg of product. HPLC purity at 254 nm: 95%. Retention time: 2.17 min (Method F). LCMS: 880.4 MH^+.

Step 3. $(1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)$-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-

benzenacyclotetradecaphane-10,12-dien-4-yl (2S,18S)-2,3,7,7-tetramethyl-4,10,15-trioxo-18-(pyridin-2-yldisulfaneyl)-16-oxa-8-thia-3,11,14-triazanonadecanoate



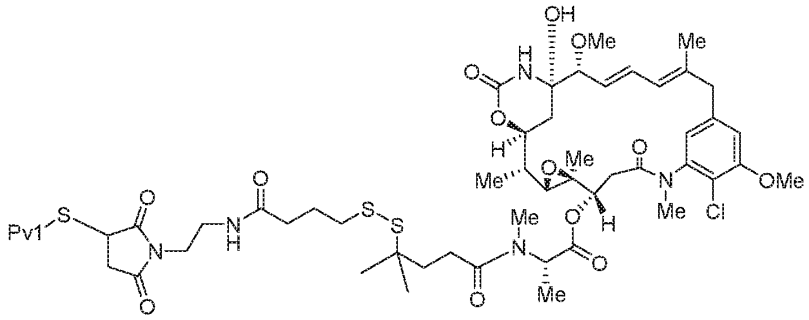
A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2-aminoethyl)amino)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate (14 mg, 0.016 mmol) in DMF (0.2 mL) was added to solid (S)-4-nitrophenyl (2-(pyridin-2-yldisulfaneyl)propyl) carbonate (6.6 mg, 0.018 mmol). Catalytic HOAt and DIEA (10 mL, 0.057 mmol) were added to the resultant solution and stirred at room temperature for 3 hours. The solution was neutralized with acetic acid (10 mL) and applied to a reverse phase column (RediSEP C18 (15.5 g)) and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to afford 18 mg (85% yield) of the title product. HPLC purity at 254 nm: 99%. Retention time: 2.85 min (Method F). LCMS: 1129.4 MNa^+.

Step 4. Synthesis of Compound 10

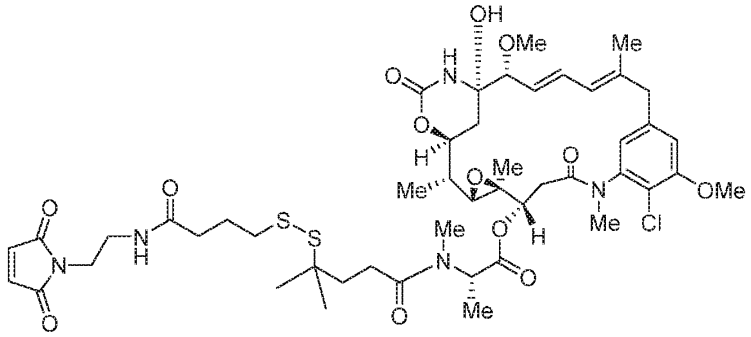
A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza -1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (2S,18S)-2,3,7,7-tetramethyl-4,10,15-trioxo-18-(pyridin-2-yldisulfaneyl)-16-oxa-8-thia-3,11,14-triazanonadecanoate (17.7 mg, 0.00857 mmol) in DMF (1 mL) was treated with sodium bicarbonate (1.8 mg, 0.0214 mmol) and water (50 mL). The resultant solution was treated with peptide, Pv1 (31.5 mg, 0.0899 mmol) and stirred at room

temperature for 3 hours, then applied to a reverse phase column, RediSep C18 (15.5 g) and eluted with a gradient of acetonitrile (30% to 70%) in water with ammonium acetate (10 mM). The fractions were combined, frozen and lyophilized to afford the product as a white solid, 18.7 mg (50%). HPLC purity at 254 nm: 99%. Retention time: 6.49 min (Method G) LCMS: 2138.0 (M+2H)/2$^+$, 1425.3 (M+3H)/3$^+$.

Example 11: Synthesis of Compound 11



Step 1. (1^{4}S,1^{6}S,3^{2}S,3^{3}S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((4-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutyl)disulfaneyl)-4-methylpentanoyl)-N-methyl-L-alaninate



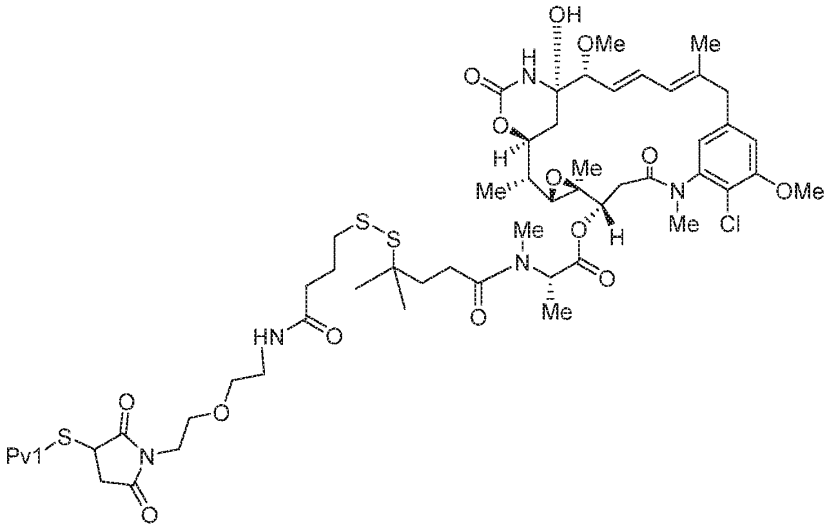
DM4 (10 mg, 0.013 mmol) and succinimidyl 4-(2-pyridyldithio)butanoate (6 mg, 0.02 mmol) were mixed in DMF (0.26 mL). Triethylamine was added (0.015 mL) and the mixture was stirred for 2 h. 1-(2-aminoethyl)-1H-pyrrole-2,5-dione hydrochloride (5 mg, 0.026 mmol) was added and after 3 h the mixture was directly loaded onto a RediSEP C18Aq (15.5 g) column and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to

