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(54) Title: ANTI-C-MET ANTIBODY AND ANTI-C-MET ANTIBODY-CYTOTOXIC DRUG CONJUGATE AND PHARMACEUTICAL USE THEREOF

(54) 发明名称 : 抗 c-Met 抗体和抗 c-Met 抗体-细胞毒性药物偶联物及其医药用途

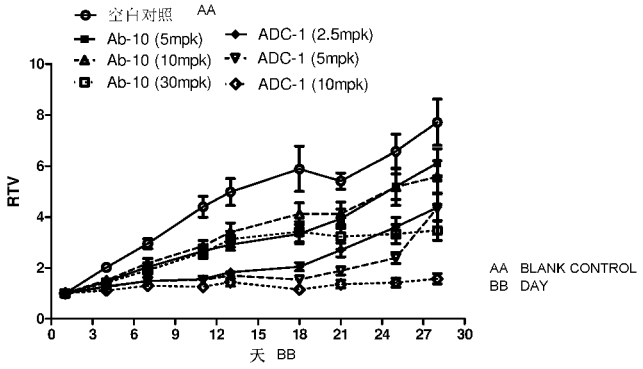

图 1

(57) **Abstract**: Provided are an anti-c-Met antibody or antigen binding fragment, and an anti-c-Met antibody-cytotoxic drug conjugate, wherein the antibody or antigen binding fragment is a chimeric antibody or a humanized antibody. Also provided are a pharmaceutical composition containing a humanized anti-c-Met antibody or antigen binding fragment, antibody-cytotoxic drug conjugate, or a pharmaceutically acceptable salt or solvent compound thereof, applied in the treatment of cancer.

(57) **摘要**: 提供了抗 c-Met 的抗体或抗原结合片段，及抗 c-Met 抗体-细胞毒性药物偶联物，所述抗体或抗原结合片段为嵌合抗体或人源化抗体，还提供了包含人源化抗 c-Met 抗体、抗原结合片段，抗体-细胞毒性药物偶联物或其可药用盐或溶剂化合物的药物组合物，可用于治疗癌症。

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— 包括国际检索报告(条约第 21 条(3))。

— 包括说明书序列表部分(细则 5.2(a))。

ANTI-C-MET ANTIBODY AND ANTI-C-MET ANTIBODY - CYTOTOXIC DRUG CONJUGATE AND PHARMACEUTICAL USE THEREOF

FIELD OF THE INVENTION

The present invention relates to a c-Met antibody, antigen binding fragment thereof; chimeric or humanized antibodies comprising CDR regions of the c-Met antibody; and c-Met antibody-cytotoxic drug conjugate thereof, a pharmaceutically acceptable salt or solvate thereof; and the pharmaceutical composition comprising the same; as well as their use as anti-cancer agents. In particular, the present invention relates to a humanized c-Met antibody and c-Met antibody - cytotoxic drug conjugate or pharmaceutically acceptable salt or solvate thereof; and their use in the preparation of a medicament for the treatment of c-Met-mediated disease or condition.

BACKGROUND OF THE INVENTION

In recent years, molecular biology and tumor pharmacology studies have shown that tyrosine kinase (Protein Tyrosine Kinases, PTKs) -related cell signal transduction pathway plays an extremely important role in tumor formation and development, more than 50% of proto-oncogenes and oncogene products have tyrosine kinase activity. The c-Met proto-oncogene belongs to the Ron subfamily of the PTKs family, and the encoded c-Met protein is a high affinity receptor for the Hepatocyte Growth Factor/Scatter Factor, HGF/SF. HGF/c-Met signaling pathway is closely related to the process of angiogenesis and tumor growth. Its sustained activation is an important cause of carcinogenesis of cells or cancer cell proliferation or hyperplasia of cancer cells. The inhibition of this pathway has become a new method of tumor targeted therapy.

The c-Met proto-oncogene is located on human chromosome 7 long arm (7q31), which is more than 120kb in size and encodes a c-Met protein precursor with a molecular weight of about 150kD, which produces a 170kD glycoprotein by local glycosylation. The glycoprotein is further cleaved into alpha subunit (50 kDa) and beta subunit (140 kDa), which are linked by disulfide to form a mature c-Met protein receptor. The heterodimer contains two strands, the beta chain has an extracellular domain, a transmembrane region (also called a membrane stretch fragment), and an intracellular domain (comprising an intracellular tyrosine kinase binding site). The alpha chain has only extracellular portion, but it is highly glycosylated and attached to the beta chain by disulfide bonds. The extracellular region of the two subunits is the recognition site of the corresponding ligand, and the intracellular domain has tyrosine

kinase activity.

C-Met activation mechanism is divided into three types: one is dependent on the activation mechanism of HGF, the second is not dependent on HGF activation mechanism, the third is through other membrane pathways, such as through the hyaluronic acid surface receptor CD44, adhesin and RON signal transduction pathway and so on. One of the most common is that dependent on the activation mechanism of HGF. The N-terminus of HGF binds to c-Met to promote the dimerization and autophosphorylation of Tyr1234 and Tyr1235 on the beta chain, and the phosphorylation of Tyr1349 and Tyr1356 near the C-terminus produces a binding site for multiple linker proteins which in turn induce P13K/Akt, Ras/Mapk, c-Src and STAT3/5-mediated activation of downstream signaling, and trigger different cellular responses, such as cell survival and activity (closely related to P13K/Akt pathway), tumor metastasis and cell proliferation (mainly mediated by Ras/Mapk). In addition, the cross-talk of c-Met with other membrane receptors has been known to promote tumor formation and metastasis. Since c-Met is the intersection of many pathways leading to tumor formation and metastasis, simultaneous interference with many pathways can be achieved relatively easily with c-Met as the target, and C-Met has become a promising target for antitumor formation and metastasis therapy.

Antibody drug conjugate(ADC) is formed by linking a monoclonal antibody or antibody fragment to a biologically active cytotoxin with a stable chemical linker, which fully utilizes the specificity binding activity of the antibody to the tumor cell or highly expressed antigen, and the high efficiency of cytotoxins, avoid the toxic side effects to normal cells. This means that antibody drug conjugates can bind tumor cells precisely and reduce the effect on normal cells, when compared with conventional chemotherapeutic agents.

ADC consists of three parts: antibodies (targeting), linkers and toxins. Among them, a good target (antibody part) determines the specificity of the ADC drug, which includes not only specific targeting binding, but also effective endocytosis.

There are three main types of inhibitors for c-Met kinase targeting: HGF and c-Met biological antagonists, HGF and c-Met antibodies, and small molecule c-Met inhibitors. The existing clinical results show that the antibodies direct targeting HGF or c-Met, or c-Met small molecule inhibition is not ideal. The ADC for c-Met may be the most effective method for treating tumor. At present, there is no c-Met ADC drug clinical research.

The present invention firstly discloses an anti-c-Met antibody ADC drug, which

not only retains the antibody-dependent cell proliferation inhibitory effect of the anti-c-Met antibody of the present invention, but also increases the effect of the potential cytotoxic drug. Because of the targeted release of toxin into tumor cells, drug toxic side effects does not increase with the increase of efficacy. The present invention provides a humanized antibody and a chimeric antibody that specifically binds human c-Met, and the humanized antibody and chimeric antibody are characterized by high affinity, high efficacy, endocytosis, good stability and free of C-Met agonist activity, etc. On the basis of these excellent properties, the present invention also provides an antibody-cytotoxic drug conjugate that specifically binds to human c-Met or a pharmaceutically acceptable salt or solvate compound thereof which retains the antibody-dependent inhibition of cell proliferation, while increases the potential effect of cytotoxic drugs and the broad spectrum of diseases to be treated. Also, because of the release of toxin into targeted tumor cells (the endocytosis of the anti-c-Met antibody of the present invention), the drug toxic side effects do not increase along with the increase of efficacy.

SUMMARY OF THE INVENTION

The present invention provides an antibody or antigen-binding fragment thereof that specifically binds to c-Met, comprising at least one CDR selected from the following sequences or the mutant sequences thereof:

heavy chain variable region HCDR sequence: SEQ ID NO: 6, SEQ ID NO:7 or SEQ ID NO:8;

and

light chain variable region HCDR sequence: SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the antibody heavy chain variable region comprises at least one HCDR region sequence selected from the following sequences or a mutant sequence thereof: SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the antibody light chain variable region comprises at least one LCDR region sequence selected from the following sequences or a mutant sequence thereof: SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the antibody comprises heavy chain variable region sequence selected from the group consisting of

SEQ ID NO: 6 (HCDR1), SEQ ID NO: 7 (HCDR2) and SEQ ID NO: 8 (HCDR3), or a mutant sequence thereof, and light chain variable region sequence selected from the group consisting of SEQ ID NO:9 (LCDR1), SEQ ID NO: 10 (LCDR2) and SEQ ID NO: 11 (LCDR3), or a mutant sequence thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the mutant sequence of CDR region are sequences which have 1-3 amino acid mutations that optimize antibody activity, wherein the mutant sequence of HCDR2 region preferably is SEQ ID NO: 12.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the c-Met antibody or the antigen-binding fragment thereof is a murine antibody or the fragment thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the heavy chain variable region sequence of the murine antibody is shown as SEQ ID NO: 4.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the light chain variable region sequence of the murine antibody is shown as SEQ ID NO: 5.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the heavy chain variable region of the murine antibody is shown as SEQ ID NO: 4, the light chain variable region of the murine antibody is shown as SEQ ID NO: 5.

In a preferred embodiment of the present invention, provided is the murine antibody or fragment thereof described above, wherein the heavy chain variable region of the antibody further comprises a heavy chain FR region derived from murine IgG1 or a variant thereof, murine IgG2 or a variant thereof, murine IgG3 or a variant thereof, or murine IgG4 or a variant thereof.

In a preferred embodiment of the present invention, provided is the murine antibody or fragment thereof described above, which further comprises a heavy chain constant region derived from murine IgG1 or a variant thereof, murine IgG2 or a variant thereof, murine IgG3 or a variant thereof, or murine IgG4 or a variant thereof.

In a preferred embodiment of the present invention, provided is the murine antibody or fragment thereof described above, wherein the light chain variable region of the antibody further comprises a light chain FR region derived from murine κ chain or a variant thereof, murine λ chain or a variant thereof.

In a preferred embodiment of the present invention, provided is the murine antibody or fragment thereof described above, which further comprises a light chain constant region derived from murine κ chain or a variant thereof, murine λ chain or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, which is a chimeric or humanized antibody or the fragment thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody heavy chain variable region further comprises a heavy chain FR region derived from human IgG1 or a variant thereof, IgG2 or a variant thereof, IgG3 or a variant thereof, or IgG4 or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody heavy chain variable region comprises heavy chain FR sequence derived from human germline heavy chain, preferably human germline heavy chain IGHV 3-33*01; comprises framework sequence of FR1, FR2, FR3 and FR4 regions of human germline heavy chain IGHV 3-33*01, or a mutant sequence thereof, preferably, the mutant sequence comprises 0-10 amino acid back-mutation(s).

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above , wherein the humanized antibody comprises a heavy chain variable region sequence shown as SEQ ID NO: 13-15 or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody light chain variable region comprises light chain FR region derived from human germline light chain, preferably human germline light chain IGKV085 or IGKV4-1*01, including a framework sequence of FR1, FR2, FR3 and FR4 regions of the human germline light chain IGKV085 or IGKV4-1*01, or a mutant sequence thereof, preferably, the mutant sequence comprises 0-10 amino acid back-mutation(s).

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody comprises a light chain variable region sequence selected from SEQ ID NO: 16-18 or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody comprises a heavy chain variable region sequence selected from SEQ ID NO: 13-15 and a light chain variable region sequence selected from SEQ ID NO: 16-18.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, which comprises a combination of a heavy chain variable region sequence and a light chain variable region sequence selected from any one of a) to c):

a) Heavy chain variable region sequence of SEQ ID NO: 13, and light chain variable region sequence of SEQ ID NO: 16;

b) Heavy chain variable region sequence of SEQ ID NO: 14, and light chain variable region sequence of SEQ ID NO: 17; or

c) Heavy chain variable region sequence of SEQ ID NO: 15, and light chain variable region sequence of SEQ ID NO: 18.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the heavy chain constant region of humanized antibody comprises a constant region derived from human IgG1 or a variant thereof, human IgG2 or a variant thereof, human IgG3 or a variant thereof, or human IgG4 or a variant thereof, preferably a constant region derived from human IgG1 or a variant thereof, human IgG2 or a variant thereof or human IgG4 or a variant thereof, most preferably a constant region derived from human IgG2 or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, which comprises a full length heavy chain sequence selected from SEQ ID NO: 23-25 or the sequences with at least 90% identity to SEQ ID NO: 23-25.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the light chain variable region of humanized antibody further comprises a light chain FR region selected from human κ or λ chain or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the light chain constant region of humanized antibody comprises a constant region selected from human κ or λ chain or a variant thereof.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, which comprises a full-length light chain sequence selected from SEQ ID NO: 26-28 or the sequences with at least 90% identity to SEQ ID NO: 26-28.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, which comprises a full length heavy chain sequence selected from SEQ ID NO: 23-25 and a full-length light chain sequence selected from SEQ ID NO: 26-28.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the humanized antibody comprises a combination of a full-length light chain sequence and a full-length heavy chain selected from any one of a) to c):

Ab-9: The heavy chain sequence of SEQ ID NO: 23 and the light chain sequence of SEQ ID NO: 26;

Ab-10: The heavy chain sequence of SEQ ID NO: 24 and the light chain sequence of SEQ ID NO: 27; or

Ab-11: The heavy chain sequence of SEQ ID NO: 25 and the light chain sequence of SEQ ID NO: 28.

In a preferred embodiment of the present invention, provided is the c-Met antibody or antigen-binding fragment thereof described above, wherein the antigen-binding fragment is Fab, Fv, scFv or F(ab')2.

The present invention further provides a DNA molecule encoding the c-Met antibody or the antigen-binding fragment thereof described above, and expressing precursor product.

The present invention further provides an expression vector comprising the DNA molecule as described above.

The present invention further provides a host cell transformed with the expression vector as described above.

In one preferred embodiment of present invention, provided is the host cell as described above, wherein said host cell is preferably mammalian cells, more preferably CHO cell.

The present invention further provides a pharmaceutical composition, comprising the c-Met antibody or the antigen-binding fragment thereof as described above; and one or more pharmaceutically acceptable excipient, diluent or carrier.

The present invention further provides use of the c-Met antibody or the antigen-binding fragment thereof according to the present invention, or the pharmaceutical composition according to the present invention in the preparation of a medicament for the treatment of c-Met-mediated disease or condition; wherein the disease or condition is preferably cancer, more preferably a cancer that expresses c-Met; most preferably a cancer selected from gastric cancer, pancreatic cancer, lung cancer, intestinal cancer, kidney cancer, melanoma, non-small cell lung cancer; most preferably gastric cancer and non-small cell lung cancer.

The present invention further provides a method for treating or preventing a c-Met-mediated disease or condition, the method comprises: administering a therapeutically effective amount of c-Met antibody or the antigen-binding fragment thereof according to the present invention, or the pharmaceutical composition according to the invention to a patient in need thereof, wherein the disease or condition is preferably cancer, more preferably a cancer that expresses c-Met; most preferably a cancer selected from gastric cancer, pancreatic cancer, lung cancer, intestinal cancer, kidney cancer, melanoma, non-small cell lung cancer; most preferably gastric cancer and non-small cell lung cancer.

The present invention further provides an antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof:

$$Ab\text{-}[(L_2)t\text{-}L_1\text{-}D)]y \qquad (I)$$

wherein:

D is a drug unit;

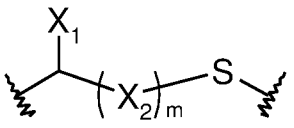
L_1 or L_2 is a linker unit;

t is 0 or 1, preferably 1;

y ranges from 1-8, preferably 2-5; y can be a decimal;

Ab is the antibody or antigen-binding fragment thereof that specifically binds to c-Met as described above.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein $-L_2-$ is the compound shown as formula ($-L_2-$):



,

wherein:

X_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, cyano, alkyl, alkoxy and cycloalkyl;

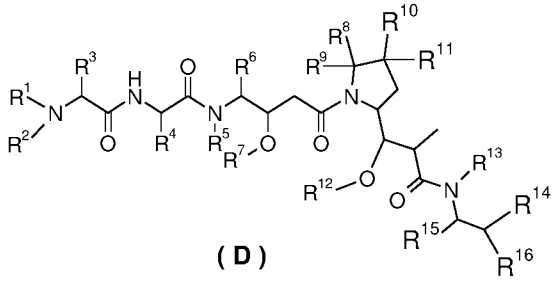
X_2 is selected from the group consisting of alkyl, cycloalkyl and heterocyclyl;

m is 0-5, preferably 1-3;

S is sulfur atom.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein the drug unit of D is a cytotoxic agent selected from toxins, chemotherapeutic agents, antibiotics, radioisotopes or nucleolytic enzyme.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein the drug unit of D has a structure of formula (D):



(D)

or a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixtures thereof,

or pharmaceutically acceptable salt thereof:

wherein:

R^1-R^7 is each selected from the group consisting of hydrogen, halogen, hydroxyl,cyano, alkyl, alkoxy and cycloalkyl;

R^8-R^{11} is each optionally selected from the group consisting of hydrogen, halogen, alkenyl, alkyl, alkoxy and cycloalkyl; preferably, at least one of R^8-R^{11} is selected from the group consisting of halogen, alkenyl, alkyl and cycloalkyl, and the rest of R^8-R^{11} are hydrogen;

or any two of R^8-R^{11} form a cycloalkyl, the rest two are each selected from the group consisting of hydrogen, alkyl and cycloalkyl;

R^{12}-R^{13} is each selected from the group consisting of hydrogen, alkyl and halogen;

R^{14} is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is optionally further substituted by substituent group selected from the group consisting of hydrogen, halogen, hydroxy, alkyl, alkoxy and cycloalkyl;

R^{15} is selected from halogen, alkenyl, alkyl, cycloalkyl or $COOR^{17}$;

R^{16} is selected from hydrogen, halogen, hydroxy, cyano, alkyl, alkoxy and cycloalkyl;

R^{17} is selected from hydrogen, alkyl and alkoxy.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein L_2 is a linker selected from Val-Cit, MC, PAB and MC-PAB, preferably MC.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein D is maytansinoid alkaloid; preferably selected from DM1, DM3 and DM4; more preferably DM1.

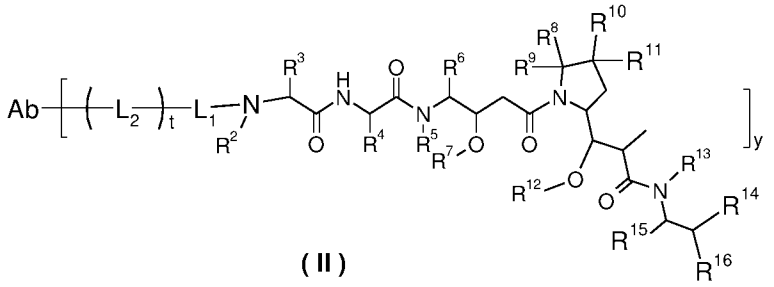
In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein L_2 is selected from the group consisting of N-succinimidyl 4- (2-pyridylthio) valerate (SPP), N-succinimidyl 4- (N-maleimidomethyl) –cyclohexane-1- carboxylic acid esters (SMCC), and N-succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB); preferably SPP or SMCC.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein D is a camptothecin

alkaloid; preferably selected from CPT, 10-hydroxy-CPT, CPT-11 (Irinotecan), SN-38 and topotecan, more preferably SN-38.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, wherein said linker L_2 is selected from the structure of Val-Cit, MC, PAB or MC-PAB; preferably MC or MC-vc-PAB.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, which is a conjugated drug of formula (II) or the pharmaceutically acceptable salt or solvate thereof :



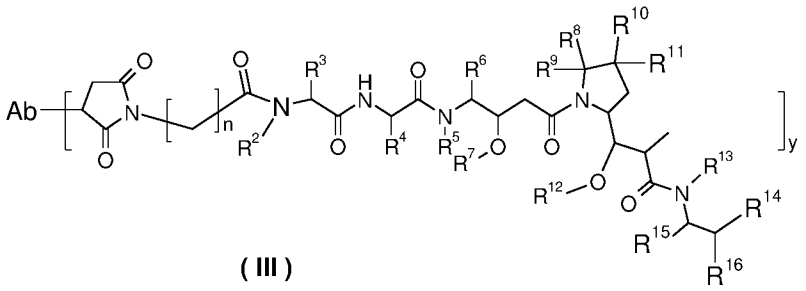
(II)

wherein:

R^2-R^{16} are as defined in formula (D);

Ab, t, y, L_1, L_2 are as defined in formula (I).

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, which is a conjugated drug of formula (III) or the pharmaceutically acceptable salt or solvate thereof:



(III)

wherein:

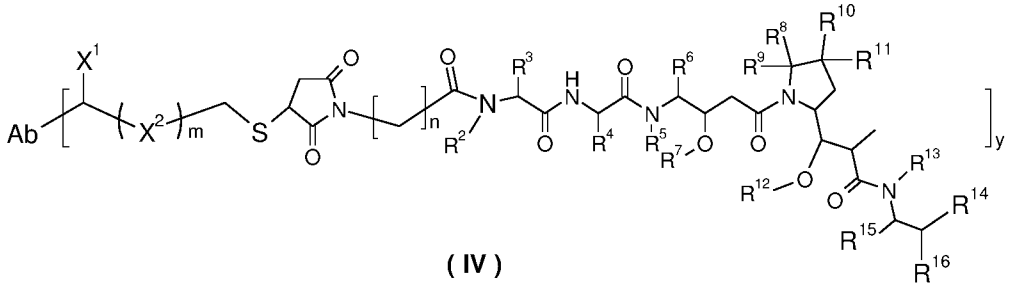
R^2-R^{16} are as defined in formula (D);

Ab, t, y, L_1, L_2 are as defined in formula (I);

n is 3-6, preferably 5.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable

salt or solvate thereof according to the invention, which is a conjugated drug of formula (IV) or the pharmaceutically acceptable salt or solvate thereof:



(IV)

wherein:

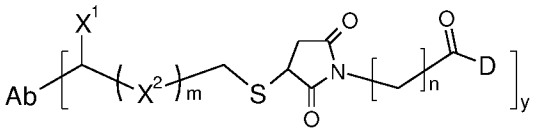
R^2-R^{16} are as defined in formula (D);

Ab, y are as defined in formula (I);

n is as defined in formula (III);

X^1, X^2, m are as defined in formula L2.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to the invention, which is a conjugated drug of formula (V) or the pharmaceutically acceptable salt or solvate thereof :



(V)

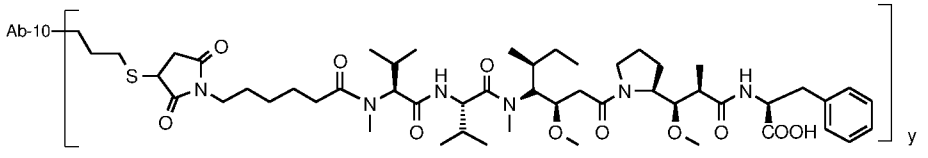
wherein:

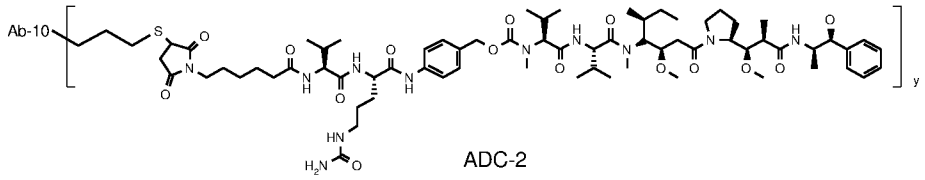
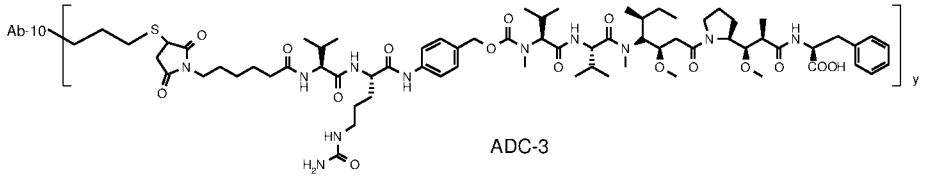
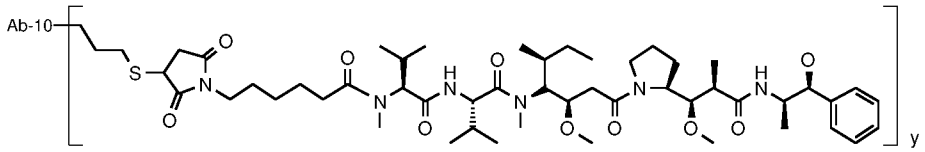
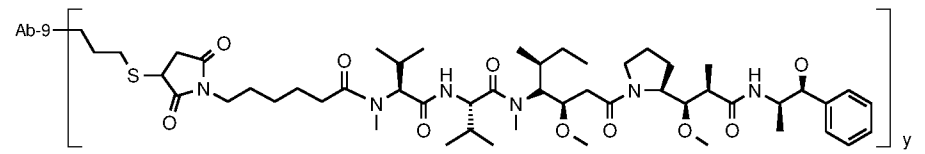
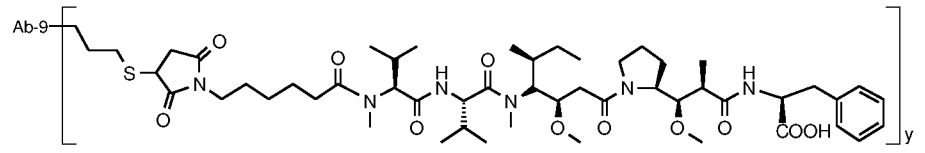
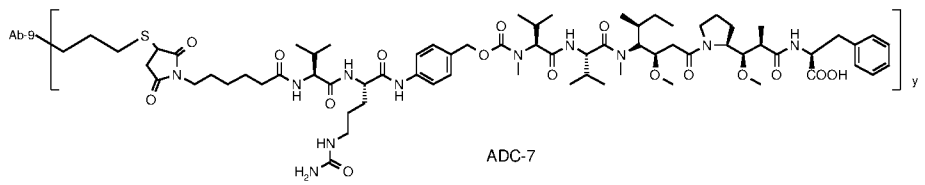
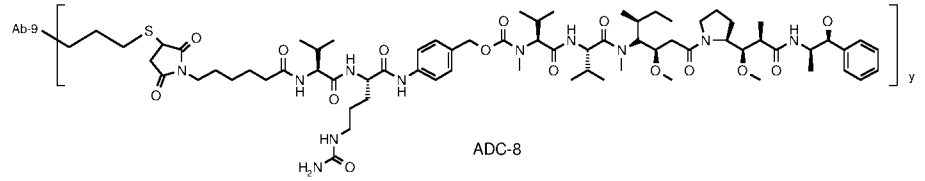
Ab, D, y are as defined in formula (I);

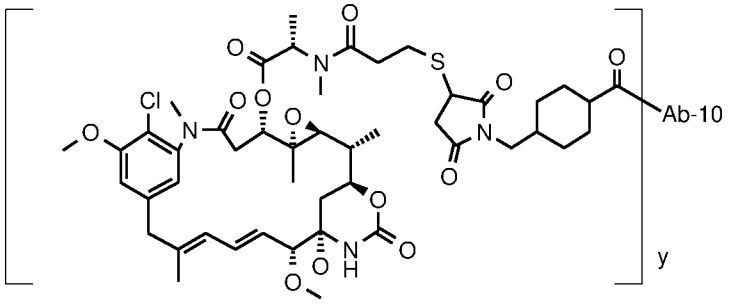
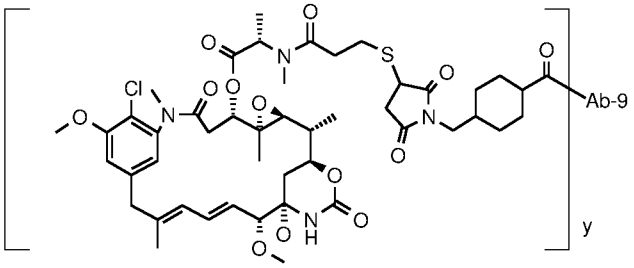
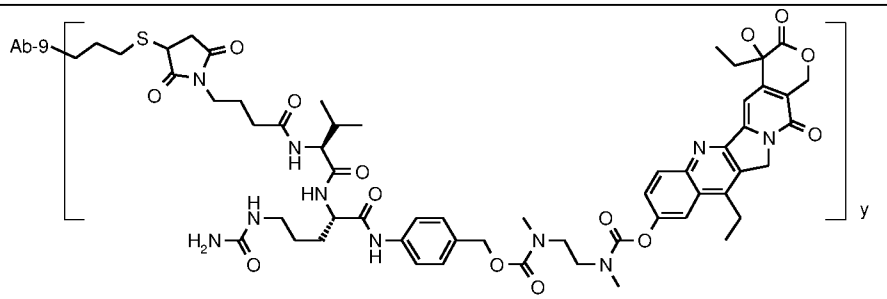
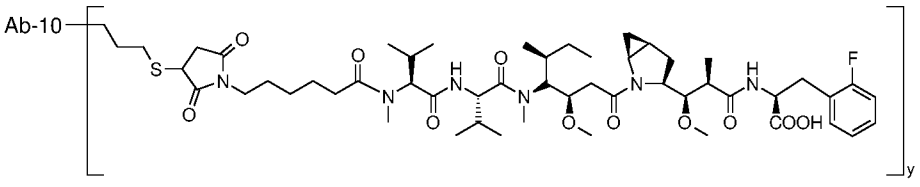
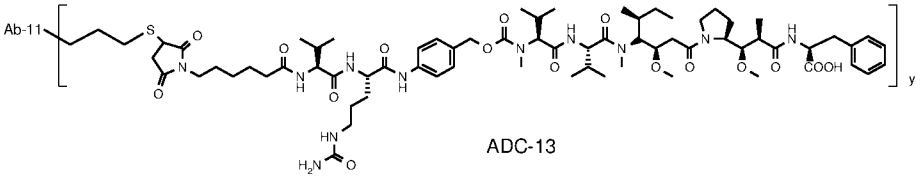
n is as defined in formula (III);

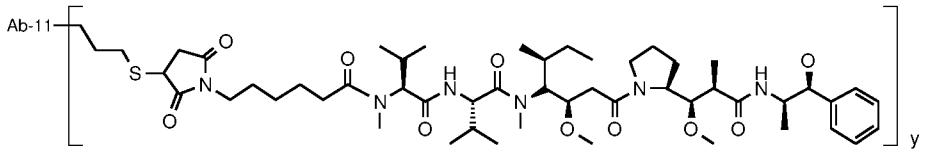
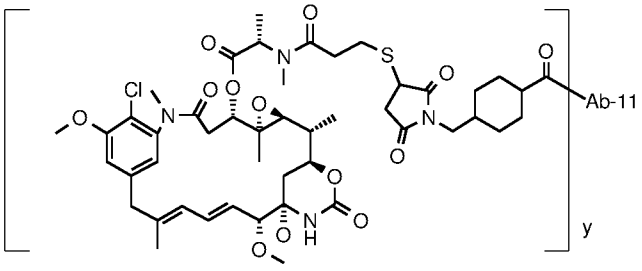
X^1, X^2, m are as defined in formula L2.

The antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to the invention include but not limited to:

No.	Structure and denomination
Example 13	 ADC-1
	Anti-c-Met antibody Ab-10 conjugated toxin MC-MMAF

Example 14	 ADC-2
	Anti-c-Met antibody Ab-10 conjugated toxin MC-VC-PAB-MMAE
Example 15	 ADC-3
	Anti-c-Met antibody Ab-10 conjugated toxin MC-VC-PAB-MMAF
Example 16	 ADC-4
	Anti-c-Met antibody Ab-10 conjugated toxin MC-MMAE
Example 17	 ADC-5
	Anti-c-Met antibody Ab-9 conjugated toxin MC-MMAE
Example 18	 ADC-6
	Anti-c-Met antibody Ab-9 conjugated toxin MC-MMAF
Example 19	 ADC-7
	Anti-c-Met antibody Ab-9 conjugated toxin MC-VC-PAB-MMAF
Example 20	 ADC-8

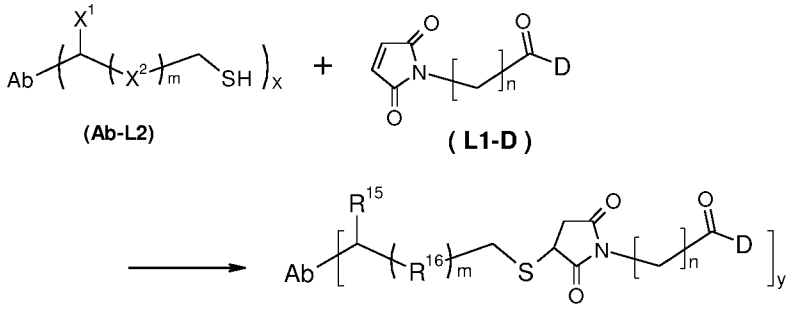
	Anti-c-Met antibody Ab-9 conjugated toxin MC-VC-PAB-MMAE
Example 21	 ADC-9
	Anti-c-Met antibody Ab-10 conjugated toxin SMCC-DM1
Example 22	 ADC-10
	Anti-c-Met antibody Ab-9 conjugated toxin SMCC-DM1
Example 23	 ADC-11
	Anti-c-Met antibody Ab-9 conjugated toxin -SN-38
Example 24	 ADC-12
	Anti-c-Met antibody Ab-10 conjugated toxin
Example 25	 ADC-13

	Anti-c-Met antibody Ab-11 conjugated toxin MC-VC-PAB-MMAF
Example 26	 ADC-14
	Anti-c-Met antibody Ab-11 conjugated toxin MC-MMAE
Example 27	 ADC-15
	Anti-c-Met antibody Ab-11 conjugated toxin SMCC-DM1

wherein Ab-9, Ab-10, Ab-11 are c-Met antibody as defined above, y is 1-8, preferably 2-5.

Wherein, y ranges from 1-8; preferably 1-4.

The present invention further provides a process of preparing the antibody-cytotoxic drug conjugate of formula (V), comprises a step of:



(Ab-L2) (L1-D)

(V)

a compound of the general formula (Ab-L2) is reacted with a compound of the general formula (L1-D) in an organic solvent to obtain a compound of the general formula (V); wherein the organic solvent is preferably acetonitrile or ethanol;

wherein:

Ab is the antibody or antigen-binding fragment thereof that specifically binds to a c-Met receptor according to the present invention;

X_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, cyano, alkyl, alkoxy and cycloalkyl;

X_2 is selected from the group consisting of alkyl, cycloalkyl and heterocyclyl;

X is 0-5, preferably 1-3,

m is 0-5, preferably 1-3.

In a preferred embodiment of the present invention, provided is the antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to the invention, which has cytotoxic activity *in vitro* or *in vivo*.

The present invention further relates to a pharmaceutical composition comprising a therapeutically effective amount of the c-Met antibody or antigen-binding fragment thereof, or antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof according the present invention, and pharmaceutically acceptable carrier, diluent or excipient.

The present invention further relates to a use of the c-Met antibody or the antigen-binding fragment thereof according to the present invention, or the antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof according the present invention, or the pharmaceutical composition comprising the same according to the present invention, in the preparation of a medicament for the treatment of c-Met-mediated disease or condition; wherein the disease or condition is preferably cancer, more preferably a cancer that expresses c-Met; most preferably a cancer selected from gastric cancer, pancreatic cancer, lung cancer, intestinal cancer, kidney cancer, melanoma, non-small cell lung cancer; most preferably gastric cancer, pancreatic cancer, non-small cell lung cancer and kidney cancer.

The present invention further relates to a method for treating or preventing a c-Met-mediated disease or condition, the method comprises administering a therapeutically effective amount of the c-Met antibody or the antigen-binding fragment thereof, or the antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof, or the pharmaceutical composition comprising the same according to the invention to a patient in need thereof, wherein the disease or condition is preferably cancer, more preferably a cancer that expresses c-Met; most preferably a cancer selected from gastric cancer, pancreatic cancer, lung cancer, intestinal cancer, kidney cancer, melanoma, non-small cell lung cancer; most preferably gastric cancer, pancreatic cancer, non-small cell lung cancer and kidney cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the tumor inhibition effect of the anti-c-Met antibody and ADC molecule of the invention, and the result shows that the new molecule of ADC can achieve complete inhibition of tumor by the attched toxin, whereas the antibody alone can not achieve such complete inhibition. The results also show that coupling of toxins does not affect $T_{1/2}$ of the ADC molecule in this invention, and the ADC drug of the present invention does not show *in vivo* toxicity in mice.

DETAILED DESCRIPTION OF THE INVENTION

1. terms

In order to make the invention more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, the single-letter code and the three-letter code for amino acids are as described in J. Biol. Chem, 243, (1968) p3558.

The term" c-Met" or "c-Met polypeptide" or "c-Met receptor" refers to a receptor tyrosine kinase that binds to a cell growth factor (HGF). In the present invention, unless specified specifically, such as murine c-Met (m-c-Met) or monkey c-Met (cyno-c-Met), term c-Met usually refers to human c-Met (h-c-Met). The human, mouse and cynomolgus monkey c-Met used in the present invention are encoded by the nucleotide sequence or polypeptide sequence provided by GenBank, for example, the human peptide was encoded by the nucleotide sequence provided in GenBank Accession No. NM_000245, or the human protein or its extracellular domain encoded by the the polypeptide sequence provided in GenBank the Accession No. NP_000236. The original single-stranded precursor proteins are cleaved after translation to produce alpha and beta subunits, which are linked by disulfide bonds to form mature receptor. Receptor tyrosine kinase c-Met involves in cell processes including, for example, the process of migration, invasion and morphogenesis of tissue regeneration associated with embryogenesis.

The term "c-Met-related disorder or condition" refers to any disease, condition or disorder originated from adverse or lack of c-Met expression, adverse regulation or lack of regulation, or deleterious activity or lack of activity, or refers to any disease, condition or disorder which could be regulated, treated or cured by modulating c-Met expression or activity. The activation of the HGF/c-Met pathway can be expected, for example, in most cancer patients, or in patients whose disease is indeed driven by changes associated with the c-Met pathway. For example, upregulation is due to different mechanisms, such as overexpression of HGF and/or c-Met, or by constitutive activation of c-Met mutations. C-Met-related disorders or conditions include, but are not limited to, such as proliferative diseases and disorders and inflammatory diseases and disorders. Proliferative diseases include, but are not limited to, for example, cancer, including, for example, gastric cancer, esophageal cancer, kidney cancer including papillary renal cell carcinoma, lung cancer, glioma,

head and neck cancer, epithelial cancer, skin cancer, leukemia, lymphoma, myeloma, brain cancer, pancreatic cancer, colorectal cancer, gastrointestinal cancer, intestinal cancer, genital cancer, urinary cancer, melanoma, prostate cancer, and other tumors known to those skilled in the art. Inflammatory diseases include, but are not limited to bacterial infections, including infections caused by *Listeria* bacteria.

"Antibody" in this invention refers to immunoglobulin, a four-peptide chain structure formed by two identical heavy chains and two identical light chains connected by interchain disulfide bond. Different immunoglobulin heavy chain constant regions exhibit different amino acid compositions and sequences, hence present different kinds of antigenicity. Accordingly, immunoglobulins can be divided into five categories, also referred as immunoglobulin isotypes, namely IgM, IgD, IgG, IgA and IgE; the corresponding heavy chains thereof are μ chain, δ chain, γ chain, α chain, ε chain, respectively. According to amino acid composition of hinge region and the number and location of heavy chain disulfide bonds, immunoglobulins can be divided into different sub-categories, for example, IgG can be divided into IgG1, IgG2, IgG3, and IgG4. Light chain can be divided into κ or λ chain, based on different constant region. Each category of Ig among these five categories involves κ or λ chain.

At the N-terminal of the antibody heavy and light chains, about 110 amino acids vary largely, which is known as variable region (V region); the amino acid sequence at the C-terminus is relative stable, which is known as constant region (C region). Variable region comprises three hypervariable regions (HVR) and four FR regions (FR) with relatively conserved sequence. Three hypervariable regions determine the specificity of the antibody, also known as complementarity determining region (CDR). Each light chain variable region (LCVR) and each heavy chain variable region (HCVR) is composed of three CDR regions and four FR regions, arranged from the amino terminal to the carboxyl terminal is: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Three light chain CDR regions refer to LCDR1, LCDR2, and LCDR3; three heavy chain CDR regions refer to HCDR1, HCDR2 and HCDR3. The number and location of CDR region amino acid residues in LCVR and HCVR regions of the antibody or antigen binding fragment herein correspond with known Kabat numbering criteria (LCDR1-3, HCDE2-3), or correspond with kabat and chothia numbering criteria (HCDR1).

The term "murine antibody" in the present invention refers to anti-human c-Met monoclonal antibody prepared from mouse according to the knowledge and skills of the field. During the preparation, test object was injected with c-Met antigen, and then

hybridoma expressing antibody which possesses desired sequence or functional characteristics was isolated. In a preferred embodiment of the present invention, the murine c-Met antibody or antigen binding fragment thereof, further comprises light chain constant region of murine κ, λ chain or a variant thereof, or further comprises heavy chain constant region of murine IgG1, IgG2, IgG3 or IgG4, or a variant thereof.

The term "chimeric antibody", is an antibody which is obtained by fusing the variable region of murine antibody to constant region of human antibody, the chimeric antibody can alleviate the murine antibody-induced immune response. To establish chimeric antibody, hybridoma secreting specific murine monoclonal antibody is firstly established, variable region gene is cloned from such murine hybridoma, and then cloned into constant region gene of human antibody for recombinant expression.

The term "humanized antibody", also known as humanized CDR-grafted antibody, refers to an antibody generated by grafting murine CDR sequences onto framework of human antibody variable region, comprising different types of sequences of human germline antibody framework. Humanized antibody avoids the strong immune antibody response induced by chimeric antibody which carrys a large number of murine components. Such framework sequences can be obtained from public DNA database covering germline antibody gene sequences or published references. For example, germline DNA sequences of human heavy and light chain variable region genes can be found in "VBase" human germline sequence database (available on website www.mrccpe.com.ac.uk/vbase), as well as found in Kabat, EA, et al, 1991 Sequences of Proteins of Immunological Interest, 5th Ed. In a preferred embodiment of the invention, the murine CDRs sequences of c-Met humanized antibody are selected from SEQ ID NO: 6, 7, 8, 9, 10, 11. Human antibody variable region frameworks were designed and selected, wherein the light chain FR region sequence of said antibody light chain variable region is derived from human germline light chain sequences, preferably selected from human germine light chain IGKV085 or IGKV 4-1*01, comprising FR1, FR2, FR3 and FR4 region of human germine light chain IGKV085 and IGKV 4-1*01; the heavy chain FR region sequence of said antibody heavy chain variable region is derived from human germline heavy chain sequences, preferably selected from human germline heavy chain IGHV 3-33*01, comprising FR1, FR2, FR3 and FR4 region of human germline heavy chain IGHV 3-33*01. To avoid decrease of activity caused by decrease of immunogenicity, a minimum of back mutation(s) could be introduced into human antibody variable region to maintain the activity.

There are multiple methods available in the art to generate humanized antibodies. For example, humanized antibodies may be produced by obtaining nucleic acid sequences encoding the HCVR and LCVR of a parent antibody (e.g., a murine antibody or antibody produced by a hybridoma) which specifically binds c-Met, and grafting such encoding nucleic acid sequences onto selected human

framework-encoding nucleic acid sequences. Optionally, a CDR region may be optimized by mutagenesis randomnly or at particular locations in order to substitute one or more amino acids in the CDR with a different amino acid prior to grafting the CDR region onto the framework region. Alternatively, a CDR region may be optimized subsequent to insertion into the human framework region using methods available to one of skilled in the art. Preferably, a "humanized antibody" has CDRs that originate from or are derived from a parent antibody (i.e., a non-human antibody, preferably a mouse monoclonal antibody), while framework and constant region, to the extent it is present, (or a significant or substantial portion thereof, i.e., at least about 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99%) are encoded by nucleic acid that occurs in the human germline immunoglobulin region (see, e.g., the International ImMunoGeneTics Database) or in recombined or mutated forms thereof, regardless of whether said antibodies are produced in a human cell. Preferably, at least two, three, four, five or six CDRs of a humanized antibody are optimized from the CDRs of a non-human parent antibody from which the humanized antibody was derived, to generate a desired property, e.g., improved specificity, affinity or neutralization, which may be identified by a screening assay, e.g., an ELISA assay. Preferably an optimized CDR in an antibody of the invention comprises at least one amino acid substitution when compared with that present in the parent antibody. When compared with CDR of parent antibodies, certain amino acid substitutions in the CDRs of humanized antibodies of the invention (see example 6 herein) decrease the likelihood of instability of the antibody (e.g., removal of Asn residues from CDR) or decrease the likelihood of immunogenicity of the antibody when administered to a human subject (e.g., as predicted by IMMUNOFILTERTM Technology).

After the CDR-encoding sequences are grafted onto the selected human framework encoding sequences, the resultant DNA sequences encoding the humanized variable heavy and variable light chain sequences are then expressed to produce a humanized antibody that binds c-Met. The humanized HCVR and LCVR may be expressed as part of a whole anti-c-Met antibody molecule, i.e., as a fusion protein with human constant domain sequences. However, the HCVR and LCVR sequences can also be expressed in the absence of constant sequences to produce a humanized anti-c-Met scFv.

References further describing methods involved in humanization of a mouse antibody that may be used include e.g., Queen et al., Proc. Natl. Acad. Sci. USA 88: 2869, 1991 and the method of Winter and co-workers [Jones et al., Nature, 321:522 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534 (1988)].

"Antigen-binding fragment" in the present invention refers to Fab fragment, Fab' fragment, $F(ab')_2$ fragment having antigen-binding activity, as well as Fv fragment scFv fragment binding with human c-Met; it comprises one or more CDR regions of

antibodies described in the present invention, selected from the group consisting of SEQ ID NOs:3 to SEQ ID NO:8. Fv fragment is a minimum antibody fragment comprising heavy chain variable region, light chain variable region, and all antigen-binding sites without a constant region. Generally, Fv antibody further comprises a polypeptide linker between the VH and VL domains, and is capable of forming a structure necessary for antigen binding. Also, different linkers can be used to connect the variable regions of two antibodies to form a polypeptide chain, named single chain antibody or single chain Fv (scFv). scFv can also be used with other antibodies such as anti-EGFR antibody to construct bispecific antibody. The term "binding with c-Met" in this inverntion, means being capable of interacting with human c-Met. The term "antigen-binding sites" in the present invention, refers to discontinuous, three-dimensional sites on the antigen, recognized by the antibody or the antigen-binding fragment of the present invention. As used herein, the term "ADCC", namely antibody-dependent cell-mediated cytotoxicity, means that the cells expressing Fc receptors directly kill the target cells coated by an antibody by recognizing the Fc segment of the antibody. ADCC effector function of the antibody can be reduced or eliminated by modifying the Fc segment in IgG. The modification refers to mutations on the antibody heavy chain constant region, such as mutations selected from N297A, L234A, L235A in IgG1; IgG2/4 chimera; F235E, or L234A/E235A mutations in IgG4.

As used herein, fusion protein described in the present invention is a protein product obtained by co-expressing two genes via recombinant DNA technology. Recombinant c-Met extracellular domain Fc fusion protein is obtained by co-expressing a c-Met extracellular domain and a human antibody Fc fragment via recombinant DNA technology. The c-Met extracellular domain refers to the moiety of c-Met outside cytomembrane.

The engineered antibody or antigen-binding fragment of the present invention may be prepared and purified using conventional methods. For example, cDNA sequences encoding a heavy chain (SEQ ID NO: 4) and a light chain (SEQ ID NO: 5) may be cloned and recombined into pEE6.4 expression vector (Lonza Biologics). The recombined immunoglobulin expression vector may then stably transfect CHO cells. As a more recommended method well known in the art, mammalian expression system will make antibodies glycosylated, typically at the highly conserved N-terminus in the FC region. Stable clones may be obtained through expression of an antibody specifically binding to human c-Met. Positive clones may be expanded in a serum-free culture medium for antibody production in bioreactors. Culture medium, into which an antibody has been secreted, may be purified by conventional techniques. For example, the medium may be conveniently applied to a Protein A or G Sepharose

FF column that has been equilibrated with a compatible buffer. The column is washed to remove nonspecific binding components. The bound antibody is eluted by PH gradient and the antibody fragments are detected by SDS-PAGE, and then collected. The antibody may be filtered and concentrated using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion or ion exchange. The obtained product may be immediately frozen, for example at -70°C, or may be lyophilized.

The term "antibody," in this invention refers to a monoclonal antibody. As used herein, the term "monoclonal antibody" or “mAb” refers to an antibody secreted by a clone derived from a single cell strain. The cell strain is not limited to eukaryotic, prokaryotic, or phage clonal cell lines. Monoclonal antibodies or antigen-binding fragments can be obtained by recombinantion, for example, hybridoma techniques, recombinant techniques, phage display techniques, synthetic techniques (such as CDR-grafting), or other techniques readily known in the art.

"Administration" and "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contacting an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition with the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" and "treatment" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contacting a agent with the cell, as well as contacting a agent with a fluid, where the fluid is in contact with the cell.

"Treat" means to administer a therapeutic agent, such as a composition comprising any of the binding compounds of the present invention, internally or externally to a patient having one or more disease symptoms for which the agent has known therapeutic activity. Typically, the therapeutic agent is administered in an amount effective to alleviate one or more disease symptoms in the treated patient or population, by inducing the regression of or inhibiting the progression of such symptom(s) to any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease symptom (also referred to "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the patient, and the ability of the drug to elicit a desired response in the patient. Whether a disease symptom has been alleviated can be assessed by any clinical measurement typically used by physicians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (e.g., a treatment method or article of

manufacture) may not be effective in alleviating the disease symptom(s) of interest in every patient, it should alleviate the target disease symptom(s) of interest in a statistically significant number of patients as determined by any statistical test known in the art such as the Student's t-test, the chi-square test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Conservative modification" or "conservative replacement or substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skilled in this art recognize that, in general, single amino acid substitution in non-essential regions of a polypeptide does not substantially alter biological activity (see, e.g., Watson et al. (1987) Molecular Biology of the Gene, The Benjamin/Cummings Pub. Co., p. 224 (4.th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity.

The term "consisting essentially of" or variation thereof as used throughout the specification and claims, indicates the inclusion of any recited elements or group of elements, and optionally inclusion of other elements, of similar or different nature than the recited elements, which do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a nonlimiting example, a binding compound which consists essentially of a recited amino acid sequence may also include one or more amino acids that do not materially affect the properties of the binding compound.

"Effective amount" encompasses an amount sufficient to ameliorate or prevent a symptom or sign of a medical condition. Effective amount also means an amount sufficient to allow or facilitate diagnosis. An effective amount for a particular patient or veterinary subject may vary depending on factors such as the condition being treated, the general health of the patient, the route and dose of administration and the severity of side effects. An effective amount can be the maximal dose or dosing regimen that avoids significant side effects or toxic effects.

"Exogenous" refers to substances that are produced outside an organism, cell, or human body, depending on the context. "Endogenous" refers to substances that are produced within a cell, organism, or human body, depending on the context.

"Homology" refers to sequence similarity between two polynucleotide sequences or between two polypeptides. When a position in both of the two compared sequences

is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared and then multiplied by 100. For example, if 6 of 10 positions in two sequences are matched or homologous when the sequences are optimally aligned, then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology.

"Optional" or "optionally" means that the event or situation that follows may but not necessarily occur, and the description includes the instances in which the event or situation does or does not occur. For example, "optionally comprises 1-3 antibody heavy chain variable regions" means that the antibody heavy chain variable region with specific sequence can be, but not necessarily, present.

"Pharmaceutical composition" refers to a mixture comprising one or more compounds according to the present invention or a physiologically/pharmaceutically acceptable salt or prodrug thereof with other chemical components, as well as additional components such as physiologically/pharmaceutically acceptable carriers and excipients. The pharmaceutical composition aims at promoting the administration to an organism, facilitating the absorption of the active ingredient and thereby exerting a biological effect.

Preparation of conventional pharmaceutical compositions can be found in Chinese pharmacopoeia.

The term "carrier" is applied for the drug of the present invention, and refers to a system that can change the manner in which a drug enters the human body, and change the *in vivo* distribution, control the release rate of the drug, and delivery of the drug to the target organ. Drug carrier release and targeting systems are capable of reducing drug degradation and loss, decreasing side effects, and improving bioavailability. For example, a macromolecular surfactant used as a carrier can self-assemble to form aggregates in various forms because of its unique amphiphilic structure, and preferred examples include micelles, emulsions, gels, liquid crystals, vesicles, etc. These aggregates not only have the ability to entrap drug molecules, but also display good membrane permeability, and can be used as excellent drug carriers.

The term "diluent" is also referred to as filler, and its main purpose is to increase the tablet weight and volume. The addition of diluent is not only to ensure a certain volume, but also to reduce the dose deviation of main components and improve the compression moldability of the drug. When pharmaceutical tablets contain an oily component, an absorbent must be added to absorb the oil material, and maintain the

"dry" state, which facilitates tablet formation.

The term "pharmaceutically acceptable salt" refers to a salt form of a ligand-cytotoxic drug conjugate of the present invention, the salt is safe and effective, and has the desired biological activity in mammals *in vivo*. The antibody-drug conjugate compound of the present invention comprises at least one amino group, by which the the antibody-drug conjugate compound can form a salt with acid.

The term "solvate" refers to a pharmaceutically acceptable solvate formed by a ligand-drug conjugate of the present invention with one or more solvent molecule(s).

The term "ligand" is a macromolecular compound which is able to recognize and bind to the target cell-associated antigens or receptors. The role of the ligand is to deliver the drug to the target cell population bound to the ligand. The ligand includes, but is not limited to, proteinaceous hormones, lectins, growth factors, antibodies and other molecules capable of binding to cells.

The therapeutic agent is a molecule or atom that is administered separately, simultaneously or successively with a binding moiety, such as an antibody or antibody fragment, or a sub-fragment thereof, and is useful for the treatment of the disease. Examples of therapeutic agents include, but are not limited to, antibodies, antibody fragments, conjugates, drugs, cytotoxic agents, apoptotic agents, toxins, nuclease (including DNase and RNase), hormones, immunomodulators, chelating agents, Boron compounds, photosensitizers or dyes, radioisotopes or radionuclides, oligonucleotides, interfering RNAs, peptides, antiangiogenic agents, chemotherapeutic agents, cytokines, chemokines, prodrugs, enzymes, binding proteins or peptides, or a combination thereof.

The conjugate is an antibody component or other targeting moiety conjugated to a therapeutic agent as described above. As used herein, the terms “conjugate” and “immunoconjugate” are used interchangeably.

The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents the function of the cell and/or causes cell death or destruction.

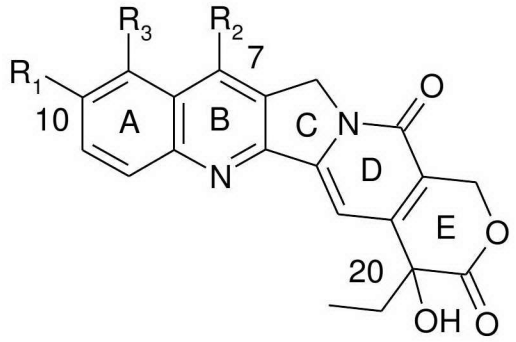
“Toxin” refers to any substance capable of adversely affecting cell growth or proliferation.

“Chemotherapeutic agent” refers to a chemical compound that can be used to treat cancer. The definition also includes anti-hormonal agents that regulate, reduce, block or inhibit the effects of hormones that promote cancer growth, and chemotherapeutic agents are often in the form of systemic or systemic treatment. Themselves can be hormones.

Auristatins are completely synthetic drugs with a relatively easily formed chemical structure that facilitates the optimization of physical properties and druggability. Auristatins derivatives used for antibody conjugation include monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), and MMAE is a synthetic pentapeptide derived from natural tubulin polymerase inhibitor dolastatin-10, synthesized by adding 2-amino-1 -phenylpropyl-1-ol at the C-terminus. MMAE inhibitory activities against a variety of human tumor cell lines are less than one nanomolar. In order to reduce the cytotoxic activity of MMAE itself, a phenylalanine is introduced at the C-terminus of dolastatin-10 in the case of MMAF. Due to the introduction of a carboxyl group in the structure, MMAF has poor capacity in passing through the membrane, and therefore the biological activity against cells is significantly decreased, but the inhibitory activity against cells after conjugation to an antibody is increased substantially (US7750116).

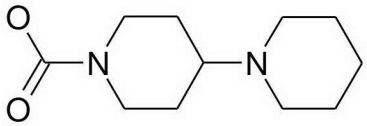
The term "tubulin inhibitor" refers to a class of compounds that exert an anti-tumor effect by inhibiting or promoting polymerization of tublin, and consequently interfering with the cell mitosis process. Non-limiting examples include maytansinoids, calicheamicins, taxanes, vincristines, colchicines, and Dolastatins/Auristatins, preferably maytansinoids or Dolastatins/Auristatins; more preferably compounds of formula D_1 or D_M.

CPT is an acronym for camptothecin, and in this application CPT is used to refer to camptothecin itself or analogs or derivatives of camptothecin. The structure of the camptothecin having the indicated number and the ring labeled with the letter A-E and some analogs thereof is provided in the following formula.



CPT: R1= R2= R3=H

10-hydroxy-CPT:R1=OH; R2= R3=H

Irinotecan (CPT-11):R1=  ; R2=ethyl; R3=H

SN-38: R1=OH; R2= ethyl; R3=H

Topotecan: R1=OH; R2=H; R3=CH-N(CH_3)$_2$

The term "intracellular metabolite" refers to a compound produced by intracellular metabolic processes or reactions of antibody-drug conjugates (ADCs). The metabolic process or reaction may be an enzymatic process, such as proteolytic cleavage of a peptide linker of an ADC, or hydrolysis of a functional group such as a hydrazone, ester or amide. Intracellular metabolites include, but are not limited to, antibodies and free drugs that undergo intracellular cleavage after entering, diffusing, ingesting or transporting into cells.

The term "of intracellular cleavage" and "intracellular cleavage" refer to intracellular metabolic processes or reactions of antibody-drug conjugates (ADCs), wherein the covalent attachment between drug moiety (D) and antibody (Ab) is cleaved (i.e. the linker is cleaved), resulting in intracellular dissociation of free drug from the antibody. The module cleaved from ADC is thus an intracellular metabolite.

The term " bioavailability" refers to the systemic availability (i.e., blood/plasma level) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates the time (rate) and the total amount (degree) required by the drug to achieve systemic circulation from the administered dose.

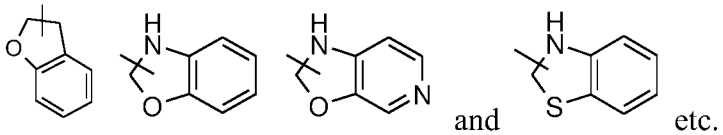
The term "cytotoxic activity" refers to cell killing, cytostatic, or growth inhibitory effects of intracellular metabolites of antibody-drug conjugates or antibody-drug conjugates. Cytotoxic activity can be expressed as the IC50 value, that is, the concentration (molar or mass) per unit volume when half of cells survive.

The term "alkyl" refers to a saturated aliphatic hydrocarbyl group, which is linear or branched chain including C_1-C_{20}, preferably an alkyl having 1 to 12 carbon atoms, more preferably an alkyl having 1 to 10 carbon atoms, most preferably an alkyl having 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 2,2-diethylhexyl, and various branched isomers thereof. The alkyl group can be substituted or unsubstituted. When substituted, the substituent group(s) can substitute at any available connection point, and the substituent group(s) is preferably one or more groups independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkyloxyl, alkylthiol, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocyclic alkyl, aryl, heteroaryl, cycloalkoxy, heterocyclic alkoxy, cycloalkylthio, heterocycloalkylthio, and oxo.

The term "Cycloalkyl" refers to a saturated or partially unsaturated monocyclic or polycyclic hydrocarbyl group. Cycloalkyl has 3 to 20 carbon atoms, preferably 3 to 12 carbon atoms, more preferably 3 to 10 carbon atoms, most preferably 3 to 8 carbon atoms. Representative examples of monocyclic cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptyl, cycloheptatrienyl, cyclooctyl, etc. Polycyclic cycloalkyl includes cycloalkyl having a spiro ring, fused ring or bridged ring.

The term "Heterocyclyl" refers to a saturated or partially unsaturated monocyclic or polycyclic hydrocarbon substituent having 3 to 20 cyclic atoms, wherein one or more cyclic atoms are heteroatoms selected from the group consisting of N, O, and $S(O)_m$ (wherein m is an integer between 0 and 2), but excluding -O-O-, -O-S- or -S-S- in the ring, and the remaining cyclic atoms are C atoms. 3 to 12 cyclic atoms are preferred, wherein 1 to 4 atoms are heteroatoms; 3 to 10 cyclic atoms are more preferred. Representative examples of monocyclic heterocyclyl include, but are not limited to, pyrrolidinyl, piperidyl, piperazinyl, morpholinyl, thiomorpholinyl, homopiperazinyl, and the like. Polycyclic heterocyclyl includes the heterocyclyl having a spiro ring, fused ring or bridged ring.

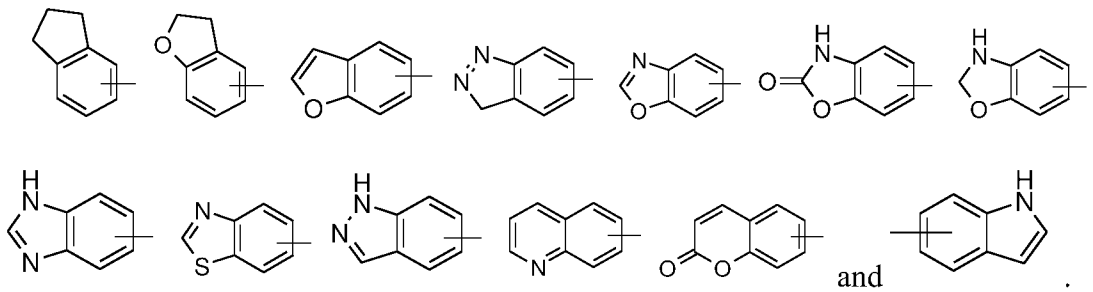
The ring of said heterocyclyl can be fused to the ring of an aryl, heteroaryl or cycloalkyl, wherein the ring bound to the parent structure is heterocyclyl. Representative examples include, but are not limited to the following groups:



etc.

