

METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER USING AN INHIBITOR OF PRM T5

CROSS-REFERENCE TO RELATED APPLICATIONS

5 **[0001]** This application claims the benefit of U.S. Appl. No. 63/535,767, filed August 31, 2023; and of U.S. Appl. No. 63/560,987, filed March 4, 2024, each of which is incorporated by reference as if fully set forth herein.

TECHNICAL FIELD

10 **[0002]** The disclosure generally relates to prostate-specific membrane antigen (PSMA) ligands, radiosensitizers, conjugates, compositions, and methods of use in the imaging and therapy of prostate cancer.

GOVERNMENT SUPPORT CLAUSE

15 **[0003]** This invention was made with government support under W81XWH-12-1-0346, W81XWH-13-1-0398, and W81XWH-16-1-0394 awarded by U.S. Army Medical Research Acquisition Activity, and CA212403 awarded by National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

20 **[0004]** Treatment-induced neuroendocrine differentiation (NED) is an emerging mechanism of treatment resistance and is a process leading to the development of neuroendocrine prostate cancer (NEPC). In NED, a subset of androgen receptor (AR)-positive prostate cancer cells trans-differentiate into AR-negative neuroendocrine-like (NE-like) cells in response to all existing treatments including radiation therapy (RT), androgen therapy (ADT), and chemotherapy. Recent increase in treatment-induced NEPC (t-NEPC) development after the use of potent next generation AR signaling inhibitors abiraterone and enzalutamide has further confirmed the clinical significance of therapy-induced NED. NEPC is now considered an aggressive variant of castration-resistant prostate cancer (CRPC), and it constitutes 17-25% of all CRPC. Presently there is no effective treatment to manage this deadly end-stage disease. Therefore, developing novel therapeutic approaches to preventing treatment-induced NED will not only sensitize prostate cancer cells to existing treatments but also prevent disease progression. In view of the foregoing, it is an object to provide such a therapy. This and other objects and advantages, as well as inventive features, will be apparent from the description.

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SUMMARY

[0005] Studies performed by the inventors have identified DNA repair enzymes (e.g., protein arginine methyltransferase 5 (PRMT5) and ataxia-telangiectasia mutated (ATM)), as regulators of fractionated ionizing radiation (FIR)-induced NED.

The inventors hypothesize that at least PRMT5 epigenetically reprograms treatment-induced NED via cofactor switch and that targeting PRMT5 can inhibit treatment-induced NED and sensitize prostate cancer cells to treatments.

[0006] To that end, the disclosure relates to conjugates of formula T-L-A in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

L is a linker, and

A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor.

[0007] The radiosensitizer can be, among other things, an inhibitor of a DNA repair enzyme (e.g., an inhibitor of a single stranded DNA repair enzyme or an inhibitor of a double stranded DNA repair enzyme). Examples of DNA repair enzymes include a protein arginine methyltransferase 5 (PRMT5) inhibitor and an ataxia-telangiectasia mutated (ATM) inhibitor. Examples of PRMT5 inhibitors include JNJ-64619178 or GSK3326595. An example of an ATM inhibitor includes AZD0156.

[0008] The conjugates of the formula T-L-A can be used in methods of treating a patient for prostate cancer or metastasis thereof with radiotherapy, which method comprises administering to the patient a conjugate of the formula T-L-A or a pharmaceutically acceptable salt thereof or a pharmaceutical composition comprising a conjugate of the formula T-L-A, whereupon the patient is treated for prostate cancer or metastasis thereof. In such methods, the conjugates of the formula T-L-A can be used in conjunction with conjugates of the formula T-L-X or a pharmaceutically acceptable salt thereof in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

L is a linker, and

X is a radiotherapeutic or radioimaging agent. Compounds of the formula T-L-(X)A are also disclosed.

DESCRIPTION OF THE DRAWINGS

[0009] The drawings illustrate generally, by way of example, but not by way of limitation, various embodiments.

[0010] **FIG. 1. Role of PRMT5 in regulating expression of androgen receptor (AR) and DNA damage response (DDR) genes in prostate cancer cells.** Protein arginine methyltransferase 5 (PRMT5) epigenetically activates transcription of AR and the genes involved in DDR such as DNA double-strand

break (DSB) repair and G2 arrest genes, contributing to prostate cancer growth and survival, radioresistance and tumor recurrence.

[0011] FIG. 2. Development of SMTDs for treatment of cancer. A. Shown are representative images from a mCRPC patient before and after two treatments with ¹⁷⁷Lu-PSMA-617. B. Structure of a novel conjugate for targeted delivery to luteinizing hormone releasing hormone receptor (LHRHR)-expressing cancer cells. Red, a small molecule LHRHR antagonist; Green, a near infrared dye; Blue, linker. C-D. Representative images showing specific tumor targeting in OVCAR3 xenograft tumors in mice. The competitor (unlabeled conjugate) was administered at 100-times excess to demonstrate specific uptake of the conjugate via LHRHR.

[0012] FIG. 3. PRMT5 promotes growth of CRPC cells via epigenetic activation of AR expression. A, Growth curve (MTT assay) of 22Rv1 cells incubated with 10 μ M PRMT5 inhibitor (BLL3.3) or equal volume of vehicle (dimethyl sulfoxide; DMSO) for 6 days. B-C, Representative Western blot analysis (B) and quantification (C) of protein expression in cell lysates from Day 6 of A. D, qPCR analysis of gene expression in cells from Day 6 of A. E, Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible PRMT5 knockdown (22Rv1-shPRMT5#1) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. F-H, Representative Western blot analysis (F) and quantification (G) of protein expression in cell lysates from Day 6 of E. H, qPCR analysis of gene expression in cells from Day 6 of E. I, Growth curve (MTT assay) of 22Rv1 cells with doxycycline-inducible scramble control expression (22Rv1-shSC) incubated in the presence (Dox (+)) or absence (Dox (-)) of doxycycline for 6 days. J-L, Representative western blot analysis (J) and quantification (K) of protein expression in cell lysates from Day 6 of I. L, qPCR analysis of gene expression in cells from Day 6 of I. M, Flow cytometry analysis of cells following PI staining at Day 6 of E (sub-G₁ cells were gated out). N, ChIP-qPCR assays for the binding of PRMT5 to the proximal or distal regions of AR promoter (N). O, ChIP-qPCR assays for the enrichment of the indicated histone markers at the proximal promoter region of AR. For MTT, western blotting, cell cycle, and qPCR analysis, statistical significance of group difference was determined for 'DMSO vs BLL3.3' or 'Dox (-) vs Dox (+)'. For ChIP-qPCR, values were normalized to the corresponding IgG control, and indicated statistical significance of group difference was determined for 'specific IP vs IgG IP'. For all experiments, results are mean $\pm$ SD from 3 independent experiments. Student *t*-test with Welch's correction was performed to determine statistical significance of group difference. *ns* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0013] FIG. 4. **PRMT5 functions as a master regulator of DDR.** In response to radiation, PRMT5 expression is upregulated and forms a complex with pICln to catalyze H4R3me2s to promote transcription of the indicated DDR genes involved in DSBs repair and G2 arrest.

5 [0014] FIG. 5. **Radiation increases PRMT5 expression in cells, xenograft tumors and prostate cancer tissues.** **A.** The indicated prostate cancer cell lines were subjected to the indicated accumulated dose of FIR (2 Gy/day, 5 days/week) and the expression of PRMT5 and the level of H4R3me2s were determined by Western blotting. **B.** Radiation-resistant sublines (IRR1, 2, 3) isolated from LNCaP
10 cells after 40 Gy of FIR maintained higher levels of PRMT5 and H4R3me2s. **C.** High PRMT5 expression in the nucleus in recurrent prostate cancer tissues after RT failure (RT-Recurrent) when compared to the primary prostate cancer tissues (RT-Primary).

[0015] FIG. 6. **Knockdown of PRMT5 sensitizes LNCaP xenograft tumors to FIR and reduces tumor recurrence.** LNCaP-shPRMT5 and LNCaP-SC cell lines
15 were injected into nude mice to establish xenograft tumors. Concurrent treatment with FIR of 40 Gy (5 Gy/each, 10 Gy/week) and PRMT5 knockdown (KD) induced by doxycycline in drinking water (1 mg/mL) were performed for 4 weeks. After the 4-week treatment, tumor growth was monitored for 7 months until all tumors in the
20 SC group died of tumor burden ($>1,500 \text{ mm}^3$). **A.** Schematic view of treatment strategy and tumor regrowth monitoring. **B.** Tumor growth over time. $P<0.0001$ (Two-way ANOVA with Sidak's Multiple Comparison Test). **C.** Percentage of tumor recurrence ($>400 \text{ mm}^3$). $P<0.0001$ (One tail Fisher's exact test). **D.** Tumor-free survival. $P<0.0001$ (Kaplan-Meier Estimate, Log-Rank test).

25 [0016] FIG. 7. **JNJ-64619178 is a potent PRMT5 inhibitor in prostate cancer cells.** **A.** CRPC cells 22Rv1 were treated 10 μM of JNJ-64619178 (JNJ) or dimethyl sulfoxide (DMSO) for the indicated days, and cell growth was measured via MTT. **B.** Total cell lysate was prepared from A at the end of 6 days for Western blotting of full-length AR (AR-FL), AR-V7, PRMT5 and β -actin. **C.** Quantified
30 expression level of AR-FL, AR-V7 and PRMT5 from B. **D.** Similar experiments were performed as described in A, and the mRNA expression of AR-FL, AR-V7 and PRMT5 was quantified by qRT-PCR. **E.** LNCaP cells were pre-treated with the indicated concentrations of JNJ or DMSO for 4 days and then subjected to 2 Gy of irradiation (IR) (IR+) or no IR (IR-). DSBs repair (γH2AX foci) at 6 hours post-IR
35 was assayed by immunofluorescence. Shown are quantified average number of foci per cell. At least 60 cells per treatment condition were counted. **F.** Similar experiments were performed, and the expression of the indicated PRMT5 target

genes was quantified by qRT-PCR. Involucrin (IVL) is a target gene repressed by PRMT5 and used as a control. All bars are the mean $\pm$ SD of 3 independent experiments. Statistical analysis was performed using Brown-Forsythe and Welch ANOVA followed by Dunnett's T3 multiple comparisons test in E. Student's *t*-test was used to determine the statistical significance between DMSO and JNJ in A, B, C, D, and F (* $P \leq 0.05$; ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$).

[0017] FIG. 8. Strategy of enhanced PSMA-based TRT for mCRPC treatment.

PSMA-based TRT molecule ^{177}Lu -PSMA-617 will be conjugated with a novel radiosensitizer JNJ-64619178 (JNJ). After specific binding to PSMA via PSMA-617 (617), the conjugate will be internalized into PSMA-expressing mCRPC cells via endocytosis. JNJ will be released in the cytoplasm and bind specifically to PRMT5 to inhibit the activity of PRMT5, which will lead to down-regulation of AR and DDR gene expression and the impairment of DSBs repair. Thus, inclusion of a radiosensitizer is expected to enhance the potency of ^{177}Lu by impairing DSBs repair.

[0018] FIG. 9 is a plot of absolute tumor volume measurement for the combination therapy with ^{177}Lu PSMA-617 **20** and compound **22**.

[0019] FIG. 10 is a plot of body weight measurement for the combination therapy with ^{177}Lu PSMA-617 **20** and compound **22**.

[0020] FIG. 11 is a survival curve for the combination therapy with ^{177}Lu PSMA-617 **20** and compound **22**.

[0021] FIG. 12 is images showing the effect of compounds **20**, **21**, and **23** on radiation-induced DNA double strand break (DSB) repair in the LNCaP cell line using γH2AX staining in 1 nM compounds for 1 hour. DAPI is the nucleus stain.

[0022] FIG. 13 is a plot showing the effect of compounds **20**, **21**, and **23** on radiation-induced DNA double strand break (DSB) repair in the LNCaP cell line using γH2AX staining in 1 nM compounds for 1 hour. Treated LNCaP cell line with compounds for 1 hour by IR 2Gy. Count foci in each cell. The plot was generated with data from three independent experiments results. γH2AX is a DNA break marker.

[0023] FIG. 14 is a plot showing the effect of compounds **20**, **21**, and **23** on radiation-induced DNA double strand break (DSB) repair in the DU145 cell line. Treated DU145 cell line with compounds for 1 hour by IR 2Gy. Count foci in each cell. The plot was generated with data from three independent experiments results. γH2AX is a DNA break marker.

[0024] FIG. 15 is a plot showing the effect of compounds **20**, **21**, and **23** on radiation-induced DNA double strand break (DSB) repair in the in PC-3 cell line.

Treated PC-3 cell line with compounds for 1 hour by IR 2Gy. Count foci in each cell. The plot was generated with data from three independent experiments results. γ H2AX is a DNA break marker.

[0025] FIG. 16 is a plot generated with data from the tumor regression analysis study described herein. The arrows signify the day of beam radiation.

[0026] FIG. 17 is a plot of body weight measurement.

DESCRIPTION

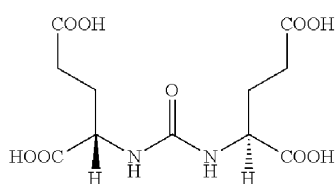
[0027] Reference will now be made in detail to certain embodiments of the disclosed subject matter. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not intended to limit the claims to the disclosed subject matter.

[0028] Provided are conjugates or compounds (where "conjugate" and "compound" are used interchangeably) of formula T-L-A and T-L-X, wherein each L can be the same or different. T is a ligand that targets prostate-specific membrane antigen (PSMA), L is a linker, X is or comprises a radiotherapeutic or (radio)imaging agent, and A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor.

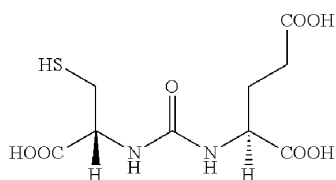
[0029] The conjugates T-L-A and T-L-X will typically be administered at different times and with different frequencies, both of which can be adjusted by a physician. For example, the two conjugates typically can be administered at different times because they may compete with the same target (e.g., PSMA) thereby affecting the uptake of each conjugate at the target site. The two conjugates also typically can be administered at different frequencies. For example, T-L-X can be administered less frequently since it comprises a radiotherapeutic (e.g., once in 6 weeks as a cycle), whereas T-L-A can be administered repeatedly during therapy to maintain the inhibition of DNA repair enzymes that repair DNA damage caused by irradiation of T-L-X.

[0030] Whether the compound of the formula T-L-A comprises a PRMT5 inhibitor or an ATM inhibitor, the compounds will prevent repair of DNA breaks induced by the irradiation of a compound of the formula T-L-X comprising a chelated radioisotope/radiotherapeutic. The compound of the formula T-L-A is therefore expected to improve the efficacy of the compound of the formula T-L-X by preventing the repair of double-stranded DNA breaks when A is a PRMT5 or an ATM inhibitor.

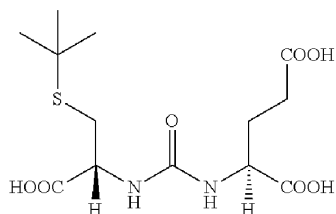
[0031] T can comprise moieties of the formulae:



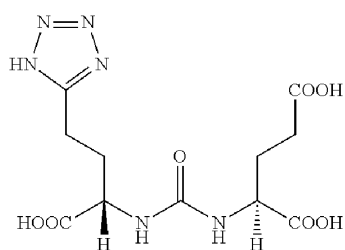
DOPA
 $IC_{50} = 47 \text{ nM}$
 $K_i = 8 \text{ nM}$



MUPA
 $IC_{50} = 6.9 \text{ nM}$



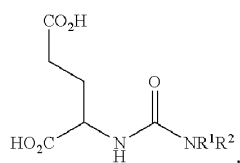
$IC_{50} = 29 \text{ nM}$



$K_i = 0.9 \text{ nM}$

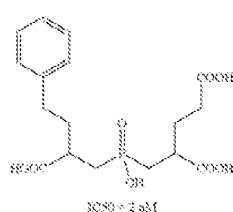
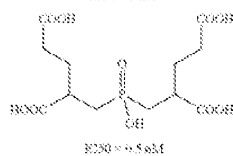
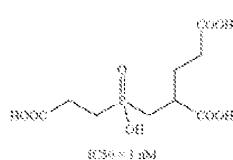
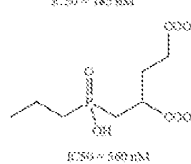
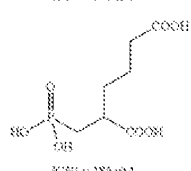
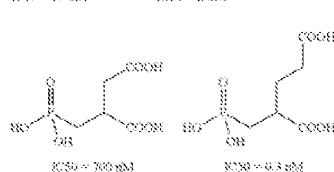
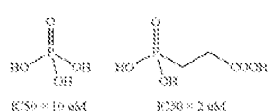
or

[0032] T can comprise:

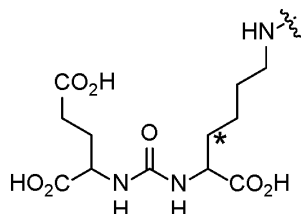


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in which R^1 and R^2 are independently selected from hydrogen or a carboxylic acid radical, which is optionally substituted, such as malonic acid, succinic acid, glutamic acid, 5-aminohexanoic acid, and adipic acid radicals, wherein the carboxylic acid radical is attached to the nitrogen bearing R^1 and R^2 via the carbon atom alpha to the/a carboxylic acid.



[0033] T can be a urea of (a) an amino dicarboxylic acid or a derivative thereof and (b) an amino dicarboxylic acid or a derivative thereof, wherein (a) and (b) can be the same or different. An example of such T groups includes:



5 wherein the asterisk indicates the carbon atom alpha to the carboxylic acid of a 5-aminohexanoic acid radical.

[0034] L can be any suitable linker. In one example, L can be a "non-releasable linker" or "non-cleavable linker." "Non-releasable linker" or "non-cleavable linker" refers to a linker that cannot be cleaved under extracellular physiological conditions (e.g., a pH-labile, an acid-labile, an oxidatively labile, or an enzyme-labile bond). However, such a linker may include bonds that can be cleaved after entry into a cell.

[0035] In another example, L can be a "releasable linker." A "releasable linker" refers to a linker that includes at least one bond that can be broken under physiological conditions (e.g., a pH-labile, acid-labile, oxidatively labile, or enzyme-labile bond). Releasable groups also include photochemically cleavable groups. Examples of photochemically cleavable groups include 2-(2-nitrophenyl)-ethan-2-ol groups, linkers containing o-nitrobenzyl, desyl, trans-o-cinnamoyl, m-nitrophenyl or benzylsulfonyl groups (see, for example, Dorman and Prestwich, Trends Biotech. 18:64-77 (2000); Greene and Wuts, Protective Groups in Organic Synthesis, 2nd ed., John Wiley & Sons, New York (1991); and U.S. Pat. Nos. 5,143,854; 5,986,076; 5,917,016; 5,489,678; and 5,405,783, all of which are hereby specifically incorporated by reference for their teachings regarding same).

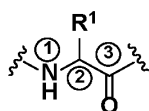
[0036] L can comprise a chain of atoms from about 3 atoms to about 30 atoms (for example about 3 atoms to about 7 atoms, about 5 atoms to about 15 atoms, about 5 atoms to about 25 atoms, about 5 atoms to about 12 atoms, about 7 atoms to about 15 atoms, about 7 atoms to about 12 atoms, about 7 atoms to about 15 atoms or about 10 atoms to about 30 atoms) in length. L can comprise a chain of atoms from about 5 Å to about 45 Å in length. L can comprise a peptide. L can comprise one or more phenylalanine residues, each of which is independently optionally substituted. L can comprise at least one phenylalanyl-phenylalanyl, in which at least one phenyl is independently optionally substituted. L can comprise

a polyoligoethylene glycol_n (POEG_n), a polyethylene glycol_n (PEG_n), or a mixture thereof, wherein n = 1-36.

[0037] In some embodiments, L can comprise at least one linker group, each linker group selected from the group consisting of polyethylene glycol (PEG), alkyl, sugar, and peptide. In some embodiments, the linker is a PEG- (e.g., pegylated-), alkyl-, sugar-, and peptide-based dual linker.

[0038] The linker can be any suitable linker. For example, in some embodiments, the linker is a hydrophilic linker, such as a linker that comprises one or more of an amino acid (which are the same or different), an alkyl chain, a PEG monomer, a PEG oligomer, a PEG polymer, or a combination of any of the foregoing. In some embodiments, the linker comprises an oligomer of peptidoglycans, glycans, or anions. For a linker that comprises one or more PEG units, all carbon and oxygen atoms of the PEG units are part of the backbone unless otherwise specified. The "backbone" of the linker L can be the shortest chain of contiguous atoms forming a covalently bonded connection between T and X and/or T and A. In some embodiments, a polyvalent linker has a branched backbone, with each branch serving as a section of backbone linker until reaching a terminus.

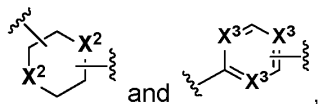
[0039] The L groups described herein can have any suitable length and chemical composition. For example, L can have a chain length of at least about 7 atoms in length. In one variation, L is at least about 10 atoms in length. In one variation, L is at least about 14 atoms in length. In another variation, L is between about 7 and about 31, between about 7 and about 24, or between about 7 and about 20 atoms in length. In another variation, L is between about 14 and about 31, between about 14 and about 24, or between about 14 and about 20 atoms in length. In another variation, L can have a chain length of at least 7 atoms, at least 14 atoms, at least 20 atoms, at least 25 atoms, at least 30 atoms, at least 40 atoms, from 1 to 15 atoms, 1 to 5 atoms, 5 to 10 atoms, 5 to 20 atoms, 10 to 40 atoms or 25 to 100 atoms. An example of an L group having a chain length of 1 to 5 atoms is a group of the formula:



wherein R¹ can be H, alkyl, arylalkyl, -alkyl-S-alkyl or arylalkyl or the side chain of any naturally- or non-naturally-occurring amino acid, and the like; and the numbers represent the atoms that are counted as being part of the chain, which in this example is 3 atoms. Examples of R¹ include H (i.e., glycine), alkyl (e.g., alanine, valine, isoleucine, and leucine), -alkyl-S-alkyl (e.g., methionine), arylalkyl (e.g.,

phenylalanine, tyrosine, tryptophan, and naphthylalanine), and the like. The atom to which R¹ is attached can be chiral and can have any suitable relative configuration, such as a D- or L-configuration.

[0040] The atoms used in forming L can be combined in all chemically relevant ways, such as chains of carbon atoms forming alkylene groups, chains of carbon and oxygen atoms forming polyoxyalkylene groups, chains of carbon and nitrogen atoms forming polyamines, and others. In addition, it is to be understood that the bonds connecting atoms in the chain can be either saturated or unsaturated, such that, for example, alkanes, alkenes, alkynes, cycloalkanes, arylenes, imides, and the like can be divalent radicals that are included in L. In addition, it is to be understood that the atoms forming the linker may also be cyclized upon each other to form saturated or unsaturated divalent cyclic radicals in the linker, such as radicals of the formulae:



wherein each X² is independently CH₂, N (when there is a bond attached to X²), NH or O and each X³ is independently N, C (when there is a bond attached to X³) or CH. In each of the foregoing and other L groups described herein the chain forming the linker can be substituted or unsubstituted.

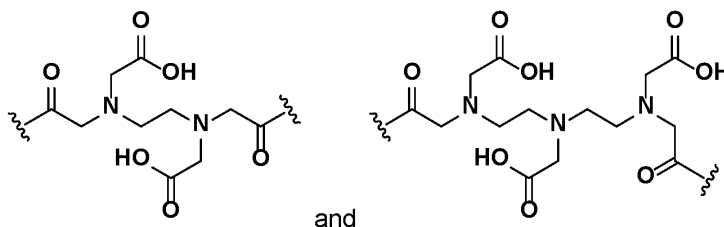
[0041] Alternatively, or in addition to chain length, L can have any suitable substituents that can affect the hydrophobicity or hydrophilicity of L. Thus, for example, L can have hydrophobic side chain group, such as an alkyl, cycloalkyl, aryl, arylalkyl, or like group, each of which is optionally substituted. If L were to include one or more amino acids, L can contain hydrophobic amino acid side chains, such as one or more amino acid side chains from phenylalanine (Phe) and tyrosine (Tyr), including substituted variants thereof, and analogs and derivatives of such side chains.

