

A pharmaceutical composition of anti-FGFR3 antibody drug conjugate and applications thereof

Technical Field

[0001] The present disclosure relates to the biopharmaceutical field, in particular, conjugates containing anti-FGFR3 antibodies and linker-payloads, and the corresponding pharmaceutical composition, preparing process and use thereof.

Background

[0002] Fibroblast growth factor (FGF) and its tyrosine kinase receptor (FGFR) play important roles in embryonic development, maintenance of homeostasis in various tissues, wound healing processes and metabolic functions. In humans, there are FGFRs (FGFR1-4) and FGFs (FGF 1-14 and FGF 16-23) with a high homology. FGFR contains an extracellular region comprising three immune domains as D1, D2 and D3, a single transmembrane region and a dividing cytoplasmic kinase moiety.

[0003] Dysregulation of signaling by FGFR1-4 is associated with several types of cancer. Genomic FGFR mutations including gene amplification, chromosomal translocation and activating mutations induce abnormal activation of the FGF pathway and promote tumor transformation. In particular, the amplification of FGFR3 is associated with the development of solid tumors such as brain cancer, bladder cancer, urothelial cancer, cervical cancer, and intrahepatic cholangiocarcinoma. Additionally, missense FGFR mutations are found in several types of cancer, and FGF-driven signaling and tumor cell proliferation can be enhanced by S249C of FGFR3. The FGFR3 fusion proteins exert a constitutive activation of the kinase domain as cancer driver alterations. The known FGFR3 fusion partners are TACC3, BAIAP2L1, AES, ELAVL3, JAKMIP1, TNIP2, and WHSC1.

[0004] Efforts have been made to develop therapeutics targeting FGFR3 to date.

[0005] B701 (Vofatamab) is a human immunoglobulin G1 monoclonal antibody against FGFR3, and clinical trials have been conducted to confirm whether it exhibits anti-tumor activity and the possibility of combination with docetaxel. When the anti-FGFR3 monoclonal antibody B-701 is administered, it specifically binds and inhibits both wild-type and mutant FGFR3 to inhibit FGFR3 phosphorylation, thereby inhibiting FGFR3 activation and FGFR3-mediated signaling pathways. Thereby, cell proliferation is inhibited, and apoptosis is induced in FGFR3-expressing tumors.

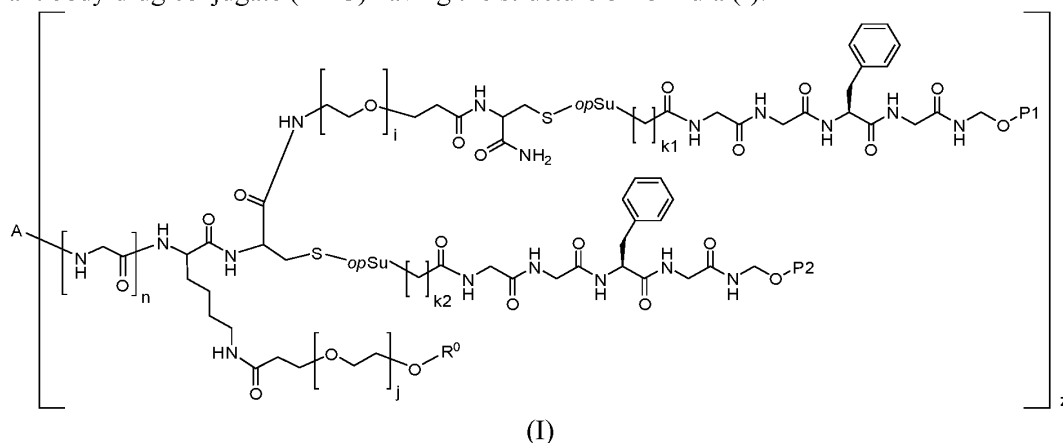
[0006] The present disclosure provides a pharmaceutical composition of an FGFR3 targeted antibody drug conjugate (ADC).

[0007] As known in the art, active pharmaceutical ingredients (APIs) determine the use of the

drugs, and the prescriptions of pharmaceutical formulation are closely related to the nature of APIs. For example, pH of the formulations can affect the stability of the APIs, and excipients not only have an impact on the solubility and dissolution of APIs, but also have a significant impact on the properties of APIs such as permeability and absorption. Thus, it is very necessary to develop formulations for specific APIs.

Summary

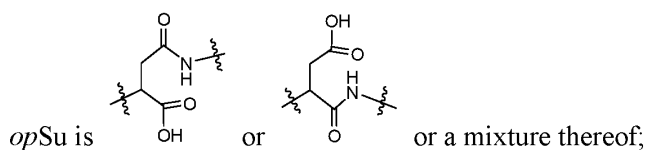
[0008] In a first aspect of the present invention, provided is a pharmaceutical composition comprising: an antibody drug conjugate (ADC), a buffer, and an optional stabilizer; where the antibody drug conjugate (ADC) having the structure of formula (I):



wherein,

A is an anti-FGFR3 antibody or an antigen binding fragment, the antibody or antigen binding fragment is modified to connect with the (Gly)_n moiety in the compound of formula (I), wherein the antibody or an antigen binding fragment comprises CDRs: a heavy chain CDR1 comprising amino acid sequence of SEQ ID NO: 9 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 9, a heavy chain CDR2 comprising amino acid sequence of SEQ ID NO: 10 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 10, a heavy chain CDR3 comprising amino acid sequence of SEQ ID NO: 11 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 11, a light chain CDR1 comprising amino acid sequence of SEQ ID NO: 12 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 12, a light chain CDR2 comprising amino acid sequence of SEQ ID NO: 13 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 13, and a light chain CDR3 comprising amino acid sequence of SEQ ID NO: 14 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 14;

z is an integer of 1 to 20; preferably 1 to 4; particularly 2;



R^0 is C_{1-10} alkyl;

n is an integer of 2 to 20;

k_1 and k_2 are independently an integer of 1 to 7;

i is an integer of 1-20;

j is an integer of 1-20;

P_1 and P_2 are independently a payload.

[0009] In some embodiments, the connection process between the modified antibody (or antigen binding fragment) and the other moiety in formula (I) is catalyzed by a ligase.

[0010] In some embodiments, the antibody or an antigen binding fragment comprises CDRs: a heavy chain CDR1 comprising amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising amino acid sequence of SEQ ID NO: 10, a heavy chain CDR3 comprising amino acid sequence of SEQ ID NO: 11, a light chain CDR1 comprising amino acid sequence of SEQ ID NO: 12, a light chain CDR2 comprising amino acid sequence of SEQ ID NO: 13, and a light chain CDR3 comprising amino acid sequence of SEQ ID NO: 14.

[0011] In some embodiments, the K_D value of the anti-FGFR3 antibody or an antigen binding fragment binding to human FGFR3 and monkey FGFR3 is less than 10 nM.

[0012] In some embodiments, the anti-FGFR3 antibody or an antigen binding fragment doesn't bind to mouse FGFR3.

[0013] In some embodiments, the payload is a cytotoxin or a fragment thereof, with an optional derivatization in order to connect the payload and the rest moiety of the ADC;

the cytotoxin is selected from the group consisting of taxanes, maytansinoids, auristatins, epothilones, combretastatin A-4 phosphate, combretastatin A-4 and derivatives thereof, indol-sulfonamides, vinblastines such as vinblastine, vincristine, vindesine, vinorelbine, vinflunine, vinglycinat, anhy-drovinblastine, dolastatin 10 and analogues, halichondrin B, eribulin, indole-3-oxoacetamide, podophyllotoxins, 7-diethylamino-3-(2'-benzoxazolyl)-coumarin (DBC), discodermolide, laulimalide, camptothecins and derivatives thereof, mitoxantrone, mitoguazone, nitrogen mustards, nitrosoureas, aziridines, benzodepa, carboquone, meturedpa, uredpa, dynemicin, esperamicin, neocarzinostatin, aclacinomycin, actinomycin, antramycin, bleomycins, actinomycin C, carabycin, carminomycin, cardinophyllin, carminomycin, actinomycin D, daunorubicin, detorubicin, adriamycin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, nogalamycin, olivomycin, peplomycin, porfiromycin, puromycin, ferric adriamycin, rodorubicin, rufocromomycin, streptozocin, zinostatin, zorubicin, trichothecene, T-2 toxin, verracurin A, bacilloporin A, anguidine, ubenimex, azaserine, 6-

diazo-5-oxo-L-norleucine, dimethyl folic acid, methotrexate, pteropterin, trimetrexate, edatrexate, fludarabine, 6-mercaptopurine, tiamiprine, thioguanine, ancitabine, gemcitabine, enocitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, floxuridine, calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone, aminoglutethimide, mitotane, trilostane, flutamide, nilutamide, bicalutamide, leuprorelin acetate, protein kinase inhibitors and a proteasome inhibitors; and/or

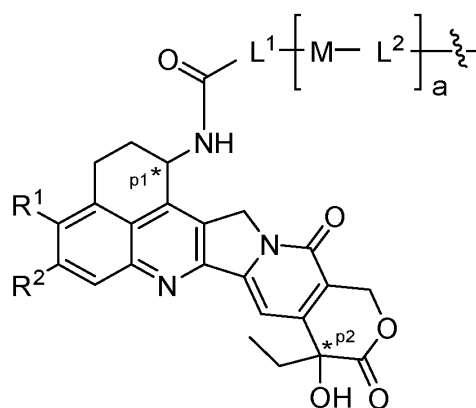
selected from vinblastines, colchicines, taxanes, auristatins, maytansinoids, calicheamicin, doxonubicin, duocarmycins, SN-38, cryptophycin analogue, deruxtecan, duocarmazine, calicheamicin, centanamycin, dolastatins, pyrrolobenzodiazepine, exatecan and derivatives thereof; and/or

selected from auristatins, especially MMAE, MMAF or MMAD; and/or

selected from exatecan and derivatives thereof, such as DX8951f; and/or

selected from DXd-(1) and DXd-(2); preferably DXd-(1).

[0014] In some embodiments, the payload has the structure of formula (II):



(II)

wherein,

a is 0 or 1;

the carbon atoms marked with p1* and p2* each is asymmetric center, and the asymmetric center is S configured, R configured or racemic;

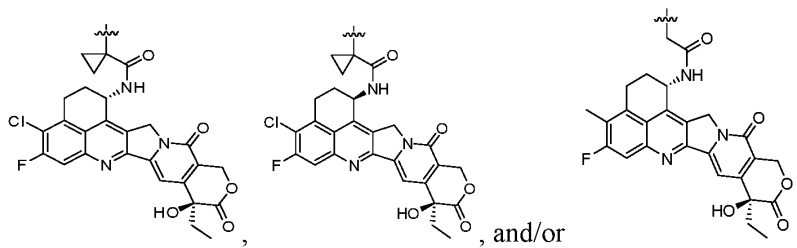
L¹ is selected from C₁₋₆ alkylene, which is unsubstituted or substituted with one substituent selected from halogen, -OH and -NH₂;

M is -CH₂-, -NH- or -O-;

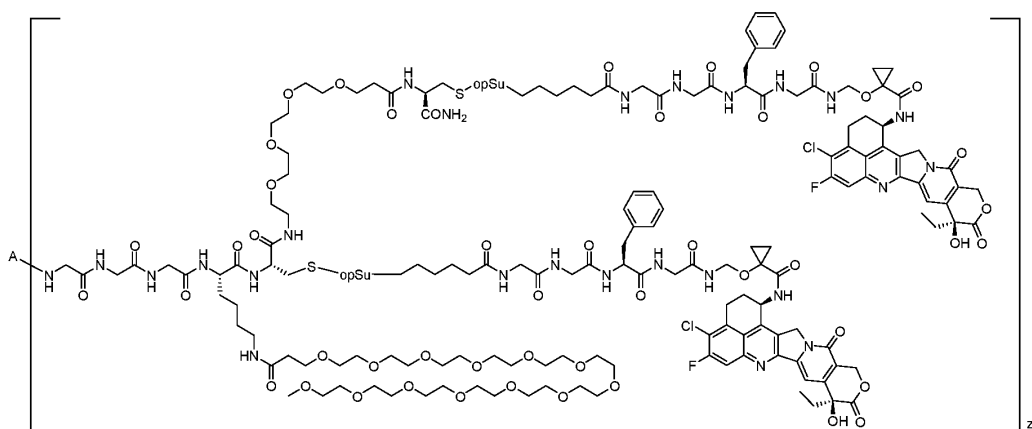
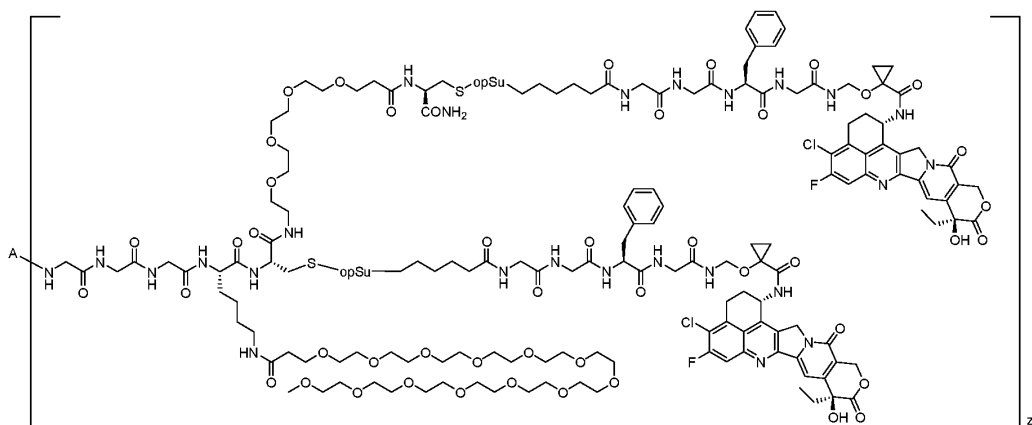
L² is C₁₋₃ alkylene;

R¹ and R² are each independently selected from hydrogen, C₁₋₆ alkyl, halogen and C₁₋₆ alkoxy.

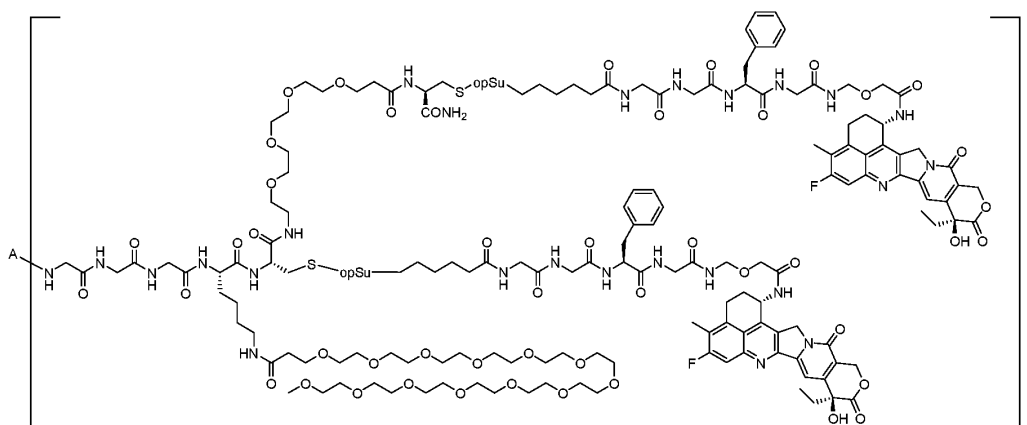
[0015] In some embodiments, wherein the payload is selected from:



[0016] In some embodiments, the ADC is selected from:



, and/or



[0017] In some embodiments, the antibody or antigen binding fragment comprises a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 15; and/or

a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 16.

[0018] In some embodiments, the antibody or antigen binding fragment comprises a VH domain comprising an amino acid sequence of SEQ ID NO: 15; and/or

a VL domain comprising an amino acid sequence of SEQ ID NO: 16.

[0019] In some embodiments, the antibody or antigen binding fragment comprises a VH domain comprising an amino acid sequence of SEQ ID NO: 15; and

a VL domain comprising an amino acid sequence of SEQ ID NO: 16.

[0020] In some embodiments, the antibody or an antigen binding fragment comprises a heavy constant domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or

a light constant domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 19.

[0021] In some embodiments, the antibody or an antigen binding fragment comprises a heavy constant domain comprising an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or

a light constant domain comprising an amino acid sequence of SEQ ID NO: 19.

[0022] In some embodiments, the antibody or an antigen binding fragment comprises a heavy constant domain comprising an amino acid sequence of SEQ ID NO: 17, a light constant domain comprising an amino acid sequence of SEQ ID NO: 19.

[0023] In some embodiments, the antibody or an antigen binding fragment comprises a heavy

constant domain comprising an amino acid sequence of SEQ ID NO: 18, a light constant domain comprising an amino acid sequence of SEQ ID NO: 19.

[0024] In some embodiments, wherein the antibody or the antigen-binding fragment comprises C-terminal modification of the heavy chain and/or C-terminal modification of the light chain. In some embodiments, the antibody, Sp and recognition sequence of the ligase donor substrate are sequentially linked; Sp is a spacer sequence selected from GA, GGGGS, GGGSGGGGS and GGGSGGGSGGGGS; the recognition sequence of the ligase donor substrate is LPXTGJ, wherein X can be any single amino acid that is natural or unnatural; J is absent, or is an amino acid fragment comprising 1-10 amino acids.

[0025] In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 20 or 21; and/or

a light chain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 22.

[0026] In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 20, and

a light chain comprising an amino acid sequence of SEQ ID NO: 22.

[0027] In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 21, and

a light chain comprising an amino acid sequence of SEQ ID NO: 22.

[0028] In some embodiments, the antibody drug conjugate has a drug to antibody ratio (DAR) of an integer or non-integer of 1 to 20, particularly 2 to 8. In some embodiments, the value of DAR is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, or the range between any two values (including the end value). In an embodiment, the antibody drug conjugate has a DAR of about 3 to about 4, preferably 3.4 to 3.9, particularly about 3.6 to about 3.7.

[0029] In some embodiments, the buffer in pharmaceutical composition is selected from citrate buffer, phosphate buffer, histidine buffer and glutamic acid buffer. In some embodiments, the concentration of the buffer is about 10-40 mM; or the concentration of the buffer is about 15-25 mM; preferably about 20 mM. In some embodiments, the concentration of the buffer is about 10 mM, about 15 mM, about 17 mM, about 18 mM, about 19 mM, about 19.5 mM, about 20 mM, about 20.4 mM, about 21 mM, about 25 mM, about 27 mM, about 32 mM, about 38 mM, about 40 mM, or the range between any two values (including the end value). In some embodiments, the pH of the buffer is about 5.0-7.0; preferably about 5.5-6.5, or about 5.9-6.1; more preferably about 6.0. In some embodiments, the pH of the buffer is about 5.0, about 5.5,

about 5.7, about 5.8, about 5.9, about 5.95, about 6.0, about 6.03, about 6.1, about 6.2, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 7.0, or the range between any two values (including the end value).

[0030] In some embodiments, the stabilizer comprises one or more of sucrose, an arginine salt, arginine, sodium chloride and sorbitol; preferably sucrose. In some embodiments, the pharmaceutical composition comprises the stabilizer, and the concentration of the stabilizer is about 2-15% (W/V) or about 100-160 mM. In some embodiments, the concentration of the stabilizer is about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, or the range between any two values (including the end value). In some embodiments, the concentration of the stabilizer is about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 137 mM, about 140 mM, about 142 mM, about 150 mM, about 160 mM, or the range between any two values (including the end value).

[0031] In some embodiments, the stabilizer is sucrose and the concentration of sucrose is about 5-15% (W/V), preferably about 6-10% (W/V); more preferably about 8% (W/V). In some embodiments, the concentration of sucrose is about 7%, about 7.5%, about 8%, or about 8.4%.

[0032] In some embodiments, the stabilizer is sorbitol and the concentration of sorbitol is about 2-8% (W/V), preferably about 3.5-6% (W/V); more preferably about 4.3%, about 4.5% or about 4.8%.

[0033] In some embodiments, the stabilizer is arginine hydrochloride and the concentration of arginine hydrochloride is about 100-160 mM, preferably about 120-160 mM; more preferably about 137 mM, about 138 mM, about 139 mM, about 140 mM, about 141 mM, or about 142 mM.

[0034] In some embodiments, the stabilizer is sodium chloride and the concentration of sodium chloride is about 100-160 mM, preferably about 120-160 mM; more preferably about 137 mM, about 138 mM, about 139 mM, about 140 mM, about 141 mM, or about 142 mM.

[0035] In some embodiments, the pharmaceutical composition further comprises a surfactant. In some embodiments, the surfactant is polysorbate. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80. In some embodiments, and the concentration of the surfactant is about 0.005-0.1% (W/V). In some embodiments, the concentration of the surfactant is about 0.005%, about 0.008%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, or the range between any two values (including the end value).

[0036] In some embodiments, the surfactant is polysorbate 80, and the concentration of the polysorbate 80 is about 0.005-0.1% (W/V), preferably about 0.005-0.05% (W/V), more preferably about 0.01-0.04% (W/V), especially about 0.01% (W/V), about 0.02% (W/V) or

about 0.04% (W/V).

[0037] In some embodiments, the concentration of the protein in ADC is about 15-60 mg/ml. In some embodiments, the concentration of the protein in ADC is about 15-30 mg/ml. In some embodiments, the concentration of the protein in ADC is about 15 mg/ml, about 16 mg/ml, about 18 mg/ml, about 19 mg/ml, about 19.6 mg/ml, about 20 mg/ml, about 20.5 mg/ml, about 21 mg/ml, about 22 mg/ml, about 28 mg/ml, about 30 mg/ml, about 35 mg/ml, about 39 mg/ml, about 44 mg/ml, about 47 mg/ml, about 50 mg/ml, about 52 mg/ml, about 55 mg/ml, about 60 mg/ml, or the range between any two values (including the end value).

[0038] In some embodiments, provided is a pharmaceutical composition comprising:

15-60 mg/ml ADC of compound (I), 10-40 mM histidine buffer (pH 5.0-7.0), 2-15% (W/V), 100-160 mM stabilizer, and 0.005-0.1% (W/V) surfactant.

[0039] In some embodiments, the pharmaceutical composition comprises: 15-60 mg/ml ADC of compound (I), 10-40 mM histidine buffer (pH 5.0-7.0), 2-15% (W/V), 100-160 mM stabilizer, and 0.005-0.1% (W/V) surfactant; wherein the stabilizer is sucrose, sorbitol, arginine hydrochloride or sodium chloride.