afford 6 mg (40% yield) of the title product. HPLC purity at 254 nm: 92%. Retention time: 2.83 min (Method F). LCMS: 1020.4 MH^+.

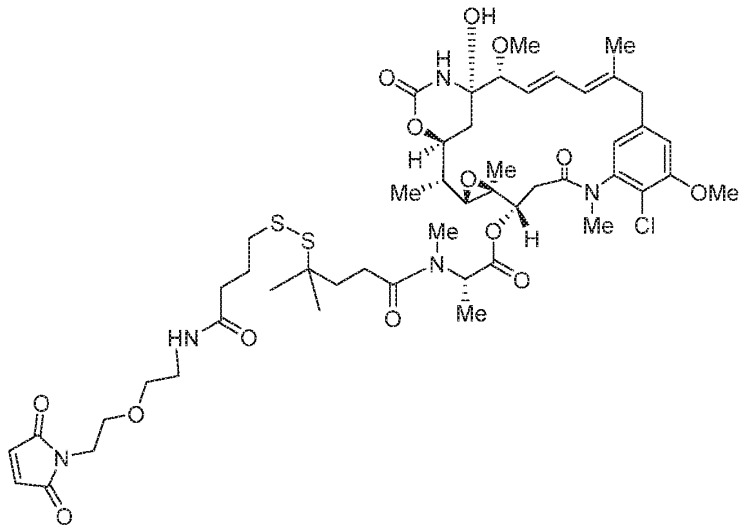
Step 2. Synthesis of Compound 11

A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((4-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutyl)disulfaneyl)-4-methylpentanoyl)-N-methyl-L-alaninate (6 mg, 0.006 mmol) and Pv1 peptide (22.3 mg, 0.006 mmol) were dissolved in DMF (0.12 mL) and treated with triethylamine (0.001 mL). After 30 minutes the reaction mixture was directly loaded onto a RediSEP C8 (15.5 g) column and eluted with a gradient of acetonitrile (35% to 75%) in water with TFA (0.05%) to afford 16 mg (64% yield) of the title compound. HPLC purity at 254 nm: 98%. Retention time: 6.19 min (Method G). LCMS: 2150.2 $(M+2H)/2^+$, 1433.3 $(M+3H)/3^+$.

Example 12. Synthesis of Compound 12



Step 1. ((1⁴S,1⁶S,3²S,3³S,2R,4S,10E,12E,14R)-8⁶-chloro-1⁴-hydroxy-8⁵,14-dimethoxy-3³,2,7,10-tetramethyl-1²,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16,16,20,21-tetramethyl-10,19-dioxo-3,6-dioxa-14,15-dithia-9,20-diazadocosan-22-oate

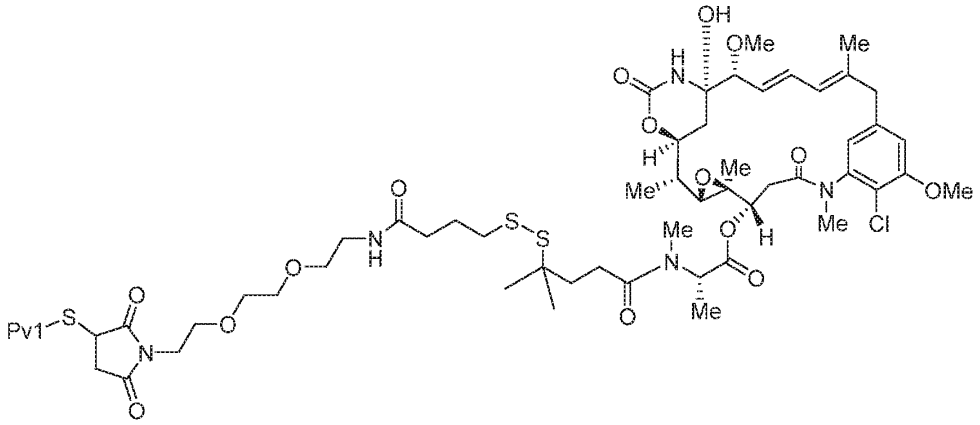


DM4 (20 mg, 0.026 mmol) and succinimidyl 4-(2-pyridyldithio)butanoate (12 mg, 0.04 mmol) were mixed in DMF (0.75 mL). Triethylamine was added (0.045 mL) and the mixture was stirred for 2 h. 1-(21-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-1H-pyrrole-2,5-dione hydrochloride (9 mg, 0.036 mmol) was added and after 3 h the mixture was directly loaded onto a RediSEP C18Aq (15.5 g) column and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to afford 17 mg (61% yield). HPLC purity at 254 nm: 99%. Retention time: 2.84 min (Method F). LCMS: 1108.4 MH^+.

