

PEPTIDE CONJUGATES OF CYTOTOXINS AS THERAPEUTICS

FIELD OF THE INVENTION

The present invention relates to peptide conjugates of cytotoxins such as topoisomerase I
5 inhibitors which are useful for the treatment of diseases such as cancer.

BACKGROUND OF THE INVENTION

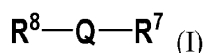
Cancer is a group of diseases characterized by aberrant control of cell growth. The annual incidence of cancer is estimated to be in excess of 1.6 million in the United States alone.
10 While surgery, radiation, chemotherapy, and hormones are used to treat cancer, it remains the second leading cause 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
15 cancer cells. One class of such agents is topoisomerase I inhibitors. Topoisomerase I enzymes function to relax supercoiled DNA and alleviate DNA helical constraints and play a role in transcriptional regulation. See Li, M., Genomics Proteomics Bioinformatics 14 (2016), 166-171. Topoisomerase I is essential for the development in the mammalian system due to its dynamic functions in DNA replication and transcription. However, due to its direct role in transcriptional
20 regulation, topoisomerase I dysfunction may lead to abnormal cellular functions. See Li, M., Genomics Proteomics Bioinformatics 14 (2016), 166-171. Thus, several human diseases such as cancer, neurodegenerative diseases, and autoimmune diseases, are linked to topoisomerase I regulation and activity.

Inhibitors of topoisomerase I have been developed and continue to be developed as anti-
25 cancer agents. In particular, topoisomerase I inhibitors are widely used for the treatment of colorectal, gastric, and other cancers. See Ogitani, Bioorg. Med. Chem. Lett. 26 (2016), 5069-5072. Although topoisomerase I inhibitors are useful in the treatment of cancer, the compounds also exhibit side effects, including neutropenia and severe diarrhea. Preferential delivery of topoisomerase inhibitors to these diseased tissues could avoid these serious side effects. Thus,
30 there is a need for more selective delivery of topoisomerase I inhibitors to diseased tissue.

SUMMARY

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



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

5 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
10 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 compounds 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.

15 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 plasma concentration of Compound 11 and released exatecan
20 after a single IV dose of 5 mg/kg of Compound 11 in a rat (data are expressed as means $\pm$ SEM).

 FIG. 2 shows a plot of the peptide concentration in tumor and bone marrow after a single IP dose of 10 mg/kg of Compound 11 in a mouse (data are expressed as means $\pm$ SEM).

 FIG. 3 shows a graph of the total bone marrow counts from the femurs of tumor bearing nude mice after dosing of 2.6 and 5.2 μ moles/kg of either Compound 11 (equivalent to 10, 20
25 mg/kg conjugate) or free exatecan (equivalent to 1.15 and 2.3 mg/kg exatecan) dosed once daily for four days (data are expressed as means $\pm$ SEM).

 FIG. 4A shows the stomachs of tumor bearing nude mice excised after dosing of vehicle or 5.2 μ moles/kg of either Compound 11 (equivalent to 20 mg/kg conjugate) or free exatecan (equivalent to 2.3 mg/kg exatecan) dosed once daily for four days.

FIG. 4B shows the stomachs of tumor bearing nude mice in situ after dosing of 5.2 μ moles/kg of either Compound 11 (equivalent to 20 mg/kg conjugate) or free exatecan (equivalent to 2.3 mg/kg exatecan) dosed once daily for four days.

FIG. 5A shows a plot of the mean tumor volume resulting from dosing equimolar
5 amounts of either free exatecan or Compound 11 in nude mice bearing HCT116 colorectal flank tumors. Animals were dosed once daily four times per week intraparentherally for three weeks.

FIG. 5B displays a Kaplan Meier survival curve for dosing equimolar amounts of either free exatecan or Compound 11 in nude mice bearing HCT116 colorectal flank tumors.

FIG. 6A shows the single agent efficacy of Compound 11 in nude mice bearing MKN45
10 HER2 negative gastric cancer flank tumors. Animals were dosed once daily four times per week intraparentherally for two weeks.

FIG. 6B displays a Kaplan Meier survival curve for dosing equimolar amounts of either free exatecan or Compound 11 in nude mice bearing MKN45 HER2 negative gastric cancer flank tumors.

FIG. 7A shows a plot of the mean tumor volume resulting from dosing Compound 11 in
15 SCID mice bearing JIMT-1 HER2 intermediate breast cancer flank tumors. Animals were dosed once daily four times per week intraparentherally for three weeks.

FIG. 7B shows a plot of the percent change in body weight in SCID mice bearing JIMT-1 HER2 intermediate breast cancer flank tumors dosed with Compound 11.

FIG. 8A shows a plot of the mean tumor volume in nude mice bearing MDA-MB-231
20 triple negative breast cancer flank tumors dosed with Compound 11. Animals were dosed once daily four times per week intraparentherally for three weeks.

FIG. 8B shows a plot of the percent change in body weight relative to day 0 in nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11.

FIG. 9A shows a plot of the mean tumor volume of nude mice bearing MDA-MB-231
25 triple negative breast cancer flank tumors dosed with Compound 11 and talazoparib. Animals were dosed once daily four times per week intraparentherally for three weeks with Compound 11 and once daily for 18 days orally with talazoparib.

FIG. 9B shows a plot of the percent change in body weight relative to day 0 of nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11 and talazoparib.

FIG. 10 shows a graph of the degradation of Compound 11 and Compound 29 resulting from treatment with 10 mM glutathione over 16 h. As shown in FIG. 10, Compound 29 is released much faster than Compound 11 under similar glutathione exposure.

DETAILED DESCRIPTION

Provided herein is a compound of Formula (I):



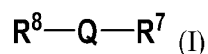
or a pharmaceutically acceptable salt thereof, wherein:

R⁷ is a peptide;

R⁸ is a small molecule topoisomerase I targeting moiety, which binds to topoisomerase I; and

15 Q is a linker, which is covalently linked to moiety R⁷ and R⁸.

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



or a pharmaceutically acceptable salt thereof, wherein:

20 R⁷ is a peptide capable of selectively delivering R⁸Q- across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.0;

R⁸ is a small molecule topoisomerase I targeting moiety, which binds to topoisomerase I; and

Q is a linker, which is covalently linked to moiety R⁷ and R⁸.

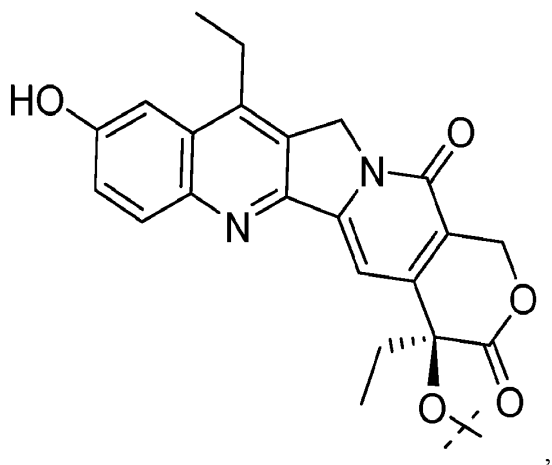
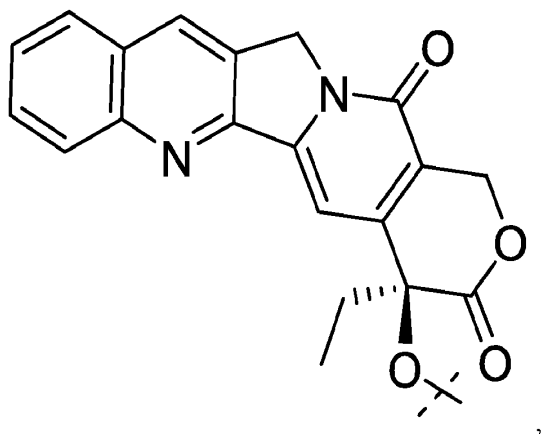
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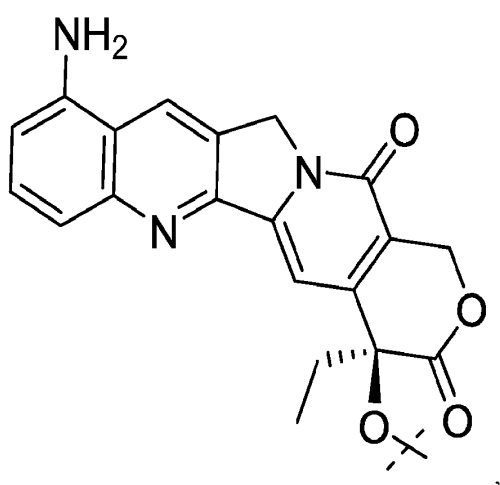
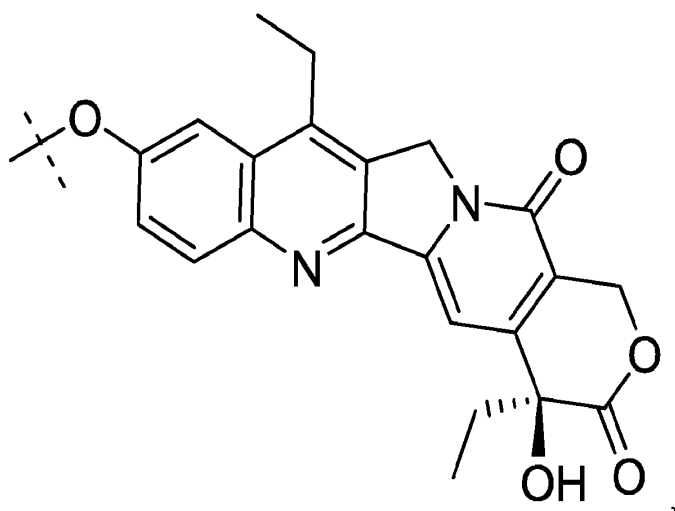


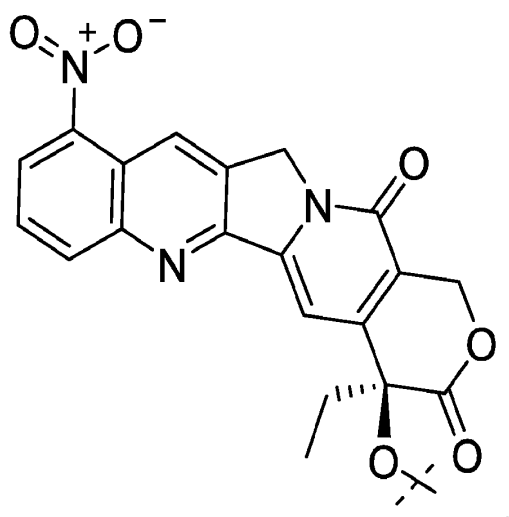
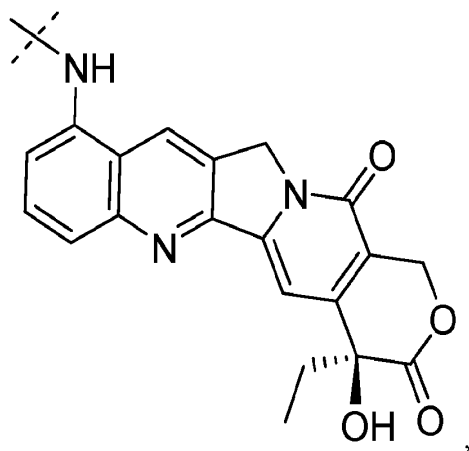
or a pharmaceutically acceptable salt thereof, wherein:

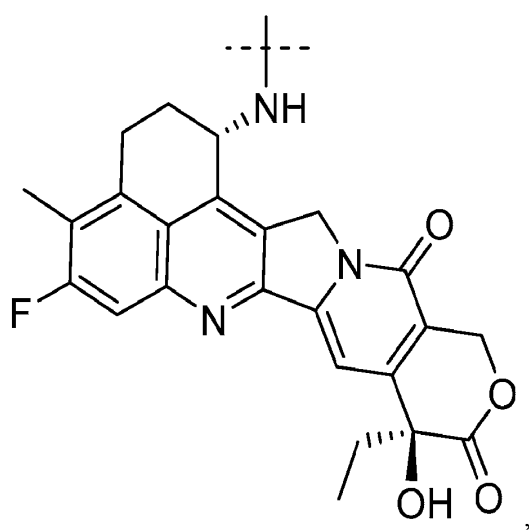
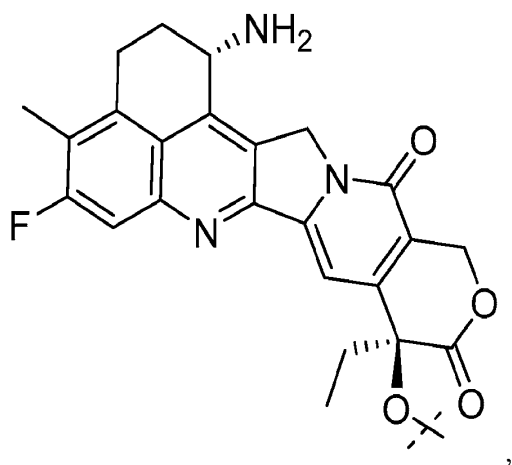
R⁷ is a peptide;

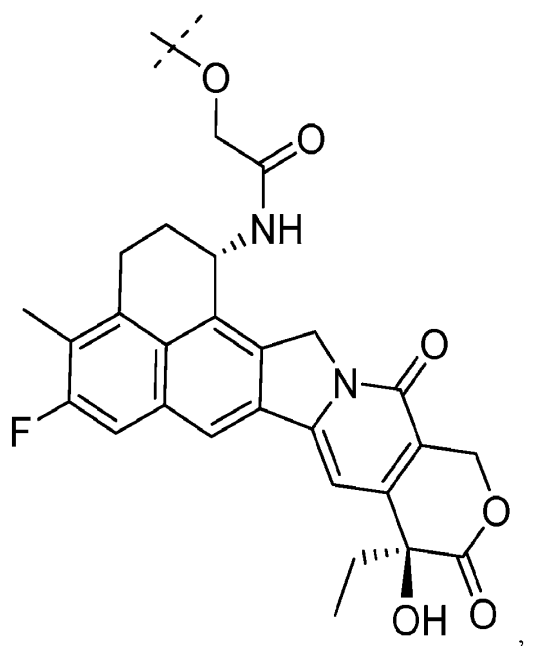
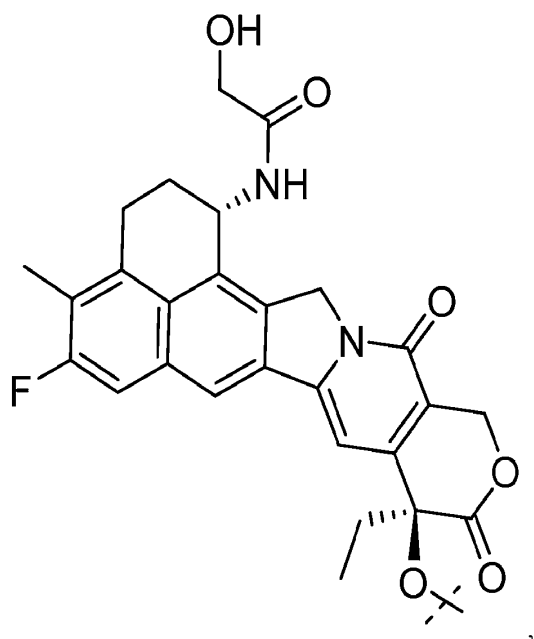
R⁸ is selected from the group consisting of:

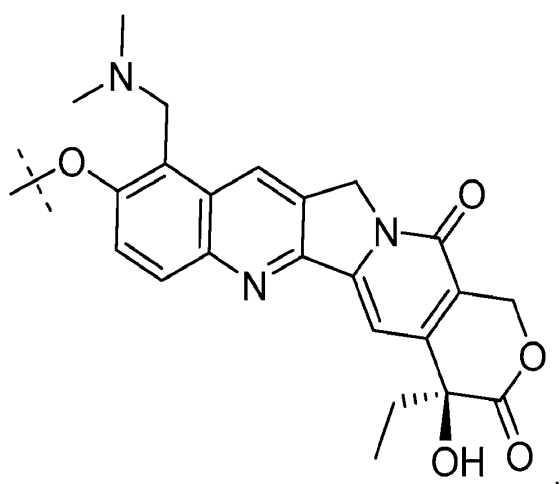
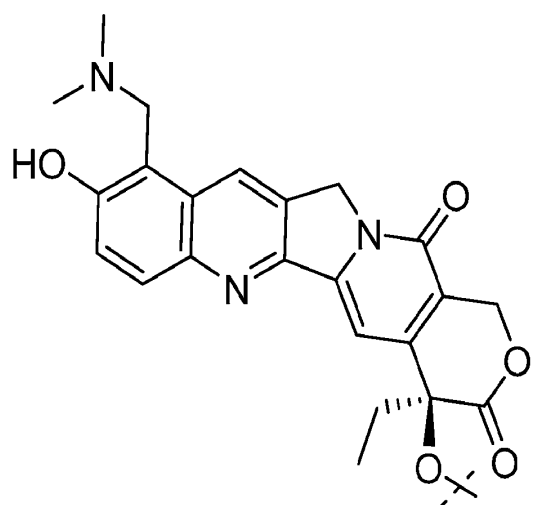


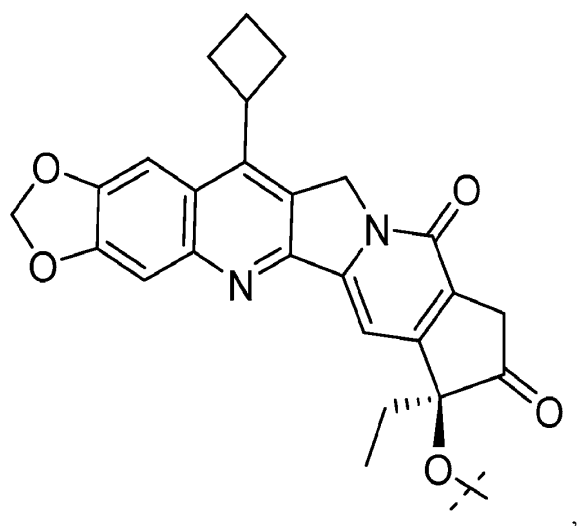
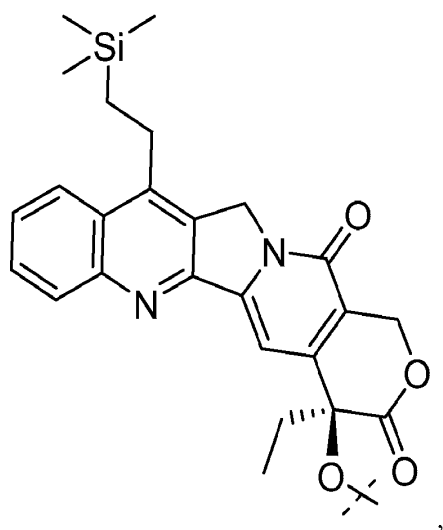


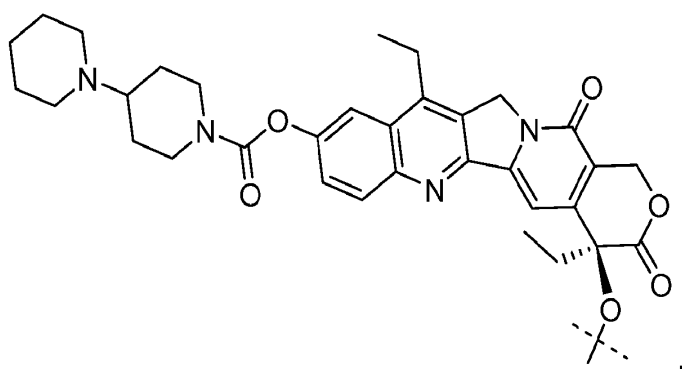
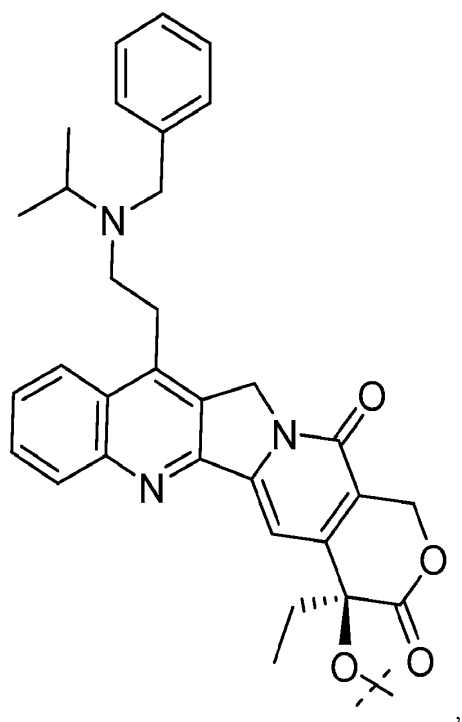


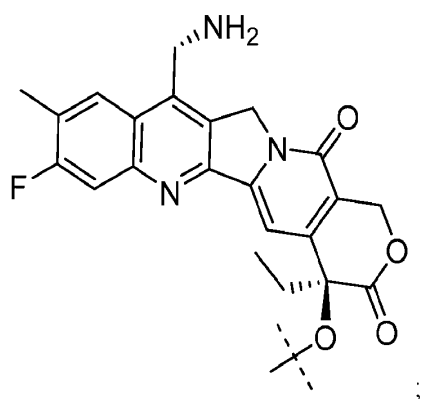
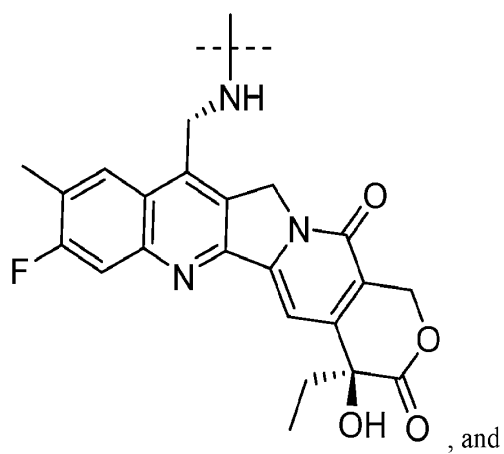












Q is a linker, which is covalently linked to moiety R^7 and R^8 .

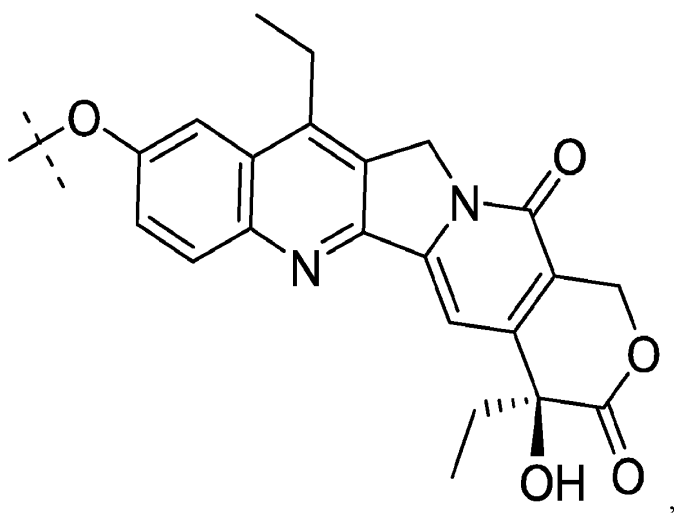
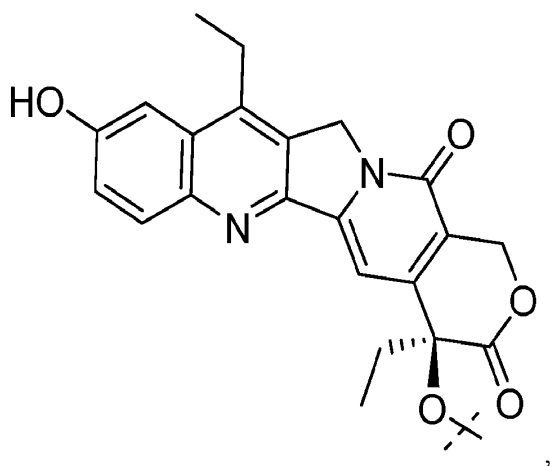
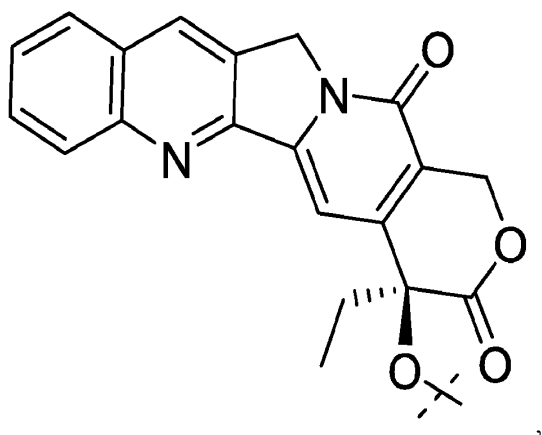
Provided herein is a compound of Formula (I):

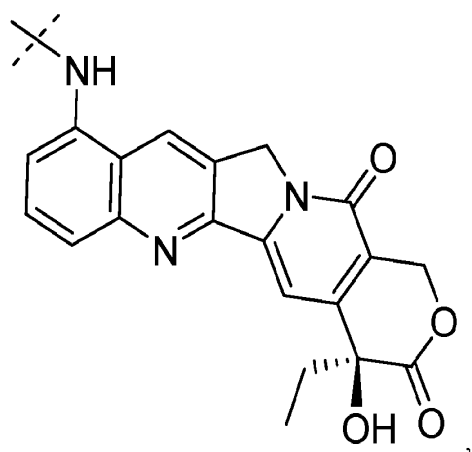
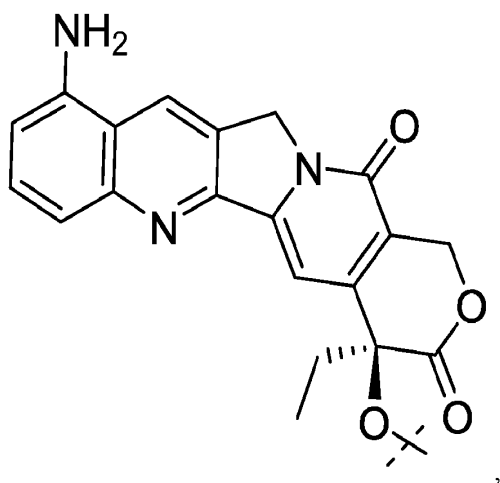


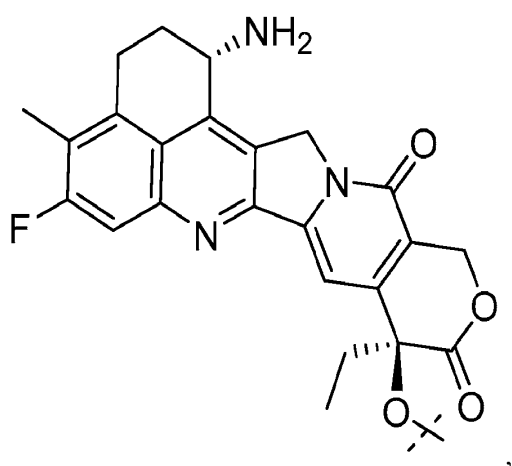
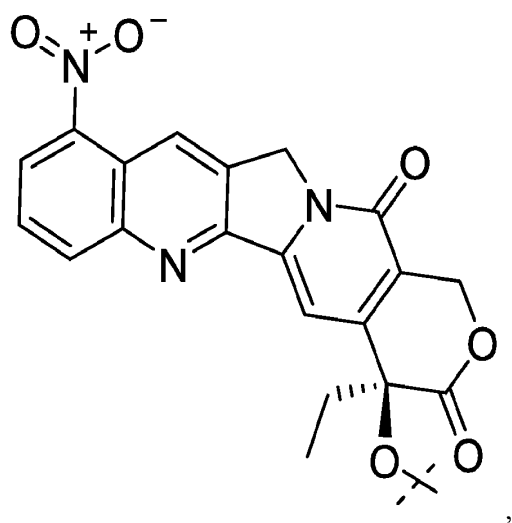
or a pharmaceutically acceptable salt thereof, wherein:

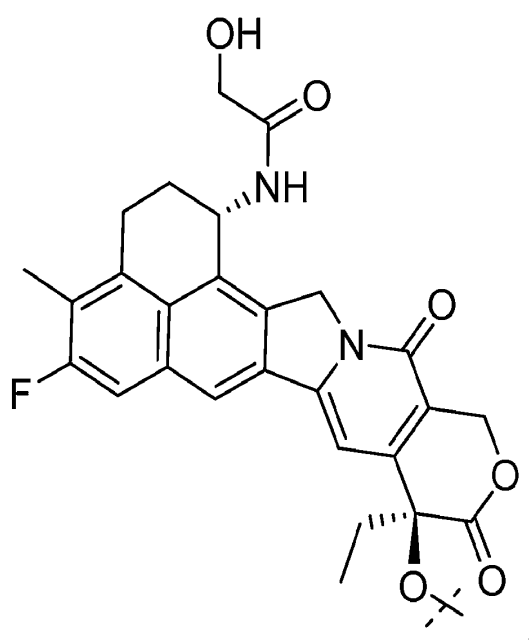
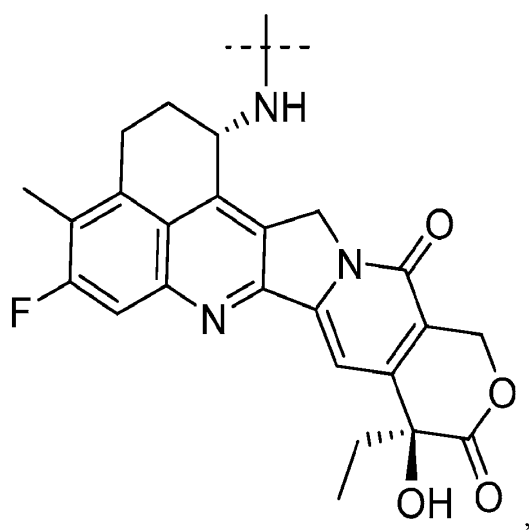
R^7 is a peptide;

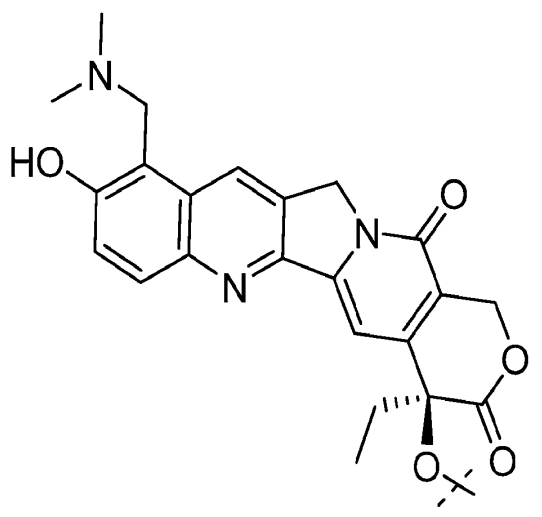
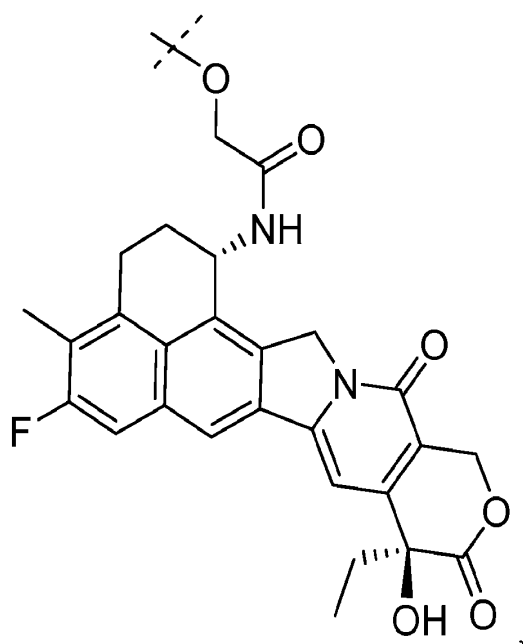
R^8 is selected from the group consisting of:

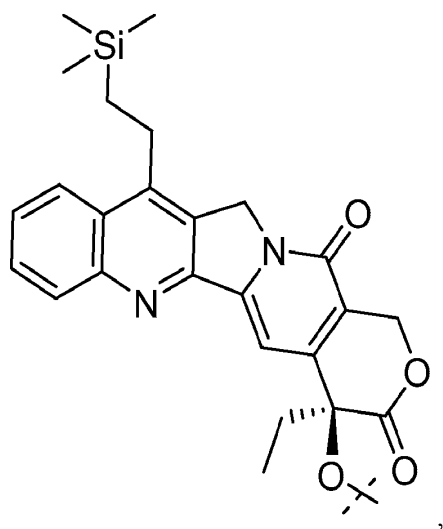
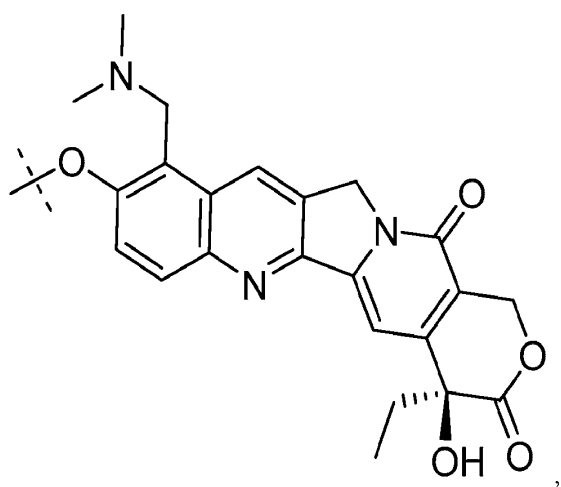


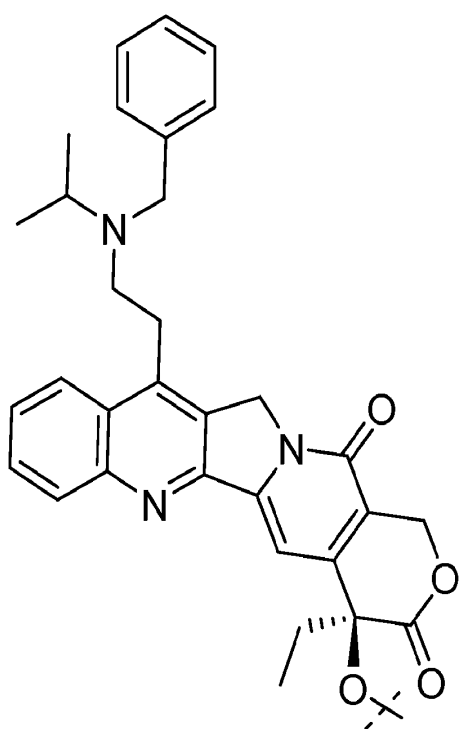
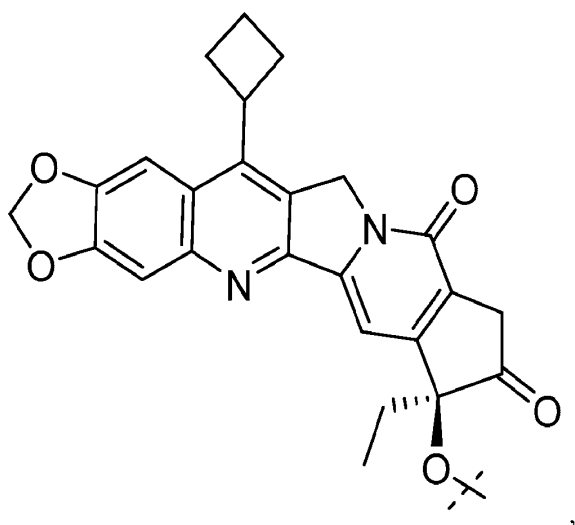










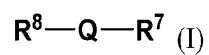


; and

5

Q is a linker, which is covalently linked to moiety R⁷ and R⁸.

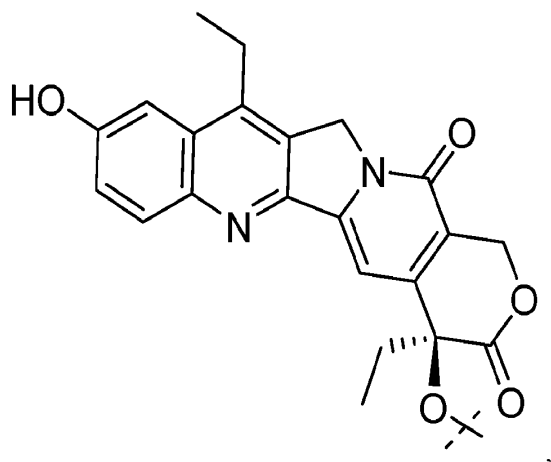
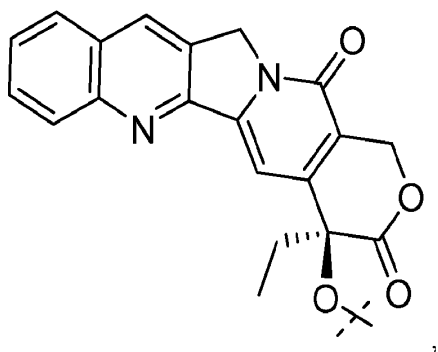
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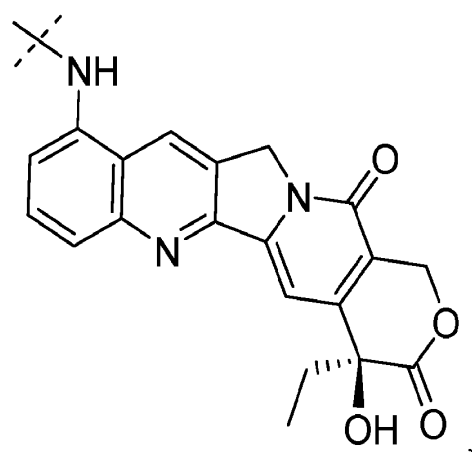
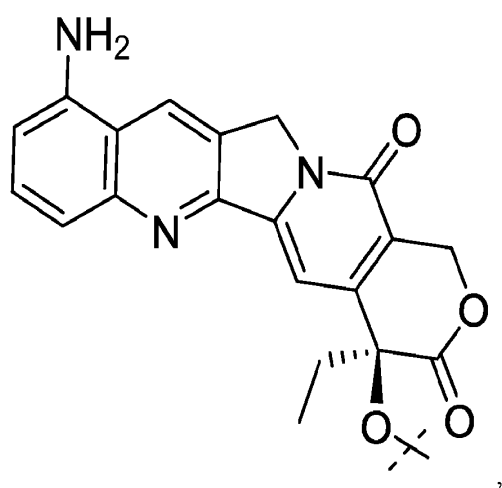
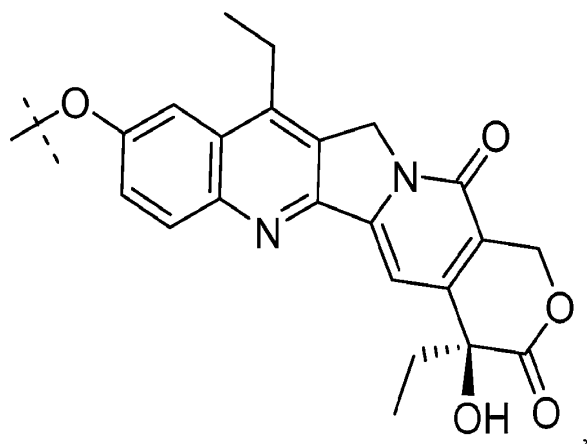


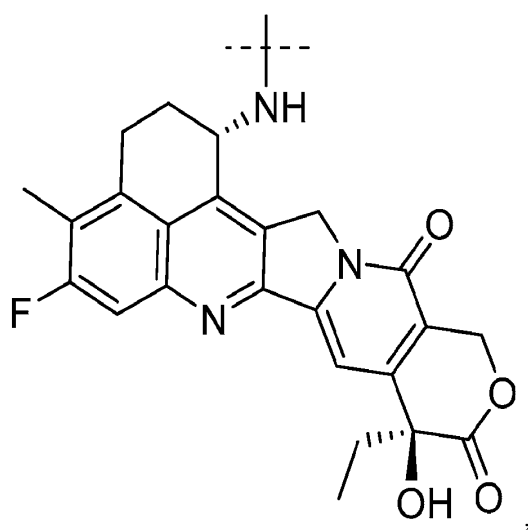
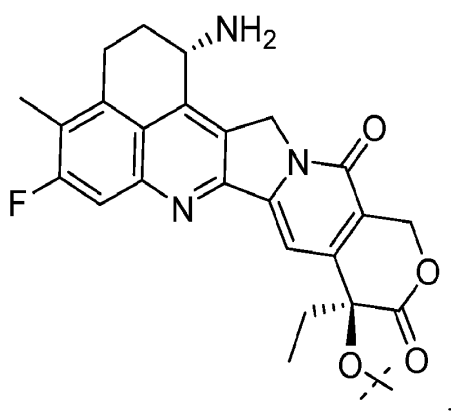
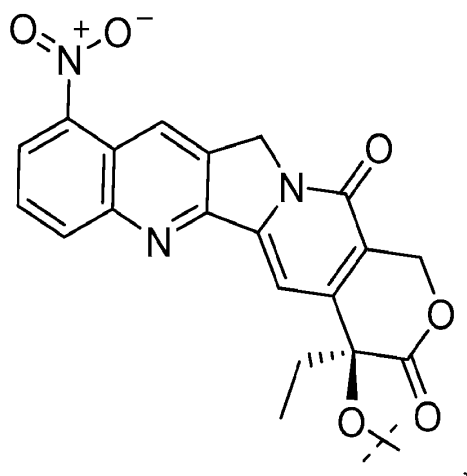
or a pharmaceutically acceptable salt thereof, wherein:

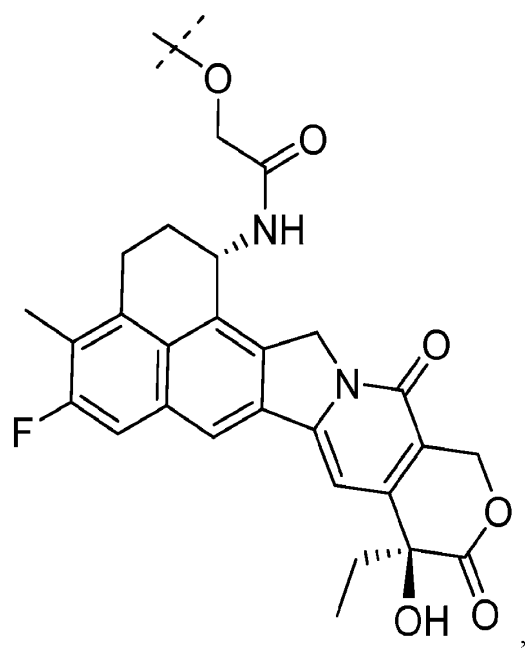
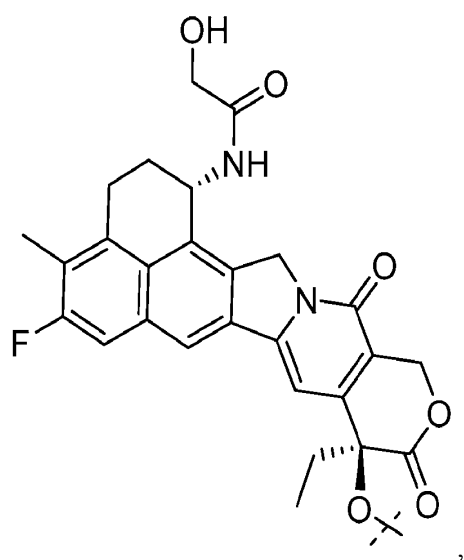
R⁷ is a peptide;

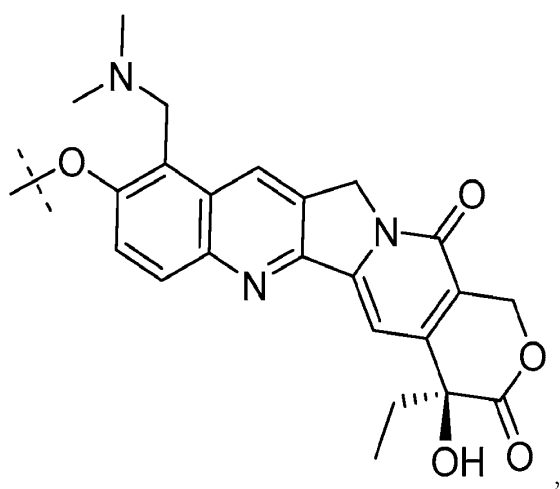
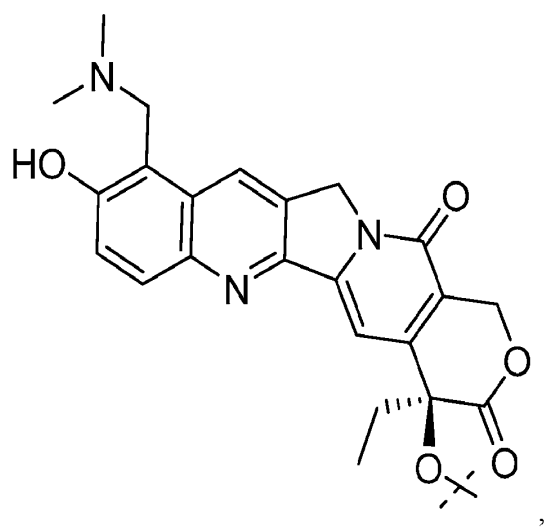
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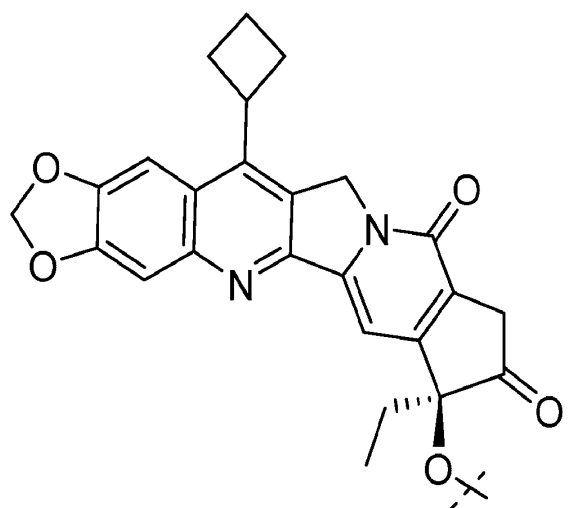
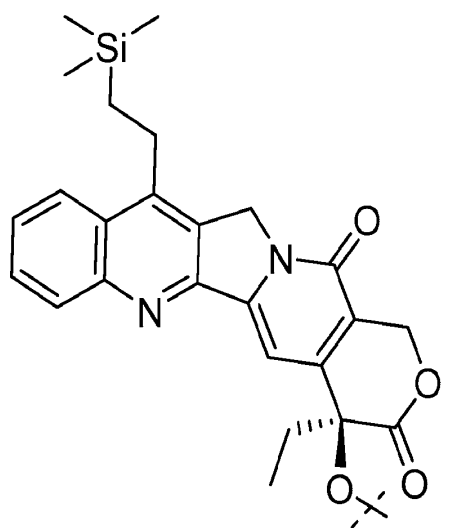


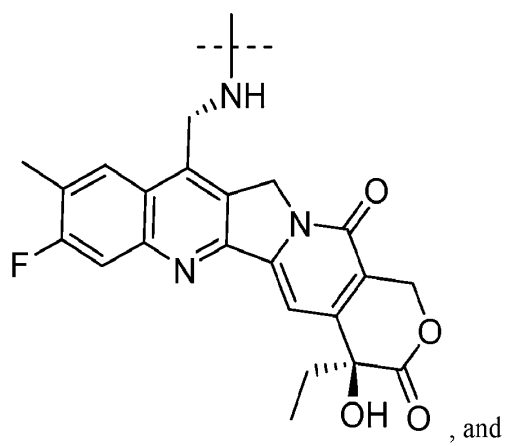
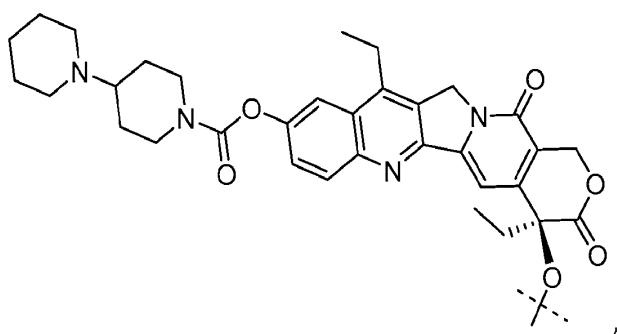
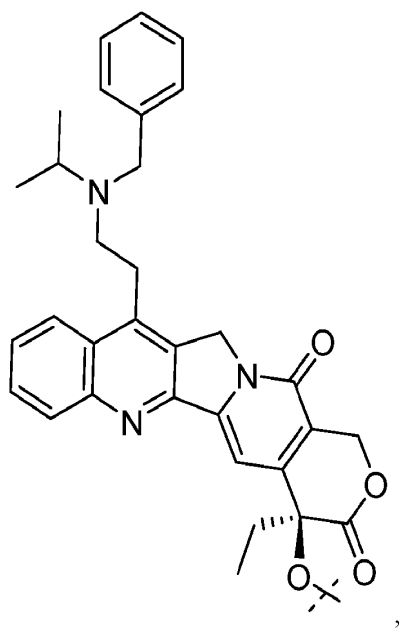


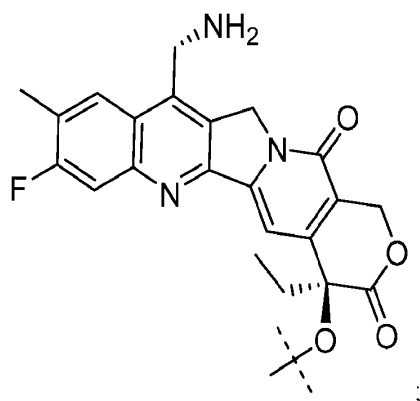




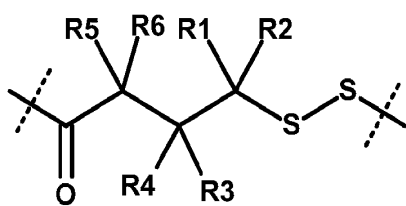
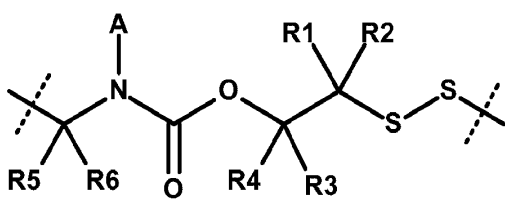
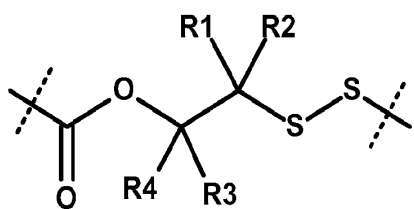




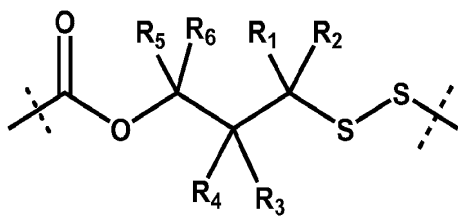


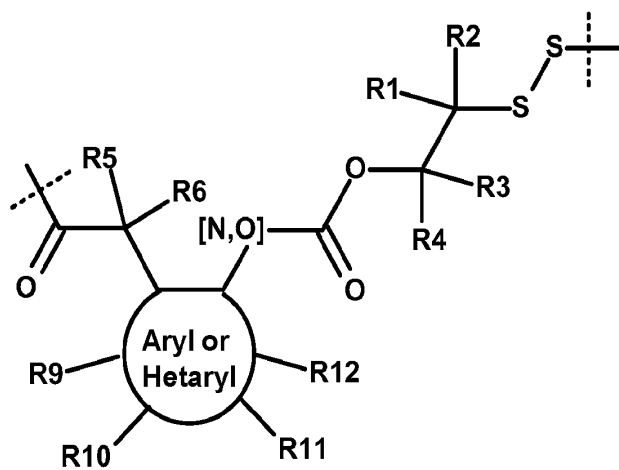
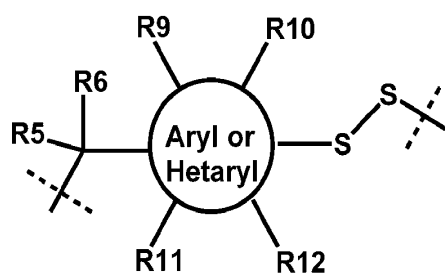
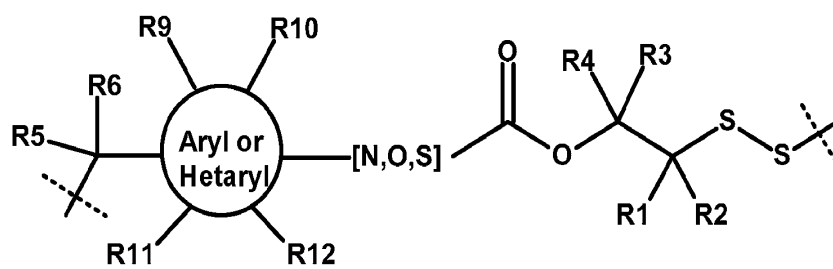
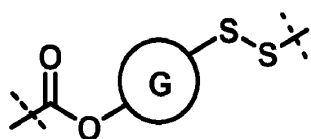


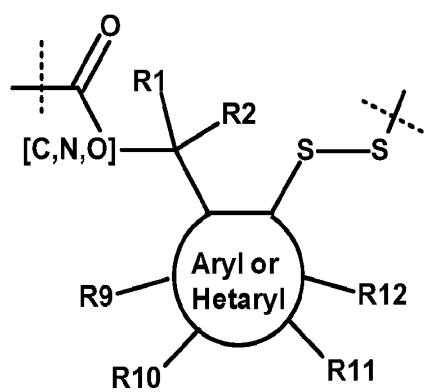
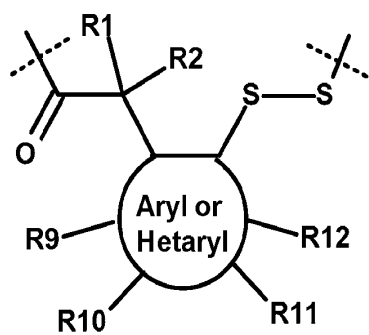
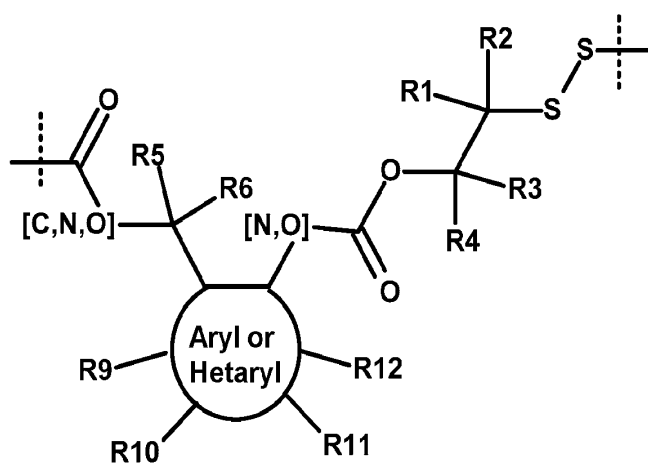
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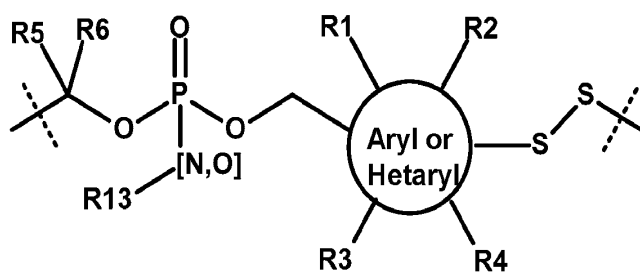
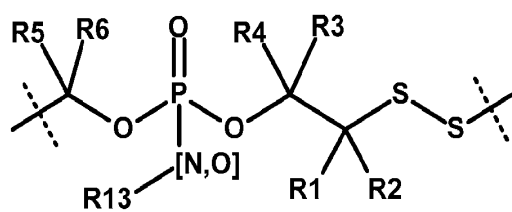
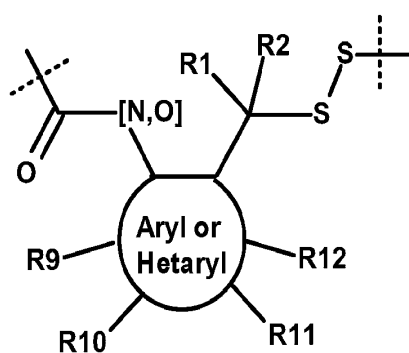
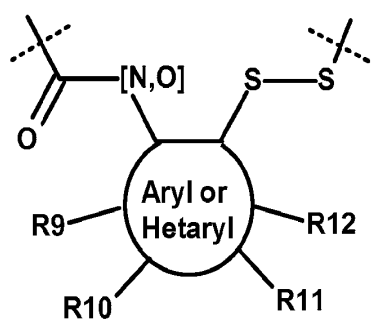


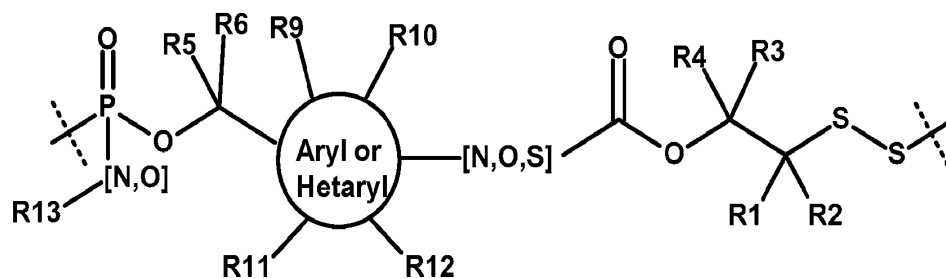
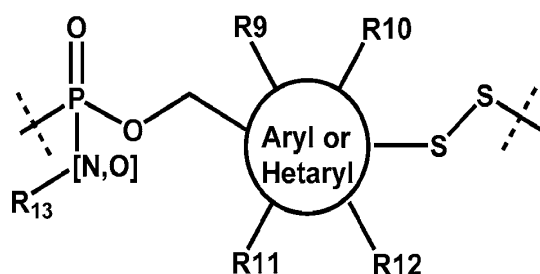
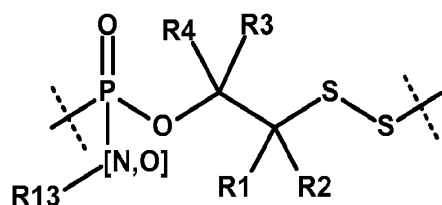
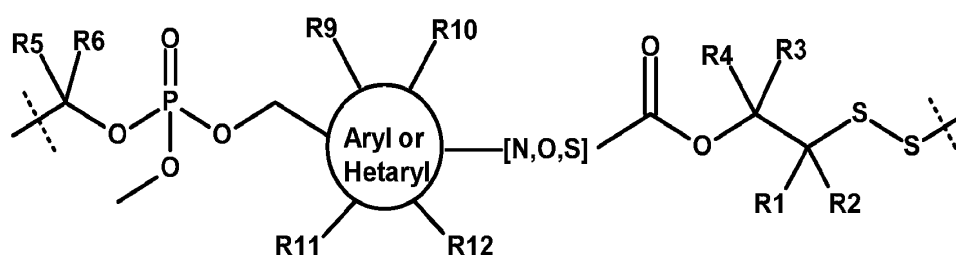
5











- 5 $R^1, R^2, R^3, R^4, R^5, R^6, R^9, R^{10}, R^{11}$, and R^{12} 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
- 10 C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, C_{3-10} cycloalkyl, 5-10 membered heteroaryl, and 4-10 membered heterocycloalkyl, are each optionally substituted with 1, 2, or 3 substituents

independently selected from halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

or R¹ and R² together with the carbon atom to which they are attached form a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with

- 5 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

or R¹ and R³ together with the carbon atoms to which they are attached form a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with

- 10 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

or R² and R³ together with the carbon atoms to which they are attached form a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with

- 15 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

or R³ and R⁴ together with the carbon atom to which they are attached form a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with

- 20 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

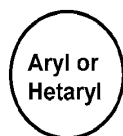
or R⁵ and R⁶ together with the carbon atom to which they are attached form a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with

- 25 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl}, C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl};

R¹³ is H or C₁₋₆ alkyl;

A is H or C₁₋₄ alkyl;

R^{a1} , R^{b1} , R^{c1} , and R^{d1} are each independently selected from H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, OH, CN, NO₂, and CO₂CH₃; wherein said C₁₋₆ alkyl and C₂₋₆ alkenyl are each optionally substituted with OH, CN, NO₂, or CO₂CH₃;



is C₆₋₁₀ 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;

Ring G is a C₃₋₁₄ cycloalkyl group or 4-14 membered heterocycloalkyl group, each optionally substituted with 1, 2, or 3 substituents independently selected from C₁₋₄ alkyl, halo, CN, NO₂, 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};

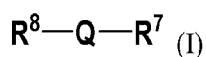
[N, O, S] is NH, O, or S;

[N, O] is NH or O;

[C, N, O] is CR^XR^Y, NH, or O; and

each R^X and R^Y are independently selected from H and C₁₋₄ alkyl.

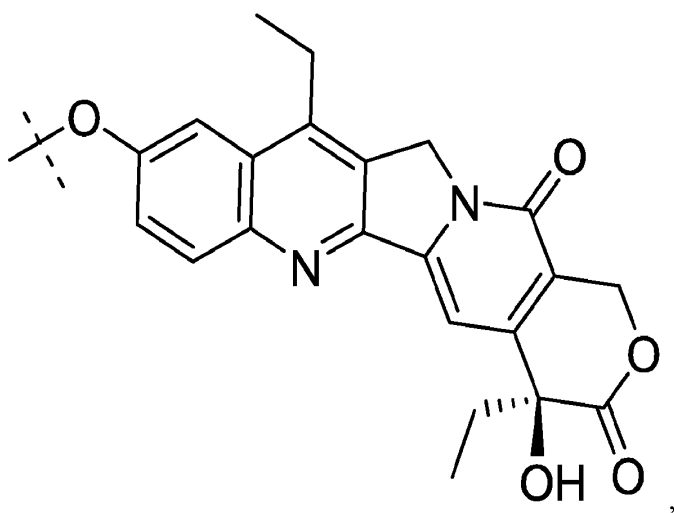
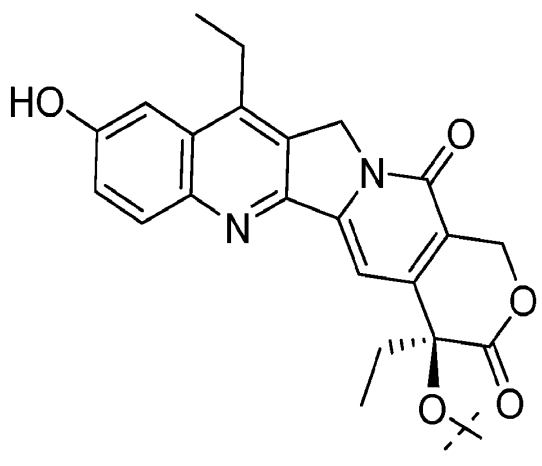
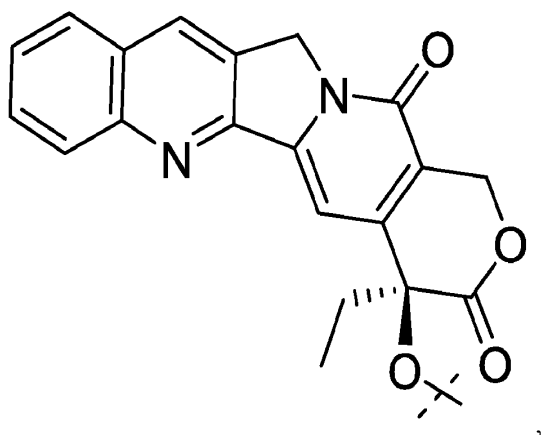
Provided herein is a compound of Formula (I):

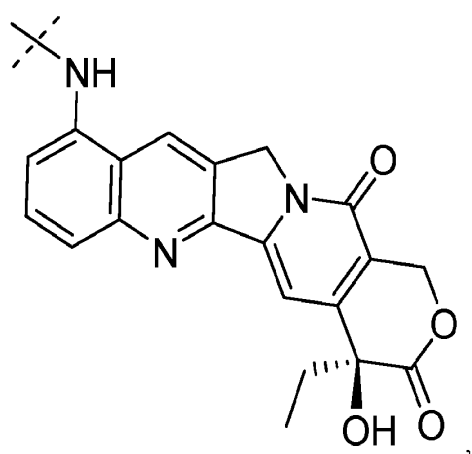
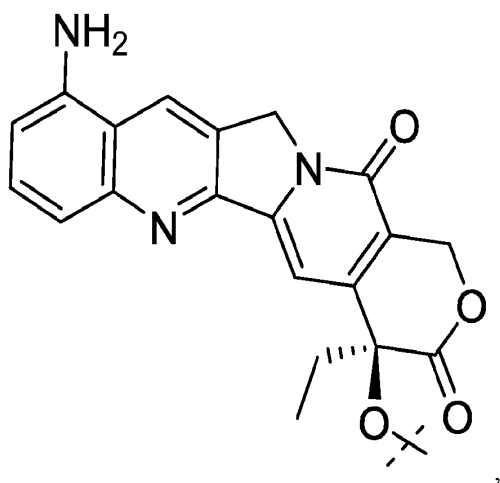


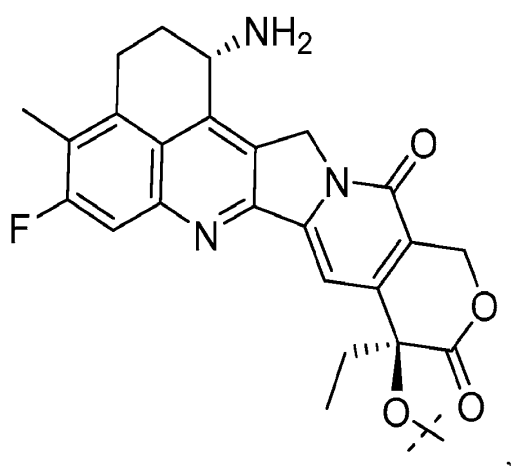
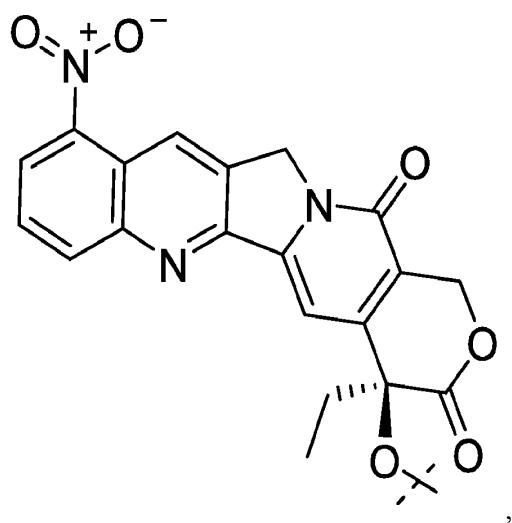
or a pharmaceutically acceptable salt thereof, wherein:

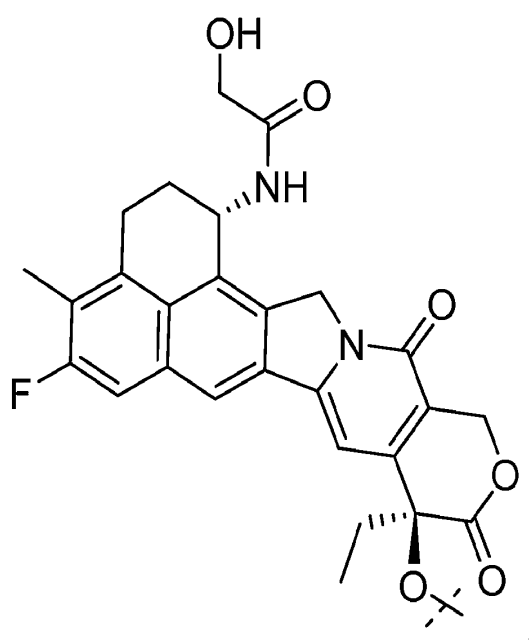
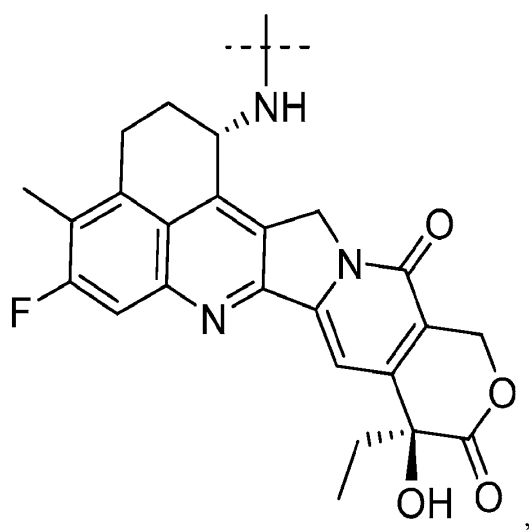
R⁷ is a peptide;

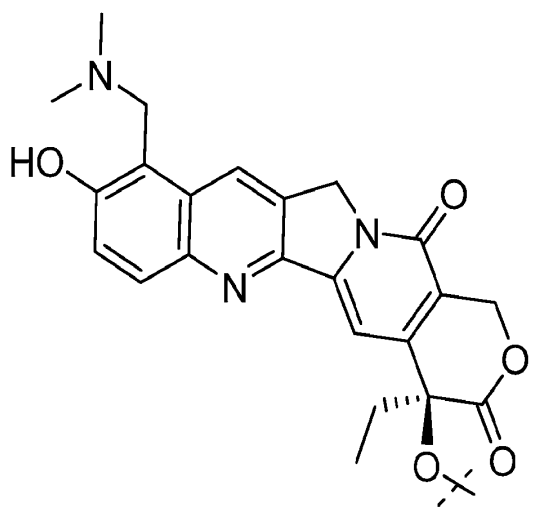
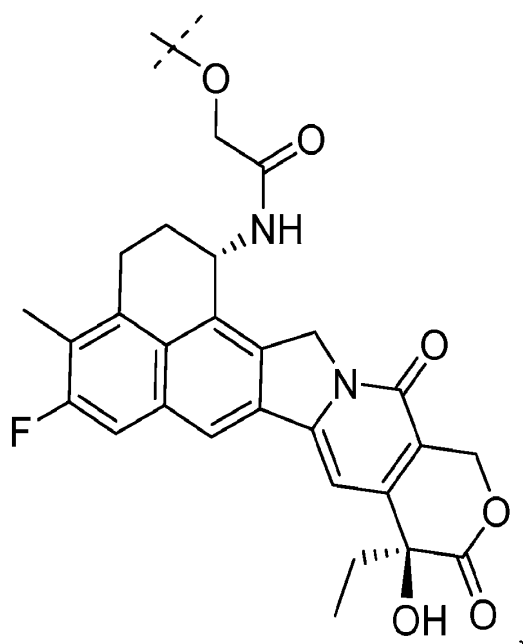
R⁸ is selected from the group consisting of:

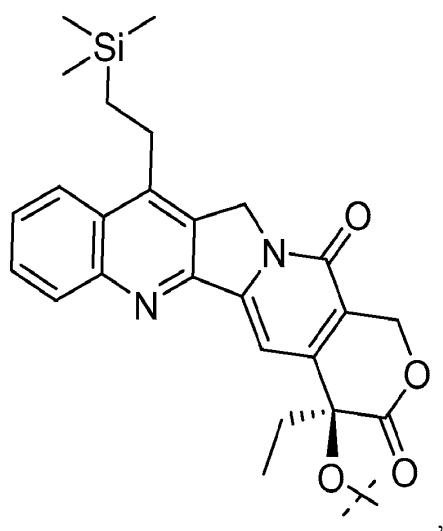
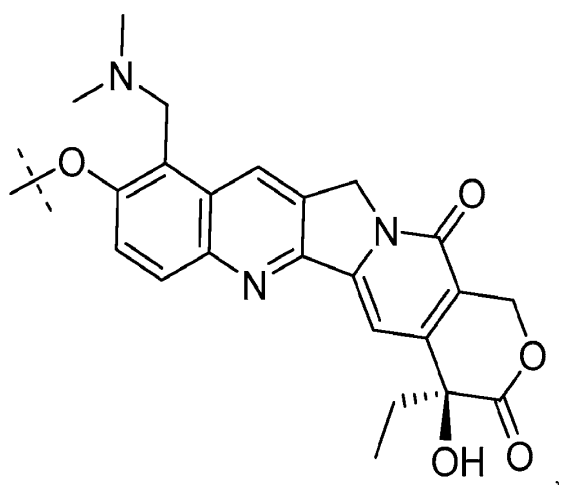


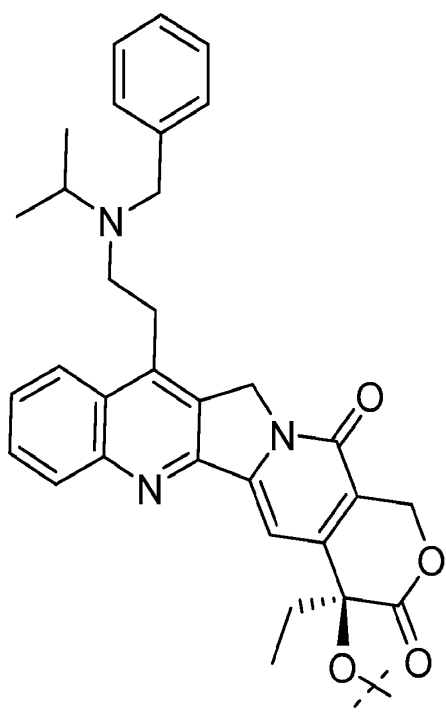
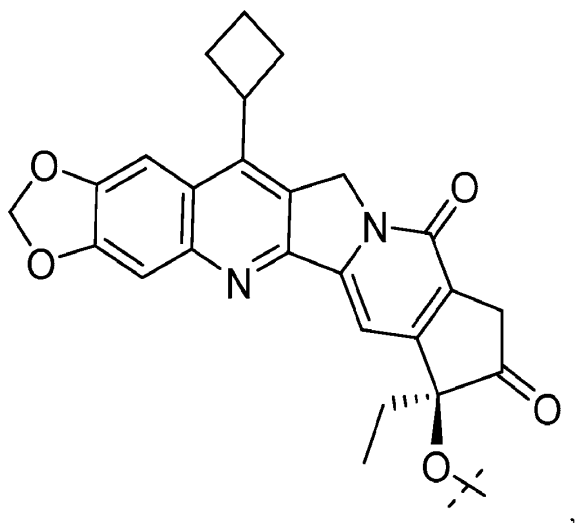




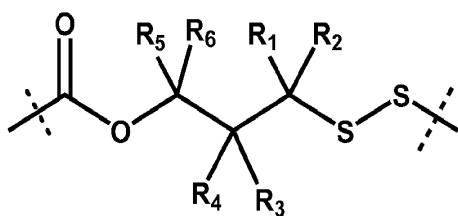
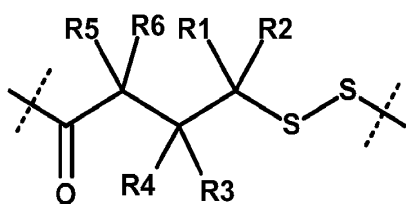
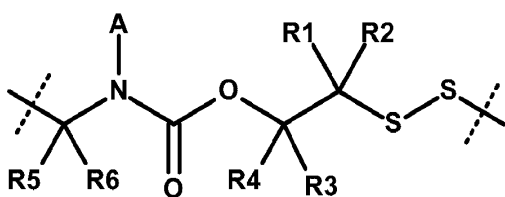
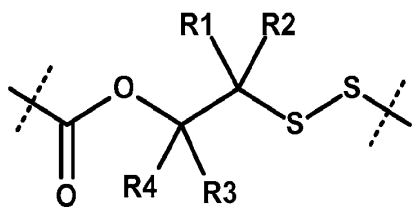




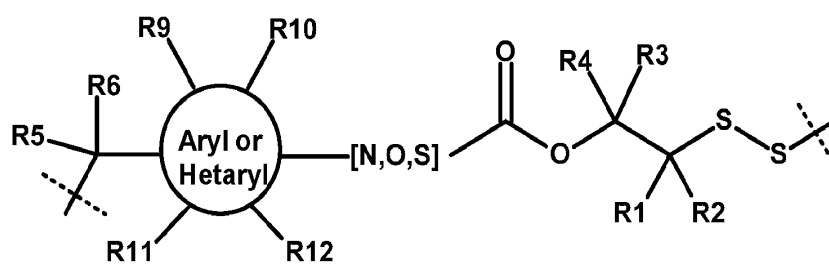


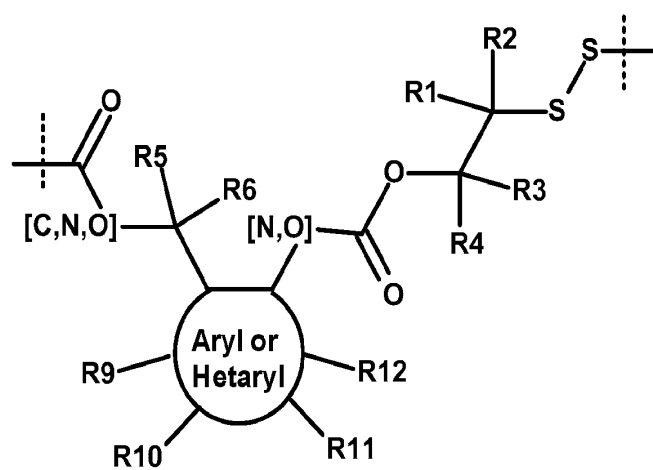
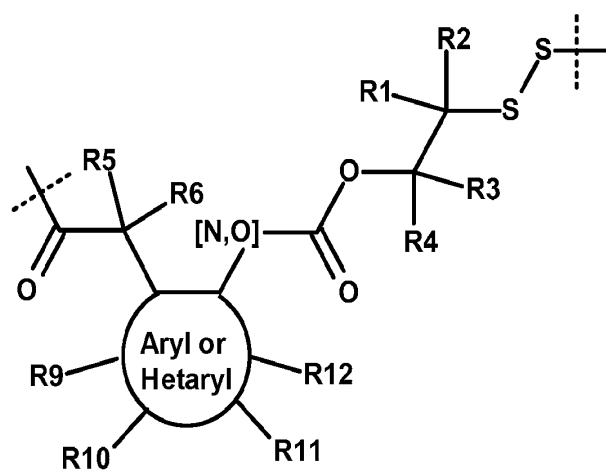
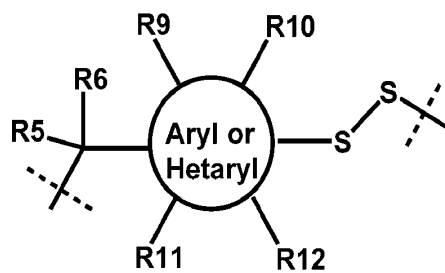


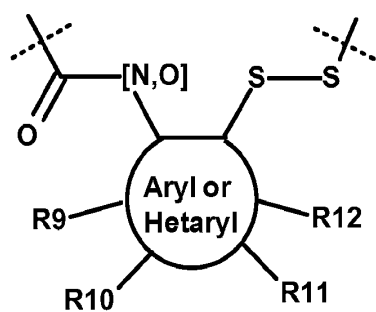
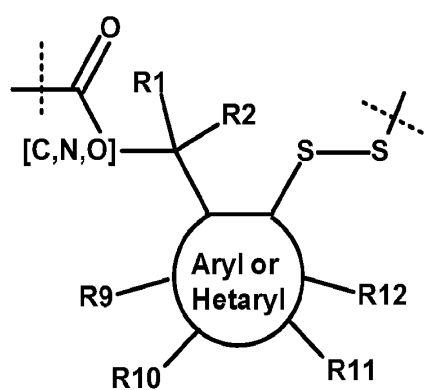
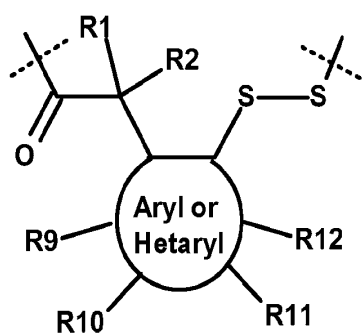
Q is selected from the group consisting of

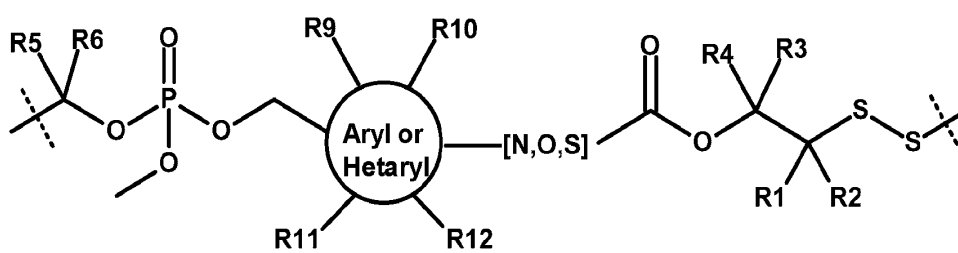
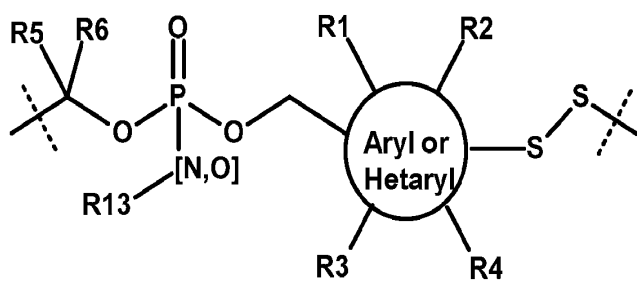
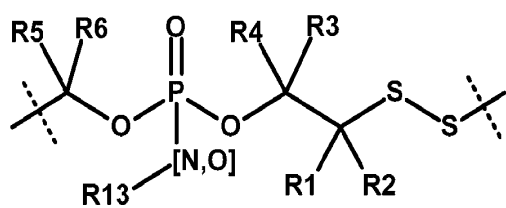
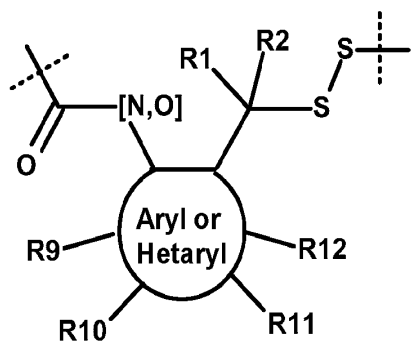


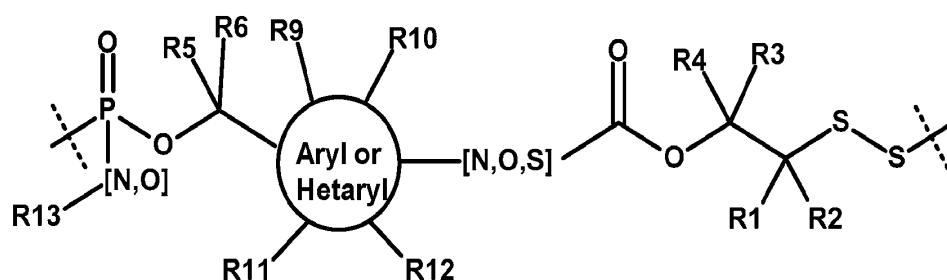
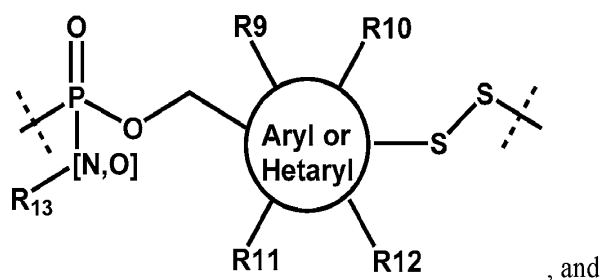
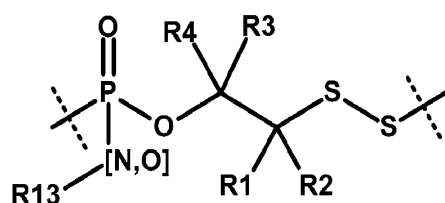
5











- $R^1, R^2, R^3, R^4, R^5, R^6, R^9, R^{10}, R^{11}$, and R^{12} are each independently selected from H,
- 5 C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, 5-10 membered heteroaryl, halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$, $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$, and $NR^{cl}C(O)NR^{cl}R^{dl}$, 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^{al} , SR^{al} , $C(O)R^{bl}$, $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$,
- 10 $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$, and $NR^{cl}C(O)NR^{cl}R^{dl}$,

or R^1 and R^2 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$, $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$, and $NR^{cl}C(O)NR^{cl}R^{dl}$.

or R^1 and R^3 together with the carbon atom to which they are attached form a 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 $NR^{c1}C(O)NR^{c1}R^{d1}$;

5 or R^2 and R^3 together with the carbon atom to which they are attached form a 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 $NR^{c1}C(O)NR^{c1}R^{d1}$;

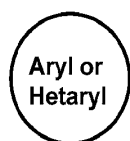
10 or R^3 and R^4 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{a1} , SR^{a1} , $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$,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

15 or R^5 and R^6 together with the carbon atom to which they are attached form a 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 $NR^{c1}C(O)NR^{c1}R^{d1}$;

R^{13} is H or C_{1-6} alkyl;

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

20 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_3 ;



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;

25 [N, O, S] is NH, O, or S;

[N, O] is NH or O;

[C, N, O] is $CR^X R^Y$, NH, or O; and

each R^X and R^Y are independently selected from H and C_{1-4} alkyl.

In some embodiments, the lefthand side of Q attaches to R⁸ and the righthand side of Q attaches to R⁷.

In some embodiments, a sulfur atom of the disulfide moiety of Q is part of a cysteine residue of R⁷.

5 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 R⁷ 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
10 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., R⁸Q-) across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.0. In some embodiments, the peptide
15 is capable of selectively delivering a conjugated moiety (e.g., R⁸Q-) 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., R⁸Q-) across a cell membrane having an acidic or hypoxic mantle having a pH less than about 5.5. In some
20 embodiments, the peptide is capable of selectively delivering a conjugated moiety (e.g., R⁸Q-) 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 R⁷ includes a cysteine residue which can form the site of attachment to a payload moiety (e.g., R⁸Q-) to be delivered across a cell membrane. In some embodiments, R⁷ is attached to Q through a cysteine residue of R⁷. In some embodiments,
25 the sulfur atom of the cysteine residue can form part of the disulfide bond of the disulfide bond-containing linker Q.

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, R⁷ is a peptide comprising at least one of the following sequences:

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1),

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

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3);

Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGKCG (SEQ ID NO. 4; Pv4); and

AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID No. 5; Pv5);

10 wherein R⁷ is attached to Q through a cysteine residue of R⁷.

In some embodiments, R⁷ 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),

15 wherein R⁷ is attached to Q through a cysteine residue of R⁷.

In some embodiments, R⁷ is a peptide comprising the sequence

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1).

In some embodiments, R⁷ is a peptide comprising the sequence

AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2).

20 In some embodiments, R⁷ is a peptide comprising the sequence

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3).

In some embodiments, R⁷ is a peptide comprising the sequence

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

In some embodiments, R⁷ is a peptide comprising the sequence

25 AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID NO. 5; Pv5).

In some embodiments, R⁷ is a peptide consisting of the sequence

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1).

In some embodiments, R⁷ is a peptide consisting of the sequence

AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO. 2; Pv2).

In some embodiments, R⁷ is a peptide consisting of the sequence
ADDQNPWARYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3).

In some embodiments, R⁷ is a peptide consisting of the sequence Ac-
AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGKCG (SEQ ID NO. 4; Pv4).

5 In some embodiments, R⁷ is a peptide consisting of the sequence
AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTC (SEQ ID NO. 5; Pv5).

In some embodiments, R⁷ is a peptide comprising at least one sequence selected from
SEQ ID NO: 6 to SEQ ID NO: 311 as shown in Table 1.

In some embodiments, R⁷ is a peptide consisting of a sequence selected from SEQ ID
10 NO: 6 to SEQ ID NO: 311 as shown in Table 1.

