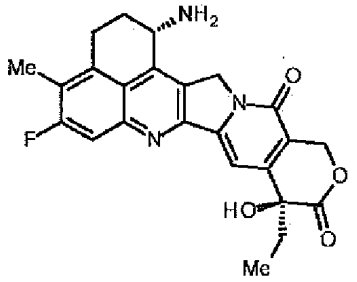
antibody-drug conjugate in which the antibody and exatecan are conjugated to each other via the linker, and confirmed an excellent antitumor effect exhibited by the conjugate to thereby complete the present disclosure.

[0011]

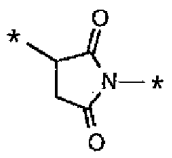
Specifically, the present invention relates to the followings.

In one aspect, the present invention refers to an antibody-drug conjugate wherein an antitumor compound represented by the following formula:



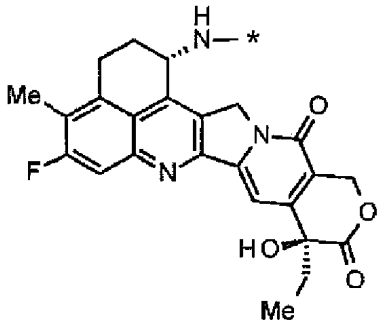

is conjugated to an antibody via a drug-linker having a structure represented by the following formula:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX), wherein -(Succinimid-3-yl-N)- has a structure represented by the following formula:



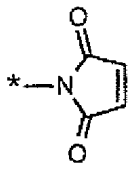

which is connected to the antibody at position 3 thereof via a thioether bond and connected to a methylene group in the linker structure containing this structure on the

nitrogen atom at position 1, and -(NH-DX) represents a group represented by the following formula:

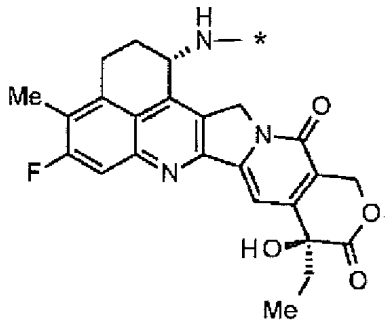



wherein the nitrogen atom of the amino group at position 1 is a connecting position. In another aspect, the present invention refers to a drug containing the antibody-drug conjugate according to the present invention, or a salt thereof. In still another aspect, the present invention refers to an antitumor drug and/or anticancer drug containing the antibody-drug conjugate according to the present invention, or a salt thereof. In another aspect, the present invention refers to a pharmaceutical composition containing the antibody-drug conjugate according to the present invention, or a salt thereof as an active component, and a pharmaceutically acceptable formulation component. Furthermore, the present invention refers to a compound of the following:

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX), wherein (maleimid-N-yl)- is a group represented by the following formula:

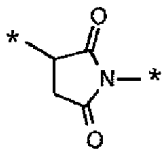


wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

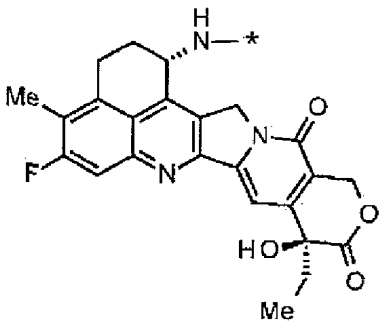


wherein the nitrogen atom of the amino group at position 1 is a connecting position. The present invention also refers to a linker having the following formula: -(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-

wherein -(Succinimid-3-yl-N)- has a structure represented by the following formula:

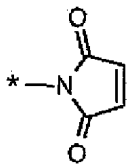


which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1. The present invention also refers to a compound of HO-$CH_2$-C(=O)-(NH-DX), wherein -(NH-DX) is a group represented by the following formula:

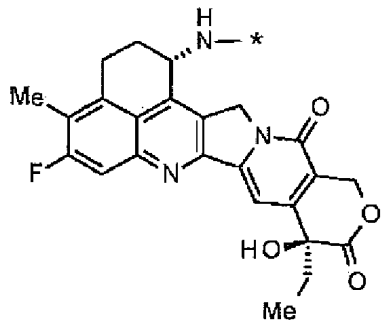


wherein the nitrogen atom of the amino group at position 1 is a connecting position. In another aspect, the present invention refers to a method for preparing an antibody-drug conjugate, wherein the antibody is treated in a reducing condition and thereafter reacted with a compound having following formula:

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX) wherein, (maleimid-N-yl)- is a group represented by the following formula:



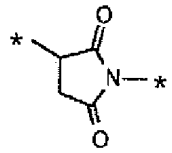
wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:



$L^c$ represents $-CH_2-$ or $-C(=O)-$,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

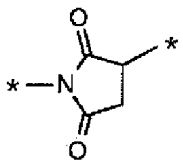
[Formula 3]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

-(N-ly-3-diminiccuS)- has a structure represented by the following formula:

[Formula 4]



which is connected to $L^2$ at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

cyc.Hex(1,4) represents a 1,4-cyclohexylene group, and when $L^2$ is $-S-(CH_2)n^6-C(=O)-$, $L^1$ is $-C(=O)$-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)-.

