

FIELD OF THE INVENTION

10 **BACKGROUND OF THE INVENTION**

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

30 The PARP enzyme family, including poly(ADP-ribose) polymerase (PARP-1) and the related enzymes PARP-2 and PARP-3 (collectively “PARP”), are important elements in the repair of DNA single-strand breaks, particularly by the base excision repair pathway. If single-strand breaks are not repaired prior to DNA replication, then double strand breaks may form. Cells with increasing numbers of double strand breaks become more dependent on

other repair pathways, such as homologous recombination, and (if single strand breaks continue unrepaired) the cells die.

Inhibitors of PARP have been developed and continue to be developed as anti-cancer agents. Because these inhibitors prevent the repair of DNA single-strand breaks, they have a variety of roles in treatment of cancer. (See, e.g., Nicola J. Curtin and Ricky A. Sharma – “PARP Inhibitors for Cancer Treatment”, Cancer Drug Discovery and Development, vol 83, Humana Press 2015.)

PARP inhibitors can be used for treatment of forms of cancer that are more dependent on PARP than normal cells (so-called “PARP-sensitive” cancers). For example, patients with homologous recombination deficiency (HRD), such as that involving the BRCA1 and BRCA2 genes, are beneficially treated with a PARP inhibitor, either as monotherapy or in combination with other agents. Since DNA repair and survival of PARP inhibited cells are heavily dependent on HR, patients carrying BRCA related mutations (who exhibit HRD) are well suited to treatment with PARP inhibitors.

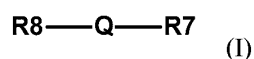
Although PARP inhibitors are useful in the treatment of cancer, the compounds also exhibit side effects. PARP side effects, which include serious hematologic and gastrointestinal adverse reactions and potentially fatal acute myeloid leukemia, are highly undesirable.

Other DNA repair inhibiting cancer therapeutics would be expected to similarly exhibit unwanted side effects when administered systemically. Such other DNA repair inhibiting cancer therapeutics include those targeting the protein kinase ataxia-telangiectasia mutated (ATM), the ATM-Rad3-related protein kinase (ATR), and the nuclear serine/threonine protein kinase DNA-PK. Cancer therapy would benefit significantly if compounds acting by these mechanisms could be delivered selectively to cancer cells and thus avoid undesired effects on normal cells.

Moreover, PARP inhibitors have potential for utility in treatment of other diseases besides cancer, including cardiovascular and inflammatory diseases, through possible roles for PARP in functions other than DNA repair, such as involvement in regulating the mitochondria-to-nucleus translocation of apoptosis-inducing-factor (AIF) or involvement in regulating the expression of proteins implicated in inflammation. (See, e.g., Pacher and Szabo – “Role of Poly(ADP-ribose) polymerase 1 (PARP-1) in Cardiovascular Diseases: The Therapeutic Potential of PARP Inhibitors, Cardiovasc Drug Rev. 2007; 25(3): 235-260). Preferential delivery of PARP inhibitors to these diseased tissues would likewise be beneficial.

SUMMARY

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



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

Broadly speaking, the invention provides (1) compounds comprising a therapeutic molecule (e.g., R⁷) and a pH-sensitive (or pH-dependent) peptide (e.g., R⁸) linked together, such as by a disulfide-containing linker or other linker which would be cleaved in the
 10 intra-cellular environment, (2) pharmaceutical compositions comprising these compounds and a pharmaceutically-acceptable carrier, (3) methods of using these compounds and compositions in the treatment of diseases and conditions in humans and other mammals involving acidic and/or hypoxic cells, and (4) methods of making the compounds and compositions, and intermediates useful in said methods.

- 15 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 diseases or conditions involving acidic or hypoxic diseased tissues by administering to a human or other mammal in
 20 need of such treatment a therapeutically effective amount of a compound of the disclosure. The disclosure also provides methods of reducing bone marrow toxicity associated with administration of an ionizing radiation or cytotoxic agent, which comprises administering to a human or other mammal a therapeutically-effective amount of a compound of the disclosure in combination with the ionizing radiation or cytotoxic agent.

- 25 The present disclosure also provides uses of the 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.

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

30 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the tumor growth delay of R⁸H-15 and Example 12 in BRCA^{-/-} Mice.

Figure 2 shows survival of R⁸H-15 and Example 12 in BRCA^{-/-} Mice.

Figure 3 shows the tumor growth delay of R⁸H-16 and Example 18 in BRCA^{-/-} Mice.

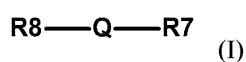
Figure 4 shows the effect of 9-Day intraperitoneal administration of Example 18 and R⁸H-16 on tumor PARylation in a murine DLD-1 BRCA2^{-/-} xenograft model.

Figure 5 shows the PARylation in bone marrow cells following intravenous administration of Example 12 or oral administration of R⁸H-15 in combination with oral administration of temozolomide (TMZ) to nude mice.

Figure 6 shows PARylation in bone marrow cells following intraperitoneal administration of Example 18 or R⁸H-16 to nude mice.

DETAILED DESCRIPTION

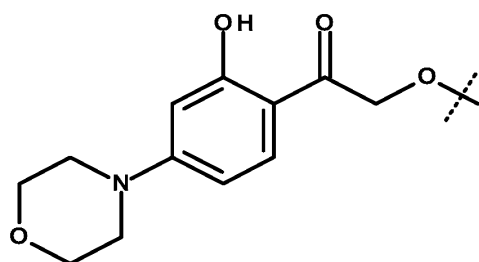
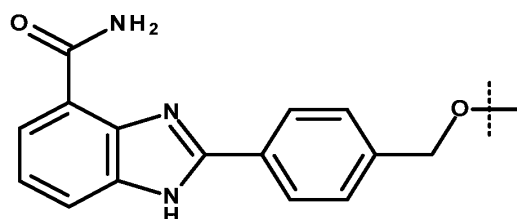
Provided herein is a compound of Formula (I):

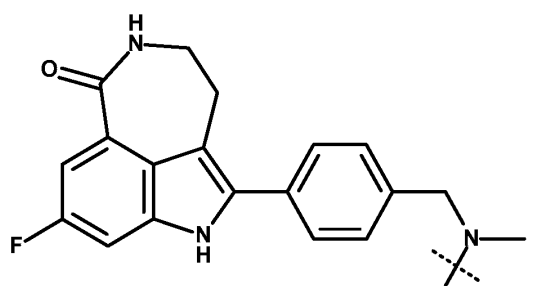
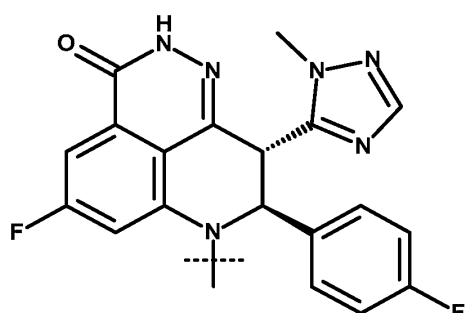
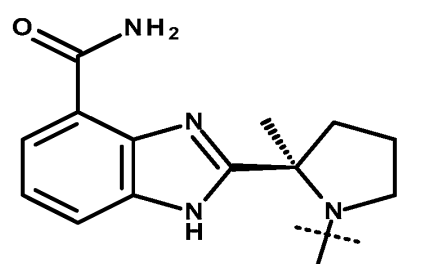
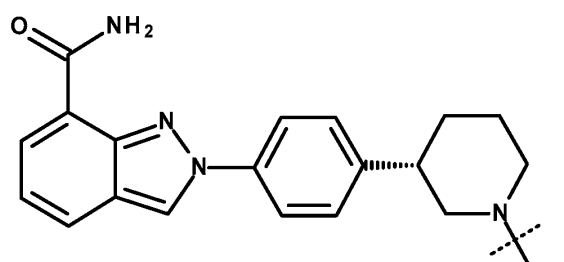


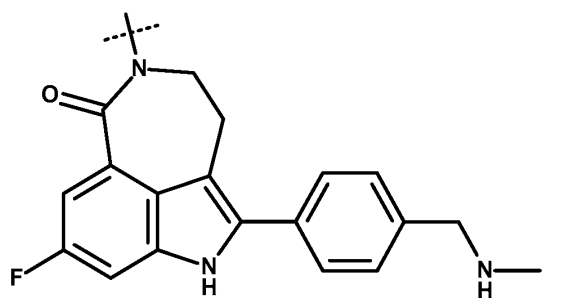
or a pharmaceutically acceptable salt thereof, wherein:

R⁷ is a peptide;

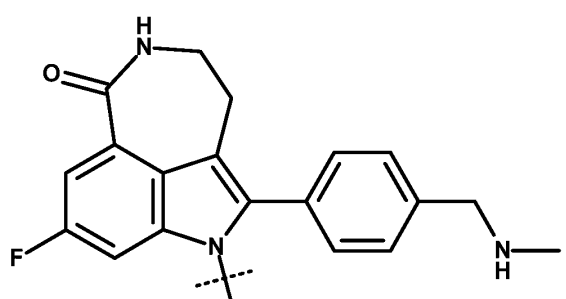
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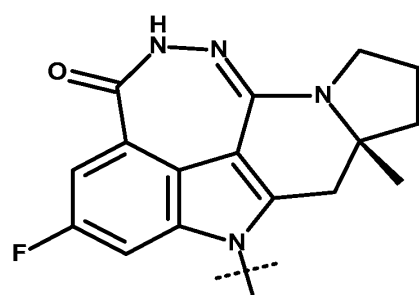




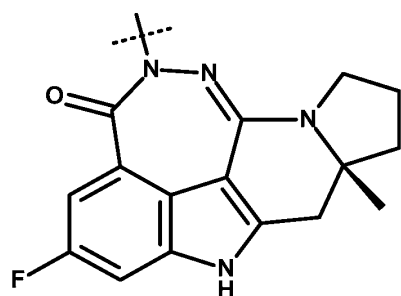
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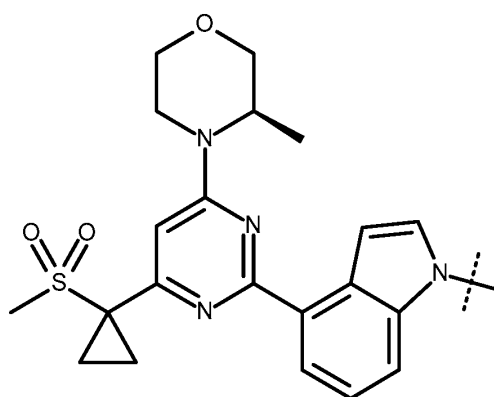
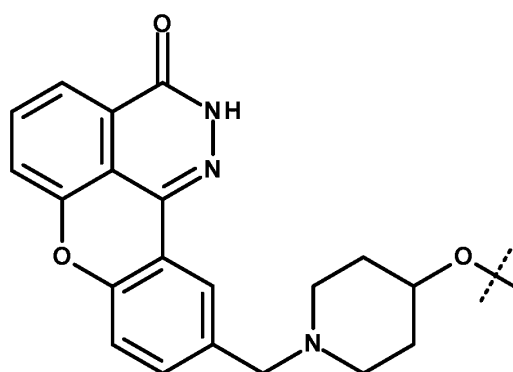
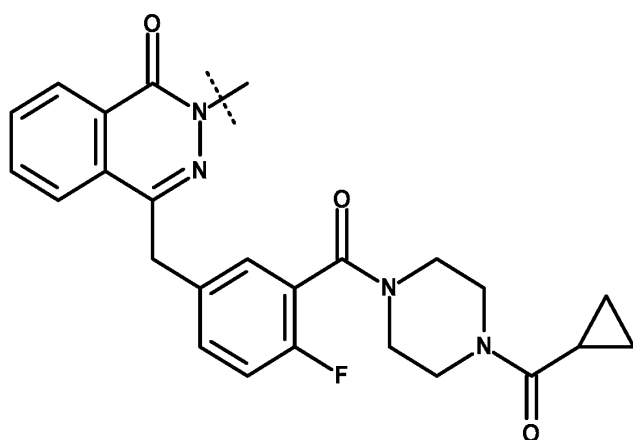


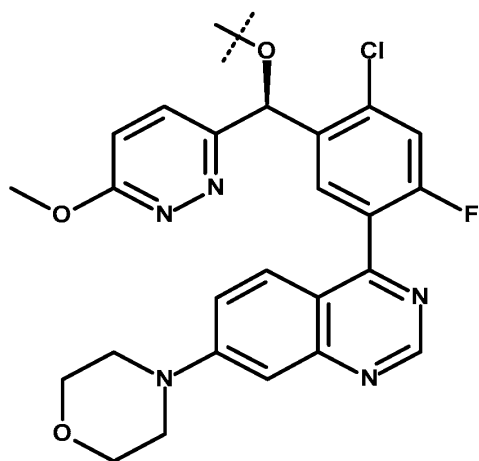
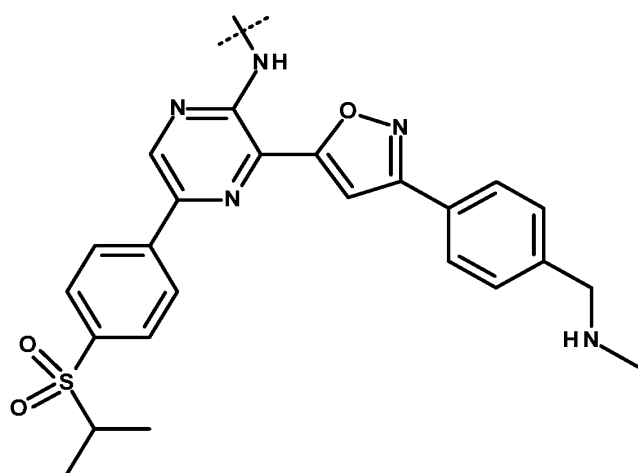
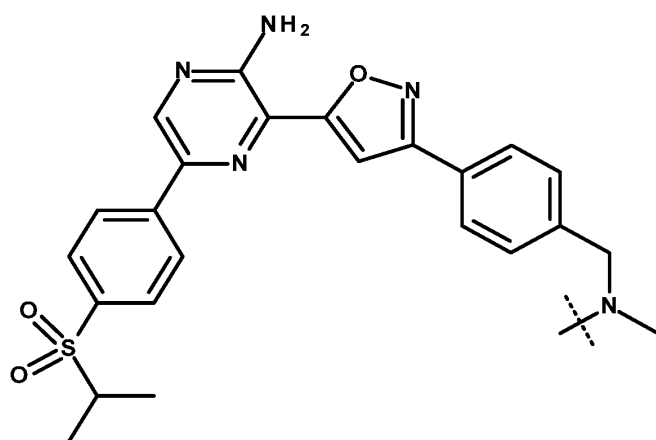
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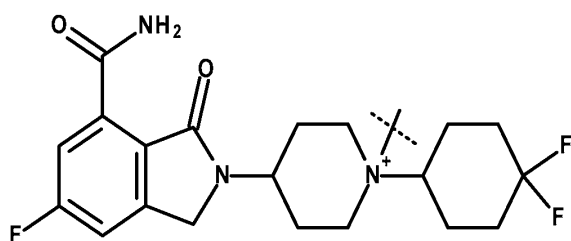
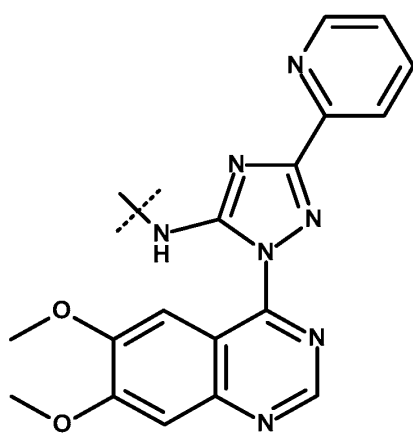
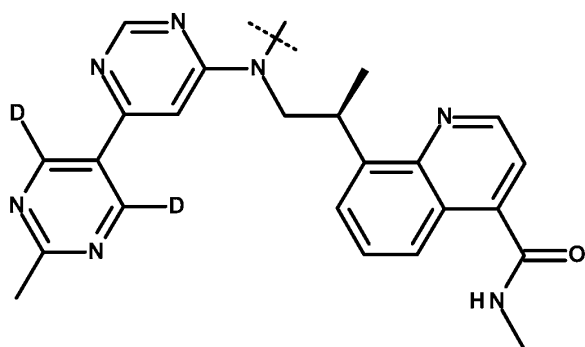


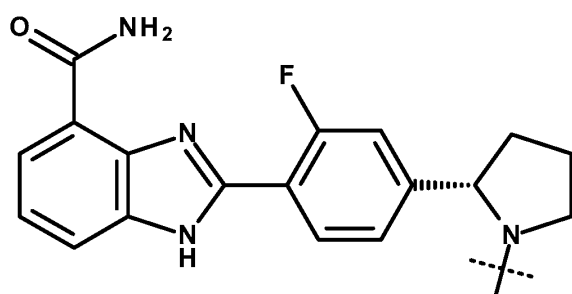
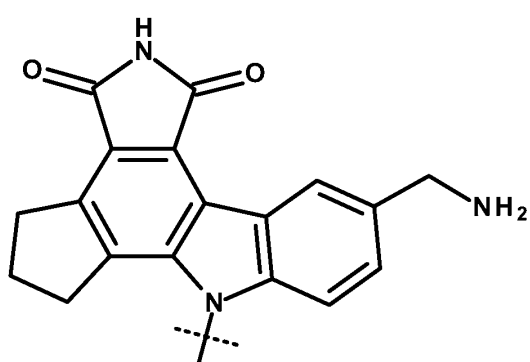
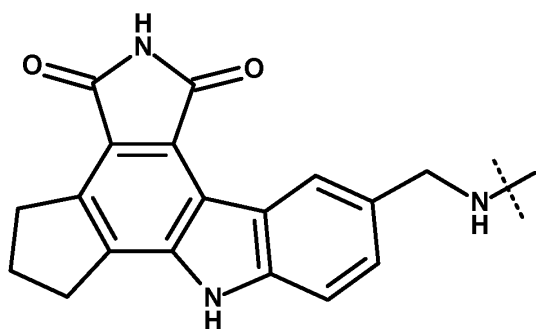
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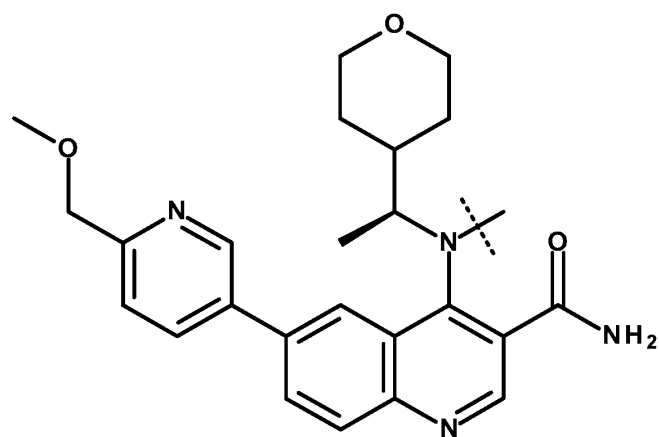
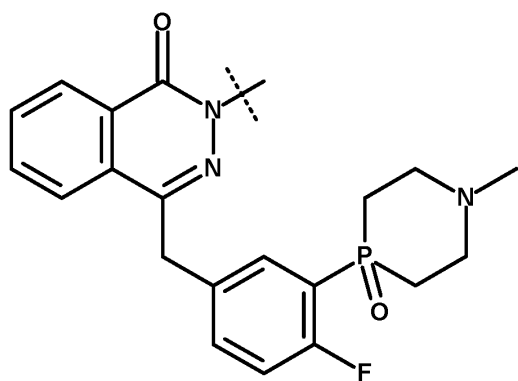
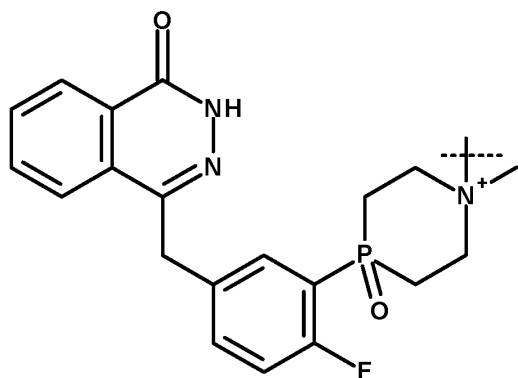
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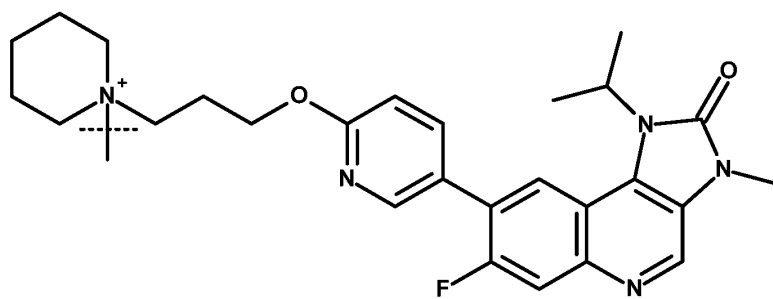
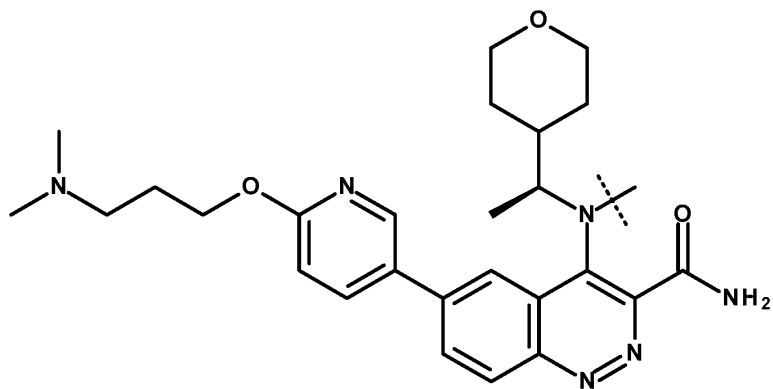
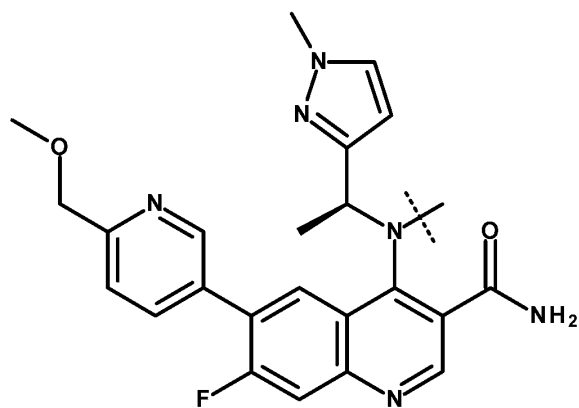


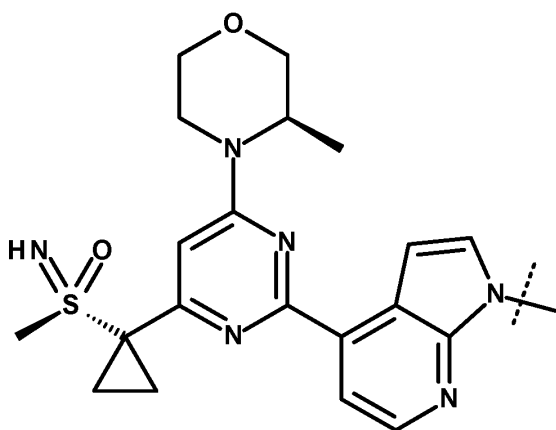
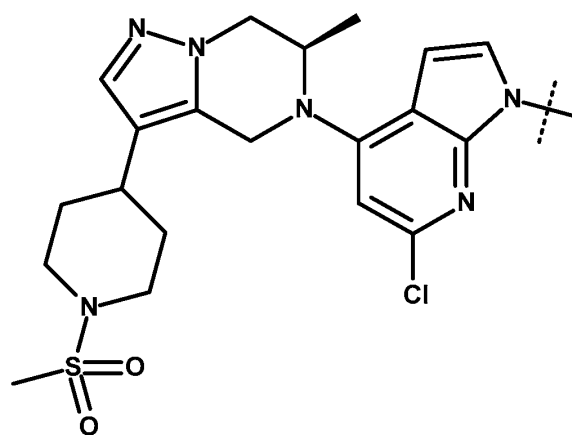
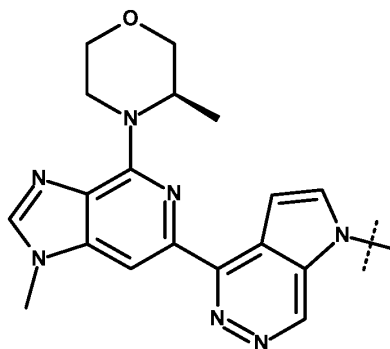




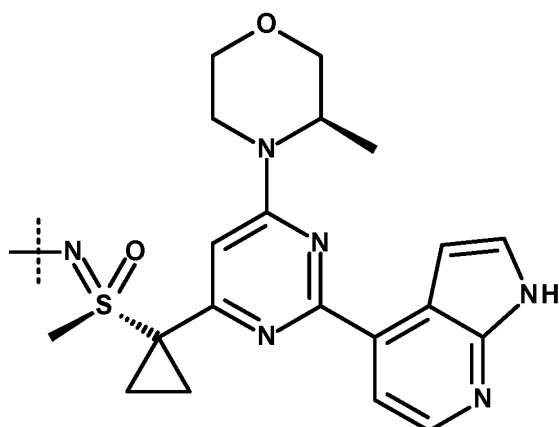




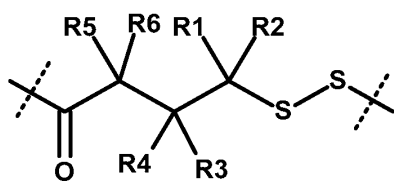
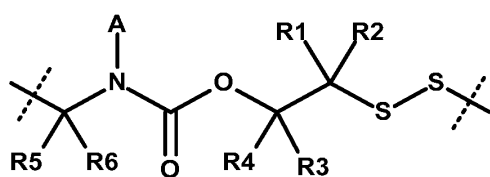
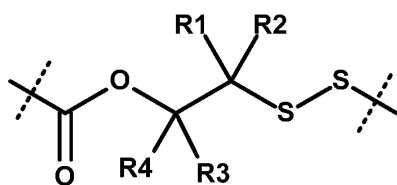




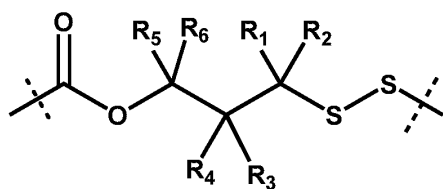
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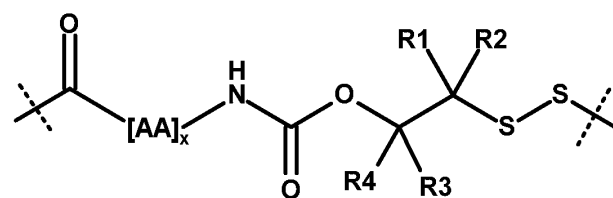
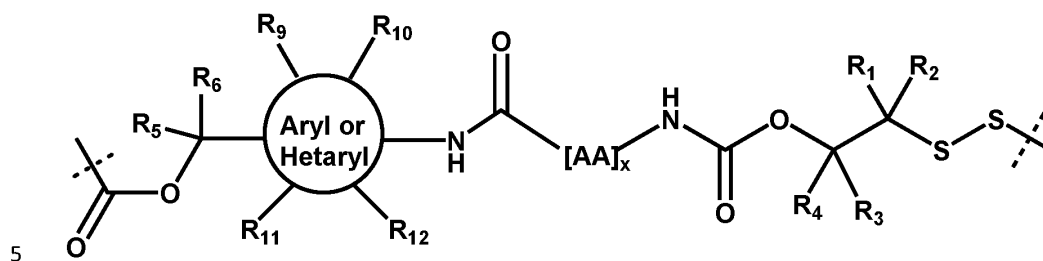
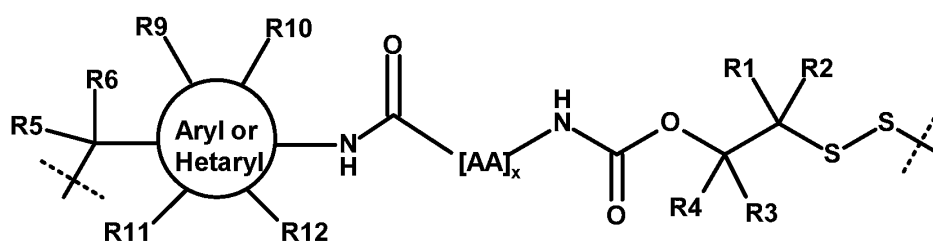
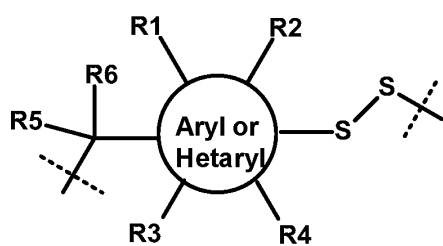
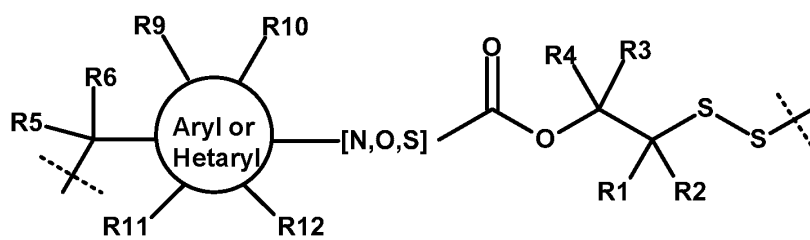


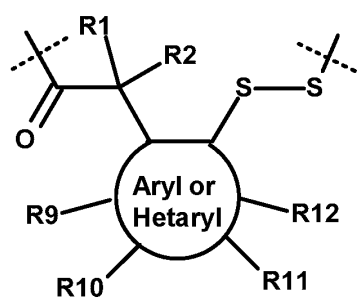
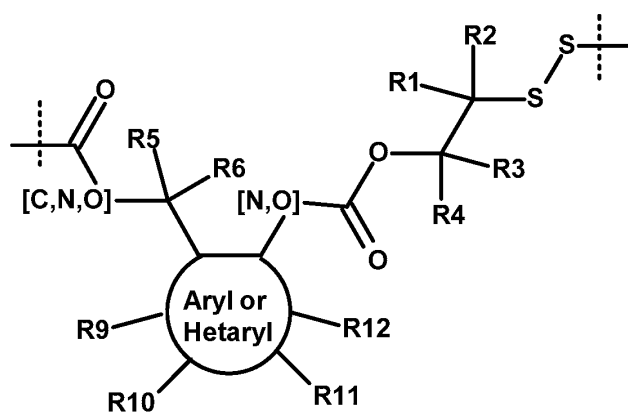
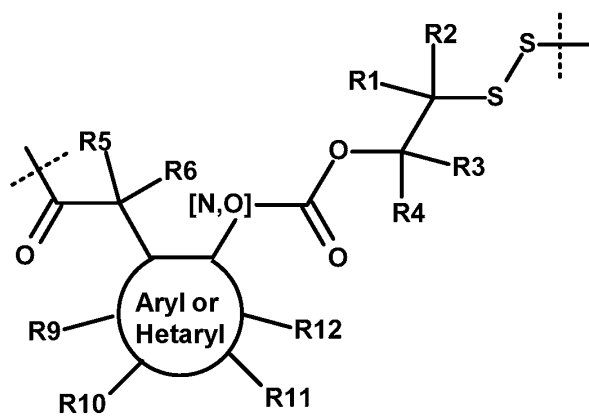
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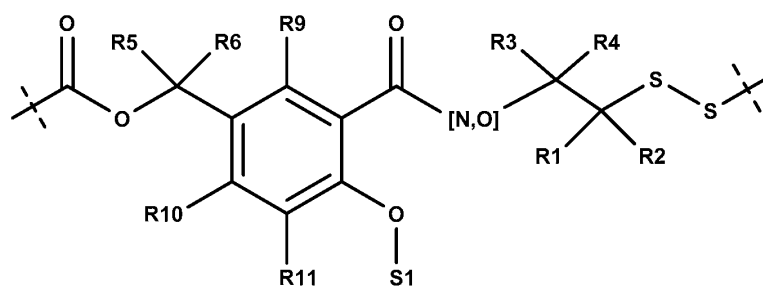
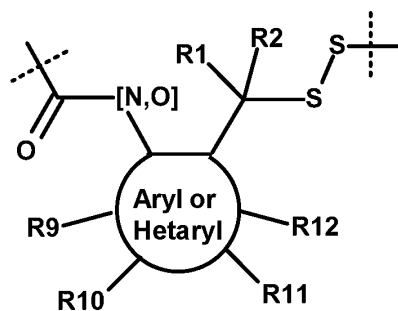
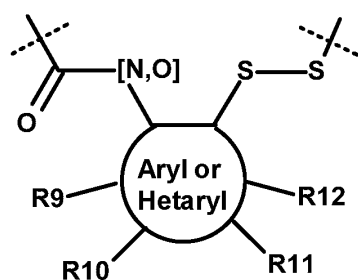
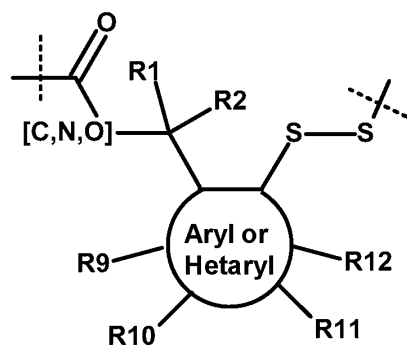


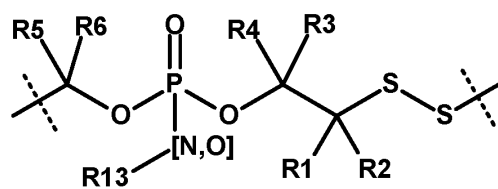
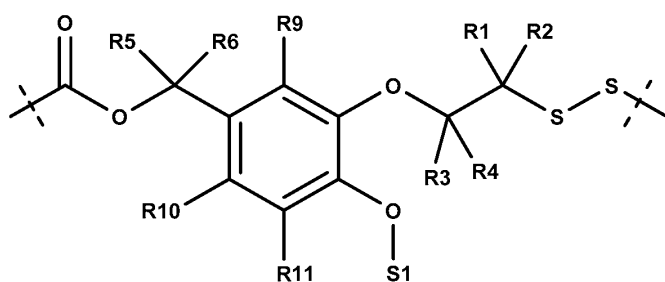
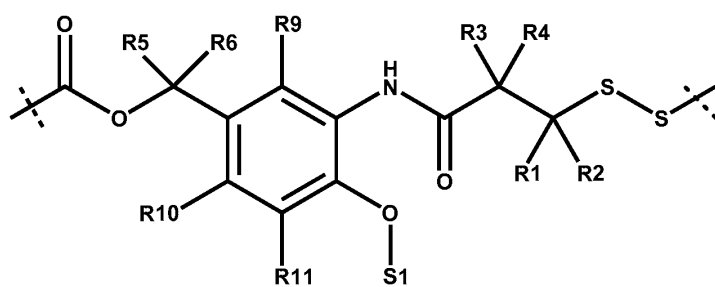
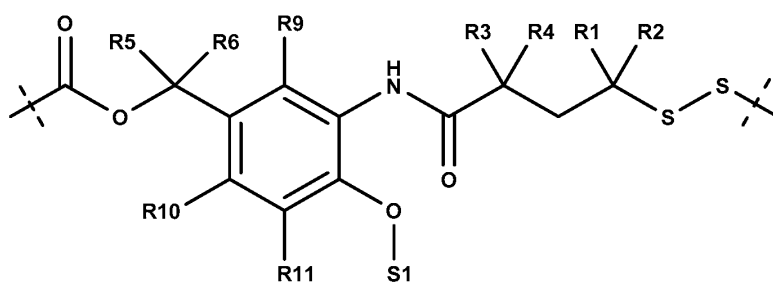
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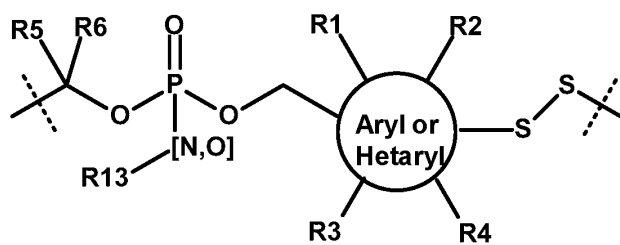


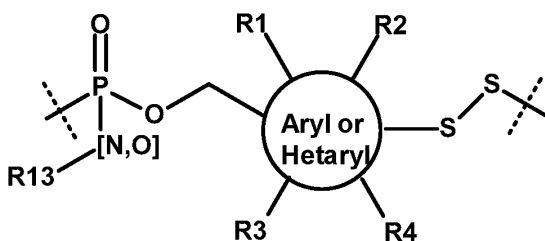
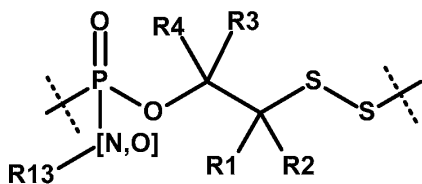
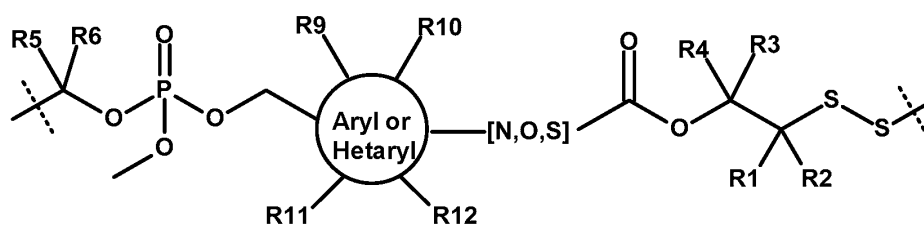




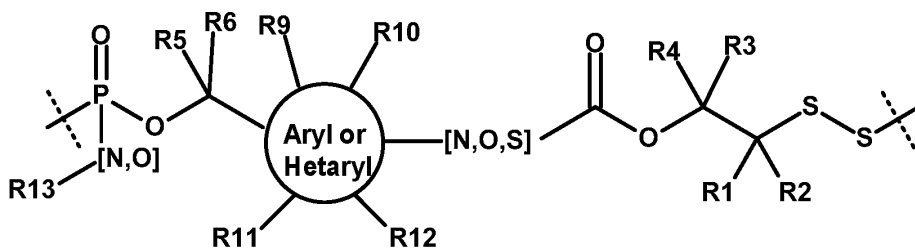


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- 5 $R^1, R^2, R^3, R^4, R^5, R^6, R^9, R^{10}, R^{11}$, and R^{12} are each independently selected from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, 5-10 membered heteroaryl, halo, CN, NO_2 , OR^{a1} , SR^{a1} , $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$, wherein said C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, and 5-10 membered heteroaryl are each optionally substituted with 1, 2, or 3 substituents
- 10 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_{3-7} 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 $NR^{c1}C(O)NR^{c1}R^{d1}$;

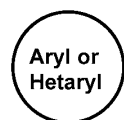
5 or R^1 and R^3 together with the carbon atom to which they are attached form a C_{3-7} cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO_2 , OR^{a1} , SR^{a1} , $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$,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

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

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

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

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



20 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;

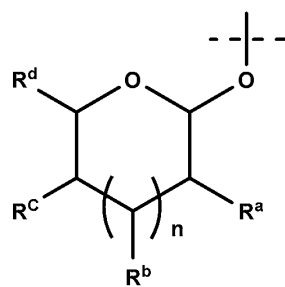
[N, O, S] is NH, O, or S;

[N, O] is NH or O;

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

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

[AA]_x is a peptide that may be cleaved by enzymatic action;



S1 is ;

each R^a , R^b , R^c , and R^d are independently selected from H, C_{1-4} alkyl, OR^{a2} , CO_2R^{a2} , and $OC(=O)R^{a2}$, wherein said C_{1-4} alkyl is optionally substituted with OR^{a2} , CO_2R^{a2} , and $OC(=O)R^{a2}$;

- 5 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 ;

R^{a2} is H or C_{1-4} alkyl; and

n is 0 or 1.

- 10 In some embodiments, the lefthand side of Q attaches to R^8 and the righthand side of Q attaches to R^7 .

In some embodiments, a sulfur atom of the disulfide moiety of Q is part of a cysteine residue of R^7 .

- Suitable peptides for use as R^7 are described, for example, in United States patents
 15 8,076,451 and 9,289,508 (which are incorporated herein by reference in their entirety), although other peptides capable of such selective insertion could be used. 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. Without being bound by theory, it is believed that the R^7 peptide reversibly folds and inserts across cell membranes
 20 in response to pH changes. The R^7 peptide can target acidic tissue and selectively translocate polar, cell-impermeable molecules across cell membranes in response to low extracellular pH. In some embodiments, R^7 is a peptide capable of selectively delivering R_sQ - across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.0. In some embodiments, R^7 is a peptide capable of selectively delivering R_sQ - across a cell membrane
 25 having an acidic or hypoxic mantle having a pH less than about 6.5. In some embodiments, R^7 is a peptide capable of selectively delivering R_sQ - across a cell membrane having an acidic or hypoxic mantle having a pH less than about 5.5. In some embodiments, R^7 is a

peptide capable of selectively delivering R₈Q- across a cell membrane having an acidic or hypoxic mantle having a pH between about 5.0 and about 6.0.

In some embodiments, R⁷ is attached to Q through a cysteine residue of R⁷. In some embodiments, the sulfur atom of the cysteine residue can form part of the disulfide bond of the disulfide bond-containing linker.

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

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1),

AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO. 2; Pv2),

and

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3);

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

AAEQNPIYWARYADWLFTTPLLDDLALLVDADEGTC (SEQ ID NO. 5; Pv5);

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),

AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO. 2; Pv2),

and

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3),

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

AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO. 2; Pv2).

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-AAEQNPIYWARYADWLFTTPLLDDLALLVDADEGKCG (SEQ ID NO. 4; Pv4).

In some embodiments, R⁷ is a peptide comprising the sequence

AAEQNPIYWARYADWLFTTPLLDDLALLVDADEGTC (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).

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

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

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

15 Table 1. Additional R⁷ Sequences

SEQ ID NO.	Sequence
6	AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG
7	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
8	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
9	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
10	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
11	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
12	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
13	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
14	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
15	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
16	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADECT
17	ACEQNPIYWARYANWLFTTPLLLLNLALLVDADEGTG
18	ACEQNPIYWARYAKWLFTTPLLLLKLALLVDADEGTG
19	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
20	AAEQNPIYWARYADWLFTTPLLLLALALLVDADEGT
21	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGT
22	AAEQNPIYWARYADWLFTTALLLLDLALLVDADEGT

23	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGT
24	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGT
25	AAEQNPIYWARYADWLFTDPLLLLDLALLVDADEGT
26	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCTG
27	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
28	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
29	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCTG
30	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCTG
31	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
32	DDDEDNPIYWARYADWLFTTPLLLHGHALLVDADEGT
33	DDDEDNPIYWARYAHLFTTPLLLHGHALLVDADEGT
34	DDDEDNPIYWARYAHLFTTPLLLHGHALLVNADEGT
35	DDDEDNPIYWARYAHLFTTPLLLHGHALLVNANECT
36	AEQNPIYWARYADFLFTTPLLLLDLALLVDADET
37	AEQNPIYFARYADWLFTTPLLLLDLALLVDADEGT
38	AEQNPIYFARYADFLFTTPLLLLDLALLVDADET
39	AKEDQNPYWARYADWLFTTPLLLLDLALLVDG
40	ACEDQNPYWARYADWLFTTPLLLLDLALLVDG
41	AEDQNPYWARYADWLFTTPLLLLDLALLVDCG
42	AEDQNPYWARYADWLFTTPLLLLELALLVECG
43	AKEDQNPYWRAYADLFTPLTLLDALLWDG
44	ACEDQNPYWRAYADLFTPLTLLDALLWDG
45	ACDDQNPWRAYLDLLFPTDLLLLDWW
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 DADEGTCTG

60	WARYADWLFTTPLLDDLALLV DADEGCT
61	GGEQNPIY WARYADWLFTTPLLDDLALLV DADEGTCG
62	ACEQNPIY WARYADWLFTTPLLDDLALLV DADEGT
63	AKEQNPIY WARYADWLFTTPLLDDLALLV DADEGT
64	AKEQNPIY WARYADWLFTTPLLDDLALLV DADECT
65	AAEQNPIY WARYADWLFTTALLDDLALLV DADEGT
66	ACAEQNPIY WARYADWLFTTGLLDDLALLV DADEGT
67	AEQNPIY WARYADFLFTTALLDDLALLV DADE_T
68	AEQNPIY FARYADWLFTTPLLDDLALLV DADEGT
69	AEQNPIY FARYADFLFTTPLLDDLALLW DADE_T
70	AKEDQNP_Y WARYADWLFTTPLLDDLALLV DG_____
71	ACEDQNP_Y WARYADWLFTTPLLDDLALLV DG_____
72	AEDQNP_Y WARYADWLFTTPLLDDLALLV DG_____
73	AEDQNP_Y WARYADWLFTTPLLLELALLV ECG_____
74	AKEDQNP_Y WRAYAD_LFT_PLTLLDLLALW DG_____
75	ACEDQNP_Y WRAYAD_LFT_PLTLLDLLALW DG_____
76	AKEDQNDP_Y WARYADWLFTTPLLDDLALLV G_____
77	TEDADVLLALDLLLLPTTFLWDAYRAWYPNQECA
78	GGEQNPIY WARYADWLFTTPLLDDLALLV DADEGT
79	AEQNPIY WARYADWLFTTPL
80	AEQNPIY WARYADWLFTTPCL
81	ACEQNPIY WARYADWLFTTPL
82	ACEQNPIY FARYADWLFTTPL
83	ACDDQNP WRAYLDLLFPTDTLLLDLLW
84	ACEEQNP WRAYLELLFPTETLLLELLW
85	ACDDQNP WARYLDWLFPTDTLLLDL
86	CDNNNP WRAYLDLLFPTDTLLLDW
87	ACEEQNP WARYLEWLFPTETLLLEL
88	ACEDQNP WARYADWLFPTTLLLD
89	ACEEQNP WARYAEWLFPTTLLLE
90	ACEDQNP WARYADLLFPTTLAW
91	ACEDQNP WARYAELLFPTTLW
92	KEDQNP WARYADLLFPTTLW
93	DDDEDNP IYWARYAHLFTTPLLHLLGALLVDADECT
94	DDDEDNPIYWARYAHLFTTPLLHLLGALLVDADECT
95	DDDEDNPIYWARYAHLFTTPLLHLLGALLVNADECT
96	DDDEDNPIYWARYAHLFTTPLLHLLGALLVNANECT

97	DDDEDNPIYWARYADWLFTTPLLLHLHGALLVDADECT
98	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGIG
99	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
100	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
101	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
102	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
103	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
104	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGTCG
105	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
106	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
107	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
108	GGEQNPIYWAQDYADWLFTTPLLLLDLALLVDADEGTCG
109	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
110	ACEQNPIYWARYANWLFTTPLLLNLALLVDADEGTG
111	DDDEDNPIYWARYAHLFTTPLLLHLHGALLVNANECT
112	DDDEDNPIYWARYAHLFTTPLLLHLHGALLVNADECT
113	DDDEDNPIYWARYADWLFTTPLLLHLHGALLVDADECT
114	DDDEDNPIYWARYAHLFTTPLLLHLHGALLVDADECT
115	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
116	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
117	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCG
118	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCG
119	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
120	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
121	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
122	GGEQNPIYWAQDYADWLFTTPLLLLDLALLVDADEGTCG
123	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
124	DDDEDNPIYWARYAHLFTTPLLLHLHGALLVNADECT
125	DDDEDNPIYWARYAHLFTTPLLLHLHGALLVNANECT
126	ACEQNPIYWARYAKWLFTTPLLLKLALLVDADEGTG
127	GGEQNPIYWAQDYADWLFTTPLLLLDLALLVDADEGTCG
128	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
129	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCG
130	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCG
131	AAEQNPIYWARYADWLFTDLPLLLLDLALLVDADEGT
132	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG
133	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG

134	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
135	AEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCTG
136	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
137	GGEQNPIYWAQDYAWLFTTPLLLLDLALLDADEGTCTG
138	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCTG
139	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
140	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTGEGTK(rhodamine)C(phalloidin)G
141	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
142	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
143	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG(phalloidin)G
144	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
145	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
146	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
147	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
148	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT
149	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
150	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
151	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
152	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG(phalloidin)G
153	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
154	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
155	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
156	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
157	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
158	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
159	DDDEDNPIYWARYADWLFTTPLLLLDGALLVNADECT
160	DDDEDNPIYWARYADWLFTTPLLLLDGALLVNADECT
161	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
162	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
163	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
164	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
165	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
166	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNANECT
167	DDDEDNPIYWARYADWLFTTPLLLLDGALLVNADECT
168	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
169	DDDEDNPIYWARYAHLFTTPLLLLDGALLVNADECT
170	GGEQNPIYWARYADWLFTTPLLLLDLALLVNANQGT

171	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
172	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
173	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
174	DDDEDNPIYWARYAHMLFTTPLLLLDGALLVDADECT
175	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNANECT
176	DDDEDNPIYWARYAHLFTTPLLLLDGALLVDADECT
177	DDDEDNPIYWARYADWLFTTPLLLLHGALLVDADECT
178	DDDEDNPIYWARYAHLFTTPLLLLHGALLVDADECT
179	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNADECT
180	DDDEDNPIYWARYAHLFTTPLLLLHGALLVNANECT
181	AAEQNPIYWARYADWLFTTGLLLLDLALLVDADEGT
182	GGEQNPIYWARYAWDLFTTPLLLLDLALLVDADEGTCTG
183	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCTG
184	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
185	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCTG
186	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCTG
187	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
188	GGEQNPIYWARYADWLFTTPLLLDLALLVDADEGTCTG
189	GGEQNPIYWARYADWLFTTPLLLDLALLVDADEGTCTG
190	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
191	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCTG
192	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
193	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCTG
194	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
195	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCTG
196	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCTG
197	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCTG
198	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCTG
199	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
200	GGEQNPIYWAQYADWLFTTPLLLLDLALLVDADEGTCTG
201	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCTG
202	AAEQNPIYWARYADWLFTTPLLLELALLVDADEGTKCG
203	EGTK(rhidamine)C(phalloidin)G
204	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
205	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG
206	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC(phalloidin)G
207	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG

208	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
209	AAEQNPIYWARYADWLFTDLPLLLLDLLALLVDADEGT
210	AAEQNPIYWARYAAWLFTTPLLLLDLALLVDADEGTCTG
211	GGEQNPIYWAQYDAWLFTTPLLLLDLALLVDADEGTCTG
212	GGEQNPIYWAQDYAWLFTTPLLLLDLALLVDADEGTCTG
213	GGEQNPIYWARYDAWLFTTPLLLLDLALLVDADEGTCTG
214	AAEQNPIYWARYAEWLFTTPLLLLDLALLVDADEGTCTG
215	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCTG
216	AAEQNPIYWARYADWLFTTPLLLALALLVDADEGTCTG
217	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTCTG
218	AAEQNPIYWARYAEWLFTTPLLLLELALLVDADEGTCTG
219	AAEQNPIYWARYADWLFTTPLLLLELALLVDADEGTKCG
220	ACEQNPIYWARYAKWLFTTPLLLKLALLVDADEGTG
221	ACEQNPIYWARYANWLFTTPLLLNLALLVDADEGTG
222	AAEQNPIYWARYADWLFTTALLLLDLALLVDADEGT
223	AEQNPIYFARYADLLFPTTLAW
224	AEQNPIYWARYADLLFPTTLAF
225	AEQNPIYWARYADLLFPTTLAW
226	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
227	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT
228	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
229	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG
230	AKEQNPIYWARYADWLFTTPLLLLDLALLVDADECT
231	CCTCTTACCTCAGTTACA
232	D-Arg8 D-Arg8-CCTCTTACCTCAGTTACA
233	D-Lys4 D-Lys4-CCTCTTACCTCAGTTACA
234	S-S-CCTCTTACCTCAGTTACA
235	S-S-CCTCTGACCTCATTTACA
236	D-Arg8-Deca D-Arg8-Deca-CCTCTTACCTCAGTTACA
237	D-Arg8-Deca-mismatch D-Arg8-Deca-CCTCTGACCTCATTTACA
238	S-S-CCTCTTACCTCAGTTACA
239	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCTG
240	AEDQNPIYWARYDWLFTTPLLLLDLALLVDCG
241	AEDQNPIYWARYADWLFTTPLLLLELALLVECG
242	AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGCT
243	ACEQNPIYWARYADWLFTTPLLLLDLALLVDADET
244	AE-QN-PI YWARYADWLFTTPLLLLDLALLV DADEGT-COOH

245	AEDQN-P- YWARYADWLFTTPLLALLDLALLV D---G--COOH
246	AEDQNDP-YWARYADWLFTTPLLALLDLALLV---G--COOH
247	AEQNPI YWARYADFLFTTPLLALLDLALLV DADET-COOH
248	AEQNPI YFARYADWLFTTPLLALLDLALLV DADET-COOH
249	AEQNPI YFARYADFLFTTPLLALLDLALLW DADET-COOH
250	AE-QN-PI YWARYADWLFTTPLLALLDLALLV DADEGCT-COOH
251	AEDQN-PI YWARYADWLFTTPLLALLDLALLV DC--G-T-COOH
252	AEDQNDPI YWARYADWLFTTPLLALLLELALLV EC--G-T-COOH
253	Chelate-ACEEQNPWARYLEWLFPTETLLEL
254	AEQNPIY WARYADWLFTTPLLALLDLALLV DADEGT-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 YWARYADWLFTTPLLALLLELALLV EC-Biotin-G-COOH
260	ACEQNPIY WARYADWLFTTPLLALLDLALLV DADEGT
261	ACEDQNPY WARYADWLFTTPLLALLDLALLV DG
262	ACEDQNPY WRAYADLFTPLTLLDLLALW DG
263	ACDDQNP WRAYLDLLFPTDTLLLDLLW
264	WRAYLELLFPTETLLELLW
265	WARYLDWLFPTDTLLLDL
266	WRAYLDLLFPTDTLLLDW
267	WARYLEWLFPTETLLEL
268	WAQYLELLFPTETLLEW
269	WRAYLELLFPTETLLEW
270	WARYADWLFPTTLLLD
271	WARYAEWLFPTTLLLE
272	ACEDQNP WARYADLLFPTTLAW
273	ACEEQNP WARYAELLFPTTLAW
274	Ac-TEDAD VLLALDLLLLPTTFLWDAYRAW YPNQECA-Am
275	CDDDDDNPNY WARYANWLFTTPLLALLNGALLV EAEET
276	CDDDDDNPNY WARYAPWLFTTPLLALLPGALLV EAEET
277	Ac-AEQNPIYWARYADWLFTTPLLALLDLALLVDADEGCT
278	Ac-AEQNPIYWARYADWLFTTPLLALLDLALLVDADEGTG
279	ACEQNPIYWARYANWLFTTPLLALLNLALLVDADEGT
280	Ac-AAEQNPIYWARYADWLFTTPLLALLLELALLVDADEGTKCG
281	DDDEDNPIYWARYADWLFTTPLLALLHGALLVDADET

282	CDDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADET
283	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVDADEGT
284	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNADEGT
285	DDDEDNPIYWARYAHWLFTTPLLLLHGALLVNANEGT
286	AKEDQNDPYWARYADWLFTTPLLLLDLALLVG
287	AEDQNPYWARYADWLFTTPLLLLELALLVCG
288	AKDDQNPWRAYLDLLFPTDTLLLDLLWC
289	ACEEQNPWRAYLELLFPTETLLLELLW
290	ACDDQNPWARYLDWLFTPTDTLLLDL
291	CDNNNPWRAYLDLLFPTDTLLLDW
292	CEEQPPWAQYLELLFPTETLLLEW
293	EEQPPWRAYLELLFPTETLLLEW
294	CDDDDDNPNYWARYANWLFTTPLLLLNALLVEAEET
295	CDDDDDNPNYWARYAPWLFTTPLLLLPALLVEAEE
296	AEQNPIYFARYADLLFPTTLAW
297	AEQNPIYWARYADLLFPTTLAF
298	AEQNPIYWARYADLLFPTTLAW
299	KEDQNPWARYADLLFPTTLW
300	ACEEQNPQAEYAEWLFTTLLLE
301	AAEEQNPWARYLEWLFTETLLLEL
302	AKEEQNPWARYLEWLFTETLLLEL
303	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
304	XXEXNPIYWAXXXXXLFTXXLLLXXXALLVXAXXXTXG
305	DAAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
306	GGEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
307	XXEXNPIYWAXXXXXLFTXXLLLXXXALLVXAXXXTGG
308	DGGEQNDPIYWARYADWLFTTPLLLLDLALLVDADEGCTXGG
309	AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTGG
310	AEDQNPYWARYDWLFTTPLLLLDLALLVDCG
311	GLAGLAGLLGLEGLLGLPLGLLEGLWLGLELEGN

In some embodiments, R⁷ is a peptide having 10 to 50 amino acids. In some
embodiments, R⁷ is a peptide having 20 to 40 amino acids. In some embodiments, R⁷ is a
peptide having 20 to 40 amino acids. In some embodiments, R⁷ is a peptide having 10 to
5 20 amino acids. In some embodiments, R⁷ is a peptide having 20 to 30 amino acids. In
some embodiments, R⁷ is a peptide having 30 to 40 amino acids.