Table 1. Additional R⁷ Sequences

SEQ ID NO.	Sequence
6	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTCG
7	AEQNPYIYARYADWLFTTPLLALLDLALLVDADEGT
8	GGEQNPYIYARYADWLFTTPLLALLDLALLVDADEGT
9	AEQNPYIYARYADWLFTTPLLALLDLALLVDADEGT
10	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTCG
11	GGEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTCG
12	ACEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTG
13	ACEQNPYIYARYADWLFTTPLLALLDLALLVDADEGT
14	AKEQNPYIYARYADWLFTTPLLALLDLALLVDADEGT
15	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGKCG
16	AKEQNPYIYARYADWLFTTPLLALLDLALLVDADECT
17	ACEQNPYIYARYANWLFTTPLLALLNLALLVDADEGTG
18	ACEQNPYIYARYAKWLFTTPLLALLKLALLVDADEGTG
19	GGEQNPYIYARYADWLFTTPLLALLDLALLVNANQGT
20	AAEQNPYIYARYADWLFTTPLLALLALALLVDADEGT
21	AAEQNPYIYARYAAWLFTTPLLALLDLALLVDADEGT
22	AAEQNPYIYARYADWLFTTALLLLDLALLVDADEGT
23	AAEQNPYIYARYADWLFTTPLLLELALLVDADEGT

24	AAEQNPIYWARYAEWLFTTPLLLLLDLALLVDADEGT
25	AAEQNPIIYWARYADWLFTDLPLLLLLDLLALLVDADEGT
26	GEQNPIYWAQYADWLFTTPLLLLLDLALLVDADEGTCG
27	GGEQNPIYWARYADWLFTTPLLLLLDLLALLVDADEGTCG
28	GGEQNPIYWARYADWLFTTPLLLLLDALLVDADEGTCG
29	GGEQNPIYWARYDAWLFTTPLLLLLDLALLVDADEGTCG
30	GGEQNPIYWARYAWDLFTTPLLLLLDLALLVDADEGTCG
31	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
32	DDDEDNPIYWARYADWLFTTPLLLLLHGALLVDADECT
33	DDDEDNPIYWARYAHWLFTTPLLLLLHGALLVDADEGCT
34	DDDEDNPIYWARYAHWLFTTPLLLLLHGALLVNADECT
35	DDDEDNPIYWARYAHWLFTTPLLLLLHGALLVNANECT
36	AEQNPIYWARYADFLFTTPLLLLLDLALLVDADET
37	AEQNPIYFARYADWLFTTPLLLLLDLALLVDADEGT
38	AEQNPIYFARYADFLFTTPLLLLLDLALLWDADET
39	AKEDQNPYWARYADWLFTTPLLLLLDLALLVDG
40	ACEDQNPYWARYADWLFTTPLLLLLDLALLVDG
41	AEDQNPYWARYADWLFTTPLLLLLDLALLVDCG
42	AEDQNPYWARYADWLFTTPLLLLLLELALLVECG
43	AKEDQNPYWRAYADLFTPLTLLDLLALWDG
44	ACEDQNPYWRAYADLFTPLTLLDLLALWDG
45	ACDDQNPWRAYLDLLFPTDTLLLDLLW
46	TEDADVLLALDLLLLPTTFLWD
47	AEQNPIYWARYADWLFTTPL
48	AEQNPIYWARYADWLFTTPCL
49	ACEQNPIYWARYADWLFTTPL
50	AEQNPIYFARYADWLFTTPL
51	KEDQNPWARYADLLFPTTLAW
52	ACEDQNPWARYADLLFPTTLAW
53	ACEDQNPWARYADWLFTTLLLLD
54	ACEEQNPWARYAELLFPTTLAW
55	ACEEQNPWARYAEWLFTTLLLLLE
56	ACEEQNPWARYLEWLFTTETLLLLLE

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 WARYADWLFTTPLLLLLELALLV 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 WARYLDWLFPDTLLLDL
86	CDNNNP WRAYLDLLFPTDTLLLDW
87	ACEEQNP WARYLEWLFPETLLLEL
88	ACEDQNP WARYADWLFPDTLLLDL
89	ACEEQNP WARYAEWLFPDTLLLLE

90	ACEDQNP WARYADLLFPTTLAW
91	ACEDQNP WARYAELLFPTTLW
92	KEDQNP WARYADLLFPTTLW
93	DDDEDNP IYWARYAHLFTTPLLHLGALLVDADECT
94	DDDEDNPIYWARYAHLFTTPLLHLGALLVDADECT
95	DDDEDNPIYWARYAHLFTTPLLHLGALLVNADECT
96	DDDEDNPIYWARYAHLFTTPLLHLGALLVNANECT
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98	ACEQNPIYWARYADWLFTTPLLHLDLALLVDADEGIG
99	ACEQNPIYWARYADWLFTTPLLHLDLALLVDADEGT
100	ACEQNPIYWARYADWLFTTPLLHLDLALLVDADEGT
101	GGEQNPIYWARYADWLFTTPLLHLDLALLVDADEGTCG
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103	GGEQNPIYWARYADWLFTTPLLHLDLALLVDADEGTCG
104	AAEQNPIYWARYAEWLFTTPLLHLDLALLVDADEGTCG
105	AAEQNPIYWARYAEWLFTTPLLHLELALLVDADEGTCG
106	GGEQNPIYWARYADWLFTTPLLHLDLALLVDADEGTCG
107	GGEQNPIYWAQYADWLFTTPLLHLDLALLVDADEGTCG
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110	ACEQNPIYWARYANWLFTTPLLHLNLALLVDADEGTG
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112	DDDEDNPIYWARYAHLFTTPLLHLGALLVNADECT
113	DDDEDNPIYWARYADWLFTTPLLHLGALLVDADECT
114	DDDEDNPIYWARYAHLFTTPLLHLGALLVDADECT
115	DDDEDNPIYWARYAHLFTTPLLHLGALLVDADECT
116	GGEQNPIYWARYADWLFTTPLLHLDLALLVNANQGT
117	AAEQNPIYWARYADWLFTTPLLHLELALLVDADEGTCG
118	AAEQNPIYWARYAEWLFTTPLLHLELALLVDADEGTCG
119	AAEQNPIYWARYADWLFTTPLLHLELALLVDADEGTCG
120	GGEQNPIYWAQYADWLFTTPLLHLDLALLVDADEGTCG
121	GGEQNPIYWAQYADWLFTTPLLHLDLALLVDADEGTCG
122	GGEQNPIYWAQYADWLFTTPLLHLDLALLVDADEGTCG

123	GGEQNPIYWARYADWLFTTPLLLLDALLVNANQGT
124	DDDEDNPIYWARYAHLFTTPLLLHLGALLVNADECT
125	DDDEDNPIYWARYAHLFTTPLLLHLGALLVNANECT
126	ACEQNPIYWARYAKWLFTTPLLLKLALLVDADEGTG
127	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
128	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
129	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
130	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
131	AAEQNPIYWARYADWLFTPLLLLDLALLVDADEGT
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133	GGEQNPIYWARYADWLFTPLLLLDLALLVDADEGTCG
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135	AEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
136	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
137	GGEQNPIYWAQDYAWLFTTPLLLLDLALLDADEGTCG
138	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
139	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
140	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG EGTK(rhodamine)C(phalloidin)G
141	AAEQNPIYWARYADWLFTTPLLLLELALLDADEGTCG
142	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
143	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
144	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
145	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
146	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
147	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
148	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
149	DDDEDNPIYWARYAHLFTTPLLLHLGALLVNADECT
150	DDDEDNPIYWARYAHLFTTPLLLHLGALLVNANECT
151	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
152	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
153	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCG
154	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
155	DDDEDNPIYWARYAHLFTTPLLLBLGALLVNADECT

156	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
157	DDDEDNPIYWARYAHLFTTPLLLLBGALLVNADECT
158	DDDEDNPIYWARYAHLFTTPLLLLBGALLVNANECT
159	DDDEDNPIYWARYADWLFTTPLLLLIBGALLVDADECT
160	DDDEDNPIYWARYADWTFTTPLLLLHGALLVDADECT
161	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
162	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
163	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
164	DDDEDNPIYWARYHWLFTTPLLLLHGALLVNANECT
165	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNANECT
166	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
167	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
168	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
169	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
170	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
171	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
172	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
173	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
174	DDDEDNPIYWARYAHMLFTTPLLLLDGALLVDADECT
175	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNANECT
176	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
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178	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
179	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
180	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNANECT
181	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
182	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCG
183	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCG
184	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCG
185	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
186	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
187	GGEQNPIYWARYADWLFTTPLLLLDALLVDADEGTCG
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189	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
190	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
191	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
192	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
193	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
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198	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
199	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
200	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
201	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
202	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGKCG
203	EGTK(rhidamine)C(phalloidin)G
204	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGKCG
205	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
206	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
207	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGKCG
208	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGKCG
209	AAEQNPIYWARYADWLFTDLPLLLLDLALLVDADEGT
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212	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCG
213	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
214	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGTCG
215	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
216	AAEQNPIYWARYADWLFTTPLLLALALLVDADEGTCG
217	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCG
218	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
219	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGKCG
220	ACEQNPIYWARYAKWLFTTPLLLKLALLVDADEGTG
221	ACEQNPIYWARYANWLFTTPLLLNLALLVDADEGTG

222	AAEQNPYIYARYADWLFTTALLLLDLALLVDADEGT
223	AEQNPIYFARYADLLFPTTLAW
224	AEQNPIYARYADLLFPTTLAF
225	AEQNPIYARYADLLFPTTLAW
226	ACEQNPIYARYADWLFTTPLLALLDLALLVDADET
227	GGEQNPIYARYADWLFTTPLLALLDLALLVDADEGT
228	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTGCG
229	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTGKCG
230	AKEQNPIYARYADWLFTTPLLALLDLALLVDADECT
231	CCTCTTACCTCAGTTACA
232	D-Arg8 D-Arg8-CCTCTTACCTCAGTTACA
233	D-Lys4 D-Lys4-CCTCTTACCTCAGTTACA
234	S-S-CCTCTTACCTCAGTTACA
235	S-S-CCTCTGACCTCATTACA
236	D-Arg8-Deca D-Arg8-Deca-CCTCTTACCTCAGTTACA
237	D-Arg8-Deca-mismatch D-Arg8-Deca-CCTCTGACCTCATTACA
238	S-S-CCTCTTACCTCAGTTACA
239	AAEQNPYIYARYADWLFTTPLLALLDLALLVDADEGTGCG
240	AEDQNPYARYDWLFTTPLLALLDLALLVDCG
241	AEDQNPYARYADWLFTTPLLLELALLVECG
242	AEQNPIYARYADWLFTTPLLALLDLALLVDADEGCT
243	ACEQNPIYARYADWLFTTPLLALLDLALLVDADET
244	AE-QN-PI YWARYADWLFTTPLLALLDLALLV DAEDEGT-COOH
245	AEDQN-P- YWARYADWLFTTPLLALLDLALLV D---G--COOH
246	AEDQNDP-YWARYADWLFTTPLLALLDLALLV----G--COOH
247	AEQNPI YWARYADFLFTTPLLALLDLALLV DAEDEGT-COOH
248	AEQNPI YFARYADWLFTTPLLALLDLALLV DAEDEGT-COOH
249	AEQNPI YFARYADFLFTTPLLALLDLALLW DAEDEGT-COOH
250	AE-QN-PI YWARYADWLFTTPLLALLDLALLV DAEDEGCT-COOH
251	AEDQN-PI YWARYADWLFTTPLLALLDLALLV DC--G-T-COOH
252	AEDQNDPI YWARYADWLFTTPLLLELALLV EC--G-T-COOH
253	Chelate-ACEEQNPWARYLEWLFPTETLLEL
254	AEQNPIY WARYADWLFTTPLLALLDLALLV DAEDEGT-COOH

255	AKEDQNPY WARYADWLFTTPLLALLDLALLV DG-COOH
256	AKEDQNDPY WARYADWLFTTPLLALLDLALLV G-COOH
257	AEQNPI YWARYADWLFTTPLLALLDLALLV DADEGC-Biotin-T-COO H
258	AEDQNP YWARYADWLFTTPLLALLDLALLV DC-Biotin-G-COOH
259	AEDQNP YWARYADWLFTTPLLLELALLV EC-Biotin-G-COOH
260	ACEQNPIY WARYADWLFTTPLLALLDLALLV DADEGT
261	ACEDQNPY WARYADWLFTTPLLALLDLALLV DG
262	ACEDQNPY WRAYADLFTPLTLLDLLALW DG
263	ACDDQNP WRAYLDLLFPTDTLLLDLLW
264	WRAYLELLFPTETLLLELLW
265	WARYLDWLFTPTDTLLLDL
266	WRAYLDLLFPTDTLLLDW
267	WARYLEWLFTPTETLLLEL
268	WAQYLELLFPTETLLLEW
269	WRAYLELLFPTETLLLEW
270	WARYADWLFTTLLLDL
271	WARYAEWLFTTLLLE
272	ACEDQNP WARYADLLFPTTLAW
273	ACEEQNP WARYAELLFPTTLAW
274	Ac-TEDAD VLLALDLLLLPTTFLWDAYRAW YPNQECA-Am
275	CDDDDDNPNY WARYANWLFTTPLLNGALLV EAEET
276	CDDDDDNPNY WARYAPWLFTTPLLPGALLV EAEET
277	Ac-AEQNPIY WARYADWLFTTPLLALLDLALLVDADEGCT
278	Ac-AEQNPIY WARYADWLFTTPLLALLDLALLVDADEGTG
279	ACEQNPIY WARYANWLFTTPLLNLALLVDADEGT
280	Ac-AAEQNPIY WARYADWLFTTPLLLELALLVDADEGTKCG
281	DDDEDNPIY WARYADWLFTTPLLHLGALLVDADET
282	CDDDEDNPIY WARYAHLFTTPLLHLGALLVDADET
283	DDDEDNPIY WARYAHLFTTPLLHLGALLVDADEGT
284	DDDEDNPIY WARYAHLFTTPLLHLGALLVNADEGT
285	DDDEDNPIY WARYAHLFTTPLLHLGALLVNANEGT
286	AKEDQNDPY WARYADWLFTTPLLALLDLALLVG
287	AEDQNPY WARYADWLFTTPLLLELALLVCG

288	AKDDQNPWAYLDLLFPTDTLLLDLLWC
289	ACEEQNPWAYLELLFPTETLLELLW
290	ACDDQNPWAYLDWLFPTDTLLLDL
291	CDNNNPWAYLDLLFPTDTLLLDW
292	CEEQPPWAQYLELLFPTETLLEW
293	EEQPPWAYLELLFPTETLLEW
294	CDDDDDNPNYWAYANWLFTTPLLNGALLVEAEET
295	CDDDDDNPNYWAYAPWLFTTPLLPGALLVEAEE
296	AEQNPIYFARYADLLFPTTLAW
297	AEQNPIYWAYADLLFPTTLAF
298	AEQNPIYWAYADLLFPTTLAW
299	KEDQNPWAYADLLFPTTLW
300	ACEEQNPQAEYAEWLFTTLLLE
301	AAEEQNPWAYLEWLFTTETLLEL
302	AKEEQNPWAYLEWLFTTETLLEL
303	AAEQNPIYWAYADWLFTTPLLDDLALLVDADEGTGG
304	XXEXNPIYWXXXXXLFTXXLLLXXXALLVXAXXXTXG
305	DAAEQNPIYWAYADWLFTTPLLDDLALLVDADEGTKGG
306	GGEQNPIYWAYADWLFTTPLLDDLALLVDADEGTGG
307	XXEXNPIYWXXXXXLFTXXLLLXXXALLVXAXXXTGG
308	DGGEQNDPIYWAYADWLFTTPLLDDLALLVDADEGCTXGG
309	AAEQNPIYWAYADWLFTTPLLDDLALLVDADEGTCG
310	AEDQNPYWAYDWLFTTPLLDDLALLVDCG
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.

- 5 In some embodiments, the peptide of R⁷ 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; Tesaro 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
5 reference in its entirety.

The term “small molecule topoisomerase I targeting moiety” or “topoisomerase I inhibitor” refers to a chemical group that binds to topoisomerase I. The small molecule topoisomerase I targeting moiety can be a group derived from a compound that inhibits the activity of topoisomerase I. Topoisomerase inhibitors include camptothecin and derivatives
10 and analogues thereof such as opotecan, irinotecan (CPT-11), silatecan (DB-67, AR-67), cositecan (BNP-1350), lurtotecan, gimatecan (ST1481), belotecan (CKD-602), rubitecan, topotecan, deruxitecan, and exatecan. Topoisomerase inhibitors are described in, for example, Ogitani, Bioorg. Med. Chem. Lett. 26 (2016), 5069-5072; Kumazawa, E., Cancer Chemother Pharmacol 1998, 42: 210-220; Tahara, M, Mol Cancer Ther 2014, 13(5): 1170-1180; Nakada,
15 T., Bioorganic & Medicinal Chemistry Letters 2016, 26: 1542-1545.

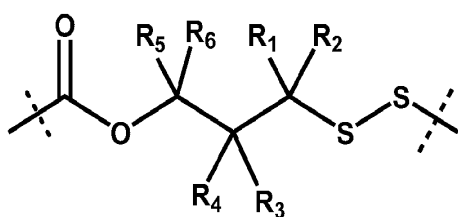
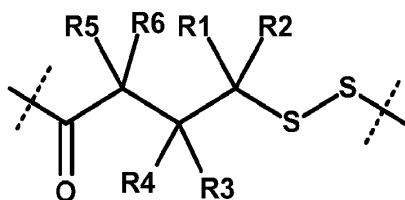
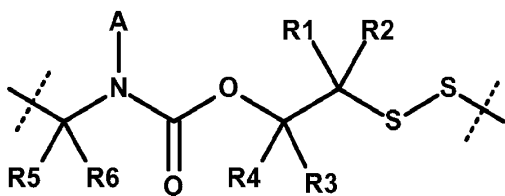
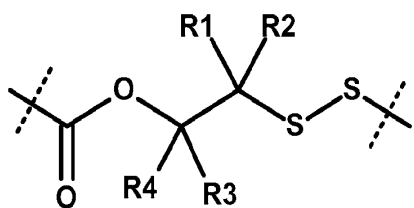
The moiety Q is a linking group, covalently connecting R⁷ and R⁸ that serves as a tether between the peptide and topoisomerase I inhibitor that may be cleaved when the conjugate or portion thereof is inside a cell. In some embodiments, Q is a chain of 1 to 40, 1 to 30, 1 to 25, 1
20 to 20, 1 to 15, 1 to 10, or 1 to 5 chain atoms, which is optionally substituted with 1-10 R^q substituents, and wherein one or more chain carbon atoms of Q 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

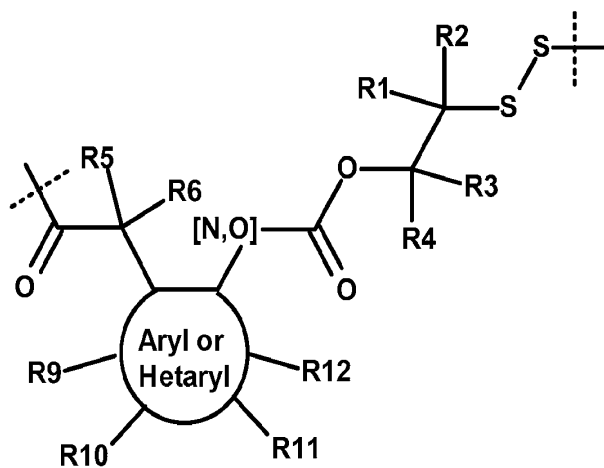
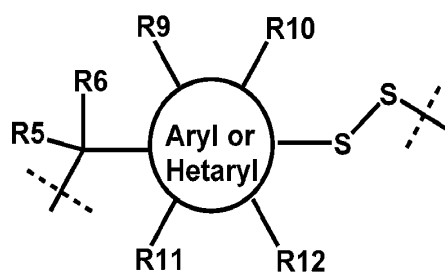
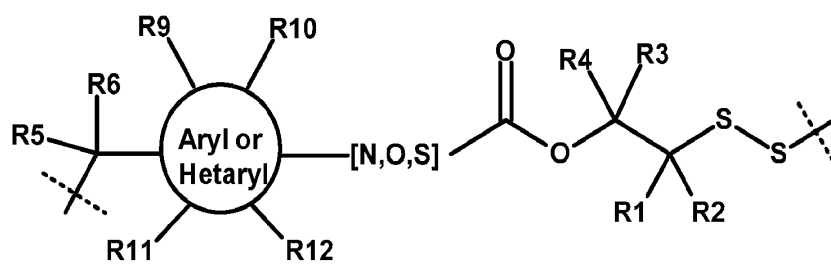
each R^q is independently selected from OH, CN, -COOH, NH₂, halo, C₁₋₆ haloalkyl, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ haloalkoxy, C₁₋₆ alkylthio, phenyl, 5-6 membered heteroaryl, 4-6
25 membered heterocycloalkyl, C₃₋₆ cycloalkyl, NH(C₁₋₆ alkyl) and N(C₁₋₆ alkyl)₂, wherein the C₁₋₆ alkyl, phenyl, C₃₋₆ cycloalkyl, 4-6 membered heterocycloalkyl, and 5-6 membered heteroaryl of R^q are each optionally substituted with halo, OH, CN, -COOH, NH₂, C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ haloalkyl, C₁₋₄ haloalkoxy, phenyl, C₃₋₁₀ cycloalkyl, 5- or 6-membered heteroaryl or 4-6 membered heterocycloalkyl; and

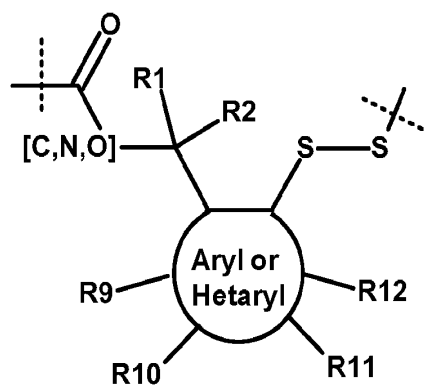
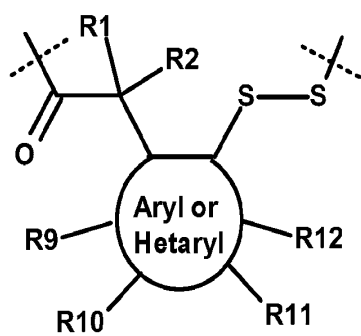
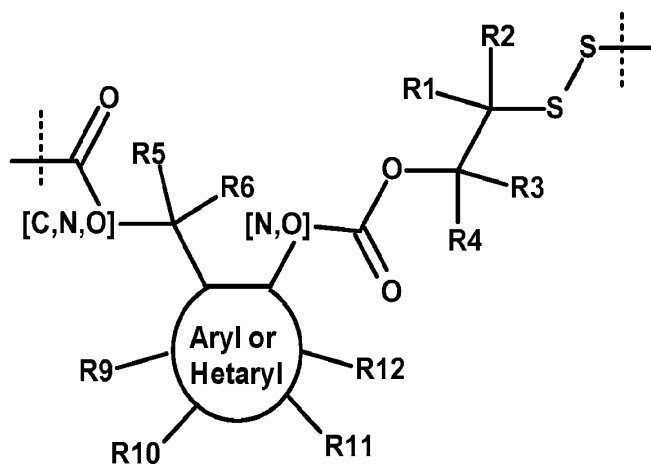
two R^q groups together with the chain atoms to which they are attached can form a phenyl, 5-6 membered heteroaryl, 4-6 membered heterocycloalkyl, or C_{3-6} cycloalkyl ring.

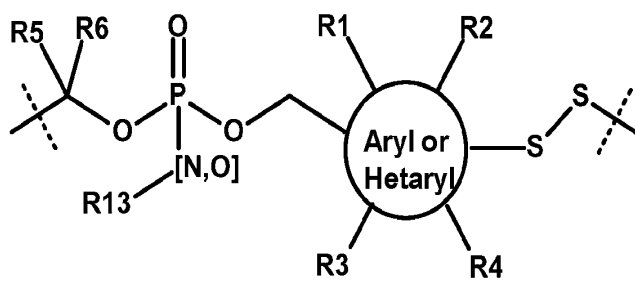
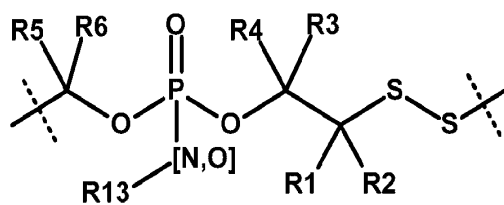
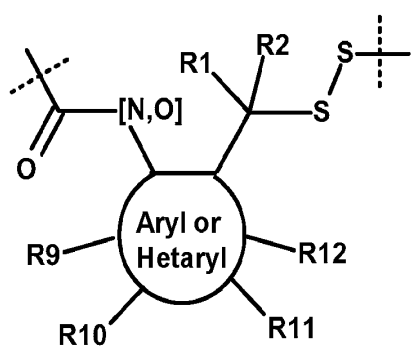
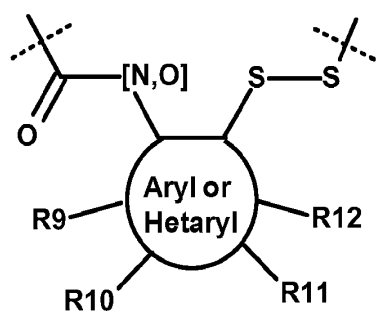
In some embodiments, R^q is independently selected from OH, CN, $-COOH$, NH_2 , halo, C_{1-6} haloalkyl, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, $NH(C_{1-6} \text{ alkyl})$ and $N(C_{1-6} \text{ alkyl})_2$.

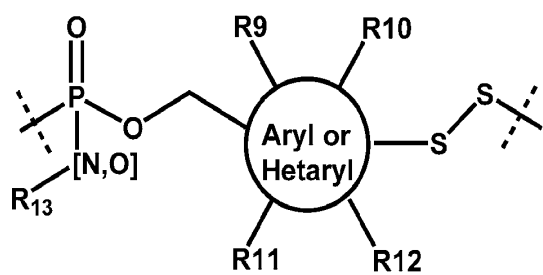
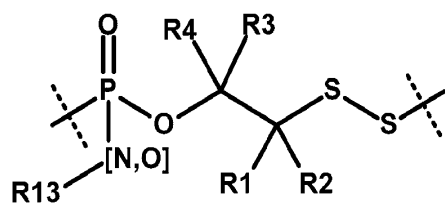
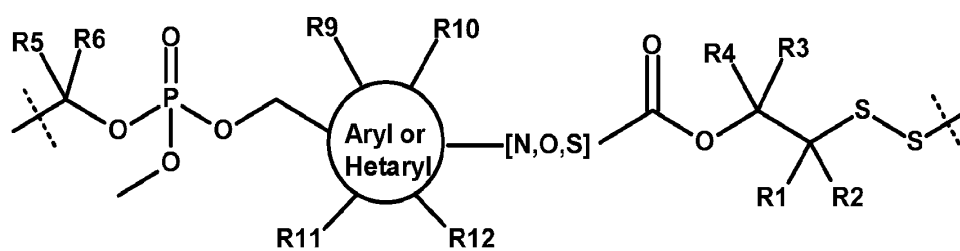
5 In some embodiments, Q is selected from:



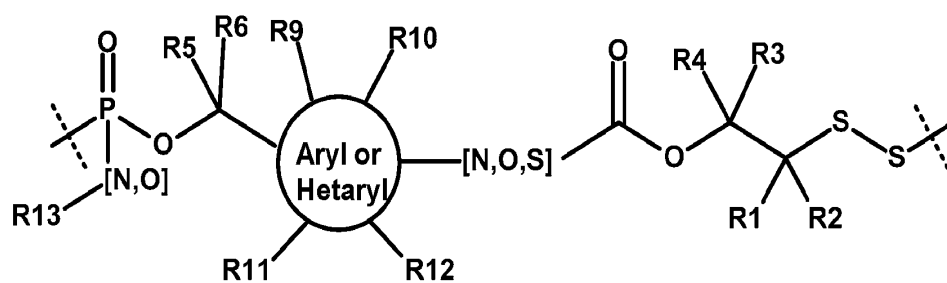




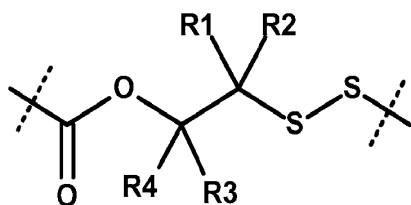




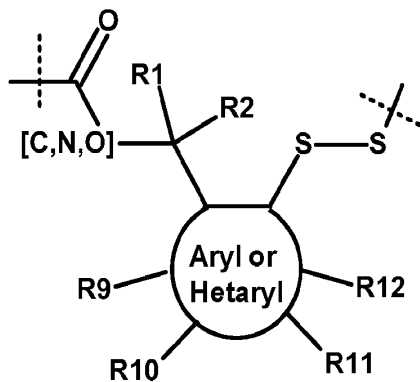
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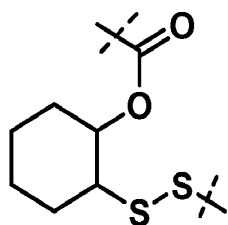
5 In some embodiments, Q is:



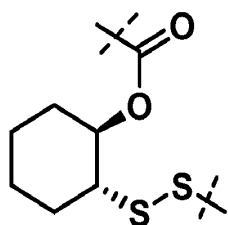
In some embodiments, Q is:



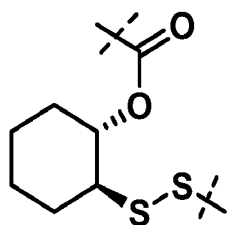
In some embodiments, Q is:



5 In some embodiments, Q is:



In some embodiments, Q is:



In some embodiments:

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

$C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$,
and $NR^{cl}C(O)NR^{cl}R^{dl}$;

or R^3 and R^4 together with the carbon atom to which they are attached form a C_{3-10}
cycloalkyl group or 4-10 membered heterocycloalkyl group, each optionally substituted with
1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$,
 $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$,
and $NR^{cl}C(O)NR^{cl}R^{dl}$.

In some embodiments:

R^1 , R^2 , R^3 , and R^4 are each independently selected from H and C_{1-4} alkyl;

or R^1 and R^2 together with the carbon atom to which they are attached form a C_{3-10}
cycloalkyl group or 4-10 membered heterocycloalkyl group, each optionally substituted with
1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$,
 $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$,
and $NR^{cl}C(O)NR^{cl}R^{dl}$;

or R^1 and R^3 together with the carbon atom to which they are attached form a C_{3-10}
cycloalkyl group or 4-10 membered heterocycloalkyl group, each optionally substituted with
1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$,
 $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$,
and $NR^{cl}C(O)NR^{cl}R^{dl}$;

or R^3 and R^4 together with the carbon atom to which they are attached form a C_{3-10}
cycloalkyl group or 4-10 membered heterocycloalkyl group, each optionally substituted with
1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$,
 $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$, $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$,
and $NR^{cl}C(O)NR^{cl}R^{dl}$.

In some embodiments, R^1 and R^2 are each independently selected from H and methyl,
and R^3 , R^4 , R^5 , and R^6 are each hydrogen.

In some embodiments, R^1 , R^2 , R^3 , and R^4 are each independently selected from H and
methyl, and R^5 , and R^6 are each hydrogen.

In some embodiments, R^1 and R^2 are each independently selected from H and methyl.

In some embodiments, R^3 and R^4 are each independently selected from H and methyl.

In some embodiments, R^1 and R^2 are each H.

In some embodiments, R^1 and R^2 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents
5 independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$, $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$,
 $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$, and $NR^{cl}C(O)NR^{cl}R^{dl}$.

In some embodiments, R^1 and R^2 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group.

In some embodiments, R^1 and R^2 together with the carbon atom to which they are
10 attached form a cyclobutyl group.

In some embodiments, R^3 and R^4 are each H.

In some embodiments, R^1 and R^3 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents
independently selected from halo, CN, NO_2 , OR^{al} , SR^{al} , $C(O)R^{bl}$, $C(O)NR^{cl}R^{dl}$, $C(O)OR^{al}$,
15 $OC(O)R^{bl}$, $OC(O)NR^{cl}R^{dl}$, $NR^{cl}R^{dl}$, $NR^{cl}C(O)R^{bl}$, $NR^{cl}C(O)OR^{al}$, and $NR^{cl}C(O)NR^{cl}R^{dl}$.

In some embodiments, R^1 and R^3 together with the carbon atom to which they are attached form a cyclopentyl, cyclohexyl, cycloheptyl, 1,2,3,4-tetrahydronaphthyl, tetrahydrofuranyl, or tetrahydropyranyl.

In some embodiments, R^1 and R^3 together with the carbon atom to which they are
20 attached form a C_{3-7} cycloalkyl group.

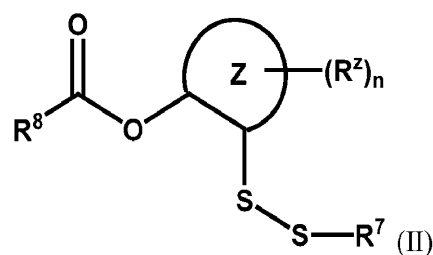
In some embodiments, R^1 and R^3 together with the carbon atom to which they are attached form a cyclohexyl group.

In some embodiments, R^2 and R^4 are each H.

In some embodiments, R^5 and R^6 are each H.

25 In some embodiments, R^9 , R^{10} , R^{11} , and R^{12} are each independently selected from H and methyl.

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



or a pharmaceutically acceptable salt thereof, wherein:

R^7 is a peptide;

R^8 is a topoisomerase I inhibitor;

5 Ring Z is a monocyclic C₅₋₇ cycloalkyl ring or a monocyclic 5-7 membered heterocycloalkyl ring;

each R^Z is independently selected from C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{b1}, C(O)NR^{cl}R^{d1}, C(O)OR^{al}, OC(O)R^{b1}, OC(O)NR^{cl}R^{d1}, NR^{cl}R^{d1}, NR^{cl}C(O)R^{b1}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{d1};

10 or two adjacent R^Z together with the atoms to which they are attached form a fused monocyclic C₅₋₇ cycloalkyl ring, a fused monocyclic 5-7 membered heterocycloalkyl ring, a fused C₆₋₁₀ 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 C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{b1}, C(O)NR^{cl}R^{d1}, C(O)OR^{al}, OC(O)R^{b1}, OC(O)NR^{cl}R^{d1}, NR^{cl}R^{d1}, NR^{cl}C(O)R^{b1}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{d1};

15 R^{al} , R^{b1} , R^{cl} , and R^{d1} are each independently selected from H, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, each optionally substituted with 1, 2, or 3 substituents independently selected from halo, OH, CN, and NO₂; and

n is 0, 1, 2, or 3.

20

In some embodiments of compounds of Formula (II), R^7 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^7 is Pv1, Pv2, Pv3, Pv4, or Pv5.

25 In some embodiments of compounds of Formula (II), R^7 is attached to the core via a cysteine residue of R^7 wherein one of the sulfur atoms of the disulfide moiety in Formula II is derived from the cysteine residue.

In some embodiments of compounds of Formula (II), R⁸ is camptothecin, opotecan, irinotecan (CPT-11), silatecan (DB-67, AR-67), cositecan (BNP-1350), lurtotecan, gimatecan (ST1481), belotecan (CKD-602), rubitecan, topotecan, deruxtecan, or exatecan.

In some embodiments of compounds of Formula (II), R⁸ is exatecan.

5 In some embodiments of compounds of Formula (II), R⁸ is attached to the core through an N atom.

In some embodiments of compounds of Formula (II), Ring Z is a monocyclic C₅₋₇ cycloalkyl ring.

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

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

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

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

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

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

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

20 In some embodiments of compounds of Formula (II), two adjacent R^Z together with the atoms to which they are attached form a fused monocyclic C₅₋₇ cycloalkyl ring, a fused monocyclic 5-7 membered heterocycloalkyl ring, a fused C₆₋₁₀ 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 C₁₋₄ alkyl, halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{bl}, C(O)NR^{cl}R^{dl},
25 C(O)OR^{al}, OC(O)R^{bl}, OC(O)NR^{cl}R^{dl}, NR^{cl}R^{dl}, NR^{cl}C(O)R^{bl}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{dl}.

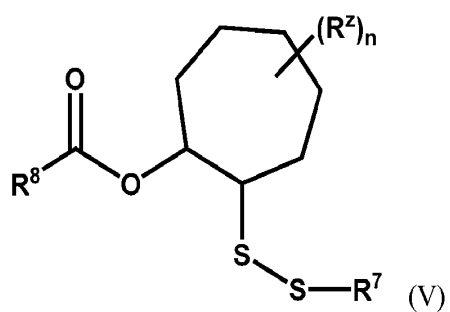
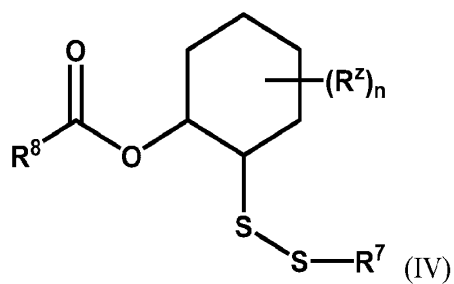
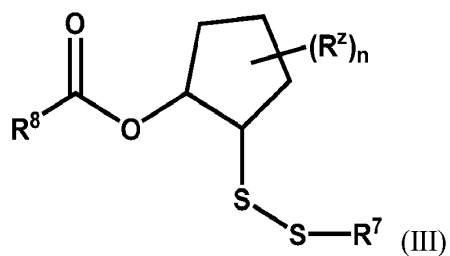
In some embodiments of compounds of Formula (II), n is 0.

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

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

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

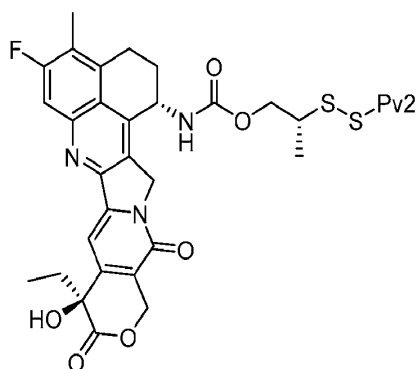
In some embodiments, the compounds of the invention is a compound of Formula (III), Formula (IV), or Formula (V):



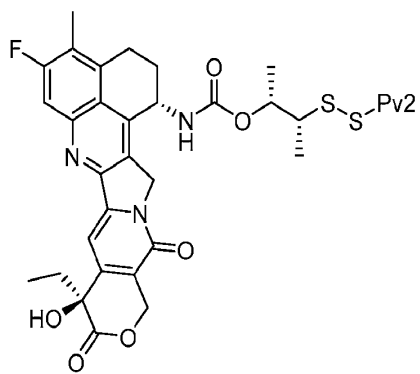
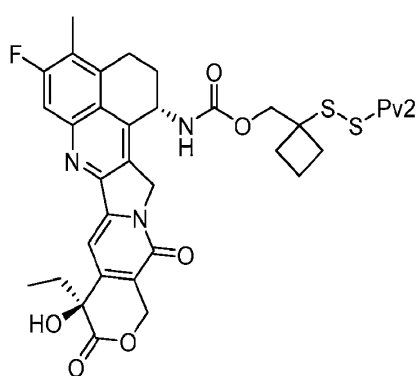
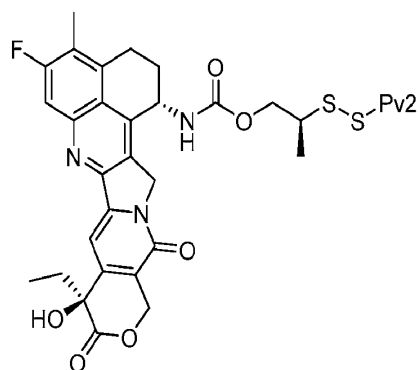
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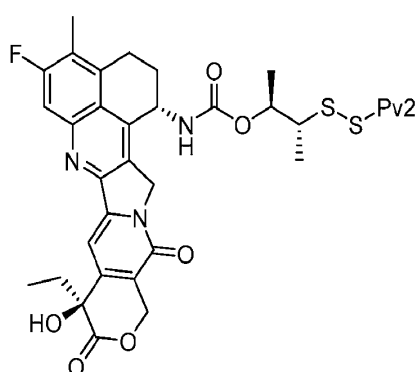
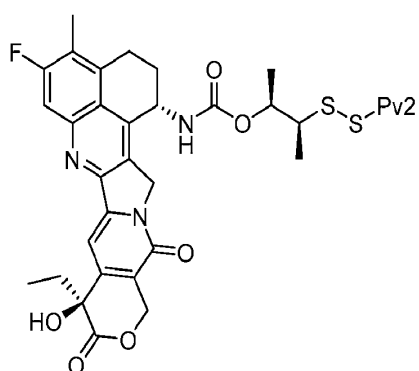
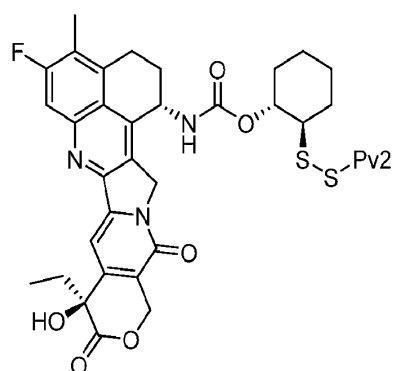
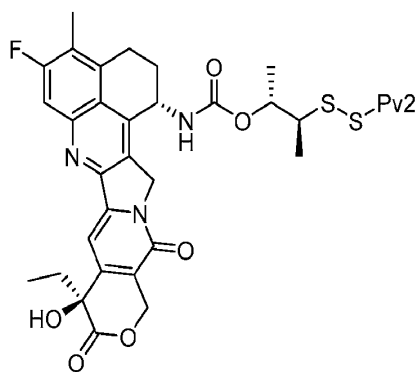
or a pharmaceutically acceptable salt thereof, wherein R^7 , R^8 , R^Z and n are defined as in any of the embodiments above for Formula (II).

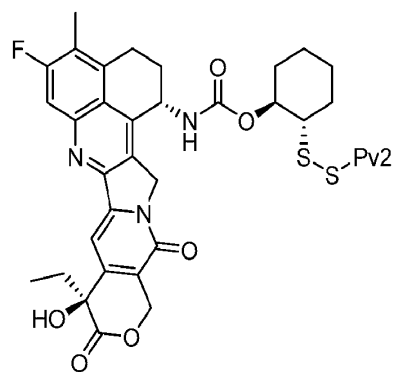
In some embodiments, the compound of formula (I) is selected from:



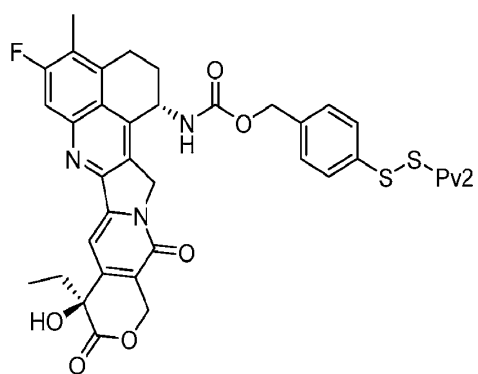
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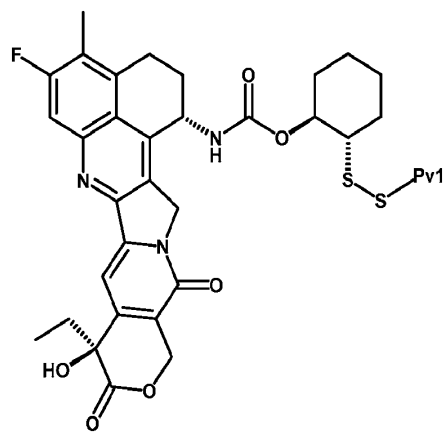
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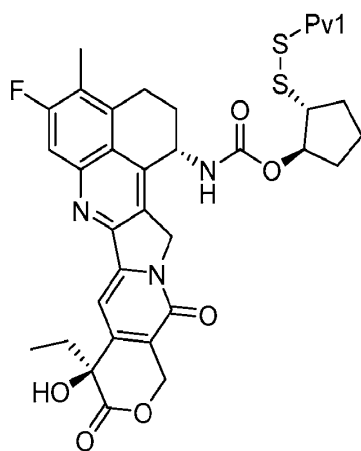
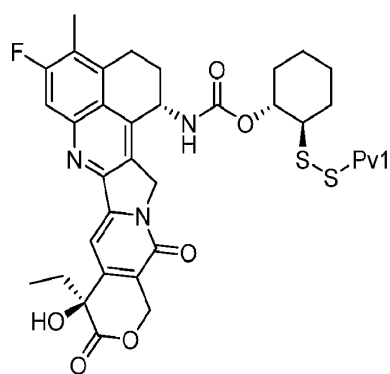
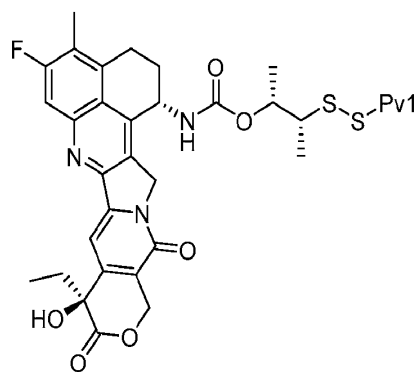
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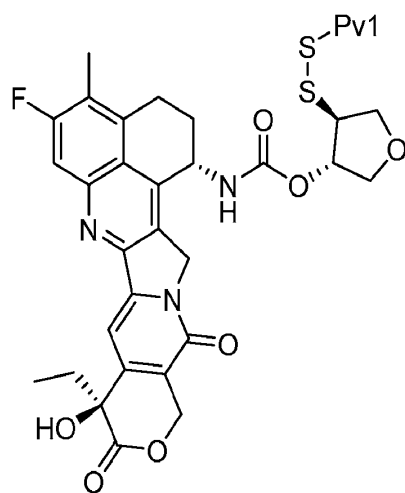
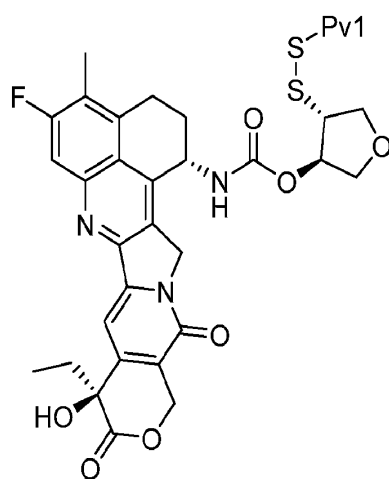
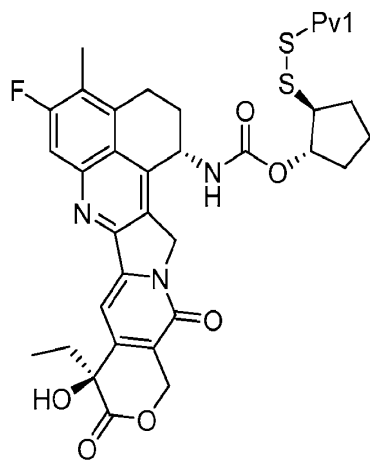
or a pharmaceutically acceptable salt of any of the aforementioned.

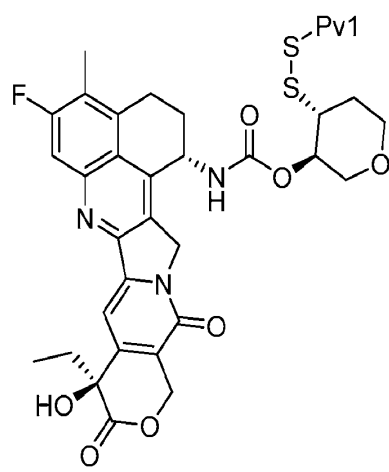
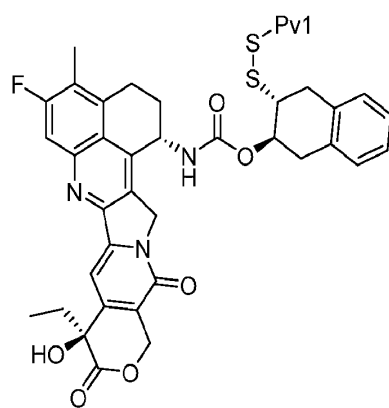
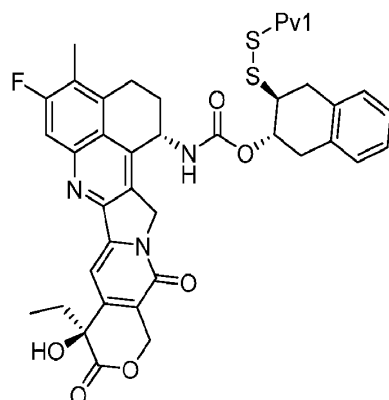
- 5 In some embodiments, the compound of formula (I) is selected from:

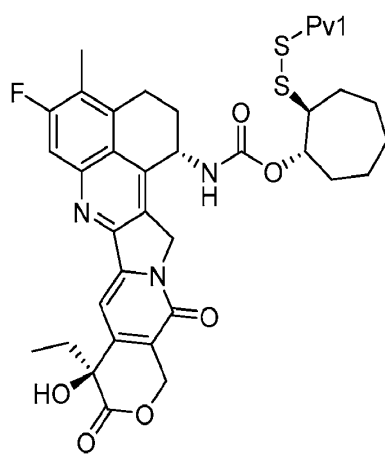
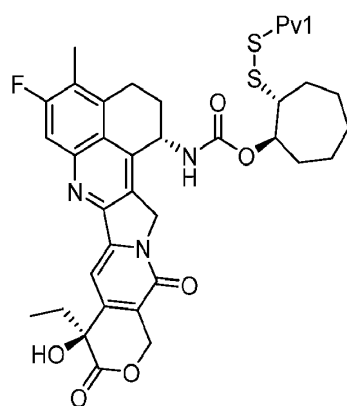
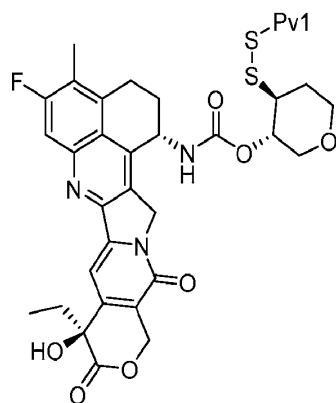


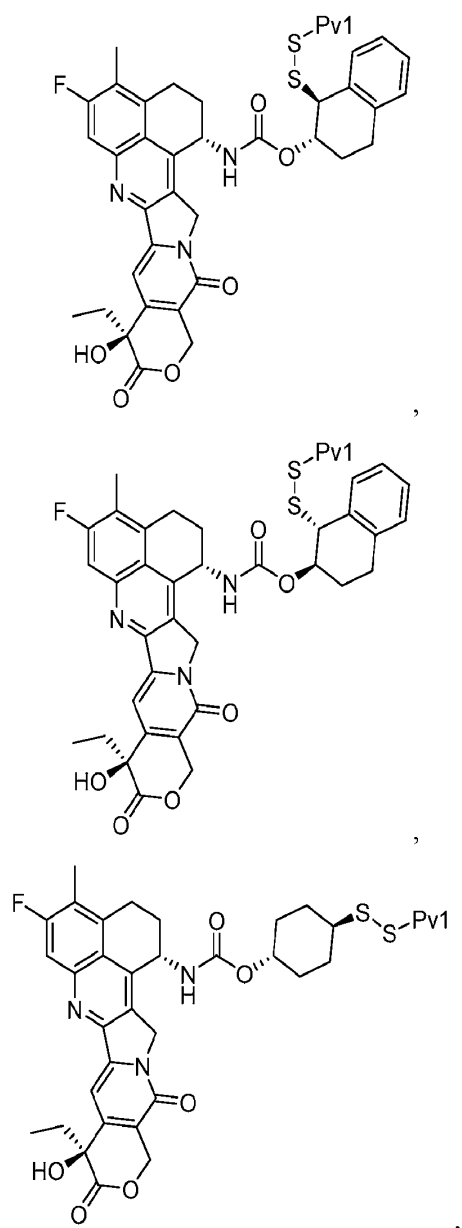
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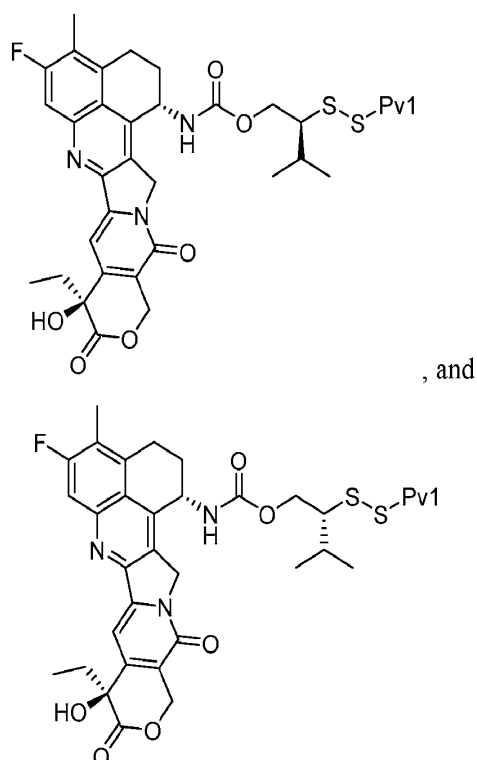




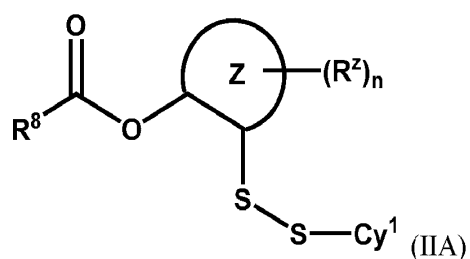








In some embodiments, provided herein is a compound having Formula (IIA):



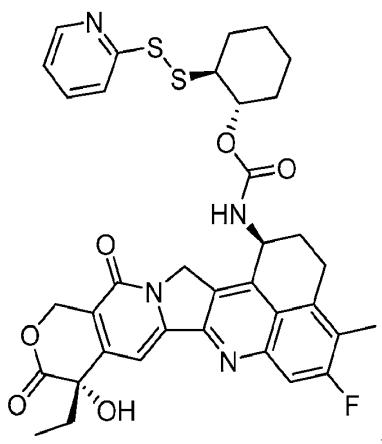
or a salt thereof, wherein:

- 5 Cy^1 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; and wherein said 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, OH, C_{1-6} alkoxy, CN, and NO_2 ;

- 10 and R^8 , Ring Z, R^Z , and n are as defined herein.