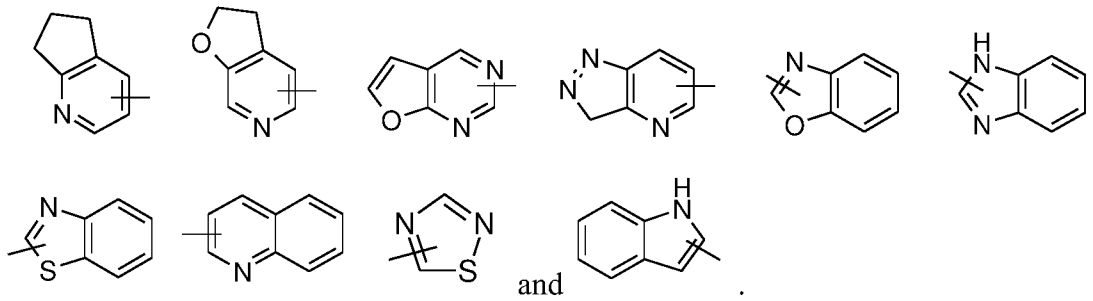
The heterocyclyl can be optionally substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more group(s) independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylsulfo, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocyclic alkyl, aryl, heteroaryl, cycloalkoxy, heterocylic alkoxy, cycloalkylthio, heterocylic alkylthio, and oxo group.

The term "aryl" refers to a 6- to 14-membered all-carbon monocyclic ring or fused polycyclic ring (that is, the rings share the adjacent carbon atom pair), which has a conjugated π-electron system. The aryl is preferably 6- to 10-membered, such as phenyl and naphthyl, preferably phenyl. The aryl ring can be fused to the ring of a heteroaryl, heterocyclyl or cycloalkyl, wherein the ring bound to the parent structure is the ring of aryl. Representative examples include, but are not limited to, the following groups:



The aryl group can be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more groups independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthiol, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio, and heterocycloalkylthio.

The term "heteroaryl" refers to a heteroaromatic system having 1 to 4 heteroatoms and 5 to 14 cyclic atoms, wherein the heteroatoms are selected from the group consisting of O, S, and N. The heteroaryl is preferably 5- to 10- membered, more preferably 5- or 6- membered, such as furyl, thienyl, pyridinyl, pyrrolyl, N-alkyl pyrrolyl, pyrimidinyl, pyrazinyl, imidazolyl, tetrazolyl, and the like. The ring of heteroaryl can be fused with the ring of an aryl, heterocyclyl or cycloalkyl, wherein the ring bound to the parent structure is the ring of heteroaryl. Representative examples include, but are not limited to, the following groups:



The heteroaryl group can be optionally substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more groups independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthiol, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio, and heterocycloalkylthio.

The term "alkoxy" refers to both an -O-(alkyl) and an -O-(unsubstituted cycloalkyl) group, wherein the alkyl is as defined above. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, butoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, and cyclohexyloxy. The alkoxy can be optionally substituted or unsubstituted. When substituted, the substituent is

preferably one or more groups independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylsulfo, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocyclic alkoxy, cycloalkylthio, and heterocyclic alkylthio.

The term "bond" refers to a covalent bond presented as "-".

The term "Hydroxy" refers to an -OH group.

The term "Halogen" refers to fluoro, chloro, bromo or iodo atoms.

The term "Amino" refers to an $-NH_2$ group.

The term "Cyano" refers to a -CN group.

The term "Nitro" refers to a $-NO_2$ group.

The term "Oxo group" refers to a =O group.

The term "optional" or "optionally" means that the event or circumstance described subsequently may, but not necessarily occur, and the description includes the instances in which the event or circumstance does or does not occur. For example, "the heterocyclic group optionally substituted with an alkyl" means that an alkyl group can be, but not necessarily, present, and the description includes a case wherein the heterocyclic group is substituted with an alkyl and a case wherein the heterocyclic group is not substituted with an alkyl.

"Substituted" refers to one or more hydrogen atoms in the group, preferably up to 5, more preferably 1 to 3 hydrogen atoms, each independently substituted with corresponding number of substituents. It is clear that the substituents only exist in their possible chemical position. The person skilled in the art is able to determine if the substitution is possible or impossible without paying excessive efforts by experiment or theory. For example, the conjugation between amino or hydroxy group having free hydrogen and carbon atoms having unsaturated bonds (such as alkene) may be unstable.

"Linker" refers to a chemical module comprising a covalent or atomic chain that covalently attaches the antibody to the drug module. In various embodiments, the linker includes: divalent radicals such as alkyldiyl, arylene, heteroarylene, such as unit like - (CR2) nO (CR2) n-, hydrocarbyloxy repeat units (e.g., polyethyleneamino, PEG, polymethyleneoxy) and aminoalkyl (e.g., polyvinylamino, Jeffamine ™), and so on; and diesters and amides including succinic acid esters, succinamides, bis Glycolate, malonate and caproamide.

Abbreviation

Linker unit

MC=6- maleimido-caproyl

Val-Cit or "vc"= Valine-citrulline (an exemplary dipeptide of a protease cleavable linker)

Citrulline=2-Amino-5-ureido pentanoic acid

PAB= P-aminobenzyloxycarbonyl (examples of "self- immolative" linker unit)

Me-Val-Cit=N- Methyl-valine-citrulline (wherein the linker peptide bond has been modified to prevent its cleavage by cathepsin B)

MC(PEG)6-OH= maleimido-caproyl -polyethylene glycol (which can be attached to antibody cysteine)

SPP=N- Succinimidyl 4- (2-pyridylthio) valerate

SPDP=N- Succinimidyl 3- (2-pyridyldithio) propionate

SMCC= Succinimidyl-4- (N-maleimidomethyl) cyclohexane-1-carboxylate

IT= imino sulfane

Cytotoxic drugs:

MMAE= Monomethyl aurantatin E (MW 718)

MMAF=variant of aurantatin E (MMAE), which has phenylalanine at the C-terminus of the drug (MW731.5)

MMAF-DMAEA= DMAEA (dimethylaminoethylamine) linked to the phenylalanine at C-terminal of MMAF(MW 801.5) by amide

MMAF-TEG= phenylalanine of MMAF is esterified by tetraethylene glycol

MMAF-NtBu= N-tert-butyl as an amide attached to the C-terminus of the MMAF

DM1=N(2')- deacetyl-N (2') - (3-mercapto-1-oxopropyl)–maytian

DM3=N(2')- deacetyl-N2- (4-mercapto-1-oxopentyl)- maytian

DM4=N(2')- deacetyl-N2- (4-mercapto-4-methyl-1-oxopentyl) - maytian

The present invention also provides an antibody-cytotoxic drug conjugate comprising any anti-c-Met antibody of the invention or other c-Met antibody showing endocytosis activity (eg, LY-2875358) conjugated to one or more cytotoxic agents, or a pharmaceutically acceptable salt or solvate compound thereof (interchangeable as "antibody-drug conjugate" or "ADC"), wherein the cytotoxic agents include examples of chemotherapeutic agents, drugs, growth inhibitors, toxins (e.g., bacterial, fungal, plant or animal-derived enzyme-active toxins or fragments thereof) or radioisotopes (i.e., radioluminescent conjugates).

In certain embodiments, the antibody-cytotoxic drug conjugate or a pharmaceutically acceptable salt or solvate compound thereof comprises an anti-c-Met antibody and a chemotherapeutic agent or other toxin. A chemotherapeutic

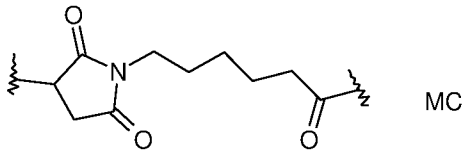
agent that can be used to produce an antibody-cytotoxic drug conjugate or a pharmaceutically acceptable salt or solvate compound thereof has been described herein (described above). Enzyme-active toxins and fragments thereof are also used, which are described in the specification.

In certain embodiments, the antibody-cytotoxic drug conjugate or a pharmaceutically acceptable salt or solvate compound thereof comprises an anti-c-Met antibody and one or more small molecule toxins including, but not limited to small molecule drugs such as camptothecin derivatives, calicheamicin, maytansinoids, dolastatin, oricotine, trichothecene and CC1065, and cytotoxic fragments of these drugs.

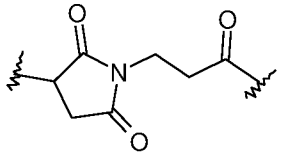
Exemplary linker L_2 includes 6-maleimidocaproyl ("MC"), maleimidopropionyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl ("PAB"), N-succinimidyl 4- (2-pyridylthio) pentanoate "SPP"), N-succinimidyl 4- (N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), and N-succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). A variety of linkers are known in the art and are described below.

The linker may be a "cleavable linker" that facilitates the release of the drug in the cell. For example, an acid labile linker (e.g., hydrazone), a protease-sensitive (e.g., peptidase-sensitive) linker, a light-labile linker, a dimethyl linker, or a disulfide-containing linker (Chari et al, Cancer Research 52: 127-131(1992); US patent No.5,208,020).

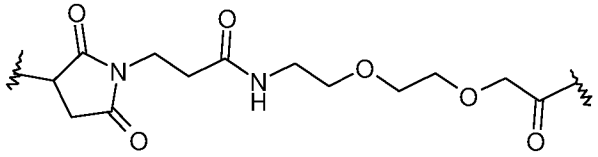
In some embodiments, the linker may be a "stretcher unit" that connects the antibody to another linker or drug module. Exemplary stretcher units are shown below (where the wavy line indicates the site to which the antibody covalently attached to):



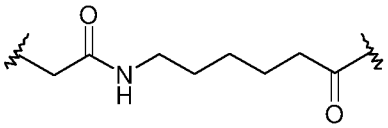
MC



MP



MPEG



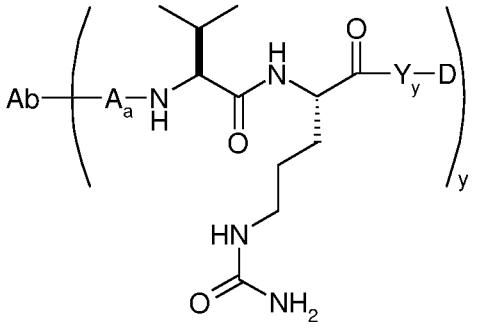
In some embodiments, the linker unit may be an amino acid unit. In one such embodiment, the amino acid unit allows the protease to cleave the linker, thereby facilitating to release the drug from the antibody-cytotoxic drug conjugate or its pharmaceutically acceptable salt or solvate compound after exposure to intracellular proteases, such as lysosomal enzymes. See the example in Doronina et al (2003) Nat. Biotechnol. 21: 778-784. Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include: valine - citrulline (VC or val - cit); alanine-phenylalanine (AF or ala-phe); phenylalanine-lysine (FK or phe-lys); or N- Methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). The amino acid units may comprise naturally occurring amino acid residues, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for their selectivity to enzymatic cleavage of specific enzymes, such as tumor-associated proteases, cathepsin B, C and D, or plasma proteases.

In some embodiments, the linker may be a “spacer” unit that connects the antibody (either directly or through the extension unit and/or the amino acid unit) to the drug module. The spacer unit may be "self-immolative" or "non-self immolative". The "non-self immolative" spacer unit refers to a portion or the whole of the spacer unit that remains the spacer unit bound to the drug module after enzymatic (protein hydrolysis) cleavage of the ADC. Examples of non-self immolative spacer units

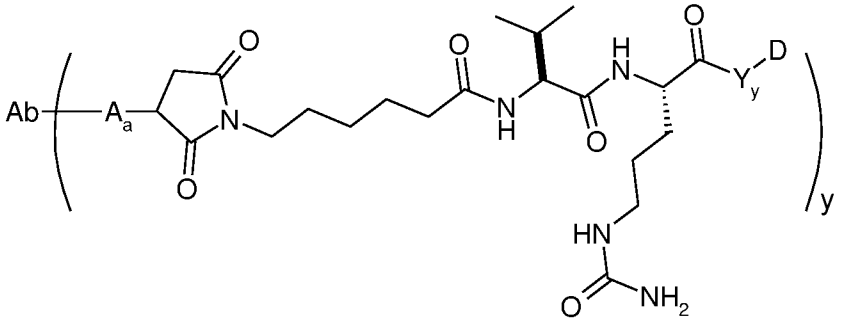
include, but are not limited to, glycine spacer units and glycine-glycine spacer units. Other combinations of peptide spacers susceptible to sequence-specific enzymatic cleavage are also contemplated. For example, enzymatic cleavage of glycine-glycine spacer unit-containing ADC by tumor-cell-associated protease will result in the release of the glycine-glycine-drug module from the remainder of the ADC. In one such embodiment, the glycine-glycine-drug module is then subjected to a separate hydrolysis step in the tumor cells, thereby cleaving the glycine-glycine spacer unit from the drug module.

The "self- immolative" spacer unit allows the release of the drug module without separate hydrolysis steps. In certain embodiments, the spacer unit of the linker comprises a p-aminobenzyl unit. In one such embodiment, p-aminobenzyl alcohol is attached to the amino acid unit via an amide bond, thereby forming a carbamate, methyl carbamate, or carbonate between benzyl alcohol and the cytotoxic agent. See, for example, in Hamann et al, (2005) Expert Opin. Ther. Patents (2005) 15: 1087-1103. In one embodiment, the spacer units are p-aminobenzyloxycarbonyl (PAB).

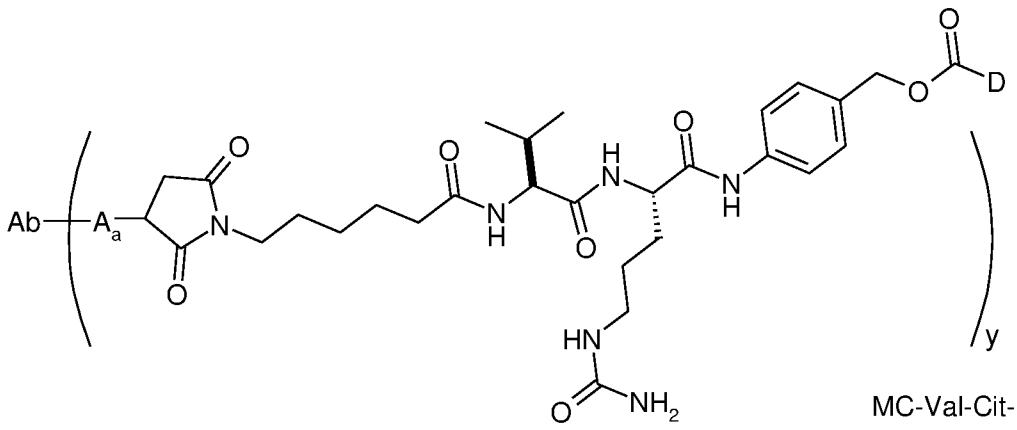
Exemplary linker unit in the present invention are as follows:



Val-Cit或VC



MC-Val-Cit



MC-Val-Cit-PAB

The linker, including the extension, the spacer, and the amino acid unit, can be synthesized by methods known in the art, such as those described in US 2005-0238649 A1.

Exemplary drug modules

Maytansine and maytansinoid alkaloids

In some embodiments, the antibody-cytotoxic drug conjugate or a pharmaceutically acceptable salt or solvate compound thereof comprises an antibody of the invention conjugated to one or more maytansinoid molecules. The maytansinoid is a mitotic inhibitor that acts by inhibiting tubulin multimerization. Maytansine was originally isolated from the Maytian tree (Maytenus serrata) from the East African shrubs (US patent No.3,896,111). It was subsequently found that certain microorganisms also generate maytansinoid alkaloids such as maytansinol and C-3 adefovir (US patent No.4,151,042).

The maytansinoid drug modules are attractive drug modules in antibody-drug conjugates because they: (i) are relatively easy to be chemically modified or derivatized from fermentation or fermentation products; (ii) readily derivatized with a functional group suitable for coupling to an antibody through a non-disulfide linker; (iii) stable in plasma; and (iv) effective for a variety of tumor cell lines.

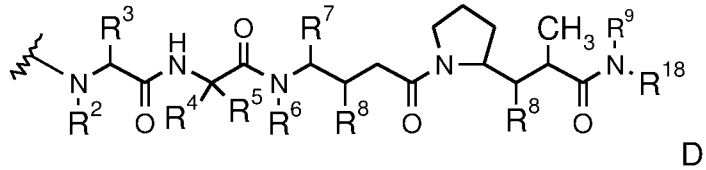
Maytansine compounds suitable for use as the maytansinoid alkaloid drug modules are well known in the art and can be isolated from natural sources according to known methods or produced using genetic engineering techniques (See Yu et al (2002) PNAS 99: 7968-7973). The maytansinol and maytansinol analogs can also be prepared according to known methods.

Exemplary embodiments of the maytansinoid alkaloid drug module include: DM1, DM3 and DM4, as disclosed herein.

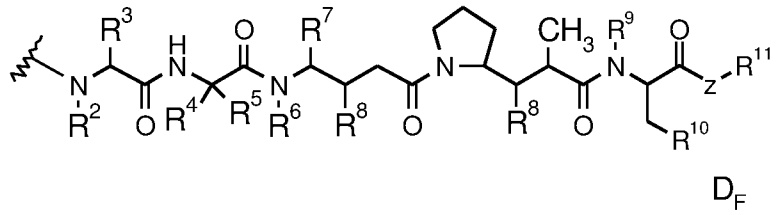
Auristatin and dolastatin

In some embodiments, the antibody-cytotoxic drug conjugate or a pharmaceutically acceptable salt or solvate compound thereof comprises an antibody of the invention conjugated to dolastatin or dolastatin peptide analogue or derivative (e.g., auristatin) (U.S. Patent No. 5,635,483; 5,780,588). Dolastatin and auristatin have been shown to interfere with microtubule kinetics, GTP hydrolysis, and nuclear and cell division (Woyke et al. (2001) Antimicrob. Agents and Chemother. 45 (12): 3580-3584), and anti-cancer activity (U.S. Patent No. 5,663,149) and antifungal activity (Pettit et al. (1998) Antimicrob. Agents Chemother. 42: 2961-2965). Dolastatin or auristatin drug modules may be attached to the antibody via the N (amino) terminus or the C (carboxy) terminus of the peptide drug module (WO02/088172).

Exemplary administration regimens of auristatin include N-terminal linked monomethyl auristatin drug modules DE and DF, which are disclosed by Senter et al, Proceedings of the American Association for CancerResearch, volume 45, abstract number 623, March 28, 2004, the disclosure of which is expressly incorporated herein by reference in its entirety.The peptide drug module may be selected from the general formulas D_E and D_F as below:



D_E



D_F

wherein the wavy lines of the D_E and D_F indicate the covalent attachment sites of the antibody or antibody-linker, and each site is independent from each other:

R^2 is selected from H and C1-C8 hydrocarbyl;

R^3 is selected from the group consisting of H, C1-C8 hydrocarbyl, C3-C8 carbocycle, aryl, C1-C8 hydrocarbyl - aryl, C1-C8 hydrocarbyl -(C3-C8 carbocycle), C3-C8 heterocycle and C1-C8 hydrocarbyl -(C3-C8 heterocycle);

R^4 is selected from the group consisting of H, C1-C8 hydrocarbyl, C3-C8 carbocycle, aryl, C1-C8 hydrocarbyl - aryl, C1-C8 hydrocarbyl -(C3-C8 carbocycle), C3-C8 heterocycle and C1-C8 hydrocarbyl-(C3-C8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 form a carbocycle of formula -(CRaRb)n-, wherein R^a and R^b are each independently selected from the group consisting of H, C1-C8 hydrocarbyl and C3-C8 carbocycle, and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H or C1-C8 hydrocarbyl;

R^7 is selected from the group consisting of H, C1-C8 hydrocarbyl, C3-C8 carbocycle, aryl, C1-C8 hydrocarbyl - aryl, C1-C8 hydrocarbyl -(C3-C8 carbocycle), C3-C8 heterocycle and C1-C8 hydrocarbyl-(C3-C8 heterocycle);

each R^8 is independently selected from the group consisting of H, OH, C1-C8 hydrocarbyl, C3-C8 carbocycle and O-(C1-C8 hydrocarbyl);

R^9 is selected from H and C1-C8 hydrocarbyl;

R^{10} is selected from aryl or C3-C8 heterocycle;

Z is selected from O, S, NH or NR^{12}-, whereinR^{12} is C1-C8 hydrocarbyl;

R^{11} is selected from the group consisting of H, C1-C20 hydrocarbyl, aryl, C3-C8 heterocycle, -$(R^{13}O)m-R^{14}$ and -$(R^{13}O)m-CH(R^{15})_2$;

m is an integer selected from 1-1000;

R^{13} is C2-C8 hydrocarbyl;

R^{14} is H or C1-C8 hydrocarbyl;

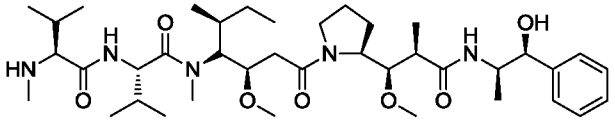
R^{15} is each independently selected from the group consisting of H, COOH, -$(CH_2)n-N(R16)_2$, -$(CH_2)n-SO_3H$ or -$(CH_2)n-SO_3$-C1-C8 hydrocarbyl;

R^{16} is each independently selected from the group consisting of H, C1-C8 hydrocarbyl or -$(CH_2)n-COOH$;

R^{18} is selected from -$C(R8)_2$-$C(R8)_2$-aryl, -$C(R8)_2$-$C(R8)_2$-(C3-C8 heterocycle) and -$C(R8)_2$-$C(R8)_2$-(C3-C8 carbocycle); and

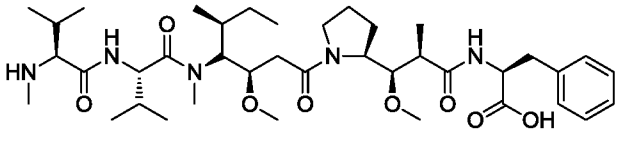
n is an integer selected from 0 to 6.

An exemplary auristatin of the formula D_E, the embodiment is MMAE, wherein the wavy line indicates a linker (L) covalently attached to the antibody-drug conjugate:



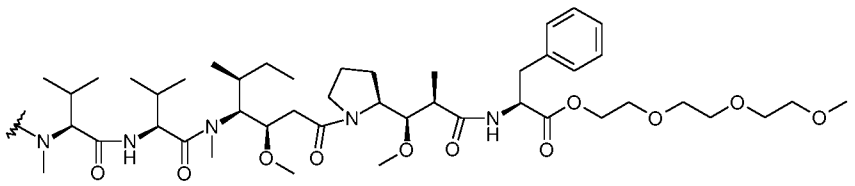
MMAE

An exemplary auristatin of the formula D_F, the embodiment is MMAF, wherein the wavy line indicates a linker (L) covalently attached to the antibody-drug conjugate (see US2005/0238649 and Doronina et al (2006) Bioconjugate Chem. 17: 114-124):



MMAF

The other drug modules comprise a MMAF derivative selected from the following, wherein the wavy line indicates a linker (L) covalently attached to the antibody-drug conjugate:



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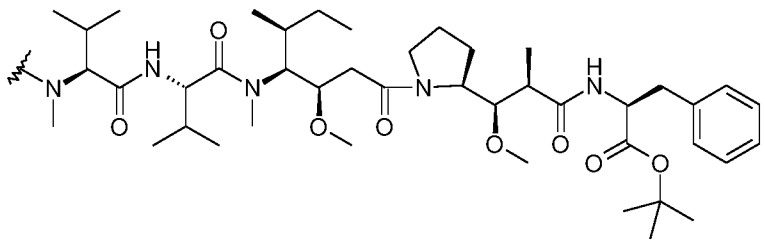

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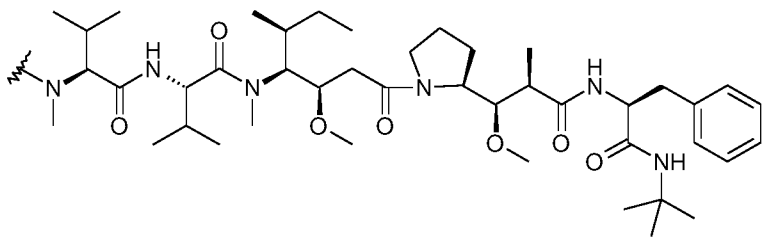

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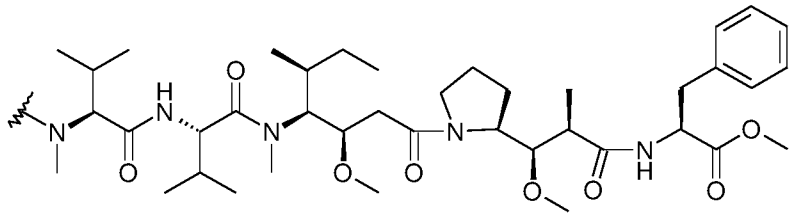
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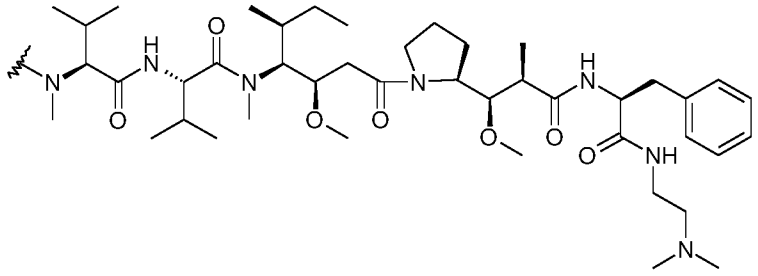
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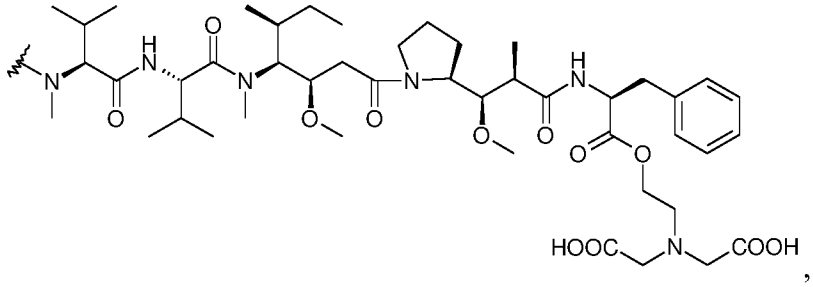
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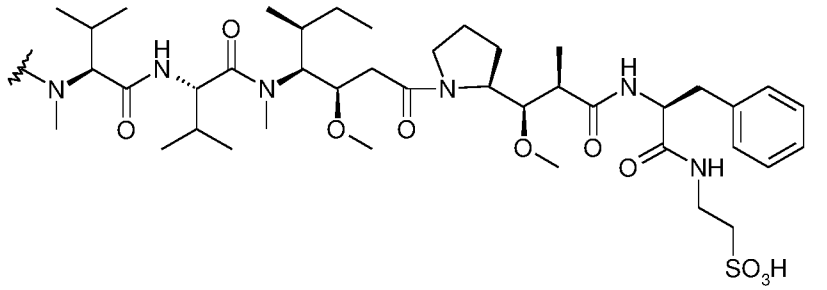
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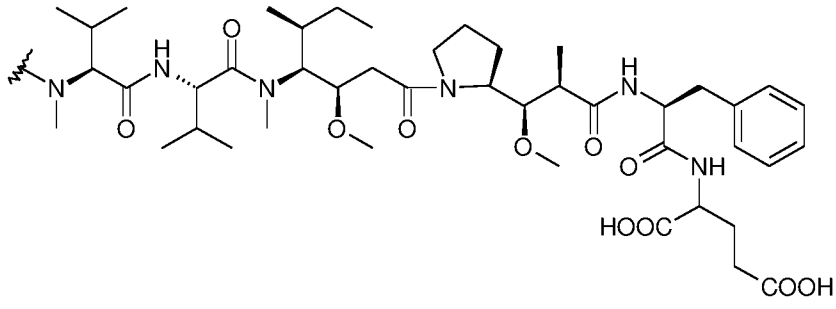
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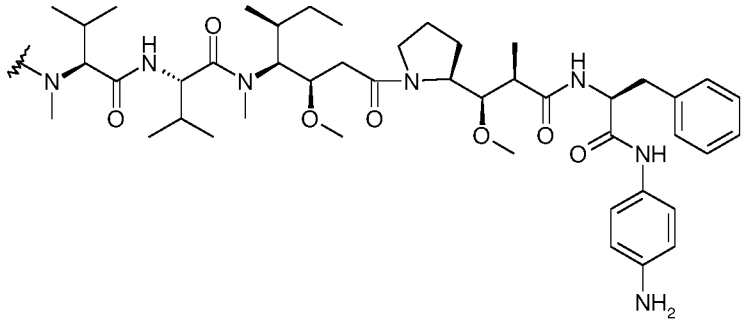
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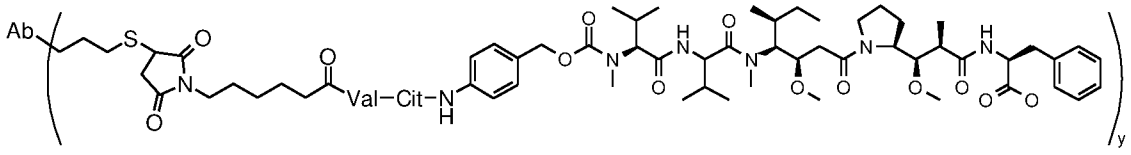
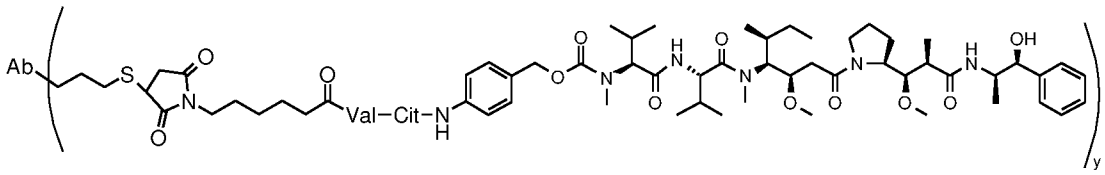
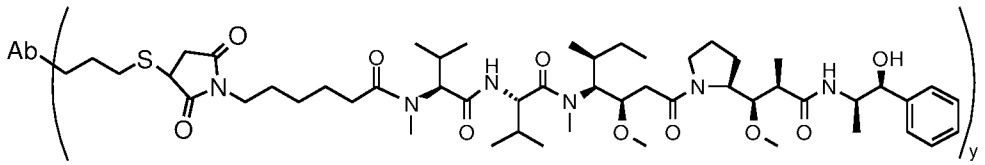
In one aspect, a hydrophilic group may be attached to a drug module at R^{11}, wherein said hydrophilic group includes, but not limited to, triethylene glycol ester (TEG), as described above. Without being limited to any particular theory, the hydrophilic groups contribute to the internalization and non-agglomeration of the drug modules. Exemplary embodiments of ADC of general formula I comprises auristatin/dolastatin or a derivative thereof are described in US2005-0238649A1 and Doronina et al (2006)Bioconjugate Chem.17:114-124, which is expressly incorporated herein by reference. Exemplary embodiments of ADCs of general formula I comprising MMAE or MMAF and various linkers have the following structure and abbreviations (wherein "Ab" is antibody; p range from 1 to about 8; "Val-Cit" is Valine-citrulline dipeptide; and "S" is sulfur atom):



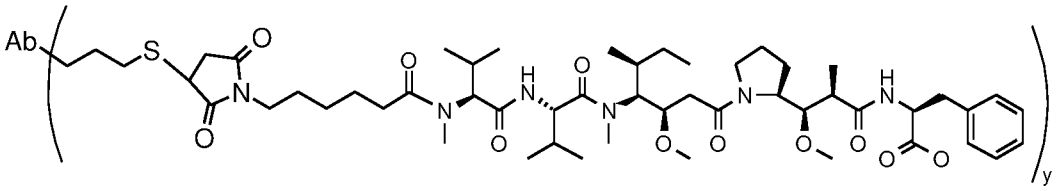
Ab- linker 1-MC-vc-PAB-MMAF



Ab- linker 1-MC-vc-PAB-MMAE



Ab- linker 1-MC- MMAE



Ab-linker1-MC- MMAF

Typically, peptide-based drug modules can be prepared by forming peptide bonds between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared according to, for example, liquid phase synthesis methods well

known in the art of peptide chemistry (see E. Schroder and K.Lübke, The Peptides, volumn 1, pp 76-136, 1965, Academic Press). The auristatin/dolastatin drug modules can be prepared according to the methods described in the following literatures: US2005-0238649A1; US patent No.5635483; US patent No.5780588; Pettit et al(1989) J. Am. Chem. Soc. 111: 5463-5465; Pettit et al (1998) Anti-Cancer Drug Design 13: 243-277; Pettit, G. R. et al, Synthesis, 1996, 719-725; Pettit et al (1996) J. Chem. Soc. Perkin Trans. 15: 859-863; and Doronina(2003) Nat. Biotechnol. 21(7): 778-784.

