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(54) Title: AMINOQUINOLINE COMPOUNDS, IMMUNOCONJUGATES, AND USES THEREOF

(57) Abrégé/Abstract:

The invention provides immunoconjugates of Formula (I) comprising an antibody linked by conjugation to one or more aminoquinoline derivatives. The invention also provides aminoquinoline derivative intermediate compositions of Formula (III) comprising a reactive functional group. Such intermediate compositions are suitable substrates for formation of the immunoconjugates through a linker or linking moiety. The invention further provides methods of treating cancer with the immunoconjugates.

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Abstract:

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AMINOQUINOLINE COMPOUNDS, IMMUNOCONJUGATES, AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This non-provisional application claims the benefit of priority to U.S. Provisional Application No. 62/895,379, filed 3 September 2019, incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates generally to an immunoconjugate comprising an antibody conjugated to one or more aminoquinoline molecules.

BACKGROUND OF THE INVENTION

New compositions and methods for the delivery of antibodies and dendritic cell adjuvants are needed in order to reach inaccessible tumors and/or to expand treatment options for cancer patients and other subjects. The invention provides such compositions and methods.

BRIEF DESCRIPTION OF THE INVENTION

The invention is generally directed to immunoconjugates comprising an antibody linked by conjugation to one or more aminoquinoline derivatives. The invention is further directed to aminoquinoline derivative intermediate compositions comprising a reactive functional group. Such intermediate compositions are suitable substrates for formation of immunoconjugates wherein an antibody may be covalently bound to aminoquinoline derivative, through a linker or linking moiety. The invention is further directed to use of such an immunoconjugates in the treatment of an illness, in particular cancer.

An aspect of the invention is an immunoconjugate comprising an antibody covalently attached to a linker which is covalently attached to one or more aminoquinoline moieties.

Another aspect of the invention is an aminoquinoline-linker compound.

Another aspect of the invention is a method for treating cancer comprising administering a therapeutically effective amount of an immunoconjugate comprising an antibody linked by conjugation to one or more aminoquinoline moieties.

Another aspect of the invention is a use of an immunoconjugate comprising an antibody linked by conjugation to one or more aminoquinoline moieties for treating cancer.

Another aspect of the invention is a method of preparing an immunoconjugate by conjugation of one or more aminoquinoline moieties with an antibody.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the invention as defined by the claims.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The invention is in no way limited to the methods and materials described.

DEFINITIONS

The term “immunoconjugate” refers to an antibody construct that is covalently bonded to an adjuvant moiety via a linker. the term “adjuvant” refers to a substance capable of eliciting an immune response in a subject exposed to the adjuvant. The phrase “adjuvant moiety” refers to an adjuvant that is covalently bonded to an antibody construct, e.g., through a linker, as described herein. The adjuvant moiety can elicit the immune response while bonded to the antibody construct or after cleavage (e.g., enzymatic cleavage) from the antibody construct following administration of an immunoconjugate to the subject.

“Adjuvant” refers to a substance capable of eliciting an immune response in a subject exposed to the adjuvant. The phrase “adjuvant moiety” refers to an adjuvant that is covalently bonded to an antibody construct, e.g., through a linker, as described herein. The adjuvant moiety can elicit the immune response while bonded to the antibody construct or after cleavage (e.g., enzymatic cleavage) from the antibody construct following administration of an immunoconjugate to the subject.

The terms “Toll-like receptor” and “TLR” refer to any member of a family of highly-conserved mammalian proteins which recognizes pathogen-associated molecular patterns and acts as key signaling elements in innate immunity. TLR polypeptides share a characteristic structure that includes an extracellular domain that has leucine-rich repeats, a transmembrane domain, and an intracellular domain that is involved in TLR signaling.

The terms “Toll-like receptor 7” and “TLR7” refer to nucleic acids or polypeptides sharing at least about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or more sequence identity to a publicly-available TLR7 sequence, e.g., GenBank accession number AAZ99026 for human TLR7 polypeptide, or GenBank accession number AAK62676 for murine TLR7 polypeptide.

The terms “Toll-like receptor 8” and “TLR8” refer to nucleic acids or polypeptides sharing at least about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or more sequence identity to a publicly-available TLR7 sequence, e.g., GenBank accession number AAZ95441 for human TLR8 polypeptide, or GenBank accession number AAK62677 for murine TLR8 polypeptide.

A “TLR agonist” is a substance that binds, directly or indirectly, to a TLR (e.g., TLR7 and/or TLR8) to induce TLR signaling. Any detectable difference in TLR signaling can indicate that an agonist stimulates or activates a TLR. Signaling differences can be manifested, for example, as changes in the expression of target genes, in the phosphorylation of signal transduction components, in the intracellular localization of downstream elements such as nuclear factor-κB (NF-κB), in the association of certain components (such as IL-1 receptor associated kinase (IRAK)) with other proteins or intracellular structures, or in the biochemical activity of components such as kinases (such as mitogen-activated protein kinase (MAPK)).

“Antibody” refers to a polypeptide comprising an antigen binding region (including the complementarity determining region (CDRs)) from an immunoglobulin gene or fragments thereof. The term “antibody” specifically encompasses monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa) connected by disulfide bonds. Each chain is composed of structural domains, which are referred to as immunoglobulin domains. These domains are classified into different categories by size and function, e.g., variable domains or regions on the light and heavy chains (V_L and V_H, respectively) and constant domains or regions on the light and heavy chains (C_L and C_H, respectively). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids, referred to as the paratope, primarily responsible for antigen recognition, i.e., the antigen binding domain. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. IgG antibodies are large molecules of about 150 kDa composed of four peptide chains. IgG antibodies contain two identical class γ heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure. The two heavy chains are linked to each other and to a light chain each by disulfide bonds. The resulting tetramer has two identical halves, which together form the Y-like shape. Each end of the fork contains an identical antigen binding domain. There are four IgG subclasses (IgG1, IgG2, IgG3, and IgG4) in humans, named in order of their abundance in serum (i.e., IgG1 is the most abundant). Typically, the antigen binding domain of an antibody will be most critical in specificity and affinity of binding to cancer cells.

"Antibody construct" refers to an antibody or a fusion protein comprising (i) an antigen binding domain and (ii) an Fc domain.

"Epitope" means any antigenic determinant or epitopic determinant of an antigen to which an antigen binding domain binds (i.e., at the paratope of the antigen binding domain). Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

The terms "Fc receptor" or "FcR" refer to a receptor that binds to the Fc region of an antibody. There are three main classes of Fc receptors: (1) FcγR which bind to IgG, (2) FcαR which binds to IgA, and (3) FcεR which binds to IgE. The FcγR family includes several members, such as FcγI (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16A), and FcγRIIIB (CD16B). The Fcγ receptors differ in their affinity for IgG and also have different affinities for the IgG subclasses (e.g., IgG1, IgG2, IgG3, and IgG4).

"Biosimilar" refers to an approved antibody construct that has active properties similar to, for example, a PD-L1-targeting antibody construct previously approved such as atezolizumab (TECENTRIQ™, Genentech, Inc.), durvalumab (IMFINZI™, AstraZeneca), and avelumab (BAVENCIO™, EMD Serono, Pfizer); a HER2-targeting antibody construct previously approved such as trastuzumab (HERCEPTIN™, Genentech, Inc.), and pertuzumab (PERJETA™, Genentech, Inc.); or a CEA-targeting antibody such as labetuzumab (CEA-CIDE™, MN-14, hMN14, Immunomedics) CAS Reg. No. 219649-07-7).

"Biobetter" refers to an approved antibody construct that is an improvement of a previously approved antibody construct, such as atezolizumab, durvalumab, avelumab, trastuzumab, pertuzumab, and labetuzumab. The biobetter can have one or more modifications (e.g., an altered glycan profile, or a unique epitope) over the previously approved antibody construct.

"Amino acid" refers to any monomeric unit that can be incorporated into a peptide, polypeptide, or protein. Amino acids include naturally-occurring α-amino acids and their stereoisomers, as well as unnatural (non-naturally occurring) amino acids and their stereoisomers. "Stereoisomers" of a given amino acid refer to isomers having the same molecular formula and intramolecular bonds but different three-dimensional arrangements of bonds and atoms (e.g., an L-amino acid and the corresponding D-amino acid). The amino acids can be glycosylated (e.g., *N*-linked glycans, *O*-linked glycans, phosphoglycans, *C*-linked glycans, or glypication) or deglycosylated. Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

Naturally-occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and *O*-phosphoserine. Naturally-occurring α-amino acids include, without limitation, alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), arginine (Arg), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), and combinations thereof. Stereoisomers of naturally-occurring α-amino acids include, without limitation, D-alanine (D-Ala), D-cysteine (D-Cys), D-aspartic acid (D-Asp), D-glutamic acid (D-Glu), D-phenylalanine (D-Phe), D-histidine (D-His), D-isoleucine (D-Ile), D-arginine (D-Arg), D-lysine (D-Lys), D-leucine (D-Leu), D-methionine (D-Met), D-asparagine (D-Asn), D-proline (D-Pro), D-glutamine (D-Gln), D-serine (D-Ser), D-threonine (D-Thr), D-valine (D-Val), D-tryptophan (D-Trp), D-tyrosine (D-Tyr), and combinations thereof.

Unnatural (non-naturally occurring) amino acids include, without limitation, amino acid analogs, amino acid mimetics, synthetic amino acids, *N*-substituted glycines, and *N*-methyl amino acids in either the L- or D-configuration that function in a manner similar to the naturally-occurring amino acids. For example, “amino acid analogs” can be unnatural amino acids that have the same basic chemical structure as naturally-occurring amino acids (i.e., a carbon that is bonded to a hydrogen, a carboxyl group, an amino group) but have modified side-chain groups or modified peptide backbones, e.g., homoserine, norleucine, methionine sulfoxide, and methionine methyl sulfonium. “Amino acid mimetics” refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally-occurring amino acid.

“Linker” refers to a functional group that covalently bonds two or more moieties in a compound or material. For example, the linking moiety can serve to covalently bond an adjuvant moiety to an antibody construct in an immunoconjugate.

“Linking moiety” refers to a functional group that covalently bonds two or more moieties in a compound or material. For example, the linking moiety can serve to covalently bond an adjuvant moiety to an antibody in an immunoconjugate. Useful bonds for connecting linking moieties to proteins and other materials include, but are not limited to, amides, amines, esters, carbamates, ureas, thioethers, thiocarbamates, thiocarbonates, and thioureas.

"Divalent" refers to a chemical moiety that contains two points of attachment for linking two functional groups; polyvalent linking moieties can have additional points of attachment for linking further functional groups. Divalent radicals may be denoted with the suffix "diyl". For example, divalent linking moieties include divalent polymer moieties such as divalent poly(ethylene glycol), divalent cycloalkyl, divalent heterocycloalkyl, divalent aryl, and divalent heteroaryl group. A "divalent cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group" refers to a cycloalkyl, heterocycloalkyl, aryl, or heteroaryl group having two points of attachment for covalently linking two moieties in a molecule or material. Cycloalkyl, heterocycloalkyl, aryl, or heteroaryl groups can be substituted or unsubstituted. Cycloalkyl, heterocycloalkyl, aryl, or heteroaryl groups can be substituted with one or more groups selected from halo, hydroxy, amino, alkylamino, amido, acyl, nitro, cyano, and alkoxy.

A wavy line (" ∿ ") represents a point of attachment of the specified chemical moiety. If the specified chemical moiety has two wavy lines (" ∿ ") present, it will be understood that the chemical moiety can be used bilaterally, i.e., as read from left to right or from right to left. In some embodiments, a specified moiety having two wavy lines (" ∿ ") present is considered to be used as read from left to right.

"Alkyl" refers to a straight (linear) or branched, saturated, aliphatic radical having the number of carbon atoms indicated. Alkyl can include any number of carbons, for example from one to twelve. Examples of alkyl groups include, but are not limited to, methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$, 1-heptyl, 1-octyl, and the like. Alkyl groups can be substituted or unsubstituted. "Substituted alkyl" groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The term “alkyldiyl” refers to a divalent alkyl radical. Examples of alkyldiyl groups include, but are not limited to, methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), and the like. An alkyldiyl group may also be referred to as an “alkylene” group.

“Alkenyl” refers to a straight (linear) or branched, unsaturated, aliphatic radical having the number of carbon atoms indicated and at least one carbon-carbon double bond, *sp*2. Alkenyl can include from two to about 12 or more carbons atoms. Alkenyl groups are radicals having “cis” and “trans” orientations, or alternatively, “E” and “Z” orientations. Examples include, but are not limited to, ethylenyl or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$). butenyl, pentenyl, and isomers thereof. Alkenyl groups can be substituted or unsubstituted. “Substituted alkenyl” groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The terms “alkenylene” or “alkenyldiyl” refer to a linear or branched-chain divalent hydrocarbon radical. Examples include, but are not limited to, ethylenylene or vinylene ($-CH=CH-$), allyl ($-CH_2CH=CH-$), and the like.

“Alkynyl” refers to a straight (linear) or branched, unsaturated, aliphatic radical having the number of carbon atoms indicated and at least one carbon-carbon triple bond, *sp*. Alkynyl can include from two to about 12 or more carbons atoms. For example, C_2-C_6 alkynyl includes, but is not limited to ethynyl ($-C\equiv CH$), propynyl (propargyl, $-CH_2C\equiv CH$), butynyl, pentynyl, hexynyl, and isomers thereof Alkynyl groups can be substituted or unsubstituted. “Substituted alkynyl” groups can be substituted with one or more groups selected from halo, hydroxy, amino, oxo (=O), alkylamino, amido, acyl, nitro, cyano, and alkoxy.

The term “alkynylene” or “alkynyldiyl” refer to a divalent alkynyl radical.

The terms “carbocycle”, “carbocyclyl”, “carbocyclic ring” and “cycloalkyl” refer to a saturated or partially unsaturated, monocyclic, fused bicyclic, or bridged polycyclic ring assembly containing from 3 to 12 ring atoms, or the number of atoms indicated. Saturated monocyclic carbocyclic rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Saturated bicyclic and polycyclic carbocyclic rings include, for example, norbornane, [2.2.2] bicyclooctane, decahydronaphthalene and adamantane. Carbocyclic groups can also be partially unsaturated, having one or more double or triple bonds in the ring. Representative carbocyclic groups that are partially unsaturated include, but are not limited to, cyclobutene, cyclopentene, cyclohexene, cyclohexadiene (1,3- and 1,4-isomers), cycloheptene, cycloheptadiene, cyclooctene, cyclooctadiene (1,3-, 1,4- and 1,5-isomers), norbornene, and norbornadiene.

The term “cycloalkyldiyl” refers to a divalent cycloalkyl radical.

"Aryl" refers to a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms (C_6–C_{20}) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system.. Aryl groups can be monocyclic, fused to form bicyclic or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. Other aryl groups include benzyl, having a methylene linking group. Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl.

The terms "arylene" or "aryldiyl" mean a divalent aromatic hydrocarbon radical of 6-20 carbon atoms (C_6–C_{20}) derived by the removal of two hydrogen atom from a two carbon atoms of a parent aromatic ring system. Some aryldiyl groups are represented in the exemplary structures as "Ar". Aryldiyl includes bicyclic radicals comprising an aromatic ring fused to a saturated, partially unsaturated ring, or aromatic carbocyclic ring. Typical aryldiyl groups include, but are not limited to, radicals derived from benzene (phenyldiyl), substituted benzenes, naphthalene, anthracene, biphenylene, indenylene, indanylene, 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthyl, and the like. Aryldiyl groups are also referred to as "arylene", and are optionally substituted with one or more substituents described herein.

The terms "heterocycle," "heterocyclyl" and "heterocyclic ring" are used interchangeably herein and refer to a saturated or a partially unsaturated (i.e., having one or more double and/or triple bonds within the ring) carbocyclic radical of 3 to about 20 ring atoms in which at least one ring atom is a heteroatom selected from nitrogen, oxygen, phosphorus and sulfur, the remaining ring atoms being C, where one or more ring atoms is optionally substituted independently with one or more substituents described below. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 4 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 6 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system. Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566. "Heterocyclyl" also includes radicals where heterocycle radicals are fused with a saturated, partially unsaturated ring, or aromatic carbocyclic or heterocyclic ring. Examples of heterocyclic rings include, but are not limited to, morpholin-4-yl, piperidin-1-yl, piperazinyl, piperazin-4-yl-2-one, piperazin-4-yl-3-one, pyrrolidin-1-yl, thiomorpholin-4-yl, S-dioxothiomorpholin-4-yl, azocan-1-yl, azetidin-1-yl, octahydropyrido[1,2-a]pyrazin-2-yl, [1,4]diazepan-1-yl, pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, homopiperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinylimidazolinyl, imidazolidinyl, 3-azabicyco[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, azabicyclo[2.2.2]hexanyl, 3H-indolyl quinolizinyl and N-pyridyl ureas. Spiro heterocyclyl moieties are also included within the scope of this definition. Examples of spiro heterocyclyl moieties include azaspiro[2.5]octanyl and azaspiro[2.4]heptanyl. Examples of a heterocyclic group wherein 2 ring atoms are substituted with oxo (=O) moieties are pyrimidinonyl and 1,1-dioxo-thiomorpholinyl. The heterocycle groups herein are optionally substituted independently with one or more substituents described herein.

The term “heterocyclyldiyl” refers to a divalent, saturated or a partially unsaturated (i.e., having one or more double and/or triple bonds within the ring) carbocyclic radical of 3 to about 20 ring atoms in which at least one ring atom is a heteroatom selected from nitrogen, oxygen, phosphorus and sulfur, the remaining ring atoms being C, where one or more ring atoms is optionally substituted independently with one or more substituents as described.

The term “heteroaryl” refers to a monovalent aromatic radical of 5-, 6-, or 7-membered rings, and includes fused ring systems (at least one of which is aromatic) of 5-20 atoms, containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur. Examples of heteroaryl groups are pyridinyl (including, for example, 2-hydroxypyridinyl), imidazolyl, imidazopyridinyl, pyrimidinyl (including, for example, 4-hydroxypyrimidinyl), pyrazolyl, triazolyl, pyrazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxadiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, tetrahydroisoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolinyl, indazolyl, indolizinyl, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, pteridinyl, purinyl, oxadiazolyl, triazolyl, thiadiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, and furopyridinyl. Heteroaryl groups are optionally substituted independently with one or more substituents described herein.

The term “heteroaryldiyl” refers to a divalent aromatic radical of 5-, 6-, or 7-membered rings, and includes fused ring systems (at least one of which is aromatic) of 5-20 atoms, containing one or more heteroatoms independently selected from nitrogen, oxygen, and sulfur.

The heterocycle or heteroaryl groups may be carbon (carbon-linked), or nitrogen (nitrogen-linked) bonded where such is possible. By way of example and not limitation, carbon bonded heterocycles or heteroaryls are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position

3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline.

By way of example and not limitation, nitrogen bonded heterocycles or heteroaryls are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline.

The terms “halo” and “halogen,” by themselves or as part of another substituent, refer to a fluorine, chlorine, bromine, or iodine atom.

The term “carbonyl,” by itself or as part of another substituent, refers to C(=O) or –C(=O)–, i.e., a carbon atom double-bonded to oxygen and bound to two other groups in the moiety having the carbonyl.

As used herein, the phrase “quaternary ammonium salt” refers to a tertiary amine that has been quaternized with an alkyl substituent (e.g., a C_1-C_4 alkyl such as methyl, ethyl, propyl, or butyl).

The terms “treat,” “treatment,” and “treating” refer to any indicia of success in the treatment or amelioration of an injury, pathology, condition (e.g., cancer), or symptom (e.g., cognitive impairment), including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the symptom, injury, pathology, or condition more tolerable to the patient; reduction in the rate of symptom progression; decreasing the frequency or duration of the symptom or condition; or, in some situations, preventing the onset of the symptom. The treatment or amelioration of symptoms can be based on any objective or subjective parameter, including, for example, the result of a physical examination.

The terms “cancer,” “neoplasm,” and “tumor” are used herein to refer to cells which exhibit autonomous, unregulated growth, such that the cells exhibit an aberrant growth phenotype characterized by a significant loss of control over cell proliferation. Cells of interest for detection, analysis, and/or treatment in the context of the invention include cancer cells (e.g., cancer cells from an individual with cancer), malignant cancer cells, pre-metastatic cancer cells, metastatic cancer cells, and non-metastatic cancer cells. Cancers of virtually every tissue are known. The phrase “cancer burden” refers to the quantum of cancer cells or cancer volume in a subject. Reducing cancer burden accordingly refers to reducing the number of cancer cells or the cancer cell volume in a subject. The term “cancer cell” as used herein refers to any cell that is a cancer cell (e.g., from any of the cancers for which an individual can be treated, e.g., isolated from an individual having cancer) or is derived from a cancer cell, e.g., clone of a cancer cell. For example, a cancer cell can be from an established cancer cell line, can be a primary cell isolated from an individual with cancer, can be a progeny cell from a primary cell isolated from an individual with cancer, and the like. In some embodiments, the term can also refer to a portion of a cancer cell, such as a sub-cellular portion, a cell membrane portion, or a cell lysate of a cancer cell. Many types of cancers are known to those of skill in the art, including solid tumors such as carcinomas, sarcomas, glioblastomas, melanomas, lymphomas, and myelomas, and circulating cancers such as leukemias.

As used herein, the term “cancer” includes any form of cancer, including but not limited to, solid tumor cancers (e.g., skin, lung, prostate, breast, gastric, bladder, colon, ovarian, pancreas, kidney, liver, glioblastoma, medulloblastoma, leiomyosarcoma, head & neck squamous cell carcinomas, melanomas, and neuroendocrine) and liquid cancers (e.g., hematological cancers); carcinomas; soft tissue tumors; sarcomas; teratomas; melanomas; leukemias; lymphomas; and brain cancers, including minimal residual disease, and including both primary and metastatic tumors.

“PD-L1 expression” refers to a cell that has a PD-L1 receptor on the cell’s surface. As used herein “PD-L1 overexpression” refers to a cell that has more PD-L1 receptors as compared to corresponding non-cancer cell.

“HER2” refers to the protein human epidermal growth factor receptor 2.

“HER2 expression” refers to a cell that has a HER2 receptor on the cell’s surface. For example, a cell may have from about 20,000 to about 50,000 HER2 receptors on the cell’s surface. As used herein “HER2 overexpression” refers to a cell that has more than about 50,000 HER2 receptors. For example, a cell 2, 5, 10, 100, 1,000, 10,000, 100,000, or 1,000,000 times the number of HER2 receptors as compared to corresponding non-cancer cell (e.g., about 1 or 2 million HER2 receptors). It is estimated that HER2 is overexpressed in about 25% to about 30% of breast cancers.

The “pathology” of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, neoplasia, premalignancy, malignancy, and invasion of surrounding or distant tissues or organs, such as lymph nodes.

As used herein, the phrases "cancer recurrence" and "tumor recurrence," and grammatical variants thereof, refer to further growth of neoplastic or cancerous cells after diagnosis of cancer. Particularly, recurrence may occur when further cancerous cell growth occurs in the cancerous tissue. "Tumor spread," similarly, occurs when the cells of a tumor disseminate into local or distant tissues and organs, therefore, tumor spread encompasses tumor metastasis. "Tumor invasion" occurs when the tumor growth spread out locally to compromise the function of involved tissues by compression, destruction, or prevention of normal organ function.

As used herein, the term "metastasis" refers to the growth of a cancerous tumor in an organ or body part, which is not directly connected to the organ of the original cancerous tumor. Metastasis will be understood to include micrometastasis, which is the presence of an undetectable amount of cancerous cells in an organ or body part that is not directly connected to the organ of the original cancerous tumor. Metastasis can also be defined as several steps of a process, such as the departure of cancer cells from an original tumor site, and migration and/or invasion of cancer cells to other parts of the body.

The phrases "effective amount" and "therapeutically effective amount" refer to a dose or amount of a substance such as an immunoconjugate that produces therapeutic effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th Edition (McGraw-Hill, 2006); and *Remington: The Science and Practice of Pharmacy*, 22nd Edition, (Pharmaceutical Press, London, 2012)). In the case of cancer, the therapeutically effective amount of the immunoconjugate may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the immunoconjugate may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR)

"Recipient," "individual," "subject," "host," and "patient" are used interchangeably and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired (e.g., humans). "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, camels, etc. In certain embodiments, the mammal is human.

The phrase "synergistic adjuvant" or "synergistic combination" in the context of this invention includes the combination of two immune modulators such as a receptor agonist, cytokine, and adjuvant polypeptide, that in combination elicit a synergistic effect on immunity relative to either administered alone. Particularly, the immunoconjugates disclosed herein comprise synergistic combinations of the claimed adjuvant and antibody construct. These synergistic combinations upon administration elicit a greater effect on immunity, e.g., relative to when the antibody construct or adjuvant is administered in the absence of the other moiety. Further, a decreased amount of the immunoconjugate may be administered (as measured by the total number of antibody constructs or the total number of adjuvants administered as part of the immunoconjugate) compared to when either the antibody construct or adjuvant is administered alone.

As used herein, the term "administering" refers to parenteral, intravenous, intraperitoneal, intramuscular, intratumoral, intralesional, intranasal, or subcutaneous administration, oral administration, administration as a suppository, topical contact, intrathecal administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to the subject.

The terms "about" and "around," as used herein to modify a numerical value, indicate a close range surrounding the numerical value. Thus, if "X" is the value, "about X" or "around X" indicates a value of from 0.9X to 1.1X, e.g., from 0.95X to 1.05X or from 0.99X to 1.01X. A reference to "about X" or "around X" specifically indicates at least the values X, 0.95X, 0.96X, 0.97X, 0.98X, 0.99X, 1.01X, 1.02X, 1.03X, 1.04X, and 1.05X. Accordingly, "about X" and "around X" are intended to teach and provide written description support for a claim limitation of, e.g., "0.98X."

ANTIBODIES

The immunoconjugate of the invention comprises an antibody. Included in the scope of the embodiments of the invention are functional variants of the antibody constructs or antigen binding domain described herein. The term "functional variant" as used herein refers to an antibody construct having an antigen binding domain with substantial or significant sequence identity or similarity to a parent antibody construct or antigen binding domain, which functional variant retains the biological activity of the antibody construct or antigen binding domain of which it is a variant. Functional variants encompass, for example, those variants of the antibody constructs or antigen binding domain described herein (the parent antibody construct or antigen binding domain) that retain the ability to recognize target cells expressing PD-L1, HER2 or CEA to a similar extent, the same extent, or to a higher extent, as the parent antibody construct or antigen binding domain.

In reference to the antibody construct or antigen binding domain, the functional variant can, for instance, be at least about 30%, about 50%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the antibody construct or antigen binding domain.

A functional variant can, for example, comprise the amino acid sequence of the parent antibody construct or antigen binding domain with at least one conservative amino acid substitution. Alternatively, or additionally, the functional variants can comprise the amino acid sequence of the parent antibody construct or antigen binding domain with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent antibody construct or antigen binding domain.

Amino acid substitutions of the inventive antibody constructs or antigen binding domains are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (e.g., Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (e.g., Asn, Gln, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (e.g., Ile, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (e.g., His, Phe, Trp, and Tyr), etc.

The antibody construct or antigen binding domain can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, e.g., other amino acids, do not materially change the biological activity of the antibody construct or antigen binding domain functional variant.

In some embodiments, the antibodies in the immunoconjugates contain a modified Fc region, wherein the modification modulates the binding of the Fc region to one or more Fc receptors.

In some embodiments, the antibodies in the immunoconjugates (e.g., antibodies conjugated to at least two adjuvant moieties) contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region that results in modulated binding (e.g., increased binding or decreased binding) to one or more Fc receptors (e.g., FcγRI (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a), and/or FcγRIIIB (CD16b)) as compared to the native antibody lacking the mutation in the Fc region. In some embodiments, the antibodies in the immunoconjugates contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region that reduce the binding of the Fc region of the antibody to FcγRIIB. In some embodiments, the antibodies in the immunoconjugates contain one or more modifications (e.g., amino acid insertion, deletion, and/or substitution) in the Fc region of the antibody that reduce the binding of the antibody to FcγRIIB while maintaining the same binding or having increased binding to FcγRI (CD64), FcγRIIA (CD32A), and/or FcRγIIIA (CD16a) as compared to the native antibody lacking the mutation in the Fc region. In some embodiments, the antibodies in the immunoconjugates contain one of more modifications in the Fc region that increase the binding of the Fc region of the antibody to FcγRIIB.

In some embodiments, the modulated binding is provided by mutations in the Fc region of the antibody relative to the native Fc region of the antibody. The mutations can be in a CH2 domain, a CH3 domain, or a combination thereof. A "native Fc region" is synonymous with a "wild-type Fc region" and comprises an amino acid sequence that is identical to the amino acid sequence of an Fc region found in nature or identical to the amino acid sequence of the Fc region found in the native antibody (e.g., cetuximab). Native sequence human Fc regions include a native sequence human IgG1 Fc region, native sequence human IgG2 Fc region, native sequence human IgG3 Fc region, and native sequence human IgG4 Fc region, as well as naturally occurring variants thereof. Native sequence Fc includes the various allotypes of Fcs (Jefferis et al., (2009) mAbs, 1(4):332-338).

In some embodiments, the mutations in the Fc region that result in modulated binding to one or more Fc receptors can include one or more of the following mutations: SD (S239D), SDIE (S239D/I332E), SE (S267E), SELF (S267E/L328F), SDIE (S239D/I332E), SDIEAL (S239D/I332E/A330L), GA (G236A), ALIE (A330L/I332E), GASDALIE

(G236A/S239D/A330L/I332E), V9 (G237D/P238D/P271G/A330R), and V11 (G237D/P238D/H268D/P271G/A330R), and/or one or more mutations at the following amino acids: E233, G237, P238, H268, P271, L328 and A330. Additional Fc region modifications for modulating Fc receptor binding are described in, for example, US 2016/0145350 and US 7416726 and US 5624821, which are hereby incorporated by reference in their entireties.

In some embodiments, the Fc region of the antibodies of the immunoconjugates are modified to have an altered glycosylation pattern of the Fc region compared to the native non-modified Fc region.

Human immunoglobulin is glycosylated at the Asn297 residue in the Cγ2 domain of each heavy chain. This N-linked oligosaccharide is composed of a core heptasaccharide, N-acetylglucosamine4Mannose3 (GlcNAc4Man3). Removal of the heptasaccharide with endoglycosidase or PNGase F is known to lead to conformational changes in the antibody Fc region, which can significantly reduce antibody-binding affinity to activating FcγR and lead to decreased effector function. The core heptasaccharide is often decorated with galactose, bisecting GlcNAc, fucose, or sialic acid, which differentially impacts Fc binding to activating and inhibitory FcγR. Additionally, it has been demonstrated that α2,6-sialyation enhances anti-inflammatory activity in vivo, while defucosylation leads to improved FcγRIIIa binding and a 10-fold increase in antibody-dependent cellular cytotoxicity and antibody-dependent phagocytosis. Specific glycosylation patterns, therefore, can be used to control inflammatory effector functions.

In some embodiments, the modification to alter the glycosylation pattern is a mutation. For example, a substitution at Asn297. In some embodiments, Asn297 is mutated to glutamine (N297Q). Methods for controlling immune response with antibodies that modulate FcγR-regulated signaling are described, for example, in U.S. Patent 7,416,726 and U.S. Patent Application Publications 2007/0014795 and 2008/0286819, which are hereby incorporated by reference in their entireties.

In some embodiments, the antibodies of the immunoconjugates are modified to contain an engineered Fab region with a non-naturally occurring glycosylation pattern. For example, hybridomas can be genetically engineered to secrete afucosylated mAb, desialylated mAb or deglycosylated Fc with specific mutations that enable increased FcRγIIIa binding and effector function. In some embodiments, the antibodies of the immunoconjugates are engineered to be afucosylated.

In some embodiments, the entire Fc region of an antibody in the immunoconjugates is exchanged with a different Fc region, so that the Fab region of the antibody is conjugated to a non-native Fc region. For example, the Fab region of cetuximab, which normally comprises an

IgG1 Fc region, can be conjugated to IgG2, IgG3, IgG4, or IgA, or the Fab region of nivolumab, which normally comprises an IgG4 Fc region, can be conjugated to IgG1, IgG2, IgG3, IgA1, or IgG2. In some embodiments, the Fc modified antibody with a non-native Fc domain also comprises one or more amino acid modification, such as the S228P mutation within the IgG4 Fc, that modulate the stability of the Fc domain described. In some embodiments, the Fc modified antibody with a non-native Fc domain also comprises one or more amino acid modifications described herein that modulate Fc binding to FcR.

In some embodiments, the modifications that modulate the binding of the Fc region to FcR do not alter the binding of the Fab region of the antibody to its antigen when compared to the native non-modified antibody. In other embodiments, the modifications that modulate the binding of the Fc region to FcR also increase the binding of the Fab region of the antibody to its antigen when compared to the native non-modified antibody.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds PD-L1.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds HER2.

In an exemplary embodiment, the immunoconjugates of the invention comprise an antibody construct that comprises an antigen binding domain that specifically recognizes and binds CEA.

In certain embodiments, immunoconjugates of the invention comprise anti-HER2 antibodies. In one embodiment of the invention, an anti-HER2 antibody of an immunoconjugate of the invention comprises a humanized anti-HER2 antibody, e.g., huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8, as described in Table 3 of US 5821337, which is specifically incorporated by reference herein. Those antibodies contain human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. The humanized antibody huMAb4D5-8 is also referred to as trastuzumab, commercially available under the tradename HERCEPTIN™ (Genentech, Inc.).

Trastuzumab (CAS 180288-69-1, HERCEPTIN®, huMAb4D5-8, rhuMAb HER2, Genentech) is a recombinant DNA-derived, IgG1 kappa, monoclonal antibody that is a humanized version of a murine anti-HER2 antibody (4D5) that selectively binds with high affinity in a cell-based assay (Kd = 5 nM) to the extracellular domain of HER2 (US 5677171; US 5821337; US 6054297; US 6165464; US 6339142; US 6407213; US 6639055; US 6719971;

US 6800738; US 7074404; Coussens et al (1985) Science 230:1132-9; Slamon et al (1989) Science 244:707-12; Slamon et al (2001) New Engl. J. Med. 344:783-792).

In an embodiment of the invention, the antibody construct or antigen binding domain comprises the CDR regions of trastuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises the framework regions of the trastuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises one or both variable regions of trastuzumab.

In another embodiment of the invention, an anti-HER2 antibody of an immunoconjugate of the invention comprises a humanized anti-HER2 antibody, e.g., humanized 2C4, as described in US 7862817. An exemplary humanized 2C4 antibody is pertuzumab (CAS Reg. No. 380610-27-5), PERJETA™ (Genentech, Inc.). Pertuzumab is a HER dimerization inhibitor (HDI) and functions to inhibit the ability of HER2 to form active heterodimers or homodimers with other HER receptors (such as EGFR/HER1, HER2, HER3 and HER4). See, for example, Harari and Yarden, Oncogene 19:6102-14 (2000); Yarden and Sliwkowski. Nat Rev Mol Cell Biol 2:127-37 (2001); Sliwkowski Nat Struct Biol 10:158-9 (2003); Cho et al. Nature 421:756-60 (2003); and Malik et al. Pro Am Soc Cancer Res 44:176-7 (2003). PERJETA™ is approved for the treatment of breast cancer.

In an embodiment of the invention, the antibody construct or antigen binding domain comprises the CDR regions of pertuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises the framework regions of the pertuzumab. In an embodiment of the invention, the anti-HER2 antibody further comprises one or both variable regions of pertuzumab.

Elevated expression of carcinoembryonic antigen (CEA, CD66e, CEACAM5) has been implicated in various biological aspects of neoplasia, especially tumor cell adhesion, metastasis, the blocking of cellular immune mechanisms, and having antiapoptosis functions. CEA is also used as a blood marker for many carcinomas. Labetuzumab (CEA-CIDE™, Immunomedics, CAS Reg. No. 219649-07-7), also known as MN-14 and hMN14, is a humanized IgG1 monoclonal antibody and has been studied for the treatment of colorectal cancer (Blumenthal, R. et al (2005) Cancer Immunology Immunotherapy 54(4):315-327). Labetuzumab conjugated to a camptothecin analog (labetuzumab govitecan, IMMU-130) targets carcinoembryonic antigen-related cell adhesion mol. 5 (CEACAM5) and is being studied in patients with relapsed or refractory metastatic colorectal cancer (Sharkey, R. et al, (2018), Molecular Cancer Therapeutics 17(1):196-203; Cardillo, T. et al (2018) Molecular Cancer Therapeutics 17(1):150-160).

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hMN-14/labetuzumab SEQ ID NO. 1 (US 6676924).

DIQLTQSPSSLSASVGDRVTITCKASQDVGTSVAWYQQKPGKAPKLLIYWTSTRHTGVPSRFSGSGSGTD
FTFTISSLQPEDIATYYCQQYSLYRSFGQGTKVEIK SEQ ID NO. 1

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hMN-14/labetuzumab SEQ ID NO. 2-8 (US 6676924).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQLTQSPSSLSASVGDRVTITC	1 - 23	23	2
CDR-L1	KASQDVGTSVA	24 - 34	11	3
LFR2	WYQQKPGKAPKLLIY	35 - 49	15	4
CDR-L2	WTSTRHT	50 - 56	7	5
LFR3	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	57 - 88	32	6
CDR-L3	QQYSLYRS	89 - 96	8	7
LFR4	FGQGTKVEIK	97 - 106	10	8

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of hMN-14/labetuzumab SEQ ID NO. 9 (US 6676924).

EVQLVESGGGVVQPGRSLRLSCSSSGFDFTTYWMSWVRQAPGKGLEWVAEIHPDSSTINYAPSLKDRFTI
SRDNSKNTLFLQMDSLRPEDTGVYFCASLYFGFPWFAYWGQGTPVTVSS SEQ ID NO. 9

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hMN-14/labetuzumab SEQ ID NO. 10-16 (US 6676924).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGVVQPGRSLRLSCSSSGFDFT	1 - 30	30	10
CDR-H1	TYWMS	31 - 35	5	11
HFR2	WVRQAPGKGLEWVA	36 - 49	14	12
CDR-H2	EIHPDSSTINYAPSLKD	50 - 66	17	13
HFR3	RFTISRDNSKNTLFLQMDSLRPEDTGVYFCAS	67 - 98	32	14
CDR-H3	LYFGFPWFAY	99 - 108	10	15
HFR4	WGQGTPVTVSS	109 - 119	11	16

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hPR1A3 SEQ ID NO. 17 (US 8642742).

DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK SEQ ID NO. 17

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hPR1A3 SEQ ID NO. 18-24 (US 8642742).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQMTQSPSSLSASVGDRVTITC	1 - 23	23	18
CDR-L1	KASAAVGTYVA	24 - 34	11	19
LFR2	WYQQKPGKAPKLLIY	35 - 49	15	20
CDR-L2	SASYRKR	50 - 56	7	21
LFR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	57 - 88	32	22
CDR-L3	HQYYTYPLFT	89 - 98	10	23
LFR4	FGQGTKLEIK	99 - 108	10	24

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hPR1A3 SEQ ID NO. 25-31 (US 8642742).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	1 - 30	30	25
CDR-H1	EFGMN	31 - 35	5	26
HFR2	WVRQAPGQGLEWMG	36 - 49	14	27
CDR-H2	WINTKTGEATYVEEFKG	50 - 66	17	28
HFR3	RVTFTTDTSTSTAYMELRSLRSDDTAVYYCAR	67 - 98	32	29
CDR-H3	WDFAYYVEAMDY	99 - 110	12	30
HFR4	WGQGTTVTVSS	111 - 121	11	31

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hMFE-23 SEQ ID NO. 32 (US 723288).

ENVLTQSPSSMSASVGDRVNIACSASSSVSYMHWFQQKPGKSPKLWIYSTSNLASGVPSRFSGSGSGTDY
SLTISSMQPEDAATYYCQQRSSYPLTFGGGTKLEIK SEQ ID NO. 32

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hMFE-23 SEQ ID NO. 33-39 (US 723288).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	ENVLTQSPSSMSASVGDRVNIAC	1 - 23	23	33
CDR-L1	SASSSVSYMH	24 - 33	10	34
LFR2	WFQQKPGKSPKLWIY	34 - 48	15	35
CDR-L2	STSNLAS	49 - 55	7	36
LFR3	GVPSRFSGSGSGTDYSLTISSMQPEDAATYYC	56 - 87	32	37
CDR-L3	QQRSSYPLT	88 - 96	9	38
LFR4	FGGGTKLEIK	97 - 106	10	39

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of hMFE-23 SEQ ID NO. 40 (US 723288).

QVKLEQSGAEVVKPGASVKLSCKASGFNIKDSYMHWLRQGPGQRLEWIGWIDPENGDTEYAPKFQGKATF
TTDTSANTAYLGLSSLRPEDTAVYYCNEGTPTGPYYFDYWGQGTLVTVSS SEQ ID NO. 40

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hMFE-23 SEQ ID NO. 41-47 (US 723288).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	QVKLEQSGAEVVKPGASVKLSCKASGFNIK	1 - 30	30	41
CDR-H1	DSYMH	31 - 35	5	42
HFR2	WLRQGPGQRLEWIG	36 - 49	14	43
CDR-H2	WIDPENGDTEYAPKFQG	50 - 66	17	44
HFR3	KATFTTDTSANTAYLGLSSLRPEDTAVYYCNE	67 - 98	32	45
CDR-H3	GTPTGPYYFDY	99 - 109	11	46
HFR4	WGQGTLVTVSS	110 - 120	11	47

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of SM3E SEQ ID NO. 48 (US 723288).

ENVLTQSPSSMSVSVGDRVTIACSASSSVPYMHWLQQKPGKSPKLLIYLTSNLASGVPSRFSGSGSGTDY
SLTISSVQPEDAATYYCQQRSSYPLTFGGGTKLEIK SEQ ID NO. 48

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of SM3E SEQ ID NO. 49-55 (US 723288).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	ENVLTQSPSSMSVSVGDRVTIAC	1 - 23	23	49
CDR-L1	SASSSVPYMH	24 - 33	10	50
LFR2	WLQQKPGKSPKLLIY	34 - 48	15	51
CDR-L2	LTSNLAS	49 - 55	7	52
LFR3	GVPSRFSGSGSGTDYSLTISSVQPEDAATYYC	56 - 87	32	53
CDR-L3	QQRSSYPLT	88 - 96	9	54
LFR4	FGGGTKLEIK	97 - 106	10	55

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of SM3E SEQ ID NO. 56 (US 723288).

QVKLEQSGAEVVKPGASVKLSCKASGFNIKDSYMHWLRQGPGQRLEWIGWIDPENGDTEYAPKFQGKATF
TTDTSANTAYLGLSSLRPEDTAVYYCNEGTPTGPYYFDYWGQGTLVTVSS SEQ ID NO. 56

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of SM3E SEQ ID NO. 57-63 (US 723288).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	QVKLEQSGAEVVKPGASVKLSCKASGFNIK	1 - 30	30	57
CDR-H1	DSYMH	31 - 35	5	58
HFR2	WLRQGPGQRLEWIG	36 - 49	14	59
CDR-H2	WIDPENGDTEYAPKFQG	50 - 66	17	60
HFR3	KATFTTDTSANTAYLGLSSLRPEDTAVYYCNE	67 - 98	32	61
CDR-H3	GTPTGPYYFDY	99 - 109	11	62
HFR4	WGQGTLVTVSS	110 - 120	11	63

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of NP-4/arcitumomab SEQ ID NO. 64-70.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	QTVLSQSPAILSASPGEKVTMTC	1 - 23	23	64
CDR-L1	RASSSVTYIH	24 - 33	10	65
LFR2	WYQQKPGSSPKSWIY	34 - 48	15	66
CDR-L2	ATSNLAS	49 - 55	7	67
LFR3	GVPARFSGSGSGTSYSLTISRVEAEDAATYYC	56 - 87	32	68
CDR-L3	QHWSSKPPT	88 - 96	9	69
LFR4	FGGGTKLEIK	97 - 106	10	70

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of NP-4/arcitumomab SEQ ID NO. 71.

EVKLVESGGGLVQPGGSLRLSCATSGFTFTDYYMNWVRQPPGKALEWLGFIGNKANGYTTEYSASVKGRF
TISRDKSQSILYLQMNTLRAEDSATYYCTRDRGLRFYFDYWGQGTTLTVSS SEQ ID NO. 71.

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of NP-4 SEQ ID NO. 72-78.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVKLVESGGGLVQPGGSLRLSCATSGFTFT	1 - 30	30	72
CDR-H1	DYYMN	31 - 35	5	73
HFR2	WVRQPPGKALEWLG	36 - 49	14	74
CDR-H2	FIGNKANGYTTEYSASVKG	50 - 68	19	75
HFR3	RFTISRDKSQSILYLQMNTLRAEDSATYYCTR	69 - 100	32	76
CDR-H3	DRGLRFYFDY	101 - 110	10	77
HFR4	WGQGTTLTVSS	111 - 121	11	78

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of M5A/hT84.66 SEQ ID NO. 79 (US 7776330).

DIQLTQSPSSLSASVGDRVTITCRAGESVDIFGVGFLHWYQQKPGKAPKLLIYRASNLESGVPSRFSGSG
SRTDFTLTISSLQPEDFATYYCQQTNEDPYTFGQGTKVEIK SEQ ID NO. 79

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of M5A/hT84.66 SEQ ID NO. 80-86 (US 7776330).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQLTQSPSSLSASVGDRVTITC	1 - 23	23	80
CDR-L1	RAGESVDIFGVGFLH	24 - 38	15	81
LFR2	WYQQKPGKAPKLLIY	39 - 53	15	82
CDR-L2	RASNLES	54 - 60	7	83
LFR3	GVPSRFSGSGSRTDFTLTISSLQPEDFATYYC	61 - 92	32	84
CDR-L3	QQTNEDPYT	93 - 101	9	85
LFR4	FGQGTKVEIK	102 - 111	10	86

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of M5A/hT84.66 SEQ ID NO. 87 (US 7776330).

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYMHWVRQAPGKGLEWVARIDPANGNSKYADSVKGRFTI
SADTSKNTAYLQMNSLRAEDTAVYYCAPFGYYVSDYAMAYWGQGTLVTVSS SEQ ID NO. 87

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of M5A/hT84.66 SEQ ID NO. 88-94 (US 7776330).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGGSLRLSCAASGFNIK	1 - 30	30	88
CDR-H1	DTYMH	31 - 35	5	89
HFR2	WVRQAPGKGLEWVA	36 - 49	14	90
CDR-H2	RIDPANGNSKYADSVKG	50 - 66	17	91
HFR3	RFTISADTSKNTAYLQMNSLRAEDTAVYYCAP	67 - 98	32	92
CDR-H3	FGYYVSDYAMAY	99 - 110	12	93
HFR4	WGQGTLVTVSS	111 - 121	11	94

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of hAb2-3 SEQ ID NO. 95 (US 9617345).

DIQMTQSPASLSASVGDRVTITCRASENIFSYLAWYQQKPGKSPKLLVYNTRTLAEGVPSRFSGSGSGTD
FSLTISSLQPEDFATYYCQHHYGTPFTFGSGTKLEIK SEQ ID NO. 95

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of hAb2-3 SEQ ID NO. 96-102 (US 9617345).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	DIQMTQSPASLSASVGDRVTITC	1 - 23	23	96
CDR-L1	RASENIFSYLA	24 - 34	11	97
LFR2	WYQQKPGKSPKLLVY	35 - 49	15	98
CDR-L2	NTRTLAE	50 - 56	7	99
LFR3	GVPSRFSGSGSGTDFSLTISSLQPEDFATYYC	57 - 88	32	100
CDR-L3	QHHYGTPFT	89 - 97	9	101
LFR4	FGSGTKLEIK	98 - 107	10	102

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of SEQ ID NO. 103 (US 9617345).

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EVQLQESGPGLVKPGGSLSLSCAASGFVFSSYDMSWVRQTPERGLEWVAYISSGGGITYAPSTVKGRFTV
SRDNAKNTLYLQMNSLTSEDTAVYYCAAHYFGSSGPFAYWGQGTLVTVSS      SEQ ID NO. 103
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In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of hAb2-3 SEQ ID NO. 104-110.

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLQESGPGLVKPGGSLSLSCAASGFVFS	1 - 30	30	104
CDR-H1	SYDMS	31 - 35	5	105
HFR2	WVRQTPERGLEWVA	36 - 49	14	106
CDR-H2	YISSGGGITYAPSTVKG	50 - 66	17	107
HFR3	RFTVSRDNAKNTLYLQMNSLTSEDTAVYYCAA	67 - 98	32	108
CDR-H3	HYFGSSGPFAY	99 - 109	11	109
HFR4	WGQGTLVTVSS	110 - 120	11	110

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable light chain (VL kappa) of A240VL-B9VH/AMG-211 SEQ ID NO. 111 (US 9982063).

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QAVLTQPASLSASPGASASLTCTLRRGINVGAYSIYWYQQKPGSPPQYLLRYKSDSDKQQGSGVSSRFSA
SKDASANAGILLISGLQSEDEADYYCMIWHSGASAVFGGGTKLTVL         SEQ ID NO. 111
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In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the light chain CDR (complementarity determining region) or light chain framework (LFR) sequences of A240VL-B9VH/AMG-211 SEQ ID NO. 112-118 (US 9982063).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
LFR1	QAVLTQPASLSASPGASASLTC	1 - 22	22	112
CDR-L1	TLRRGINVGAYSIY	23 - 36	14	113
LFR2	WYQQKPGSPPQYLLR	37 - 51	15	114

CDR-L2	YKSDSDKQQGS	52 - 62	11	115
LFR3	GVSSRFSASKDASANAGILLISGLQSEDEADYYC	63 - 96	34	116
CDR-L3	MIWHSGASAV	97 - 106	10	117
LFR4	FGGGTKLTVL	107 - 116	10	118

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of B9VH SEQ ID NO. 119 (US 9982063).

EVQLVESGGGLVQPGRSLRLSCAASGFTVSSYWMHWVRQAPGKGLEWVGFIRNKANGGTTEYAASVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGLRFYFDYWGQGTTVTVSS SEQ ID NO. 119

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of SEQ ID NO. 120-126 (US 9982063).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGRSLRLSCAASGFTVS	1 - 30	30	120
CDR-H1	SYWMH	31 - 35	5	121
HFR2	WVRQAPGKGLEWVG	36 - 49	14	122
CDR-H2	FIRNKANGGTTEYAASVKG	50 - 68	19	123
HFR3	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR	69 - 100	32	124
CDR-H3	DRGLRFYFDY	101 - 110	10	125
HFR4	WGQGTTVTVSS	111 - 121	11	126

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the Variable heavy chain (VH) of E12VH SEQ ID NO. 127 (US 9982063).

EVQLVESGGGLVQPGRSLRLSCAASGFTVSSYWMHWVRQAPGKGLEWVGFILNKANGGTTEYAASVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGLRFYFDYWGQGTTVTVSS SEQ ID NO. 127

In an embodiment of the invention, the CEA-targeting antibody construct or antigen binding domain comprises the heavy chain CDR (complementarity determining region) or heavy chain framework (HFR) sequences of SEQ ID NO. 128-134 (US 9982063).

Region	Sequence Fragment	Residues	Length	SEQ ID NO.
HFR1	EVQLVESGGGLVQPGRSLRLSCAASGFTVS	1 - 30	30	128
CDR-H1	SYWMH	31 - 35	5	129
HFR2	WVRQAPGKGLEWVG	36 - 49	14	130
CDR-H2	FILNKANGGTTEYAASVKG	50 - 68	19	131
HFR3	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR	69 - 100	32	132
CDR-H3	DRGLRFYFDY	101 - 110	10	133
HFR4	WGQGTTVTVSS	111 - 121	11	134

In some embodiments, the antibody construct further comprises an Fc domain. In certain embodiments, the antibody construct is an antibody. In certain embodiments, the antibody construct is a fusion protein. The antigen binding domain can be a single-chain variable region fragment (scFv). A single-chain variable region fragment (scFv), which is a truncated Fab fragment including the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated using routine recombinant DNA technology techniques. Similarly, disulfide-stabilized variable region fragments (dsFv) can be prepared by recombinant DNA technology. The antibody construct or antigen binding domain may comprise one or more variable regions (e.g., two variable regions) of an antigen binding domain of an anti-PD-L1 antibody, an anti-HER2 antibody, or an anti-CEA antibody, each variable region comprising a CDR1, a CDR2, and a CDR3.

In some embodiments, the antibodies in the immunoconjugates contain a modified Fc region, wherein the modification modulates the binding of the Fc region to one or more Fc receptors.

In some embodiments, the Fc region is modified by inclusion of a transforming growth factor beta 1 (TGFβ1) receptor, or a fragment thereof, that is capable of binding TGFβ1. For example, the receptor can be TGFβ receptor II (TGFβRII). In some embodiments, theTGFβ receptor is a human TGFβ receptor. In some embodiments, the IgG has a C-terminal fusion to a TGFβRII extracellular domain (ECD); e.g., amino acids 24-159 of SEQ ID NO: 9 of US 9676863, incorporated herein. An "Fc linker" may be used to attach the IgG to the TGFβRII extracellular domain, for example, a G_4S_4G Fc linker. The Fc linker may be a short, flexible peptide that allows for the proper three-dimensional folding of the molecule while maintaining the binding-specificity to the targets. In some embodiments, the N-terminus of the TGFβ receptor is fused to the Fc of the antibody construct (with or without an Fc linker). In some embodiments, the C-terminus of the antibody construct heavy chain is fused to the TGFβ receptor (with or without an Fc linker). In some embodiments, the C-terminal lysine residue of the antibody construct heavy chain is mutated to alanine.

In some embodiments, the antibodies in the immunoconjugates are glycosylated.

In some embodiments, the antibodies in the immunoconjugates is a cysteine-engineered antibody which provides for site-specific conjugation of an adjuvant, label, or drug moiety to the antibody through cysteine substitutions at sites where the engineered cysteines are available for conjugation but do not perturb immunoglobulin folding and assembly or alter antigen binding and effector functions (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al. (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; US 2012/0121615; WO 2009/052249). A "cysteine engineered antibody" or "cysteine engineered antibody variant" is an antibody in which one or more residues of an antibody are substituted with cysteine residues. Cysteine-engineered antibodies can be conjugated to the aminoquinoline adjuvant moiety as an aminoquinoline-linker compound with uniform stoichiometry (e.g., up to 2 aminoquinoline moieties per antibody in an antibody that has a single engineered cysteine site).

In some embodiments, cysteine-engineered antibodies used to prepare the immunoconjugates of Table 3 have a cysteine residue introduced at the 149-lysine site of the light chain (LC K149C). In other embodiments, the cysteine-engineered antibodies have a cysteine residue introduced at the 118-alanine site (EU numbering) of the heavy chain (HC A118C). This site is alternatively numbered 121 by Sequential numbering or 114 by Kabat numbering. In other embodiments, the cysteine-engineered antibodies have a cysteine residue introduced in the light chain at G64C or R142C according to Kabat numbering, or in the heavy chain at D101C, V184C or T205C according to Kabat numbering.

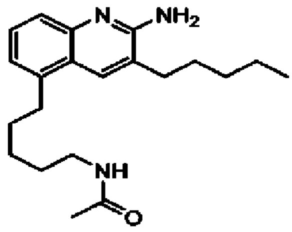
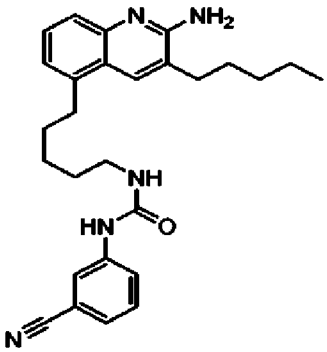
AMINOQUINOLINE ADJUVANT COMPOUNDS

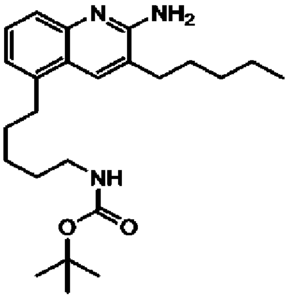
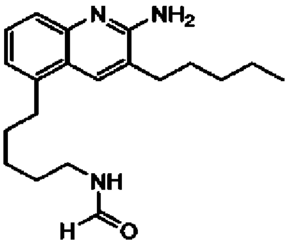
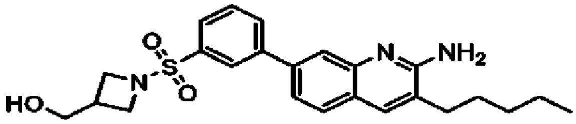
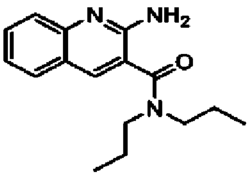
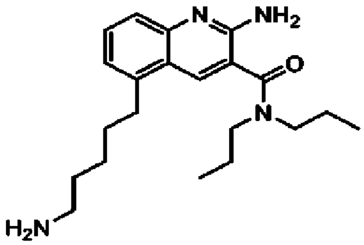
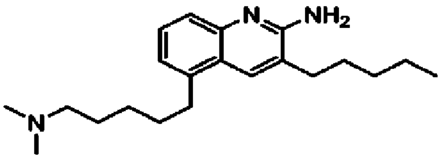
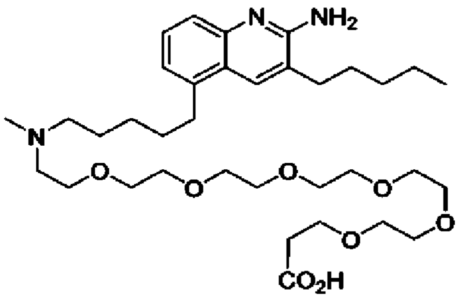
The immunoconjugate of the invention comprises an aminoquinoline adjuvant moiety. The adjuvant moiety described herein is a compound that elicits an immune response (i.e., an immunostimulatory agent). Generally, the adjuvant moiety described herein is a TLR agonist. TLRs are type-I transmembrane proteins that are responsible for the initiation of innate immune responses in vertebrates. TLRs recognize a variety of pathogen-associated molecular patterns from bacteria, viruses, and fungi and act as a first line of defense against invading pathogens. TLRs elicit overlapping yet distinct biological responses due to differences in cellular expression and in the signaling pathways that they initiate. Once engaged (e.g., by a natural stimulus or a synthetic TLR agonist), TLRs initiate a signal transduction cascade leading to activation of nuclear factor-κB (NF-κB) via the adapter protein myeloid differentiation primary response gene 88 (MyD88) and recruitment of the IL-1 receptor associated kinase (IRAK). Phosphorylation of IRAK then leads to recruitment of TNF-receptor associated factor 6 (TRAF6), which results in the phosphorylation of the NF-κB inhibitor I-κB. As a result, NF-κB enters the cell nucleus and initiates transcription of genes whose promoters contain NF-κB binding sites, such as cytokines. Additional modes of regulation for TLR signaling include TIR-domain containing adapter-inducing interferon-β (TRIF)-dependent induction of TNF-receptor associated factor 6 (TRAF6) and activation of MyD88 independent pathways via TRIF and TRAF3, leading to the phosphorylation of interferon response factor three (IRF3). Similarly, the MyD88 dependent pathway also activates several IRF family members, including IRF5 and IRF7 whereas the TRIF dependent pathway also activates the NF-κB pathway.

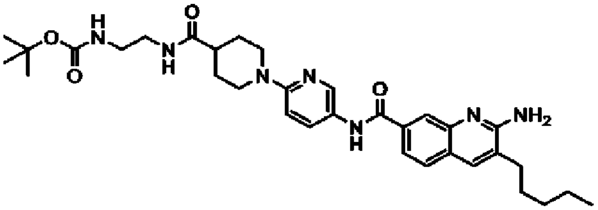
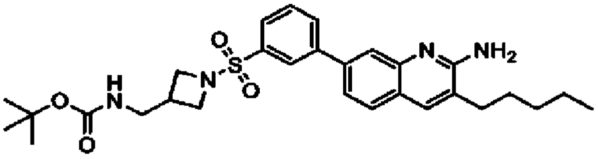
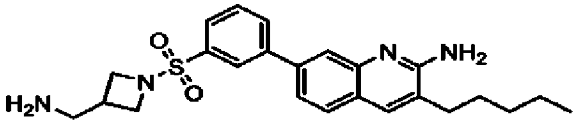
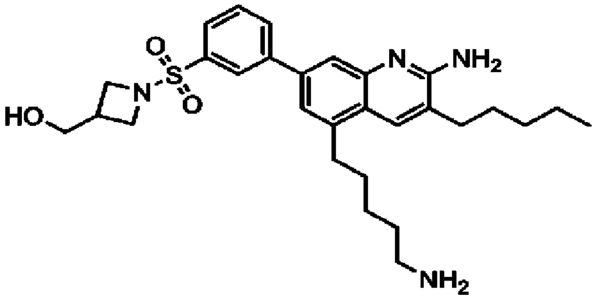
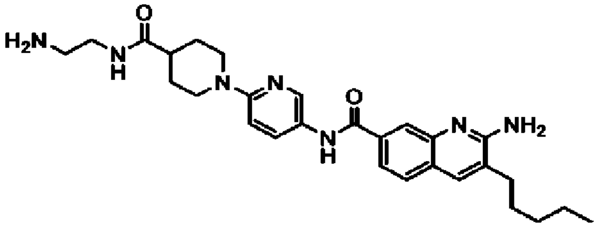
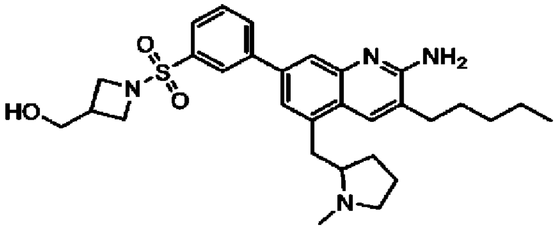
Typically, the adjuvant moiety described herein is a TLR7 and/or TLR8 agonist. TLR7 and TLR8 are both expressed in monocytes and dendritic cells. In humans, TLR7 is also expressed in plasmacytoid dendritic cells (pDCs) and B cells. TLR8 is expressed mostly in cells of myeloid origin, i.e., monocytes, granulocytes, and myeloid dendritic cells. TLR7 and TLR8 are capable of detecting the presence of “foreign” single-stranded RNA within a cell, as a means to respond to viral invasion. Treatment of TLR8-expressing cells, with TLR8 agonists can result in production of high levels of IL-12, IFN-γ, IL-1, TNF-α, IL-6, and other inflammatory cytokines. Similarly, stimulation of TLR7-expressing cells, such as pDCs, with TLR7 agonists can result in production of high levels of IFN-α and other inflammatory cytokines. TLR7/TLR8 engagement and resulting cytokine production can activate dendritic cells and other antigen-presenting cells, driving diverse innate and acquired immune response mechanisms leading to tumor destruction.

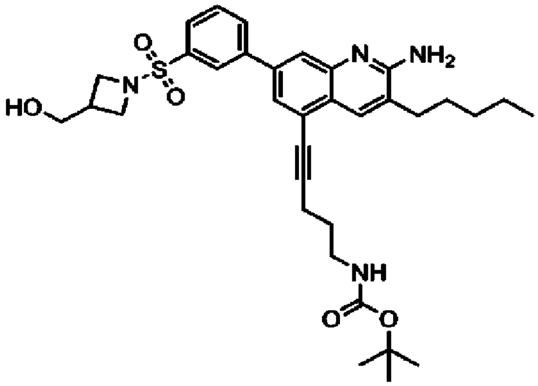
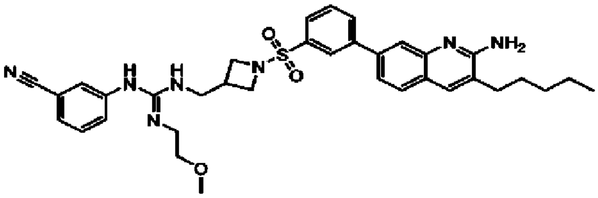
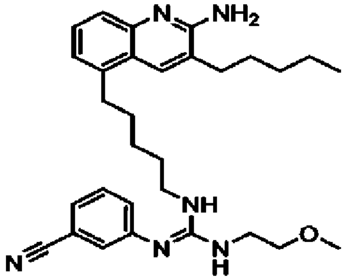
Exemplary aminoquinoline compounds (AQ) of the invention are shown in Table 1. Each compound was characterized by mass spectrometry and shown to have the mass indicated. Activity against HEK293 NFKB reporter cells expressing human TLR7 or human TLR8 was measured according to Example 31. The aminoquinoline compounds of Table 1 demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

Table 1 Aminoquinoline compounds (AQ)

AQ No.	Structure	MW	HEK293 hTLR7 EC50 (nM)	HEK293 hTLR8 EC50 (nM)
AQ-1		341.49	252	>9000
AQ-2		443.59	>9000	>9000

AQ-3		399.57	>9000	>9000
AQ-4		327.47	>9000	>9000
AQ-5		439.57	>9000	562
AQ-6		271.36	>9000	>9000
AQ-7		356.51	>9000	>9000
AQ-8		327.51	>9000	1023
AQ-9		649.86	ND	ND

AQ-10		603.76	>9000	>9000
AQ-11		538.7	>9000	>9000
AQ-12		438.59	>9000	1481
AQ-13		524.72	>9000	>9000
AQ-14		503.64	>9000	>9000
AQ-15		536.73	>9000	2454

AQ-16		620.8	>9000	>9000
AQ-17		639.8	>9000	>9000
AQ-18		500.7	>9000	>9000

AMINOQUINOLINE-LINKER COMPOUNDS

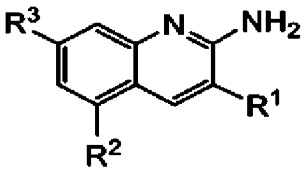
The immunoconjugates of the invention are prepared by conjugation of an antibody with an aminoquinoline-linker compound. The aminoquinoline-linker compounds comprise an aminoquinoline moiety covalently attached to a linker unit. The linker units comprise functional groups and subunits which affect stability, permeability, solubility, and other pharmacokinetic, safety, and efficacy properties of the immunoconjugates. The linker unit includes a reactive functional group which reacts, i.e. conjugates, with a reactive functional group of the antibody. For example, a nucleophilic group such as a lysine side chain amino of the antibody reacts with an electrophilic reactive functional group of the aminoquinoline-linker compound to form the immunoconjugate. Also, for example, a cysteine thiol of the antibody reacts with a maleimide or bromoacetamide group of the aminoquinoline-linker compound to form the immunoconjugate.

Electrophilic reactive functional group suitable for the aminoquinoline-linker compounds include, but are not limited to, N-hydroxysuccinimidyl (NHS) esters and N-hydroxysulfosuccinimidyl (sulfo-NHS) esters (amine reactive); carbodiimides (amine and carboxyl reactive); hydroxymethyl phosphines (amine reactive); maleimides (thiol reactive);

halogenated acetamides such as *N*-iodoacetamides (thiol reactive); aryl azides (primary amine reactive); fluorinated aryl azides (reactive via carbon-hydrogen (C-H) insertion); pentafluorophenyl (PFP) esters (amine reactive); tetrafluorophenyl (TFP) esters (amine reactive); imidoesters (amine reactive); isocyanates (hydroxyl reactive); vinyl sulfones (thiol, amine, and hydroxyl reactive); pyridyl disulfides (thiol reactive); and benzophenone derivatives (reactive via C-H bond insertion). Further reagents include, but are not limited, to those described in Hermanson, *Bioconjugate Techniques* 2nd Edition, Academic Press, 2008.

The invention provides solutions to the limitations and challenges to the design, preparation and use of immunoconjugates. Some linkers may be labile in the blood stream, thereby releasing unacceptable amounts of the adjuvant/drug prior to internalization in a target cell (Khot, A. et al (2015) Bioanalysis 7(13):1633–1648). Other linkers may provide stability in the bloodstream, but intracellular release effectiveness may be negatively impacted. Linkers that provide for desired intracellular release typically have poor stability in the bloodstream. Alternatively stated, bloodstream stability and intracellular release are typically inversely related. In addition, in standard conjugation processes, the amount of adjuvant/drug moiety loaded on the antibody, i.e. drug loading, the amount of aggregate that is formed in the conjugation reaction, and the yield of final purified conjugate that can be obtained are interrelated. For example, aggregate formation is generally positively correlated to the number of equivalents of adjuvant/drug moiety and derivatives thereof conjugated to the antibody. Under high drug loading, formed aggregates must be removed for therapeutic applications. As a result, drug loading-mediated aggregate formation decreases immunoconjugate yield and can render process scale-up difficult.

Exemplary embodiments include an aminoquinoline-linker compound of Formula III:



III

where one of R^1, R^2 and R^3 is attached to L;

R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^2 is selected from the group consisting of:

H;

C_1-C_8 alkyl;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^4 is selected from the group consisting of C_6-C_{20} aryl and C_1-C_8 alkyl;

R^5 is selected from the group consisting of H and C_1-C_8 alkyl;

or two R^5 groups form a 5- or 6-membered heterocyclyl ring; and

R^3 is selected from the group consisting of H, $-C(=O)NR^5R^6$, and phenyl, where phenyl is substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, $-CN$, $-CH_3$, $-CF_3$, $-CO_2H$, $-NH_2$, $-NHCH_3$, $-NO_2$, $-OH$, $-OCH_3$, $-SCH_3$, $-S(O)_2CH_3$, $-S(O)_3H$, and R^7;

R^6 is independently selected from the group consisting of

H;

C_1-C_8 alkyl;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_{20}$ heterocyclyl$)$;

$-(C_2-C_{20}$ heterocyclyldiyl$)-*$;

$-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$--(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-OH$;

$-(C_1-C_{20}$ heteroaryldiyl$)-(C_2-C_{20}$ heterocyclyldiyl$)-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1-C_{20}$ heteroaryldiyl$)-NR^5-*$;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

R^7 is selected from the group consisting of:

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)–*;

–C(=O)–(C_2-C_{20} heterocyclyl);

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–NR^5–*;

–C(=O)N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–NR^5–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)N(R^5)$_2$;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

where * indicates the attachment site of L;

L is the linker selected from the group consisting of:

–(PEP)–C(=O)–(PEG)–C(=O)–Q;

$-NR^5-$(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–NR^5–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–$N^+(R^5)_2$–(PEG)–C(=O)–Q;

–C(=O)–(PEG)–C(=O)–Q;

–C(=O)–$CH(AA_1)$–NR^5–C(=O)–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–C(=O)–$CH(AA_1)$–NR^5–(PEG)–C(=O)–Q;

–C(=O)O–(C_1-C_{12} alkyldiyl)–SS–(PEG)–C(=O)–Q;

–C(=O)–(C_1-C_{12} alkyldiyl)–SS–(PEG)–C(=O)–Q;

–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–Q;

–(MCgluc)–(C(=O)–(PEG)–OCH_2–(C_1-C_{20} heteroaryldiyl)–$CH_2CH_2OCH_2CH_2$–C(=O)–Q;

–(PEP)–C(=O)–$(CH_2)_m$–C(=O)–Q; and

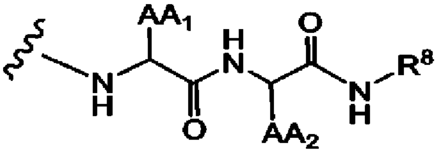
–(PEP)–C(=O)–$(CH_2)_m$–Q;

where

PEG has the formula:

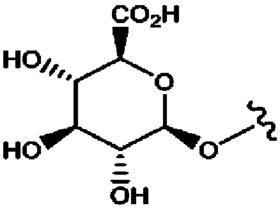
$-(CH_2CH_2O)_n-(CH_2)_m-$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

PEP has the formula:



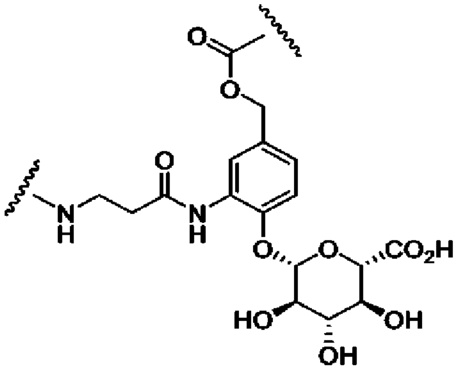
where AA_1 and AA_2 are independently selected from an amino acid side chain, or AA_1 or AA_2 and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

R^8 is selected from the group consisting of C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl substituted with $-CH_2O-C(=O)-$, and optionally with:

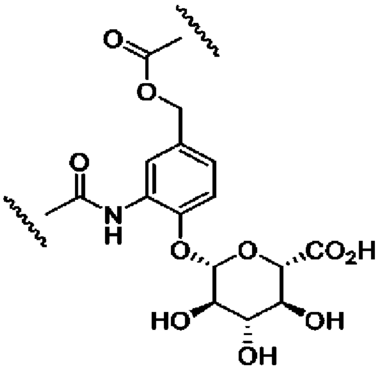


; and

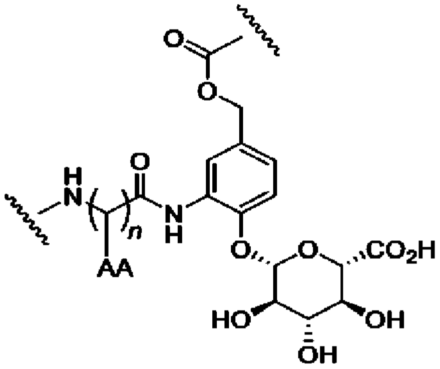
MCgluc is selected from the groups:



;



; and

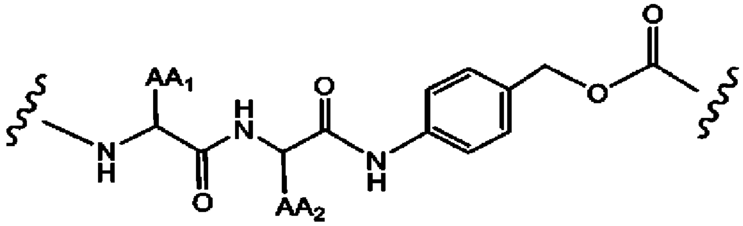


where n is 1 to 8, and AA is an amino acid side chain; and

Q is selected from the group consisting of N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, maleimide, and phenoxy substituted with one or more groups independently selected from F, Cl, NO_2, and SO_3^-;

where alkyl, alkyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $-CH_3$, $-CH_2CH_3$, $-CH=CH_2$, $-C\equiv CH$, $-C\equiv CCH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2CH_2OH$, $-C(CH_3)_2OH$, $-CH(OH)CH(CH_3)_2$, $-C(CH_3)_2CH_2OH$, $-CH_2CH_2SO_2CH_3$, $-CH_2OP(O)(OH)_2$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CH_2CHF_2$, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NO_2$, $=O$, $-OH$, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$.

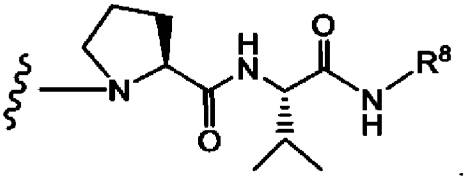
An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein PEP has the formula:



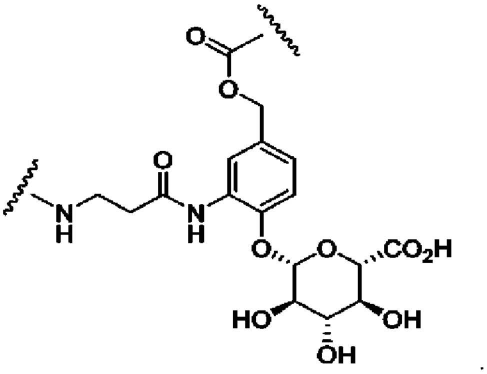
wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 or AA_2 with an adjacent nitrogen atom form a 5-membered ring to form a proline amino acid.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein PEP has the formula:



An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein MCgluc has the formula:



An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid, including where AA_1 or AA_2 with an adjacent nitrogen atom form a 5-membered ring to form a proline amino acid.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$,

$-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 and the adjacent nitrogen atom form a proline amino acid, and AA_2 is $-CH(CH_3)_2$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^1 is attached to L.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^2 is attached to L.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^3 is attached to L.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^1 is selected from the group consisting of:

C_1-C_8 alkyl;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$; and
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$;

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^2 is selected from the group consisting of:

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$; and
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^6 is selected from the group consisting of:

C_1-C_8 alkyl;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$;
$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^6 is selected from the group consisting of:

$-(C_6\text{-}C_{20}\text{ aryldiyl})-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)N(R^5)_2$;

$-(C_6\text{-}C_{20}\text{ aryldiyl})-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-(C_6\text{-}C_{20}\text{ aryldiyl})-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;

$-(C_6\text{-}C_{20}\text{ aryldiyl})-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$; and

$-(C_6\text{-}C_{20}\text{ aryldiyl})-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-OH$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein R^7 is selected from the group consisting of:

$-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$; and

$-S(=O)_2-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-OH$.

An exemplary embodiment of the aminoquinoline-linker compound includes wherein L is selected from the group consisting of:

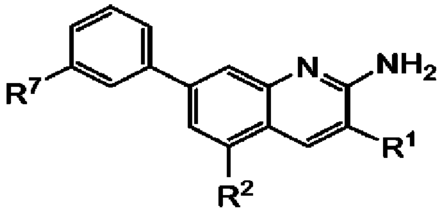
$-(PEP)-C(=O)-(PEG)-C(=O)-Q$;

$-NR^5-(PEG)-C(=O)-Q$;

$-C(=O)-(PEG)-C(=O)-Q$; and

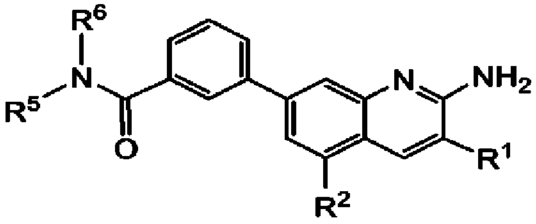
$-(PEG)-C(=O)-Q$.

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AQ is selected from Formula IIIa:



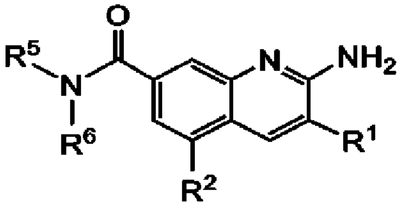
IIIa .

An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AQ is selected from Formula IIIb:



IIIb .

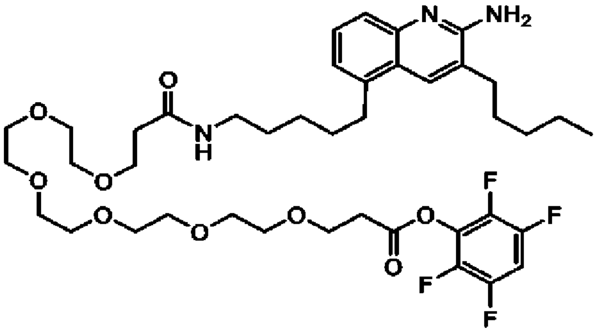
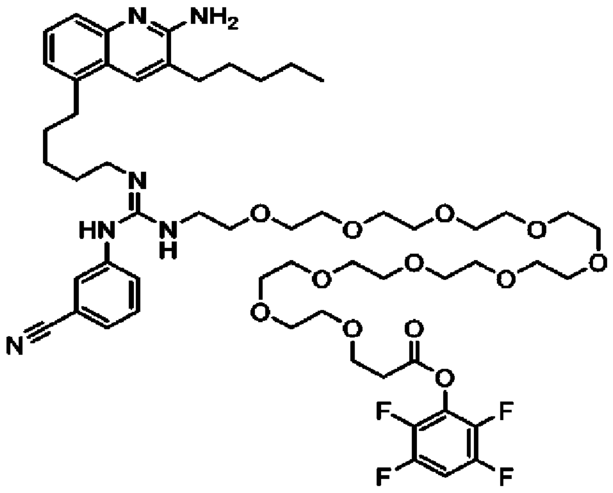
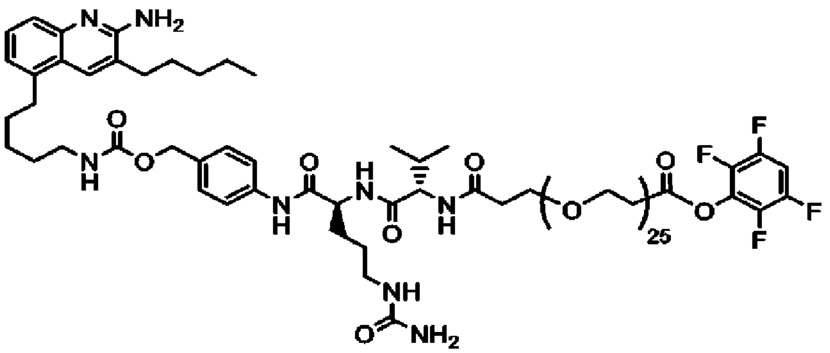
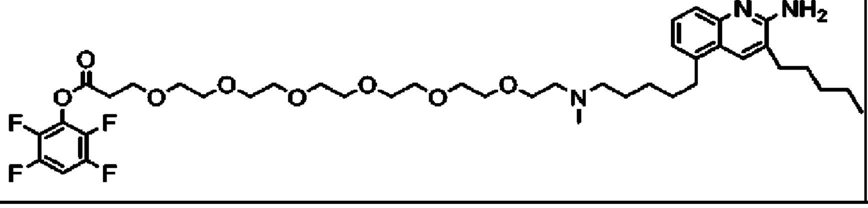
An exemplary embodiment of the aminoquinoline-linker compound of Formula III includes wherein AQ is selected from Formula IIIc:

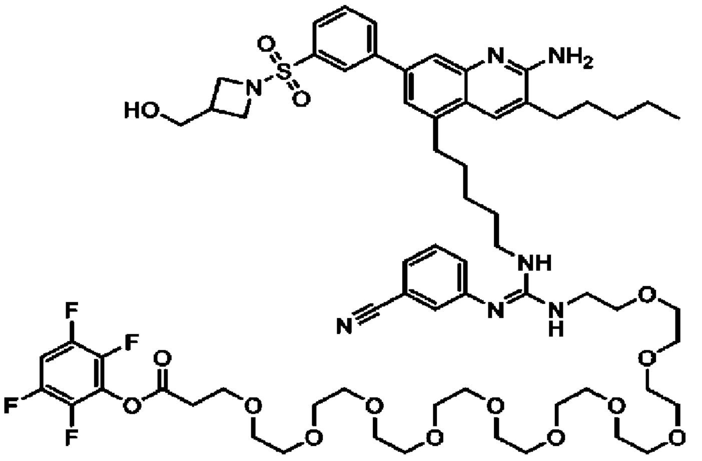
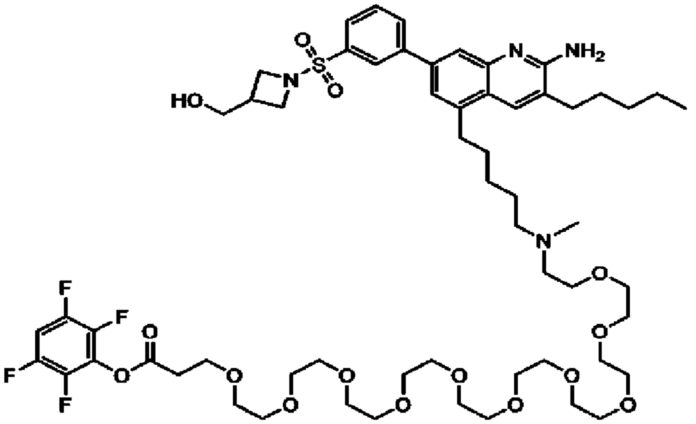
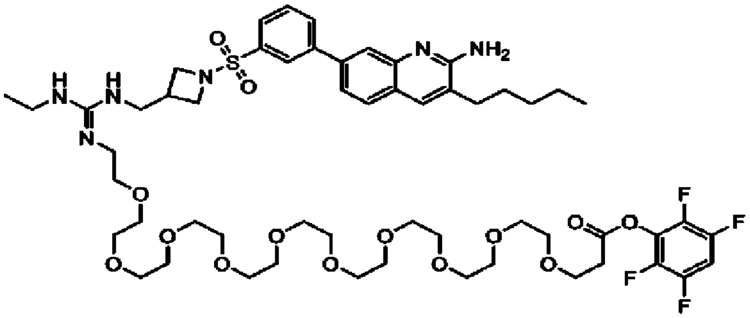
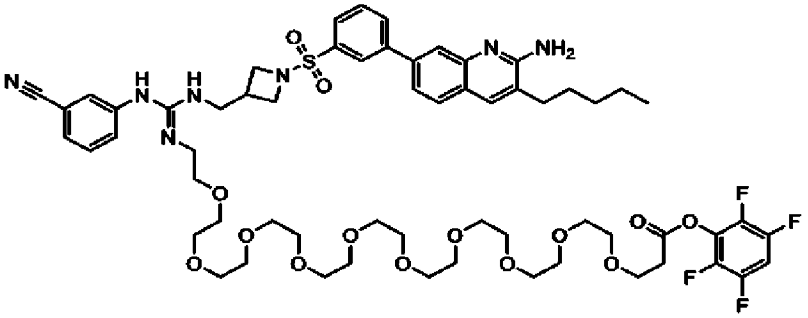


IIIc .

An exemplary embodiment of the aminoquinoline-linker compound of Formula III is selected from the Table 2 compounds. Each compound was characterized by mass spectrometry and shown to have the mass indicated. The aminoquinoline-linker compounds of Table 2 demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

Table 2 Aminoquinoline-linker Formula III compounds

AQ-L	Structure	MW
AQ-L1		811.9
AQ-L2		1103.3
AQ-L3		2054.4
AQ-L4		797.92

AQ-L5		1328.5
AQ-L6		1199.4
AQ-L7		1169.3
AQ-L8		1242.4

IMMUNOCONJUGATES

Exemplary embodiments of immunoconjugates comprise an antibody covalently attached to a divalent linker which is covalently attached to one or more aminoquinoline moieties, and having Formula I:

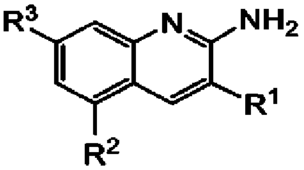
$$\text{Ab–[L–AQ]}_p \qquad \text{I}$$

or a pharmaceutically acceptable salt thereof,

wherein:

Ab is the antibody;

AQ is the aminoquinoline moiety having Formula II:



II

where one of R^1, R^2 and R^3 is attached to L;

R^1 is selected from the group consisting of:

- C_1-C_8 alkyl;
- –(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;
- –(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;
- –(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)R^5$;
- –(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)OR^5$;
- –(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)N(R^5)_2$;
- –(C_1-C_{12} alkyldiyl)–NR^5–*;
- –(C_2-C_6 alkenyldiyl)–$NR^5C(=NR^4)NR^5$–*;
- –(C_2-C_6 alkenyldiyl)–$N(R^5)_2$;
- –(C_2-C_6 alkenyldiyl)–NR^5–*;
- –(C_2-C_6 alkynyldiyl)–$NR^5C(=NR^4)NR^5$–*;
- –(C_2-C_6 alkynyldiyl)–$N(R^5)_2$;
- –(C_2-C_6 alkynyldiyl)–NR^5–*;
- –(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;
- –(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;
- –(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;
- –$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;
- –$C(=O)NR^5$–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^2 is selected from the group consisting of:

H;

C_1-C_8 alkyl;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(NR^5)=N-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)R^5$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)OR^5$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)N(R^5)_2$;

$-(C_2-C_6$ alkenyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_6$ alkenyldiyl$)-N(R^5)_2$;

$-(C_2-C_6$ alkenyldiyl$)-NR^5-*$;

$-(C_2-C_6$ alkynyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_6$ alkynyldiyl$)-N(R^5)_2$;

$-(C_2-C_6$ alkynyldiyl$)-NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-(C_2-C_{20}$ heterocyclyldiyl);

$-(C_1-C_{12}$ alkyldiyl$)-(C_1-C_{20}$ heteroaryldiyl);

$-(C_1-C_{12}$ alkyldiyl$)-(C_6-C_{20}$ aryldiyl);

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^4 is selected from the group consisting of C_6-C_{20} aryl and C_1-C_8 alkyl;

R^5 is selected from the group consisting of H and C_1-C_8 alkyl;

or two R^5 groups form a 5- or 6-membered heterocyclyl ring; and

R^3 is selected from the group consisting of H, $-C(=O)NR^5R^6$, and phenyl, where phenyl is substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, $-CN$, $-CH_3$, $-CF_3$, $-CO_2H$, $-NH_2$, $-NHCH_3$, $-NO_2$, $-OH$, $-OCH_3$, $-SCH_3$, $-S(O)_2CH_3$, $-S(O)_3H$, and R^7;

R^6 is independently selected from the group consisting of

H;

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

––(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{20} heteroaryldiyl)–NR^5–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)N(R^5)_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

R^7 is selected from the group consisting of:

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–C(=O)–*;

–C(=O)–(C_2-C_{20} heterocyclyl);

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-(C_2-C_{20}$ heterocyclyldiyl$)-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-NR^5-*$;

$-C(=O)N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-NR^5-*$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-OH$;

where * indicates the attachment site of L;

L is the linker selected from the group consisting of:

$-C(=O)-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-NR^5-$;

$-C(=O)-(PEG)-NR^5-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-N^+(R^5)_2-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-C(=O)-$;

$-C(=O)-(PEG)-C(=O)NR^5CH(AA_1)C(=O)-$;

$-C(=O)-(PEG)-NR^5CH(AA_1)C(=O)-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-SS-(C_1-C_{12}$ alkyldiyl$)-OC(=O)-$;

$-C(=O)-(PEG)-SS-(C_1-C_{12}$ alkyldiyl$)-C(=O)-$;

$-C(=O)-(PEG)-$;

$-C(=O)-(C_1-C_{12}$ alkyldiyl$)-C(=O)-(PEP)-$;

$-C(=O)-CH_2CH_2OCH_2CH_2-(C_1-C_{20}$ heteroaryldiyl$)-CH_2O-(PEG)-C(=O)-$(MCgluc)$-$; and

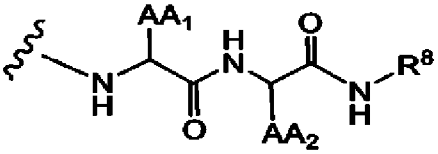
$-$(succinimidyl)$-(CH_2)_m-C(=O)-(PEP)-$;

where

PEG has the formula:

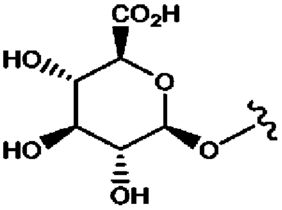
$-(CH_2CH_2O)_n-(CH_2)_m-$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

PEP has the formula:



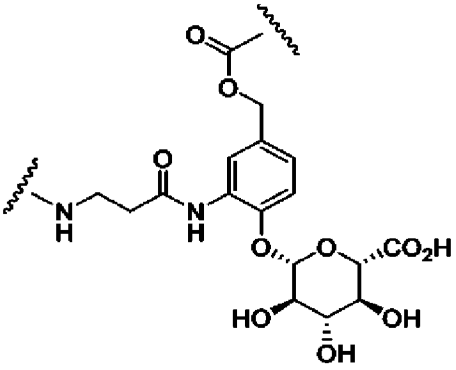
where AA_1 and AA_2 are independently selected from an amino acid side chain, or AA_1 or AA_2 and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment;

R^8 is selected from the group consisting of C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl substituted with $-CH_2O-C(=O)-$, and optionally with:

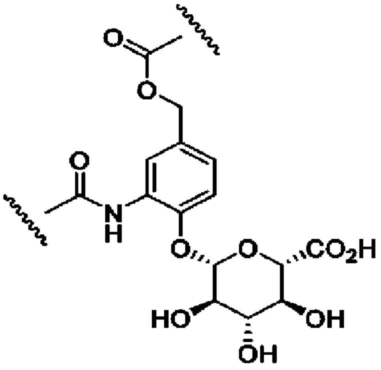


; and

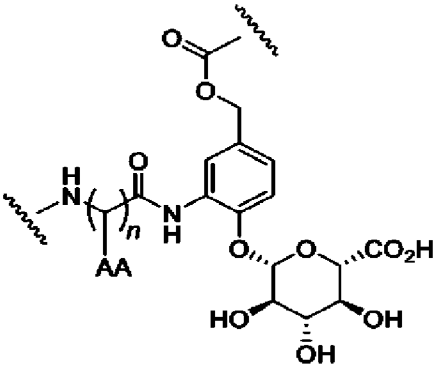
MCgluc is selected from the groups:



;



; and



where n is 1 to 8, and AA is an amino acid side chain;

where alkyl, alkyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $-CH_3$, $-CH_2CH_3$, $-CH=CH_2$, $-C\equiv CH$, –

$C{\equiv}CCH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2CH_2OH$, $-C(CH_3)_2OH$, $-CH(OH)CH(CH_3)_2$, $-C(CH_3)_2CH_2OH$, $-CH_2CH_2SO_2CH_3$, $-CH_2OP(O)(OH)_2$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CH_2CHF_2$, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NO_2$, $=O$, $-OH$, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$; and

p is an integer from 1 to 8.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds PD-L1.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, and avelumab, or a biosimilar or a biobetter thereof.

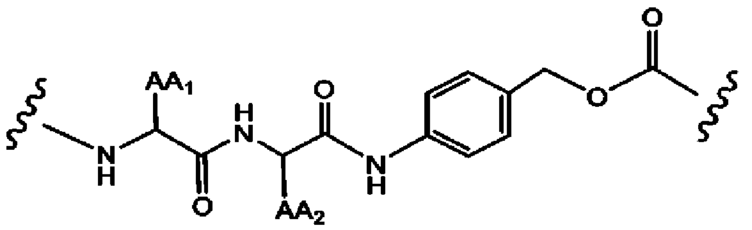
An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds HER2.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is selected from the group consisting of trastuzumab and pertuzumab, or a biosimilar or a biobetter thereof.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is an antibody construct that has an antigen binding domain that binds CEA.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein the antibody is labetuzumab, or a biosimilar or a biobetter thereof.

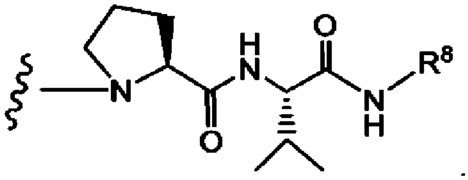
An exemplary embodiment of the immunoconjugate of Formula I includes wherein PEP has the formula:



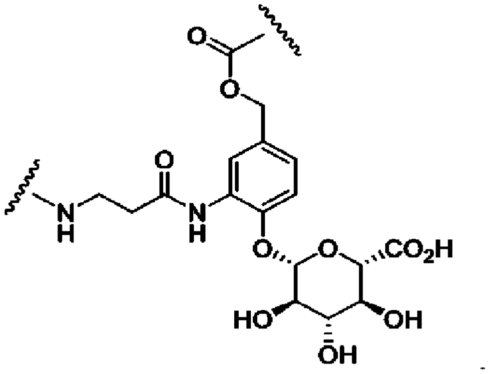
wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 or AA_2 with an adjacent nitrogen atom form a 5-membered ring proline amino acid.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein PEP has the formula:



An exemplary embodiment of the immunoconjugate of Formula I includes wherein MCgluc has the formula:



An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and the adjacent nitrogen atom form a proline amino acid, and AA_2 is $-CH(CH_3)_2$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^1 is attached to L.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 is attached to L.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^3 is attached to L.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$; and

–(C_1-C_{12} alkyldiyl)–NR^5–*;

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^2 is selected from the group consisting of:

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$; and

–(C_1-C_{12} alkyldiyl)–NR^5–*.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^6 is selected from the group consisting of:

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^6 is selected from the group consisting of:

–(C_6-C_{20} aryldiyl)–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)N(R^5)_2$;

–(C_6-C_{20} aryldiyl)–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_6-C_{20} aryldiyl)–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_6-C_{20} aryldiyl)–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–(C_6-C_{20} aryldiyl)–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein R^7 is selected from the group consisting of:

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)– $NR^5C(=NR^4)N(R^5)_2$;

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)– $NR^5C(=NR^4)NR^5$–*;

–$S(=O)_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein L is selected from the group consisting of:

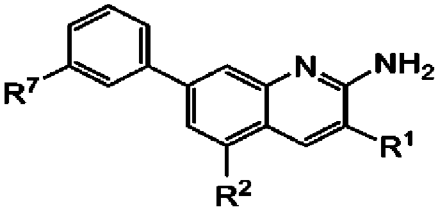
–(PEP)–C(=O)–(PEG)–C(=O)–Q;

$-NR^5$–(PEG)–C(=O)–Q;

–C(=O)–(PEG)–C(=O)–Q; and

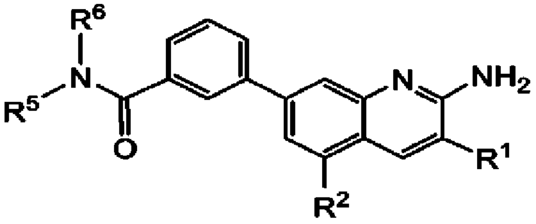
–(PEG)–C(=O)–Q.

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AQ is selected from Formula IIa:



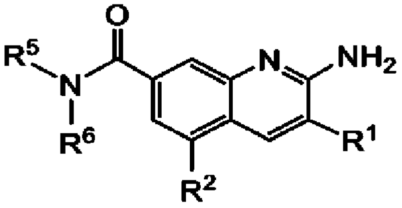
IIa .

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AQ is selected from Formula IIb:



IIb .

An exemplary embodiment of the immunoconjugate of Formula I includes wherein AQ is selected from Formula IIc:



IIc .

The invention includes all reasonable combinations, and permutations of the features, of the Formula I embodiments.

In certain embodiments, the immunoconjugate compounds of the invention include those with immunostimulatory activity. The immunoconjugate compounds of the invention selectively deliver an effective dose of an aminoquinoline drug to tumor tissue, whereby greater selectivity (*i.e.*, a lower efficacious dose) may be achieved while increasing the therapeutic index ("therapeutic window") relative to unconjugated aminoquinoline.

Drug loading is represented by p, the number of aminoquinoline moieties per antibody in an immunoconjugate of Formula I. Drug (aminoquinoline) loading may range from 1 to about 8 drug moieties (D) per antibody. Immunoconjugates of Formula I include mixtures or collections of antibodies conjugated with a range of drug moieties, from 1 to about 8. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of reactive or available amino acid side chain residues such as lysine and cysteine. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein. In such aspects, p may be 1, 2, 3, 4, 5, 6, 7, or 8, and ranges thereof, such as from 1 to 8 or from 2 to 5. In any such aspect, p and n are equal (i.e., p = n = 1, 2, 3, 4, 5, 6, 7, or 8, or some range there between). Exemplary immunoconjugate compounds of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al. (2012) *Methods in Enzym.* 502:123-138). In some embodiments, one or more free cysteine residues are already present in an antibody forming intrachain disulfide bonds, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

For some immunoconjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments described herein, an antibody may have only one or a limited number of cysteine thiol groups, or may have only one or a limited number of sufficiently reactive thiol groups, to which the drug may be attached. In other embodiments, one or more lysine amino groups in the antibody may be available and reactive for conjugation with an aminoquinoline-linker compound of Formula II. In certain embodiments, higher drug loading, *e.g.* p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain immunoconjugate compounds. In certain embodiments, the average drug loading for an immunoconjugate ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

The loading (drug/antibody ratio) of an immunoconjugate may be controlled in different ways, and for example, by: (i) limiting the molar excess of the aminoquinoline-linker intermediate compound relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive denaturing conditions for optimized antibody reactivity.

It is to be understood that where more than one nucleophilic group of the antibody reacts with a drug, then the resulting product is a mixture of immunoconjugate compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual immunoconjugate molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, *e.g.* hydrophobic interaction chromatography (*see, e.g.*, McDonagh et al. (2006) Prot. Engr. Design & Selection 19(7):299-307; Hamblett et al. (2004) Clin. Cancer Res. 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous immunoconjugate with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Table 3 shows exemplary embodiments of the immunoconjugate of Formula I. The immunoconjugates of Table 3 demonstrate the surprising and unexpected property of TLR8 agonist selectivity which may predict useful therapeutic activity to treat cancer and other disorders.

Table 3 Immunoconjugates (IC)

Immunoconjugate No.	AQ-linker Table 2	Ab Antigen	DAR	Myeloid TNFα Secretion EC50 [nM]
IC-1	AQ-L1	rituximab CD20	1.68	>1000
IC-2	AQ-L1	anti-h/rHER2	2.36	>1000
IC-3	AQ-L1	avelumab PD-L1	2.24 2.44	>1000
IC-4	AQ-L3	trastuzumab HER2	1.35	88

IC-5	AQ-L2	trastuzumab HER2	1.96	132
IC-6	AQ-L4	trastuzumab HER2	2.68	298
IC-7	AQ-L5	trastuzumab HER2	2.25	143
IC-8	AQ-L7	trastuzumab HER2	2.53	119

* DAR = drug (adjuvant) to antibody ratio

COMPOSITIONS OF IMMUNOCONJUGATES

The invention provides a composition, e.g., a pharmaceutically or pharmacologically acceptable composition or formulation, comprising a plurality of immunoconjugates as described herein and optionally a carrier therefor, e.g., a pharmaceutically or pharmacologically acceptable carrier. The immunoconjugates can be the same or different in the composition, i.e., the composition can comprise immunoconjugates that have the same number of adjuvants linked to the same positions on the antibody construct and/or immunoconjugates that have the same number of aminoquinoline adjuvants linked to different positions on the antibody construct, that have different numbers of adjuvants linked to the same positions on the antibody construct, or that have different numbers of adjuvants linked to different positions on the antibody construct.

In an exemplary embodiment, a composition comprising the immunoconjugate compounds comprises a mixture of the immunoconjugate compounds, wherein the average drug (aminoquinoline) loading per antibody in the mixture of immunoconjugate compounds is about 2 to about 5.

A composition of immunoconjugates of the invention can have an average adjuvant to antibody construct ratio (DAR) of about 0.4 to about 10. A skilled artisan will recognize that the number of aminoquinoline adjuvants conjugated to the antibody construct may vary from immunoconjugate to immunoconjugate in a composition comprising multiple immunoconjugates of the invention, and, thus, the adjuvant to antibody construct (e.g., antibody) ratio can be measured as an average, which may be referred to as the drug to antibody ratio (DAR). The adjuvant to antibody construct (e.g., antibody) ratio can be assessed by any suitable means, many of which are known in the art.

The average number of adjuvant moieties per antibody (DAR) in preparations of immunoconjugates from conjugation reactions may be characterized by conventional means such as mass spectrometry, ELISA assay, and HPLC. The quantitative distribution of immunoconjugates in a composition in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous immunoconjugates where p is a certain value from immunoconjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

In some embodiments, the composition further comprises one or more pharmaceutically or pharmacologically acceptable excipients. For example, the immunoconjugates of the invention can be formulated for parenteral administration, such as IV administration or administration into a body cavity or lumen of an organ. Alternatively, the immunoconjugates can be injected intra-tumorally. Compositions for injection will commonly comprise a solution of the immunoconjugate dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and an isotonic solution of one or more salts such as sodium chloride, e.g., Ringer's solution. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed, including synthetic monoglycerides or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These compositions desirably are sterile and generally free of undesirable matter. These compositions can be sterilized by conventional, well known sterilization techniques. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

The composition can contain any suitable concentration of the immunoconjugate. The concentration of the immunoconjugate in the composition can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. In certain embodiments, the concentration of an immunoconjugate in a solution formulation for injection will range from about 0.1% (w/w) to about 10% (w/w).

METHOD OF TREATING CANCER WITH IMMUNOCONJUGATES

The invention provides a method for treating cancer. The method includes administering a therapeutically effective amount of an immunoconjugate as described herein, and such as a composition as described herein, to a subject in need thereof, e.g., a subject that has cancer and is in need of treatment for the cancer. The method includes administering a therapeutically effective amount of an immunoconjugate (IC) selected from Table 3.

It is contemplated that the immunoconjugate of the present invention may be used to treat various hyperproliferative diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary hyperproliferative disorders include benign or malignant solid tumors and hematological disorders such as leukemia and lymphoid malignancies.

In another aspect, an immunoconjugate for use as a medicament is provided. In certain embodiments, the invention provides an immunoconjugate for use in a method of treating an individual comprising administering to the individual an effective amount of the immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described herein.

In a further aspect, the invention provides for the use of an immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer, the method comprising administering to an individual having cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described herein.

Carcinomas are malignancies that originate in the epithelial tissues. Epithelial cells cover the external surface of the body, line the internal cavities, and form the lining of glandular tissues. Examples of carcinomas include, but are not limited to, adenocarcinoma (cancer that begins in glandular (secretory) cells such as cancers of the breast, pancreas, lung, prostate, stomach, gastroesophageal junction, and colon) adrenocortical carcinoma; hepatocellular carcinoma; renal cell carcinoma; ovarian carcinoma; carcinoma in situ; ductal carcinoma; carcinoma of the breast; basal cell carcinoma; squamous cell carcinoma; transitional cell carcinoma; colon carcinoma; nasopharyngeal carcinoma; multilocular cystic renal cell carcinoma; oat cell carcinoma; large cell lung carcinoma; small cell lung carcinoma; non-small cell lung carcinoma; and the like. Carcinomas may be found in prostrate, pancreas, colon, brain (usually as secondary metastases), lung, breast, and skin. In some embodiments, methods for treating non-small cell lung carcinoma include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, methods for treating breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, methods for treating triple-negative breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof).

Soft tissue tumors are a highly diverse group of rare tumors that are derived from connective tissue. Examples of soft tissue tumors include, but are not limited to, alveolar soft part sarcoma; angiomatoid fibrous histiocytoma; chondromyoxid fibroma; skeletal chondrosarcoma; extraskeletal myxoid chondrosarcoma; clear cell sarcoma; desmoplastic small round-cell tumor; dermatofibrosarcoma protuberans; endometrial stromal tumor; Ewing's sarcoma; fibromatosis (Desmoid); fibrosarcoma, infantile; gastrointestinal stromal tumor; bone giant cell tumor; tenosynovial giant cell tumor; inflammatory myofibroblastic tumor; uterine leiomyoma; leiomyosarcoma; lipoblastoma; typical lipoma; spindle cell or pleomorphic lipoma; atypical lipoma; chondroid lipoma; well-differentiated liposarcoma; myxoid/round cell liposarcoma; pleomorphic liposarcoma; myxoid malignant fibrous histiocytoma; high-grade malignant fibrous histiocytoma; myxofibrosarcoma; malignant peripheral nerve sheath tumor; mesothelioma; neuroblastoma; osteochondroma; osteosarcoma; primitive neuroectodermal tumor; alveolar rhabdomyosarcoma; embryonal rhabdomyosarcoma; benign or malignant schwannoma; synovial sarcoma; Evan's tumor; nodular fasciitis; desmoid-type fibromatosis; solitary fibrous tumor; dermatofibrosarcoma protuberans (DFSP); angiosarcoma; epithelioid hemangioendothelioma; tenosynovial giant cell tumor (TGCT); pigmented villonodular synovitis (PVNS); fibrous dysplasia; myxofibrosarcoma; fibrosarcoma; synovial sarcoma; malignant peripheral nerve sheath tumor; neurofibroma; pleomorphic adenoma of soft tissue; and neoplasias derived from fibroblasts, myofibroblasts, histiocytes, vascular cells/endothelial cells, and nerve sheath cells.

A sarcoma is a rare type of cancer that arises in cells of mesenchymal origin, e.g., in bone or in the soft tissues of the body, including cartilage, fat, muscle, blood vessels, fibrous tissue, or other connective or supportive tissue. Different types of sarcoma are based on where the cancer forms. For example, osteosarcoma forms in bone, liposarcoma forms in fat, and rhabdomyosarcoma forms in muscle. Examples of sarcomas include, but are not limited to, askin's tumor; sarcoma botryoides; chondrosarcoma; ewing's sarcoma; malignant hemangioendothelioma; malignant schwannoma; osteosarcoma; and soft tissue sarcomas (e.g., alveolar soft part sarcoma; angiosarcoma; cystosarcoma phyllodesdermatofibrosarcoma protuberans (DFSP); desmoid tumor; desmoplastic small round cell tumor; epithelioid sarcoma; extraskeletal chondrosarcoma; extraskeletal osteosarcoma; fibrosarcoma; gastrointestinal stromal tumor (GIST); hemangiopericytoma; hemangiosarcoma (more commonly referred to as "angiosarcoma"); kaposi's sarcoma; leiomyosarcoma; liposarcoma; lymphangiosarcoma;

malignant peripheral nerve sheath tumor (MPNST); neurofibrosarcoma; synovial sarcoma; and undifferentiated pleomorphic sarcoma).

A teratoma is a type of germ cell tumor that may contain several different types of tissue (e.g., can include tissues derived from any and/or all of the three germ layers: endoderm, mesoderm, and ectoderm), including, for example, hair, muscle, and bone. Teratomas occur most often in the ovaries in women, the testicles in men, and the tailbone in children.

Melanoma is a form of cancer that begins in melanocytes (cells that make the pigment melanin). Melanoma may begin in a mole (skin melanoma), but can also begin in other pigmented tissues, such as in the eye or in the intestines.

Merkel cell carcinoma is a rare type of skin cancer that usually appears as a flesh-colored or bluish-red nodule on the face, head or neck. Merkel cell carcinoma is also called neuroendocrine carcinoma of the skin. In some embodiments, methods for treating Merkel cell carcinoma include administering an immunoconjugate containing an antibody construct that is capable of binding PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars thereof, or biobetters thereof). In some embodiments, the Merkel cell carcinoma has metastasized when administration occurs.

Leukemias are cancers that start in blood-forming tissue, such as the bone marrow, and cause large numbers of abnormal blood cells to be produced and enter the bloodstream. For example, leukemias can originate in bone marrow-derived cells that normally mature in the bloodstream. Leukemias are named for how quickly the disease develops and progresses (e.g., acute versus chronic) and for the type of white blood cell that is affected (e.g., myeloid versus lymphoid). Myeloid leukemias are also called myelogenous or myeloblastic leukemias. Lymphoid leukemias are also called lymphoblastic or lymphocytic leukemia. Lymphoid leukemia cells may collect in the lymph nodes, which can become swollen. Examples of leukemias include, but are not limited to, Acute myeloid leukemia (AML), Acute lymphoblastic leukemia (ALL), Chronic myeloid leukemia (CML), and Chronic lymphocytic leukemia (CLL).

Lymphomas are cancers that begin in cells of the immune system. For example, lymphomas can originate in bone marrow-derived cells that normally mature in the lymphatic system. There are two basic categories of lymphomas. One category of lymphoma is Hodgkin lymphoma (HL), which is marked by the presence of a type of cell called the Reed-Sternberg cell. There are currently 6 recognized types of HL. Examples of Hodgkin lymphomas include nodular sclerosis classical Hodgkin lymphoma (CHL), mixed cellularity CHL, lymphocyte-depletion CHL, lymphocyte-rich CHL, and nodular lymphocyte predominant HL.

The other category of lymphoma is non-Hodgkin lymphomas (NHL), which includes a large, diverse group of cancers of immune system cells. Non-Hodgkin lymphomas can be further divided into cancers that have an indolent (slow-growing) course and those that have an aggressive (fast-growing) course. There are currently 61 recognized types of NHL. Examples of non-Hodgkin lymphomas include, but are not limited to, AIDS-related Lymphomas, anaplastic large-cell lymphoma, angioimmunoblastic lymphoma, blastic NK-cell lymphoma, Burkitt's lymphoma, Burkitt-like lymphoma (small non-cleaved cell lymphoma), chronic lymphocytic leukemia/small lymphocytic lymphoma, cutaneous T-Cell lymphoma, diffuse large B-Cell lymphoma, enteropathy-type T-Cell lymphoma, follicular lymphoma, hepatosplenic gamma-delta T-Cell lymphomas, T-Cell leukemias, lymphoblastic lymphoma, mantle cell lymphoma, marginal zone lymphoma, nasal T-Cell lymphoma, pediatric lymphoma, peripheral T-Cell lymphomas, primary central nervous system lymphoma, transformed lymphomas, treatment-related T-Cell lymphomas, and Waldenstrom's macroglobulinemia.

Brain cancers include any cancer of the brain tissues. Examples of brain cancers include, but are not limited to, gliomas (e.g., glioblastomas, astrocytomas, oligodendrogliomas, ependymomas, and the like), meningiomas, pituitary adenomas, and vestibular schwannomas, primitive neuroectodermal tumors (medulloblastomas).

Immunoconjugates of the invention can be used either alone or in combination with other agents in a therapy. For instance, an immunoconjugate may be co-administered with at least one additional therapeutic agent, such as a chemotherapeutic agent. Such combination therapies encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the immunoconjugate can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Immunoconjugates can also be used in combination with radiation therapy.

The immunoconjugates of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof are known to be useful in the treatment of cancer, particularly breast cancer, especially triple negative (test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer, bladder cancer, and Merkel cell carcinoma. The immunoconjugate described herein can be used to treat the same types of cancers as atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof, particularly breast cancer, especially triple negative (test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer, bladder cancer, and Merkel cell carcinoma.

The immunoconjugate is administered to a subject in need thereof in any therapeutically effective amount using any suitable dosing regimen, such as the dosing regimens utilized for atezolizumab, durvalumab, avelumab, biosimilars thereof, and biobetters thereof. For example, the methods can include administering the immunoconjugate to provide a dose of from about 100 ng/kg to about 50 mg/kg to the subject. The immunoconjugate dose can range from about 5 mg/kg to about 50 mg/kg, from about 10 μg/kg to about 5 mg/kg, or from about 100 μg/kg to about 1 mg/kg. The immunoconjugate dose can be about 100, 200, 300, 400, or 500 μg/kg. The immunoconjugate dose can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. The immunoconjugate dose can also be outside of these ranges, depending on the particular conjugate as well as the type and severity of the cancer being treated. Frequency of administration can range from a single dose to multiple doses per week, or more frequently. In some embodiments, the immunoconjugate is administered from about once per month to about five times per week. In some embodiments, the immunoconjugate is administered once per week.

In another aspect, the invention provides a method for preventing cancer. The method comprises administering a therapeutically effective amount of an immunoconjugate (e.g., as a composition as described above) to a subject. In certain embodiments, the subject is susceptible to a certain cancer to be prevented. For example, the methods can include administering the immunoconjugate to provide a dose of from about 100 ng/kg to about 50 mg/kg to the subject. The immunoconjugate dose can range from about 5 mg/kg to about 50 mg/kg, from about 10 μg/kg to about 5 mg/kg, or from about 100 μg/kg to about 1 mg/kg. The immunoconjugate dose can be about 100, 200, 300, 400, or 500 μg/kg. The immunoconjugate dose can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg. The immunoconjugate dose can also be outside of these ranges, depending on the particular conjugate as well as the type and severity of the cancer being treated. Frequency of administration can range from a single dose to multiple doses per week, or more frequently. In some embodiments, the immunoconjugate is administered from about once per month to about five times per week. In some embodiments, the immunoconjugate is administered once per week.

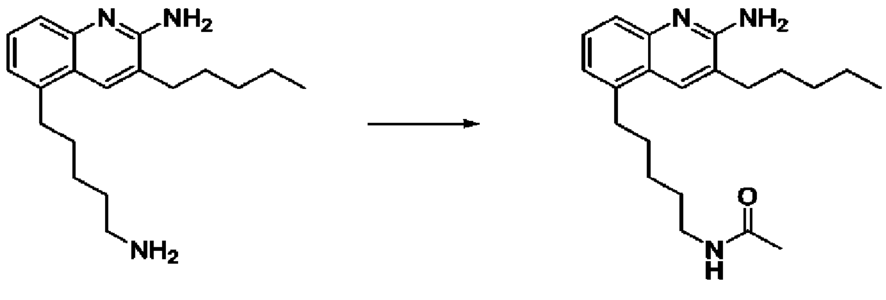
Some embodiments of the invention provide methods for treating cancer as described above, wherein the cancer is breast cancer. Breast cancer can originate from different areas in the breast, and a number of different types of breast cancer have been characterized. For example, the immunoconjugates of the invention can be used for treating ductal carcinoma *in* *situ;* invasive ductal carcinoma (*e.g.*, tubular carcinoma; medullary carcinoma; mucinous carcinoma; papillary carcinoma; or cribriform carcinoma of the breast); lobular carcinoma *in situ*; invasive lobular carcinoma; inflammatory breast cancer; and other forms of breast cancer such as triple negative (test negative for estrogen receptors, progesterone receptors, and excess HER2 protein) breast cancer. In some embodiments, methods for treating breast cancer include administering an immunoconjugate containing an antibody construct that is capable of binding HER2 (e.g. trastuzumab, pertuzumab, biosimilars, or biobetters thereof) and PD-L1 (e.g., atezolizumab, durvalumab, avelumab, biosimilars, or biobetters thereof). In some embodiments, methods for treating colon cancer lung cancer, renal cancer, pancreatic cancer, gastric cancer, and esophageal cancer include administering an immunoconjugate containing an antibody construct that is capable of binding CEA, or tumors over-expressing CEA (e.g. labetuzumab, biosimilars, or biobetters thereof).

In some embodiments, the cancer is susceptible to a pro-inflammatory response induced by agonism of the TLR7 and/or TLR8 receptors.

EXAMPLES

Preparation of Aminoquinoline compounds (AQ)

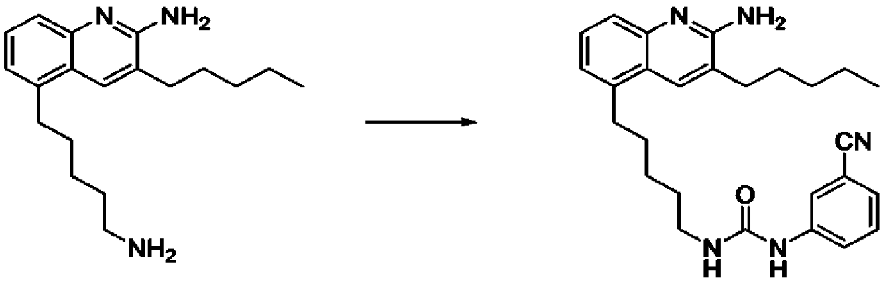
Example 1 Preparation of N-(5-(2-amino-3-pentylquinolin-5-yl)pentyl)acetamide, AQ-1



A281 A393

A vial was charged with 5-(5-aminopentyl)-3-pentylquinolin-2-amine (24.3 mg, 0.07 mmol), diisopropylethylamine (37 µL (microliters), 0.21 mmol), acetic anhydride (6.7 µL, 0.07 mmol) and 0.5 mL dimethylformamide (DMF). The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic (TFA) acid. The purified fractions were combined and lyophilized to afford 28.6 mg of AQ-1. LC/MS [M+H] 342.25 (calculated); LC/MS [M+H] 342.38 (observed).

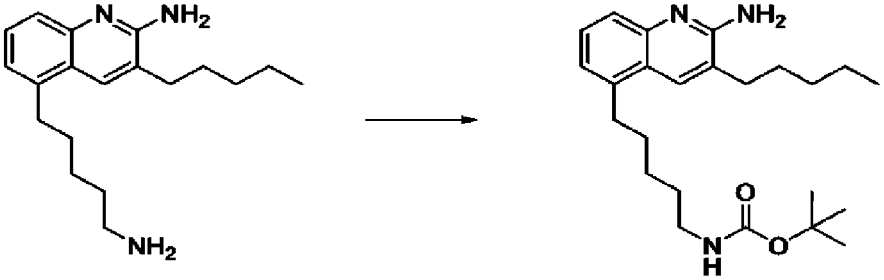
Example 2 Preparation of 1-(5-(2-amino-3-pentylquinolin-5-yl)pentyl)-3-(3-cyanophenyl)urea, AQ-2



A281 **A394**

A vial was charged with 5-(5-aminopentyl)-3-pentylquinolin-2-amine (24.3 mg, 0.07 mmol), diisopropylethylamine (37 mL, 0.21 mmol), 3-cyanophenyl isocyanate (10.1 mg, 0.07 mmol) and 1 mL DMF. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 19.9 mg of AQ-2. LC/MS [M+H] 444.28 (calculated); LC/MS [M+H] 444.84 (observed).

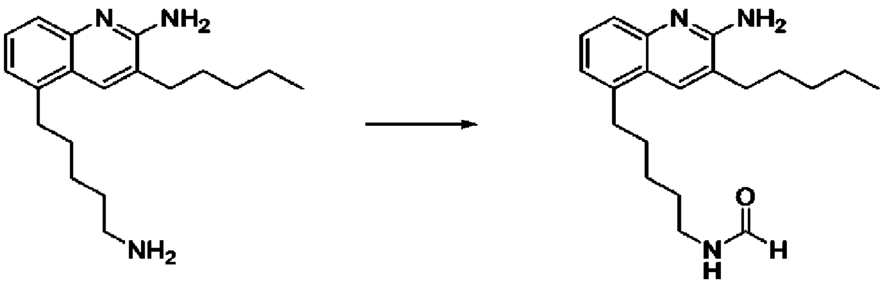
Example 3 Preparation of tert-butyl (5-(2-amino-3-pentylquinolin-5-yl)pentyl)carbamate, AQ-3



A395

A vial was charged with 5-(5-aminopentyl)-3-pentylquinolin-2-amine (22 mg, 0.064 mmol), diisopropylethylamine (37 µL, 0.21 mmol), di-tert-butyl carbonate (15 µL, 0.07 mmol) and 0.7 mL DCM. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 16.6 mg of AQ-3. LC/MS [M+H] 400.30 (calculated); LC/MS [M+H] 400.79 (observed).

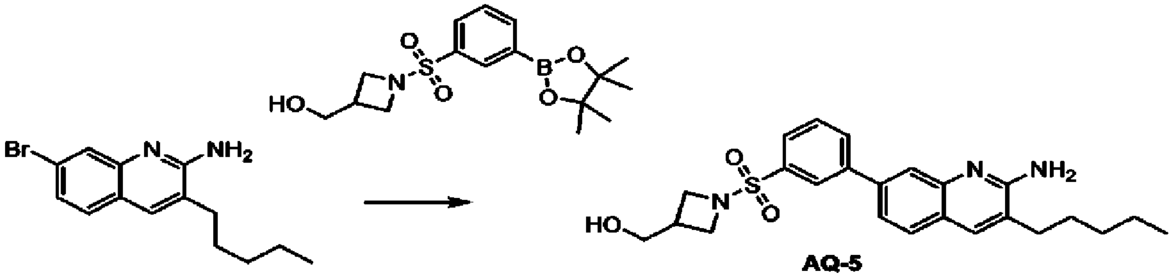
Example 4 Preparation of N-(5-(2-amino-3-pentylquinolin-5-yl)pentyl)formamide AQ-4



A396

A vial was charged with 5-(5-aminopentyl)-3-pentylquinolin-2-amine (22 mg, 0.064 mmol), diisopropylethylamine (37 µL, 0.21 mmol), di-tert-butyl carbonate (15 µL, 0.07 mmol) and 0.7 mL DCM. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 6.4 mg of AQ-4. LC/MS [M+H] 328.24 (calculated); LC/MS [M+H] 328.36 (observed).

Example 5 Preparation of (1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methanol, AQ-5

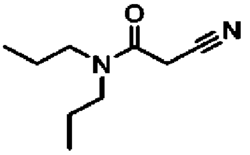


AQ-5

To a mixture of 7-bromo-3-pentyl-quinolin-2-amine (0.1 g, 341.06 µmol, 1 eq) and [1-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]sulfonylazetidin-3-yl]methanol (144.57 mg, 409.27 µmol (micromoles), 1.2 eq) in dioxane (3 mL) and H_2O (0.5 mL) were added $Pd(dppf)Cl_2$ (24.96 mg, 34.11 µmol, 0.1 eq) and K_2CO_3 (94.27 mg, 682.12 µmol, 2 eq) at 25 °C under N_2. The mixture was stirred at 90 °C for 1 hour. LCMS showed the reaction was completed and major as desired. The mixture was concentrated in vacuum. The residue was purified by prep-HPLC (column: Welch Xtimate C18 150*25mm*5um;mobile phase: [water(10mM NH_4HCO_3)-ACN];B%: 40%-60%,10.5min) to afford AQ-5 (98 mg, 222.95 µmol, 65.37% yield) as off-white solid. ^1H NMR (DMSO-d_6, 400 MHz) δ 8.14 (d, J = 2.8 Hz, 1H), 8.01 (s, 1H), 7.72-7.86 (m, 5H), 7.49-7.58 (m, 1H), 6.36 (s, 2H), 4.66 (t, J = 5.2 Hz, 1H), 3.78 (t, J = 8.2 Hz, 2H), 3.46-3.57 (m, 2H), 3.21 (t, J = 5.6 Hz, 2H), 2.58 (br t, J = 7.6 Hz, 2H), 2.44-2.48 (m, 1H), 1.60-1.66 (m, 2H), 1.33-1.39 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H). LC/MS [M+H] 439.19 (calcd), LC/MS [M+H] 440.2 (observed).

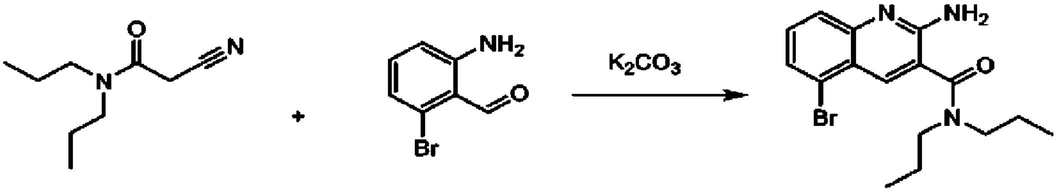
Example 6 Synthesis of 2-amino-N,N-dipropylquinoline-3-carboxamide (1.7 mg, 0.0062 mmol, 4.3%). 2-amino-N,N-dipropylquinoline-3-carboxamide, AQ-6

Preparation of 2-cyano-N,N-dipropylacetamide



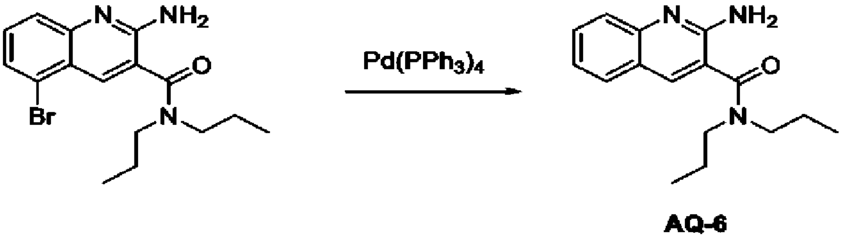
Dipropylamine (1.84 ml, 13.43 mmol, 2.5 eq.) and 2-cyanoacetic acid (0.46 g, 5.37 mmol, 1 eq.) were combined in a mixture of 4 ml DCM and 2 ml DMF. HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (3.06 mg, 8.06 mmol, 1.5 eq.) was added and the reaction stirred at room temperature. Upon completion, the reaction mixture was concentrated and purified by preparatory HPLC to give 2-cyano-N,N-dipropylacetamide as a light brown oil (0.80 g, 4.76 mmol, 89% yield). LC/MS [M+H] 169.13 (calculated); LC/MS [M+H] 169.09 (observed).

Preparation of 2-amino-5-bromo-N,N-dipropylquinoline-3-carboxamide



2-Cyano-N,N-dipropylacetamide (0.10 g, 0.59 mmol, 1 eq.), 2-amino-6-bromobenzaldehyde (0.18 g, 0.89 mmol, 1.5 eq.), and potassium carbonate (0.41 g, 2.97 mmol, 5 eq.) were stirred in 1.5 ml dimethylsulfoxide (DMSO) overnight. The reaction was diluted with acetonitrile, filtered, and then purified by HPLC to give 2-amino-5-bromo-N,N-dipropylquinoline-3-carboxamide (0.051 g, 0.14 mmol, 24%). LC/MS [M+H] 350.09/352.08 (calculated); LC/MS [M+H] 350.23/352.18 (observed).

Preparation of AQ-6

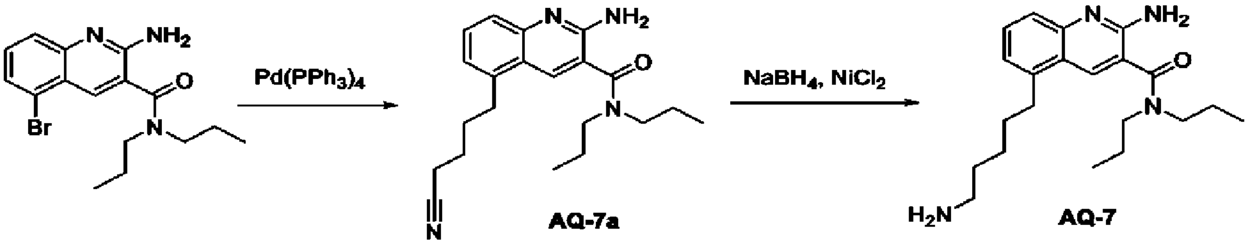


AQ-6

2-Amino-5-bromo-N,N-dipropylquinoline-3-carboxamide (0.051 g, 0.14 mmol, 1 eq.) and Pd(PPh$_3$)$_4$ (8.7 mg, 0.0075 mmol, 0.05 eq.) were dissolved in a solution of cyanobutylzinc bromide in THF (2.88 ml, 0.5 M, 10 eq.). The reaction was heated to 70 °C and monitored by

LCMS. Upon consumption of starting material, the reaction was concentrated and purified by HPLC affording AQ-6: LC/MS [M+H] 272.18 (calculated); LC/MS [M+H] 272.24 (observed).

Example 7 Synthesis of 2-amino-5-(5-aminopentyl)-N,N-dipropylquinoline-3-carboxamide, AQ-7



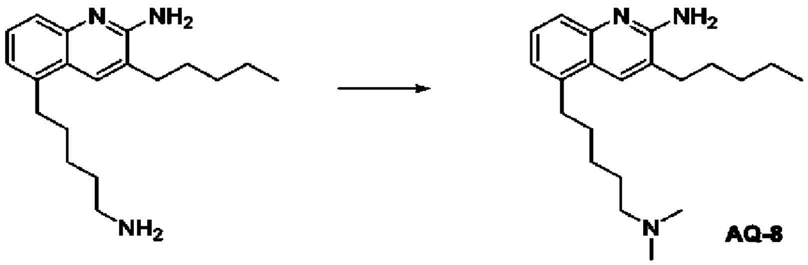
Preparation of 2-Amino-5-(4-cyanobutyl)-N,N-dipropylquinoline-3-carboxamide, AQ-7a

2-Amino-5-bromo-N,N-dipropylquinoline-3-carboxamide (0.051 g, 0.14 mmol, 1 eq.) and $Pd(PPh_3)_4$ (8.7 mg, 0.0075 mmol, 0.05 eq.) were dissolved in a solution of cyanobutylzinc bromide in THF (2.88 ml, 0.5 M, 10 eq.). The reaction was heated to 70 °C and monitored by LCMS. Upon consumption of starting material, the reaction was concentrated and purified by HPLC. AQ-7a was isolated as the primary product (21.4 mg, 0.061 mmol, 42%). LC/MS [M+H] 353.23 (calculated); LC/MS [M+H] 353.23 (observed).

Preparation of 2-amino-5-(5-aminopentyl)-N,N-dipropylquinoline-3-carboxamide, AQ-7

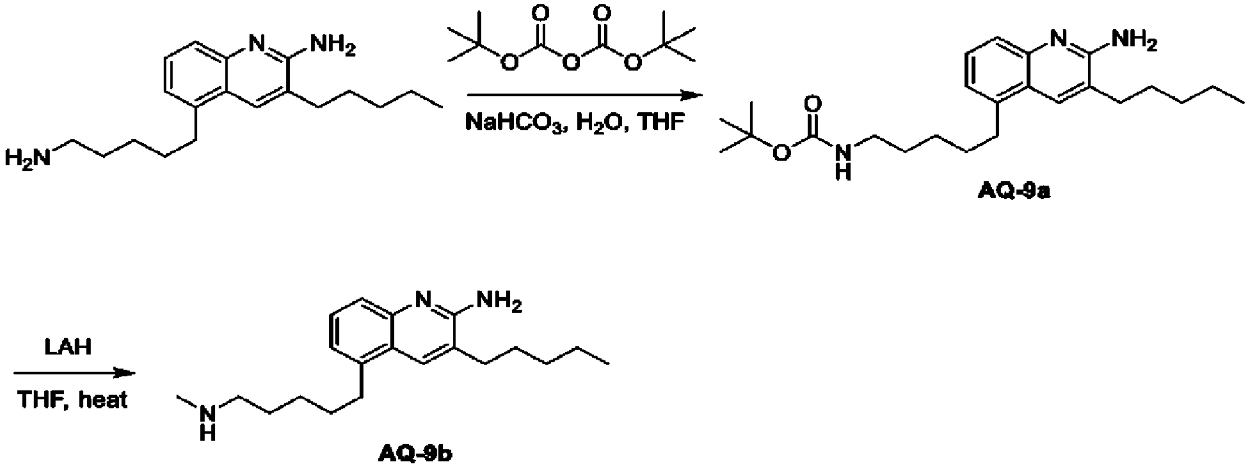
2-Amino-5-(4-cyanobutyl)-N,N-dipropylquinoline-3-carboxamide (14.4 mg, 0.06 mmol, 1 eq.) was dissolved in 2 ml methanol. Nickel chloride hexahydrate (21.4 mg, 0.06 mmol, 1 eq.) was added, followed by sodium borohydride (11.5 mg, 0.30 mmol, 5 eq.). Immediate formation of black precipitate was observed, and the exothermic reaction was stirred at ambient temperature. Upon consumption of starting material by LCMS, the reaction was filtered, concentrated, and purified by HPLC to give AQ-7 (4.5 mg, 0.013 mmol, 21%). LC/MS [M+H] 357.26 (calculated); LC/MS [M+H] 357.42 (observed).

Example 8 Preparation of 5-(5-(dimethylamino)pentyl)-3-pentylquinolin-2-amine AQ-8



To a solution of 5-(5-aminopentyl)-3-pentylquinolin-2-amine formate salt (34.5 mg, 0.1 mmol, 1 eq) in methanol (4 mL) was added 37% w/w formaldehyde in water (100 µL) then sodium cyanoborohydride (25 mg, 0.4 mmol, 4 eq.). After 20 minutes, the solvent was removed and the residue was treated with 10% sodium carbonate for 10 minutes. The crude product was purified by reverse phase HPLC to obtain AQ-8 (14.1 mg, 0.032 mmol, 32%) trifluoroacetate salt after removal of solvent. LC/MS [M+H] 328.27 (calculated); LC/MS [M+H] 328.84 (observed).

Example 9 Preparation of A27-(2-amino-3-pentylquinolin-5-yl)-22-methyl-4,7,10,13,16,19-hexaoxa-22-azaheptacosanoic acid hydrochloride, AQ-9

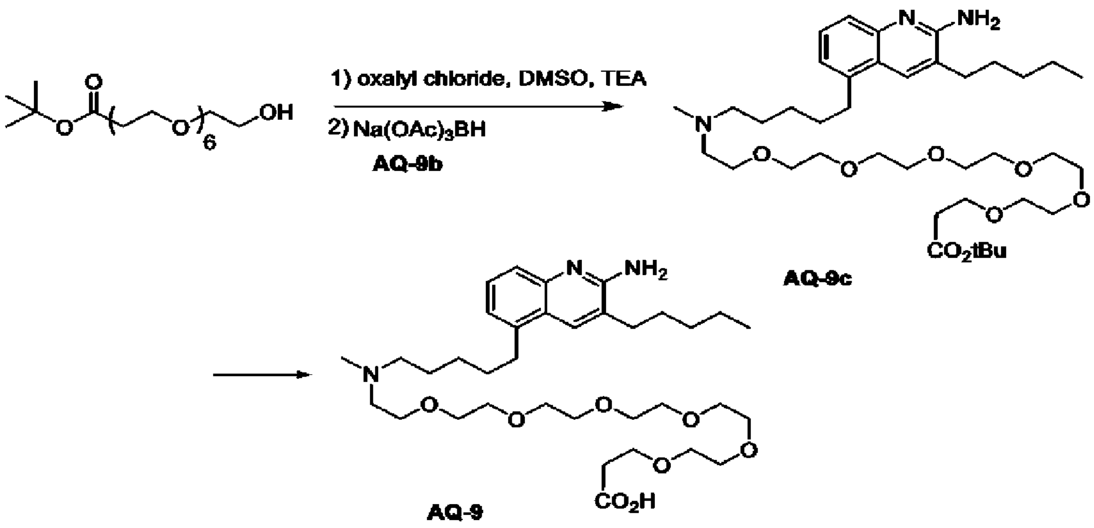


Preparation of AQ-9b

To a solution of 5-(5-aminopentyl)-3-pentylquinolin-2-amine formate salt (172 mg, 0.5 mmol, 1 eq) in THF (2 mL) and water (2 mL) was added a solution of sodium bicarbonate (63 mg, 0.75 mmol. 1.5 eq.) in water (1 mL). A solution of di-tert-butyl dicarbonate (131 mg, 0.6 mmol, 1.2 eq) dissolved in THF (1 mL) was added dropwise. The mixture was allowed to stir at room temperature for 45 minutes then portioned between ethyl acetate (25 mL) and water (25 mL). The organic layer was washed with brine (25 mL), then dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (ethyl acetate/hexanes) to obtain tert-butyl (5-(2-amino-3-pentylquinolin-5-yl)pentyl)carbamate AQ-9a (161 mg, 0.4 mmol, 81%) as a glassy solid after removal of solvent.. LC/MS [M+H] 400.29 (calculated); LC/MS [M+H] 400.41 (observed).