[0040] In some embodiments, the pharmaceutical composition comprises: 15-30 mg/ml ADC of compound (I), 15-26 mM histidine buffer (pH 5.8-6.1), 2.7-12% (W/V), 123-160 mM stabilizer, and 0.008-0.1% (W/V) polysorbate; wherein the stabilizer is sucrose, sorbitol, arginine hydrochloride or sodium chloride.

[0041] In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.02% (W/V) polysorbate 80. In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 4.5% (W/V) sorbitol and 0.02% (W/V) polysorbate 80. In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 140 mM arginine hydrochloride and 0.02% (W/V) polysorbate 80. In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 140 mM sodium chloride and 0.02% (W/V) polysorbate 80. In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.01% (W/V) polysorbate 80. In some embodiments, the pharmaceutical composition comprises: 20 mg/ml ADC of compound (I), 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.04% (W/V) polysorbate 80.

[0042] In a second aspect, provided is use of the pharmaceutical composition in the manufacture of a medicament for treating a disease; wherein the disease is FGFR3-mediated disease.

[0043] In some embodiments, the disease includes tumor overexpressing FGFR3, tumor with

FGFR3 gene fusion or tumor with FGFR3 gene mutation. In some embodiments, the disease includes FGFR3-positive tumor. In some embodiments, the disease includes tumor overexpressing FGFR3 or tumor with FGFR3 alteration. In some embodiments, the disease includes tumor with FGFR3 infusion (such as TACC3 fusion, intracellular fusion) or tumor with FGFR3 gene mutation (such as Y373C, G380R, S371C, S249C or R248C). In some embodiments, the disease is selected from the group consisting of: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma, multiple myeloma, head and neck cancer. In some embodiments, the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, or intrahepatic cholangiocarcinoma. In some embodiments, the disease is glioblastoma, bladder cancer, or multiple myeloma.

[0044] In some embodiments, the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, multiple myeloma or intrahepatic cholangiocarcinoma.

[0045] In a third aspect, provided is a method for treating a subject suffering a disease or preventing disease progression, comprises administering the pharmaceutical composition to the subject, and the disease is FGFR3-mediated disease.

[0046] In some embodiments, the disease includes tumor overexpressing FGFR3 or tumor with FGFR3 gene mutation. In some embodiments, the disease includes FGFR3-positive tumor. In some embodiments, the disease includes tumor overexpressing FGFR3 or tumor with FGFR3 alteration. In some embodiments, the disease includes tumor with FGFR3 infusion (such as TACC3 fusion, intracellular fusion) or tumor with FGFR3 gene mutation (such as Y373C, G380R, S371C, S249C or R248C). In some embodiments, the disease is selected from the group consisting of: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma,

ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma, multiple myeloma, head and neck cancer. In some embodiments, the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, or intrahepatic cholangiocarcinoma. In some embodiments, the disease is glioblastoma, bladder cancer, or multiple myeloma. In some embodiments, the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, multiple myeloma or intrahepatic cholangiocarcinoma.

Brief Description of the Drawings

[0047] Figure 1.1 and Figure 1.2 show in vivo efficacy test in glioblastoma PDX models.

[0048] Figure 2 shows bystander killing effect of ADC19 and ADC20.

Detailed Description

[0049] The specific embodiments are provided below to illustrate technical contents of the present disclosure. Those skilled in the art can easily understand other advantages and effects of the present disclosure through the contents disclosed in the specification. The present disclosure can also be implemented or applied through other different specific embodiments. Various modifications and variations can be made by those skilled in the art without departing from the spirit of the present disclosure.

Definitions

[0050] Unless otherwise defined hereinafter, all technical and scientific terms used herein have the same meaning as commonly understood by those skilled in the art. The techniques used herein refer to those that are generally understood in the art, including the variants and equivalent substitutions that are obvious to those skilled in the art. While the following terms are believed to be readily comprehensible by those skilled in the art, the following definitions are set forth to better illustrate the present disclosure. When a trade name is present herein, it refers to the corresponding commodity or the active ingredient thereof. All patents, published patents applications and publications cited herein are hereby incorporated by reference.

[0051] When a certain amount, concentration, or other value or parameter is set forth in the form of a range, a preferred range, or a preferred upper limit or a preferred lower limit, it should

be understood that it is equivalent to specifically revealing any range formed by combining any upper limit or preferred value with any lower limit or preferred value, regardless of whether the said range is explicitly recited. Unless otherwise stated, the numerical ranges listed herein are intended to include the endpoints of the range and all integers and fractions (decimals) within the range. For example, the expression “i is an integer of 1 to 20” means that i is any integer of 1 to 20, for example, i can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Other similar expressions such as j, k1, k2, n, k and z should also be understood in a similar manner.

[0052] “Conservative amino acid substitution” refers to the replacement of one amino acid residue with another amino acid residue having a side chain (R group) of similar chemical nature (e.g., charge or hydrophobicity). Generally, conservative amino acid substitutions do not substantially alter the functional properties of the protein. Examples of amino acid classes with a side chain of similar chemical nature include: 1) an aliphatic side chain: glycine, alanine, valine, leucine and isoleucine; 2) an aliphatic hydroxyl side chain: serine and threonine; 3) an amide-containing side chain: asparagine and glutamine; 4) an aromatic side chain: phenylalanine, tyrosine and tryptophan; 5) a basic side chain: lysine, arginine and histidine; and 6) an acidic side chain: aspartic acid and glutamic acid.

[0053] Unless otherwise stated herein, singular forms like “a” and “the” include the plural forms. The expression “one or more” or “at least one” may mean 1, 2, 3, 4, 5, 6, 7, 8, 9 or more.

[0054] The terms “about” and “approximately”, when used in connection with a numerical variable, generally mean that the value of the variable and all values of the variable are within experimental error (for example, within a 95% confidence interval for the mean) or within $\pm 10\%$ of a specified value, or a wider range.

[0055] The term “optional” or “optionally” means the event described subsequent thereto may, but not necessarily happen, and the description includes the cases wherein said event or circumstance happens or does not happen.

[0056] The expression “comprising” or similar expressions “including”, “containing” and “having” are open-ended, and do not exclude additional unrecited elements, steps, or ingredients. The expression “consisting of” excludes any element, step, or ingredient not designated. The expression “consisting essentially of” means that the scope is limited to the designated elements, steps or ingredients, plus elements, steps or ingredients that are optionally present that do not substantially affect the essential and novel characteristics of the claimed subject matter. It should be understood that the expression “comprising” encompasses the expressions “consisting essentially of” and “consisting of”.

[0057] As used herein, the concentration “%” represents a mass volume concentration in the unit of g/ml. For example, 8% sucrose solution represents that 8 g of sucrose is dissolved in the

solvent to form 100 ml of solution, which means that the solution contains 8 g sucrose per 100 ml.

[0058] The amount of buffer in the present disclosure refers to the total amount of the buffer pair in the buffer system constituting the buffer. In some embodiments, molar concentration is used as the unit of the amount of the buffer, and its numerical value refers to the molar concentration of the buffer pair in the buffer system of the buffer.

[0059] The term “targeting molecule” refers to a molecule that has an affinity for a particular target (e.g., receptor, cell surface protein, cytokine, etc.). A targeting molecule can deliver the payload to a specific site *in vivo* through targeted delivery. A targeting molecule can recognize one or more targets. The specific target site is defined by the targets it recognizes. For example, a targeting molecule that targets a receptor can deliver a payload to a site containing a large number of the receptors. Examples of targeting molecules include, but are not limited to antibodies, antibody fragments, binding proteins for a given antigen, antibody mimics, scaffold proteins having affinity for a given target, ligands, and the like.

[0060] As used herein, the term “antibody” is used in a broad way and particularly includes an intact monoclonal antibody, a polyclonal antibody, a monospecific antibody, a multispecific antibody (e.g., a bispecific antibody), and an antibody fragment, as long as they have the desired biological activity. The antibody may be of any subtype (such as IgG, IgE, IgM, IgD, and IgA) or subclass, and may be derived from any suitable species. In some embodiments, the antibody is of human or murine origin. The antibody may also be a fully human antibody, humanized antibody or chimeric antibody prepared by recombinant methods.

[0061] Monoclonal antibodies are used herein to refer to antibodies obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies constituting the population are identical except for a small number of possible natural mutations. Monoclonal antibodies are highly specific for a single antigenic site, multiple antigenic sites or different epitopes of the same antigen. The word “monoclonal” refers to that the characteristics of the antibody are derived from a substantially homogeneous population of antibodies and are not to be construed as requiring some particular methods to produce the antibody.

[0062] An intact antibody or full-length antibody essentially comprises the antigen-binding variable region(s) as well as the light chain constant region(s) (C_L) and heavy chain constant region(s) (C_H), which could include C_H1, C_H2, C_H3 and/or C_H4, depending on the subtype of the antibody. An antigen-binding variable region (also known as a fragment variable region, Fv fragment) typically comprises a light chain variable region (V_L) and a heavy chain variable region (V_H). A constant region can be a constant region with a native sequence (such as a constant region with human native sequences) or an amino acid sequence variant thereof. The variable region recognizes and interacts with the target antigen. The constant region can be

recognized by and interacts with the immune system.

[0063] As used herein, the term “heavy chain constant region (C_H)” includes amino acid sequences derived from an intact antibody or full-length antibody heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a C_{H1} domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a C_{H2} domain, a C_{H3} domain, a C_{H4} domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a C_{H1} domain; a polypeptide chain comprising a C_{H1} domain, at least a portion of a hinge domain, and a C_{H2} domain; a polypeptide chain comprising a C_{H1} domain and a C_{H3} domain; a polypeptide chain comprising a C_{H1} domain, at least a portion of a hinge domain, and a C_{H3} domain, or a polypeptide chain comprising a C_{H1} domain, at least a portion of a hinge domain, a C_{H2} domain, and a C_{H3} domain.

[0064] As used herein, “C_L” refers to a constant region of a light chain.

[0065] The subunit structures and three dimensional configuration of the constant regions of the various antibody classes are well known. As used herein, the term “V_H domain” includes the amino terminal variable domain of an antibody heavy chain and the term “C_{H1} domain” includes the first (most amino terminal) constant region domain of an antibody heavy chain. The C_{H1} domain is adjacent to the V_H domain and is amino terminal to the hinge region of an antibody heavy chain molecule.

[0066] As used herein, “V_L” refers to a variable region of a light chain.

[0067] An antibody fragment may comprise a portion of an intact antibody, preferably its antigen binding region or variable region. Examples of antibody fragments include Fab, Fab', F(ab')₂, Fd fragment consisting of V_H and C_{H1} domains, Fv fragment, single-domain antibody (dAb) fragment, and isolated complementarity determining region (CDR). The Fab fragment is an antibody fragment obtained by papain digestion of a full-length immunoglobulin, or a fragment having the same structure produced by, for example, recombinant expression. A Fab fragment comprises a light chain (comprising a V_L and a C_L) and another chain, wherein the said other chain comprises a variable domain of the heavy chain (V_H) and a constant region domain of the heavy chain (C_{H1}). The F(ab')₂ fragment is an antibody fragment obtained by pepsin digestion of an immunoglobulin at pH 4.0-4.5, or a fragment having the same structure produced by, for example, recombinant expression. The F(ab')₂ fragment essentially comprises two Fab fragments, wherein each heavy chain portion comprises a few additional amino acids, including the cysteines that form disulfide bonds connecting the two fragments. A Fab' fragment is a fragment comprising one half of a F(ab')₂ fragment (one heavy chain and one light chain). The antibody fragment may comprise a plurality of chains joined together, for example, via a disulfide bond and/or via a peptide linking unit. Examples of antibody fragments also include single-chain Fv (scFv), Fv, dsFv, diabody, Fd and Fd' fragments, and other fragments, including

modified fragments. An antibody fragment typically comprises at least or about 50 amino acids, and typically at least or about 200 amino acids. An antigen-binding fragment can include any antibody fragment that, when inserted into an antibody framework (e.g., by substitution of the corresponding region), can result in an antibody that immunospecifically binds to the antigen.

[0068] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

[0069] As used herein, “HCDR” refers to a complementarity determining region of a heavy chain.

[0070] As used herein, “LCDR” refers to a complementarity determining region of a light chain.

[0071] Antibodies according to the present disclosure can be prepared using techniques well known in the art, such as the following techniques or a combination thereof: recombinant techniques, phage display techniques, synthetic techniques, or other techniques known in the art. For example, a genetically engineered recombinant antibody (or antibody mimic) can be expressed by a suitable culture system (e.g., *E. coli* or mammalian cells). The engineering of antibody can refer to, for example, the introduction of a ligase-specific recognition sequence at its terminals.

[0072] As used herein, the term “targeting molecule-drug conjugate” is referred to as “conjugate”. Examples of conjugates include, but are not limited to, antibody-drug conjugates.

[0073] A small molecule compound refers to a molecule with a size comparable to that of an organic molecule commonly used in medicine. The term does not encompass biological macromolecules (e.g., proteins, nucleic acids, etc.), but encompasses low molecular weight peptides or derivatives thereof, such as dipeptides, tripeptides, tetrapeptides, pentapeptides, and

the like. Typically, the molecular weight of the small molecule compound can be, for example, about 100 to about 2000 Da, about 200 to about 1000 Da, about 200 to about 900 Da, about 200 to about 800 Da, about 200 to about 700 Da, about 200 to about 600 Da, about 200 to about 500 Da.

[0074] Linking unit refers to a functional group that covalently bonds two or more moieties in a compound or material. For example, the linking unit can serve to covalently bond adjuvant moieties of targeting molecule(s) and/or payload(s).

[0075] A spacer is a structure that is located between different structural modules and can spatially separate the structural modules. The definition of spacer is not limited by whether it has a certain function or whether it can be cleaved or degraded *in vivo*. Examples of spacers include but are not limited to amino acids and non-amino acid structures, wherein non-amino acid structures can be, but are not limited to, amino acid derivatives or analogues. “Spacer sequence” refers to an amino acid sequence serving as a spacer, and examples thereof include but are not limited to a single amino acid such as Leu, Gln, etc., a sequence containing a plurality of amino acids, for example, a sequence containing two amino acids such as GA, etc., or, for example, GGGGS, GGGSGGGGS, GGGSGGGSGGGGS, etc. Other examples of spacers include, for example, self-immolative spacers such as PABC (p-benzyloxycarbonyl), and the like.

[0076] The term “alkyl” refers to a straight or branched saturated aliphatic hydrocarbon group consisting of carbon atoms and hydrogen atoms, which is connected to the rest of the molecule through a single bond. The alkyl group may contain 1 to 20 carbon atoms, referring to C₁-C₂₀ alkyl group, for example, C₁-C₄ alkyl group, C₁-C₃ alkyl group, C₁-C₂ alkyl, C₃ alkyl, C₄ alkyl, C₃-C₆ alkyl. Non-limiting examples of alkyl groups include but are not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, tert-butyl, isopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neopentyl, 1,1-dimethylpropyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 2-ethylbutyl, 1-ethylbutyl, 3,3-dimethylbutyl, 2,2-dimethyl butyl, 1,1-dimethylbutyl, 2,3-dimethylbutyl, 1,3-dimethylbutyl or 1,2-dimethylbutyl, or their isomers. A bivalent radical refers to a group obtained from the corresponding monovalent radical by removing one hydrogen atom from a carbon atom with free valence electron(s). A bivalent radical has two connecting sites which are connected to the rest of the molecule. For example, an “alkylene” or an “alkylidene” refers to a saturated divalent hydrocarbon group, either straight or branched. Examples of alkylene groups include but are not limited to methylene (-CH₂-), ethylene (-C₂H₄-), propylene (-C₃H₆-), butylene (-C₄H₈-), pentylene (-C₅H₁₀-), hexylene (-C₆H₁₂-), 1-methylethylene (-CH(CH₃)CH₂-), 2-methylethylene (-CH₂CH(CH₃)-), methylpropylene, ethylpropylene, and the like.

[0077] As used herein, when a group is combined with another group, the connection of the

groups may be linear or branched, provided that a chemically stable structure is formed. The structure formed by such a combination can be connected to other moieties of the molecule via any suitable atom in the structure, preferably via a designated chemical bond. For example, when describing a combination of a C₁₋₄ alkylene with one of the groups including -CH₂-, -NH-, -(CO)-, -NH(CO)-, -(CO)NH-, the C₁₋₄ alkylene may form a linear connection with the above groups, such as C₁₋₄ alkylene-CH₂-, C₁₋₄ alkylene-NH-, C₁₋₄ alkylene-(CO)-, C₁₋₄ alkylene-NH(CO)-, C₁₋₄ alkylene-(CO)NH-, -CH₂-C₁₋₄ alkylene, -NH-C₁₋₄ alkylene, -(CO)-C₁₋₄ alkylene, -NH(CO)-C₁₋₄ alkylene, -(CO)NH-C₁₋₄ alkylene. The resulting bivalent structure can be further connected to other moieties of the molecule.

[0078] The term “acidic buffer” refers to a buffer with pH<7, for example, the buffer with pH = 4.0-6.0, pH=4.0, pH=4.5, pH=5.0, pH=5.5 or pH=6.0.

[0079] The “stabilizer” refers to a chemical that can increase the stability of solutions, colloids, solids, mixtures, etc., and has the functions of slowing down the reaction, maintaining chemical balance, reducing surface tension, and preventing light, thermal decomposition or oxidative decomposition, for example, the stabilizer may be sucrose, trehalose dihydrate, sorbitol, and the combinations thereof.

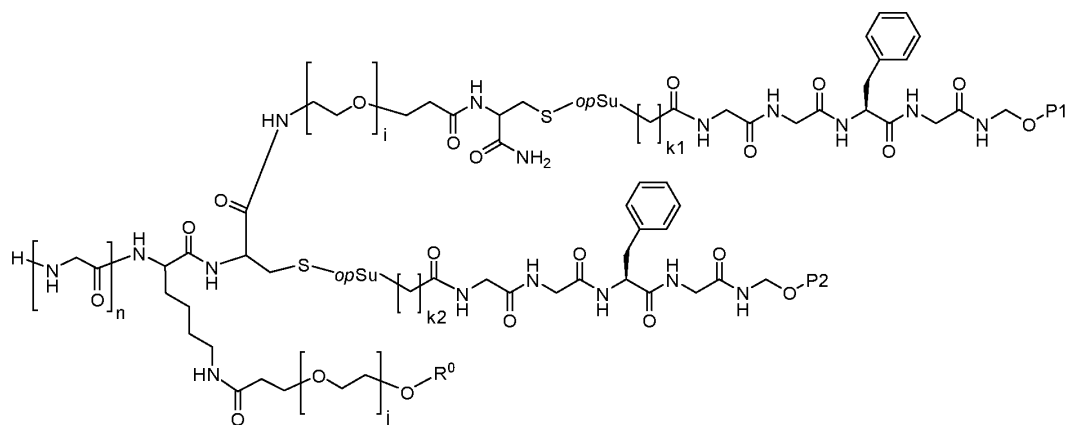
[0080] As used herein, “surfactant” refers to a substance that can obviously change the interface state of the solution system by adding a small amount. Examples of surfactant include, but not limited to, tween 20 (PS 20), tween 80 (PS 80), span 20 (SP 20), span 80 (SP 80).

[0081] As used herein, “T_m” refers to melting temperature indicating the temperature at which protein begins to denature. T_{m1} is the melting temperature of the CH₂ domain; T_{m2} is the melting temperature of the Fab domain, and T_{m3} is the melting temperature of the CH₃ domain. As used herein, “Tagg” refers to aggregation temperature indicating the temperature at which protein begins to aggregate.

[0082] As used herein, the expressions “antibody-conjugated drug”, “ADC” and “antibody-drug conjugate” has the same meaning.

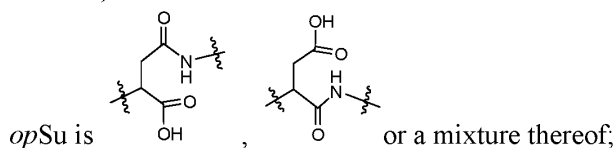
Compound of Formula (III)

[0083] In some embodiments, the linker-payload intermediate of the ADC has the structure of formula (III):



(III)

wherein,



R^0 is C_{1-10} alkyl;

n is an integer of 2 to 20;

k_1 and k_2 are independently an integer of 1 to 7;

i is an integer of 1-100;

j is an integer of 1-100;

P_1 and P_2 are independently payloads having the structure of formula (II).

[0084] In an embodiment, R^0 is C_{1-6} alkyl. In a preferred embodiment, R^0 is C_{1-3} alkyl. In a particular embodiment, R^0 is methyl.

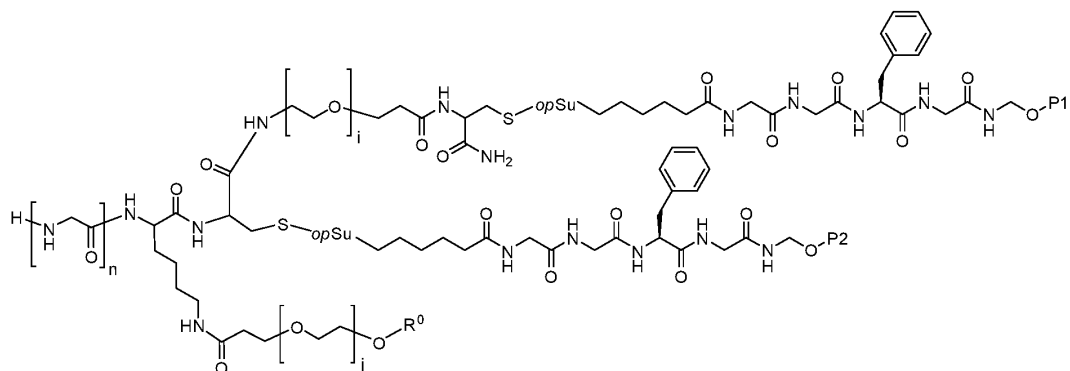
[0085] In an embodiment, n is an integer of 2 to 5. In a particular embodiment, n is 3.

[0086] In an embodiment, k_1 and k_2 are independently 1, or 3 or 5. In a particular embodiment, k_1 and k_2 are independently 5.

[0087] In an embodiment, i is independently an integer of 1 to 20, preferably 1 to 12, more preferably 2 to 8. In a particular embodiment, i is 4.

[0088] In an embodiment, j is independently an integer of 1 to 20, preferably 1 to 12, more preferably 8 to 12, especially 8 or 12. In a particular embodiment, j is 12.