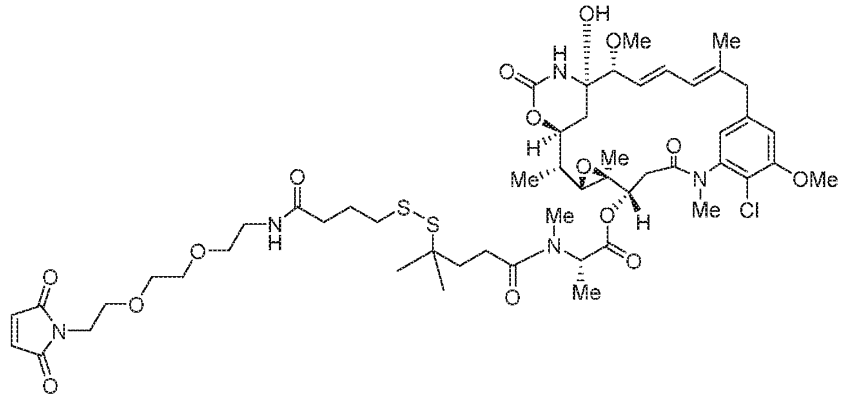
Step 2. Synthesis of Compound 12.

A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16,16,20,21-tetramethyl-10,19-dioxo-3,6-dioxa-14,15-dithia-9,20-diazadocosan-22-oate (15 mg, 0.014 mmol) and Pv1 peptide (52 mg, 0.015 mmol) were dissolved in DMF (0.28 mL) and treated with triethylamine (0.006 mL). After 30 minutes the reaction mixture was directly loaded onto a RediSEP C8 (15.5 g) column and eluted with a gradient of acetonitrile (35% to 60%) in water with TFA (0.05%) to afford 25 mg (34% yield). HPLC purity at 254 nm: 98%. Retention time: 6.26 min (Method G). LCMS: 2194.0 $(M+2H)/2^+$, 1463.0 $(M+3H)/3^+$.

Example 13. Synehsis of Compound 13



Step 1. 1⁴S,1⁶S,3²S,3³S,2R,4S,10E,12E,14R)-8⁶-chloro-1⁴-hydroxy-8⁵,14-dimethoxy-3³,2,7,10-tetramethyl-1²,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-13,13,17,18-tetramethyl-7,16-dioxo-3-oxa-11,12-dithia-6,17-diazanonadecan-19-oate

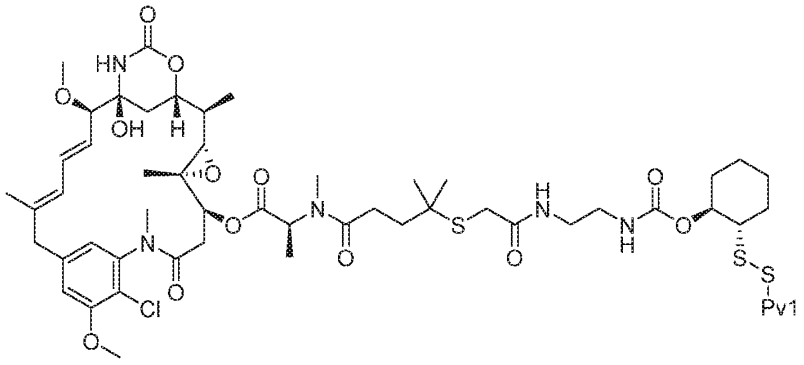


DM4 (20 mg, 0.026 mmol) and succinimidyl 4-(2-pyridyldithio)butanoate (12 mg, 0.04 mmol) were mixed in DMF (0.75 mL). Triethylamine was added (0.045 mL) and the mixture was stirred for 2 h. 1-(2-(2-aminoethoxy)ethyl)-1H-pyrrole-2,5-dione hydrochloride (5 mg, 0.024 mmol) was added and after 3 h the mixture was directly loaded onto a RediSEP C18Aq (15.5 g) column and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to afford 13 mg (41% yield) of the title product. HPLC purity at 254 nm: 94%. Retention time: 2.85 min (Method F). LCMS: 1064.4 MH^+.