In particular, the auristatin/dolastatin drug modules of the general formula DF, such as MMAF and derivatives thereof, can be prepared using the methods described in US2005-0238649A1 and Doronina et al. (2006) Bioconjugate Chem. 17: 114-124. The auristatin/dolastatin drug modules of the general formula DE, such as MMAE and derivatives thereof, can be prepared by the method described in Doronina et al. (2003) Nat. Biotech. 21: 778-784. The drug-linker modules of MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF and MC-vc-PAB-MMAE can be conveniently synthesized by conventional methods such as those described in Doronina et al. (2003) Nat. Biotech. 21: 778-784 and U.S. Patent Application Publication No. US2005/0238649A1, and then conjugated to the antibody of interest.

Drug load

The drug load (loading) is expressed by y, that is, the average number of drug modules per antibody in the molecule of formula I. The drug load can range from 1 to 20 drug modules (D) per antibody. The ADC of Formula I includes a collection of antibodies conjugated to a range of (1-20) drug modules. The average number of drug modules per antibody in ADC preparation obtained from coupling reaction can be characterized by conventional means such as mass spectrometry, ELISA assay, and HPLC. It is also possible to determine the quantitative distribution of ADCs in the aspect of y. In some cases, homogeneous ADCs with certain p values are isolated from ADCs of other drug loads, and then purified and characterized, this can be achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody-drug conjugates, y may be limited by the number of attachment sites on the antibody. For example, if the cysteine thiol is attached, as in the above illustrative embodiment, the antibody may have only one or several cysteine thiol groups, or may only have one or more reactive thiol group which can be attached to the linker. In certain embodiments, a higher drug load, such as y >5, may cause aggregation, insolubility, toxicity, or loss of cell permeability of certain

antibody-drug conjugates. In certain embodiments, the drug load of the ADC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8 ; about 3.2 to about 3.7; about 3.2 to about 3.6; about 3.3 to about 3.8; or about 3.3 to about 3.7. In fact, for some ADCs, it has been shown that the optimal ratio of each antibody drug module may be less than 8 and may be from about 2 to about 5. See US2005-0238649A1 (its entire content is incorporated herein by reference).

In certain embodiments, a drug module having less than the theoretical maximum is coupled to the antibody in the coupling reaction. The antibody may comprise, for example, a lysine residue that does not react with a drug-linker intermediate or a linker agent, as discussed below. Only the most reactive lysine groups can react with amine-reactive linker agents. In general, the antibody does not contain a number of free and reactive cysteine thiol groups, which can be linked to a drug module; in fact, most of the cysteine thiol groups in the antibody are present in the form of a disulfide bridge. In certain embodiments, the antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonyl ethyl phosphine (TCEP) under partially or completely reductive conditions to produce a reactive cysteine thiol group. In certain embodiments, the antibody is placed under denaturing conditions to expose a reactive nucleophilic group, such as lysine or cysteine.

The drug load (drug/antibody ratio, DAR) of the ADC can be controlled in different ways, for example by: (i) limiting the molar excess of the drug-linker intermediate or linker agent; (ii) limiting the time or temperature of the coupling reaction; (iii) limiting the modification of the cysteine thiol or restricting the reductive condition; (iv) engineered the amino acid sequence of the antibody by recombinant techniques, such that the number and location of cysteine residues are altered in order to control the number and/or position of the linker-drug attachment (such as the thioMab or the thioFab prepared as those described in the present invention and WO2006/034488 (incorporated herein by reference in its entirety)).

It is to be understood that if more than one nucleophilic group is reacted with a drug-linker intermediate or with a linker and subsequent drug module agents, the resulting product is an ADC compound mixture having one or more drug modules attached to a distribution of the antibody. The average number of drugs per antibody can be calculated from the mixture by ELISA assay which involves antibody-specific and drug-specific antibodies. The various ADC molecules in the mixture can be identified by mass spectrometry, and separated by HPLC, for example, hydrophobic

interaction chromatography. In certain embodiments, a homogeneous ADC with a single load value can be isolated from the coupling mixture by electrophoresis or chromatography.

Methods for preparing antibody-cytotoxic drug conjugates or pharmaceutically acceptable salts or solvate compounds thereof

The ADC of general formula I can be prepared by several routes using organic chemical reactions, conditions and agents known to those skilled in the art, including: (1) the nucleophilic group of the antibody reacts with the divalent linker agent via a covalent bond to form Ab-L, followed by reaction with the drug module D; and (2) the nucleophilic group of the drug module reacts with the divalent linker agent via a covalent bond to form D-L, followed by reaction with the nucleophilic group of the antibody. The exemplary method for preparing the ADC of Formula I via the latter route is described in US2005-0238649A1, which is expressly incorporated herein by reference.

Nucleophilic groups of antibodies include, but are not limited to: (i) an N-terminal amine group; (ii) a side chain amine group such as lysine; (iii) a side chain thiol group such as cysteine; and (iv) a hydroxyl or amino group of saccharide in the glycosylated antibody. Amines, thiols and hydroxyl groups are nucleophilic and are capable of reacting with the electrophilic groups on the linker module to form covalent bonds, and the linker agents include: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) hydrocarbyl and benzyl halides, such as haloacetamides; (iii) aldehydes, ketones, carboxyl groups and maleimide groups. Some antibodies have a reducible interchain disulfide, that is a cysteine bridge. The antibody can be completely or partially reduced by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonyl ethylphosphine (TCEP) to provide coupling reactivity with the linker. Each cysteine bridge will theoretically form two reactive thiol nucleophiles. Alternatively, the sulfhydryl group may be introduced into the antibody via modification of the lysine residue, for example by reacting the lysine residue with 2-imin sulfane (Traut reagent), resulting in the conversion of the amine to the thiol.

The antibody-drug conjugates of the present invention can also be produced by the reaction between an electrophilic group on an antibody (such as an aldehyde or ketone carbonyl group) and a linker or a nucleophilic group on a drug. Useful nucleophilic groups on the linker include, but are not limited to: hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate and arylhydrazide. In

one embodiment, the saccharide of the glycosylated antibody can be oxidized with, for example, a periodate oxidant to form an aldehyde or ketone group that can react with the amine group of the linker or drug module. The resulting imine Schiff base may form a stable linkage or may be reduced with, for example, a borohydride agent to form a stable amine linkage. In one embodiment, the reaction of the carbohydrate moiety of the glycosylated antibody with galactose oxidase or sodium metaperiodate may produce a carbonyl group (aldehyde group and keto group) in the antibody, which may be reacted with a suitable group on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, an antibody comprising an N-terminal serine or threonine residue may react with sodium metaperiodate, resulting in the formation of an aldehyde at the first amino acid (Geoghegan and Stroh,(1992) Bioconjugate Chem. 3: 138-146; US5362852). Such aldehydes can react with the drug module or the linker nucleophile.

Nucleophilic groups on the drug module include, but are not limited to: amine, thiol, hydroxy, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate and arylhydrazide groups, which can react with the electrophilic groups on the linker module to form covalent bonds. And the linker agents include: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) hydrocarbyl and benzyl halides, such as haloacetamides; (iii) aldehydes, ketones, carboxyl groups, and maleimide groups.

The compounds of the present invention clearly cover but are not limited to the ADC prepared by the following crosslinking agents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB and SVSB (succinimidyl- (4-vinylsulfone) benzoate), which are commercially available (such as Pierce Biotechnology,Inc., Rockford, IL., U.S.A, · refer to the 2003-2004 Application Manual and product catalog (2003-2004 Applications Handbook and Catalog) page 467-498).

Antibody-cytotoxic drug conjugates or their pharmaceutically acceptable salts or solvate compounds containing antibodies and cytotoxic agents can also be prepared using a variety of bifunctional protein coupling agents, such as N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4- (N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), aminosulfane (IT), imidates (such as dimethyl adipamide HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis azide compounds (such as bis (p-azidobenzoyl) hexamethylene

diamine), bis diazo derivatives (such as bis (p-diazo benzoyl) -ethylenediamine), diisothiocyanate (such as toluene 2,6-Diisocyanate) and dual active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, ricin immunotoxins can be prepared as described in Vitetta et al., Science 238: 1098 (1987). The carbon-14 labeled 1-isothiocyanate benzyl-3- methyl diethylene triamine pentaacetic acid (MX-DTPA) is an exemplary chelating agent for coupling a radioactive nucleotide to an antibody. See WO 94/11026.

Alternatively, a fusion protein comprising an antibody and a cytotoxic agent can be prepared by, for example, recombinant techniques or peptide synthesis. The recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic moiety of the conjugate respectively, either adjacent to each other or separated by a region encoding a linker peptide, wherein said linker peptide does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor"; (such as streptavidin) for pre-targeting the tumor, the antibody-receptor conjugate is administered to a patient, followed by the use of a scavenger which removes the unbound conjugates from circulation. Then, a " ligand" (e.g., avidin) coupled to a cytotoxic agent (such as a radioactive nucleotide) is administrated. The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention is further described with reference to examples. However, the scope of the present invention is not limited thereto.

In the examples of the present invention, where specific conditions are not described, the experiments are generally conducted under conventional conditions, or under conditions proposed by the manufacturers of material or product. See Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory; Current Protocols in Molecular Biology, Ausubel et al, Greene Publishing Associates, Wiley Interscience, NY. Where the source of the agents is not specifically given, the agents are commercially available.

EXAMPLE

Example 1. Antigenic antibody Clonal expression

The antibodies (light and heavy chains) and antigens used in the present

invention are constructed by overlapping extension PCR methods known in the art. The DNA fragment obtained by overlapping extension PCR was inserted into the expression vector pEE6.4 (Lonza Biologics) using HindIII/BstBI, and expressed in 293F cells (Invitrogen, Cat # R790-07). The resulting recombinant protein is used for immunization or screening. The c-Met gene template is derived from origene Corporation (article number RC217003). The DNA sequence to be cloned and expressed is as follows.

Human c-Met extracellular region (ECD) and murine Fc region fusion protein (human c-Met ECD-mFc) DNA sequence:

atgaaggcccccgctgtgcttgcacctggcatcctcgtgctcctgtttaccttggtgcagaggagcaatggggagtgtaaag
aggcactagcaaagtccgagatgaatgtgaatatgaagtatcagcttcccaacttcaccgcggaaacacccatccagaatg
tcattctacatgagcatcacattttccttggtgccactaactacatttatgttttaaatgaggaagaccttcagaaggttgctgagt
acaagactgggcctgtgctggaacacccagattgtttcccatgtcaggactgcagcagcaaagccaatttatcaggaggtg
tttggaaagataacatcaacatggctctagttgtcgacacctactatgatgatcaactcattagctgtggcagcgtcaacaga
gggacctgccagcgacatgtctttccccacaatcatactgctgacatacagtcggaggttcactgcatattctccccacagat

agaagagcccagccagtgtcctgactgtgtggtgagcgccctgggagccaaagtcctttcatctgtaaaggaccggttcat
caacttctttgtaggcaataccataaattcttcttatttcccagatcatccattgcattcgatatcagtgagaaggctaaaggaaa
cgaaagatggttttatgtttttgacggaccagtcctacattgatgttttacctgagttcagagattcttaccccattaagtatgtcc
atgcctttgaaagcaacaattttatttacttcttgacggtccaaagggaaactctagatgctcagacttttcacacaagaataatc
aggttctgttccataaactctggattgcattcctacatggaaatgcctctggagtgtattctcacagaaaagagaaaaaagag
atccacaaagaaggaagtgtttaatatacttcaggctgcgtatgtcagcaagcctggggcccagcttgctagacaaatagga

gccagcctgaatgatgacattcttttcggggtgttcgcacaaagcaagccagattctgccgaaccaatggatcgatctgcca
tgtgtgcattccctatcaaatatgtcaacgacttcttcaacaagatcgtcaacaaaaacaatgtgagatgtctccagcatttttac
ggacccaatcatgagcactgctttaataggacacttctgagaaattcatcaggctgtgaagcgcgccgtgatgaatatcgaa
cagagtttaccacagctttgcagcgcgttgacttattcatgggtcaattcagcgaagtcctcttaacatctatatccaccttcatt
aaaggagacctcaccatagctaatcttgggacatcagagggtcgcttcatgcaggttgtggtttctcgatcaggaccatcaa
cccctcatgtgaattttctcctggactcccatccagtgtctccagaagtgattgtggagcatacattaaaccaaaatggctaca

cactggttatcactgggaagaagatcacgaagatcccattgaatggcttgggctgcagacatttccagtcctgcagtcaatg
cctctctgccccaccctttgttcagtgtggctggtgccacgacaaatgtgtgcgatcggaggaatgcctgagcgggacatg
gactcaacagatctgtctgcctgcaatctacaaggttttcccaaatagtgcacccctgaaggagggacaaggctgaccata

tgtggctgggactttggatttcggaggaataataaatttgatttaaagaaaactagagttctccttggaaatgagagctgcacc
ttgactttaagtgagagcacgatgaatacattgaaatgcacagttggtcctgccatgaataagcatttcaatatgtccataattat
ttcaaatggccacgggacaacacaatacagtacattctcctatgtggatcctgtaataacaagtatttcgccgaaatacggtc
ctatggctggtggcactttacttactttaactggaaattacctaaacagtgggaattctagacacatttcaattggtggaaaaac
atgtactttaaaaagtgtgtcaaacagtattcttgaatgttataccccagcccaaaccatttcaactgagtttgctgttaaattgaa
aattgacttagccaaccgagagacaagcatcttcagttaccgtgaagatcccattgtctatgaaattcatccaaccaaatctttt
attagtggtgggagcacaataacaggtgttgggaaaaacctgaattcagttagtgtcccgagaatggtcataaatgtgcatg
aagcaggaaggaactttacagtggcatgtcaacatcgctctaattcagagataatctgttgtaccactccttccctgcaacag
ctgaatctgcaactccccctgaaaaccaaagccttttcatgttagatgggatcctttccaaatactttgatctcatttatgtacat
aatcctgtgtttaagccttttgaaaagccagtgatgatctcaatgggcaatgaaaatgtactggaaattaagggaaatgatatt
gaccctgaagcagttaaaggtgaagtgttaaaagttggaaataagagctgtgagaatatacacttacattctgaagccgtttta
tgcacggtccccaatgacctgctgaaattgaacagcgagctaaatatagagtggaagcaagcaatttcttcaaccgtccttg
gaaaagtaatagttcaaccagatcagaatttcaca (SEQ ID NO: 1)

Human c-Met extracellular Sema region and Flag-His tag (Human c-Met Sema-Flis) DNA sequence:

atgaaggcccccgctgtgcttgcacctggcatcctcgtgctcctgtttaccttggtgcagaggagcaatggggagtgtaaag
aggcactagcaaagtccgagatgaatgtgaatatgaagtatcagcttcccaacttcaccgcggaaacacccatccagaatg
tcattctacatgagcatcacattttccttggtgccactaactacatttatgttttaaatgaggaagaccttcagaaggttgctgagt
acaagactgggcctgtgctggaacacccagattgtttcccatgtcaggactgcagcagcaaagccaatttatcaggaggtg
tttggaaagataacatcaacatggctctagttgtcgacacctactatgatgatcaactcattagctgtggcagcgtcaacaga
gggacctgccagcgacatgtctttccccacaatcatactgctgacatacagtcggaggttcactgcatattctccccacagat
agaagagcccagccagtgtcctgactgtgtggtgagcgccctgggagccaaagtcctttcatctgtaaaggaccggttcat
caacttctttgtaggcaataccataaattcttcttatttcccagatcatccattgcattcgatatcagtgagaaggctaaaggaaa
cgaaagatggttttatgtttttgacggaccagtcctacattgatgttttacctgagttcagagattcttaccccattaagtatgtcc
atgcctttgaaagcaacaattttatttacttcttgacggtccaaagggaaactctagatgctcagacttttcacacaagaataatc
aggttctgttccataaactctggattgcattcctacatggaaatgcctctggagtgtattctcacagaaaagagaaaaaagag
atccacaaagaaggaagtgtttaatatacttcaggctgcgtatgtcagcaagcctggggcccagcttgctagacaaatagga
gccagcctgaatgatgacattcttttcggggtgttcgcacaaagcaagccagattctgccgaaccaatggatcgatctgcca
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ggacccaatcatgagcactgctttaataggacacttctgagaaattcatcaggctgtgaagcgcgccgtgatgaatatcgaa
cagagtttaccacagctttgcagcgcgttgacttattcatgggtcaattcagcgaagtcctcttaacatctatatccaccttcatt
aaaggagacctcaccatagctaatcttgggacatcagagggtcgcttcatgcaggttgtggtttctcgatcaggaccatcaa
cccctcatgtgaattttctcctggactcccatccagtgtctccagaagtgattgtggagcatacattaaaccaaaatggctaca

cactggttatcactgggaagaagatcacgaagatcccattgaatggcttgggctgcagacatttccagtcctgcagtcaatg
cctctctgccccacccctttgttcagtgtggctggtgccacgacaaatgtgtgcgatcggaggaatgcctgagcgggacatg
gactcaacagatctgtctgcctgcaatctacaaggactacaaggacgacgacgacaagcatgtccaccatcatcaccatca
ctgattcgaa (SEQ ID NO: 2)

Human c-Met ECD his tag (Human c-Met ECD-His) recombinant protein DNA sequence:

atgaaggcccccgctgtgcttgcacctggcatcctcgtgctcctgtttaccttggtgcagaggagcaatggggagtgtaaag
aggcactagcaaagtccgagatgaatgtgaatatgaagtatcagcttcccaacttcaccgcggaaacacccatccagaatg
tcattctacatgagcatcacattttccttggtgccactaactacatttatgttttaaatgaggaagaccttcagaaggttgctgagt
acaagactgggcctgtgctggaacacccagattgtttcccatgtcaggactgcagcagcaaagccaatttatcaggaggtg
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gggacctgccagcgacatgtctttccccacaatcatactgctgacatacagtcggaggttcactgcatattctccccacagat
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caacttctttgtaggcaataccataaattcttcttatttcccagatcatccattgcattcgatatcagtgagaaggctaaaggaaa
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aggttctgttccataaactctggattgcattcctacatggaaatgcctctggagtgtattctcacagaaaagagaaaaaagag
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gccagcctgaatgatgacattcttttcggggtgttcgcacaaagcaagccagattctgccgaaccaatggatcgatctgcca
tgtgtgcattccctatcaaatatgtcaacgacttcttcaacaagatcgtcaacaaaaacaatgtgagatgtctccagcatttttac
ggacccaatcatgagcactgctttaataggacacttctgagaaattcatcaggctgtgaagcgcgccgtgatgaatatcgaa
cagagtttaccacagctttgcagcgcgttgacttattcatgggtcaattcagcgaagtcctcttaacatctatatccaccttcatt
aaaggagacctcaccatagctaatcttgggacatcagagggtcgcttcatgcaggttgtggtttctcgatcaggaccatcaa
cccctcatgtgaattttctcctggactcccatccagtgtctccagaagtgattgtggagcatacattaaaccaaaatggctaca
cactggttatcactgggaagaagatcacgaagatcccattgaatggcttgggctgcagacatttccagtcctgcagtcaatg
cctctctgccccacccctttgttcagtgtggctggtgccacgacaaatgtgtgcgatcggaggaatgcctgagcgggacatg
gactcaacagatctgtctgcctgcaatctacaaggttttcccaaatagtgcacccctgaaggagggacaaggctgaccata
tgtggctgggactttggatttcggaggaataataaatttgatttaaagaaaactagagttctccttggaaatgagagctgcacc
ttgactttaagtgagagcacgatgaatacattgaaatgcacagttggtcctgccatgaataagcatttcaatatgtccataattat
ttcaaatggccacgggacaacacaatacagtacattctcctatgtggatcctgtaataacaagtatttcgccgaaatacggtc
ctatggctggtggcactttacttactttaactggaaattacctaaacagtgggaattctagacacatttcaattggtggaaaaac
atgtactttaaaaagtgtgtcaaacagtattcttgaatgttataccccagcccaaaccatttcaactgagtttgctgttaaattgaa
aattgacttagccaaccgagagacaagcatcttcagttaccgtgaagatcccattgtctatgaaattcatccaaccaaatctttt
attagtggtgggagcacaataacaggtgttgggaaaaacctgaattcagttagtgtcccgagaatggtcataaatgtgcatg

aagcaggaaggaactttacagtggcatgtcaacatcgctctaattcagagataatctgttgtaccactccttccctgcaacag
ctgaatctgcaactccccctgaaaaccaaagccttttcatgttagatgggatcctttccaaatactttgatctcatttatgtacat
aatcctgtgtttaagccttttgaaaagccagtgatgatctcaatgggcaatgaaaatgtactggaaattaagggaaatgatatt
gaccctgaagcagttaaaggtgaagtgttaaaagttggaaataagagctgtgagaatatacacttacattctgaagccgtttta
tgcacggtccccaatgacctgctgaaattgaacagcgagctaaatatagagtggaagcaagcaatttcttcaaccgtccttg
gaaaagtaatagttcaaccagatcagaatttcacacaccatcatcaccatcactgattcgaa (SEQ ID NO: 3)

Example 2. Binding assay of antibody and antigen (ELISA)

This experiment uses enzyme linked immunosorbent assay (ELISA) to detect affinity of c-Met antibody to c-Met antigen *in vitro* (including supernatant of hybridoma or recombinantly expressed monoclonal antibodies).

Experimental procedures: Coating buffer (PBS; Hyclone, Cat No.: SH30256.01B) was used to dilute antigen (human c-Met-His, example 1) to 2μg/ml, which was added to 96-well plate with 100μl/well (Costar 9018, Cat No.:03113024) and incubated overnight at 4℃. The next day, the 96-well plate was restored to room temperature and was washed three times with washing buffer (PBS+0.05%Tween 20 (Sigma, Cat No.:P1379). Blocking buffer was added at 200μl/well (PBS+1%BSA (Roche, Cat No.:738328) and the plate was incubated at 37°C for 1 hours. The plate was then washed three times with washing buffer. Anti c-Met antibody to be tested was added to the 96-well plate and was incubated for 1 hour at room temperature. The plate was then washed three times with washing buffer. Secondary antibody (Goat anti-Mouse IgG(H+L)(HRP)(Thermo, No.:31432) diluted with blocking buffer (10000× dilution) was added to the 96-well plate at 100μl/well and the plate was incubated for 1 hour at room temperature. The plate was then washed three times and TMB chromogenic substrate (eBioscience REF:00-4201-56) was added to the 96-well plate at 100μl/well. Stop solution 2N H_2SO_4 was added to the 96-well plate at 100μl/well. Read the plate with plate reader at 450nm.

Example 3. Production of murine monoclonal antibody cell line of anti-human c-Met

This invention obtain murine anti-huamn c-Met monoclonal cell line by immunizing mice, fusion of spleen cell and screening of hybridoma. This method is well-known in this field. Recombinantly expressed antigen (human c-Met ECD-mFc, human c-Met Sema-flis, see example 1) was diluted to 1mg/ml with PBS (Hyclone, Cat No.:SH30256.01B) and emulsified with Freund's adjuvant (The first

immunization was performed with Freund's complete adjuvant, and the other booster immunizations were performed with Freund's incomplete adjuvant); and injected into Balb/C mice subcutaneously (5 mice/group) with each mouse inoculated 100μg antigen, booster immunizations were given every two weeks. Since the first booster immunization, mice serum was collected during 7 to 10 days after each booster immunization, and its titer was detected by ELISA (Methods were in Example 2).

After immunization, mice with serum titer higher than $1:10^5$ were selected for fusion. Mice B-cells and myeloma cells (SP2/0, ATCC number:CRL-1581^{TM}) were prepared respectively in aseptic condition and counted. The two kinds of cells were mixed in proportion with B-cells : SP2/0 of 1:4 and then were centrifuged (1500r/min,7min). Supernatant was discarded and 1ml of 50% polyethylene glycol (Supplier: SIGMA, Catalogue# RNBB306) was added. Next, 1ml serum-free RPMI1640 (Supplier: GIBCO, Catalogue#C22400) was used for termination and centrifuged for 10 minutes. Supernatant was then discarded. The pellet was resuspended in RPMI1640 which comprises hybridoma cell growth factor (Supplier: Roche, Catalogue# 1363735001), serum (Supplier: GIBCO, Catalogue#C20270) and HAT (Supplier: Invitrogen, Catalogue# 21060-017). B-cells were plated on the plate at 10^5/well and each well is 100μl. The plate was placed in cell incubator at 37℃. 3 days later, 100μl of RPMI1640 which comprises hybridoma cell growth factor, serum and HT (Supplier: Invitrogen, Catalogue# 11067-030) was added in each well. After 2 to 4 days, each well was replaced for 150μl RPMI1640 comprising hybridoma cell growth factor, serum and HT. Next day, positive clone was detected by ELISA (see Methods in Example 2). The results are shown in Table 1.

Table 1. Detection of hybridoma fusion in human c-Met immunized mice

Clone No.	Detection Results (OD450)
Negative control	0.07
Ab-1	1.48
Ab-2	1.38
Ab-3	1.29
Ab-4	1.6
Ab-5	1.64
Ab-6	1.75

Ab-7	1.58
Ab-8	1.24

Example4. Inhibitory effect of anti-human c-Met monoclonal antibody on proliferation of gastric cancer cell MKN45.

The clones above were selected and further cultured to obtain monoclone. After verification of binding activity by ELISA, monoclones were selected for cultivation, and the resulting supernatant was subjected to cell viability assay. According to the experiment principle, anti-human c-Met antibody of the invention is able to inhibit phosphorylation of c-Met expressed on the surface of human gastric cancer cell MKN45, thereby inhibit the proliferation of MNK45 cells.

Human gastric cancer cells (MKN45, JCRB, JCRB0254, P11) were added to a 96-well cell culture plate (costar, #3799) at 1 $\times 10^5$ cells/mL and 50 μl/well. The medium is RPMI1640 medium: (GIBCO, cat#11835) +10%FBS (GIBCO-10099141). Anti-human c-Met antibody to be tested was added at 50 μl/well and was cultured for 5 days in incubator at 37℃ (Manufacture: SANYO; Equipment No. TINC035). CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7573) was used, and the proliferation of cells were detected according to the instructions. The plate was read by PerkinElmer plate reader, TREA001-RDA-IBA100. The following formula was used to calculate the percentage proliferation of cells: % proliferation of cells = (1 - cell reads in experimental group/cell reads in untreated group) × 100%. The results are in Table 2.

Table 2. Anti-human c-Met mAb cell viability

Clone No.	MKN45 inhibition percentage (%)
Blank Control	0.01
Ab-1	59.2
Ab-2	58.4
Ab-3	59.6
Ab-4	54.9
Ab-5	77.4
Ab-6	70.8
Ab-7	56.4
Ab-8	53.8

Example 5. Anti c-Met antibody sequence cloning

The single cell line Ab-5 with good viability obtained from example 4 was selected for cDNA sequence cloning. Mab was recombinantly expressed and subjected to various activity tests. The variable regions of heavy chain and light chain of antibody gene were amplified by reverse transcription PCR, and ligated to vector to obtain heavy and light chain sequence of monoclonal antibody by sequencing. First of all, RNA purification kit (Qiagen company, No.74134, see the instructions for this procedure) was used to extract all cell RNAs of the active single cell line from example 4. Next, cDNA single chain was prepared by the cDNA synthesis kit (Invitrogen company, No.18080-051), which is Oligo-dT primers cDNA reverse transcription. The product was served as template, and the variable region sequence of the antibody heavy and light chain was synthesized by PCR method. The products of PCR were cloned to TA vector pMD-18T and then sequenced. Obtained heavy and light chain sequence of antibody were separately cloned to expression vectors (see example 1), and monoclonal antibody was recombinantly expressed to prove its activity (see example 2 and 4), followed by humanization.

Sequence of mice hybridoma cell monoclonal antibody Ab-5:

Heavy chain variable region:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLP**NYGVH**WVRQSPGKGLEWLG**VIW**
SGGSTNYAAAFVSRLRISKDNSKSQVFFEMNSLQADDTAVYYCAR**NHDNPY**
NYAMDYWGQGTTVTVSS (SEQ ID NO: 4)

Light chain variable region:

DIVLTQSPGSLAVYLGQRATISC**RANKSVSTSTYNYLH**WYQQKPGQPPKLLI
YLASNLASGVPARFSGSGSGTDFTLNIHPLEEEDAATYYC**QHSRDLPPT**FGA
GTKLELKR (SEQ ID NO: 5)

The amino acid residues of VH/VL CDR of anti-human c-Met antibodies are determined and annotated by the Kabat numbering system.

CDR sequences of mouse-origin in the invention are shown in Table 3:

Table 3. CDR sequence of Mouse-origin anti-sclerostin antibody

Antibody	Ab-5

Heavy chain CDR1	NYGVH (SEQ ID NO: 6)
Heavy chain CDR2	VIWSGGSTNYAAAFVS (SEQ ID NO: 7)
Heavy chain CDR3	NHDNPYNYAMDY (SEQ ID NO: 8)
Light chain CDR1	RANKSVSTSTYNYLH (SEQ ID NO: 9)
Light chain CDR2	LASNLAS (SEQ ID NO: 10)
Light chain CDR3	QHSRDLPPT (SEQ ID NO: 11)

Example 6. Humanization of anti c-Met antibody

The mouse-origin anti c-Met monoclonal antibody heavy and light chain sequence obtained from example 5 was aligned against antibody data base for homology, and a humanized antibody model was established then. Depending on the model, the optimal humanized c-Met monoclonal antibody was selected as the preferred molecule of the invention according to reversion mutation. Crystal structure showing similar homology with the obtained murine candidiate molecules was selected from the published database of mice Fab crystal structure model (e.g. PDB database), and Fab crystal structure with high resolution (such as, less than 2.5 Å) was selected; and mouse Fab model was established. The murine antibody heavy and light chain sequences of the invention were aligned against the sequences in model, the consistent sequence was maintained and then the structure model of mouse antibody of the invention could be obtained. The inconsistent amino acids might be potential sites for reverse mutation. Swiss-pdb viewer software was used to run the mouse antibody structure model to optimize energy (minimization). Reverse mutation was performed at different amino acid sites other than those in CDRs of the model, and the resultant humanized antibody was aligned against that before being humanized to detect the activity. Humanized antibody with good activity was maintained. And then, the CDR region was further optimized, including avoiding glycosylation, deamination, oxidation sites and so on. CDR region of the optimized humanized anti c-Met antibody is shown in table 4:

Table 4. CDR sequence of optimized anti c-Met antibody

Antibody	**Optimized humanized antibody**
Heavy Chain CDR1	NYGVH (SEQ ID NO: 6)
Heavy Chain CDR2	VIWSGGSTNYAAAFVS (SEQ ID NO: 7)

Heavy Chain CDR3	NHDNPYNYAMDY (SEQ ID NO: 8)
Light Chain CDR1	RADKSVSTSTYNYLH (SEQ ID NO: 12)
Light Chain CDR2	LASNLAS (SEQ ID NO: 10)
Light Chain CDR3	QHSRDLPPT (SEQ ID NO: 11)

Variable regions of humanized heavy and light chain sequences are shown below:

1. Heavy chain variable region

Ab-9

QVTLKESGPVLVKPTETLTLTCTVSGFSLP**NYGVH**WVRQPPGKALEWLA**VI**
WSGGSTNYAAAFVSRLRISKDTSKSQVVFTMNNMDPVDTATYYCAR**NHDN**
PYNYAMDYWGQGTTVTVSS (SEQ ID NO: 13)

Ab-10

QVQLVESGGGVVQPGRSLRLSCAASGFSLS**NYGVH**WVRQAPGKGLEWLA**VI**
WSGGSTNYAAAFVSRLTISKDNSKNTVYLQMNSLRAEDTAVYYCAR**NHDN**
PYNYAMDYWGQGTTVTVSS (SEQ ID NO: 14)

Ab-11

QVQLVESGGGVVQPGRSLRLSCAASGFTLP**NYGVH**WVRQAPGKGLEWLA**VI**
WSGGSTNYAAAFVSRLTISKDNSKNTVYLQMNSLRAEDTAVYYCAR**NHDN**
PYNYAMDYWGQGTTVTVSS (SEQ ID NO: 15)

2. Light chain variable regions

Ab-9

DIVLTQSPASLAVSPGQRATITC**RANKSVSTSTYNYLH**WYQQKPGQPPKLLIY
LASNLASGVPARFSGSGSGTDFTLTINPVEANDTANYYC**QHSRDLPPT**FGQG
TKLEIKR (SEQ ID NO: 16)

Ab-10

DIVLTQSPDSLAVSLGERATINC**RADKSVSTSTYNYLH**WYQQKPGQPPKLLIY
LASNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QHSRDLPPT**FGQG
TKLEIKR (SEQ ID NO: 17)

Ab-11

DIVLTQSPDSLAVSLGERATINC**RANKSVSTSTYNYLH**WYQQKPGQPPKLLIY
LASNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QHSRDLPPT**FGQG
TKLEIKR (SEQ ID NO: 18)

The humanized heavy and light chain sequences are recombined with IgG Fc regions to obtain the humanized anti c-Met monoclonal antibody of the invention. The

Fc sequence used was selected optionally from the following sequences:

Heavy chain constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK (SEQ ID NO: 19)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPA
PIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK (SEQ ID NO: 20)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPC
PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS
SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN
HYTQKSLSLSLGK (SEQ ID NO: 21)

Light chain constant region:

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C (SEQ ID NO: 22)

The above antibodies were cloned, expressed and purified by gene cloning and recombination expression, respectively. The humanized antibodies with best activity Ab-9, Ab-10, Ab-11 were finally selected through ELISA (Example 2) and *in vitro* binding activity assay (Example 7). The sequence is shown as below:

Ab-9 humanized antibody:

Heavy chain:

QVTLKESGPVLVKPTETLTLTCTVSGFSLPNYGVHWVRQPPGKALEWLA
VIWSGGSTNYAAAFVSRLRISKDTSKSQVVFTMNNMDPVDTATYYCARNHD
NPYNYAMDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNV
DHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH
QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 23)

Light chain:

DIVLTQSPASLAVSPGQRATITCRANKSVSTSTYNYLHWYQQKPGQPPKL
LIYLASNLASGVPARFSGSGSGTDFTLTINPVEANDTANYYCQHSRDLPPTFG
QGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO: 26)

Ab-10 humanized antibody:

Heavy chain:

QVQLVESGGGVVQPGRSLRLSCAASGFSLSNYGVHWVRQAPGKGLEWL
AVIWSGGSTNYAAAFVSRLTISKDNSKNTVYLQMNSLRAEDTAVYYCARNH
DNPYNYAMDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN
VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVV
HQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 24)

Light chain:

DIVLTQSPDSLAVSLGERATINCRADKSVSTSTYNYLHWYQQKPGQPPKL
LIYLASNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRDLPPTFGQ
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC (SEQ ID NO: 27)

Ab-11 humanized antibody:

Heavy chain:

QVQLVESGGGVVQPGRSLRLSCAASGFTLPNYGVHWVRQAPGKGLEWL
AVIWSGGSTNYAAAFVSRLTISKDNSKNTVYLQMNSLRAEDTAVYYCARNH
DNPYNYAMDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN
VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVV
HQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 25)

Light chain:

DIVLTQSPDSLAVSLGERATINCRANKSVSTSTYNYLHWYQQKPGQPPKL
LIYLASNLASGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSRDLPPTFGQ
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC (SEQ ID NO: 28)

Example 7. *In vitro* activity detection of binding of anti c-Met humanized antibody

The humanized antibody of the invention was detected for its *in vitro* activity by ELISA (Example 2), and also detected for its binding with cell line MKN45 showing c-Met over-expression, and for its affinity to c-Met antigen (BIACore detection). The results are shown in table 5 and table 6.

FACS method was used to detect the binding activity of c-Met humanized antibody with cell line MKN45 showing c-Met over-expression.

MKN45 cells (JCRB, Cat No.: JCRB0254) were resuspended in the RPMI1640 medium (GIBCO, Cat No.: 11835-030) which contains 10% (v/v) fetal calf serum (FBS GIBCO, Cat No.: 10099-141) and Penicillin/ Streptomycin Solution (GIBCO, Cat No.: 15070-063) to reach 10000,000 cells/mL. 2mL of resuspended MKN45 cells was added to 96-well microtitier plate (Corning, Cat No.: 3799) at 150000 cells/well, 8 concentrations of c-Met antibody (5 times gradient dilution, starting from 20μg/ml) were added to corresponding wells and the final volume was 100μl, the plate was incubated for 1 hour at 4°C. FACS buffer (PBS comprising 2.5% (v/v) FBS (Hyclone, Cat: SH30256.01B)) was added. It was centrifuged under 4°C at 1300rmp for 4 minutes, then the supernatant was discarded. This procedure was repeated for three times. 100μl of secondary antibodies (Fluorescence labeled goat-anti-mouse secondary antibodies with 1:200 dilution, Biolegend, Cat No. 405307; Fluorescence

labeled anti-human secondary antibody with 1:30 dilution, Biolegend, Cat No. 409304) were added to each well, and the plate was incubated for 1 hour at 4°C. FACS buffer was added and centrifuged under 4°C at 1300rpm for 4 minutes, and then the supernatants were discarded; this procedure was repeated for three times. 200μl FACS buffer was added to resuspend the cells, the sample was prepared and detected by flow cytometry (BD FACS Array).

The affinity of c-Met antibody to c-Met antigen Sema-His was detected by surface plasmon resonance (SPR) in the invention.

Anti-mouse IgG (GE Life Sciences catalog # BR-1008-38) or anti-human IgG (GE Life Sciences catalog # BR-1008-39) antibodies were respectively diluted to 30μg/ml and 50μg/ml by sodium acetate solution pH 5.0 (GE Healthcare, Cat#BR-1003-51). Amino coupling kit (GE Life Sciences, Cat#BR100050) was immobilized onto the test channels and control channels on CM5 chip (GE Life Sciences catalog # BR-1000-12), and the coupling level was set at 15000RU. Running buffere PBS (Hyclone, Cat#SH30256.01B) + 0.05% P20 (GE Life Sciences, Cat#BR-1000-54) were used to dilute c-Met antibody to 1.5μg/ml. Antigen Sema-His was diluted to 200nM with running buffer, and then diluted at 1:2 dilution with the same buffer until 0.78nM is reached. The diluted antibody passed through test channel for 1 minute at a speed of 30μl/min, and the antigen passed through the test channels and control channels for 3 minutes with the same speed. after 10 minutes of dissociation, the flow speed was adjusted to 10μl/min, and regeneration buffer passed through test channels and control channels for 3 minutes. Data was fitted by BiaEvaluation 4.1 after double deduction, and the fitting model is 1:1 Langmuir model.

Table5. Binding activity of humanized anti c-Met antibody

Humanized antibody	Ab-9	Ab-10	Ab-11
ELISA detection (EC_{50}, nM)	0.13	0.39	0.2

Table6. Binding activity of humanized anti c-Met antibody with MKN45; and affinity of humanized anti c-Met antibody to antigen

Humanized antibody	MKN45/FCAS Binding activity (nM)	affinity to antigen Biacore(nM)
Ab-9	1.6	4
Ab-10	1.23	8

The above results show that the binding activity of humanized antibody with antigen is within 0.13-8 nM, and the results may vary depending on the detection methods used. The results show that humanized anti c-Met antibody maintains

binding activity of parent antibody prior to humanization.

Example 8. *in vitro* function and cell viability evaluation of anti c-Met humanized antibody.

To detect the function of the antibody of the invention, a test of blocking the binding between c-Met ligand (hepatocyte growth factor, HGF) and c-Met, as well as inhibition test of cell proliferation (Example 4) were performed to evaluate antibodies in example 7.

Binding of HGF with c-Met results in tyrosine phosphorylation of c-Met molecules and activation of c-Met signaling pathway. The activity of anti c-Met antibody of the invention in blocking HGF from binding with the receptor c-Met protein (i.e. IC_{50}) is measured by ELISA.

c-Met ECD-mFc (Example 1) was diluted in PBS (Hyclone, Cat #SH30256.01B) with a final concentration of 2μg/ml, and then 96-well ELISA plate (Costar, cat#2592) was coated with c-Met ECD-mFc at room temperature overnight. Plate was washed with PBST (PBS+0.05% tween 20, Simga, Cat#P1379) for 3 times on plate washer (Suppler: BioTex; Model: ELX405; S/N: 251504). 300μl blocking solution PBS+1%BSA (Roche, Cat #738328) was added to 96-well plate and the plate was incubated for 60 minutes at 37°C. After the plate was washed with PBST for 3 times, 50 μl ofantibody diluted by blocking solution was added to 96-well plate and the plate was incubated for 90 minutes at 37°C. 50μl of human HGF (Sino Biological, #10463-HNAS) which has been diluted with blocking solution to a final concentration of 20ng/ml was added to the 96-well plate containing c-Met antibody, and the plate was incubated for 120 minutes at room temperature. After the plate was washed with PBST for 3 times, 100μl of biotin-labeled anti HGF antibody (R&D, Cat #BAF294) which has been diluted with blocking solution to a final concentration of 100ng/ml was added to the 96-well plate and incubated for 90 minutes. After the plate was washed with PBST for 3 times, 100μl of horseradish peroxidase (ebioscience, #18-4100-51) diluted with blocking solution was added to the 96-well plate and incubated for 30 minutes. After the plate was washed with PBST for 3 times, 100μl

substrate (ebioscience, cat#00-4201-56) was added to the plate and incubated for 10 minutes at room temperature. 100μl stop solution (2N H_2SO_4) was added and data was read by 450nM microplate reader (Supplier: Moleculer Devices; Model: MNR0643; Equip ID: TMRP001). Data analysis was performed by SoftMax Pro v5. The results are shown in table7.

Table 7. in vitro function and cell viability evaluation of anti c-Met humanized antibody

Humanized antibody	Activity of inhibition of HGF/c-Met binding (IC_{50}, nM)	Activity of inhibition of MKN45 cells proliferation	
		IC_{50} (nM)	Maximum inhibition rate (%)
Ab-9	1.42	0.52	33
Ab-10	1.50	0.55	30

The above results show that the humanized antibody of the invention not only retains the binding activity with antigen, but also block the binding between antigen and ligand. It also shows the inhibition of growth activity of cancer cells.

Example 9. Agonist activity evaluation of anti c-Met humanized antibody

Anti c-Met antibody blocks HGF/c-Met binding, and may activate c-Met signal. This means that c-Met antibody has agonist activity. The agonist activity of anti c-Met is not desired in the invention. To detect whether the antibody of the invention has agonist activity, three experiments were performed including c-Met phophorylation, proliferation of metastatic human clear renal cell carcinoma (caki-1), and human lung cancer H441 cell migration. The detection and evaluation have done.

The binding of HGF to c-Met activates tyrosine phosphorylation of c-Met molecule and c-Met signaling pathway. Therefore, the activation of c-Met by HGF was used as a positive control of agonist experiment, and human lung cancer cell line A459 was used to evaluate the induced phophorylation of c-Met tyrosine residue 1349.

A549 cells were suspended in solution containing Ham's F12K, 2mM glutamine (Invitrogen, #21127-022), and 10% (v/v) FBS (GIBCO, #10099141). 0.2mL cell suspension was taken and added to a 96-well plate (Corning, # 3599), and the cell

concentration is 60000 cells/well. The plate was incubated for 24 hours under 5% CO_2 at 37°C . After 24 hours, the medium in 96-well plate was discarded, and 100μl low serum medium (Ham's F12K+ 2mM glutamine + 0.5% FBS) was added. The cells were starved for 6 hours under 5% CO_2 at 37°C . Antibodies were diluted by using the above low serum medium to a final concentration of 20μg/ml. HGF concentration in positive control is 200ng/ml, and was incubated for 15 minutes at 37°C. After the medium was discarded, 50μl cell lysis solution (10 mM Tris, 150 mM NaCl, 2 mM EDTA), 50nM NaF, 1% (v/v) TRITON-X 100, protease inhibitors (Roche cat # 05892791001), phosphatase inhibitors cocktail II (Sigma #P5726) and phosphatase inhibitors cocktail III (Sigma #P0044) were added into the plate. After cell lysis, c-Met tyrosine phosphorylation was detected by ELISA. c-Met capture antibody (CST, cat#3148s) was diluted by PBS at 1:1000 dilution, and then added to 96-well ELISA plate (costar, cat#9018) At 100μl per well. The plate was incubated at 4°C overnight. The plate was washed for 3 times with TBS-T and 300μl blocking solution (TBS-T plus 2% (w/v) BSA) was added, the plate was incubated for 1 hour. The plate was washed for 3 times with TBS-T. 75μl cell blocking solution and 25 cell lysate were added and the plate was incubated at 4°C overnight. The plate was washed for 3 times with TBS-T, and pY1349 c-Met antibody (cell signal, #3133) was diluted with blocking solution at 1:1000 dilution, 100μl per well. After 2-hour of incubation at room temperature, the plate was washed for 4 times with TBST, and the HRP labeled goat anti rabbit polyclonal antibody (cell signaling, cat#7074) was diluted with blocking solution at 1:12000. 100μl per well was incubated for 1 hour at room temperature. The plate was washed with TBS-T for 5 times and 100μl of TMB (ebioscience#TMB, 004201) was added to each well, then 100μl stop solution (2N H_2SO_4) was added. Data was read by 450nM microplate reader (Supplier: Moleculer Devices; Model: MNR0643; Equip ID: TMRP001). SoftMax Pro v5 was used for data analysis. The results are shown in table 8.

Caki-1 cells express hepatocyte growth factor receptor c-Met, and HGF can bind c-Met to stimulate Caki-1 cells proliferation. Therefore, agonist activity of anti c-Met antibody could be evaluated, since both ofthe humanized anti c-Met antibody of the invention and HGF stimulate Caki-1 cells.

Caki-1 cells (Shanghai Branch of Chinese Academy of Science, TCHu135, P12) were added to 96-well culture plate (costar, #3799) at 1000/well. The medium was McCoy's 5A (invitrogen, #16600) + 10%FBS (GIBCO-10099141), and the condition

was at 37°C for 24 hours. Afterwards, cells were starved for 24 hours (The medium for cell starvation is McCoy's 5A and 0.5% FBS). After starvation, cells were treated with gradient diluted anti c-Met antibodies (The highest concentration is 20μg/ml) and positive controls for 5 days, and cell proliferation was detected by cell proliferation assay kit (CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7573). The plate was read by plate reader (Manufacture: PerkinElmer device No.: TREA001-RDA-IBA100). Percentage proliferation of the cell was calculated: proliferation % = reads obtained from cells in test group/ reads obtained from cells in untreatedgroup × 100%. The results show that this antibody has no effect on proliferation of Caki-1 cells; data are shown in table 8.

If anti c-Met antibody have agonist activity, migration ability of cells could be affected. H441 cell line expressing c-Met is used in the invention to evaluate the ability of c-Met antibody to affect cell migration.

H441 cells (ATCC, Cat No.: HTB-174) were resuspended to 500000cells/ml in RPMI 1640 medium (GIBCO, Cat No.: 11835-030) which contains 10% (v/v) FBS (GIBCO, Cat No.: 10099-141) and penicillin-streptomycin (GIBCO, Cat No.: 15070-063). The resuspended H441 cells were added to a 12-well culture plate (Costar, Cat No.3513) at 1ml/well, and were cultured for 3 days at 37°C in 5% CO_2. Cells were washed twice with PBS and was added into RPMI 1640 medium containing 0.5% FBS, and were cultured for 16 hours at 37°C in 5% CO_2. Scratch the bottom of each well with 5ml tips and wash the plate once with medium containing low concentration FBS, and then add 1ml medium RPMI 1640 containing low concentration of serum. Mark the plate and take photos for randomly selected scratch areas under inverted microscope at 4×magnification. This time was set as starting point. Cells were treated with 10μg/ml c-Met antibody or HGF control (200ng/ml) for 16 hours at 37°C in 5% CO_2. After that, take photos for the marked scratch areas under inverted microscope at 4×magnification, and this time was set as the time after migration. The percentage of cell migration is measured as the migration distance relative to starting point divided by migration distance of the test group relative to starting point, and then multiplied by 100. The results are shown in table 8.

Table 8. Evaluation of agonist activity of humanized anti c-Met antibody

Humanized antibody	Activation of c-Met phosphorylation (%)*	Stimulation of proliferation of Caki-1	H441 migration (%) #
Ab-9	29.9	none	53
Ab-10	33.7	none	34

*: Antibody concentration was 20μg/ml, the activation of c-Met phosphorylation by HGF (200ng/ml) was set as 100%. #: Antibody concentration was 20μg/ml, H441 migration% affected by HGF (200ng/ml) was set as 100%.

From the above results, the humanized antibody shows low (c-Met phosphorylation, H441 migration experiment results) or none (No stimulation of Caki-1 proliferation was observed at 20μg/ml of antibody) agonist activity.

Example 10. Pharmacological evaluation of anti c-Met antibody *in vivo*

To evaluate antitumor activity of the antibody, human gastric cancer MKN45 cell model obtained by subcutaneous xenograft in BALB/c nude mouse is used for detection.

MKN45 cells were monolayer cultured on RPMI-1640 medium (10% FBS), and the culture environment was at 37°C in 5% CO_2. Count and collect cells during the logarithmic phase. Cells were resuspended in PBS to proper concentration, and 0.1ml 3×10^6 cells were subcutaneously inoculated in mouse right wing (BALB/c nude mouse, female, 10-week old, 22-28g, from Shanghai SLAC experimental animal ltd. Licence No. 2007000548777; Environment: SPF grade). When the tumor's average volume reached 114 mm^3, weight was weighted and tumor's volume was measured, and then mice were separated into groups and administrated. Control group was treated with PBS, antibody therapy group was treated with 5mg/kg antibody of the invention and the frequency is once a week and twice per time. Tumor's volume and weight were measured twice a week and terminate the experiment at day 25. The formula of calculating tumor size: tumor volume (mm^3) = 0.5× (tumor length × tumor diameter^2). The formula of calculating inhibition rate: inhibition rate = (V0-VT)/V0 × 100%, and V_0, V_T is respectively the volume of tumor at the beginning and the end of the experiment.

The results shows that the inhibition rate of antibody Ab-9, Ab-10 are 56% and 64%, respectively. There was no obvious change on mice weight during the experiment (22-24g). This results show that the humanized anti c-Met antibody could inhibit the growth of tumor in vivo.

Example 11. Endocytosis of anti c-Met antibody

The antibody of the invention could bind to human c-Met, and has very good *in vitro* activity and inhibits tumor activity *in vivo*. Besides, the antibody does not have or has very weak agonist activity. In order to detect whether the antibody could be uptaken into the cell along with human c-Met once bound to human c-Met, human gastric cancer cell MKN45 (JCRB, Cat No.: JCRB0254) expressing c-Met was used for evaluation.

MKN45 cells were resuspended to 10000,000 cells/mL in RPMI 1640 medium (GIBCO, Cat No.: 11835-030), which contains 10%(v/v) FBS (GIBCO, Cat No.: 10099-141) and penicillin-streptomycin (GIBCO, Cat No.: 15070-063). 2mL resuspended MKN45 cells were added to 96-well microtiter plate at 250,000 cells/well, and 10μg/ml of c-Met antibody was added to corresponding wells and the final volume is 100μl, the plate was incubated at 4°C for 1 hour. FACS buffer (phosphate buffer solution including 2.5% fetal bovine serum; Hyclone, Cat: SH30256.01B) was added and centrifuged at 4°C, 1300rpm for 4 minutes. The supernatant was discarded and this procedure was repeated three times. 100μl secondary antibody solution (Fluorescence labeled goat anti mouse secondary antibodies at 1:200 dilution, Biolegend, Cat No. 405307; Fluorescence labeled anti-human secondary antibody at 1:30 dilution, Biolegend, Cat No. 409304) was added into each well. the plate was incubated at 4°C for 1 hour. FACS buffer was added and centrifuged at 4°C, 1300rpm for 4 minutes. The supernatant was discarded and this procedure was repeated three times. Complete cell culture medium (RPMI 1640 medium with 10% FBS) was added and was incubated at 37°C in 5%CO_2 for 0, 0.5, 1, 2, 4 hours. 5μl 7-AAD (Biolegend, Cat:420403) was added to100μl FACS buffer each well, and was incubated at 4°C for 30 minutes. FACS buffer was added and was centrifuged at 4°C, 1300rpm for 4 minutes. The supernatant was discarded and this procedure was repeated three times. 200μl Stripping buffer (0.05 M glycine, pH 3.0; 0.1 M NaCl, mixed in accordance with 1:1 (v/v)) was added to each well. The cells were resuspended and were incubated for 7 minutes at room temperature. The cells was centrifuged at room temperature at 1300rpm for 4 minutes, and the supernatant was discarded. 200μl neutralizing wash buffer (0.15M trihydroxymethyl aminomethane, pH 7.4) was added to each well, The cells were resuspended and were centrifuged at room temperature at 1300rpm for 4 minutes, and the supernatant was discarded. 200μl FACS buffer was added and cells were resuspended. Samples were prepared and detected by flow cytometry (BD FACS Calibur). The results are shown

in table 9.

Endocytosis of c-Met antibody %= (intensity of fluorescence at various time points – average intensity of fluorescence at time 0)/average intensity of fluorescence at time 0.

Table 9. Evaluation of cell endocytosis of humanized anti c-Met antibody (endocytosis%)

Humanized antibody	0h	0.5h	1h	2h	4h
hIgG (control)*	0	-0.9	-4.4	-4.9	3.6
Ab-9	0	26	32	32	31
Ab-10	0	24	38	53	59

*control group: Experimental error is from 4.9% to 3.6%, and it is considered as no endocytosis.

The table 9 shows that the antibody of the invention has good endocytosis without agonist activity. Once bound with target cells, both antibodies and receptors would be rapidly untaken into target cells, and maximum value was reached within 2-4 hours.

Example 12. Analysis of biophysical stability of anti c-Met antibody

To evaluate the biophysical stability of the antibody of the invention, such as the presence of glycosylation and deamination and stability, LC-MS analysis was used to evaluate the anti c-Met antibody.

Molecular weight of heavy and light chain was detected by LC-MS for the analysis of glycosylation. Deamination was analyzed by LC-MS at 4°C for a long time (at least 3 months), or 40°C for 21 days under accelerated condition. Samples treated with different conditions were taken and diluted to 2mg/ml with pH7.2 Tris-HCl, and added into 10mM TCEP and 6M urea (AMRESCO, Cat# $0378)_3$, then the samples were incubated for 20 minutes at 7°C. IAA(Sigma-Aldrich, Cat#I1149) with a final concentration of 20mM was added and was incubated for 15 minutes in darkness to protect sulfhydryl group. pH was adjusted by pH7.2 Tris-HCl, and protease (Sigma-Aldrich, Cat# T6567) was added at a proportion by weight of 10:1 (protein: enzyme). The samples were incubated at 37°C for 25 minutes, and then formic acid with a final concentration of 0.1% (Fluca, Cat#94318) was added to terminate the reactions. Samples were centrifuged and analyzed by LC-MS.

BiopharmaLynx was used to analyze the presence of deamination. Extracted Ion Chromatogram (EIC) diagram was obtained from LC-MS data by searching native peptide comprising deamination site and modified product, and then extracting parent ion. Peak area was obtained by integration, and the percentage of deamination and oxidation were calculated. The results are shown in Table 10.

Table10. Evaluation of physical stability of the humanized anti c-Met antibody of the invention

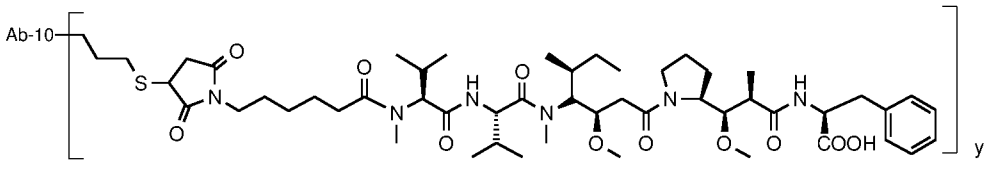
The antibody of the invention	Molecular weight of light chain*		Analysis of deamination#	
	Detected value	Estimated value	4°C, 3.5 months	40°C, 21days
Ab-9	25940	23907	0.66	
Ab-10	23828	23832		0.3

*: Heavy chains all involve glycosylation, and molecular weight was as expected. #: Proportion of deamination molecules (%). 0.66-1.0% is within the background of detection.

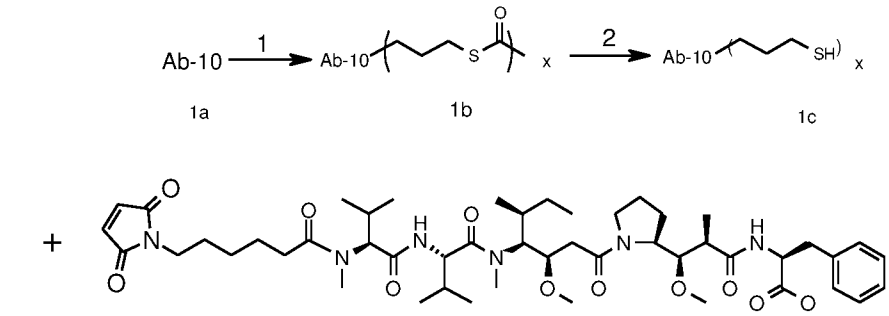
The above results shows that the antibody of the invention is stable and has good physical properties.

Example 13. anti-c-Met antibody Ab-10 conjugated toxin MC-MMAF

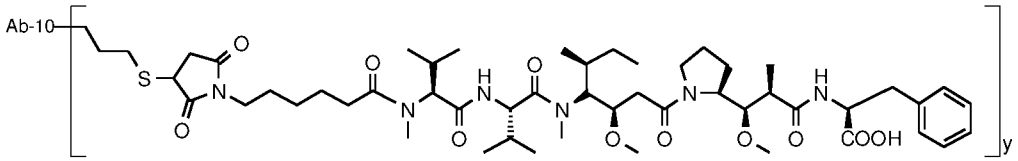
The anti-c-Met antibodies of the present invention have inhibitory activity of receptor binding, without agonist activity, show activity of endocytosis in targeted cells as well as physical stability. These properties make the antibodies of the invention particularly suitable for the preparation of ADC drugs when conjugated to toxins for the treatment of c -Met expressing cancer. The coupling process is shown below:



ADC-1



MC-MMAF



ADC-1

Step1. Thioacetic acid S-(3-carbonyl propyl) ester (0.7 mg, 5.3μmol) was dissolved in 0.9mL acetonitrile solution. The acetonitrile solution of thio acetic acid S-(3-carbonyl propyl) ester prepared above was added into acetic acid/sodium acetate buffer pH 4.3 (10.35mg/ml, 9.0ml, 0.97mmol) containing Ab-10 monoclonal antibody, and 1.0mL sodium borohydride aqueous solution (14.1mg, 224μmol) was added with shaking for 2 hours at 25°C. At the end of reaction, desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution pH 6.5), product **1b** solution was collected and was concentrated to 10mg/ml directly for the next reaction.

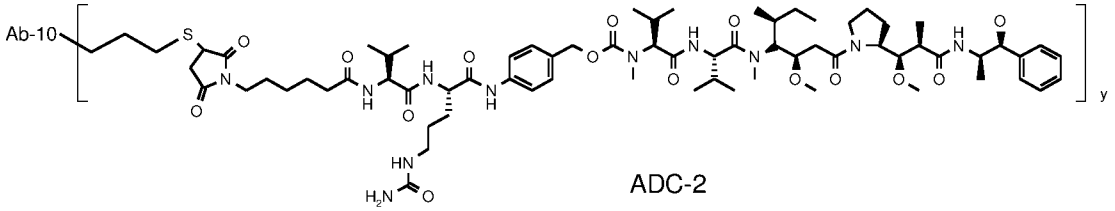
Step 2. 0.35ml 2.0M of hydroxylamine hydrochloride solution was added into 11.0mL of 1b solution with shaking for 30 minutes at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution pH 6.5), the captioned product Ab-10 monoclonal antibody-propyl mercaptan **1c** solution was collected (6.17mg/ml, 14.7mL).