[0013]

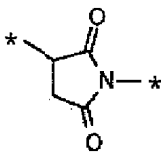
The present disclosure further relates to each of the followings.

wherein $R^2$ and $R^3$ each represents a hydrogen atom,

$L^c$ represents -C(=O)-,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

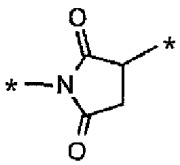
[Formula 6]



which is connected to the antibody at position 3 thereof and is connedted to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

-(N-ly-3-diminiccuS)- has a structure represented by the following formula:

[Formula 7]



which is connected to $L^2$ at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

cyc.Hex(1,4) represents a 1,4-cyclohexylene group, and when $L^2$ is -S-$(CH_2)n^6$-C(=O)-, $L^1$ is -C(=O)-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)-.

[0016]

-$CH_2$-C(=O)-NH-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

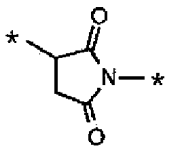
-C(=O)-$CH_2CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

-C(=O)-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)-S-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

[0018]

Wherein, -(Succinimid-3-yl-N)- has a structure represented by the following formula:

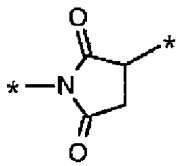
[Formula 8]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

-(N-ly-3-diminiccuS)- has a structure represented by the following formula:

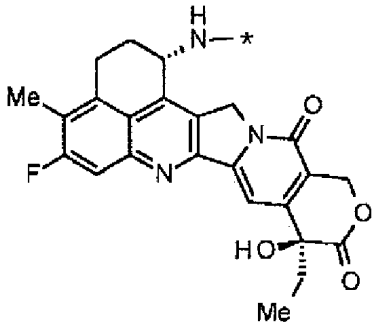
[Formula 9]



which is connected to $L^2$ at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1, cyc.Hex(1,4) represents a 1,4-cyclohexylene group,

-(NH-DX) represents a group represented by the following formula:

[Formula 10]




wherein the nitrogen atom of the amino group at position 1 is the connectig position, and

-GGFG- represents a peptide residue of -Gly-Gly-Phe-Gly-.

[0019]

[17] The antibody-drug conjugate according to any one of [1] to [9] and [11] to [14], wherein the drug-linker structure moiety having a drug connected to $-L^1-L^2-L^p-NH-(CH_2)n^1-L^a-L^b-L^c-$ is one drug-linker structure selected from the following group:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX)

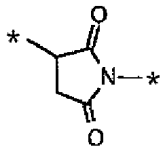
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX)

-(Succinimid-3-yl-N)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX).

[0020]

In the above, -(Succinimid-3-yl-N)- has a structure represented by the following formula:

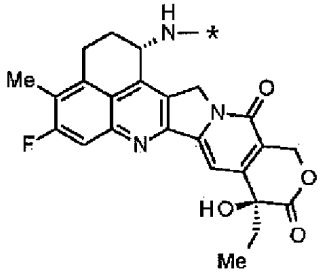
[Formula 11]



which is connected to the antibody at position 3 thereof and connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1, and

-(NH-DX) represents a group represented by the following formula:

[Formula 12]

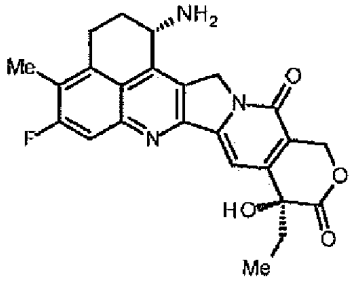


wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0021]

[18] An antibody-drug conjugate wherein an antitumor compound represented by the following formula:

[Formula 13]



is conjugated to an antibody via a linker having a structure represented by the following formula:

$-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-L^b-L^c-$.

Here, the antibody is connected to the terminal of $L^1$, the antitumor compound is connected to the terminal of $L^c$,

wherein

$n^1$ represents an integer of 0 to 6,

$L^1$ represents -(Succinimid-3-yl-N)-$(CH_2)n^2$-C(=O)- and is connected to the antibody via a thioether bond which is formed at a disulfide bond site present in a hinge part of the antibody,

wherein $n^2$ represents an integer of 2 to 8,

$L^2$ represents -NH-$(CH_2-CH_2-O)n^5-CH_2-CH_2$-C(=O)- or a single bond,

wherein $n^5$ represents an integer of 1 to 6,

$L^P$ represents a tetrapeptide residue of GGFG,

$L^a$ represents -O- or a single bond,

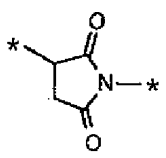
$L^b$ represents $-CR^2(-R^3)-$ or a single bond,

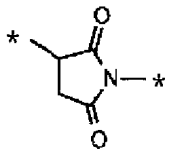
wherein $R^2$ and $R^3$ each represents a hydrogen atom,

$L^c$ represents -C(=O)-, and

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

[Formula 14]

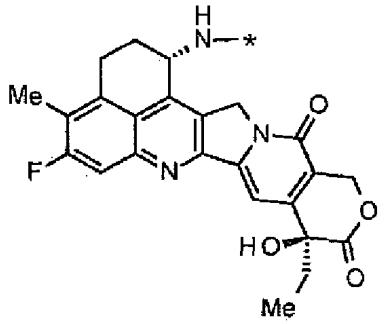




which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1, and

-(NH-DX) represents a group represented by the following formula:

[Formula 16]



wherein the nitrogen atom of the amino group at position 1 is a connection position.

[0024]

[24] The antibody-drug conjugate according to [23], wherein the drug-linker structure moiety having a drug connected to $-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-L^b-L^c-$ is one drug-linker structure selected from the following group:

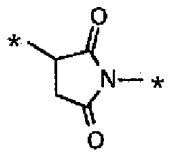
-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX)

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX)

-(Succinimid-3-yl-N)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX).

In the above, -(Succinimid-3-yl-N)- has a structure represented by the following formula:

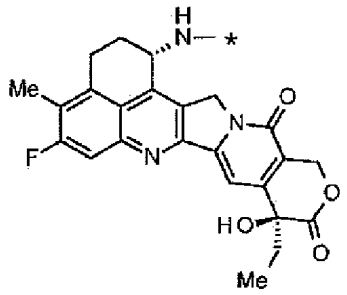
[Formula 17]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1, and

-(NH-DX) represents a group represented by the following formula:

[Formula 18]



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0025]

[25] The antibody-drug conjugate according to any one of [1] to [24], wherein an average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 1 to 10.

wherein $n^7$ represents an integer of 1 to 6, $R^1$ represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^8-COOH$, or $-(CH_2)n^9-OH$, $n^8$ represents an integer of 1 to 4, $n^9$ represents an integer of 1 to 6,

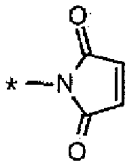
$L^b$ represents $-CR^2(-R^3)-$, $-O-$, $-NR^4-$, or a single bond,

wherein $R^2$ and $R^3$ each independently represent a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^a-NH_2$, $-(CH_2)n^b-COOH$, or $-(CH_2)n^c-OH$, $R^4$ represents a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, $n^a$ represents an integer of 0 to 6, $n^b$ represents an integer of 1 to 4, $n^c$ represents an integer of 1 to 4, provided that when $n^a$ is 0, $R^2$ and $R^3$ are not the same as each other,

$L^c$ represents $-CH_2-$ or $-C(=O)-$,

(maleimid-N-yl)- is a group represented by the following formula:

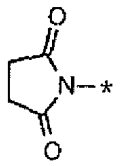
[Formula 19]



whherein the nitrogen atom is a connecting position,

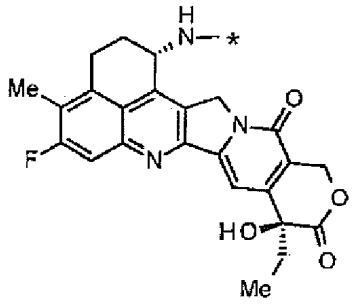
(Pyrrolidine-2,5-dione-N-yl) is a group represented by the following formula:

[Formula 20]



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

[Formula 21]




wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0029]

[40] The drug-linker intermediate compound according to [39], wherein $L^c$ is -C(=O)-.

[41] The drug-linker intermediate compound according to [39] or [40], wherein $L^p$ is a peptide residue consisting of 4 amino acids.

[42] The drug-linker intermediate compound according to any one of [39] to [41], wherein $L^p$ is -GGFG-.

[43] The drug-linker intermediate compound according to any one of [39] to [42], wherein $-NH-(CH_2)n^1-L^a-L^b-$ is

$-NH-CH_2CH_2-$,

$-NH-CH_2CH_2CH_2-$,

$-NH-CH_2CH_2CH_2CH_2-$,

$-NH-CH_2CH_2CH_2CH_2CH_2-$,

$-NH-CH_2-O-CH_2-$, or

$-NH-CH_2CH_2-O-CH_2-$.

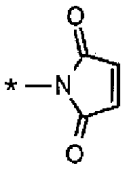
(Pyrrolidine-2,5-dione-N-yl)-O-C(=O)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX), or

(Pyrrolidine-2,5-dione-N-yl)-O-C(=O)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX).

[0031]

In the above, (maleimid-N-yl)- is a group represented by the following formula:

[Formula 22]

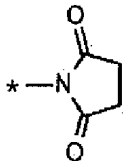


wherein the nitrogen atom is a connecting position,

X represents a halogen atom,

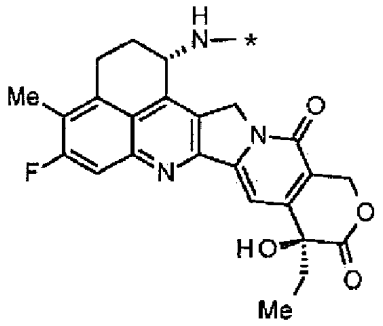
(Pyrrolidine-2,5-dione-N-yl)- is a group represented by the following formula:

[Formula 23]



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

[Formula 24]



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0032]

[51] A compound of the following:

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

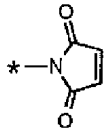
(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX) (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX) (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX)

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX) (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX) (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX) (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX), or (maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX).

[0033]

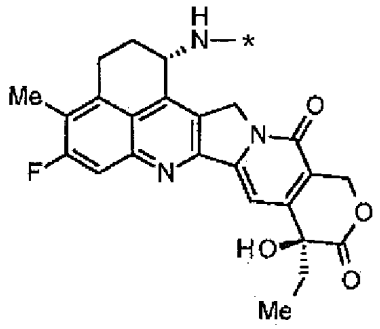
In the above, (maleimid-N-yl)- is a group represented by the following formula: [Formula 25]



wherein the nitrogen atom is a connecting position, and

-(NH-DX) is a group represented by the following formula:

[Formula 26]



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0034]

[52] A compound of the following:

(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

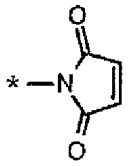
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX) or

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX).

[0035]

In the above, (maleimid-N-yl)- is a group represented by the following formula:

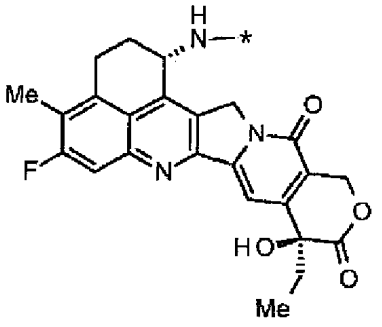
[Formula 27]



wherein the nitrogen atom is a connecting position, and

-(NH-DX) is a group represented by the following formula:

[Formula 28]



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0036]

[53] A compound selected from the following group:

$NH_2$-$CH_2CH_2$-C(=O)-(NH-DX),

$NH_2$-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

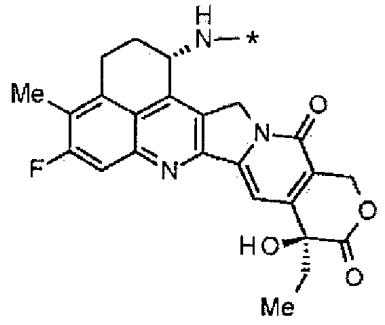
$NH_2$-$CH_2$-O-$CH_2$-C(=O)-(NH-DX),

$NH_2$-$CHCH_2$-O-$CH_2$-C(=O)-(NH-DX), and

HO-$CH_2$-C(=O)-(NH-DX)

wherein -(NH-DX) is a group represented by the following formula:

[Formula 29]



$L^P$ represents a peptide residue consisting of 2 to 7 amino acids selected from phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, and aspartic acid,

$n^1$ represents an integer of 0 to 6,

$L^a$ represents $-C(=O)-NH-$, $-NR^1-(CH_2)n^7-$, $-O-$, or a single bond,

wherein $n^7$ represents an integer of 1 to 6, $R^1$ represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^8-COOH$, or $-(CH_2)n^9-OH$, $n^8$ represents an integer of 1 to 4, $n^9$ represents an integer of 1 to 6,

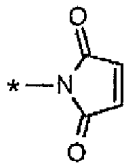
$L^b$ represents $-CR^2(-R^3)-$, $-O-$, $-NR^4-$, or a single bond,

wherein $R^2$ and $R^3$ each independently represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^a-NH_2$, $-(CH_2)n^b-COOH$, or $-(CH_2)n^c-OH$, $R^4$ represents a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, $n^a$ represents an integer of 0 to 6, $n^b$ represents an integer of 1 to 4, $n^c$ represents an integer of 1 to 4, provided that when $n^a$ is 0, $R^2$ and $R^3$ are not the same as each other,

$L^c$ represents $-CH_2-$ or $-C(=O)-$,

(maleimid-N-yl)- is a group represented by the following formula:

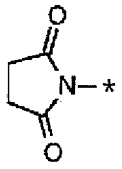
[Formula 33]



wherein the nitrogen atom is a connecting position,

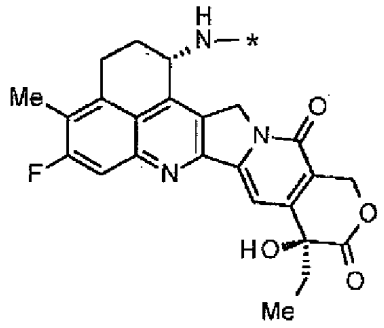
(Pyrrolidine-2,5-dione-N-yl) is a group represented by the following formula:

[Formula 34]



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

[Formula 35]



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0042]

[58] The production method according to [57], wherein the method for conjugating a drug-linker moiety to an antibody is

a method of reducing the antibody and thereafter forming a thioether bond by the reaction with the compound in which Q is a maleimidyl group or X-$CH_2$-C(=O)-NH-,

a method of forming an amide bond by the reaction with the compound in which Q is (Pyrrolidine-2,5-dione-N-yl)-O-C(=O)-, or

a method of reacting the antibody with a compound represented by the formula $Q^1$-$L^{1a}$-$Q^2$

[wherein $Q^1$ represents (Pyrrolidine-2,5-dione-N-yl)-O-C(=O)-, (3-Sulfo-pyrrolidine-2,5-dione-N-yl)-O-C(=O)-, $R^Q$-O-C(=N)-, or O=C=N-,

$L^{1a}$- represents -cyc.Hex(1,4)-$CH_2$-, an alkylene group having 1 to 10 carbon atoms, a phenylene group, -$(CH_2)n^4$-C(=O)-, -$(CH_2)n^{4a}$-NH-C(=O)-$(CH_2)n^{4b}$-, or -$(CH_2)n^{4a}$-NH-C(=O)-cyc.Hex(1,4)-$CH_2$-,