To a solution of AQ-9a (161 mg, 0.4 mmol, 1 eq) in anhydrous THF (5 mL) was added solid lithium aluminum hydride (76 mg, 2 mmol, 5 eq.). The mixture was heated at reflux for 10 minutes. After cooling, solid sodium bicarbonate (0.5 g. 6 mmol, 15 eq.) was added then slowly water (100 µL) and the resulting suspension was stirred vigorously for 5 minutes. The suspension was filtered through a plug of Celite and the grey solid was washed with DCM (20 mL) to obtain 5-(5-(methylamino)pentyl)-3-pentylquinolin-2-amine AQ-9b (94 mg, 0.3 mmol, 75%) as a golden film. This material was used without further purification. LC/MS [M+H] 400.29 (calculated); LC/MS [M+H] 400.41 (observed).

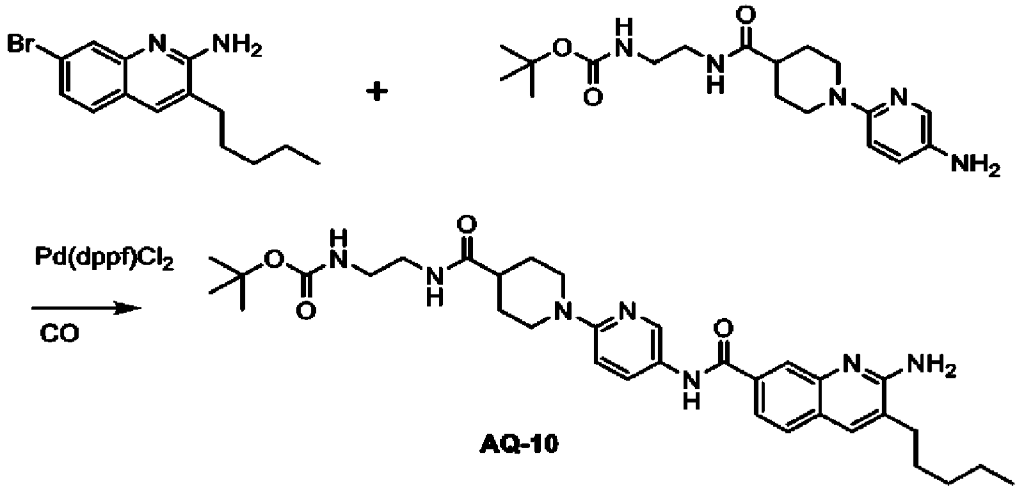
Preparation of AQ-9.



To a solution of oxalyl chloride (127 mg, 1 mmol, 3.3 eq) in DCM (3 mL) at -78 °C was added dropwise DMSO (142 µL, 2 mmol, 6.6 eq). After 15 minutes of stirring at -78 °C a solution of hydroxyl-PEG6-t-butyl ester (123 mg, 0.3 mmol, 1 eq) in DCM was added. After an additional 15 minutes, triethylamine (420 µL, 3 mmol, 10 eq) was added. The mixture was stirred at -78 °C for 15 minutes then warmed to room temperature for 30 minutes. This suspension was added to a mixture of 5-(5-(methylamino)pentyl)-3-pentylquinolin-2-amine AQ-9b obtained from the previous step and Na(OAc)$_3$BH (212 mg, 1 mmol, 3.3 eq) in DMF (2 mL) and the mixture was heated gently with a heat gun then stirred for 30 minutes. The solvent was removed under reduced pressure and the reside was stirred with 10% sodium carbonate for 10 minutes. The crude product was purified by reverse phase HPLC (acetonitrile/water) to obtain tert-butyl 27-(2-amino-3-pentylquinolin-5-yl)-22-methyl-4,7,10,13,16,19-hexaoxa-22-azaheptacosanoate trifluoroacetate salt, AQ-9c (103 mg, 0.013 mmol, 43%) after removal of solvent. LC/MS [M+H] 706.49 (calculated); LC/MS [M+H] 706.72 (observed).

To AQ-9c (103 mg, 0.015 mmol, 1 eq.) in dioxane (2 mL) was added 3M HCl (2 mL) and the solution was heated to reflux for 30 minutes. The solvent was removed and the product, 27-(2-amino-3-pentylquinolin-5-yl)-22-methyl-4,7,10,13,16,19-hexaoxa-22-azaheptacosanoic acid hydrochloride AQ-9, was dried by evaporation with acetonitrile (4x5 mL) and used without further purification. LC/MS [M+H] 650.43 (calculated); LC/MS [M+H] 650.62 (observed).

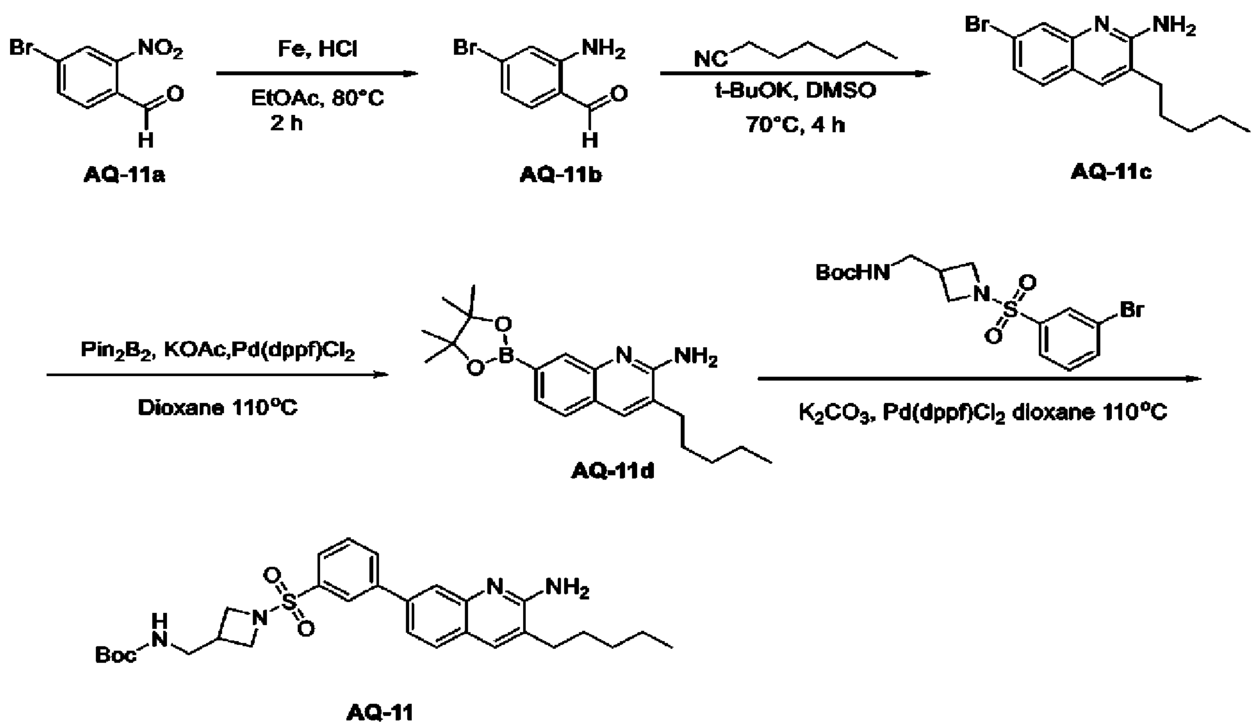
Example 10 Preparation of tert-butyl N-[2-[[1-[5-[(2-amino-3-pentyl-quinoline-7-carbonyl)amino]-2-pyridyl]piperidine-4-carbonyl]amino]ethyl]carbamate AQ-10



AQ-10

To a solution of tert-butyl N-[2-[[1-(5-amino-2-pyridyl)piperidine-4-carbonyl]amino]ethyl] carbamate (619.80 mg, 1.71 mmol, 2.5 eq), 7-bromo-3-pentyl-quinolin-2-amine (200 mg, 682.12 µmol, 1 eq) and Et_3N (207.07 mg, 2.05 mmol, 284.83 µL, 3 eq) in DMF (5 mL) was added Pd(dppf)Cl_2 (49.91 mg, 68.21 µmol, 0.1 eq) under N_2. The suspension was degassed under vacuum and purged with CO several times. The mixture was stirred under CO (50 psi) at 80°C for 16 hours. The mixture was concentrated in vacuum. The residue was purified by prep-HPLC (column: Welch Xtimate C18 150*25mm*5um;mobile phase: [water(10mM NH_4HCO_3)-ACN];B%: 40%-60%,10.5min) to afford AQ-10 (70 mg, 115.94 µmol, 17.00% yield) as gray solid. ^1H NMR (MeOD 400 MHz) δ 8.41 (d, J = 2.2 Hz, 1H), 8.09 (s, 1H), 7.87-7.97 (m, 2H), 7.69-7.83 (m, 2H), 6.88 (d, J= 9.2 Hz, 1H), 4.30 (d, J = 13.2 Hz, 2H), 3.13-3.29 (m, 4H), 2.84-2.96 (m, 2H), 2.63-2.74 (m, 2H), 2.38-2.49 (m, 1H), 1.67-1.93 (m, 5H), 1.40-1.46 (m, 13H), 0.91-1.01 (m, 3H). LC/MS [M+H] 604.4 (calculated); LC/MS [M+H] 604.4 (observed).

Example 11 Preparation of tert-butylN-[[1-[3-(2-amino-3-pentyl-7-quinolyl)phenyl]sulfonylazetidin-3-yl]methyl] carbamate, AQ-11



Preparation of 2-amino-4-bromo-benzaldehyde AQ-11b

To a mixture of 4-bromo-2-nitro-benzaldehyde AQ-11a (20 g, 86.95 mmol, 1 eq) in EtOH (200 mL) was added HCl (4 M, 6.52 mL, 0.3 eq) and iron powder (14.57 g, 260.85 mmol, 3 eq) at 15°C under N_2. The mixture was stirred at 80°C for 2 h. TLC showed the reaction was finished. The mixture was concentrated and adjusted pH to 8 ~ 9 with aq.$NaHCO_3$. Then the mixture was extracted with EtOAc (60 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=5/1) to afford AQ-11b (15 g, crude) as yellow solid. ^{1}H NMR ($CDCl_3$, 400MHz) δ 9.82 (s, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.88 (dd, J = 1.6, 8.4 Hz, 1H), 6.85 (s, 1H), 6.18 (s, 2H).

Preparation of 7-bromo-3-pentyl-quinolin-2-amine AQ-11c

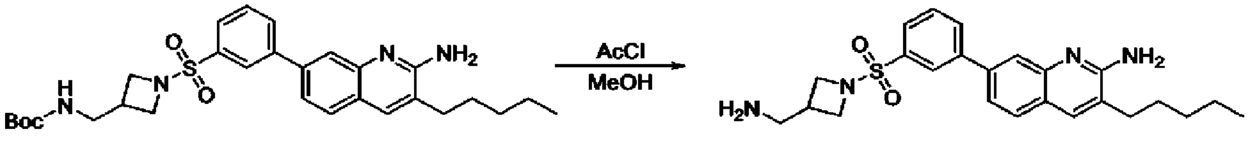
To a mixture of 2-amino-4-bromo-benzaldehyde AQ-11b (15 g, 74.99 mmol, 1 eq) and heptanenitrile (12.51 g, 112.48 mmol, 15.44 mL, 1.5 eq) in DMSO (80 mL) was added t-BuOK (16.83 g, 149.98 mmol, 2 eq) at 15°C. The mixture was stirred at 70°C for 4 h. The mixture was diluted with water and extracted with EtOAc (100 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=5/1) to afford AQ-11c (13.5 g, 46.04 mmol, 61.40% yield) as yellow solid. ^{1}H NMR (DMSO-d_6, 400MHz) δ 7.71 (s, 1H), 7.58-7.53 (m, 2H), 7.26-7.23 (m, 1H), 6.48 (s, 2H), 2.56-2.52 (m, 2H), 1.65-1.56 (m, 2H), 1.35-1.32 (m, 4H), 0.90-0.85 (m, 3H)

Preparation of 3-pentyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinolin-2-amine AQ-11d

To a mixture of 7-bromo-3-pentyl-quinolin-2-amine AQ-11c (0.2 g, 682.12 µmol, 1 eq) in dioxane (4 mL) was added Pin_2B_2 (207.86 mg, 818.55 µmol, 1.2 eq) KOAc (100.42 mg, 1.02 mmol, 1.5 eq) and $Pd(dppf)Cl_2$ (49.91 mg, 68.21 µmol, 0.1 eq) at 15°C under N_2. The mixture was stirred at 90°C for 2 h. LCMS showed the reaction was finished. The mixture was filtered and concentrated to afford AQ-11d (0.23 g, crude) as black solid.

To a mixture of 3-pentyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinolin-2-amine AQ-11d (0.23 g, 675.94 µmol, 1 eq) and tert-butyl N-[[1-(3-bromophenyl)sulfonylazetidin-3-yl]methyl] carbamate (273.96 mg, 675.94 µmol, 1 eq) in dioxane (6 mL) was added K_2CO_3 (326.97 mg, 2.37 mmol, 3.5 eq) H_2O (1 mL) and $Pd(dppf)Cl_2$ (49.46 mg, 67.59 µmol, 0.1 eq) at 15 °C under N_2. The mixture was stirred at 90 °C for 2 h. LCMS showed the reaction was finished. The mixture was diluted with water and extracted with EtOAc(30 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purification by prep-HPLC(column: Welch Xtimate C18 150*25mm*5um;mobile phase: [water (10mM NH_4HCO_3)-ACN];B%: 65%-95%,10.5min) to give AQ-11 (0.17 g, 315.57 µmol, 46.69% yield) as light yellow solid. ^1H NMR (DMSO-d_6, 399MHz) δ 8.14 (d, J = 2.8 Hz, 1H), 8.00 (s, 1H), 7.82-7.78 (m, 2H), 7.77-7.73 (m, 3H), 7.52 (dd, J = 1.6, 8.4 Hz, 1H), 6.34 (s, 2H), 3.76 (t, J = 8.4 Hz, 2H), 3.51-3.45 (m, 2H), 3.29 (s, 2H), 2.90 (t, J = 6.4 Hz, 2H), 2.67 (d, J = 2.0 Hz, 1H), 2.62-2.56 (m, 2H), 1.64 (d, J = 7.6 Hz, 2H), 1.36 (d, J = 4.0 Hz, 2H), 1.31 (s, 9H), 0.92-0.86 (m, 3H). LCMS (ESI): mass calcd. for $C_{29}H_{38}N_4O_4S$ 538.26, m/z found 539.2 $[M+H]^+$.

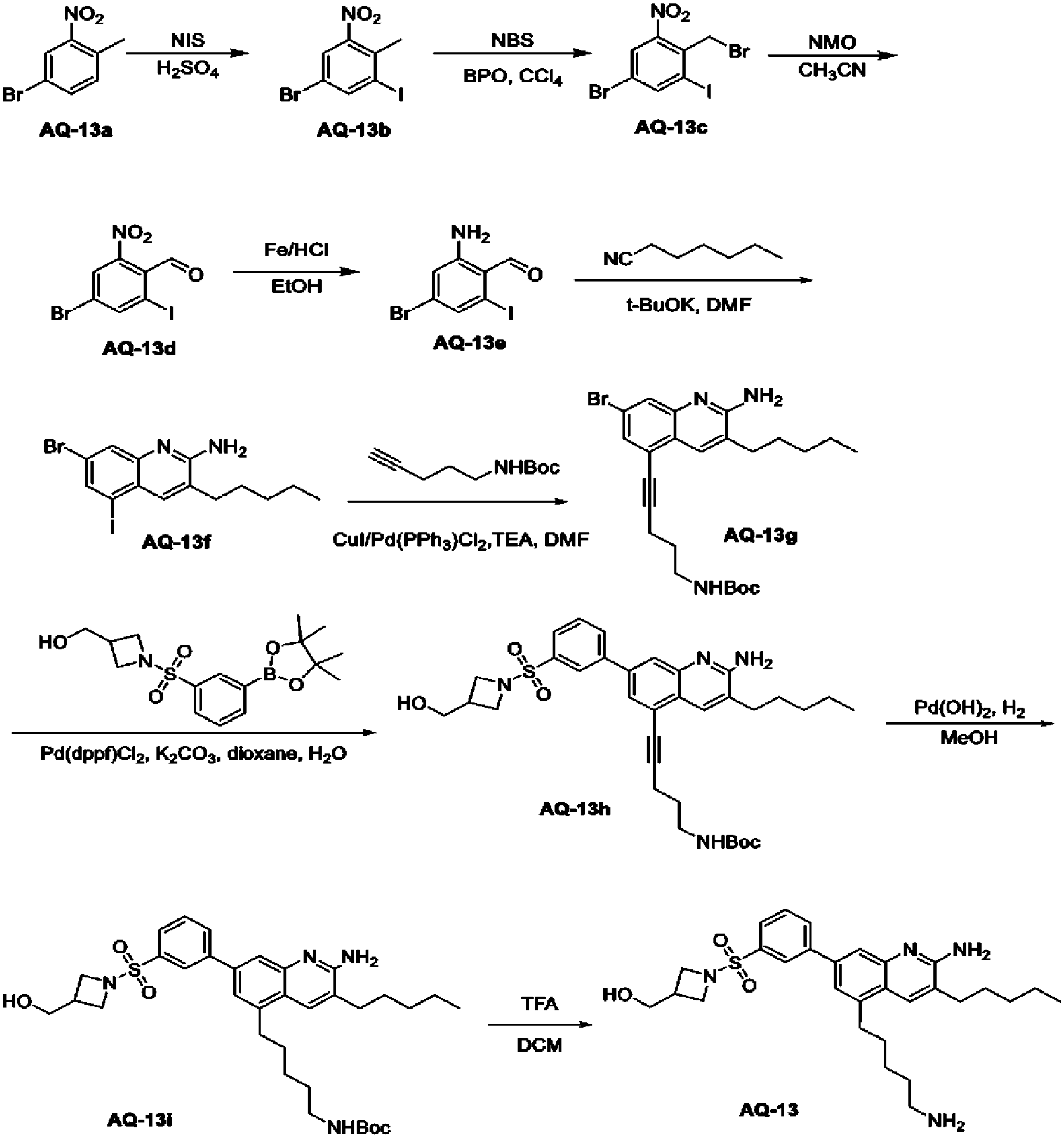
Example 12 Preparation of 7-[3-[3-(aminomethyl)azetidin-1-yl] sulfonylphenyl]-3-pentyl-quinolin-2-amine AQ-12



AQ-11 AQ-12

To a mixture of tert-butyl N-[[1-[3-(2-amino-3-pentyl-7-quinolyl)phenyl]sulfonylazetidin-3-yl] methyl]carbamate AQ-11 (0.95 g, 1.76 mmol, 1 eq) in MeOH (2 mL) was added acetyl chloride (692.15 mg, 8.82 mmol, 629.23 µL, 5 eq) at 15 °C. The mixture was stirred at 50°C for 0.5 h. LCMS showed the reaction was finished. The mixture was diluted with aq.$NaHCO_3$ and adjusted pH to 8 ~ 9. The mixture was concentrated. The residue was purified by prep-HPLC(column: Welch Xtimate C18 100*25mm*3um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-40%,12min) to give AQ-12 (0.35 g, 798.02 µmol, 45.25% yield) as white solid. ^{1}H NMR (MeOD-d$_4$, 400MHz) δ 8.26 (s, 1H), 8.18-8.11 (m, 2H), 8.01 (d, J = 8.4 Hz, 1H), 7.98-7.92 (m, 2H), 7.88-7.82 (m, 2H), 3.97 (t, J = 8.4 Hz, 2H), 3.66 (dd, J = 5.6, 8.4 Hz, 2H), 3.06 (d, J = 7.6 Hz, 2H), 2.77-2.72 (m, 2H), 2.68-2.74 (m, 1H), 1.81-1.73 (m, 2H), 1.52-1.40 (m, 4H), 1.01-0.91 (m, 3H). LCMS (ESI): mass calcd. for $C_{24}H_{30}N_4O_2S$ 438.21, m/z found 439.2 [M+H]$^+$.

Example 13 Preparation of AQ-13



Preparation of 5-bromo-1-iodo-2-methyl-3-nitro-benzene AQ-13b

To a solution of 4-bromo-1-methyl-2-nitro-benzene AQ-13a (45 g, 208.30 mmol, 1 eq) in H_2SO_4 (300 mL) was added NIS (84.36 g, 374.94 mmol, 1.8 eq) at 0 °C under N_2. The mixture was stirred at 0 °C for 1 h. TLC indicated reactant was consumed completely and one new spot formed. The mixture was poured into ice water (2000 mL) while stirring vigorously, and extracted with EtOAc (300 mL x 3). The organic layer was washed with brine (200 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 100/1) to give AQ-13b (54 g, 157.93 mmol, 75.82% yield) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21 (d, J = 2.0 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 2.55 (s, 3H).

Preparation of 5-bromo-2-(bromomethyl)-1-iodo-3-nitro-benzene AQ-13c

To a solution of 5-bromo-1-iodo-2-methyl-3-nitro-benzene AQ-13b (53.5 g, 156.47 mmol, 1 eq) in CCl_4 (500 mL) was added NBS (41.77 g, 234.70 mmol, 1.5 eq) and benzoyl peroxide, BPO (3.79 g, 15.65 mmol, 0.1 eq) at 20 °C under N_2. The mixture was stirred at 90 °C for 24 h. TLC indicated reactant was consumed completely and two new spots formed. The mixture was filtered and concentrated. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate = 50/1, 10/1) to give AQ-13c (24 g, 57.03 mmol, 36.45% yield) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8. 29 (d, J = 1.6 Hz 1H), 8.04 (d, J = 2.0 Hz, 1H), 4.82 (s, 2H).

Preparation of 4-bromo-2-iodo-6-nitro-benzaldehyde AQ-13d

To a solution of 5-bromo-2-(bromomethyl)-1-iodo-3-nitro-benzene AQ-13c (37 g, 87.92 mmol, 1 eq) in CH_3CN (300 mL) was added N-methylmorpholine N-oxide, NMO (20.60 g, 175.85 mmol, 18.56 mL, 2 eq). The mixture was stirred at 25 °C for 12 h. TLC indicated reactant was consumed completely and one new spot formed. The mixture was diluted with water (1000 mL) and extracted with EtOAc (300 mL x 3). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~50% Ethyl acetate/Petroleum ether gradient at 65 mL/min) to give AQ-13d (25 g, 70.24 mmol, 79.89% yield) as off-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.00 (s, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.14 (d, J = 2.0 Hz, 1H).

Preparation of 2-amino-4-bromo-6-iodo-benzaldehyde AQ-13e

To a solution of 4-bromo-2-iodo-6-nitro-benzaldehyde AQ-13d (25 g, 70.24 mmol, 1 eq) in EtOH (500 mL) was added Fe (11.77 g, 210.73 mmol, 3 eq) and then a solution of HCl (12 M, 1.17 mL, 0.2 eq) in H_2O (100 mL) was added to the reaction mixture at 25 °C. The mixture was stirred at 85 °C for 1 h. TLC indicated reactant was consumed completely and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove EtOH, and then the residue was added ethylacetate (EtOAc) (100 mL) and water (100 mL). The pH was adjusted to ~ 8 by progressively adding of aq.$NaHCO_3$, the mixture was filtered and extracted with EtOAc (80 mL x 3). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel chromatography (ISCO®; 30 g SepaFlash® Silica Flash Column, Eluent of 0~70% Ethyl acetate/Petroleum ethergradient @ 100 mL/min) to give AQ-13e (20 g, 61.36 mmol, 87.36% yield) as yellow solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 10.04 (s, 1H), 7.38 (d, J = 1.6 Hz, 1H), 6.84 (d, J = 1.6 Hz, 1H), 6.50 (br, s, 2H).

Preparation of 7-bromo-5-iodo-3-pentyl-quinolin-2-amine AQ-13f

To a solution of 2-amino-4-bromo-6-iodo-benzaldehyde AQ-13e (17 g, 52.16 mmol, 1 eq) and heptanenitrile (8.70 g, 78.24 mmol, 10.74 mL, 1.5 eq) in DMF (200 mL) was added t-BuOK (11.71 g, 104.32 mmol, 2 eq) at 25 °C. The mixture was stirred at 70 °C for 2 h. TLC indicated reactant was consumed completely and one new spot formed. The mixture was diluted with water (500 mL), and extracted with EtOAc (150 mL x 3). The organic layer was washed wit brine (80 mL x 3), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~80% Ethyl acetate/Petroleum ether gradient at 100 mL/min) to give AQ-13f (6 g, 14.32 mmol, 27.45% yield) as yellow solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.89(d, J = 1.6 Hz, 1H), 7.81 (s, 1H), 7.78 (d, J = 1.6 Hz, 1H), 5.43 (br, s, 2H), 2.63-2.52 (m, 2H), 1.81-1.68 (m, 2H), 1.49-1.35 (m, 4H), 1.01-0.89 (m, 3H).

Preparation of tert-butyl (5-(2-amino-7-bromo-3-pentylquinolin-5-yl) pent-4-yn-1-yl)carbamate AQ-13g

A mixture of 7-bromo-5-iodo-3-pentyl-quinolin-2-amine AQ-13f (2 g, 4.77 mmol, 1 eq), tert-butyl N-pent-4-ynylcarbamate (961.93 mg, 5.25 mmol, 1.1 eq), $Pd(PPh_3)_2Cl_2$ (167.48 mg, 238.61 μmol, 0.05 eq), CuI (181.77 mg, 954.43 μmol, 0.2 eq) in TEA (7 mL) and DMF (20 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90 °C for 1 h under N_2 atmosphere. TLC indicated Reactant was consumed completely. The reaction mixture was quenched by addition of H_2O (100 mL) at 0 °C, and then extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (30 mL x 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0/1). Product AQ-13g (1.6 g, 3.37 mmol, 70.67% yield) was obtained as a brown solid. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.04 (s, 1H), 7.76 (s, 1H), 7.46 (d, J = 1.6 Hz, 1H), 4.97 (br s, 2H), 4.73 (br s, 1H), 3.38-3.32 (m, 2H), 2.63-2.57 (m, 4H), 1.93-1.66 (m, 6H), 1.51-1.35 (m, 11H), 0.94 (br t, J= 6.8 Hz, 3H).

Preparation of tert-butyl N-[5-[2-amino-7-[3-[3-(hydroxymethyl)azetidin-1-yl]sulfonylphenyl]-3-pentyl-5-quinolyl]pent-4-ynyl]carbamate, AQ-13h

A mixture of tert-butyl N-[5-(2-amino-7-bromo-3-pentyl-5-quinolyl)pent-4-ynyl]carbamate, AQ-13g (0.6 g, 1.26 mmol, 1 eq), [1-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]sulfonylazetidin-3-yl]methanol (893.46 mg, 2.53 mmol, 2 eq), Pd(dppf)Cl_2 (46.27 mg, 63.23 μmol, 0.05 eq), K_2CO_3 (349.58 mg, 2.53 mmol, 2 eq) in dioxane (10 mL) and H_2O (1 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90 °C for 2 h under N_2 atmosphere. LC-MS showed AQ-13g was consumed. Several new peaks were shown on LC-MS and ~22% of desired compound AQ-13h was detected. The reaction mixture was quenched by addition of H_2O (30 mL) at 0 °C, and then extracted with EtOAc (20 mL x 3). The combined organic layers were washed with brine (10 mL x 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0/1) and (SiO_2, EtOAc: MeOH = 1:0 to 5:1). Compound AQ-13h (0.31 g, 499.36 μmol, 39.49% yield) was obtained as a yellow solid. ^{1}H NMR (MeOD, 400 MHz) δ 8.16 (s, 1H), 8.13-8.03 (m, 2H), 7.89-7.85 (m, 1H), 7.83-7.78 (m, 1H), 7.76 (s, 1H), 7.64 (d, J = 1.2 Hz, 1H), 4.59 (br s, 2H), 3.88 (t, J = 8.4 Hz, 2H), 3.62 (dd, J = 6.0, 8.0 Hz, 2H), 3.43 (d, J = 6.0 Hz, 2H), 2.73-2.54 (m, 5H), 1.87 (quin, J = 7.2 Hz, 2H), 1.80-1.69 (m, 2H), 1.44 (s, 13H), 0.97 (br t, J = 6.8 Hz, 3H).

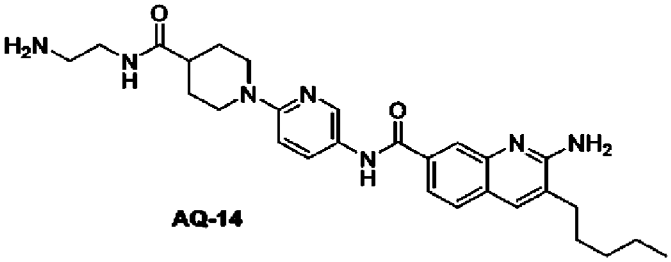
Preparation of tert-butyl N-[5-[2-amino-7-[3-[3-(hydroxymethyl)azetidin-1-yl] sulfonylphenyl]-3-pentyl-5-quinolyl]pentyl]carbamate, AQ-13i

To a solution of tert-butyl N-[5-[2-amino-7-[3-[3-(hydroxymethyl)azetidin-1-yl]sulfonylphenyl] -3-pentyl-5-quinolyl]pent-4-ynyl]carbamate, AQ-13h (310 mg, 499.36 μmol, 1 eq) in MeOH (10 mL) was added Pd$(OH)_2$/C (20%, 0.1 g) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (50 psi) at 25 °C for 12 h. LC-MS showed the reactant was consumed completely and one main peak with desired mass was detected. The mixture was filtered and concentrated to give AQ-13i (270 mg, 432.12 μmol, 86.53% yield) as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (s, 1H), 7.97 (br d, J = 7.6 Hz, 1H), 7.93 (s, 1H), 7.89-7.79 (m, 2H), 7.71-7.64 (m, 1H), 7.38 (s, 1H), 5.27-5.14 (m, 1H), 4.60-4.50 (m, 1H), 3.98-3.88 (m, 2H), 3.73-3.65 (m, 2H), 3.62-3.56 (m, 2H), 3.21-3.08 (m, 2H), 3.04 (br t, J = 7.6 Hz, 2H), 2.71-2.60 (m, 3H), 1.79-1.74 (m, 6H), 1.58-1.51 (m, 2H), 1.43 (s, 13H), 0.95 (br t, J = 7.2 Hz, 3H).

Preparation of [1-[3-[2-amino-5-(5-aminopentyl) -3-pentyl-7-quinolyl]phenyl]sulfonylazetidin-3-yl]methanol, AQ-13

To a solution of tert-butyl N-[5-[2-amino-7-[3-[3-(hydroxymethyl)azetidin-1-yl] sulfonylphenyl]-3-pentyl-5-quinolyl]pentyl]carbamate, AQ-13i (150 mg, 240.06 μmol, 1 eq) in DCM (5 mL) was added TFA (1.54 g, 13.51 mmol, 1 mL, 56.26 eq) at 20 °C. The mixture was stirred at 20 °C for 1 h. LC-MS showed AQ-13i remained. Several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with CH_3CN (10 mL) and H_2O (1 mL) and adjusted to pH = 9 with aq. LiOH at 0 °C. The mixture was stirred for 1 h at 20 °C. The mixture was filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Welch Xtimate C18 100*25mm*3um;mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%,12min). Compound AQ-13 (60 mg, 93.94 μmol, 39.13% yield, TFA) was obtained as a white solid. ^1H NMR (MeOD-d_4, 400 MHz) δ 8.36 (s, 1H), 8.14-8.09 (m, 2H), 7.94-7.90 (m, 1H), 7.86-7.80 (m, 2H), 7.68 (d, J = 1.6 Hz, 1H), 3.87 (t, J = 8.0 Hz, 2H), 3.63 (dd, J = 6.0, 8.0 Hz, 2H), 3.43 (d, J = 6.0 Hz, 2H), 3.17 (t, J = 7.6 Hz, 2H), 2.99-2.90 (m, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.64-2.53 (m, 1H), 1.87-1.69 (m, 6H), 1.62-1.52 (m, 2H), 1.50-1.41 (m, 4H), 1.00-0.93 (m, 3H). LCMS (ESI): mass calcd. for $C_{29}H_{40}N_4O_3S$ 524.28, m/z found 525.3 $[M+H]^+$.