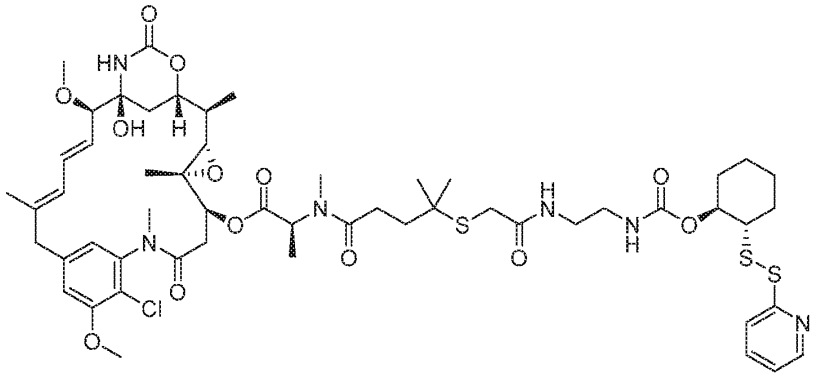
Step 2. Synthesis of Compound 13

A solution of (14S,16S,32S,33S,2R,4S,10E,12E,14R)-86-chloro-14-hydroxy-85,14-dimethoxy-33,2,7,10-tetramethyl-12,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16,16,20,21-tetramethyl-10,19-dioxo-3,6-dioxa-14,15-dithia-9,20-diazadocosan-22-oate (18 mg, 0.017 mmol) and Pv1 peptide (63 mg, 0.019 mmol) were dissolved in DMF (0.34 mL) and treated with triethylamine (0.007 mL). After 30 minutes the reaction mixture was directly loaded onto a RediSEP C8 (15.5 g) column and eluted with a gradient of acetonitrile (35% to 60%) in water with TFA (0.05%) to afford 25 mg (34% yield) of the title compound. HPLC purity at 254 nm: 99%. Retention time: 6.24 min (Method G). LCMS: 2172.0 $(M+2H)/2^+$, 1448.7 $(M+3H)/3^+$.

Example 14. Synthesis of Compound 14



Step 1. ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-9,9,13,14-tetramethyl-1,6,12-trioxo-1-(((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl)oxy)-8-thia-2,5,13-triazapentadecan-15-oate

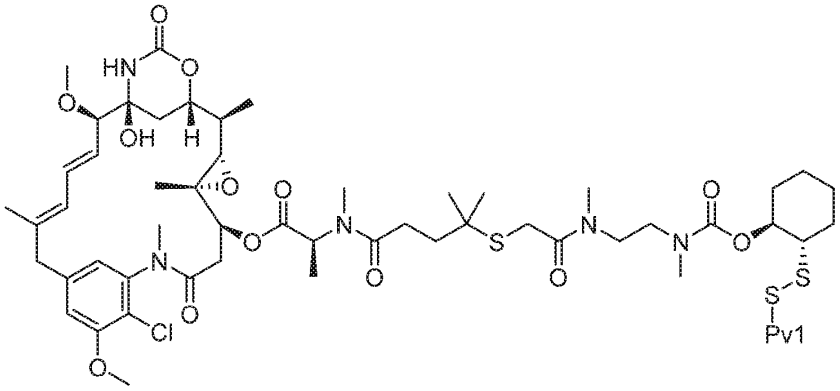


A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2-aminoethyl)amino)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate (14 mg, 0.016 mmol; Example 10, Step 2) in DMF (0.2 mL) was added to solid 4-nitrophenyl ((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl) carbonate (7.2 mg, 0.018 mmol). Catalytic HOAt and DIEA (10 mL, 0.057 mmol) were added to the resultant solution and stirred at room temperature for 3 hours. The solution was neutralized with acetic acid (10 mL) and applied to a reverse phase column, RediSEP C18 (15.5 g) and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to afford 15 mg (80% yield) of the title compound. HPLC purity at 254 nm: 98%. Retention time: 3.05 min (Method F). LCMS: 1147.4 MH^+.

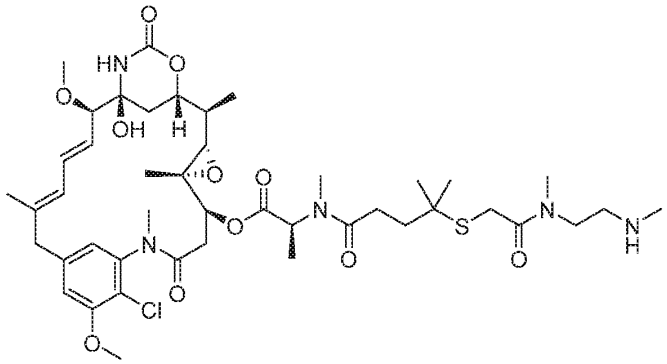
Step 2. Synthesis of Compound 14

A solution of (1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R)-8^6-chloro-1^4-hydroxy-8^5,14-dimethoxy-3^3,2,7,10-tetramethyl-1^2,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-9,9,13,14-tetramethyl-1,6,12-trioxo-1-(((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl)oxy)-8-thia-2,5,13-triazapentadecan-15-oate (17 mg, 0.015 mmol) and Pv1 peptide (47 mg, 0.013 mmol) were dissolved in DMF (0.34 mL) and treated with triethylamine (0.007 mL). After 30 minutes the reaction mixture was directly loaded onto a RediSEP C8 (15.5 g) column and eluted with a gradient of acetonitrile (35% to 60%) in water with TFA (0.05%) to afford 28 mg (37% yield). HPLC purity at 254 nm: 99%. Retention time: 7.36 min (Method G). LCMS: 2158.0 $(M+2H)/2^+$, 1439.0 $(M+3H)/3^+$.