$Q^2$ represents (maleimid-N-yl), a halogen atom, or -S-S-(2-Pyridyl),

$R^Q$ represents an alkyl group having 1 to 6 carbon atoms,

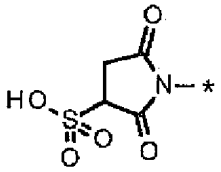
$n^4$ represents an integer of 1 to 8,

$n^{4a}$ represents an integer of 0 to 6, $n^{4b}$ represents an integer of 1 to 6,

(3-Sulfo-pyrrolidine-2,5-dione-N-yl)- is a group represented by the following formula:

[0043]

[Formula 36]

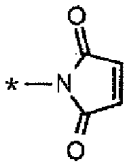


[0044]

[0047]

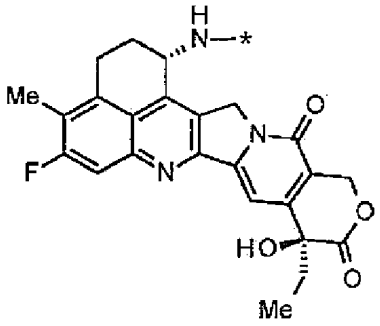
In the above, (maleimid-N-yl)- is a group represented by the following formula:

[Formula 37]



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

[Formula 38]




wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[0048]

[68] An antibody-drug conjugate obtained by forming a thioether bond at a sulfide bond site present in a hinge part of an antibody, wherein the antibody is treated in a reducing condition and thereafter reacted with a compound selected from the compound group shown below:

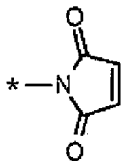
(maleimid-N-yl)-$CH_2CH_2$-C(=O)-NH-$CH_2CH_2O$-$CH_2CH_2O$-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-(NH-DX),

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX), or

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-O-$CH_2$-C(=O)-(NH-DX).

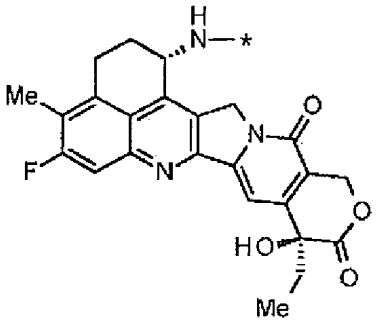
In the above, (maleimid-N-yl)- is a group represented by the following formula:

[Formula 39]



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:

[Formula 40]




wherein the nitrogen atom of the amino group at position 1 is a connceting position.

[0049]

[69] The antibody-drug conjugate according to [67] or [68], wherein an average number of units of the selected one drug-linker structure conjugated per antibody is in a range of from 1 to 10.

wherein $n^7$ represents an integer of 1 to 6, $R^1$ represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^8-COOH$, or $-(CH_2)n^9-OH$, $n^8$ represents an integer of 1 to 4, $n^9$ represents an integer of 1 to 6,

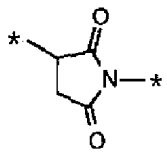
$L^b$ represents $-CR^2(-R^3)-$, $-O-$, $-NR^4-$, or a single bond,

wherein $R^2$ and $R^3$ each independently represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^a-NH_2$, $-(CH_2)n^b-COOH$, or $-(CH_2)n^c-OH$, $R^4$ represents a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, $n^a$ represents an integer of 0 to 6, $n^b$ represents an integer of 1 to 4, $n^c$ represents an integer of 1 to 4, provided that when $n^a$ is 0, $R^2$ and $R^3$ are not the same each other,

$L^c$ represents $-CH_2-$ or $-C(=O)-$,

-(Succinimid-3-yl-N)- has a structure represented by the following formula:

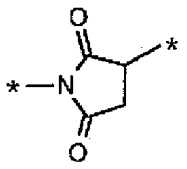
[Formula 41]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

-(N-ly-3-diminiccuS)- has a structure represented by the following formula:

[Formula 42]



which is connected to $L^2$ at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1,

cyc.Hex(1,4) represents a 1,4-cyclohexylene group, and when $L^2$ is -S-$(CH_2)n^6$-C(=O)-, $L^1$ is -C(=O)-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)-.

[0051]

[77] The linker according to [76], which is selected from the following group, provided that the left terminal is a connectiong position with the antibody and the right terminal is a connecting position with the antitumor compound:

-(Succinimid-3-yl-N)-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2$-C(=O)-

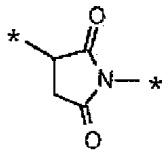
-(Succinimid-3-yl-N)-$CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2CH_2CH_2$-C(=O)-

In the linker having a structure represented by -(Succinimid-3-yl-N)-$(CH_2)n^2$-C(=O)- of $L^1$, "-(Succinimid-3-yl-N)-" has a structure represented by the following formula:

[0096]

[Formula 44]

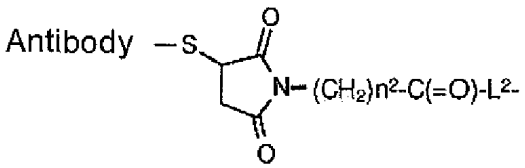


[0097]

Position 3 of the above partial structure is a connecting position to the antibody. The bond to the antibody at position 3 is characterized by bonding with thioether formation. On the other hand, the nitrogen atom at position 1 of the structure moiety is connected to the carbon atom of methylene which is present within the linker including the structure. Specifically, -(Succinimid-3-yl-N)-$(CH_2)n^2$-C(=O)-$L^2$- is a structure represented by the following formula (herein, "antibody-S-" originates from an antibody).