Step 3. MC-MMAF (1.1mg,. 1.2μmol; prepared by method published in PCT patent WO2005081711) was dissolved in 0.3ml acetonitrile and was added in 3.0ml of Ab-10 monoclonal antibody-propyl mercaptan 1c solution (6.17mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution pH 6.5). The PBS buffer solution of captioned product ADC-1 (3.7mg/ml, 4.7ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 148119.2 (M_{Ab}+0D), 149278.1 (M_{Ab}+1D), 150308.1 (M_{Ab}+2D), 151314.1 (M_{Ab}+3D). The toxin: antibody ratio (DAR) was

calculated by analysis and the average value is y=1.7.

Example 14. Anti-c-Met antibody Ab-10 conjugated toxin MC-VC-PAB-MMAE

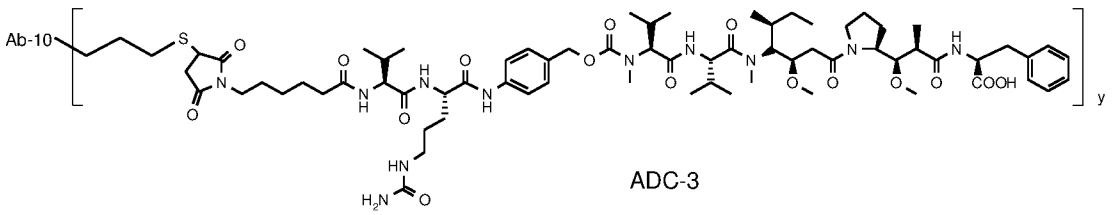


ADC-2

MC-VC-PAB-MMAE (1.6mg,. 1.2μmol; prepared as method disclosed in PCT patent WO2004010957) was dissolved in 0.3ml acetonitrile and was added into 3.0ml of Ab-10 monoclonal antibody-propyl mercaptan 1c solution (6.17mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-2 (3.6mg/ml, 4.8ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak:148118.4 (M_{Ab}+0D), 149509.2 (M_{Ab}+1D), 150903.1 (M_{Ab}+2D), 152290.4 (M_{Ab}+3D), 153680.7 (M_{Ab}+4D). The toxin: antibody ratio (DAR) was calculated by analysis and the average value is y=1.8.

Example 15. Anti-c-Met antibody Ab-10 conjugated toxin MC-VC-PAB-MMAF

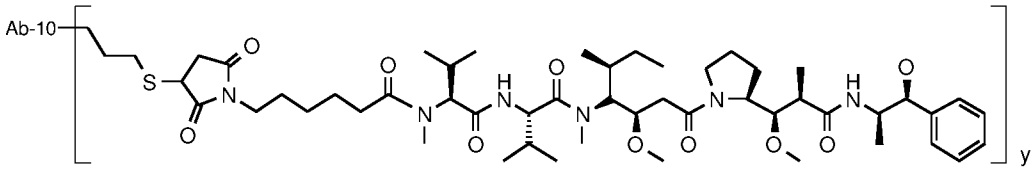


ADC-3

MC-VC-PAB-MMAF (1.6mg,. 1.2μmol; prepared as method disclosed in PCT patent WO2005081711) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-10 monoclonal antibody-propyl mercaptan 1c solution(6.17mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-3 (3.5mg/ml, 4.9ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 148119.1 (M_{Ab}+0D), 149525.3 (M_{Ab}+1D), 150930.7 (M_{Ab}+2D), 152335.2 (M_{Ab}+3D), 153739.8 (M_{Ab}+4D). The toxin: antibody ratio (DAR) was calculated by analysis and the average value is y=1.6.

Example16. Anti-c-Met antibody Ab-10 conjugated toxin MC-MMAE

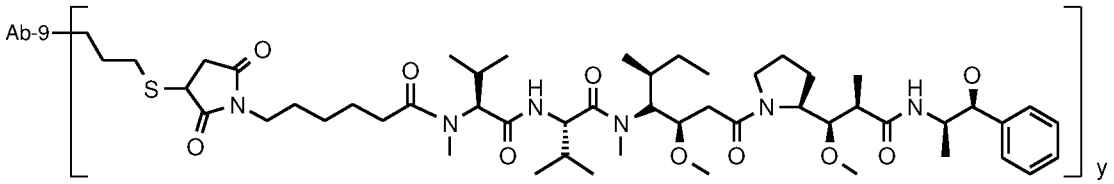


ADC-4

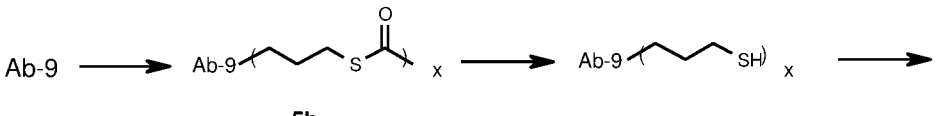
MC-MMAE (1.2mg, 1.2μmol; prepared as method disclosed in patent application US7/750/116B1) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-10 monoclonal antibody-propyl mercaptan 1c solution (6.17mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-4 (3.4mg/ml, 5.0 ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 148118.6(M_{Ab}+0D), 149104.3(M_{Ab}+1D), 150090.1(M_{Ab}+2D), 151075.8(M_{Ab}+3D). The toxin: antibody ratio (DAR) was calculated by analysis and the average value is y=1.6.

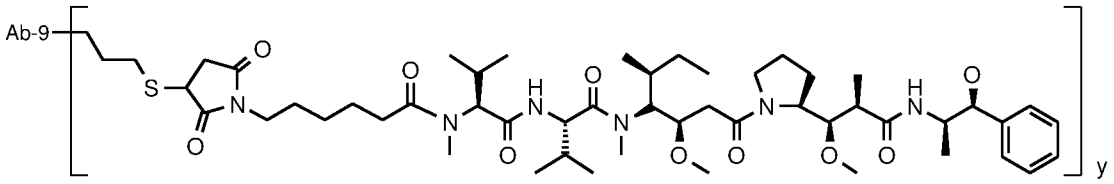
Example17. Anti-c-Met antibody Ab-9 conjugated toxin MC-MMAE



ADC-5



5b **5c**



ADC-5

Step1. Thioacetic acid S-(3-carbonyl propyl) ester (0.7 mg, 5.3μmol) was dissolved in 0.9mL acetonitrile solution. The acetonitrile solution of thio acetic acid S-(3-carbonyl propyl) ester prepared above was added into acetic acid/sodium acetate

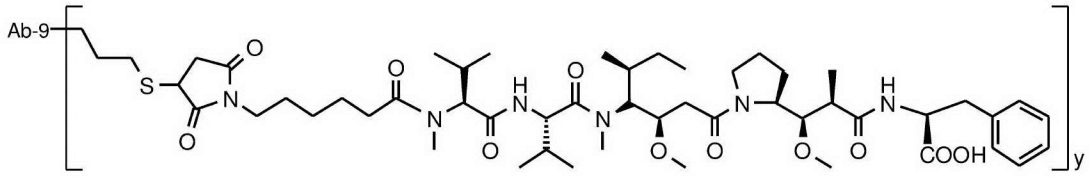
buffer (10.85mg/ml, 9.0ml, 0.976mmol) containing Ab-9 monoclonal antibody, and 1.0mL sodium borohydride aqueous solution (14.1mg, 224μmol) was added with shaking for 2 hours at 25℃. At the end of reaction, desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5), and the product 5b solution was collected and concentrated to 10mg/ml directly for the next reaction.

Step 2. 0.35ml 2.0M of hydroxylamine hydrochloride solution was added into 11.0mL of 5b solution with shaking for 30 minutes at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5), the captioned product Ab-9 monoclonal antibody-propyl mercaptan 5c solution was collected (6.2mg/ml, 15.0mL).

Step3. MC-MMAE (1.1mg,. 1.2μmol) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-9 monoclonal antibody-propyl mercaptan 5c solution (6.2mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-5 (3.8 mg/ml, 4.6 ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 150530.9(M_{Ab}+0D), 151915.7(M_{Ab}+1D), 153333.6(M_{Ab}+2D), 154763.4(M_{Ab}+3D), 156271.9(M_{Ab}+4D). The toxin: antibody ratio (DAR) was calculated by analysis and the average value is y=1.5.

Example 18. Anti-c-Met antibody Ab-9 conjugated toxin MC-MMAF



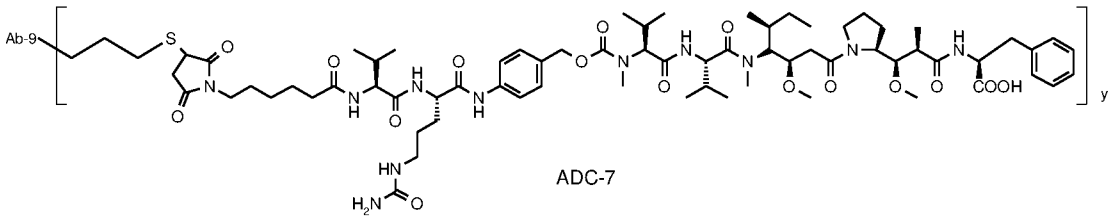
ADC-6

MC-MMAF (1.1mg,. 1.2μmol) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-9 monoclonal antibody-propyl mercaptan 5c solution(6.17mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-6 (3.8mg/ml, 4.6ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 150537.8(M_{Ab}+0D), 152087.9(M_{Ab}+1D),

153486.5(M_{Ab}+2D), 154911.7(M_{Ab}+3D), 156499.9(M_{Ab}+4D). The toxin: antibody ratio (DAR) was calculated by analysis and the average value is y=1.7.

Example19. Anti-c-Met antibody Ab-9 conjugated toxin MC-VC-PAB-MMAF

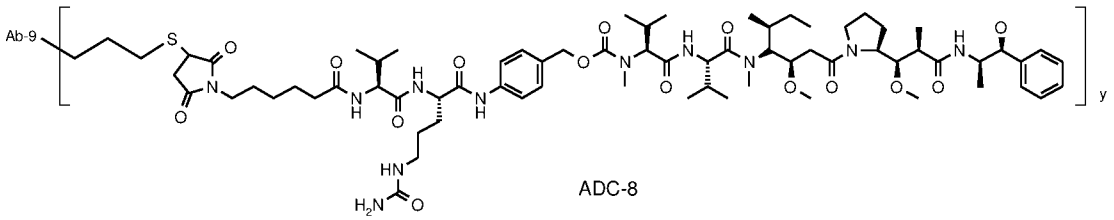


ADC-7

MC-VC-PAB-MMAF (1.6mg,. 1.2μmol) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-9 monoclonal antibody-propyl mercaptan 5c solution (6.2mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-7 (3.8mg/ml, 4.6ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 150537.8(M_{Ab}+0D), 152087.9(M_{Ab}+1D), 153486.5(M_{Ab}+2D), 154911.7(M_{Ab}+3D), 156499.9(M_{Ab}+4D). The toxin: antibody ratio (DAR) was gained by analysis and the average value is y=1.8.

Example20. Anti-c-Met antibody Ab-9 conjugated toxin MC-VC-PAB-MMAE

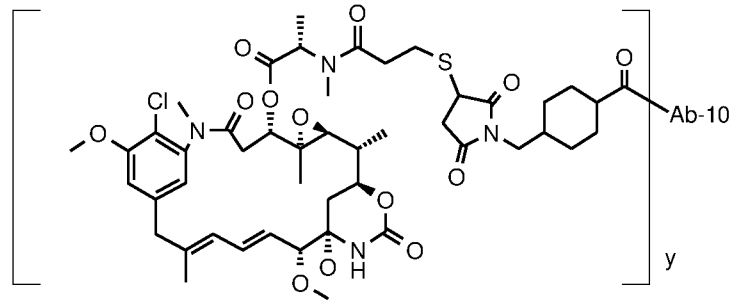


ADC-8

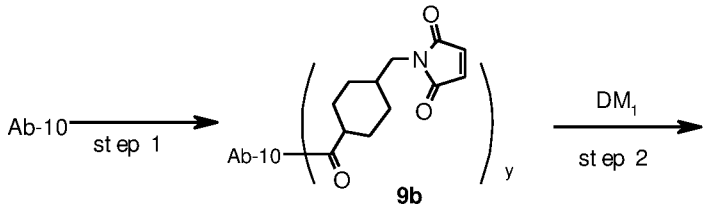
MC-VC-PAB-MMAE (1.6mg,. 1.2μmol) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-9 monoclonal antibody-propyl mercaptan 5c solution (6.2mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-8 (3.8mg/ml, 4.6ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 150508.6(M_{Ab}+0D), 151903.6(M_{Ab}+1D), 153314.5(M_{Ab}+2D), 154747.8(M_{Ab}+3D), 156039.5M_{Ab}+4D). The toxin: antibody ratio (DAR) was gained by analysis and the average value is y=1.6.

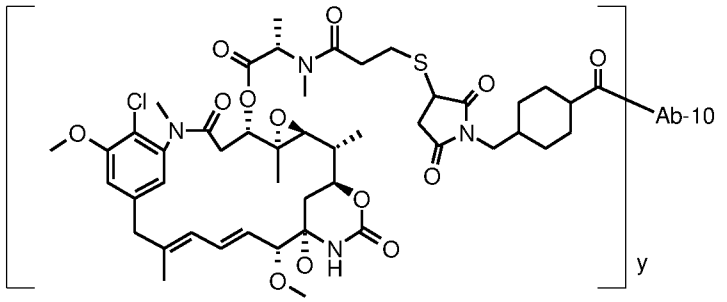
Example21. Anti-c-Met antibody Ab-10 conjugated toxin SMCC-DMI



ADC-9



9b



ADC-9

Step1

SMCC (Succinimidyl 4-(N-Maleimidomethyl)cyclohexane-1- carboxylate; 1.65mg, 4.94μmol, perchased from Shanghai Hanhong Biochemical Company, Batch No. BH-4857-111203) was dissolved in 0.9mL acetonitrile solution. The aceonitrile solution of SMCC prepared above was added into pH 6.5 PBS buffer (10.15mg/ml, 9.0mL, 0.62μmol,) containing Ab-10 monoclonal antibody with shaking for 2 hours at 25°C. After the reaction, desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5), and the product 9b solution was collected and was concentrated to 10mg/ml (8.3mg/ml, 11ml) for next reaction.

Step2

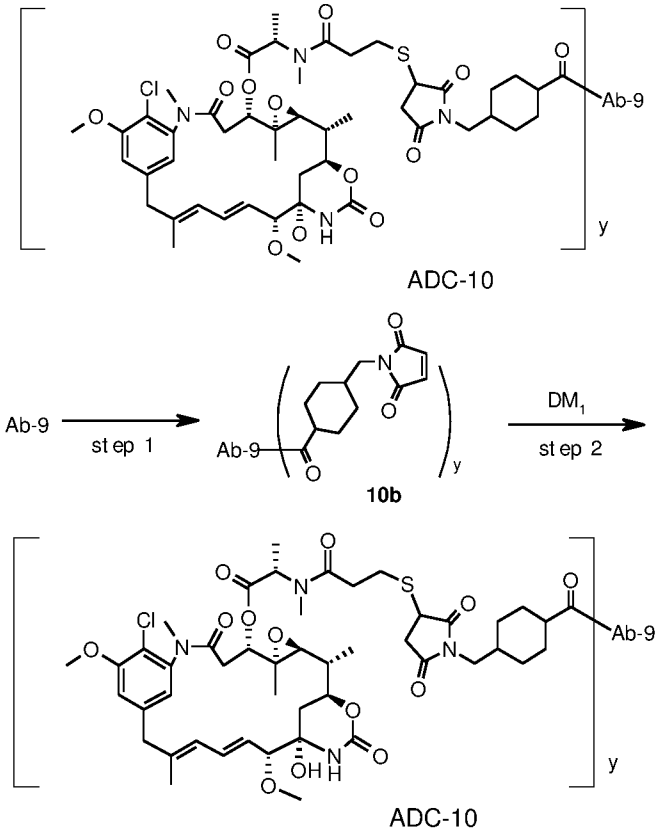
Ethanol solution of 3.0 mg L-DM1 (3.0 mg L-DM1/1.1 ml ethanol) (prepared by known methods publish ed in *Journal of Medicinal Chemistry. 2006, 49, 4392-4408*) was added to 9b solution (11.0ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase:

0.05M of PBS solution which pH is 6.5), the product ADC-9 solution was collected (6.3mg/ml, 14.0mL) and stored at 4°C.

Q-TOF LC/MS: characteristic peak: 148119.6(M_{Ab}+0D), 149078.1(M_{Ab}+1D), 149836.4 (M_{Ab}+2D), 150593.7(M_{Ab}+3D), 151552.5(M_{Ab}+4D).

The average value is y=2.3.

Example22. Anti-c-Met antibody Ab-9 conjugated toxin SMCC-DM1



Step1

SMCC (Succinimidyl 4-(N-Maleimidomethyl)cyclohexane-1-carboxylate; 1.65mg, 4.94μmol) was dissolved in 0.9mL acetonitrile solution. The aceonitrile solution of SMCC prepared above was added into pH 6.5 PBS buffer (10.15mg/ml, 9.0mL, 0.62μmol,) containing Ab-9 monoclonal antibody with shaking for 2 hours at 25°C. After the reaction, desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5), and the product 10b solution was collected and was concentrated to 10mg/ml (8.3mg/ml, 11ml) for next reaction.

Step2

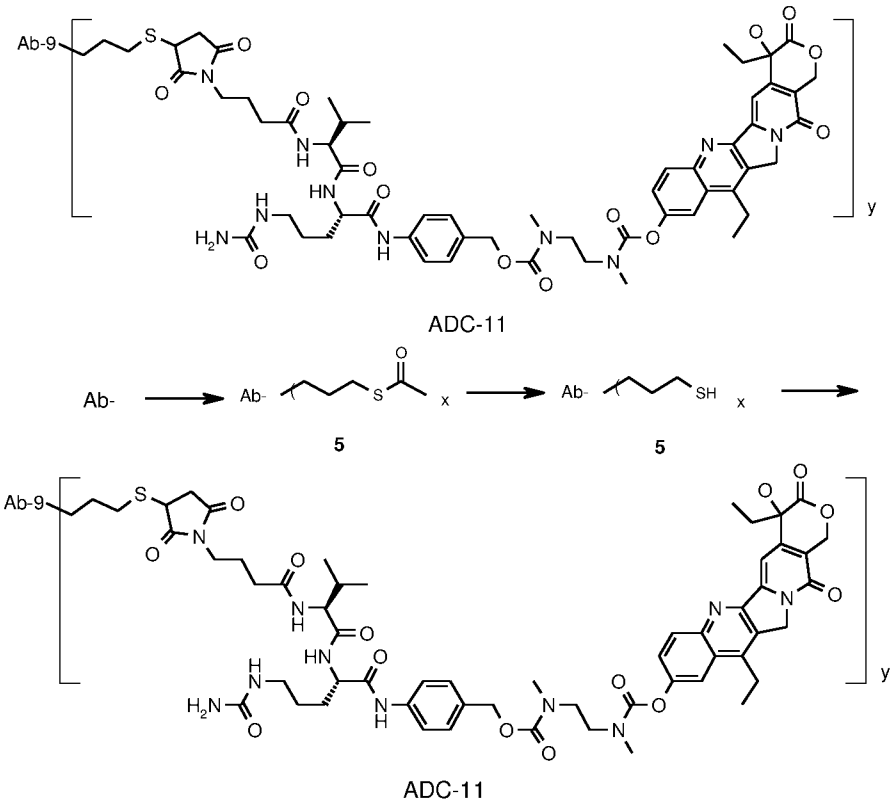
Ethanol solution of 3.0 mg L-DM1 (3.0 mg L-DM1/1.1 ml ethanol) was added to 9b solution (11.0ml) with shaking for 4 hours at 25°C, and then desalination and

purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5), the product ADC-10 solution was collected (6.3mg/ml, 14.0mL) and stored at 4°C.

Q-TOF LC/MS: characteristic peak: 150534.2(M_{Ab}+0D), 151492.6(M_{Ab}+1D), 152451.7(M_{Ab}+2D), 153409.7(M_{Ab}+3D), 154368.1(M_{Ab}+4D).

The average value is y=2.2.

Example23. Anti-c-Met antibody Ab-9 conjugated toxin SN-38



MC-VC-PAB-SN-38 (1.3mg,. 1.2μmol) was dissolved in 0.3ml acetonitrile and was added in 3.0ml Ab-9 monoclonal antibody-propyl mercaptan 5c solution (6.2mg/ml) with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-11 (3.7mg/ml, 4.5ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

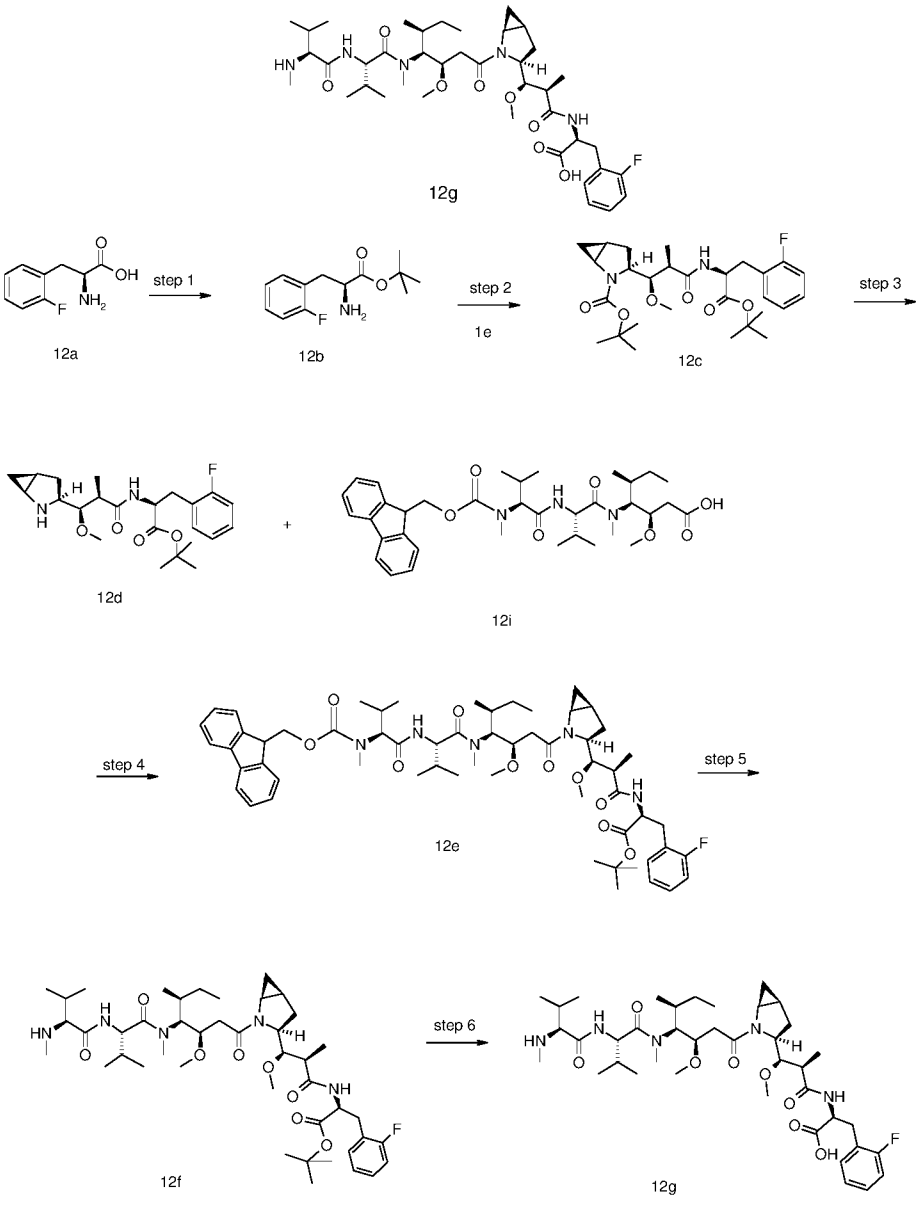
Q-TOF LC/MS: characteristic peak: 150537.1(M_{Ab}+0D), 151786.6(M_{Ab}+1D), 152948.6(M_{Ab}+2D), 154161.7(M_{Ab}+3D), 155365.9(M_{Ab}+4D), 156477.8(M_{Ab}+5D).

The average value is y=2.6.

Example24. Anti-c-Met antibody Ab-10 conjugated toxin

1. Preparation of toxin

(*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2-((3*R*,4*S*,5*S*)-4-((*S*)-*N*,3-dimethyl-2-((*S*)-3-methyl-2-(methyl amino) butyramide) butyramide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl)propionic acid



Step1. (*S*)-tert-butyl-2-amino-3-(2-fluorophenyl) propanoic acid

(*S*)-2-amino-3-(2-fluorophenyl)propanoic acid **12a** (400 mg, 2.18 mmol, prepared according to the known method in "*Advanced Synthesis & Catalysis*, 2012, 354(17), 3327-3332") was dissolved in 10mL of tert-butyl acetate. Perchloric acid

(300 mg(70%), 3.3 mmol) was added and stirred at room temperature for 16 hours. 6ml of water was added after reaction, and solution was separated. The organic phase was washed with saturated sodium bicarbonate solution (5ml). The aqueous phase was adjusted to pH=8 with saturated sodium bicarbonate solution, and then was extracted with dichloromethane (5ml ×3), and combined with the organic phase. The reaction mixture was then washed successively with water (3ml) and saturated sodium chloride solution (5ml), dried with anhydrous sodium sulfate, filtered; the filtrate was concentrated under reduced pressure. The crude product (S)-tert-butyl 2-amino-3-(2-fluorophenyl) propanoic acid **12b** was obtained (390mg, yellow, oily) which was subjected to the next reaction directly without purification.

Step2.

(1S,3S,5S)-tert-butyl 3-((1R,2R)-3(((S)-1-(t-butoxy)-3-(2-fluorophenyl)-1-carbonylpropyl-2-)amino)-1-methoxy-2-methyl-3-carbonyl propyl)-2-azabicyclo [3.1.0] hexane-2-carboxylic acid

(2R,3R)-3-((1S,3S,5S)-2-(tert-butoxycarbonyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methyl propionate **12e** (100mg, 0.334mmol) was dissolved in 6ml the mixture of dichloromethane and dimethylformamide (V/V=5:1), and then crude product (S)-tert-butyl 2- amino-3-(2-fluorophenyl) propionate **12b** (80mg, 0.334mmol) was added. *N,N*-diisopropylethylamine (0.29ml, 1.67mmol) and 2-(7-Azabenzotriazol)-N,N,N',N'-tetramethyluronium hexafluorophosphate (152.3mg, 0.40mmol) were added into that mixture. The mixture was stirred for 1 hour under argon atmosphere at room temperature. After reaction, 10ml water was added and stirred. Layer of dichloromethane was washed by saturated sodium chloride solution (10 ml), and dried with anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residues were purified by silica gel column chromatography using eluent system B to obtain the captioned product of (1S,3S,5S)-tert-butyl-3-((1R,2R)-3-(((S)-1-(t-butoxy)-3(2-fluorophenyl)-1-carbonylpropyl-2-yl)amino)-1-methoxy-2-methyl-3-carbonyl propyl)-2-azabicyclo[3.1.0] hexane-2-carboxylic acid **12c** (173 mg, clear liquid). The yield is 99.5%.
MS m/z (ESI): 521.2 [M+1]

Step3.

(S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S),-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropionamide)-3-(2-fluorophenyl) propionic acid

(1S,3S,5S)-tert-butyl-3-((*1R,2R*)-3-(((S)-1-(t-butoxy)-3(2-fluorophenyl)-1-carbonylpropyl-2-yl)amino)-1-methoxy-2-methyl-3-carbonyl propyl)-2-azabicyclo[3.1.0]hexane-2-carboxylic acid **12c** (173mg, 0.33mmol) was dissolved in 2ml dioxane, and 5.6M

hydrogen chloride dioxane solution (0.21ml, 1.16mmol) was added. The mixture was stirred for 1 hour under argon atmosphere at room temperature, and was placed in 0℃ refrigerator for 12 hours. After reaction, the reaction mixture was concentrated under reduced pressure, and 5ml dichloromethane was added to dilute the reaction mixture. 10ml saturated sodium bicarbonate solution was added and the mixture was stirred for 10 minutes. The product was separated and the aqueous phase was extracted by dichloromethane (5ml×3). Dichloromethane layer was combined and was washed by saturated sodium chloride solution (10ml), dried with anhydrous sodium sulfate, filtered. The filtrate was concentrated under reduced pressure. The crude product (S)-tert-butyl-2-((*2R,3R*)-3-((*1S,2S,5S*)-2-azabicyclo[3.1.0]hexane-3 -yl)-3-methoxy-2-methylpropionamide)-3-(2-fluorophenyl) propionic acid **12d** (77mg, yellow liquid) was obtained and directly subjected to the next reaction without purification.
MS m/z (ESI):421.2 [M+1]

Step4.
(S)-tert-butyl-2-((*2R,3R*)-3-((*1S,3S,5S*)-2-((*5S,8S,11S,12R*)-11-((S)- secbutly)-1-(9H-fluorene-9-yl)-5,8-diisopropyl-12-methoxy-4,10-dimethyl-3,6,9-tricarbonyl-2-oxygen -4,7,10-triazatetradecyl-14-acyl)-2-azabicyclo[3.1.0] hexane-3-yl)-3-methoxy-2-methylpropane amide)-3-(2-fluorophenyl) propionic acid

Crude product (S)-tert-butyl-2-((2R,3R)-3-((1S,2S,5S)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropionamide)-3-(2-fluorophenyl) propionic acid **12d** (77mg, 0.183mmol) and (5S,8S,11S,12R)-11-((S)-secbutly)-1-(9H-fluorene-9-yl)-5,8-diisopropyl-12-methoxy-4,10-dimethyl-3,6,9-tricarbonayl-2-oxo-4,7,10-triazatetra decyl-14-carboxylic acid **12i** (116.8mg, 0.183mmol, prepared by methods published in patent application "WO 2013072813") were dissolved in 6ml mixture of dichloromethane and dimethylformamide (V/V=5:1). *N,N*-diisopropylethylamine (0.16ml, 0.915mmol) and 2-(7-Azabenzotriazol)-N,N,N',N'-tetramethyluronium hexafluorophosphate (84mg, 0.22mmol) were added into that mixture. The reaction mixture was stirred for 1 hour under argon atmosphere at room temperature. After reaction, 10ml water was added and stirred. Layer of dichloromethane was washed by saturated sodium chloride solution (10 ml), and dried with anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residues were purified by silica gel column chromatography using eluent system B to obtain the captioned product of (S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S)-2-((5S,8S,11S,12R) -11-((S)-secbutly)-1-(9H-fluorene-9-yl)-5,8-diisopropyl-12-methoxy-4,10-dimethyl-3, 6,9-tricarbonayl-2-oxo-4,7,10-triazatetradecyl-14-acyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropionamide)-3-(2-fluorophenyl) propionic acid **12e** (190.5mg, yellow viscous) with yield of 100%.