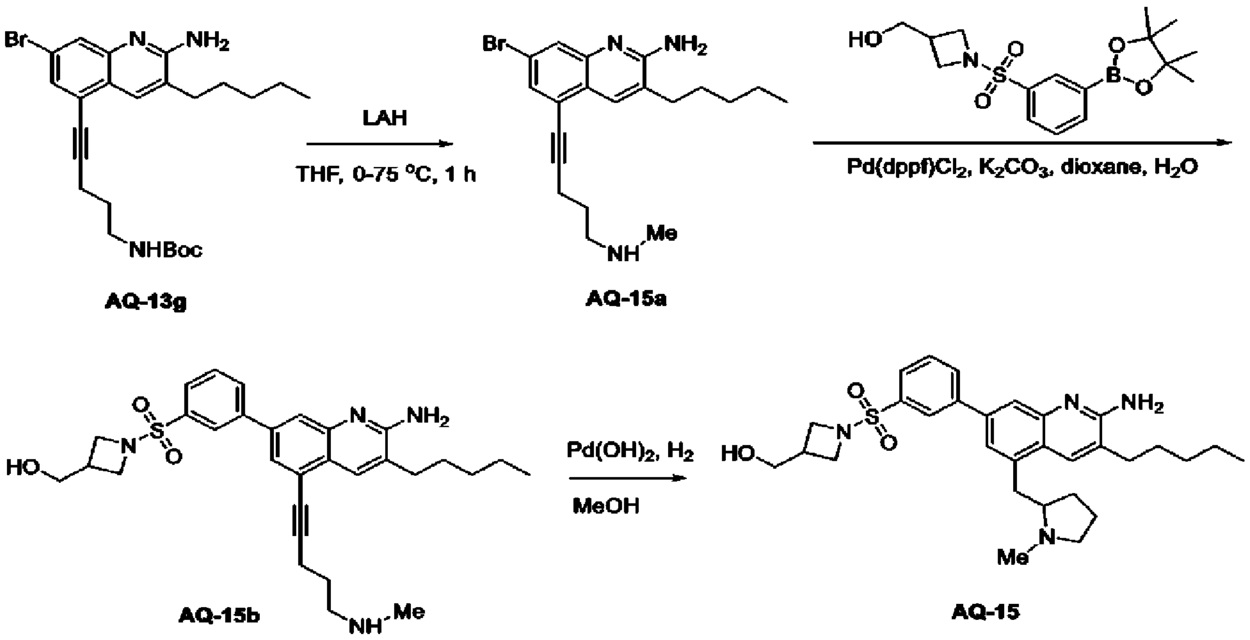
Example 14 Preparation of 2-amino-N-(6-(4-((2-aminoethyl)carbamoyl)piperidin-1-yl)pyridin-3-yl)-3-pentylquinoline-7-carboxamide, AQ-14



AQ-14

A vial was charged with tert-butyl N-[2-[[1-[5-[(2-amino-3-pentyl-quinoline-7-carbonyl)amino]-2-pyridyl]piperidine-4-carbonyl]amino]ethyl]carbamate AQ-10 (65 mg, 0.11 mmol), 0.5 mL TFA and 1 mL DCM. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 501 mg of AQ-14. LC/MS [M+H] 504.31 (calculated); LC/MS [M+H] 504.51 (observed).

Example 15 Preparation of [1-[3-[2-amino-5-[(1-methylpyrrolidin-2-yl)methyl]-3-pentyl-7-quinolyl]phenyl] sulfonyl azetidin-3-yl]methanol, AQ-15



Preparation of 7-bromo-5-[5-(methylamino)pent-1-ynyl]-3-pentyl-quinolin-2-amine AQ-15a

To a solution of tert-butyl (5-(2-amino-7-bromo-3-pentylquinolin-5-yl) pent-4-yn-1-yl)carbamate AQ-13g (0.59 g, 1.24 mmol, 1 eq) in THF (18 mL) was added LAH (141.58 mg, 3.73 mmol, 3 eq) at 0 °C. The mixture was stirred at 75 °C for 1 h. LC-MS showed Reactant was consumed. Several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was quenched by addition of H_2O (0.15 mL) at 0 °C, and then added 15% NaOH (0.15 mL) at 0°C. The reaction was stirred at 25 °C for 30 min. The mixture was filtered through celite and washed with THF (10 mL x 6), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Ethyl acetate/MeOH = 1/0 to 1/1). Compound AQ-15a (0.12 g, 309.00 µmol, 24.85% yield) was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (s, 1H), 7.50 (s, 1H), 7.18 (d, J = 1.2 Hz, 1H), 4.86-4.81 (m, 2H), 3.71 (t, J = 6.0 Hz, 2H), 3.32 (t, J = 6.8 Hz, 2H), 2.92 (s, 3H), 2.62-2.53 (m, 3H), 2.00-1.89 (m, 2H), 1.81-1.67 (m, 6H), 0.95-0.91 (m, 3H). LC/MS [M+H] 388.1/390.1 (calculated); LC/MS [M+H] 388.2/390.2 (observed).

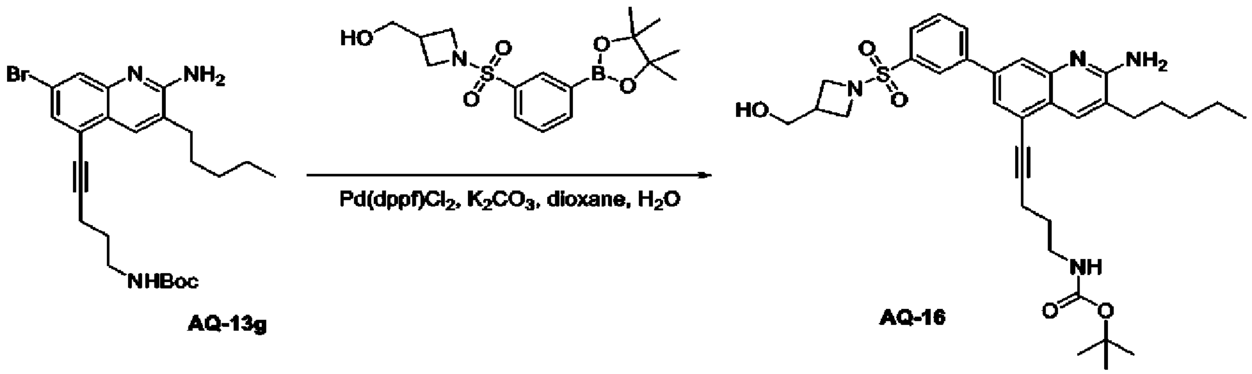
Preparation of [1-[3-[2-amino-5-[5-(methylamino)pent-1-ynyl]-3-pentyl- 7-quinolyl]phenyl]sulfonylazetidin-3-yl]methanol AQ-15b

A mixture of 7-bromo-5-[5-(methylamino)pent-1-ynyl]-3-pentyl-quinolin-2-amine, AQ-15a (120 mg, 309.00 µmol, 1 eq), [1-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl] sulfonylazetidin-3-yl]methanol (120.07 mg, 339.91 µmol, 1.1 eq), Pd(dppf)Cl_2 (22.61 mg, 30.90 µmol, 0.1 eq) and K_2CO_3 (85.42 mg, 618.01 µmol, 2 eq) in dioxane (2 mL) and H_2O (0.2 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90 °C for 2 h under N_2 atmosphere. LC-MS showed Reactant was consumed. Several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was quenched by addition of H_2O (10 mL) at 0 °C, and then extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (5 mL x 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Ethyl acetate/MeOH = 1/0 to 1/1). Compound AQ-15b (70 mg, 130.91 μmol, 42.37% yield) was obtained as a yellow oil. LC/MS [M+H] 535.3 (calculated); LC/MS [M+H] 535.1 (observed).

Preparation of AQ-15

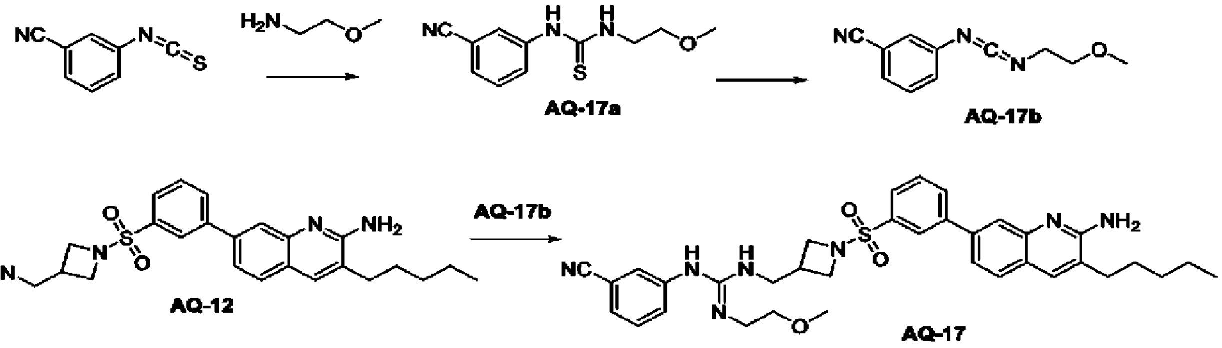
To a solution of [1-[3-[2-amino-5-[5-(methylamino)pent-1-ynyl]-3-pentyl-7-quinolyl] phenyl]sulfonylazetidin-3-yl]methanol, AQ-15b (70 mg, 130.91 μmol, 1 eq) in MeOH (3 mL) was added $Pd(OH)_2/C$ (20%, 20 mg) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (50 psi) at 25 °C for 12 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by prep-HPLC (neutral condition; column: Welch Xtimate C18 150*25mm*5um;mobile phase: [water(10mM NH_4HCO_3)-ACN];B%: 48%-68%,10.5min). AQ-15 (10 mg, 18.63 μmol, 14.23% yield) was obtained as a white solid. ^1H NMR (MeOD-d_4, 400 MHz) δ 8.15-8.05 (m, 2H), 8.01 (s, 1H), 7.90-7.84 (m, 1H), 7.83-7.75 (m, 1H), 7.73 (s, 1H), 7.48 (s, 1H), 3.88 (t, J = 8.4 Hz, 2H), 3.67-3.56 (m, 3H), 3.43 (d, J = 6.4 Hz, 2H), 3.23-3.15 (m, 1H), 2.97-2.88 (m, 1H), 2.71 (br t, J = 7.2 Hz, 3H), 2.62-2.49 (m, 4H), 2.43-2.32 (m, 1H), 1.90-1.62 (m, 5H), 1.51-1.38 (m, 4H), 0.96 (br t, J = 7.2 Hz, 3H). LC/MS [M+H] 537.3 (calculated); LC/MS [M+H] 537.3 (observed).

Example 16 Preparation of tert-butyl N- [5- [2-amino-7- [3-[3-(hydroxymethyl)azetidin-1-yl]sulfonylphenyl]-3-pentyl-5-quinolyl]pent-4-ynyl]carbamate, AQ-16



To a mixture of [1-[3- (4,4,5,5- tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl] sulfonylazetidin-3-yl] methanol (44.67 mg, 126.47 μmol, 1.2 eq) and tert-butyl (5-(2-amino-7-bromo-3-pentylquinolin-5-yl) pent-4-yn-1-yl)carbamate, AQ-13g (0.05 g, 105.39 μmol, 1 eq) in dioxane (5 mL) and H_2O (1 mL) was added Pd(dppf)Cl_2 (2.31 mg, 3.16 µmol, 0.03 eq) and K_2CO_3 (29.13 mg, 210.78 µmol, 2 eq) at 25°C. The mixture was stirred at 90 °C for 12 hours. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by prep-HPLC (column: Welch Xtimate C18 150 x 25mm x 5um; mobile phase: [water (10mM NH_4HCO_3)-ACN]; B%: 58%-88%, 10.5 min). AQ-16 (0.024 g, 37.20 µmol, 35.30% yield, 96.227% purity) was obtained as a white solid. ^1H NMR (MeOD, 400 MHz) δ 8.19 (s, 1H), 8.14-8.08 (m, 2H), 7.88 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.79 (s, 1H), 7.67 (s, 1H), 3.90 (t, J = 8.0 Hz, 2H), 3.64 (t, J = 6.0 Hz, 2H), 3.44 (d, J = 6.4 Hz, 1H), 3.30-3.38 (m, 2H), 2.72-2.62 (m, 5H), 1.94-1.84 (m, 2H), 1.83-1.71 (m, 2H), 1.55-1.31 (m, 13H), 0.99 (t, J = 6.8 Hz, 3H). LC/MS [M+H] 621.3 (calculated); LC/MS [M+H] 621.3 (observed).

Example 17 Preparation of 1-((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)-3-(3-cyanophenyl)-2-(2-methoxyethyl)guanidine, AQ-17



Preparation of 1-(3-cyanophenyl)-3-(2-methoxyethyl)thiourea, AQ-17a

A 20mL vial was charged with 2-methoxyethan-1-amine (476 mg, 2.98 mmol), 3-isothiocyanatobenzonitrile (256 µL, 2.98 mmol) and 10 mL DCM. The reaction was maintained for 4 hours, concentrated then purified by silica gel flash chromatography using a gradient of 2-10% MeOH:DCM. The fractions containing product were pooled and concentrated to afford 752 mg of AQ-17a. LC/MS [M+H] 236.09 (calculated); LC/MS [M+H] 236.13 (observed).

Preparation of 3-((((2-methoxyethyl)imino)methylene)amino)benzonitrile, AQ-17b

A vial was charged with 59 mg of AQ-17a (0.25 mmol), triethylamine (104µL, 0.75 mmol) and 2 mL DCM. To this vial was added 2-chloro-1-methylpyridinium iodide (77 mg, 0.30 mmol). The reaction was maintained for three hours. The crude reaction was concentrated under reduced pressure and purified by silica gel flash chromatography using a 25-75% gradient of Ethyl Acetate:Hexanes affording 46 mg of the desired carbodiimide, AQ-17b. LC/MS [M+H] 202.10 (calculated); LC/MS [M+H] 202.19 (observed).

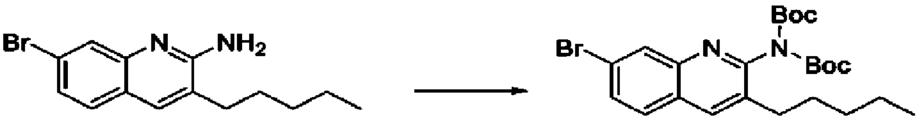
Preparation of (E)-1-((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)-3-(3-cyanophenyl)-2-(2-methoxyethyl)guanidine, AQ-17

A vial was charged with AQ-12 (9.7 mg, 0.018 mmol), AQ-17b (3.5mg, 0.018 mmol), triethylamine (7.3 µL (microliters), 0.054 mmol) and 200 µL DMF. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 501 mg of AQ-17. LC/MS [M+H] 640.31 (calculated); LC/MS [M+H] 640.55 (observed).

Example 18 Preparation of 1-(5-(2-amino-3-pentylquinolin-5-yl)pentyl)-3-(3-cyanophenyl)-2-(2-methoxyethyl)guanidine, AQ-18

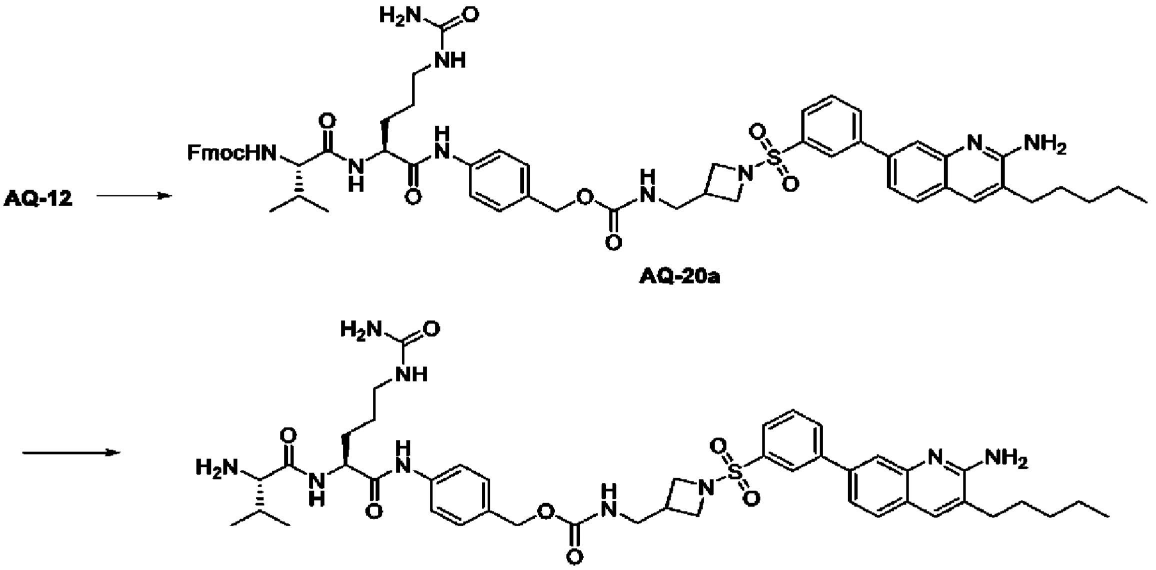
AQ-18 was synthesized using the method described in Example 18 for AQ-17. LC/MS [M+H] 501.33 (calculated); LC/MS [M+H] 501.52 (observed).

Example 19 Preparation of tert-butyl N-(7-bromo-3-pentyl-2-quinolyl)-N-tert-butoxycarbonyl-carbamate, AQ-19



To a mixture of 7-bromo-3-pentyl-quinolin-2-amine (12.5 g, 42.63 mmol, 1 eq), Et_3N (8.63 g, 85.27 mmol, 11.87 mL, 2 eq) and DMAP (520.84 mg, 4.26 mmol, 0.1 eq) in DCM (200 mL) was added Boc_2O (27.91 g, 127.90 mmol, 29.38 mL, 3 eq) slowly at 15°C. The mixture was stirred at 15°C for 2 h. TLC showed the reaction was finished. The mixture was diluted with water (200 mL) and extracted with DCM (100 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, Petroleum ether/Ethyl acetate=3/1) to afford tert-butyl N-(7-bromo-3-pentyl-2-quinolyl) -N-tert-butoxycarbonyl-carbamate (18 g, 36.48 mmol, 85.57% yield) as yellow solid. ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.22 (d, J =2.0 Hz, 1H), 7.99 (s, 1H), 7.70-7.63 (m, 2H), 2.69-2.64 (m, 2H), 1.74-1.70 (m, 2H), 1.42-1.39 (m, 22H), 0.95-0.90 (m, 3H). LCMS (ESI): mass calcd. for $C_{24}H_{33}BrN_2O_4$ 492.16, m/z found 493.2$[M+H]^+$.

Example 20 Preparation of 4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl ((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)carbamate, AQ-20



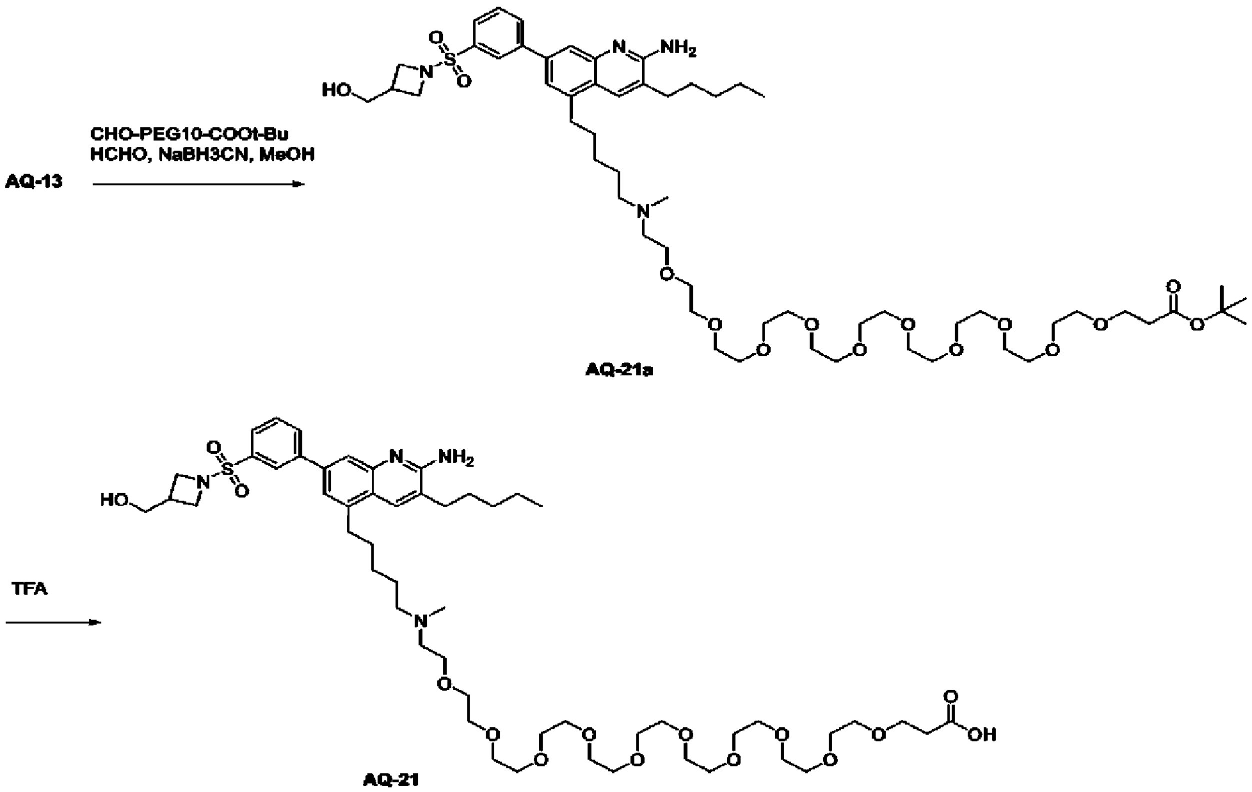
Preparation of 9H-fluoren-9-ylmethyl (2S)-2-[[(1S)-1-[[4-[[1-[3-(2-amino-3-pentyl -7-quinolyl)phenyl]sulfonylazetidin-3-yl]methylcarbamoyloxymethyl]phenyl]carbamoyl]-4-ureido-butyl]carbamoyl]-3-methyl-butanoate, AQ-20a

To a mixture of 7-[3-[3-(aminomethyl)azetidin-1-yl]sulfonylphenyl]-3-pentyl-quinolin-2-amine, AQ-12 (52.57 mg, 119.87 μmol, 1 eq) and [4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate (91.92 mg, 119.87 μmol, 1 eq) in DMF (2 mL) was added DIEA (30.98 mg, 239.75 μmol, 41.76 μL, 2 eq) at 15 °C. The mixture was stirred at 15°C for 0.5 h. LCMS showed the reaction was finished. The mixture was poured into water(20 mL), then filtered to give the crude product AQ-20a (0.12 g, 114.15 μmol, 95.23% yield) as yellow solid.

Preparation of AQ-20

Solid 4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl, AQ-20a ((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)carbamate (53 mg, 50 μmol, 1 eq.) was dissolved in 1:1 v/v diethylamine/DMF (3 mL) and heated gently with a heat gun until all of the starting material had disappeared by LC/MS. The solvent was removed under reduced pressure then dried by repeated evaporation with toluene (5x3 mL). AQ-20 was obtained and used in the next reaction without purification. LC/MS [M+H] 844.41 (calculated); LC/MS [M+H] 844.63 (observed).

Example 21 Preparation of 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[2-amino-7-[3-[3-(hydroxymethyl) azetidin-1-yl]sulfonylphenyl]-3-pentyl-5-quinolyl]pentyl-methyl-amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid, AQ-21



Preparation of Preparation of tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[5-[2-amino-7-[3-[3-(hydroxymethyl)azetidin-1-yl]sulfonylphenyl]-3-pentyl-5-quinolyl]pentyl-methyl-amino] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate, AQ-21a

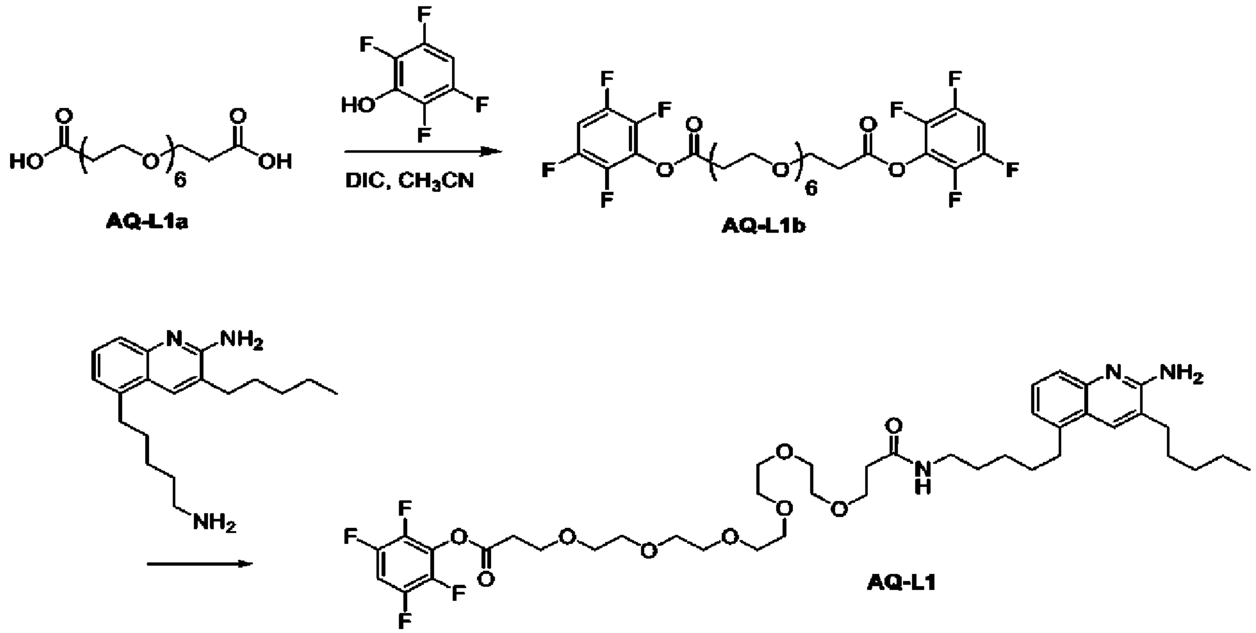
To a solution of [1-[3-[2-amino-5-(5-aminopentyl)-3-pentyl-7-quinolyl]phenyl] sulfonylazetidin-3-yl]methanol, AQ-13 (0.26 g, 173.43 μmol, 1 eq) in MeOH (5 mL) were added tert-butyl 3-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-oxoethoxy)ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoate (152.10 mg, 260.14 μmol, 1.5 eq) and AcOH (10.41 mg, 173.43 μmol, 9.92 μL, 1 eq) at 25 °C. After addition, the mixture was stirred at 25 °C for 15 min, and then $NaBH_3CN$ (21.80 mg, 346.85 μmol, 2 eq) was added at 25 °C. The resulting mixture was stirred at 25 °C for 12 h. Formaldehyde, HCHO (84.45 mg, 1.04 mmol, 77.48 μL, 37% purity, 6 eq) was added to the reaction mixture at 25 °C. After addition, the mixture was stirred at 25 °C for 15 min, and then $NaBH_3CN$ (21.80 mg, 346.85 μmol, 2 eq) was added at 25 °C. The resulting mixture was stirred at 25 °C for 2 h and then quenched with aq. $NaHCO_3$ at 0 °C and concentrated under reduced pressure. The residue was purified by prep-HPLC (neutral condition: column: Welch Xtimate C18 150*25mm*5um;mobile phase: [water(10mM NH_4HCO_3)-ACN];B%: 55%-85%,10.5min). AQ-21a (80 mg, 72.24 μmol, 41.65% yield) was obtained as a light yellow oil. LC/MS [M+H] 1107.7 (calculated); LC/MS [M+H] 1107.7 (observed).

Preparation of AQ-21

To a solution of AQ-21a (60 mg, 54.18 µmol, 1 eq) in CH_3CN (0.5 mL) and H_2O (0.1 mL) was added TFA (185.33 mg, 1.63 mmol, 120.34 µL, 30 eq) at 25 °C. The mixture was stirred at 70 °C for 1 h. LC-MS showed the reactant was consumed. Several new peaks were shown on LC-MS and desired compound was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved with CH_3CN (10 mL) and H_2O (1 mL) and adjusted to pH = 8 with aq. $NaHCO_3$ at 0 °C. The mixture was stirred for 1 h at 25 °C. The mixture was adjusted to pH = 7 with 1 N HCl at 0 °C. The mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (TFA condition: column: Nano-micro Kromasil C18 100*30mm 8um;mobile phase: [water(0.1%TFA)-ACN];B%: 20%-40%, 10min). The product was concentrated under reduced pressure at 30 °C and then lyophilized to give crude product which contained ~8% trifluoroacetate ester . The crude product was dissolved with CH3CN (5 mL) and H_2O (1 mL) and adjusted to pH = 8 with aq. $NaHCO_3$ at 0 °C. The mixture was stirred at 25 °C for 30 min. The mixture was adjusted to pH = 6 with 1 N HCl at 0 °C, and then concentrated under reduced pressure to give a residue. The residue was washed with CH3CN (5 mL x 3), filtered and the filtrate was concentrated under reduced pressure and then lyophilized to give a pure AQ-21 (25 mg, 22.98 µmol, 42.42% yield, HCl) as a colorless oil. ^{1}H NMR (MeOD-d_4, 400 MHz) δ 8.43 (s, 1H), 8.17-8.11 (m, 2H), 7.95 (d, J = 7.6 Hz, 1H), 7.88-7.82 (m, 2H), 7.74 (s, 1H), 3.92-3.85 (m, 2H), 3.83-3.78 (m, 2H), 3.74-3.54 (m, 42H), 3.48-3.38 (m, 4H), 3.28-3.18 (m, 2H), 2.91 (s, 3H), 2.85-2.77 (m, 2H), 2.67-2.56 (m, 1H), 2.52 (t, J = 6.0 Hz, 2H), 1.92-1.72 (m, 6H), 1.60-1.41 (m, 6H), 1.02-0.93 (m, 3H). LC/MS [M+H] 1051.6 (calculated); LC/MS [M+H] 1051.5 (observed).

Preparation of Aminoquinoline-linker Formula III compounds (AQ-L)

Example 22 Preparation of 2,3,5,6-tetrafluorophenyl 28-(2-amino-3-pentylquinolin-5-yl)-22-oxo-4,7,10,13,16,19-hexaoxa-23-azaoctacosanoate trifluoroacetic acid salt, AQ-L1



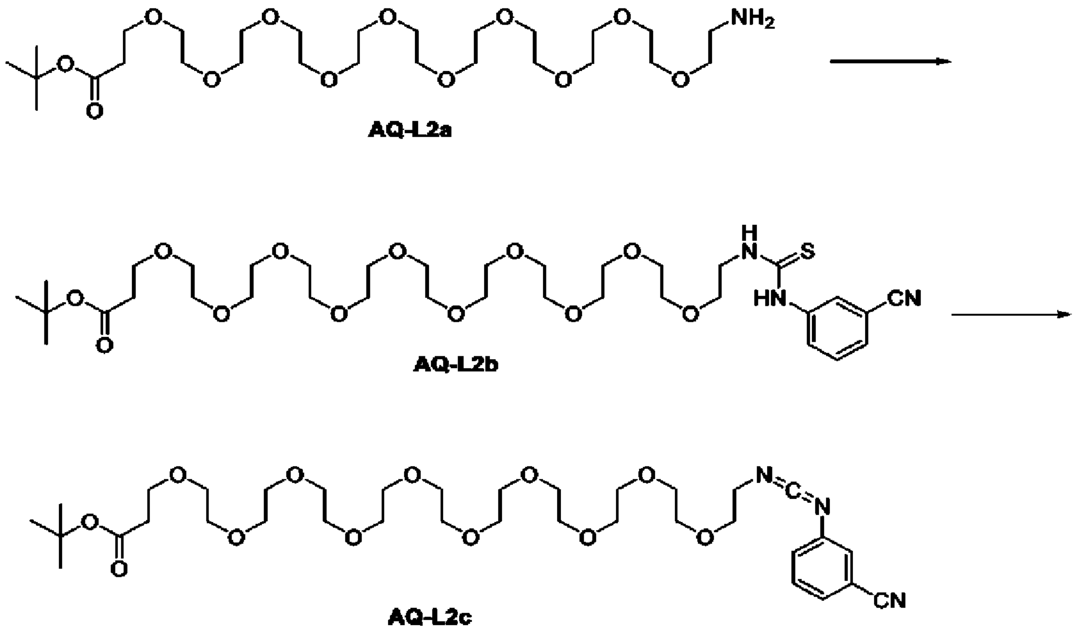
Preparation of AQ-L1b

To bis-PEG6-acid, AQ-L1a (68.5 mg, 0.2 mmol, 1 eq) in acetonitrile (1 mL) was added a mixture of 2,3,5,6-tetrafluorophenol (73.1 mg, 0.44 mmol, 2.2 eq) and diisopropylcarbodiimide, DIC (82 µL, 0.52 mmol, 2.6 eq). The mixture was heated to 50 °C for 15 minutes and then the solvent was removed to obtain PEG6-bis-(2,3,5,6-tetrafluorophenyl) ester AQ-L1b that was used without purification.

Preparation of AQ-L1

To a solution of 5-(5-aminopentyl)-3-pentylquinolin-2-amine formate salt (69.1 mg, 0.2 mmol, 1 eq) in DMF (2 mL) added diisopropylethylamine (0.14 mL, 0.8 mmol, 4 eq.) then a solution of PEG6-bis-(2,3,5,6-tetrafluorophenyl) ester, AQ-L1b in DMF (2 mL) and the mixture was heated to 50 °C for 45 minutes. The crude product was purified by reverse phase HPLC (acetonitrile/water) to obtain AQ-L1 (0.0914 mg, 0.098 mmol, 49%) as a yellow syrup after concentration. LC/MS [M+H] 812.40 (calculated); LC/MS [M+H] 812.64 (observed).