[0098]

[Formula 45]




[0099]

In the formula, $n^2$ is an integer of 2 to 8, and preferably 2 to 5.

[0100]

In the linker having a structure represented by -$CH_2$-C(=O)-NH-$(CH_2)n^3$-C(=O)- of $L^1$, $n^3$ is an integer of 1 to 8, preferably 2 to 6. This linker is connected to the antibody at its carbon atom of terminal methylene and has the following structure for connecting by thioether formation, as with the preceding linker (herein, "antibody-S-" originates from an antibody).

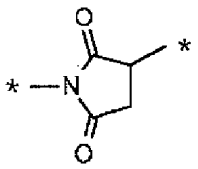
Antibody-S-$CH_2$-C(=O)-NH-$(CH_2)n^3$-C(=O)-$L^2$-.

[0101]

In the linker having a structure represented by -C(=O)-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)- of $L^1$, "-(N-ly-3-diminiccuS)-" has a structure represented by the following formula:

[0102]

[Formula 46]



[0103]

In this structure moiety, the nitrogen atom at position 1 is connected to the carbon atom of methylene present in the linker structure containing this structure. The carbon atom at position 3 is connected to the terminal sulfur atom of -S-$(CH_2)n^6$-C(=O)- of $L^2$ in the linker. This moiety -S-$(CH_2)n^6$-C(=O)- of $L^2$ in the linker forms a combined linker structure only with -C(=O)-cyc.Hex(1,4)-$CH_2$-(N-ly-3-diminiccuS)- of $L^1$ in

each reaction formula. Specifically, they are referred to as a "compound of the formula (1)", a "compound (1)", or the like. The compounds with numbers other than those are also described similarly.

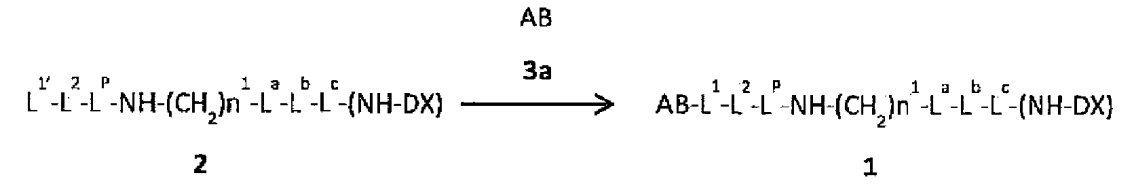
[0129]

1. Production method 1

The antibody-drug conjugate represented by the formula (1) in which the antibody is bound to the linker structure via thioether can be produced by the following method, for example.

[0130]

[Formula 48]

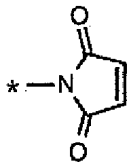



[0131]

[In the formula, AB represents an antibody with a sulfhydryl group, and $L^{1'}$ represents $L^1$ linker structure in which the linker terminal is a maleimidyl group (formula shown below)

[0132]

[Formula 49]



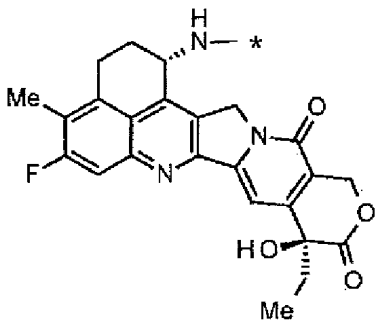
[0133]

(in the formula, the nitrogen atom is the connecting position)

or the terminal is halogen, and represents a group in which the -(Succinimid-3-yl-N)- moiety in -(Succinimid-3-yl-N)-$(CH_2)n^2$-C(=O)- of $L^1$ is a maleimidyl group or a halogen-$CH_2$C(=O)NH-$(CH_2)n^3$-C(=O)- group in which terminal methylene in -$CH_2$C(=O)NH-$(CH_2)n^3$-C(=O)- of $L^1$ is halogenated to form haloacetamide. Further, the -(NH-DX) represents a structure represented by the following formula:

[0134]

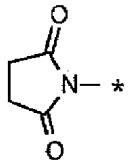
[Formula 50]




[0135]

and it represents a group that is derived by removing one hydrogen atom of the amino group at position 1 of exatecan. Further, the compound of the formula (1) in the above reaction formula is described as a structure in which one structure moiety from drug to the linker terminal connects to one antibody. However, it is only the description given for the sake of convenience, and there are actually many cases in which a plurality of the structure moieties are connected to one antibody molecule. The same applies to the explanation of the production method described below.]

[Formula 53]

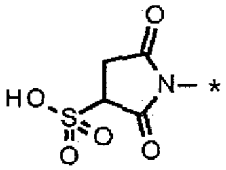


[0147]

wherein the nitrogen atom as a connecting position, and (3-Sulfo-pyrrolidine-2,5-dione-N-yl)- is a group represented by the following formula:

[0148]

[Formula 54]



[0149]

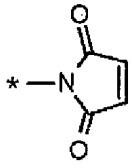
wherein the nitrogen atom is a connectiong position, and this sulfonic acid is capable of forming a lithium salt, sodium salt, or potassium salt, and preferably sodium salt,

cyc.Hex(1,4) represents a 1,4-cyclohexylene group,

(maleimid-N-yl) is a group represented by the following formula:

[0150]

[Formula 55]



[0151]

wherein the nitrogen atom is a connecting position,

$L^a$ represents $-C(=O)-NH-$, $-NR^1-(CH_2)n^7-$, $-O-$, or a single bond,

wherein $n^7$ represents an integer of 1 to 6, $R^1$ represents a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^8-COOH$, or $-(CH_2)n^9-OH$, $n^8$ represents an integer of 1 to 4, $n^9$ represents an integer of 1 to 6,

$L^b$ represents $-CR^2(-R^3)-$, $-O-$, $-NR^4-$, or a single bond,

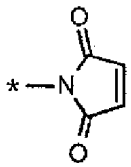
wherein $R^2$ and $R^3$ each independently represent a hydrogen atom, an alkyl group having 1 to 6 carbon atoms, $-(CH_2)n^a-NH_2$, $-(CH_2)n^b-COOH$, or $-(CH_2)n^c-OH$, $R^4$ represents a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, $n^a$ represents an integer of 0 to 6, $n^b$ represents an integer of 1 to 4, $n^c$ represents an integer of 1 to 4, provided that when $n^a$ is 0, $R^2$ and $R^3$ are not the same as each other,

$L^c$ represents $-CH_2-$ or $-C(=O)-$,

(maleimid-N-yl)- is a group having a structure represented by the following formula:

[0193]

[Formula 67]



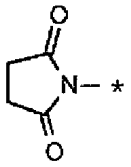
[0194]

(in the formula, the nitrogen atom is the connecting position), (Pyrrolidine-2,5-dione-N-yl)- is a group

having a structure represented by the following formula:

[0195]

[Formula 68]

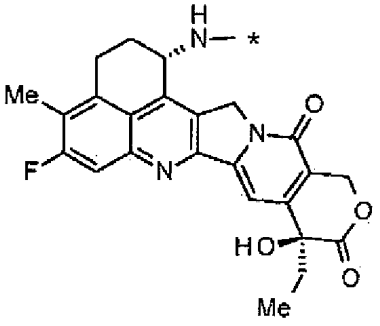


[0196]

(in the formula, the nitrogen atom is the connecting position), -(NH-DX) is a group having a structure represented by the following formula:

[0197]

[Formula 69]



[0198]

(in the formula, the nitrogen atom of the amino group at position 1 is the connceting position).

[0199]

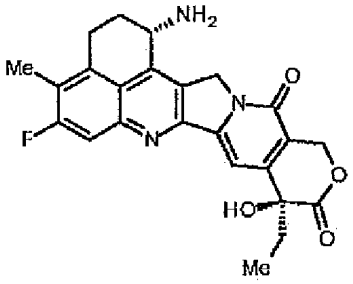
A compound in which $L^C$ is -C(=O)- is preferred as a production intermediate.

As for the peptide residue of $L^P$, a compound of an amino acid residue consisting of an amino acid selected from phenylalanine, glycine, valine, lysine, citrulline,

## Claims

[Claim 1]

An antibody-drug conjugate wherein an antitumor compound represented by the following formula:

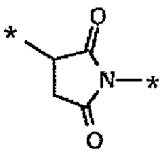


is conjugated to an antibody via a drug-linker having a structure represented by the following formula:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX),

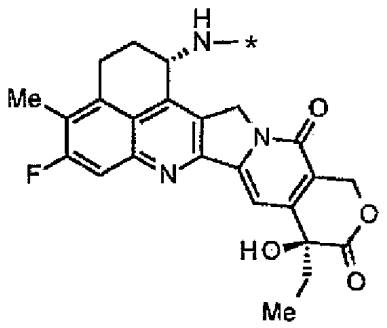
wherein

-(Succinimid-3-yl-N)- has a structure represented by the following formula:



which is connected to the antibody at position 3 thereof via a thioether bond and connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1, and

-(NH-DX) represents a group represented by the following formula:



wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[Claim 2]

The antibody-drug conjugate according to claim 1, wherein an average number of the drug-linker structure conjugated per antibody is in a range of from 2 to 8.

[Claim 3]

The antibody-drug conjugate according to claim 1, wherein an average number of the drug-linker structure conjugated per antibody is in a range of from 3 to 8.

[Claim 4]

The antibody-drug conjugate according to claim 1, wherein the antibody is an anti-B7-H3 antibody, an anti-CD30 antibody, an anti-CD33 antibody, or an anti-CD70 antibody.

[Claim 5]

The antibody-drug conjugate according to claim 1, wherein the antibody is an anti-B7-H3 antibody.

[Claim 6]

The antibody-drug conjugate according to claim 5, wherein the anti-B7-H3 antibody has a CDRH1 comprising an amino acid sequence represented by SEQ ID NO: 3, a CDRH2 comprising an amino acid sequence represented by SEQ ID NO: 4, and a CDRH3 comprising an amino acid sequence represented by SEQ ID NO: 5 as heavy chain complementarity determining regions, and a CDRL1 comprising an amino acid sequence represented by SEQ ID NO: 6, a CDRL2 comprising an amino acid sequence represented by SEQ ID NO: 7, and a CDRL3 comprising an amino acid sequence represented by SEQ ID NO: 8 as light chain complementarity determining regions.