MS m/z (ESI): 1040.6 [M+1]

Step5.
(S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S)-2-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl) propionic acid

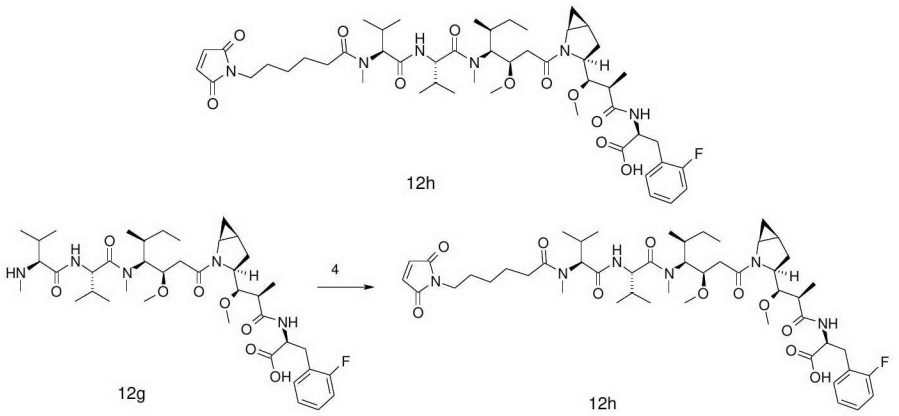
(S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S)-2-((5S,8S,11S,12R)-11-((S)-secbutly)-1-(9H-fluorene-9-yl)-5,8-diisopropyl-12-methoxy-4,10-dimethyl-3,6,9-tricarbonayl-2-oxo-4,7,10-triazatetradecyl-14-acyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methyl propionamide)-3-(2-fluorophenyl) propionic acid **12e** (190.5mg, 0.183mmol) was dissolved in 1.5ml dichloromethane and 2 ml diethylamine was added. The mixture was stirred for 3 hours under argon atmosphere at room temperature. After reaction, the reaction mixture was concentrated under reduced pressure and the crude captioned product
(S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S)-2-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido) -3-(2-fluorophenyl)propionic acid **12f** (150mg, yellow viscous) was obtained. Products were directly subjected to the next reaction without purification.
MS m/z (ESI): 818.5 [M+1]

Step6.
(*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2-((3*R*,4*S*,5*S*)-4-((*S*)-*N*,3-dimethyl-2-((*S*)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl)propionic acid
The crude product (S)-tert-butyl-2-((2R,3R)-3-((1S,3S,5S)-2-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl)propionic acid **12f** (150mg, 0.183mmol) was dissolved in 1ml dioxane, 3ml of 5.6M hydrogen chloride dioxane. The mixture was stirred for 12 hours under argon atmosphere at room temperature. After reaction, the reaction solution was concentrated under reduced pressure with ether solvent. The residues were purified by high performance liquid chromatography to obtain the captioned product (*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2-((3*R*,4*S*,5*S*)-4-((*S*)-*N*,3-dimethyl-2-((*S*)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl) propionic acid **12g** (28mg, white powder) with yield of 20%.
MS m/z (ESI): 762.7[M+1]

^{1}H NMR (400 MHz, CD_3OD): δ 7.38-7.18(m, 2H), 7.13-7.01(m, 2H), 4.80-4.67(m, 2H), 4.30-4.15(m, 1H), 4.13-4.01(m, 1H), 3.96-3.83(m, 2H), 3.75-3.60(m, 2H), 3.42-3.11(m, 9H), 3.06-2.95(m, 1H), 2.70-2.58(m, 4H), 2.28-2.01 (m, 4H), 1.88-1.70(m, 3H), 1.57-1.25 (m, 4H), 1.22-0.95(m, 18H), 0.92-0.80(m, 4H), 0.78-0.65(m, 1H).

2. Preparation of toxin intermediates

(*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2-((3*R*,4*S*,5*S*)-4-((*S*)-2-((*S*)-2-(6-(2,5-dicarbonyl-2,5-dihydro-1H-pyrrol-1-yl)-*N*-methyl hexanamide)-3-methyl butanamide)-*N*,3-dimethyl butanamide)-3-methoxy-5- methylheptanoyl)-2- azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2- fluorophenyl)propionic acid



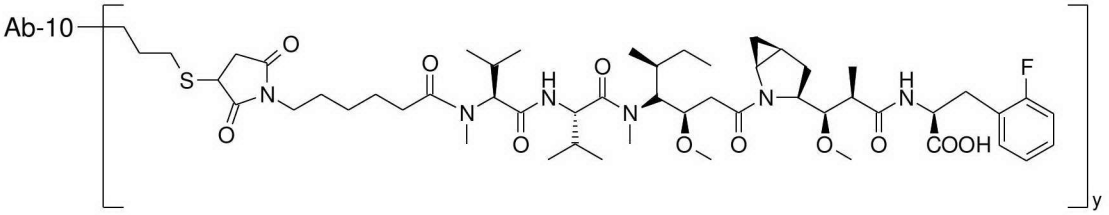
(*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2- ((3*R*,4*S*,5*S*)-4-((*S*)-*N*,3-dimethyl-2-((*S*)-3-methyl-2-(methylamino)butanamide)butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2- fluorophenyl)propionic acid **12g** (25mg, 0.033mmol) was dissolved in 3ml dichloromethane and *N,N*-diisopropylethylamine (0.029ml, 0.164mmol) was added into. The reaction system was under argon atmosphere, and the dichloromethane solution of 6-(2,5-dicarbonyl-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl chloride **4b** (11.3mg, 0.049mmol) prepared above was dropwise added into the mixture in ice-bath, and the reaction was performed for 3 hours at room temperature. After the reaction, 5ml water was added and the mixture was stirred for 20 minutes. The mixture was allowed to stand until layers formed, and the organic layer phase was dried with anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure. The residues were purified by high performance liquid chromatography to obtained the captioned product, (*S*)-2-((2*R*,3*R*)-3-((1*S*,3*S*,5*S*)-2-((3*R*,4*S*,5*S*)-4-((*S*)-2-((*S*)-2-(6-(2,5-dicarbonyl-2,5-dihydro-1H-pyrrol -1-yl)-*N*-methyl hexanamide)-3-methyl butanamide)-*N*,3-dimethyl butanamide)-3-methoxy-5-methylheptanoyl)-2-azabicyclo[3.1.0]hexane-3-yl)-3-methoxy-2-methylpropanamido)-3-(2-fluorophenyl) propionic acid **12h** (7mg, yellow

viscous). The yield is 22.4%.

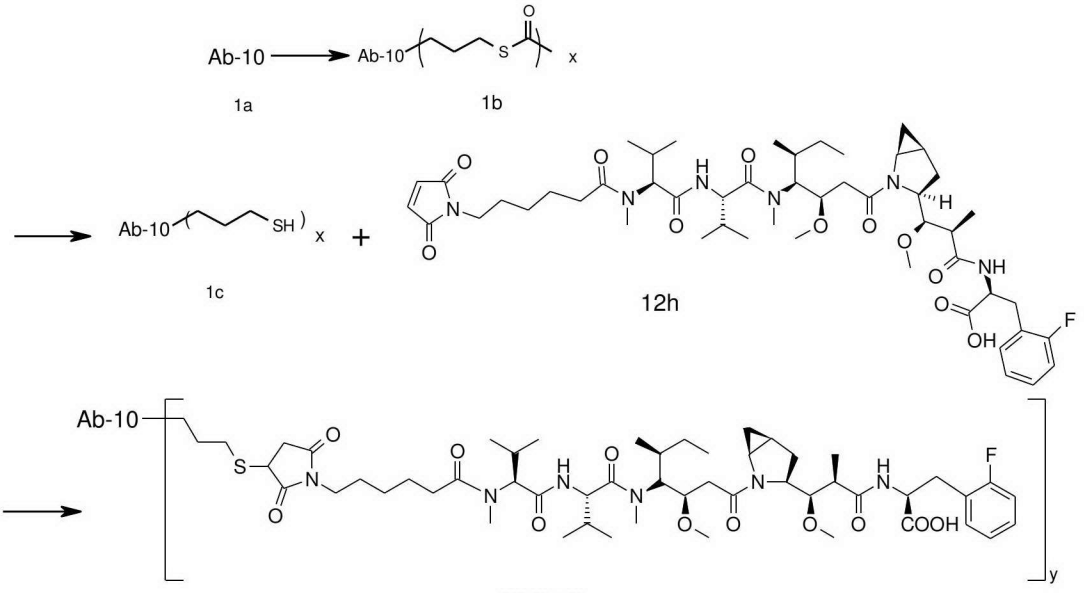
MS m/z (ESI): 955.4 [M+1]

^{1}H NMR (400 MHz, CD_3OD): δ 7.36-7.30(m, 1H), 7.29-7.21(m, 1H), 7.17-7.02(m, 2H), 6.83-6.79(m, 2H), 4.81-4.71(m, 2H), 4.69-4.55 (m, 2H), 4.25-4.15(m, 1H), 4.13-4.04(m, 1H), 3.96-3.85(m, 2H), 3.70-3.61(m, 1H), 3.55-3.46(m, 3H), 3.40-3.21 (m, 4H), 3.18-3.10(m, 2H), 3.07-2.96(m, 4H), 2.67-2.56(m, 2H), 2.54-2.34(m, 3H), 2.29-2.17(m, 2H), 2.10-1.99(m, 1H), 1.89-1.57(m, 7H), 1.52-1.28(m, 6H), 1.21-1.11 (m, 4H), 1.07-0.96(m, 6H), 0.95-0.81(m, 12H), 0.80-0.69(m, 1H).

3. Preparation of antibody-toxin conjugate



ADC-12

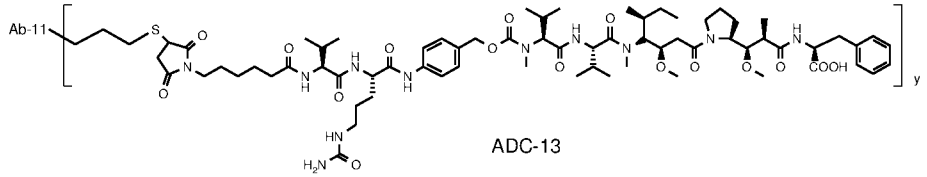
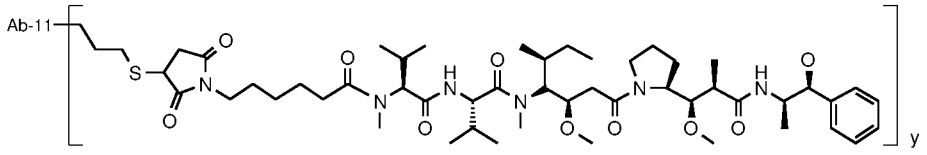
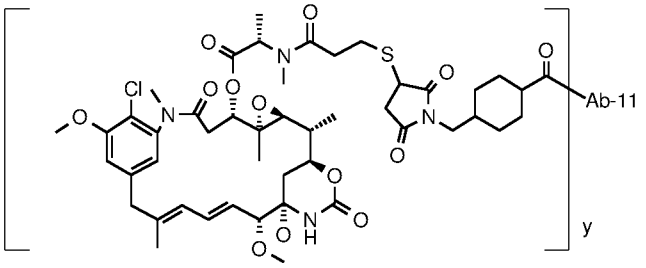


ADC-12

Compound **12h** (1.2mg, 1.2μmol) was dissolved in 0.3ml acetonitrile. Ab-10 monoclonal antibody-propylmercaptan **1c** solution (6.17mg/ml) was added with shaking for 4 hours at 25°C, and then desalination and purification were done on Sephadex G25 gel column (Elution phase: 0.05M of PBS solution which pH is 6.5). The PBS buffer solution of captioned product ADC-12 (3.3mg/ml, 5.0 ml) was obtained by filtration through 0.2μm filter under aseptic condition, and then stored at 4°C.

Q-TOF LC/MS: characteristic peak: 148119.6(M_{Ab}+0D), 149150.5 (M_{Ab}+1D), 150221.1 (M_{Ab}+2D), 151265.1(M_{Ab}+3D) , 152314.3(M_{Ab}+4D).
Average value:y=1.6.

Referring to examples 13-24, ADC compounds of examples 25-27 were prepared.

Example 25	 ADC-13
	Anti-c-Met antibody Ab-11 conjugated toxin MC-VC-PAB-MMAF
Example 26	 ADC-14
	Anti-c-Met antibody Ab-11 conjugated toxin MC-MMAE
Example 27	 ADC-15
	Anti-c-Met antibody Ab-11 conjugated toxin SMCC-DM1

Test Examples of Anti-c-Met Antibody Toxin Conjugate (ADC) Molecular

Test Example 1. Stability Evaluation of anti-c-Met Antibody Toxin Conjugate (ADC) Molecular

The toxin intermediates and toxins of the ADC molecules of the invention or other c-Met antibody-toxin conjugates (e.g., LY-2875358-ADC) with endocytosis were evaluated for the stability of free toxins in PBS, human and monkey plasma.

The toxin intermediates and toxins of the example compounds ADC-1 and ADC-12 were diluted to 500μg/mL with PBS, human or monkey plasma (Suzhou Xishan Zhongke Pharmaceutical Research and Development Co., Ltd., animal production license number: SCXK (Su) 2012-0009), samples were incubated at 37°C for 7 days, and concentrations of free toxins and toxin intermediates were measured at

day 0, 3, and 7. 20μl of internal control (camptothecin, Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd., batch number 090107, 100ng/ml) and 150μl acetonitrile were added to 50μl of human, monkey plasma or PBS sample containing drug, the samples were vortexed for 3 min and centrifuged at 15000 rpm for 10 min, 80μL of the supernatant was taken and mixed with 80μL of 0.2% formic acid, and then 10μL of the sample was taken for injection. Standard curve method was performed as the following: 50μl of blank human, monkey plasma or PBS sample was added into 50μl of working solution, respectively; 20μl of internal control (camptothecin, 100ng/ml) and 100μl acetonitrile were added, the samples were vortexed for 3 min and then centrifuged at 15000 rpm for 10 min; 80μL of the supernatant was taken and mixed with 80μL of 0.2% formic acid; 10μL of the sample was taken for injection.

The Shimadzu LC-30AD ultra-high performance liquid chromatography system (Shimadzu Corporation), UPLC-MS/MS mass spectrometer API4000 triple quadrupole tandem mass spectrometer (AB SCIEX) were used, with the followingchromatographic condition settings column: Waters XBridge™ BEH300 C18 (100 mm×4.6 mm i.d., 3.5 mm), the mobile phase was 0.2% formic acid-acetonitrile (gradient elution). The results were shown in Table 11 and Table 12.

Table 11. Evaluation of plasma stability of toxin intermediates and toxin drugs of ADC-1 of the present invention

Free toxin/ Solvent	MC-MMAF			MMAF		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
PBS	0.02*	0.03	0.19	ND	ND	ND
human plasma	ND	0.05	0.1	ND	ND	ND
Monkey plasma	ND	ND	ND	ND	ND	ND

*: The test value is the percentage of free toxins in the sample. 0.01-0.19% is within the range of detection background; ND: Not detectable, undetectable.

Table 12. Evaluation of plasma stability of toxin intermediates and toxin drugs of ADC-12 of the present invention

Free toxin/ Solvent	Toxin intermediates 12h			Toxin 12g		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
PBS	ND	ND	ND	ND	ND	ND
human	ND	ND	ND	ND	ND	ND

plasma						
Monkey plasma	ND	ND	ND	ND	ND	ND

ND:Not detectable, undetectable..

The above results show that the toxin intermediates and toxins of the ADC-1, ADC-12 in the present invention are stable in various solvents (PBS, human plasma, monkey plasma, etc.). No degradation products, free toxins and toxin intermediates (toxin -linker) were detected after incubation at 37 ° C for 0 day, 3 days and 7 days.

Test Example 2. Evaluation of the Activity *in vitro* of Anti-c-Met Antibody Toxin Conjugate (ADC)

The *in vitro* activity of the ADC-1, ADC-12 of the present invention was evaluated by FACS (detection the binding activity with c-Met positive cell) and endocytosis (method see Example 11). The result was shown in Table 13.

Table 13. The *in vitro* activity of the ADC molecule of the present invention

Humanized antibody	MKN45/FCAS binding activity (nM)	Endocytosis of cell (%)*
Ab-10	1.01	32.7
ADC-1	1.22	32.9
ADC-12	0.48	31.8

*: The data was expressed as endocytosis ratio at 1 hour.

The above results show that the antibody of the invention conjugated to toxin still maintains the binding activity and endocytosis activity of the antibody.

Test Example 3. Cytotoxicity Test of Anti-c-Met Antibody Toxin Conjugate (ADC)

In order to assess the toxic effects of the ADC molecules of the present invention on cells, the ATP toxicity test was performed. ATP is an indicator of the metabolism of living cells, and the detection of ATP can reflect the effect of the toxicity of molecules on cells.

HepG2 cells (Chinese Academy of Sciences cell bank, Cat# TCHu 72) were cultivated in EMEM complete medium containing 10% FBS, MKN45 cells were cultivated in RPMI1640 complete medium containing 10% FBS. The digestion was performed for 2-3 min by adding 2-3 ml trypsin, 10-15ml complete medium was added to elute the digested cells when the digestion was complete, the cells were centrifuged at 1000rpm for 3min. Discard the supernatant, then 10-20 ml complete medium was added to resuspend the cells to obtain single cell suspension, the cell

density was adjusted to 4×10^4 cells/ml. 0.1 ml of the above cell suspension was added to each well of a 96-well cell culture plate and cultivated in an incubator at 37 °C in 5% CO_2. After 24 hours, the medium was removed, and 90μl of EMEM medium containing 2% FBS or RPMI1640 medium containing 2% FBS was added to each well, the test sample (the compound and toxin of Example 13) was diluted with PBS to a gradient of different concentrations, 10μl of the sample was added to each well, the plate was incubated in a incubator at 37 °C in 5% CO_2 for 72 hours. Detection was performed cccording to the instruction of CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, Cat # G7571). The chemiluminescence was measured by a microplate reader (VICTOR 3, PerkinElmer) and the result was analyzed by GraphPad Prism (version 5.0) software. The result was shown in Table 14.

Table 14. Cytotoxicity of ADC molecules of the invention and corresponding free toxins

Test sample	IC_{50}(nM) in MKN45 cell	IC_{50}(nM) in HepG2 cell
ADC-1	0.51	ND
MMAF	0.85	4.88
ADC-12	0.59	ND
12g	79.4	400.8

ND: No activity was detected; NA: Not applicable.

Discussion: The above results show that the cytotoxicity of ADC-1 and ADC-12 to c-Met positive cells MKN45 is the same (IC_{50} is 0.51nM and 0.59nM, respectively). However, the cytotoxicity effect of each toxin moiety on c-Met positive cells was different, there exists 93-fold difference between these two ADCs (79.4/0.85).

The ADC-1 and ADC-12 of the present invention have no cytotoxic effect on c-Met-negative HepG2 cells, indicating that the ADC compound has a specific targeting effect. However, the cytotoxicity effect of each toxin moiety on c-Met-negative HepG2 cells was different, there exists 82-fold difference between these two ADCs (400.8/4.88).

These results show that ADC-1 and ADC-12 have a specific targeting effect and can inhibit the proliferation of c-Met positive cells without toxic effect on non-specific (normal cells) cells. The difference between ADC-1 and ADC-12 is that the toxicity of free toxins of ADC-1 and ADC 12 on target cells and that on non target cells is different. The cytotoxicity of toxin moiety of ADC-12 on c-Met positive cells and negative HepG2 cells is 93 times and 82 times lower than that of ADC-1, respectively. Thus, when the molecule reaches the target cells, the non-specific toxic

effect of ADC-12 is weaker than that of ADC-1, if free toxin is released. Therefore, the toxic side effect is smaller and the safety is good.

Test Example 4. Proliferation inhibition Effect of Anti-c-Met Antibody Toxin Conjugate (ADC) Molecule on Tumor cell

These results indicate that ADC-1 (example 13) can specifically kill tumor target cells expressing c-Met. In order to detect the proliferation inhibition of toxic effects on tumor cell, the molecules of the present invention were tested on a variety of tumor cells; the inhibitory effect of the sample on cell proliferation was measured by CCK method, and the *in vitro* cell activity of the ADC molecule of the present invention was evaluated according to IC_{50} value.

The cells used and the corresponding medium are shown in Table 15 below. Cell proliferation was measured using the Cell Counting Kit (Dojindo chem Co. Cat # CK04) (according to the instructions).

The digestion was performed for 2-3min by adding 2-3ml trypsin, 10-15ml complete medium was added to elute the digested cells when the digestion was complete, the cells were centrifuged at 1000rpm for 3min. Discard the supernatant, then 10-20ml complete medium was added to resuspend the cells to obtain single cell suspension, the cell density was adjucted to 4×10^4 cells/ml. 0.1 ml of the above cell suspension was added to each well of a 96-well cell culture plate and cultivated in an incubator at 37 °C in 5% CO_2. After 24 hours, the medium was removed, and 90μl of medium containing 2% FBS was added to each well, the test sample was diluted with PBS to a gradient of different concentrations, 10μl of the sample was added to each well and the plate was incubated in a incubator at 37 °C in 5% CO_2 for 72 hours. 10μl of CCK8 was added to each well and incubated for 2 hours in an incubator. OD450 was detected by a microplate reader (VICTOR 3, PerkinElmer) and data was analyzed using GraphPad Prism (version 5.0) software. The results are shown in Table 16.

Table 15. The culture medium used in this example

Cell line	Medium	Manufacturer, No
MKN45	RPMI1640+10%FBS	JCRB , JCRB0254
SNU5	IMDM+10%FBS	ATCC, Cat# CRL-5973™
BxPC3	RPMI1640+10%FBS	Chinese Academy of Sciences cell bank (Cat# TCHu 12)
Caki-1	McCOY's 5A+10%FBS	Chinese Academy of Sciences cell bank, Cat# TCHu135
NCI-H1993	RPMI1640+10%FBS	ATCC, Cat# CRL-5909™
PC9	DMEM+10%FBS	Shanghai bioleaf Biological Technology Co.,

		Ltd
NCI-H596	DMEM+10%FBS	Shanghai bioleaf Biological Technology Co., Ltd

Table 16. Inhibitory effect of molecule of present invention on proliferation of different cancer cells

Cell line	Tumor type	ADC-1		Ab-10	
		IC_{50}(nM)	**Maximum inhibition rate (%)**	**IC_{50}(nM)**	**Maximum inhibition rate (%)**
MKN45	gastric cancer	0.17	67	0.15	41
SNU5	gastric cancer	0.24	75	0.10	70
BxPC3	Pancreatic cancer	6.9	71	>1000	0
Caki-1	Renal cell carcinoma	6.5	61	>1000	0
PC9	Non-small cell lung cancer	5.6	37	>1000	0
NCI-H596	Non-small cell lung cancer	103.9	56	13.7	13
NCI-H1993	Non-small cell lung cancer	2.8	60	1.1	40

The result of Table 16 shows that the anti-c-Met antibody of the present invention has a relatively good activity on gastric cancer cell lines MKN45 and SUN. However, as for other tumor cells with low c-Met expressing or absence of c-Met expressing, such as lung cancer cells, the activity is very weak, or absent. Because of the ADC-1 of the present invention has additional toxins, it has a good activity on tumor cells expressing c-Met including gastric cancer cell line MKN45 and SUN, especially on lung cancer, pancreatic cancer and renal cell carcinoma cells on which c-Met antibodies have no or very weak effects .

Test Example 5. Evaluation of the efficacy *in vivo* of Anti-c-Met Antibody Toxin Conjugate (ADC) Molecule

1. Test purpose

In order to better evaluate the antitumor efficacy of the anti-c-Met antibody and ADC molecule of the present invention, parallel comparison experiment of antibody Ab-10 and ADC-1 were performed by using the method of example 10. In contrast to Example 10, this test was performed by administration of single dose. This

experiment would be terminated, until the tumor inhibition effect exhibited a tendency to recover.

2. The antibody to be tested

Ab-10 (5mg/kg), the stock solution (2.18mg/ml) was diluted with PBS to a final concentration of 0.5mg/ml.

Ab-10 (10mg/kg), the stock solution (2.18mg/ml) was diluted with PBS to a final concentration of 1mg/ml.

Ab-10 (30mg/kg), the stock solution (2.18mg/ml) was diluted with PBS to a final concentration of 3mg/ml.

ADC-1 (2.5mg/kg), the stock solution (10mg/ml) was diluted with PBS to a final concentration of 0.25mg/ml.

ADC-1 (5mg/kg), the stock solution (10mg/ml) was diluted with PBS to a final concentration of 0.5mg/ml.

ADC-1 (10mg/kg), the stock solution (10mg/ml) was diluted with PBS to a final concentration of 1mg/ml.

All animals were administrated by tail vein injection, and the administration volume was 0.2 ml/mouse.

3. Test procedures

Nude mice were inoculated subcutaneously in the right rib with MKN-45 cells (1×10^6/mouse). When the average volume of tumor reached (150.19 + 8.44) mm^3, animals were randomly divided into different administration groupswith each group of 8 mice. The specific dosing regimen is shown in Table 17.

Mice were measured for tumor volume and body weight twice a week, and data was recorded.

Excel statistical software: mean value is calculated as avg; SD is calculated as STDEV; SEM is calculated as STDEV/SQRT; P value between different groups is calculated as TTEST.

Tumor volume (V) is calculated as: $V=1/2\times L_{length}\times L_{width}^2$

Tumor Inhibition Rate (%) = $(V_0 - V_T)/ V_0 * 100\%$

Wherein V_0 and V_T represent the tumor volume at the beginning of the experiment and at the end of the experiment, respectively.

4. Result

Table 17. Therapeutic efficacy of administering compounds to MKN-45 nude mice

Compound	Mean tumor volume (mm^3)	Mean tumor volume (mm^3)	Relative tumor volume (mm^3)	Relative tumor volume	Tumor Inhibition Rate	P Value (d18) (vs blank)	Tumor Inhibition Rate	P Value (d28) (vs blank)

									(mm^3)			
Group	D0	SEM	D28	SEM	D18	SEM	D28	SEM	D18%		D28%	
Solvent	150.13	9.84	1123.71	128.33	5.89	0.88	7.71	0.91	-	-	-	-
Ab-10 (5 mg/kg)	149.42	8.73	904.68	75.30	3.34	0.38	6.13	0.55	*43.32	0.0188	20.54	0.1575
Ab-10 (10 mg/kg)	149.66	8.46	827.75	90.31	4.12	0.44	5.58	0.63	29.98	0.0943	27.62	0.0755
Ab-10 (30 mg/kg)	157.84	9.66	551.96	79.43	3.42	0.40	3.47	0.39	*41.94	0.0230	**55.02	0.00075
ADC-1 (2.5 mg/kg)	149.83	9.08	661.41	88.25	2.05	0.16	4.38	0.53	**65.23	0.0015	**43.23	0.0094
ADC-1 (5 mg/kg)	150.10	7.99	656.60	192.02	1.54	0.11	4.35	1.27	**73.85	0.0002	*43.54	0.04915
ADC-1 (10 mg/kg)	143.92	6.49	228.44	32.31	1.15	0.11	1.57	0.20	**80.49	0.0001	**79.63	0.00001

**p<0.01 * p<0.05

Conclusion: The antibody and ADC compound of the invention have obvious effect on nude mice with MKN-45 xenograft tumor.

ADC-1 and ADC-12 were compared in parallel with the same test method described above in order to evaluate the *in vivo* efficacy of the ADC-12 of the present invention. The same dose is 3mg/kg, administration of single dose. The result is shown in Table 18.

Table 18. The inhibition effect of ADC-1 and ADC-12 on tumor

Inhibition Rate (%)	Day 11	Day 15	Day 18	Day 21
ADC-1	42.8	44.7	35.4	27.1
ADC-12	44.6	54.5	50.5	50.4

The result above shows that the tumor inhibition rate of ADC-1 and ADC-12 was similar on day 11, but the efficiency of ADC-1 was decreased 15 days later (27.1% on day 21), while the antitumor effect of ADC-12 was maintained at the level of theeleventh day (50.4%).

Test Example 6. Effect of ADC-12 on nude mice with subcutaneous tumor xenograft **of human lung cancer NCI-H1993**

1. Test purpose

Evaluation and comparison of the efficacy of ADC-12 and Ab-10 antibody solution on nude mice with subcutaneous tumor xenograft of human lung cancer NCI-H1993.

2. Drug preparation

ADC-12 was dissolved 20 mg/ml with injection water, aliquoted and stored in a refrigerator at -80°C. The sample was diluted with normal saline containing 0.1%

BAS to corresponding concentration before use. The stock concentration of Ab-10 is 16.3mg/ml, which was diluted with normal saline containing 0.1% BAS, and then aliquoted and stored in a -80°C refrigerator.

3. Experimental animal

BALB/cA-nude mice, 6-7 weeks, ♀, purchased from Shanghai Ling Chang Biological Technology Co., Ltd. Production license number: SCXK (Shanghai) 2013-0018; animal certificate number 2013001814303. Feeding environment: SPF grade.

4. Test procedures

Nude mice were inoculated subcutaneously with human lung cancer cell NCI-H1993. When the volume of tumor reached 100-150mm^3, animals were randomly divided into groups (D0). The administration regimen and dosage are shown in Table 19. Mice were measured for tumor volume and body weight 2-3 times a week, and data was recorded. The tumor volume (V) is calculated as:

$V = 1/2 \times a \times b^2$, wherein a and b represent the length and width, respectively.

$T/C\ (\%) = (T-T_0)/(C-C_0) \times 100$, wherein T and C represent the tumor volume at the end of the experiment. T_0 and C_0 represent the tumor volume at the beginning of the experiment.

5. Result

ADC-12 is an anti-c-Met antibody-toxin conjugate. ADC-12 (1, 3, 10mg/kg, IV, D0) inhibited the growth of subcutaneous xenograft tumor of c-Met over-expressing human lung caner NCI-H1993 in nude mice in a dose-dependent manner, and the tumor inhibition rates were 45%, 63%, 124%, respectively; 70% mice of the 10 mg/kg dose group exhibited partial regression (D21); Ab-10 antibody stock solution is antibody used for the preparation of ADC-12, the tumor inhibitory rate of Ab-10 antibody (30 mg/kg, IV, twice a week for 6 times) was 42% for NCI-H1993; The tumor bearing mice were well tolerated to the above drugs, and no symptoms such as loss of weight were observed. By comparison, the effect of ADC-12 on NCI-H1993 is obviously stronger than that of Ab-10 antibody stock solution.

Table 19. Effect of ADC-12 and Ab-10 antibody stock solution on subcutaneous xenograft tumor of human lung cancer NCI-H1993 in nude mice

Compound	Administration	Mean tumor volume (mm^3)		Mean tumor volume (mm^3)		%T/C	Tumor Inhibition Rate	P Value (d21) (vs blank)	partial regression
Group		D0	SEM	D21	SEM	D21	D21%		

Solvent	D0	136.7	±3.4	1851.3	±116.4	-	-	-	0
ADC-12 (1 mg/kg)	D0	132.5	±5.3	1075.1	±56.8	55	45	0.000	0
ADC-12 (3 mg/kg)	D0	127.3	±3.1	760.8	±96.6	37	63	**0.000	0
ADC-12 (10 mg/kg)	D0	132.7	±2.9	100.6	±16.3	-24	124	**0.000	7
Ab-10 (10 mg/kg)	D0,3,7,10,14,17	129.7	±3.8	1124.0	±63.3	58	42	0.000	0

D0: time for the first administration; P value, as compared with solvent; **P<0.01, as compared with that of 30mg/kg group of Ab-10 antibody solution; Student's t test was used. The number of mice at the beginning of the experiment: n = 10.