Example 15. Synthesis of Compound 15



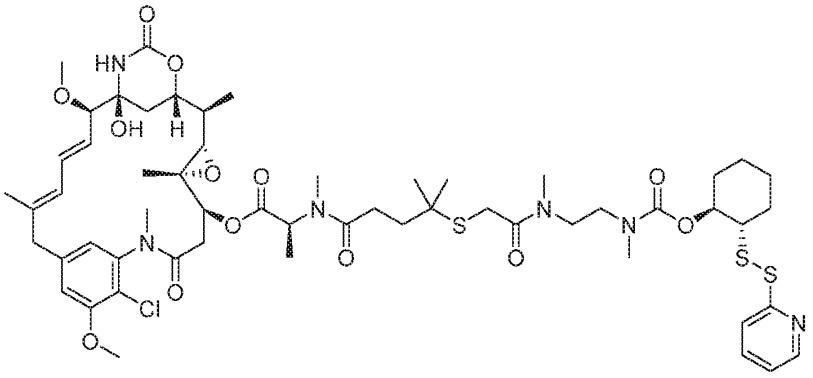
Step 1. ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-5,9,9,13,14-pentamethyl-6,12-dioxo-8-thia-2,5,13-triazapentadecan-15-oate



25 mg of ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate (0.027 mmol) and 40 mg of N1-((3-methoxyphenyl)diphenylmethyl)-N1,N2-dimethylethane-1,2-diamine (0.11 mmol, 4 eq) were dissolved in dioxane (1 mL). After 3 h, the reaction appeared to be complete on LC/MS. 0.05 mL of TFA was added and the mixture was loaded onto a 15.5 g C18Aq column and purified via standard 5-100% B gradient (A: water w. 0.05% TFA; B: ACN w. 0.05% TFA). Product

containing fractions were lyophilized to afford 22 mg of product (75% yield). HPLC purity at 254 nm: 98%. Retention time: 2.25 min (Method F). LCMS: 908.4 MH^+.

Step 2. ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-2,5,9,9,13,14-hexamethyl-1,6,12-trioxo-1-(((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl)oxy)-8-thia-2,5,13-triazapentadecan-15-oate



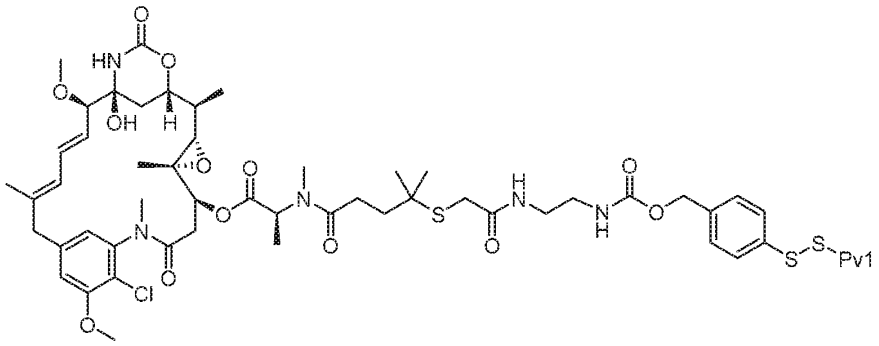
A solution of ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-5,9,9,13,14-pentamethyl-6,12-dioxo-8-thia-2,5,13-triazapentadecan-15-oate (15 mg, 0.016 mmol) in DMF (0.2 mL) was added to solid 4-nitrophenyl ((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl) carbonate (6.6 mg, 0.018 mmol). Catalytic HOAt and DIEA (10 mL, 0.057 mmol) were added to the resultant solution and stirred at room temperature for 3 hours. The solution was neutralized with acetic acid (10 mL) and applied to a reverse phase column, RediSEP C18 (15.5 g) and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%) to afford 16 mg (82% yield) of the title product. HPLC purity at 254 nm: 97%. Retention time: 3.36 min (Method F). LCMS: 1175.5 MH^+.

Step 3. Synthesis of Compound 15

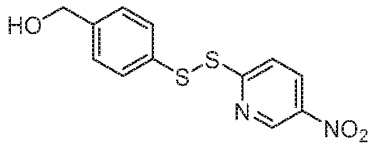
A solution of ($1^4S,1^6S,3^2S,3^3S,2R,4S,10E,12E,14R$)-$8^6$-chloro-$1^4$-hydroxy-$8^5$,14-dimethoxy-$3^3$,2,7,10-tetramethyl-$1^2$,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-

benzenacyclotetradecaphane-10,12-dien-4-yl (S)-2,5,9,9,13,14-hexamethyl-1,6,12-trioxo-1-(((1S,2S)-2-(pyridin-2-yldisulfaneyl)cyclohexyl)oxy)-8-thia-2,5,13-triazapentadecan-15-oate (16 mg, 0.014 mmol) and Pv1 peptide (52 mg, 0.015 mmol) were dissolved in DMF (0.28 mL) and treated with triethylamine (0.008 mL). After 30 minutes the reaction mixture was directly loaded onto a RediSEP C8 (15.5 g) column and eluted with a gradient of acetonitrile (35% to 60%) in water with TFA (0.05%) to afford 32 mg (44% yield) of the title compound. HPLC purity at 254 nm: 99%. Retention time: 6.86 min (Method G). LCMS: 2172.0 $(M+2H)/2^+$, 1448.4 $(M+3H)/3^+$.

