

methyl-L-alanine derivatives are found to be much more cytotoxic than the corresponding esters of simple carboxylic acid and to be 100 times more cytotoxic than their epimers corresponding to N-methyl-D-alanine (U.S. Pat. Nos. 4,137,230; 4,260,608; Kawai, *et al.*, *Chem. Pharm. Bull.* 32: 3441-3451 (1984); Widdison, *et al.*, *J. Med. Chem.* 49:4392-4408 (2006)).

[0006] Maytansinoids were expected to have the capacity to treat many different cancers due to their highly toxic nature and the *in vitro* activities against multiple cancer cell lines. However, the toxicity also made this class of compounds unfavorable in human clinical trials as the side effects were intolerable for many patients (Issel *et al.*, *Cancer Treat. Rev.* 5:199-207 (1978)). Accordingly, targeted delivery of cytotoxic compounds to cancer cells by conjugating toxic drugs to monoclonal antibodies (ADC for antibody drug conjugate) is proposed in order to reduce the side effects.

[0007] Antibody drug conjugates (ADCs) are composed of three key elements: antibody, linker, and drug. The selection of a particular antibody and drug will have a great impact on the efficacy and safety depending on the particular disease. Linker stability and the method by which the drug is conjugated to the antibody plays a critical role in the success or failure of the ADC drug development.

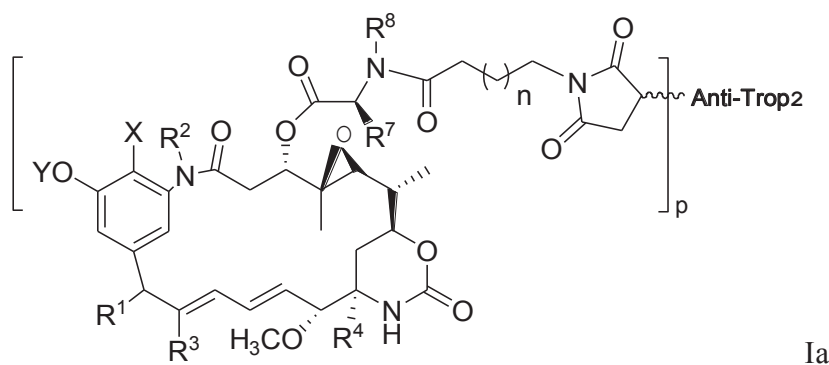
[0008] The efficacy of an ADC depends in part on combination of a variety of parameters, involving not only the specificity of the antibody and the potency of drugs, but also the linker's stability or sensitivity to cleavage, the cell surface triggered the internalization, trafficking, and subsequent release of the active cytotoxic payload. Thus, ADC comprising different drug linkers or with different antibodies against the same target can vary significantly in their utility.

SUMMARY

[0009] In one aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of anti-Trop2 antibody BAT0806, BAT0807 or BAT0808, wherein the amino acid sequence for BAT0806 or BAT0807 is expressed in CHO-BAT cells, and the amino acid sequence for BAT0808 is expressed in CHO-BAT-KF cells. In another aspect, the present disclosure refers to the use of an effective amount of anti-Trop2 antibody BAT0806, BAT0807 or BAT0808 in the manufacture of a medicament for treating cancer, wherein the amino acid

sequence for BAT0806 or BAT0807 is expressed in CHO-BAT cells, and the amino acid sequence for BAT0808 is expressed in CHO-BAT-KF cells. In yet another aspect, the present disclosure refers to the use of an anti-Trop2 antibody BAT0806, BAT0807 or BAT0808 in the manufacture of a medicament for use in combinatorial therapy for treating a tumor, wherein said medicament is to be administered in combination with a second or third antibody against immunoinhibitory receptor to produce more effective treatment.

[0009a] In another aspect, the present disclosure refers to a compound of Formula Ia:



or a salt thereof,

wherein X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C₁ - C₆ alkyl, C₃ - C₆ cycloalkyl, and -C(=O)R₅;

R₁ is selected from the group consisting of hydrogen, -OH, -OC(=O)R₅ and -OR₅;

R₂ is hydrogen or C₁ - C₆ alkyl;

R₃ is methyl, -CH₂OH, or -CH₂C(=O)R₆;

R₄ is -OH or -SH;

R₅ is C₁ - C₆ alkyl or benzyl;

R₆ is C₁ - C₆ alkyl, phenyl or benzyl;

R₇ is hydrogen, C₁ - C₆ alkyl or an amino acid side chain;

R₈ is hydrogen or C₁ - C₆ alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

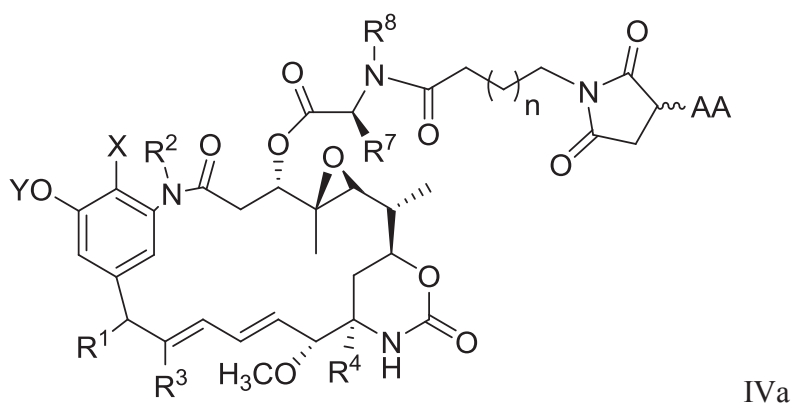
p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

Anti-TROP2 is an anti-TROP2 (TACSTD2) antibody.

[0009b] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a compound as described herein. In yet another aspect, the present disclosure refers

to the use of an effective amount of a compound as described herein or use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition in a patient in need thereof. In yet another aspect, the present disclosure refers to the use of the anti-Trop2 antibody as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for use in combinatorial therapy for treating a tumor, wherein said medicament is to be administered in combination with a second or third antibody enhancing immune surveillance activity to produce more effective treatment.

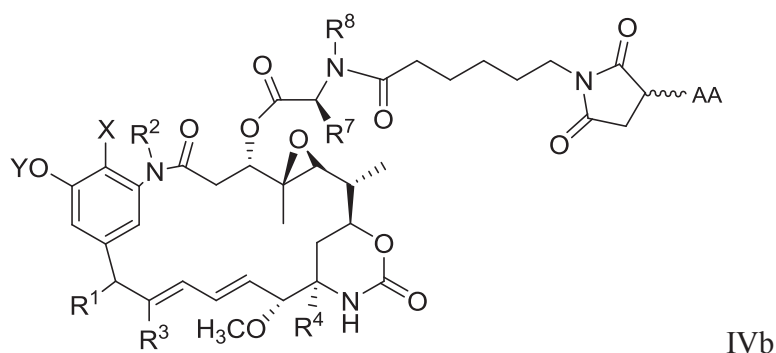
[0009c] In yet another aspect, the present disclosure refers the use of an effective amount of a compound of Formula IVa:



or a salt thereof, in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition characterized by TROP2 positive cells in a patient in need thereof, wherein the compound of Formula IVa is generated as a result of a metabolic chemical reaction following administration of the compound as described herein, or a pharmaceutically acceptable

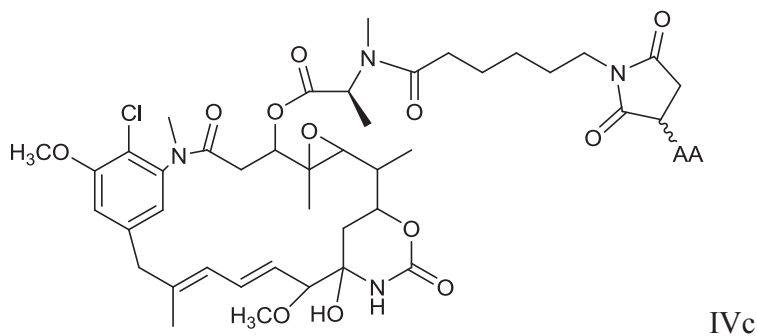
salt thereof, to the patient, wherein AA is an amino acid, thiolated amino acid, or cysteine.

[0009d] In yet another aspect, the present disclosure refers to the use of an effective amount of a compound of Formula IVb:



or a salt thereof, in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition characterized by TROP2 positive cells in a patient in need thereof, wherein the compound of Formula IVb is generated as a result of a metabolic chemical reaction following administration of the compound as described herein, or a pharmaceutically acceptable salt thereof, to the patient, wherein AA is an amino acid or thiolated amino acid, or cysteine.

[0009e] In yet another aspect, the present disclosure refers to the use of an effective amount of a compound of Formula IVc:

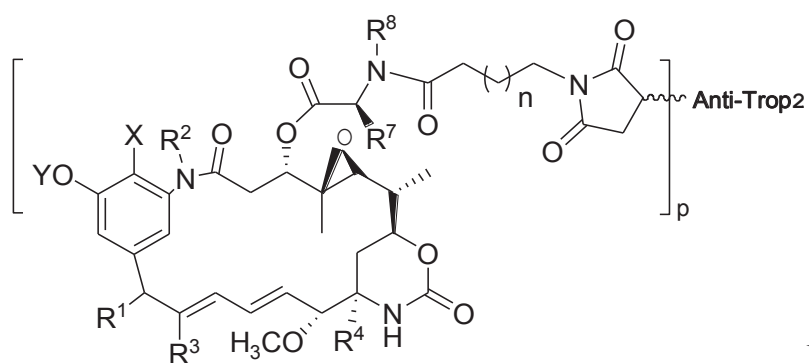
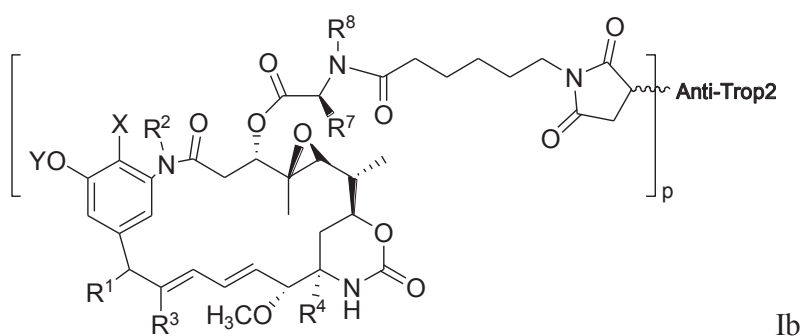


or a salt thereof, in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition characterized by TROP2 positive cells in a patient in need thereof, wherein the compound of Formula IVc is generated as a result of a metabolic chemical reaction following administration of the compound as described herein, or a pharmaceutically acceptable salt thereof, to the patient, wherein AA is an amino acid or thiolated amino acid, or cysteine.

In one example, provided herein is an anti-TROP2 antibody that is conjugated with maytansinoid molecules, thus targeting diseased cells or tissues. The anti-TROP2 antibody binds to an antigen in the diseased cells or tissues. A drug conjugated to the antibody exerts a cytotoxic, cytostatic, or immunosuppressive effect on the antigen-expressing cells to treat or prevent recurrence of TROP2 -positive cancers. The high affinity of the antibody drug conjugate ensures that the cytotoxic maytansinoid targets the tumor cells. Otherwise, the highly toxic maytansinoid will become systemically bound to unintended targets which results in very high and often unacceptable toxicity. The present technology provides a method to treat cancers by exerting cellular inhibitory or killing effect of maytansinoid on the TROP2 positive cells, while minimizing the undesirable side effects of maytansinoid, such as bystander killing effects on antigen negative cells.

[0010] In another example, provided herein is an anti-TROP2 antibody conjugated with a maytansinoid compound, wherein the maytansinoid compound is linked to an anti-TROP2 antibody via a linker that is not acid labile, not peptidase cathepsin sensitive, and does not contain a disulfide bond and provides stability during circulation while being able to release the drug once inside the cells. Such linkers are contemplated to provide stability to the conjugated molecule prior to endocytosis, such as during circulation, to prevent premature degradation of the linker and release of the toxic drug, thus minimize the toxic effect of the drug. In some examples, the maytansinoid-linker portion of the conjugate is N2'-deacetyl- N2'-(6-maleimido-1-oxo-hexyl)-maytansine (3AA-MDC or batansine), or a derivative thereof. In some examples, the conjugate has a drug load of at least one drug molecule per antibody for improved safety and activity. Surprisingly, the antibody remain sufficiently stable for targeted delivery of the drug to target cells despite the high drug load.

[0011] In some examples, provided herein is a maytansinoid linker anti-TROP2 antibody conjugate of Formula Ia or Ib:



or a pharmaceutically acceptable salt or solvate thereof,

wherein

X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C₁ - C₆ alkyl, C₃ - C₆ cycloalkyl, and -C(=O)R⁵;

R¹ is selected from the group consisting of hydrogen, -OH, -OC(=O)R⁵ and -OR⁵;

R² is hydrogen or C₁ - C₆ alkyl;

R³ is methyl, -CH₂OH, or -CH₂C(=O)R⁶;

R⁴ is -OH or -SH;

R⁵ is C₁ - C₆ alkyl or benzyl;

R⁶ is C₁ - C₆ alkyl, phenyl or benzyl;

R⁷ is hydrogen, C₁ - C₆ alkyl or an amino acid side chain;

R⁸ is hydrogen or C₁ - C₆ alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

Anti-TROP2 is an anti-TROP2 antibody

[0012] In another example, provided herein is a composition comprising the above-described maytansinoid linked anti-TROP2 antibody conjugate, such as a compound of Formula Ia.

[0013] In another example, provided herein is a method of preparing the above-described maytansinoid linked anti-TROP2 antibody conjugate which method comprises contacting an anti-TROP2 antibody with one or more maytansinoid compounds described herein capable of being conjugated to the anti-TROP2 antibody.

[0014] In another example, provided herein is a method for targeting a maytansinoid to TROP2 antigen positive cells or tissues with an anti-TROP2 antibody conjugated with maytansinoids described herein.

[0015] In some examples, the anti-TROP2 antibody provided by the present disclosure may be BAT0806, which comprises an amino acid sequence of the anti-Trop2 light chain shown in SEQ ID NO:1, and the anti-TROP2 heavy chain shown in SEQ ID NO:2, wherein the anti-TROP2 antibody or TROP2 antigen binding unit is expressed through an expression vector by CHO-BAT. The host cell line was derived from Chinese hamster ovary cell line CHO-K1 (ATCC#CCL-61), and was adapted to suspension growth. In some examples, the anti-Trop2 antibody provided by the present disclosure may be BAT0807, which comprises an amino acid sequence of the anti-TROP2 light chain shown in SEQ ID NO:3, and the anti-TROP2 heavy chain shown in SEQ ID NO:4, wherein the anti-TROP2 antibody or TROP2 antigen binding unit is expressed through a expression vector by CHO-BAT. The host cell line was derived from from Chinese hamster ovary cell line CHO-K1(ATCC#CCL-61), and was adapted to suspension growth. In other examples, the anti-TROP2 antibody provided by the present disclosure may be BAT0808, which comprises an amino acid sequence of the anti-Trop2 light chain shown in SEQ ID NO:3, and the anti-TROP2 heavy chain shown in SEQ ID NO:4, wherein the anti-TROP2 antibody or TROP2 antigen binding unit is expressed by a expression vector by CHO-BAT-KF. The host cell line was knocked out the α -(1,6)-fucosyltransferase, which was characterized by the expressed antibody has a fucose content of 0-5% and an ADCC-enhanced effect. The host cell was deposited at the China Type Culture Collection with Accession No.: CCTCC NO: C2017127.

In some examples, the anti-TROP2 antibody provided by the present disclosure may be other TROP2 antigen binding units.

[0016] In another example, provided herein is a method for treatment of proliferative disorders such as tumors, inflammatory or immunologic diseases such as graft rejections, and other diseases that can be treated by targeted therapy in a subject in need of the treatment, wherein the disease is characterized by cells comprising an antigen that binds to an anti-TROP2 antibody, said method comprising administering to the subject an effective amount of the anti-TROP2 antibody drug conjugate described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1A and 1B show measuring the aggregation rate of BAT0806 and Batanine-0806 through the dimensional exclusion chromatography.

[0018] FIG. 2A and 2B show measuring the aggregation rate of BAT0808 and Batanine-0808 through the dimensional exclusion chromatography.

[0019] FIG. 3 shows the map of the Sephadex G25 (M) column separating the antibody drug conjugate Batansine-0806.

[0020] FIG.4 shows the map of the Sephadex G25 (M) column separating the antibody drug conjugate BAT0806-CL2A-SN-38.

[0021] FIG.5 shows the map of the Sephadex G25 (M) column separating the antibody drug conjugate Batansine-0808.

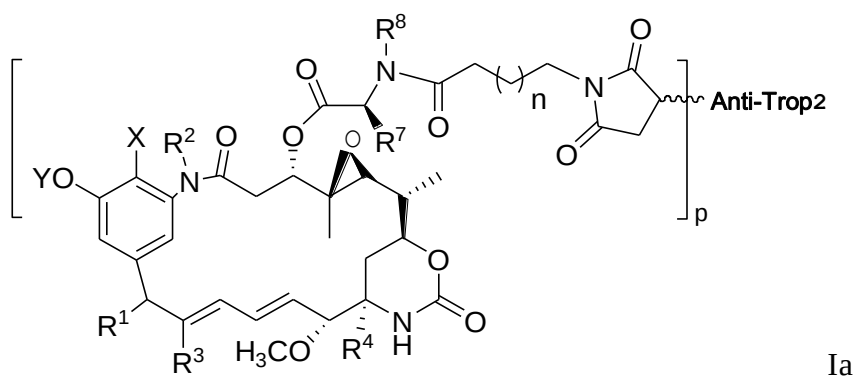
[0022] FIG. 6 shows the Proliferation Inhibitory effect of DM1 and Cys-3AA-MDC towards LO2 cells.

[0023] FIG. 7 shows the Proliferation Inhibitory effect of Batansine-0806, Batansine-0807 and Batansine-0808 towards MDA-MB-468 cells.

[0024] FIG. 8 shows the Proliferation Inhibitory effect of Batansine-0806, Batansine-0807 and Batansine-0808 towards A431 cells

What is claimed is:

1. A pharmaceutical composition comprising an effective amount of anti-Trop2 antibody BAT0806, BAT0807 or BAT0808, wherein the amino acid sequence for BAT0806 or BAT0807 is expressed in CHO-BAT cells, and the amino acid sequence for BAT0808 is expressed in CHO-BAT-KF cells.
2. Use of an effective amount of anti-Trop2 antibody BAT0806, BAT0807 or BAT0808 in the manufacture of a medicament for treating cancer, wherein the amino acid sequence for BAT0806 or BAT0807 is expressed in CHO-BAT cells, and the amino acid sequence for BAT0808 is expressed in CHO-BAT-KF cells.
3. The use of claim 2 wherein said cancer is selected from the group consisting of Trop2 positive cancers, including but not limited to triple negative breast cancers, glioblastoma, medulloblastoma, Urothelial Carcinoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer, Kaposi's sarcoma, pancreatic cancer and lung cancers.
4. Use of an anti-Trop2 antibody BAT0806, BAT0807 or BAT0808 in the manufacture of a medicament for use in combinatorial therapy for treating a tumor, wherein said medicament is to be administered in combination with a second or third antibody against immuno-inhibitory receptor to produce more effective treatment.
5. A compound of Formula Ia:



or a salt thereof,

wherein

X is hydrogen or halo;

Y is selected from the group consisting of hydrogen, C₁ - C₆ alkyl, C₃ - C₆ cycloalkyl, and -C(=O)R⁵;

R¹ is selected from the group consisting of hydrogen, -OH, -OC(=O)R⁵ and -OR⁵;

R² is hydrogen or C₁ - C₆ alkyl;

R³ is methyl, -CH₂OH, or -CH₂C(=O)R⁶;

R⁴ is -OH or -SH;

R⁵ is C₁ - C₆ alkyl or benzyl;

R⁶ is C₁ - C₆ alkyl, phenyl or benzyl;

R⁷ is hydrogen, C₁ - C₆ alkyl or an amino acid side chain;

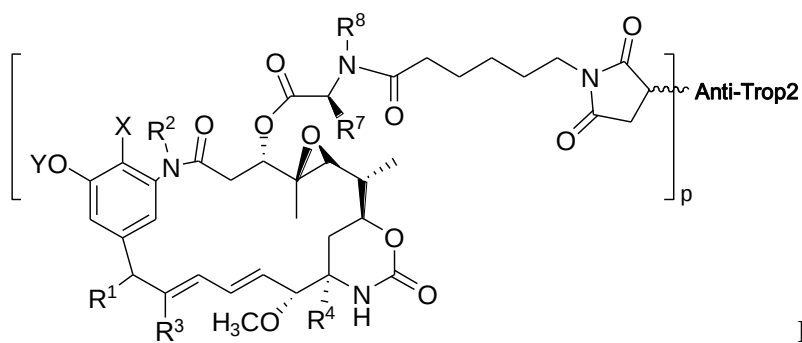
R⁸ is hydrogen or C₁ - C₆ alkyl;

n is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

p is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; and

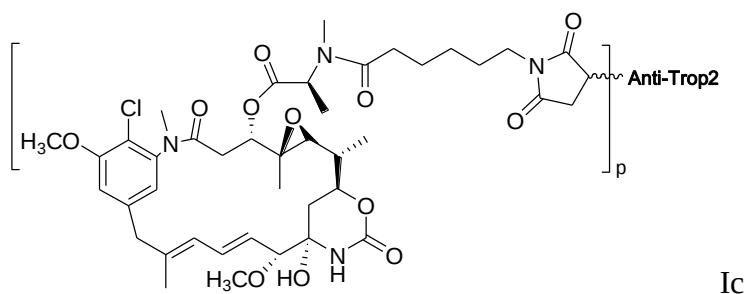
Anti-TROP2 is an anti-TROP2 (TACSTD2) antibody.

6. The compound of claim 5, which is a compound of Formula Ib



or a pharmaceutically acceptable salt or solvate thereof.

7. The compound of claim 5, which is a compound of Formula Ic



or a pharmaceutically acceptable salt or solvate thereof,

wherein Anti-TROP2 is an anti-TROP2 (TACSTD2) antibody.

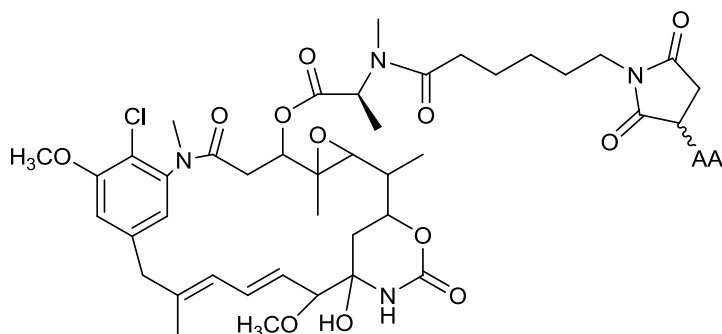
8. The compound of claim 7, wherein the linked drug N2'-deacetyl-N2'-(6-maleimido-1-oxo-hexyl)maytansine) is covalently bonded to the antibody.
9. The compound of any one of claims 5-8, wherein the anti-TROP2 antibody is an antibody comprising BAT0806, BAT0807 or BAT0808 or an antigen-binding fragment thereof.
10. A pharmaceutical composition comprising a compound of any one of claims 5-9.
11. Use of an effective amount of a compound of any one of claims 5-9 or use of the pharmaceutical composition of claim 10 in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition in a patient in need thereof.
12. Use of the anti-Trop2 antibody of claims 5-9 or the pharmaceutical composition of claim 10 in the manufacture of a medicament for use in combinatorial therapy for treating a tumor, wherein said medicament is to be administered in combination with a second or third antibody enhancing immune surveillance activity to produce more effective treatment.
13. Use of an effective amount of a compound of Formula IVa:



14. Use of an effective amount of a compound of Formula IVb:



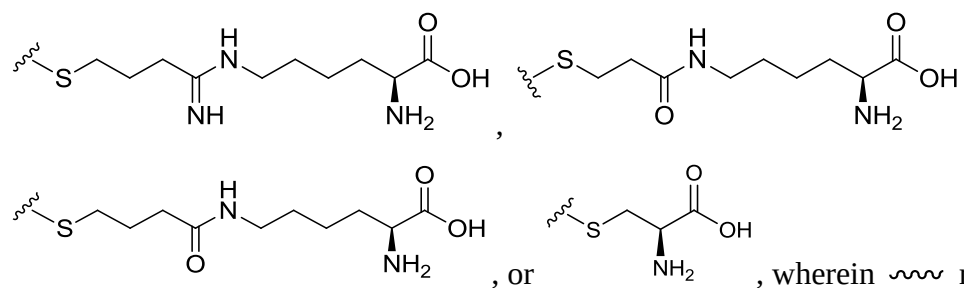
15. Use of an effective amount of a compound of Formula IVc:



IVc

or a salt thereof, in the manufacture of a medicament for treating a proliferative, inflammatory or immunologic disease or condition characterized by TROP2 positive cells in a patient in need thereof, wherein the compound of Formula IVc is generated as a result of a metabolic chemical reaction following administration of the compound of claim 7, or a pharmaceutically acceptable salt thereof, to the patient, wherein AA is an amino acid or thiolated amino acid, or cysteine.

16. The use of any one of claims 12-14, wherein AA is



, or , wherein $\sim$ represents a point of connection to the rest of the molecule.