

MCL-1 INHIBITOR COMPOUNDS AND USE IN ANTIBODY DRUG CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to US Provisional Application No. 63/600,479, filed November 17, 2023, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present disclosure relates to inhibitors of induced myeloid leukemia cell differentiation protein (MCL-1), compositions comprising such compounds, antibody drug conjugates (ADCs) comprising such compounds, and methods of using such compounds, compositions, and ADCs.

BACKGROUND

[0003] Myeloid cell leukemia-1 or MCL1 is a member of the Bcl2 family of anti-apoptotic proteins and is an attractive target for cancer therapy. MCL1 is found at the outer membrane of the mitochondria and is involved in a series of protein-protein interactions with the pro-apoptotic BH3 proteins (BIM, PUMA, NOXA) to regulate cell apoptosis. Inhibition of MCL1 by a BH3-mimetic therapeutic agent ultimately results in release of the BCL effectors BAK and BAX, which oligomerize to form pores in the mitochondrial membrane, resulting in cytochrome c release into the cytosol followed by cell apoptosis. MCL1 is amplified in around 10% of human cancers, is broadly expressed in both hematological and solid tumors and is upregulated as a resistance mechanism to chemotherapy and targeted therapies. Therefore MCL1 is a target of high interest for potentially treating a variety of cancers, including AML.

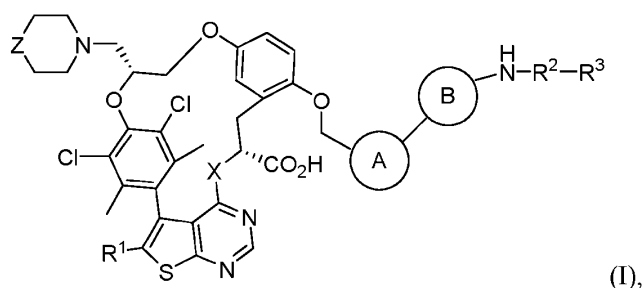
[0004] Acute myeloid leukemia (or AML) is a type of blood cell cancer that originates in the bone marrow and spreads to the blood and potentially other organs, ultimately interfering with the body's ability to produce blood cells. AML can progress rapidly if untreated, ultimately resulting in death. Recent advances in targeted treatments have allowed subsets of patients to receive newer therapies such as the BCL2 inhibitor venetoclax (venclexta) or the antibody-drug conjugate Mylotarg. However, the treatment of the majority of patients with AML has not

significantly changed in the last 40 years, and long-term survival rates from the disease remain low.

[0005] One of the biggest challenges to the successful application of antibody-drug conjugates (ADCs) for treating cancer patients has been the toxicities encountered at (or even below) efficacious doses. The resulting narrow or nonexistent therapeutic indices can be a result of many factors. The first generation of antibody-drug conjugates (such as Mylotarg) have been limited in their therapeutic use because of the instability of the linker connecting the drug or payload to the antibody. Premature release of the cytotoxic payload both decreases the efficacy and increases the off-target toxicity. Yet even ADCs with stable linkers have still found narrow therapeutic indices, potentially caused by non-specific uptake (e.g. pinocytosis) by the ADC in normal tissue. Despite decades of work in the space of targeted therapies, including ADCs, for different types of cancer (including AML), there still remains significant unmet need that directly impacts the lives of patients. One method to improve the safety profile around ADCs is the utilization of novel payloads with improved selectivity between tumor cells and normal tissue.

BRIEF SUMMARY OF THE INVENTION

[0006] In some aspects, provided herein is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

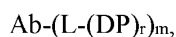
each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-OH$.

[0007] In further aspects, provided herein are subformulae of Formula (I), and exemplified compounds of Formula (I). Further provided are conjugates comprising the compounds of Formula (I), and methods of using said compounds and conjugates. Thus, in another aspect, provided herein is a conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

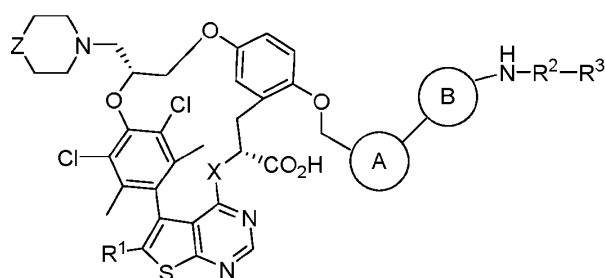
L is a linker;

DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

wherein the drug payload is a compound of Formula (I):



(I),

or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

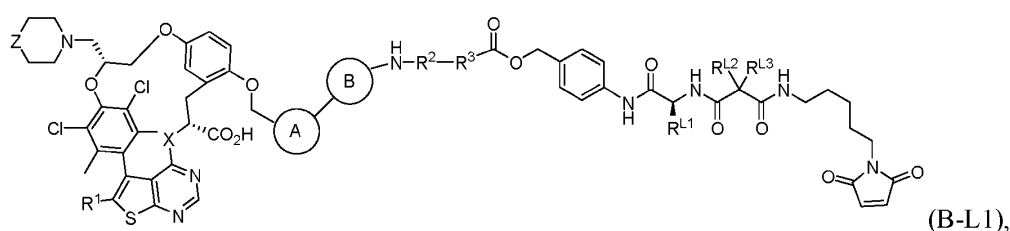
p and q are independently integers from 1 to 8; and

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

[0008] In some aspects of the ADCs provided herein, the linker is a peptide linker or a peptidomimetic linker. In certain aspects, the antibody binds to one or more to one or more tumor-associated antigens or cell-surface receptors selected from the group consisting of CLL1, CD71, Trop2, MSLN, NaPi2b, Ly6E, EpCAM, STEAP1, STEAP2, TrpM4, CD21, CD79a, CD72, MUC16, HER2, CD33, CD22, CD79b, LIV1, CD123, CD74, BCMA, and FcRH5.

[0009] In additional aspects, provided herein is a pharmaceutical composition comprising any of the conjugates provided herein, and a pharmaceutically acceptable excipient. Further provided are methods of treating a disorder in a subject in need thereof, by administering to the subject a therapeutically effective amount of a conjugate as provided herein, or a pharmaceutical composition as provided herein. In some aspects, the disorder is a cancer, tumor, or other malignancy.

[0010] In addition, provided herein is a drug payload-linker conjugate, wherein the drug payload-linker conjugate is of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-OH$; each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂, or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

Z is N - C_{1-6} alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are

attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or
haloheterocycloalkyl;
each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;
each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and
p and q are independently integers from 1 to 8.

[0011] Additional aspects of these compounds, conjugates, compositions, and methods, and additional aspects, are described in more detail herein. It is to be understood that one, some, or all of the properties of the various aspects and embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** Provides a reaction scheme for the synthesis of macrocycle intermediate 1g, as described in Example 2.

[0013] **FIG. 2** provides the structures of comparative Compound A, Compound B, and Compound C, which are evaluated in certain biological examples provided herein.

[0014] **FIG. 3A** and **3B** are graphs demonstrating the cytotoxic potency of different concentrations of Compound A, Compound B, and Compound C, as free drug on the viability of SK-BR-3 adenocarcinoma cells (**FIG. 3A**) and CAMA-1 human breast cancer cells (**FIG. 3B**) as described in Biological Example 1.

[0015] **FIG. 4A** and **4B** are graphs demonstrating the cytotoxic potency of different concentrations of Compound A, Compound B, and Compound C as free drug on the viability of SU-DHL5 (**FIG. 4A**) and SU-DHL10 (**FIG. 4B**) acute myeloid leukemia (AML) cell lines, as described in Biological Example 3.

[0016] **FIGS. 5A, 5B, and 5C** are graphs demonstrating the cytotoxic potency of different concentrations of Compound A, Compound B, and Compound 100 on *in vitro* viability of MOLM-16 (**FIG. 5A**), HL-60 (**FIG. 5B**), and OCI-AML3 (**FIG. 5C**) AML cell lines.

[0017] **FIGS. 6A, 6B, and 6C** are graphs demonstrating the cytotoxic potency of Compound A, Compound B, and Compound 100 as ADCs conjugated to the anti-CD33 antibody 15G15 via

the sq-Cit linker on *in vitro* viability of the AML cell lines EOL-1 (FIG. 6A), MV-4-11 (FIG. 6B), and NOMO-1 (FIG. 6C), as described in Biological Example 5.

[0018] FIG. 7 is a graph demonstrating the cytotoxic potency of Compound B, Compound C, and Compound 100 conjugated to 7C2 antibody via a sq-Cit linker, on *in vitro* SK-BR-2 adenocarcinoma cells.

[0019] FIG. 8A is a size exclusion chromatography (SEC) analysis trace of an exemplary anti-Her2 7C2-sqCit linker-Compound 100 ADC, prepared according to Example 11.

[0020] FIG. 8B is an LC-MS analysis of the light chain of an exemplary anti-Her2 7C2-sqCit linker-Compound 100 ADC, prepared according to Example 11.

[0021] FIG. 8C is an LC-MS analysis of the heavy chain of an exemplary anti-Her2 7C2-sqCit linker-Compound 100 ADC, prepared according to Example 11.

[0022] FIG. 9 is a graph demonstrating cytotoxicity of exemplary anti-CD33-sqCit ADCs comprising payloads described herein, on *in vitro* MV-4-11 cells as described in Biological Example 7.

[0023] FIG. 10 is a graph demonstrating the cytotoxicity of anti-CD33-sqCit ADCs comprising an exemplary payload or comparison payload; non-target ADCs comprising the same payloads; and vehicle, in an *in vivo* MV4-11 assay in NSG mice as described in Biological Example 8. The graph presents % tumor burden evaluated at 9 days post-ADC dose.

[0024] FIG. 11 is a graph demonstrating the dose response to an exemplary DAR6 ADC using the anti-CD33 antibody 15G15, the sq-Cit linker, and payload Compound 100, evaluated in an *in vivo* MV4-11 assay in NSG mice as described in Biological Example 9. The graph presents bone marrow tumor burden (as live tumor cells / 50k) at day 7.

[0025] FIG. 12 is a graph demonstrating cytotoxicity of anti-CD33-sqCit ADCs comprising an exemplary payload or comparison payload, in an *in vivo* subcutaneous MV4-11 assay in SCID mice, as described in Biological Example 10. The graph presents the tumor volume over time.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Disclosed herein are compounds of Formula (X) and (I), or a pharmaceutically acceptable salt thereof, and antibody drug conjugates (ADCs) comprising said compounds. The

ADCs provided herein may be useful in the treatment of cancer, such as liquid cancers. The compounds provided herein demonstrate surprisingly improved cytotoxic potency as payloads in ADCs, compared to previously known cytotoxic macrocycle compounds. In particular, in some instances the compounds provided herein demonstrate poor cytotoxicity as free drugs, but high cytotoxicity as ADCs. In comparison to other, previously known macrocycle compounds, in some instances the compounds provided herein are worse at cell killing as free drugs, but comparable or better at cell killing as ADCs. This inversion of cytotoxic activity in free drug vs. as an ADC is surprisingly unexpected.

Definitions

[0027] Compounds, including conjugates, described herein (e.g., of Formula (X), (I), or (A)) or a pharmaceutically acceptable salt thereof may exist in one or more stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the subject matter disclosed herein. Likewise, it is understood that a compound or salt may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the subject matter disclosed herein. It is to be understood that the subject matter disclosed herein includes combinations and subsets of the particular groups described herein. The scope of the subject matter disclosed herein includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. It is to be understood that the subject matter disclosed herein includes combinations and subsets of the particular groups defined herein.

[0028] The subject matter disclosed herein also includes isotopically-labelled forms of the compounds described herein, such as wherein one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds described herein (and pharmaceutically acceptable salts thereof) include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine, iodine, and chlorine, such as ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{15}N , ^{17}O , ^{18}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , ^{36}Cl , ^{123}I , and ^{125}I .

[0029] As described herein, compounds, including conjugates, of the present disclosure may optionally be substituted with one or more substituents, such as those illustrated generally herein,

or as exemplified by particular classes, subclasses, and species of the present disclosure. In general, the term “substituted” refers to the replacement of a hydrogen atom in a given structure with a specified substituent. In some embodiments, more than one hydrogen atom is replaced with a specified substituent (e.g. when two hydrogen atoms are replaced with one oxo substituent). Combinations of substituents envisioned by the present disclosure are typically those that result in the formation of stable or chemically feasible compounds.

[0030] As used herein, the terms “including,” “containing,” and “comprising” are used in their open, non-limiting sense.

[0031] The articles “a” and “an” as used in this disclosure may refer to one or more than one (e.g., to at least one) of the grammatical object of the article. By way of example, “an element” may mean one element or more than one element.

[0032] The term “antibody” herein is used in the broadest sense and includes monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity (Miller et al (2003) *Jour. of Immunology* 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs (complementary determining regions) on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In some aspects, however, the immunoglobulin is of human, murine, or rabbit origin.

[0033] The term "antibody fragment(s)" as used herein comprises a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; minibodies (Olafsen et al (2004) *Protein Eng. Design & Sel.* 17(4):315-323), fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0034] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the subject matter described herein may be made by the hybridoma method first described by Kohler et al (1975) *Nature*, 256:495, or may be made by recombinant DNA methods (see for example: US 4816567; US 5807715). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) *Nature*, 352:624-628; Marks et al (1991) *J. Mol. Biol.*, 222:581-597; for example.

[0035] The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the

desired biological activity (US 4816567; and Morrison et al (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855). Chimeric antibodies of interest herein include “primatized” antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.*, Old World Monkey, Ape, etc.) and human constant region sequences.

[0036] The term “chimeric” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0037] The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

[0038] The term “intact antibody” as used herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more “effector functions” which refer to those biological activities attributable to the Fc constant region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

[0039] The term “Fc region” as used herein refers to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions.

[0040] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0041] A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

[0042] The term “free cysteine amino acid” as used herein refers to a cysteine amino acid residue which has been engineered into a parent antibody, has a thiol functional group (-SH), and is not paired as an intramolecular or intermolecular disulfide bridge.

[0043] The term “amino acid” as used herein means glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine, cysteine, methionine, lysine, arginine, histidine, tryptophan, aspartic acid, glutamic acid, asparagine, glutamine or citrulline.

[0044] “Alkyl”, as used herein, refers to an unbranched or branched saturated hydrocarbon chain. In some embodiments, if not otherwise described, alkyl comprises 1 to 12 carbon atoms (C₁-C₁₂alkyl), 1 to 8 carbon atoms (C₁-C₈alkyl), 1 to 6 carbon atoms (C₁-C₆alkyl), or 1 to 4 carbon atoms (C₁-C₄alkyl). Examples of alkyl groups may include methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, 2-pentyl, isopentyl, neopentyl, n-hexyl, 2-hexyl, 3-hexyl, and 3-methyl pentyl. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons may be encompassed. Thus, for example, “butyl” can include n-butyl, sec-butyl, isobutyl and t-butyl, and “propyl” can include n-propyl and isopropyl.

[0045] “Alkoxy”, as used herein, refers to a group -OR, wherein R is alkyl as used herein. In some embodiments, if not otherwise described, alkoxy comprises 1 to 12 carbon atoms (C₁-C₁₂alkoxy), 1 to 8 carbon atoms (C₁-C₈alkoxy), 1 to 6 carbon atoms (C₁-C₆alkoxy), or 1 to 4 carbon atoms (C₁-C₄alkoxy). Examples of alkoxy groups may include but are not limited to methoxy, ethoxy, propoxy, and butoxy.

[0046] “Alkyne” refers to an unsaturated unbranched or branched univalent hydrocarbon chain or combination thereof, having at least one site of acetylenic unsaturation (*i.e.*, having at

least one moiety of the formula $C\equiv C$) having the number of carbon atoms designated (*i.e.*, C₂₋₁₀ means two to ten carbon atoms). Particular alkynyl groups include those with 2 to 8 carbon atoms (a “C₂-C₈ alkynyl”), 2 to 6 carbon atoms (a “C₂-C₆ alkynyl”), having 2 to 4 carbon atoms (a “C₂-C₄ alkynyl”). Examples of an alkyne group include, but are not limited to, groups such as ethynyl (or acetylenyl), prop-1-ynyl, prop-2-ynyl (or propargyl), but-1-ynyl, but-2-ynyl, and but-3-ynyl. In particular, alkyne includes prop-1-yne.

[0047] “Cycloalkyl” as used herein refers to a monocyclic or polycyclic saturated or partially unsaturated, non-aromatic hydrocarbon. In some embodiments, unless otherwise described, cycloalkyl comprises 3 to 12 carbon atoms (C₃-C₁₂cycloalkyl), 3 to 8 carbon atoms (C₃-C₈cycloalkyl), 3 to 6 carbon atoms (C₃-C₆cycloalkyl), or 3 to 5 carbon atoms (C₃-C₅cycloalkyl). In some embodiments, cycloalkyl is a saturated monocyclic or polycyclic hydrocarbon. In other embodiments, cycloalkyl comprises one or more double bonds (e.g., a non-aromatic monocyclic hydrocarbon comprising one or two double bonds). Polycyclic cycloalkyl groups may include spiro, fused, or bridged polycyclic moieties wherein each ring is a saturated or partially unsaturated, non-aromatic hydrocarbon. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, octahydropentalenyl, spiro[3.3]heptanyl, and the like.

[0048] “Halocycloalkyl” as used herein refers to a cycloalkyl group in which one or more hydrogen atoms is replaced with a halo, wherein each halo is independently selected. In certain embodiments, one to four hydrogen atoms is replaced with a halo, wherein each halo is independently selected. Thus, halocycloalkyl includes, for example, a C₃-C₆ membered cycloalkyl in which one or more hydrogen atoms is independently substituted with a fluoro, chloro, iodo, or bromo.

[0049] “Halo” or Halogen” includes fluoro, chloro, bromo, and iodo. Where a moiety is substituted with more than one halogen, it may be referred to by using a prefix corresponding to the number of halogen moieties attached, e.g., dihaloaryl, dihaloalkyl, trihaloaryl etc. refer to aryl and alkyl substituted with two (“di”) or three (“tri”) halo groups, which may be but are not necessarily the same halo; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl.

[0050] “Haloalkyl” refers to an alkyl group in which one or more hydrogen atoms is replaced with a halo, wherein each halo is independently selected. Thus, haloalkyl includes, for example,

C₁-C₆alkyl in which one or more hydrogen atoms is independently substituted with a fluoro, chloro, iodo, or bromo.

[0051] “Haloalkoxy”, as used herein, refers to an alkoxy group in which one or more hydrogen atoms is replaced with a halo, wherein each halo is independently selected. Thus, haloalkoxy includes, for example, C₁-C₆alkoxy in which one or more hydrogen atoms is independently substituted with a fluoro, chloro, iodo, or bromo. Haloalkoxy may include, for example, C₁-C₁₂haloalkoxy, C₁-C₈haloalkoxy, C₁-C₆haloalkoxy, or C₁-C₄haloalkoxy. In some embodiments, haloalkoxy is fluoromethoxy or fluoroethoxy, such as trifluoromethoxy or trifluoroethoxy.

[0052] “Heteroaryl” as used herein refers to a monocyclic or polycyclic group comprising at least one aromatic ring, wherein the aromatic ring comprises at least one ring heteroatom. In some embodiments, the heteroatom is independently selected from the group consisting of N, O, and S. Unless otherwise specified, a heteroaryl group may comprise 5, 6, 7, 8, 9, 10, 11, 12, or more ring atoms, where ring atoms refer to the sum of carbon and heteroatoms in the one or more rings (e.g., be a 5-membered, 6-membered, 7-membered, 8-membered, 9-membered, 10-membered, 11-membered, or 12-membered heteroaryl). Heteroaryl may also include polycyclic groups with at least one aromatic ring comprising at least one ring heteroatom, fused to a non-aromatic hydrocarbon ring (e.g. 5,6,7,8-tetrahydroquinolinyl; 4,5,6,7-tetrahydroisobenzofuranyl). Heteroaryl may also include polycyclic groups comprising at least one aromatic ring comprising at least one ring heteroatom, fused to an aromatic hydrocarbon ring (e.g., quinolinyl, quinoxalinyl, benzothiazolyl). Heteroaryl may include polycyclic groups with two fused aromatic rings, wherein each ring comprises at least one ring heteroatom (e.g., naphthyridinyl). Wherein the heteroaryl is a polycyclic group, the attachment point to another moiety (e.g., to the rest of a formula) may occur on any ring. Examples of heteroaryl groups include, but are not limited to, pyrrolyl, imidazolyl, triazolyl, furanyl, oxazolyl, thiophenyl, thiazolyl, pyridinyl, pyrimidinyl, pyrazinyl, quinolinyl, and indolyl.

[0053] “Heterocycloalkyl” as used herein refers to a saturated or an unsaturated non-aromatic cyclic group having a single ring or multiple condensed rings, and having from 1 to 14 annular (i.e., ring) carbon atoms and from 1 to 6 annular (i.e., ring) heteroatoms, such as nitrogen, phosphorus, sulfur or oxygen, and the like. A heterocycle comprising more than one ring may be fused, spiro, or bridged, or any combination thereof. In fused ring systems, one or more may be

fused rings can be cycloalkyl. Particular heterocyclyl groups are 3- to 14-membered rings having 1 to 13 annular carbon atoms and 1 to 6 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur; 3- to 12-membered rings having 1 to 11 annular carbon atoms and 1 to 6 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur; 3- to 10-membered rings having 1 to 9 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur; 3- to 8-membered rings having 1 to 7 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur; and 3- to 6-membered rings having 1 to 5 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur. In one variation, heterocycloalkyl comprises a monocyclic 3-, 4-, 5-, 6- or 7-membered ring having from 1 to 2, 1 to 3, 1 to 4, 1 to 5 or 1 to 6 annular carbon atoms and 1 to 2, 1 to 3 or 1 to 4 annular heteroatoms independently selected from nitrogen, phosphorus, oxygen and sulfur. In particular embodiments, heterocycloalkyl is a 3 to 8 membered, saturated, monocyclic ring comprising one or two ring heteroatoms selected from S, O, and N. In some embodiments, the heterocycloalkyl is a 3 to 6 membered, saturated, monocyclic ring comprising one or two ring heteroatoms selected from S, O, and N.

[0054] “Haloheterocycloalkyl” as used herein refers to a heterocycloalkyl group in which in which one or more hydrogen atoms is replaced with a halo, wherein each halo is independently selected. In certain embodiments, one to four hydrogen atoms is replaced with a halo, wherein each halo is independently selected. Thus, haloheterocycloalkyl includes, for example, a 3 to 8 membered heterocycloalkyl in which one or more hydrogen atoms is independently substituted with a fluoro, chloro, iodo, or bromo.

[0055] A “patient” or “subject” may encompass both mammals and non-mammals. Examples of mammals may include, but are not limited to, any member of the class *Mammalia*: humans; nonhuman primates such as chimpanzees, monkeys, baboons, or rhesus monkeys, as well as other apes and monkey species; farm animals such as cattle, horses, sheep, goats, and swine; companion animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs; and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. In some embodiments, the patient or subject is a human.

[0056] The terms “effective amount” or “therapeutically effective amount” refers to an amount of a compound, pharmaceutically acceptable salt thereof, or pharmaceutical composition, sufficient to produce a desired therapeutic outcome, such as reducing the severity of duration of, stabilizing the severity of, or eliminating one or more signs, symptoms, or causes of a disorder. For therapeutic use, beneficial or desired results may include, for example, decreasing one or more symptoms resulting from the disorder (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes presenting during development of the disorder, increasing the quality of life of those suffering from the disorder, decreasing the dose of other medications required to treat the disorder, enhancing effect of another medication, delaying the progression of the disorder, and/or prolonging survival of patients. In some embodiments, the compound is a conjugate as provided herein, such as an ADC.

[0057] The term “excipient” as used herein refers to an inert or inactive substance that may be used in the production of a drug or pharmaceutical composition, such as a tablet containing a compound (including of a conjugate, such as an ADC) as described herein (or pharmaceutically acceptable salt) as an active ingredient. Various substances may be embraced by the term excipient, including without limitation any substance used as a diluent, filler or extender, binder, disintegrant, humectant, coating, emulsifier or dispersing agent, compression/encapsulation aid, cream or lotion, lubricant, solution for parenteral administration, material for chewable tablets, sweetener or flavoring, suspending/gelling agent, or wet granulation agent. In some cases, the term “excipient” encompasses pharmaceutically acceptable carriers.

[0058] “Pharmaceutically acceptable salts” includes salts which are generally safe and not biologically or otherwise undesirable, and includes those which are acceptable for veterinary use as well as human pharmaceutical use. Such salts may be prepared by any suitable method, for example, treatment of the free acid with an inorganic or organic base (e.g., if the compound is a free acid), or treatment of the free base with an inorganic or organic acid (e.g., if the compound is a free base). Suitable pharmaceutically acceptable salts may include, for example, those derived from inorganic acids, organic acids, a pyranosidyl acid, an amino acid, an aromatic acid, a sulfonic acid, or the like. Suitable pharmaceutically acceptable salts may also include, for example, those derived from organic bases (such as an amine, e.g., a primary, secondary or tertiary amine), an alkali metal hydroxide, or alkaline earth metal hydroxide, or the like.

Illustrative examples of suitable salts include, but are not limited to, organic salts derived from amino acids (such as glycine or arginine); ammonia; primary, secondary, and tertiary amines; cyclic amines (such as piperidine, morpholine, and piperazine); and inorganic salts. In some embodiments, the compound is a conjugate as provided herein, such as an ADC or a drug payload-linker conjugate.

[0059] The term “peptidomimetic” as used herein means a non-peptide chemical moiety. Peptides are short chains of amino acid monomers linked by peptide (amide) bonds, the covalent chemical bonds formed when the carboxyl group of one amino acid reacts with the amino group of another. The shortest peptides are dipeptides, consisting of 2 amino acids joined by a single peptide bond, followed by tripeptides, tetrapeptides, etc. A peptidomimetic chemical moiety includes non-amino acid chemical moieties. A peptidomimetic chemical moiety may also include one or more amino acid that are separated by one or more non-amino acid chemical units. A peptidomimetic chemical moiety may not contain in any portion of its chemical structure, two or more adjacent amino acids that are linked by peptide bonds.

[0060] Numerical ranges, as used herein, may include sequential integers. For example, a range expressed as “from 0 to 5” would include 0, 1, 2, 3, 4 and 5.

[0061] As used herein, the term “unsubstituted” may mean that the specified group bears no substituents beyond the moiety recited (e.g., where valency is satisfied by hydrogen).

[0062] As used herein, the terms “treat” or “treatment” are meant to indicate a postponement of development of one or more disorders; preventing the development of one or more disorders; and/or reducing severity of one or more symptoms of a disorder that will or are expected to develop. Thus, these terms may include ameliorating one or more existing disorder symptoms; preventing one or more additional symptoms; ameliorating or preventing the underlying causes of one or more symptoms; inhibiting the disorder, e.g., arresting the development of the disorder; relieving the disorder; causing regression of the disorder; relieving a symptom caused by the disorder; or stopping or alleviating the symptoms of the disorder.

[0063] As used herein, the term “about,” when referring to a value is meant to encompass variations of, for example, in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$, and in some

embodiments $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

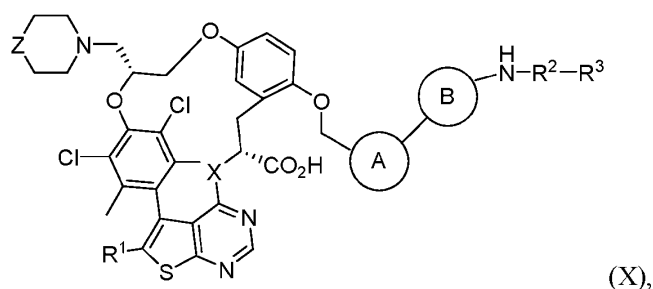
[0064] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit, unless the context clearly dictates otherwise, between the upper and lower limit of the range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these small ranges which may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0065] Chemical names can be generated based on the compound structures provided herein following naming conventions known to one of skill in the art, for example those provided by the International Union of Pure and Applied Chemistry (IUPAC). Chemical names may also be generated, for example, using ChemDraw® software, such as ChemDraw® version 19.1. For any disagreement between the structure and the name, the structure controls.

[0066] Compounds described herein (including conjugates), or a pharmaceutically acceptable salt thereof, may exist in one or more stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the subject matter disclosed herein. Likewise, it is understood that a compound or salt may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the subject matter disclosed herein. It is to be understood that the subject matter disclosed herein includes combinations and subsets of the particular groups described herein. Unless otherwise specified, the scope of the subject matter disclosed herein includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. It is to be understood that the subject matter disclosed herein includes combinations and subsets of the particular groups defined herein.

1. Compounds of Formula (X)

[0067] Provided herein is a compound of Formula (X):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

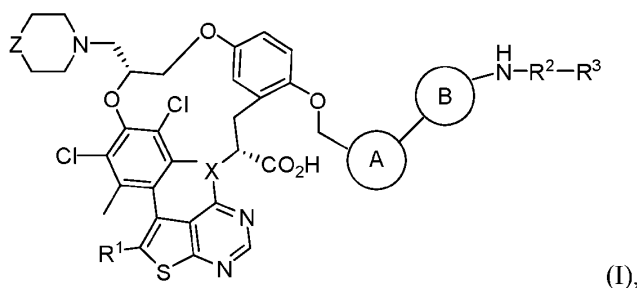
each R^{2a} and each R^{2b} is independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8;

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, alkoxy, and haloalkoxy.

[0068] In some embodiments, the compound of Formula (X) is a compound of Formula (I), or a pharmaceutically acceptable salt thereof. Provided herein is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} is independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8;

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

[0069] In some embodiments of the compound of Formula (X) or (I):

X is O or NH;

Z is N-CH₃, N-CH₂CH₃, or NH;

Ring A is phenyl or 6-membered heteroaryl comprising one to three ring N atoms;

Ring B is phenyl or 6-membered heteroaryl comprising one to three ring N atoms;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is C₁-C₆alkyl, C₃-C₈cycloalkyl, 3- to 8-membered heterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, C₁-C₆alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form C₃-C₈cycloalkyl, C₃-C₈halocycloalkyl, 3- to 8-membered heterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S, or 3- to 8-membered haloheterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S;

each R^{2a} and each R^{2b} is independently C₁-C₆alkyl or C₁-C₆haloalkyl;

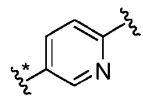
each R^{2c} is independently hydrogen, C₁-C₆alkyl, or C₁-C₆haloalkyl;

p and q are independently integers from 1 to 8;

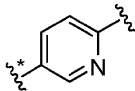
R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently C₁-C₆alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

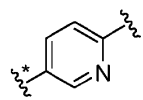
[0070] In some embodiments of the compound of Formula (X) or (I), or a pharmaceutically acceptable salt thereof, Ring A and Ring B are independently phenyl or heteroaryl comprising one or two ring N atoms. In some embodiments, both Ring A and Ring B are independently heteroaryl comprising one or two ring N atoms. In some embodiments, Ring A is pyrimidine. In

certain embodiments, Ring B is phenyl or pyridine. In some embodiments, Ring B is phenyl or

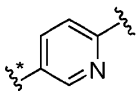


, wherein the * indicates the attachment point to Ring A. In some embodiments, Ring A is pyrimidine and Ring B is phenyl, pyrimidine, or pyridine. In some embodiments, Ring A is pyrimidine and Ring B is phenyl or pyridine. In some embodiments, Ring A is pyrimidine

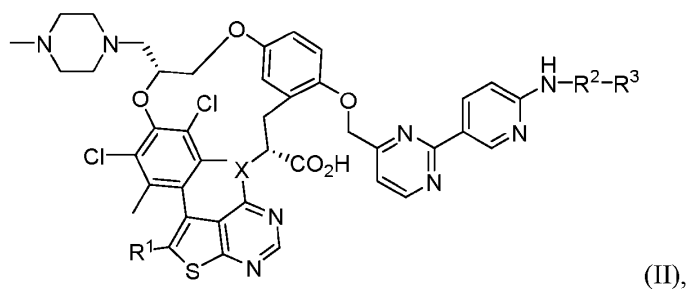
and Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A. In yet further embodiments, Ring A is pyrimidine and Ring B is phenyl or



, wherein the * indicates the attachment point to Ring A. In still further

embodiments, Ring A is pyrimidine and Ring B is , wherein the * indicates the attachment point to Ring A. In certain embodiments, Z is N-H. In other embodiments, Z is N-CH₃. In still further embodiments, Z is N-CH₂CH₃.

[0071] In some embodiments, the compound of Formula (X) or (I) is a compound of Formula (II):



or a pharmaceutically acceptable salt thereof, wherein:

X, R¹, R², and R³ are as defined for Formula (I).

[0072] In some embodiments of Formula (II), or a pharmaceutically acceptable salt thereof:

X is O;

Z is N-CH₃, N-CH₂CH₃, or NH;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is C_1 - C_6 alkyl, C_3 - C_8 cycloalkyl, 3- to 8-membered heterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S, or $-R^{2a}(OR^{2a})_p$ -, wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, C_1 - C_6 alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form C_3 - C_8 cycloalkyl, C_3 - C_8 halocycloalkyl, 3- to 8-membered heterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S, or 3- to 8-membered haloheterocycloalkyl comprising one to three ring heteroatoms independently selected from O, N, and S;

each R^{2a} and each R^{2b} is independently C_1 - C_6 alkyl or C_1 - C_6 haloalkyl;

each R^{2c} is independently hydrogen, C_1 - C_6 alkyl, or C_1 - C_6 haloalkyl;

p and q are independently integers from 1 to 8;

R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently C_1 - C_6 alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-OH$.

[0073] In some embodiments of the compound of Formula (X) or (I), or the compound of Formula (II), or a pharmaceutically acceptable salt thereof, R^1 is phenyl substituted by halo. In certain embodiments, R^1 is phenyl substituted by fluoro, such as by one fluoro. In some embodiments, X is O.

[0074] In some embodiments of the compound of Formula (X) or (I), or the compound of Formula (II), or a pharmaceutically acceptable salt thereof, R^2 is C_1 - C_6 alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of $-OH$ and halo. In some embodiments, R^2 is C_2 - C_4 alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of $-OH$ and halo. In some embodiments, R^2 is C_2 - C_4 alkyl, unsubstituted or substituted with one or more fluoro. In yet

further embodiments, R^2 is unsubstituted C₂-C₄alkyl, such as $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$. In still further embodiments, R^2 is ethylene, unsubstituted or substituted with one or more substituents independently selected from the group consisting of $-\text{OH}$ and halo (such as fluoro). In certain embodiments, R^2 is $-\text{CH}_2\text{CH}_2-$.

[0075] In some embodiments of the compound of Formula (X), or a pharmaceutically acceptable salt thereof, R^3 is $-\text{NH}_2$, $-\text{NHR}^{3a}$, $-\text{N}(\text{R}^{3a})_2$, or $-\text{N}^+(\text{R}^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-\text{OH}$, alkoxy, and haloalkoxy. In some embodiments, each R^{3a} is independently C₁₋₆alkyl, wherein each C₁₋₆alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-\text{OH}$, C₁₋₆alkoxy, and C₁₋₆haloalkoxy. In some embodiments, each R^{3a} is independently C₁₋₆alkyl, wherein each alkyl is unsubstituted or substituted with halo, $-\text{OH}$, or $-\text{OCH}_3$. In some embodiments of the compound of Formula (X) or (I), or the compound of Formula (II), or a pharmaceutically acceptable salt thereof, R^3 is $-\text{NH}_2$, $-\text{NHR}^{3a}$, $-\text{N}(\text{R}^{3a})_2$, or $-\text{N}^+(\text{R}^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-\text{OH}$. In some embodiments, R^3 is $-\text{NH}_2$, $-\text{NHR}^{3a}$, $-\text{N}(\text{R}^{3a})_2$, or $-\text{N}^+(\text{R}^{3a})_3$, wherein each R^{3a} is independently unsubstituted C₁-C₆alkyl. In some embodiments, R^3 is $-\text{NH}_2$ or $-\text{NH}(\text{C}_1\text{-C}_6\text{alkyl})$, such as $-\text{NH}_2$ or $-\text{NH}(\text{CH}_3)$.

[0076] In some embodiments of the compound of Formula (I), or a pharmaceutically acceptable salt thereof:

Z is $\text{N-CH}_2\text{CH}_3$ or N-CH_3 ;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or pyridine;

R^1 is phenyl substituted by one fluoro;

R^2 is C₁-C₆alkyl unsubstituted or substituted with fluoro;

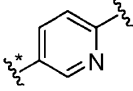
R^3 is $-\text{NH}_2$ or $-\text{NH}(\text{C}_1\text{-C}_6\text{alkyl})$; and

X is O.

[0077] In some embodiments of the compound of Formula (I), or a pharmaceutically acceptable salt thereof:

Z is N-C₁₋₆alkyl;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

R¹ is phenyl substituted by one fluoro;

R² is C₁-C₆alkyl unsubstituted or substituted with fluoro;

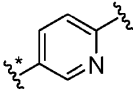
R³ is -NH₂ or -NH(C₁₋₆alkyl); and

X is O.

[0078] In some embodiments of the compound of Formula (I), or a pharmaceutically acceptable salt thereof:

Z is N-CH₂CH₃ or N-CH₃;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

R¹ is phenyl substituted by one fluoro;

R² is C₁-C₆alkyl unsubstituted or substituted with fluoro;

R³ is -NH₂ or -NH(C₁₋₆alkyl); and

X is O.

[0079] In some embodiments of the compound of Formula (I), or a pharmaceutically acceptable salt thereof:

Z is N-CH₃;

Ring A is pyrimidine;

Ring B is pyridine;

R¹ is phenyl substituted by one fluoro;

R² is unsubstituted C₁-C₆alkyl;

R³ is -NH₂; and

X is O.

{0080} In some embodiments of the compound of Formula (II), or a pharmaceutically acceptable salt thereof:

R¹ is phenyl substituted by one fluoro;

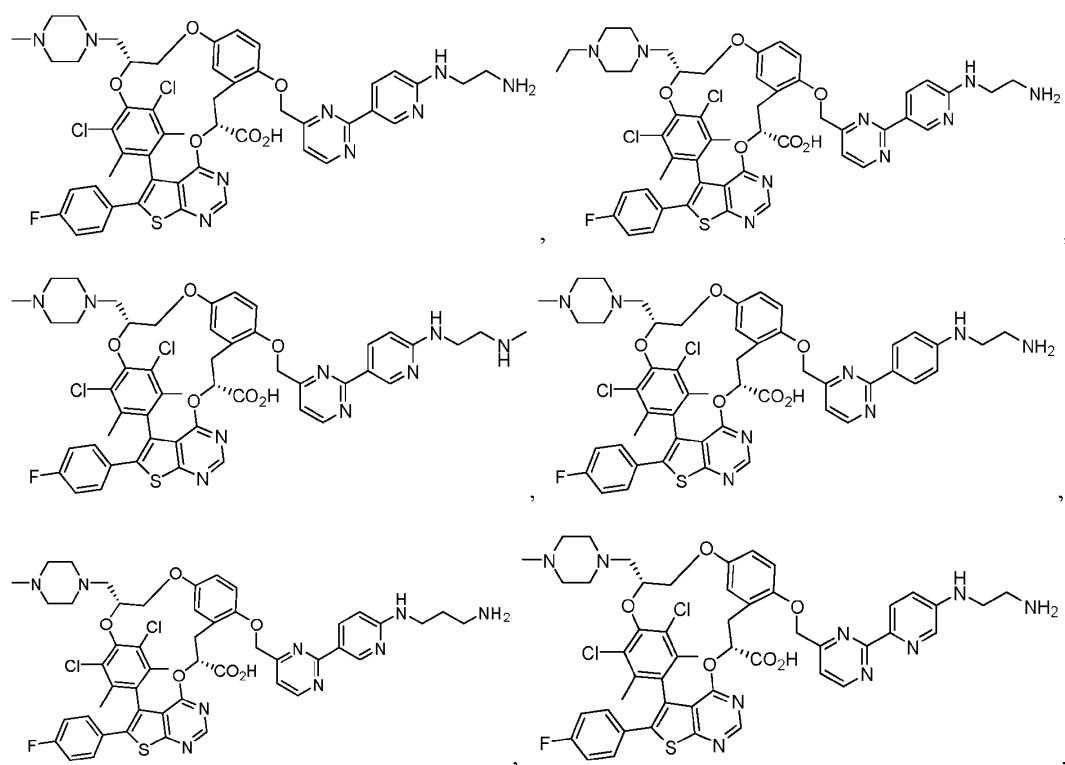
R² is unsubstituted C₁-C₆alkyl;

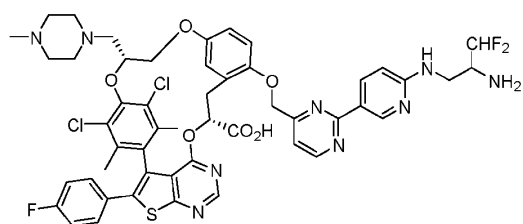
R³ is -NH₂; and

X is O.