Example 16. Synthesis of Compound 16

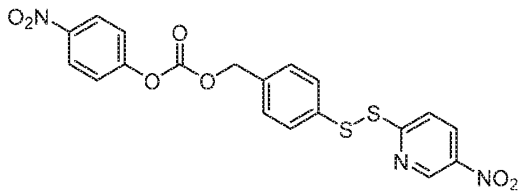


Step 1. (4-((5-nitropyridin-2-yl)disulfaneyl)phenyl)methanol



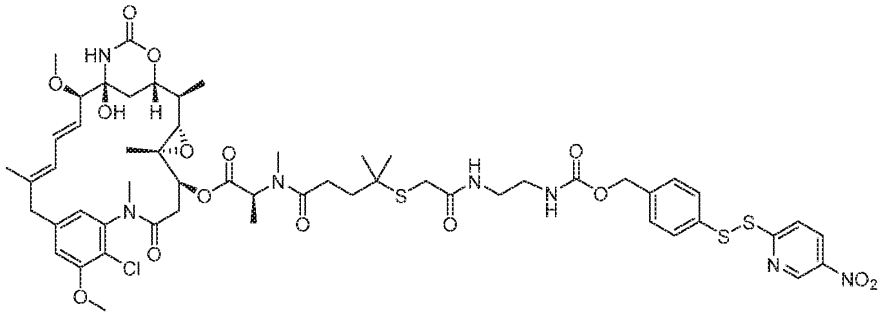
A solution of (4-mercaptophenyl)methanol (0.74 g, 4.83 mmol) in THF (10 mL) was treated with 5-nitro-2-((4-nitrophenyl)disulfaneyl)pyridine (1.0 g, 3.23 mmol). The resultant suspension was stirred at room temperature for 2 hours, and the solvent was evaporated in vacuo. The residue was dissolved in DCM and applied to a RediSep silica gel column and eluted with a gradient of ethyl acetate (10% to 60%) in hexanes to afford the product (0.499 g, 52% yield). HPLC purity at 254 nm: 90%. Retention time: 2.72 min (Method F). MS data, 295.1 $(M+H)^+$. ^{1}HNMR(DMSO-d_6) d 9.18 (s, 1H), 8.58 (d of d, 1H), 8.02 (d, 1H), 7.56 (d, 2H), 7.34 (d, 2H), 5.24 (t, 1H) and 4.47 (d, 2H).

Step 2. 4-nitrophenyl (4-((5-nitropyridin-2-yl)disulfaneyl)benzyl) carbonate



A solution of 4-nitrophenyl chloroformate (255 mg, 1.26 mmol) in THF (5 mL) was cooled on an ice-bath and treated with a solution (4-((5-nitropyridin-2-yl)disulfaneyl)phenyl)methanol (220 mg, 0.748 mmol), triethyl amine (0.7 mL, 5.03 mmol), and 4-dimethyaminopyridine (45 mg, 0.368 mmol) in THF (5 mL) added over about 15 minutes. The ice-bath was removed, and the solution was stirred at room temperature for one hour and stored in a freezer overnight. The solvent was evaporated in vacuo, and the residue was dissolved in DCM, applied to a RediSep silica gel column (12 g) and eluted with a gradient of ethyl acetate (2% to 100%) in hexanes. The product was purified further by reverse phase chromatography on a RediSep C18 cartridge (50 g) eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%), to afford the product, 55 mg (16%). HPLC purity at 254 nm: >99%. Retention time: 3.77 min (Method F). MS data, 460.7 $(M+H)^+$. ^{1}HNMR($CDCl_3$) d 9.29 (d, 1H), 8.39 (d of d, 1H), 8.28 (d of d, 2H), 7.83 (d of d, 1H), 7.55 (d of d, 2H), 7.44 (d of d), 7.36 (d of d, 2H) and 5.26 (d, 2H).

Step 3. (14S,16S,32S,33S,2R,4S,10E,12E,14R)-86-chloro-14-hydroxy-85,14-dimethoxy-33,2,7,10-tetramethyl-12,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-11,11,15,16-tetramethyl-1-(4-((5-nitropyridin-2-yl)disulfaneyl)phenyl)-3,8,14-trioxo-2-oxa-10-thia-4,7,15-triazaheptadecan-17-oate



A solution of (14S,16S,32S,33S,2R,4S,10E,12E,14R)-86-chloro-14-hydroxy-85,14-dimethoxy-33,2,7,10-tetramethyl-12,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl N-(4-((2-((2-aminoethyl)amino)-2-oxoethyl)thio)-4-methylpentanoyl)-N-methyl-L-alaninate (15 mg, 0.017 mmol; Example 10, Step 2) in DMF (1 mL) was added to solid 4-nitrophenyl (4-((5-nitropyridin-2-yl)disulfaneyl)benzyl) carbonate (26 mg, 0.0566 mmol). Catalytic HOAt and DIEA (10 mL, 0.057 mmol) were added to the resultant solution and stirred at room temperature for 3 hours. The solution was neutralized with acetic acid (7 mL, 0.122 mmol) and applied to a reverse phase column, RediSEP C18 (15.5 g) and eluted with a gradient of acetonitrile (30% to 95%) in water with acetic acid (0.05%). Further purification on a silica gel column, RediSep (4 g), using a gradient of methanol (0.2% to 6%) in DCM as the eluant, afforded the title product (10.3 mg, 50% yield). HPLC purity at 254 nm: >99%. Retention time: 3.16 min (Method F). MS data, 1182.3 $(M+H-H_2O)^+$, 1201.3 $(M+H)^+$, 1222.3 $(M+Na)^+$.

Step 4. Synthesis of Compound 16

A solution of (14S,16S,32S,33S,2R,4S,10E,12E,14R)-86-chloro-14-hydroxy-85,14-dimethoxy-33,2,7,10-tetramethyl-12,6-dioxo-7-aza-1(6,4)-oxazinana-3(2,3)-oxirana-8(1,3)-benzenacyclotetradecaphane-10,12-dien-4-yl (S)-11,11,15,16-tetramethyl-1-(4-((5-nitropyridin-2-yl)disulfaneyl)phenyl)-3,8,14-trioxo-2-oxa-10-thia-4,7,15-triazaheptadecan-17-oate (10.3 mg, 0.00857 mmol) in DMF (1 mL) was treated with sodium bicarbonate (1.8 mg, 0.0214 mmol) and water (50 mL). The resultant solution was treated with peptide, Pv1 (31.5 mg, 0.0899 mmol) and stirred at room temperature for 3 hours, then applied to a reverse phase column, RediSep C18 (15.5 g) and eluted with a gradient of acetonitrile (30% to 70%) in water with ammonium acetate (10 mM). The fractions were combined, frozen and lyophilized to afford the product as a white solid, 18.7 mg (50%). HPLC purity at 254 nm: 99%. Retention time: 6.63 min (Method G). MS data, 2162.4 $(M+2H)/2^+$, 1441.8 $(M+3H)/3^+$, 1082.6 $(M+4H)/4^+$, 1435.7 $(M+3H-H_2O)/3^+$.

Example A. Growth Delay Assay

Cells were plated in 96 well black walled-clear bottom plates (Griener), DLD-1 WT cells at 2500 cells per well, FaDu, and HeLa cells at 5000 cells per well, and HCT116 at 3000 cells per well, in growth media containing 10% FBS. Cells were allowed to adhere at room temperature for 60 minutes before returning to a 37C, 5% CO_2 incubator. After 24 hours, media was removed and replaced with fresh growth media containing various drug concentrations. Each drug concentration was added in triplicate. Non-drug treated controls contained growth media only. Cells were returned to the incubator. Ninety-six hours after addition of drug, cells were fixed with 4% paraformaldehyde for 20 minutes and stained with Hoechst at 1 µg/mL. The plates were imaged on a Cytation 5 auto imager (BioTek) and cells were counted using CellProfiler (http://cellprofiler.org). The percent cell growth delay was calculated and data plotted using GraphPad Prism

Table 5: Growth Delay Assay data

Example	DLD-1 (IC_{50}, nM)	HCT116 (IC_{50}, nM)	FaDu (IC_{50}, nM)	HeLa (IC_{50}, nM)
R^2SH-1 (see Table 2)	9.8	4.3	2.8	2.6
R^2SH-2 (see Table 2)	0.45	0.20	0.13	0.02
1	60.5	21.8	10.4	7.4
2	114	21.1	15.4	8.0
3	45.1	19.3	10.1	6.9
4	11.7	2.5	1.4	0.85
5	9.4	2.7	2.3	0.95
6	8.3	3.3	5.2	1.8
7	NC*	3.4	4.6	2.0
8	9.8	3.0	3.4	1.6

NC* = Not calculated

Example B: Effect on In Vitro Tubulin Polymerization

A fluorescence-based tubulin polymerization assay (Cytoskeleton Cat# BK011P) was performed to quantitate the impact of unconjugated DM4 and Compound 5 on in vitro tubulin polymerization. DM4 and Compound 5 were prepared as 10 mM stocks in DMSO then diluted at 10X to 200, 50 and 5 μM in ultrapure distilled water for a final DMSO concentration of 0.2%. Kit reagents were defrosted rapidly then kept cold on ice to prevent premature polymerization. A tubulin reaction mixture was prepared on ice by mixing purified porcine brain tubulin, GTP, and lycerol buffer all in 1X kit buffer for final concentrations of 2 mg/mL tubulin, 1 mM GTP and 15% glycerol. 5 μL of DM4, Compound 5 or DMSO control were added to a pre-warmed black, half-well reaction plate at 37 °C for no longer than 1 minute, to warm, but not allow for evaporation. 50 μL of tubulin reaction mixture was rapidly added to each well and immediately placed in a pre-warmed Cytation 5 imaging reader (BioTek). A kinetic reading was performed at 360 excitation/450 emission for 2 hours at 37 °C, with readings every 2.5 minutes to follow the enhancement of fluorescence due to incorporation of a fluorescent reporter into microtubules as polymerization occurs.

FIG. 1 shows a plot of the effect of free DM4 and Compound 5 on in vitro β-tubulin polymerization (in terms of relative fluorescence units) at 0.5 μM, 5 μM, and 20 μM.