Suitable therapeutic molecules (e.g., R⁸) for use in the invention include those which have undesirable side effects when delivered systemically because of their possible deleterious effect on normal tissue. Example therapeutic molecules are DNA repair inhibiting compounds such as inhibitors of PARP, ATR, DNK-PK, and ATM. Such DNA
 5 repair inhibiting compound are useful for treatment of cancer and other diseases in which inhibition of DNA repair would be desirable.

Three PARP inhibitors (olaparib, rucaparib, and niraparib) are currently commercially available and others are in development, such as AG-014699 (Agouron/Pfizer), KU-0059436 (KuDOS/AstraZeneca), INO-1001 (Inotek/Genentech),
 10 NT-125 (now E-7449; Eisai; 3H-Pyridazino[3,4,5-de]quinazolin-3-one, 8-[(1,3-dihydro-2H-isoindol-2-yl)methyl]-1,2-dihydro-), 2X-121 (2X Oncology; 3H-pyridazino[3,4,5-de]quinazolin-3-one, 8-[(1,3-dihydro-2H-isoindol-2-yl)methyl]-1,2-dihydro-), and ABT-888 (Abbvie). PARP inhibitors are disclosed in (for example) United States patents 6,100,283; 6,310,082; 6,495,541; 6,548,494; 6,696,437; 7,151,102; 7,196,085; 7,449,464;
 15 7,692,006; 7,781,596; 8,067,613; 8,071,623; and 8,697,736, which patents are incorporated herein by reference in their entirety.

In some embodiments, R¹ and R² are each independently selected from H and methyl, and R³, R⁴, R⁵, and R⁶ are each hydrogen.

In some embodiments, R¹ and R² are each independently selected from H and methyl.

20 In some embodiments, R¹ and R² are each H.

In some embodiments, R³ and R⁴ are each H.

In some embodiments, R⁵ and R⁶ are each H.

In some embodiments, R⁹, R¹⁰, R¹¹, and R¹² are each independently selected from H and methyl.

25 In some embodiments, the enzyme capable of cleaving [AA]_x is Cathepsin B, matrix-metalloproteinases (MMP), DPPIV, glycoprotein, peptidase, or caspase. In some embodiments, [AA]_x is a peptide having two to twelve (x is 2 to 12) amino acid (AA) residues. In some embodiments, [AA]_x is a peptide having two to ten (x is 2 to 10) amino acid (AA) residues. In some embodiments, [AA]_x is a peptide having two to five (x is 2 to 5)
 30 amino acid (AA) residues. In some embodiments, [AA]_x is a peptide having six to nine (x is 6 to 9) amino acid (AA) residues. In some embodiments, [AA]_x is a peptide having three to eight (x is 3 to 8) amino acid (AA) residues.

Peptide linkers (e.g., [AA]_x) that are capable of being cleaved by proteins are described in Yang, Y., Acta Pharmaceutica Sinica B 2011, 1(3), 143-159; Choi, K.,

Theranostics 2012, 2, 156-178; and Anderson, C., Ind. Eng. Chem. Res. 2017, 56, 4761-5777.

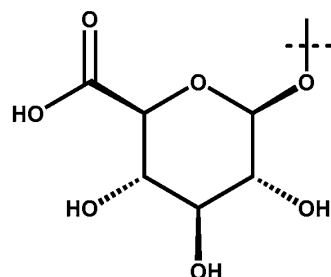
Peptide linkers (e.g., [AA]_x) that are capable of being cleaved by DPPIV are described in Diez-Torrubia, A., J. Med. Chem. 2010, 53, 559-572; Garcia-Aparicio, C., J. Med. Chem. 2006, 49, 5339-5351; Diez-Torrubia, A., ChemMedChem 2012, 7, 618-628; Dahan, A., Mol. Pharmaceutics 2014, 11, 4385-4394; Wickstrom, M., Oncotarget 2017, 8, 66641-66655; and Simplicio, A. L., Molecules, 2008, 13, 519-547.

Peptide linkers (e.g., [AA]_x) that are capable of being cleaved by cathepsin B are described in Caculitan, N., Cancer Res. 2017, 77(24), 7027-7037; Zhong, Y-J, International Journal of Oncology 2013, 42, 373-383; and Fan, P., Drug Metabolism and Disposition 2016, 44, 1253-1261.

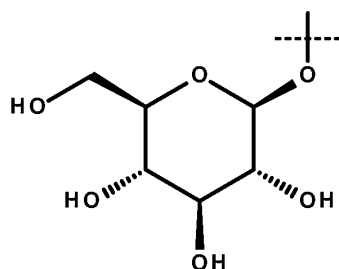
Peptide linkers (e.g., [AA]_x) that are capable of being cleaved by MMP (e.g., MMP-9) are described in Kalafatovic, D., Biomaterials 98 (2016), 192-202; Kim, HS, Gene Therapy 2013, 20, 378-385; and Yao, W., Trends in Pharmacological Sciences 2018, 39, 766-781.

In some embodiments, [AA]_x is -Pro_Gly-, -Val_Cit-, -Gly_Pro_Leu_Gly_Leu_Ala_Gly_Asp_Asp-, -Gly_Pro_GLeu_Gly_Val_Arg_Gly, or -Ser_Ser_Lys_Leu_Gly-.

In some embodiments, S1 is a group having the following structure:



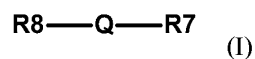
In some embodiments, S1 is a group having the following structure:



The S1 group can be a carbohydrate that can be cleaved by glucuronidase. Such carbohydrate groups are used in the art as glucuronide prodrugs, and are described in Grinda, M. Med. Chem. Commun. 2012, 3, 68-70; Herceg, V., Biorganic Chemistry 2018, 78, 372-380; Adiyala P., Bioorganic Chemistry 2018, 76, 288-293; and Kolakowski, R., Angew. Chem. Int. Ed. 2016, 55, 7948-7951.

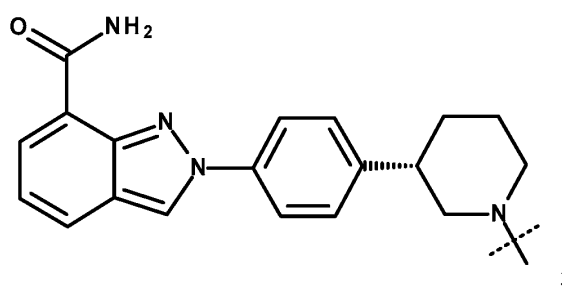
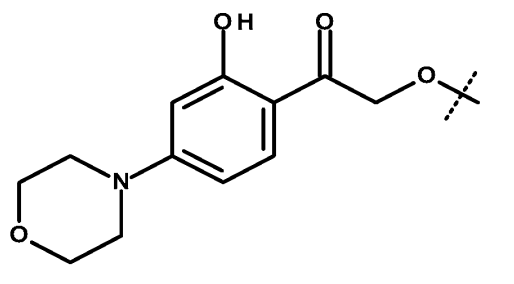
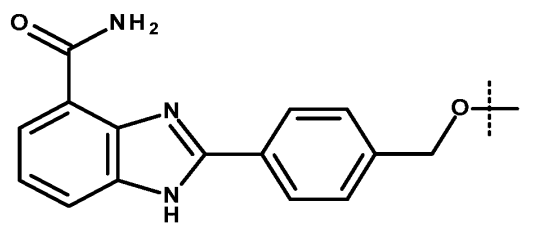
5 Chem. Int. Ed. 2016, 55, 7948-7951.

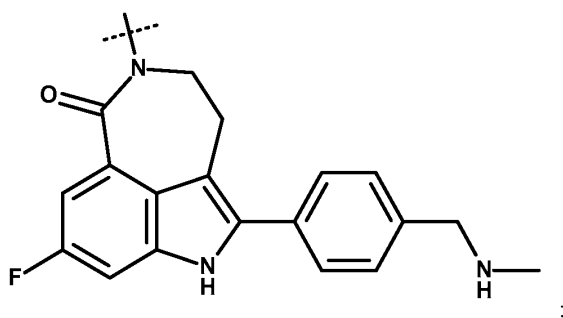
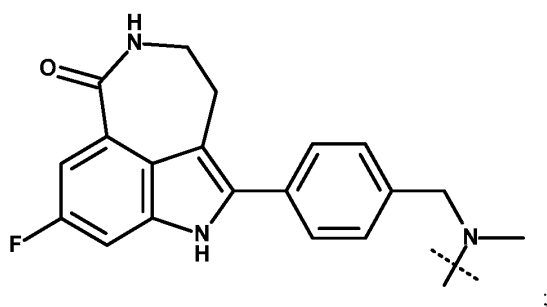
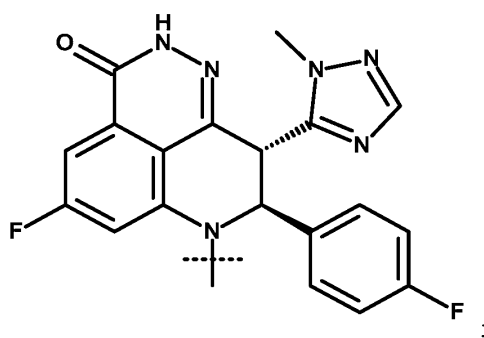
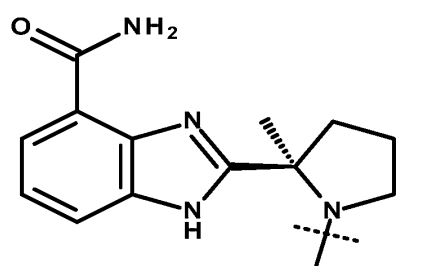
Also provided herein is a compound of formula (I)

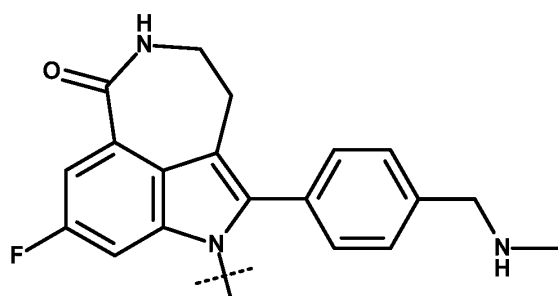


and the pharmaceutically acceptable salts thereof, wherein

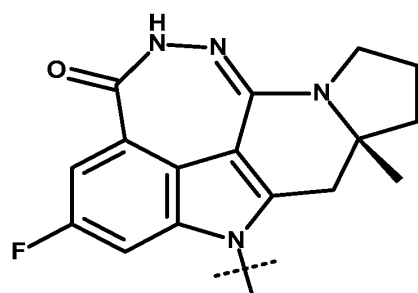
R⁸ is a member selected from the group consisting of:



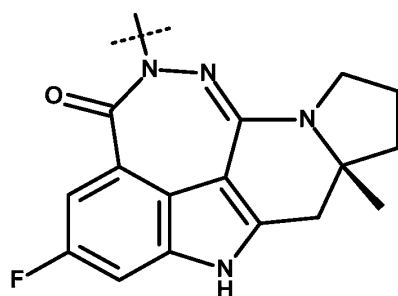




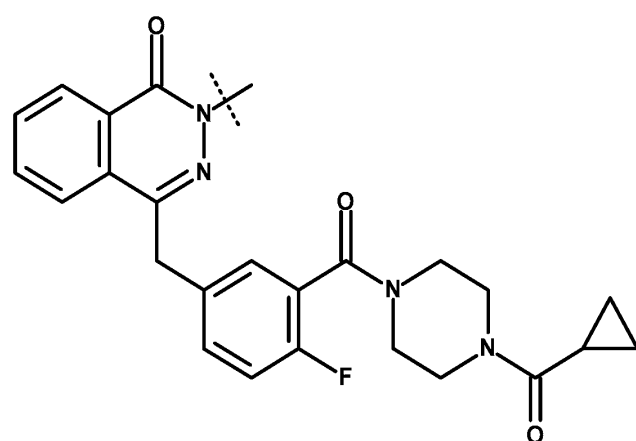
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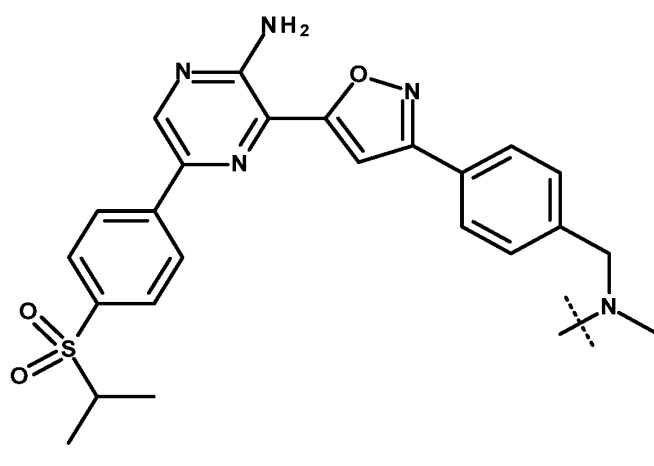
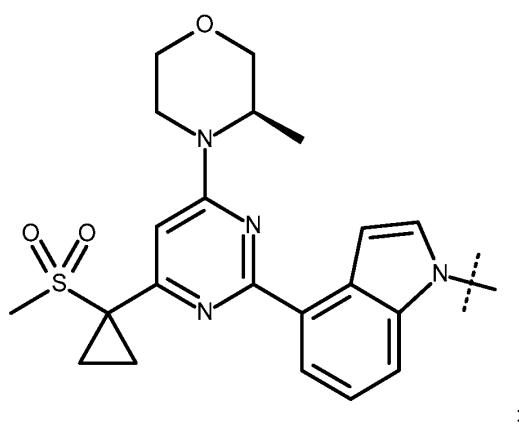
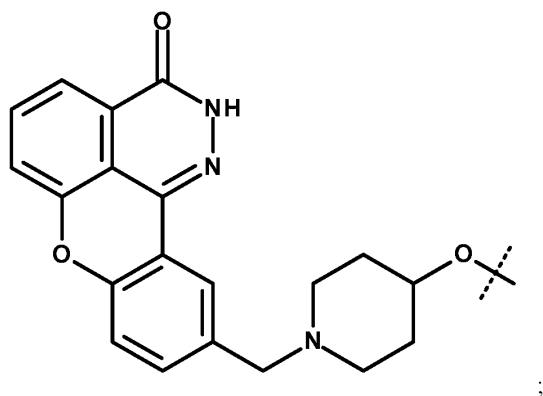
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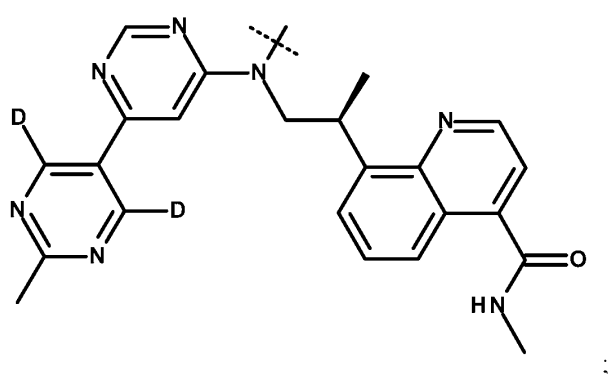
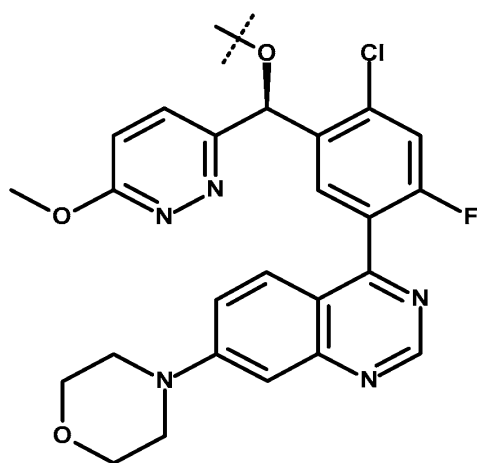
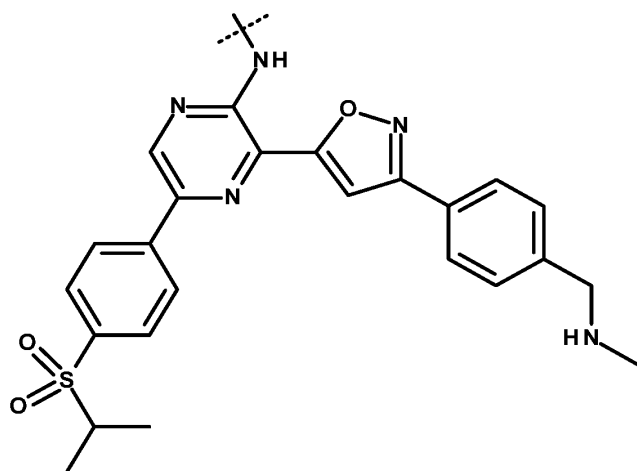


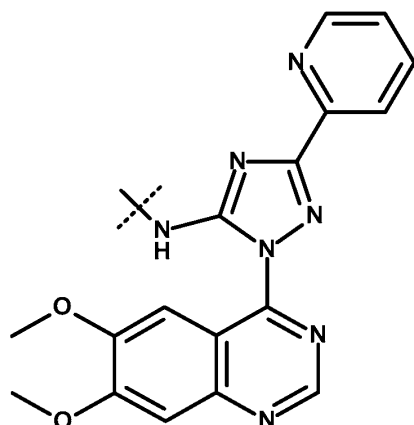
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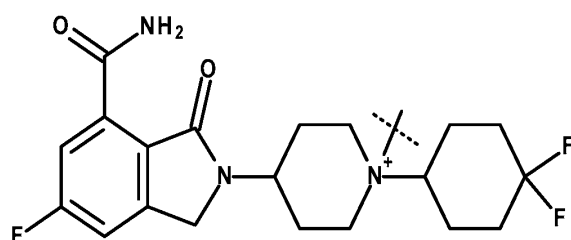
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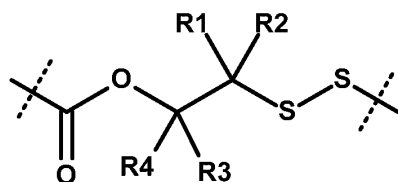


;and

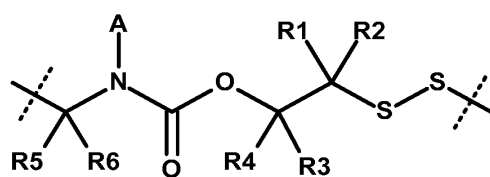


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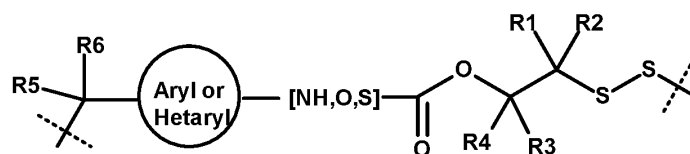
Q is a member selected from the group consisting of



;



and



wherein R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 are independently selected from H, optionally substituted C_1 - C_4 alkyl, optionally substituted C_1 - C_4 alkenyl, optionally substituted C_1 - C_4 alkoxy, amino optionally substituted with one or two C_1 - C_4 alkyl substituents, halo, nitro or hydroxyl, and wherein $[NH, O, S]$ means a linking NH, O, or S moiety,

5 and

R^7 is a peptide capable of selectively delivering R^8Q across a cell membrane having an acidic or hypoxic mantle in a pH dependent fashion.

In some embodiments, R^7 is a member selected from the group consisting of ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1),

10 AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO. 2; Pv2),

and

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3),

wherein R^7 and Q are attached through a cysteine residue.

15

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.

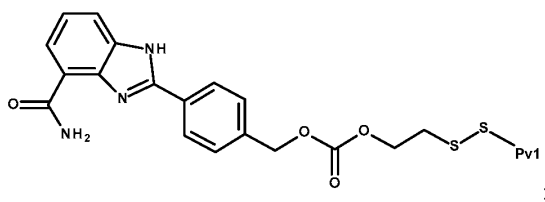
In some embodiments, R^7 is ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO. 1; Pv1).

20 In some embodiments, R^7 is

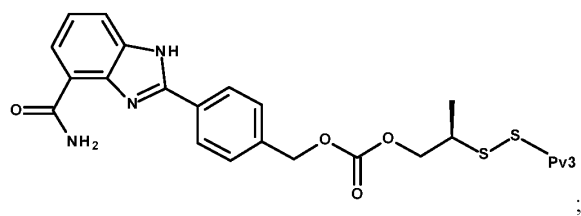
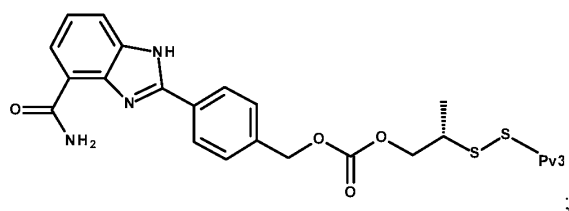
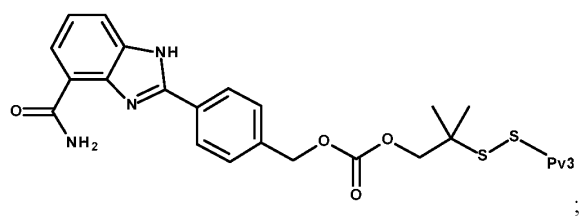
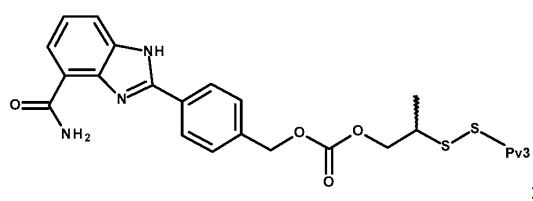
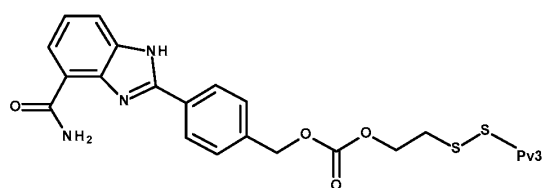
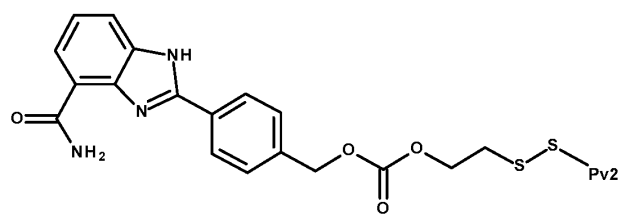
AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO. 2; Pv2).

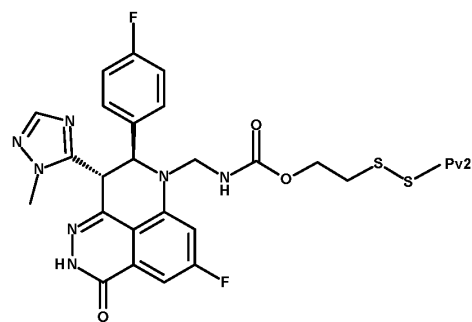
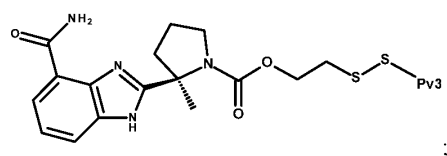
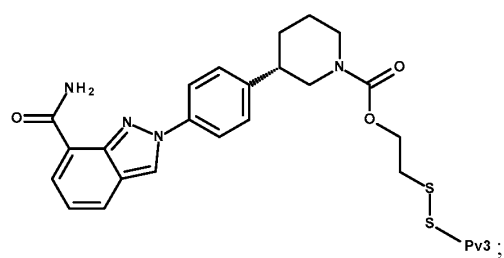
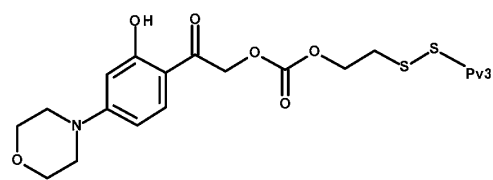
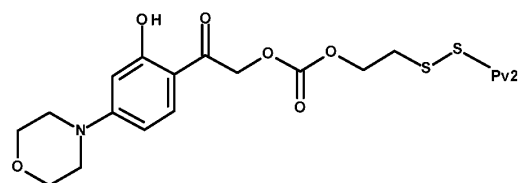
In some embodiments, R^7 is ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO. 3; Pv3).

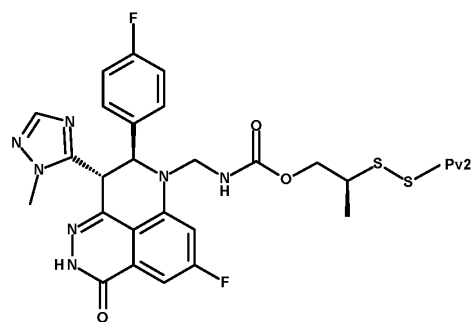
In some embodiments, the compound of formula (I) is selected from:



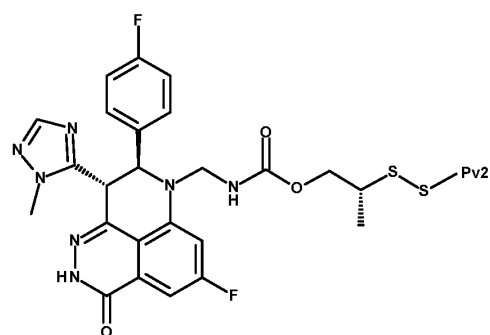
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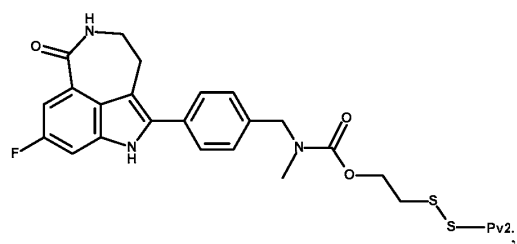




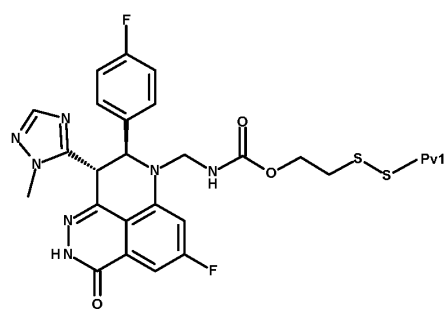
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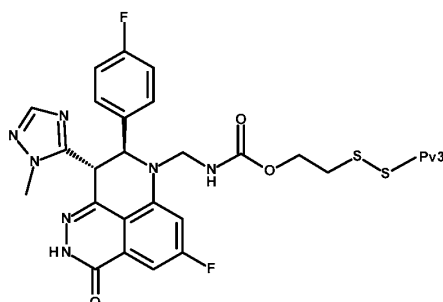
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; and



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

The Amino acids are represented by the IUPAC abbreviations, as follows: Alanine (Ala; A), Arginine (Arg; R), Asparagine (Asn; N), Aspartic acid (Asp; D), Cysteine (Cys; C), Glutamine (Gln; Q), Glutamic acid (Glu; E), Glycine (Gly; G), Histidine (His; H), Isoleucine (Ile; I), Leucine (Leu; L), Lysine (Lys; K), Methionine (Met; M), Phenylalanine (Phe; F), Proline (Pro; P), Serine (Ser; S), Threonine (Thr; T), Tryptophan (Trp; W), Tyrosine (Tyr; Y), Valine (Val; V). The term “Pv1” means ADDQNPWRAYLDLLFPTDTLLLDLLWCG, which is the the peptide of SEQ ID No. 1. The term “Pv2” means AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG, which is the peptide of SEQ ID No. 2. The term “Pv3” means ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG, which is the peptide of SEQ ID No. 3. The term “Pv4” means Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGKCG, which is the peptide of SEQ ID NO. 4. The term “Pv5” means AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC, which is the peptide of SEQ ID NO. 5. In the compounds of the invention, the peptides R⁷ are attached to the disulfide linker by the cysteine moiety.

The term “acidic and/or hypoxic mantle” refers to the environment of the cell in the diseased tissue in question having a pH lower than 7.0 and preferably lower than 6.5. An acidic or hypoxic mantle more preferably has a pH of about 5.5 and most preferably has a pH of about 5.0. The compounds of formula (I) insert across a cell membrane having an acidic and/or hypoxic mantle in a pH dependent fashion to insert 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 or hypoxic mantle surrounding the cell and not across the cell membrane of "normal" cells, which do not have an acidic or hypoxic mantle.

The terms "pH-sensitive" or "pH-dependent" as used herein to refer to the peptide R⁷ or to the mode of insertion of the peptide R⁷ or of the compounds of the invention across a cell membrane, means that the peptide has a higher affinity to a cell membrane lipid bilayer having an acidic or hypoxic mantle than a membrane lipid bilayer at neutral pH. Thus, the compounds of the invention preferentially insert through the cell membrane to insert 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 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, 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 C₁₋₄, C₁₋₆ and the like.

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

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

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

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

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

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

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

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

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

The term "haloalkyl" as used herein refers to an alkyl group in which one or more of the hydrogen atoms has been replaced by a halogen atom. The term " C_{n-m} haloalkyl" refers to a C_{n-m} alkyl group having *n* to *m* carbon atoms and from at least one up to $\{2(n \text{ to } m)+1\}$ 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 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 haloalkoxy group has 1 to 6, 1 to 4, or 1 to 3 carbon atoms.

5 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.

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

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

15 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
20 atoms. In some embodiments aryl groups have 10 carbon atoms. In some embodiments, the aryl group is phenyl.

The term "heteroaryl" or "heteroaromatic," employed alone or in combination with other terms, refers to a monocyclic or polycyclic aromatic heterocycle having at least one heteroatom ring member selected from sulfur, oxygen and nitrogen. In some embodiments,
25 the heteroaryl ring has 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, any ring-forming N in a heteroaryl moiety can be an N-oxide. In some embodiments, the heteroaryl has 5-14 ring atoms including carbon atoms and 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl has 5-10 ring atoms
30 including carbon atoms and 1, 2, 3 or 4 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl has 5-6 ring atoms and 1 or 2 heteroatom ring members independently selected from nitrogen, sulfur and oxygen. In some embodiments, the heteroaryl is a five-membered or six-membered

heteroaryl ring. In other embodiments, the heteroaryl is an eight-membered, nine-membered or ten-membered fused bicyclic heteroaryl ring.

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

5 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
10 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.
15 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
20 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
25 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
30 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 2pyrrolidinyl; 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.

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

In some embodiments, the compounds of the invention have the (*R*)-configuration. In other embodiments, the compounds have the (*S*)-configuration. In compounds with more
10 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
15 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
20 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
25 or substituted with isotopes of the atoms in natural or non-natural abundance. In some embodiments, the compound includes at least one deuterium atom. For example, one or more hydrogen atoms in a compound of the present disclosure can be replaced or substituted by deuterium. In some embodiments, the compound includes two or more deuterium atoms. In some embodiments, the compound includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 deuterium
30 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.

5 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).

10 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.

15 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
20 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
25 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.

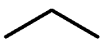
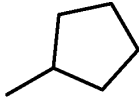
30 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation,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

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

- 5 The present invention also includes pharmaceutically acceptable salts of the compounds described herein. The term "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues
10 such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present invention include the non-toxic salts of the parent compound formed, *e.g.*, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods.
- 15 Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, alcohols (*e.g.*, methanol, ethanol, iso-propanol or butanol) or acetonitrile (MeCN) are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th Ed.,
20 (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.

- 25 As understood in the art, the symbol  is used in structural formulas to indicate the bond that is the point of attachment of the moiety containing it to the adjacent portion of the compound. According to a similar convention, pendant carbon atoms and their attached

hydrogen atoms may not be explicitly expressed. Thus, the symbol  represents a

methyl group, the symbol  represents an ethyl group, the symbol  represents a cyclopentyl group, and so on. The orientation of a substituent group in a

molecule or moiety is represented by the convention that the symbol  represents a bond in which the group is projecting out of the plane of the page toward the reader, while the symbol  represents a bond in which the group is projecting behind the plane of the page away from the reader, and the symbol  represents a bond in which the group has indeterminate orientation (i.e., the compound is diastereomeric).

All terms as used herein in this specification, unless otherwise stated, shall be understood in their ordinary meaning as known in the art. For example, “C₁₋₄alkyl” is a saturated aliphatic hydrocarbon monovalent radical containing 1-4 carbons such as methyl, ethyl, *n*-propyl, 1-methylethyl (isopropyl), *n*-butyl or *t*-butyl; “C₁₋₄ alkoxy” is a C₁₋₄ alkyl with a terminal oxygen, such as methoxy, ethoxy, propoxy, butoxy. All alkyl, alkenyl and alkynyl groups shall be understood as being branched or unbranched, cyclized or uncyclized where structurally possible and unless otherwise specified. Other more specific definitions are as follows:

The term “C_{1-n}-alkyl”, wherein *n* is an integer from 2 to 6, either alone or in combination with another radical denotes an acyclic, saturated, branched or linear hydrocarbon radical with 1 to *n* C atoms. For example the term C₁₋₅-alkyl embraces the radicals H₃C-, H₃C-CH₂-, H₃C-CH₂-CH₂-, H₃C-CH(CH₃)-, H₃C-CH₂-CH₂-CH₂-, H₃C-CH₂-CH(CH₃)-, H₃C-CH(CH₃)-CH₂-, H₃C-C(CH₃)₂-, H₃C-CH₂-CH₂-CH₂-CH₂-, H₃C-CH₂-CH₂-CH(CH₃)-, H₃C-CH₂-CH(CH₃)-CH₂-, H₃C-CH(CH₃)-CH₂-CH₂-, H₃C-CH₂-C(CH₃)₂-, H₃C-C(CH₃)₂-CH₂-, H₃C-CH(CH₃)-CH(CH₃)- and H₃C-CH₂-CH(CH₂CH₃)-.

The term “C_{3-n}-cycloalkyl”, wherein *n* is an integer 4 to 7, either alone or in combination with another radical denotes a cyclic, saturated, unbranched hydrocarbon radical with 3 to *n* C atoms. For example, the term C₃₋₇-cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

The term “heteroatom” as used herein shall be understood to mean atoms other than carbon such as O, N, S and P.

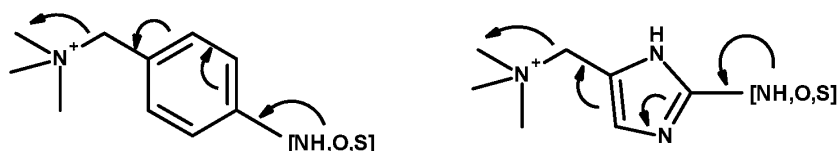
In some embodiments, alkyl groups or carbon chains, one or more carbon atoms can be optionally replaced by heteroatoms: O, S or N. It shall be understood that if N is not represented as substituted then it is NH, and that the heteroatoms may replace either terminal carbon atoms or internal carbon atoms within a branched or unbranched carbon chain. Such

groups can be substituted as herein above described by groups such as oxo to result in definitions such as but not limited to: alkoxy carbonyl, acyl, amido and thioxo.

In some embodiments, the term "aryl" as used herein, either alone or in combination with another radical, denotes a carbocyclic aromatic monocyclic group containing 6 carbon atoms which may be further fused to a second 5- or 6-membered carbocyclic group which may be aromatic, saturated or unsaturated. Aryl includes, but is not limited to, phenyl, indanyl, indenyl, naphthyl, anthracenyl, phenanthrenyl, tetrahydronaphthyl and dihydronaphthyl.

In some embodiments, the term "heteroaryl" (sometimes abbreviated "hetaryl") means an aromatic 5 to 6-membered monocyclic heteroaryl or an aromatic 7 to 11-membered heteroaryl bicyclic ring where at least one of the rings is aromatic, wherein the heteroaryl ring contains 1-4 heteroatoms such as N, O and S. Non-limiting examples of 5 to 6-membered monocyclic heteroaryl rings include furanyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, pyrazolyl, pyrrolyl, imidazolyl, tetrazolyl, triazolyl, thienyl, thiadiazolyl, pyridinyl, pyrimidinyl, pyridazinyl, pyrazinyl, triazinyl, and purinyl. Non-limiting examples of 7 to 11-membered heteroaryl bicyclic heteroaryl rings include benzimidazolyl, quinolinyl, dihydro-2H-quinolinyl, tetrahydroquinolinyl, isoquinolinyl, quinazolinyl, indazolyl, thieno[2,3-d]pyrimidinyl, indolyl, isoindolyl, benzofuranyl, dihydrobenzofuranyl, benzopyranyl, benzodioxolyl, benzoxazolyl and benzothiazolyl.

As used in Q, the definition of "aryl and hetaryl" are as described above. They are preferably phenyl or imidazolyl such as the following moieties that in conjunction with [NH,O,S] chemically undergo immolation to release R₃H as described below.



In some embodiments, the term "heterocycl" means a stable non-aromatic 4-8 membered monocyclic heterocyclic radical or a stable nonaromatic 6 to 11-membered fused bicyclic, bridged bicyclic or spirocyclic heterocyclic radical. The 5 to 11-membered heterocycle consists of carbon atoms and one or more, preferably from one to four heteroatoms chosen from nitrogen, oxygen and sulfur. The heterocycle may be either saturated or partially unsaturated. Non-limiting examples of nonaromatic 4-8 membered monocyclic heterocyclic radicals include tetrahydrofuranyl, azetidiny, pyrrolidinyl, pyranyl,

tetrahydropyranyl, dioxanyl, thiomorpholinyl, 1,1-dioxo-1 λ^6 -thiomorpholinyl, morpholinyl, piperidinyl, piperazinyl, and azepinyl. Non-limiting examples of nonaromatic 6 to 11-membered fused bicyclic radicals include octahydroindolyl, octahydrobenzofuranyl, and octahydrobenzothiophenyl. Non-limiting examples of nonaromatic 6 to 11-membered
5 bridged bicyclic radicals include 2-azabicyclo[2.2.1]heptanyl, 3-azabicyclo[3.1.0]hexanyl, and 3-azabicyclo[3.2.1]octanyl. Non-limiting examples of nonaromatic 6 to 11-membered spirocyclic heterocyclic radicals include 7-aza-spiro[3,3]heptanyl, 7-spiro[3,4]octanyl, and 7-aza-spiro[3,4]octanyl. The term “heterocyclyl” or is intended to include all the possible isomeric forms.

10 The term “halogen” and the corresponding term “halo” as used in the present specification shall be understood to mean bromine, chlorine, fluorine or iodine, or the corresponding bromo, chloro, fluoro, or iodo. The definitions “halogenated”, “partially or fully halogenated”; partially or fully fluorinated; “substituted by one or more halogen atoms”, includes for example, mono, di or tri halo derivatives on one or more carbon atoms. For alkyl,
15 non-limiting examples would be -CH₂CHF₂, -CF₃, etc.

Each alkyl, cycloalkyl, heterocycle, aryl or heteroaryl, or the analogs thereof, described herein shall be understood to be optionally partially or fully halogenated.

As used herein, “nitrogen” or “N” and “sulfur” or “S” includes any oxidized form of nitrogen and sulfur and the quaternized form of any basic nitrogen. For example, for an -S-
20 C₁₋₆ alkyl radical, unless otherwise specified, this shall be understood to include -S(O)-C₁₋₆ alkyl and -S(O)₂-C₁₋₆ alkyl, likewise, -S-R_a may be represented as phenyl-S(O)_m- when R_a is phenyl and where m is 0, 1 or 2.

Unless specifically indicated, throughout the specification and the appended claims, a given chemical formula or name shall encompass tautomers and all stereo, optical and
25 geometrical isomers (e.g. enantiomers, diastereomers, E/Z isomers, etc.) and racemates thereof as well as mixtures in different proportions of the separate enantiomers, mixtures of diastereomers, or mixtures of any of the foregoing forms where such isomers and enantiomers exist, as well as salts, including pharmaceutically acceptable salts thereof and solvates thereof such as for instance hydrates including solvates of the free compounds or
30 solvates of a salt of the compound. In the peptides R₇, the amino acids may be all of L configuration, all of D configuration, or a mixture of D and L configuration.

Compounds of the invention also include their isotopically-labelled forms. An isotopically-labelled form of a compound of the present invention is identical to said active agent but for the fact that one or more atoms of said compound have been replaced by an

atom or atoms having an atomic mass or mass number different from the atomic mass or mass number of said atom which is usually found in nature. Examples of isotopes which are readily available commercially and which can be incorporated into a compound of the present invention in accordance with well established procedures, include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, *e.g.*, ^2H (deuterium or "D"), ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively. A compound of the present invention, a prodrug thereof, or a pharmaceutically acceptable salt of either which contains one or more of the above-mentioned isotopes and/or other isotopes of other atoms is contemplated to be within the scope of the present invention.

10 The invention includes pharmaceutically acceptable derivatives of compounds of formula (I). The term "pharmaceutically acceptable derivative" refers to any pharmaceutically acceptable salt or ester, or any other compound which, upon administration to a patient, is capable of providing (directly or indirectly) a compound useful for the invention, or a pharmacologically active metabolite or pharmacologically active residue
15 thereof. A pharmacologically active metabolite shall be understood to mean any compound of the invention capable of being metabolized enzymatically or chemically. This includes, for example, hydroxylated or oxidized derivative compounds of the formula (I).

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts
20 thereof. 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. For example, such salts include acetates, ascorbates, benzenesulfonates, benzoates, besylates, bicarbonates, bitartrates, bromides/hydrobromides, edetates, camsylates, carbonates, chlorides/hydrochlorides, citrates,
25 edisylates, ethane disulfonates, estolates esylates, fumarates, gluceptates, gluconates, glutamates, glycolates, glycolylarsnates, hexylresorcinates, hydrabamines, hydroxymaleates, hydroxynaphthoates, iodides, isothionates, lactates, lactobionates, malates, maleates, mandelates, methanesulfonates, methylbromides, methylnitrates, methylsulfates, mucates, napsylates, nitrates, oxalates, pamoates, pantothenates, phenylacetates,
30 phosphates/diphosphates, polygalacturonates, propionates, salicylates, stearates, subacetates, succinates, sulfamides, sulfates, tannates, tartrates, teoclates, toluenesulfonates, triethiodides, ammonium, benzathines, chloroprocaines, cholines, diethanolamines, ethylenediamines, meglumines and procaines. Further pharmaceutically acceptable salts can be formed with cations from metals like aluminium, calcium, lithium, magnesium, potassium, sodium, zinc

and the like. (See, e.g., Pharmaceutical Salts, Birge, S.M. et al., J. Pharm. Sci., (1977), 66, 1-19).

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a sufficient amount of the appropriate base or acid in water or in an organic diluent like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile, or a mixture thereof.

Salts of other acids than those mentioned above which for example are useful for purifying or isolating the compounds of the present invention (e.g. trifluoro acetate salts) also comprise a part of the invention.

In addition, within the scope of the invention are prodrugs of the compounds of the formula (I). Prodrugs include those compounds that, upon simple chemical transformation, are modified to produce compounds of the invention. Simple chemical transformations include hydrolysis, oxidation and reduction. Specifically, when a prodrug is administered to a patient, the prodrug may be transformed into a compound disclosed hereinabove, thereby imparting the desired pharmacological effect. By reserving the right to proviso out or exclude any individual members of any such group, including any sub-ranges or combinations of sub-ranges within the group, that can be claimed according to a range or in any similar manner, less than the full measure of this disclosure can be claimed for any reason. Further, by reserving the right to proviso out or exclude any individual substituents, analogs, compounds, ligands, structures, or groups thereof, or any members of a claimed group, less than the full measure of this disclosure can be claimed for any reason.

The terms "a," "an," and "the" are intended to include plural alternatives, e.g., at least one, unless otherwise specified. For instance, the disclosure of "a therapeutic agent" or "a compound" is meant to encompass one, or mixtures or combinations of more than one, therapeutic agent or compound, respectively.

While compositions and methods are described in terms of "comprising" various components or steps, the compositions and methods can also "consist essentially of" or "consist of" the various components or steps. For example, a pharmaceutical composition described herein can comprise; alternatively, can consist essentially of; or alternatively, can consist of; (i) a therapeutically effective amount of a compound, or a pharmaceutically acceptable salt thereof, and (ii) a pharmaceutically acceptable diluent, excipient, or carrier.

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
5 synthetic routes, such as those in the Schemes below.