[0042] L can comprise portions that are neutral under physiological conditions. But L can comprise portions that can be protonated or deprotonated to carry one or more positive or one or more negative charges, respectively. Or L can comprise neutral portions and portions that can be protonated to carry one or more positive charges. Examples of neutral portions include poly hydroxyl groups, such as sugars, carbohydrates, saccharides, inositols, and the like, and/or polyether groups, such as polyoxyalkylene groups including polyoxyethylene, polyoxypropylene, and the like. Examples of portions that can be protonated to carry one or more positive charges include amino groups, such as

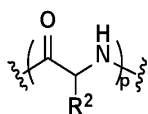
polyaminoalkylenes including ethylene diamines, propylene diamines, butylene diamines and the like, and/or heterocycles including pyrrolidines, piperidines, piperazines, and other amino groups, each of which can be optionally substituted. Examples of portions that can be deprotonated to carry one or more negative

charges include carboxylic acids, such as aspartic acid, glutamic acid, and longer chain carboxylic acid groups, and sulfuric acid esters, such as alkyl esters of sulfuric acid.

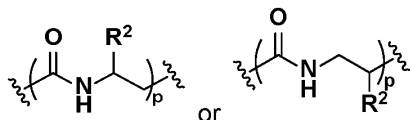
[0043] Illustrative polyoxyalkylene groups include those of a specific length range from about 4 to about 20 polyoxyalkylene (e.g., polyethylene glycol) groups. Illustrative alkyl sulfuric acid esters may also be introduced with click chemistry directly into the backbone. Illustrative L groups comprising polyamines include L groups comprising EDTA and DTPA radicals:



(poly)peptides:



β -amino acids, and the like:



and combinations thereof, wherein each R^2 is independently H, alkyl, arylalkyl, heterocyclylalkyl, ureido, aminoalkyl, alkylthio or amidoalkyl, such as in the side chains of naturally-occurring amino acids like alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine threonine, asparagine, methionine, lysine, arginine, and histidine. Non-naturally occurring amino acids are also contemplated herein.

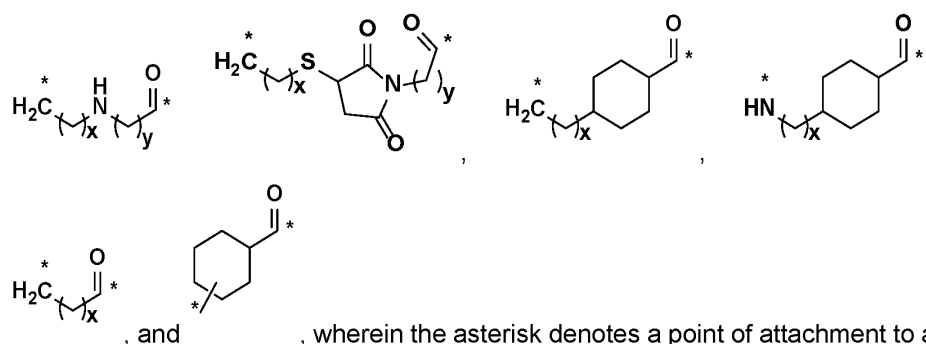
[0044] The L groups can have any suitable molecular weight, such as from about 30 g/mol to about 1,000 g/mol, from about 30 g/mol to about 300 g/mol, about 100 g/mol to about 500 g/mol or about 150 g/mol to about 600 g/mol.

[0045] The terms "non-releasable linker" or "non-cleavable linker" are used interchangeably. As used herein, they refer to a linker that cannot be cleaved under

extracellular physiological conditions (e.g., a pH-labile, acid-labile, oxidatively labile, or enzyme-labile bond). However, such a linker may include bonds that can be cleaved after entry into a cell.

[0046] L can comprise carbonyl, thionocarbonyl, alkylene, cycloalkylene, aminoalkylene, alkylenecycloalkyl, alkylenecycloalkylenecarbonyl, aminoalkylenecycloalkylenecarbonyl, alkylenecarbonyl, cycloalkylenecarbonyl, carbonylalkylcarbonyl, 1-alkylenesuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl, alkylenesulfoxyl, sulfonylalkyl, alkylenesulfoxylalkyl, alkylenesulfonylalkyl, carbonyltetrahydro-2H-pyranyl, carbonyltetrahydrofuryl, 1-(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl or 1-(carbonyltetrahydrofuryl)succinimid-3-yl, each of which is optionally substituted, and combinations thereof. In this example, L can further comprise an additional nitrogen (e.g., -NR³-, wherein R³ can be H or alkyl) such that L comprises alkylenecarbonyl, cycloalkylenecarbonyl, carbonylalkylcarbonyl or 1-(carbonylalkyl)succinimid-3-yl groups, each of which can be optionally substituted, bonded to the nitrogen to form an amide. Alternatively, L can further comprise a sulfur atom and alkylene or cycloalkylene groups, each of which can be optionally substituted with carboxy, and can be bonded to the sulfur to form a thiol. In yet another example, L comprises a sulfur atom and 1-alkylenesuccinimid-3-yl and 1-(carbonylalkyl)succinimid-3-yl groups bonded to the sulfur to form a succinimid-3-ylthiol.

[0047] L can include alkyleneaminoalkylenecarbonyl, alkylene-thio-(carbonylalkylsuccinimid-3-yl), alkylenecycloalkylenecarbonyl, aminoalkylenecycloalkylenecarbonyl, alkylenecarbonyl, cycloalkylenecarbonyl and the like and combinations thereof, as further illustrated by the following formulae:



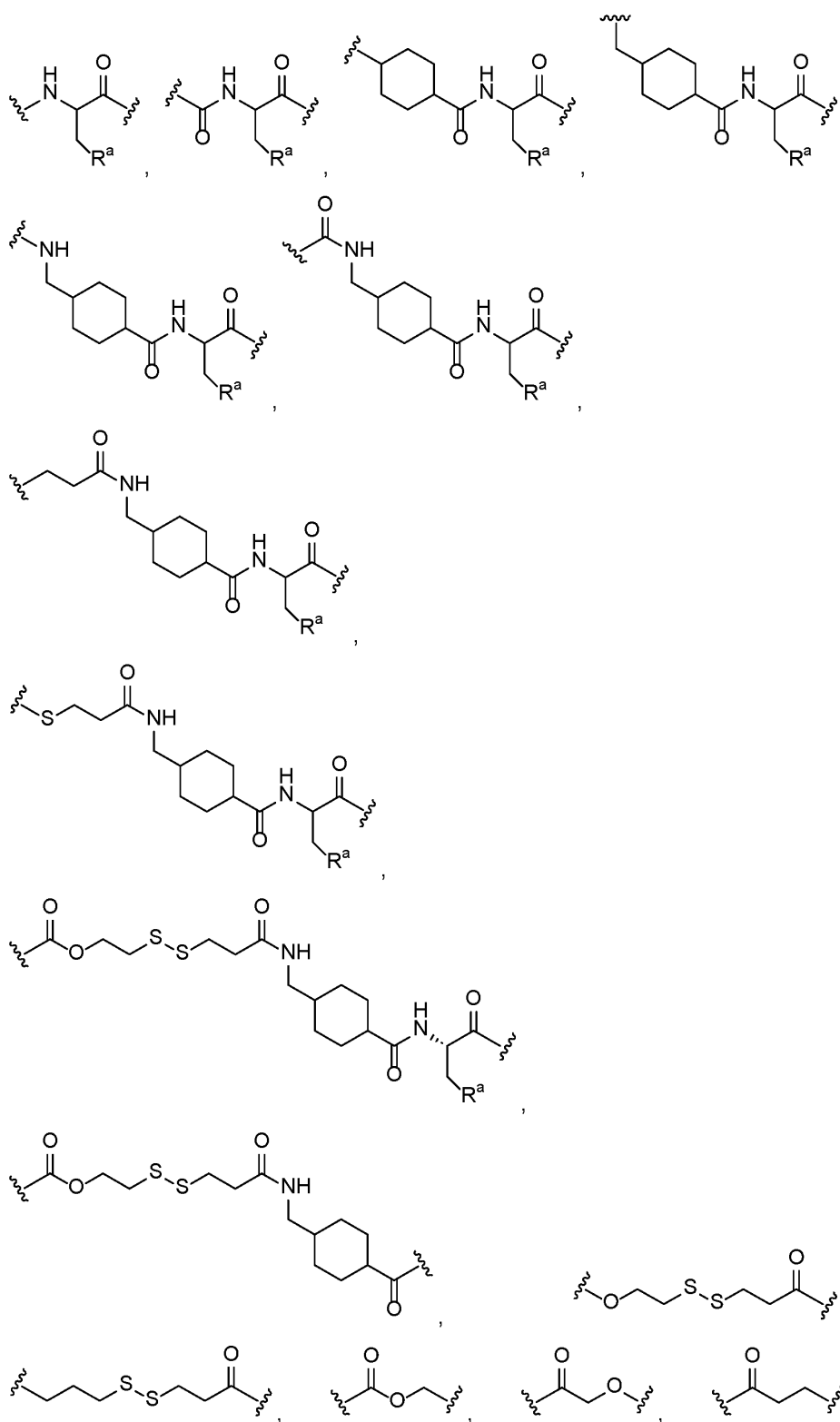
, and , wherein the asterisk denotes a point of attachment to a group present in L, in T, in A, or in X; and wherein x and y are each independently 1, 2, 3, 4, or 5.

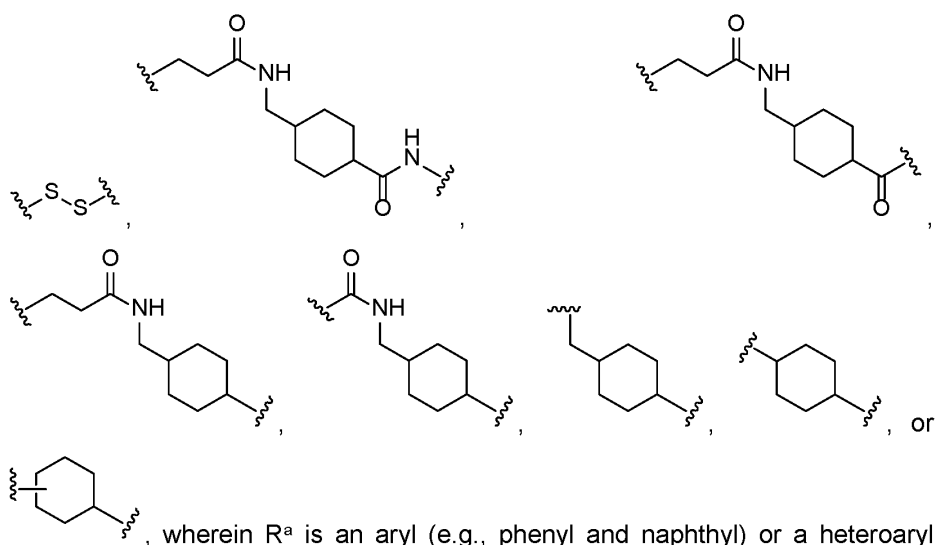
[0048] L can have any suitable assortment of atoms in the chain, including C (e.g., -CH₂-, C(O)), N (e.g., NH, NR⁴, wherein R⁴ is, e.g., H, alkyl, alkylaryl, and the like),

O (e.g., -O-), P (e.g., -O-P(O)(OH)O-), and S (e.g., -S-). For example, the atoms used in forming L can be combined in all chemically relevant ways, such as chains of carbon atoms forming alkyl groups, chains of carbon and oxygen atoms forming polyoxyalkyl groups, chains of carbon and nitrogen atoms forming polyamines, and others, including rings, such as those that form aryl and heterocyclyl groups (e.g., triazoles, oxazoles, and the like). In addition, the bonds connecting atoms in the chain in L can be either saturated or unsaturated, such that for example, alkanes, alkenes, alkynes, cycloalkanes, arylenes, imides, and the like can be divalent radicals that are included in L. Further, the chain forming L can be substituted, e.g., with an -N(R⁴)₂ group, or unsubstituted.

[0049] Additional examples of L include L groups that include the groups 1-alkylsuccinimid-3-yl, carbonyl, thionocarbonyl, alkyl, cycloalkyl, alkylcycloalkyl, alkylcarbonyl, cycloalkylcarbonyl, carbonylalkylcarbonyl, 1-alkylsuccinimid-3-yl, 1-(carbonylalkyl)succinimid-3-yl, alkylsulfoxyl, sulfonylalkyl, alkylsulfoxylalkyl, alkylsulfonylalkyl, carbonyltetrahydro-2H-pyranyl, carbonyltetrahydrofuryl, 1-(carbonyltetrahydro-2H-pyranyl)succinimid-3-yl, and 1-(carbonyltetrahydrofuryl)succinimid-3-yl, wherein each group can be substituted or unsubstituted. Any of the aforementioned groups can be L or can be included as a portion of L. In some instances, any of the aforementioned groups can be used in combination (or more than once) (e.g., -alkyl-C(O)-alkyl) and can further comprise an additional nitrogen (e.g., alkyl-C(O)-NH-, -NH-alkyl-C(O)- or -NH-alkyl-), oxygen (e.g., -alkyl-O-alkyl-) or sulfur (e.g., -alkyl-S-alkyl-). Examples of such L groups are alkylcarbonyl, cycloalkylcarbonyl, carbonylalkylcarbonyl, 1-(carbonylalkyl)succinimid-3-yl, and succinimid-3-ylthiol, wherein each group can be substituted or unsubstituted.

[0050] L can comprise one or more of the following groups, in any suitable combination:





5 **[0051]** Conjugates of the formula T-L-A may comprise releasable linkers for L if, e.g., release of A *in vivo* is desired. Releasable linkers for L are well-known in the art.

10 **[0052]** L can be a "releasable linker" that is cleavable by an enzyme. The enzyme can be cathepsin, metalloproteinase, esterase, phosphatase, or pyrophosphatase. L can be cleavable by a reactive oxygen species (ROS). L can be p-aminophenol ether. L can be cleavable by hypoxic activation. L can be a quinone, a nitroaromatic, an aliphatic N-oxide, or a hetero-aromatic N-oxide.

15 **[0053]** The cleavable bond or bonds can be present in the interior of a cleavable linker and/or at one or both ends of a cleavable linker. It should be appreciated that such physiological conditions resulting in bond breaking include standard chemical hydrolysis reactions that occur, for example, at physiological pH, or as a result of compartmentalization into a cellular organelle such as an endosome having a lower pH than cytosolic pH. Illustratively, the bivalent linkers can undergo cleavage under other physiological or metabolic conditions, such as by the action

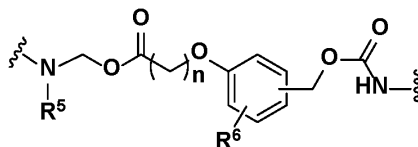
20 of a glutathione mediated mechanism. It is appreciated that the lability of the cleavable bond can be adjusted by including functional groups or fragments within the bivalent linker L that are able to assist or facilitate such bond breakage, also termed anchimeric assistance. The lability of the cleavable bond can also be adjusted by, for example, substitutional changes at or near the cleavable bond,

25 such as including alpha branching adjacent to a cleavable disulfide bond, increasing the hydrophobicity of substituents on silicon in a moiety having a silicon-oxygen bond that can be hydrolyzed, homologating alkoxy groups that form part

of a ketal or acetal that can be hydrolyzed, and the like. In addition, it is appreciated that additional functional groups or fragments can be included within the bivalent linker L that are able to assist or facilitate additional fragmentation of the PSMA binding drug linker conjugates after bond breaking of the releasable linker, when present.

[0054] In one example, L can comprise one or more releasable linkers that cleave under the conditions described herein by a chemical mechanism involving beta elimination. Such releasable linkers include beta-thio, beta-hydroxy, and beta-amino substituted carboxylic acids and derivatives thereof, such as esters, amides, carbonates, carbamates, and ureas. Such linkers also include 2- and 4-thioarylesters, carbamates, and carbonates.

[0055] An example of a releasable linker includes a linker of the formula:

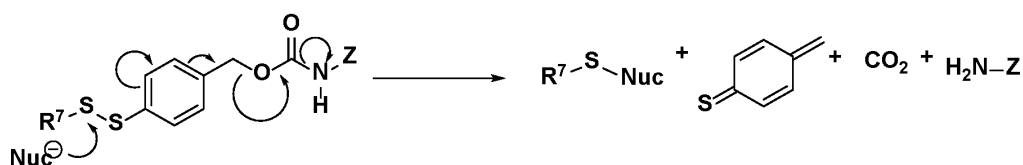
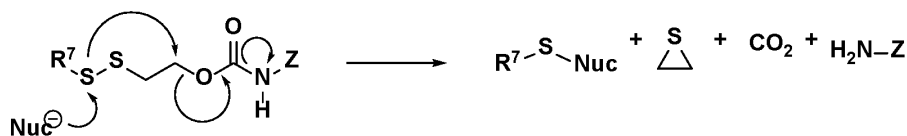
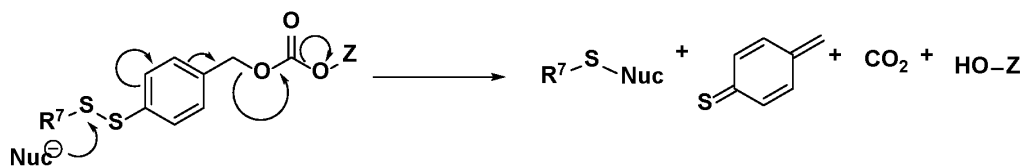
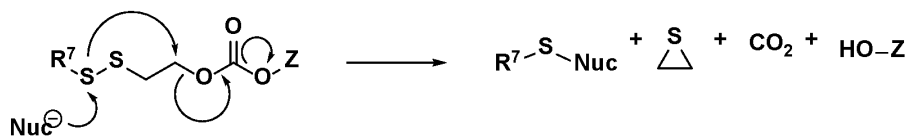


wherein n is an integer selected from 0, 1, 2, and 3, R⁵ is H or alkyl, R⁶ is hydrogen, or a substituent, including a substituent that can stabilize a positive charge inductively or by resonance on the aryl ring, such as alkoxy, and the like. The releasable linker can be further substituted.

[0056] Assisted cleavage of releasable portions of L can include mechanisms involving benzylium intermediates, benzyne intermediates, lactone cyclization, oxonium intermediates, beta-elimination, and the like. In addition to fragmentation subsequent to cleavage of a releasable portion of L, the initial cleavage of the releasable linker can be facilitated by an anchimerically assisted mechanism. Thus, in the example of a releasable portion of L given above, the hydroxyalkanoic acid, which may cyclize, facilitates cleavage of the methylene bridge, by for example an oxonium ion, and facilitates bond cleavage or subsequent fragmentation after bond cleavage of the releasable linker. Alternatively, acid catalyzed oxonium ion-assisted cleavage of the methylene bridge can begin a cascade of fragmentation of this illustrative bivalent linker, or fragment thereof. Alternatively, acid-catalyzed hydrolysis of the carbamate may facilitate the beta elimination of the hydroxyalkanoic acid, which may cyclize, and facilitate cleavage of the methylene bridge by, for example, an oxonium ion. It is appreciated that other chemical mechanisms of bond breakage or cleavage under the metabolic, physiological, or cellular conditions may initiate such a cascade of fragmentation. It is appreciated that other chemical mechanisms of bond breakage or cleavage

under the metabolic, physiological, or cellular conditions can initiate such a cascade of fragmentation.

[0057] Illustrative mechanisms for cleavage of the bivalent linkers include the following 1,4 and 1,6 fragmentation mechanisms for carbonates and carbamates:



10 wherein Nuc⁻ is an exogenous or endogenous nucleophile, glutathione, or bio-reducing agent, and the like, and one of R⁷ and Z is T (or X) connected through other portions of the bivalent linker, and the other is X (or T) connected through other portions of the bivalent linker. The location of R⁷ and Z can be switched such that, e.g., the resulting products are Z-S-Nuc and HO-R⁷ or H₂N-R⁷.

15 **[0058]** Although the above fragmentation mechanisms are depicted as concerted mechanisms, any number of discrete steps can take place to effect the ultimate fragmentation of the bivalent linker to the final products shown. For example, the bond cleavage can also occur by acid-catalyzed elimination of the carbamate moiety, which can be anchimerically assisted by the stabilization provided by either

20 the aryl group of the beta sulfur or disulfide illustrated in the above examples. In those variations of this embodiment, the releasable linker is the carbamate moiety. Alternatively, the fragmentation can be initiated by a nucleophilic attack on the disulfide group, causing cleavage to form a thiolate. The thiolate can intermolecularly displace a carbonic acid or carbamic acid moiety and form the

25 corresponding thiocyclopropane. In the case of the benzyl-containing bivalent

linkers, following an illustrative breaking of the disulfide bond, the resulting phenyl thiolate can further fragment to release a carbonic acid or carbamic acid moiety by forming a resonance stabilized intermediate. In any of these cases, the releasable nature of the illustrative bivalent linkers can be realized by whatever mechanism can be relevant to the chemical, metabolic, physiological, or biological conditions present.

[0059] As described above, therefore, releasable linkers can comprise a disulfide group. Further examples of releasable linkers comprised in L can include divalent radicals comprising alkyleneaziridin-1-yl, alkylencarbonylaziridin-1-yl, carbonylalkylaziridin-1-yl, alkylensulfoxylaziridin-1-yl, sulfoxylalkylaziridin-1-yl, sulfonylalkylaziridin-1-yl, or alkylensulfonylaziridin-1-yl groups, wherein each of the releasable linkers is optionally substituted. Additional examples of releasable linkers comprise can include divalent radicals comprising methylene, 1-alkoxyalkylene, 1-alkoxycycloalkylene, 1-alkoxyalkylenecarbonyl, 1-alkoxycycloalkylenecarbonyl, carbonylarylcabonyl, carbonyl(carboxyaryl)carbonyl, carbonyl(biscarboxyaryl)carbonyl, haloalkylenecarbonyl, alkylene(dialkylsilyl), alkylene(alkylarylsilyl), alkylene(diarylsilyl), (dialkylsilyl)aryl, (alkylarylsilyl)aryl, (diarylsilyl)aryl, oxycarbonyloxy, oxycarbonyloxyalkyl, sulfonyloxy, oxysulfonylalkyl, iminoalkylidenyl, carbonylalkylideniminyl, iminocycloalkylidenyl, carbonylcycloalkylideniminyl, alkylenethio, alkylenearylthio or carbonylalkylthio groups, wherein each of the releasable linkers can be optionally substituted.

[0060] Additional examples of releasable linkers comprised in L can include an oxygen atom and methylene, 1-alkoxyalkylene, 1-alkoxycycloalkylene, 1-alkoxyalkylenecarbonyl or 1-alkoxycycloalkylenecarbonyl groups, wherein each of the releasable linkers can be optionally substituted. Alternatively, the releasable linker can include an oxygen atom and a methylene group, wherein the methylene group can be substituted with an optionally substituted aryl, and the releasable linker can be bonded to the oxygen to form an acetal or ketal. Further, the releasable linker can include an oxygen atom and a sulfonylalkyl group, and the releasable linker can be bonded to the oxygen to form an alkylsulfonate.

[0061] Additional examples of releasable linkers comprised in L can include a nitrogen (e.g., -NR⁵-, wherein R⁵ is H or alkyl) and iminoalkylidenyl, carbonylalkylideniminyl, iminocycloalkylidenyl, and carbonylcycloalkylideniminyl groups, wherein each of the releasable linkers can be optionally substituted, and the releasable linker can be bonded to the nitrogen to form an hydrazone. In an alternate configuration, the hydrazone can be acylated with a carboxylic acid

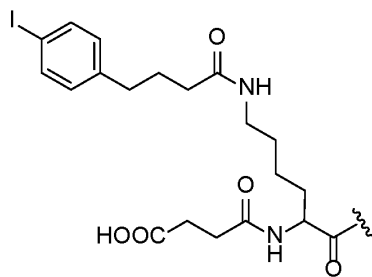
derivative, an orthoformate derivative, or a carbamoyl derivative to form various acylhydrazone releasable linkers.