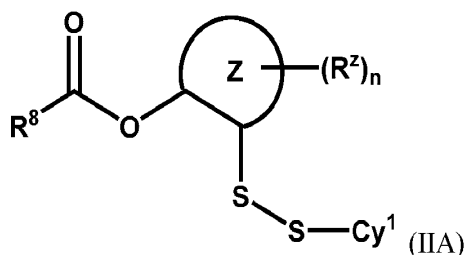
In some embodiments, Cy¹ is 5-10 membered heteroaryl. In some embodiments, Cy¹ is pyridinyl. In some embodiments, Cy¹ is phenyl.

In some embodiments, the compound of Formula (IIA) has the the structure:



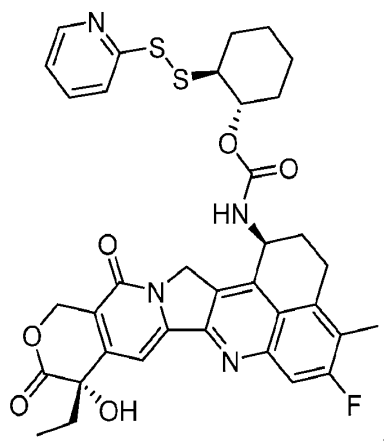
5 or a salt thereof.

In some embodiments, provided herein is a compound of Formula (IIA):



or a salt thereof, for use in preparing a compound of the invention (e.g., a compound of Formula (I) or Formula (II)), wherein Cy¹, R⁸, Ring Z, R^Z, R^{a1}, R^{b1}, R^{c1}, R^{d1}, and n are as defined herein.

10 In some embodiments, provided herein is a compound having the structure:



or a salt thereof, for use in preparing a compound of the invention (e.g., a compound of Formula (I) or Formula (II)).

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 ADDQNPNWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO:1).

The term “Pv2” means AEQNPIYWARYADWLFTTPLLLDLALLVDADECG (SEQ ID NO:2).

The term “Pv3” means ADDQNPNWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO:3).

The term “Pv4” means AcAAEQNPIYWARYADWLFTTPLLLDLALLVDADEGKCG (SEQ ID NO:4).

The term “Pv5” means AAQNPIYWARYADWLFTTPLLALLVDADEGTC (SEQ ID NO:5).

In the compounds of the invention, the peptides R⁷ are attached to the disulfide moiety in the linker Q by an amino acid residue comprising a sulfur atom, such as a cysteine residue.

5 Typically, the sulfur atom of the disulfide moiety in the linker Q which is the point of attachment to peptide R⁷ is derived from an amino acid residue of the peptide, such as from a cysteine residue.

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
10 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 R⁸Q into the cell, whereupon the disulfide linker is cleaved to deliver free R⁸H. Since the compounds of formula (I) are pH-dependent, they preferentially insert across a cell membrane only in the presence of an acidic
15 or hypoxic mantle surrounding the cell and not across the cell membrane of “normal” cells, which do not have an acidic or hypoxic mantle. An example of a cell having an acidic or hypoxic mantle is a cancer cell.

The terms “pH-sensitive” or “pH-dependent” as used herein to refer to the peptide R⁷ or to the mode of insertion of the peptide R⁷ or of the compounds of the invention across a cell
20 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 R⁸Q to the interior of the cell (and thus deliver R⁸H as described above) when the cell membrane lipid bilayer has an acidic or hypoxic mantle (a “diseased” cell) but does not insert through a
25 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 R⁷ forming a helical configuration, which facilitates membrane insertion.

It is further appreciated that certain features of the invention, which are, for clarity,
30 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 "C₁₋₆ alkyl" is specifically intended to individually disclose (without limitation) methyl, ethyl, C₃ alkyl, C₄ alkyl, C₅ alkyl and C₆ 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.

5 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 10 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 15 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 20 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 25 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 30 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-
5 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
10 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
15 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 $\{2(n \text{ to } m)+1\}$ 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
20 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 $-O\text{-haloalkyl}$, 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
25 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.

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

10 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.

15 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
20 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
25 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; azetidyl; 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 β -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.

10 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 30 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 be 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
5 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
10 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
15 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
20 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
25 (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.

30

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.

5 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
10 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.

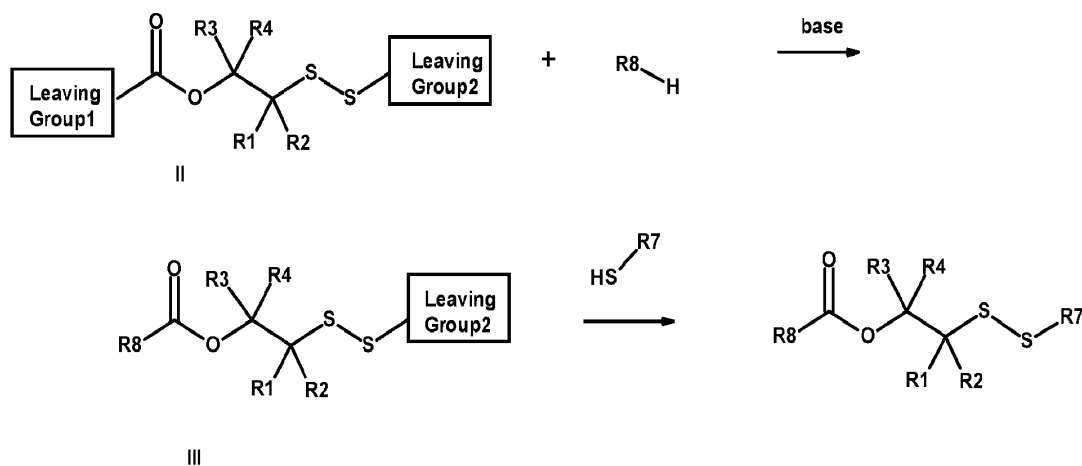
Preparation of compounds of the invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of
15 appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups is 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);
20 Petursson *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.*, ¹H or ¹³C), infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), mass spectrometry or by chromatographic methods such as high performance liquid
25 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.

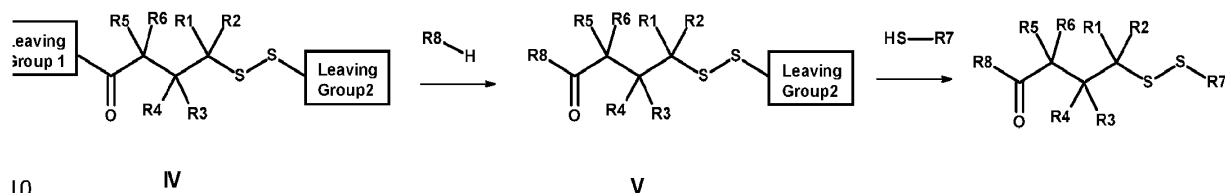
Compounds of Formula (I) can be prepared, *e.g.*, using a process as illustrated in the schemes below.

Scheme 1: Synthesis of Carbonate and Carbamate Linked Compounds



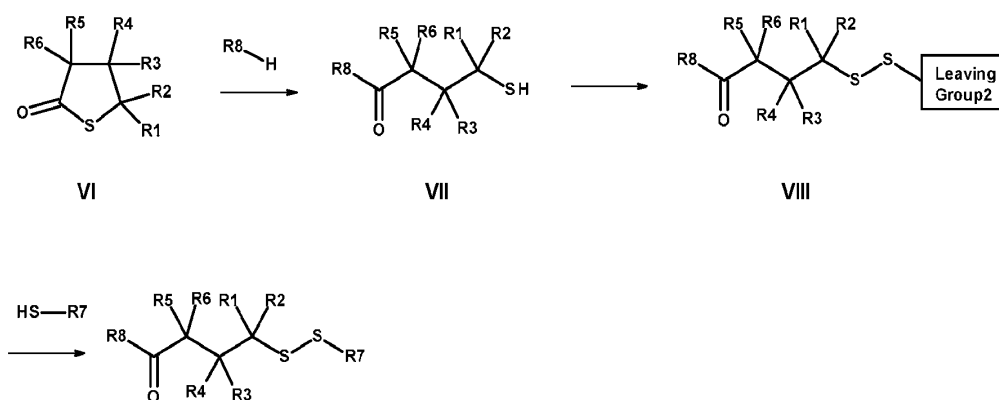
- 5 Intermediate II, which is flanked by orthogonal leaving groups, can be reacted with a nucleophilic R^8H compound to give Intermediate III. Intermediate III can then be reacted with a thiol containing peptide ($HS-R^7$) that participates in a disulfide exchange reaction to give the final compound. Suitable leaving groups are described below.

Scheme 2: Synthesis 1 of Thio Propionate Linked Conjugates



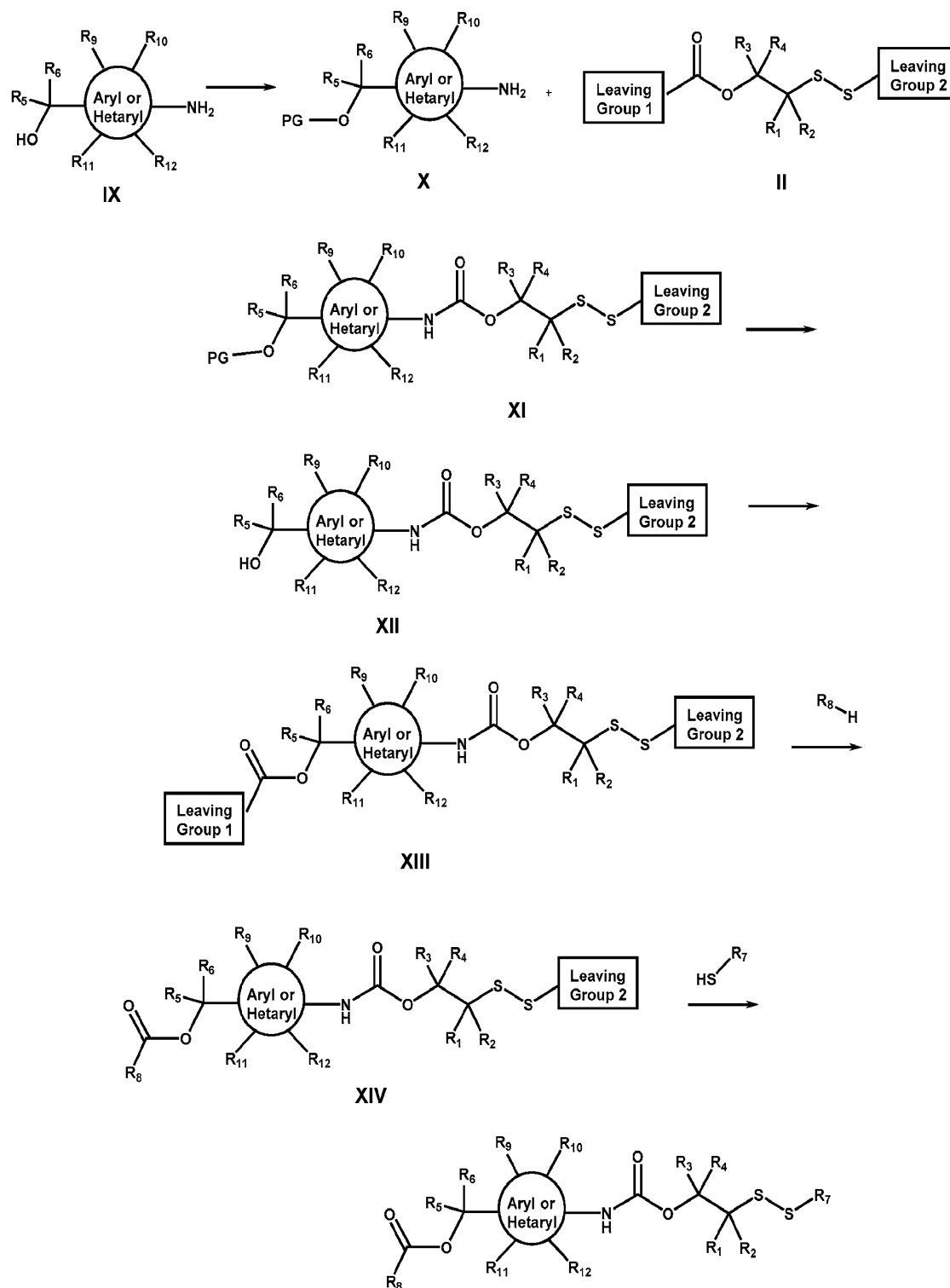
Propionate disulfide IV with previously installed Leaving Groups 1 and 2 can be reacted selectively with nucleophilic R^8-H to give V. This compound can then be reacted with R^7-SH to provide the desired conjugate.

Scheme 3: Synthesis 2 of Thio Propionate Linked Conjugates



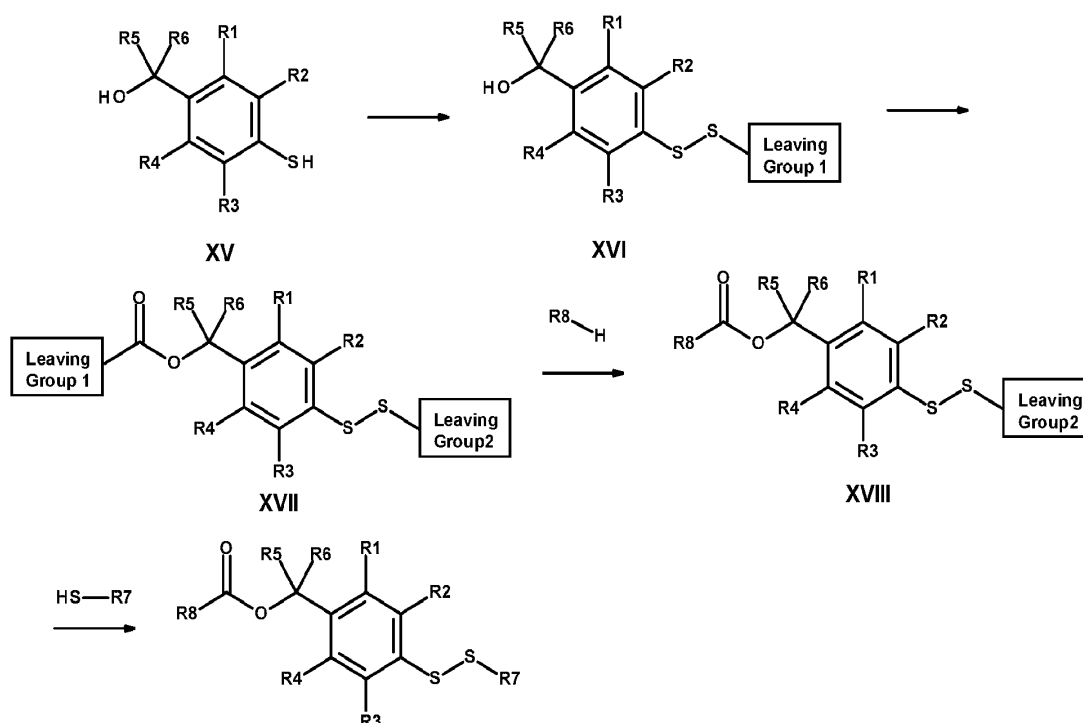
- Thionoester VI can be reacted with nucleophilic R^8-H to give propionate thiol VII. This compound can engage in a disulfide exchange reaction to provide Intermediate VIII. This
- 5 compound can be treated with R^7-SH to provide the desired conjugate.

Scheme 4: Synthesis 1 of Para Benzyl-Linked Conjugates



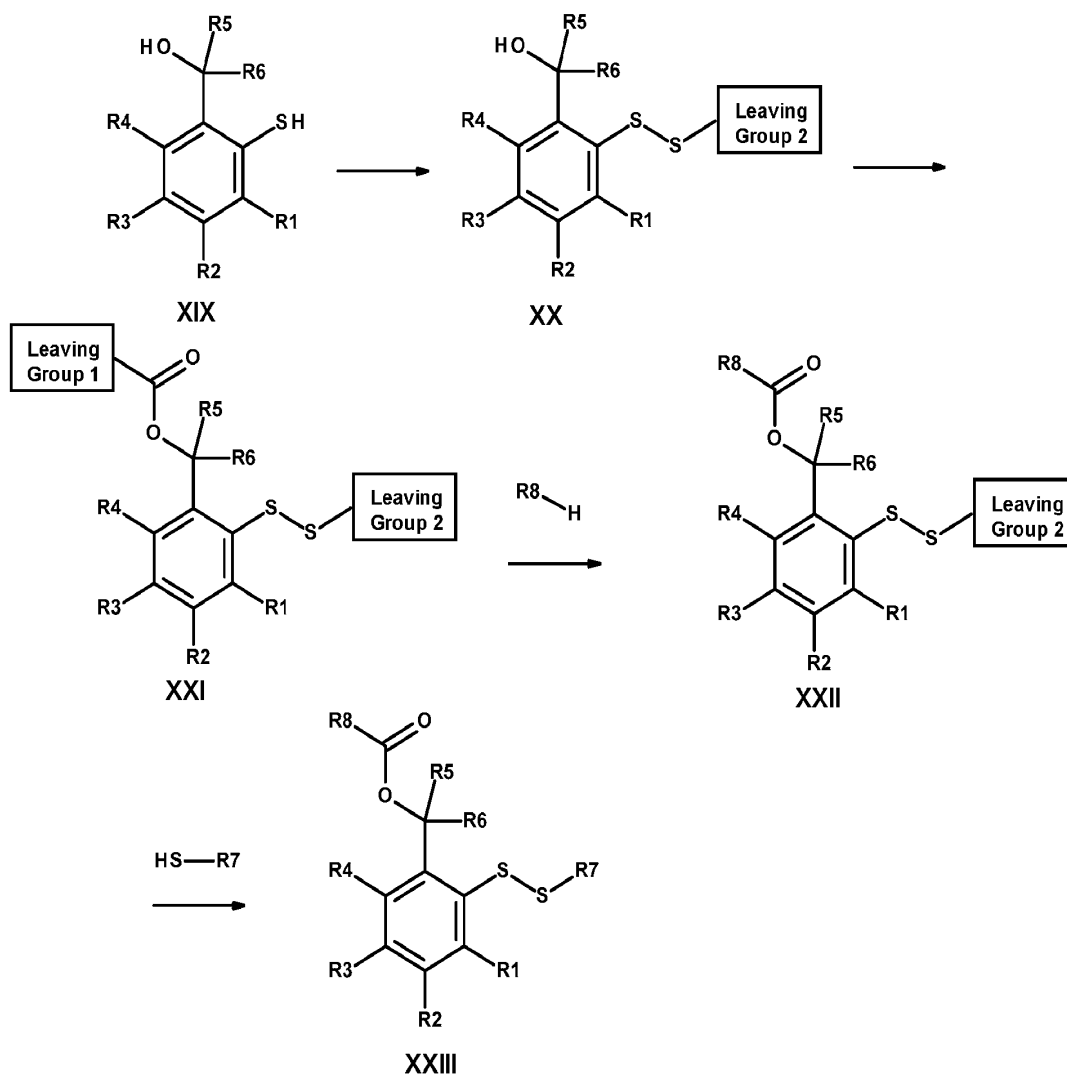
The alcohol group of para aminobenzyl alcohol IX can be selectively protected to give Intermediate X. This intermediate can then be reacted at the aniline position with Intermediate II to provide aryl carbamate XI. The protecting group can be removed giving free alcohol XII, which can be treated with an activating agent to provide Intermediate XIII, containing orthogonal leaving groups. Reaction of Intermediate XIII with R^8-H can provide Intermediate XIV, followed by treatment with R^7-SH can give the desired para benzyl-linked conjugate.

Scheme 5: Synthesis of Para Benzyl-Linked Conjugates



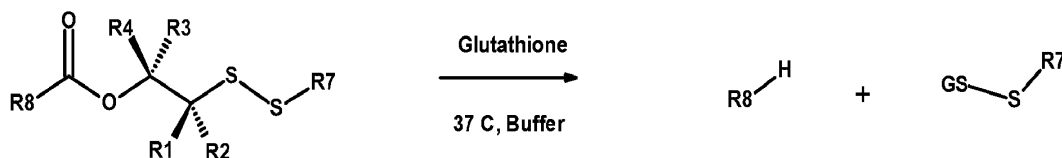
4-Mercapto benzyl alcohol XV can be reacted in a disulfide exchange reaction to give 4-mercapto benzyl alcohol disulfide XVI containing Leaving Group 2. The remaining benzyl alcohol can be treated with an appropriate carbonyl compound to provide activated compound XVII. This intermediate can be further reacted selectively with nucleophilic R^8-H to provide Intermediate XVIII, which can be treated with R^7-SH to give the desired conjugate.

Scheme 6: Synthesis of Ortho Benzyl-Linked Conjugates



2-Mercapto benzyl alcohol XXIII can be reacted as previously described to give the desired conjugate.

5 Scheme 7: Cleavage of Peptide Conjugates



Cleavage of the final compound to release R⁸-H can be achieved by treating the compound with an excess of glutathione (GSH) in a buffer with incubation at 37°C. Reversed phase HPLC analysis at a desired time course is used to follow the course of the cleavage.

The peptides R⁷ 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 peptides R⁷ may also be produced by fermentation, for example, by modification of *E. coli*. Protein production in *E. coli* can be controlled to produce recombinant polypeptides having a sequence of an R⁷ peptide disclosed herein. Recombinant polypeptide production in *E. coli* is described in the following references: Zhao, Q., Xu, W., Xing, L. *et al.* Recombinant production of medium- to large-sized peptides in *Escherichia coli* using a cleavable self-aggregating tag. *Microb Cell Fact* 15, 136 (2016); de Marco, Recombinant polypeptide production in *E. coli*: towards a rational approach to improve the yields of functional proteins; *Microbial Cell Factories* 2013, 12:101; and Kleiner-Grote G.M., Risse, J.M., Friehs, K; Secretion of recombinant proteins from *E. coli*; *Eng. Life Sci.* 2018, 18, 532–550, each of which is incorporated by reference in its entirety.

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

5 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 or neurodegenerative disease. Hypoxia and acidosis are physiological markers of many disease processes, including cancer. In cancer, hypoxia is one
10 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 often 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
15 therapeutic index of cytotoxic agents is to leverage this physiological characteristic to afford selective delivery of compound to hypoxic cells over healthy tissue.

 In the methods of treatment of the invention, 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
20 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.

25 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,
30 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, 5 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 10 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, 15 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.

20 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 25 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 30 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
5 cancers.

The compounds of the invention (e.g., a compound of formula (I)) comprising a topoisomerase I targeting moiety derived from a topoisomerase I inhibitor (e.g., exatecan) can exhibit certain therapeutic advantages over the topoisomerase I inhibitor itself. For example, administration of a compound of formula (I) can show reduced toxicity (e.g., bone marrow or
10 gastric toxicity) as compared with administration of the corresponding topoisomerase I inhibitor (e.g., exatecan). In some embodiments, the bone marrow toxicity is measured by total bone marrow count from samples of the subject (e.g., total bone marrow count in femurs of a mouse). In some embodiments, bone marrow toxicity is measured by PARylation in bone marrow tissue. In some embodiments, bone marrow toxicity is measured according to total nucleated bone
15 marrow cells. In some embodiments, gastric toxicity is assessed using photographs of the stomachs of the subject (e.g., a mouse) taken both *in situ* and *ex vivo*.

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
20 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 or gastric toxicity, when administered together with a compound of formula (I), or a pharmaceutically acceptable salt thereof, as compared with when
25 administered in combination with the corresponding topoisomerase inhibitor (e.g., R⁸-H).

Suitable chemotherapeutic or other anti-cancer agents include, for example, alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazines) such as uracil mustard, chlormethine, cyclophosphamide (CytoxanTM), 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 (TAXOLTM), mithramycin, deoxycoformycin, mitomycin-C, L-asparaginase, interferons (especially IFN- α), etoposide, and teniposide.

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

Also suitable are cytotoxic agents such as, for example, epipodophyllotoxin; 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, lapatinib and neratinib. Antibodies against the EGFR include but are not limited to cetuximab, panitumumab and necitumumab. Inhibitors of c-Met
5 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
10 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
15 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.

20 Agents against the PI3 kinase include but are not limited to pirlaralisib, 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,
25 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
30 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.

5 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
10 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
15 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, " $\mu\text{g/ml}$ " (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
20 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
25 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:

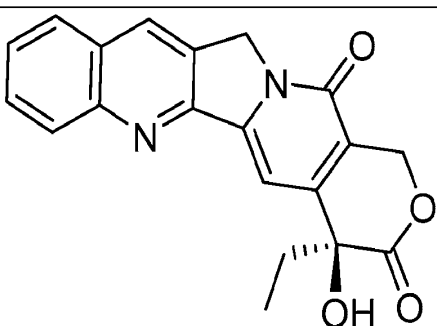
- Brine: a saturated NaCl solution in water
- DCM: dichloromethane
- TFA: trifluoroacetic acid

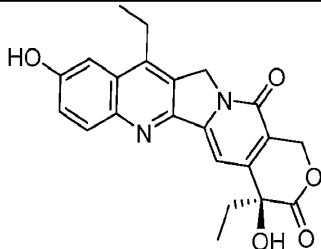
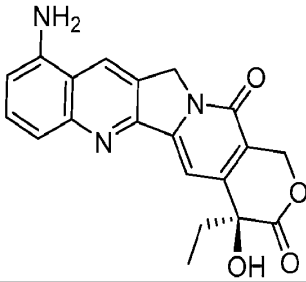
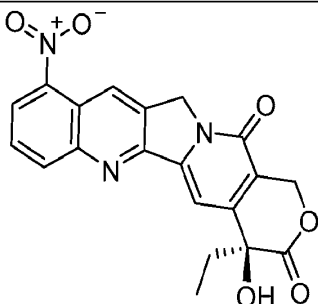
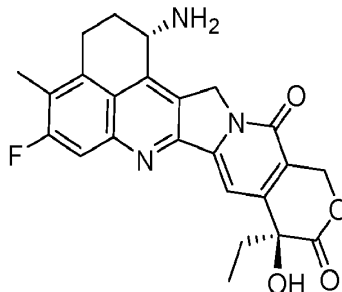
- DIPEA: diisopropylethylamine
- DMA: dimethylacetamide
- DME: dimethoxyethane
- DMF: dimethylformamide
- 5 • DMSO: methylsulfoxide
- DTT: dithiothreitol
- MSD: mass spec detector
- Et₂O: ethyl ether
- EtOAc: ethyl acetate
- 10 • EtOH: ethyl alcohol
- HATU: O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
hexafluorophosphate
- HOBt: 1-hydroxybenzotriazole
- RP: reverse phase
- 15 • HPLC: high performance liquid chromatography
- IPA: isopropanol
- LAH: lithium aluminum hydride
- N-BuLi: n-butyl lithium
- LC-MS: liquid chromatography-mass spectrometry
- 20 • LDA: lithium diisopropylethylamide
- Me: methyl
- MeOH: methanol
- MTBE: methyl t-butyl ether
- NMP: N-methylpyrrolidine
- 25 • Ph: phenyl
- PNPC: para-nitrophenylchloroformate
- RT or rt: room temperature
- SFC: supercritical fluid chromatography
- TBAI: tetrabutylammonium iodide
- 30 • TBME: tert-butylmethyl ether

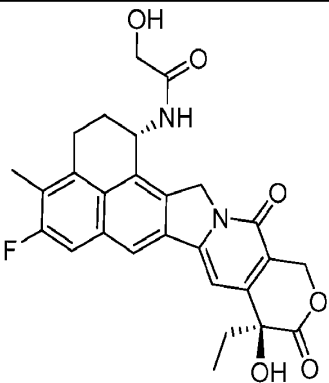
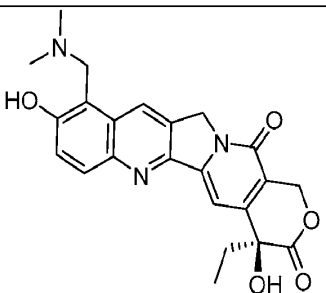
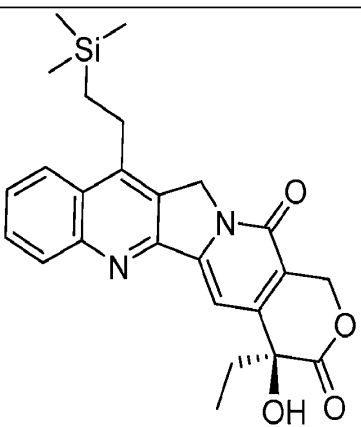
- tBu: tertiary butyl
- THF: tetrahydrofuran
- TEA: triethylamine
- TMEDA: tetramethylethylenediamine
- 5 • GSH: Glutathione
- GS: Glutathione bonded at sulfur
- LiOH: lithium hydroxide
- DPPA: diphenyl phosphoryl azide
- Sn(Bu)₂(Laurate)₂: dibutyltin dilaurate
- 10 • PBS: phosphate buffered saline
- ACN: acetonitrile
- AcOH: acetic acid
- EEDQ: *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
- DMAP: 4-dimethylaminopyridine
- 15 • EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

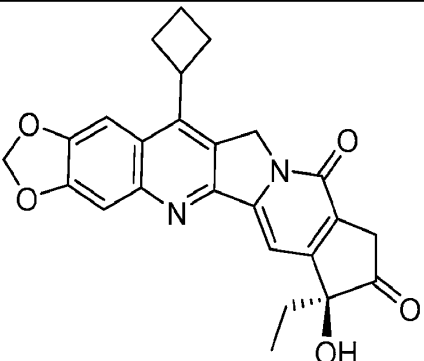
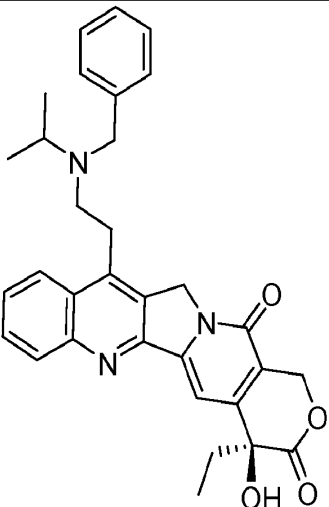
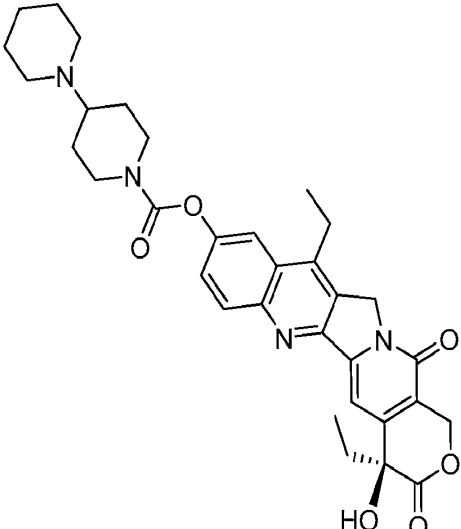
The source of the starting materials employed in the Examples are set forth below in the following tables.

Table 2. Starting materials for R⁸

R ⁸ Code	R ⁸ H Structure	Synthesis Reference or Purchased
R ⁸ H-1		Medchem Express HY-16560

R ⁸ H-2		MedKoo 406280
R ⁸ H-3		AstaTech F11420
R ⁸ H-4		AstaTech 42333
R ⁸ H-5		Medchem Express HY-13631A

R ⁸ H-6		Medchem Express HY-13631D
R ⁸ H-7		AstaTech 21428
R ⁸ H-8		Medchem Express HY-14812

R ⁸ H-9		US 20030105109 A1
R ⁸ H-10		WO 9902530 A1
R ⁸ H-11		Medchem Express: Cat. No.: HY-16562

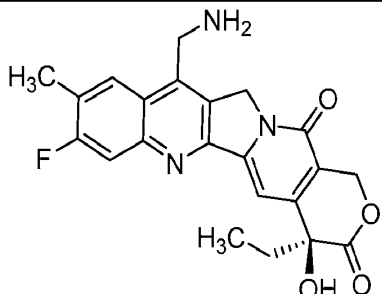
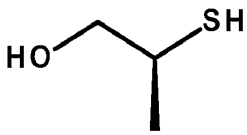
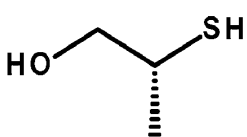
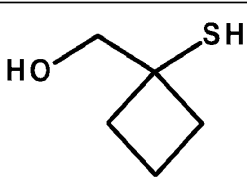
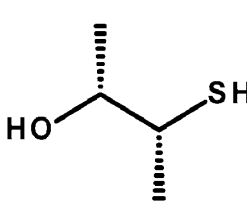
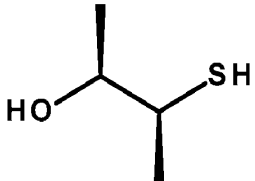
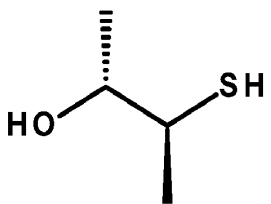
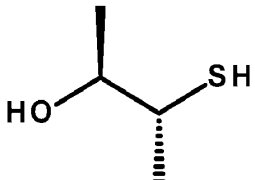
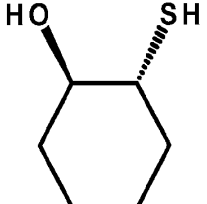
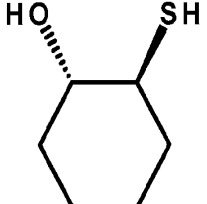
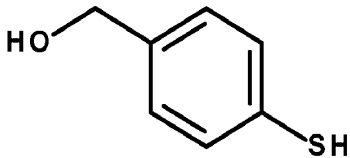
R ⁸ H-12		Widdison <i>et al.</i> , <i>ACS Medicinal Chemistry Letters</i> 2019 10 (10), 1386-1392
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Table 3. Starting Materials for Linkers

Linker Code	Linker Structure	Synthetic Reference or Purchased
L-1		Synthesized WO2013055987A1
L2		Synthesized WO2013055987A1
L3		Synthesized ACS Med. Chem. Lett. 2016, 7, 988–993
L4		R, R*

L5		S, S*
L6		R, S*
L7		S, R*
L8		R, R*
L9		S, S*
XV-1		Combiblocks OR-5865

*Absolute configuration randomly assigned

The HPLC methods employed are set forth below:

HPLC Methods

A: Sunfire C18 150x4.6mm; H₂O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 1ml/min;
Wavelength = 217 nM.

5 B: Ace Equivalence 250x4.6mm; H₂O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 1ml/min;
Wavelength = 217 nM.

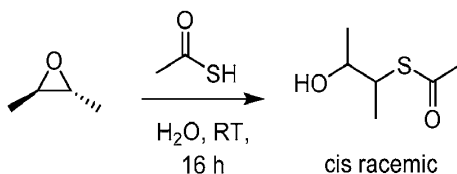
C: Sunfire C18 150x30mm; H₂O/Acetonitrile w/ TFA modifier (0.05%); Flow rate: 30ml/min;
Wavelength = 217 nM.

10 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).

15 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 ($m/z=3$).

Synthesis of *cis*-S-(3-hydroxybutan-2-yl) ethanethioate (L-4 and L-5)



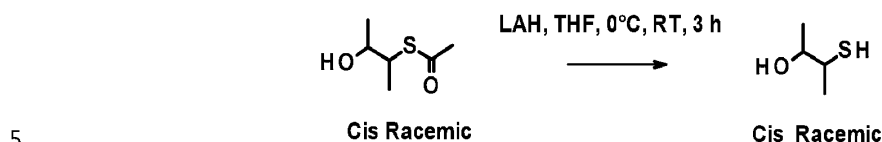
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To a stirred solution of trans-2,3-dimethyloxirane (5.0 g, 69.3 mmol) in water (50 mL) was added thioacetic acid (5.8 mL, 76.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with sat. sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (200 mL). The organic layer was

25 dried over anhydrous sodium sulphate and then evaporated under reduced pressure to afford *cis*-

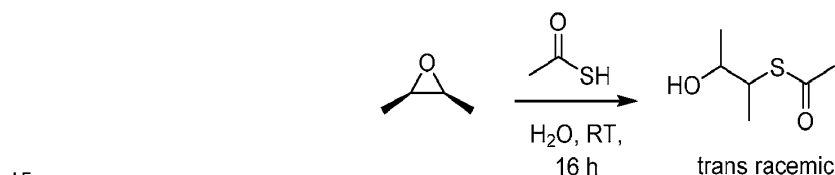
S-(3-hydroxybutan-2-yl) ethanethioate as an oily compound (4.0 g, crude). MS m/z 149.0 $[M+H]^+$.

Synthesis of *cis*-3-Mercaptobutan-2-ol



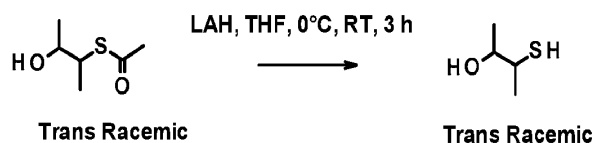
To a stirred solution of S-(3-hydroxybutan-2-yl) ethanethioate (4 g, 26.9 mmol) in THF (40 mL) was added lithium aluminum hydride (1M solution in THF) (27 mL, 26.9 mmol) drop wise at 0° C. The Reaction mixture was gradually allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was quenched slowly with 1N HCl at 0°C and pH was
 10 adjusted to 2-3. The reaction mixture was extracted in ethyl acetate (50 mL) and the organic layer was dried over anhydrous sodium sulphate and evaporated off to obtain *cis*-3-mercaptoputan-2-ol as a crude oily compound.

Synthesis of *trans*-S-(3-hydroxybutan-2-yl) ethanethioate (L-6 and L-7)



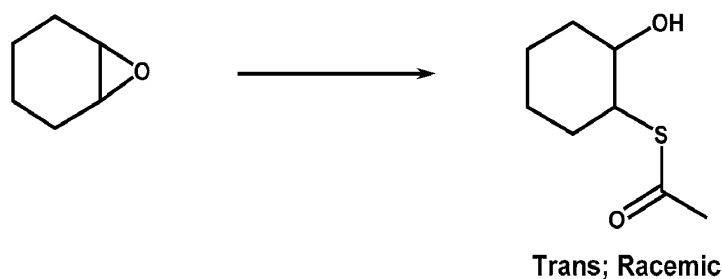
To a stirred solution of cis-2,3-dimethyloxirane (1.0 g, 13.9 mmol) in water (15 mL) was added thioacetic acid (1.1 mL, 15.6 mmol) at room temperature and stirred for 16 h. The reaction mixture was quenched with sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (20mL). The organic layer was dried over anhydrous sodium sulphate and then
 20 evaporated under reduced pressure to afford *trans*-S-(3-hydroxybutan-2-yl) ethanethioate as yellow oil (0.7 g crude).

Synthesis of *trans*-3-mercaptoputan-2-ol



To a stirred solution of *trans*-S-(3-hydroxybutan-2-yl) ethanethioate (700 mg, 4.72 mmol) in THF (10 mL) was added lithium aluminum hydride (1M solution in THF) (4.8 mL, 4.72 mmol) drop wise at 0°C and stirred at the same temperature for 3 h. The reaction mixture was quenched with 1N HCl at 0°C then pH was adjusted to 2-3. The reaction mixture was extracted with CH₂Cl₂ (10 mL). The organic layer was dried over anhydrous sodium sulphate and taken directly for next step.

Synthesis of *trans*-S-(2-hydroxycyclohexyl) ethanethioate (L-8 and L-9)

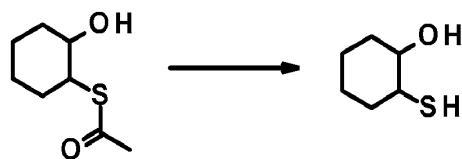


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To a stirred solution of 7-oxabicyclo[4.1.0]heptane (5.0 g, 51.0 mmol) in water (50.0 mL) was added thioacetic acid (4.92 mL, 61.0 mmol). The reaction mixture was stirred for 16 h at room temperature. Progress of the reaction was monitored by TLC (20% EtOAc/Hexane). After completion of reaction, the reaction mixture was diluted with diethyl ether. The organic layer was separated and washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford *trans*-S-(2-hydroxycyclohexyl) ethanethioate as a brown color liquid (3.8 g crude).

15

Synthesis of *trans*-2-mercaptocyclohexan-1-ol



Trans Racemic

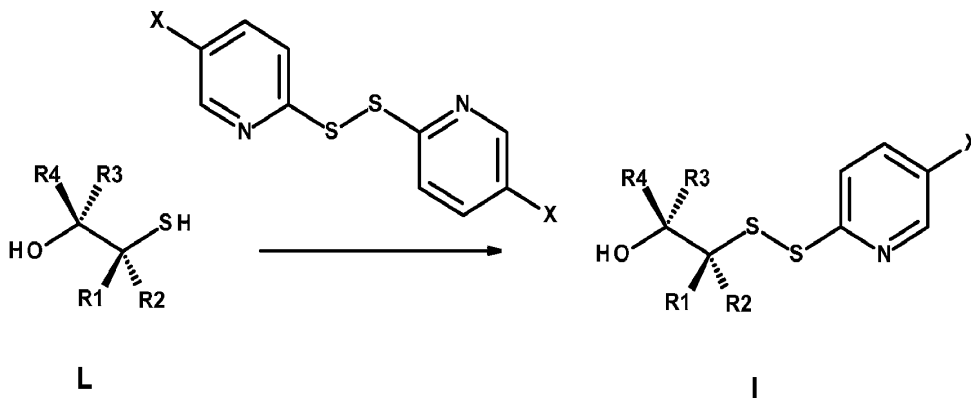
Trans Racemic

To a stirred solution of *trans*-S-(2-hydroxycyclohexyl) ethanethioate (3.8 g, 21.8 mmol) in THF (20.0 mL) was added 1M LiAlH₄ in THF (21.8 mL, 21.8 mmol) at 0°C. The reaction mixture was gradually allowed to warm to room temperature and stirred for 1 h.

- 5 Progress of the reaction was monitored by TLC (20% EtOAc/Hex). Upon completion of the reaction, the reaction mixture was cooled to room temperature and quenched with 1.0 N HCl (30 mL). The reaction mixture was extracted in CH₂Cl₂ (30.0 mL). The organic layer was washed with brine solution (30.0 mL), concentrated and crude *trans*-2-sulfanylcyclohexanol taken for next step. (2.88 g, crude).