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

Preparation of compounds of the invention can involve the protection and
15 deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups is described, *e.g.*, in Kocienski, *Protecting Groups*, (Thieme, 2007); Robertson, *Protecting Group Chemistry*, (Oxford University Press, 2000); Smith *et al.*, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*,
20 6th Ed. (Wiley, 2007); 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).

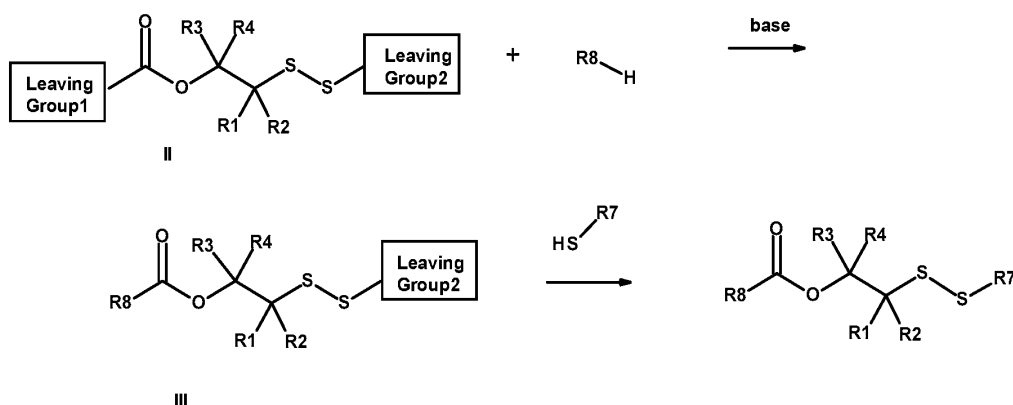
Reactions can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear
25 magnetic resonance spectroscopy (*e.g.*, ¹H or ¹³C), infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), mass spectrometry or by chromatographic methods such as high performance liquid 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
30 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.

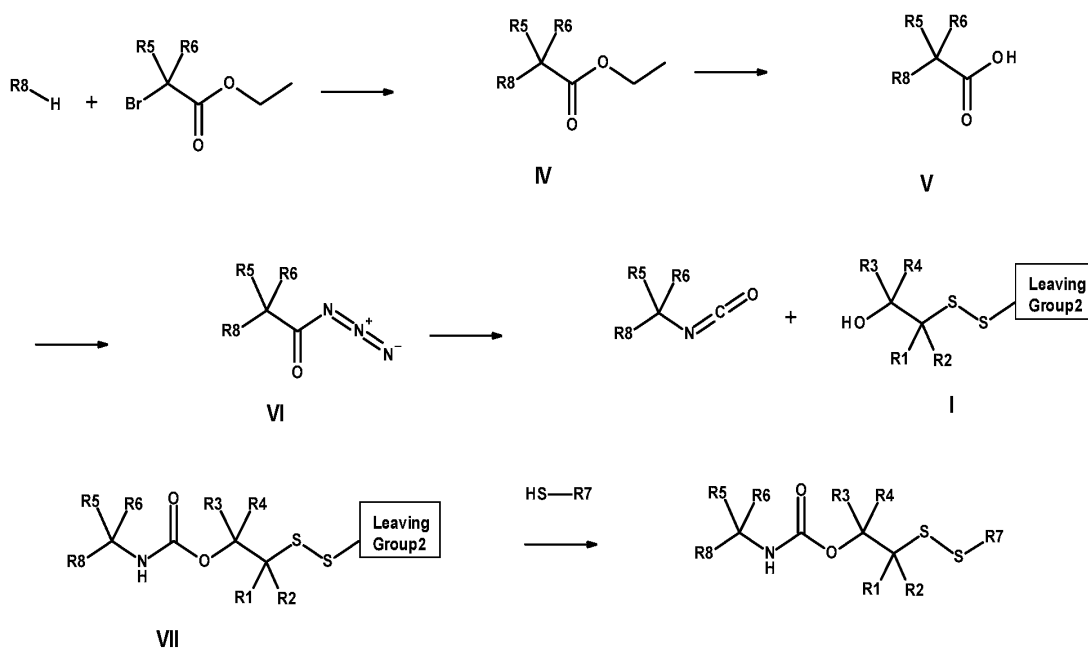
As shown in Scheme 1, Intermediate II, which is flanked by orthogonal leaving groups, can be reacted with a nucleophilic R^8H compound to give intermediate III. Intermediate III is then reacted with a thiol containing peptide ($HS-R^7$) that participates in a disulfide exchange reaction to give the final compound. Suitable leaving groups are described below.

Scheme 1: Synthesis of Carbonate and Carbamate Linked Compounds



The synthesis of aminated linked compounds is shown in Scheme 2. A nucleophilic R^8H compound is reacted with a bromoacetic acid derivative to provide intermediate IV. This ester containing intermediate is hydrolyzed under basic conditions to give intermediate acid V. The resulting acid is transformed to the acyl azide intermediate and then subjected to Curtius rearrangement conditions. The corresponding transient isocyanate is trapped with hydroxy containing intermediate I to give intermediate VII. Intermediate VII is then reacted with a thiol containing peptide ($HS-R^7$) that participates in a disulfide exchange reaction to give the final compound.

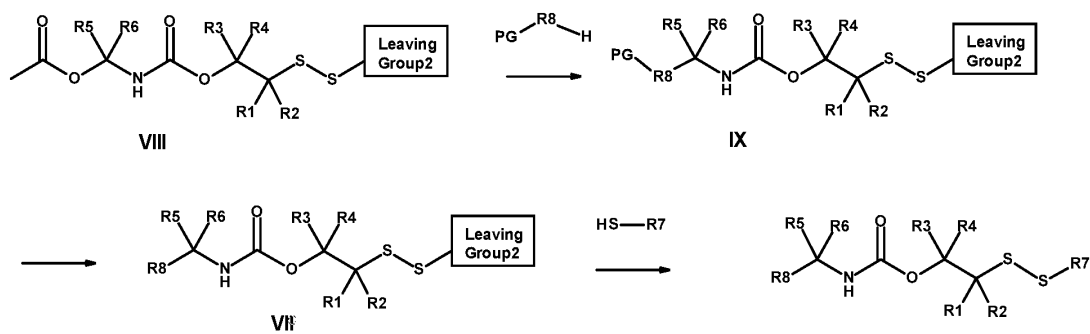
Scheme 2: Synthesis of Amino Linked Compounds



An alternative synthesis of amino linked conjugates is shown in Scheme 3.

- 5 Nucleophilic R^8-H , which can include a protecting group (PG), is reacted with AcO-hemi-aminal carbamate **VIII** with a pre-installed linked Leaving Group 2 to give Intermediate **IX**. This compound is treated with conditions to remove the protecting group and the resulting compound **VII** is reacted with R^7-SH to give the desired conjugate.

Scheme 3: Synthesis 2 of Amino Linked Conjugates



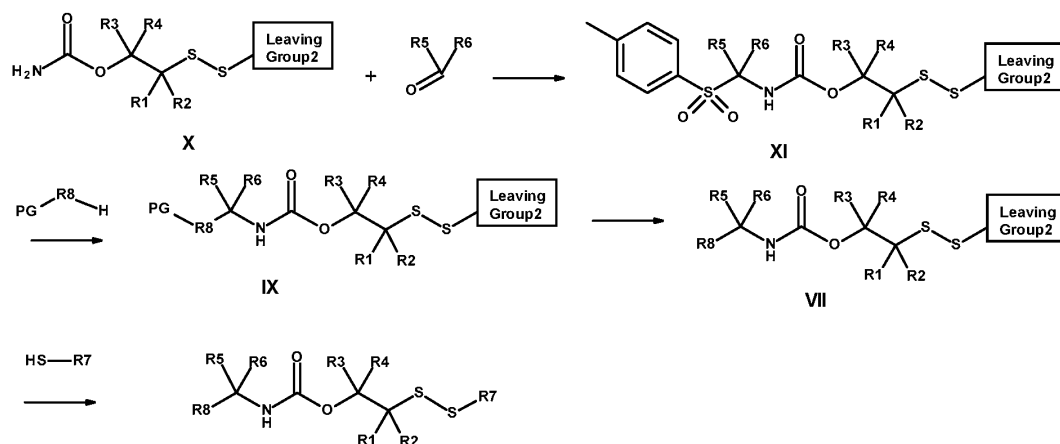
10

A further alternative synthesis of amino linked conjugates is shown in Scheme 4.

Primary carbamate **X** with a pre-installed linked Leaving Group 2 is reacted with a carbonyl compound and p-toluene sulfinate sodium salt to give sulfonyl carbamate **XI**. This is further

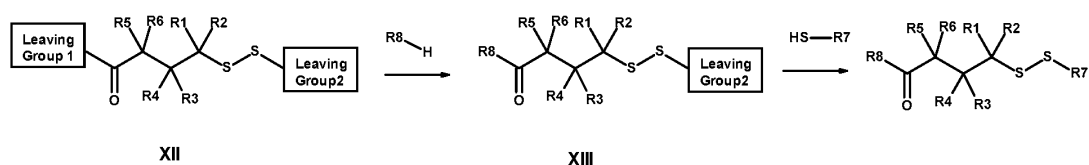
treated with nucleophilic R^8 -H with a protecting group (PG) to give IX. This compound is treated as previously described to give the desired conjugate.

Scheme 4: Synthesis 3 of Amino Linked Conjugates



An exemplary synthesis of thiopropionate linked conjugates is shown in Scheme 5. Propionate disulfide XII with previously installed Leaving Groups 1 and 2 is reacted selectively with nucleophilic R^8 -H to give XIII. This compound is further reacted with R^7 -SH to provide the desired conjugate.

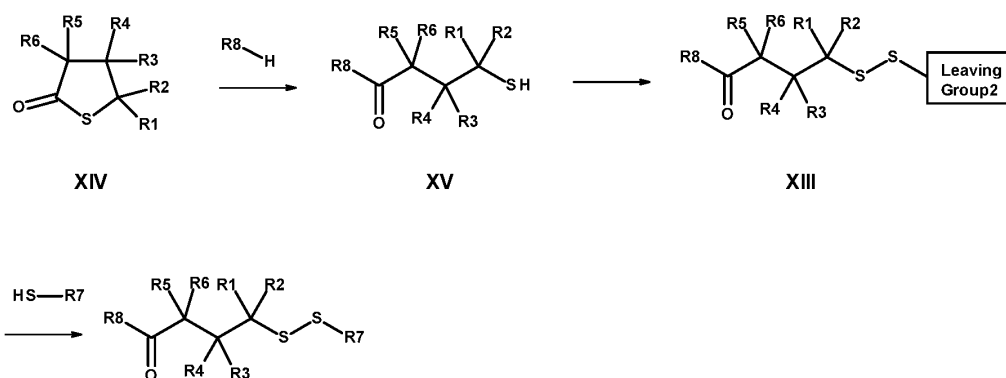
Scheme 5: Synthesis 1 of Thiopropionate Linked Conjugates



An alternative synthesis of thiopropionate linked conjugates is shown in Scheme 6.

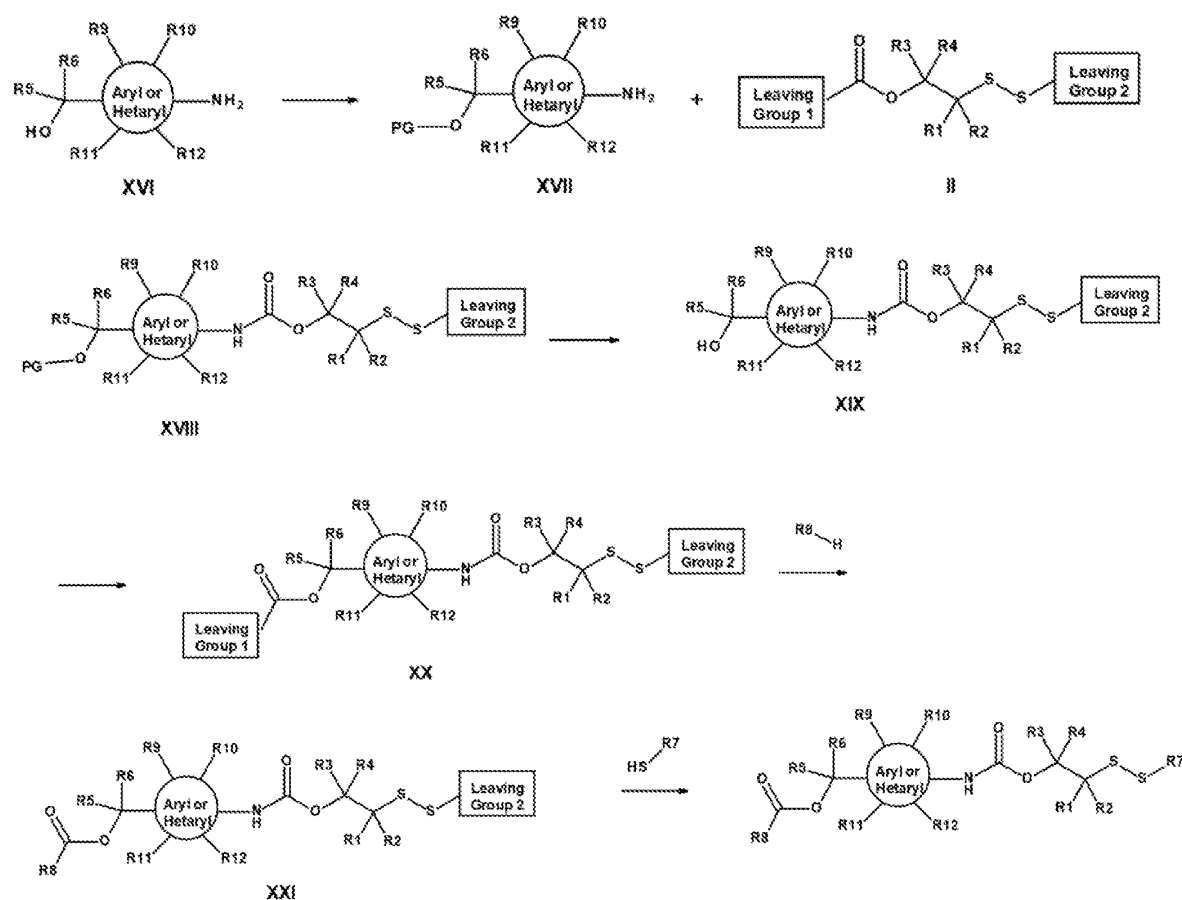
- 15 Thionoester XIV is reacted with nucleophilic R^8 -H to give propionate thiol XV. This compound engages in a disulfide exchange reaction to provide XIII. This compound is treated with R^7 -SH to provide the desired conjugate.

Scheme 6: Synthesis 2 of Thio Propionate Linked Conjugates



- The synthesis of para benzoic-linked conjugates is depicted in Scheme 7. Para amino benzoic alcohol XVI is selectively protected at the oxygen to give XVII. This is then reacted at the aniline position with II to provide aryl carbamate XVIII. The protecting group is
- 5 removed giving free OH XIX which is treated with an activating agent to provide XX which contains orthogonal leaving groups. Reaction of XX with R^8-H to give XXI followed by R^7-SH give provides the desired conjugate.

Scheme 7: Synthesis 1 of Para Benzoic-Linked Conjugates

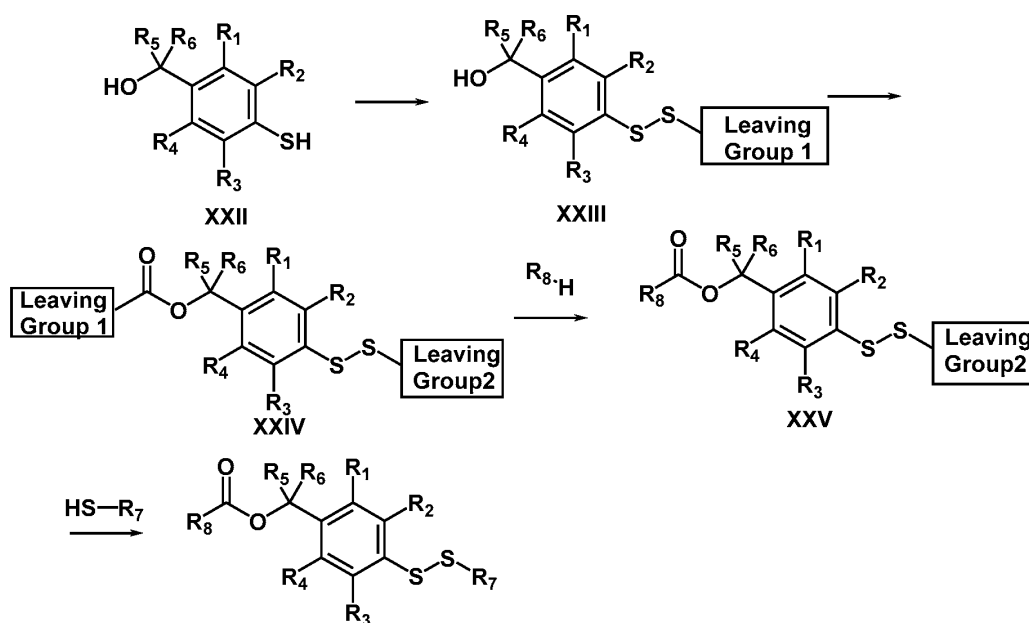


5

An alternative synthesis of para benzoic-linked conjugates is shown in Scheme 8. 4-Mercapto benzoic alcohol XXII is reacted in a disulfide exchange reaction to give 4-mercapto benzoic alcohol disulfide XXI containing Leaving Group 2. The remaining benzyl alcohol is treated to provide activated compound XXIV. This is further reacted selectively with

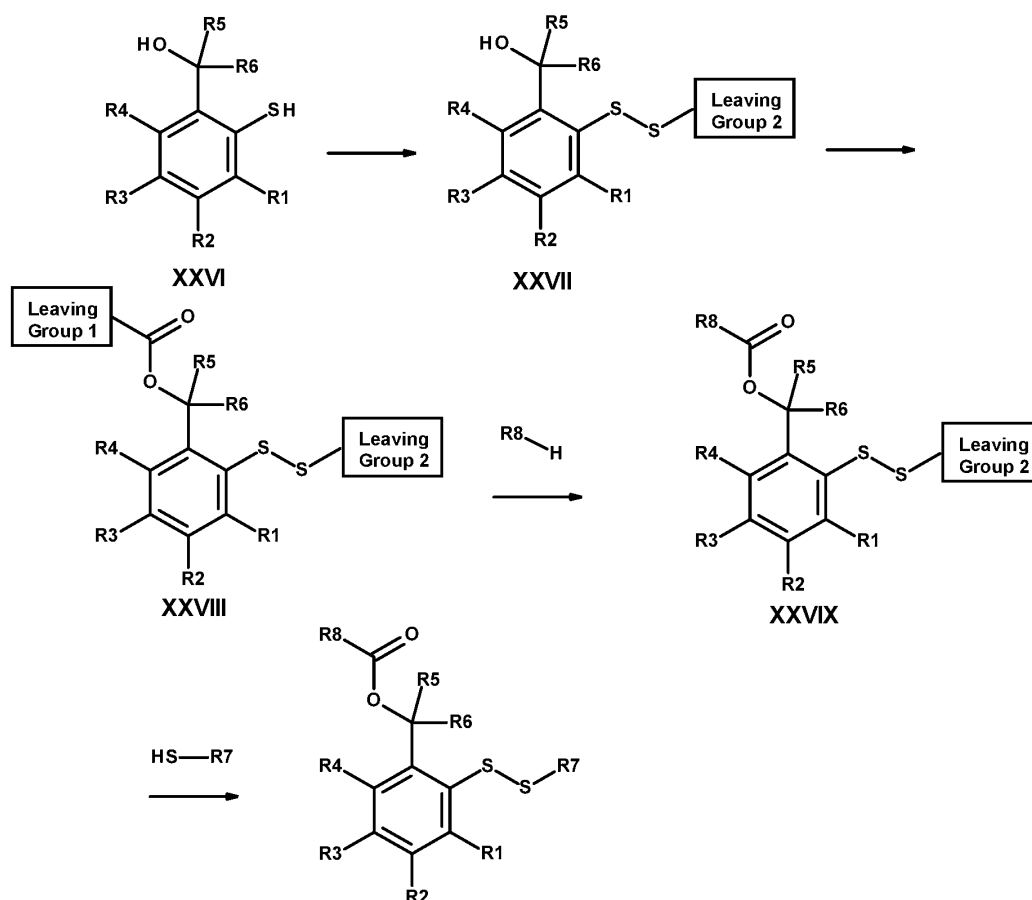
10 nucleophilic R⁸-H and resulting XXV with R⁷-SH to give the desired conjugate.

Scheme 8: Synthesis 2 of Para Benzoic-Linked Conjugates



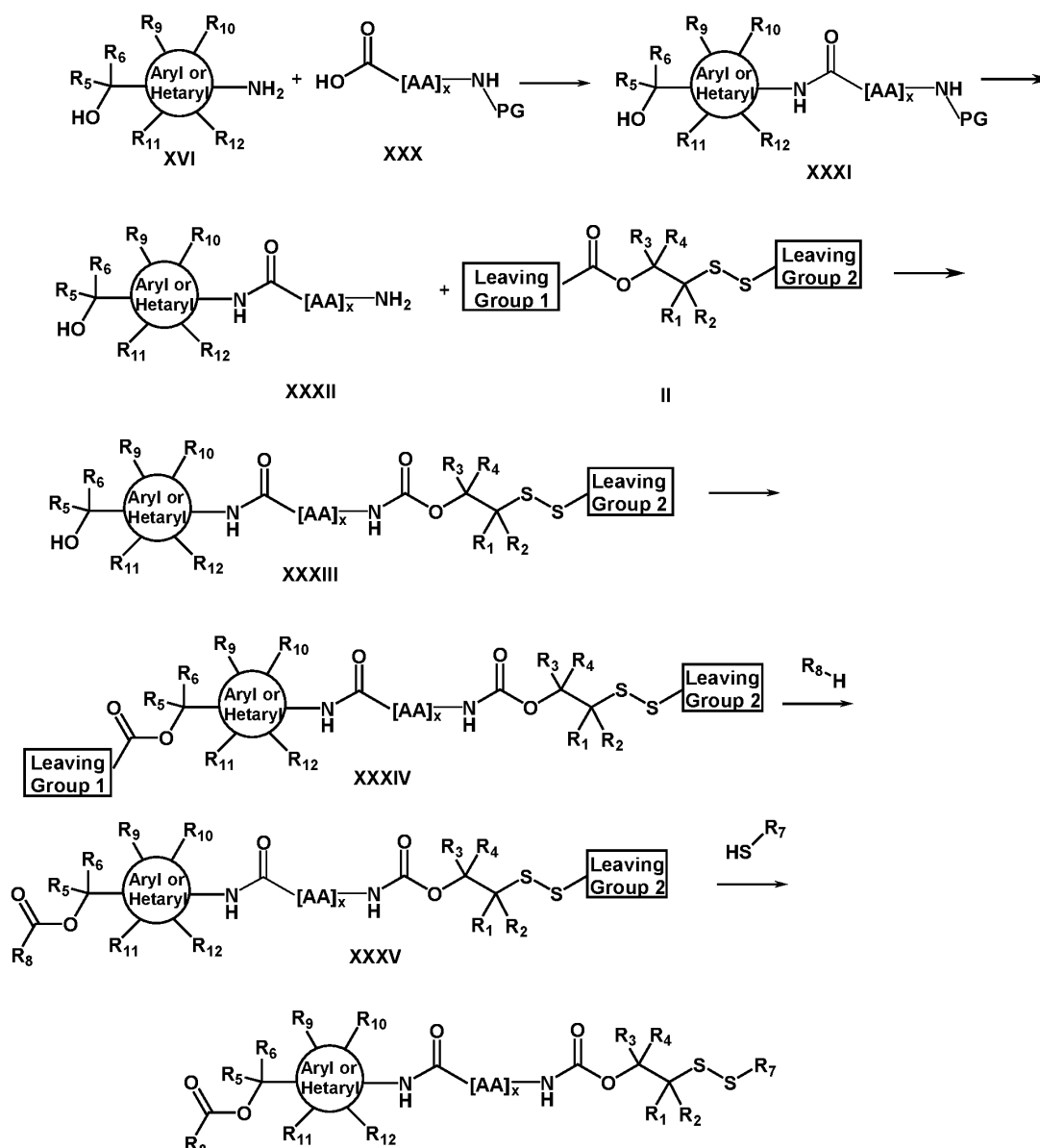
- The synthesis of ortho benzoic-linked conjugates is shown in Scheme 9. 2-Mercapto
- 5 benzoic alcohol XXVIII is reacted as previously described for Scheme 8 to give the desired conjugate.

Scheme 9: Synthesis of Ortho Benzoic-Linked Conjugates



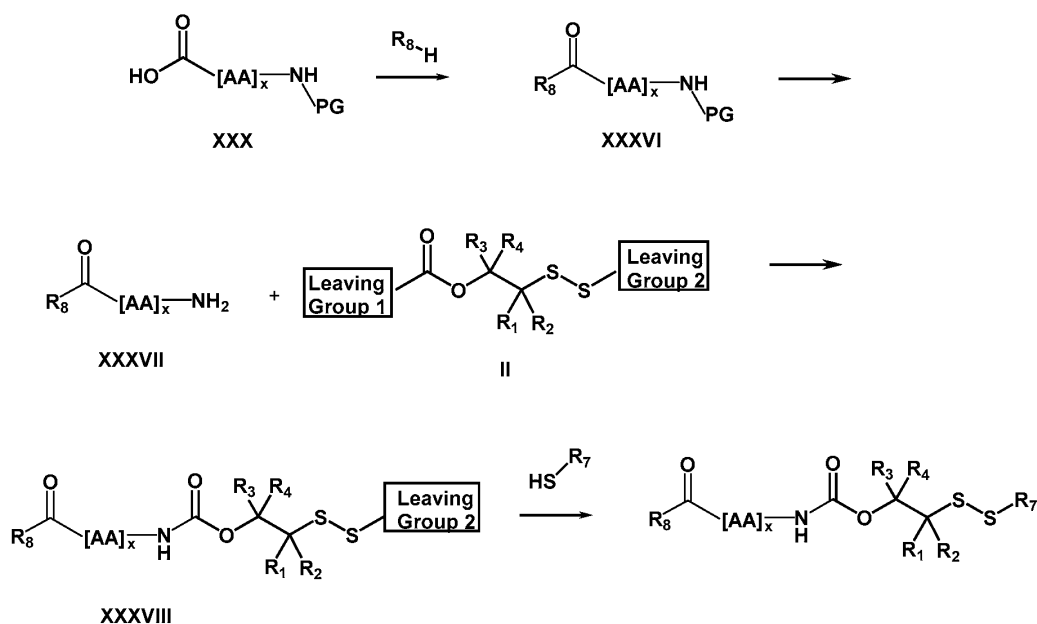
- The synthesis of amino acid benzoic carbamate-linked conjugates is shown in Scheme 10. Para amino benzoic/heterobenzoic alcohol XVI can be selectively coupled to N-protected amino acid or peptide XXX to give XXXI. The protecting group can be removed to provide XXXII which is then reacted with II to give carbamate XXXIII with Leaving Group 2 installed. The alcohol can be selectively reacted to give carbonate XXXIV which has Leaving Group 1 present. Leaving Group 1 can be subsequently displaced with R⁸-H to give XXXV which is reacted with R⁷-SH to provide the desired amino acid benzoic carbamate-linked conjugate.

Scheme 10: Synthesis of Amino Acid Benzoic Carbamate-Linked Conjugates



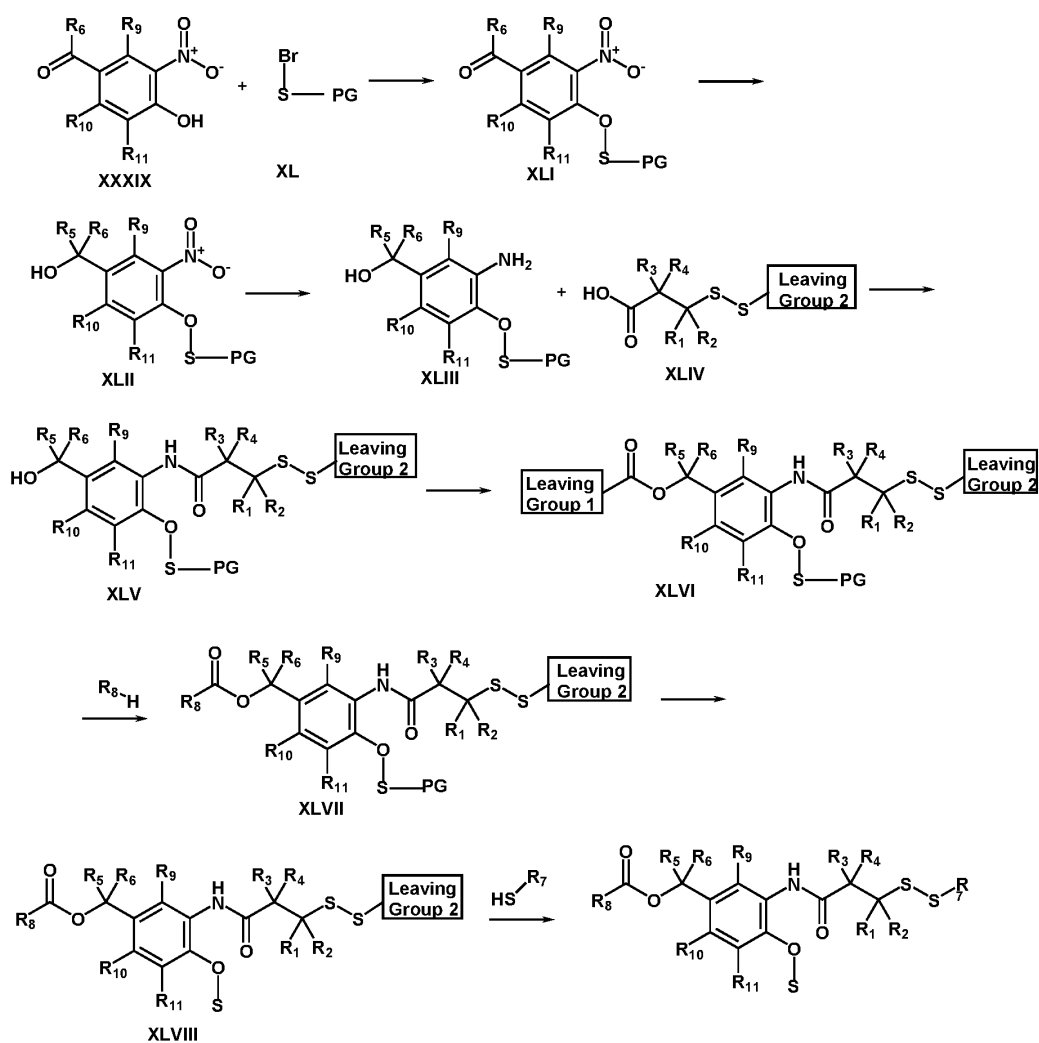
The synthesis of amino acid-linked conjugates is shown in Scheme 11. N-Protected amino acid or peptide XXX can be coupled to R⁸-H to give XXXVI. The protecting group can be removed to give XXXVII which can be subsequently reacted with II, displacing Leaving Group 1 to give XXXVIII. This intermediate can then be treated with R⁷-SH to provide the conjugate.

Scheme 11: Synthesis of Amino Acid-Linked Conjugates



- The synthesis of benzoic alcohol-linked, glucuronide conjugates is shown in Scheme 12. 2-Nitro phenol XXXIX can be coupled with protected glucuronide XL to give XLI. This intermediate can be reduced selectively at the carbonyl to give alcohol XLII and then the
- 5 nitro group can be reduced to provide aniline XLIII. This intermediate can be further coupled with propionic acid derivative XLIV to give amide XLV which contains Leaving Group 2. XLV can then be reacted at the alcohol to install Leaving Group 2, giving XLVI. Leaving Group 1 can be selectively displaced with R⁸-H to give XLVII. The protecting groups on the glucuronide can be removed and XLVIII can be reacted with R⁷-SH to give the conjugate.

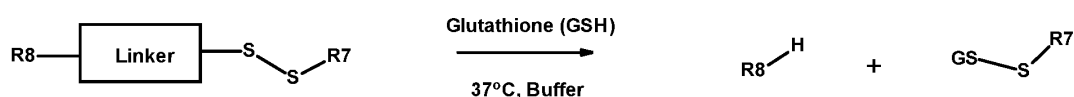
Scheme 12: Synthesis of Benzoic Alcohol-Linked, Glucuronide Conjugates



Cleavage of the final compound to release R⁸H 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.

Scheme 13: Cleavage of Peptide Conjugates



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
5 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
10 depends on attachment of the first amino acid of the chain to a solid polymer by a covalent bond, followed by the addition of the succeeding protected amino acids, one at a time, in a stepwise manner until the desired sequence is assembled. Finally, the protected peptide is removed from the solid resin support and the protecting groups are cleaved off.

The amino acids may be attached to any suitable polymer. The polymer must be
15 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.

20 *Methods of Use*

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, stroke, myocardial infarction, or long-term neurodegenerative disease. In some embodiments, the cancer is PARP-sensitive. In some embodiments, the cancer is associated with abnormal
25 expression or activity of ATM. In some embodiments, the cancer is associated with abnormal expression or activity of ATM. In some embodiments, the cancer is associated with abnormal expression or activity of DNA-PK. In some embodiments, the cancer is a BRCA-mutated breast cancer. In some embodiments, the cancer is germline BRCA-mutated ovarian cancer.

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

appreciated by those of skill in the art. Either method of treatment (single agent or combination with other forms of therapy) may be administered as a course of treatment involving multiple doses or treatments over a period of time. Also provided herein is a method of reducing bone marrow toxicity associated with administration of an ionizing radiation or cytotoxic agent, which comprises administering to a human or other mammal a therapeutically-effective amount of a compound of the disclosure, or a pharmaceutically acceptable salt thereof, in combination with the ionizing radiation or cytotoxic agent. The reduction in bone marrow toxicity can be relative to bone marrow toxicity observed when the ionizing radiation or cytotoxic agent is administered together with R⁸H instead of the compound of the invention having the same R⁸ (e.g., where the R⁸H compound corresponds to the protonated R⁸ moiety of the R⁸-Q-R⁷ compound of the invention). In some embodiments, toxicity can be measured by PARylation in bone marrow tissue. In some embodiments, toxicity can be measured according to total nucleated bone marrow cells. In some embodiments, the cytotoxic agent can be any of those listed herein. In some embodiments, the cytotoxic agent is temozolomide (TMZ).

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

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

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

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

15 In certain embodiments, a compound of formula (I) or a pharmaceutically-acceptable salt thereof may be used in combination with a chemotherapeutic agent, a targeted cancer therapy, an immunotherapy or radiation therapy. The agents can be combined with the present compounds in a single dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms. In some embodiments, the
20 chemotherapeutic agent, targeted cancer therapy, immunotherapy or radiation therapy is less toxic to the patient, such as by showing reduced bone marrow toxicity, when administered together with a compound of formula (I), or a pharmaceutically acceptable salt thereof, as compared with when administered in combination with the corresponding free DNA repair inhibiting compound (e.g., R⁸-H) such as an inhibitor of PARP, ATR, DNK-PK, or ATM as
25 described herein.

Suitable chemotherapeutic or other anti-cancer agents include, for example, alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes) such as uracil mustard, chlormethine, cyclophosphamide (CytosanTM), ifosfamide, melphalan, chlorambucil, pipobroman,
30 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).

5 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.

10 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,
15 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, epidophyllotoxin; an
20 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
25 (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
30 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. megestrol 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 may be used in combination with the compounds of the invention. These include onartumzumab, tivantinib, and INC-280. Agents against Abl (or Bcr-Abl) include imatinib, dasatinib, nilotinib, and ponatinib and those against Alk (or EML4-ALK) include crizotinib.

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

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

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

Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR, e.g., 1996 edition, Medical Economics Company, Montvale, NJ), the disclosure of which is incorporated herein by reference as if set forth in its entirety.

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

Said therapeutically effective amount can be obtained from a clinical trial, an animal model, or an *in vitro* cell culture assay. It is known in the art that the effective amount suitable for human use can be calculated from the effective amount determined from an animal model or an *in vitro* cell culture assay. For instance, as reported by Reagan-Shaw et al., FASEB J. 2008: 22(3) 659-61, "µg/ml" (effective amount based on *in*

vitro cell culture assays) = "mg/kg body weight/day" (effective amount for a mouse).

Furthermore, the effective amount for a human can be calculated from the effective amount for a mouse based on the fact that the metabolism rate of mice is 6 times faster than that of humans.

- 5 As an example of treatment using a compound of formula (I) as monotherapy, a therapeutically-effective dosage of a compound of formula (I) wherein R⁸H is a PARP inhibitor may be administered to a patient suffering from a PARP dependent cancer, such as a female suffering from BRCA-mutated breast cancer, germ line BRCA-mutated ovarian cancer, or fallopian tube cancer or to a patient suffering from primary peritoneal
- 10 cancer, squamous cell lung cancer, or non-small cell lung cancer, or to a patient suffering from stroke, myocardial infarction, or long-term neurodegenerative disease. As another example, a therapeutically-effective dosage of a compound of formula (I) wherein R⁸H is a DNA repair inhibitor targeting the protein kinase ataxia-telangiectasia mutated (ATM), the ATM-Rad3-related protein kinase (ATR), or the nuclear serine/threonine protein kinase
- 15 DNA-PK could be administered to a patient suffering from a cancer in which inhibition of one or more of the above proteins would be therapeutically useful.

- 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
- 20 involving a therapeutically-effective amount of ionizing radiation or a cytotoxic agent. In the context of this treatment regimen, the term "therapeutically-effective" amount should be understood to mean effective in the combination therapy. It will be understood by those of skill in the cancer-treatment field how to adjust the dosages to achieve the optimum therapeutic outcome.

- 25 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
- 30 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.

5 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.

10 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
15 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), the intent is to recite that the therapeutically effective amount can be equal to about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about
20 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 26 mg/kg, about 27 mg/kg, about 28 mg/kg, about 29 mg/kg, about 30 mg/kg, about 31 mg/kg, about 32 mg/kg, about 33 mg/kg,
25 about 34 mg/kg, about 35 mg/kg, about 36 mg/kg, about 37 mg/kg, about 38 mg/kg, about 39 mg/kg, about 40 mg/kg, about 41 mg/kg, about 42 mg/kg, about 43 mg/kg, about 44 mg/kg, about 45 mg/kg, about 46 mg/kg, about 47 mg/kg, about 48 mg/kg, about 49 mg/kg, or about 50 mg/kg. Additionally, the therapeutically effective amount can be within any sub-range included within about 1 mg/kg to about 50 mg/kg (for example, the amount can be in a range
30 from about 2 mg/kg to about 10 mg/kg), and this also includes any combination of ranges between about 1 mg/kg and about 50 mg/kg (for example, the amount can be in a range from about 1 mg/kg to about 5 mg/kg or from about 20 mg/kg to about 35 mg/kg). Likewise, all other ranges disclosed herein should be interpreted in a manner similar to this example.

Formulation, Dosage Forms and Administration

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

EXAMPLES

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

- Brine: a saturated NaCl solution in water
- DCM: dichloromethane
- TFA: trifluoroacetic acid
- DIPEA: diisopropylethylamine
- DMA: dimethylacetamide

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

- 5
- LiOH: lithium hydroxide
 - DPPA: diphenyl phosphoryl azide
 - Sn(Bu)₂(Laurate)₂: dibutyltin dilaurate
 - PBS: phosphate buffered saline
 - ACN: acetonitrile
 - AcOH: acetic acid
 - EEDQ: *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
 - DMAP: 4-dimethylaminopyridine
 - EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

10 HPLC Methods

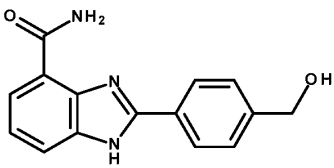
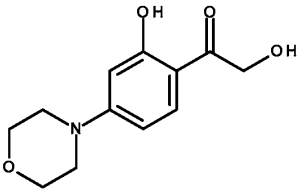
All HPLC methods use reverse phase conditions: H₂O/Acetonitrile with TFA modifier (0.05%); Flow rate: 1 mL/min; Wavelength = 217 nm. Column conditions are described below:

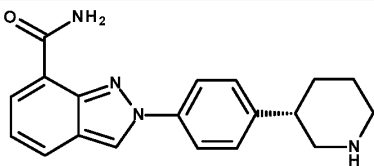
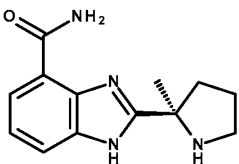
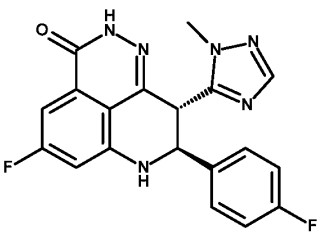
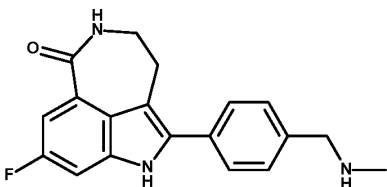
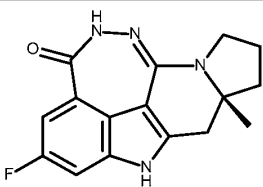
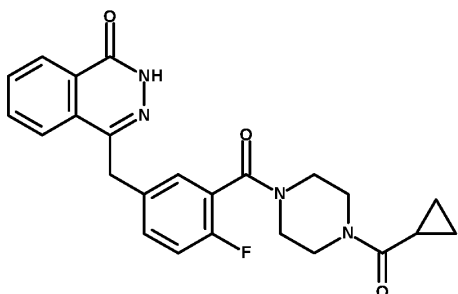
- 15
- A: Sunfire C18 150x4.6mm
- B: Ace Equivalence 250x4.6 mm
- C: Sunfire C18 150x30 mm

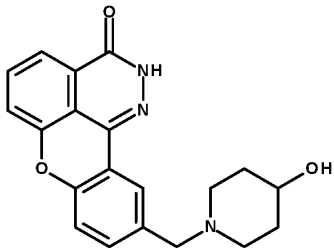
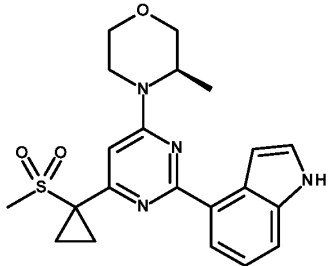
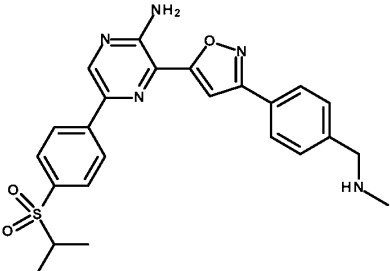
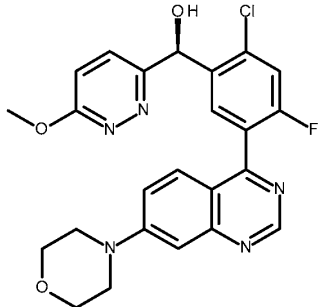
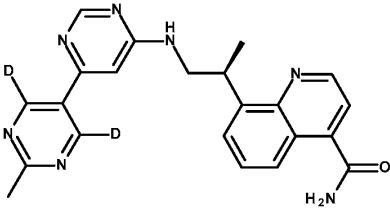
R⁸H Intermediates

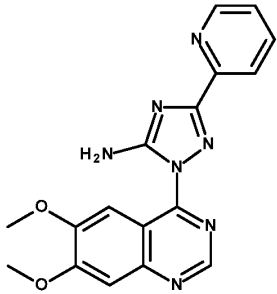
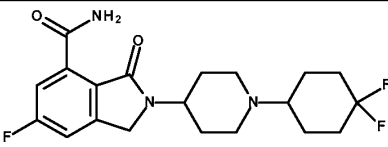
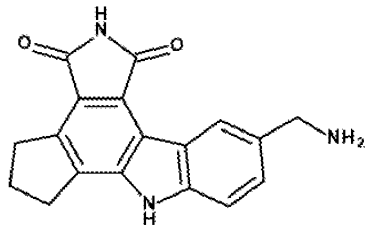
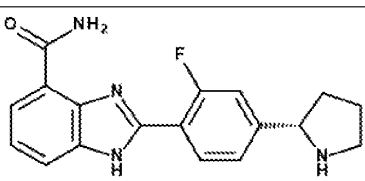
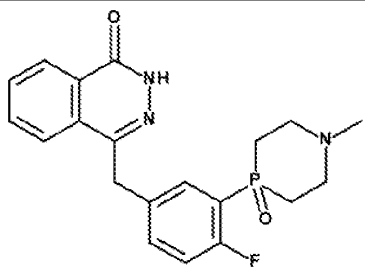
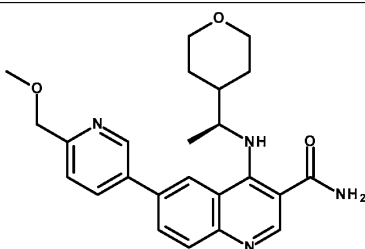
The R⁸H groups used throughout the Examples were purchased or synthesized as indicated in Table 2.

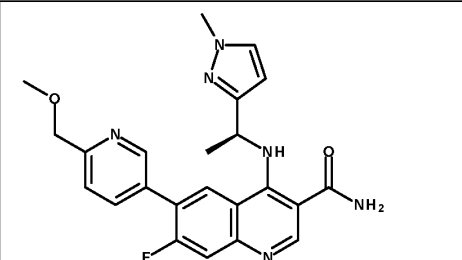
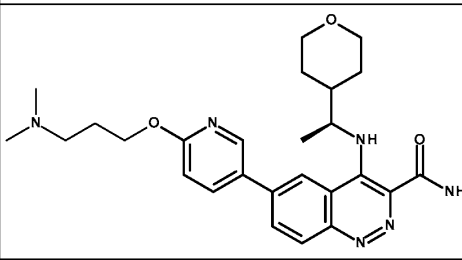
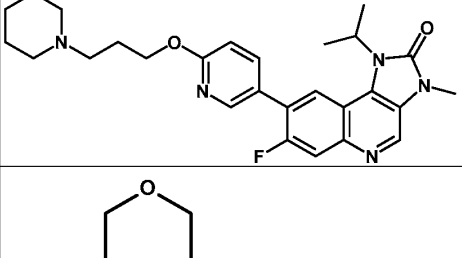
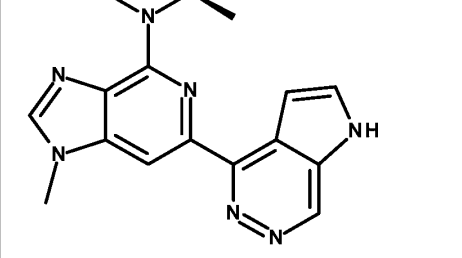
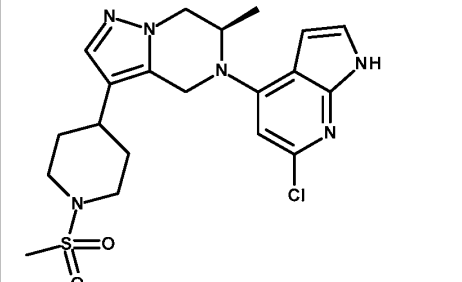
20 Table 2: R⁸H Intermediates

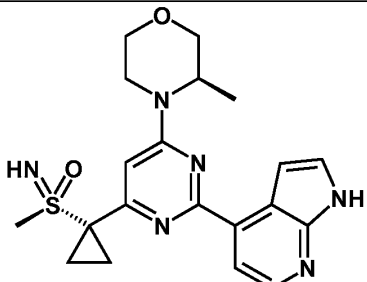
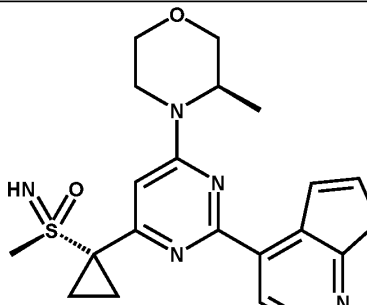
R ⁸ Code	R ⁸ H Structure	Synthesis Reference or Purchased	Class
R ⁸ H-1		Synthesized - Bioorganic & Medicinal Chemistry Letters 14 (2004) 2433–2437; J. Med. Chem. 2000, 43, 4084-4097	PARP
R ⁸ H-2		Synthesized - WO0220500A2	DNA-PK

R⁸H-3		Astatech 42568	PARP
R⁸H-4		MedKoo 203115	PARP
R⁸H-15		Chemscene CS-0937	PARP
R⁸H-16		Astatech 81136	PARP
R⁸H-17		MentonChem MC7030606	PARP
R⁸H-18		Selleckchem S1060	PARP

R⁸H-19		MedKoo 205150	PARP
R⁸H-20		Advanced Chemblocks 10278	ATR
R⁸H-21		Astatech 42043	ATR
R⁸H-22		Medchem Express HY-10619B	DNA-PK
R⁸H-23		Synthesized - WO2013163190A1	DNA-PK

R⁸H-24		Astatech 40964	ATM
R⁸H-25		MedKoo 407142	PARP
R⁸H-26		MedKoo 406357	PARP
R⁸H-27		MedKoo 406362	PARP
R⁸H-28		Synthesized WO2012166983A1	PARP
R⁸H-29		Synthesized J. Med. Chem. 2016, 59, 6281–6292	ATM

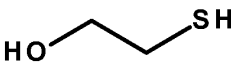
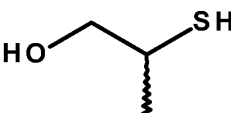
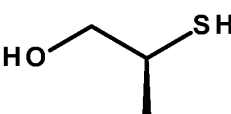
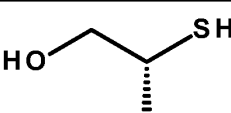
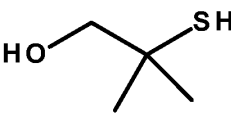
R⁸H-30		Synthesized J. Med. Chem. 2016, 59, 6281–6292	ATM
R⁸H-31		ACS Med. Chem. Lett. 2018, 9, 809–814	ATM
R⁸H-32		J. Med. Chem. 2018, 61, 3823–3841.	ATM
R⁸H-33		Synthesized ACS Med. Chem. Lett. 2015, 6, 42–46	ATR
R⁸H-34		Synthesized ACS Med. Chem. Lett. 2015, 6, 37–41	ATR

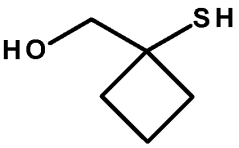
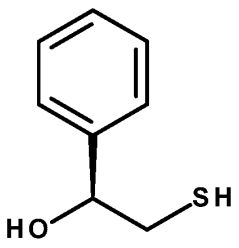
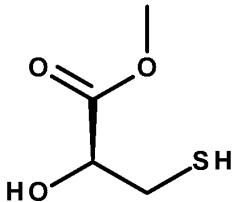
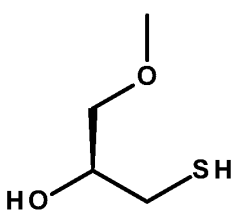
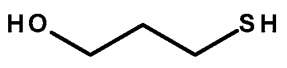
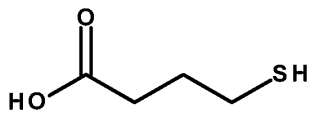
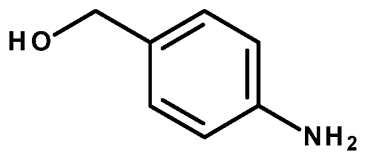
R⁸H-35		Oncotarget, 6, 42, 44289-44305	ATR
R⁸H-36		Oncotarget, 6, 42, 44289-44305	ATR

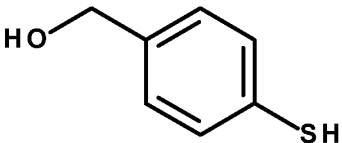
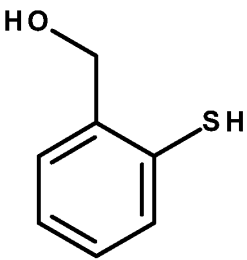
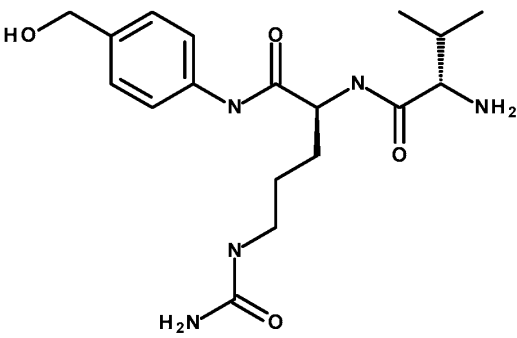
Linkers

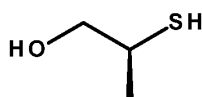
Linkers used herein were either purchased or synthesized as shown below in Table 3:

Table 3: Commercially Available Linkers

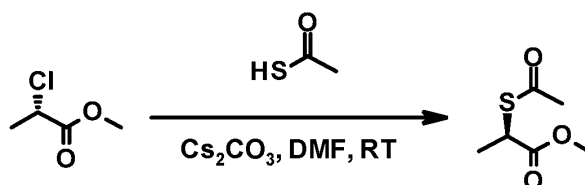
Linker Code	Linker Structure	Synthetic Reference or Purchased
L1		Alfa Aesar A15890
L2		Enamine EN300-9515
L4		Synthesized - WO2013055987A1
L5		Synthesized - WO2013055987A1
L3		Enamine EN300-220914

L6		Synthesized - ACS Med. Chem. Lett. 2016, 7, 988–993
L7		Synthesized Synlett, 2005, 20 3063
L8		Synthesized Synlett, 2005, 20 3063
L9		Synthesized Synlett, 2005, 20 3063
L10		TCI M1206
L11		Aurum GN23710
Linker XVI-1		Combiblocks OR-0735

Linker XXII-1		Combiblocks OR-5865
Linker XXVI-1		Oakwood 001239
Linker XXXI-1		Aurum DS18383

Linker L4: (2R)-2-sulfanylpropan-1-ol

Step 1: Methyl (2R)-2-acetylsulfanylpropanoate

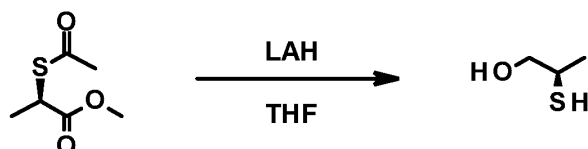


5

Thioacetic acid (2.24 g, 29.4 mmol) and cesium carbonate (7.98 g, 24.5 mmol) were taken up in 40 mL of dry DMF. The mixture was stirred at RT for 30 min before the methyl (2S)-2-chloropropanoate (3.00 g, 24.5 mmol) was added. The mixture was then stirred for an additional 3 h. Diethyl ether (150 mL) and water (150 mL) were added and the layers

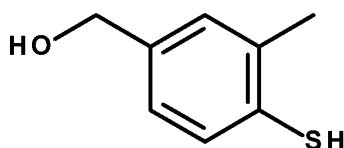
separated. The aqueous layer was washed with additional ether and the combined organics dried over Na₂SO₄. The organic layer was concentrated and the residue purified by column chromatography (SiO₂, 0-10% EtOAc/Hexanes) to give 3.50 g, 88% of methyl (2R)-2-acetylsulfanylpropanoate.

5 *Step 2: (2R)-2-sulfanylpropan-1-ol*



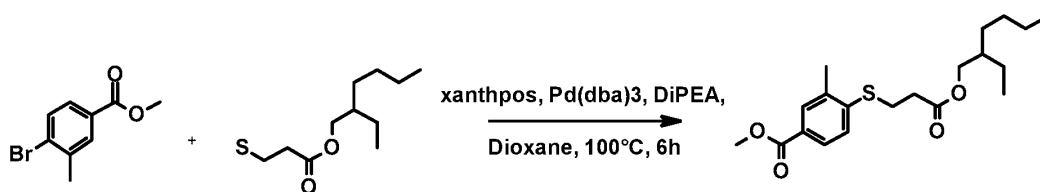
A solution of methyl (2R)-2-acetylsulfanylpropanoate (3.50 g, 21.6 mmol) in THF (30 mL) was added dropwise to a suspension of LAH (4.10 g, 108 mmol) in THF (60 mL) at 0 °C. After addition was complete, the mixture was stirred at RT for 3h. The reaction was
 10 cooled to 0 °C, then quenched by adding 2N HCl (~75 mL) dropwise. After complete addition, the mixture was stirred at RT for 1h. The mixture was extracted with CH₂Cl₂ (5 X 100 mL) and dried with Na₂SO₄. The crude (2R)-2-sulfanylpropan-1-ol, 1.60 g, 81% was carried on without further purification.

Linker XXII-2: (3-methyl-4-sulfanyl-phenyl)methanol



15

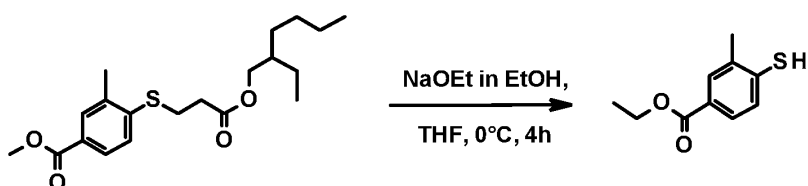
Step 1: 4-((3-((2-ethylhexyl)oxy)-3-oxopropyl)thio)-3-methylbenzoate



To a stirred solution of methyl 4-bromo-3-methylbenzoate (0.20 g, 0.88 mmol) and 2-ethylhexyl 3-mercaptopropanoate (0.21 g, 0.96 mmol) in 1,4-dioxane (2 mL), DIPEA (0.32
 20 mL, 1.80 mmol), Xanthphos (0.005 g, 0.009 mmol) were added. The reaction mixture was purged with argon for 5 min. Pd(dba)₃ (0.008 g, 0.009 mmol) was added and the reaction

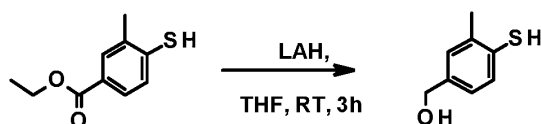
mixture was heated at 100°C for 6 h. After completion of the reaction, the reaction mixture was quenched with water (10 mL), and extracted into ethyl acetate (20 mL). The organic layer was washed with sodium chloride solution (20 mL) and dried over Na₂SO₄ to afford the crude product. The crude product was purified by flash chromatography (SiO₂, 0-5% ethyl acetate/hexane) to afford 4-((3-((2-ethylhexyl)oxy)-3-oxopropyl)thio)-3-methylbenzoate as a yellow color liquid (0.2 g, 62% yield). MS m/z 367.1 [M+H]⁺.

Step 2: Ethyl 4-mercapto-3-methylbenzoate



A stirred solution of methyl 4-((3-((2-ethylhexyl)oxy)-3-oxopropyl)thio)-3-methylbenzoate (0.20 g, 0.54 mmol) in THF (5 mL) was cooled to 0°C. Sodium ethoxide (35 wt. %, 2 mL) was added and the mixture was stirred for 4h at room temperature. After completion of the reaction, the reaction mixture was acidified with 2N HCl (10 mL) and concentrated. The resulting residue was extracted into ethyl acetate (20 mL). The organic layer was washed with water (20 mL), saturated sodium chloride solution (20 mL) and dried over Na₂SO₄ to afford ethyl 4-mercapto-3-methylbenzoate as a colorless liquid (80 mg, 80% yield). MS m/z 197.1 [M+H]⁺.

Step 3: (3-methyl-4-sulfanyl-phenyl)methanol



XXII-2

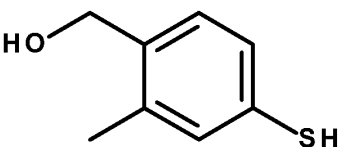
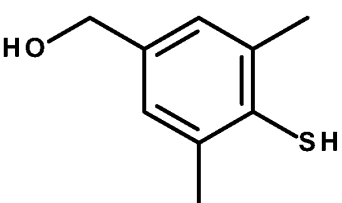
To a stirred solution of ethyl 4-mercapto-3-methylbenzoate (0.60 g, 3.07 mmol) in THF (20 mL) maintained at 0°C was slowly added a 1M solution of LAH in THF (7.80 mL, 7.80 mmol). The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the reaction mixture was cooled to 0°C and quenched with water (20 mL). Temperature was maintained below 20 °C during quenching. After completion of quenching, the pH was adjusted to 2-3 with 2N HCl. The resulting residue was extracted into ethyl

acetate (20 mL) and the organic layer was washed with sodium chloride solution (20 mL). The organic phase was dried over Na₂SO₄ to afford the crude product. The crude product was purified by flash chromatography (SiO₂, 0-20% ethyl acetate/hexane) to afford (3-methyl-4-sulfanyl-phenyl)methanol as a colorless liquid (0.45 g, 90% yield). MS m/z 153.0 [M-H]⁻.

5

XXII-3 and XXII-4, as shown in Table 4 below, were synthesized in an analogous manner to Linker XXII-2.

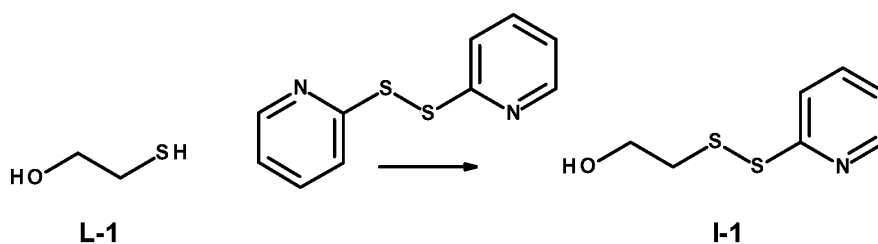
Table 4: Additional Linkers

Linker Code	Linker Structure	m/z found [M-H]
XXII-3		153.1
XXII-4		167.0

10

Intermediates

Intermediate I-1: 2-(2-pyridyldisulfanyl)ethanol



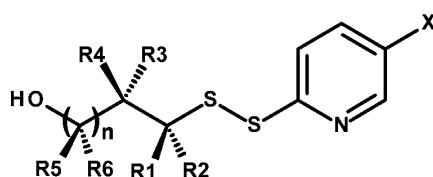
To 2-(2-pyridyldisulfanyl)pyridine (4.693 g, 21.3mmol) in 40 ml of degassed (N₂) MeOH was added 2-sulfanylethanol (0.498mL, 7.10mmol) in a drop-wise fashion. The mixture was stirred for 2-20 h under N₂. The mixture was concentrated to dryness and directly purified (SiO₂, 0-5% EtOAc/CH₂Cl₂) to give 3 fractions; (F1 mix SM A and product; F2 product; F3 2-

15

thiopyridine/product mix). The impure material was purified again (SiO₂, 0-50% EtOAc/Hexanes) to give 1.17 g, 88% yield of 2-(2-pyridyldisulfanyl)ethanol. MS m/z found 188.4 [M+H]⁺

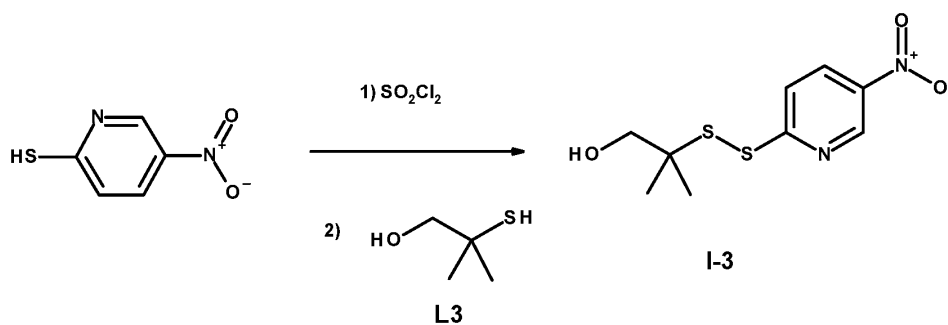
- 5 The following Intermediates shown in Table 5 were prepared analogously to Intermediate I-1.

Table 5. Intermediates



Intermediate	R ¹ , R ²	R ³ , R ⁴	n	R ⁵ , R ⁶	X	MH ⁺
I-2	Me-H (rac)	H, H	0	---	H	202.1
I-7	H, H	H, Ph	0	---	H	264.1
I-8	H, H	H, CO ₂ Me	0	----	H	246.1
I-9	H, H	H, CH ₂ OMe	0	--	H	232.0
I-10	H, H	H, H	1	H, H	H	367.0

- 10 **Intermediate I-3: 2-methyl-2-[(5-nitro-2-pyridyl)disulfanyl]propan-1-ol**

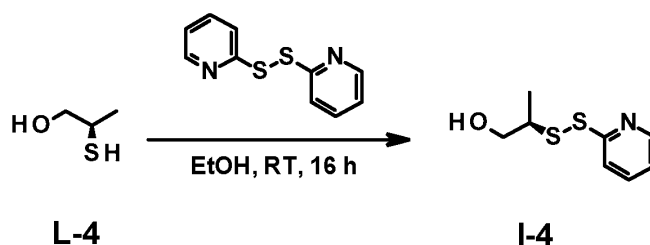


- 15 SO₂Cl₂ (0.382 mL, 4.71 mmol) was added drop-wise to a stirred suspension of 5-nitropyridine-2-thiol (668 mg, 4.28 mmol) in dry DCM (15 mL) at 4° C under an N₂ atmosphere. The reaction mixture turned from a yellow suspension to a yellow solution and was allowed to warm to room temperature with stirring for 2 hours, after which time the mixture was concentrated to provide a yellow solid. The solid was re-dissolved in DCM (15

mL) and treated drop-wise with a solution of 2-methyl-2-sulfanyl-propan-1-ol (454 mg, 4.28 mmol) in dry DCM (10 mL) at 4° C under an N₂ atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 20 hours. The reaction was monitored by LC/MS for the desired product mass. To the mixture was added 50 mL of H₂O and the diluted mixture was treated with ammonium hydroxide solution. The reaction mixture was diluted with 50 mL of EtOAc, partitioned, and separated. The organic phase was dried with MgSO₄, filtered, and concentrated to give the crude product. The crude mixture was purified (SiO₂, 0-50% EtOAc/hexanes) to give 721 mg, 65% yield of 2-methyl-2-[(5-nitro-2-pyridyl)disulfanyl]propan-1-ol. MS m/z found 261.7 [M+H]⁺.

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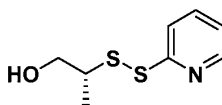
Intermediate I-4: (2R)-2-(2-pyridyldisulfanyl)propan-1-ol



To 2-(2-pyridyldisulfanyl)pyridine (5.00 g, 22.7 mmol) in 40 ml of MeOH degassed with 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 found 202.1 [M+H]⁺

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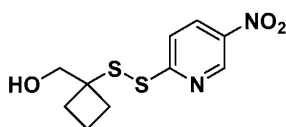
Intermediate I-5



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Intermediate I-5 was prepared in an analogous fashion to Intermediate I-4 from L-5.

Intermediate I-6

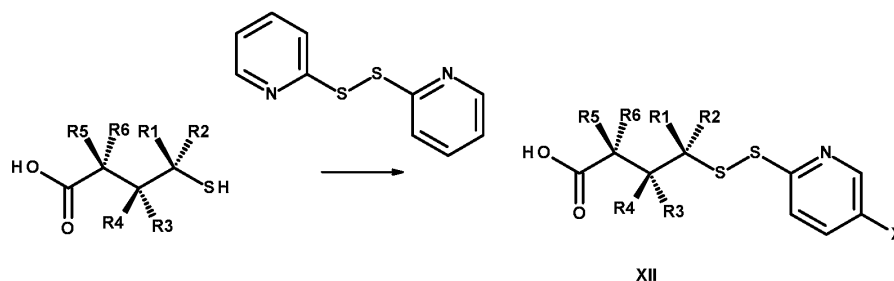


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Intermediate I-6 was prepared analogously to Intermediate I-3 from Linker L-6.

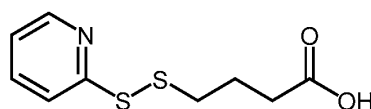
Synthesis of Intermediates XII

Table 6. Intermediates XII



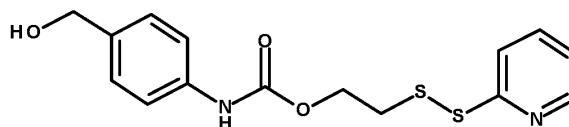
Int.	R ¹ , R ²	R ³ , R ⁴	R ⁵ , R ⁶	X	MH ⁺
XII-1	H, H	H, H	H, H	H	230.0

5

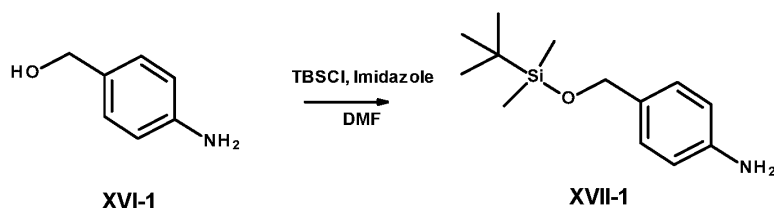
Synthesis of Intermediate XII-1: 4-(2-pyridyldisulfanyl)butanoic acid

To 2-(2-pyridyldisulfanyl)pyridine (1120 mg, 5.08 mmol) in 20 ml of degassed (N₂) MeOH was added 4-sulfanylbutanoic acid (500 mg, 4.16 mmol) in a drop-wise fashion. The mixture was stirred for 16 h under N₂. The mixture was concentrated to dryness and purified by reverse-phase chromatography (Sunfire C18, 30x150 mm, 5-95% CH₃CN/H₂O, 0.05% TFA) to give 187 mg, 19.6% of 4-(2pyridyldisulfanyl)butanoic acid. MS m/z found 230.0 [M+H]⁺.

15 **Intermediate XIX-1: 2-(2-pyridyldisulfanyl)ethyl N-[4-(hydroxymethyl)phenyl]carbamate**

**XIX-1**

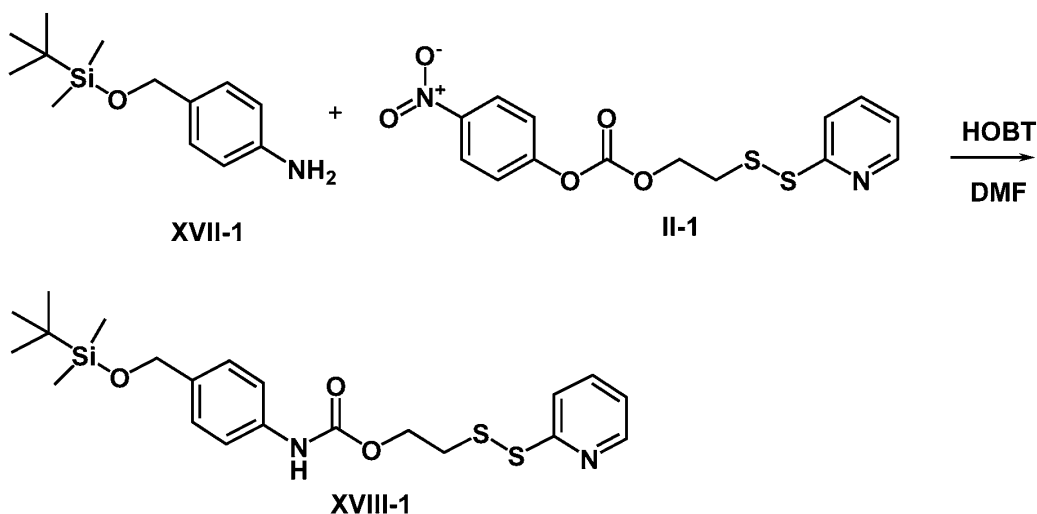
Step 1: Synthesis of 4-[[tert-butyl(dimethyl)silyl]oxymethyl]aniline



A solution of (4-aminophenyl)methanol (5.00 g, 40.6 mmol) in anhydrous DMF (10 mL) was added dropwise within 30 min to a stirred solution of tert-butyl-chloro-dimethylsilane (7.34 g, 48.7 mmol) and imidazole (7.90 mL, 81.2 mmol) in anhydrous DMF (40 mL). After stirring at room temperature for 16 h, the reaction mixture was poured into water (400 mL) and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and dried over MgSO₄. After removing the volatiles in vacuo, the residue was purified by column chromatography (SiO₂, 0-25% EtOAc/hexanes) affording 4-[[tert-butyl(dimethyl)silyl]oxymethyl]aniline (7.91 mg, 33.3 mmol, yield: 82.1 %).

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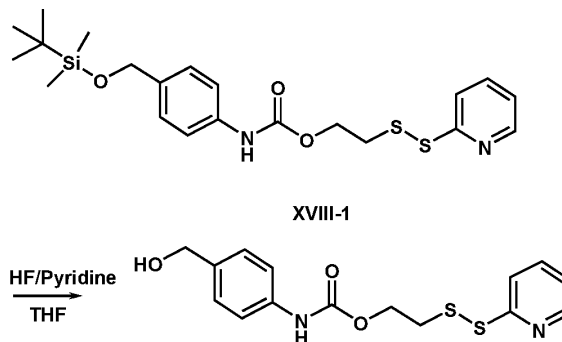
Step 2: Synthesis of 2-(2-pyridyldisulfanyl)ethylN-[4-[[tert-butyl(dimethyl)silyl]oxymethyl]phenyl]carbamate



To (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate (200 mg, 0.57 mmol) in 2 mL of DMF was added 4-[[tert-butyl(dimethyl)silyl]oxymethyl]aniline (202 mg, 0.85 mmol), 1-hydroxybenzotriazole hydrate (104 mg, 0.68 mmol) and 200 mg of activated 4 Å Mol sieves. The mixture was stirred for 18 h. The solids were filtered off and the celite plug rinsed with 2 mL of DMF. The filtrate was concentrated to dryness and the residue purified by column chromatography (SiO₂, 0-50% EtOAc/hexanes) to afford 2-(2-

pyridyldisulfanyl)ethyl N-[4-[[tert-butyl(dimethyl)silyl]oxymethyl]phenyl]carbamate (244 mg, 0.54 mmol, yield: 95.4 %).

Step 3: 2-(2-pyridyldisulfanyl)ethyl N-[4-(hydroxymethyl) phenyl]carbamate



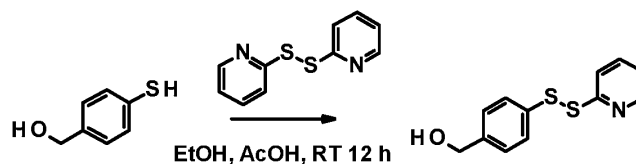
XIX-1

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2-(2-Pyridyldisulfanyl)ethyl N-[4-[[tert-butyl(dimethyl)silyl]oxymethyl]phenyl]-carbamate (194 mg, 0.430 mmol) was dissolved in 2 mL of THF and cooled to 0 °C under N₂. HF-pyridine (780 µL, 8.89 mmol) was added and the solution continued to stir at 0 °C for 1h. Water (3 mL) was added, and the reaction was neutralized with sat. NaHCO₃ and extracted with CH₂Cl₂ (3X50 mL). The organic layer was concentrated and purified by column chromatography (SiO₂, 0-75% EtOAc/hexane) to give 2-(2-pyridyldisulfanyl)ethyl N-[4-(hydroxymethyl) phenyl]carbamate (99.1 mg, 0.29 mmol, yield: 68.4 %).

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Intermediate XXIII-1: [4-(2-pyridyldisulfanyl)phenyl]methanol



XXII-1

XXIII-1

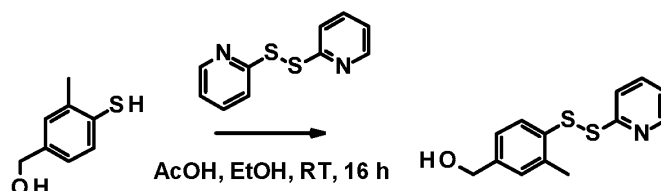
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A stirred solution of 1,2-di(pyridin-2-yl)disulfane (2.68 g, 12.1 mmol) in a mixture of AcOH : ethanol (5 mL, 1:10) solvent was degassed under N₂. This was followed by addition of 4-mercaptophenylmethanol (0.74 g, 5.2 mmol) in a mixture of AcOH/ethanol (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

20

purified by column chromatography (SiO₂, 60-70% EtOAc/hexanes) to afford [4-(2-pyridyldisulfanyl)phenyl]methanol as a colourless liquid (800 mg, 61% yield).

Intermediate XXIII-2: [3-methyl-4-(2-pyridyldisulfanyl)phenyl]methanol



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XXII-2

XXIII-2

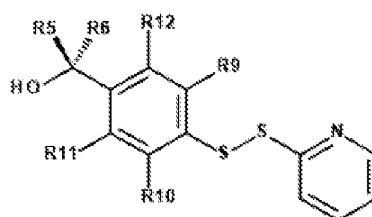
A stirred solution of 1,2-di(pyridin-2-yl)disulfane (1.10 g, 4.90 mmol) dissolved in acetic acid/ethanol (1:10, 37 mL) was purged with N₂ for 5 min. To this, a solution of 4-mercapto-3-methylphenylmethanol (0.51 g, 3.30 mmol) in acetic acid/ethanol (1:10, 18 mL) was added drop-wise over a period of 20 min. The resulting reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the mixture was concentrated under reduced pressure to afford the crude product. The crude product was purified by flash chromatography (SiO₂, 0-70 % EtOAc/hexane gradient) to afford [3-methyl-4-(2-pyridyl disulfanyl)phenyl]methanol as a colorless liquid (0.69 g, 80 % yield). MS m/z found 264.0 [M+H]⁺.

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Intermediates XXIII-3 and XXIII-4

Intermediates XXIII-3 and XXIII-4 as shown in Table 7 were prepared analogously to XXIII-2.

Table 7. Intermediates XXIII



XXIII

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Intermediate	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	MH ⁺
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XXIII-3	H, H	H, H, H, Me	264.2
XXIII-4	H, H	Me, Me, H, H	278.0

Intermediates XXXIII from Intermediates XXXII

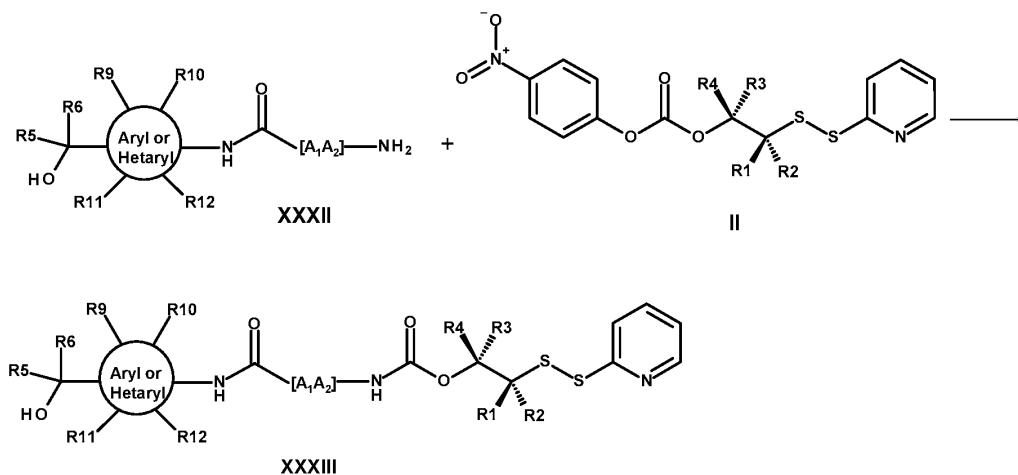
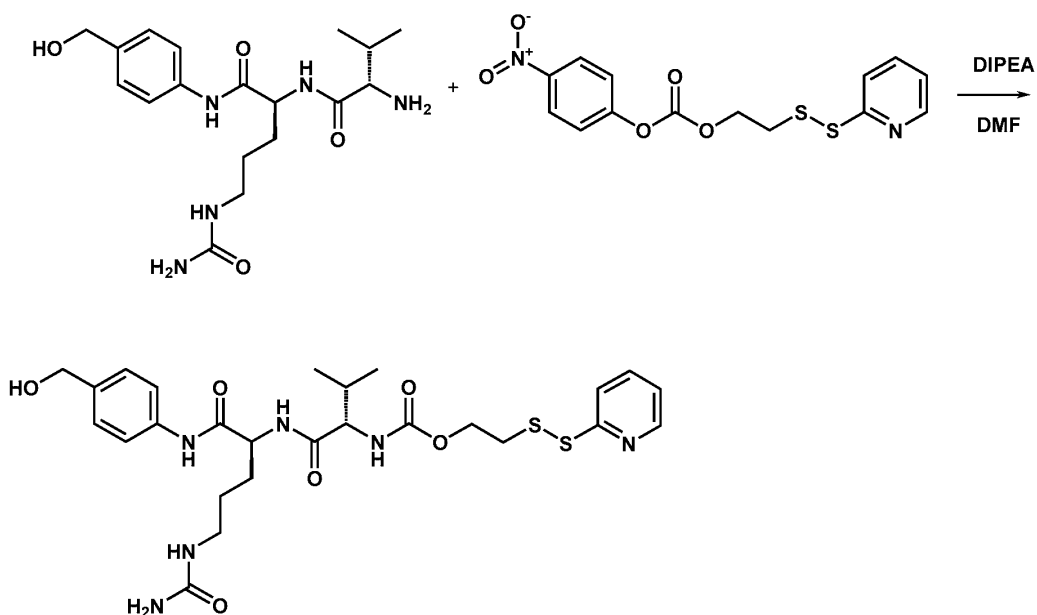


Table 8. Intermediates XXXIII

Int.	R ₁ , R ₂ , R ₃ , R ₄ ,	R ₅ , R ₆	R ₉ , R ₁₀ , R ₁₁ , R ₁₂	A ₁ , A ₂	MH ⁺
XXXIII-1	H, H, H, H	H, H	H, H, H, H	Cit, Val	593.2

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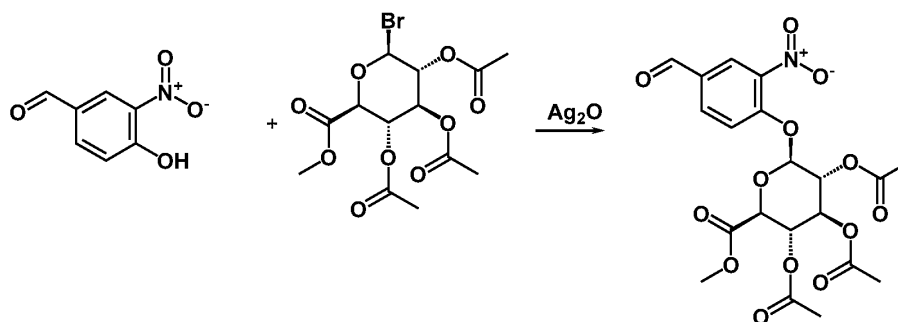
Synthesis of Intermediate XXXIII-1: 2-(2-pyridyldisulfanyl)ethyl N-[(1S)-1-[[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbonyl]-4-ureido-butyl]carbonyl]-2-methyl-propyl]carbamate



To (2S)-2-[[[(2S)-2-amino-3-methyl-butanoyl]amino]-N-[4-(hydroxymethyl)phenyl]-5-ureido-pentanamide (100 mg, 0.264 mmol) in 2 mL of dry DMF under N₂ is added *N,N*-Diisopropylethylamine (0.122 mL, 0.659 mmol) and (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate (92.9 mg, 0.264 mmol). The mixture is stirred for 16 h under N₂. The mixture is concentrated to a solid. The crude residue is purified on an SiO₂ column (12g, 0-10% MeOH/CH₂Cl₂) to give 2-(2-pyridyldisulfanyl)ethyl *N*-[(1S)-1-[[[(1S)-1-[[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate (149 mg, 0.251 mmol, yield: 95.4 %). MS *m/z* found 593.2 [M+H]⁺.

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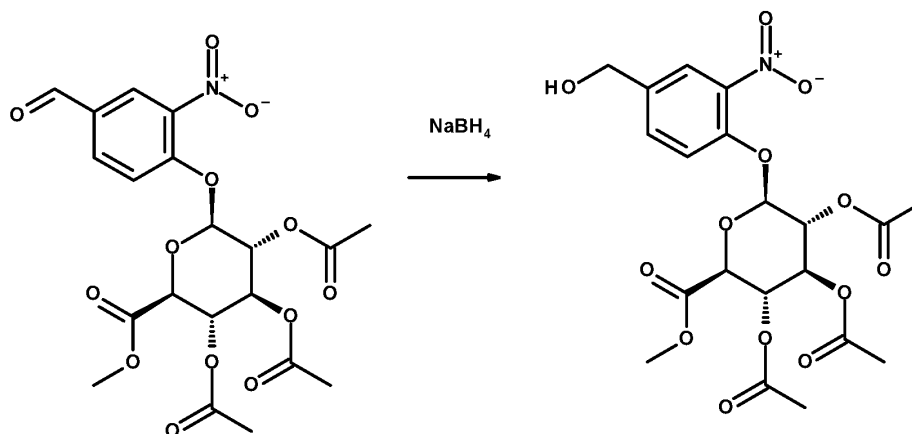
Synthesis of XLI-1: Methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-(4-formyl-2-nitro-phenoxy)tetrahydropyran-2-carboxylate



The title compound was prepared as outlined in US 2017/0145044 A1.

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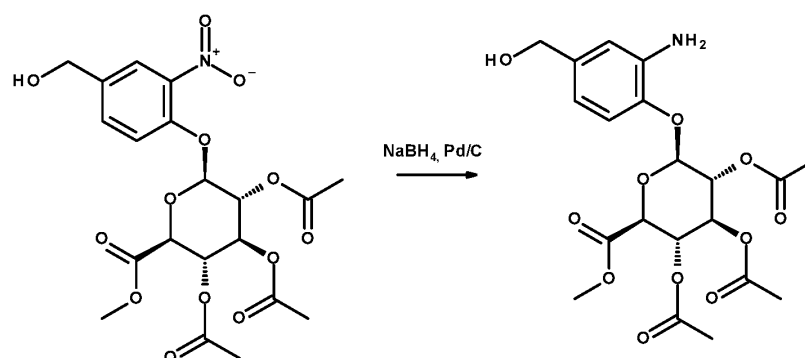
Synthesis of XLII-1: Methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-[4-(hydroxymethyl)-2-nitro-phenoxy]tetrahydropyran-2-carboxylate



The title compound was prepared as outlined in WO 2011/066418 A1.

5

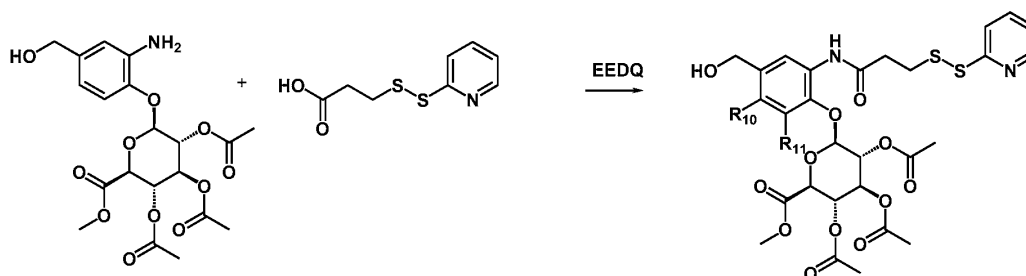
Synthesis of XLIII-1: Methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-[2-amino-4-(hydroxymethyl)phenoxy]tetrahydropyran-2-carboxylate



Methyl (2S, 3S, 4S, 5R)-3,4,5-triacetoxy-6-[4-(hydroxymethyl)-2-nitro-
 10 phenoxy]tetrahydropyran-2-carboxylate (361 mg, 0.74 mmol) was dissolved in MeOH and to
 it was added Pd/C (50.0 mg, 0.47 mmol) followed by NaBH₄ (84.4 mg, 2.23 mmol). The
 reaction mixture was stirred at RT for 15 min. LC-MS indicated the desired product had
 formed. The reaction mixture was filtered through celite and then quenched with sat. NH₄Cl.
 The product was extracted with DCM, EtOAc and the organic layers combined. They were
 15 washed with brine and concentrated. The crude product was partitioned in half and one
 portion was purified by RP HPLC (20-95% ACN/H₂O) to give methyl (2S,3S,4S,5R,6S)-

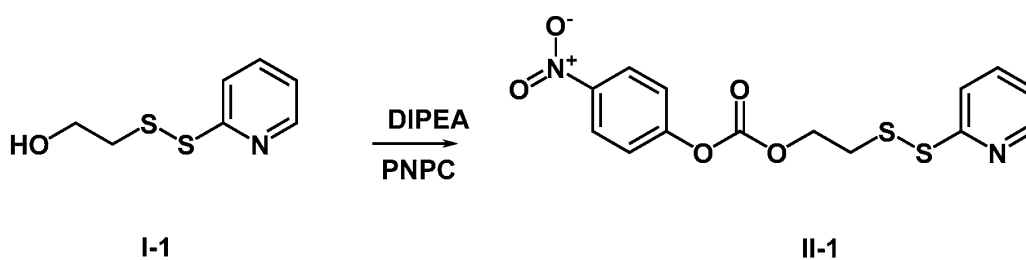
3,4,5-triacetoxy-6-[2-amino-4-(hydroxymethyl)phenoxy]tetrahydropyran-2-carboxylate (180 mg, 0.40 mmol, 53.1% yield). MS m/z found 456.2 $[M+H]^+$.

Synthesis of XLV-1: Methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-[3-R10-2-R11-4-(hydroxymethyl)-6-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate



EEDQ (130 mg, 0.527 mmol) was added to 3-(2-pyridyldisulfanyl)propanoic acid (56.7 mg, 0.263 mmol) dissolved in DCM and was stirred for 20 min under N_2 . The methyl (2S,3S,4S,5R)-3,4,5-triacetoxy-6-[2-amino-4-(hydroxymethyl)phenoxy]tetrahydropyran-2-carboxylate (120 mg, 0.263 mmol) was dissolved in DCM and added to the mixture and stirred overnight. LC-MS indicated the desired product had been formed. The reaction mixture was concentrated and purified by reverse phase chromatography (20-95% ACN/ H_2O) to give 63 mg of methyl (2S,3S,4S,5R)-3,4,5-triacetoxy-6-[4-(hydroxymethyl)-2-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate (63.0 mg, 0.0965 mmol, yield: 36.6 %). MS m/z found 653.2 $[M+H]^+$.

Intermediate II-1: (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate



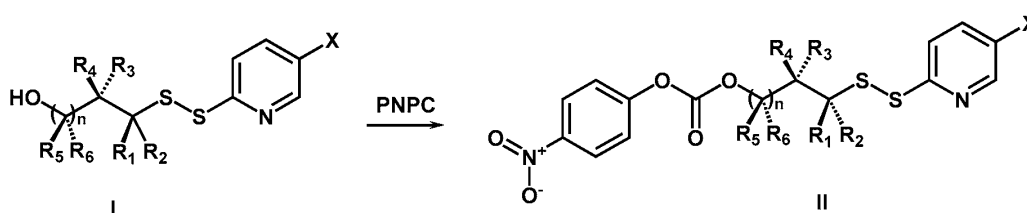
To 2-(2-pyridyldisulfanyl)ethanol (517mg, 2.76mmol) in 20 ml CH_2Cl_2 under N_2 at $4^\circ C$ was added N,N -Diisopropylethylamine (1.02mL, 5.52mmol) and (4-nitrophenyl) carbonochloridate (834mg, 4.14mmol). The mixture was stirred for 16 h under N_2 . The mixture was dissolved into 40 mL of EtOAc and quenched with 20 mL of sat. NH_4Cl . The

mixture was washed with 2x20 mL H₂O and 1x20 mL sat. brine. The crude mixture was purified via SiO₂ column, eluting from 0-50% EtOAc/hexanes to give (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate (482 mg, 50% yield).

5 Intermediates II-2, II-3, and II-5

Intermediates II-2, II-3, and II-5 were prepared analogously to Intermediate II-1 using the appropriate intermediates I-2, I-3, and I-5, as shown below:

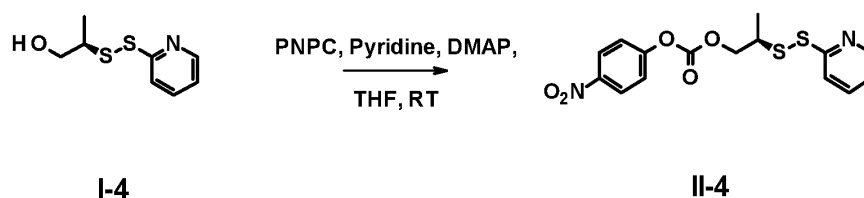
Table 9. Additional Intermediates



Intermediate	R ¹ , R ²	R ³ , R ⁴	n	R ⁵ , R ⁶	X	MH ⁺
II-2	Me-H (rac)	H, H	0	---	H	367.1
II-5	H, Me	H, H	0	---	H	367.1
II-3	Me, Me	H, H	0	---	NO ₂	

10

Intermediate II-4: (4-nitrophenyl) [(2R)-2-(2-pyridyldisulfanyl)propyl] carbonate



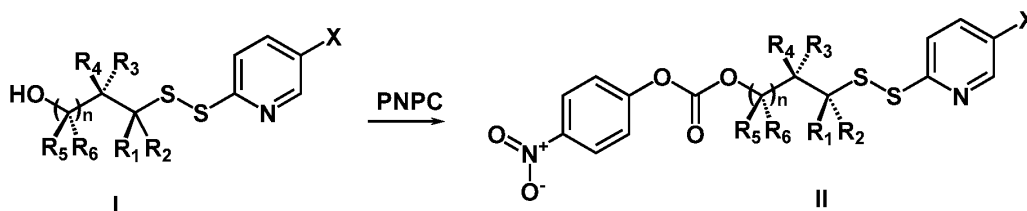
To (2R)-2-(2-pyridyldisulfanyl)propan-1-ol (0.39 g, 1.94 mmol) in THF under N₂ was added pyridine (0.16 mL, 1.94 mmol) and the (4-nitrophenyl) carbonochloridate (0.59 g, 2.91 mmol). The mixture was stirred for 16 h under N₂. The mixture was diluted with EtOAc and quenched with 20 mL of sat. NH₄Cl. The 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]⁺.

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Intermediates II-6 through II-9

Intermediates II-6, II-7, II-8, and II-9 were prepared analogously to Intermediate II-4, as shown below in Table 10:

Table 10. Additional Intermediates



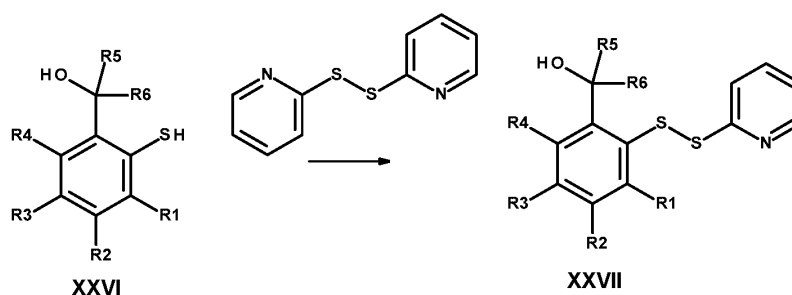
Intermediate	R ¹ , R ²	R ³ , R ⁴	n	R ⁵ , R ⁶	X	MH ⁺
II-6	-CH ₂ CH ₂ CH ₂ - (R ¹ and R ² form a cyclobutyl)	H, H	0	---	NO ₂	438.0
II-7	H, H	H, Ph	0	---	H	429.1
II-8	H, H	H, CO ₂ Me	0	---	H	411.0
II-9	H, H	H, CH ₂ OMe	0	---	H	397.1
II-10	H, H	H, H	1	H, H	H	495.1

5

Synthesis of Intermediate XXVII-1

Intermediate XXVII-1 is prepared analogously to Intermediates XXII.

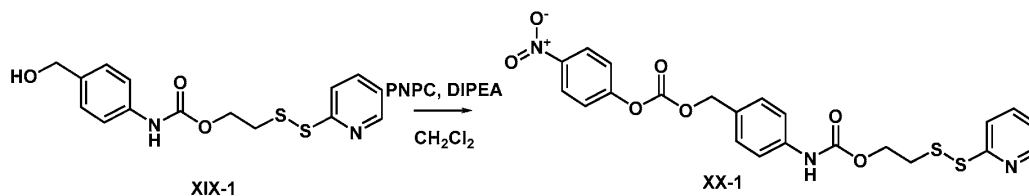
Table 11. Intermediates XXVII



Int.	R ¹ , R ² , R ³ , R ⁴	R ⁵ , R ⁶	MH ⁺
XXVII-1	H, H, H, H	H, H	250.1

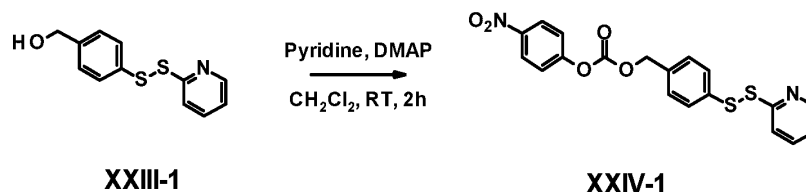
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Intermediate XX-1: (4-nitrophenyl) [4-[2-(2-pyridyldisulfanyl)ethoxycarbonylamino]phenyl]methyl carbonate



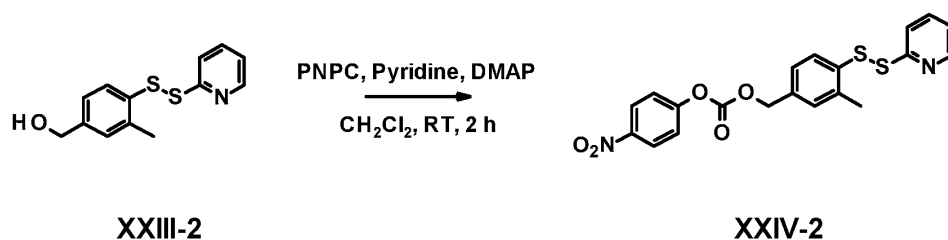
To 2-(2-pyridyldisulfanyl)ethyl N-[4-(hydroxymethyl)phenyl]carbamate (136 mg, 0.41 mmol) in 5 ml CH₂Cl₂ under N₂ was added DIPEA (0.15 mL, 0.81 mmol) and (4-nitrophenyl) carbonochloride (122 mg, 0.61 mmol). The mixture was stirred for 16 h under N₂. The mixture was dissolved into 20 mL of EtOAc and quenched with 20 mL of sat. NH₄Cl. The mixture was washed with 2x20 mL H₂O and 1x20 mL sat. brine. The crude mixture was purified via column chromatography (SiO₂, 0-25% EtOAc/hexanes) to afford (4-nitrophenyl) [4-[2-(2-pyridyldisulfanyl)ethoxycarbonylamino]phenyl]methyl carbonate (44.2 mg, 0.09 mmol, yield: 21.8 %).

Intermediate XXIV-1: (4-nitrophenyl) [4-(2-pyridyldisulfanyl)phenyl]methyl carbonate



To a stirred solution of (4-(pyridin-2-yl)disulfanyl)phenyl)methanol (0.40 g, 1.60 mmol) in CH₂Cl₂ (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 Na₂SO₄ and concentrated. The crude product was purified by column chromatography (SiO₂, 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 [M+H]⁺

Intermediate XXIV-2: [3-methyl-4-(2-pyridyldisulfanyl)phenyl]methyl (4-nitrophenyl) carbonate

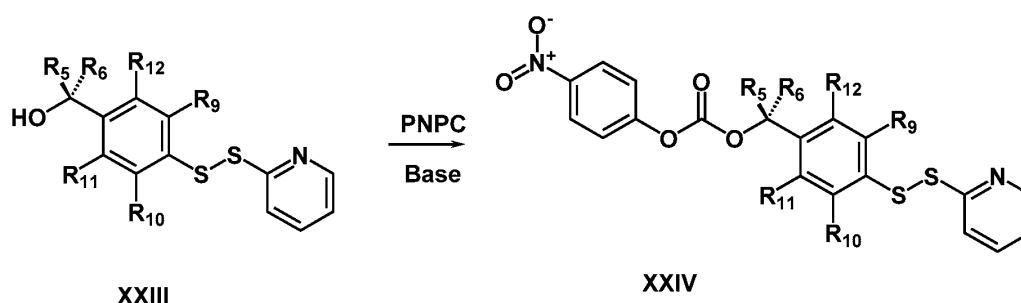


To a stirred solution of (3-methyl-4-(pyridin-2-yl)disulfaneyl)phenyl)methanol (0.69 g, 2.60 mmol) dissolved in CH_2Cl_2 (10 mL) was added 4-nitrophenyl chloroformate (1.05 g, 5.2 mmol), pyridine (0.43 mL, 5.2 mmol), and a catalytic amount of DMAP (0.005 g) at 0°C .

- 5 The mixture was allowed to stir for 2 h at room temperature. After completion of the reaction, the reaction mixture was quenched with 1.5 N HCl. The organic layer was separated, washed with sodium chloride solution (10 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (SiO_2 , 20-30% EtOAc/hexanes) to afford [3-methyl-4-(2-pyridyl)disulfaneyl]phenyl]methyl (4-nitrophenyl) carbonate (700 mg, 62 % yield). MS m/z 429.0 $[\text{M}+\text{H}]^+$.
- 10

The following intermediate were prepared analogously to Intermediates XXIV-1 and XXIV-2 as shown below in Table 12:

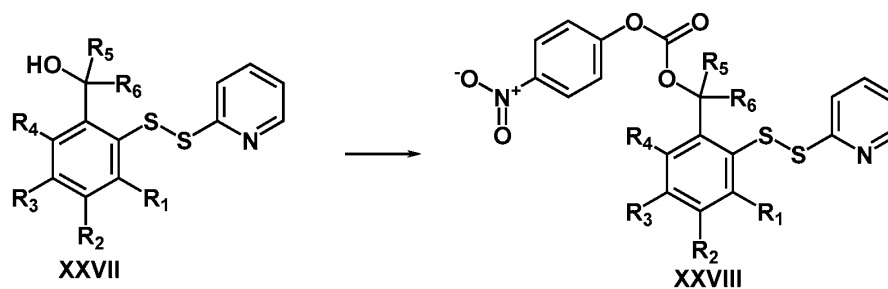
Table 12. Additional Intermediates



Intermediate	R^5, R^6	$\text{R}^9, \text{R}^{10}, \text{R}^{11}, \text{R}^{12}$	X	MH ⁺
XXIV-3	H, H	H, H, Me, H	H	429.0
XXIV-4	H, H	Me, Me, H, H	H	443.0

Intermediates XXVIII

Table 13. Intermediates XXVIII

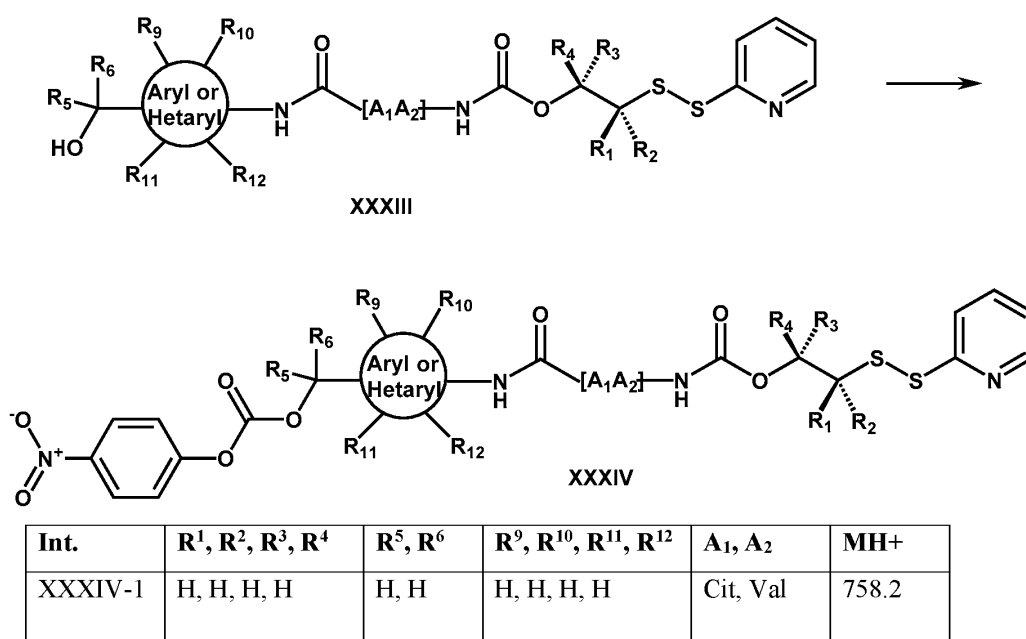


Int.	R ¹ , R ² , R ³ , R ⁴	R ⁵ , R ⁶	MH ⁺
XXVIII-1	H, H, H, H	H, H	415.0

Intermediate XXVIII-1 was prepared analogously to Intermediates XXIV.

Intermediates XXXIV

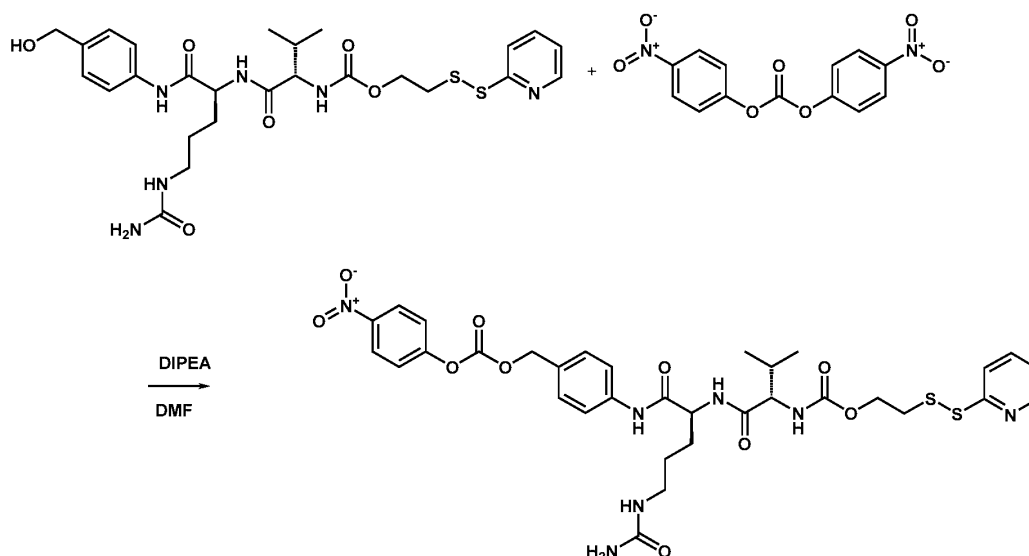
5 Table 14. Intermediates XXXIV



Int.	R ¹ , R ² , R ³ , R ⁴	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	A ₁ , A ₂	MH ⁺
XXXIV-1	H, H, H, H	H, H	H, H, H, H	Cit, Val	758.2

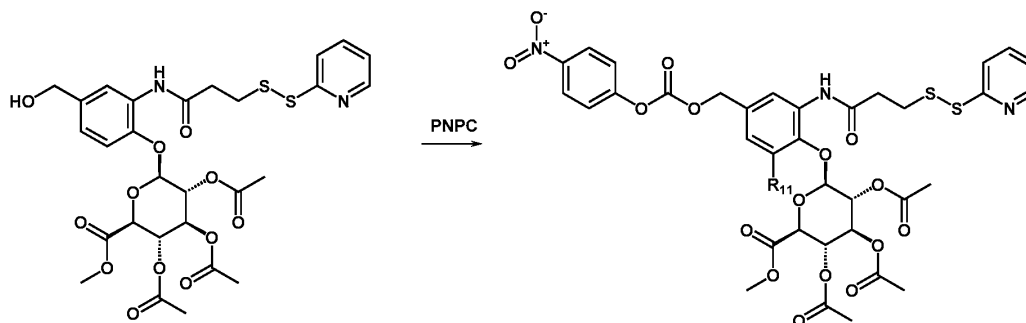
Synthesis of XXXIV-1: [4-[(2S)-2-[(2S)-3-methyl-2-[2-(2-pyridyldisulfanyl)-ethoxycarbonylamino]butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate

10



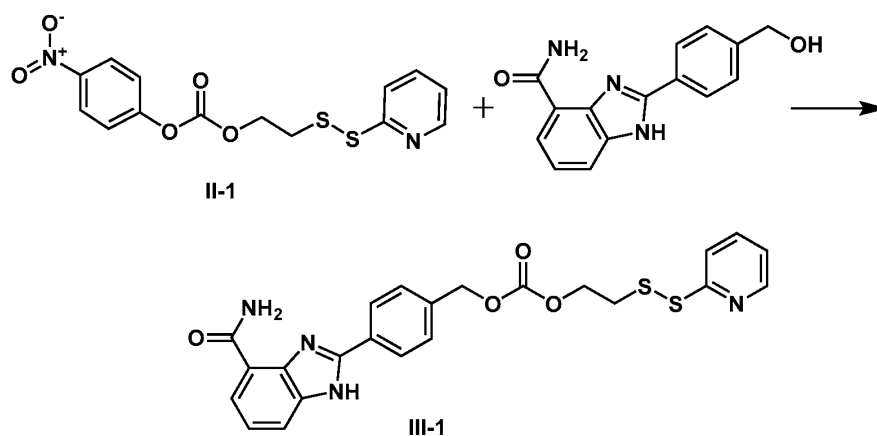
2-(2-pyridyldisulfany)ethyl *N*-[(1*S*)-1-[[4-(hydroxymethyl)phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-2-methyl-propyl]carbamate (149 mg, 0.251 mmol) was dissolved into 2 mL of dry DMF and cooled to 4 °C. To this is added bis(4-nitrophenyl) carbonate (153 mg, 0.503 mmol) and *N,N*-Diisopropylethylamine (0.0928 mL, 0.503 mmol). The mixture was allowed to warm to RT over 2 h. The progress of the reaction was monitored by LC-MS. The mixture was concentrated and purified (25g SiO₂, 0-10% MeOH/CH₂Cl₂) to give 4-[[[(2*S*)-2-[[[(2*S*)-3-methyl-2-[2-(2-pyridyldisulfany)ethoxycarbonylamino]butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate (139 mg, yield: 73.1 %). MS *m/z* found 758.2 [*M*+H]⁺.

Synthesis of XLVI-1: Methyl (2*S*,3*S*,4*S*,5*R*,6*S*)-3,4,5-triacetoxy-6-[2-*R*11-4-[(4-nitrophenoxy)carbonyloxymethyl]-6-[3-(2-pyridyldisulfany)propanoylamino]-phenoxy]tetrahydropyran-2-carboxylate



Methyl (2S,3S,4S,5R)-3,4,5-triacetoxy-6-[4-(hydroxymethyl)-2-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate (102 mg, 0.15 mmol) was dissolved in THF and to it was added pyridine (0.02 mL, 0.19 mmol) followed by (4-nitrophenyl) carbonochloridate (63.0 mg, 0.313 mmol). The reaction mixture was stirred under N₂ overnight. LC-MS indicated a complete reaction. The mixture was diluted with 100 mL EtOAc and quenched with 50 mL of sat. NH₄Cl. The mixture was washed with 50 mL sat. brine. The organic layer was dried with NaSO₄ and concentrated. The crude mixture was purified by column chromatography (50-100% EtOAc/Hexanes) to give (58.0 mg, 0.07 mmol, 45.4%). MS m/z found 818.2 [M+H]⁺.

Intermediate III-1: [4-(4-carbamoyl-1H-benzimidazol-2-yl)phenyl]methyl 2-(2-pyridyldisulfanyl)ethyl carbonate

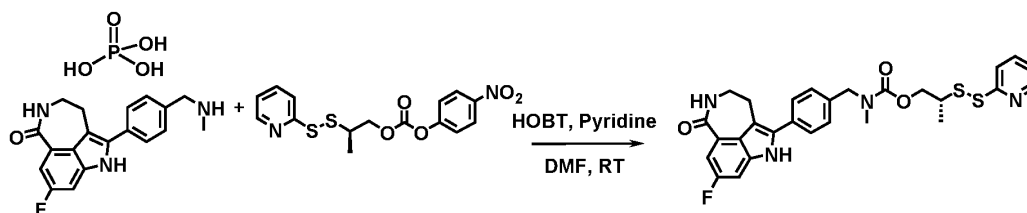


To 2-[4-(hydroxymethyl)phenyl]-1H-benzimidazole-4-carboxamide (150mg, 0.561 mmol) in 4 mL of dry DMF under N₂ was added N,N-diisopropylethylamine (0.207 mL, 1.12 mmol), DMAP (68.6 mg, 0.561 mmol), and (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate (241 mg, 0.684 mmol). The mixture was stirred for 16 h., and then diluted with 50 mL of EtOAc and successively washed with 1x20 mL of sat. NH₄Cl,

3x30 mL of sat. NaHCO₃, 3x30 mL of H₂O, and 1x20 mL of sat. brine. The organic phase was dried with MgSO₄, filtered, and concentrated. The crude residue was purified (SiO₂, 50-100% EtOAc/hexanes) to give 260 mg, 97% yield of [4-(4-carbamoyl-1H-benzimidazol-2-yl)phenyl]methyl-2-(2-pyridyldisulfanyl)ethyl carbonate.

5

Intermediate III-4: [(2R)-2-(2-pyridyldisulfanyl)propyl] N-[[4-(6-fluoro-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate



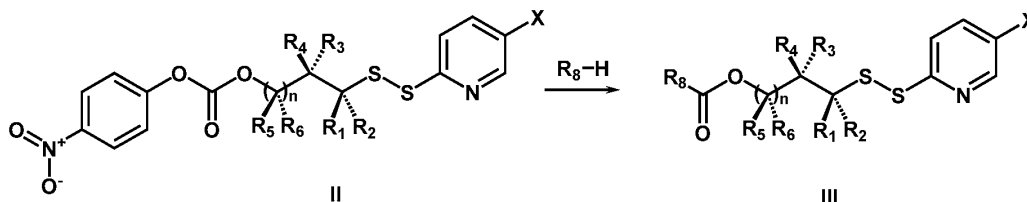
To a mixture of HOBt (48.0 mg, 0.31 mmol), pyridine (0.11 mL, 1.31 mmol), finely ground molecular sieve 4 Å (250 mg), and the 6-fluoro-2-[4-(methylaminomethyl)phenyl]-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-9-one phosphate (110 mg, 0.26 mmol) in 5 mL of anhydrous DMF was added the (4-nitrophenyl) [(2R)-2-(2-pyridyldisulfanyl)propyl] carbonate [Intermediate II-4] (105 mg, 0.29 mmol). After stirring for 16 h at room temperature, the molecular sieves were filtered off and the solvent removed in vacuo. The residue was then adsorbed onto SiO₂ and purified by column chromatography (SiO₂, 0-10% MeOH/CH₂Cl₂) to afford [(2R)-2-(2-pyridyldisulfanyl)propyl] N-[[4-(6-fluoro-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate (115 mg, 80% yield) MS m/z 551.1 (M+H)⁺.

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Intermediates III-2, III-3, and III-5 through III-15

The following intermediates were prepared analogously to Intermediates III-1 and III-4 as shown below in Table 15.

Table 15. Additional Intermediates

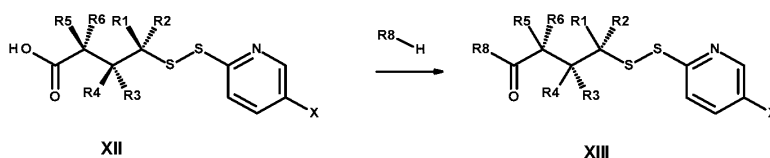


25

Intermediate	R ⁸ H	R ¹ , R ²	R ³ , R ⁴	n	R ⁵ , R ⁶	X	MH ⁺
III-2	R ⁸ H-1	Me-H (rac)	H, H		---	H	495.1
III-3	R ⁸ H -1	Me, Me	H, H		---	NO ₂	
III-5	R ⁸ H -1	Me, H	H, H		---	H	496.1
III-6	R ⁸ H -2	H, H	H, H		---	H	451.1
III-7	R ⁸ H -3	H, H	H, H		---	H	534.2
III-8	R ⁸ H -4	H, H	H, H		---	H	458.1
III-9	R ⁸ H -16	H, H	H, H		---	H	536.4
III-10	R ⁸ H -16	H, Me	H, H		---	H	551.1
III-11	R ⁸ H -16	Me, H	H, H		---	H	551.1
III-12	R ⁸ H -16	-CH ₂ CH ₂ CH ₂ -	H, H		---	NO ₂	622.2
III-13	R ⁸ H -16	H, H	Ph, H		---	H	613.2
III-14	R ⁸ H -16	H, H	CO ₂ Me, H		---	H	595.2
III-15	R ⁸ H -16	H, H	CH ₂ OMe		---	H	581.2
III-16	R ₈ H-3	H, Me	H, H	0	---	H	548.1
III-17	R ₈ H-3	Me, H	H, H	0	---	H	548.1
III-18	R ₈ H-4	H, Me	H, H	0	---	H	472.1
III-19	R ₈ H-4	Me, H	H, H	0	---	H	472.1
III-20	R ₈ H-16	H, H	H, H	1	H, H	H	495.2

Ester Linked Intermediates XIII

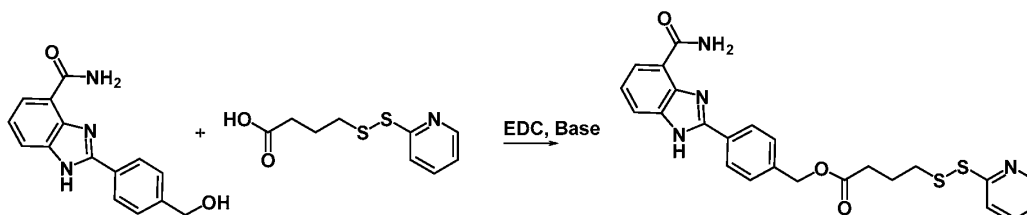
Table 16. Intermediates XIII



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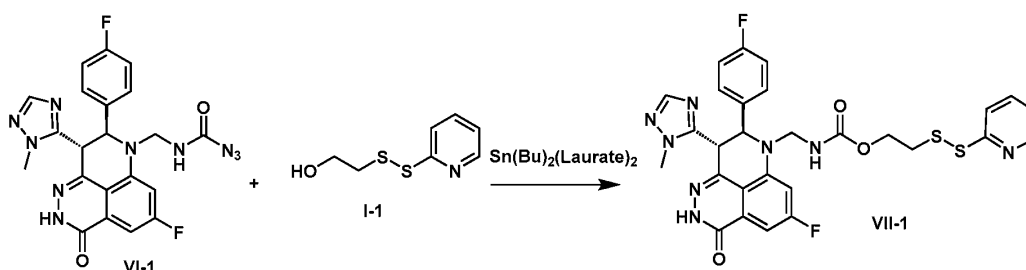
Int.	R ⁸	R ¹ , R ²	R ³ , R ⁴	R ⁵ , R ⁶	X	MH ⁺
XIII-1	R ⁸ H-1	H, H	H, H	H, H	H	479.0

Synthesis of XIII-1: [4-(4-carbamoyl-1H-benzimidazol-2-yl)phenyl]methyl 4-(2-pyridyldisulfanyl)butanoate



To a mixture of 4-(2-pyridyldisulfanyl)butanoic acid TFA salt (81.2 mg, 0.236 mmol), EDC HCl (47.8 mg, 0.250 mmol), and DIEA (0.0986 mL, 0.576 mmol) in 2 mL of DMF was added 2-[4-(hydroxymethyl)phenyl]-1H-benzimidazole-4-carboxamide (51.3 mg, 0.192 mmol). The mixture was stirred overnight and monitored by LC-MS. The mixture was concentrated and purified with SiO₂ chromatography (0-10% MeOH/CH₂Cl₂) to give (83.1 mg, 0.17 mmol, yield: 90.4 %) MS m/z found 479.0 [M+H]⁺.

Intermediate VII-1: 2-(2-pyridyldisulfanyl)ethyl N-[(11S,12R)-7-fluoro-11-(4-fluorophenyl)-12-(2-methyl-1,2,4-triazol-3-yl)-4-oxo-2,3,10-triazatricyclo[7.3.1.0^{5,13}]trideca-1,5,7,9(13)-tetraen-10-yl]methyl]carbamate

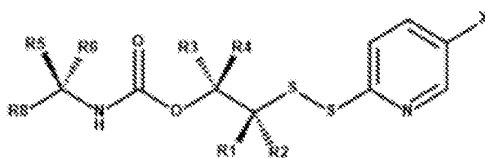


The 2-[(11S,12R)-7-fluoro-11-(4-fluorophenyl)-12-(2-methyl-1,2,4-triazol-3-yl)-4-oxo-2,3,10-triazatricyclo[7.3.1.0^{5,13}]trideca-1,5,7,9(13)-tetraen-10-yl]acetyl azide, V-1, (170 mg, 0.367 mmol) was dissolved in dry DMF (2 mL) and 2-(2-pyridyldisulfanyl)ethanol (137 mg, 0.734 mmol) is added. The reaction was heated to 65° C for 2h. Catalytic dibutyltin dilaurate (40 uL) was added and the reaction mixture stirred overnight at 65° C. The mixture was concentrated and purified by column chromatography (0-10%MeOH/DCM) to give 3 peaks. NMR indicated peak 2 was the desired product. MALDI showed 3 masses of 624 (desired product), 646 (product+23) and 380 (BMN). Yield: 80 mg of Intermediate VII-1: 2-(2-pyridyldisulfanyl)ethyl N-[(11S,12R)-7-fluoro-11-(4-fluorophenyl)-12-(2-methyl-1,2,4-triazol-3-yl)-4-oxo-2,3,10-triazatricyclo[7.3.1.0^{5,13}]trideca-1,5,7,9(13)-tetraen-10-yl]methyl]carbamate.

Intermediates VII-2 through VII-5

Intermediates VII-2, VII-3 and VII-4 were prepared using R⁸H-15 and intermediates II-4, II-5 and II-6, respectively. Intermediate VII-5 was prepared using R⁸H-17 and intermediate II-1 as shown in Table 17.

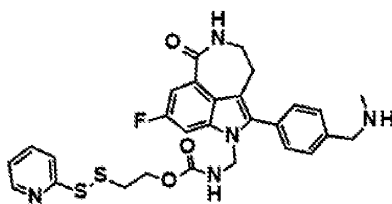
5 Table 17. Additional Intermediates



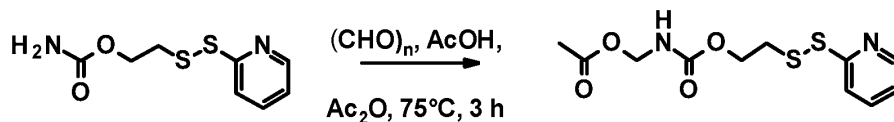
VII

Int.	R ⁸ H	R ¹ , R ²	R ³ , R ⁴	R ⁵ , R ⁶	X	MH ⁺
VII-2	R ⁸ H -15	H, Me	H, H	H, H	H	637.2
VII-3	R ⁸ H-15	Me, H	H, H	H, H	H	637.2
VII-4	R ⁸ H -15	-CH ₂ CH ₂ CH ₂ - (R ¹ and R ² form a cyclopropyl)	H, H	H, H	NO ₂	708.2
VII-5	R ⁸ H-17	H, H	H, H	H, H	H	541.1

Intermediate VII-6: 2-(2-pyridyldisulfanyl)ethyl N-[[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]methyl]carbamate



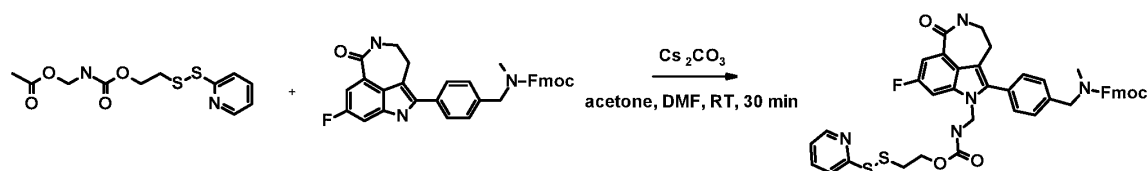
Step 1: (((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl acetate



To a stirred solution of 2-(pyridin-2-yl)ethanethiol (0.10 g, 0.43 mmol) in acetic acid (0.59 mL) was added paraformaldehyde (0.01 g, 0.47 mmol) and acetic

anhydride (1.78 mL). The mixture was heated at 75°C for 3 h. After completion of reaction, the reaction was quenched with water (20 mL) and extracted with ethyl acetate (20 mL x 2). The combined organic layer was washed with water (20 mL) and brine solution (20 mL). The organic phase was dried over anhydrous Na₂SO₄ and concentrated. The crude product was
 5 purified by column chromatography (SiO₂, 40-50% of ethyl acetate/hexanes) to afford (((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl acetate as a yellow liquid (120 mg, 70%). MS m/z 303.3 (M+H)⁺

Step 2: (9H-fluoren-9-yl)methyl(4-(8-fluoro-1-oxo-6-(((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl)-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl)(methyl)carbamate



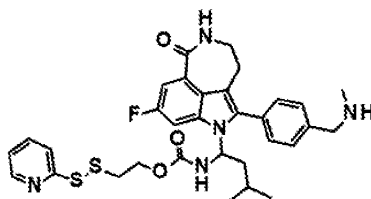
To a stirred solution of (9H-fluoren-9-yl)methyl (4-(8-fluoro-1-oxo-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl)(methyl)carbamate (0.05 g, 0.09 mmol) in
 15 acetone (1.0 mL) was added cesium carbonate (0.06 g, 0.18 mmol) and stirred for 5 min at room temperature under N₂ atmosphere. Then (((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl acetate (0.03 g, 0.09 mmol) and DMF (0.1 mL) were added and further stirred for 30 min at room temperature. The reaction was quenched with water (25 mL) and then extracted with 10% of MeOH/CH₂Cl₂ mixture (50 mL x 2). The
 20 combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude product which was purified by flash column chromatography using 60-70% of EtOAc/hexanes mixture to afford (9H-fluoren-9-yl)methyl(4-(8-fluoro-1-oxo-6-(((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl)-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl)(methyl)carbamate (12 mg, 17 %
 25 yield); MS m/z 788.8 (M+H)⁺.

Step 3: 2-(2-pyridyl)disulfanyl ethyl N-[[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]methyl]carbamate



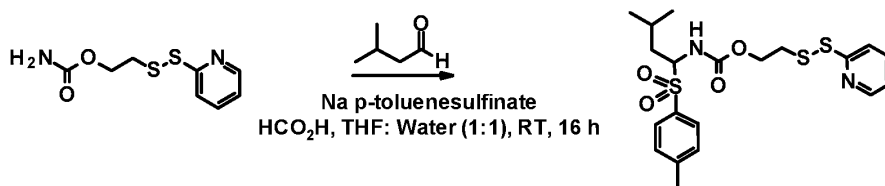
(9H-fluoren-9-yl)methyl(4-(8-fluoro-1-oxo-6-(((2-(pyridin-2-yl)disulfanyl)ethoxy)carbonyl)amino)methyl)-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl(methyl)carbamate (0.15 g, 0.19 mmol) was stirred with 20% of diethylamine in DMF (1.4 mL) for 1 h under N₂ at room temperature. The reaction mixture was concentrated to dryness and purified by prep-HPLC [Column: Inertsil ODS 3V (250mm X 20mm X 5mic); Mobile phase-A-0.1% ammonia in H₂O; Mobile phase-B-ACN] to afford 2-(2-pyridyl)disulfanyl)ethyl N-[[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]methyl]carbamate as a colorless solid (30 mg, 28% yield). MS m/z 566.4 (M+H)⁺.

Intermediate VII-7: 2-(2-pyridyl)disulfanyl)ethyl N-[1-[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]-3-methyl-butyl]carbamate



15

Step 1: 2-(pyridin-2-yl)disulfanyl) ethyl(3-methyl-1-(phenylsulfonyl)butyl)carbamate

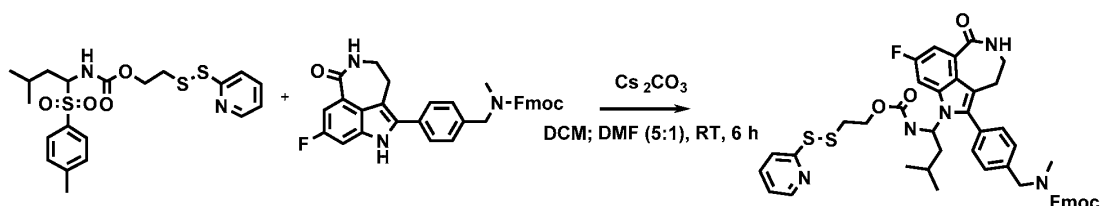


To a stirred solution of 2-(pyridin-2-yl)disulfanyl)ethyl carbamate (2.50 g, 0.01 mol) in THF:water (19 mL, 1:1) were added sodium p-toluenesulfonate (1.93 g, 0.01 mol), 3-methylbutanal (1.26 mL, 0.01 mol) and then formic acid (2.5 mL). The mixture was stirred for 16 h at room temperature under N₂. The reaction mixture was concentrated to dryness and

20

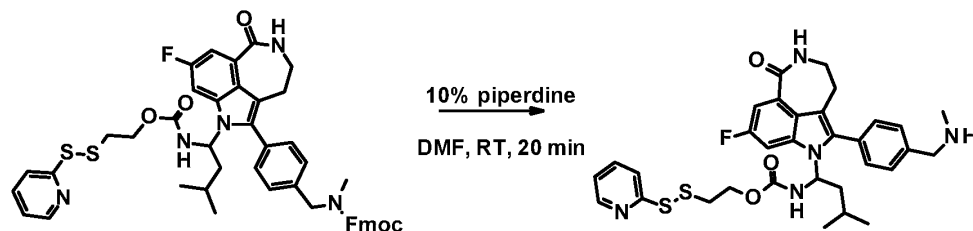
then purified by flash column chromatography (SiO₂, 40-70% EtOAc/hexanes) to afford 2-(pyridin-2-yl)disulfanyl ethyl(3-methyl-1-(phenylsulfonyl)butyl)carbamate (1.7 g, 41% yield). MS m/z 455.1 [M+H]⁺.

- 5 *Step 2: (9H-fluoren-9-yl)methyl 4-(8-fluoro-6-(3-methyl-1-(((2-(pyridin-2-yl)disulfanyl) ethoxy) carbonyl)amino)butyl)-1-oxo-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl(methyl) carbamate*



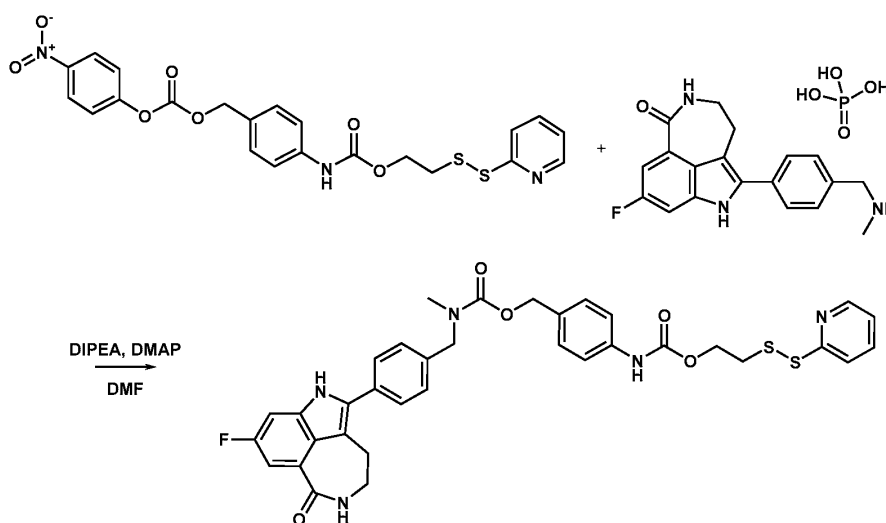
- To a stirred suspension of (9H-fluoren-9-yl)methyl 4-(8-fluoro-1-oxo-2,3,4,6-
 10 tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl(methyl)carbamate (0.5 g, 0.92 mmol) and cesium carbonate (0.89 g, 2.74 mmol) in CH₂Cl₂:DMF (5:1, 12 mL) was added 2-(pyridin-2-yl)disulfanyl ethyl(3-methyl-1-(phenylsulfonyl)butyl)carbamate (0.80 g, 1.83 mmol) portion wise over 4 h. The mixture was stirred an additional 2 h under N₂ at room temperature. The reaction was monitored by TLC. The reaction mixture was diluted with water (25 mL) and
 15 DCM (100 mL). The organic layer was separated, washed with water, brine. The organic phase was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified by flash column chromatography (SiO₂, 90-100% EtOAc/hexanes) to afford (9H-fluoren-9-yl)methyl 4-(8-fluoro-6-(3-methyl-1-(((2-(pyridin-2-yl)disulfanyl) ethoxy) carbonyl)amino)butyl)-1-oxo-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl(methyl) carbamate (0.35 g, 45% yield). MS m/z 844.1 [M+H]⁺.
 20

Step 3: 2-(2-pyridyl)disulfanyl ethyl N-[1-[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]-3-methyl-butyl]carbamate



(9H-fluoren-9-yl)methyl 4-(8-fluoro-6-(3-methyl-1-(((2-(pyridin-2-yl)disulfanyl)ethoxy) carbonyl)amino)butyl)-1-oxo-2,3,4,6-tetrahydro-1H-azepino[5,4,3-cd]indol-5-yl)benzyl(methyl) carbamate (0.30 g, 0.355 mmol) was stirred with 10% piperidine in DMF (1.4 mL) for 20 min at room temperature under N₂. The reaction mixture was concentrated to dryness and then triturated with diethyl ether to obtain a colorless solid. The resultant solid was further purified by prep HPLC [Column: Inertsil ODS 3V (250mm X 20mm X 5mic), Mobile phase-A-0.1% ammonia in H₂O: Mobile phase-B-ACN] to afford 2-(2-pyridyl)disulfanyl)ethyl N-[1-[6-fluoro-2-[4-(methylaminomethyl)phenyl]-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-3-yl]-3-methyl-butyl]carbamate (0.07 g, 30% yield). MS m/z 622.0 [M+H]⁺.

Intermediate XXI-1: [4-[2-(2-pyridyl)disulfanyl)ethoxycarbonylamino] phenyl]methyl N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4(13),5,7-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate

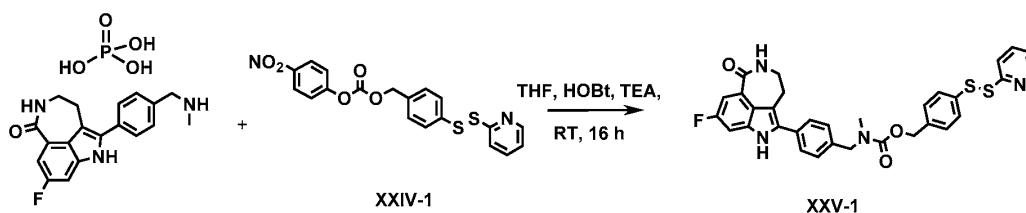


To 2-[4-(methylaminomethyl)phenyl]-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4(13),5,7-tetraen-9-one;phosphoric acid (36.0 mg, 0.09 mmol) in 2 mL of dry DMF under N₂ was added DIPEA (0.03 mL, 0.18 mmol), DMAP (10.9 mg, 0.09 mmol) and (4-nitrophenyl) [4-[2-(2-pyridyl)disulfanyl)ethoxycarbonyl amino] phenyl]methyl carbonate (44.8 mg, 0.09 mmol). The mixture was stirred for 16 h. The mixture was diluted with 20 mL of EtOAc, washed with 1x20 mL of sat. NH₄Cl, 2x20 mL of sat. NaHCO₃, 3x30 mL of H₂O and 1x20 mL of sat. brine. The mixture was dried with MgSO₄, filtered and concentrated.

The crude residue was purified by column chromatography (SiO₂, 0-5% MeOH/CH₂Cl₂) to give [4-[2-(2-pyridyldisulfanyl) ethoxycarbonylamino]phenyl]methyl N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}] trideca-1,4(13),5,7-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate (44.2 mg, 0.06 mmol, yield: 75.4 %).

5

Intermediate XXV-1: [4-(2-pyridyldisulfanyl)phenyl]methyl N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate

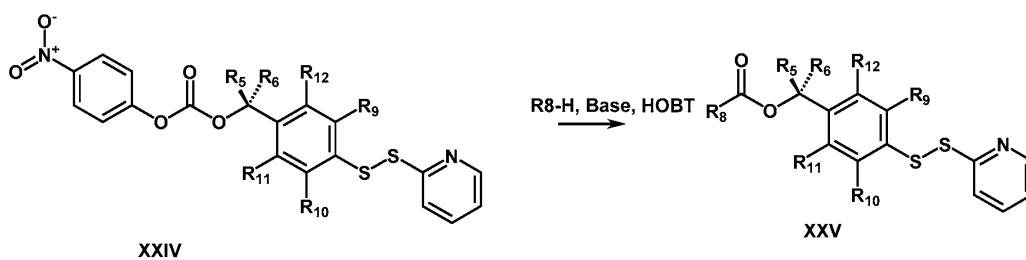


To a stirred solution of 2-[4-(methylaminomethyl)phenyl]-3,10-diazatricyclo[6.4.1.0^{4,13}] trideca-1,4(13),5,7-tetraen-9-one;phosphoric acid (1.00 g, 3.09 mmol) in THF (20 mL) under N₂ was added TEA (1.40 mL, 3.04 mmol), HOBt (0.21 g, 1.50 mmol) and 4-nitrophenyl (4-(pyridin-2-yl)disulfanyl)benzyl carbonate (1.40 g, 3.40 mmol). The mixture was stirred under N₂ for 16 h at room temperature. The reaction mixture was concentrated and the crude purified by flash chromatography (SiO₂, 0-5% MeOH/CH₂Cl₂) to afford [4-(2-pyridyldisulfanyl)phenyl]methyl N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}] trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate as a colourless solid (1.13 g, 59% yield). MS m/z 599.0 (M+H)⁺.

20 Intermediates XXV-2 through XXV-5

Intermediates XXV-2 through XXV-5 were prepared analogously to Intermediate XXV-1 using the appropriate R₈-H compound and intermediates XXIV-2 through XXIV-5 as shown below in Table 18.

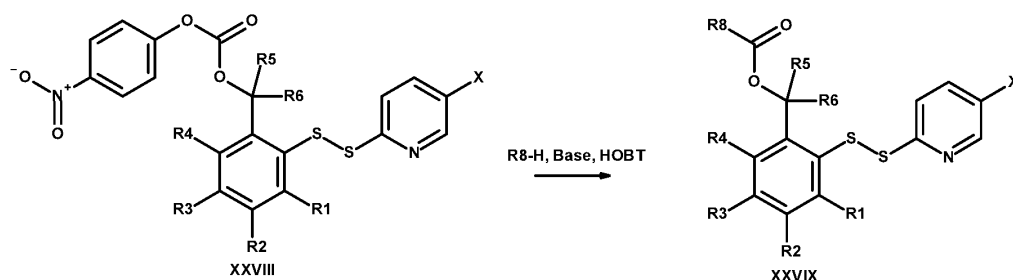
Table 18. Additional Intermediates



Int.	R ⁸ H	R ⁵ , R ⁶	R ⁹ , R ¹⁰ , R ¹¹ , R ¹²	X	MH ⁺
XXV-2	R ⁸ H-16	H, H	Me, H, H, H	H	613.2
XXV-3	R ⁸ H-16	H, H	H, H, Me, H	H	613.0
XXV-4	R ⁸ H-16	H, H	Me, Me, H, H	H	627.0
XXV-5	R ⁸ H-3	H, H	H, H, H, H	H	596.2
XXV-6	R ⁸ H-4	H, H	H, H, H, H	H	520.2
XXV-7	R ⁸ H-21	H, H	H, H, H, H	H	739.2

Synthesis of Intermediates XXVIX from XXVII

5 Table 19. Intermediates XXVIX



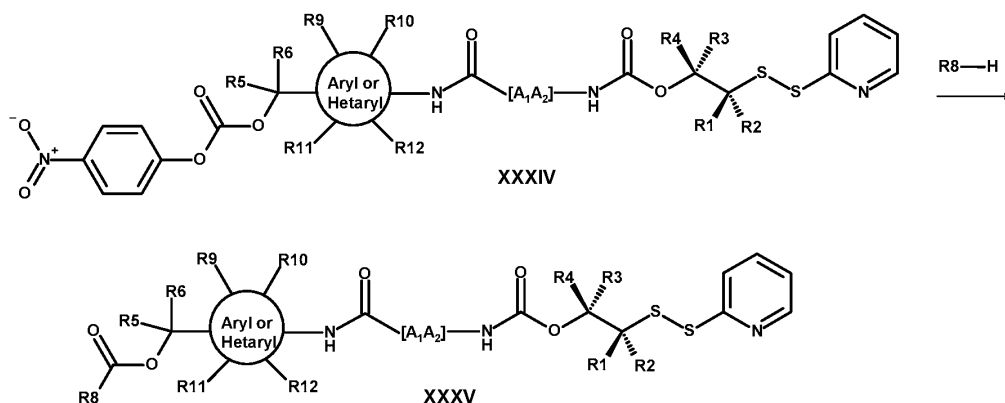
Int.	R ⁸	R ¹ , R ²	R ³ , R ⁴	R ⁵ , R ⁶	X	MH ⁺
XXVIX-1	R ⁸ H-16	H, H	H, H	H, H	H	599.1

Intermediate XXVIX-1 was prepared from Intermediate XXVIII-1 analogously to Intermediate XXV-1.

10

Intermediates XXXV from XXXIV

Table 20. Intermediates XXXV



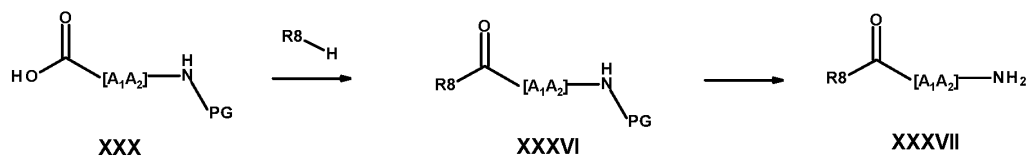
Int.	R ⁸	R ¹ , R ² , R ³ , R ⁴	R ⁵ , R ⁶	R ⁹ , R ₁₀ , R ₁₁ , R ₁₂	A ₁ , A ₂	MH ⁺
XXXV-1	R ₈ H-16	H, H, H, H	H, H	H, H, H, H	Cit, Val	942.4

Intermediate XXXV-1 was prepared from Intermediate XXXIV-1 analogously to Intermediate XXV-1.

5

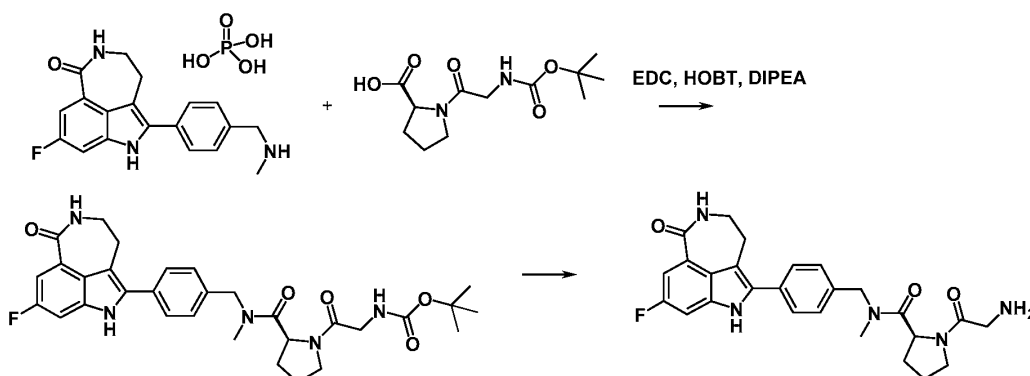
Intermediates XXXVII from XXX

Table 21. Intermediates XXXVII



Int.	R ⁸	R ¹ , R ² , R ³ , R ⁴	A ₁ , A ₂	X	MH ⁺
XXXVII-1	R ⁸ H-16	H, H, H, H	Pro, Gly	H	478.2

- 10 **Synthesis of XXXVII-1: (2S)-1-(2-aminoacetyl)-N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methylpyrrolidine-2-carboxamide**



Step 1. Synthesis of tert-butyl-N-[2-[(2S)-2-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-2-yl]phenyl]methyl-methyl-carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]carbamate

- 5 1-[2-(tert-Butoxycarbonylamino)acetyl]pyrrolidine-2-carboxylic acid (0.16 g, 0.59 mmol) was dissolved in DMF and to it was added 1-hydroxybenzotriazole hydrate (80.0 %, 114 mg, 0.59 mmol) and EDC HCl (114 mg, 0.59 mmol). The solution was stirred at RT for 15 min before the 6-fluoro-2-[4-(methylaminomethyl)phenyl]-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-9-one;phosphoric acid (200 mg, 0.475 mmol) and N,N-diisopropylethylamine (0.44 mL, 2.37 mmol) were added. The solution was then heated to 65 °C overnight. LC-MS indicated a complete reaction. The reaction mixture was diluted with EtOAc, washed with sat. NH₄Cl, water, and brine. The crude [1-[2-(tert-butoxycarbonylamino)acetyl]pyrrolidin-2-yl] N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-2-yl]phenyl)methyl]-N-methyl-
- 10 carbamate (141 mg, 0.24 mmol, yield: 50.0 %) was carried on as is. MS m/z 478.2 (M+H minus BOC)⁺.

Step 2. Synthesis of (2S)-1-(2-aminoacetyl)-N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-2-yl]phenyl)methyl]-N-methyl-

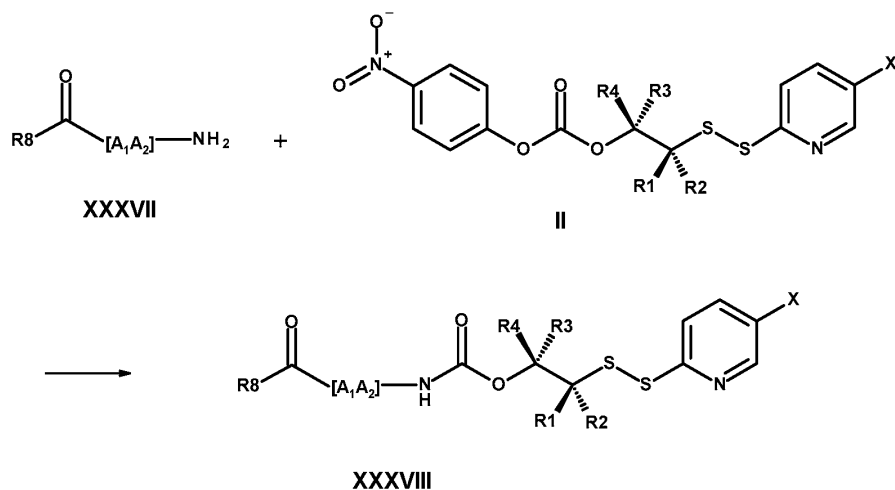
20 pyrrolidine-2-carboxamide

- The tert-Butyl-N-[2-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-2-yl]phenyl)methyl-methyl-carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]carbamate (141 mg, 0.24 mmol) was dissolved in DCM and 1 mL HCl (4.00 M, 0.12 mL, 0.48 mmol) in dioxane was added. The reaction mixture was stirred at RT overnight.
- 25 LC-MS indicated a complete reaction. The reaction mixture was concentrated and purified by reverse phase chromatography (20-85% ACN/H₂O). to give 68 mg of 1-(2-aminoacetyl)-N-

[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-pyrrolidine-2-carboxamide (68.0 mg, 0.142 mmol, yield: 58.3 %). MS *m/z* 478.2 (M+H)⁺.

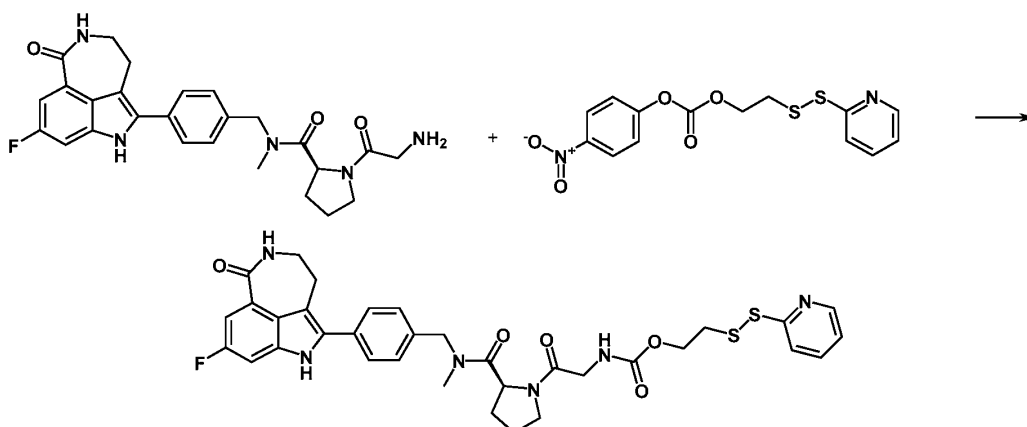
5 Intermediates XXXVIII

Table 22. Intermediates XXXVIII



Int.	R8	R1, R2, R3, R4,	A1, A2	X	MH ⁺
XXXVIII-1	R ₈ H-16	H, H, H, H	Pro, Gly	H	691.2

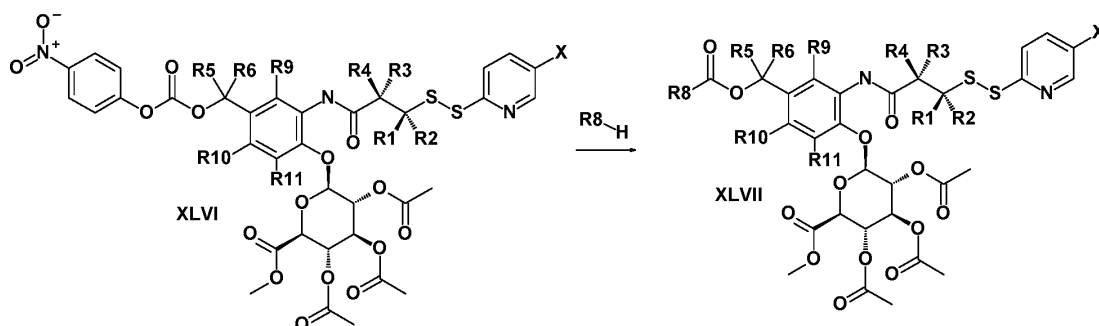
10 **Synthesis of XXXVIII-1: 2-(2-pyridyldisulfanyl)ethyl N-[2-[(2S)-2-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl-methyl-carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]carbamate**



To 1-(2-aminoacetyl)-N-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0⁴,13]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-pyrrolidine-2-carboxamide (68.0 mg, 0.142 mmol) in 2 mL of dry DMF was added DMAP (17.4 mg, 0.142 mmol), (4-nitrophenyl) 2-(2-pyridyldisulfanyl)ethyl carbonate (50.2 mg, 0.142 mmol) and N,N-diisopropylethylamine (27.6 mg, 0.214 mmol). The mixture stirred for 16 h and then diluted with 50 ml of EtOAc, washed with 1x20 mL of sat. NH₄Cl, 3x30 mL of H₂O and 1x20 mL of sat. brine. The mixture was dried with MgSO₄, filtered and concentrated. The crude residue was purified on an SiO₂ column (0-3% MeOH/DCM) to give 30 mg of 2-(2-pyridyldisulfanyl)ethyl N-[2-[2-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0⁴,13]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl-methyl-carbamoyl]pyrrolidin-1-yl]-2-oxo-ethyl]carbamate (30.0 mg, 0.0434 mmol, yield: 30.5%). MS m/z 691.2 (M+H)⁺.