[Claim 7]

The antibody-drug conjugate according to claim 5, wherein the anti-B7-H3 antibody has a heavy chain variable region and

a light chain variable region selected from the group consisting of

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 13,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 14,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 15,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 16,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 17,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 18,

a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 9 and a light chain variable region comprising an amino

acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 19,
a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 12 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 13,
a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 12 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 14,
a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 12 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 15, and
a heavy chain variable region comprising an amino acid sequence described in amino acid positions 20 to 141 in SEQ ID NO: 12 and a light chain variable region comprising an amino acid sequence described in amino acid positions 21 to 128 in SEQ ID NO: 16.

[Claim 8]

The antibody-drug conjugate according to claim 5, wherein the anti-B7-H3 antibody comprises a heavy chain and a light chain selected from the group consisting of
a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 13,
a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 14,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 15,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 16,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 17,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 18,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 9 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 19,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 12 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 13,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 12 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 14,

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 12 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 15, and

a heavy chain comprising an amino acid sequence described in amino acid positions 20 to 471 in SEQ ID NO: 12 and a light chain comprising an amino acid sequence described in amino acid positions 21 to 233 in SEQ ID NO: 16.

[Claim 9]

The antibody-drug conjugate according to claim 8, wherein the anti-B7-H3 antibody lacks an amino acid at the carboxyl terminus of the amino acid sequence represented by SEQ ID NO: 9 or 12 in the heavy chain.

[Claim 10]

A drug containing the antibody-drug conjugate according to claim 1, or a salt thereof.

[Claim 11]

An antitumor drug and/or anticancer drug containing the antibody-drug conjugate according to claim 1, or a salt thereof.

[Claim 12]

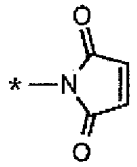
A pharmaceutical composition containing the antibody-drug conjugate according to claim 1, or a salt thereof as an active component, and a pharmaceutically acceptable formulation component.

[Claim 13]

A compound of the following:

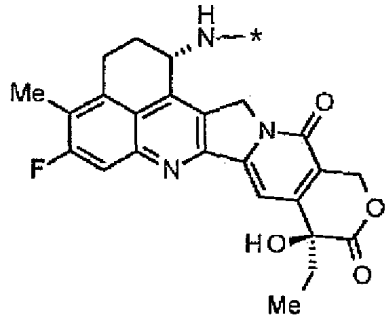
(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX),

wherein (maleimid-N-yl)- is a group represented by the following formula:



wherein the nitrogen atom is a connecting position, and

-(NH-DX) is a group represented by the following formula:



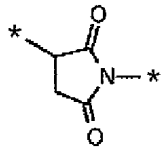
wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[Claim 14]

A linker having the following formula:

-(Succinimid-3-yl-N)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-

wherein -(Succinimid-3-yl-N)- has a structure represented by the following formula:



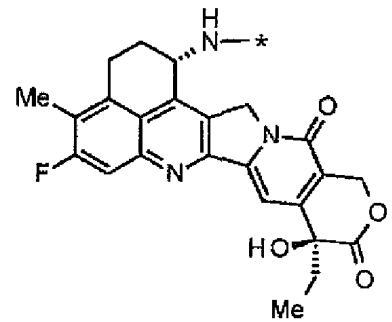
which is connected to an antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1.

[Claim 15]

A compound of

HO-$CH_2$-C(=O)-(NH-DX),

wherein -(NH-DX) is a group represented by the following formula:



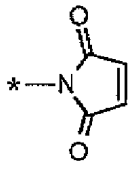
wherein the nitrogen atom of the amino group at position 1 is a connecting position.

[Claim 16]

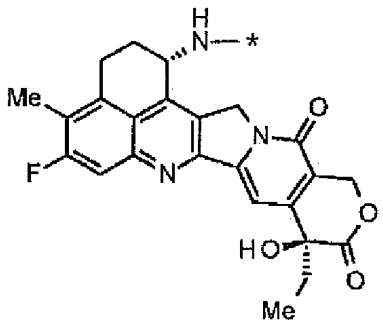
A method for preparing an antibody-drug conjugate, wherein the antibody is treated in a reducing condition and thereafter reacted with a compound having following formula:

(maleimid-N-yl)-$CH_2CH_2CH_2CH_2CH_2$-C(=O)-GGFG-NH-$CH_2$-O-$CH_2$-C(=O)-(NH-DX)

wherein, (maleimid-N-yl)- is a group represented by the following formula:



wherein the nitrogen atom is a connecting position, and -(NH-DX) is a group represented by the following formula:




wherein the nitrogen atom of the amino group at position 1 is a connceting position.

# ANTIBODY-DRUG CONJUGATE

Abstract

As an antitumor drug which is excellent in terms of antitumor effect and safety, there is provided an antibody-drug conjugate in which an antitumor compound represented by the following formula is conjugated to an antibody via a linker having a structure represented by the following formula: $-L^1-L^2-L^P-NH-(CH_2)n^1-L^a-L^b-L^c-$ wherein the antibody is connected to the terminal of $L^1$, and the antitumor compound is connected to the terminal of $L^c$ with the nitrogen atom of the amino group at position 1 as a connecting position.

No Suitable Figure