[0089] In an embodiment, the compound of formula (III) has the structure of formula (III-1)

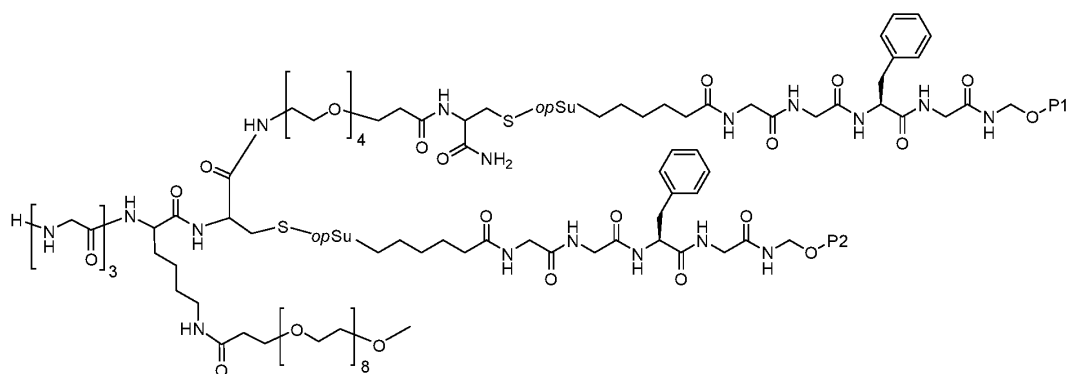


(III-1)

wherein,

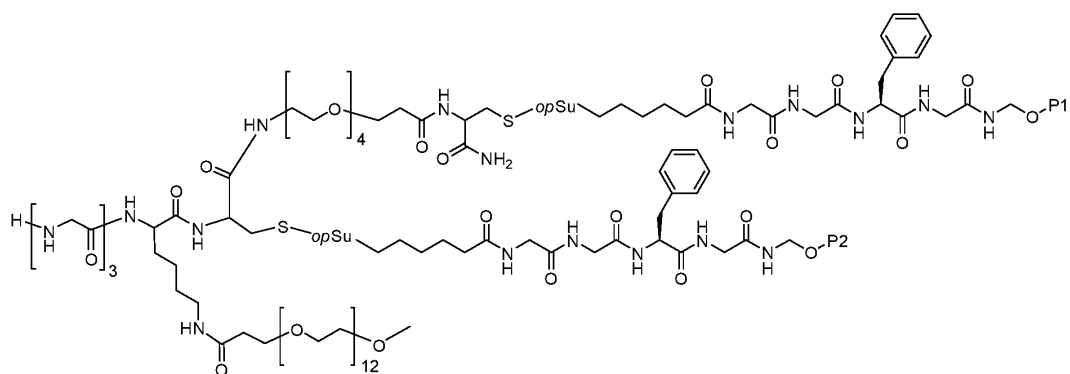
P1, P2, R⁰, *opSu*, n, i and j are as defined in formula (III).

[0090] In an embodiment, the compound of formula (III) is selected from the group consisting of:



(III-2)

and

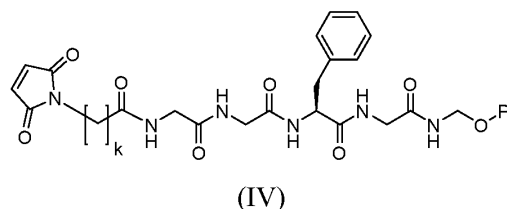


(III-3).

Preparation of the Compound of Formula (III)

[0091] In an embodiment, compound of formula (III) can be synthesized by connecting a linker with a payload or by connecting a serial of suitable building blocks. Such building blocks can be easily designed by retrosynthetic analysis, and any reaction known in the art can be used.

[0092] In an embodiment, provided is a compound having the structure of formula (IV):



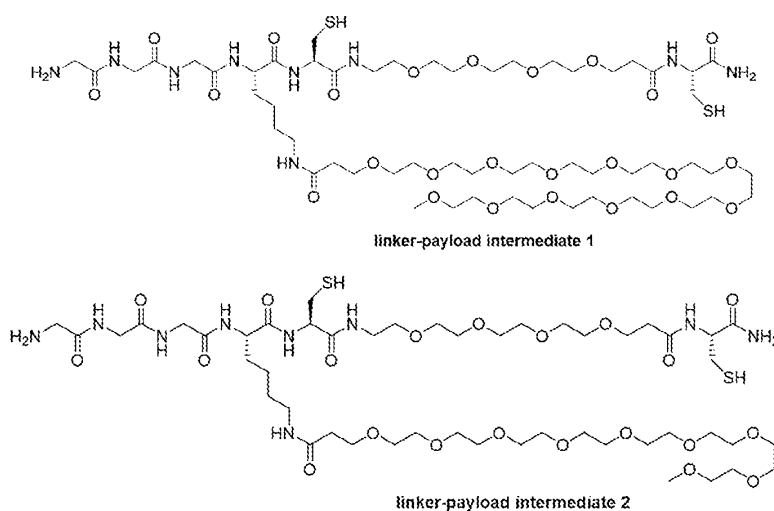
wherein,

k is an integer of 1 to 7;

P is a payload having the structure of formula (II), wherein the structure of formula (II) is as defined above.

[0093] In some embodiments, k is 1, 2, 3, 4, 5, 6, or 7. In an embodiment, k is 1, or 3 or 5. In a particular embodiment, k is 5.

[0094] The compound of formula (III) can be synthesized using the compound of formula (II)/(IV) and other necessary building blocks, using a method similar to the synthetic method as disclosed in EP2907824A (e.g., synthetic method for formula (2) or (2b) of EP2907824A). Suitable building blocks include but not limited to Linker-payload intermediate 2 and Linker-payload intermediate 1.



[0095] Then the maleimide group of formula (IV) therein can be reacted to a thiol group on another building block. The resulting thiosuccinimide is unstable under physiological

conditions and is liable to reverse Michael addition which leads to cleavage at the connection site. Moreover, when another thiol compound is present in the system, thiosuccinimide may also undergo thiol exchange with the other thiol compound. Both of these reactions cause the fall-off of the payload and result in toxic side effects. The thiosuccinimide is then subjected to ring opening reaction. The compound of formula (III) can then be obtained.

[0096] Method of ring opening reaction can be found in WO2015165413A1. The compound comprising ring-opened succinimide moiety can be purified by semi-preparative/preparative HPLC or other suitable separation means to obtain with high purity and defined composition, regardless of the efficiency of the succinimide ring opening reaction.

[0097] In the present disclosure, when applied in the linker-payload (linker–small molecule intermediate), the ring-opened succinimide structure no longer undergoes reverse Michael addition or thiol exchange, and thus the product is more stable.

Moiety Comprising Recognition Sequence of the Ligase Acceptor and Donor Substrate

[0098] In an embodiment, the (Gly)_n moiety of the compound of formula (III) is a recognition sequence of a ligase donor substrate, which facilitates enzyme-catalyzed coupling of compound of formula (III) with the an antibody or an antigen binding fragment under the catalysis of the ligase. The antibody or the antigen binding fragment is optionally modified and comprises the corresponding recognition sequence of a ligase acceptor substrate.

[0099] In an embodiment, the ligase is a transpeptidase. In an embodiment, the ligase is selected from the group consisting of a natural transpeptidase, an unnatural transpeptidase, variants thereof, and the combination thereof. Unnatural transpeptidase enzymes can be, but are not limited to, those obtained by engineering of natural transpeptidase. In a preferred embodiment, the ligase is selected from the group consisting of a natural Sortase, an unnatural Sortase, and the combination thereof. The species of natural Sortase include Sortase A, Sortase B, Sortase C, Sortase D, Sortase *L. plantarum*, etc. (US20110321183A1). The type of ligase corresponds to the ligase recognition sequence and is thereby used to achieve specific conjugation between different molecules or structural fragments.

[0100] In an embodiment, the (Gly)_n moiety of the compound of formula (III) is a recognition sequence of a ligase acceptor substrate; and the antibody or the antigen binding fragment is optionally modified and comprises the corresponding recognition sequence of a ligase donor substrate.

[0101] In some embodiments, the ligase is a Sortase selected from Sortase A, Sortase B, Sortase C, Sortase D and Sortase *L. plantarum*.

[0102] In a particular embodiment, the ligase is Sortase A from *Staphylococcus aureus*. Accordingly, the ligase recognition sequence of the ligase donor substrate may be the typical

recognition sequence LPXTG of the enzyme, wherein X can be any single amino acid that is natural or unnatural. In yet another particular embodiment, the recognition sequence of the ligase donor substrate is LPXTGJ, wherein X can be any single amino acid that is natural or unnatural; J is absent, or is an amino acid fragment comprising 1-10 amino acids, optionally labeled. In an embodiment, J is absent, and the recognition sequence of the ligase donor substrate is as shown in SEQ ID NO: 1. In yet another embodiment, J is an amino acid fragment comprising 1-10 amino acids, wherein each amino acid is independently any natural or unnatural amino acid, and the recognition sequence of the ligase donor substrate is as shown in SEQ ID NO: 2. In another embodiment, J is (Gly)_m, wherein m is an integer of 1 to 10. In yet another particular embodiment, the recognition sequence of the ligase donor substrate is LPETG (SEQ ID NO: 3). In another particular embodiment, the recognition sequence of the ligase donor substrate is LPETGG (SEQ ID NO: 4).

[0103] In an embodiment, the ligase is Sortase B from *Staphylococcus aureus* and the corresponding donor substrate recognition sequence can be NPQTN (SEQ ID NO: 5). In another embodiment, the ligase is Sortase B from *Bacillus anthracis* and the corresponding donor substrate recognition sequence can be NPKTG (SEQ ID NO: 6).

[0104] In yet another embodiment, the ligase is Sortase A from *Streptococcus pyogenes* and the corresponding donor substrate recognition sequence can be LPXTGJ (SEQ ID NO: 1 or SEQ ID NO: 2), wherein J is as defined above. In another embodiment, the ligase is Sortase subfamily 5 from *Streptomyces coelicolor*, and the corresponding donor substrate recognition sequence can be LAXTG (SEQ ID NO: 7).

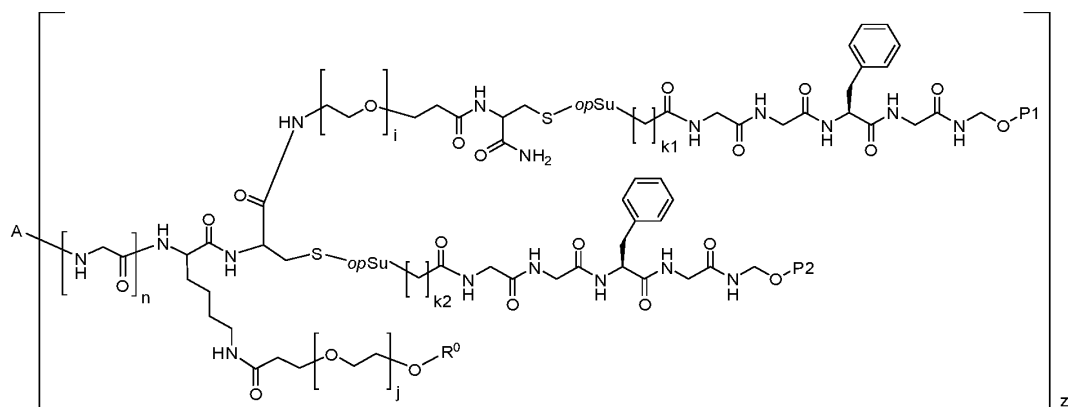
[0105] In yet another embodiment, the ligase is Sortase A from *Lactobacillus plantarum* and the corresponding donor substrate recognition sequence can be LPQTSEQ (SEQ ID NO: 8).

[0106] The ligase recognition sequence can also be other totally new recognition sequence for transpeptidase optimized by manual screening.

Conjugates and Preparation thereof

[0107] Furthermore, the payload-bearing compound (compound of formula (III)) which has the moiety comprising ligase acceptor substrate recognition sequence can be conjugated with anti-FGFR3 or an antigen binding fragment comprising a ligase donor substrate recognition sequence.

[0108] In yet another aspect, provided is a conjugate having the structure of formula (I):



(I)

wherein,

A is an anti-FGFR3 antibody or an antigen binding fragment thereof;

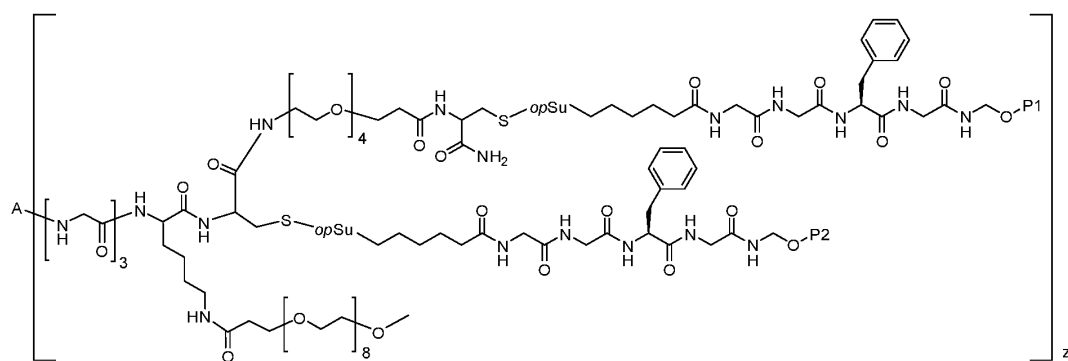
z is an integer of 1 to 20;

P1, P2, R⁰, opSu, n, k1, k2, i and j are as defined as above.

[0109] In an embodiment, the antibody or antigen binding fragment is modified to connect with the (Gly)_n moiety in the compound of formula (III).

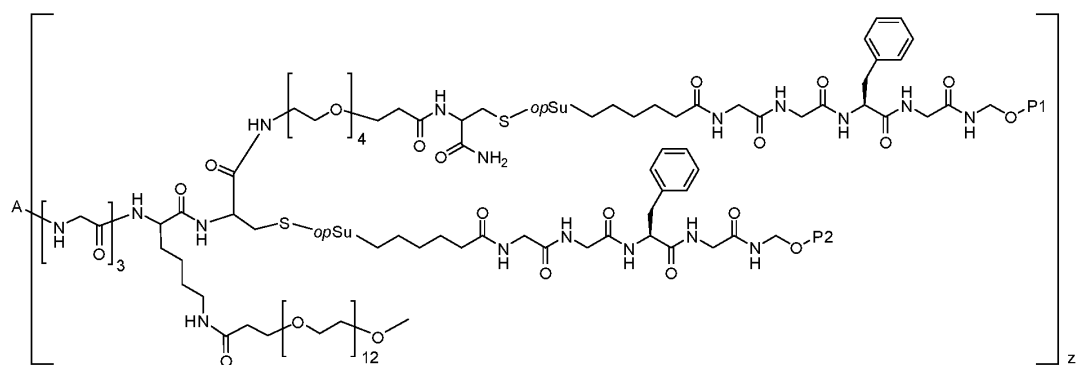
[0110] In an embodiment, z is about 1 to 20. In some embodiments, z is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, or the range between any two values (including the end value) .

[0111] In an embodiment, the compound of formula (I) is selected from the group consisting of:



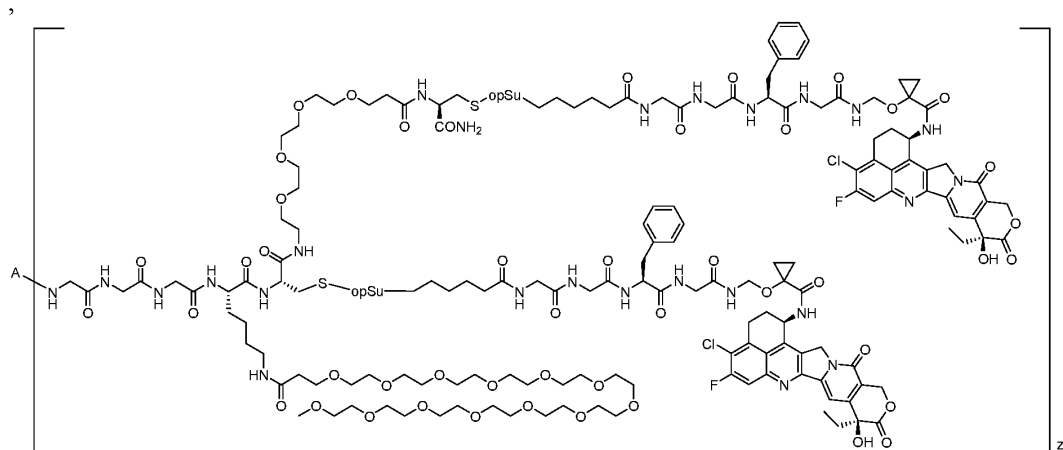
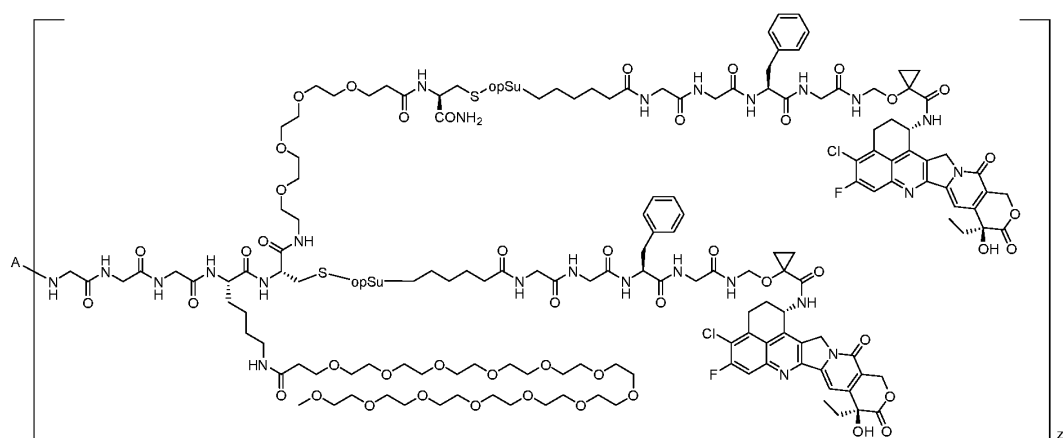
(I-1)

and

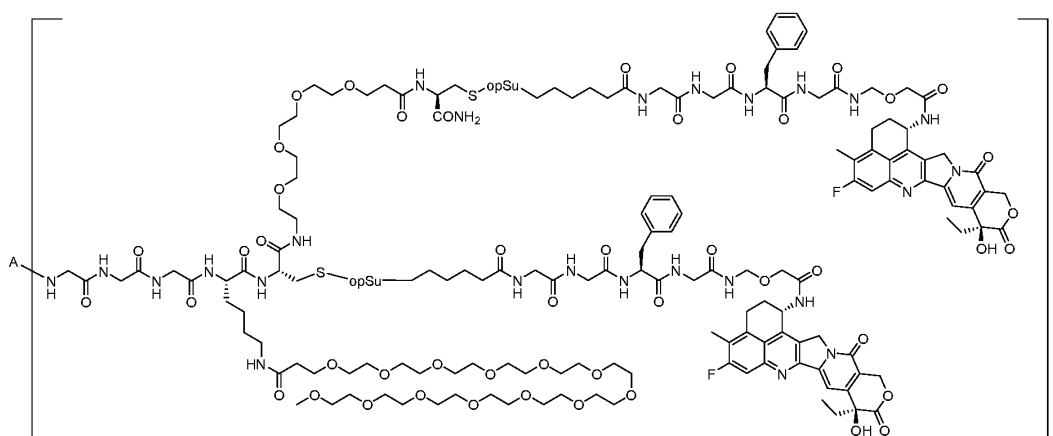


(I-2).

[0112] In an embodiment, the compound of formula (I) is selected from:



, and/or



Antibody or the antigen binding fragment

[0113] In an embodiment, the antibody or the antigen binding fragment (shown as “A” in formula (I)) is an anti-FGFR3 antibody or an antigen binding fragment thereof. In some embodiments, the antibody or an antigen binding fragment comprises CDRs: a heavy chain CDR1 (HCDR1) comprising amino acid sequence of SEQ ID NO: 9 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 9, a heavy chain CDR2 (HCDR2) comprising amino acid sequence of SEQ ID NO: 10 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 10, a heavy chain CDR3 (HCDR3) comprising amino acid sequence of SEQ ID NO: 11 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 11, a light chain CDR1 (LCDR1) comprising amino acid sequence of SEQ ID NO: 12 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 12, a light chain CDR2 (LCDR2) comprising amino acid sequence of SEQ ID NO: 13 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 13, and a light chain CDR3 (LCDR3) comprising amino acid sequence of SEQ ID NO: 14 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 14.

[0114] In some embodiments, the antibody or the antigen binding fragment comprises HCDR1 of SEQ ID NO: 9, HCDR2 of SEQ ID NO: 10, HCDR3 of SEQ ID NO: 11, LCDR1 of SEQ ID NO: 12, LCDR2 of SEQ ID NO: 13, LCDR3 of SEQ ID NO: 14.

[0115] In some embodiments, the KD value of the anti-FGFR3 antibody or an antigen binding fragment binding to human FGFR3 and/or monkey FGFR3 is less than 10 nM. In some embodiments, the KD value of the anti-FGFR3 antibody or an antigen binding fragment binding to human FGFR3 and/or monkey FGFR3 is about 9.9 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4.4 nM, about 3.9 nM, about 2 nM, about 1 nM, about 0.9

nM, about 0.7 nM, about 0.5 nM, about 0.3 nM, about 0.2 nM, about 0.1 nM, or the range between any two values (including the end value).

[0116] In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain variable domain (VH) comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 15, and/or a light chain variable domain (VL) comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain variable domain comprising an amino acid sequence having at least about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 15, and the antibody or an antigen binding fragment comprises a light chain variable domain comprising an amino acid sequence having at least about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the antibody or an antigen binding fragment comprises VH of SEQ ID NO: 15, and/or VL of SEQ ID NO: 16. In some embodiments, the antibody or an antigen binding fragment comprises VH of SEQ ID NO: 15, and VL of SEQ ID NO: 16.

[0117] In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain constant domain (CH) comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 18, and/or a light chain constant domain (CL) comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 19. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain constant domain comprising an amino acid sequence having at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 18, and a light chain constant domain comprising an amino acid sequence having at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 19. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain constant domain of SEQ ID NO: 17, and a light chain constant domain of SEQ ID NO: 19. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain constant domain of SEQ ID NO: 18, and a light chain constant domain of SEQ ID NO: 19.

[0118] In some embodiments, the antibody or an antigen binding fragment comprises a heavy

chain comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 21, and/or a light chain comprising an amino acid sequence having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain comprising an amino acid sequence having at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 21, and/or a light chain comprising an amino acid sequence having at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100% (the range between any two values (including the end value)) sequence identity to the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain of SEQ ID NO: 20 and a light chain comprising an amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or an antigen binding fragment comprises a heavy chain of SEQ ID NO: 21 and a light chain comprising an amino acid sequence of SEQ ID NO: 22.

The above-mentioned sequences are listed below (Divided according to Kabat):

[0119] SEQ ID NO: 1: LPXTG
 [0120] SEQ ID NO: 2: LPXTGXXXXXXXXXX, up to 9 Xs could be deleted at amino acids 6-15
 [0121] SEQ ID NO: 3: LPETG
 [0122] SEQ ID NO: 4: LPETGG
 [0123] SEQ ID NO: 5: NPQTN
 [0124] SEQ ID NO: 6: NPKTG
 [0125] SEQ ID NO: 7: LAXTG
 [0126] SEQ ID NO: 8: LPQTSEQ
 [0127] SEQ ID NO: 9: SYDMS
 [0128] SEQ ID NO: 10: GIYSGDGSIIYADSVKG
 [0129] SEQ ID NO: 11: DGPMNEESRFDY
 [0130] SEQ ID NO: 12: SGSSSNIGNNYVS
 [0131] SEQ ID NO: 13: ADSKRPS
 [0132] SEQ ID NO: 14: ASWDYSLSGYV
 [0133] SEQ ID NO: 15:
 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSGIYSGDGS
 IYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGPMNEESRFDYWGQ

GTLVTVSS

[0134] SEQ ID NO: 16:

QSVLTQPPSASGTPGQRTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYADSKRPSGV
PDRFSGSKSGTSASLAISGLRSEDEADYYCASWDYSLSGYVFGGGTKLTVLG

[0135] SEQ ID NO: 17:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[0136] SEQ ID NO: 18:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP

[0137] SEQ ID NO: 19:

QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC

[0138] SEQ ID NO: 20:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSGIYSGDGS
IYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGMNEESRFDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LSDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[0139] SEQ ID NO: 21:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSGIYSGDGS
IYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGMNEESRFDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP

[0140] SEQ ID NO: 22:

QSVLTQPPSASGTPGQRVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYADSKRPSGV
PDRFSGSKSGTSASLAISGLRSEDEADYYCASWDYSLSGYVFGGGTKLTVLGQPKAA
PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNK
YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC

[0141] In an embodiment, the antibody or the antigen-binding fragment thereof may comprise terminal modification. A terminal modification refers to a modification at the C-terminal or N-terminal of the heavy chain or light chain of the antibody, which for example comprises a ligase recognition sequence. In another embodiment, the terminal modification may further comprise a spacer Sp comprising 2-100 amino acids, wherein the antibody, Sp and the ligase recognition sequence are sequentially linked. In a preferred embodiment, Sp is a spacer sequence containing 2-20 amino acids. In a particular embodiment, Sp is a spacer sequence selected from GA, GGGGS (SEQ ID NO: 23), GGGGSGGGGS (SEQ ID NO: 24), and GGGGSGGGGSGGGGS (SEQ ID NO: 25), especially GA.

[0142] In some embodiments, the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 26, and/or a light chain of SEQ ID NO: 27. In some embodiments, the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 26 and a light chain of SEQ ID NO: 27. In some embodiments, the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 26 and a light chain of SEQ ID NO: 22. In some embodiments, the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 20 and a light chain of SEQ ID NO: 27.

[0143] SEQ ID NO: 26:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSGIYSGDGS
IYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDGP MNEESRFDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVL
DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGALPETGG

[0144] SEQ ID NO: 27:

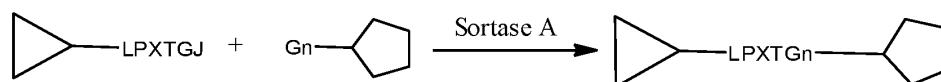
QSVLTQPPSASGTPGQRTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYADSKRPSGV
PDRFSGSKSGTSASLAISGLRSEDEADYYCASWDYSLSGYVFGGGTKLTVLGQPKAA
PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK
YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECGALPETGG

Preparation of the Conjugate

[0145] The conjugates (i.e., the compound of formula (I)) of the present disclosure can be prepared by any method known in the art. In some embodiments, the conjugate is prepared by the ligase-catalyzed site-specific conjugation of an antibody or an antigen binding fragment and a compound of formula (III), wherein the antibody or the antigen binding fragment thereof is modified by a ligase recognition sequence.

[0146] The antibody or the antigen binding fragment thereof and the compound of formula (III) are linked to each other via the ligase-specific recognition sequences of the substrates. The recognition sequence depends on the particular ligase employed. In an embodiment, the antibody or the antigen binding fragment thereof is an antibody with recognition sequence-based terminal modifications introduced at the C-terminal of the light chain and/or C-terminal of the heavy chain, and the antibody or the antigen binding fragment thereof is conjugated with the rest moiety in formula (I), under the catalysis of the wild type or optimized engineered ligase or any combination thereof, and under suitable catalytic reaction conditions.

[0147] In a specific embodiment, the ligase is Sortase A and the conjugation reaction can be represented by the following scheme:



[0148] The triangle represents a portion of an antibody; the pentagon represents a portion of a compound of formula (III); and G_n represents the $(Gly)_n$ moiety. n , X and J are respectively as defined above. When conjugated with G_n , which is the corresponding recognition sequence of the acceptor substrate, the upstream peptide bond of the glycine in the LPXTGJ sequence is cleaved by Sortase A, and the resulting intermediate is linked to the free N-terminal of G_n to generate a new peptide bond. The resulting amino acid sequence is LPXTG $_n$. The sequences G_n and LPXTGJ are as defined above.

[0149] The compound of formula (III) of the present disclosure has defined structure, defined composition and high purity, so that when the conjugation reaction with an antibody is conducted, fewer impurities are introduced or no other impurities are introduced. When such an intermediate is used for the ligase-catalyzed site-specific conjugation with a modified

antibody containing a ligase recognition sequence, a homogeneous ADC with highly controllable quality is obtained.

Metabolism of the Conjugate in a physiological environment

[0150] When a part or whole linker is cleaved in tumor cells, the payload is released. As the linker is cleaved at a connecting position to the antitumor compound, the antitumor compound is released in its intrinsic structure to exhibit its intrinsic antitumor effect.

[0151] In an embodiment, the GGFG (Gly-Gly-Phe-Gly) (SEQ ID NO: 28) moiety comprised by the compound of formula III) can be cleaved by lysosomal enzymes (such as cathepsin B and/or cathepsin L).

[0152] In an embodiment, the compound of formula (III) comprises a self-immolative spacer. In an embodiment, the self-immolative spacer is an acetal or a heteroacetal. In an embodiment, the -GGFG-NH-CH₂-O- moiety comprised by the compound of formula (III) represents a combination of a restriction enzyme site and a self-immolative spacer, which would cleave in the cell and release the aimed molecule (such as the antitumor compound).

Pharmaceutical Composition and Pharmaceutical Preparation

[0153] Another object of the disclosure is to provide a pharmaceutical composition comprising the conjugate of the present disclosure, and at least one pharmaceutically acceptable carrier.

[0154] The pharmaceutical composition of the present disclosure may be administered in any manner as long as it achieves the effect of preventing, alleviating, preventing or curing the symptoms of a human or animal. For example, various suitable dosage forms can be prepared according to the administration route, especially injections such as lyophilized powder for injection, solution for injection, or sterile powder for injection.

[0155] The term "pharmaceutically acceptable" means that when contacted with tissues of the patient within the scope of normal medical judgment, no undue toxicity, irritation or allergic reaction, etc. shall arise, having reasonable advantage-disadvantage ratios and effective for the intended use.

[0156] The term pharmaceutically acceptable carrier refers to those carrier materials which are pharmaceutically acceptable and which do not interfere with the bioactivities and properties of the conjugate. Examples of aqueous carriers include but are not limited to buffered saline, and the like. The pharmaceutically acceptable carrier also includes carrier materials which brings the composition close to physiological conditions, such as pH adjusting agents, buffering agents, toxicity adjusting agents and the like, and sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, and the like. In some embodiments, The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle that is administered with an active ingredient for treatment. Such pharmaceutical carriers may be sterile liquids, such as water and oils, including

oils originated from petroleum, animal, plant or synthesis, such as peanut oil, soybean oil, mineral oil and sesame oil. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline and solutions of glucose in water or glycerol can also be used as a liquid carrier, particularly for injection. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, skimmed milk powder, glycerol, propylene, glycol, water, ethanol and the like. If desired, the composition may also comprise a small amount of a wetting agent, an emulsifier, or a pH buffering agent such as acetates, citrates or phosphates. Antibacterials such as benzyl alcohol or methylparaben, antioxidants such as ascorbic acid or sodium bisulfite, chelating agents such as ethylenediamine tetraacetic acid, and tonicity adjusting agents such as sodium chloride or dextrose are also contemplated. Such compositions may be in the form of solutions, suspensions, emulsions, tablets, pills, capsules, pulvises, sustained-release formulations and the like. The composition may be formulated as a suppository using conventional binders and carriers such as triglycerides. Oral formulations may comprise standard carriers such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose and magnesium carbonate of pharmaceutical grade. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, which is incorporated herein by reference. Such composition will comprise a clinically effective dose of an antibody, preferably in purified form, together with a suitable amount of a carrier to provide a dosing form suitable for the patient. The formulation should be suitable for the administration mode. The parent formulation may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0157] In an embodiment, the pharmaceutical composition of the present disclosure has a drug to antibody ratio (DAR) of an integer or non-integer of about 1 to about 20, such as about 1 to about 10, about 1 to about 8, about 1 to about 6, about 1 to about 4. In a particular embodiment, the conjugate of the present disclosure has a DAR of about 4.

[0158] In one embodiment, the pharmaceutical composition comprises an ADC, buffer, and optional one or more stabilizers.

[0159] In one embodiment, the buffer in the pharmaceutical composition is an acidic buffer.

[0160] In one embodiment, the buffer is selected from citrate buffer, phosphate buffer, histidine buffer and glutamic acid buffer. In one embodiment, the buffer is histidine buffer. In one embodiment, the citrate buffer comprises citric acid and sodium citrate, the histidine buffer comprises L-histidine and L-histidine hydrochloride.

[0161] In one embodiment, the concentration of buffer is about 10-40 mM. In a preferred embodiment, the concentration of buffer is about 10 mM, about 14 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 26 mM, about 30 mM,

about 33 mM, about 37 mM or about 40 mM. In a preferred embodiment, the concentration of buffer is about 20 mM. In one embodiment, the concentration of buffer is about 15-25 mM. In one embodiment, the concentration of buffer is about 15 mM, about 16.5 mM, about 17 mM, about 18.3 mM, about 19 mM, about 21 mM, about 22 mM, about 23 mM or about 25 mM. In a preferred embodiment, the concentration of buffer is about 20 mM.

[0162] In one embodiment, the buffer in the pharmaceutical composition is about 20 mM histidine buffer.

[0163] In one embodiment, the pH of the buffer in the pharmaceutical composition is about 5.0-7.0; preferably 5.5-6.5; more preferably 6.0. In one embodiment, the pH of the buffer in the pharmaceutical composition is about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9 or about 7.0.

[0164] In one embodiment, the pH of the pharmaceutical composition is about 5.0-7.0; preferably 5.5-6.5; more preferably 6.0. In one embodiment, pH of the pharmaceutical composition is about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9 or about 7.0.

[0165] In one embodiment, the stabilizers in the pharmaceutical composition comprise one or more of sucrose, an arginine salt, arginine, sodium chloride and sorbitol; preferably sucrose.

[0166] In one embodiment, the stabilizer is sucrose and the amount of the stabilizer based on the total amount of pharmaceutical composition is about 5% to about 15% (W/V), preferably about 6% to about 10% (W/V); more preferably about 8% (W/V). In one embodiment, the stabilizer is sucrose and the amount of the stabilizer based on the total amount of pharmaceutical composition is about 6% (W/V), about 7% (W/V), about 8% (W/V), about 9% (W/V), or about 10% (W/V). In one embodiment, the stabilizer in the pharmaceutical composition is sucrose and the amount of sucrose based on the total amount of pharmaceutical composition is about 8% (W/V).

[0167] In one embodiment, the stabilizer is sorbitol and the amount of the stabilizer based on the total amount of pharmaceutical composition is about 2% to about 8% (W/V), preferably about 3.5% to about 6% (W/V); more preferably about 4.5% (W/V). In one embodiment, the stabilizer is sorbitol and the amount of the stabilizer based on the total amount of pharmaceutical composition is about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, or about 5.5% (W/V).

[0168] In one embodiment, the stabilizer is one or more of an arginine salt, arginine and sodium

chloride, and the concentration of the stabilizer based on the total volume of pharmaceutical composition is about 100 to about 160 mM, preferably about 120 to about 160 mM; more preferably about 140 mM. In a preferred embodiment, the arginine salt is arginine hydrochloride.

[0169] In one embodiment, the pharmaceutical further comprises surfactant. In one embodiment, the surfactant is tween 20 (PS20 or polysorbate 20) and/or tween 80 (PS80 or polysorbate 80). In one embodiment, the surfactant is tween 80 (PS80). In one embodiment, the amount of the surfactant based on the total amount of pharmaceutical composition is about 0.005% to about 0.4% (W/V), preferably about 0.005% to about 0.1% (W/V), or about 0.005% to about 0.05% (W/V), or about 0.005% to about 0.1% (W/V); more preferably about 0.01% to about 0.04% (W/V); especially about 0.01%, about 0.02%, or about 0.04% (W/V). In one embodiment, the amount of the surfactant based on the total amount of pharmaceutical composition is about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.10%, about 0.11%, about 0.12%, about 0.13%, about 0.14%, about 0.15%, about 0.16%, about 0.17%, about 0.18%, about 0.19%, about 0.20%, about 0.21%, about 0.22%, about 0.23%, about 0.24%, about 0.25%, about 0.26%, about 0.27%, about 0.28%, about 0.29%, about 0.30%, about 0.31%, about 0.32%, about 0.33%, about 0.34%, about 0.35%, about 0.36%, about 0.37%, about 0.38%, about 0.39%, about 0.40% (W/V).

[0170] In one embodiment, the surfactant is PS 80, and the amount of the surfactant based on the total amount of pharmaceutical composition is about 0.01%, about 0.02%, or about 0.04% (W/V).

Treatment Method and Use

[0171] In another aspect, also provided is use of pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating FGFR3-mediated disease. Specifically, the FGFR3-mediated disease is an FGFR3-positive tumor, more specifically brain cancer, bladder cancer, urothelial cancer, cervical cancer, or intrahepatic cholangiocarcinoma.

[0172] In some embodiments, the disease includes tumor overexpressing FGFR3 or tumor with FGFR3 gene mutation. In some embodiments, the disease is selected from the group consisting of: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendothelial sarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, pancreatic cancer, breast cancer, thyroid cancer, endometrial cancer, melanoma, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous carcinoma, papillary carcinoma, papillary

adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchial carcinoma, renal cell carcinoma, liver cancer, bile duct cancer, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung cancer, small cell lung cancer, bladder cancer, epithelial cancer, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma and retinoblastoma. In some embodiments, the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, or intrahepatic cholangiocarcinoma. In some embodiments, the disease is glioblastoma. In a preferred embodiment, the conjugate of the present disclosure formed by conjugation of the anti-FGFR3 antibody and the small molecule cytotoxin can specifically bind to FGFR3 on the surface of the tumor cell and selectively kill the FGFR3-expressing tumor cells.

Beneficial effects

[0173] The present disclosure utilizes a linker with unique structure and uses a ligase to catalyze the conjugation of the targeting molecule and the payload. The conjugate of the present disclosure has good homogeneity and high activity. Furthermore, the toxicity of the linker-payload intermediate is much lower than that of the free payload, and thus the manufacture process of the drug is less detrimental, which is advantageous for industrial production.

[0174] The conjugate of the present disclosure achieves at least one of the following technical effects:

- (1) High inhibitory activity against target cells, and strong bystander killing effect.
- (2) Good physicochemical properties (e.g., solubility, physical and/or chemical stability).
- (3) Good pharmacokinetic properties (e.g., good stability in plasma, appropriate half-life and duration of action).
- (4) High specificity and good safety (low toxicity on non-target normal cells or tissues, and/or fewer side effects, wider treatment window), etc.
- (5) Highly modular design, simple assembly of multiple drugs.

[0175] The drug can prevent the patient from resisting to FGFR3-targeting therapy, and can overcome low response rate of current FGFR3-directed therapies. And the formulation of the present disclosure has better stability, and the formulation can ensure above technical effects of the ADCs of the present disclosure.

Examples

[0176] In order to more clearly illustrate the objects and technical solutions, the present disclosure is further described below with reference to specific examples. It is to be understood that the examples are not intended to limit the scope of the disclosure. The specific experimental

methods which were not mentioned in the following examples were carried out according to conventional experimental method.

[0177] Unless otherwise stated, the instruments and reagents used in the examples are commercially available. The reagents can be used directly without further purification.

MS: Thermo Fisher Q Exactive Plus, Waters 2795-Quattro micro triple quadrupole mass spectrometer

HPLC : Waters 2695, Agilent 1100, Agilent 1200

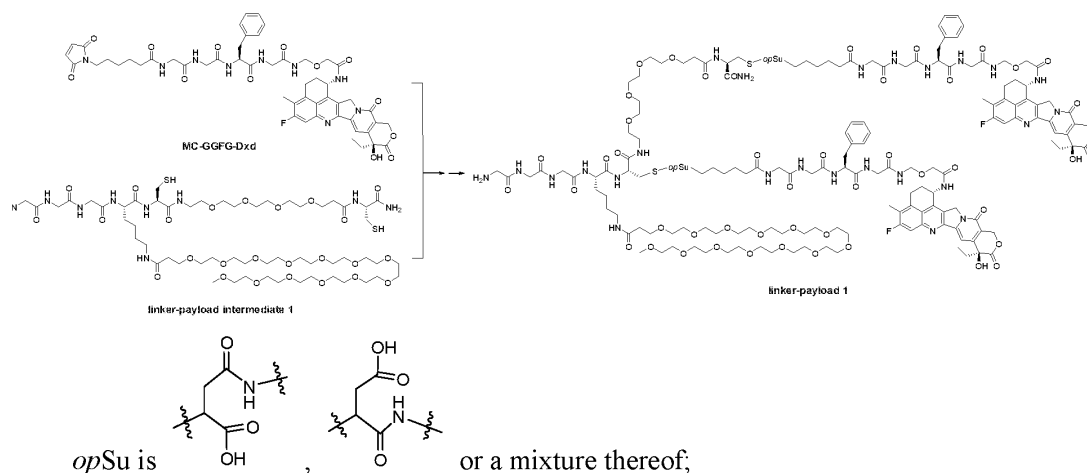
Semi-preparative HPLC: Lisure HP plus 50D

Flow Cytometry: CytoFLEX S

HIC-HPLC: Butyl-HIC; mobile phase A: 25 mM PB, 2M (NH₄)₂SO₄, pH 7.0; mobile phase B: 25 mM PB, pH 7.0; flow rate: 0.8 ml/min; acquisition time: 25 min; injection amount: 20 µg; column temperature: 25 °C; detection wavelength: 280 nm; sample chamber temperature: 8 °C.

[0178] SEC-HPLC: column: TSK-gel G3000 SWXL, TOSOH 7.8 mm ID × 300 mm, 5 µm; mobile phase: 0.2 M KH₂PO₄, 0.25 M KCl, pH 6.2; flow rate: 0.5 ml/min; acquisition time: 30 min; injection volume: 50 µl; column temperature: 25 °C; detection wavelength; 280 nm; sample tray temperature: 8 °C. In some cases, the order of carrying out the foregoing reaction schemes may be varied to facilitate the reaction or to avoid unwanted reaction products. The following examples are provided so that the invention might be more fully understood. These examples are illustrative only and should not be construed as limiting the invention in any way.

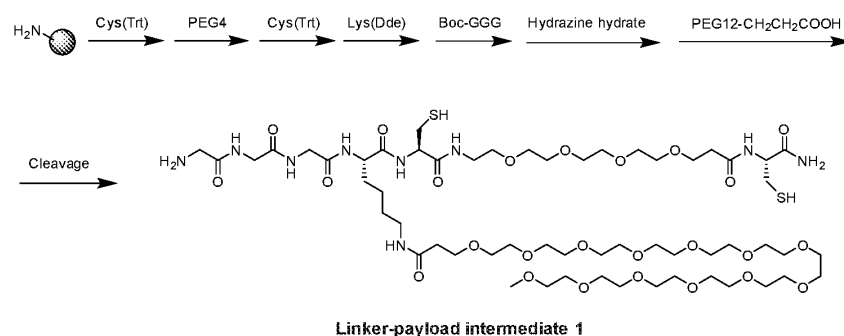
Example 1 Preparation of Linker-payload 1



Preparation of intermediate MC-GGFG-DXd

[0179] The intermediate MC-GGFG-DXd is commercial available or prepared following the procedures as described in EP2907824. This compound is used to prepare linker-payload 1.

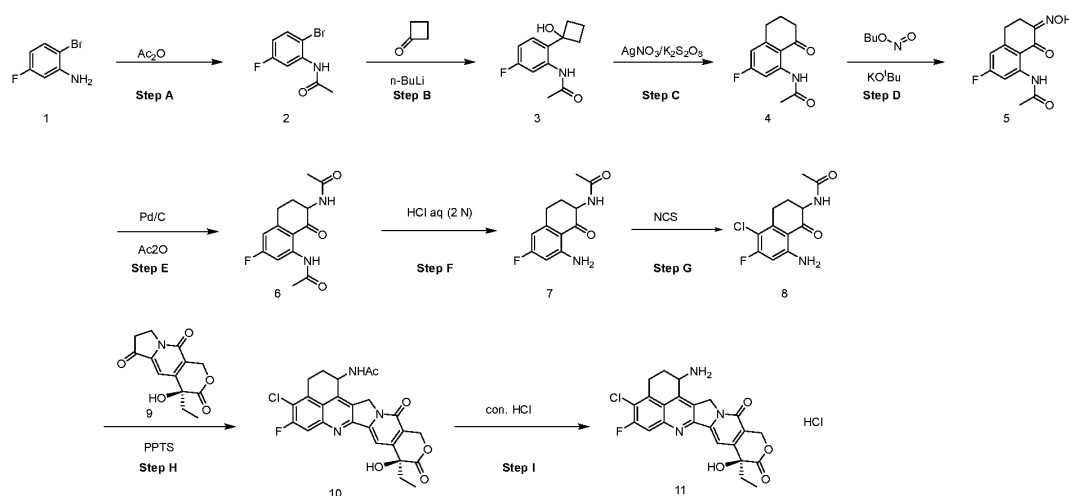
Preparation of Linker-payload intermediate 1



[0180] **Linker-payload intermediate 1** can be synthesized by a conventional solid phase polypeptide synthesis using Rink-amide-MBHA-resin. Fmoc was used to protect the amino acid in the linking unit. The coupling reagent was selected from HOBT, HOAt/DIC, DCC, EDCI or HATU. After synthesis, the product was cleaved from resin using TFA/TIS/H₂O solution. The product was purified by prep-HPLC, lyophilized and stored for use. LCMS m/z: [M-H]⁺ = 1382.6.