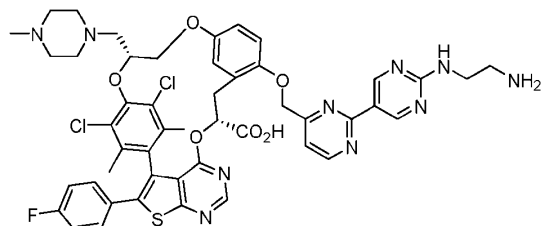
[0081] In some embodiments of the compound of Formula (X) or (I), or a pharmaceutically acceptable salt thereof: X is O, Y is N, R¹ is phenyl substituted with one fluoro, R² is alkyl, and R³ is -NH₂.

[0082] In some embodiments, the compound of Formula (X) or (I) is selected from the group consisting of:





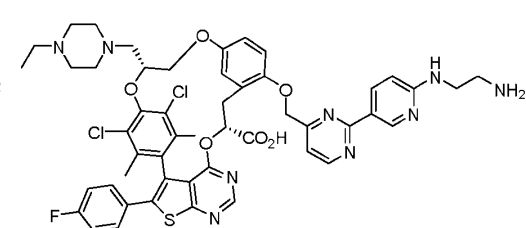
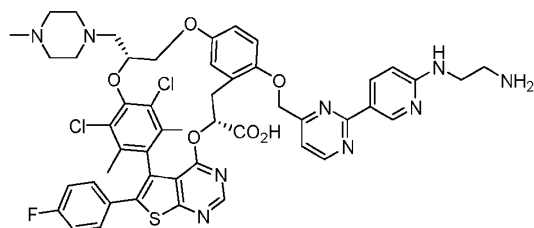
, and



, or is a pharmaceutically acceptable salt

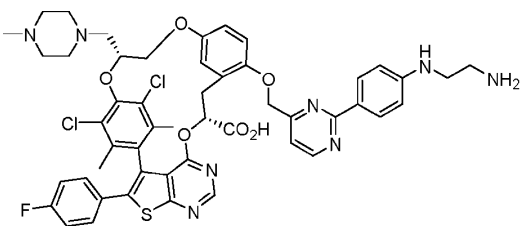
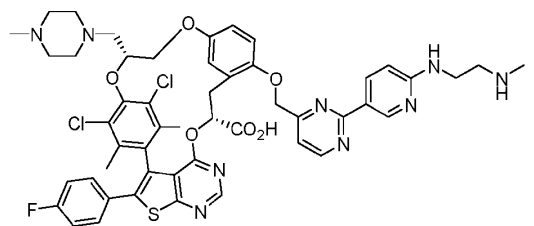
thereof.

[0083] In some embodiments, the compound of Formula (X) is selected from the group consisting of:



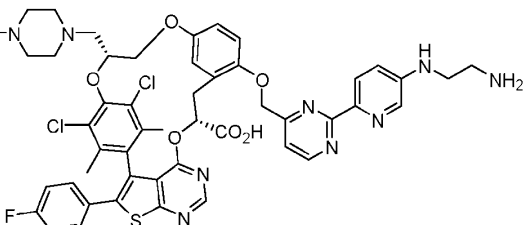
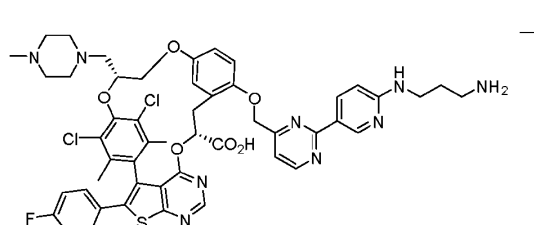
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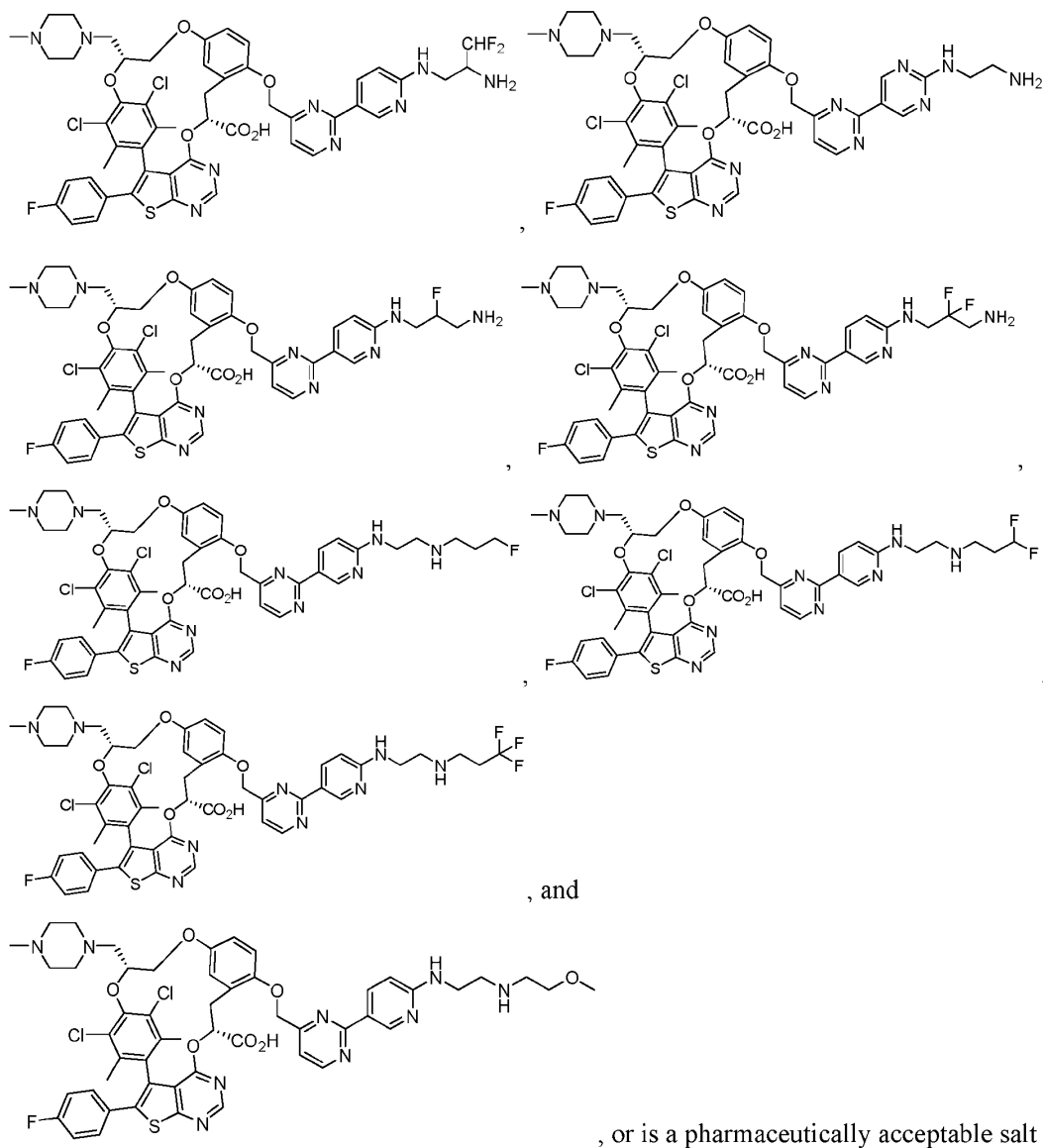
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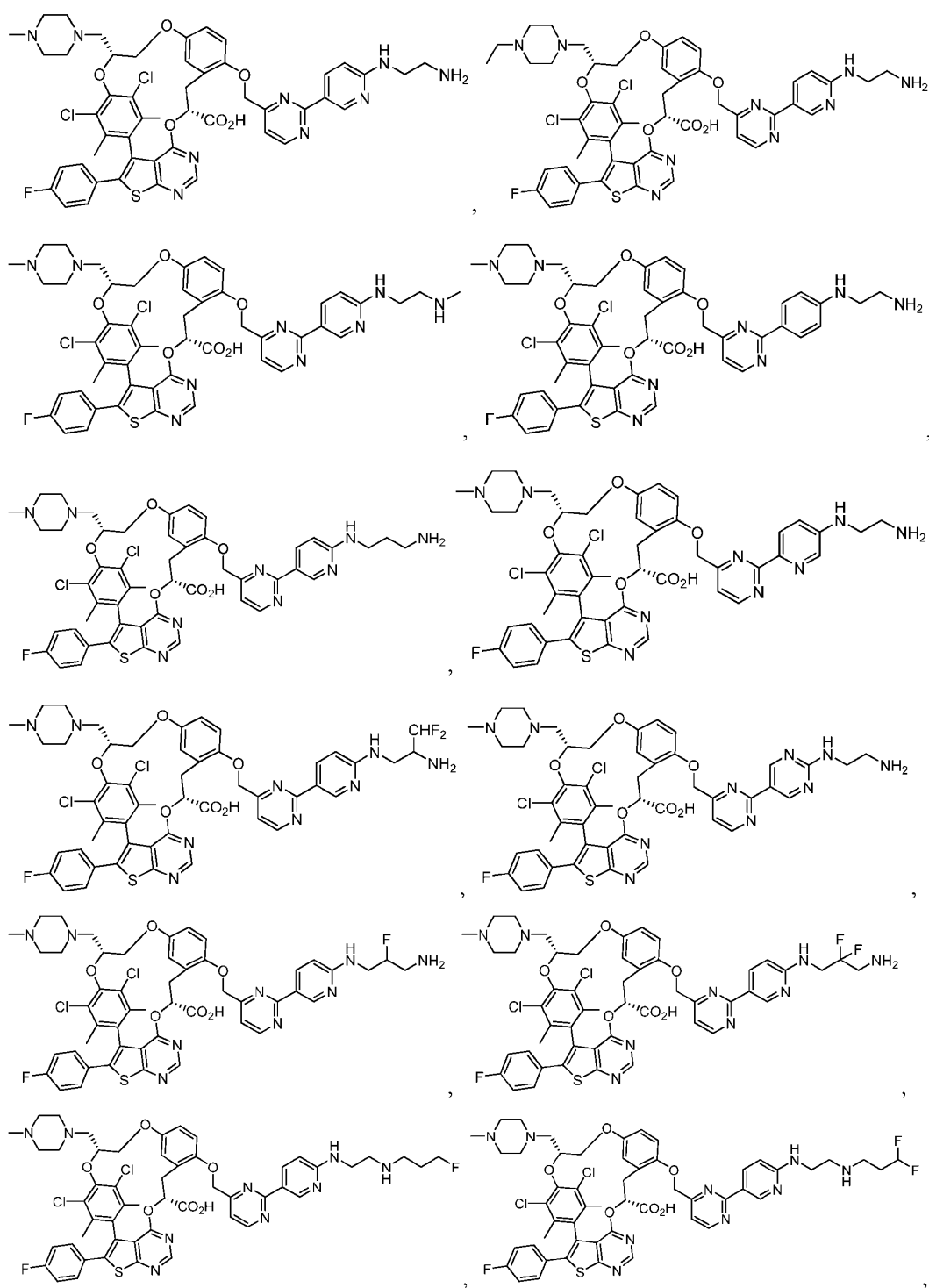
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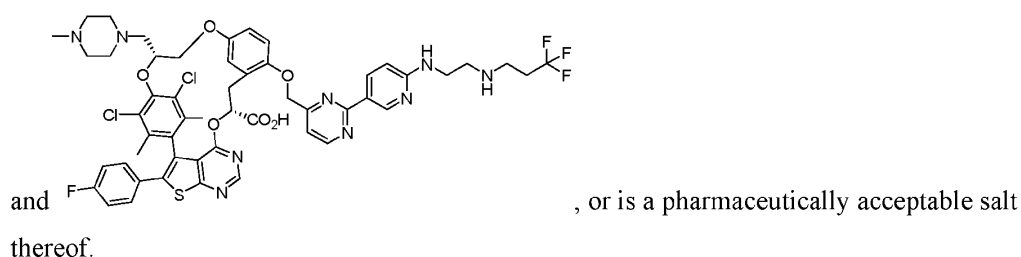
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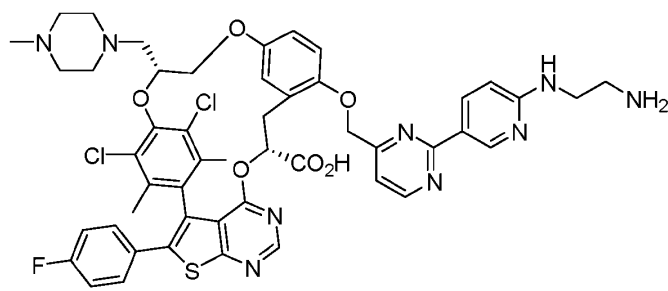
thereof.

[0084] In some embodiments, the compound of Formula (I) is selected from the group consisting of:





[0085] In certain embodiments, the compound of Formula (X), (I), or (II) is:

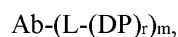


or a pharmaceutically acceptable salt thereof.

2. Conjugates Comprising a Payload

[0086] Further provided herein are conjugates comprising a compound of Formula (I) as a payload. The conjugates provided herein may comprise a single payload, or a multiplicity of payloads, attached to an antibody via a linker. In some embodiments, the payload is a compound of Formula (II). In some embodiments, the conjugate is an antibody drug conjugate (ADC).

[0087] In some embodiments, provided herein is a conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

L is a linker;

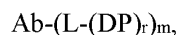
DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

wherein DP is a compound of Formula (X), or a pharmaceutically acceptable salt thereof.

[0088] In some embodiments, the compound of Formula (X) is a compound of Formula (I). Thus, in some embodiments, provided herein is a conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

L is a linker;

DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

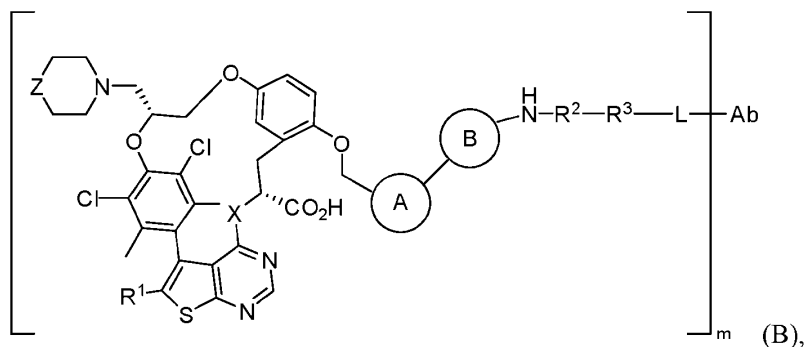
wherein DP is a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

[0089] The drug payload may be any of the compounds or embodiments of Formula (X) or (I) as provided herein. For example, in some embodiments the drug payload is a compound of Formula (X) or (I) wherein Ring A and Ring B are independently phenyl or heteroaryl comprising one or two ring N atoms. In some embodiments, both Ring A and Ring B are independently heteroaryl comprising one or two ring N atoms. In some embodiments, Ring A is pyrimidine. In certain embodiments, Ring B is pyridine. In some embodiments, Ring A is pyrimidine and Ring B is pyridine. In still further embodiments, Ring A is pyrimidine and Ring B is phenyl, pyrimidine, or pyridine. In certain embodiments, Z is N-H. In other embodiments, Z is N-C₁₋₆alkyl, such as for example N-CH₃ or N-CH₂CH₃. In still further embodiments, Z is N-CH₃. In some embodiments, the drug payload is a compound of Formula (II), or a pharmaceutically acceptable salt thereof. In some embodiments of the drug payload, R² is C₁-C₆alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of -OH and halo. In some embodiments, R² is C₂-C₄alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of -OH and halo. In some embodiments, R² is C₂-C₄alkyl, unsubstituted or substituted with one or more fluoro. In yet further embodiments, R² is unsubstituted C₂-C₄alkyl, such as -CH₂CH₂-, -CH₂CH₂CH₂-, or -CH₂CH₂CH₂CH₂-. In still further embodiments, R² is ethylene, unsubstituted or substituted with one or more substituents independently selected from the group consisting of -OH and halo. In certain embodiments, R² is -CH₂CH₂-. In some embodiments, R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents

independently selected from the group consisting of halo, -OH, alkoxy, and haloalkoxy. In some embodiments, R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH. In some embodiments, R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently C_1 - C_6 alkyl, unsubstituted or substituted with one to four independently selected halo or C_1 - C_6 alkoxy. In some embodiments, R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently unsubstituted C_1 - C_6 alkyl. In some embodiments, R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently C_1 - C_6 alkyl unsubstituted or substituted with one to four halo. In some embodiments, R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently C_1 - C_4 alkyl unsubstituted or substituted with one to four fluoro. In still further embodiments, R^3 is $-NHR^{3a}$, wherein each R^{3a} is independently C_1 - C_4 alkyl unsubstituted or substituted with one to four fluoro. In some embodiments, R^3 is $-NH_2$ or $-NH(C_1-C_6alkyl)$, such as $-NH_2$ or $-NH(CH_3)$. In some embodiments, r is an integer from 1 to 2; and m is an integer from 1 to 10. In certain embodiments, r is 1, and m is an integer from 2 to 8, such as 2, 3, 4, 5, 6, 7, or 8.

[0090] The one or more drug payloads in the ADC of Formula (A) may be attached to the antibody through one or more linkers, wherein the linker attaches to the drug payload at any chemically feasible site. In embodiments of the ADC comprising a compound of Formula (X), Formula (I), or any of the subformulae or compounds thereof, the attachment of a linker to the compound requires forming one or more covalent bonds to the compound, and the structure of the compound is accordingly adjusted – such as by replacing a hydrogen atom with a bond to the linker, or adding a bond to the linker through a chemically feasible site and adding a charge. In some embodiments, the linker site is a N atom. In certain embodiments, the linker is attached through a single covalent bond through variable Z and the conjugate comprises the compound wherein variable Z is N (replacing a hydrogen atom with a bond to the linker) or $N^+C_1-C_6alkyl$ (wherein a bond is formed with the tertiary amine, converting it to a quaternary amine). In other embodiments, this is through a single covalent bond to variable R^3 , and variable R^3 in the conjugate is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$. A person of skill in the art would readily recognize how to adjust the structure of the compound when covalent attachment of the compound to a linker through a different site occurs.

[0091] Thus, provided herein are ADCs of Formula (A), wherein the ADC of Formula (A) is an ADC of Formula (B):



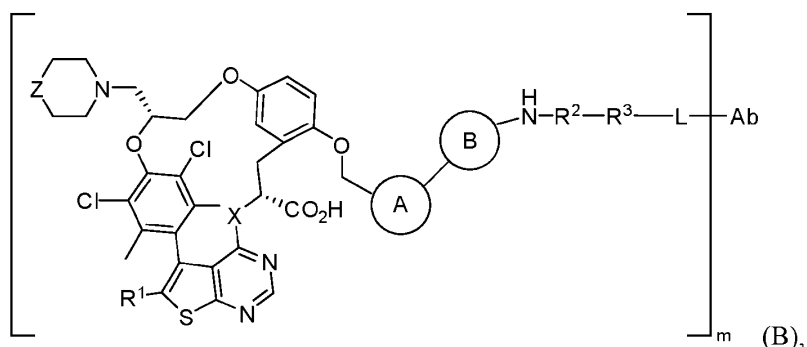
or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (X).

[0092] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and the ADC of Formula (B) is:



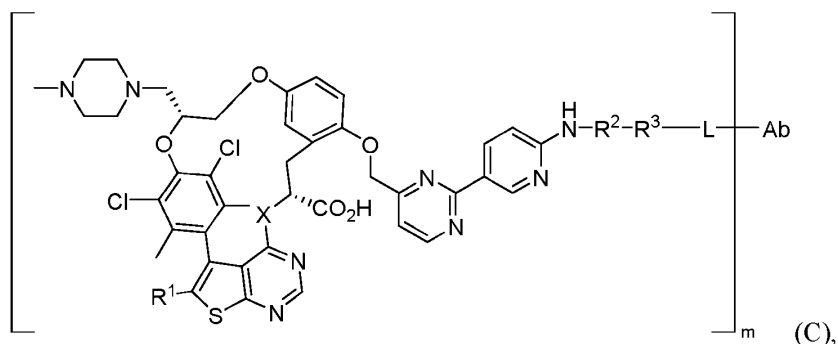
or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (I).

[0093] Further provided herein are ADCs of Formula (A), wherein the ADC of Formula (A) is an ADC of Formula (C):



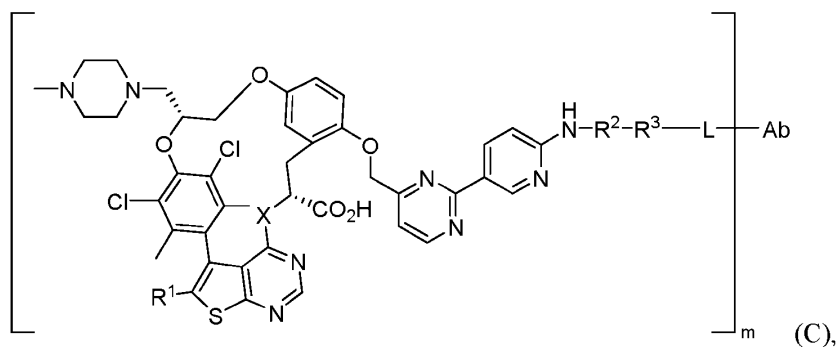
or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X , R^1 , R^2 , and R^{3a} are as defined for Formula (X).

[0094] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and the ADC of Formula (C) is:



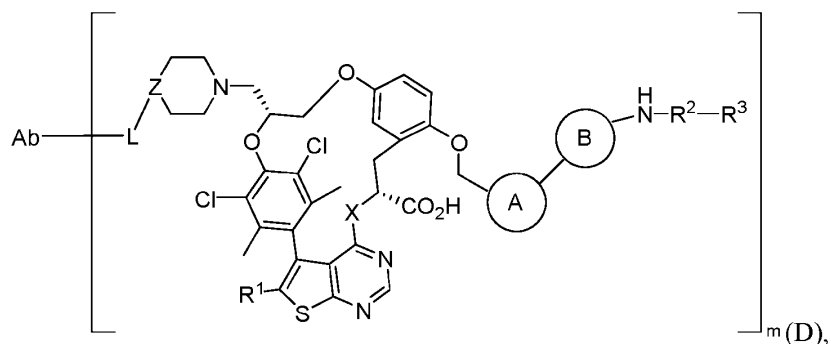
or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X , R^1 , R^2 , and R^{3a} are as defined for Formula (I).

[0095] In still further embodiments, provided herein are ADCs of Formula (A), wherein the ADC of Formula (A) is an ADC of Formula (D):



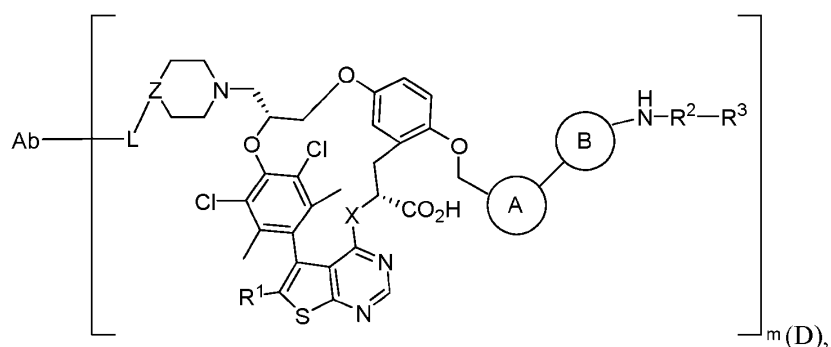
or a pharmaceutically acceptable salt thereof, wherein:

m is an integer from 1 to 10;

Z is N^+ -C₁₋₆alkyl or N; and

X , R^1 , R^2 , and R^3 are as defined for Formula (X).

[0096] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and the ADC of Formula (D) is:



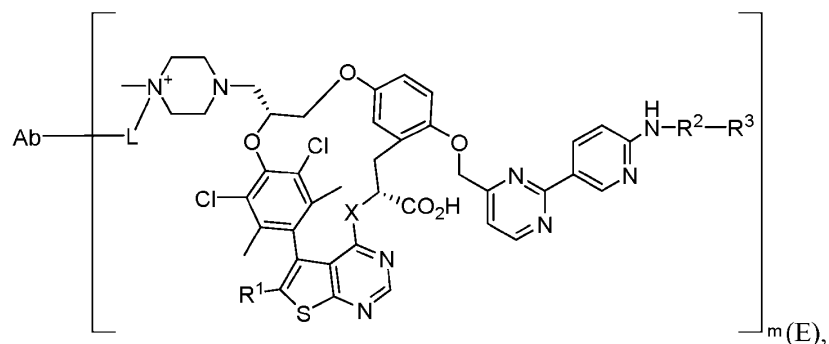
or a pharmaceutically acceptable salt thereof, wherein:

m is an integer from 1 to 10;

Z is N^+ -C₁₋₆alkyl or N; and

X , R^1 , R^2 , and R^3 are as defined for Formula (I).

[0097] In yet further embodiments, provided herein are ADCs of Formula (A), wherein the ADC of Formula (A) is an ADC of Formula (E):

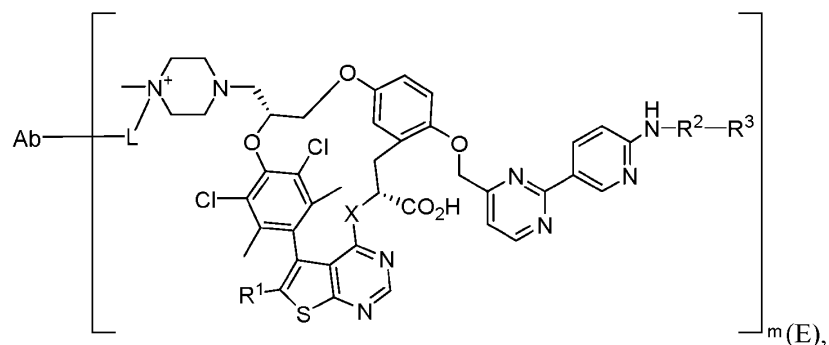


or a pharmaceutically acceptable salt thereof, wherein:

m is an integer from 1 to 10; and

X , R^1 , R^2 , and R^3 are as defined for Formula (X).

[0098] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and the ADC of Formula (E) is:



or a pharmaceutically acceptable salt thereof, wherein:

m is an integer from 1 to 10; and

X , R^1 , R^2 , and R^3 are as defined for Formula (I).

[0099] Each of the embodiments or combinations of embodiments described herein for compounds of Formula (X), (I), or (II) apply to ADCs comprising said compounds of Formula (X), (I), or (II), respectively, including the ADCs of Formulae (A), (B), (C), (D), and (E). The antibody and linker components of said antibodies are described in further detail below.

a. Antibody (Ab)

[0100] As described herein, the compounds of Formula (I) (including compounds of Formula (II)) may be used in combination with an antibody (Ab) and linker to form an antibody drug

conjugate (ADC), such as the ADCs of Formulae (A), (B), (C), (D), or (E). In such embodiments, a single or multiplicity of the compound of Formula (I) is covalently attached to the Ab through a linker to form the ADC. Wherein a multiplicity of the compound of Formula (X) or (I) is used, each compound may be attached to the antibody through a separate linker. In ADCs, the non-antibody, non-linker chemical group (such as the compound of Formula (X) or (I), including those of Formula (II)), is commonly referred to as the “payload”, “drug”, or “drug payload”.

[0101] As described herein, an antibody, such as a monoclonal antibody (mAb) is used to deliver the payload to target cells, e.g., cells that express the specific protein that is targeted by the antibody. The antibody portion of an ADC can target a cell that expresses an antigen, such as a cell-surface antigen.

[0102] In particular embodiments, the antibody may be mutated to reduce effector function. Examples of mutations that modulate the Fc effector function include LALAPG mutations and NG2LH mutations.

[0103] In particular embodiments, the antibody is a THIOMAB™, such as THIOMAB™ antibodies previously described in WO2016/04856. In certain embodiments, combinations are contemplated, such that any antibody target can be combined with any suitable combination of THIOMAB™ mutations with or without any Fc effector modulation including LALAPG or NG2LH mutations.

[0104] In some embodiments, the antibody is a human antibody. Such human antibodies may include those that are, for example, described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008). In some embodiments, the antibody is a library-derived antibody. A variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-

1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0105] In some embodiments, the antibody is a chimeric and humanized antibody. Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0106] In some embodiments, the antibody is a multispecific antibody, *e.g.* a bispecific antibody. The term "multispecific antibody" as used herein refers to an antibody comprising an antigen binding domain that has polyepitopic specificity (*i.e.*, is capable of binding to two, or more than two, different epitopes on one molecule or is capable of binding to epitopes on two, or more than two, different molecules). The term "bispecific antibody" as used herein refers to a multispecific antibody comprising an antigen-binding domain that is capable of binding to two different epitopes on one molecule or is capable of binding to epitopes on two different molecules. A bispecific antibody may also be referred to herein as having "dual specificity" or as being "dual specific." Exemplary bispecific antibodies may bind both protein and any other antigen. Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168, WO2009/089004, US2009/0182127, US2011/0287009, Marvin and Zhu, *Acta Pharmacol. Sin.* (2005) 26(6):649-658, and Kontermann (2005) *Acta Pharmacol. Sin.*, 26:1-9). Multispecific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific

antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991). Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies” or “dual-variable domain immunoglobulins” (DVDs) are also included herein (see, e.g., US 2006/0025576A1, and Wu et al. *Nature Biotechnology* (2007)).). The antibody or fragment herein also includes a “Dual Acting FAB” or “DAF” comprising an antigen binding site that binds to a target protein as well as another, different antigen (see, US 2008/0069820, for example).

[0107] In some embodiments, the antibody is an antibody fragment. Antibody fragments may include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells.

[0108] In some embodiments, the antibody is an antibody variant. In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing

appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[0109] The antibody used in the ADCs as provided herein may, in some embodiments, be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. Referring to antibody affinity, in some embodiments the antibody binds to one or more tumor-associated antigens or cell-surface receptors.

[0110] In certain embodiments, the tumor-associated antigen or cell surface receptor is selected from the group consisting of CLL1, CD71, Trop2, MSLN, NaPi2b, Ly6E, EpCAM, STEAP1, STEAP2, TrpM4, CD21, CD79a, CD72, MUC16, HER2, CD33, CD22, CD79b, LIV1, CD123, CD74, BCMA, and FcRH5. Accordingly, in certain embodiments, the ADC as provided herein may comprise an antibody which is an: anti-CLL1, anti-CD71, anti-Trop2, anti-MSLN, anti-NaPi2b, anti-Ly6E, anti-EpCAM, anti-STEAP1, anti-STEAP2, anti-TrpM4, anti-CD21, anti-CD79a, anti-CD72, anti-MUC16, anti-HER2, anti-CD33, anti-CD22 antibody, anti-CD79b antibody, anti-LIV1 antibody, anti-CD123 antibody, anti-CD74 antibody, anti-BCMA antibody, or anti-FcRH5 antibody.

[0111] In some embodiments, the ADC comprises an anti-Trop2 antibody. Trop2 (trophoblast antigen 2) is a transmembrane glycoprotein that is an intracellular calcium signal transducer that is differentially expressed in many cancers. It signals cells for self-renewal, proliferation, invasion, and survival. Trop 2 is also known as cell surface glycoprotein Trop-2/Trop2, gastrointestinal tumor-associated antigen GA7331, pancreatic carcinoma marker protein GA733-1/GA733, membrane component chromosome 1 surface marker 1 M1S1, epithelial glycoprotein-1, EGP-1, CAA1, Gelatinous Drop-Like Corneal Dystrophy GDLD, and TTD2. In some embodiments of an ADC comprising an anti-Trop2 antibody, the anti-Trop2 antibody is humanized. In one embodiment, the anti-Trop2 antibody is one described in US-2014/0377287 or US-2015/0366988. In some embodiments, the antibody is a cysteine engineered variant of a disclosed antibody.

[0112] In some embodiments, the ADC comprises an anti-Her2 antibody. Anti-Her2 antibodies are described in more detail in PCT/US1997/018385 and US7862817. In some embodiments, the ADC comprises the anti-Her2 antibody 7C2, described in more detail in PCT/US1997/018385, which is hereby incorporated by reference in its entirety. In some embodiments of an ADC comprising an anti-Her2 antibody, the anti-Her2 antibody is humanized. In some embodiments, the antibody is a cysteine engineered variant of a disclosed antibody.

[0113] In some embodiments, the ADC comprises an anti-CD33 antibody. CD33 is a member of the sialic acid binding, immunoglobulin-like lectin family, and is a 67 kDa glycosylated transmembrane protein. CD33 is expressed on most myeloid and monocytic leukemia cells in addition to committed myelomonocytic and erythroid progenitor cells. It is not seen on the earliest pluripotent stem cells, mature granulocytes, lymphoid cells, or nonhematopoietic cells (Sabbath et al., (1985). Clin. Invest. 75:756-56; Andrews et al., (1986) Blood 68:1030-5). CD33 contains two tyrosine residues on its cytoplasmic tail, each of which is followed by hydrophobic residues similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM) seen in many inhibitory receptors. In some embodiments, the CD33 antibody is one described in PCT/US2014/069874, such as the 15G15 antibody described in PCT/US2014/069874, which is hereby incorporated by reference in its entirety. In some embodiments, the antibody is a cysteine engineered variant of a disclosed antibody.

[0114] In still further embodiments, the ADC comprises an anti-CD22 antibody. CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814, Genbank accession No. AK026467); Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Claim 1; Fig 1); Cross-references: MIM:107266; NP_001762.1; NM_001771_1. In some embodiments, the ADC comprises the anti-CD22 antibody 10F4v3, described in more detail in PCT/US2007/069889, which is hereby incorporated by reference in its entirety. In some embodiments of an ADC comprising an anti-CD22 antibody, the anti-CD22 antibody is humanized. In some embodiments, the antibody is a cysteine engineered variant of a disclosed antibody.

b. Linker

[0115] As described herein, the compounds of Formula (X) or (I) (including compounds of Formula (II)) may be used in combination with an antibody to form an antibody drug conjugate (ADC), such as those of Formula (A), (B), or (C); or (D) or (E). In such embodiments, a single or multiplicity of the compound of Formula (X) or (I) is covalently attached to the Ab through a linker to form the ADC. Wherein a multiplicity of the compound of Formula (X) or (I) is used, each compound may be attached to the antibody through a separate linker.

[0116] A “linker” (L) is a bifunctional or multifunctional moiety that can be used to link one or more drug moieties (D) to an antibody (Ab) to form an ADC, wherein the drug moiety is a compound of Formula (X) or (I) (and may also be called a payload, or drug payload, e.g., “DP”). In some embodiments, ADCs can be prepared using a Linker having reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an Ab can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

[0117] Linkers for use in the methods and compositions provided herein may include cleavable linkers (such as peptide, hydrazone, disulfide, peptidomimetic, and glucuronide) and non-cleavable (such as thioether).

[0118] Methods of attaching linkers to antibodies are well known in the art, and include the use of reactive functional groups on the linker such as NHS esters, isothiocyanates, haloacetamides, mixed disulfides, and maleimides. Thus, the ADCs provided herein include those in which the Ab is covalently attached to a linker through a thio-succinimide, disulfide, ester, amide, or triazole functional group.

[0119] In some embodiments, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Non-limiting exemplary such reactive functionalities include maleimide, haloacetamides, α -haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. *See, e.g.*, the conjugation method at page 766 of Klussman, et al (2004), *Bioconjugate Chemistry* 15(4):765-773, and the Examples herein. Thus, in some embodiments, the Ab includes a reactive cysteine thiol to which the linker forms a bond, to attached the payload to the Ab.

[0120] In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Non-limiting examples of such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0121] A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl (“MC”), maleimidopropanoyl (“MP”), valine-citrulline (“val-cit” or “vc”), alanine-phenylalanine (“ala-phe”), p-aminobenzyloxycarbonyl (a “PAB”), N-Succinimidyl 4-(2-pyridylthio) pentanoate (“SPP”), 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (“MCC”), 1-(5-aminopentyl)-1H-pyrrole-2,5-dione, cyclobutane-1,1-dicarbaldehyde (“sq”), or cyclobutane-1,1-dicarbaldehyde-citrulline (“sq-cit”). Various linker components are known in the art, some of which are described below.

[0122] A linker may be a “cleavable linker,” facilitating release of a drug. Non-limiting exemplary cleavable linkers include acid-labile linkers (e.g., comprising hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photolabile linkers, disulfide-containing linkers (Chari et al., Cancer Research 52:127-131 (1992); US 5208020), and b-glucuronide linkers (e.g., cleavable by b-glucuronidase). Cleavable linkers include peptide linkers that can be hydrolyzed by lysosomal enzymes, such as lysosomal cysteine proteases and lysosomal thiol reductases. Such cleavable linkers may include those comprising a Valine-Citrulline (Val-Cit) dipeptide, which can be cleaved by Cathepsin B (see, e.g., US 6,214,345). Cleavable linkers further include peptidomimetic linkers, non-peptide linkers that have certain properties of peptides. Cleavable linkers include those that contain a disulfide bond, and may be known as disulfide linkers. The disulfide bond (which may be known alternatively as a disulfide bridge) may occur at any location in the linker, including at the attachment point of the linker to another component of the ADC, such as attachment of the linker to the antibody, or linker to the payload, or linker to the masking moiety, depending on the configuration of the ADC. Disulfide linkers may be cleaved via reduction, thiol-disulfide exchange, or through enzymatic cleavage. Intracellular enzymatic cleavage may occur, for example, by action of enzymes of the thioredoxin family.

Other types of enzyme-cleavable linkers exist, including b-glucuronide linkers, those with a b-glucuronide glycosidic bond that is cleavable by the lysosomal enzyme b-glucuronidase.

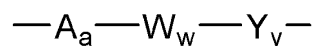
[0123] In some embodiments, the linker of the ADC as provided herein is a cleavable linker, wherein the cleavable linker comprises a cleavable peptide bond, disulfide bond, or b-glucuronide glycosidic bond. In some embodiments, the cleavable linker is a peptidomimetic linker.

[0124] In some embodiments, the payload is linked to the antibody through a non-peptide, peptidomimetic linker that is cleavable by lysosomal enzymes. For example, the amide bond in the middle of a dipeptide (e.g. Val-Cit) may be replaced with an amide mimic; and/or entire amino acid (e.g., valine amino acid in Val-Cit dipeptide) may be replaced with a non-amino acid moiety (e.g., cycloalkyl dicarbonyl structures (for example, ring size = 4 or 5)).

[0125] In other embodiments, the antibody is linked to the rest of the ADC through a non-cleavable linker. Non-cleavable linkers include linkers comprising a peptide wherein the peptide is not cleavable by lysosomal proteases. Non-cleavable linkers also include linkers which do not comprise a peptide, and which are not cleavable by lysosomal proteases.

[0126] In still further embodiments, the linker comprises a functional group by which it is covalently attached to the Ab, a spacing element, and a functional group by which it is covalently attached to the rest of the ADC (e.g. to the payload, such as a compound of Formula (I)). For example, the linker may be covalently attached to the Ab through a thio-succinimide, disulfide, ester, amide, or triazole functional group; comprise a spacing element; and then be attached to the compound of Formula (I) through a thio-succinimide, disulfide, ester, amide, or triazole functional group. In certain embodiments, the spacing element comprises an alkyl chain, or an ether. In certain embodiments, the spacing element comprises C₁-C₁₀ alkyl, or comprises polyethylene glycol (PEG). In some embodiments, the spacing element comprises [-O-CH₂CH₂-]₁₋₁₀. Linkers for use in the methods and compositions provided herein include, for example, maleimide-PEG_n-succinimidyl esters, wherein n is an integer from 1 to 20, such as from 1 to 10, or from 3 to 6.

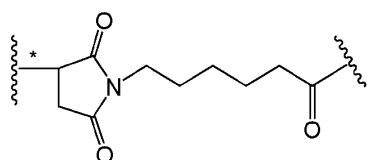
[0127] In certain embodiments, a linker has the following Formula X:



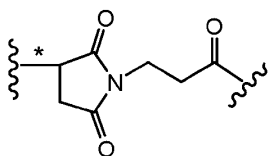
Formula X

wherein A is a “stretcher unit”, and a is an integer from 0 to 1; W is an “amino acid unit”, and w is an integer from 0 to 12; Y is a “spacer unit”, and y is 0, 1, or 2; and Ab, D, and p are defined as above for Formula X. Exemplary embodiments of such linkers are described in U.S. Patent No. 7,498,298, which is expressly incorporated herein by reference.

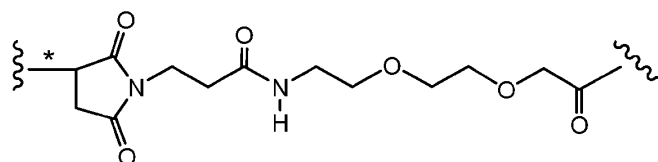
[0128] In some embodiments, a linker component comprises a “stretcher unit” that links an antibody to another linker component or to a drug moiety. Non-limiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components; and in some embodiments the * indicates the point of attachment to the antibody):



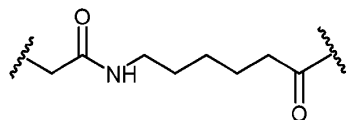
MC ;



MP ;



mPEG ; and

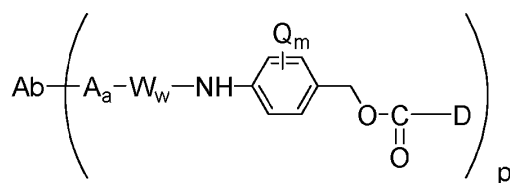


[0129] In some embodiments, a linker component comprises an “amino acid unit”. In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular

proteases, such as lysosomal enzymes (Doronina *et al.* (2003) *Nat. Biotechnol.* 21:778-784). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

[0130] In some embodiments, a linker component comprises a “spacer” unit that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be “self-immolative” or a “non-self-immolative.” A “non-self-immolative” spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

[0131] A “self-immolative” spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann *et al.* (2005) *Expert Opin. Ther. Patents* (2005) 15:1087-1103). In some embodiments, the spacer unit is p-aminobenzylloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:

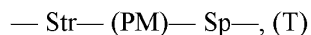


wherein Q is -C₁-C₈ alkyl, -O-(C₁-C₈ alkyl), -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; p ranges from 1 to about 20; and D is the drug payload (DP). In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

[0132] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Patent No. 7,375,078; Hay *et al.*, (1999) *Bioorg. Med. Chem. Lett.* 9:2237) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues *et al.*, (1995) *Chemistry Biology* 2:223), appropriately substituted bicyclic[2.2.1] and bicyclic[2.2.2] ring systems (Storm *et al.*, (1972) *J. Amer. Chem. Soc.* 94:5815) and 2-aminophenylpropionic acid amides (Amsberry *et al.*, (1990) *J. Org. Chem.* 55:5867). Linkage of a drug to the α -carbon of a glycine residue is another example of a self-immolative spacer that may be useful in ADC (Kingsbury *et al.*, (1984) *J. Med. Chem.* 27:1447).

[0133] In some embodiments, the linker is a peptidomimetic linker, such as a peptidomimetic linker described in WO2015/095227 A2, which is incorporated herein by reference in its entirety. In some embodiments, the linker is referred to as an MC-sq-Ala linker, or an MC-sq-Cit-PAB linker. In some embodiments and examples provided herein, it is described simply as the “sq-Cit” linker. For any confusion or discrepancy between nomenclature and structure, the structures provided herein dominate.

[0134] In some embodiments, the linker has the following Formula (T):

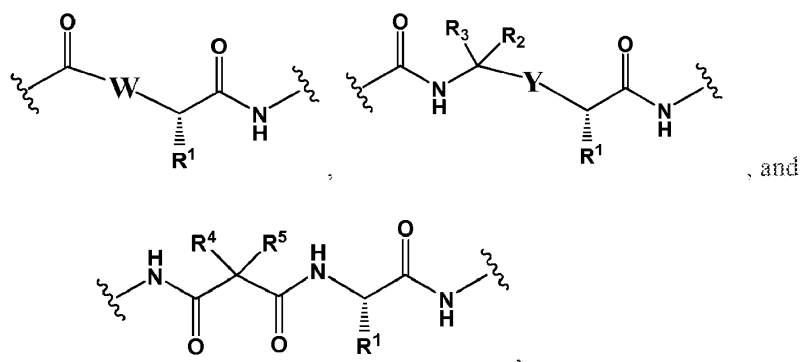


wherein

Str is a stretcher unit covalently attached to Ab;

Sp is a bond or spacer unit covalently attached to a drug moiety;

PM is a non-peptide chemical moiety selected from the group consisting of:



W is -NH-heterocycloalkyl- or heterocycloalkyl;

Y is heteroaryl, aryl, -C(O)C₁-C₆alkylene, C₁-C₆alkenyl, C₁-C₆alkylene or -C₁-C₆alkylene-NH-;

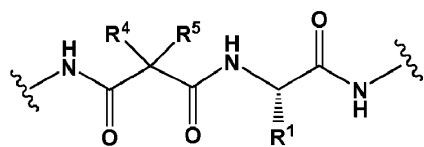
each R¹ is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂;

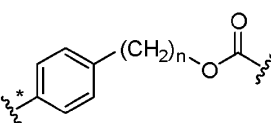
R³ and R² are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R³ and R² together may form a C₃-C₇cycloalkyl; and

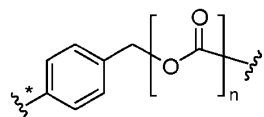
R⁴ and R⁵ are each independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl, heteroarylalkyl, (C₁-C₁₀alkyl)OCH₂-, or R⁴ and R⁵ together may form a C₃-C₇cycloalkyl ring.

[0135] In some such embodiments, the PM is covalently attached to the Str unit through the left-hand side of the chemical moiety as shown, that is, through a bond to the carbon of the C(O) portion of the PM in the first two instances, or a bond to the nitrogen of the NHC(O)(CR⁴R³)- portion of the PM in the last instance.

[0136] In some embodiments of ADCs comprising the linker of Formula (T), the PM is

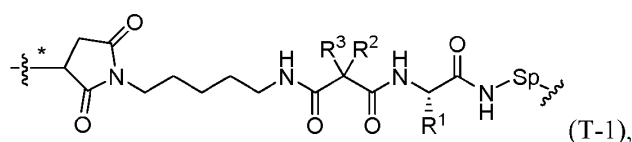


linker of Formula (T), Sp is a spacer of the formula , wherein each n is independently an integer from 1 to 6 and * indicates the point of attachment to the PM unit of the linker. In some embodiments of the linker of Formula (T), Sp is a spacer of the formula



, wherein n is 0 or 1 and * indicates the point of attachment to the PM unit of the linker.

[0137] In some embodiments of the ADCs provided herein, such as the ADCs of Formula (A), (B), or (C), the linker is of the Formula (T-1):



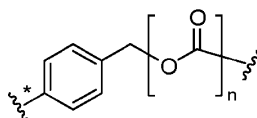
wherein:

each R¹ is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂;

R³ and R² are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R³ and R² together may form a C₃-C₇cycloalkyl; and

* indicates the point of attachment to the Ab; and

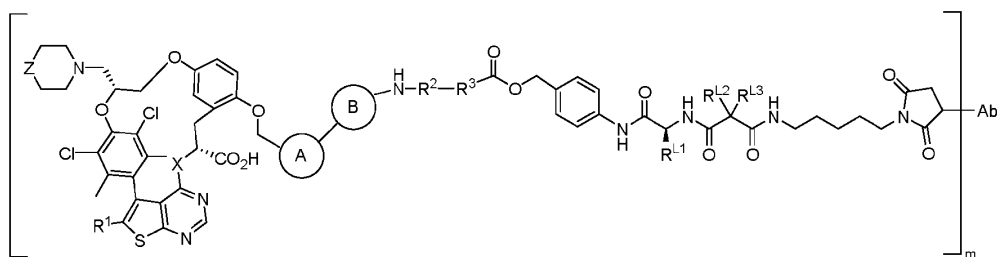
Sp is a spacer of the formula



wherein n is 0 or 1, and

* indicates the point of attachment to the remainder of the linker.

[0138] Thus, in some embodiments, provided herein is an ADC of Formula (B-1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10;

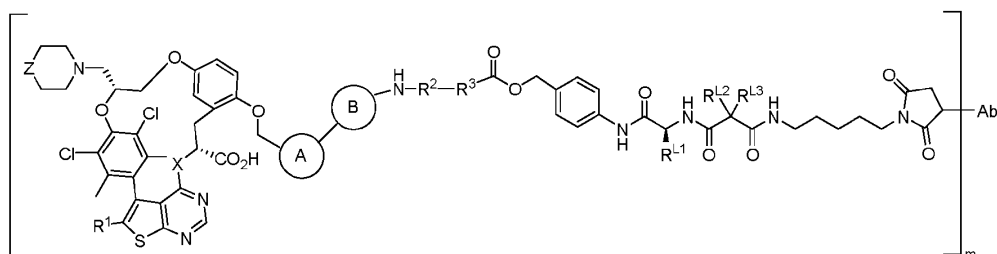
X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (X);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl; and

Ab is an antibody.

[0139] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and provided herein is an ADC of Formula (B-1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10;

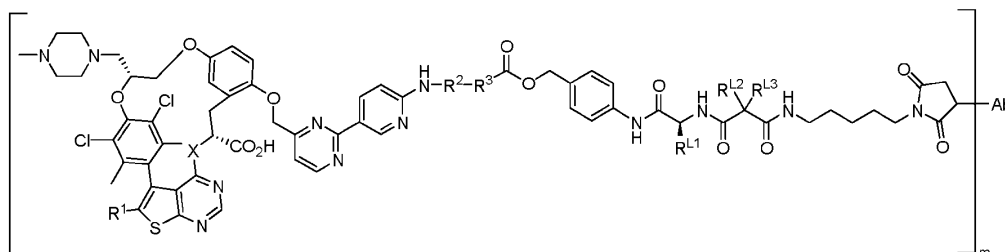
X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (I);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl; and

Ab is an antibody.

[0140] Further provided herein is an ADC of Formula (C-1):



or a pharmaceutically acceptable salt thereof, wherein:

R³ is -NH-, -NR^{3a}-, or -N⁺(R^{3a})₂-;

m is an integer from 1 to 10;

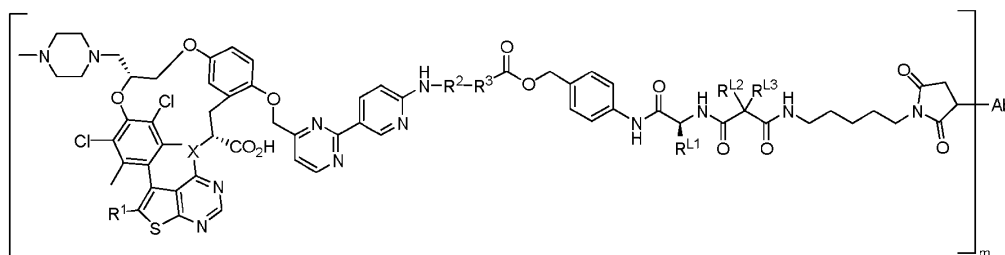
X, R¹, R², and R^{3a} are as defined for Formula (X);

each R^{1.1} is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C₃-C₇cycloalkyl; and

Ab is an antibody.

[0141] In some embodiments, the compound of Formula (X) is a compound of Formula (I), and provided herein is an ADC of Formula (C-1):



or a pharmaceutically acceptable salt thereof, wherein:

$$R^3 \text{ is } -NH-, -NR^{3a}-, \text{ or } -N^+(R^{3a})_2-$$

m is an integer from 1 to 10;

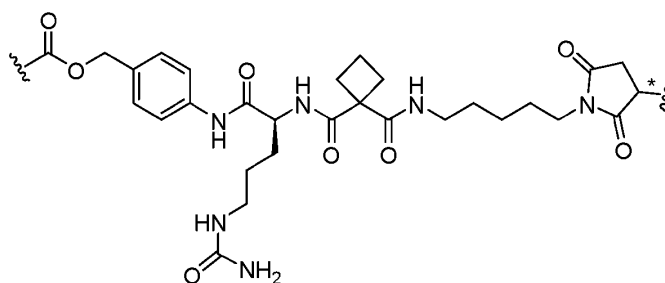
X, R¹, R², and R^{3a} are as defined for Formula (I);

each R^{L1} is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C₃-C₇cycloalkyl; and Ab is an antibody.

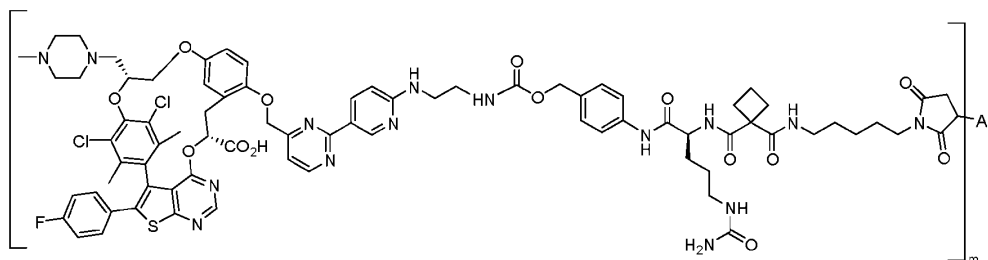
[0142] In some embodiments of the ADCs of Formula (B-1) and (C-1), R^{L1} is C₁-C₁₀alkylNHC(NH)NH₂, and R^{L3} and R^{L2} together form a C₃-C₇cycloalkyl. In some embodiments, R^{L1} is C₁-C₁₀alkylNHC(NH)NH₂, and R^{L3} and R^{L2} together form C₄cycloalkyl.

[0143] In some embodiments, the linker is of the structure:



wherein * indicates the site of covalent attachment to the antibody.

[0144] In some embodiments, the ADC has the structure:

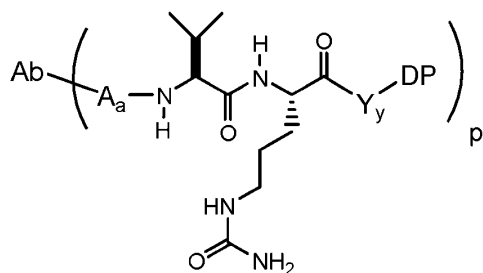


wherein m is an integer from 1 to 10, and Ab is an antibody as described herein.

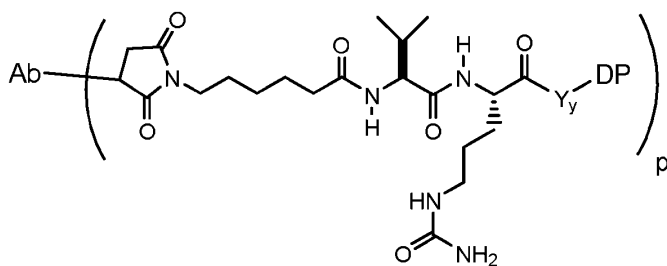
[0145] In some embodiments of the various ADCs provided herein, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun *et al.*, (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun *et al.*, (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, *i.e.*, loading, which is related

to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

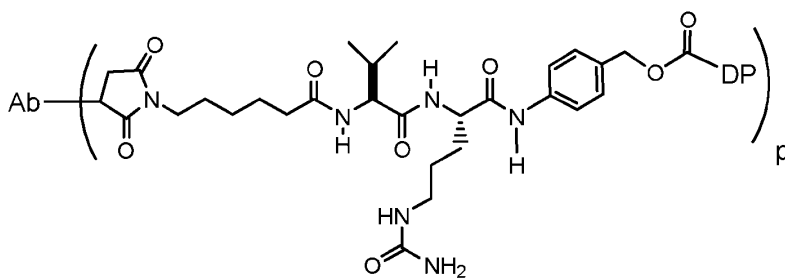
[0146] Non-limiting exemplary linkers are shown below in the context of an ADC of Formula (A) as provided herein:



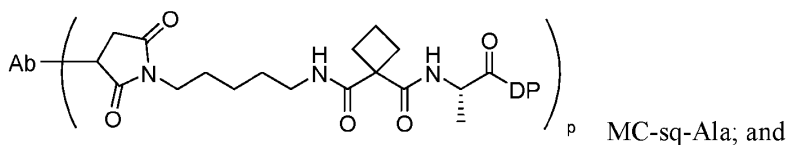
val-cit ;



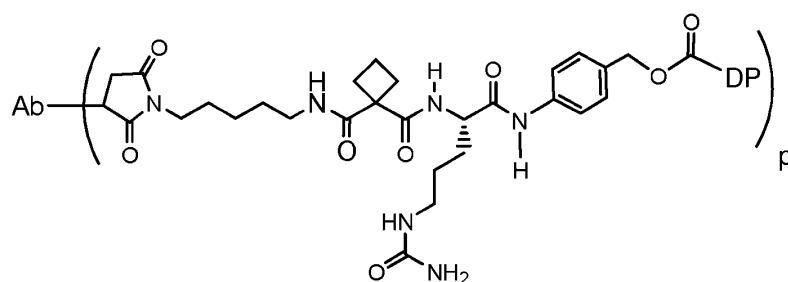
MC-val-cit ;



MC-val-cit-PAB ;

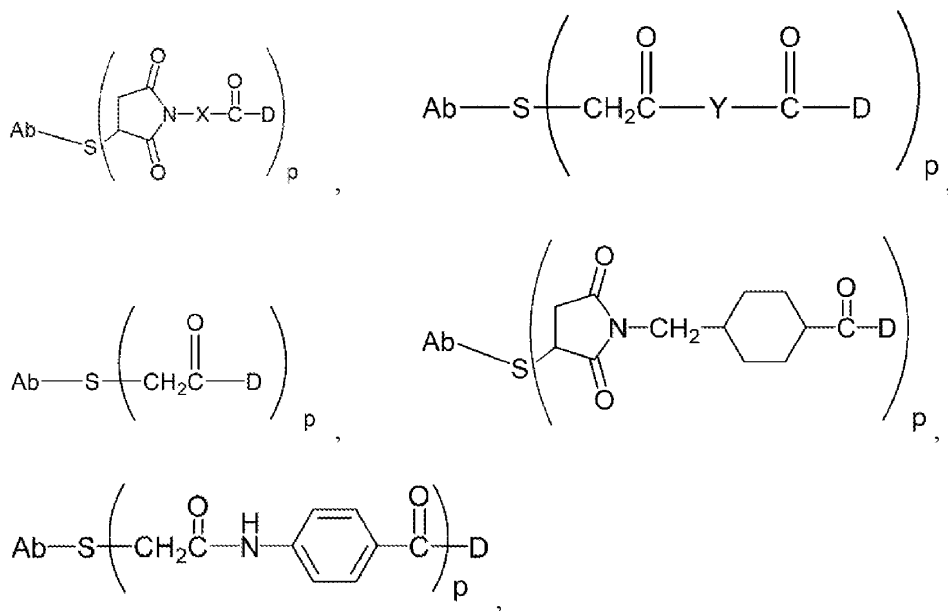


MC-sq-Ala; and

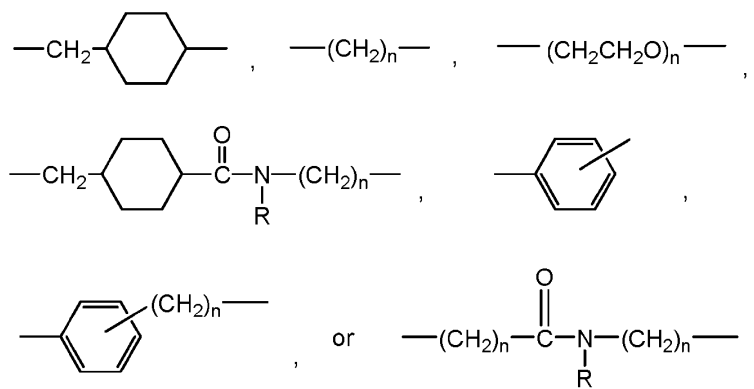


MC-sq-Cit-PAB.

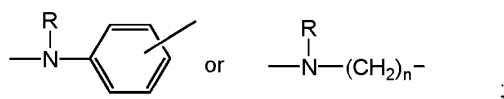
[0147] Further non-limiting exemplary ADCs include the following structures where -S- is part of the antibody:



where X is:



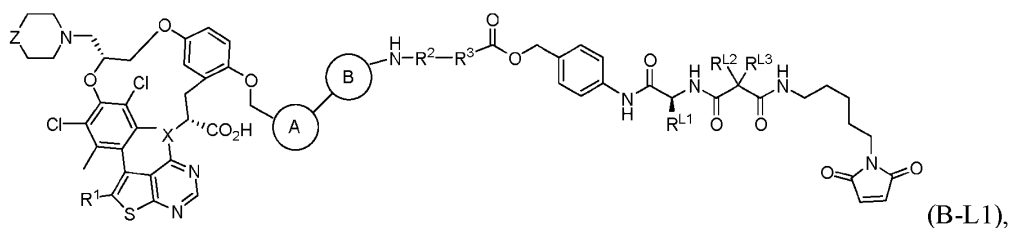
Y is:



each R is independently H or C₁–C₆ alkyl; n is 1 to 12; and D is the drug payload (DP) of Formula (A).

[0148] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a non-limiting example, a charged substituent such as sulfonate ($-\text{SO}_3^-$) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with DP, or DP-L (drug payload-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)^a is coupled to drug payload-(linker portion)^b to form the ADC of Formula (A). In some such embodiments, the antibody comprises more than one (linker portion)^a substituents, such that more than one drug is coupled to the antibody in the ADC of Formula (A). In other embodiments, the linker is first coupled to the drug payload, and then the drug payload-linker (DP-L) is coupled to the Ab. In some embodiments, multiple DP-L are coupled to the Ab.

[0149] Provided in some embodiments is a drug payload-linker conjugate of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

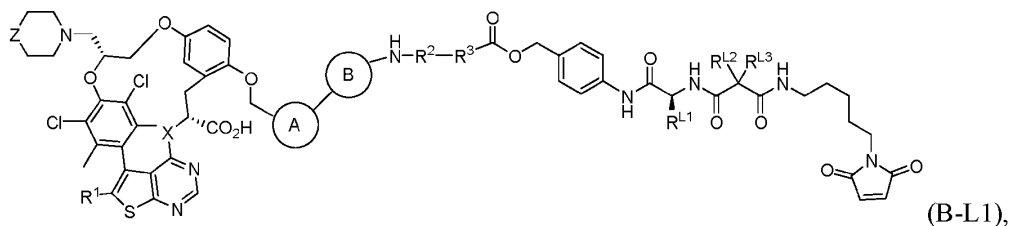
R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

X, R^1 , R^2 , R^{3a} , Z, Ring A, and Ring B are as defined for Formula (X);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂; and

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl.

[0150] Also provided in some embodiments is a drug payload-linker conjugate of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

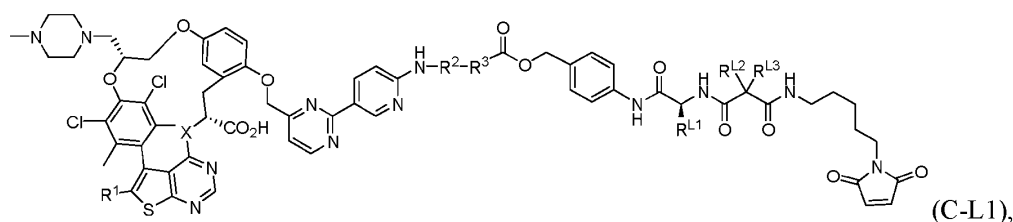
R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

X, R^1 , R^2 , R^{3a} , Z, Ring A, and Ring B are as defined for Formula (I);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂; and

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl.

[0151] Further provided herein is a drug payload-linker conjugate of Formula (C-L1):



or a pharmaceutically acceptable salt thereof, wherein:

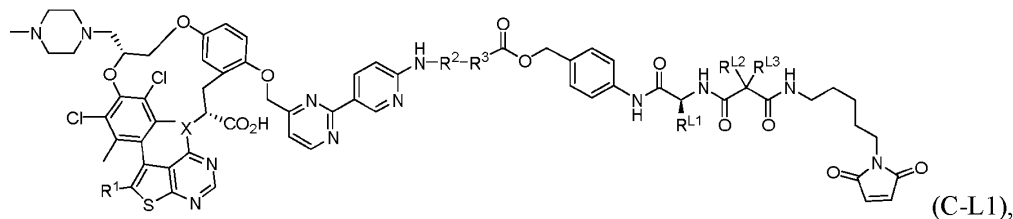
R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

X , R^1 , R^2 , and R^{3a} are as defined for Formula (X);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂; and

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl.

[0152] Also provided herein is a drug payload-linker conjugate of Formula (C-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

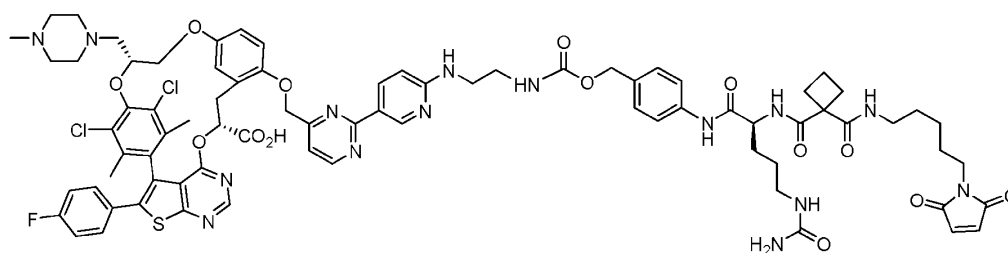
X , R^1 , R^2 , and R^{3a} are as defined for Formula (I);

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂; and

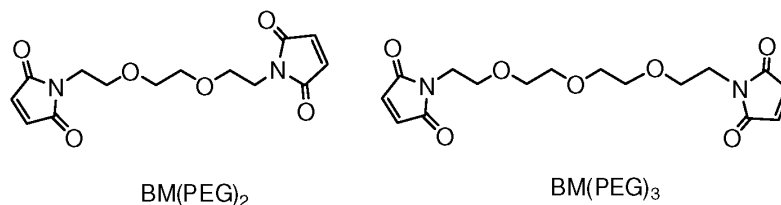
R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl.

[0153] In some embodiments of the drug payload-linker conjugates of Formula (B-L1) and (C-L1), X is O. In further embodiments of the drug payload-linker conjugates of Formula (B-L1) and (C-L1), X is O, R^{L1} is C_1 - C_{10} alkylNHC(NH)NH₂, and R^{L3} and R^{L2} together form a C_3 - C_7 cycloalkyl. In some embodiments, R^{L1} is C_1 - C_{10} alkylNHC(NH)NH₂, and R^{L3} and R^{L2} together form C_4 cycloalkyl.

[0154] Provided herein is a drug payload-linker conjugate of the structure:



[0155] The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β-maleimidopropoxy)-N-hydroxy succinimide ester (BMPS), N-(ε-maleimidocaproxy) succinimide ester (EMCS), N-[γ-maleimidobutyryloxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido)propionate (SBAP), succinimidyl iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(β-maleimidopropionamido)hexanoate] (SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone)benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4 Bismaleimidyl-2,3-dihydroxybutane (BMDDB), bismaleimidohexane (BMH), bismaleimidoethane (BMOE), BM(PEG)₂ (shown below), and BM(PEG)₃ (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bis-maleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.



[0156] Certain useful linker reagents can be obtained from various commercial sources, such as Pierce Biotechnology, Inc. (Rockford, IL), Molecular Biosciences Inc. (Boulder, CO), or synthesized in accordance with procedures described in the art; for example, in Toki *et al.*, (2002) *J. Org. Chem.* 67:1866-1872; Dubowchik *et al.*, (1997) *Tetrahedron Letters*, 38:5257-60; Walker, M.A. (1995) *J. Org. Chem.* 60:5352-5355; Frisch *et al.*, (1996) *Bioconjugate Chem.* 7:180-186; US 6214345; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

c. Drug Payload Loading of ADCs

[0157] The drug loading is the average number of drug moieties per antibody. The ADCs, provided herein, such as those of Formulae (A), (B), (B-1), (C), (C-1), (D), or (E), may include drug loading ranging from 1 to 10 drug molecules (D) per antibody (Ab). Thus, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 drug moieties may be covalently attached to the antibody, wherein the drug moiety is a drug payload of Formula (I) (including compounds of Formula (II)). A composition of ADC may include antibodies conjugated with a range of drugs, from 1 to 10, to form a composition with a range of drug loading. The average drug loading per antibody in a composition of ADC, including in a pharmaceutical composition such as those described herein, is often referred to as the drug/antibody ratio, or “DAR”. The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy (including LCMS), ELISA assay, electrophoresis, or HPLC. The quantitative distribution of ADC in terms of m may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where m is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC, electrophoresis, or LC-MS.

[0158] For some antibody-drug conjugates, the number of drug moieties per antibody “m” may be limited by the number of attachment sites on the antibody. For example, an antibody may

have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. Higher drug loading, e.g. $m > 5$, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

[0159] Typically, fewer than the theoretical maximum of drug moieties is conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the linker-drug intermediate or linker reagent. Also, in some embodiments only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent or linker-drug intermediate. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions.

[0160] The DAR of an ADC (drug loading as drug/antibody ratio) may be controlled in several different manners, including: (i) limiting the molar excess of linker-drug intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification. Where more than one nucleophilic or electrophilic group of the antibody reacts with a linker-drug intermediate, or linker reagent followed by drug moiety reagent, then the resulting product may be a mixture of ADC molecules with a distribution of drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Thus, the DAR of an ADC composition may not be an integer, but rather an average of the existing molecules in the composition. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (m) may be isolated, however, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody. Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more drug moieties and where the drug moieties may be attached to the antibody at various amino acid residues.

3. Methods of Preparing Compounds

[0161] The compounds disclosed herein may be prepared by methods known in the art of organic synthesis as set forth in part by the following synthetic schemes. In the schemes described herein, it is well understood that protecting groups for sensitive or reactive groups are employed where necessary in accordance with general principles of chemistry. Protecting groups are manipulated according to standard methods of organic synthesis (T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis," Third edition, Wiley, New York 1999). These groups are removed at a convenient stage of the compound synthesis using methods that are readily apparent to those skilled in the art. The selection processes, as well as the reaction conditions and order of their execution, shall be consistent with the preparation of compounds of disclosed herein. The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, and/or enzymatic processes.

[0162] Those skilled in the art will recognize if a stereocenter exists in the compounds disclosed herein. In some embodiments, compounds of the disclosure can exist as enantiomeric or diastereomeric stereoisomers. Accordingly, the present disclosure includes both possible stereoisomers (unless specified in the synthesis) and includes not only racemic compounds but the individual enantiomers and/or diastereomers as well. When a compound is desired as a single enantiomer or diastereomer, it may be obtained by stereospecific synthesis or by resolution of the final product or any convenient intermediate. For example, enantiomerically pure compounds of the disclosure can be prepared using enantiomerically pure chiral building blocks. Alternatively, racemic mixtures of the final compounds or a racemic mixture of an advanced intermediate can be subjected to chiral purification as described herein to deliver the desired enantiomerically pure intermediates or final compounds. In the instances where an advanced intermediate is purified into its individual enantiomers, each individual enantiomer can be carried on separately to deliver the final enantiomerically pure compounds of the disclosure. Resolution of the final product, an intermediate, or a starting material may be affected by any suitable method known in the art. See, for example, "Stereochemistry of Organic Compounds," by E. L. Eliel, S. H. Wilen, and L. N. Mander (Wiley Interscience, 1994).

4. Pharmaceutical Compositions

[0163] Provided herein is a pharmaceutical composition comprising an ADC of Formula (A) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In

some embodiments, the pharmaceutical composition comprises an ADC of Formula (B) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition comprises an ADC of Formula (B-1) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In certain embodiments, the pharmaceutical composition comprises an ADC of Formula (C) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In still further embodiments, the pharmaceutical composition comprises an ADC of Formula (C-1) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In still further embodiments, the ADC is of Formula (D), or (E).

[0164] Pharmaceutical compositions of ADCs are typically prepared for parenteral administration, such as through bolus, intravenous, or intratumor injection. Such pharmaceutical compositions may be prepared in an aqueous solution, or as a lyophilized form for reconstitution into an aqueous solution for administration. In some embodiments, the pharmaceutical composition is a liquid for intravenous administration. Further information regarding the preparation of pharmaceutical compositions, including those for parenteral administration, aqueous solutions, or as a lyophilized form for reconstitution into an aqueous solution for administration, may be found for example in Remington's Pharmaceutical Sciences (2020) 23rd edition, Adejare, A. Ed. Such pharmaceutically acceptable excipients may include, for example, buffering agents; pyrogen-free water; isotonic saline; Ringer's solution; and phosphate buffer solutions. Preservatives and antioxidants can also be present in the pharmaceutical composition, according to the judgment of the formulator.

5. Methods of Use

[0165] Provided herein are methods of treating a disorder in a subject in need thereof, comprising administering a therapeutically effective amount of an ADC as described herein to the subject. Further provided are methods of treating a disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an ADC as described herein, and a pharmaceutically acceptable excipient. The ADC of the methods herein, including administering an ADC or administering a pharmaceutical composition comprising an ADC and a pharmaceutically acceptable excipient, may be an ADC of Formula (A), Formula (B), Formula (B-1), Formula (C), or Formula (C-1).

For example, in some embodiments of the methods herein, the ADC is of Formula (B), or Formula (B-1). In other embodiments, the ADC is of Formula (C), or Formula (C-1). In still further embodiments, the ADC is of Formula (D), or (E). In some embodiments, the subject is a human.

[0166] Also provided is a compound for use in treating a disorder in a subject in need thereof, wherein the compound is an ADC as described herein (e.g., of Formula (A), (B), (B-1), (C), (C-1), (D), or (E)). Further provided is use of a compound as described herein, in treating a disorder in a subject in need thereof, wherein the compound is an ADC as described herein (e.g., of Formula (A), (B), (B-1), (C), (C-1), (D), or (E)). Still further provided is the use of a compound as described herein for the manufacture of a medicament for the treatment of a disorder in a subject in need thereof, wherein the compound is an ADC as described herein (e.g., of Formula (A), (B), (B-1), (C), (C-1), (D), or (E)). In some embodiments, the subject is a human.

[0167] In some embodiments, the disorder is a cancer, tumor or other malignancy. As used herein, cancers, tumors, and malignancies, refer to disorders, or to cells or tissues associated with the disorders, characterized by aberrant or abnormal cell proliferation, differentiation and/or migration often accompanied by an aberrant or abnormal molecular phenotype that includes one or more genetic mutations or other genetic changes associated with oncogenesis, expression of tumor markers, loss of tumor suppressor expression or activity and/or aberrant or abnormal cell surface marker expression. In some embodiments, the disorder is selected from benign or malignant solid tumors and hematological disorders such as leukemia and lymphoid malignancies. In some embodiments, cancers, tumors, and malignancies may include sarcomas, lymphomas, leukemias, solid tumors, blastomas, gliomas, carcinomas, melanomas and metastatic cancers, although without limitation thereto.

[0168] More generally, provided herein is a method of treating a hyperproliferative disease, such as cancer, in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an ADC as provided herein. In some embodiments, the ADC is of Formula (A), such as of Formula (B), Formula (B-1), Formula (C), Formula (C-1), Formula (D), or Formula (E). Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell, small-cell lung cancer, non-small cell

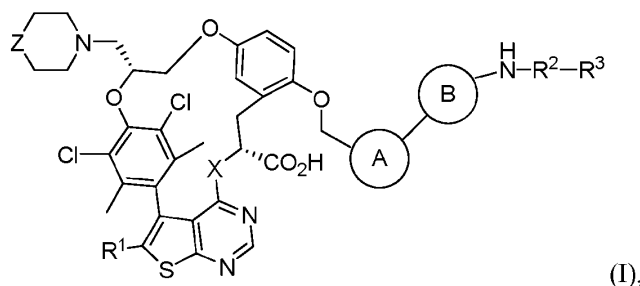
lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), or myeloid cell leukemia (MCL). Also provided are compounds for use in treating a disorder, use of a compound for treating a disorder, and use of a compound as described herein for the manufacture of a medicament for the treatment of a disorder, wherein the compound is an ADC as described herein (e.g., of Formula (A), (B), (B-1), (C), (C-1), (D), or (E)), and the disorder is as described herein. In some embodiments, the subject is a human.

[0169] In certain embodiments, an ADC as provided herein comprising an anti-CD33 antibody is used in a method of treating a of treating hematological malignancies such as non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), or myeloid cell leukemia (MCL), including B-cell related cancers and proliferative disorders. In some embodiments, an ADC as provided herein comprising an anti-HER2 antibody is used in a method of treating a HER2+ cancer, such as HER2+ breast or gastric cancer.

[0170] For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 -20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above.

ENUMERATED EMBODIMENTS

[0171] Embodiment 1. A compound, wherein the compound is of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

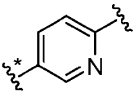
each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

[0172] Embodiment 2. The compound of Embodiment 1, or a pharmaceutically acceptable salt thereof, wherein Ring A and Ring B are independently phenyl or heteroaryl comprising one or two ring N atoms.

[0173] Embodiment 3. The compound of Embodiment 1, or a pharmaceutically acceptable salt

thereof, wherein Ring A is pyrimidine and Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A.

[0174] Embodiment 4. The compound of any one of Embodiments 1 to 3, or a pharmaceutically acceptable salt thereof, wherein R¹ is phenyl substituted by one fluoro.

[0175] Embodiment 5. The compound of any one of Embodiments 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R² is C₁-C₆alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of -OH and halo.

[0176] Embodiment 6. The compound of any one of Embodiments 1 to 5, or a pharmaceutically acceptable salt thereof, wherein Z is N-CH₃ or N-CH₂CH₃.