[0062] Additional examples of releasable linkers comprised in L can include an oxygen atom and alkylene(dialkylsilyl), alkylene(alkylarylsilyl), alkylene(diarylsilyl), (dialkylsilyl)aryl, (alkylarylsilyl)aryl or (diarylsilyl)aryl groups, wherein each of the releasable linkers can be optionally substituted, and the releasable linker can be bonded to the oxygen to form a silanol.

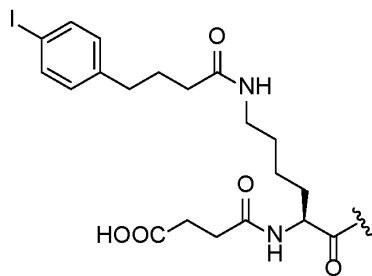
[0063] Additional examples of releasable linkers comprised in L can include two independent nitrogens (e.g., $-NR^5-$) and carbonylarylcarbonyl, carbonyl(carboxyaryl)carbonyl, carbonyl(biscarboxyaryl)carbonyl, and the releasable linker can be bonded to the heteroatom nitrogen to form an amide and also bonded to Z or R^7 via an amide bond.

[0064] Additional examples of releasable linkers comprised in L can include an oxygen atom, a nitrogen (e.g., $-NR^5-$), and a carbonylarylcarbonyl, carbonyl(carboxyaryl)carbonyl, carbonyl(biscarboxyaryl)carbonyl, and the releasable linker can form an amide, and also be bonded to Z or R^7 via an amide bond.

[0065] The linker L can further comprise a group that improves tumor uptake of, e.g., the conjugates of the formula T-L-A, though conjugates of the formula T-L-X can also comprise such groups attached to L. Examples of groups that improve tumor uptake of the conjugates of the formula T-L-A and/or T-L-X include albumin binding groups of the formula:



, such as a group of the formula:

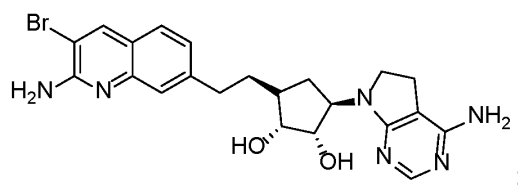


. Such groups can be linked to L via any suitable substituent present on L, including an $-NR^4-$ group, wherein R^4 is H or alkyl, or an

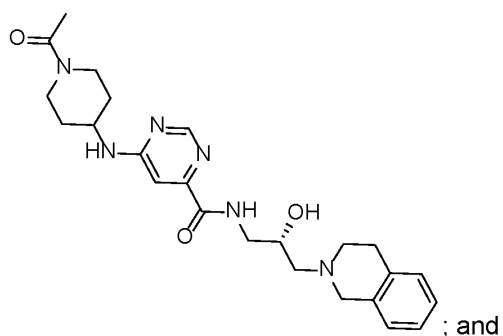
-O- group; or via a group on L comprising an -NR⁴- group, wherein R⁴ is H or alkyl, or an -O- group.

[0066] A can comprise a radiosensitizer. The radiosensitizer can be selected from the group consisting of a topoisomerase inhibitor, hypoxia-activated anthraquinone (AQ4N), an alkylating agent, and an inhibitor of a DNA repair enzyme. The topoisomerase inhibitor can be camptothecin or topotecan. The alkylating agent can be temozolomide. The inhibitor of a DNA repair enzyme can be an inhibitor of PRMT5, ATM or DNA-PK.

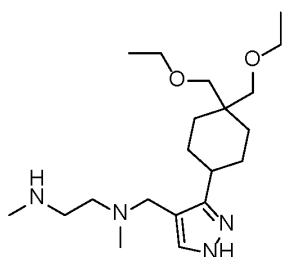
[0067] A can comprise a radiosensitizer, such as a protein arginine methyltransferase 5 (PRMT5) inhibitor, for inhibiting double stranded DNA repair. An example of a PRMT5 inhibitor that can serve as the basis for a radiosensitizer is JNJ-64619178, which has the formula:



GSK3326595, which has the formula:

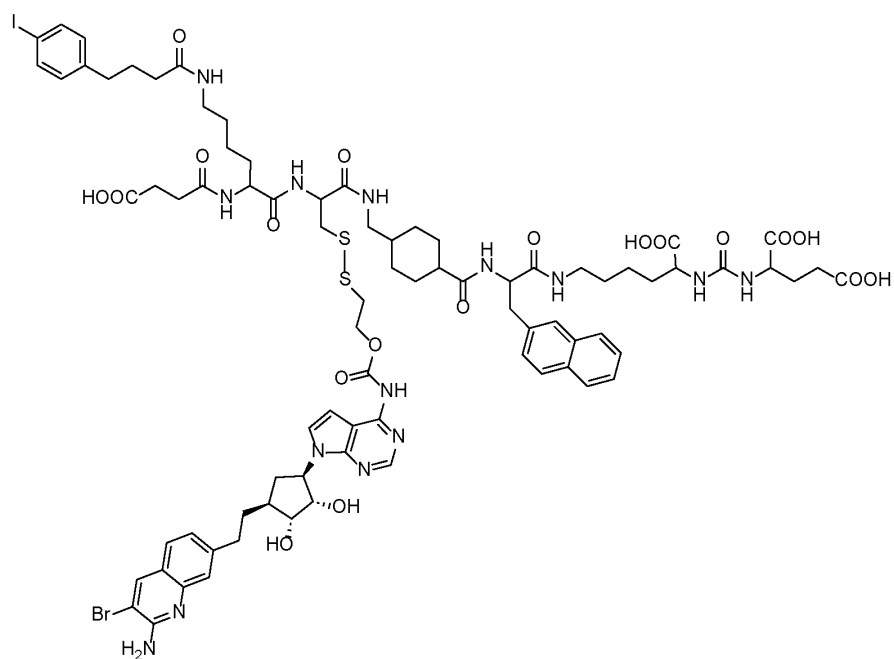


GSK336871, which has the formula:



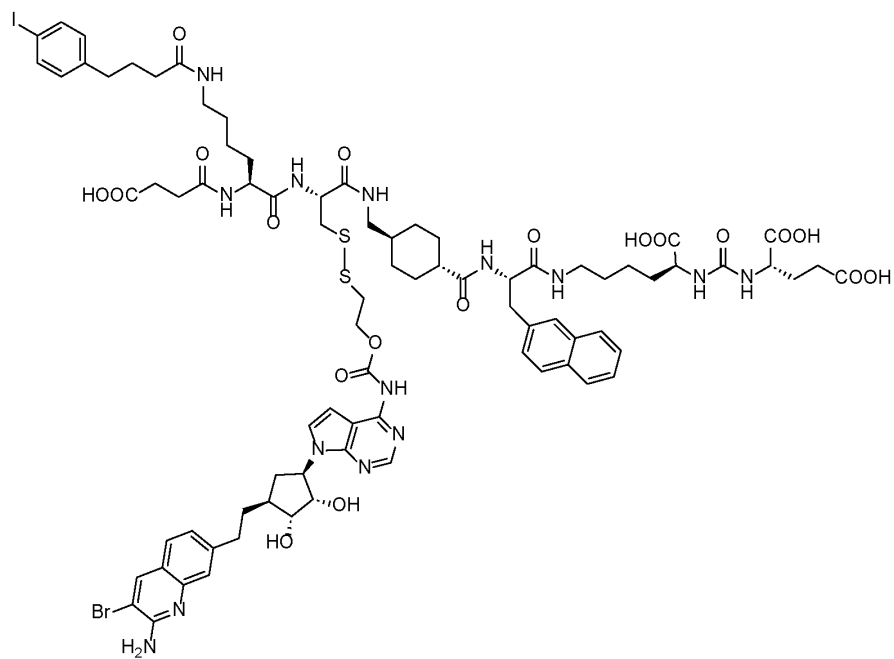
or pharmaceutically acceptable salts of each of the foregoing.

[0068] Thus, for example, a conjugate of the formula T-L-A includes conjugates of the formulae:



or

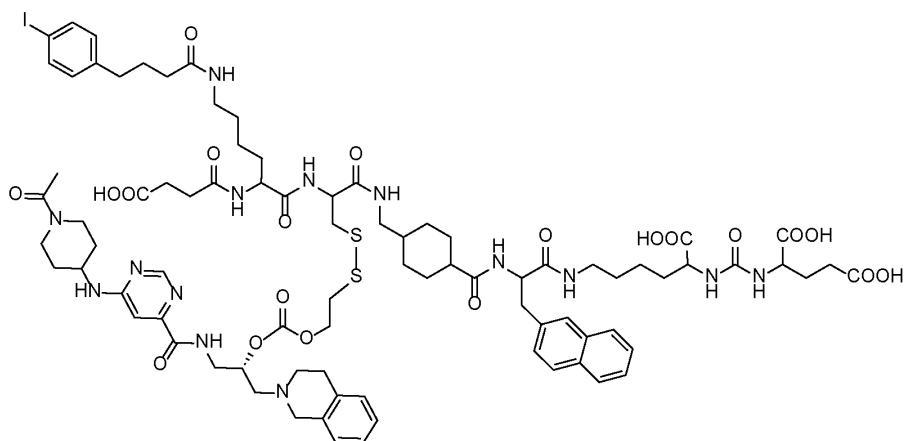
a pharmaceutically acceptable salt thereof, such as:



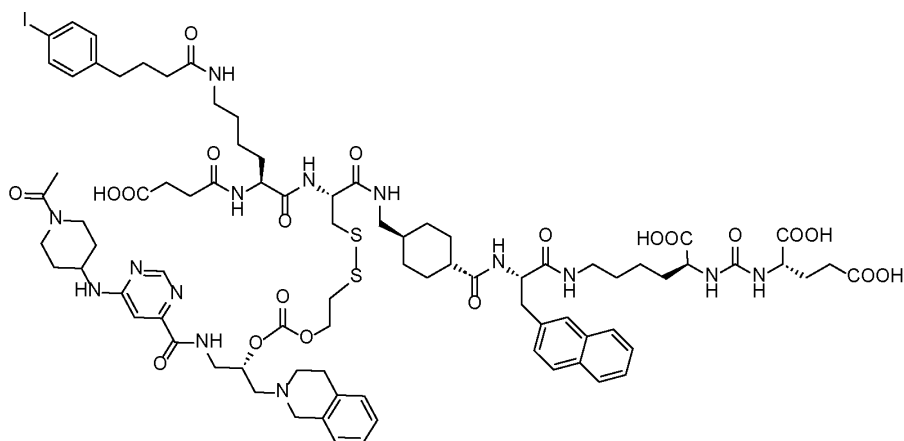
or

a pharmaceutically acceptable salt thereof;

and



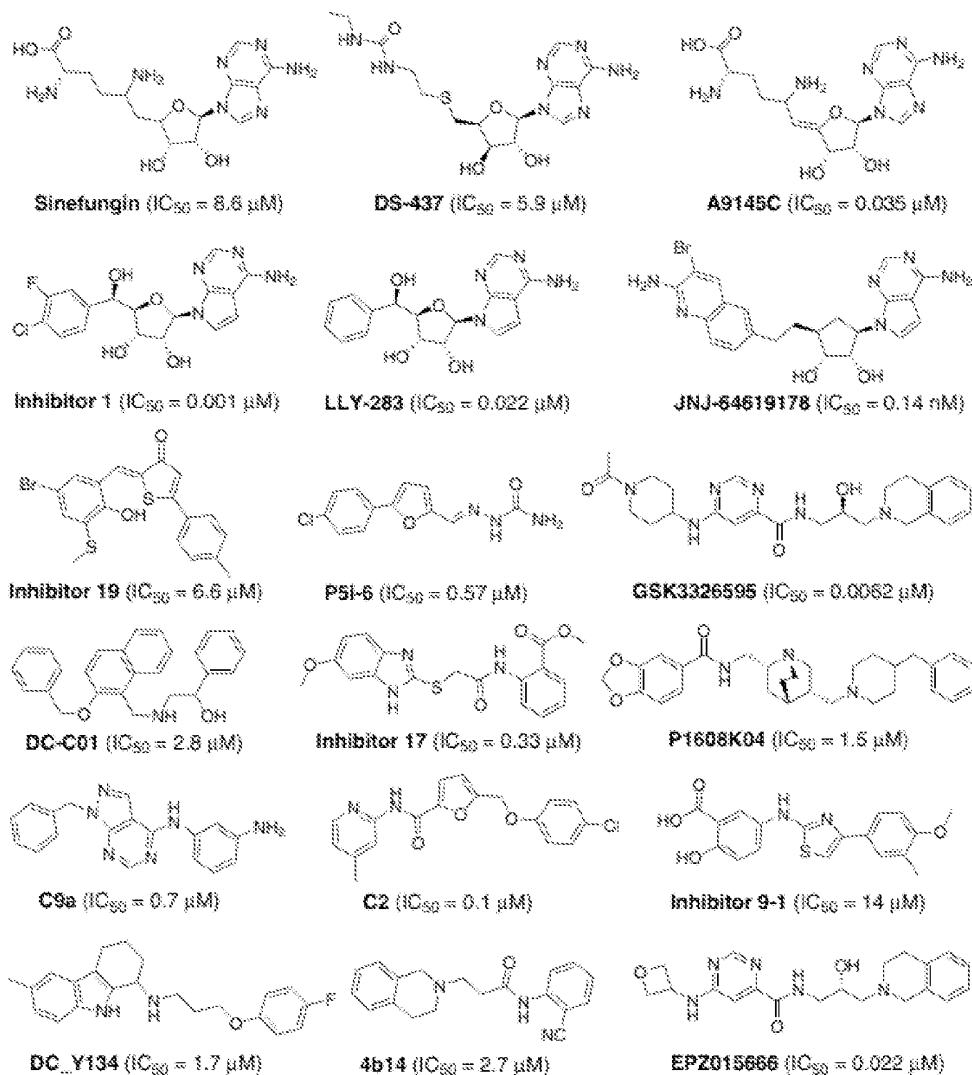
or a pharmaceutically acceptable salt thereof, such as:



or a pharmaceutically acceptable salt thereof.

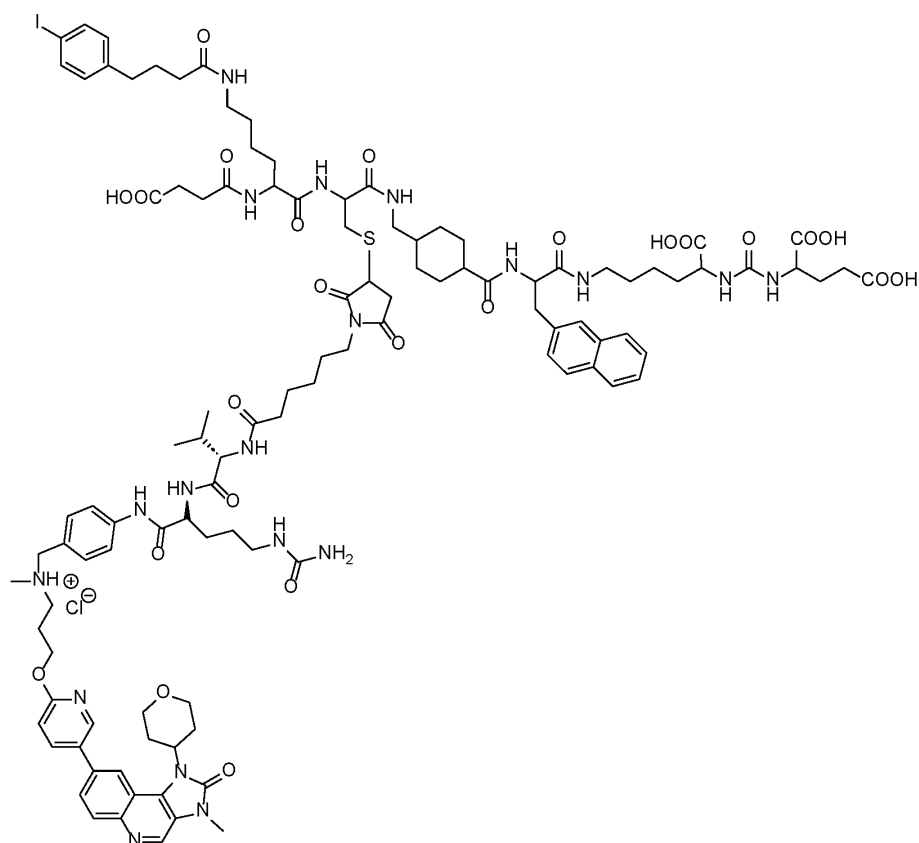
5 [0069] These conjugate are examples of conjugates targeting dually prostate-specific membrane antigen (PSMA) as a targeted radionuclide therapy (TRT) and PRMT5 as a PRMT5 inhibitor. The first conjugate comprises a fragment of JNJ-64619178, and the second comprises a fragment of GSK3326595.

10 [0070] Other PRMT5 inhibitors that can be comprised in A include those described in Zhu, *et al.*, *Journal of Computer-Aided Molecular Design* 33: 775–785 (2019), which is incorporated by reference as if fully set forth herein, and include JNJ-64619178 and GSK3326595:

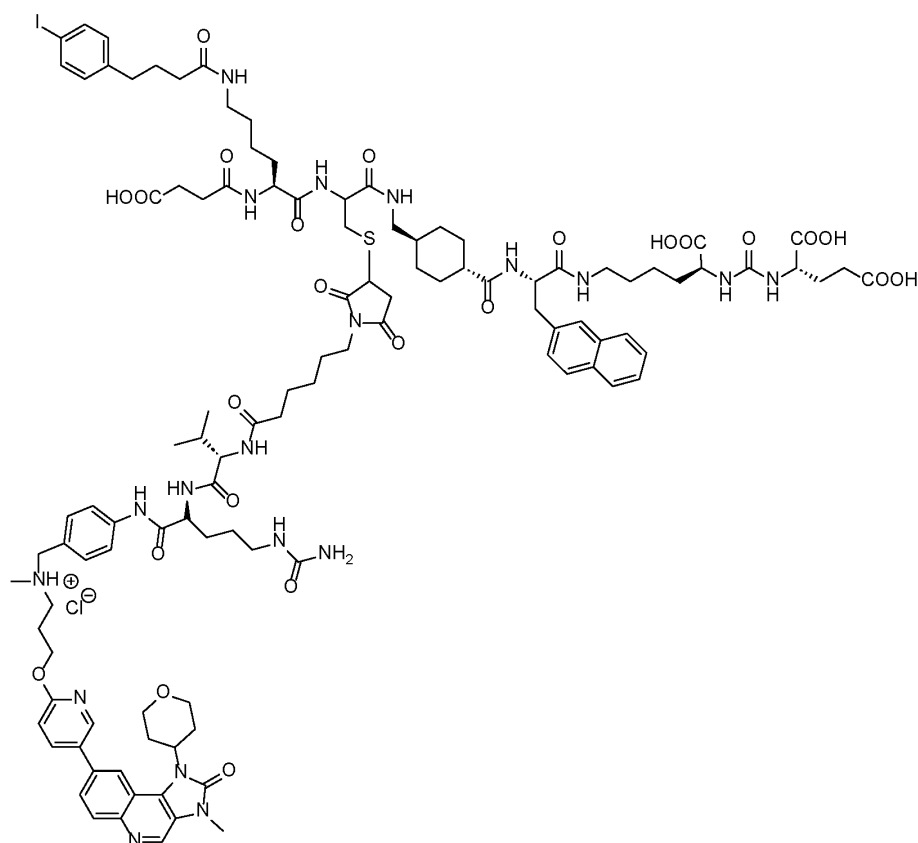


[0071] A can comprise a radiosensitizer, such as an ataxia-telangiectasia mutated (ATM) inhibitor, for inhibiting double stranded DNA repair. Examples of ATM inhibitors that can serve as the basis for a radiosensitizer include Cinobufagin, AZD0156, AZD1390, AZ32, KU-60019, KU-55933, Wortmannin, CP-466722, AZ31, Chloroquine diphosphate, Mirin, Torin 2, CGK 733, and ETP-46464. Additional ATM inhibitors include AZD-7648, VE-821, Camonsertib, Lartesertib, Ro 90-7501, ATM Inhibitor-1, ATM Inhibitor-2, ATM Inhibitor-3, ATM Inhibitor-4, AO11, and ATR-IN-15.

[0072] Thus, for example, a conjugate of the formula T-L-A includes conjugates of the formulae:



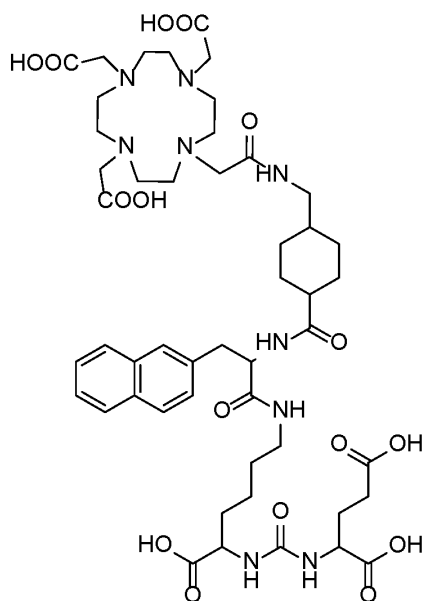
or a pharmaceutically acceptable salt thereof, such as:



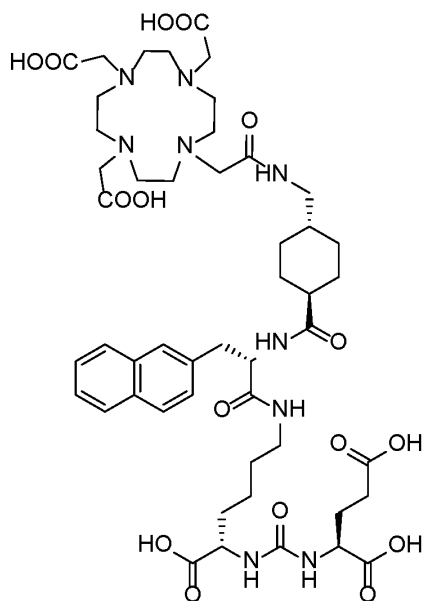
or a pharmaceutically acceptable salt thereof.

[0073] A can comprise a radiosensitizer, such as DNA protein kinase (DNA-PK) inhibitor. Examples of DNA-PK inhibitors that can serve as the basis for a radiosensitizer include Nedisertib, AZD7648, KU57748, Torin 2, PI-103, NU7026, Samotolisib, PIK 90, CC-115, PIK-75 hydrochloride, VX-984, KU-0060648, BAY-8400, DNA-PK-IN-2, DNA-PK-IN-1, DNA-PK-IN-4, DNA-PK-IN-5, DNA-PK-IN-6, XRD-0394, NU5455, STL127705, PI-103 hydrochloride, LTURM34, ZL-2201 free base, DNA-PK-IN-14, DNA-PK-IN-10, DNA-PK-IN-11, and DNA-PK-IN-13.

[0074] An example of a conjugate of the formula T-L-X includes a compound of the formula:



or a pharmaceutically acceptable salt thereof, such as:



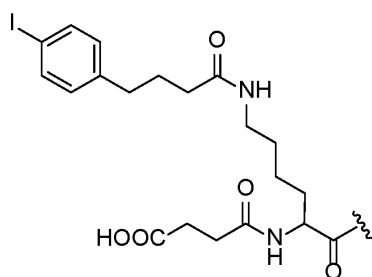
which is sometimes called "PSMA-617."

- 5 **[0075]** X can comprise a radioisotope bound to a chelator, such that X is or comprises a radiotherapeutic or (radio)imaging agent, selected from the group consisting of:

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or a derivative thereof;

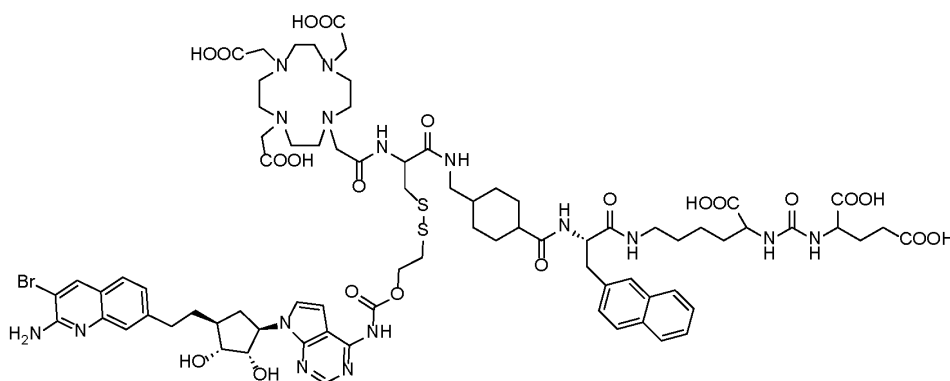
- 10 TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) or a derivative thereof;

- SarAr (1-N-(4-Aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine or a derivative thereof;
- NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) or a derivative thereof;
- NETA (4-[2-(bis-carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl) acetyc acid or a derivative thereof
- TRAP (1,4,7-triazacyclononane-1,4,7-tris[methyl(2-carboxyethyl)phosphinic acid] or a derivative thereof;
- HBED (N,N0-bis(2-hydroxybenzyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof;
- 2,3-HOPO (3-hydroxypyridin-2-one) or a derivative thereof;
- PCTA (3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9,-triacetic acid) or a derivative thereof;
- DFO (desferrioxamine) or a derivative thereof;
- DTPA (diethylenetriaminepentaacetic acid) or a derivative thereof;
- OCTAPA (N,N0-bis(6-carboxy-2-pyridylmethyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof; or H2-MACROPA (N,N'-bis[(6-carboxy-2-pyridimethyl]-4,13-diaza-18-crown-6) or a derivative thereof;
- H2dedpa (1,2-[[carboxy]-pyridin-2-yl]-methylamino]ethane or a derivative thereof; and
- EC20-head comprising β -l-diaminopropionic acid, aspartic acid, and cysteine.
- [0076]** X can comprise a radioisotope selected from the group consisting of ^{18}F , ^{44}Sc , ^{47}Sc , ^{52}Mn , ^{55}Co , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , $^{114\text{m}}\text{In}$, $^{117\text{m}}\text{Sn}$, ^{124}I , ^{125}I , ^{131}I , ^{149}Tb , ^{153}Sm , ^{152}Tb , ^{155}Tb , ^{161}Tb , ^{177}Lu , ^{186}Re , ^{188}Re , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{224}Ra , ^{225}Ac , ^{225}Ab , and ^{227}Th .
- [0077]** In view of the above, also provided is a conjugate of formula T-L-(X)A in which:
- T is a ligand that targets prostate-specific membrane antigen (PSMA),
- L is a linker,
- A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor; and
- X is a radiotherapeutic or radioimaging agent.
- [0078]** A and X can be connected to L.
- [0079]** The conjugate can further comprise an albumin binding group. The albumin binding group can have the formula:

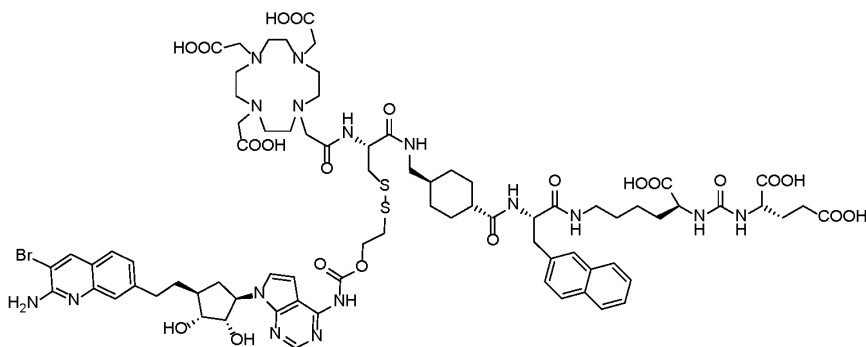


[0080] The albumin binding group can be linked to L via a substituent or group present on L, and wherein the albumin binding group is optionally linked to L as described above. T is as defined above. L is as defined above. A is as defined above.

[0081] An example of a compound of the formula T-L-(X)A is a compound of the formula:



, such as a compound of the formula:



[0082] The above compounds can be synthesized in accordance with methods known in the art.

[0083] Further provided is a pharmaceutical composition comprising an above conjugate (for example, a conjugate of the formula T-L-A, formula T-L-X, and combinations thereof) and a pharmaceutically acceptable carrier. Still further provided is a pharmaceutical composition comprising a conjugate of the formula

T-L-(X)A. Pharmaceutical compositions can be prepared in accordance with methods known in the art. Combinations of conjugates or combinations of conjugates and other active agents also can be combined with a pharmaceutically acceptable carrier.

5 **[0084]** Still further provided is a method of radio-imaging a patient for prostate cancer or metastasis thereof. The method comprises (i) administering to the patient a conjugate of the formula T-L-X or a pharmaceutical composition comprising same and (ii) obtaining a radio-image of the patient. Even still further provided is a method of radio-imaging a patient for prostate cancer or metastasis
10 thereof. The method comprises (i) administering to the patient a conjugate of the formula T-L-(X)A or a pharmaceutical composition comprising same and (ii) obtaining a radio-image of the patient.

[0085] Even still further provided is a method of treating a patient for prostate cancer or metastasis thereof with radiotherapy. The method comprises
15 administering to the patient a conjugate (for example, a conjugate of the formula T-L-A, formula T-L-X, and combinations thereof) or a pharmaceutical composition comprising same. The method can further comprise administering to the patient an immune checkpoint inhibitor. The immune checkpoint inhibitor can be selected from the group consisting of an inhibitor of T lymphocyte-associated protein 4 (CTLA-4), an inhibitor of programmed cell death protein 1 (PD-1), an inhibitor of programmed cell death ligand 1 (PD-L1), an inhibitor of lymphocyte activation gene-3 (LAG-3), an inhibitor of T cell immunoglobulin and mucin-domain containing-3 (TIM-3), an inhibitor of T cell immunoglobulin and ITIM domain (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), and B- and T-lymphocyte attenuator (BTLA). The immune checkpoint inhibitor can be selected
20 from the group consisting of nivolumab (Opdivo), pembrolizumab (Keytruda), ipilimumab (Yervoy), atezolizumab, avelumab, and durvalumab.

[0086] Yet even still further provided is a method of treating a patient for prostate cancer or metastasis thereof with radiotherapy. The method comprises
30 administering to the patient a conjugate of formula T-L-(X)A or a pharmaceutical composition comprising same.

[0087] PSMA is not restricted absolutely to prostate tissue. PSMA is expressed in other cancers, more specifically in the neovasculature associated with these cancers (Silver et al., Clin Cancer Res 3: 81-85 (1997); and Liu et al., Cancer Res 57: 3629-3634 (1997)). Consistent, strong expression of PSMA in the
35 neovasculature of conventional (clear cell) renal cell, transitional cell of the bladder, testicular-embryonal, neuroendocrine, colon, and breast has been

reported (Chang et al., Clin Cancer Res 5: 2674-2681 (1999), supra). Anti-PSMA mAbs also consistently bind duodenal epithelial (brush border) cells and proximal tubule cells in the kidney (Liu et al. (1997), supra; and Chang et al. (1999), supra). Thus, the above method(s) of treating a patient (i.e., for prostate cancer or metastasis thereof) may be useful in the treatment of other cancers.

[0088] The conjugates of the formula T-L-A, formula T-L-X, and combinations thereof, can also be used in the methods, wherever a “compound of targeted radionuclide therapy (TRT)” is used. Accordingly, conjugates of the formula T-L-A, formula T-L-X, and combinations thereof, can be used in a method for treating a patient of cancer comprising the step of administering a therapeutic effective amount of a compound of targeted radionuclide therapy (TRT), wherein said compound of targeted radionuclide therapy (TRT) also function as an inhibitor for protein arginine methyltransferase 5 (PRMT5); a method for treating a patient of metastatic castration-resistant prostate cancer (mCRPC) comprising the step of administering a therapeutic effective amount of a compound targeting dually PSMA as a targeted radionuclide therapy (TRT) and PRMT5 as a PRMT5 inhibitor; a method for treating a patient of cancer comprising the step of administering a therapeutic effective amount of a targeted radionuclide therapy (TRT) together with a therapeutic effective amount of an inhibitor for PRMT5; or a method for treating a patient of mCRPC comprising the step of administering a therapeutic effective amount of a PSMA-based TRT together with a therapeutic effective amount of an inhibitor for PRMT5, wherein the TRT is optionally a PSMA-based TRT. The methods can improve the efficacy of targeted radio therapy and reduces toxicities due to uptake of higher radiation doses to other PSMA-expressing tissues, primarily salivary gland.

[0089] Also provided is a pharmaceutical composition for treating a patient of mCRPC comprising a compound targeting dually PSMA as a TRT and PRMT5 as a PRMT5 inhibitor, together with one or more diluents, excipients or carriers, wherein said compound having a formula of, or a pharmaceutically acceptable salt thereof; a pharmaceutical composition for treating a patient of cancer comprising the step of administering a therapeutic effective amount of a TRT together with a therapeutic effective amount of an inhibitor for PRMT5; and a pharmaceutical composition for treating a patient of cancer comprising the step of administering a therapeutic effective amount of a TRT together with a therapeutic effective amount of an inhibitor for PRMT5 as a radio sensitizer. JNJ-64619178 can, in some embodiments, be the PRMT5 inhibitor, but it can be substituted by any other type of PRMT5 inhibitors. JNJ-64619178 is also used as a radiosensitizer and this

can be substituted by any other type of radiosensitizer such as AR inhibitors or those that inhibit key DNA damage response proteins. The proposed approach can be used for hormone-naïve prostate cancer that expresses PSMA if the proposed approach is superior to the existing treatments (surgery, radiotherapy or radiotherapy plus androgen derivation therapy). The proposed approach can be used to treat any other type of cancers if targeted molecule PSMA-617 is replaced with other targeted molecules. The validated effect of JNJ-64619178 or any other PRMT5 inhibitors can also be used alone in combination with other radiotherapy and chemotherapy. PRMT5 also promotes the repair of DSB induced by chemotherapy (e.g., etoposide). Thus, PRMT5 inhibitors such as JNJ-64619178 or other PRMT5 inhibitors can be also used as a chemosensitizer for combination therapy of cancer.

[0090] The terms “substituted,” “substituent,” and “functional group” refer to a group that can be or is substituted onto a molecule or onto another group (e.g., on an aryl or an alkyl group). Examples of substituents include, but are not limited to, a halogen (e.g., F, Cl, Br, and I), OR, OC(O)N(R)₂, CN, NO, NO₂, ONO₂, azido, CF₃, OCF₃, R, O (oxo), S (thiono), C(O), S(O), methylenedioxy, ethylenedioxy, N(R)₂, SR, SOR, SO₂R, SO₂N(R)₂, SO₃R, -(CH₂)₀₋₂P(O)(OR)₂, C(O)R, C(O)C(O)R, C(O)CH₂C(O)R, C(S)R, C(O)OR, OC(O)R, C(O)N(R)₂, OC(O)N(R)₂, C(S)N(R)₂, (CH₂)₀₋₂N(R)C(O)R, (CH₂)₀₋₂N(R)C(O)OR, (CH₂)₀₋₂N(R)N(R)₂, N(R)N(R)C(O)R, N(R)N(R)C(O)OR, N(R)N(R)CON(R)₂, N(R)SO₂R, N(R)SO₂N(R)₂, N(R)C(O)OR, N(R)C(O)R, N(R)C(S)R, N(R)C(O)N(R)₂, N(R)C(S)N(R)₂, N(COR)COR, N(OR)R, C(=NH)N(R)₂, C(O)N(OR)R, or C(=NOR)R wherein each R can be, independently, hydrogen, alkyl, acyl, cycloalkyl, aryl, aralkyl, heterocyclyl, heteroaryl, or heteroarylalkyl, wherein any alkyl, acyl, cycloalkyl, aryl, aralkyl, heterocyclyl, heteroaryl, or heteroarylalkyl or two R groups bonded to a nitrogen atom or to adjacent nitrogen atoms can together with the nitrogen atom or atoms form a heterocyclyl, which can be mono- or independently multi-substituted.

[0091] The term “alkyl” as used herein refers to substituted or unsubstituted straight chain and branched mono- or divalent alkyl groups and cycloalkyl groups having from 1 to 40 carbon atoms (C₁-C₄₀), 1 to about 20 carbon atoms (C₁-C₂₀), 1 to 12 carbons (C₁-C₁₂), 1 to 8 carbon atoms (C₁-C₈), or, in some embodiments, from 1 to 6 carbon atoms (C₁-C₆). Examples of straight chain alkyl groups include those with from 1 to 8 carbon atoms such as methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, and n-octyl groups. Examples of branched alkyl groups include, but are not limited to, isopropyl, iso-butyl, sec-butyl, t-butyl, neopentyl, isopentyl, and 2,2-dimethylpropyl groups. As used herein, the term “alkyl”

encompasses n-alkyl, isoalkyl, and *ante*-isoalkyl groups as well as other branched chain forms of alkyl. Representative substituted alkyl groups can be substituted one or more times with any of the groups listed herein, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

5 [0092] The term “alkenyl” as used herein refers to substituted or unsubstituted straight chain and branched mono- or divalent alkenyl groups and cycloalkenyl groups having at least one double bond and having from 1 to 40 carbon atoms (C_1 - C_{40}), 1 to about 20 carbon atoms (C_1 - C_{20}), 1 to 12 carbons (C_1 - C_{12}), 1 to 8 carbon atoms (C_1 - C_8), or, in some embodiments, from 1 to 6 carbon atoms (C_1 - C_6).
10 Examples of straight chain alkenyl groups include those with from 1 to 8 carbon atoms such as $-CH=CH-$, $-CH=CHCH_3$, and $-CH_2CH=CHCH_2-$ groups, wherein the double bonds can have an E- or Z-configuration. And when there are multiple bonds, each double bond can, independently, have an E- or a Z-configuration. Examples of branched alkenyl groups include, but are not limited to, $-CH=C(CH_3)-$ and $CH_2C=CH(CH_3)$ groups. Representative substituted alkenyl groups can be substituted one or more times with any of the groups listed herein, for example, amino, hydroxy, cyano, carboxy, nitro, thio, alkoxy, and halogen groups.

15 [0093] The term “cycloalkyl” as used herein refers to substituted or unsubstituted cyclic alkyl groups such as, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl groups. In some embodiments, the cycloalkyl group can have 3 to about 8-12 ring members, whereas in other embodiments the number of ring carbon atoms range from 3 to 4, 5, 6, or 7. Cycloalkyl groups can have any number of carbon atoms, e.g., 3 to 8 carbon atoms (C_3 - C_8), 3 to 6 carbon atoms (C_3 - C_6), and 4 to 8 carbon atoms (C_4 - C_8). Cycloalkyl groups further include polycyclic cycloalkyl groups such as, but not limited to, norbornyl, adamantyl, bornyl, camphenyl, isocamphenyl, and carenyl groups, and fused rings such as, but not limited to, decalinyl, and the like.

20 [0094] The term “cycloalkylalkyl” as used herein refers to substituted or unsubstituted alkyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group as defined herein is replaced with a bond to a cycloalkyl group as defined herein. Representative cycloalkylalkyl groups include, but are not limited to, cyclopentylalkyl.

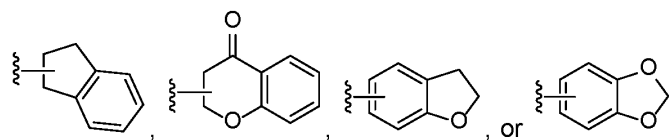
25 [0095] The term “alkylcycloalkyl” as used herein refers to substituted or unsubstituted cycloalkyl groups as defined herein in which a hydrogen of a cycloalkyl group as defined herein is replaced with a bond to an alkyl group as defined herein. Representative alkylcycloalkyl groups include, but are not limited to, alkylcyclopropyl.

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[0096] The term “acyl” as used herein refers to a group containing a carbonyl moiety wherein the group is bonded via the carbonyl carbon atom. The carbonyl carbon atom is also bonded to another carbon atom, which can be part of a substituted or unsubstituted alkyl, aryl, aralkyl cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl group or the like. In the special case wherein the carbonyl carbon atom is bonded to a hydrogen, the group is a “formyl” group, an acyl group as the term is defined herein. An acyl group can include 0 to about 12-40, 6-10, 1-5 or 2-5 additional carbon atoms bonded to the carbonyl group. An acryloyl group is an example of an acyl group. An acyl group can also include heteroatoms within the meaning here. A nicotinoyl group (pyridyl-3-carbonyl) is an example of an acyl group within the meaning herein. Other examples include acetyl, benzoyl, phenylacetyl, pyridylacetyl, cinnamoyl, and acryloyl groups and the like. When the group containing the carbon atom that is bonded to the carbonyl carbon atom contains a halogen, the group is termed a “haloacyl” group. An example is a trifluoroacetyl group.

[0097] The term “heterocyclylcarbonyl” is an example of an acyl group that is bonded to a substituted or unsubstituted heterocyclyl group, as the term “heterocyclyl” is defined herein. An example of a heterocyclylcarbonyl group is a prolyl group, wherein the prolyl group can be a D- or an L-prolyl group.

[0098] The term “aryl” as used herein refers to substituted or unsubstituted cyclic aromatic hydrocarbons that do not contain heteroatoms in the ring. Thus, aryl groups include, but are not limited to, phenyl, azulenyl, heptalenyl, biphenyl, indacenyl, fluorenyl, phenanthrenyl, triphenylenyl, pyrenyl, naphthacenyl, chrysenyl, biphenylenyl, anthracenyl, and naphthyl groups. In some embodiments, aryl groups contain about 6 to about 14 carbons (C_6-C_{14}) or from 6 to 10 carbon atoms (C_6-C_{10}) in the ring portions of the groups. Aryl groups can be unsubstituted or substituted, as defined herein. “Aryl” and the phrase “aryl group” includes fused ring species including those that include fused aromatic and non-aromatic groups. Accordingly, “aryl” and the phrase “aryl group” include groups of the formula:



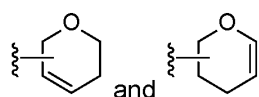
, each of which can be substituted or unsubstituted, such as hydroxy substituted.

[0099] Representative substituted aryl groups can be mono-substituted or substituted more than once, such as, but not limited to, 2-, 3-, 4-, 5-, or 6-

substituted phenyl or 2-8 substituted naphthyl groups, which can be substituted with carbon or non-carbon groups such as those listed herein.

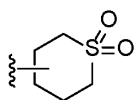
[00100] The terms “aralkyl” and “arylalkyl” refer to alkyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to an aryl group as defined herein. Representative aralkyl groups include benzyl and phenylethyl groups and fused (cycloalkylaryl)alkyl groups such as 4-ethyl-indanyl. Aralkenyl groups are alkenyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to an aryl group as defined herein.

[00101] The term “heterocyclyl” or “heterocyclo” refers to substituted or unsubstituted aromatic and non-aromatic ring compounds containing 3 or more ring members, of which one or more (e.g., 1, 2 or 3) is a heteroatom such as, but not limited to, N, O, and S. Thus, a heterocyclyl can be a cycloheteroalkyl or a heteroaryl or, if polycyclic, any combination thereof. In some embodiments, heterocyclyl groups include 3 to about 20 ring members, whereas other such groups have 3 to about 15 ring members. In some embodiments, heterocyclyl groups include heterocyclyl groups that include 3 to 8 carbon atoms (C_3-C_8), 3 to 6 carbon atoms (C_3-C_6), 3 to 5 carbon atoms (C_3-C_5) or 6 to 8 carbon atoms (C_6-C_8). A heterocyclyl group designated as a C_2 -heterocyclyl can be a 5-ring with two carbon atoms and three heteroatoms, a 6-ring with two carbon atoms and three heteroatoms and so forth. Likewise, a C_4 -heterocyclyl can be a 5-ring with one heteroatom, a 6-ring with two heteroatoms, and so forth. The number of carbon atoms plus the number of heteroatoms equals the total number of ring atoms. A heterocyclyl ring can also include one or more double bonds, such as in the group 3,6-dihydro-2H-pyran and 3,4-dihydro-2H-pyran, having the formula:

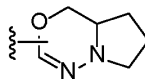


and , respectively, each of which can be substituted.

[00102] A heteroaryl ring is an embodiment of a heterocyclyl group. The phrase “heterocyclyl group” includes fused ring species including those that include fused aromatic and non-aromatic groups. Representative heterocyclyl groups include, but are not limited to tetrahydro-2H-thiopyran-1,1-dioxide, having the formula:

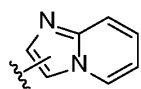


, which can be substituted, 4a,5,6,7-tetrahydro-4H-pyrrolo[1,2-

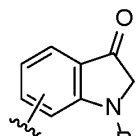


d[1,3,4]oxadiazinyl, having the formula:

, which can be substituted, pyrrolidinyl, pyrrolidinone (e.g., pyrrolidin-2-one), azetidiny, piperidiny, piperazinyl, morpholinyl, chromanyl, indolinonyl, isoindolinonyl, furanyl, pyrrolidinyl, pyridinyl, pyrazinyl, pyrimidinyl, triazinyl, thiophenyl, tetrahydrofuranyl, pyrrolyl, oxazolyl, oxadiazolyl, imidazolyl, imidazo[1,2-a]pyridinyl, having the formula:

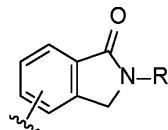


, which can be substituted, triazyolyl, tetrazolyl, benzoxazoliny, thiazolyl, benzthiazoliny, and benzimidazoliny groups. Examples of indolinonyl groups include groups having the general formula:



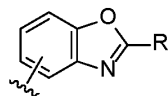
, wherein R is as defined herein.

[00103] Examples of isoindolinonyl groups include groups having the general formula:



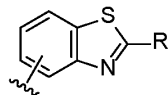
, wherein R is as defined herein.

[00104] Examples of benzoxazoliny groups include groups having the general formula:



, wherein R is as defined herein.

[00105] Examples of benzthiazoliny groups include groups having the general formula:



, wherein R is as defined herein.

[00106] In some embodiments, the group R in benzoxazoliny and benzthiazoliny groups is an N(R)₂ group. In some embodiments, each R is hydrogen or alkyl, wherein the alkyl group is substituted or unsubstituted. In some

embodiments, the alkyl group is substituted with a heterocyclyl group (e.g., with a pyrrolidinyl group).

[00107] The term “heterocyclylalkyl” refers to alkyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group as defined herein is replaced with a bond to a heterocyclyl group as defined herein. Representative heterocyclylalkyl groups include, but are not limited to, furan-2-yl methyl, furan-3-yl methyl, pyridine-3-yl methyl, tetrahydrofuran-2-yl methyl, and indol-2-yl propyl.

[00108] The term “heterocyclylalkoxy” refers to alkyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group as defined herein is replaced with a bond to a heterocyclyl group as defined herein and the alkyl group is attached to an oxygen. Representative heterocyclylalkoxy groups include, but are not limited to, $-O-(CH_2)_q$ heterocyclyl, wherein q is an integer from 1 to 5. In some embodiments, heterocyclylalkoxy groups include $-O-(CH_2)_q$ morpholinyl such as $-O-CH_2CH_2$ -morpholine.

[00109] The term “heteroarylalkyl” refers to alkyl groups as defined herein in which a hydrogen or carbon bond of an alkyl group is replaced with a bond to a heteroaryl group as defined herein.

[00110] The term “alkoxy” refers to an oxygen atom connected to an alkyl group, including a cycloalkyl group, as are defined herein. Examples of linear alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, and the like. Examples of branched alkoxy include, but are not limited to, isopropoxy, sec-butoxy, tert-butoxy, isopentyloxy, isohexyloxy, and the like. Examples of cyclic alkoxy include, but are not limited to, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like. An alkoxy group can include one to about 12-20 or about 12-40 carbon atoms bonded to the oxygen atom, can further include double or triple bonds, and can also include heteroatoms. For example, an allyloxy group is an alkoxy group within the meaning herein. A methoxyethoxy group is also an alkoxy group within the meaning herein, as is a methylenedioxy group in a context where two adjacent atoms of a structure are substituted therewith.

[00111] The terms “amine,” “amine group,” “amino,” and “amino group” refer to a substituent of the form $-NH_2$, $-NHR$, $-NR_2$, or $-NR_3^+$, wherein each R is defined herein, and protonated forms of each, except for $-NR_3^+$, which cannot be protonated. Accordingly, any compound substituted with an amino group can be viewed as an amine. An “amino group” within the meaning herein can be a primary, secondary, tertiary, or quaternary amino group.

[00112] An "alkylamino" group includes a monoalkylamino, dialkylamino, and trialkylamino group. An example of a "alkylamino" is -NH-alkyl and -N(alkyl)₂.

[00113] An example of a "cycloalkylamino" group is -NH-cycloalkyl and -N(cycloalkyl)₂.

5 [00114] An example of a "cycloalkyl heterocycloamino" group is -NH-(heterocyclo cycloalkyl), wherein the heterocyclo group is attached to the nitrogen and the cycloalkyl group is attached to the heterocyclo group.

[00115] An example of a "heterocyclo cycloamino" group is -NH-(cycloalkyl heterocycle), wherein the cycloalkyl group is attached to the nitrogen and the
10 heterocyclo group is attached to the cycloalkyl group.

[00116] The term "amido" refers to a group of the formula -C(O)NR₂, wherein R is defined herein.

[00117] The terms "halo," "halogen," and "halide" group, by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine,
15 bromine, or iodine atom.

[00118] The term "haloalkyl" group includes mono-halo alkyl groups, poly-halo alkyl groups, wherein all halo atoms can be the same or different, and per-halo alkyl groups, wherein all hydrogen atoms are replaced by halogen atoms, such as fluoro. Examples of haloalkyl include trifluoromethyl, 1,1-dichloroethyl,
20 1,2-dichloroethyl, 1,3-dibromo-3,3-difluoropropyl, perfluorobutyl, -CF(CH₃)₂ and the like.

[00119] The terms "treat," "treating," "treated," or "treatment" (with respect to a disease or condition) is an approach for obtaining beneficial or desired results including and preferably clinical results and includes, but is not limited to, one or
25 more of the following: improving a condition associated with a disease, curing a disease, lessening severity of a disease, delaying progression of a disease, alleviating one or more symptoms associated with a disease, increasing the quality of life of one suffering from a disease, prolonging survival and/or prophylactic or preventative treatment.

30 [00120] An "effective amount" refers to any amount that is sufficient to achieve a desired biological effect. Combined with the teachings provided herein, by choosing among the various active conjugates or compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and mode of administration, an effective prophylactic or
35 therapeutic treatment regimen can be planned which does not cause substantial unwanted toxicity and yet is effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the

disease or condition being treated, the particular compound being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular compound and/or other therapeutic agent without necessitating undue experimentation. A maximum dose can be used, that is, the highest safe dose according to some medical judgment. Multiple doses per day can be used to achieve appropriate systemic levels of compounds. Appropriate systemic levels can be determined by, for example, measurement of the patient's peak or sustained plasma level of the drug. "Dose" and "dosage" are used interchangeably herein.

[00121] Generally, daily oral doses of a compound are, for human subjects, from about 0.01 milligrams/kg per day to 1,000 milligrams/kg per day. Oral doses in the range of 0.5 to 50 milligrams/kg, in one or more administrations per day, can yield therapeutic results. Dosage can be adjusted appropriately to achieve desired drug levels, local or systemic, depending upon the mode of administration. For example, intravenous administration can vary from one order to several orders of magnitude lower dose per day. If the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localized delivery route) can be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of the compound.

[00122] A "therapeutically effective amount" (or "effective amount") of a compound with respect to use in treatment, refers to an amount of the compound in a preparation which, when administered as part of a desired dosage regimen (to a mammal, such as a human) 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 or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

[00123] For any compound therapeutically effective amount can be initially determined from animal models. A therapeutically effective dose can also be determined from human data for compounds which have been tested in humans and for compounds which are known to exhibit similar pharmacological activities, such as other related active agents. Higher doses may be required for parenteral administration. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other

methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

[00124] The formulations can be administered in pharmaceutically acceptable solutions, which can routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients. For use in therapy, an effective amount of the compound can be administered to a subject by any mode that delivers the compound to the desired surface. Administering a pharmaceutical composition can be accomplished by any means known to the skilled artisan. Routes of administration include, but are not limited to, intravenous, intramuscular, intraperitoneal, intravesical (urinary bladder), oral, subcutaneous, direct injection (for example, into a tumor or abscess), mucosal (e.g., topical to eye), inhalation, and topical.

[00125] For intravenous and other parenteral routes of administration, a compound can be formulated as a lyophilized preparation, as a lyophilized preparation of liposome-intercalated or -encapsulated active compound, as a lipid complex in aqueous suspension, or as a salt complex. Lyophilized formulations are generally reconstituted in suitable aqueous solution, e.g., in sterile water or saline, shortly prior to administration.

[00126] For oral administration, the compounds can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well-known in the art. Such carriers enable the compounds to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone (PVP). If desired, disintegrating agents can be added, such as the cross-linked PVP, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations can also be formulated in saline or buffers, e.g., EDTA for neutralizing internal acid conditions, or can be administered without any carriers.

[00127] Also contemplated are oral dosage forms of the compounds. The compounds can be chemically modified so that oral delivery of the derivative is

efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the compound itself, where said moiety permits (a) inhibition of acid hydrolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the compounds and increase in circulation time in the body. Examples of such moieties include polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, PVP and polyproline. Abuchowski and Davis, "Soluble Polymer-Enzyme Adducts," In: Enzymes as Drugs, Hocenberg and Roberts, eds., Wiley-Interscience, New York, N.Y., pp. 367-383 (1981); Newmark et al., J Appl Biochem 4:185-189 (1982). Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. For pharmaceutical usage, as indicated above, polyethylene glycol moieties are suitable.

[00128] The location of release of a compound hereof can be the stomach, the small intestine (e.g., the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations, which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. The release can avoid the deleterious effects of the stomach environment, either by protection of the compound or by release of the compound beyond the stomach environment, such as in the intestine.

[00129] To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and shellac. These coatings can be used as mixed films.

[00130] A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings which make the tablet easier to swallow. Capsules can consist of a hard shell (such as gelatin) for delivery of dry therapeutic (e.g., powder); for liquid forms, a soft gelatin shell can be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

[00131] The compound can be included in the formulation as fine multi-particulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder,

lightly compressed plugs or even as tablets. Therapeutic agent could be prepared by compression.

[00132] Colorants and flavoring agents may all be included. For example, the compound can be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

[00133] One may dilute or increase the volume of the compound with an inert material. These diluents can include carbohydrates, especially mannitol, α-lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts also can be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

[00134] Disintegrants can be included in the formulation of therapeutic agent into a solid dosage form. Materials used as disintegrates include, but are not limited to, starch, including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrant is the insoluble cationic exchange resin. Powdered gums can be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

[00135] Binders can be used to hold the compound together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). PVP and hydroxypropylmethyl cellulose (HPMC) can both be used in alcoholic solutions to granulate therapeutic agent.

[00136] An anti-frictional agent can be included in the formulation of therapeutic to prevent sticking during the formulation process. Lubricants can be used as a layer between therapeutic agent and the die wall, and these can include, but are not limited to, stearic acid, including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants can also be used, such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

[00137] Glidants, which can improve the flow properties of the drug during formulation and aid rearrangement during compression, can be added. The glidants can include starch, talc, pyrogenic silica and hydrated silicoaluminate.

[00138] To aid dissolution of therapeutic agent into the aqueous environment a surfactant can be added as a wetting agent. Surfactants can include anionic detergents, such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents which can be used include benzalkonium chloride and benzethonium chloride. Potential non-ionic detergents that can be included in the formulation as surfactants include lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the compound or derivative thereof either alone or as a mixture in different ratios.

[00139] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. Microspheres formulated for oral administration can also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

[00140] For buccal administration, the compositions can take the form of tablets or lozenges formulated in conventional manner.

[00141] For topical administration, the compound can be formulated as solutions, gels, ointments, creams, suspensions, etc. as are well-known in the art. Systemic formulations include those designed for administration by injection, e.g., subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal oral or pulmonary administration.

[00142] For administration by inhalation, compounds can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable

gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

5 **[00143]** Also contemplated is pulmonary delivery of the compounds (or salts thereof). The compound is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. Other reports of inhaled molecules include Adjei et al., Pharm Res 7:565-569 (1990); Adjei et al., Int J Pharmaceutics 63:135-144 (1990) (leuprolide acetate); Braquet et al., J Cardiovasc Pharmacol 13(suppl. 5):143-146 (1989) (endothelin-1); Hubbard et al., Ann Int Med 3:206-212 (1989) (a1-antitrypsin); Smith et al., 1989, J Clin Invest 84:1145-1146 (a-1-proteinase); Oswein et al., 1990, "Aerosolization of Proteins," Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, (recombinant hepatocyte growth hormone); Debs et al., 1988, J Immunol 140:3482-3488 (interferon-gamma and tumor necrosis factor alpha) and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor; incorporated herein by reference). A method and composition for pulmonary delivery of drugs for systemic effect is described in U.S. Pat. No. 5,451,569 (specifically incorporated herein by reference for its disclosure regarding same), issued Sep. 19, 1995, to Wong et al.

20 **[00144]** Contemplated for use are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

25 **[00145]** Nasal delivery of a pharmaceutical composition is also contemplated. Nasal delivery allows the passage of a pharmaceutical composition to the blood stream directly after administering therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

30 **[00146]** The compounds, when it is desirable to deliver them systemically, can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, 35 stabilizing and/or dispersing agents.

[00147] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethylcellulose, sorbitol, or dextran. Optionally, the suspension can also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[00148] Alternatively, the active compounds can be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00149] The compounds can also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00150] In addition to the formulations described above, a compound can also be formulated as a depot preparation. Such long-acting formulations can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00151] The pharmaceutical compositions also can comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[00152] Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer R, Science 249:1527-1533 (1990).

[00153] The compound and optionally one or more other therapeutic agents can be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

[00154] Suitable buffering agents include acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

[00155] Pharmaceutical compositions contain an effective amount of a compound as described herein and optionally one or more other therapeutic agents included in a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also can be commingled with the compounds, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

[00156] Therapeutic agent(s), including specifically, but not limited to, a compound, can be provided in particles. "Particles" as used herein means nanoparticles or microparticles (or in some instances larger particles) that can consist in whole or in part of the compound or the other therapeutic agent(s) as described herein. The particles can contain therapeutic agent(s) in a core surrounded by a coating, including, but not limited to, an enteric coating. Therapeutic agent(s) also can be dispersed throughout the particles. Therapeutic agent(s) also can be adsorbed into the particles. The particles can be of any order release kinetics, including zero-order release, first-order release, second-order release, delayed release, sustained release, immediate release, and any

combination thereof, etc. The particle can include, in addition to therapeutic agent(s), any of those materials routinely used in the art of pharmacy and medicine, including, but not limited to, erodible, nonerodible, biodegradable, or nonbiodegradable material or combinations thereof. The particles can be microcapsules which contain the compound in a solution or in a semi-solid state. The particles can be of virtually any shape.

[00157] Both non-biodegradable and biodegradable polymeric materials can be used in the manufacture of particles for delivering therapeutic agent(s). Such polymers can be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired. Bioadhesive polymers of particular interest include bioerodible hydrogels described in Sawhney et al., *Macromolecules* 26:5823-2787 (1993), the teachings of which are specifically incorporated by reference herein. These include polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

[00158] Therapeutic agent(s) can be contained in controlled-release systems. The term "controlled release" refers to any drug-containing formulation in which the manner and profile of drug release from the formulation are controlled. This refers to immediate as well as non-immediate release formulations, with non-immediate release formulations including, but not limited to, sustained release and delayed release formulations. The term "sustained release" (also referred to as "extended release") refers to a drug formulation that provides for gradual release of a drug over an extended period of time, and that can result in substantially constant blood levels of a drug over an extended time period. The term "delayed release" refers to a drug formulation in which there is a time delay between administration of the formulation and the release of the drug therefrom. "Delayed release" may or may not involve gradual release of drug over an extended period of time, and thus may or may not be "sustained release."

[00159] Use of a long-term sustained release implant can be particularly suitable for treatment of chronic conditions. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 7 days, and up to 30-60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

[00160] As used herein, the term “salts” and “pharmaceutically acceptable salts” refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic groups such as amines; and alkali or organic salts of acidic groups such as carboxylic acids. Pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic, and the like.

[00161] Pharmaceutically acceptable salts can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. In some instances, 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, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton, Pa., 1990, the disclosure of which is hereby incorporated by reference.

[00162] The term “solvate” means a compound, or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of solvent bound by non-covalent intermolecular forces. Where the solvent is water, the solvate is a hydrate.

[00163] The term “prodrug” means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide an active compound, particularly a compound of the invention. Examples of prodrugs include, but are not limited to, derivatives and metabolites of a compound of the invention that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Specific prodrugs of compounds with carboxyl functional groups are the lower alkyl esters of the carboxylic acid. The carboxylate esters are conveniently formed by esterifying any of the carboxylic acid moieties present on

the molecule. Prodrugs can typically be prepared using well-known methods, such as those described by Burger's Medicinal Chemistry and Drug Discovery 6th ed. (Donald J. Abraham ed., 2001, Wiley) and Design and Application of Prodrugs (H. Bundgaard ed., 1985, Harwood Academic Publishers GmbH).

5 **[00164]** Further, in each of the foregoing and following embodiments, it is to be understood that the formulae include and represent not only all pharmaceutically acceptable salts of the compounds, but also include any and all hydrates and/or solvates of the compound formulae or salts thereof. It is to be appreciated that certain functional groups, such as the hydroxy, amino, and like
10 groups form complexes and/or coordination compounds with water and/or various solvents, in the various physical forms of the compounds. Accordingly, the above formulae are to be understood to include and represent those various hydrates and/or solvates. In each of the foregoing and following embodiments, it is also to be understood that the formulae include and represent each possible isomer, such
15 as stereoisomers and geometric isomers, both individually and in any and all possible mixtures. In each of the foregoing and following embodiments, it is also to be understood that the formulae include and represent any and all crystalline forms, partially crystalline forms, and non-crystalline and/or amorphous forms of the compounds.

20 **[00165]** The term "pharmaceutically acceptable carrier" is art-recognized and refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof. Each carrier must be "acceptable" in the sense of being compatible with the subject
25 composition and its components and not injurious to the patient. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt;
30 (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17)
35 isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer

solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

[00166] The term “administering” includes all means of introducing the compounds and compositions described herein to the patient, including, but are not limited to, oral (po), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, inhalation, buccal, ocular, sublingual, vaginal, rectal, and the like. The compounds and compositions may be administered in unit dosage forms and/or formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles.

[00167] Illustrative formats for oral administration include tablets, capsules, elixirs, syrups, and the like. Illustrative routes for parenteral administration include intravenous, intraarterial, intraperitoneal, epidural, intraurethral, intrasternal, intramuscular and subcutaneous, as well as any other art recognized route of parenteral administration.

[00168] Illustrative means of parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques, as well as any other means of parenteral administration recognized in the art. Parenteral formulations are typically aqueous solutions, which may contain excipients such as salts, carbohydrates and buffering agents (preferably at a pH in the range from about 3 to about 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. Parenteral administration of a compound is illustratively performed in the form of saline solutions or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, a solubilizer such as ethanol can be applied.

[00169] The dosage of each compound of the claimed combinations depends on several factors, including: the administration method, the condition to be treated, the severity of the condition, whether the condition is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular patient may affect the dosage regimen used.

[00170] In the methods the individual components of a co-administration, or combination can be administered by any suitable means, contemporaneously, simultaneously, sequentially, separately or in a single pharmaceutical formulation. Where the co-administered compounds or compositions are administered in separate dosage forms, the number of dosages administered per day for each compound may be the same or different. The compounds or compositions may be administered via the same or different routes of administration. The compounds or compositions may be administered according to simultaneous or alternating regimens, at the same or different times during the course of the therapy, concurrently in divided or single forms.

[00171] The term "therapeutically effective amount" refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well-known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

[00172] Depending upon the route of administration, a wide range of permissible dosages are contemplated, including doses falling in the range from about 1 µg/kg to about 1 g/kg. The dosages may be single or divided and may administered according to a wide variety of protocols, including q.d. (once a day), b.i.d. (twice a day), t.i.d. (three times a day), or even every other day, once a week, once a month, once a quarter, and the like. In each of these cases the described therapeutically effective amounts correspond to the instance of administration, or alternatively to the total daily, weekly, month, or quarterly dose, as determined by the dosing protocol.

[00173] An effective amount of any one or a mixture of the compounds described herein can be determined by the attending diagnostician or physician by the use of known techniques and/or by observing results obtained under analogous circumstances. In determining the effective amount or dose, a number of factors are considered by the attending diagnostician or physician, including, but not limited to the species of mammal, including human, its size, age, and general health, the specific disease or disorder involved, the degree of or involvement or the severity of the disease or disorder, the response of the individual patient, the particular compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, the use of concomitant medication, and other relevant circumstances.

[00174] The term "patient" includes human and non-human animals such as companion animals (dogs and cats and the like) and livestock animals. Livestock animals are animals raised for food production. The patient to be treated is preferably a mammal, in particular a human being.

[00175] The following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art.

[00176] The term "about" can allow for a degree of variability in a value or range, for example, within 20%, within 10%, within 5%, or within 1% of a stated value or of a stated limit of a range.

[00177] The terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. In addition, it is to be understood that the phraseology or terminology employed herein, and not otherwise defined, is for the purpose of description only and not of limitation. Any use of section headings is intended to aid reading of the document and is not to be interpreted as limiting. Further, information that is relevant to a section heading may occur within or outside of that particular section. Furthermore, all publications, patents, and patent documents referred to in this document are incorporated by reference herein in their entirety, as though individually incorporated by reference. In the event of inconsistent usages between this document and those documents so incorporated by reference, the usage in the incorporated reference should be considered supplementary to that of this document; for irreconcilable inconsistencies, the usage in this document controls.

[00178] The term “substantially” refers to a majority of, or mostly, as in at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, 99.99%, or at least about 99.999% or more.

5 [00179] The term “substantially not” as used herein refers to less than about 30%, 25%, 20%, 15%, 10%, 5%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.001%, or at less than about 0.0005% or less or about 0% or 0%.

10 [00180] In the methods, the steps can be carried out in any order without departing from the principles of the invention, except when a temporal or operational sequence is explicitly recited. Furthermore, specified steps can be carried out concurrently unless explicit claim language recites that they be carried out separately. For example, a claimed step of doing X and a claimed step of doing Y can be conducted simultaneously within a single operation, and the resulting process will fall within the literal scope of the claimed process.

15 [00181] Those skilled in the art will appreciate that many modifications to the described embodiments are possible without departing from the spirit and scope of the present disclosure. Thus, the description is not intended and should not be construed to be limited to the examples given but should be granted the full breadth of protection afforded by the appended claims and equivalents thereto. In addition, it is possible to use some of the features of the present disclosure without
20 the corresponding use of other features. Accordingly, the foregoing description of or illustrative embodiments is provided for the purpose of illustrating the principles of the present disclosure and not in limitation thereof and can include modification thereto and permutations thereof.

25 [00182] The disclosure also relates to the subject matter of the following Clauses listed in no particular order of importance:

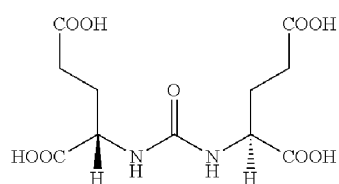
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T is a ligand that targets prostate-specific membrane antigen (PSMA),

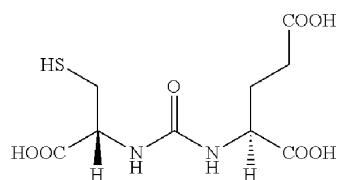
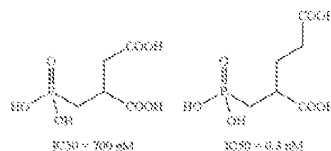
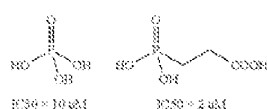
L is a linker, and

A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor.

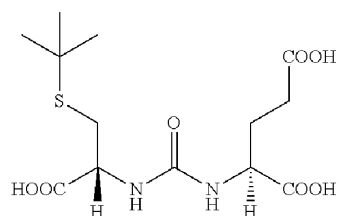
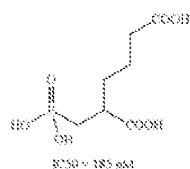
2. The conjugate of Clause 1, wherein T is:



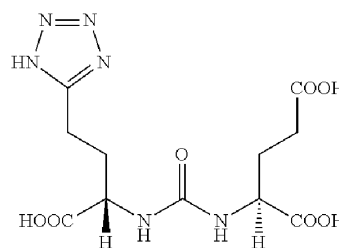
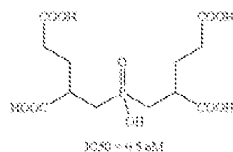
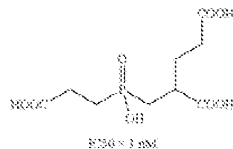
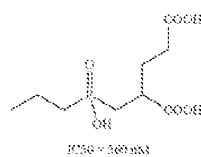
DOPA
IC₅₀ = 47 nM
K_i = 8 nM



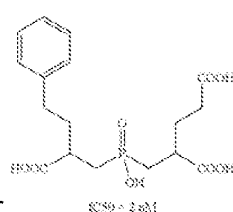
MUPA
IC₅₀ = 6.9 nM



IC₅₀ = 29 nM

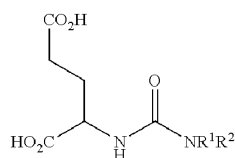


K_i = 0.9 nM



or

3. The conjugate of Clause 1, wherein T is:



, in which R¹ and R² are independently selected from hydrogen, carboxylic acid, which is optionally substituted, malonic acid, succinic acid, glutamic acid, and adipic acid.

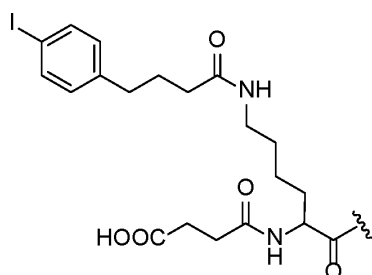
4. The conjugate of Clause 3, wherein the substituted carboxylic acid is thiolacetic acid or thiopropionic acid.

5. The conjugate of Clause 1, wherein T is a urea of (a) an amino dicarboxylic acid or a derivative thereof and (b) an amino dicarboxylic acid or a derivative thereof, wherein (a) and (b) can be the same or different.
6. The conjugate of any one of Clauses 1-5, wherein L comprises a chain of atoms from about 3 atoms to about 30 atoms in length.
7. The conjugate of any one of Clauses 1-6, wherein L comprises a chain of atoms from about 5 Å to about 45 Å in length.
8. The conjugate of any one of Clauses 1-7, wherein L comprises a peptide.
9. The conjugate of any one of Clauses 1-8, wherein L comprises one or more phenylalanine residues, each of which is independently optionally substituted.
10. The conjugate of any one of Clauses 1-9, wherein L comprises at least one phenylalanyl-phenylalanyl, in which at least one phenyl is independently optionally substituted.
11. The conjugate of any one of Clauses 1-10, wherein L comprises a polyoligoethylene glycoln (POEGn), a polyethylene glycoln (PEGn), or a mixture thereof, wherein n = 1-36.
12. The conjugate of any one of Clauses 1-11, wherein L comprises a disulfide.
13. The conjugate of Clause 1, wherein the PRMT5 inhibitor comprises a radical of JNJ-64619178 or a radical of GSK3326595.
14. The conjugate of Clause 1, wherein the ATM inhibitor comprises a radical of AZD0156.
15. The conjugate of Clause 1, wherein the DNA-PK inhibitor comprises a radical of Nedisertib, AZD7648, KU57748, Torin 2, PI-103, NU7026, Samotolisib, PIK 90, CC-115, PIK-75 hydrochloride, VX-984, KU-0060648, BAY-8400, DNA-PK-IN-2, DNA-PK-IN-1, DNA-PK-IN-4, DNA-PK-IN-5, DNA-PK-IN-6, XRD-0394, NU5455,

STL127705, PI-103 hydrochloride, LTURM34, ZL-2201 free base, DNA-PK-IN-14, DNA-PK-IN-10, DNA-PK-IN-11 or DNA-PK-IN-13.

16. The conjugate of any one of Claims 1-15 further comprising an albumin binding group.

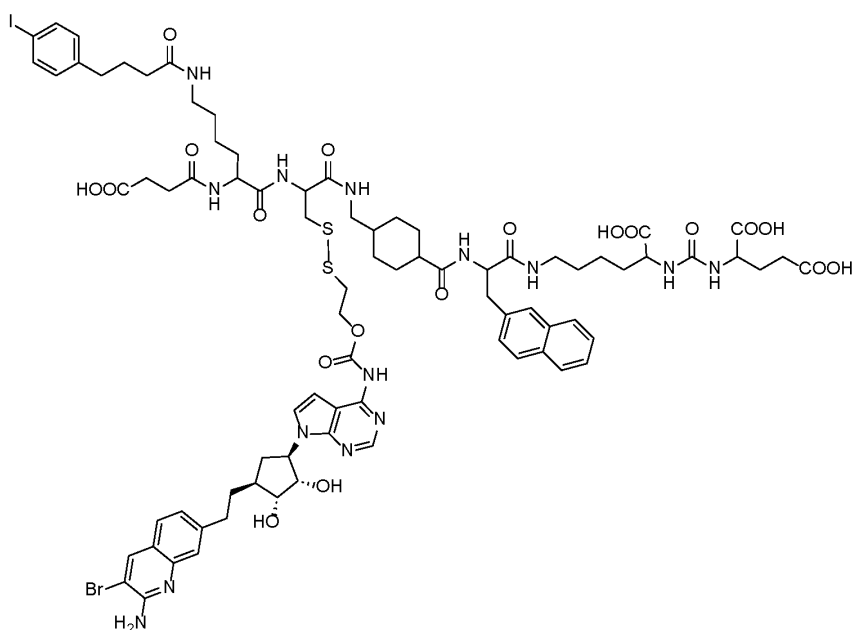
17. The conjugate of Claim 16, wherein the albumin binding group is of the formula:

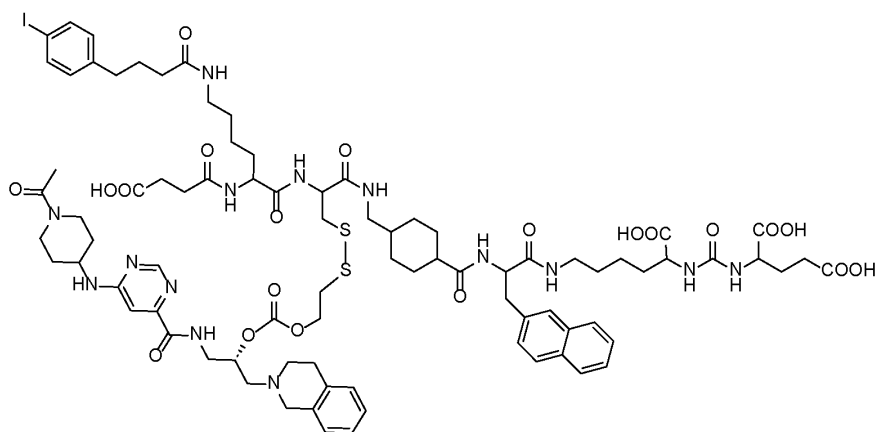


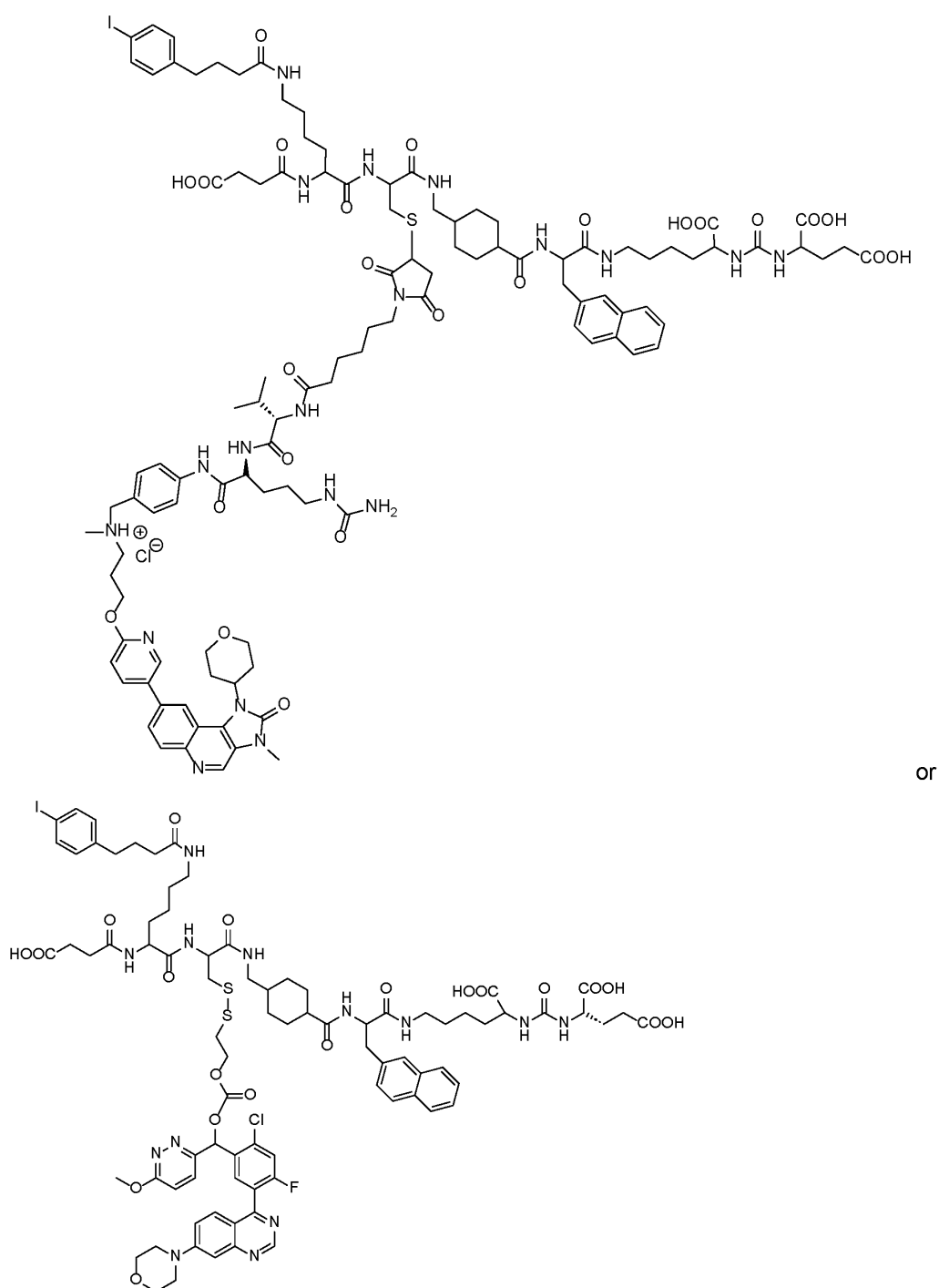
18. The conjugate of Claim 16, wherein the albumin binding group is linked to L via a substituent or group present on L.

19. The conjugate of Claim 16, wherein the albumin binding group is linked to L via an -NR⁴- group, wherein R⁴ is H or alkyl; or an -O- group.

20. A conjugate of the formula:







21. A conjugate of formula T-L(X)A in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

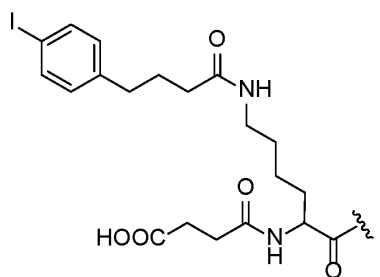
L is a linker,

A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor; and X is a radiotherapeutic or radioimaging agent.

22. The conjugate of Clause 21, wherein A and X are connected to L.

23. The conjugate of Clause 21 or 22, further comprising an albumin binding group.

24. The conjugate of Clause 23, wherein the albumin binding group is of the formula:



25. The conjugate of Clause 21 or 22, wherein the albumin binding group is linked to L via a substituent or group present on L, and wherein the albumin binding group is optionally linked to L as in Clause 19 or 20.

26. The conjugate of any one of Clauses 21-25, wherein T is as defined in Clauses 2-5.

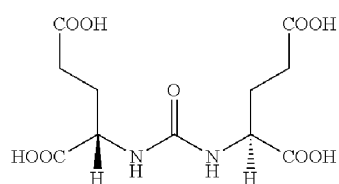
27. The conjugate of any one of Clauses 21-26, wherein L is as defined in Clauses 6-12.

28. The conjugate of any one of Clauses 21-27, wherein A is as defined in Clauses 13-15.

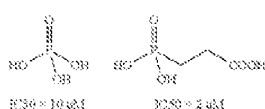
29. A pharmaceutical composition comprising a conjugate of any one of Clauses 1-20, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising a conjugate of any one of Clauses 21-28, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

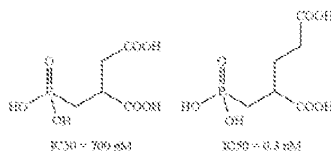
31. A method of treating a patient for prostate cancer or metastasis thereof with radiotherapy, which method comprises administering to the patient a conjugate of any of Clauses 1-20, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of Clause 29, whereupon the patient is treated for prostate cancer or metastasis thereof.
32. The method of Clause 31, further comprising administering a conjugate of the formula T-L-X or a pharmaceutically acceptable salt thereof to the patient before or after administering a conjugate of any of Clauses 1-20 or the pharmaceutical composition thereof in which:
T is a ligand that targets prostate-specific membrane antigen (PSMA),
L is a linker, and
X is a radiotherapeutic or radioimaging agent.
33. The method of Clause 32, wherein T is:



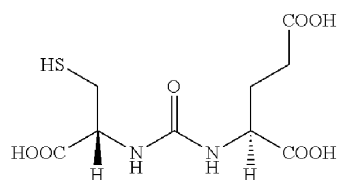
DOPA
IC₅₀ = 47 nM
K_i = 8 nM



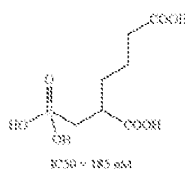
IC₅₀ = 10 nM IC₅₀ = 2 nM



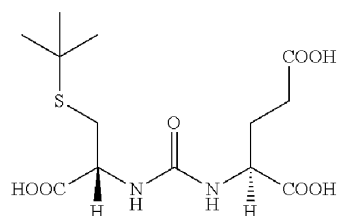
IC₅₀ = 709 nM IC₅₀ = 0.3 nM



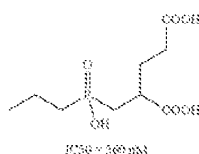
MUPA
IC₅₀ = 6.9 nM



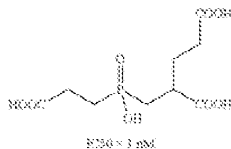
IC₅₀ = 185 nM



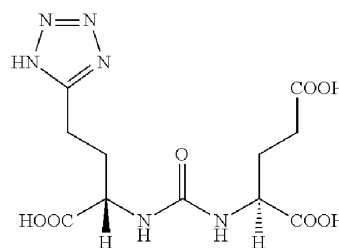
IC₅₀ = 29 nM



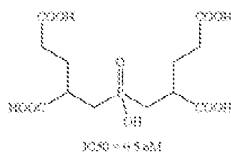
IC₅₀ = 569 nM



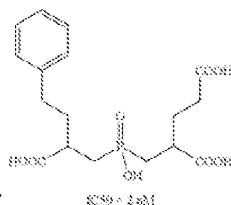
IC₅₀ = 1 nM



K_i = 0.9 nM



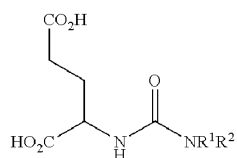
IC₅₀ = 4.5 nM



IC₅₀ = 2 nM

or

34. The method of Clause 32, wherein T is:



, in which R¹ and R² are independently selected from hydrogen, carboxylic acid, which is optionally substituted, malonic acid, succinic acid, glutamic acid, and adipic acid.

35. The method of Clause 32, wherein the substituted carboxylic acid is thiolacetic acid or thiopropionic acid.

36. The method of Clause 32, wherein T is a urea of (a) an amino dicarboxylic acid or a derivative thereof and (b) an amino dicarboxylic acid or a derivative thereof, wherein (a) and (b) can be the same or different.
37. The method of Clause 32, wherein L comprises a chain of atoms from about 3 atoms to about 30 atoms in length.
38. The method of Clause 32, wherein L comprises a chain of atoms from about 5 Å to about 45 Å in length.
39. The method of Clause 32, wherein L comprises a peptide.
40. The method of Clause 32, wherein L comprises one or more phenylalanine residues, each of which is independently optionally substituted.
41. The method of Clause 32, wherein L comprises at least one phenylalanyl-phenylalanyl, in which at least one phenyl is independently optionally substituted.
42. The method of Clause 32, wherein L comprises a polyoligoethylene glycoln (POEGn), a polyethylene glycoln (PEGn), or a mixture thereof, wherein n = 1-36.
43. The method of Clause 32, wherein X comprises a radioisotope bound to a chelator selected from the group consisting of:
- DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or a derivative thereof;
 - TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) or a derivative thereof;
 - SarAr (1-N-(4-Aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine or a derivative thereof;
 - NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) or a derivative thereof;
 - NETA (4-[2-(bis-carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl) acetyc acid or a derivative thereof
 - TRAP (1,4,7-triazacyclononane-1,4,7-tris[methyl(2-carboxyethyl)phosphinic acid) or a derivative thereof;

- HBED (N,N0-bis(2-hydroxybenzyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof;
 - 2,3-HOPO (3-hydroxypyridin-2-one) or a derivative thereof;
 - PCTA (3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9,-triacetic acid) or a derivative thereof;
 - DFO (desferrioxamine) or a derivative thereof;
 - DTPA (diethylenetriaminepentaacetic acid) or a derivative thereof;
 - OCTAPA (N,N0-bis(6-carboxy-2-pyridylmethyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof; or H2-MACROPA (N,N'-bis[(6-carboxy-2-pyridylmethyl)-4,13-diaza-18-crown-6] or a derivative thereof;
 - H2dedpa (1,2-[[carboxy)-pyridin-2-yl]-methylamino]ethane or a derivative thereof; and
 - EC20-head comprising β -l-diaminopropionic acid, aspartic acid, and cysteine.
44. The method of Clause 32, wherein X comprises a radioisotope selected from the group consisting of ^{18}F , ^{44}Sc , ^{47}Sc , ^{52}Mn , ^{55}Co , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , $^{114\text{m}}\text{In}$, $^{117\text{m}}\text{Sn}$, ^{124}I , ^{125}I , ^{131}I , ^{149}Tb , ^{153}Sm , ^{152}Tb , ^{155}Tb , ^{161}Tb , ^{177}Lu , ^{186}Re , ^{188}Re , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{224}Ra , ^{225}Ac , and ^{227}Th .
45. The method of Clause 32, further comprising administering to the patient an immune checkpoint inhibitor.
46. The method of Clause 45, wherein the immune checkpoint inhibitor is selected from the group consisting of an inhibitor of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), an inhibitor of programmed cell death protein 1 (PD-1), and an inhibitor of programmed cell death ligand 1 (PD-L1).
47. The method of Clause 45, wherein the immune checkpoint inhibitor is selected from the group consisting of nivolumab (Opdivo), pembrolizumab (Keytruda), ipilimumab (Yervoy), atezolizumab, avelumab, and durvalumab.
48. A method of treating a patient for prostate cancer or metastasis thereof with radiotherapy, which method comprises administering to the patient a conjugate of any of Clauses 21-28, or a pharmaceutically acceptable salt thereof, or the

pharmaceutical composition of Clause 30, whereupon the patient is treated for prostate cancer or metastasis thereof.

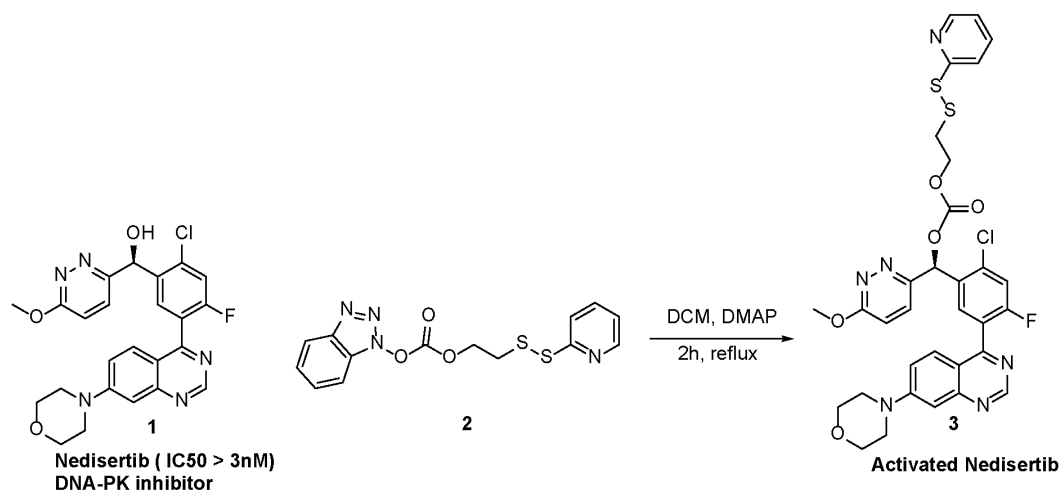
EXAMPLES

[00183] The disclosure can be better understood by reference to the following examples which are offered by way of illustration. The disclosure is not limited to the examples given herein.

Example 1: Synthesis of ATM inhibitors

[00184] Synthesis of activated DNAPKcs 3 (Scheme 1, below).

Heterobifunctional linker 2 (0.078 mmol, 0.027g) was added to a solution of compound 1 (0.052 mmol, 0.025g) and DMAP (0.052 mmol, 0.006 g) in DCM at room temperature under nitrogen atmosphere. The reaction mixture was stirred at reflux temperature for 3h. LCMS was monitored to observe the progress of the reaction. After 3h, the reaction was quenched by adding DCM. The product was purified by column chromatography using 5% DCM methanol mixture as mobile phase to obtain the pure product 3 as greenish solid (20 mg, yield 55%, t_R = 4.337 min). Compound 3 LCMS (+ESI) calcd for $[M+H]^+$ ($C_{32}H_{28}ClFN_6O_5S_2$)⁺ 695.1 found 695.1.



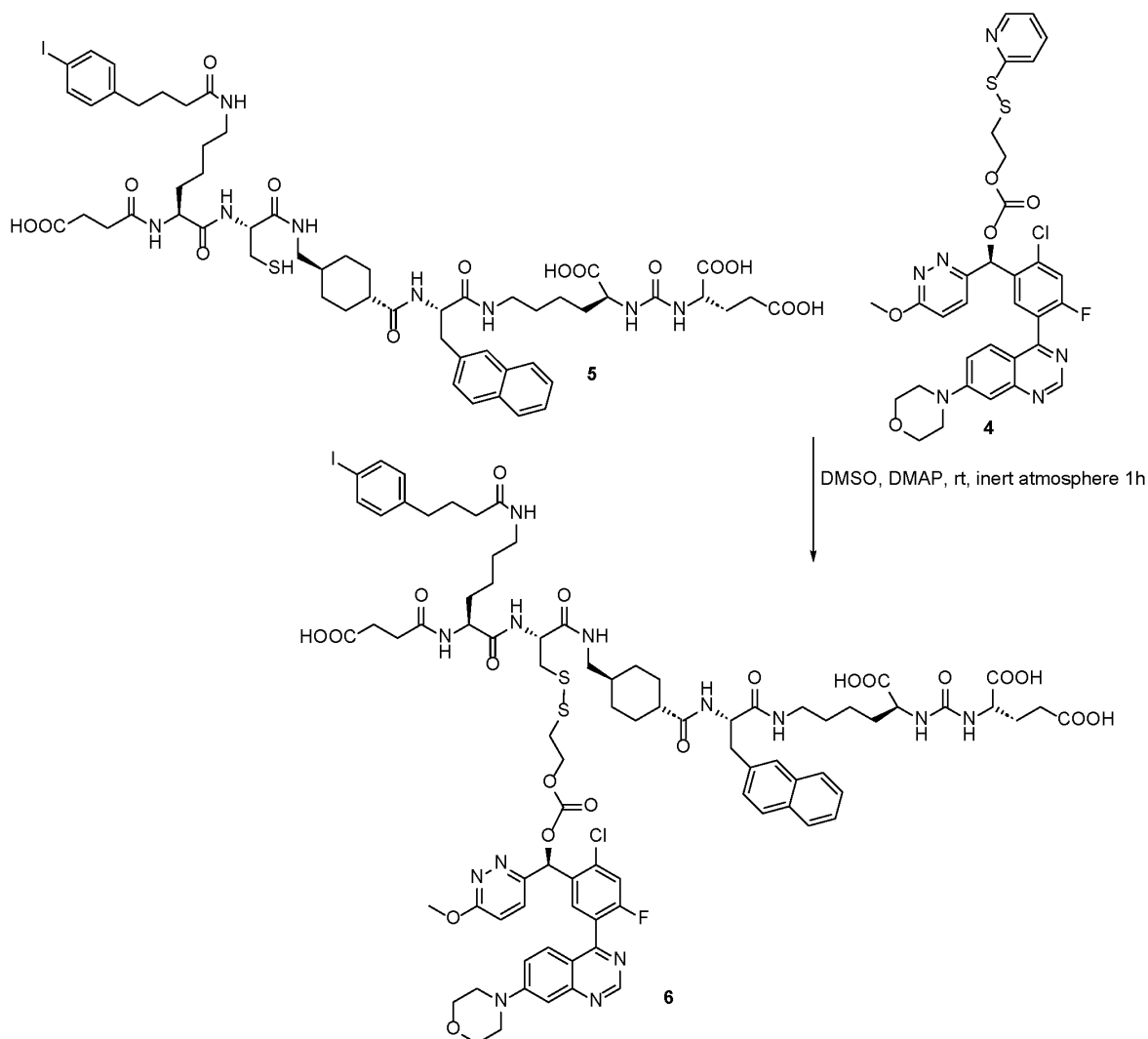
Scheme 1

[00185] Synthesis of conjugate 6 (Scheme 2, below). Compound 4 (1eqv) was dissolved in DMSO (1mL). DMAP (1eqv) was added to the reaction mixture. The reaction mixture was degassed continuously with Argon. Compound 5 (1eqv) was dissolved in DMSO and added to the reaction mixture portion wise (3 parts) with 20 mins interval. LCMS was monitored after 1h. After completion of the reaction the product 6 was purified using reverse phase HPLC using 10 mmolar ammonium acetate (pH=5) and acetonitrile. Pure fractions of 6 were collected using automatic fraction

collector, acetonitrile was evaporated under reduced pressure and after lyophilization pure bioconjugate **6** was obtained.

[00186] Conjugate **6** LCMS (+ESI) calcd for $[M+H]^+$ ($C_{83}H_{98}ClFIN_{13}O_{20}S_2$)⁺ 1841.5 found 1841.5. The product was isolated as yellow solid. (Yield 60%; t_R = 3.748 min).

5

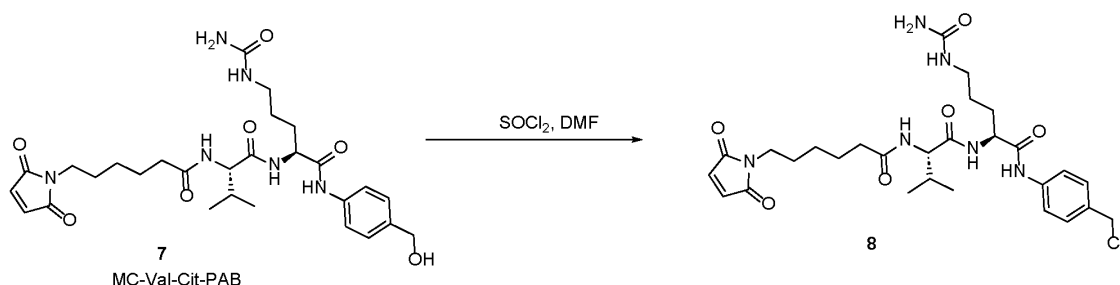


Scheme 2

[00187] **Synthesis of compound 8 (Scheme 3, below).** MC-Val-Cit-PAB-OH, **7** (0.087 mmol, 50 mg) was suspended in DMF (500 μ L) and heated to 80 °C to dissolve all solids. After cooling to 0 °C, thionyl chloride (50 μ L) was added portion wise. Following the addition, the reaction was held at 0 °C for 2-5 h. Reaction progression was monitored through LCMS. The crude reaction mixture was purified by column chromatography using 5% DCM methanol mixture as mobile phase to obtain the pure

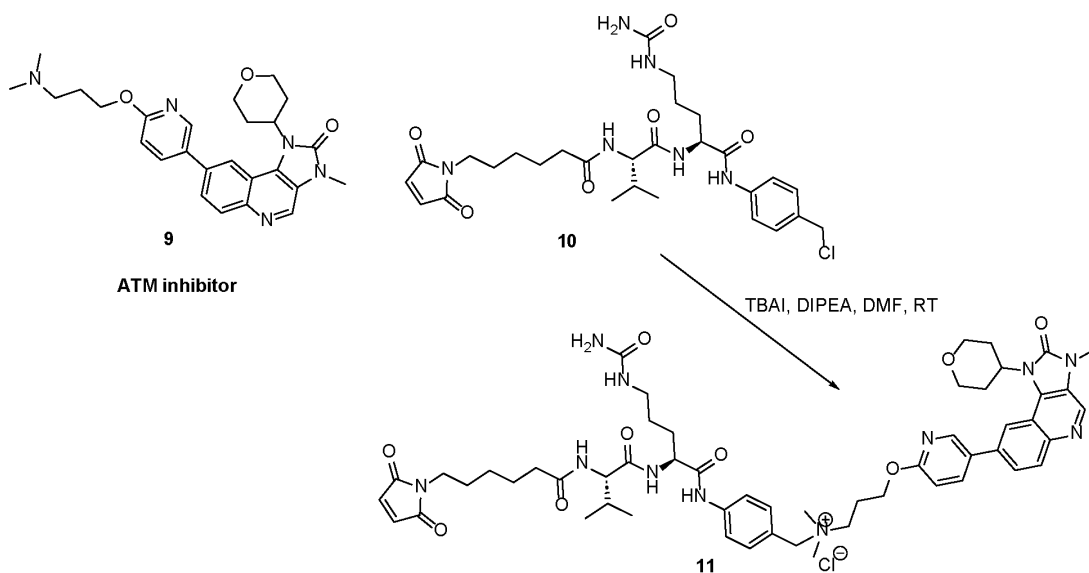
10

product **8** as yellow solid. (31 mg, yield 60%, t_R = 3.980 min). Compound **8** LCMS (+ESI) calcd for $[M+H]^+$ ($C_{28}H_{39}ClN_6O_6$)⁺ 591.3 found 591.3.



Scheme 3

- 5 **[00188] Synthesis of compound 11 (activation of ATM inhibitor; Scheme 5, below).** In a small vial, the tertiary amine **9** (1eq) and **10** N-[(1S)-1-[[[(1S)-1-[[4-(chloromethyl)phenyl]carbonyl]-4-ureido-butyl]carbonyl]-2-methyl-propyl]-6-(2,5-dioxypyrrol-1-yl)hexanamide (1.1 eq) were combined in DMF at room temperature. To the solution was added 0.5 eq of tetrabutylammonium iodide followed by N,N-diisopropylethylamine (2.5 eq) and the mixture stirred until all starting material amine
- 10 was consumed or until decomposition was observed by LCMS (3-48 h). The mixture was diluted with 1.5 mL of DMF and injected directly on HPLC for purification eluting with 20-60% acetonitrile:0.1% formic acid in water to afford the quaternary salt product. The pure product **11** was obtained as white solid (yield 45%, t_R = 3.980 min). Compound **11** LCMS (+ESI) calcd for $[M+H]^+$ ($C_{54}H_{70}N_{11}O_9$)⁺ 1016.5 found 1016.5.
- 15

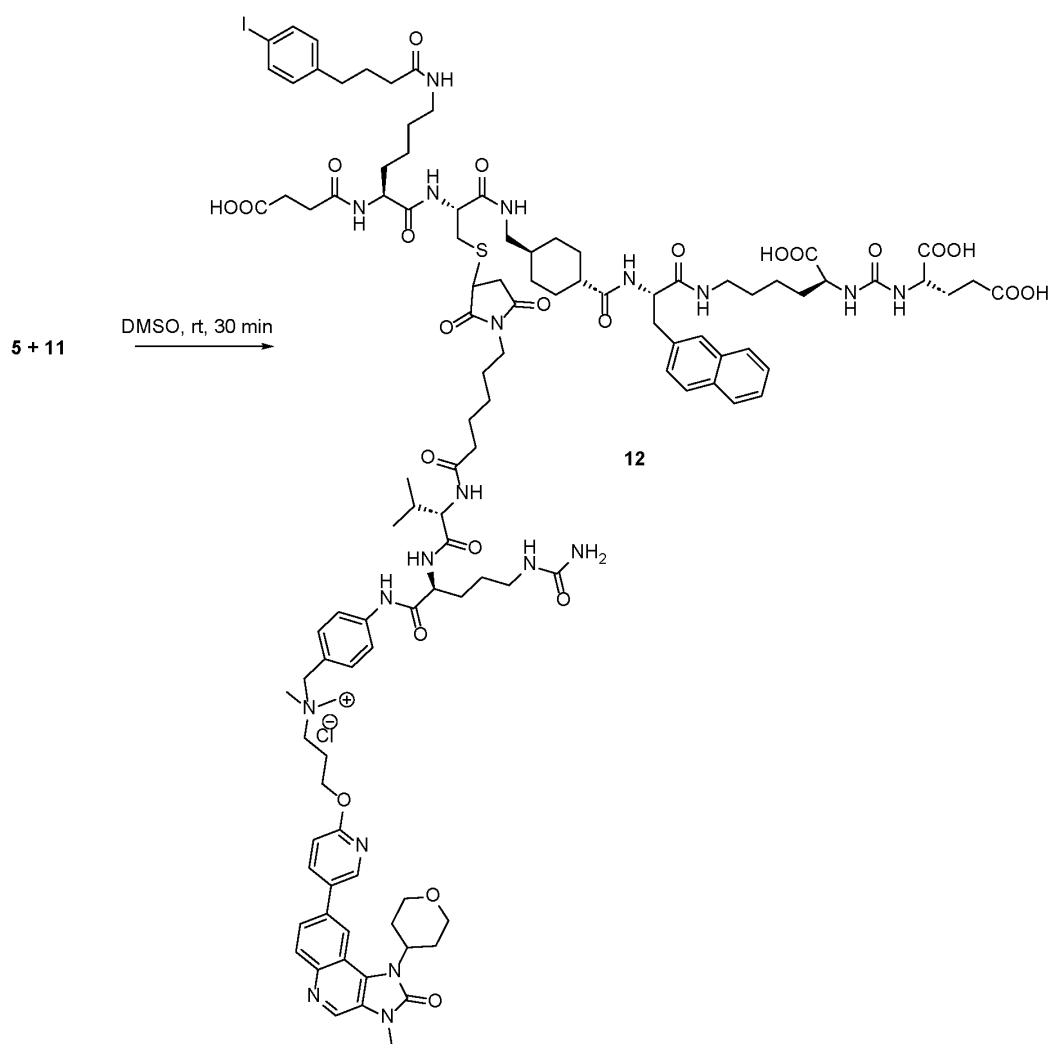


Scheme 4

- [00189] Synthesis of conjugate 12 (Scheme 5, below).** Compound **11** (1eqv) was dissolved in DMSO (1mL). The reaction mixture was degassed continuously with

Argon. Compound **5** (1eqv) was dissolved in DMSO and added to the reaction mixture. LCMS was monitored after 1h. After completion of the reaction the product **12** was purified using reverse phase HPLC using 10 mmolar ammonium acetate (pH=5) and acetonitrile. Pure fractions of **12** were collected using automatic fraction collector, acetonitrile was evaporated under reduced pressure and after lyophilization pure bioconjugate **12** was obtained.

[00190] Conjugate **12** LCMS (+ESI) calcd for $(M+H/2)^+$ $[(C_{110}H_{145}IN_{19}O_{24}S)^+]/2$ 1138.2 found 1138.2. The product was isolated as yellow solid. (Yield 60%; t_R = 3.678 min).



Scheme 5

Example 2

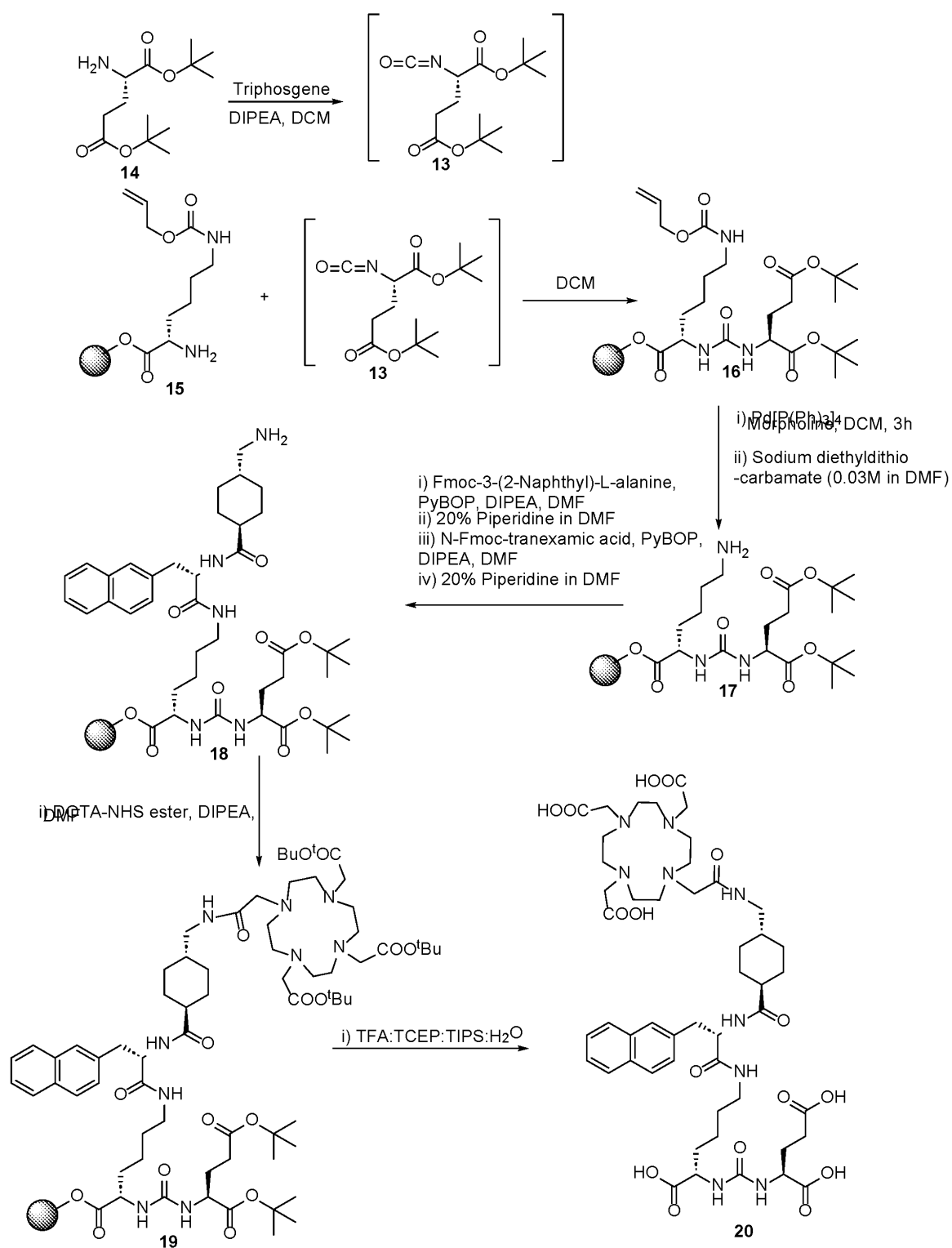
[00191] **Synthesis of Competition ligand 20 (Scheme 6, below).** In the first step, the isocyanate **13** of the glutamyl moiety was generated in situ by adding mixture

of Bis(tert-butyl)-L-glutamate hydrochloride **14** (2.1 mmol, 0.62g, 3 eqv) and 1 mL of DIPEA in 5 mL of dry DCM to a solution of triphosgene (0.7 mmol, 0.207g, 1eqv) in 5 mL dry DCM at -78°C. The reaction mixture was stirred at -78°C for 1h and further agitated another 1.5h in room temperature. The generated isocyanate **13** was transferred to a peptide vessel containing H-L Lys(Alloc)-2-Cl-trityl resin **15** (0.35 mmol, 500mg, resin loading 0.7mmol/g). The reaction continued for 16h with argon bubbling at room temperature to form the urea moiety **16**. The resin beads were washed with DCM (3 × 4 mL). Completion of the reaction was confirmed by performing the Kaiser test. After that, allyloxy protecting group was removed by agitating the resin bead using 100mg of tetrakis(triphenylphosphine) palladium (0) and 500μL of morpholine and 6mL of dry DCM for 3h. After deprotection resin beads were washed with DCM (3 × 4 mL) dried under vacuum and Completion of deprotection was confirmed by performing the Kaiser test. The resin was further washed with Sodium diethyldithiocarbamate (0.03M in DMF) to remove excess palladium complex to form **17**. The following coupling of Fmoc-naphthyl alanine (0.7mmol, 0.306g) and Fmoc-transexamic acid (0.7mmol, 0.266g) were performed using PyBOP (0.7mmol, 0.364g) DIPEA(0.5 mL) and DMF(6 mL). After each peptide coupling Fmoc group was deprotected using 20% piperidine in DMF for 30min to form **18**. Finally, DOTA-tris(tBu)ester NHS ester(0.428g, 0.525mmol) carboxylic acid coupled with compound **18** to form the conjugate **19**.

[00192] General procedure for peptide cleavage from resin beads. A mixture of 14.25 mL trifluoroacetic acid (TFA), 0.375 mL triisopropylsilane (TIPS), and 0.375 mL H₂O was prepared, and 7.5 mL of this cocktail solution was added to resin beads and argon was bubbled through the solution for 30 min. Same procedure was repeated one more time using remaining 7.5 mL (30min) of cocktail solution. The collected mother liquor from cleavage was evaporated under reduced pressure and the concentrated viscous liquid was precipitated in ice cold diethyl ether to form crude compound **20**.

[00193] Analytical HPLC method. The Product formation was further confirmed by LCMS. The purity of bio-conjugate **20** was analyzed using Agilent 1260 Infinity II system. Typically, a solution of bio-conjugate **20** in DMF was injected via autosampler and eluted using XBridge® Shield RP18 3.5μm, 3.0×50mm column at a flow rate of 0.750mL/min (mobile phase, A = 10mmolar ammonium bicarbonate at pH 7 and B = acetonitrile). The initial mobile phase is 95% buffer and 5% acetonitrile. The percentage of acetonitrile gradually increase from 5%-95% in a period of 4.5min and came back to its initial composition within another 2.5min. The chromatogram of **20** was recorded on the variable wavelength detector at 220 nm with t_R = 2.6min.

5 [00194] **RP-Combi flush method for purification.** The crude conjugate **20** was purified with 15.5g C18Aq High performance column with a flow rate of 30mL/min. (A= 20mmolar ammonium acetate pH=7 and B= acetonitrile). The acetonitrile percentage gradually increased from 0-50 percent over a period of 25 min to get the pure compound. The chromatogram was recorded at 220nm. Pure fractions of **20** were collected using automatic fraction collector, acetonitrile was evaporated under reduced pressure and after lyophilization pure bioconjugate **20** was obtained.



Scheme 6

Example 3

[00195] Radiolabeling. PSMA-617 **20** was dissolved in NH₄OAc buffer (10mMolar, pH 6.0) and labeled with [¹⁷⁷Lu]Lu⁺³ (National Isotope Development Center).

[00196] Animal Husbandry. Mice were provided normal rodent chow and water and maintained on a standard 12-h light–dark cycle. All animal procedures were approved by the Purdue Animal Care and Use Committee.

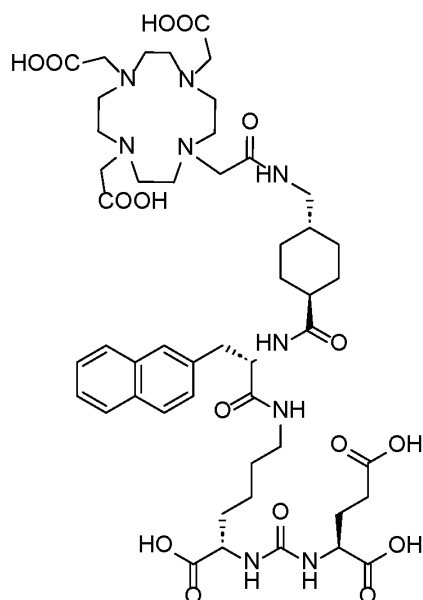
[00197] Tumor Models Athymic nude mice were inoculated on their shoulders with 5×10⁵ LNCaP cells, using 150μL of PBS and metrigel (2:1).

[00198] Radiotherapy. Mice bearing LNCaP tumors were randomly divided into control and treatment groups to ensure equal starting tumor volumes. Each cohort received a single intravenous injection of either vehicle alone or vehicle with ¹⁷⁷Lu-radiolabeled PSMA-617 **20** conjugate on day 0. Mice bearing LNCaP tumors were distributed in 5 different groups and there were 5 mice in each group. The groups are as follows:

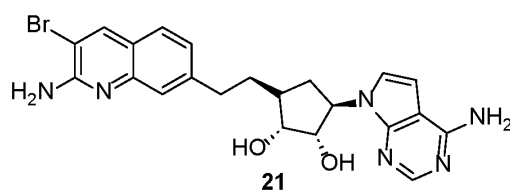
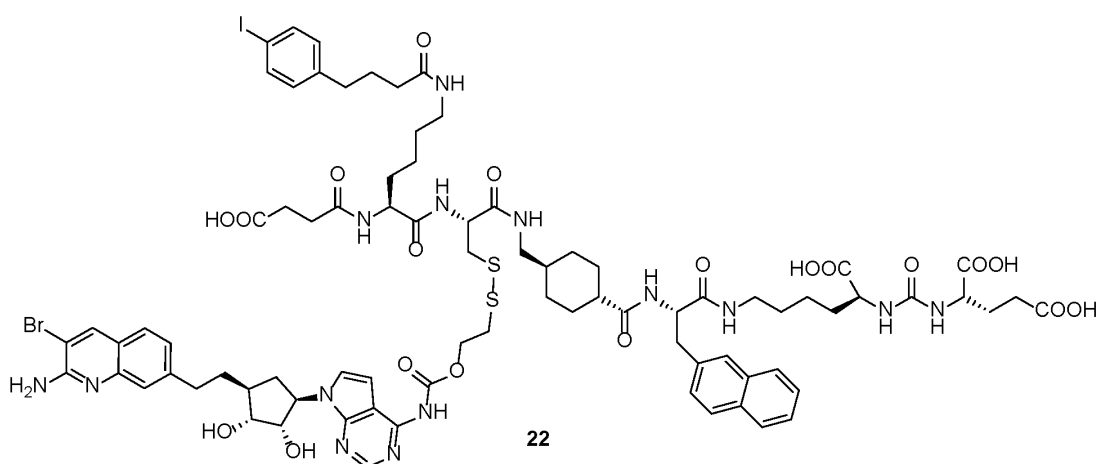
- untreated or control group;
- treated with 9.25 MBq of ¹⁷⁷Lu PSMA-617 **20**; treated with 9.25 MBq of ¹⁷⁷Lu PSMA-617 and 10 nmoles of compound **21** (daily for 28 days); and
- treated with 9.25 MBq of ¹⁷⁷Lu PSMA 617 and 10 nmoles of compound **22** (daily for 28 days);

except the untreated group every group received 9.25 MBq of ¹⁷⁷Lu PSMA-617 **20**.

[00199] For ease of reference, the structure of each compound is as follows:



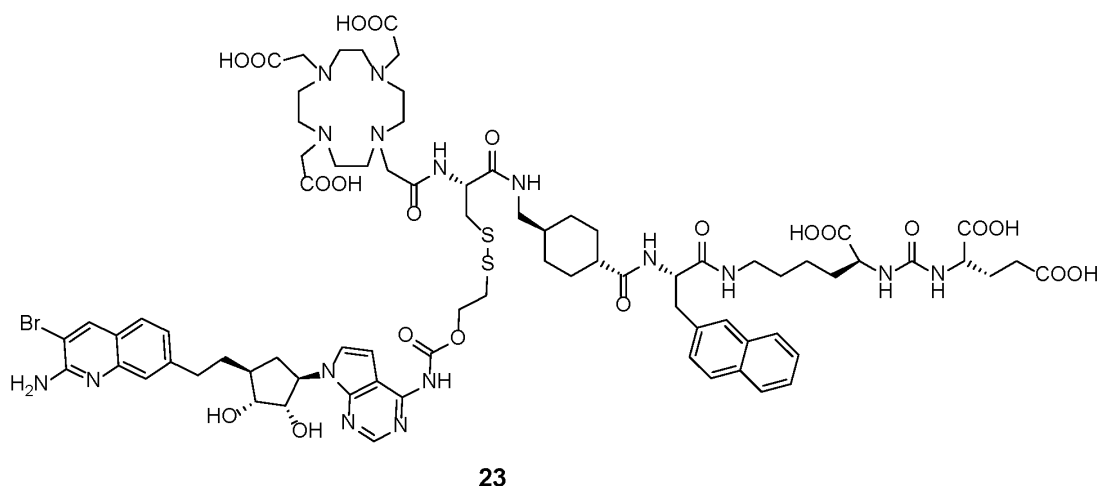
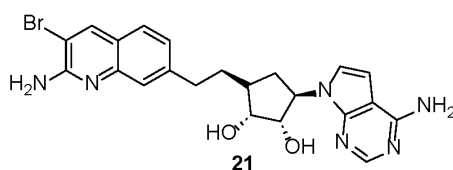
Compound **20**:

Compound **21**:Compound **22**:

[00200] The tumor growth was measured using a caliper in two perpendicular directions. The mice were euthanized on reaching one of the predefined endpoint criteria according to the regulations of the Institutional Animal Care and Use Committee. Data show that the combination therapy with ^{177}Lu PSMA-617 **20** and compound **22** showed 81% tumor growth inhibition (**FIG. 9**) relative to control. There is no significant body weight loss during the therapy study. See **FIG. 10** for data from combination therapy with ^{177}Lu PSMA-617 **20** and compound **22**. The survival curves show that the mice survived about 30 days longer with the combination therapy relative to control. See **FIG. 11** for combination therapy with ^{177}Lu PSMA-617 **20** and compound **22**.; and **FIG. 14** for combination therapy with ^{177}Lu PSMA-617 **29** and compound **34**.

Example 4

[00201] **γH2AX staining and fluorescence imaging.** LNCaP (PSMA⁺), DU145 (PSMA⁺), PC3 (PSMA⁻) cells were grown in T-75 flask in RPMI 1640 medium supplemented with 1% penicillin streptavidin and 10% FBS at 37°C. The cells were with 5% CO₂ in the humidified incubator. For the experiment cells were seeded to 6 cm dish (4x10⁵ cells/dish) with a coverglass and 2mL of media was added to each well. 2μl of fresh working solution of 3 different compounds (PSMA-617 **20**, JNJ64619178 **21**, PSMA-617-JNJ **23**):



with 3 different concentrations (10 nM, 1 nM, 0.1 nM) was added to each well compound. The cells were incubated for 1h. The cells treated with IR:2Gy after adding compounds for 1h. The medium was changed immediately after IR. After radiation cells were incubated for 6 hours to recover the damaged DNA before harvesting.

[00202] Cells were rinsed with 1XPBS solution once and fixed with 3.7% formaldehyde solution. The cells were incubated for 20 minutes at room temperature. The cells were rinsed with 1XPBS solution 3 times than 0.2% of Triton X-100 solution was added to each well, incubated for 5 minutes at room temperature and again rinsed with 1XPBS for 3 times. The cells were blocked with 5% milk in 1XPBS solution for 1h at room temperature. Cells were incubated with primary antibody [Phospho-Histone H2A.X (ser139) (20E3)] solution (1:400 in 5% milk PBS solution) in the cover glass (100 μ L) for overnight at 40°C. Next day the cells were washed with 1XPBS 3 times. After that secondary antibody with DAPI solution was added to the cells and (anti-rabbit- Alexa Fluors: 1:2000, DAPI of final concentration 1mg/mL in 5% milk PBS solution). Incubate for 1 hour at room temperature. Cells were washed with 1XPBS 3 times and Cells on coverslips were mounted on glass slides using the ProLong® Antifade Kit (Invitrogen). Images were captured using inverted fluorescence microscope under oil immersion (60 x objective) (Nikon Instruments Melville, NY, USA) and through NIS Elements software. Incubation of LNCaP, DU145 and PC-3 cells with different compounds has been done for 1 hour and result showed that the double stranded DNA breakage repair is significantly lower (more foci) with compound **23** at

1 nM for both LNCaP (PSMA+), DU145 (PSMA+). See FIGS. 12-14. PC-3 cell lines do not express PSMA so the effect is not significant which further demonstrates the specificity of compound 23. See FIG. 15.

[00203] The results shown in FIGS. 12-14 demonstrate that compound 23, which comprises a PRMT5 inhibitor, significantly decreases DSB repair relative to vehicle (i.e., DMSO), the PRMT5 inhibitor alone (21), and to PSMA-617 (20), which carries ¹⁷⁷Lu but does not comprise a PRMT5 inhibitor.

Example 5

[00204] **Tumor model and therapeutic study.** Five-week-old male nu/nu mice were inoculated subcutaneously with LNCaP cells (5.0×10^6 cells/mouse in 100 μ L PBS and 50 μ L Matrigel) on their shoulders. Growth of the tumors was measured in two perpendicular directions every 2 days using a caliper (body weights were monitored on the same schedule), and the volumes of the tumors were calculated as $0.5 \times L \times W^2$ (L longest axis and W axis perpendicular to L in millimeters). Once tumors reached between 150-200 mm³ in volume, animals will be treated. xenograft tumors were subjected to FIR (5 Gy/fraction) 12 mice divided into following four different groups:

- Group 1 Untreated: 3 mice
- Group 2 Tumor bearing mice treated with external beam radiation (IR): 3 mice.
- Group 3 Tumor bearing mice treated with only compound 23 (10 nmol): 3 mice.
- Group 4 Tumor bearing mice treated with external beam radiation (IR) + compound 23 (10 nmol): 3 mice.

For group 4 compound 23 was administered 1 hour before the external beam radiation.

[00205] Data show that the combination therapy with compound 23 showed tumor growth inhibition (FIG. 16) relative to control. There is no significant body weight loss during the therapy study. See FIG. 17.

What is claimed is:

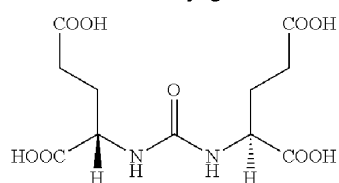
1. A conjugate of formula T-L-A in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

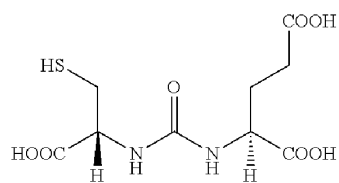
L is a linker, and

A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor.

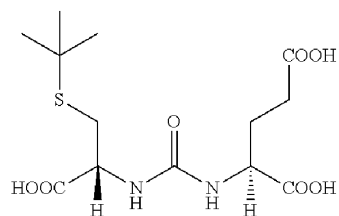
2. The conjugate of claim 1, wherein T is:



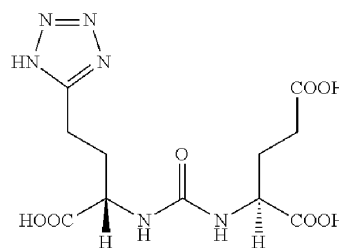
DUPA
IC₅₀ = 47 nM
K_i = 8 nM



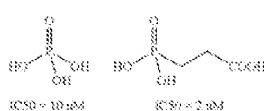
MUPA
IC₅₀ = 6.9 nM



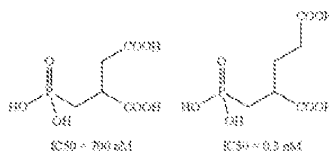
IC₅₀ = 29 nM



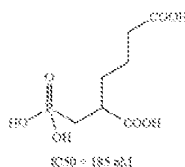
K_i = 0.9 nM



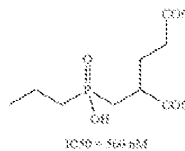
IC₅₀ = 10 nM



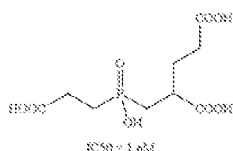
IC₅₀ = 2 nM



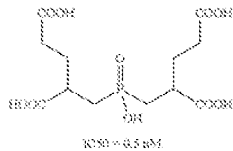
IC₅₀ = 700 nM



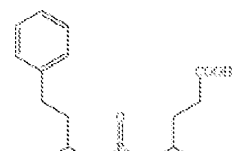
IC₅₀ = 0.2 nM



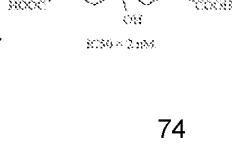
IC₅₀ = 185 nM



IC₅₀ = 564 nM



IC₅₀ = 1 nM



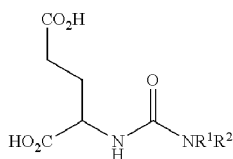
IC₅₀ = 0.5 nM

or



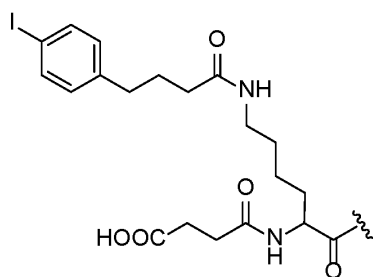
IC₅₀ = 2 nM

3. The conjugate of claim 1, wherein T is:

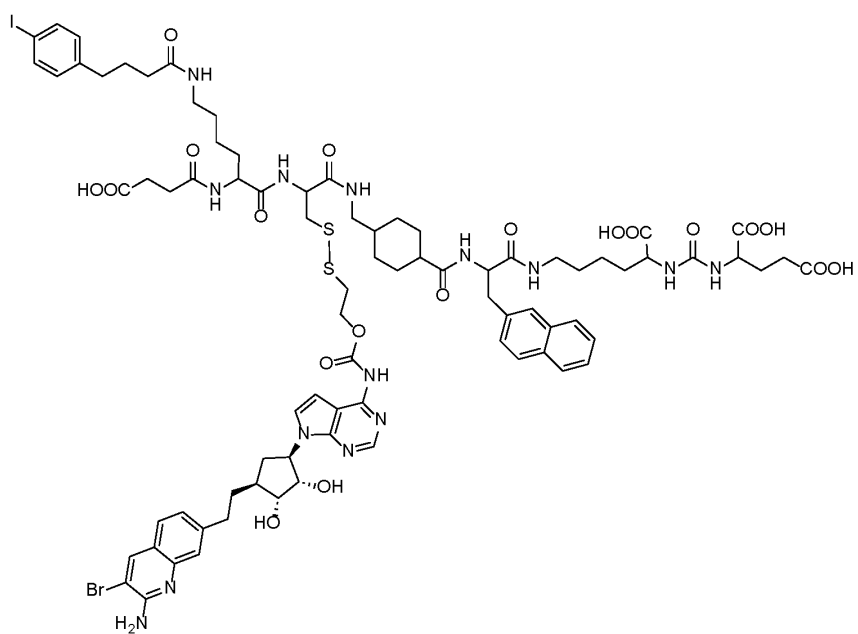


- , in which R^1 and R^2 are independently selected from hydrogen, carboxylic acid, which is optionally substituted, malonic acid, succinic acid, glutamic acid, and adipic acid.
4. The conjugate of claim 3, wherein the substituted carboxylic acid is thiolacetic acid or thiopropionic acid.
5. The conjugate of claim 1, wherein T is a urea of (a) an amino dicarboxylic acid or a derivative thereof and (b) an amino dicarboxylic acid or a derivative thereof, wherein (a) and (b) can be the same or different.
6. The conjugate of any one of claims 1-5, wherein L comprises a chain of atoms from about 3 atoms to about 30 atoms in length.
7. The conjugate of any one of claims 1-6, wherein L comprises a chain of atoms from about 5 Å to about 45 Å in length.
8. The conjugate of any one of claims 1-7, wherein L comprises a peptide.
9. The conjugate of any one of claims 1-8, wherein L comprises one or more phenylalanine residues, each of which is independently optionally substituted.
10. The conjugate of any one of claims 1-9, wherein L comprises at least one phenylalanyl-phenylalanyl, in which at least one phenyl is independently optionally substituted.
11. The conjugate of any one of claims 1-10, wherein L comprises a polyoligoethylene glycoln (POEGn), a polyethylene glycoln (PEGn), or a mixture thereof, wherein $n = 1-36$.
12. The conjugate of any one of claims 1-11, wherein L comprises a disulfide.

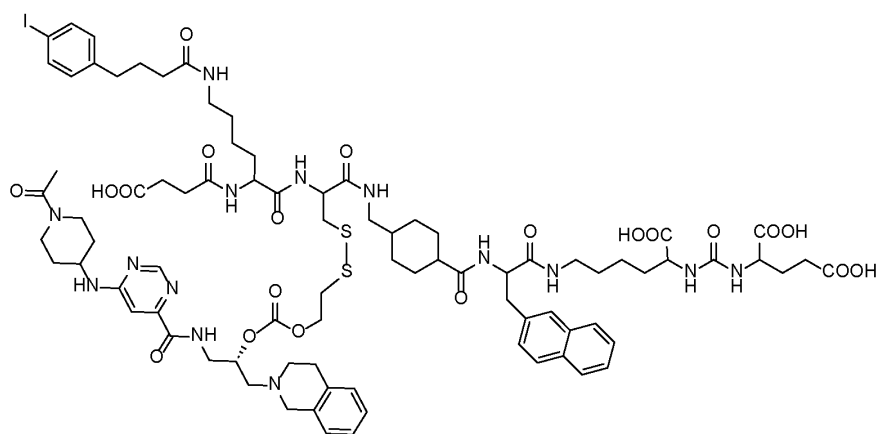
13. The conjugate of claim 1, wherein the PRMT5 inhibitor comprises a radical of JNJ-64619178 or a radical of GSK3326595.
14. The conjugate of claim 1, wherein the ATM inhibitor comprises a radical of AZD0156.
15. The conjugate of claim 1, wherein the DNA-PK inhibitor comprises a radical of Nedisertib, AZD7648, KU57748, Torin 2, PI-103, NU7026, Samotolisib, PIK 90, CC-115, PIK-75 hydrochloride, VX-984, KU-0060648, BAY-8400, DNA-PK-IN-2, DNA-PK-IN-1, DNA-PK-IN-4, DNA-PK-IN-5, DNA-PK-IN-6, XRD-0394, NU5455, STL127705, PI-103 hydrochloride, LTURM34, ZL-2201 free base, DNA-PK-IN-14, DNA-PK-IN-10, DNA-PK-IN-11 or DNA-PK-IN-13.
16. The conjugate of any one of claims 1-15 further comprising an albumin binding group.
17. The conjugate of claim 16, wherein the albumin binding group is of the formula:



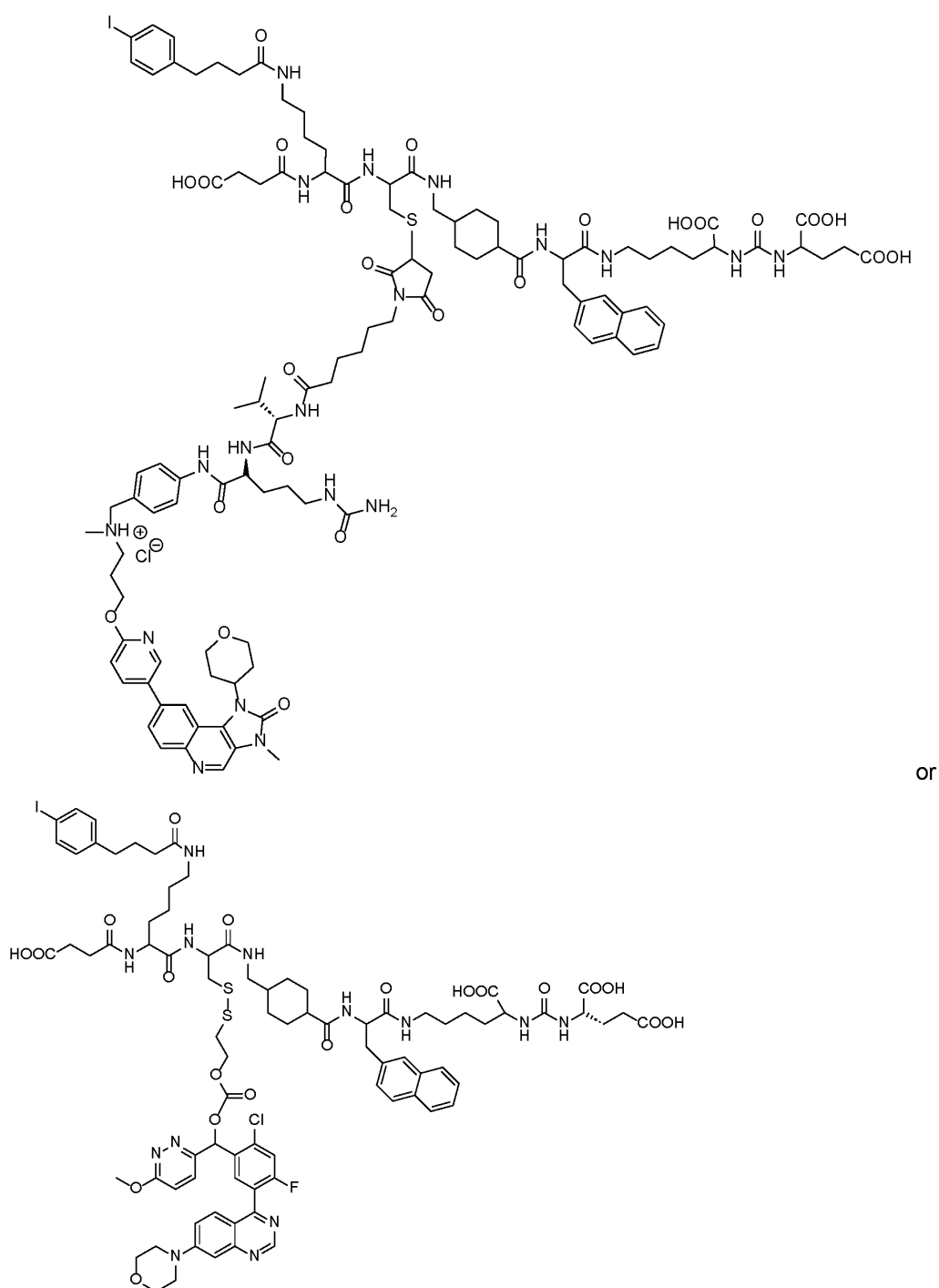
18. The conjugate of claim 16, wherein the albumin binding group is linked to L via a substituent or group present on L.
19. The conjugate of claim 16, wherein the albumin binding group is linked to L via an -NR⁴- group, wherein R⁴ is H or alkyl; or an -O- group.
20. A conjugate of the formula:



1



1



21. A conjugate of formula T-L(X)A in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

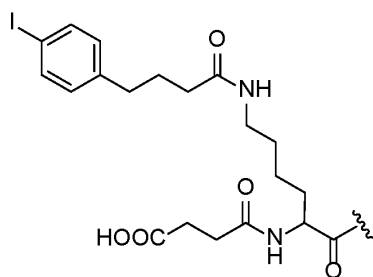
L is a linker,

A comprises an inhibitor of a DNA repair enzyme, wherein the inhibitor of a DNA repair enzyme is a protein arginine methyltransferase 5 (PRMT5) inhibitor, an ataxia-telangiectasia mutated (ATM) inhibitor or a DNA protein kinase (DNA-PK) inhibitor; and X is a radiotherapeutic or radioimaging agent.

22. The conjugate of claim 21, wherein A and X are connected to L.

23. The conjugate of claim 21 or 22, further comprising an albumin binding group.

24. The conjugate of claim 23, wherein the albumin binding group is of the formula:



25. The conjugate of claim 21 or 22, wherein the albumin binding group is linked to L via a substituent or group present on L, and wherein the albumin binding group is optionally linked to L as in claim 19 or 20.

26. The conjugate of any one of claims 21-25, wherein T is as defined in claims 2-5.

27. The conjugate of any one of claims 21-26, wherein L is as defined in claims 6-12.

28. The conjugate of any one of claims 21-27, wherein A is as defined in claims 13-15.

29. A pharmaceutical composition comprising a conjugate of any one of claims 1-20, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising a conjugate of any one of claims 21-28, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

31. A method of treating a patient for prostate cancer or metastasis thereof with radiotherapy, which method comprises administering to the patient a conjugate of

any of claims 1-20, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 29, whereupon the patient is treated for prostate cancer or metastasis thereof.

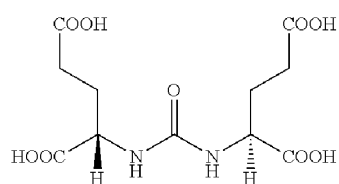
32. The method of claim 31, further comprising administering a conjugate of the formula T-L-X or a pharmaceutically acceptable salt thereof to the patient before or after administering a conjugate of any of claims 1-20 or the pharmaceutical composition thereof in which:

T is a ligand that targets prostate-specific membrane antigen (PSMA),

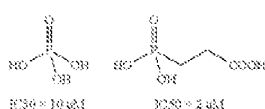
L is a linker, and

X is a radiotherapeutic or radioimaging agent.

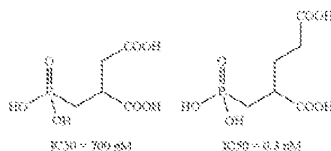
33. The method of claim 32, wherein T is:



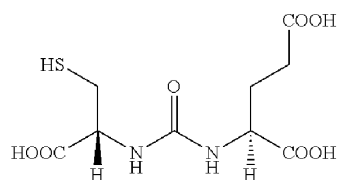
DOPA
IC₅₀ = 47 nM
K_i = 8 nM



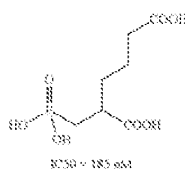
IC₅₀ = 10 nM IC₅₀ = 2 nM



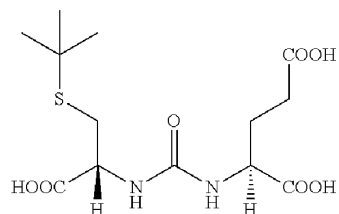
IC₅₀ = 709 nM IC₅₀ = 0.3 nM



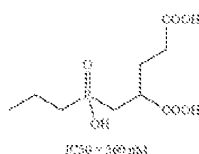
MUPA
IC₅₀ = 6.9 nM



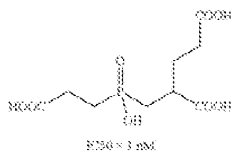
IC₅₀ = 185 nM



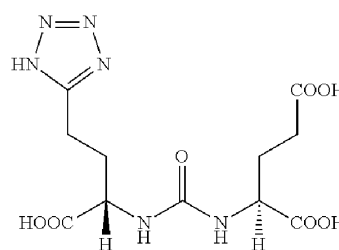
IC₅₀ = 29 nM



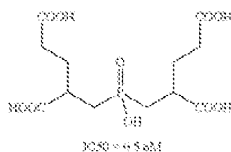
IC₅₀ = 569 nM



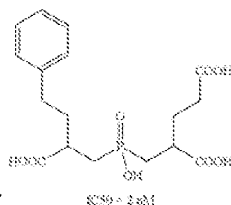
IC₅₀ = 1 nM



K_i = 0.9 nM



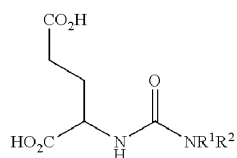
IC₅₀ = 4.5 nM



IC₅₀ = 2 nM

or

34. The method of claim 32, wherein T is:



, in which R¹ and R² are independently selected from hydrogen, carboxylic acid, which is optionally substituted, malonic acid, succinic acid, glutamic acid, and adipic acid.

35. The method of claim 32, wherein the substituted carboxylic acid is thiolacetic acid or thiopropionic acid.

36. The method of claim 32, wherein T is a urea of (a) an amino dicarboxylic acid or a derivative thereof and (b) an amino dicarboxylic acid or a derivative thereof, wherein (a) and (b) can be the same or different.
37. The method of claim 32, wherein L comprises a chain of atoms from about 3 atoms to about 30 atoms in length.
38. The method of claim 32, wherein L comprises a chain of atoms from about 5 Å to about 45 Å in length.
39. The method of claim 32, wherein L comprises a peptide.
40. The method of claim 32, wherein L comprises one or more phenylalanine residues, each of which is independently optionally substituted.
41. The method of claim 32, wherein L comprises at least one phenylalanyl-phenylalanyl, in which at least one phenyl is independently optionally substituted.
42. The method of claim 32, wherein L comprises a polyoligoethylene glycoln (POEGn), a polyethylene glycoln (PEGn), or a mixture thereof, wherein n = 1-36.
43. The method of claim 32, wherein X comprises a radioisotope bound to a chelator selected from the group consisting of:
- DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or a derivative thereof;
 - TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) or a derivative thereof;
 - SarAr (1-N-(4-Aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-eicosane-1,8-diamine or a derivative thereof;
 - NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) or a derivative thereof;
 - NETA (4-[2-(bis-carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl) acetyc acid or a derivative thereof
 - TRAP (1,4,7-triazacyclononane-1,4,7-tris[methyl(2-carboxyethyl)phosphinic acid) or a derivative thereof;

- HBED (N,N0-bis(2-hydroxybenzyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof;
 - 2,3-HOPO (3-hydroxypyridin-2-one) or a derivative thereof;
 - PCTA (3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-triene-3,6,9,-triacetic acid) or a derivative thereof;
 - DFO (desferrioxamine) or a derivative thereof;
 - DTPA (diethylenetriaminepentaacetic acid) or a derivative thereof;
 - OCTAPA (N,N0-bis(6-carboxy-2-pyridylmethyl)-ethylenediamine-N,N0-diacetic acid) or a derivative thereof; or H2-MACROPA (N,N'-bis[(6-carboxy-2-pyridylmethyl)-4,13-diaza-18-crown-6] or a derivative thereof;
 - H2dedpa (1,2-[[carboxy)-pyridin-2-yl]-methylamino]ethane or a derivative thereof; and
 - EC20-head comprising β -l-diaminopropionic acid, aspartic acid, and cysteine.
44. The method of claim 32, wherein X comprises a radioisotope selected from the group consisting of ^{18}F , ^{44}Sc , ^{47}Sc , ^{52}Mn , ^{55}Co , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{86}Y , ^{89}Zr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , $^{114\text{m}}\text{In}$, $^{117\text{m}}\text{Sn}$, ^{124}I , ^{125}I , ^{131}I , ^{149}Tb , ^{153}Sm , ^{152}Tb , ^{155}Tb , ^{161}Tb , ^{177}Lu , ^{186}Re , ^{188}Re , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{224}Ra , ^{225}Ac , and ^{227}Th .
45. The method of claim 32, further comprising administering to the patient an immune checkpoint inhibitor.
46. The method of claim 45, wherein the immune checkpoint inhibitor is selected from the group consisting of an inhibitor of cytotoxic T lymphocyte-associated protein 4 (CTLA-4), an inhibitor of programmed cell death protein 1 (PD-1), and an inhibitor of programmed cell death ligand 1 (PD-L1).
47. The method of claim 45, wherein the immune checkpoint inhibitor is selected from the group consisting of nivolumab (Opdivo), pembrolizumab (Keytruda), ipilimumab (Yervoy), atezolizumab, avelumab, and durvalumab.
48. A method of treating a patient for prostate cancer or metastasis thereof with radiotherapy, which method comprises administering to the patient a conjugate of any of claims 21-28, or a pharmaceutically acceptable salt thereof, or the

pharmaceutical composition of claim 30, whereupon the patient is treated for prostate cancer or metastasis thereof.