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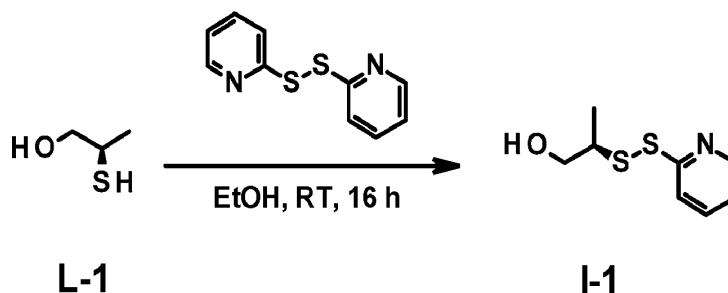
Synthesis of Intermediate I from L



Int.	R ¹ , R ²	R ³ , R ⁴	X	Method/Ref.	MH ⁺
I-1	H, Me	H, H	H	WO2013055987A1	202.1
I-2	Me, H	H, H	H	WO2013055987A1	202.1
I-3	-CH ₂ CH ₂ CH ₂ -	H, H	NO ₂	ACS Med. Chem. Lett. 2016, 7, 988–993	272.9

I-4	H, Me	Me, H	H		216.1
I-5	Me, H	H, Me	H		216.1
I-6	Me, H	Me, H	H		215.9
I-7	H, Me	H, Me	H		216.2
I-8	$\begin{array}{c} \text{CH}_2, \text{H} \quad \text{CH}_2, \text{H} \\ \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$		H		
I-9	$\begin{array}{c} \text{H}, \text{CH}_2 \quad \text{H}, \text{CH}_2 \\ \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$		H		

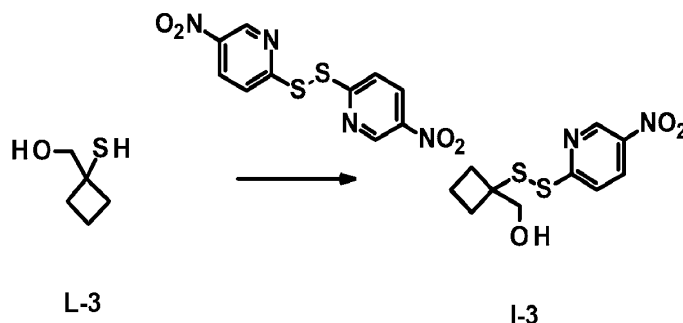
Synthesis of Intermediate I-1: (2R)-2-(2-pyridyldisulfanyl)propan-1-ol



To 2-(2-pyridyldisulfanyl)pyridine (5.00 g, 22.7 mmol) in 40 ml of MeOH degassed with
 5 N₂ was added (2R)-2-sulfanylpropan-1-ol (0.75 g, 8.14 mmol) in a drop-wise fashion. The
 mixture was stirred for 2 h under N₂. The mixture was concentrated to dryness and directly
 loaded onto a SiO₂ flash column and eluted with 0-50% EtOAc/Hexanes to give 1.17 g, 71% of
 (2R)-2-(2-pyridyldisulfanyl)propan-1-ol. MS m/z 202.1 [M+H]⁺.
 Intermediate I-2 was prepared from L-2 in an analogous manner

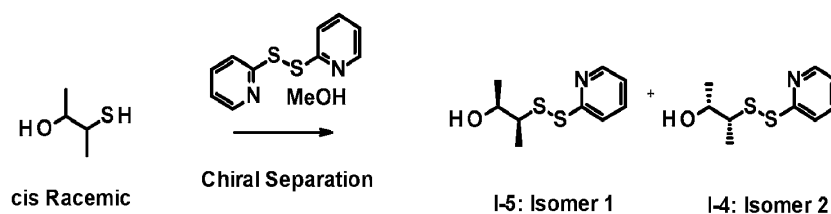
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Synthesis of Intermediate I-3: [1-[(5-nitro-2-pyridyl)disulfanyl]cyclobutyl]methanol



To a solution of 5-nitro-2-[(5-nitro-2-pyridyl)disulfany]pyridine (17.4 g, 56.0 mmol) in degassed (N₂) MeOH (100 mL) was added (1-mercaptocyclobutyl)methanol (8.3 mL, 70.0 mmol) (degassed with N₂) in a dropwise manner and stirred for 16 h at room temperature under N₂ atmosphere. The reaction mixture was concentrated to dryness under vacuum. The resultant crude was purified by column chromatography using 30% EtOAc/hexanes to afford [1-[(5-nitro-2-pyridyl)disulfany]cyclobutyl]methanol as a yellow liquid (9.0 g, 46% yield). MS m/z 272.9 [M+H]⁺.

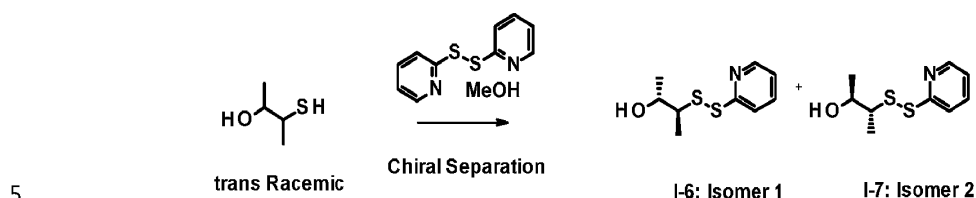
Synthesis of I-4 and I-5: 3-(pyridin-2-yl)disulfany]butan-2-ol Isomer 1 and Isomer 2



A stirred solution 2,2'-dipyridyldisulfide (520 mg, 2.35 mmol) in MeOH (15 mL) was purged with nitrogen gas for 5 min. Nitrogen gas purged solution of *cis*-3-mercaptobutan-2-ol (500 mg) in CH₂Cl₂ (10 mL) was added to it at 0° C. The reaction mixture was gradually allowed to warm to room temperature and stirred for 16h. The reaction mixture was concentrated under reduced pressure to afford crude material which was purified by column chromatography using 30-40 % EA/hexane. The racemic product was separated with Chiral Prep HPLC (CHIRALPAK IG; 100mm X 4.6mm X 3mic; Mobile phase : nHexane :Ethanol 80:20 with 0.1% DEA; Flow rate : 1.0 mL/min) to separate the respective enantiomers. Solvents were removed to obtain

(2S,3S)-3-(2-pyridyldisulfanyl)butan-2-ol* (140 mg, Isomer-1) MS m/z 216.1 [M+H]⁺ and (2R,3R)-3-(2-pyridyldisulfanyl)butan-2-ol (140 mg, Isomer-2). MS m/z 216.1 [M+H]⁺.

Synthesis of I-6 and I-7: 3-(pyridin-2-yl)disulfanylbutan-2-ol Isomer 1 and Isomer 2

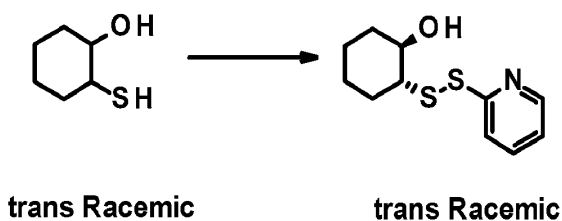


A stirred solution 2,2-dipyridyldisulfide (520 mg, 2.35 mmol) in MeOH (15 mL) was purged with nitrogen gas for 5 min. Nitrogen gas purged solution of *cis*-3-mercaptoputan-2-ol (500 mg) in CH₂Cl₂ (10 mL) was added to it at 0° C. The reaction mixture was gradually allowed to warm to room temperature and stirred for 16h. The reaction mixture was concentrated under reduced pressure to afford crude material which was purified by column chromatography using 30-40 % EA/hexane. The racemic product was separated with Chiral Prep HPLC (Column: CHIRALPAK IG (100mm X 4.6mm X 3mic) Mobile phase : nHexane :Ethanol with 0.1% DEA (80:20) Flow rate : 1.0 mL/min) to separate the respective enantiomers. Solvents were removed to obtain (2R,3S)-3-(2-pyridyldisulfanyl)butan-2-ol* (0.6 g, Isomer-I) MS m/z 215.9 [M+H]⁺ and (2S,3R)-3-(2-pyridyldisulfanyl)butan-2-ol* (0.6 g, Isomer-II) MS m/z 216.2 [M+H]⁺ as oily compounds.

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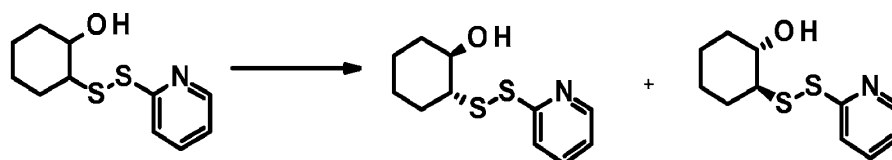
Synthesis Intermediate I-6: Trans-2-(pyridin-2-yl)disulfanylcyclohexan-1-ol



To a solution of 1,2-di(pyridin-2-yl)disulfane (2.41 g, 10.9 mmol) in MeOH (degassed with N₂) (30 mL) was added *trans*-2-sulfanylcyclohexanol (2.88 g, 21.0 mmol) (degassed with N₂) dropwise and stirred for 16 h at room temperature under N₂ atmosphere. The reaction mixture was concentrated to dryness under vacuum. The resultant crude was purified by column

20

chromatography using 30% of EtOAc/hexanes to afford trans-2-(pyridin-2-ylldisulfanyl)cyclohexan-1-ol as a yellow color liquid.



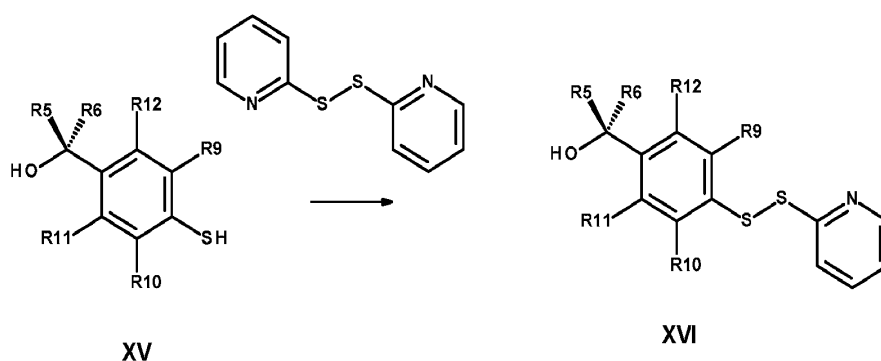
Trans Racemic

I-8: Isomer 1

I-9: Isomer 2

- 5 Chiral separation was done by chiralpak IG (100 mm X 4.6 mm X 3 mic) using n-hexane: IPA with 0.1% diethylamine (80:20) to afford (1R,2R)-2-(2-pyridyldisulfanyl)cyclohexanol* Isomer-1 (350 mg) and (1S,2S)-2-(2-pyridyldisulfanyl)cyclohexanol* Isomer-2 (400 mg).

Intermediate XV from XXI



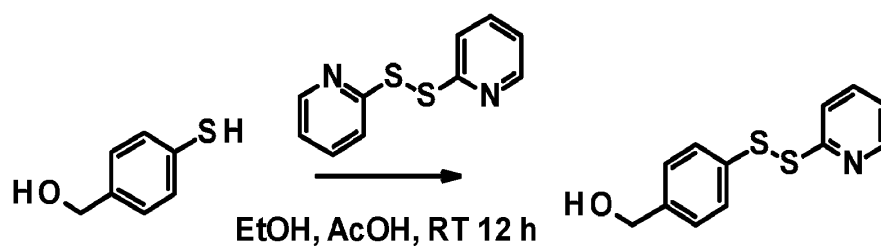
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XV

XVI

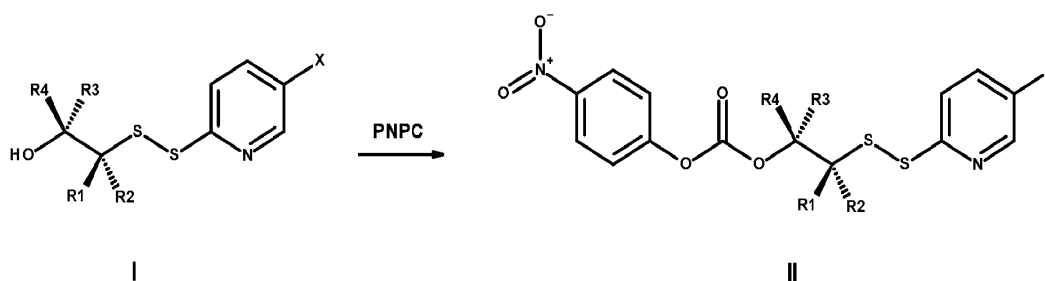
Int.	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	MH ⁺
XV-1	H, H	H, H, H, H	250.1

Synthesis of Intermediate XV-1: [4-(2-pyridyldisulfanyl)phenyl]methanol

**XV-1****XVI-1**

A stirred solution of 1,2-di(pyridin-2-yl)disulfane (2.68 g, 12.1 mmol) in mixture of AcOH : ethanol (5 mL, 1:10) solvent was degassed under N₂. This was followed by addition of 4-mercaptophenyl)methanol (0.74 g, 5.2 mmol) in mixture of AcOH/ethanol (5 mL) solvent drop-
 wise over 20 min and stirred for 12 h under N₂ atmosphere at room temperature. The reaction
 was concentrated under reduced pressure to afford the crude product which is purified by column
 chromatography (SiO₂, 60-70% EtOAc/hexanes) to afford [4-(2-
 pyridyl]disulfanyl)phenyl]methanol as a colourless liquid (800 mg, 61% yield).

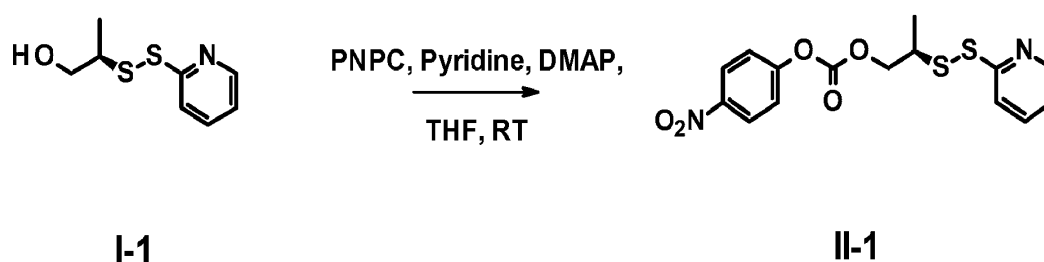
10 Carbonate Leaving Group Intermediate II from Intermediate I



Int.	R ¹ , R ²	R ³ , R ⁴	X	MH ⁺
II-1	H, Me	H, H	H	367.1
II-2	Me, H	H, H	H	367.1
II-3	-CH ₂ CH ₂ CH ₂ -	H, H	NO ₂	438.0
II-4	H, Me	Me, H	H	381.0
II-5	Me, H	H, Me	H	381.0
II-6	Me, H	Me, H	H	381.0

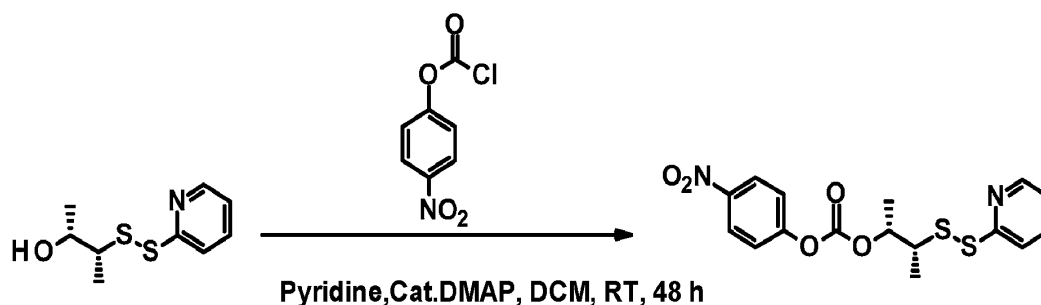
II-7	H, Me	H, Me	H	381.0
II-8	$\begin{array}{c} \text{CH}_2, \text{H} \quad \text{CH}_2, \text{H} \\ \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$		H	407.0
II-9	$\begin{array}{c} \text{H}, \text{CH}_2 \quad \text{H}, \text{CH}_2 \\ \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$		H	406.7

Synthesis of II-1: (4-nitrophenyl) [(2R)-2-(2-pyridyldisulfanyl)propyl] carbonate



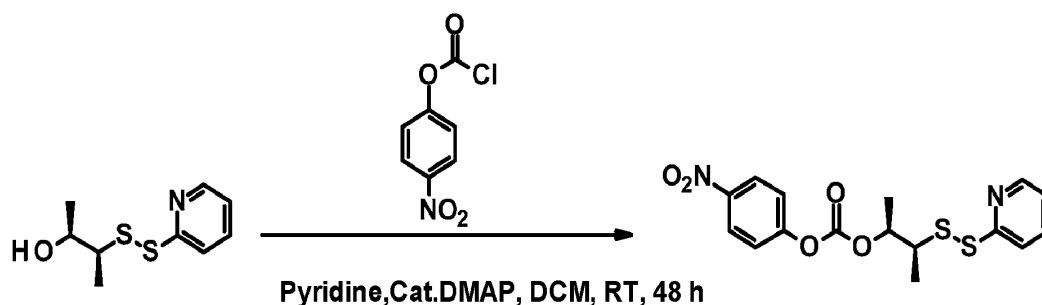
To (2R)-2-(2-pyridyldisulfanyl)propan-1-ol (0.39 g, 1.94 mmol) in THF under N₂ was added pyridine (0.16 mL, 1.94 mmol) and the (4-nitrophenyl) carbonochloridate (0.59 g, 2.91 mmol). The mixture was stirred for 16 h under N₂. The mixture was diluted with EtOAc and quenched with 20 mL of sat. NH₄Cl. The mixture was washed with water and brine and the organic layer concentrated. The crude mixture was purified by column chromatography (SiO₂, 0-50% EtOAc/Hexanes) to afford 0.59 g, 83% of (4-nitrophenyl) [(2R)-2-(2-pyridyldisulfanyl)propyl] carbonate. MS m/z found 367.1 [M+H]⁺. Intermediates II-2 and II-3 were synthesized analogously to II-1.

Synthesis of II-4: 4-nitrophenyl((2R,3R)-3-(pyridin-2-yl)disulfanyl)butan-2-yl carbonate



To a stirred solution of (2R,3R)-3-(pyridin-2-yl)disulfanyl butan-2-ol (140mg, 0.651 mmol) in CH₂Cl₂ (2.0 mL) was added pyridine (0.11 mL, 1.43 mmol), 4-nitrophenyl carbonochloridate (150 mg, 0.781 mmol) and catalytic amount of 4-dimethylaminopyridine at room temperature. The reaction vessel was sealed and stirred at RT for 48h. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and then washed with water (10 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford crude which is purified by column chromatography using 30-40% ethyl acetate/hexane. The fractions were concentrated off to obtain crude which was further purified over C18 reverse phase column. The pure fractions are concentrated to obtain 4-nitrophenyl((2R,3R)-3-(pyridin-2-yl)disulfaneyl)butan-2-yl) carbonate (70 mg, 28 %) as an oily compound. MS m/z 381.0 [M+H]⁺.

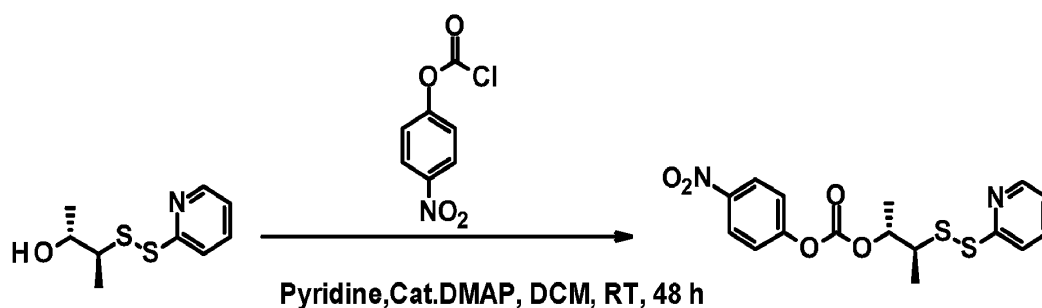
Synthesis of II-5: 4-nitrophenyl((2S,3S)-3-(pyridin-2-yl)disulfanyl)butan-2-yl) carbonate



To a stirred solution of (2S,3S)-3-(pyridin-2-yl)disulfanylbutan-2-ol (80mg, 0.372 mmol) in CH₂Cl₂ (1.0 mL) was added pyridine (0.066 mL, 0.818 mmol), 4-nitrophenylcarbonochloridate (89 mg, 0.446 mmol) and catalytic amount of 4-dimethylaminopyridine at room temperature. The reaction vessel was sealed and stirred at RT for 48h. The reaction mixture was diluted with CH₂Cl₂ (5 mL) and then washed with water (5 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude titular product which was purified by column chromatography using 30-40% ethyl acetate/Hexane. The fractions were concentrated to obtain crude which was further purified over C18 reverse phase column. The pure fractions are concentrated to obtain 4-nitrophenyl ((2S,3S)-3-(pyridin-2-yl)disulfanyl)butan-2-yl) carbonate (140 mg, 58 %) as an oily compound. MS m/z 381,0 [M+H]⁺.

25

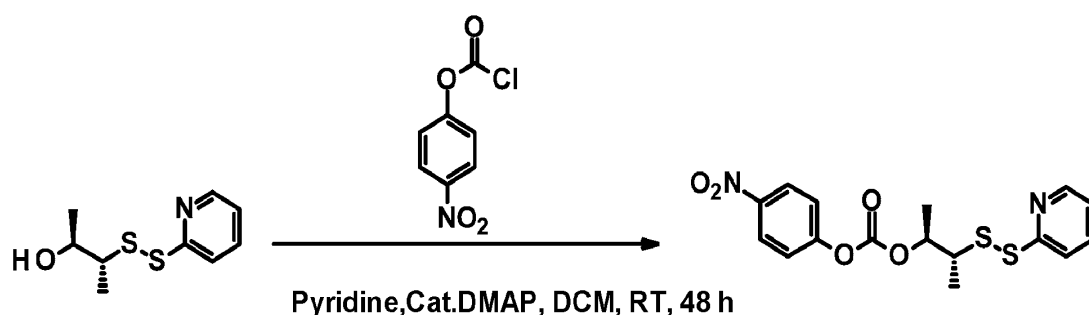
Synthesis of II-6: 4-nitrophenyl((2R,3S)-3-(pyridin-2-yl)disulfanyl)butan-2-yl carbonate



To a stirred solution of (2R,3S)-3-(pyridin-2-yl)disulfanylbutan-2-ol (0.4 g, 1.86 mmol) in CH₂Cl₂ (10 mL) was added pyridine (0.36 mL, 4.09 mmol), 4-nitrophenyl carbonochloridate (0.44 g, 2.32 mmol) and catalytic amount of 4-dimethylaminopyridine at 0° C. The reaction vessel was sealed and stirred at room temperature for 48h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with water (20 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford crude which was purified by silica gel flash column chromatography using 30-40% ethyl acetate/hexane. The compound eluted out as a mixture in 30 % EtOAc:Hexane. The fractions were concentrated to obtain crude which is further purified over C18 reverse phase column. The pure fractions were evaporated off to obtain 4-nitrophenyl ((2R,3S)-3-(pyridin-2-yl)disulfanyl)butan-2-yl carbonate (0.17 g, 24.2 %) as an oily compound. MS m/z 381.0 [M+H]⁺.

15

Synthesis of II-7: 4-nitrophenyl((2S,3R)-3-(pyridin-2-yl)disulfanyl)butan-2-yl carbonate



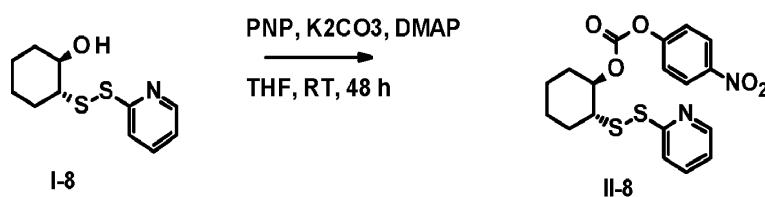
To a stirred solution of (2S,3R)-3-(pyridin-2-yl)disulfanylbutan-2-ol (0.4 g, 1.86 mmol) in CH₂Cl₂ (10 mL) was added pyridine (0.36 mL, 4.09 mmol), 4-nitrophenyl carbonochloridate

20

(0.44 g, 2.32 mmol) and catalytic amount of 4-dimethylaminopyridine at 0° C. The reaction vessel was sealed and stirred at room temperature for 48h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with water (20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford crude which was purified by silica gel flash column chromatography using 30-40% ethyl acetate/hexane. The compound eluted out as a mixture in 30 % EtOAc:Hexane. The fractions were concentrated to obtain crude which is further purified over C18 reverse phase column. The pure fractions were concentrated to obtain 4-nitrophenyl ((2S,3R)-3-(pyridin-2-yl)disulfanyl)butan-2-yl carbonate (0.18 g, 26 %) as an oily compound. MS m/z 381.0 [M+H]⁺.

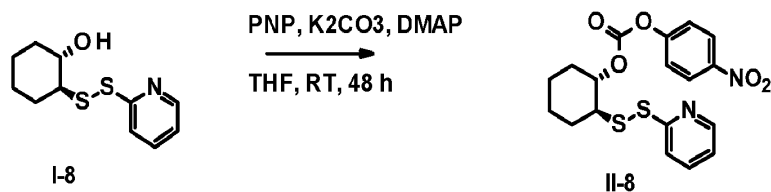
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Synthesis of II-8: (4-nitrophenyl) [(1R,2R)-2-(2-pyridyl)disulfanyl]cyclohexyl carbonate



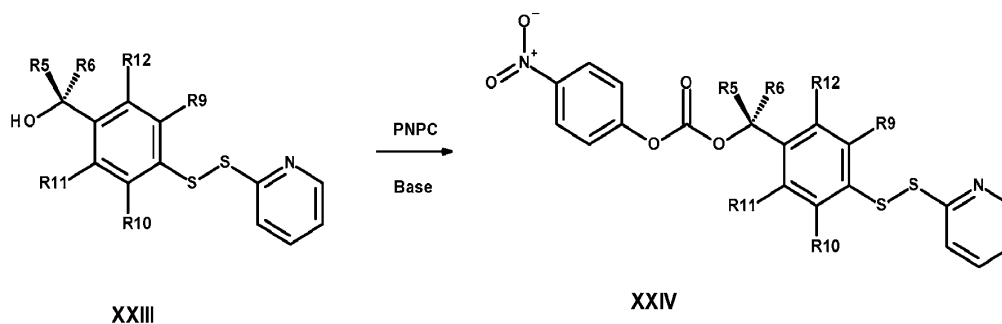
To a solution of (1R,2R)-2-(2-pyridyl)disulfanyl]cyclohexanol* (130.0 mg, 0.5 mmol) in THF (3.0 mL) was added potassium carbonate (0.20 g, 1.5 mmol), catalytic amount of DMAP and 4-nitrophenyl chloroformate (0.21 g, 0.10 mmol) at room temperature. The reaction vessel was sealed and stirred at RT for 48 h. Progress of the reaction was monitored by TLC (20% EtOAc/Hex). After completion of reaction, the reaction mixture was quenched with water (20.0 mL) and extracted with EtOAc (20.0 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude product which was purified by column chromatography using 20-30% of EtOAc/hexanes to afford 4-nitrophenyl (4-nitrophenyl) [(1R,2R)-2-(2-pyridyl)disulfanyl]cyclohexyl carbonate* as an off white solid (89 mg, 40 % yield). MS m/z 407.0 [M+H]⁺.

Synthesis of II-9: (4-nitrophenyl) [(1S,2S)-2-(2-pyridyl)disulfanyl]cyclohexyl carbonate



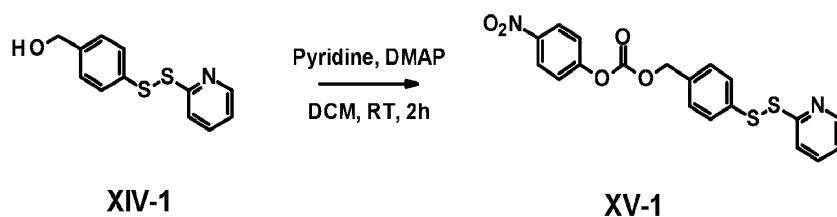
To a solution of (1S,2S)-2-(2-pyridyldisulfanyl)cyclohexanol* (0.42 g, 1.7 mmol) in THF (10.0 mL) was added potassium carbonate (0.70g, 5.1 mmol), catalytic amount of DMAP and 4-nitrophenyl chloroformate (0.69 g, 3.4 mmol) at room temperature. The reaction vessel was sealed and stirred at RT for 48 h. Progress of the reaction was monitored by TLC (20% EtOAc/Hex). After completion of reaction, the reaction mixture was quenched with water (20.0 mL) and extracted with EtOAc (20.0 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude product which was purified by column chromatography using 20-30% of EtOAc/hexanes to afford 4-nitrophenyl [(1R,2R)-2-(2-pyridyldisulfanyl)cyclohexyl] carbonate* as an off white solid (250 mg, 35 % yield). MS m/z 406.7 [M+H]⁺.

Carbonate Leaving Group Intermediate XV from XIV



Intermediate	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	X	MH ⁺
XV-1	H, H	H, H, H, H	H	415.0

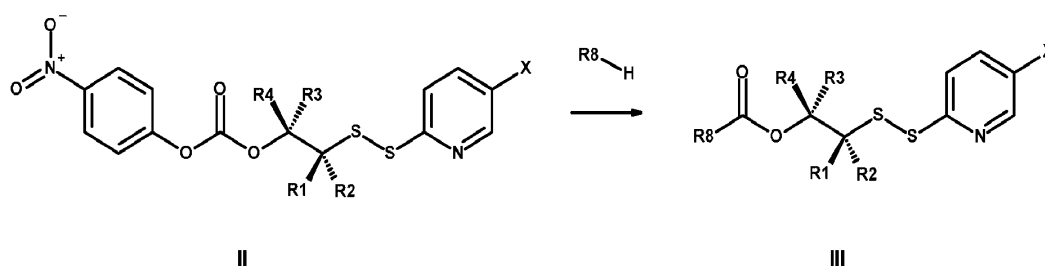
Synthesis of XV-1: (4-nitrophenyl) [4-(2-pyridyldisulfanyl)phenyl]methyl carbonate



To a stirred solution of (4-(pyridin-2-yl)disulfanyl)phenylmethanol (0.40 g, 1.60 mmol) in CH_2Cl_2 (10 mL) were added 4-nitrophenyl chloroformate (0.65 g, 3.2 mmol), pyridine (0.25 mL, 3.20 mmol), catalytic amount of DMAP (0.005 g) at 0°C . The mixture was allowed to stir for 2 h at room temperature. The reaction mixture was quenched with 1.5 N HCl solution. The organic layer was separated and washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2 , 20-30% of EtOAc/hexanes) to afford (4-nitrophenyl) [4-(2-pyridyldisulfanyl)phenyl]methyl carbonate as a colourless liquid (600 mg, 91% yield); MS m/z 415.0 $[\text{M}+\text{H}]^+$.

10

Carbonate and Carbamate Linked Intermediates III

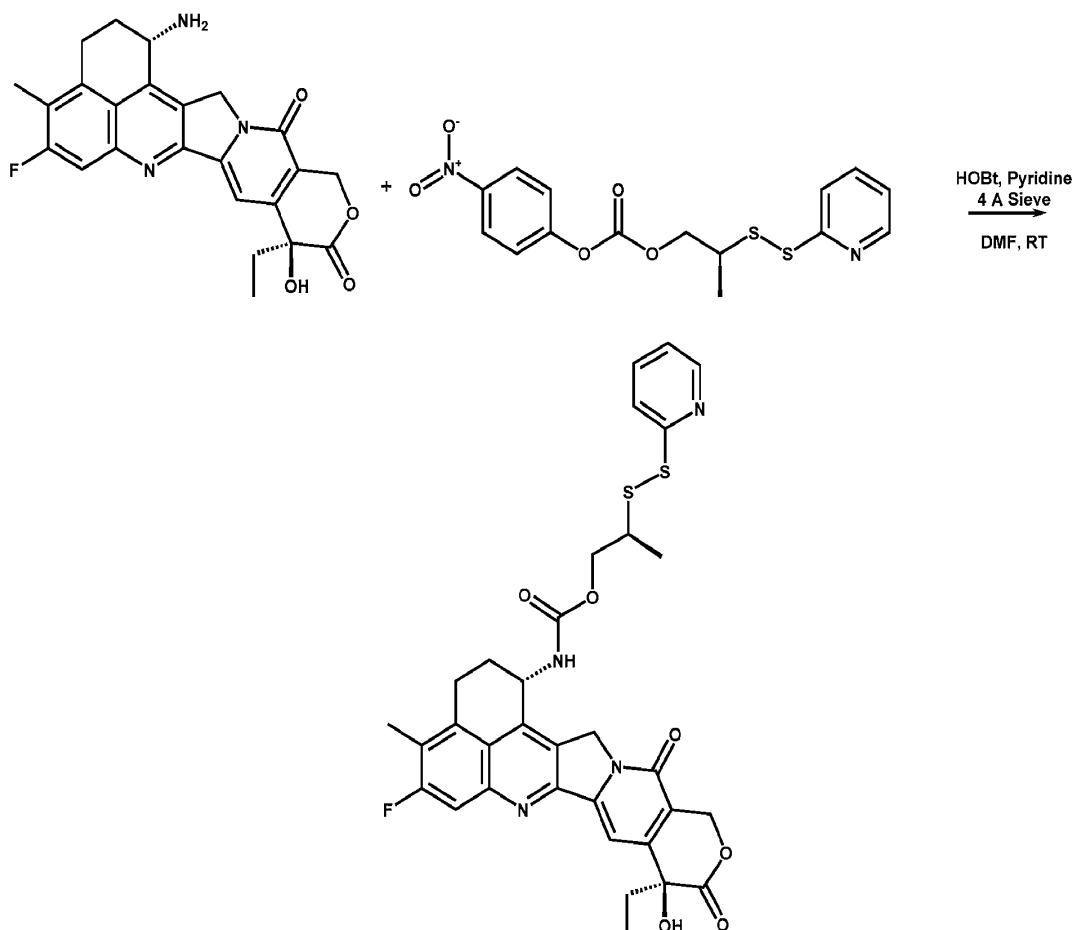


Intermediate	R^8H	R^1, R^2	R^3, R^4	X	MH^+
III-1	$\text{R}^8\text{H-5}$	H, Me	H, H	H	663.0
III-2	$\text{R}^8\text{H-5}$	Me, H	H, H	H	663.0
III-3	$\text{R}^8\text{H-5}$	$-\text{CH}_2\text{CH}_2\text{CH}_2-$	H, H	NO_2	734.0
III-4	$\text{R}^8\text{H-5}$	H, Me	Me, H	H	677.0
III-5	$\text{R}^8\text{H-5}$	Me, H	H, Me	H	677.1
III-6	$\text{R}^8\text{H-5}$	Me, H	Me, H	H	677.0
III-7	$\text{R}^8\text{H-5}$	H, Me	H, Me	H	677.1

III-8	R ⁸ H-5	$\begin{array}{c} \text{CH}_2, \text{H} \quad \text{CH}_2, \text{H} \\ \quad \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$	H	703.0
III-9	R ⁸ H-5	$\begin{array}{c} \text{H}, \text{CH}_2 \quad \text{H}, \text{CH}_2 \\ \quad \quad \\ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \end{array}$	H	703.1

Synthesis of III-1: [(2S)-2-(2-pyridyldisulfanyl)propyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15

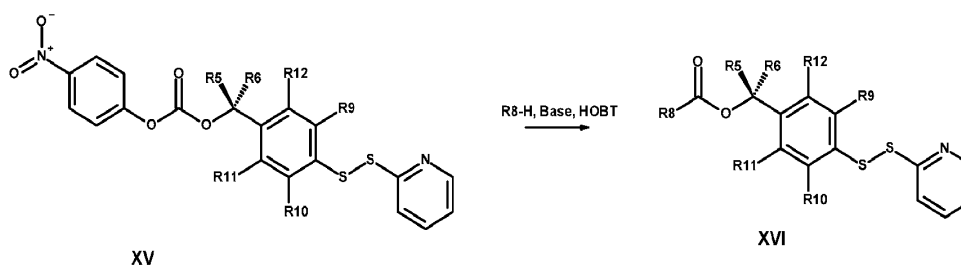
5 diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracosa-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate



To a mixture of 1-hydroxybenzotriazole hydrate (8.64 mg, 0.0564 mmol), finely ground molecular sieve 4 Å (50 mg) (10S,23S)-23-amino-10-ethyl-18-fluoro-10-hydroxy-19-methyl-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaene-5,9-dione; methanesulfonic acid (25.0 mg, 0.0470 mmol) and Pyridine (0.0190 mL, 0.235 mmol) in 2 mL of anhydrous DMF was added (4-nitrophenyl) [(2S)-2-(2-pyridyldisulfanyl)propyl] carbonate (19.0 mg, 0.0517 mmol). After stirring for 16 h at room temperature the mixture was filtered and the solution concentrated. The residue was purified by column chromatography (0-5% MeOH/DCM) to give [(2S)-2-(2-pyridyldisulfanyl)propyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (29.0 mg, yield: 93.0 %). MS m/z 663.0 [M+H]⁺.

Intermediates III-2 through III-9 are prepared from II-2 through II-9 analogously to III-1.

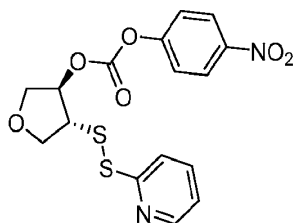
Carbonate and Carbamate Linked Intermediates XVI



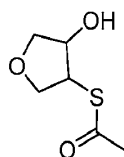
Int.	R ⁸ H	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	X	MH ⁻
XVI-1	R ⁸ H-5	H, H	H, H, H, H	H	711.0

Intermediate XVI-1 is prepared from XV-1 analogously to III-1.

Synthesis of 4-nitrophenyl (*trans*-(3RS,4RS)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-yl) carbonate

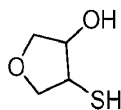


Step 1: Synthesis of racemic trans-(4-hydroxytetrahydrofuran-3-yl) ethanethioate



To a stirred solution of 3,6-dioxabicyclo[3.1.0]hexane (5.0 g, 0.051 mol) in water (40.0 mL) was added thioacetic acid (4.98 mL, 0.069 mol) and the resulting reaction mixture was stirred at room temperature for 16 h. Progress of the reaction was monitored by TLC (20% EtOAc/Hexane). Upon completion of the reaction, the reaction mixture was diluted with diethyl ether and washed with 10% sodium bicarbonate solution. The organic layer was separated and washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product, which was purified by column chromatography using 20% EtOAc : *n*-Hexane to obtain the title product as a brown colour liquid (4.0 g, yield 42%).
¹HNMR (400 MHz, CDCl₃): δ 4.35 - 4.28 (m, 2H), 4.02 - 3.98 (m, 1H), 3.81 - 3.73 (m, 2H), 3.69 - 3.62 (m, 1H), 2.37 (s, 3H).

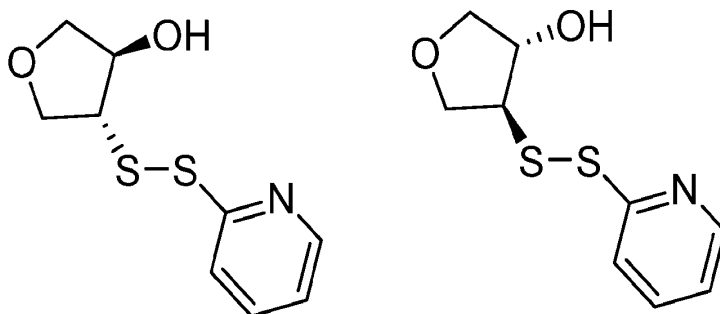
Step 2: Synthesis of Racemic trans-4-mercaptotetrahydrofuran-3-ol



To a stirred solution of racemic trans-(4-hydroxytetrahydrofuran-3-yl) ethanethioate (4.0 g, 24.7 mmol) in dry THF (20.0 mL) under nitrogen atmosphere was added LAH (1M in THF) (27.1 mL, 27.1 mmol) in dropwise manner at 0 °C. The reaction mixture was gradually allowed to warm to room temperature and stirred for 2 h. Progress of the reaction was monitored by TLC (20% EtOAc : *n*-Hexane). Upon completion of the reaction, the reaction mixture was cooled to room temperature and quenched with 1.0 N HCl (50 mL). The reaction mixture was extracted into DCM (3 x 20 mL), the organic layer was washed with brine solution (20 mL), dried over

anhydrous sodium sulfate, filtered, partially distilled and taken as such to the next step. (2.9 g, crude).

Step 3: Synthesis of *trans*-(4*RS*,3*RS*)-4-(pyridin-2-yldisulfanyl)tetrahydrofuran-3-ol and *trans*-(4*SR*,3*SR*)-4-(pyridin-2-yldisulfanyl)tetrahydrofuran-3-ol



Isomer 1 *trans* (1*RS*,2*RS*)

Isomer 2 *trans* (1*SR*, 2*SR*)

To a solution of 2-(pyridin-2-yldisulfanyl)pyridine (0.9 g, 21.7 mmol) in MeOH (degassed with N₂) (10 mL) was added 4-sulfanyloxolan-3-ol (2.9 g, 24.1 mmol) (degassed with N₂) dropwise and stirred at room temperature under nitrogen atmosphere for 16 h. The reaction mixture was concentrated to dryness under vacuum. The resultant crude was purified by flash column chromatography using 30% of EtOAc : *n*-Hexane to afford the title compound 4-(pyridin-2-yldisulfanyl)oxolan-3-ol (racemic mixture) as a yellow oil. The isomers were separated by Chiral preparative HPLC.

Chiral preparative HPLC Conditions:

Column : Chiralpak IA (250 mm X 20 mm X 5 mic)

Mobile phase : EtOH with 0.1% DEA (90:10)

Flow rate : 19 mL/min

Separated fractions of resolved isomers were collected from chiral prep. HPLC and evaporated under reduced pressure to afford the title compounds as Isomer 1 (600mg) and

Isomer 2 (620 mg).

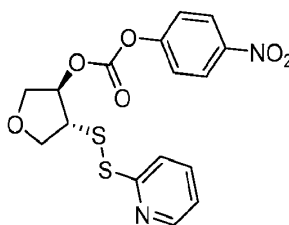
Isomer 1: (*trans*-(4*RS*,3*RS*)-4-(pyridin-2-yldisulfanyl)tetrahydrofuran-3-ol): LC-MS *m/z* calcd. for C₉H₁₁NO₂S₂, 229; found 230 [M+H]⁺. ¹H-NMR (400 MHz, CDCl₃): δ 8.53 - 8.52 (m,

1H), 7.67 - 7.63 (m, 1H), 7.56 (d, $J = 8.0$ Hz, 1H), 7.23 - 7.19 (m, 1H), 4.45 - 4.48 (m, 1H), 4.25 (t, $J = 8.8$ Hz, 1H), 4.12 (t, $J = 6.8$ Hz, 1H), 3.74 - 3.67 (m, 2H), 3.48 - 3.41 (m, 1H).

Isomer 2: (*trans*-(4*SR*,3*SR*)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-ol): LC-MS m/z calcd. for $C_9H_{11}NO_2S_2$, 229; found 230 $[M+H]^+$. ^1H -NMR (400 MHz, $CDCl_3$): δ 8.54 - 8.53 (m, 1H), 7.68 - 7.64 (m, 1H), 7.56 (d, $J = 8.0$ Hz, 1H), 7.23 - 7.20 (m, 1H), 4.49 - 4.45 (m, 1H), 4.25 (t, $J = 7.6$ Hz, 1H), 4.12 - 4.10 (m, 1H), 3.74 - 3.67 (m, 2H), 3.47 - 3.44 (m, 1H).

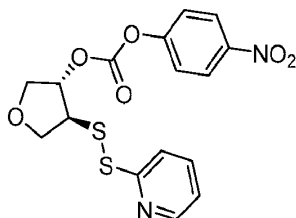
The absolute stereochemistry of the isomers was arbitrarily assigned.

Step 4 : Synthesis of 4-nitrophenyl (*trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-yl carbonate



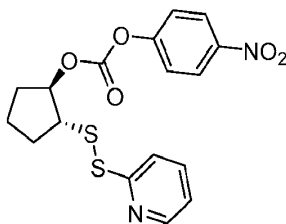
To a stirred solution of *trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-ol (0.61 g, 2.69 mmol) in DMF (10 mL) under nitrogen atmosphere was added DIPEA (1.45 mL, 8.08 mmol) and bis(4-nitrophenyl) carbonate (1.64 g, 5.38 mmol) at room temperature. The reaction vessel was sealed and stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC (20% EtOAc : *n*-Hexane). Upon completion of the reaction, the reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 x 10 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography using 20-30% of EtOAc : *n*-Hexane to afford 4-nitrophenyl (*trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-yl carbonate as an off-white solid (790 mg, 77% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (d, $J = 4.4$ Hz, 1H), 8.27 (d, $J = 8.8$ Hz, 2H), 7.67 - 7.59 (m, 2H), 7.36 (d, $J = 8.8$ Hz, 2H), 7.15 (t, $J = 5.2$ Hz, 1H), 5.44 - 5.43 (m, 1H), 4.40 - 4.25 (m, 2H), 4.03 (d, $J = 11.2$ Hz, 1H), 3.92 - 3.86 (m, 1H), 3.85 - 3.79 (m, 1H); LC-MS m/z calcd. for $C_{16}H_{14}N_2O_6S_2$, 394; found 395 $[M+H]^+$.

Synthesis of 4-nitrophenyl (*trans*-(3SR,4SR)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-yl carbonate



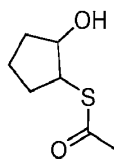
To a stirred solution of *trans*-(3SR,4SR)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-ol
 5 (550 mg, 2.46 mmol) in DMF (10.0 mL) under nitrogen was added DIPEA (1.32 mL, 7.38 mmol) and bis(4-nitrophenyl) carbonate (1.5 g, 4.92 mmol) at room temperature. The reaction vessel was sealed and stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC (20% EtOAc : *n*-Hexane). Upon completion of the reaction, the reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 x 10 mL). The organic
 10 layer was separated, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography using 20-30% of EtOAc : *n*-Hexane to afford 4-nitrophenyl (*trans*-(3SR,4SR)-4-(pyridin-2-yl)disulfanyl)tetrahydrofuran-3-yl carbonate as an off-white solid (0.6 g, 70 % yield). ¹HNMR (400 MHz, CDCl₃): δ 8.85 (d, *J* = 4.4 Hz, 1H), 8.26 (d, *J* = 8.8 Hz, 2H),
 15 7.68 - 7.59 (m, 2H), 7.35 (d, *J* = 8.8 Hz, 2H), 7.14 (t, *J* = 5.2 Hz, 1H), 5.44 - 5.43 (m, 1H), 4.40 - 4.25 (m, 2H), 4.03 (d, *J* = 11.2 Hz, 1H), 3.92 - 3.86 (m, 1H), 3.85 - 3.79 (m, 1H); LC-MS *m/z* calcd for C₁₆H₁₄N₂O₆S₂, 394; found 395 [M+H]⁺.

Synthesis of 4-nitrophenyl (*trans*-(1RS,2RS)-2-(pyridin-2-yl)disulfanyl)cyclopentyl carbonate



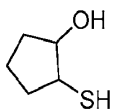
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Step 1: Synthesis of racemic trans-(5-hydroxycyclopentan-1-yl) ethanethioate



To a stirred solution of 6-oxabicyclo[3.1.0]hexane (3.0 g, mmol) in water (30 mL) was added thioacetic acid (3 mL, 39.2 mmol) at room temperature and stirred for 16 h. The reaction mixture was quenched with sat. sodium bicarbonate solution and extracted with ethyl acetate (3 x 10 mL). The organic layer was separated and washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford racemic trans-(5-hydroxycyclopentan-1-yl) ethanethioate as an oily compound (2.6 g, crude). LC-MS m/z calcd for C₇H₁₂O₂S, 160.2; found 143.3 [M+H - 17]⁺.