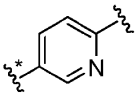
[0177] Embodiment 7. The compound of any one of Embodiments 1 to 6, or a pharmaceutically acceptable salt thereof, wherein R³ is -NH₂ or -NHCH₃.

[0178] Embodiment 8. The compound of any one of Embodiments 1 to 7, or a pharmaceutically acceptable salt thereof, wherein X is O.

[0179] Embodiment 9. The compound of Embodiment 1, or a pharmaceutically acceptable salt thereof, wherein:

Z is N-CH₂CH₃ or N-CH₃;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

R¹ is phenyl substituted by one fluoro;

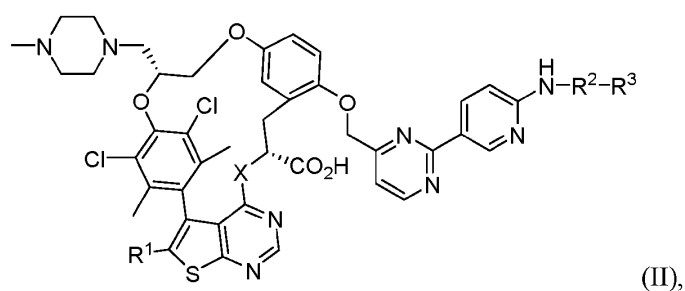
R² is C₁-C₆alkyl unsubstituted or substituted with fluoro;

R³ is -NH₂ or -NH(C₁₋₆alkyl); and

X is O.

[0180] Embodiment 10. The compound of Embodiment 1, or a pharmaceutically acceptable salt thereof, wherein X is O, R¹ is phenyl substituted with one fluoro, R² is alkyl, and R³ is -NH₂.

[0181] Embodiment 11. The compound of Embodiment 1, wherein the compound is of Formula (II):

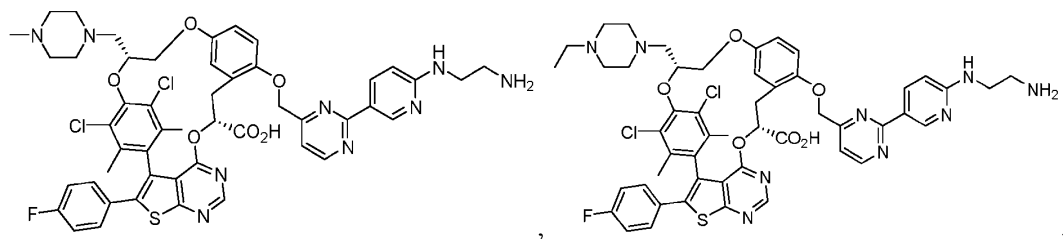


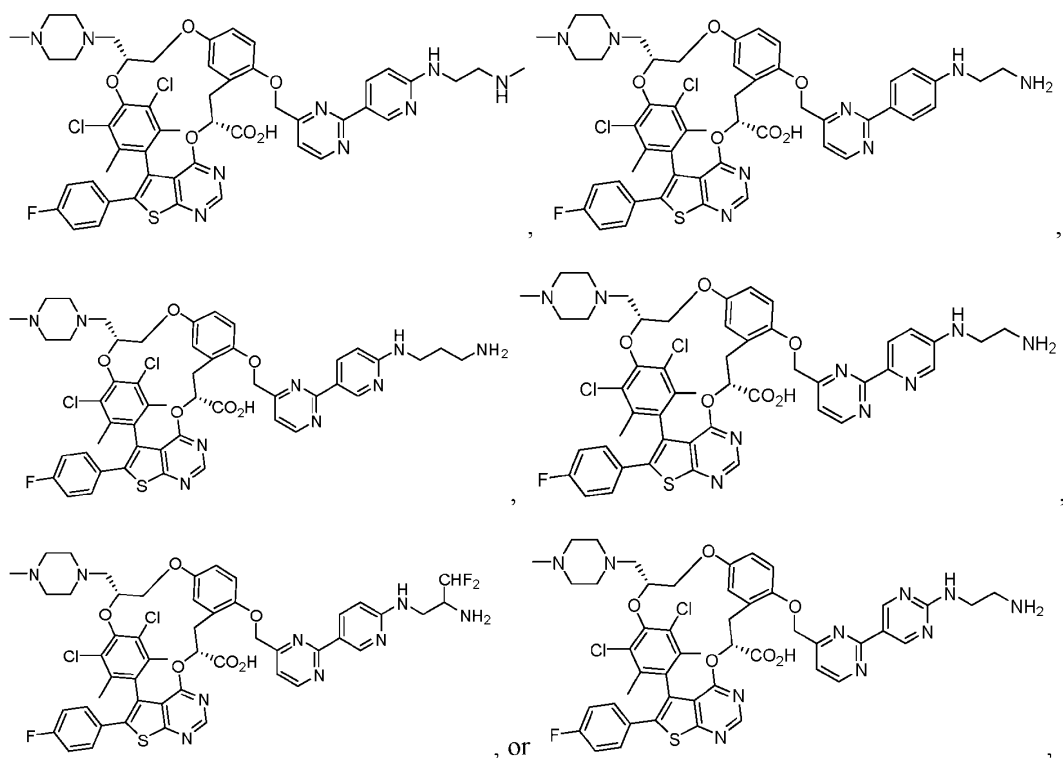
or a pharmaceutically acceptable salt thereof, wherein:

X, R¹, R², and R³ are as defined for Formula (I).

[0182] Embodiment 12. The compound of Embodiment 11, or a pharmaceutically acceptable salt thereof, wherein R² is C₁-C₆alkyl unsubstituted or substituted with fluoro; and R³ is -NH₂ or -NH(C₁-C₆alkyl).

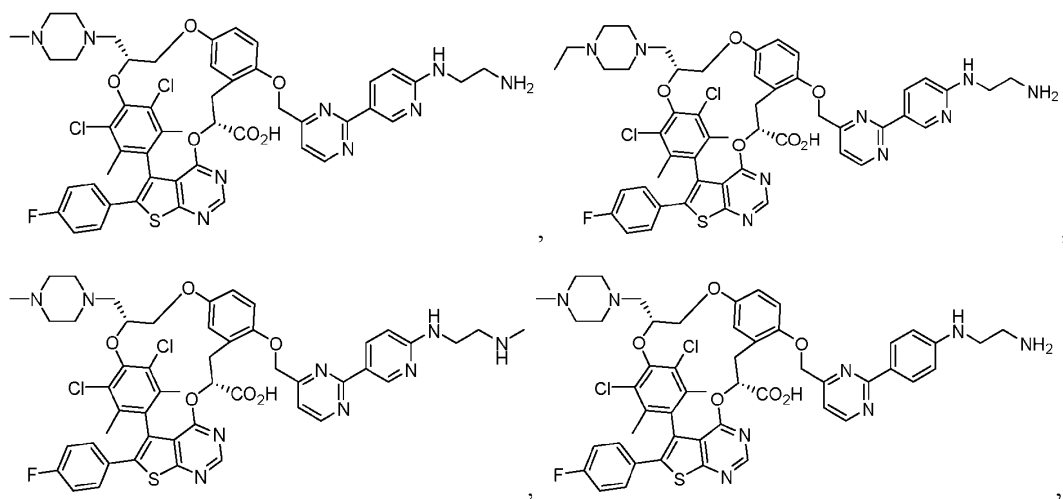
[0183] Embodiment 13. The compound of Embodiment 1, or a pharmaceutically acceptable salt thereof, wherein the compound is:

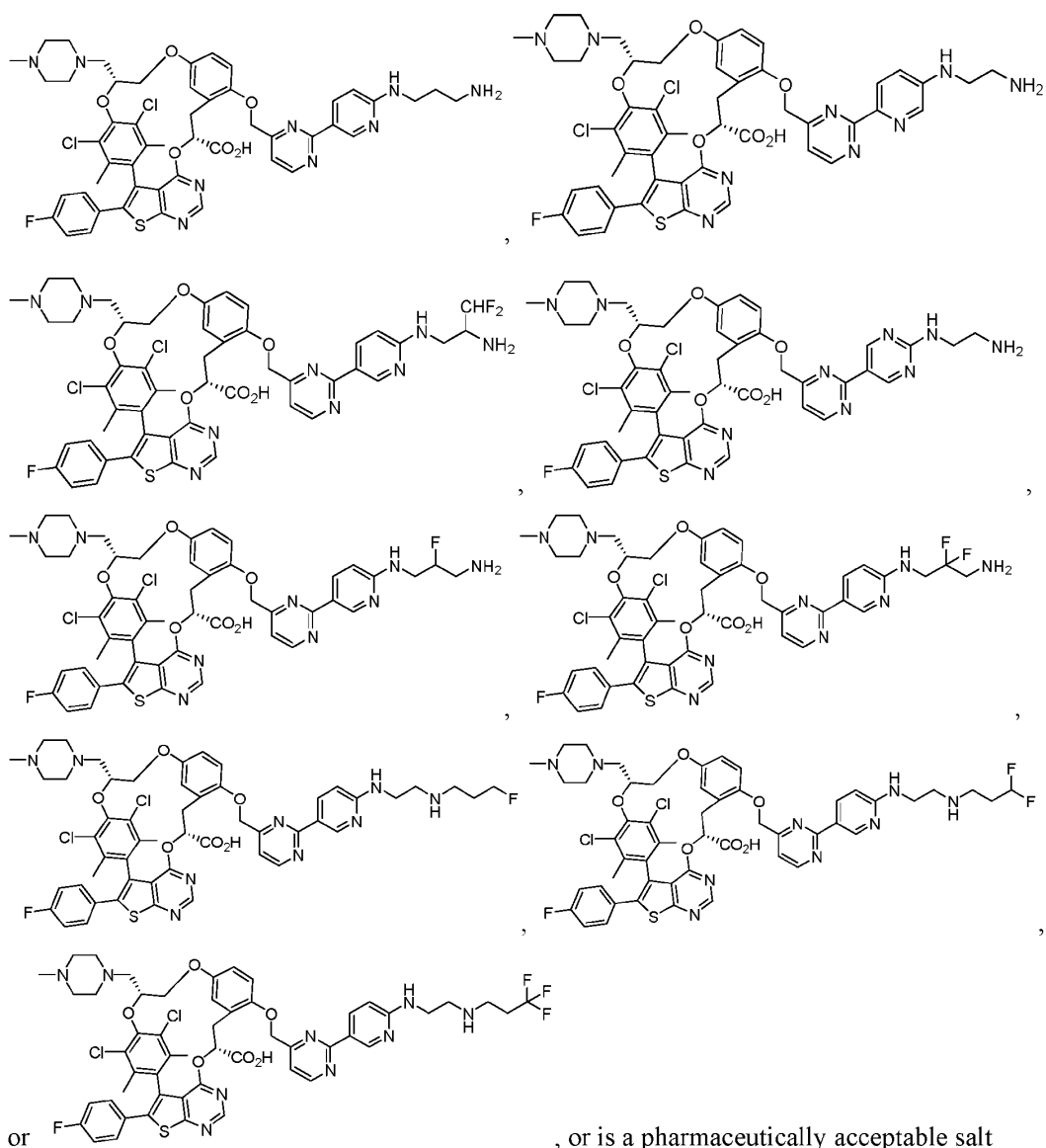




or is a pharmaceutically acceptable salt thereof.

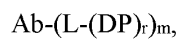
[0184] Embodiment 13a. The compound of Embodiment 1, or a pharmaceutically acceptable salt thereof, wherein the compound is:





or , or is a pharmaceutically acceptable salt thereof.

[0185] Embodiment 14. A conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

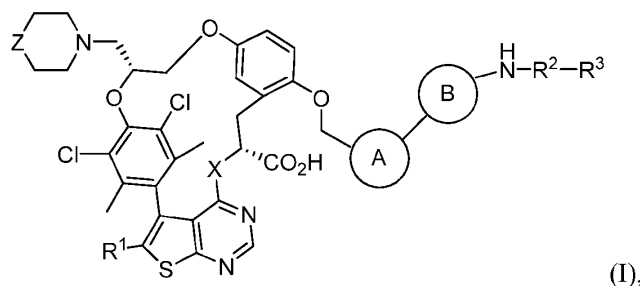
L is a linker;

DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

wherein the drug payload is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

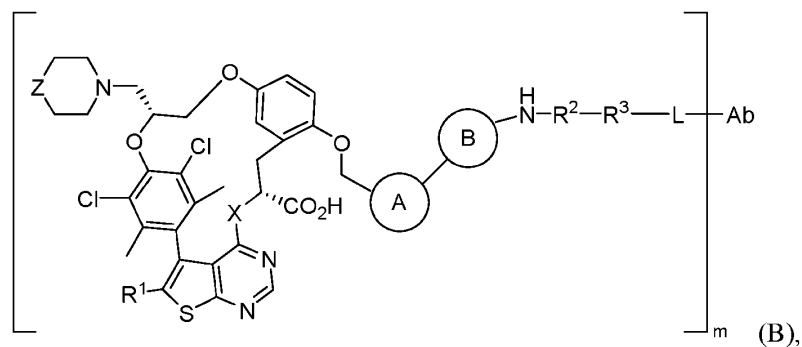
each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

[0186] Embodiment 15. The conjugate of Embodiment 14, wherein the conjugate of Formula (A) is a conjugate of Formula (B):



or a pharmaceutically acceptable salt thereof, wherein:

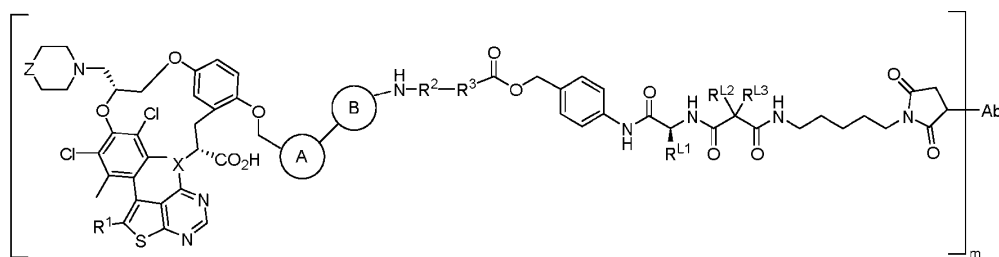
R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (I).

[0187] Embodiment 16. The conjugate of Embodiment 14 or 15, or a pharmaceutically acceptable salt thereof, wherein the linker is a peptide linker or a peptidomimetic linker.

[0188] Embodiment 17. The conjugate of any one of Embodiments 14 to 16, wherein the conjugate is of Formula (B-1):



R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10;

X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (I);

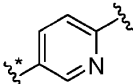
each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂; and

R^{L3} and R^{L2} are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C₃-C₇cycloalkyl.

[0189] Embodiment 18. The conjugate of any one of Embodiments 14 to 17, wherein:

Z is N-CH₂CH₃ or N-CH₃;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

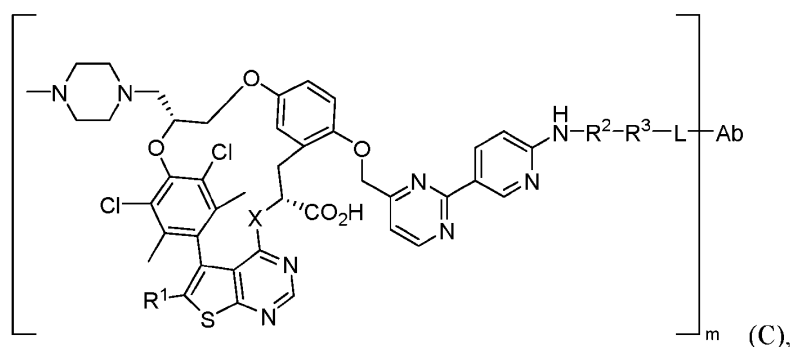
R^1 is phenyl substituted by one fluoro;

R^2 is C₁-C₆alkyl unsubstituted or substituted with fluoro;

R^3 is -NH₂ or -NH(C₁-6alkyl); and

X is O.

[0190] Embodiment 19. The conjugate of Embodiment 15, wherein the conjugate of Formula (A) is a conjugate of Formula (C):



or a pharmaceutically acceptable salt thereof, wherein:

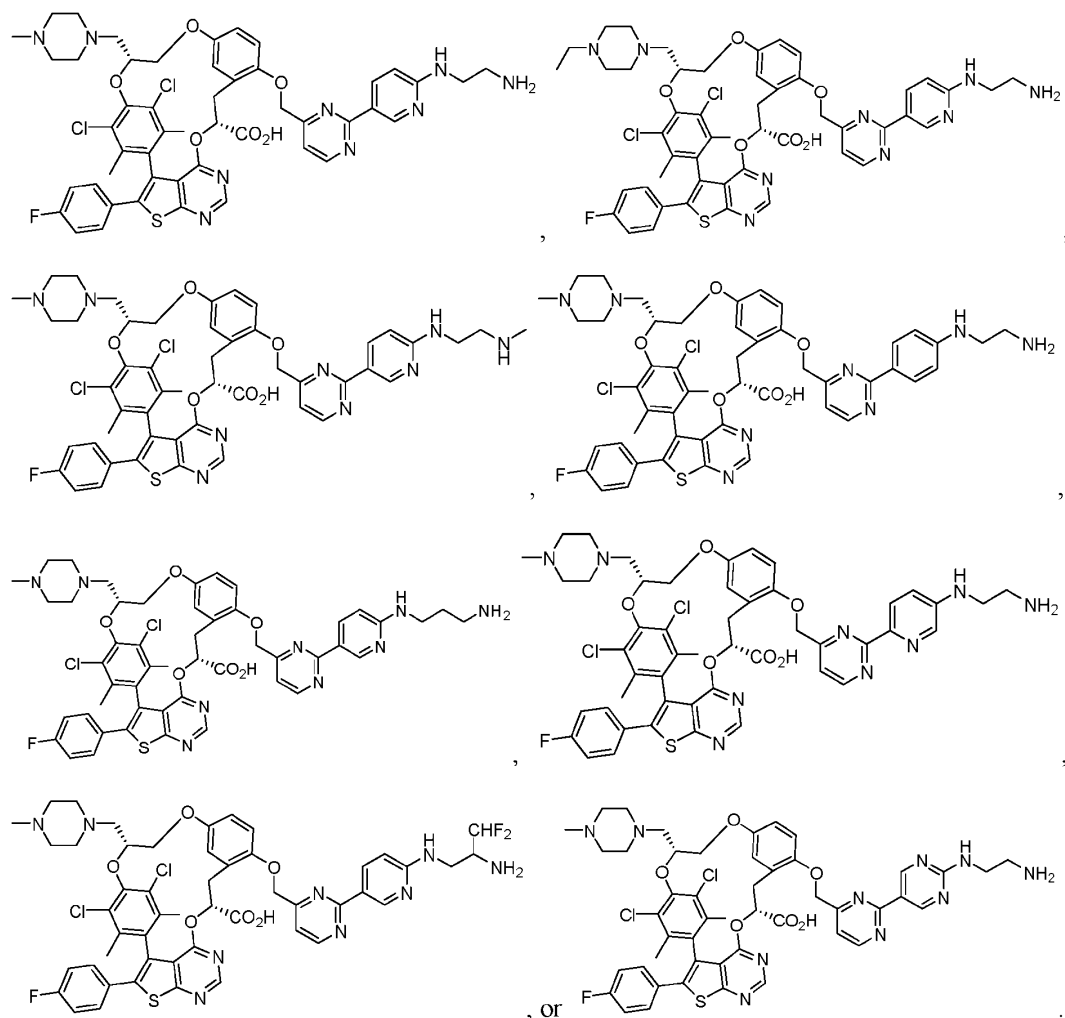
R^3 is -NH, -NR^{3a}, or -N⁺(R^{3a})₂;

m is an integer from 1 to 10; and

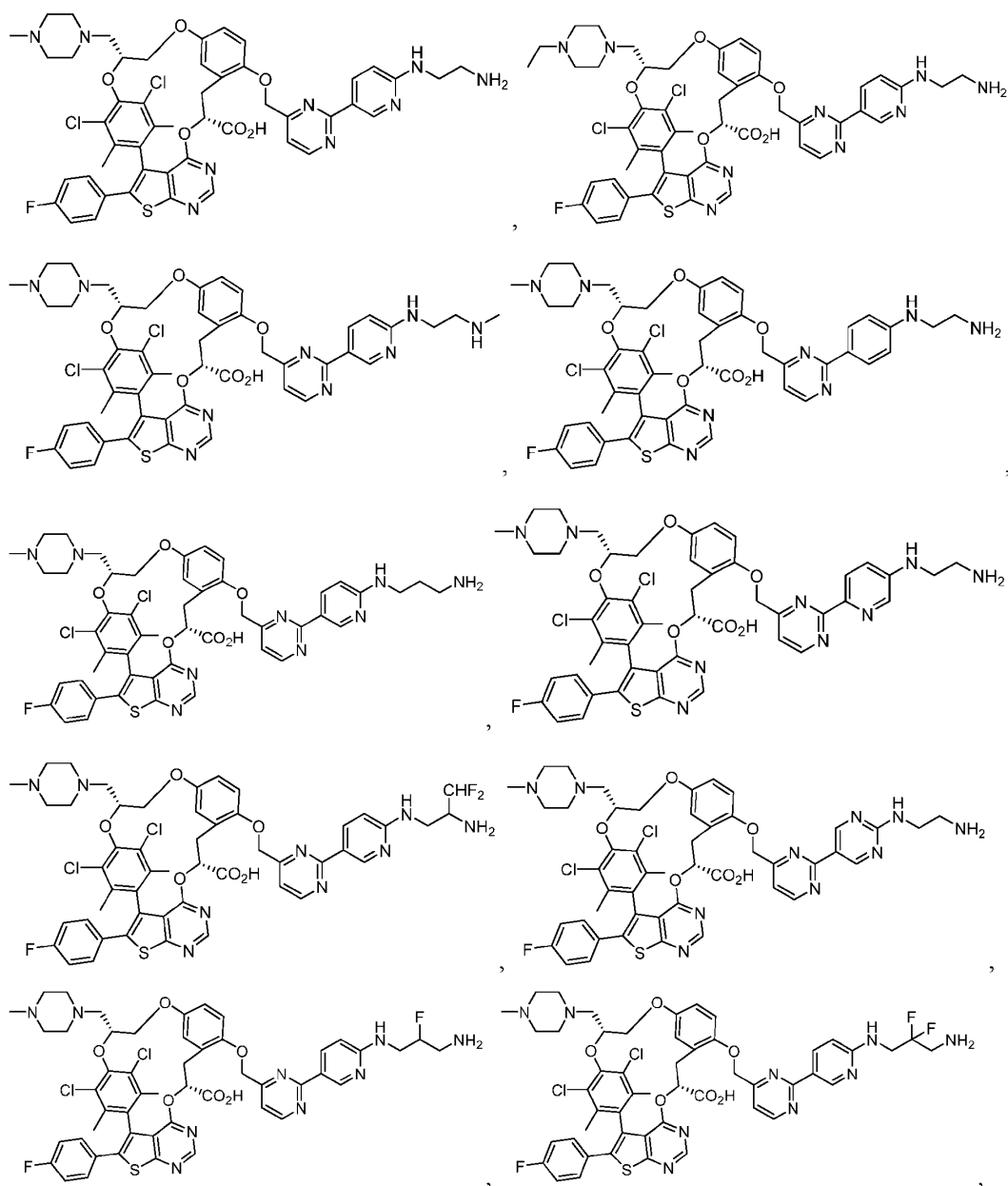
and X, R^1 , R^2 , and R^{3a} are as defined for Formula (I).

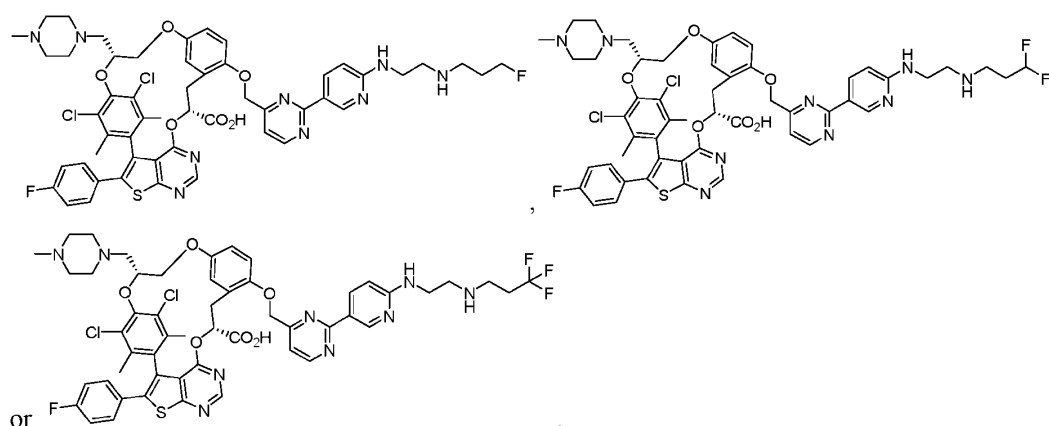
[0191] Embodiment 20. The conjugate of Embodiment 19, or a pharmaceutically acceptable salt thereof, wherein R^2 is C₁-C₆alkyl unsubstituted or substituted with fluoro; and R^3 is -NH₂ or -NH(C₁-6alkyl).

[0192] Embodiment 21. The conjugate of any one of Embodiments 14 to 17, or a pharmaceutically acceptable salt thereof, wherein the drug payload is:

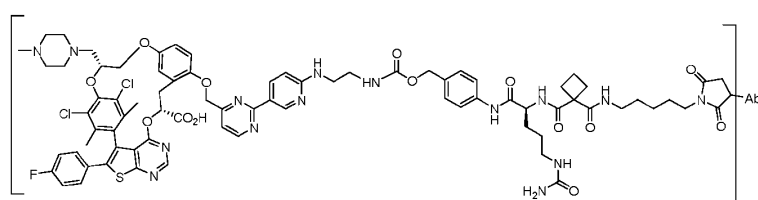


[0193] Embodiment 21a. The conjugate of any one of Embodiments 14 to 17, or a pharmaceutically acceptable salt thereof, wherein the drug payload is:





[0194] Embodiment 22. The conjugate of Embodiment 14, wherein the conjugate is of the structure:



[0195] Embodiment 23. The conjugate of any one of Embodiments 14 to 22, wherein the antibody binds to one or more to one or more tumor-associated antigens or cell-surface receptors selected from the group consisting of CLL1, CD71, Trop2, MSLN, NaPi2b, Ly6E, EpCAM, STEAP1, STEAP2, TrpM4, CD21, CD79a, CD72, MUC16, HER2, CD33, CD22, CD79b, LIV1, CD123, CD74, BCMA, and FcRH5.

[0196] Embodiment 24. A pharmaceutical composition comprising the conjugate of any one of Embodiments 14 to 23, and a pharmaceutically acceptable excipient.

[0197] Embodiment 25. A method of treating a disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a conjugate of any one of Embodiments 14 to 23.

[0198] Embodiment 26. The method of Embodiment 25, wherein the disorder is a cancer, tumor, or other malignancy.

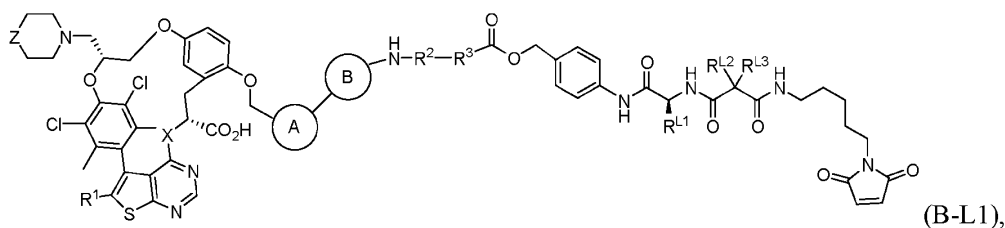
[0199] Embodiment 27. The method of Embodiment 26, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).

[0200] Embodiment 28. A compound for use in a method of treating a disorder in a subject in need thereof, wherein the compound is a conjugate of any one of Embodiments 14 to 23.

[0201] Embodiment 29. The compound for use of Embodiment 28, wherein the disorder is a cancer, tumor, or other malignancy.

[0202] Embodiment 30. The compound for use of Embodiment 29, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).

[0203] Embodiment 31. A drug payload-linker conjugate, wherein the drug payload-linker conjugate is of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-OH$; each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH $_2$ or C_1 - C_{10} alkylNHC(O)NH $_2$;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

Z is N- C_{1-6} alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

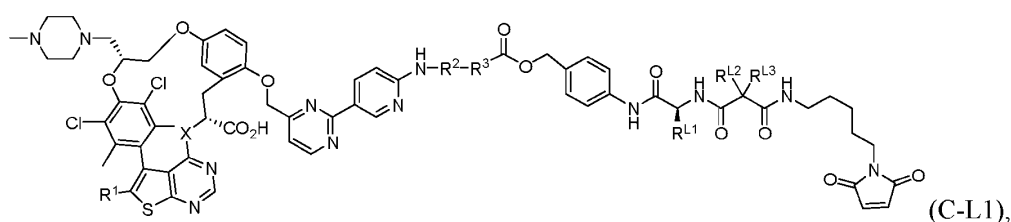
R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

[0204] Embodiment 32. The drug payload-linker conjugate of Embodiment 31, wherein the drug payload-linker intermediate is of Formula (C-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

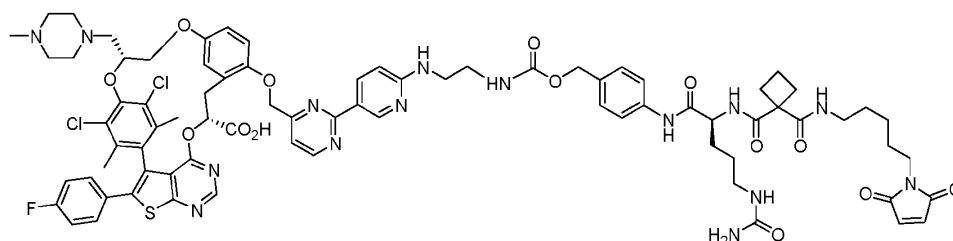
R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

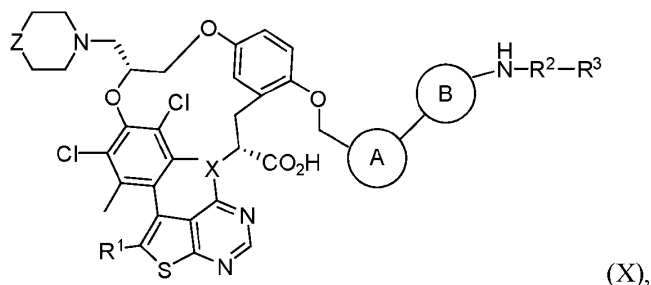
each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

[0205] Embodiment 33. The drug payload-linker conjugate of Embodiment 31, wherein the drug payload-linker intermediate is of the structure:



[0206] Embodiment 34. A compound of Formula (X):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

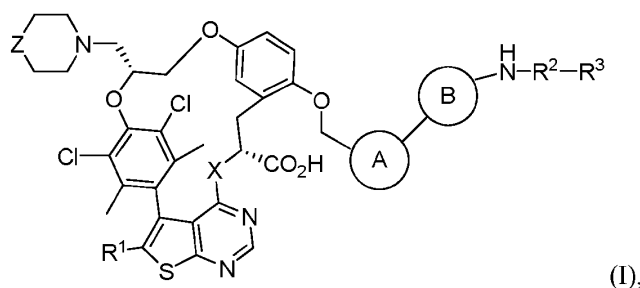
each R^{2a} and each R^{2b} is independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8;

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, alkoxy, and haloalkoxy.

[0207] Embodiment 35. The compound of claim 34, wherein the compound of Formula (X) is a compound of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-, wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

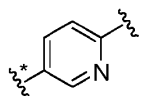
each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and -OH.

[0208] Embodiment 36. The compound of Embodiment 34 or 35, or a pharmaceutically acceptable salt thereof, wherein Ring A and Ring B are independently phenyl or heteroaryl comprising one or two ring N atoms.

[0209] Embodiment 37. The compound of Embodiment 34 or 35, or a pharmaceutically acceptable salt thereof, wherein Ring A is pyrimidine and Ring B is phenyl, pyrimidine, or



, wherein the * indicates the attachment point to Ring A.

[0210] Embodiment 38. The compound of any one of Embodiments 34 to 37, or a pharmaceutically acceptable salt thereof, wherein R¹ is phenyl substituted by one fluoro.

[0211] Embodiment 39. The compound of any one of Embodiments 34 to 38, or a pharmaceutically acceptable salt thereof, wherein R² is C₁-C₆alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of -OH and halo.

[0212] Embodiment 40. The compound of any one of Embodiments 34 to 39, or a pharmaceutically acceptable salt thereof, wherein Z is N-CH₃ or N-CH₂CH₃.

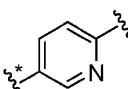
[0213] Embodiment 41. The compound of any one of Embodiments 34 to 40, or a pharmaceutically acceptable salt thereof, wherein R³ is -NH₂ or -NHCH₃.

[0214] Embodiment 42. The compound of any one of Embodiments 34 to 41, or a pharmaceutically acceptable salt thereof, wherein X is O.

[0215] Embodiment 43. The compound of Embodiment 34 or 35, or a pharmaceutically acceptable salt thereof, wherein:

Z is N-CH₂CH₃ or N-CH₃;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

R¹ is phenyl substituted by one fluoro;

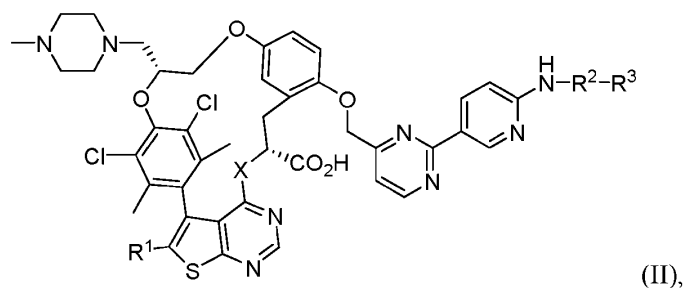
R^2 is C_1 - C_6 alkyl unsubstituted or substituted with fluoro;

R^3 is $-NH_2$ or $-NH(C_1$ - C_6 alkyl); and

X is O.

[0216] Embodiment 44. The compound of Embodiment 34 or 35, or a pharmaceutically acceptable salt thereof, wherein X is O, R^1 is phenyl substituted with one fluoro, R^2 is alkyl, and R^3 is $-NH_2$.

[0217] Embodiment 45. The compound of Embodiment 34 or 35, wherein the compound is of Formula (II):

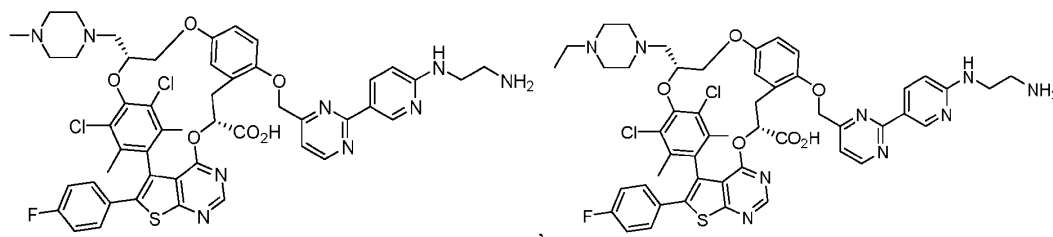


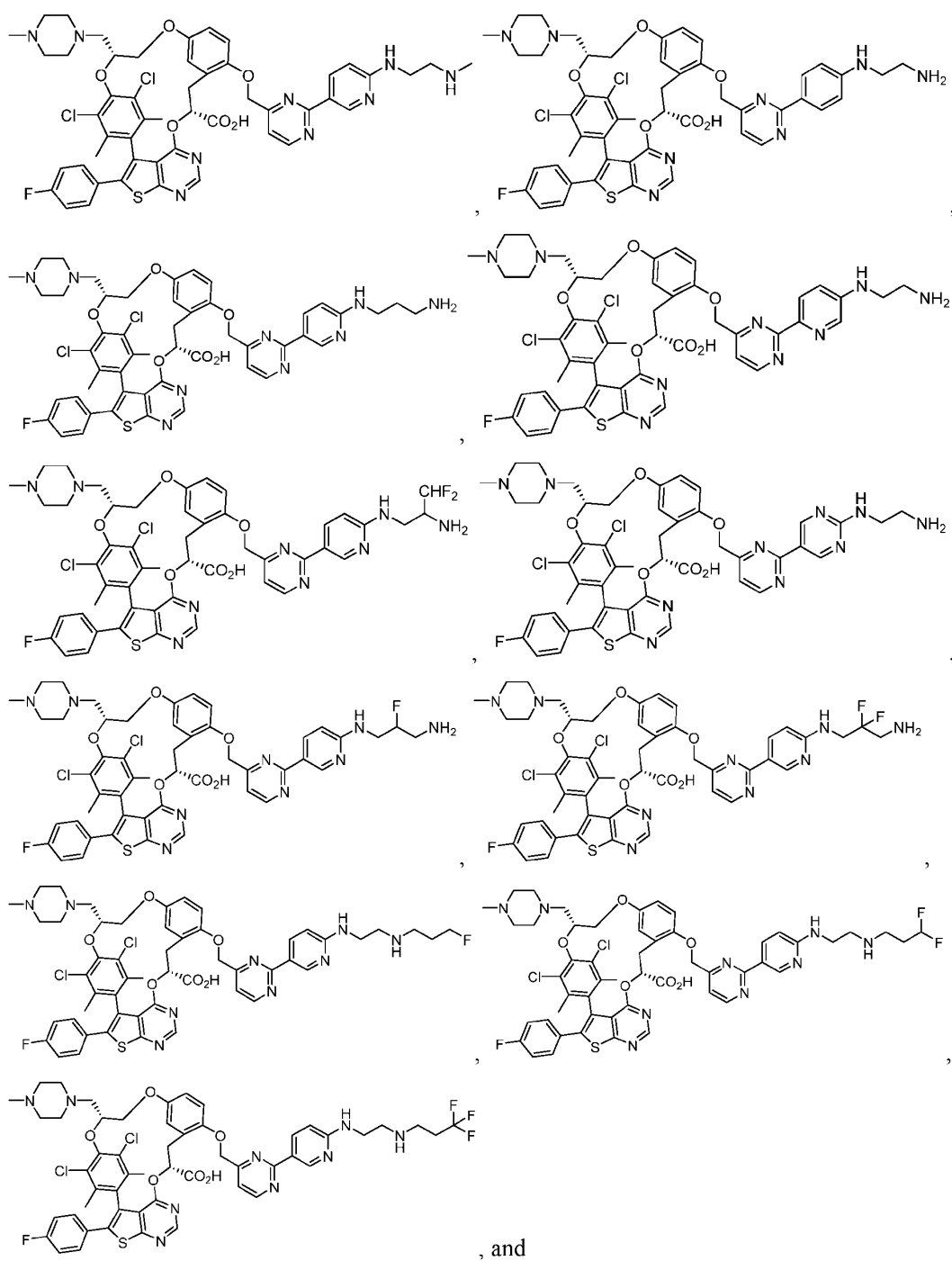
or a pharmaceutically acceptable salt thereof, wherein:

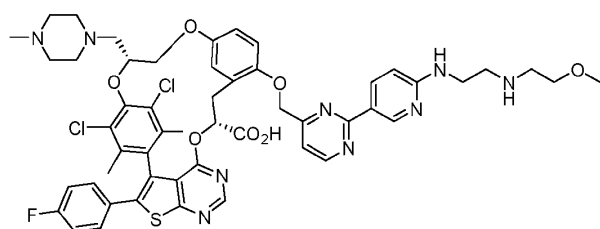
X, R^1 , R^2 , and R^3 are as defined for Formula (I).