Discuss: ADC-12 (1, 3, 10mg/kg, IV, D0) inhibited the growth of subcutaneous xenograft tumor of c-Met over-expressing human lung cancer NCI-H1993 in nude mice in a dose-dependent manner, resulting in partial tumor regression; Ab-10 antibody stock solution (30 mg/kg, IV, twice a week for 6 times) was also effective for NCI-H1993; The effect of ADC-12 on NCI-H1993 is obviously stronger than that of Ab-10 antibody stock solution. The tumor bearing mice were well tolerated to the above drugs.

CLAIMS:

1. An antibody or antigen-binding fragment thereof that specifically binds to c-Met, comprising at least one CDR region selected from the following sequences or the mutant sequence thereof:

antibody heavy chain variable region HCDR sequence: SEQ ID NO: 6, SEQ ID NO:7 or SEQ ID NO:8;

and

antibody light chain variable region HCDR sequence: SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11.

2. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 1, wherein the antibody heavy chain variable region comprises at least one HCDR region sequence selected from the following sequences or mutant sequence thereof: SEQ ID NO: 6, SEQ ID NO: 7 or SEQ ID NO: 8.

3. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 1, wherein the antibody light chain variable region comprises at least one LCDR region sequence selected from the following sequences or a mutant sequence thereof: SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 11.

4. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1-3, wherein the antibody comprises heavy chain variable regions shown as sequence SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, or a mutant sequence thereof; and light chain variable regions shown as sequence SEQ ID NO:9, SEQ ID NO: 10 and SEQ ID NO: 11, or a mutant sequence thereof.

5. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1-4, wherein the mutant sequence of CDR region is a sequence which has 1-3 amino acid mutations that optimize antibody activity, wherein the mutant sequence of HCDR2 region preferably is SEQ ID NO: 12.

6. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1-5, wherein the antibody or the antigen-binding fragment thereof that specifically binds to c-Met receptor is a murine antibody or the fragment thereof.

7. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 6, wherein the heavy chain variable region sequence of the murine antibody is shown as SEQ ID NO: 4.

8. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 6, wherein the light chain variable region sequence of the murine antibody is shown as SEQ ID NO: 5.

9. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 6 to 8, wherein the heavy chain variable region of the murine antibody is shown as SEQ ID NO: 4, the light chain variable region of the murine antibody is shown as SEQ ID NO: 5.

10. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1-5, wherein the antibody or the antigen-binding fragment thereof is a chimeric antibody or the fragment thereof.

11. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 10, wherein the humanized antibody heavy chain variable region comprises a heavy chain FR region derived from human germline heavy chain sequence, preferably human germline heavy chain IGHV 3-33*01; wherein said heavy chain FR region comprises framework sequence of FR1, FR2, FR3 and FR4 regions of the human germline heavy chain IGHV 3-33*01, or a mutant sequence thereof, preferably, the mutant sequence comprises 0-10 amino acid back-mutation(s).

12. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 11, wherein the humanized antibody comprises a heavy chain variable region sequence selected from SEQ ID NO: 13-15 or variant thereof.

13. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 10, wherein the humanized antibody light chain variable region comprises a light chain FR region derived from human germline light chain sequence, preferably human germline light chain IGKV085 or IGKV4-1*01; wherein said light chain FR region comprises framework sequence of FR1, FR2, FR3 and FR4 regions of the human germline light chain IGKV085 or IGKV4-1*01, or a mutant sequence thereof, preferably, the mutant sequence comprises 0-10 amino acid back-mutation(s).

14. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 13, wherein the humanized antibody comprises a light chain variable region sequence selected from SEQ ID NO: 16-18 or variant thereof.

15. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 10 to 14, wherein the humanized antibody comprises a heavy chain variable region sequence selected from SEQ ID NO: 13-15 and a light chain variable region sequence selected from SEQ ID NO: 16-18.

16. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1-5 and 10-15, which comprises a combination of a heavy chain variable region sequence and a light chain variable region sequence selected from any one of a) to c):

a) Heavy chain variable region sequence of SEQ ID NO: 13, and light chain variable region sequence of SEQ ID NO: 16;

b) Heavy chain variable region sequence of SEQ ID NO: 14, and light chain variable region sequence of SEQ ID NO: 17; or

c) Heavy chain variable region sequence of SEQ ID NO: 15, and light chain variable region sequence of SEQ ID NO: 18.

17. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 10-16, wherein the heavy chain constant region of humanized antibody comprises a constant region derived from human IgG1 or a variant thereof, human IgG2 or a variant thereof, human IgG3 or a variant thereof, or human IgG4 or a variant thereof, preferably a constant region derived from human IgG1 or a variant thereof, human IgG2 or a variant thereof or human IgG4 or a variant thereof, most preferably a constant region derived from human IgG2 or a variant thereof.

18. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 17, which comprises a full length heavy chain sequence selected from SEQ ID NO: 23-25 or the sequences with at least 90% identity to SEQ ID NO: 23-25.

19. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 10-16, wherein the light chain constant region of humanized antibody comprises a constant region selected from human κ or λ or a variant thereof.

20. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to claim 19, which comprises a full-length light chain sequence selected from SEQ ID NO: 26-28 or the sequences with at least 90% identity to SEQ ID NO: 26-28.

21. The antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 10-20, wherein the humanized antibodie comprises a combination of a full-length light chain sequence and a full-length heavy chain selected from:

Ab-9: The heavy chain sequence of SEQ ID NO: 23 and the light chain sequence of SEQ ID NO: 26;

Ab-10: The heavy chain sequence of SEQ ID NO: 24 and the light chain sequence of SEQ ID NO: 27; or

Ab-11: The heavy chain sequence of SEQ ID NO: 25 and the light chain sequence of SEQ ID NO: 28.

22. A DNA molecule encoding the antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one claims of 1 to 21.

23. An expression vector comprising the DNA molecule according to claim 22.

24. A host cell transformed with the expression vector according to claim 23, wherein the host cell is preferably mammalian cell, more preferably CHO cell.

25. A pharmaceutical composition, comprising the antibody or the antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1 to 21, and one or more pharmaceutically acceptable excipient, diluent or carrier.

26. An antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof:

$$Ab-[(L_2)t-L_1-D)]y \quad (I)$$

wherein:

D is a drug unit;

L_1, L_2 is a linker unit;

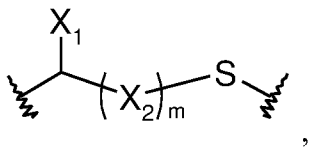
t is 0 or 1, preferably 1;

y is selected from 1-8, preferably 2-5;

Ab is the antibody or antigen-binding fragment thereof that specifically binds to

c-Met receptor according to any one of claims 1-21.

27. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, wherein -L_2- is shown as formula (-L_2-):



,

wherein:

X_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, cyano, alkyl, alkoxy and cycloalkyl;

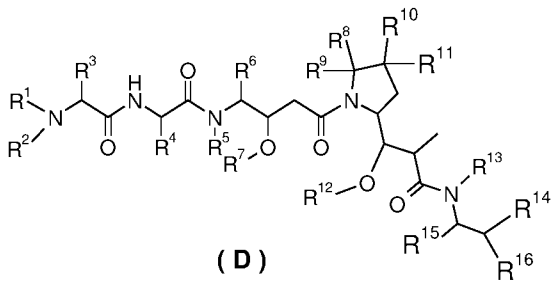
X_2 is selected from the group consisting of alkyl, cycloalkyl and heterocyclyl;

m is 0-5, preferably 1-3;

S is sulfur atom.

28. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, wherein the drug unit of D is a cytotoxic agent selected from toxins, chemotherapeutic agents, antibiotics, radioisotopes and nucleolytic enzyme.

29. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, wherein D is shown as formula (D):



(D)

or a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixtures thereof, or pharmaceutically acceptable salt thereof:

wherein:

R^1-R^7 is each selected from the group consisting of hydrogen, halogen, hydroxyl, cyano, alkyl, alkoxy and cycloalkyl;

R^8-R^{11} is each optionally selected from the group consisting of hydrogen,

halogen, alkenyl, alkyl, alkoxy and cycloalkyl; preferably, at least one of R^8-R^{11} is selected from the group consisting of halogen, alkenyl, alkyl and cycloalkyl, and the rest of R^8-R^{11} are hydrogen;

or any two of R^8-R^{11} form a cycloalkyl, the rest two are each selected from the group consisting of hydrogen, alkyl and cycloalkyl;

R^{12}-R^{13} are each selected from the group consisting of hydrogen, alkyl and halogen;

R^{14} is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is optionally further substituted by substituent group selected from the group consisting of hydrogen, halogen, hydroxy, alkyl, alkoxy and cycloalkyl;

R^{15} is selected from halogen, alkenyl, alkyl, cycloalkyl or $COOR^{17}$;

R^{16} is selected from hydrogen, halogen, hydroxy, cyano, alkyl, alkoxy and cycloalkyl;

R^{17} is selected from hydrogen, alkyl and alkoxy.

30. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 29, wherein L_2 comprises a linker selected from Val-Cit, MC, PAB and MC-PAB, preferably MC.

31. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, wherein D is maytansinoid alkaloids; preferably DM1, DM3 or DM4; more preferably DM1.

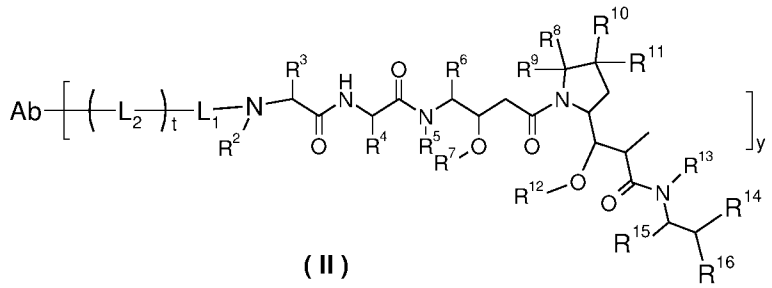
32. The antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 31, wherein L_2 is selected from the group consisting of N-succinimidyl 4-(2-pyridylthio) valerate (SPP), N-succinimidyl 4-(N-maleimidomethyl) - cyclohexane-1-Carboxylic acid ester (SMCC), and N-succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB); preferably SPP or SMCC.

33. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, wherein D is a camptothecin alkaloid; preferably selected from CPT, 10-hydroxy-CPT, CPT-11 (Irinotecan), SN-38 and topotecan, more preferably SN-38.

34. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 33, wherein said linker L_2

comprises structure selected from Val-Cit, MC, PAB or MC-PAB; preferably MC or MC-vc-PAB.

35. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, which is conjugated drug of formula (II) or the pharmaceutically acceptable salt or solvate thereof :



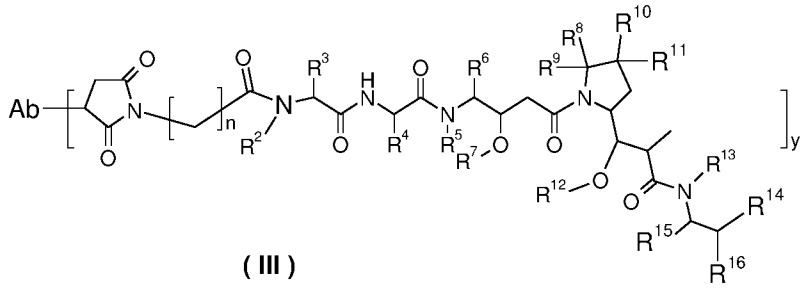
(II)

wherein:

R^2-R^{16} are as defined in claim 27;

Ab, t, y, L_1, L_2 are as defined in claim 24.

36. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, which is a conjugated drug of formula (III) or the pharmaceutically acceptable salt or solvate thereof :



(III)

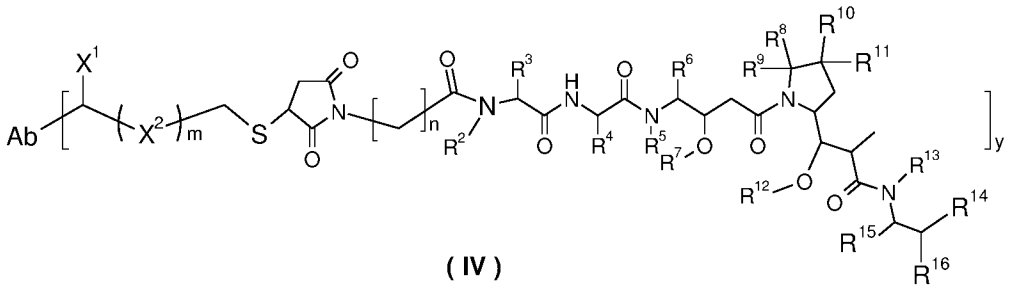
wherein:

R^2-R^{16} are as defined in claim 27;

Ab, t, y, L_1, L_2 are as defined in claim 24;

n is 3-6, preferably 5.

37. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, which is a conjugated drug of formula (IV) or the pharmaceutically acceptable salt or solvate thereof :



(IV)

wherein:

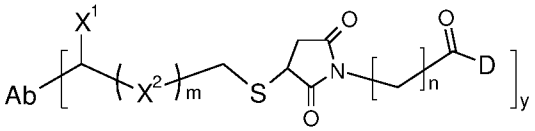
R^2-R^{16} are as defined in claim 27;

Ab, y are as defined in claim 24;

n is as defined in claim 34;

X^1, X^2, m are as defined in claim 25.

38. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to claim 26, which is a conjugated drug of formula (V) or the pharmaceutically acceptable salt or solvate thereof :



(V)

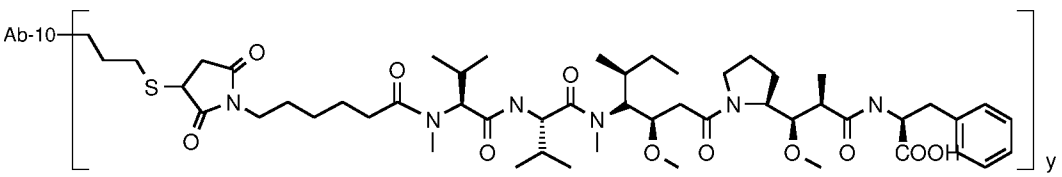
wherein:

Ab, D, y are as defined in claim 24;

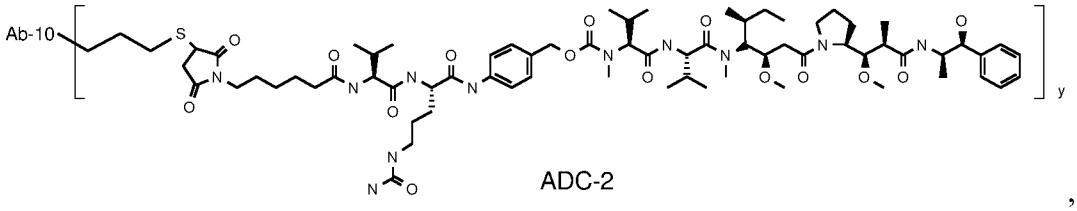
n is as defined in claim 34;

X^1, X^2, m are as defined in claim 25.

39. The antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 26-38, wherein the antibody-cytotoxic drug conjugate or the pharmaceutically acceptable salt or solvate thereof is selected from the group consisting of :



ADC-1 ,



ADC-2

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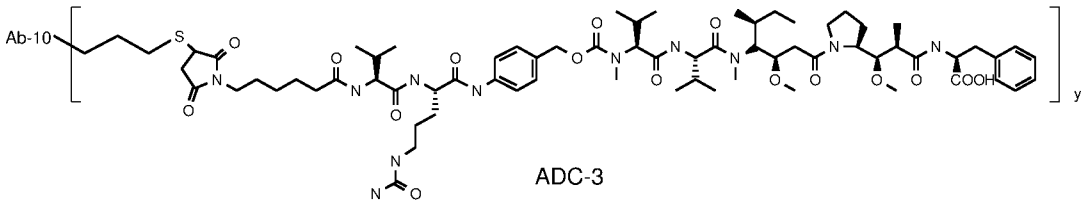

ADC-3

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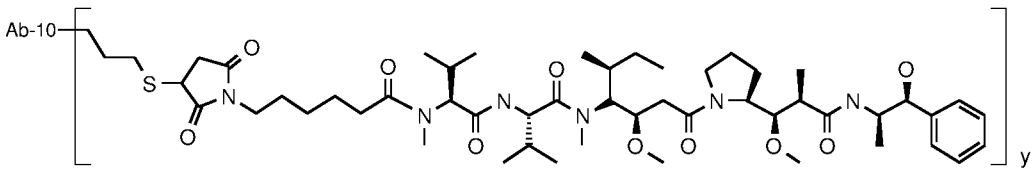

ADC-4

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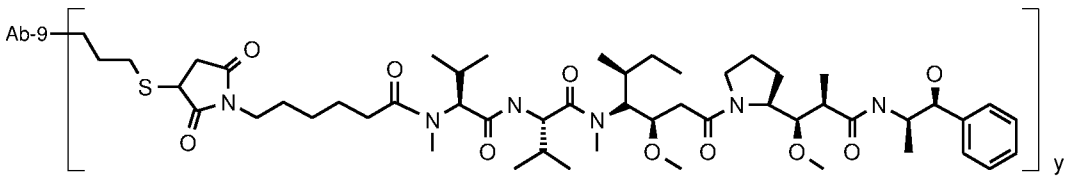

ADC-5

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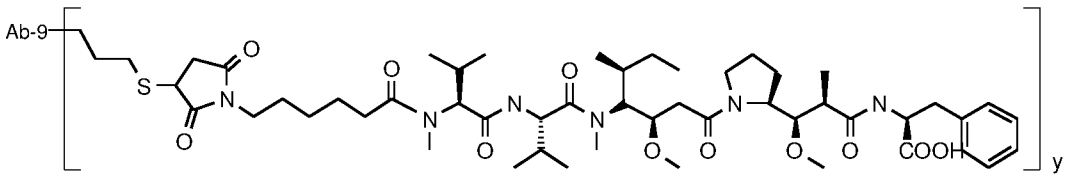

ADC-6

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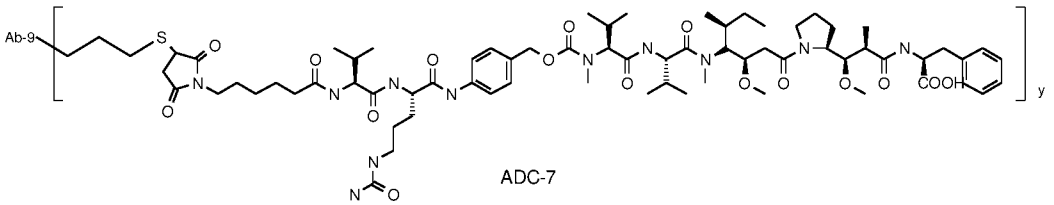

ADC-7

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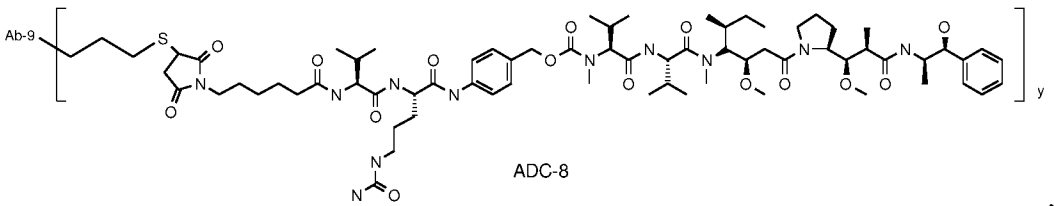

ADC-8

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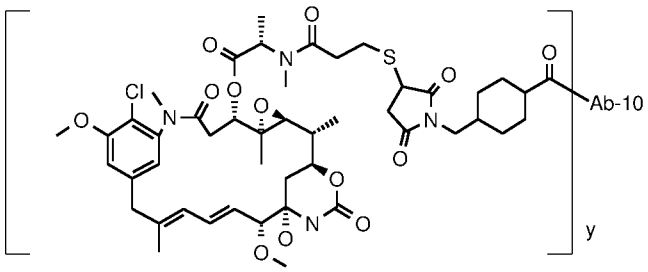

ADC-9

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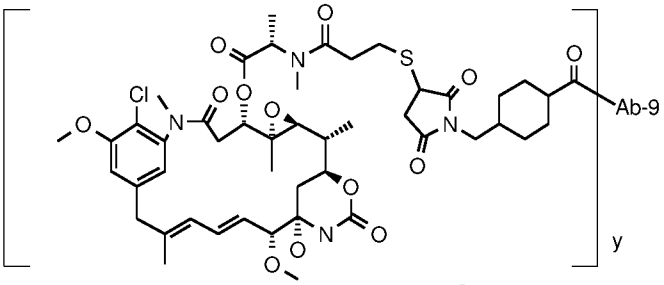

ADC-10

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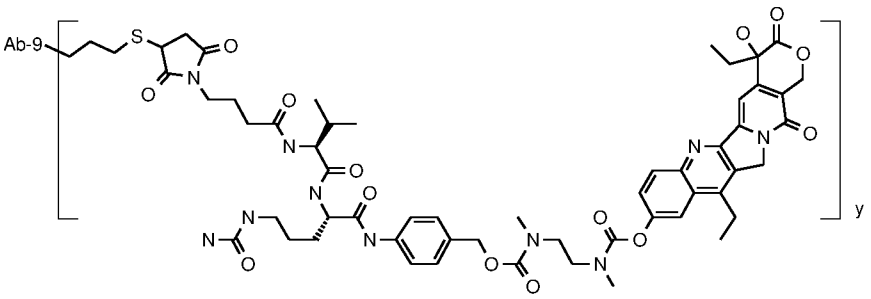

ADC-11

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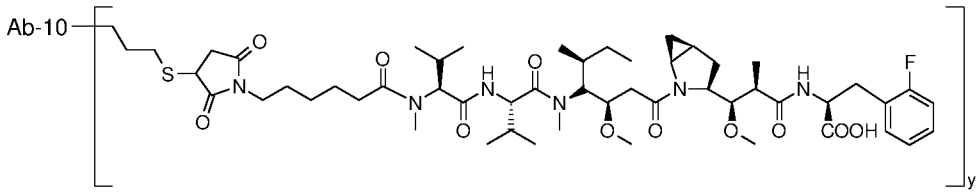

ADC-12

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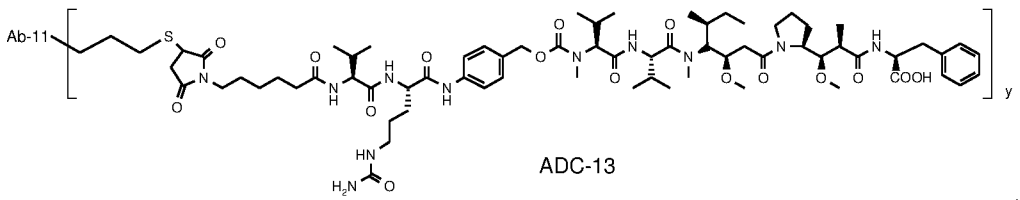

ADC-13

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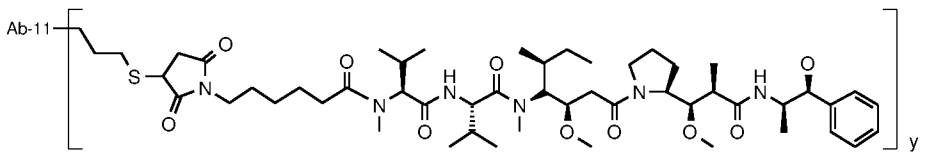

ADC-14

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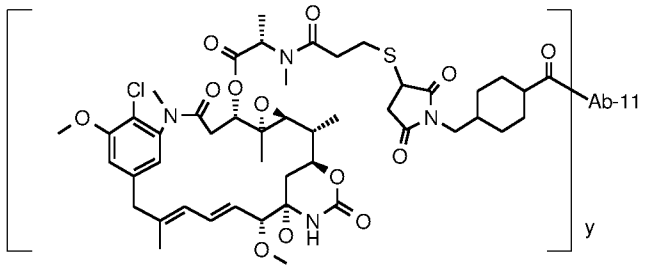
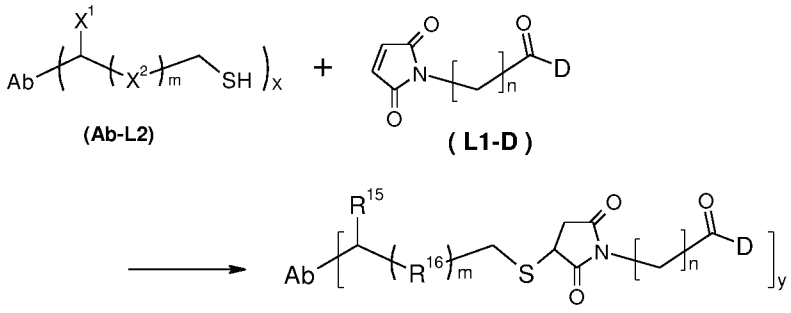


ADC-15 ;

wherein Ab-9, Ab-10, Ab-11 are as defined in claim 21, y ranges from 1-8, preferably 2-5.

40. A process of preparing the conjugated drug of formula (V) or the pharmaceutically acceptable salt or solvate thereof according to claim 38, comprises a step of:



(Ab-L2) (L1-D)

(V)

a compound of the general formula (Ab-L2) is reacted with a compound of the general formula (L1-D) in an organic solvent to obtain a compound of the general formula (V); the organic solvent is preferably acetonitrile or ethanol;

wherein:

Ab is an antibody or antigen-binding fragment thereof that specifically binds to c-Met receptor according to any one of claims 1 to 21;

X_1 is selected from the group consisting of hydrogen, halogen, hydroxyl, cyano, alkyl, alkoxy and cycloalkyl;

X_2 is selected from the group consisting of alkyl, cycloalkyl and heterocyclyl;

X is 0-5, preferably 1-3,

m is 0-5, preferably 1-3.

41. A pharmaceutical composition comprising the antibody-cytotoxic drug conjugate of formula (I) or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 26-39, and pharmaceutically acceptable excipient, diluent or carrier.

42. The use of any one of the following in the preparation of a medicament for

treatment of c-Met-mediated disease or condition:

the antibody or antigen-binding fragment thereof which specifically binds to c-Met receptor according to any one of claims 1 to 21, or the pharmaceutical composition according to claim 25, or the antibody-cytotoxic drug conjugate of formula (I) or a pharmaceutically acceptable salt or solvate thereof according to any one of claims 26-39, or the pharmaceutical composition according to claim 41,

wherein the disease or condition is preferably cancer; more preferably a cancer that expresses c-Met; most preferably a cancer selected from gastric cancer, pancreatic cancer, lung cancer, intestinal cancer, kidney cancer, melanoma, non-small cell lung cancer; most preferably gastric cancer, pancreatic cancer, non-small cell lung cancer and kidney cancer.

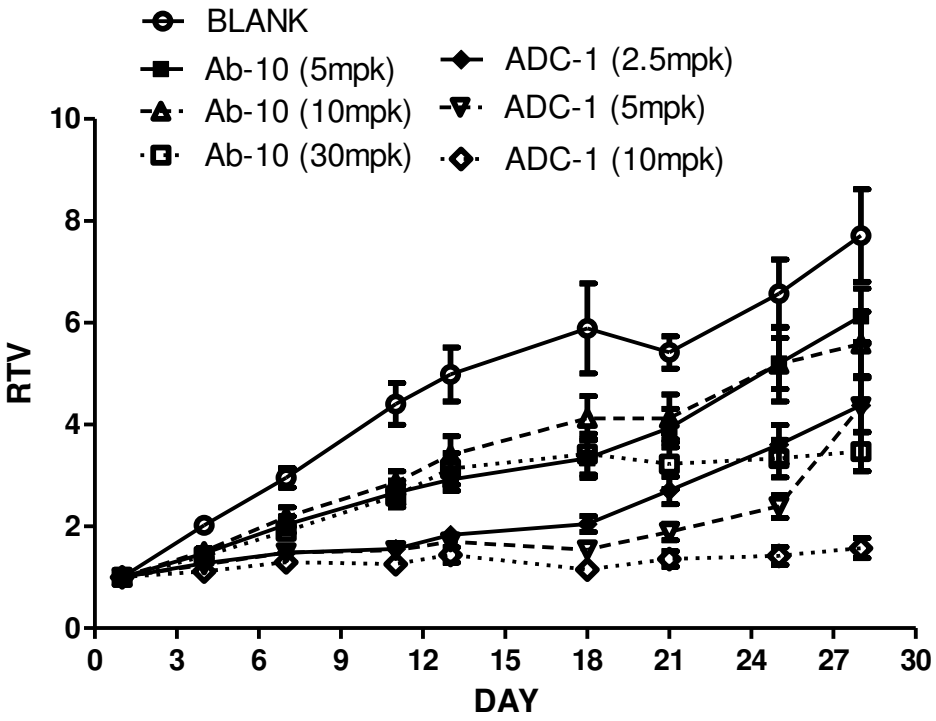

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Figure 1

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Gly Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
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Ser Arg Leu Arg Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
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Glu Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
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Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Ala
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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
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Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
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Ser Arg Leu Arg Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val Phe
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Thr Met Asn Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
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35 40 45

Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
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Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
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Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
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Gly Thr Thr Val Thr Val Ser Ser
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35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn
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20 25 30

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35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
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35 40 45

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Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
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Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys

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Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
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210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 20
<211> 326
<212> PRT
<213> ÈËÔ´

<400> 20

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys

275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 21
<211> 327
<212> PRT
<213> ÈËÔ´

<400> 21

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
100 105 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
115 120 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
130 135 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145 150 155 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
165 170 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
180 185 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
195 200 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
210 215 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225 230 235 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
245 250 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320

Leu Ser Leu Ser Leu Gly Lys
325

<210> 22
<211> 106
<212> PRT
<213> ÈËÔ´

<400> 22

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 23
<211> 446
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-9ÖØÁ´

<400> 23

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Pro Asn Tyr
20 25 30

Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
50 55 60

Ser Arg Leu Arg Ile Ser Lys Asp Thr Ser Lys Ser Gln Val Val Phe
65 70 75 80

Thr Met Asn Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Arg Asn His Asp Asn Pro Tyr Asn Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val
210 215 220

Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
290 295 300

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 24
<211> 446
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-10ÖØÁ´

<400> 24

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Asn Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asn His Asp Asn Pro Tyr Asn Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val
210 215 220

Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
290 295 300

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 25
<211> 446
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-11ÖØÁ´

<400> 25

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Pro Asn Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Ala Val Ile Trp Ser Gly Gly Ser Thr Asn Tyr Ala Ala Ala Phe Val
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Asn His Asp Asn Pro Tyr Asn Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val
210 215 220

Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
290 295 300

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 26
<211> 218
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-9ÇáÁ´

<400> 26

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Pro Gly
1 5 10 15

Gln Arg Ala Thr Ile Thr Cys Arg Ala Asn Lys Ser Val Ser Thr Ser
20 25 30

Thr Tyr Asn Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn
65 70 75 80

Pro Val Glu Ala Asn Asp Thr Ala Asn Tyr Tyr Cys Gln His Ser Arg
85 90 95

Asp Leu Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 27
<211> 218
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-10ÇáÁ´

<400> 27

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Asp Lys Ser Val Ser Thr Ser
20 25 30

Thr Tyr Asn Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Asp Leu Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 28
<211> 218
<212> PRT
<213> ÈË¹¤ÐòÁÐ

<220>
<223> Ab-11ÇáÁ´

<400> 28

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Arg Ala Asn Lys Ser Val Ser Thr Ser
20 25 30

Thr Tyr Asn Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Ala Ser Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln His Ser Arg
85 90 95

Asp Leu Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215