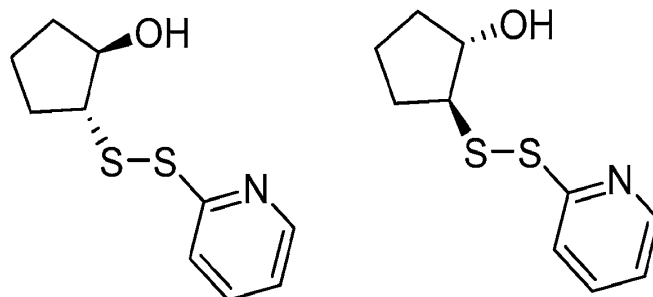
10 *Step 2: Synthesis of Racemic trans-2-mercaptocyclopentan-1-ol*



To a stirred solution of racemic trans-(5-hydroxycyclopentan-1-yl) ethanethioate (2.6 g, 16.2 mmol) in THF (20 mL) at 0 °C under nitrogen atmosphere, was added LAH (1Min THF) (24 mL, 24.3 mmol) in dropwise manner. The reaction mixture was gradually allowed to warm to room temperature and stirred for 2 h. Progress of the reaction was monitored by TLC (20% EtOAc : n-Hexane). Upon completion of the reaction, the reaction mixture was cooled to room temperature and quenched with 1N HCl solution and extracted in DCM. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated partially and the crude racemic trans-2-mercaptocyclopentan-1-ol carried forward to the next step (1.9 g, crude).

20

Step 3: Synthesis of trans-(1RS, 2RS)-2-(pyridin-2-yldisulfanyll)cyclopentan-1-ol and trans-(1SR, 2SR)-2-(pyridin-2-yldisulfanyll)cyclopentan-1-ol



Isomer 1 trans (1RS,2RS)

Isomer 2 trans (1SR, 2SR)

To a stirred solution of 2-(pyridin-2-yl)disulfanylpipridine (2.1 g, 9.65 mmol) in MeOH
 5 (10 mL) under nitrogen atmosphere was added racemic *trans*-2-mercaptocyclopentan-1-ol (1.9 g,
 16.1 mmol) in a dropwise manner at 0 °C. The reaction mixture was gradually allowed to warm
 to room temperature and stirred for 16 h. After completion of the reaction, the reaction mixture
 was concentrated to dryness under vacuum. The resultant crude was purified by silica gel flash
 column chromatography. The compound was eluted out in 15 % EtOAc : *n*-Hexane. Fractions
 10 containing the desired product were combined and evaporated under reduced pressure to afford
 the title compound (racemic mixture) as a yellow liquid. The isomers were separated by Chiral
 preparative HPLC.

Chiral preparative HPLC conditions:

Column : Chiralpak IA (250 mm X 20 mm X 5 mic)
 15 Mobile phase : EtOH with 0.1% DEA (70:30)
 Flow rate : 19 mL/min

Separated fractions of separated isomers were collected from chiral prep. HPLC and evaporated
 under reduced pressure to afford the title compounds as Isomer 1 (300mg) and Isomer 2 (300
 mg) as a colourless oil.

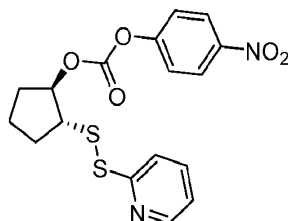
20 Isomer 1 (*trans*-(1RS, 2RS)-2-(pyridin-2-yl)disulfanyll)cyclopentan-1-ol): LC-MS *m/z*
 calcd for C₁₀H₁₃NOS₂, 227.34; found 228.1 [M+H]⁺. ¹HNMR (400 MHz, CDCl₃): δ 8.51 -

8.50 (m, 1H), 7.61 - 7.57 (m, 1H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.17 - 7.14 (m, 1H), 4.03 - 3.97 (m, 1H), 3.0 - 2.87 (m, 1H), 2.11 - 2.02 (m, 3H), 1.75 - 1.65 (m, 4H).

Isomer 2 (*trans*-(1SR, 2SR)-2-(pyridin-2-ylidisulfanyl)cyclopentan-1-ol): LC-MS m/z calcd for $C_{10}H_{13}NOS_2$, 227.34; found 228.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 8.51 - 8.50 (m, 1H), 7.61 - 7.57 (m, 1H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.17 - 7.14 (m, 1H), 4.03 - 3.97 (m, 1H), 3.0 - 2.87 (m, 1H), 2.11 - 2.02 (m, 3H), 1.75 - 1.65 (m, 4H).

The absolute stereochemistry of the isomers was arbitrarily assigned.

Step 4: Synthesis of 4-nitrophenyl ((1R,2R)-2-(pyridin-2-ylidisulfanyl)cyclopentyl)carbonate



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To a stirred solution of *trans*-(1SR,2RS)-2-(pyridin-2-ylidisulfanyl)cyclopentan-1-ol (0.3g, 1.34 mmol) in DMF (10 mL) under nitrogen atmosphere was added DIPEA (0.65 mL, 3.96 mmol) and Bis(4-nitrophenyl) carbonate (0.8 g, 2.64 mmol) at room temperature. The reaction vessel was sealed and stirred at room temperature for 16 h. The reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 x 10 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product which was purified by silica gel flash column chromatography. The compound eluted out in 10 % EtOAc : *n*-Hexane as a mixture. The fractions were evaporated off to obtain crude compound which was purified over reverse phase column chromatography. Fractions containing the product were evaporated under reduced pressure to obtain 4-nitrophenyl (*trans*-(1SR,2RS)-2-(pyridin-2-ylidisulfanyl)cyclopentyl) carbonate as a colourless oil (305 mg, 59 %).

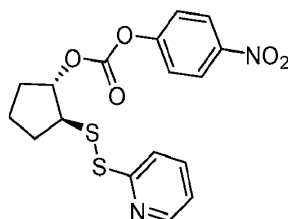
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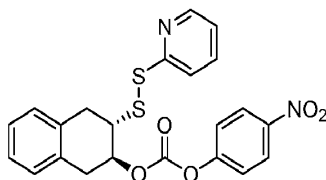
^1H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, $J = 4.1$ Hz, 1H), 8.25 (d, $J = 6.8$ Hz, 2H), 7.66 - 7.62 (m, 2H), 7.34 (d, $J = 6.4$ Hz, 2H), 7.10 - 7.08 (m, 1H), 5.29 - 5.10 (m, 1H), 3.52 - 3.45 (m, 1H), 2.32 - 2.28 (m, 2H), 1.9 - 1.76 (m, 4H). LC-MS m/z calcd for $C_{17}H_{16}N_2O_5S_2$, 392.44; found 393.0 $[M+H]^+$.

Synthesis of 4-nitrophenyl (*trans*-(1*SR*,2*SR*)-2-(pyridin-2-yl)disulfanyl)cyclopentyl carbonate

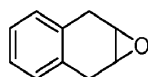


To a stirred solution of (1*SR*,2*SR*)-2-(pyridin-2-yl)disulfanyl)cyclopentan-1-ol (0.26 g, 1.14 mmol) in DMF (10.0 mL) under nitrogen atmosphere was added DIPEA (0.57 mL, 3.43 mmol) and bis(4-nitrophenyl) carbonate (0.7 g, 2.29 mmol) at room temperature. The reaction vessel was sealed and stirred at room temperature for 16 h. The reaction mixture was quenched with water (20.0 mL) and extracted with EtOAc (3 x 10 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford the crude product which was purified by silica gel flash column chromatography. The compound eluted out in 10 % EtOAc : *n*-Hexane as a mixture. The fractions were evaporated off to obtain crude compound which was purified over reverse phase column chromatography. Fractions containing the product were evaporated under reduced pressure to obtain 4-nitrophenyl (*trans*-(1*SR*,2*SR*)-2-(pyridin-2-yl)disulfanyl)cyclopentyl carbonate (330 mg, 73.5 %) as a colorless oil. ¹HNMR (400 MHz, CDCl₃): δ 8.46 (d, *J* = 4 Hz, 1H), 8.25 (d, *J* = 6.8 Hz, 2H), 7.66 – 7.62 (m, 2H), 7.34 (d, *J* = 6.4 Hz, 2H), 7.10 – 7.08 (m, 1H), 5.29 – 5.10 (m, 1H), 3.52 – 3.45 (m, 1H), 2.32 – 2.28 (m, 2H), 1.9 – 1.76 (m, 4H). LC-MS *m/z* calcd for C₁₇H₁₆N₂O₅S₂, 392.44; found 393.0 [M+H]⁺.

Synthesis 4-nitrophenyl (*trans*-(2*RS*, 3*RS*)-3-(pyridin-2-yl)disulfanyl)-1, 2, 3, 4-tetrahydronaphthal en-2-yl) carbonate



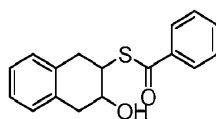
Step 1: Synthesis of 1*aH*, 2*H*, 7*H*, 7*aH*-naphtho [2, 3-*b*] oxirene



To a stirred solution of 1,4-dihydronaphthalene (100 mg, 768 μmol) in dichloromethane (2.00 ml) under nitrogen atmosphere at 0 °C was added 3-chlorobenzene-1-carboxoperoxoic acid (199 mg, 1.5 eq., 1.15 mmol) lot wise and stirred for 16 h at RT. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was filtered and extracted with dichloromethane, washed with sodium bicarbonate solution, followed by water and brine. The two layers were separated and the combined organic layer was dried over sodium sulfate, filtered and evaporated to get the crude product, which was purified by silica gel flash column chromatography. Product was eluted out in 10% EtOAc and *n*-Hexane, (product is UV Inactive), fractions were collected and dried under the vacuum to obtain 1aH,2H,7H,7aH-naphtho[2,3-b]oxirene (85.0 mg, 581 μmol) as an oily compound.

¹HNMR (400 MHz, CDCl₃): δ 7.14 (t, *J* = 3.2 Hz, 2H), 7.05 (t, *J* = 3.2 Hz, 2H), 3.48 (s, 2H), 3.32 (d, *J* = 17.6 Hz, 2H), 3.19 (d, *J* = 17.6 Hz, 2H).

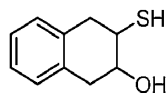
Step 2: Synthesis of Racemic [trans-(3-hydroxy-1, 2, 3, 4-tetrahydronaphthalen-2-yl) sulfanyl] (phenyl) methanone



To a stirred solution of 1aH,2H,7H,7aH-naphtho[2,3-b]oxirene (100 mg, 684 μmol) in ethoxyethane (4.00 mL), under nitrogen atmosphere was added aluminium oxide (1.00 g) (acidic). The solution was cooled to 0 °C. Then, thiobenzoic acid (482 mg, 5.1 eq., 3.49 mmol) was added to the reaction mixture and stirred at RT for 24 h. After completion of reaction (progress of the reaction was monitored by TLC), the reaction mixture was filtered and washed with sodium bicarbonate solution, followed by washing with water and brine solution to give the crude product. The crude product was purified by silica gel flash column chromatography using and the product was eluted out in 20% EtOAc : *n*-Hexane to obtain racemic [*trans*-(3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)sulfanyl](phenyl)methanone (125 mg, 440 μmol) as a colourless liquid.

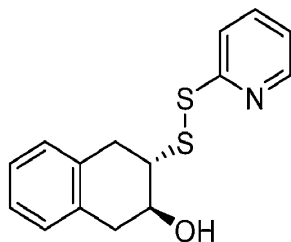
¹HNMR (400 MHz, DMSO): δ 7.89 (d, *J* = 7.2 Hz, 2H), 7.66 (t, 1H), 7.53 (d, *J* = 7.2 Hz, 2H), 7.09 (m, 4H), 5.39 (s, 1H), 4.00 (s, 2H), 3.42 (d, *J* = 17.6 Hz, 1H), 3.12 (t, *J* = 16 Hz, 1H), 2.81 (t, *J* = 18.4 Hz, 2H).

Step 3: Synthesis of Racemic *trans*-3-sulfanyl-1, 2, 3, 4-tetrahydronaphthalen-2-ol

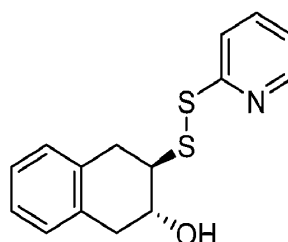


To a stirred solution of [(3-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)sulfanyl] (phenyl)
 5 methanone (115 mg, 404 μmol) in methanol (3.00 mL) was added K_2CO_3 (113 mg, 2 eq., 809 μmol) and the reaction mixture was stirred for 0.5 h at RT. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was concentrated (to remove methanol) and then acidified with 1N HCl solution until the pH reached 2-3, to obtain racemic *trans*-3-sulfanyl-1,2,3,4-tetrahydronaphthalen-2-ol (70.0 mg, 388 μmol) which was
 10 taken further as such for the next step.

Step 4: Synthesis of *trans*-(2*RS*, 3*RS*)-3-(pyridin-2-yldisulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol and *trans*-(2*SR*, 3*SR*)-3-(pyridin-2-yldisulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol



Isomer 1 *trans* (2*RS*,3*RS*)



Isomer 2 *trans* (2*SR*, 3*SR*)

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To a stirred solution of racemic *trans*-3-sulfanyl-1,2,3,4-tetrahydronaphthalen-2-ol (350 mg, 1.94 mmol) in methanol (2.50 mL) under nitrogen atmosphere was added 2-(pyridin-2-yl)disulfanylpyridine (428 mg, 1 eq., 1.94 mmol) and stirred at RT for 16 h. Progress of the reaction was monitored by TLC and LC-MS. After completion of the reaction, the reaction mass
 20 was concentrated and then diluted with DCM, washed with water followed by brine and dried over sodium sulfate. The crude product obtained was purified by silica gel flash column chromatography. The desired product was eluted out in 20% EtOAc : Hexane. The product was re-purified by reverse phase column chromatography (10-20% of 0.1% Formic acid in water/

Acetonitrile). Fractions containing the desired product were collected and evaporated off under vacuum to obtain 3-(pyridin-2-ylidisulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol (350 mg, 1.21 mmol) as a yellow solid. The isomers were separated by chiral preparative HPLC.

¹HNMR (400 MHz, DMSO): δ 8.44 (d, J = 4.4 Hz, 1H), 7.79 (d, J = 3.2 Hz, 2H), 7.26 - 7.24 (m, 1H), 7.06 (s, 4H), 5.61 (s, 1H), 3.91 - 3.80 (m, 1H), 3.31 - 3.19 (m, 2H), 3.13 - 3.07 (m, 1H), 2.92 - 2.84 (m, 1H), 2.75 - 2.65 (m, 1H).

Prep. conditions:

Column : CHIRALPAK IA (250 mm X 420 mm X 5 mic)

Mobile phase : *n*-Hexane : Ethanol with 0.1% DEA (50:50)

Flow rate : 19 mL/min

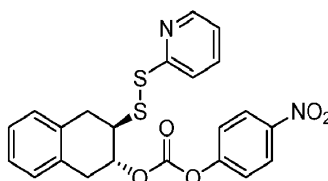
The isomers were separated and the respective fractions were collected from chiral prep. HPLC were combined and evaporated to afford the respective isomers. Isomer 1 was collected first and assigned as *trans*-(2RS, 3RS)-3-(pyridin-2-ylidisulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol. Isomer 2 was collected second and assigned as *trans*-(2SR, 3SR)-3-(pyridin-2-ylidisulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol. The absolute stereochemistry of the isomers was arbitrarily assigned.

Step 5: Synthesis of 4-nitrophenyl (trans-(2RS, 3RS)-3-(pyridin-2-ylidisulfanyl)-1, 2, 3, 4-tetrahydronaphthal en-2-yl) carbonate

To a stirred solution of *trans*-(2RS, 3RS)-3-(pyridin-2-ylidisulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol (150 mg, 518 μ mol) in Dimethylformamide (3.00 ml, 38.7 mmol) was added bis(4-nitrophenyl) carbonate (315 mg, 2 eq., 1.04 mmol) followed by N,N-Diisopropylethylamine (271 μ L, 3 eq., 1.55 mmol). The reaction mixture was stirred at RT for 12 h. After completion of the reaction, the reaction mass was quenched with water, extracted with DCM (3 x 5), combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure to afford crude product. The crude product was purified by silica gel flash column chromatography (0-40% EtOAc : *n*-Hexane) and also re-purified by reverse phase column chromatography (10-50% of 0.1% formic acid in water : ACN), to give (*trans*-(2RS, 3RS)-3-(pyridin-2-ylidisulfanyl)-1, 2, 3, 4-tetrahydronaphthal en-2-yl) carbonate (133 mg, 293 μ mol) as an off white solid.

¹HNMR (400 MHz, DMSO): δ 8.44 (d, 1H), 8.30 (d, $J = 9.2$ Hz, 2H), 7.80 - 7.76 (m, 2H), 7.54 (d, $J = 9.2$ Hz, 2H), 7.26 - 7.24 (m, 1H), 7.14 - 7.06 (m, 4H), 5.21 - 5.19 (m, 1H), 3.78 - 3.77 (m, 1H), 3.45 - 3.25 (m, 2H), 3.10 - 3.01 (m, 2H).

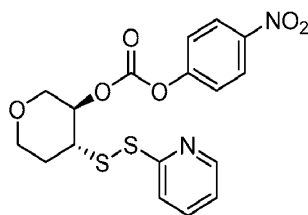
5 Synthesis of 4-nitrophenyl (*trans*-(2*SR*,3*SR*)-3-(pyridin-2-yl)disulfanyl)-1, 2, 3, 4-tetrahydronaphthal en-2-yl) carbonate



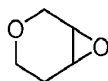
To a stirred solution of *trans*-(2*SR*, 3*SR*)-3-(pyridin-2-yl)disulfanyl)-1, 2, 3, 4-tetrahydronaphthalen-2-ol (130 mg, 449 μ mol) in dimethylformamide (2.60 ml, 33.6 mmol) was added bis(4-nitrophenyl) carbonate (273 mg, 2 eq., 898 μ mol) followed by diisopropylethylamine (13.0 mL, 3 eq., 74.6 mmol). The reaction mixture was stirred at RT for 12 h. After completion of the reaction (progress of the reaction was monitored by TLC), reaction mass was quenched with water, extracted with DCM (3 x 5), the combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure to afford crude product which was purified by flash column chromatography (0-40% EtOAc : *n*-Hexane). The product was re-purified by reverse phase column chromatography (10-50% of 0.1% formic acid in water: ACN) to give 4-nitrophenyl (*trans*-(2*SR*,3*SR*)-3-(pyridin-2-yl)disulfanyl)-1, 2, 3, 4-tetrahydronaphthal en-2-yl) carbonate (30.0 mg, 66.0 μ mol) as an off white solid.

¹HNMR (400 MHz, DMSO): δ 8.44 (d, 1H), 8.30 (d, $J = 9.2$ Hz, 2H), 7.80 - 7.76 (m, 2H), 7.54 (d, $J = 9.2$ Hz, 2H), 7.26 - 7.24 (m, 1H), 7.14 - 7.06 (m, 4H), 5.21 - 5.19 (m, 1H), 3.78 - 3.77 (m, 1H), 3.45 - 3.25 (m, 2H), 3.10 - 3.01 (m, 2H).

Synthesis of 4-nitrophenyl (*trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyl)oxan-3-yl) carbonate

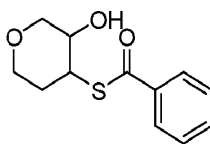


Step 1: Synthesis of 3,7-dioxabicyclo[4.1.0]heptane



To a stirred solution of 3,6-dihydro-2H-pyran (2.0 g, 23.8 mmol) in dichloromethane (20.0 mL) at 0 °C was added 3-chlorobenzene-1-carboperoxoic acid (4.92 g, 1.2 eq., 28.5 mmol) slowly in portions and stirred under nitrogen atmosphere for 16 h at room temperature. Progress of the reaction was monitored by TLC. After completion of reaction, the reaction mass was quenched with sat. sodium bicarbonate solution, and the organic layer was separated and washed with water followed by brine solution, dried over anhydrous sodium sulfate, filtered and evaporated to give the title compound 3,7-dioxabicyclo[4.1.0]heptane (1.00 g, 9.99 mmol) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) : 4.03 - 3.94 (m, 2H), 3.55 - 3.49 (m, 1H), 3.46 - 3.41 (m, 1H), 3.35 (m, 1H), 3.18 (m, 1H), 2.00 (m, 2H).

Step 2: Synthesis of Racemic [*trans*-(3-hydroxytetrahydropyran-4-yl) sulfanyl](phenyl)methanone:

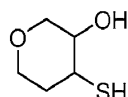


To a stirred solution of 3,7-dioxabicyclo[4.1.0]heptane (1.00 g, 9.99 mmol) in ethoxyethane (40 mL) at room temperature was added benzenecarbothioic S-acid (5.88 mL, 5 eq., 49.9 mmol) followed by silanediol (3.00 g, 5 eq., 49.9 mmol) and the reaction mixture was stirred at room temperature for 12 h. Progress of the reaction monitored by TLC, upon completion of the starting material, the reaction mass was quenched with sat. sodium bicarbonate solution and then extracted with ethyl acetate (2 x 10 mL). The combined organic layers was dried over anhydrous sodium sulfate, filtered and then evaporated under reduced pressure to afford crude product which was purified by flash column chromatography (0-30% EtOAc : *n*-Hexane). Compound was eluted at 20% EtOAc : *n*-Hexane. Pure fractions were collected and evaporated to give racemic [*trans*-(3-hydroxytetrahydropyran-4-yl) sulfanyl](phenyl)methanone (2.0 g, 8.39 mmol).

LC-MS m/z calculated C₁₂H₁₄O₃S; 238.3, found 239.1 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃): δ 7.43 (d, *J* = 7.6 Hz, 2H), 7.09 (t, *J* = 17.2 Hz, 1H), 6.93 (t, *J* = 8.0 Hz, 2H), 3.55 (dd, *J* = 4.0 Hz, 4.0 Hz, 1H), 3.37 (d, *J* = 11.2 Hz, 1H), 3.28 - 3.24 (m, 1H), 3.22 - 3.18 (m, 1H), 3.01 (t, *J* = 10.8 Hz, 1H), 2.83 (t, *J* = 12.4 Hz, 1H), 1.36 - 1.27 (m, 1H), 1.27 (s, 2H).

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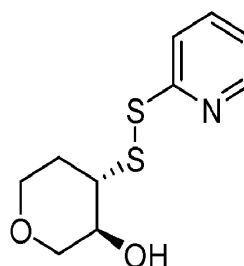
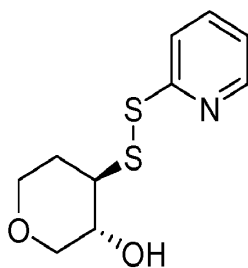
Step 3: Synthesis of Racemic trans- 4-sulfanyloxan-3-ol



To a stirred solution of racemic [*trans*-3-hydroxytetrahydropyran-4-yl)sulfanyl](phenyl)methanone (2.50 g, 10.5 mmol) in dichloromethane (25 mL) at room temperature was added hydrazine hydrate (5.15 mL, 10 eq., 105 mmol) slowly and the reaction mixture was stirred for 1 h. Progress of the reaction was monitored by TLC, upon completion of the reaction, the reaction mass was quenched with 1N HCl so that the pH was adjusted to 2-3. The two layers were separated and the organic layer was dried over sodium sulphate, filtered and partially evaporated and the crude racemic *trans*- 4-sulfanyltetrahydropyran-3-ol was taken for the next step.

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Step 4: Synthesis of trans-(3RS,4RS)-4-(pyridin-2-yldisulfanyl)tetrahydropyran-3-ol and trans-(3SR,4SR)-4-(pyridin-2-yldisulfanyl)tetrahydropyran-3-ol



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Isomer 1 trans (2RS,3RS)

Isomer 2 trans (2SR, 3SR)

To a stirred solution of 2-(pyridin-2-yl)disulfanylpuridine (1.85 g, 0.8 eq., 8.41 mmol) in Methanol (40 mL) was added racemic *trans*-4-sulfanyltetrahydropyran-3-ol (1.41 g, 10.5 mmol) in DCM at 0°C and then reaction mixture was stirred at room temperature for 12 h. Upon completion of the reaction, the completed reaction mass was evaporated under reduced pressure to afford crude which was purified by flash column chromatography. Product was eluted out in 20% EtOAc : *n*-Hexane, pure fractions were collected and evaporated to afford the title product 4-(pyridin-2-yl)disulfanyloxan-3-ol (racemic mixture). The isomers were separated by Chiral preparative HPLC.

Chiral preparative HPLC conditions:

Column : CHIRALPAK IA (250 mm X 20 mm X 5 mic)

Mobile phase : *n*-Hexane : IPA with 0.1% DEA (90:10)

Flow rate : 19 mL/min

The isomers were separated and the respective fractions were collected from chiral prep. HPLC.

The fractions were combined and evaporated to afford the respective isomers.

(Isomer 1 - 350 mg, Isomer 2 - 350 mg) LC-MS *m/z* calculated C₁₀H₁₃NO₂S₂; 243.34, found 244 [M+H]⁺.

Isomer 1 (*trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyltetrahydropyran-3-ol):

¹H-NMR (400 MHz, DMSO): δ 8.53 (s, 1H), 7.60 (t, *J* = 6.40 Hz, 1H), 7.40 (d, *J* = 7.2 Hz, 1H), 7.23 (t, *J* = 20.8 Hz, 1H), 4.28 - 4.06 (m, 1H), 3.94 (d, *J* = 12 Hz, 1H), 3.54 - 3.40 (m, 3H), 3.33 - 3.21 (m, 1H), 3.07 - 2.74 (m, 1H), 2.04 - 1.94 (m, 2H).

Isomer 2 (*trans*-(3*SR*,4*SR*)-4-(pyridin-2-yl)disulfanyltetrahydropyran-3-ol)

¹H-NMR (400 MHz, DMSO): δ 8.52 (d, *J* = 2.8 Hz, 1H), 7.61 (t, *J* = 6.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.18 (t, *J* = 5.2 Hz, 1H), 4.12 - 4.09 (m, 1H), 3.94 (d, *J* = 12 Hz, 1H), 3.53 - 3.47 (m, 1H), 3.47 - 3.37 (m, 1H), 3.25 (t, *J* = 10.4 Hz, 1H), 2.80 - 2.73 (m, 1H), 1.96 - 1.42 (m, 1H), 1.20 (d, *J* = 6.0 Hz, 2H).

The absolute stereochemistry of the isomers was arbitrarily assigned.

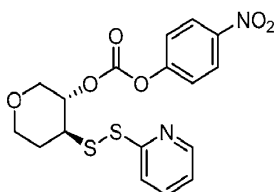
Step 5: Synthesis of 4-nitrophenyl (trans-(3RS,4RS)-4-(pyridin-2-yl)disulfanyltetrahydropyran-3-yl)carbonate

To a stirred solution of (*trans*-(3*RS*,4*RS*)-4-(pyridin-2-yl)disulfanyltetrahydropyran-3-ol)

(300 mg, 1.23 mmol) in DMF (8 mL) was added bis(4-nitrophenyl) carbonate (750 mg, 2 eq., 2.47 mmol) and then followed by di-isopropylethylamine (644 μ L, 3 eq., 3.70 mmol) at room temperature for 12 h. Upon completion of the reaction, the reaction mass was partitioned between water and DCM. The organic layer was separated and washed with brine solution and dried over sodium sulfate, filtered and evaporated under reduced pressure to afford crude which was purified by flash column chromatography. The desired compound elutes at 25% EtOAc : *n*-Hexane as a mixture. The mixture was purified by reverse phase column chromatography (10-60% of 0.1% formic acid in water/ ACN). Fractions containing the desired product were combined and evaporated to afford 4-nitrophenyl (*trans*-(3*SR*,4*SR*)-4-(pyridin-2-yl)disulfanyl)tetrahydropyran-3-yl) carbonate (270 mg, 0.66 mmol). LC-MS *m/z* calculated $C_{17}H_{16}N_2O_6S_2$; 408.4, found 409.1 $[M+H]^+$; ^1H -NMR (400 MHz, $CDCl_3$): δ 8.46 (d, 1H), 8.28 (d, J = 8.8 Hz, 2H), 7.64 - 7.52 (m, 2H), 7.41 (d, J = 8.8 Hz, 2H), 7.09 (s, 1H), 4.87 (d, J = 2.8 Hz, 1H), 4.25 - 4.18 (m, 1H), 3.91 (d, J = 11.6 Hz, 1H), 3.52 - 3.42 (m, 1H), 3.20 (d, J = 2.8 Hz, 1H), 2.21 (d, J = 12.4 Hz, 1H), 1.98 (d, J = 7.6 Hz, 1H), 1.25 (s, 1H).

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Synthesis of 4-nitrophenyl (*trans*-(3*SR*,4*SR*)-4-(pyridin-2-yl)disulfanyl)tetrahydropyran-3-yl) carbonate

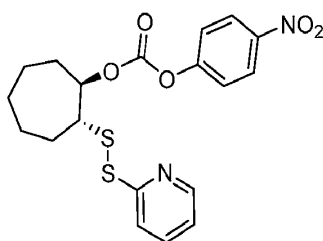


To a stirred solution of (*trans*-(3*SR*,4*SR*)-4-(pyridin-2-yl)disulfanyl)tetrahydropyran-3-ol) (340 mg, 1.40 mmol) in DMF (8 mL) was added bis(4-nitrophenyl) carbonate (850 mg, 2 eq., 2.79 mmol) followed by di-isopropylethylamine (730 μ L, 3 eq., 4.19 mmol) at room temperature for 12 h. Upon completion of the starting material, the reaction mixture was partitioned between water and DCM. The organic layer was separated and washed with brine solution, dried over sodium sulfate, filtered and evaporated under reduced pressure to afford the crude product which was purified by flash column chromatography (0-40% EtOAc : *n*-Hexane). The desired product was eluted out as mixture and then re-purified by reverse phase column chromatography (10-50% of 0.1% formic acid in water/ ACN). Fractions containing the desired product were

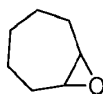
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combined and evaporated to afford 4-nitrophenyl (*trans*-(3SR,4SR)-4-(pyridin-2-yl)disulfanyl)tetrahydropyran-3-yl carbonate (300 mg, 735 μ mol). LC-MS *m/z* calculated $C_{17}H_{16}N_2O_6S_2$; 408.4, found 409.1 $[M+H]^+$; ^1H -NMR (400 MHz, DMSO): δ 8.46 (d, 1H), 8.28 (d, $J = 8.0$ Hz, 2H), 7.66 - 7.58 (m, 2H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.09 (s, 1H), 4.87 (d, $J = 3.6$ Hz, 1H), 4.25 - 4.22 (m, 1H), 3.91 (d, $J = 11.6$ Hz, 1H), 3.52 - 3.42 (m, 2H), 3.20 (d, $J = 3.6$ Hz, 1H), 2.21 (d, $J = 12.0$ Hz, 1H), 1.98 - 1.95 (m, 1H).

Synthesis of 4-nitrophenyl (*trans*-(1RS,2RS)-2-(pyridin-2-yl)disulfanyl) cycloheptyl) carbonate

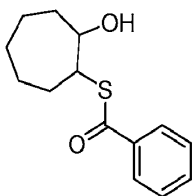


10 *Step 1: Synthesis of 8-oxabicyclo[5.1.0]octane*



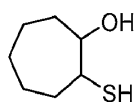
To a stirred solution of cycloheptene (1.0 g, 10.4 mmol) in dichloromethane (10 mL) was added 3-chlorobenzene-1-carboxoperoxoic acid (2.15 g, 1.2 eq., 12.5 mmol) at 0°C. The reaction mixture was stirred at 0 °C for 1 h and then for 16 h at room temperature. Progress of the reaction monitored by TLC. Upon completion of the reaction, the reaction mixture was quenched slowly with aqueous sat. sodium bicarbonate solution and the mixture was stirred vigorously for about 30 min. The two layers were separated, the organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford the desired product as a colourless liquid (700 mg, 6.24 mmol). ^1H -NMR (400 MHz, $CDCl_3$): δ 3.07 (s, 2H), 1.93 - 1.86 (m, 4H), 1.60 - 1.43 (m, 4H), 1.21 - 1.17(m, 2H).

Step 2: Synthesis of Racemic [trans-(2-hydroxycycloheptyl)sulfanyl](phenyl)methanone



To a stirred solution of 8-oxabicyclo[5.1.0]octane (3.00 g, 26.7 mmol) in toluene (60 mL) at room temperature under nitrogen atmosphere was added benzenecarbothioic S-acid (4.72 mL, 1.5 eq., 40.1 mmol), followed by 2-methylpropan-2-aminium chloride (293 mg, 0.1 eq., 2.67 mmol). The reaction mixture was stirred at 50 °C for 16 h (progress of the reaction was monitored by TLC). Upon completion of the reaction, the reaction mixture was quenched with sat. sodium bicarbonate solution and then extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford the crude product, which was purified by flash column chromatography. The desired product was eluted out in 20% EtOAc : *n*-Hexane, pure fractions were collected and evaporated to afford the title compound racemic [*trans*-(2-hydroxycycloheptyl)sulfanyl](phenyl)methanone (3.0 g, 12.0 mmol). LC-MS *m/z* calculated C₁₄H₁₈O₂S; 250.4, ¹H-NMR (400 MHz, CDCl₃) : δ 7.96 (d, *J* = 8.0 Hz, 2H), 7.57 (t, *J* = 6.8 Hz, 1H), 7.4 (t, *J* = 7.6 Hz, 2H), 3.88 - 3.85 (m, 1H), 3.81 - 3.77 (m, 1H), 2.09 - 2.05 (m, 1H), 2.04 - 1.62 (m, 8H), 1.55 - 1.53 (m, 2H).

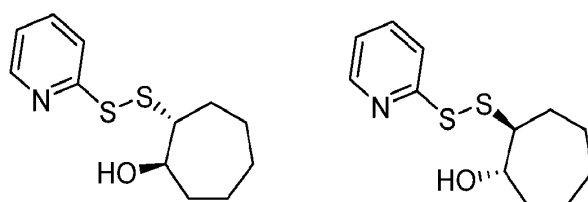
Step 3: Synthesis of Racemic trans-4-sulfanylcycloheptan-3-ol



To a stirred solution of racemic [*trans*-(2-hydroxycycloheptyl)sulfanyl](phenyl)methanone (2.80 g, 11.2 mmol) in dichloromethane (25 mL) at room temperature, under nitrogen atmosphere was added 1,4-disulfanylbutane-2,3-diol (173 mg, 0.1 eq., 1.12 mmol), followed by hydrazine hydrate (1.37 mL, 2.5 eq., 28.0 mmol). The reaction mixture was stirred at room temperature for 3 h (progress of the reaction monitored by TLC). Upon completion of the reaction, the reaction mixture was quenched with 1N HCl and extracted with DCM (2 x 30 mL). The organic layers were combined and dried over sodium

sulfate, filtered, the organic layer was partially evaporated and the crude racemic *trans*-4-sulfanylcycloheptan-3-ol directly taken for the next step.

Step 4: Synthesis of *trans*-(1*RS*, 2*RS*)-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol and *trans*-(1*SR*, 2*SR*)-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol

Isomer 1 *trans* (1*RS*, 2*RS*)Isomer 2 *trans* (1*SR*, 2*SR*)

To a stirred solution of 2-(pyridin-2-ylidisulfanyl)pyridine (1.73 g, 0.7 eq., 7.85 mmol) in methanol (25 mL) under nitrogen atmosphere at 0 °C was added racemic *trans*-4-sulfanylcycloheptan-3-ol (1.64 g, 11.2 mmol) in DCM and the reaction mixture was stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC and LCMS and the reaction mass was evaporated under reduced pressure. The crude product was purified by flash column chromatography and the desired product was eluted out in 20% EtOAc : *n*-Hexane. As the product was collected as a mixture, it was re-purified by reverse phase column chromatography (10-50% of 0.1% formic acid in water : acetonitrile) to afford racemic *trans*-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol (1.5 g, 52%) (Racemic mixture). The isomers were separated by chiral preparative HPLC. (Isomer-1: 550 mg, Isomer-2: 550 mg).

Chiral preparative HPLC conditions:

Column : CHIRALPAK IA (250 mm X 20 mm X 5 mic)

Mobile phase : *n*-Hexane : IPA with 0.1% DEA (90:10)

Flow rate : 19 mL/min

The isomers were separated and the respective fractions were collected from chiral prep. HPLC.

The fractions were evaporated separately, to afford the respective isomers.

Isomer 1 (*trans*-(1RS, 2RS)-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol):

LC-MS *m/z* calculated C₁₂H₁₇NOS₂; 255.4, found 256.2 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃): δ 8.49 (s, 1H), 7.56 (d, *J* = 6.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.13 (s, 1H), 6.17 (s, 1H), 3.51 (m, 1H), 2.75 - 2.73 (m, 1H), 2.08 - 1.95 (m, 2H), 1.82 - 1.67 (m, 4H), 1.57 - 1.25 (m, 4H).

5 **Isomer 2** (*trans*-(1SR, 2SR)-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol):

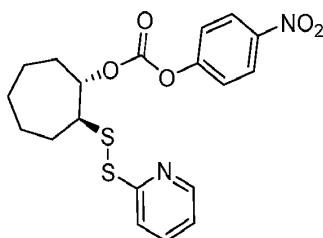
LC-MS *m/z* calculated C₁₂H₁₇NOS₂; 255.4, found 256.2 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃): δ 8.50 (d, *J* = 4.40 Hz, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 8.40 Hz, 1H), 7.13 (t, *J* = 6.4 Hz, 1H), 6.18 (s, 1H), 3.53 - 3.49 (m, 1H), 2.77 - 2.72 (m, 1H), 2.11 - 2.08 (m, 1H), 2.00 - 1.96 (m, 1H), 1.84 - 1.67 (m, 4H), 1.59 - 1.45 (m, 4H).

10 The absolute stereochemistry of the isomers was arbitrarily assigned.

Step 5: Synthesis of 4-nitrophenyl (trans-(1RS,2RS)-2-(pyridin-2-ylidisulfanyl) cycloheptyl) carbonate

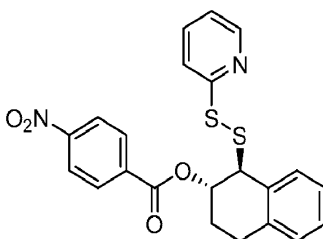
To a stirred solution of *trans*-(1RS, 2RS)-2-(pyridin-2-ylidisulfanyl)cycloheptan-1-ol (500 mg, 1.96 mmol) in DMF (10 mL) under nitrogen atmosphere was added bis(4-nitrophenyl) carbonate (1.49 g, 2.5 eq., 4.89 mmol) followed by diisopropylethylamine (1.02 mL, 3 eq., 5.87 mmol) at room temperature. The reaction mixture was stirred for 12 h. Upon completion of the reaction, the reaction mixture was partitioned between water and DCM. The two layers were separated and the organic layer was washed with brine solution, dried over sodium sulfate, filtered and evaporated under reduced pressure to afford the crude product which was purified by flash column chromatography. The desired product was eluted out in 23% EtOAc : *n*-Hexane as a mixture. The mixture was re-purified by reverse phase column chromatography (10-60% of 0.1% formic acid in water/ ACN) to afford the title product 4-nitrophenyl (*trans*-(1RS,2RS)-2-(pyridin-2-ylidisulfanyl) cycloheptyl) carbonate (450 mg, 1.07 mmol). LC-MS *m/z* calculated C₁₉H₂₀N₂O₅S₂; 420.5, found 421.3 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃): δ 8.45 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.09 (m, 1H), 5.04 - 5.03 (m, 1H), 3.22 (m, 1H), 2.15 - 2.00 (m, 3H), 1.87 - 1.79 (m, 2H), 1.72 - 1.63 (m, 4H), 1.54 - 1.49 (m, 2H).

30 Synthesis of 4-nitrophenyl (*trans*-(1SR,2SR)-2-(pyridin-2-ylidisulfanyl) cycloheptyl) carbonate

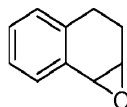


To a stirred solution of *trans*-(1SR,2SR)-2-(pyridin-2-yl)disulfanylcycloheptan-1-ol (580 mg, 2.27 mmol) in DMF (10 mL) under nitrogen atmosphere was added bis(4-nitrophenyl) carbonate (1.73 g, 2.5 eq., 5.68 mmol) followed by di-isopropylethylamine (1.38 mL, 3.5 eq., 7.95 mmol). The reaction mixture was stirred at room temperature for 12 h. Upon completion of the reaction, monitored by TLC, the reaction mixture was partitioned between water and DCM. The two layers were separated and the combined organic layer was washed with brine solution, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography. The desired product was eluted out in 23 - 25% EtOAc : *n*-Hexane as a mixture. The product was re-purified by reverse phase column chromatography (10-60% of 0.1% formic acid in water/ ACN) to afford the title compound 4-nitrophenyl (*trans*-(1SR,2SR)-2-(pyridin-2-yl)disulfanylcycloheptan-1-yl) carbonate (450 mg, 1.07 mmol). LC-MS *m/z* calculated C₁₉H₂₀N₂O₅S₂; 420.5, found 421.3 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃): δ 8.46 (s, 1H), 8.27 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.12 (m, 1H), 5.04 - 5.03 (m, 1H), 3.23 (m, 1H), 2.12 - 2.00 (m, 2H), 1.87 - 1.79 (m, 3H), 1.63 - 1.49 (m, 6H).

Synthesis of 4-nitrophenyl (*trans*-(1RS,2RS)-1-(pyridin-2-yl)disulfanylcycloheptan-1-yl) carbonate

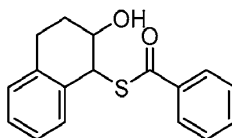


Step 1: Synthesis of 1aH,2H,3H,7bH-naphtho[1,2-b]oxirene



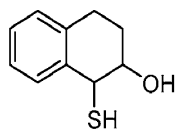
To a stirred solution of 1,2-dihydronaphthalene (2.0 g, 15.4 mmol) in dichloromethane (75 mL) was added a saturated solution of sat. sodium hydrogen carbonate (75 mL). The mixture was cooled to 0°C. To this mixture was added portion-wise 3-chlorobenzene-1-carboperoxoic acid (5.30 g, 2 eq., 30.7 mmol) over a period of 30 min. After the addition, the reaction mass was allowed to stir at room temperature for 16 h. The reaction was monitored by TLC. After, the reaction completion, the two layers were separated and the organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford 1aH,2H,3H,7bH-naphtho[1,2-b]oxirene (2.77 g). The crude obtained was used directly for the next step without any further purification.

Step 2: Synthesis of racemic [trans-(2-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)sulfanyl] (phenyl) methanone



To a stirred solution of 1aH,2H,3H,7bH-naphtho[1,2-b]oxirene (2.25 g, 15.4 mmol) in ethoxyethane (20 mL) was added silanediol (4.50 g, 74.9 mmol) and benzenecarbothioic S-acid drop-wise (9.06 mL, 5 eq., 77.0 mmol). The mixture was allowed to stir at room temperature for 16 h. The progress of the reaction was monitored by TLC and LCMS. After reaction completion, the reaction mixture was quenched with sat. sodium carbonate solution (25 mL) and extracted with ethyl acetate (2 x 20mL). The combined organic layer was washed with water, brine and dried over sodium sulfate and concentrated under reduced pressure to obtain a crude which was purified by column chromatography to afford racemic [trans-(2-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)sulfanyl] (phenyl) methanone as an yellow liquid (1.57 mg, 35.87%)

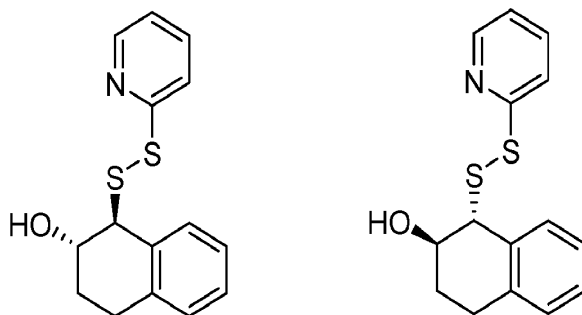
Step 3: Synthesis of Racemic trans-1-sulfanyl-1,2,3,4-tetrahydronaphthalen-2-ol



To a stirred solution of racemic *trans*-(2-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)sulfanyl](phenyl)methanone (1.40 g, 4.92 mmol) in dichloromethane (25.0 mL) was added (2R,3R)-1,4-disulfanylbutane-2,3-diol (144 mg, 0.19 eq., 935 μ mol) and hydrazine hydrate (60.4 μ L, 0.25 eq., 1.23 mmol). The reaction mass was stirred at room temperature for 3 h. The reaction was monitored by TLC. After reaction completion, the reaction mixture was quenched with HCl solution (pH=1~2). The DCM layer was separated and dried over sodium sulfate, filtered and concentrated under reduced pressure to afford racemic *trans*-1-sulfanyl-1,2,3,4-tetrahydronaphthalen-2-ol which taken as such for the next step.

10

Step 4: Synthesis of trans-(1RS,2RS)-1-(pyridin-2-ylidisulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol and trans-(1SR,2SR)-1-(pyridin-2-ylidisulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol



Isomer 1 trans (1RS, 2RS)

Isomer 2 trans (1SR, 2SR)

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To a stirred solution of 2-(pyridin-2-ylidisulfanyl)pyridine (867 mg, 0.8 eq., 3.94 mmol) in methanol (5 mL) at 0°C. To this, was added drop-wise racemic *trans*-1-sulfanyl-1,2,3,4-tetrahydronaphthalen-2-ol in DCM taken from the previous step. The reaction was allowed to stir at RT for 16 h. The reaction was monitored by LCMS and TLC. After reaction completion, the reaction mass was concentrated under reduced pressure to afford a crude which was purified by

20

column chromatography to afford racemic 1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol as a yellow oil which was further purified by reverse phase column chromatography to afford a colorless oil (380 mg, 26.69%). The racemic product obtained was separated by chiral chromatography to afford Isomer-1: 130 mg; Isomer-2: 190 mg.

5 Prep. conditions:

Column : CHIRALPAK IA (250 mm X 420 mm X 5 mic)

Mobile phase : *n*-Hexane : Ethanol with 0.1% DEA (50:50)

Flow rate : 19 mL/min

Isomer-1(*trans*-(1*RS*,2*RS*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol):

10 LC-MS *m/z* calculated C₁₅H₁₅NOS₂; 289.4, found 290.1 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃):
δ 8.07(d, *J* = 7.2 Hz, 2H), 7.61 - 7.57(m, 1H), 7.48 - 7.44 (m, 2H), 7.36 - 7.34 (m, 1H), 7.18-7.13
(m, 3H), 4.98 (d, *J*=4.4 Hz, 1H), 4.24 (m, 1H), 3.07-2.99 (m, 1H), 2.91-2.80 (m, 1H).

Isomer-2(*trans*-(1*SR*,2*SR*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol)

LC-MS *m/z* calculated C₁₅H₁₅NOS₂; 289.4, found 290.1 [M+H]⁺; ¹H-NMR (400 MHz, CDCl₃):
15 δ 8.55 (d, *J*=4.0 Hz, 1H), 7.77 (d, *J*=8.0 Hz, 1H), 7.58 (t, *J*=7.6 Hz, 1H), 7.37(d, *J*=8.4 Hz, 1H),
7.26-7.17 (m, 3H), 7.08 (d, *J*=7.2 Hz, 1H), 4.15 (d, *J*=8.0 Hz, 1H), 3.97-3.93 (m, 1H), 2.89 (d,
J=4.8 Hz, 2H), 2.32-2.28 (m, 1H), 1.97-1.87 (m, 2H).

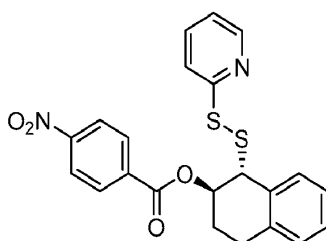
The absolute stereochemistry of the isomers was arbitrarily assigned.

20 *Step 5. Synthesis of 4-nitrophenyl (trans*-(1*RS*,2*RS*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-yl) carbonate

To a stirred solution of *trans*-(1*RS*,2*RS*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol (170 mg, 587 μmol) in N,N-dimethylformamide (2.50 mL) was added bis(4-nitrophenyl) carbonate (447 mg, 2.5 eq., 1.47 mmol) followed by diisopropylethylamine
25 (307 μL, 3 eq., 1.76 mmol) drop-wise at RT. The reaction mixture was stirred at RT for 12 h in a sealed tube. The reaction was monitored by TLC and LCMS. After reaction completion, the reaction mass was partitioned between water (5 mL) and DCM (5 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to afford crude, which was purified by flash column chromatography (0-40% EA in hexane) and also re-purified by reverse
30 phase column chromatography (10-70% of 0.1% formic acid in water/ ACN) to give 4-

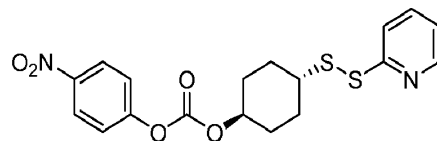
nitrophenyl (*trans*-(1*SR*,2*RS*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-yl) carbonate (70.0 mg, 154 μ mol) as a colorless gummy solid (70 mg, 26.22%). LC-MS *m/z* calculated $C_{22}H_{18}N_2O_4S_2$; 454.5 found 455.3 $[M+H]^+$; ^1H -NMR (400 MHz, $CDCl_3$): δ 8.73(d, $J=20.4$ Hz, 1H), 8.22(d, $J=8.4$ Hz, 2H), 7.67(s, 2H), 7.50(m, 1H), 7.32(d, $J=8.4$ Hz, 2H), 7.25-7.16(m, 4H), 5.51(s, 1H), 4.52(s, 1H), 3.01-2.85(m, 2H), 2.63(m, 1H), 2.26-2.22(m, 1H).

Synthesis of 4-nitrophenyl (*trans*-(1*SR*,2*SR*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-yl) carbonate

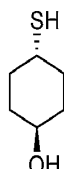


To a stirred solution of *trans*-(1*SR*,2*SR*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol (120 mg, 415 μ mol) in *N,N*-dimethylformamide (1.50 mL) was added bis(4-nitrophenyl) carbonate (315 mg, 2.5 eq., 1.04 mmol) followed by diisopropylethylamine (217 μ L, 3 eq., 1.24 mmol) drop-wise at RT. The reaction mixture was stirred at RT for 12 h in a sealed tube. The progress of the reaction was monitored by TLC and LCMS. After reaction completion, the reaction mass was partitioned between water (5 mL) and DCM (5 mL), the organic layer was dried over sodium sulfate and evaporated under reduced pressure to afford crude which was purified by flash column chromatography (0-40% EA in hexane) and also re-purified by reverse phase column chromatography (10-70% of 0.1% formic acid in water/ ACN) to give 4-nitrophenyl (*trans*-(1*SR*,2*SR*)-1-(pyridin-2-yl)disulfanyl)-1,2,3,4-tetrahydronaphthalen-2-yl) carbonate (65.0 mg, 143 μ mol) as a colorless gummy solid (65mg, 34.49%). LC-MS *m/z* calculated $C_{22}H_{18}N_2O_4S_2$; 454.5 found 455.3 $[M+H]^+$; ^1H -NMR (400 MHz, $CDCl_3$): δ 8.55(m, 1H), 8.22(d, $J=7.6$ Hz, 2H), 7.69(s, 2H), 7.51(m, 1H), 7.32(d, $J=7.6$ Hz, 2H), 7.25-7.16(m, 4H), 5.51(s, 1H), 4.52(s, 1H), 3.01-2.86(m, 2H), 2.62(m, 1H), 2.26(m, 1H).

Synthesis of 4-nitrophenyl (*trans*-4-(pyridin-2-yl)disulfanyll)cyclohexyl) carbonate

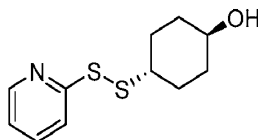


Step 1: Synthesis of trans-4-mercaptocyclohexan-1-ol



To a stirred solution of 7-oxabicyclo[2.2.1]heptane (1.00 g, 10.2 mmol) in ethanol (10 mL) was added 4-methylbenzene-1-sulfonic acid (2.63 g, 1.5 eq., 15.3 mmol), thiourea (1.16 g, 1.5 eq., 15.3 mmol) and the reaction mass was heated to 80 °C for 24 h. Then, the reaction mass was cooled to room temperature and 50% aqueous sodium hydroxide solution (1.30 g, 3.2 eq., 32.6 mmol) was added to the reaction mass and heated at 100 °C for 2 h. After completion of the reaction, the reaction mass was cooled to room temperature, concentrated under reduced pressure and acidify with 10% H₂SO₄ solution. Then, the reaction mass was extracted with DCM and taken-up for the next step as such.

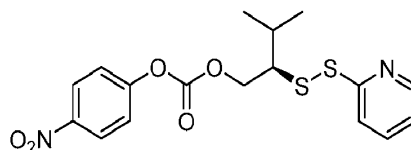
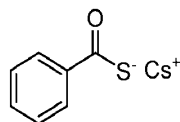
Step 2: Synthesis of trans-4-(pyridin-2-ylthio)cyclohexan-1-ol



A stirred solution of 2-(pyridin-2-ylthio)pyridine (1.60 g, 0.8 eq., 7.26 mmol) in methanol (10.0 mL) at 0 °C was added the organic layer from (step 1) 4-sulfanylcyclohexan-1-ol (1.20 g, 9.08 mmol). Upon completing the addition, the reaction mass allowed to stir at room temperature for 16 h. After completion of the reaction, the reaction mass was concentrated and the crude product was purified by column chromatography (using 0-40% EtOAc : *n*-Hexane) to give the desired product. The product was re-purified by reverse phase column chromatography using 0.1% Formic acid and ACN. Fractions containing the desired product were collected and concentrated under reduced pressure the afford the title product as a yellow oil (1.60 g, 73 % yield). LC-MS *m/z* calculated for C₁₁H₁₅NOS₂, 241; found 242 [M+H]⁺.

Step 3: Synthesis of 4-nitrophenyl (trans-4-(pyridin-2-yl)disulfanyl)cyclohexyl carbonate

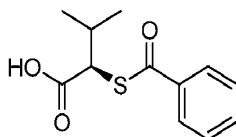
To a stirred solution of trans-4-(pyridin-2-yl)disulfanyl)cyclohexan-1-ol (400 mg, 1.66 mmol) in N,N-dimethylformamide (3 mL) under Nitrogen atmosphere was added bis(4-nitrophenyl) carbonate (907 mg, 1.8 eq., 2.98 mmol), ethylbis(propan-2-yl)amine (892 μ L, 3 eq., 4.97 mmol) and stirred for at room temperature for 16 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was quenched with water (15 mL) and extracted with DCM (3 x 10 mL). The two layers were separated and the combined organic layer was washed with water followed by brine solution, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product obtained was purified by flash column chromatography (0-30% EtOAc : *n*-Hexane). The product was re-purified by reverse phase column chromatography using 0.1% formic acid and ACN. Fractions containing the desired product were collected and concentrated under reduced pressure the afford 4-nitrophenyl (*trans*-4-(pyridin-2-yl)disulfanyl)cyclohexyl carbonate as a yellow oil (0.3 g, 73 % yield). LC-MS *m/z* calculated for C₁₈H₁₈N₂O₅S₂, 407; found 407 [M+H]⁺; ¹HNMR (400 MHz, CDCl₃): δ 8.49 - 8.42 (m, 1H), 8.26 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.65 - 7.60 (m, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.12 - 7.05 (m, 1H), 4.75 - 4.65 (m, 1H), 2.98 - 2.87 (m, 1H), 2.28 - 2.18 (m, 4H), 1.68 - 1.50 (m, 4H).

20 Synthesis of (2R)-3-methyl-2-(pyridin-2-yl)disulfanyl butyl 4-nitrophenyl carbonate*Step 1. Synthesis of cesium benzoysulfanide*

To a stirred solution of benzenecarbothioic S-acid (5.00 g, 36.2 mmol) in methanol (40.0 mL) was added cesium carbonate (7.72 g, 1.1 eq., 39.8 mmol) in portions over 10-15 min, under nitrogen atmosphere. The reaction mixture was stirred at RT for 2 h. After completion of the

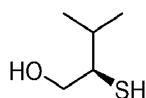
reaction (as judged by TLC), the reaction mixture was concentrated under reduced pressure. The solid residue was diluted with 10 mL of acetone and the white solid (CsHCO_3) was filtered off. This process was repeated two times to ensure all CsHCO_3 was removed. Acetone was then concentrated to afford cesium benzoylsulfanide (9.50 g, 35.2 mmol) as a colorless solid. ^1H NMR (400 MHz, CD_3OD): δ 8.08 (d, J = 6.8 Hz, 2H), 7.37 - 7.27 (m, 3H).

Step 2. Synthesis of (2R)-2-(benzoylsulfanyl)-3-methylbutanoic acid



To a stirred solution of (2S)-2-bromo-3-methylbutanoic acid (2.00 g, 11.0 mmol) in N,N-dimethylformamide (14.0 mL) was added cesium benzoylsulfanide (2.98 g, 11.0 mmol). The reaction mixture was stirred at RT for 20 h. Progress of the reaction was monitored by TLC, after completion of the reaction, the reaction mixture was diluted with di ethyl ether (3 x 15 mL) and washed with water (3 x 15 mL). The ethereal layer was separated, dried over sodium sulfate, and concentrated under reduced pressure. The residue obtained was recrystallized from *n*-hexanes to afford (2R)-2-(benzoylsulfanyl)-3-methylbutanoic acid (2.50 g, 10.5 mmol) as an oily compound. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 12.93 (s, 1H), 7.92 (d, J = 7.2 Hz, 2H), 7.69 (t, J = 7.6 Hz, 1H), 7.55 (t, J = 7.2 Hz, 2H), 4.14 (d, J = 6.8 Hz, 1H), 2.30 - 2.22 (m, 1H), 1.01 - 0.89 (m, 6H).

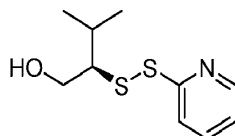
Step 3. Synthesis of (2R)-3-methyl-2-sulfanylbutan-1-ol



To a stirred solution of (2R)-2-(benzoylsulfanyl)-3-methylbutanoic acid (2.50 g, 10.5 mmol) in ethoxyethane (50.0 mL) at 0 °C was added lithiumaluminiumhydride (52.5 mL, 5 eq., 52.5 mmol) in drop wise manner under nitrogen atmosphere. After completion off the addition, the ice-bath was removed and the reaction mixture was stirred at room temperature for 2 h. Progress of the reaction was monitored by TLC. After the completion of the starting material, the reaction mixture was cooled in an ice-bath and quenched with 1.0 N HCl (30 mL) at 0°C. The

reaction mixture was extracted with DCM (20 mL) and the remaining gel-like material from the LAH reduction was washed with diethyl ether (10 mL). The combined organic layer was dried over sodium sulfate, filtered and carried out further to the next step.

5 *Step 4. Synthesis of (2R)-3-methyl-2-(pyridin-2-yldisulfanyl) butan-1-ol*



To a stirred solution of (2R)-3-methyl-2-sulfanylbutan-1-ol (1.20 g, 9.98 mmol) in MeOH (5 mL) was added 2-(pyridin-2-yldisulfanyl)pyridine (1.76 g, 0.8 eq., 7.99 mmol) under nitrogen atmosphere and stirred at room temperature for 16 h. Progress of the reaction was
10 monitored by TLC and LC-MS. After completion of reaction, the reaction mass was concentrated, and then extracted with DCM. The two layers were separated and the combined organic layer was washed with water followed by brine and dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel flash column chromatography (using 12 g column), which was eluted out in 50% EtOAc : *n*-Hexanes and also re-purified by
15 reverse phase column chromatography (10-20% of 0.1% formic acid in water/ Acetonitrile). Fractions containing the product were collected and evaporated off under vacuum to obtain the title product. The product was re-purified by Prep. HPLC.

Prep. HPLC Conditions:

Column : X-BridgeC-18 (250mm X 4.6mm X 5mic)
20 Mobile phase(A) : 0.1% Ammonia in water
Mobile phase(B) : Acetonitrile
Flow rate : 19 mL/min
Gradient B: 0/10,12/60,22/95,25/95,27/10,30/10

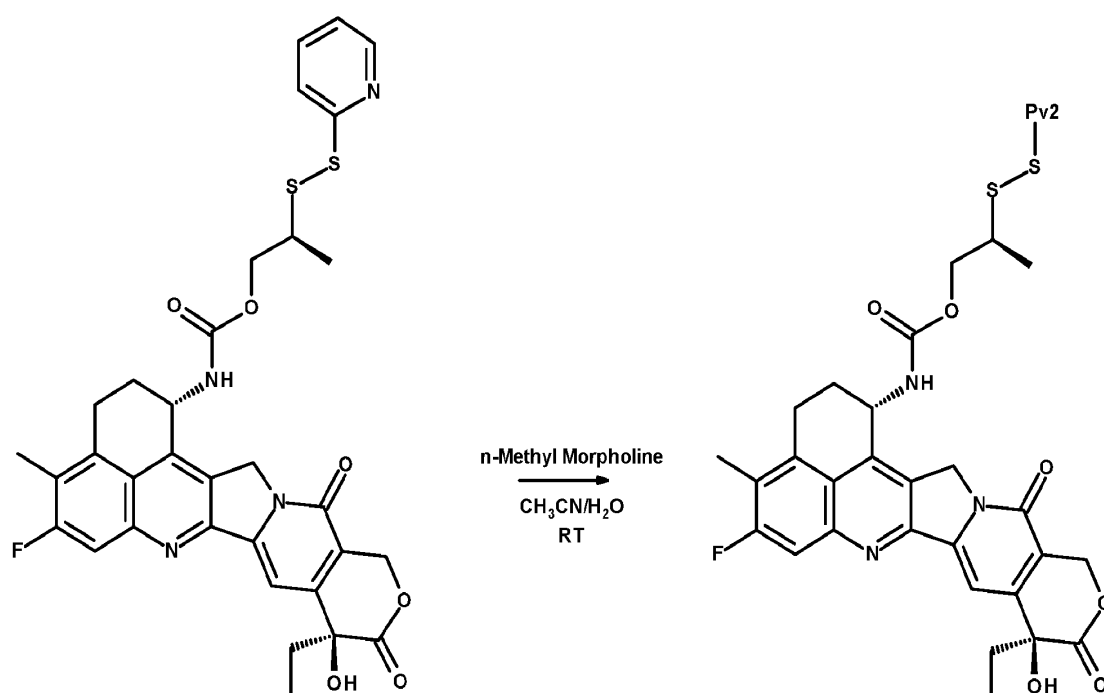
25 Fractions collected from Prep. HPLC were combined and evaporated to afford the title product 3-(pyridin-2-yldisulfanyl)-1,2,3,4-tetrahydronaphthalen-2-ol (350 mg, 1.21 mmol) as a yellow solid. ¹HNMR (400 MHz, CDCl₃): δ 8.49 (d, *J* = 4 Hz, 1H), 7.55 - 7.54 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 6.4 Hz, 1H), 3.82 (dd, *J* = 12.4 Hz, 1H), 3.66 - 3.60 (m, 1H), 2.75 - 2.70 (m, 1H), 2.01 - 1.92 (m, 1H), 1.10 - 1.01 (m, 7H).

Step5. Synthesis of (2R)-3-methyl-2-(pyridin-2-yl-disulfanyl) butyl 4-nitrophenyl carbonate

To a stirred solution of (2R)-3-methyl-2-(pyridin-2-yl-disulfanyl)butan-1-ol (800 mg, 3.49 mmol) in N,N-dimethylformamide (2.50 mL) was added bis(4-nitrophenyl) carbonate (2.12 g, 2 eq., 6.98 mmol) followed by diisopropylethylamine (1.82 mL, 3 eq., 10.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. After the reaction was completed, the reaction mass was partitioned between water and DCM. The two layers were separated and the organic layer was dried over sodium sulfate, filtered and evaporated under reduced pressure to afford the crude product, which was purified by flash column chromatography (0-40% EtOAc : *n*-Hexanes). The product was re-purified by reverse phase chromatography (10-70% of 0.1% formic acid in water/ ACN) to obtain the title product (2R)-3-methyl-2-(pyridin-2-yl-disulfanyl)butyl 4-nitrophenyl carbonate (600 mg, 1.52 mmol) as a colorless gum. ¹HNMR (400 MHz, CDCl₃): δ 8.45 (d, *J* = 4.0 Hz, 1H), 8.26 (d, *J* = 9.2 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.35 (d, *J* = 9.2 Hz, 2H), 7.08 (t, *J* = 6.8 Hz, 1H), 4.59 - 4.48 (m, 2H), 3.08 (q, *J* = 6.0 Hz, 1H), 2.21 - 2.13 (m, 1H), 1.14 - 1.06 (m, 6H).

From step 2, same procedure was followed to synthesize (2S)-3-methyl-2-(pyridin-2-yl-disulfanyl) butyl 4-nitrophenyl carbonate using (2R)-2-bromo-3-methylbutanoic acid.

Synthesis of the compound of Example 2 from Intermediate III-2

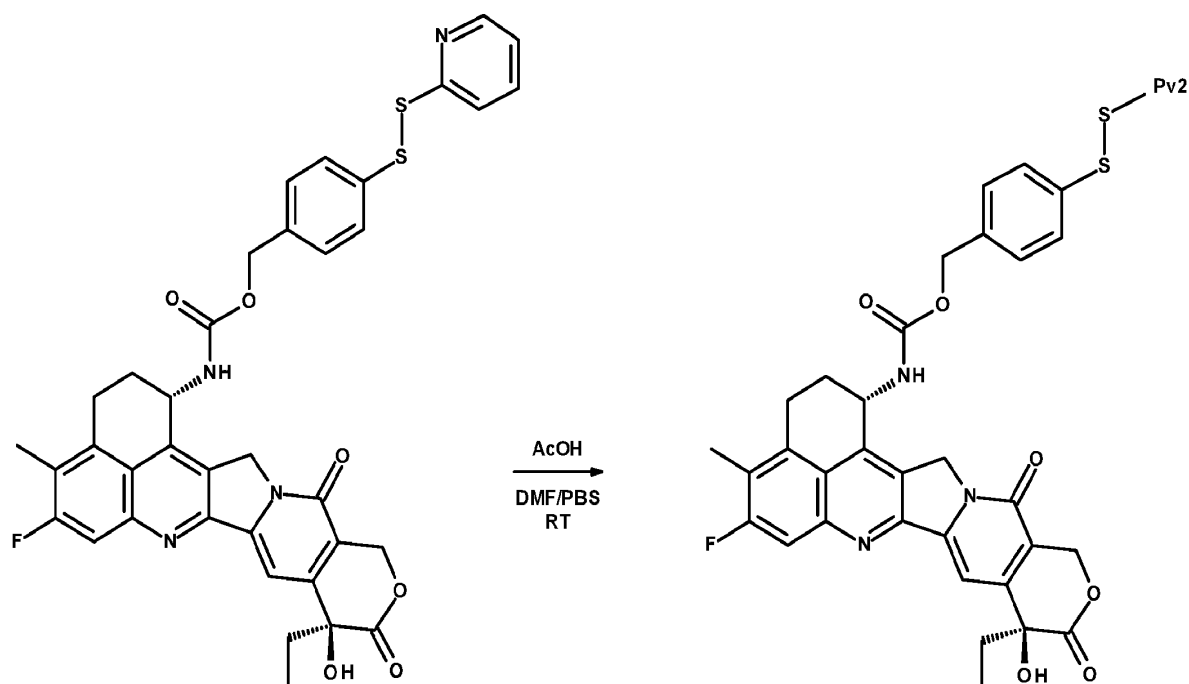


In a vial with Pv2 (25.0 mg, 0.061 mmol; as a free flowing solid), [(2S)-2-(2-pyridyl)disulfanyl]propyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (6.03 mg, 0.091 mmol), was added 1 mL of CH₃CN and 0.5 mL of water. To this was added N-Methyl morpholine (22.7 mg, 0.224 mmol). The mixture was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-85% CH₃CN/H₂O+0.05% TFA, 15 min) to give the desired product (13.0 mg, yield: 47.0 %).

10

The compounds of Examples 1 and 3-9 (see Table 4 below) were synthesized analogously as the compound of Example 2, from Intermediates III-1 and III-3 to III-9, respectively.

15 Synthesis of the compound of Example 10 from Intermediate XVI-1



DMF and PBS were degassed using a N₂ stream for 30 min. In a separate vial was placed Pv2 (25.0 mg, 0.061 mmol; as a free flowing solid), [4-(2-pyridyldisulfanyl)phenyl]methyl N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-

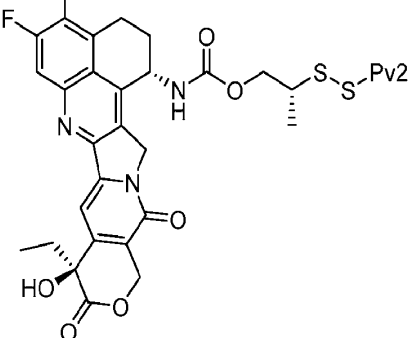
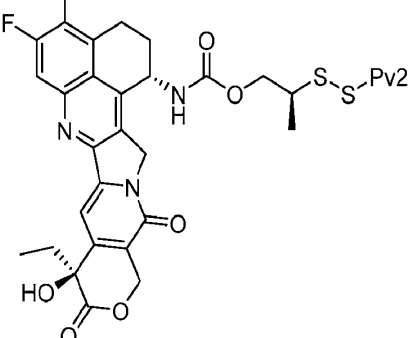
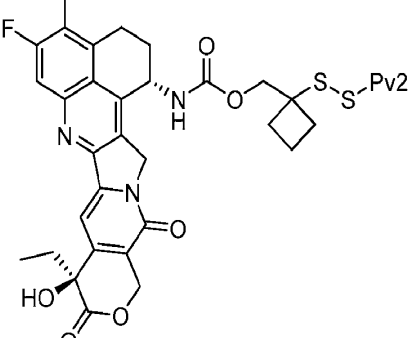
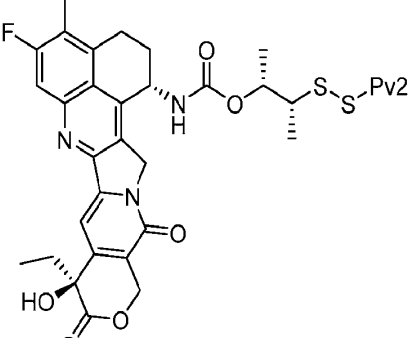
5 1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (6.5 mg, 0.09 mmol), 1.5 mL of DMF and 0.5 mL of PBS. To this was added CH₃CO₂H (0.0347 mL, 0.606 mmol). The mixture was stirred at RT overnight. LC-MS indicated a complete reaction. The reaction mixture was purified by reverse phase HPLC (PrepSlope_4min, 30-100% CH₃CN/H₂O+0.05%TFA,18 min) to give the

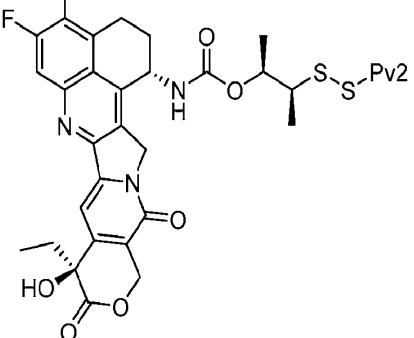
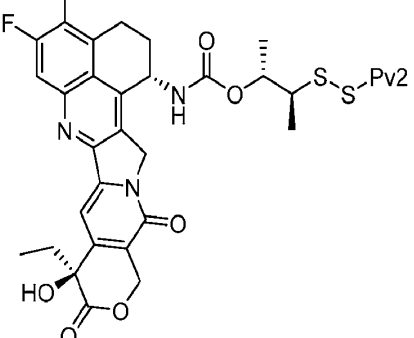
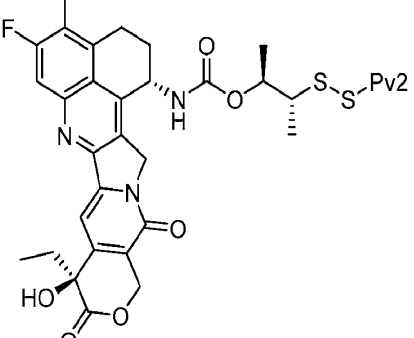
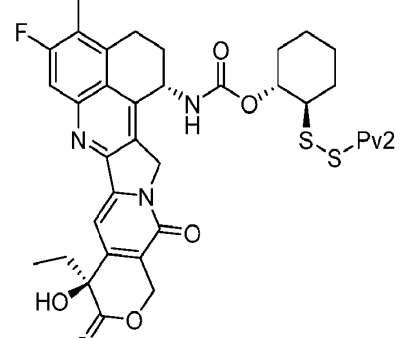
10 desired product (3.0 mg, yield: 10.7 %).

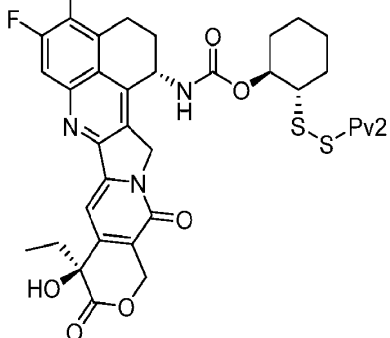
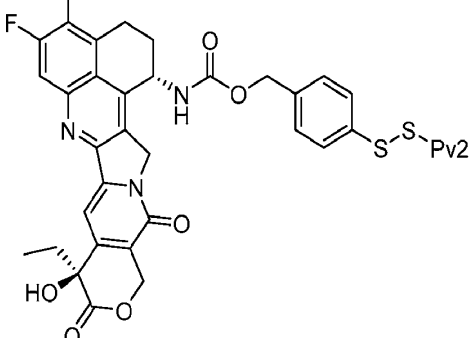
Compounds of the invention and analytical data are presented below.

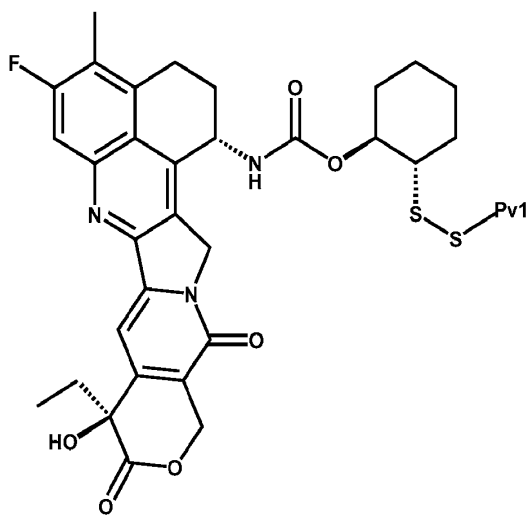
Table 4. Example Compounds

Example	Structure	MS A: Maldi-TOF (M+) B: ESI (m/z=3)	Column %ACN/H ₂ O Run Time Retention Time

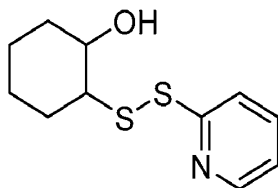
1		B: 1521.3	A 2-95% 11 min 7.4 min
2		B: 1521.7	A 2-95% 11 min 7.4 min
3		B: 1530.6	A 2-95% 11 min 7.5 min
4		B: 1526.5	A 2-95% 11 min 7.5 min

5		B: 1526.1	A 2-95% 11 min 7.5 min
6		B: 1525.8	A 2-95% 11 min 7.4 min
7		B: 1526.4	A 2-95% 11 min 7.5 min
8		B: 1534.8	A 2-95% 11 min 7.6 min

9		B: 1534.6	A 2-95% 11 min 7.7 min
10		B: 1537.6	A 2-95% 11 min 7.5 min

Example 11: Synthesis of Compound 11

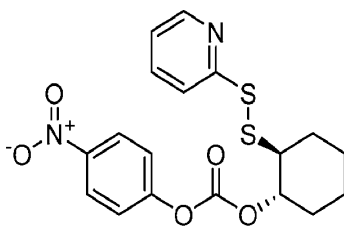
Step 1. Synthesis of 2-(pyridine-2-yl)disulfanyl)cyclohexan-1-ol



To a solution of 1,2-di(pyridine-2-yl)disulfane (15.2 g, 68.9 mmol) in MeOH (degassed with N₂) (30 mL) was added (1-mercaptocyclobutyl)methanol (11.4 g, 86.2 mmol) (degassed with N₂) dropwise and stirred for 16 h at room temperature under an N₂ atmosphere. The reaction mixture was concentrated to dryness under vacuum. The resultant crude material was purified by column chromatography using 30% EtOAc/hexanes to afford the title compound as a yellow liquid. ¹HNMR (400 MHz, CDCl₃): δ 8.54-8.53 (m, 1H), 7.60-7.56 (m, 1H), 7.40-7.38 (m, 1H), 7.17-7.14 (m, 1H), 3.38-3.34 (m, 1H), 2.62-2.57 (m, 1H), 2.11-2.02 (m, 1H), 1.75-1.74 (m, 2H), 1.61-1.60 (m, 1H), 1.42-1.24 (m, 4H).

The title compound was subjected to chiral preparative HPLC conditions (Chiralpak IG : 250 mm x 20 mm x 5 mic; *n*-Hexane : IPA with 0.1% Diethylamine (80:20); 19 mL/min; 25 °C (Room Temperature)). (1R,2R)-2-(pyridin-2-yl)disulfanylcyclohexan-1-ol (4.5 g, 18.6 mmol) eluted first (retention time: 3.9 minutes), followed by (1S,2S)-2-(pyridin-2-yl)disulfanylcyclohexan-1-ol (retention time: 11.3 minutes). The absolute stereochemistry was confirmed by comparison of the product of Step 2 with chiral material having a reported absolute stereochemistry (see Monaco, M. R.; J. Am. Chem. Soc. 2014, 136, 49, 16982-16985).

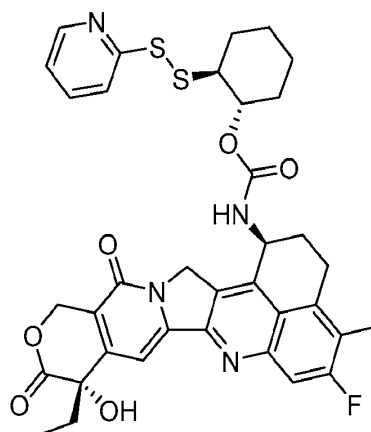
Step 2. Synthesis of 4-nitrophenyl ((1S,2S)-2-(pyridin-2-yl)disulfanylcyclohexyl) carbonate.



To a solution of (1R,2R)-2-(pyridin-2-yl)disulfanylcyclohexan-1-ol (4.5 g, 18.6 mmol) in DMF (90.0 mL) was added DIPEA (10.3 mL, 56.0 mmol) and bis(4-nitrophenyl) carbonate (11.35 g, 27.3 mmol) at room temperature. The reaction vessel was sealed and stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC (20% EtOAc/hexanes).

After completion of the reaction, the reaction mixture was quenched with water (20.0 mL) and extracted with EtOAc (20.0 mL). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the crude product, which was purified by column chromatography using 20-30% EtOAc/hexanes to afford the title product as an off-white solid (5.0 g, 66% yield). ¹HNMR (400 MHz, CDCl₃): δ 8.44 (d, *J* = 4 Hz, 1H), 8.28 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.61-7.57 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 9.6 Hz, 2H), 7.08-7.05 (t, *J* = 5.2 Hz, 1H), 4.85-4.74 (m, 1H), 3.03-2.92 (m, 1H), 2.28 (d, *J* = 9.6 Hz, 1H), 2.20-2.12 (m, 1H), 1.85-1.62 (m, 3H), 1.45-1.25 (m, 3H). LC-MS *m/z* calculated: 406.7; found: 407.4 [M+H]⁺.

Step 3. Synthesis of [(1S,2S)-2-(2-pyridyldisulfanyl)cyclohexyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate.



To (10S,23S)-23-amino-10-ethyl-18-fluoro-10-hydroxy-19-methyl-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-5,9-dione methanesulfonic acid (250 mg, 0.470 mmol) in 10 mL of dry DMF was added (1R,2R)-2-(pyridin-2-yl)disulfanyl)cyclohexan-1-ol (from Step 2; 191 mg, 0.470 mmol), N,N-diisopropylethylamine (122 mg, 0.941 mmol) and DMAP (115 mg, 0.941 mmol). The mixture was stirred at room temperature overnight. LC-MS indicated that the desired coupling product had formed. The reaction mixture was then diluted with EtOAc, washed with saturated aqueous

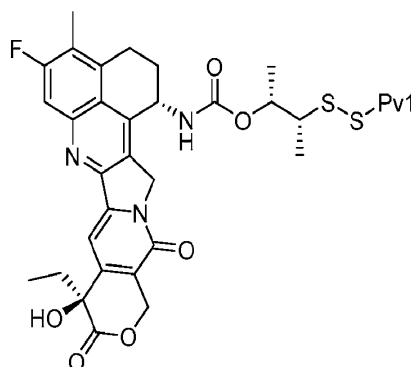
NH₄Cl, H₂O, and brine. The mixture was dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by column chromatography using 0-5% MeOH/dichloromethane to give 240 mg of the desired product in 72.6% yield (240 mg).

5 *Step 4. Coupling with Pv1 (Compound 11)*

In a vial was added Pv1 (275 mg, .0811 mmol), the compound of Step 3 (74.1 mg, 0.105 mmol), acetonitrile (10 mL) and water (5 mL). n-Methylmorpholine (0.303 g, .0030 mol) was added to this mixture. The mixture was stirred at room temperature overnight. LC-MS indicated that the desired coupled product had been formed.

10 The reaction mixture was purified directly by reverse phase HPLC (20-85% acetonitrile/water, 0.5% acetic acid on a Sunfire Prep C18 column (10 μm, 50x150 mm), retention time: 7.022 min) to give 213 mg of the desired product in 68% yield (213 mg). ESI (M+3H/3)³⁺: 1291.6

15 **Example 12: Synthesis of Compound 12**



20 *Step 1. Synthesis of [(1R,2R)-1-methyl-2-(2-pyridyldisulfanyl)propyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate*

To a mixture of 1-hydroxybenzotriazole hydrate (8.64 mg, 0.0564 mmol), finely ground molecular sieve 4 Å (50 mg), and (10S,23S)-23-amino-10-ethyl-18-fluoro-10-hydroxy-19-methyl-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaene-5,9-dione;methanesulfonic acid (25.0 mg, 0.0470 mmol)

and pyridine (0.0190 mL, 0.235 mmol) in 2 mL of anhydrous DMF was added [(1R,2R)-1-methyl-2-(2-pyridyldisulfanyl)propyl] (4-nitrophenyl) carbonate (19.7 mg, 0.470 mmol) (see Synthesis of II-4: 4-nitrophenyl((2R,3R)-3-(pyridin-2-yl)disulfanyl)butan-2-yl) carbonate). After stirring for 16 h at room temperature the mixture was filtered, and the solution was concentrated.

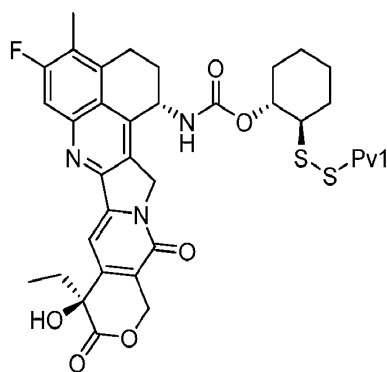
5 The residue was then purified by column chromatography (0-5% MeOH/DCM) to give the title compound (35.0 mg, 0.0517 mmol, yield: 110 %).

Step 2. Coupling with Peptide Pv1 (Compound 12)

In a vial was placed peptide Pv1 (50.0 mg, 14.7e-5 mol), [(1R,2R)-1-methyl-2-(2-pyridyldisulfanyl)propyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.013 g, 1.92e-5 mol), 2 mL of ACN and 1 mL of water. To this was added N-methylmorpholine (0.060 mL, 0.000545 mol). The mixture was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse

15 phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-85% ACN/H₂O+0.05% TFA, 13 min; retention time: 6.95 min) to give Compound 12 (0.0350 g, 9.10e-6 mol, yield: 61.8 %). ESI (M+3H/3)³⁺: 1281.9

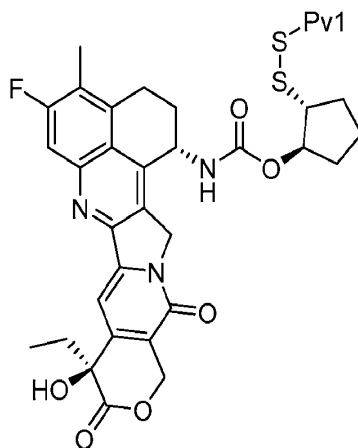
Example 13: Synthesis of Compound 13



Compound 13 was made in an analogous fashion to Compound 11, replacing ((1S,2S)-2-(pyridin-2-yl)disulfanyl)cyclohexyl) carbonate with ((1R,2R)-2-(pyridin-2-yl)disulfanyl)cyclohexyl) carbonate in Step 2. Sunfire Prep C18 column (10 μm, 50x150 mm)

(20-85% acetonitrile/water, 0.5% acetic acid); retention time: 6.609 minutes. ESI (M+3H/3)³⁺: 1290.3

Example 14: Synthesis of Compound 14



5

Step 1. Synthesis of (4-Nitrophenyl) [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cyclopentyl] carbonate

The title compound was synthesized according to analogous synthetic methods described in the synthesis of Compound 11, using the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-2-(2-pyridyldisulfanyl)cyclopentyl assigned as *trans*-(1RS,2RS)-2-(2-pyridyldisulfanyl)cyclopentan-1-ol.

Step 2. Synthesis of [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cyclopentyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl] carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol), DMAP (23.0 mg, 0.188 mmol), and (4-nitrophenyl) [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cyclopentyl] carbonate (40.6 mg, 0.103 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated

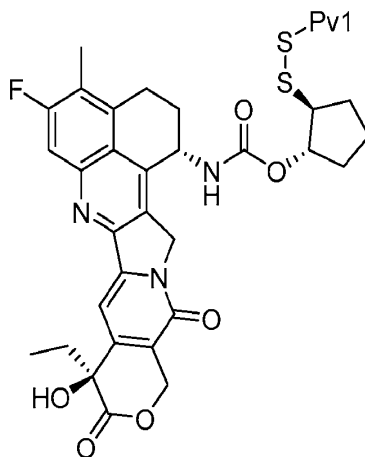
NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-5% MeOH/DCM) to give the title compound (33.0 mg, 0.0479 mmol, yield: 50.9 %).

5 *Step 3. Coupling with Peptide Pv1 (Compound 14)*

In a vial was placed peptide Pv1 (50.0 mg, 1.47e-5 mol), [*trans*-(1*RS*,2*RS*)-2-(2-pyridyldisulfanyl)cyclopentyl] N-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0₂,14.0₄,13.0₆,11.0₂₀,2₄]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.0124 g, 1.80e-5 mol), 2 mL of ACN and
 10 1 mL of water. To this was added N-methylmorpholine (0.060 mL, 0.000545 mol). The mixture was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-90% ACN/H₂O+0.05% TFA, 16 min; retention time: 6.761 min) to give Compound 14 (0.0360 g, 9.34e-6 mol, yield: 63.3 %). ESI (M+3H/3)³⁺: 1286.3.

15

Example 15: Synthesis of Compound 15



*Step 1. Synthesis of (4-nitrophenyl) [trans-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cyclopentyl] carbonate*

20 The title compound was synthesized from the second stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-2-(2-pyridyldisulfanyl)cyclopentyl, assigned

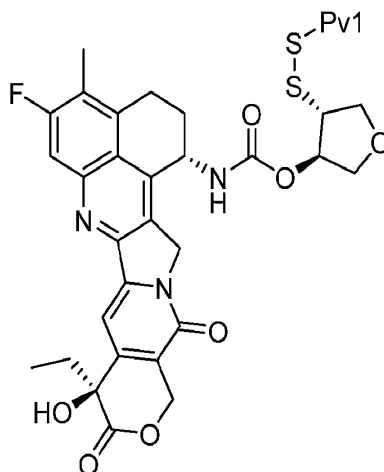
as *trans*-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cyclopentan-1-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [trans-(1SR,2SR)-2-(2-pyridyldisulfanyl)cyclopentyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol), DMAP (23.0 mg, 0.188 mmol), and (4-nitrophenyl) [trans-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cyclopentyl] carbonate (38.2 mg, 0.0974 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-5% MeOH/DCM) to give the title compound (29.0 mg, 0.0421 mmol, yield: 44.8 %).

Step 3. Coupling with Peptide Pv1 (Compound 15)

In a vial was placed peptide Pv1 (50.0 mg, 1.47e-5 mol), *trans*-[(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cyclopentyl] N-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.0124 g, 1.80e-5 mol), 2 mL of ACN and 1 mL of water. To this was added N-methylmorpholine (0.060 mL, 0.000545 mol). The mixture was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-90% ACN/H₂O+0.05% TFA, 16 min; retention time: 6.883 min) to give Compound 15 (0.0280 g, 7.26e-6 mol, yield: 49.3 %). ESI (M+3H/3)³⁺: 1285.9.

Example 16: Synthesis of Compound 16

Step 1. Synthesis of (4-nitrophenyl) [*trans*-(3*RS*,4*RS*)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] carbonate

The title compound was synthesized from the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-ol, assigned as *trans*-(3*RS*,4*RS*)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [*trans*-(3*RS*,4*RS*)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] *N*-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0²,14.0⁴,13.0⁶,11.0²⁰,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl] carbamate

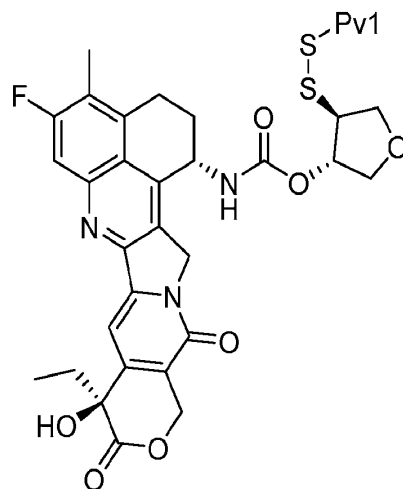
To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol), DMAP (23.0 mg, 0.188 mmol), and (4-nitrophenyl) [*trans*-(3*RS*,4*RS*)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] carbonate (38.2 mg, 0.0969 mmol) in 2 mL of anhydrous DMF was added *N,N*-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH_4Cl , 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the

residue was purified by column chromatography (0-5% MeOH/DCM) to give the title compound (40.0 mg, 0.0579 mmol, yield: 61.6 %).

Step 3. Coupling with Peptide Pv1 (Compound 16)

- 5 In a vial was placed peptide Pv1 (50.0 mg, 1.47e-5 mol), [*trans*-(3RS,4RS)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-
- 1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.0124 g, 1.80e-5 mol), 2 mL of ACN and 1 mL of water. To this was added N-Methylmorpholine (0.060 mL, 0.000545 mol). The mixture
- 10 was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-80% ACN/H₂O+0.05% TFA, 15 min; retention time: 6.633 min) to give Compound 16 (0.0290 g, 7.52e-6 mol, yield: 51.0 %). ESI (M+3H/3)³⁺: 1286.4.

15 **Example 17: Synthesis of Compound 17**



Step 1. Synthesis of (4-nitrophenyl) [trans-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] carbonate

- The title compound was synthesized from the second stereoisomer to be eluted from the
- 20 chiral chromatography separation of racemic *trans*-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-ol,

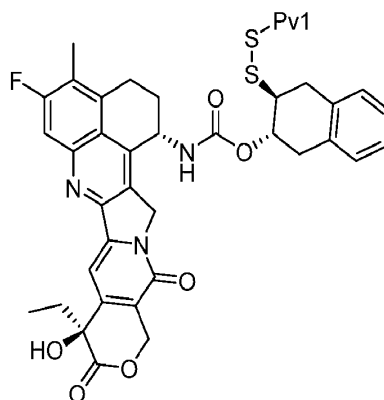
assigned as *trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [trans-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol), DMAP (23.0 mg, 0.188 mmol), and (4-nitrophenyl) [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] carbonate (38.2 mg, 0.0969 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-5% MeOH/DCM) to give [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (31.0 mg, 0.0449 mmol, yield: 47.7 %).

Step 3. Coupling with Peptide Pv1 (Compound 17)

In a vial was placed peptide Pv1 (50.0 mg, 1.47e-5 mol), [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydrofuran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.0124 g, 1.80e-5 mol), 2 mL of ACN and 1 mL of water. To this was added N-methylmorpholine (0.060 mL, 0.000545 mol). The mixture was stirred overnight at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-85% ACN/H₂O+0.05% TFA, 13 min; retention time: 6.670 min) to give Compound 17 (0.0170 g, 4.41e-6 mol, yield: 29.9 %). ESI (M+3H/3)³⁺: 1286.7.

Example 18: Synthesis of Compound 18

Step 1. Synthesis of (4-nitrophenyl) [trans-(2RS,3RS)-3-(2-pyridyldisulfanyl)tetralin-2-yl] carbonate

- 5 The title compound was synthesized from the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-3-(2-pyridyldisulfanyl)tetralin-2-ol, assigned as *trans*-(2RS,3RS)-3-(2-pyridyldisulfanyl)tetralin-2-ol, using analogous synthetic methods described in the synthesis of Compound 11.