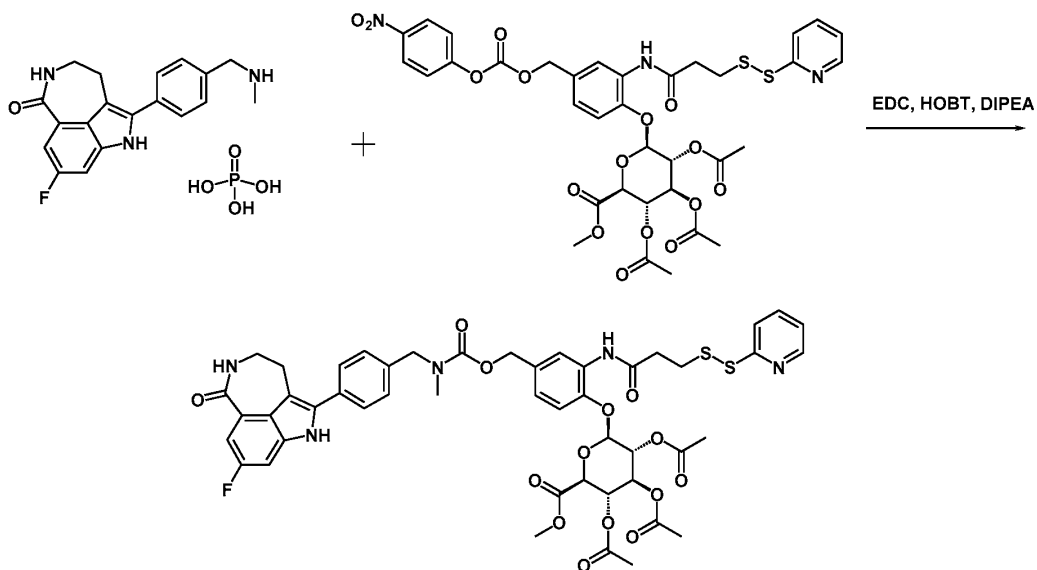
Intermediates XLVIII from XLVI

Table 23. Intermediates XLVIII



Int.	R8	R1, R2, R3, R4,	R5, R6	R9, R10, R11, R12	X	MH ⁺
XLVII-1	R ₈ H-16	H, H, H, H	H, H	H, H, H, H	H	1002.1

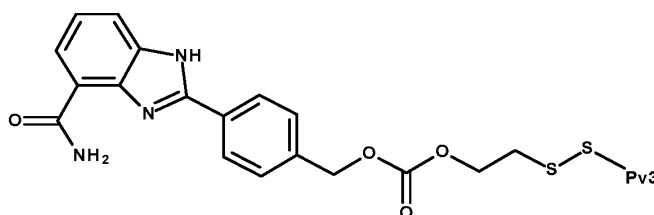
Synthesis of XLVII-1: Methyl (2S,3S,4S,5R,6S)-3,4,5-triacetoxy-6-[3-R10-2-R11-4-[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0⁴,13]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl-methyl-carbamoyl]oxymethyl]-6-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate



To a mixture of 1-hydroxybenzotriazole hydrate (13.0 mg, 0.0851 mmol), finely ground molecular sieve 4 Å (100 mg), and 6-fluoro-2-[4-(methylaminomethyl)phenyl]-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-9-one;phosphoric acid (35.9 mg, 0.0851 mmol) in 2 mL of anhydrous DMF was added methyl (2S,3S,4S,5R)-3,4,5-triacetoxy-6-[4-[(4-nitrophenoxy)carbonyloxy methyl]-2-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate (58.0 mg, 0.0709 mmol). After stirring for 16 h at room temperature, the molecular sieve was filtered off and the solvent was removed in vacuo. The reaction mixture was diluted with EtOAc, washed with sat. NH₄Cl, water and brine. The organic layer was dried with NaSO₄ and concentrated. The crude residue was purified by column chromatography (0-3% MeOH/DCM) to give 35 mg of methyl (2S,3S,4S,5R)-3,4,5-triacetoxy-6-[4-[[[4-(6-fluoro-9-oxo-3,10-diazatricyclo[6.4.1.0.4,13]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl-methyl-carbamoyl]oxymethyl]-2-[3-(2-pyridyldisulfanyl)propanoylamino]phenoxy]tetrahydropyran-2-carboxylate (43.0 mg, 0.0429 mmol, yield: 60.5 %) with a slight unknown impurity. MS m/z 1002.1 (M+H)⁺.

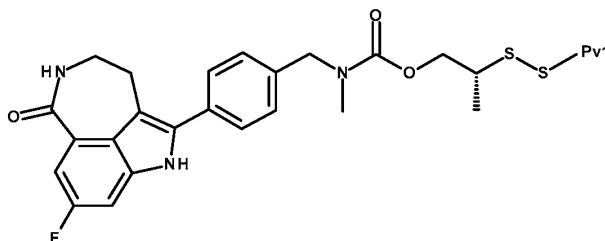
Conjugated Compounds

Example 3



One 8 mL vial containing PBS and one 8 mL vial containing DMF were degassed with bubbling N₂ for 1 h. In a separate vial was placed Peptide Variant 3 ("Pv3," 50.0 mg, 1.31e-5mol). The degassed 1.0 mL of PBS and a 3.0 mL portion of DMF were added to [4-(4-carbamoyl-1H-benzimidazol-2-yl)phenyl]methyl 2-(2-pyridyldisulfanyl)ethyl carbonate (18.9 mg, 3.92e-5 mol). To the mixture was added CH₃CO₂H (0.05 mL 0.000873mol). The mixture was placed under N₂ and stirred for at RT for 16h. The mixture was monitored for progress with RP HPLC until complete consumption of the starting peptide and remaining pyridyl disulfide (Ace Equivalence 250x4.6mm, 50% isocratic, 25min run). The crude reaction mixture was purified by preparative RP HPLC (Sunfire 30x150 mm; CH₃CN/H₂O (0.1% TFA) gradient, 16 min run) to give 28.6 mg, 54% yield of Example 3.

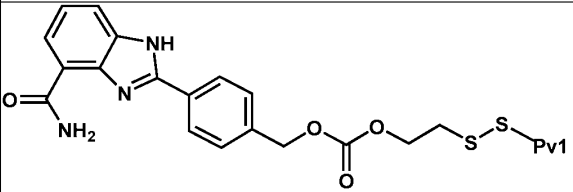
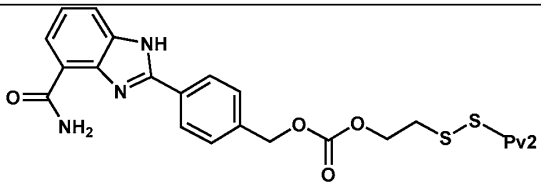
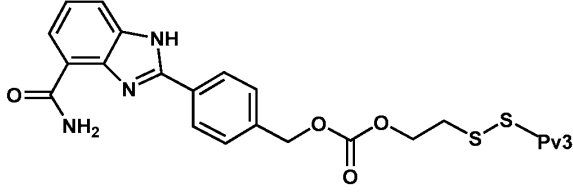
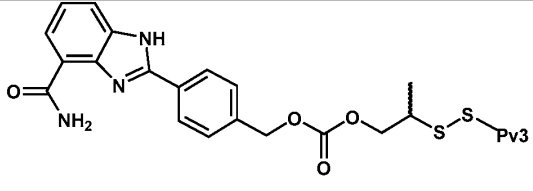
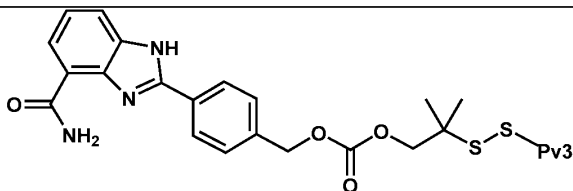
Example 18

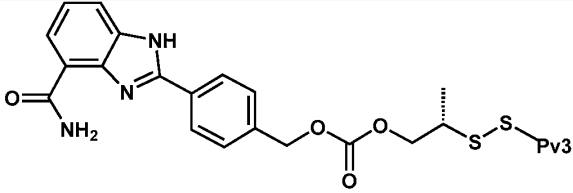
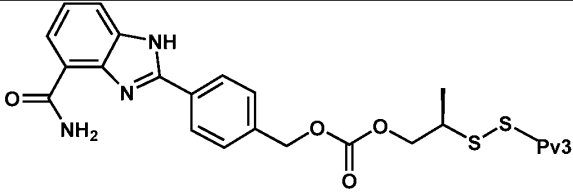
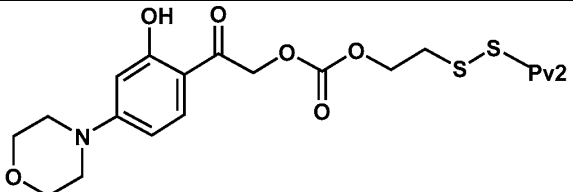
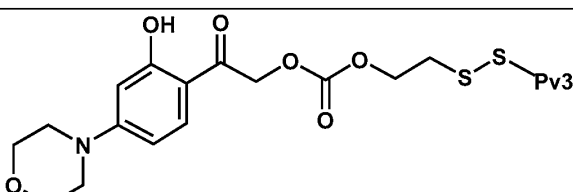
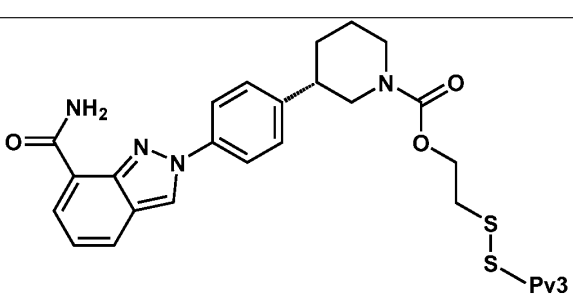
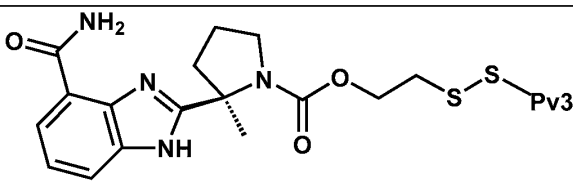


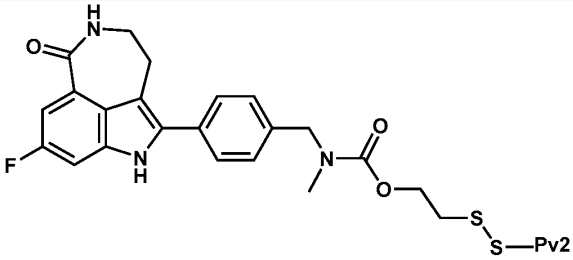
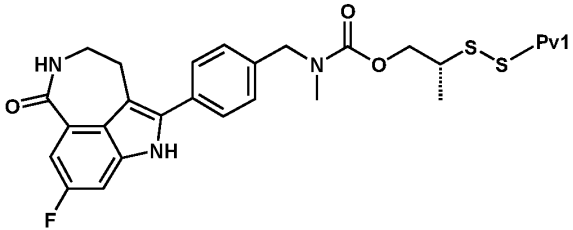
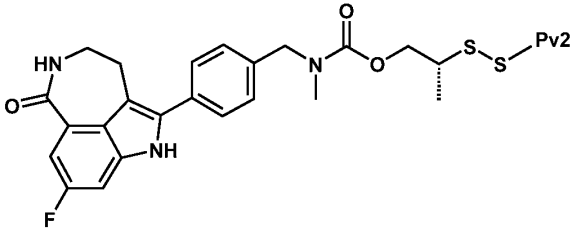
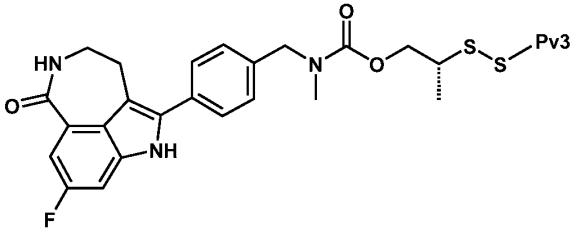
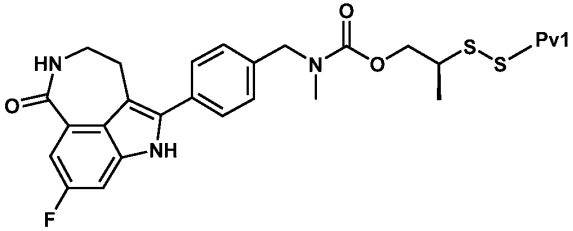
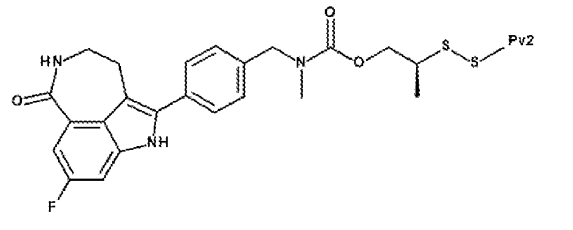
Two separate 8 mL vials containing PBS and DMF, respectively, were degassed with bubbling N₂ for 1 h. In a separate vial was placed Pv1 (50.0 mg, 0.02 mmol). To this vial was added [(2R)-2-(2-pyridyldisulfanyl)propyl] N-[[4-(6-fluoro-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-2-yl)phenyl]methyl]-N-methyl-carbamate [Intermediate III-4] (16.2 mg, 0.03 mmol), 1.33 mL of PBS and 4 mL of DMF followed by acetic acid (4.2 µL, 0.08 mmol). The mixture was placed under N₂ and stirred at RT overnight. The mixture was purified by prep HPLC (40-72% CH₃CN/H₂O, 15 min) to give 29.0 mg, 0.008 mmol, 53% of example 18.

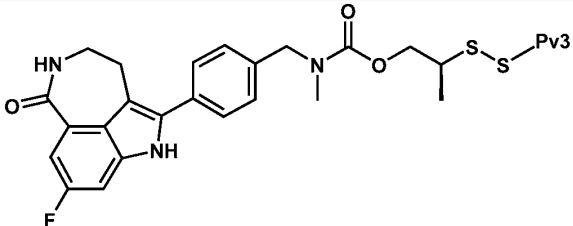
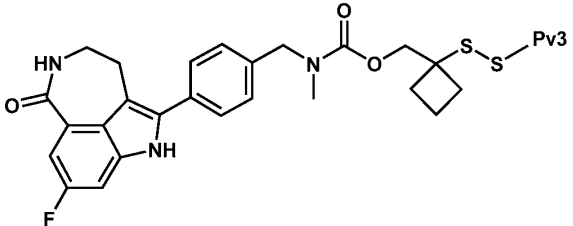
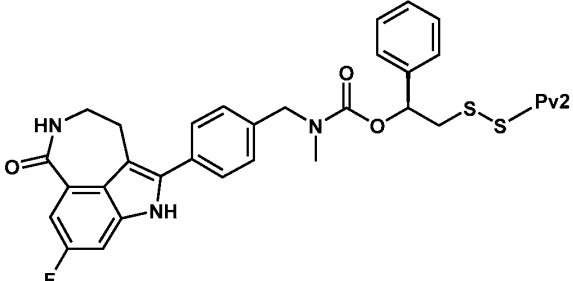
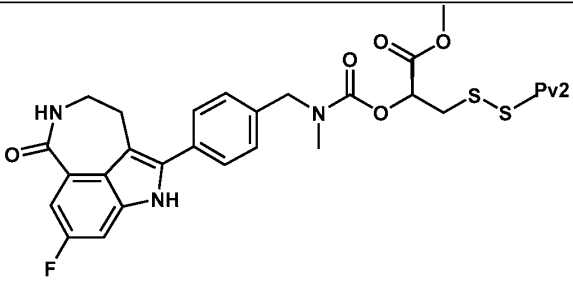
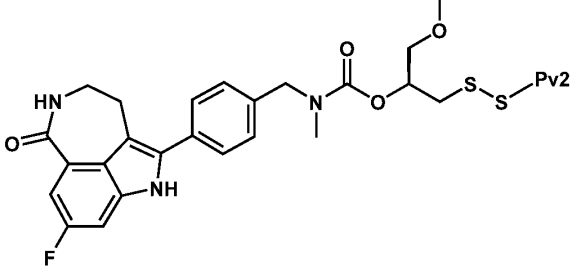
The following compounds were prepared analogously to Example 18 using the appropriate Intermediates and peptides.

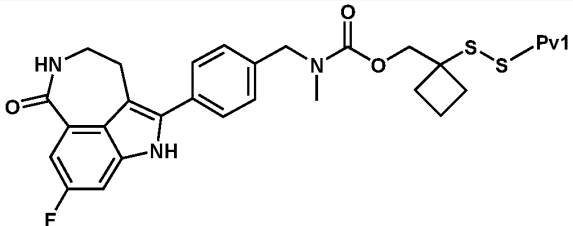
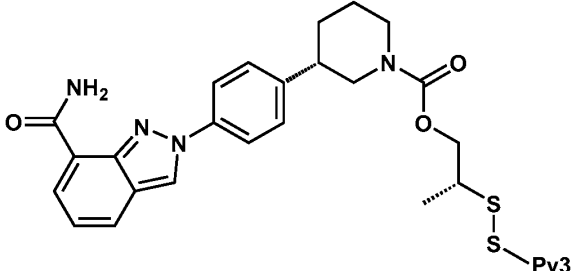
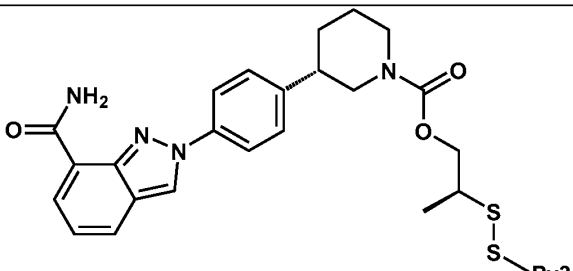
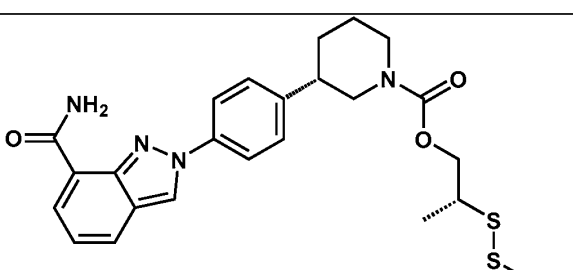
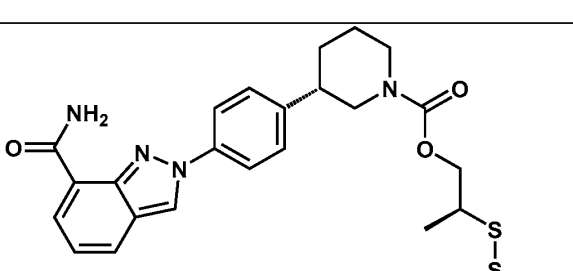
Table 24. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H ₂ O Run Time RT
1		A: 3649.5	A 25-75% 10 min 6.9 min
2		A: 4381.8	A 25-75% 10 min 7.7 min
3		A: 4081.8	B 20-80% 15 min 15.6 min
4		A: 4094.2	<u>Diastereomer</u> <u>1</u> A 45-55% 10 min 6.3 min <u>Diastereomer</u> <u>2</u> A 45-55% 10 min 6.6 min
5		A: 4107.7	A 30-50% 10 min 6.6 min

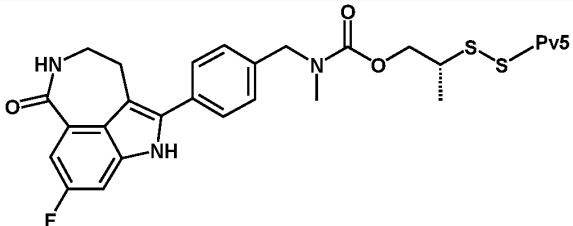
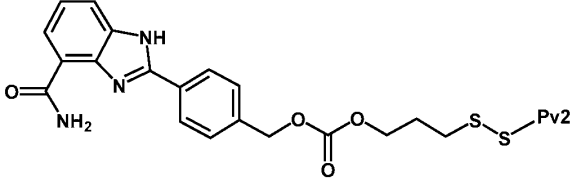
6		A: 4095.0	A 45-55% 10 min 6.3 min
7		A: 4094.7	A 45-55% 10 min 6.6 min
8		A: 4350.8	A 50% iso 10 min 5.5 min
9		A: 4049.6	A 25-75% 10 min 8.5 min
10		A: 4133.0	A 50% iso 10 min 6.5 min
11		A: 4055.3	A 25-75% 10 min 6.7 min

15		A: 4436.2	A 10-95% 10 min 9.1 min
18		A: 3718.3	C 40-80% 15 min 7.4 min
19		A: 4450.3	C 45-71% 11 min 7.9 min
20		A: 4147.2	C 40-80% 15 min 6.9 min
21		A: 3718.6	C 30-45% 15 min 6.0 min
22		A: 4449.8	C 45-71% 11 min 8.3 min

23		A: 4148.9	C 40-72% 12 min 7.0
24		A: 4477.3	C 30-50% 13 min 6.3 min
25		A: 4511.8	C 30-50% 11 min 7.5 min
26		A: 4493.8	C 48-64% 11 min 8.0 min
27		A: 4478.6	C 52-60% 11 min 8.5 min

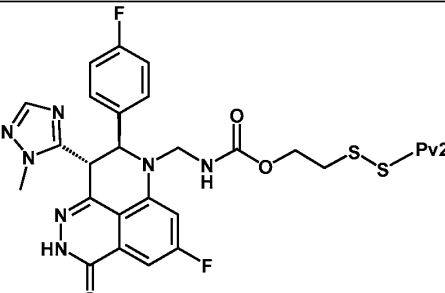
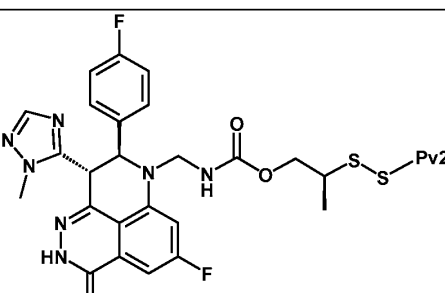
44		A: 3744.3	C 50-85% 13 min 4.4 min
45		A: 4145.3	C 25-95% 15 min 8.5 min
46		A: 4146.0	C 25-95% 15 min 8.5 min
47		A: 4446.5	C 35-95% 12 min 7.3 min
48		A: 4446.5	C 35-95% 12 min 7.3 min

49		A: 4434.2	C 35-91% 11 min 7.2 min
51		A: 4069.7	C 20-65% 10 min 8.2 min
52		A: 4070.3	C 20-65% 10 min 8.2 min
53		A: 4357.6	C 35-91% 12 min 7.3 min
54		A: 4370.6	C 35-70% 11 min 7.4 min
55		A: 4369.9	C 35-70% 11 min 7.4 min
57		B: 1617.1	C 25-75% 15 min 11.5 min

58		B: 1542.1	C 25-75% 15 min 11.7 min
61		B: 1465.5	C 40-64% 10 min 6.3 min

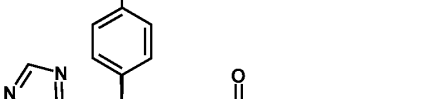
The following compounds were prepared analogously to Example 18 from the appropriate VII Intermediates and peptides.

Table 25. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H ₂ O Run Time RT
12		A: 4522.3	A 40-80% 8.2 min
13		A: 4536.5	A 40-80% 10 min 8.4 min

14		A: 4535.7	A 40-80% 10 min 8.4 min
16		A: 3790.0	A 40-80% 10min 7.1 min
17		A: 4220.1	A 40-80% 10 min 6.7 min
33		A: 4462.8	C 40-58% 9 min 6.8 min
34		A: 4520.7	<u>Diastereomer 1</u> C 40-67% 12 min 9.8 min

35		A: 4521.4	<u>Diastereomer 2</u> C 40-57% 12 min 10.2 min
38		A: 4440.5	C 40-60% 12 min 8.5 min
39		A: 4559.6	C 50-80% 15 min 6.5 min
40		A: 3804.0	C 40-62% 12 min 8.6 min
41		A: 4234.7	C 40-70% 11 min 6.5 min

42		A: 3805.3	C 40-62% 12 min 8.5 min
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Synthesis of the Final Compounds from Intermediates XII

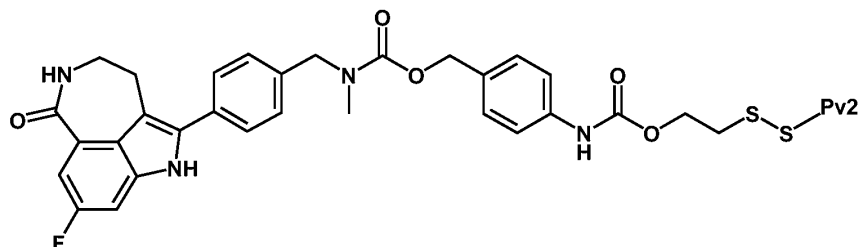
The following compound was synthesized from Intermediate XII-1.

Table 26. Additional Examples

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H₂O Run Time RT
60	 The structure shows a benzimidazole core. At position 2 of the imidazole ring, there is a phenyl substituent. This phenyl ring has a propyl chain attached at its para position, which terminates in a disulfide (-S-S-) linkage connected to a pyridine ring.	B: 1460.8	C 20-65% 2 min 11.1 min

5

Example 32

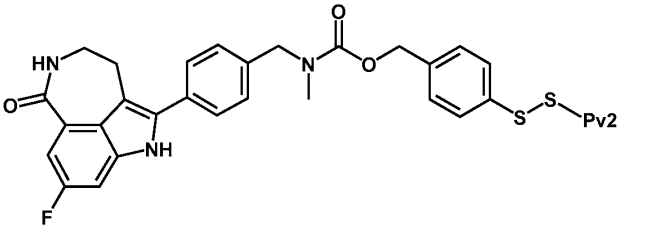
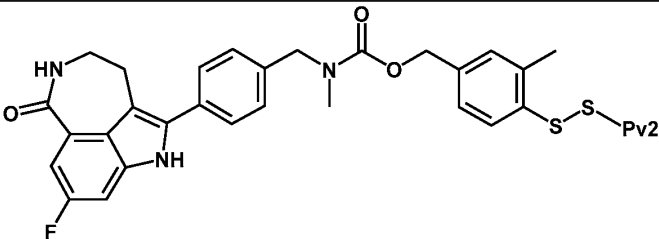
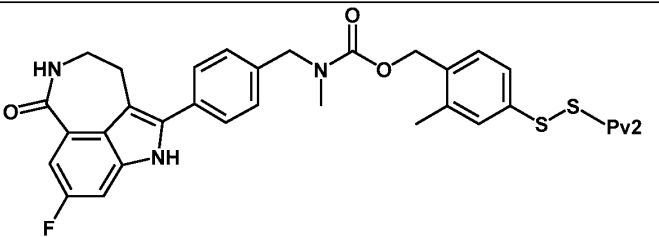
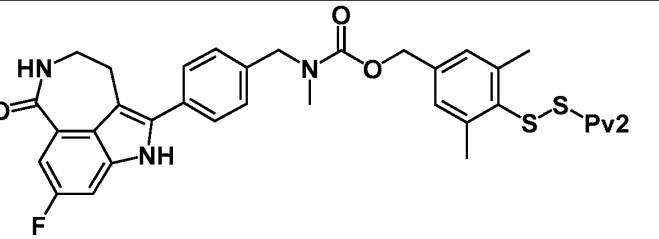
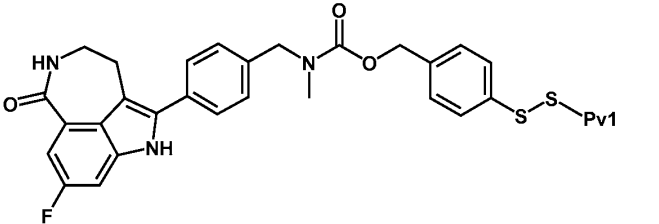


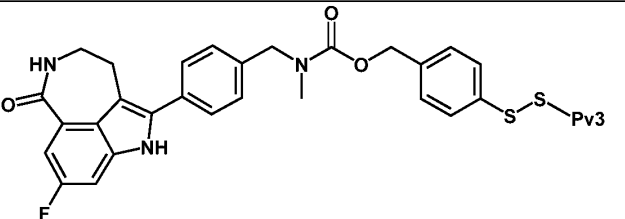
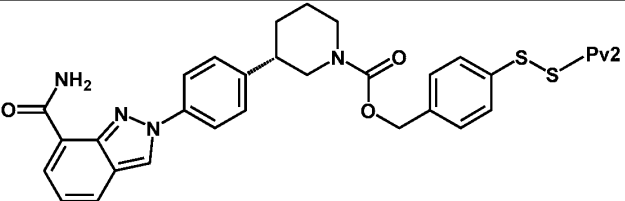
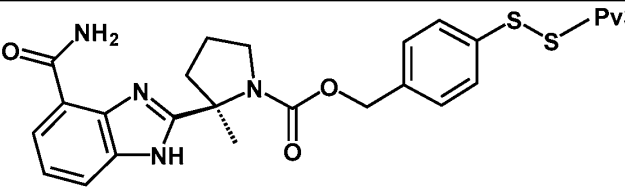
Example 32 was synthesized from Intermediate XXI and peptide Pv2. The MS (MALDI-TOF) found was 4582.9. The compound was purified using 30-50% acetonitrile in water, Conditions 3, eluting at 11 and 8.3 min.

Synthesis of Compounds from Intermediates XXV

The following compounds were synthesized analogously to Example 18 from Intermediates XXV.

15 Table 27. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H ₂ O RT
28		A: 4497.8	C 50-80% 15 min 6.5 min
29		A: 4510.9	C 50-100% 20 min 7.4 min
30		A: 4510.3	C 50-100% 20 min 7.3 min
31		A: 4526.2	C 50-100% 20 min 7.0 min
36		A: 3767.3	C 40-90% 15 min 7.0

37		A: 4196.8	C 40-90% 15 min 6.4 min
50		B: 1397.6	C 20-95% 16 min 9.5 min
56		B: 1373.5	C 35-60% 15 min 9.3 min

Synthesis of the Final Compounds from Intermediates XXV

The following compound was synthesized analogously to Example 18 from Intermediate XXV.

5 Table 28. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H ₂ O RT
59		B: 1499.8	C 50-92% 16 min 6.1 min

Synthesis of the Final Compounds from Intermediates XXXV

The following compound was synthesized analogously to Example 18 from Intermediate XXXV-1.

10 Table 29. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/ H ₂ O RT
62		1614.4	C 40-68% 10 min 8.4 min

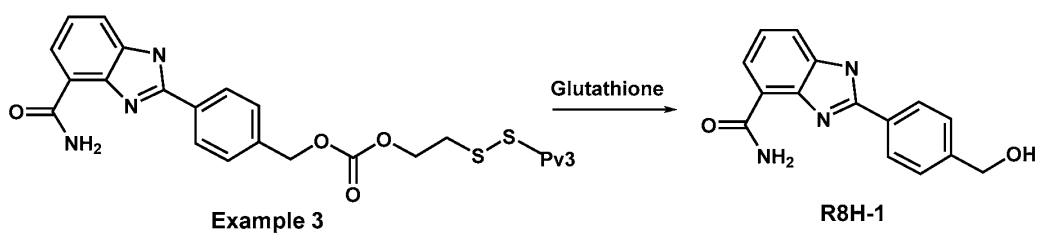
Synthesis of the Final Compounds from Intermediates XXXVIII

The following compound was synthesized analogously to Example 18 from Intermediates XXXVIII-1.

5 Table 30. Example Compounds

Example	Structure	MS A: Maldi-TOF B: m/z=3	Column %ACN/H ₂ O RT
64		B: 1531.8	C 35-75% 10 min 7.6 min

Example A. Cleavage of Compounds



To (2S)-2-amino-5-[[[(1R)-2-(carboxymethylamino)-2-oxo-1-(sulfanylmethyl)ethyl] 10 amino]-5-oxo-pentanoic acid (4.90 mg, 0.0159 mmol) was added 2 mL of 1M Tris HCl buffer (pH 7.0) to create an 8 mM solution. An aliquot of 1 mL of this solution was added to Example 3 (0.400 mg, 9.81x10⁻⁵ mmol) to create a 100 μM solution. This mixture was

heated at 37° C with time points taken at 15 minute intervals to measure for conjugate integrity. A steady loss of conjugate is observed with commensurate appearance of 2-[4-(hydroxymethyl)phenyl]-1~{H}-benzimidazole-4-carboxamide. The cleavage was complete by the 60 min time point as observed/confirmed by MSD. HPLC conditions: ES Industries
 5 Sonoma 4.6x50 mm; 5-100% CH₃CN/H₂O (0.1% TFA); 5.5 min run Conjugate RT: 3.71 Product RT: 2.24.

Other compounds were similarly cleaved to give the appropriate R⁸H molecules.

The crude cleavage solution containing the cleaved compounds of the invention was assayed as described below in Examples B and C to assess the amount of cleaved R⁸H. In
 10 addition to the cleaved compounds, assays were also performed on several of the R⁸ starting materials set out in Table 2.

Example B. Enzymatic Assay 1 of cleaved Compounds (PARP)

Analysis was performed using Trevigen's HT F Homogeneous PARP Inhibition
 15 Assay Kit (#4690-096-K) as follows: Purified PARP enzyme was incubated in the presence of a serially diluted PARP inhibitor (e.g., a compound of the Examples cleaved according to Example A) in duplicate and 1 uM NAD for thirty minutes at room temperature in black, round-bottom 96-well plates. An equal volume of cycling mix was added to each well and the reaction was incubated for one hour at room temperature. The reactions were stopped
 20 with the addition of Stop Solution and the plates were read on a BioTek Cytation 5 plate reader using the fluorescence (544 nm excitation and 590 nm emission) endpoint. Results were plotted in GraphPad Prism and are given below.

Table 31. HT F Homogeneous PARP Inhibition Assay of Cleaved Compounds

Assayed Compound	IC₅₀ Enzymatic Assay 1 (nM)
R ⁸ H-1	96.8
Example 3 (cleaved)	104
Example 6 (cleaved)	202
Example 7 (cleaved)	111

25 Example C. Enzymatic Assay 2 of cleaved Compounds (PARP)

Analysis was performed using Trevigen's HT Universal Chemiluminescent PARP Assay Kit (#4676-096-K) as follows: Purified PARP enzyme was incubated in the presence of a serially diluted PARP inhibitor (e.g., a compound of the Examples cleaved according to Example A) in duplicate for ten minutes at room temperature in rehydrated 96-well stripwells

containing bound histones. An equal volume of 1x PARP cocktail containing activated DNA was added to each well and the reaction was incubated for one hour at room temperature. The wells were washed twice with 1x PBS + 0.1% Triton X-100 and twice with PBS. 50 ul/well of diluted streptavidin – HRP was added and the wells were incubated for one hour at room temperature. The wells were washed twice with 1x PBS + 0.1% Triton X-100 and washed twice with PBS. The liquid was removed, 100 ul/well 1:1 PeroxyGlow A/ PeroxyGlow B was added and the chemiluminescent readings were measured on a BioTek Cytation 5 plate reader using the luminescence fiber endpoint. Results were plotted in GraphPad Prism and are shown below.

10 Table 32. HT Universal Chemiluminescent PARP Assay of Cleaved Compounds

Assayed Compound	IC ₅₀ Enzymatic Assay 2 (nM)
R ⁸ H -1	6.4
R ⁸ H-3	3.9
R ⁸ H-4	1.3
R ⁸ H-15	1.0
R ⁸ H-16	0.8
R ⁸ H-17	1.0
Example 1 (Cleaved)	12.1
Example 2 (Cleaved)	9.52
Example 3 (Cleaved)	9.15
Example 6 (Cleaved)	9.29
Example 7 (Cleaved)	6.45
Example 10 (Cleaved)	64.9
Example 11 (Cleaved)	1.08
Example 12 (Cleaved)	0.47
Example 13 (Cleaved)	1.0
Example 14 (Cleaved)	0.5
Example 15 (Cleaved)	6.47
Example 16 (Cleaved)	0.61
Example 17 (Cleaved)	0.41
Example 18 (Cleaved)	1.1
Example 19 (Cleaved)	2.2
Example 20 (Cleaved)	1.7
Example 21 (Cleaved)	2.2
Example 22 (Cleaved)	1.8
Example 23 (Cleaved)	2.5
Example 24 (Cleaved)	7.6
Example 25 (Cleaved)	3.4
Example 26 (Cleaved)	2.0
Example 27 (Cleaved)	3.9
Example 28 (Cleaved)	2.4
Example 29 (Cleaved)	6.4
Example 30 (Cleaved)	2.9

Example 31 (Cleaved)	3.3
Example 32 (Cleaved)	43.9
Example 33 (Cleaved)	2.3
Example 34 (Cleaved)	2.5
Example 35 (Cleaved)	3.1
Example 36 (Cleaved)	2.2
Example 37 (Cleaved)	3.1
Example 38 (Cleaved)	2.8
Example 39 (Cleaved)	3.0
Example 40 (Cleaved)	1.1
Example 41 (Cleaved)	1.9
Example 42 (Cleaved)	1.4
Example 43 (Cleaved)	1.4
Example 44 (Cleaved)	1.5
Example 45 (Cleaved)	3.2
Example 46 (Cleaved)	3.7
Example 47 (Cleaved)	2.4
Example 48 (Cleaved)	2.1
Example 49 (Cleaved)	26
Example 50 (Cleaved)	2.0
Example 51 (Cleaved)	0.9
Example 52 (Cleaved)	1.1
Example 53 (Cleaved)	1.0
Example 54 (Cleaved)	0.6
Example 55 (Cleaved)	0.7
Example 56 (Cleaved)	0.8

Example D. Elisa Parylation Assay

Twelve--well tissue culture plates were seeded with HeLa cells and incubated at 37°C in 5% CO₂ to yield 80% confluent cell monolayers the following day. The monolayers were treated with a three-fold dilution series of either free drug or conjugate at a desired pH for 1 hour at 37°C. The monolayers were aspirated and each well received 150 ul of RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Plates were incubated on ice for 10 minutes and then frozen. Upon thawing, the lysates were supplemented with Mg and incubated with DNase for 90 minutes at 37°C. The samples were clarified by centrifugation at 12,000 x g for 5' at 4°C and the cleared lysate was transferred to a clean tube and protein determinations were performed using a BCA Protein Assay.

Analysis was performed using Trevigen's HT PARP in vivo Pharmacodynamic Assay II (#4520-096-K) as follows: Duplicate 25 ul sample lysates were loaded on pre-coated/pre-blocked ELISA stripwells and incubated for 16 hours at 4°C in tandem with serially diluted purified PAR standards. The wells were washed 4x with PBST and incubated with 50 ul/well of PAR polyclonal detecting antibody in antibody diluent for 2 hours at room temperature.

The wells were washed 4x with PBST and incubated with 50 ul/well Goat anti-rabbit IgG-HRP conjugate in antibody diluent for 1 hour at room temperature. The wells were washed 4x with PBST, incubated with 100 ul/well of 1:1 PARP PeroxyGlow A and PARP PeroxyGlow B and read in a BioTek Cytation 5 using the luminescence fiber endpoint.

- 5 Results were plotted as raw luminescence units or as PAR (pg/ml) using values calculated from the standard curve and are presented below.

Table 33. Parylation Assay Results

Example	IC ₅₀ Elisa PARP Inhibition(nM) pH 5.5	IC ₅₀ Elisa PARP Inhibition(nM) pH 6.2	IC ₅₀ Elisa PARP Inhibition(nM) pH 7.4	IC ₅₀ Elisa PARP Inhibition(nM) pH 8.0
1	115			587
2	93, 135			1290, 1400
3	151			616
6	239			456
7	282			542
10	785			3075
11	IC			9102
12	101	159	186	116

Cells are treated as previously described at pH 7.4 and incubated for 16 h.

- 10 Table 34. Parylation Assay Results

Compound	IC ₅₀ Elisa PARP Inhibition(nM) pH 7.4 (BRCA-/-) DLD-1 cells, 16 h	IC ₅₀ Elisa PARP Inhibition(nM) pH 7.4 SW620 cells, 16 h
18	2401	
19	2381	
20	2196	2767
21	3545	
22	3877	
23	1425	
28	712	2197
29	1314	
30	497	
31	1140	
32	4995	
34	2092	
35	1580	
36	271	1174
37	439	1386
40	522	
41	459	
42	540	
43	380	

51	4178	
54	1857	
55	2928	
56	103	

Example E. Growth Delay Assay

Cells were plated in 96 well black walled-clear bottom plates (Griener), DLD-1 WT cells at 2500 cells per well, DLD-1 BRCA2-/- at 5000 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 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 calculated and data plotted using GraphPad Prism.

Table 35. Growth Delay Assay Summary

Example	IC ₅₀ Growth Delay (nM) pH 7.4, BRCA (-/-) DLD-1, 96h
12	162
13	51
14	72
16	84
17	50
39	104
40	64
41	43
42	53
43	46

The above results demonstrate that the compounds of the invention are cleaved to release free R⁸H when subjected to conditions replicating those within a cell and exhibit inhibition of the biological target, which in this particular assay is PARP. Based on these results one of skill in the art would readily recognize the compounds of the invention to be useful for treatment of diseases involving acidic or hypoxic diseased tissue, and particularly for treating PARP mutated cancers.

Example F. In Vivo Tumor Growth Delay*Study Design (R⁸H-15, Example 12)*

Female Nude mice arrived at the facility at 6 weeks of age and were housed 5 per cage on Alpha-Dri bedding in a disposable IVC caging system (Innovive). After an acclimation period of 5-10 days, DLD-1 BRCA2^{-/-} cells were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5x10⁶ cells in 100μL. Xenograft tumor growth was monitored and caliper measurements were obtained twice weekly. When xenografts reached a minimal volume of 100mm³, mice were administered an intraperitoneal (IP) dose of vehicle, Example 12 (6.4, 20 or 50mg/kg), or an oral dose of R⁸H-15 (0.3mg/kg) once daily for 8 days. All mice were administered an oral dose of 10mg/kg temozolomide (TMZ) prepared in 20% glucose immediately after dosing. Caliper measurements were obtained twice weekly to evaluate compound effect on tumor growth. Monitoring of tumor growth continued after the 8-day dosing period for another 7 weeks (wash out period). Mice were euthanized if body weight loss exceeded 20% or if tumor volume increased to 4x the original size. Kaplan-Meier analysis was used to evaluate survival rate based on death or removal from study.

Study Design (R⁸H-16, Example 18)

Female Nude mice arrived at the facility at 6 weeks of age and were housed 5 per cage on Alpha-Dri bedding in a disposable IVC caging system (Innovive). After an acclimation period of 5-10 days, DLD-1 BRCA2^{-/-} cells were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5x10⁶ cells in 100μL. Xenograft tumor growth was monitored and caliper measurements were obtained twice weekly. When xenografts reached a minimal volume of 100mm³, mice were administered an intraperitoneal (IP) dose of vehicle, Example 18 (8.8, 17.7 or 44.2 mg/kg), or R⁸H-16 (1, 2, 5 mg/kg), once daily for 8 days. All mice were administered an oral dose of 10 mg/kg temozolomide (TMZ) prepared in 20% glucose immediately after dosing for 5 days. Caliper measurements were obtained twice weekly to evaluate compound effect on tumor growth. Monitoring of tumor growth continued after the 8-day dosing period for another 8-day (wash out period). Mice were euthanized if body weight loss exceeded 20% or if tumor volume increased to 4x the original size.

DLD-1 BRCA2^{-/-} Cell Preparation for Implantation (R⁸H-15, Example 12; R⁸H-16, Example 18)

Matrigel was thawed overnight on ice at 4° C prior to prepare for DLD-1 BRCA2^{-/-} cell implantation and was kept on ice during all preparatory steps. Cells were passaged
5 between one and three days prior to preparation for implantation. Growth medium was replaced every 2-3 days as needed to maintain cell viability. On the day of implantation, cells were trypsinized, washed with complete media and pelleted by centrifugation at 1200rpm for 5 minutes. The supernatant was decanted and cells were washed three times with sterile PBS and pelleted by centrifugation. During the final centrifugation, viability was determined
10 using trypan blue exclusion. Cells were resuspended in sterile PBS at a concentration of 5x10⁶ cells/ 50µL. Prior to implantation, cells were mixed 1:1 with Matrigel for a final concentration of 5x10⁶ cells/ 100 µL. The Matrigel/ cell mixture was kept on ice in a conical tube until used for implantation.

DLD-1 BRCA2^{-/-} Cell Implantation (R⁸H-15, Example 12; R⁸H-16, Example 18)

15 Matrigel/ DLD-1 BRCA2^{-/-} cells (5x10⁶ cells/ 100µL) kept on ice in a conical tube were drawn into sterile 1cc syringes fitted with sterile 27-gauge needles. Excess Matrigel/ cell mixture was expelled back into the conical tube leaving an injection volume of 100µL in each syringe. Filled syringes were kept on ice until time of implantation to avoid polymerization of the Matrigel in the syringe. Cells were subcutaneously implanted into the
20 left flank of each mouse and upon the development of palpable xenografts, caliper measurements were obtained twice weekly. Compound evaluation proceeded when xenografts reached a minimal volume of 100mm³.

Compound Administration (R⁸H-15, Example 12)

Intraperitoneal doses of 6.4, 20 and 50mg/kg Example 12 were prepared in 8%
25 PEG400 + 2% Tween 80 in PBS as described above and were administered once daily for 8 days. Mice were dosed at a volume of 12mL/kg (300µL per 25g mouse). Oral doses of 0.3mg/kg R⁸H-15 (BMN673) were prepared in 0.5% methyl cellulose and were administered once daily for 8 days at volume of 10mL/kg (250µL per 25g mouse) for comparison. Immediately after compound administration, all mice were administered an oral dose of 10
30 mg/kg temozolomide (TMZ) in 20% glucose.

Compound Administration (R⁸H-16, Example 18)

Example 18 was dissolved in 100% dimethyl sulfoxide (DMSO) to yield a 0.1 mg/ μ L stock solution. Intraperitoneal (IP) doses of 8.83, 17.66 or 44.15mg/kg were prepared fresh daily by diluting 14, 29 or 72 μ L of the stock, respectively, with 1950 μ L of a vehicle
5 comprised of 8% PEG400 + 2% Tween 80 in PBS. The doses were vortexed to achieve homogenous suspensions and were administered at a concentration of 12mg/mL (300 μ L per 25g mouse), once daily for 8 days. Each day, 2.5mg of R⁸H-16 was suspended in 6mL of a vehicle comprised of 8% PEG400 + 2% Tween 80 in PBS. The suspension was sonicated for 10 minutes to result in a homogeneous 5 mg/kg intraperitoneal dose which was further
10 diluted 1 to 5 and 1 to 2.5 with vehicle to result in 1 and 2mg/kg doses, respectively. All doses were vortexed and administered at a concentration of 12mL/kg (300 μ L per 25g mouse) once daily for 8 days. Oral doses of 50 mg/kg temozolomide (TMZ) were prepared fresh daily by suspending 70 mg of compound in 14mL of a 20% glucose vehicle. The final 5 mg/mL doses were sonicated for 15 minutes until homogenous suspensions were achieved.
15 Mice were orally administered TMZ at 10mL/kg (250 μ L per 25g mouse) once daily for 5 days immediately following administration of Example 18 or R⁸H-16.

Tumor Growth

Tumor growth was monitored and caliper measurements were obtained twice weekly over a dosing period of 8 days to evaluate compound efficacy against tumor growth rate.
20 Growth measurements continued for another 7 weeks after dosing ceased. Mice were removed from study when tumor volume exceeded 4x its original size.

Statistical Analysis

Analysis of variance (ANOVA) was used to test for significant differences between groups. Post-hoc Bonferroni multiple comparison test analysis was used to determine
25 significant differences among means. All statistical analysis was accomplished using Graph Pad Prism 7.03 software. Kaplan-Meier analysis was used to evaluate survival rate based on death or removal from study when body weight loss exceeded 20% from initial body weight.

Figure 1 shows the tumor growth delay of R⁸H-15 and Example 12 in BRCA^{-/-} Mice.

Figure 2 shows survival of R⁸H-15 and Example 12 in BRCA^{-/-} Mice.

30 Figure 3 shows the tumor growth delay of R⁸H-16 and Example 18 in BRCA^{-/-} Mice.

Example G. Tumor Parylation

Study Design (R⁸H-16, Example 18)

Female Nude mice arrived at the facility at 6 weeks of age and were housed 5 per cage on Alpha-Dri bedding in a disposable IVC caging system (Innovive). Human DLD-1 BRACA2^{-/-} cells were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5x10⁶ cells in 100μL. Caliper measurements were obtained twice weekly and treatment began when xenografts reached a minimal volume of 250mm³. Intraperitoneal (IP) doses of Example 18 (8.83, 17.66 or 44.15 mg/kg) or R⁸H-16 (2 or 10mg/kg) were administered once daily for 9 days in an 8% PEG400 + 2% Tween 80 vehicle. Mice were also administered an oral dose of 20% glucose. The eighth dose was administered in the evening about 12 hours before the ninth dose. Serum and tissue samples were collected 2 hours after administration of the ninth dose. Compound effect against PARylation was determined in tumor and bone marrow.

Compound Administration (R⁸H-16, Example 18)

Example 18 was dissolved in 100% dimethyl sulfoxide (DMSO) to yield a 0.1mg/μL stock solution. Intraperitoneal (IP) doses of 8.8, 17.7 or 44.2 mg/kg were prepared fresh daily by diluting 13, 26 or 66 μL of the stock, respectively, with 1800 μL of a vehicle comprised of 8% PEG400 + 2% Tween 80 in PBS. The doses were vortexed to achieve homogenous suspensions. Intraperitoneal (IP) doses of 10mg/kg R⁸H-16 were prepared fresh daily by suspending 5 mg of R⁸H-16 in 6mL of a vehicle comprised of 8% PEG400 + 2% Tween 80 in PBS. The doses were vortexed to achieve homogenous suspensions. IP doses of 2mg/kg were prepared by diluting the 10mg/kg dose 1:5 with vehicle. Intraperitoneal (IP) doses of Example 18 or R⁸H-16 were administered once daily for 9 days at a volume of 12mL/kg (300μL per 25g mouse).

Tissue Collection (R⁸H-16, Example 18)

Following blood collection, mice were euthanized by cervical dislocation under anesthesia. Xenograft tumors were removed, weighed, and cut into smaller pieces with a scalpel blade. Random 100 mg tumor samples were collected in cryo-tubes, snap frozen in liquid nitrogen and stored at -80° C until processed. PARylation measurements were made in tumor homogenates.

PARylation in Tumor Homogenates (R⁸H-16, Example 18)

Frozen tumor samples were homogenized in a RIPA buffer containing protease and phosphatase inhibitors (1 mg/mL) and 300 μL aliquots were brought up to a 1% SDS final

concentration. Samples were heated at 100°C for 5 minutes, quenched on ice and clarified by centrifugation at 14,000 x g for 5 minutes. Tumor samples were diluted 1:10 in RIPA/HALT buffer for protein quantification using a Pierce Rapid Gold BCA Protein Assay kit. Samples were diluted to 200 µg/mL (10 µg/well) in sample buffer and 50 µL aliquots (10µg/well) were loaded on pre-coated/pre-blocked ELISA strip wells. Following a 16-hour incubation at 4°C, samples were washed 4x with PBST (1L PBS + 1mL Tween 20) and incubated with 50µL PAR detecting antibody for 2 hours at room temperature. Samples were washed 4x with PBST and incubated with 50µL goat anti-rabbit IgG-HRP conjugate for 1 hour at room temperature. Following a final 4x wash with PBST, 100µL of PeroxyGlow™ was added to each sample and luminescence was determined on a BioTek Cytation 5 using luminescence fiber endpoint and strip well area plate definition.

Figure 4 shows the effect of 9-Day intraperitoneal administration of Example 18 and R⁸H-16 on tumor PARylation in a murine DLD-1 BRCA2^{-/-} xenograft model.

15 **Example H. Bone Marrow Selectivity**

Study Design (R⁸H-15, Example 12)

Female Nude mice arrived at the facility at 6 weeks of age and were housed 5 per cage on Alpha-Dri bedding in a disposable IVC caging system (Innovive). After an acclimation period of 5-10 days, DLD-1 BRCA2^{-/-} tumor cells were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5x10⁶ cells in 100µL. Tumor growth was monitored and caliper measurements were obtained twice weekly. When tumors reached a minimal volume of 100mm³, mice were administered an oral dose of 50mg/kg Temozolomide (TMZ) alone or in combination with either an oral dose of 0.3mg/kg R⁸H-15 or an intravenous dose of 10 mg/kg Example 12, once daily for 2 days. On Day 3, fed mice were euthanized by cervical dislocation under anesthesia and tumors were removed, weighed and snap frozen in liquid nitrogen. Femurs were removed and bone marrow was extruded with PBS into 50 mL conical tubes for isolation of bone marrow cells by centrifugation. Myelotoxicity was evaluated by bone marrow cell count and by PARylation inhibition as determined by Elisa. Compound effectiveness against tumor cell viability was determined by an Elisa PARylation assay.

Study Design (R⁸H-16, Example 18)

Female Nude mice arrived at the facility at 6 weeks of age and were housed 5 per cage on Alpa-Dri bedding in a disposable IVC caging system (Innovive). Human DLD-1

BRACA2^{-/-} cells were diluted 1:1 in Phenol Red-free Matrigel and subcutaneously implanted into the left flank of each mouse at a density of 5x10⁶ cells in 100 µL. Caliper measurements were obtained twice weekly and treatment began when xenografts reached a minimal volume of 250mm³. Intraperitoneal (IP) doses of Example 18 (8.83, 17.66 or 44.15mg/kg) or R⁸H-16 (2 or 10mg/kg) were administered once daily for 9 days in an 8% PEG400 + 2% Tween 80 vehicle. Mice were also administered an oral dose of 20% glucose. The eighth dose was administered in the evening about 12 hours before the ninth dose. Serum and tissue samples were collected 2 hours after administration of the ninth dose. Compound effect against PARylation was determined in bone marrow.

10 *Compound Administration (R⁸H-15, Example 12)*

5 mg of Exmple 12 was dissolved in 50µL of 100% dimethyl sulfoxide (DMSO) to yield a 0.1mg/µL stock solution. The final 10mg/kg intravenous dose was prepared by diluting 25µL of the stock with 2475µL of sterile PBS. The dose was gently mixed to result in a clear injectable 1mg/mL dosing solution. Doses were administered once daily for 2 days by tail vein injection at 10mL/kg (200µL per 20g mouse). Mice were dosed in combination with an oral 50g/kg dose of Temozolomide. An oral dose of 3mg/kg R⁸H-15 was prepared by suspending 1.5 mg of compound in 5mL of a 10% Dimethyl Acetamide (DMAc) + 6% Solutol + 84% PBS vehicle. The suspension was further diluted 1:10 in the vehicle. The final 0.3mg/kg dose was sonicated for 15 minutes until a homogenous 0.3mg/mL suspension was achieved. Mice were orally dosed at 10mL/kg (200µL per 20g mouse) once daily for 2 days in combination with an oral 50mg/kg dose of Temozolomide.

Compound Administration (R⁸H-16, Example 18)

Compound administration was as described in the *Compound Administration (R⁸H-16, Example 18)* section of Example F.

25 *Bone Marrow Collection (R⁸H-15, Example 12)*

Following tumor collection, 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 RPMI + 2% fetal bovine serum (FBS). Bone marrow was homogenized by gentle pipetting and filtered through 100 µm nylon mesh filters. Bone marrow cells were pelleted by centrifugation at 1200 rpm for 5 minutes at 4°C. Cells were washed with 5 mL of PBE (PBS + 0.2% bovine serum albumin + 2mM EDTA) and were re-pelleted by centrifugation as described above. Cells were re-suspended in 3 mL 1X RBC Lysis Buffer and incubated at

room temperature for 2-5 minutes. PBE was added to a final volume of 25 mL and cells were pelleted by centrifugation. Cells were re-suspended in 5 mL PBE, passed through a 40 μ m nylon mesh filter, and harvested by centrifugation. Cells were re-suspended in 1 mL PBE. Cell concentration and viability were determined using trypan blue exclusion on a TC-20 cell counter (Biorad) prior to preparation for PARylation analysis. Following cell count, 1.5mL bone marrow cells were pelleted by centrifugation at 1200rpm (300rcf) for 5 minutes at 4°C. Supernatant was collected and stored at -80°C for possible analysis of drug concentration. The remaining 2.5mL of cells were pelleted by centrifugation at 1200rpm (300rcf) for 5 minutes at 4°C. Pelleted cells were lysed in RIPA buffer containing protease and phosphatase inhibitors (100 μ L buffer per 10⁶ cells) and stored at -20°C for measurement of PARylation in bone marrow cells as determined by an Elisa PARylation assay.

PARylation in Bone Marrow Cells (R⁸H-15, Example 12)

Bone marrow cells isolated from femurs on Day 3 were lysed in RIPA buffer containing protease/phosphatase inhibitors. The homogenized samples were brought to 1% SDS final concentration, heated at 100 °C for 5 minutes, quenched on ice and clarified by centrifugation at 12,000 x g for 5 minutes at 4 °C. PARylation analysis was performed using Trevigen's HT PARP in vivo Pharmacodynamic Assay II. Briefly, duplicate 10 μ g samples were loaded on pre-coated/pre-blocked ELISA strip wells as 50 μ L volumes in sample buffer and incubated for 16 hours at 4 °C in tandem with serially diluted purified PAR standards. The wells were washed 4x with PBST and incubated with 50 μ L/well of PAR polyclonal detecting antibody in antibody diluent for 2 hours at room temperature. The wells were washed 4x with PBST and incubated with 50 μ L/well goat anti-rabbit IgG-HRP conjugate in antibody diluent for 1 hour at room temperature. The wells were washed 4x with PBST, incubated with 100 μ g/well of 1:1 PARP PeroxyGlow A and PARP PeroxyGlow B and read in a BioTek Cytation 5 using the luminescence fiber endpoint. Data were plotted as PAR (pg/mL) using values calculated from the standard curve.

Bone Marrow Collection (R⁸H-16, Example 18)

Following tumor collection, 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 (FBS). 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 3mL of

lysis buffer for 2 minutes at room temperature. PBS was added to a volume of 25mL and cells were re-pelleted by centrifugation as described above. Cell pellets were suspended in 5mL of PBS and cell count was assessed by trypan blue exclusion on a TC-20 cell counter (BioRad). A subset of 2.5×10^6 cells from each sample was collected into a microfuge tube
5 for measurement of PARylation.

PARylation in Bone Marrow Cells (R⁸H-16, Example 18)

Bone marrow cells (2.5×10^6 cells per pellet) were lysed in 500μL of RIPA/HALT buffer and incubated on ice for 15 minutes with periodic vortexing. Cell lysates were brought up to a 1% SDS final concentration, heated at 100°C for 5 minutes and quenched on ice.
10 Cellular debris was removed by centrifugation at 14,000 x g for 5 minutes 4°C and pelleted cells were resuspended in buffer at a concentration of 250,000 cells/ 50μL. Bone marrow cell samples were loaded on pre-coated/pre-blocked ELISA strip wells and incubated for 16 hours at 4°C. Samples were washed 4x with PBST (1L PBS + 1mL Tween 20) and incubated with 50μL PAR detecting antibody for 2 hours at room temperature. Samples were washed 4x
15 with PBST and incubated with 50μL goat anti-rabbit IgG-HRP conjugate for 1 hour at room temperature. Following a final 4x wash with PBST, 100μL of PeroxyGlow™ was added to each sample and luminescence was determined on a BioTek Cytation 5 using luminescence fiber endpoint and strip well area plate definition.

Statistical Analysis

20 Analysis of variance (ANOVA) was used to test for significant differences between groups. Post-hoc Bonferroni multiple comparison test analysis was used to determine significant differences among means. All statistical analysis was accomplished using Graph Pad Prism 7.03 software.

Figure 5 shows the PARylation in bone marrow cells following intravenous
25 administration of Example 12 or oral administration of R⁸H-15 in combination with oral administration of temozolomide (TMZ) to nude mice.

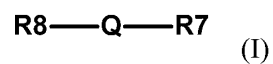
Figure 6 shows PARylation in bone marrow cells following intraperitoneal administration of Example 18 or R⁸H-16 to nude mice.

30 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.

What is claimed is:

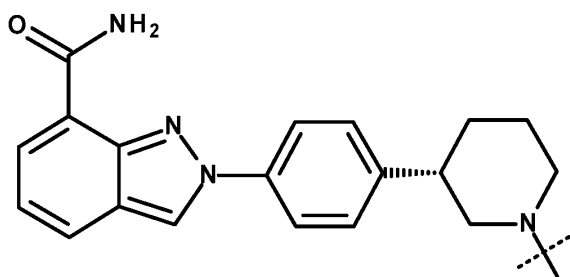
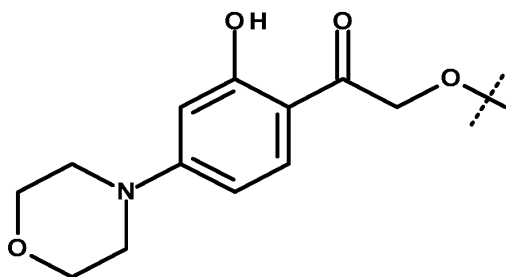
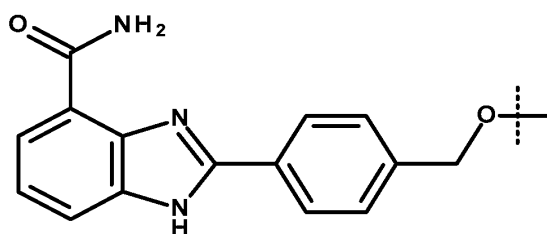
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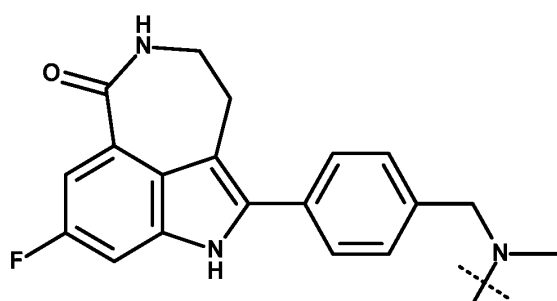
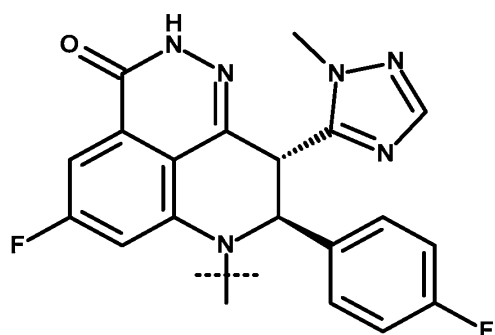
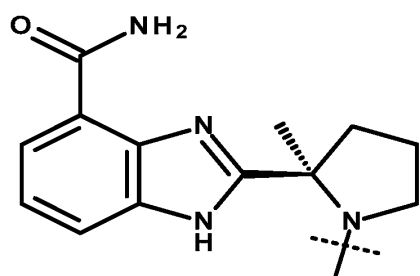


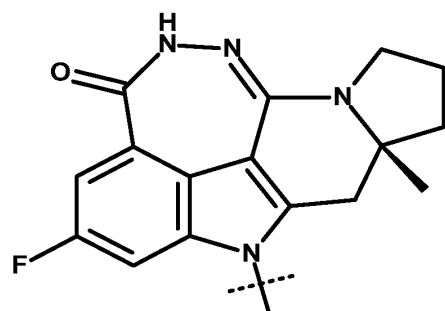
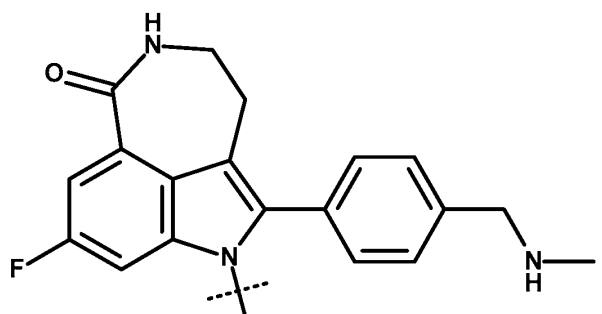
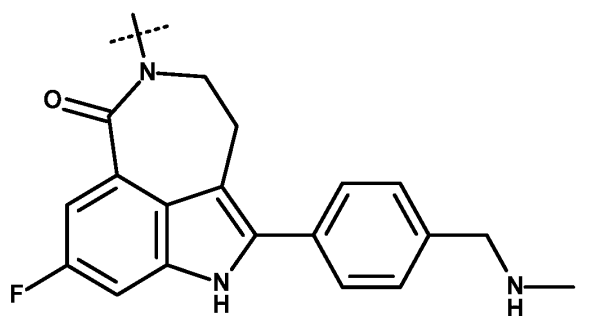
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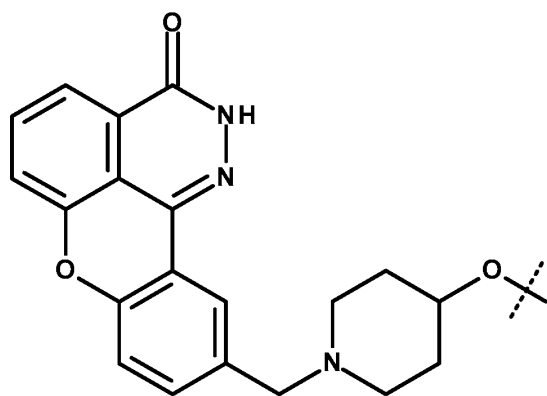
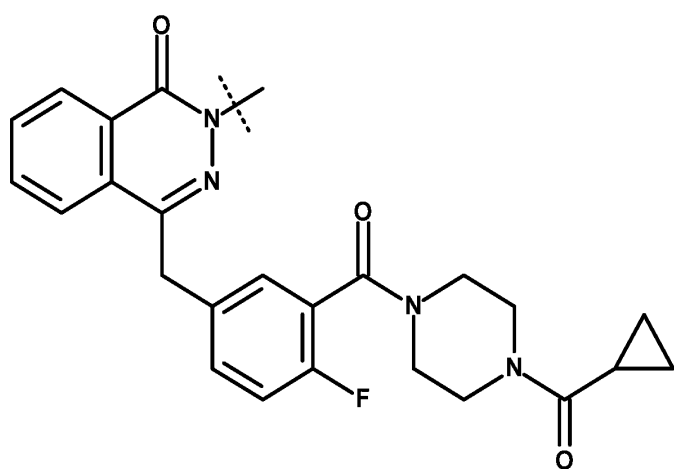
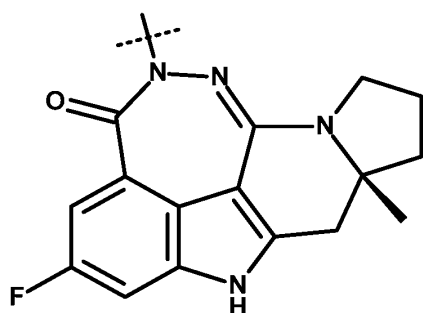
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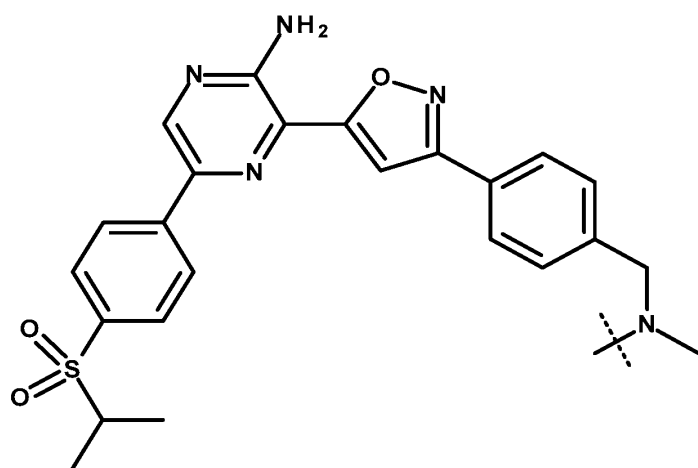
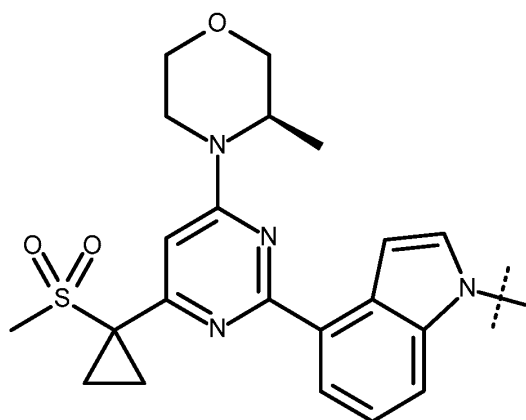
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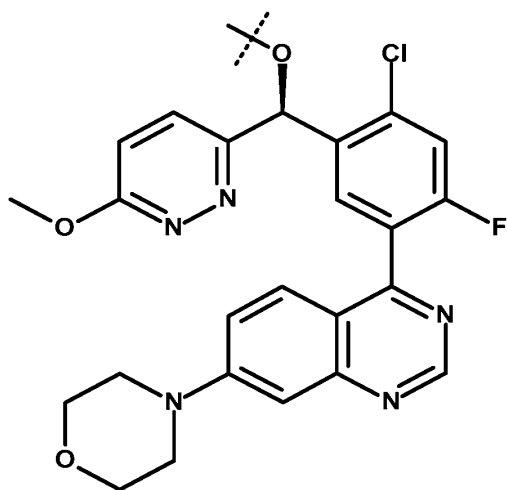
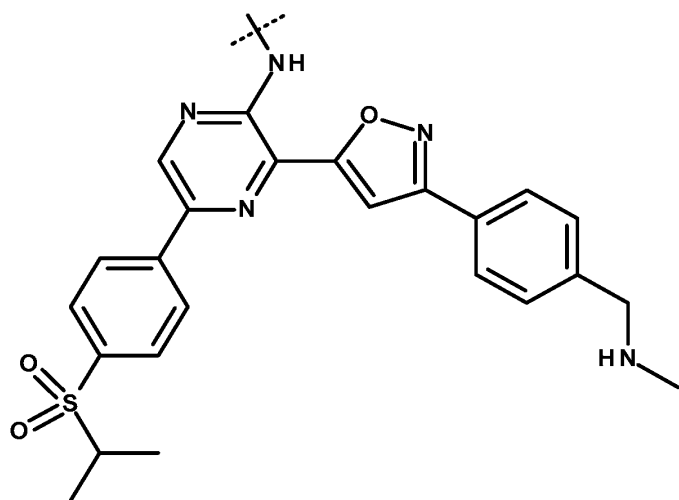


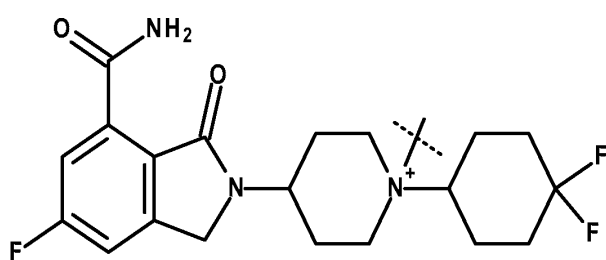
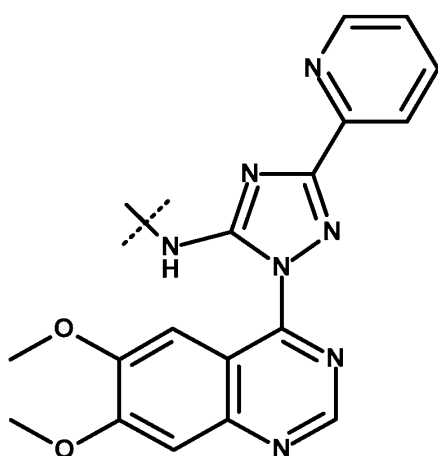
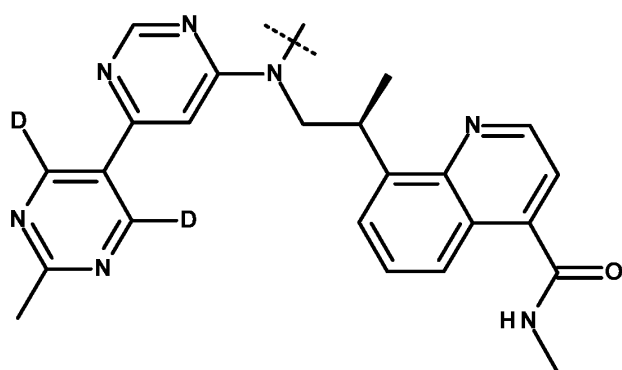


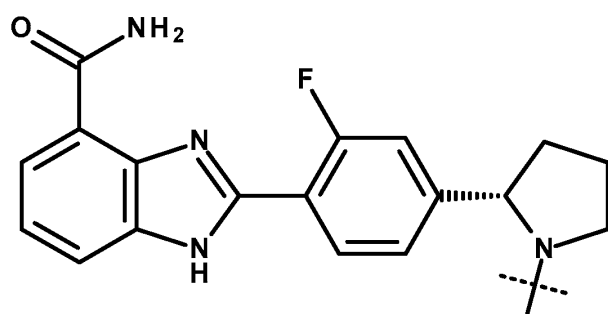
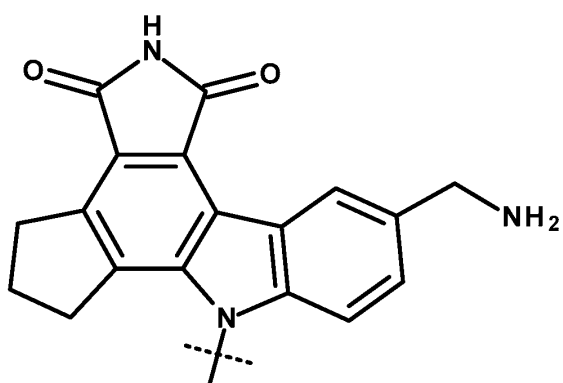
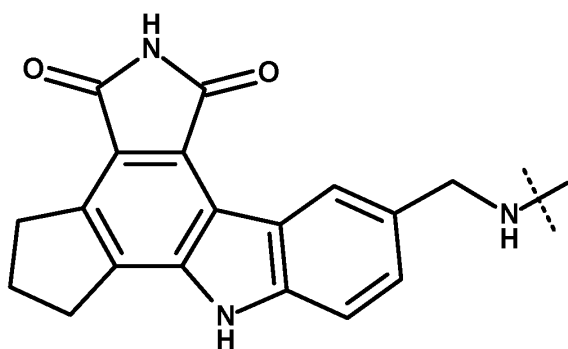


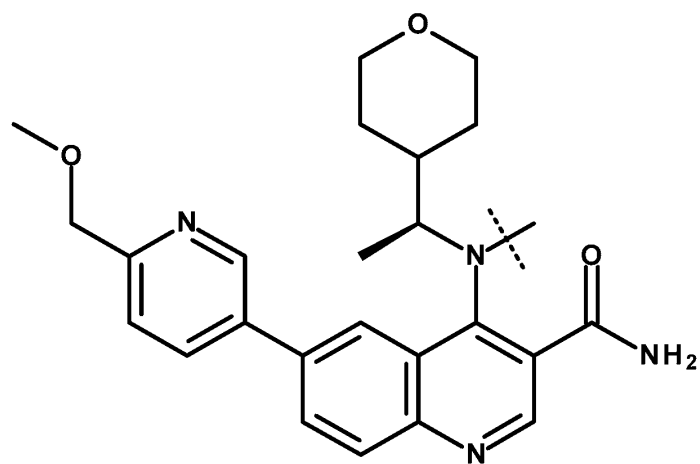
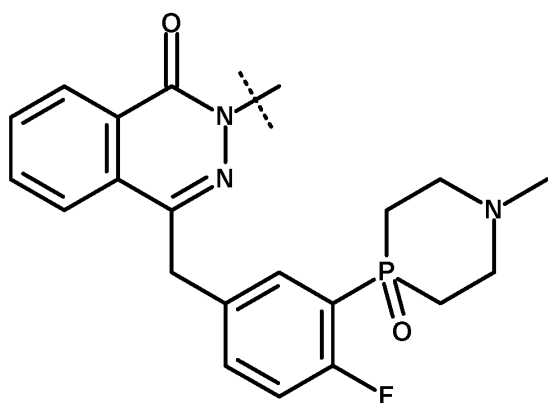
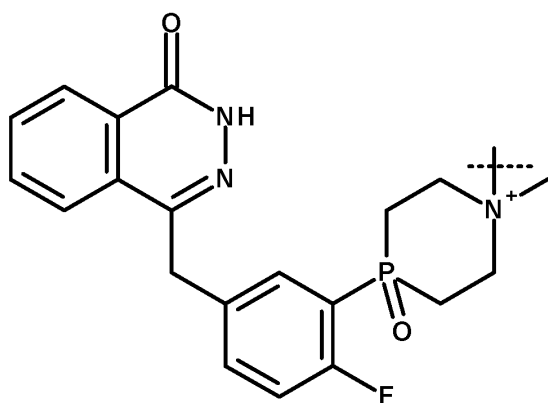


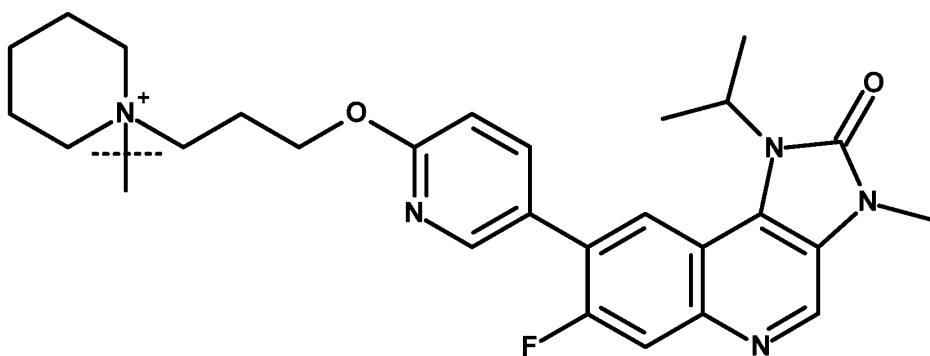
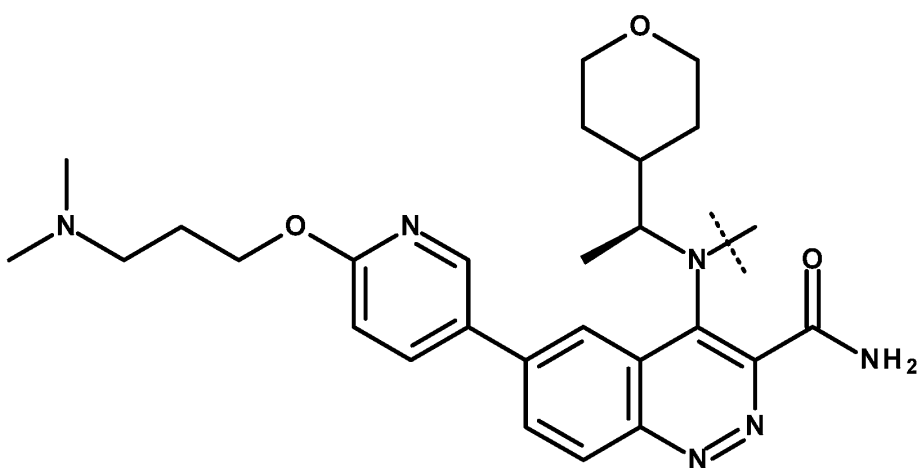
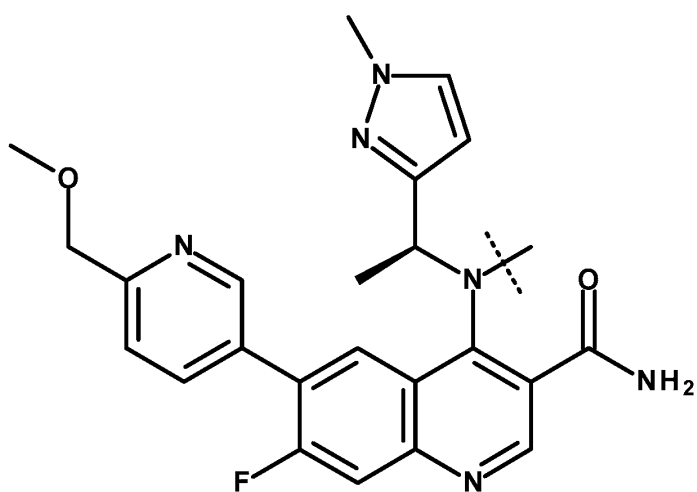


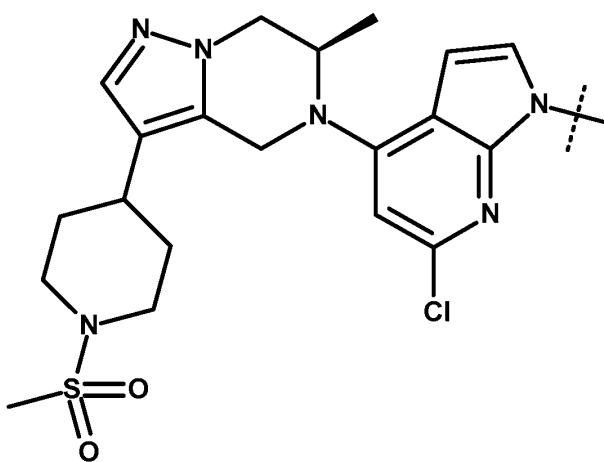
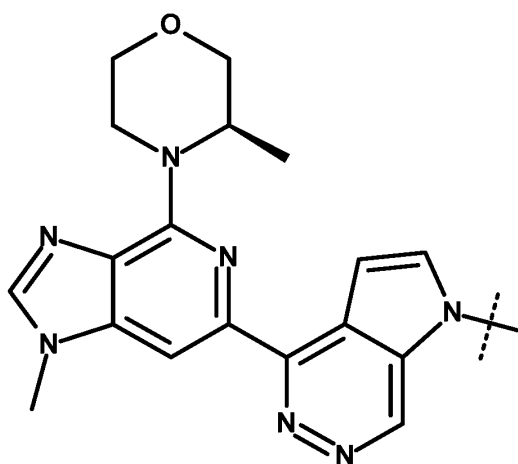


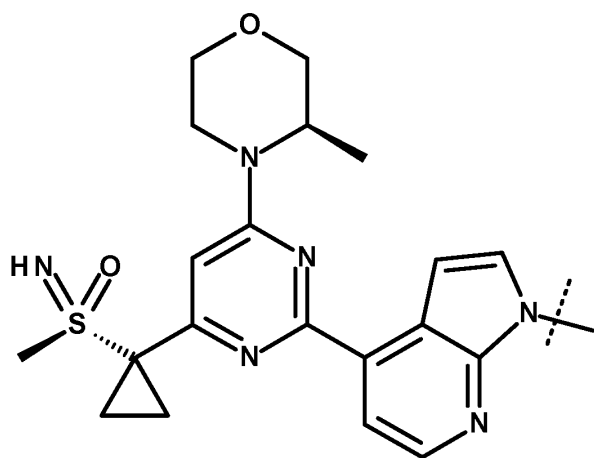




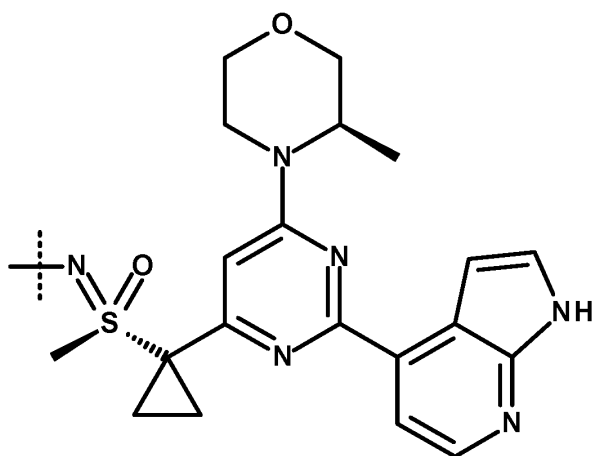






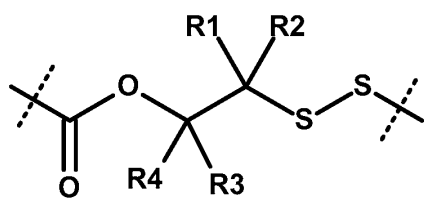


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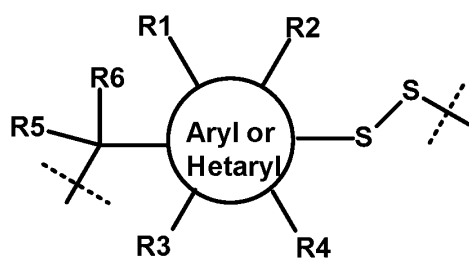
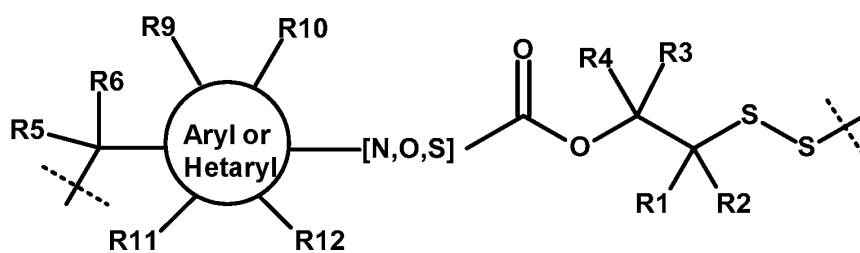
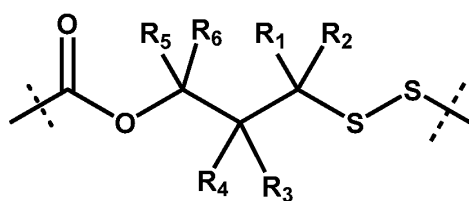
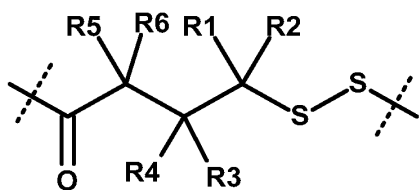
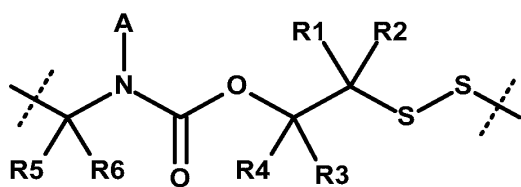


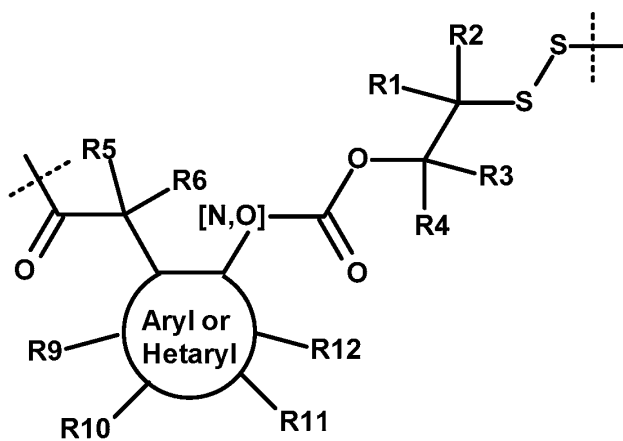
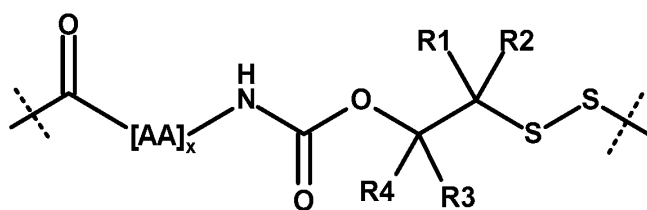
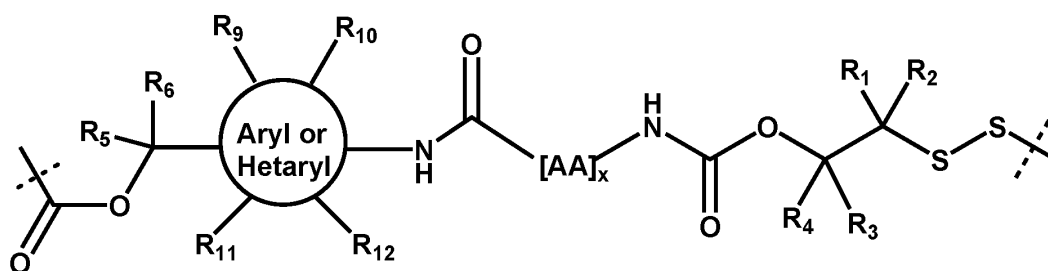
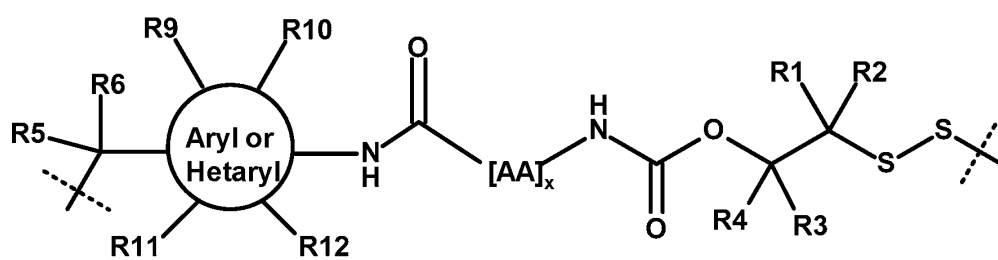
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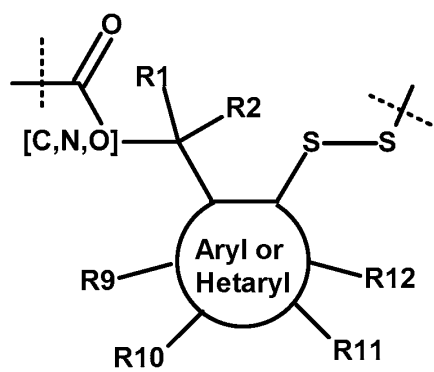
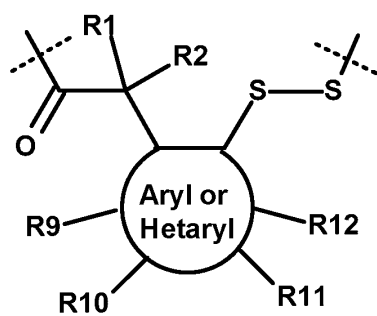
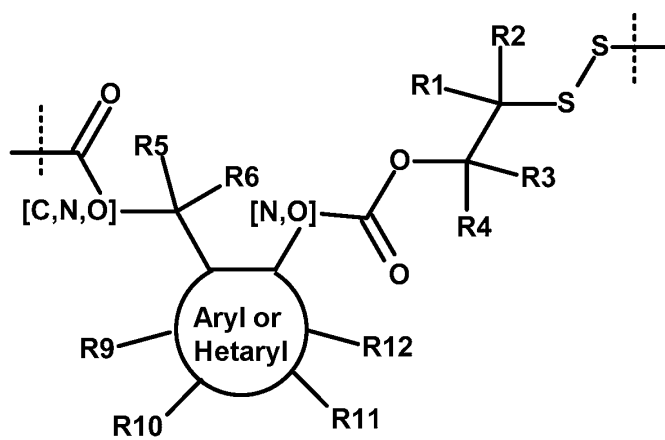
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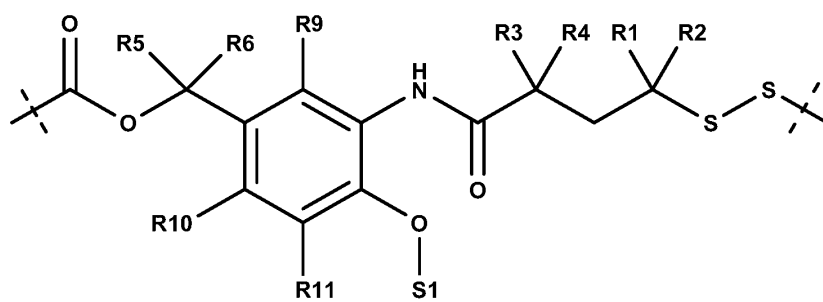
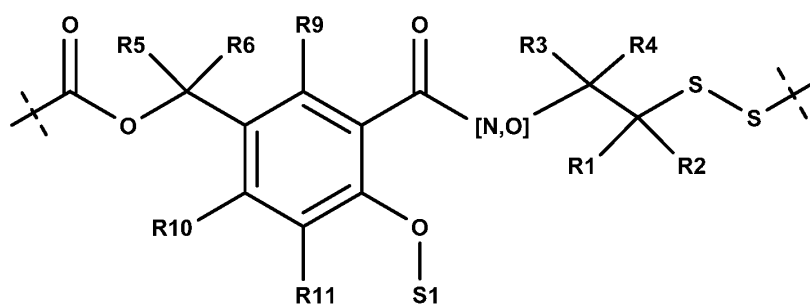
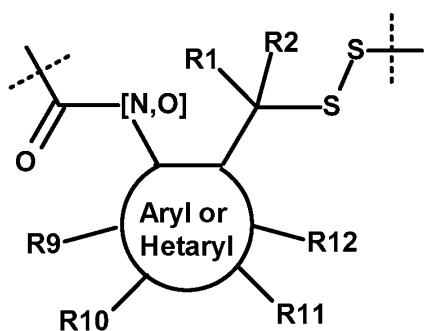
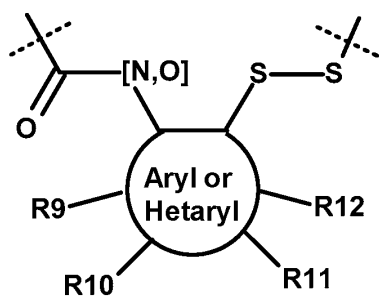


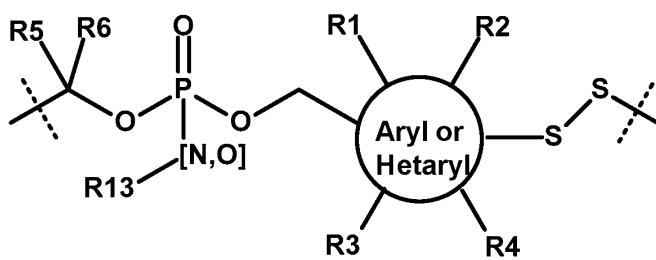
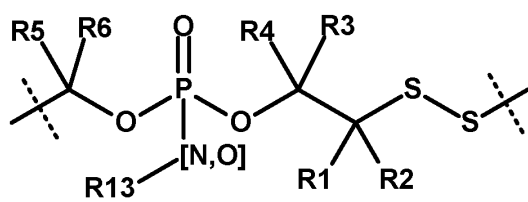
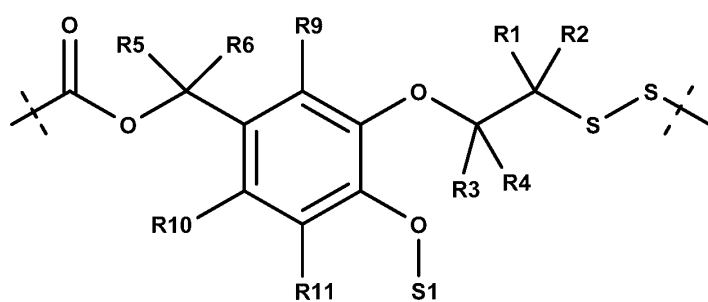
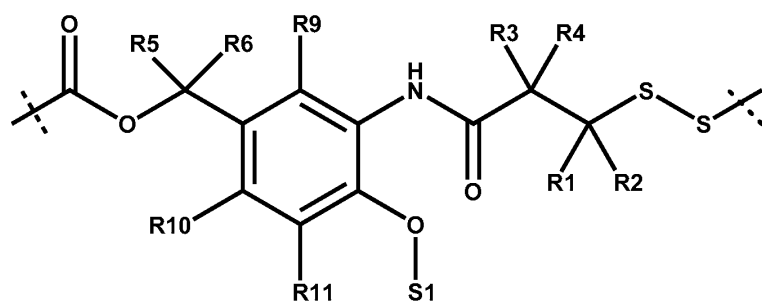
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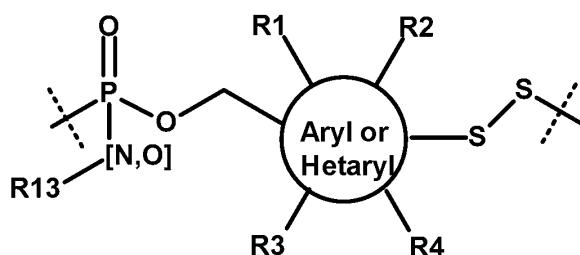
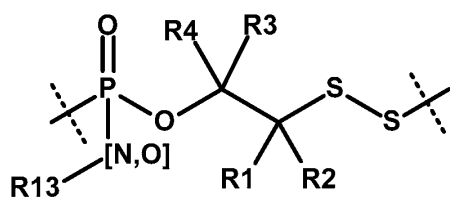
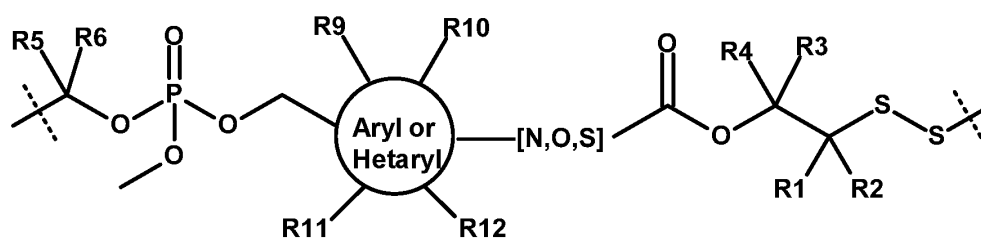




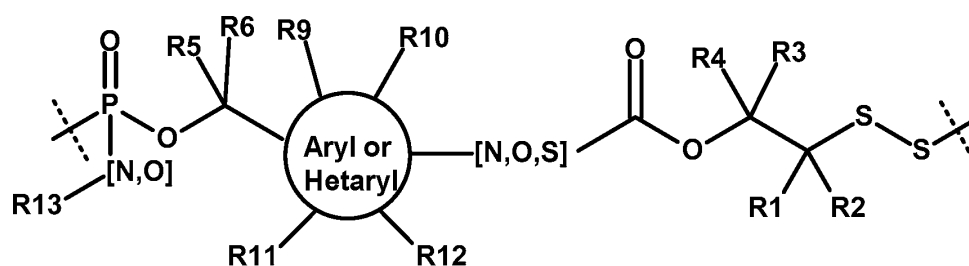








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$R^1, R^2, R^3, R^4, R^5, R^6, R^9, R^{10}, R^{11},$ and R^{12} are each independently selected from H, C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, 5-10 membered heteroaryl, halo, CN, NO_2 , OR^{a1} , SR^{a1} , $C(O)R^{b1}$, $C(O)NR^{c1}R^{d1}$, $C(O)OR^{a1}$, $OC(O)R^{b1}$, $OC(O)NR^{c1}R^{d1}$, $NR^{c1}R^{d1}$, $NR^{c1}C(O)R^{b1}$, $NR^{c1}C(O)OR^{a1}$, and $NR^{c1}C(O)NR^{c1}R^{d1}$, wherein said C_{1-4} alkyl, C_{1-4} alkenyl, C_{6-10} aryl, and 5-10 membered heteroaryl are each optionally substituted with 1, 2, or 3 substituents

independently selected from halo, CN, 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},

or R¹ and R² together with the carbon atom to which they are attached form a C₃₋₇ cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from 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};

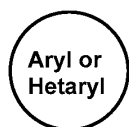
or R¹ and R³ together with the carbon atom to which they are attached form a C₃₋₇ cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from halo, CN, NO₂, OR^{al}, SR^{al}, C(O)R^{b1}, C(O)NR^{cl}R^{d1}, C(O)OR^{al}, OC(O)R^{b1}, OC(O)NR^{cl}R^{d1}, NR^{cl}R^{d1}, NR^{cl}C(O)R^{b1}, NR^{cl}C(O)OR^{al}, and NR^{cl}C(O)NR^{cl}R^{d1};

or R³ and R⁴ together with the carbon atom to which they are attached form an C₃₋₇ cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from 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};

or R⁵ and R⁶ together with the carbon atom to which they are attached form an C₃₋₇ cycloalkyl group optionally substituted with 1, 2, or 3 substituents independently selected from 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}

R¹³ is H or C₁₋₆ alkyl;

A is H or C₁₋₄ alkyl;



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;

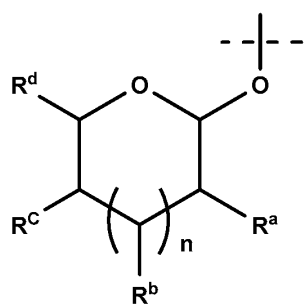
[N, O, S] is NH, O, or S;

[N, O] is NH or O;

[C, N, O] is CR^xR^y, NH, or O;

each R^x and R^y are independently selected from H and C₁₋₄ alkyl;

[AA]_x is a peptide that may be cleaved by enzymatic action;



S1 is ;

each R^a , R^b , R^c , and R^d are independently selected from H, C_{1-4} alkyl, OR^{a2} , CO_2R^{a2} , and $OC(=O)R^{a2}$, wherein said C_{1-4} alkyl is optionally substituted with OR^{a2} , CO_2R^{a2} , and $OC(=O)R^{a2}$;

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 ;

R^{a2} is H or C_{1-4} alkyl; and

n is 0 or 1.

2. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^7 is a peptide capable of selectively delivering R^8Q^- across a cell membrane having an acidic or hypoxic mantle having a pH less than about 6.0.

3. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^7 is a peptide comprising at least one of the following sequences:

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO: 1; Pv1);

AEQNPIYWARYADWLFTTPLLDDLALLVDADECG (SEQ ID NO: 2; Pv2);

ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO: 3; Pv3);

Ac-AAEQNPIYWARYADWLFTTPLLDDLALLVDADEGKCG (SEQ ID NO: 4; Pv4); and

AAEQNPIYWARYADWLFTTPLLDDLALLVDADEGTC (SEQ ID No. 5; Pv5);

and wherein R^7 is attached to Q through a cysteine residue of R^7 .

4. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^7 is a peptide comprising at least one of the following sequences:

ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO: 1; Pv1),
AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO: 2; Pv2), and
ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO: 3; Pv3),
and wherein R⁷ is attached to Q through a cysteine residue of R⁷.

5. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R⁷ is a peptide comprising the sequence: ADDQNPWRAYLDLLFPTDTLLLDLLWCG (SEQ ID NO: 1; Pv1).

6. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R⁷ is a peptide comprising the sequence: AEQNPIYWARYADWLFTTPLLLLDLALLVDADECG (SEQ ID NO: 2; Pv2).

7. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R⁷ is a peptide comprising the sequence: ADDQNPWRAYLDLLFPTDTLLLDLLWDADECG (SEQ ID NO: 3; Pv3).

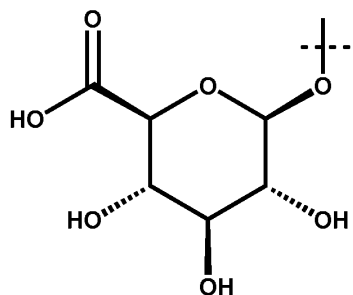
8. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R⁷ is a peptide comprising the sequence: Ac-AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTKCG (SEQ ID NO: 4; Pv4).

9. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R⁷ is a peptide comprising the sequence: AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTC (SEQ ID No. 5; Pv5).

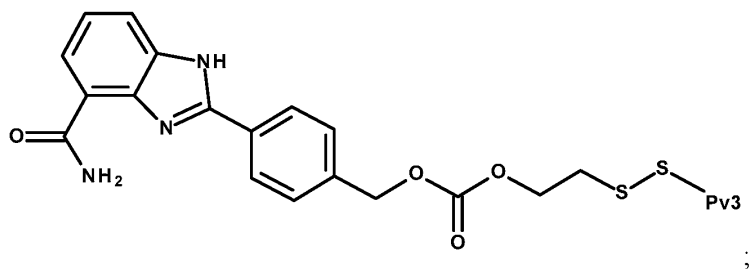
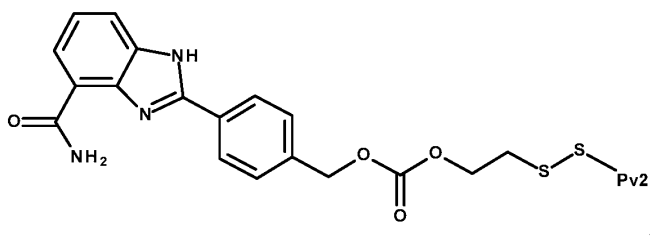
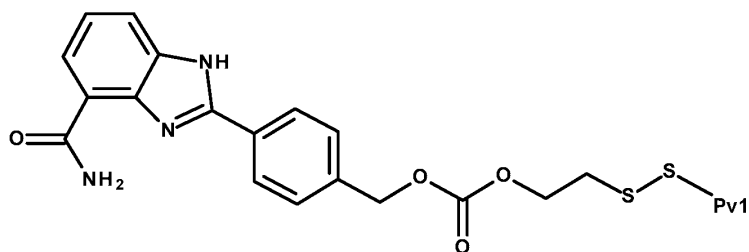
10. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein R¹ and R² are each independently selected from H and methyl, and R³, R⁴, R⁵, and R⁶ are each H.

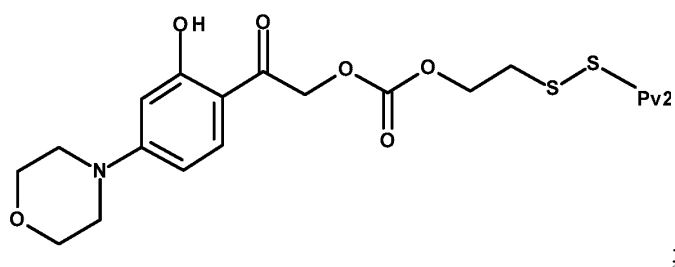
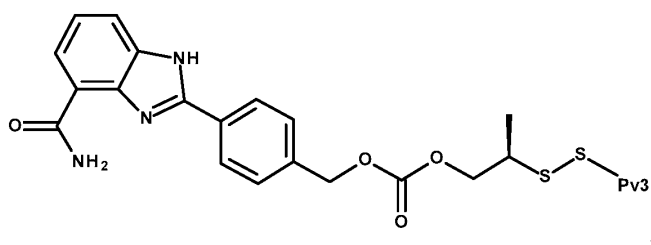
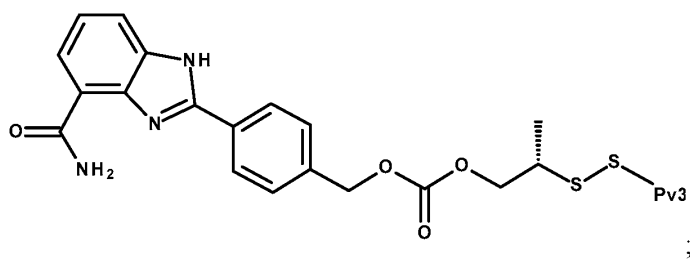
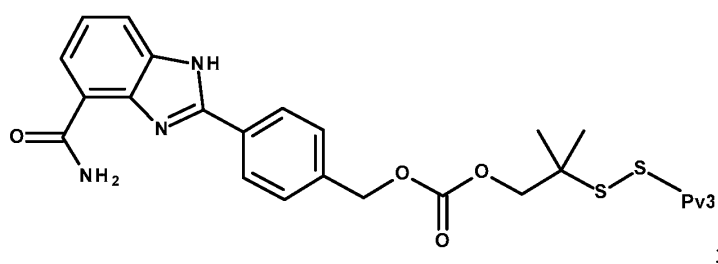
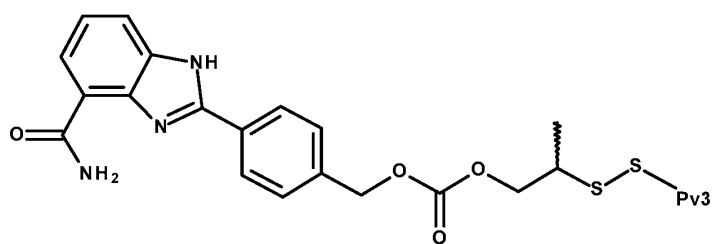
11. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein R¹ and R² are each independently selected from H and methyl.

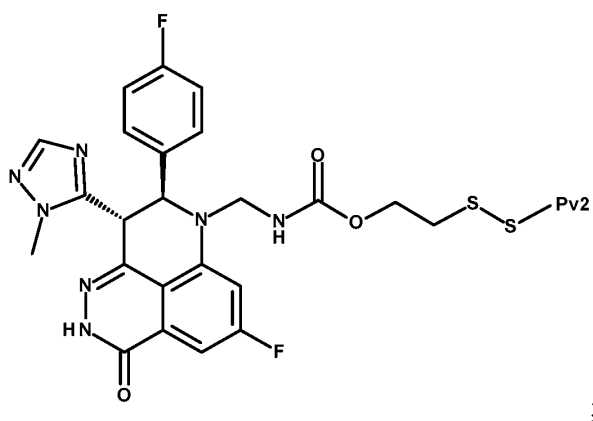
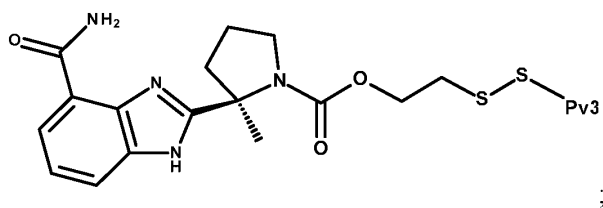
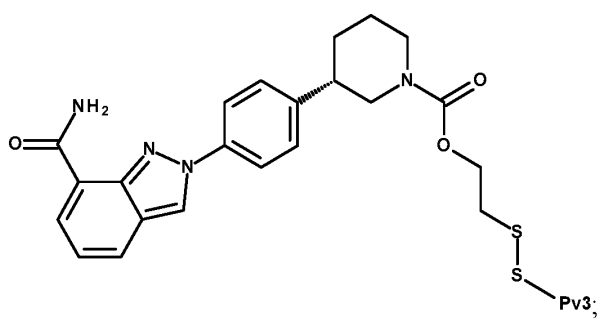
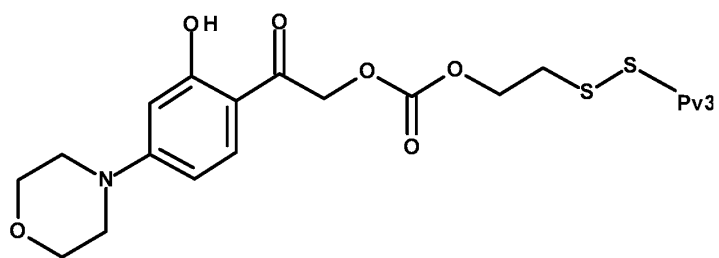
12. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein R¹ and R² are each H.
13. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein R³ and R⁴ are each H.
14. The compound of any one of claims 1-9, or a pharmaceutically acceptable salt thereof, wherein R⁵ and R⁶ are each H.
15. The compound of any one of claims 1-14, or a pharmaceutically acceptable salt thereof, wherein R⁹, R¹⁰, R¹¹, and R¹² are each independently selected from H and methyl.
16. The compound of any one of claims 1-15, or a pharmaceutically acceptable salt thereof, wherein the enzyme capable of cleaving [AA]_x is Cathepsin B, MMPXX, DPPIV, glycoprotein, peptidase, or caspase.
17. The compound of any one of claims 1-15, or a pharmaceutically acceptable salt thereof, wherein [AA]_x is a peptide having two to ten amino acid residues.
18. The compound of any one of claims 1-15, or a pharmaceutically acceptable salt thereof, wherein [AA]_x is -Pro_Gly-, -Val_Cit-, -Gly_Pro_Leu_Gly_Leu_Alanyl_Gly_Asp_Asp-, -Gly_Pro_GLeu_Gly_Val_Arg_Gly, or -Ser_Ser_Lys_Leu_Gly-.
19. The compound of any one of claims 1-18, or a pharmaceutically acceptable salt thereof, wherein S1 is a group having the following structure:

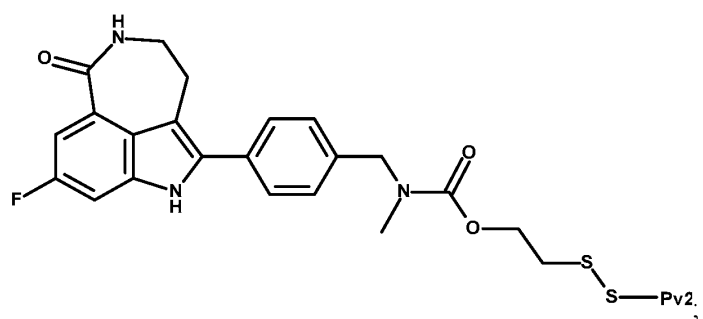
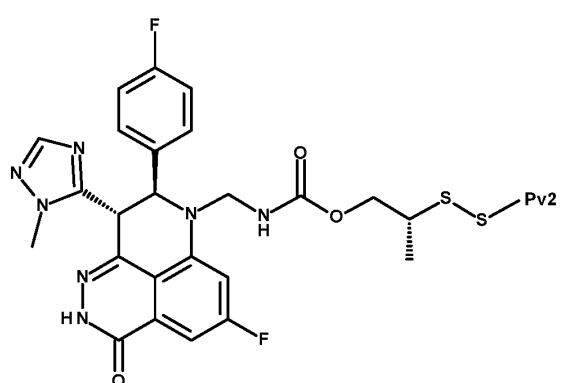
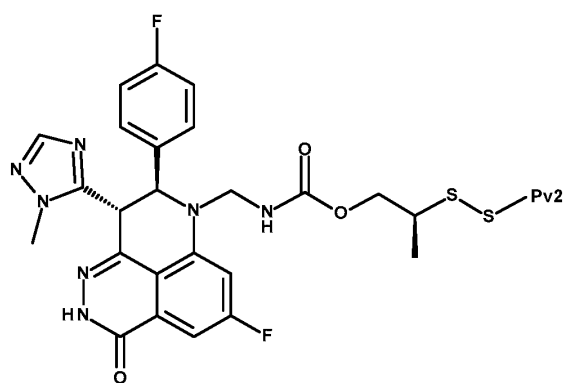


20. The compound of claim 1, or a pharmaceutically acceptable salt thereof, which is selected from:

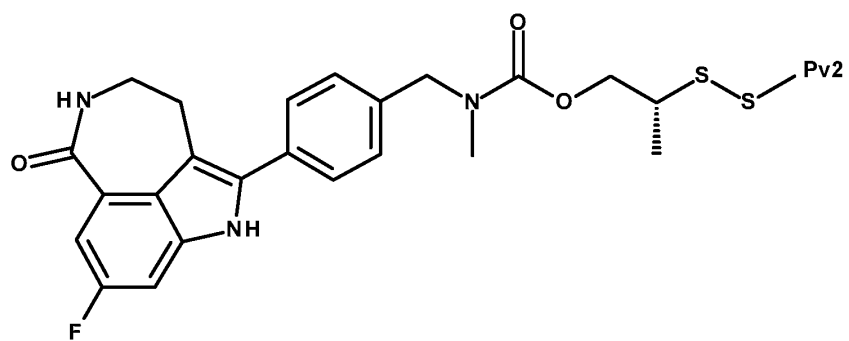




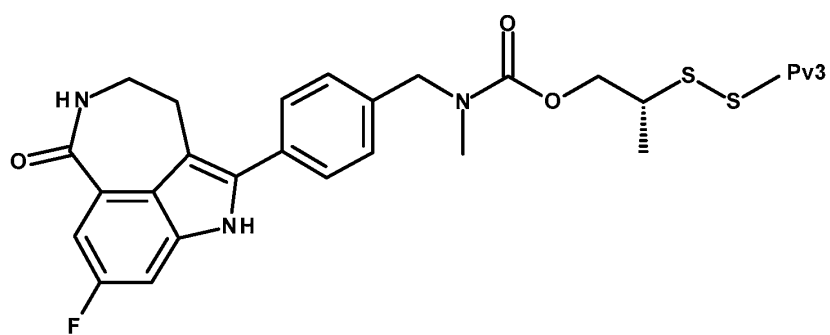




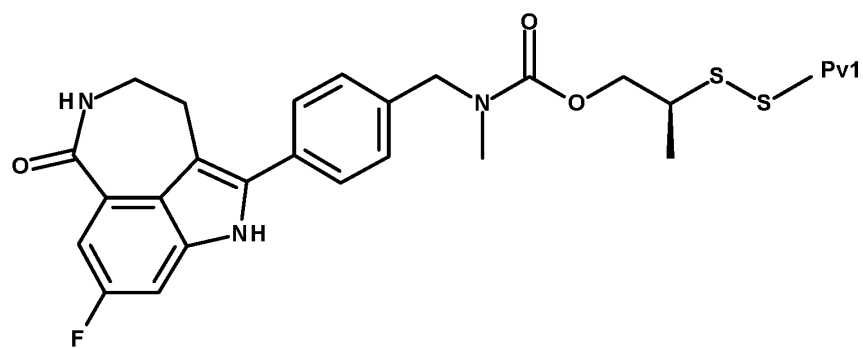




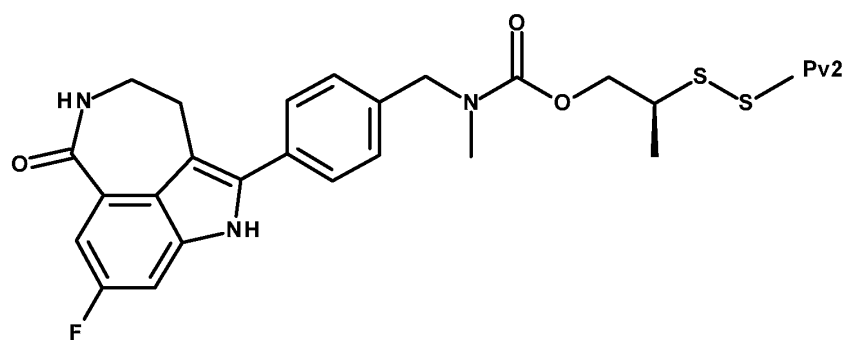
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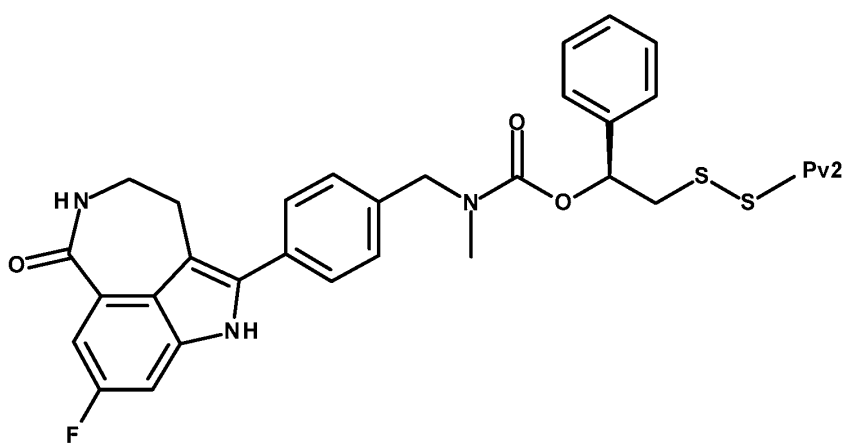
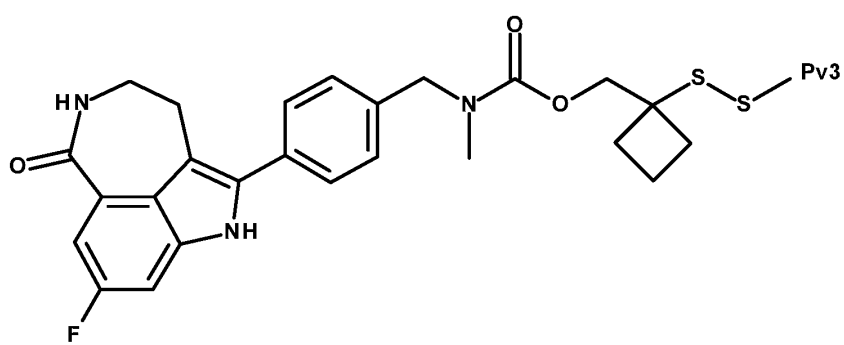
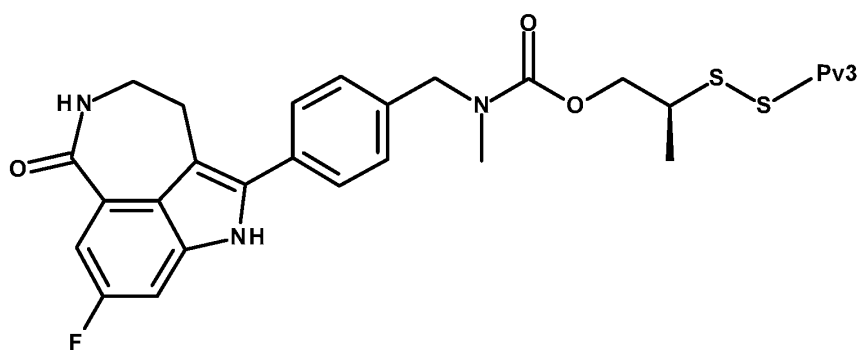
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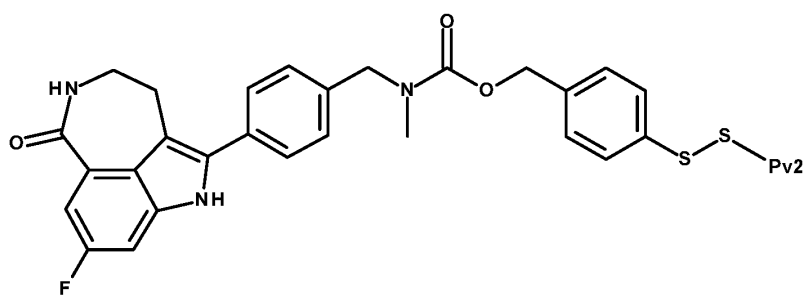
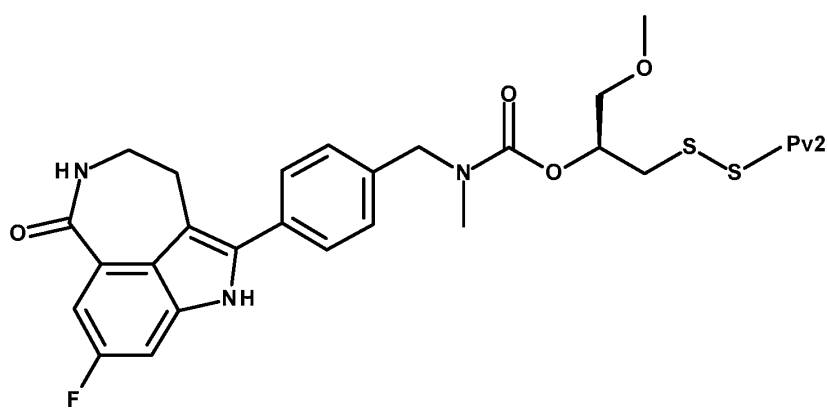
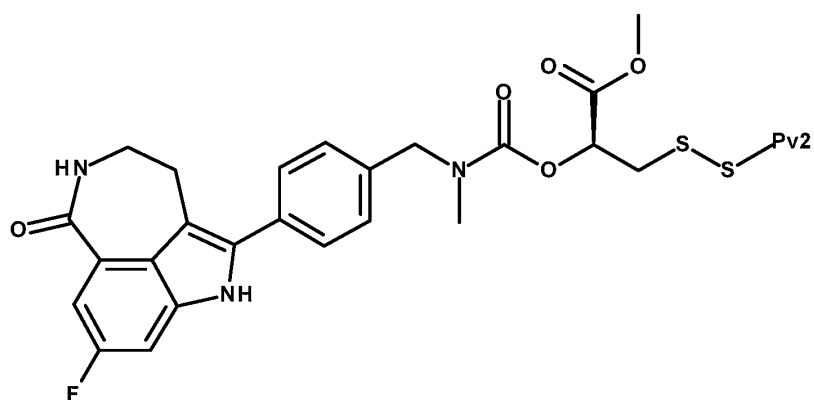


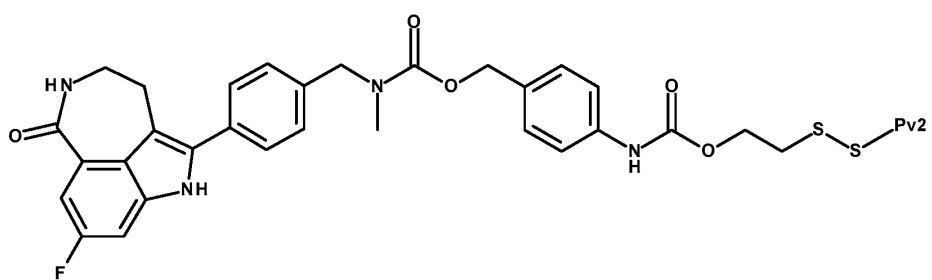
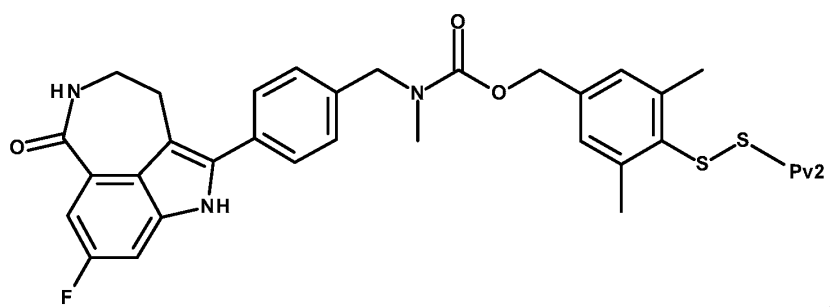
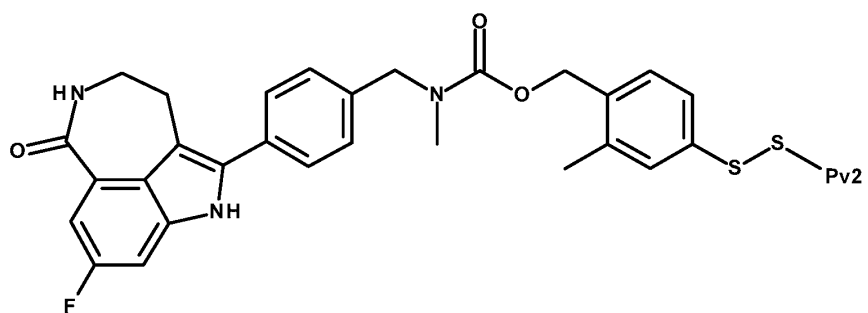
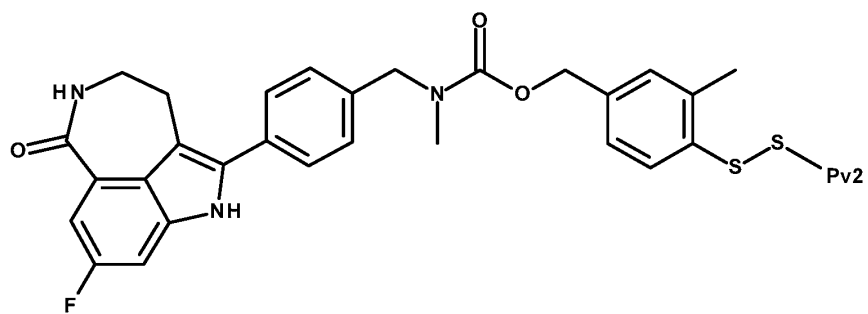
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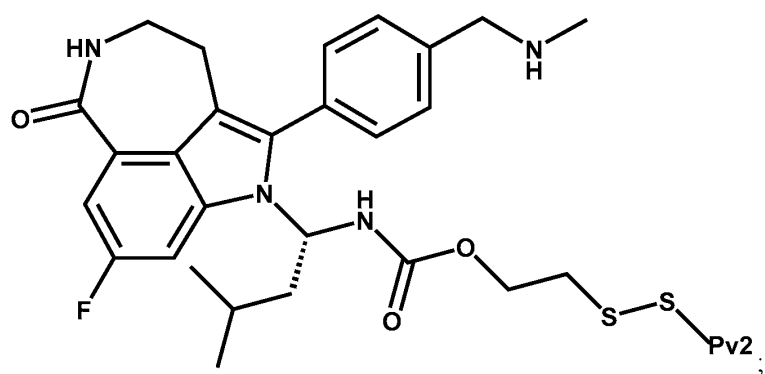
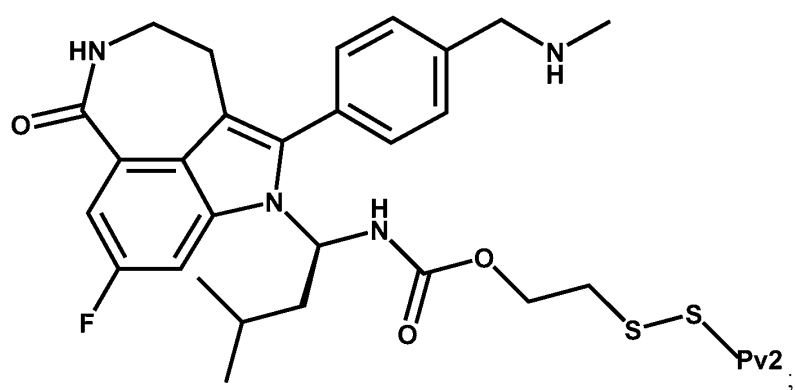
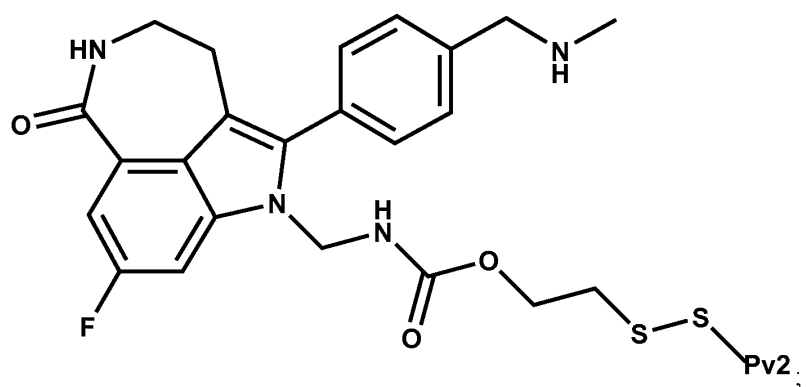


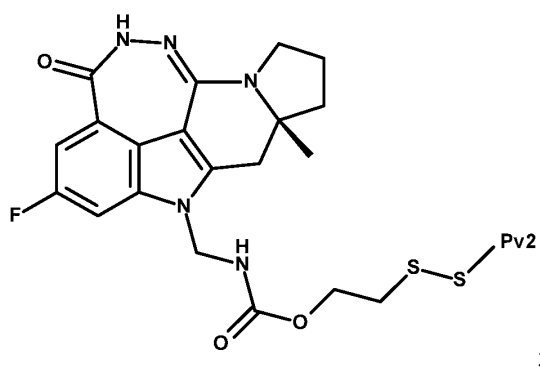
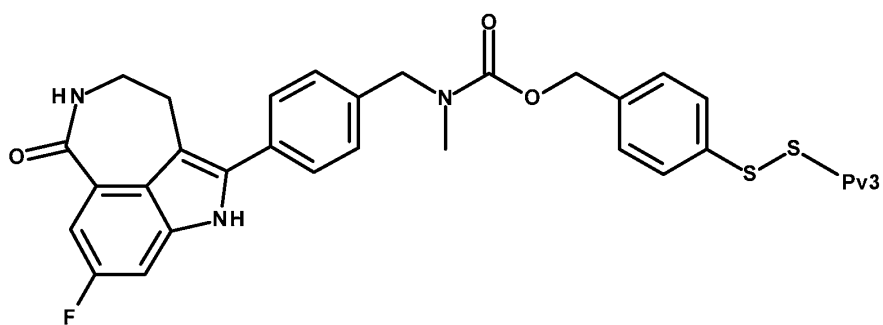
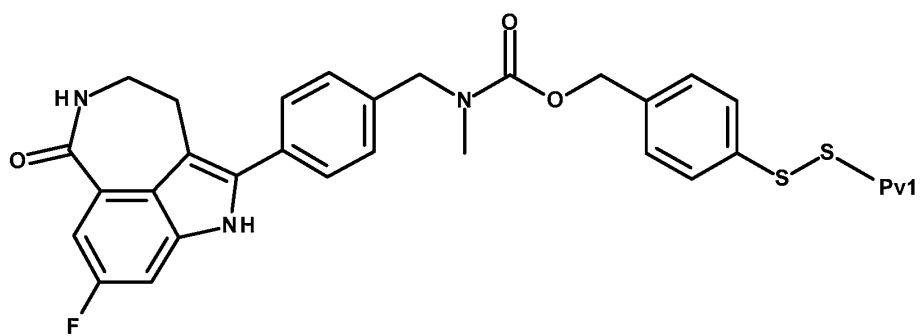
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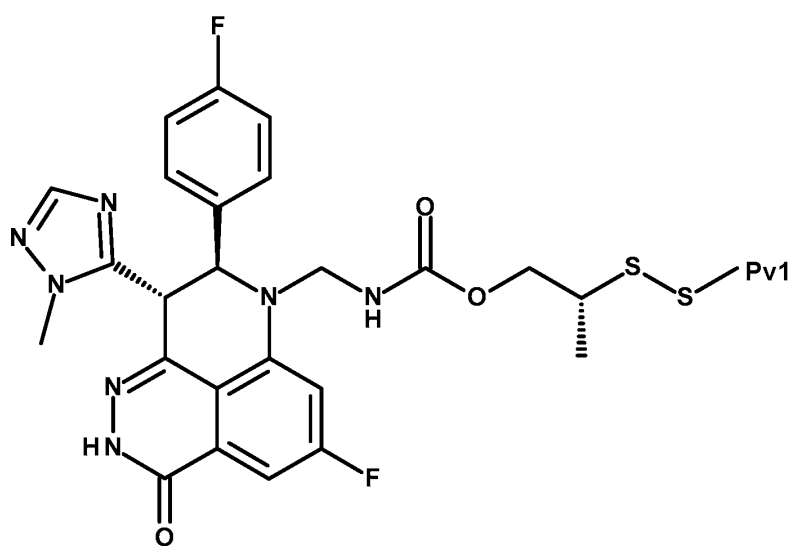
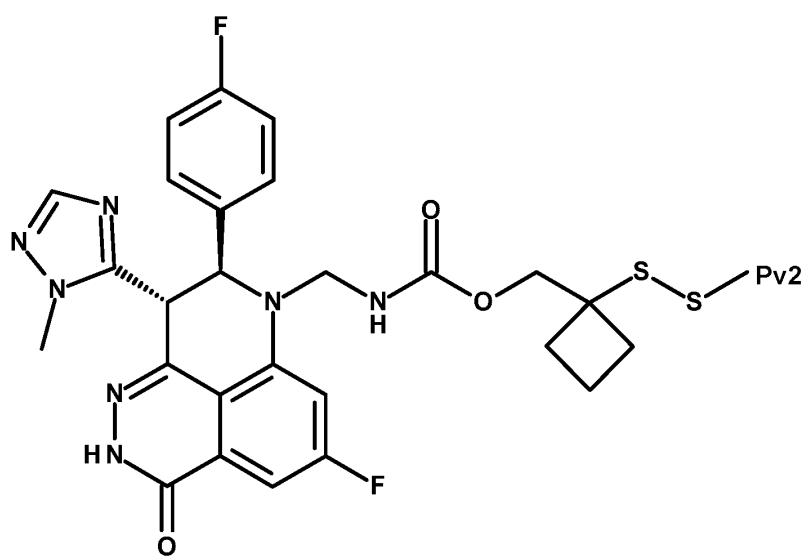


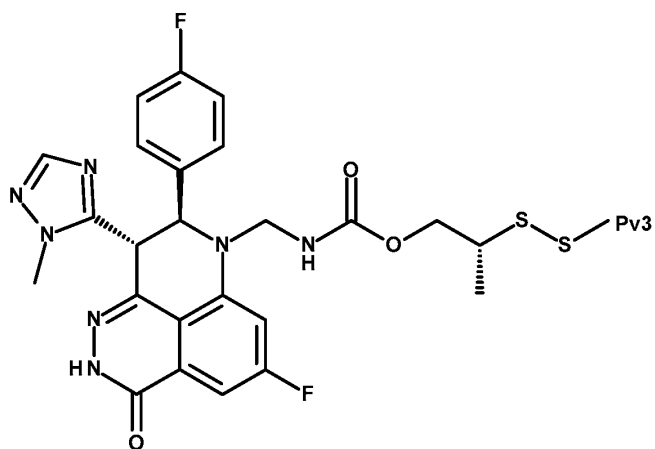




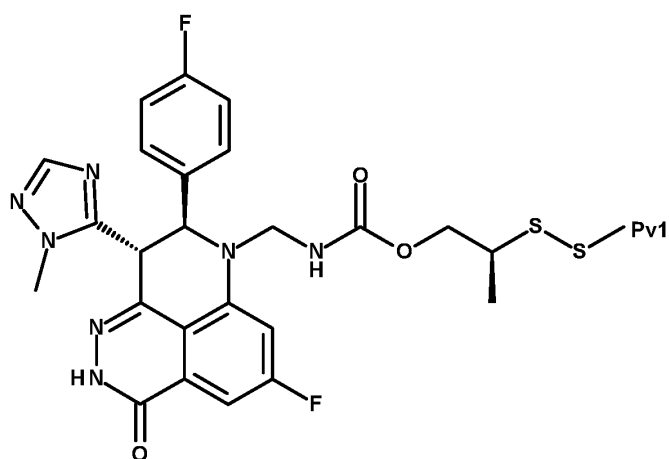




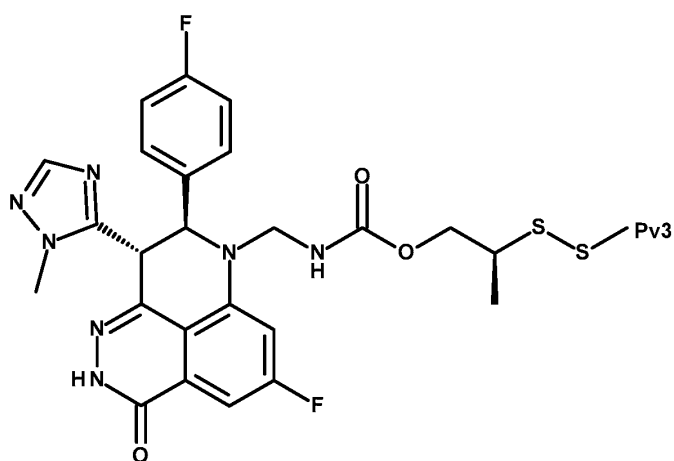




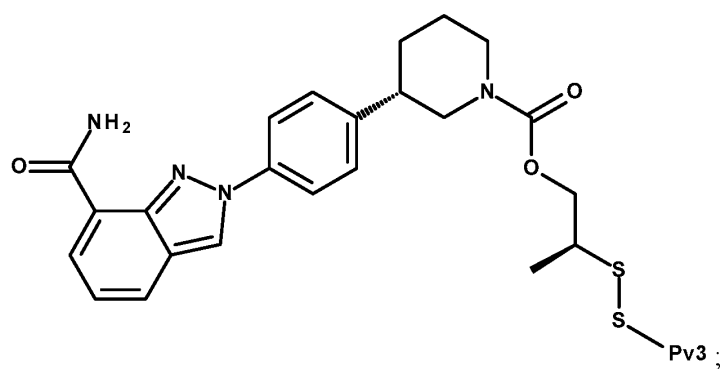
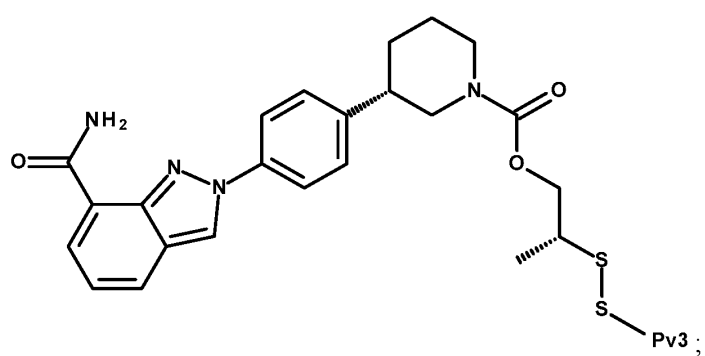
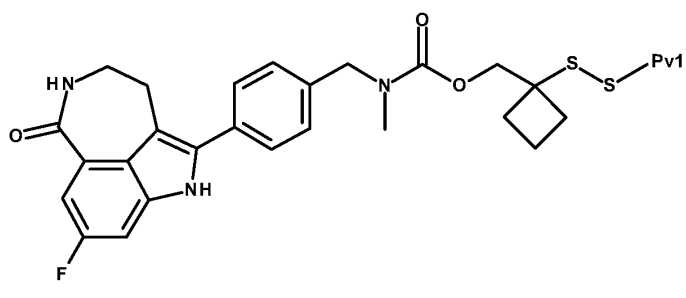
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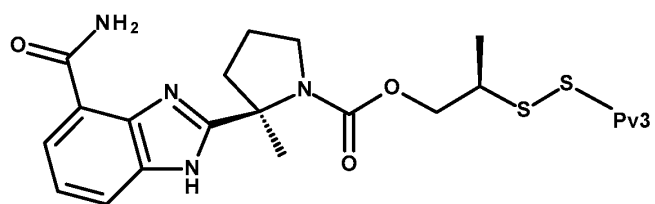


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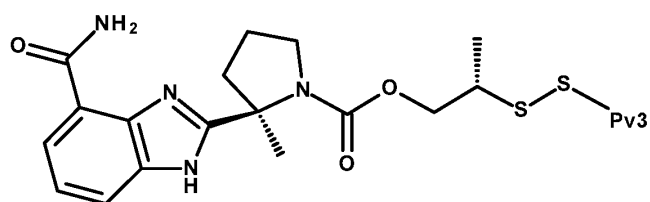


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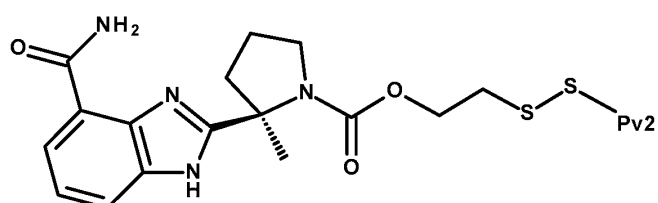




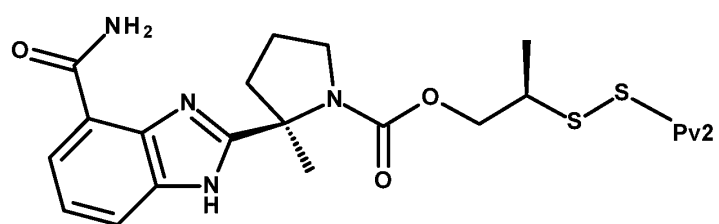
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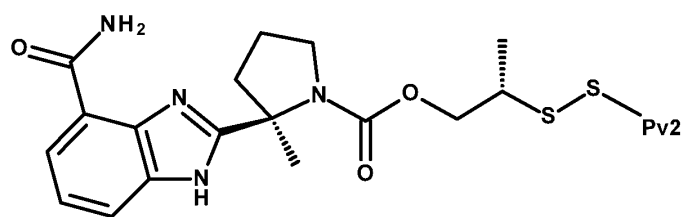
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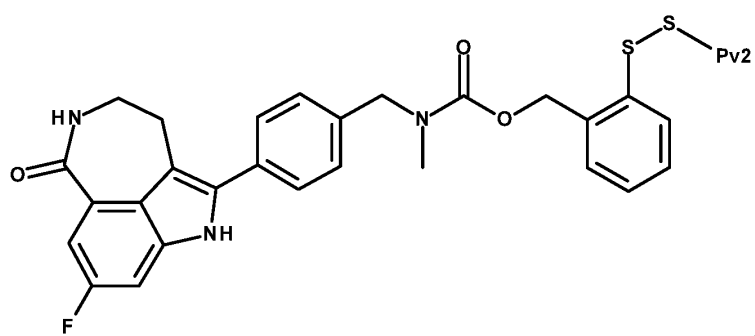
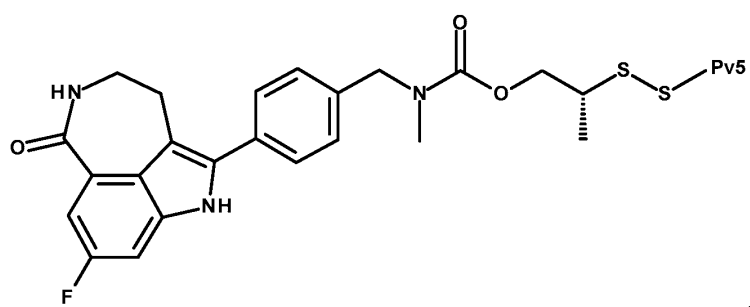
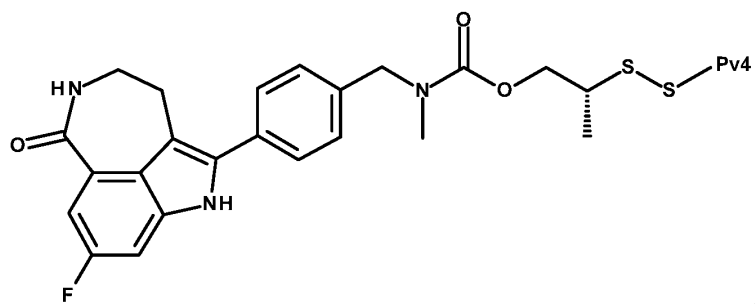
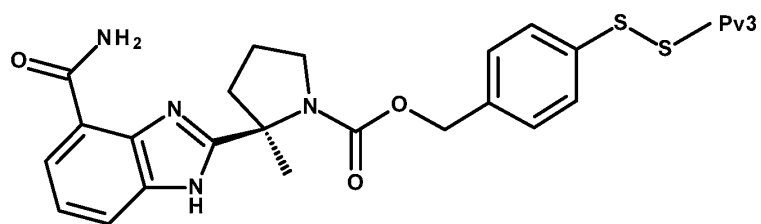
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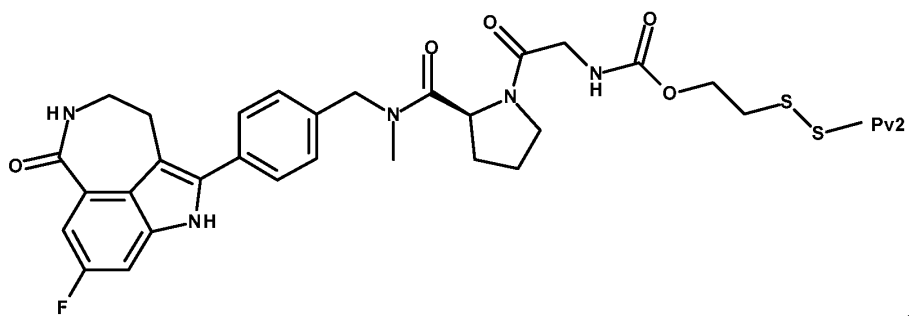
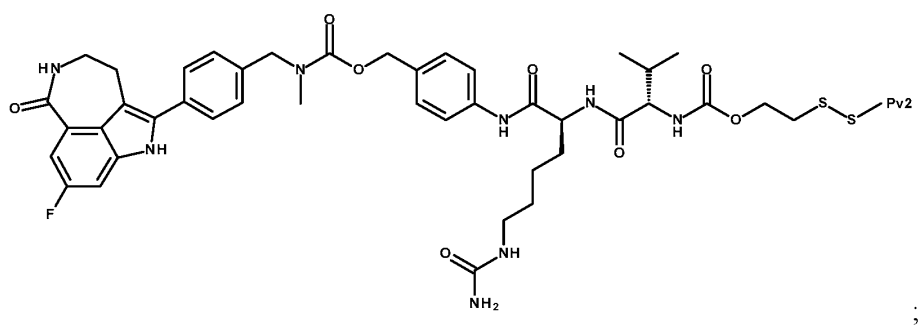
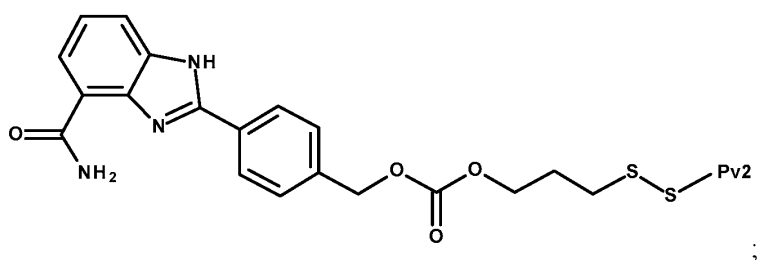
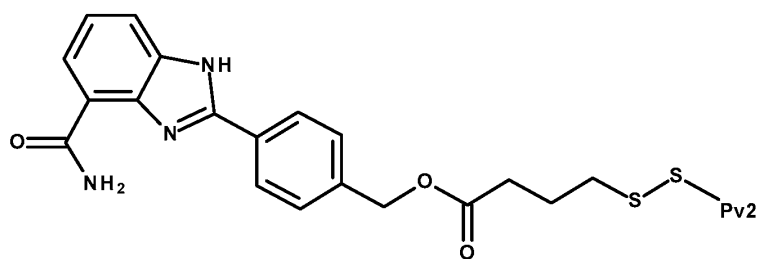


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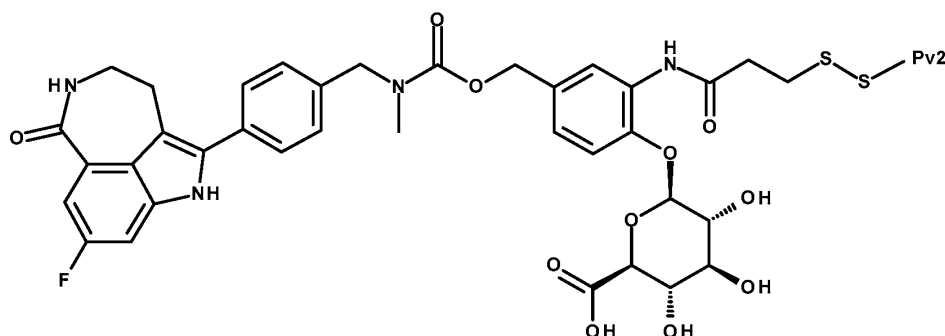


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; and



21. A pharmaceutical composition that comprises a compound of any one of claims 1-20, or a pharmaceutically acceptable salt thereof.
22. A method of treating a disease or condition involving acidic or hypoxic diseased tissue in an afflicted human or other mammal in need of such treatment which comprises administering to the afflicted human or other mammal a therapeutically-effective amount of the compound of any one of claims 1-20, or a pharmaceutically acceptable salt thereof.
23. The method of claim 22, wherein the disease or condition is selected from cancer, stroke, myocardial infarction, and long-term neurodegenerative disease.
24. The method of claim 23, wherein the disease or condition is cancer.
25. The method of claim 24, wherein the cancer is PARP-sensitive.
26. The method of claim 24, wherein the cancer is associated with abnormal expression or activity of ATM.
27. The method of claim 24, wherein the cancer is associated with abnormal expression or activity of DNA-PK.
28. The method of claim 24, wherein the cancer is BRCA-mutated breast cancer.

29. The method of claim 24, wherein the cancer is germline BRCA-mutated ovarian cancer.
30. The method of claim 23, which further comprises administering a therapeutically-effective amount of ionizing radiation or a cytotoxic agent to said afflicted human or other mammal.
31. A method of reducing bone marrow toxicity associated with administration of an ionizing radiation or cytotoxic agent, which comprises administering to a human or other mammal a therapeutically-effective amount of the compound of any one of claims 1-20, or a pharmaceutically acceptable salt thereof, in combination with the ionizing radiation or cytotoxic agent.