Example C: Kinetic Analysis of Conjugate Binding

Binding experiments were performed using a Biacore S200 instrument. A Series S sensor chip with pre-immobilized streptavidin was conditioned with 1 M NaCl in 50 mM NaOH. Biotin-labeled human tubulin derived from HeLa cells was immobilized to the sensor chip at a concentration of 125 μg/mL in HBS-P+ buffer at a flow rate of 10 μl/min. A final 3000 RU (response units) of protein was directly immobilized to the chip. After tubulin immobilization, the sensor chip was washed with 50% isopropanol, 50 mM NaOH and 1 M NaCl and subsequently allowed to equilibrate in assay buffer for 4 hours. A streptavidin-biotin capture blank (reference FC) was used to monitor non-specific binding.

To collect kinetic binding data, Compound 5 diluted in assay buffer was injected over the flow cells at concentrations ranging from 100 μM to 0.048 μM and 50 μM to 0.024 μM, at a flow rate of 60 μL/ minute and a temperature of 25 °C. The complex was allowed to dissociate for 60

seconds. Binding of compound to tubulin was monitored in real time to obtain on (Ka) and off (Koff) rates. The affinity constant (KD) was calculated by steady state kinetics.

FIG. 2 depicts the kinetic analysis of Compound 5 binding to β-tubulin in vitro as determined by Biacore surface plasmon resonance. Compound 5 is able to bind to β-tubulin with a similar KD as free DM4 (3.55 μM) and slower on/off rates relative to free DM4.

Example D: Efficacy of Compound 5 in a Mouse Colorectal Cancer Model

Six-week-old female athymic nude *Foxn*^{nu} mice were obtained from Taconic Labs (Cat# NCRNU-F) and were housed 5 per cage on Alpha-Dri bedding in a disposable caging system. Human HCT116 cells derived from colorectal carcinoma were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of $2.5x10^6$ cells in 100 μL. When xenografts reached a mean volume of 100-200 mm^3, mice were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 0.21, 0.29, 0.35, 0.42 μmole/kg Compound 5 (equivalent to 1.1, 1.4, 1,7, or 2 mg/kg Compound 5) or 0.42 μmole/kg unconjugated DM4 (equivalent to 0.33 mg/kg unconjugated DM4). Doses were prepared by diluting 0.1 mg/μL DMSO stocks in 5% mannitol in citrate buffer and were administered QDX4 with a two day interval between the second and third doses, at a volume of 12 mL/kg (300 μL per 25 g mouse). Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: Volume = $\pi/6$ x (length) x $(width)^2$. Body weight of animals was measured at the same time as tumor volume assessment. Animals were removed from the study due to death, tumor size exceeding 2000 mm^3 or loss of >20% body weight. Kaplan-Meier analysis was used to evaluate survival rate based on death or removal from study.

FIG. 3A shows a plot of the mean tumor volume in nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5.

FIG. 3B shows the percent change in body weight of nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5 relative to day 0.

FIG. 4 depicts a Kaplan-Meier plot of nude mice bearing HCT116 colorectal flank tumors dosed with DM4 or Compound 5. Animals were removed from the study due to either death, tumor size exceeding 2000 mm^3 or due to loss of greater than 20% body weight. Free

DM4 induced the spontaneous death of half of the DM4 group of animals during the post-dosing period. As shown in FIG. 4, Compound 5 safely delivers amounts of DM4 in vivo that otherwise result in systemic toxicity and death when dosed as free DM4.

Example E: Effect of Compound 6 on Lung Metastases in a Mouse Lung Cancer Model

Mouse 4T1-iRFP cancer cells derived from mouse mammary carcinoma and transfected with near infrared fluorescent protein (iRFP) were cultured as a monolayer at 37 °C in a humidified atmosphere with 5% CO_2. Cells were passaged between one and three days prior to implantation and media was replaced every 2-3 days as needed to maintain cell viability. Cells were not allowed to exceed 80% confluency. On the day of implantation, cells were trypsinized, washed with complete media and pelleted by centrifugation at 1200 rpm for 5 minutes. The supernatant was decanted, and cells were washed three times with sterile PBS and pelleted by centrifugation. During the final centrifugation, viability was determined using trypan blue exclusion. Cells were resuspended in sterile PBS a final concentration of $5x10^5$ cells/ 100 µL. Cells were drawn into sterile 1cc tuberculin syringes with a 27-gauge needle. Air bubbles were removed, and excess cell mixture was expelled back into the conical tube leaving an injection volume of 100 µL in each syringe. The 100 µL of cells were injected directly into the medial tail vein of six-week-old female athymic nude *Foxn*^{nu} mice (Taconic Labs Cat# NCRNU-F).

Three days after cell injection, mice were administered intraperitoneal doses of vehicle or 2.5 mg/kg Compound 6 once daily for 2 days followed by 2 days of no treatment, followed by a single dose of Compound 6, for a total of three total doses of Compound 6. Eleven days after injection, mice were euthanized, and the lungs were removed for imaging using the LI-COR PEARL Trilogy small animal imager to visualize and quantitate lung metastasis and evaluate compound effect on tumor growth.

FIG. 5A depicts the ventral view and extracted lungs of nude mice inoculated with 4T1-RFP fluorescent cells via tail vein injection and imaged 11 days after inoculation and after 3 doses of vehicle or Compound 6.

FIG. 5B depicts a graph of the fluorescent signal from extracted lungs of 4T1-RFP inoculated mice after 3 doses of vehicle or Compound 6.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference, including without limitation all patent, patent applications, and publications, cited in the present application is incorporated herein by reference in its entirety.