- 10 *Step 2. Synthesis of [trans-(2RS,3RS)-3-(2-pyridyldisulfanyl)tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate*

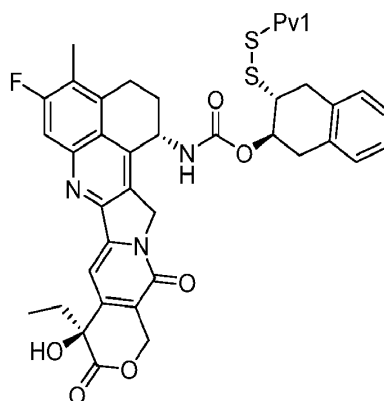
- To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP
 15 (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [trans-(2RS,3RS)-3-(2-pyridyldisulfanyl)tetralin-2-yl] carbonate (32.1 mg, 0.0705 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by
 20 column chromatography (0-3% MeOH/DCM) to give the title compound (26.0 mg, 0.0346 mmol, yield: 73.6 %).

Step 3. Coupling with Peptide Pv1 (Compound 18)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6 mol), [*trans*-(2RS,3RS)-3-(2-pyridyl)disulfanyl]tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00719 g, 9.58e-6 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time: 6.851 min) to give Compound 18 (0.0080 g, 2.04e-6 mol, yield: 27.7 %). ESI (M+3H/3)³⁺: 1307.4.

10

Example 19: Synthesis of Compound 19



Step 1. Synthesis of (4-nitrophenyl) [trans-(2SR,3SR)-3-(2-pyridyl)disulfanyl]tetralin-2-yl] carbonate

15 The title compound was synthesized from the second stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-3-(2-pyridyl)disulfanyl]tetralin-2-ol, assigned as *trans*-(2SR,3SR)-3-(2-pyridyl)disulfanyl]tetralin-2-ol, using analogous synthetic methods described in the synthesis of Compound 11.

20 *Step 2. Synthesis of [trans-(2SR,3SR)-3-(2-pyridyl)disulfanyl]tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-*

diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

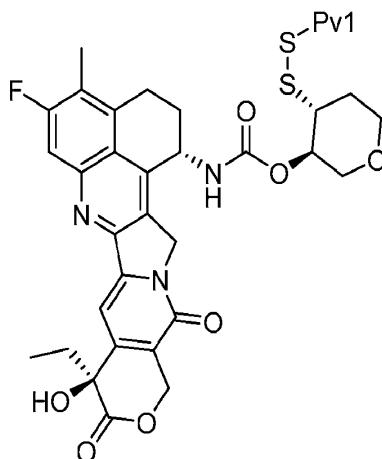
To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [*trans*-(2SR,3SR)-3-(2-pyridyldisulfanyl)tetralin-2-yl] carbonate (32.1 mg, 0.0705 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 µL, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give [*trans*-(2SR,3SR)-3-(2-pyridyldisulfanyl)tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (10.0 mg, 0.0133 mmol, yield: 28.3 %).

Step 3. Coupling with Peptide Pv1 (Compound 19)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6 mol), [*trans*-(2SR,3SR)-3-(2-pyridyldisulfanyl)tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.010 g, 1.33e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18,

PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time: 6.855) to give Compound 19 (0.0060 g, 1.33e-5 mol, yield: 20.8 %). ESI (M+3H/3)³⁺: 1307.6.

Example 20: Synthesis of Compound 20



5

Step 1. Synthesis of (4-nitrophenyl) [trans-(3RS,4RS)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] carbonate

The title compound was synthesized from the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-4-(2-pyridyldisulfanyl)tetrahydropyran-3-ol, assigned as *trans*-(2RS,3RS)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-ol, using analogous synthetic methods described in the synthesis of Compound 11.

10

Step 2. Synthesis of [trans-(3RS,4RS)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

15

To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [trans-(3RS,4RS)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] carbonate (23.1 mg, 0.0564 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue

20

was purified by column chromatography (0-3% MeOH/DCM) to give [*trans*-(3RS,4RS)-4-(2-pyridyl)disulfanyl]tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (30.0 mg, 0.0426 mmol, yield: 90.5 %).

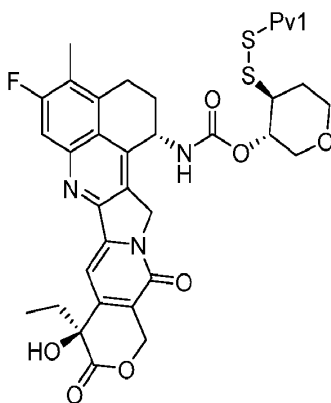
5

Step 3. Coupling with Peptide Pv1 (Compound 20)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6 mol), [*trans*-(3RS,4RS)-4-(2-pyridyl)disulfanyl]tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00779 g, 1.11e-5 mol), , 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 30-85% ACN/H₂O+0.05% TFA, 13 min; retention time: 6.380) to give Compound 20 (0.0060 g, 1.55e-6 mol, yield: 21.0 %). ESI (M+3H/3)³⁺: 1292.3.

15

Example 21: Synthesis of Compound 21



Step 1. Synthesis of (4-nitrophenyl) [trans-(3SR,4SR)-4-(2-pyridyl)disulfanyl]tetrahydropyran-3-yl] carbonate

20

The title compound was synthesized from the second stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-4-(2-pyridyl)disulfanyl]tetrahydropyran-3-ol,

assigned as *trans*-(2SR,3SR)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [trans-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

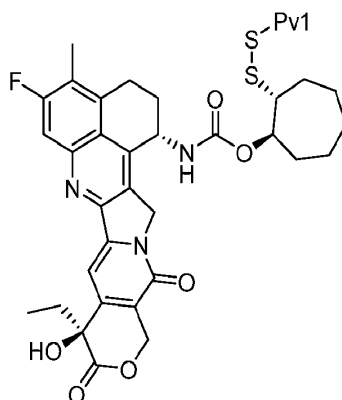
To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] carbonate (23.1 mg, 0.0564 mmol) in 2 mL of anhydrous DMF was added N,N-Diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (25.0 mg, 0.0355 mmol, yield: 75.4 %).

Step 3. Coupling with Peptide Pv1 (Compound 21)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-(3SR,4SR)-4-(2-pyridyldisulfanyl)tetrahydropyran-3-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00779 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18,

PrepSlope_4 min, 20-70% ACN/H₂O+0.05% TFA, 17 min; retention time: 6.765 min) to give Compound 21 (0.021 g, 5.42e-6 mol, yield: 73.6 %). ESI (M+3H/3)³⁺: 1291.1.

Example 22: Synthesis of Compound 22



5

Step 1. Synthesis of (4-nitrophenyl) [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cycloheptyl] carbonate

The title compound was synthesized from the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-2-(2-pyridyldisulfanyl)cycloheptan-1-ol, assigned as *trans*-(1RS,2RS)-2-(2-pyridyldisulfanyl)cycloheptan-1-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cycloheptyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [trans-(1RS,2RS)-2-(2-pyridyldisulfanyl)cycloheptyl] carbonate (23.7 mg, 0.0564 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue

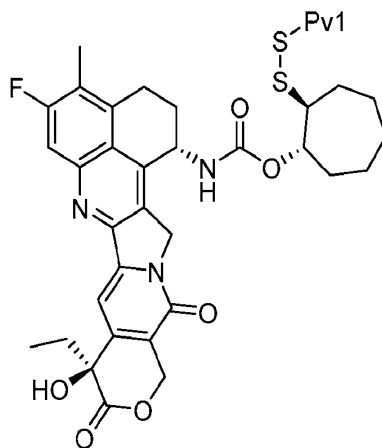
20

was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (29.0 mg, 0.0405 mmol, yield: 86.0 %).

Step 3. Coupling with Peptide Pv1 (Compound 22)

- 5 In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-(1RS,2RS)-2-(2-pyridyldisulfanyl)cycloheptyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0.2,14.0.4,13.0.6,11.0.20,24]tetracos-
- 1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00792 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The
- 10 mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-70% ACN/H₂O+0.05% TFA, 17 min; retention time: 6.868 min) to give Compound 22 (0.020 g, 5.15e-6 mol, yield: 69.9 %). ESI (M+3H/3)³⁺: 1296.3.

15 **Example 23: Synthesis of Compound 23**



Step 1. Synthesis of (4-nitrophenyl) [trans-(1SR,2SR)-2-(2-pyridyldisulfanyl)cycloheptyl] carbonate

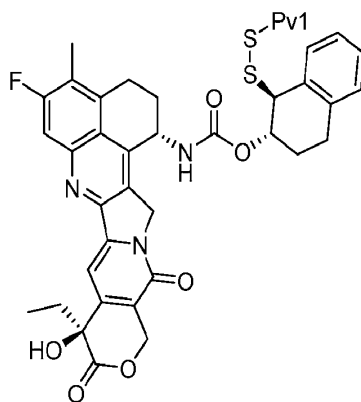
- The title compound was synthesized from the second stereoisomer to be eluted from the
- 20 chiral chromatography separation of racemic *trans*-2-(2-pyridyldisulfanyl)cycloheptan-1-ol, assigned as *trans*-(1SR,2SR)-2-(2-pyridyldisulfanyl)cycloheptan-1-ol, using analogous synthetic methods described in the synthesis of Compound 11.

Step 2. Synthesis of [*trans*-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cycloheptyl] N-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [*trans*-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cycloheptyl] carbonate (23.7 mg, 0.0564 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (31.0 mg, 0.0432 mmol, yield: 91.9 %).

Step 3. Coupling with Peptide Pv1 (Compound 23)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-(1*SR*,2*SR*)-2-(2-pyridyldisulfanyl)cycloheptyl] N-[(10*S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00792 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-88% ACN/H₂O+0.05% TFA, 17 min; retention time 7.178 min) to give Compound 23 (0.020 g, 5.15e-6 mol, yield: 69.9 %). ESI (M+3H/3)³⁺: 1296.0.

Example 24: Synthesis of Compound 24

Step 1. Synthesis of (4-nitrophenyl) [trans-1-(1RS,2RS)-1-(2-pyridyldisulfanyl)tetralin-2-yl] carbonate

- 5 The title compound was synthesized from the first stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-1-(2-pyridyldisulfanyl)tetralin-2-ol, assigned as *trans*-(1RS,2RS)-1-(2-pyridyldisulfanyl) tetralin-2-ol, using analogous synthetic methods described in the synthesis of Compound 11.

- 10 *Step 2. Synthesis of [trans-(1RS,2RS)-1-(2-pyridyldisulfanyl)tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate*

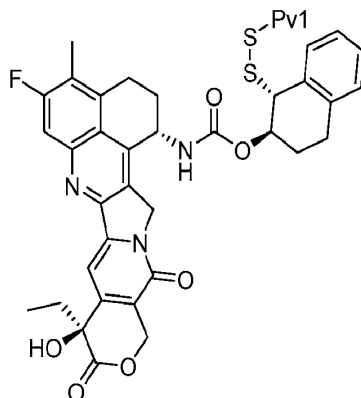
- 15 To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [trans-1-(1RS,2RS)-2-pyridyldisulfanyl]tetralin-2-yl] carbonate (32.1 mg, 0.0705 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by
20 column chromatography (0-3% MeOH/DCM) to give the title compound (20.0 mg, 0.0266 mmol, yield: 56.6 %).

Step 3. Coupling with Peptide Pv1 (Example 24)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-1-(1*RS*,2*RS*)-1-(2-pyridyl)disulfanyl]tetralin-2-yl] N-[(1*0S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0²,14.0⁴,13.0⁶,11.0²⁰,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.0083 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time 6.968) to give Compound 24 (0.012 g, 3.06e-6 mol, yield: 41.6 %). ESI (M+3H/3)³⁺: 1307.2

10

Example 25: Synthesis of Compound 25



*Step 1. Synthesis of (4-nitrophenyl) [trans-(1*SR*,2*SR*)-1-(2-pyridyl)disulfanyl]tetralin-2-yl] carbonate*

The title compound was synthesized from the second stereoisomer to be eluted from the chiral chromatography separation of racemic *trans*-1-(2-pyridyl)disulfanyl]tetralin-2-ol, assigned as *trans*-(1*SR*,2*SR*)-1-(2-pyridyl)disulfanyl] tetralin-2-ol, using analogous synthetic methods described in the synthesis of Compound 11.

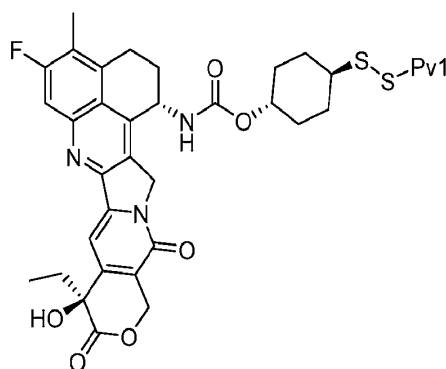
*Step 2. Synthesis of [trans-(1*SR*,2*SR*)-1-(2-pyridyl)disulfanyl]tetralin-2-yl] N-[(1*0S*,23*S*)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0²,14.0⁴,13.0⁶,11.0²⁰,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate*

To a mixture of exatecan mesylate [CAS: 169869-90-3] (25 mg, 0.0470 mmol), DMAP (11.5 mg, 0.0941 mmol), and (4-nitrophenyl) [*trans*-(1SR,2SR)-1-(2-pyridyldisulfanyl)tetralin-2-yl] carbonate (32.1 mg, 0.0705 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (18 μ L, 0.941 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (22.0 mg, 0.0293 mmol, yield: 62.3 %).

Step 3. Coupling with Peptide Pv1 (Compound 25)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-(1SR,2SR)-1-(2-pyridyldisulfanyl)tetralin-2-yl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.0²,14.0⁴,13.0⁶,11.0²⁰,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl] carbamate (0.0083 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time: 6.944) to give Compound 25 (0.013 g, 3.32e-6 mol, yield: 45.0 %). ESI (M+3H/3)³⁺: 1307.0

Example 26: Synthesis of Compound 26



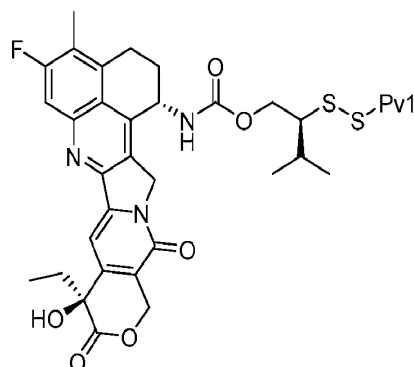
Step 1. Synthesis of [*trans*-4-(2-pyridyldisulfanyl)cyclohexyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol) and (4-nitrophenyl) [4-(2-pyridyldisulfanyl)cyclohexyl] carbonate (synthesized from commercial *trans*-4-mercaptocyclohexan-1-ol) (42.1 mg, 0.103 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (45.0 mg, 0.0640 mmol, yield: 68.1 %).

Step 2. Coupling with Peptide Pv1 (Compound 26)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [*trans*-4-(2-pyridyldisulfanyl)cyclohexyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00777 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time: 6.593 min) to give Compound 26 (0.028 g, 7.23e-6 mol, yield: 98.2 %). ESI (M+3H/3)³⁺: 1291.0.

Example 27: Synthesis of Compound 27

Step 1. Synthesis of [(2S)-3-methyl-2-(2-pyridyldisulfanyl)butyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-

5 *diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate*

To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol) and [(2S)-3-methyl-2-(2-pyridyldisulfanyl)butyl] (4-nitrophenyl) carbonate (synthesized from L-valine, cf J. Org. Chem. 1990,55, 2286-2288) (40.8 mg, 0.103 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (48.0 mg, 0.0695 mmol, yield: 73.9 %).

15

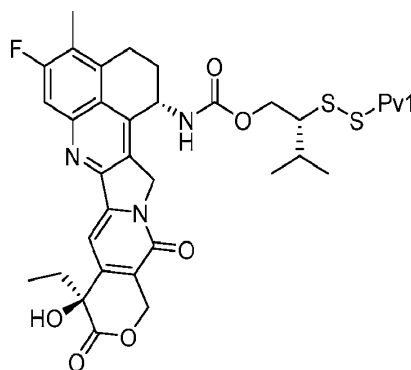
Step 2. Coupling with Peptide Pv1 (Compound 27)

In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6 mol), [(2S)-3-methyl-2-(2-pyridyldisulfanyl)butyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00764 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20

20

min; retention time: 6.773 min) to give Compound 27 (0.024 g, 6.22e-6 mol, yield: 84.4 %). ESI (M+3H/3)³⁺: 1286.8.

Example 28: Synthesis of Compound 28



5

Step 1. Synthesis of [(2R)-3-methyl-2-(2-pyridyldisulfanyl)butyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate

10 To a mixture of exatecan mesylate [CAS: 169869-90-3] (50 mg, 0.0941 mmol) and (4-nitrophenyl) (2R)-3-methyl-2-(2-pyridyldisulfanyl)butyl carbonate (synthesized from D-valine, cf J. Org. Chem. 1990,55, 2286-2288) (40.8 mg, 0.103 mmol) in 2 mL of anhydrous DMF was added N,N-diisopropylethylamine (35 μ L, 0.188 mmol). After stirring for 16 h at room temperature the mixture was diluted with EtOAc (50 mL), washed with 30 mL of saturated

15 NH₄Cl, 30 mL of water, and 20 mL of brine. The organic layer was concentrated and the residue was purified by column chromatography (0-3% MeOH/DCM) to give the title compound (41.0 mg, 0.0594 mmol, yield: 63.1 %).

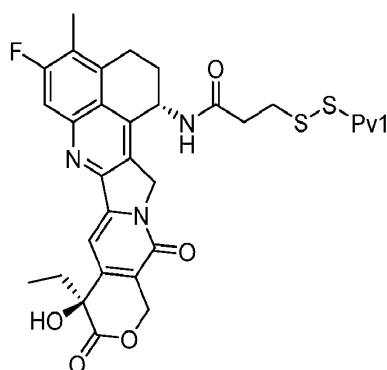
Step 2. Coupling with Peptide Pv1 (Compound 28)

20 In a vial was placed peptide Pv1 (25.0 mg, 7.37e-6), [(2R)-3-methyl-2-(2-pyridyldisulfanyl)butyl] N-[(10S,23S)-10-ethyl-18-fluoro-10-hydroxy-19-methyl-5,9-dioxo-8-oxa-4,15-diazahexacyclo[14.7.1.02,14.04,13.06,11.020,24]tetracos-1,6(11),12,14,16(24),17,19-heptaen-23-yl]carbamate (0.00764 g, 1.11e-5 mol), 1 mL of ACN and 0.5 mL of water. To this was added N-methylmorpholine (0.030 mL, 0.000273 mol). The mixture was stirred for 65 h at

RT. LC-MS indicated a complete reaction. The reaction mixture was purified directly by reverse phase HPLC (Waters SunfirePrep C18, PrepSlope_4 min, 20-95% ACN/H₂O+0.05% TFA, 20 min; retention time: 6.708 min) to give Compound 28 (0.012 g, 3.08e-6 mol, yield: 41.8 %). ESI (M+3H/3)³⁺: 1287.8.

5

Example 29: Synthesis of Compound 29



Analytical methods: Chromatographic purities were determined on an Agilent 1200 Series, 1100 Series or 6130 Series LC/MS system using a Merck Chromolith RP-18e analytical HPLC column (monolithic, 50 × 2 mm) and the following analytical HPLC method: injection volume 5
 10 μL; flow rate 1 mL/min; 5→95% acetonitrile in water with 0.05% AcOH (Method A) or 0.05% TFA (Method B) over 5 mins; Agilent diode array detector at λ = 254, 220 or 195 nm; room temperature.

15 *Step 1. Preparation of N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)-3-(pyridin-2-yl)disulfaneyl)propanamide*

A solution of 2,5-dioxopyrrolidin-1-yl 3-(pyridin-2-yl)disulfaneyl)propanoate (180 mg, 0.576 mmol), in DMF (4 mL) was added to solid exatecan mesylate [CAS: 169869-90-3] (80
 20 mg, 0.150 mmol) then added aqueous PBS buffer (4 mL, pH=7.4, 50 mM) and sonicated ~5 minutes. The cloudy mixture was stirred at ambient temperature for 2 hours, and the reaction was determined to be about 25% complete. Ammonium acetate (11 mg, 0.143 mmol) was added with an additional 2 mL of DMF, and the resultant mixture was stirred at ambient temperature for 18 hours. The mixture was made acidic with TFA (80 mL, 0.98 mmol), and divided into 2

equal portions. Each individual portion was purified on a Redi-Sep C₁₈ 50 g cartridge and eluted with a gradient of acetonitrile (5% to 95%) in water with TFA (0.05% v/v). Combined fractions were frozen and lyophilized to afford the title compound as a pale yellow solid (42 mg, 44%). HPLC purity at 254 nm: 97%. Retention time: 2.50 min (Method A). MS data, 633.2 (M+H)⁺.

5

Step 2. Coupling with Peptide Pv1 (Compound 29)

Solid peptide Pv1 (168.4 mg, 0.0480 mmol) was added to solid N-((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)-3-(pyridin-2-yl)disulfaneyl)propanamide (30.5 mg, 0.0482 mmol) and dissolved in DMF (2 mL) with sonication (~1 minute) and flushed with nitrogen. 4-Methylmorpholine (20 mL, 0.182 mmol) was added and the solution kept at ambient temperature for 18 h. The solution was made acidic with acetic acid (17 mL, 0.296 mmol), applied to a Biotage C₁₈ 300A 25 g reverse phase column, and eluted with a gradient of acetonitrile (25% to 95%) in water with TFA (0.05% v/v).

10 Combined fractions were frozen and lyophilized to afford a pale yellow solid. The product was dissolved in DMSO (3 mL) and 1 mL portions of the solution were individually purified on a Biotage C₁₈ 300A 25 g reverse phase column, eluted with a gradient (25% to 95%) of a solution (acetonitrile/water/2-propanol, 3/2/1) in water with ammonium acetate (10 mM). Combined fractions were frozen and lyophilized to afford a pale yellow solid, which was dissolved in

15 water/acetonitrile (2/1) with 0.4% TFA, transferred into a tared vial and lyophilized to a solid, Compound 29 (128 mg, 66%). HPLC purity at 254 nm: >95%. Retention time: 3.19 min (Method B) MS data: 1900.6 (M+2H/2)²⁺, 1267.3 (M+3H/3)³⁺.

20

Example A. Growth Delay Assay

25 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% CO₂ incubator. After 24 hours, media was removed and replaced with fresh growth media containing various drug

30 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 ug/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

5 calculated and data plotted using GraphPad Prism

Compound	DLD-1 (IC50, nM)	HCT116 (IC50, nM)	FaDu (IC50, nM)	HeLa (IC50, nM)
R ⁸ H-5	0.13	0.05	0.04	0.12
1	1.8	0.38	0.21	0.3
2	4.0	0.66	0.39	0.51
3	IC*	8.33	5.9	7.6
6	13.9	1.0	0.76	0.62
5	0.83	0.12	0.06	0.07
4	0.80	0.10	0.06	0.07

*IC = Incomplete curve.

Compound	HCT-116 (IC50, nM)
11	22.6
12	2.6
13	21.0
14	4.7
15	1.7
16	1.7
17	0.7
18	2.9
19	1.8
20	7.9
21	3.1
22	5.9
23	11.9
24	3.4
25	2.9
26	87.0
27	0.7
28	1.1
29	69.0

Example B: Plasma Pharmacokinetics of Compound 11 in a Rat Model

Animal Dosing

Male Sprague Dawley rats underwent jugular vein cannulation and insertion of a vascular access button (VAB, Instech Labs Cat # VABR1B/22) at Envigo Labs prior to shipment.

- 5 Magnetic, aluminum caps (Instech Labs Cat # Cat #VABRC) were used to protect the access port for the jugular catheters allowing the animals to be housed 2 per cage on corn cob bedding for 4-5 days prior to the study. Rats were administered a single intravenous dose of 5mg/kg Compound 11 prepared in a vehicle of 5% mannitol in citrate buffer. At 1, 2, 4, 8, 24 and 30 hours following compound administration, blood (250 μ L) was collected into K2EDTA filled
10 microtainers from fed rats. Plasma was isolated by centrifugation and 100 μ L aliquots were transferred to 96-well polypropylene plates on dry ice. Samples were stored at -80 °C until processed for quantification of total peptide by ELISA and released exatecan by LC-MS/MS.

ELISA Measurement of Total Peptide Plasma Concentrations

- 15 96-Well plates were coated with 100 μ L/ well of 0.1 μ M BSA-labelled peptide prepared in 0.2 M Carbonate-Bicarbonate Buffer, pH 9.4 and incubated overnight at 4 °C. Plates were washed 4x with an ELISA wash buffer (PBS + 0.05% Tween 20), incubated for 2 hours at room temperature with Blocking Buffer (PBS + 5% dry milk + 0.05% Tween 20) (300 μ L/ well) and washed again 4x with ELISA wash buffer. Concurrently, 2x Compound 11 standards in control
20 plasma and study plasma samples were pre-incubated with 1-10 ng/mL of a primary antibody specific for the Pv1 peptide for 30 minutes at room temperature. Pre-incubated samples were added to pre-coated, pre-blocked assay plates at 100 μ L/ well and incubated for 1 hour at room temperature. Plates were washed 4x with ELISA wash buffer and incubated with 100 μ L/ well of a secondary goat anti-mouse IgG HRP antibody (1:5,000 in antibody diluent) for 1 hour at room
25 temperature. Plates were washed 4x with ELISA wash buffer and incubated with 100 μ L/ well of SuperSignal substrate at room temperature with gentle shaking for 1 minute. Luminescence was read from the plate on a BioTek Cytation 5 plate reader.

LC-MS/MS Measurement of Exatecan Plasma Concentrations

For quantification of exatecan, a 20 μ L plasma sample was added to a polypropylene autosampler vial. 20 μ L PPT-IS (ACN:H₂O (50:50)+0.5 % FA containing 1000 ng/mL internal standard) and 20 μ L diluent (ACN:H₂O (50:50)+0.5 % FA) was added to each sample. Followed by addition of 120 μ L of ACN + 5% FA. The vials were capped and vortexed for 2 minutes. The samples were centrifuged for 5-10 minutes at 3700 rpm then analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS).

FIG. 1 shows a plot of the plasma concentration of Compound 11 and released exatecan after a single IV dose of 5mg/kg of Compound 11 in a rat (data are expressed as means $\pm$ SEM). As shown in FIG. 1, less than 0.002% of the exatecan warhead was released after 30h in circulation. FIG. 1 demonstrates that Compound 11 is stable in plasma for at least 30 h.

Example C: Tumor and Bone Marrow Pharmacokinetics of Compound 11 in a Mouse Model

Animal Dosing

Six-week-old female athymic nude *Foxn1^{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 (Innovive). Human HCT116 cancer 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 2.5×10^6 cells in 100 μ L. When xenografts reached a minimal volume of 300 mm³, mice were administered a single intraperitoneal injection of 10 mg/kg Compound 11 prepared in a vehicle of 5% mannitol in citrate. Tumor and bone marrow samples were collected from fed, anesthetized mice at 1, 2, 4, 8, 16, 24, 32 and 48 hours after compound administration. Total peptide concentrations in tumor and bone marrow were determined via ELISA.

ELISA Measurement of Total Peptide Tissue Concentrations

96-well plates were coated with 100 μ L/ well of 0.1 μ M BSA-labelled peptide prepared in 0.2 M Carbonate-Bicarbonate Buffer, pH 9.4 and incubated overnight at 4°C. Plates were washed 4x with an ELISA wash buffer (PBS + 0.05% Tween 20), incubated for 2 hours at room

temperature with Blocking Buffer (PBS + 5% dry milk + 0.05% Tween 20) (300 μ L/ well) and washed again 4x with ELISA wash buffer. Concurrently, 2x Compound 11 standards (in respective tissue matrix) or sample tumor homogenates or bone marrow samples diluted with antibody diluent (PBS + 2% dry milk + 0.05% Tween 20), were pre-incubated with 1-10 ng/mL of a primary antibody specific for the Pv1 peptide for 30 minutes at room temperature. Pre-incubated samples were added to pre-coated, pre-blocked assay plates at 100 μ L/ well and incubated for 1 hour at room temperature. Plates were washed 4x with ELISA wash buffer and incubated with 100 μ L/ well of a secondary goat anti-mouse IgG HRP antibody (1:5,000 in antibody diluent) for 1 hour at room temperature. Plates were washed 4x with ELISA wash buffer and incubated with 100 μ L/ well of SuperSignal substrate at room temperature with gentle shaking for 1 minute. Luminescence was read from the plate on a BioTek Cytation 5 plate reader.

FIG. 2 shows a plot of the peptide concentration in tumor and bone marrow after a single IP dose of 10 mg/kg of Compound 11 in a mouse (data are expressed as means $\pm$ SEM). FIG. 2 demonstrates Compound 11 effectively targets tumors.

Example D: Bone Marrow Toxicity Study in a Mouse Model

Animal Dosing

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 (Innovive). Human HCT116 cancer 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 2.5×10^6 cells in 100 μ L. When xenografts reached a minimal volume of 200 mm³, mice were administered intraperitoneal doses of vehicle or 2.6 or 5.2 μ moles/kg of either unconjugated exatecan (equivalent to 1.15 or 2.3 mg/kg exatecan, respectively) or Compound 11 (equivalent to 10 or 20 mg/kg Compound 11, respectively). Compounds were administered once daily for 4 days.

Bone Marrow Collection

Tumor bearing mice were euthanized by cervical dislocation 6 hours after the last dose. Femurs were removed, and bone marrow was extruded into 50 mL conical tubes by flushing the bones with a 23-gauge needle fitted on a 5cc syringe containing PBS + 2% fetal bovine serum.

5 Bone marrow was homogenized by gentle pipetting and filtered through 100 μ m nylon mesh filters and cells were pelleted by centrifugation at 1200 rpm for 5 minutes at 4 °C. Red blood cells were lysed with 3 mL of lysis buffer for 2 minutes at room temperature. PBS was added to a volume of 25 mL and cells were re-pelleted by centrifugation as described above. Cell pellets were suspended in 5 mL of PBS and cell count was assessed by trypan blue exclusion. The cell

10 counts from four independent studies were averaged and plotted.

FIG. 3 shows a graph of the total bone marrow counts from the femurs of tumor bearing nude mice after dosing of 2.6 and 5.2 μ moles/kg of either Compound 11 (equivalent to 10, 20mg/kg conjugate) or free exatecan (equivalent to 1.15 and 2.3mg/kg exatecan) dosed once

15 daily for four days. (data are expressed as means $\pm$ SEM). Compound 11 did not display the bone marrow toxicity that limits the clinical utility of exatecan.

Example E: Gastric Toxicity Study in a Mouse Model

20 *Animal Dosing and Stomach Imaging*

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 (Innovive). 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

25 2.5×10^6 cells in 100 μ L. When xenografts reached a minimal volume of 300 mm³, mice were administered intraperitoneal doses of vehicle, or 5.2 μ moles/kg of either unconjugated exatecan (equivalent to 2.3 mg/kg exatecan) or Compound 11 (equivalent to 20 mg/kg Compound 11). Compounds were administered once daily for 4 days. At 6 hours after administration of the last dose, mice were euthanized by cervical dislocation and gross necropsy was conducted.

30 Photographs were taken of the stomachs both *in situ* and *ex vivo*.

FIG. 4A shows the stomachs of tumor bearing nude mice excised after dosing of vehicle or 5.2 μ moles/kg of either Compound 11 (equivalent to 20 mg/kg conjugate) or free exatecan (equivalent to 2.3 mg/kg exatecan) dosed QDx4. FIG. 4B shows the stomachs of tumor bearing nude mice in situ after dosing of 5.2 μ moles/kg of either Compound 11 (equivalent to 20 mg/kg conjugate) or free exatecan (equivalent to 2.3 mg/kg exatecan) dosed once daily for four days. Compound 11 did not display the gastric toxicity that limits the clinical utility of exatecan.

Example F: Efficacy of Compound 11 in a HCT116 Colorectal Cancer Model

Six-week-old female athymic nude *Foxntm* 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 2.5×10^6 cells in 100 μ L. When xenografts reached a mean volume of 100-200 mm³, mice were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 2.6 or 5.2 μ mole/kg of either unconjugated exatecan (equivalent to 1.15 or 2.3 mg/kg exatecan, respectively) or Compound 11 (equivalent to 10 or 20 mg/kg Compound 11, respectively). Doses were prepared by diluting 0.1 mg/ μ L DMSO stocks in 5% mannitol in citrate buffer and were administered QDX4/week for three weeks at a volume of 12mL/kg (300 μ L per 25 g mouse). Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: Volume = $\pi/6 \times (\text{length}) \times (\text{width})^2$. Animals were removed from the study due to death, tumor size exceeding 2000mm³ or loss of >20% body weight. The below table shows the dosing schedule of various treatment groups.

Group	Treatment	Dose	Dosing Schedule	Administration Route	Number of Mice
1	Vehicle (5% mannitol in citrate buffer)	NA	QDx4/wk x 3	i.p.	8
2	Compound 11	10 mg/kg	QDx4/wk x 3	i.p.	8

3	Compound 11	20 mg/kg	QDx4/wk x 3	i.p.	8
4	exatecan	1.15 mg/kg	QDx4/wk x 3	i.p.	8
5	exatecan	2.3 mg/kg	QDx4/wk x 3	i.p.	8

FIG. 5A shows a plot of the mean tumor volume resulting from dosing equimolar amounts of either free exatecan or Compound 11 in nude mice bearing HCT116 colorectal flank tumors. Animals were dosed once daily four times per week intraperitoneally for three weeks.

FIG. 5B displays a Kaplan Meier survival curve for dosing equimolar amounts of either free exatecan or Compound 11 in nude mice bearing HCT116 colorectal flank tumors. Data are expressed as means $\pm$ SEM. These data demonstrate that Compound 11 demonstrates potent anti-tumor activity in a pre-clinical colorectal cancer model.

Example G: Efficacy of Compound 11 in a MKN45 HER2 Negative Gastric Cancer Model

Six-week-old female athymic nude *Foxn1tm* 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 MKN45 cells derived from gastric carcinoma were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 2×10^6 cells in 100 μ L. When xenografts reached a mean volume of 100-200 mm³, mice were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 2.6 or 5.2 μ mole/kg of either unconjugated exatecan (equivalent to 1.15 or 2.3 mg/kg exatecan, respectively) or Compound 11 (equivalent to 10 or 20 mg/kg Compound 11, respectively). Doses were prepared by diluting 0.1 mg/ μ L DMSO stocks in 5% mannitol in citrate buffer and were administered QDX4/week for two weeks at a volume of 12mL/kg (300 μ L per 25 g mouse). Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: Volume = $\pi/6 \times (\text{length}) \times (\text{width})^2$. Animals were removed from the study due to death, tumor size exceeding 2000 mm³, or loss of >20% body weight. The following table shows the dosing schedule of the various treatment groups.

Group	Treatment	Dose	Dosing Schedule	Administration Route	Number of Mice
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1	Vehicle (5% mannitol in citrate buffer)	NA	QDx4/wk x 2	i.p.	8
2	Compound 11	2.5 mg/kg	QDx4/wk x 2	i.p.	8
3	Compound 11	5 mg/kg	QDx4/wk x 2	i.p.	8
4	Compound 11	10 mg/kg	QDx4/wk x 2	i.p.	8
5	Compound 11	20 mg/kg	QDx4/wk x 2	i.p.	8
6	exatecan	1.15 mg/kg	QDx4/wk x 2	i.p.	8
7	exatecan	2.3 mg/kg	QDx4/wk x 2	i.p.	8

FIG. 6A shows the single agent efficacy of Compound 11 in nude mice bearing MKN45 HER2 negative gastric cancer flank tumors. Animals were dosed once daily four times per week intraperitoneally for two weeks. FIG. 6B displays a Kaplan Meier survival curve for dosing equimolar amounts of either free exatecan or Compound 11 in nude mice bearing MKN45 HER2 negative gastric cancer flank tumors. Data are expressed as means $\pm$ SEM. These data demonstrate that Compound 11 demonstrates potent anti-tumor activity in a pre-clinical gastric cancer model.

Figure 6B. Kaplan-Meier analysis was used to evaluate survival rate based on death or removal from study.

Example H: Efficacy of Compound 11 in a J1MT-1 HER2 Intermediate Breast Cancer Model

Five to six-week-old female NOD.SCID mice were obtained from Beijing Anikeeper Biotech Co., Ltd (Beijing, China). Human J1MT-1 cells derived from breast carcinoma were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5×10^6 cells in 100 μ L. When xenografts reached a mean volume of 100 mm³, mice were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 2.6 or 5.2 μ mole/kg of Compound 11 (equivalent to 10 or 20 mg/kg Compound 11, respectively). Doses were prepared by diluting 0.1

mg/ μ L DMSO stocks in 5% mannitol in citrate buffer and were administered QDX4/week for three weeks at a volume of 12 mL/kg (300 μ L per 25 g mouse). Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: Volume = $\pi/6 \times$ (length) $\times$ (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 mm³, or loss of >20% body weight. The following table shows the dosing schedule for various treatment groups.

Group	Treatment	Dose	Dosing Schedule	Administration Route	Number of Mice
1	Vehicle (5% mannitol in citrate buffer)	NA	QDx4/wk x 3	i.p.	8
2	Compound 11	10 mg/kg	QDx4/wk x 3	i.p.	8
3	Compound 11	20 mg/kg	QDx4/wk x 3	i.p.	8

FIG. 7A shows a plot of the mean tumor volume resulting from dosing Compound 11 in SCID mice bearing JIMT-1 HER2 intermediate breast cancer flank tumors. Animals were dosed once daily four times per week intraperitoneally for three weeks. FIG. 7B shows a plot of the percent change in body weight in SCID mice bearing JIMT-1 HER2 intermediate breast cancer flank tumors dosed with Compound 11. Data are expressed as means $\pm$ SEM. These data demonstrate that Compound 11 demonstrates potent anti-tumor activity in a pre-clinical breast cancer model.

Example I: Efficacy of Compound 11 in a MDA-MB-231 Triple Negative Breast Cancer Model

Three to four-week-old female athymic nude *Foxn1*^{nu} mice were obtained from Envigo Labs. Human MDA-MB-231 cells derived from breast adenocarcinoma were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 2×10^6 cells in 100 μ L. When xenografts reached a mean volume of 50-100 mm³, mice

were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 5, 10, or 20 mg/kg Compound 11. Doses were prepared by diluting 0.1 mg/ μ L DMSO stocks in 5% mannitol in citrate buffer and were administered QDX4/week for three weeks at a volume of 12 mL/kg (300 μ L per 25 g mouse). Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: $\text{Volume} = \pi/6 \times (\text{length}) \times (\text{width})^2$. Body weight of animals was measured at the same time as tumor volume assessment. Animals were removed from the study due to either death, tumor size exceeding 2000 mm³ or due to loss of >20% body weight. The following table shows the dosing schedule of various treatment groups.

Group	Treatment	Dose	Dosing Schedule	Administration Route	Number of Mice
1	Vehicle (5% mannitol in citrate buffer)	NA	NA	i.p.	8
2	Compound 11	5 mg/kg	QDx4/wk x 3	i.p.	9
3	Compound 11	10 mg/kg	QDx4/wk x 3	i.p.	9
4	Compound 11	20 mg/kg	QDx4/wk x 3	i.p.	9

FIG. 8A shows the a plot of the mean tumor volume in nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11. Animals were dosed once daily four times per week intraparenterally for three weeks. FIG. 8B shows a plot of the percent change in body weight relative to day 0 in nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11. Data are expressed as means $\pm$ SEM. These data demonstrate that Compound 11 demonstrates potent anti-tumor activity in a pre-clinical breast cancer model.

Example J: Combination Efficacy of Compound 11 and Talazoparib in a MDA-MB-231 Triple Negative Breast Cancer Model

Three to four-week-old female athymic nude *Foxntm* mice were obtained from Envigo Labs. Human MDA-MB-231 cells derived from breast adenocarcinoma were diluted 1:1 in

Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 2×10^6 cells in 100 μ L. When xenografts reached a mean volume of 50-100 mm^3 , mice were randomized into groups and treated as detailed in the table below. Mice were administered intraperitoneal (IP) doses of vehicle or 5 mg/kg Compound 11 alone or in combination with an oral (PO) dose of 0.33 mg/kg talazoparib. Doses were prepared by diluting 0.1 mg/ μ L DMSO stocks in 5% mannitol in citrate buffer. Compound 11 was administered QDX4/week for three weeks at a volume of 12 mL/kg (300 μ L per 25 g mouse) and talazoparib was administered once daily for 15 days. Xenograft tumors were measured by calipers and volume was calculated using the equation for ellipsoid volume: $\text{Volume} = \pi/6 \times (\text{length}) \times (\text{width})^2$. Body weight of animals was measured at the same time as tumor volume assessment. Animals were removed from the study due to either death, tumor size exceeding 2000 mm^3 or due to loss of >20% body weight. The following table shows the dosing schedule of various treatment groups.

Group	Treatment	Dose	Dosing Schedule	Administration Route	Number of Mice
1	None	NA	NA	NA	9
2	Talazoparib	0.33 mg/kg	QDx15	p.o.	9
3	Compound 11	5 mg/kg	QDx4/wk x 3	i.p.	10
4	Talazoparib Compound 11	0.33 mg/kg 5 mg/kg	QDx15 QDx4/wk x 3	p.o. i.p.	8

FIG. 9A shows a plot of the mean tumor volume of nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11 and talazoparib. Animals were dosed once daily four times per week intraperitoneally for three weeks with Compound 11 and once daily for 18 days orally with talazoparib. FIG. 9B shows a plot of the percent change in body weight relative to day 0 of nude mice bearing MDA-MB-231 triple negative breast cancer flank tumors dosed with Compound 11 and talazoparib.

Example K: Glutathione Cleavage Study

A 20 mM stock of conjugate was prepared in 100% DMSO. The stocks were subsequently diluted in 100 mM Tris, pH 7.5, to yield an intermediate dilution of 500 μ M followed by an additional dilution of 1:5 in 100mM Tris, pH 7.5 to give a final concentration of

100 μ M of conjugate. 100 mM glutathione was prepared immediately prior to use in H₂O and diluted 1:10 in challenge samples for a final glutathione challenge concentration of 10 mM. Samples were mixed by inversion and incubated at 37 °C for up to 24 hrs. 50 μ L samples were aliquoted into siliconized microfuge tubes at time 0, 4, and 24 hours and immediately frozen at -80 °C.

Samples were thawed and extracted as follows: 8 μ L of 25% phosphoric acid followed by 117 μ L of 100% acetonitrile/0.1%TFA were added to each sample, mixed and centrifuged at 13000xG for 10 minutes. The supernatant was pipetted into 0.2 mL HPLC vials and placed on a Perkin Elmer Flexar HPLC autosampler. The following table summarizes HPLC conditions:

HPLC	Perkin Elmer Flexar Binary pump, auto sampler, UV detector		
Column	Waters BioResolve RP mAb Polyphenyl Column, 450 Å, 2.7 μ m, 4.6 mm X 150 mm		
Guard Coulmn	Waters BioResolve RP mAb Polyphenyl VanGuard Cartridge w/holder, 450 Å, 2.7 μ m, 3.9 mm x 5 mm		
Detection Wavelength	217 nm		
Column temperature	37°C		
Pressure Limits	Min: 0 PSI, Max: 3050 PSI		
Mobile phase			
Mobile phase A	0.05% TFA in water		
Mobile phase B	0.05% TFA in Acetonitrile		
Flow rate	0.8 mL/min		
Injection volume	10.0 μ L		
Run time	14.0 minutes		
Gradient program	Time (minutes)	%A	%B
	0.0	80	20
	0.5	80	20
	10.0	0	100
	11.0	80	20
	14.0	80	20

Data was analyzed by calculating the percentage reduction of compound (area of retention time peak of cleaved conjugate/area of retention time peak of conjugate at time 0) X 100.

FIG. 10 shows a graph of the degradation of Compound 11 and Compound 29 resulting from treatment with 10 mM glutathione over 16 h. As shown in FIG. 10, Compound 29 is released much faster than Compound 11 under similar glutathione exposure.

The table below summarizes the degradation data for the 10 mM glutathione exposure conditions described above for Compounds 11 to Compound 29 measured at 4 h and 24 h.

Glutathione Challenge (10 mM)		
Compound	%Remaining at 4 h	%Remaining at 24 h
11	50.1	10.2
12	ND	ND
13	ND	ND
14	14.9	6.5
15	19.4	8.3
16	3.4	2.0
17	3.4	1.7
18	60.1	13.0
19	ND	ND
20	5.2	8.0
21	9.5	12.6
22	65.8	12.3
23	61.4	13.2
24	20.8	4.2
25	42.0	4.1
26	75.8	32.1
27	18.0	0.0
28	40.5	0.0
29	3.1	5.9

Example L: Plasma Stability Studies

A 20 mM stock of conjugate was prepared in 100% DMSO. The stocks were subsequently diluted in 100 mM Tris, pH 7.5 to yield an intermediate dilution of 500 μ M and then diluted 1:5 directly into rat plasma to yield a final concentration of 100 μ M of conjugate. Samples were mixed by inversion and incubated at 37 °C for up to 24 h. 50 μ L samples were aliquoted into siliconized microfuge tubes at time 0, 4, and 24 h and immediately frozen at -80 °C.

Samples were thawed and extracted as follows: 8 μ L of 25% phosphoric acid followed by 117 μ L of 100% Acetonitrile/0.1% TFA was added to each sample, mixed and centrifuged at 13000xG for 10 minutes. The supernatant was pipetted into 0.2 mL HPLC vials and placed on a Perkin Elmer Flexar HPLC autosampler. The following table summarizes HPLC conditions:

5

HPLC	Perkin Elmer Flexar Binary pump, auto sampler, UV detector		
Column	Waters BioResolve RP mAb Polyphenyl Column, 450 Å, 2.7 μ m, 4.6 mm X 150 mm		
Guard Coulmn	Waters BioResolve RP mAb Polyphenyl VanGuard Cartridge w/holder, 450 Å, 2.7 μ m, 3.9 mm x 5 mm		
Detection Wavelength	217 nm		
Column temperature	37°C		
Pressure Limits	Min: 0 PSI, Max: 3050 PSI		
Mobile phase			
Mobile phase A	0.05% TFA in water		
Mobile phase B	0.05% TFA in Acetonitrile		
Flow rate	0.8 mL/min		
Injection volume	10.0 μ L		
Run time	14.0 minutes		
Gradient program	Time (minutes)	%A	%B
	0.0	80	20
	0.5	80	20
	10.0	0	100
	11.0	80	20
	14.0	80	20

Data was analyzed by calculating the percentage reduction of compound (area of retention time peak of incubated conjugate/area of retention time peak of conjugate at time 0) X 100. The results of the study are shown in the table below.

Compound	Plasma Stability	
	%Remaining at 4hrs	%Remaining at 24hrs
11	108.2	107.2
12	ND	ND

13	ND	ND
14	106.1	105.9
15	104.9	103.3
16	115.6	106.1
17	106.8	105.0
18	104.0	93.0
19	ND	ND
20	98.7	95.8
21	101.0	98.2
22	106.0	105.0
23	92.0	92.0
24	87.0	87.0
25	103.0	104.0
26	105.0	112.0
27	117.0	109.0
28	106.0	106.0
29	116.0	110.0

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.