[0218] Embodiment 46. The compound of Embodiment 45, or a pharmaceutically acceptable salt thereof, wherein R^2 is C_1 - C_6 alkyl unsubstituted or substituted with fluoro; and R^3 is $-NH_2$ or $-NH(C_1$ - C_6 alkyl).

[0219] Embodiment 47. The compound of Embodiment 34, or a pharmaceutically acceptable salt thereof, wherein the compound is:

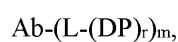






, or is a pharmaceutically acceptable salt thereof.

[0220] Embodiment 48. A conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

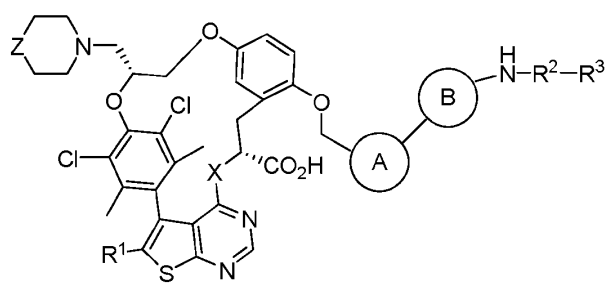
L is a linker;

DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

wherein the drug payload is a compound of Formula (X):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents

independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

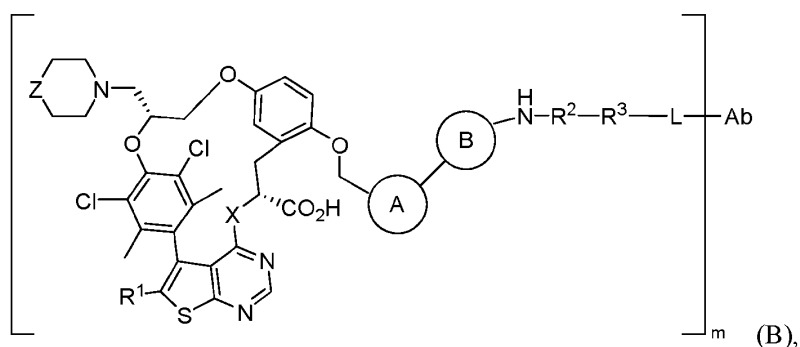
each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R³ is -NH₂, -NHR^{3a}, -N(R^{3a})₂, or -N⁺(R^{3a})₃, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, -OH, alkoxy, and haloalkoxy.

[0221] Embodiment 49. The conjugate of Embodiment 48, wherein the conjugate of Formula (A) is a conjugate of Formula (B):



or a pharmaceutically acceptable salt thereof, wherein:

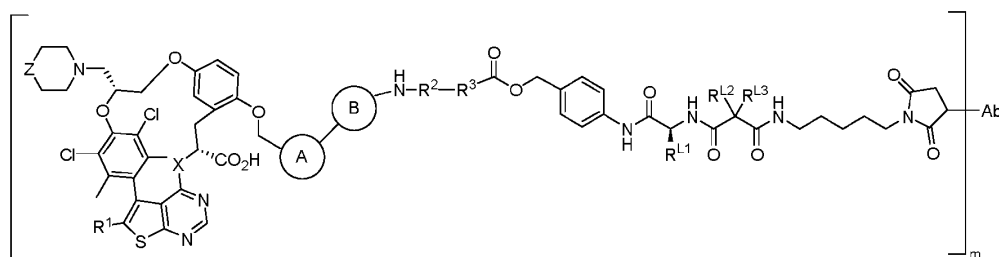
R³ is -NH-, -NR^{3a}-, or -N⁺(R^{3a})₂-;

m is an integer from 1 to 10; and

X, R¹, R², R^{3a}, Z, Ring A, and Ring B are as defined for Formula (X).

[0222] Embodiment 50. The conjugate of Embodiment 48 or 49, or a pharmaceutically acceptable salt thereof, wherein the linker is a peptide linker or a peptidomimetic linker.

[0223] Embodiment 51. The conjugate of any one of Embodiments 48 to 50, wherein the conjugate is of Formula (B-1):



R^3 is $-\text{NH}-$, $-\text{NR}^{3a}-$, or $-\text{N}^+(\text{R}^{3a})_2-$;

m is an integer from 1 to 10;

X , R^1 , R^2 , R^{3a} , Z , Ring A, and Ring B are as defined for Formula (X);

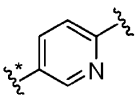
each R^{L1} is independently $\text{C}_1\text{-C}_{10}\text{alkyl}$, $\text{C}_1\text{-C}_{10}\text{alkenyl}$, $\text{C}_1\text{-C}_{10}\text{alkylNHC(NH)NH}_2$ or $\text{C}_1\text{-C}_{10}\text{alkylNHC(O)NH}_2$; and

R^{L3} and R^{L2} are each independently H , $\text{C}_1\text{-C}_{10}\text{alkyl}$, $\text{C}_1\text{-C}_{10}\text{alkenyl}$, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a $\text{C}_3\text{-C}_7\text{cycloalkyl}$.

[0224] Embodiment 52. The conjugate of any one of Embodiments 48 to 51, wherein:

Z is $\text{N-CH}_2\text{CH}_3$ or N-CH_3 ;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

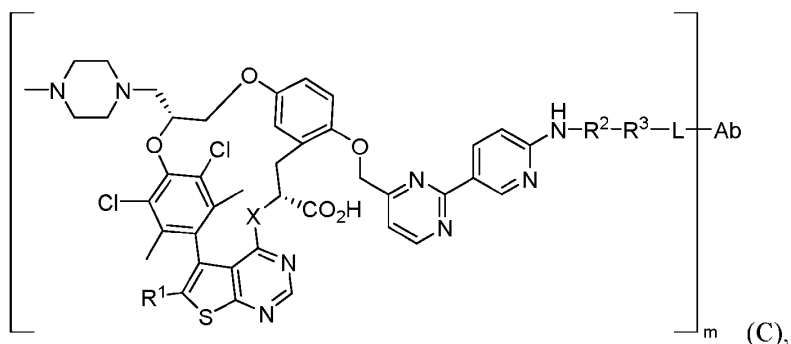
R^1 is phenyl substituted by one fluoro;

R^2 is $\text{C}_1\text{-C}_6\text{alkyl}$ unsubstituted or substituted with fluoro;

R^3 is $-\text{NH}_2$ or $-\text{NH}(\text{C}_1\text{-C}_6\text{alkyl})$; and

X is O .

[0225] Embodiment 53. The conjugate of Embodiment 48, wherein the conjugate of Formula (A) is a conjugate of Formula (C):



or a pharmaceutically acceptable salt thereof, wherein:

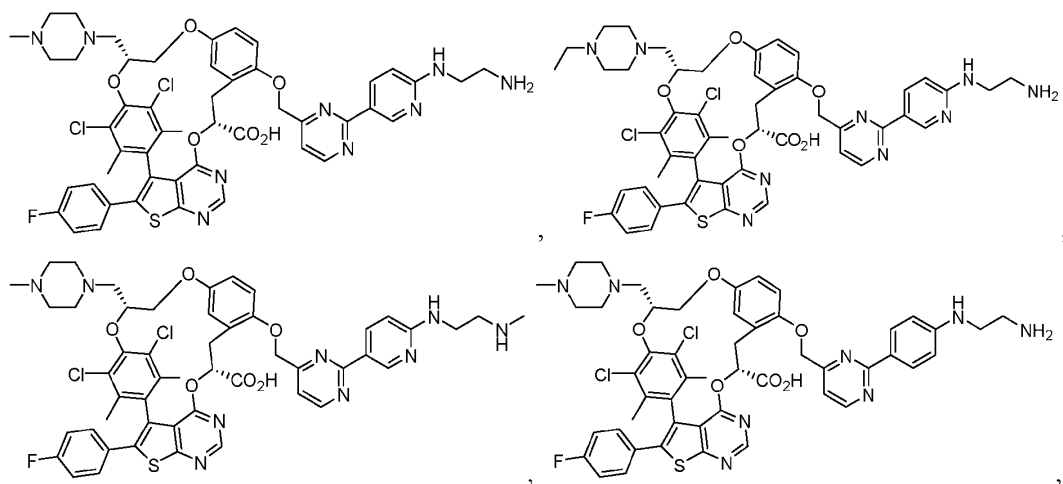
R^3 is $-NH$, $-NR^{3a}$, or $-N^+(R^{3a})_2$;

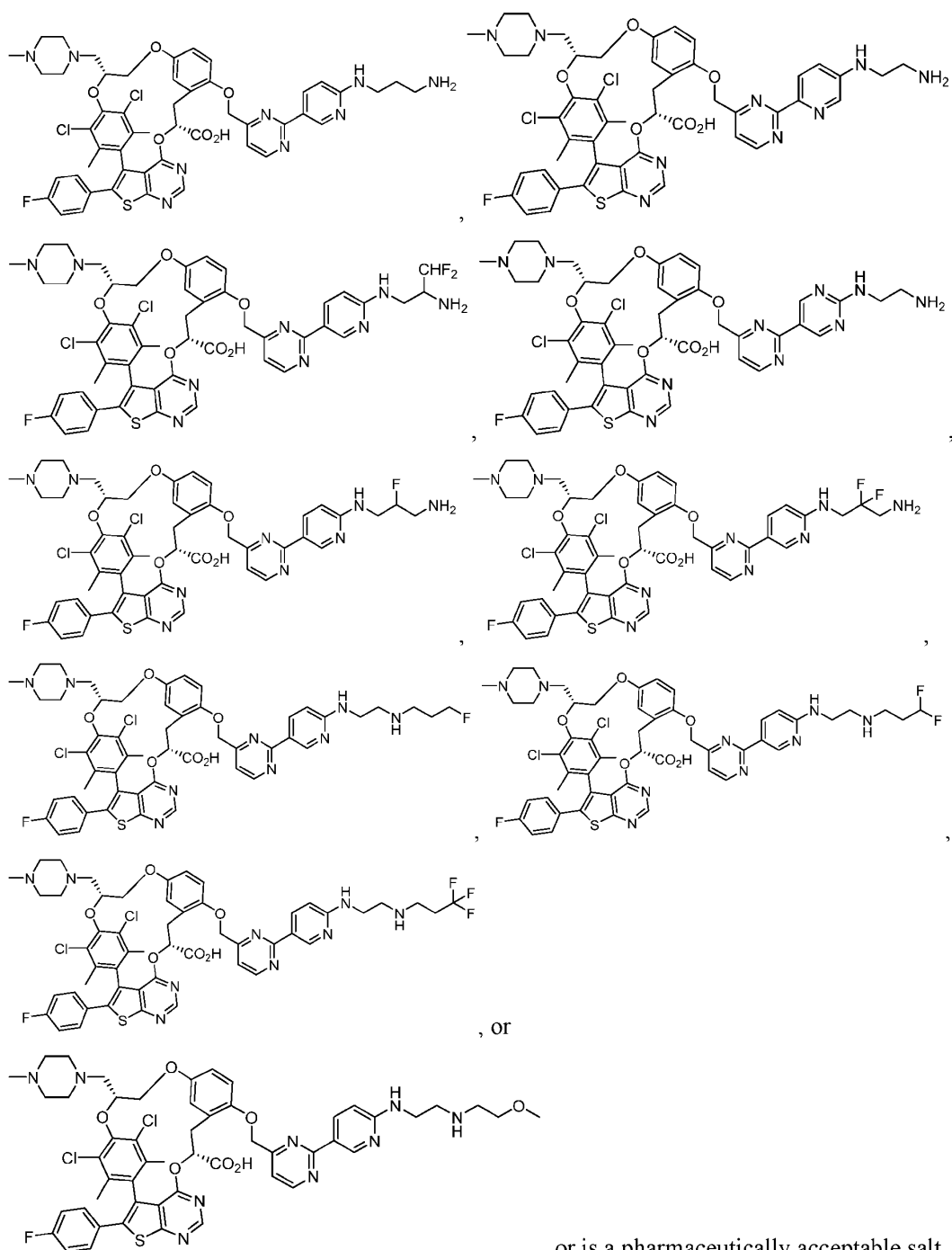
m is an integer from 1 to 10; and

and X , R^1 , R^2 , and R^{3a} are as defined for Formula (X).

[0226] Embodiment 54. The conjugate of Embodiment 53, or a pharmaceutically acceptable salt thereof, wherein R^2 is C_1 - C_6 alkyl unsubstituted or substituted with fluoro; and R^3 is $-NH_2$ or $-NH(C_1$ - C_6 alkyl).

[0227] Embodiment 55. The conjugate of any one of Embodiments 48 to 54, or a pharmaceutically acceptable salt thereof, wherein the drug payload is:

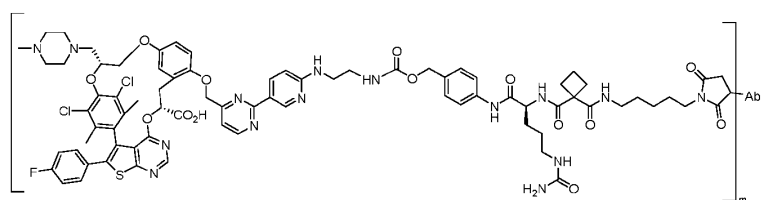




, or is a pharmaceutically acceptable salt

thereof.

[0228] Embodiment 56. The conjugate of Embodiment 48, wherein the conjugate is of the structure:



[0229] Embodiment 57. The conjugate of any one of Embodiments 48 to 56, wherein the antibody binds to one or more to one or more tumor-associated antigens or cell-surface receptors selected from the group consisting of CLL1, CD71, Trop2, MSLN, NaPi2b, Ly6E, EpCAM, STEAP1, STEAP2, TrpM4, CD21, CD79a, CD72, MUC16, HER2, CD33, CD22, CD79b, LIV1, CD123, CD74, BCMA, and FcRH5.

[0230] Embodiment 58. A pharmaceutical composition comprising the conjugate of any one of Embodiments 48 to 57, and a pharmaceutically acceptable excipient.

[0231] Embodiment 59. A method of treating a disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a conjugate of any one of Embodiments 48 to 57.

[0232] Embodiment 60. The method of Embodiment 59, wherein the disorder is a cancer, tumor, or other malignancy.

[0233] Embodiment 61. The method of Embodiment 59, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic

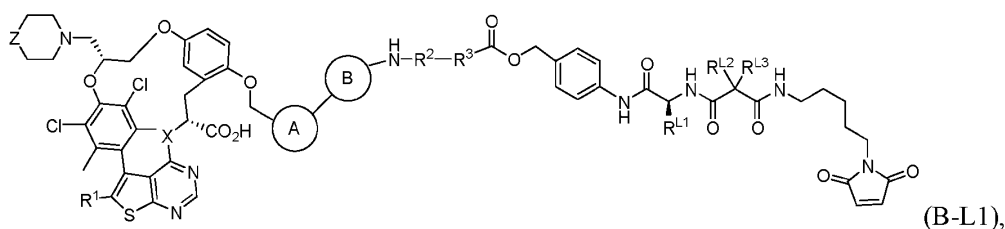
lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).

[0234] Embodiment 62. A compound for use in a method of treating a disorder in a subject in need thereof, wherein the compound is a conjugate of any one of Embodiments 48 to 57.

[0235] Embodiment 63. The compound for use of Embodiment 62, wherein the disorder is a cancer, tumor, or other malignancy.

[0236] Embodiment 64. The compound for use of Embodiment 62, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).

[0237] Embodiment 65. A drug payload-linker conjugate, wherein the drug payload-linker conjugate is of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-OH$, alkoxy, and haloalkoxy;

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

Z is N- C_1 -6alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

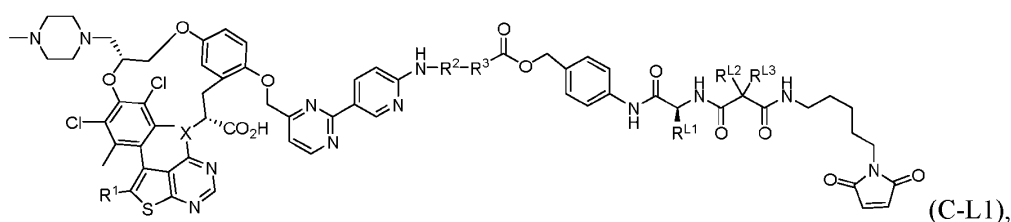
R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

[0238] Embodiment 66. The drug payload-linker conjugate of Embodiment 65, wherein the drug payload-linker intermediate is of Formula (C-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more

substituents independently selected from the group consisting of halo, -OH, alkoxy, and haloalkoxy.

each R^{L1} is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C₃-C₇cycloalkyl;

X is O or NH;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

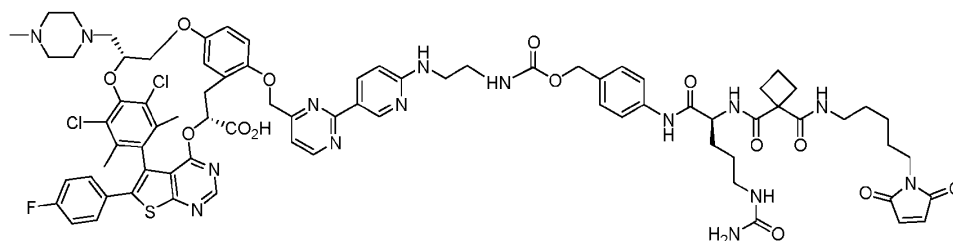
R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

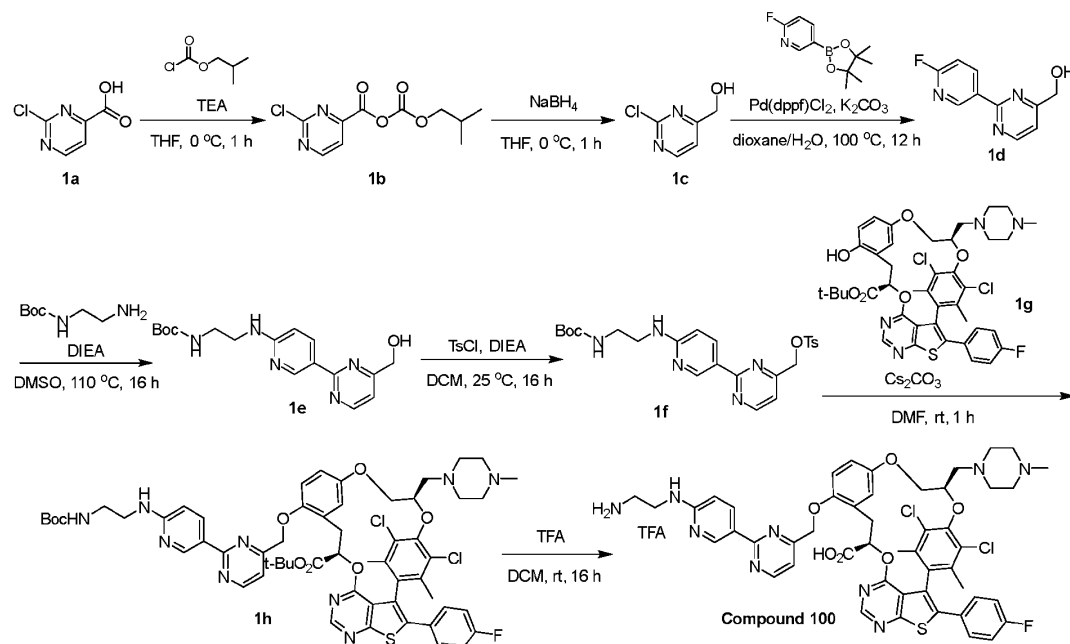
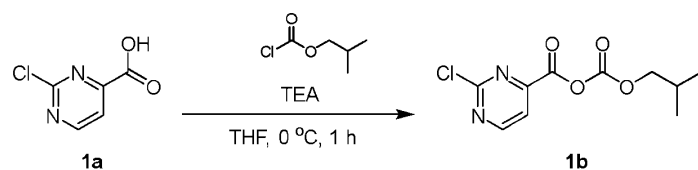
[0239] Embodiment 67. The drug payload-linker conjugate of Embodiment 65, wherein the drug payload-linker intermediate is of the structure:



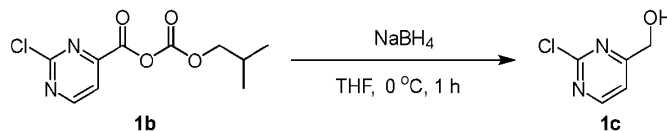
EXAMPLES

[0240] Abbreviations used in the following examples may include:

ACN: acetonitrile
DCM: dichloromethane
DEA: diethylamine
DIPEA: N,N-diisopropylethylamine
DMAP: 4-dimethylaminopyridine
DMF: dimethylformamide
DMSO: dimethylsulfoxide
EtOAc: ethyl acetate
EtOH: ethanol
HOAc: acetic acid
HPLC: high performance liquid chromatography
IPA: isopropanol
LCMS: liquid chromatography-mass spectrometry
MeOH: methanol
MsCl: methanesulfonyl chloride
MTBE: methyl tert-butyl ether
NBS: N-bromosuccinimide
NMR: nuclear magnetic resonance
PTSA: p-toluenesulfonic acid
TBAF: tetra-n-butylammonium fluoride
TBSCl: tert-butyldimethylsilyl chloride
TEA: triethylamine
TFA: trifluoroacetic acid
THF: tetrahydrofuran
TLC: thin layer chromatography
prep-TLC: preparative thin layer chromatography
SFC: supercritical fluid chromatography

Synthetic Example 1: Synthesis of Compound 100**Step 1: Preparation of intermediate 1b**

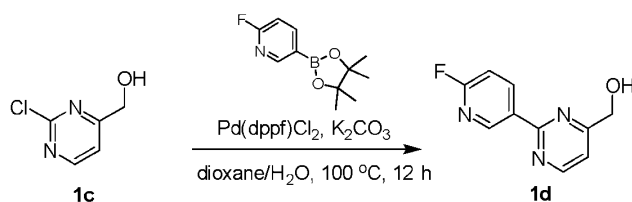
[0241] To a solution of 2-chloropyrimidine-4-carboxylic acid (10 g, 63.1 mmol) and triethylamine (9.7 mL, 69.4 mmol) in Tetrahydrofuran (120 mL) was added isobutyl chloroformate (9.0 mL, 69.4 mmol) slowly at 0 °C. The mixture was stirred at 0 °C for 1 h. The reaction mixture was filtered and the filtrate was directly used for the next step.

Step 2: Preparation of intermediate 1c

[0242] To a solution of 2-chloropyrimidine-4-carboxylic (isobutyl carbonic) anhydride (16.3 g, 63.0 mmol) in Tetrahydrofuran (200 mL) and water (20 mL) was added sodium borohydride (4.7 g, 124.8 mmol) at 0 °C dropwise. Then the mixture was stirred at 0 °C for 1 hour. The reaction

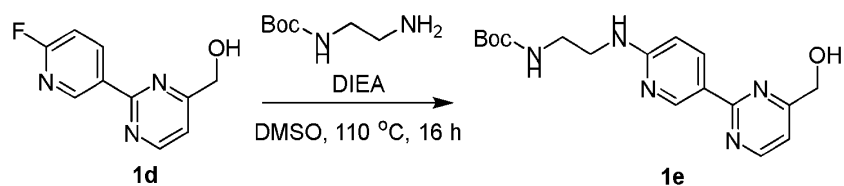
mixture was quenched with aqueous NH_4Cl (20 mL) and diluted with water (100 mL), extracted with ethyl acetate (100 mL x 2), washed with brine (50 mL x 2), dried over Na_2SO_4 , filtered and concentrated to residue which was purified by silica gel chromatography (solvent gradient: 0-50% ethyl acetate in petroleum ether) to afford (2-chloropyrimidin-4-yl)methanol (3 g, 33% yield) as a yellow solid.

Step 3: Preparation of intermediate 1d



[0243] To a solution of (2-chloropyrimidin-4-yl) methanol (4.5 g, 31.1 mmol), 2-fluoropyridine-5-boronic acid pinacol ester (8.3 g, 37.4 mmol) and K_2CO_3 (12.9 g, 93.4 mmol) in dioxane (120 mL) and water (12 mL) was added Pd(dppf)Cl_2 (2.3 g, 3.1 mmol). The reaction mixture was degassed with N_2 for three times. The resulting mixture was stirred at 100 °C for 12 hours. The mixture was filtered and concentrated to residue which was purified by silica gel chromatography (solvent gradient: 0-50% ethyl acetate in petroleum ether) to afford (2-(6-fluoropyridin-3-yl)pyrimidin-4-yl)methanol (4.7 g, 74% yield) as a white solid. ^1H NMR (400MHz, DMSO-d_6) δ 9.15 (d, J = 2.4 Hz, 1H), 8.92 (d, J = 5.2 Hz, 1H), 8.85 - 8.80 (m, 1H), 7.56 (d, J = 5.2 Hz, 1H), 7.36 - 7.33 (m, 1H), 5.73 (t, J = 6.0 Hz, 1H), 4.67 - 4.63 (m, 2H).

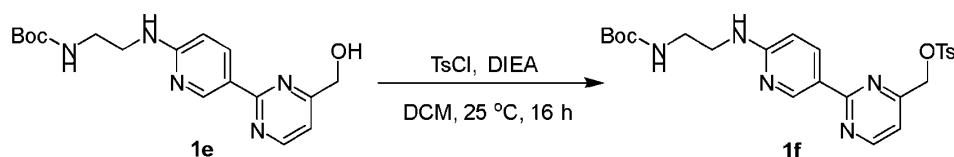
Step 4: Preparation of intermediate 1e



[0244] To a solution of [2-(6-fluoro-3-pyridyl) pyrimidin-4-yl] methanol (3.0 g, 14.6 mmol), tert-butyl-N-(2-aminoethyl) carbamate (6.9 mL, 43.8 mmol) in DMSO (70 mL) was added N, N-Diisopropylethylamine (12.7 mL, 73.1 mmol) at 25 °C, the mixture was heated to 110 °C and stirred for 16 hours. The reaction mixture was diluted with water (30 mL), extracted with ethyl acetate (50 mL x 2), washed with brine (50 mL x 2), dried over Na_2SO_4 , filtered and concentrated to residue which was purified by silica gel chromatography (solvent gradient: 0-5%

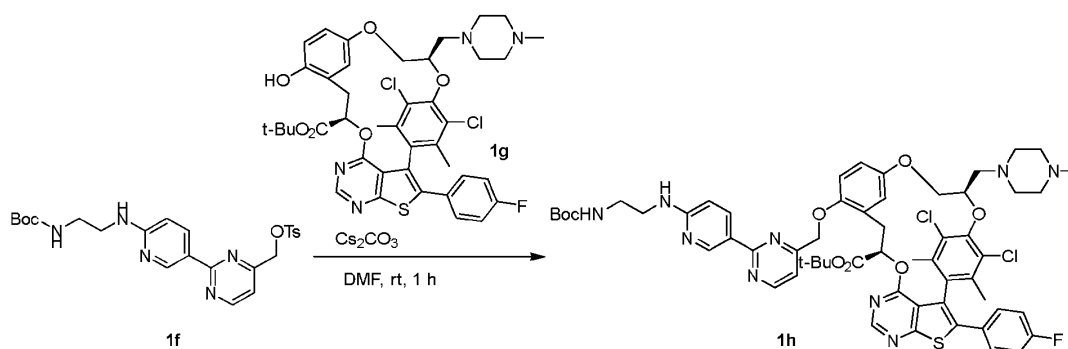
methanol in dichloromethane) to afford tert-butyl (2-((5-(4-(hydroxymethyl)pyrimidin-2-yl)pyridin-2-yl)amino)ethyl)carbamate (4.3 g, 85% yield) as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6): δ 8.98 (d, J = 2.0 Hz, 1H), 8.74 (d, J = 5.2 Hz, 1H), 8.26 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 5.2 Hz, 1H), 7.09 (t, J = 5.2 Hz, 1H), 6.89 (t, J = 5.2 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 5.60 (t, J = 6.0 Hz, 1H), 4.57 (d, J = 6.0 Hz, 2H), 3.44 (d, J = 7.2 Hz, 2H), 3.11 (d, J = 6.4 Hz, 2H), 1.38 (s, 9H).

Step 5: Preparation of intermediate 1f



[0245] To a solution of tert-butyl N-[2-[[5-[4-(hydroxymethyl) pyrimidin-2-yl]-2-pyridyl] amino]ethyl] carbamate (1 g, 2.9 mmol), N, N-Diisopropylethylamine (2.02 mL, 11.6 mmol) in dichloromethane (10 mL) was added TsCl (1.1 g, 5.8 mmol), the mixture was stirred at 25 °C for 16 hours. The mixture was concentrated on vacuo and purified by silica gel chromatography (solvent gradient: 0-2% methanol in dichloromethane) to afford (2-(6-((2-((tert-butoxycarbonyl)amino)ethyl)amino)pyridin-3-yl)pyrimidin-4-yl)methyl 4-methylbenzenesulfonate (660 mg, 46% yield) as a white solid. LCMS (5-95AB, 1.5 min): R_t = 0.823 min, m/z = 500.2[M+H] $^+$.

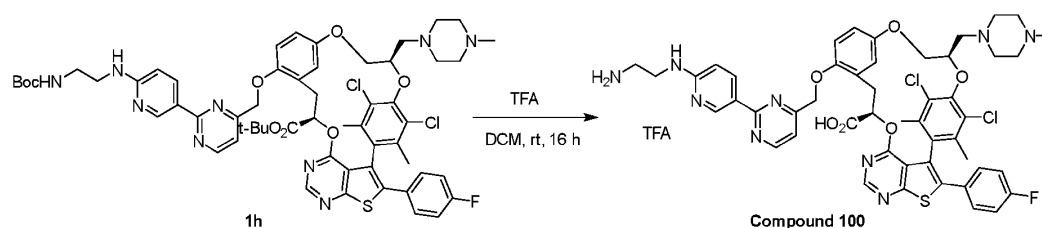
Step 6: Preparation of intermediate 1h



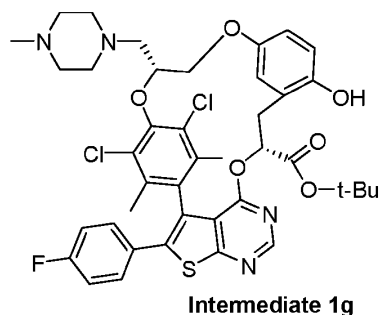
[0246] To a solution of tert-butyl rac-(11R,20R)-23,26-dichloro-3-(4-fluorophenyl)-14-hydroxy-24,25-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.1.13,17.09,28]octacosal(25),2,5(28),6,8,13,15,17(27),22(26),23-

decaene-11-carboxylate (300 mg, 0.37 mmol), [2-[6-[2(tert-butoxycarbonylamino)ethylamino]-3-pyridyl]pyrimidin-4-yl]methyl 4-methylbenzenesulfonate (204 mg, 0.41 mmol) and Cs_2CO_3 (302 mg, 0.93 mmol) in DMF (4 mL) was stirred at 25 °C for 1 hour. The mixture was poured into water (20 mL), extracted with ethyl acetate (20 mL $\times$ 2) and washed with brine (10 mL $\times$ 3), then concentrated and purified by flash chromatography column (0-6% methanol in dichloromethane) to afford intermediate 1h (320 mg, 76% yield) as a white solid. LCMS (5-95 AB, 1.5 min): R_t = 0.930 min, m/z = 1136.5 $[\text{M}+\text{H}]^+$.

Step 7: Preparation of Compound 100



[0247] To a solution of tert-butyl (11R,20R)-14-[[2-[6-[2-(tert-butoxycarbonylamino)ethylamino]-3-pyridyl]pyrimidin-4-yl]methoxy]-23,26-dichloro-3-(4-fluorophenyl)-24,25-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.1.13,17.09,28]octacos-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylate (320 mg, 0.28 mmol) in dichloromethane (2 mL) was added TFA (3 mL), the mixture was stirred at 25 °C for 16 hours. The mixture was concentrated to afford the crude product. It was purified by prep-HPLC (15 – 45% / water (TFA) - ACN) to afford **Compound 100** (320 mg, 94% yield) as a yellow solid. ^1H NMR (400MHz, DMSO- d_6) δ 9.57 (s, 1H), 9.03 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 5.2 Hz, 1H), 8.76 (s, 1H), 8.39 - 8.34 (m, 1H), 7.86 (s, 3H), 7.42 (d, J = 5.2 Hz, 1H), 7.24 - 7.11 (m, 4H), 6.93 - 6.89 (m, 1H), 6.86 - 6.80 (m, 1H), 6.69 (d, J = 8.8 Hz, 1H), 6.28 - 6.23 (m, 1H), 5.80 (d, J = 2.4 Hz, 1H), 5.24 - 5.09 (m, 2H), 4.96 - 4.94 (m, 1H), 4.52 - 4.41 (m, 2H), 3.63 - 3.58 (m, 5H), 3.41 - 3.40 (m, 2H), 3.23 - 3.21 (m, 1H), 3.13 - 2.98 (m, 6H), 2.86 - 2.84 (m, 2H), 2.80 (s, 3H), 1.97 (s, 6H). LCMS (5-95 AB, 1.5 min): R_t = 0.797 min, m/z = 980.4 $[\text{M}+\text{H}]^+$.

Example 2: Synthesis of macrocycle intermediate 1g

[0248] A synthetic scheme for the production of macrocycle intermediate 1g is provided in **FIG. 1**. References to intermediate compounds by number in this example correspond with the numbering in **FIG. 1**.

[0249] *Step 1, Preparation of 5,6-diiodo-3H-thieno[2,3-d]pyrimidin-4-one (intermediate 2):* A mixture of 3H-thieno[2,3-d]pyrimidin-4-one (10 g, 65.7 mmol), periodic acid (12.71 g, 65.7 mmol), Iodine (38.41 g, 151.1 mmol) and sulfuric acid (1.0 mL, 65.7 mmol) in acetic acid (150 mL) and water (35 mL) was stirred at 110 °C for 3 hours under N₂. TLC (50% ethyl acetate in petroleum ether, R_f = 0.4) showed a new pot. The mixture was cooled to room temperature, then MTBE (100 mL) was added and stirred further at 10 °C for 30 minutes. The precipitate was filtered off and washed with a mixture of MTBE and ethanol (100 mL, 2:1), then washed with MTBE (50 mL x 3) and dried under vacuum to afford 5,6-diiodo-3H-thieno[2,3-d]pyrimidin-4-one (18.92 g, 71% yield) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.67 (br, 1H), 8.15 (s, 1H).

[0250] *Step 2, Preparation of 4-chloro-5,6-diiodo-thieno[2,3-d]pyrimidine (intermediate 3):* To a mixture of 5,6-diiodo-3H-thieno[2,3-d]pyrimidin-4-one (10.00 g, 24.8 mmol) in phosphorus oxychloride (50 mL) was added N,N-Dimethylaniline (1.2 mL, 9.3 mmol), the mixture was stirred at 105 °C for 2 h. The resulting suspension was cooled to room temperature and 100 mL of hexane was added and it was stirred for 20 minutes. The precipitate was filtered off, washed with hexane (50 mL x 3) and water (50 mL x 3), dried under vacuum to afford 4-chloro-5,6-diiodo-thieno[2,3-d]pyrimidine (9.23 g, 88% yield) as a grey solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.88 (s, 1H).

[0251] *Step 3, Preparation of 4-chloro-5-iodo-thieno[2,3-d]pyrimidine (intermediate 4):* To a solution of 4-chloro-5,6-diiodo-thieno[2,3-d]pyrimidine (8.80 g, 20.7 mmol) in Tetrahydrofuran (100 mL) was added Tert-Butylmagnesiumchloride (20.70 mL, 20.7 mmol) over 20 minutes at -20 °C, the mixture was stirred at 0 °C for 2 h. The reaction was quenched with water (7 mL) and concentrated under reduced pressure. The crude product was sonicated in a mixture of acetonitrile and water (100 mL, 3/1) and the solid was collected by filtration to afford 4-chloro-5-iodo-thieno[2,3-d]pyrimidine (5.19 g, 84%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.96 (s, 1H), 8.46 (s, 1H).

[0252] *Step 4, Preparation of 4-chloro-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (intermediate 5):* A mixture of 4-chloro-5-iodo-thieno[2,3-d]pyrimidine (5.00 g, 16.9 mmol), (2,6-dimethyl-4-methoxyphenyl)boronic acid (6.07 g, 33.7 mmol), [2-(2-aminophenyl)phenyl]-chloro-palladium;bis(1-adamantyl)-butyl-phosphane (2.39 g, 3.37 mmol), cesium carbonate (10.99 g, 33.7 mmol) in toluene (30 mL) and water (6 mL) was stirred at 100 °C for 16 hours under N₂. The mixture was diluted with ethyl acetate (160 mL), washed with brine (50 mL x 2) and concentrated to give crude product which was purified by column chromatography (solvent gradient: 0-4% ethyl acetate in petroleum ether) to afford 4-chloro-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (1.47 g, 29% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 8.89 (s, 1H), 7.35 (s, 1H), 6.70 (s, 2H), 3.86 (s, 3H), 1.99 (s, 6H).

[0253] *Step 5, Preparation of 4-chloro-6-iodo-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (intermediate 6):* To a solution of 4-chloro-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (3.60 g, 11.8 mmol) in Tetrahydrofuran (40 mL) was added (diisopropylamino)lithium (6.50 mL, 13.0 mmol) dropwise at -78 °C under argon atmosphere, the mixture was stirred for 15 minutes. Iodine (5.99 g, 23.6 mmol) in Tetrahydrofuran (20 mL) was added dropwise, then the cooling bath was removed and the mixture was stirred at 20 °C for 3 h. The TLC (10% ethyl acetate in petroleum ether, R_f=0.3) indicated the reaction was completed. The reaction was quenched by aqueous NH₄Cl (10 mL) and extracted with ethyl acetate (30 mL x 3), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give the crude product which was purified by column chromatography (solvent gradient: 0-10% ethyl acetate in petroleum ether) to afford 4-chloro-6-iodo-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (2.90 g, 52% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 8.82 (s, 1H), 6.72 (s, 2H), 3.87 (s, 3H), 1.93 (s, 6H).

[0254] *Step 6, Preparation of 4-chloro-3-(3,5-dichloro-4-methoxy-2,6-dimethylphenyl)-2-iodothieno[3,2-c]pyridine (intermediate 7):* To a solution of 4-chloro-6-iodo-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (2.95 g, 6.8 mmol) in Acetonitrile (50 mL) was added NCS (3.66 g, 27.4 mmol) at 26 °C. The reaction mixture was stirred at 90 °C for 3 hours. TLC (10 % ethyl acetate in petroleum ether, R_f = 0.4) showed a new spot. The reaction was quenched with aqueous Na_2SO_3 (30 mL) and extracted with ethyl acetate (50 mL x 3). The organic layer was washed with brine (50 mL) and concentrated to residue which was purified by column chromatography (solvent gradient: 0 - 5% ethyl acetate in petroleum ether) to afford the 4-chloro-3-(3,5-dichloro-4-methoxy-2,6-dimethylphenyl)-2-iodothieno[3,2-c]pyridine (2.1 g, 59% yield) as a yellow solid.