Preparation of linker-payload 1

[0181] **Linker-payload intermediate 1** and MC-GGFG-DXd (molar ratio ~1:2) were weighed and dissolved in water and DMF, respectively, and then thoroughly mixed to give a mixture, which was reacted at 0-40°C for 0.5-30h. Once the reaction was completed, the reaction mixture was directly added with an appropriate amount of Tris Base solution or other solution that promotes the ring-opening reaction, and the reaction was performed at 0-40°C for another 0.2-20h. After the reaction was completed, the product was purified by semi-preparative/preparative HPLC and lyophilized to obtain **linker-payload 1**. LCMS m/z: [(M+3H)/3]⁺ = 1163.3.

Example 2 Preparation of Linker-payload 2**Preparation of Intermediate 11**

[0182] **Step A:** N-(2-bromo-5-fluorophenyl)acetamide: To a stirred solution of acetic anhydride (214 g, 2.10 mol) in acetic acid (500 mL) was added con. H_2SO_4 (3 mL), followed with 2-bromo-5-fluoroaniline (100 g, 526.27 mmol) in portions at room temperature. The mixture was stirred for 3 h, then poured into 2000 mL ice-water. A precipitate was formed, which was collected by filtration and dried in vacuo at room temperature to afford N-(2-bromo-5-fluorophenyl)acetamide (105 g) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.68 (dd, $J = 8.9, 6.0$ Hz, 1H), 7.61 (ddd, $J = 10.7, 5.3, 3.1$ Hz, 1H), 7.02 (ddd, $J = 8.9, 8.0, 3.1$ Hz, 1H), 2.11 (s, 3H). LCMS m/z 232.0(M+H).

[0183] **Step B:** N-(5-fluoro-2-(1-hydroxycyclobutyl)phenyl)acetamide: To a stirred solution of N-(2-bromo-5-fluorophenyl)acetamide (105 g, 452.48 mmol) in THF (1000 mL) was added n-BuLi (594 mL, 1.6 M in n-hexane, 950.22 mmol) dropwise over 1 h at -78°C . After completion, the mixture was stirred for 0.5 h under N_2 . Then a solution of cyclobutanone (38.06 g, 542.98 mmol) in THF (50 mL) was added dropwise at -78°C over 0.5 h, the mixture was stirred at -78°C to room temperature for 6 h. The mixture was poured into 500 mL saturated NH_4Cl aq at 0°C . Extracted with ethyl acetate (500 mL x 3), washed with brine (250 mL x 2), dried over Na_2SO_4 and concentrated. The mixture was triturated with (PE/EA=1:1, 100 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to afford N-(5-fluoro-2-(1-hydroxycyclobutyl)phenyl)acetamide (24 g) as a yellow solid. LCMS m/z 206.1(M-18+H), 246.1(M+Na).

[0184] **Step C:** N-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide: To a stirred mixture of N-(5-fluoro-2-(1-hydroxycyclobutyl)phenyl)acetamide (24 g, 107.50 mmol) in CH_2Cl_2 (170 mL) and water (170 mL) was added silver nitrate (AgNO_3) (5.48 g, 32.25 mmol)

and potassium persulfate ($K_2S_2O_8$) (58.12 g, 215.01 mmol), the mixture was stirred at 30 °C for 6 h. The mixture was filtered on Celite and washed with CH_2Cl_2 (100 mL), the filtrate was concentrated and purified by FCC(EA/PE=0-40%) to afford N-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (14 g) as a light yellow solid. LCMS m/z 222.1(M+H).

[0185] **Step D:** N-(3-fluoro-7-(hydroxyimino)-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide: To a stirring mixture of N-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (14 g, 63.28 mmol) in THF (500 mL) at 0 °C was added 1-butyl nitrite (8.48 g, 63.28 mmol), followed with t-BuOK (8.52 g, 75.94 mmol). The mixture was stirred at 0 °C for 2 h. After completion, the mixture was acidified by HCl (2 N) to adjust pH=3. The mixture was extracted by ethyl acetate (200 mL x 3), washed by brine (100 mL x 2), dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was triturated with tert-butyl methyl ether (200 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to afford N-(3-fluoro-7-(hydroxyimino)-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (12 g) as a yellow solid. LCMS m/z 251.1(M+H).

[0186] **Step E:** N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide: To a solution of N-(3-fluoro-7-(hydroxyimino)-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (12 g, 47.96 mmol) in acetic anhydride (90 mL) and THF (90 mL) was added 10% Pd/C (1 g), the mixture was stirred at 25 °C under H_2 atmosphere for 16 h. After cooling to 0 °C, Et_3N (20 mL) was added dropwise, the mixture was stirred at 0 °C for 1 h. Filtered on Celite, the filtrate was poured into ice-water (500 mL). Extracted with ethyl acetate (500 mL x 3), washed with brine (250 mL x 2), dried over Na_2SO_4 and concentrated. The residue was triturated with tert-butyl methyl ether (120 mL) for 10 mins, filtered and the cake was collected and dried in vacuo to give N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide (7.9 g) as a yellow solid. LCMS m/z 279.1(M+H).

[0187] **Step F:** N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide: To a solution of N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide (7.9 g, 28.39 mmol) in MeOH (150 mL) was added HCl aq (2 N, 150 mL), the mixture was stirred at 50 °C for 7 h. After cooling to 0 °C, Sat. $NaHCO_3$ aq was added dropwise to adjust pH = 8. Extracted with ethyl acetate (200 mL x 3), washed with brine (200 mL x 2), dried over Na_2SO_4 and concentrated under reduced pressure to give N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-diyl)diacetamide (6.0 g) as a yellow solid. 1H NMR (400 MHz, Chloroform-d) δ 6.57 (s, 3H), 6.18 (td, J = 11.1, 2.4 Hz, 2H), 4.52 (dt, J = 13.3, 5.0 Hz, 1H), 3.13 (ddd, J = 17.5, 13.0, 4.6 Hz, 1H), 3.00 – 2.81 (m, 1H), 2.69 (dtd, J = 9.4, 4.6, 2.5 Hz, 1H), 2.09 (s, 3H), 1.79 (qd, J = 13.0, 4.3 Hz, 1H). LCMS m/z 237.1(M+H).

[0188] **Step G:** N-(8-amino-5-chloro-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide: To a solution of N,N'-(3-fluoro-8-oxo-5,6,7,8-tetrahydronaphthalene-1,7-

diyl)diacetamide (4.0 g, 16.93 mmol) in DMF (80 mL) was added NCS (2.26 g, 16.93 mmol) in portions at 0 °C, the mixture was stirred at room temperature for 16 h. The mixture was poured into 200 mL ice-water. A precipitate was formed, which was collected by filtration and dried in vacuo at room temperature to afford N-(8-amino-5-chloro-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (4.0 g) as a yellow solid. ¹H NMR (400 MHz, DMSO-d₆) δ 8.11 (d, J = 8.0 Hz, 1H), 7.71 (s, 2H), 6.62 (d, J = 11.9 Hz, 1H), 4.53 (ddd, J = 13.0, 8.0, 4.7 Hz, 1H), 3.18 – 3.04 (m, 1H), 2.91 (ddd, J = 17.5, 12.4, 4.8 Hz, 1H), 2.21 – 2.08 (m, 1H), 1.99 – 1.83 (m, 4H). LCMS m/z 271.0(M+H).

[0189] **Step H:**

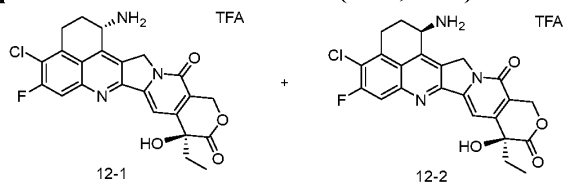
N-((9S)-4-chloro-9-ethyl-5-fluoro-9-hydroxy-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide: To a mixture of N-(8-amino-5-chloro-6-fluoro-1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (4.0 g, 14.78 mmol) in toluene (400 mL) was added (S)-4-ethyl-4-hydroxy-7,8-dihydro-1H-pyrano[3,4-f]indolizine-3,6,10(4H)-trione (4.28 g, 16.25 mmol), pyridinium p-Toluenesulfonate (1.11 g, 4.43 mmol) and o-cresol (10 mL), the mixture was heated to reflux under N₂ for 24 h. The solvent was removed by reduced pressure and the mixture was purified by FCC(THF/CH₂Cl₂=0-60%) to afford N-((9S)-4-chloro-9-ethyl-5-fluoro-9-hydroxy-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (4.1 g) as a brown solid. LCMS m/z 498.1(M+H).

[0190] **Step I:**

(9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione: A mixture of N-((9S)-4-chloro-9-ethyl-5-fluoro-9-hydroxy-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)acetamide (2.0 g, 4.02 mmol) in 20 mL con. HCl aq was stirred at 70 °C under N₂ for 36 h. The mixture was concentrated under reduced pressure to give crude

(9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione hydrochloride (2 g) as a brown solid. LCMS (ESI) m/z 456.1 (M+H).

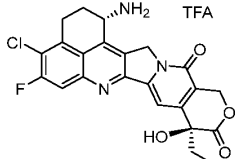
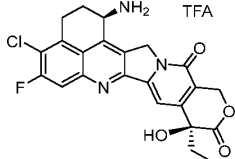
Preparation of Intermediate 12 (12-1, 12-2)



[0191] **12-1** and **12-2** were prepared by prep-HPLC from (9S)-1-amino-4-chloro-9-ethyl-5-fluoro-9-hydroxy-1,2,3,9,12,15-hexahydro-10H,13H-

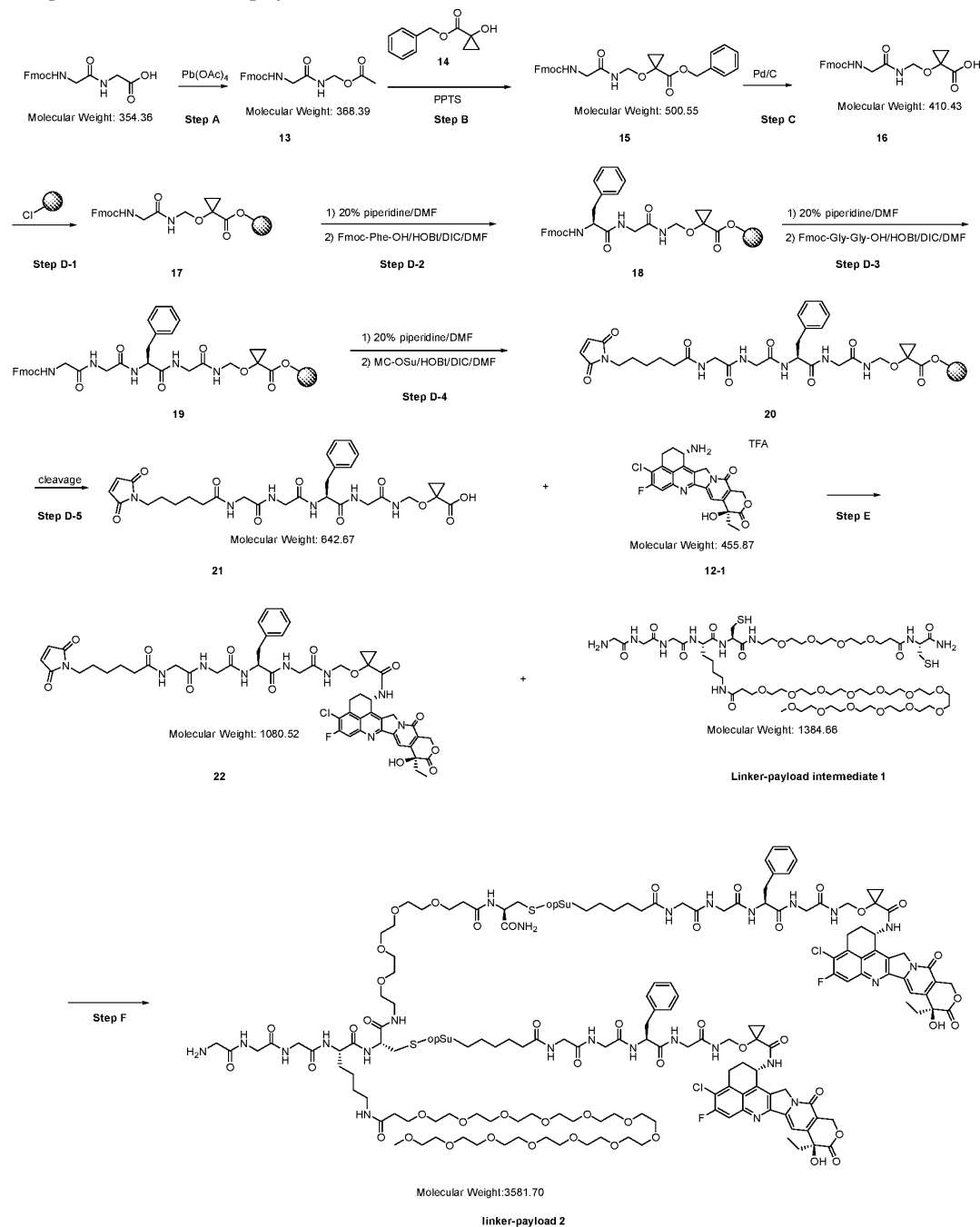
benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione hydrochloride (intermediate **11**) as TFA salt.

Table 1

Number	Structure	¹ HNMR	MS (M+H)	Retention time on HPLC (min)
12-1		¹ H NMR (400 MHz, DMSO-d ₆) δ 8.51 (d, <i>J</i> = 4.8 Hz, 3H), 8.17 (d, <i>J</i> = 10.2 Hz, 1H), 7.38 (s, 1H), 6.56 (s, 1H), 5.74 (d, <i>J</i> = 19.4 Hz, 1H), 5.52 – 5.40 (m, 3H), 5.16 (s, 1H), 3.44 (dd, <i>J</i> = 16.3, 4.1 Hz, 1H), 3.19 (t, <i>J</i> = 13.9 Hz, 1H), 2.57 (d, <i>J</i> = 14.0 Hz, 1H), 2.26 (t, <i>J</i> = 14.3 Hz, 1H), 1.89 (hept, <i>J</i> = 7.0 Hz, 2H), 0.89 (t, <i>J</i> = 7.3 Hz, 3H).	456.0	1.395
12-2		¹ H NMR (400 MHz, DMSO-d ₆) δ 8.56 (s, 2H), 8.17 (d, <i>J</i> = 10.1 Hz, 1H), 7.38 (s, 1H), 6.57 (s, 1H), 5.74 (d, <i>J</i> = 19.4 Hz, 1H), 5.53 – 5.28 (m, 3H), 5.16 (s, 1H), 3.59 – 3.01 (m, 2H), 2.35 – 2.19 (m, 2H), 2.00 – 1.78 (m, 2H), 0.88 (t, <i>J</i> = 7.3 Hz, 3H).	456.0	1.541

Conditions of HPLC above: Equipment: Agilent 1200; Chromatographic column: Waters XBridge C18 4.6*50mm, 3.5μm; Flow: 2.0mL/min; Gradient elute: 5.0%-95.0%-95.0%-5.0%-5.0%, 0.00min-1.50min-2.50min-2.52min-3.00min; Temperature : 40°C; Phase : A: Acetonitrile, B: H₂O (0.05% TFA); Wavelength: 214 nm/254 nm.

Preparation of linker-payload 2

Synthesis of **13** (Step A)

[0192] 4.33 g Fmoc-Gly-Gly-OH and 6.84 g Pb(OAc)₄ were weighed and added into a 500 ml single-neck round bottom flask. Anhydrous THF/Toluene (120/40 ml) was added under nitrogen atmosphere and stirred for dissolving. Then 1.16 mL of pyridine was added to the reaction

system. The reaction system was heated to 80°C and refluxed for 5hr under nitrogen atmosphere. Samples were taken and detected by HPLC to monitor the reaction.

[0193] The reaction system was cooled to room temperature, filtered, and the filter cake was washed with EA for 3 times. The filtrates were combined and concentrated to dryness. Column chromatography was performed (PE: EA = 100: 0 ~ 50: 100) to give about 2000 mg of the target product in white solid with a yield of 44%.

Synthesis of **15** (Step B)

[0194] 200 mg **13** was weighed and added into a 100 ml single-neck round bottom flask. Then 15 ml THF was added and stirred for dissolving. Then **14** (312mg, 3.0 eq) and TsOH·H₂O (15 mg, 0.15 eq) were added to the reaction system. The reaction system was reacted overnight at room temperature. Samples were taken and detected by TLC (PE/EA=1: 1) to monitor the reaction. The raw material basically disappeared, and a new point was detected.

[0195] Saturated sodium bicarbonate solution was added to quench reaction. Extraction was conducted with EA for 3 times. The organic phase was combined and washed with saline, dried with anhydrous magnesium sulfate and concentrated. The crude product was purified by column chromatography (PE: EA = 5: 1 ~ 1: 1) to give about 80 mg of the target product in colorless oil with a yield of 29%. LCMS m/z: [M+H]⁺ = 501.1

Synthesis of **16** (Step C)

[0196] 200 mg of **15** was weighed and added into a 100 ml single-neck round bottom flask. Then 10 ml of EtOH and 5 ml of EA were added with complete dissolution. Then 40 mg of palladium carbon was added to the reaction system under nitrogen atmosphere, and the reaction system was purged with hydrogen gas for three times. The reaction system was kept under hydrogen atmosphere and stirred for 0.5 hr at room temperature. Samples were taken and detected by TLC (DCM/MeOH=10: 1) to monitor the reaction. The raw material basically disappeared, and a new point was detected.

[0197] The reaction system was filtered, and the filter cake was washed with EA for 3 times. The filtrates were combined and concentrated to dryness to give 200 mg product in white solid with 100% yield. The product can be directly used in the next reaction without purification. LCMS m/z: [M-H]⁻ = 409.4.

Synthesis of **21** (Step D)

Step D-1

[0198] 2.0 g of dichlororesin was weighed and placed in a polypeptide synthesis tube. DCM (10 ml) was added and swelled at room temperature for 30 minutes. The solvent was removed by vacuum suction. The resin was washed twice with DCM, with a volume of 7 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. Then **16**(200 mg) was weighed and added into a 50 ml centrifuge tube. DCM (about 10 ml) was added. the

solid was dissolved by shaking. Added to the above resin. Stirring was conducted to soak all the resin in the solution (if there was resin attached to the tube wall, a small amount of DCM was used to wash the tube wall). Stirring was conducted for 4-5 hours. After the reaction was complete, an appropriate amount of methanol was added. Stirring was conducted for 30 min. The solvent was removed by vacuum suction. The resin was washed with DMF once, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating qualified for the next coupling step.

Step D-2

[0199] The deprotection was conducted twice by adding 10 mL readymade 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were dark blue.

[0200] To a 50 mL centrifuge tube was added 563 mg Fmoc-Phe-OH, 197 mg HOBt. Then about 7 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIC was added. Activated for 10-30 minutes to give the activated reaction solution.

[0201] 3 molar equivalent of activated reaction solution added to the resin. Stirring was conducted to soak the resin completely in the solution (if there was resin attached to the tube wall, a small amount of DCM was used to wash the tube wall). Stirring was conducted for 2-3 hours. After the reaction was complete, the solvent was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating qualified for the next coupling step.

Step D-3

[0202] The deprotection was conducted twice by adding 10 mL readymade 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were

dark blue.

[0203] To a 50 mL centrifuge tube was added 531 mg Fmoc-GG-OH, 197mg HOBt. Then about 10 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIC was added. Activated for 10-30 minutes to give the activated reaction solution.

[0204] 3 molar equivalent of activated reaction solution was added to the resin. Stirring was conducted to soak the resin completely in the solution (if there was resin attached to the tube wall, a small amount of DCM was used to wash the tube wall). Stirring was conducted for 2-3 hours. After the reaction was complete, the reaction solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating qualified for the next coupling step.

Step D-4

[0205] The deprotection was conducted twice by adding 10 mL readymade 20% piperidine/DMF solution and reacting for 10 minutes for each time. After the reaction was complete, the solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. Both the resin and solution were dark blue. Then, 462 mg MC-OSu was placed in a 50 mL centrifuge tube, about 10 mL DMF was added. The solid was dissolved by shaking. Then 0.24 mL DIEA was added to the resin. Stirring was conducted to soak the resin completely in the solution (if there was resin attached to the tube wall, a small amount of DCM was used to wash the tube wall). Stirring was conducted for 2-3 hours. After the reaction was complete, the reaction solution was removed by vacuum suction. The resin was washed with DMF twice, methanol once, DMF once, methanol once and DMF twice in sequence, with a volume of 10 mL and a time length of 1 minute for each wash. The solvent was removed by vacuum suction. A small amount of dry resin was taken for ninhydrin detection. The resin was colorless and transparent, and the solution was yellowish, indicating qualified for the next coupling step.

Step D-5

[0206] The resin was washed twice with 10 mL of methanol. Then the solvent was removed thoroughly by vacuum suction. The resin was poured out and weighed. The lysis buffer was prepared in a 250 mL conical flask, wherein: the ratio of TFE/DCM was 80%/20%, and the volume was 7-8 times of the weight of peptide resin. The lysis buffer was added into the peptide resin, shaken well. The resin was fully soaked in the lysis buffer, and lysis was carried out at

room temperature for 2-3 hours. The lysis buffer was then filtered out using a simple filter made of a syringe, and the resin was washed with 1-2 ml DCM and discarded. Then 150 mL precooled anhydrous ether was added to the lysis buffer, shaken well and then stood for 20-30 minutes. Using a 50 mL centrifuge tube, the above system was centrifuged in a centrifuge at 3500 rpm for 3 minutes, and the supernatant was poured out and discarded. The solid was shaken with precooled anhydrous ether, washed once under ultrasound, centrifuged at 3500rpm for 3 minutes, and the supernatant was poured out and discarded. The solid was placed in a centrifuge tube and allowed to air dry overnight, and then subjected to preparative purification to give 125 mg of product in white solid with a yield of 40%. LCMS m/z: $[M-H]^- = 641.5$.

Synthesis of **22 (Step E)**

[0207] 150 mg of raw material **21** and 55 mg of TSTU were weighed and added into a 10 mL single-neck round bottom flask, and anhydrous DMF (3 mL) was added under nitrogen atmosphere and stirred for 20 min. Then 18 mg **12-1** and 20 μ l DIEA were added in sequence to the reaction system. Stirring was conducted at room temperature for 2-8 hr under nitrogen atmosphere. Samples were taken and detected by HPLC to monitor the reaction. The raw material peak completely disappeared, and new peaks were detected.

[0208] The reaction system was subjected to preparative purification, and the target product was collected and lyophilized to give about 22mg of product in yellowish solid. LCMS m/z: $[M+H]^+ = 1081.0$.

Synthesis of **linker-payload 2 (Step F)**

[0209] **22** (30mg) was weighed and added into a 10 ml single-neck round bottom flask, purified water (2ml) was added. Stirring was conducted for dissolving. DMF solution (2 ml) containing **Linker-payload** intermediate **1** (19.5 mg) was added to the reaction system and stirred. After reacting overnight, HPLC was used to monitor the reaction until all of the raw material had converted into intermediates. The reaction mixture was directly added with an appropriate amount of Tris Base solution or other solution that promotes the ring-opening reaction, and the reaction was performed at 0-40°C for another 0.2-20h. The reaction was monitored by HPLC until all the intermediates were consumed and then quenched by acetic acid solution.

[0210] The reaction system was subjected to preparative purification, and the target product was collected and lyophilized to give about 25mg of **linker-payload 2** with yellowish solid. LCMS m/z: $[(M+3H)/3]^+ = 1194.4$

Example 3 Construction of antibody and the ADC

1. Production of the anti-FGFR3 antibody

[0211] The anti-FGFR3 antibody consists of two vectors as heavy and light chains, respectively, in each mammalian expression system. Anti-FGFR3 antibody was produced using the Expi293 transient mammalian expression system (Gibco, A14635, Carlsbad, CA, USA) via co-

transfection of the above-mentioned vector. After transfection, culture supernatants were purified using the ÄKTA protein purification system (GE Healthcare Life Sciences, Uppsala, Sweden) with HiTrap Mabselect SuRe (GE Healthcare Life Sciences, 11-0034-93, Uppsala, Sweden). After purification, concentration was performed with an Amicon® Ultra Centrifugal Filter (Merck Millipore, MA, USA). SEC-HPLC shows that the purity of the obtained antibody is more than 98.5%.

[0212] The anti-FGFR3 antibodies thus obtained are shown in the table below. The CDRs are highlighted with underlines and the constant regions are shown in *italic*.

Table 2

Na me	Heavy or light chain	Amino acids Sequence
A9	Heavy chain	EVQLLES GGGLVQPGGSLRL SCAASGFTFSS YDMSWVRQAPGKGLEWVS GIYSGDGSIIYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGPMNE <i>ESRFDYWGQGT</i> LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV <i>SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK</i> <i>VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED</i> <i>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</i> <i>KALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE</i> <i>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYT</i> <i>QKSLSLSPGK</i>
	Light chain	QSVLTQPPSASGTPGQRVTISCSGSSSNIGNNYSWYQQLPGTAPKLLIY ADSK RPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCASWDYSLSGYVFGGGTKL TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE TTTPSKQSNNKYAASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTEC
A9Q	Heavy chain	EVQLLES GGGLVQPGGSLRL SCAASGFTFSS YDMSWVRQAPGKGLEWVS GIYSGDGSIIYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGPMNE <i>ESRFDYWGQGT</i> LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV <i>SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK</i> <i>VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED</i> <i>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN</i> <i>KALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE</i> <i>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYT</i> <i>QKSLSLSPGK</i>
	Light chain	QSVLTQPPSASGTPGQRVTISCSGSSSNIGNNYSWYQQLPGTAPKLLIY ADSK RPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCASWDYSLSGYVFGGGTKL TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE TTTPSKQSNNKYAASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECGALPET GG

Note: the upstream peptide bond of GG in the LPETGG sequence is cleaved by Sortase A, and the resulting intermediate is linked to the free N-terminal of G₃ to generate a new peptide bond.

2. ADC preparation

[0213] The linker-payload intermediates were respectively conjugated to an antibody in a site-specific manner by a ligase to form an ADC. The method for conjugation reaction can be found

in WO2015165413A1. The resulting ADCs are as listed in the following table.

Table 3

Name of ADC	Linker-Payload	Antibody
ADC19	Linker-Payload 1	A9Q
ADC20	Linker-Payload 2	A9Q

Example 4 In vitro cytotoxicity assay

[0214] 1) 3D single spheroid model was formed by isolating cancer cells derived from glioblastoma patients. Two types of patient-derived cells with FGFR3 overexpression (AMB-BT-0050T, AMB-BT-0112T) and FGFR3 non-expression derived cells (AMB-BT-0013T), sampled from patients suffering from glioblastoma, were incubated overnight to form single spheroids (3D), followed by treatment of ADC (incubated for a week), and then spheroid size and volume were quantified. The results are shown in the table below, indicating that for both the conjugates ADC19 and ADC20, the cytotoxicity on FGFR3 positive cells are significantly higher than that to FGFR3 negative cells, and thus the ADCs are highly specific for FGFR3.

Table 4.1

Cell	Type	FGFR3 expression	IC50 (nM)	
			ADC19	ADC20
AMB-BT-0050T	GBM PDCs	+++	1	0.4
AMB-BT-0112T		++	10	21
AMB-BT-0013T		-	400	700

GBM is abbreviation of glioblastoma, PDCs is abbreviation of patient-derived cells.

[0215] 2) 3D single spheroid model was formed from bladder cell line RT112 (DSMZ, ACC418), which is FGFR3 overexpression. RT112 were seeded in cell spheroid culture plate and incubated overnight to form single spheroids (3D), followed by treatment of ADC (incubated for a week), and then spheroid size and volume were quantified. The results are shown in the table 4.2 below, indicating that for both the conjugates ADC19 and ADC20 had significantly cytotoxicity on FGFR3 positive bladder cancer cells.

Table 4.2

Cell	Type	FGFR3 expression	IC50 (nM)	
			ADC19	ADC20
RT112	Bladder	++	227	10

Example 5 In vivo efficacy test in glioblastoma PDX models

[0216] 1) For evaluating the survival rate in an orthotopic mouse model of brain tumor with target expression, AMB-BT-0050T patient-derived cells were sub-cultured and mixed 2.0×10^5 cells with the medium. 7-wk-old female BALB/c nude mice were used for intracranial transplantation. The prepared patient-derived cells were injected into the brains of mice by stereotactic intracranial injection at a depth of 3.2 mm at a position of 1.7 mm left and 0.5 mm above the bregma. Mice were housed with a 12-h light /12-h dark cycle and ad libitum access to food and water. Therapeutics administration is as follows. TMZ (temozolomide) was injected through oral administration every day for 5 times. ADCs were administered only once (single injection) or once a week for four weeks (multi-injection) via intravenous injection from the 7th day after model production to each group. The mice were sacrificed either when 20% body weight loss or neurological symptoms (lethargy, ataxia, and seizures) were observed and the results are shown in Figure 1.1. The survival of mice is evaluated through MST (Median Survival Time) and ILS (Increase in Life Span); MST, the time point at which the probability of survival equals 50%; ILS (Increase in Life Span), $ILS (\%) = [(median \text{ survival time of treated group}) / (median \text{ survival time of control group}) - 1] \times 100$.

[0217] The results show that anti-FGFR3 ADCs could inhibit the progression of brain tumors and prolong the survival time.

[0218] 2) To furtherly evaluate the survival rate in an orthotopic mouse model of brain tumor with target expression, in AMB-BT-0050T PDX model, mice were grouped and treated with (1) vehicle, (2) TMZ (temozolomide), 20 mg/kg; (3) ADC20 20 mg/kg; (4) combination of TMZ and ADC20. TMZ was injected through oral administration every day for 3 times. ADCs were administered once via intravenous injection. The results are shown in Figure 1.2. The survival of mice is evaluated through MST and ILS.

[0219] The results show both monotherapy of ADC20 and combo of ADC20 with TMZ could inhibit the progression of brain tumors and prolong the survival time. Combination of ADC and TMZ (standard of care for GBM) can significantly prolong the survival time.

Example 6 In vivo efficacy test in bladder cancer CDX models

[0220] To evaluate the in vivo anti-tumor efficacy of ADC19 and ADC20 in mice bearing bladder cancer FGFR3-high CDX model, several types of FGFR3 overexpressed bladder cancer

CDX models were used.

[0221] 1) RT112 cells in exponential growth stage were collected and counted for tumor inoculation. 0.2 mL of matrix gel buffer (PBS: Matrigel = 1:1) was used to subcutaneously inject 10×10^6 cells into the right flank of SPF female BALB/c nude mice aged 6-8 weeks.

[0222] The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 a \times b^2$ (wherein a is the long diameter of the tumor and b is the short diameter of the tumor). When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group, ADC19 5 mg/kg group and ADC20 5 mg/kg group. The day of first administration was defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week. The experiment was end on day 27, and the tumor growth inhibition rate (TGI) was calculated as follows: $TGI (\%) = [1 - (\text{the mean tumor volume of the treatment group on the end day} - \text{the mean tumor volume of the treatment group on the first day}) / (\text{the mean tumor volume of the vehicle group on the end day} - \text{the mean tumor volume of the vehicle group on the first day})] \times 100\%$.

[0223] Table 5.1 showed on the end day (day 27), the mean tumor volumes of ADC19 5 mg/kg group, ADC20 5 mg/kg group were 48mm³ and 32mm³ respectively; TGI were 106.35% and 107.08% respectively.

Table 5.1

Group	Tumor volume (mm ³) ^a	TGI ^b (%)
Vehicle	2,307 ± 336	--
ADC19 5 mg/kg	48 ± 9	106.35
ADC20 5 mg/kg	32 ± 7	107.08

a. Mean ± SEM; measured on the end day;

b. $TGI (\%) = [1 - (T_{27} - T_0) / (V_{27} - V_0)] \times 100\%$. T_0 is the mean tumor volume of the treatment group on the first day of administration, and T_{27} is the mean tumor volume of the treatment group at day 27 after administration; V_0 is the mean tumor volume of the vehicle group on the first day of administration, V_{27} is the mean tumor volume of the vehicle group at the day 27 after administration.

[0224] The results show both ADC19 and ADC20 have excellent anti-tumor efficacy in FGFR3 overexpressed RT112 bladder cancer CDX model.

[0225] 2) For evaluation the dose-dependent antitumor efficacy in bladder cancers, the RT112 cells bearing BALB/c nude mice were randomly grouped and treated by solvent; ADC20 8 mg/kg single dose and ADC20 8 mg/kg, QW, 2 times. The experiment was end on day 33, and the tumor growth inhibition rate (TGI) was calculated as follows: $TGI (\%) = [1 - (\text{the mean}$

tumor volume of the treatment group on the end day – the mean tumor volume of the treatment group on the first day)/(the mean tumor volume of the vehicle group on the end day -the mean tumor volume of the vehicle group on the first day)] $\times$ 100%. The ADC groups continued to be observed until day 84.

[0226] Table 5.2 showed on the end day (day 33), the mean tumor volumes of ADC20 single dose group, ADC20 repeat dose group were 36 mm³ and 16 mm³ respectively; TGI were 106.34% and 107.65% respectively. And cause completed response (CR) in repeated dose group.

Table 5.2

Group	Tumor volume (mm ³)	TGI (%)
Vehicle	1,564 $\pm$ 244	--
ADC20 8 mg/kg, single	36 $\pm$ 45	106.34
ADC20 8 mg/kg, repeat	16 $\pm$ 16	107.65

[0227] The results show either single dose or repeated dose of ADC20 has excellent anti-tumor efficacy in FGFR3 overexpressed RT112 bladder cancer CDX model, and repeated dose of ADC20 caused CR in this bladder cancer CDX model.

[0228] 3) SW780 cells in exponential growth stage were collected and counted for tumor inoculation. 0.2 mL of matrix gel buffer (PBS: Matrigel = 1:1) was used to subcutaneously inject 10 $\times$ 10⁶ cells into the right flank of SPF female NOD SCID mice aged 6-8 weeks.

[0229] The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 a \times b^2$. When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group, ADC19 5 mg/kg group and ADC20 5 mg/kg group. The day of first administration was defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week. The experiment was end on day 20, and the tumor growth inhibition rate (TGI) was calculated as follows: TGI (%)=[1 – (the mean tumor volume of the treatment group on the end day – the mean tumor volume of the treatment group on the first day)/(the mean tumor volume of the vehicle group on the end day -the mean tumor volume of the vehicle group on the first day)] $\times$ 100%.

[0230] Table 5.3 showed on the end day (day 20), the mean tumor volumes of ADC19 5 mg/kg group, ADC20 5 mg/kg group were 1,109mm³ and 526mm³ respectively; TGI were 40.80% and 76.66% respectively.

Table 5.3

Group	Tumor volume (mm ³)	TGI (%)
Vehicle	1,771 ± 414	--
ADC19 5 mg/kg	1,109 ± 100	40.80
ADC20 5 mg/kg	526 ± 128	76.66

[0231] The results show both ADC19 and ADC20 have potential anti-tumor efficacy in FGFR3 overexpressed SW780 bladder cancer CDX model.

[0232] 4) UM-UC1 cells in exponential growth stage were collected and counted for tumor inoculation. 0.1 mL of matrix gel buffer (PBS: Matrigel = 1:1) was used to subcutaneously inject 10×10^5 cells into the right flank of SPF female Balb/c nude mice aged 6-8 weeks.

[0233] The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 \times a \times b^2$. When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group and ADC20 8 mg/kg group. The day of first administration was defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week. The experiment was end on day 17, and the tumor growth inhibition rate (TGI) was calculated as follows: $TGI (\%) = [1 - (\text{the mean tumor volume of the treatment group on the end day} - \text{the mean tumor volume of the treatment group on the first day}) / (\text{the mean tumor volume of the vehicle group on the end day} - \text{the mean tumor volume of the vehicle group on the first day})] \times 100\%$.

[0234] Table 7.4 showed on the end day (day 17), the mean tumor volume of ADC20 8 mg/kg group was 402mm³; TGI was 82.71%.

Table 5.4

Group	Tumor volume (mm ³)	TGI (%)
Vehicle	1,735 ± 394	--
ADC20 8 mg/kg	402 ± 104	82.71

[0235] The results show that ADC20 has anti-tumor efficacy in FGFR3 overexpressed UM-UC1 bladder cancer CDX model.

Example 7 In vivo efficacy test in multiple myeloma CDX models

[0236] To evaluate the in vivo anti-tumor efficacy of ADC19 and ADC20 in mice bearing multiple myeloma FGFR3 overexpressed CDX model, KMS11 cells in exponential growth stage were collected and counted for tumor inoculation. 0.2 mL of matrix gel buffer (PBS:

Matrigel = 1:1) was used to subcutaneously inject 10×10^6 cells into the right flank of SPF female CB17.SCID mice aged 6-8 weeks.

[0237] The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 \times a \times b^2$. When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group, ADC19 5 mg/kg group and ADC20 5 mg/kg group. The day of first administration was defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week. The experiment was end on day 32, and the tumor growth inhibition rate (TGI) was calculated as follows: $TGI (\%) = [1 - (\text{the mean tumor volume of the treatment group on the end day} - \text{the mean tumor volume of the treatment group on the first day}) / (\text{the mean tumor volume of the vehicle group on the end day} - \text{the mean tumor volume of the vehicle group on the first day})] \times 100\%$.

[0238] Table 6 showed on the end day (day 32), the mean tumor volumes of ADC19 5 mg/kg group, ADC20 5 mg/kg group were 9mm³ and 11mm³ respectively; TGI were 108.41% and 108.31% respectively.

Table 6		
Group	Tumor volume (mm ³)	TGI (%)
Vehicle	2,161 ± 263	--
ADC19 5 mg/kg	9 ± 0	108.41
ADC20 5 mg/kg	11 ± 1	108.31

[0239] The results show both ADC19 and ADC20 have excellent anti-tumor efficacy in FGFR3 overexpressed KMS11 multiple myeloma CDX model.

Example 8 Bystander killing effect of ADC20 in RT112/U87MG

[0240] Far-Red labelled FGFR3-positive RT112 cells and CFSE labelled FGFR3-negative U87MG cells were seeded onto 96 well round bottom plate with 1.0×10^4 cells for each cell type. The cells were incubated overnight for stable adhesion to plate, followed by treatment with ADCs or PBS (incubation for a week). After incubation, the cells were centrifuged at 2300 rpm for 2 min, and then supernatant was discarded. The cells were washed once with PBS, detached, and resuspended into flow cytometry staining buffer containing LIVE/DEAD staining dye. Finally, the total amount of FGFR3-positive and FGFR3-negative cells and their viability were detected and analyzed by Flow cytometer (Novocyte, Agilent).

[0241] As shown in Figure 2, ADC19 and ADC20 both have bystander killing effect, and ADC20 has a more effective bystander killing effect than ADC19.

Example 9 In vivo efficacy test in glioblastoma PDX models

[0242] To evaluate the in vivo anti-tumor efficacy of ADC20 in mice bearing glioblastoma FGFR3-high PDX model, several types of FGFR3 overexpressed glioblastoma PDX models were used.

[0243] 1) AMB-BT-0039T cells with FGFR3-TACC3 fusion (GBM, patients-derived cells) in exponential growth stage were collected and counted for tumor inoculation. 0.1 mL of matrix gel buffer (PBS: Matrigel = 1:1) was used to subcutaneously inject 1×10^6 cells into the right flank of SPF female BALB/c nude mice aged 6-8 weeks. The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 a \times b^2$ (wherein a is the long diameter of the tumor and b is the short diameter of the tumor). When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group, and ADC20 8 mg/kg group. The day of first administration was defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week. The experiment was end on day 24, and the tumor growth inhibition rate (TGI) was calculated as follows: $TGI (\%) = [1 - (\text{the mean tumor volume of the treatment group on the end day} - \text{the mean tumor volume of the treatment group on the first day}) / (\text{the mean tumor volume of the vehicle group on the end day} - \text{the mean tumor volume of the vehicle group on the first day})] \times 100\%$.

[0244] Table 7 showed on the end day (day 24), the mean tumor volumes of ADC20 8 mg/kg group was 100mm³ and TGI was 100.03%.

Table 7

Group	Tumor volume (mm ³) ^a	TGI ^b (%)
Vehicle	2,274 ± 592	--
ADC20 8 mg/kg	100 ± 18	100.03

a. Mean ± SEM; measured on the end day;

b. $TGI (\%) = [1 - (T_{24} - T_0) / (V_{24} - V_0)] \times 100\%$. T₀ is the mean tumor volume of the treatment group on the first day of administration, and T₂₄ is the mean tumor volume of the treatment group at day 24 after administration; V₀ is the mean tumor volume of the vehicle group on the first day of administration, V₂₄ is the mean tumor volume of the vehicle group at the day 24 after administration.

[0245] 2) AMB-BT-0112T cells with FGFR3-TACC3 fusion (GBM, patients-derived cells) in exponential growth stage were collected and counted for tumor inoculation. 0.1 mL of matrix gel buffer (PBS: Matrigel = 1:1) was used to subcutaneously inject 1×10^6 cells into the right flank of SPF female BALB/c nude mice aged 6-8 weeks.

[0246] The tumor diameter was measured with a caliper and the tumor volume was calculated according to the formula $V = 0.5 a \times b^2$ (wherein a is the long diameter of the tumor and b is the short diameter of the tumor). When the mean tumor volume was about 100-300 mm³, the mice were randomized into vehicle group, and ADC20 8 mg/kg group. The day of first administration is defined as day 0. Mice in the vehicle group were given the solvent of ADC drugs with the same frequency and administration route. The tumor volume of mice in each group was measured twice a week.

[0247] Table 8 showed on the end day (day 33), the mean tumor volumes of ADC20 8 mg/kg group was 216mm³ and TGI was 95.08%.

Table 8

Group	Tumor volume (mm ³) ^a	TGI ^b (%)
Vehicle	2,344 ± 242	--
ADC20 8 mg/kg	216 ± 52	95.08

[0248] There are other examples in patent PCT/CN2023/103152 which are incorporated herein by reference in their entireties.

Example 10 pH Buffer Screening for the formulation comprising an ADC

[0249] Sample: ADC20

[0250] 9 groups of formulation buffers with different pH (pH values were 5.0, 5.5, 6.0, 6.5, 7.0) was prepared, and the 9 groups of formulation buffers were as follows (Table 9). The sample was buffer exchanged using the 9 groups of buffers by Big tuna (Unchained labs, XPDT-C-014), and the protein concentration was adjusted to about 20 mg/ml. And then the stability of sample was examined at 25 °C and 40 °C in different pH buffers.

[0251] Components of glutamate buffer comprise L-glutamic acid and sodium L-glutamate; Components of citrate buffer comprise citric acid monohydrate and trisodium citrate dihydrate; Components of histidine buffer comprise L-histidine and L-histidine monohydrochloride; Components of phosphate buffer (PB buffer) comprise sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate.

Table 9

Formula No.	Buffer	pH
FP-1	20 mM Glutamate	5.0
FP-2	20 mM Glutamate	5.5
FP-3	20 mM Citrate	5.5
FP-4	20 mM Citrate	6.0
FP-5	20 mM Histidine	5.5

FP-6	20 mM Histidine	6.0
FP-7	20 mM Histidine	6.5
FP-8	20mM PB	6.5
FP-9	20mM PB	7.0

[0252] 1) T_m of the start sample by DSC

[0253] The T_m values of proteins in solution were determined using a MicroCal PEAQ DSC (Malvern, MAL1268569) at an instrument-programmed controlled temperature. Prior to analysis, protein samples were first diluted to 1 mg/mL using its reference buffer. 400 μ L of each reference buffer was added to the odd wells of a 96-well plate and 400 μ L of sample was added to the even wells of the same plate. The experimental parameters were set so that the scan temperature risen from 20-100°C at a rate of 200°C/h. Thermal spectra were analysed in MicroCal PEAQ DSC automated data analysis software.

Table 10.1

No.	DSC			
	T _m Onset (°C)	T _m ₁ (°C)	T _m ₂ (°C)	T _m ₃ (°C)
FP-1	54.5	64.1	75.1	83.5
FP-2	57.9	67.9	74.9	82.6
FP-3	54.9	65.8	73.4	82.1
FP-4	57.6	69.7	73.8	82.1
FP-5	52.3	62.0	74.0	82.7
FP-6	54.8	65.3	74.3	82.9
FP-7	56.7	67.7	74.1	82.9
FP-8	59.5	69.3	73.9	82.7
FP-9	60.7	68.1	73.8	82.7

[0254] T_m onset of all samples in 9 formulations are > 52°C, indicating good thermal stability at room temperature and stress condition temperature 40°C.

[0255] 2) Appearance, pH and protein concentration

[0256] Appearance: the appearance of samples, including clarity, color and visible particles was examined against black and white background using YB-2 light box (clarity detector, Tianda Tianfa).

[0257] pH: pH was measured using a pH meter with a glass electrode. The pH meter was calibrated every day prior to use with three different standard buffers (pH 4.01, 7.00 and 9.21).

The slope of calibration was confirmed to be between 95.0% - 105.0%, and the zero drift between -60.0 mv to +60.0 mv.

[0258] Protein concentration: Stunner (Unchained labs) was used to measure the ultraviolet absorption spectrum (UV 280 nm and 360 nm) of the protein. The protein concentration of the sample was calculated based on the extinction coefficient via the slope spectroscopy technology.

Table 10.2

No.	T0			40°C-1W			40°C-2W			25°C-2W		
	Appearance	pH	Conc. (mg/ml)	Appearance	pH	Conc. (mg/ml)	Appearance	pH	Conc. (mg/ml)	Appearance	pH	Conc. (mg/ml)
FP-1	C,SO,FP	5.1	20.1	C,SO,EFP	5.0	20.3	C,SO,EFP	5.1	20.2	C,SO,EFP	5.1	20.4
FP-2	C,SO,FP	5.6	19.4	C,SO,FP	5.6	19.5	C,SO,FP	5.7	19.4	C,SO,EFP	5.7	19.3
FP-3	C,SO,FP	5.5	20.0	C,SO,FP	5.5	20.0	C,SO,EFP	5.6	20.0	C,SO,FP	5.5	19.9
FP-4	C,SO,FP	6.0	19.8	C,SO,EFP	6.0	19.9	C,SO,NEFP	6.0	19.8	C,SO,EFP	6.0	19.8
FP-5	C,SO,FP	5.4	19.7	C,SO,FP	5.5	19.9	C,SO,FP	5.5	19.9	C,SO,FP	5.5	19.7
FP-6	C,SO,FP	6.0	19.9	C,SO,FP	6.1	20.1	C,SO,EFP	6.1	20.1	C,SO,EFP	6.0	20.0
FP-7	C,SO,FP	6.5	19.6	C,SO,FP	6.5	19.7	C,SO,NEFP	6.5	19.8	C,SO,EFP	6.5	19.7
FP-8	C,SO,FP	6.5	19.7	C,SO,FP	6.5	20.0	C,SO,NEFP	6.5	20.0	C,SO,EFP	6.5	19.9
FP-9	C,SO,FP	6.9	19.8	C,SO,FP	7.0	19.9	C,SO,NEFP	7.0	19.8	C,SO,EFP	7.0	19.8

Note: T0: time of start; 1W: one week; 2W: two weeks; C: colorless; SY: slightly yellow; Y: yellow; CL: clear liquid; SO: slightly opalescent liquid; FP: free of visible particles (number=0); EFP: essentially free of visible particles (number≤3); NEFP: not essentially free of visible particles (number>3).

Compared with the T0 point, the pH and protein concentration results of each prescription did not change significantly. FP-4, FP-7, FP-8, and FP-9 have more than 3 visible particles under the conditions of 40°C-2W.

[0259] 3) Purity by SEC-HPLC (Aggregate %)

[0260] Size-exclusion chromatography HPLC (SEC-HPLC) separates proteins by a combination of their hydrodynamic size, diffusion coefficient, and surface properties. Soluble proteins enter the stationary phase of the column, traveling between the void of the particles and the pores of the matrix. Larger proteins above the exclusion limit of the column do not efficiently enter the pores and will elute in the void volume. Proteins below the exclusion limit will enter the pores and separate based on hydrodynamic radius, where larger species typically have shorter retention times and smaller species have longer retention times. Following separation, the relative percentages of monomer species, High Molecular Weight Species (HMWS) and Low Molecular Weight Species (LMWS) are quantified via UV detection.

[0261] Sample was injected into a TSKgel G3000SWXL Column (7.8×300 mm, 5 µm). The analysis was performed on the Waters Arc system or Agilent 1260 system with a UV detector (detection wavelength: 280 nm). The chromatographic program was a 20 min isocratic gradient with a mobile phase of 50 mM phosphate buffer containing 300 mM sodium chloride (pH 6.8 ± 0.1) at a flow rate of 1.0 mL/min.

Table 10.3-1

No.	T0			40°C-1W			40°C-2W		
	Main peak (%)	HMWS (%)	LMWS (%)	Main peak (%)	HMWS (%)	LMWS (%)	Main peak %	HMWS (%)	LMWS (%)
FP-1	96.4	3.6	-	95.1	4.3	0.7	93.8	5.3	0.9
FP-2	96.2	3.8	-	95.1	4.4	0.5	94.0	5.4	0.6
FP-3	96.1	3.9	-	94.6	5.0	0.4	93.4	6.1	0.5
FP-4	95.9	4.1	-	94.5	5.1	0.4	93.5	6.1	0.4
FP-5	96.3	3.7	-	95.2	4.1	0.6	94.2	5.0	0.8
FP-6	96.2	3.8	-	95.3	4.2	0.5	94.5	4.9	0.6
FP-7	96.2	3.8	-	95.1	4.4	0.5	94.1	5.3	0.6
FP-8	95.7	4.3	-	93.9	5.7	0.4	92.4	7.0	0.5
FP-9	95.6	4.4	-	93.6	6.0	0.5	92.2	7.3	0.6

Table 10.3-2

No.	T0			25°C-2W		
	Main peak (%)	HMWS (%)	LMWS (%)	Main peak (%)	HMWS (%)	LMWS (%)
FP-1	96.4	3.6	-	96.0	3.6	0.5
FP-2	96.2	3.8	-	95.8	3.6	0.6

FP-3	96.1	3.9	-	95.3	4.2	0.4
FP-4	95.9	4.1	-	95.1	4.5	0.4
FP-5	96.3	3.7	-	95.8	3.7	0.5
FP-6	96.2	3.8	-	95.6	3.9	0.5
FP-7	96.2	3.8	-	95.4	4.1	0.5
FP-8	95.7	4.3	-	94.5	5.1	0.5
FP-9	95.6	4.4	-	94.1	5.4	0.5

[0262] The SEC performance of 9 formulations is good. Compared with other formulations, the SEC purity of FP-6 formulation dropped less at 40°C-2W.

[0263] 4) DAR

[0264] DAR value analysis was performed by hydrophobic chromatography. The analytical column was TSK gel Butyl NPR (4.6mm×3.5 cm, particle size 2.5 μm); 50 mmol/L phosphate buffer + 1.5 mol/L ammonium sulfate solution (pH 7.0) was used as mobile phase A. Mobile phase B consisted of 50 mmol/L phosphate solution (pH 7.0) mixed with isopropanol at a volume ratio of 75:25. The flow rate was 1 mL per minute, the column temperature was 30 °C, and the detection wavelength was 280 nm. The test sample was diluted with purified water to a solution containing about 5 mg per 1 mL, as the test solution. Injection volume was 5 μL. Elution gradient was performed according to the table below.

Time (minute)	Phase A (%)	Phase B (%)
0.00	90.0	10.0
1.00	90.0	10.0
8.00	65.0	35.0
12.10	0.0	100.0
15.00	0.0	100.0
15.10	90.0	10.0
17.00	90.0	10.0

Table 10.4

No.	T0	40°C-1W	40°C-2W	25°C-2W
FP-1	3.65	3.61	3.58	3.61
FP-2	3.62	3.59	3.56	/
FP-3	3.64	3.62	3.59	3.61
FP-4	3.64	3.62	3.60	3.61
FP-5	3.64	3.61	3.59	3.61
FP-6	3.64	3.62	3.59	3.61

FP-7	3.64	3.61	3.58	3.61
FP-8	3.64	3.61	3.59	3.61
FP-9	3.64	3.61	3.58	3.61

Note: “/” means no detecting.

[0265] There were no significant differences in DAR results among the prescriptions.

[0266] 5) Concentration of free drug

[0267] This method used high-performance liquid chromatography-ultraviolet detector to detect residual free drug and its related substances in ADC samples. After protein precipitation, the samples were loaded to a column, and the residual drug and its related substances were eluted by gradient mobile phase. The concentrations of residual drug and its related substances of ADC samples were calculated by external standard quantification method. The sample preparation was following:

[0268] Added 50 μ L of each ADC sample to 100 μ L of protein precipitator (10.0 g NaCl, 75 mL of methanol, and 25 mL of acetonitrile), and mixed well at room temperature, then centrifuged for 10 min at 13,000 rpm. Finally, the supernatant was taken immediately into a sample tube for analysis. Linker-payload 2 (LP2) and payload stock solution were diluted. Agilent 1260 HPLC system with a UV detector was used for analysis.

Table 10.5

No.	T0			40°C-1W			40°C-2W			25°C-2W		
	LP 2	pay load	Total free drug	LP 2	pay load	Total free drug	LP 2	pay load	Total free drug	LP 2	pay load	Total free drug
FP-1	3.4	1.4	4.8	15.0	6.7	21.7	18.2	11.1	29.3	10.7	3.2	13.9
FP-2	3.3	3.3	6.6	11.4	6.3	17.6	15.4	8.8	24.2	7.9	27.4	/
FP-3	2.2	1.7	3.9	10.8	4.8	15.6	15.2	8.0	23.2	15.2	2.6	17.8
FP-4	1.8	1.4	3.1	7.5	3.6	11.1	12.4	6.0	18.4	6.5	2.3	8.8
FP-5	2.8	1.4	4.2	12.6	5.6	18.2	16.1	8.6	24.7	8.3	2.5	10.8
FP-6	2.4	1.4	3.8	9.6	3.8	13.4	13.2	6.1	19.3	6.3	2.1	8.4
FP-7	2.4	1.6	4.0	8.2	3.8	12.0	11.8	6.0	17.8	5.8	2.2	8.1
FP-8	2.1	1.8	3.8	6.6	4.3	10.9	8.3	5.8	14.1	4.6	2.3	6.9
FP-9	1.6	1.9	3.5	3.9	5.0	8.8	6.6	8.0	14.6	3.5	3.1	6.6

Note: “/” means no detecting.

[0269] As pH rises, free drug rises less. The RP-HPLC performance of FP-2, FP-3, FP-4, FP-6, FP-7, FP-8 and FP-9 are better.

[0270] 6) Reduced Calliper-SDS

[0271] The reduced sample denaturing solution for the reduced sample was prepared by mixing sample buffer with 10% SDS and 1M dithiothreitol at the volume ratio of 100:10:4. Diluted samples (diluted to 1.0 mg/mL with ultrapure water) were mixed well with the sample denaturing solution at a ratio of 2:7 individually. Subsequently, these samples were incubated at 70°C for 10 min and then mixed well and centrifuged. A certain volume of ultrapure water was added (equivalent to 5 times volume of sample denaturing solution), mixed well and centrifuged. After that, the sample plate was analyzed with the LabChip® GXII HT instrument. At last, the raw data was analyzed with Empower.

Table 10.6

No.	T0			40°C-1W			40°C-2W			25°C-2W		
	Purity (%)	LC %	HC %	Purity (%)	LC %	HC %	Purity (%)	LC %	HC %	Purity (%)	LC %	HC %
FP-1	97.5	53.1	44.5	96.5	52.8	43.6	96.2	52.5	43.7	97.6	52.9	44.7
FP-2	97.4	53.1	44.4	97.3	52.8	44.4	96.2	51.9	44.3	97.4	51.6	45.7
FP-3	97.4	53.2	44.2	97.4	52.9	44.6	97.1	52.2	44.9	97.6	53	44.6
FP-4	97.3	53.1	44.1	97.2	52.6	44.6	97.1	52.1	45	97.5	52.9	44.6
FP-5	97.4	53.1	44.3	97.3	52.7	44.6	96.5	52.2	44.4	97.5	52.8	44.8
FP-6	97.4	53.0	44.3	97.4	52.7	44.7	97.1	52.3	44.8	97.5	52.7	44.8
FP-7	97.4	53.0	44.4	97.2	52.4	44.8	97.0	51.7	45.3	97.3	52.6	44.7
FP-8	97.3	52.9	44.4	97.1	52.5	44.6	96.8	51.4	45.4	97.3	52.9	44.4
FP-9	97.1	52.5	44.6	96.4	52.3	44.1	95.8	51.1	44.7	97.2	52.5	44.8

[0272] Note: HC is the abbreviation for heavy chain, LC is the abbreviation for light chain.

[0273] The purity of F1, F2, and F9 decreased slightly.

[0274] Based on the results, this ADC20 molecule performed better in the buffer (20 mM histidine, pH6.0).

Example 11 Stabilizer / Surfactant Screening for the formulation comprising an ADC

[0275] Sample: ADC20

[0276] 6 formulations with different excipient were prepared, and the composition of 6 formulations were as follows in table 11. The sample was buffer-exchanged into 20 mM pH 6.0 histidine buffer with ultrafiltration tube, and then concentrated to 27.4 mg/ml. Appropriate amounts of the stock solutions of 40% (w/w) sucrose, 13.5% (w/w) sorbitol, 700 mM Arg-HCl, 700 mM NaCl, 5% (w/w) PS80, and 20 mM pH 6.0 histidine buffer were compounded according to Table 11. After compounding, each formulation was filtrated with the 0.22 µm

PVDF filter in bio-safety hood, and then filled into 20 mL vials with the filling volume of 3 mL per vial. Samples were tested under different stress conditions.

Table 11

Formula No.	Buffer	Excipient
FS-1	20 mM Histidine, pH6.0	8% sucrose, 0.02% PS80
FS-2	20 mM Histidine, pH6.0	4.5 % sorbitol, 0.02% PS80
FS-3	20 mM Histidine, pH6.0	140 mM Arg-HCl, 0.02% PS80
FS-4	20 mM Histidine, pH6.0	140 mM NaCl, 0.02% PS80
FS-5	20 mM Histidine, pH6.0	8% sucrose, 0.01% PS80
FS-6	20 mM Histidine, pH6.0	8% sucrose, 0.04% PS80

[0277] 1) T_m and osmolality of the start sample by DSC

[0278] The osmolality of the samples was measured using an osmometer. An appropriate volume of sample was added to the centrifuge tube to avoid air bubbles and placed in the osmometer for testing.

Table 12.1

No.	DSC				Osmolality (mOsm/kg)
	T _{Onset} (°C)	T _{m1} (°C)	T _{m2} (°C)	T _{m3} (°C)	
FS-1	59.9	69.9	76.2	85.7	300
FS-2	58.3	69.2	75.9	85.2	296
FS-3	57.2	67.9	73.9	82.8	279
FS-4	57.5	68.3	74.0	83.0	299
FS-5	57.2	69.7	76.1	85.0	298
FS-6	58.5	69.8	76.1	85.2	295

[0279] The osmolality range is from 279 to 300, with the FS-3 formulation slightly lower, and there is no significant difference between the remaining formulations.

[0280] 2) Appearance

Table 12.2

No.	T0	Agitation-3D	Agitation-7D	F/T-3X	F/T-5X	40°C-2W	40°C-4W
FS-1	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP
FS-2	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP

FS-3	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP
FS-4	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP
FS-5	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP
FS-6	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, FP	C, SO, EFP

Note: Agitation means shaking under condition of 300 rpm, 25°C; F/T means Freeze/Thaw (from -40°C to room temperature); 3X means 3 cycles; 5X means 5 cycles; 4W means 4 weeks; 2W means 2 weeks; 3D means 3 days; 7D means 7 days.

[0281] There were no significant differences in the appearance results among the formulations.

[0282] 3) Purity by SEC-HPLC (Aggregate %):

Table 12.3-1

No.	T0			Agitation-3D			Agitation-7D		
	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %
FS-1	96.0	4.0	ND	96.1	3.9	ND	96.1	3.9	ND
FS-2	96.0	4.0	ND	96.1	3.9	ND	96.0	4.0	ND
FS-3	96.0	4.0	ND	96.2	3.8	ND	96.1	3.9	ND
FS-4	95.9	4.1	ND	96.0	4.0	ND	95.9	4.1	ND
FS-5	96.0	4.0	ND	96.1	3.9	ND	96.1	3.9	ND
FS-6	96.0	4.0	ND	96.1	3.9	ND	96.1	3.9	ND

Table 12.3-2

No.	T0			F/T-3X			F/T-5X		
	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %
FS-1	96.0	4.0	ND	96.0	4.0	ND	96.0	4.0	ND
FS-2	96.0	4.0	ND	96.0	4.0	ND	96.0	4.0	ND
FS-3	96.0	4.0	ND	96.0	4.0	ND	96.0	4.0	ND
FS-4	95.9	4.1	ND	95.8	4.2	ND	95.8	4.2	ND
FS-5	96.0	4.0	ND	96.0	4.0	ND	96.0	4.0	ND
FS-6	96.0	4.0	ND	96.0	4.0	ND	96.0	4.0	ND

Table 12.3-3

No.	T0			40°C-2W			40°C-4W		
	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %	Main peak %	HMWS %	LMWS %
FS-1	96.0	4.0	ND	94.9	4.7	0.4	93.4	5.9	0.7
FS-2	96.0	4.0	ND	94.6	5.0	0.4	92.7	6.6	0.7
FS-3	96.0	4.0	ND	94.7	4.9	0.4	92.8	6.5	0.7
FS-4	95.9	4.1	ND	94.2	5.4	0.4	92.0	7.3	0.6
FS-5	96.0	4.0	ND	94.9	4.7	0.4	93.6	5.8	0.7
FS-6	96.0	4.0	ND	94.9	4.7	0.4	93.4	6.0	0.7

[0283] There was no significant change in SEC purity of each formulation under the freezing, thawing and shaking conditions.

[0284] 4) DAR

Table 12.4

No.	T0	Agitation-3D	Agitation-7D	F/T-3X	F/T-5X	40°C-2W	40°C-4W
FS-1	3.5	3.5	3.5	3.5	3.5	3.5	3.5
FS-2	3.5	3.5	3.5	3.5	3.5	3.5	3.5
FS-3	3.5	3.5	3.5	3.5	3.5	3.5	3.4
FS-4	3.5	3.5	3.5	3.5	3.5	3.5	3.5
FS-5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
FS-6	3.5	3.5	3.5	3.5	3.5	3.5	3.5

[0285] The DAR results of each formulation did not change significantly.

[0286] 5) Reduced Calliper-SDS

Table 12.5-1

No.	T0			Agitation-3D			Agitation-7D		
	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%
FS-1	97.5	51.6	46.0	97.6	51.8	45.8	97.2	51.4	45.7
FS-2	97.6	51.4	46.1	97.5	51.7	45.8	97.4	52.0	45.4
FS-3	97.6	51.5	46.1	97.6	51.7	45.8	97.4	51.7	45.7
FS-4	97.5	51.6	45.8	97.6	51.8	45.7	97.6	51.8	45.8

FS-5	97.4	51.6	45.8	97.2	51.7	45.5	97.2	51.7	45.6
FS-6	97.5	51.7	45.8	97.2	51.6	45.6	97.3	51.7	45.7

Table 12.5-2

No.	T0			F/T-3X			F/T-5X		
	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%
FS-1	97.5	51.6	46.0	97.5	51.7	45.7	97.4	51.1	46.4
FS-2	97.6	51.4	46.1	97.5	51.6	45.9	97.4	50.9	46.4
FS-3	97.6	51.5	46.1	96.9	52.7	44.2	97.0	50.3	46.7
FS-4	97.5	51.6	45.8	97.5	51.7	45.9	97.3	50.6	46.7
FS-5	97.4	51.6	45.8	97.1	51.5	45.6	97.5	51.4	46.1
FS-6	97.5	51.7	45.8	97.4	51.7	45.8	97.5	51.3	46.2

Table 12.5-3

No.	T0			40°C-2W			40°C-4W		
	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%	Purity (%)	LC%	HC%
FS-1	97.5	51.6	46.0	97.1	51.6	45.6	95.8	49.1	46.6
FS-2	97.6	51.4	46.1	97.1	51.5	45.6	95.6	49.2	46.4
FS-3	97.6	51.5	46.1	97.2	51.3	45.9	95.8	48.5	47.3
FS-4	97.5	51.6	45.8	96.9	51.7	45.3	95.8	48.9	46.9
FS-5	97.4	51.6	45.8	97.0	51.5	45.5	95.8	48.9	47.0
FS-6	97.5	51.7	45.8	96.7	51.2	45.5	95.6	48.7	46.9

[0287] Reduced Calliper-SDS results did not change significantly among formulations. The reduced Calliper-SDS results at 40°C-4W were slightly lower, and there were no obvious difference between the formulations.

Summary

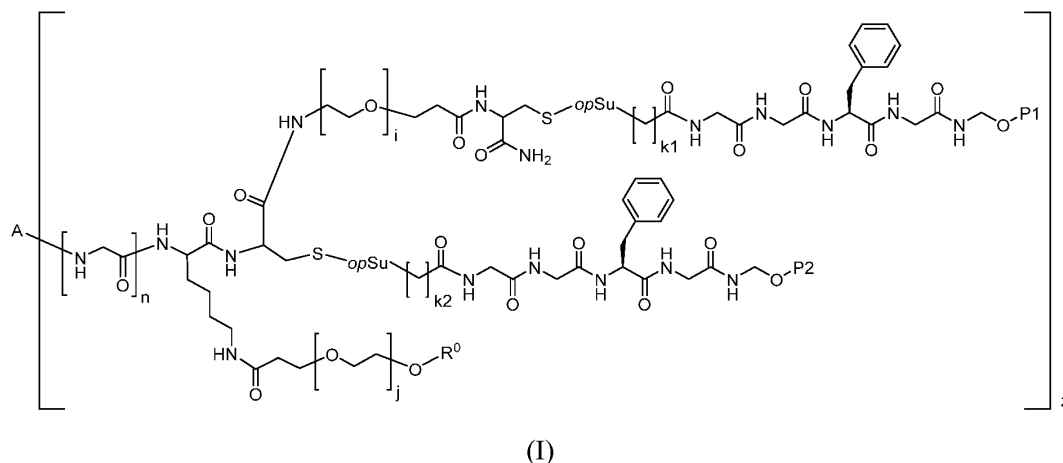
FS-1 formulation is better compared to other formulations.

Acknowledgement

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Claims

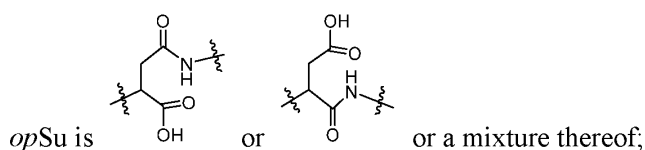
1. A pharmaceutical composition, comprising:
 an antibody drug conjugate (ADC),
 a buffer, and
 an optional stabilizer;
 wherein the ADC has the structure of formula (I):



wherein,

A is an anti-FGFR3 antibody or an antigen binding fragment thereof, the antibody or antigen binding fragment is modified to connect with the (Gly)_n moiety in the compound of formula (I), wherein the antibody or an antigen binding fragment comprises CDRs: a heavy chain CDR1 comprising amino acid sequence of SEQ ID NO: 9 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 9, a heavy chain CDR2 comprising amino acid sequence of SEQ ID NO: 10 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 10, a heavy chain CDR3 comprising amino acid sequence of SEQ ID NO: 11 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 11, a light chain CDR1 comprising amino acid sequence of SEQ ID NO: 12 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 12, a light chain CDR2 comprising amino acid sequence of SEQ ID NO: 13 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 13, and a light chain CDR3 comprising amino acid sequence of SEQ ID NO: 14 or having one to three conservative amino acid substitutions compared to SEQ ID NO: 14;

z is an integer of 1 to 20; preferably 1 to 4; particularly 2;



R^0 is C_{1-10} alkyl;

n is an integer of 2 to 20;

k_1 and k_2 are independently an integer of 1 to 7;

i is an integer of 1-20;

j is an integer of 1-20;

P_1 and P_2 are independently a payload.

2. The pharmaceutical composition of claim 1, wherein

R^0 is C_{1-3} alkyl, preferably methyl; and/or

n is an integer of 2 to 5, preferably 3; and/or

k_1 and k_2 are independently 1, 3 or 5;

i is 4; and/or

j is 8 or 12.

3. The pharmaceutical composition of claim 1 or 2, wherein the connection process between the modified antibody or antigen binding fragment and the $(Gly)_n$ moiety in compound of formula (I) is catalyzed by a ligase.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein the payload is a cytotoxin or a fragment thereof, with an optional derivatization in order to connect the payload and the rest moiety of the ADC;

the cytotoxin is selected from the group consisting of: taxanes, maytansinoids, auristatins, epothilones, combretastatin A-4 phosphate, combretastatin A-4 and derivatives thereof, indol-sulfonamides, vinblastines such as vinblastine, vincristine, vindesine, vinorelbine, vinflunine, vinglycinates, anhy-drovinblastine, dolastatin 10 and analogues, halichondrin B, eribulin, indole-3-oxoacetamide, podophyllotoxins, 7-diethylamino-3-(2'-benzoxazolyl)-coumarin (DBC), discodermolide, laulimalide, camptothecins and derivatives thereof, mitoxantrone, mitoguazone, nitrogen mustards, nitrosoureas, aziridines, benzodepa, carboquone, meturedepa, uredepa, dynemicin, esperamicin, neocarzinostatin, aclacinomycin, actinomycin, antramycin, bleomycins, actinomycin C, carabycin, carminomycin, cardinophyllin, carminomycin, actinomycin D, daunorubicin, detorubicin, adriamycin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, nogalamycin, olivomycin, peplomycin, porfiromycin, puromycin, ferric adriamycin, rodorubicin, rufocromomycin, streptozocin, zinostatin, zorubicin, trichothecene, T-2 toxin, verracurin A, bacilloporin A, anguidine, ubenimex, azaserine, 6-diazo-5-oxo-L-norleucine, dimethyl folic acid, methotrexate, pteropterin, trimetrexate, edatrexate, fludarabine, 6-mercaptopurine, tiampirine, thioguanine, ancitabine, gemcitabine,

enocitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, floxuridine, calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone, aminoglutethimide, mitotane, trilostane, flutamide, nilutamide, bicalutamide, leuprorelin acetate, protein kinase inhibitors and a proteasome inhibitors; and/or

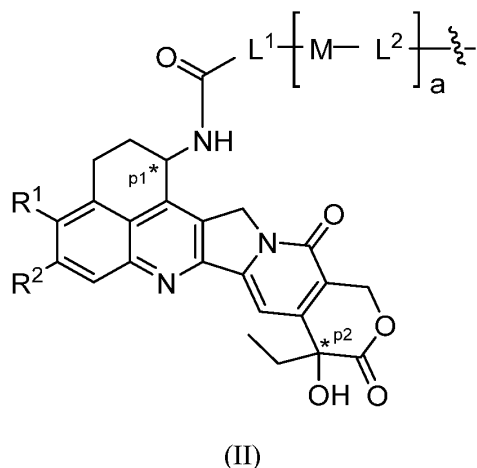
selected from vinblastines, colchicines, taxanes, auristatins, maytansinoids, calicheamicin, doxonubicin, duocarmycins, SN-38, cryptophycin analogue, deruxtecan, duocarmazine, calicheamicin, centanamycin, dolastatins, pyrrolobenzodiazepine, exatecan and derivatives thereof; and/or

selected from auristatins, especially MMAE, MMAF or MMAD; and/or

selected from exatecan and derivatives thereof, such as DX8951f; and/or

selected from DXd-(1) and DXd-(2); preferably DXd-(1).

5. The pharmaceutical composition of any one of claims 1 to 4, wherein the payload has the structure of formula (II):



wherein,

a is 0 or 1;

the carbon atoms marked with p1* and p2* each is asymmetric center, and the asymmetric center is S configured, R configured or racemic;

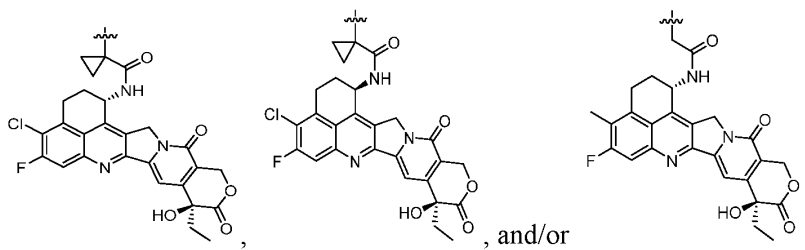
L¹ is selected from C₁₋₆ alkylene, which is unsubstituted or substituted with one substituent selected from halogen, -OH and -NH₂;

M is -CH₂-, -NH- or -O-;

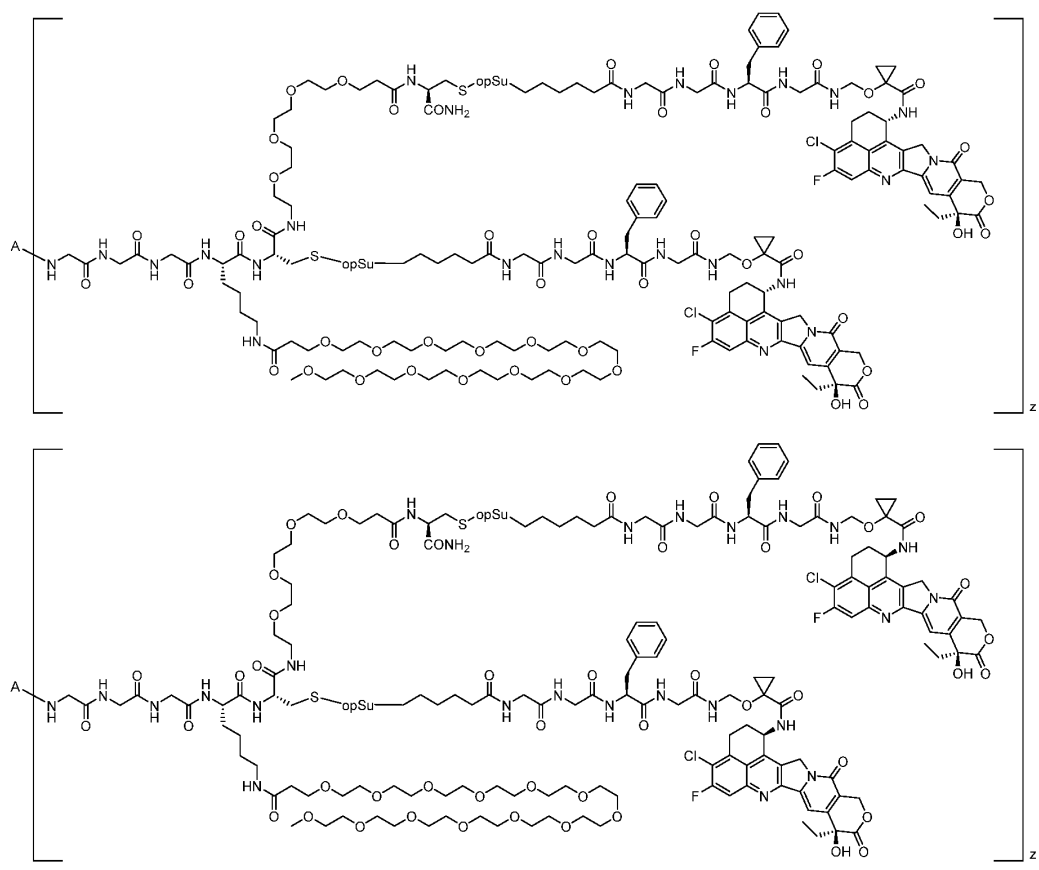
L² is C₁₋₃ alkylene;

R¹ and R² are each independently selected from hydrogen, C₁₋₆ alkyl, halogen and C₁₋₆ alkoxy.

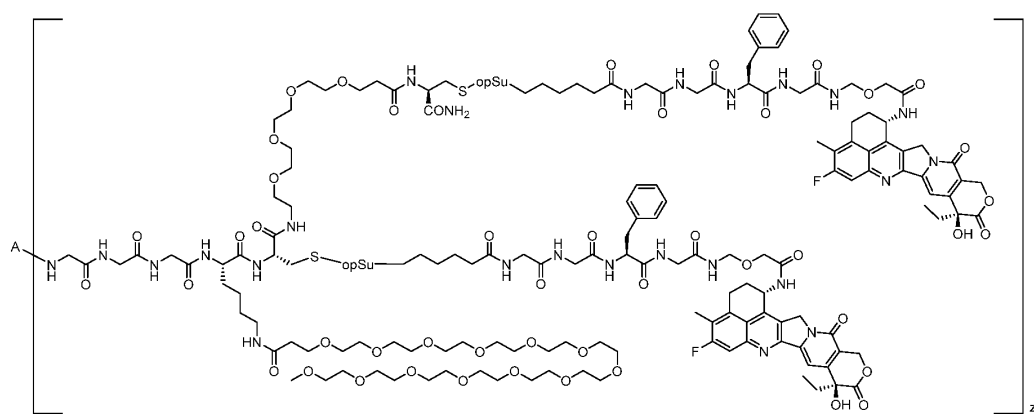
6. The pharmaceutical composition of any one of claims 1 to 5, wherein the payload is selected from:



7. The pharmaceutical composition of any one of claims 1 to 6, which is selected from:



and/or



8. The pharmaceutical composition of any one of claims 1 to 7, wherein
the antibody or antigen binding fragment comprises a VH domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 15; and/or

a VL domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 16.

9. The pharmaceutical composition of any one of claims 1 to 8, wherein
the antibody or an antigen binding fragment comprises a heavy constant domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or

a light constant domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 19.

10. The pharmaceutical composition of any one of claims 1 to 9, wherein
the antibody or an antigen binding fragment comprises a heavy chain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 20 or 21; and/or

a light chain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 22.

11. The pharmaceutical composition of any one of claims 1 to 10, wherein the ligase is sortase.

12. The pharmaceutical composition of any one of claims 1 to 11, wherein the antibody or the antigen-binding fragment comprises C-terminal modification of the heavy chain and/or C-terminal modification of the light chain, the antibody, Sp and recognition sequence of the ligase donor substrate are sequentially linked; Sp is a spacer sequence selected from GA, GGGGS, GGGGSGGGGS and GGGGSGGGGSGGGGS; the recognition sequence of the ligase donor

substrate is LPXTGJ, wherein X can be any single amino acid that is natural or unnatural; J is absent, or is an amino acid fragment comprising 1-10 amino acids.

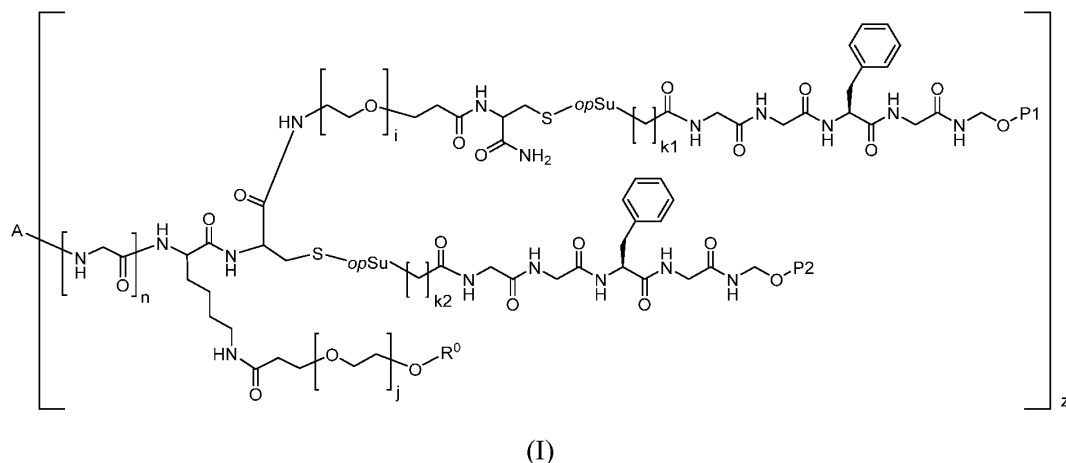
13. A pharmaceutical composition, comprising:

an antibody drug conjugate (ADC),

a acidic buffer, and

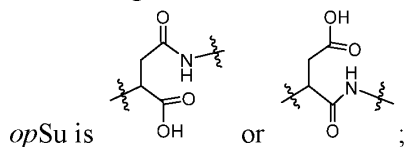
an optional stabilizer;

wherein the ADC has the structure of formula (I):



wherein,

z is an integer of 1 to 4;



R^0 is C_{1-3} alkyl;

n is an integer of 2 to 5;

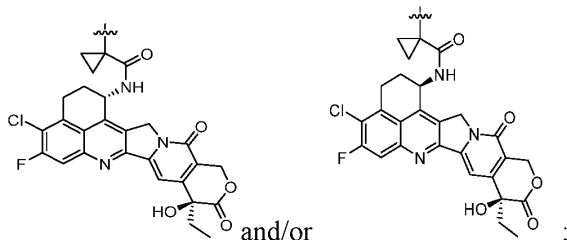
k1 and k2 are independently 1, or 3 or 5;

i is an integer of 1-20;

j is an integer of 1-20;

P1 and P2 are independently a payload;

preferably, P1 and P2 are:



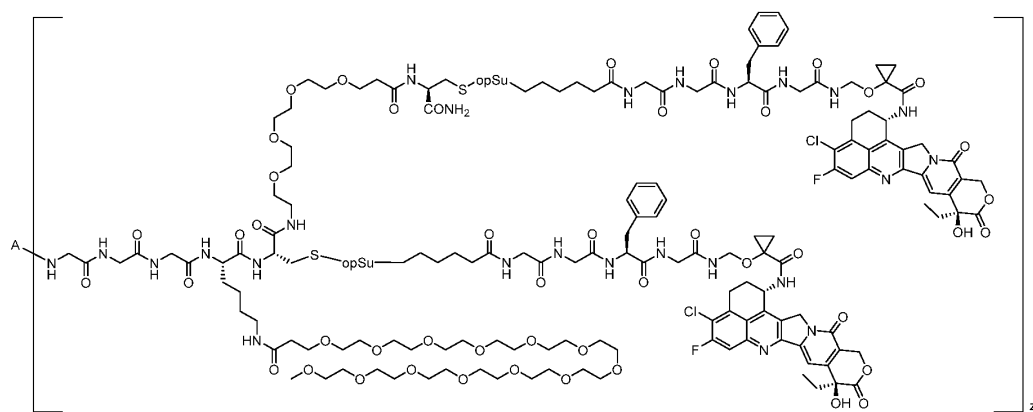
A is an anti-FGFR3 antibody or an antigen binding fragment thereof, the antibody or antigen binding fragment is modified to connect with the (Gly)_n moiety in the compound of formula (I), wherein

the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 26, a light chain of SEQ ID NO: 27; and/or

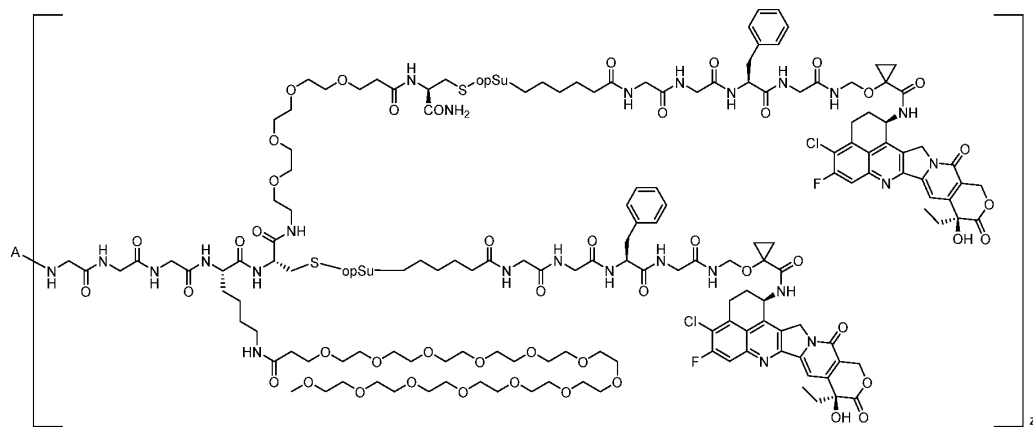
the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 26, a light chain of SEQ ID NO: 22; and/or

the modified antibody or the antigen-binding fragment thereof comprises a heavy chain of SEQ ID NO: 20, a light chain of SEQ ID NO: 27.

14. The pharmaceutical composition of any one of claims 1 to 13, which is selected from:



and/or



15. The pharmaceutical composition of any one of claims 1 to 14, wherein the pharmaceutical composition has a drug to antibody ratio (DAR) of an integer or non-integer of 1 to 20, particularly 2-8.

16. The pharmaceutical composition of any one of claims 1 to 15, wherein the KD value of the anti-FGFR3 antibody or an antigen binding fragment binding to human FGFR3 and monkey FGFR3 is less than 10 nM.

17. The pharmaceutical composition of any one of claims 1 to 16, wherein the buffer is selected from citrate buffer, phosphate buffer, histidine buffer and glutamic acid buffer.

18. The pharmaceutical composition of any one of claims 1-17, wherein the concentration of the buffer is 10-40 mM; or the concentration of the buffer is 15-25 mM, preferably 20 mM.

19. The pharmaceutical composition of any one of claims 1-18, wherein the pH of the buffer is 5.0-7.0; preferably 5.5-6.5, or 5.9-6.1; more preferably 6.0.

20. The pharmaceutical composition of any one of claims 1-18, wherein the stabilizer comprises one or more of sucrose, an arginine salt, arginine, sodium chloride and sorbitol; preferably sucrose.

21. The pharmaceutical composition of any one of claims 1-20, wherein the concentration of the stabilizer is 2-15% (W/V) or 100-160 mM; or

the stabilizer is sucrose and the concentration of sucrose is 5-15% (W/V), preferably 6-10% (W/V), more preferably 8% (W/V); or

the stabilizer is sorbitol and the concentration of sorbitol is 2-8% (W/V), preferably 3.5-6% (W/V), more preferably 4.5% (W/V); or

the stabilizer is arginine hydrochloride and the concentration of arginine hydrochloride is 100-160 mM, preferably 120-160 mM, more preferably 140 mM; or

the stabilizer is sodium chloride and the concentration of sodium chloride is 100-160 mM, preferably 120-160 mM, more preferably 140 mM.

22. The pharmaceutical composition of any one of claims 1-21, wherein the pharmaceutical composition further comprises a surfactant.

23. The pharmaceutical composition of claim 22, wherein the surfactant is polysorbate, and the concentration of the surfactant is 0.005-0.1% (W/V);

preferably, the surfactant is polysorbate 80, and the concentration of the polysorbate 80 is 0.005-0.1% (W/V), preferably 0.005-0.05% (W/V), more preferably 0.01-0.04% (W/V), especially 0.01% (W/V), 0.02% (W/V) or 0.04% (W/V).

24. The pharmaceutical composition of claim 1-23, wherein the concentration of the protein in ADC is 15-60 mg/ml.

25. The pharmaceutical composition of any one of claims 1-24, comprising:

15-60 mg/ml ADC, 10-40 mM histidine buffer (pH 5.0-7.0), 2-15% (W/V) or 100-160 mM stabilizer, and 0.005-0.1% (W/V) surfactant;

or

15-60 mg/ml ADC, 10-40 mM histidine buffer (pH 5.0-7.0), 2-15% (W/V) or 100-160 mM stabilizer, and 0.005-0.1% (W/V) surfactant; wherein the stabilizer is sucrose, sorbitol, arginine hydrochloride or sodium chloride;

or

15-30 mg/ml ADC, 15-26 mM histidine buffer (pH 5.8-6.1), 2.7-12% (W/V) or 123-160 mM stabilizer, and 0.008-0.1% (W/V) polysorbate; wherein the stabilizer is sucrose, sorbitol, arginine hydrochloride or sodium chloride;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.02% (W/V) polysorbate 80;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 4.5% (W/V) sorbitol and 0.02% (W/V) polysorbate 80;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 140 mM arginine hydrochloride and 0.02% (W/V) polysorbate 80;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 140 mM sodium chloride and 0.02% (W/V) polysorbate 80;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.01% (W/V) polysorbate 80;

or

20 mg/ml ADC, 20 mM histidine buffer (pH 6.0), 8% (W/V) sucrose and 0.04% (W/V) polysorbate 80.

26. Use of the pharmaceutical composition of any one of claims 1-25 in the manufacture of a medicament for treating a disease; wherein the disease is FGFR3-mediated disease.

27. The use of claim 26, wherein the disease includes tumor overexpressing FGFR3, tumor with FGFR3 gene fusion or tumor with FGFR3 gene mutation.

28. The use of claim 27, wherein the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, multiple myeloma or intrahepatic cholangiocarcinoma.

29. A method for treating a subject suffering a disease or preventing disease progression, comprises administering the pharmaceutical composition of any one of claims 1-25 to the subject, and the disease is FGFR3-mediated disease.

30. The method of claim 29, wherein the disease includes tumor overexpressing FGFR3 or tumor with FGFR3 gene mutation.

31. The method of claim 30, wherein the disease is selected from: brain cancer, bladder cancer, urothelial cancer, cervical cancer, multiple myeloma or intrahepatic cholangiocarcinoma.