Example 23 Preparation of AQ-L2


AQ-L2a

AQ-L2b

AQ-L2c

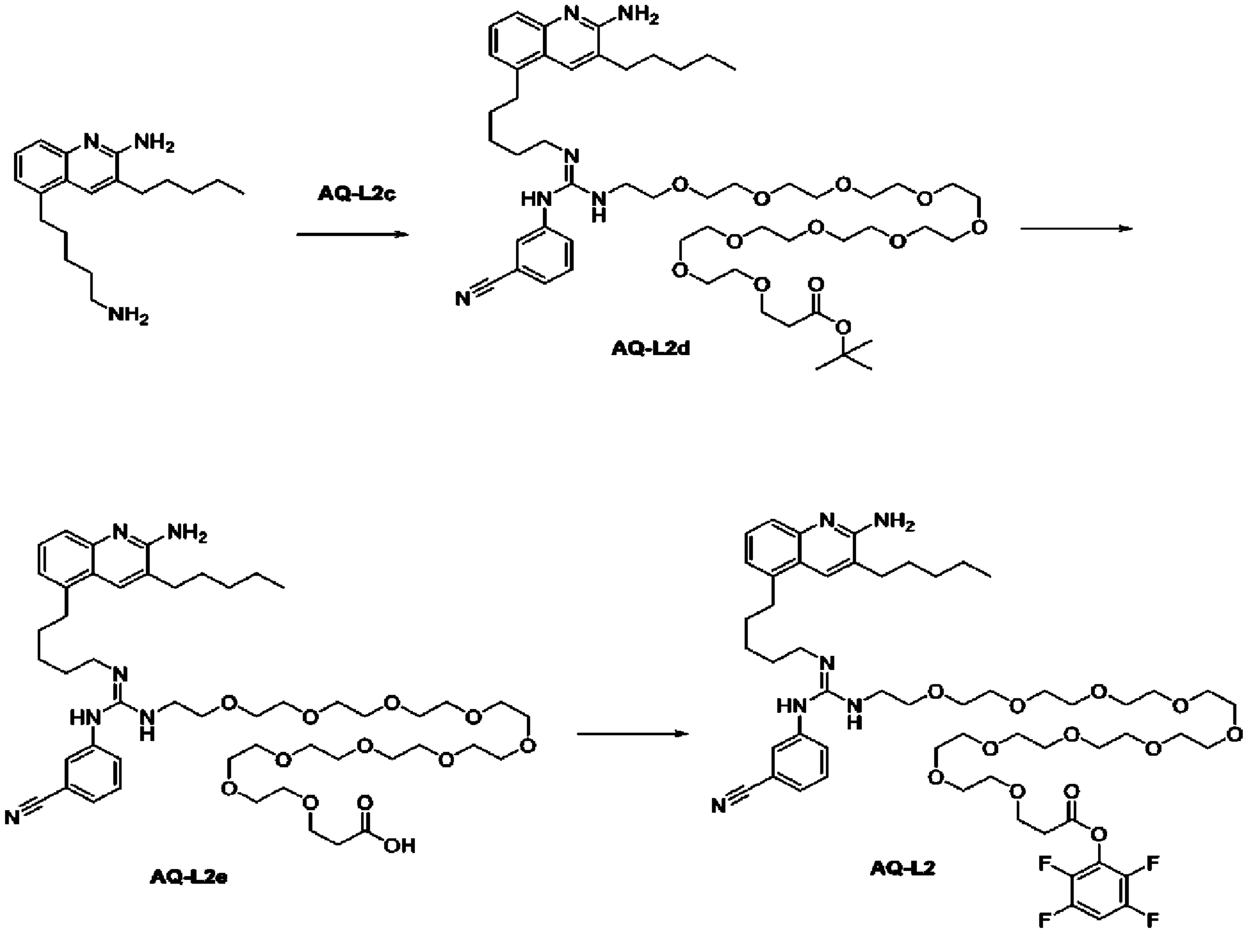
Preparation of tert-butyl 1-((3-cyanophenyl)amino)-1-thioxo-5,8,11,14,17,20,23,26,29,32-decaoxa-2-azapentatriacontan-35-oate, AQ-L2b

A vial was charged with tert-butyl 1-amino-3,6,9,12,15,18,21,24,27,30-decaoxatritriacontan-33-oate, AQ-L2a (378 mg, 0.645 mmol), 3-cyanophenyl isothiocyanate (103 mg, 0.645 mmol) and 7 mL DCM. The reaction was maintained for 2 h, then purified by reverse phase preparative HPLC utilizing a 25-75% gradient of acetonitrile:water containing 0.1% trifluoroacetic acid. The purified fractions were combined and lyophilized to afford 501 mg of AQ-L2b. LC/MS [M+H] 746.39 (calculated); LC/MS [M+H] 746.69 (observed).

Preparation of tert-butyl 1-((3-cyanophenyl)imino)-5,8,11,14,17,20,23,26,29,32-decaoxa-2-azapentatriacont-1-en-35-oate, AQ-L2c

A vial was charged with 107 mg of thiourea (0.143 mmol), triethylamine (60µL, 0.429 mmol) and 1.3 mL DCM. To this vial was added 2-chloro-1-methylpyridinium iodide (44 mg, 0.172 mmol). The reaction was sonicated 2 min. The reaction was still heterogenous; 100 ul DMF was added and the reaction was stirred for 1h at which no thiourea remained, only carbodiimide, by LCMS. The crude reaction was concentrated under reduced pressure and azeotroped thrice with toluene. The crude material purified by silica gel flash chromatography using a 25-75% gradient of MeCN:Ethyl Acetate affording 68.6 mg of the carbodiimide AQ-L2c in 67% yield. LC/MS [M+H] 712.40 (calculated); LC/MS [M+H] 712.67 (observed).

Preparation of tert-butyl 41-(2-amino-3-pentylquinolin-5-yl)-35-((3-cyanophenyl)amino)4,7,10,13,16,19,22,25,28,31-decaoxa-34,36-diazahentetracont-35-enoate, AQ-L2d

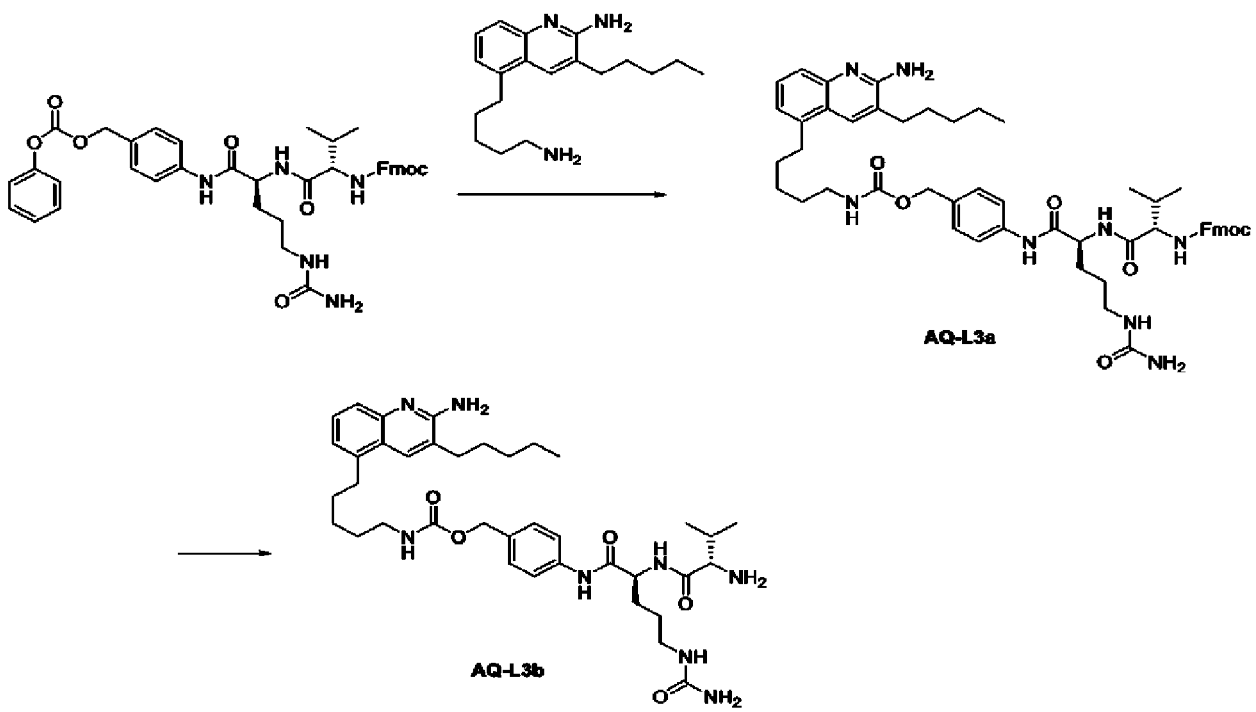


AQ-L2d was prepared from AQ-L2c and 5-(5-aminopentyl)-3-pentylquinolin-2-amine formate salt according to the method described in Example 25. LC/MS [M+H] 1011.64 (calculated); LC/MS [M+H] 1011.97 (observed).

AQ-L2e was prepared from AQ-L2d according to the methods described in Example 25. LC/MS [M+Na] 977.56 (calculated); LC/MS [M+Na] 977.70 (observed).

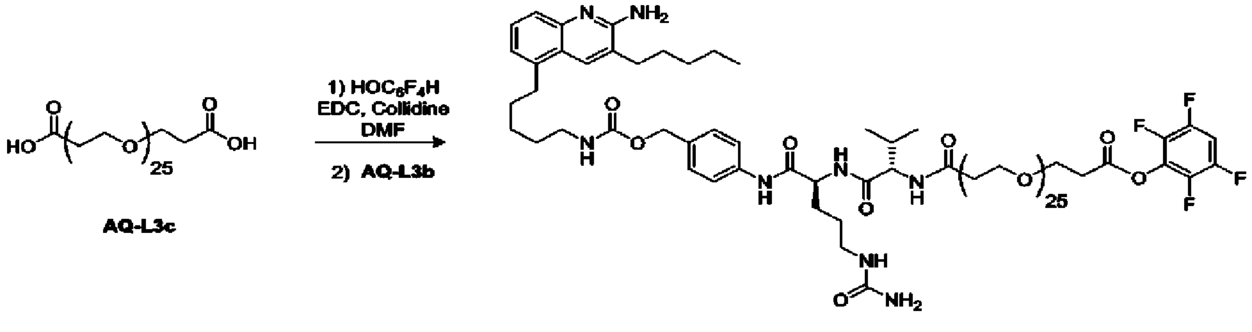
AQ-L2 was prepared from AQ-L2e according to the method described in Example 25. LC/MS [M+H] 1103.57 (calculated); LC/MS [M+H] 1103.71 (observed).

Example 24 Preparation of 2,3,5,6-tetrafluorophenyl (6S,9S)-1-amino-6-((4-((((5-(2-amino-3-pentylquinolin-5-yl)pentyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)-9-isopropyl-1,8,11-trioxo-14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86-pentacosaoxa-2,7,10-triazanonaoctacontan-89-oate, AQ-L3



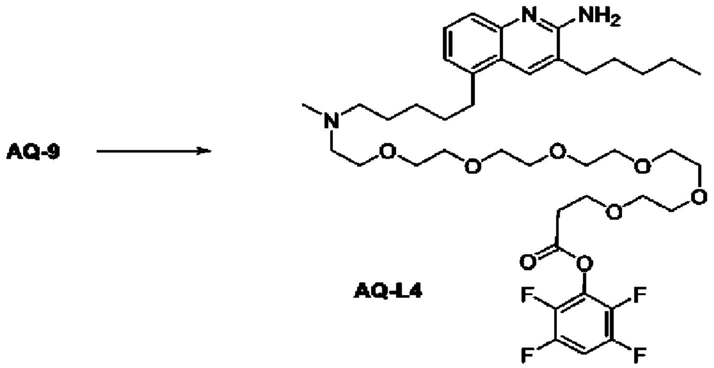
AQ-L3a was prepared from 5-(5-aminopentyl)-3-pentylquinolin-2-amine formate salt and [4-[[(2S)-2-[[(2S)-2-(9H-fluoren-9- ylmethoxycarbonylamino)-3-methyl-butanoyl]amino]-5-ureido-pentanoyl]amino]phenyl]methyl (4-nitrophenyl) carbonate using the procedure described in Example 20. LC/MS [M+H] 927.51 (calculated); LC/MS [M+H] 927.51 (observed).

AQ-L3b was prepared using the procedure described in Example 20. LC/MS [M+H] 705.45 (calculated); LC/MS [M+H] 705.61 (observed).



AQ-L3 was prepared using the procedure described in Example 24. LC/MS [M+H] 2054.40 (calculated); LC/MS [M+H] 2054.11 (observed).

Example 24 Preparation of 2,3,5,6-tetrafluorophenyl 27-(2-amino-3-pentylquinolin-5-yl)-22-methyl-4,7,10,13,16,19-hexaoxa-22-azaheptacosanoate trifluoroacetate salt, AQ-L4

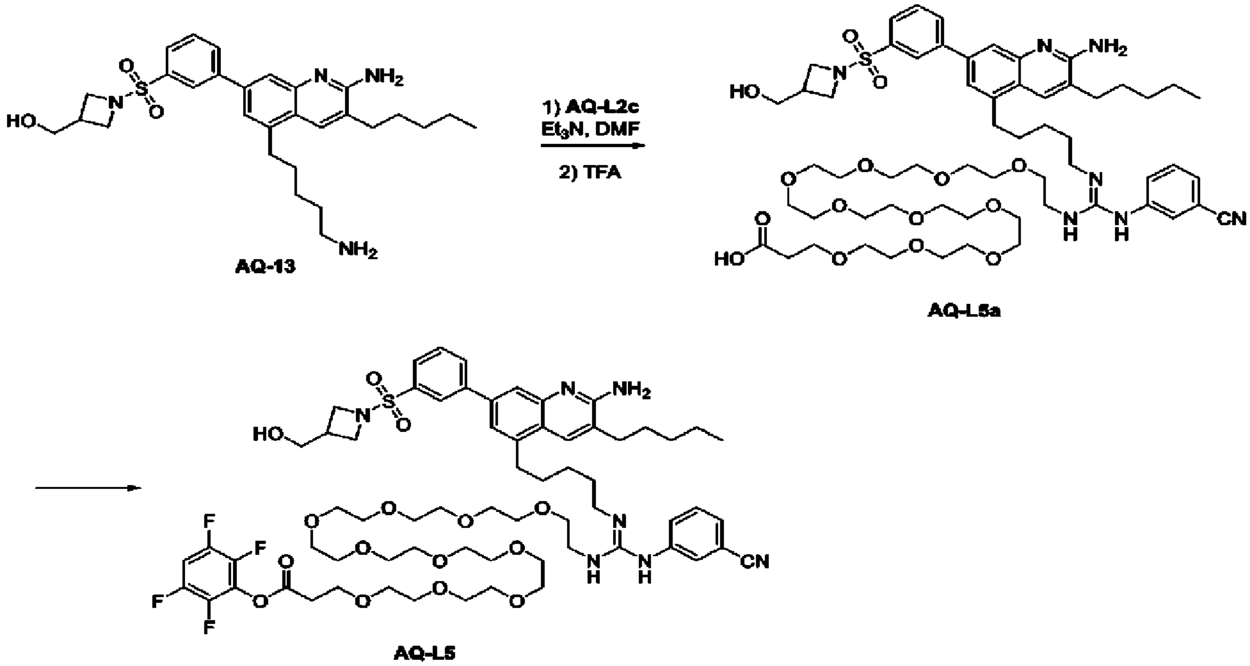


AQ-9

AQ-L4

To the hydrochloride salt of AQ-9 (55 mg, 80 µmol, 1 eq) was added a mixture of 2,3,5,6-tetrafluorophenol (67 mg, 0.40 mmol, 5 eq) and diisopropylcarbodiimide (63 µL, 0.40 mmol, 5 eq) in acetonitrile (2.5 mL). The mixture was gently heated with a heat gun then stirred for 45 minutes at room temperature. The crude product was purified by reverse phase HPLC (acetonitrile/water) to obtain AQ-L4 (41 mg, 51 µmol, 64%) after removal of solvent. LC/MS [M+H] 798.42 (calculated); LC/MS [M+H] 798.66 (observed).

Example 25 Preparation of 2,3,5,6-tetrafluorophenyl (E)-41-(2-amino-7-(3-((3-(hydroxymethyl)azetidin-1-yl)sulfonyl)phenyl)-3-pentylquinolin-5-yl)-35-((3-cyanophenyl)imino)-4,7,10,13,16,19,22,25,28,31-decaoxa-34,36-diazahentetracontanoate, AQ-L5



AQ-13

AQ-L5a

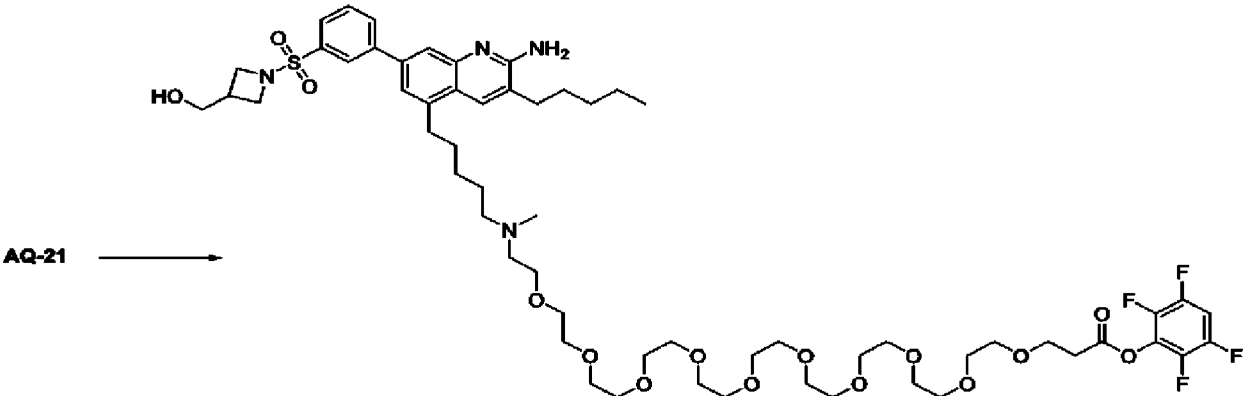
AQ-L5

(1-((3-(2-Amino-5-(5-aminopentyl)-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methanol, AQ-13 (0.1 g, 0.19 mmol, 1 eq.) and tert-butyl 1-((3-cyanophenyl)imino)-5,8,11,14,17,20,23,26,29,32-decaoxa-2-azapentatriacont-1-en-35-oate, AQ-L2c (0.14 g, 0.19 mmol, 1 eq.) were dissolved in DMF. Triethylamine (0.08 ml, 0.57 mmol, 3 eq.) was added, and the reaction was stirred at ambient temperature. Upon consumption of amine starting material, the reaction was concentrated and purified by HPLC. The isolated t-butyl ester product was taken up in minimal TFA for 10 minutes, then concentrated to give (E)-41-(2-amino-7-(3-((3-(hydroxymethyl)azetidin-1-yl)sulfonyl)phenyl)-3-pentylquinolin-5-yl)-35-((3-cyanophenyl)imino)-4,7,10,13,16,19,22,25,28,31-decaoxa-34,36-diazahentetracontanoic acid, AQ-L5a (0.15 g, 0.13 mmol, 67%). LC/MS [M+H] 1180.62 (calculated); LC/MS [M+H] 1181.05 (observed).

Preparation of AQ-L5

AQ-L5a (0.15 g, 0.127 mmol, 1 eq.) and tetrafluorophenol, TFP (0.032 g, 0.19 mmol, 1.5 eq.) were dissolved in 2 ml DMF. Collidine (0.083 ml, 0.64 mmol, 5 eq.) was added, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, EDC-HCl (0.049 g, 0.25 mmol, 2 eq.). The reaction was stirred at room temperature until complete, then concentrated and purified by HPLC to give AQ-L5 (0.063 g, 0.055 mmol, 43%). LC/MS [M+H] 1328.61 (calculated); LC/MS [M+H] 1329.07 (observed).

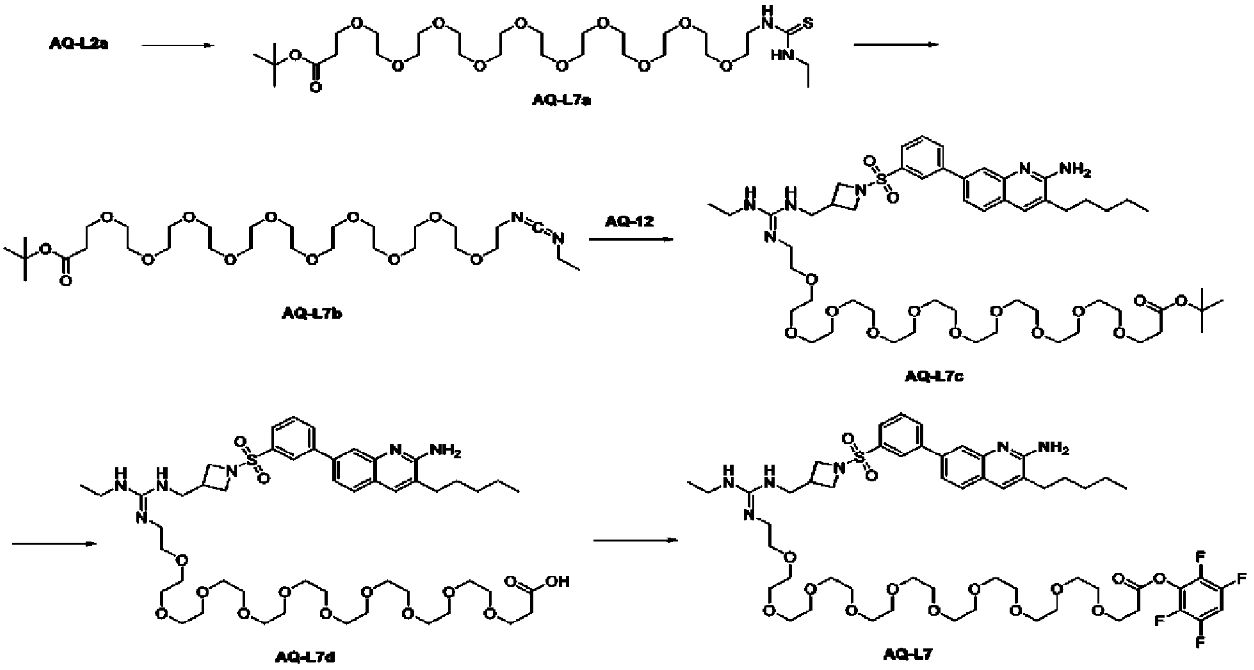
Example 26 Preparation of 2,3,5,6-tetrafluorophenyl 39-(2-amino-7-(3-((3-(hydroxymethyl)azetidin-1-yl)sulfonyl)phenyl)-3-pentylquinolin-5-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoate, AQ-L6



AQ-L6

39-(2-Amino-7-(3-((3-(hydroxymethyl)azetidin-1-yl)sulfonyl)phenyl)-3-pentylquinolin-5-yl)-34-methyl-4,7,10,13,16,19,22,25,28,31-decaoxa-34-azanonatriacontanoic acid, AQ-21 (0.1 g, 0.095 mmol, 1 eq.) and tetrafluorophenol, TFP (0.032 g, 0.19 mmol, 2 eq.) were dissolved in 2 ml DMF. Collidine (0.063 ml, 0.48 mmol, 5 eq.) was added, followed by EDC-HCl (0.036 g, 0.19 mmol, 2 eq.). The reaction was stirred at room temperature for 2 hours, then concentrated and purified by HPLC to give AQ-L6 (0.049 g, 0.040 mmol, 43%). LC/MS [M+H] 1199.58 (calculated); LC/MS [M+H] 1199.98 (observed).

Example 27 Preparation of 2,3,5,6-tetrafluorophenyl (Z)-4-(((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)amino)-8,11,14,17,20,23,26,29,32,35-decaoxa-3,5-diazaoctatriacont-4-en-38-oate, AQ-L7



AQ-L7a was prepared according to the method described in Example 23. LC/MS [M+H] 673.39 (calculated); LC/MS [M+H] 673.91 (observed).

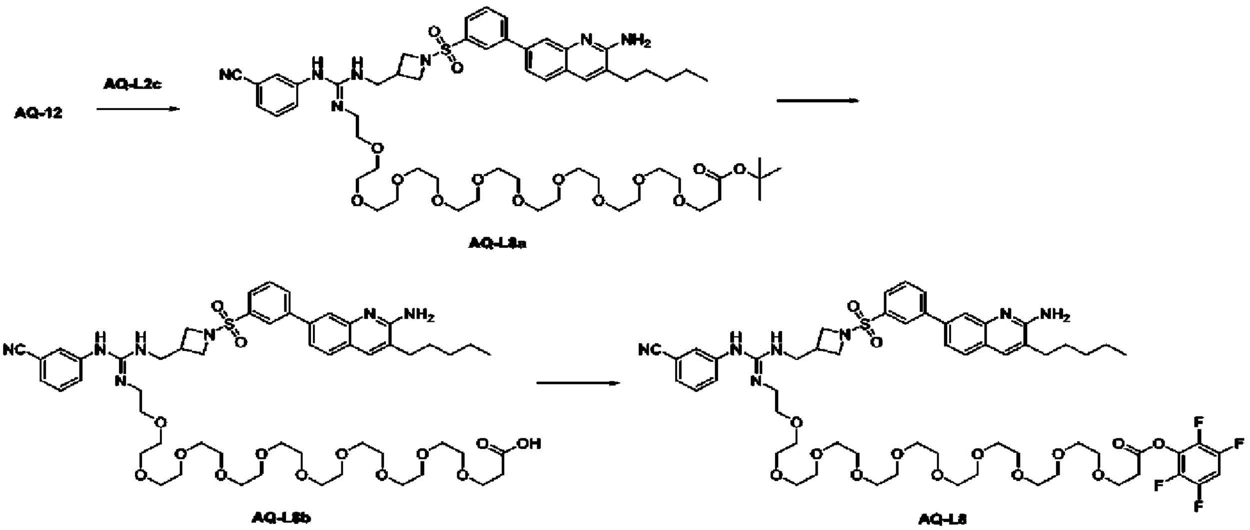
AQ-L7b was prepared according to the method described in Example 23 and used directly in the subsequent step without further purification.

AQ-L7c was prepared according to the method described in Example 23. LC/MS [M+H] 1077.62 (calculated); LC/MS [M+H] 1077.89 (observed).

AQ-L7d was prepared according to the method described in Example 23. LC/MS [M+H] 1021.55 (calculated); LC/MS [M+H] 1021.77 (observed).

AQ-L7 was prepared according to the method described in Example 23. LC/MS [M+H] 1169.55 (calculated); LC/MS [M+H] 1169.88 (observed).

Example 28 Preparation of 2,3,5,6-tetrafluorophenyl (E)-1-(1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)-3-((3-cyanophenyl)amino)-7,10,13,16,19,22,25,28,31,34-decaoxa-2,4-diazaheptatriacont-3-en-37-oate, AQ-L8

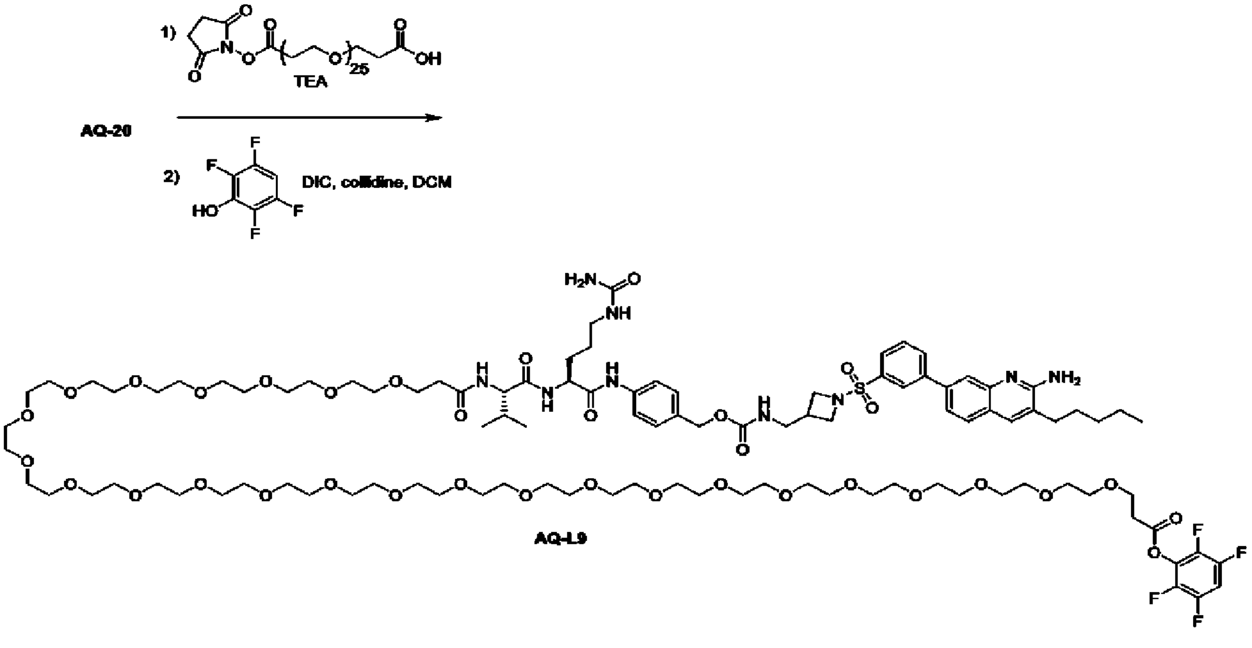


AQ-L8a was prepared according to the method described in Example 23. LC/MS [M+H] 1150.61 (calculated); LC/MS [M+H] 1150.95 (observed).

AQ-L8b was prepared according to the method described in Example 23. LC/MS [M+H] 1094.55 (calculated); LC/MS [M+H] 1094.69 (observed).

AQ-L8 was prepared according to the method described in the procedures of Example 23. LC/MS [M+H] 1242.54 (calculated); LC/MS [M+H] 1242.98 (observed).

Example 29 Preparation of 2,3,5,6-tetrafluorophenyl (6S,9S)-1-amino-6-((4-(((((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)carbamoyl)oxy)methyl)phenyl)carbamoyl)-9-isopropyl-1,8,11-trioxo-14,17,20,23,26,29,32,35,38,41,44,47,50,53,56,59,62,65,68,71,74,77,80,83,86-pentacosaoxa-2,7,10-triazanonaoctacontan-89-oate, AQ-L9



To a solution of 4-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)benzyl ((1-((3-(2-amino-3-pentylquinolin-7-yl)phenyl)sulfonyl)azetidin-3-yl)methyl)carbamate, AQ-20 (40 mg) in DMF (2 mL) was added a solution of acid-PEG25-NHS ester (assumed 66 mg, 50 μmol) and triethylamine (21 μL, 0.15 mmol, 3 eq) in DMF (1 mL). The mixture was gently heated with a heat gun then stirred for 30 minutes at room temperature to obtain NHS. To the crude product was added water (1 mL) and the mixture was heated gently until the NHS ester that was formed was hydrolyzed. Solvent was removed by evaporation and then further addition and evaporation of toluene (5x4 mL). To the crude acid was added a mixture of 2,3,5,6-tetrafluorophenol (66 mg, 0.4 mmol) and DIC (50 mg, 0.4 mmol, 8 eq.) and collidine (48 mg, 0.4 mmol, 8 eq) in DMF (2 mL). The mixture was gently heated until almost all of the starting material had disappeared by LC/MS. Crude product was purified by reverse phase HPLC (acetonitrile/water) to obtain AQ-L8 (10.9 mg, 5 μmol, 10%) after removal of solvent.. LC/MS [M+H] 2193.08 (calculated); LC/MS [M+H] 2193.30 (observed).

Example 30 Preparation of Immunoconjugates (IC)

In an exemplary procedure, an antibody is buffer exchanged into a conjugation buffer containing 100 mM boric acid, 50 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid at pH 8.3, using G-25 SEPHADEX™ desalting columns (Sigma-Aldrich, St. Louis, MO). The eluates are then each adjusted to 6 mg/ml using the buffer and then sterile filtered. The antibody at 6 mg/ml is pre-warmed to 30 °C and rapidly mixed with 2-20 (e.g., 7-10) molar equivalents of aminoquinoline-linker compound of Formula II. The reaction is allowed to proceed for 16 hours at 30 °C and the immunoconjugate compound is separated from reactants by running over two successive G-25 desalting columns equilibrated in phosphate buffered saline (PBS) at pH 7.2 to provide the Immunoconjugate (IC) of Table 3. Adjuvant-antibody ratio (DAR) is determined by liquid chromatography mass spectrometry analysis using a C4 reverse phase column on an ACQUITY™ UPLC H-class (Waters Corporation, Milford, Massachusetts) connected to a XEVO™ G2-XS TOF mass spectrometer (Waters Corporation).

For conjugation, the antibody may dissolved in a physiological buffer system known in the art that will not adversely impact the stability or antigen-binding specificity of the antibody. Phosphate buffered saline may be used. The aminoquinoline-linker intermediate compound is dissolved in a solvent system comprising at least one polar aprotic solvent as described elsewhere herein. In some such aspects, aminoquinoline-linker intermediate is dissolved to a concentration of about 5 mM, 10 mM, about 20 mM, about 30 mM, about 40 mM or about 50 mM, and ranges thereof such as from about 50 mM to about 50mM or from about 10 mM to about 30 mM in pH 8 Tris buffer (e.g., 50 mM Tris). In some aspects, the aminoquinoline-linker intermediate is dissolved in DMSO or acetonitrile, or in DMSO. In the conjugation reaction, an equivalent excess of aminoquinoline-linker intermediate solution is diluted and combined with chilled antibody solution (e.g. from about 1 °C to about 10 °C). The aminoquinoline-linker intermediate solution may suitably be diluted with at least one polar aprotic solvent and at least one polar protic solvent, examples of which include water, methanol, ethanol, n-propanol, and acetic acid. In some particular aspects the aminoquinoline-linker intermediate is dissolved in DMSO and diluted with acetonitrile and water prior to admixture with the antibody solution. The molar equivalents of aminoquinoline-linker intermediate to antibody may be about 1.5:1, about 3:1, about 5:1, about 10:1 about 15:1 or about 20:1, and ranges thereof, such as from about 1.5:1 to about 20:1 from about 1.5:1 to about 15:1, from about 1.5:1 to about 10:1,from about 3:1 to about 15:1, from about 3:1 to about 10:1, from about 5:1 to about 15:1 or from about 5:1 to about 10:1. The reaction may suitably be monitored for completion by methods known in the art, such as LC-MS, and the reaction is typically complete in from about 1 hour to about 24 hours. After the reaction is complete, a reagent may be added to the reaction mixture to quench the reaction and/or cap unreacted antibody thiol groups. An example of a suitable capping reagent is ethylmaleimide.

Following conjugation, the immunoconjugates may be purified and separated from unconjugated reactants and/or conjugate aggregates by purification methods known in the art such as, for example and not limited to, size exclusion chromatography, hydrophobic interaction chromatography, ion exchange chromatography, chromatofocusing, ultrafiltration, centrifugal ultrafiltration, and combinations thereof. For instance, purification may be preceded by diluting the immunoconjugate, such in 20 mM sodium succinate, pH 5. The diluted solution is applied to a cation exchange column followed by washing with, e.g., at least 10 column volumes of 20 mM sodium succinate, pH 5. The conjugate may be suitably eluted with a buffer such as PBS.

Example 31 HEK Reporter Assay

HEK293 reporter cells expressing human TLR7 or human TLR8 were purchased from Invivogen and vendor protocols were followed for cellular propagation and experimentation. Briefly, cells were grown to 80-85% confluence at 5% CO_2 in DMEM supplemented with 10% FBS, Zeocin, and Blasticidin. Cells were then seeded in 96-well flat plates at $4x10^4$cells/well with substrate containing HEK detection medium and immunostimulatory molecules. Activity was measured using a plate reader at 620-655 nm wavelength.

