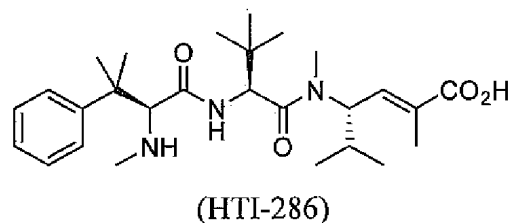
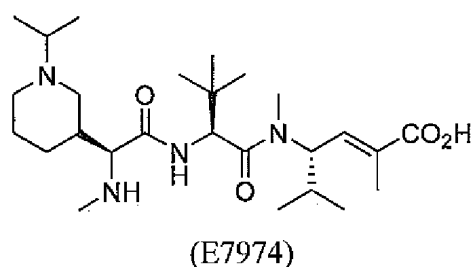


in patients with solid malignancies," presented at American Society of Clinical Oncology Annual Meeting; June 1-5, 2007; Chicago, Ill; both summarized in Rocha-Lima *et al.*, *Cancer*, September 1, 2012 pp. 4262-4270). However, no results have been reported for HTI-286 to date.

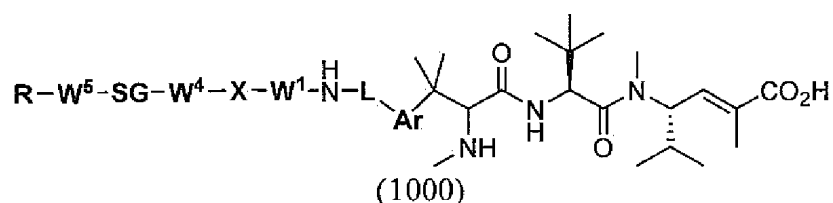


[0005] In addition, conjugation of HTI-286 at the C-terminus to a gastrin decapeptide VLALAEEEAYGWNleDF-NH₂ for tumor targeting is described in Tarsova *et al.*, United States patent application publication number US 2005/0171014 A1. Reported activity, however, was very weak.

SUMMARY

[0006] Provided herein are hemiasterlin derivatives, conjugates thereof, compositions comprising the derivatives or conjugates thereof, methods of producing the derivatives and conjugates thereof, and methods of using the derivatives, conjugates, and compositions for the treatment of cell proliferation. The derivatives, conjugates, and compositions are useful in methods of treatment and prevention of cell proliferation and cancer, methods of detection of cell proliferation and cancer, and methods of diagnosis of cell proliferation and cancer.

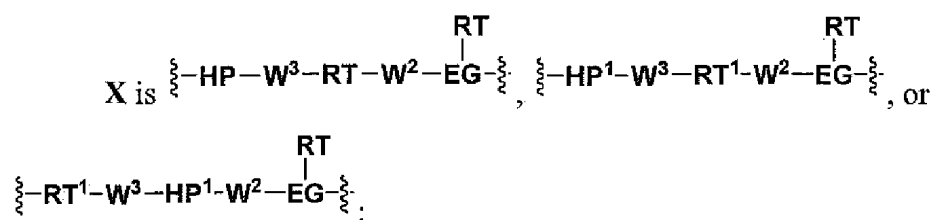
[0007] In one aspect, the present invention refers to a compound according to Formula 1000:



or a pharmaceutically acceptable salt, solvate, stereoisomer, or tautomer thereof, wherein:

Ar is a divalent five- or six-membered, substituted or unsubstituted, monocyclic aryl or heteroaryl ring or a divalent eight-, nine- or ten-membered, substituted or unsubstituted, fused bicyclic aryl or heteroaryl ring;

L is absent or -CH₂-;



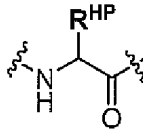
W^1 , W^2 , W^3 , W^4 , and W^5 are each independently a single bond, absent, or a divalent attaching group;

EG is absent or an eliminator group;

each RT is a release trigger group, in the backbone of Formula 1000 or bonded to EG , wherein each RT is optional;

RT^1 is a release trigger group, or a cleavable linker, or RT^1 is absent;

HP is a single bond, absent, or a divalent hydrophilic group;

HP^1 is a single bond, absent, a divalent hydrophilic group, or  where R^{HP} is a monovalent hydrophilic group;

SG is a single bond, absent, or a divalent spacer group; and

R is hydrogen, a terminal conjugating group, or a divalent residue of a terminal conjugating group;

or, in the alternative, W^1 , W^2 , W^3 , W^4 , W^5 , EG , RT , HP , SG , and R combine to form $-\text{H}$.

[0008] In one aspect, the present invention refers to a conjugate comprising the compound as described herein, or a pharmaceutically acceptable salt, solvate, or tautomer thereof, linked to a second compound. In one example, there is disclosed a conjugate comprising a compound described herein (e.g., a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2, 101-111b, or 1-8b) linked to a second compound.

[0009] In another aspect, the present invention refers to a pharmaceutical composition comprising the compound or conjugate as described herein; and a pharmaceutically acceptable excipient, carrier, or diluent. In one example, there is disclosed a pharmaceutical composition comprising:

a compound (e.g., a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2, 101-111b, or 1-8b) or conjugate (e.g., a conjugate according to any of Formulas C1-C17b, E1, F1-F17b, and G1-G17b) as described herein; and

a pharmaceutically acceptable excipient, carrier, or diluent.

[0010] In yet another aspect, the present invention refers to the compound, conjugate, or pharmaceutical composition as described herein for use in therapy. In a further aspect, the present invention refers to use of an effective amount of the compound, conjugate, or pharmaceutical composition as described herein in the manufacture of a medicament for inhibiting tubulin polymerization. In one example, there is disclosed a method of inhibiting tubulin polymerization in a subject in need thereof comprising administering an effective amount of a compound (*e.g.*, a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2, 101-111b, or 1-8b), conjugate (*e.g.*, a conjugate according to any of Formulas C1-C17b, E1, F1-F17b, and G1-G17b), or composition comprising a compound or conjugate, as described herein, to the subject.

[0011] In one aspect, the present invention refers to use of an effective amount of the compound, conjugate, or pharmaceutical composition as described herein in the manufacture of a medicament for treating cell proliferation or cancer. In one example, there is disclosed a method of treating cell proliferation or cancer in a subject in need thereof comprising administering an effective amount of a compound (*e.g.*, a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2, 101-111b, or 1-8b), conjugate (*e.g.*, a conjugate according to any of Formulas C1-C17b, E1, F1-F17b, and G1-G17b), or composition comprising a compound or conjugate, as described herein, to the subject.

[0012] In another aspect, the present invention refers to a method of producing a conjugate, comprising contacting the compound as described herein with a second compound under conditions suitable for conjugating the compound as described herein with the second compound; wherein the second compound comprises an alkyne, strained alkene, tetrazine, thiol, maleimide, carbonyl, oxyamine, or azide. In one example, there is disclosed a method of producing a conjugate, comprising contacting a compound described herein (*e.g.*, a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2 or 101-111b) with a second compound under conditions suitable for conjugating the second compound with the compound described herein (*e.g.*, a compound according to any of Formulas 1000-1000b, 1001-1001b, 1002-1002b, and I-XIXb-2 or 101-111b); wherein the second compound comprises a modified amino acid comprising an alkyne, strained alkene, tetrazine, thiol, maleimide, carbonyl, oxyamine, or azide.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Figure 1 provides results of a cell killing assay described in detail herein. Racemic [R/S,S,S] Compound 1 is evaluated as a conjugate on the trastuzumab heavy chain at F404 and also on the light chain at S7. All other conjugates in Figure 1 are on the trastuzumab heavy chain at F404. The relative cell viability of SKBR3 cells is plotted against concentration for trastuzumab (exes), trastuzumab F404 [S,S,S] Compound 1 conjugate (filled squares), trastuzumab F404 racemic [R/S,S,S] Compound 1 conjugate (split squares), trastuzumab S7 racemic [R/S,S,S] Compound 1 conjugate (split circles), and a trastuzumab auristatin (MMAF) conjugate (open triangles).

[0014] Figure 2a provides results of a cell killing assay described in detail herein. In Figure 2a relative cell viability is plotted against concentration of [S,S,S] Compound 1 (filled

squares) and [R,S,S] Compound 1(open squares) for SKBR3 cells in panel (a), MDA-MB-453 cells in panel (b), and MDA-MB-468 cells in panel (c).

[0015] Figure 2b provides results of a cell killing assay described in detail herein. In Figure 2b relative cell viability is plotted against concentration of [S,S,S] Compound 1 (filled squares) and [R,S,S] Compound 1(open squares) for HTC116 cells in panel (a), HT29 cells in panel (b), and SKCO1 cells in panel (c).

[0016] Figure 2c provides results of a cell killing assay described in detail herein. In Figure 2c relative cell viability is plotted against concentration of [S,S,S] Compound 1 (filled

65. The conjugate of any of claims 35-62, wherein **COMP** is a residue of an antibody chain.

66. A pharmaceutical composition comprising the compound or conjugate of any one of claims 1-65; and a pharmaceutically acceptable excipient, carrier, or diluent.

67. The compound, conjugate, or pharmaceutical composition of any of claims 1-66 for use in therapy.

68. Use of an effective amount of the compound, conjugate, or pharmaceutical composition of any one of claims 1-66 in the manufacture of a medicament for inhibiting tubulin polymerization.

69. Use of an effective amount of the compound, conjugate, or pharmaceutical composition of any one of claims 1-66 in the manufacture of a medicament for treating cell proliferation or cancer.

70. The use of claim 69 where the cancer is small cell lung cancer, non-small cell lung cancer, ovarian cancer, platinum-resistant ovarian cancer, ovarian adenocarcinoma, endometrial cancer, breast cancer, breast cancer which overexpresses Her2, triple-negative breast cancer, a lymphoma, large cell lymphoma; diffuse mixed histiocytic and lymphocytic lymphoma; follicular B cell lymphoma, colon cancer, colon carcinoma, colon adenocarcinoma, colorectal adenocarcinoma, melanoma, prostate cancer, or multiple myeloma.

71. A method of producing a conjugate, comprising contacting the compound of any of claims 1-34 with a second compound under conditions suitable for conjugating the compound of any of claims 1-34 with the second compound; wherein the second compound comprises an alkyne, strained alkene, tetrazine, thiol, maleimide, carbonyl, oxyamine, or azide.

72. The method of claim 71, wherein the second compound comprises a tetrazine; and **R** comprises a strained alkene.

73. The method of claim 71 or 72, wherein **R** is:

