

SUMMARY

[0007] One aspect pertains to an anti-human epidermal growth factor receptor (hEGFR) antibody drug conjugate comprising the structure of Formula (I) conjugated to an antibody Ab:

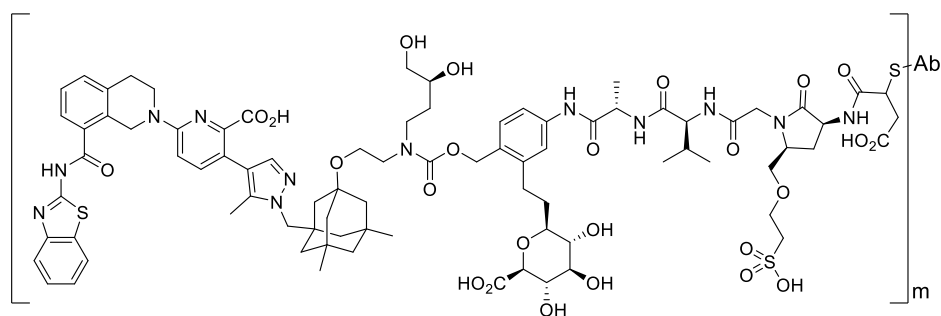


[0007a] Another aspect pertains to an anti-human epidermal growth factor receptor (hEGFR) antibody-drug conjugate comprising the following structure:

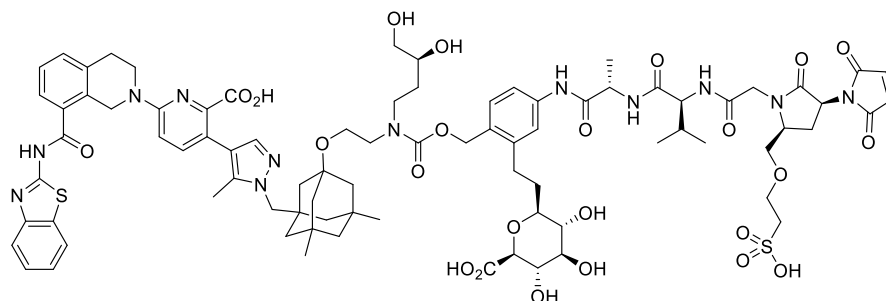


wherein Ab is an IgG1 anti-hEGFR antibody comprising a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5; and wherein m is 2.

[0008] Another aspect pertains to an anti-human Epidermal Growth Factor Receptor antibody-drug conjugate comprising the structure of formula (II) conjugated to an antibody Ab:



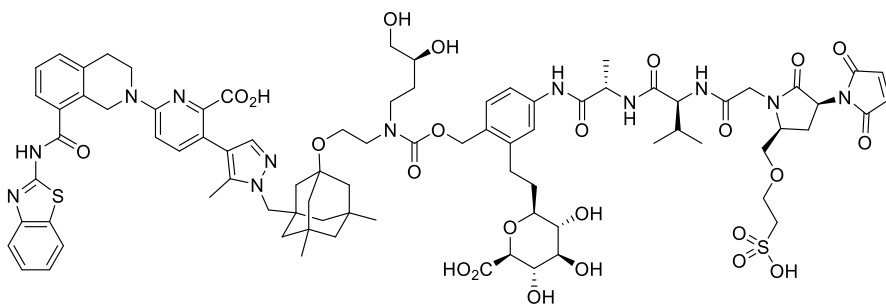
(II),



(IV),

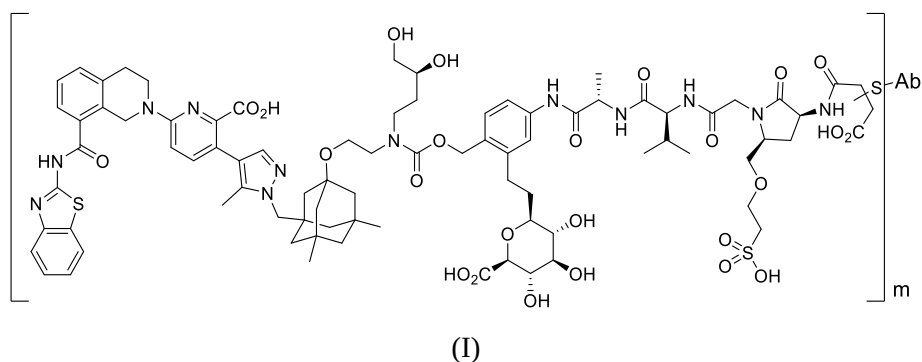
to form an antibody-drug conjugate comprising a drug-linker conjugated to the anti-EGFR antibody, wherein the anti-EGFR antibody comprises a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5. In embodiments, the drug-linker is conjugated to the antibody through C220 of the heavy chain (i.e., C219 of SEQ ID NO: 1).

[0010a] Another aspect pertains to a method of producing an antibody drug conjugate (ADC) comprising a step of conjugating a monoclonal human IgG₁ anti-EGFR antibody with a synthon comprising the structure:



to form an antibody-drug conjugate comprising a drug-linker conjugated to the anti-EGFR antibody, wherein the anti-EGFR antibody comprises a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5; and wherein said drug-linker is conjugated to said antibody through C220 of the heavy chain.

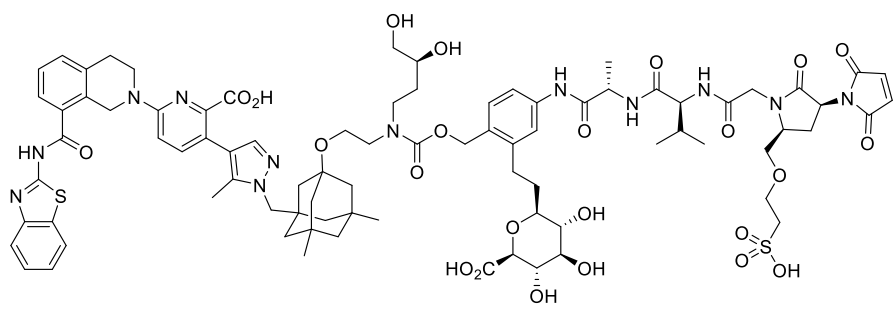
[0010b] Another aspect pertains to a process for the preparation of an anti-human Epidermal Growth Factor Receptor antibody-drug conjugate comprising the following structure:



wherein Ab is an IgG1 anti-human epidermal growth factor receptor antibody comprising a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5; and wherein m is 2; the method comprising the process comprising:

treating an antibody in a buffered aqueous solution with an effective amount of a disulfide reducing agent for about 16-24 hours;

adding to the reduced antibody solution a solution of dimethyl acetamide comprising a synthon having the following structure:

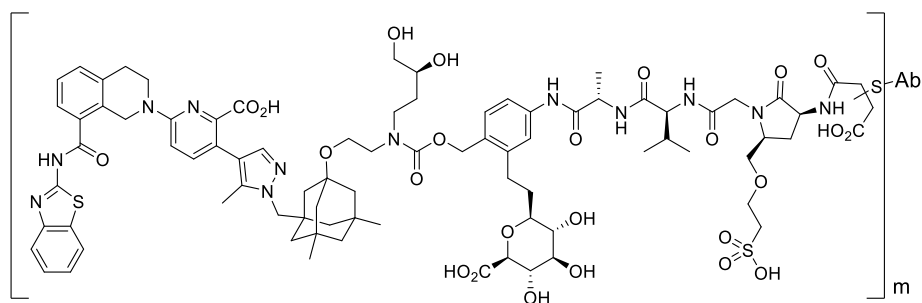


allowing the reaction to run to form the ADC;

wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a Succinimide as measured by electron spray mass spectrometry; and

wherein the ADC is optionally purified by hydrophobic interaction chromatography.

[0011] Another aspect pertains to a process for the preparation of an antibody drug conjugate (ADC) according to Formula (I):



(I),

wherein Ab is an IgG1 anti-human epidermal growth factor receptor antibody comprising a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5; and

m is 2;

the process comprising:

treating an antibody in a buffered aqueous solution with an effective amount of a disulfide reducing agent for about 16-24 hours;

adding to the reduced antibody solution a solution of dimethyl acetamide comprising a synthon having the following structure:

the drug-linker is conjugated to the anti-EGFR antibody through C220 of the heavy chain (i.e., C219 of SEQ ID NO: 1).

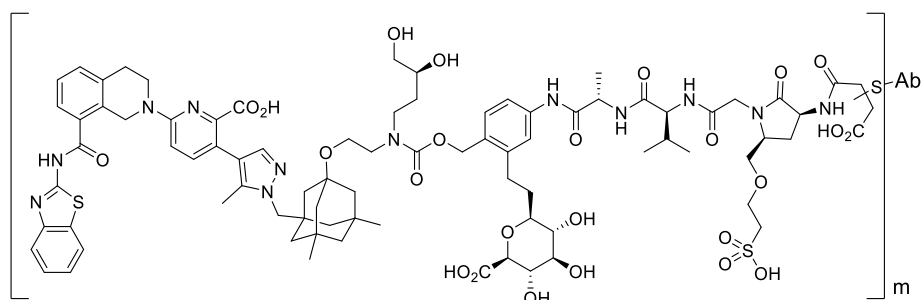
[0012a] Another aspect pertains to an antibody-drug conjugate prepared as described herein.

[0012b] Another aspect pertains to the use of the antibody-drug conjugate as described herein in the manufacture of a medicament for treating non-small cell lung cancer.

[0012c] Another aspect pertains to a pharmaceutical composition comprising an effective amount of the antibody-drug conjugate as described herein and a pharmaceutically acceptable carrier.

[0012d] Another aspect pertains to an antibody-drug conjugate as described herein for use in therapy.

[0013] Another aspect pertains to a process for the preparation of an antibody drug conjugate (ADC) according to Formula (I):



(I),

wherein Ab is an IgG1 anti-human epidermal growth factor receptor antibody comprising a heavy chain comprising the amino acid sequence set forth as NO: 1 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 5; and

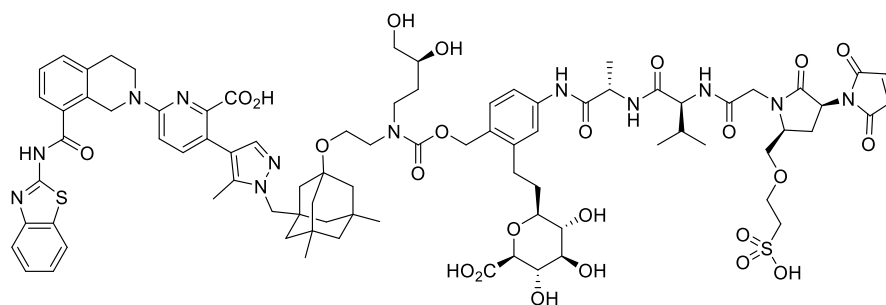
m is 2;

the process comprising:

treating an antibody in a buffered aqueous solution of about pH 7.4 with an effective amount of a disulfide reducing agent at about 4 °C for about 16-24 hours;

allowing the reduced antibody solution to warm to ambient temperature;

adding to the reduced antibody solution a solution of dimethyl acetamide comprising a synthon having the following structure:



(IV);

allowing the reaction to run for about 60 minutes to form the ADC;

quenching with about 2 equivalents of *N*-acetyl-L-cysteine;

purifying with hydrophobic interaction Chromatography (HIC);

purifying with tangential flow filtration (TFF);

hydrolyzing the succinimide with a pH buffer of about 8-9; and