[0255] *Step 7, Preparation of 2,6-dichloro-4-(4-chloro-6-iodo-thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethyl-phenol (intermediate 8):* A mixture of 4-chloro-5-(3,5-dichloro-4-methoxy-2,6-dimethyl-phenyl)-6-iodo-thieno[2,3-d]pyrimidine (4.20 g, 8.4 mmol) and aluminum chloride (3.36 g, 25.2 mmol) in 1,2-Dichloroethane (30 mL) was stirred at 70 °C for 12 hours under N_2 . TLC (10% ethyl acetate in petroleum ether, R_f = 0.3) showed the reaction was completed. The reaction was quenched with saturated NaHCO_3 (3 mL) and the mixture was stirred for 2 minutes. Then Saturated NH_4Cl (15 mL) was added. It was extracted with ethyl acetate (50 mL), washed with brine (50 mL) and concentrated to give crude product which was purified by column chromatography (solvent gradient: 0-10% ethyl acetate in petroleum ether) to afford 2,6-dichloro-4-(4-chloro-6-iodo-thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethyl-phenol (2.4 g, 59% yield) as a yellow solid. LCMS (AB_5-95_1.5min): R_T = 1.038 min, M/Z =484.8 $[\text{M}+\text{H}]^+$.

[0256] *Step 8, Preparation of (R)-3-(allyloxy)-2-(2,6-dichloro-4-(4-chloro-6-iodothieno[2,3-d]pyrimidin-5-yl)-3,5-dimethylphenoxy)propyl acetate (intermediate 10):* To a solution of triphenylphosphine (3.50 g, 13.2 mmol), (S)-3-(allyloxy)-2-hydroxypropyl acetate (2.31 g, 13.2 mmol) and 2,6-dichloro-4-(4-chloro-6-iodo-thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethyl-phenol (3.25 g, 6.6 mmol) in toluene (50 mL) was added Di-Tert-Butyl Azodicarboxylate (0.84 mg, 3.6 mmol) slowly under nitrogen atmosphere at 0 °C. The mixture was stirred at 50 °C for 16 h. The TLC (10% ethyl acetate in petroleum ether, R_f =0.2) indicated the reaction was completed. The mixture was diluted with ethyl acetate (150 mL) and washed with brine (30 mL x 2), the organic layer was dried over anhydrous sodium sulfate, filtrated and concentrated under vacuum to give crude product which was purified by column chromatography (solvent gradient: 0-6% ethyl

acetate in petroleum ether) to afford (R)-3-(allyloxy)-2-(2,6-dichloro-4-(4-chloro-6-iodothieno[2,3-d]pyrimidin-5-yl)-3,5-dimethylphenoxy)propyl acetate (4.1 g, 95% yield) as a white solid. LCMS (AB, 5-95, 1.5min): $R_T = 1.172$ min, $m/z = 642.8$ $[M+H]^+$.

[0257] *Step 9, Preparation of (R)-3-(allyloxy)-2-(2,6-dichloro-4-(4-chloro-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethylphenoxy)propyl acetate (intermediate 12):* A mixture of 4-chloro-6-iodo-5-(4-methoxy-2,6-dimethyl-phenyl)thieno[2,3-d]pyrimidine (5 g, 7.8 mmol), 4-fluorophenylboronic acid (2.2 g, 15.6 mmol), tris(dibenzylideneacetone)dipalladium(0) (713 mg, 0.78 mmol), 2-Di-Tert-Butylphosphino-2',4',6'-Triisopropylbiphenyl (331 mg, 0.78 mmol) and K_3PO_4 (3.3 g, 15.6 mmol) in Tetrahydrofuran (40 mL) and water (10 mL) was stirred at 65 °C for 16 hours under N_2 . TLC (10% ethyl acetate in petroleum ether, $R_f = 0.4$) showed the reaction was completed. The mixture was diluted with ethyl acetate (160 mL), washed with brine (50 mL x 2) and concentrated to give crude product which was purified by column chromatography (solvent gradient: 0-8% ethyl acetate in petroleum ether) to afford (R)-3-(allyloxy)-2-(2,6-dichloro-4-(4-chloro-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethylphenoxy)propyl acetate (4.0 g, 84% yield) as a yellow solid. LCMS (AB_5-95_1.5min): $R_T = 1.189$ min, $M/Z = 610.8$ $[M+H]^+$.

[0258] *Step 10, Preparation of (R)-tert-butyl 2-((5-(4-(((R)-1-acetoxy-3-(allyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyl)dimethylsilyl)oxy)phenyl)propanoate (intermediate 14):* To a solution of (R)-tert-butyl 3-(2-(benzyloxy)-5-((tert-butyl)dimethylsilyl)oxy)phenyl-2-hydroxypropanoate (2.92 g, 6.36 mmol) and cesium carbonate (4.15g, 12.7 mmol) in dry tertiary butanol (40 mL) was added (R)-3-(allyloxy)-2-(2,6-dichloro-4-(4-chloro-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-5-yl)-3,5-dimethylphenoxy)propyl acetate (3.88 g, 6.36 mmol) at 25 °C, the mixture was stirred for 12 h at 65 °C. The TLC (10% ethyl acetate in petroleum ether, $R_f = 0.3$) indicated the reaction was completed. The resulting homogeneous mixture was diluted with ethyl acetate (100 mL) and washed with brine (50 mL), then dried over anhydrous Na_2SO_4 , filtrated and concentrated to afford the crude product which was purified by column chromatography (solvent gradient: 0-10% ethyl acetate in petroleum ether) to afford (R)-tert-butyl 2-((5-(4-(((R)-1-acetoxy-3-(allyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-

butyldimethylsilyl)oxy)phenyl)propanoate (2.4 g, 37% yield) as colorless oil. LCMS (R₁₀-80_1.5min): R_T = 1.396 min, M/Z=1031.3 [M+H]⁺.

[0259] *Step 11, Preparation of (R)-tert-butyl 2-((5-(4-(((S)-1-(allyloxy)-3-hydroxypropan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (intermediate 15):* To a solution of (R)-tert-butyl 2-((5-(4-(((R)-1-acetoxy-3-(allyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (2.4 g, 2.32 mmol) in ethanol (20 mL) was added sodium ethanolate (31.6 mg, 0.46 mmol) at 25 °C, the mixture was stirred for 12 h. The TLC (10% ethyl acetate in petroleum ether, R_f=0.2) indicated the reaction was completed. The resulting homogeneous mixture was diluted with ethyl acetate (100 mL) and washed with brine (50 mL), then dried over anhydrous Na₂SO₄, filtrated and concentrated to afford the crude product which was purified by column chromatography (solvent gradient: 0-8% ethyl acetate in petroleum ether) to afford (R)-tert-butyl 2-((5-(4-(((S)-1-(allyloxy)-3-hydroxypropan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (1.9 g, 83% yield) as a white solid. LCMS (10-80, R, 1.5min): R_T = 1.363 min, m/z=989.4 [M+H]⁺.

[0260] *Step 12, Preparation of (R)-tert-butyl 2-((5-(4-(((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (intermediate 16):* To a solution of (R)-tert-butyl 2-((5-(4-(((S)-1-(allyloxy)-3-hydroxypropan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (5.5 g, 5.5 mmol) in dry Dichloromethane (50 mL) was added 1,4-diazabicyclo[2.2.2]octane (DABCO; 3.10 mL, 27.8 mmol) and p-Toluenesulfonylchloride (4.24 g, 22.2 mmol) at 0 °C, the reaction was stirred for 12 h at 25 °C. The TLC (10% ethyl acetate in petroleum ether, R_f=0.3) indicated the reaction was completed. The resulting homogeneous mixture was diluted with ethyl acetate (100 mL) and washed with brine (50 mL), then dried over anhydrous Na₂SO₄, filtrated and concentrated to afford the crude product which was purified by column chromatography (solvent gradient: 0-8% ethyl acetate in petroleum ether) to afford (R)-tert-butyl 2-((5-(4-(((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-

3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (5.5 g, 86% yield) as a white solid. LCMS (85-100, AB, 2min): $R_T = 1.357$ min, $m/z = 1143.2$ $[M+H]^+$.

[0261] *Step 13, Preparation of (R)-tert-butyl 2-((5-(4-((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-hydroxyphenyl)propanoate (intermediate 17):* To a solution of (R)-tert-butyl 2-((5-(4-((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)propanoate (5.5 g, 4.8 mmol) in dry Tetrahydrofuran (50 mL) was slowly added tetra-butylammoniumfluoride (5.30 mL, 5.3 mmol) at 0 °C, the mixture was stirred for 2 h at 25 °C. The TLC (25% ethyl acetate in petroleum ether, $R_f = 0.4$) indicated the reaction was completed. The resulting homogeneous mixture was diluted with ethyl acetate (100 mL) and washed with brine (50 mL), then dried over anhydrous Na_2SO_4 , filtrated and concentrated to afford the crude product which was purified by column chromatography (solvent gradient: 0-15% ethyl acetate in petroleum ether) to afford (R)-tert-butyl 2-((5-(4-((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-hydroxyphenyl)propanoate (4.8 g, 97% yield) as colorless oil.

[0262] *Step 14, Preparation of intermediate 18:* To a solution of (R)-tert-butyl 2-((5-(4-((R)-1-(allyloxy)-3-(tosyloxy)propan-2-yl)oxy)-3,5-dichloro-2,6-dimethylphenyl)-6-(4-fluorophenyl)thieno[2,3-d]pyrimidin-4-yl)oxy)-3-(2-(benzyloxy)-5-hydroxyphenyl)propanoate (4.8 g, 4.7 mmol) in dry N,N-Dimethylformamide (50 mL) was added cesium carbonate (7.59 g, 23.3 mmol) at 25 °C, the mixture was stirred for 2 h. The TLC (10% ethyl acetate in petroleum ether, $R_f = 0.2$) indicated the reaction was completed. The resulting homogeneous mixture was diluted with ethyl acetate (100 mL) and washed with brine (50 mL), then dried over anhydrous Na_2SO_4 , filtrated and concentrated to afford the crude product which was purified by column chromatography (solvent gradient: 0-10% ethyl acetate in petroleum ether) to afford title compound (3.2 g, 80% yield) as a white solid. LCMS (AB, 5-95, 1.5min): $R_T = 1.367$ min, $m/z = 858.6$ $[M+H]^+$.

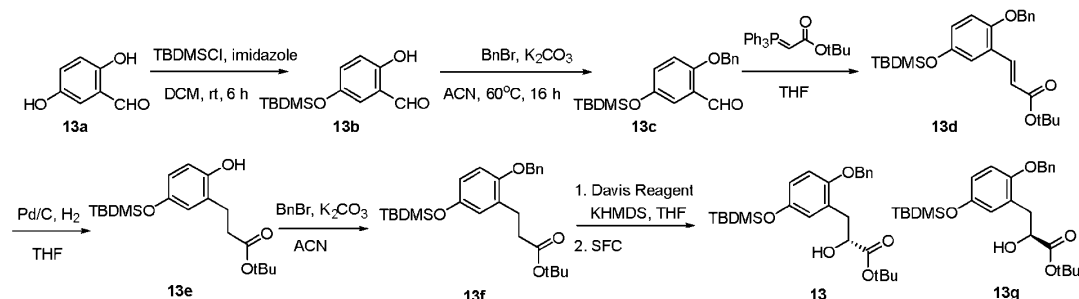
[0263] *Step 15, Preparation of intermediate 19:* To a mixture of intermediate 18 (2.50 g, 2.9 mmol) from step 14 in Dichloromethane (20 mL) and Methyl alcohol (10 mL) was added 1,3-

dimethylbarbituric acid (3.64 g, 23.3 mmol) and $\text{Pd}(\text{Ph}_3\text{P})_4$ (1.35 g, 1.2 mmol) at 25 °C, the reaction mixture was degassed with N_2 for three times and then stirred for 16 h. The mixture was concentrated and purified by column chromatography (solvent gradient: 0-30% ethyl acetate in petroleum ether) to afford intermediate 19 (3.8 g, 96 % yield) as a white solid. LCMS (5-95, AB, 1.5min): $R_T = 1.235$ min, $m/z = 819.1$ $[\text{M}+\text{H}]^+$.

[0264] *Step 16, Preparation of intermediate 20:* To a mixture of intermediate 19 from step 15 (3.80 g, 2.8 mmol) and 1,4-diazabicyclo[2.2.2]octane (DABCO; 2.45mL, 22.3 mmol) in Dichloromethane (30 mL) was added p-toluenesulfonylchloride (2.66 g, 13.9 mmol) at 25 °C, the mixture was stirred for 16 h. The TLC (30% ethyl acetate in petroleum ether, $R_f = 0.5$) showed the desired product. The resulting mixture was concentrated to afford the crude which was purified by column chromatography (solvent gradient: 0-15% ethyl acetate in petroleum ether) to afford intermediate 20 (2.2 g, 81% yield) as a white solid. LCMS (5-95, AB, 1.5min_220&254): $R_T = 1.303$ min, $m/z = 972.8$ $[\text{M}+\text{H}]^+$.

[0265] *Step 17, Preparation of intermediate 22:* To a mixture of intermediate 20 (2.20 g, 2.26 mmol) from step 17 in dry Acetonitrile (30 mL) was added KI (0.75 g, 4.53 mmol), 1-methylpiperazine (100 mL, 905.4 mmol) and cesium carbonate (1.47 g, 4.52 mmol), the mixture was stirred at 60 °C for 16 h. The solvent was concentrated under vacuum. The residue was purified by column chromatography (solvent gradient: 0-10% methanol in dichloromethane) to afford intermediate 22 (2.01 g, 98% yield) as a yellow solid. LCMS (5-95AB, 1.5min_220&254): $R_T = 0.970$ min, $m/z = 899.3$ $[\text{M}+\text{H}]^+$.

[0266] *Step 18, Preparation of Intermediate 1g:* To a mixture of intermediate 22 (2 g, 2.22 mmol) from step 17 in Tetrahydrofuran (30 mL) was added 10% palladium on carbon (2.36 g, 2.22 mmol) at 25 °C, the mixture was stirred for 16 h under H_2 (15 psi). TLC (10% MeOH in DCM, $R_f = 0.4$) showed the desired product. The mixture was filtered and concentrated to afford macrocycle Intermediate 1g (1.50 g, 83% yield) as a white solid. LCMS (5-95AB, 1.5min_220&254): $R_T = 0.962$ min, $m/z = 809.4$ $[\text{M}+\text{H}]^+$.

Example 3: Synthesis of alcohol intermediate 13

[0267] *Step 1, Preparation of intermediate 13b:* To a solution of 2,5-dihydroxybenzaldehyde (20 g, 144.8 mmol) and imidazole (19.72 g, 289.6 mmol) in Dichloromethane (300 mL) was added TBSCl (21.82 g, 144.8 mmol), the mixture was stirred at 25 °C for 16 h. TLC (10% ethyl acetate in petroleum ether, $R_f = 0.3$) showed the starting material was consumed. The mixture was diluted with water (150 mL), washed with brine (200 mL x 3), the organic solution was concentrated to give the crude product which was purified by column chromatography (solvent gradient: 0-10% ethyl acetate in petroleum ether) to afford 5-((tert-butyldimethylsilyl)oxy)-2-hydroxybenzaldehyde (30.5 g, 72% yield) as yellow oil.

[0268] *Step 2, Preparation of intermediate 13c:* To a solution of 5-[(tert-butyldimethyl)silyl]oxy-2-hydroxy-benzaldehyde (22 g, 87.2 mmol) and K_2CO_3 (14.46 g, 104.6 mmol) in Acetonitrile (200 mL) was added benzyl bromide (7.76 mL, 65.2 mmol), the mixture was stirred at 60 °C for 16 h. TLC (5% ethyl acetate in petroleum ether, $R_f = 0.5$) showed the reaction was completed. The mixture was partitioned between ethyl acetate (200 mL) and H_2O (100 mL), the organic solution was concentrated and purified by column chromatography (solvent gradient: 0-3% ethyl acetate in petroleum ether) to afford 2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)benzaldehyde (28.2 g, 94% yield) as yellow oil.

[0269] *Step 3, Preparation of intermediate 13d:* To a solution of 2-benzyloxy-5-[(tert-butyldimethyl)silyl]oxy-benzaldehyde (35.8 g, 104.5 mmol) in Tetrahydrofuran (300 mL) was added (tert-butoxycarbonylmethylene)triphenylphosphorane (35.8 g, 3.21 mmol) at 0 °C, the mixture was stirred at 25 °C for 16 h. TLC (30% ethyl acetate in petroleum ether, $R_f = 0.4$) showed the starting material was consumed and a new spot was found. The mixture was partitioned between ethyl acetate (300 mL) and H_2O (150 mL), the organic solution was concentrated and purified by column chromatography (solvent gradient: 0-2% ethyl acetate in

petroleum ether) to afford (E)-tert-butyl 3-(2-(benzyloxy)-5-((tert-butyltrimethylsilyl)oxy)phenyl)acrylate (35.1 g, 76% yield) as a yellow oil. LCMS (5-95, AB, 1.5min): $R_T = 1.3\text{min}$, $m/z = 881.0$ $[2M+H]^+$.

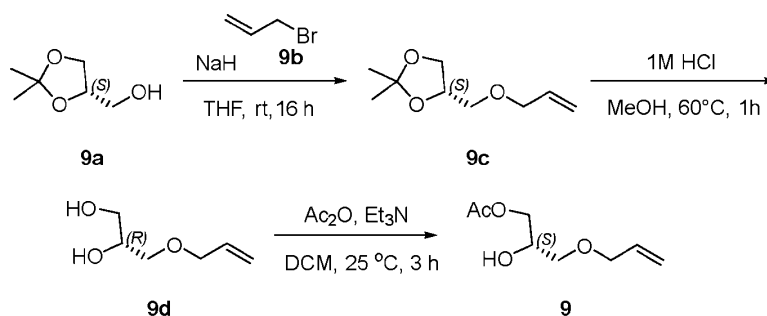
[0270] *Step 4, Preparation of intermediate 13e:* To a solution of (E)-tert-butyl 3-(2-(benzyloxy)-5-((tert-butyltrimethylsilyl)oxy)phenyl)acrylate (35 g, 79.4 mmol) in Tetrahydrofuran (300 mL) was added 10% palladium on carbon (12.68 g), then the mixture was stirred at 25 °C for 16 h under H_2 (30 psi). TLC (20% ethyl acetate in petroleum ether, $R_f = 0.4$) showed the starting material was consumed. The mixture was filtered and concentrated to afford tert-butyl 3-(5-((tert-butyltrimethylsilyl)oxy)-2-hydroxyphenyl)propanoate (23.2 g, 82% yield) as a yellow solid. 1H NMR (400 MHz, $CDCl_3$): δ 6.95 (br s, 1H), 6.59 (d, $J = 8.4$ Hz, 1H), 6.47 - 6.39 (m, 2H), 2.65 - 2.61 (m, 2H), 2.46 - 2.43 (m, 2H), 1.25 (s, 9H), 0.81 (s, 9H), 0.01 (m, 6H).

[0271] *Step 5, Preparation of intermediate 13f:* To a solution of tert-butyl 3-(5-((tert-butyltrimethylsilyl)oxy)-2-hydroxyphenyl)propanoate (23 g, 65.2 mmol) in Acetonitrile (200 mL) was added potassium carbonate (9.02 g, 65.2 mmol) and benzyl bromide (7.76 mL, 65.2 mmol), the mixture was stirred at 80 °C for 16 h. TLC (20% ethyl acetate in petroleum ether, $R_f = 0.8$) showed the reaction was completed. The mixture was partitioned between ethyl acetate (200 mL) and H_2O (100 mL), the organic solution was concentrated and purified by column chromatography (solvent gradient: 0-3% ethyl acetate in petroleum ether) to afford tert-butyl 3-(2-(benzyloxy)-5-((tert-butyltrimethylsilyl)oxy)phenyl)propanoate (15.4 g, 54% yield) as a colorless oil. 1H NMR (400 MHz, $CDCl_3$): δ 7.44 - 7.32 (m, 5H), 6.75 (d, $J = 8.4$ Hz, 1H), 6.69 (d, $J = 2.8$ Hz, 1H), 6.65 - 6.59 (m, 1H), 5.03 (s, 2H), 2.91 (t, $J = 8.0$ Hz, 2H), 2.53 (t, $J = 8.0$ Hz, 2H), 1.43 (s, 9H), 0.98 (s, 9H), 0.17 (s, 6H).

[0272] *Step 6, Preparation of Intermediates 13 and 13g:* To a mixture of tert-butyl 3-(2-(benzyloxy)-5-((tert-butyltrimethylsilyl)oxy)phenyl)propanoate (5.00 g, 11.3 mmol) in THF (50 mL) was added potassium bis(trimethylsilyl)amide (17.20 mL, 17.2 mmol) dropwise at -78 °C under nitrogen atmosphere. The mixture was stirred for 40 min. To the above mixture was added 3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine (4.10 g, 15.3 mmol) in THF (20 mL) dropwise at -78 °C. The mixture was stirred for 3h at -65 °C. Then the reaction was warmed to 25°C. TLC (20% ethyl acetate in petroleum ether, $R_f = 0.4$) showed the starting material was consumed and a new spot was formed. The mixture was partitioned between ethyl acetate (100 mL) and H_2O

(50 mL), the organic solution was concentrated and purified by column chromatography (solvent gradient: 0-20% ethyl acetate in petroleum ether) to afford the racemic mixture (2.10 g, 38.9% yield) as a yellow oil. The racemic mixture was separated by SFC (Daicel Chiralpak AS (250mm*50mm, 10μm); Mobile phase: 20% of ethanol (0.1% ammonia hydroxide) in CO₂) to afford tert-butyl (R)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)-2-hydroxypropanoate (intermediate 13; 1.05 g, peak 2, the desired isomer, R_T=2.769 min). ¹H NMR (400 MHz, CDCl₃): δ 7.46 - 7.32 (m, 5H), 6.79 (d, J = 8.8 Hz, 1H), 6.74 (d, J = 2.8 Hz, 1H), 6.67 (dd, J = 8.8, 4.4 Hz, 1H), 5.04 (s, 2H), 4.40 - 4.37 (m, 1H), 3.15 (dd, J = 14.0, 4.8 Hz, 1H), 2.89 - 2.84 (m, 2H), 1.41 (s, 9H), 0.98 (s, 9H), 0.17 (s, 6H). Also obtained was the stereoisomer tert-butyl(S)-3-(2-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)phenyl)-2-hydroxypropanoate (intermediate 13g; 0.94 g, peak 1, R_T=2.535 min). ¹H NMR (400 MHz, CDCl₃): δ 7.46 - 7.32 (m, 5H), 6.79 (d, J = 8.8 Hz, 1H), 6.74 (d, J = 2.8 Hz, 1H), 6.67 (dd, J = 8.8, 4.4 Hz, 1H), 5.04 (s, 2H), 4.40 - 4.37 (m, 1H), 3.15 (dd, J = 14.0, 4.8 Hz, 1H), 2.89 - 2.84 (m, 2H), 1.41 (s, 9H), 0.98 (s, 9H), 0.17 (s, 6H).

Example 4: Preparation of intermediate 9



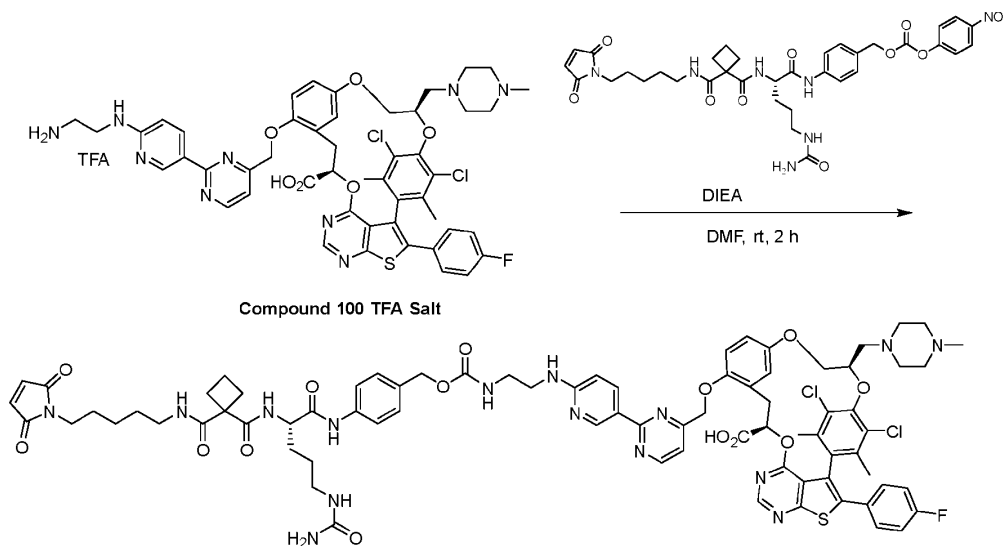
[0273] *Step 1, Preparation of intermediate 9c:* To a mixture of sodium hydride (12.11 g, 302.7 mmol) in tetrahydrofuran (500 mL) was added (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (18.70 mL, 151.3 mmol) at 0 °C for 30 min under nitrogen, the reaction mixture was stirred at 0 °C for 0.5 hour. Then 3-bromoprop-1-ene (19.64 mL, 227 mmol) was added dropwise at 0 °C and stirred at 25 °C for 16 hours. The TLC (10 % ethyl acetate in petroleum ether, R_f=0.5) indicated the reaction was completed. The reaction was quenched with water (500 mL), extracted with ethyl acetate (500 mL) and washed with brine (500 mL). The organic layer was dried over

anhydrous sodium sulfate, filtrated and concentrated under vacuum to afford crude (S)-4-((allyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (26 g) as a yellow oil for next step directly.

[0274] *Step 2, Preparation of intermediate 9d:* To a solution of (S)-4-((allyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (26.00 g, 151 mmol) in methanol (300 mL) was added 1 M hydrogen chloride (24.40 mL, 24.4 mmol), the mixture was stirred at 60 °C for 1 h. TLC (25% ethyl acetate in petroleum ether, $R_f=0.6$) showed the start material was consumed and a new spot was found. The mixture was concentrated on vacuo to afford the crude (R)-3-(allyloxy)propane-1,2-diol (19 g) as a colorless oil for next step directly.

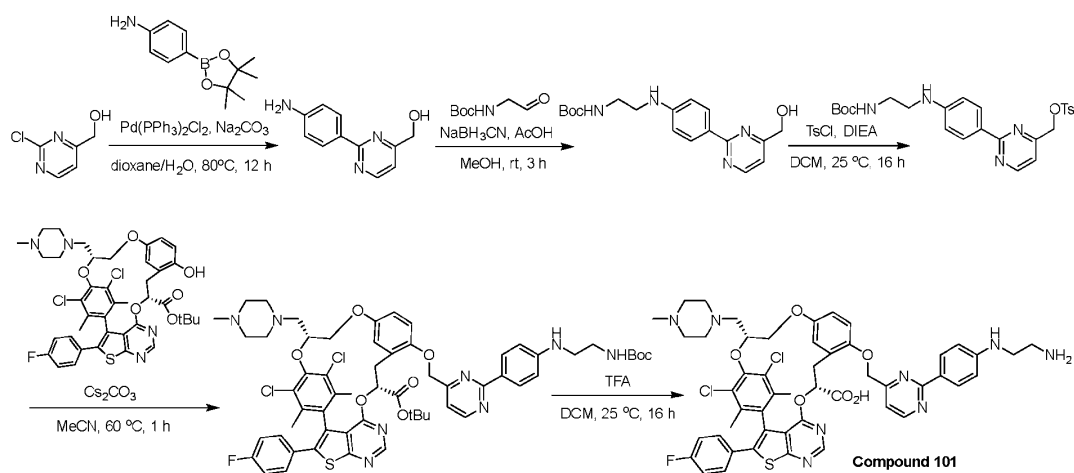
[0275] *Step 3, Preparation of intermediate 9:* To a solution of (R)-3-(allyloxy)propane-1,2-diol (3.00 g, 22.7 mmol) and triethylamine (4.80 mL, 34.0 mmol) in dichloromethane (50 mL) was added acetic anhydride (2.2 mL, 22.7 mmol), the reaction mixture was stirred at 25 °C for 3 h. TLC (50% ethyl acetate in petroleum ether, $R_f=0.5$) showed the start material was consumed and a new spot was found. The mixture was concentrated to residue which was purified by column chromatography (solvent gradient: 0 - 25% ethyl acetate in petroleum ether) to afford (S)-3-(allyloxy)-2-hydroxypropyl acetate (2.05 g, 51% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): δ 5.81 - 5.98 (m, 1 H), 5.16 - 5.35 (m, 2 H), 4.09 - 4.24 (m, 2 H), 4.05 - 4.02 (m, 3 H), 3.40 - 3.60 (m, 2 H), 2.09 (s, 3 H).

Example 5: Preparation of sqCit linker-Compound 100 conjugate



[0276] Compound 100 was used to prepare a linker-drug conjugate, which was further used to prepare ADCs. The linker used for all conjugates was the square-cit (sq-Cit, or sqCit) peptidomimetic linker described in more detail in PCT/US2014/070654. This linker was activated to form the nitrophenyl carbonate, also as described in PCT/US2014/070654. To conjugate this activated linker to Compound 100, to a solution of *rac*-(11R,20R)-14-[[2-[6-(2-aminoethylamino)-3-pyridyl]pyrimidin-4-yl]methoxy]-23,26-dichloro-3-(4-fluorophenyl)-24,25-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacos-1(24),2,5(28),6,8,13,15,17(27),22,25-decaene-11-carboxylic acid TFA salt (Compound 100; 650 mg, 0.54 mmol) and (4-nitrophenyl) [4-[[*rac*-(2S)-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutanecarbonyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl carbonate (593 mg, 0.81 mmol) in DMF (4 mL) was added DIEA (0.28 mL, 1.61 mmol) at 25 °C, then the mixture was stirred for 2 hours. The reaction solution was purified by prep-HPLC (15 – 45% / water (TFA)-ACN) to afford the title compound (676.3 mg, 74% yield) as a white solid. LCMS (5-95 AB, 1.5 min): $R_t = 0.858$ min, $m/z = 1576.54$ $[M+H]^+$.

Example 6: Synthesis of Compound 101



[0277] *Preparation of [2-(4-aminophenyl)pyrimidin-4-yl]methanol*: To a solution of (2-chloropyrimidin-4-yl) methanol (2.0 g, 13.84 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenylamine (3.64 g, 16.6 mmol) and sodium carbonate (4.4 g, 41.51 mmol) in dioxane (80 mL) and water (8 mL) was added 1,1'-bis(diphenylphosphino)ferrocene palladium dichloride

(1.01 g, 1.38 mmol) at 25 °C, the mixture was stirred at 80 °C for 12 h. The reaction solution was concentrated to give the crude product which was purified by flash chromatography on silica gel eluting (50% ethyl acetate in petroleum ether) to afford [2-(4-aminophenyl)pyrimidin-4-yl]methanol (0.75 g, 27% yield) as a white solid. ¹H NMR (400MHz, DMSO-*d*₆) δ = 8.69 (d, *J* = 5.2 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 5.2 Hz, 1H), 6.61 (d, *J* = 8.8 Hz, 2H), 5.63 (s, 2H), 5.57 (t, *J* = 6.0 Hz, 1H), 4.55 (d, *J* = 5.6 Hz, 2H).

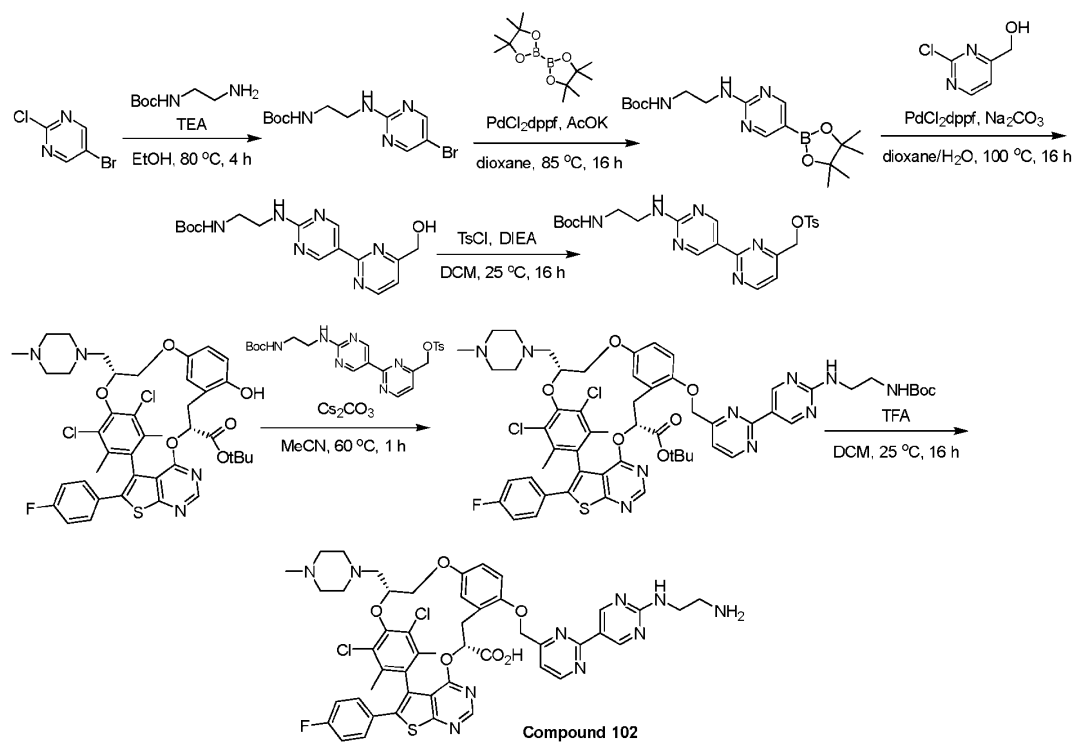
[0278] *Preparation of tert-butyl N-[2-[4-[4-(hydroxymethyl)pyrimidin-2-yl]anilino]ethyl]carbamate:* To a solution of [2-(4-aminophenyl)pyrimidin-4-yl]methanol (300 mg, 1.49 mmol), tert-butyl *N*-(2-oxoethyl)carbamate (0.36 mL, 1.94 mmol) and acetic acid (44.76 mg, 0.75 mmol) in methyl alcohol (12 mL) was added NaBH₃CN (474 mg, 2.24 mmol). The reaction was stirred at 25 °C for 3 h. The reaction solution was quenched with saturated NaHCO₃ solution (10 mL), extracted with dichloromethane (10 mL x 2). The organic layer was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (eluting 0-6% methanol in dichloromethane) to give tert-butyl *N*-[2-[4-[4-(hydroxymethyl)pyrimidin-2-yl]anilino]ethyl]carbamate (180 mg, 35% yield) as a yellow oil. ¹H NMR (400MHz, DMSO-*d*₆) δ = 8.70 (d, *J* = 5.2 Hz, 1H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 5.2 Hz, 1H), 6.91 (s, 1H), 6.64 (d, *J* = 8.8 Hz, 2H), 6.20 (s, 1H), 5.57 (t, *J* = 6.0 Hz, 1H), 4.55 (d, *J* = 6.0 Hz, 2H), 3.23 - 3.10 (m, 4H), 1.39 (s, 9H).

[0279] *Preparation of [2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methyl 4-methylbenzenesulfonate:* To a solution of tert-butyl *N*-[2-[4-[4-(hydroxymethyl)pyrimidin-2-yl]anilino]ethyl]carbamate (130 mg, 0.38 mmol) and *N,N*-Diisopropylethylamine (0.52 mL, 3.01 mmol) in dichloromethane (5 mL) was added TsCl (430 mg, 2.26 mmol) at 25 °C. The mixture was stirred for 16 h. The reaction solution was concentrated to give the crude product which was purified by Pre-TLC (50% ethyl acetate in petroleum ether, R_f=0.5) to afford [2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methyl 4-methylbenzenesulfonate (15 mg, 8% yield) as a white solid. LCMS (5-95AB/1.5min): R_t=1.000 min, m/z=499.2 [M+H]⁺.

[0280] *Preparation of tert-butyl (11R,20R)-14-[[2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methoxy]-2³,2⁶-dichloro-3-(4-*

fluorophenyl)-2⁴,2⁵-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacosa-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylate: Macrocycle intermediate 1g was prepared following the procedure of Example 2. A mixture of intermediate 1g (20 mg, 0.02 mmol), [2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methyl 4-methylbenzenesulfonate (12.93 mg, 0.03 mmol) and Cs₂CO₃ (24 mg, 0.07 mmol) in acetonitrile (2 mL) was stirred at 60 °C for 1 h. The reaction solution was concentrated to give the crude product which was purified by prep-TLC (10% methyl alcohol in dichloromethane) to afford tert-butyl (11R,20R)-14-[[2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methoxy]-2³,2⁶-dichloro-3-(4-fluorophenyl)-2⁴,2⁵-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacosa-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylate (15 mg, 54% yield) as a white solid. LCMS (5-95AB/1.5min): Rt = 1.066 min, m/z = 1135.5 [M+H]⁺.

[0281] *Preparation of Compound 101*: A solution of tert-butyl (11R,20R)-14-[[2-[4-[2-(tert-butoxycarbonylamino)ethylamino]phenyl]pyrimidin-4-yl]methoxy]-2³,2⁶-dichloro-3-(4-fluorophenyl)-2⁴,2⁵-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacosa-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylate (15 mg, 0.01 mmol) in TFA (1 mL) and dichloromethane (1 mL) was stirred at 25 °C for 16 h. The reaction solution was concentrated to give the crude product which was purified by pre-HPLC (acetonitrile 18-48/0.025% TFA in water) to afford (11R,20R)-14-[[2-[4-(2-aminoethylamino)phenyl]pyrimidin-4-yl]methoxy]-2³,2⁶-dichloro-3-(4-fluorophenyl)-2⁴,2⁵-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacosa-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylic acid (10 mg, 69% yield; Compound 101) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.50 - 9.36 (m, 1H), 8.77 (s, 1H), 8.76 (d, *J* = 4.8 Hz, 1H), 8.26 - 8.19 (m, 2H), 7.90 - 7.72 (m, 3H), 7.39 - 7.28 (m, 1H), 7.24 - 7.12 (m, 4H), 6.94 (s, 1H), 6.87 - 6.81 (m, 1H), 6.75 - 6.69 (m, 2H), 6.41 - 6.24 (m, 2H), 5.83 - 5.78 (m, 1H), 5.25 - 5.07 (m, 2H), 4.98 (s, 1H), 4.55 - 4.49 (s, 2H), 3.69 - 3.57 (m, 2H), 3.27 - 3.19 (m, 3H), 3.15 - 2.93 (m, 8H), 2.93 - 2.77 (m, 6H), 2.01 (s, 3H), 1.97 (s, 3H). LCMS (5-95AB/1.5min): Rt = 0.844 min, m/z = 979.4 [M+H]⁺.

Example 7: Synthesis of Compound 102

[0282] *Preparation of tert-butyl (2-((5-bromopyrimidin-2-yl)amino)ethyl)carbamate:* To a solution of 5-bromo-2-chloropyrimidine (1.0 g, 5.17 mmol) in EtOH (20 mL) was added TEA (2 mL, 14.35 mmol) and *tert*-butyl (2-aminoethyl)carbamate (0.98 mL, 6.19 mmol) at 25 °C, the mixture was stirred at 80 °C for 4 h. The reaction was concentrated and purified by column chromatography (silica gel, 100-200 mesh, 0-30% ethyl acetate in petroleum ether) to give *tert*-butyl (2-((5-bromopyrimidin-2-yl)amino)ethyl)carbamate (1.4 g, 85% yield) as a white solid. ¹H NMR (400M Hz, DMSO-*d*₆): δ = 8.35 (s, 2H), 7.39 (t, *J* = 5.2 Hz, 1H), 6.84 (t, *J* = 5.2 Hz, 1H), 3.28 - 3.21 (m, 2H), 3.10 - 3.04 (m, 2H), 1.36 (s, 9H).