Example 32 Assessment of Immunoconjugate Activity *In Vitro*

This example shows that Immunoconjugates of the invention are effective at eliciting myeloid activation, and therefore are useful for the treatment of cancer.

Isolation of Human Antigen Presenting Cells: Human myeloid antigen presenting cells (APCs) were negatively selected from human peripheral blood obtained from healthy blood donors (Stanford Blood Center, Palo Alto, California) by density gradient centrifugation using a ROSETTESEP™ Human Monocyte Enrichment Cocktail (Stem Cell Technologies, Vancouver, Canada) containing monoclonal antibodies against CD14, CD16, CD40, CD86, CD123, and HLA-DR. Immature APCs were subsequently purified to >90% purity via negative selection using an EASYSEP™ Human Monocyte Enrichment Kit (Stem Cell Technologies) without CD16 depletion containing monoclonal antibodies against CD14, CD16, CD40, CD86, CD123, and HLA-DR.

Myeloid APC Activation Assay: 2 x 10^5 APCs were incubated in 96-well plates (Corning, Corning, NY) containing iscove's modified dulbecco's medium, IMDM (Lonza) supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL (micrograms per milliliter) streptomycin, 2 mM L-glutamine, sodium pyruvate, non-essential amino acids, and where indicated, various concentrations of unconjugated (naked) PD-L1 or HER2 antibodies and immunoconjugates of the invention (as prepared according to the Example above). Trastuzumab (anti-HER2) and avelumab (anti-PD-L1) were used as the antibody constructs. Cells and cell-free supernatants were analyzed after 18 hours via ELISA to measure TNFα secretion as a readout of a proinflammatory response.

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

CLAIMS:

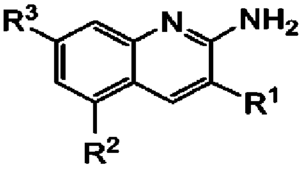
1. An immunoconjugate comprising an antibody covalently attached to a divalent linker which is covalently attached to one or more aminoquinoline moieties, and having Formula I:

$$\mathrm{Ab{-}[L{-}AQ]_p} \qquad \mathrm{I}$$

or a pharmaceutically acceptable salt thereof, wherein:

Ab is the antibody;

AQ is the aminoquinoline moiety having Formula II:



II

where one of R^1, R^2 and R^3 is attached to L;

R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)C(=O)R^5$;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)C(=O)OR^5$;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)C(=O)N(R^5)_2$;

$-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$;

$-(C_2\text{-}C_6\text{ alkenyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-(C_2\text{-}C_6\text{ alkenyldiyl})-N(R^5)_2$;

$-(C_2\text{-}C_6\text{ alkenyldiyl})-NR^5-*$;

$-(C_2\text{-}C_6\text{ alkynyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-(C_2\text{-}C_6\text{ alkynyldiyl})-N(R^5)_2$;

$-(C_2\text{-}C_6\text{ alkynyldiyl})-NR^5-*$;

$-(C_2\text{-}C_{20}\text{ heterocyclyldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-(C_1\text{-}C_{20}\text{ heteroaryldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;

$-(C_6\text{-}C_{20}\text{ aryldiyl})-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5-*$;

$-C(=O)NR^5-(C_1\text{-}C_{12}\text{ alkyldiyl})-NR^5C(=NR^4)NR^5-*$;

$-C(=O)NR^5-(C_1\text{-}C_{12}\text{ alkyldiyl})-N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^2 is selected from the group consisting of:

H;

C_1-C_8 alkyl;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(NR^5)=N-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)R^5$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)OR^5$;

$-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(=O)N(R^5)_2$;

$-(C_2-C_6$ alkenyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_6$ alkenyldiyl$)-N(R^5)_2$;

$-(C_2-C_6$ alkenyldiyl$)-NR^5-*$;

$-(C_2-C_6$ alkynyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2-C_6$ alkynyldiyl$)-N(R^5)_2$;

$-(C_2-C_6$ alkynyldiyl$)-NR^5-*$;

$-(C_1-C_{12}$ alkyldiyl$)-(C_2-C_{20}$ heterocyclyldiyl$)$;

$-(C_1-C_{12}$ alkyldiyl$)-(C_1-C_{20}$ heteroaryldiyl$)$;

$-(C_1-C_{12}$ alkyldiyl$)-(C_6-C_{20}$ aryldiyl$)$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

R^4 is selected from the group consisting of C_6-C_{20} aryl and C_1-C_8 alkyl;

R^5 is selected from the group consisting of H and C_1-C_8 alkyl;

or two R^5 groups form a 5- or 6-membered heterocyclyl ring; and

R^3 is selected from the group consisting of H, $-C(=O)NR^5R^6$, and phenyl, where phenyl is substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, $-CN$, $-CH_3$, $-CF_3$, $-CO_2H$, $-NH_2$, $-NHCH_3$, $-NO_2$, $-OH$, $-OCH_3$, $-SCH_3$, $-S(O)_2CH_3$, $-S(O)_3H$, and R^7;

R^6 is independently selected from the group consisting of

H;

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_2-C_{20} heterocyclyl);

–(C_2-C_{20} heterocyclyldiyl)–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

—(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{20} heteroaryldiyl)–NR^5–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–(C_6-C_{20} aryldiyl)–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

R^7 is selected from the group consisting of:

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)–*;

–C(=O)–(C_2-C_{20} heterocyclyl);

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-(C_2-C_{20}$ heterocyclyldiyl$)-C(=O)NR^5-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-NR^5-*$;

$-C(=O)N(R^5)_2$;

$-C(=O)NR^5-(C_1-C_{20}$ heteroaryldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-NR^5-*$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)C(NR^5)=N-*$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2-C_{20}$ heterocyclyldiyl$)-(C_1-C_{12}$ alkyldiyl$)-OH$;

where * indicates the attachment site of L;

L is the linker selected from the group consisting of:

$-C(=O)-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-NR^5-$;

$-C(=O)-(PEG)-NR^5-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-N^+(R^5)_2-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-C(=O)-$;

$-C(=O)-(PEG)-C(=O)NR^5CH(AA_1)C(=O)-$;

$-C(=O)-(PEG)-NR^5CH(AA_1)C(=O)-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-SS-(C_1-C_{12}$ alkyldiyl$)-OC(=O)-$;

$-C(=O)-(PEG)-SS-(C_1-C_{12}$ alkyldiyl$)-C(=O)-$;

$-C(=O)-(PEG)-$;

$-C(=O)-(C_1-C_{12}$ alkyldiyl$)-C(=O)-(PEP)-$;

$-C(=O)-CH_2CH_2OCH_2CH_2-(C_1-C_{20}$ heteroaryldiyl$)-CH_2O-(PEG)-C(=O)-$(MCgluc)$-$; and

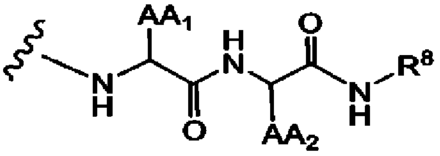
$-$(succinimidyl)$-(CH_2)_m-C(=O)-(PEP)-$;

where

PEG has the formula:

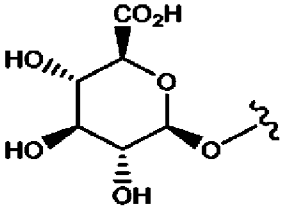
$-(CH_2CH_2O)_n-(CH_2)_m-$; m is an integer from 1 to 5, and n is an integer from 2 to 50;

PEP has the formula:



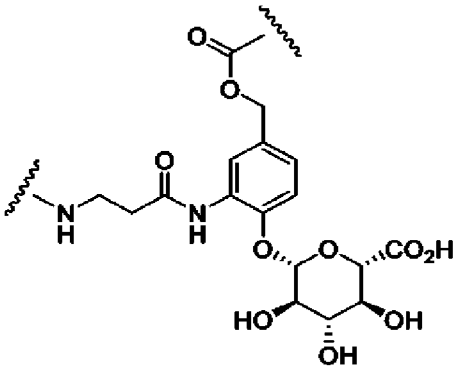
where AA_1 and AA_2 are independently selected from an amino acid side chain, or AA_1 or AA_2 and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment and;

R^8 is selected from the group consisting of C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl substituted with $-CH_2O-C(=O)-$, and optionally with:

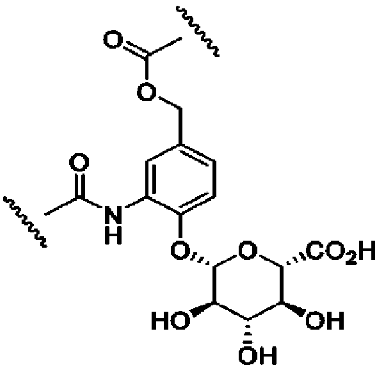


; and

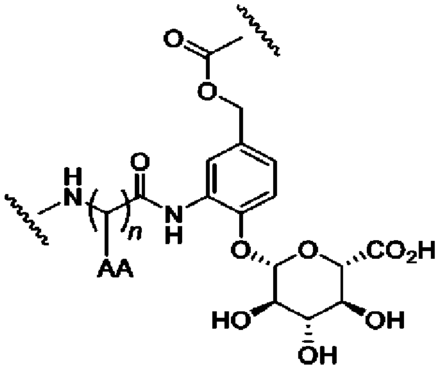
MCgluc is selected from the groups:



;



; and



where n is 1 to 8, and AA is an amino acid side chain;

where alkyl, alkyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, $-CN$, $-CH_3$, $-CH_2CH_3$, $-CH=CH_2$, $-C\equiv CH$, –

$C{\equiv}CCH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2CH_2OH$, $-C(CH_3)_2OH$, $-CH(OH)CH(CH_3)_2$, $-C(CH_3)_2CH_2OH$, $-CH_2CH_2SO_2CH_3$, $-CH_2OP(O)(OH)_2$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CH_2CHF_2$, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NO_2$, $=O$, $-OH$, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$; and

p is an integer from 1 to 8.

2. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds PD-L1.

3. The immunoconjugate of claim 2 wherein the antibody is selected from the group consisting of atezolizumab, durvalumab, and avelumab, or a biosimilar or a biobetter thereof.

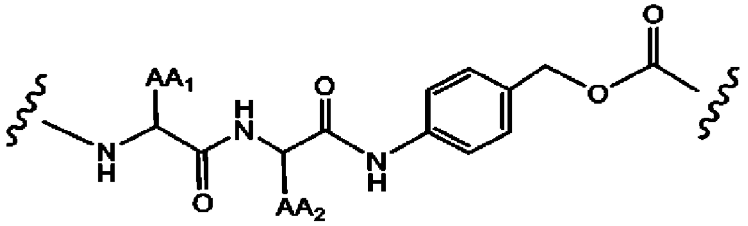
4. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds HER2.

5. The immunoconjugate of claim 4 wherein the antibody is selected from the group consisting of trastuzumab and pertuzumab, or a biosimilar or a biobetter thereof.

6. The immunoconjugate of claim 1 wherein the antibody is an antibody construct that has an antigen binding domain that binds CEA.

7. The immunoconjugate of claim 6 wherein the antibody is labetuzumab, or a biosimilar or a biobetter thereof.

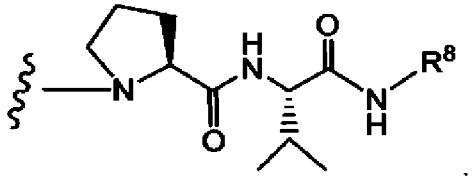
8. The immunoconjugate of any one of claims 1 to 7 wherein PEP has the formula:



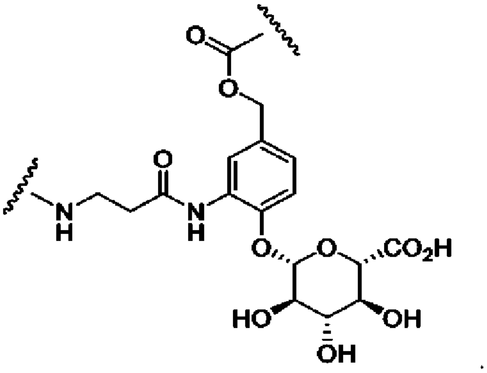
wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

9. The immunoconjugate of any one of claims 1 to 7 wherein AA_1 or AA_2 with an adjacent nitrogen atom form a 5-membered ring proline amino acid.

10. The immunoconjugate of any one of claims 1 to 7 wherein PEP has the formula:



11. The immunoconjugate of any one of claims 1 to 7 wherein MCgluc has the formula:



12. The immunoconjugate of any one of claims 1 to 7 wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

13. The immunoconjugate of any one of claims 1 to 7 wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$.

14. The immunoconjugate of claim 13 wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

15. The immunoconjugate of any one of claims 1 to 7 wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

16. The immunoconjugate of any one of claims 1 to 7 wherein R^1 is attached to L.

17. The immunoconjugate of any one of claims 1 to 7 wherein R^2 is attached to L.

18. The immunoconjugate of any one of claims 1 to 7 wherein R^3 is attached to L.

19. The immunoconjugate of any one of claims 1 to 7 wherein R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

$-(C_1$-C_{12} alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$; and

$-(C_1$-C_{12} alkyldiyl$)-NR^5-*$.

20. The immunoconjugate of any one of claims 1 to 7 wherein R^2 is selected from the group consisting of:

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$; and

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$.

21. The immunoconjugate of any one of claims 1 to 7 wherein R^6 is selected from the group consisting of:

$C_1\text{-}C_8$ alkyl;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$.

22. The immunoconjugate of any one of claims 1 to 7 wherein R^6 is selected from the group consisting of:

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$.

23. The immunoconjugate of any one of claims 1 to 7 wherein R^7 is selected from the group consisting of:

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$.

24. The immunoconjugate of any one of claims 1 to 7 wherein L is selected from the group consisting of:

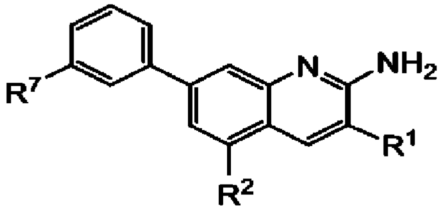
$-C(=O)-(PEG)-C(=O)-(PEP)-$;

$-C(=O)-(PEG)-NR^5-$;

$-C(=O)-(PEG)-C(=O)-$; and

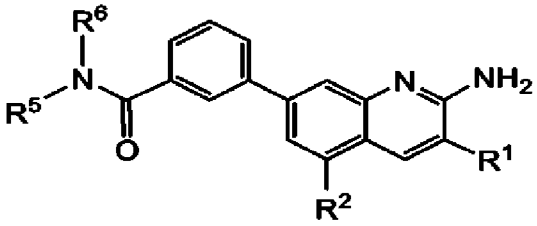
$-C(=O)-(PEG)-$.

25. The immunoconjugate of any one of claims 1 to 7 wherein AQ is selected from Formula IIa:



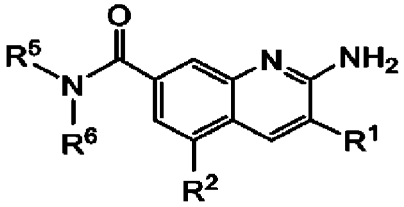
IIa .

26. The immunoconjugate of any one of claims 1 to 7 wherein AQ is selected from Formula IIb:



IIb .

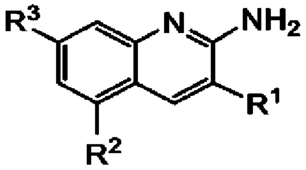
27. The immunoconjugate of any one of claims 1 to 7 wherein AQ is selected from Formula IIc:



IIc .

28. An immunoconjugate selected from Table 3.

29. An aminoquinoline-linker compound of Formula III:



III

where one of R^1, R^2 and R^3 is attached to L;

R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_1-C_{12} alkyldiyl)–$N(R^5)_2$;

–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)R^5$;

–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)OR^5$;

–(C_1-C_{12} alkyldiyl)–$N(R^5)C(=O)N(R^5)_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_2-C_6 alkenyldiyl)–$NR^5C(=NR^4)NR^5$–*;

–(C_2-C_6 alkenyldiyl)–N(R^5)$_2$;

–(C_2-C_6 alkenyldiyl)–NR^5–*;

–(C_2-C_6 alkynyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_2-C_6 alkynyldiyl)–N(R^5)$_2$;

–(C_2-C_6 alkynyldiyl)–NR^5–*;

–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_6-C_{20} aryldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

R^2 is selected from the group consisting of:

H;

C_1-C_8 alkyl;

–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)N(R^5)–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)C(NR^5)=N–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–N(R^5)C(=O)R^5;

–(C_1-C_{12} alkyldiyl)–N(R^5)C(=O)OR^5;

–(C_1-C_{12} alkyldiyl)–N(R^5)C(=O)N(R^5)$_2$;

–(C_2-C_6 alkenyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_2-C_6 alkenyldiyl)–N(R^5)$_2$;

–(C_2-C_6 alkenyldiyl)–NR^5–*;

–(C_2-C_6 alkynyldiyl)–NR^5C(=NR^4)NR^5–*;

–(C_2-C_6 alkynyldiyl)–N(R^5)$_2$;

–(C_2-C_6 alkynyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–(C_2-C_{20} heterocyclyldiyl);

–(C_1-C_{12} alkyldiyl)–(C_1-C_{20} heteroaryldiyl);

–(C_1-C_{12} alkyldiyl)–(C_6-C_{20} aryldiyl);

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5C(=NR^4)NR^5–*;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

R^4 is selected from the group consisting of C_6-C_{20} aryl and C_1-C_8 alkyl;

R^5 is selected from the group consisting of H and C_1-C_8 alkyl;

or two R^5 groups form a 5- or 6-membered heterocyclyl ring; and

R^3 is selected from the group consisting of H, $-C(=O)NR^5R^6$, and phenyl, where phenyl is substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, $-CN$, $-CH_3$, $-CF_3$, $-CO_2H$, $-NH_2$, $-NHCH_3$, $-NO_2$, $-OH$, $-OCH_3$, $-SCH_3$, $-S(O)_2CH_3$, $-S(O)_3H$, and R^7;

R^6 is independently selected from the group consisting of

H;

C_1-C_8 alkyl;

$-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$;

$-(C_1$-C_{12} alkyldiyl$)-NR^5-*$;

$-(C_1$-C_{12} alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2$-C_{20} heterocyclyl$)$;

$-(C_2$-C_{20} heterocyclyldiyl$)-*$;

$-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$;

$--(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-NR^5-*$;

$-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-OH$;

$-(C_1$-C_{20} heteroaryldiyl$)-(C_2$-C_{20} heterocyclyldiyl$)-C(=O)NR^5-(C_1$-C_{12} alkyldiyl$)-NR^5-*$;

$-(C_1$-C_{20} heteroaryldiyl$)-NR^5-*$;

$-(C_1$-C_{20} heteroaryldiyl$)-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$;

$-(C_6$-C_{20} aryldiyl$)-S(=O)_2-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-(C_6$-C_{20} aryldiyl$)-S(=O)_2-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_6$-C_{20} aryldiyl$)-S(=O)_2-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$;

$-(C_6$-C_{20} aryldiyl$)-S(=O)_2-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-NR^5-*$; and

$-(C_6$-C_{20} aryldiyl$)-S(=O)_2-(C_2$-C_{20} heterocyclyldiyl$)-(C_1$-C_{12} alkyldiyl$)-OH$;

R^7 is selected from the group consisting of:

$-(C_1$-C_{12} alkyldiyl$)-N(R^5)_2$;

–(C_1-C_{12} alkyldiyl)–NR^5–*;

–(C_1-C_{12} alkyldiyl)–NR^{5}C(=NR^4)NR^5–*;

–C(=O)–*;

–C(=O)–(C_2-C_{20} heterocyclyl);

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^{5}C(=NR^4)NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_2-C_{20} heterocyclyldiyl)–C(=O)NR^5–(C_1-C_{12} alkyldiyl)–NR^5–*;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–NR^5–*;

–C(=O)N(R^5)$_2$;

–C(=O)NR^5–(C_1-C_{20} heteroaryldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–NR^5–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^{5}C(=NR^4)N(R^5)$_2$;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^{5}C(=NR^4)NR^5–*;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–N(R^5)$_2$;

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–NR^5–*; and

–S(=O)$_2$–(C_2-C_{20} heterocyclyldiyl)–(C_1-C_{12} alkyldiyl)–OH;

where * indicates the attachment site of L;

L is the linker selected from the group consisting of:

–(PEP)–C(=O)–(PEG)–C(=O)–Q;

–NR^5–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–NR^5–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–N$^+$(R^5)$_2$–(PEG)–C(=O)–Q;

–C(=O)–(PEG)–C(=O)–Q;

–C(=O)–CH(AA$_1$)–NR^5–C(=O)–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(PEG)–C(=O)–CH(AA$_1$)–NR^5–(PEG)–C(=O)–Q;

–C(=O)O–(C_1-C_{12} alkyldiyl)–SS–(PEG)–C(=O)–Q;

–C(=O)–(C_1-C_{12} alkyldiyl)–SS–(PEG)–C(=O)–Q;

–(PEG)–C(=O)–Q;

–(PEP)–C(=O)–(C_1-C_{12} alkyldiyl)–C(=O)–Q;

–(MCgluc)–(C(=O)–(PEG)–OCH_2–(C_1-C_{20} heteroaryldiyl)–$CH_2CH_2OCH_2CH_2$–C(=O)–Q;

–(PEP)–C(=O)–$(CH_2)_m$–C(=O)–Q; and

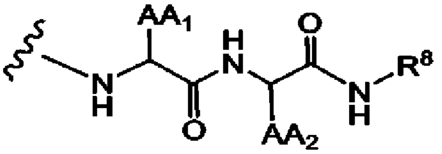
–(PEP)–C(=O)–$(CH_2)_m$–Q;

where

PEG has the formula:

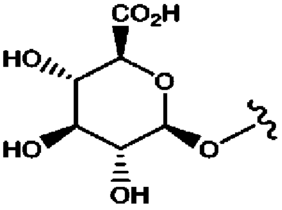
–$(CH_2CH_2O)_n$–$(CH_2)_m$–; m is an integer from 1 to 5, and n is an integer from 2 to 50;

PEP has the formula:



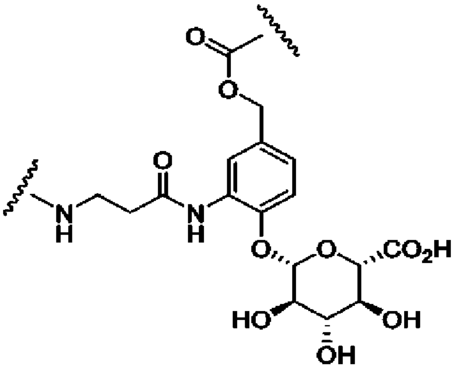
where AA_1 and AA_2 are independently selected from an amino acid side chain, or AA_1 or AA_2 and an adjacent nitrogen atom form a 5-membered ring proline amino acid, and the wavy line indicates a point of attachment and;

R^8 is selected from the group consisting of C_6-C_{20} aryldiyl and C_1-C_{20} heteroaryldiyl substituted with –CH_2O–C(=O)–, and optionally with:

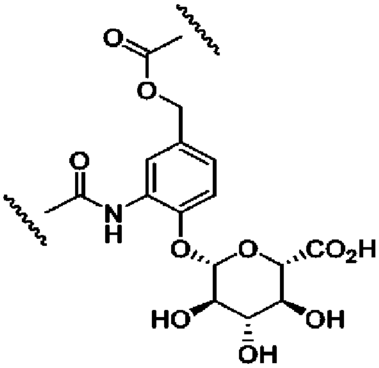


; and

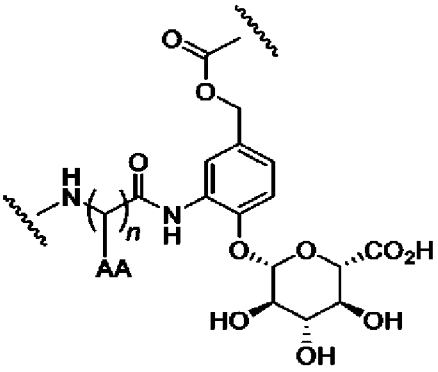
MCgluc is selected from the groups:



;



; and

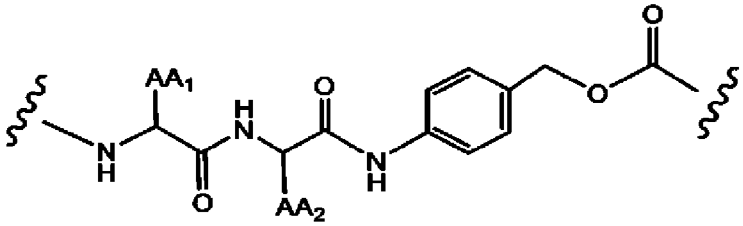


where n is 1 to 8, and AA is an amino acid side chain; and

Q is selected from the group consisting of N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, maleimide, and phenoxy substituted with one or more groups independently selected from F, Cl, NO_2, and SO_3^-;

where alkyl, alkyldiyl, aryl, aryldiyl carbocyclyl, carbocyclyldiyl, heterocyclyl, heterocyclyldiyl, heteroaryl, and heteroaryldiyl are optionally substituted with one or more groups independently selected from F, Cl, Br, I, –CN, $-CH_3$, $-CH_2CH_3$, $-CH=CH_2$, $-C\equiv CH$, $-C\equiv CCH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$, $-CH_2OH$, $-CH_2OCH_3$, $-CH_2CH_2OH$, $-C(CH_3)_2OH$, $-CH(OH)CH(CH_3)_2$, $-C(CH_3)_2CH_2OH$, $-CH_2CH_2SO_2CH_3$, $-CH_2OP(O)(OH)_2$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CH_2CHF_2$, $-CH(CH_3)CN$, $-C(CH_3)_2CN$, $-CH_2CN$, $-CH_2NH_2$, $-CH_2NHSO_2CH_3$, $-CH_2NHCH_3$, $-CH_2N(CH_3)_2$, $-CO_2H$, $-COCH_3$, $-CO_2CH_3$, $-CO_2C(CH_3)_3$, $-COCH(OH)CH_3$, $-CONH_2$, $-CONHCH_3$, $-CON(CH_3)_2$, $-C(CH_3)_2CONH_2$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-NHCOCH_3$, $-N(CH_3)COCH_3$, $-NHS(O)_2CH_3$, $-N(CH_3)C(CH_3)_2CONH_2$, $-N(CH_3)CH_2CH_2S(O)_2CH_3$, $-NO_2$, =O, –OH, $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_2CH_2N(CH_3)_2$, $-O(CH_2CH_2O)_n-(CH_2)_mCO_2H$, $-O(CH_2CH_2O)_nH$, $-OP(O)(OH)_2$, $-S(O)_2N(CH_3)_2$, $-SCH_3$, $-S(O)_2CH_3$, and $-S(O)_3H$.

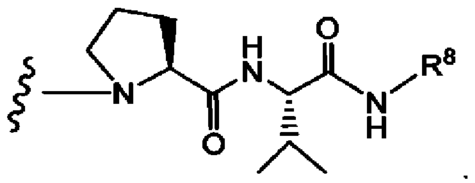
30. The aminoquinoline-linker compound of claim 29 wherein PEP has the formula:



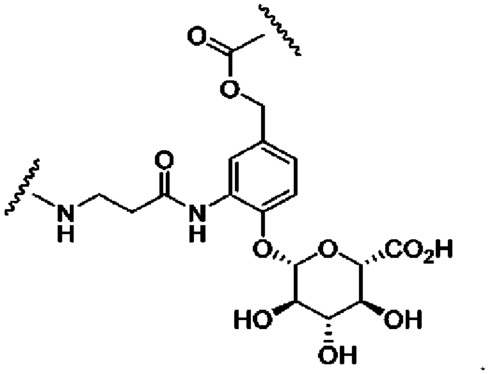
wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

31. The aminoquinoline-linker compound of claim 29 wherein AA_1 or AA_2 with an adjacent nitrogen atom form a 5-membered ring to form a proline amino acid.

32. The aminoquinoline-linker compound of claim 29 wherein PEP has the formula:



33. The aminoquinoline-linker compound of claim 29 wherein MCgluc has the formula:



34. The aminoquinoline-linker compound of claim 29 wherein AA_1 and AA_2 are independently selected from a side chain of a naturally-occurring amino acid.

35. The aminoquinoline-linker compound of claim 29 wherein AA_1 and AA_2 are independently selected from H, $-CH_3$, $-CH(CH_3)_2$, $-CH_2(C_6H_5)$, $-CH_2CH_2CH_2CH_2NH_2$, $-CH_2CH_2CH_2NHC(NH)NH_2$, $-CHCH(CH_3)CH_3$, $-CH_2SO_3H$, and $-CH_2CH_2CH_2NHC(O)NH_2$.

36. The aminoquinoline-linker compound of claim 35 wherein AA_1 is $-CH(CH_3)_2$, and AA_2 is $-CH_2CH_2CH_2NHC(O)NH_2$.

37. The aminoquinoline-linker compound of claim 29 wherein AA_1 and AA_2 are independently selected from GlcNAc aspartic acid, $-CH_2SO_3H$, and $-CH_2OPO_3H$.

38. The aminoquinoline-linker compound of claim 29 wherein R^1 is attached to L.

39. The aminoquinoline-linker compound of claim 29 wherein R^2 is attached to L.

40. The aminoquinoline-linker compound of claim 29 wherein R^3 is attached to L.

41. The aminoquinoline-linker compound of claim 29 wherein R^1 is selected from the group consisting of:

C_1-C_8 alkyl;

$-(C_1$-C_{12} alkyldiyl)$-NR^5C(=NR^4)NR^5-*$;

$-(C_1$-C_{12} alkyldiyl)$-N(R^5)_2$; and

$-(C_1$-C_{12} alkyldiyl)$-NR^5-*$.

42. The aminoquinoline-linker compound of claim 29 wherein R^2 is selected from the group consisting of:

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$; and

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$.

43. The aminoquinoline-linker compound of claim 29 wherein R^6 is selected from the group consisting of:

$C_1\text{-}C_8$ alkyl;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$;

$-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$.

44. The aminoquinoline-linker compound of claim 29 wherein R^6 is selected from the group consisting of:

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)N(R^5)_2$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5C(=NR^4)NR^5-*$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-(C_6\text{-}C_{20}$ aryldiyl$)-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$.

45. The aminoquinoline-linker compound of claim 29 wherein R^7 is selected from the group consisting of:

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)- NR^5C(=NR^4)N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)- NR^5C(=NR^4)NR^5-*$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-N(R^5)_2$;

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-NR^5-*$; and

$-S(=O)_2-(C_2\text{-}C_{20}$ heterocyclyldiyl$)-(C_1\text{-}C_{12}$ alkyldiyl$)-OH$.

46. The aminoquinoline-linker compound of claim 29 wherein L is selected from the group consisting of:

$-(PEP)-C(=O)-(PEG)-C(=O)-Q$;

$-NR^5-(PEG)-C(=O)-Q$;

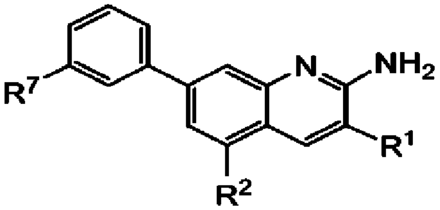
$-C(=O)-(PEG)-C(=O)-Q$; and

$-(PEG)-C(=O)-Q$.

47. The aminoquinoline-linker compound of claim 29 wherein Q is phenoxy substituted with one or more F.

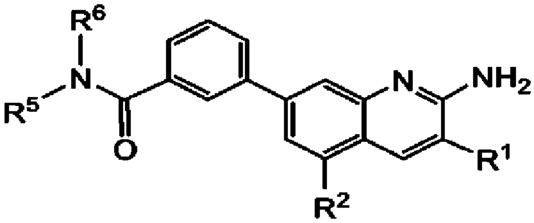
48. The aminoquinoline-linker compound of claim 29 wherein Q is 2,3,5,6-tetrafluorophenoxy.

49. The aminoquinoline-linker compound of claim 29 wherein AQ is selected from Formula IIIa:



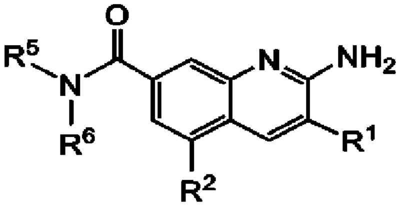
IIIa .

50. The aminoquinoline-linker compound of claim 29 wherein AQ is selected from Formula IIIb:



IIIb .

51. The aminoquinoline-linker compound of claim 29 wherein AQ is selected from Formula IIIc:



IIIc .

52. The aminoquinoline-linker compound of claim 29 selected from Table 1.

53. A method for treating cancer comprising administering a therapeutically effective amount of an immunoconjugate according to any one of claims 1 to 7, to a patient in need thereof.

54. The method of claim 53, wherein the cancer is susceptible to a pro-inflammatory response induced by TLR7 and/or TLR8 agonism.

55. The method of claim 53, wherein the cancer is a PD-L1-expressing cancer.

56. The method of claim 53, wherein the cancer is a HER2-expressing cancer.

57. The method of claim 53, wherein the cancer is a CEA-expressing cancer.

58. The method of any one of claims 53-57, wherein the cancer is selected from bladder cancer, urinary tract cancer, urothelial carcinoma, lung cancer, non-small cell lung cancer, Merkel cell carcinoma, colon cancer, colorectal cancer, gastric cancer, and breast cancer.

59. The method of claim 58, wherein the breast cancer is triple-negative breast cancer.

60. The method of claim 58, wherein the Merkel cell carcinoma cancer is metastatic Merkel cell carcinoma.

61. The method of claim 58, wherein the gastric cancer is HER2 overexpressing gastric cancer.

62. The method of claim 58, wherein the cancer is gastroesophageal junction adenocarcinoma.

63. Use of an immunoconjugate according to any one of claims 1 to 7 for treating cancer.

64. A method of preparing an immunoconjugate of Formula I of claim 1 wherein an aminoquinoline-linker compound of Formula III of claim 29 is conjugated with the antibody.

65. An immunoconjugate of claim 1 prepared by conjugation of an antibody with the aminoquinoline-linker compound of claim 29.

66. The immunoconjugate of claim 1 prepared by conjugation of an antibody with an aminoquinoline-linker compound of Table 2.