[0283] *Preparation of tert-butyl (2-((5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)amino)ethyl)carbamate:* A mixture of *tert*-butyl (2-((5-bromopyrimidin-2-yl)amino)ethyl)carbamate (1.4 g, 4.41 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.7 g, 6.69 mmol), PdCl₂dppf (328 mg, 0.44 mmol) and AcOK (1.3 g, 13.25 mmol) in dioxane (20 mL) was stirred at 85 °C for 16 h. The reaction was concentrated and

purified by column chromatography (silica gel, 100-200 mesh, 10-80% ethyl acetate in petroleum ether) to afford tert-butyl (2-((5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)amino)ethyl)carbamate (1.4 g, 87% yield) as a white solid.

[0284] *Preparation of tert-butyl (2-((4-(hydroxymethyl)-[2,5'-bipyrimidin]-2'-yl)amino)ethyl)carbamate:* A mixture of tert-butyl (2-((5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)amino)ethyl)carbamate (0.3 g, 0.82 mmol), (2-chloropyrimidin-4-yl)methanol (100 mg, 0.69 mmol), PdCl₂dppf (52 mg, 0.07 mmol) and Na₂CO₃ (220 mg, 2.08 mmol) in dioxane (5 mL) and water (0.5 mL) was stirred at 100 °C for 16 h. The reaction solution was concentrated and purified by flash chromatography column (0-2% MeOH in DCM) to afford tert-butyl (2-((4-(hydroxymethyl)-[2,5'-bipyrimidin]-2'-yl)amino)ethyl)carbamate (0.2 g, 84% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 9.28 (s, 2H), 8.68 (d, *J* = 5.2 Hz, 1H), 7.14 (d, *J* = 5.2 Hz, 1H), 5.94 (s, 1H), 5.11 - 4.95 (m, 1H), 4.79 (s, 2H), 3.65 (q, *J* = 6.0 Hz, 2H), 3.47 - 3.36 (m, 2H), 1.45 (s, 9H).

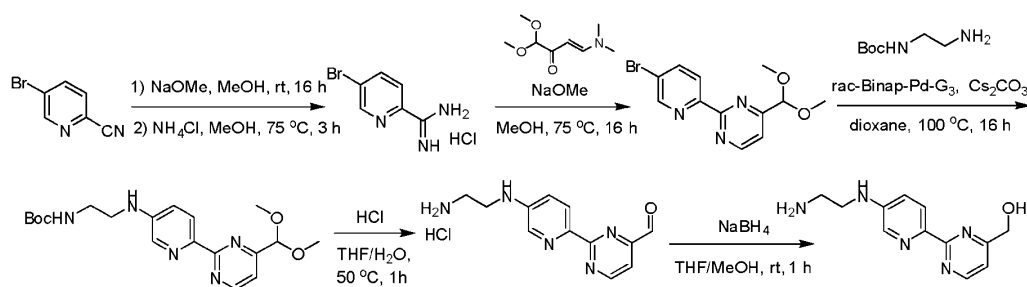
[0285] *Preparation of (2'-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-[2,5'-bipyrimidin]-4-yl)methyl 4-methylbenzenesulfonate:* To a solution of tert-butyl (2-((4-(hydroxymethyl)-[2,5'-bipyrimidin]-2'-yl)amino)ethyl)carbamate (200 mg, 0.58 mmol) and DIEA (0.4 mL, 2.31 mmol) in DCM (5 mL) was added TsCl (220 mg, 1.15 mmol), the mixture was stirred at 25 °C for 16 h. The mixture was concentrated and purified by flash chromatography column (0-2% MeOH in DCM) to afford (2'-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-[2,5'-bipyrimidin]-4-yl)methyl 4-methylbenzenesulfonate (0.2 g, 69% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ = 9.21 (s, 2H), 8.77 - 8.72 (m, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.29 - 7.27 (m, 1H), 5.93 (s, 1H), 5.12 (s, 2H), 4.96 (s, 1H), 3.67 - 3.63 (m, 2H), 3.43 - 3.39 (m, 2H), 2.46 (s, 3H), 1.45 (s, 9H). LCMS (5-95AB/1.5min): Rt = 0.879 min, m/z = 501.1 [M+H]⁺.

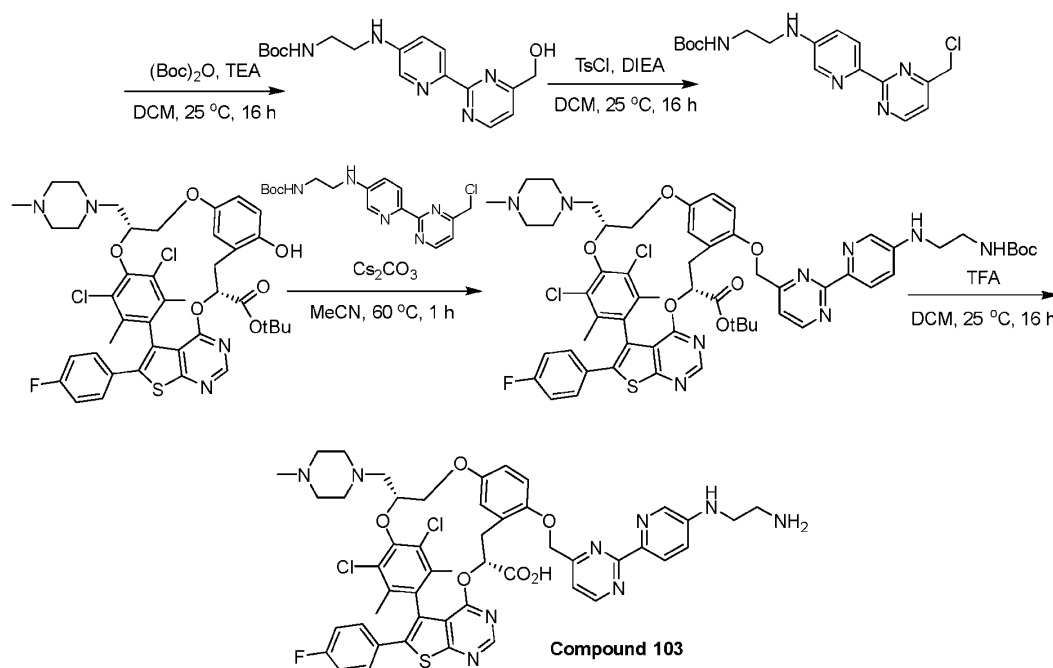
[0286] *Preparation of tert-butyl (4R,9R)-6'-((2'-((2-((tert-butoxycarbonyl)amino)ethyl)amino)-[2,5'-bipyrimidin]-4-yl)methoxy)-1³,1⁵-dichloro-2⁶-(4-fluorophenyl)-1²,1⁶-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidin-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylate:* Macrocycle intermediate 1g was prepared following the procedure of Example 2. A mixture of intermediate 1g (30 mg, 0.03 mmol), [2-[2-[2-(tert-butoxycarbonylamino)ethylamino]pyrimidin-5-yl]pyrimidin-4-yl)methyl 4-methylbenzenesulfonate (20.4 mg, 0.045 mmol) and Cs₂CO₃ (30 mg, 0.09 mmol) in MeCN (1

mL) was stirred at 60 °C for 1 h. The mixture was concentrated and purified by prep TLC (10% MeOH in DCM) to give tert-butyl (4*R*,9*R*)-66-((2'-((2-(tert-butoxycarbonyl)amino)ethyl)amino)-[2,5'-bipyrimidin]-4-yl)methoxy)-13,15-dichloro-26-(4-fluorophenyl)-12,16-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidina-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylate (40 mg, 94% yield) as a white solid. LCMS (5-95AB/1.5min): *R*_t=1.066 min, *m/z*=1136.6 [M+H]⁺.

[0287] Preparation of Compound 102: To a solution of tert-butyl (11*R*,20*R*)-14-[[2-[2-[2-(tert-butoxycarbonylamino)ethylamino]pyrimidin-5-yl]pyrimidin-4-yl]methoxy]-23,26-dichloro-3-(4-fluorophenyl)-24,25-dimethyl-20-[(4-methylpiperazin-1-yl)methyl]-10,18,21-trioxa-4-thia-6,8-diazapentacyclo[20.2.2.12,5.113,17.09,28]octacos-1(25),2,5(28),6,8,13,15,17(27),22(26),23-decaene-11-carboxylate (20 mg, 0.02 mmol) in DCM (1 mL) was added TFA (0.3 mL), the mixture was stirred at 25 °C for 16 h. The mixture was concentrated and purified by pre-HPLC (acetonitrile 18-48/0.025% TFA in water) to give (4*R*,9*R*)-6⁶-((2'-((2-aminoethyl)amino)-[2,5'-bipyrimidin]-4-yl)methoxy)-1³,1⁵-dichloro-2⁶-(4-fluorophenyl)-1²,1⁶-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidina-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylic acid (12 mg, 70% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.20 (s, 2H), 8.84 (d, *J* = 5.2 Hz, 1H), 8.77 (s, 1H), 7.92 - 7.78 (m, 4H), 7.52 - 7.45 (m, 1H), 7.23 - 7.11 (m, 4H), 6.94 - 6.89 (m, 1H), 6.86 - 6.81 (m, 1H), 6.27 - 6.21 (m, 1H), 5.82 - 5.78 (m, 1H), 5.25 - 5.10 (m, 2H), 5.00 - 4.92 (m, 1H), 4.52 - 4.15 (m, 3H), 3.47 - 3.34 (m, 4H), 3.30 - 3.16 (m, 2H), 3.13 - 2.93 (m, 7H), 2.87 - 2.80 (m, 5H), 1.97 (s, 6H). LCMS (5-95AB/1.5min): *R*_t= 0.797min, *m/z*=981.2 [M+H]⁺.

Example 8: Synthesis of Compound 103





[0288] *Preparation of 5-bromopicolinimidamide hydrochloride salt:* To a solution of 5-bromo-2-pyridinecarbonitrile (1.8 g, 10 mmol) in MeOH (50 mL) was added NaOMe (32.4 mg, 0.6 mmol), the mixture was stirred at 25 °C for 16 h. Then NH_4Cl (630 mg, 11.7 mmol) was added. The reaction solution was stirred at 75 °C for 3 h. The mixture was concentrated and EtOH (30 mL) was added subsequently. The suspension was stirred at 80 °C for 0.5 h, filtered and concentrated to give the residue which was triturated with dichloromethane (20 mL) and filtered to give 5-bromopicolinimidamide hydrochloride salt (2 g, 87% yield) as a white solid.

[0289] *Preparation of 2-(5-bromopyridin-2-yl)-4-(dimethoxymethyl)pyrimidine:* To a solution of 5-bromopyridine-2-carboximidine hydrochloride salt (1 g, 4.23 mmol) in MeOH (30 mL) was added (*E*)-4-(dimethylamino)-1,1-dimethoxybut-3-en-2-one (878 mg, 5.07 mmol) and NaOMe (685 mg, 12.68 mmol), the mixture was stirred at 75 °C for 16 h under N_2 atmosphere. The reaction was concentrated and diluted with ethyl acetate (50 mL). The solution was washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated to give the crude product which was purified by column chromatography (silica gel, 100-200 mesh, 10-60% ethyl acetate in petroleum ether) to give 2-(5-bromopyridin-2-yl)-4-(dimethoxymethyl)pyrimidine (1 g, 76% yield) as a white solid.

[0290] *Preparation of tert-butyl (2-((6-(4-(dimethoxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate:* A mixture of 2-(5-bromopyridin-2-yl)-4-(dimethoxymethyl)pyrimidine (500 mg, 1.61 mmol), tert-butyl (2-aminoethyl)carbamate (0.38 mL, 2.42 mmol), Cs₂CO₃ (1.3 g, 4 mmol) and rac-Binap-Pd-G3 (160 mg, 0.16 mmol) in dioxane (10 mL) was stirred at 100 °C for 16 h under N₂ atmosphere. The reaction solution was concentrated to give the crude product which was purified by column chromatography (silica gel, 100-200 mesh, 0-10% MeOH in DCM) to afford the crude tert-butyl (2-((6-(4-(dimethoxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (300 mg, 60% purity) as yellow solid. LCMS (5-95AB/1.5min): Rt = 0.802 min, m/z=390.1 [M+H]⁺.

[0291] *Preparation of 2-(5-((2-aminoethyl)amino)pyridin-2-yl)pyrimidine-4-carbaldehyde:* A mixture of tert-butyl (2-((6-(4-(dimethoxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (300 mg, 0.77 mmol) and con. HCl (1 mL) in THF (4 mL) and water (3 mL) was stirred at 50 °C for 1 h. The reaction solution was concentrated to give 2-(5-((2-aminoethyl)amino)pyridin-2-yl)pyrimidine-4-carbaldehyde which was used for next step directly.

[0292] *Preparation of [2-[5-(2-aminoethylamino)-2-pyridyl]pyrimidin-4-yl]methanol:* To a solution of 2-[5-(2-aminoethylamino)-2-pyridyl]pyrimidine-4-carbaldehyde (300 mg, 1.23 mmol) in MeOH (3 mL) and THF (5 mL) was added NaBH₄ (100 mg, 2.64 mmol) at 0 °C, the mixture was stirred at 25 °C for 1 h. The reaction solution was quenched with aqueous NH₄Cl (2 mL) and concentrated to give crude [2-[5-(2-aminoethylamino)-2-pyridyl]pyrimidin-4-yl]methanol (300 mg) which was used for next step directly.

[0293] *Preparation of tert-butyl (2-((6-(4-(hydroxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate:* To a solution of [2-[5-(2-aminoethylamino)-2-pyridyl]pyrimidin-4-yl]methanol (300 mg, 1.22 mmol) in DCM (10 mL) was added (Boc)₂O (267 mg, 1.22 mmol) and TEA (0.51 mL, 3.67 mmol), the mixture was stirred at 25 °C for 16 h. The reaction solution was concentrated to give the crude product which was purified by pre-TLC (10% methanol in dichloromethane, R_f=0.5) to afford tert-butyl (2-((6-(4-(hydroxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (70 mg, 17% yield) as a yellow oil. LCMS (5-95AB/1.5min RT = 0.7 min, m/z=346.1 [M+H]⁺.

[0294] *Preparation of tert-butyl (2-((6-(4-(chloromethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate:* To a solution of tert-butyl (2-((6-(4-(hydroxymethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (70 mg, 0.2 mmol) and DIEA (0.14 mL, 0.81 mmol) in DCM (2 mL) was added TsCl (77 mg, 0.41 mmol), the mixture stirred at 25 °C for 16 h. The reaction solution was concentrated on vacuum and purified by flash chromatography column (0-2% MeOH in DCM) to afford tert-butyl (2-((6-(4-(chloromethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (20 mg, 27% yield) as a yellow oil. LCMS (5-95AB/1min): Rt = 0.475 min, m/z = 364.2 [M+H]⁺.

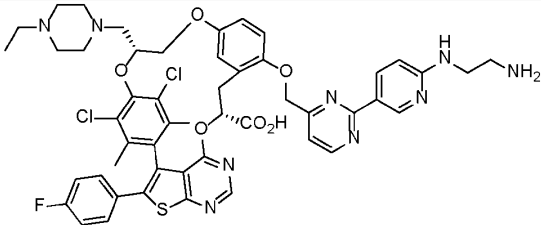
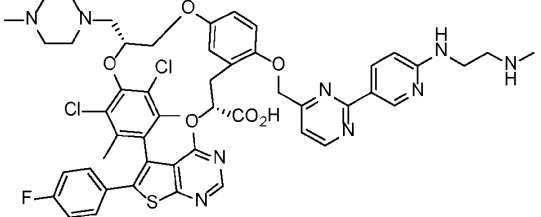
[0295] *Preparation of tert-butyl (4R,9R)-6'-((2-(5-((2-((tert-butoxycarbonyl)amino)ethyl)amino)pyridin-2-yl)pyrimidin-4-yl)methoxy)-1³,1⁵-dichloro-2⁶-(4-fluorophenyl)-1²,1⁶-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidina-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylate:* Macrocycle intermediate 1g was prepared following the procedure of Example 2. A mixture of intermediate 1g (20 mg, 0.02 mmol), tert-butyl (2-((6-(4-(chloromethyl)pyrimidin-2-yl)pyridin-3-yl)amino)ethyl)carbamate (10 mg, 0.025 mmol) and Cs₂CO₃ (20 mg, 0.06 mmol) in MeCN (1 mL) was stirred at 60 °C for 1 h. The mixture was concentrated and purified by prep TLC (10 % MeOH in DCM) to give tert-butyl (4R,9R)-6'-((2-(5-((2-((tert-butoxycarbonyl)amino)ethyl)amino)pyridin-2-yl)pyrimidin-4-yl)methoxy)-1³,1⁵-dichloro-2⁶-(4-fluorophenyl)-1²,1⁶-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidina-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylate (10 mg, 36% yield) as a white solid.

[0296] *Preparation of Compound 103:* A mixture of tert-butyl (4R,9R)-6'-((2-(5-((2-((tert-butoxycarbonyl)amino)ethyl)amino)pyridin-2-yl)pyrimidin-4-yl)methoxy)-1³,1⁵-dichloro-2⁶-(4-fluorophenyl)-1²,1⁶-dimethyl-9-((4-methylpiperazin-1-yl)methyl)-3,7,10-trioxa-2(5,4)-thieno[2,3-d]pyrimidina-1(1,4),6(1,3)-dibenzenacyclodecaphane-4-carboxylate (10 mg, 0.01 mmol) and TFA (0.3 mL) in DCM (1 mL) was stirred at 25 °C for 16 h. The mixture was concentrated and purified by pre-HPLC (acetonitrile 18-48/0.025% TFA in water) to give compound 103 (2.4 mg, 28% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.92 - 8.85 (m, 1H), 8.76 (s, 1H), 8.33 - 8.28 (m, 1H), 8.19 - 8.08 (m, 1H), 7.85 - 7.80 (m, 2H), 7.57 - 7.49 (m, 1H), 7.27 - 7.11 (m, 6H), 6.93 - 6.83 (m, 2H), 6.29 - 6.20 (m, 1H), 5.83 - 5.68 (m, 1H),

5.30 - 5.17 (m, 2H), 5.01 - 4.87 (m, 1H), 4.56 - 4.38 (m, 3H), 3.12 - 2.95 (m, 11H), 2.86 - 2.74 (m, 7H), 2.06 (s, 3H) 1.95 (s, 3H).

Example 9: Preparation of additional Payload Compounds

[0297] Additional payload compounds were prepared generally following the procedures outlined in Examples 1-8. For compound 104, the synthesis of intermediate 1g was generally followed while replacing 1-methylpiperazine with 1-ethylpiperazine. For compounds 105-113, the synthesis of intermediate 1e was generally followed while replacing tert-butyl (2-aminoethyl)carbamate with the alternative Boc-protected amine.

Cmpd #	Structure, ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆)	
104		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ = 9.58 - 9.34 (m, 1H), 9.03 (d, <i>J</i> = 4.0 Hz, 1H), 8.80 (d, <i>J</i> = 4.0 Hz, 1H), 8.76 (s, 1H), 8.34 - 8.36 (m, 1H), 7.91 - 7.82 (m, 3H), 7.44 - 7.39 (m, 2H), 7.22 - 7.13 (m, 4H), 6.92 - 6.90 (m, 1H), 6.84 - 6.82 (m, 1H), 6.67 (d, <i>J</i> = 8.0 Hz, 1H), 6.27 - 6.24 (m, 1H), 5.81 - 5.77 (m, 1H), 5.23 - 5.11 (m, 2H), 4.99 - 4.93 (m, 1H), 4.39 - 4.53 (m, 3H), 3.17 - 3.12 (m, 9H), 3.06 - 2.98 (m, 5H), 2.89 - 2.83 (m, 2H), 1.99 - 1.95 (m, 6H), 1.22 (t, <i>J</i> = 7.2 Hz, 3H).
105		Was not characterized as a free drug, taken into linker conjugation step directly

106		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ = 9.01 (s, 1H), 8.81 (d, <i>J</i> = 5.2 Hz, 1H), 8.77 (s, 1H), 8.43 - 8.35 (m, 1H), 7.80 (br s, 3H), 7.43 (d, <i>J</i> = 4.8 Hz, 1H), 7.26 - 7.07 (m, 4H), 6.94 - 6.87 (m, 1H), 6.87 - 6.81 (m, 1H), 6.77 - 6.71 (m, 1H), 6.29 - 6.24 (m, 1H), 5.80 (d, <i>J</i> = 2.8 Hz, 1H), 5.26 - 5.11 (m, 2H), 5.00 - 4.88 (m, 1H), 4.54 - 4.38 (m, 2H), 3.66 - 3.60 (m, 4H), 3.28 - 3.17 (m, 2H), 3.16 - 3.02 (m, 4H), 3.02 - 2.92 (m, 2H), 2.92 - 2.81 (m, 4H), 2.80 (s, 3H), 2.01 - 1.94 (m, 6H), 1.90 - 1.79 (m, 2H).
107		¹ H NMR (400 MHz, METHANOL- <i>d</i> ₄) δ = 9.126 (s, 1H), 8.76 (d, <i>J</i> = 5.0 Hz, 1H), 8.77 (s, 1H), 8.43 - 8.35 (m, 1H), 7.53 (d, <i>J</i> = 5.2 Hz, 1H), 7.16 - 7.10 (m, 2H), 7.05 - 7.03 (m, 2H), 6.87 - 6.78 (m, 3H), 6.26 - 6.25 (m, 2H), 5.97 - 5.96 (m, 1H), 5.18 - 5.16 (m, 3H), 4.52 - 4.51 (m, 1H), 4.35 - 4.31 (m, 1H), 4.07 - 3.92 (m, 1H), 3.79 - 3.77 (m, 1H), 3.76 - 3.75 (m, 1H), 3.65 - 3.55 (m, 1H), 3.52 - 3.32 (m, 4H), 3.30 - 3.07 (m, 4H), 3.05 - 2.93 (m, 3H), 2.90 (s, 3H), 2.12 (s, 3H), 1.97 (s, 3H).
108		¹ H NMR (400 MHz, DMSO- <i>d</i> ₆): δ 9.02 (d, <i>J</i> = 2.4 Hz, 1H), 8.79 (d, <i>J</i> = 5.2 Hz, 1H), 8.77 (s, 1H), 8.36 - 8.33 (m, 1H), 8.09 - 8.05 (m, 3H), 7.54 - 7.52 (m, 1H), 7.41 (d, <i>J</i> = 5.2 Hz, 1H), 7.20 - 7.13 (m, 4H), 6.92 - 6.89 (m, 1H), 6.84 - 6.82 (m, 1H), 6.70 (d, <i>J</i> = 8.4 Hz, 1H), 6.26 - 6.24 (m, 1H), 5.79 (d, <i>J</i> = 2.8 Hz, 1H), 5.23 - 5.10 (m, 2H), 4.99 - 4.86 (m, 2H), 4.48 - 4.41 (m, 2H), 3.35 - 3.18 (m, 7H), 3.11 - 2.96 (m, 6H), 2.88 - 2.82 (m, 2H), 2.80 (s, 3H), 1.97 (s, 6H). LCMS (5-95/1.5min): R _T =0.789 min, m/z=1012.5 [M+H] ⁺ .

109		LCMS (5-95AB/1.5min): R _T = 0.806 min, m/z=1030.4 [M+H] ⁺
110		¹ H NMR (400 MHz, MeOD): δ 9.09 (d, J = 2.0 Hz, 1H), 8.76 (d, J = 5.2 Hz, 1H), 8.64 (s, 1H), 8.52 - 8.50 (m, 1H), 7.53 (d, J = 5.2 Hz, 1H), 7.14 - 7.11 (m, 2H), 7.05 - 7.00 (m, 2H), 6.86 - 6.80 (m, 1H), 6.78 - 6.76 (m, 2H), 6.26 - 6.23 (m, 1H), 5.97 - 5.96 (m, 1H), 5.18 - 5.16 (m, 3H), 4.91 - 4.84 (m, 3H), 4.69 - 4.68 (m, 1H), 4.66 - 4.58 (m, 2H), 4.30 - 4.25 (m, 1H), 3.74 - 3.73 (m, 2H), 3.64 - 3.62 (m, 1H), 3.52 - 3.48 (m, 2H), 3.47 - 3.40 (m, 3H), 3.31 - 3.30 (m, 3H), 3.28 - 2.93 (m, 3H), 2.92 - 2.91 (m, 4H), 2.18 - 2.13 (m, 5H), 1.96 (s, 3H). LCMS (5-95/1.5min): R _T =0.741 min, m/z=521.1 [M/2+H] ⁺ .
111		¹ H NMR (400 MHz, DMSO-d ₆): δ 13.16 (s, 1H), 9.44 (s, 1H), 9.05 (d, J = 2.0 Hz, 1H), 8.80 (d, J = 5.2 Hz, 1H), 8.76 (s, 1H), 8.37 - 8.33 (m, 1H), 7.44 - 7.39 (m, 2H), 7.23 - 7.13 (m, 4H), 6.91 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 9.0 Hz, 1H), 6.67 (d, J = 8.8 Hz, 1H), 6.38 - 6.09 (m, 2H), 5.80 (d, J = 2.4 Hz, 1H), 5.19 - 5.07 (m, 2H), 4.95 - 4.94 (m, 1H), 4.49 - 4.43 (m, 2H), 3.63 - 3.60 (m, 2H), 3.43 - 3.37 (m, 2H), 3.25 - 3.14 (m, 6H), 3.08 - 2.95 (m, 4H), 2.86 - 2.78 (m, 5H), 2.29 - 2.17 (m, 2H), 1.97 (s, 6H). LCMS (5-95AB/1.5min): R _T = 0.794min, m/z=1058.6 [M+H] ⁺ .

112		^1H NMR (400 MHz, DMSO-d_6): δ 9.05 (d, $J = 2.0$ Hz, 1H), 8.80 (d, $J = 4.8$ Hz, 1H), 8.76 (s, 1H), 8.37 - 8.34 (m, 1H), 7.42 - 7.41 (m, 2H), 7.22 - 7.14 (m, 4H), 6.92 - 6.89 (m, 1H), 6.84 - 6.79 (m, 1H), 6.68 (d, $J = 8.8$ Hz, 1H), 6.26 - 6.23 (m, 1H), 5.79 (d, $J = 2.8$ Hz, 1H), 5.18 - 5.14 (m, 2H), 4.98 - 4.90 (m, 1H), 4.45 - 4.44 (m, 2H), 3.65 - 3.60 (m, 6H), 3.30 - 3.22 (m, 8H), 3.03 - 2.84 (m, 6H), 2.80 (s, 3H), 2.76 - 2.70 (m, 2H), 1.97 (s, 6H). LCMS (5-95/1.5min): $R_T=0.788$ min, $m/z=1076.3$ [M+H]$^{+}$.
113		^1H NMR (400 MHz, DMSO-d_6): δ 9.04 (d, $J = 2.0$ Hz, 1H), 8.82 - 8.79 (m, 2H), 8.76 (s, 1H), 8.37 - 8.34 (m, 1H), 7.47 - 7.39 (m, 2H), 7.24 - 7.13 (m, 4H), 6.92 - 6.90 (m, 1H), 6.85 - 6.84 (m, 1H), 6.67 (d, $J = 9.2$ Hz, 1H), 6.26 - 6.24 (m, 1H), 5.80 - 5.75 (m, 1H), 5.24 - 5.10 (m, 2H), 4.99 - 4.90 (m, 1H), 4.54 - 4.37 (m, 2H), 3.71 - 3.58 (m, 2H), 3.42 - 3.35 (m, 4H), 3.33 (s, 3H), 3.29 - 3.12 (m, 8H), 3.09 - 2.97 (m, 4H), 2.90 - 2.82 (m, 2H), 2.80 (s, 3H), 1.97 (s, 6H). LCMS (5-95AB/1.5min): $R_T=0.815$min, $m/z=1038.5$ [M+H]$^{+}$.

Example 10: General Procedure for linker-drug intermediates

[0298] Additional linker-drug intermediates were prepared using the activated sq-Cit peptidomimetic linker and other payload drugs, generally following the procedure described in Example 5 for Compound 100.

Example 11: General Procedure for Conjugation of linker-drug Intermediates to Antibodies

[0299] The cysteine-engineered antibody (THIOMAB™) selected to be in the ADC in 10 mM succinate, pH 5, 150 mM NaCl, 2 mM EDTA, was pH-adjusted to pH 7.5-8.5 with 1M Tris. 10-18 equivalents of the 10mM linker-drug intermediate with a thiol-reactive maleimide group was dissolved in DMF or DMSO and added to the reduced, reoxidized, and pH-adjusted antibody. The reaction is incubated at room temperature for 37 °C and monitored until completion (1 to about 24 hours), as determined by LC-MS analysis of the reaction mixture. When the reaction was complete, the Ab-Linker-Payload conjugate was purified by one or any combination of several methods, the goal being to remove remaining unreacted linker-drug intermediate and aggregated protein (if present at significant levels). For example, the conjugate may have been diluted with 20 mM histidine-acetate, pH 5.5 until final pH was approximately 5.5 and purified by S cation exchange chromatography using either HiTrap S columns connected to an Akta purification system (GE Healthcare) or S maxi spin columns (Pierce). Alternatively, the conjugate may have been purified by gel filtration chromatography using an S200 column connected to an Akta purification system or Zeba spin columns. Alternatively, dialysis may have been used.

[0300] For the conjugates discussed and characterized in these Examples, the Ab-Linker-Payload conjugates were formulated into 20 mM His/acetate, pH 5, with 240 mM sucrose using either gel filtration or dialysis. The purified conjugate was concentrated by centrifugal ultrafiltration and filtered through a 0.2-µm filter under sterile conditions and frozen for storage.

[0301] The resulting conjugates were characterized to determine protein concentration by BCA assay, aggregation level by analytical SEC, and DAR by LC-MS, as described below.

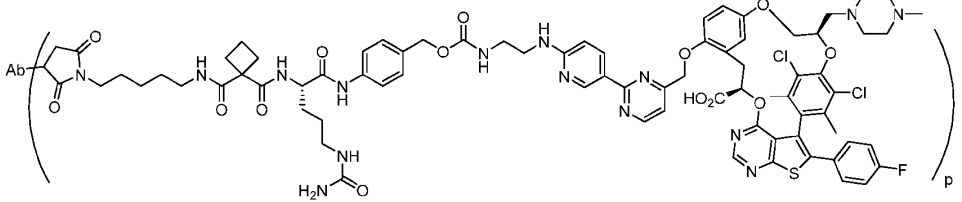
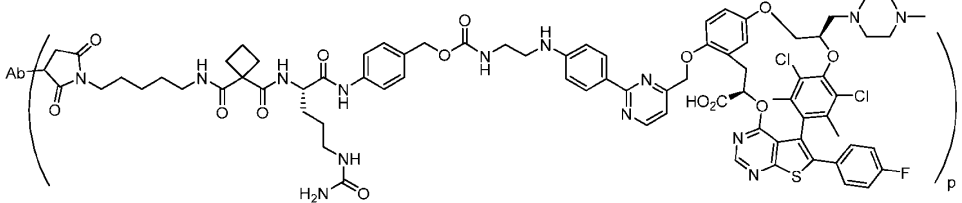
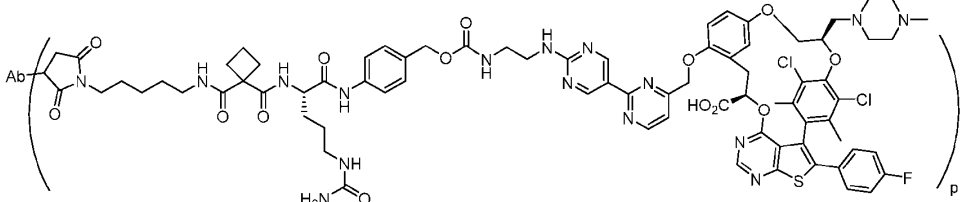
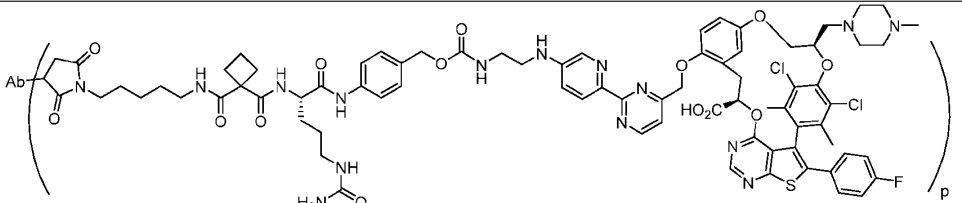
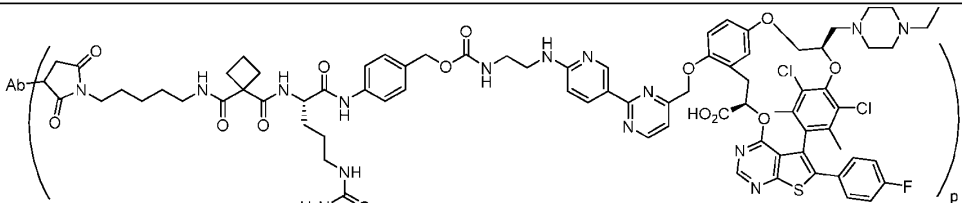
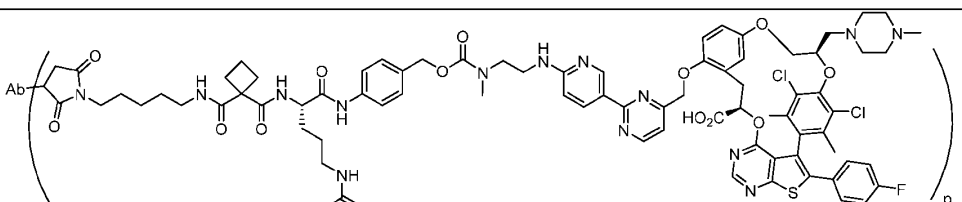
[0302] SEC: Size exclusion chromatography was performed on Ab-Linker-Payload conjugates using a Shodex KW802.5 column in 0.2M potassium phosphate pH 6.2 with 0.25 mM potassium chloride and 15% IPA at a flow rate of 0.65 ml/min. Aggregation state of the conjugate was determined by integration of eluted peak area absorbance at 280 nm.

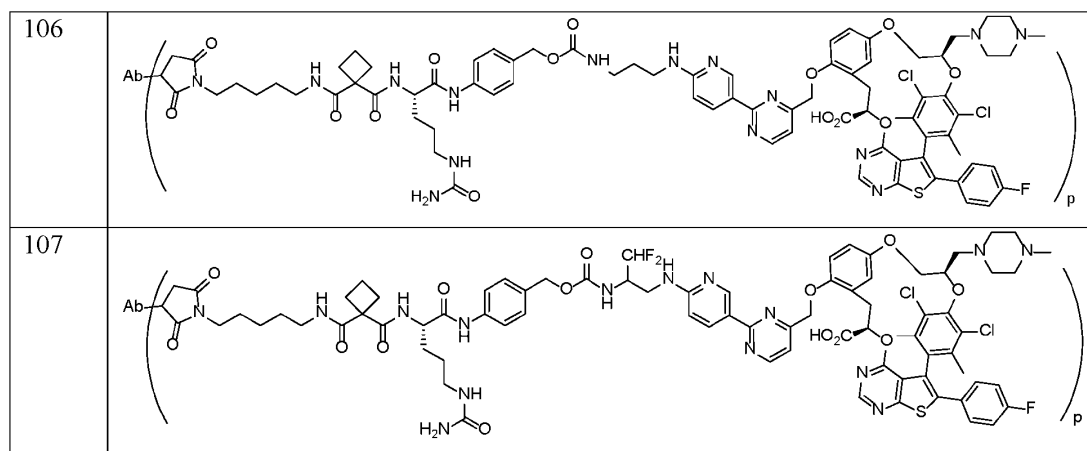
[0303] LC-MS: LC-MS analysis was performed on conjugates using an Agilent TOF 6530 ESI instrument. As an example, the Ab-Linker-Payload conjugates would be treated with 25 mM DTT at 37°C for 15 minutes resulting in reduction between heavy and light antibody chains. The

resulting cleavage fragments were loaded onto a 1000Å (Angstrom), 8 µm (micron) PLRP-S (highly cross-linked polystyrene) column heated to 80 °C and eluted with a gradient of 30% B to 40% B in 10 minutes. Mobile phase A was H₂O with 0.05% TFA and mobile phase B was acetonitrile with 0.05% TFA. The flow rate was 0.5ml/min. Protein elution was monitored by UV absorbance detection at 280nm prior to electrospray ionization and MS analysis. The obtained m/z spectra were deconvoluted using Mass Hunter™ software (Agilent Technologies) to calculate the mass of the antibody fragments. Chromatographic resolution of the unconjugated LC, HC, and conjugated fragments was observed. In some instances, linker drug in-source fragmentation was observed. These fragments were taken into account for final characterization and calculation of the Drug-to-Ab ratio. Ratio was calculated through analysis of ratios of peak intensities for each fragment observed.

[0304] *Specific Example for anti-Her2 7C2 ADC:* The anti-Her2 7C2 antibody was prepared as described in PCT/US1997/018385. The sqCit linker-Compound 100 conjugate was prepared following Example 5 above. To the anti-Her2-7C2 antibody in 10 mM succinate, pH 5, 150 mM NaCl, 2 mM EDTA, was added 4 equivalents of sqCit linker-Compound 100 conjugate dissolved in DMF or DMSO (concentration = 10 mM). This mixture was pH-adjusted to pH 7.5-8.5 with 1M Tris and was incubated at room temperature and monitored until completion as determined by LC-MS. When the conjugation reaction was complete, the resulting Anti Her2-7C2-sqCit-Compound 100 conjugate was purified to remove unreacted linker-drug intermediate and was analyzed by Size Exclusion Chromatography (SEC) to determine the yield, Drug-to-Ab ratio (DAR), and percentage of aggregation. The SEC analysis trace of the Anti Her2-7C2-sqCit-Compound 100 is shown in **FIG. 8A**, and the yield was 64.83%. The LC-MS analysis of the light chain and the heavy chain of the conjugate are shown in **FIG. 8B** and **FIG. 8C**, respectively. The Drug-to-Ab ratio in conjugate was calculated to be 1.9; the aggregation was determined to be 0.46%.

[0305] ADCs of Compound 100, and additional payload Compounds 101-107, were prepared and characterized in Biological Examples provided herein. The general structure of the ADCs is provided below, demonstrating the payload and attachment point of the sq-Cit linker. The particular Ab used and the DAR, if determined, is provided in the specific examples.

Cmpd #	Structure
100	
101	
102	
103	
104	
105	



Biological Example 1: Assessment of comparative compounds on *in vitro* viability of adenocarcinoma and breast cancer cells

[0306] Comparative compounds A, B, and C were assessed for cytotoxicity in SK-BR-3 adenocarcinoma cells, and separately cytotoxicity in CAMA-1 breast cancer cells after 5 days of incubation, as free drugs. **FIG. 2** provides the structures of the assessed compounds.

Comparative compound A was prepared as described in PCT/US2018/000183. Comparative compound B was prepared as described in PCT/EP2014/078947. Comparative compound C was prepared as described in PCT/US2018/000180.

[0307] Human breast cancer cell lines were obtained from the American Type Culture Collection (ATCC). Cells were plated in RPMI supplemented with 10% FBS, 2 mmol/L L-glutamine in black-walled 96-well plates (4000 per well for SK-BR-3 cell line; 8000 per well for CAMA1 cell line) and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO₂. Medium was then removed and replaced by fresh culture medium RPMI containing different concentrations of free drugs or conjugates (dose range = 0.0005 to 10 mM or mg/mL). After 5 days, 100 uL Cell Titer-Glo reagent (Promega Corp.) was added to the wells for 10 min at room temperature and the luminescent signal was measured using an EnVision Multilabel Plate Reader (PerkinElmer). Treatment groups for all cell assays were in quadruplicate. A DMSO control was included in all studies.

[0308] **FIG. 3A** is a graph of the viability vs. compound concentration for SK-BR-3 adenocarcinoma cells. **FIG. 3B** is a graph of the viability vs. compound concentration for CAMA-1 breast cancer cells. A summary of the IC₅₀ for each tested compound in each cell line is provided in Table 1a. Compound A demonstrated the highest cytotoxic potency (as measured by lowest IC₅₀) in both cell lines. The acyclic Compound B was the second most potent molecule in both cell lines. Compound C was the least potent molecule evaluated.

Table 1a. Summary of IC₅₀ in two different cell lines, for comparative acyclic and cyclic MCL1 inhibitors, with IC₅₀ reported in nanomolar units.

	SK-BR-3 IC ₅₀ (nM)	CAMA-1 IC ₅₀ (nM)
Compound A	0.2 (est.)	4.6
Compound B	4.9	11.6
Compound C	16.3	26

[0309] Cyclic MCL1 inhibitors prepared according to the Examples provided herein were evaluated in SK-BR-3 cells, following the same procedure. The IC₅₀ of those free compounds in SK-BR-3 cell is provided in Table 1b. Each tested molecule demonstrated a higher IC₅₀ than the compounds in Table 1a, with the majority of molecules demonstrating drastically worse inhibition. Compound 100 had an IC₅₀ of 0.43 μ M, over 25x less active than Compound C (16.3 nM) and 2000x worse than Compound A (0.2 nM, estimated).

Table 1b. Summary of IC₅₀ in SK-BR-3 cells, for selected cyclic MCL1 inhibitors described herein, with IC₅₀ reported in micromolar units.

Compound #	SK-BR-3 IC ₅₀ (μ M)
100	0.43
101	0.2
104	0.43
106	0.28
107	0.16
108	0.31
109	0.14
110	0.28
111	0.13
112	0.093
113	0.21

Biological Example 2: General Procedure for *in vitro* acute myeloid leukemia (AML) and non-Hodgkin's lymphoma (NHL) cell assays

[0310] The *in vitro* cell assays evaluating the cytotoxic activity of free compounds and compounds as conjugates were performed following the same general procedure.

[0311] All human acute myeloid leukemia and non-Hodgkin's lymphoma cell lines (AML: MV-4-11, EOL-1, Molm-13, Nomo-1, HL-60 and OCL-AML-3; NHL: Su-DHL-5 and Su-DHL-10) were originally obtained from ATCC or DSMZ cell banks. The cells were cultured in PRMI-1640 culture medium supplemented with 10% FBS and 2% glutamine. 2000-4000 cells were seeded in a 96-well plate at 100 μ L/well and incubated overnight at 37°C in a standard tissue incubator. The cells were then exposed to various concentrations of MCL1 inhibitors (free drug) or antibody drug conjugates (ADCs) reagents using 1:3 serial dilutions from stock solutions. After incubation 5 days for free drug and 7 days for antibody drug conjugates (ADCs), the cell titer-Glo reagent (Promega, Madison WI) was added to the wells at 100 μ L/well, and after a 10 min incubation at room temperature, the Luminescent signal was recorded. Data represent the one of three separate experiments (each performed in duplicates).

Biological Example 3: Assessment of comparative compounds on *in vitro* viability of lymphocyte cells

[0312] Comparative compounds A, B, and C were assessed for cytotoxicity of SU-DHL5 and SU-DHL10 cells as free drugs, following the General Procedure for *in vitro* NHL cell assays described above. **FIG. 4A** and **FIG. 4B** is a graph of the viability vs. compound concentration for SU-DHL5 and SU-DHL10, respectively. The IC₅₀ for each small molecule inhibitor in each cell line is summarized in Table 2. In this assessment, Compound A was the most cytotoxic, Compound C the next most cytotoxic, and Compound B least cytotoxic in the SU-DHL5 cell line; and Compound A most cytotoxic, Compound B next most cytotoxic, and Compound C least cytotoxic in the SU-DHL10 cell line.

Table 2. Summary of IC₅₀ in two different cell lines, for comparative acyclic and cyclic MCL1 inhibitors.

	SU-DHL5 IC ₅₀ (nM)	SU-DHL10 IC ₅₀ (nM)
Compound A	0.27	0.07
Compound B	1.57	0.29
Compound C	0.8	0.45

[0313] The results of Biological Examples 1 and 3 demonstrate that across four cell lines, in two types of cancer models, Compound A was the most cytotoxic molecule.

Biological Example 4: Assessment of comparative compounds and Compound 100 on *in vitro* viability of acute myeloid leukemia (AML) cell lines

[0314] The cytotoxicity of Compound 100, Compound A, and Compound B were assessed in the AML cell lines MOLM-16, HL-60, OCI-AML3, and SU-DHL-10 as free drugs, following the General Procedure for *in vitro* AML cell assays described above.

[0315] FIGS. 5A-5C are graphs of cell viability vs. compound concentration for MOLM-16, HL-60, and OCI-AML3, respectively. As a free drug, Compound 100 was significantly less potent as a cytotoxic agent than either Compound A or Compound B across all four cell lines – over 90x less potent in MOLM-16; over 250x less potent in HL-60; over 30x less potent in OCI-AML3; and over 70x less potent in SU-DHL-10.

Table 3. Summary of IC₅₀ in four different AML cell lines, for comparative compounds A and B, and Compound 100

	MOLM-16 IC ₅₀ (uM)	HL-60 IC ₅₀ (uM)	OCI-AML3 IC ₅₀ (uM)	SU-DHL-10 IC ₅₀ (uM)
Compound A	0.014	< 1 nM	0.0015	< 1 nM
Compound B	0.012	< 1 nM	0.012	< 1 nM
Compound 100	1.13	0.29	3.8	0.07

Biological Example 5: Assessment of anti-CD33 ADCs comprising payload Compound A, Compound B, or Compound 100 in AML cell lines

[0316] ADCs using the anti-CD33 antibody 15G15, the sq-Cit linker, and payloads Compound 100, compound A, or compound B were prepared following the procedures set out in Examples 5 and 11. The 15G15 antibody is disclosed in more detail in PCT/US2014/069874.

[0317] The three different ADCs were evaluated for cytotoxicity in *in vitro* AML cell lines EOL-1, MV-4-11, and NOMO-1, following the General Procedure for *in vitro* AML cell assays described above.

[0318] FIGS. 6A-6C are graphs of cell viability vs. compound concentration for EOL-1, MV-4-11, and NOMO-1, respectively. The ADC comprising Compound 100 as a payload had significantly improved cytotoxicity in the AML cell line EOL-1, as shown in FIG. 6A. It

maintained high cytotoxic potency in the cell line MV-4-11, shown in **FIG. 6B**. In cell line NOMO-1, the Compound 100 ADC demonstrated decreased cytotoxic potency, but was still better than the ADCs with either Compound A or Compound B as payloads. As described in earlier examples, the free drug Compound 100 was significantly less potent than comparator compounds. When Compound 100 was used as a payload in an ADC however, the ADC showed comparable or better cytotoxic potency than ADCs using comparator Compounds A or B. This significant gain of cytotoxicity when converting from a free drug to an ADC is surprising and unexpected.

Biological Example 6: Assessment of anti-Her2 7C2-sq-Cit-Payload ADCs using Compound B, Compound C, or Compound 100 as the Payload

[0319] ADCs using the anti-HER2 antibody 7C2, the sq-Cit linker, and payloads Compound B, Compound C, or Compound 100 were prepared according to the procedures set out in Examples 5 and 11. The 7C2 antibody is disclosed in more detail in PCT/US1997/018385. The ADC potencies were evaluated for cytotoxicity *in vitro* in the breast cancer cell line SK-BR-3 following the procedure in Biological Example 1 for SK-BR-3 cell assays described above.

FIG. 7 is a graph of the activity of each ADC. As shown in the figure and in the below table, the ADC using Compound 100 as a payload has significantly better cytotoxic activity compared to ADCs using the same antibody, same linker, but payload Compound C or Compound B. The DAR (drug loading) of the Compound 100 and Compound C ADCs was approximately 2; however the DAR of the Compound B ADC was about three times that, at approximately 6 drug molecules per antibody. Even with this higher drug loading, the ADC comprising Compound B was still significantly less cytotoxic than that comprising Compound 100. This demonstrates the surprisingly improved cytotoxic potency of ADCs comprising Compound 100 as a payload in the breast cancer cell line SK-BR-3. The ADC using compound 100 has better EC₅₀ and maximum cell killing than either of Compound C or B. Without wishing to be bound by theory, this may be a result of improved cell retention of Compound 100 compared to Compounds B and C.

Table 4. Activity of 7C2-sq-Cit-Payload ADCs on SK-BR-3 breast cancer cell line.

	DAR	SK-BR-3 (IC ₅₀ ng/mL)
Compound 100-sqCit-7C2	2	9.5
Compound C-sqCit-7C2	2	37

Compound B-sqCit-7C2	6	35
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Biological Example 7: Assessment of anti-CD33 ADCs comprising various exemplified payload compounds on *in vitro* MV4-11 cell line

[0320] ADCs using the anti-CD33 antibody 15G15, the sq-Cit linker, and payload Compounds 100-107 were prepared following the procedures set out in Examples 5 and 11. The cytotoxicity of these ADCs were assessed using an *in vitro* MV4-11 assay following the procedure described in Biological Example 2. A graph of the results is shown in **FIG. 9**. The ADC comprising Compound 100 as the payload had a DAR of approximately 6; all other ADCs had a DAR of approximately 4.

Biological Example 8: Comparison of anti-CD33 ADCs on tumor burden in *in vivo* disseminated AML model

[0321] ADCs using the anti-CD33 antibody 15G15, the sq-Cit linker, and payload Compounds 100 (ADC-100) or Compound B (ADC-B) were prepared following the procedures set out in Examples 5 and 11, with a DAR of 6. Non-target anti-gD ADCs were also made, for comparison – gD is a viral protein, not expressed on the tumor cells. The cytotoxicity of these targeted ADCs at 30 mg/kg or 10 mg/kg, the non-target ADCs at 30 mg/kg, and vehicle (control) were assessed using an *in vivo* MV4-11 assay in NSG mice.

[0322] Mice were preconditioned with 20 mg/kg busulfan (Busilvex, Pierre Fabre, France) by intraperitoneal injection 24 h before inoculation of leukemic cells. Busulfan was pharmaceutical grade and was diluted in sterile saline. Mice were then inoculated with MV-411 luciferase/GFP+ leukemic cells (2 million) aseptically (cells were suspended in HBSS and injected intravenously into recipient mice in 100 uL). Animals were monitored with Bioluminescence imaging once per week to track tumor progression over time. When tumors were detectable by the imaging, animals were randomized into groups and treatments began (referred to Day 0 of the study). All animals received an intraperitoneal injection of 30 mg/kg anti-ragweed to minimize nonspecific ADC uptake through Fc receptors expressed on the tumor cells. Four hours later, a single intravenous injection of the ADCs or vehicle (20 mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) was administered to mice through the tail vein. At the end of study, whole blood was collected from animals to assess treatment effects.

[0323] A graph of the results is shown in FIG. 10, as % tumor burden evaluated at 9 days post-ADC dose. As shown in FIG. 10, both anti-CD33 ADCs showed anti-tumor activity, with ADC-100 more effective at reducing blood tumor burden. The corresponding non-target conjugates had minimal effect compared to the vehicle control.

Biological Example 9: Assessment of dose-response of Compound 1 ADC on tumor burden in *in vivo* disseminated AML model

[0324] The DAR6 ADC using the anti-CD33 antibody 15G15, the sq-Cit linker, and payload Compound 100 was evaluated in an *in vivo* MV4-11 assay in NSG mice to evaluate dose response. The general procedure of Biological Example 8 above was followed, except at the end of the study, bone marrow from the femur was collected to assess treatment effect. The bone marrow tumor burden (as live tumor cells / 50k) at day 7 is shown in FIG. 11. The average of the data is tabulated in Table 5.

Table 5. Average of live tumor cell count at day 7, as graphed in FIG. 11.

Dose range (mg/kg)	# Live Tumor (Average)	% of vehicle
Vehicles	10,857	100.0%
10 mg/kg	2,215	20.4%
3 mg/kg	5,058	46.6%
1 mg/kg	5,244	48.3%
0.3 mg/kg	6,385	58.8%
0.1 mg/kg	6,624	61.0%

[0325] This data illustrates that the 15G15-Compound 100 ADC demonstrated dose response inhibition in an *in vivo* disseminated AML model. Even at the lowest dose of 0.1 mg/kg, a reduction in tumor cells was observed (relative to vehicle).

Biological Example 10: Assessment of anti-CD33 ADCs on tumor burden in *in vivo* subcutaneous model

[0326] ADCs using the anti-CD33 antibody 15G15, the sq-Cit linker, and payload Compound 100 (ADC-100) or Compound B (ADC-B) were prepared following the procedures set out in Examples 5 and 11, with DAR 6. The cytotoxicity of these ADCs at 30 mg/kg vs. vehicle or unmodified antibody (Ab) were assessed using an *in vivo* subcutaneous MV4-11 assay in SCID

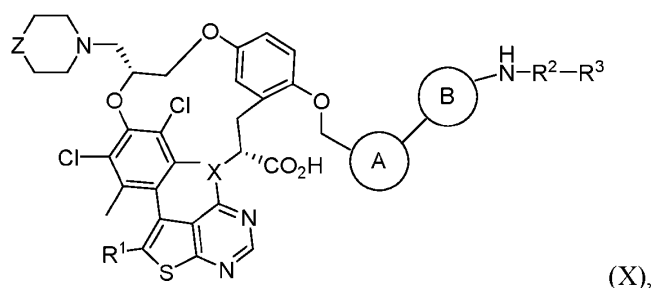
mice. Mice were inoculated with MV4-11 leukemic cells (10 million in 0.1mL HBSS) subcutaneously in the flank area. When tumors reached approximately 200 mm³ size, animals were randomized into groups and treatments began (referred to Day 0 of study). All animals received an intraperitoneal injection of 30 mg/kg anti-ragweed to minimize nonspecific ADC uptake through Fc receptors expressed on the tumor cells. Four hours later, a single intravenous injection of the ADCs or vehicle (20 mM histidine acetate, 240 mM sucrose, 0.02% Tween 20, pH5.5) was administered to mice through the tail vein. Tumors were measured in two dimensions (length and width, each perpendicular to the other) using the caliper (Model 54-10-111; Fred V. Fowler Co.) and tumor volumes were calculated using the following formula: tumor volume (mm³) = (length x width²) x 0.5.

[0327] A graph of the tumor volume over time is shown in **FIG. 12**. As shown in **FIG. 12**, the ADC comprising Compound 100 was less effective at reducing tumor volume in the subcutaneous model compared to the ADC comprising Compound B. Without wishing to be bound by theory, this in combination with the results from Example 8 illustrates the unexpected advantages of Compound 100 as an ADC in disseminated (or liquid) cancer, compared to in solid tumors. Without wishing to be bound by theory, this may be because Compound 100 comprises an overall net charge, which inhibits the ability to passively diffuse across membranes. This may lead to decreased bystander effect (activity in non-targeted neighboring cells) which might lead to diminished activity in a solid tumor with lower tumor penetration or antigen heterogeneity. However, this may also lead to improved safety by not killing neighboring “healthy” cells and also increase activity in liquid tumors by the charged payload being retained in the targeted cancer cell (compared to a free diffusible neutral payload). The overall net charge is, in part, a result of the presence of the amine functional group.

CLAIMS

What is claimed is:

1. A compound of Formula (X):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R¹ is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R² is alkyl, cycloalkyl, heterocycloalkyl, or -R^{2a}(OR^{2a})_p-; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, -OH, -OR^{2b}, and -O(R^{2b}O)_qR^{2c}, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

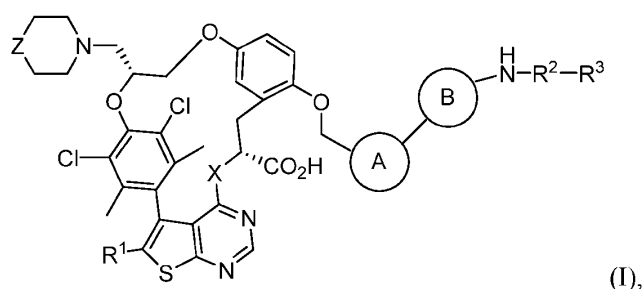
each R^{2a} and each R^{2b} is independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8;

R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-OH$, alkoxy, and haloalkoxy.

2. The compound of claim 1, wherein the compound is of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

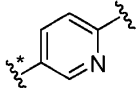
each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo and $-OH$.

3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein Ring A and Ring B are independently phenyl or heteroaryl comprising one or two ring N atoms.

4. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein Ring

A is pyrimidine and Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A.

5. The compound of any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R^1 is phenyl substituted by one fluoro.

6. The compound of any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, wherein R^2 is C_1 - C_6 alkyl, unsubstituted or substituted with one or more substituents independently selected from the group consisting of $-OH$ and halo.

7. The compound of any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, wherein Z is $N-CH_3$ or $N-CH_2CH_3$.

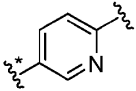
8. The compound of any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein R^3 is $-NH_2$ or $-NHCH_3$.

9. The compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, wherein X is O.

10. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein:

Z is $N-CH_2CH_3$ or $N-CH_3$;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

R¹ is phenyl substituted by one fluoro;

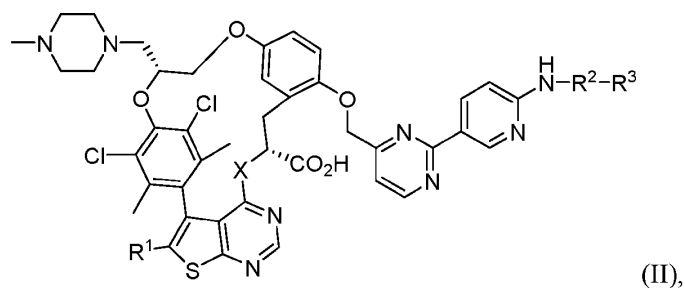
R² is C₁-C₆alkyl unsubstituted or substituted with fluoro;

R³ is -NH₂ or -NH(C₁₋₆alkyl); and

X is O.

11. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein X is O, R¹ is phenyl substituted with one fluoro, R² is alkyl, and R³ is -NH₂.

12. The compound of claim 2, wherein the compound is of Formula (II):

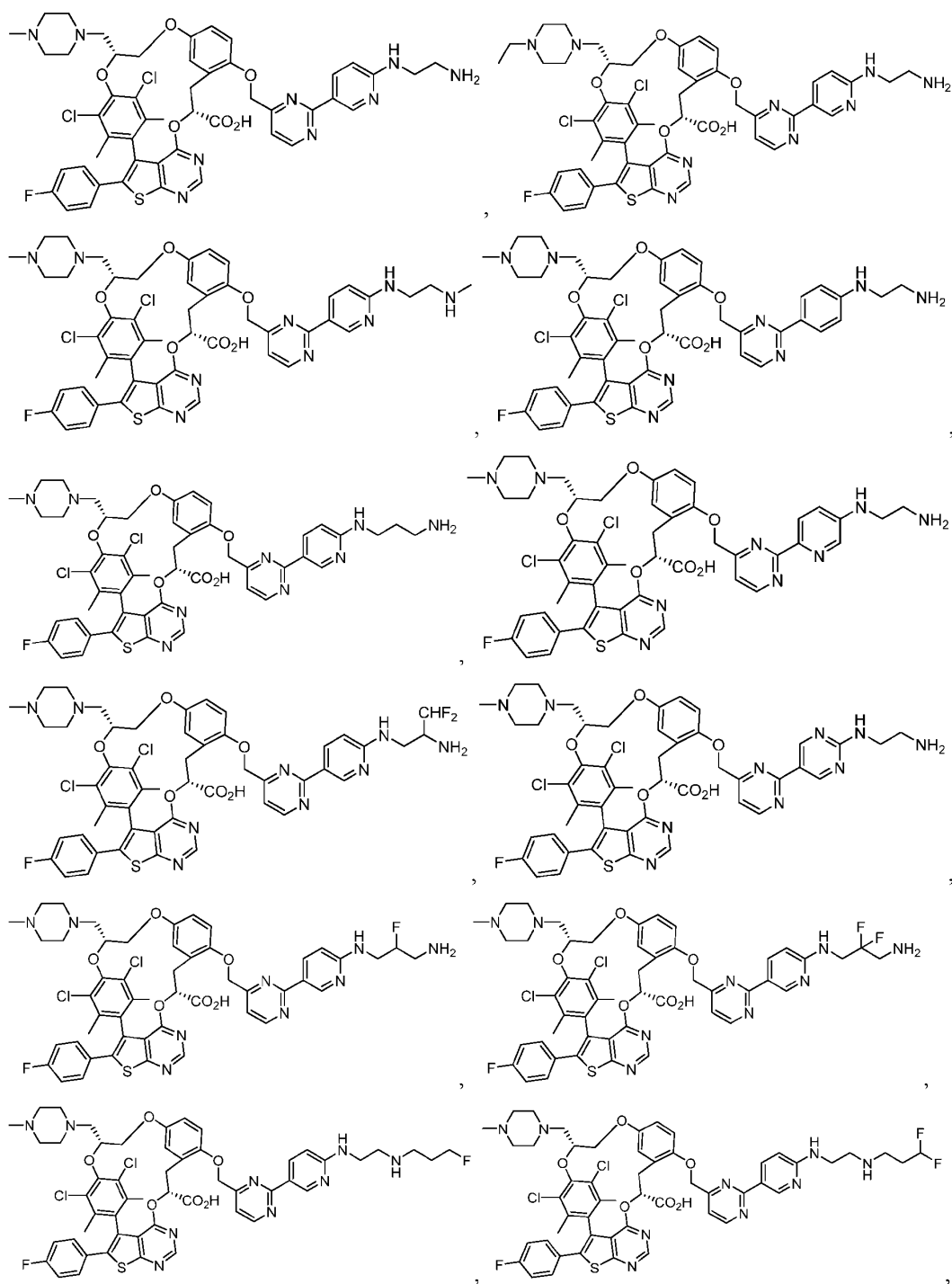


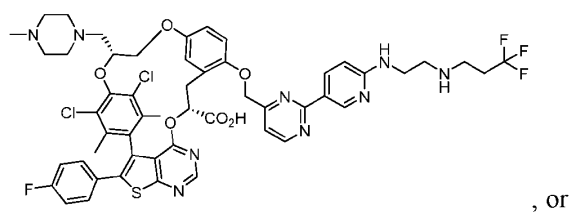
or a pharmaceutically acceptable salt thereof, wherein:

X, R¹, R², and R³ are as defined for Formula (X).

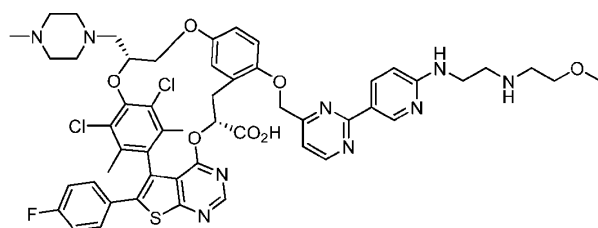
13. The compound of claim 12, or a pharmaceutically acceptable salt thereof, wherein R² is C₁-C₆alkyl unsubstituted or substituted with fluoro; and R³ is -NH₂ or -NH(C₁₋₆alkyl).

14. The compound of claim 1, wherein the compound is:





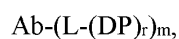
, or



, or is a pharmaceutically acceptable salt

thereof.

15. A conjugate of Formula (A):



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody;

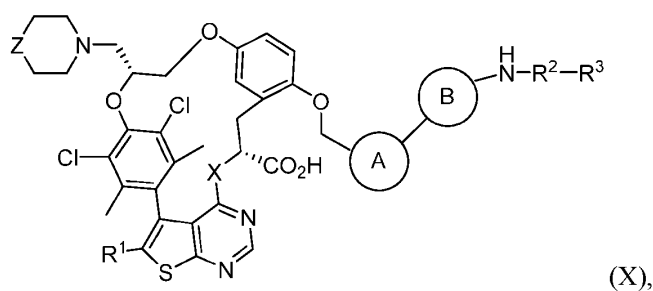
L is a linker;

DP is a drug payload;

r is an integer from 1 to 8; and

m is an integer from 1 to 10;

wherein the drug payload is a compound of Formula (X):



(X),

or a pharmaceutically acceptable salt thereof, wherein:

X is O or NH;

Z is N-C₁₋₆alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

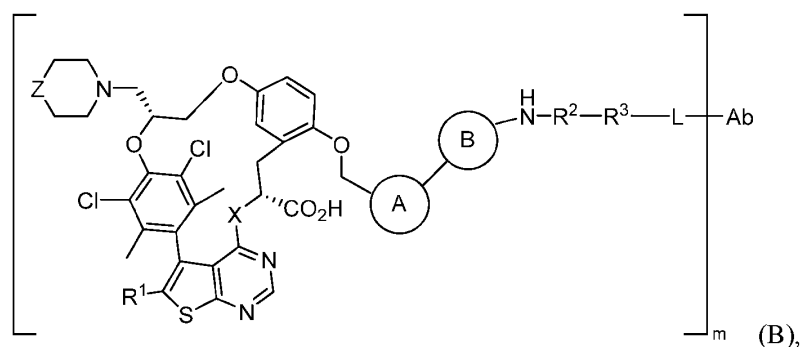
each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl;

p and q are independently integers from 1 to 8; and

R^3 is $-NH_2$, $-NHR^{3a}$, $-N(R^{3a})_2$, or $-N^+(R^{3a})_3$, wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-OH$, alkoxy, and haloalkoxy.

16. The conjugate of claim 15, wherein the conjugate of Formula (A) is a conjugate of Formula (B):



or a pharmaceutically acceptable salt thereof, wherein:

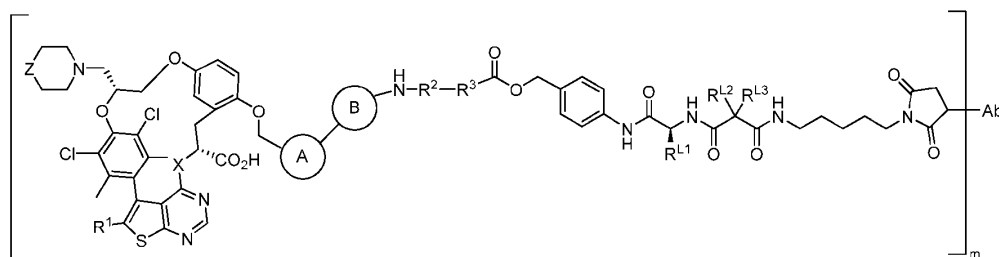
R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$;

m is an integer from 1 to 10; and

X, R¹, R², R^{3a}, Z, Ring A, and Ring B are as defined for Formula (X).

17. The conjugate of claim 15 or 16, or a pharmaceutically acceptable salt thereof, wherein the linker is a peptide linker or a peptidomimetic linker.

18. The conjugate of any one of claims 15 to 17, wherein the conjugate is of Formula (B-1):



R³ is -NH-, -NR^{3a}-, or -N⁺(R^{3a})₂-;

m is an integer from 1 to 10;

X, R¹, R², R^{3a}, Z, Ring A, and Ring B are as defined for Formula (X);

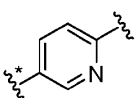
each R^{L1} is independently C₁-C₁₀alkyl, C₁-C₁₀alkenyl, C₁-C₁₀alkylNHC(NH)NH₂ or C₁-C₁₀alkylNHC(O)NH₂; and

R^{L3} and R^{L2} are each independently H, C₁-C₁₀alkyl, C₁-C₁₀alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C₃-C₇cycloalkyl.

19. The conjugate of any one of claims 15 to 18, wherein:

Z is N-CH₂CH₃ or N-CH₃;

Ring A is pyrimidine;

Ring B is phenyl, pyrimidine, or , wherein the * indicates the attachment point to Ring A;

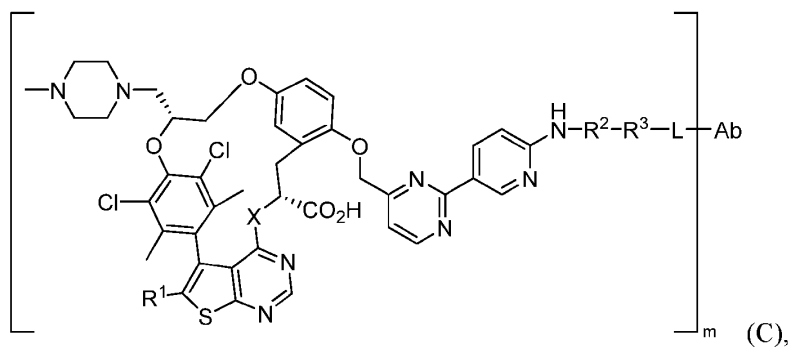
R¹ is phenyl substituted by one fluoro;

R² is C₁-C₆alkyl unsubstituted or substituted with fluoro;

R³ is -NH₂ or -NH(C₁₋₆alkyl); and

X is O.

20. The conjugate of claim 16, wherein the conjugate of Formula (A) is a conjugate of Formula (C):



or a pharmaceutically acceptable salt thereof, wherein:

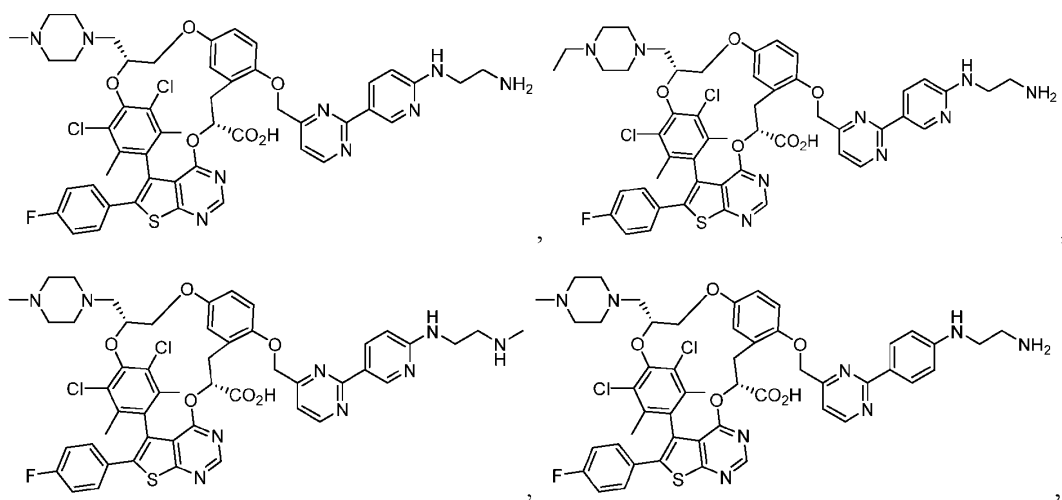
R^3 is $-NH$, $-NR^{3a}$, or $-N^+(R^{3a})_2$;

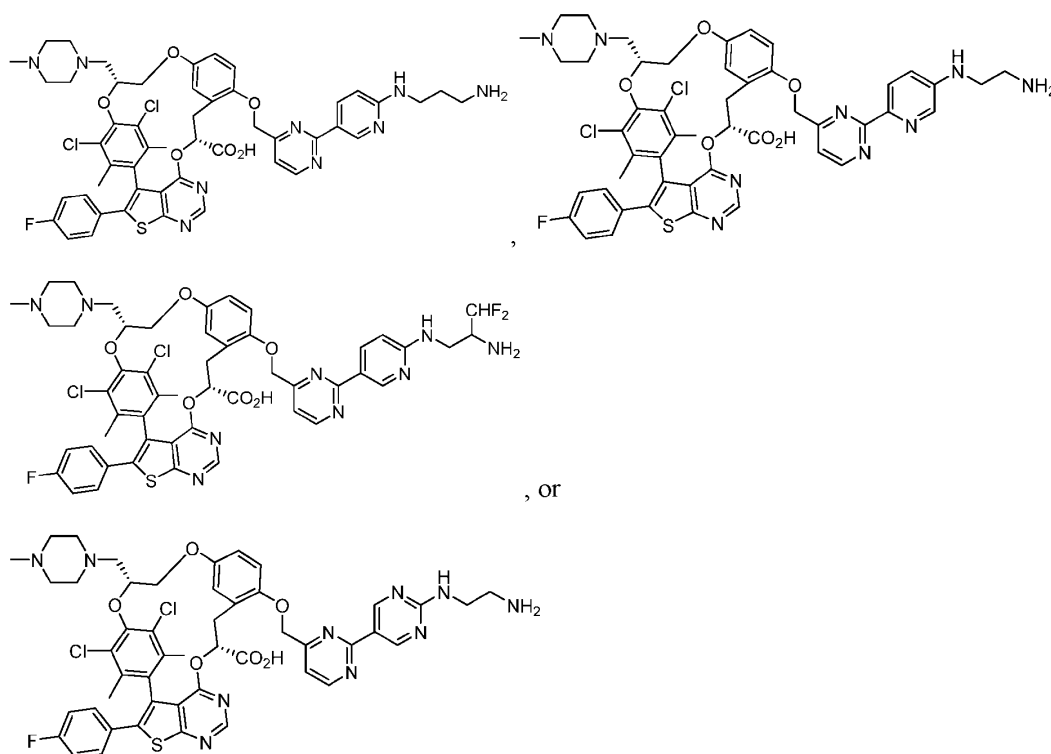
m is an integer from 1 to 10; and

and X , R^1 , R^2 , and R^{3a} are as defined for Formula (X).

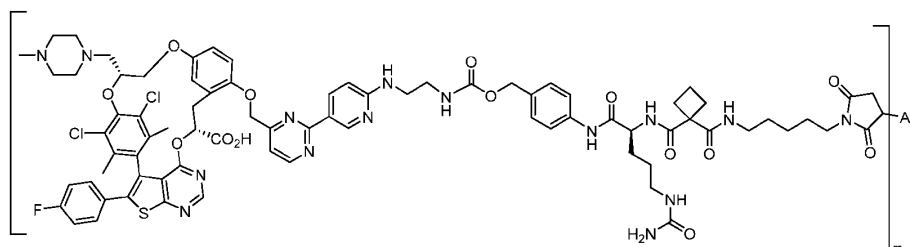
21. The conjugate of claim 20, or a pharmaceutically acceptable salt thereof, wherein R^2 is C_1 - C_6 alkyl unsubstituted or substituted with fluoro; and R^3 is $-NH_2$ or $-NH(C_1$ - C_6 alkyl).

22. The conjugate of any one of claims 15 to 18, or a pharmaceutically acceptable salt thereof, wherein the drug payload is:





23. The conjugate of claim 15, wherein the conjugate is of the structure:

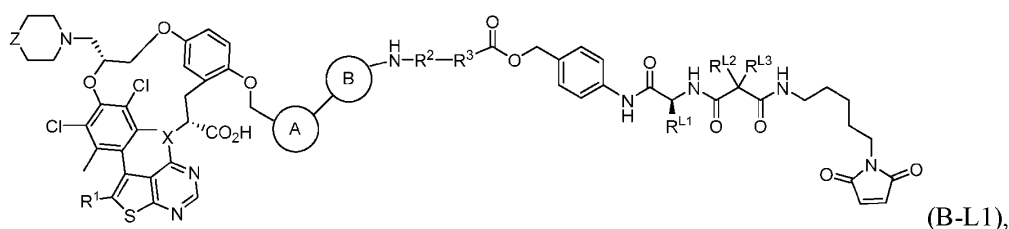


24. The conjugate of any one of claims 15 to 23, wherein the antibody binds to one or more to one or more tumor-associated antigens or cell-surface receptors selected from the group consisting of CLL1, CD71, Trop2, MSLN, NaPi2b, Ly6E, EpCAM, STEAP1, STEAP2, TrpM4, CD21, CD79a, CD72, MUC16, HER2, CD33, CD22, CD79b, LIV1, CD123, CD74, BCMA, and FcRH5.

25. A pharmaceutical composition comprising the conjugate of any one of claims 15 to 24, and a pharmaceutically acceptable excipient.
26. A method of treating a disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a conjugate of any one of claims 15 to 24.
27. The method of claim 26, wherein the disorder is a cancer, tumor, or other malignancy.
28. The method of claim 27, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).
29. A compound for use in a method of treating a disorder in a subject in need thereof, wherein the compound is a conjugate of any one of claims 15 to 24.
30. The compound for use of claim 29, wherein the disorder is a cancer, tumor, or other malignancy.
31. The compound for use of claim 30, wherein the disorder is selected from the group consisting of squamous cell, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial carcinoma, uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma,

head and neck cancer, non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL).

32. A drug payload-linker conjugate, wherein the drug payload-linker conjugate is of Formula (B-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each R^{3a} is independently alkyl, wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-OH$, alkoxy, and haloalkoxy;

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

Z is N - C_{1-6} alkyl or NH;

Ring A is phenyl or 6-membered heteroaryl;

Ring B is phenyl or 6-membered heteroaryl;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are

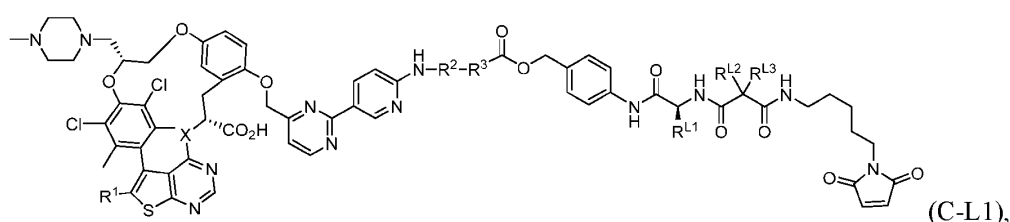
attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or
haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

33. The drug payload-linker conjugate of claim 32, wherein the drug payload-linker intermediate is of Formula (C-L1):



or a pharmaceutically acceptable salt thereof, wherein:

R^3 is $-NH-$, $-NR^{3a}-$, or $-N^+(R^{3a})_2-$; wherein each alkyl is independently unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, $-OH$, alkoxy, and haloalkoxy;

each R^{L1} is independently C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, C_1 - C_{10} alkylNHC(NH)NH₂ or C_1 - C_{10} alkylNHC(O)NH₂;

R^{L3} and R^{L2} are each independently H, C_1 - C_{10} alkyl, C_1 - C_{10} alkenyl, arylalkyl or heteroarylalkyl, or R^{L3} and R^{L2} together may form a C_3 - C_7 cycloalkyl;

X is O or NH;

R^1 is alkyne or phenyl, wherein the phenyl is unsubstituted or substituted with 1 to 3 independently selected halo;

R^2 is alkyl, cycloalkyl, heterocycloalkyl, or $-R^{2a}(OR^{2a})_p-$; wherein the alkyl, cycloalkyl, or heterocycloalkyl is unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, alkyl, $-OH$, $-OR^{2b}$, and $-O(R^{2b}O)_qR^{2c}$, or two substituents together with the atoms to which they are attached form cycloalkyl, halocycloalkyl, heterocycloalkyl, or haloheterocycloalkyl;

each R^{2a} and each R^{2b} are independently alkyl or haloalkyl;

each R^{2c} is independently hydrogen, alkyl, or haloalkyl; and

p and q are independently integers from 1 to 8.

34. The drug payload-linker conjugate of claim 32, wherein the drug payload-linker intermediate is of the structure:

